Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary information for

A solution-based single-molecule study of surface-bound PBIs: solvent-mediated environmental effects on molecular flexibility

Ji-Eun Lee, Ye Ri Han, Sujin Ham, Chul-Ho Jun* and Dongho Kim*

Corresponding Author
* junch@yonsei.ac.kr, dongho@yonsei.ac.kr

1. General

Flash column chromatography was performed on silica gel E. Merck 230-400 mesh. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Advance II/DPX 400(400 MHz ^{1}H, 100 MHz ^{13}C) spectrometers with chemical shifts reported relative to residual $CDCl_3$. ^{1}H NMR spectra were reported as follows: chemical shift, multiplicity (br=broad, s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet), number of protons. Infrared spectra were recorded on a Nicolet Impact 400 spectrometer. High resolution mass spectra (HRMS) and Elementary Analyses (EA) were recorded by the Yonsei Center for Research Facilities.

2. Materials

The following chemicals were obtained as a reagent grade and used without further purification [chlorodimethylsilane, *2*-propanol, magnesium tunning, allyl chloride, 10-undecen-1-ol, 3-chloro-2-methylpropene, copper iodide (I), *n*-butylamine, chlorosulfonic acid and perylene-3,4,9,10-tetracarboxylic dianhydride(CAS no. 128-69-8) were purchased from Sigma-Aldrich Co.; propargylamine was purchased from Sejinci co.; 6-chlorohex-1-ene was purchased from Alfa Aesar; H_2PtCl_6•$6H_2O$ was purchased from Pressure Chemical Co.]. Cover glass was purchased from Fisher Scientific (Fisherfinest Premium Cover Glass) which was treated with 10% w/v NaOH (Sigma-Aldrich, 50% in H_2O) for immobilization use.

3. Synthesis

*1,6,7,12-tetrachloroperylene-3,4,9,10-tetracarboxylic dianhydride (**1**)* (CAS no. 156028-26-1): A mixture of perylene-3,4,9,10-tetracarboxylic dianhydride (1 g, 2.5 mmol), iodine (0.17 g, 0.68 mmol) and chlorosulfonic acid (6.55 ml, 98.5 mmol) were stirred at 70 ^{o}C for 20 hours under nitrogen atmosphere . The reaction mixture was cooled down to room temperature and poured into ice cold water slowly. The obtained solid was filtered with water in a reduced pressure and dried in *vacuo* to give **1** (red solid, 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 8.74 (s, 4H).

*2-butyl-5,6,12,13-tetrachloro-9-(prop-2-yn-1-yl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**2**)*: To a 5 ml pressure vial, 1,6,7,12-tetrachloroperylene-3,4,9,10-tetracarboxylic dianhydride (**1**) (0.2 g, 0.38 mmol) and dried toluene (4 ml) were added. Then *n*-butyl amine (33 mg, 0.42 mmol) and propargyl amine (23 mg, 0.42 mmol) were added to a reaction mixture and stirred at 110 ^{o}C for 24 hours. After the reaction, the solvent was totally evaporated and purified by column chromatography (dichloromethane: *n*-hexane = 2: 1) to give **2** (red-brown solid, 15% yield). m.p. > 250 °C; ^{1}H NMR (400 MHz, $CDCl_3$ δ): 8.72 (s, 2H), 8.68 (s, 2H), 4.99 (s, 2H), 4.21 (t, J = 7.6 Hz, 2H), 2.23 (t, J = 2.4 Hz, 1H), 1.76-1.69 (m, 2H), 1.51-1.42 (m, 2H), 0.99 (t, J = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 162.4, 161.7, 135.8, 135.5, 133.5, 133.1, 131.7, 131.6, 129.2, 128.6, 123.6, 123.5, 123.4, 122.9, 78.0, 71.4, 40.9, 30.3, 29.9, 20.5, 14.0; IR spectrum (KBr): 3305, 3275, 3094, 3054, 2957, 2923, 2853, 2127, 1701, 1667, 1587, 1494, 1432, 1416, 1390, 1338, 1286, 1236, 1178, 1159, 927, 885, 775, 715, 546 cm^{-1}; HRMS (ESI) calcd for $C_{31}H_{16}Cl_4N_2O_4$: $[M+Na]^+$, 642.9756, found 642.8709 and Anal. Calcd for C, 59.83; H, 2.59; N, 4.50; found: C, 60.24; H, 3.51; N, 4.58.

General procedure I: preparation of (3-chloropropyl)dimethyl(2-methylallyl)silane

(3-chloropropyl)dimethyl(2-methylallyl)silane (CAS no. 928139-40-6): To a two-neck round bottom flask preloaded with reflux condenser, chlorodimethylsilane (9.46 g, 100 mmol) and 10% 2-propanol solution (0.2 ml) $H_2PtCl_6.6H_2O$ (40 mg, 0.08 mmol) were added and the resulting solution was stirred for 30 minutes. Then, allyl chloride (8.42 g, 110 mmol) was added dropwise and stirred at room temperature for 12 hours. After the reaction, under reduced pressure, unreacted allyl chloride and chlorodimethylsilane were removed by distillation to give crude chloro(3-chloropropyl)dimethylsilane. Without further

purification, the crude chloro(3-chloropropyl)dimethylsilane was added dropwise to methallylmagnesium chloride solution in THF and stirred at 0 ^{o}C for 6 hours. To the reaction mixture, saturated NH_4Cl (aq) solution was added and extracted with diethyl ether. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed in *vacuo*. The crude product was purified by column chromatography (n-hexane = 100%) to give (3-chloropropyl)dimethyl(2-methylallyl)silane (colorless liquid, 64% of overall yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 4.53 (d, *J* = 46.2 Hz, 2H), 3.48 (t, *J* = 6.8 Hz, 2H), 1.80-1.72 (m, 2H), 1.70 (s, 3H), 1.54 (s, 2H), 0.65-0.61 (m, 2H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 143.4, 108.7, 48.0, 27.8, 27.1, 25.4, 13.2, -2.9.

(6-chlorohexyl)dimethyl(2-methylallyl)silane: General procedure I was used employing 6-chlorohex-1-ene to give the desired product (colorless liquid, 16% of overall yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 4.51 (d, *J* = 45.6 Hz, 2H), 3.53 (t, *J* = 6.8 Hz, 2H), 1.79-1.72 (m, 2H), 1.70 (s, 3H), 1.52 (s, 2H), 1.44-1.39 (m, 2H), 1.33-1.28 (m, 4H), 0.54-0.50 (m, 2H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 144.1, 108.3, 45.4, 33.0, 32.8, 27.3, 26.8, 25.5, 23.9, 15.5, -2.8; IR spectrum (neat): 3596, 3048, 2924, 2861, 1637, 1450, 1372, 1251, 1161, 1060, 1002, 841, 725, 654 cm^{-1}; Anal. Calcd for $C_{12}H_{25}ClSi$: C, 61.89; H, 10.82; found: C, 58.19; H, 11.76.

(11-chloroundecyl)dimethyl(2-methylallyl)silane: General procedure I was used employing 11-chloroundec-1-ene to give the desired product (colorless liquid, 85% of overall yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 4.51 (d, *J* = 45.2 Hz, 2H), 3.53 (t, *J* = 6.8 Hz, 2H), 1.80-1.73 (m, 2H), 1.70 (s, 3H), 1.52 (s, 2H), 1.43-1.38 (m, 2H), 1.27-1.26 (m, 14H), 0.53-0.49 (m, 2H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 144.2, 108.2, 45.4, 33.9, 32.9, 29.8, 29.79, 29.70, 29.5, 29.1, 27.4, 27.1, 25.5, 24.0, 15.6, -2.8; IR spectrum (neat): 3714, 3073, 2982, 2880, 2836, 1637, 1464, 1455, 1373, 1280, 1248, 1161, 998, 973, 869, 843, 723, 656 cm^{-1}; Anal. Calcd for $C_{17}H_{35}ClSi$: C, 67.39; H, 11.64; found: C, 66.66; H, 13.28.

General procedure II: preparation of (3-azidopropyl)dimethyl(2-methylallyl)silane (**3a**)

*(3-azidopropyl)dimethyl(2-methylallyl)silane **(3a)*** (CAS no. 1273569-11-1): To a 100 ml round bottom flask, (3-chloropropyl)dimethyl(2-methylallyl)silane (2.07 g, 10.8 mmol) and DMF (30 ml) were added and the solution was stirred. Sodium azide (2.12 g, 32.5 mmol) was added to the mixture and the reaction was stirred at 80 ^{o}C for 4 hours. After the reaction, the reaction mixture was cooled down to the room temperature and H_2O was added. The solution was extracted with diethyl ether and the organic layer was dried over anhydrous $MgSO_4$ then solvents were reduced by evaporation. Purification by column chromatography (n-hexane = 100%) to give **3a** (colorless liquid, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 4.53 (d, *J* = 47.6 Hz, 2H), 3.23 (t, *J* = 6.8 Hz, 2H), 1.71 (s, 3H), 1.64-1.55 (m, 2H), 1.54 (s, 2H), 0.60-0.56 (m, 2H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 143.6, 108.7, 54.7, 27.2, 25.4, 23.8, 12.7, -2.9.

*(6-azidohexyl)dimethyl(2-methylallyl)silane **(3b)***: General procedure II was used employing (6-chlorohexyl)dimethyl(2-methylallyl)silane to give the desired product (colorless liquid, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 4.52 (d, *J* = 45.6 Hz, 2H), 3.25 (t, *J* = 7.0 Hz, 2H), 1.70 (s, 3H), 1.61-1.55 (m, 2H), 1.52 (s, 2H),1.36-1.28 (m, 6H), 0.54-0.50 (m, 2H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 144.0, 108.3, 51.7, 33.3, 29.0, 27.4, 26.6, 25.4, 23.9, 15.5, -2.8; IR spectrum (neat): 3710, 3077, 2946, 2864, 2097, 1638, 1453, 1251, 1162, 842, 724 cm^{-1}; Anal. Calcd for $C_{12}H_{25}N_3Si$: C, 60.20; H, 10.52; N, 17.55; found: C, 58.47; H, 11.84; N, 16.93.

*(11-azidoundecyl)dimethyl(2-methylallyl)silane **(3c)***: General procedure II was used employing (11-chloroundecyl)dimethyl(2-methylallyl)silane to give the desired product (colorless liquid, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$ δ): 4.51 (d, *J* = 44.8 Hz, 2H), 3.25 (t, *J* = 7.0 Hz, 2H), 1.70 (s, 3H), 1.63-1.55 (m, 2H), 1.52 (s, 2H), 1.37-1.26 (m, 16H), 0.53-0.49 (m, 2H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 144.1, 108.2, 51.7, 33.8, 29.8, 29.77, 29.71, 29.5, 29.3, 29.0,

27.4, 26.9, 25.4, 24.0, 15.6, -2.8; IR spectrum (neat): 3075, 2950, 2901, 2850, 2094, 1637, 1464, 1455, 1413, 1373, 1348, 1280, 1248, 1161, 869, 843, 721, 660 cm^{-1}; Anal. Calcd for $C_{17}H_{35}N_3Si$: C, 65.96; H, 11.40; N, 13.57; found: C, 65.89; H, 13.08; N, 13.88.

General procedure III: preparation of 2-butyl-5,6,12,13-tetrachloro-9-((1-(3-(dimethyl(2-methylallyl)silyl)propyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4a**)

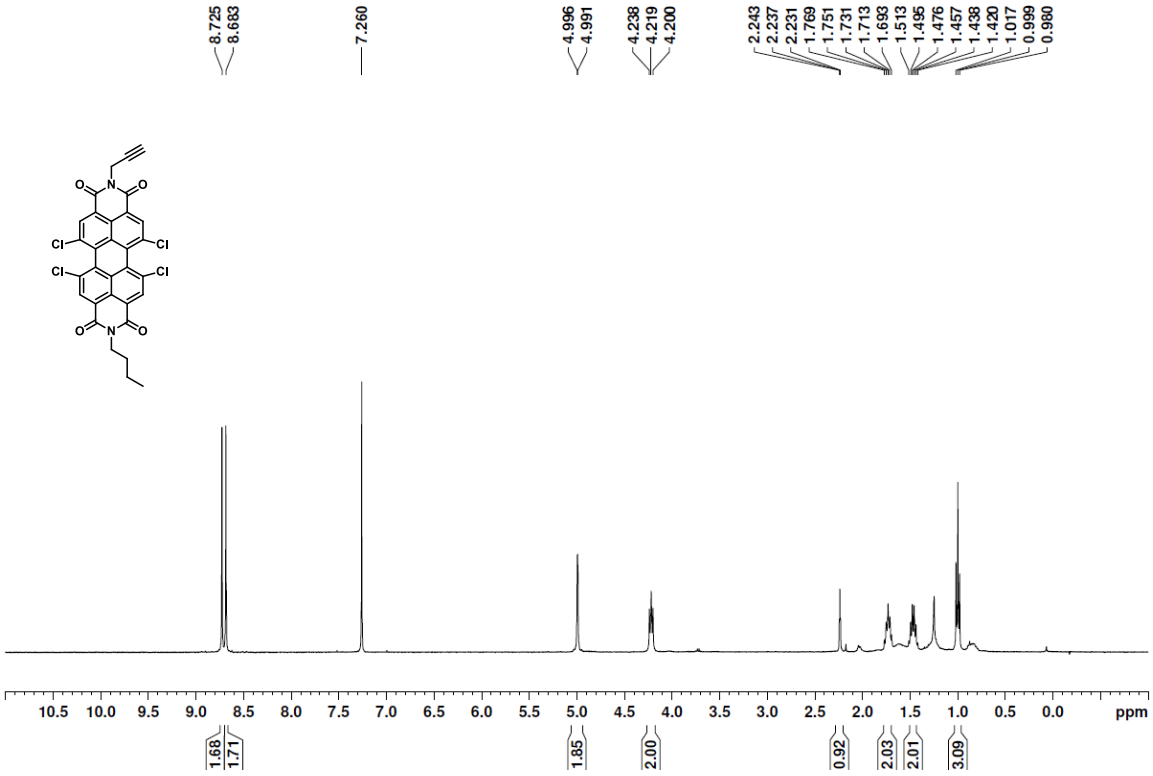
*2-butyl-5,6,12,13-tetrachloro-9-((1-(3-(dimethyl(2-methylallyl)silyl)propyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4a**)*: To a 1 ml pressure vial, 2-butyl-5,6,12,13-tetrachloro-9-(prop-2-yn-1-yl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**2**) (30 mg, 0.05 mmol) was added and dissolved in THF. Then, (3-azidopropyl)dimethyl(2-methylallyl)silane (**3a**) (19.7 mg, 0.1 mmol) and copper iodide (1.8 mg, 0.01 mmol) were added and stirred at 80 ^oC for 4 hours. After the reaction, reaction mixture was concentrated and purified by column chromatography (*n*-hexane: ethyl acetate = 2: 1) to give **4a** (red solid, 91% yield). m.p. 144.6–146.9 °C; ^1H NMR (400 MHz, $CDCl_3$ δ): 8.69 (s, 2H), 8.67 (s, 2H), 7.65 (s, 1H), 5.53 (s, 2H), 4.47 (d, *J* = 55.4 Hz, 2H), 4.27 (t, *J* = 7.2 Hz, 2H), 4.21 (t, *J* = 7.6 Hz, 2H), 1.91-1.85 (m, 2H), 1.76-1.69 (m, 2H), 1.65 (s, 3H), 1.50 (s, 2H), 1.49-1.43 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.54-0.49 (m, 2H), 0.00 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 162.4, 162.2, 143.4, 142.8, 135.6, 135.5, 133.4, 133.1, 131.65, 131.61, 129.0, 128.7, 123.55, 123.51, 123.46, 123.42, 123.1, 108.7, 53.5, 40.9, 35.7, 30.3, 27.0, 25.5, 25.4, 20.5, 14.0, 12.4, -2.9; IR spectrum (neat): 3059, 2957, 2929, 2872, 1705, 1667, 1589, 1501, 1433, 1391, 1351, 1288, 1237, 1172, 1049, 924, 909, 840, 805, 742, 685, 512 cm^{-1}; HRMS (ESI) calcd for $C_{40}H_{35}Cl_4N_5O_4Si$: $[M+Na]^+$, 840.1105 found 840.1122.

*2-butyl-5,6,12,13-tetrachloro-9-((1-(6-(dimethyl(2-methylallyl)silyl)hexyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4b**)*: General procedure III was used employing (6-azidohexyl)dimethyl(2-methylallyl)silane (**3b**) to give the desired product (red solid, 83% yield). m.p. 137.8–139.9 °C; ^1H NMR (400 MHz, $CDCl_3$ δ): 8.69 (s, 2H), 8.66 (s, 2H), 7.66 (s, 1H), 5.53 (s, 2H), 4.49 (d, *J* = 47.6 Hz, 2H), 4.30 (t, *J* = 7.2 Hz, 2H), 4.21 (t, *J* = 7.6 Hz, 2H), 1.88-1.84 (m, 2H), 1.76-1.68 (m, 5H), 1.49-1.43 (m, 4H), 1.31-1.24 (m, 6H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.50-0.46 (m, 2H), -0.02 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 162.4, 162.2, 144.0, 142.9, 135.7, 135.5, 133.4, 133.1, 131.67, 131.63, 129.0, 128.7, 123.57, 123.54, 123.4, 123.3, 123.2, 108.3, 50.6, 40.9, 35.8, 33.1, 30.5, 30.3, 27.3, 26.4, 25.5, 23.8, 20.5, 15.5, 14.0, -2.8; IR spectrum (neat): 3145, 3071, 2957, 2920, 2854, 1706, 1667, 1589, 1495, 1433, 1391, 1287, 1236, 1171, 1048, 1021, 924, 909, 845, 805, 743, 685, 545 cm^{-1}; HRMS (ESI) calcd for $C_{43}H_{41}Cl_4N_5O_4Si$: $[M+Na]^+$, 882.1574 found 882.1578.

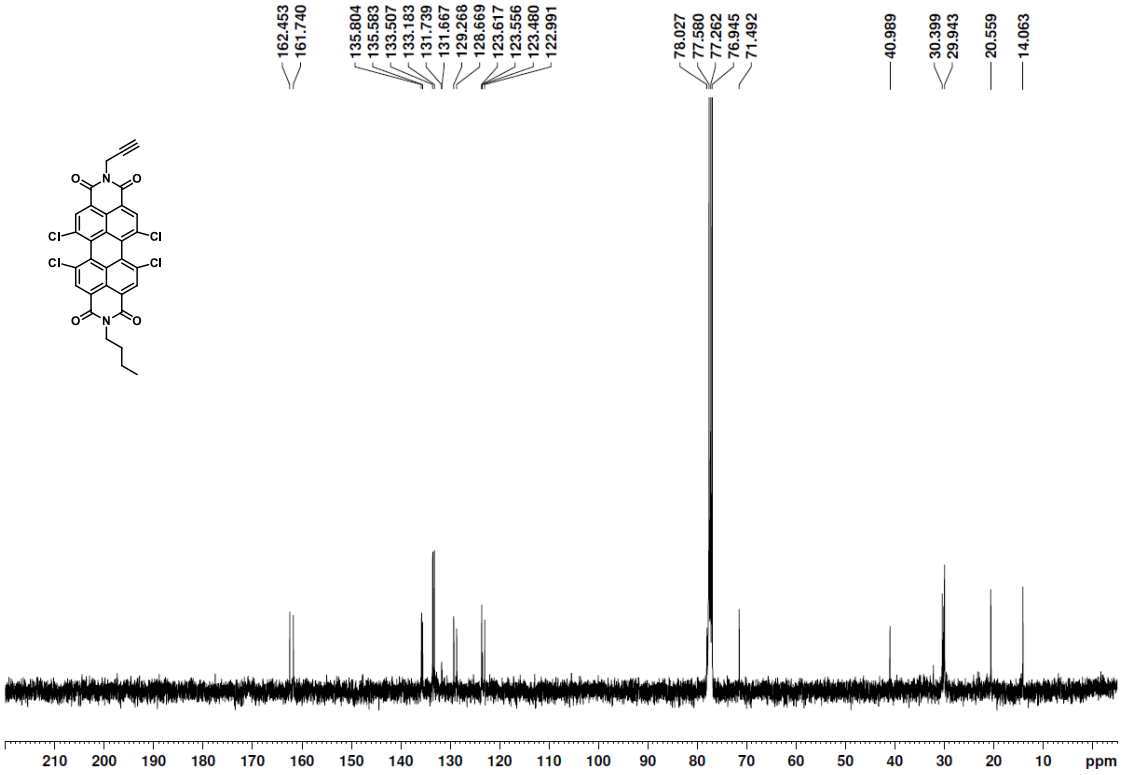
*2-butyl-5,6,12,13-tetrachloro-9-((1-(11-(dimethyl(2-methylallyl)silyl)undecyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4c**)*: General procedure III was used employing (11-azidoundecyl)dimethyl(2-methylallyl)silane (**3c**) to give the desired product (red solid, 87% yield). m.p. 108.8–111.2 °C; ^1H NMR (400 MHz, $CDCl_3$ δ): 8.69 (s, 2H), 8.67 (s, 2H), 7.66 (s, 1H), 5.53 (s, 2H), 4.50 (d, *J* = 44.8 Hz, 2H), 4.30 (t, *J* = 7.2 Hz, 2H), 4.21 (t, *J* = 7.4 Hz, 2H), 1.88-1.85 (m, 2H), 1.76-1.69 (m, 5H), 1.51-1.41 (m, 4H), 1.30-1.22 (m, 16H), 0.99 (t, *J* = 7.4 Hz, 3H), 0.51-0.47 (m, 2H), -0.01 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$ δ): 162.4, 162.2, 144.2, 142.9, 135.7, 135.5, 133.4, 133.1, 131.67, 131.63, 129.0, 128.7, 123.58, 123.53, 123.4, 123.3, 123.2, 108.2, 50.6, 40.9, 35.8, 33.8, 30.5, 30.3, 29.76, 29.75, 29.6, 29.5, 29.2, 27.4, 26.7, 25.5, 24.0, 20.5, 15.6, -2.8; IR spectrum (neat): 3072, 2922, 2852, 1706, 1667, 1589, 1501, 1463, 1433, 1391, 1287, 1236, 1158, 1048, 871, 842, 805, 746, 685, 545 cm^{-1}; HRMS (ESI) calcd for $C_{48}H_{51}Cl_4N_5O_4Si$: $[M+Na]^+$, 952.2357 found 952.2362.

^{1}H and ^{13}C NMR spectra for new compounds

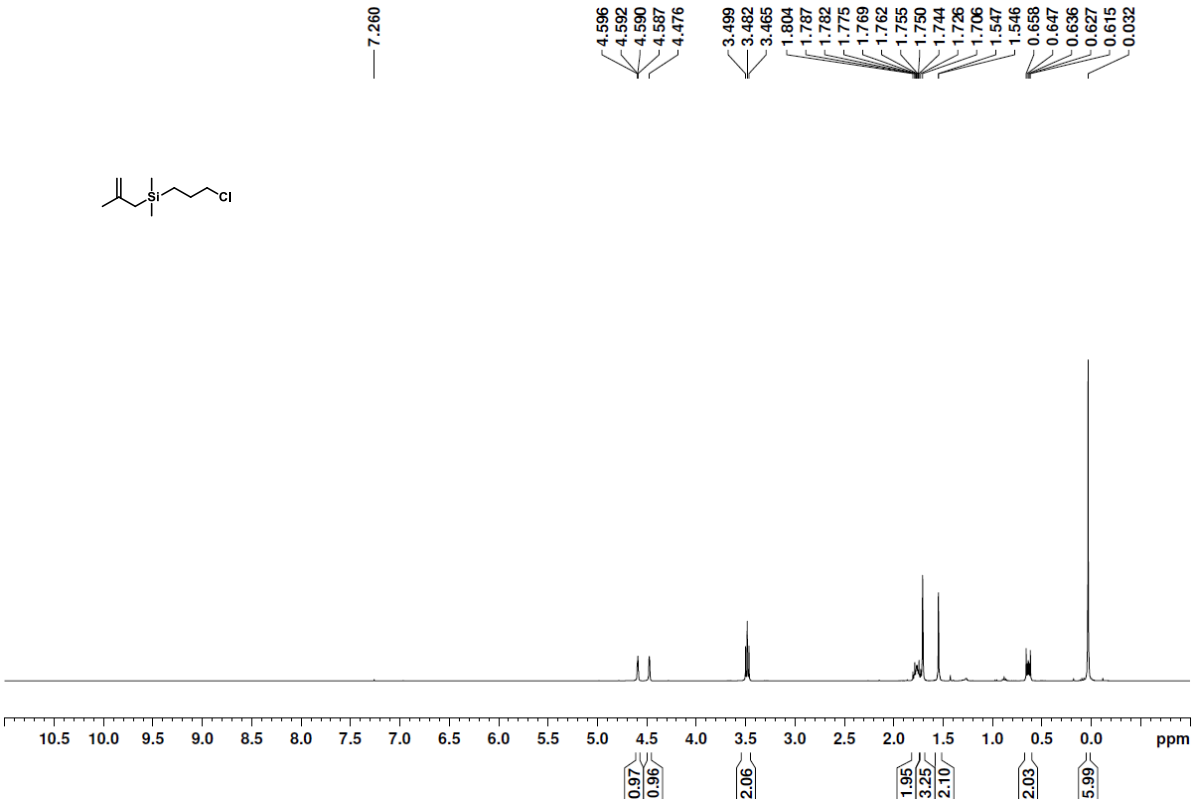
1. ^{1}H NMR of 2-butyl-5,6,12,13-tetrachloro-9-(prop-2-yn-1-yl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**2**)



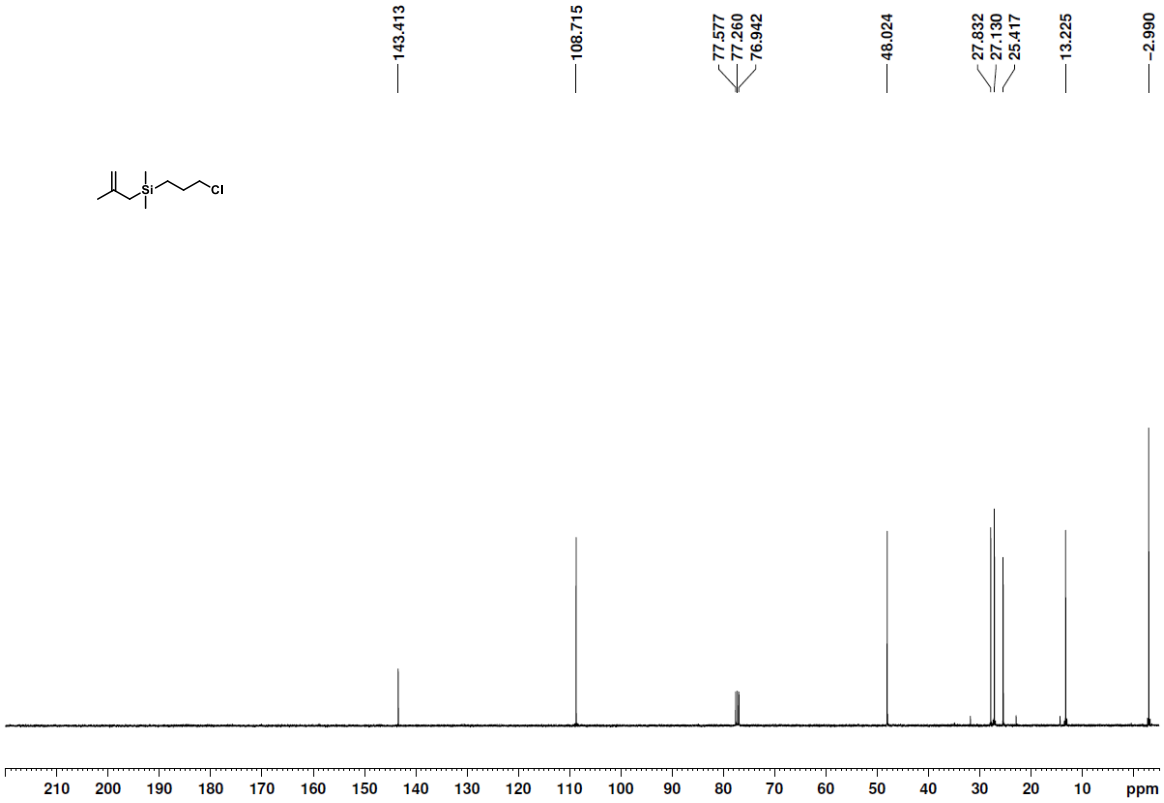
2. ^{13}C NMR of 2-butyl-5,6,12,13-tetrachloro-9-(prop-2-yn-1-yl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**2**)



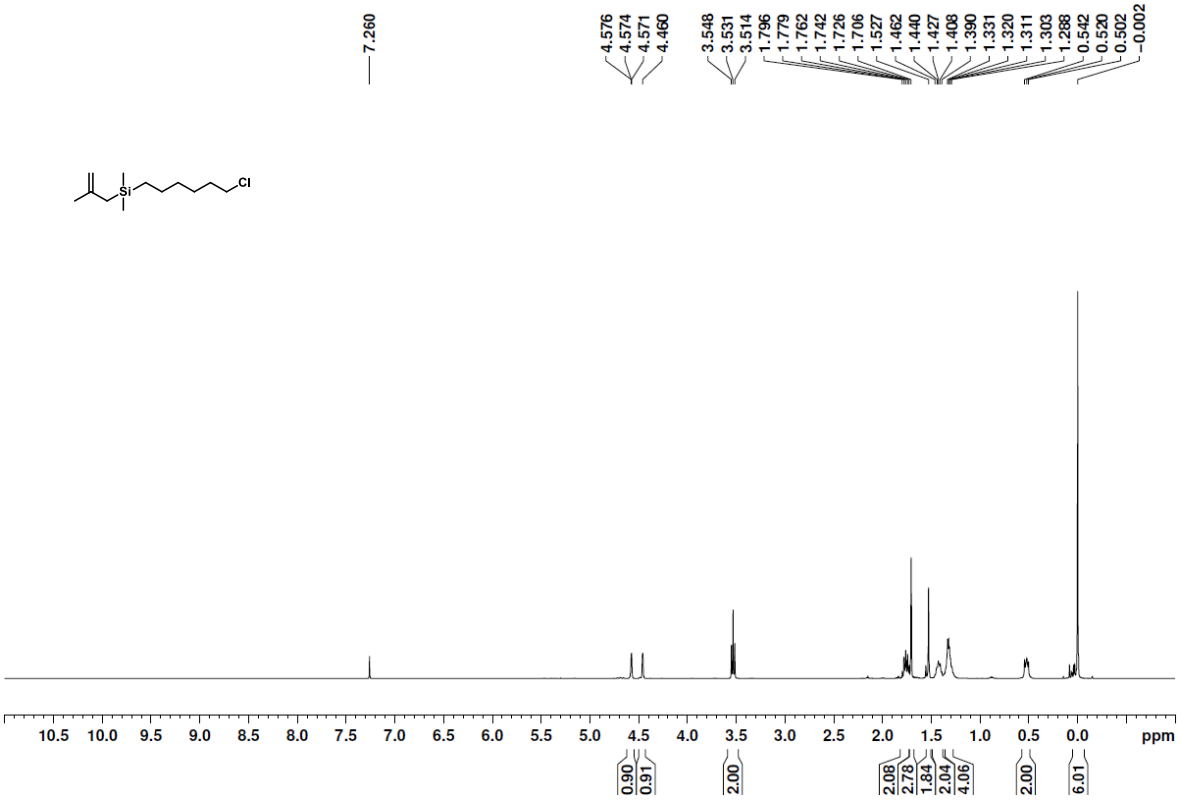
3. ^{1}H NMR of (3-chloropropyl)dimethyl(2-methylallyl)silane



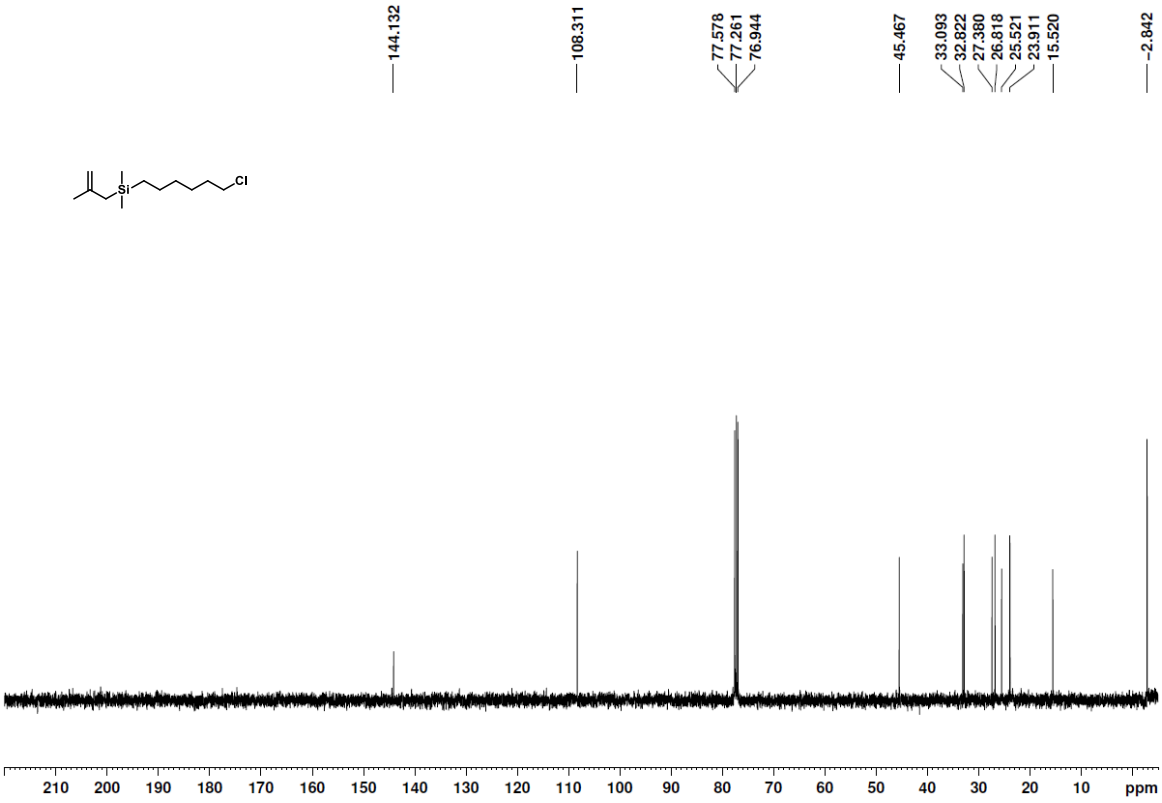
4. ^{13}C NMR of (3-chloropropyl)dimethyl(2-methylallyl)silane



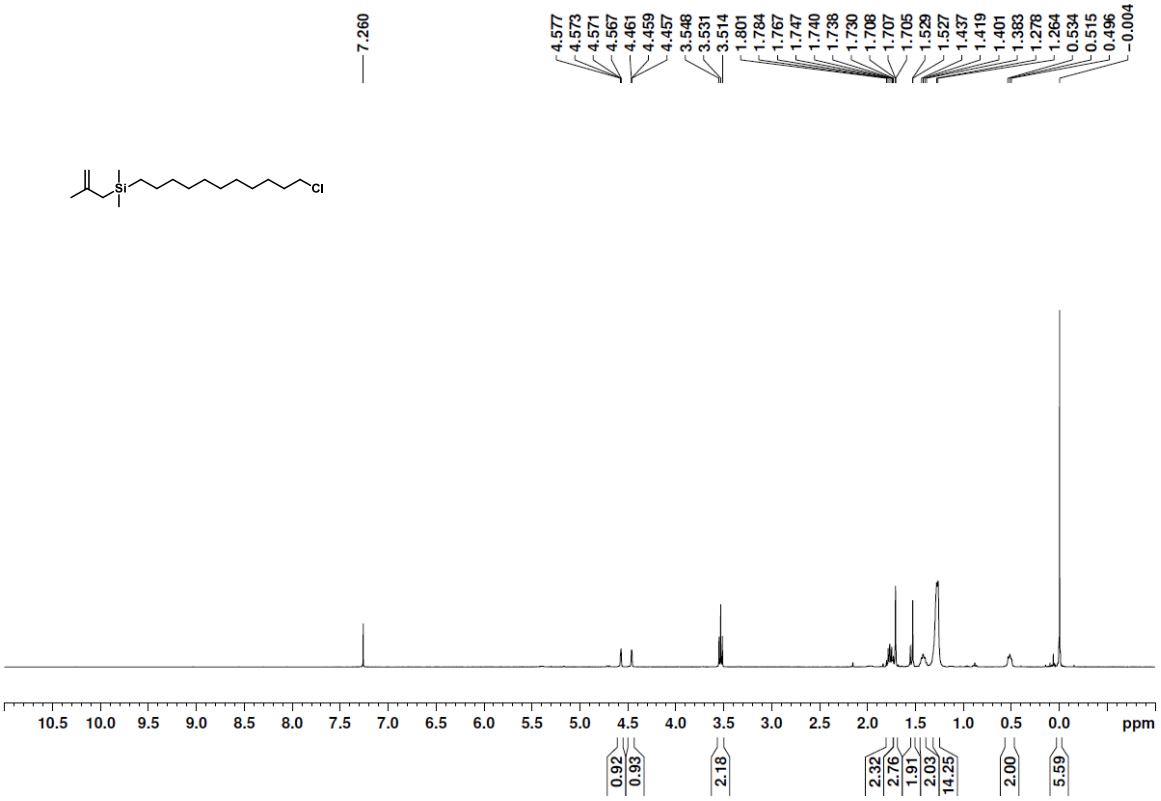
5. ^{1}H NMR of (6-chlorohexyl)dimethyl(2-methylallyl)silane



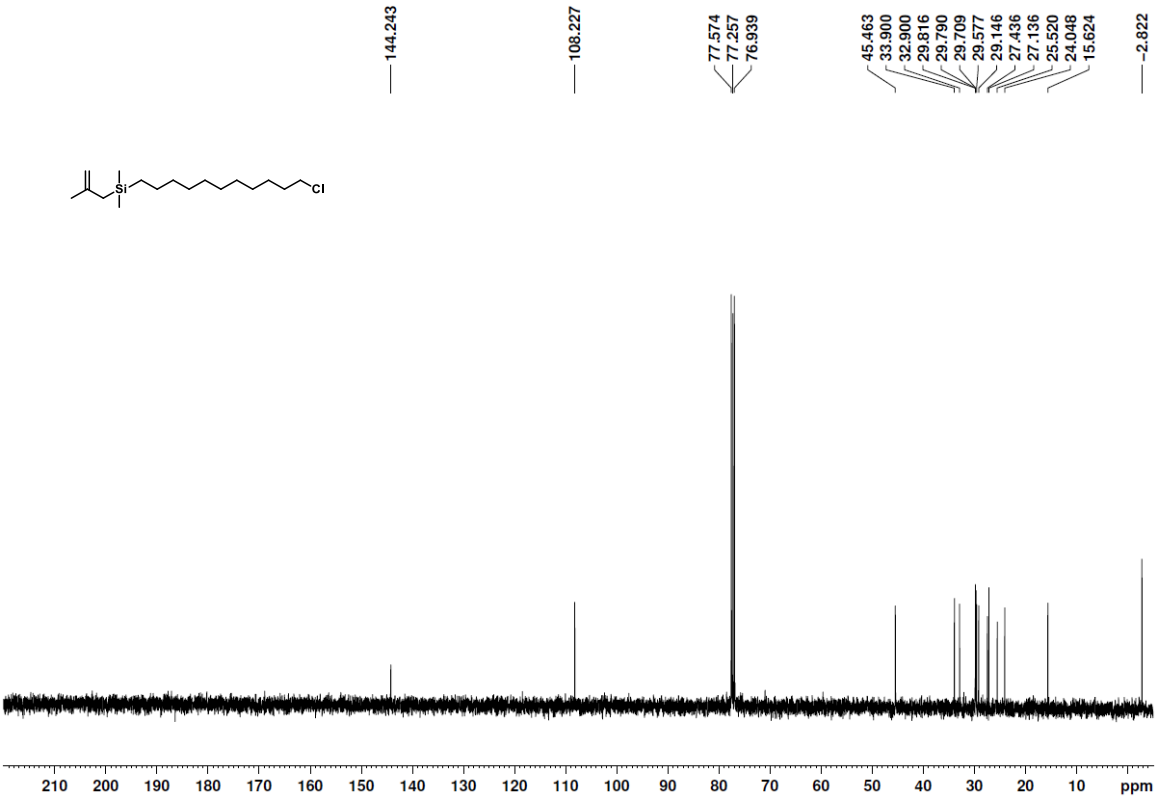
6. ^{13}C NMR of (6-chlorohexyl)dimethyl(2-methylallyl)silane



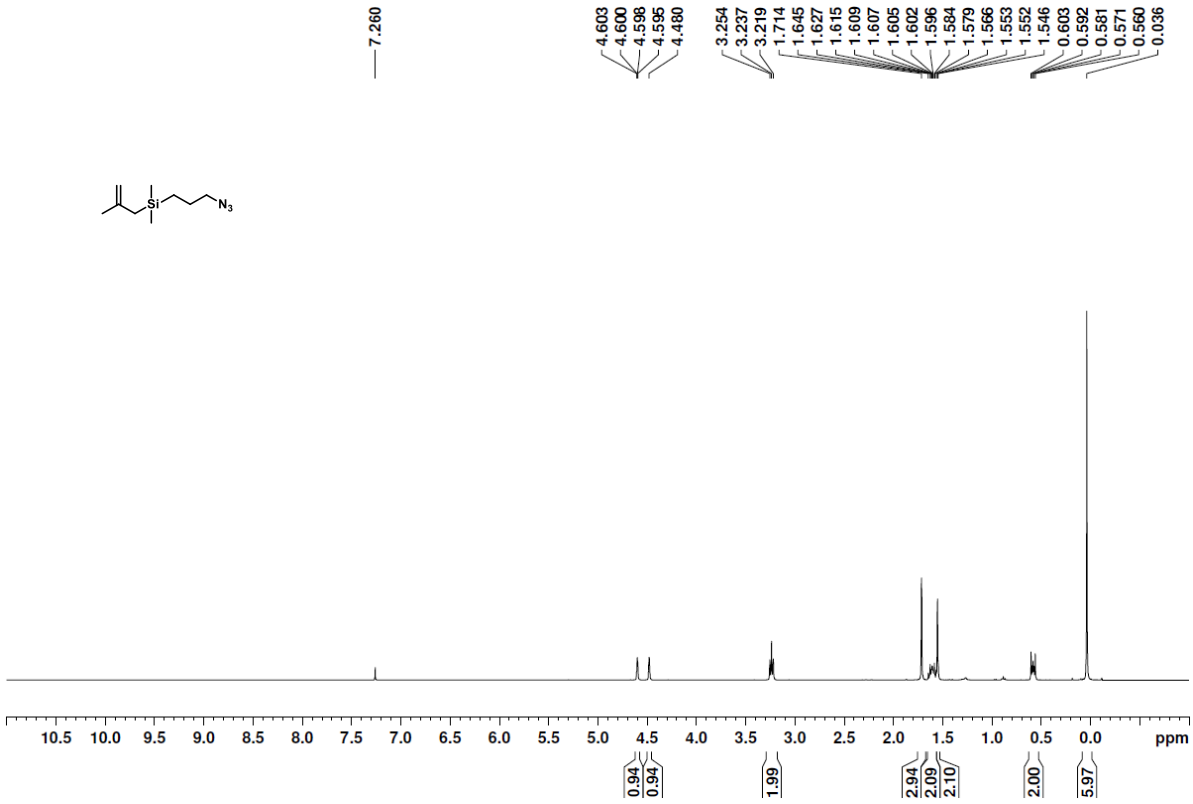
7. ^{1}H NMR of (11-chloroundecyl)dimethyl(2-methylallyl)silane



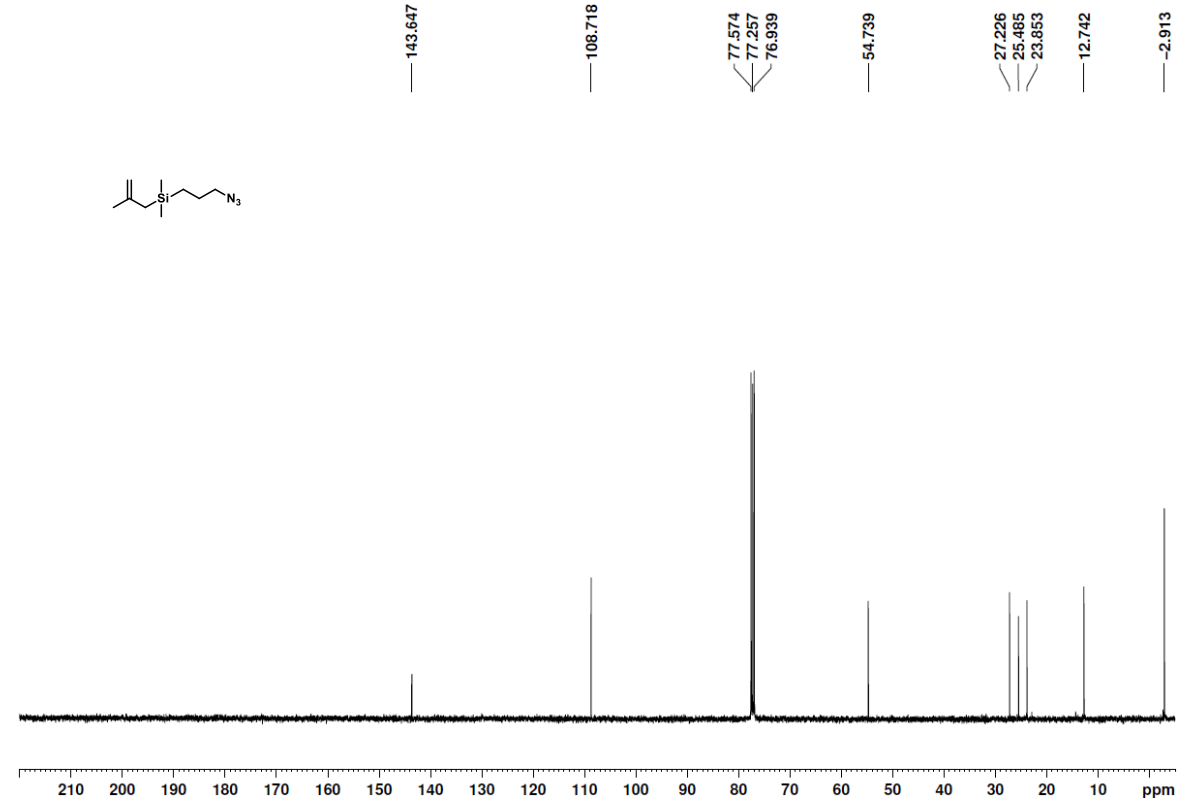
8. ^{13}C NMR of (11-chloroundecyl)dimethyl(2-methylallyl)silane



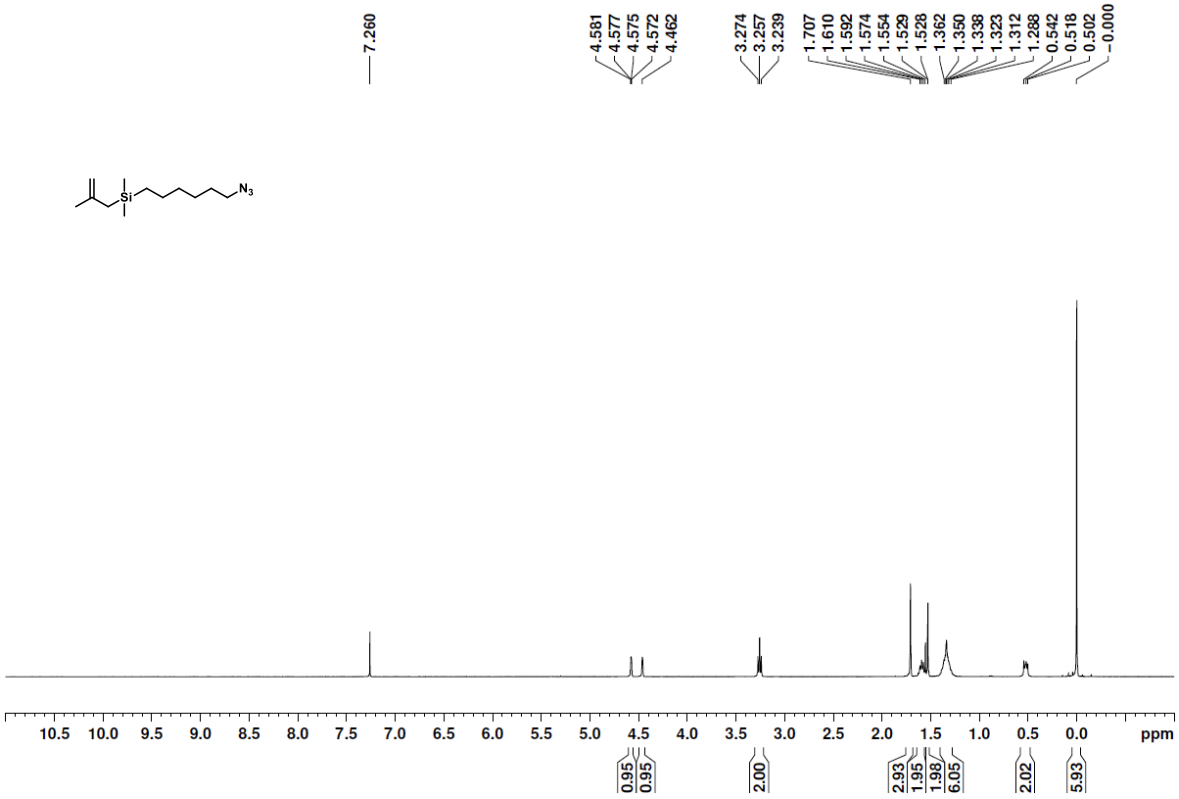
9. ^{1}H NMR of (3-azidopropyl)dimethyl(2-methylallyl)silane (**3a**)



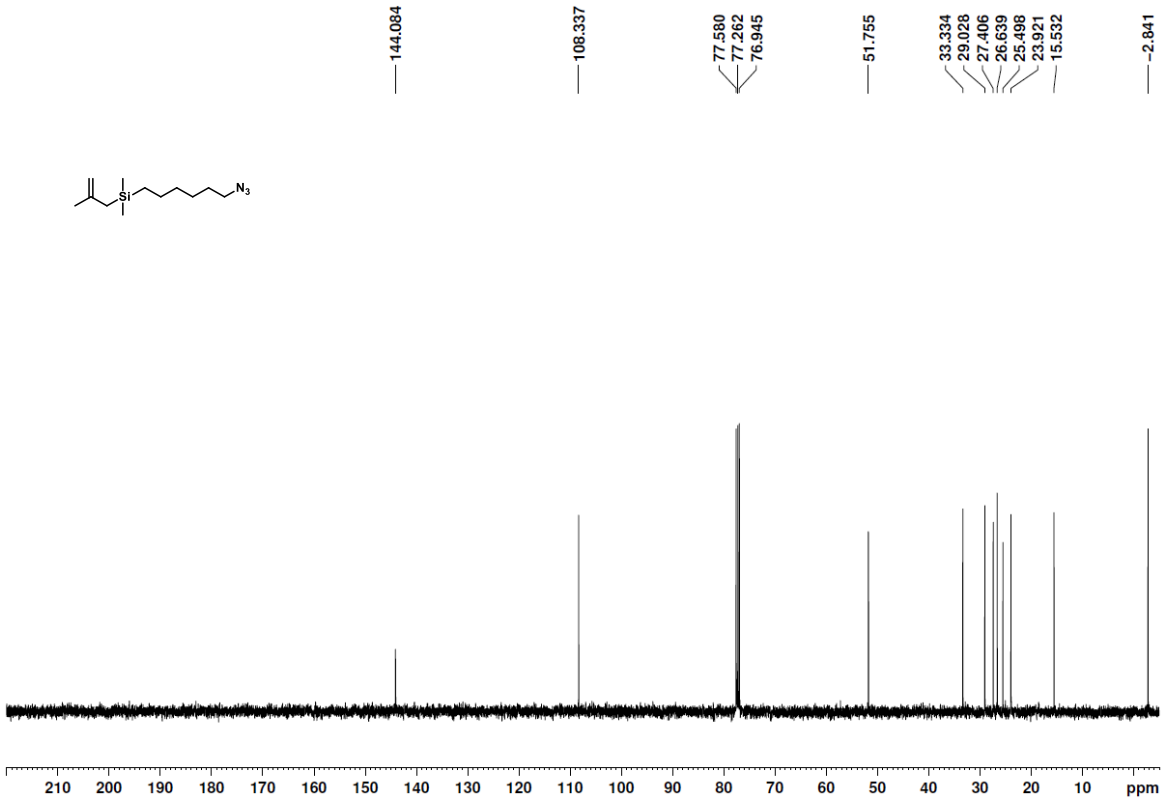
10. ^{13}C NMR of (3-azidopropyl)dimethyl(2-methylallyl)silane (**3a**)



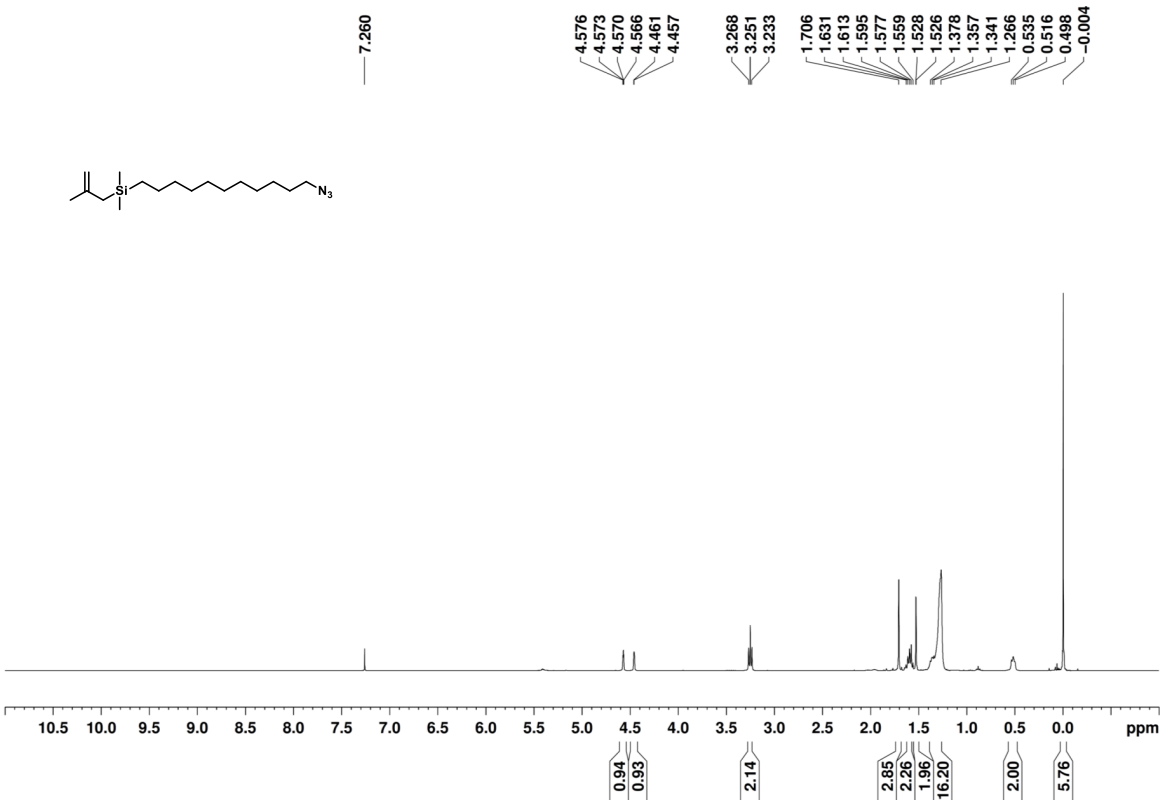
11. ^{1}H NMR of (6-azidohexyl)dimethyl(2-methylallyl)silane (**3b**)



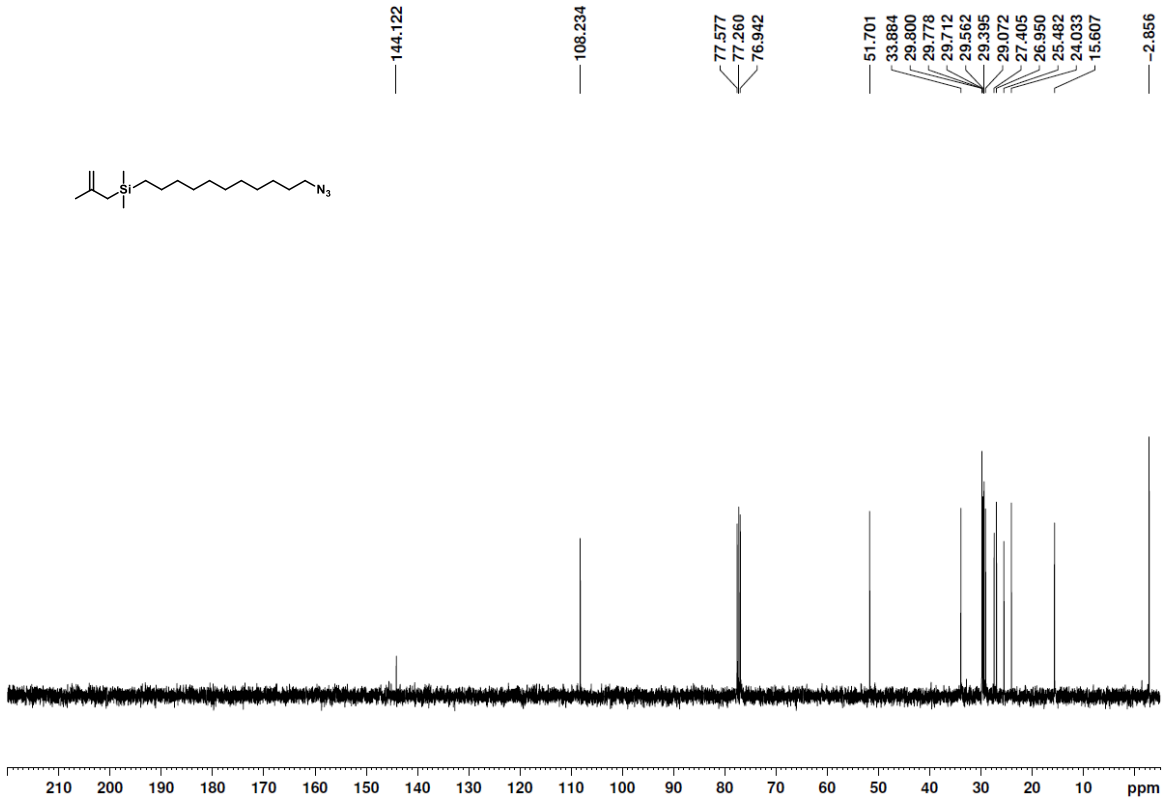
12. ^{13}C NMR of (6-azidohexyl)dimethyl(2-methylallyl)silane (**3b**)



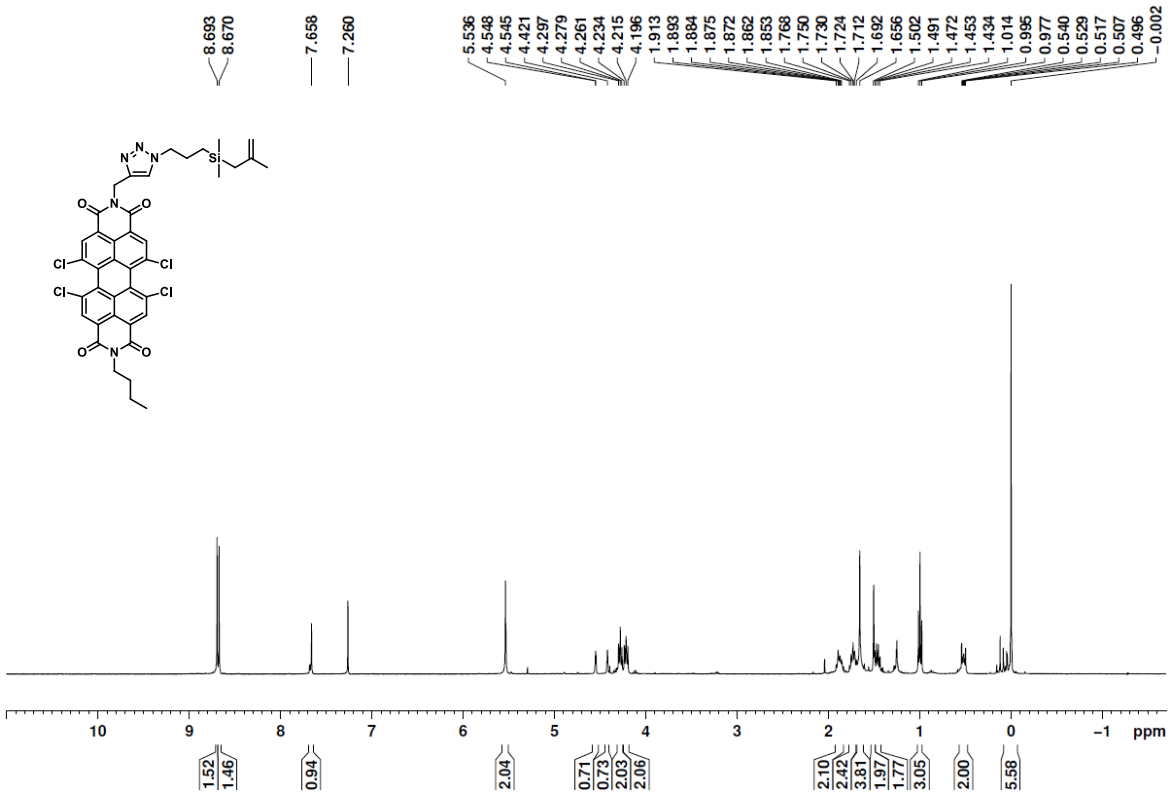
13. ^{1}H NMR of (11-azidoundecyl)dimethyl(2-methylallyl)silane (**3c**)



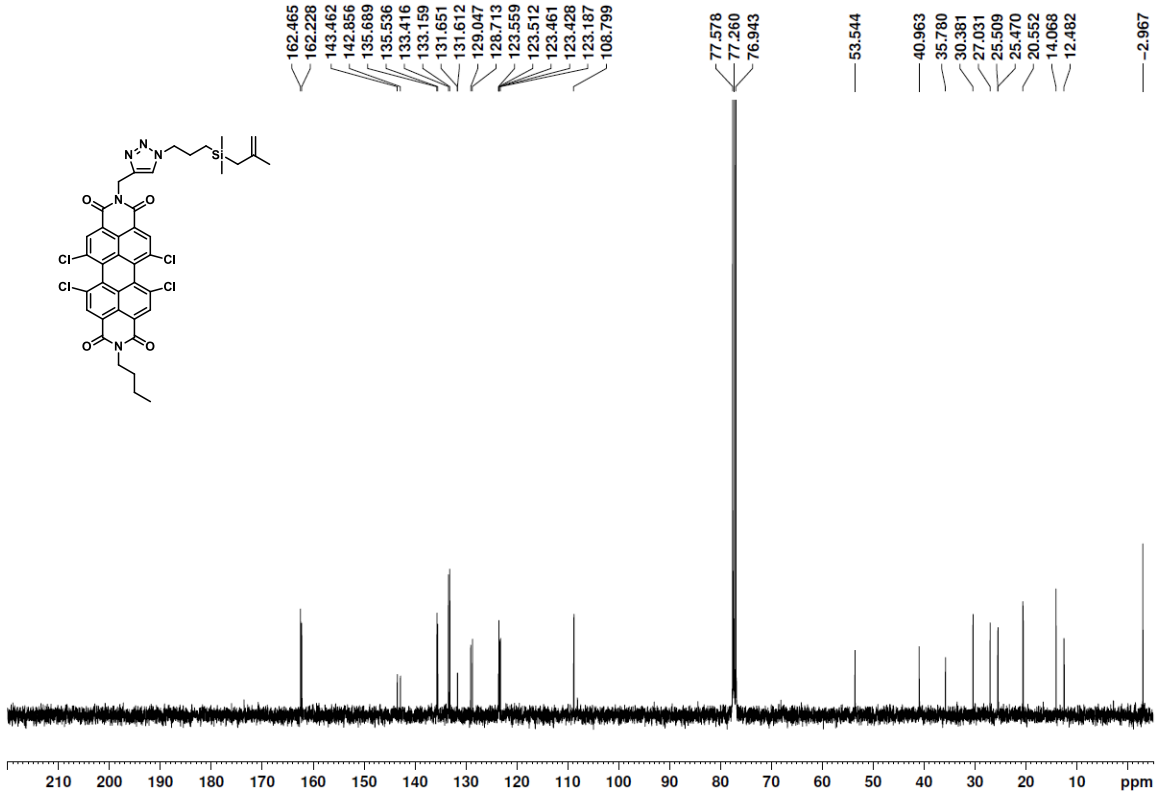
14. ^{13}C NMR of (11-azidoundecyl)dimethyl(2-methylallyl)silane(**3c**)



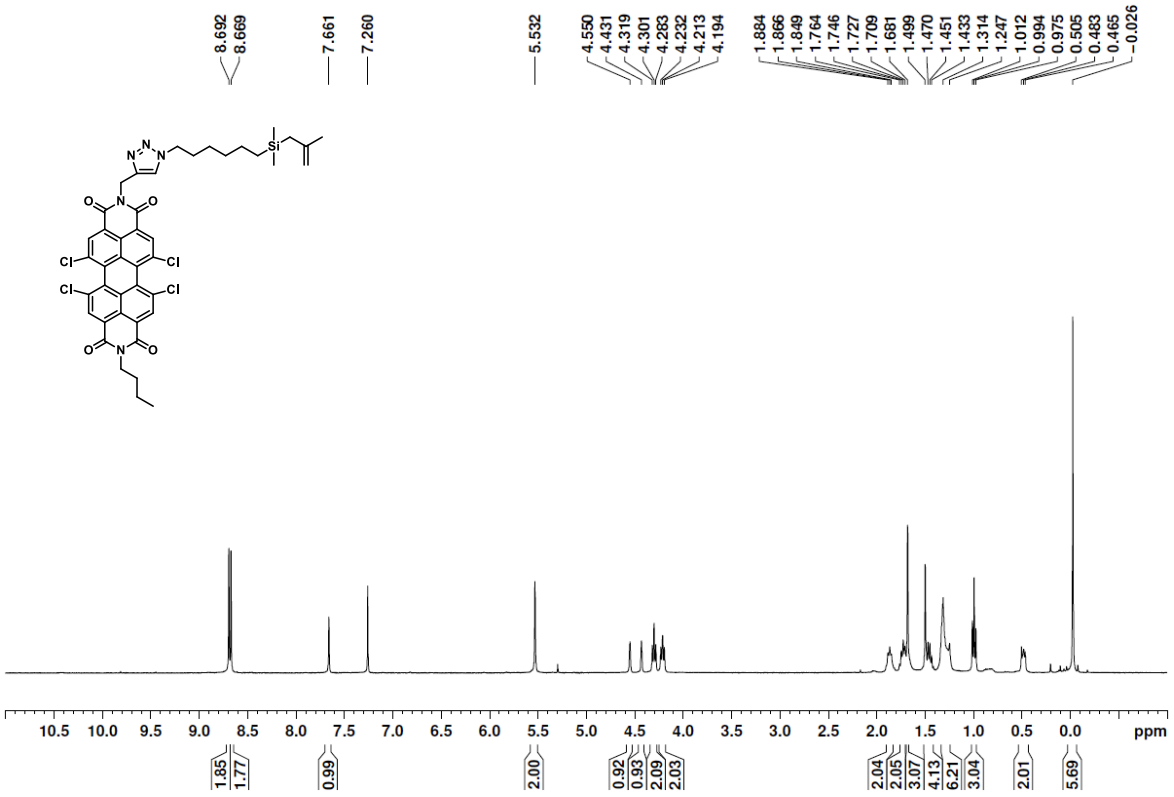
15. ^{1}H NMR of 2-butyl-5,6,12,13-tetrachloro-9-((1-(3-(dimethyl(2-methylallyl)silyl)propyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4a**)



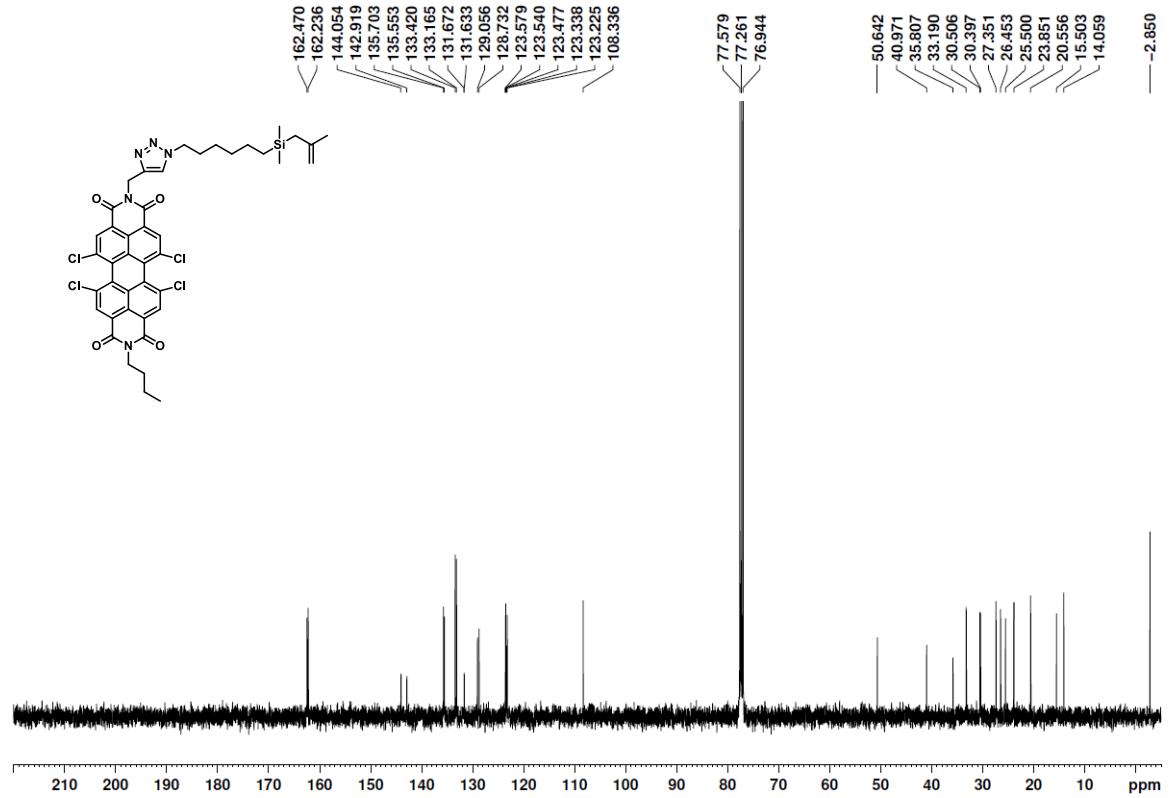
16. ^{13}C NMR of 2-butyl-5,6,12,13-tetrachloro-9-((1-(3-(dimethyl(2-methylallyl)silyl)propyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4a**)



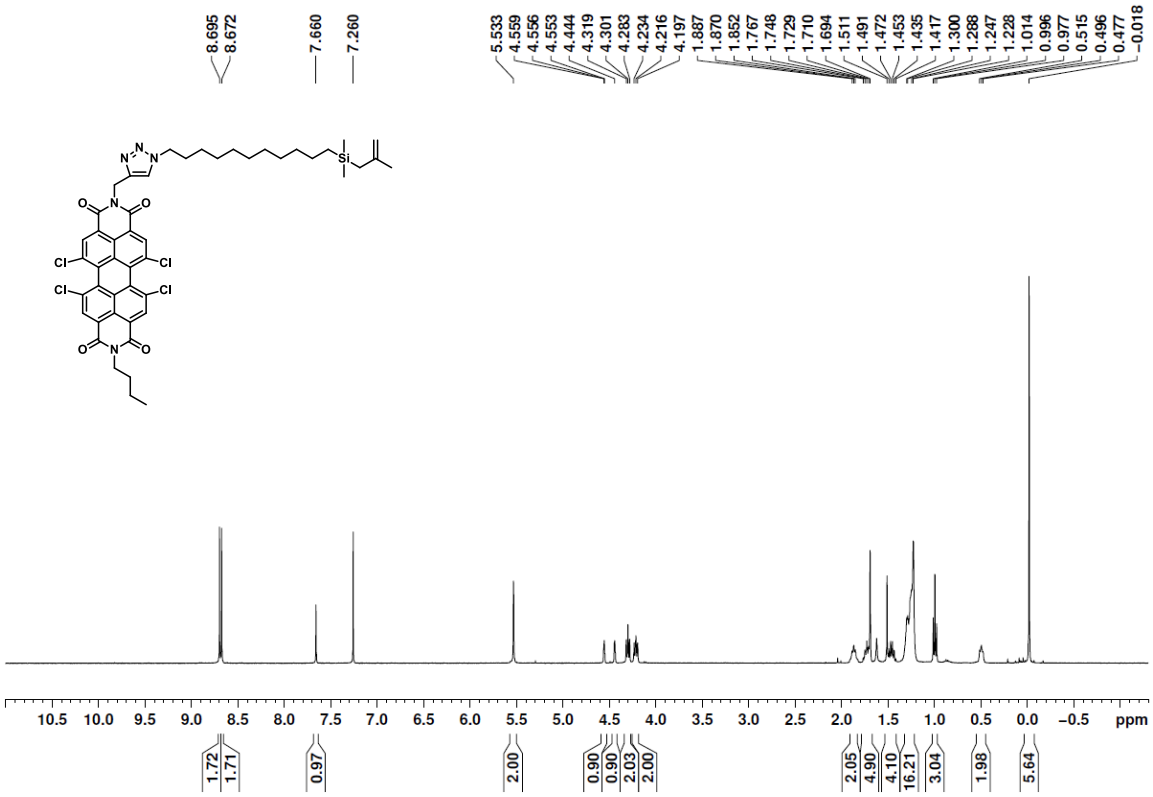
17. ^{1}H NMR of 2-butyl-5,6,12,13-tetrachloro-9-((1-(6-(dimethyl(2-methylallyl)silyl)hexyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4b**)



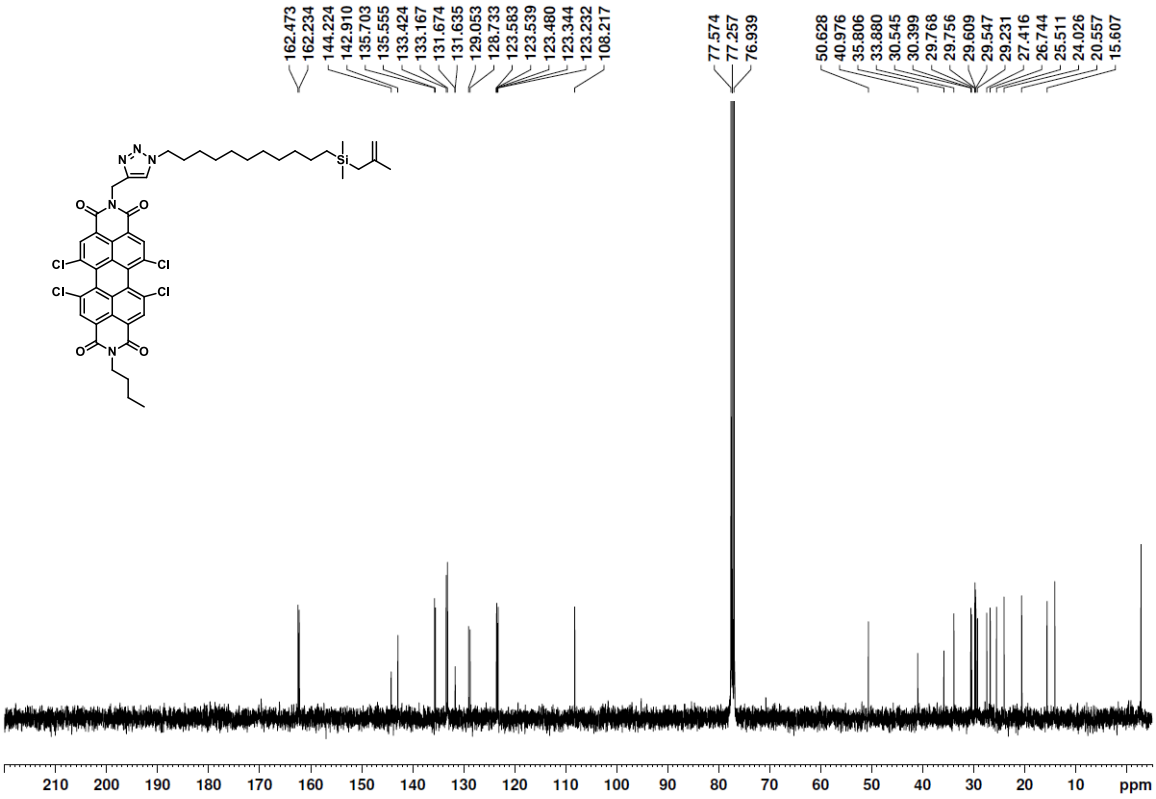
18. ^{13}C NMR of 2-butyl-5,6,12,13-tetrachloro-9-((1-(6-(dimethyl(2-methylallyl)silyl)hexyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4b**)



19. ^{1}H NMR of 2-butyl-5,6,12,13-tetrachloro-9-((1-(11-(dimethyl(2-methylallyl)silyl)undecyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4c**)



20. ^{13}C NMR of 2-butyl-5,6,12,13-tetrachloro-9-((1-(11-(dimethyl(2-methylallyl)silyl)undecyl)-1H-1,2,3-triazol-4-yl)methyl)anthra[2,1,9-def:6,5,10-d'e'f']diisoquinoline-1,3,8,10(2H,9H)-tetraone (**4c**)



4. Experimental Section

Ensemble Spectroscopy

Steady-state absorption and fluorescence (485 nm excitation wavelength) of the functionalized PBIs (**4a**, **4b**, and **4c**) in cyclohexane, toluene, chloroform, dichloromethane, acetonitrile, and methanol (Sigma-Aldrich, spectrophotometric grade) were measured using a Cary5000 (Varian) UV/vis spectrometer and F-2500 (Hitachi) fluorometer at room temperature.

Sample Preparation

For single-molecule detection in the solution phase, we prepared glass surface-bound PBI systems (**SPBI**, **MPBI**, and **LPBI**) with the anchoring linkers of different lengths acting as a single anchoring point that isolates the molecule onto a glass surface. The cover glasses were cleaned by sonicating them once in acetone for 30 minutes, treated with 10% w/v NaOH solution twice for 5 minutes to generate hydroxyl-functionalized glass surface and then washed with Milli-Q water twice. The contact angle was around 3~8°. To immobilize the samples (**4a**, **4b**, and **4c**) onto the treated glass surface, the functionalized PBI (**4a**, **4b**, and **4c**) in acetonitrile (~10^{-11} M) is reacted with hydroxyl-functionalized glass surface in the presence of 10 mol% of trifluoromethanesulfonic acid (TfOH) catalyst. After 12 hours, this glass surface is rinsed thoroughly with acetonitrile solvent and Milli-Q water. The cover glass immobilized samples are combined with the customized chamber for the single-molecule experiments in the solution phase. After adding solvent in the chamber, the chamber is covered with the cleaned cover glass by applying epoxy to the outside base of the chambers. To avoid oxidation or degradation, all the processes of the above preparation were conducted in glove-box under Ar gas.

Single-Molecule Fluorescence Spectroscopy

Detection of single-molecule fluorescence was performed with a confocal microscope (TE2000-U, Nikon) equipped with a sample scanning stage (XE-120, Park Systems) at room temperature under ambient conditions. The sample was excited by a circularly polarized light from a picosecond pulsed diode laser (LDH-P-C-485, Picoquant, 485 nm, 10 MHz repetition rate, 70 ps pulse width) prepared by a Berek compensator (5540, New Focus). Line filter (z485/10x, Chroma) The laser beam was focused onto the sample via an oil immersion objective lens (Plan Fluor, 1.3 NA, 100x, Nikon) giving irradiation power of ~ 200 W/cm^2 at the sample. Fluorescence was collected using the same objective, passed through a dichroic mirror (z488rdc, Chroma), filtered with a notch filter (HNPF-485.0-1.0, Kaiser Optical Systems Inc.) as well as long pass filters (FF01-488/LP-25 and FF01-496/LP-25, Semrock), and focused on an avalanche photodiode (APD) (SPCM-AQR-16-FC, EG&G) and a EMCCD (ProEM:512B EMCCD, PI) coupled with a spectrograph (SP-150, Acton) at the same time. After an individual molecule was positioned in the laser focus, the fluorescence signal detected by the APD was registered by a time-correlated single photon counting (TCSPC) PC card (SPC 830, Becker & Hickl) operated in first-in-first-out regime, in which the arrival time after the beginning of the acquisition and the time lag with respect to the excitation pulse were stored for each detected photon. The full width at half maximum (FWHM) of the overall instrumental response function was about 250 ps. These data were processed using BIFL data analyzer software (Scientific Software Technologies Center) to obtain the FITs with a user-defined binning time, and the time-resolved fluorescence decays using photons belonging to a user-defined region in the trajectories.

Defocused fluorescence imaging measurements were performed using a wide-field fluorescence microscopic system consisting of an inverted optical microscope (IX71, Olympus) equipped with an oil immersion objective (1.4 NA, 100x, Plan Fluorite, Olympus) and a highly sensitive, cooled, 512 X 512 pixels EMCCD camera (Andor, iXon Ultra). For excitation, 448 nm light from a continuous wave diode laser (Cyan 488, Spectra Physics). The circular polarized laser beam, prepared by a Berek compensator (5540, New Focus), was sent to the microscope after passing through a laser line filter (FF01-488/20, Semrock), collimating lens and a dichroic mirror (Di02-R488-25x36, Chroma Technology), and was focused onto the back-focal plane of the objective to achieve a wide-field illumination (Köhler illumination mode). The defocused fluorescence image was obtained by shifting the sample plane by 0.9 μm toward the objective from the focus position. The defocused image was magnified 3.4 times with a relay lens and spectrally filtered with a notch filter (HNPF-488.0-1.0, Kaiser Optical Systems Inc.) and longpass filters (FF01-488/LP-25 and FF01-496/LP-25, Semrock). The image integration time was 1 s in order to improve the signal-to-noise ratio. The defocused fluorescence images were analyzed using a pattern matching routine written in the Matlab software to determine the rough transition dipole moment orientation by calculating two-dimensional correlation coefficients (*r*) of the defocused images obtained experimentally and theoretically using equation (1)[1]

$$r = \frac{\sum_m \sum_n (A_{mn} - \bar{A})(B_{mn} - \bar{B})}{\sqrt{\left(\sum_m \sum_n (A_{mn} - \bar{A})^2\right)\left(\sum_m \sum_n (B_{mn} - \bar{B})^2\right)}} \quad (1)$$

where $\bar{A}$ and $\bar{B}$ are the means of *A* and *B*, respectively. Furthermore, we have double-checked the defined angles of defocused images every seconds, one by one by comparing with the calculated images to ensure the orientations of the images.

Water Contact Angle Measurements

Contact angle measurements were conducted using KSV/Theta lite 100.

5. Supporting Figures

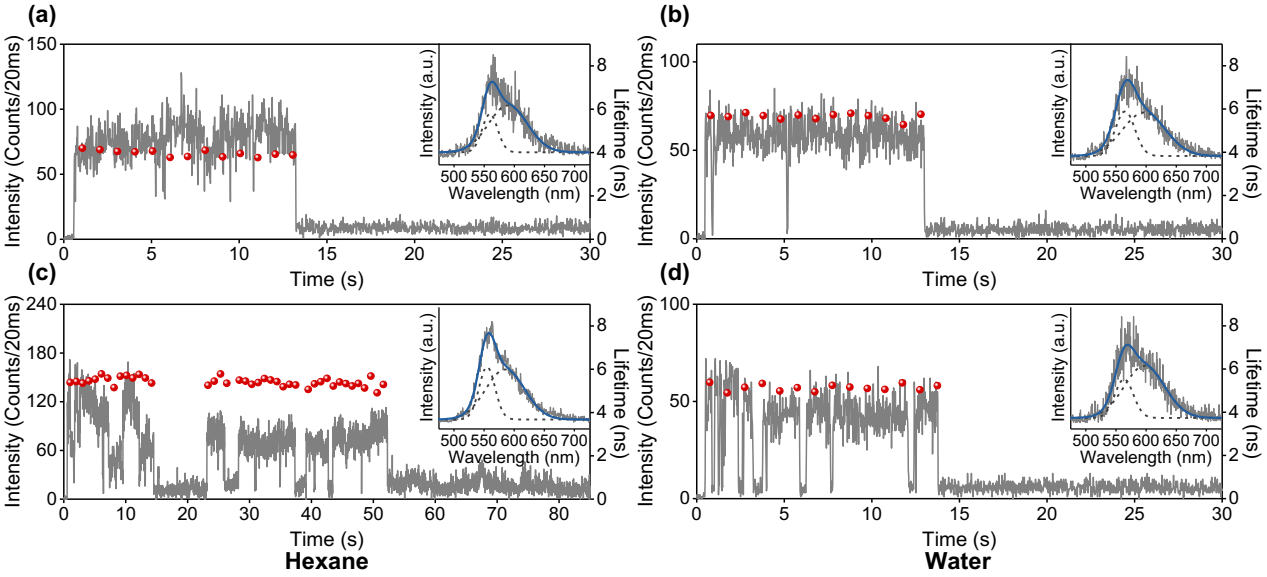


Figure S1. Representative FITs, fluorescence lifetimes (red dots in the FITs), and emission spectra of **MPBI** in hexane (a and c) and water (b and d). (a) One-step photobleaching behavior, spectral peak position = 559 nm (inset) in hexane, (b) one-step photobleaching behavior, spectral peak position = 565 nm (inset) in water, (c) stepwise photobleaching behavior, spectral peak position = 554 nm (inset) in hexane, (d) stepwise photobleaching behavior, spectral peak position = 563 nm (inset) in water.

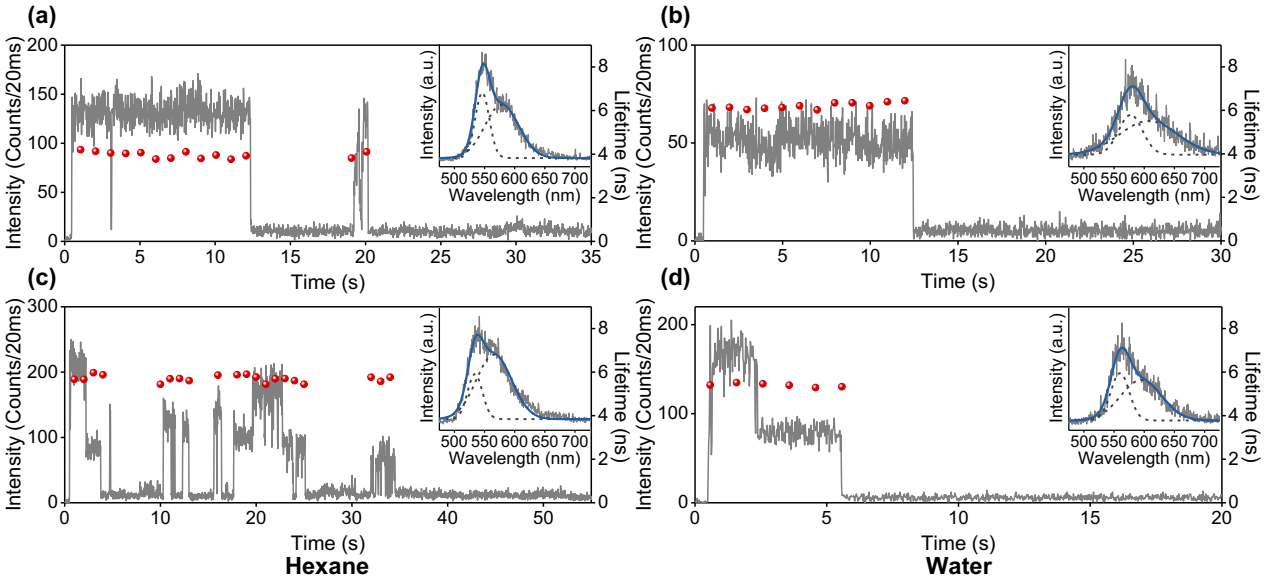


Figure S2. Representative FITs, fluorescence lifetimes (red dots in the FITs), and emission spectra of **LPBI** in hexane (a and c) and water (b and d). (a) One-step photobleaching behavior, spectral peak position = 546 nm (inset) in hexane, (b) one-step photobleaching behavior, spectral peak position = 577 nm (inset) in water, (c) stepwise photobleaching behavior, spectral peak position = 534 nm (inset) in hexane, (d) stepwise photobleaching behavior, spectral peak position = 559 nm (inset) in water.

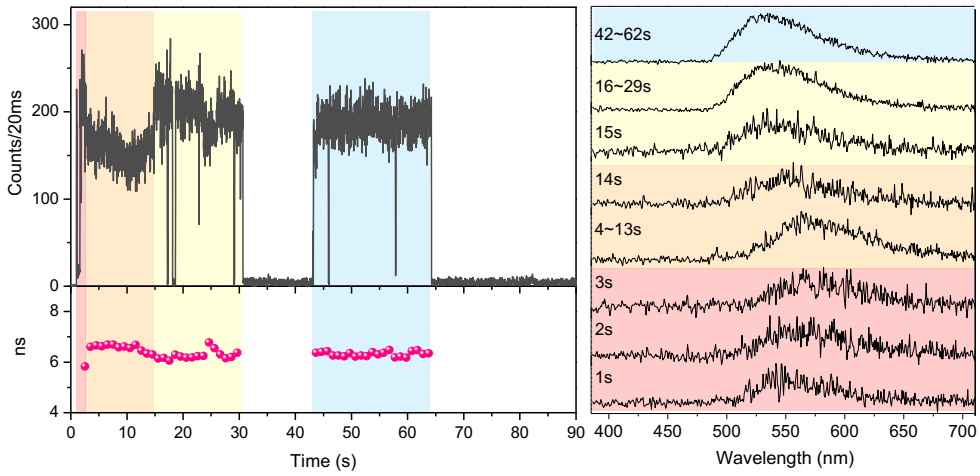


Figure S3. An example showing conformational change of a single **LPBI** molecule in hexane with changing the fluorescence lifetime and spectrum according to the fluorescence intensity fluctuations. The ratio of this case is 5.1%, 9.1%, and 12. 8 % in hexane, and 4.8%, 5.7%, and 6.2 % in water for **SPBI**, **MPBI**, and **LPBI**, respectively.

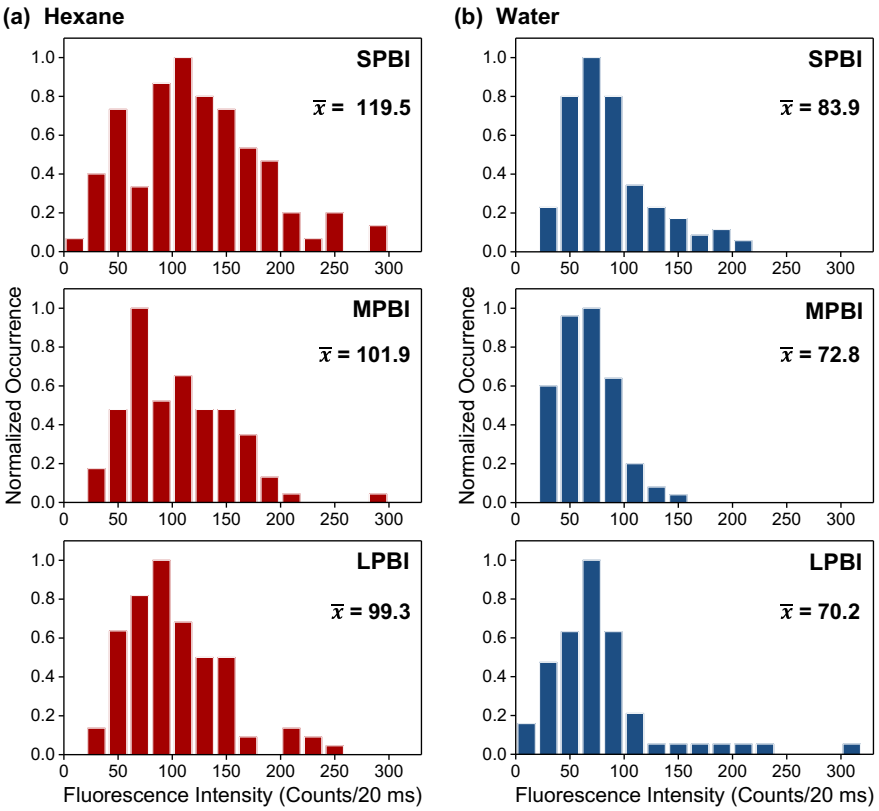


Figure S4. Comparison of the brightness of the PBI molecules by calculating the average fluorescence intensity at the first emissive level in their FITs. The brightness of **SPBI**, **MPBI**, and **LPBI** molecules in hexane (a) shows 1.4 times higher fluorescence level than in water (b).

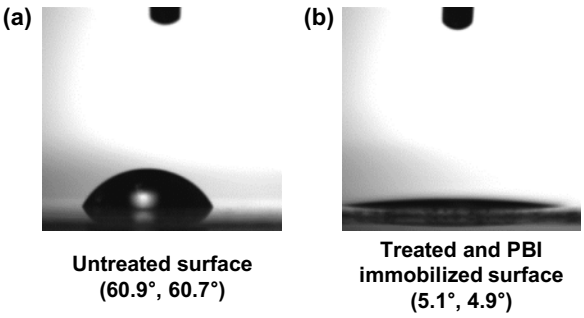


Figure S5. Photographs demonstrating the contact angle of water droplet at the surface of (a) untreated glass and (b) glass after immobilization onto the treated surface. A contact angle of the treated glass was around 5°. The contact angle of the glass surface after the immobilization of the functional molecules is much smaller than the another report of the glass surface immobilized organic molecules[2] since we used highly diluted solution for the immobilization onto the treated surface.

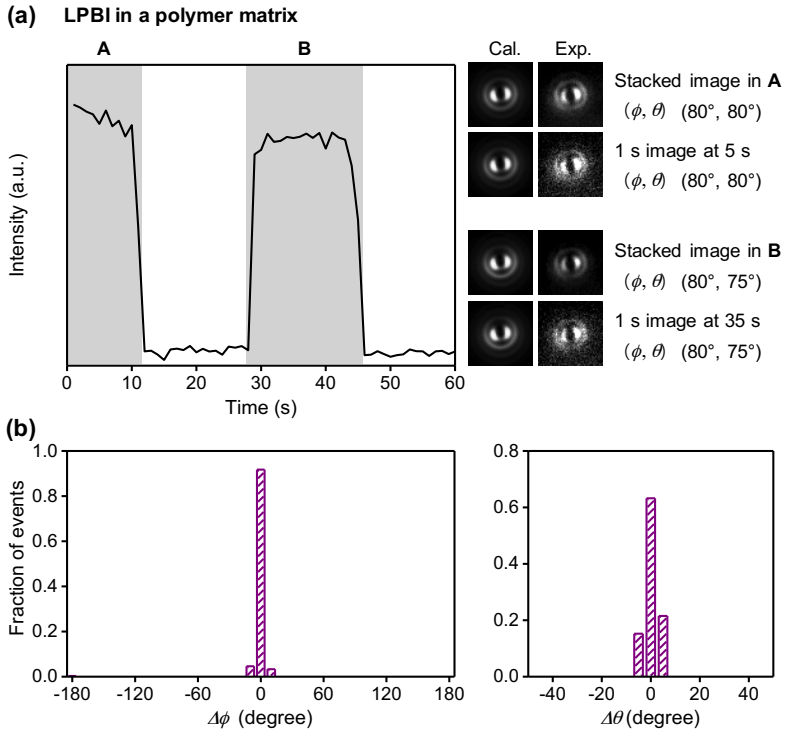


Figure S6. (a) An example of defocused wide-field image of a single **LPBI** molecule with its FIT in a polymer matrix, (b) The histograms of in-plane angle deviations ($\Delta\phi$) and out-of-plane angle deviations ($\Delta\theta$) of **LPBI** molecules in the polymer matrix. Because the **LPBI** molecules are embedded in the polymer matrix, we cannot see large angle deviations ($\Delta\varphi$ and $\Delta\theta$ of PBIs in the range of ± 0–10° and ± 0–5°). This result supports that the PBI molecule shows the flexible motion in a solution-phase, and the motions of the PBI molecules are affected by the interactions with local environments.

5. References

(1) S. Habuchi, T. Oba, M. Vacha, *Phys. Chem. Chem. Phys.* **2011**, *13*, 7001–7007.
(2) Y.-R.Yeon, Y. J. Park, J.-S. Lee, J.-W. Park, S.-G. Kang, C.-H. Jun, *Angew. Chemie. Int. Ed.* **2008**, *47*, 109–112.