A Multi-responsive Switchable Diarylethene and Its Application in Bioimaging

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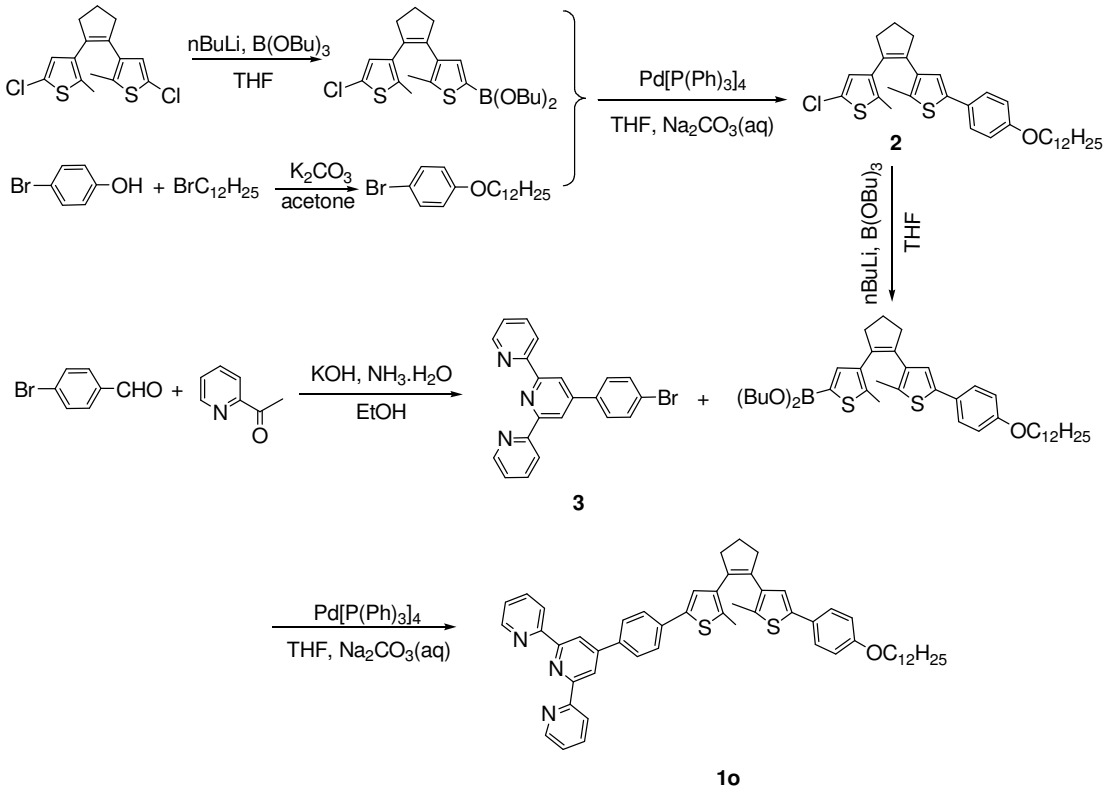
Supporting Information

EXPERIMENTAL

General

All starting materials were obtained from commercial supplies and used as received. *n*-Butyl lithium, tetrakis (triphenylphosphine) palladium, 1-(pyridin-2-yl)ethanone were purchased from Sigma-Aldrich. 4-bromophenol, 4-bromobenzaldehyde, 1-bromododecane and other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai). Column chromatography was carried out on silica gel (200-300 mesh). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Mercuryplus-Varian instrument. Proton chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). MALDI-TOF-MS was recorded on BIFLEX III MALDI-TOF mass spectroscopy instrument (Bruker Daltonics Inc.). Melting points were determined on a hot-plate melting point apparatus XT4-100A.

Synthesis and characterizations



Scheme S1 Synthetic routes of **1o**

4'-(4-bromophenyl)-2, 2':6', 2''-terpyridine (3) was prepared according to reference 1. To a 30 mL C_2H_5OH solution of 4-bromobenzaldehyde (2.0 g, 10.9 mmol), a 40 mL C_2H_5OH solution of 2-acetyl-pyridine (2.42 g, 20.0 mmol), KOH (1.7 g) and 29 mL concentrated NH_4OH (aq) were added at 0 °C. The reaction mixture was refluxed for 1 day. The oil-bath was then removed and the reaction mixture was stirred at room temperature for 1 h. The slight yellow precipitate which was formed upon cooling was filtered and was washed sequentially with C_2H_5OH. The pure product was then obtained by column chromatography on activated basic Al_2O_3 (hexane/CH_2Cl_2 1:1) as a white solid (1.64 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.69 (m, 6H), 7.87 (t, J = 7.7, 2H), 7.65 (d, J = 8.4, 2H), 7.63 (d, J = 8.4, 2H), 7.35 (m, 2H).

1-(5-chloro-2-methylthien-3-yl)-2-[2-methyl-5-(4-(dodecyloxy) benzene-4-yl) thien-3-yl] cyclopentene (2) was prepared according to reference 2. To 25 mL anhydrous THF solution of 1, 2-Bis(5-chloro-2-methylthien-3-yl) cyclopentene (1.0 g, 3.04 mmol), *n*-BuLi (1.9 mL of 1.6 M solution in hexane, 3.04 mmol) was added dropwise under Ar atmosphere at -5 °C. The mixture was stirred for 15 minutes at room temperature and $B(OBu)_3$ (1.2 mL, 3.4 mmol) was added in one portion. This resulting reddish solution was stirred for 6 hours at room temperature and was added to a flask containing 1-bromo-4-(dodecyloxy) benzene (1.0 g, 2.9 mmol), $Pd(PPh_3)_4$ and 10 mL Na_2CO_3 solution (20 wt %) at 50 °C. The mixture was refluxed under Ar atmosphere for 18 hours. The pure product was obtained by column chromatograph (petroleum ether) as a yellow solid (660 mg, 41%) M.p: 50 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40 (d, *J* = 8.9, 2H), 6.88 (s, 1H), 6.86 (d, *J* = 2.9, 2H), 6.63 (s, 1H), 3.96 (t, *J* = 6.6, 2H), 2.80 (t, *J* = 7.4, 2H), 2.74 (t, *J* = 7.4, 2H), 2.04 (m, 2H), 1.96 (s, 3H), 1.87 (s, 3H), 1.78 (m, 2H), 1.26-1.46 (m, 18H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.146, 14.194, 14.329, 22.701, 22.887, 26.023, 29.239, 29.360, 29.399, 29.583, 29.604, 29.641, 29.666, 31.920, 38.332, 38.443, 68.072, 114.732, 122.575, 124.861, 126.492, 126.800, 127.048, 133.201, 133.278, 133.473, 135.146, 135.376, 136.135, 139.787, 158.410.

1-[2-methyl-(5-(2, 6-di (pyridin-2-yl) pyridin-4-yl) benzene-4-yl) thien-3-yl]-2 -[2-methyl-5-(4-(dodecylcoxy) benzene-4-yl) thien-3-yl] cyclopentene (1): To 6.0 mL anhydrous THF solution of **2** (0.3 g, 0.54 mmol), *n*-BuLi (0.4 mL of 1.6 M solution in hexane, 0.64 mmol) was added under Ar atmosphere at -5 °C. The mixture was stirred for 45 minutes at room temperature and $B(OBu)_3$ (0.40 mL, 1.15 mmol) was added in one portion. This resulting reddish solution was stirred for 6 hours at room temperature and was added to a flask containing **3** (0.23 g, 0.59 mmol), $Pd(PPh_3)_4$, 3 mL Na_2CO_3 solution (20 wt %) at 50 °C. The reaction was refluxed under Ar atmosphere for 19 hours. The pure product was obtained as a white solid (156 mg, 35%) by column chromatograph (petroleum ether: triethylamine = 18:1) and was washed with petroleum ether/ CH_3OH. M.p: 162 °C, ^{1}H NMR (400 MHz,

$CDCl_3$) δ = 8.75 (t, J = 3.2, 4H), 8.68 (d, J = 7.9, 2H), 7.90 (m, 4H), 7.63 (d, J = 8.4, 2H), 7.42 (d, J = 8.8, 2H), 7.37 (m, 2H), 7.15 (s, 1H), 6.93 (s, 1H), 6.87 (d, J = 8.8, 2H), 3.96 (t, J = 6.5, 2H), 2.86 (d, J = 6.7, 4H), 2.10 (m, 2H), 2.02 (s, 3H), 2.00 (s, 3H), 1.78 (m, 2H), 1.26-1.47 (m, 18H), 0.88 (t, J = 6.8, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 14.394, 14.672, 14.795, 22.944, 23.266, 26.272, 29.494, 29.600, 29.647, 29.824, 29.845, 29.880, 29.907, 32.161, 38.743, 68.327, 114.992, 118.670, 121.630, 123.002, 124.089, 124.834, 125.851, 126.779, 127.403, 127.923, 133.584, 134.505, 135.178, 135.442, 135.472, 136.691, 137.160, 139.019, 139.956, 149.338, 149.807, 156.140, 156.445, 158.627. MALDI-TOF-MS m/z: 828.4. Anal. Caled for $C_{54}H_{57}N_3OS_3$: C, 78.31; H, 6.94; N, 5.07. Found: C, 78.48; H, 6.96; N, 5.06.

Technology

UV-visible spectra were obtained with a Shimadzu 2550 spectrometer (Japan). Edinburgh 900 spectrometer was used for fluorescence spectra. The UV and visible irradiations were carried out on a CHF-XM 550W power system (China) by using suitable band-pass filter (Omega). Confocal fluorescence imaging was performed with an OLYMPUS ZX81 laser scanning microscopy and a 60x oil-immersion objective lens. Excitation at 405 nm was carried out with a semiconductor laser, and emission was collected from 420 to 520 nm.

The fluorescent quantum yield means the ratio amount of emissive photons /amount of absorbed photons: $Q_S = Q_R (I_S A_R n^2_S)/(I_R A_S n^2_R)$. Here Q means the fluorescent quantum yield; I refers to the fluorescent integrated area; A is the absorbance at the excitation wavelength; and n is the refractive index of the solvent. (Subscript R is the reference and subscript S is the sample)

Table S1. Absorption Characteristics and Photochromic Quantum Yields of **1o**

Compound	λ_{max}^{Abs}/nm ($\varepsilon \times 10^4$, THF) (Open)	λ_{max}^{Abs}/nm ($\varepsilon \times 10^4$, THF) (PSS)	Photochromic quantum yields (THF)[a]		Ratio (PSS) (Open: Closed)[b]
			$\Phi_{o\text{-}c}$ (λ/nm)	$\Phi_{c\text{-}o}$ (λ/nm)	
1o	289 (3.43)	550 (1.70)	0.23 (365)	0.22 (549)	1: 0.78
		305 (3.10)	0.21 (380)	0.02 (650)	

[a] Calculated from absorption spectral change.

[b] Obtained from the result of ^{1}H NMR.

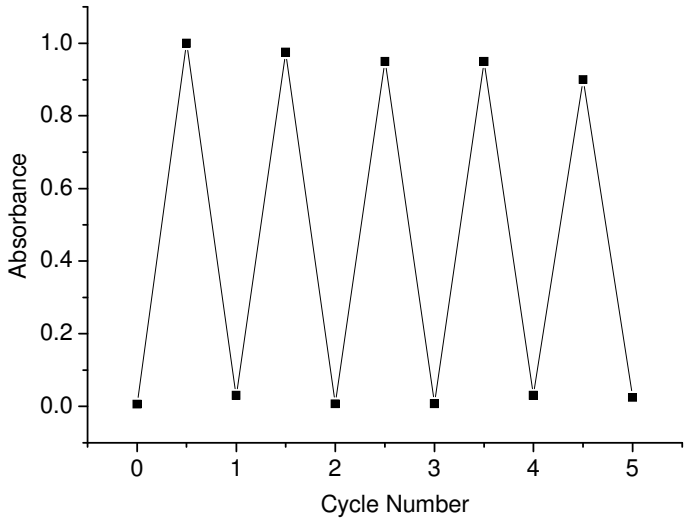


Figure S1 The absorption at 550 nm of **1** (1×10^{-5} M in THF) upon the alternative irradiation of 365 nm for 4 min and 549 nm for 2 h.

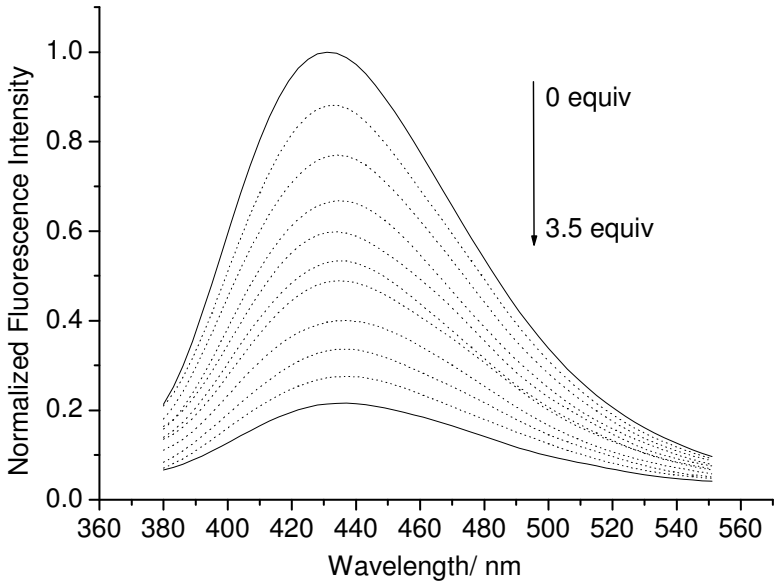


Figure S2 Fluorescence intensity changes of **1o** in THF (1×10^{-5} M) by adding Cu^{2+} from 0 equiv to 3.5 equiv.

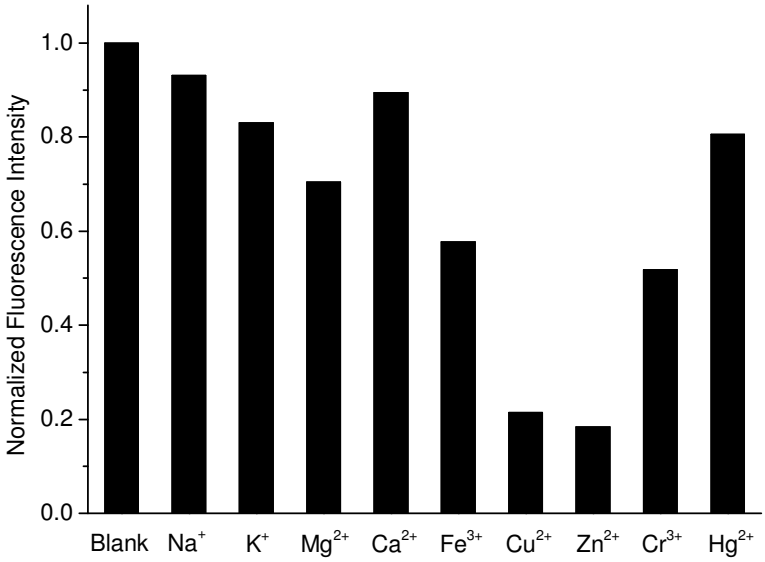


Figure S3 Fluorescence intensity changes (centered at 440 nm) of **1o** in THF (1×10^{-5} M) by adding various metal ions (5×10^{-4} M, 3.5 equiv)

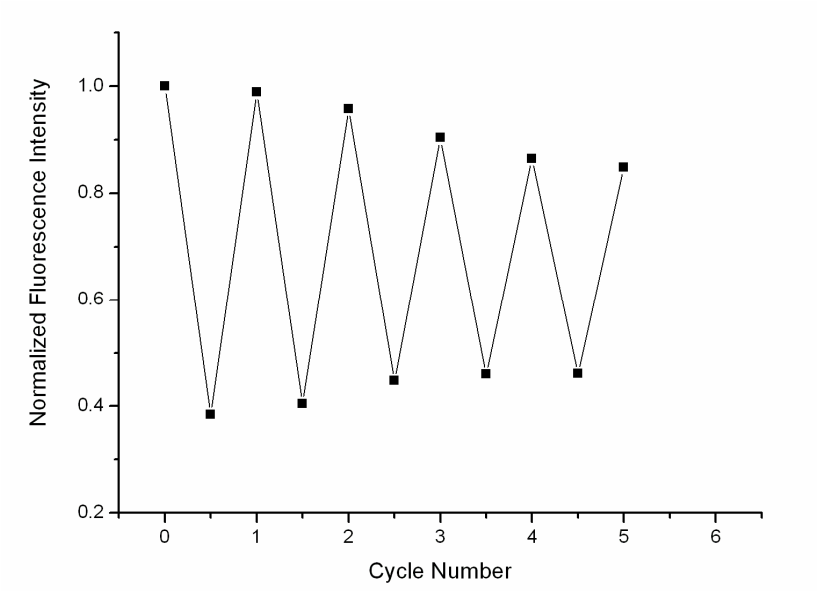


Figure S4 The fluorescent intensity at 440 nm of **1o** (1×10^{-5} M in THF) at the alternative addition of 1.0 equiv $Zn(NO_3)_2$ (1×10^{-3} M in water) and 1.0 equiv EDTA (1×10^{-3} M in water).

Cytotoxicity Assay.

MTT assay: The cytotoxicity study was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in human nasopharyngeal epiclermal carcinoma cell line KB. Cells growing in log phase were seeded into 96-well cell-culture plate at 1×10^4/well. The cells were incubated for 24 h at 37 ^oC under 5% CO_2. The compound **1o** (20 μL/well) at concentrations of 1, 10, 100 μM, was added to the wells of the treatment group, and 20 μL/well DMSO diluted in RPMI 1640 (DMSO: RPMI 1640 = 5: 100, v/v) to the control group, respectively. The cells were incubated for 6, 8, 12, 24 h at 37 ^oC under 5% CO_2; 5 mg/mL MTT (10 μL/well) in PBS solution was added to each well and incubated at 37 ˚C for 4 hours. Formazan extraction was performed with sodium dodecyl sulfate (SDS) and the quantity determined colorimetrically by using a Mutil reader (TECAN, Infinite M200), which was used to measure the OD570 nm (absorbance value) with the correction of interference at 690 nm. The following formula was used to calculate the viability of cell growth: Viability (%) = (mean of absorbance value of treatment group/mean absorbance value of control) × 100. The results are expressed as an average over five nominally identical measurement.

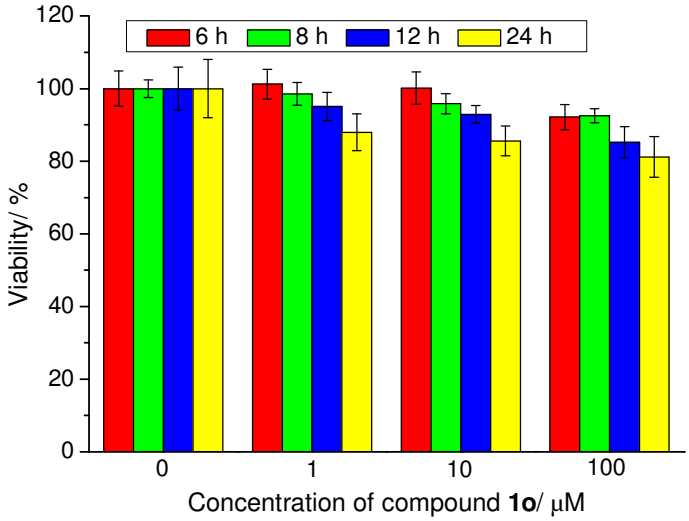


Figure S5. Cell viability values (%) estimated by MTT assay in KB, which were cultured in the presence of 1-100 μM **1o** at 37 °C for 6 ~24 hours.

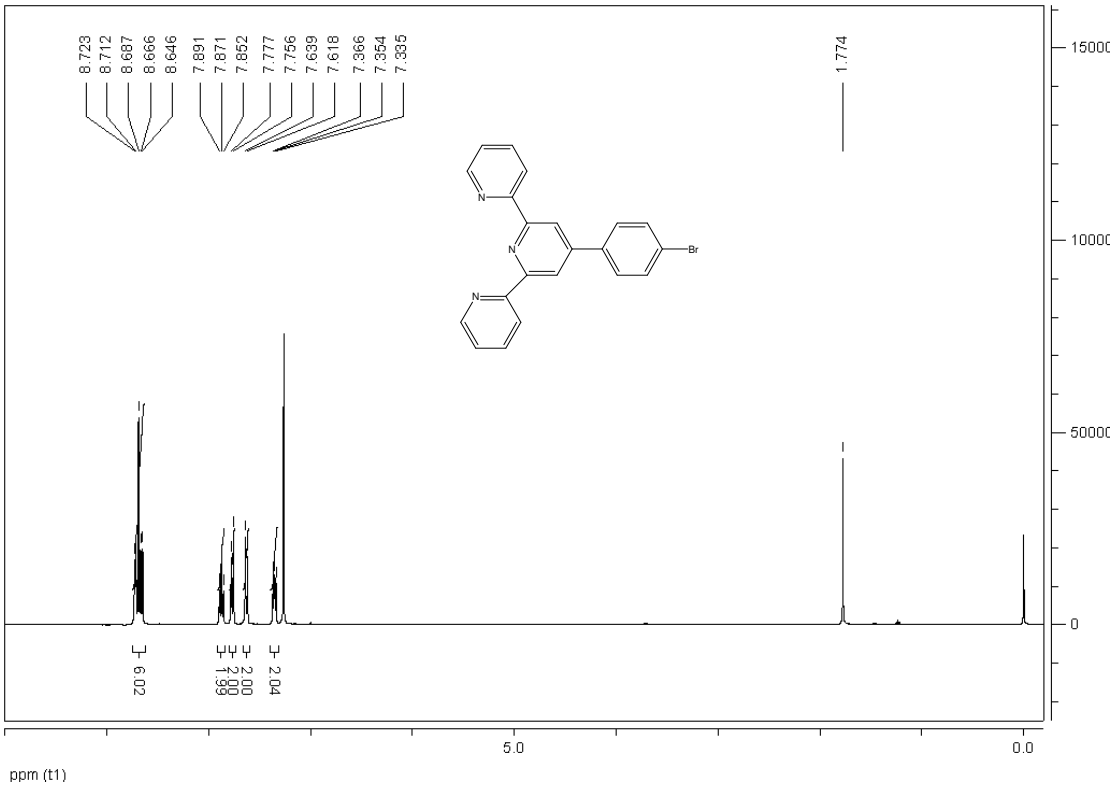
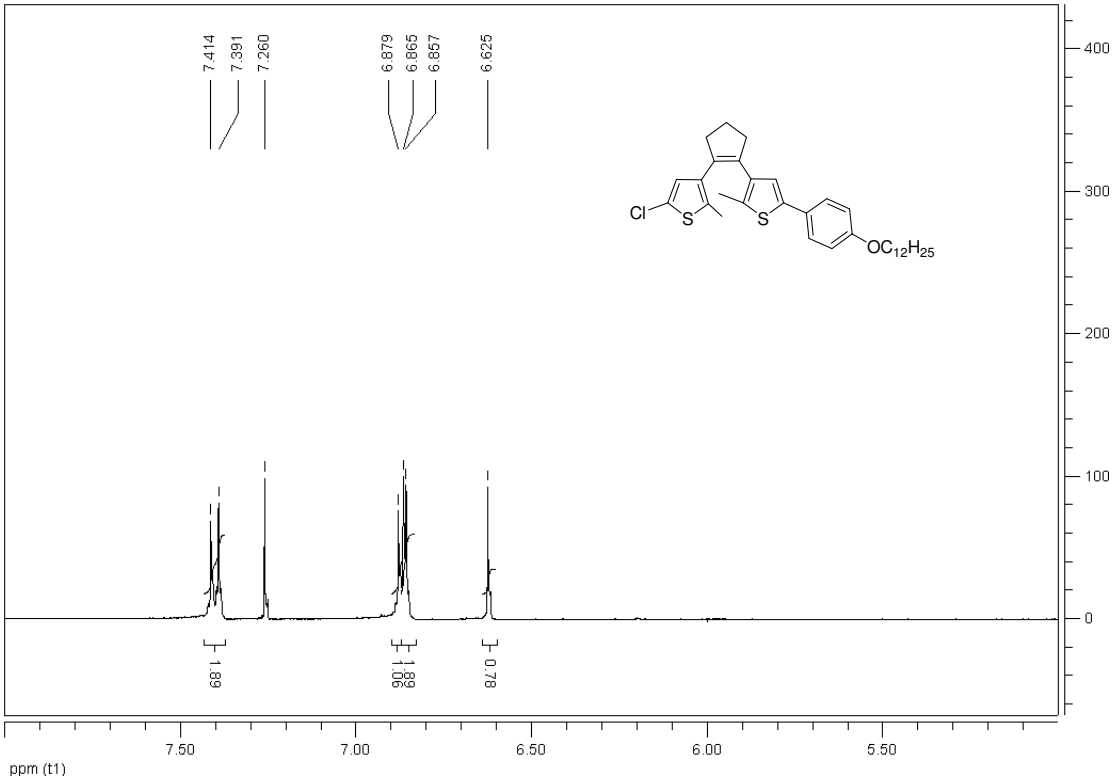


Figure S6. ^{1}H NMR spectrum of **3** in $CDCl_3$



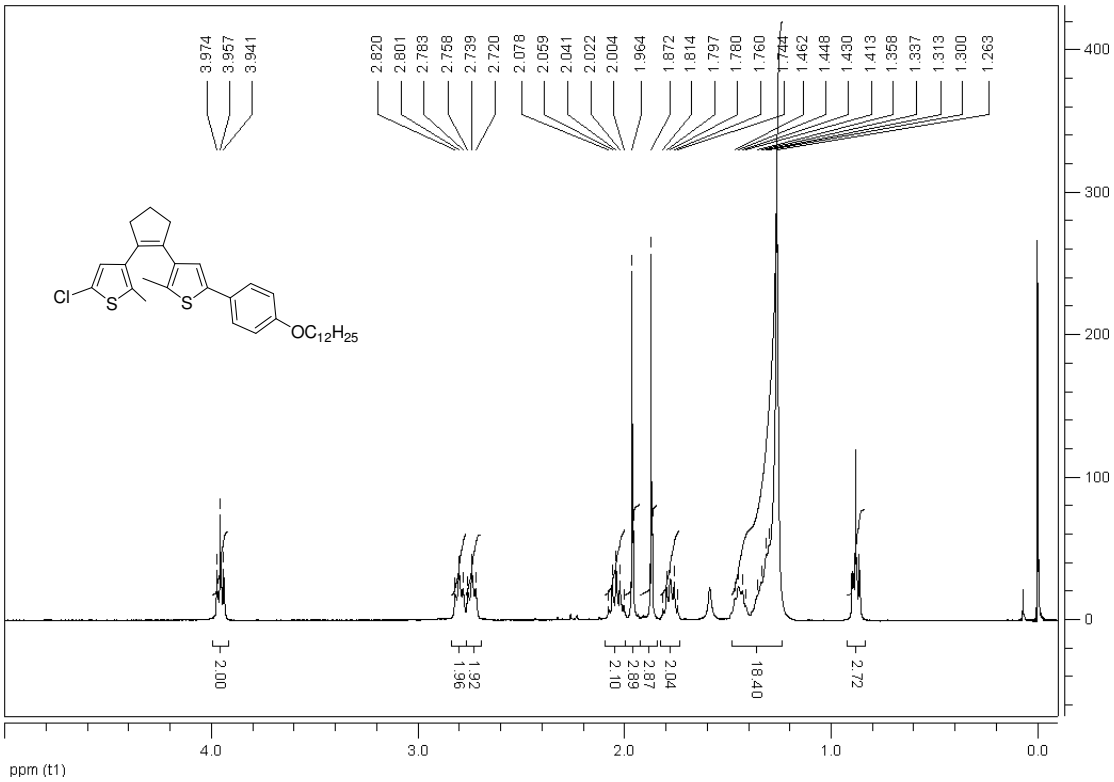


Figure S7. ^{1}H NMR spectrum of **2** in $CDCl_3$

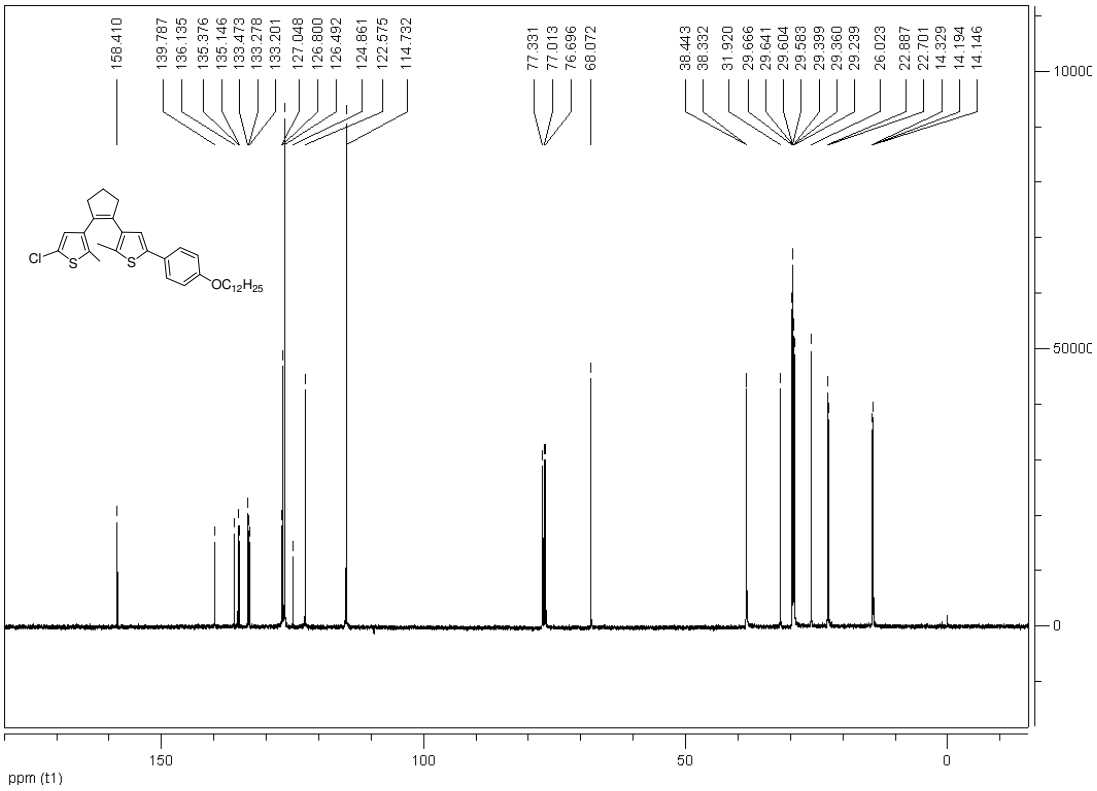


Figure S8. ^{13}C NMR spectrum of **2** in $CDCl_3$

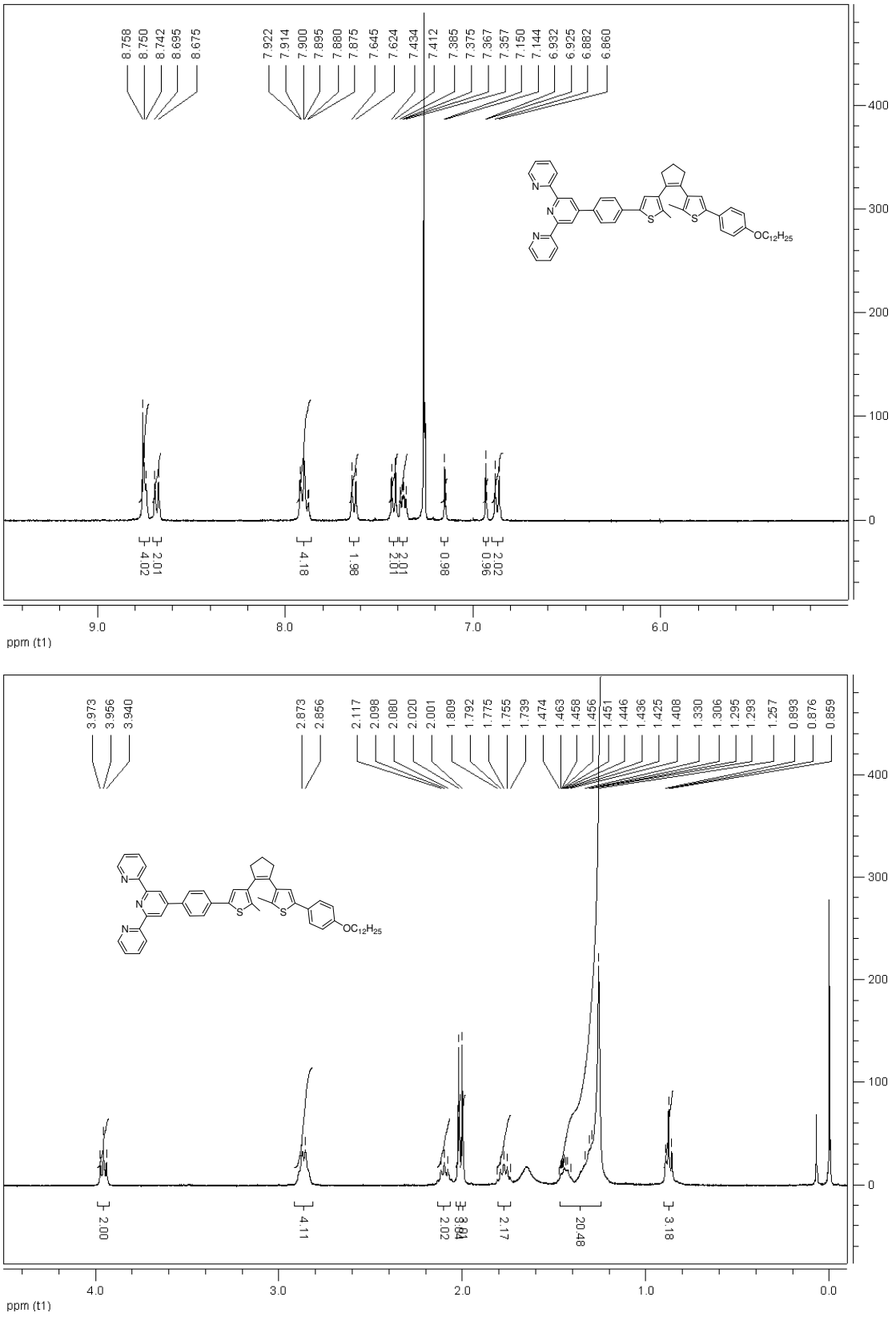


Figure S9. ^{1}H NMR spectrum of **1o** in $CDCl_3$

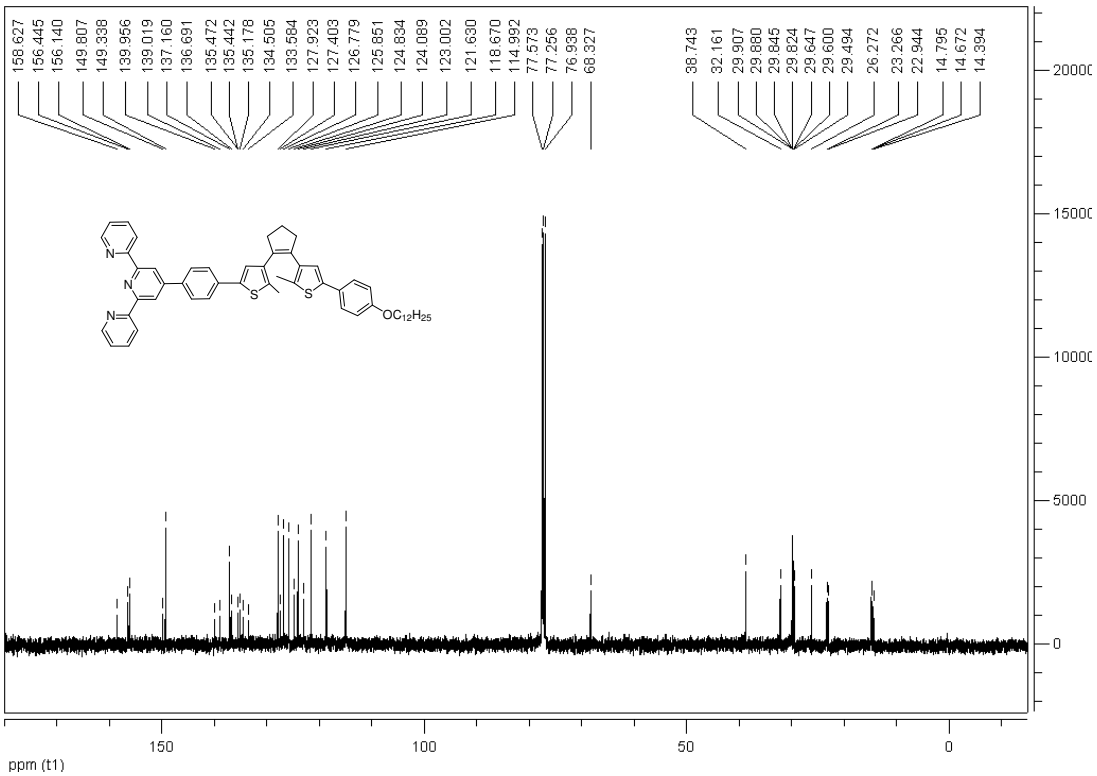


Figure S10. ^{13}C NMR spectrum of **1o** in $CDCl_3$

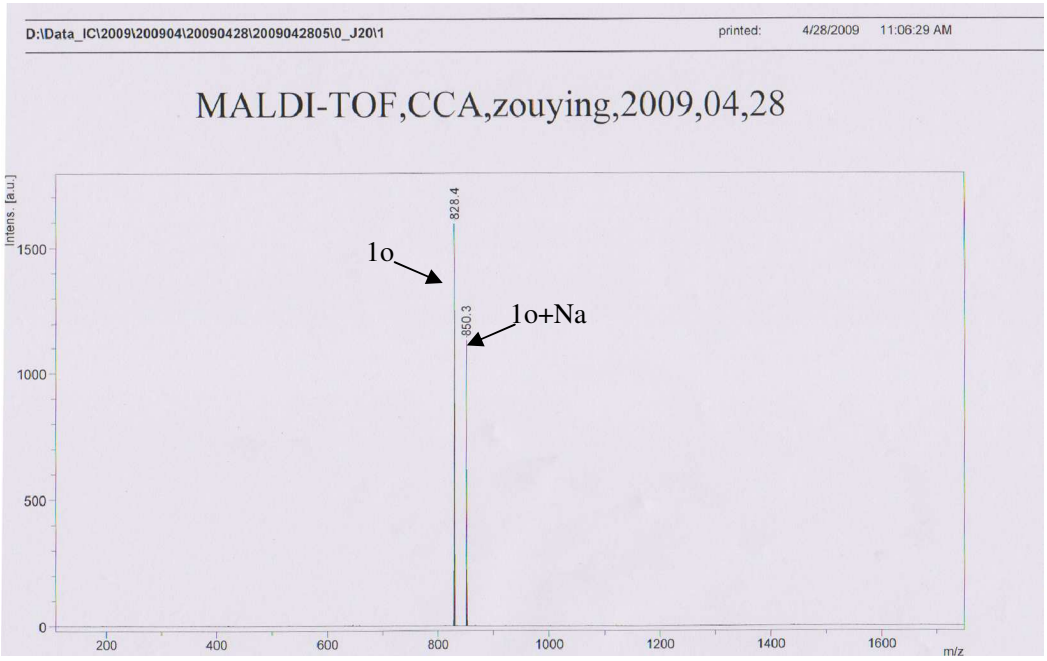


Figure S11. Maldi-tof-mass spectrum of **1o**

References

1) Han, F. S.; Higuchi, M.; Kurth D. G. *Org. Lett.* **2007**, *9*, 559-562.
2) Lucas, L. N.; de Jong, J. J. D.; van Esch, J. H.; Kellogg, R. M.; Feringa, B. L. *Eur. J. Org. Chem.*, **2003,** 155-166.