SUPPORTING INFORMATION

4-Cyanoindole-2′-deoxyribonucleoside (4CIN): A Universal Fluorescent Nucleoside Analogue

Kellan T. Passow and Daniel A. Harki

Department of Medicinal Chemistry, University of Minnesota, Minneapolis, Minnesota 55455, United States

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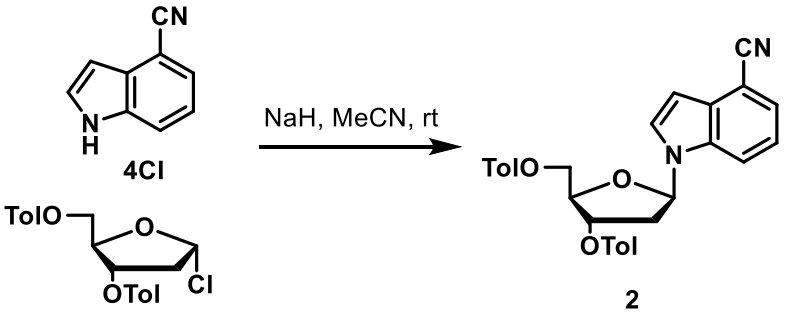
I. Nucleoside and Phosphoramidite Synthesis.

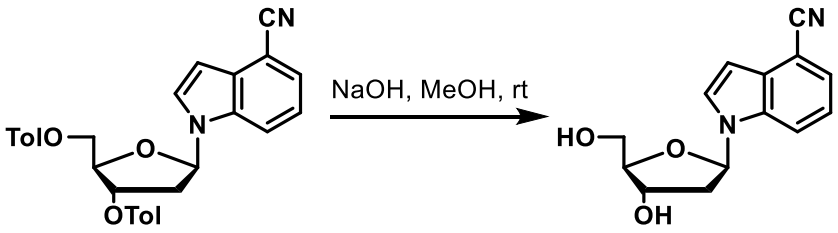
General. Reactions were performed in flame-dried glassware under inert gas (N_2 or Ar) and stirred using a Teflon-coated magnetic stir bar. Reaction solvents acetonitrile (MeCN) and dichloromethane (DCM) were dried by passage over a column of activated alumina using a solvent purification system (MBraun). Other solvents including MeOH and pyridine (anhydrous) were purchased as ACS grade or higher. N,N-diisopropylethylamine (DIPEA) was vacuum distilled from 4 Å molecular sieves and stored with 4Å molecular sieves under argon until use. Water used for the fluorescence experiments was purified using a MilliQ system (MilliporeSigma). 3,5-di-O-toluoyl-α-1-chloro-2-deoxy-D-ribofuranose (**1**) was purchased from Carbosynth. All other chemicals were used as received. Reactions were monitored using thin layer chromatography using EMD Chemicals Silica Gel 60 F_{254} glass plates (250 µm thickness) and visualized with UV irradiation at either 254 nm or 365 nm. Silica gel chromatography was accomplished using a Teledyne-Isco Combiflash Rf-200 instrument using Redisep Rf High Performance silica gel columns from Teledyne-Isco. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and ^{31}P NMR (202 MHz) were collected on a Bruker Advance NMR spectrometer at room temperature. NMR chemical shifts (δ) are recorded relative to TMS (0.05% v/v; δ = 0.0) for ^{1}H NMR and the solvent signal for ^{13}C NMR (δ = 77.0 for $CDCl_3$, and δ = 39.7 for $(CD_3)_2SO$). High resolution mass spectra were obtained at the Analytical Biochemistry Core Facility at the University of Minnesota Masonic Cancer Center using an LTP Orbitrap Velos Mass Spectrometer (Thermo Fisher).

Quantum yield standards and other important compounds used in this study were purchased and analyzed by HPLC (see below) to determine their purity (in parentheses): 2-amino-9-(2′-deoxy-β-D-ribofuranosyl)purine (*e.g.,* 2-aminopurine-2′-deoxyribonucleoside, **2APN**) from Carbosynth (>99%), L-tryptophan from Fluka Chemical (>99%); quinine hemisulfate monohydrate (**QS**) from Alfa Aesar (96%); and 4-cyanoindole (**4CI**) from Sigma Aldrich (>99%). Compound purities of purchased and synthesized molecules were determined by analytical HPLC analysis using an Agilent 1200 series instrument with a diode array detector monitoring 215, 254, or 300 nm. A Zorbax SB-C18 column (4.6 mm x 150 mm x 5.0 µm, Agilent Technologies) was used. A two solvent system was used as the eluents: solvent A = distilled and deionized H_2O (containing 0.1% TFA) and solvent B: MeCN (containing 0.1% TFA). The gradient (1 mL / min flow rate) consisted of: A:B 90:10 from 0-2 minutes, followed by a linear gradient to A:B 20:80 from 2-24 minutes, a second gradient to A:B 5:95 from 24-26 minutes, and an isocratic A:B 5:95 from 26-30 minutes.



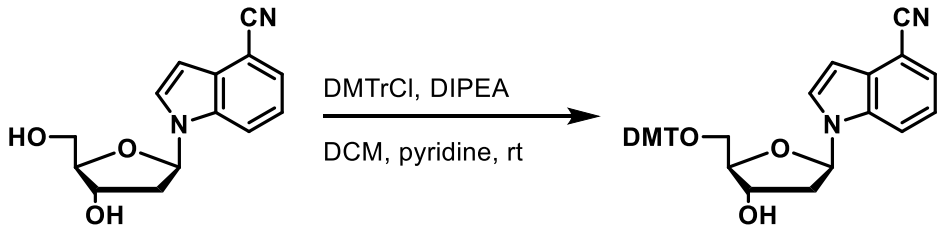
*3′,5′-di-O-toluoyl-protected 4CIN **2**.* Compound **2** was synthesized in a manner similar to a previous report.[1] 4-cyanoindole **4CI** (1.0 g, 7.0 mmol, 1 eq) was dissolved in anhydrous MeCN (150 mL) and

NaH (60% disp. oil) (340 mg, 8.40 mmol, 1.2 eq) was added. This mixture was stirred for 30 minutes at room temperature. 3,5-di-O-toluoyl-α-1-chloro-2-deoxy-D-ribofuranose **1** from Carbosynth (3.3 g, 8.4 mmol, 1.2 eq) was added to this mixture in small portions, and allowed to stir overnight. The reaction is diluted in DCM to approximately 200 mL, filtered through a pad of celite, concentrated *in vacuo*, and the residue purified by chromatography on silica gel with a gradient of 0-60% EtOAc in hexanes to afford **2** (2.5 g, 5.1 mmol, 72% yield with minor impurities that are removed in the subsequent step) as a pale yellow foam. An analytical sample was acquired using preparative thin layer chromatography (Silicycle 60Å F_{254} glass backed plates with a 2000 µm thickness) with a 10% EtOAc in toluene eluent. The β-anomer was confirmed by comparison to ^{1}H NMR data previously reported for 4CIN;[1] additionally, the coupling constants for H1'α-H2'α (*J* = ~5.6 Hz) and H1'α-H2'β (*J* = ~8 Hz) are consistent with β-configuration nucleosides (see reference 2). ^{1}H NMR (500 MHz, $CDCl_3$): 7.98 (d, *J* = 8.0 Hz, 2H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.75 (d, *J* = 8.4 Hz, 1H), 7.48 – 7.42 (m, 2H), 7.29 (d, *J* = 7.9 Hz, 2H), 7.24 (d, *J* = 8.0 Hz, 2H), 7.15 (app t, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 3.3 Hz, 1H), 6.45 (dd, *J* = 8.3, 5.6 Hz, 1H), 5.77 – 5.70 (m, 1H), 4.69 (dd, *J* = 12.0, 3.6 Hz, 1H), 4.62 (dd, *J* = 12.0, 3.7 Hz, 1H), 4.60 – 4.56 (m, 1H), 2.91 – 2.83 (m, 1H), 2.70 (ddd, *J* = 14.3, 5.8, 2.1 Hz, 1H), 2.44 (s, 3H), 2.43 (s, 3H) δ ppm. ^{13}C NMR (126 MHz, $CDCl_3$): 166.1, 166.0, 144.6, 144.2, 135.5, 130.6, 129.8, 129.6, 129.3, 129.3, 126.7, 126.7, 126.43, 125.7, 121.8, 118.4, 114.9, 103.5, 102.4, 85.7, 82.1, 74.7, 63.9, 38.0, 21.74, 21.70 δ ppm. HRMS-ESI^{+} (m/z) calc'd $[M+Na]^{+}$ for $C_{30}H_{26}N_2O_5Na$: 517.1739, found: 517.1725.



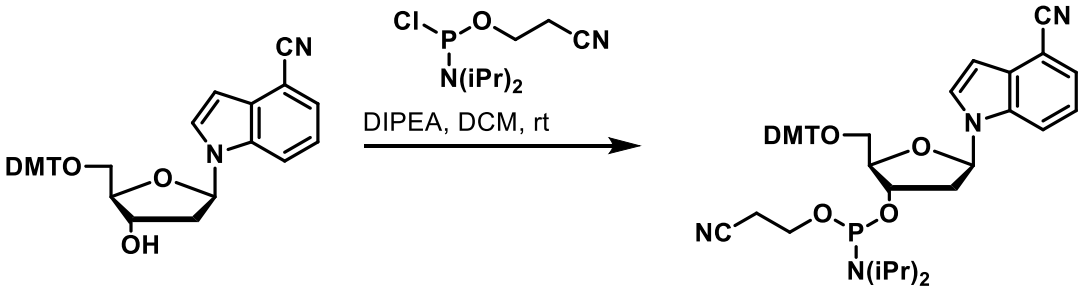
2 **4CIN**

4-Cyanoindole-2'-deoxyribonucleoside (4CIN). **4CIN** was synthesized in a manner similar to a previous report:[1] **2** (2.3 g, 4.6 mmol) was dissolved in a solution of NaOH in MeOH (1% wt/v; 100 mL) and was stirred for 30 minutes. The solution was evaporated *in vacuo*, and residue purified by chromatography on silica gel with a gradient of 0-10% MeOH in DCM to afford **4CIN** (1.0 g, 3.9 mmol, 85% yield). ^{1}H NMR (500 MHz, DMSO-d_6): 8.02 (d, *J* = 8.4 Hz, 1H), 7.91 (d, *J* = 3.3 Hz, 1H), 7.59 (d, *J* = 7.3 Hz, 1H), 7.30 (app t, *J* = 7.9 Hz, 1H), 6.67 (m, 1H), 6.46 (app t, *J* = 6.9 Hz, 1H), 5.31 (d, *J* = 3.9 Hz, 1H), 4.93 (app t, *J* = 5.4 Hz, 1H), 4.40 – 4.34 (m, 1H), 3.87 – 3.81 (m, 1H), 3.60 – 3.47 (m, 2H), ~2.5 (obstructed by DMSO, 1H; Visible in D_2O), 2.31 – 2.23 (m, 1H) δ ppm. ^{13}C NMR (126 MHz, DMSO-d_6): 135.2, 129.3, 128.9, 125.1, 121.4, 118.3, 115.8, 101.5, 100.3, 87.2, 84.7, 70.5, 61.6, 40.3 (CH_2 obstructed by DMSO, visible in DEPT135) δ ppm. HRMS-ESI^{+} (m/z) calc'd $[M + Na]^{+}$ for $C_{14}H_{14}N_2O_3Na$: 281.0902, found: 281.0897.



4CIN **3**

*5'-O-dimethoxytrityl protected 4CIN **3**.* **4CIN** (85 mg, 0.33 mmol) was co-evaporated with anhydrous pyridine (2 mL, 3X) before being dissolved in an anhydrous mixture of pyridine:DCM (1:1, 9 mL). Fresh distilled DIPEA (0.086 mL, 0.49 mmol, 1.5 eq) was added to the mixture. 4,4'-Dimethoxytrityl chloride (DMTrCl) powder (197 mg, 0.581 mmol, 1.75 eq) was added slowly to the mixture, and then put under N_2 and stirred for 3 hours. MeOH was added to quench the reaction, and then the solution was concentrated *in vacuo*. The residue was directly purified by chromatography on silica gel using a gradient of 0-70% EtOAc in hexanes to yield **3** as a yellow foam (148 mg, 0.264 mmol, 80% yield). ^{1}H NMR (500 MHz,$CDCl_3$): 7.74 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 7.3 Hz, 1H), 7.42 – 7.36 (m, 3H), 7.35 – 7.18 (m, 8H), 7.14 (app t, *J* = 7.9 Hz, 1H), 6.88 – 6.74 (m, 4H), 6.72 – 6.66 (m, 1H), 6.38 (app t, *J* = 6.7 Hz, 1H), 4.66 – 4.59 (m, 1H), 4.14 – 4.06 (m, 1H), 3.76 (s, 6H), 3.36 (dd, *J* = 10.3, 4.1 Hz, 1H), 3.32 (dd, *J* = 10.2, 4.6 Hz, 1H), 2.67 – 2.57 (m, 1H), 2.46 – 2.38 (m, 1H) δ ppm. ^{13}C NMR (126 MHz,$CDCl_3$): 158.6, 144.5, 135.62, 135.58, 135.53, 130.4, 130.1, 128.1, 127.9, 127.1, 127.0, 125.5, 121.6, 118.6, 115.1, 113.2, 103.1, 101.8, 86.6, 85.5, 85.2, 72.5, 63.8, 55.2, 40.3 δ ppm. HRMS-ESI$^+$ (m/z) calc'd [M + Na]$^+$ for $C_{35}H_{32}N_2O_5Na$: 583.2209, found: 583.2195.



3 **4**

*5'-O-dimethoxytrityl protected 4CIN phosphoramidite **4.*** **3** (148 mg, 0.264 mmol, 1 eq) was dried under high vacuum for 24 hours before being dissolved in anhydrous DCM (3 mL). Freshly distilled DIPEA (0.138 mL, 0.793 mmol, 3 eq) was added, followed by a dropwise addition of 2-cyanoethyl N,N'-diisopropylchlorophosphoramidite (0.089 mL, 0.40 mmol, 1.5 eq) and then stirring under N_2 for 1 hour. The reaction was concentrated *in vacuo* and directly purified by chromatography on deactivated silica gel (deactivated by washing with 2 column volumes of 1% Et_3N in hexanes) using a gradient of 0-30% EtOAc in cyclohexane to yield **4** as a white foam (a diastereomeric mixture) (144 mg, 0.189 mmol, 72% yield). ^{1}H NMR (500 MHz, $CDCl_3$): 7.82 (d, *J* = 3.3 Hz, resolved diastereomer, 0.5H), 7.80 (d, *J* = 3.3 Hz, resolved diastereomer, 0.5H), 7.47 (d, *J* = 7.4 Hz, 1H), 7.44 (app t, *J* = 3.8 Hz, 1H), 7.39 (app t, *J* =

6.6 Hz, 2H), 7.31 – 7.18 (m, 7H), 7.14 (app t, *J* = 7.7 Hz, 1H), 6.81 – 6.73 (m, 4H), 6.70 (app t, *J* = 4.0 Hz, 1H), 6.42 – 6.34 (m, 1H), 4.78 – 4.69 (m, 1H), 4.29 – 4.21 (m, 1H), 3.95 – 3.81 (m, 1H), 3.80 – 3.75 (m, 6H), 3.74 – 3.65 (m, 1H), 3.65 – 3.54 (m, 2H), 3.42 – 3.24 (m, 2H), 2.79 – 2.43 (m, 4H), 1.21 – 1.16 (m, 9H), 1.11 (d, *J* = 6.8 Hz, 3H) δ ppm. ^{13}C NMR (126 MHz, $CDCl_3$): 158.54, 158.51, 144.51 (br), 135.67, 135.61, 135.59, 135.57, 135.56, 135.53, 130.51, 130.49, 130.15, 130.13, 130.09, 128.24, 128.18, 127.82, 127.80, 127.25, 127.21, 126.91, 126.87, 125.47, 125.46, 121.56, 121.53, 118.61, 118.58, 117.55, 117.43, 115.35, 115.26, 113.09 (br), 103.24, 103.18, 101.75, 101.69, 86.48, 86.46, 85.56, 85.28, 85.25, 85.02, 84.97, 74.0 ($J_{C\text{-}P}$ = 17.6 Hz, one diastereomer), 73.5 ($J_{C\text{-}P}$ = 17.6 Hz, one diastereomer) 63.45, 63.34, 58.52, 58.35 ($J_{C\text{-}P}$ = 12.6 Hz, one diastereomer), 58.19 ($J_{C\text{-}P}$ = 12.6 Hz, one diastereomer), 58.37, 55.21, 55.19, 43.30 ($J_{C\text{-}P}$ = 5.0 Hz, one diastereomer), 43.20 ($J_{C\text{-}P}$ = 5.0 Hz, one diastereomer), 39.70, 39.67, 26.89, 24.6-24.5 (complex), 24.49, 20.42, 20.41 ($J_{C\text{-}P}$ = 7.6 Hz, one diastereomer) 20.37, 20.23 ($J_{C\text{-}P}$ = 7.6 Hz, one diastereomer) δ ppm.^{31}P NMR (202 MHz, $CDCl_3$): 148.88, 148.76 δ ppm. HRMS-ESI$^+$ (m/z) calc'd $[M + Na]^+$ for $C_{44}H_{49}N_4O_6PNa$: 783.3287, found:783.3273.

II. Solid-Phase DNA Synthesis. Oligonucleotides containing **4CIN** were synthesized using previously described methods.[3]

III. HPLC Purification and LC-MS Analysis of DNA. Oligonucleotides were purified using previously described methods.[3] Purchased DNAs from Integrated DNA Technologies and were ordered as either desalted or HPLC purified, checked for purity upon receipt, and purified again by HPLC if necessary. See section **XII** for characterization data of DNA oligonucleotides.

IV. UV Absorption Spectroscopy and Steady State Fluorescence Measurements. UV spectra were collected on an Agilent Cary 100 UV-vis spectrophotometer (Simple Reads software, with SBW 1.5 nm) at room temperature. Steady state fluorescence measurements were collected on a Varian Cary Eclipse fluorescence spectrophotometer (Scan software; excitation slit at 2.5 nm, emission slit at 5 nm in most cases). Samples were dissolved in the described solvent or buffer solution in clean 10 mm path length quartz cuvettes (Starna Cells, Inc.).

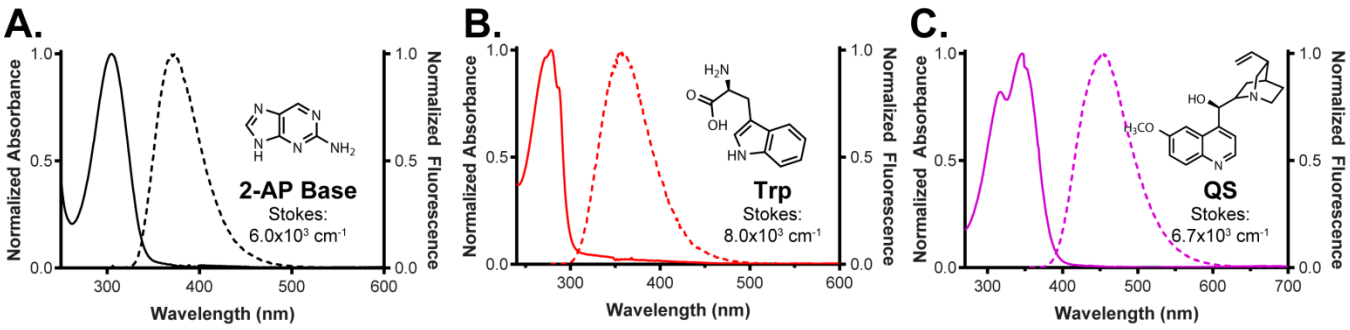


Figure S1. Absorption and emission spectra of additional molecules used in this study as references or quantum yield (QY) standards. A. 2-AP Base, B. L-Tryptophan (**Trp**), C. Quinine Sulfate (**QS**). Normalized absorbance is shown on the left axis (solid line) and the normalized emission shown on the right axis (dotted line). Emission spectra were collected by exciting the molecules at absorption maximum. All compounds were dissolved in distilled and deionized H_2O except **QS** which was dissolved in aqueous 0.105M $HClO_4$, pH = 1.

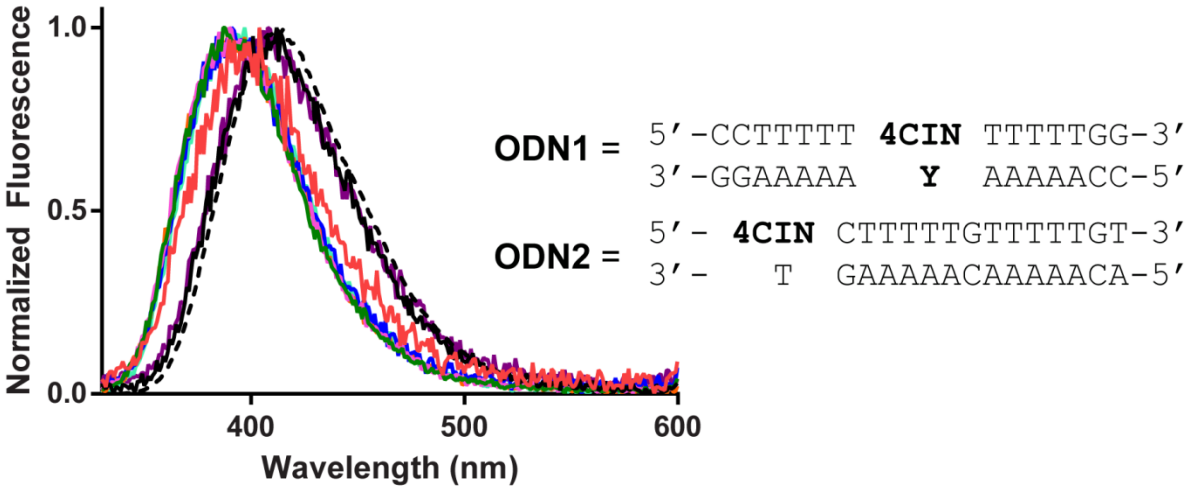


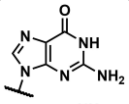
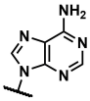
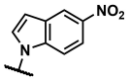
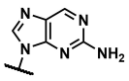
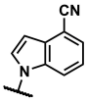
Figure S2. Fluorescence emission spectra of **4CIN**-modified DNAs in PBS pH = 7 excited at 305 nm. **4CIN** monomer emission shown as black dotted line. Single strand **ODN1** (top strand; red) $\lambda_{em,\ max}$ = 404 nm. Single strand **ODN2** (top strand; solid black) $\lambda_{em,\ max}$ = 412 nm. **ODN1** duplexes (**Y = A, C, T, G, Ab** shown in green, pink, blue, orange, teal all overlapping) show $\lambda_{em,\ max}$ = 380-390 nm and **ODN2** duplex (purple, overlap with black curves) shows $\lambda_{em,\ max}$ = 408 nm.

V. UV Thermal Melting Analysis. Conducted using previously described methods.[3]

Table S1. UV Thermal Analysis of Modified Oligonucleotides.

ODN1 = 5′-CCTTTTT **X** TTTTTGG-3′
3′-GGAAAAA **Y** AAAAACC-5′

ODN2 = 5′- **X** CTTTTTGTTTTTGT-3′
3′- **Z** GAAAAACAAAAACA-5′

nucleobases		Y = A	Y = C	Y = G	Y = T	Z = T
X = Guanosine[a]		45.9 (± 0.8)	57.3 (± 0.5)	47.4 (± 0.7)	48.5 (± 0.5)	ND
X = Adenosine		43.2 (± 0.7)	42.5 (± 0.5)	47.1 (± 0.3)	52.6 (± 0.5)	53.5 (± 0.5)
X = 5-NO_2-indole[a]		46.2 (± 0.6)	49.8 (± 0.5)	44.7 (± 0.3)	45.8 (± 0.4)	54.9 (± 0.6)
X = **2APN**		44.5 (± 0.5)	45.5 (± 0.9)	43.4 (± 0.3)	49.8 (± 0.5)	52.0 (± 0.02)
X = **4CIN**		43.4 (± 0.6)	43.3 (± 0.9)	41.4 (± 0.8)	43.3 (± 0.9)	52.0 (± 0.05)

Melting experiments performed in 10 mM aqueous sodium cacodylate, 10 mM KCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$, pH 7.0. Mean ± SD (n = 4) are shown. a. X = G and X = 5-NO_2-indole in **ODN1** data from supporting information reference 3.

VI. Quantum Yield Calculations. Quantum yields (QYs) of molecules and oligonucleotides were determined using the method by Würth, et. al.[4] for the determination of relative fluorescence quantum yields ("Procedure 1"). Since the compounds described herein had maximum absorbances less than 350 nm, the use of quinine sulfate (**QS**), a well characterized QY standard, was used as the reference standard for measurements above 300 nm as prescribed in the protocol (Φ = 0.59 in aqueous 0.105M $HClO_4$).[4] For measurements made below 300 nm, the amino acid tryptophan (**Trp**) was used as a standard (Φ = 0.15 in H_2O). 4-cyanoindole (**4CI**) was also used as a convenient reference, whose QY measurements were reported by Hilaire et. al.[5]

In brief, 1 mL each of target sample and reference sample are prepared in clean quartz cuvettes (Starna Cells, Inc. 10 mm path length) in pairs such that absorbance at the target wavelength is around 0.05 (maximum of 0.1 recommended). Absorbance was measured, followed by total fluorescence by exciting at the target wavelength and then calculating the area under the fluorescence emission curve using the fluorometer software. Absorbance was measured on an Agilent Cary 100 UV-vis spectrophotometer (Simple Reads software, with SBW 1.5 nm) and fluorescence data was collected and analyzed on a Varian Cary Eclipse fluorescence spectrophotometer (Scan software; excitation slit at 1.5 nm, emission slit at 10 nm). Quantum yield Φ was then calculated using Equations 1-3:[4]

$$Equation\ 1\text{: } f = 1 - 10^{-A(\lambda_{ex})}$$

$$Equation\ 2\text{: } F = \int_{\lambda_{ex}} I_c \cdot \lambda_{em} \cdot d\lambda_{em}$$

$$Equation\ 3\text{: } \Phi_{f,x} = \Phi_{f,st} \cdot \frac{F_x}{F_{st}} \cdot \frac{f_{st}}{f_x} \cdot \frac{\eta_x^2(\lambda_{em})}{\eta_{st}^2(\lambda_{em})}$$

where *f* is the absorption factor; λ_{ex} is the excitation wavelength; $A(\lambda_{ex})$ is the absorbance measured at wavelength λ_{ex}; *F* is the relative integral photon flux emitted (the integration of the fluorescence emission curve collected on the fluorometer); λ_{em} is the emission wavelength; η is the refractive index of the solvent used at the emission wavelength (λ_{em}) where η for all water-based solutions was retrieved from reference 6 and η for tetrahydrofuran (THF) was retrieved from the Sigma Aldrich website;[7] and Φ is the quantum yield. In all cases, subscript *x* refers to the sample solution and subscript *st* refers to the standard solution (**Trp** or **QS**).[4] QYs are calculated in a pairwise fashion between one sample and one standard cuvette. The reported QY values and standard deviations are the mean of a minimum of three replicates and reported as mean ± the standard deviation as calculated in Microsoft Excel.

VII. Molar Extinction Coefficient Calculations. The extinction coefficient of **4CIN** was calculated as follows: a known mass of **4CIN** was measured in dry glass vials and then dissolved in the appropriate solvent such that the exact concentration of the solution was known. Then, Beer's Law plots were constructed by taking the solution and diluting it in a serial manner such that 6 samples of a known concentration could be measured at 305 nm using a UV spectrometer (all absorbances < 1.0) using quartz cuvettes (Starna Cells, Inc., 10 mm path length). The slope of the line absorbance vs concentration (in molar, M) is the extinction coefficient ε_{305} in $M^{-1}cm^{-1}$. This process was repeated three times with three different stock solutions, the slopes averaged, and standard deviation calculated using Microsoft Excel for each solvent tested.

VIII. Measuring Fluorescence Lifetimes. Fluorescence lifetimes were measured using a custom time-resolved fluorescence spectrometer ("Fargoland") at the University of Minnesota Biophysical Technology Center (instrument design described in reference 8). The laser excitation was set to 355 nm, and the emission filter used was for 470 nm. Samples were kept under 10 μM in concentration to limit self-quenching and filtering effects. The IRF (instrument response function) was calculated using water (where the fluorescent lifetime (τ) is 0 ns), followed by each sample solution in their respective solvents such that signal was between 20-100 mV. Lifetime calculations were made using the custom "Fargoland" software using a global curve fitting.

IX. Circular Dichroism Analysis of Duplex DNA. Circular dichroism (CD) spectroscopy was performed on a JASCO J-815 CD spectropolarimeter. Wavelengths 310nm – 200 nm were measured at a rate of 50 nm/minute at data increments of 1 nm, and run at room temperature with a 1.00 nm bandwidth. Three scans were performed per sample and averaged by the accompanying software. Approximately 15 nmol of each DNA duplex was annealed in PBS pH = 7 prior to measuring. Sample was diluted into PBS (approximately 200 μL total volume) for running on the instrument in a quartz cuvette (Starna Cells, Inc., 1 mm path length). Spectra reported as ellipticity (θ) in millidegrees (mdeg).

X. References.

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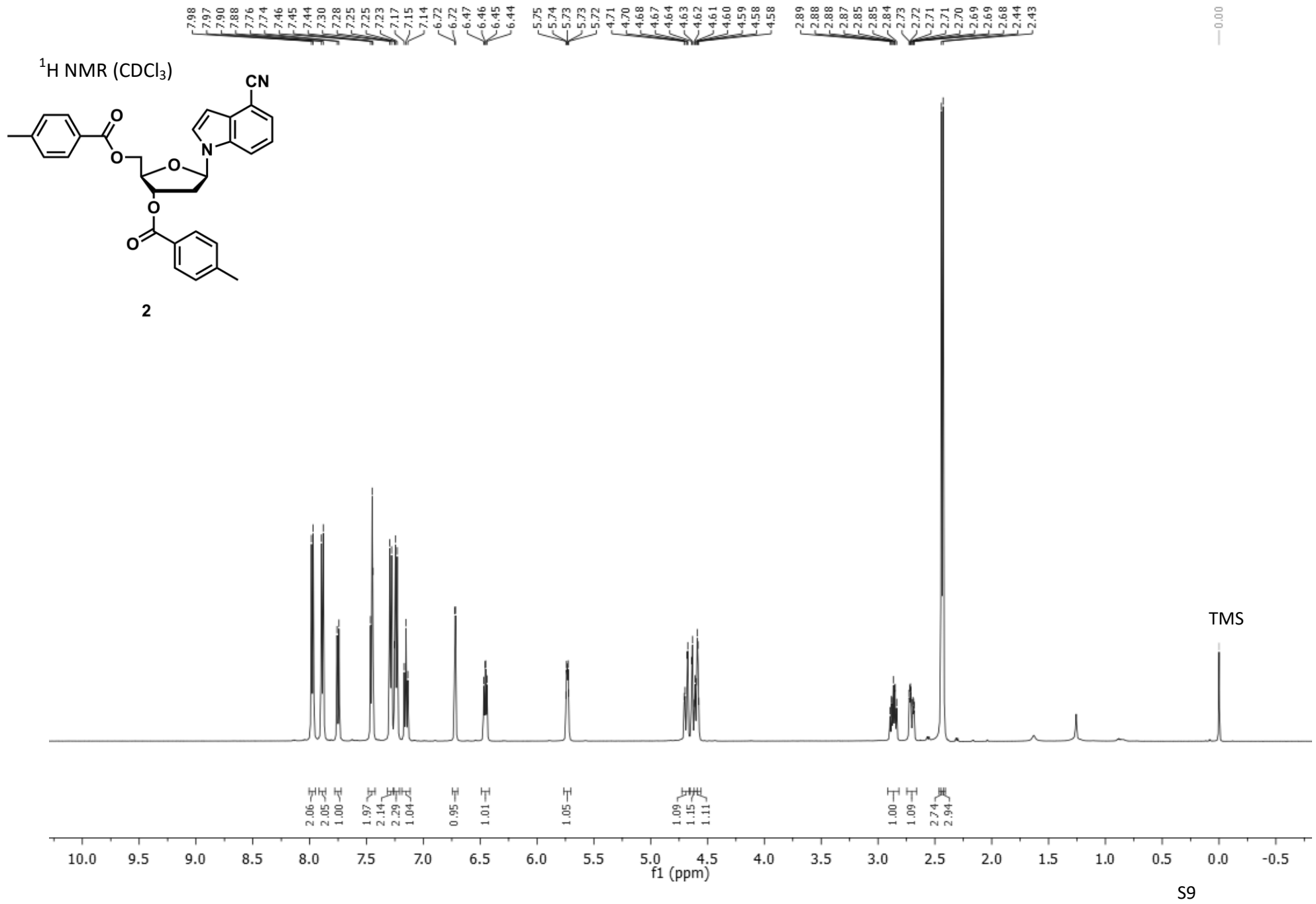
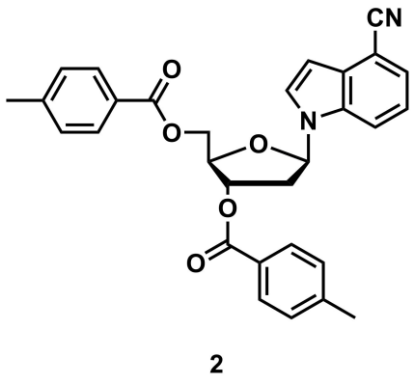
8. Muretta, J. M.; Kyrychenko, A.; Ladokhin, A. S.; Kast, D. J.; Gillispie, G. D.; Thomas, D. D. *Rev. Sci. Instrum.* **2010**, *81,103101*.

XI. ^{1}H, ^{13}C, and ^{31}P NMR Spectra of Synthesized Compounds

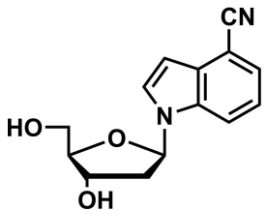


^{13}C NMR ($CDCl_3$)



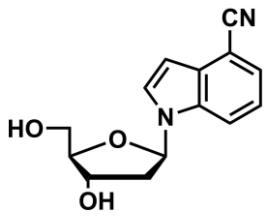
2

^{1}H NMR (DMSO-d_6)



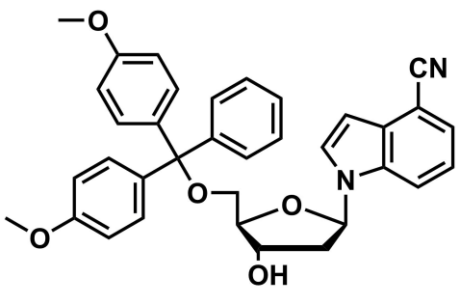
4CIN

^{13}C NMR (DMSO-d_6)



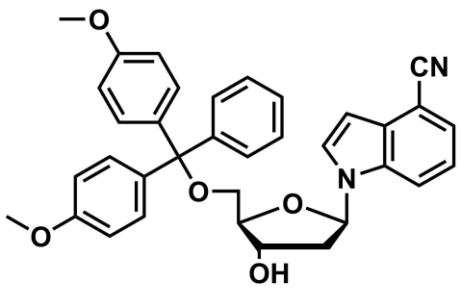
4CIN

^{1}H NMR ($CDCl_3$)

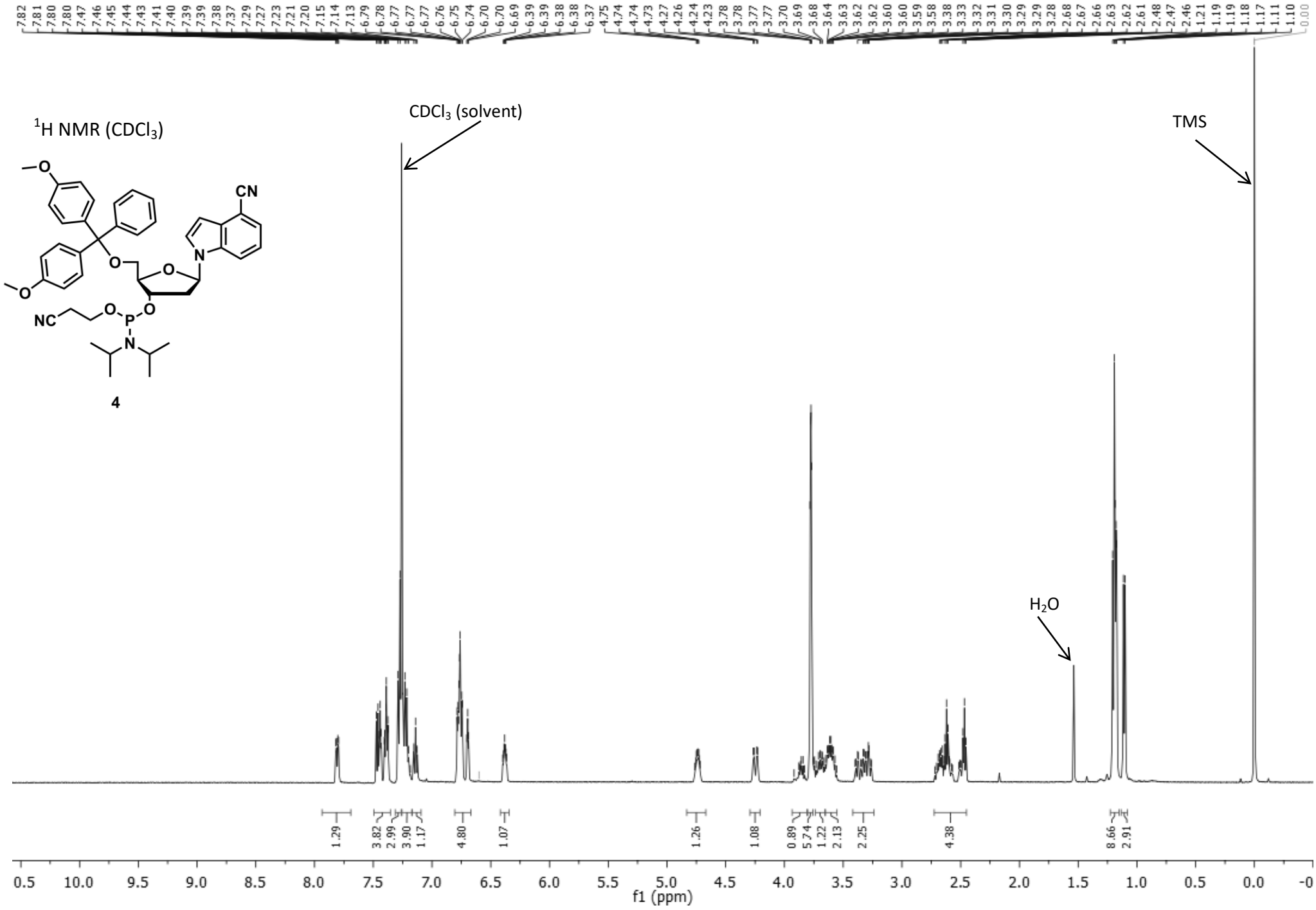


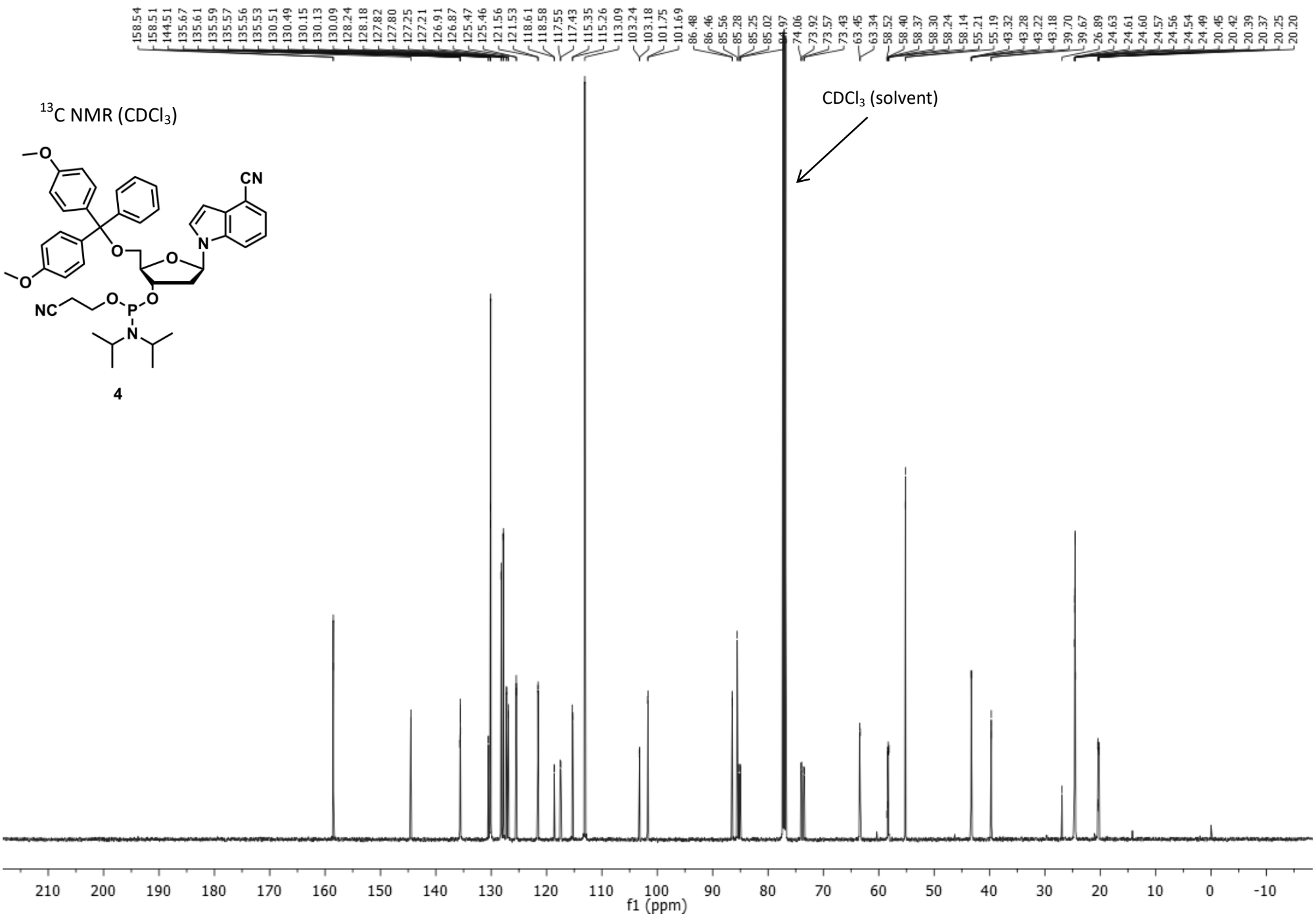
3

^{13}C NMR ($CDCl_3$)

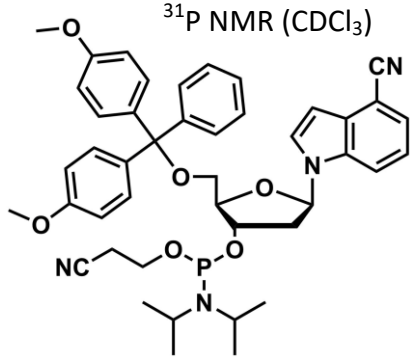


3

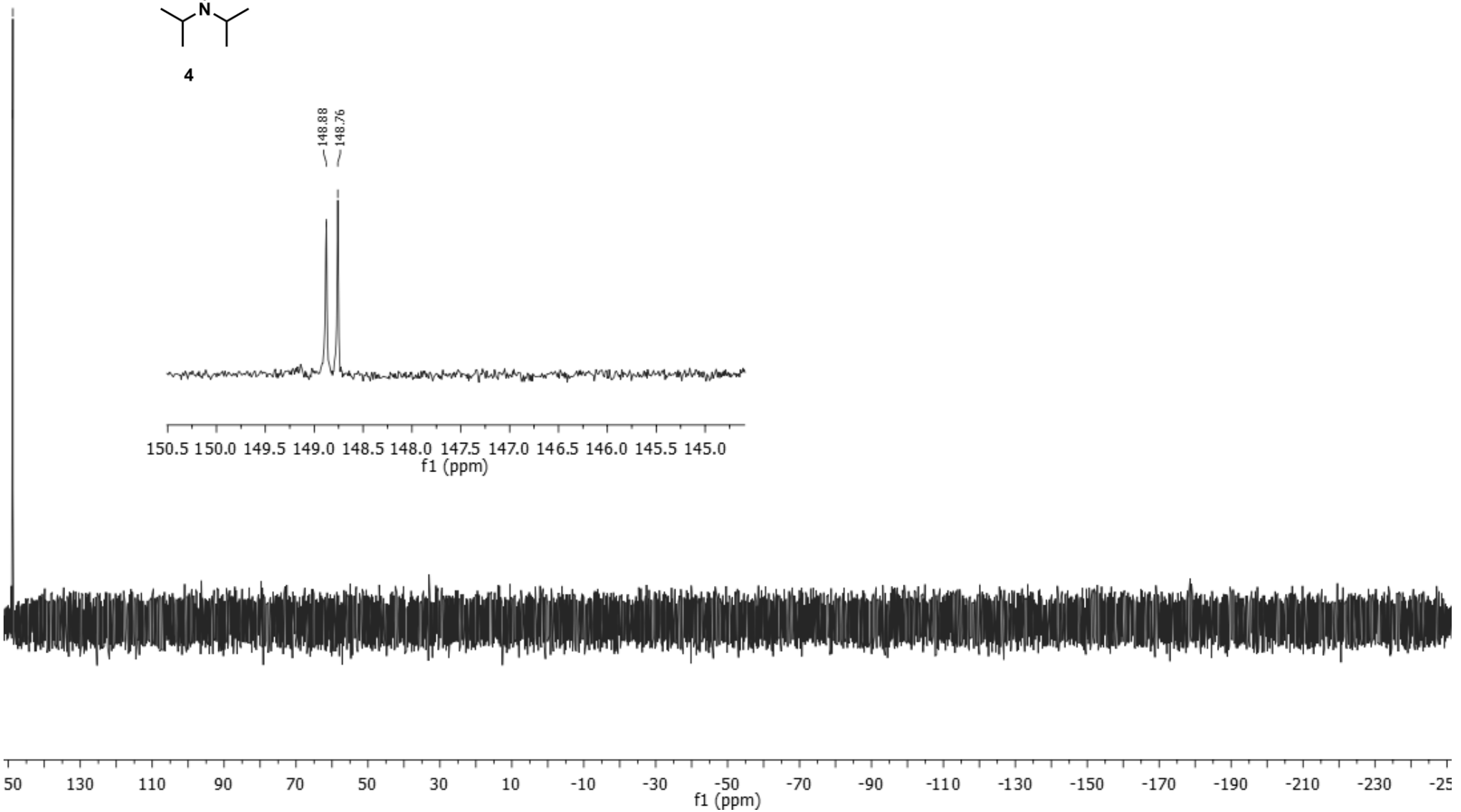

^{1}H NMR ($CDCl_3$)
$CDCl_3$ (solvent)
TMS
H_2O
4
f1 (ppm)


158.54
158.51
144.51
135.67
135.61
135.59
135.57
135.56
135.53
130.51
130.49
130.15
130.13
130.09
128.24
128.18
127.82
127.80
127.25
127.21
126.91
126.87
125.47
125.46
121.56
121.53
118.61
118.58
117.55
117.43
115.35
115.26
113.09
103.24
103.18
101.75
101.69
86.48
86.46
85.56
85.28
85.25
85.02
84.97
74.06
73.92
73.57
73.43
63.45
63.34
58.52
58.40
58.37
58.30
58.24
58.14
55.21
55.19
43.32
43.28
43.22
43.18
39.70
39.67
26.89
24.63
24.61
24.60
24.57
24.56
24.54
24.49
20.45
20.42
20.39
20.37
20.25
20.20
^{13}C NMR ($CDCl_3$)
$CDCl_3$ (solvent)
CN
NC
O
N
P
4
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

^{31}P NMR ($CDCl_3$)



4

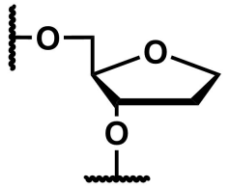


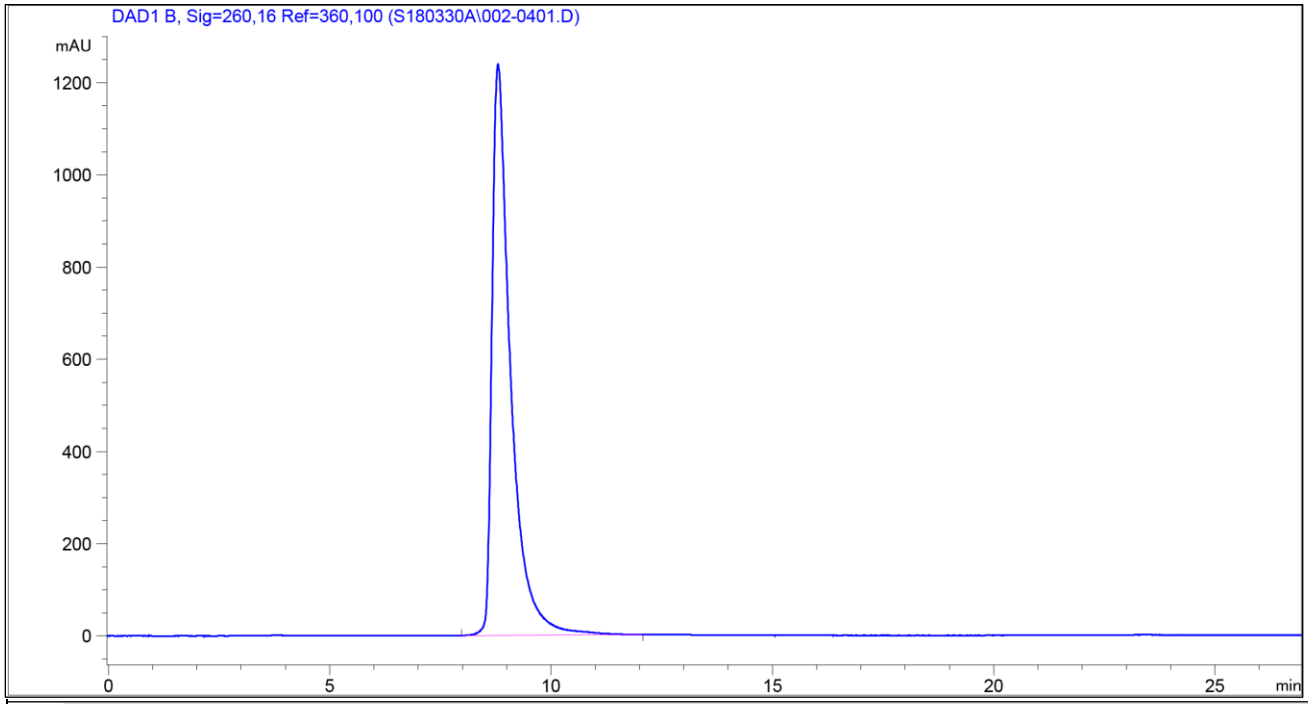
XI. LC-MS Traces and Masses of Oligonucleotides

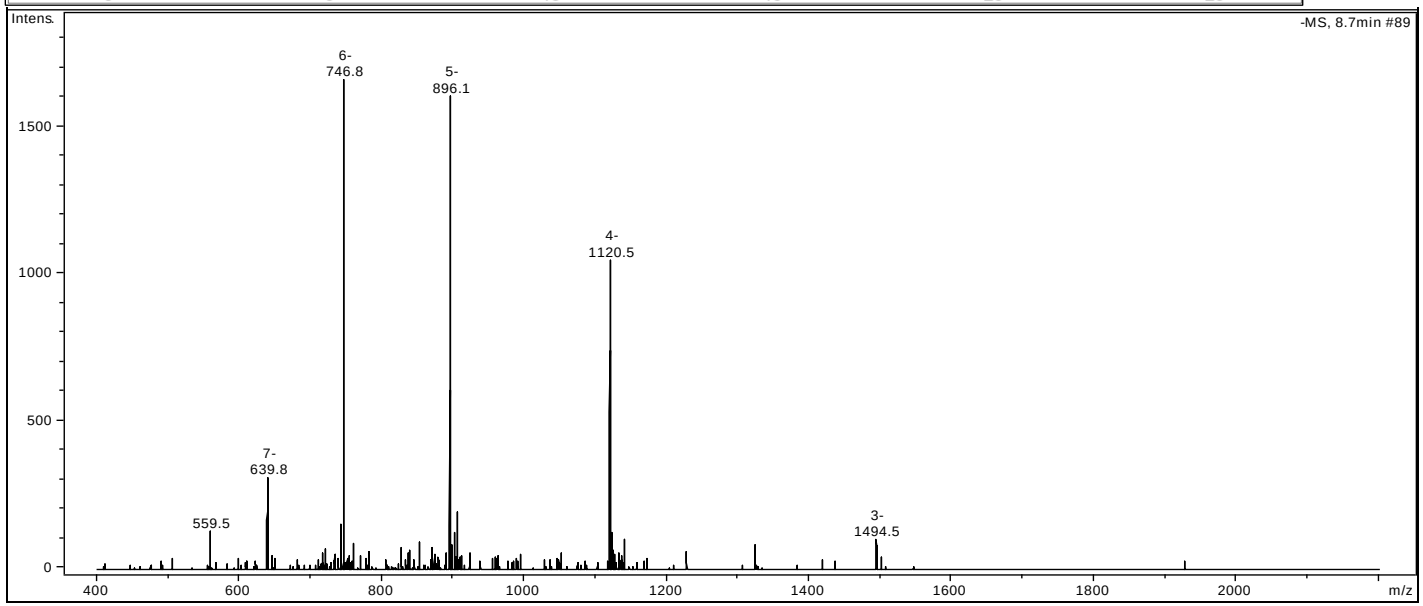
(From Integrated DNA Technologies)

5' – CCAAAAA(Ab)AAAAGG - 3' ; m/z calculated: 4486.0, m/z found: 4486.1; Integrated Purity: >99%

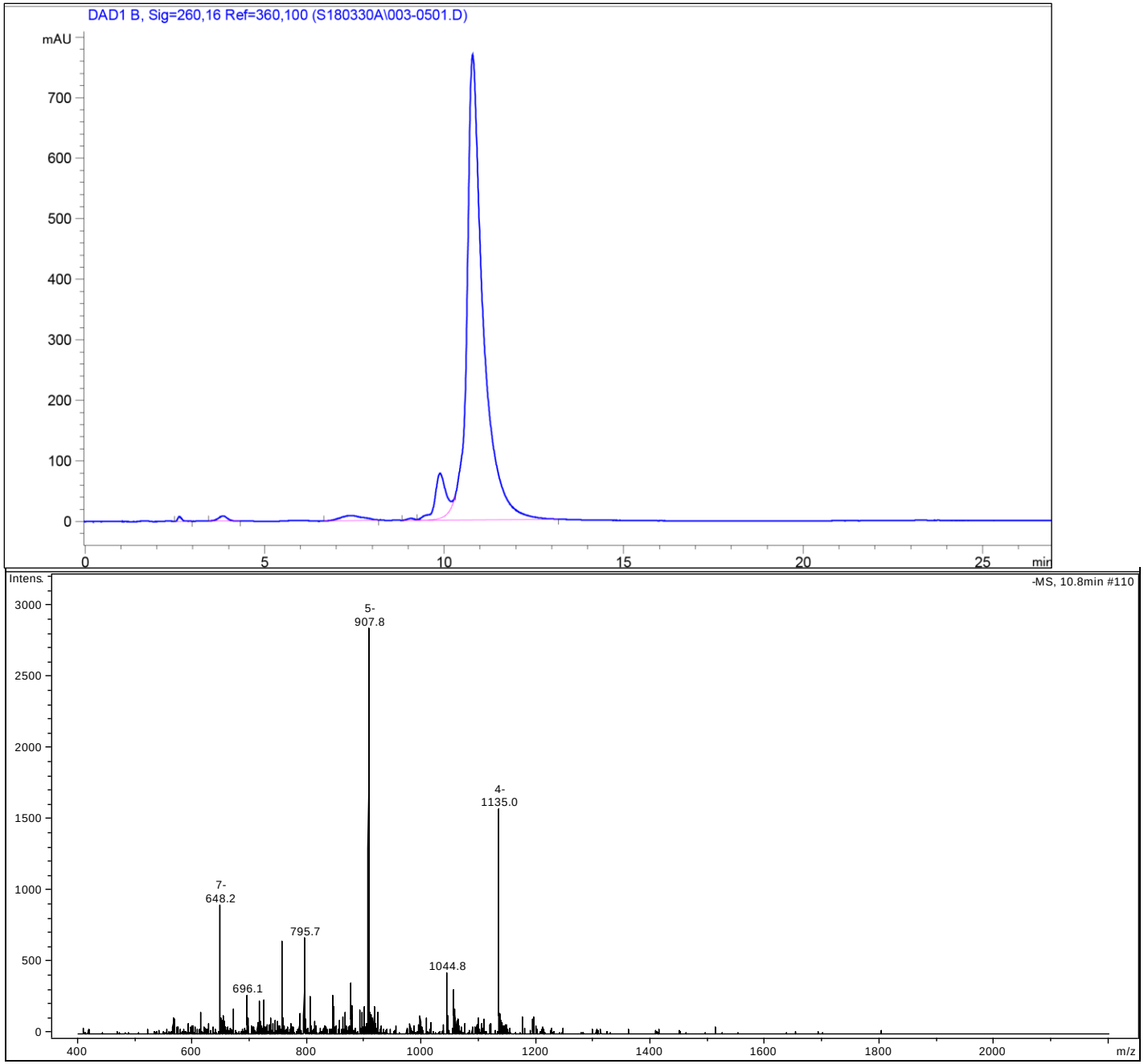
Ab =



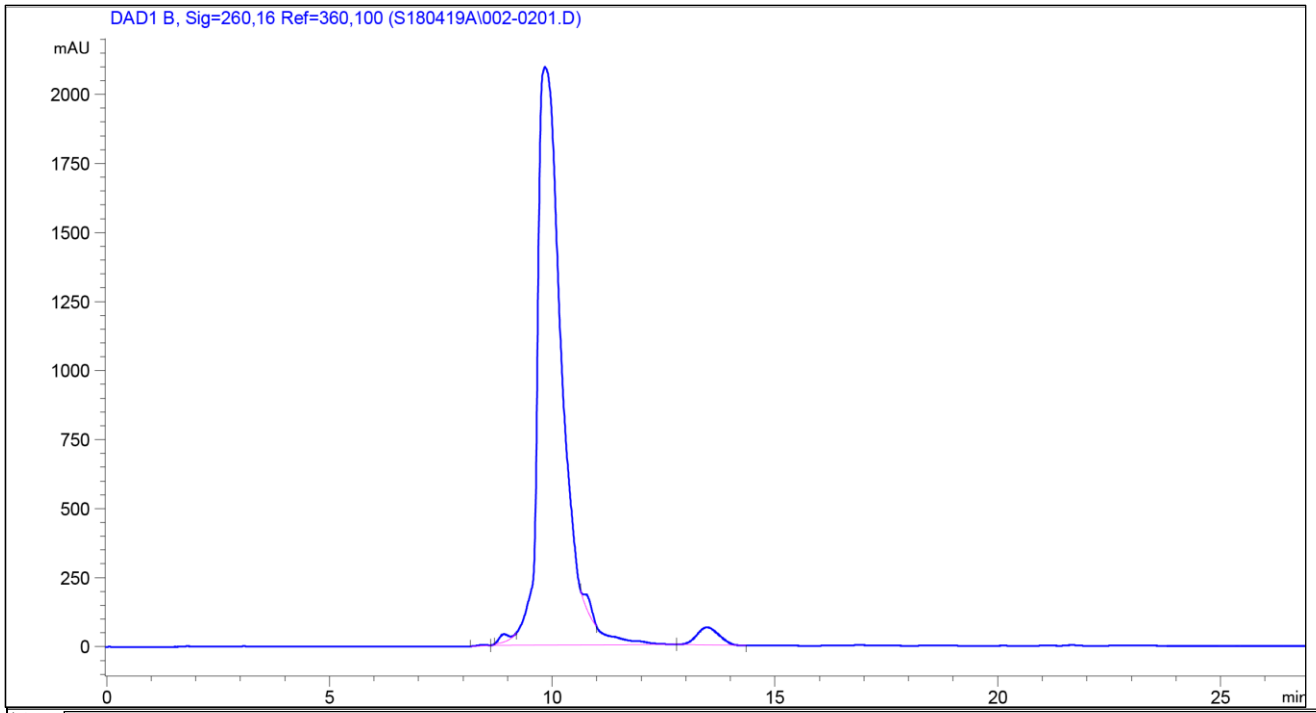


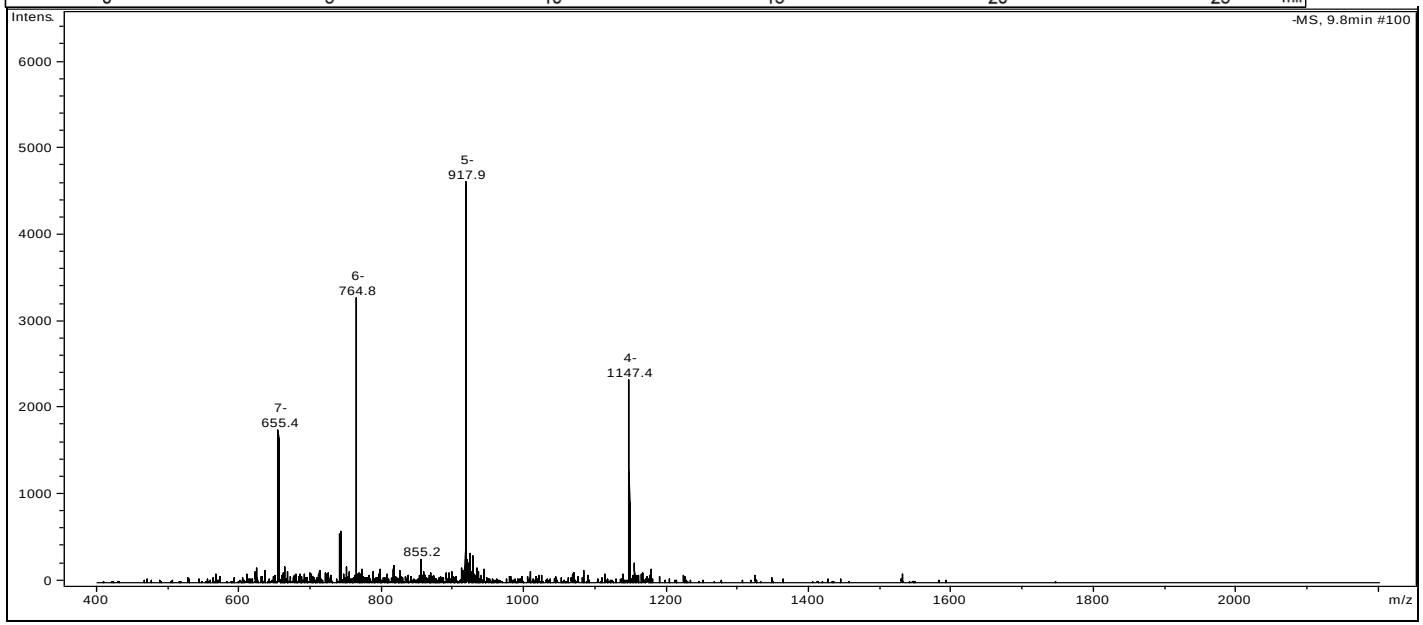


5’ – (2APN)CTTTTTGTTTTTGT – 3’ ; m/z calculated: 4543.9, m/z found: 4544.1; Integrated Purity: 92%

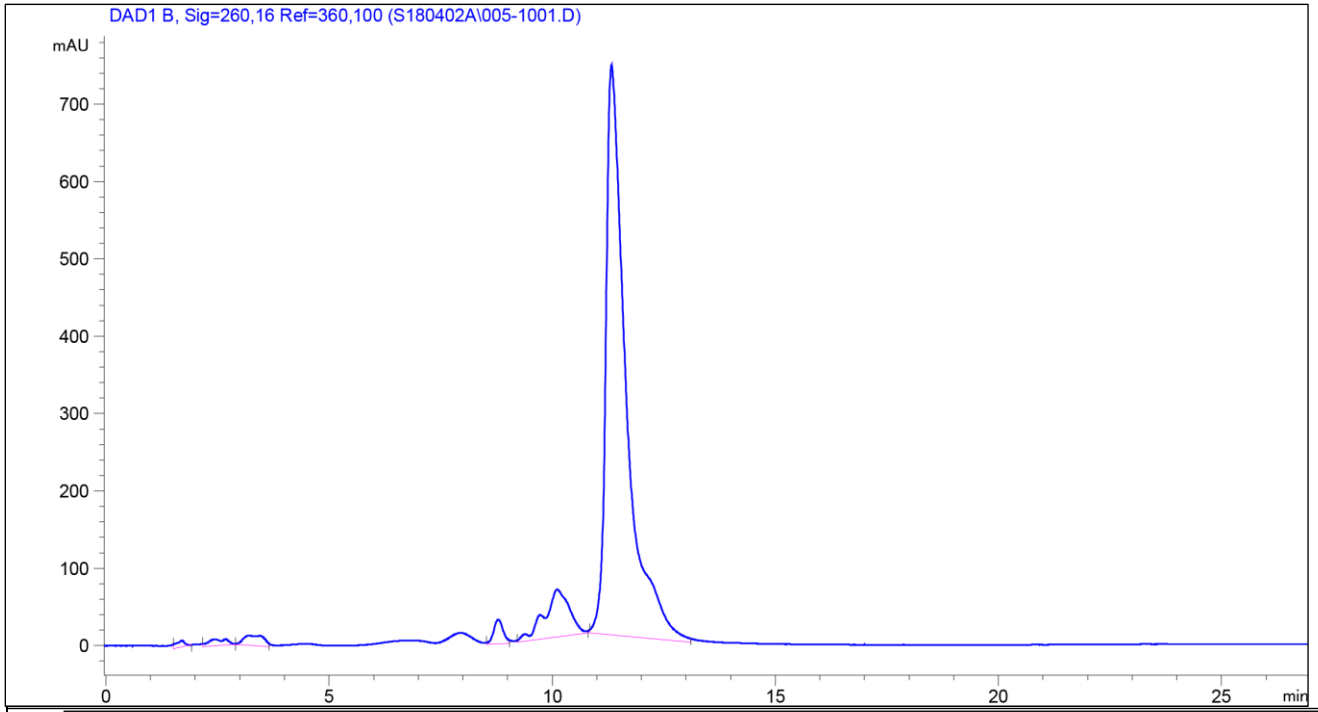


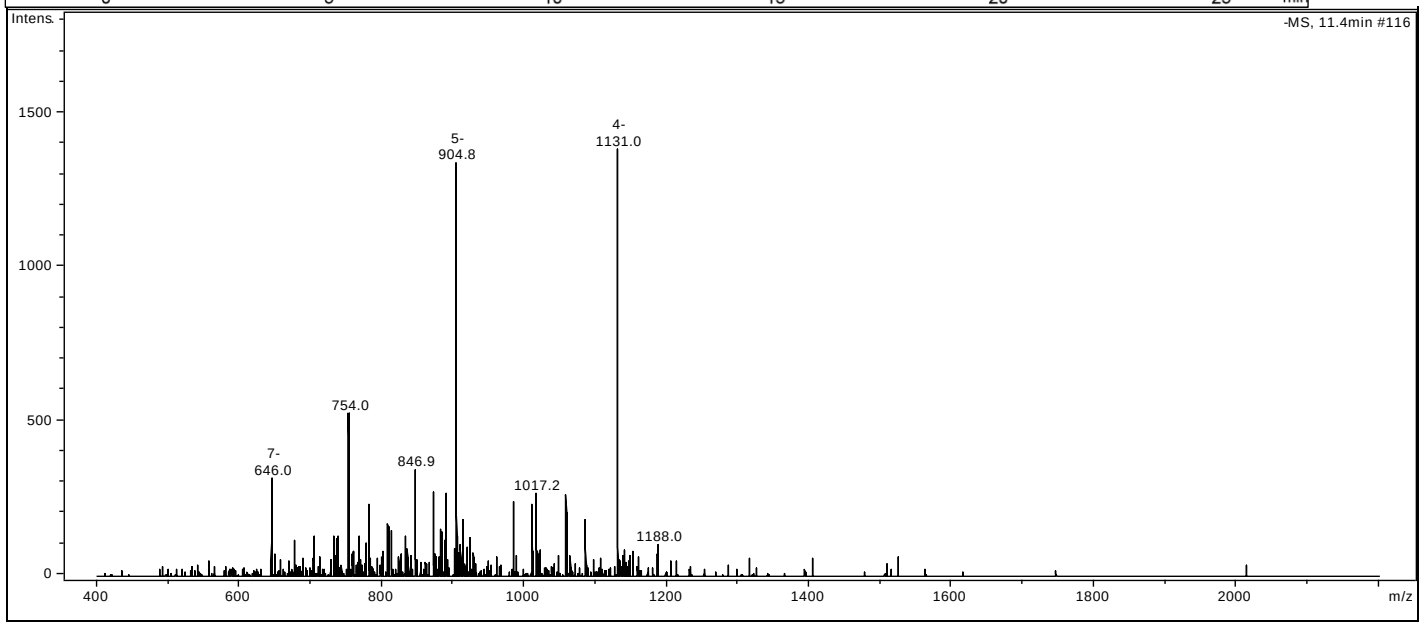
5' – ACAAAAACAAAAAGT – 3'; m/z calculated: 4594.1, m/z found: 4593.8; Integrated Purity: 97%



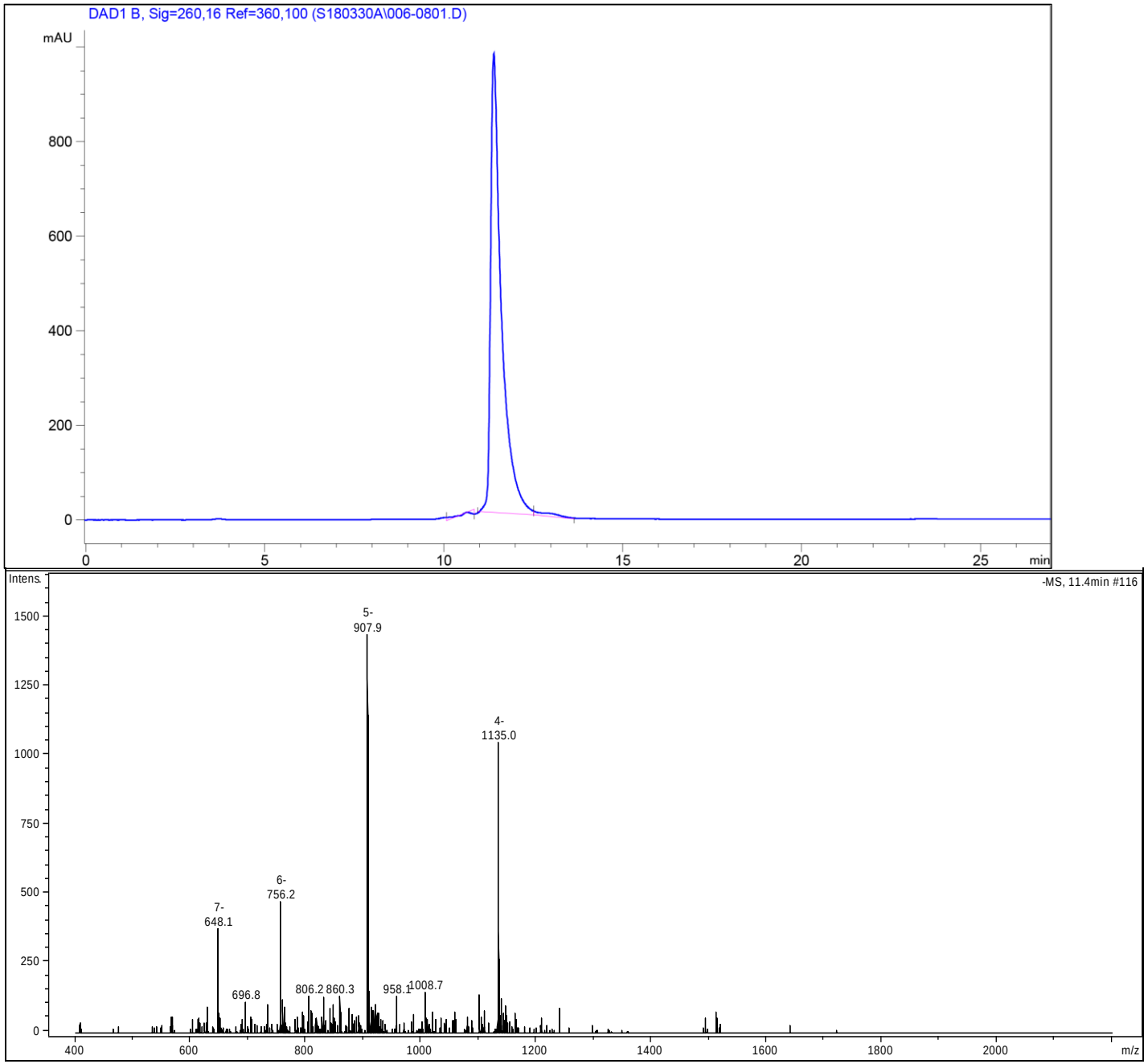


5’ – CCTTTTT(2APN)TTTTTGG – 3’; m/z calculated: 4529.0, m/z found: 4528.6; Integrated Purity: 86%

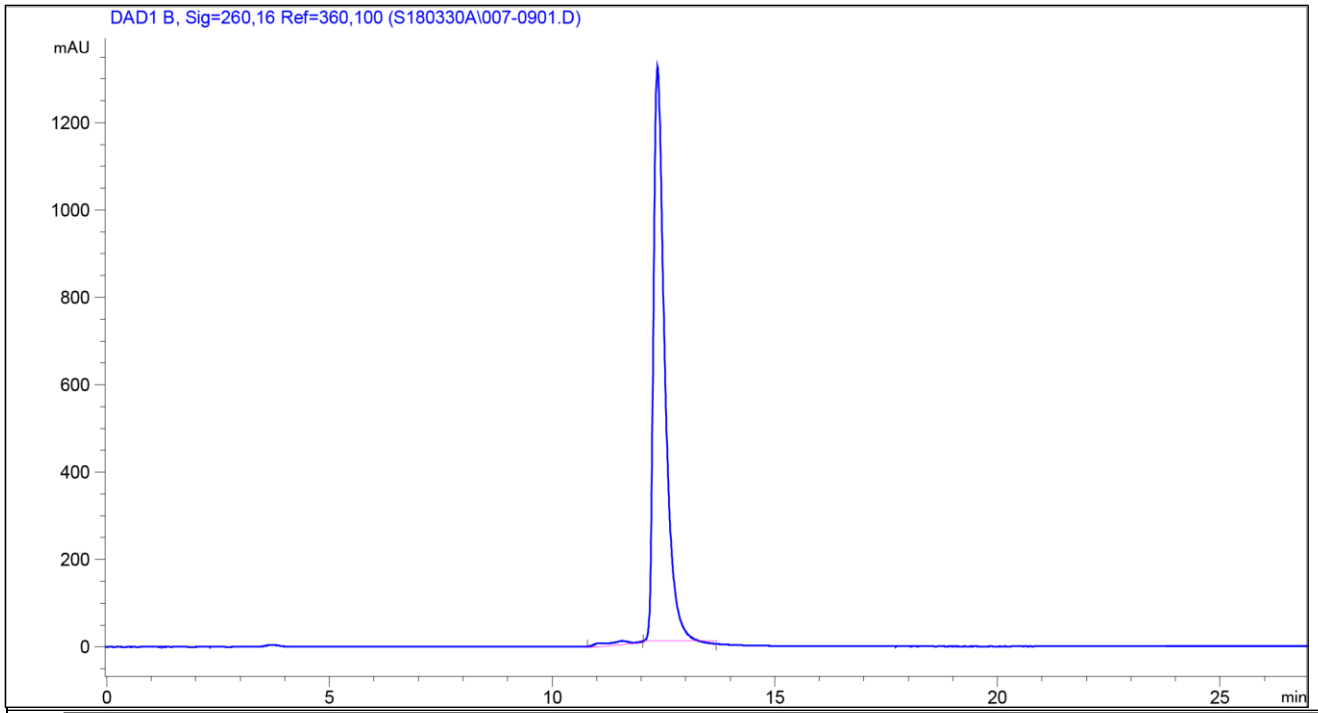


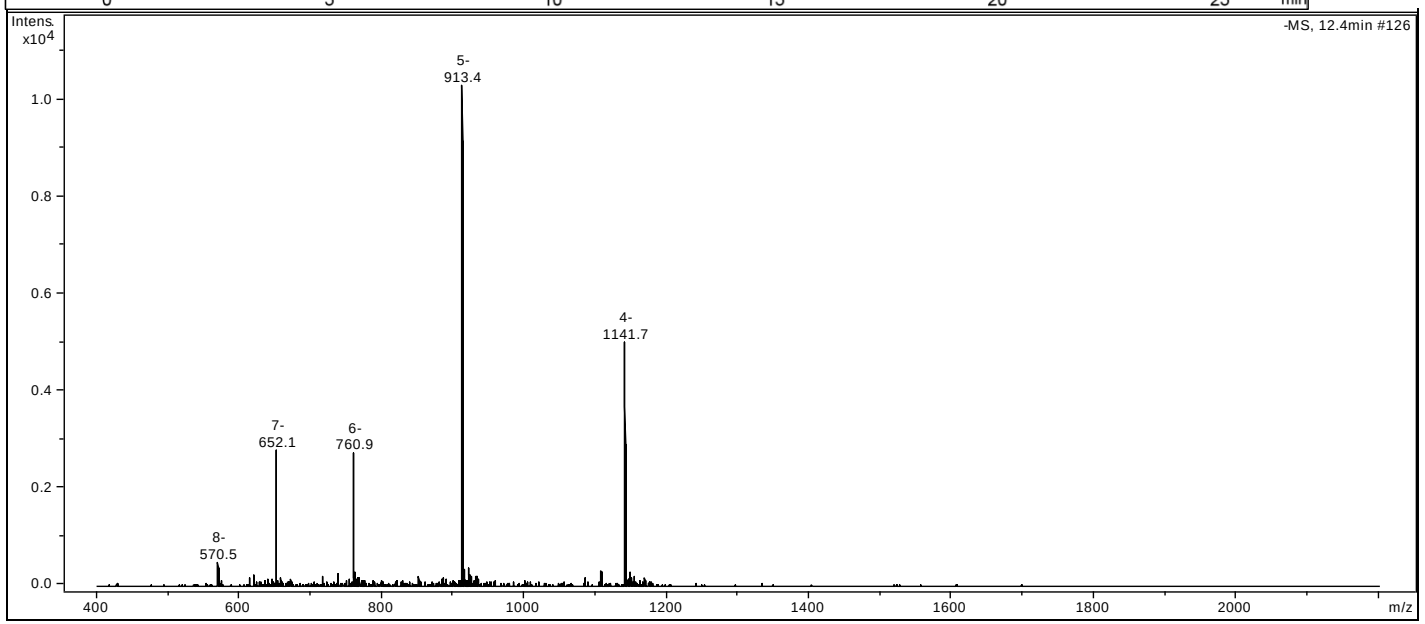


5’ – ACTTTTTGTTTTTGT – 3’; m/z calculated: 4544.0, m/z found: 4544.1; Integrated Purity: 98%

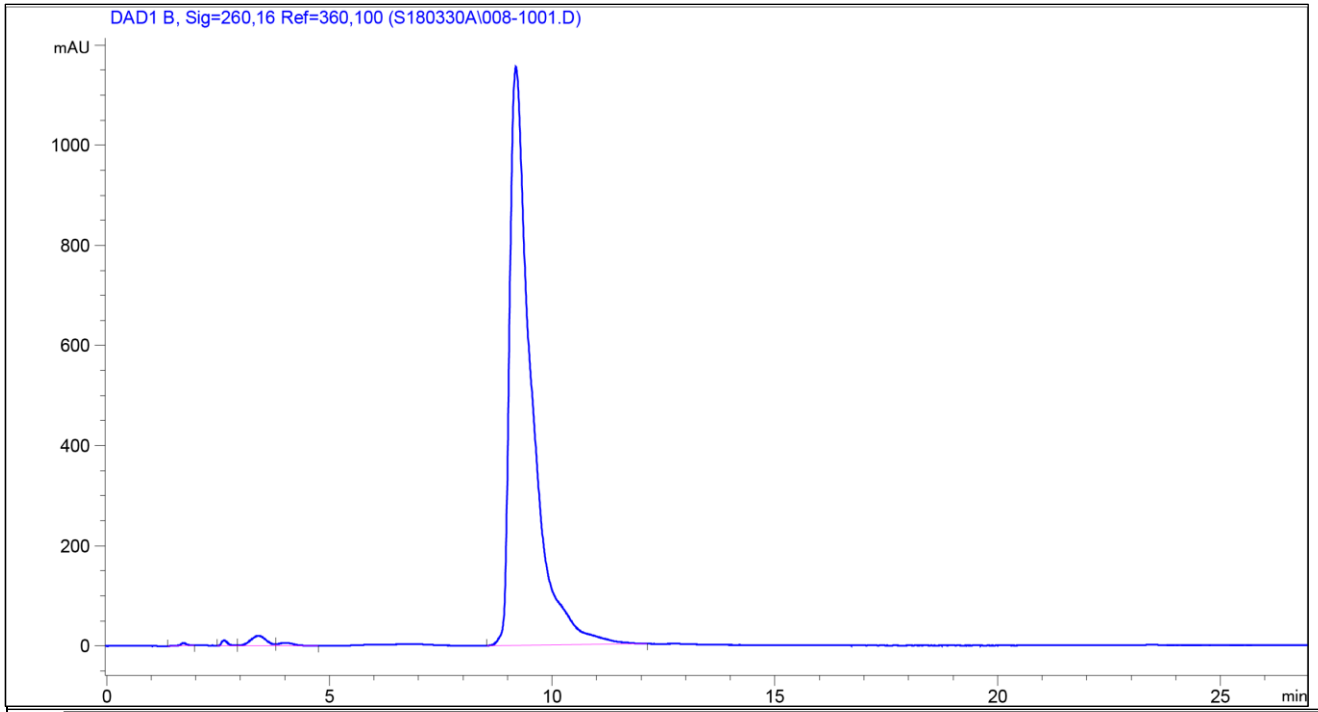


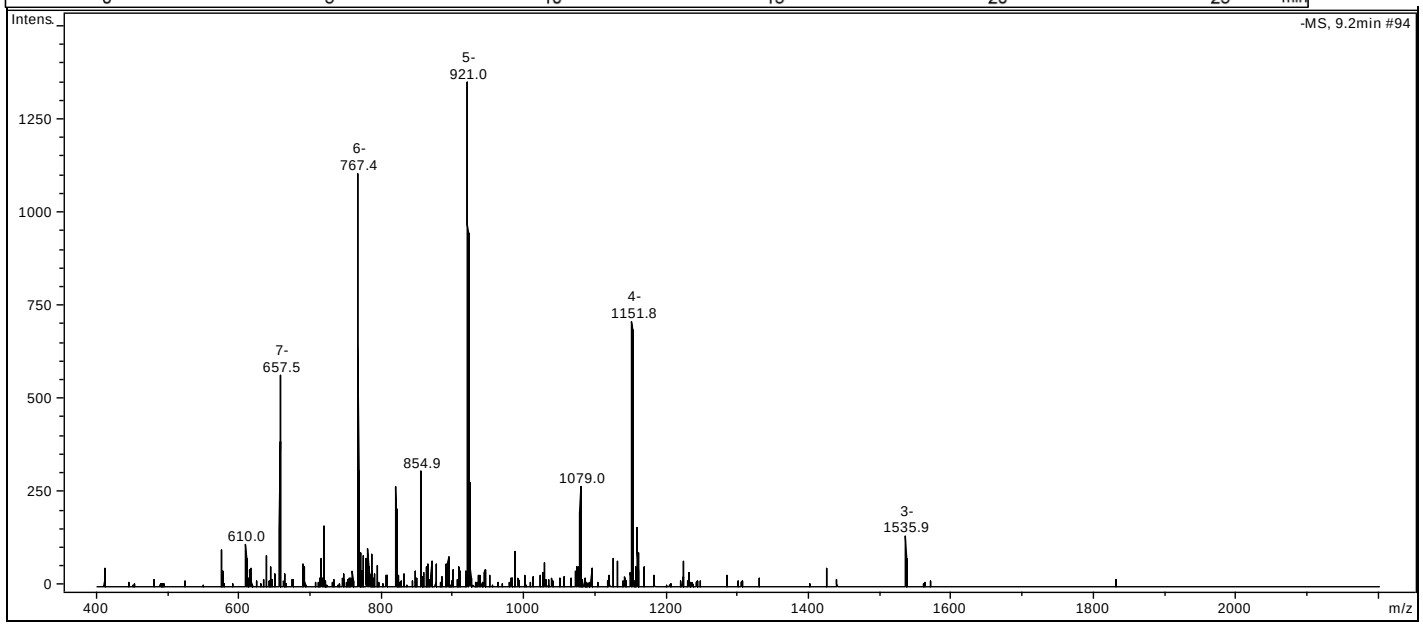
5' – (5NO$_2$)CTTTTTGTTTTTGT – 3'; m/z calculated: 4570.9, m/z found: 4570.9; Integrated Purity: 97%



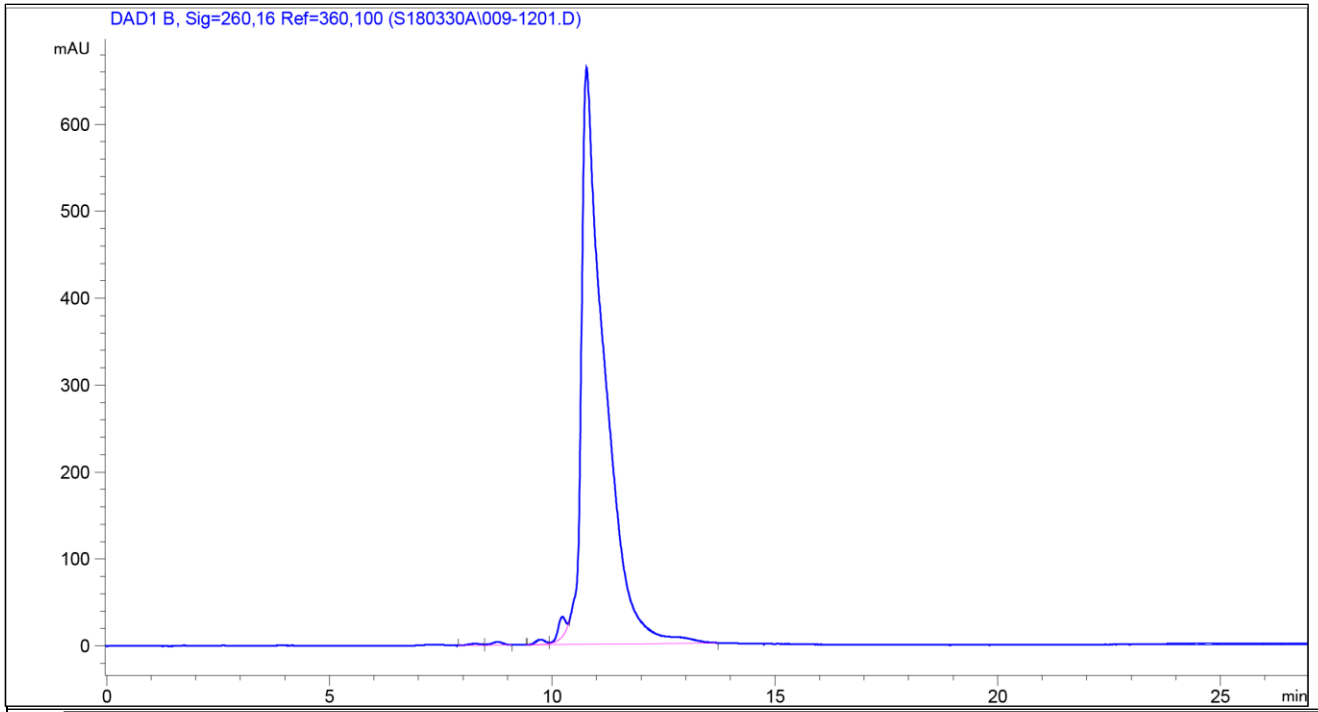


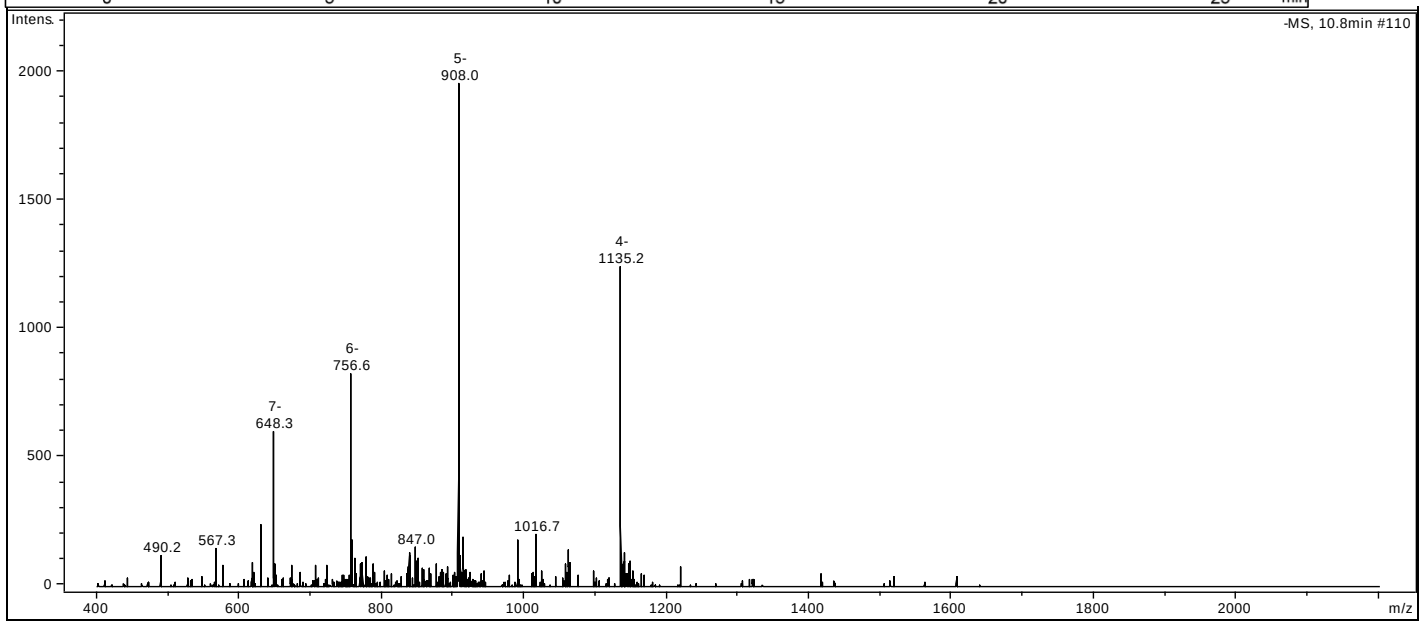
5’ – CCAAAAATAAAAAGG – 3’; m/z calculated: 4610.1, m/z found: 4611.3; Integrated Purity: 98%



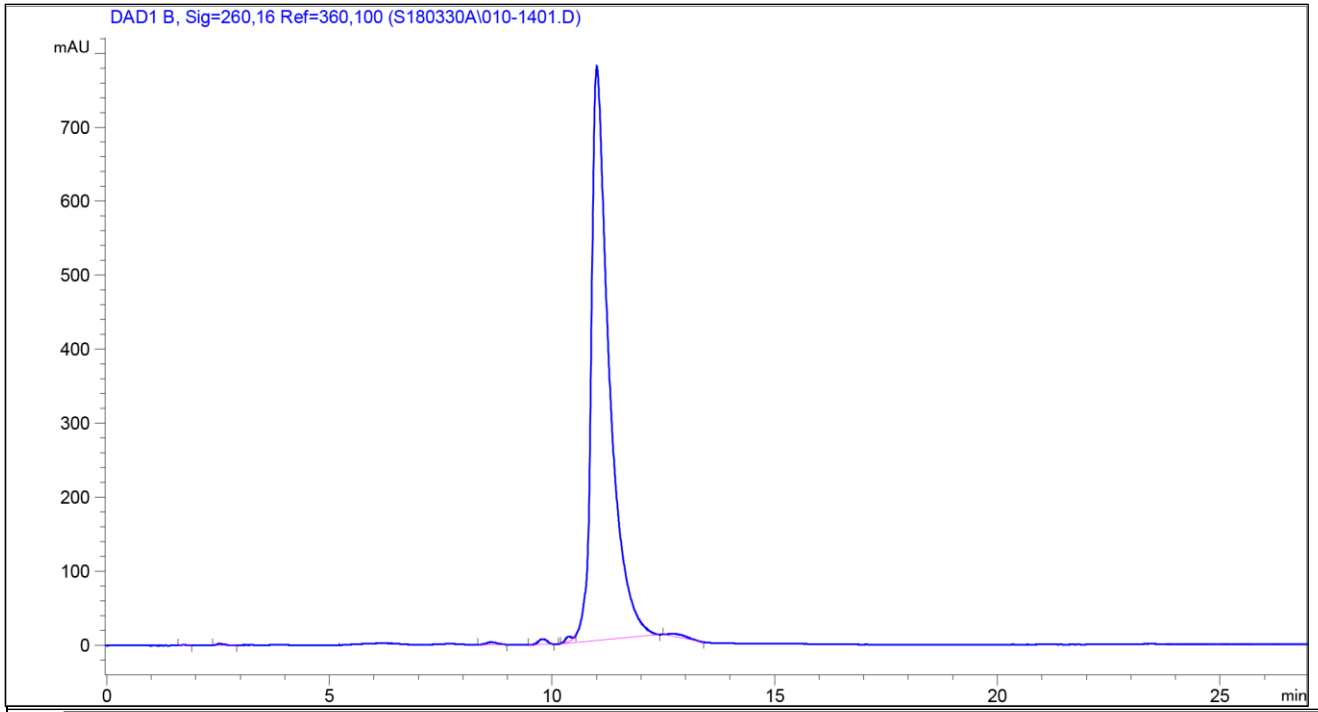


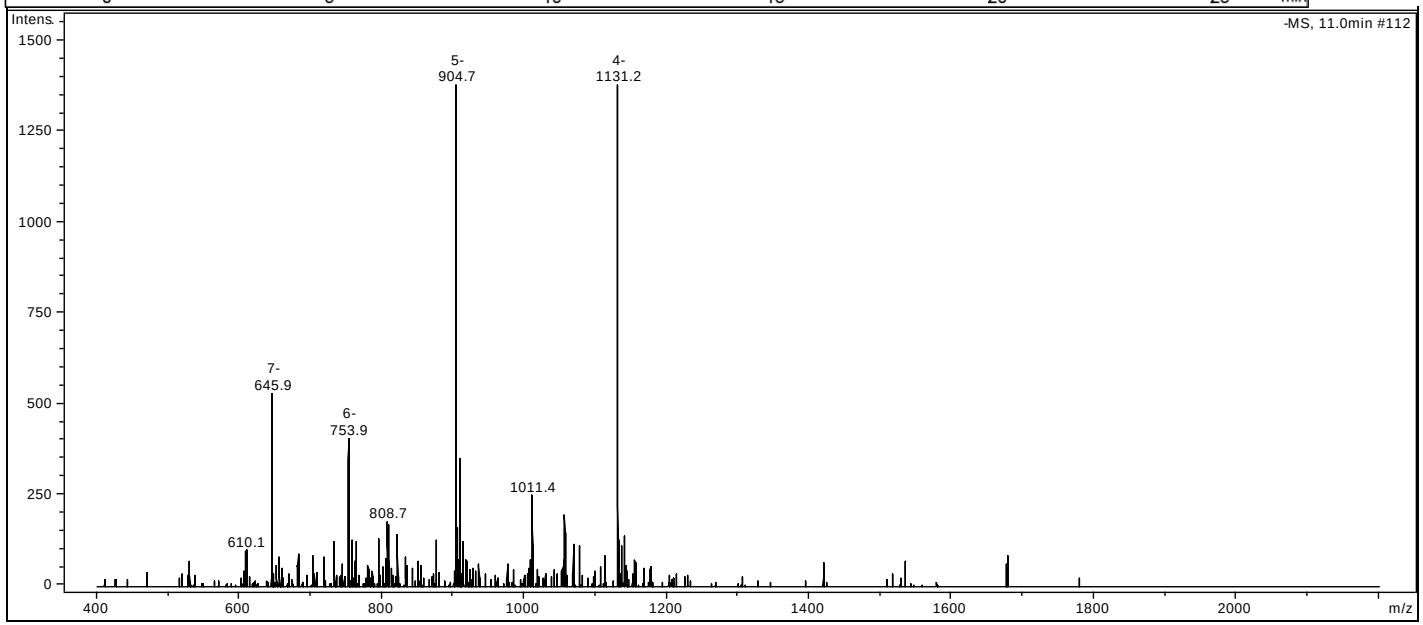
5’ – CCTTTTTGTTTTTGG – 3’; m/z calculated: 4545.0, m/z found: 4544.6; Integrated Purity: 98%



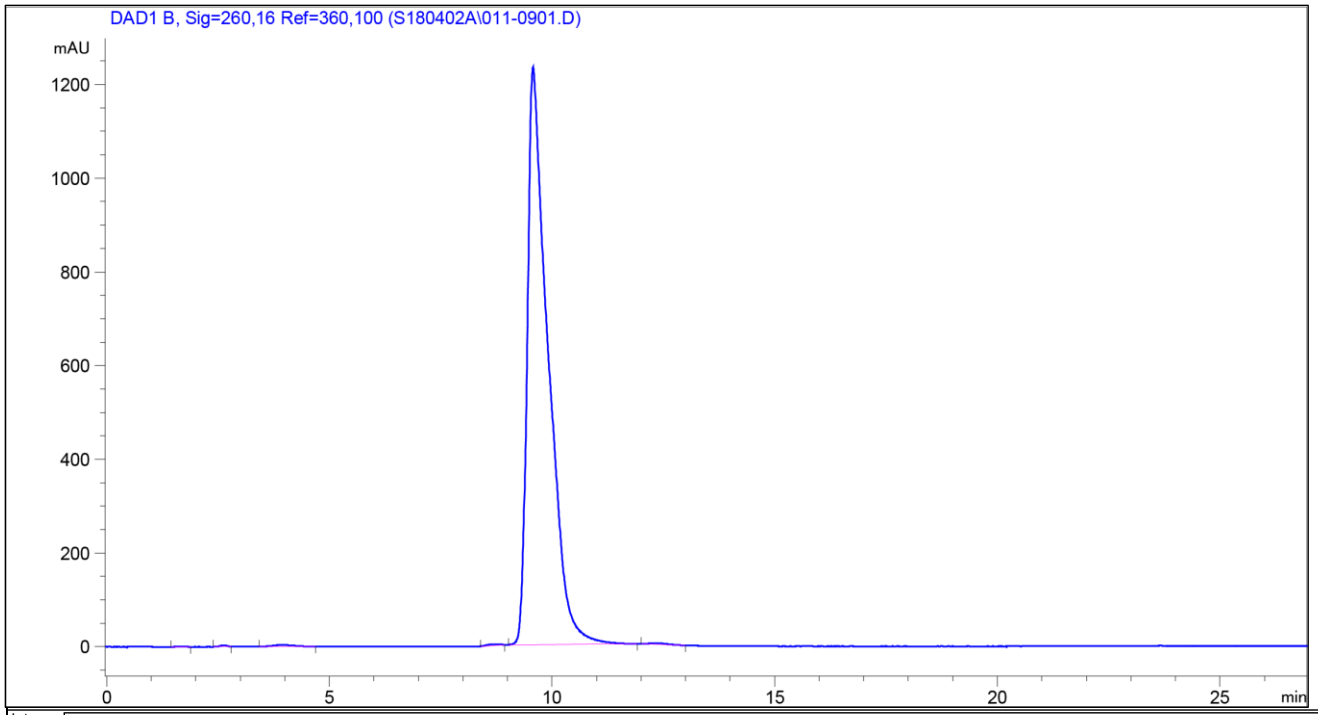


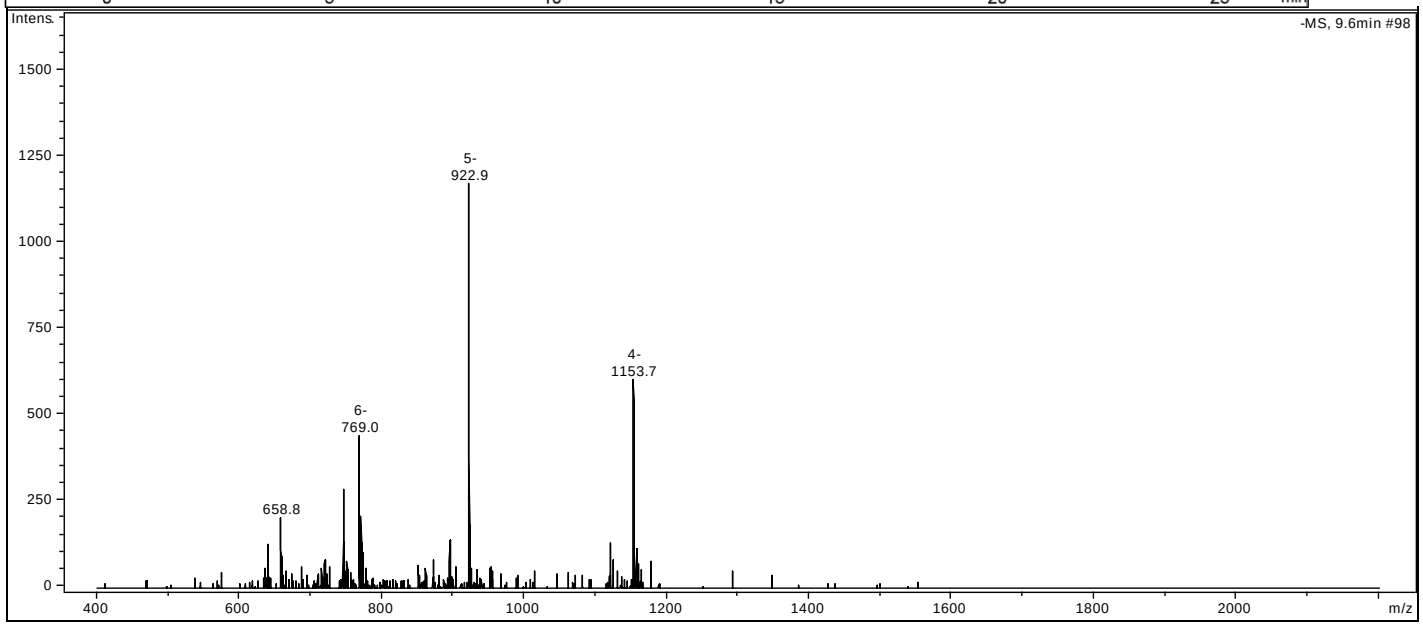
5’ – CCTTTTTATTTTTGG – 3’; m/z calculated: 4529.0, m/z found: 4528.7; Integrated Purity: 96%



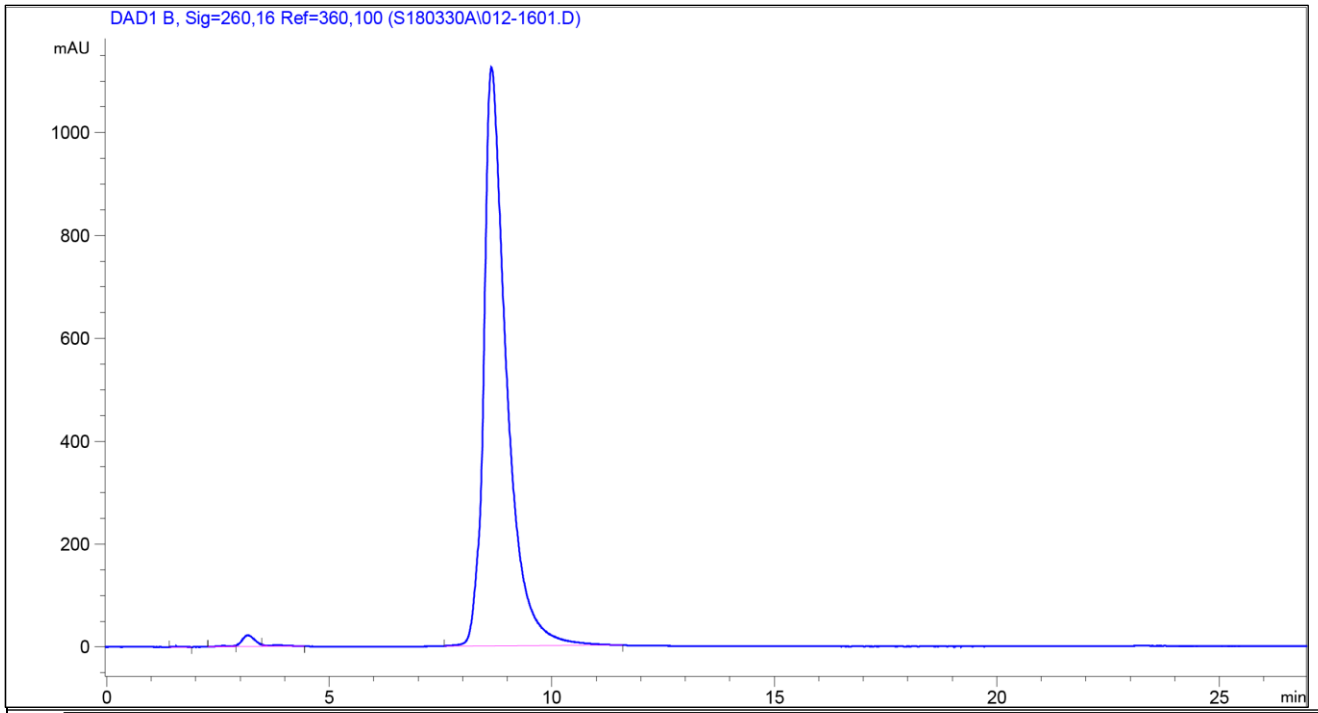


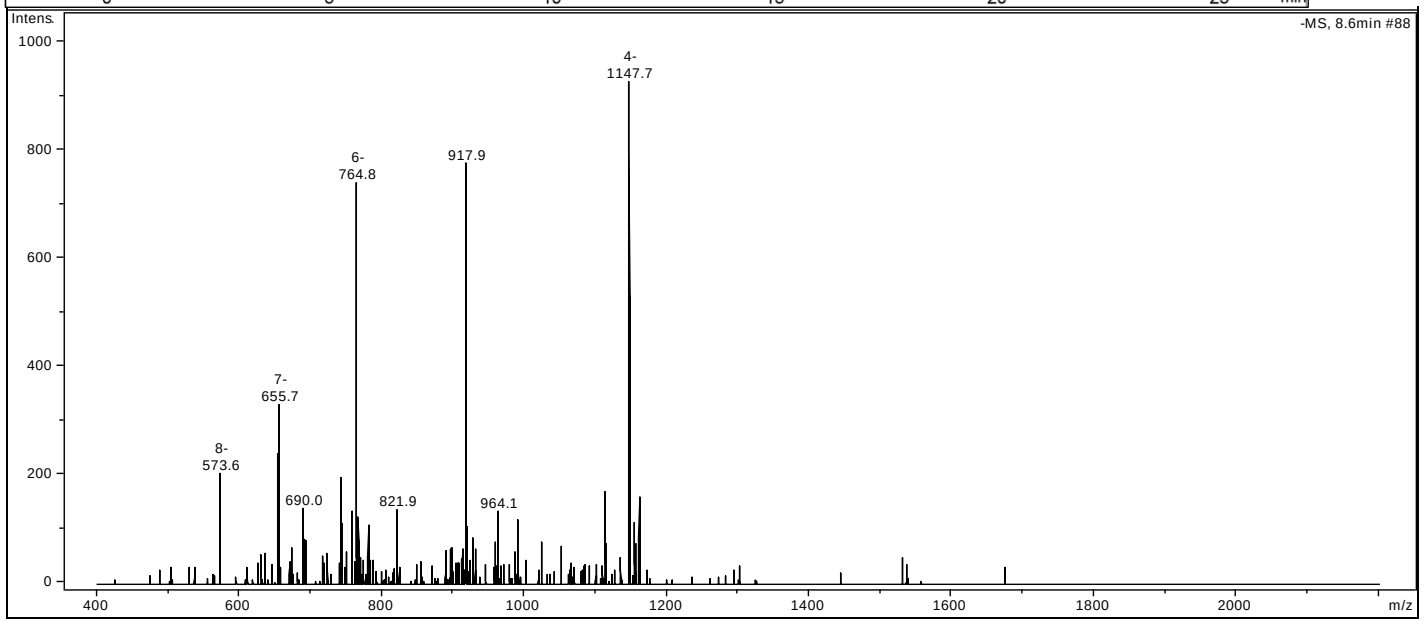
5’ – CCAAAAAAAAAAAGG – 3’; m/z calculated: 4619.1, m/z found: 4618.9; Integrated Purity: 99%



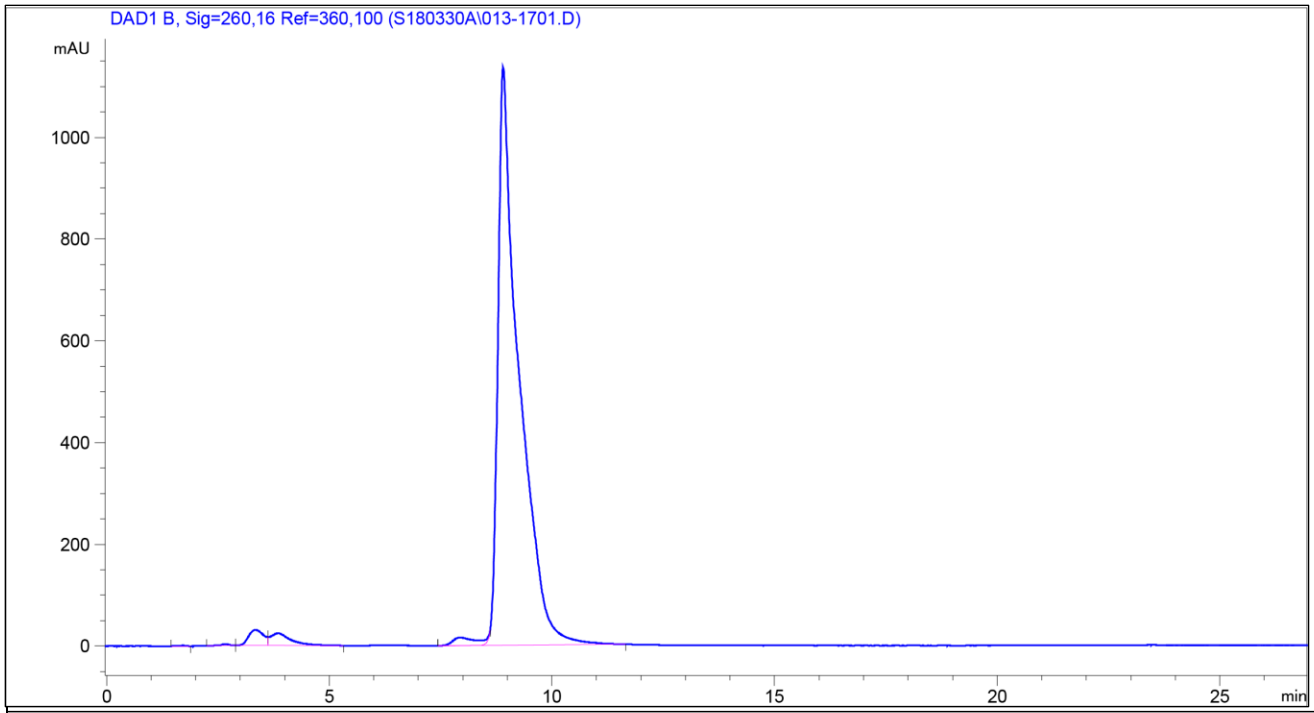


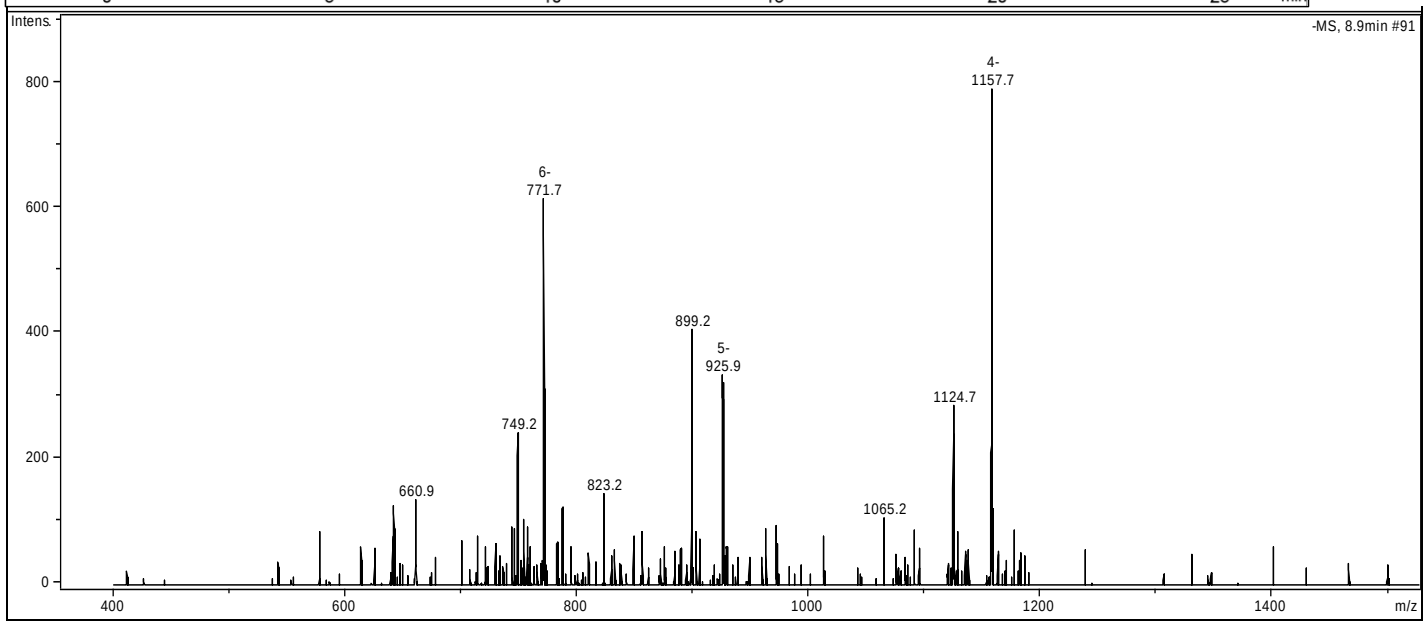
5’ – CCAAAAACAAAAAGG – 3’; m/z calculated: 4594.1, m/z found: 4594.9; Integrated Purity: 98%



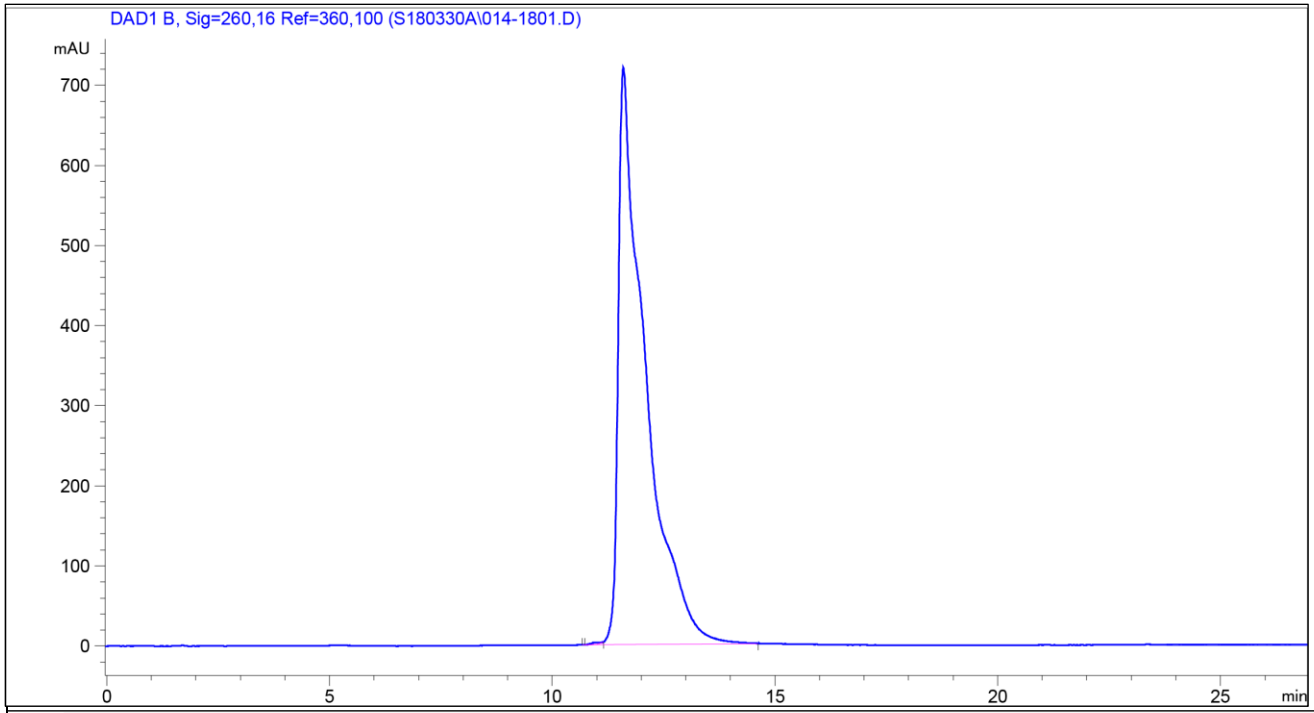


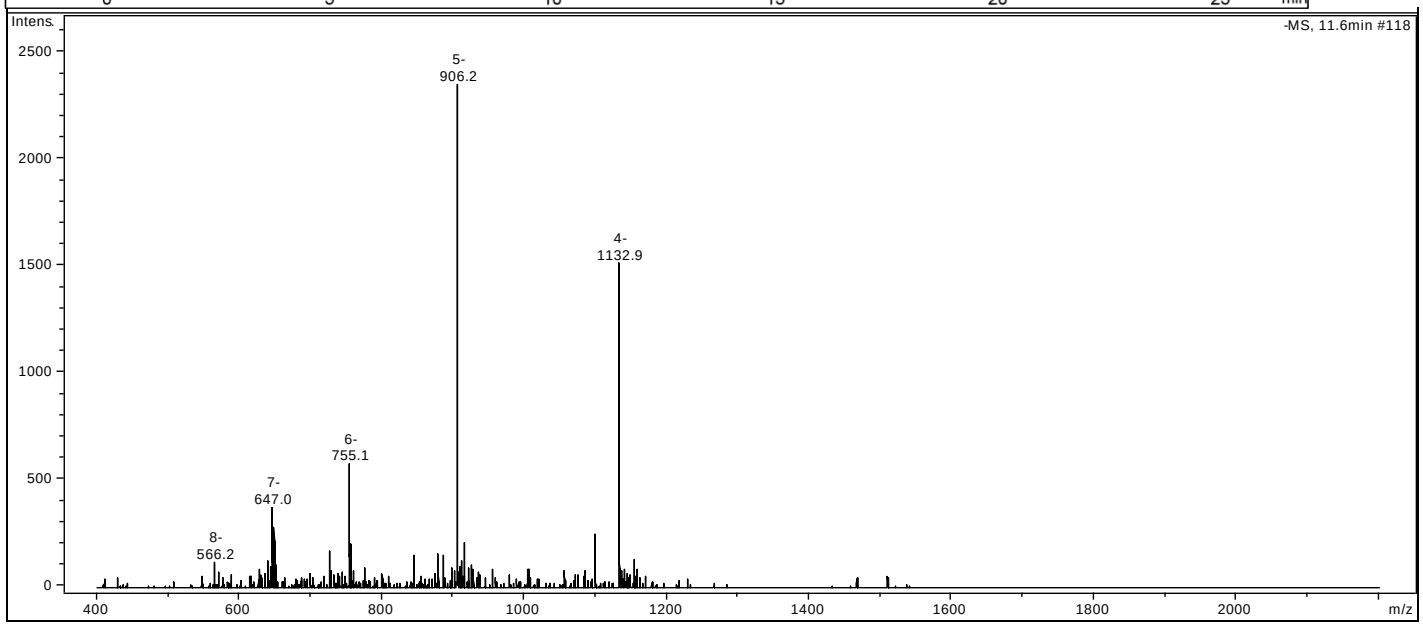
5’ – CCAAAAAGAAAAAGG – 3’; m/z calculated: 4635.1, m/z found: 4634.9; Integrated Purity: 94%





5’ - CCTTTTT(4CIN)TTTTTGG – 3’; m/z calculated 4535.9, m/z found: 4535.8; Integrated Purity: >99%





5’ – (4CIN)CTTTTTGTTTTTGT – 3’; m/z calculated: 4550.9, m/z found: 4550.9; Integrated Purity: >99%