Supplementary Information

Bioinspired Bifunctional Catalyst: Amphiphilic Organometallic Catalyst for Ring-Closing Metathesis Forming Liquid Droplets in Aqueous Media

Miki Mori,[a] Hiroka Sugai,[b] Kohei Sato,[a, ‡] Asuki Okada,[c] Takashi Matsuo,[c]

and Kazushi Kinbara*[a, b]

[a]*School of Life Science and Technology, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan. E-mail: kinbara.k.aa@m.titech.ac.jp*
[b]*Research Center for Autonomous Systems Materialogy (ASMat), Institute of Innovative Research, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan*
[c]*Division of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama-cho, Ikoma, Nara 630-0192, Japan*

[‡]*Present address: Department of Chemistry, School of Science, Kwansei Gakuin University, 1 Gakuen Uegahara, Sanda-shi, Hyogo 669-1330, Japan*

Table of Contents

1. General

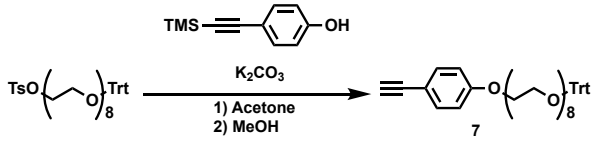
Unless otherwise noted, all commercial reagents were used as received. ^{1}H and ^{13}C NMR spectra were recorded on Varian model 400-MR spectrometer or JEOL model JNM-ECZL400S spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The chemical shifts were determined with respect to tetramethylsilane (TMS) or a residual non-deuterated solvent as an internal reference. Electrospray ionization time-of-flight (ESI-TOF) mass spectrometry was performed on a Bruker model microTOF Ⅱ spectrometer. High-performance liquid chromatography (HPLC) was performed at room temperature using a 10 mm I.D. x 250 mm or 20 mm I.D. x 250 mm Cosmosil 5SL-Ⅱ packed column on a Shimadzu HPLC prominence system, equipped with a UV-Vis detector. Dynamic light scattering (DLS) was performed on a Malvern model Zetasizer Nano ZSP spectrophotometer using a disposable plastic micro cuvette (ZEN0040). Confocal laser scanning microscopic observation and phase contrast microscopic observation were carried out on an Olympus model FV1000 microscope. A 0.1 mm thick silicon-based spacer was placed between a cover glass and a coverslip for imaging and micrographs were analyzed using ImageJ.[S1]

2. Materials

Potassium hexamethyldisilazide (KHMDS; 0.5 M toluene solution), Grubbs 1st Generation catalyst, 1,6-heptadiene-4-ol, Hoveyda-Grubbs 2nd generation catalyst (**HGII**), and Amberlite® IRA 410 chloride form were purchased from Sigma Aldrich. Octaethylene glycol monododecyl ether, diethyl diallylmalonate, and Nile Red were purchased from TCI. *N*,*N*-diallyl-4-methylbenzenesulfonamide was purchased from BLD Pham. Triton X-100 was purchased from Nacalai Tesque.

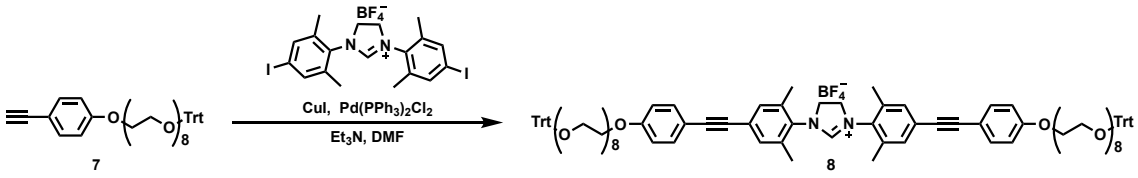
3. Synthesis

1) Synthesis of **7**



To a solution of Trt-PEG$_8$-Ts[S2,S3] (1.43 g, 1.86 mmol) in dry acetone (16 mL) were added 4-((trimethylsilyl)ethynyl)phenol (535 mg, 1.86 mmol) and K_2CO_3 (1.25 g, 9.04 mmol) at room temperature under Ar, and the resulting mixture was refluxed at 60 ºC for 6 h. The reaction mixture was evaporated and dried under reduced pressure. Dry MeOH (14 mL) was added to the residue under Ar, and the resulting solution was stirred at room temperature for 14 h. The reaction mixture was evaporated to dryness under reduced pressure. Sat. $NaHCO_3$ aq. was added, and the resulting mixture was then extracted with $CHCl_3$ for three times. The organic extract was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography with Hex/EtOAc (2/8 to 0/10) to afford **7** (1.31 g, 1.83 mmol) in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$ containing 0.03% TMS) δ 7.47-7.44 (m, 6H), 7.40 (d, J = 8.8 Hz, 2H), 7.30-7.26 (m, 6H), 7.21 (t, J = 7.2 Hz, 3H), 6.84 (d, J = 8.8 Hz, 2H), 4.10 (t, J = 4.6 Hz, 2H), 3.83 (t, J = 4.6 Hz, 2H), 3.72-3.61 (m, 26H), 3.23 (t, J = 5.2 Hz, 2H), 2.99 (s, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$ containing 0.03% TMS) δ 159.0, 144.0, 133.4, 128.8, 128.6, 127.6, 126.8, 114.4, 114.2, 86.4, 83.5, 77.2, 75.8, 70.7, 70.6, 70.6, 70.5, 70.5, 70.4, 69.5, 67.3, 63.2 ppm; HRMS (ESI-TOF-MS) *m/z* calculated for $C_{43}H_{52}O_9Na$ $[M+Na]^+$: 735.3504; found: 735.3525.

2) Synthesis of **8**

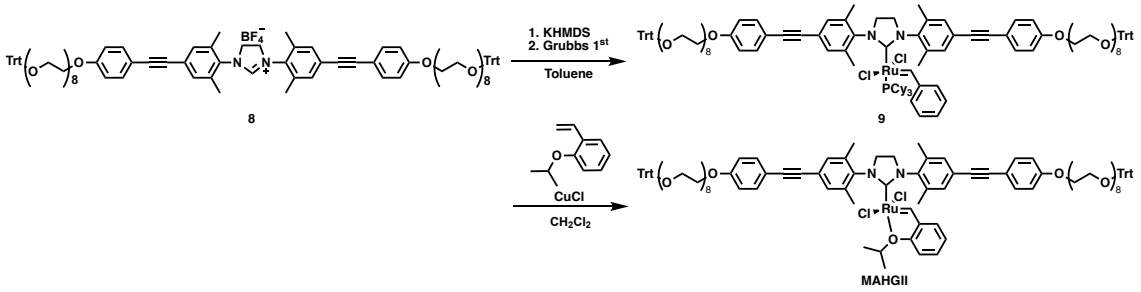


To a degassed solution of **7** (502 mg, 0.70 mmol) in a mixture of dry Et_3N (2.8 mL) and dry DMF (2.0 mL) were added $Pd(PPh_3)_2Cl_2$ (17 mg, 0.024 mmol), CuI (5 mg, 0.026 mmol), and 1,3-bis(4-iodo-2,6-dimethylphenyl)-4,5-dihydro-1*H*-imidazol-3-ium tetrafluoroborate (162 mg, 0.26 mmol) under Ar and the resulting mixture was stirred for 2.5 h at room temperature. The reaction mixture was dried under reduced pressure and the residue was subjected to column chromatography on silica gel with $CHCl_3$/MeOH (96/4). The brown gummy crude product was purified by normal phase HPLC to afford **8** in 91% yield (426 mg, 0.24 mmol). ^{1}H NMR(400 MHz, $CDCl_3$ containing 0.03% TMS, 25 ºC) δ 8.66 (s, 1H), 7.50-7.43 (m, 16H), 7.32-7.20 (m, 22H), 6.90 (d, *J* = 8.8 Hz, 4H), 4.75 (br, 4H), 4.14 (t, *J* = 4.8 Hz, 4H), 3.86 (t, *J* = 4.6 Hz, 4H), 3.75-3.61 (m, 52H), 3.22 (t, *J* = 5.2 Hz, 4H), 2.47 (s, 12H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$ containing 0.03% TMS, 25ºC) δ 159.1, 158.3, 143.9, 135.5, 133.1, 131.9, 131.7, 128.5, 127.6, 126.8, 125.9, 114.6, 91.4, 86.7, 86.3, 77.2, 70.7, 70.6, 70.5, 70.5, 70.4, 70.4, 69.4, 67.3, 63.1, 52.2, 18.5 ppm; HRMS (ESI-TOF-MS) *m/z* calculated for $C_{105}H_{123}N_2O_{18}$ $[M–BF_4]^+$: 1699.8765; found:1699.8787.

3) Ion exchange of **8**

The Amberlite® IRA-410 chloride form (16 g) was sequentially washed with H_2O, sat. NH_4BF_4 aq., and H_2O. Then the eluent was exchanged to H_2O/acetone/MeOH (1/1/1), and subsequently to acetone/MeOH (1/1). Then, the acetone/MeOH (1/1) solution of **8** (310 mg) was flushed through the resin. The solvent was evaporated and dried under reduced pressure to afford 278 mg of ion exchanged **8**.

4) Synthesis of **MAHGII**



Compound **8** (278 mg) was dried overnight with molecular sieve 3A in dry CH_2Cl_2 (6 mL) under Ar. The resulting solution was transferred to a Schlenk flask using cannula, which was dried under reduced pressure and lyophilized prior to use. Then, to a solution of **8** (278 mg, 0.16 mmol) in dry toluene (1.0 mL) in a Schlenk flask was added 0.5 M KHMDS in dry toluene (0.35 mL, 0.18 mmol), and the resulting mixture was stirred for 5 min at room temperature under Ar. To the reaction mixture was added Grubbs 1st generation catalyst (121 mg, 0.15 mmol), and the resulting mixture was stirred at 50 ºC for 3 h under a gentle stream of Ar. After the solvent was evaporated, the residue was dried under reduced pressure and subjected to column chromatography on silica gel with $CHCl_3$/MeOH (98/2) and to afford a red gummy crude product containing **9**. While the ^{1}H NMR spectrum of the crude mixture suggested the presence of **9** and a byproduct (hydrolyzed compound of **8** generated from unreacted carbene), this mixture was used without further purification. To a solution of the crude mixture of **9** (233 mg) in dry CH_2Cl_2 (5 mL) were added 2-isopropoxystyrene (46 mg, 0.28 mmol) and CuCl (22 mg, 0.22 mmol), and the resulting mixture was refluxed at 45 ºC for 1.5 h under Ar. The solvent was evaporated to dryness under reduced pressure. The resulting residue was subjected to column chromatography on silica gel with $CHCl_3$/MeOH (98/2). The gummy product was further purified by normal phase HPLC to allow isolation of **MAHGII** (52 mg, 0.026 mmol) as a green gummy solid in 17% yield over 2 steps. ^{1}H NMR (400 MHz,

$CDCl_3$ containing 0.03% TMS) δ 16.59 (s, 1H), 7.52-7.44 (m, 17H), 7.42 (s, 4H), 7.33-7.27 (m, 12H), 7.24-7.18 (m, 6H), 7.05-7.03 (dd, *J* = 7.6 Hz, 1.2 Hz 1H), 6.93-6.86 (m, 5H), 6.80 (d, *J* = 8.4 Hz, 1H), 4.96-4.89 (m, 1H), 4.19-4.13 (m, 8H), 3.88 (t, *J* = 4.8 Hz, 4H), 3.75-3.56 (m, 52H), 3.23 (t, *J* = 5.2 Hz, 4H), 2.52 (br, 12H), 1.32 (d, *J* = 6.4 Hz, 6H) ppm; ^{13}C NMR, (101 MHz, $CDCl_3$ containing 0.03% TMS) δ 212.1, 158.9, 152.3, 145.1, 144.0, 133.1, 131.5, 129.8, 128.6, 127.7, 126.8, 124.3, 123.0, 122.4, 115.3, 114.6, 112.8, 90.0, 87.7, 86.4, 77.2, 75.2, 70.8, 70.7, 70.6, 70.6, 70.5, 70.5, 70.5, 69.5, 67.4, 63.2, 51.3, 21.1, 19.4 ppm; HRMS (ESI-TOF-MS) *m/z* calculated for $C_{115}H_{134}Cl_2N_2O_{19}RuNa$ $[M+Na]^+$: 2041.7894; found: 2041.7882.

4. Analytical data

1) NMR spectra

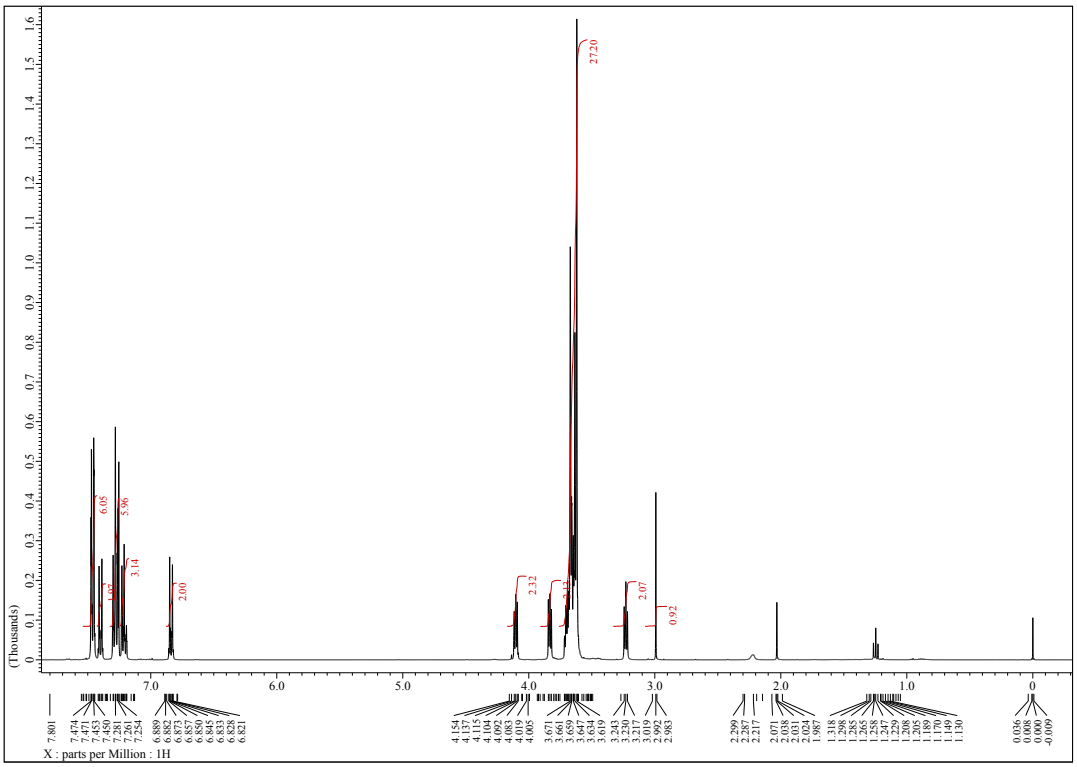


Fig. S1 ^1H NMR spectrum of **7** in $CDCl_3$.

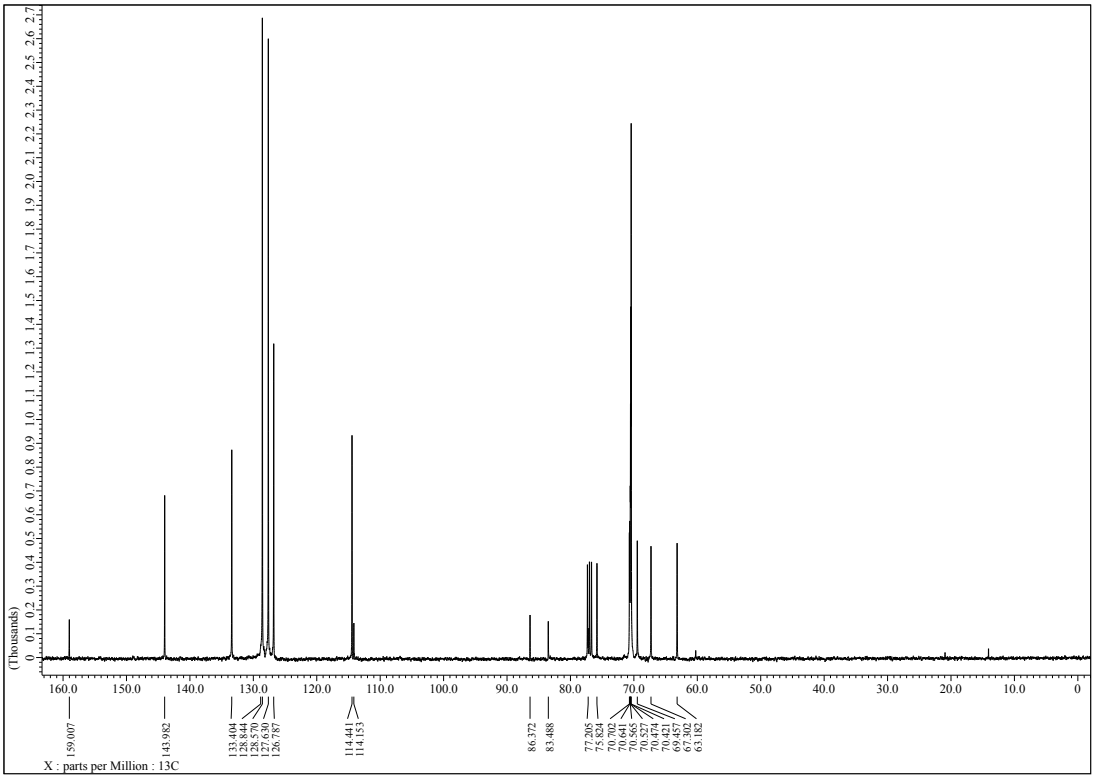


Fig. S2 ^{13}C NMR spectrum of **7** in $CDCl_3$.

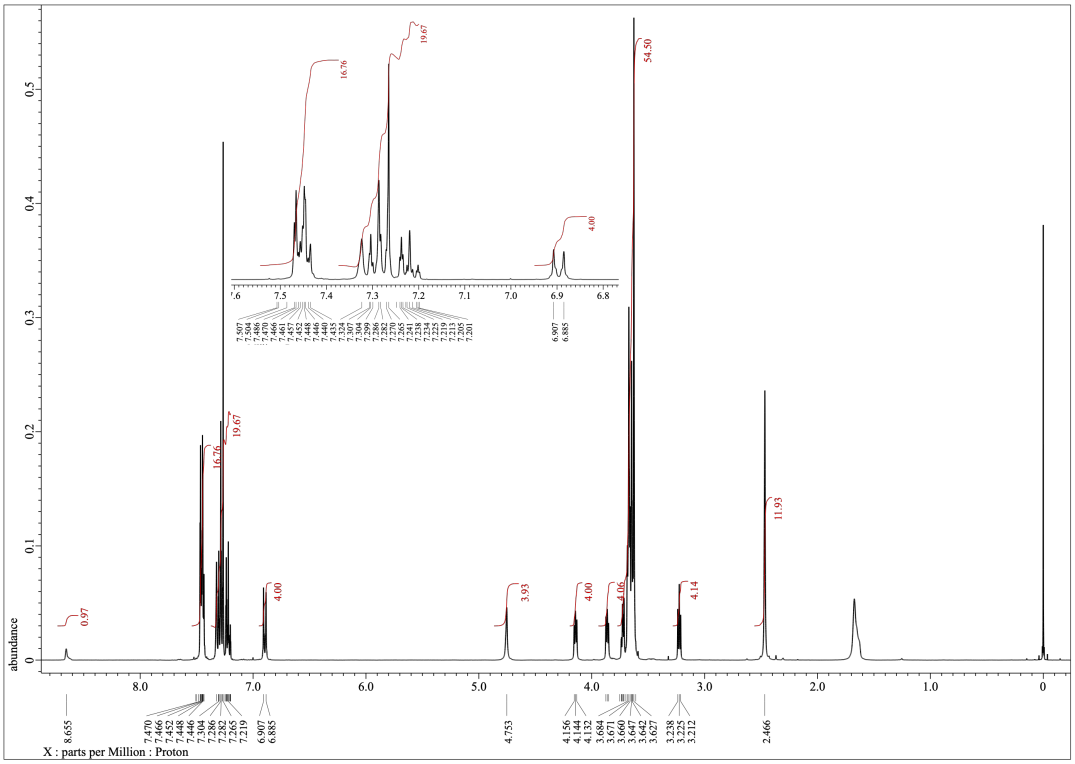


Fig. S3 ^{1}H NMR spectrum of **8** in $CDCl_3$.

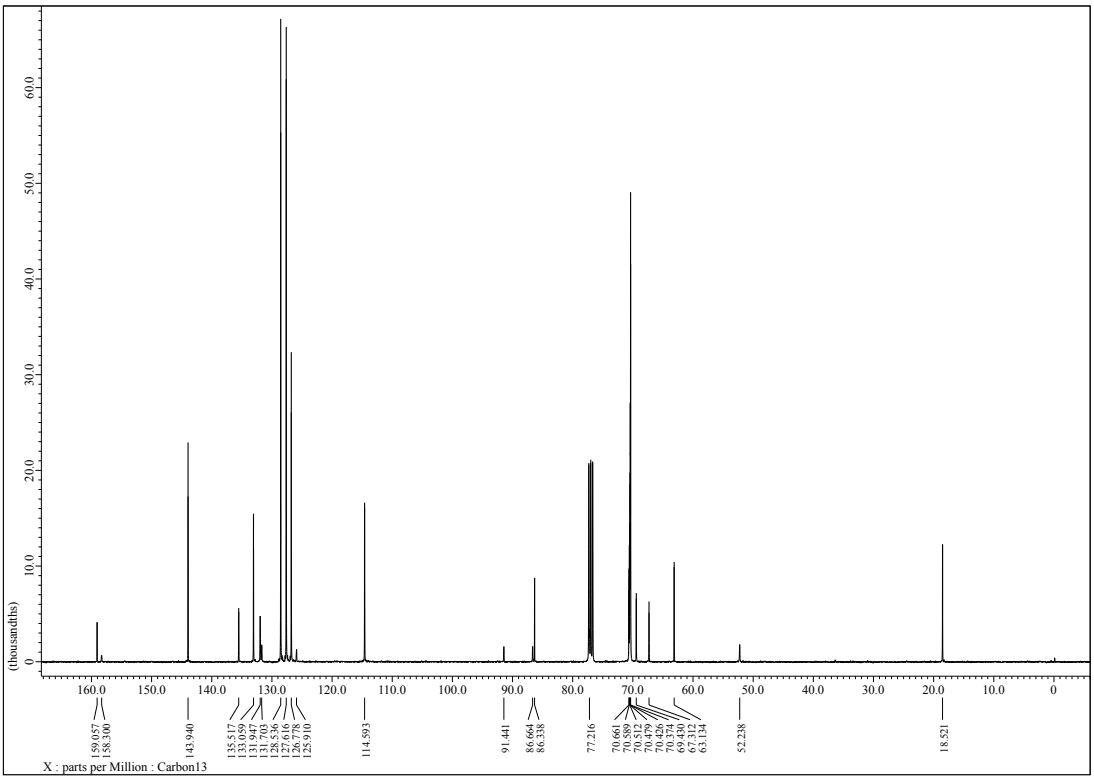


Fig. S4 ^{13}C NMR spectrum of **8** in $CDCl_3$.

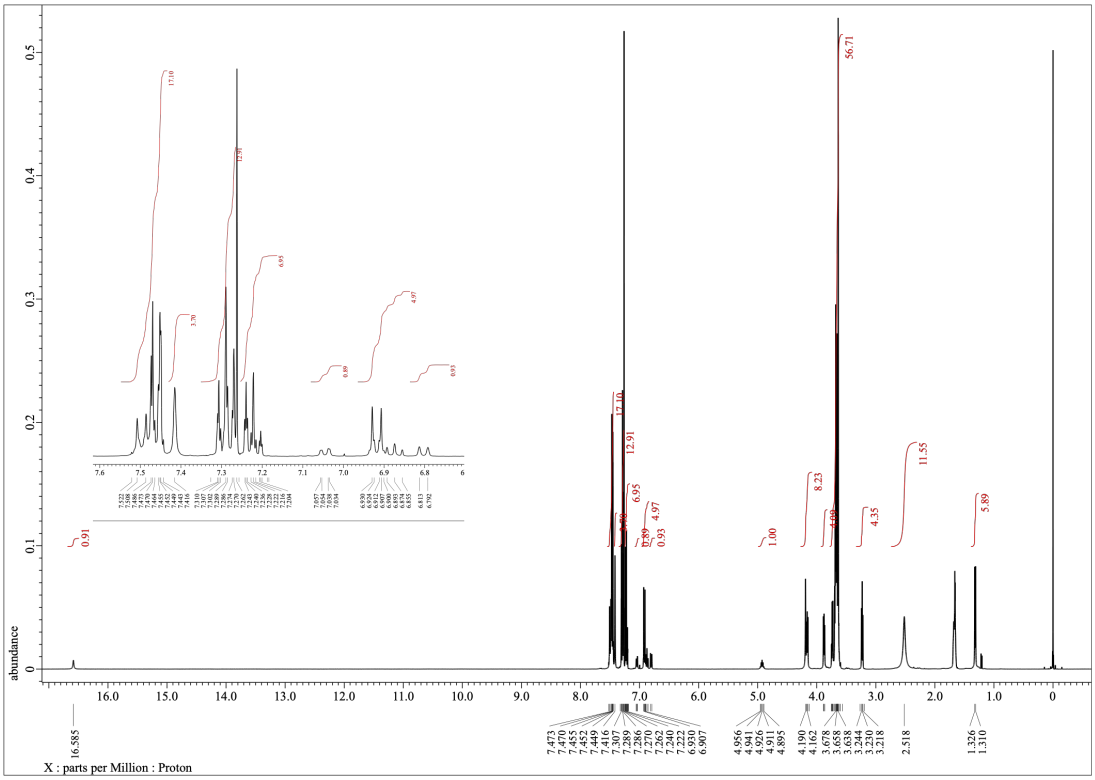


Fig. S5 ^{1}H NMR spectrum of **MAHGII** in $CDCl_3$.

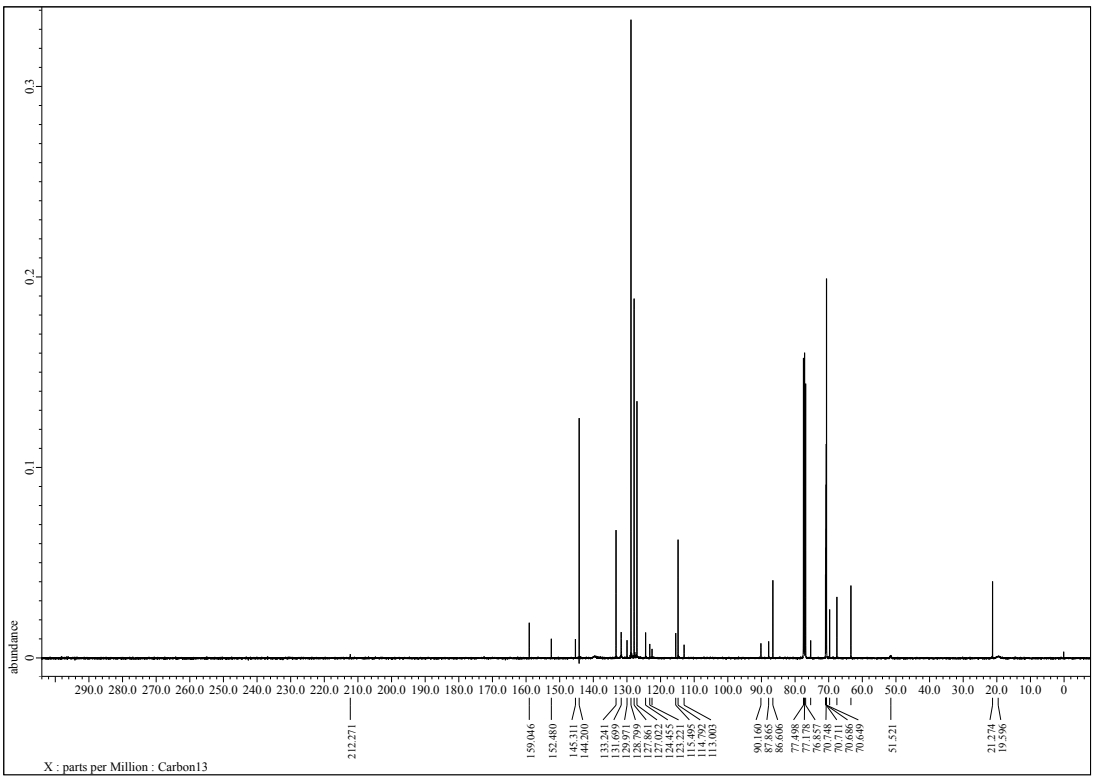


Fig. S6 ^{13}C NMR spectrum of **MAHGII** in $CDCl_3$.

2) High-resolution mass spectrometry

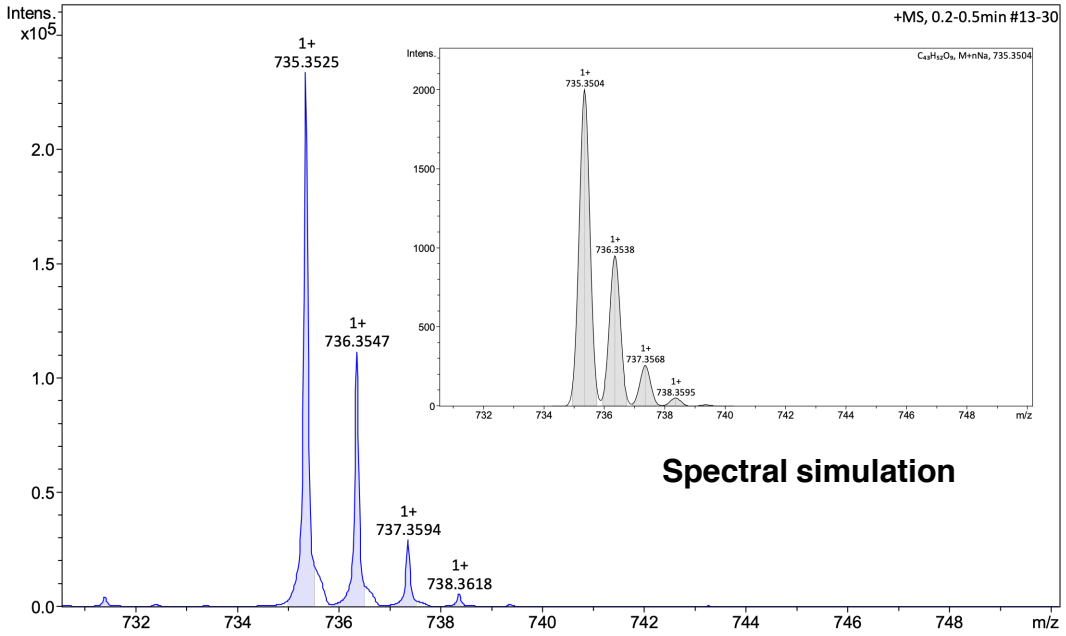


Fig. S7 High-resolution ESI-TOF-MS spectrum of **7** and simulated spectrum (inset).

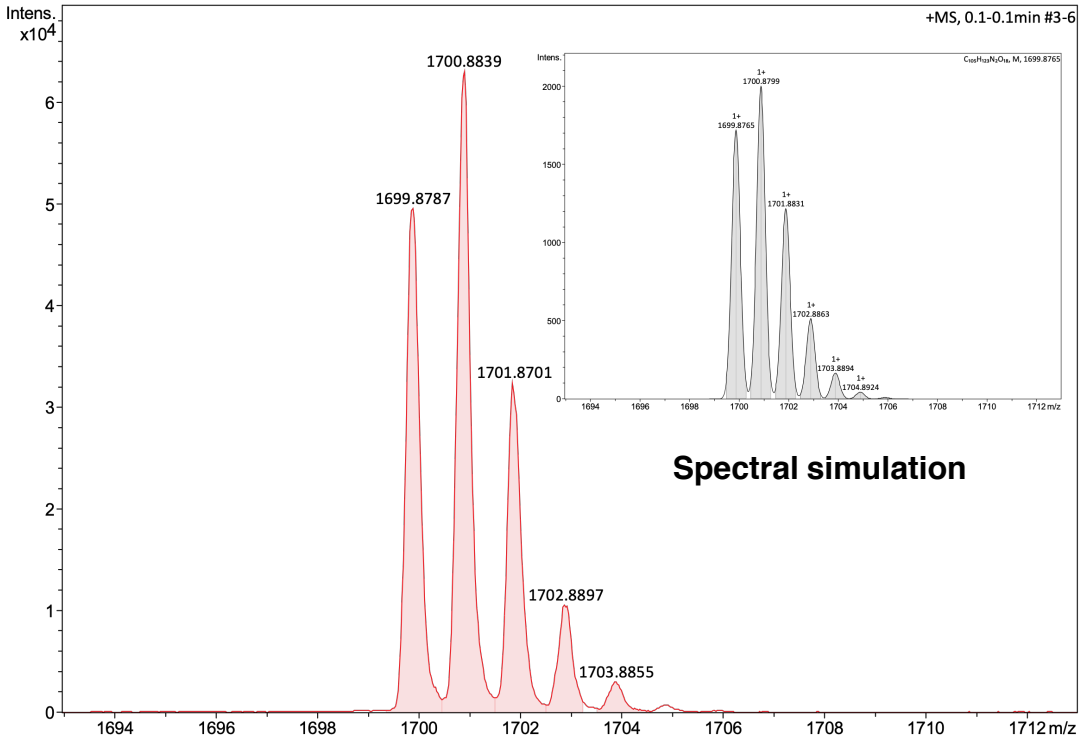


Fig. S8 High-resolution ESI-TOF-MS spectrum of **8** and simulated spectrum (inset).

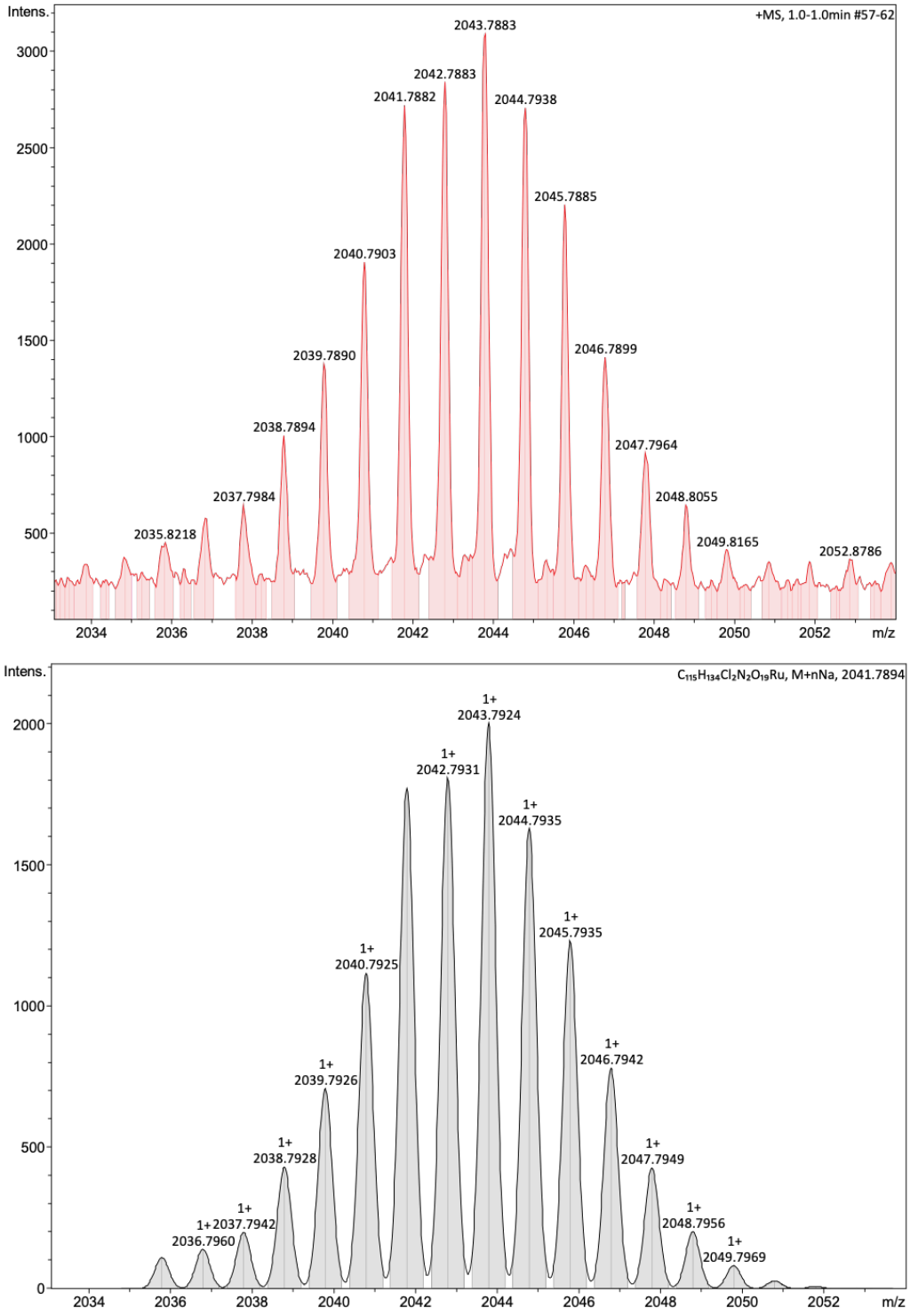


Fig. S9 High-resolution ESI-TOF-MS spectrum of **MAHGII** (top) and spectral simulation (bottom).

5. Methods

1) RCM reaction in CD_2Cl_2

To CD_2Cl_2 (400 μL) in an NMR tube was added a 50 mM CD_2Cl_2 solution (50 μL) of substrate and a 500 μM CD_2Cl_2 solution (50 μL) of a catalyst so that the final concentrations of substrate and catalyst were 5 mM and 50 μM, respectively. The reaction was monitored by ^1H NMR spectroscopy at room temperature under open air.

2) Phase contrast microscopy

To a mixture of 100 mM KCl aq. (200 μL) with 0.5 v/v% DMF was added a DMF solution (1 μL) of 40 mM **MAHGII** under stirring. The mixture was deposited on a glass slip with a silicone spacer and sandwiched with another cover glass slip for time-lapse imaging.

3) Confocal laser scanning microscopy

To an aqueous solution of 100 mM KCl (200 μL) was added a DMF solution (1 μL) of 40 mM **MAHGII** under stirring. 100 μL of the resulting dispersion was transferred to a vial followed by addition of 2 mM DMF solution (0.5 μL) of Nile Red. The mixture was deposited on a glass slip with a silicone spacer and sandwiched with another cover glass slip for the confocal laser microscopic observation at λ_{ex} = 559 nm, λ_{obsd} = 612 nm.

4) DLS measurement of **MAHGII** droplets in 100 mM KCl aq.

To an aqueous solution of 100 mM KCl (150 μL) containing 0 or 2 mM surfactant was added a DMF solution (1.5 μL) of 20 mM **MAHGII** under stirring. The resulting suspension was transferred to a 40 μL disposable plastic microcuvette, and an intensity-based particle size profile was obtained.

5) DLS measurement of **MAHGII** droplets in the presence of 1,6-heptadien-4-ol (**5**)

To an aqueous solution of 100 mM KCl (200 μL) was added a DMF solution (1 μL) of 40 mM **MAHGII**, followed by addition of a DMF solution (1 μL) of **5** (1 M) under stirring at room temperature. The resulting suspension was transferred to a 40 μL disposable plastic microcuvette, and an intensity-based particle size profile was obtained.

6) RCM reaction in 100 mM KCl D_2O in the presence and absence of surfactants

To a 0, 1, 2, or 4 mM solution (500 μL) of a surfactant in 100 mM KCl D_2O was added a DMF solution (2.5 μL) of 40 mM **MAHGII** or **HGII** and the resulting mixtures were stirred for 10 min at room temperature. To the resulting mixture was added 1 M DMF solution (2.5 μL) of **5** and the resulting suspension was heated to 50 ºC for 4 h on the water bath under open air without stirring. The reaction mixture was cooled down to room temperature and transferred to an NMR tube for ^{1}H NMR spectroscopy. The conversions for the reactions were calculated based on the relative integration values of allylic protons of **5** and **6**, where other compounds than the starting material and RCM product were not detected.

6. Supplementary data

1) Catalytic activity of **HGII** in CD_2Cl_2

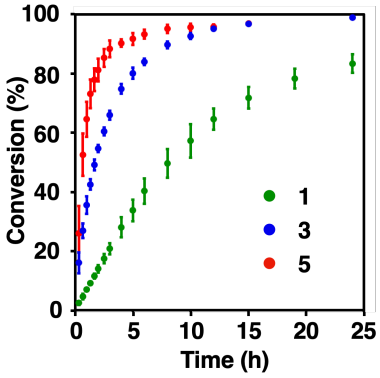


Fig. S10 Time versus conversion profile of RCM reaction of **1** (green), **3** (blue) and **5** (red) catalyzed by commercially available Hoveyda-Grubbs 2nd generation catalyst (**HGII**) under the same reaction conditions for that with **MAHGII** described in the main text. [**HGII**] = 50 μM, [substrates] = 5 mM in CD_2Cl_2 at room temperature. All reactions were performed in triplicate and the error bars represent the standard deviation.

2) Distribution of Nile Red in **MAHGII** droplets.

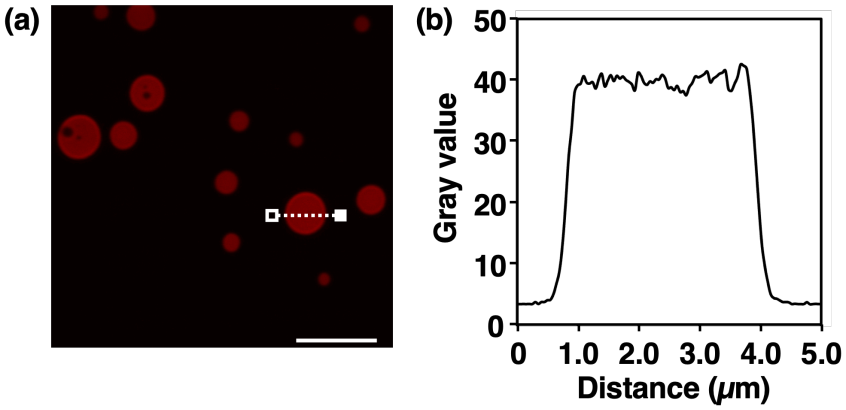


Fig. S11 (a) Confocal laser scanning microscopic image of Nile Red accumulated in **MAHGII** droplets. [**MAHGII**] = 200 μM, [Nile Red] = 10 μM in 100 mM KCl aq. with 1 v/v% DMF. Scale bar: 5 μm. (b) Gray values of Nile Red emission obtained along the white dotted line from the empty to filled squares in (a).

3) Microscopic image of **MAHGII** droplets in the presence of substrate **5**.

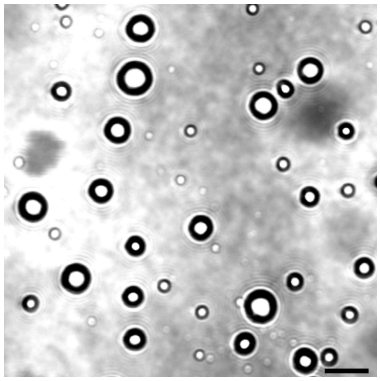


Fig. S12 Phase contrast micrograph of **MAHGII** droplet in the presence of **5**. [**MAHGII**] = 200 μM, [**5**] = 5 mM in 100 mM KCl aq. with 1 v/v% DMF.

4) DLS profiles of substrate **5** and a mixture of **MAHGII** and **5**

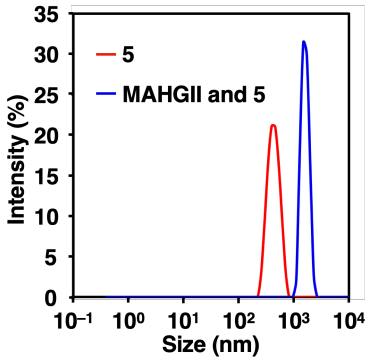


Fig. S13 DLS profile of substrate **5** (red) and **MAHGII** in the presence of **5**. [**MAHGII**] = 200 μM, [**5**] = 5 mM in 100 mM KCl aq. with 1 v/v% DMF.

5) Catalytic activity of **HGII** in the presence and absence of surfactants in aqueous environments

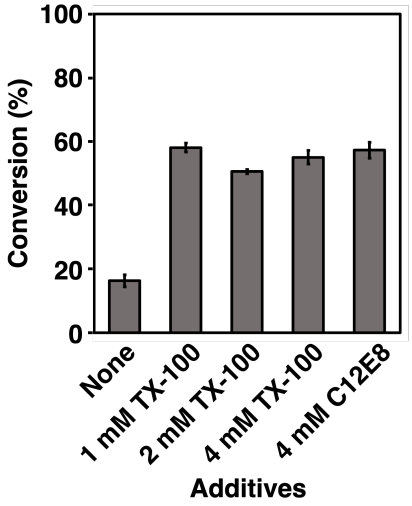


Fig. S14 Conversions for RCM reactions of **5** catalyzed by **HGII** in the presence and absence of surfactants in 100 mM KCl D_2O with 1 v/v% DMF.

6) ^{1}H NMR spectra of RCM reactions of **5** in $CDCl_3$

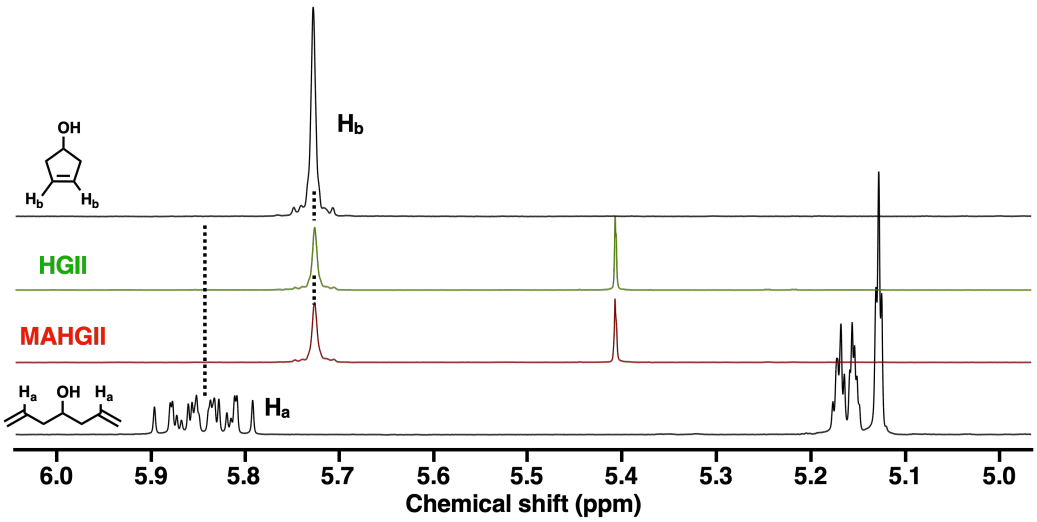


Fig. S15 ^{1}H NMR spectra after RCM reaction of 5 mM substrate **5** with 4 mol% **MAHGII** (red) or **HGII** (green) in $CDCl_3$ at 50 ºC for 4 h without stirring under open air. The spectra at the bottom and the top show the chemical shifts of allylic protons of substrate (**5**) and product (**6**), respectively with the indication of allylic protons H_a and H_b at the left of spectra, which were used to evaluate the conversions.

7. References

S1 W. S. Rasband, *ImageJ*, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2012.

S2 A. M. Wawro, T. Muraoka and K. Kinbara, *Polym. Chem.* 2016, **7**, 2389–2394.

S3 A. M. Wawro, T. Muraoka, M. Kato and K. Kinbara, *Org. Chem. Front.* 2016, **3**, 1524–1534.