Supporting Information

Growth Inhibitory Activity of a Bis-benzimidazole-Bridged Arene Ruthenium Metalla-Rectangle and Prism

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Experimental Section:

Material and methods:

The donors **L1 and L2** were prepared according to literature methods.[1] Deuterated solvents were purchased from Cambridge Isotope Laboratory (Andover, MA). NMR spectra were recorded on a Bruker 300 MHz spectrometer. ^{1}H and ^{13}C NMR chemical shifts are reported relative to residual solvent (H and C) signals. HR-ESI-Mass spectra were recorded on a Micromass Quattro II triple- quadrupole mass-spectrometer using electrospray ionization and analyzed using the MassLynx software suite. UV-Vis spectra were recorded on Cary 100 Conc. Fluorescence studies were carried out on a HORIBA FluoroMax-4 fluorometer.

A single crystal was mounted on a loop and data were collected at 100 K on an ADSC Quantum 210 CCD diffractometer with a synchrotron radiation (λ = 0.75000 Å) at Supramolecular

Crystallography Beamline 2D, Pohang Accelerator Laboratory (PAL), Pohang, Korea. The raw data were processed and scaled using the program HKL2000. The structure was solved by direct methods, and the refinements were carried out with full-matrix least-squares on F^2 with appropriate software implemented in SHELXTL program package. X-Ray data for **4**: $C_{80}H_{104}F_{12}N_8O_{20}Ru_4S_4$, M = 2258.23, Triclinic, *P*-1 (no. 2), a = 15.657(3) Å, b = 16.600(3) Å, c = 19.979(4) Å, α = 95.93(3)°, β = 109.49(3)°, γ = 102.26(3)°, V = 4697(2) Å^3, Z = 2, T = 100 K, μ(synchrotron) = 0.927 mm^{-1}, ρ_{calc} = 1.597 g/cm^3, 22156 reflections measured, 11262 unique (R_{int} = 0.0292), R_1 = 0.0829, wR_1 = 0.3058 for 5591 reflections ($I > 2\sigma(I)$), R_1 = 0.0952, wR_2 = 0.3203 (all data), GOF = 1.192, 1158 parameters and 104 restraints. All the non-hydrogen atoms were refined anisotropically. Hydrogen atoms were added in their geometrically-ideal positions except OH groups of methanol. CCDC 907587 (**4**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Stability of Rectangles in DMSO. For stability studies, molecular rectangle **5** was dissolved in DMSO, and the sample was analyzed by ^{1}H NMR spectroscopy immediately after dissolution and after 48 h. No change was observed even after 48 h, thus attesting to the stability of molecular rectangle in DMSO. Further, the UV-Visible spectrum for **4**, **5** and **6** was recorded in DMSO:Water (1:1) solution, which remains same even after 48 h of dissolution. The result confirms the stability of metalla rectangle in DMSO: Water solution.

Cancer Cell Growth Inhibition Assay (MTS assay)

Human cancer cell lines, Colo320, A549, H1299, and MCF7 were purchased from the Korean Cell Line Bank (KCLB-Seoul, Korea). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (FBS) and 100 μg of penicillin-streptomycin at 37°C in a humidified atmosphere of 5% CO_2. For the MTS cell proliferation assay, cells were plated in triplicate at 5.0×10^4 cells/well in 96-well culture plates in RPMI 1640 medium. Compounds were preincubated in DMEM-10 or DMSO for indicated times and added to cells. After 24 h of incubation, MTS (CellTiter 96® Aqueous One Solution reagent, Promega, USA) was added to each well according to the manufacturer's instructions. Absorbance at 490nm was determined for each well using a Victor 1420 multilabel counter (EG&Gwallac, Turku, Finland). The percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC_{50} values were obtained by fitting values to sigmoidal dose-response curves using the routines provided in GraphPad Prism.

TUNEL staining

TUNEL staining was conducted using an *in situ* cell death detection kit, TMR Red, according to the protocol supplied by the manufacturer (Roche Molecular Biochemicals). Briefly, cells were plated in 60-mm dishes at 2 x 10^5cells/mL DMEM. On the following day, the cells were treated with 10 μM cisplatin or 10 μM rectangle 5, harvested, and fixed with 2% paraformaldehyde solution and permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate. After washing twice with PBS (137 mM NaCl; 2.7 mM KCl; 4.3 mM Na_2HPO_4 $7H_20$; 1.4 mM KH_2PO_4; pH 7.2), cells were incubated in a TUNEL reaction mixture containing terminal deoxynucleotidyl transferase and tetramethyl-rhodamine-dUTP. Cells were analyzed for fluorescence intensity using a FACS flow cytometer (Becton Dickinson, Inc.).

Molecular Clip 4. A mixture of $[Ru_2(p\text{-}Pr^iC_6H_4Me)_2(m\text{-}Cl)Cl]_2$ **1** (184.0 mg, 0.3 mmol), Bisbenzimidazole **2** (70.2 mg, 0.3 mmol) and CH_3COONa (50.1 mg, 0.6 mmol) was suspended in 50 ml of ethanol and refluxed overnight. The dark brown precipitate of **3** was filtered, washed with diethyl ether, and dried *in vacuo* (yield 91 mg, 72 %). Further, solid $Ag[O_3SCF_3]$ (64.2 mg, 0.25 mmol) was added to a solution of compound **3** (91.0 mg, 0.117 mmol) in methanol (30ml). The mixture was stirred at room temperature for 2 h, and then filtered. The filtrate was concentrated and pure product **4** was precipitated by addition of diethyl ether as a yellow brown solid. Isolated yield: 70% with respect to **1**. Anal. Calcd for $C_{36}H_{36}F_6N_4O_6Ru_2S_2$: C, 43.20; H, 3.63; N, 5.60. Found: C, 43.01; H, 3.68; N,5.49. ^{1}H NMR [300 MHz, CD_3NO_2]: δ (ppm) 8.00 (m, 4H, Hbibz), 7.50 (m, 4H, Hbibz), 6.43 (d, *J* = 6.0 Hz, 4H, Hcym), 6.30 (d, *J* = 6.0 Hz, 4H, Hcym), 2.59 (sept, 2H, C*H*$(CH_3)_2$), 2.24 (s, 6H, CH_3), 1.02 (d, *J* = 6.6 Hz, 12H,CH(C*H*$_3)_2$); ^{13}C NMR [75 MHz, CD_3NO_2]: δ (ppm) 157.4, 145.4, 128.8, 126.1, 123.0, 116.2, 81.0, 78.8, 31.1, 20.9, 17.5 ; MS (ESI) for **4** ($C_{36}H_{36}F_6N_4O_6Ru_2S_2$): 852.0 $[M - OTf]^+$.

Metalla-rectangle 5. Acceptor clip **4** (10.0 mg, 0.01mmol) and dipyridyl donor **L1** (2.42 mg, 0.01 mmol) were stirred in nitromethane-methanol (1:1, 2 mL) at 50ºC for 12 hour after which the solution was concentrated and diethyl ether added to precipitate the pure rectangle **5**. Isolated yield: 89%. Anal. Calcd for $C_{96}H_{92}F_{12}N_{16}O_{16}Ru_4S_4$: C, 46.37; H, 3.73; N, 9.01. Found: C, 46.11; H, 3.61; N,9.15. ^{1}H NMR [300 MHz, CD_3NO_2]: δ (ppm). ^{1}H NMR [300 MHz, CD_3NO_2]: δ (ppm) 9.57 (s, 4H, NH), 8.03 (m, 8H, Hbibz), 7.68 (d, *J* = 7.2 Hz, 8H, Hα), 7.58 (m, 8H, Hbibz), 7.14 (d, *J* = 7.2 Hz, 8H, Hβ), 6.59 (d, *J* = 6.3Hz, 8H, Hcym), 6.11 (d, *J* = 6.3 Hz, 8H, Hcym), 2.60 (sept, 4H, $CH(CH_3)_2$), 1.77 (s, 12H, CH_3), 0.94 (d, *J* = 6.9Hz, 24H,$CH(CH_3)_2$); ^{13}C NMR [75 MHz, CD_3NO_2]: δ (ppm) 158.7, 157.9, 155.0, 146.7, 145.8, 125.0, 117.3, 116.5, 104.3,

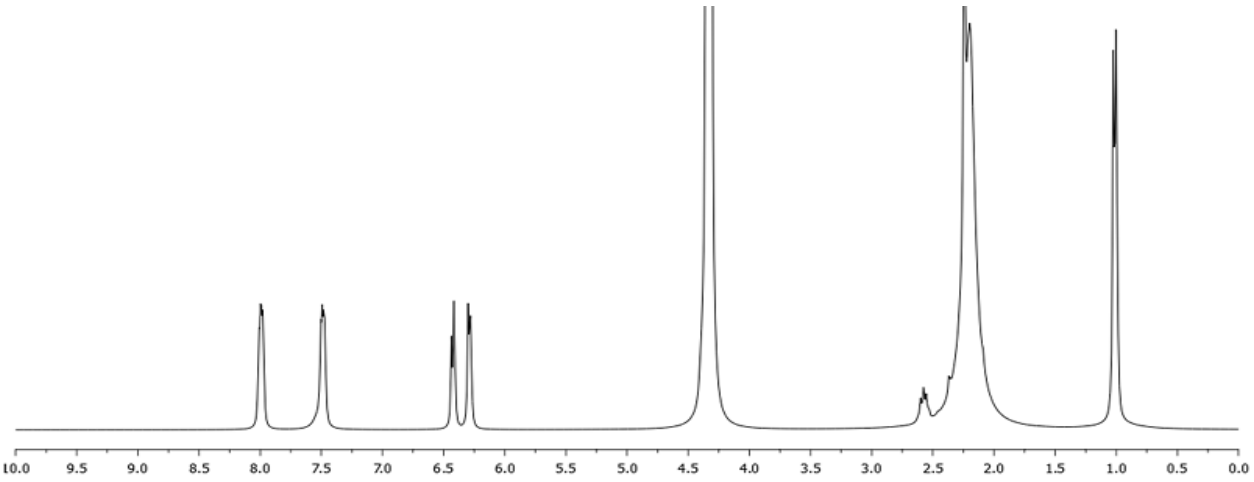
103.8, 87.0, 79.5, 32.6, 22.6, 18.3 ; MS (ESI) for **5** ($C_{96}H_{92}F_{12}N_{16}O_{16}Ru_4S_4$): 1094.1 [M – 2OTf]$^{2+}$, 679.8 [M – 3OTf]$^{3+}$.

Metalla-prism 6. Acceptor clip **4** (9.00 mg, 0.01 mmol) and tripyridyl donor **L2** (2.28 mg, 0.006 mmol) were stirred in nitromethane-methanol (1:1, 2 mL) at 50ºC for 12 hours after which the solution was concentrated and diethyl ether added to precipitate the pure **6**. Isolated yield: 87%. Anal. Calcd for $C_{162}H_{138}F_{18}N_{18}O_{18}Ru_6S_6$: C, 51.67; H, 3.69; N, 6.70. Found: C, 51.51; H, 3.55; N, 6.80. ^{1}H NMR [300 MHz, CD_3NO_2]: δ (ppm) 8.08 (m, 12H, Hbibz), 7.85 (d, *J* = 6.6 Hz, 12H, Hα), 7.64 (m, 12H, Hbibz), 7.29 (s, 6H, Hbz), 6.93 (d, *J* = 7.2 Hz, 12H, Hβ), 6.64 (d, *J* = 6.0Hz, 12H, Hcym), 6.17 (d, *J* = 6.0 Hz, 12H, Hcym), 2.61 (sept, 6H, $CH(CH_3)_2$), 1.81(s, 18H, CH_3), 0.96 (d, *J* = 6.9Hz, 36H,$CH(CH_3)_2$); ^{13}C NMR [75 MHz, CD_3NO_2]: δ (ppm) 156.5, 153.1, 144.5, 133.3, 127.0, 126.5, 123.8, 123.1, 122.4, 118.8, 116.1, 103.1, 102.6, 95.3, 85.9, 78.3, 31.2, 21.2, 16.9 ; MS (ESI) for **6** ($C_{162}H_{138}F_{18}N_{18}O_{18}Ru_6S_6$): 1106.2 [M – 3OTf]$^{3+}$, 604.1 [M – 5OTf]$^{5+}$.

Molecular Modeling

The structure of a model of molecular rectangle **5** was predicted by density functional theory calculations using the Gaussian 09 computational software suite.[2] The individual Ru donor and dipyridyl acceptor were initially optimized and these fragments were then combined into the full rectangle using GaussView 5, followed by subsequent optimization of the complete self-assembly. The restricted b3lyp optimization was run with organic atoms (C, H, N, O) calculated using the 6-31G** basis set and Ru centers using the LANL2DZ basis set. The methyl and isopropyl groups of the cymene ligands were omitted in the model for computational cost considerations.

(a)



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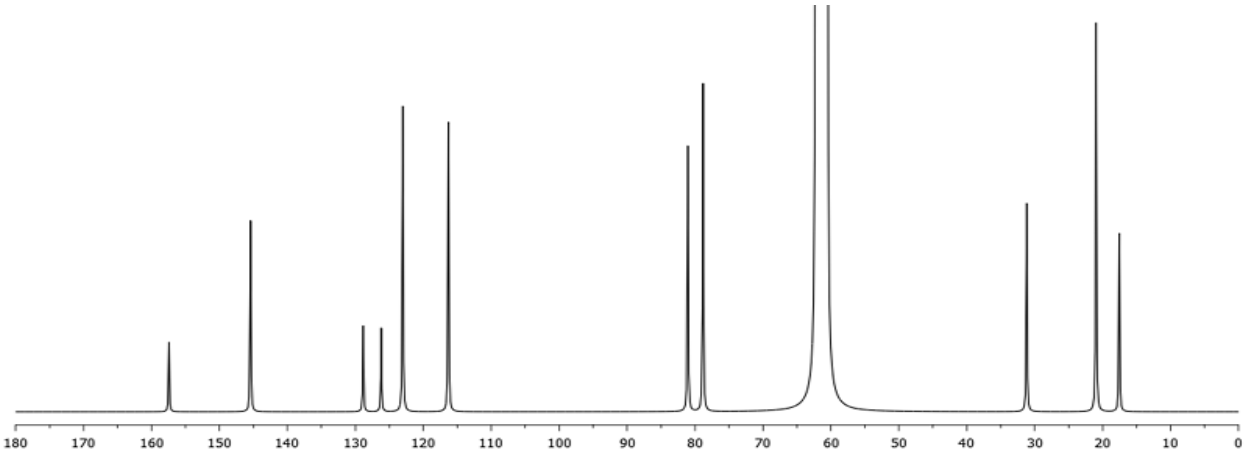

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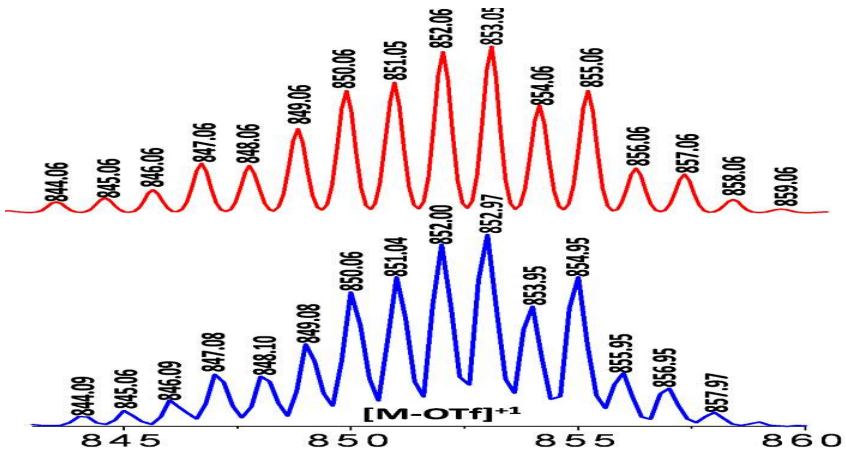


Figure 1. ^{1}H (a), ^{13}C NMR (b) and HR-ESI-MS (c) spectra of the molecular clip **4**.

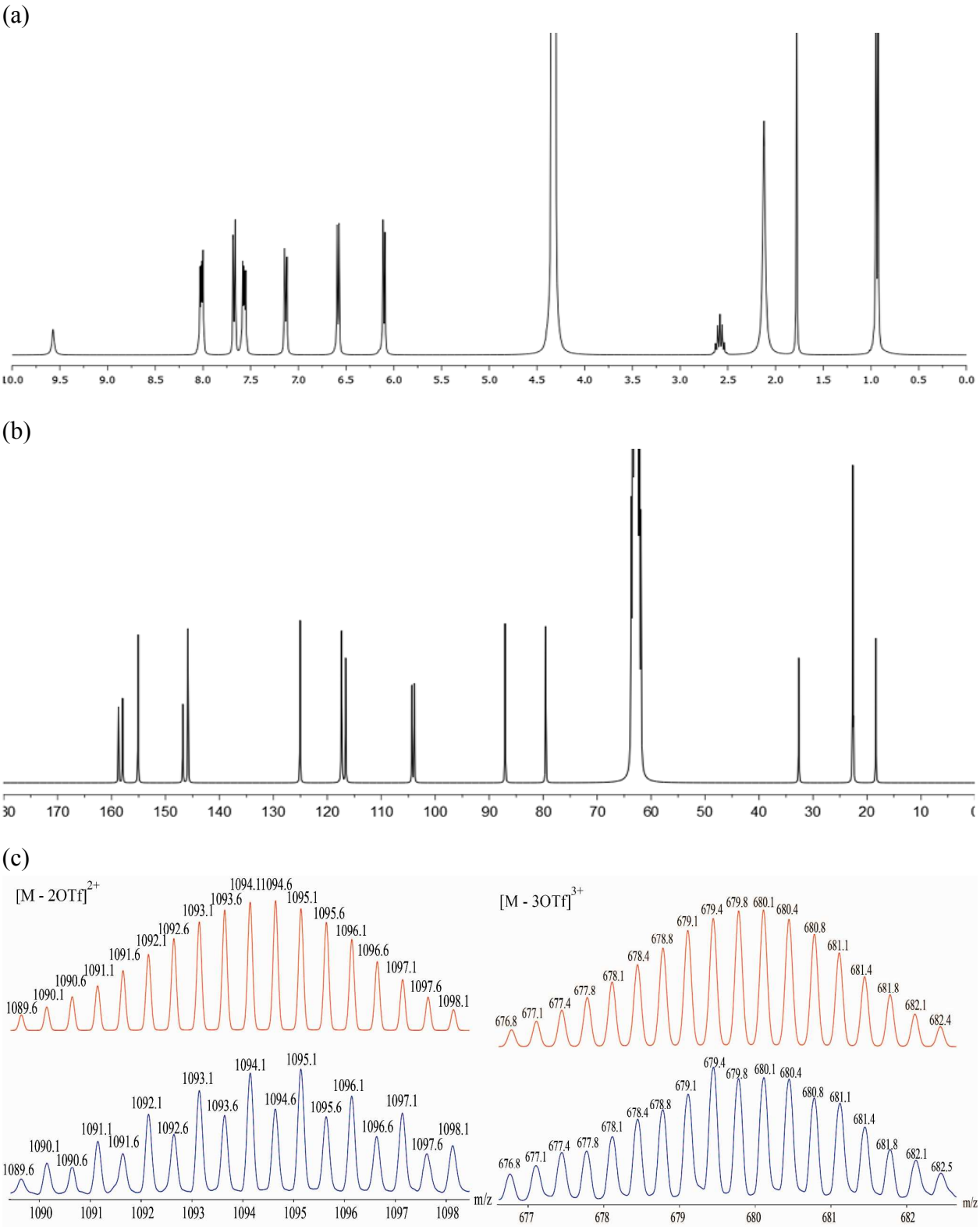
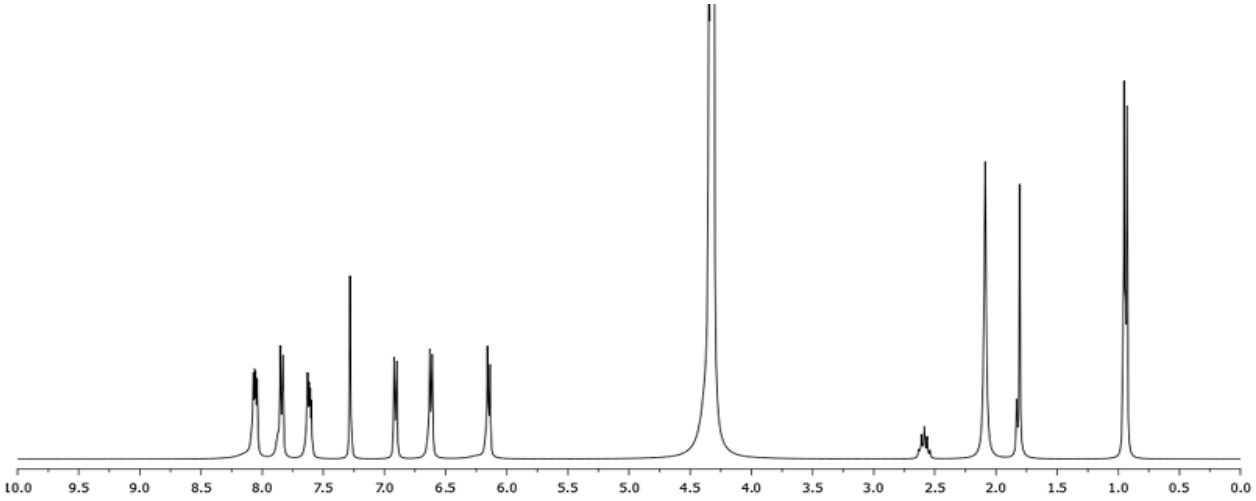


Figure 2. ^{1}H (a), ^{13}C NMR (b) and HR-ESI-MS (c) spectra of the molecular-rectangle **5**.

(a)



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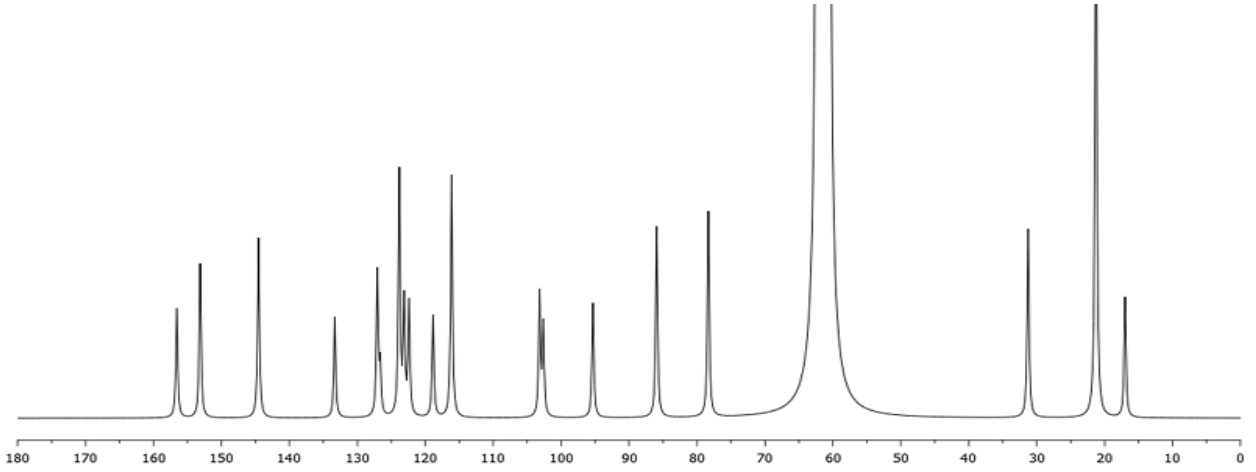

(c)

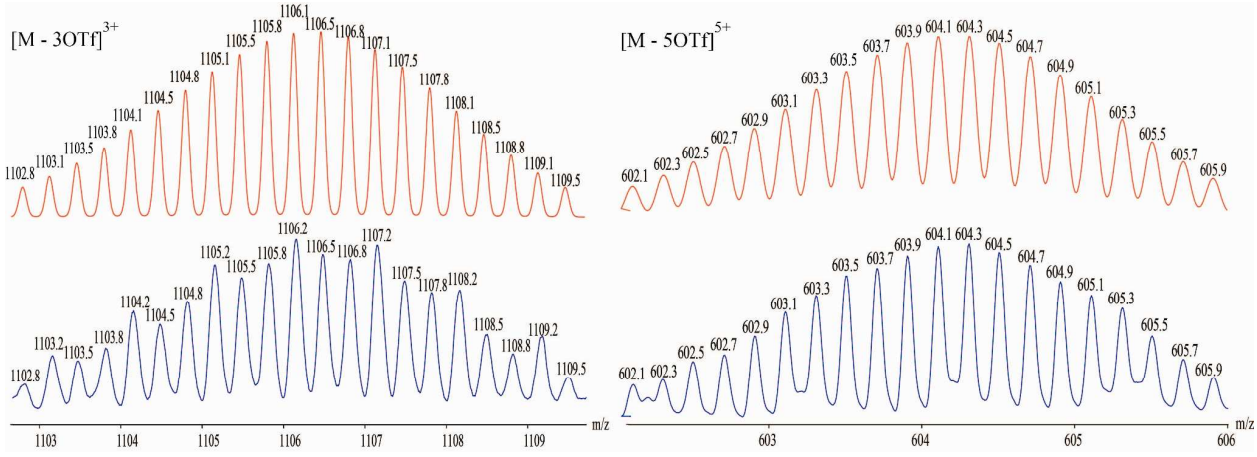

Figure 3. ^{1}H (a), ^{13}C NMR (b) and HR-ESI-MS (c) spectra of the metalla-prism **6**.

Table S1. Atomic Coordinates for the DFT Geometry Optimization of Rectangle **5**.

Atom	X	Y	Z
C	-1.354	5.281	2.864
C	-1.529	3.909	2.943
C	-0.403	3.069	2.97
C	0.866	3.669	2.917
C	0.946	5.052	2.84
H	-2.208	5.945	2.825
H	-2.535	3.504	2.982
H	1.767	3.072	2.936
H	1.911	5.541	2.784
N	-0.604	1.691	3.032
C	0.334	0.697	3.024
H	-1.56	1.343	3.065
O	1.549	0.822	2.993
C	-0.334	-0.697	3.024
O	-1.549	-0.822	2.993
N	0.604	-1.691	3.032
H	1.56	-1.343	3.065
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H	2.535	-3.504	2.982
H	-1.911	-5.541	2.784
H	2.208	-5.945	2.825
N	0.135	-5.861	2.819
Ru	-0.067	-7.988	2.802
N	-1.439	-7.68	1.142
N	1.333	-7.944	1.142
C	-1.582	-9.1	4.114
C	1.248	-9.163	4.27
C	-0.848	-10.081	3.367
C	0.552	-10.106	3.441
C	0.524	-8.211	5.007
C	-0.908	-8.179	4.935
C	-2.767	-7.647	0.718
C	-0.75	-7.691	0
C	2.643	-8.168	0.718
C	0.654	-7.824	0

H	-2.661	-9.06	4.025
H	2.33	-9.177	4.31
H	-1.374	-10.766	2.711
H	1.116	-10.809	2.838
H	1.05	-7.479	5.611
H	-1.464	-7.431	5.488
C	-3.972	-7.604	1.43
C	-2.767	-7.647	-0.718
N	-1.439	-7.68	-1.142
C	2.643	-8.168	-0.718
C	3.832	-8.364	1.43
N	1.333	-7.944	-1.142
C	-5.158	-7.571	0.705
H	-3.985	-7.597	2.515
C	-3.972	-7.604	-1.43
Ru	-0.067	-7.988	-2.802
C	3.832	-8.364	-1.43
C	5.002	-8.562	0.705
H	3.844	-8.364	2.515
C	-5.158	-7.571	-0.705
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H	-2.208	5.945	-2.825
H	-2.535	3.504	-2.982
H	1.767	3.072	-2.936
H	1.911	5.541	-2.784
N	-0.604	1.691	-3.032
C	0.334	0.697	-3.024
H	-1.56	1.343	-3.065
O	1.549	0.822	-2.993
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N	0.604	-1.691	-3.032
H	1.56	-1.343	-3.065
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C	-0.866	-3.669	-2.917
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H	-1.911	-5.541	-2.784
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Reference:

1. (a) Kikuchi, T.; Murase, T.; Sato, S.; Fujita, M. *Supramolecular. Chem*. **2008**, *20*, 81. (b) Tzeng, B.-I.; Chen, Y. –F.; Wu, C. –C.; Hu, C. –C.; Chang, Y. –T.; Chen, C. –K. *New J. Chem.* **2007**, *31*, 202.

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