

Phenylene–Vinylene Macrocycles as Artificial Transmembrane Transporters

Xinyu Hu,^{a,b} Chao Yu,^b Kenji Okochi,^b Yinghua Jin,^b Zhenning Liu,^a and Wei Zhang^{b,*}

^a Key Laboratory of Bionic Engineering (Ministry of Education), Jilin University, Changchun 130022, P. R. China

^b Department of Chemistry and Biochemistry, University of Colorado Boulder, CO 80309, USA

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Materials and general synthetic methods

Reagents and solvents were purchased from commercial suppliers and used without further purification, unless otherwise indicated. Tetrahydrofuran (THF), toluene, CH₂Cl₂ and dimethylformamide (DMF) were purified by the MBRAUN solvent purification system. **S-1**,¹ **S-2**,² **S-3**,³ **S-4**,² **S-5**,⁴ macrocycles **2b–2d**,^{2, 4} and alkyne metathesis catalyst⁵ were synthesized following literature reported procedures.

All reactions were conducted under dry nitrogen in oven-dried glassware, unless otherwise specified. All the olefin/alkyne metathesis reactions were conducted in the glove box. Solvents used in the olefin/alkyne metathesis were dried over 4 Å molecular sieves. Solvents were evaporated using a rotary evaporator after workup. Unless otherwise specified, the purity of the compounds was ≥ 95 % based on ¹H NMR spectral integration.

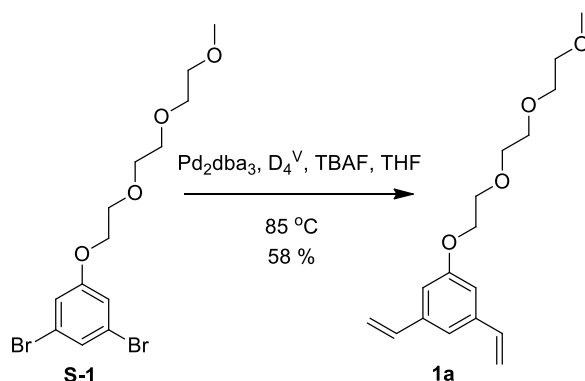
Flash column chromatography was performed by using a 100–150 times weight excess of flash silica gel 32–63 µm from Dynamic Absorbants Inc. Fractions were analyzed by TLC using TLC silica gel F254 250 µm precoated-plates from Dynamic Absorbants Inc.

MALDI-TOF mass spectra were obtained on the Voyager-DE™ STR Biospectrometry Workstation using sinapinic acid (SA) as the matrix. The high resolution mass spectra were obtained on Waters SYNAPT G2 High Definition Mass Spectrometry System. Analyte molecules were diluted in methanol, chloroform, or acetonitrile/water mixture to final concentrations of 10 ppm or lower. The solution was injected into the electrospray ionization (ESI) source at a rate of 5 µL/min. Either ESI⁺ or ESI[−] mode was used based on the molecular properties. Accurate mass analysis was performed by using the Lock Mass calibration feature with the instrument.

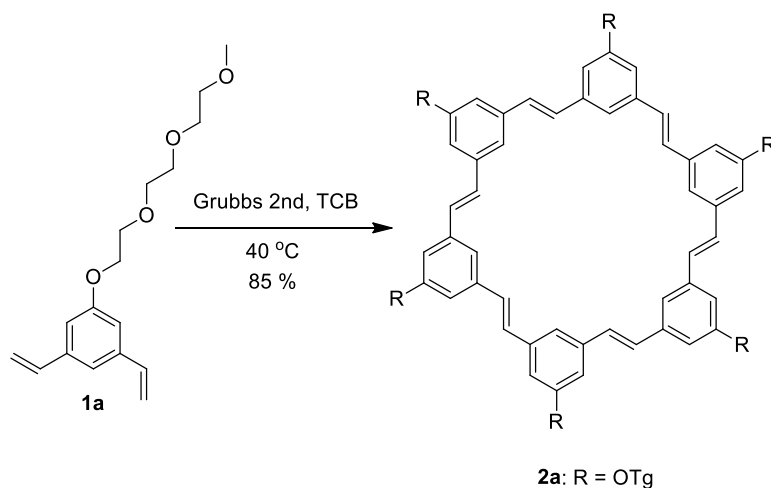
NMR spectra were taken on Inova 400 and Inova 500 spectrometers. CDCl₃ (7.26 ppm) was used as internal reference for ¹H NMR spectra. CDCl₃ (77.16 ppm) was used as internal reference for ¹³C NMR spectra. ¹H NMR data were reported in the following order: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (*J*, Hz), number of protons.

Fluorescence spectra were conducted by HITACHI F-2500 fluorescence spectrophotometer.

Synthetic procedures

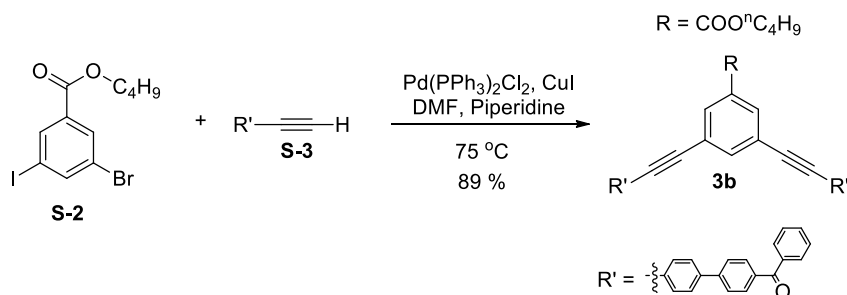


Synthesis of 1a: To a Schlenk tube were added **S-1** (995 mg, 2.50 mmol) and Pd_2dba_3 (229 mg, 0.250 mmol). TBAF (1.0 M solution in THF, 10.0 mL) and THF (25.0 mL) were added into the tube. D_4^{V} (0.88 mL, 2.50 mmol) was added and the mixture was stirred at 85 °C for 14 h. The mixture was cooled to room temperature and diethyl ether (40 mL) was added. The mixture was run through a short silica pad and all the solvents were evaporated. The residue was further purified by flash column chromatography (ethyl acetate/hexanes, 1/5, v/v) to afford product as a colorless oil (441 mg, 58%): ^1H NMR (300 MHz, CDCl_3) δ 7.03 (td, $J = 1.5, 0.7$ Hz, 1H), 6.88 (d, $J = 1.5$ Hz, 2H), 6.73 – 6.60 (m, 2H), 5.78 – 5.66 (m, 2H), 5.30 – 5.20 (m, 2H), 4.19 – 4.10 (m, 2H), 3.90 – 3.81 (m, 2H), 3.74 (ddd, $J = 5.9, 3.7, 1.2$ Hz, 2H), 3.71 – 3.63 (m, 4H), 3.57 – 3.52 (m, 2H), 3.37 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 159.31, 139.11, 136.72, 72.03, 70.94, 70.78, 70.68, 69.86, 67.55, 59.14; HR-ESI (m/z): $[\text{M}+\text{Li}]^+$ calcd. for $\text{C}_{17}\text{H}_{24}\text{O}_4\text{Li}$, 299.1835; found: 299.1837.

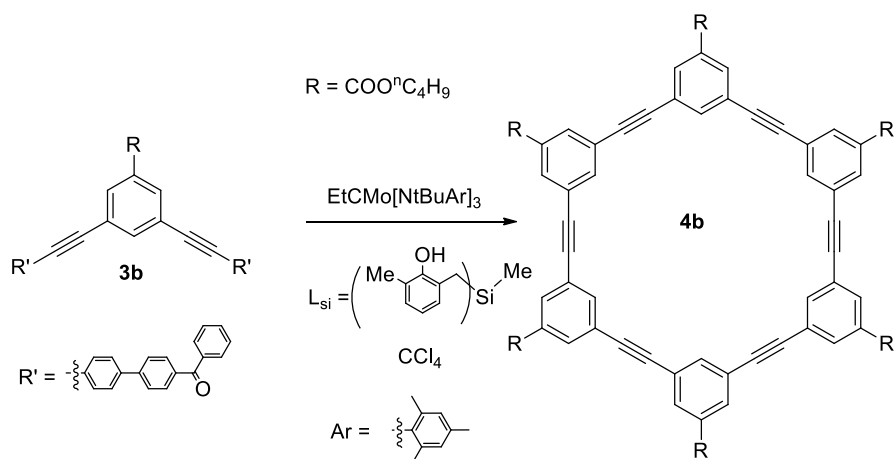


Synthesis of 2a: To a Schlenk tube were added **1a** (100 mg, 0.342 mmol), Grubbs 2nd generation catalyst (29.0 mg, 0.0342 mmol), and 1,2,4-trichlorobenzene (10 mL). The mixture was stirred at 40 °C for 11 h. All the solvents were removed and the residue was dissolved in dichloromethane (50 mL). The organic solution was washed with water (50 mL) and dried over anhydrous Na_2SO_4 . The residue was purified by flash column chromatography (MeOH/DCM, 2/98, v/v) to afford product as a white solid (77 mg, 85%): ^1H NMR (300 MHz, CDCl_3) δ 7.39 (s, 1H), 7.14 (s, 2H), 7.00 (s, 2H), 4.24 (t, $J = 4.9$ Hz, 2H), 3.92 (t, $J = 4.9$ Hz, 2H), 3.83 – 3.76 (m, 2H), 3.75 – 3.64 (m,

4H), 3.60 – 3.54 (m, 2H), 3.39 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 159.65, 138.64, 128.70, 118.16, 112.23, 72.11, 71.05, 70.88, 70.77, 69.95, 67.72, 59.22; MALDI-TOF (m/z): $[\text{M}+\text{H}]^+$ calcd. for $\text{C}_{90}\text{H}_{121}\text{O}_{24}$, 1585.82; found: 1586.66.

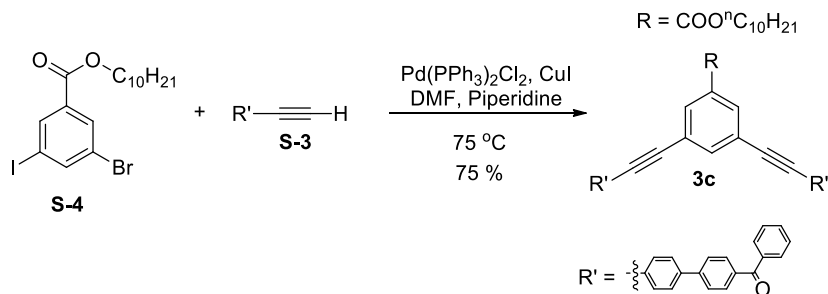


Synthesis of 3b: To a Schlenk tube were added **S-2** (766 mg, 2.00 mmol), **S-3** (1412 mg, 5.00 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (112 mg, 0.160 mmol), and CuI (3.8 mg, 0.020 mmol). Dimethylformamide (DMF, 29 mL) and piperidine (6.0 mL) were added into the tube and the mixture was stirred at 75 °C for 18 h. All the solvents were removed and the residue was dissolved in dichloromethane (200 mL). The solution was washed with water (3×100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and then concentrated to give the crude product, which was further purified by flash column chromatography (CH_2Cl_2 /hexane, 9/1, v/v) to afford **3b** as a yellow solid (1.31 g, 89%): ^1H NMR (500 MHz, CDCl_3) δ 8.17 (d, $J = 1.7$ Hz, 2H), 7.90 (dd, $J = 8.2, 2.1$ Hz, 5H), 7.85 – 7.81 (m, 4H), 7.74 – 7.57 (m, 14H), 7.50 (t, $J = 7.9$ Hz, 4H), 4.37 (t, $J = 6.7$ Hz, 2H), 1.79 (p, $J = 7.0$ Hz, 2H), 1.51 (h, $J = 7.5$ Hz, 2H), 1.01 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 196.15, 165.33, 144.10, 140.11, 138.22, 137.68, 136.66, 132.51, 132.39, 132.28, 131.33, 130.85, 130.05, 128.40, 127.33, 126.89, 124.09, 122.57, 90.70, 88.99, 65.47, 30.82, 19.34, 13.88. HR-ESI (m/z): $[\text{M}+\text{Li}]^+$ calcd. for $\text{C}_{53}\text{H}_{38}\text{O}_4\text{Li}$, 745.2932; found: 745.2952.

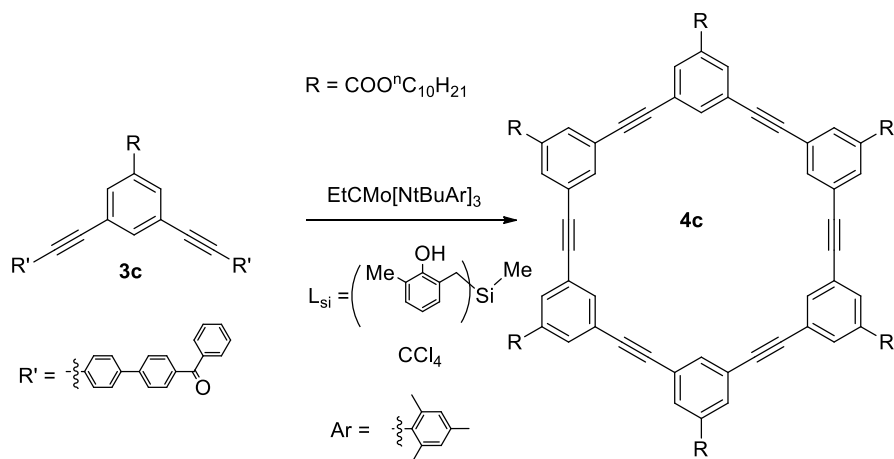


Synthesis of 4b: The triphenolsilane ligand L_{Si} (2.2 mg, 0.0054 mmol) and the molybdenum precursor (3.6 mg, 0.0054 mmol) were premixed in dry carbon tetrachloride (0.3 mL) and stirred for 20 minutes to generate the catalyst in situ. Then the catalyst solution was added to a solution of **3b** (66.5 mg, 0.09 mmol) in CCl_4 (5 mL) and the mixture was stirred at 55 °C for overnight. All the solvents were removed and the crude mixture was purified by flash column chromatography (CH_2Cl_2 /hexane,

9/1, v/v) to provide the pure product as a white solid (10.4 mg, 58%): ^1H NMR (500 MHz, CDCl_3) δ 8.13 (d, J = 1.6 Hz, 12H), 7.81 (t, J = 1.6 Hz, 6H), 4.39 (t, J = 5.9 Hz, 12H), 1.83 (m, 12H), 1.54 (m, 12H), 1.04 (t, J = 7.4 Hz, 18H). The NMR data is consistent with the literature report.⁶

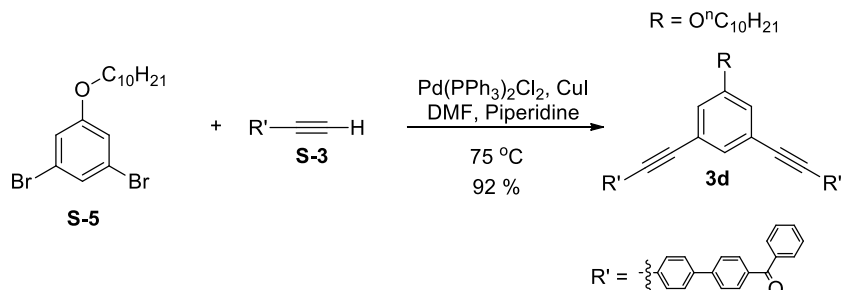


Synthesis of 3c: To a Schlenk tube were added **S-4** (934 mg, 2.00 mmol), **S-3** (1412 mg, 5.00 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (112 mg, 0.160 mmol), and CuI (3.8 mg, 0.020 mmol). Dimethylformamide (DMF, 29 mL) and piperidine (6.0 mL) were added into the tube and the mixture was stirred at 75 °C for 18 h. All the solvents were removed and the residue was dissolved in dichloromethane (200 mL). The solution was washed with water (3×100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and then concentrated to give the crude product, which was further purified by flash column chromatography (CH_2Cl_2 /hexane, 9/1, v/v) to afford **3c** as a yellow solid (1.23 g, 75%): ^1H NMR (500 MHz, CDCl_3) δ 8.18 (d, J = 1.6 Hz, 2H), 7.94 – 7.89 (m, 5H), 7.86 – 7.82 (m, 4H), 7.77 – 7.59 (m, 14H), 7.54 – 7.49 (m, 4H), 4.37 (t, J = 6.7 Hz, 2H), 1.81 (p, J = 6.9 Hz, 2H), 1.52 – 1.20 (m, 14H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, CDCl_3) δ 196.31, 165.46, 144.24, 140.25, 137.77, 136.77, 132.59, 132.46, 132.38, 131.43, 130.94, 130.14, 128.47, 127.43, 127.00, 124.17, 122.66, 90.71, 89.03, 65.88, 32.03, 29.70, 29.67, 29.46, 28.84, 26.16, 22.83, 14.26. HR-ESI (m/z): $[\text{M}+\text{Li}]^+$ calcd. for $\text{C}_{59}\text{H}_{50}\text{O}_4\text{Li}$, 829.3870; found: 829.3909.

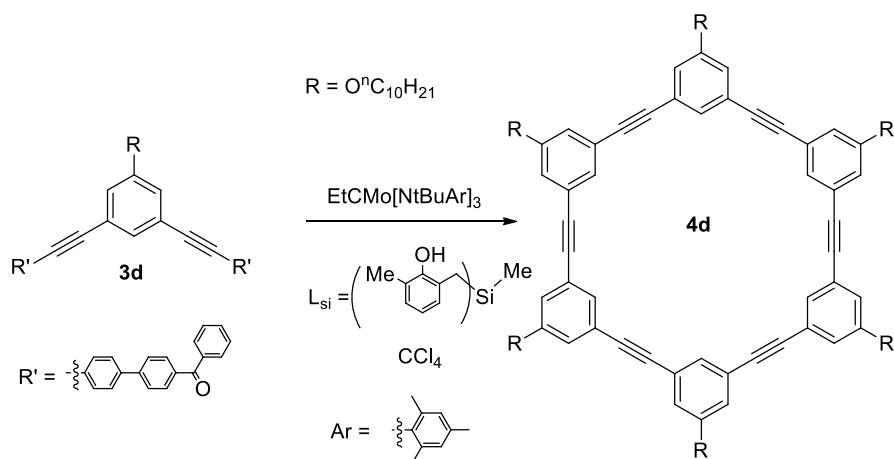


Synthesis of 4c: The triphenolsilane ligand L_{Si} (1.5 mg, 0.0036 mmol) and the molybdenum precursor (2.4 mg, 0.0036 mmol) were premixed in dry carbon tetrachloride (200 μL) and stirred for 20 minutes to generate the catalyst in situ. Then the catalyst solution was added to a solution of **3c** (49.5 mg, 0.06 mmol) in CCl_4 (3 mL) and the mixture was stirred at 55 °C for overnight. All the solvents were removed

and the crude mixture was purified by flash column chromatography (CH₂Cl₂/hexane, 9/1, v/v) to provide the pure product as a white solid (10.0 mg, 58%): ¹H NMR (500 MHz, CDCl₃) δ 8.18 (d, *J* = 1.6 Hz, 12H), 7.85 (t, *J* = 1.6 Hz, 6H), 4.39 (t, *J* = 5.9 Hz, 12H), 1.84 (m, 12H), 1.37 (m, 84H), 0.89 (t, *J* = 6.9 Hz, 18H). The NMR data is consistent with the literature report.⁷



Synthesis of 3d: To a Schlenk tube were added **S-5** (784 mg, 2.00 mmol), **S-3** (1412 mg, 5.00 mmol), Pd(PPh₃)₂Cl₂ (112 mg, 0.160 mmol), and CuI (3.8 mg, 0.020 mmol). Dimethylformamide (DMF, 29 mL) and piperidine (6.0 mL) were added into the tube and the mixture was stirred at 75 °C for 18 h. All the solvents were removed and the residue was dissolved in dichloromethane (200 mL). The solution was washed with water (3 × 100 mL) and brine (100 mL). The organic layer was dried over anhydrous Na₂SO₄ and then concentrated to give the crude product, which was further purified by flash column chromatography (CH₂Cl₂/hexane, 9/1, v/v) to afford **3d** as a light green solid (1.46 g, 92%): ¹H NMR (500 MHz, CDCl₃) δ 7.89 (dd, *J* = 8.2, 1.5 Hz, 4H), 7.86 – 7.82 (m, 4H), 7.73 – 7.57 (m, 14H), 7.52 – 7.47 (m, 4H), 7.37 (d, *J* = 1.4 Hz, 1H), 7.09 (d, *J* = 1.4 Hz, 2H), 3.98 (t, *J* = 6.5 Hz, 2H), 1.80 (p, *J* = 6.8 Hz, 2H), 1.51 – 0.88 (m, 14H), 0.91 (t, *J* = 6.9 Hz, 3H). ¹³C NMR (75 MHz, CDCl₃) δ 196.05, 158.92, 144.10, 139.73, 137.65, 136.52, 132.44, 132.29, 130.80, 130.00, 128.35, 127.22, 126.81, 124.40, 122.92, 117.92, 90.03, 89.51, 68.33, 31.97, 29.67, 29.65, 29.46, 29.41, 29.23, 26.08, 22.76, 14.22. HR-ESI (*m/z*): [M+Li]⁺ calcd. for C₅₈H₅₀O₃Li, 801.3922; found: 801.3954.



Synthesis of 4d: The triphenolsilane ligand L_{Si} (1.5 mg, 0.0036 mmol) and the molybdenum precursor (2.4 mg, 0.0036 mmol) were premixed in dry carbon tetrachloride (0.2 mL) and stirred for 20 minutes to generate the catalyst in situ. Then

the catalyst solution was added to a solution of **3d** (48.0 mg, 0.06 mmol) in CCl₄ (3 mL) and the mixture was stirred at 55 °C for overnight. All the solvents were removed and the crude mixture was purified by flash column chromatography (CH₂Cl₂/hexane, 9/1, v/v) to provide the pure product as a white solid (5.7 mg, 47%): ¹H NMR (500 MHz, CDCl₃) δ 7.34 (t, *J* = 1.5 Hz, 6H), 7.05 (d, *J* = 1.4 Hz, 12H), 4.01 (t, *J* = 6.6 Hz, 12H), 1.82 (m, 12H), 1.35 (m, 84H), 0.91 (t, *J* = 6.8 Hz, 18H). The NMR data is consistent with the literature report.⁸

Preparation of HPTS-entrapped large unilamellar vesicles

1,2-diacyl-sn-glycero-3-phosphocholine (PC, 100 mg/ml, 0.15 mL) and 3-β-hydroxy-5-cholestene (CH, 3.75 mg) were dissolved in CHCl₃ (10 mL) in a round-bottom flask. The solvent was removed under reduced pressure (5 min, 20 °C) to produce a uniform thin film. The film was dried under high vacuum for 3 h at room temperature. Then the film was hydrated with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer solution (1.5 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.0) containing a pH sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS, 0.1 mM) in thermostatic shaker-incubator at 37 °C for 2 h to give a milky suspension. The mixture was then subjected to ten freeze-thaw cycles: freeze in liquid N₂ for 30 s, warm it up at 37 °C for 1.5 min, then gentle vortex mixing for 3 min at room temperature. The vesicle suspension was extruded through polycarbonate membrane (0.22 μm) to produce homogeneous suspension of large unilamellar vesicles (LUVs) with encapsulated HPTS. The suspension of LUVs was divided into two equal aliquots and dialyzed for 36 h with gentle stirring (200 r/min) using membrane tube (MWCO = 8000–14000) against the same HEPES buffer solution (300 mL, without HPTS) for six times to remove free HPTS.

Determination of proton transport activity through HPTS assay

Procedure a:

The prepared LUVs suspension (6 μL, 13.3 mM, internal buffer: 10 mM HEPES, 100 mM NaCl, pH = 7.0) was added to HEPES buffer solution (total volume 800 μL, 10 mM HEPES, 100 mM NaCl, pH = 7.6). The solution of macrocycle in THF (1.0 mM) was added with gentle mixing. The fluorescence intensity was immediately measured as a function of time to investigate the channeling activity of the macrocycles. Fluorescence intensity of HPTS (*I*₁) was continuously monitored at 510 nm (excitation at 454 nm) for 30 min. Aqueous solution of Triton X-100 (16 μL, 20% v/v) was added to achieve the maximum changes in dye fluorescence emission (*I*₂). The collected data were then normalized into the fractional change in fluorescence intensity according to the following equation: $R(\%) = \frac{I_1 - I_0}{I_2 - I_0} \times 100$, where *I*₀ is the initial intensity.⁹

Procedure b:

The prepared LUVs suspension (6 μ L, 13.3 mM, internal buffer: 10 mM HEPES, 100 mM NaCl, pH = 7.0) was added to the HEPES buffer solution (total volume 800 μ L, 10 mM HEPES, 100 mM NaCl, pH = 7.0). The solution of macrocycle in THF (1 mM) was added into the above LUVs suspension and the incubated at room temperature for 30 min. After incubation, the fluorescence intensity was recorded for 100s (excitation at 454 nm, emission at 510 nm), at which time the transmembrane pH gradient was generated by the rapid addition aqueous NaOH solution (0.5 M, 2 μ L). The fluorescence emission was then continuously monitored for additional 1700s. Aqueous solution of Triton X-100 (16 μ L, 20% v/v) was added to achieve the maximum changes in dye fluorescence emission. The fluorescence emission data were analyzed as described in procedure a.

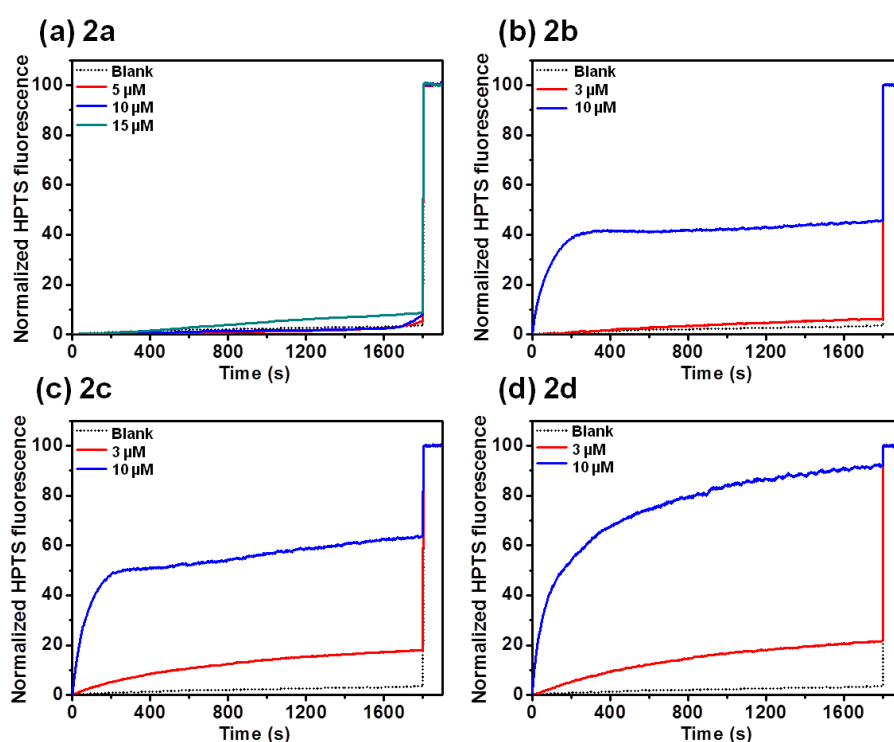


Figure S1. Normalized fluorescence traces of HPTS emission in the presence of **2a** (a), **2b** (b), **2c** (c), and **2d** (d). The procedure **a** for HPTS assay was followed.

Calcein-encapsulated large unilamellar vesicles

PC (100 mg/mL, 0.10 mL) and CH (2.5 mg) were dissolved in CHCl_3 (20 mL). The solution was evaporated under reduced pressure (10 min, 20 $^{\circ}\text{C}$), and further dried under high vacuum for 3 h. The lipid film was then hydrated with HEPES buffer solution (1.0 mL, 10 mM HEPES, 100 mM NaCl, pH = 7.4) containing calcein (40 mM) at 37 $^{\circ}\text{C}$ for 2 h in thermostatic shaker-incubator to give a milky suspension. Ten freeze-thaw cycles (freeze in liquid nitrogen for 30 s, warm it up at 37 $^{\circ}\text{C}$ for 1.5 min, and then gentle vortex mixing for 3 min) were performed. The LUVs suspension was extruded through polycarbonate membrane (0.22 μm) to produce homogeneous suspension of LUVs. The suspension of LUVs was divided into two equal aliquots

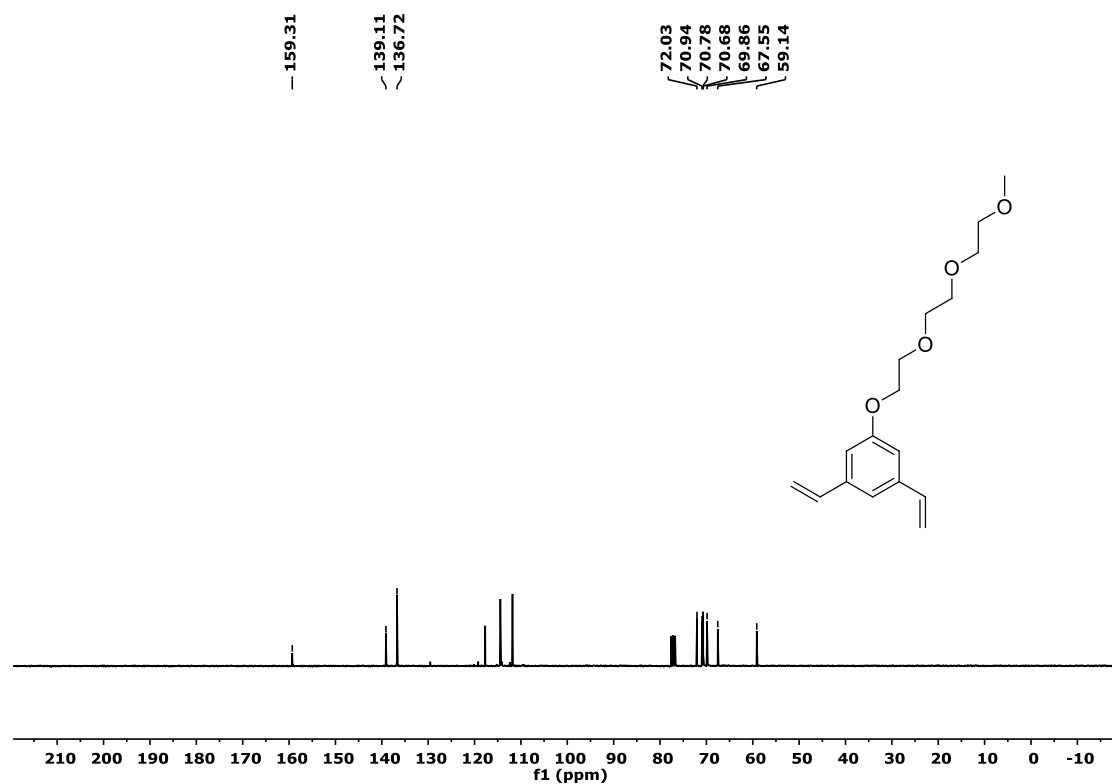
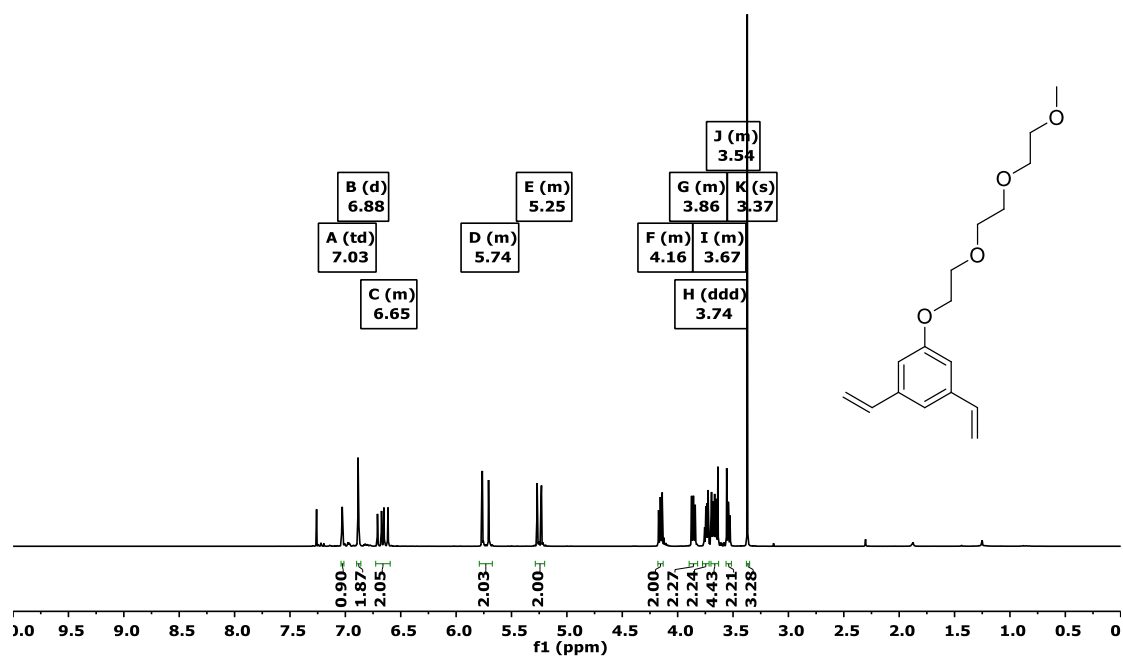
and dialyzed for 36 h with gentle stirring (200 r/min) using membrane tube (MWCO = 8000–14000) against the same HEPES buffer solution (300 mL, without calcein) for six times to remove free calcein.

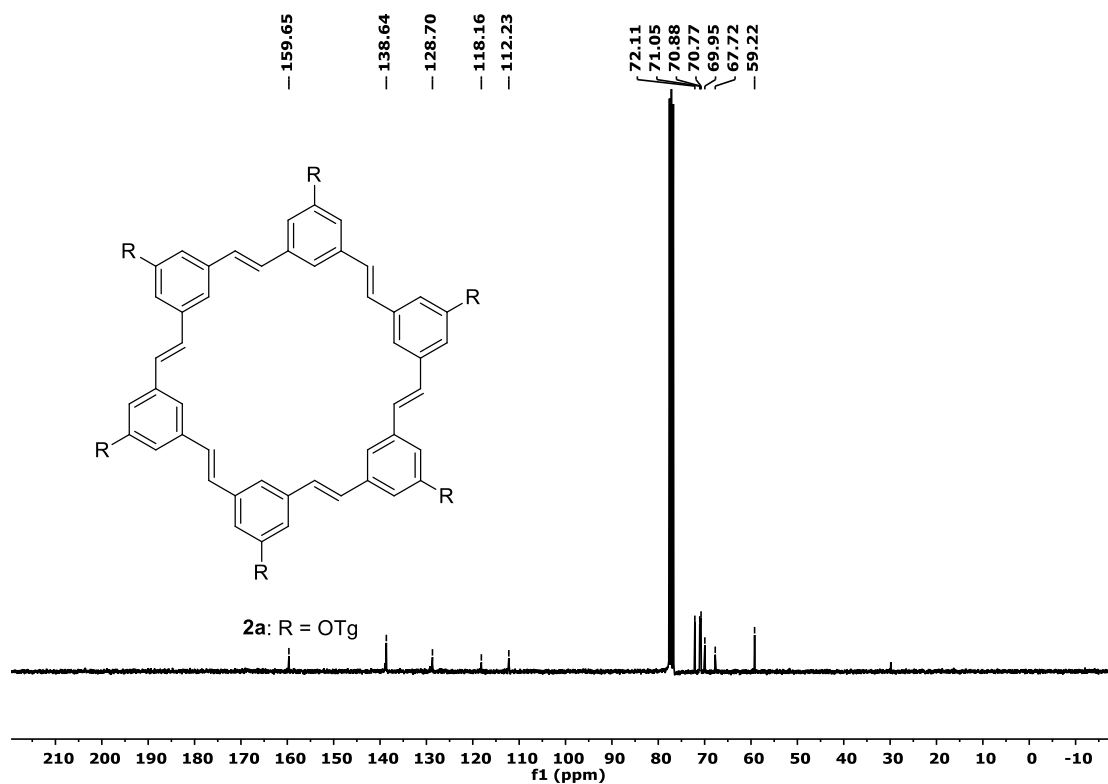
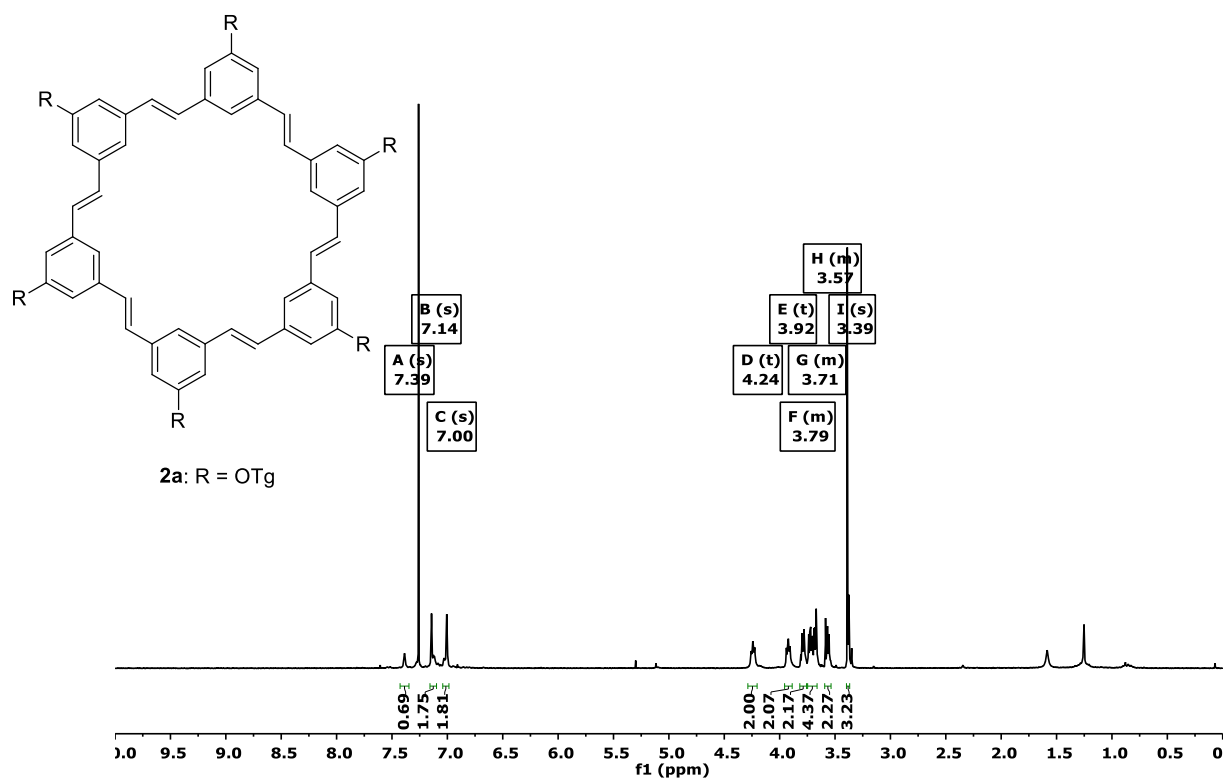
Determination of calcein transport through calcein assay

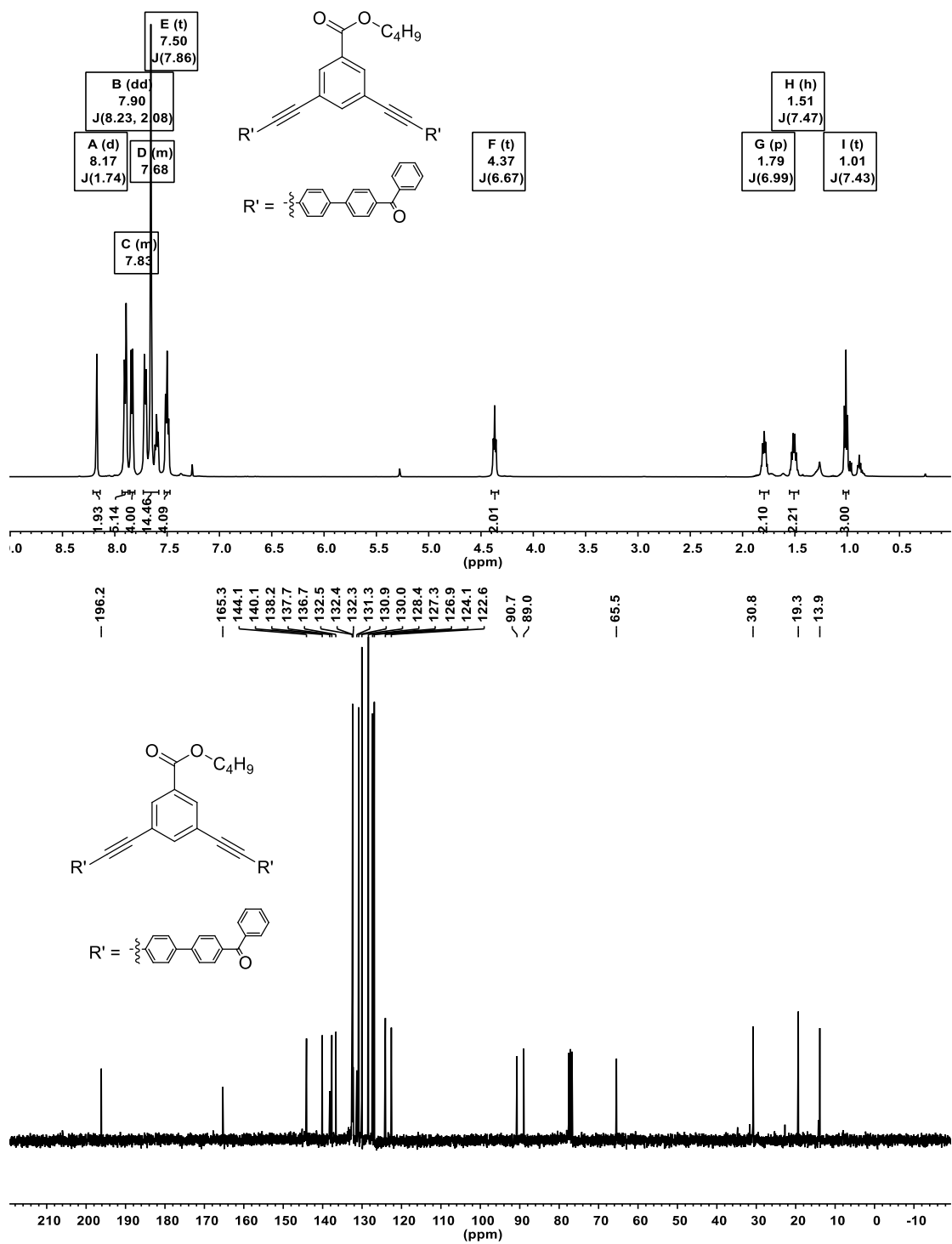
The above suspension of LUVs with entrapped-calcein (6 μ L) was added to HEPES buffer solution (total volume 800 μ L, 10 mM HEPES, 100 mM NaCl, pH = 7.4), followed by the solution of macrocycle (1.0 mM) in THF with gentle mixing. Fluorescence intensity of calcein (I_1) was continuously monitored at 505 nm (excitation at 493 nm) for 30 min. Then, aqueous solution of Triton X-100 (16 μ L, 20% v/v) was added to the cuvette to achieve the maximum changes in dye fluorescence emission (I_2) at the end of experiment. The collected data were normalized into the fractional change in fluorescence according to the following equation: $R(\%) =$

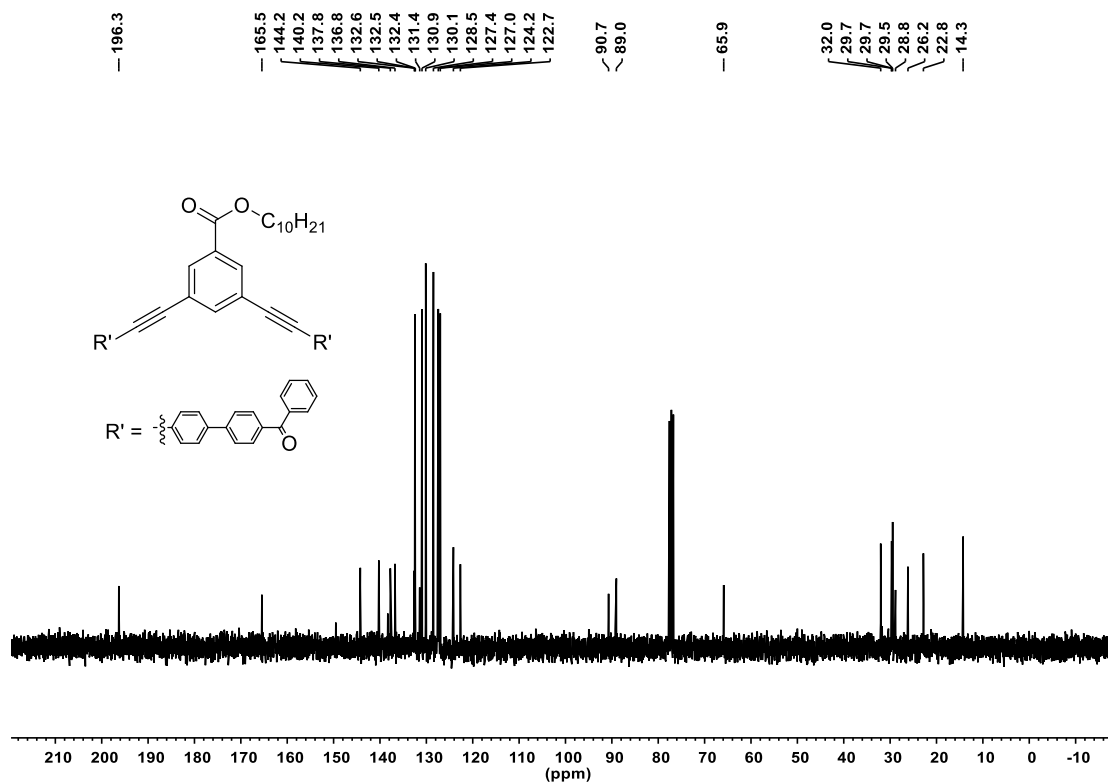
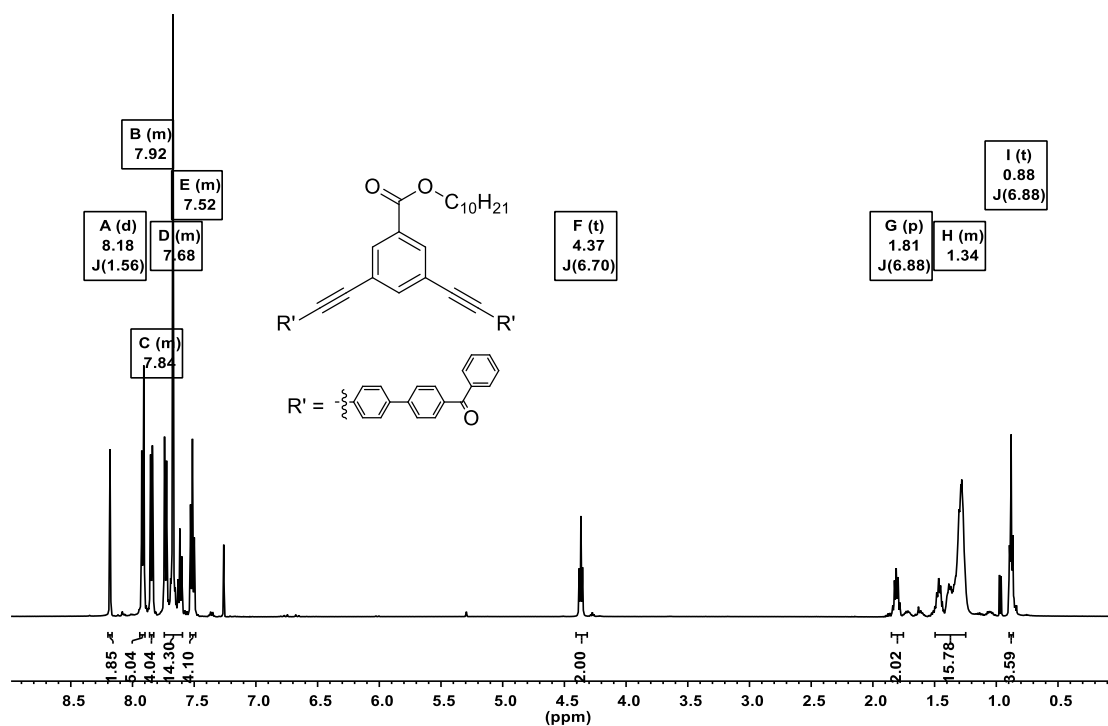
$$\frac{I_1 - I_0}{I_2 - I_0} \times 100, \text{ where } I_0 \text{ is the initial intensity.}^{10}$$

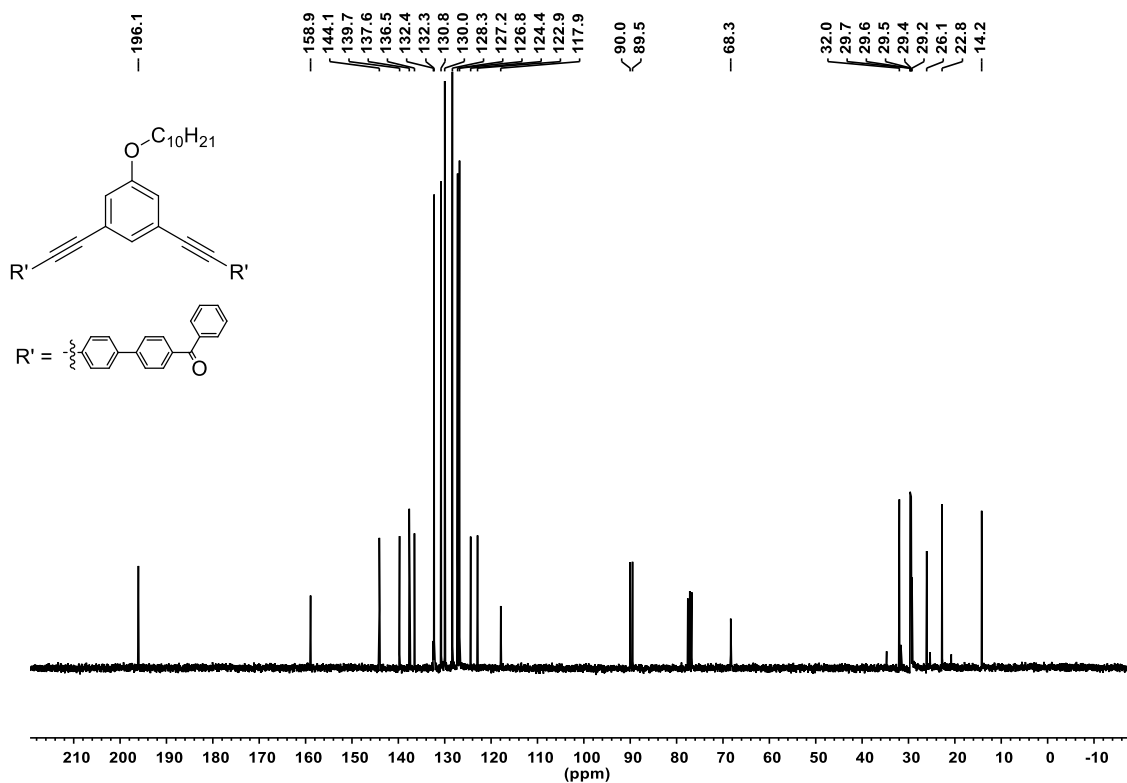
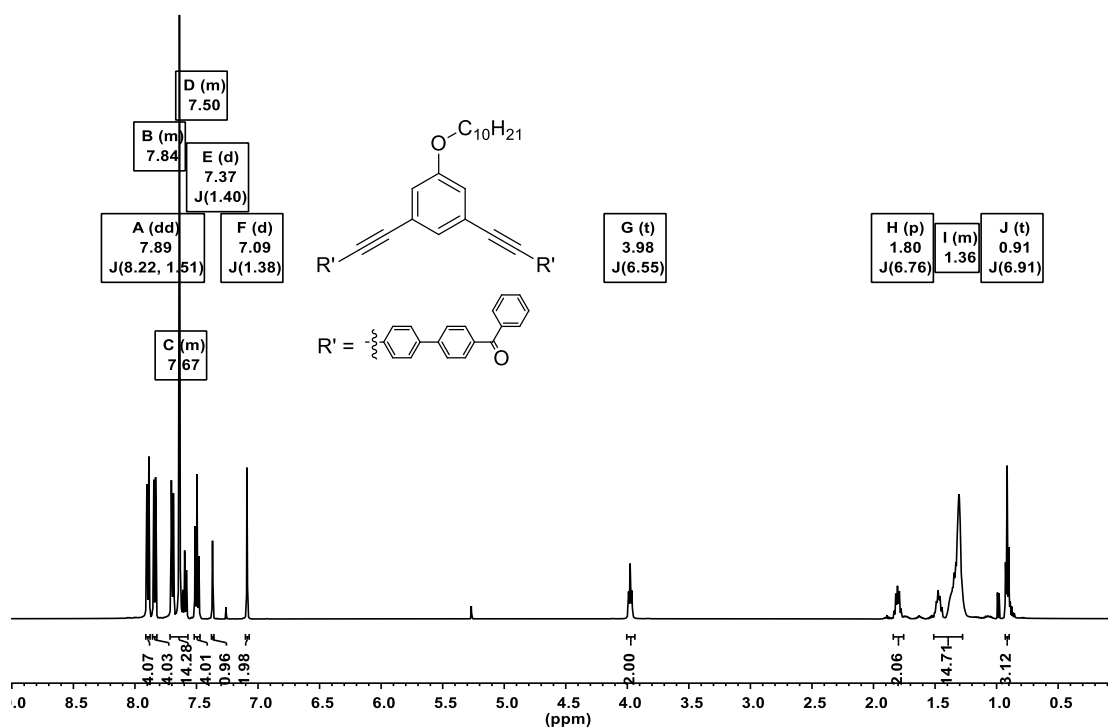
NMR spectra











References

1. W. Zhang and J. S. Moore, *J. Am. Chem. Soc.*, 2004, **126**, 12796-12796.
2. C. Zhang, C. Yu, H. Long, R. J. Denman, Y. Jin and W. Zhang, *Chem. Eur. J.*, 2015, **21**, 16935-16940.
3. C. Zhang, Q. Wang, H. Long and W. Zhang, *J. Am. Chem. Soc.*, 2011, **133**, 20995-21001.
4. Y. Jin, A. Zhang, Y. Huang and W. Zhang, *Chem. Commun.*, 2010, **46**, 8258-8260.
5. H. Yang, Z. Liu and W. Zhang, *Adv. Synth. Catal.*, 2013, **355**, 885-890.
6. J. Zhang and J. S. Moore, *J. Am. Chem. Soc.*, 1992, **114**, 9701-9702.
7. J. Zhang, D. J. Pesak, J. L. Ludwick and J. S. Moore, *J. Am. Chem. Soc.*, 1994, **116**, 4227-4239.
8. A. S. Shetty, J. Zhang and J. S. Moore, *J. Am. Chem. Soc.*, 1996, **118**, 1019-1027.
9. X. B. Hu, Z. X. Chen, G. F. Tang, J. L. Hou and Z. T. Li, *J. Am. Chem. Soc.*, 2012, **134**, 8384-8387.
10. M. Danial, C. M. N. Tran, K. A. Jolliffe and S. Perrier, *J. Am. Chem. Soc.*, 2014, **136**, 8018-8026.