Supporting Information

Luminescent Carbon Dot Mimics Assembled on DNA

Ke Min Chan, Wang Xu, Hyukin Kwon, Anna M. Kietrys and Eric T. Kool*

Department of Chemistry, Stanford University, Stanford, CA 94305 (USA)

Email: kool@stanford.edu

*Author to whom correspondence should be addressed.

Table of Contents

Experimental Methods

Chemicals and reagents

Solvents and reagents for DNA-CA monomer synthesis were purchased from Sigma-Aldrich, Merck Millipore, TCI America, Alfa Aesar, Combi-Blocks, Praxair, ArkPharm and Fisher Scientific. DNA synthesis reagents were purchased from Glen Research and Merck Millipore. All reactions were conducted under argon atmosphere unless otherwise noted. Merck 60Å 200-425 mesh silica gel was used for column chromatography.

General Instrumentation

^{1}H and ^{13}C NMR spectra were measured with Varian NMR spectrometers (Inova 300, Varian 400 and Mercury 400). For optical spectra, DNA-CAs were prepared as ~1 μM solutions in water (Molecular Biology Grade, Corning). Absorption spectra were measured with Cary 100 Bio UV-Vis spectrometer, fluorescence spectra obtained by Jobin Yvon-Spex Fluorolog 3 spectrometer, and fluorescence lifetime measurements were made with a PTI EasyLife LS spectrometer. Mass spectra were obtained using ESI or MALDI-TOF ionization modes at the Stanford University Mass Spectrometry Facility. HPLC was performed with a Shimadzu LC-20AD equipped with SPD-M20A diode array detector and Phenomenex Jupiter reverse phase C5 column. Dynamic light scattering measurements were made with a Malvern Instruments Nanoseries ZS90 Zetasizer. Epifluorescence microscopy for cell bioimaging and library screening were conducted on a Nikon Eclipse 80i microscope equipped with Nikon Plan Fluor 4x – 40x objective, and QIClick digital CCD camera, with a high pressure 100W high pressure mercury lamp as the excitation source (365 nm mercury plasma emission line), 340-380 nm excitation filter and > 420 nm long-pass emission filter. Photographs of aqueous phase DNA-CAs, DNA-CAs in hydrogel and flexible DNA-CA display were captured with an iPhone 6S+ camera, with a 365 nm gel transilluminator UV source (VWR LM-20E) as backlight.

DNA-CA library construction

The DNA-CA library was synthesized on amine-functionalized polyethylene glycol coated polystyrene beads (Merck NovaSyn TG amino resin, 130μm diameter) using a previously reported protocol.[1] The surface of each bead carries a unique DNA-CA sequence, attached via a 10-hydroxy-decanoic acid linker to the amino moiety of the bead, along with a cleavable low molecular weight binary chemical tag that uniquely identifies the DNA-CA sequence.[2-3] In total, the library contained a theoretical 1296 unique DNA-CAs sequences. Overall average stepwise coupling yield for the library tetrameric DNA-CA sequences was 95%. After library synthesis, the DNA-CAs were deprotected with 50 mM potassium carbonate in methanol, washed with EDTA in DMF, water, acetonitrile, DCM and dried under argon gas stream.

Library screening procedure

Library beads were adhered to a double-sided tape attached to a microscope slide, clustered in zones containing about 20-30 beads each (Figure S3 shows images of several zones). The attached beads on each slide were moistened with 200μL water for 5 minutes before each visualization under epifluorescence microscope with a 365 nm excitation source. Images of the fluorescent beads in each zone were captured and fed into a custom machine vision-based bead detector program (Figure S4), which extracts the RGB values, luminosity value, and position of each bead. The code for this program is provided in a separate document. To select for photostability, approximately 2000 beads were tracked over a period of four days, with a 5-minute exposure to 365 nm UV per day. At the end of the four-day period, beads exhibiting highest brightness and photostability were selected, and sequenced by decoding of the cleavable tags. For DNA-CA sequence decoding, single beads were submerged in 3 μL of CAN solution (0.5 M ceric ammonium nitrate in water:acetonitrile 1:1) and 3 μL decane in a sealed capillary tube as described.[3] The capillary tube was sonicated for 3 hours, centrifuged, and the organic decane layer containing cleaved tags were derivatized with N,O-Bis(trimethylsilyl)acetamide and analyzed with GC-ECD following the literature procedure.[3]

Resynthesis and characterization of high brightness and photostable DNA-CA sequences

Selected high-brightness and high-photostability DNA-CA sequences from the library screening effort were resynthesized via standard oligonucleotide synthesis on an ABI 394 DNA synthesizer. The DNA-CA tetramers were synthesized at 1 μmol scale on 3'-phosphate CPG columns (Glen Research), deprotected with 50 mM potassium carbonate in methanol, preparatory HPLC purified with a C5 reverse phase column, using pH 7.0 adjusted 50 mM triethylammonium acetate buffer and acetonitrile as eluents. The purified sequences were dried by vacuum concentrator, reconstituted in molecular biology grade water, characterized by MALDI-TOF mass spectrometry, and optical properties measured.

Flexible DNA-CA display fabrication

0.1 μmol of each DNA-CA was mixed with 20 μmol Varathane water-based polyurethane and screen-printed on flexible cellulose acetate substrate and left to air dry. The emissive display was pumped with a 365 nm UV backlight (gel transilluminator) for imaging.

DNA-CA-containing collagen hydrolysate hydrogel synthesis

To create hydrogel solutions, 3 g dextrose was mixed with 0.5 g collagen hydrolysate powder in 10 mL water. The suspension was allowed to stand for 20 minutes, and then heated in a microwave oven until all solids were dissolved. 0.1 μmol of each DNA-CA was mixed with 100 μL melted hydrogel solution and left to set in plastic molds at 5 °C. The emissive hydrogel was excited with a 365 nm UV backlight (gel transilluminator) for imaging.

Cell culture and imaging studies

HeLa and HEK293T cells were seeded at a concentration of 3×10^4 cells per well to 96-well plate in supplemented DMEM culture medium (10% FBS, Gibco, Penicillin Streptomycin, Thermo Fisher Scientific) and incubated for 16 h at 37°C and 5% CO_2 with 95% humidity. Old medium was removed and cells washed once with 1xPBS pH 7.4 (Gibco). To detach cells, 20 μL of 0.25% Trypsin (Gibco) was added and incubated for 5 minutes at 37°C and 5% CO_2 with 95% humidity. Cells in each well were suspended in 50μL fresh DMEM supplemented medium, and collected for centrifugation (3 mins, 800 RCF) at room temperature, the supernatant was discarded and cells resuspended in 20μL fresh DMEM medium lacking phenol red (Gibco). A suspension of live cells was incubated with various DNA-CA dyes at 0.1 to 3 μM concentrations from 5 min up to 3 h for bioimaging. Cells were also incubated with 0.1% aqueous methyl green to visualize cell nuclei. Visualization and imaging of cells was carried out under an epifluorescence microscope at room temperature.

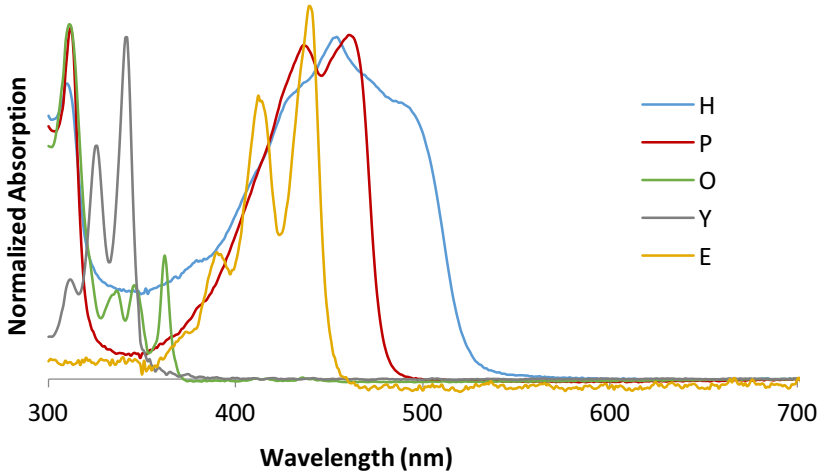


Figure S1a. UV-Vis absorption spectra of DNA-CA monomers. The spectra of DMT-protected P, H and O (molecules **6**, **14** and **20**) were measured in ethanol, and diols Y and E in methanol.

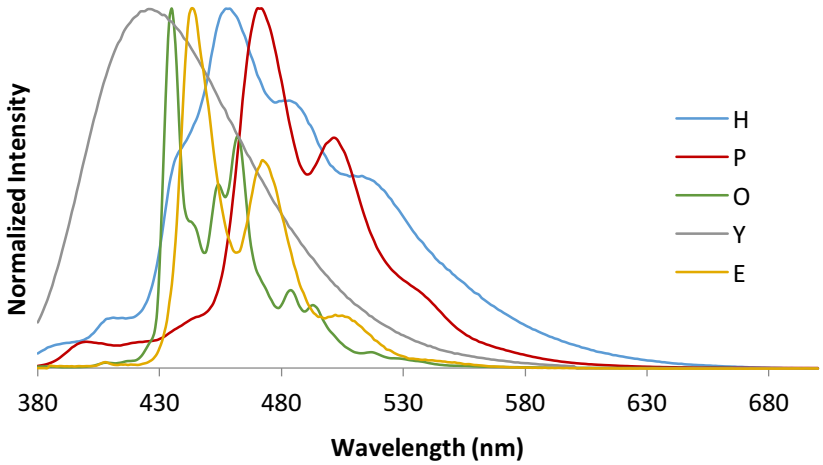


Figure S1b. Emission spectra of DNA-CA monomers. The spectra of DMT-protected P, H and O (molecules **6**, **14** and **20**) were measured in ethanol, and diols Y and E in methanol.

DNA-CA monomer	Solvent	Extinction coefficient at 365 nm	Extinction coefficient at absorption maximum (Absorption maximum wavelength)	Quantum yield at 365 nm excitation
Y (diol)	Methanol	500 $mol^{-1}L^{-1}cm^{-1}$	28200 $mol^{-1}L^{-1}cm^{-1}$ (342 nm)	0.31
E (diol)	Methanol	200 $mol^{-1}L^{-1}cm^{-1}$	2400 $mol^{-1}L^{-1}cm^{-1}$ (440 nm)	0.11
O (molecule **4**)	Ethanol	11800 $mol^{-1}L^{-1}cm^{-1}$	23500 $mol^{-1}L^{-1}cm^{-1}$ (363 nm)	0.10
P (molecule **12**)	Ethanol	11800 $mol^{-1}L^{-1}cm^{-1}$	79600 $mol^{-1}L^{-1}cm^{-1}$ (437 nm) 82800 $mol^{-1}L^{-1}cm^{-1}$ (461 nm)	0.96
H (molecule **18**)	Ethanol	1200 $mol^{-1}L^{-1}cm^{-1}$	42400 $mol^{-1}L^{-1}cm^{-1}$ (465 nm)	0.20

Figure S1c. Molar absorptivity and quantum yield of DNA-CA monomers in ethanol.

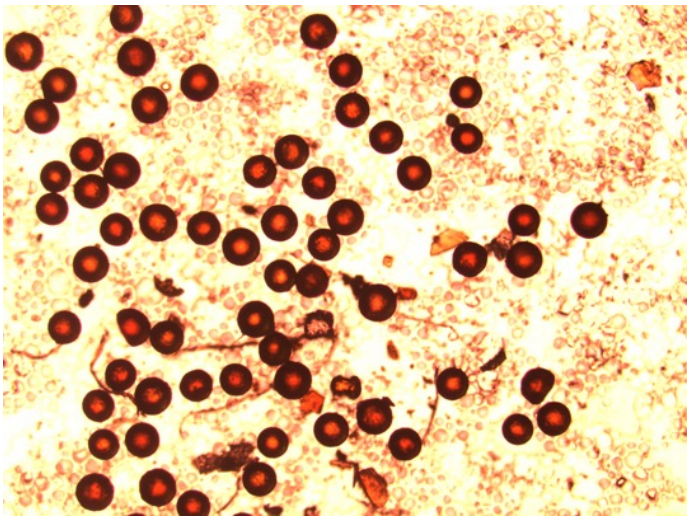


Figure S2. Bright field microscopy image of randomized library beads. Each bead is surface functionalized with a unique DNA-CA sequence along with its corresponding tag code.

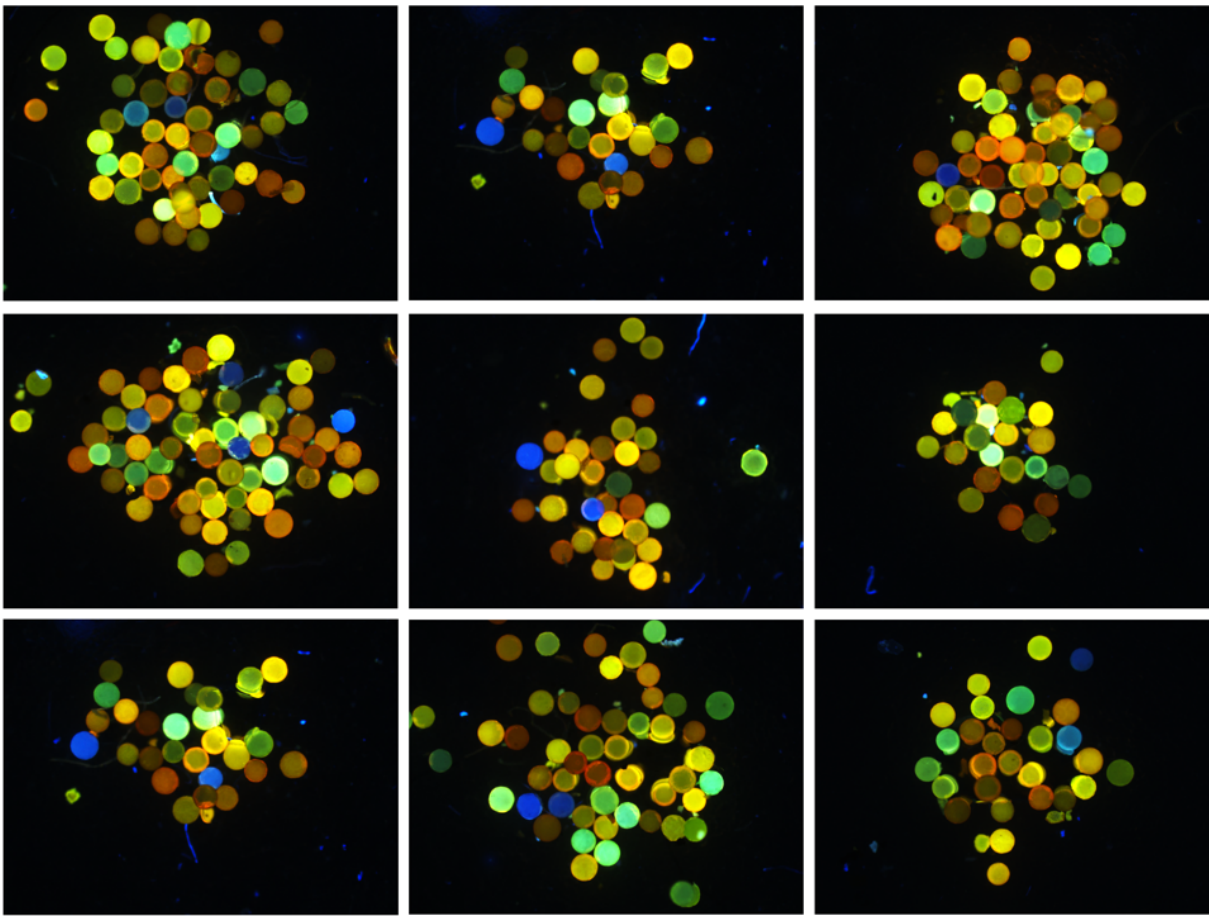


Figure S3. Visible light fluorescence of water-immersed randomized library beads under epifluorescence microscope, with single excitation at 365 nm. DNA-CA fluorescence occurs in the visible spectrum with varied emission colors and brightness levels. Nine images shown are of different subsets of library.

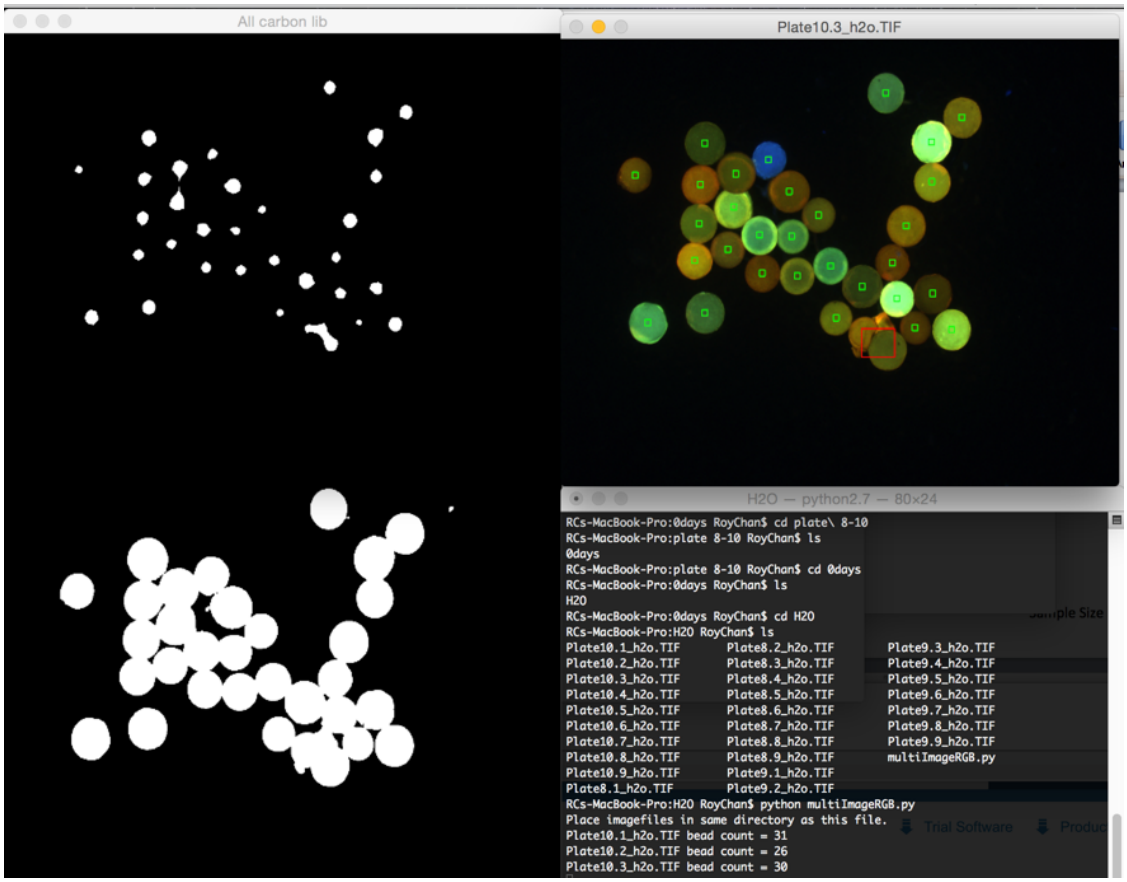


Figure S4. Screenshot of our machine vision based library-bead detector program (see separate file for code). Upper right quadrant: DNA-CA beads under 365 nm excitation. Average fluorescence response (RGB values) of each bead was sampled from a 15x15 pixel grid at the center of the bead (green boxes) in automated fashion. Red boxes indicate spurious regions excluded by the machine vision program in the calculation of library color profile. Upper left quadrant: Visualization of the positions of the bead centers - the centroid of each contour was taken as the bead center. Lower left quadrant: Image thresholding was performed to keep the bright bead regions of each image, then edge regions closest to zero pixels were eroded to preserve only bead centers.

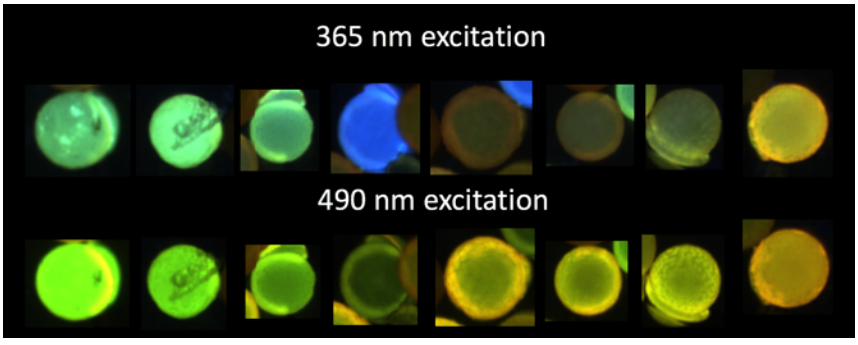


Figure S5. Comparison of selected library beads under two different excitation wavelengths, showing changes in emission color. Top row: beads under 365 nm exposure. Bottom row: Same beads under 490 nm exposure.

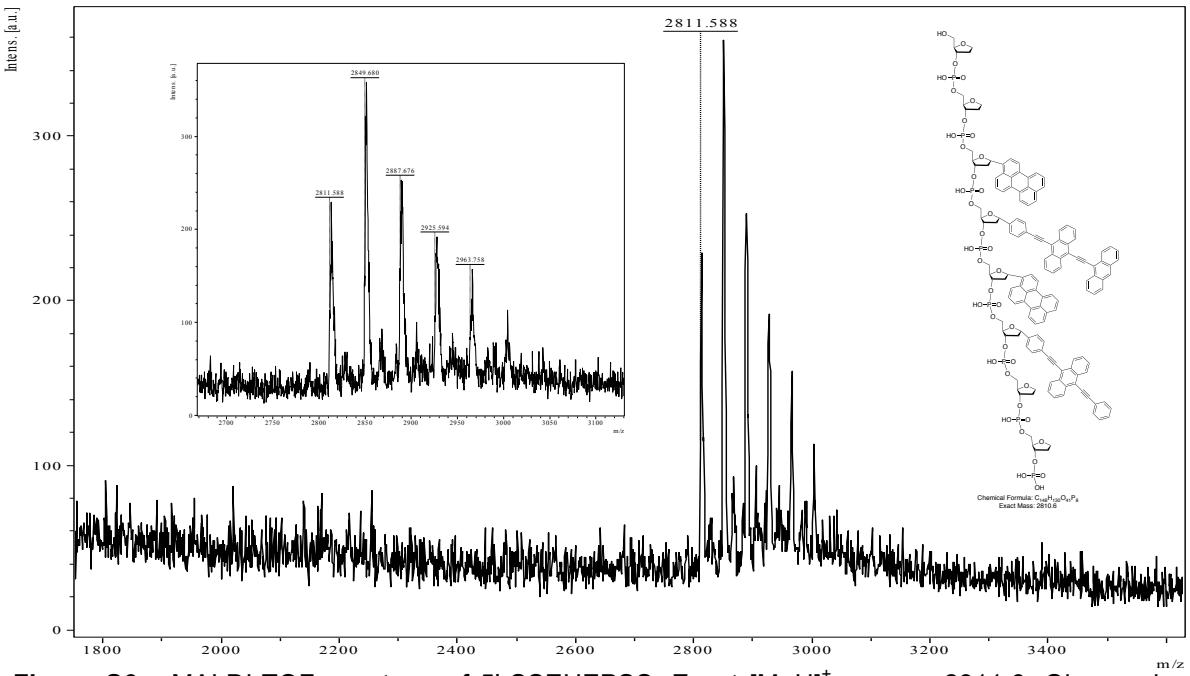


Figure S6a. MALDI-TOF spectrum of 5'-SSEHEPSS. Exact $[M+H]^+$ mass = 2811.6. Observed mass = 2811.6.

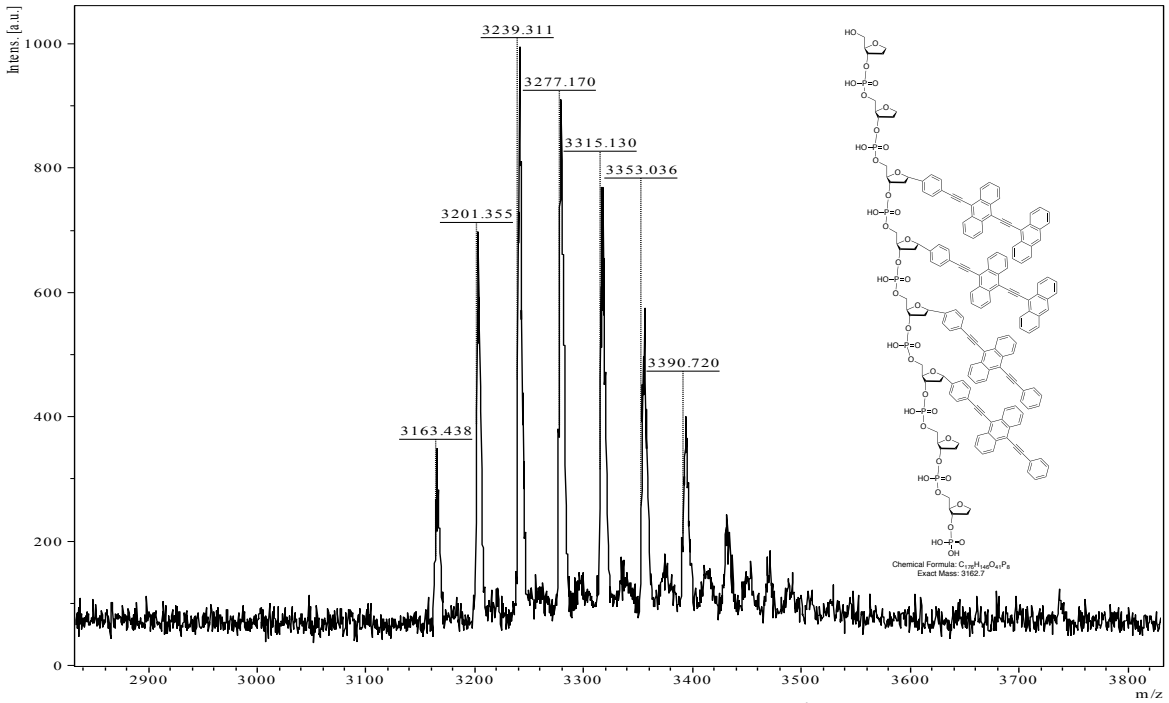


Figure S6b. MALDI-TOF spectrum of 5'-SSHHPPSS. Exact $[M+H]^+$ mass = 3163.7. Observed mass = 3163.4.

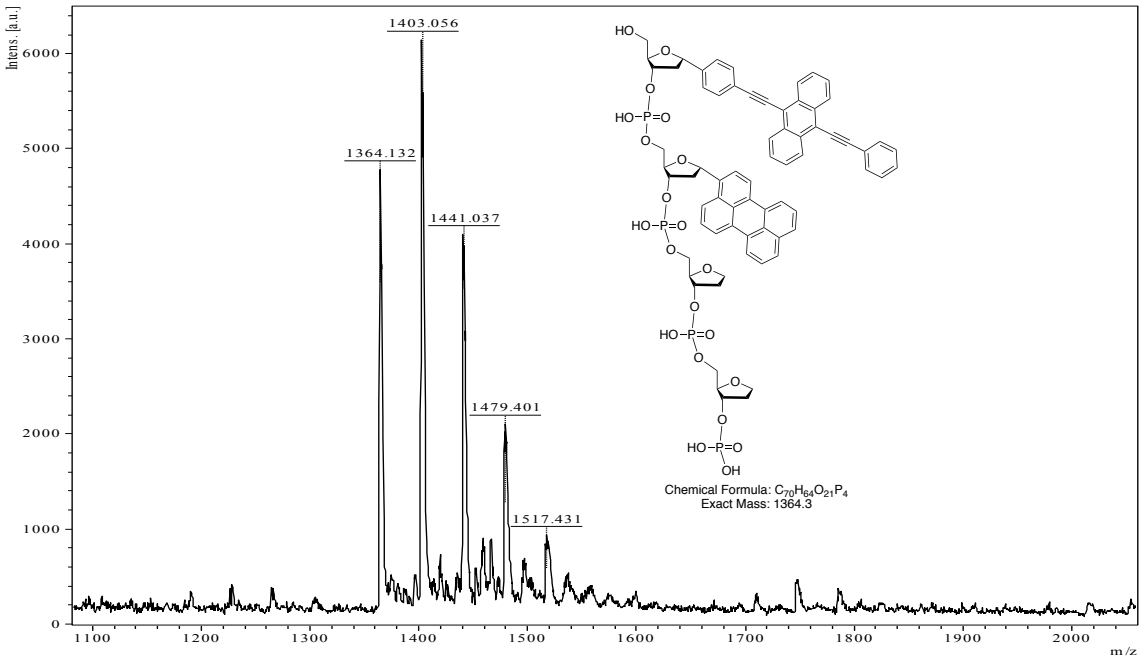


Figure S6c. MALDI-TOF spectra of 5'-PESS. Expected $[M]^+$ = 1364.3. Observed mass = 1364.1.

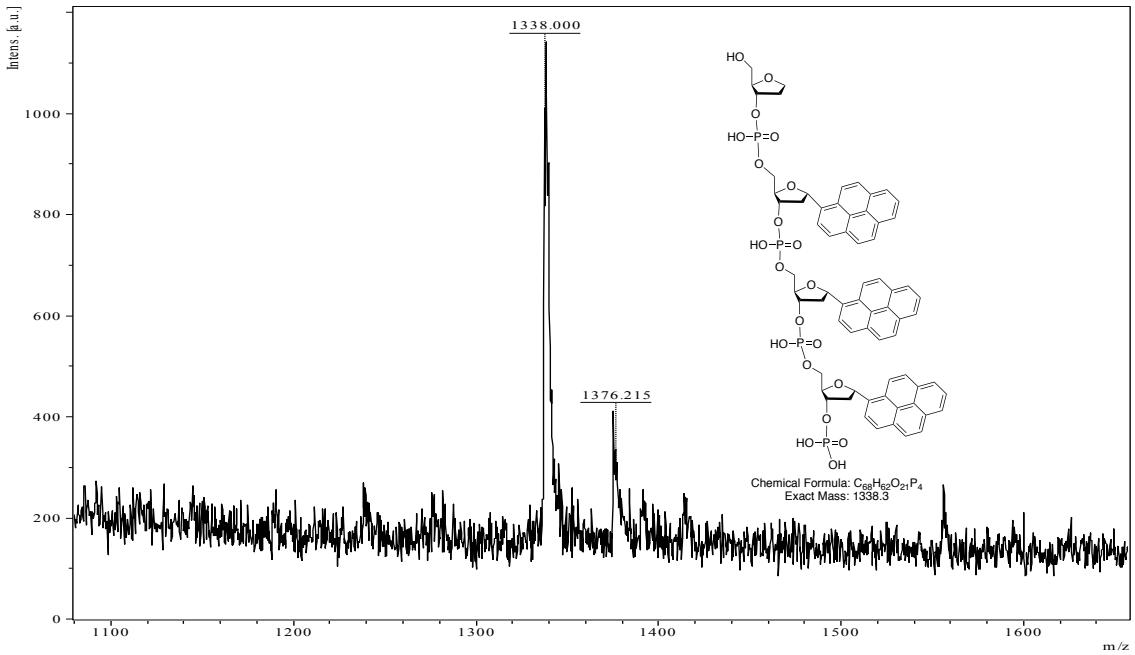


Figure S6d. MALDI-TOF spectra of SYYY. Expected $[M]^+$ = 1338.3. Observed mass = 1338.0.

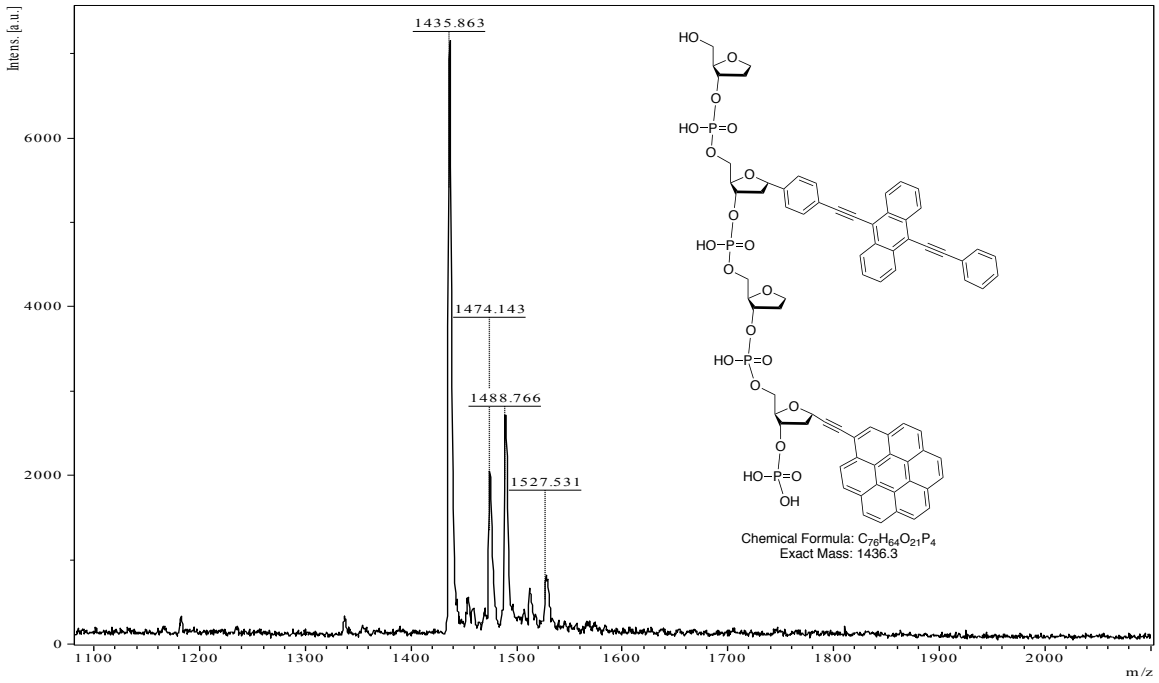


Figure S6e. MALDI-TOF spectra of SPSO. Expected $[M]^+$ = 1436.3. Observed mass = 1435.9

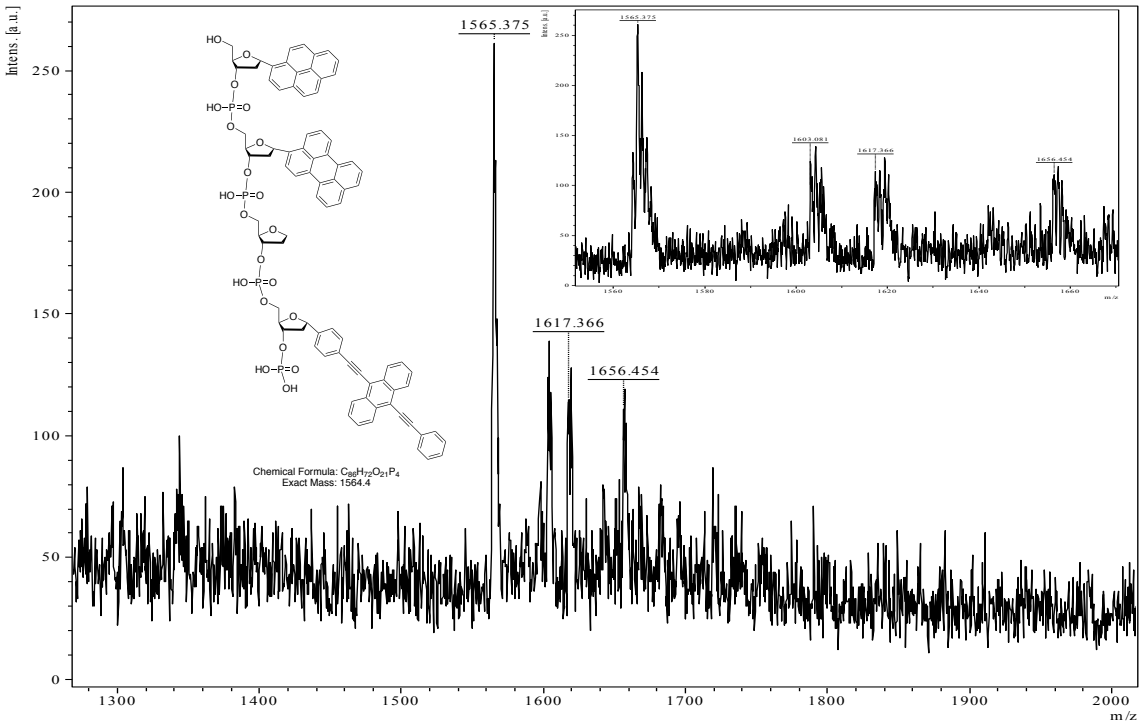


Figure S6f. MALDI-TOF spectra of YESP. Expected $[M+H]^+$ = 1565.4. Observed mass = 1565.4.

DNA-CA sequence (5' -> 3')	Chemical formula	Observed MALDI-TOF m/z	Expected ion	Exact mass
OYOY	$C_{104}H_{74}O_{21}P_4$	1783.0	$[M]^{\cdot+}$	1782.4
PSYO	$C_{92}H_{72}O_{21}P_4$	1635.6	$[M+H]^+$	1636.4
PYYO	$C_{108}H_{80}O_{21}P_4$	1837.9	$[M+H]^+$	1837.4
SOSY	$C_{62}H_{56}O_{21}P_4$	1261.0	$[M+H]^+$	1261.2
PYYY	$C_{98}H_{78}O_{21}P_4$	1715.8	$[M+H]^+$	1715.4
YHPP	$C_{134}H_{98}O_{21}P_4$	2165.2	$[M]^{\cdot+}$	2166.6

Figure S7. Table of observed MALDI-TOF masses for additional resynthesized DNA-CAs

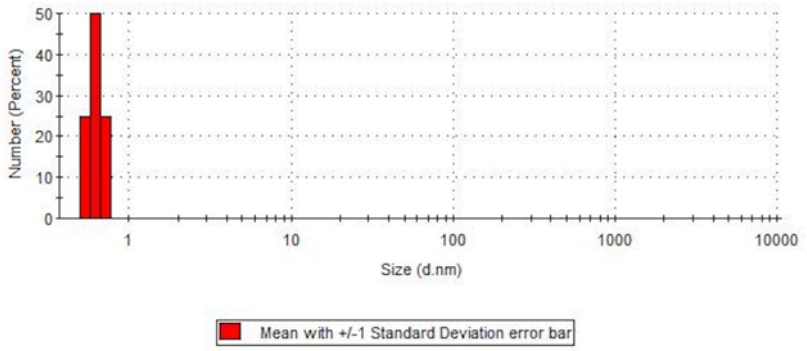


Figure S8a. Particle size distribution of 0.1 μM SPSO in water (Dynamic light scattering measurement).

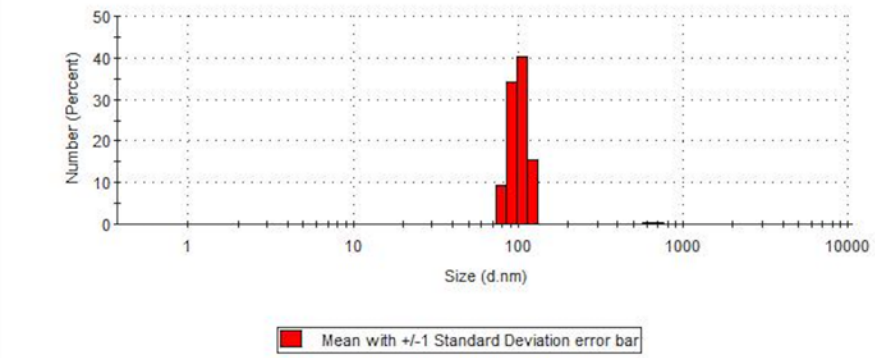


Figure S8b. Particle size distribution of 10 μM SPSO in water (Dynamic light scattering measurement).

....

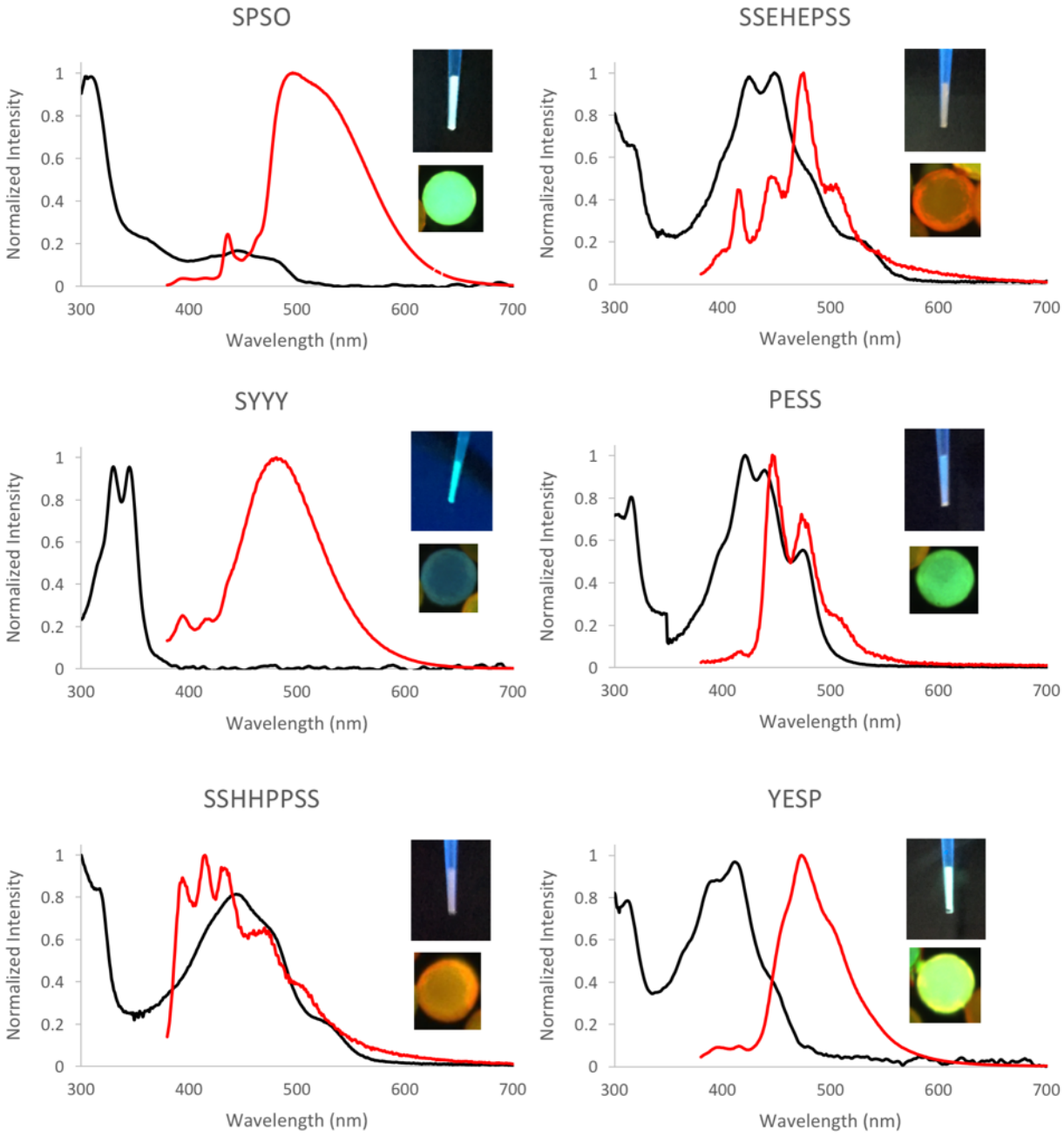


Figure S9. UV-Vis absorption (black curves) and visible range emission at 365 nm excitation (red curves) spectra of selected high photostability DNA-CAs. Inset images show corresponding library bead fluorescence, and solution phase fluorescence of aqueous DNA-CAs in pipette tips.

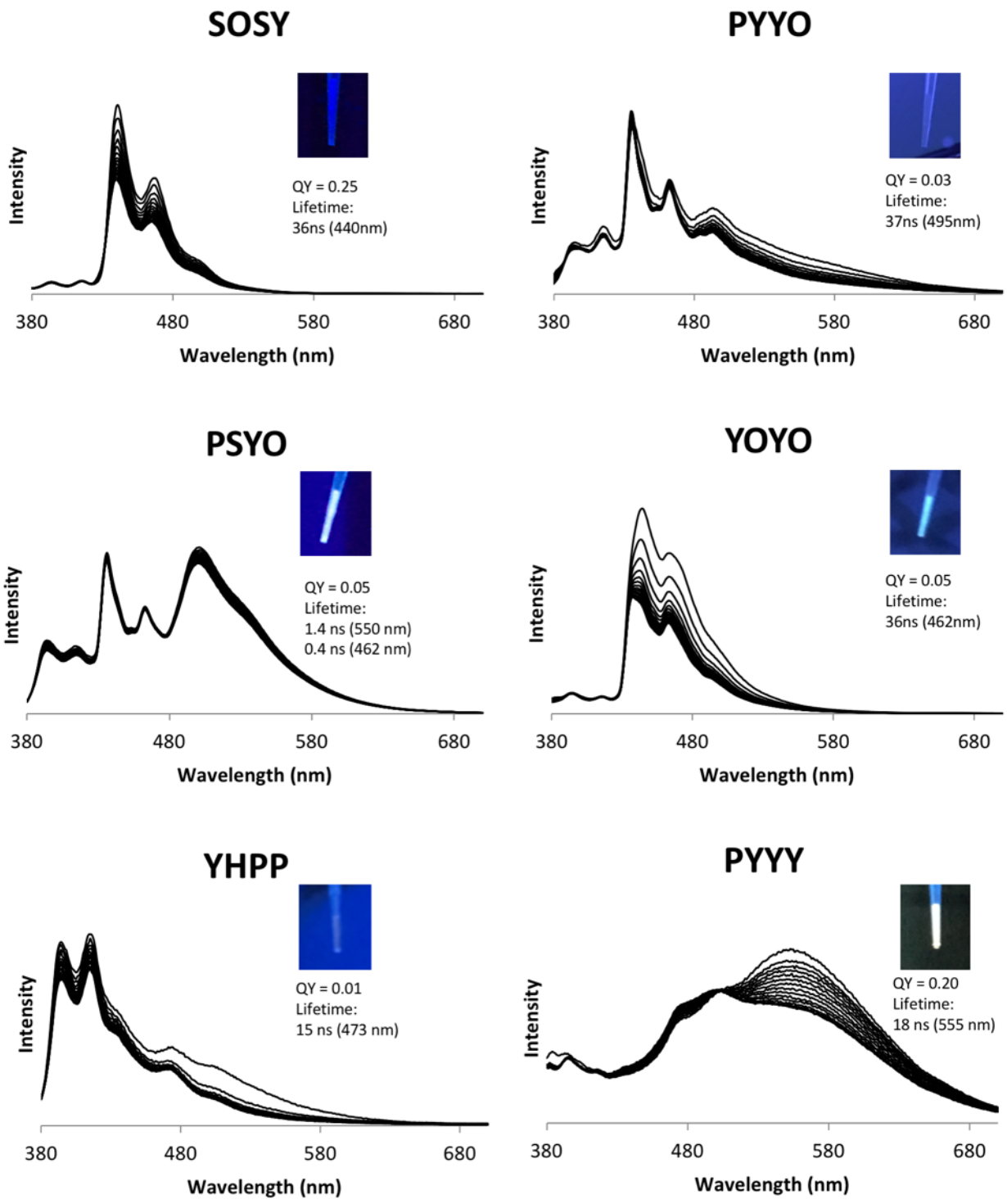


Figure S10. Optical properties of an additional selection of DNA-CAs that were resynthesized in aqueous soluble form. Some of these sequences were prone to photobleaching and were not selected for bioimaging. Black curves are emission spectra of aqueous phase DNA-CAs, measured at 1 min intervals over a period of 15 min of 365 nm UV exposure. Inset images show visible light emission of solution phase DNA-CAs in pipette tips, excited with 365 nm light.

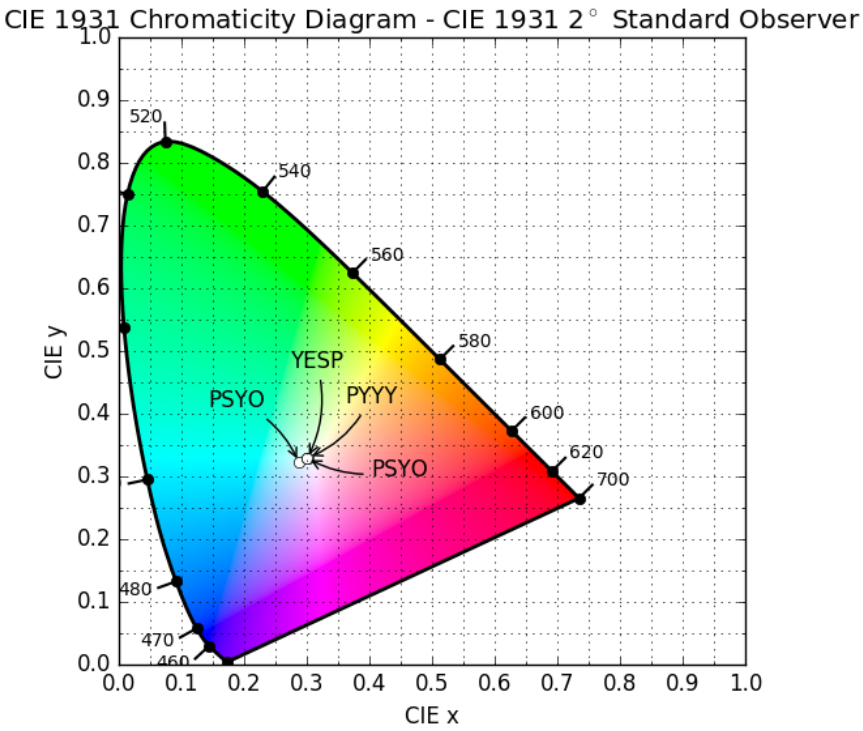


Figure S11. CIE 1931 chromaticity plot of white emitting aqueous phase DNA-CAs. Sequences of 4 aqueous phase DNA-CAs with CIE coordinates that are very close to the ideal white emission color (0.33, 0.33): PSYO (0.29,0.33), PYYY (0.30,0.33), SPSO (0.30,0.33), and YESP (0.30, 0.33) are shown.

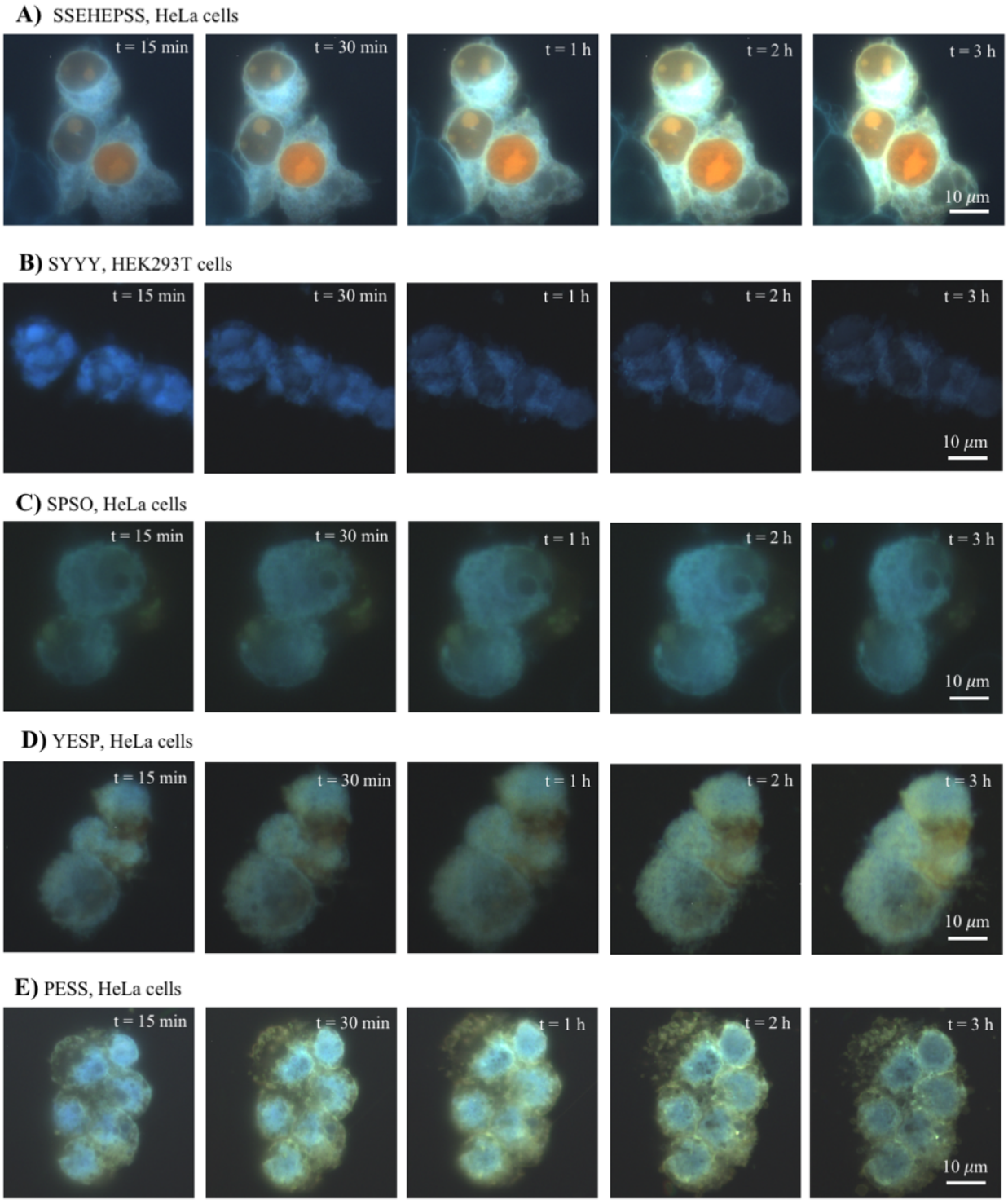


Figure S12. A-E) 3-hour time course images of human cells (HeLa / HEK293T) incubated in DNA-CA solution (0.1μM to 10μM DNA-CA in DMEM).

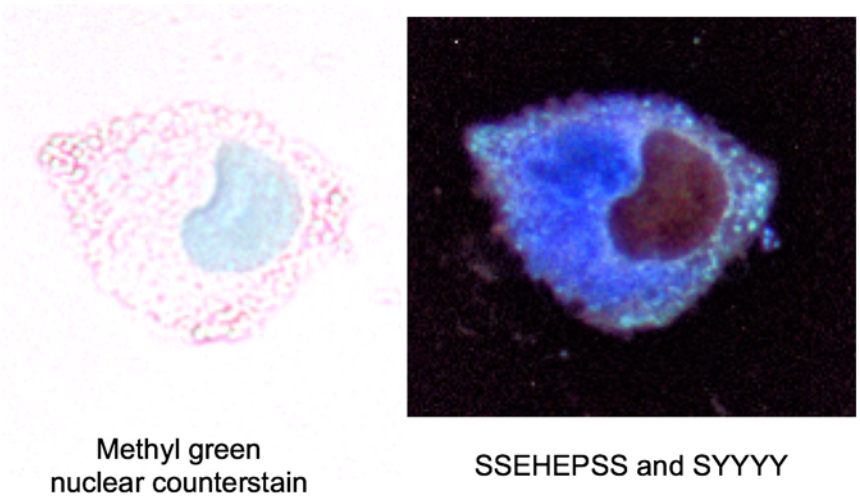


Figure S13. HeLa cell nuclear staining with SSEHEPSS (1μM in DMEM) and nuclear counterstaining with methyl green. Left: brightfield image of methyl green counterstained HeLa cell. Right: Fluorescence of SYYY (blue) and SSEHEPSS (orange) with 365 nm excitation.

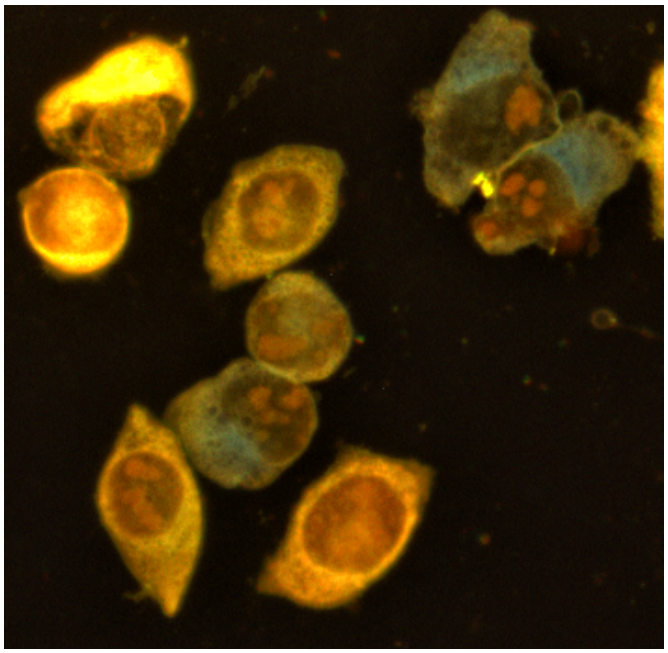


Figure S14. Concentration dependent emission of PESS. Stained HeLa cells produce emission in two colors, green and yellow, depending on degree of cell uptake. Distinct staining of subcellular compartments (orange-yellow) was also observed.

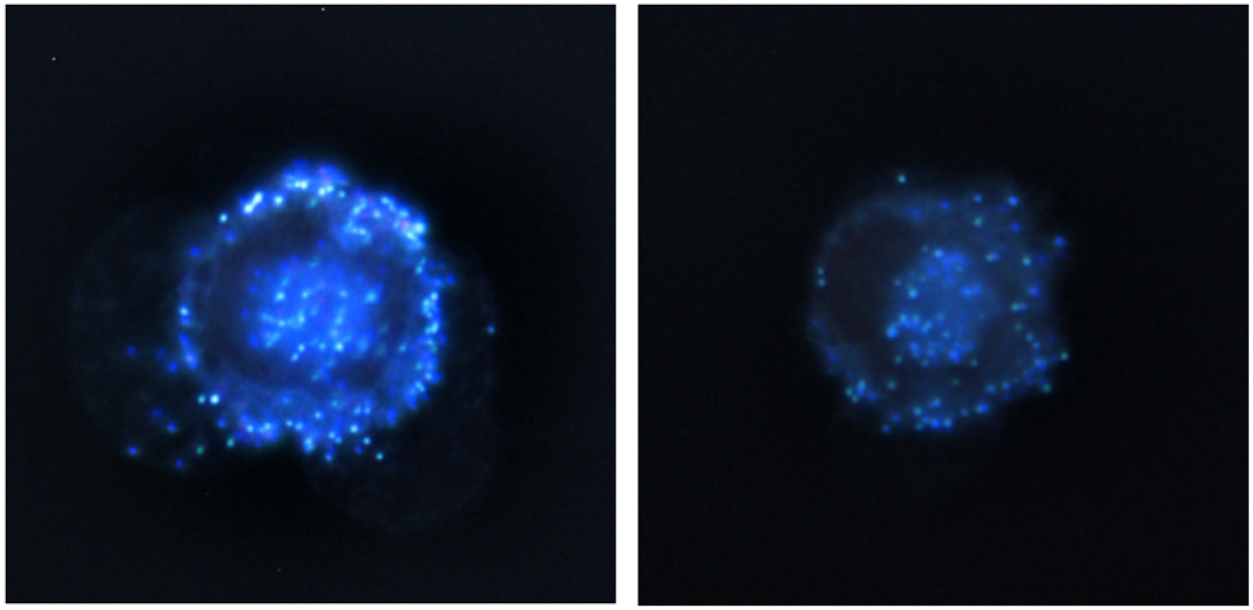
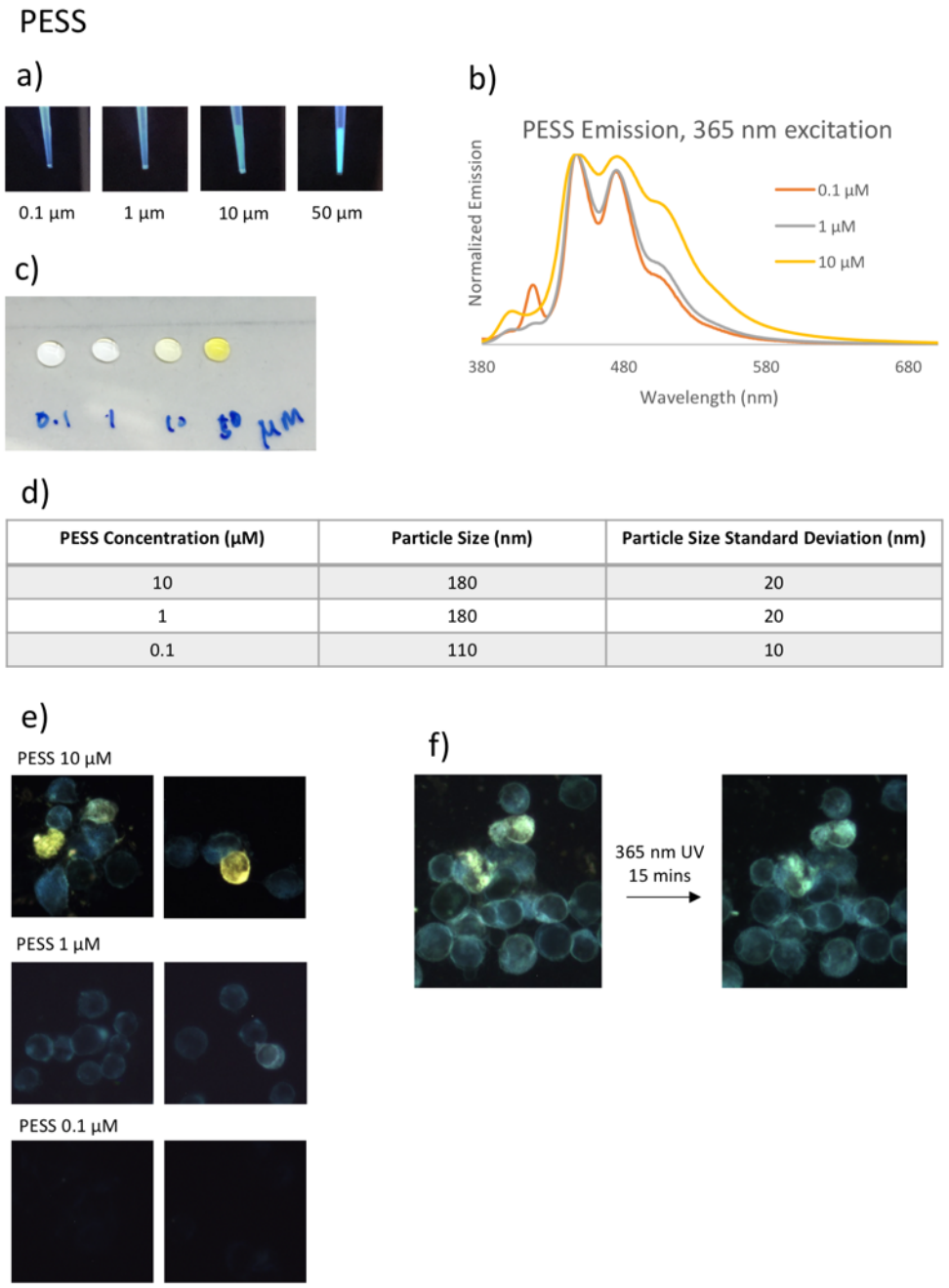
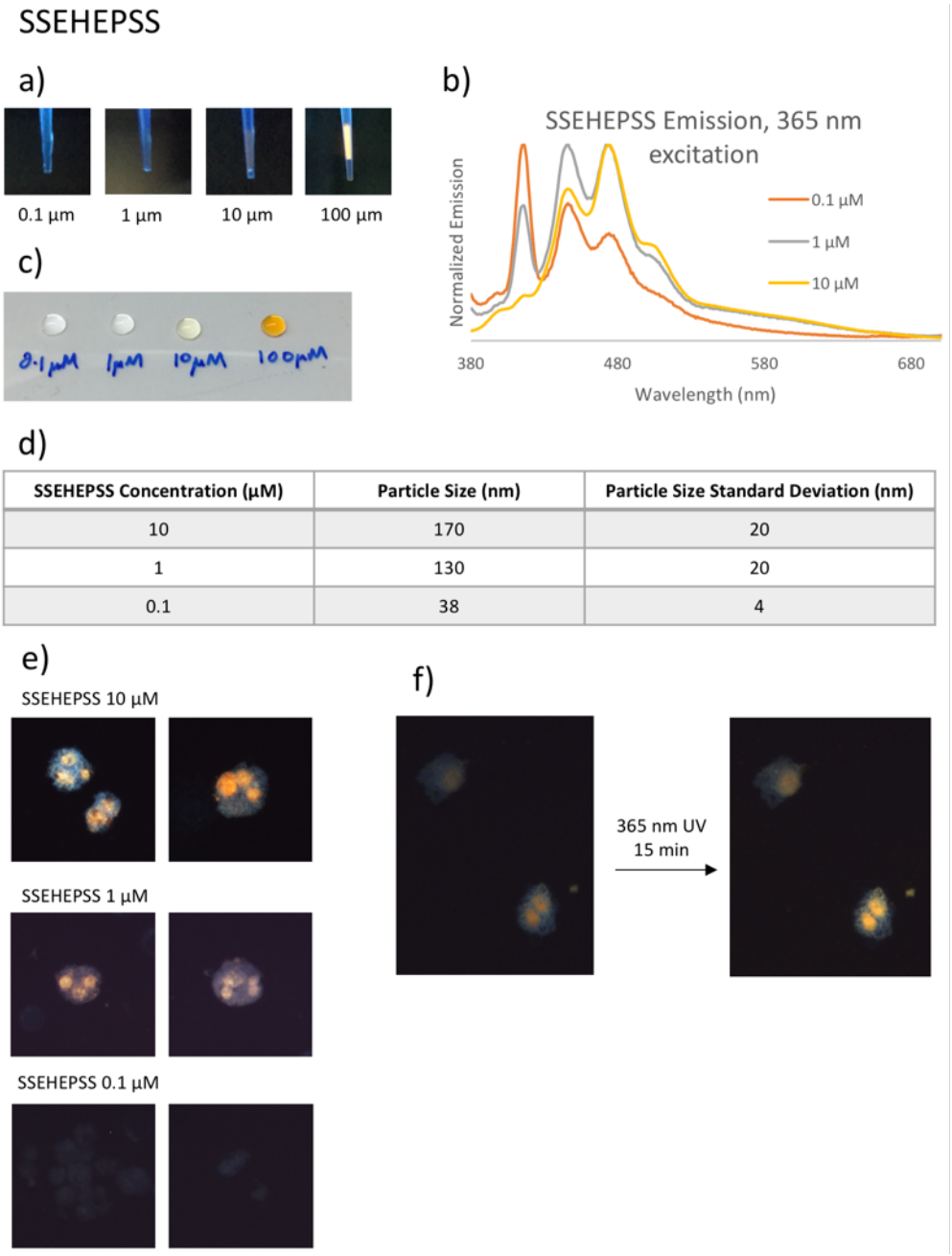


Figure S15. Individual HeLa cells stained with SYYY (20 min incubation). The punctate appearance of fluorescence suggests an endosomal uptake mechanism, although more study will be needed to draw conclusions.



PESS Concentration (µM)	Particle Size (nm)	Particle Size Standard Deviation (nm)
10	180	20
1	180	20
0.1	110	10

Figure S16. Influence of PESS concentration on its emissive properties, solution phase particle sizes, and cell labelling studies. a) PESS emission at various concentrations in a pipette tip, under 365 nm excitation. b) Normalized emission spectra of PESS at three concentrations, 0.1, 1, and 10, in water. c) Visible light image of aqueous PESS solutions at 0.1, 1, 10, and 50 µM. d) Dynamic light scattering particle size measurements of PESS at the 3 concentrations used for HEK293T cell staining in e). e): Different views of HEK293T cells incubated for 20 min in aqueous PESS solutions at varied concentrations (in 1 X PBS), visualized under epifluorescence microscope with 365 nm UV excitation. At 10 µM PESS concentration, cells exhibited emission colors ranging from green to yellow, likely due to differential uptake of the DNA-CA. At 1 µM PESS, no yellow emitting cells were observed. f) Before and after images of PESS stained HEK293T cells (30 min incubation in 10 µM PESS in 1 X PBS) exposed to 365 nm UV for 15 min. Cells exhibiting yellow emission turned green in emission color, whereas green emitting cells experienced full retention of their original emission in both color and intensity.



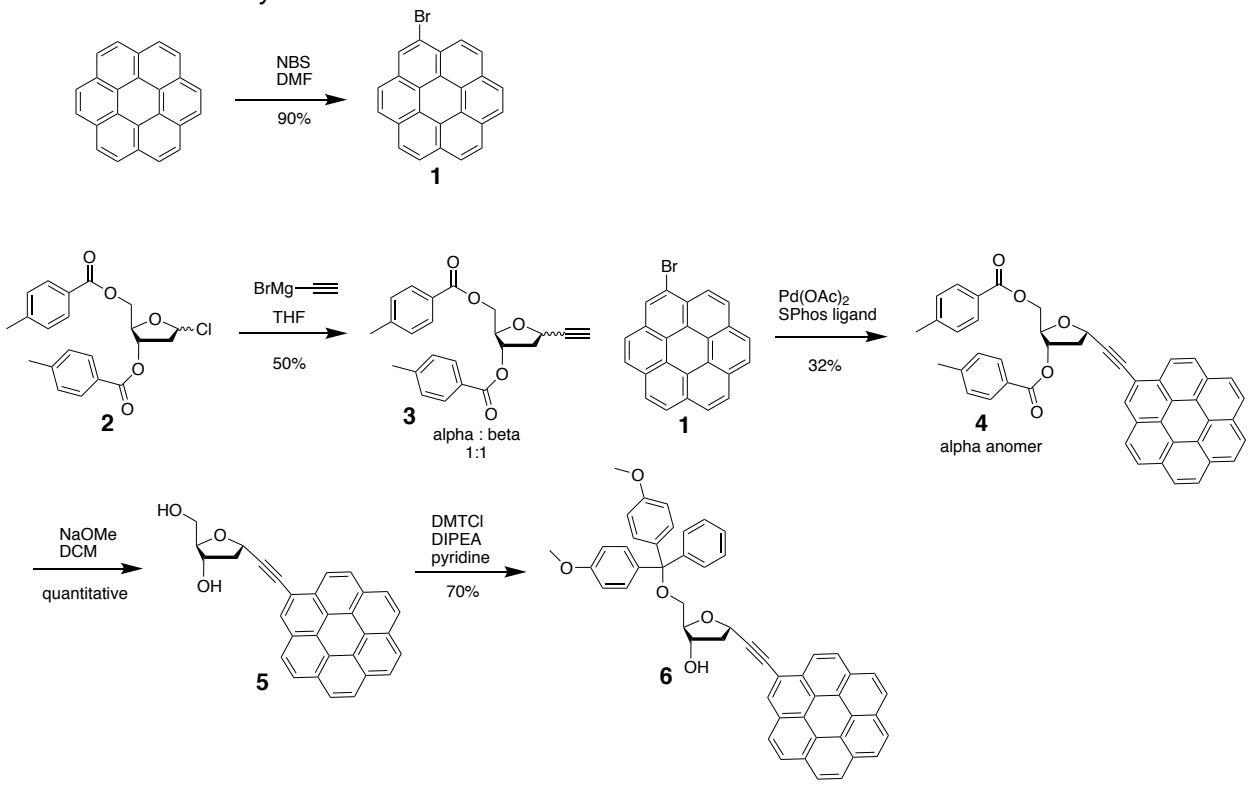
SSEHEPSS Concentration (µM)	Particle Size (nm)	Particle Size Standard Deviation (nm)
10	170	20
1	130	20
0.1	38	4

Figure S17. Influence of SSEHEPSS concentration on its emissive properties, solution phase particle sizes, and cell labelling studies. a) SSEHEPSS emission at various concentrations in a pipette tip, under 365 nm excitation. b) Normalized emission spectra of SSEHEPSS at three concentrations, 0.1, 1, and 10 µM, in water. c) Visible light image of aqueous SSEHEPSS solutions at 0.1, 1, 10, and 100 µM. d) Dynamic light scattering particle size measurements of SSEHEPSS at the 3 concentrations used for HeLa cell staining in e). e) Different views of HeLa cells incubated for 20 min in aqueous SSEHEPSS solutions at varied concentrations (in 1 X PBS), visualized under epifluorescence microscope with 365 nm UV excitation. At 1 and 10 µM SSEHEPSS concentrations, bright orange fluorescence was observed in cell nuclei, while cytoplasm regions gained white emission. At 0.1 µM, cell fluorescence was too faint to discern from background. f) Before and after images of SSEHEPSS stained HeLa cells (30 min incubation in 10 µM SSEHEPSS in 1 X PBS) exposed to 365 nm UV for 15 min. No fluorescence bleaching was observed for SSEHEPSS in stained HeLa cells, but instead a gain in brightness intensity was noted.

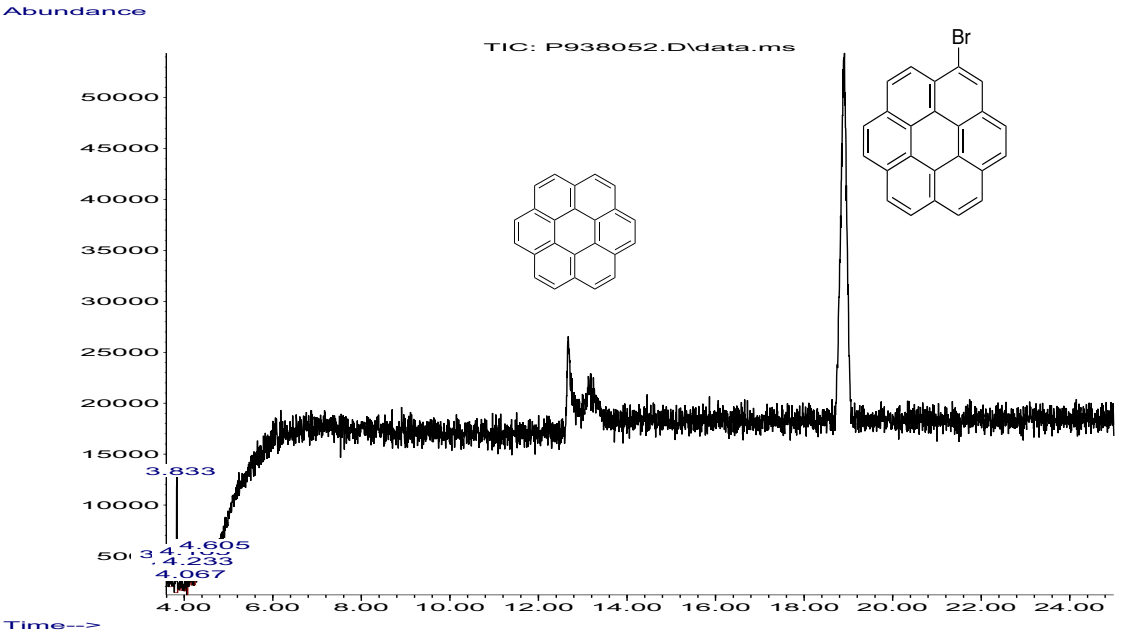
Synthetic Procedures for DNA-CA Monomers

Pyrene monomer (Y) and pereylene monomer (E) phosphoramidites were synthesized through synthetic routes previously developed by our laboratory.[4-5] Spacer monomer (S) phosphoramidite was purchased from Glen Research. Coronene monomer (O), bisphenylethynyl-anthracene monomer (P) and phenylethynyl-anthracenylethynyl-anthracene monomer (H) were synthesized via newly developed protocols as follows.

Coronene monomer synthesis:



1-Bromocoronene **1** - 0.691 g (2.3 mmol) of solid coronene (98% purity) was stirred with 1.63 g (9.2 mmol) of N-bromosuccinimide and 37 mg (0.22 mmol) azobisisobutyronitrile (AIBN) over 2 weeks at room temperature in 40 mL dry DMF. The reaction was monitored by analyzing THF-diluted samples of the reaction mixture with GC-MS, using a high temperature elution program. The reaction slurry was titrated with an additional portion of 1.6 g N-bromosuccinimide at the end of the first week to drive the reaction to completion. After 2 weeks, or when product profile was mostly mono-bromocoronene, water was added to reaction mixture to precipitate the product. The filamentous dirty brown crystals were washed sequentially with water, cold acetone, and then cold DCM and dried under high vacuum. GCMS yield of product appeared to be >90% (see GC chromatogram below). Observed GC-EI m/z = 378.0, 380.0, expected $[M]^{\cdot+}$ = 378.0, 380.0.



Hoffer's Chlorosugar **2** - Synthesized in 3 steps from deoxyribose via a previously reported protocol.[6]

(2R,3S)-5-ethynyl-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate **3** - 16 g (41 mmol) of chlorosugar **2** was dissolved in 200 mL dry THF and stirred continuously while 80 mL of 0.5 M (40 mmol) ethynylmagnesium chloride solution was added dropwise to the reaction solution over 20 min. Reaction was monitored with TLC (product was a bright active UV active spot, which stained well with $KMnO_4$ stain but not with vanillin stain), Rf = 0.4 with hexane:ethyl acetate 10:1. Upon completion of reaction, the solution was quenched with solid NH_4Cl, solvent evaporated, and purified with column chromatography. Product was a clear syrup. NMR analysis shows 1:1 alpha:beta anomeric ratio.

(2R,3S,5S)-5-(coronen-1-ylethynyl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate **4** – 2g (5.2 mmol)of sugar **3**, 500 mg (1.32 mmol) of 1-bromocoronene **1**, 2.24 g (6.88 mmol) of Cs_2CO_3, and 105 mg (0.257 mmol) Buchwald SPhos catalyst were premixed in round bottom flask with 100 mL THF, and the solution degassed by sparging with argon gas for 20 minutes. After degassing, 58 mg (0.257 mmol) solid $Pd(OAc)_2$ catalyst was added, followed by further argon sparging for an additional 10 minutes. While a tight seal was maintained (THF boiling point 68˚C) on the reaction flask, the reaction temperature was raised to 80˚C and left to react for 24 hours. For the workup, organic phase was washed with water, dried with brine and then anhydrous $MgSO_4$, followed by column purification with gradient elution Hexane:EA 4:1 -> Hexane:EA 1:1 -> pure DCM. 450 mg (50 % yield) yellow foam product was collected of which 290 mg (32 % yield) was the alpha anomer. Observed MS-AP+ m/z = 669.4, calculated $[M+Na]^+$ mass = 669.8. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.76 (d, *J* = 8.5 Hz, 1H), 8.45 (s, 1H), 8.42 – 8.10 (m, 9H), 8.05 – 7.95 (m, 4H), 7.26 – 7.20 (m, 2H), 6.86 – 6.73 (m, 2H), 5.72 (ddd, *J* = 6.9, 3.0, 2.1 Hz, 1H), 5.64 (dd, *J* = 7.9, 2.7 Hz, 1H), 5.03 – 4.92 (m, 1H), 4.82 – 4.67 (m, 2H), 3.01 (ddd, *J* = 13.9, 7.9, 6.8 Hz, 1H), 2.83 (dt, *J* = 13.9, 2.5 Hz, 1H), 2.38 (s, 3H), 2.02 (s, 3H).

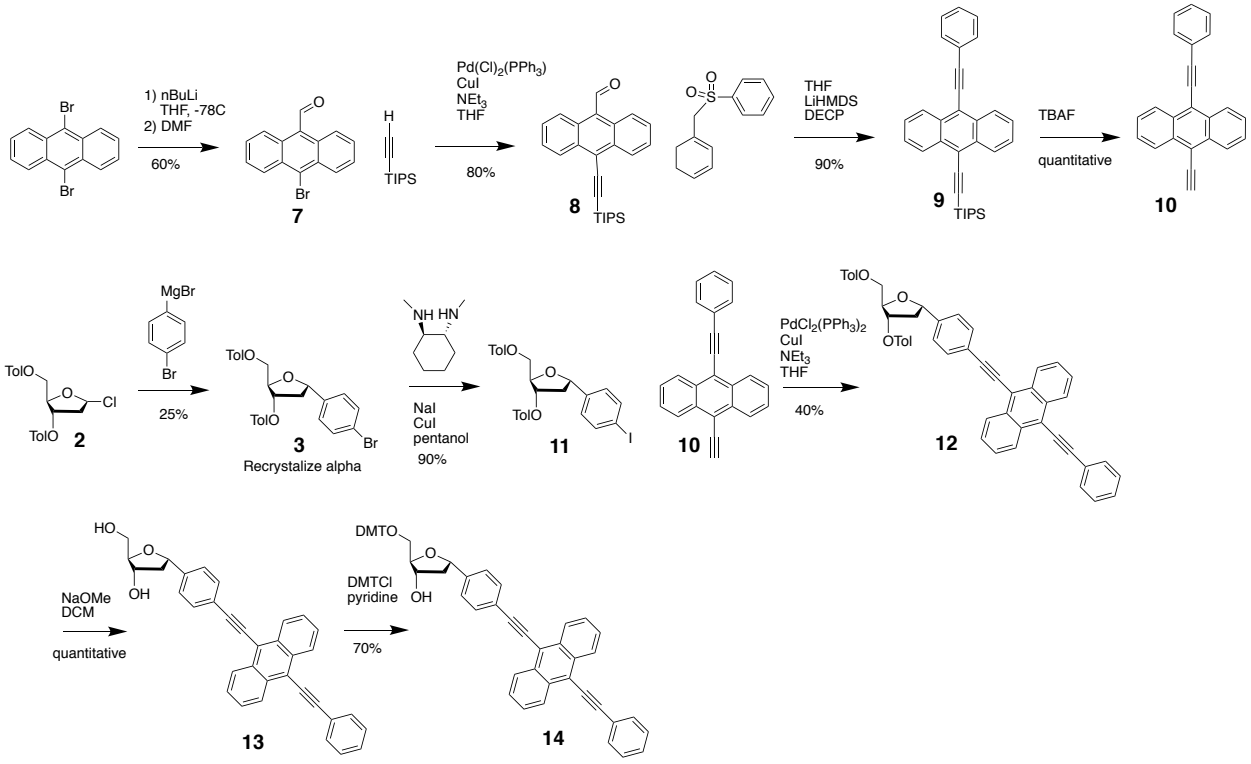
(2R,3S,5S)-5-(coronen-1-ylethynyl)-2-(hydroxymethyl)tetrahydrofuran-3-ol **5** – Sugar **4** was dissolved in dry DCM, with gentle heat applied via heat gun to fully dissolve sugar. 3 equivalents of sodium methoxide (0.5 M in methanol) was then added to the solution. Reaction was monitored by TLC with eluent DCM:methanol 95:5, and left to stir overnight. On completion of reaction, the solvent was evaporated, leaving a yellow precipitate which was collected, washed with water, and dried under high vacuum. The reaction yield was nearly quantitative. Due to solubility issues, the crude product was used for the next reaction.

(2R,3S,5S)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(coronen-1-ylethynyl)tetrahydrofuran-3-ol **6** –Starting material **5** was co-evaporated with dry pyridine for azeotropic removal of trace water to attain

exceptional dryness. 140 mg (0.318 mmol) of **5** was dissolved in 5 mL dry pyridine solvent under dry atmosphere, followed by addition of 320 uL (1.84 mmol) anhydrous N,N-Diisopropylethylamine, and finally 320 mg (0.94 mmol) of 4,4′-dimethoxytriphenylmethyl chloride on continuous stirring. The reaction was monitored with TLC and upon completion quenched with methanol to avoid the doubly functionalized product. TLC and column purification eluent ratio was DCM:hexane:THF (3 solvent system) 4.8:5:0.2 -> 5:4.5:0.5. Average product yield was 70%. High resolution mass spectrometry observed m/z = 765.2611, formula $C_{52}H_{38}O_5Na$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.88 (d, *J* = 8.6 Hz, 1H), 8.61 (s, 1H), 8.51 – 8.28 (m, 9H), 7.57 – 7.51 (m, 2H), 7.43 (dd, *J* = 8.9, 2.1 Hz, 4H), 7.35 (dd, *J* = 8.5, 6.9 Hz, 2H), 7.30 – 7.21 (m, 2H), 6.92 – 6.85 (m, 4H), 5.49 (dd, *J* = 7.8, 4.1 Hz, 1H), 4.55 (dp, *J* = 7.3, 3.7 Hz, 2H), 3.79 (s, 6H), 3.49 – 3.35 (m, 2H), 2.88 (ddd, *J* = 13.0, 7.9, 6.3 Hz, 1H), 2.61 (dt, *J* = 13.3, 3.8 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.70, 144.99, 136.15, 136.12, 130.27, 130.25, 129.25, 128.56, 128.36, 128.24, 128.21, 128.14, 128.10, 127.78, 127.11, 127.04, 126.17, 126.13, 125.97, 125.79, 125.75, 125.71, 125.66, 125.28, 123.76, 121.54, 121.51, 121.39, 121.30, 121.25, 117.55, 113.39, 104.89, 94.08, 86.57, 85.68, 85.20, 77.47, 77.16, 76.84, 75.16, 68.75, 64.57, 55.38, 42.79, 25.74, 12.70.

The DMT-protected sugar **6** was converted to the phosphoramidite derivative under argon by reaction with 3 equivalents of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite in dry DCM containing 6 equivalents of anhydrous N,N-diisopropylethylamine. This phosphoramidite product was purified with a short silica column (hexane:ethyl acetate 4:1), and used immediately for DNA coupling.

Bisphenylethynyl-anthracene monomer synthesis



Synthesis of anthracene derivatives **7**, **8**, **9** and **10** were accomplished by the protocol developed by Toyota et. al.[7]

(2R,3S,5S)-5-(4-Bromophenyl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate **3** was synthesized using a modified procedure originally developed by Hocek.[8] – Added to a flame dried round bottom flask containing 105 mg (4.4 mmol) dry magnesium chips was 1.03 g (4.4 mmol) dibromobenzene, and 5 mL dry THF. The mixture was sonicated at room temperature until all magnesium dissolved. The solution became cloudy upon generation of the Grignard reagent. Grignard solution was

diluted with 50 mL dry THF. 1.7 g (4.4mmol) Chlorosugar **2** was added to the room temperature reaction solution in one shot, with vigorous immediate stirring to ensure quick dissolution of the chlorosugar. Reaction was left to stir overnight, and upon completion quenched with aqueous NH_4Cl, and the organic fraction was dried with brine and anhydrous sodium sulfate. Solvent was evaporated from the organic fraction, and product purified with column chromatography hexane:ethyl acetate 20:1. TLC product spot at Rf = 0.1 with hexane:ethyl acetate 20:1 were the closely eluting alpha and beta anomers. The alpha anomer crystallized out of the hexane:ethyl acetate fraction on prolonged standing. The hexane:ethyl acetate solvent was partially evaporated to induce further crystallization, and the pure alpha product crystals were collected with vacuum filtration on a glass frit filter. Average product yield for the alpha anomer was 25%.

(2R,3S,5S)-5-(4-Iodophenyl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate **11** – 1 g of sugar **3**, 2 g NaI, 200 mg CuI, 300µL of trans-N,N′-dimethylcyclohexane-1,2-diamine and 20 mL butanol solvent were added to a 100 mL volume sealed tube and left to stir at 130˚C overnight. The reaction mixture was quenched with aqueous ammonia, filtered, and purified with column chromatography. The product was a white solid. Observed MS-ESI+ m/z = 557.2, calculated $[M+H]^+$ mass = 557.1. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 (d, *J* = 8.2 Hz, 2H), 7.62 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.5 Hz, 2H), 7.33 – 7.26 (m, 2H), 7.26 – 7.21 (m, 2H), 7.21 – 7.13 (m, 2H), 5.58 (ddd, *J* = 6.4, 3.5, 2.6 Hz, 1H), 5.33 (dd, *J* = 7.6, 5.4 Hz, 1H), 4.67 (td, *J* = 4.7, 2.7 Hz, 1H), 4.56 (dd, *J* = 4.7, 2.1 Hz, 2H), 2.91 (ddd, *J* = 14.1, 7.7, 6.6 Hz, 1H), 2.40 (d, *J* = 6.2 Hz, 6H), 2.27 (ddd, *J* = 13.7, 5.3, 3.5 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 166.38, 166.02, 144.09, 143.93, 142.56, 137.49, 129.79, 129.72, 129.66, 129.24, 129.18, 127.60, 127.11, 126.77, 92.60, 82.45, 79.71, 76.36, 64.59, 40.32, 21.77.

((2R,3S,5S)-3-((4-Methylbenzoyl)oxy)-5-(4-((10-(phenylethynyl)anthracen-9-yl)ethynyl)phenyl)tetrahydrofuran-2-yl)methyl 4-methylbenzoate **12** – 870 mg (1.56 mmol) of sugar **11**, 461 mg (1.56 mmol) of alkyne **10** were added to a Schlenk flask under argon, followed by 30 mL THF, and 3 mL trimethylamine. The reaction solution was argon sparged for 20 minutes before the addition of 6.9 mg CuI and 50 mg $PdCl_2(PPh_3)_2$ catalysts, after which a further 10 minutes of argon sparging was conducted. The reaction was then left to stir at 80˚C overnight. The product was purified with column chromatography. Average product yield is 40%. Observed MS-ESI+ m/z = 753.4, calculated $[M+Na]^+$ mass = 753.3. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.70 (dtd, *J* = 7.0, 2.6, 0.8 Hz, 4H), 7.99 (d, *J* = 8.2 Hz, 2H), 7.82 – 7.74 (m, 4H), 7.69 – 7.61 (m, 6H), 7.54 – 7.49 (m, 2H), 7.49 – 7.39 (m, 3H), 7.31 – 7.22 (m, 2H), 7.19 (dd, *J* = 8.6, 0.7 Hz, 2H), 5.63 (dt, *J* = 6.3, 3.2 Hz, 1H), 5.47 (dd, *J* = 7.6, 5.2 Hz, 1H), 4.73 (td, *J* = 4.6, 2.6 Hz, 1H), 4.61 (dd, *J* = 4.5, 1.0 Hz, 2H), 2.98 (ddd, *J* = 14.0, 7.8, 6.6 Hz, 1H), 2.42 (s, 3H), 2.41 – 2.34 (m, 2H), 2.32 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 166.61, 166.27, 144.30, 144.12, 143.69, 132.32, 131.98, 131.91, 129.98, 129.87, 129.41, 129.31, 128.94, 128.80, 127.51, 127.43, 127.29, 127.05, 126.96, 125.97, 123.62, 122.53, 102.67, 102.54, 86.69, 82.67, 80.21, 76.57, 64.82, 40.60, 21.93, 21.88.

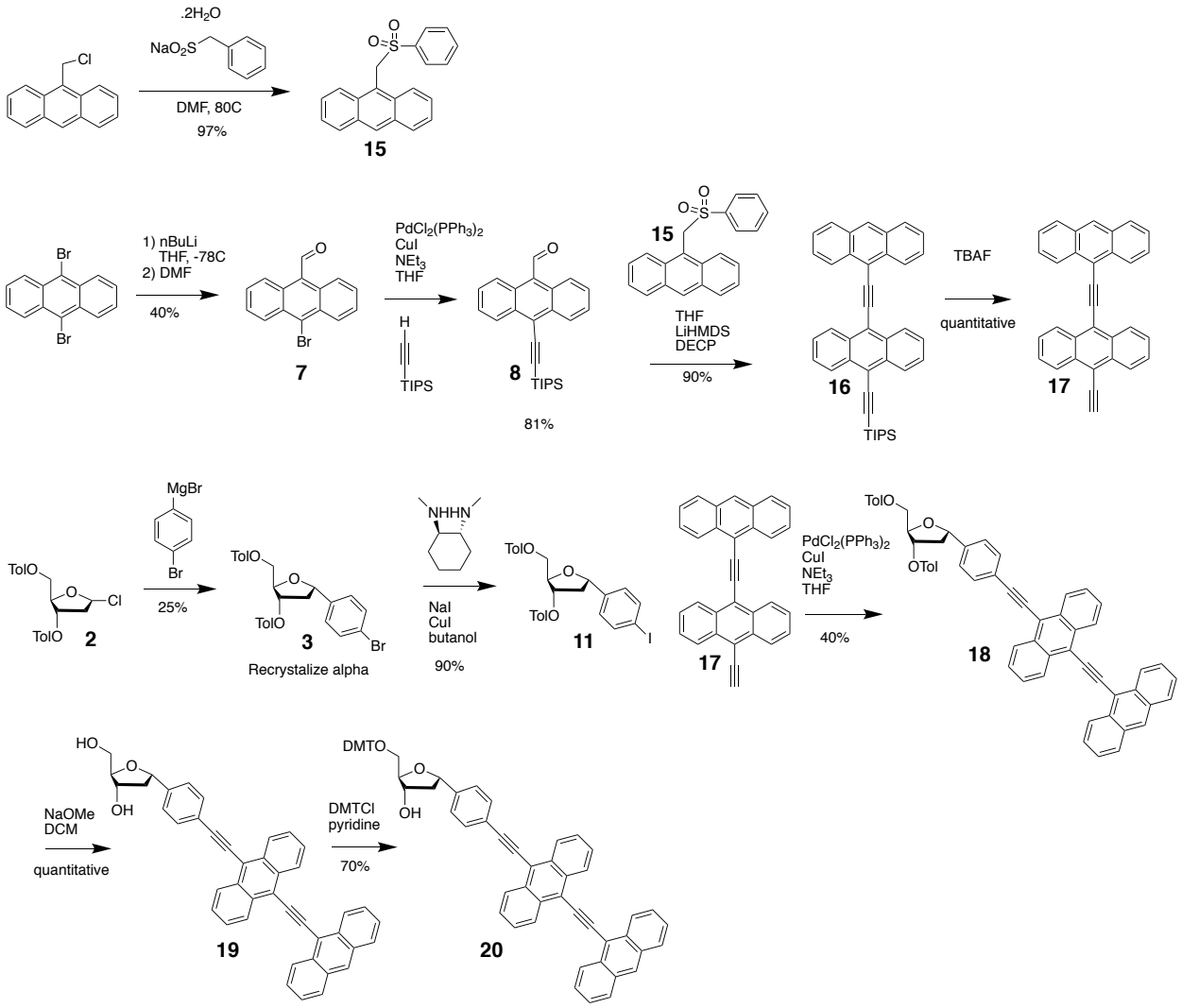
(2R,3S,5S)-2-(Hydroxymethyl)-5-(4-((10-(phenylethynyl)anthracen-9-yl)ethynyl)phenyl)tetrahydrofuran-3-ol **13** - Sugar **12** was dissolved in dry DCM, followed by the addition of 3 equivalents of sodium methoxide (0.5M in methanol). Reaction was monitored by TLC with eluent hexane:ethylacetate 4:1, and stirred for several hours until completion. Reaction mixture was eluted through a short plug of silica to remove salts. The silica plug was flushed with DCM:THF:methanol 1:1:0.5 to ensure all product was collected. Solvent was then evaporated and the product purified with a longer silica column using eluent DCM 100% -> DCM:THF:methanol 1:1:0.5 (3 solvent system). Product yield was quantitative.

(2R,3S,5S)-2-((Bis(4-methoxyphenyl)(phenyl)methoxy)methyl)-5-(4-((10-(phenylethynyl)anthracen-9-yl)ethynyl)phenyl)tetrahydrofuran-3-ol **14** - Starting material **13** was coevaporated with dry pyridine to attain exceptional dryness via azeotropic removal of water. 230 mg (0.465 mmol) of **14** was dissolved in 5 mL dry pyridine solvent under dry atmosphere, followed by addition of 485 uL (2.79 mmol) anhydrous N,N-Diisopropylethylamine, and finally 472 mg (1.395 mmol) of 4,4′-Dimethoxytriphenylmethyl chloride on continuous stirring. Reaction was monitored with TLC and quenched with methanol upon reaction completion to avoid doubly functionalized product. TLC and column purification eluent ratio was DCM:hexane:ethyl acetate (3 solvent system) 1:3:0.5. Average product yield was 70%. Observed MS-ESI- m/z = 797.6, calculated $[M]^-$ mass = 796.6. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.75 – 8.64 (m, 4H), 7.82 – 7.73 (m, 4H), 7.65 (dd, *J* = 6.7, 3.2 Hz, 4H), 7.52 – 7.42 (m, 5H), 7.29 (ddd, *J* = 4.7, 1.3, 0.8 Hz, 5H), 7.20 – 7.10 (m, 4H), 6.88 – 6.77 (m, 4H), 5.26 – 5.14 (m, 1H), 4.59 – 4.47 (m, 1H), 4.15 (q, *J* = 5.0 Hz, 1H), 3.88

(dd, *J* = 11.4, 4.2 Hz, 1H), 3.80 (s, 6H), 3.77 (d, *J* = 4.3 Hz, 1H), 2.76 (dt, *J* = 13.2, 6.8 Hz, 1H), 2.10 (ddd, *J* = 12.8, 8.2, 6.9 Hz, 1H).

The DMT-protected sugar **14** was converted to the phosphoramidite derivative under argon by reaction with 3 equivalents of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite in dry DCM containing 6 equivalents of anhydrous base N,N-diisopropylethylamine. This phosphoramidite product was purified with a short silica column (hexane:ethyl acetate 4:1), and used immediately for DNA coupling.

Phenylethynyl-anthracenylethynyl-benzene monomer synthesis



9-((Phenylsulfonyl)methyl)anthracene **15** – 1 g (4.41 mmol) of 9-(Chloromethyl)anthracene was mixed with 1.094 g (6.62 mmol) of sodium benzenesulfinate dehydrate in 8mL DMF and stirred at 80˚C for 10 minutes. TLC with hexane:ethyl acetate 4:1 eluent indicated completion of reaction. Product solution was poured onto 20 mL water and the slurry stirred for 10 minutes. Crude product precipitate was collected on a glass frit with vacuum filtration. The crude product was recrystallized in DCM-hexane to obtain a fluffy yellow solid as the pure product. 92% product yield (1.84 g) obtained after recrystallization. Observed MS-ESI+ m/z = 355.1, calculated $[M+Na]^+$ mass = 355.1. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.49 (s, 1H), 8.05 – 7.94 (m, 4H), 7.57 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.50 – 7.35 (m, 5H), 7.31 – 7.26 (m, 2H), 5.46 (s, 2H).

Synthetic intermediates **7**, **8**, **16**, and **17** were made via known protocol developed by Toyota et. al.[7]

9-(Anthracen-9-ylethynyl)-10-ethynylanthracene **17** - ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.01 – 8.92 (m, 2H), 8.88 (dq, *J* = 8.7, 1.0 Hz, 2H), 8.73 – 8.64 (m, 2H), 8.53 (s, 1H), 8.14 – 8.05 (m, 2H), 7.73 – 7.63 (m, 6H), 7.57 (ddd, *J* = 7.9, 6.5, 1.2 Hz, 2H), 4.13 (s, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 132.98, 132.91, 132.21, 131.44, 129.06, 128.54, 127.53, 127.34, 127.25, 127.24, 126.95, 125.97, 117.41, 97.41, 94.57, 90.05.

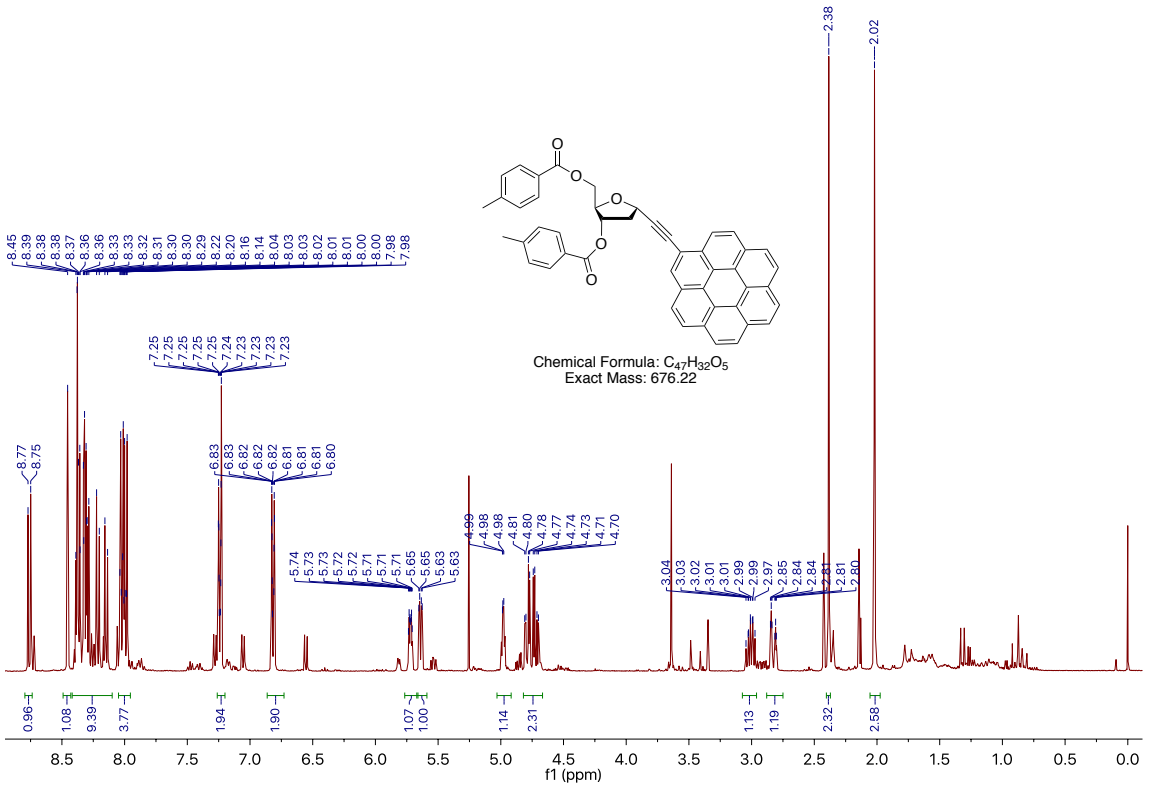
(2R,3S,5S)-5-(4-((10-(Anthracen-9-ylethynyl)anthracen-9-yl)ethynyl)phenyl)-2-(((4-methylbenzoyl)oxy)methyl)tetrahydrofuran-3-yl 4-methylbenzoate **18** – 1.1 g (2.00 mmol) of sugar **11**, 800 mg (2.00 mmol) of alkyne **17** were added to a Schlenk flask under argon, followed by 30 mL THF, and 3 mL trimethylamine. The reaction solution was argon sparged for 20 minutes before the addition of 6.9 mg CuI and 50 mg $PdCl_2(PPh_3)_2$ catalysts, after which a further 10 minutes of argon sparging was conducted. The reaction was left to stir at 80˚C overnight. The product was purified with column chromatography. Average product yield was 40%. Observed MS-ESI+ m/z = 853.5, calculated $[M+Na]^+$ mass = 853.29. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.02 – 8.93 (m, 2H), 8.93 – 8.86 (m, 2H), 8.78 – 8.71 (m, 2H), 8.49 (s, 1H), 8.13 – 8.03 (m, 2H), 8.03 – 7.96 (m, 2H), 7.85 – 7.76 (m, 2H), 7.75 – 7.61 (m, 8H), 7.61 – 7.49 (m, 4H), 7.27 (d, *J* = 8.0 Hz, 2H), 7.21 (d, *J* = 8.0 Hz, 2H), 5.64 (dt, *J* = 6.4, 3.1 Hz, 1H), 5.48 (dd, *J* = 7.6, 5.3 Hz, 1H), 4.75 (td, *J* = 4.6, 2.6 Hz, 1H), 4.63 (dd, *J* = 4.6, 1.2 Hz, 2H), 2.99 (ddd, *J* = 14.0, 7.7, 6.6 Hz, 1H), 2.43 (s, 3H), 2.41 – 2.35 (m, 1H), 2.33 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 166.53, 166.19, 144.21, 144.04, 143.63, 132.96, 132.39, 132.34, 131.92, 131.44, 129.90, 129.80, 129.33, 129.24, 129.03, 128.42, 127.57, 127.50, 127.22, 127.16, 126.99, 126.89, 125.92, 125.90, 122.45, 118.83, 117.53, 102.64, 99.42, 97.72, 86.71, 82.59, 80.13, 76.50, 64.75, 40.53, 21.85, 21.81.

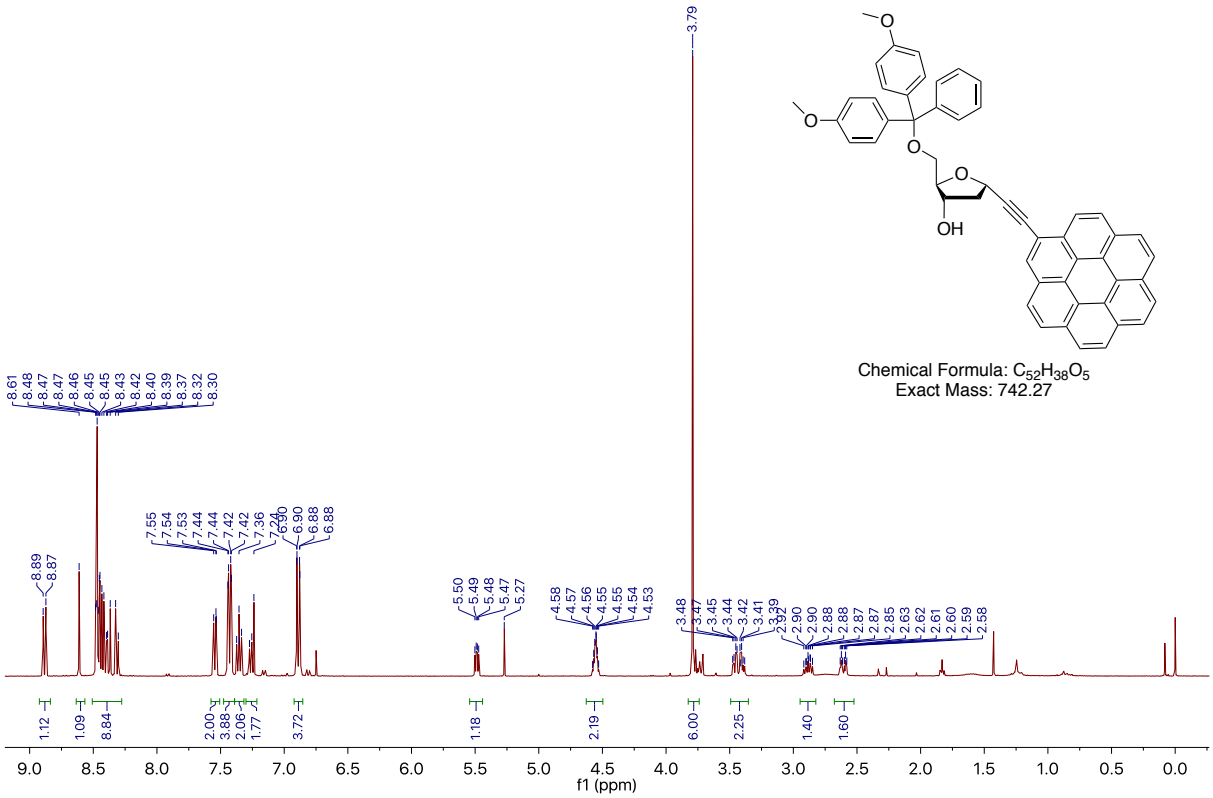
(2R,3S,5S)-5-(4-((10-(Anthracen-9-ylethynyl)anthracen-9-yl)ethynyl)phenyl)-2-(hydroxymethyl)tetrahydrofuran-3-ol **19** – Sugar **19** was dissolved in dry DCM before the addition of 3 equivalents of sodium methoxide (0.5M in methanol). Reaction was monitored by TLC with eluent hexane:ethylacetate 1:1. Upon completion of the reaction, solvent was evaporated, product dissolved in pure ethyl acetate, loaded onto short silica plug, and the plug flushed with hexane:ethylacetate 1:1 to preferentially elute impurities. Once impurities were removed from the silica plug, the plug was flushed with DCM:methanol 1:1 to recover product. Solvent was evaporated from the product solution, and the crude brown solid product washed with water and dried under high vacuum for next reaction. Product yield was quantitative.

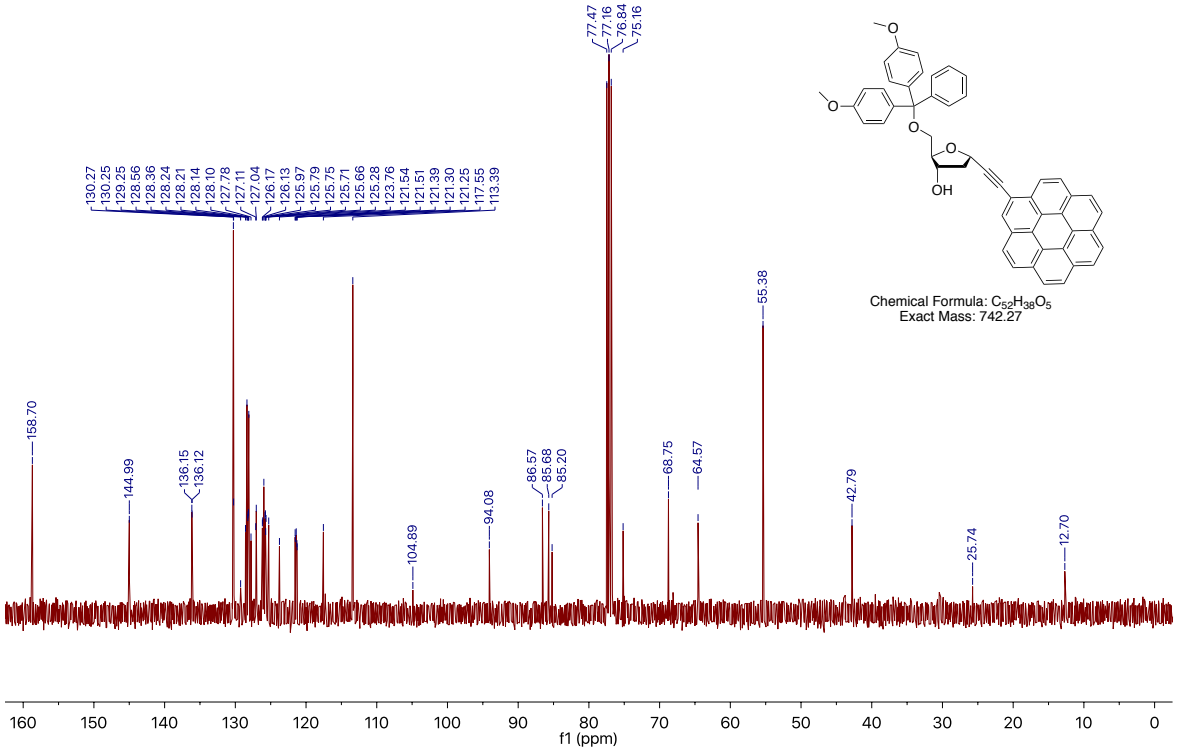
(2R,3S,5S)-5-(4-((10-(Anthracen-9-ylethynyl)anthracen-9-yl)ethynyl)phenyl)-2-((bis(4-methoxyphenyl)(phenyl)methoxy)methyl)tetrahydrofuran-3-ol **20** - Starting material **19** was coevaporated with dry pyridine to attain exceptional dryness via azeotropic removal of water. 253 mg (0.426 mmol) of **19** was dissolved in 5 mL dry pyridine solvent under dry atmosphere, followed by addition of 442 uL (2.54 mmol) anhydrous N,N-diisopropylethylamine, and finally 433 mg (1.27 mmol) of 4,4′-Dimethoxytriphenylmethyl chloride on continuous stirring. Reaction was monitored with TLC and quenched with methanol upon completion to avoid doubly functionalized product. TLC and column purification eluent ratio was DCM:hexane:ethyl acetate (3 solvent system) 1:3:0.5. Average product yield was 70%. Observed MS-AP+ m/z = 919.6, calculated $[M+Na]^+$ mass = 919.3. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.98 – 8.91 (m, 2H), 8.89 (dt, *J* = 8.7, 1.0 Hz, 2H), 8.80 – 8.68 (m, 2H), 8.49 (s, 1H), 8.06 (d, *J* = 8.4 Hz, 2H), 7.83 – 7.74 (m, 2H), 7.66 (dddd, *J* = 11.4, 6.7, 2.4, 1.2 Hz, 6H), 7.60 – 7.43 (m, 6H), 7.42 – 7.35 (m, 4H), 7.32 (td, *J* = 7.4, 1.1 Hz, 2H), 7.24 (t, *J* = 1.8 Hz, 2H), 6.91 – 6.82 (m, 4H), 5.21 (t, *J* = 7.4 Hz, 1H), 4.47 (q, *J* = 6.1 Hz, 1H), 4.24 (q, *J* = 5.2 Hz, 1H), 3.80 (s, 6H), 3.42 (dd, *J* = 9.6, 4.5 Hz, 1H), 3.28 (dd, *J* = 9.5, 6.0 Hz, 1H), 2.76 (dt, *J* = 13.3, 6.8 Hz, 1H), 2.13 – 2.05 (m, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.65, 144.90, 144.04, 136.06, 136.02, 132.92, 132.35, 132.30, 131.97, 131.41, 130.21, 130.19, 129.25, 129.01, 128.38, 128.26, 128.06, 127.53, 127.51, 127.21, 127.15, 127.02, 126.97, 126.05, 125.91, 118.86, 117.53, 113.33, 113.27, 102.69, 99.32, 97.73, 86.67, 86.56, 84.77, 79.66, 75.28, 64.86, 60.56, 55.38, 43.33, 14.34.

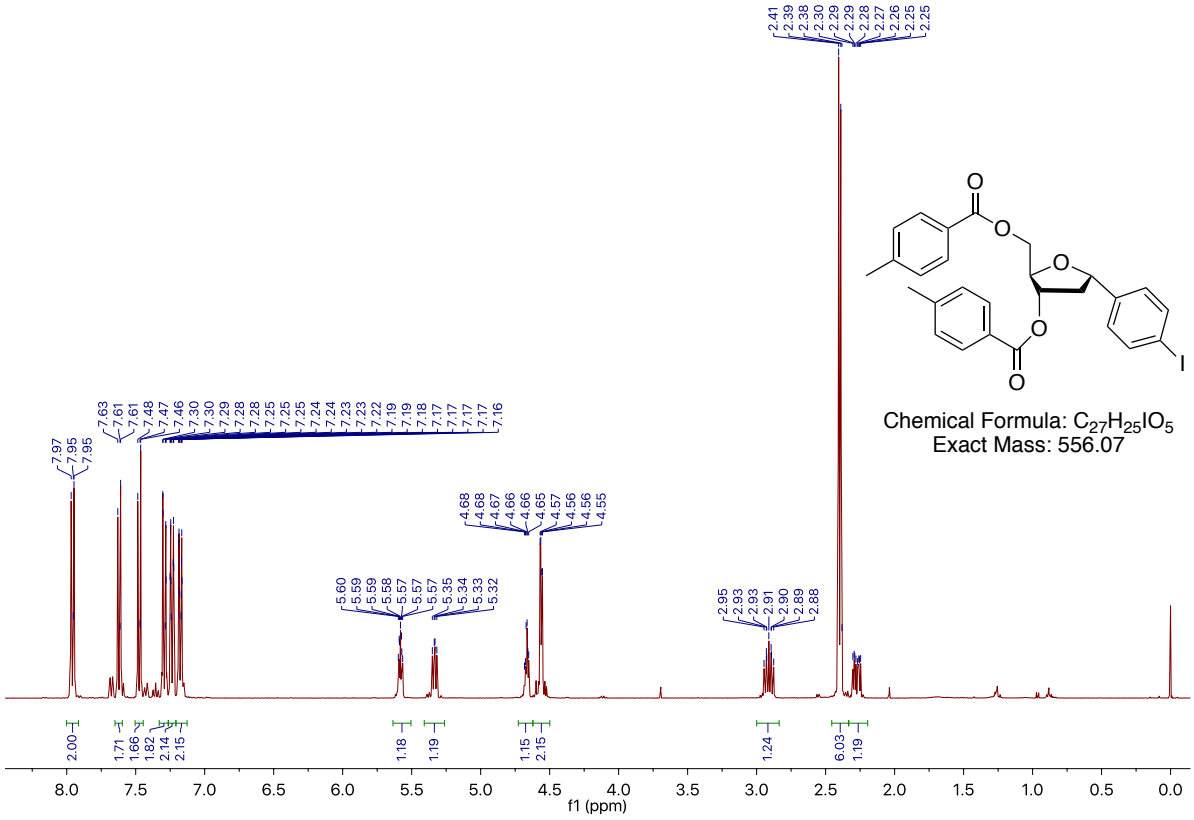
The DMT-protected sugar **20** was converted to a phosphoramidite derivative under argon by reaction with 3 equivalents of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite in dry DCM containing 6 equivalents of anhydrous base N,N-diisopropylethylamine. This phosphoramidite product was purified with a short silica column (hexane:ethyl acetate 4:1), and used directly for DNA coupling.

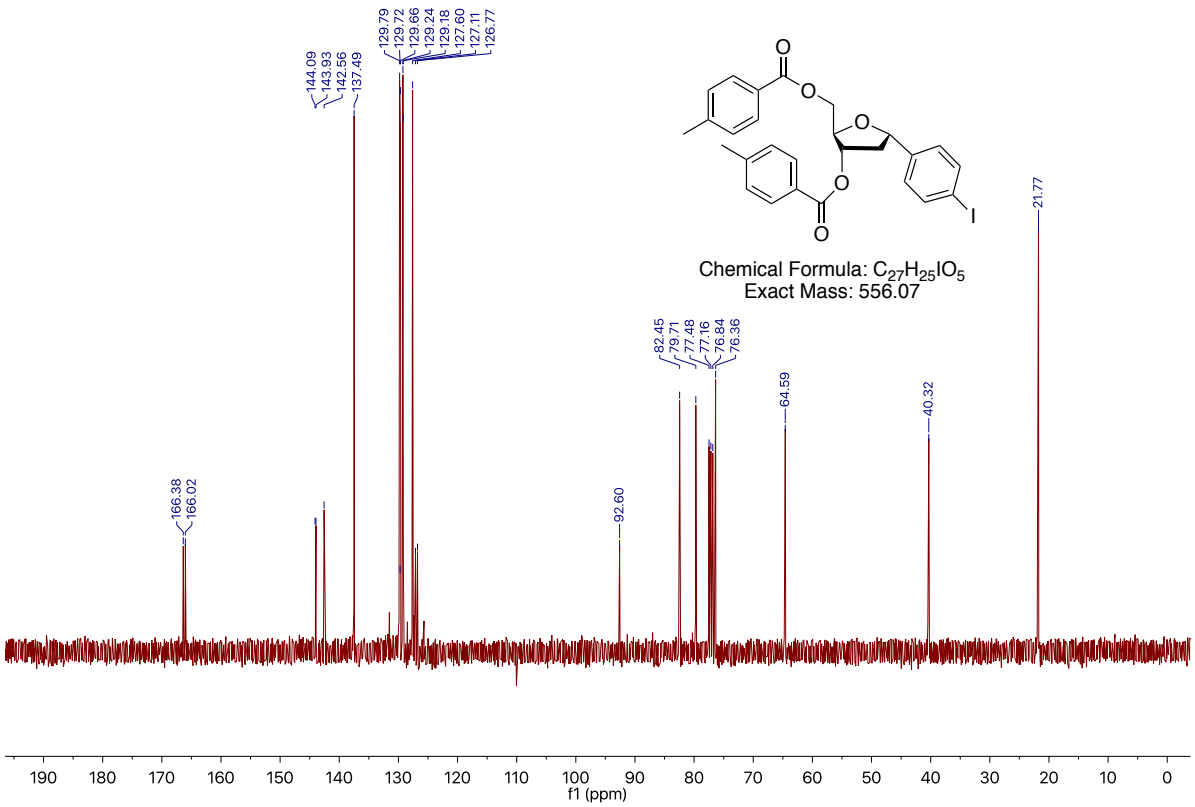
^{1}H and ^{13}C NMR Spectra of Key Synthetic Intermediates

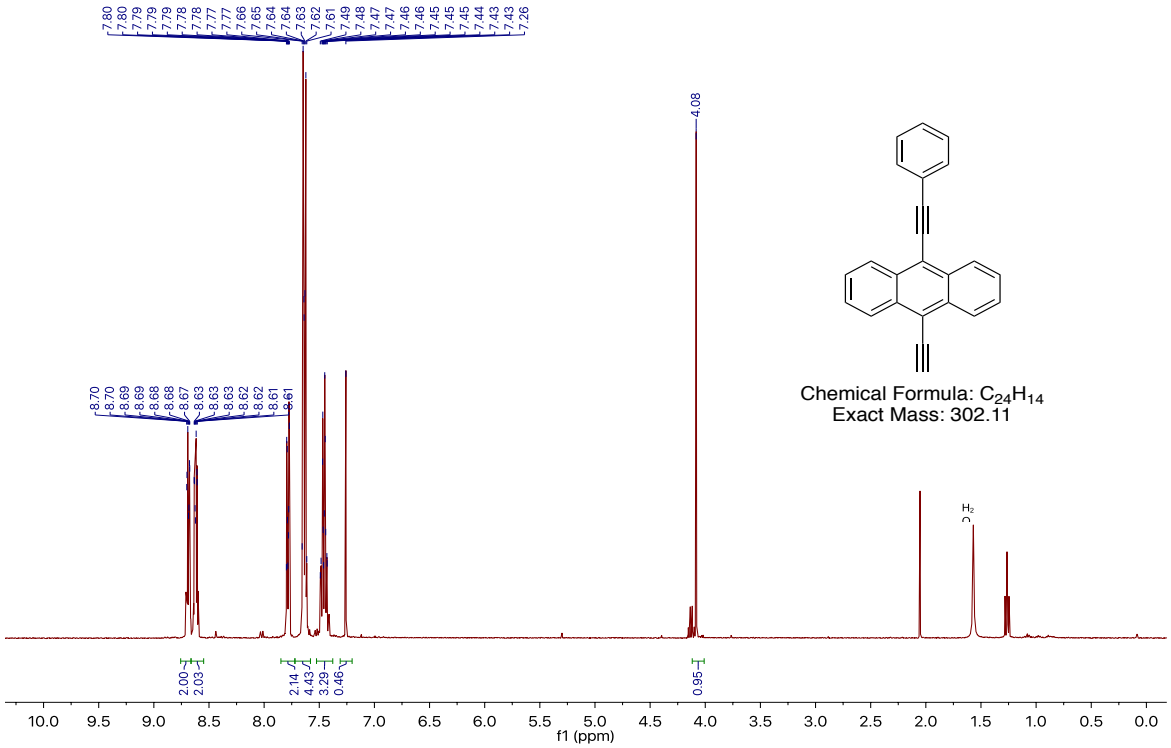


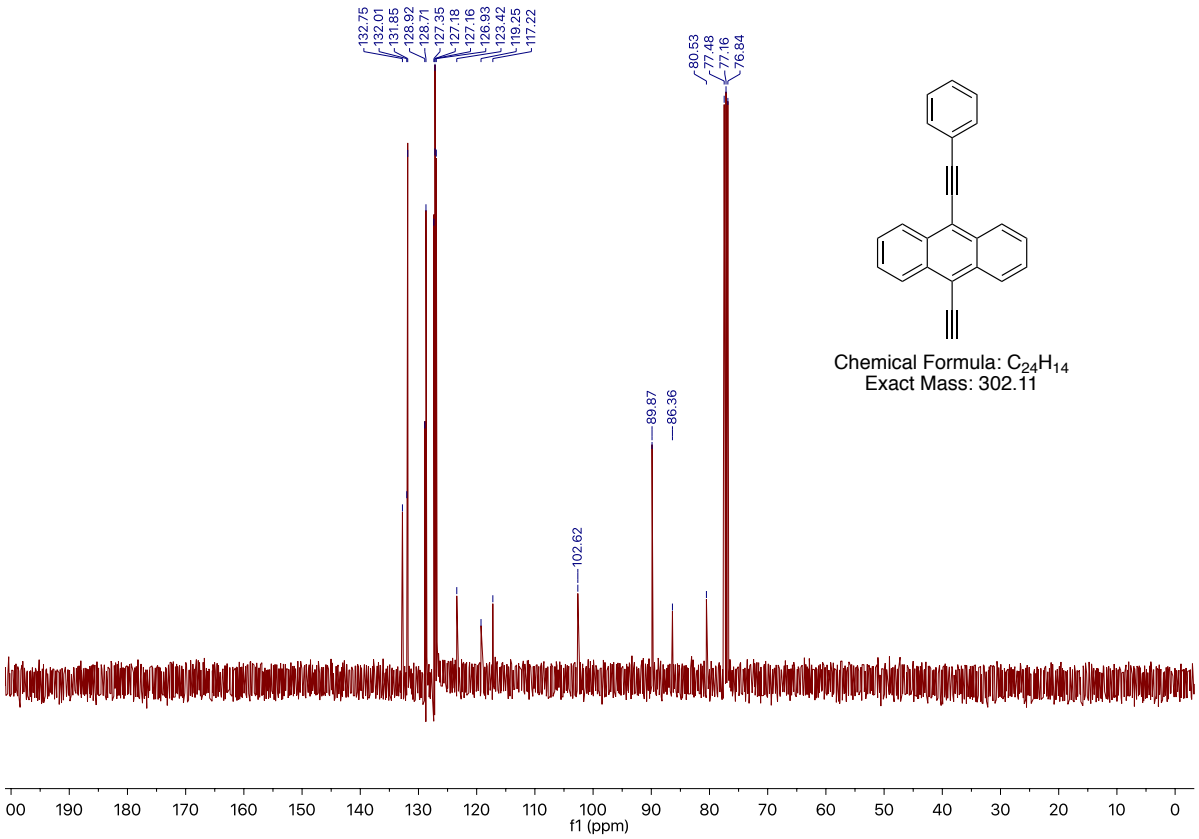


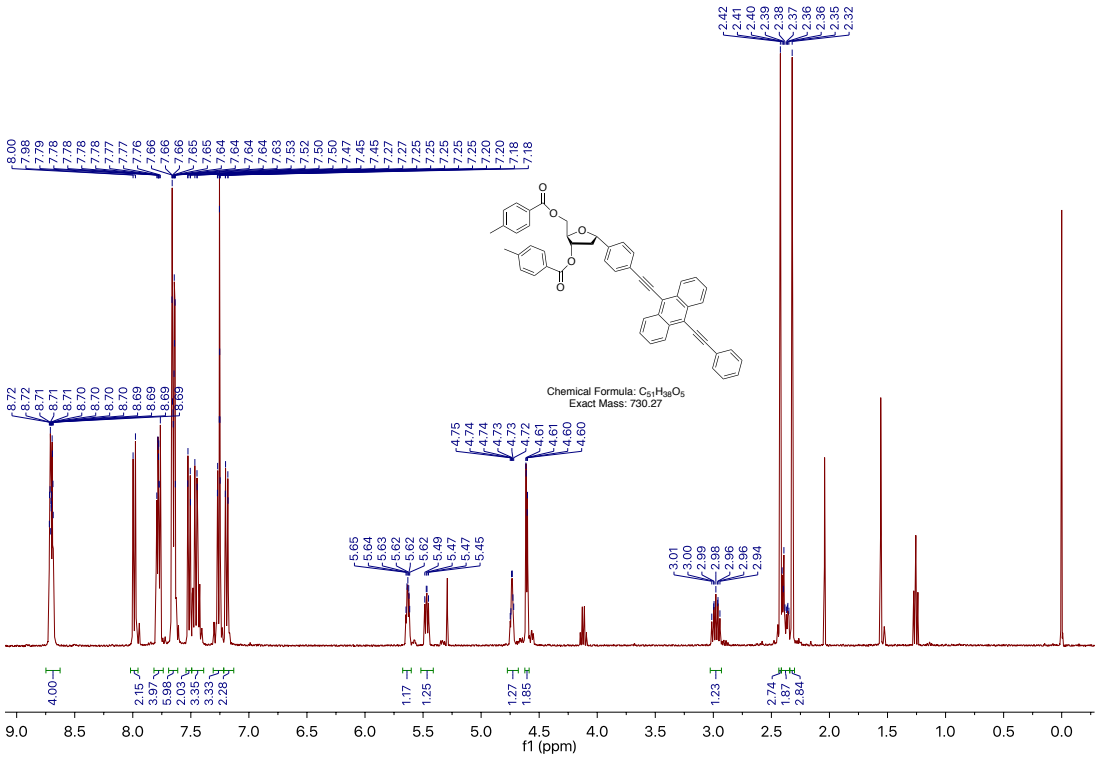

Chemical Formula: C52H38O5
Exact Mass: 742.27
f1 (ppm)

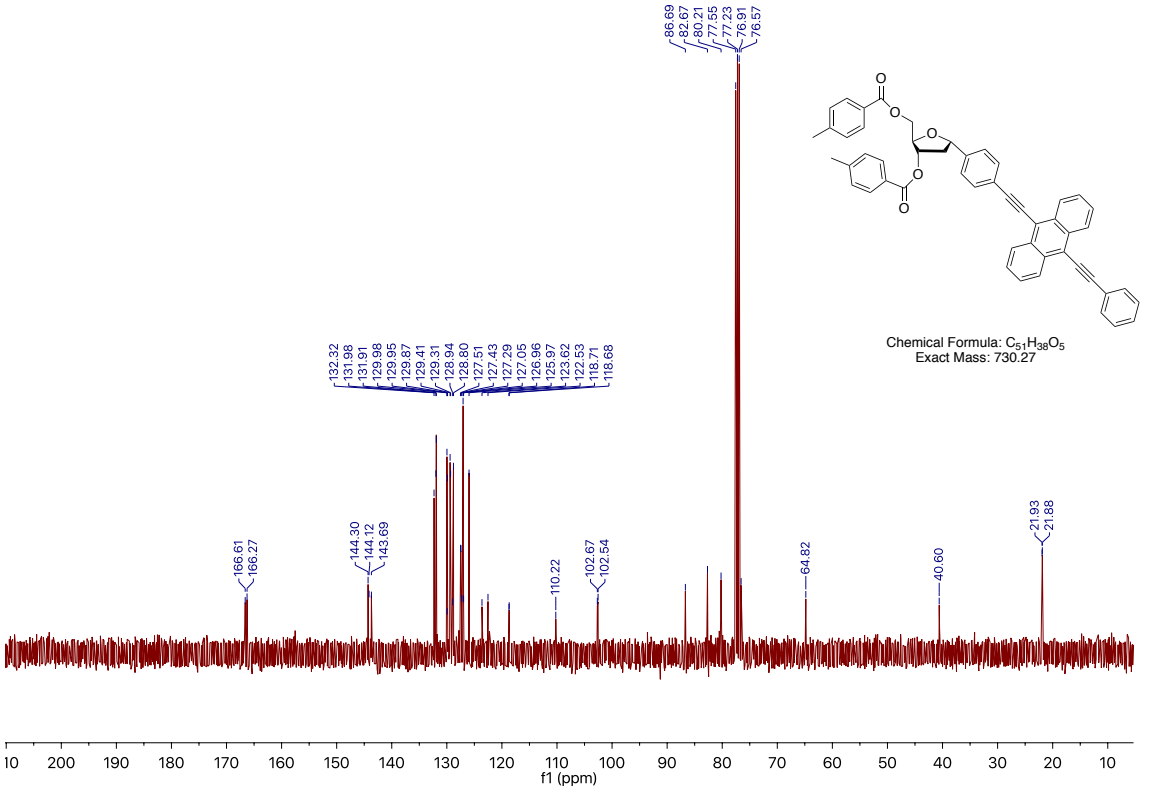

Chemical Formula: C27H25IO5
Exact Mass: 556.07
f1 (ppm)

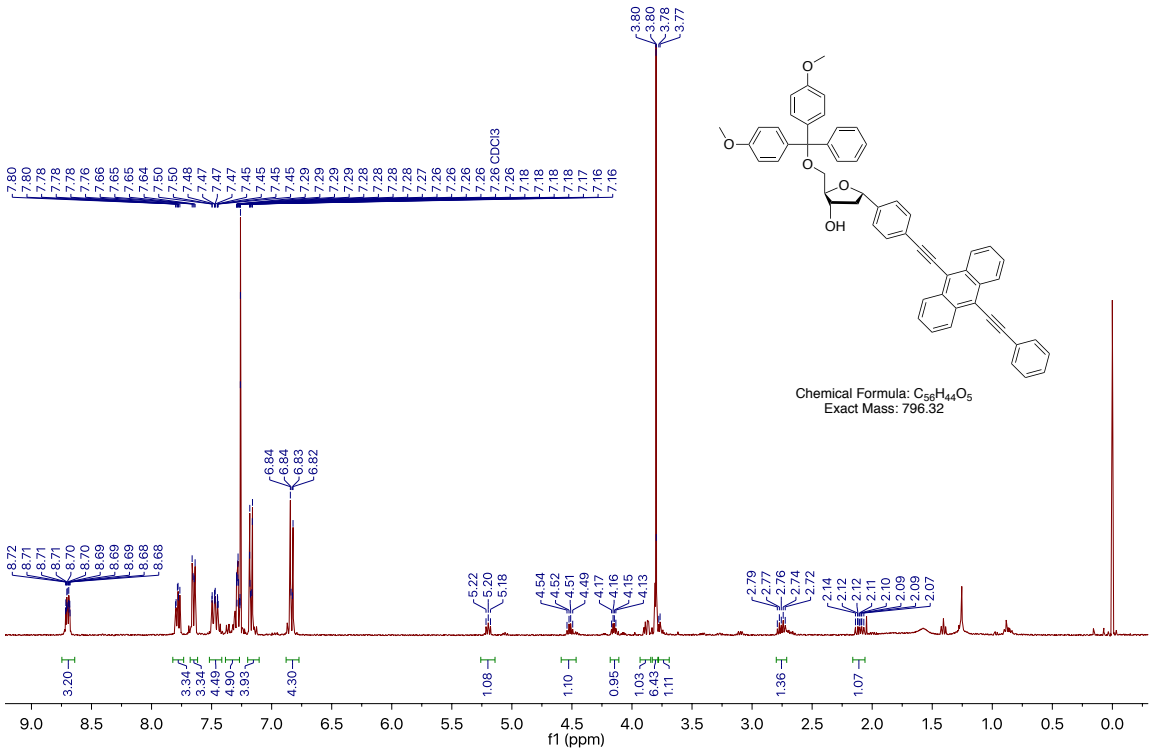

144.09
143.93
142.56
137.49
129.79
129.72
129.66
129.24
129.18
127.60
127.11
126.77
166.38
166.02
92.60
82.45
79.71
77.48
77.16
76.84
76.36
64.59
40.32
21.77
Chemical Formula: C27H25IO5
Exact Mass: 556.07
f1 (ppm)

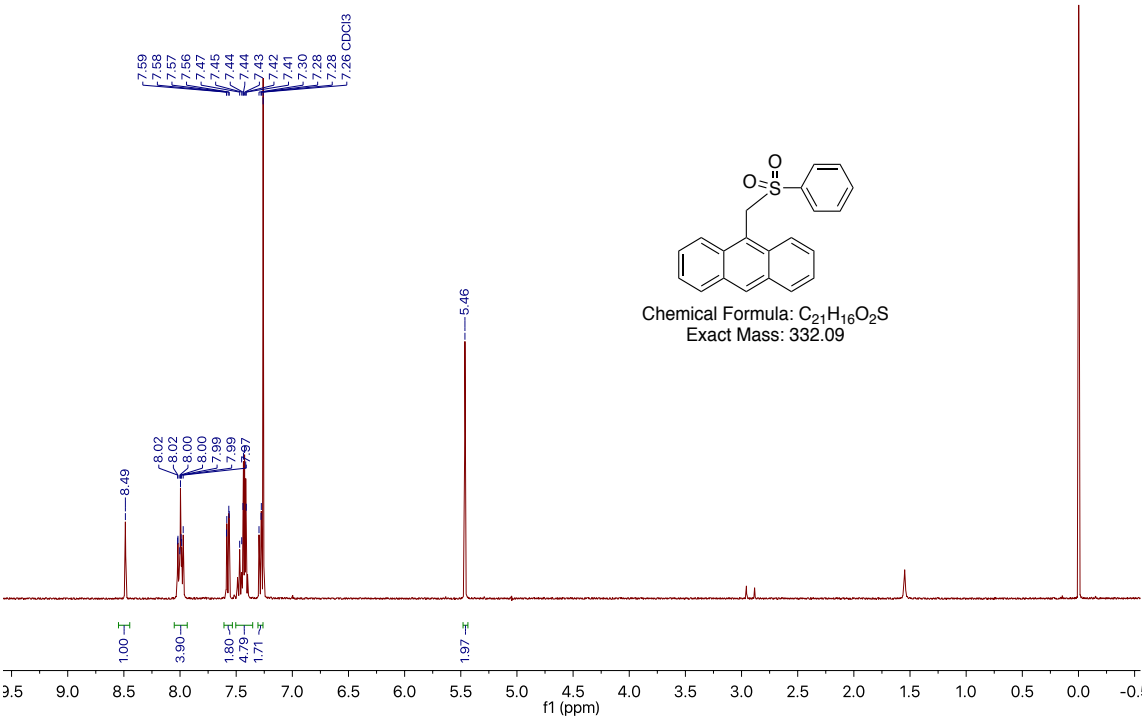

4.08
Chemical Formula: C24H14
Exact Mass: 302.11
2.00
2.03
2.14
4.43
3.29
0.46
0.95
f1 (ppm)


132.75
132.01
131.85
128.92
128.71
127.35
127.18
127.16
126.93
123.42
119.25
117.22
80.53
77.48
77.16
76.84
89.87
86.36
102.62
Chemical Formula: C24H14
Exact Mass: 302.11
f1 (ppm)

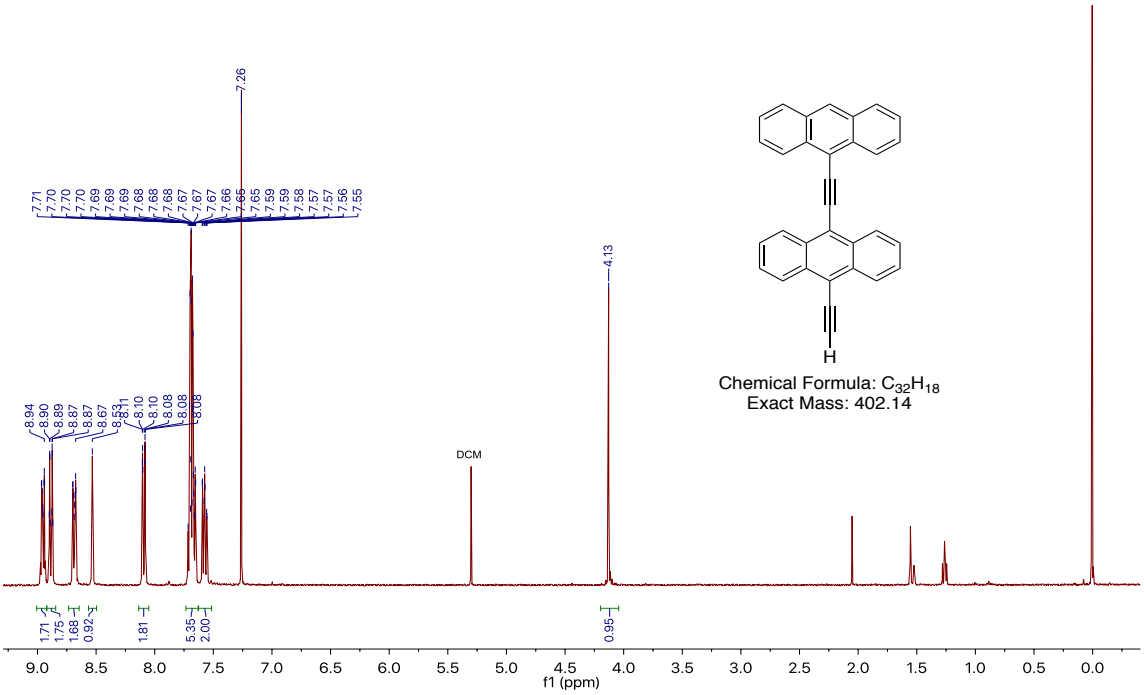

Chemical Formula: C51H38O5
Exact Mass: 730.27
f1 (ppm)


86.69
82.67
80.21
77.55
77.23
76.91
76.57
132.32
131.98
131.91
129.98
129.95
129.87
129.41
129.31
128.94
128.80
127.51
127.43
127.29
127.05
126.96
125.97
123.62
122.53
118.71
118.68
166.61
166.27
144.30
144.12
143.69
110.22
102.67
102.54
64.82
40.60
21.93
21.88
Chemical Formula: C51H38O5
Exact Mass: 730.27
f1 (ppm)

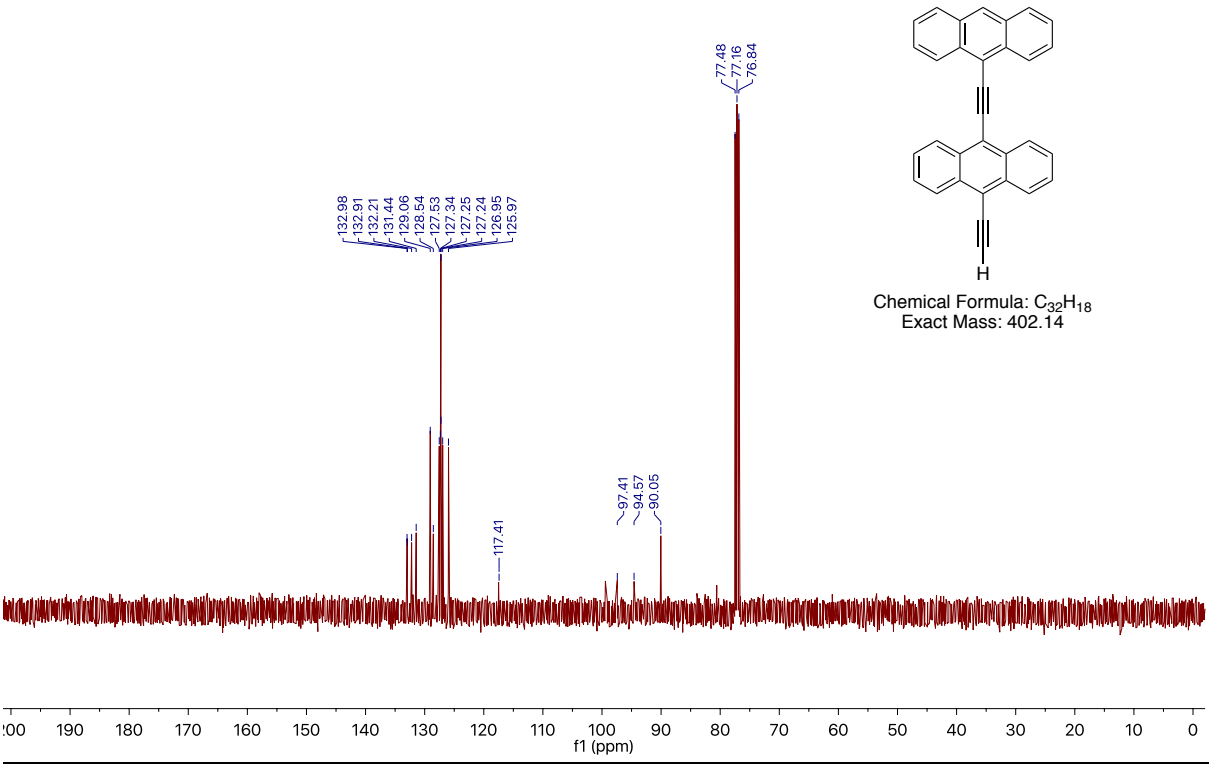

3.80
3.80
3.78
3.77
7.80
7.80
7.78
7.78
7.78
7.76
7.66
7.65
7.65
7.64
7.50
7.50
7.48
7.47
7.47
7.47
7.45
7.45
7.45
7.45
7.29
7.29
7.29
7.29
7.28
7.28
7.28
7.28
7.27
7.26
7.26
7.26
7.26
7.26
CDCl3
7.26
7.18
7.18
7.18
7.17
7.16
7.16
6.84
6.84
6.83
6.82
8.72
8.71
8.71
8.71
8.70
8.70
8.69
8.69
8.69
8.68
8.68
5.22
5.20
5.18
4.54
4.52
4.51
4.49
4.17
4.16
4.15
4.13
2.79
2.77
2.76
2.74
2.72
2.14
2.12
2.12
2.11
2.10
2.09
2.09
2.07
OH
Chemical Formula: C56H44O5
Exact Mass: 796.32
3.20
3.34
3.34
4.49
4.90
3.93
4.30
1.08
1.10
0.95
1.03
6.43
1.11
1.36
1.07
f1 (ppm)

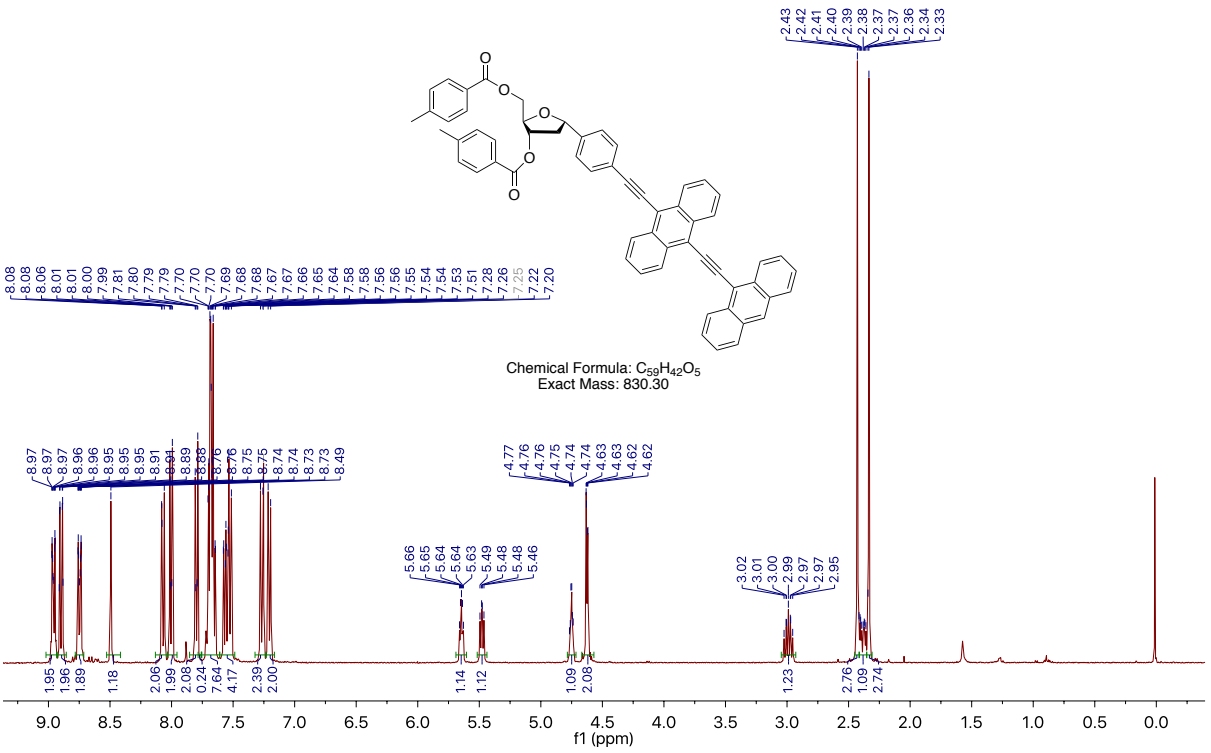


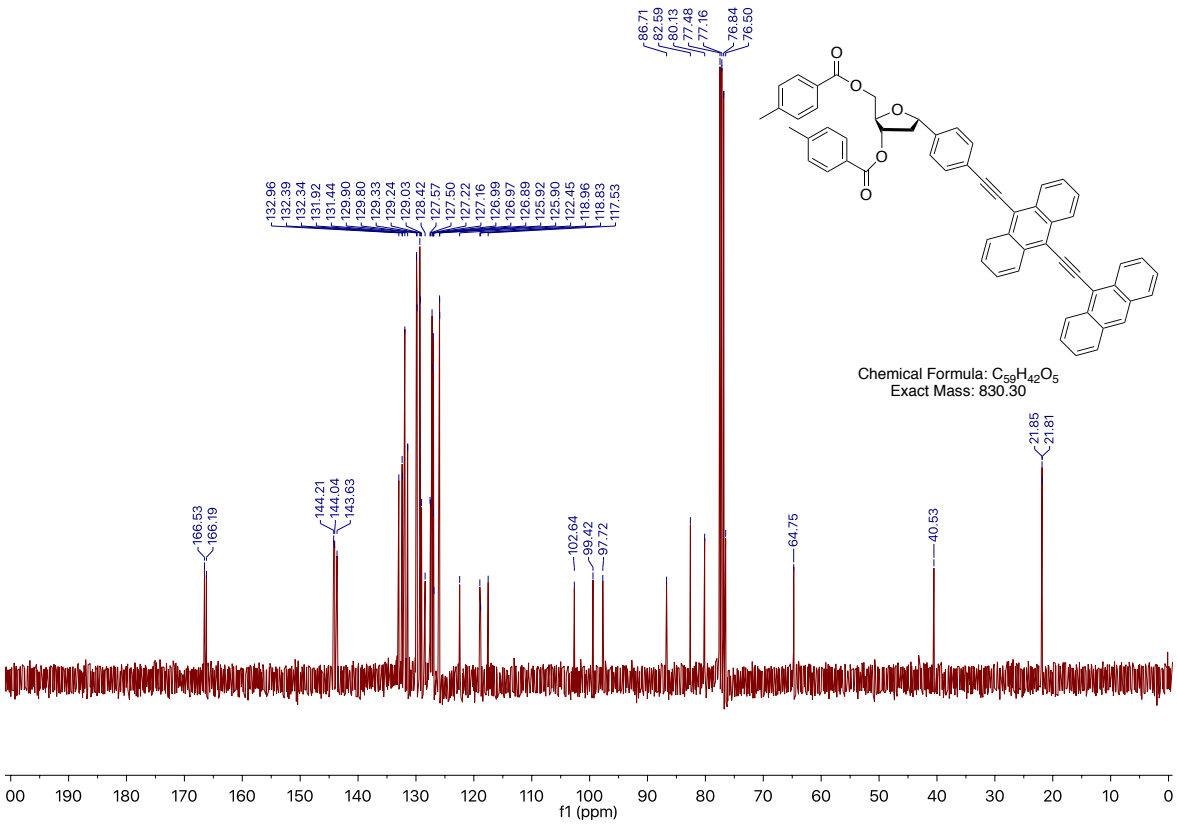
Chemical Formula: $C_{21}H_{16}O_2S$
Exact Mass: 332.09

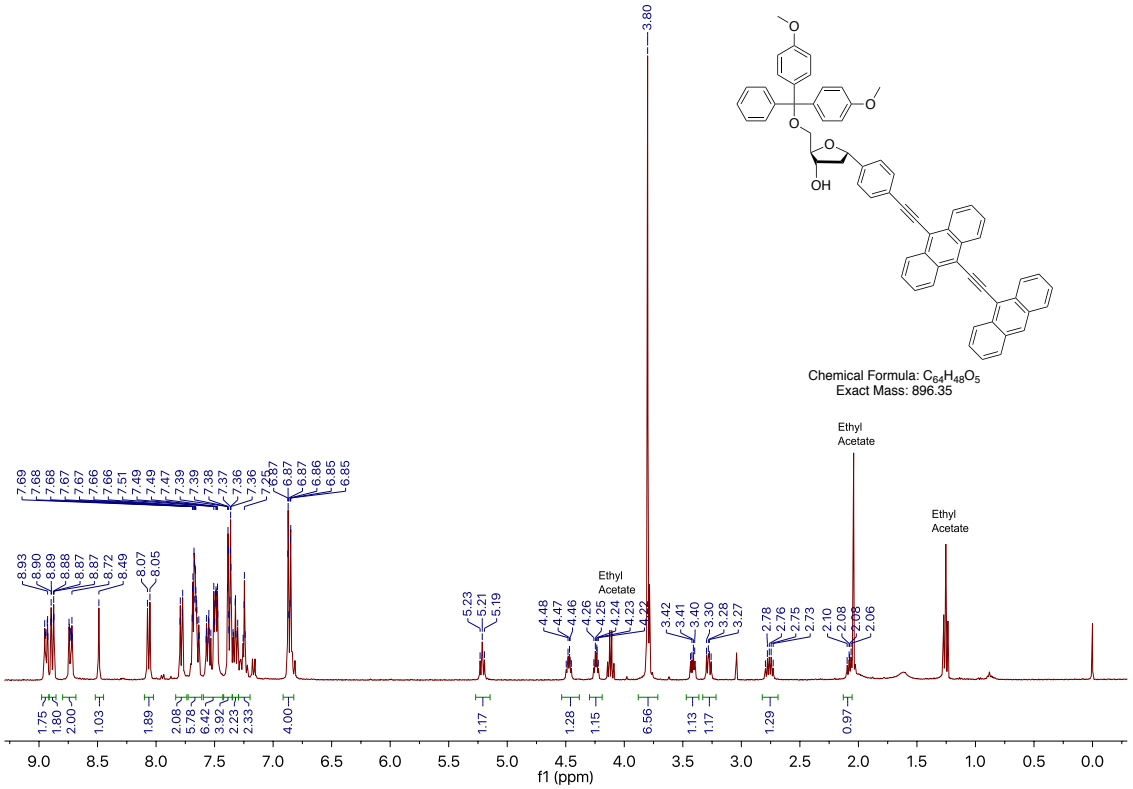


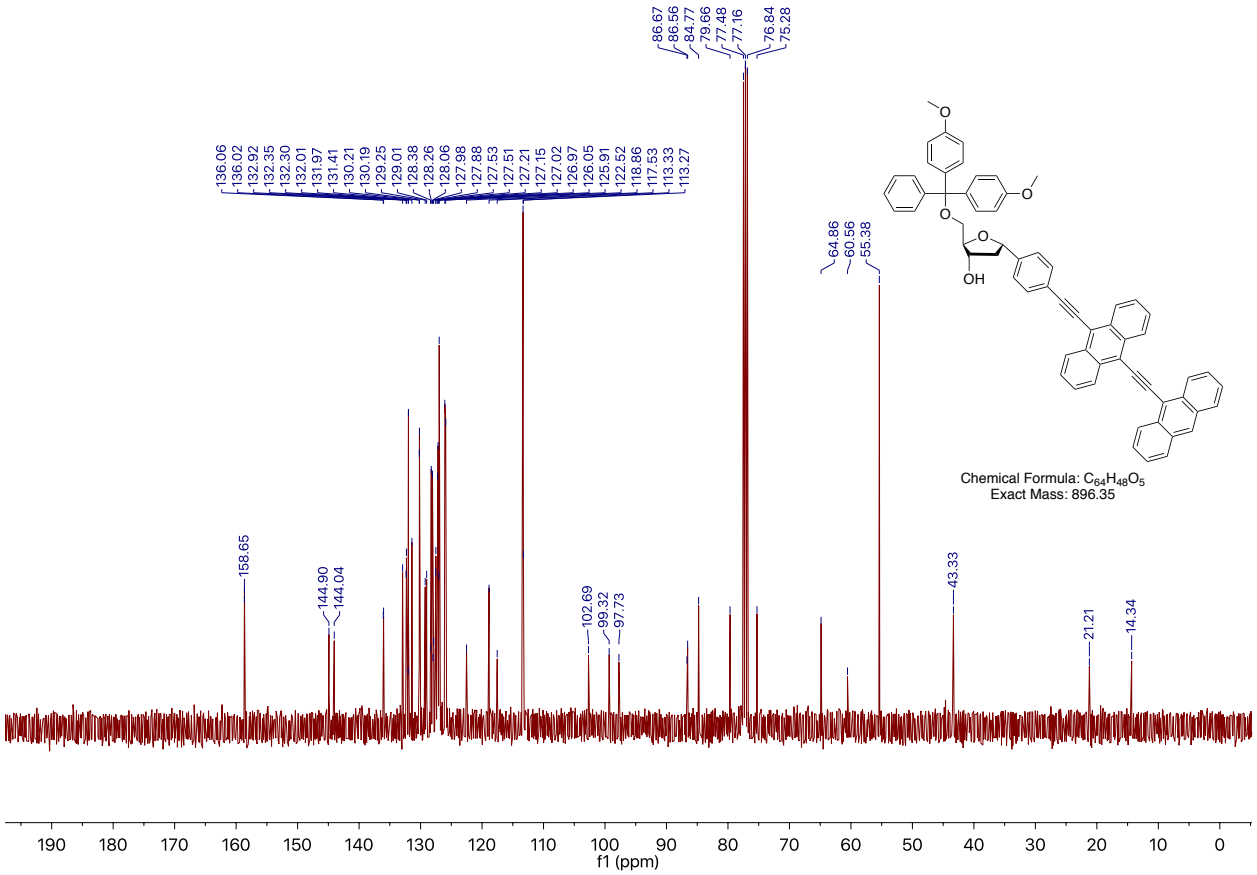
Chemical Formula: $C_{32}H_{18}$
Exact Mass: 402.14


77.48
77.16
76.84
132.98
132.91
132.21
131.44
129.06
128.54
127.53
127.34
127.25
127.24
126.95
125.97
117.41
97.41
94.57
90.05
H
Chemical Formula: C32H18
Exact Mass: 402.14
f1 (ppm)


Chemical Formula: C59H42O5
Exact Mass: 830.30
f1 (ppm)


Chemical Formula: C59H42O5
Exact Mass: 830.30
f1 (ppm)


Chemical Formula: C64H48O5
Exact Mass: 896.35
Ethyl Acetate
Ethyl Acetate
Ethyl Acetate
f1 (ppm)


86.67
86.56
84.77
79.66
77.48
77.16
76.84
75.28
136.06
136.02
132.92
132.35
132.30
132.01
131.97
131.41
130.21
130.19
129.25
129.01
128.38
128.26
128.06
127.98
127.88
127.53
127.51
127.21
127.15
127.02
126.97
126.05
125.91
122.52
118.86
117.53
113.33
113.27
64.86
60.56
55.38
158.65
144.90
144.04
102.69
99.32
97.73
43.33
21.21
14.34
O
O
O
O
OH
Chemical Formula: C64H48O5
Exact Mass: 896.35
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

References

1. Gao, J.; Strässler, C.; Tahmassebi, D.; Kool, E. T., *J. Am. Chem. Soc.* **2002,** *124*, 11590-11591.
2. Ohlmeyer, M. H.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C., *Proc. Natl. Acad. Sci. U.S.A.* **1993,** *90*, 10922-10926.
3. Nestler, H. P.; Bartlett, P. A.; Still, W. C., *J. Org. Chem.* **1994,** *59*, 4723-4724.
4. Gao, J.; Watanabe, S.; Kool, E. T., *J. Am. Chem. Soc.* **2004,** *126*, 12748-12749.
5. Ren, R. X. F.; Chaudhuri, N. C.; Paris, P. L.; Rumney; Kool, E. T., *J. Am. Chem. Soc.* **1996,** *118*, 7671-7678.
6. Rolland, V.; Kotera, M.; Lhomme, J., *Synth. Commun* **1997,** *27*, 3505-3511.
7. Toyota, S.; Mamiya, D.; Yoshida, R.; Tanaka, R.; Iwanaga, T.; Orita, A.; Otera, J., *Synthesis* **2013,** *45*, 1060-1068.
8. Hocek, M.; Pohl, R.; Klepetářová, B., *Eur. J. Org. Chem.* **2005,** *2005*, 4525-4528.