Supplementary Information for:

A Water-soluble PEGylated RGD-functionalized Bisbithiophenyl Diketopyrrolopyrrole as a Photoacoustic Sonophore

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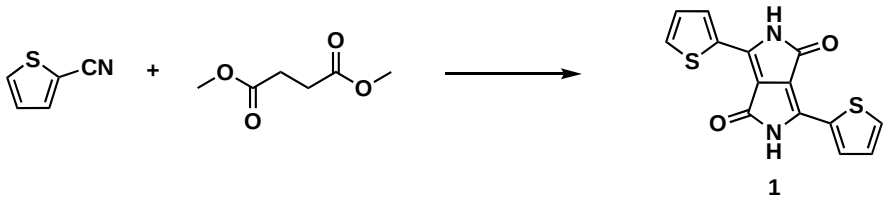
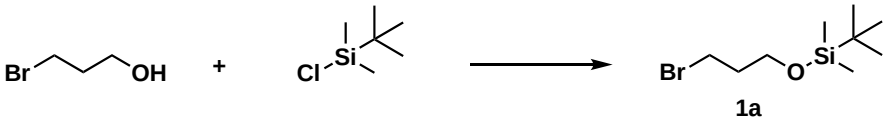


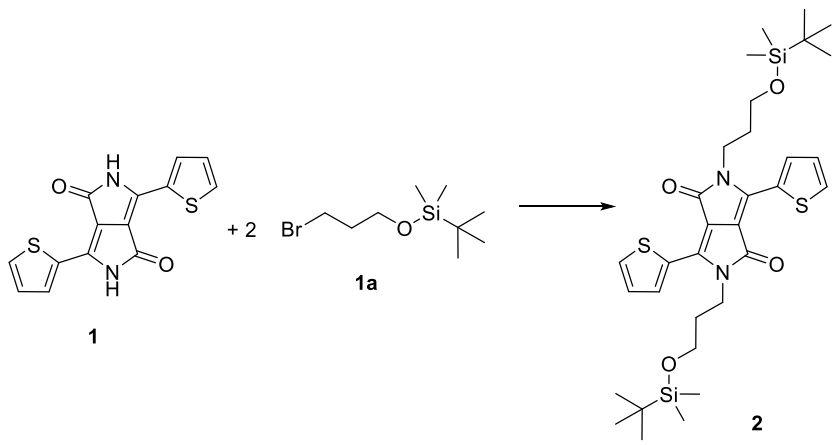
1

3,6-Di(thiophen-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (1)
1 is synthesized according to a described procedure (see also manuscript: WO2008000664). In short, potassium tert-butylate (20.00 g, 178.24 mmol, 3.57 eq) is dissolved in 2-methyl-2-butanol (190 mL) in a round flask under argon atmosphere. Then 2-thiophenecarbonitrile (20.92 mL, 149.78 mmol, 3 eq) is injected using a syringe in one portion. The mixture is heated to 110 °C and a solution of dimethyl succinate (9.80 mL, 49.93 mmol, 1 eq) in 2-methyl-2-butanol (60 mL) is slowly added over 6 hours. After the addition is completed, the reaction is stirred at the same temperature over night. The reaction mixture is cooled to 65 °C, diluted with 200 mL of methanol and 100 mL of acetic acid and refluxed for another 10 min. The suspension is filtered and the filter cake is washed with hot methanol and water twice. The solid is dried *in vacuo* and used directly without further purification. Yield: 69 % (dark purple solid).



1a

(3-Bromopropxy)(*tert*-butyl)dimethylsilane (1a)
3-Bromo-1-propanol (15.00 mL, 165.88 mmol, 1 eq), triethylamine (27.59 mL, 199.05 mmol, 1.2 eq) and 4-(dimethylamino)pyridine (0.405 g, 3.32 mmol, 0.02 eq) are dissolved in 150 mL DCM. The reaction mixture is cooled to 0 °C and purged with argon. *Tert*-Butyldimethylsilyl chloride (30.00 g, 199.05 mmol, 1.2 eq) is added in portions, and then the cooling bath is removed. After the reaction temperature warms up to ambient temperature, the mixture is stirred over night. The reaction is quenched by a saturated sodium bicarbonate solution (50 mL), extracted by DCM twice, washed with brine and dried over Na_2SO_4.The crude product is purified by column chromatography (DCM:Aceton, 8.5:1.5).
Yield: 89 % (colorless liquid).
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.73 (t, 2H, CH_2), 3.51 (t, 2H, CH_2), 2.03 (p, 2H, CH_2-), 0.89 (t, 9H, CH_3), 0.06 (s, 6H, CH3) ppm.
^{13}C-NMR (101 MHz, $CDCl_3$): δ = 60.41 (s), 35.54 (s), 30.69 (s), 25.90 (s), 18.29 (s), -5.39 (s) ppm.
Elemental analysis: C9H21BrOSi, calcd (%): C, 42.68; H, 8.36; Br, 31.55; O, 6.32; Si, 11.09; measd (%): C 41.93, H 8.48.



1 1a 2

2,5-*bis*(3-((*tert*-butyldimethylsilyl)oxy)propyl)-3,6-di(thiophen-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (2)

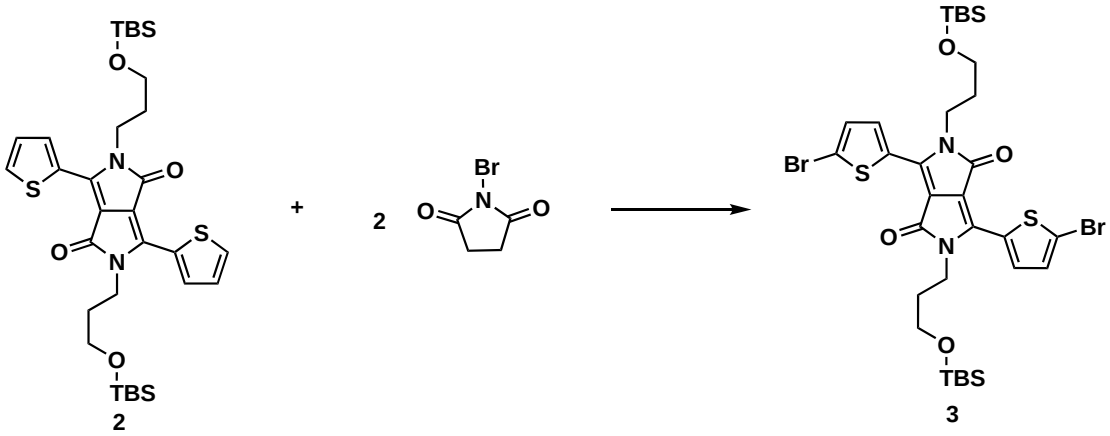
Compound **1** (3.22 g, 10.71 mmol, 1 eq), anhydrous potassium carbonate (5.96 g, 42.28 mmol, 4 eq) and sodium iodide (3.21 g, 21.43 mmol, 2 eq) are dissolved in dry DMF (60 mL) and heated to 145 °C for 2 hours under argon protection. Then compound **2** (12.48 g, 49.86 mmol, 4.6 eq) is purged with argon and injected by syringe for 6 h. After completion, the reaction mixture is stirred over night at 145 °C. The solution is cooled to room temperature and poured into 500 mL of ice-water, then filtered. The filter cake is washed with water and methanol several times, dried *in vacuo* and purified by column chromatography ($CHCl_3$).

Yield: 19 % (golden red solid).

^{1}H-NMR (400 MHz, $CDCl_3$) : δ = 8.73 (dd, 2H, C*H*), 7.56 (dd, 2H, C*H*), 7.20 (t, 2H, C*H*), 4.12 (t, 4H, CH_2), 3.70 (t, 4H, CH_2), 1.91 (m, 4H, CH_2), 0.84 (s, 18H, CH_3), 0.00 (s, 12H, CH_3) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 161.42 (s), 139.91 (s), 134.58 (s), 130.75 (s), 129.89 (s), 128.56 (s), 107.74 (s), 60.85 (s), 40.02 (s), 32.67 (s), 25.95 (s), 18.31 (s), -5.39 (s) ppm.

Elemental analysis: C32H48N2O4S2Si2, calcd (%):C, 59.59; H, 7.50; N, 4.34; O, 9.92; S, 9.94; Si, 8.71; measd (%): C 57.24, H 7.52, N 4.34, S 9.95.



3,6-*bis*(5-bromothiophen-2-yl)-2,5-*bis*(3((*tert*-butyldimethylsilyl)oxy)propyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (3)

Compound **2** (1.00 g, 1.55 mmol, 1 eq), is dissolved in 100 mL DCM, cooled to 0 °C and purged with argon. The solution is protected from light and *N*-bromosuccinimide (0.58 g, 3.26 mmol, 2.1 eq) is added in one portion.

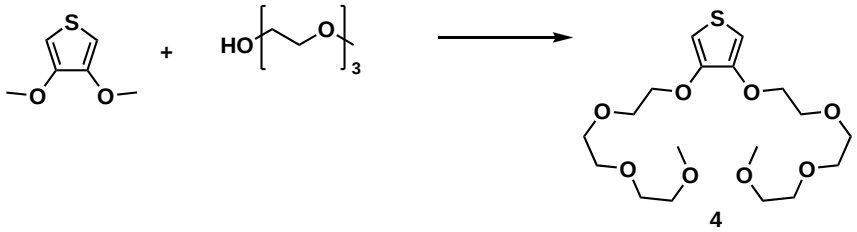
After two days, the mixture is poured into 300 mL methanol. The precipitation is filtered off, washed by hot methanol and water twice each time and dried in vacuum.

Yield: 77 % (dark red solid).

^{1}H-NMR (400 MHz, $CDCl_3$) : δ = 8.58 (d, 2H, C*H*), 7.21 (d, 2H, C*H*), 4.11 (t, 4H, CH_2), 3.76 (t, 4H, CH_2), 1.95 (m, 4H, CH_2), 0.92 (s, 18H, CH_3), 0.08 (s, 12H, CH_3) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 161.05 (s), 139.79 (s), 134.80 (s), 131.74 (s), 131.56 (s), 119.44 (s), 107.75 (s), 60.69 (s), 40.31 (s), 32.56 (s), 26.00 (s), 18.34 (s), -5.39 (s) ppm.

Elemental analysis: C32H46Br2N2O4S2Si2, calcd (%):C, 47.87; H, 5.78; Br, 19.91; N, 3.49; O, 7.97; S, 7.99; Si, 7.00; measd (%): C 45.79.51, H 5.89, N 3.47, S 8.00.

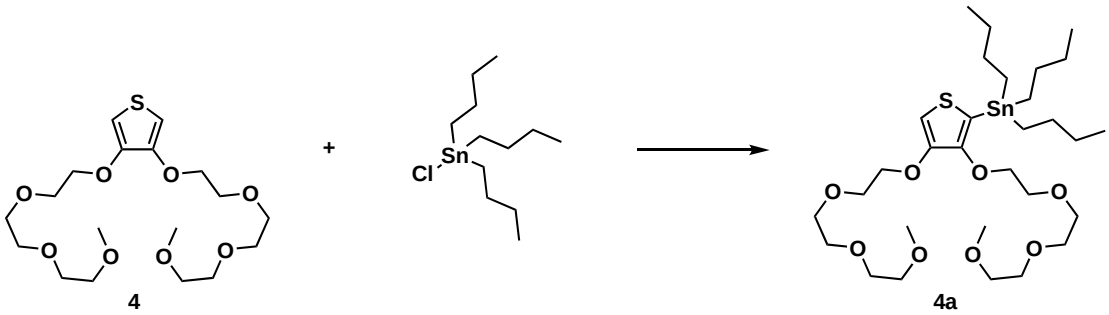


3,4-Di[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]thiophene (4)

3,4-dimethoxythiophene (1.814 g, 12.58 mmol, 1.5 mL) is dissolved in triethylene glycol monomethyl ether (5.507 g, 33.54 mmol, 5.4 mL, 2.7 eq) and degassed by bubbling with argon (15 min). Then *p*-toluene sulfonic acid is added to the reaction mixture and the solution is stirred for three days at 90 °C under constant nitrogen flow. The raw product is purified using column chromatography (ethyl acetat: dimethoxyethane / 9.5:0.5).

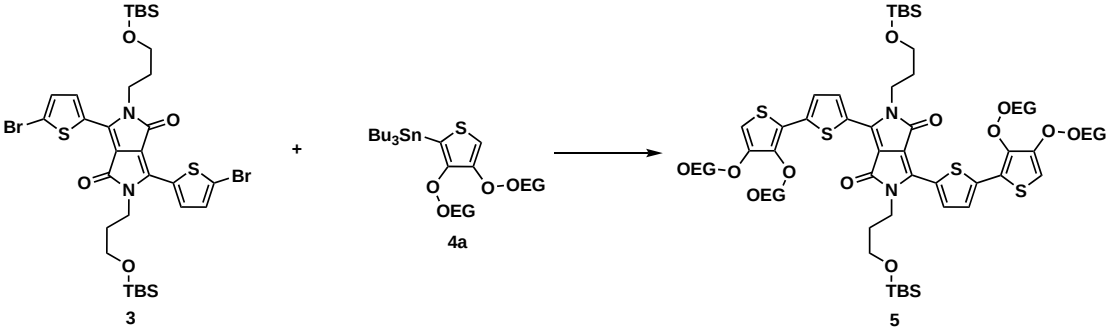
Yield: 56 % (yellow liquid).

^{1}H-NMR (CDCl3, 400 MHz): δ = 6.20 (s, 2H, C*H*), 4.11 (m, 4H, CH_2), 3.81 (m, 4H, CH_2), 3.70 (m, 4H, CH_2), 3.64 (m, 8H, CH_2), 3.51 (m, 4H, CH_2), 3.34 (s, 6H, CH_3) ppm.
^{13}C-NMR (101 MHz, $CDCl_3$): δ = 147.26 (s), 98.05 (s), 72.08 (s), 70.80 (s), 70.70 (s), 69.98 (s), 69.65 (s), 59.19 (s) ppm.
Elemental analysis: C18H32O8S, calcd (%): C 52.92; H, 7.90; O, 31.33; S, 7.85; measd (%): C 53.17, H 7.69, S 7.96.



4 **4a**

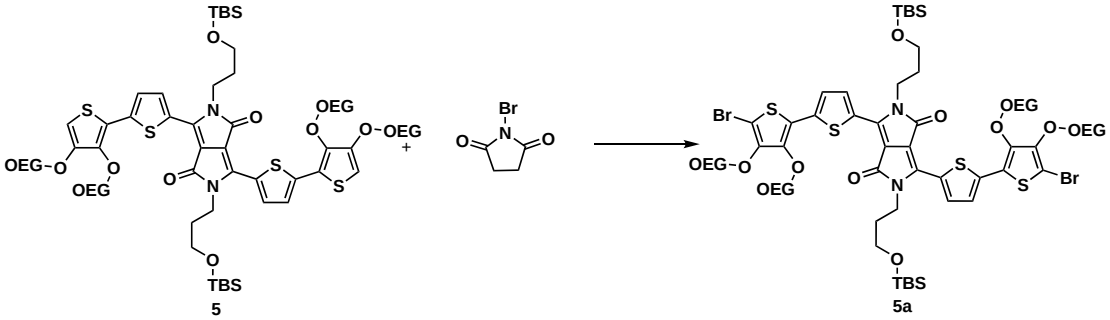
(3,4-bis(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)thiophene-2-yl)tributylstannane (4a)
Compound **4a** (2.07 g, 5.07 mmol, 1eq) is dissolved in 100 mL of dry diethyl ether under inert conditions. The solution is cooled to -78 °C and *N,N,N',N'*-tetrametyhlethylenediamine (0.84 mL, 5.57 mmol, 1.1 eq) is added dropwise. After 10 min, butyllithium (3.48 mL, 1.6 mol/L in hexane, 5.57 mmol, 1.1 eq) is added slowly. The reaction mixture is stirred at room temperature for two hours and subsequently cooled down to -78 °C. Tributyltin chloride (1.51 mL, 5.57 mmol, 1.1 eq) is added in one portion and then the cooling bath is removed. The reaction mixture is stirred over night at room temperature, quenched by adding water (50 mL) and extracted by diethyl ether. The crude product is purified by column chromatography (Ethyl acetate)
Yield: 55 % (yellow liquid).
^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 6.46 (s, 1H, C*H*), 4.11 (m, 4H, CH_2), 3.83 (m, 2H, CH_2), 3.73 (m, 2H, CH_2), 3.71 (m, 4H, CH_2), 3.65 (m, 8H, CH_2), 3.54 (m, 4H, CH_2), 3.34 (s, 6H, CH_3), 1.54 (m, 6H, CH_2), 1.33 (m, 6H, CH_2), 1.10 (m, 6H, CH_2), 0.88 (m, 9H, CH_3) ppm.
^{13}C-NMR (101 MHz, $CDCl_3$): δ = 147.8 (s), 97.1 (s), 95.1 (s), 72-70 (m), 59.4 (s), 29-27 (m), 16.9 (s), 13.8 (s) ppm.
Elemental analysis: $C_{30}H_{58}O_8SSn$, calcd (%): C, 51.66; H, 8.38; O, 18.35; S, 4.60; Sn, 17.02; measd (%): C 50.12, H 8.85, S 4.39.



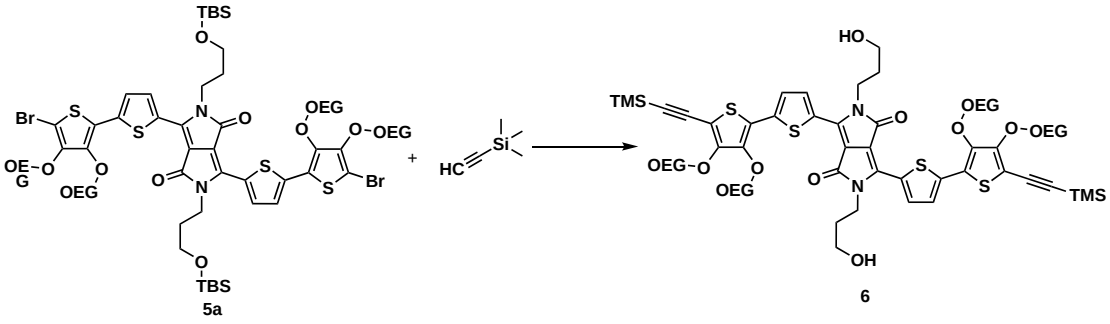
3 **4a** **5**

3,6-*bis*(3',4'-*bis*(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-[2,2'-bithiophen]-5-yl)-2,5-*bis*(3-((*tert*-butyldimethylsilyl)oxy)propyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (5)
Compound **3** (0.374 g, 0.465 mmol, 1 eq) and compound **4** (0.975 g, 1.37 mmol, 3 eq) is dissolved in dry toluene (50 mL) and dry DMF (15 mL) under inert conditions. Subsequently, tetrakis(triphenylphosphine)palladium(0) (0.054 g, 0.047 mmol) is added and the reaction mixture is stirred at 120 °C for 7 days with a constant nitrogen flow. A color change from pink to purple to dark blue is visible. The reaction is quenched with 30 mL of water and the crude product is purified by column chromatography (DCM:Acetone, 7:3)
Yield: 97 % (golden blue solid).
^{1}H-NMR (CDCl3, 400 MHz): δ = 8.98 (s, 1H, C*H*), 8.79 (s, 1H, C*H*), 7.43 (m, 1H, C*H*), 7.36 (m, 1H, C*H*), 6.20 (s, 2H, C*H*), 4.38 (m, 4H, CH_2), 4.28 (m, 4H, CH_2), 4.18 (m, 4H, CH_2), 4.13 (m, 4H, CH_2), 3.83 (m, 8H, CH_2), 3.63 (m, 16H, CH_2), 3.58 (m, 8H, CH_2), 3.50 (m, 8H, CH_2), 3.33 (m, 12H, CH_3), 2.00 (m, 4H, CH_2), 0.86 (m, 18H, CH_3), 0.03 (m, 6H, CH_3) ppm.

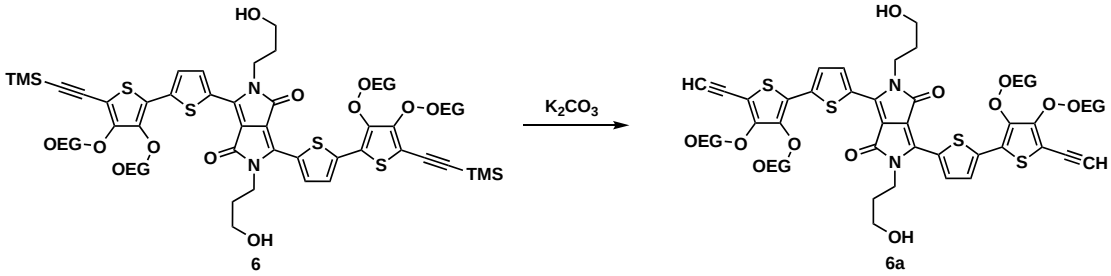
^{13}C-NMR (101 MHz, $CDCl_3$): δ = 165.1 (s), 152.5 (s), 147.7 (s), 143-141 (m), 136.4 (s), 132.5 (s), 128.0 (s), 115-113 (m), 94.8 (s), 73-70 (m), 60.5 (s), 60 (s), 41.5 (s), 33.3 (s), 30.5 (s), 25.8 (s) -2.3 (s) ppm.
Elemental analysis: $C_{68}H_{108}N_2O_{20}S_4Si_2$, calcd (%):C, 56.02; H, 7.47; N, 1.92; O, 21.95; S, 8.80; Si, 3.85; meads (%): C 55.97, H 7.99, N 1.56, S 8.81.



3,6-*bis*(5'-bromo-3',4'-*bis*(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-[2,2'-bithiophen]-5-yl)-2,5-*bis*(3-((*tert*-butyldimethylsilyl)oxy)propyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (5a)
Compound **5** (0.431 g, 0.295 mmol, 1 eq), is dissolved in 30 mL DCM, cooled to 0 °C and purged with argon. The solution is protected from light and *N*-bromosuccinimide (0.116 g, 0.650 mmol, 2.2 eq) is added in one portion. After two days, the mixture is evaporated and dissolved in toluene. The organic phase is washed twice with water and the crude product is purified by column chromatography (DCM:Aceton, 1:1)
Yield: 71 % (dark purple blue solid).
^{1}H-NMR (400 MHz, $CDCl_3$) : δ = 8.92 (d, 1H, C*H*), 8.71 (d, 1H, C*H*), 7.28 (d, 1H, C*H*), 7.22 (d, 1H, C*H*), 4.32 (m, 4H, CH_2), 4.24 (m, 8H, CH_2), 4.13 (m, 4H, CH_2), 3.76 (m, 8H, CH_2), 3.66-3.49 (m, 24H, CH_2), 3.44 (m, 8H, CH_2), 3.30 (m, 12H, CH_3), 1.94 (m, 4H, CH_2), 0.84 (m, 18H, CH_3), 0.01 (m, 6H, CH_3) ppm.
^{13}C-NMR (101 MHz, $CDCl_3$): δ = 165.1 (s), 156.1 (s), 150 (s), 143-140 (m), 136 (s), 133 (s), 127.9 (s), 117 (s), 113.5 (s), 85 (s), 73-70 (m), 61-59 (m), 41.5 (s), 33 (s), 30.5 (s), 25.5 (s), -2.3 (s) ppm.
Elemental analysis: $C_{68}H_{106}Br_2N_2O_{20}S_4Si_2$, calcd (%):C, 50.55; H, 6.61; Br, 9.89; N, 1.73; O, 19.80; S, 7.94; Si, 3.48; measd (%): C 56.19, H 6.72, N 1.29, S 7.16.



3,6-*bis*(3',4'-*bis*(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-5'-((trimethylsilyl)ethynyl)-[2,2'-bithiophen]-5-yl)-2,5-*bis*(3-hydroxylpropyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (6)
Compound **5a** (290.6 mg, 0.210 mmol, 1 eq), is dissolved in THF (30 mL) and degassed by bubbling with argon. While bubbling with argon, copper(I) iodide (4.0 mg, 0.02 mmol, 0.1 eq), bis(triphenylphosphine)palladium (II) dichloride (14.7 mg, 0.02 mmol, 0.01 eq) and dry diisopropylamine (20 mL) is added. Subsequently trimethylsilylacetylene (65 µL, 0.462 mmol, 2.2 eq) is added in one portion with a syringe. The reaction mixture is protected from light and stirred at room temperature over night. The solvent is evaporated and 30 ml of DCM is added and washed with a saturated with a NH_4Cl-solution and with water. The crude product is purified by column chromatography (DCM:Aceton, 1:1)
The product is obtained as a purple-blue solid (yield: 36 %), and immediately deprotected to form **6a**.
^{1}H-NMR (400 MHz, $CDCl_3$) : δ = 9.01 (d, 2H, C*H*), 7.40 (d, 2H, C*H*), 4.60 (t, 4H, CH_2), 4.40 (t, 4H, O*H*), 4.30 (t, 4H, CH_2), 3.81 (m, 8H, CH_2), 3.71 (m, 32H, CH_2), 3.52 (m, 8H, CH_2), 3.35 (m, 12H, CH_3), 2.01 (m, 4H, CH_2), 0.32 (m, 9H, CH_3) ppm.



3,6-*bis*(5'-ethynyl-3',4'-*bis*(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)-[2,2'-bithiophen]-5-yl)-2,5-*bis*(3-hydroxylpropyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (6a)

Compound **6** (52.0 mg, 0.037 mmol, 1 eq), is dissolved in methanol (60 mL) and DCM (30 mL). The solution is degassed by bubbling with argon for 15 min. Subsequently, potassium carbonate (21.2 mg, 0.152 mmol, 4.2 eq) is added to the reaction mixture and stirred for five hours in the dark. The blue solution is washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$ and the solvent is removed under pressure.

Yield: quant. (purple blue solid).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.01 (d, 1H, C*H*), 7.68-7.43 (m, 2H, C*H*), 7.39 (d, 1H, C*H*), 4.58 (t, 4H, CH_2), 4.40 (t, 4H, O*H*), 4.30 (t, 4H, CH_2), 3.82 (m, 8H, CH_2), 3.60-3.55 (m, 34H, CH_2 + O*H*), 3.49 (m, 8H, CH_2), 3.35 (m, 12H, CH_3), 2.03 (m, 4H, CH_2) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 165 (s), 148.5 (s), 144 (s), 143 (s), 141.5 (s), 136.5 (s), 132.5 (s), 128 (s), 113.5 (s), 109 (s), 94 (s), 75.5 (s), 73.5 (s), 73-70 (m), 60-58 (m), 41 (s), 32 (s) ppm.

Elemental analysis: $C_{60}H_{80}N_2O_{20}S_4$, calcd (%): C, 56.41; H, 6.31; N, 2.19; O, 25.05; S, 10.04; measd (%): C 56.62, H 6.91, N 2.20, S 10.10.

Thiol-yne click chemistry (RGD-7 + RAD-7)

Compound **6a** (5.3 mg, 0.004 mmol, 1 eq) and 2,2-dimethoxy-2-phenylacetophenone (1.7 mg, 0.007 mmol, 0.17 eq) are dissolved in 2:1 methanol-water (1.2 mL) and degassed by bubbling with argon. Subsequently, either cyclo(-Arg-Gly-Asp-D-Phe-Cys) acetate salt (c(RGDfC)) or cyclo(-Arg-Ala-Asp-D-Phe-Cys) trifluoroacetate salt (c(RADfC)) (5 mg, 0.008 mmol, 2 eq) is added and the solution is irradiated (λ_{max} 365 nm) at room temperature for five hours. Chloroform (5 mL) is added and the solution is washed with water twice. The aqueous phase is freeze-dried yielding blue solid products.

Yield (RGD-**7**): 97 %, yield (RAD-**7**): 74 %.

Analysis for RAD-**7**:

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.01 (d), 7.68-7.43 (m), 7.4-7.2 (br), 6.62 (s), 6.29 (s), 6.22 (s), 5.58 (s), 4.9 - 4.8 (br), 4.58 (br), 4.40 (br), 4.0 (t), 3.7 (s), 3.60-3.55 (br), 3.35 (s), 3.2 (br), 2.8-2.5 (br), 2.03 (m), 1.9 (s), 1.40 (m). 1.15 (m) ppm.

^{13}C-NMR (101 MHz, $CDCl_3$): δ = 174-168 (m), 165 (s), 158 (s), 149 (s), 142.5 (s), 141.5 (s), 138-135 (m), 132.5 (s), 129-125 (m), 120-118 (m, br), 114 (s), 73-70 (m), 65.5 (s), 62-55 (m), 44-33 (m, br), 32 (s), 28 (s), 25 (s) ppm.

Elemental analysis: $C_{107}H_{146}N_{18}O_{34}S_6$, calcd (%): C, 53.09; H, 6.08; N, 10.41; O, 22.47; S, 7.95; measd (%): C 54.45, H 6.98, N 9.47, S 7.14.

UV-Vis Spectroscopy

Absorptions are measured with a Varian Cary 50 Bio UV-Visible spectrophotometer. The software is Varian UV Cary Scan 3.00(303).

Photoluminescence Spectroscopy

Photoluminescence spectroscopy measurements are conducted on a Horiba Jobin-Yvon FluoroMax-4 spectrofluorometer with a 150 W xenon lamp, ozone free. The software has been FluorEssence™ version 3.8.2.2, Origin version 8.6001.The conjugated polymers are dissolved in either toluene or water.

Photoacoustics

Photoacoustic excitation spectra were recorded using a VEVO LAZR photoacoustic imaging system equipped with a LZ250 transducer (VisualSonics, Amsterdam NL). The system delivers peak energies of 45 +/- 5 mJ, with a pulse duration of 4-6 ns at tunable wavelengths between 680 – 970 nm (2 nm step size). High resolution ultrasound images and sprectra were acquired at 21 MHz.

Cell culture

To test the cytotoxicity of different DPP molecules (DPP, DPP + TMS, DPP + RGD and DPP + RDG), a proliferation assay is performed. Prior to cell culture experiments, the DPP samples are sterilized using UV for 30 min. The samples are dissolved in water and 1 M PBS is added to the different suspensions to obtain a final salt concentration of 0.1 M PBS. Sequentially, the different DPP molecules are diluted in cell culture media at different concentrations (2.3, 4.5, 9.0, 18.0, 36.1 and 72.2 µg/mL). L929 mouse fibroblasts are

seeded in a 96-well TCPS plate at a density of 50,000 cells/mL, 5,000 cells per well and incubated at 37°C with 5% CO_2 in RPMI 1640, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. After 24 h of culture, the media is removed and the solutions with different particle concentrations are added (n=3). As a positive control, cell culture media without DPP is added to the cells. After an incubation time of 48 h, an MTS proliferation assay (MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, Promega, USA) is performed. The absorption is measured with the Synergy HT plate-reader (BioTek, USA) using 490 nm and the levels are normalized to the positive control.

1H-NMR

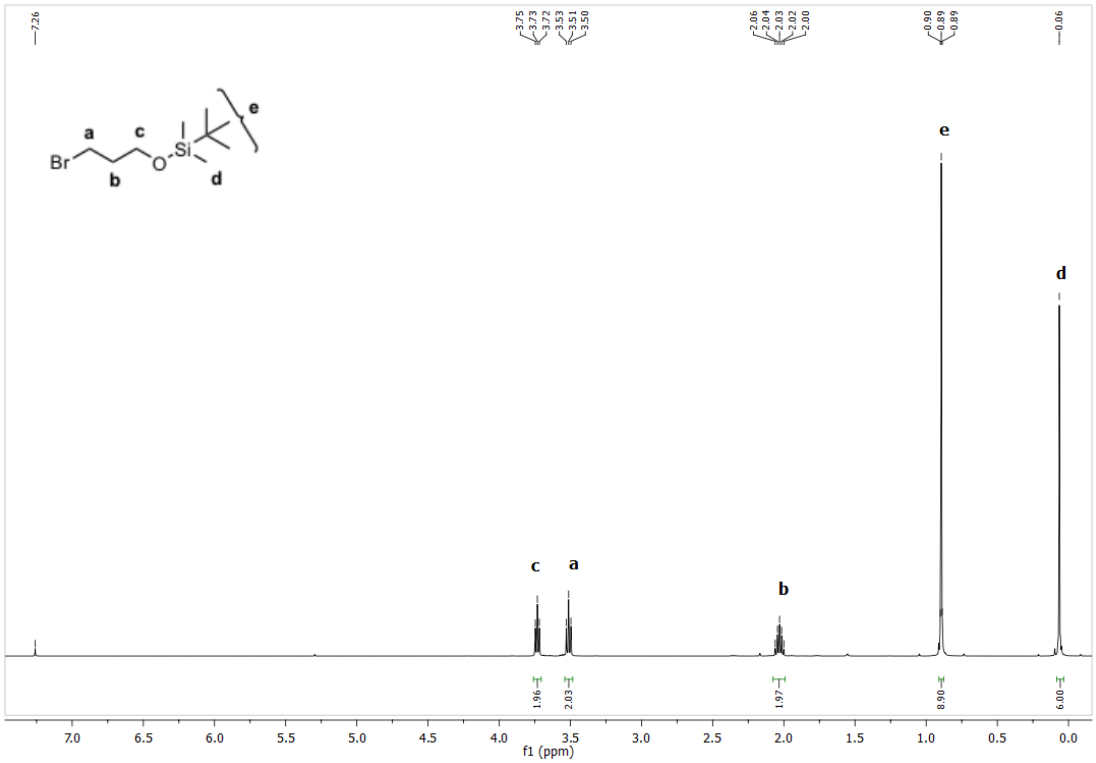


Figure S1: ^{1}H-NMR of **1a**.

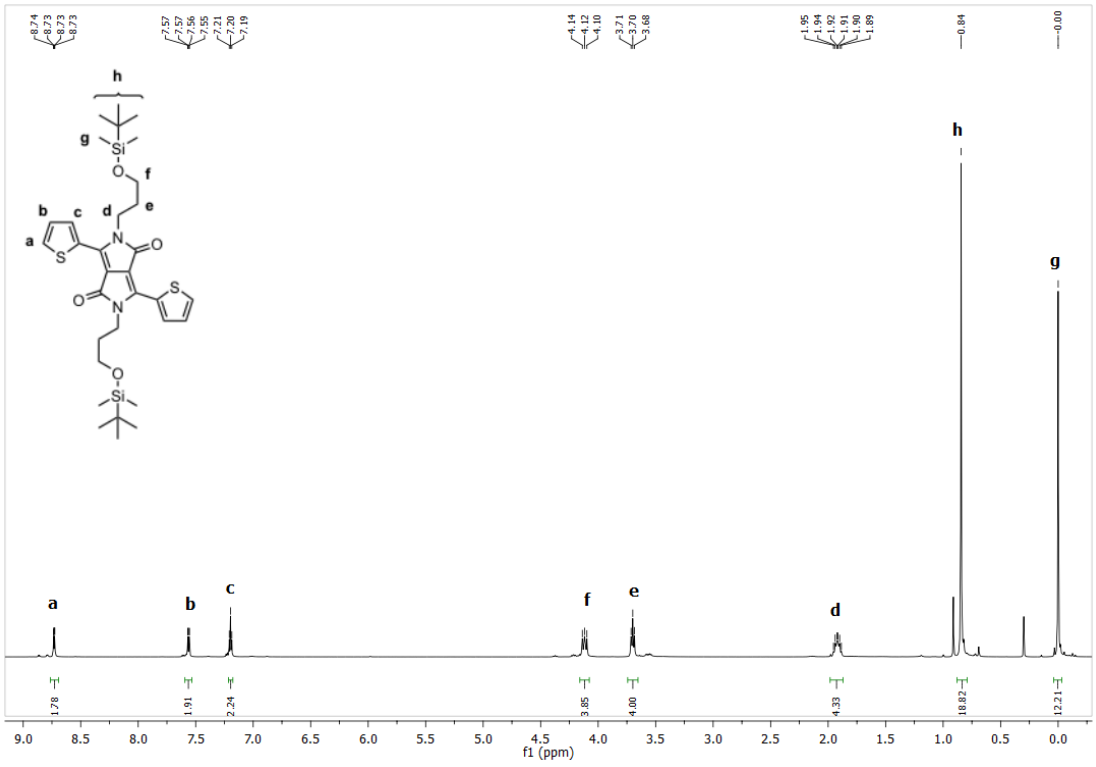


Figure S2: ^{1}H-NMR of **2**.

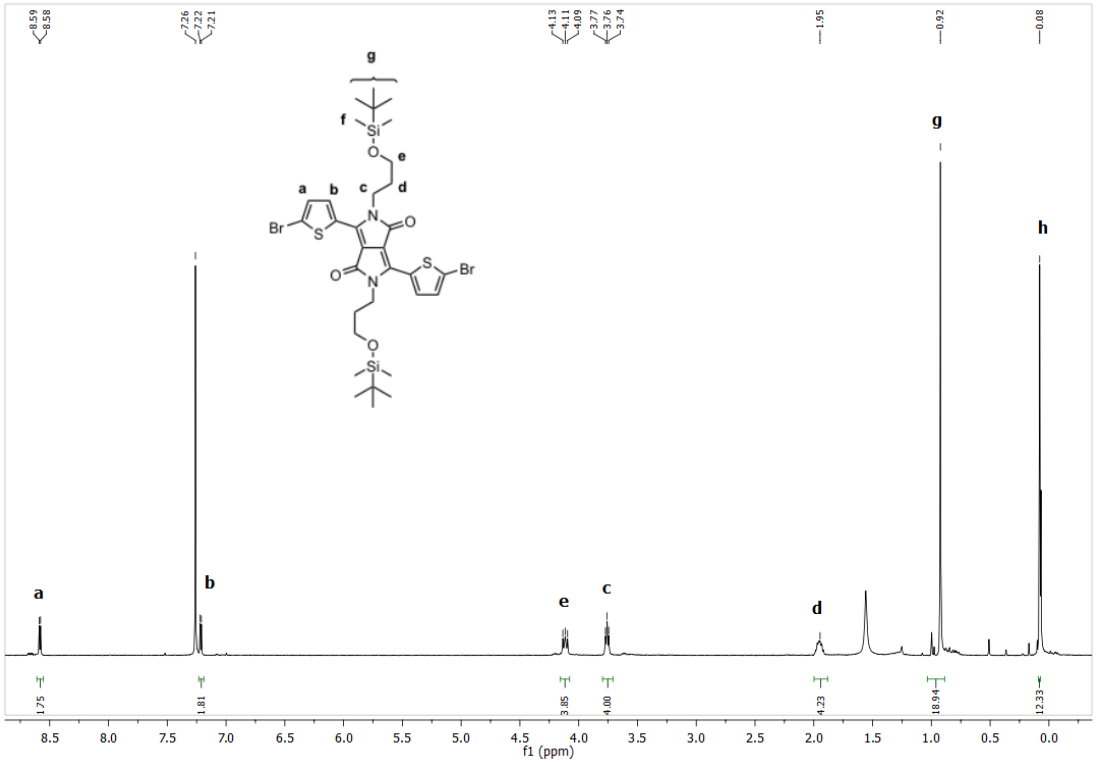


Figure S3: ^{1}H-NMR of **3**.

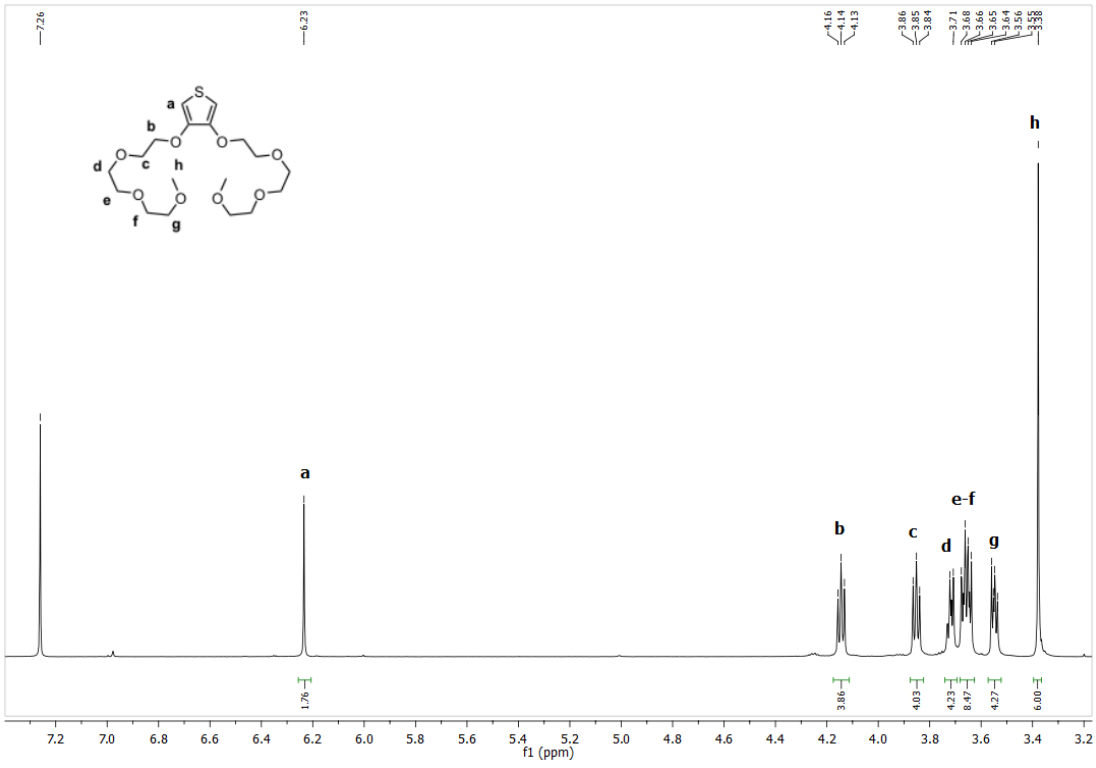


Figure S4: ^{1}H-NMR of **4**.

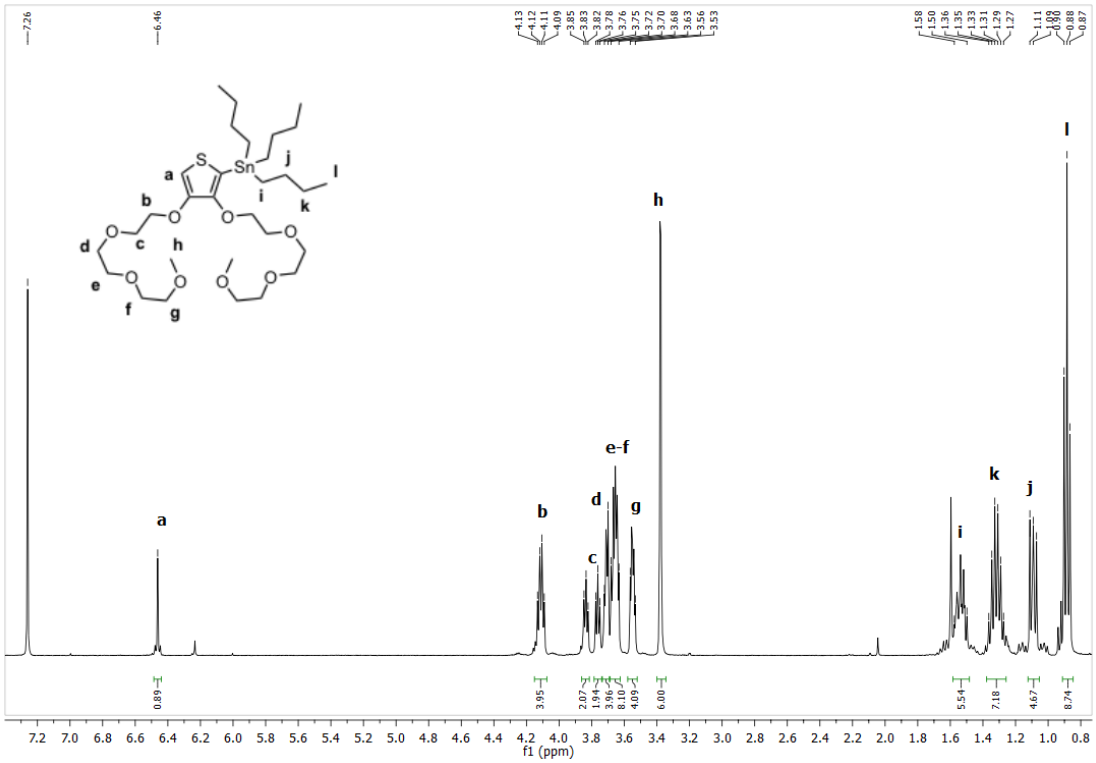


Figure S5: ^{1}H-NMR of **4a**.

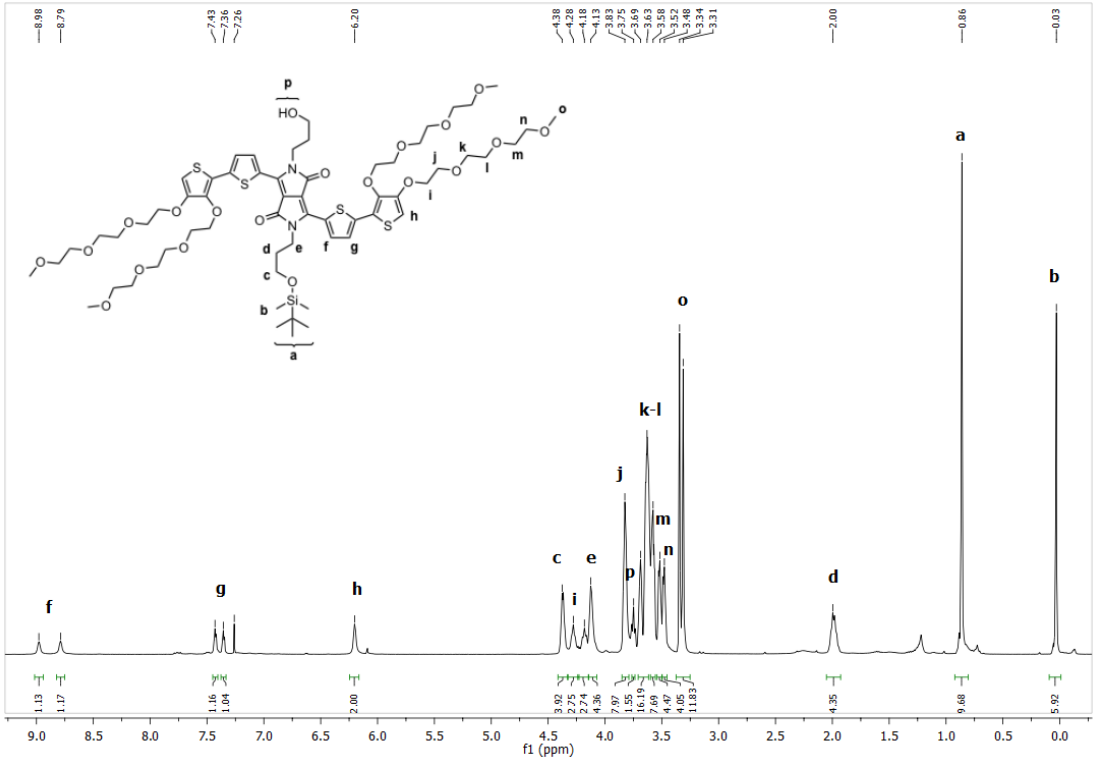


Figure S6: ^{1}H-NMR of **5**.

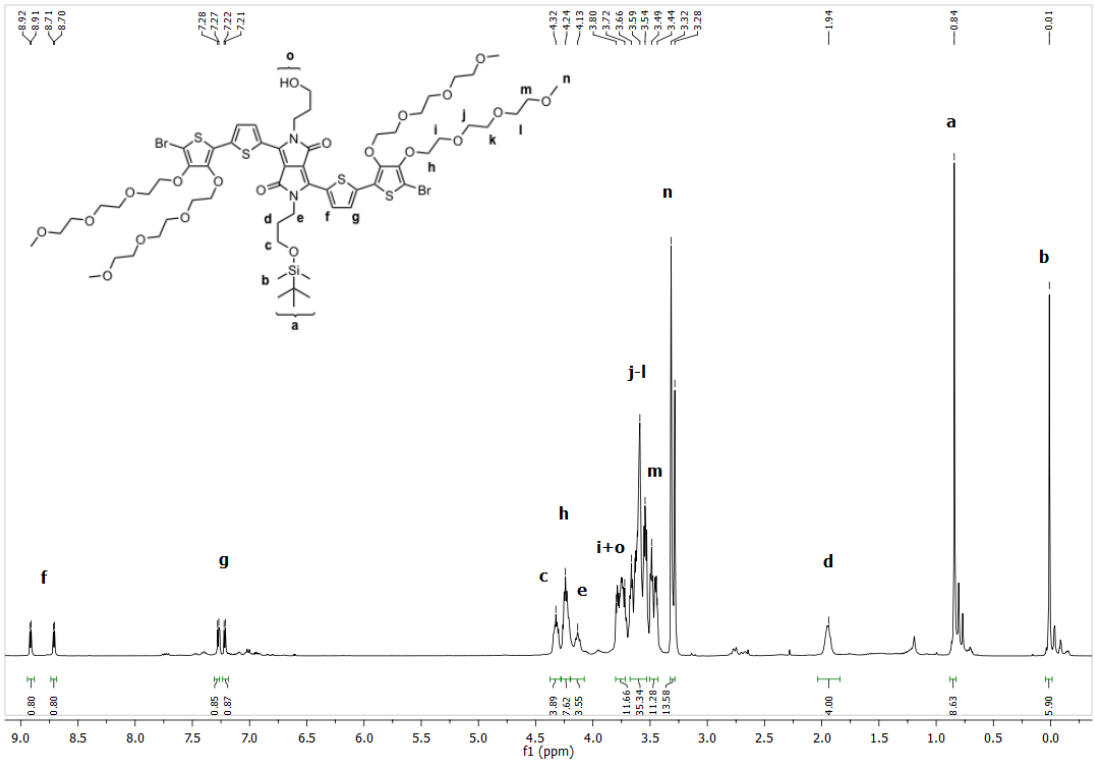


Figure S7: ^{1}H-NMR of **5a**.

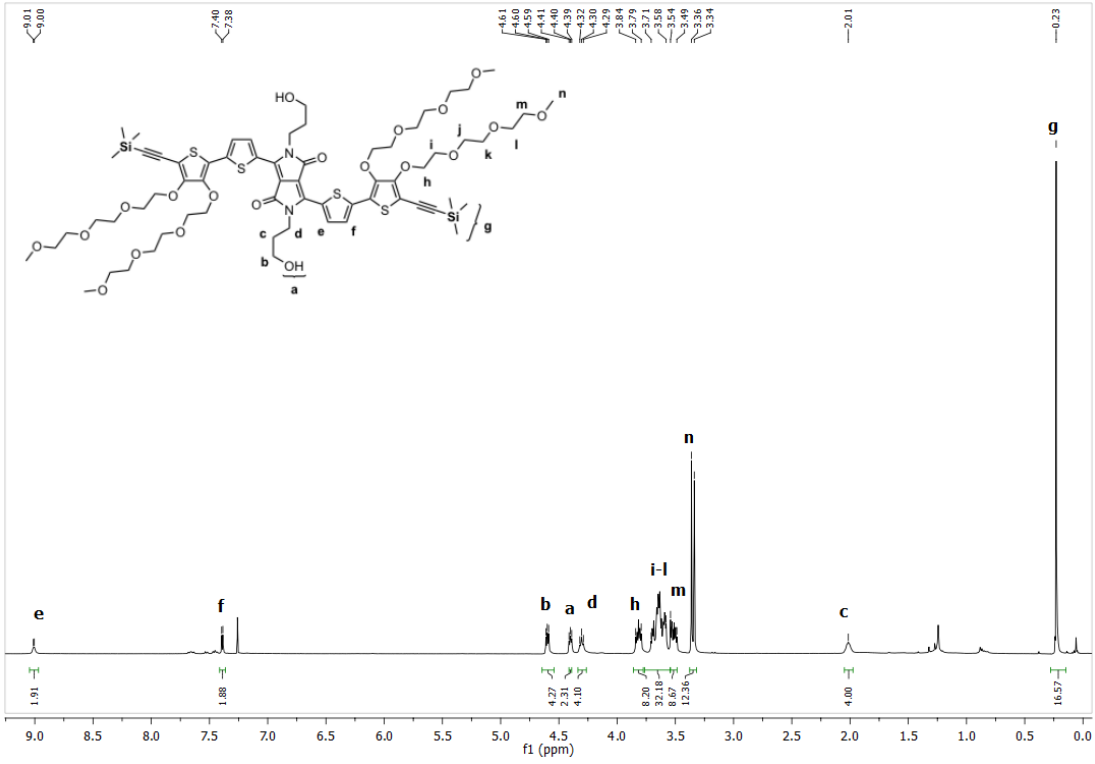


Figure S8: ^{1}H-NMR of **6**.

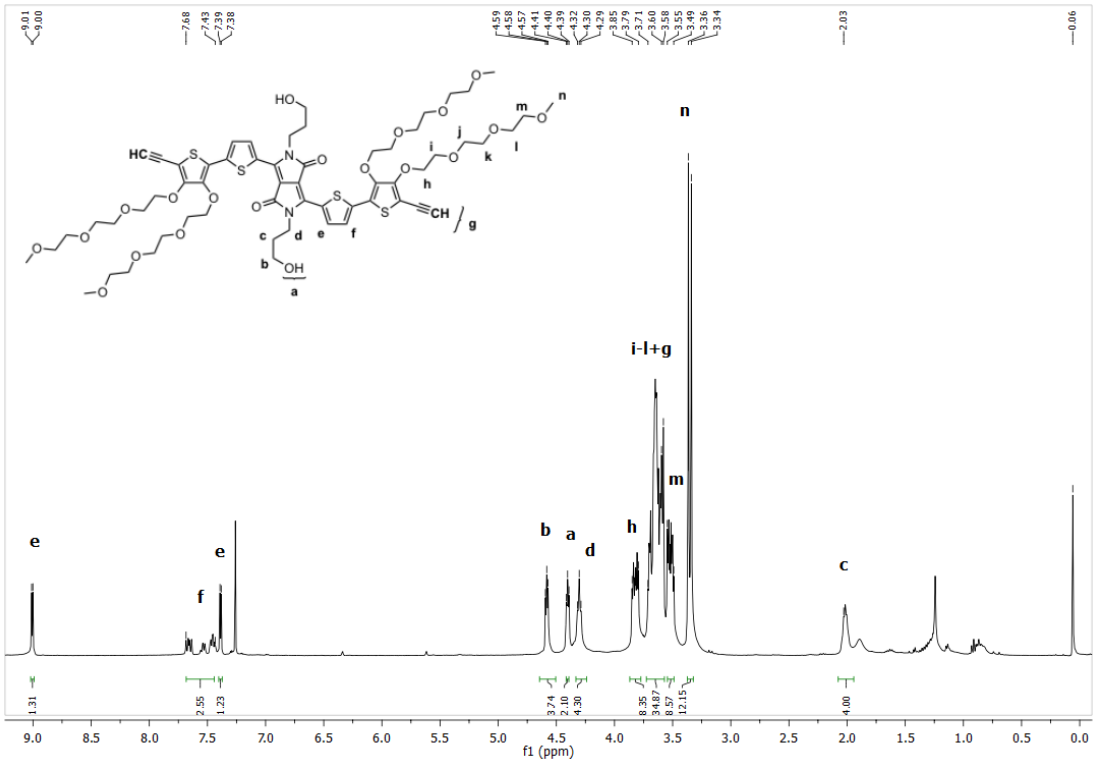


Figure S9: ^{1}H-NMR of **6a**.

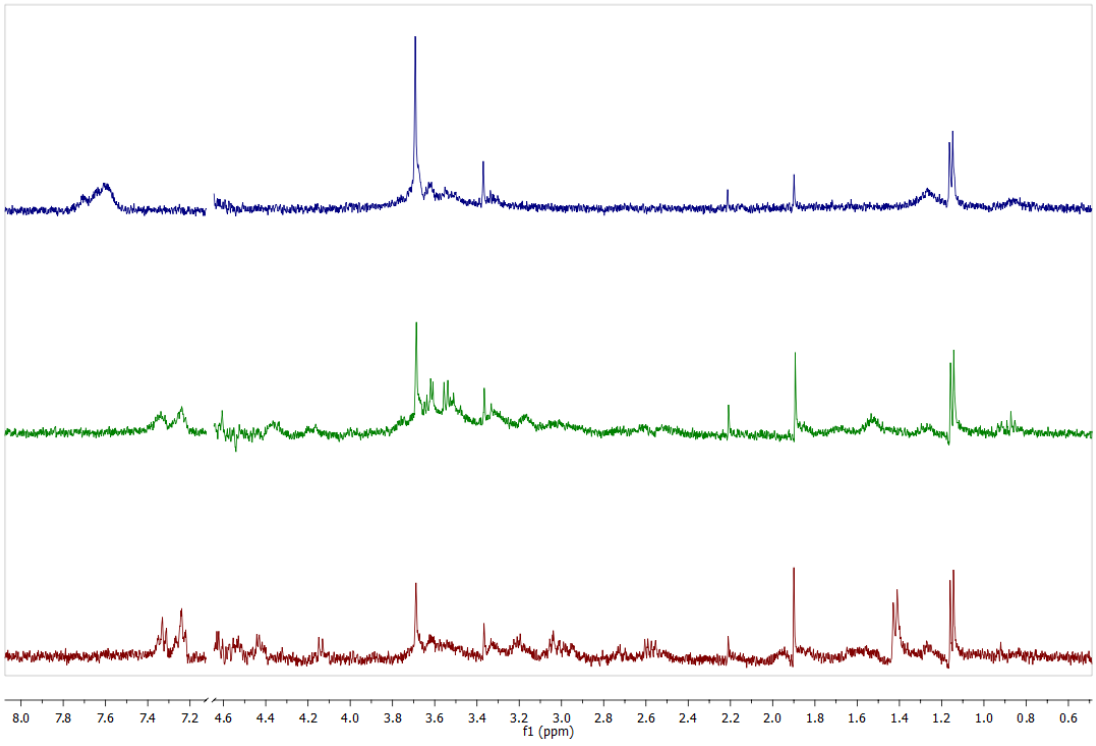


Figure S10: ^{1}H-NMR in D_2O of **6a** (blue), **RGD-7** (green) and **RAD-7** (red).