

Supporting Information

Two-photon Fluorescent *Bombyx Mori* Silk by Molecular Recognised Functionalization

Naibo Lin,^{abc} Guoyang William Toh,^c Yan Feng,^a X.Y. Liu,^{*abc} and Hongyao Xu^{*a}

^a College of Material Science and Engineering & State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Donghua University, Shanghai, 201620, China.

^b Research Institute for Biomimetics and Soft Matter & College of Materials, Xiamen University, Xiamen, 361005, China.

^c MIT-Singapore Alliance, Department of Physics, National University of Singapore, 2 Science Drive 3, Singapore, 117542, Singapore.

Materials

Unless otherwise noted, all commercial reagents were used as received. 4-vinylbenzyl chloride, triethyl phosphite, Palladium(II) acetate was purchased from Aldrich, kept under an inert atmosphere in a glove box, and used as received without further purification. Dimethyl sulfoxide (DMSO) was dried over anhydrous MgSO_4 and freshly distilled prior to use. Triethylamine was purchased from Shanghai Chemical Reagent Company and distilled before use. 2, 7-dibromofluorene was obtained from TEIKO International LTD. 1-Bromooctane, tetrabutyl ammonium bromide, 4-Methyl benzaldehyde, 4-Methoxy benzaldehyde, 4-Nitrobenzaldehyde, NaOH, MgSO_4 , hexane, Triphenylphosphine, p-Nitrobenzyl bromide, formaldehyde, dichloromethane, anhydrous methanol, toluene, petroleum ether and ethyl acetate and p-methylstyrene were all purchased from Shanghai Chemical Reagent Company. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin streptomycin solution were from Biochrom AG. D-(+)-Glucose, sodium bicarbonate, paraformaldehyde (PFA) and Triton X-100 were purchased from Sigma Aldrich. Phalloidin-Alexa 488 was from Invitrogen, and 4',6'-di-amidino-2-phenyl-indol (DAPI) was from Vector laboratories.

Synthesis of 2,7-Dibromo-9,9-dioctyl-9H-fluorene.

1-Bromooctane (5.60 g, 29.0 mmol) was added by using a syringe to a mixture of 2,7-dibromo-9H-fluorene (3.88g, 12.0mmol), tetrabutyl ammonium bromide (0.0225 g, 0.0975mmol) and 3.75 mL of 50% aqueous NaOH in DMSO (50.0 mL). The reaction mixture was stirred at room temperature for 3h. The mixture was poured into H_2O (500 mL), and then was extracted three times with dichloromethane. The combined organic layers were dried over anhydrous MgSO_4 and decolor by active carbon. The solvent was removed under reduced pressure. The crude product was purified by recrystallization by hexane as a solvent to yield a colorless crystal (89%). IR (KBr), ν (cm^{-1}): 2955, 2920, 2851 (CH_2 , CH_3); 1467, 1448, 1416, 812 (Ar). ^1H NMR (400 MHz, CDCl_3), δ (TMS, ppm): 7.51 (d, 2H, $J = 7.7$ Hz), 7.45 (m, 4H), 1.90 (m, 4H, $-\text{CH}_2(\text{CH}_2)_6\text{CH}_3$), 1.25-0.86 (m, 20H, $-\text{CH}_2(\text{CH}_2)_5\text{C}_2\text{H}_5$), 0.83 (t, 6H, $J = 7.2$ Hz, $-(\text{CH}_2)_7\text{CH}_3$), 0.58 (s, 4H, $-(\text{CH}_2)_6\text{CH}_2\text{CH}_3$).

Synthesis of 4-Ethenyl-4'-nitro-1,2-stilbene.

Triethyl phosphite (5.5 mL, 30 mmol) and 4-vinylbenzyl chloride (3.9 mL, 25 mmol) were refluxed at 110 °C for 6 h. After evaporating the excess raw materials, the resulting product was dissolved into 20 mL anhydrous THF, NaH (1.2 g, 50 mmol) was then added slowly with stirring for 30 minutes at 0 °C. To this solution, 20 mL THF solution containing

4-nitrobenzaldehyde (25 mmol) was added slowly and stirred for 3 h. A little of water was added dropwise to above mixture, the precipitate was filtered and washed with water and ethanol. The crude product was recrystallized from ethanol twice to give crystals with 53 % yield. IR (KBr), ν (cm^{-1}): 3021, 997, 927, 972 (=C-H), 1629 (C=C), 1589, 850 (Ar), 1514, 1338 (NO_2). ^1H NMR (400 MHz, CDCl_3), δ (TMS, ppm): 8.24 (d, $J = 8.8$ Hz, 2H, Ar-H ortho to NO_2), 7.65 (d, $J = 8.8$ Hz, 2H, Ar-H meta to NO_2), 7.54 (d, $J = 8.4$ Hz, 2H, Ar-H meta to vinyl), 7.46 (d, $J = 8.4$ Hz, 2H, Ar-H ortho to vinyl), 7.30-7.14 (q, $J = 18.7$ Hz, 2H, -CH=CH-), 6.79-6.72 (q, $J = 9.5$ Hz, 1H, -CH=CH₂), 5.82 (d, $J = 17.6$ Hz, 1H, -CH=CH₂), 5.33 (d, $J = 11.2$ Hz, 1H, -CH=CH₂).

Synthesis of 4-Ethenyl-4'-methyl-1,2-stilbene.

This was prepared as above from 4-methylbenzaldehyde. The product was white crystal. Yield: 44 %. IR (KBr), ν (cm^{-1}): 3021, 970, 992, 898 (=C-H), 2915, 2855 (CH_3), 1624 (C=C), 1513, 835 (Ar). ^1H NMR (400 MHz, CDCl_3), δ (TMS, ppm): 7.48 (d, $J = 8.4$ Hz, 2H, Ar-H meta to CH_3), 7.43 (t, $J = 13.6$ Hz, 4H, Ar-H-CH=CH₂), 7.19 (d, $J = 8.0$ Hz, 2H, Ar-H ortho to CH_3), 7.08 (d, $J = 6.0$ Hz, 2H, -CH=CH-), 6.74 (q, $J = 18.2$ Hz, 1H, -CH=CH₂), 5.77 (d, $J = 17.6$ Hz, 1H, -CH=CH₂), 5.26 (d, $J = 11.2$ Hz, 1H, -CH=CH₂), 2.39 (s, 3H, Ar-CH₃).

Synthesis of 2,7-Bis((E)-4-((E)-4-nitrostyryl)styryl)-9,9-dioctyl-9H-fluorene (NF).

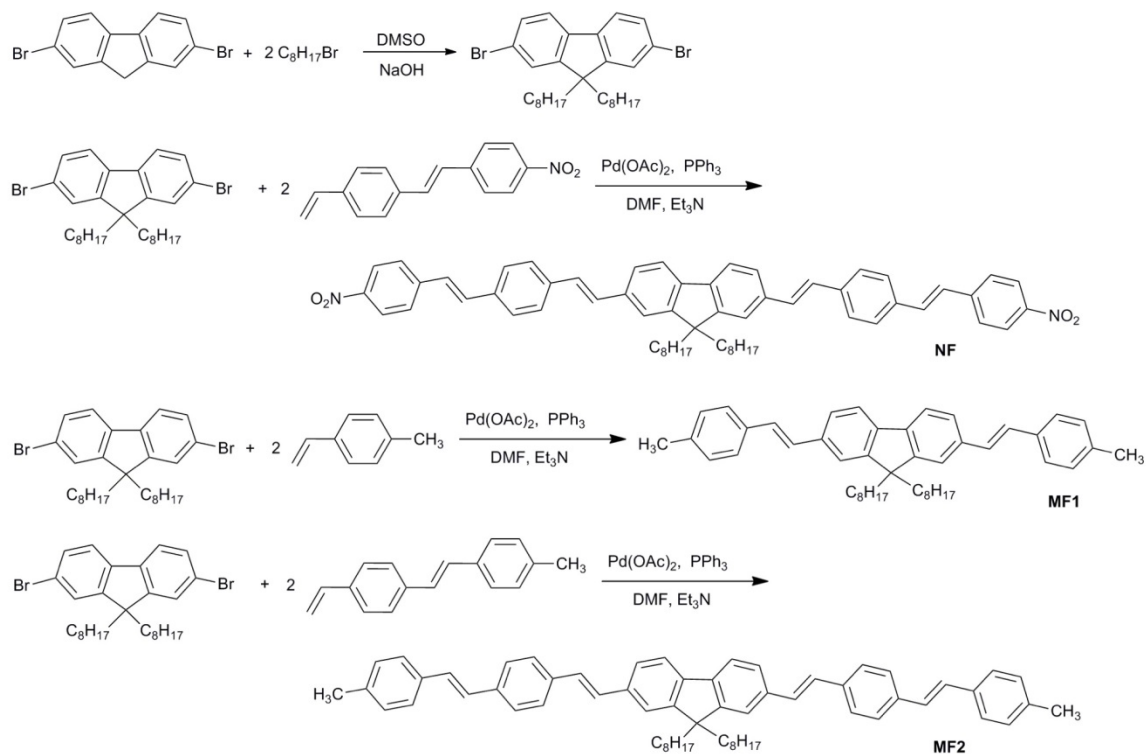
7-Dibromo-9,9-dioctylfluorene (2 mmol), $\text{Pd}(\text{OAc})_2$ (8.96 mg, 4×10^{-2} mmol), triphenylphosphine (40 mg, 0.15 mmol), 4-ethenyl-4'-nitro-1,2-stilbene (4 mmol), Et_3N (8 mL) and DMF (40 mL) were combined in a screw cap vial and heated to 110 °C for 72 h. The reaction mixture was cooled to room temperature and undergone the process of distillation. The mixture was extracted with CH_2Cl_2 , dried over MgSO_4 , and concentrated. The crude product was purified by column chromatography with petroleum ether/ CH_2Cl_2 (30:1). Yield: 67 %. mp 204.1 °C. IR (KBr), ν (cm^{-1}): 3023, 962 (=C-H), 2924, 2851 (CH_2 , CH_3), 1586, 1464, 839 (Ar), 1514, 1338 (NO_2). ^1H NMR (400 MHz, CDCl_3), δ (TMS, ppm): 8.25 (d, $J = 8.8$ Hz, 4H, Ar-H), 7.71-7.66 (q, $J = 7.7$ Hz, 6H, Ar-H), 7.62-7.52 (m, 12H, Ar-H), 7.33-7.17 (m, 8H, -CH=CH-), 2.06 (m, 4H, -CH₂(CH₂)₆CH₃), 1.22-1.10 (m, 20H, -CH₂(CH₂)₅C₂H₅), 0.82 (t, $J = 7.0$ Hz, 6H, -(CH₂)₇CH₃), 0.71 (m, 4H, -(CH₂)₆CH₂CH₃); ^{13}C NMR (75 MHz, CDCl_3), δ (ppm): 151.7, 146.7, 143.9, 140.9, 138.2, 136.2, 135.3, 132.9, 130.1, 127.5, 127.3, 126.9, 126.8, 126.0, 125.9, 124.2, 120.9, 120.1 (Ar and -CH=CH-), 55.1 (C4), 40.5, 31.8, 30.0, 29.2, 23.8, 22.6, 14.1 (-(CH₂)₇CH₃); Anal. calcd for $\text{C}_{61}\text{H}_{64}\text{N}_2\text{O}_4$: C 82.40, H 7.25, N 3.15; found: C 82.38, H 7.29, N 3.14.

Synthesis of 2,7-Bis((E)-4-methylstyryl)-9,9-dioctyl-9H-fluorene (MF1).

This compound was prepared as the similar approach of **NF**, using 4-methylstyrene instead of 4-ethenyl-4'-nitro-1,2-stilbene, as the starting material. Yield: 50%. mp: 121 °C. IR (KBr), ν (cm^{-1}): $\nu=2927, 2852(\text{m}; \text{CH}), 1604, 1464, 819(\text{m}; \text{Ar})$. $^1\text{H NMR}$ (400 MHz, CDCl_3), δ (TMS, ppm): 7.67 (d, $J = 4$ Hz, 2H, Ar-*H*), 7.47 (m, 8H, Ar-*H*), 7.19 (t, $J = 7.0$ Hz, 4H, -CH=CH-), 2.39 (s, $J = 2.08$ Hz, 6H, Ar- CH_3), 2.03 (t, 4H, $\text{CH}_2(\text{CH}_2)_6\text{CH}_3$), 1.08-1.29 (m, 20H, $-\text{CH}_2(\text{CH}_2)_5\text{C}_2\text{H}_5$), 0.83 (t, $J = 7.6$ Hz, 6H, $-(\text{CH}_2)_7\text{CH}_3$), 0.69 (s, 4H, $-(\text{CH}_2)_6\text{CH}_2\text{CH}_3$). Anal. calcd for $\text{C}_{47}\text{H}_{58}$: C 90.61, H: 9.38; found: C 90.51, H 9.27.

Synthesis of 2,7-Bis((E)-4-(E)-4-methylstyryl)styryl)-9,9-dioctyl-9H-fluorene (MF2).

This compound was prepared as the similar approach of **NF**, using 4-ethenyl-4'-methyl-1,2-stilbene instead of 4-ethenyl-4'-nitro-1,2-stilbene, as the starting material. Yield: 59 %. mp 213.5 °C. IR (KBr), ν (cm^{-1}): 3021, 964 (=C-H), 2926, 2852 (CH_2, CH_3), 1513, 1465, 824 (Ar). $^1\text{H NMR}$ (400 MHz, CDCl_3), δ (TMS, ppm): 7.68 (d, $J = 7.6$ Hz, 2H, Ar-*H*), 7.56-7.44 (m, 16H, Ar-*H*), 7.21 (m, 8H, -CH=CH-), 7.11 (d, $J = 7.6$ Hz, 4H, Ar-*H*), 2.39 (s, 6H, Ar- CH_3), 2.05 (m, 4H, $-\text{CH}_2(\text{CH}_2)_6\text{CH}_3$), 1.17-1.05 (m, 20H, $-\text{CH}_2(\text{CH}_2)_5\text{C}_2\text{H}_5$), 0.82 (t, $J = 7.2$ Hz, 6H, $-(\text{CH}_2)_7\text{CH}_3$), 0.71 (m, 4H, $-(\text{CH}_2)_6\text{CH}_2\text{CH}_3$). $^{13}\text{C NMR}$ (75 MHz, CDCl_3), δ (ppm): 151.6, 140.7, 137.6, 136.8, 136.4, 134.6, 129.5, 129.1, 128.5, 127.6, 127.4, 126.8, 126.5, 125.7, 120.8, 120.0 (Ar and -CH=CH-), 55.0 (C4), 40.5, 31.8, 30.1, 29.2, 23.8, 22.6, 14.1 ($-(\text{CH}_2)_7\text{CH}_3$), 21.3 (Ar- CH_3); Anal. calcd for $\text{C}_{63}\text{H}_{70}$: C 91.47, H 8.53; found: C 91.51; H 8.49.



Scheme S1. Synthesis of NF, MF1, and MF2

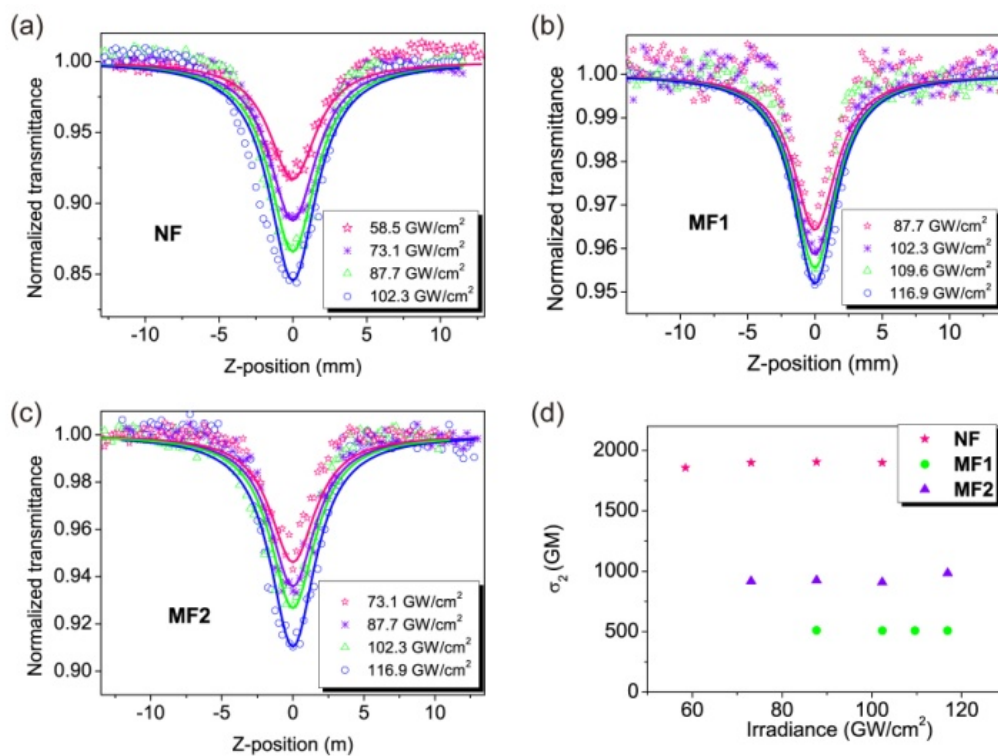


Figure S1. Open-aperture Z-scans of 1-mm-thick solutions of a) NF, b) MF1, and c) MF2 with different irradiances in THF, performed at the same concentration of 1.0×10^{-4} M. The

solid lines are the theoretical fit for pure two-photon absorption. d) Irradiance independence of the TPA cross-section profile of **NF**, **MF1**, and **MF2**.

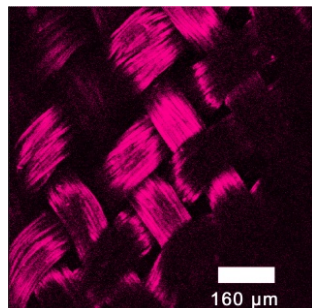


Figure S2. Cross-section Two-photon fluorescence (TPF) image of **NF** scaffold

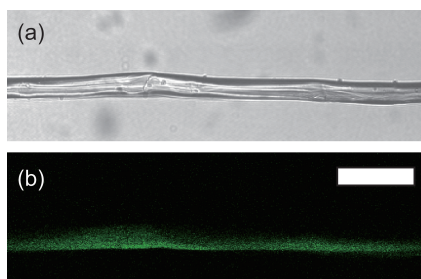


Figure S3. a) Grey image of **MF1** fiber cross-section under normal light. b) confocal TPF images of **MF1** fiber cross-section under laser at the wavelength 800 nm. Scan bar: 68 μm .

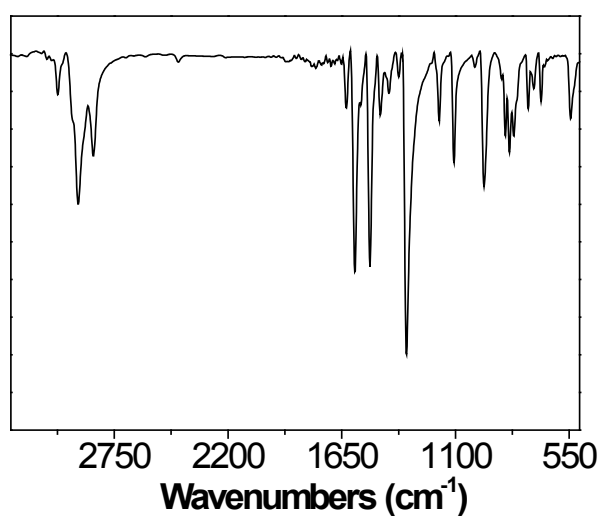


Figure S4. FTIR spectrum of 2,7-Bis((E)-4-((E)-4-nitrostyryl)styryl)-9,9-dioctyl-9H-fluorene (**NF**)

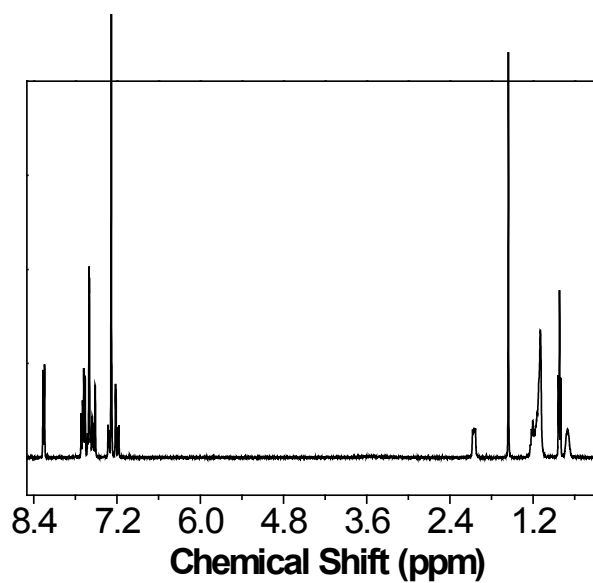


Figure S5. ^1H NMR spectrum of
2,7-Bis((E)-4-((E)-4-nitrostyryl)styryl)-9,9-dioctyl-9H-fluorene (**NF**)

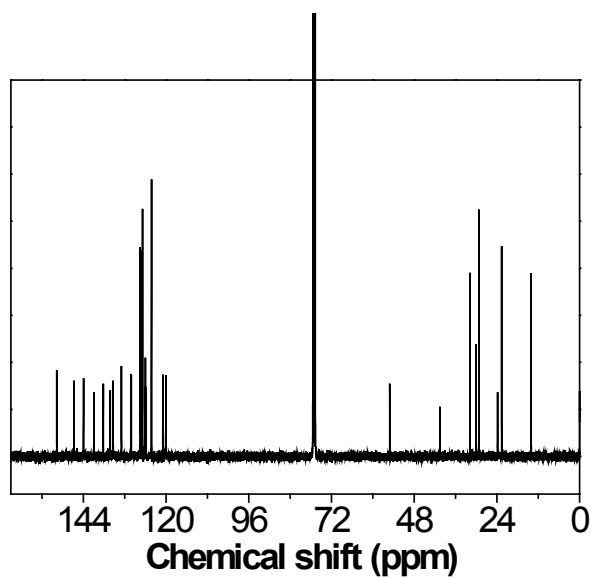


Figure S6. ^{13}C NMR spectrum of
2,7-Bis((E)-4-((E)-4-nitrostyryl)styryl)-9,9-dioctyl-9H-fluorene (**NF**)

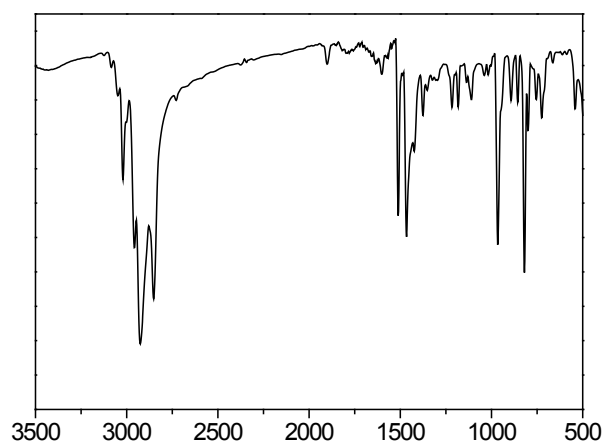


Figure S7. FTIR spectrum of 2,7-dimethyl styrene-9,9-dioctylfluorene(MF1)

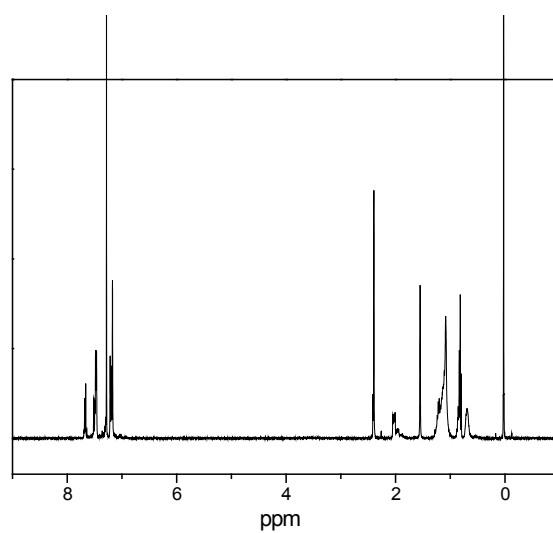


Figure S8. ¹H-NMR spectrum of 2,7-dimethyl styrene-9,9-dioctylfluorene (MF1)

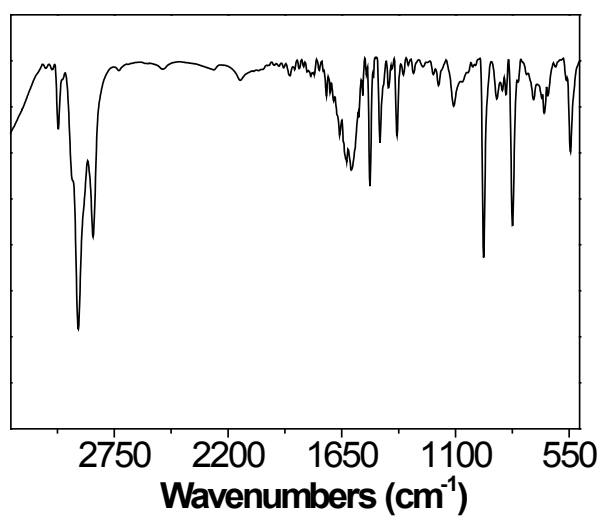


Figure S9. FTIR spectrum of
2,7-Bis((E)-4-((E)-4-methylstyryl)styryl)-9,9-dioctyl-9H-fluorene (**MF2**)

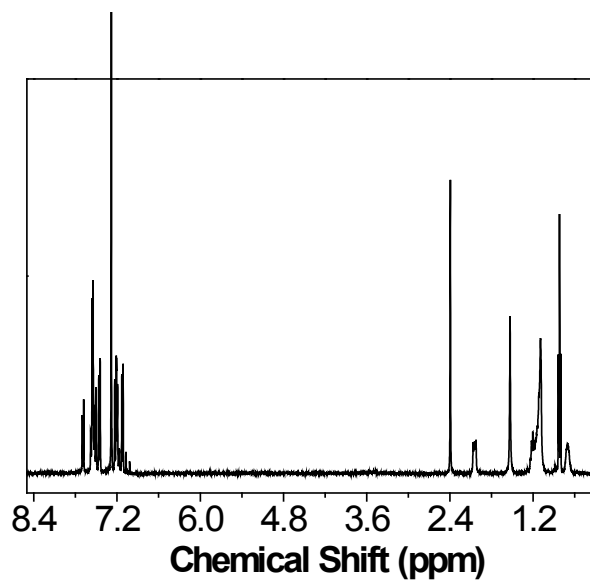


Figure S10. ¹H NMR spectrum
of 2,7-Bis((E)-4-((E)-4-methylstyryl)styryl)-9,9-dioctyl-9H-fluorene (**MF2**)

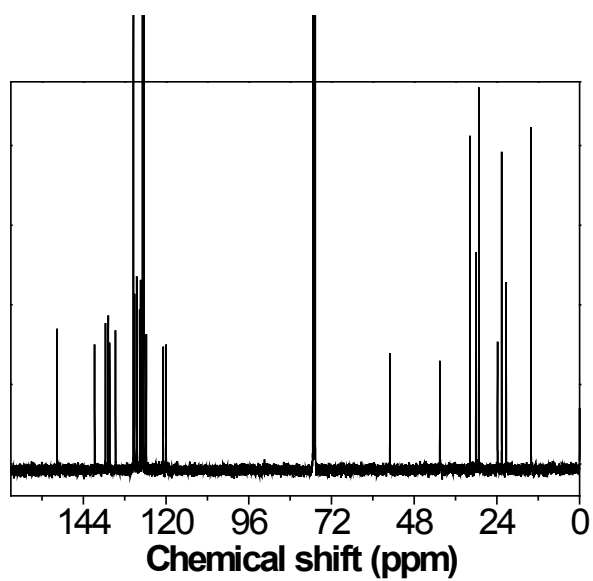


Figure S11. ^{13}C NMR spectra of
2,7-Bis((E)-4-((E)-4-methylstyryl)styryl)-9,9-dioctyl-9H-fluorene (**MF2**)