Supporting information:

Synthesis and ion transport activity of oligoesters containing an environment-sensitive fluorophore

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Synthetic experimental details

General procedures

Most chemicals and solvents were used as received from known suppliers, except THF which was dried and distilled before use. NMR spectra were collected on a 300 MHz Bruker or 500 MHz Varian instrument. UV spectra were run on a Cary 5 UV-VIS spectrometer in a 10 x 10 mm quartz cell. ESI Mass spectra were recorded on a Waters MicroMass Q-TOF instrument running in negative ion mode. HPLC was performed using an HP Series 1100 instrument, with either a Macherey-Nagel "Nucleosil" RP C18 analytical (4 mm x 250 mm) or a Grace Davison "Alltima" RP C18 semi-prep (10 mm x 150 mm) column. Solvents used (ACN, CH_3OH; HPLC-grade) were filtered through a Milipore sub-micrometre filter before use. HPLC elution was monitored at various UV wavelengths (typically 254, 280 and 220 nm) and fluorometrically (λex = 310, λem = 330 nm). Fluorescence spectra were run on a PTI QM-2 instrument at T = 20^0C in 10 x 10 mm quartz cells equipped with a micro stir rod.

Sonogashira coupling [1]: To a round bottomed flask equipped with a septum, 1.2–1.5 equivalents (in relation to the alkyne starting material) of the iodo-containing reactant and 6–10% CuI were dissolved in dry DMF, which was then deoxygenated under vacuum. The alkyne reactant was then added to the flask, which was kept in the dark. $Pd(PPh_3)_4$ (3–5%) was then added, followed by 2–5 equivalents of NEt_3. The reaction was then stirred under N_2 at temperatures ranging from rt to 50^0C, for 10–24 h, depending on the reagents used. Reactions were monitored by TLC (silica gel, EtOAc/hexanes as eluent, visualized by UV, *p*-anisaldehyde and/or vanillin stain). Once complete, reactions were cooled if necessary, diluted with EtOAc and washed with saturated EDTA (2–3 times), H_2O (once), saturated NaCl (once), dried over sodium sulfate, and concentrated under vacuum. Unless noted otherwise, the crude products were purified by column chromatography on silica gel, typically using EtOAc/hexanes as eluent.

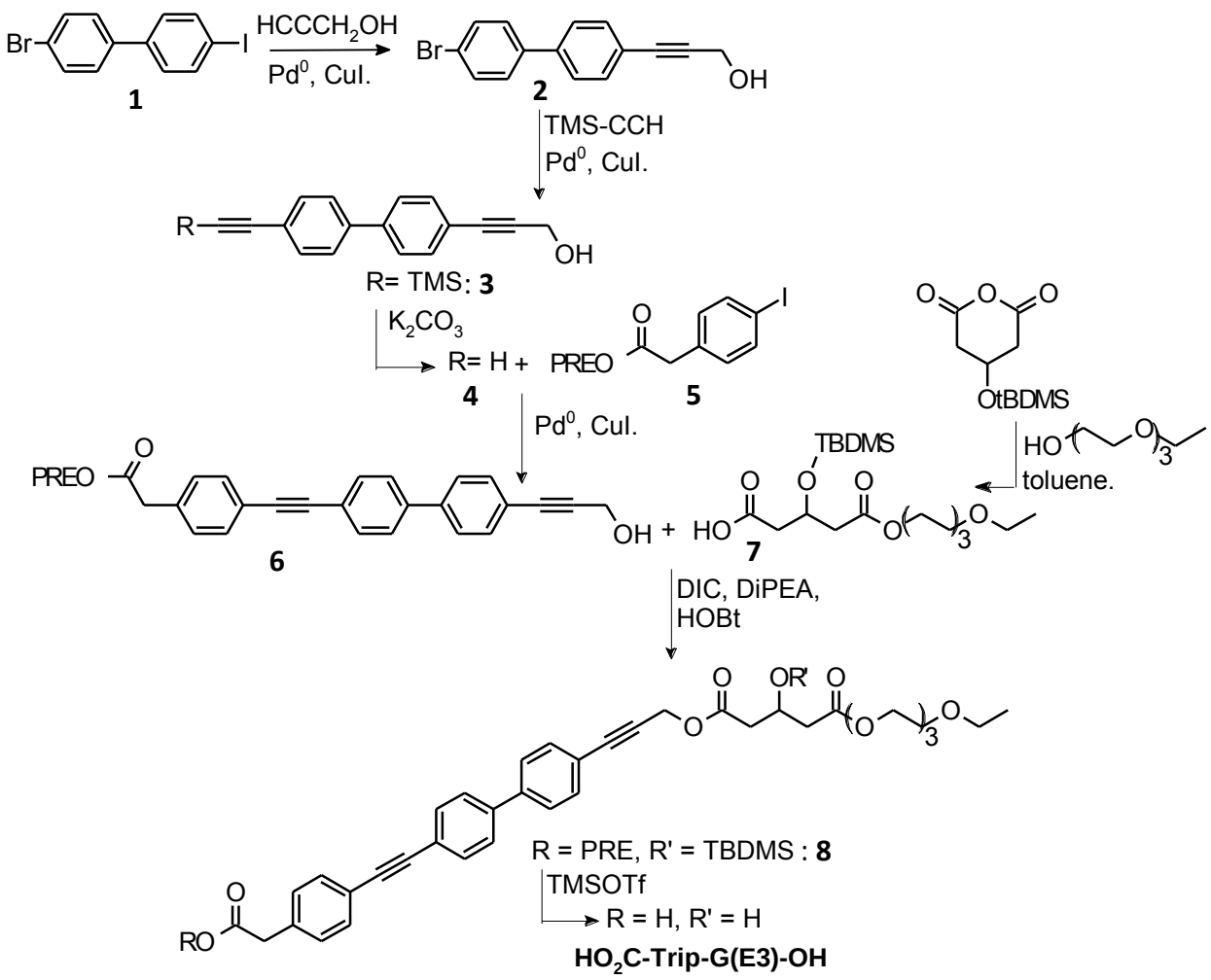
Ester coupling [2]: To a solution of 1.3–2 equivalents of either the alcohol or acid building block in relation to 1 equivalent of the other (excess reagent choice determined by ease of synthesis or availability) in THF or DMF (depending on substrate) were added 1.3–2 eq. of DIC, HOBt and 2.6–4 eq. of DIPEA. The reaction was sealed under an atmosphere of N_2, and stirred for 16–24h at temperatures varying between rt and 50^0C. Reaction completion was monitored by TLC. Once complete, the reaction was cooled (if necessary), filtered to remove DIU, and diluted with DCM or EtOAc. The organic phase was extracted with H_2O (twice), saturated $NaHCO_3$ (twice), rinsed with

sat. NaCl (once), dried with anhydrous sodium sulfate, and concentrated under vacuum. Unless noted otherwise, the crude product was purified by column chromatography on silica gel, typically using EtOAc/hexanes as eluent.

THP removal: pTsOH (5–25%) was added to a solution of compound in ~10–30% CH_3OH: DCM, which was stirred at rt for 1–3 h, as monitored by TLC. Once complete, the reaction was diluted with DCM, washed with H_2O (once), saturated $NaHCO_3$ (twice), and saturated NaCl (once), then dried over sodium sulfate and concentrated under vacuum. If necessary, further purification was carried out as noted.

Prenyl deprotection*:* Adapted [3]: 0.025–0.1 equivalents TMSOTf were added to the compound dissolved in DCM, which was stirred at rt. Once complete (as monitored by TLC, generally < 1 h), the reaction mixture was diluted further into DCM, washed with H_2O, saturated $NaHCO_3$ and saturated NaCl, then dried over sodium sulfate and concentrated under vacuum. Further purification was carried out as noted.

Synthetic details for new compounds:



Scheme S1: Synthesis of HO_2C-**Trip-G(E3)**-OH

1: Adapted [4]. 1.0 equivalent (6.97mmol, 2.32g) I_2O_5, 1.6 equivalents 4-bromo-4'-iodobiphenyl, 3.5 equivalents I_2, in 5mL CCl_4, 50% Aq. H_2SO_4 in nitrobenzene (10mL) were stirred at 90^0C for 48hrs. After this time, the reaction mixture was cooled and diluted with CH_3OH, from which white shiny crystals precipitated. These were filtered to yield 2.05g (78%) of **1**. MP= 169-170^0C (lit. 166 – 168^0C). NMR ($CDCl_3$): agrees with literature.

2: Sonogashira coupling conditions: 1.0 equivalent (2.5g, 6.96mmol) of **1** (4-Bromo, 4'-Iodobiphenyl), 2.0 equivalents (0.805mL) propargyl alcohol, 5% $Pd(PPh_3)_4$, 10% CuI and 2 equivalents NEt_3 were stirred in THF at rt for 20 hrs. Standard work-up followed by silica gel chromatography (elution at 20%

EtOAc/hexanes) led to 1.78g (89%) of the product as creamy white needles. MP= 153-154^{0}C. NMR ($CDCl_3$) ^{1}H: 7.55 (dt, 2H, *J*= 8, 2Hz), 7.49 (s, 4H), 7.42 (dt, 2H, *J*= 8, 2 Hz), 4.51 (d, 2H, *J*= 6Hz), 1.68 (t, 1H, *J*= 6Hz). ^{13}C: 139.9, 139.1, 132.2, 131.9, 128.6, 126.8, 121.9, 121.8, 88.1, 85.4, 51.7.

3: Sonogashira coupling conditions; 1.0 equivalent (0.4g, 1.39mmol) of **2**, 2.0 equivalents (0.392mL) TMS-acetylene, 5% $Pd(PPh_3)_4$, 10% CuI and 2 equivalents NEt_3 were stirred in DMF at 70^{0}C for 48 hrs. Standard work-up followed by silica gel chromatography (elution at ~15% EtOAc/hexanes) led to 296mg (71%) of **3** as dull yellow flakes. MP= 158-160^{0}C. NMR ($CDCl_3$) ^{1}H: 7.52 (m, 8H), 4.51 (s, 2H), 0.26 (s, 9H). ^{13}C: 140.3, 140.1, 132.5, 132.2, 126.8, 126.7, 122.5, 121.8, 104.8, 95.3, 88.1, 85.5, 51.7, -0.1.

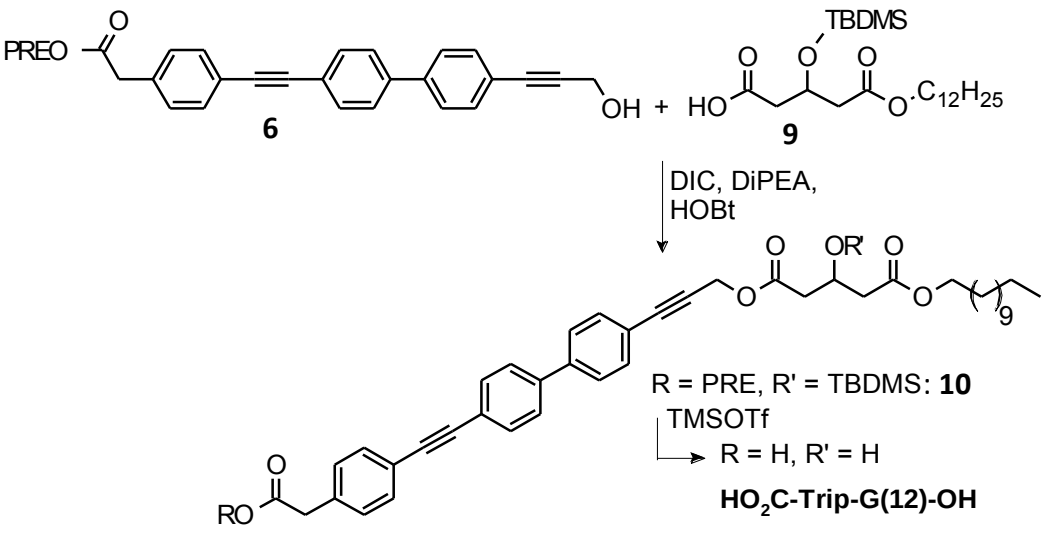
4: TMS deprotection: 1.0 equivalent (280mg, 9.2mmol) of **3** and 1.0 equivalent (127mg) K_2CO_3 were stirred in 1:1 DCM: CH_3OH for 3.5 hrs. Once complete, the reaction was diluted with DCM, washed with 1M Aq. HCl (twice), H_2O (twice), sat. NaCl (once), dried over sodium sulfate and rotovapped. This yielded 186mg (87%) of **4** as a tan powder, which was used without further purification. NMR ($CDCl_3$) ^{1}H: 7.52 (m, 8H), 4.52 (s, 2H), 3.14 (s, 1H), 1.72 (s, br, 1H). ^{13}C: 140.5, 140.2, 132.6, 132.2, 126.9, 126.8, 121.9, 121.4, 88.1, 85.5, 83.4, 78.1, 51.7.

6: Sonogashira coupling conditions; 1.0 equivalent (150mg, 6.47mmol) of **4**, 1.4 equivalents (298mg) **5** [5] 5% $Pd(PPh_3)_4$, 10% CuI and 2 equivalents NEt_3 were stirred in Et_2O at rt for 16 hrs. Standard work-up, purification by silica gel chromatography (elution at ~ 40% EtOAc/hexanes) yields 218mg (77%) as a pale yellow solid. MP= 140^{0}C (decomp). NMR ($CDCl_3$) ^{1}H: 7.52 (m, 10H), 7.27 (d, 1H, *J*= 8Hz), 5.33 (m, 1H), 4.59 (d, 2H, *J*= 7Hz), 4.52 (s, 2H), 3.63 (s, 2H), 1.75 (s, 3H), 1.69 (s, 3H). ^{13}C: 171.2, 140.3, 139.9, 139.4, 134.4, 132.2, 132.1, 131.7, 129.4, 126.9, 122.6, 121.9, 121.8, 118.3, 90.2, 89.2, 88.1, 85.5, 61.9, 51.7, 41.3, 25.8, 18.0.

7: 1.0 equivalent (0.523mL, 3.7mmol) of tri(ethylene glycol) monoethyl ether and 1.1 equivalents glutaric anhydride were refluxed in toluene for 16hr. After completion, the reaction was rotovapped to remove toluene, re-dissolved in DCM, washed with H_2O, NaCl (sat), dried over sodium sulfate and concentrated under vacuum. The crude product was then further purified by silica get chromatography (1:1 DCM:hexanes + 1% AcOH) to yield 1.21g (81%) of **7** as a clear, pale blue oil, which solidified below 0^{0}C. NMR ($CDCl_3$) ^{1}H: 4.52 (p, 1H, *J*= 6Hz), 4.21 (m, 2H), 3.69 – 3.48 (m, 12H), 2.57 (m, 4H), 1.18 (t, 3H, *J* = 7Hz), 0.82 (s, 9H), 0.05 (s, 6H). ^{13}C: 170.8, 70.6, 70.5, 70.4, 69.7, 68.9, 66.6, 66.1, 63.6, 42.3, 42.2, 17.9, 15.1, -4.9, -4.9.

8: Ester coupling conditions; 1.0 equivalent (0.2g, 4.61mmol) of **6**, 1.2 equivalents (243 mg) of **7**, 1.3 equivalents DIC, HOBt and 2.6 equivalents DiPEA were stirred in ACN at rt for 24 hrs. Standard work-up and purification by silica gel chromatography (elution at ~30% EtOAc/hexanes) yields 276mg (71%) of the product as a yellow oil. NMR ($CDCl_3$) ^{1}H: 7.57 – 7.48 (m, 10H), 7.27 (d, 2H, *J*= 8Hz), 5.33 (m, 1H), 4.92 (s, 2H), 4.58 (m, 3H), 4.22 (td, 2H, *J*= 6, 1Hz), 3.70 – 3.47 (m, 14H), 2.63 (m, 4H), 1.75 (s, 3H), 1.68 (s, 3H), 1.19 (t, 3H, *J*= 7Hz), 0.84 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H). ^{13}C: 171.2, 170.8, 170.3, 140.6, 139.8, 139.4, 134.4, 132.4, 132.1, 131.7, 129.4, 126.9, 126.8, 122.7, 121.9, 118.3, 90.2, 89.2, 86.3, 83.7, 70.7, 70.6, 69.8, 69.0, 66.6, 66.1, 63.6, 61.9, 52.8, 42.3, 42.3, 41.3, 25.8, 25.7, 18.0, 17.9, 15.2, -4.8, -4.9.

HO_2C-**Trip-G(E3)**-OH**:** Prenyl deprotection conditions; 1.0 equivalent (0.2g, 0.24mmol) of **8** and 5µL TMSOTf were stirred in DCM for 0.5hr. The reaction was then diluted with DCM and washed with H_2O, during this time the solution went from a dark green to a pale yellow. The mixture was then washed with sat. NaCl, dried over sodium sulfate and concentrated under vacuum. The crude product (125mg, ~80% yield) was then purified by silica gel chromatography (elution at 98% Et_2O/2% AcOH). Purification was poor, as the compound did not cleanly elute from the column, leading to a poor recovery (~50% yield, 78mg) of the purified compound as a pale yellow, waxy solid. The compound was further purified by HPLC (semi-prep column, 75% CH_3OH: 25% ACN) to yield a virtually unchanged pale yellow, waxy solid. UV (CH_3OH); λ_{max}Abs=315nm (ε=90 806 $M^{-1}cm^{-1}$). Fluorescence (CH_3OH); λ_{max}Ex= 325nm, λ_{max}Em= 370nm. NMR ($CDCl_3$) ^{1}H: 7.57 – 7.48 (m, 10H), 7.27 (d, 2H, *J*= 8Hz), 4.95 (s, 2H), 4.52 (p, 1H, *J*= 6Hz), 4.27 (m, 2H), 3.72 – 3.48 (m, 14H), 2.62 (m, 4H), 1.19 (t, 3H, *J*= 7Hz). ^{13}C: 175.6, 171.6, 170.9, 140.6, 139.9, 133.7, 132.4, 132.1, 131.8, 129.5, 126.9, 126.8, 122.6, 122.3, 121.3, 90.1, 89.4, 86.5, 83.5, 70.6, 70.5, 69.8, 68.9, 66.7, 64.7, 63.8, 53.1, 40.8, 40.7, 40.6, 15.1. MS (-ve ESI): calc'd for $C_{38}H_{39}O_{10}$ = 655.2549 amu, obtained = 655.2659 amu. Overall yield over 7 steps starting from **1** = 16%.

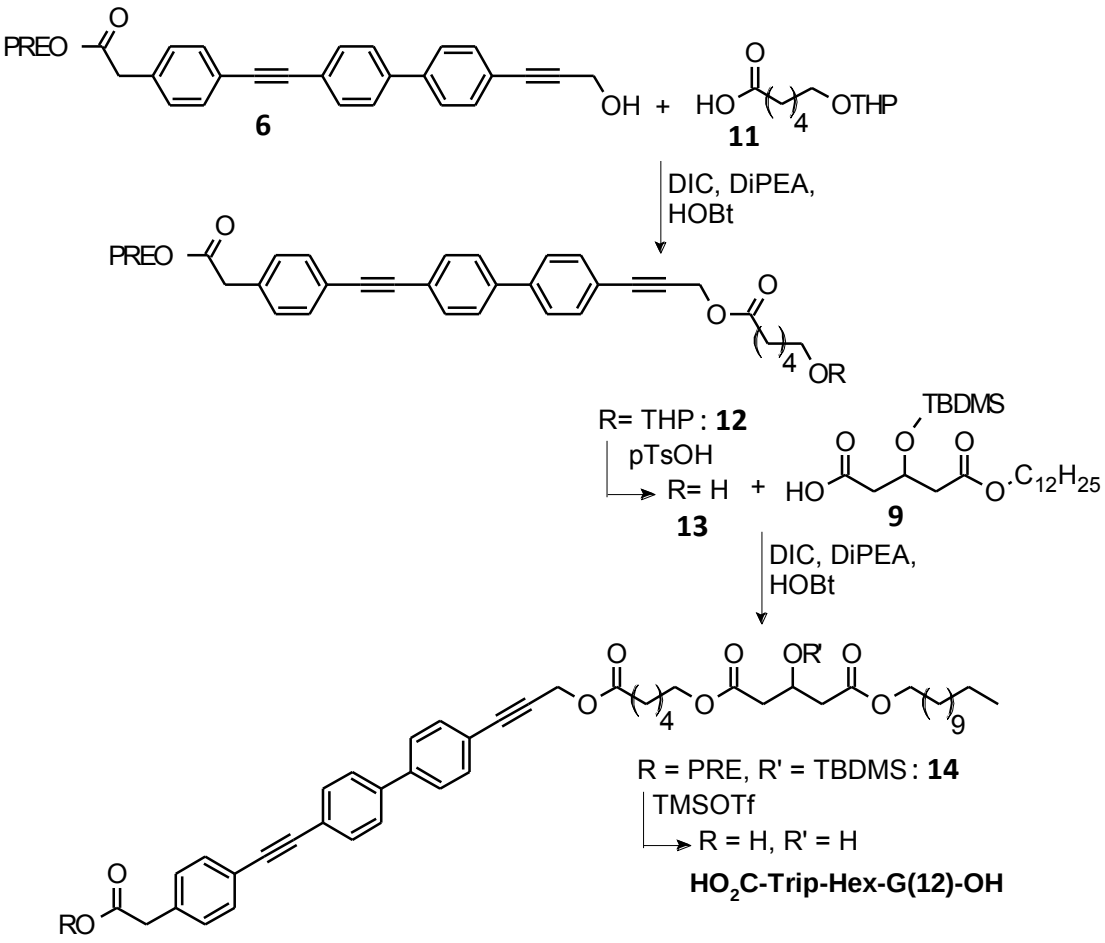


Scheme S2: Synthesis of HO_2C-**Trip-G(12)**-OH

10: Ester coupling conditions; 1.0 equivalent (0.2g, 0.46mmol) of **6**, 1.3 equivalents (0.26g) **9** [6] , 1.3 equivalents DIC, HOBt and 2.6 equivalents of DIPEA were stirred at rt in ACN for 18hr. Standard work-up, purification by silica gel chromatography (elution ~7% EtOAc:hexanes) yields 285mg (73%) of a clear, colourless oil, which solidified under vacuum to give a white foam. MP < rt. NMR ($CDCl_3$): ^{1}H: 7.58 – 7.48 (m, 10H), 7.27 (d, 2H, *J*= 8Hz), 5.33 (m, 1H), 4.92 (s, 2H), 4.60 (m, 3H), 4.05 (m, 2H), 3.63 (s, 2H), 2.65 (d, 2H, *J*= 6Hz), 2.57 (d, 2H, *J*= 6Hz), 1.75 (s, 3H), 1.69 (s, 3H), 1.61 (m, 2H), 1.25 (s, 18H), 0.86 (m, 12H), 0.10 (s, 3H), 0.08 (s, 3H). ^{13}C: 171.2, 170.9, 170.4, 140.5, 139.8, 139.4, 134.4, 132.4, 132.1, 131.7, 129.4, 126.9, 126.8, 122.7, 121.9, 121.4, 118.4, 90.2, 89.2, 86.3, 83.8, 66.2, 64.8, 61.9, 52.8, 42.5, 42.3, 41.3, 31.9, 29.6, 29.6, 29.5, 29.3, 29.3, 28.6, 25.9, 25.8, 25.7, 22.7, 18.0, 17.9, 14.1, -4.9.

HO_2C-**Trip-G(12)**-OH: Prenyl deprotection conditions; 1.0 equivalent (0.2g, 0.24mmol) **10** and 0.1 equivalent TMSOTf were stirred in DCM for 20 mins. Upon addition of TMSOTf, the reaction mixture turned from a pale yellow solution to a dark bluish-green, which reverted back to the pale yellow color upon quenching with water. After washing the organic layer with water twice, it was dried over sodium sulfate and rotary evaporated. The crude residue was then re-suspended in hexanes and sonicated, from which a pale greenish-yellow solid precipitated. This product was then purified by silica gel chromatography (elution at 20% acetone/hexanes, 2% AcOH added to all eluting solvents), to yield 130mg (79%) of a white solid. The product was then further purified by RP-HPLC (semi-prep column, 1:1 ACN:CH_3OH) to yield transparent crystals. UV (CH_3OH); λ_{max}Abs=313nm (ε=90 800 $M^{-1}cm^{-1}$). Fluorescence (CH_3OH); λ_{max}Ex= 325nm, λ_{max}Em= 370nm. NMR ($CDCl_3$) ^{1}H (500MHz): 7.57 – 7.49 (m,

10H), 7.28 (d, 2H, *J*= 8Hz), 4.96 (s, 2H), 4.49 (p, 1H, *J*= 6Hz), 4.09 (t, 2H, *J*= 7Hz), 3.67 (s, 2H), 2.63 (m, 2H), 2.56 (d, 2H, *J*= 6Hz), 1.64 – 1.53 (m, 3H), 1.24 (s, 18H), 0.86 (t, 3H, *J*= 7Hz). ^{13}C (125MHz): 173.9, 172.1, 171.2, 140.9, 140.2, 133.8, 132.7, 132.4, 132.1, 129.7, 127.1, 127.0, 122.9, 122.6, 121.6, 90.4, 89.7, 86.8, 83.7, 65.4, 64.9, 53.4, 40.7, 40.6, 32.1, 29.9, 29.8, 29.7, 29.6, 29.5, 28.8, 26.1, 22.9, 14.3, MS (-ve ESI): calc'd for $C_{42}H_{47}O_7$ = 663.3327 amu, obtained = 663.3408 amu. Overall yield over 7 steps from **1** = 19%



Scheme S3: Synthesis of HO_2C-**Trip-Hex-G(12)**-OH

12: Ester coupling conditions; 1.0 equivalent (0.18g, 0.42mmol) **6**, 2.0 equivalents (0.18g) **11** [7], 2 equivalents DIC, HOBt and 3.5 equivalents DIPEA were stirred in THF for 14 hrs at rt. Standard work-up, purification by silica gel chromatography (elution at 10% EtOAc/hexanes) gave 0.152mg (58%) of **12** as a white semi-solid. NMR ($CDCl_3$): ^{1}H: 7.57 – 7.48 (m, 10H), 7.25 (d, 2H, *J*= 8Hz), 5.32 (m, 1H), 4.92 (s, 2H), 4.28 (d, 2H, *J*= 7Hz), 4.55 (m, 1H), 3.84 (m, 1H), 3.72 (m, 1H), 3.62 (s, 2H), 3.46 m, 1H), 3.37 (m, 1H), 2.39 (t, 2H, *J*= 7Hz), 1.74 (s, 3H), 1.68 (s, 3H), 1.64 – 1.39 (m, 12H). ^{13}C: 172.9, 171.0, 140.5, 139.8, 139.3,

134.4, 132.4, 132.0, 131.7, 129.3, 126.8, 126.7, 122.6, 121.9, 121.4, 118.3, 98.8, 90.2, 89.2, 86.1, 83.9, 67.2, 62.3, 61.9, 52.6, 41.2, 33.9, 30.7, 29.3, 25.8, 25.7, 25.4, 24.6, 19.6, 17.9.

13: THP removal conditions; 1.0 equivalent (0.14g, 0.22mmol) **12** and 0.2 equivalents TsOH were stirred in 20% CH_3OH:DCM for 2 hrs at rt. Standard work up led to 108 mg (89%) of the desired compound, which was used directly in the next reaction without further purification.

14: Ester coupling conditions; 1.0 equivalent (0.1g, 0.182mmol) **13**, 1.5 equivalents DIC, HOBt, 3 equivalents of DIPEA and 1.5 equivalents (0.12g) of **9** were stirred in THF for 48hrs at 40^0C. TLC monitoring indicated no further reaction occurred after approximately 16hrs. Standard workup, purification by silica gel chromatography (elution at ~10% EtOAc/hexanes) led to 102mg (58%) of the product as a clear colorless oil. NMR ($CDCl_3$): ^{1}H: 7.52 – 7.42 (m, 10H), 7.21 (d, 2H, *J*= 8Hz), 5.27 (m, 1H), 4.88 (s, 2H), 4.53 (d, 2H, *J*= 7Hz), 4.48 (t, 2H, *J*= 7Hz), 3.99 (m, 4H), 3.57 (s, 2H), 2.48 (m, 4H), 2.34 (t, 2H, *J*= 7Hz), 1.69 – 1.52 (m, 11H), 1.19 (s, br, 22H), 0.81 (m, 12H), -0.01 (s, 6H). ^{13}C: 172.7, 171.2, 171.1, 171.0, 140.6, 139.8, 139.4, 134.4, 132.4, 132.1, 131.7, 129.4, 126.9, 126.8, 122.7, 121.9, 121.4, 118.3, 90.2, 89.2, 86.2, 83.9, 66.3, 64.7, 64.3, 61.9, 52.7, 42.5, 42.5, 41.3, 33.9, 31.9, 29.6, 29.5, 29.5, 29.3, 29.2, 28.5, 28.2, 25.9, 25.8, 25.6, 25.5, 24.5, 22.7, 18.0, 17.9, 14.1, -4.9.

HO_2C-**Trip-Hex-G(12)**-OH: Prenyl deprotection conditions; 1.0 equivalent (60mg, 0.062mmol) **14** and 0.2 equivalents TMSOTf were stirred in DCM for 40 mins at rt. Upon addition of TMSOTf, the reaction mixture turned from a pale yellow solution to a dark green, which reverted back to the pale yellow color upon quenching with water. After washing the organic layer with water twice, it was dried over sodium sulfate and rotary evaporated. The crude residue was then re-suspended in 1:1 EtOH: hexanes and sonicated, from which a pale yellow solid precipitated to yield 43mg (73%) of crude product. This was then further purified by RP-HPLC (semi-prep column, 3:1 ACN:CH_3OH) to yield white crystals. UV (CH_3OH); λ_{max}Abs=312nm (ε=90 806 $M^{-1}cm^{-1}$). Fluorescence (CH_3OH); λ_{max}Ex= 327nm, λ_{max}Em= 365nm. NMR ($CDCl_3$) ^{1}H: 7.57 – 7.49 (m, 10H), 7.28 (d, 2H, *J*= 8Hz), 4.93 (s, 2H), 4.44 (p, 1H, *J*= 6Hz), 4.10 (m, 4H), 3.67 (s, 2H), 2.53 (m, 4H, *J*= Hz), 2.40 (t, 2H, *J*= 7Hz), 1.75 – 1.57 (m, 6H), 1.42 (m, 2H), 1.25 (s, 18H), 0.87 (t, 3H, *J*= 7Hz). ^{13}C: 175.4, 172.8, 171.9, 171.8, 140.6, 139.9, 133.5, 132.4, 132.1, 131.8, 129.5, 126.9, 126.8, 122.6, 122.3, 121.4, 90.0, 89.4, 86.2, 83.9, 65.0, 64.8, 64.6, 52.8, 40.6, 33.9, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 28.5, 28.2, 25.9, 25.4, 24.4, 22.7, 14.1. MS (-ve ESI): calc'd for $C_{48}H_{58}O_9$ =778.4037 amu, obtained = 778.4346 amu. Overall yield over 9 steps from **1** = 7%

Transport assay & fluorescence experimental details

General procedures

All final compounds were purified by HPLC prior to use. Stock concentrations of approximately 1 – 5mM in THF were stored under N_2 in the freezer when not in use, and periodically re-checked by HPLC. Steady-state fluorescence spectra were collected on a PTI QM instrument at T= 20^0C in 10 x 10 mm quartz cells equipped with a stir rod (volume ~2mL), or 1 x 10 mm quartz cells (no stir rod, volume ~0.8mL). For general fluorescence experiments, the excitation wavelength was ~320nm, emission wavelength was ~370nm (solvent-dependent), excitation and emission slits were both 1nm, integration time= 0.1s, step size 1nm. Other parameters for particular experiments are as noted.

For studies in organic solvents, the solvents used were of spectral or higher quality, and were purged with N_2. For all aqueous studies except those involving $CuSO_4$, the aqueous buffer consisted of 10mM $Na_3PO_4.12H_2O$, 100mM NaCl, pH=6.4. For quenching studies with $CuSO_4$ in aqueous solution 10mM Bis-Tris, 100mM NaCl, pH=6.4 was used (as noted). Vesicles were prepared and sized as reported previously [2, 8], in certain cases, the vesicles were made with the same buffer both internal and external to the vesicle rather than containing the HPTS dye. Alternatively, for certain experiments, the compound was pre-incorporated into the vesicle bilayer by adding a solution of the compound of interest to the lipid mix before continuing the preparation as usual. An injection port was used to add solutions to the cuvette while the experiment was running.

BILAYER CLAMP ASSAY

As previously described [9, 10]. A model BC-525A bilayer clamp (Warner Instrument Corp.) was used for planar bilayer experiments, ClampEx 8 and ClampFit 10 (Axon Instruments) were the software used for acquisition and analysis, respectively. Cups used were made of polystyrene and had 250µm diameter apertures. The lipid used in all cases was diphytanoyl phosphatidylcholine (diPhyPC) (Avanti Polar lipids). A stock solution of 25mg/mL lipid in $CHCl_3$ was dried under N_2 and then re-suspended in 200µL decane. For compounds that had to be pre-loaded into the lipid, 0.1 – 1mol% compound in $CHCl_3$ was added to the lipid mix and then dried down. The electrolytes used were 1M KCl, CsCl or NMe_4Cl in 10mM HEPES, 10mM TRIS, pH 7 (unadjusted). The aperture was primed with 0.5-1µL of decane/lipid, excess solvent was removed by blowing N_2 over the aperture. The cup was then placed into the electrolyte-filled holding cell, consisting of 5mL and 3mL chambers, and salt bridges (KNO_3/Agar) and electrodes

(Ag/AgCl) were attached. Bilayers were formed by brushing on 1- 1.5µL of the decane/lipid mix over the aperture, and were monitored for stability, capacitance and resistance for at least 20 minutes before test compound was added. Test compounds were added either by injection from an organic solution (typically no more than 1-10µL of solution) or by breaking the lipid-only bilayer and brushing on the compound-preloaded lipid mix. All data were hardware filtered (8- pole Bessel filter, 1 kHz) and data was collected in a survey mode using the Gap-free protocol. Bilayers were tested repeatedly for capacitance and resistance, and no bilayer was used for more than 2 hrs. Once formed, 'activity' from pristine bilayers was never observed. Each experiment was carried out in each electrolyte at least twice, with two different cups.

HPTS ASSAY

Vesicle preparation: A chloroform solution of 8:1:1 PC:PA:cholesterol (Avanti Polar lipids) was dried *in vacuo* in a pear-shaped flask and then left on the vacuum line overnight. For compounds that were pre-loaded into the vesicle, a solution of the test compound of interest was added to the initial $CHCl_3$ lipid solution at 0.1 – 1mol%, and then prepared as described. The 50 mg lipid film was hydrated with 1 mL of internal buffer solution (10 µM HPTS, 10 mM $Na_3PO_4 \cdot 12H_2O$, 100 mM NaCl in deionized H_2O, pH 6.4, adjusted with conc. H_3PO_4). The suspension was frozen under liquid nitrogen and subsequently thawed at room temperature over ten minutes (3 times). The mixture was then sonicated in an ice bath for 20 seconds with 2 second pulses (at 50% duty cycle and 20% power output) 3 times, with a 30s rest between cycles. The unilamellar vesicles were then left to anneal overnight. The vesicle solution was then sized 19 times through a 400nm polycarbonate Nucleopore filter using a LiposoFast membrane extrusion apparatus (Avestin) (0.5mL x 2) and purified on a PD-10 Sephadex G-25 column (GE Healthsystems) using an external buffer solution (10mM $Na_3PO_4 \cdot 12H_2O$, 100mM NaCl, pH=6.4). The first three cloudy drops were discarded but thereafter the cloudy fraction was collected and diluted to 5.00mL using the external buffer solution. A typical preparation of this vesicle stock solution contained 200 ±20nm diameter vesicles (determined by dynamic light scattering, Brookhaven Instruments, ZetaPALS particle sizing software) and a lipid concentration of typically 7 mg/mL [7]. The vesicle solution was stored at 5^0C and used within 24 hours of preparation.

Typical experiment: in a typical experiment, 100µL of vesicles were added to the fluorescence cuvette. 2.00mL of external buffer (10mM $Na_3PO_4 \cdot 12H_2O$, 100mM NaCl, pH=6.4) and a solution of the test compound in THF or MeOH was then added. The solution was placed in the fluorimeter and left to equilibrate for 3 minutes. An excitation ratio was started (Ex1= 403nm, Ex2= 460nm, Em= 510nm, slits =

3nm, Integration 1s, duration 600s). At t=60s, 50µL of a 0.5M aqueous NaOH solution was added through the injection port (continuous monitoring, no pause). At t=540s, the experiment was paused and 50µL of a 0.5% aqueous solution of Triton-X 100 was added. The experiment was then re-started after 30s of stirring time. The data was analysed as reported previously [8].

CF ASSAY

Vesicle preparation: Modified from published procedures [11]: 0.45g 5(6)-Carboxyfluorescein, (CF) was added to ~5 mL deionized water, solvated by titration of 1M potassium hydroxide to pH 7.5 (to form K^+CF^-), evaporated *in vacuo* and further dried under vacuum for 48 hours. The CF salt was diluted with CF buffer (10 mM Tris·HCl, 0.04 M KCl in deionized H_2O), to KCF solution of 0.1 M (10 mL) (pH 7.5 with 1M HCl). To a 50 mL round bottom flask, 4 mL of lipid stock (8:1:1 PC:PA:Cholesterol in $CHCl_3$) was dried as noted previously. The lipid was re-suspended in diethyl ether (6 mL) and 2mL of the KCF solution was then added. Sonication was used to disperse the two phases to a cloudy orange dispersion (power = 2.5, probe tip at the interface of the two phases). This dispersion was evaporated slowly under vacuum until bubbling from ether removal stopped. Then 1 mL external buffer (10 mM Tris·HCl, 0.14 M KCl in deionized H_2O, pH 7.5 with HCl) was added and rotary evaporation of the suspension continued to remove any excess ether for 30 min. The liposomes were sized with the membrane extrusion apparatus 19 times (500µL vesicle solution x 3) and then size-exclusion filtered as noted. The cloudy fraction, after the first four cloudy drops, was collected, for a total volume of vesicle suspension of ~1.5mL. The diameter of the resulting vesicles was ~200nm (measured by dynamic light scattering). The vesicle solution was stored in the fridge and used within 12 hours.

Typical experiment: 160 µL external buffer (10 mM Tris·HCl, 0.14 M KCl, pH 7.5) and 30 µL test solution (compound in THF or MeOH or 5% aqueous Triton X-100) was added to a 1.5mL Eppendorf tube and vortexed briefly. To each tube, 20µL CF vesicle suspension was added, vortexed for 10 seconds, and allowed to incubate at room temperature for 30 min. Each sample was then diluted to 5% in external buffer (1.5mL total volume, 0.6mL solution used for each trial). Samples were excited at 475nm (slits= 2 nm, integration = 1s) and the fluorescence emission scan was measured from 500-550nm in a 1 x 10mm quartz cell at T=20^0C. The average emission intensity at λ_{max} (~515 nm) was determined for each sample concentration. The percentage of CF released was calculated as $I(\%)=[(I_{sample}-I_{MeOH\ blank})/(I_{triton}-I_{blank})$ and plotted against test compound concentration.

PYRENE ASSAY [12, 13]: As reported previously [2, 8]. Pyrene was purified by column chromatography and sublimation, and dissolved in deoxygenated, HPLC grade MeOH at 0.625mg/mL. This was then diluted to 0.025mg/mL to make the stock pyrene solution used for the assay. In a typical experiment, 25µL of test compound in MeOH, 9µL stock pyrene solution and 0.5mL aqueous buffer (10 mM Na_3PO_4, 100 mM NaCl in deionized water, pH 6.4) were added to a 1 x 10 mm quartz fluorescence cuvette, and allowed to equilibrate at T= 20^0C for 3 minutes. Scans were taken with an excitation wavelength of 331 nm and the emission collected between 365-400 nm (excitation slit = 3 nm, emission = 1.5nm, integration = 0.5s, step size= 0.25 s^{-1}). Spectra were also collected for solutions that contained all components except pyrene (25µL MeOH blank). A ratio of the emission intensity at λ_{max} ~ 373 nm (I_1) and λ_{max} ~ 383 nm (I_3) was calculated and plotted against test compound concentration.

QUENCHING ASSAYS: For solution quenching studies, experiments were carried out in 10 x 10mm quartz fluorescence cuvettes, T=20^0C, excitation slit = emission slit = 3nm for Dip-containing compounds and 1nm for Trip-containing oligomers. Solvents used were either MeOH or aqueous (10mM Bis-Tris, 100mM NaCl, pH= 6.4 or 100mM NaCl), solutions of quencher ($CuSO_4$) were made in the appropriate solvent. Steady-state scans of compound without copper and with increasing concentrations of copper were taken at time intervals of 1, 5 and 10minutes, the fluorescence intensity in the absence (I_O) and presence (I_Q) of quencher were then plotted as a ratio (I_O/I_Q) versus copper concentration (Stern-Volmer analysis).

PARTITIONING COEFFICIENT ASSAY: From [14]. To a 10 x 10mm fluorescence cuvette a constant concentration of test compound in THF (no more than 20µL) was added to increasing concentrations of lipid vesicles prepared as noted and containing 10mM BisTris, 100mM NaCl, pH= 6.4 both inside and out. The volume of aqueous buffer was modified to maintain a constant volume of 2.00mL. The solutions were left to equilibrate at T=20^0C for 3 minutes, and then steady-state spectra were collected. The fluorescence intensity without vesicles (I_o) and in the presence of vesicles (I_i) were then plotted against the concentration of lipid (as vesicles).

Supporting information; fluorescence

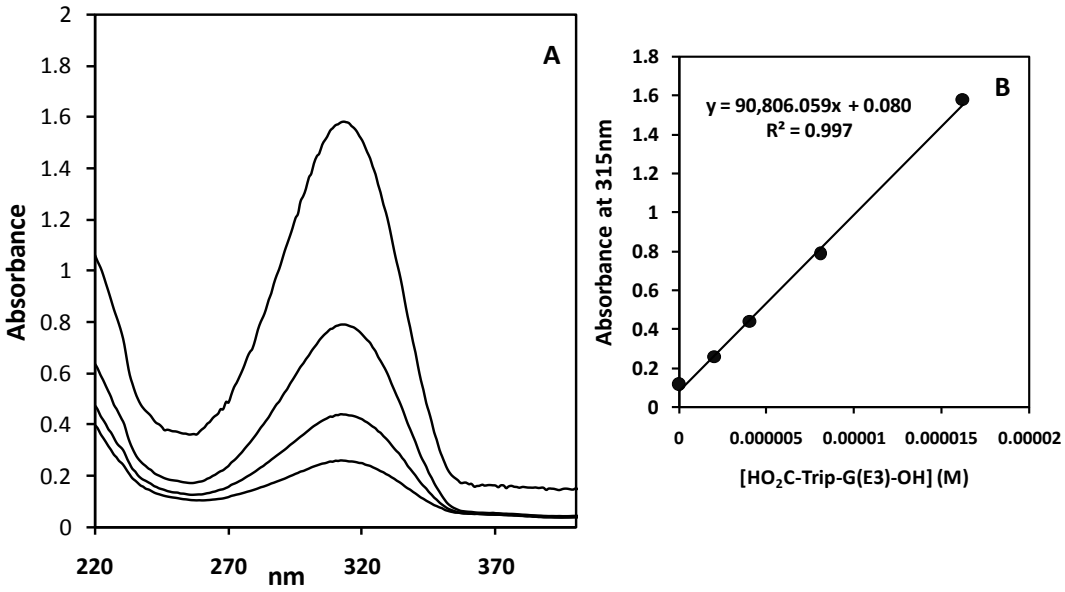


Figure S1: UV absorption data for HO_2C-**Trip-G(E3)**-OH in MeOH. **A**: raw data, **B**: absorption at maximum wavelength as a function of concentration; determination of ε. Other Trip compounds had very similar extinction coefficients and spectra.

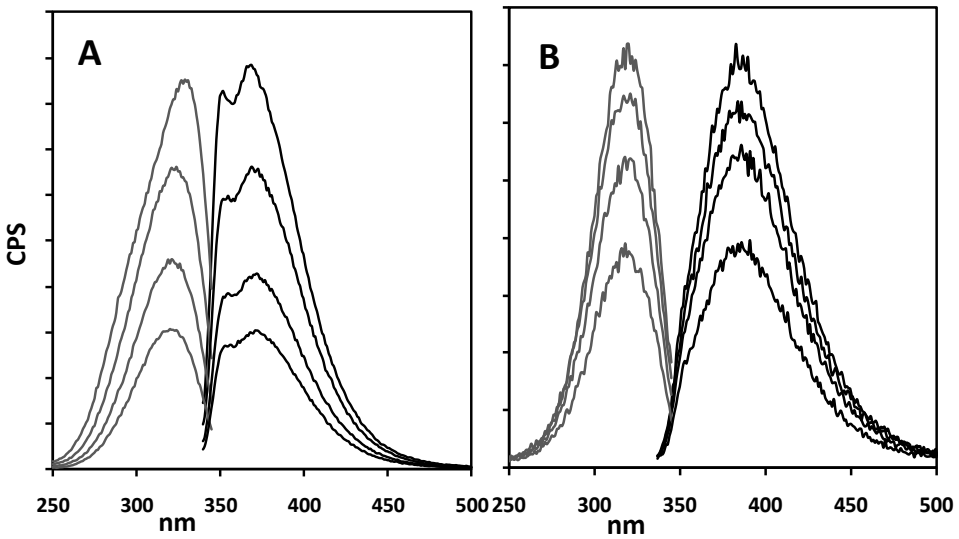


Figure S2: Fluorescence excitation (grey lines) and emission (black lines) spectra of HO_2C-**Trip-G(E3)**-OH in **A**: MeOH and **B**: aqueous buffer (10mM BisTris, 100mM NaCl, pH 6.4). From top to bottom, [cmpd]= 16, 8, 4, 2µM. The scale in **B** is 10% of **A**. Other compounds had very similar spectra in MeOH, while the aqueous fluorescence was nearly undetectable.

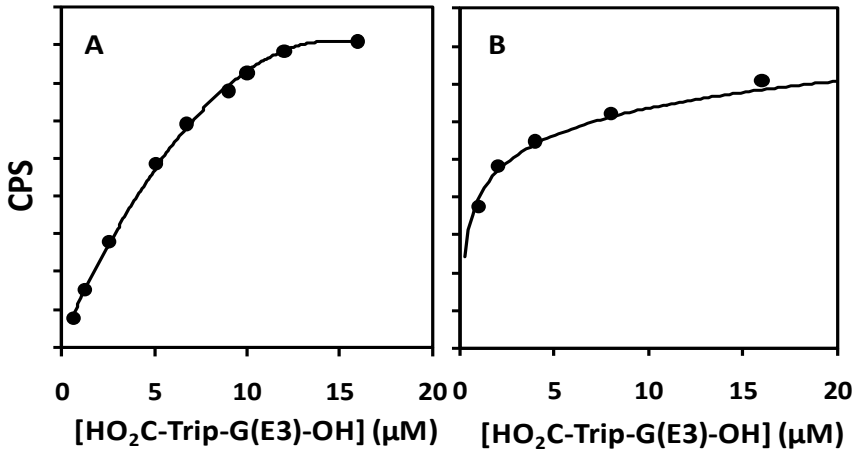


Figure S3: Fluorescence emission intensity as a function of concentration of HO_2C-**Trip-G(E3)**-OH in **A**: MeOH, **B**: aqueous buffer (10mM BisTris, 100mM NaCl, pH 6.4). The lines are to guide the eye.

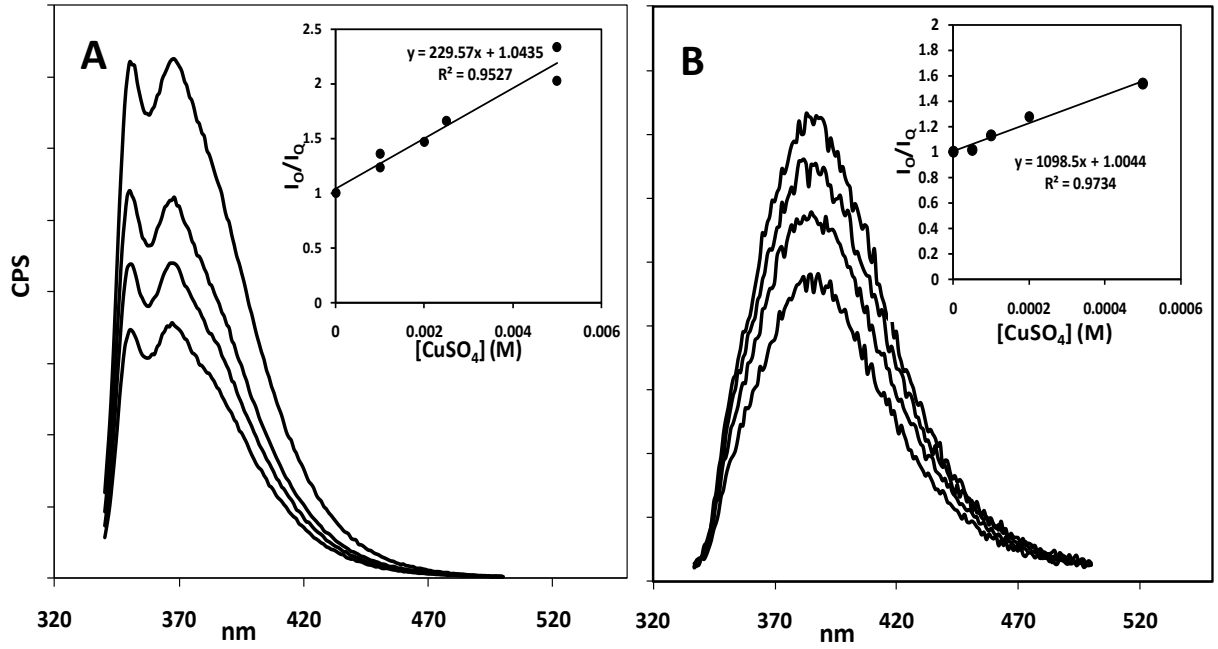


Figure S4: Quenching of 16μM HO_2C-**Trip-G(E3)-OH** by $CuSO_4$ in **A**: MeOH; [$CuSO_4$] from top to bottom = 0, 2, 3, 4mM. INSET: Stern-Volmer analysis of the data, K_{SV}= 229M^{-1}. **B**: aqueous buffer (10mM BisTris, 100mM NaCl, pH = 6.4), [$CuSO_4$] from top to bottom = 50, 100, 200, 500μM in aqueous BisTris buffer. INSET: Stern-Volmer analysis of the data, K_{SV}= 1098 M^{-1}.

PROPERTY	DIP	TRIP
Extinction coefficient ($M^{-1}cm^{-1}$)	20 000	90 000
Absorption: λMax (nm)	289	313
Excitation: λMax (nm)	305	325
Emission: λMax (nm)	320	365
CPS at λMax_{EM}[a]	300 000	>3 000 000
Quenched by $CuSO_4$ / K_{SV}	Yes / $1000M^{-1}$	Yes / $250M^{-1}$

Table S1: Comparison of representative photophysical parameters of the Dip and Trip fluorophores in methanolic solution. a= The reported values are for 16μM HO_2C-**Trip-G(E3)**-OH compared with the same concentration of HO_2C-**Dip-Hex-Hex-(G12)**-OH [2] with same slit widths (3nm); the CPS are above the instrument limit for the Trip compound.

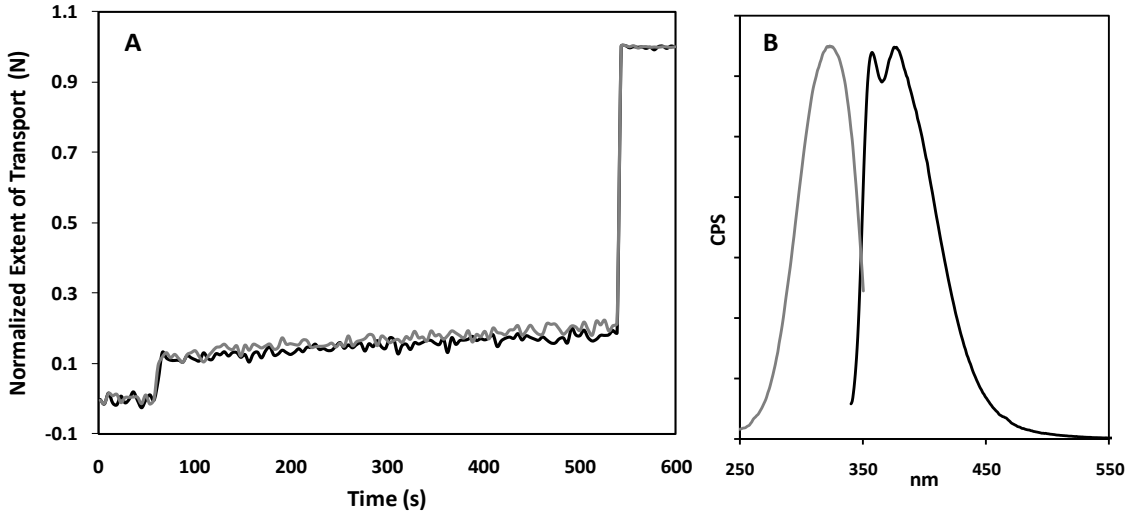


Figure S5: Transport and fluorescence of 0.5% HO_2C-**Trip-Hex-G(12)**-OH pre-loaded into lipid vesicles. **A**: HPTS scans of 100μL of compound-containing vesicles (black line) compared with THF blank (grey line) in 2mL phosphate buffer (10mM Na_3PO_4, 100mM NaCl, pH 6.4. **B**: Fluorescence excitation (grey line) and emission (black line) spectra of same solution, vesicles were visibly fluorescent under UV light.

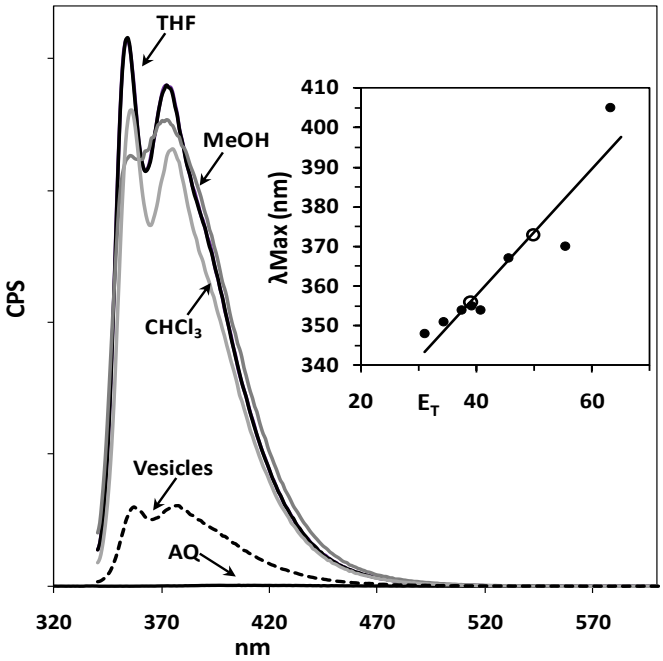


Figure S6: Solvent effects for HO_2C-**Trip-6-G(12)**-OH. Fluorescence emission spectra (Ex~325nm) for 17uM compound in selected solvents, and pre-loaded into vesicles. INSET: λMax as a function of solvent polarity. Open circles= tested solvents, black circles= fit of vesicle wavelengths onto linear relationship.

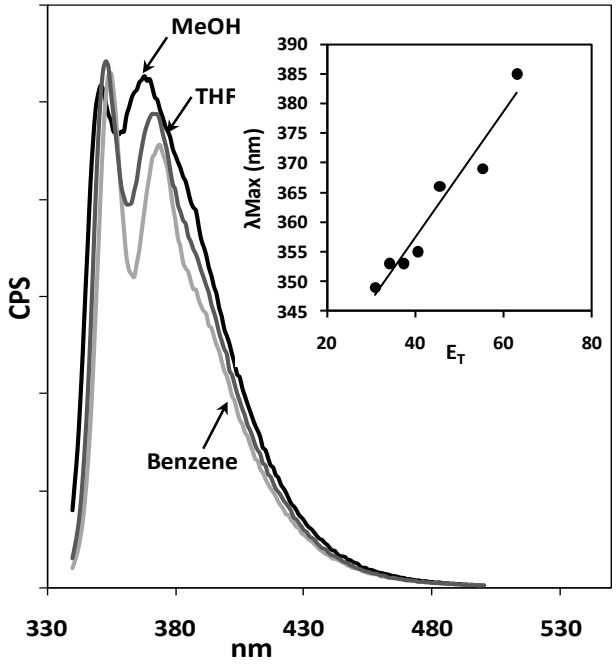


Figure S7: Solvent effects for HO_2C-**Trip-G(E3)**-OH. Fluorescence emission spectra (Ex~325nm) for 16µM compound in selected solvents. INSET: λMax as a function of solvent polarity.

SOLVENT	E_T	λMax_{EM} (nm)	
		HO_2C-Trip-Hex-G(12)-OH	HO_2C-Trip-G(E3)-OH
Water	63.1	405	385
MeOH	55.4	370	369
ACN	45.6	367	366
DCM	40.7	354	355
$CHCl_3$	39.1	355	n/a
THF	37.4	354	353
Benzene	34.3	351	353
Hexanes	31	348	349
VESICLES		356, 373	356, 375
PREDICTED E_T IN VESICLES		39,50[a]	39, 57[b]

Table S2: Solvent effects for Trip isomers. *a*= compound pre-loaded into vesicles, *b*= compound incubated with vesicles for 10mins.

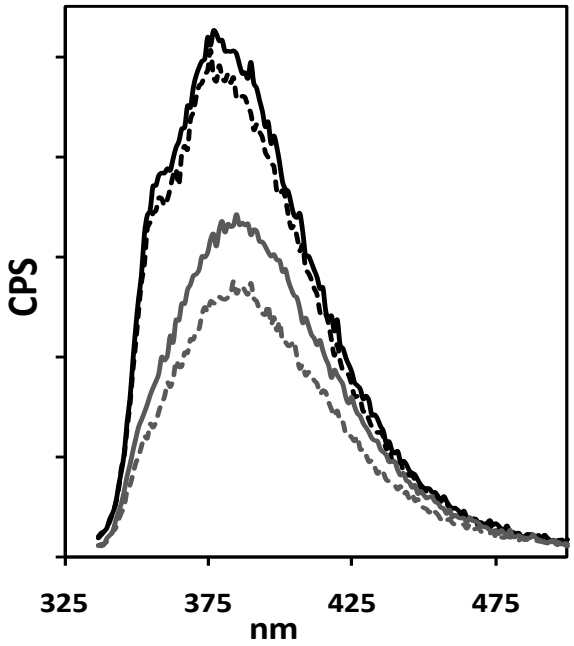


Figure S8: Fluorescence emission spectra of 15µM HO_2C-**Trip-G(E3)**-OH in aqueous buffer (10mM BisTris, 100mM NaCl, pH 6.4) in the presence (black) or absence (grey) of lipid vesicles, with the addition of 200µM $CuSO_4$ (dashed lines). Ex= 325nm.

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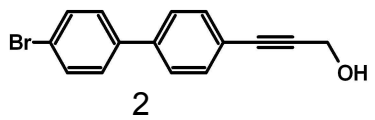

2

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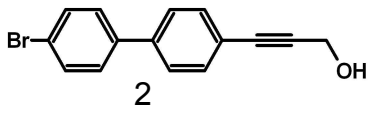

2

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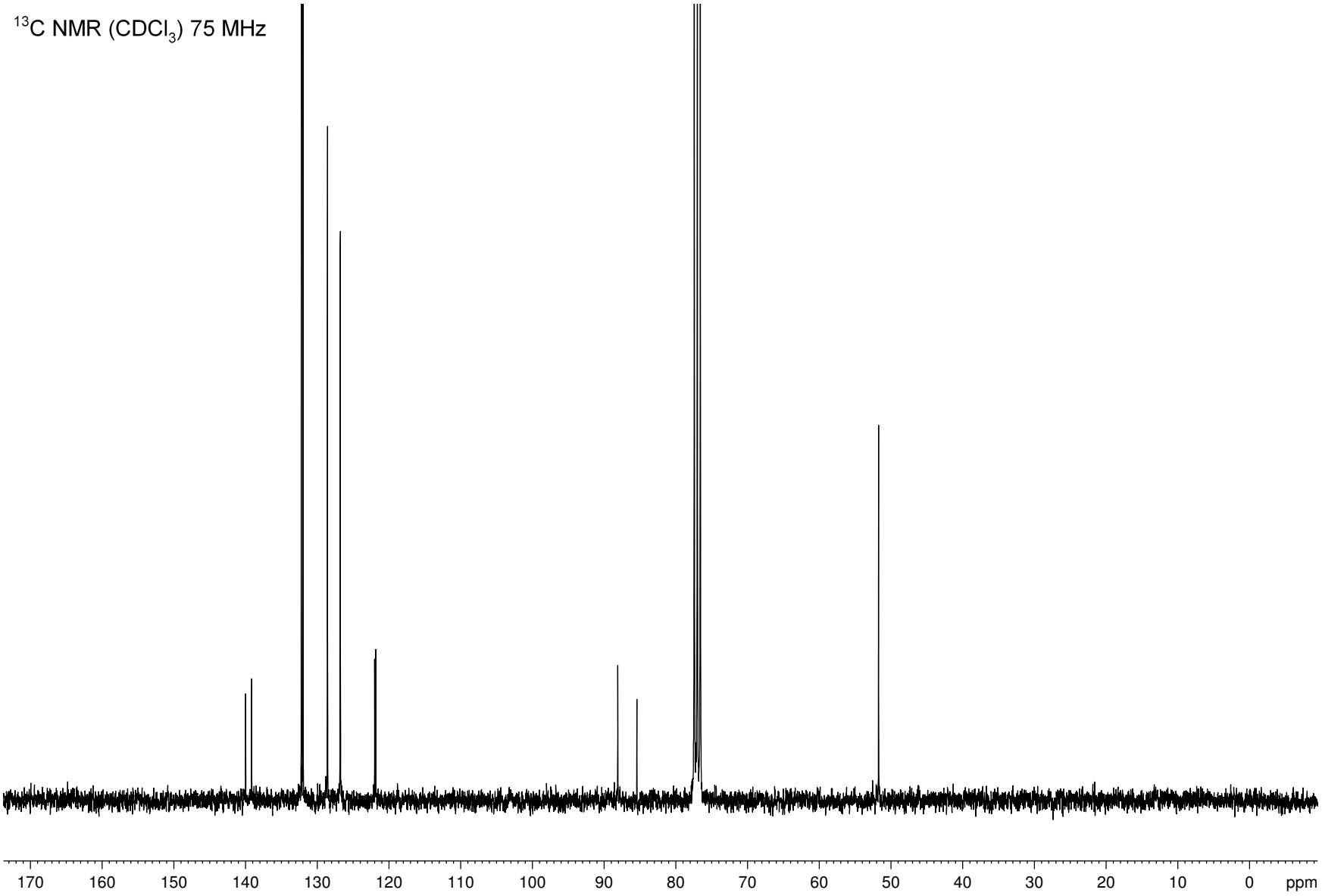

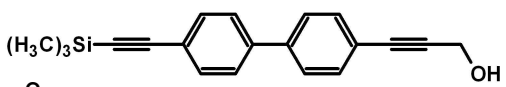

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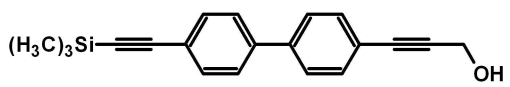

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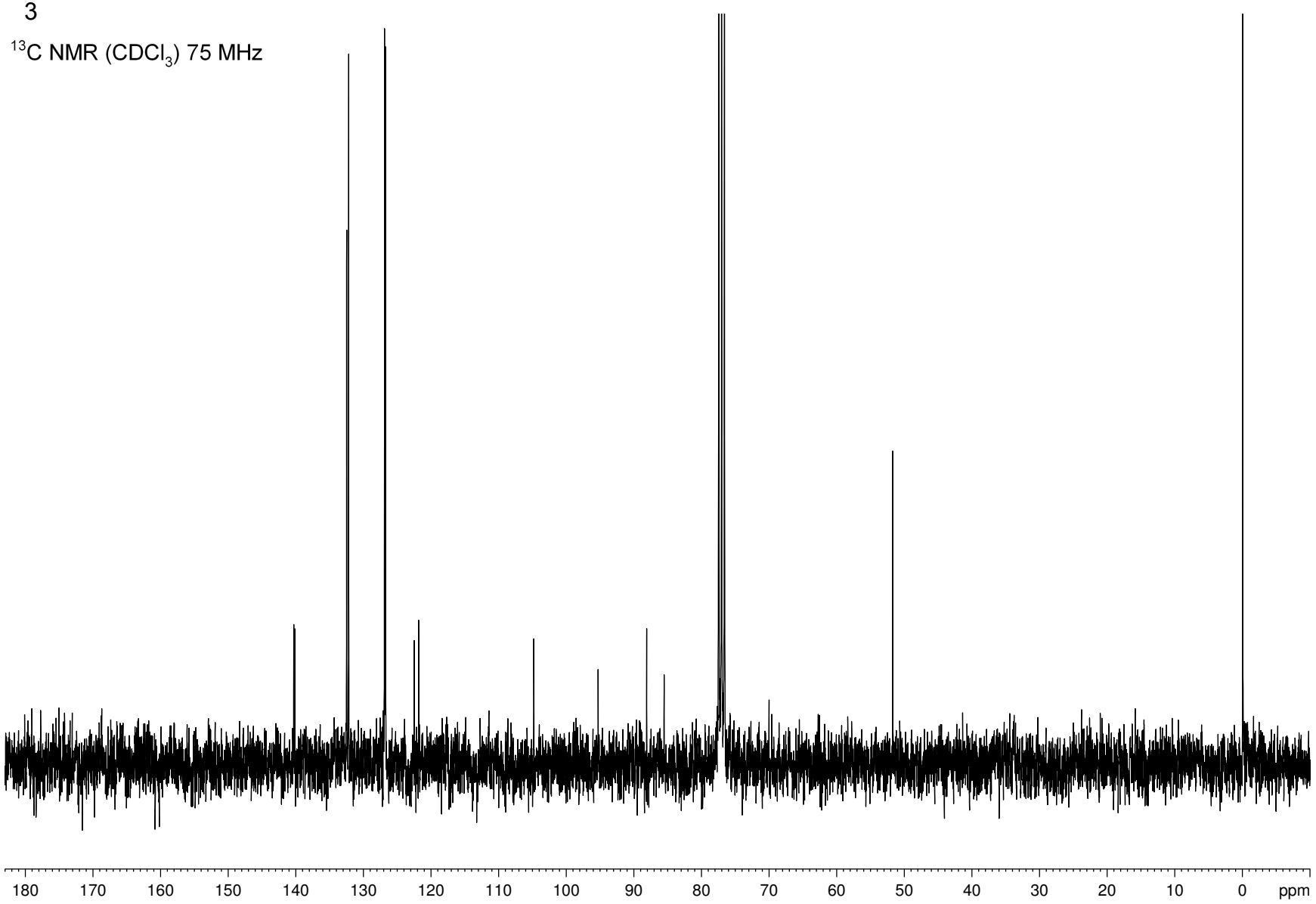

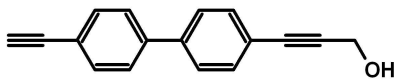

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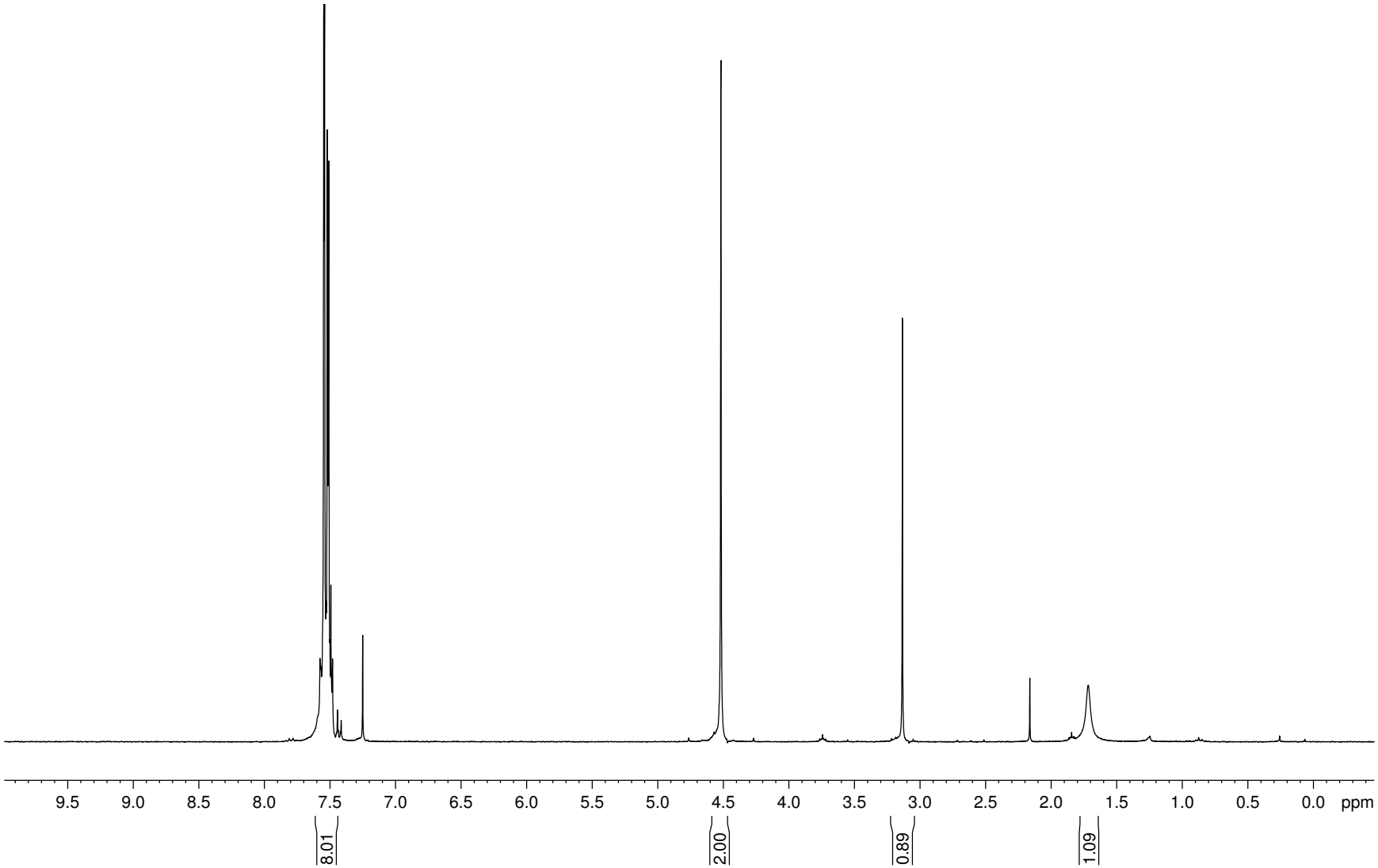

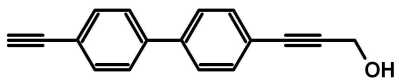

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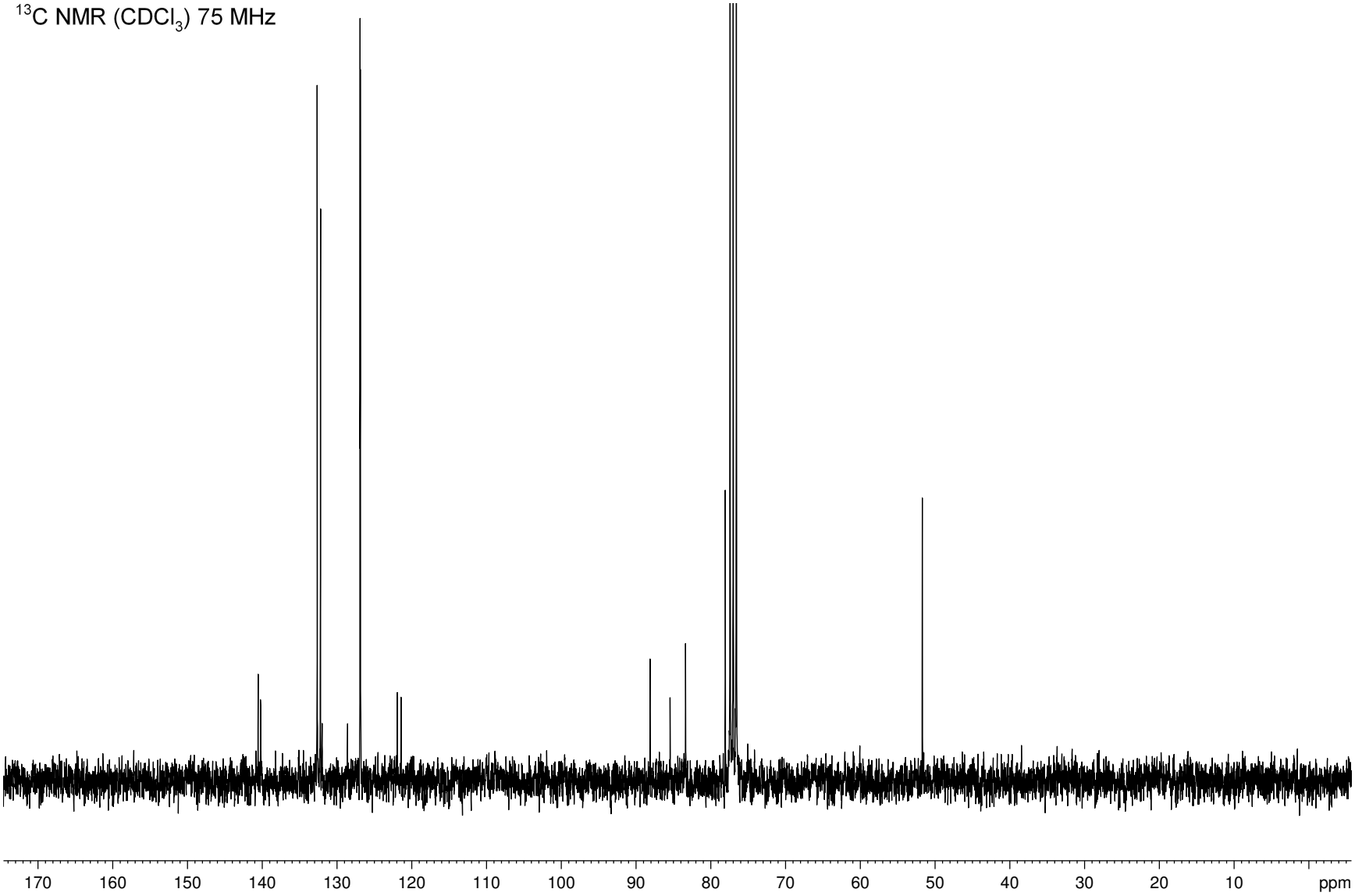

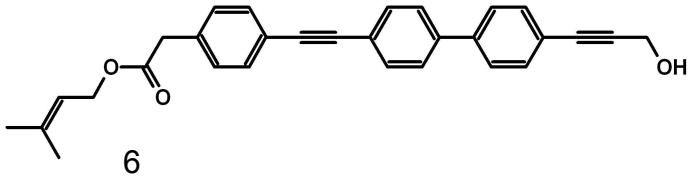

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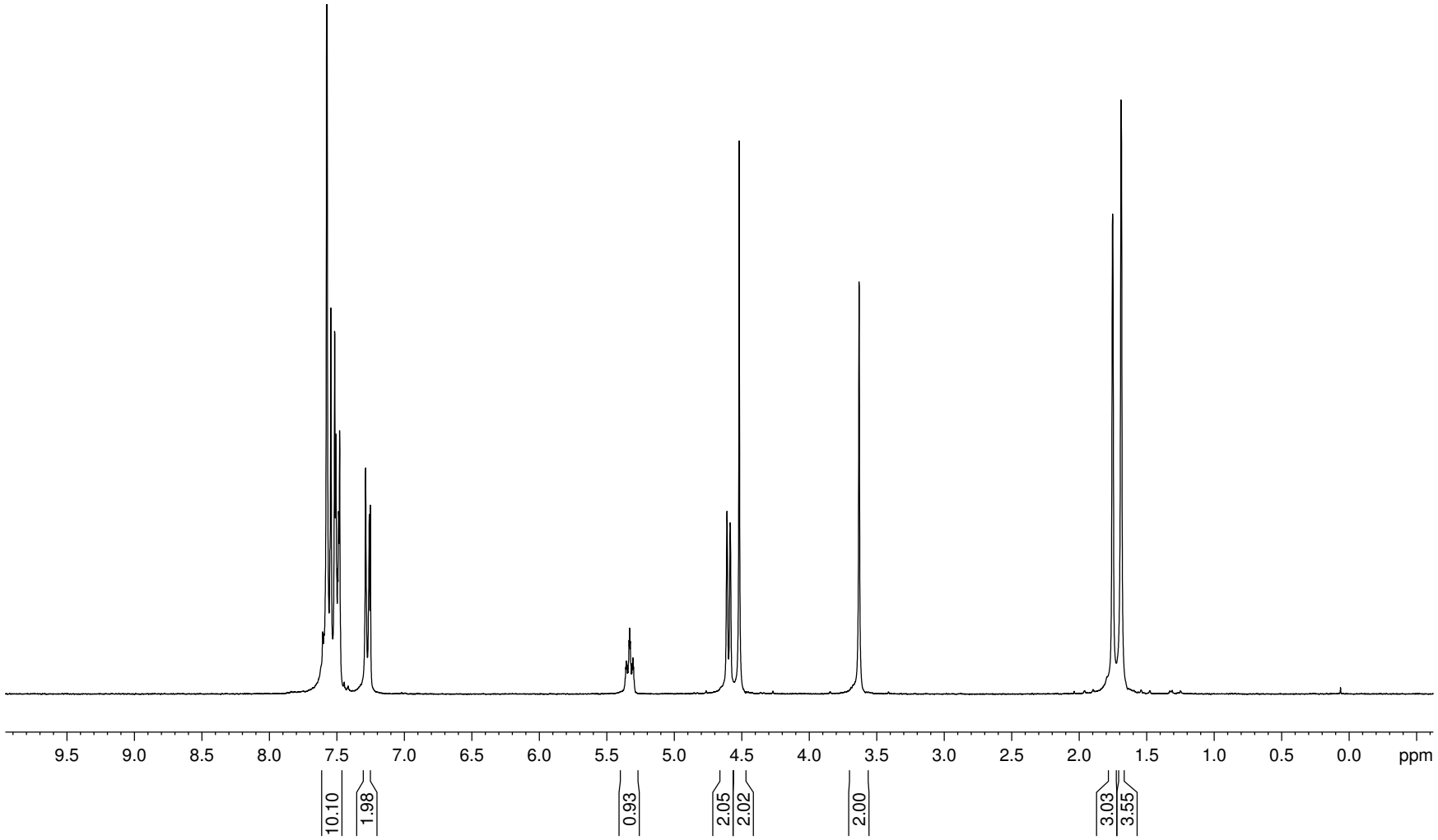

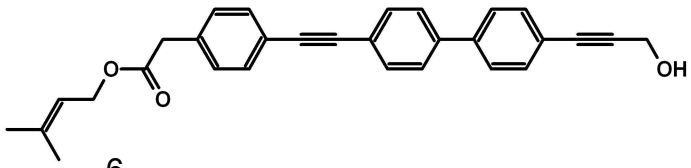

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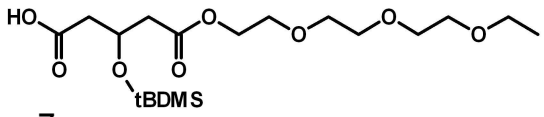

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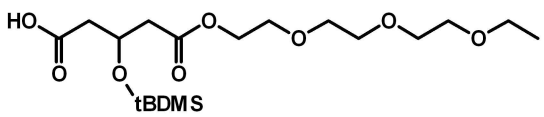

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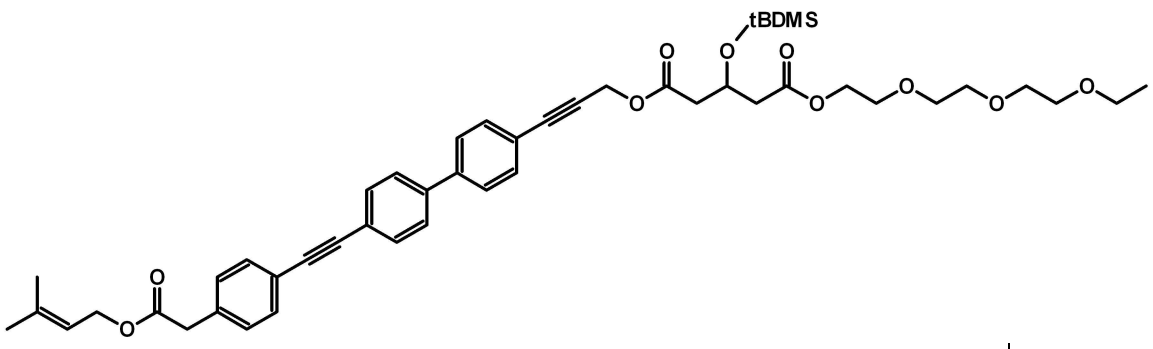

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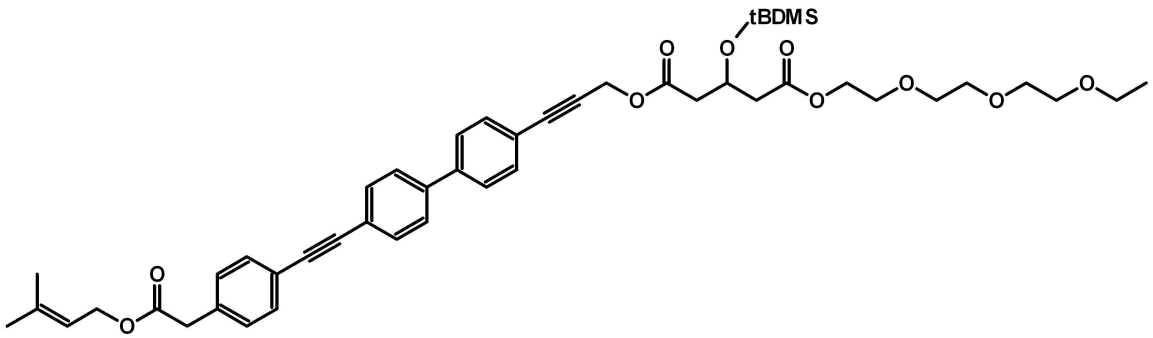

8

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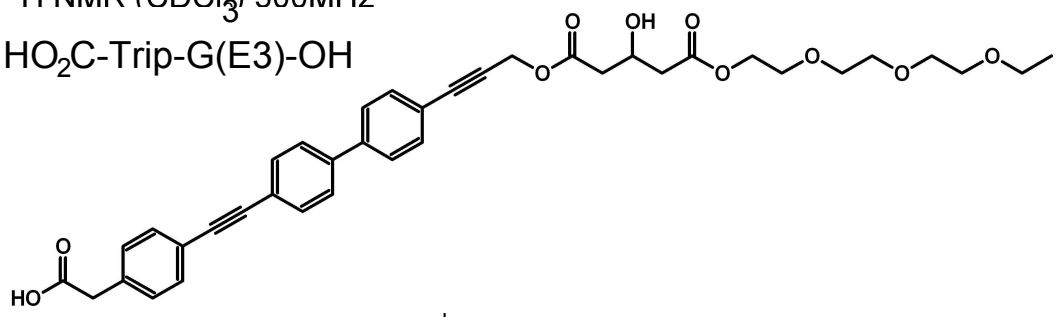
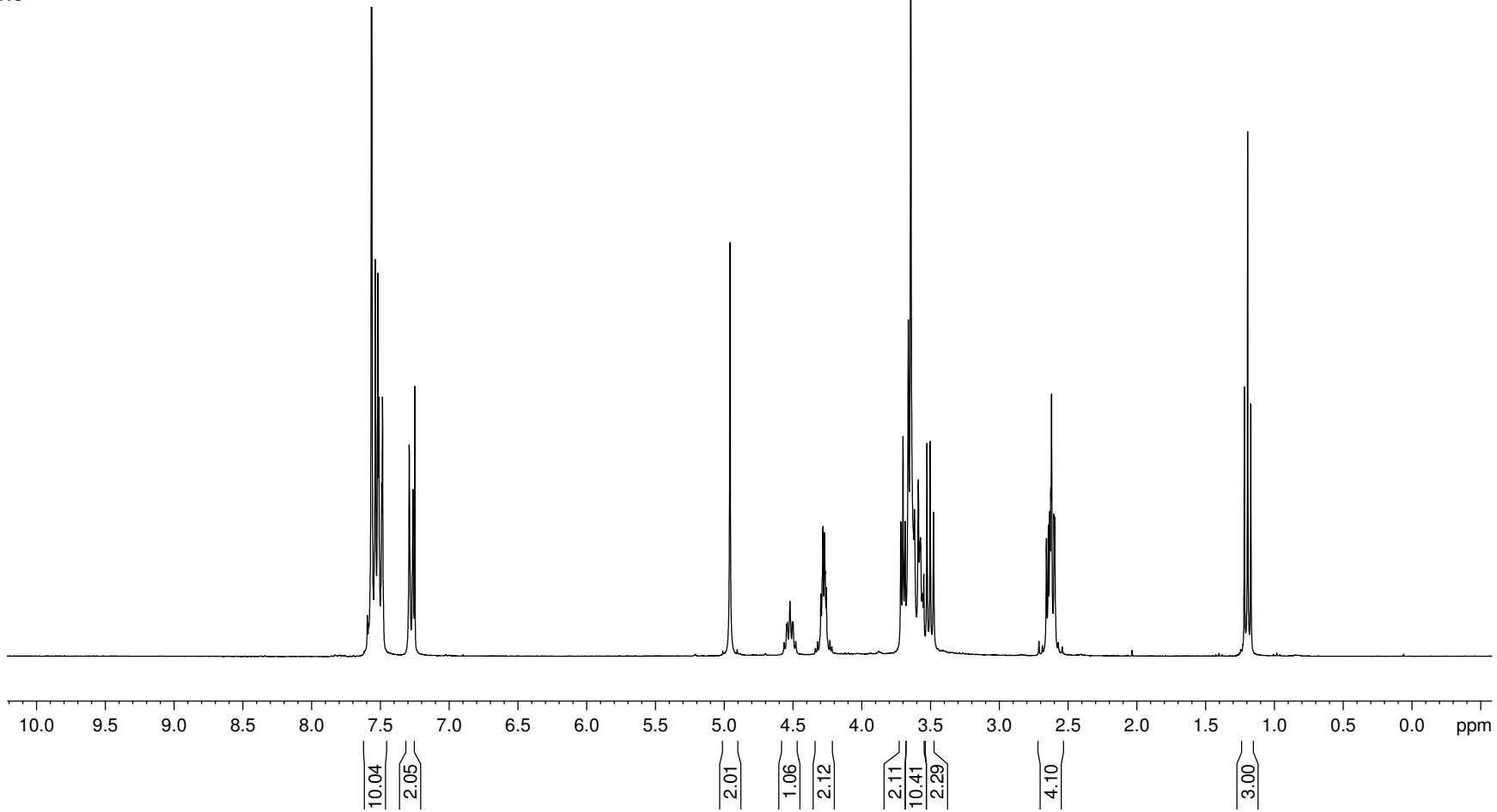
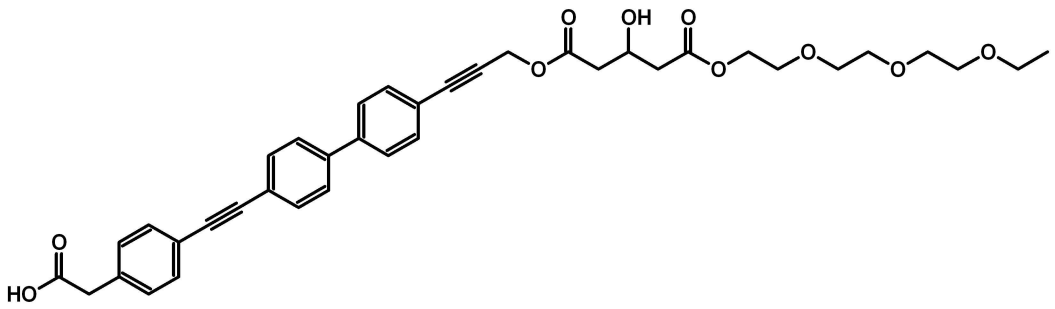
HO_2C-Trip-G(E3)-OH







HO_2C-Trip-G(E3)-OH

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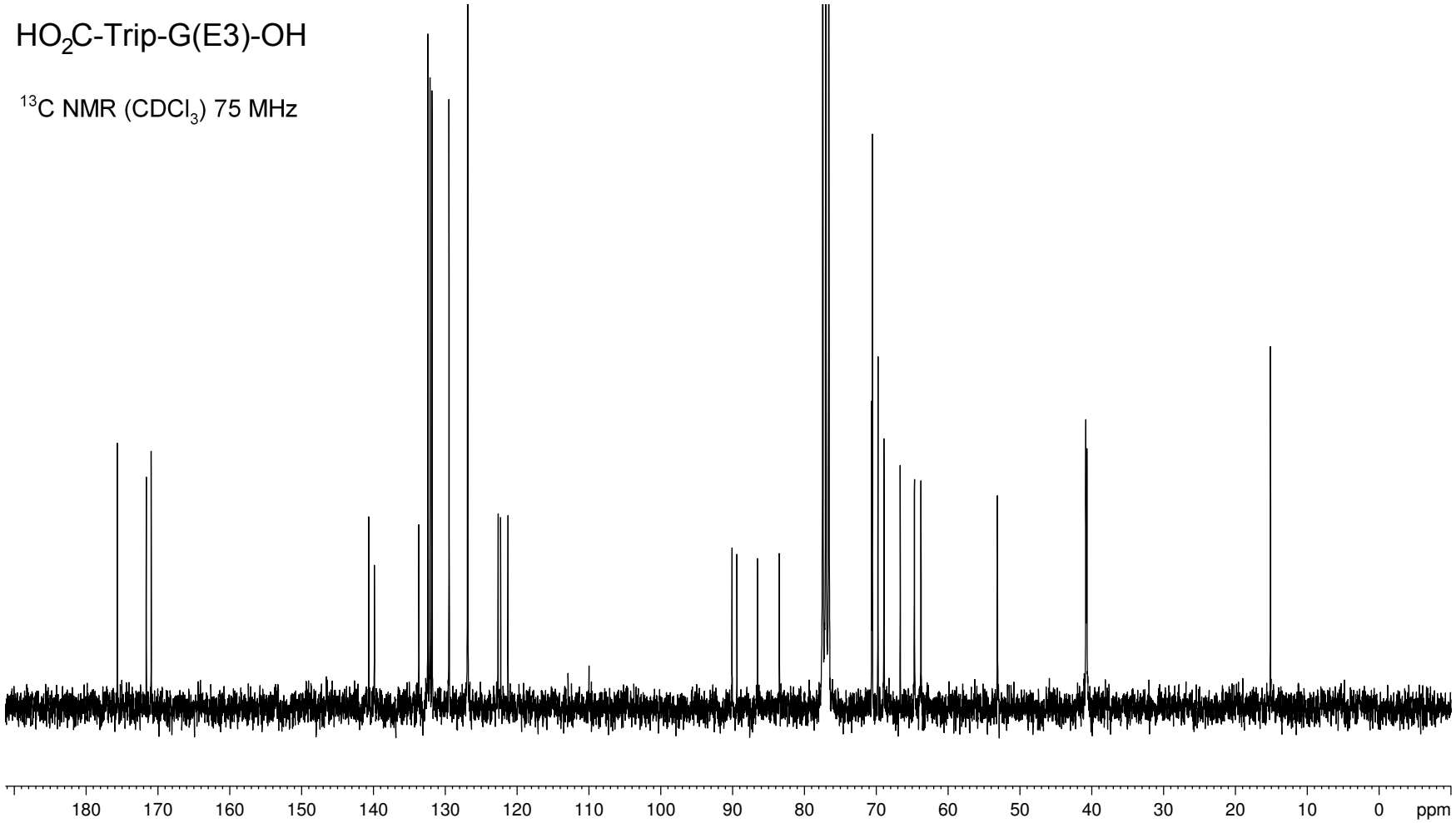

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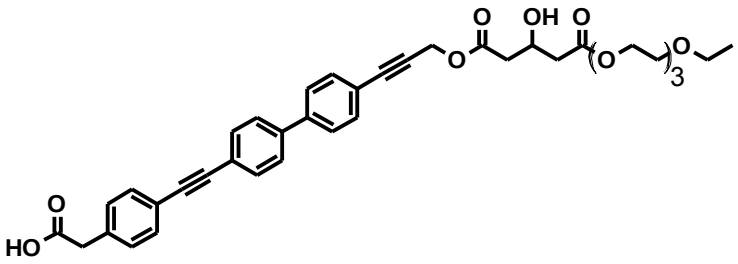
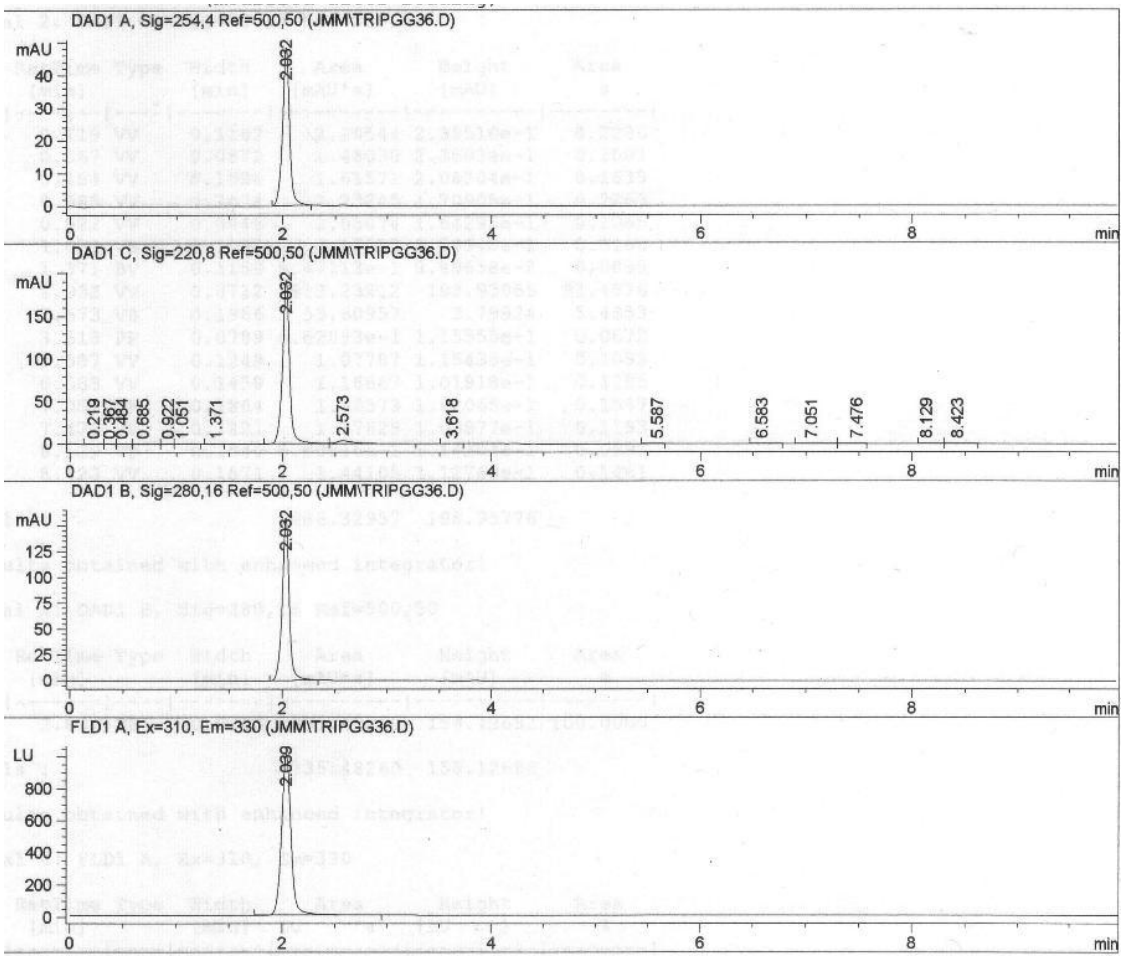
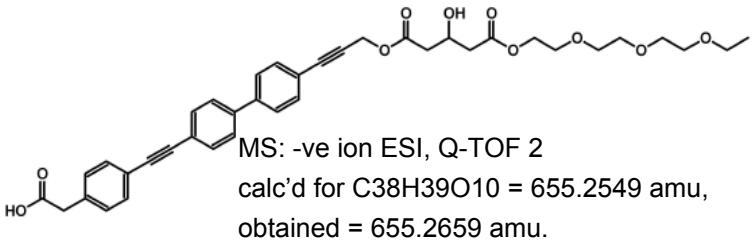


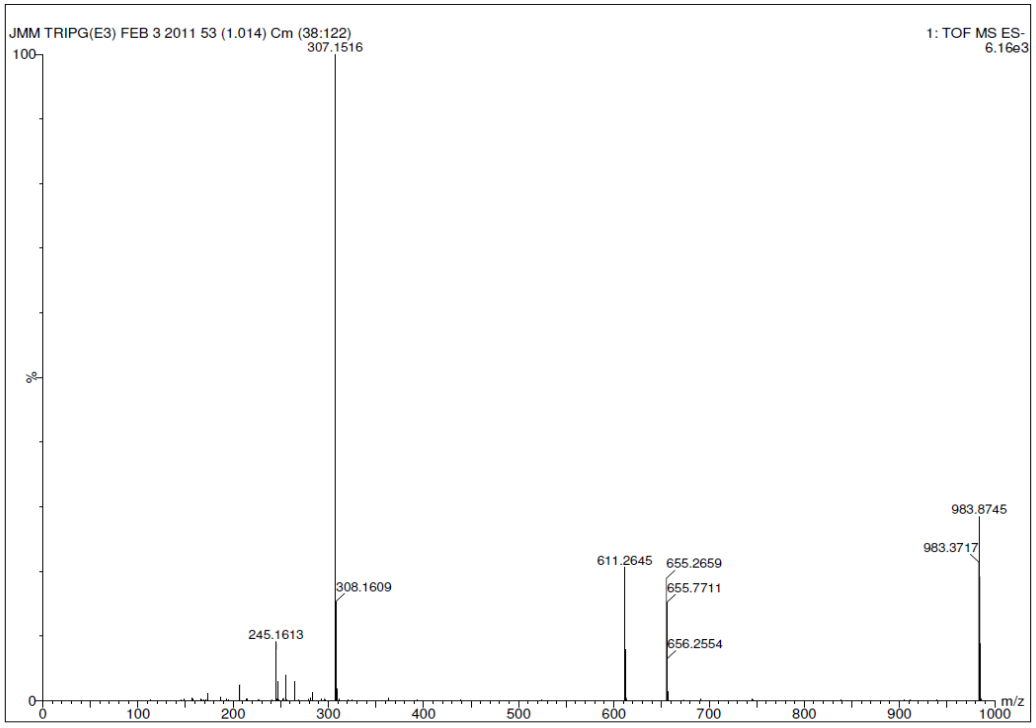


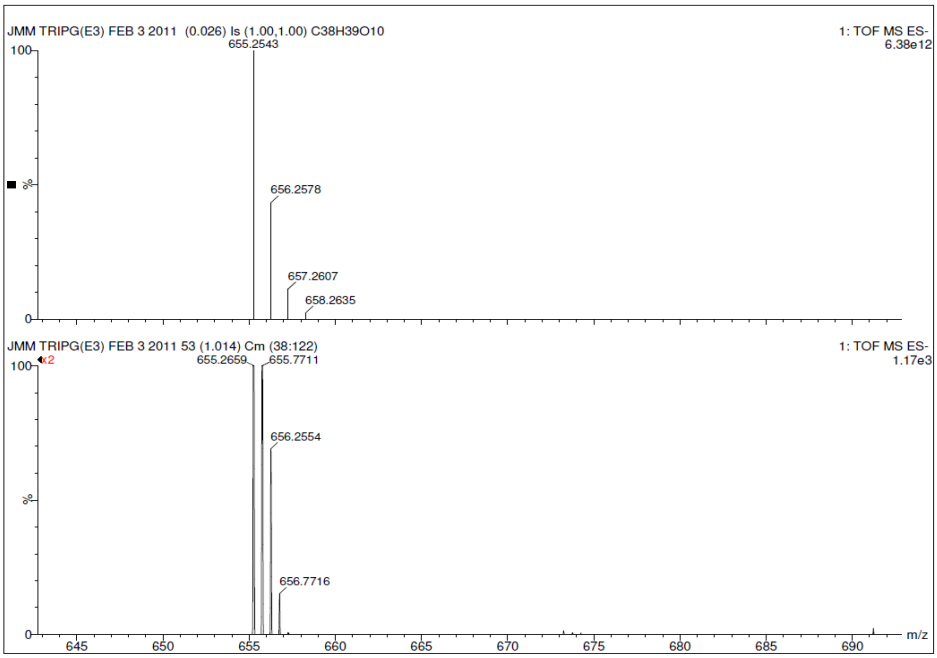
-HPLC trace of sample used for fluorescence and transport studies
-CONDITIONS: HP series 1100 HPLC
-Machery-Nagel RP C18 “Nucleosil” analytical column (4 mm x 250mm)
-3:1 CH_3OH:ACN as eluting solvents, flow 1mL/min

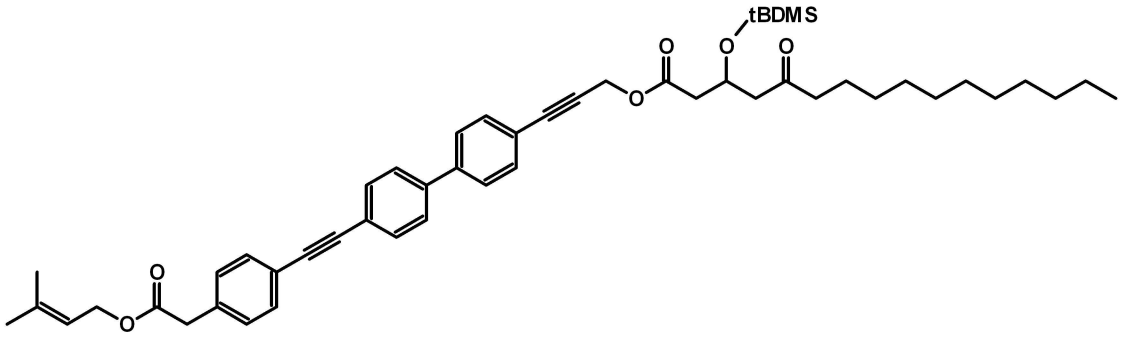
HO_2C-Trip-G(E3)-OH



MS: -ve ion ESI, Q-TOF 2
calc'd for C38H39O10 = 655.2549 amu,
obtained = 655.2659 amu.

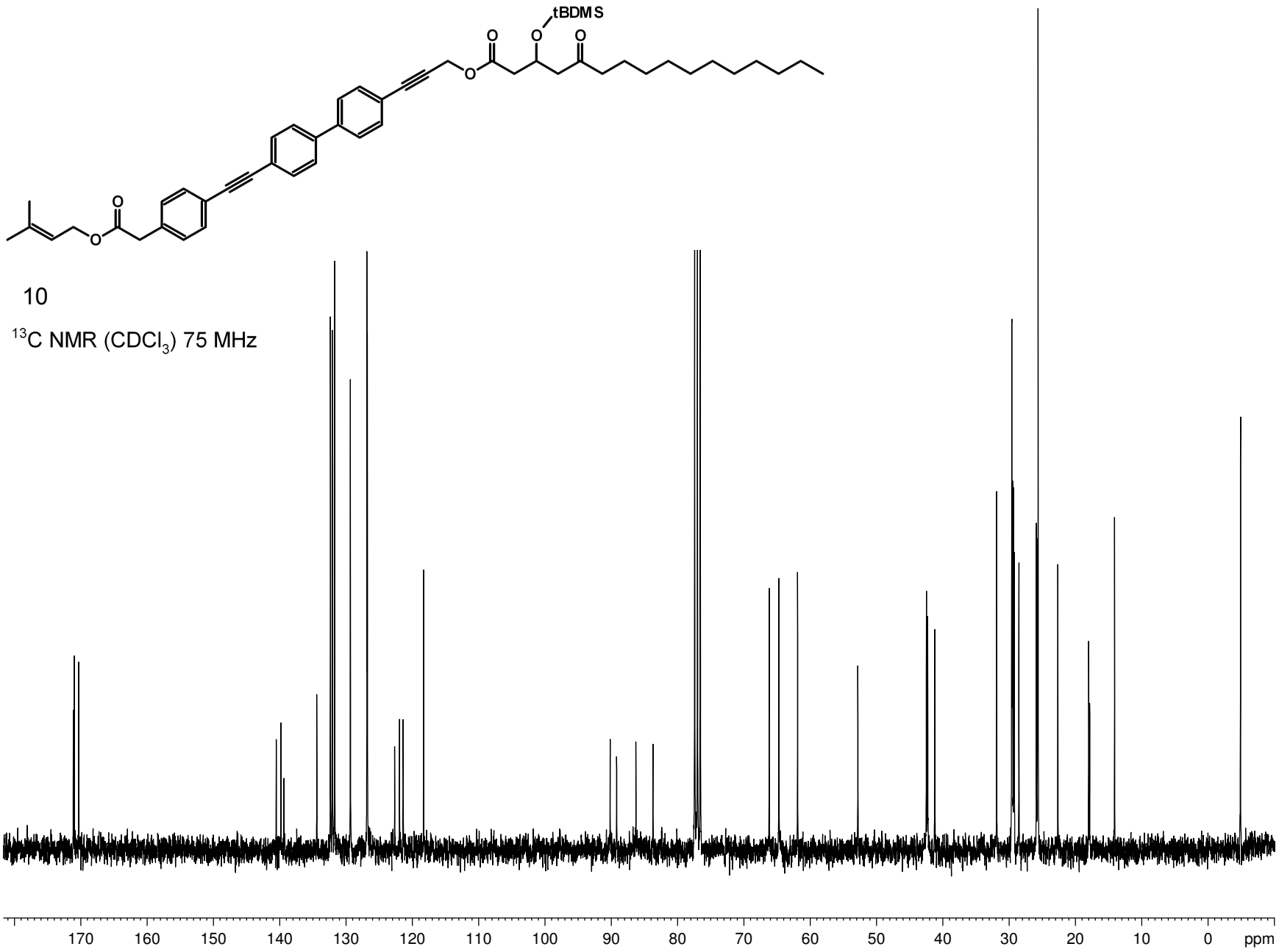


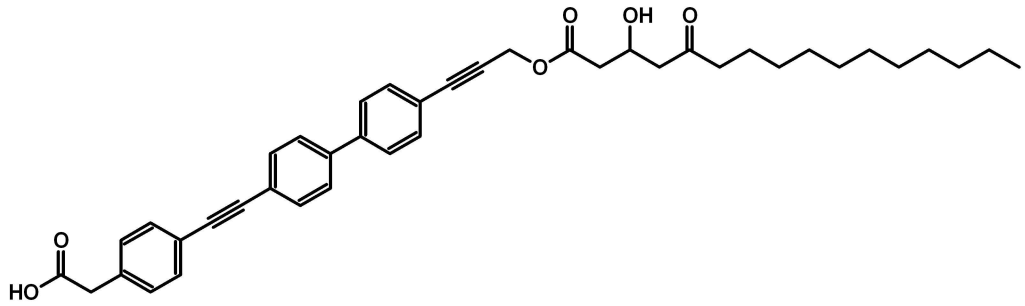




10

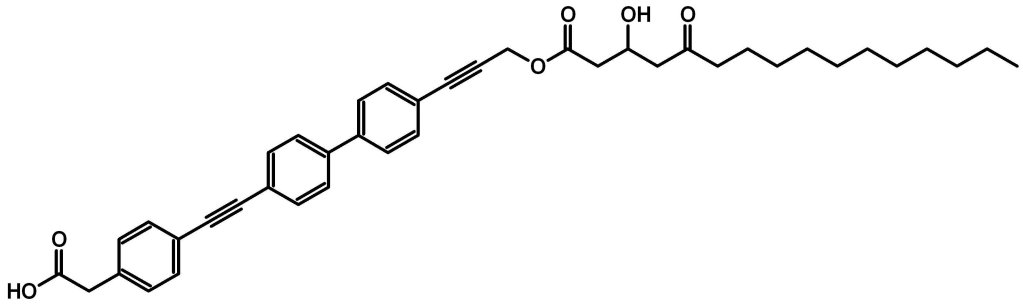
^{1}H NMR ($CDCl_3$) 300MHz





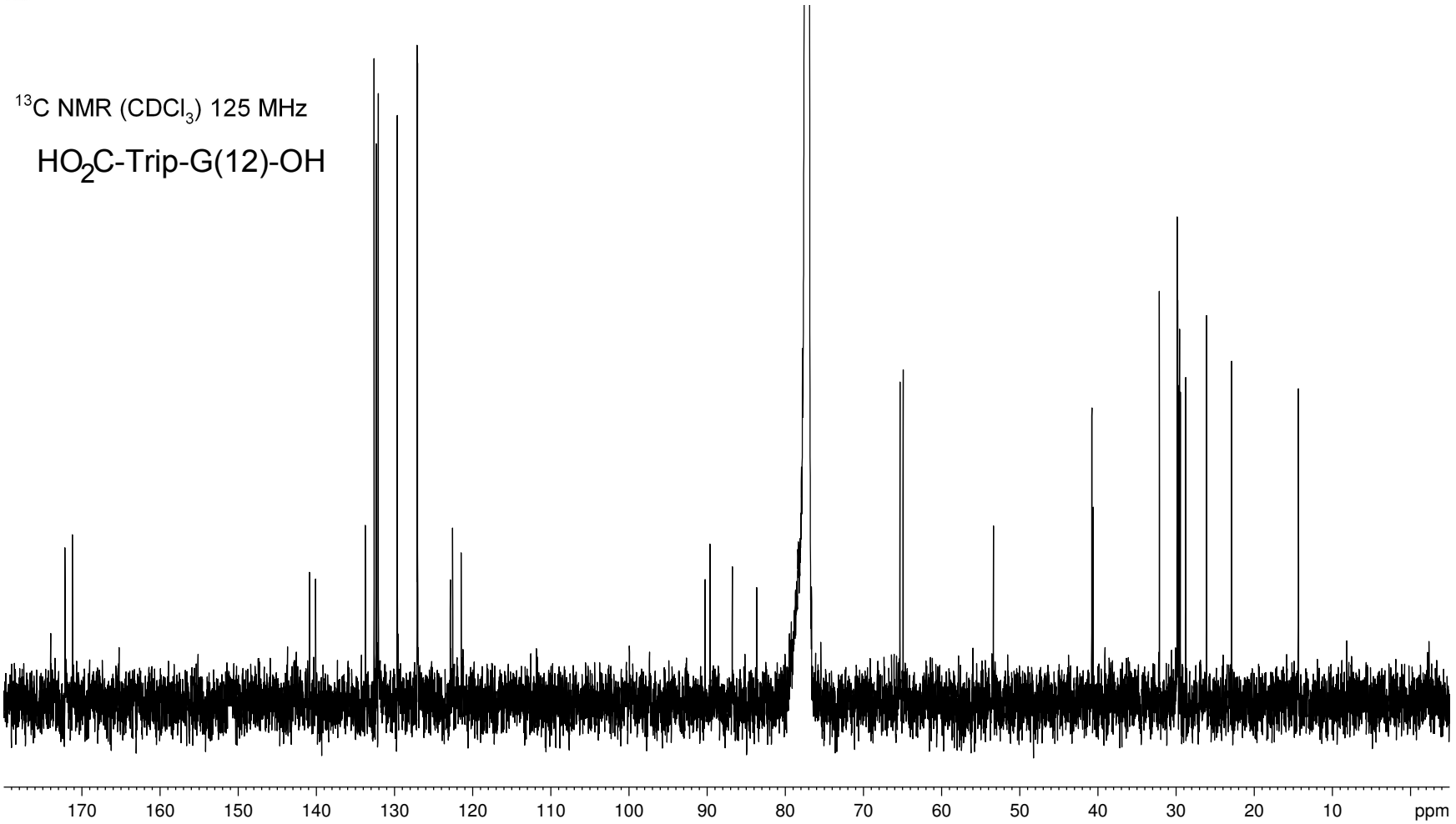
^{1}H NMR ($CDCl_3$) 500MHz

HO_2C-Trip-G(12)-OH

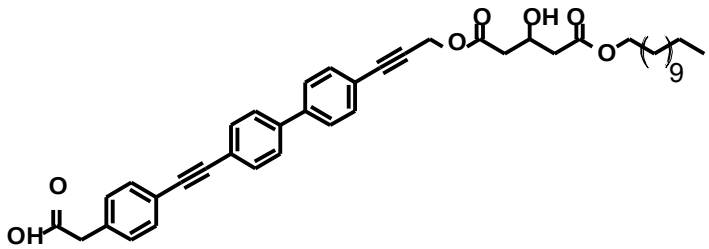


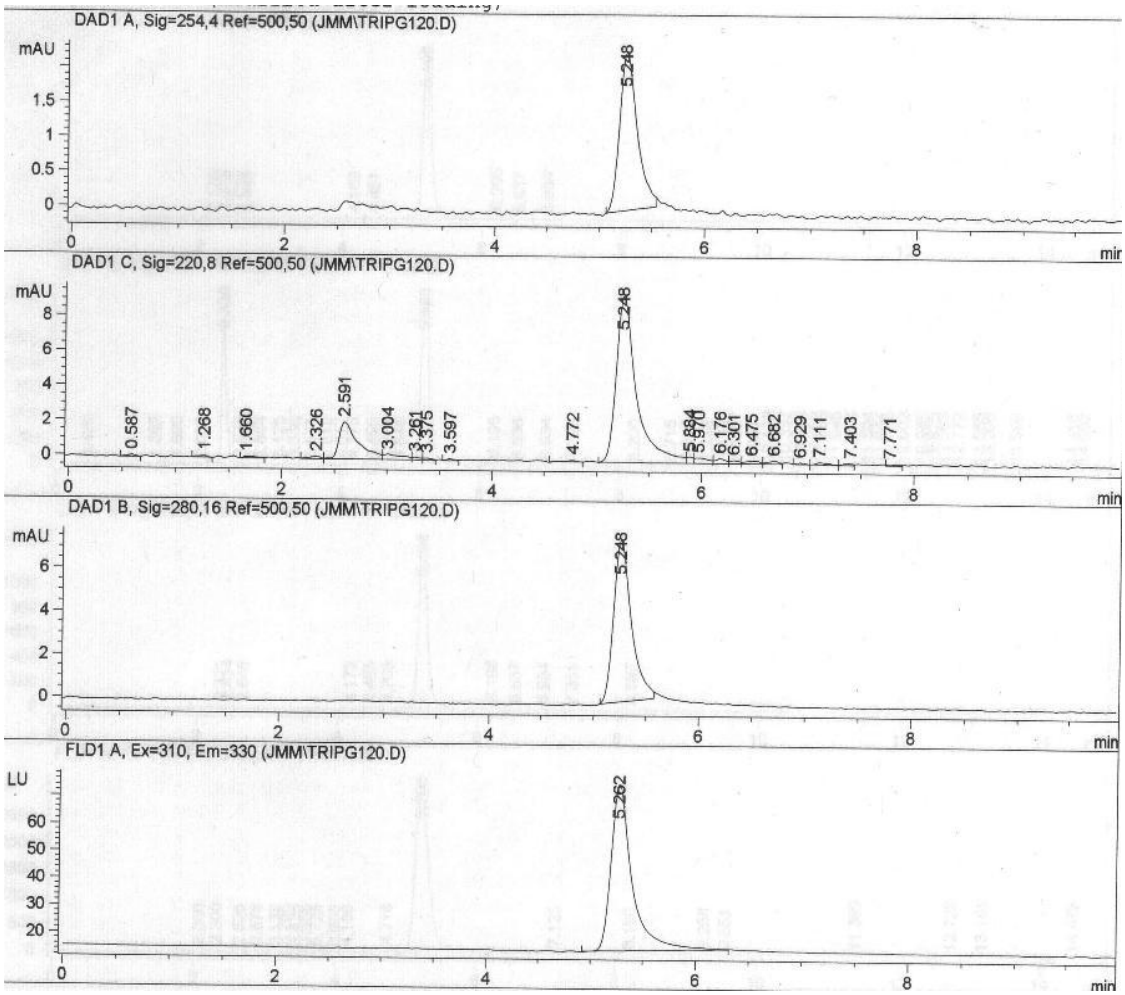
^{13}C NMR ($CDCl_3$) 125 MHz

HO_2C-Trip-G(12)-OH



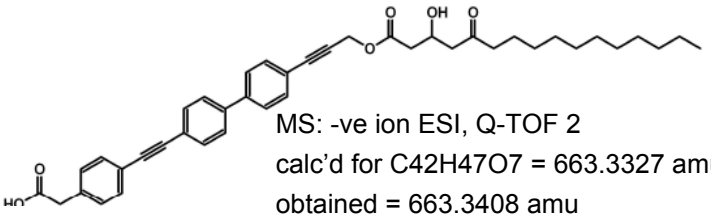
HO_2C-Trip-G(12)-OH



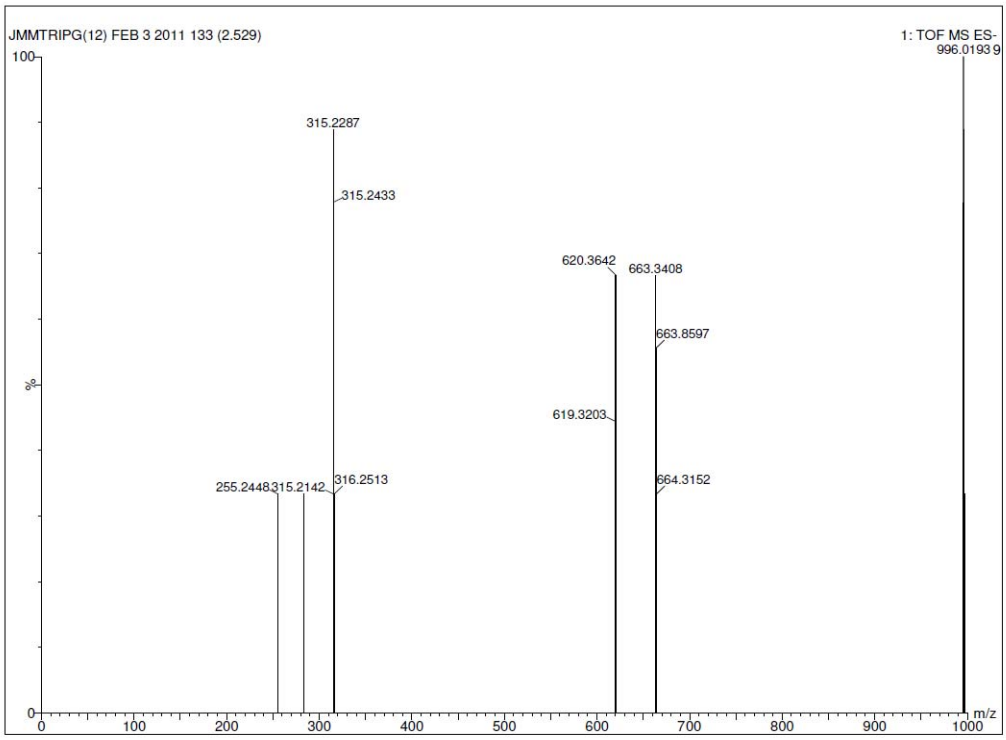


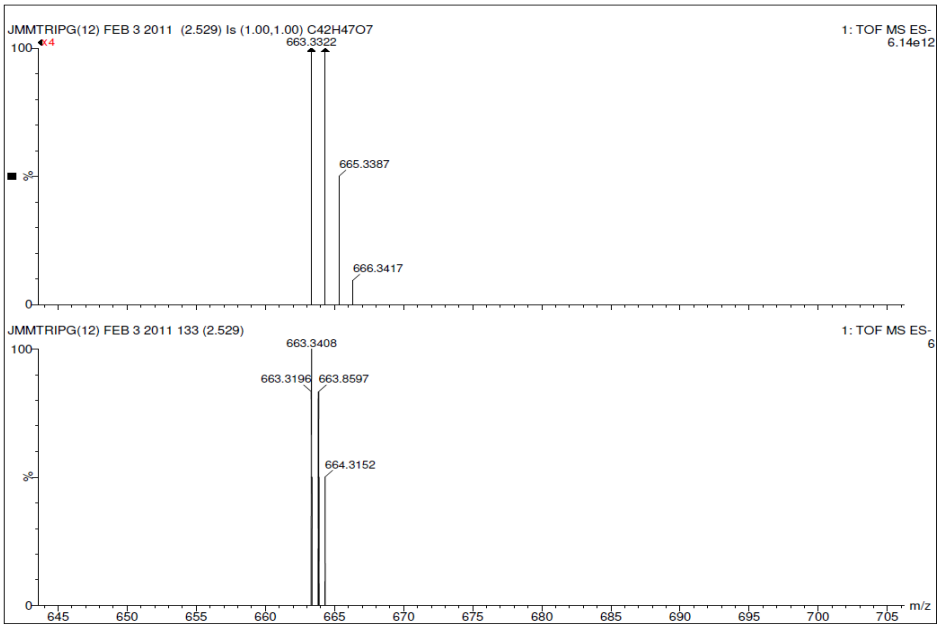
-HPLC trace of sample used for fluorescence and transport studies
-CONDITIONS: HP series 1100 HPLC
-Machery-Nagel RP C18 “Nucleosil” analytical column (4 mm x 250mm)
-1:1 CH_3OH:ACN as eluting solvents, flow 1mL/min

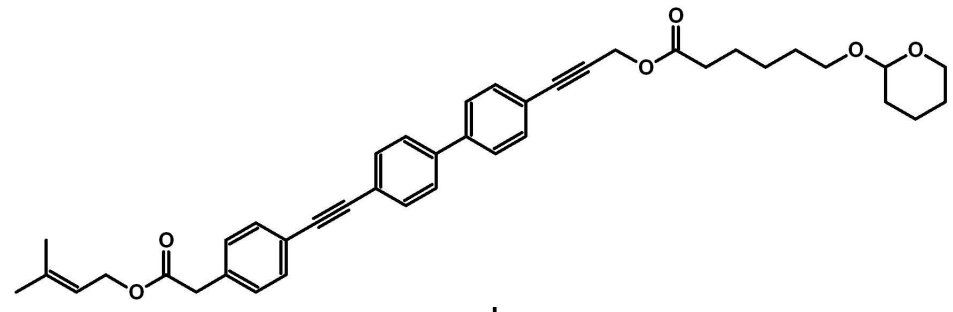
HO_2C-Trip-G(12)-OH



MS: -ve ion ESI, Q-TOF 2
calc'd for C42H47O7 = 663.3327 amu,
obtained = 663.3408 amu

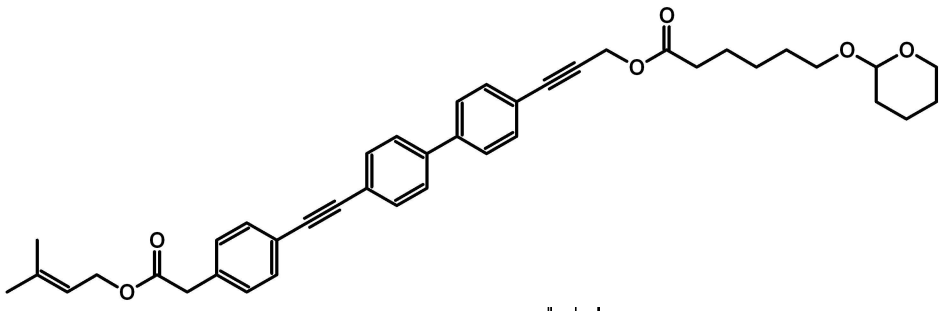






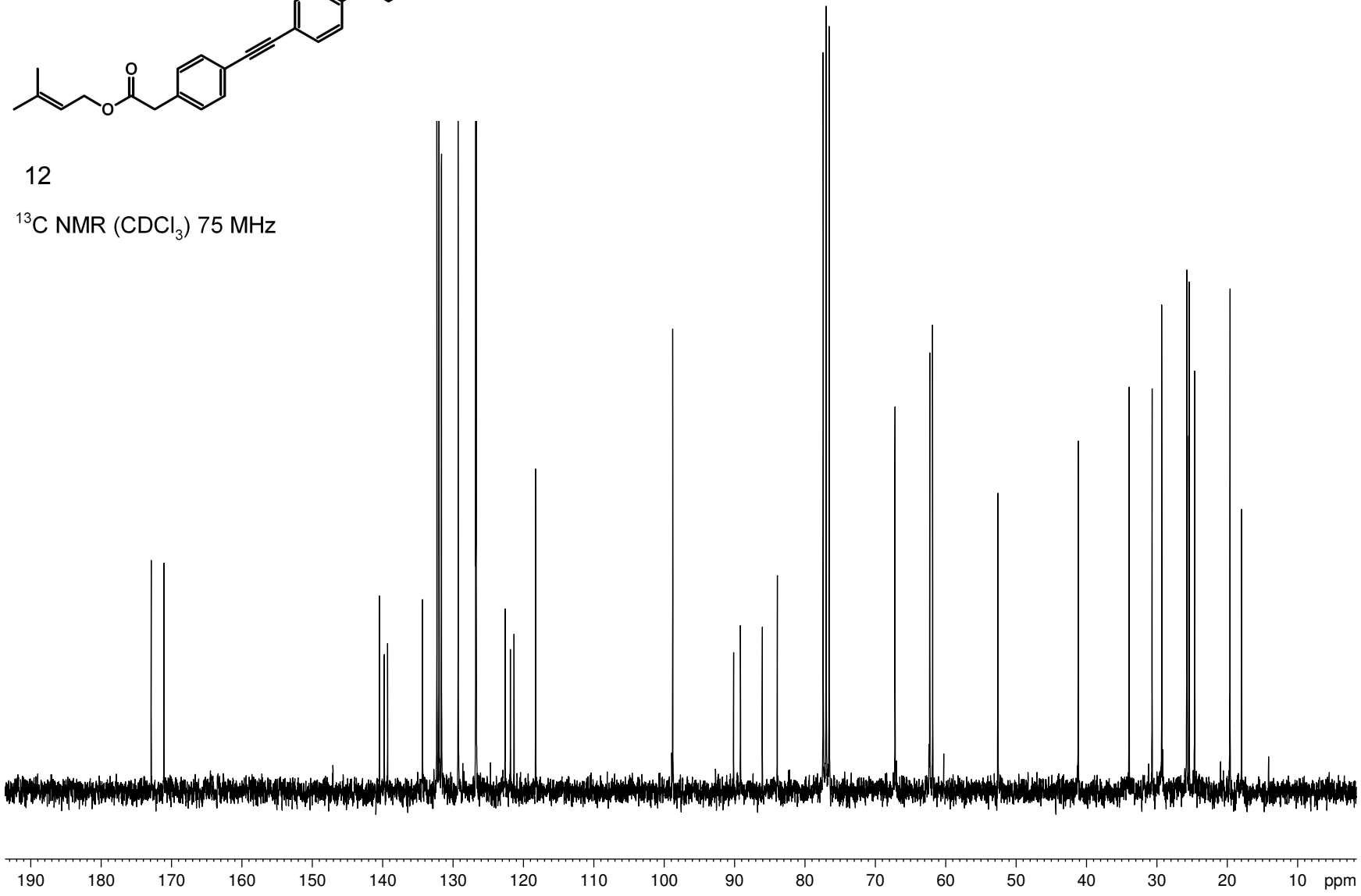
12

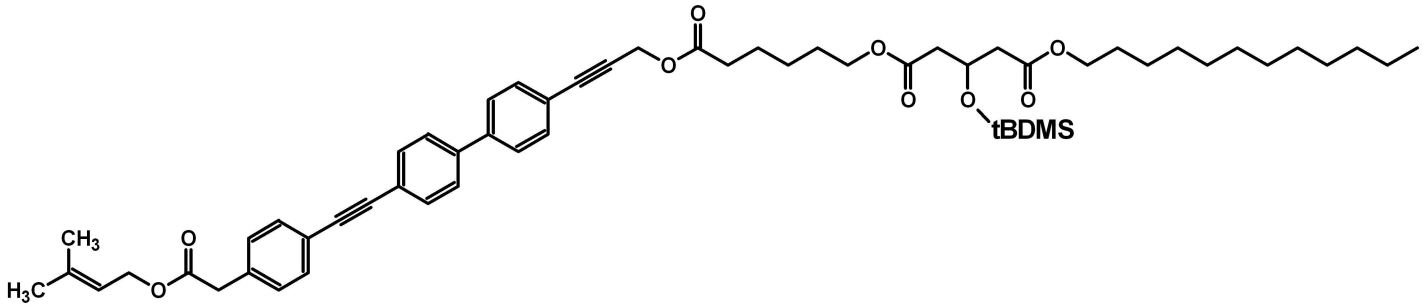
^{1}H NMR ($CDCl_3$) 300MHz



12

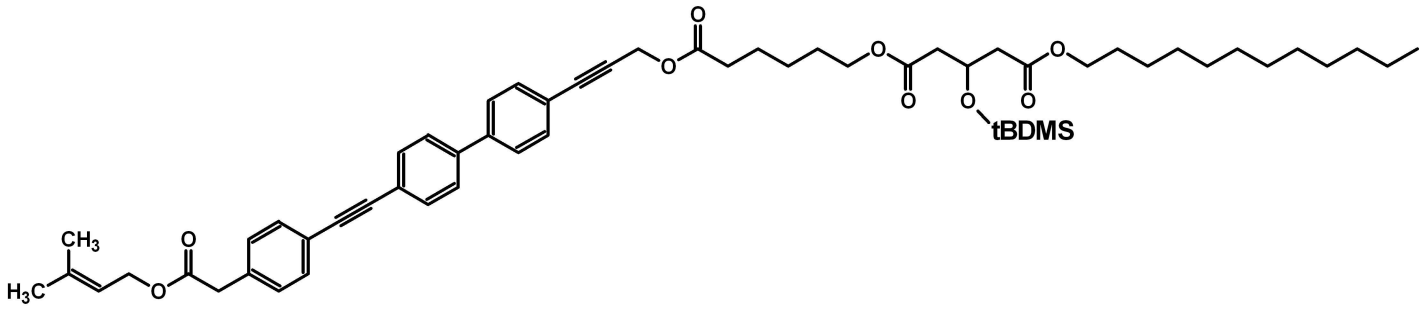
^{13}C NMR ($CDCl_3$) 75 MHz





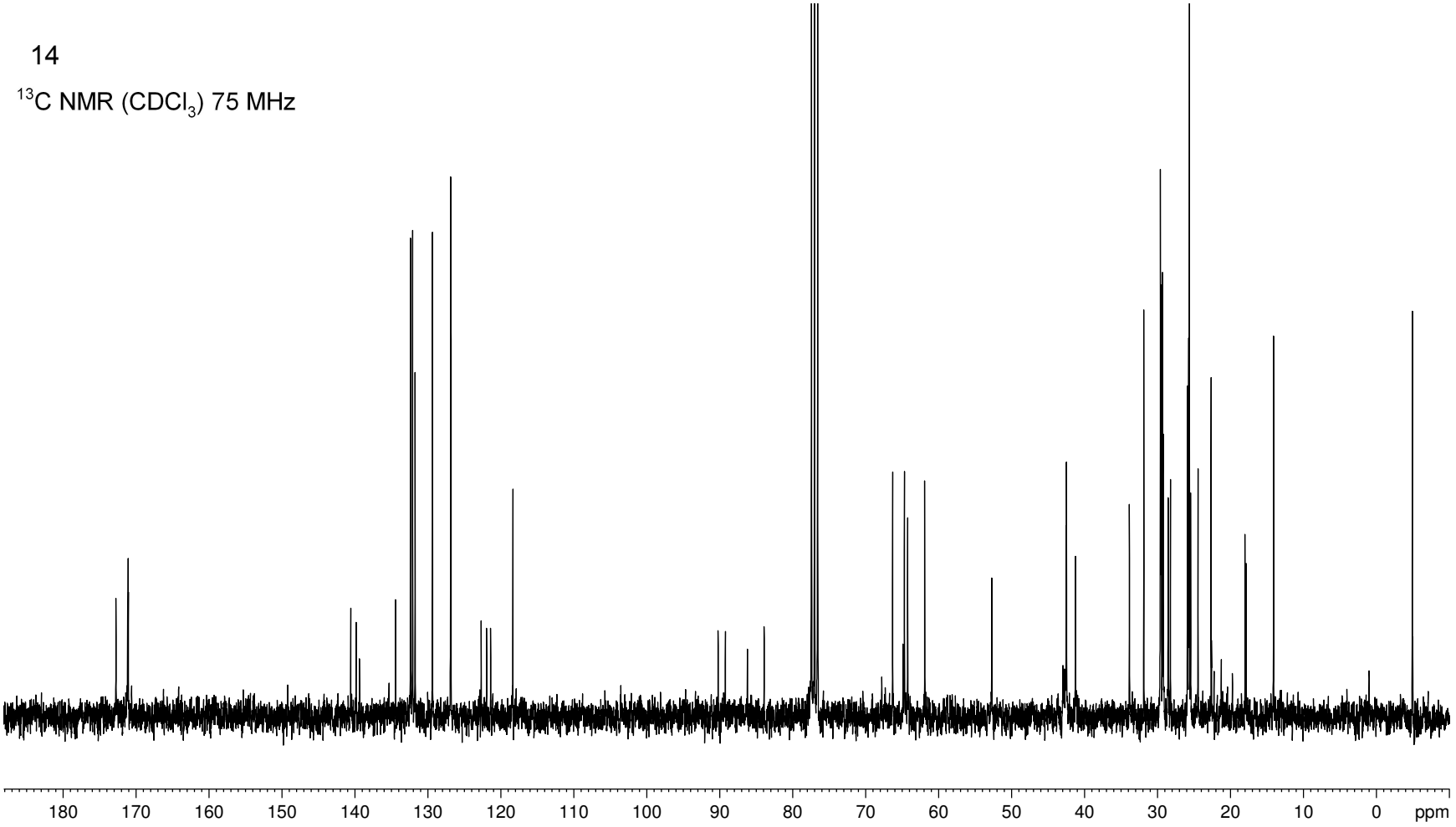
14

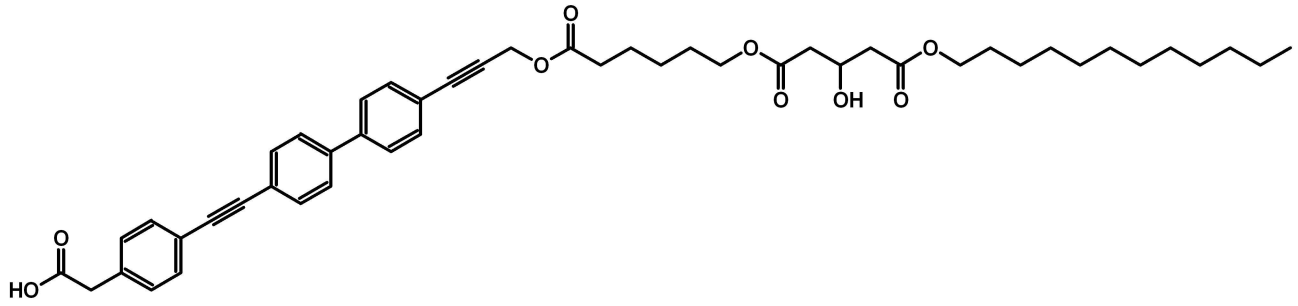
^{1}H NMR ($CDCl_3$) 300MHz



14

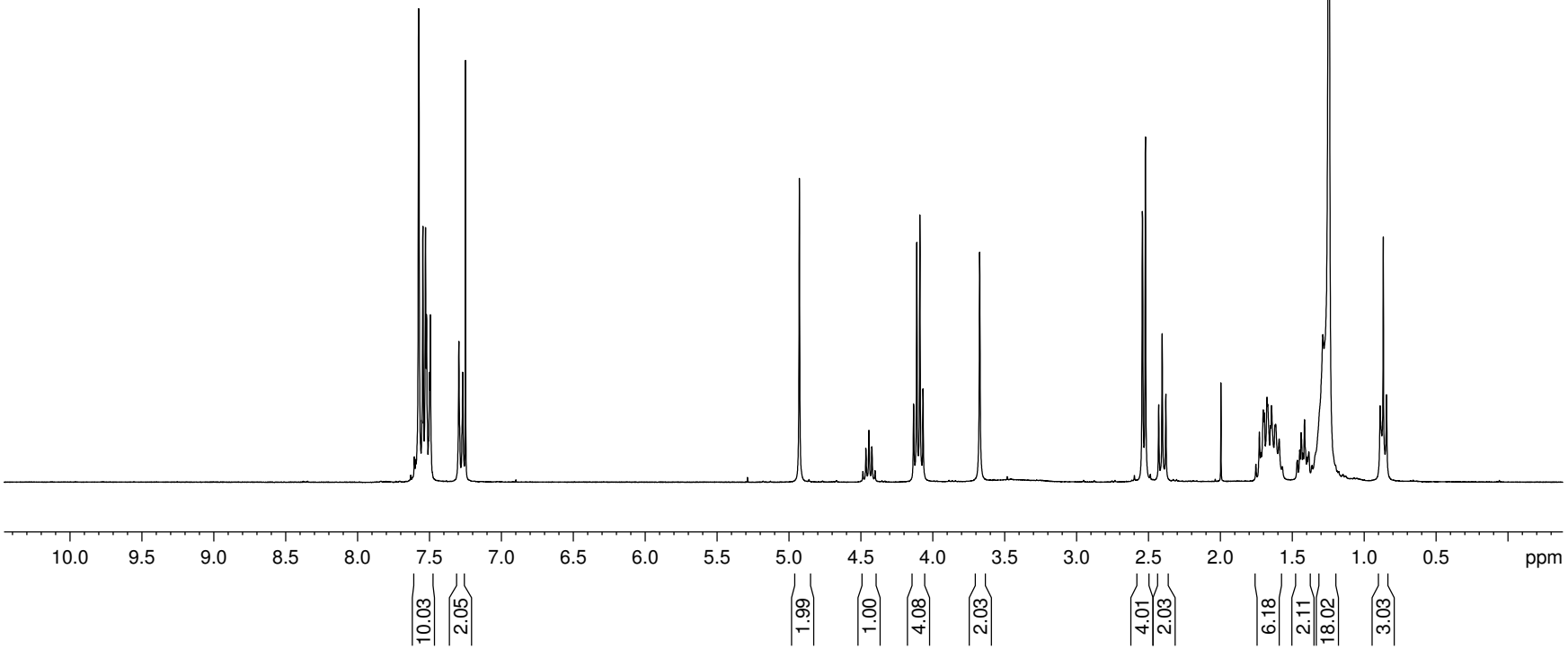
^{13}C NMR ($CDCl_3$) 75 MHz

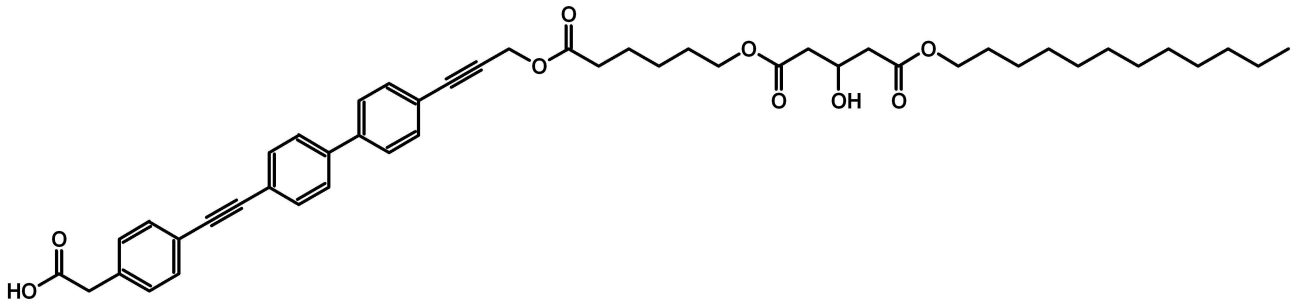




^{1}H NMR ($CDCl_3$) 300MHz

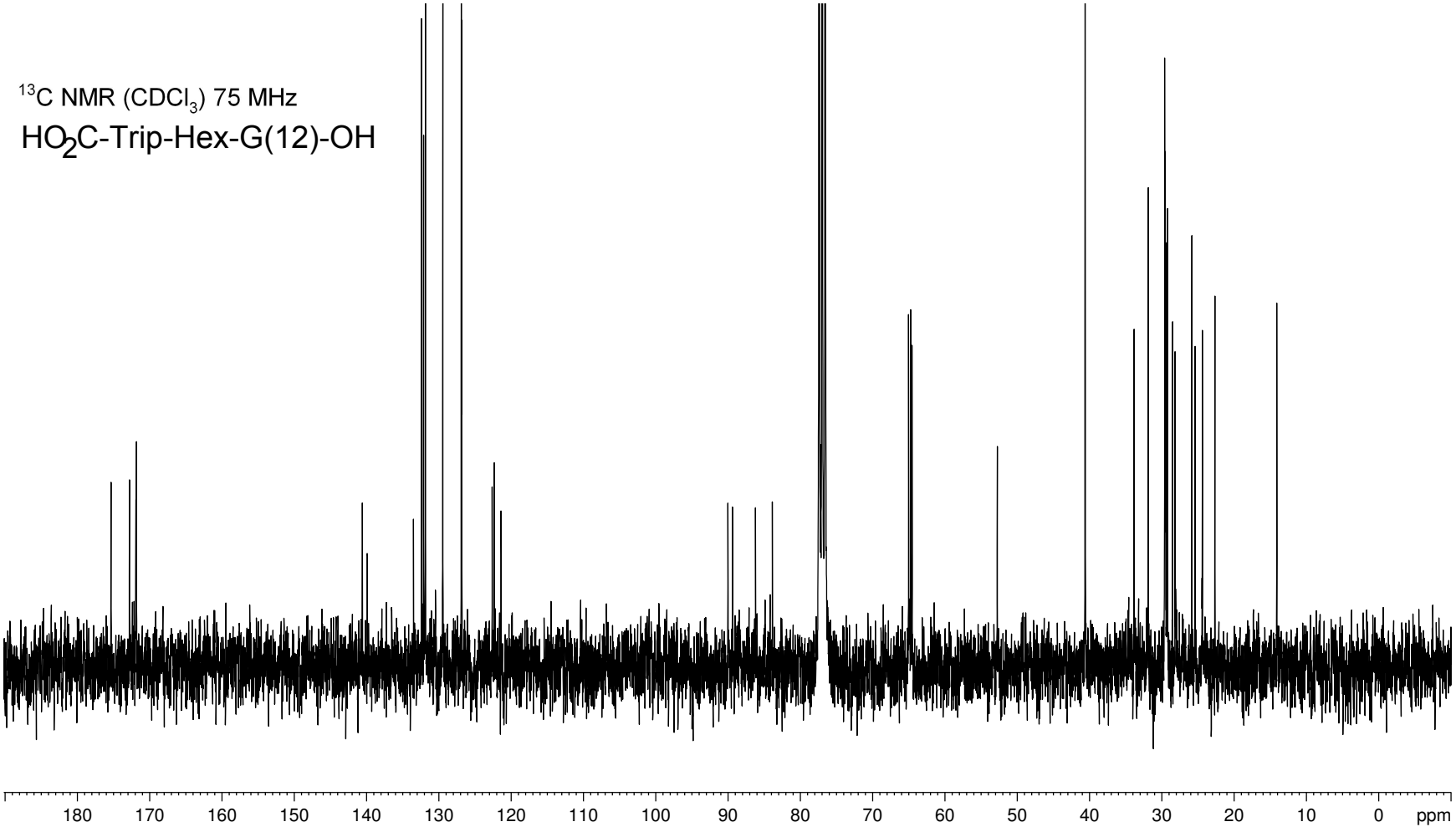
HO_2C-Trip-Hex-G(12)-OH



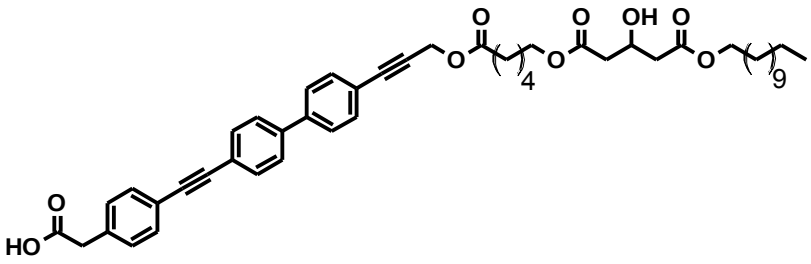


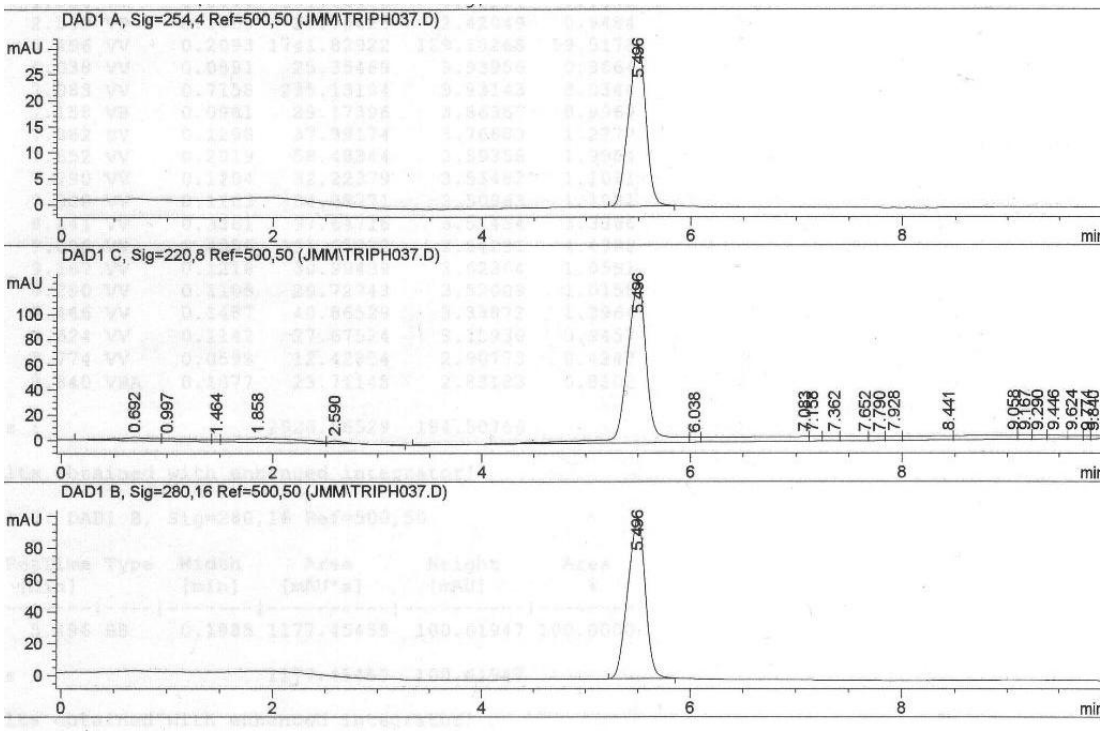
^{13}C NMR ($CDCl_3$) 75 MHz

HO_2C-Trip-Hex-G(12)-OH



HO_2C-Trip-Hex-G(12)-OH

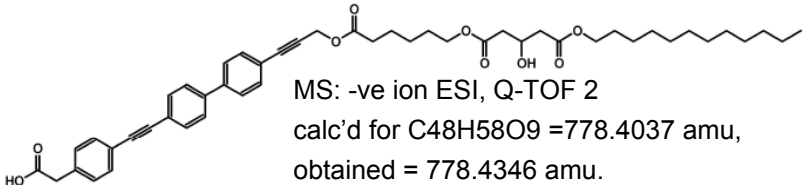




-HPLC trace of sample used for fluorescence and transport studies
-CONDITIONS: HP series 1100 HPLC
-Machery-Nagel RP C18 “Nucleosil” analytical column (4 mm x 250mm)
-3:1 CH_3OH:ACN as eluting solvents, flow 1mL/min

-as the fluorescence response was very high for this compound, the detector was saturated and therefore fluorescence detection was not utilized in this instance.

HO_2C-Trip-Hex-G(12)-OH



MS: -ve ion ESI, Q-TOF 2
calc'd for C48H58O9 =778.4037 amu,
obtained = 778.4346 amu.