Electronic Supplementary Information

Title Self-assembly and binding properties of a metallomacrocycle having two interactive binding subcavities

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Contents

1. Syntheses of Ligand and Metallomacrocycle

General Experimental Methods: All reagents were, unless otherwise noted, used as purchased. Chloroform and dichloromethane were distilled under nitrogen from phosphorus pentoxide, acetonitrile from calcium hydride, tetrahydrofuran from Na/benzophenone, and pyridine, triethylamine and *N*,*N*-diisopopylethylamine from KOH. Melting points were uncorrected. All NMR spectra were recorded on a DRX-500 spectrometer and chemical shifts were reported in ppm downfield relative to TMS (0 ppm) for ^{1}H NMR, and the residual solvent peak ($CHCl_3$: 77 ppm) for ^{13}C NMR spectrum. Infrared spectra were obtained with a Nicolet impact 410 FT-IR spectrometer and ESI-mass spectra were obtained with a VG Quattro mass spectrometer.

***N*-(4-iodo-2,6-diisopropylphenyl)-*N'*-(3,5-dimethylpyridin-4-yl)pyridine-2,6-dicarboxamide (5):** To a solution of pyridine-2,6-dicarbonyl dichloride **2** (2.89 g, 14.2 mmol) in CH_2Cl_2 (30 mL) was added dropwise a solution of 4-iodo-2,6-diisopropylaniline **3**[1] (4.30 g, 14.2 mmol, 1 equiv) and *N*,*N*-diisopropylethylamine (5.00 mL, 28.6 mmol, 2 equiv) in CH_2Cl_2 (30 mL) for 1.5 h at 0 ^{o}C (ice-water bath) under argon, and he solution was stirred for 4 h at room temperature. 4-Amino-3,5-lutidine **4**[2] (1.73 g, 14.2 mmol, 1 equiv) was added at 0 ^{o}C then the mixture was stirred at room temperature for 3 h. The organic solution was washed with saturated $NaHCO_3$ solution and brine, and dried over anhydrous $MgSO_4$. After concentration, the residue was purified by column chromatography ($CHCl_3$:Acetone=1:1) to give **5** as a white solid (5.15 g, 65%): mp 270–271 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.54 (s, 1H; NH), 9.05 (s, 1H; NH), 8.57 (d, *J* = 7.8 Hz, 1H), 8.51 (d, *J* = 7.8 Hz, 1H), 8.27 (s, 2H), 8.19 (t, *J* = 7.8 Hz, 1H), 7.53 (s, 2H), 3.12 (m, 2H), 2.21 (s, 6H), 1.21 (d, *J* = 6.8 Hz, 12H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 162.9, 161.0, 149.5, 149.2, 148.8, 148.2, 141.6, 139.9, 133.3, 131.1, 130.0, 126.7, 126.0, 95.2, 29.1, 23.7, 15.7; IR (KBr) 3287, 1693 cm^{-1}; Anal. Calcd for $C_{26}H_{29}IN_4O_2$: C, 56.12; H, 5.25; N, 10.07. Found: C, 56.50; H, 5.28; N, 9.96.

***N*-(4-(triisopropylsilyl-ethynyl)-2,6-diisopropylphenyl)-*N'*-(3,5-dimethylpyridin-4-yl)pyridine-2,6-dicarboxamide (6)**: Compound **5** (3.50 g, 6.28 mmol), PPh_3 (164 mg, 0.63 mmol, 0.1 equiv), CuI (24 mg,

(1) S. Kajigaeshi, T. Kakinami, H. Yamasaki, S. Fujisaki and T. Okamoto, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 600-602.
(2) (a) M. Malinowski and Ł. Kaczmarek, *J. Prakt. Chem.* **1988**, 154-158. (b) J. M. Essery and K. Schofield, *J. Chem. Soc.* **1960**, 4953-4959.

0.13 mmol, 0.02 equiv), triisopropylsilylacetylene (1.26 mL, 6.28 mmol, 1 equiv) and bis(dibenzylideneacetone) palladium(0) (76 mg, 0.13 mmol, 0.02 equiv) were added to a Schlenk tube. The solution was degassed by evacuating air then flushing N_2 gas (3 times). THF (30 mL) and Et_3N (30 mL) were added to the Schlenk tube and degassed again (3 times). The solution was stirred under nitrogen at 60–70 ^oC for 7 h. The reaction mixture was filtered through Celite and evaporated. The residue was dissolved in $CHCl_3$ (50 mL), and washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous $MgSO_4$. After concentration, the residue was purified by column chromatography ($CHCl_3$:Acetone=1:1) to give **6** as a white solid (3.24 g, 85%): mp 264–265 ^oC; ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.67 (s, 1H; NH), 9.06 (s, 1H; NH), 8.57 (d, *J* = 7.7 Hz, 1H), 8.50 (d, *J* = 7.7 Hz, 1H), 8.26 (s, 2H), 8.18 (t, *J* = 7.7 Hz, 1H), 7.31(s, 2H), 3.17 (m, 2H), 2.19 (s, 6H), 1.23 (d, *J* = 6.8 Hz, 12H), 1.15 (s, 21 H); ^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) 162.9, 161.0, 149.5, 149.3, 148.2, 146.4, 141.7, 139.8, 131.5, 130.0, 127.7, 126.6, 125.8, 123.9, 107.5, 90.5, 29.2, 23.7, 18.9, 15.6, 11.5; IR (KBr) 3346, 2159, 1684 cm^{-1}; Anal. Calcd for $C_{37}H_{50}N_4O_2Si$: C, 72.74; H, 8.25; N, 9.17. Found: C, 72.76; H, 8.41; N, 9.03.

***N*-(4-ethynyl-2,6-diisopropylphenyl)-*N'*-(3,5-dimethylpyridin-4-yl)pyridine-2,6-dicarboxamide (7)**: To a solution of **6** (3.24 g, 5.30 mmol) in THF (100 mL) and water (6.0 mL), 1.0 M THF solution of tetrabutylammonium fluoride (6.36 mL, 6.36 mmol, 1.2 equiv) was added. After being stirred at 70–72 ^oC for 12 h, the solution was concentrated and diluted with brine (100 mL). The residue was extracted with $CHCl_3$ (50 mL x 3). The organic layer was washed with brine, and dried over $MgSO_4$. After concentration, the residue was purified by column chromatography ($CHCl_3$:Acetone=1:1) to give the product **7** as a white solid (3.24 g, 85%): mp 268–269 ^oC (dec); ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.57 (s, 1H; NH), 9.06(s, 1H; NH), 8.57(d, *J* = 7.7 Hz, 1H), 8.51(d, *J* = 7.7 Hz, 1H), 8.28 (s, 2H), 8.19 (t, *J* = 7.7 Hz, 1H), 7.37 (s, 2H), 3.17 (m, 2H), 3.09 (s, 1H), 2.20 (s, 6H), 1.23 (d, *J* = 6.8 Hz, 12H); ^{13}C NMR(126 MHz, $CDCl_3$): δ (ppm) 162.9, 161.0, 149.6, 149.2, 148.2, 146.7, 141.6, 139.9, 131.9, 130.0, 127.9, 126.6, 126.0, 122.4, 84.0, 29.2, 23.7, 15.7; IR (KBr) 3334, 3253, 1696 cm^{-1}; Anal. Calcd for $C_{28}H_{30}N_4O_2$: C, 73.98; H, 6.65; N, 12.33. Found: C, 73.64; H, 6.72; N, 12.17.

Ligand (8): A solution of **7** (2.86 g, 6.29 mmol) and $Cu(OAc)_2$ (2.51 g, 12.6 mmol, 2.0 equiv) in pyridine (60 mL) was stirred at 60–65 ^oC for 20 h, then $Cu(OAc)_2$ (2.51 g, 12.6 mmol, 2.0 equiv) was added. After being stirred 4 h, ice water (100 mL) was added, and the mixture was extracted with $CHCl_3$ (30 mL x 3). The organic layer was washed with 25% acetic acid (100 mL) and 25% $NaHCO_3$ solution (120 mL), then dried over anhydrous $MgSO_4$. After concentration, the residue was purified by column chromatography (MeOH:$CHCl_3$:EtOAc=1:10:10) to give ligand **8** as a white solid (1.30 g, 85%): mp >300 ^oC; ^1H NMR (500 MHz, 97:3 $CDCl_3/CD_3CN$): δ (ppm); 9.66 (s, 2H; NH), 9.59 (s, 2H; NH), 8.52 (m, 4H), 8.38 (s, 4H), 8.19 (t, *J* = 7.7 Hz, 2H), 7.42 (s, 4H), 3.17 (m, 4H), 2.30 (s, 12H), 1.22 (d, *J* = 6.6 Hz, 24H); ^{13}C NMR (126 MHz, 97:3 $CDCl_3/CD_3CN$): δ (ppm) 162.7, 161.2, 149.3, 148.5, 148.1, 147.1, 141.7, 139.4, 132.5, 130.1, 127.8, 125.8, 125.6, 121.6, 81.8, 73.7, 28.8, 23.3, 15.2; IR (KBr) 3288, 1696 cm^{-1} ; HRMS-MALDI (*m/z*): $[M + H]^+$ calcd for $C_{56}H_{58}N_8O_4$, 907.466; found, 907.462.

Metallomacrocycle (1): A solution of ligand **8** (0.70 g, 0.77 mmol) and $Pd(dppp)OTf_2$[3] (0.63 g, 0.77 mmol, 1 equiv) in CH_2Cl_2 (10 mL) was stirred at room temperature for 4 h under argon then filtered. Hexane was added and the solidified product **1** was washed with hexane repeatedly. A pale yellow solid (1.30 g, 95 %): mp 263–264; ^1H NMR (500 MHz, 97:3 $CDCl_3/CD_3CN$): δ (ppm) 9.44 (s, 4H; NH), 9.31 (s, 4H; NH), 8.71 (s, 8H), 8.47 (d, *J* = 7.7 Hz, 4H), 8.41 (d, *J* = 7.7 Hz, 4H), 8.14 (t, *J* = 7.7 Hz, 4H), 7.63 (br s, 16H), 7.44-7.29 (m, 32H), 3.14 (br s, 8H), 2.98 (m, 8H), 2.22 (m, 4H), 2.02 (s, 24H), 1.09 (br s, 48H); ^{13}C NMR (126 MHz, 97:3 $CDCl_3/CD_3CN$): δ (ppm) 162.7, 160.6, 149.1, 148.7, 147.6, 145.1, 139.6, 133.9, 132.5, 129.6, 128.0, 126.3, 125.6, 125.2, 125.0, 124.8, 122.1, 116.5, 81.8, 74.6, 28.8, 23.4, 21.6, 17.5, 15.4; IR (KBr) 3265, 1684, 1158, 1099, 1025 cm^{-1}; ESI-MS *m/z* (% relative intensity, ion): 1574 (100, $[M - 2OTf^-]^{2+}$), 1000 (65, $[M - 3OTf^-]^{3+}$), 712 (70, $[M - 4OTf^-]^{4+}$); Anal. Calcd for $C_{170}H_{168}F_{12}N_{16}O_{20}P_4Pd_2S_4 \cdot 4H_2O$: C, 58.00; H, 5.04; N, 6.37; S, 3.64. Found: C, 57.78; H, 5.07; N, 6.27; S, 3.61.

(3) P. J. Stang, D. H. Cao, S. Saito and A. M. Arif, *J. Am. Chem. Soc.* **1995**, *117*, 6273-6283.

2. Binding Studies[4]

^{1}H NMR Titrations: Chloroform was stored over 4 Å molecular sieves, and filtered through basic alumina prior to use. A 5 mM solution of host (**1**) and a 50 mM solution of guest (**9**) in 97:3 $CDCl_3/CD_3CN$ (1.5–2.0 mL) were separately prepared at 23±1 °C. A 500 μL of the host solution was transferred to an NMR tube, and an initial NMR spectrum was taken to determine the initial chemical shift (δ_{free}) of the free host. Aliquots of the guest solution (10 μL initially, then 15–30 μL, and finally 50–160 μL) were added to the host solution. The spectrum was recorded after each addition and overall 20–21 data points were obtained. The association constants (K_1 and K_2) were determined by fitting the titration curves plotting chemical shift of the host NH signals against the concentration of the guest, using HOSTEST program.[5] EQNMR[6] program gave similar results.

Hill Plots: Hill coefficient(h) was obtained from the slope of the plotting log[Y/(1-Y)] versus $\log[G]_{free}$. Data from titration experiments were used as it is.

$$H + 2\,G \rightleftharpoons HG_2 \qquad K = \frac{[HG_2]}{[H][G]^2}$$

$$\log\frac{Y}{1-Y} = h\log[G]_{free} + \log K, \quad Y = \frac{[HG_2]}{2[H]_t}$$

$[G]_{free}$: concentration of the free guest, $[H]_t$: total concentration of the host

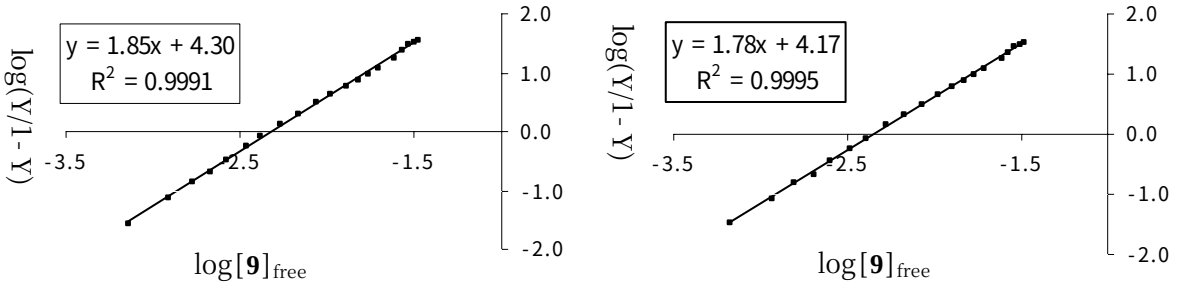


Figure S1. Hill plots of **1** and **9**, NH^1 (left) and NH^2 (right) were observed.

(4) (a) K. A. Connors, *Binding Constants*; John Wiley & Sons: New York, 1987. (b) H.-H. Schneider and A. K. Yatsimirsky, *Principles and Methods in Supramolecular Chemistry*; John Wiley & Sons; New York, 2000.
(5) C. S. Wilcox, and N. M. Glagovich, HOSTEST, v5.60; Department of Chemistry, The University of Pittsburgh: Pittsburgh, PA, 1997

Job's plots: Stock solutions of host **1**(10.0 mM) and guest **9** (10.0 mM) in 97:3 $CDCl_3/CD_3CN$ (4 mL) were prepared separately. Eleven NMR tubes were filled with total 500 μL solution of the host and guest in the following ratios (μL, host:guest): 500:0, 450:50, 400:100, 350:150, 300:200, 250:250, 200:300, 150:350, 100:400, 50:450, 0:500. The ^{1}H NMR spectra were obtained for each tube, and the host NH signal and guest aromatic proton signal were used to calculate the complex concentration, $[HG] = [H]_t \times [(\delta_{obsd} - \delta_{free}) / (\delta_{max} - \delta_{free})]$. These values were plotted against the mol fraction of the host and the resulting curve showed a maximum at the mol fraction of ~ 0.33 of host indicative of host-guest 1:2 binding.

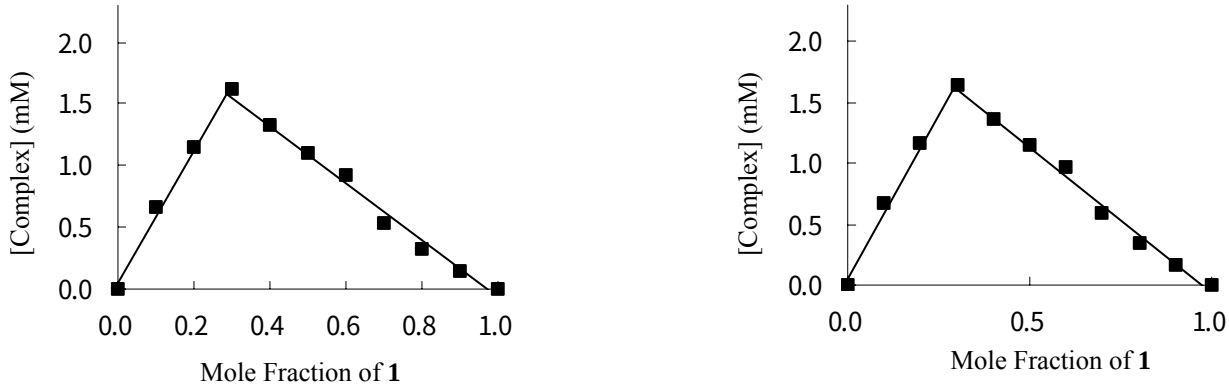


Figure S2. Job's plots between metallomacrocycle **1** and guest **9**, based on NH^1 of **1** (left) and NH^2 of **1**(right).

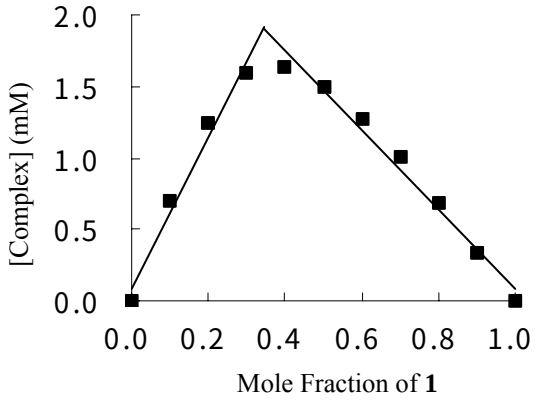
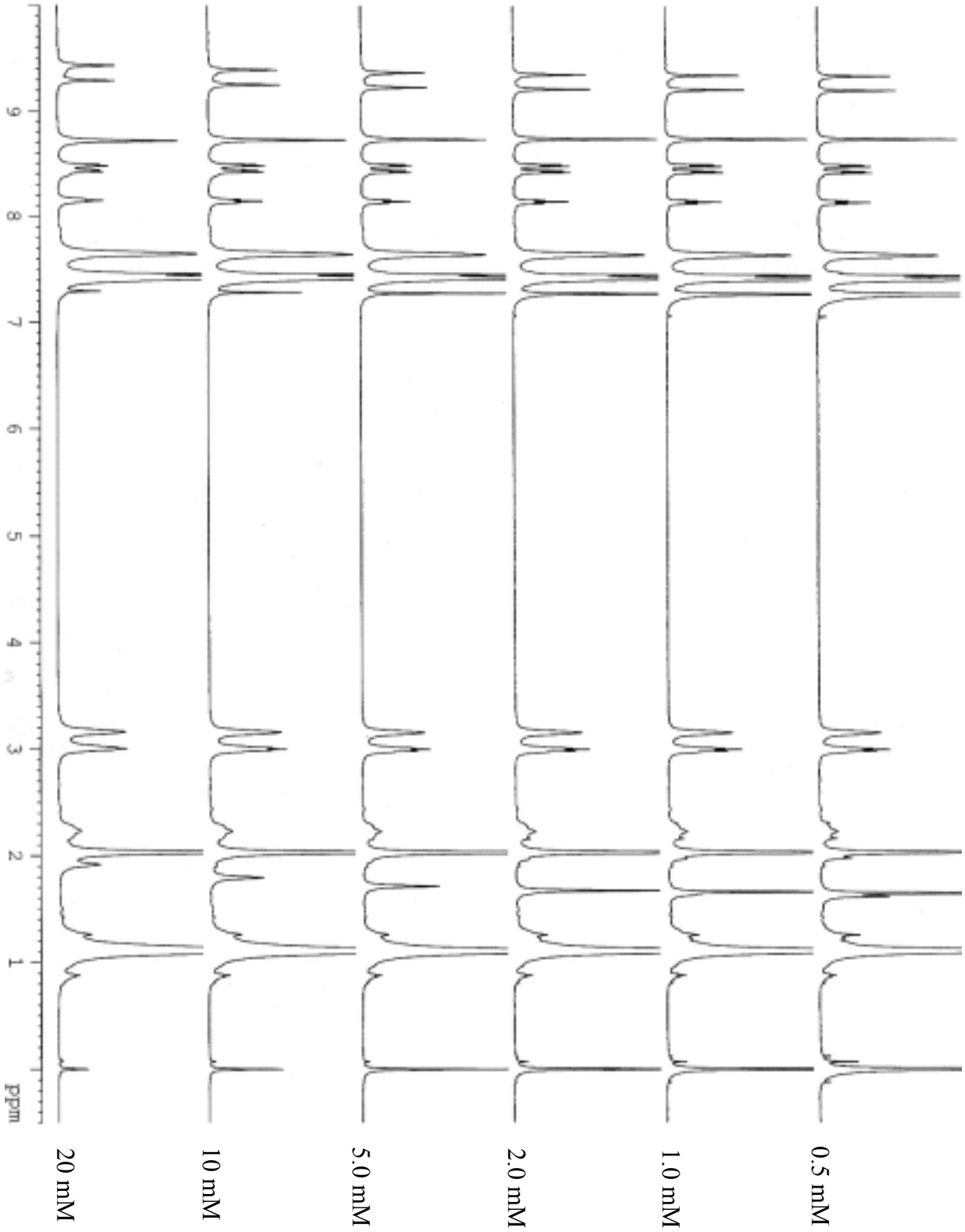


Figure S3. Job's plots between metallomacrocycle **1** and guest **9**, based aromatic proton of **9**.

(6) M. Hynes, J. *J. Chem. Soc. Dalton Trans.* **1993**, 311-312.

3. Concentration-Dependent ^{1}H NMR Spectra of **1** (97:3 $CDCl_3/CD_3CN$, 25 ^{o}C)



4. Modeling Structure of **1**

The energy-minimized structure was generated with MM3[*7] force field and $CHCl_3$ solvation parameters in MacroModel[8] Version 7.1 using Silicon Graphics Indigo2. 1000 separate search steps were performed in Monte Carlo conformational search. Bond stretching and angle bending parameters for Pd were implemented in MM3[*] force field[9] and Pd center was constrained according to the X-ray crystal structure.[3]

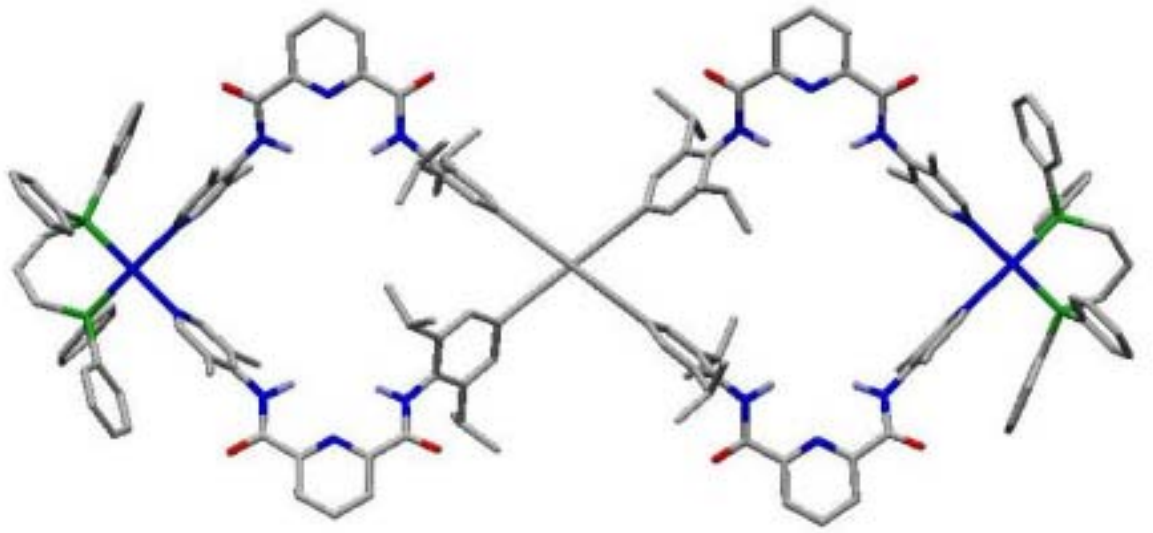


Figure S4. Energy-minimized structure of **1** generated by MM3[*] force field.

(7) N. L. Allinger, Y. H. Yuh and J. H. Lii, *J. Am. Chem. Soc*. **1989**, *111*, 8551-8556.
(8) F. Mohamedi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G.. Chang, T. Hendrickson and W. C. Still, *J. Comp. Chem.* **1990**, *11*, 440-467.
(9) H. Hagelin, B. Åkermark and P.-O. Norrby, *Organometallics* **1999**, *18*, 2884-2895.

5. VPO[10] Experiments of **1**

The experiments were performed in CH_2Cl_2 at 25 ^oC over a concentration range 3.6–13.0 g/kg (sample/solvent) using a Knauer K-7000 twin-thermistor hanging-drop vapor pressure osmometer. Total 6 different stock solutions were prepared and 2–3 measurements were made at each concentration. A calibration curve was generated using benzil as the standard (MW = 210.23) under the same condition.

Table S1. VPO data for benzil in CH_2Cl_2 at 25 ^oC.

Entry	Concn of benzil (mmol/kg)	VPO reading
1	2.0	2.0
2	2.5	2.3
3	3.0	2.9
4	3.5	3.4
5	4.0	3.8

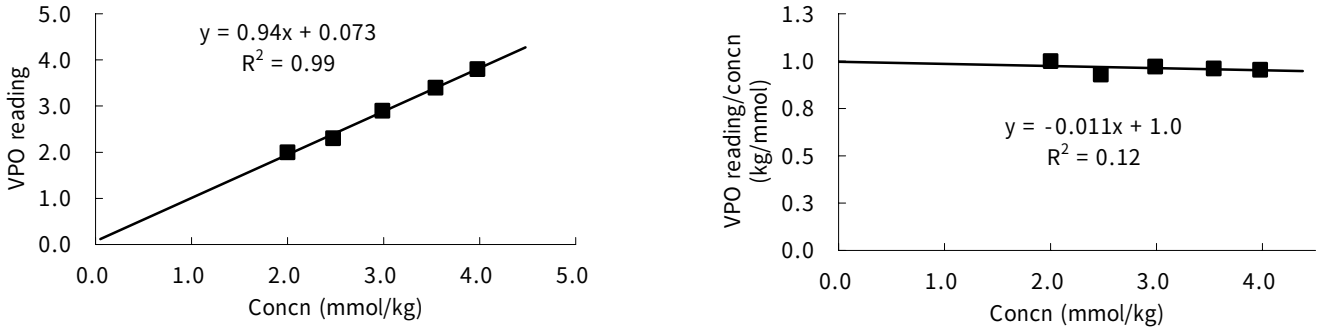


Figure S5. Plots of VPO reading vs concentration (left) and VPO reading/concentration vs concentration (right) for the standard, benzil.

10 (a) E. E. Schrier, *J. Chem. Edu.* **1968**, *45*, 176-180. (b) C. T. Seto and G. M. Whitesides, *J. Am. Chem. Soc.* **1993**, *115*, 1330-1340. (c) A. S. Shetty, J. Zhang and J. S. Moore, *J. Am. Chem. Soc.* **1996**, *118*, 1019-1027.

Table S2. VPO data for **1** in CH_2Cl_2 at 25 ^oC.

Entry	Concn of **1** (g/kg)	VPO reading	Calcd concn of **1*** (mmol/kg)	Calcd MW
1	3.6	1.1	1.0	3400
2	5.0	1.4	1.4	3500
3	7.1	1.9	2.0	3600
4	9.1	2.4	2.5	3700
5	11.0	2.9	3.0	3600
6	13.0	3.2	3.3	3900

* Molal concentrations of **1** were calculated from the calibration curve equation: y = 0.94 x + 0.073,

Molal concn = (VPO reading – 0.073)/0.94

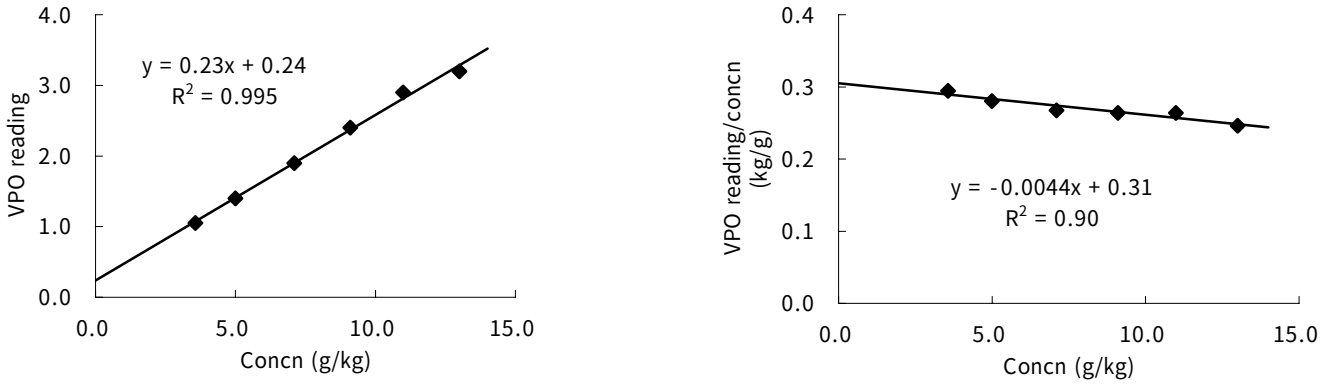


Figure S6. Plots of VPO reading vs concentration (left) and VPO reading/concentration vs concentration (right) for **1.**

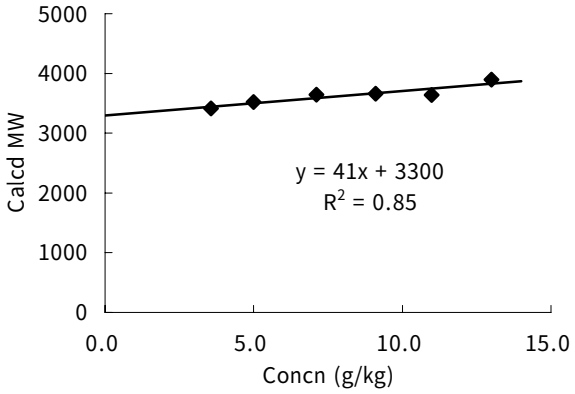


Figure S7. Plots of calculated molecular weight vs concentration of **1**.

6. ESI-Mass Spectra of Metallomacrocycle and Complexes

Table S3. ESI-mass spectral data of **1** in 50% (v/v) $CH_3CN/CHCl_3$.

m/z	Ion	Intensity
1574	$[M–2OTf]^{2+}$	100%
1000	$[M–3OTf]^{3+}$	65%
712*	$[M–4OTf]^{4+}$	70%

*The peaks for $[(M–4OTf)/2]^{2+}$ have been overlapped with $[M–4OTf]^{4+}$ at *m/z* = 712.

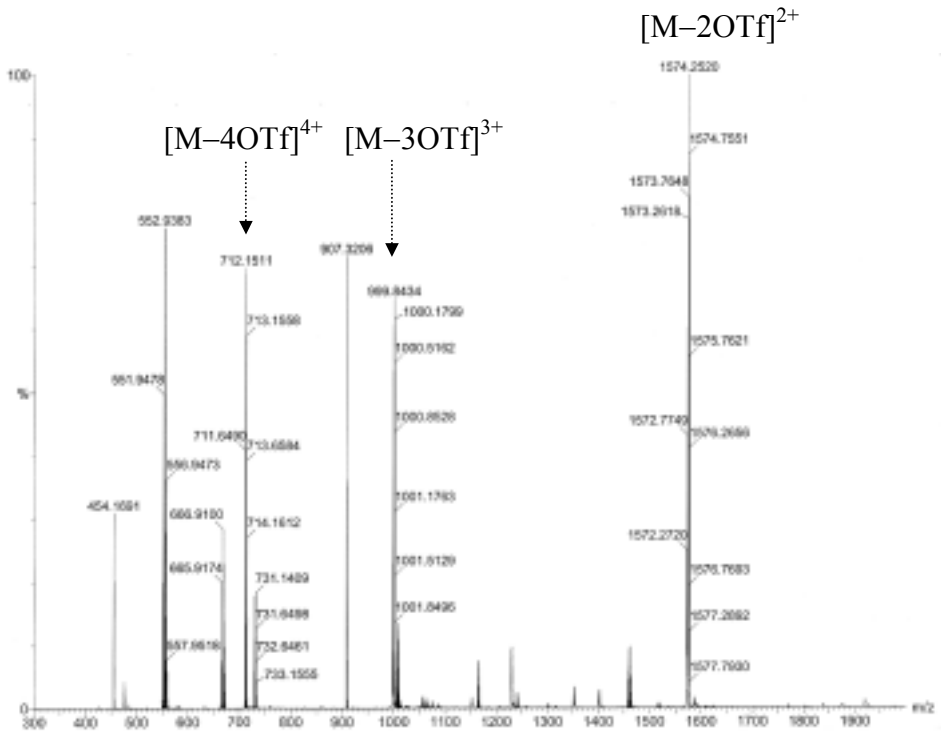


Figure S8. Observed ESI-mass spectrum of **1**.

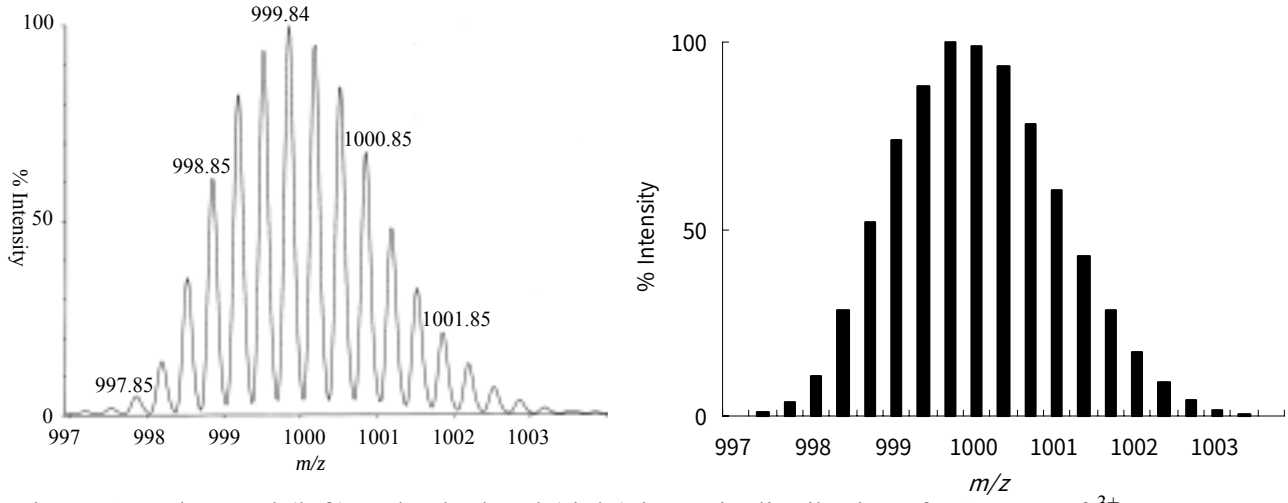


Figure S9. Observed (left) and calculated (right) isotopic distributions for $[M–3OTf]^{3+}$.

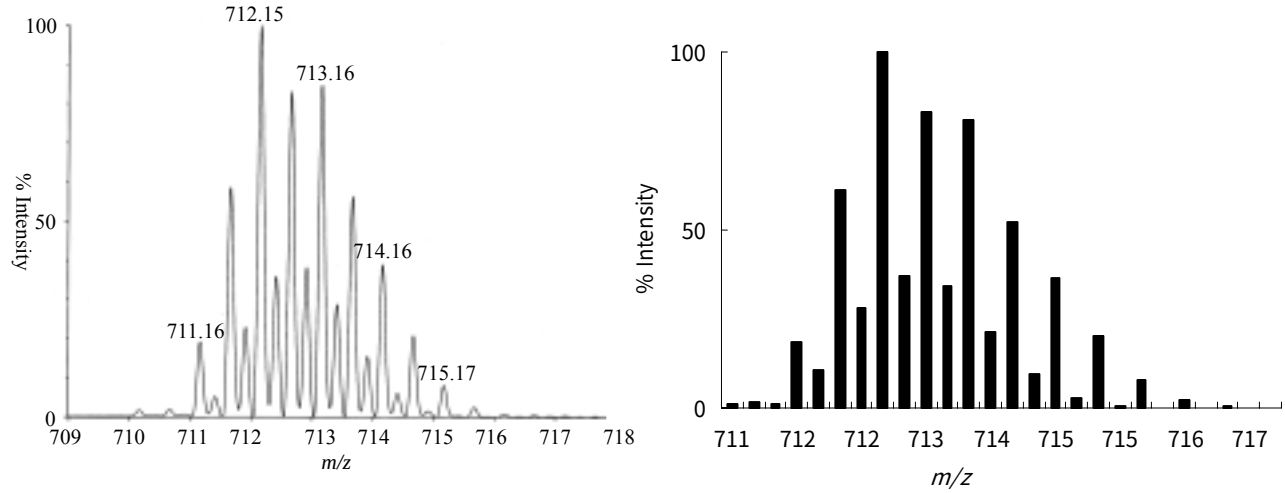


Figure S10. Observed (left) and calculated (right) isotopic distributions for two overlapped species, $[M–4OTf]^{4+}$ and $[(M–4OTf)/2]^{2+}$.

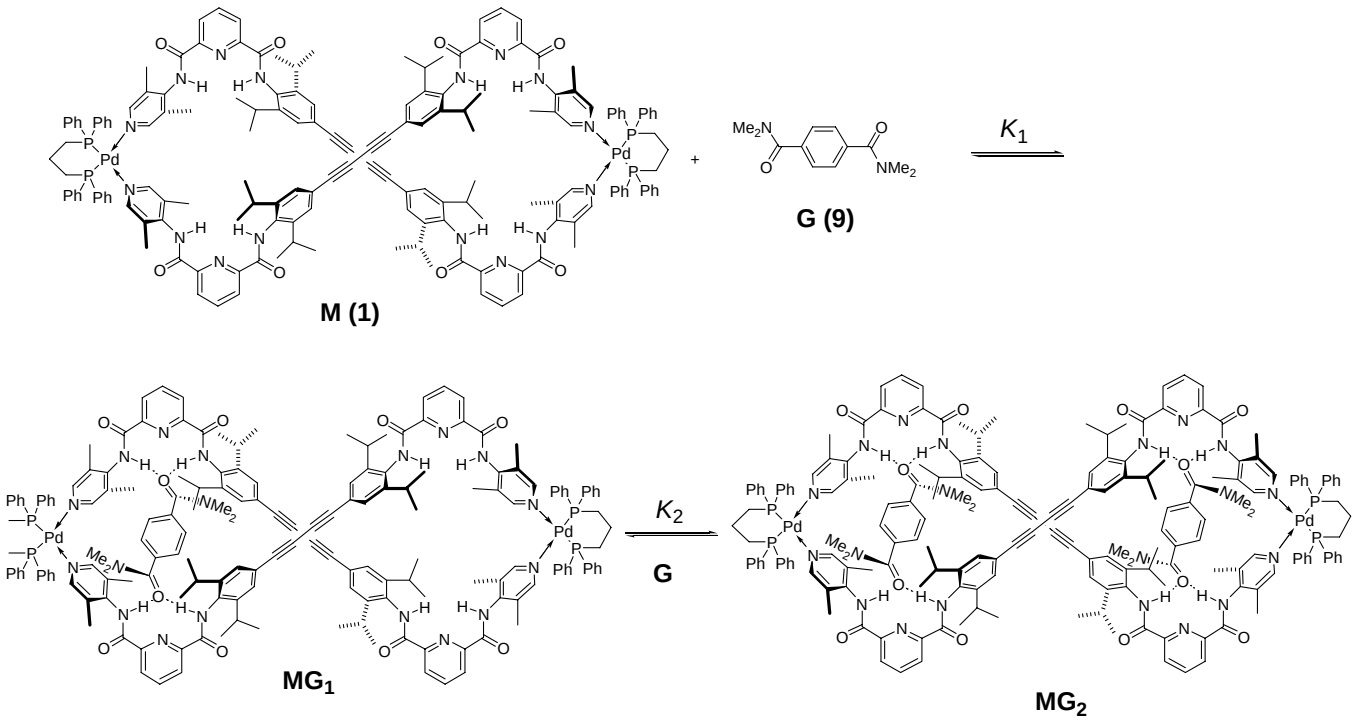


Table S4. ESI-mass spectral data of **1** in the presence of guest **9** (~10 equiv) in 3 and 50% (v/v) $CH_3CN/CHCl_3$*.

% CH_3CN	**MG_2** (1:2 complex)			**MG_1** (1:1 complex)			**M** (**1**)		
in $CHCl_3$	*m/z*	Ion	Intensity	*m/z*	Ion	Intensity	*m/z*	Ion	Intensity
3%	1794	$[MG_2–2OTf]^{2+}$	7%	1684	$[MG_1–2OTf]^{2+}$	7%	1574	$[M–2OTf]^{2+}$	3%
	1146	$[MG_2–3OTf]^{3+}$	25%	1073	$[MG_1–3OTf]^{3+}$	38%	1000	$[M–3OTf]^{3+}$	13%
	823	$[MG_2–4OTf]^{4+}$	32%	768	$[MG_1–4OTf]^{4+}$	4%	712	$[M–4OTf]^{4+}$	80%
50%	1794	$[MG_2–2OTf]^{2+}$	10%	1684	$[MG_1–2OTf]^{2+}$	2%	1574	$[M–2OTf]^{2+}$	0%
	1146	$[MG_2–3OTf]^{3+}$	56%	1073	$[MG_1–3OTf]^{3+}$	53%	1000	$[M–3OTf]^{3+}$	14%
	823	$[MG_2–4OTf]^{4+}$	13%	768	$[MG_1–4OTf]^{4+}$	4%	712	$[M–4OTf]^{4+}$	1%

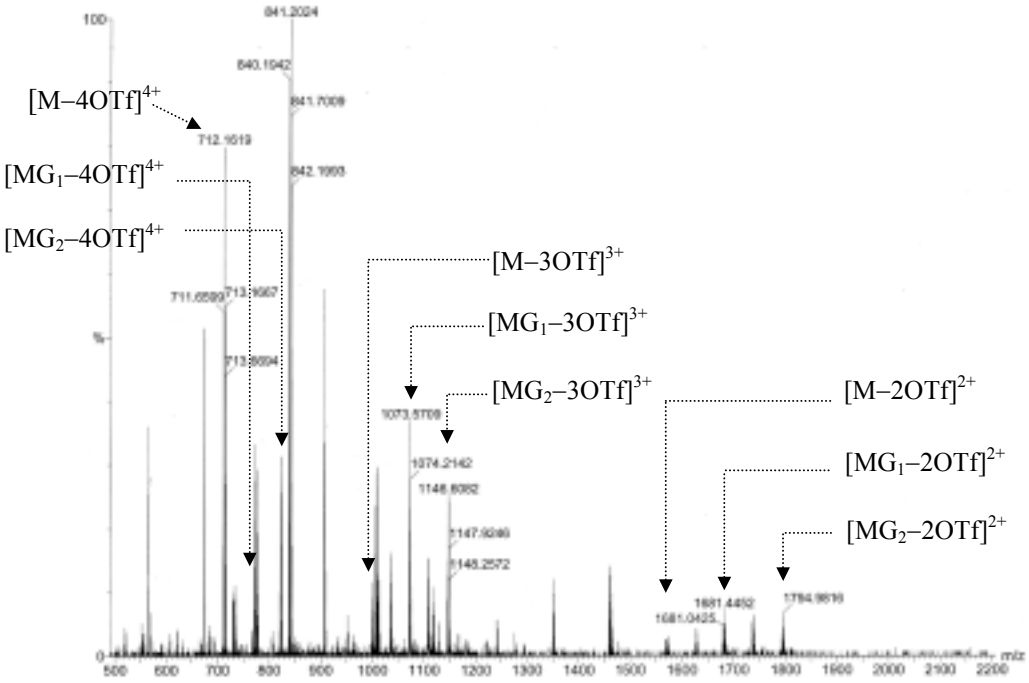


Figure S11. ESI-mass spectrum of **1** and **9** in 3% (v/v) $CH_3CN/CHCl_3$.

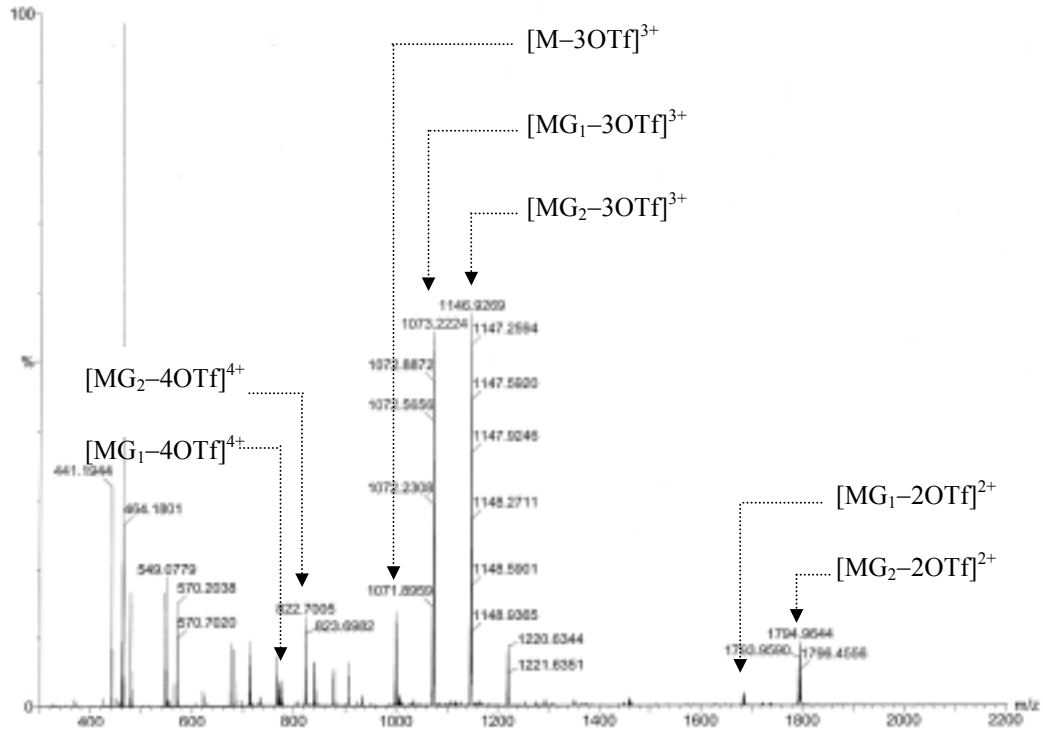


Figure S12. ESI-mass spectrum of **1** and **9** in 50% (v/v) $CH_3CN/CHCl_3$.

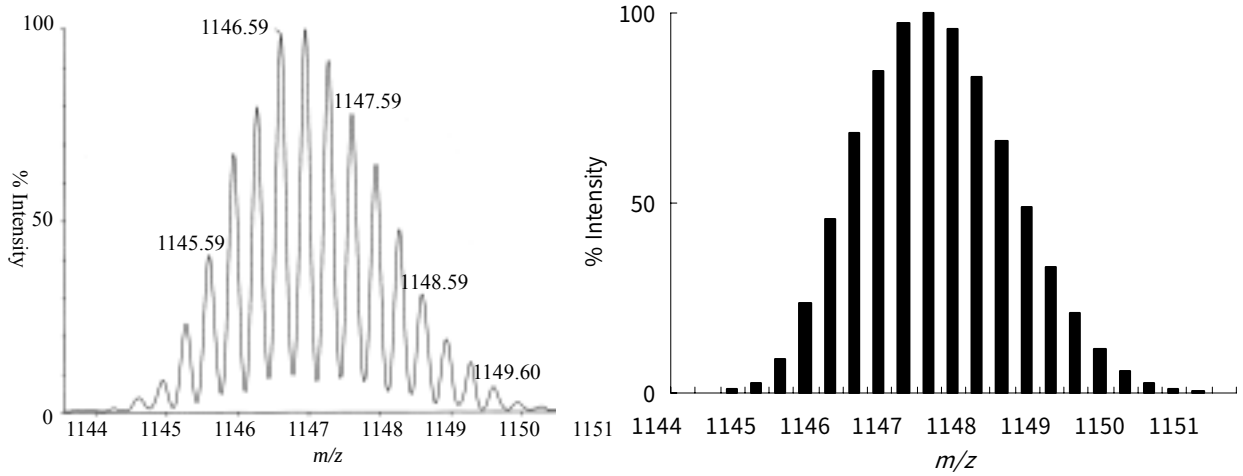


Figure S13. Observed (left) and calculated (right) isotopic distributions for $[MG_2–3OTf]^{3+}$ in 50% (v/v) $CH_3CN/CHCl_3$.

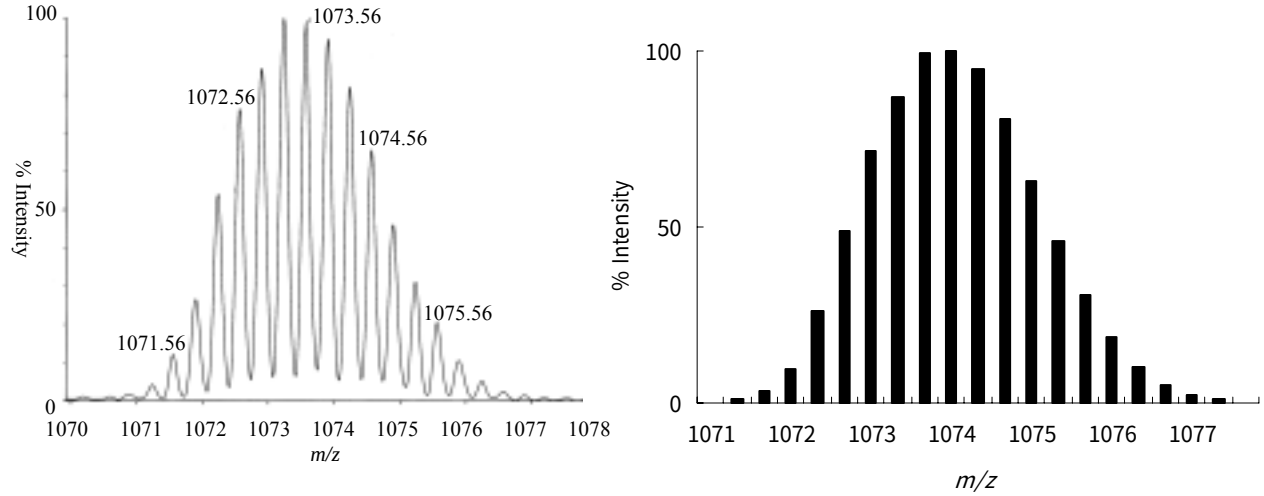


Figure S14. Observed (left) and calculated (right) isotopic distributions for $[MG_1–3OTf]^{3+}$ in 50% (v/v) $CH_3CN/CHCl_3$.