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Supporting information

Cyclopropenone-caged Sondheimer Diyne (Dibenzo[*a,e*]cyclooctadiyne): A Photoactivatable Linchpin for Efficient SPAAC Crosslinking

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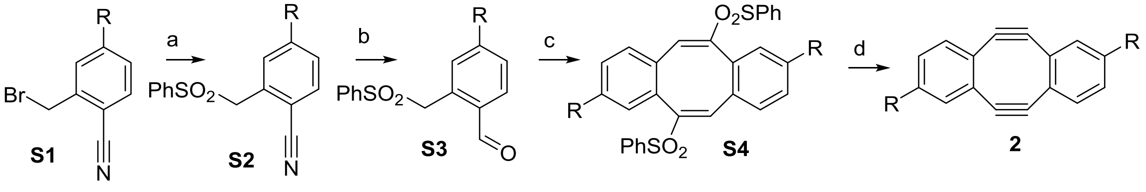
General Methods. Tetrahydrofuran was freshly distilled from sodium/ benzophenone ketyl prior to use. Dichloromethane was freshly distilled from CaH_2 prior to use. Solutions were prepared using HPLC grade water and methanol. Flash chromatography was performed using 40-63 μm silica gel. Electronic spectra were recorded using Cary 300 Bio UV-Vis spectrometer. Photolyses were conducted using a Rayonet photoreactor equipped with sixteen 4W 350 or 420 nm fluorescent lamps. A handheld lamp equipped with 2 fluorescent UV lamps (4W, 350 nm) was employed for the irradiation of 96-well plates.[*] The quantum yields of photolysis were measured against the 4-nitroveratrole actinometer.[1] All NMR spectra were recorded on a 400M Hz spectrometer in deuterochloroform and referenced to TMS unless otherwise noted. Dynamic light scattering analysis was performed using a Zetasizer Nano S90 size analyzer (Malvern Corp).

Materials: All reagents were purchased from Sigma Aldrich or VWR and used as received unless otherwise noted. Amino-binding 96-well plates were obtained from G-Biosciences. Rhodamnie B – azide,[2] PEG_{5000}-azide,[3] PEG_{5000}-propargyl ether,[4] 4-butoxy-2-methylbenzonitrile,[5] 4-methoxy-2-methylbenzonitrile,[6] 1-azido-3-iodopropane,[7] abd N-{2-[2-(2-azidoethoxy)ethoxy]-ethyl}-2-iodoacetamide[8] were prepared following the procedures reported previously.

Scheme S1. Syntheses of dibenzo[*a,e*]cyclooctadiynes



R= H (a); OBu (b); $O(CH_2CH_2O)_4TBDPS$ (e)

[a]*Reagents and conditions*: (a) DMF, 70°C, $PhSO_2Na$; (b) DIBAL, THF, -78°C; (c) $ClP(O)O_2Et_2$, LHMDS, -78°C; (d) LDA, -78°C

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[*] Regular Rayonet lamps has been employed. According to manufacturer information, emission spectrum of these fluorescent lamps has roughly Goussian shape. The 420 nm has emission maximium at 419 nm and half hight width of 38 nm, while 350 nm has λ_{max} at 350 nm and hhw of 58 nm. Eight of such lamps in a linear array produce at a 18 cm distance from the meter produce radiation dose of ca 73 W m^{-2} and 55 W m^{-2} correspondingly (www.lutzchem.com).

2-[(phenylsulfonyl)methyl]benzonitrile[9] **(S2a).** Sodium benzenesulfinate dihydrate (4.80 g, 24.0 mmol) was added to a solution of 2-(bromomethyl)benzonitrile (3.92 g, 20.0 mmol), in DMF (30mL). The mixture was stirred at 80°C for 2 h, cooled to r.t., diluted with ethyl acetate, washed with distilled water, and solvents evaporated *in vacuo*. The residue was recrystallized from EtOAc/hexanes to give 4.78g (93%) as a white solid. ^{1}H NMR: 4.57 (s, 2H,), 7.27 (m, 4H), 7.62 (m, 5H). ^{13}C NMR: 137.66, 134.27, 132.92, 132.82, 132.21, 131.70, 129.58, 129.29, 128.70, 116.56, 114.95, 60.55.

2-[(phenylsulfonyl)methyl]benzaldehyde (**S3a**).9 DIBAL (1.0 M in hexane, 102 mL, 102 mmol) was added to a solution of ortho-(phenylsulfonylmethyl)benzonitrile (**S2a**, 11.42 g, 44.4 mmol) in DCM (444 mL) at 78°C, and stirred for 2 h. A saturated aqueous solution of NH_4Cl was added to the mixture, solution diluted with DCM, washed with 1M HCl, and the solvent was evaporated *in vacuo*. The residue was filtered through a short silica gel plug and recrystallized from DCM/hexane to give 6.50g (58%) as a white solid. ^{1}H NMR: 5.03 (s, 2H,), 7.43 (m, 3H), 7.55 (m, 3H), 7.69 (m, 3H), 9.83 (s, 1H). ^{13}C NMR: 191.89, 138.33, 134.71, 134.30, 133.82, 133.75, 133.48, 129.43, 128.88, 128.81, 128.65, 57.70.

5,11-bis(phenylsulfonyl)-Dibenzo[a,e][8]annulene (**S4a**).9 A THF solution of LHMDS (38.4mL, 38.4 mmol) was added dropwise to a mixture of aldehyde **S3a** (5.00g, 19.21 mmol) and diethyl phosphorochloridate (3.98g, 23.05 mmol) in THF (291mL) at -78^{0}C and stirred for 30 min. The reaction mixture was slowly warmed to r.t., stirred for 1.5 h, quenched with a saturated aqueous solution of NH_4Cl, and diluted with EtOAc. The organic layer was washed with brine, the solvents evaporated *in vacuo*, and the residue purified via flash chromatography (DCM in Hexanes, gradient 1:5 -> 1:1) to yield 2.48g (53%) of **S4a** as a white solid. ^{1}H NMR: 7.61 (t, J=7.2Hz, 2H), 7.49-7.40 (m, 9H), 7.35 (s, 2H), 7.24 (m, 4H), 6.98-6.95 (d, J=6.6Hz, 2H). ^{13}C NMR: 144.76, 138.89, 138.86, 135.62, 133.83, 130.71, 129.33, 128.96, 128.91, 128.30, 128.08, 126.93.

5,6,11,12-tetradehydrodibenzo[a,e][8]annulene (**2a**).9 A THF solution of n-BuLi (23.3 mL, 53.7 mmol) was added dropwise to a solution of diisopropylamine (7.67 mL, 54.7 mmol) in THF (200 mL) at -78°C and stirred for 1 h. A solution of the **S4a** (2.60 g, 5.37 mmol) in THF (15 mL) was added to the reaction mixture, stirred for 2 h, and quenched with a saturated aqueous solution of NH_4Cl. The mixture was diluted with DCM, washed with distilled water and brine, dried over $MgSO_4$, and the solvents evaporated *in vacuo*. The crude product was triturated with DCM to give 1.01g (94%) of **2a** as a bright yellow solid. ^{1}H NMR: 6.92 (m, 4H), 6.74 (m, 4H). ^{13}C NMR: 132.90, 129.02, 126.88, 109.31.

1,1,6,6-Tetrafluorodibenzo[a,e]dicyclopropa[c,g][8]annulene (**4a**). A solution of diyne **2a** (0.207 g, 1.03 mmol), $TMSCF_3$ (0.607 mL, 4.14 mmol), NaI (0.682 g, 4.55 mmol) in THF (5 mL) was heated in a pressure vessel for 2 h at at 110^{0}C. The reaction was quenched by adding saturated aqueous sodium bicarbonate solution (20 mL), diluted with DCM (30 mL) and EtOAc (100 mL), washed with with deionized water and brine. The organic phase was dried over anhydrous potassium carbonate, partially concentrated, and cooled to -40°C. The resulting crystals were separated and washed with EtOAc to afford 0.186g (59%) of yellow solid, m.p. = 183 - 185^{0}C. ^{1}H NMR: 6.75 (dd, J=8.8, 3.3Hz, 4H), 6.44 (dd, J=8.8, 3.3Hz, 4H. ^{19}F NMR: -116.82. ^{13}C NMR: 132.65, 132.05, 125.37, 125.13 (t, J=12.1Hz), 100.05 (t, J=27.5Hz). **4a** used in the next step without further purification.

Dibenzo[*a*,*e*]dicyclopropa[*c*,*g*][8]annulene-1,6-dione (**1a**). The crude dicyclopropene **4a** (0.155g, 0.516mmol) was loaded onto a silica gel column (1:1 DCM/hexane + 0.5% H_2O), spread by recycling the eluent, and allowed to react overnight. The remaining starting material was flushed with DCM, and then the bis-cyclopropenone was eluted with 10%MeOH/DCM to give 0.107g (81%) as a bright yellow solid. M.p. (dec)= 259^{0}C. ^{1}H NMR: 7.82 (dd, J = 5.6, 3.3 H-z, 4H), 7.54 (dd, J = 5.6, 3.3 Hz, 4H). ^{13}C NMR: 152.99, 148.86, 135.76, 134.60, 123.25. ESI HRMS cald. ($M+H^+$): $C_{18}H_9O_2$ 257.0297, found: 257.0603; IR (cm^{-1}): 3040, 2960, 1854, 1619, 1430, 1322, 1258, 1017, 801, 766.

1,8-dibutyldibenzo[3,4:7,8]cycloocta[1,2-*d*:5,6-*d*]bis([1,2,3])triazole) (**5**) and **1,10-dibutyldibenzo[3,4:7,8]cycloocta[1,2-*d*:5,6-*d*]bis([1,2,3])triazole)** (**6**). A solution of dicyclopropenone **1a** (120 mg, 0.468 mmol) in DCM/MeOH (1:10, 100 mL) and butyl azide (93 mg, 0.937 mmol) was irradiated at 350 nm. After 15 min irradiation, the bis-cyclopropenone peak at 360 nm had disappeared. The solution was stirred overnight and separated via column chromatography (DCM - MeOH gradient, 1:0 -> 200:1) to give

81 mg (43%) of the head-to-tail (**5**) and 64 mg (34%) of the head-to-head adducts, both as yellow solids.

Bis-triazole **5**, m.p.= 175 - 179^{0}C; ^{1}H NMR: 7.68 (d, J=7.6Hz, 2H), 7.45 (m, 4H), 7.20 (d, J=7.5Hz, 2H), 4.20 (m, 4H), 1.58 (m, 4H), .98 (m, 4H), .67 (t, J=7.4Hz, 6H). ^{13}C NMR: 144.92, 134.49, 132.74, 131.26, 130.00, 129.39, 128.82, 126.80, 48.32, 31.74, 19.30, 13.14.

Bis-triazole **6**, m.p.= 189 - 190^{0}C. ^{1}H NMR: 7.62 (dd, J=9.2, 2.3Hz, 2H), 7.53 (dd, J=9.2, 2.3Hz, 2H), 7.42 (dd, J=9.2, 2.3Hz, 2H), 7.32 (dd, J=9.2, 2.3Hz, 2H), 4.25 (m, 4H), 1.77 (m, 4H), 1.21 (m, 4H), .82 (t, J=7.4Hz, 6H). ^{13}C NMR: 145.96, 133.27, 130.98, 130.66, 130.37, 128.94, 128.46, 48.21, 32.18, 19.75, 13.41. ESI HRMS cald. (M+H$^+$): $C_{24}H_{27}N_6$ 399.2292, found: 399.2288.

2-(Bromomethyl)-4-butoxybenzonitrile (**S1b**). A mixture of 4-butoxy-2-methylbenzonitrile (20.5 g, 108 mmol), NBS (28.9 g, 163 mmol), and AIBN (1.78 g, 10.8 mmol) in cyclohexane (157 mL) was refluxed for 3 h under inert atmosphere, cooled to r.t., filtered, and washed with water and brine. The reaction mixture was concentrated under vacuum and purified by flash chromatography (Hexanes to 5% EtOAc in hexanes) to give 16.8 g (57%) of **S1b** as a yellow oil. ^{1}H NMR: 7.53 (d, J=8.6Hz, 1H,), 6.98 (d, J=2.4Hz, 1H), 6.85 (dd, J=8.6, 2.4Hz, 1H) 4.54 (s, 2H), 3.98 (t, J=6.5Hz, 2H), 1.75 (m, 2H), 1.46 (m, 2H), 0.94 (t, J=7.4Hz, 3H). ^{13}C NMR: 162.58, 142.93, 134.77, 116.46, 115.05, 103.53, 68.30, 30.84, 29.60, 19.10, 13.76.

4-Butoxy-2-((phenylsulfonyl)methyl)benzonitrile (**S2b**). A mixture of 2-(bromomethyl)-4-butoxybenzonitrile (**S1b**, 4.33 g, 16.15 mmol) and sodium benzenesulfinate (3.18 g, 19.4 mmol) in DMF (25 mL) was stirred for 3 h under inert atmosphere at 80°C. The crude mixture was cooled to r.t., diluted with ether, and washed with water and brine. The mixture was concentrated under vacuum and purified by flash chromatography (25% EtOAc-hexanes) to yield 4.23g (80%) of **S2b** as a white solid. ^{1}H NMR: 7.72 (m, 2H), 7.64 (t, 1H), 7.48 (m, 2H), 7.42 (d, J=8.7Hz, 1H), 7.06 (d, J=2.4Hz, 1H), 6.90 (dd, J=8.7, 2.4Hz, 1H), 4.49 (s, 2H), 3.99 (t, J=6.5Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), .97 (t, J=7.5Hz, 3H). ^{13}C NMR: 162.29, 137.59, 134.22, 133.51, 129.22, 128.67, 117.75, 117.03, 115.96, 105.51, 68.39, 60.52, 30.89, 19.08, 13.75.

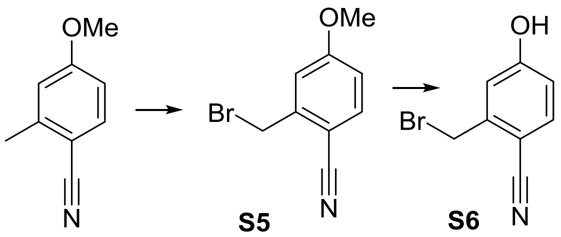
4-Butoxy-2-((phenylsulfonyl)methyl)benzaldehyde (**S3b**). A DIBAL solution (1.0 M in Hexanes, 25.7 mL, 25.7 mmol) was added dropwise to a solution of 4-butoxy-2-((phenylsulfonyl)methyl)benzonitrile (**S2b**, 5.65 g, 17.15 mmol) in DCM (170mL) at -78°C under inert atmosphere. The mixture was stirred for 2 h and quickly quenched with a saturated aqueous solution of NH_4Cl, diluted with DCM, and washed with 1M HCl followed by brine. The mixture was concentrated under vacuum and purified by flash chromatography (25% EtOAc in hexanes) to produce 4.36g (76%) of **S3b** as a white solid, m.p.= 99 - 102^{0}C. ^{1}H NMR: 9.59 (s, 1H), 7.64 (d, J=8.1Hz, 2H,), 7.56 (m, 2H), 7.39 (t, J=7.8Hz, 2H), 6.93 (dd, J=8.5, 2.4Hz, 1H), 6.88 (s, 1H), 4.98 (s, 2H), 3.96 (t, J=6.5Hz, 2H), 1.73 (m, 2H), 1.45 (m, 2H), .94 (t, J=7.4Hz, 3H).^{13}C NMR: 190.61, 162.87, 138.26, 137.19, 133.74, 131.10, 128.75, 128.63, 127.61, 119.76, 114.77, 68.26, 57.55, 30.99, 19.08, 13.78. ESI HRMS cald. (M+H)+: $C_{18}H_{21}O_4S$ 333.1155, found: 333.1161

(5E,11E)-2,8-Dibutoxy-6,12-bis(phenylsulfonyl)dibenzo[*a,e*][8]annulene (**S4b**). A solution of LHMDS (29.2 mL, 29.2 mmol) was added dropwise to a solution of 4-butoxy-2-((phenylsulfonyl)methyl)benzaldehyde (**S3b**, 4.86 g, 14.6 mmol) and diethyl chlorophosphate (3.03g, 17.5mmol) in THF (443 mL) at -78°C under inert atmosphere. The reaction mixture solution was stirred for 30 min, warmed to r.t., and stirred for 2 h. The mixture was diluted with ethyl acetate and washed with 1 M HCl, followed by water, then brine. The mixture was concentrated under vacuum and purified by flash chromatography (30% EtOAc in Hexanes). The crude product was recrystallized from hexanes – EtOAc to give 1.55 g (34%) of **S4b** as a white solid, m.p.= 192 - 195^{0}C. ^{1}H NMR: 7.59 (m, 2H,), 7.42 (m, 8H), 7.31 (s, 2H), 6.95-6.75 (m, 6H), 3.84 (t, J=6.5Hz, 4H), 1.68 (m, 4H), 1.40 (m, 4H), .91 (t, J=7.4Hz, 6H). ^{13}C NMR: 162.97, 148.01, 143.43, 143.25, 137.80, 134.57, 133.02, 132.36, 132.15, 131.60, 120.67, 119.82, 71.92, 35.19, 23.24, 17.94. ESI HRMS calcd. (M+H)+: $C_{36}H_{37}O_6S_2$ 629.2026, found: 629.2029.

2,8-Dibutoxy-5,6,11,12-tetradehydrodibenzo[a,e][8]annulene (**2b**). A solution of n-BuLi (12.9 mL, 29.7 mmol) was added dropwise to a solution of diisopropylamine (4.30 mL, 30.2 mmol) in THF (100 mL) at -78°C. The reaction mixture was stirred for 1 h and a solution of **S4b** (1.86 g, 2.96 mmol) in THF (20 mL) was added. The mixture was stirred for 2 h and quenched with a saturated aqueous solution of NH_4Cl. The reaction mixture was diluted with EtOAc, washed with distilled water and brine, dried over $MgSO_4$, and evaporated in vacuo. The crude product was purified by flash chromatography (2:3 DCM/hexanes) to **y**ield 530 mg (52%) of **2b** as a yellow solid, m.p. (decmp)= 134^0C. ^{1}H NMR: 6.63 (d, J=8.4Hz, 2H), 6.37 (dd, J=8.4, 2.6Hz, 2H), 6.32 (d, J=2.6Hz, 2H), 3.84 (t, J=6.5Hz, 4H), 1.70 (m, 4H), 1.42 (m, 4H), 0.94 (t, J=7.4Hz, 6H). ^{13}C NMR: 159.82, 134.82, 127.77, 123.54, 114.92, 112.48, 110.13, 107.29, 67.81, 31.05, 19.11, 13.76. HRMS calcd. ($C_{24}H_{24}O_2$) 344.1776, found: 344.1774.

3,8-Dibutoxydibenzo[*a,e*]dicyclopropa[*c,g*][8]annulene-1,6-dione (**1b**). A solution of **2b**, (0.512 g, 1.49 mmol), $TMSCF_3$ (0.881 mL, 5.95 mmol), NaI (0.936g, 6.24 mmol), and THF (5 mL) was heated at 110°C for 2 h in a pressure vessel. The reaction was quenched by adding saturated sodium bicarbonate solution (20 mL), diluted with DCM (30 mL) and EtOAc (100 mL), and washed with deionized water and brine. The organic phase was dried over anhydrous K_2CO_3, partially concentrated, and purified by flash chromatography (DCM-hexanes). The reaction yielded 400 mg (60%) of 3,8-dibutoxy-1,1’6,6’-tetrafluorodibenzo[a,e]dicyclopropa[c,g][8]annulene (**4b**) as yellow solid. ^{1}H NMR: 7.20 (d, J=8.4Hz, 2H), 6.81 (d, J=2.5Hz, 2H), 6.69 (dd, J=8.4, 2.5Hz, 2H), 3.97 (t, J=6.4Hz, 4H), 1.76 (m, 4H), 1.47 (m, 4H), .97 (t, J=7.4Hz, 6H). ^{18}F NMR: -116.48. ^{13}C NMR: 162.56, 133.78, 127.48, 125.68 (t, J=11.2Hz), 121.30 (t, J=11.7Hz), 118.70, 117.06, 116.32, 100.33, 68.27, 30.98, 19.09, 13.75.

Crude **4b** (661 mg, 1.48 mmol) was loaded onto a silica gel column pre-packed with “wet” hexanes (1% of water), spread throughout the column, and incubated for 72 h. After 72 h, the unreacted starting material and mono-adduct were eluted with 20% EtOAc in hexanes and polarity was increased to 5% MeOH/DCM to elute the target compound producing 433 mg (73%) of **1b** as a bright yellow solid, m.p. (decmp) = 201^0C. ^{1}H NMR: 7.70 (d, J=8.5Hz, 2H), 7.26 (m, 2H), 6.90 (dd, J=8.5, 2.5Hz, 2H), 4.04 (t, J=6.5Hz, 4H), 1.70 (m, 4H), 1.42 (m, 4H), .98 (t, J=7.4Hz, 6H). ^{13}C NMR 164.12, 152.87, 149.39, 143.73, 137.79, 125.45, 121.49, 118.82, 115.50, 68.79, 30.88, 19.02, 13.69. HRMS cald. (M+H)+: $C_{26}H_{25}O_4$ 401.1747, found: 401.1738. IR (cm^{-1}): 2953, 2871, 1838, 1631, 1585, 1557, 1326, 1233, 1093, 1065, 1020, 973, 884, 682.



2-(Bromomethyl)-4-methoxybenzontrile (**S5**). AIBN (1.22 g, 7.41 mmol) was added to a suspension of 4-methoxy-2-methylbenzonitrile (5.45 g, 37 mmol) and NBS (9.89 g, 55.5 mmol) in carbon tetrachloride (185 mL) and the mixture was refluxed fo 3 h. The reaction was quenched with aqueous sodium thiosulfate solution, washed with water, and the solvents evaporated in vacuo. The crude solid was purified by flash chromatography (20% EtOAc in hexanes) to give 5.3 g (63%) **S5** as a clear oil. ^{1}H NMR: 7.59 (d, J=8.6Hz, 1H), 7.03 (d, J=2.3Hz, 1H), 6.91 (dd, J=8.6, 2.3Hz, 1H), 4.58 (s, 2H), 3.87 (s, 3H). ^{13}C NMR: 162.99, 143.06, 134.88, 117.20, 116.02, 114.71, 103.91, 55.79, 29.49.

2-(Bromomethyl)-4-hydroxybenzonitrile (**S6**)**.** Boron tribromide (6.05 g, 250 mmol) was added to a solution of the **S5** (5.45 g, 22.1 mmol) in DCM (120 mL) at -10°C. After 30 min the reaction mixture was slowly warmed to r.t., stirred for 4 days, quenched with water, and washed with saturated aqueous sodium bicarbonate solution. The solvents were evaporated and the residue purified through a short silica gel plug to give 4.32 g (82%) of **S6** as a white solid. ^{1}H NMR: 10.78 (s, 1H), 7.64 (d, J=8.5Hz, 1H), 7.01 (d, J=2.2Hz, 2H), 6.84 (dd, J=8.5, 2.3Hz, 1H), 4.66 (s, 2H). ^{13}C NMR: 162.05, 143.62, 135.77, 118.00, 116.80, 101.88, 31.31. ESI HRMS cald. (M-H^{-}): C_8H_5BrNO 209.9560, found: 209.9565.

2-(Bromomethyl)-4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)benzonitrile (**S1e**). DIAD (4.12 g, 20.4 mmol) was added to a solution of 2-(bromomethyl)-4-hydroxybenzonitrile (**S6**, 4.32 g, 20.4 mmol), triphenylphosphine (5.88 g, 22.4 mmol), and 2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-ol (8.81 g, 20.4 mmol) in THF (204 mL) at 0°C. The reaction mixture was warmed to r.t. and stirred for 12 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (20% EtOAc in hexanes) to give 10.3 g (81%) of **S1e** as a clear oil. ^{1}H NMR: 7.67 (m, 4H), 7.56 (d, J=8.6Hz, 1H), 7.39 (m, 6H), 7.04 (d, J=2.3Hz, 1H), 6.89 (dd, J=8.6, 2.4Hz, 1H), 4.56 (s, 2H), 4.15 (t, J=4.7Hz, 2H), 3.85 (t, J=4.7Hz, 2H), 3.80 (t, J=5.3Hz, 2H), 3.64 (m 10H), 1.04 (s, 9H). ^{13}C NMR: 162.24, 143.02, 135.58, 134.80, 133.65, 129.60, 127.62, 117.15, 116.64, 115.19, 104.05, 72.43, 70.92, 70.76, 70.73, 70.68, 69.33, 67.96, 63.42, 26.81, 19.19. ESI HRMS cald. (M+Na)+: $C_{32}H_{40}BrNNaO_5Si$ 648.1751, found: 648.1754.

4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)-2-(2-(phenylsulfonyl)ethyl)benzonitrile (**S2e**). A solution of 2-(bromomethyl)-4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)benzonitrile (**S1c**, 10.3g, 16.6 mmol) and sodium benzenesulfinate (3.24 g, 20.0 mmol) in DMF (110 mL) was heated at 80°C and for 2 h. The solution was cooled to r.t., diluted in ether, and washed with water and brine. Solvent was evaporated in vacuum and the residue purified by flash chromatography (30%EtOAc in hexanes) to give 6.04 g (53%) of **S2e** as a clear oil. ^{1}H NMR: 7.74-7.68 (m, 7H), 7.50 (t, J=7.8Hz, 2H), 7.38 (m, 7H), 7.14 (s, 1H), 6.95 (dd, J=8.6, 2.4Hz, 1H), 4.51 (s, 2H), 4.17 (t, J=4.7Hz, 2H), 3.87 (t, J=4.7Hz, 2H), 3.82 (t, J=5.3Hz, 2H), 3.72-3.62 (m, 10H), 1.05 (s, 9H). ^{13}C NMR: 161.97, 137.52, 135.59, 134.28, 134.21, 133.63, 133..59, 129.62, 129.26, 128.71, 127.63, 117.86, 116.91, 116.13, 105.94, 96.11, 72.44, 70.93, 70.76, 70.73, 70.67, 69.28, 68.06, 63.43, 60.48, 26.82, 19.20. ESI HRMS cald. (M-H)-: $C_{38}H_{44}NO_7SSi$ 686.2613, found: 686.2594.

4-((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)-2-(2-(phenylsulfonyl)ethyl)benzaldehyde (**S3e**). A DIBAL solution (20.0 mL, 20.0 mmol) was added dropwise to solution of **S2e** (5.00 g, 7.27 mmol) in DCM (22 mL) at -78°C under inert atmosphere. The mixture was stirred for 2 h and quickly quenched with a saturated aqueous ammonium chloride solution. The crude mixture was diluted with DCM, and washed with 1M HCl and brine. The solvents were removed in vacuum and the residue purified by flash chromatography (25% EtOAc in hexanes) to give 3.71 g (74%) **S3e** as a yellow oil.^{1}H NMR: 9.64 (s, 1H), 7.74-7.68(m, 6H), 7.59 (t, 2H), 7.49-7.38 (m, 8H), 7.01 (m, 2H), 5.03 (s, 2H), 4.20 (t, J=4.7Hz, 2H), 3.87 (t, J=4.6Hz, 2H), 3.82 (t, J=5.3Hz, 2H), 3.72-3.62 (m, 10H), 1.05 (s, 9H). ^{13}C NMR: 190.63, 162.52, 138.29, 137.24, 135.59, 133.75, 133.65, 131.18, 129.60, 128.77, 128.71, 127.93, 127.62, 119.87, 114.98, 72.43, 70.93, 70.76, 70.67, 69.32, 67.96, 63.42, 57.52, 26.81, 19.19. ESI HRMS cald. (M-H)-: $C_{38}H_{45}O_8SSi$ 689.2610, found: 689.2588

(((5E,11E)-6,12-Bis(phenylsulfonyl)dibenzo[a,e][8]annulene-1,8-diyl)bis(oxy)bis-(2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecane) (**S4e**). A solution of LiHMDS (1.0M in THF, 10.74 mL, 10.74 mmol) was added dropwise to a solution of **S3e** (3.71 g, 5.37 mmol) and ClP(O)(OEt)2 (0.93 mL, 6.44 mmol) in THF (160 mL) at -78°C. The reaction mixture was stirred at -78°C for 30 min, then at r.t. for 2 h, and quenched with a saturated aqueous solution of NH_4Cl. The mixture was diluted with ethyl acetate, washed with water and brine, and then evaporated *in vacuo*. The residue was purified by chromatography (AcOEt-hexanes 1:1) to give 1.3g (36%) of **S4e** as a clear oil. ^{1}H NMR: 7.68 (m, 8H), 7.62 (m, 2H), 7.5-7.33 (m, 20H), 7.31 (s, 2H), 7.00 (m, 2H), 6.84 (m, 4H), 4.02 (m, 4H), 3.80 (m, 8H), 3.63 (m, 22H), 1.04 (s, 18H). ^{13}C NMR: 158.53, 143.99, 139.19, 139.11, 135.58, 133.69, 130.47, 129.52, 128.51, 128.21, 128.06, 127.87, 127.60, 116.74, 115.81, 72.42, 70.82, 70.74, 70.69, 70.63, 69.43, 67.56, 63.42, 26.81, 19.18. . ESI HRMS cald. (M+H)+: $C_{76}H_{88}O_{14}S_2Si_2$ 1345.5232, found: 1345.5237.

2,8-bis((2,2-dimethyl-3,3-diphenyl-4,7,10,13-tetraoxa-3-silapentadecan-15-yl)oxy)-5,6,11,12-tetradehydrodibenzo[a,e][8]annulene (**2e**). A solution of n-BuLi (8.88 mL, 20.4 mmol) was dropwise added to a solution diisopropylamine (2.97 mL, 20.8 mmol) in THF (82mL) at -78°C. The reaction mixture was stirred for 1 h and a solution of **S4e** (2.04g, 1.52 mmol) in THF (10ml) was added. The mixture was stirred for 2 h and quenched with a saturated aqueous solution of NH_4Cl. The mixture was diluted with DCM, washed with distilled water and brine, dried over $MgSO_4$, and the solvents were evaporated *in vacuo*. The crude product was purified by flash chromatography (1:1 hexanes - EtOAc) to give 1.22 g (56%) of the diyne **2e** as a bright yellow solid. ^{1}H NMR: 7.69 (m, 10H), 7.37 (m, 10H), 6.62 (d, J=8.4Hz, 2H), 6.39 (dd, J=8.4, 2.6Hz, 2H), 6.35 (d, J=2.6Hz, 2H) 4.01 (t, J=4.8Hz, 4H), 3.81 (m, 8H), 3.67 (m, 20H), 1.05 (s, 18H). ^{13}C NMR: 159.47, 135.58, 134.84, 133.70, 129.58, 127.74, 127.60, 124.05, 115.07, 112.70, 110.12, 107.37, 72.43, 70.85, 70.75, 70.71, 70.65, 69.45, 67.54, 63.43, 26.82, 19.19. ESI HRMS cald. (M+H)+: $C_{64}H_{77}O_{10}Si_2$ 1061.5055, found: 1061.5057.

(((((((5,6,11,12-Tetradehydrodibenzo[*a,e*][8]annulene-2,8-diyl)bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy)) diethanol (**2c**)**.** A solution of TBAF (2.87 mL, 2.87 mmol) was dropwise added to a solution of **2e** (1.22 g, 1.15 mmol) in THF (12 mL) at r.t. and stirred for 45 min, and quenched with saturated aqueous ammonium chloride solution. The organic layer was washed with brine, dried over $MgSO_4$, and the solvent evaporated *in vacuo*. The crude product was purified by flash chromatography (DCM - MeOH, gradient from 0.5% to 5%) to give 400 mg (60%) of **2c** as a bright yellow solid. ^{1}H NMR: 6.81 (d, J=8.4Hz, 2H), 6.59 (dd, J=8.4, 2.6Hz, 2H), 6.52 (d, J=2.6Hz, 2H), 4.56 (s, 2H), 4.04 (m, 4H), 3.69 (m, 4H), 3.51 (m, 20H), 3.41 (m, 4H). ^{13}C NMR: 159.47, 133.89, 128.18, 122.59, 114.98, 113.33, 110.01, 107.22, 72.31, 69.87, 69.80, 69.75, 69.73, 68.65, 67.48, 60.19. ESI HRMS cald. (M+H)+: $C_{32}H_{41}O_{10}$ 585.2655, found: 585.2696.

(((((((5,6,11,12-Tetradehydro-dibenzo[a,e][8]annulene-2,8-diylbis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis (ethane-2,1-diyl) diacetate (**2d**)**.** Acetic anhydride (0.52 mL, 5.5 mmol) and DMAP (17 mg, 0.137 mmol) were added to a solution of triethylamine (2.5 mL) and **2c** (400 mg, 0.68 mmol) at r.t., stirred for 3 h, diluted with DCM (200 mL), washed with distilled water and brine, the organic layer dried over $NaSO_4$, and the solvents evaporated *in vacuo*. The crude product was purified by flash chromatography (1:1 hexanes-EtOAc) to give 360 mg (80%) of **2d** as a bright yellow solid. ^{1}H NMR: 6.67 (d, J=8.4Hz, 2H), 6.42 (dd, J=8.4, 2.6Hz, 2H), 6.37 (d, J=2.6Hz, 2H), 4.23 (t, J=4.8Hz, 4H), 4.04 (t, J=4.7Hz, 4H), 3.80 (m, 4H), 3.69 (m, 20H), 2.08 (s, 3H). ^{13}C NMR: 170.99, 159.47, 134.84, 127.75, 124.06, 115.08, 112.71, 110.11, 107.36, 70.85, 70.64, 70.61, 70.57, 69.48, 69.13, 67.55, 63.57, 20.94. ESI HRMS cald. (M+H)+: $C_{36}H_{45}O_{12}$ 669.2911, found: 669.2902.

(((((((1,1,6,6-Tetrafluoro-dibenzo[*a,e*]dicyclopropa[c,g][8]annulene-3,8-diyl)bis (oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl))bis(oxy))bis(ethane-2,1-diyl) diacetate (**4d**). A solution of **2d** (360 mg, 0.538 mmol), $TMSCF_3$ (0.207 mL, 1.40 mmol), and NaI (0.218 g, 1.45 mmol) in THF (8 mL) were heated at 110°C for 2 h in a pressure vessel. The reaction was quenched by a saturated sodium bicarbonate solution (20 mL), diluted with DCM (200 mL), washed with deionized water and brine. The organic phase was dried over anhydrous K_2CO_3, concentrated, and purified on a silica gel column (50% EtOAc in hexanes with 1% of triethylamine) to give 93 mg (23%) of **4d** as a yellow solid. ^{1}H NMR: 7.26 (d, J=8.5Hz, 2H), 6.88 (d, J=2.4Hz, 2H), 6.77 (dd, J=8.4,2.6Hz, 2H), 4.23 (t, J=4.8Hz, 4H), 4.17 (t, J=4.7Hz, 4H), 3.87 (t, J=4.6Hz, 4H), 3.67 (m, 20H), 2.08 (s, 3H).: ^{19}F-NMR: -116.57 ^{13}C-NMR: 171.01, 162.21, 133.78, 127.46, 121.66, 118.91, 117.49, 116.55, 70.93, 70.65, 70.62, 70.51, 69.13, 69.40, 67.98, 63.56, 20.94. ESI HRMS cald. (M+Na)+: $C_{38}H_{44}F_4NaO_{12}$ 791.2666, found: 791.2660.

3,8-bis(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)dibenzo[*a,e*]dicyclopropa [*c,g*][8]annulene-1,6-dione (**1c**). A solution of **4d** (93 mg, 0.121 mmol) and potassium carbonate (42 mg, 0.302mmol) in 2:1 MeOH:DCM (3ml) was stirred at r.t. for 1 h. The reaction mixture was filtered and evaporated *in vacuo*. The crude mixture was hydrolyzed immediately during chromatography (1%MeOH/DCM) to give 41 mg (53%) of the bis-cyclopropenone **1c** as a yellow solid, m.p. (decmp) = 137-139°C. ^{1}H NMR: 7.65 (d, J=8.2Hz, 2H), 7.17 (m, 4H), 4.56 (t, J=5.5Hz, 2H), 4.27 (m, 4H), 3.76 (m, 4H), 3.59-3.39 (m, 24H). ^{13}C NMR: 163.83, 152.95, 149.18, 143.80, 137.86, 125.35, 121.78, 119.15, 115.78, 72.55, 70.68, 70.56, 70.27, 69.27, 68.44, 61.61. ESI HRMS cald. (M+H)+: $C_{34}H_{41}O_{12}$ 641.2598, found: 641.2591. IR (cm^{-1}): 3348, 2876, 1861, 1827, 1604, 1585, 1346, 1258, 1106, 1068, 956, 848.

Azido-BSA.[10] Derivatization of BSA with 1-azido-3-iodopropane in PBS (pH = 7.4) did not produce significant incorporation of azido groups into protein structure. Increasing the pH resulted in the incorporation of several azide units. Thus, a solution of 1-azido-3-iodopropane (4.2 mg, 19 μmol) in acetonitrile (1 mL) was added to a solution of BSA (131 mg, 2 μmol) in Tris buffer (6 mL, pH 8.1) and stirred for 12 h. The resulting protein was purified by spin filtration (MWCO=10,000 amu) and lyophilized to give 100 mg (76%) of azido-BSA as a white powder. Conjugation of the azido-BSA produced by this method with PEG_{5000} using both SPAAC and CuAAC techniques (*vide infra*) indicate that it contained a mixture of unmodified BSA (60%) and BSA molecules derivatized with one (24%), two (10%), three (4%), and four (2%) azide groups. Using 100 equivalents of 1-azido-3-iodopropane at pH= 8.1 produces a mixture of BSA derivatives with a higher fraction of multi-labelled molecules (native – 19%, one azide – 26%, two –

23%, three - 17%, four – 10%, five – 5%). BSA can be functionalized at pH=7.4 using stronger electrophile, N-{2-[2-(2-azidoethoxy)ethoxy]-ethyl}-2-iodoacetamide, but this reaction also resulted in incorporation of multiple azide units per protein molecule.

Methods

The nanocrystalline suspensions of photo-DIBOD 1a were prepared employing reprecipitation technique.[11] Photo-DIBOD **1a** (1 mg, 3.9 μmol) was taken up in 0.5 mL DCM and diluted in MeOH (4 mL). The DCM was evaporated by heating in a sonicator at 40°C for 30 min. The nanocrystalline suspension was prepared by by slow addition of the methanol solution into 10 mL of PBS buffer (7 mM, pH 7.4) containing 15 mM of SDS under sonication. Dynamic light scattering analysis of three independently prepared samples produced mean values of 509 nm, 753 nm, 453 nm.

Photo-DIBOD **1a** nanocrystalline suspensions for BSA labeling experiments were prepared without the use of SDS by the direct addition of **1a** in 2 mL of methanol to PBS solution of protein under sonication.

Stability of Sondheimer diyne in aqueous buffer. A suspension of the diyne **2a** (0.96 mM) in a 20% MeOH/PBS (pH 7.4) containing 15 mM of SDS was prepared by re-pricipitation technique. Decomposition was followed by disappearance of absorbance of **2a** at 350 nm. The half lifetime of decomposition was 10 ± 2 minutes.

Photo-conjugation azido-BSA with Rhodamine B – azide. The mixture of the nanocrystalline suspension of **1a** (1 mg, 3.90 μmol), azido-BSA (25.8 mg, 0.390 μmol), and Rhodamine B - azide (2.06 mg, 3.90 μmol) in PBS (10 mL) was irradiated for 8 min at 350 nm and stirred overnight in the dark. The resulting protein was concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. The BSA-Rhodamine conjugate, as well as controls, were resolved on 12% SDS-PAGE gel and visualized through in-gel fluorescence using a GE Typhoon scanner with excitation wavelength fixed at 532 nm and emission wavelength fixed at 580 nm.

Photo-conjugation azido-BSA with biotin-azide. The mixture of the nanocrystalline suspension of **1a** (1 mg, 3.90 μmol), azido-BSA (25.8 mg, 0.390 μmol), and biotin azide (1.74mg, 3.90 μmol) in PBS (6 mL) was irradiated for 5 min at 350 nm, and incubated overnight. The mixture was spin filtered (MWCO=10,000amu), purified on a PD-10 column, and lyophilized. Experimental and control samples were dissolved in Dulbecco's Phosphate Buffered saline (DPBS) and the concentration of BSA in each sample was determined by bicinchoninic acid assay (BCA). Each 2 μg (or 6 μg) of the samples were loaded and separated on 8% SDS-PAGE gel and then transferred to nitrocellulose membrane. Immunoblotting was done by anti-biotin antibody conjugated with Horseradish peroxidase (HRP) (1:100,000, Jackson Immunology Lab). For the loading control, the nitrocellulose membrane was stained by 0.5% Ponceau S in 1 % acetic acid.

Conjugation of azido-BSA and PEG$_{5000}$-azide using photo-DIBOD. The mixture of the nanocrystalline suspension of **1a** (2 mg, 7.8 μmol), azido-BSA (25.8 mg, 0.390 μmol), and PEG$_{5000}$ azide (19.51 mg, 3.90 μmol) in PBS (10 mL) was irradiated for 8 min at 350 nm, then stirred overnight. The resulting protein solution was concentrated by spin filtration (MWCO 10,000), purified on a PD-10 Sephadex column, and lyophilized. The MALDI-TOF analysis of the BSA-PEG$_{5000}$ conjugate indicated that it contained a mixture of unmodified BSA (60%) and BSA molecules derivatized with one (24%), two (10%), three (4%), and four (2%) PEG$_{5000}$ fragments (Figure S1).

Stirring the mixture containing the nanocrystalline suspension of **1a**, azido-BSA, and PEG$_{5000}$-azide in PBS overnight, as well as incubation of azido-BSA with PEG$_{5000}$-azide, didn't produce any BSA-PEG$_{5000}$ conjugate (Fig. S2).

Copper-catalyzed click (CuAAC) conjugation of azido-BSA and propargyl-PEG$_{5000}$. A solution azido-BSA (13.2 mg, 0.20 μmol), propargyl-PEG$_{5000}$ (10 mg, 2.0 μmol), ascorbic acid (7.0 μg, 0.040 μmol) and $CuSO_4$ (3.2 μg, 0.020 μmol) in PBS (7 mM, 6 mL) was stirred for 12 h, concentrated to 1.5 mL by spin filtration (MWCO 10,000), purified over a PD-10 column, and lyophilized. The MALDI-TOF analysis of the protein shows that it contains a mixture of unmodified BSA and BSA molecules derivatized with one, two, three, and four PEG$_{5000}$ fragments (Fig. S3). The ratio of these proteins (60 : 23 : 11 : 4 : 2) was almost identical to photo-DIBOD pegylation experiment.

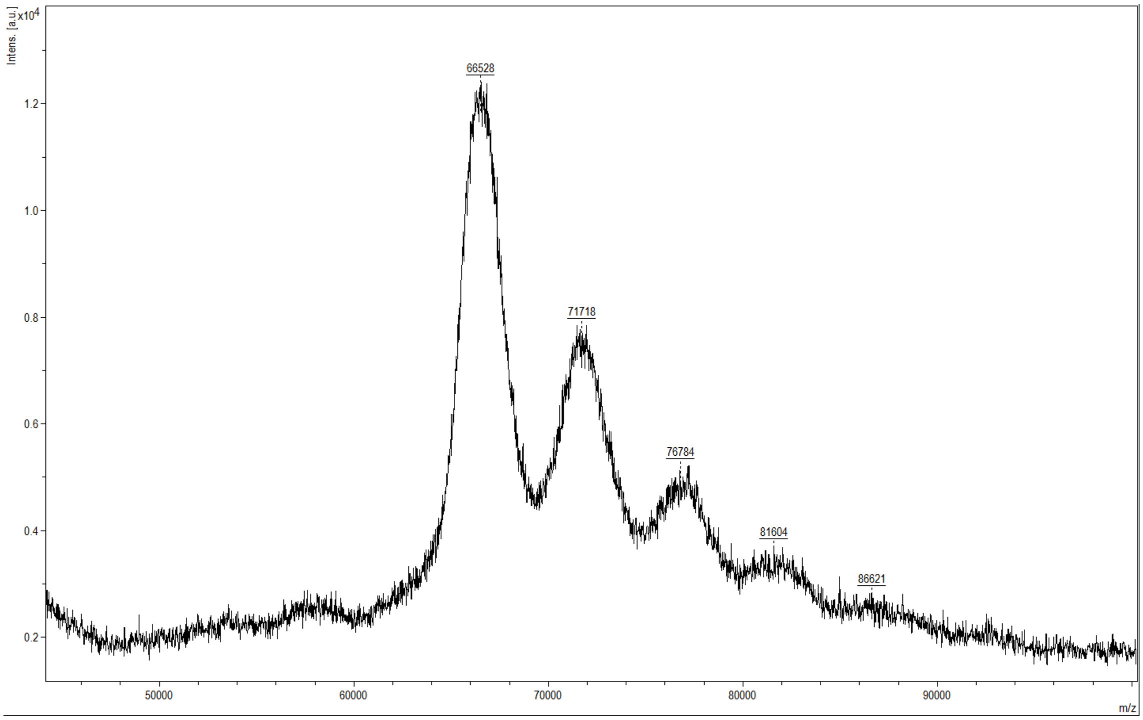


Figure S1. Photo-derivatization of azido-BSA-azide with PEG$_{5000}$-azide using **1a**.

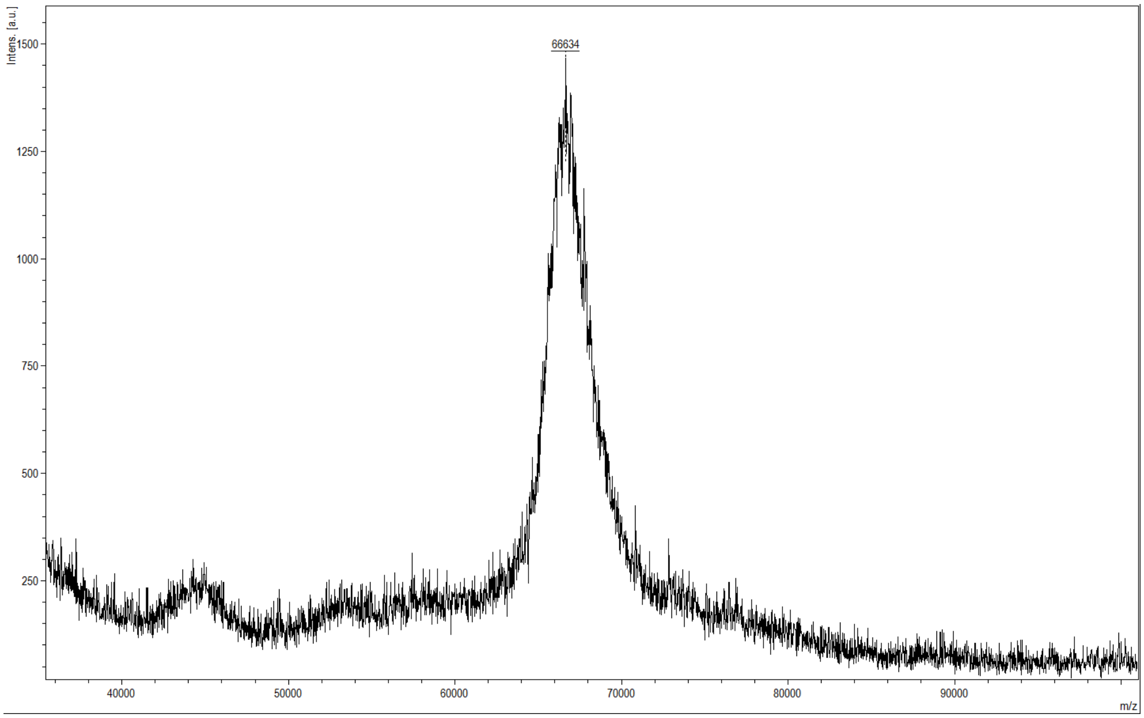


Figure S2. Control experiment: photoDIBOD, azido-BSA, and PEG$_{5000}$-azide incubated in the dark.

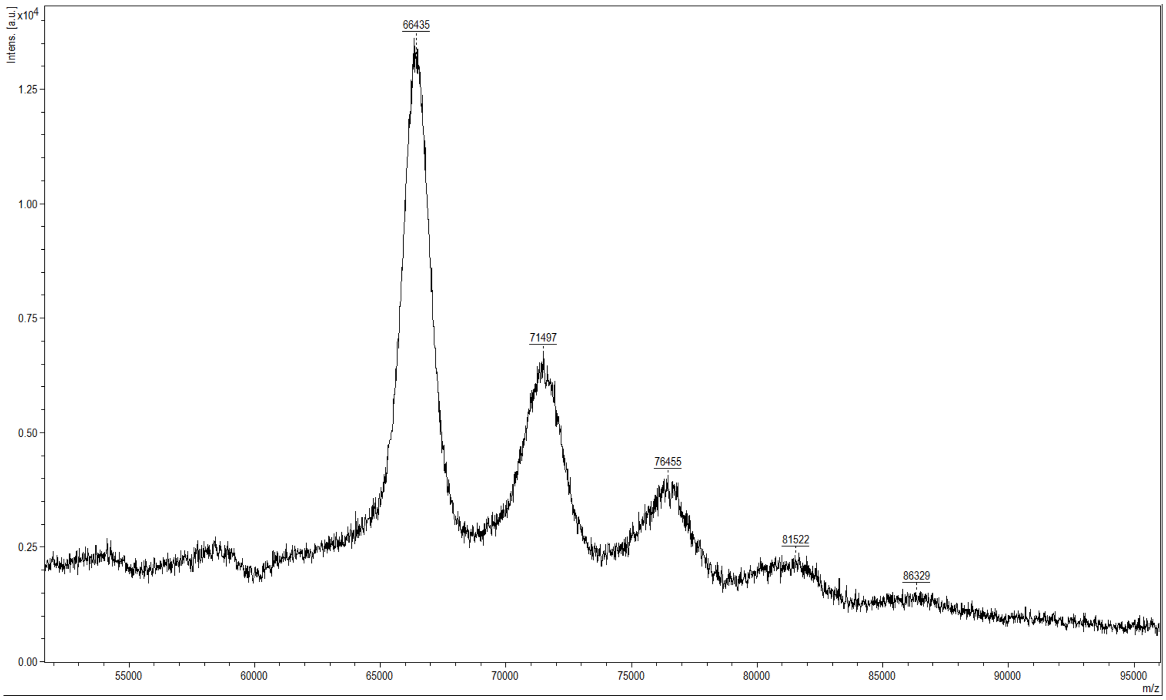


Figure S3. CuAAC-conjugation of azido-BSA with propargyl-PEG$_{5000}$

Table S1. Fluorescent intensity of wells on the Rhodamine B-azide derivatized 96-well plate

Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1a	+	+	+	+	+	-	-	-	-
Irradiation (min)	5	-	8	5	-	-	-	-	-
Incubation (hours)	1	1	5	5	5	-	-	-	-
well 1	468	181	1080	1201	305	80	60	87	38
well 2	500	94	1312	758	254	42	52	53	61
well 3	543	125	1623	1110	253	57	41	75	116
well 4	658	121	1817	1193	409	95	54	28	36
well 5	685	83	2044	1089	153	51	64	59	51
well 6	597	95	2386	1472	239	49	52	35	58
well 7	788	106	3611	1893	476	64	30	38	56
well 8	703	236	1977	1376	257	44	61	5	64
Average	618	130	1981	1261	293	60	52	48	60

Functionalization of 96-well plates with Rhodamine-B azide. Amino binding 96-well plates were incubated overnight in a 46 mM solution of 3-azidopropylamine in MeOH. The wells in lanes 1-5 were loaded with 140 μL of 0.39 mM suspension of photo-DIBOD **1a** in MeOH and 140 μL of 0.03 mM Rhodamine B-azide in MeOH, irradiated for various time intervals with a 350 nm lamp, incubated for various intervals then thoroughly washed. Florescent readout at 580 nm was conducted in a plate reader using 510 nm excitation source. The values fluorescence intensity counts per well are presented (in the Table S1.

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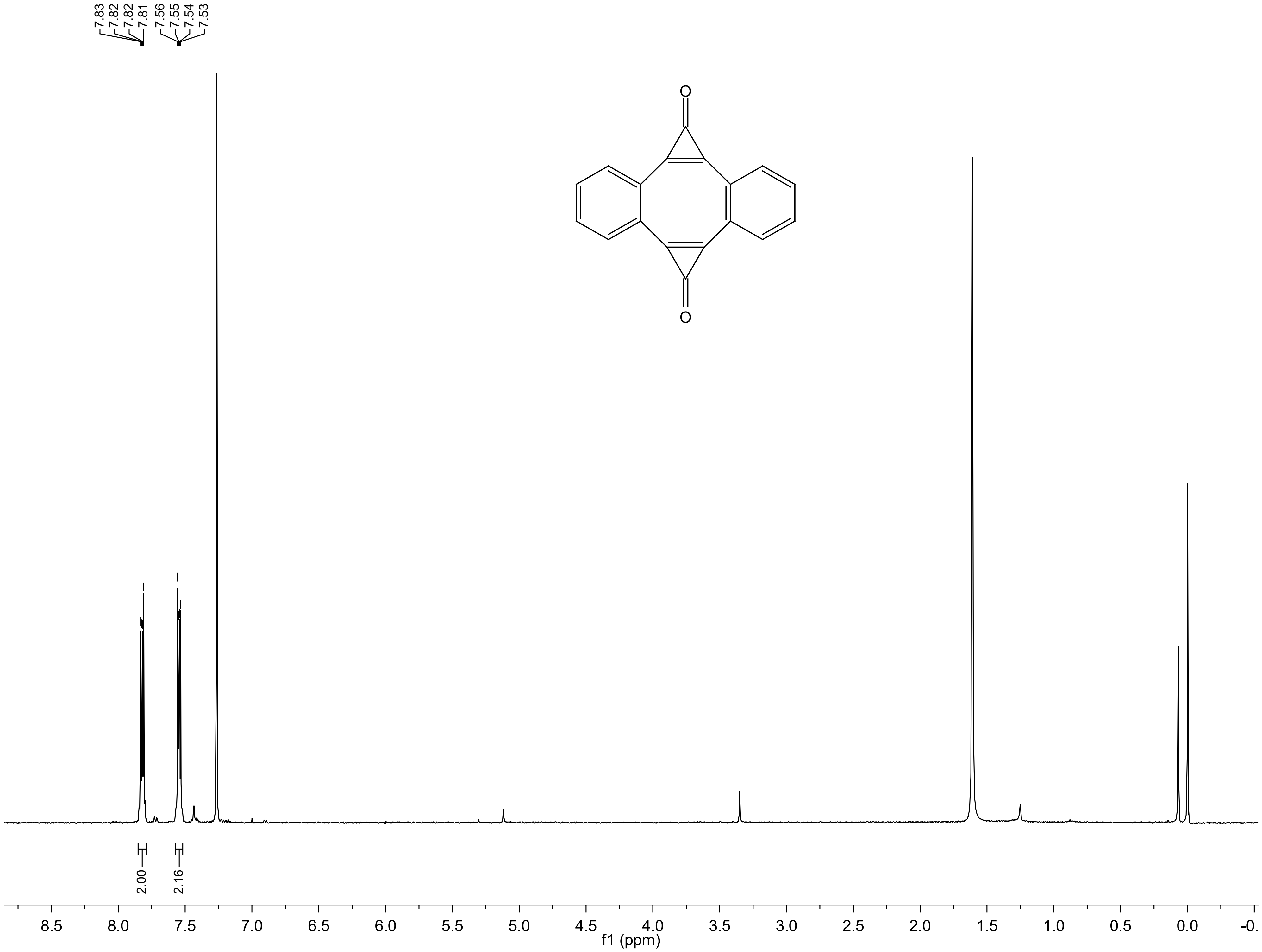
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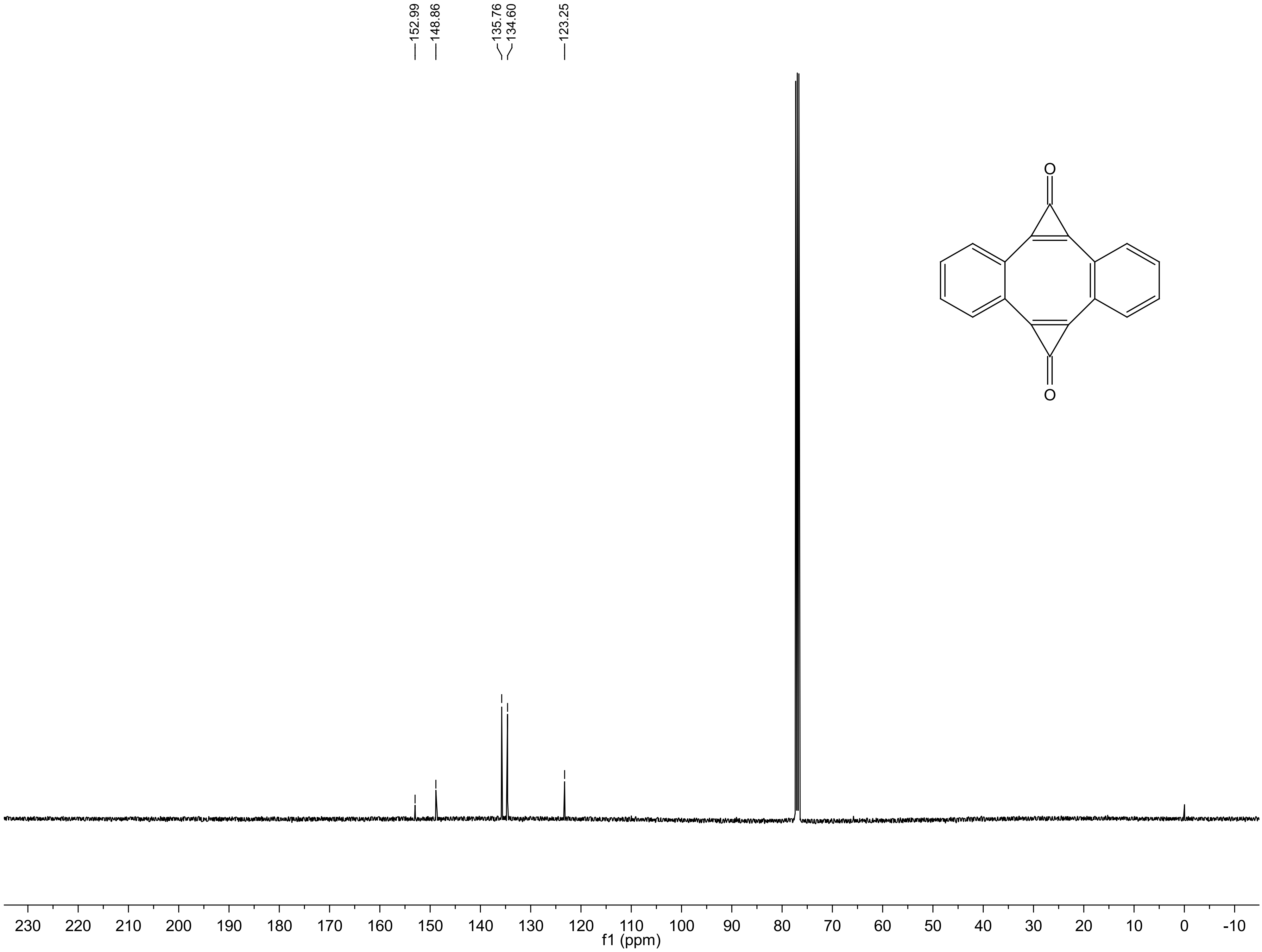
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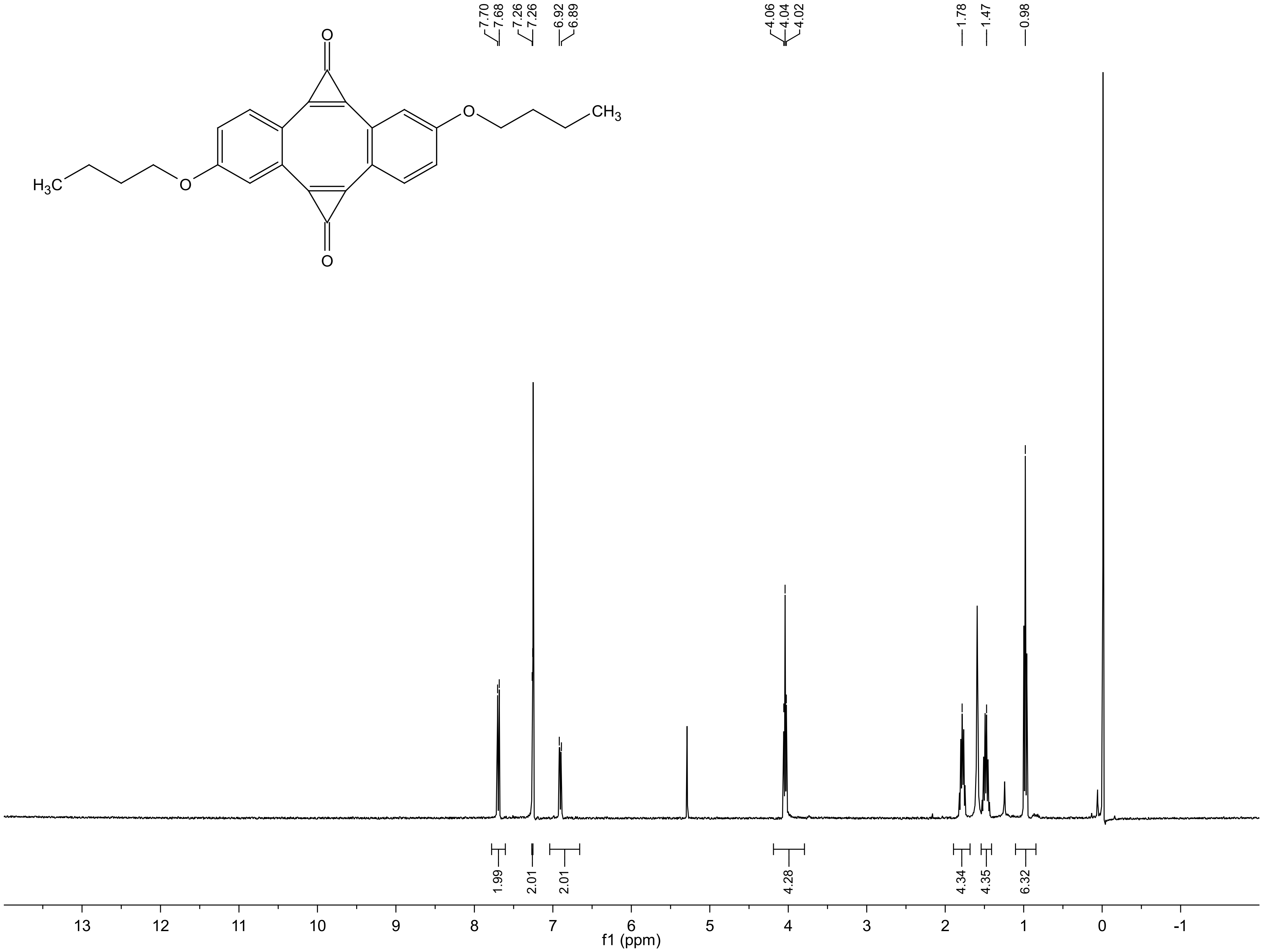
f1 (ppm)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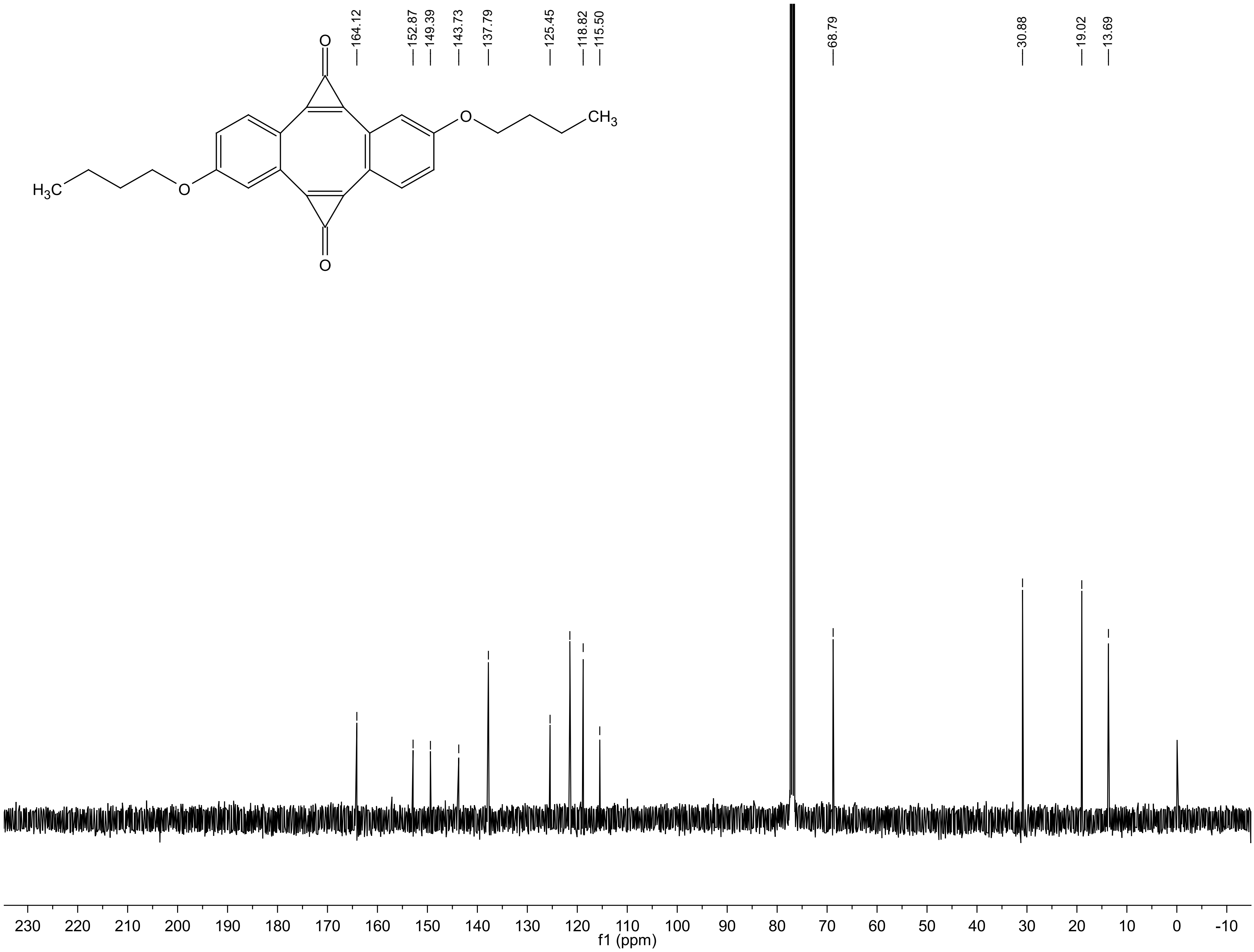
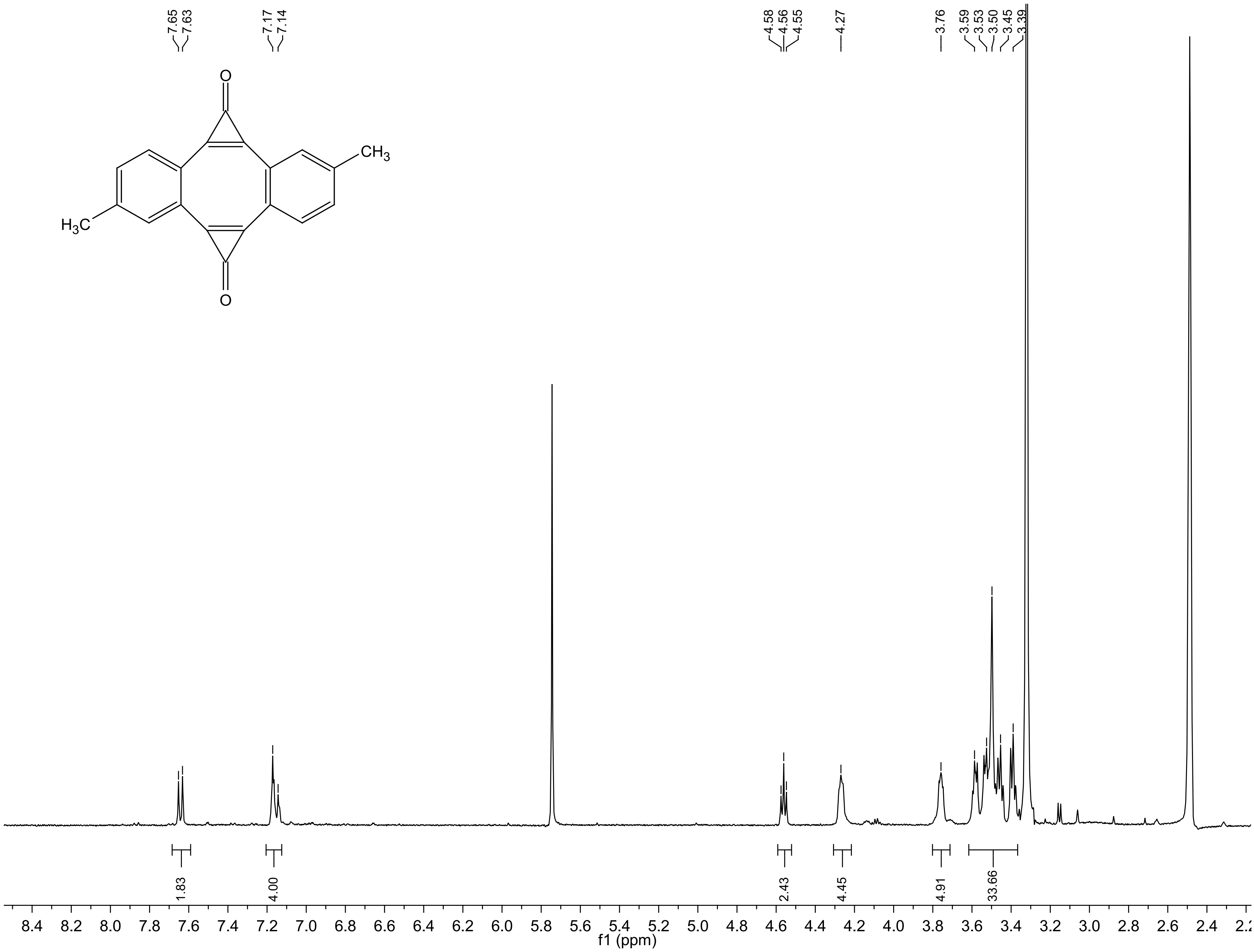
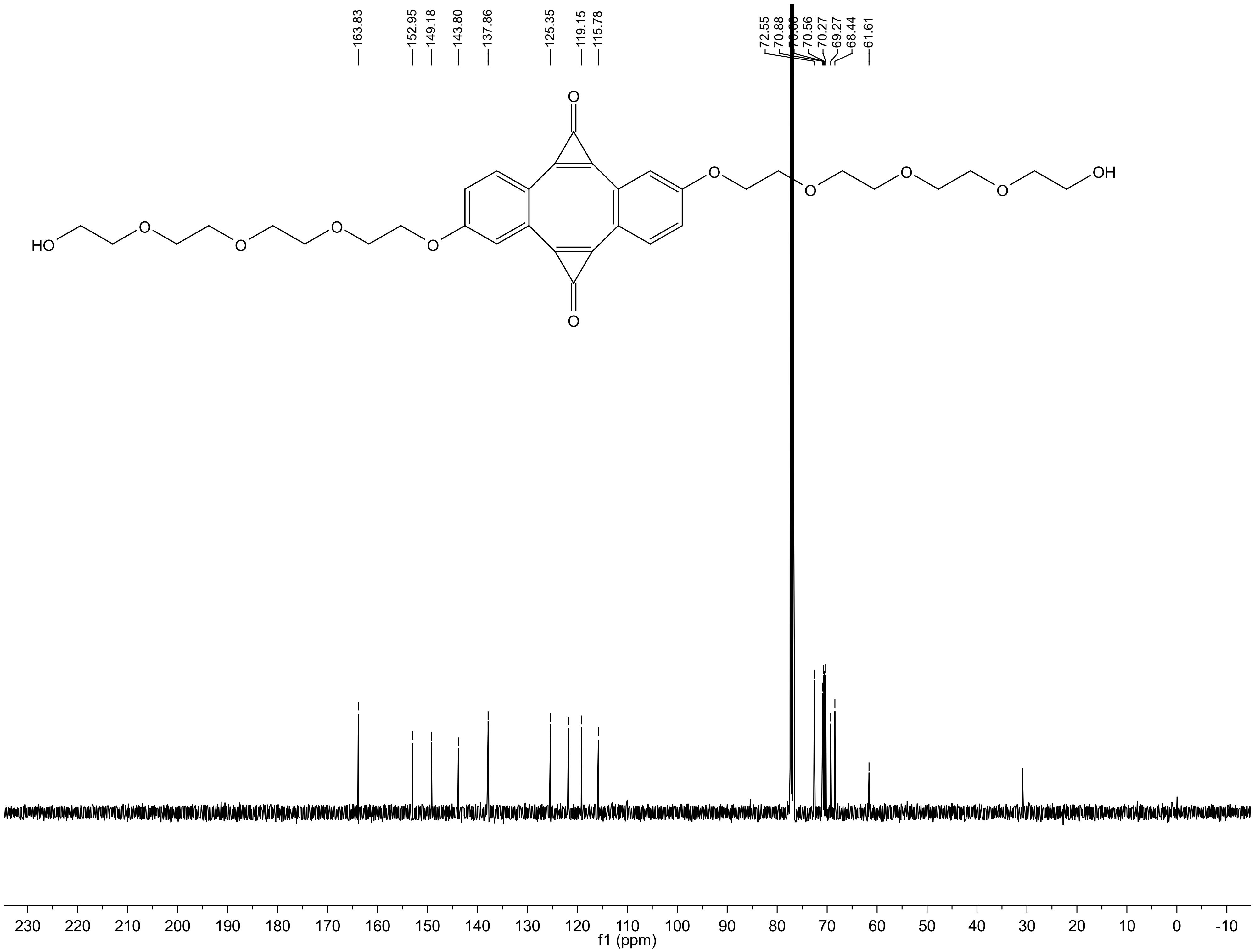
O
O
O
CH_3
H_3C
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)



O
O
O
O
H$_3$C
CH$_3$
164.12
152.87
149.39
143.73
137.79
125.45
118.82
115.50
68.79
30.88
19.02
13.69
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


7.65
7.63
7.17
7.14
4.58
4.56
4.55
4.27
3.76
3.59
3.53
3.50
3.45
3.39
O
O
CH3
H3C
1.83
4.00
2.43
4.45
4.91
33.66
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
f1 (ppm)



163.83
152.95
149.18
143.80
137.86
125.35
119.15
115.78
72.55
70.88
70.56
70.27
69.27
68.44
61.61
O
O
HO
O
O
O
O
O
O
O
O
OH
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)