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Supplementary Information

Synthesis and Fundamental Studies of a Photoresponsive Oligonucleotide-Upconverting Nanoparticle Covalent Conjugate

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Abstract: Photo-mediated systems present a highly attractive route for controlling the intracellular delivery of their cargo. Of particular interest, oligonucleotides are a promising class of molecule that are highly versatile for intracellular applications, but lack the necessary *in vivo* stability on their own. A novel, greener synthetic route to a photocleavable phosphoramidite was developed. The amidite was incorporated into an oligonucleotide by solid-phase synthesis, which was covalently linked to UV-emissive lanthanide-doped upconverting nanoparticles (UCNPs) through click chemistry. The nanosystem was fully characterized for energy transfer dynamics between the photocleavable oligonucleotide and UCNP using 976 nm excitation. The practical and green synthesis of the photocleavable phosphoramidite, combined with the fundamental understanding of the interactions between the oligonucleotide and nanoparticle during excitation paves the way for future *in vitro* applications for this type of system.

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N,N′-Diisopropylamino cyanoethyl phosphonamidic chloride (Cl-POCEN(*i*Pr)$_2$) and dimethoxytrityl chloride (DMTr-Cl) were purchased from ChemGenes Corporation (Wilmington, MA). 2-Amino-1,3-propanediol (serinol) was purchased from TCI America (Portland, OR). 2-Nitrobenzaldehyde was purchased from Alfa Aesar (Ward Hill, MA). 5'-*O*-Dimethoxytrityl-2'-deoxyribonucleoside-3'-*O*-(β-cyanoethyl-*N,N′*-diisopropyl) phosphoramidites and protected 2'-deoxyribonucleoside controlled pore glass supports (500 Å) were purchased from Glen Research (Sterling, VA). All other chemicals and solvents were purchased from Sigma Aldrich (Milwaukee, WI) or EMD Chemicals Inc. (Gibbstown, NJ). Flash column chromatography was performed using silica gel 60 (230–400 mesh) purchased from Canadian Life Science (Pointe-Claire, QC). Thin layer chromatography (TLC) was carried out with pre-coated TLC plates (Merck, Kieselgel 60 F_{254}, 0.25 mm), purchased from EMD Chemicals Inc. (Gibbstown, NJ), with UV light and 0.5 % w/v potassium permanganate aqueous solution (followed by gentle heating) used for visualization. NMR spectra were recorded on a Varian 500 MHz NMR spectrometer at room temperature. ^{1}H NMR spectra were recorded at a frequency of 500.0 MHz and chemical shifts were reported in parts per million (ppm). 1,3,5-Trimethoxybenzene was used an internal standard for estimating yield by NMR. ^{13}C NMR spectra (^{1}H decoupled) were recorded at a frequency of 125.7 MHz and chemical shifts were reported in ppm. The NMR spectra were calibrated using the proton or carbon signals of residual, nondeuterated solvent peaks: δ_H 7.26 and δ_C 77.0 for $CDCl_3$, δ_H 2.50 and δ_C 39.52 for $(CD_3)_2SO$ and δ_H 2.05 and δ_C 29.84 for $(CD_3)_2CO$. ^{31}P NMR spectra (^{1}H decoupled) were recorded at a frequency of 202.3 MHz and chemical shifts were reported in ppm with H_3PO_4 used as an external standard. High resolution mass spectrometry was done using a 7T-LTQ FT ICR mass spectrometer (Thermo Scientific) at the Concordia University Centre for Structural and Functional Genomics. The mass spectrometer was operated in full scan, positive ion detection mode.

Reagents and general methods for the preparation and characterization of UCNPs

All reagents were used without further purification. Lithium trifluoroacetate (97 % purity), Y_2O_3 (99.999 % purity), Yb_2O_3 (99.999 % purity) and Tm_2O_3 (99.999 % purity) were purchased from Alfa Aesar (Ward Hill, MA). Trifluoroacetic acid (ReagentPlus 99 % purity), IGEPAL-CO520 (M_n 441), oleic acid (technical grade, 90 %), 1-octadecene (technical grade, 90 %), tetraethyl orthosilicate (reagent grade 98%), ammonium hydroxide (28-30% NH_3 basis), dimethylformamide (99.8 % purity), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99 % purity) and sodium chloride (ACS reagent, ≥99.0 % purity) were purchased from Sigma Aldrich (Milwaukee, WI). Azidopropyltriethoxysilane was purchased from SelectLab Chemicals GmbH (Münster, DE). 3 mm, 300 mesh copper grids coated with 10 nm thick Formvar film and stabilized with 1 nm thick evaporated carbon film were purchased from Electron Microscopy Sciences (Hatfield, PA).

Transmission electron microscopy was performed on a JEOL JEM2100F microscope operating at 200 kV at Le CM^2 microscopy facility at Ecole Polytechnique in Montreal, QC.

Powder X-ray diffractograms (PXRD) were obtained using a Scintag XDS-2000 diffractometer equipped with a Cu Kα source operating at 45 kV and 40 mA and a Si(Li) Peltier-cooled solid state detector. Data was collected using a 2θ scan range of 10 - 90° with a step size of 0.02° and dwell time of 0.5 s.

Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR-FTIR) was performed on solid state samples on a Thermo Scientific Nicolet iS5 spectrometer equipped with the iD5 accessory with a laminate-diamond crystal window. Spectra were recorded with a resolution of 0.4 cm^{-1} and 64 scans.

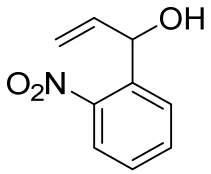
Upconversion lifetimes were recorded using a 976 nm excitation from a Coherent 6-pin 15 fiber-coupled F6 series laser diode coupled to a fiber with a 100 μm core diameter (output power of 4 W/cm^2 and a pulse width of 1 ms). Emissions were collected from 10 mg/ml dispersions of the nanoparticle samples in a 1 cm pathlength quartz cuvette (Thorlabs) or from solid powders in an Eppendorf tube, perpendicular to the incident excitation beam. UV emissions were dispersed using an Oriel 77250 1/8 m monochromator (2400 grooves/mm) and collected with a Hamamatsu R4632 photomultiplier tube. Signals were enhanced with an SR440 Stanford Research Systems preamplifier and reported through an SR400 Stanford Research Systems gated photon counter.

Upconversion emission spectroscopy was performed using a FERGE BRX-VR UV-NIR spectrograph from Princeton Instruments equipped with a 295 grooves/mm grating blazed at 545 nm. Emissions were collected perpendicular to the excitation source using a 600 μm core diameter optical fiber from Ocean Optics. Excitation of the samples was performed using the same fiber-coupled 976 nm laser used for the lifetime emission spectroscopy experiments, on continuous-wave mode at 0.460 W power output (4.6 W/cm^2).

UV-Visible absorption spectroscopy was performed using a Cary 5000 UV-VIS-NIR absorption spectrometer operating with a resolution of 1 nm, source changeover at 350 nm, and acquisition speed of 600 nm/minute. All spectra were recorded on solutions in a 1 cm pathlength quartz cuvette from Thorlabs.

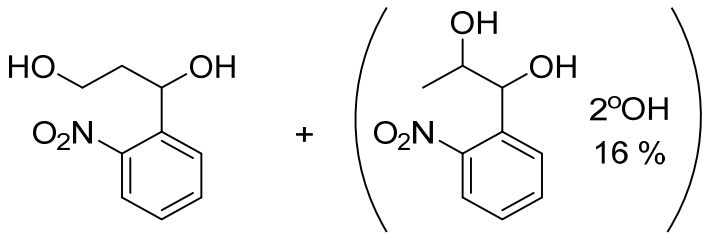
Chemical synthesis of small molecules

1-(2-nitrophenyl)prop-2-en-1-ol – allylic alcohol **1**



To a flame-dried round bottom flask, 2-nitrobenzaldehyde (0.3024 g; 2.0 mmol) was dissolved in anhydrous THF (20 ml). The solution was cooled to -78 ºC and the atmosphere exchanged with argon. To this solution was added vinylmagnesium bromide (1M in THF; 4 ml; 4 mmol) dropwise over 5 minutes. The reaction mixture was left stirring at -78 ºC under argon for 3 hours, and was subsequently quenched by slowly injecting saturated NH_4Cl (10 ml). The reaction mixture was left to come up to room temperature over 30 minutes and subsequently diluted with brine (20 ml). The aqueous layer was extracted with ethyl acetate (3 x 20 ml). The combined organic extracts were washed with brine (2 x 20 ml), dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo* to afford allylic alcohol **1** as a yellow oil (70 % yield; estimated by NMR). The crude material was used without further purification. $\mathbf{R_f}$ (SiO_2 TLC): 0.84 (1:1 EtOAc/hexanes, v/v). $\boldsymbol{\lambda_{max\ (MeCN)}}$: 256 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.91-7.89 (dd, *J* = 8.2, 1.4 Hz, 1H, Ar), 7.77-7.75 (dd, *J* = 7.6, 1.5 Hz, 1H, Ar), 7.65-7.62 (td, *J* = 7.6, 1.4 Hz, 1H, Ar), 7.45-7.22 (m, 1H, Ar), 6.10-6.04 (ddd, *J* = 17.1, 10.5, 4.9 Hz 1H, C=C-H), 5.79-5.78 (d, 1H, *J* = 4.9 Hz, HO-C-H), 5.42-5.38 (dt, *J* = 17.1, 1.4 Hz, 1H, C=C-H), 5.26-5.24 (dt, *J* = 10.5, 1.4 Hz, 1H, C=C-H), 2.69 (s broad, 1H, OH). **^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 148.22, 137.93, 137.49, 133.49, 128.76, 128.40, 124.44, 116.11, 69.86. **HRMS** (ESI-MS) *m/z* calculated for $C_9H_9NO_3$: 179.0582; compound degrades to a nitroso ketone under MS conditions.

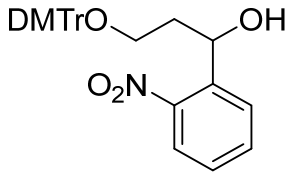
1-(2-nitrophenyl)propane-1,3-diol – diol **2**



To a stirring solution of allylic alcohol **1** (0.208 g; 1.16 mmol) in anhydrous DCM (10 ml) at 0 ºC was added triethylamine (0.484 ml; 3.47 mmol), followed by trimethylsilylchloride (0.441 ml;

3.47 mmol). The reaction was lifted from the ice bath and stirred for 1 hour, coming up to room temperature. The reaction was then quenched by addition of methanol (5 ml) and the mixture concentrated *in vacuo*. The crude material was washed with THF (3x 10 ml) and filtered to afford the blocked intermediate. The filtrate was concentrated *in vacuo*, the residue was dissolved in anhydrous THF (5.4 ml) and the solution cooled to 0 ºC under argon atmosphere. To this solution was added BH_3-THF (1M in THF, 4.6 ml; 4.63 mmol) dropwise over 5 minutes. The reaction mixture was stirred overnight (19 hours), slowly coming up to room temperature. Once again, the reaction mixture was cooled to 0 ºC before addition of NaOH (3 M in H_2O; 3 ml; 9 mmol), followed by 30 % H_2O_2 (3 ml; 29.4 mmol). After 10 minutes on ice, the flask was lifted and left to come up to room temperature over 50 minutes. Sodium bisulfite (3 M in H_2O; 5 ml) was then slowly added to quench excess H_2O_2. The reaction mixture was extracted with ethyl acetate (2x 30 ml), and the combined organic extracts washed with brine (30 ml), dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by flash column chromatography using EtOAc/hexanes (6:4; v/v) to afford 0.169 g (74 %) of diol **2** as a yellowish brown oil. $\mathbf{R_f}$ (SiO_2 TLC): 0.21 (1:1 EtOAc/hexanes, v/v). $\boldsymbol{\lambda_{max\ (MeCN)}}$: 258 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.95-7.91 (m, 2H, Ar), 7.69-7.65 (m, 1H, Ar), 7.45-7.42 (m, 1H, Ar), 5.53-5.51 (dt, *J* = 9.1, 3.0 Hz, 1H), 4.03-3.94 (m, 2H,), 3.50-3.49 (d, *J* = 3.0 Hz, 1H, OH), 2.19-2.17 (t, *J* = 4.6 Hz, 1H, OH), 2.15-2.09 (m, 1H), 2.04-1.96 (m, 1H). **^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 147.29, 139.86, 133.59, 128.10, 128.04, 124.28, 69.40, 61.57, 39.52. **HRMS** (ESI-MS) *m/z* calculated for $C_9H_{12}NO_4^+$: 198.0761; found 198.0760 $[M + H]^+$.
2ºOH regioisomer: $\mathbf{R_f}$ (SiO_2 TLC): 0.42 (1:1 EtOAc/hexanes, v/v). **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.93-7.87 (m, 2H, Ar), 7.65-7.61 (m, 1H, Ar), 7.43-7.40 (m, 1H, Ar), 5.39 (app s, 1H), 4.15 (app s, 1H), 3.35 (s, 1H), 2.69 (s, 1H), 1.07-1.06 (d, *J* = 6.4 Hz, 3H).

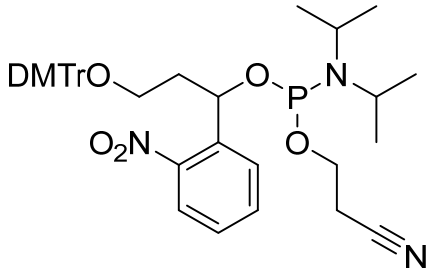
3-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-(2-nitrophenyl)propan-1-ol – pre-amidite **3**



To a stirring solution of diol **2** (0.120 g; 0.61 mmol), triethylamine (0.220 ml; 1.58 mmol) and DMAP (0.007 g; 10 mol%) in anhydrous DCM (6 ml) at 0 ºC was added DMTr-Cl (0.271 g; 0.80 mmol). After stirring for 10 minutes on ice, the flask was raised and left to come up to RT over 50 minutes. The reaction mixture was quenched with methanol (10 ml) and concentrated *in vacuo*. Without workup, the crude material was purified by flash column chromatography using hexanes/EtOAc (8:2; v/v) (with 2 % NEt_3, v/v) to afford 0.259 g (85 %) of pre-amidite **3** as a colorless foam. $\mathbf{R_f}$ (SiO_2 TLC): 0.65 (3:2 hexanes/EtOAc, v/v). $\boldsymbol{\lambda_{max\ (MeCN)}}$: 234 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.91-7.89 (d, *J* = 6.9 Hz, 1H, Ar), 7.85-7.84 (d, *J* = 8.0 Hz, 1H, Ar), 7.61-7.58 (t, *J* = 8.0 Hz, 1H, Ar), 7.45-7.43 (d, *J* = 7.7 Hz, 2H, Ar), 7.40-7.37 (t, *J* = 6.9 Hz, 1H, Ar), 7.35-7.30 (m, 6H, Ar), 7.25-7.22 (t, *J* = 7.3 Hz, 1H, Ar), 6.86-6.85 (d, *J* = 8.8 Hz, 4H, Ar), 5.45-5.43 (dt, *J* = 8.6, 2.9 Hz, 1H, HO-C-H), 4.02-4.01 (d, *J* = 2.9 Hz, 1H, OH), 3.80 (s, 6H, 2 x OCH_3), 3.48-3.49 (m, 2H), 2.21-2.16 (m, 1H), 2.01-1.93 (m, 1H). **^{13}C NMR** (125.7 MHz, $CDCl_3$,

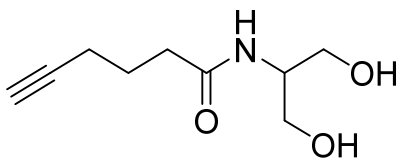
ppm): δ 158.58, 147.47, 144.49, 139.86, 135.73, 135.65, 133.36, 129.96, 129.94, 128.31, 128.01, 127.98, 127.82, 126.93, 124.28, 113.26, 87.20, 69.53, 62.57, 55.22, 37.95. **HRMS** (ESI-MS) *m/z* calculated for $C_{30}H_{29}NO_6Na^+$: 522.1887; found 522.1882 $[M + Na]^+$.

3-(bis(4-methoxyphenyl)(phenyl)methoxy)-1-(2-nitrophenyl)propyl (2-cyanoethyl) diisopropyl phosphoramidite – photocleavable phosphoramidite **4**



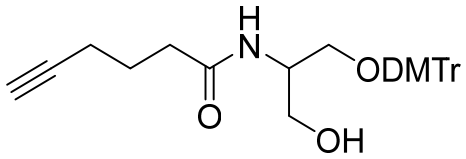
To a stirring solution of pre-amidite **3** (0.173 g, 0.346 mmol) in anhydrous DCM (4 ml) was added DIPEA (0.181 ml, 1.04 mmol), followed by the dropwise addition of Cl-POCEN(*i*Pr)$_2$ (0.155 ml, 0.693 mmol). After 25 minutes, the solvent was evaporated *in vacuo*. The content was diluted with EtOAc (40 ml) and washed with 3 % (aq., w/v) $NaHCO_3$ (40 ml) and brine (40 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by short flash column chromatography using EtOAc/hexanes (1:1, v/v) (with 0.5 % NEt_3, v/v) to afford 0.200 g (83%) of PCP **4** as a colorless foam. $\mathbf{R_f}$ (SiO_2 TLC) diastereomers: 0.70, 0.77 (3:2 hexanes/EtOAc, v/v). $\boldsymbol{\lambda_{max\ (MeCN)}}$: 234 nm. **^{1}H NMR** (500 MHz, acetone-d_6, ppm): δ 7.95-7.93 (m, 1H, Ar), 7.84-7.81 (m, 1H, Ar), 7.74-7.69 (m, 1H, Ar), 7.56-7.45 (m, 3H, Ar), 7.38-7.28 (m, 6H, Ar), 7.24-7.19 (m, 1H, Ar), 6.90-6.85 (m, 4H, Ar), 5.63-5.49 (m, 1H), 3.79-3.78 (d, *J* = 4.4 Hz, 6H, 2 x OCH_3), 3.67-3.52 (m, 3H), 3.46-3.39 (m, 1H), 3.35-3.27 (m, 2H), 2.60-2.57 (t, *J* = 6.1 Hz, 1H), 2.54-2.42 (m, 1H), 2.33-2.21 (m, 1H), 2.17-2.07 (m, 1H), 1.13-1.05 (m, 9H), 0.82-0.81 (d, *J* = 6.8 Hz, 3H). **^{13}C NMR** (125.7 MHz, acetone-d_6, ppm): δ 159.50, 148.77, 148.66, 146.49, 146.47, 139.68, 139.59, 137.32, 137.28, 137.26, 134.10, 133.97, 130.96, 130.85, 130.22, 130.14, 129.25, 129.10, 129.05, 128.92, 128.50, 128.48, 127.40, 127.38, 124.76, 124.72, 118.72, 118.50, 113.81, 113.78, 113.77, 86.98, 86.89, 70.01, 69.88, 68.62, 68.48, 61.19, 60.73, 60.07, 59.92, 59.50, 59.34, 55.50, 55.47, 43.94, 43.84, 43.82, 43.72, 40.24, 40.20, 40.10, 40.05, 24.97, 24.91, 24.85, 24.82, 24.76, 24.40, 24.35, 20.72, 20.67, 20.53, 20.47. **^{31}P NMR** (202.3 MHz, acetone-d_6, ppm): δ 149.65, 148.19. **HRMS** (ESI-MS) *m/z* calculated for $C_{39}H_{47}N_3O_7P^+$: 700.3146; found 700.3146 $[M + H]^+$.

N-(1,3-dihydroxypropan-2-yl)hex-5-ynamide – amide **5**



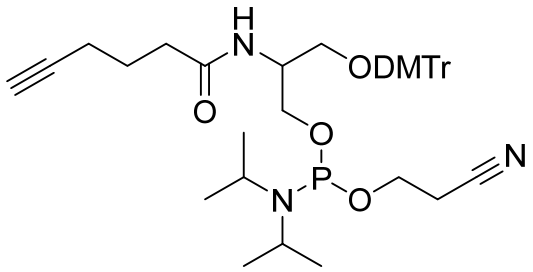
To a stirring solution of 5-hexynoic acid (0.299 g; 2.67 mmol) in ethyl acetate (20 ml) at 0 ºC and under argon atmosphere was added *N*-methylmorpholine (0.580 ml; 5.27 mmol), followed by isobutyl chloroformate (0.350 ml; 2.70 mmol). The reaction was left stirring at 0 ºC for 40 minutes to generate the mixed anhydride intermediate before adding serinol (0.201 g; 2.2 mmol). After addition, the flask was raised from the ice bath and the reaction mixture left to come up to room temperature overnight (21 hours). The reaction mixture was quenched with methanol (20 ml) and concentrated *in vacuo*. Without workup, the crude material was purified by flash column chromatography using 8 % MeOH in EtOAc to afford 0.363 g (89 %) of amide **5** as a colorless solid. $\mathbf{R_f}$ (SiO_2 TLC): 0.36 (10 % MeOH in EtOAc). $\boldsymbol{\lambda_{max\ (MeCN)}}$: < 200 nm. **^{1}H NMR** (500 MHz, DMSO-d_6, ppm): δ 7.50-7.48 (d, *J* = 8.0 Hz, 1H, NH), 4.57-4.55 (t, *J* = 5.5 Hz, 2H, 2 x OH), 3.72-3.66 (dt, *J* = 8.0, 5.5 Hz, 1H, N-C-H), 3.39-3.37 (t, *J* = 5.5 Hz, 4H), 2.77-2.76 (t, *J* = 2.7 Hz, 1H), 2.18-2.13 (m, 4H), 1.68-1.62 (p, *J* = 7.3 Hz, 2H). **^{13}C NMR** (125.7 MHz, DMSO-d_6, ppm): δ 171.41, 84.21, 71.40, 60.23, 52.82, 34.24, 24.38, 17.39. **HRMS** (ESI-MS) *m/z* calculated for $C_9H_{16}NO_3^+$: 186.1125; found 186.1126 $[M + H]^+$.

N-(1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxypropan-2-yl)hex-5-ynamide – pre-amidite **6**



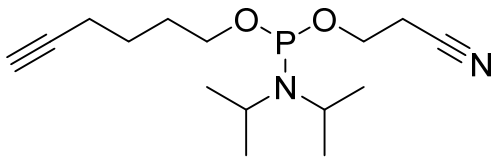
To a stirring solution of amide **5** (0.100 g; 0.540 mmol) and triethylamine (0.151 ml; 1.08 mmol) in anhydrous THF (3 ml) at 0 ºC and under argon atmosphere was added a solution of DMTr-Cl (0.184 g; 0.543 mmol) and DMAP (0.0066 g; 10 mol%) in anhydrous THF (2 ml) portionwise (0.5 equiv. at a time). After each 0.5 equiv. DMTr-Cl addition (1 ml), the reaction was left stirring at 0 ºC for 30 minutes. After 1 hour on ice, the reaction was lifted and left to come up to room temperature over 1.5 hours. The reaction mixture was quenched with methanol (10 ml) and concentrated *in vacuo*. Without workup, the crude material was purified by flash column chromatography using EtOAc/TEA (99:1; v/v) to afford 0.175 g (67 %) of pre-amidite **6** as a yellowish white foam. $\mathbf{R_f}$ (SiO_2 TLC): 0.56 (100 % EtOAc). $\boldsymbol{\lambda_{max\ (MeCN)}}$: 235 nm. **^{1}H NMR** (500 MHz, $CDCl_3$, ppm): δ 7.40-7.39 (d, *J* = 7.8 Hz, 2H, Ar), 7.31-7.29 (m, 6H, Ar), 7.24-7.21 (t, *J* = 7.3 Hz, 1H, Ar), 6.85-6.83 (d, *J* = 8.6 Hz, 4H, Ar), 6.04-6.02 (d, *J* = 7.7 Hz, 1H, NH), 4.11-4.06 (m, 1H), 3.83-3.79 (m, 7H), 3.70-3.67 (m, 1H), 3.35-3.29 (m, 2H), 2.79 (s broad, 1H, OH), 2.33-2.30 (t, *J* = 7.1 Hz, 2H), 2.27-2.24 (td, *J* = 7.1, 2.5 Hz, 2H), 1.97-1.96 (t, *J* = 2.5 Hz, 1H), 1.87-1.81 (p, *J* = 7.1 Hz, 2H). **^{13}C NMR** (125.7 MHz, $CDCl_3$, ppm): δ 172.66, 158.61, 144.41, 135.49, 135.47, 129.91, 129.89, 127.98, 127.92, 127.00, 113.28, 86.56, 83.39, 69.31, 64.18, 63.33, 55.21, 51.20, 35.02, 24.07, 17.79. **HRMS** (ESI-MS) *m/z* calculated for $C_{30}H_{33}NO_5Na^+$: 510.2251; found 510.2255 $[M + Na]^+$.

3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-(hex-5-ynamido)propyl (2-cyanoethyl) diisopropyl phosphoramidite – alkyne phosphoramidite **7**



To a stirring solution of pre-amidite **6** (0.083 g, 0.170 mmol) in anhydrous THF (2 ml) was added DIPEA (86 µL, 0.492 mmol), followed by the dropwise addition of Cl-POCEN(*i*Pr)$_2$ (100 µL, 0.448 mmol). After 30 minutes, the reaction mixture was taken up in ethyl acetate (40 ml) and washed with 3 % (aq., w/v) $NaHCO_3$ (40 ml) and brine (40 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by short flash column chromatography using EtOAc/hexanes (6:4, v/v) (with 2 % NEt_3, v/v) to afford 0.081 g (70 %) of alkyne phosphoramidite **7** as a colorless semi-solid. $\mathbf{R_f}$ (SiO_2 TLC): 0.74 (1:1 EtOAc/hexanes, v/v). $\boldsymbol{\lambda_{max\,(MeCN)}}$: 235 nm. **^{1}H NMR** (500 MHz, acetone-d$_6$, ppm): δ 7.49-7.40 (m, 2H, Ar), 7.36-7.28 (m, 6H, Ar), 7.25-7.20 (m, 1H, Ar), 6.99-6.87 (m, 4H, Ar), 6.84-6.82 (m, 1H), 4.41-4.28 (m, 1H), 3.88-3.83 (m, 1H), 3.79-3.71 (m, 9H), 3.65-3.56 (m, 2H), 3.31-3.21 (m, 2H), 2.70-2.67 (t, J = 6.0 Hz, 2H), 2.34-2.19 (m, 5H), 1.82-1.74 (m, 2H), 1.18-1.12 (m, 12H). **^{13}C NMR** (125.7 MHz, acetone-d$_6$, ppm): δ 172.04, 172.01, 159.58, 159.52, 146.29, 137.02, 136.99, 136.97, 136.94, 136.87, 131.01, 130.98, 129.04, 129.02, 128.55, 127.49, 119.02, 113.85, 86.72, 84.58, 84.56, 70.23, 63.37, 63.32, 63.22, 63.02, 59.67, 59.59, 59.53, 59.45, 55.50, 50.84, 50.78, 49.82, 43.80, 43.78, 43.70, 43.68, 35.40, 35.38, 35.31, 25.49, 24.99, 24.93, 24.86, 20.80, 20.75, 18.43. **^{31}P NMR** (202.3 MHz, acetone-d$_6$, ppm): δ 147.60, 147.34. **HRMS** (ESI-MS) *m/z* calculated for $C_{39}H_{50}N_3O_6PNa^+$: 710.3329; found 710.3334 $[M + Na]^+$.

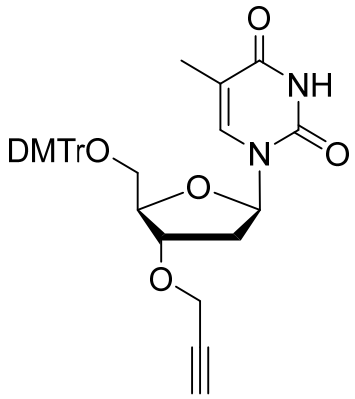
2-cyanoethyl hex-5-yn-1-yl diisopropylphosphoramidite – control alkyne phosphoramidite **8**



To a stirring solution of 5-hexyn-1-ol (0.100 g, 1.02 mmol) in anhydrous THF (8 ml) was added DIPEA (0.532 ml, 3.06 mmol), followed by the dropwise addition of Cl-POCEN(*i*Pr)$_2$ (0.500 ml, 2.24 mmol). After 20 minutes, the reaction mixture was taken up in ethyl acetate (40 ml) and washed with 3 % (aq., w/v) $NaHCO_3$ (40 ml) and brine (40 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by short flash column chromatography using EtOAc/hexanes (1:1, v/v) (with 2 % NEt_3, v/v) to

afford 0.236 g (78 %) of control alkyne phosphoramidite as a clear oil. $\mathbf{R_f}$ (SiO_2 TLC): 0.90 (1:1 EtOAc/hexanes, v/v). $\lambda_{max\ (MeCN)}$: 203 nm. **^{1}H NMR** (500 MHz, acetone-d_6, ppm): δ 3.89-3.78 (m, 2H), 3.74-3.61 (m, 4H), 2.76-2.73 (t, *J* = 6.0 Hz, 2H), 2.32-2.31 (t, *J* = 2.7 Hz, 1H), 2.24-2.21 (td, *J* = 7.0, 2.7 Hz, 2H), 1.75-1.70 (m, 2H), 1.65-1.59 (m, 2H), 1.20-1.18 (dd, *J* = 6.8, 3.2 Hz, 12H). **^{13}C NMR** (125.7 MHz, acetone-d_6, ppm): δ 118.94, 84.74, 69.97, 63.77, 63.63, 59.42, 59.28, 43.74, 43.64, 31.02, 30.97, 25.93, 24.91, 24.85, 20.79, 20.73, 18.44. **^{31}P NMR** (202.3 MHz, acetone-d_6, ppm): δ 147.10. **HRMS** (ESI-MS) *m/z* calculated for $C_{15}H_{28}N_2O_2P^+$: 299.1883; found 299.1885 $[M + H]^+$.

5'-*O*-DMTr-3'-*O*-propargyl thymidine (**9**)



To a flame-dried round bottom flask was added NaH (0.065 g; 2.71 mmol) and anhydrous THF (6 ml). The suspension was cooled to 0 ºC and the atmosphere exchanged with argon. To this suspension was added 5'-*O*-DMTr thymidine (0.302 g; 0.554 mmol). The reaction mixture was left to react for 10 minutes before being raised from the ice bath and left to come up to room temperature over 20 minutes. After being placed back on ice for 5 minutes, propargyl bromide (0.125 ml; 1.40 mmol) was injected into the reaction mixture dropwise. The reaction mixture was then sonicated for 1.5 hours at room temperature, before being quenched with methanol (10 ml) and concentrated *in vacuo*. The content was taken up in DCM (40 ml) and washed with water (20 ml) and brine (20 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated *in vacuo*. The crude material was purified by flash column chromatography using EtOAc/hexanes (8:2, v/v) (with 1 % NEt_3, v/v) to afford 0.248 g (77 %) of 5'-*O*-DMTr-3'-*O*-propargyl thymidine as a colorless foam. $\mathbf{R_f}$ (SiO_2 TLC): 0.82 (8:2 EtOAc/hexanes, v/v). $\lambda_{max\ (MeCN)}$: 235 nm. **HRMS** (ESI-MS) *m/z* calculated for $C_{34}H_{24}N_2O_7Na^+$: 605.2258; found 605.2256 $[M + Na]^+$. Other spectral data were in accordance with the literature.[1]

Oligonucleotide solid-phase synthesis

All oligonucleotide sequences were assembled with an Applied Biosystems Model 3400 synthesizer on a 2 μmol scale using standard β-cyanoethyl phosphoramidite cycles supplied by the manufacturer with slight modifications to coupling times as described below. The oligonucleotides

were prepared using commercially available 5'-*O*-dimethoxytrityl-thymidine-3'-*O*-(β-cyanoethyl-*N,N'*-diisopropyl) phosphoramidite, which was dissolved in anhydrous acetonitrile at a concentration of 0.1 M. The photocleavable (**4**) and alkyne (**7**) phosphoramidites used in the oligonucleotides were prepared in anhydrous acetonitrile at a concentration of 0.12 M. The control alkyne phosphoramidite (**8**) was dissolved in an anhydrous acetonitrile/THF (2:1; v/v) mixture (to help with solubility) at a concentration of 0.10 M. Oligonucleotide sequence assembly was carried out as previously described.[2,3] The capping step of the assembly was performed using acetic anhydride/pyridine/tetrahydrofuran 1:1:8 (v/v/v; solution A) and 1-methyl-imidazole/ tetrahydrofuran 16:84 (w/v; solution B). The coupling times for the photocleavable phosphoramidite **4**, alkyne phosphoramidite **7** and control alkyne phosphoramidite **8** were extended to 900 seconds, 600 seconds and 600 seconds, respectively (compared to 120 seconds for the thymidine phosphoramidites). All oligonucleotides were synthesized without final acid treatment (DMTr-on).

Oligonucleotide deprotection and purification

The solid-phase column was removed from the synthesizer and the CPG-bound oligonucleotide was treated with 10 % diethylamine in acetonitrile (1 ml) for 5 minutes, washed with acetonitrile (20 ml) and dried *in vacuo*. The CPG was then transferred to a 2 ml Teflon lined screw cap vial. The oligonucleotides were deprotected and cleaved from the solid support by treatment with 1 ml NH_4OH/EtOH (3:1; v/v) for 30 minutes at 55 °C. After the incubation period, the supernatant was transferred to a separate screw cap vial and the CPG washed twice with 200 µL aqueous acetonitrile (50%, v/v). To the photocleavable oligonucleotide (PCO) sample was added 500 µL of 50 mM Tris (25 µmol). The crude oligonucleotides were lyophilized in a speed-vac concentrator.

All oligonucleotides were purified by ion exchange (IEX) HPLC using a Dionex DNAPAC PA-100 column (0.4 cm x 25 cm) purchased from Dionex Corp (Sunnyvale, CA). The column was eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl). The column was monitored at 260 nm for analytical runs (0.1-1.0 OD_{260} injected) and 260 nm and 280 nm for preparative runs (20-30 OD_{260} injected). The purified oligonucleotides were desalted using C-18 SEP PAK cartridges (Waters). To the purified and desalted PCO samples were added 50 µL of 50 mM Tris (2.5 µmol) before lyophilisation to retain the 5'-*O*-DMTr group. The purified oligonucleotide samples were stored in 18 MΩ H_2O at -20 ºC.

Oligonucleotide characterization by LC-MS

LC-MS analyses of oligonucleotides were obtained at the Concordia University Centre for Biological Applications of Mass Spectrometry (CBAMS) using an Agilent 1100 LC system coupled to a Thermo LTQ Orbitrap Velos mass spectrometer equipped with a heated electrospray ion source in negative mode. A Spursil C18-EP column (50x2.1 mm and 3 µm particle diameter, Dikma Technologies) was used and oligonucleotides were eluted using a 20 minute gradient at an

initial flow rate of 250 µL/min with mobile phase A (10 mM ammonium acetate and 1 mM ammonium fluoride water solution) and B (acetonitrile). The gradient started at 2% B and held for 3 min, linear gradients were achieved to 50% B at 8 min, to 90% B at 10 min, then followed by isocratic with 90% B for 2 min. The column was reconditioned from 13 min with 2% B at a flow rate of 400 µL/min for 5 min and at 250 µL/min for extra 2 min. Dried samples were reconstituted in 50 µL of mobile phase A and the injection volume was 10 µL. The divert valve was set at 0 min to the waste, and at 4.0 min to the detector. MS spectra (*m/z* 300-2000) were acquired in the Orbitrap at a resolution of 60,000. The uncharged monoisotopic mass of oligonucleotides were calculated using Thermo FreeStyle™ software (v1.7 SP2).

UCNP synthesis

$LiYF_4$: 25% Yb^{3+}, 0.2% Tm^{3+} was prepared according to our previously established protocol.[4] The reaction was carried out on a 2.5 mmol scale. Briefly, Y_2O_3 (211.1 mg; 1.87 mmol), Yb_2O_3 (123.2 mg; 0.625 mmol) and Tm_2O_3 (1 mg; 0.005 mmol) were added to a 250 ml 3-neck round bottom flask, and mixed with 5 ml H_2O and 5 mL trifluoroacetic acid. This mixture was refluxed at 80 ºC for approximately 6 hours, or until the solution turned from opaque white to transparent and colorless. The resulting solution was evaporated to yield a dry white powder containing the lanthanide-trifluoroacetate precursors. Lithium trifluoroacetate (299 mg; 2.5 mmol), oleic acid (20 ml) and 1-octadecene (20 ml) were added to the flask containing the precursors. The resulting mixture was heated to 120 ºC under vacuum (0 mbar) for 30 minutes. The vacuum was then removed and an argon flow was introduced. The solution was then heated to 315 ºC for 1 hour at a rate of 10 ºC/min. The solution was then removed from the heating mantle and allowed to cool to room temperature under argon flow. The resulting yellow solution was then divided in two and transferred to two 50 ml centrifuge tubes. 30 ml of ethanol was added to each tube and the resulting mixture was centrifuged for 15 minutes at 4,000 RPM. The supernatant was then discarded and the pellet was suspended in 10 ml of n-hexanes, and the nanoparticles were precipitated with 40 ml ethanol. The resulting solution was again centrifuged for 15 minutes and this process was repeated three times to yield purified, oleate-capped $LiYF_4$: 25% Yb^{3+}, 0.2% Tm^{3+} upconverting nanoparticles.

UCNP SiO_2-N_3 coating

25 mg of oleate-capped $LiYF_4$ UCNPs were dispersed in 6 ml *n*-hexanes and sonicated for 10 minutes to ensure complete dispersion. The solution was then set to stir at 900 RPM at room temperature, and 100 µL of IGEPAL-CO520 was added to the solution. The resulting mixture was sonicated for 10 minutes, after which 200 µL of IGEPAL-CO520 was again added to the solution. The mixture was left to stir for 10 minutes. Another 200 µL of IGEPAL-CO520 was added to the solution, followed by 80 µL of NH_4OH. This mixture was sonicated for 20 minutes, until a transparent solution was obtained. The solution was then set to stir at 900 RPM and 20 µL tetraethyl orthosilicate (TEOS) was added at a rate of 4 µL/hour in 5 steps. The resulting solution was left to stir for 48 hours, after which 25 µL of azidopropyltriethoxysilane (AzPTES) was added to the solution at a rate of 5 µL/hour in 5 steps. The solution was left to stir an additional 24 hours.

To precipitate and purify the nanoparticles, 10 ml of acetone was added to the mixture and the resulting solution was centrifuged at 13,300 xG for 10 minutes. The supernatant was discarded and the pellet was redispersed in 1 ml H_2O and then precipitated with 1 ml of ethanol. The mixture was centrifuged at 13,300 xG for 10 minutes. This process was repeated 3 times to yield azide silica-coated UCNPs (AzSiUCNPs).

Click reaction between AzSiUCNPs and 5'-*O*-DMTr-3'-*O*-propargyl thymidine

10 mg of AzSiUCNPs were dispersed in 2 ml DMF and sonicated for 10 minutes to ensure dispersion of the UCNPs. The solution was bubbled with argon for 10 minutes and then 200 µL of 5 mM sodium ascorbate, 200 µL of 0.75 mM $CuSO_4$ and 100 µL of PMDETA were added. The solution was stirred at 900 RPM for 10 minutes and then 10 mg of 5'-*O*-DMTr-3'-*O*-propargyl thymidine (dT) was added to the mixture. The mixture was left to stir for 4 hours at room temperature. The DMTr-dT-UCNPs were collected by centrifugation at 13,300 xG for 10 minutes. To purify the nanoparticle pellet, the supernatant was discarded and the pellet was dispersed in 2 ml DMF and centrifuged again. This process was repeated 5 times to yield a purified pellet of DMTr-dT-UCNPs. The resulting pellet was dried and used for azide quantification.

PCO-UCNP/Control-UCNP click reaction

The protocols for clicking the alkyne-functionalized, photocleavable oligonucleotide (PCO) and the control oligonucleotide were identical, except for the amount of $CuSO_4$ and PMDETA added. 5 mg of AzSiUCNPs were dispersed in 10 ml 0.4 M NaCl and sonicated for 10 minutes. The solution was then put to stir at 500 RPM and room temperature and degassed by bubbling argon through the solution for 5 minutes and then placed under vacuum for 10 minutes (0 mbar). To this dispersion, 400 µL of 0.2 M sodium citrate and 100 µL of PMDETA were added for the CT-UCNPs (or 300 µL for the PCO-UCNPs) and again the solution was degassed using the same protocol. To this mixture, 2 mg of PCO were added (approximately 50 OD_{260}) and the solution was again degassed, this time for 30 minutes under vacuum (0 mbar). 100 µL (CT-UCNPs) or 300 µL (PCO-UCNPs) of 50 mM $CuSO_4$ was then added to the solution, which went from light yellow to dark blue. The mixture was covered with foil to shield it from light and allowed to stir for 4 hours. The resulting solution was centrifuged at 13,300 xG for 10 minutes. The supernatant was discarded, and the pellets were purified by dispersing in water and centrifuging at 13,300 xG for 5 minutes. The process was repeated 6 times to remove as much salt as possible and yield purified PCO-UCNPs and CT-UCNPs. These nanoparticles were stored as a pellet in water at -20 ºC.

TEM prep for oleate-capped UCNPS and AzSiUCNPs

A 1 mg/ml dispersion of oleate-capped UCNPs in hexanes was sonicated for 15 minutes. A 100 µL aliquot of the solution was deposited onto a carbon-Formvar Cu TEM grid and allowed to dry. The same procedure was utilized for AzSiUCNPs, except the solution was prepared in water.

Triphenylphosphine-Ninhydrin assay of UCNPs

This procedure was modified from the one reported by Cegielska *et al*.[5] 200 µL of a 1 mg/ml solution of nanoparticles (pre- and post-click reaction) were deposited onto a filter paper and left to dry in air at room temperature. This was repeated 3 times in the same spot to ensure adequate deposition of nanoparticles. 200 µL of 10 % (w/v) triphenylphosphine in DCM was then deposited on the dried nanoparticle spots, and again left to dry. 200 µL of 5 % (w/v) ninhydrin in acetone was then deposited on the same spots, and again left to dry. Finally, the dried filter paper was gently heated with a heat gun for 5 minutes and any color change was observed.

Trityl assay for the determination of azide sites on the AzSiUCNPs

Between 0.1-0.2 mg of dried DMTr-dT-UCNPs were dispersed in 500 µL of a solution of 3 % trichloroacetic acid in dichloromethane. The resulting orange pellet was then sonicated for 10 minutes to ensure the trityl cation was released into solution. The solution was centrifuged at 13,300 xG for 10 minutes and the supernatant was then isolated and evaluated by UV-visible absorption spectroscopy. Assuming the click reaction was quantitative, the number of trityl cations in solution is equal to the number of azide sites in the sample, which was correlated to the number of azides per nanoparticle.

Determination of PCO loading on AzSiUCNPs

The trityl assay was repeated to determine the number of oligonucleotides clicked to the AzSiUCNP surface. In this case, the number of trityl cations is assumed to be equal to the number of oligonucleotides on the UCNP surface. This value was then used to quantify the number of PCO per nanoparticle.

PCO cleavage experiment

Experiments performed with CT-UCNPs and PCO-UCNPs were done in an identical manner. A 0.1 mg/ml solution of nanoparticles was prepared in ultrapure (18 MΩ) water in a 2 ml Eppendorf tube, and thoroughly vortexed to ensure dispersion. This solution was then placed in an ice bath (to prevent excess heating of the solution) and irradiated with continuous-wave 976 nm excitation (0.622 W/cm^2). At the corresponding time points (10, 20, 30, 45, 60 and 75 minutes), the solution was centrifuged at 13,300 xG for 5 minutes and the supernatant was evaluated by UV-Visible absorption spectroscopy. The supernatant was then returned to the centrifuge tube and the pellet was vortexed to re-disperse the nanoparticles. This process was repeated for each of the time points.

The theoretical absorption maximum of the PCO in solution (λ = 260 nm) was calculated based on the determined PCO loading on the UCNPs and the extinction coefficient of the oligonucleotide

(146400 L mol^{-1} cm^{-1}). The ratio of the absorption maximum at each observed time to the theoretical maximum was used to determine the percent cleavage.

Denaturing polyacrylamide gel electrophoresis (PAGE) analysis

The gel consisted of 20% acrylamide (19:1, w/w, acrylamide to bis-acrylamide) and 7 M urea in a pH 8.0 TBE buffer (89 mM Tris base, 89 mM boric acid, 0.2 mM ethylenediaminetetraacetic acid (EDTA)) with dimensions 10 cm x 7.5 cm x 0.75 mm (L x H x W). The gel was run for 1 hour 15 minutes at 200 V using the TBE buffer described above. Markers for the gel were a mixture of bromophenol blue (8 nucleotides) and xylene cyanol FF (28 nucleotides). Oligonucleotides were prepared by lyophilizing 0.1 OD_{260} of sample and dissolving in 10 μL formamide. The supernatant of the irradiated PCO-UCNP and CT-UCNP were lyophilized in a speed-vac concentrator and dissolved in 10 μL formamide. The gel was visualized by placing it on a TLC plate (Merck, Kieselgel 60 F_{254}, 0.25 mm) and irradiating with 254 nm light (UV shadowing).

Figure S1: IEX HPLC traces of crude dT_{18} and PCO. (**a**) Crude dT_{18} sequence prior to coupling photocleavable phosphoramidite **4** and alkyne phosphoramidite **7**. (**b**) Crude PCO after lyophilization with Tris. (**c**) Crude PCO after lyophilization in the absence of Tris.
The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

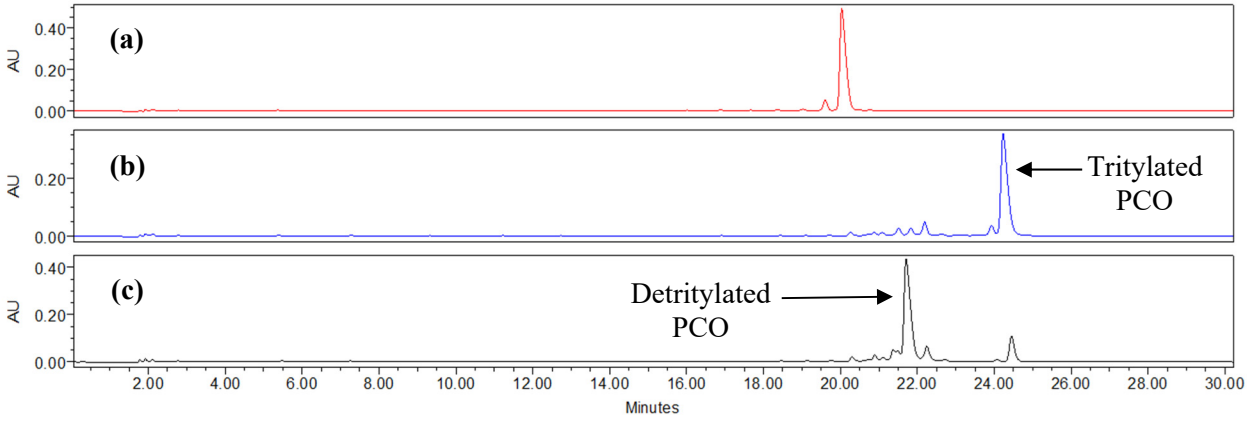


Figure S2: IEX HPLC trace of purified and desalted PCO.
The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl)

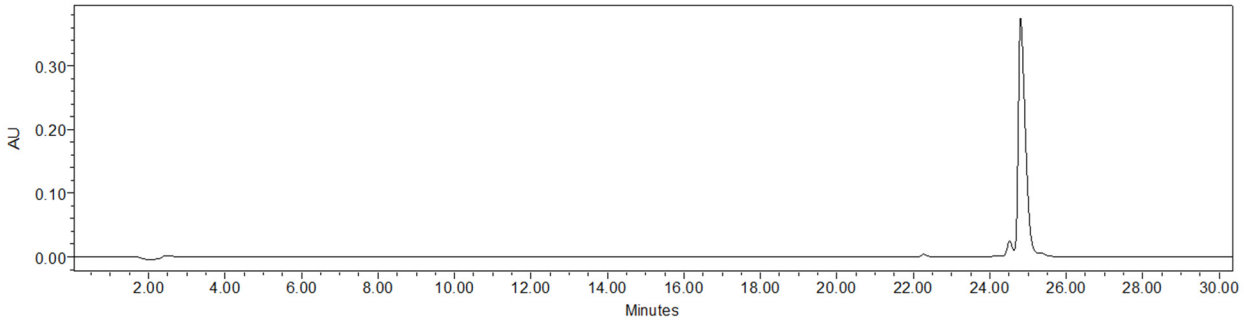


Figure S3: ESI-MS spectrum of PCO (expected mass: 6219.09). *Also observed the DMTr-on alkyne photocleavable dT_{17} sequence (expected mass: 5915.04)

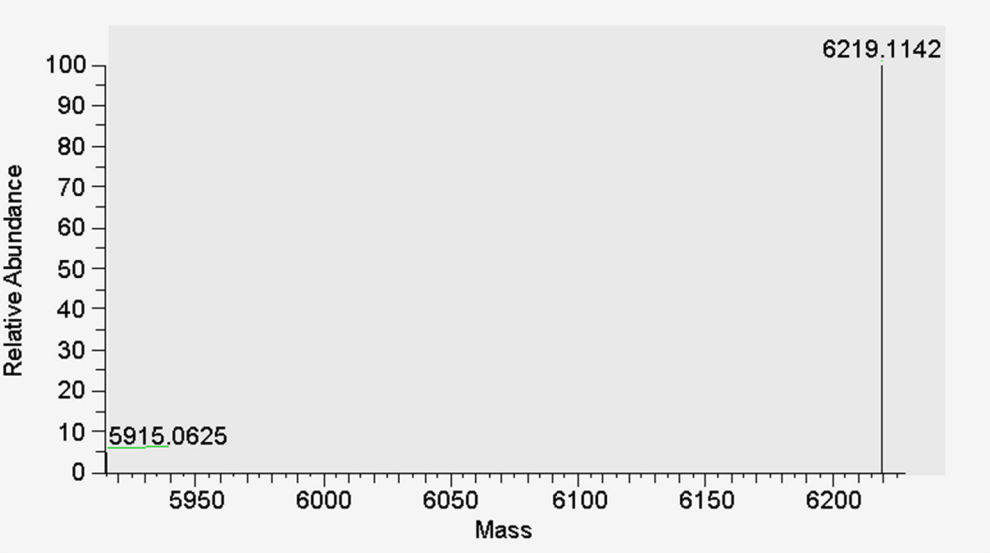


Figure S4: IEX HPLC trace of purified and desalted control alkyne oligonucleotide.

The column was monitored at 260 nm and eluted at room temperature using a linear gradient of 0-52 % buffer B over 30 minutes (buffer A: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile; buffer B: 100 mM Tris HCl, pH 7.5, 10 % acetonitrile, 1 M NaCl).

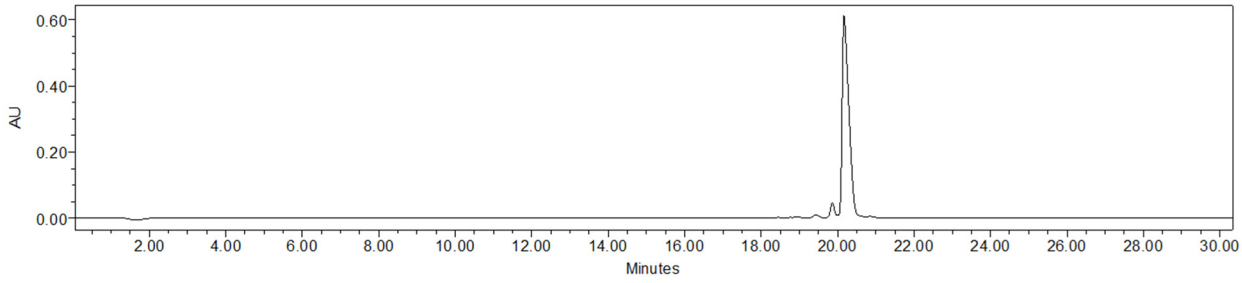


Figure S5: ESI-MS spectrum of control alkyne oligonucleotide (expected mass: 5570.90)

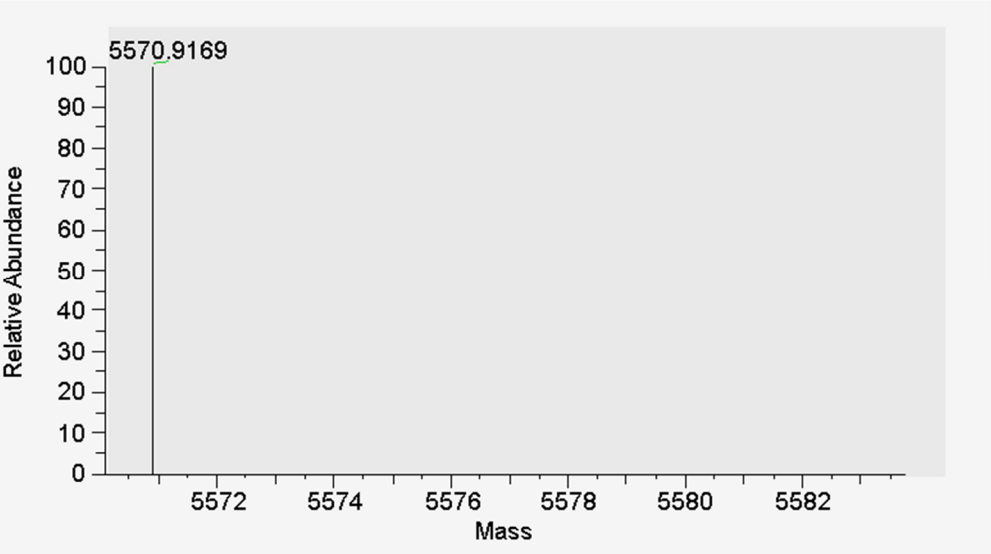


Figure S6: FT-IR spectra of UCNPs. AzSiUCNPs (black trace) and oleate-capped UCNPs (red trace).

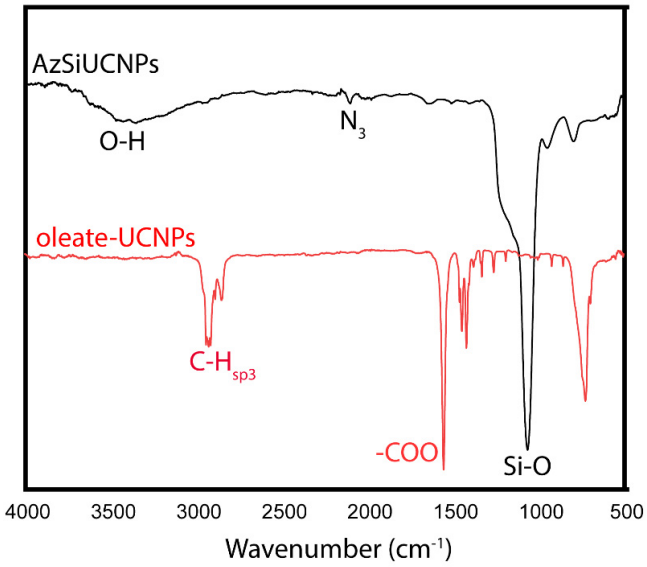


Figure S7: Ninhydrin/Triphenylphosphine assay of UCNPs. Pre-click (left) and post-click (right) reactions to qualitatively examine the presence of azides, evidenced by a yellow color.

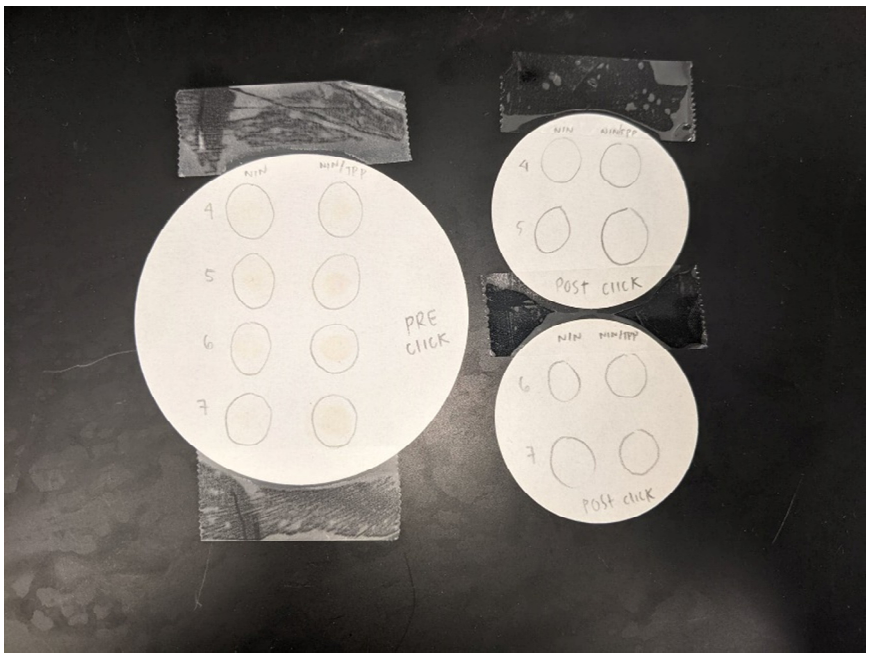
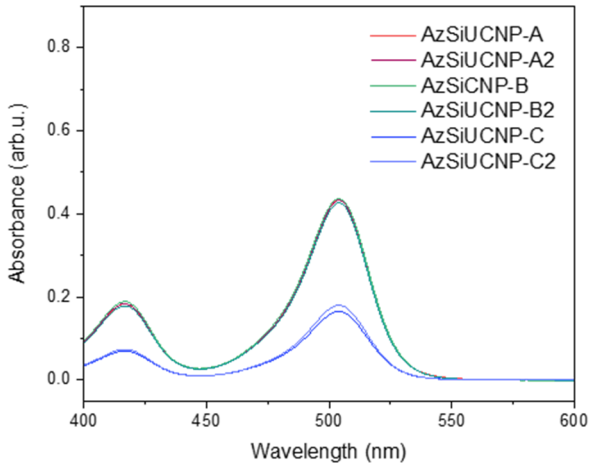


Table S1: Quantitative determination of the number of azide groups per nanoparticle using the trityl assay

Sample	Abs 504	Mass NPs (mg)	Molecules	Loading (µmol/g)	Av. Loading (µmol/g)	#NPs	#DMTr/NP	Av. #DMTr/NP
AzSiUCNP-A	0.43481	0.182	6.89E+15	62.87	60.98	5.98E+11	1.15E+04	**1.12E+04**
AzSiUCNP-A2	0.43341	0.193	6.86E+15	59.09		6.35E+11	1.08E+04	
AzSiUCNP-B	0.43627	0.168	6.91E+15	68.34	63.10	5.52E+11	1.25E+04	**1.16E+04**
AzSiUCNP-B2	0.4266	0.194	6.76E+15	57.86		6.38E+11	1.06E+04	
AzSiUCNP-C	0.16524	0.066	2.62E+15	65.88	71.13	2.17E+11	1.21E+04	**1.30E+04**
AzSiUCNP-C2	0.17998	0.062	2.85E+15	76.39		2.04E+11	1.40E+04	

Figure S8: UV-Visible absorption spectra of the DMTr-dT-UCNPs supernatant after acid treatment. Used to quantify the number of azides per nanoparticle.



Sample calculations for nanoparticle mass and oligonucleotide quantification

Transmission electron microscopy values were used to obtain the static sizes of the nanoparticles, with and without the silica shell. Without the silica shell, the diameter of the nanoparticles along the long axis was 97.5 nm, and 52.3 nm along the short axis. With the silica shell, the long diameter measured 107.2 nm, and the short axis measured 72 nm. These values were used to first calculate the volume of a single nanoparticle with a square bipyramidal morphology, and then the known densities of the materials were used to calculate the nanoparticle mass.

Density[6] SiO_2 : 2.65 g/cm^3

Density[7] $LiYF_4$: 3.97 g/cm^3

$Altitude_{square\ pyramid}$ $2a^2 = c^2$ $a = \sqrt{c^2/2} = 36.98\ nm$ $V_{square\ bipyramid} = \frac{1}{3}Bh$

$B = a^2 = 1367.65\ nm^2$ $h = \frac{NP\ long\ diagonal}{2} = 48.75\ nm$

$$V_{square\ bipyramid} = \frac{2}{3}Bh = 4.45x10^{-17}cm^3 \qquad V_{square\ bipyramid} = \frac{2}{3}Bh = 9.26x10^{-17}cm^3$$

$$V_{silicaNP} - V_{NP} = 4.92x10^{-17}cm^3$$

$$NP\ mass\ (M) = D * V = 1.76x10^{-16}g$$

$$Silica\ mass\ (M) = D * V = 1.276x10^{-16}g$$

$$Total\ mass = 3.04\ x10^{-16}g\ per\ nanoparticle$$

Absorbance of 0.1 mg PCO-UCNP sample using trityl assay: 0.2227

Absorption coefficient of trityl cation: 76 mL cm^{-1} µmol^{-1}

$$\frac{A}{\varepsilon l} = c = 0.00293\ \mu mol\ DMTr = \mu mol\ DNA = 1.76x10^{15}\ molecules\ oligonucleotide$$

$$\frac{Molecules\ DNA}{\left(\frac{total\ mass\ NPs}{mass\ per\ NP}\right)} = 5366\ oligonucleotides/NP$$

Figure S9: Upconversion emission spectra of AzSiUCNPs (black), PCO-UCNPs (red) and CT-UCNPs (blue) to evaluate energy transfer percentages. (1 mg/mL in hexanes, 976 nm excitation)

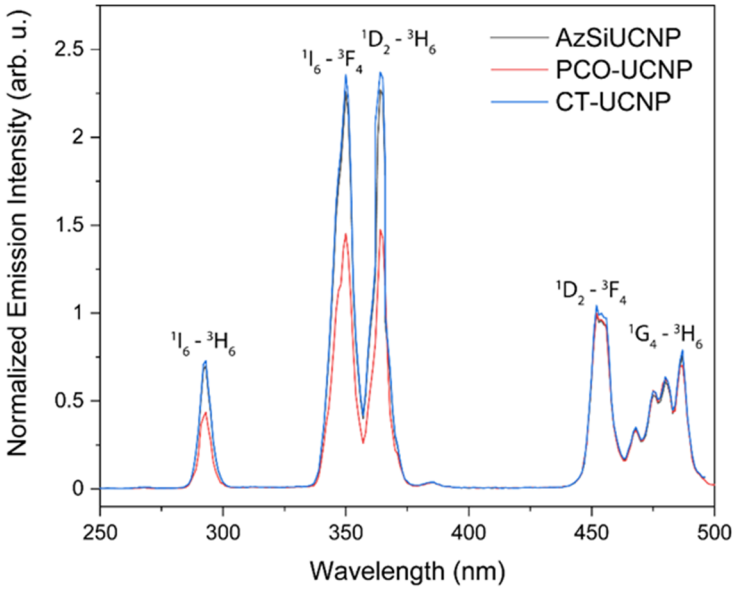


Figure S10: Size distributions of the silica shell at the sides (blue) and the apices (red) of the nanoparticles.

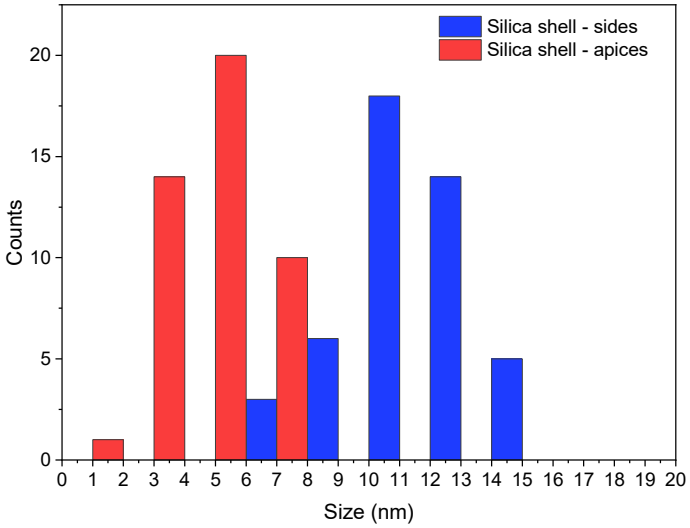
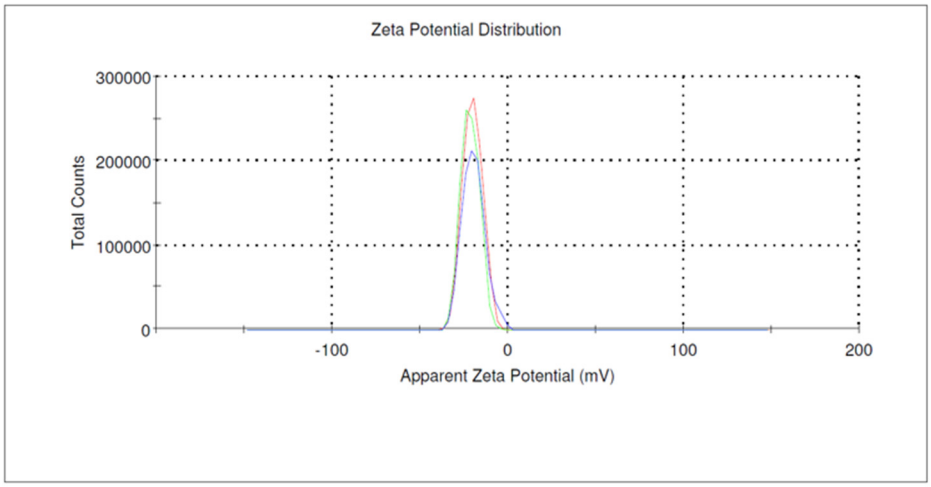


Figure S11: Zeta potential of A) AzSiUCNPs prior to the click reaction and B) of PCO-UCNPs after clicking to the AzSiUCNPs.

A

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -20.3	Peak 1:	-20.3	100.0	5.63
Zeta Deviation (mV): 5.63	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0195	Peak 3:	0.00	0.0	0.00
Result quality : Good				



B

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -33.3	Peak 1:	-33.3	100.0	5.62
Zeta Deviation (mV): 5.62	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0174	Peak 3:	0.00	0.0	0.00
Result quality : Good				

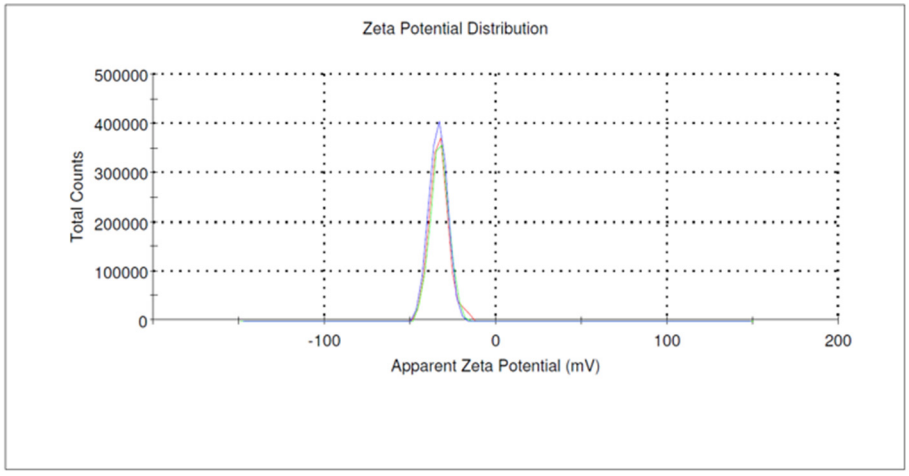


Figure S12: Denaturing PAGE analysis of PCO-UCNP and CT-UCNP samples after irradiation. Lane 1: PCO; Lane 2: dT_{18}; Lane 3: PCO-UCNP after 976 nm excitation for 75 minutes; Lane 4: CT-UCNP after 976 nm excitation for 75 minutes; Lane 5: control alkyne oligonucleotide.

Figure S13: ESI-MS spectrum of photoreleased 5'-phosphate dT_{18} oligonucleotide (expected mass: 5490.84)

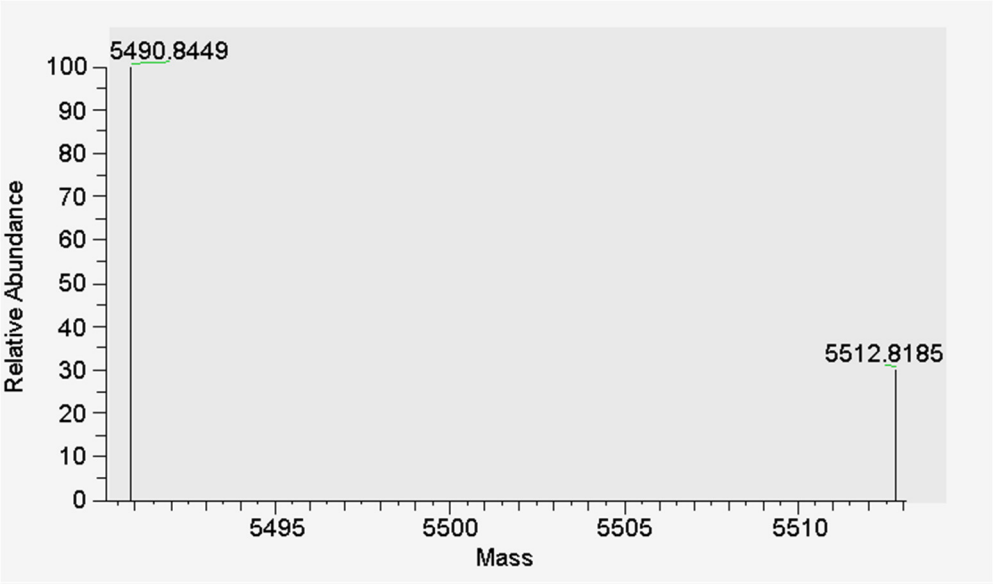


Figure S14: 500 MHz ^{1}H NMR spectrum of crude diol **2** without TMS blocking (in $CDCl_3$)

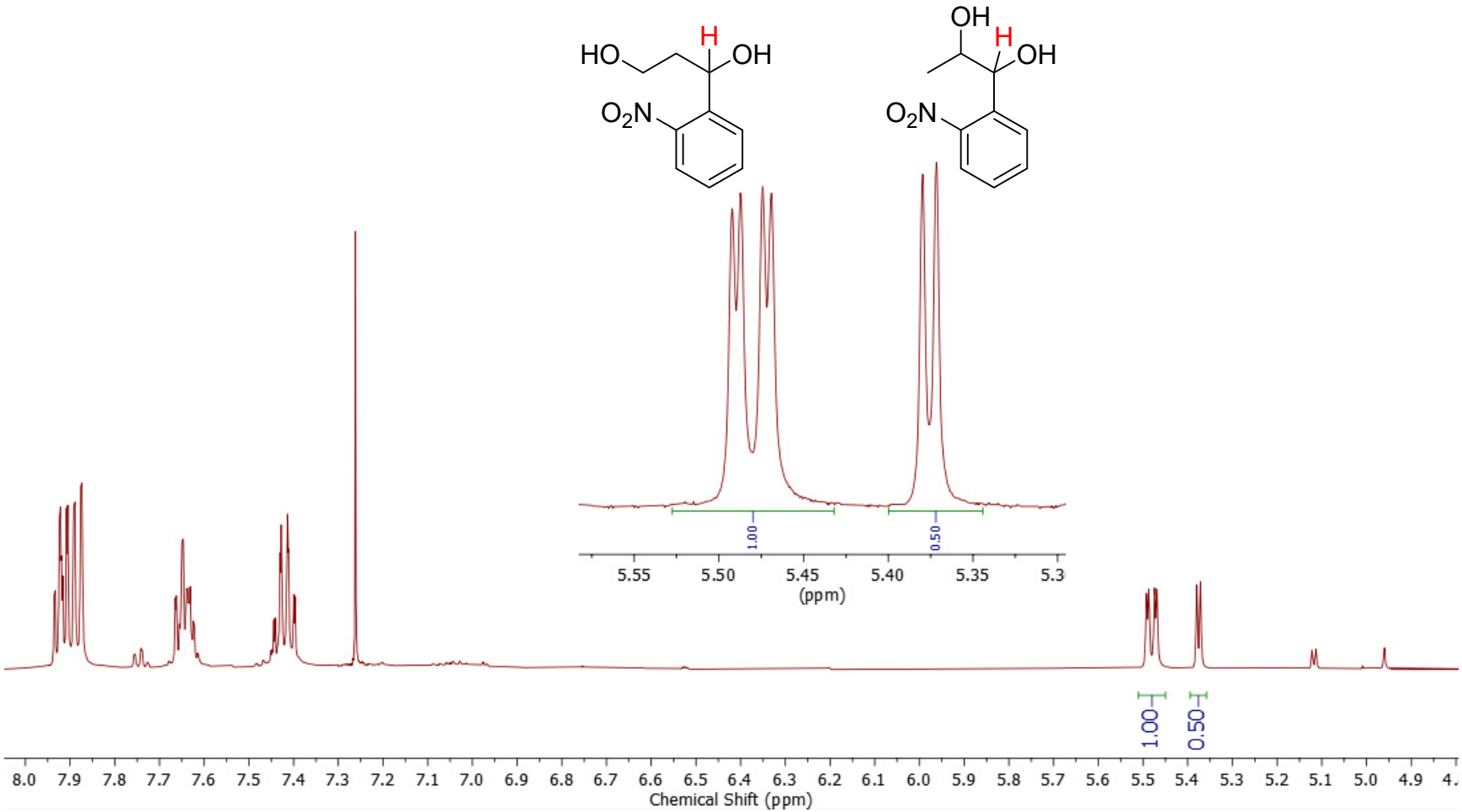


Figure S15: 500 MHz ^{1}H NMR spectrum of crude diol **2** with TMS blocking (in $CDCl_3$)

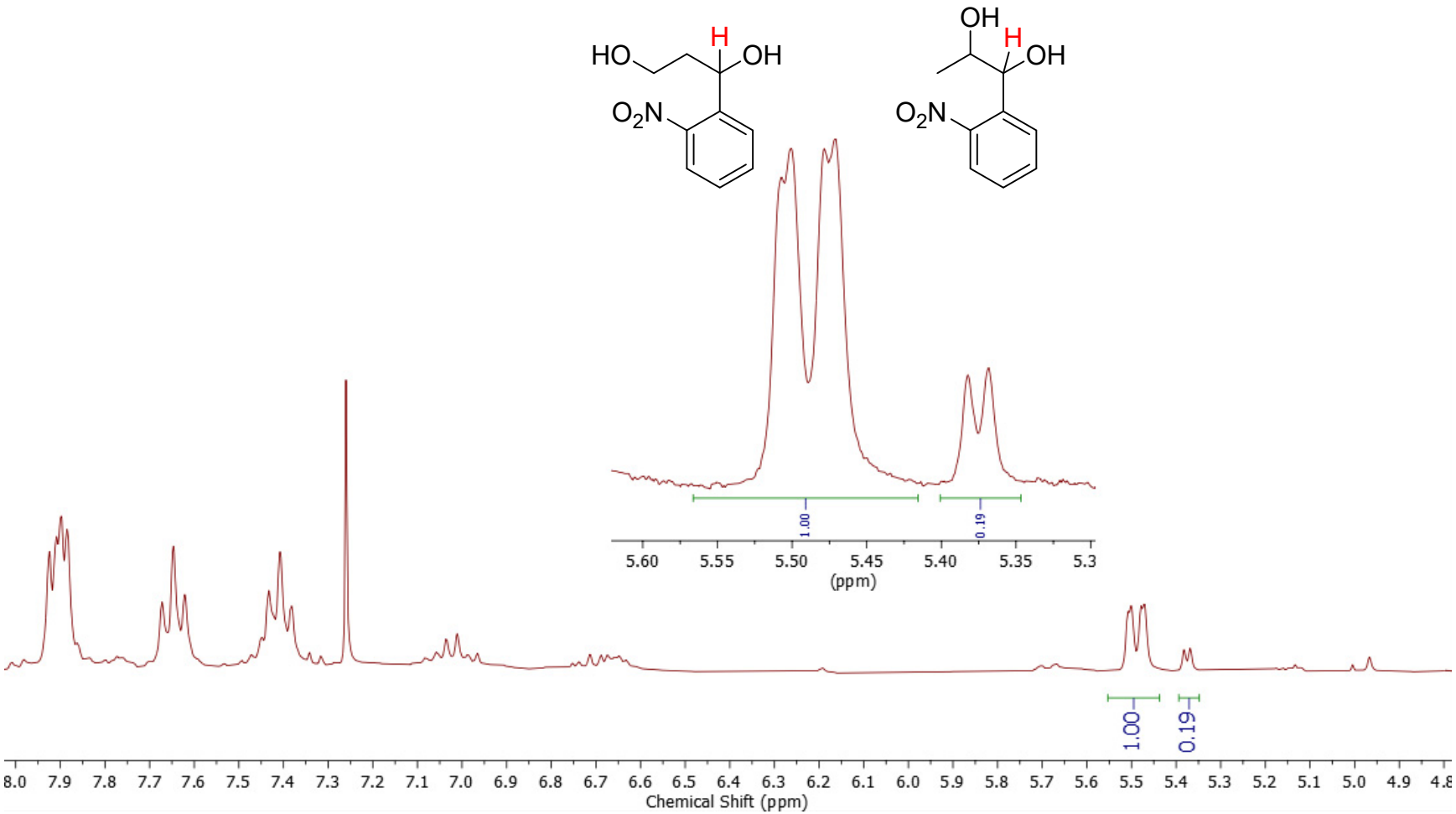


Figure S16: 500 MHz ^{1}H NMR spectrum of allylic alcohol **1** (in $CDCl_3$)

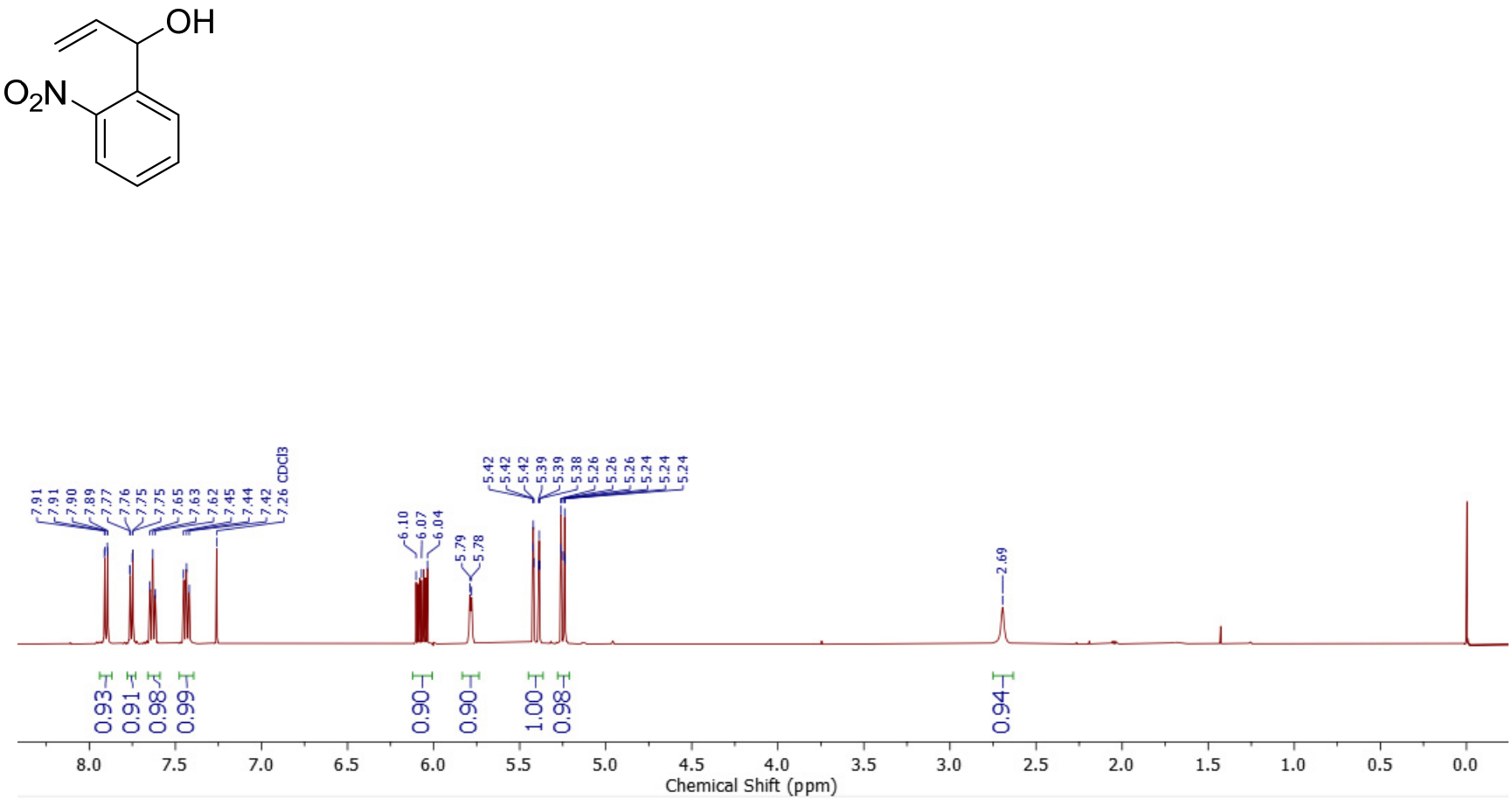


Figure S17: 125.7 MHz ^{13}C NMR spectrum of allylic alcohol **1** (in $CDCl_3$)

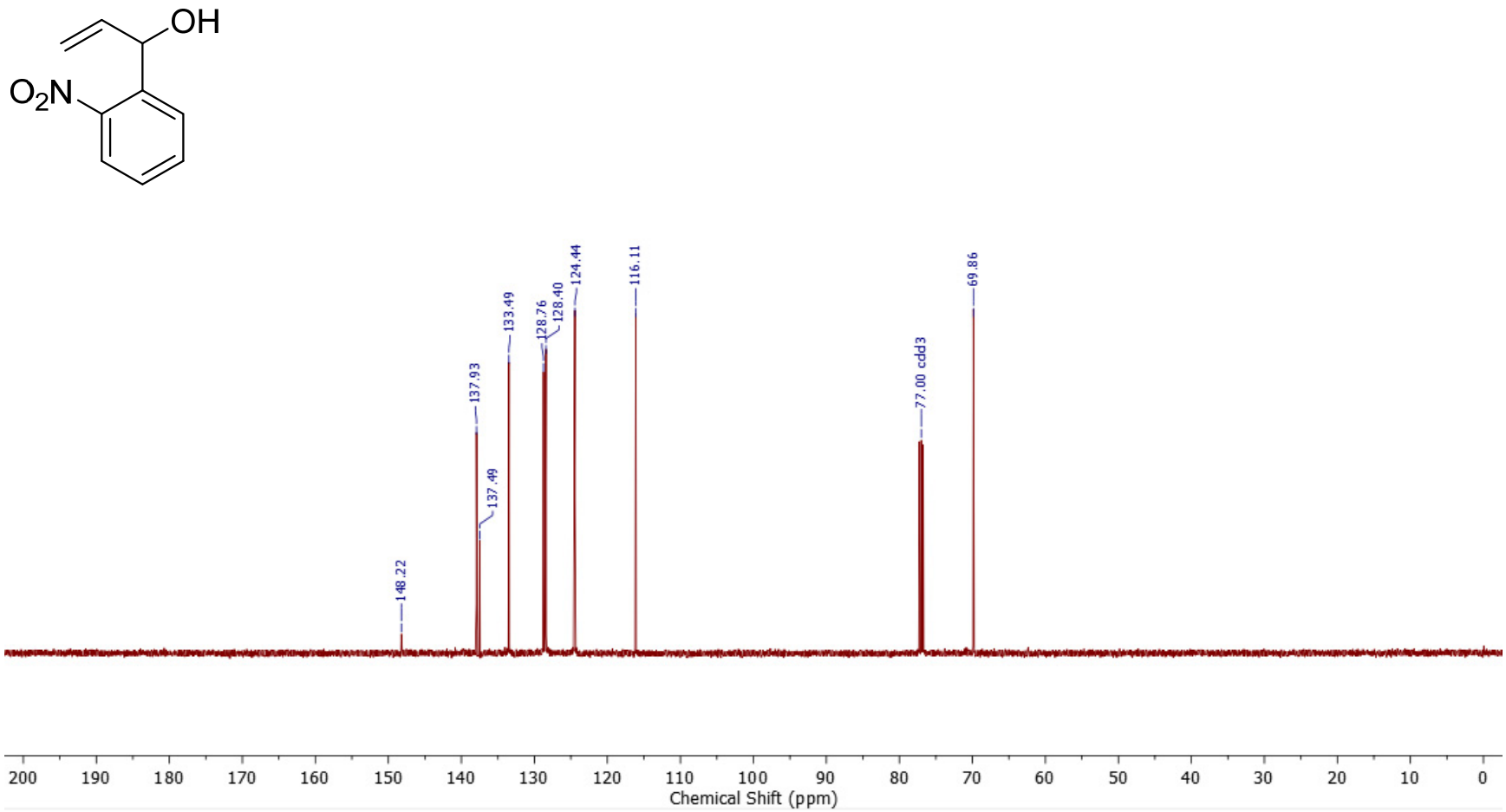


Figure S18: 500 MHz ^{1}H NMR spectrum of diol **2** (in $CDCl_3$)

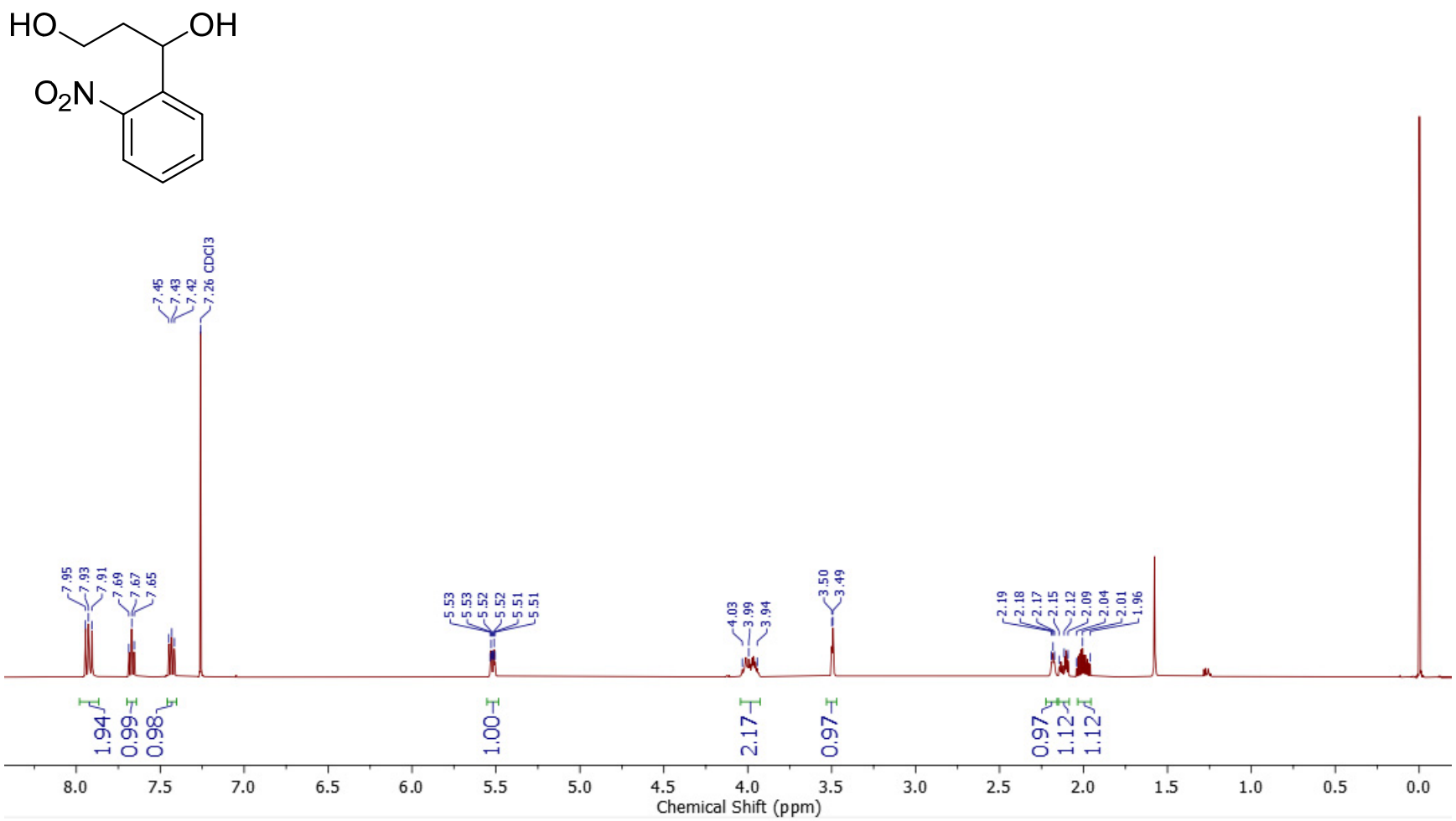


Figure S19: 125.7 MHz ^{13}C NMR spectrum of diol **2** (in $CDCl_3$)

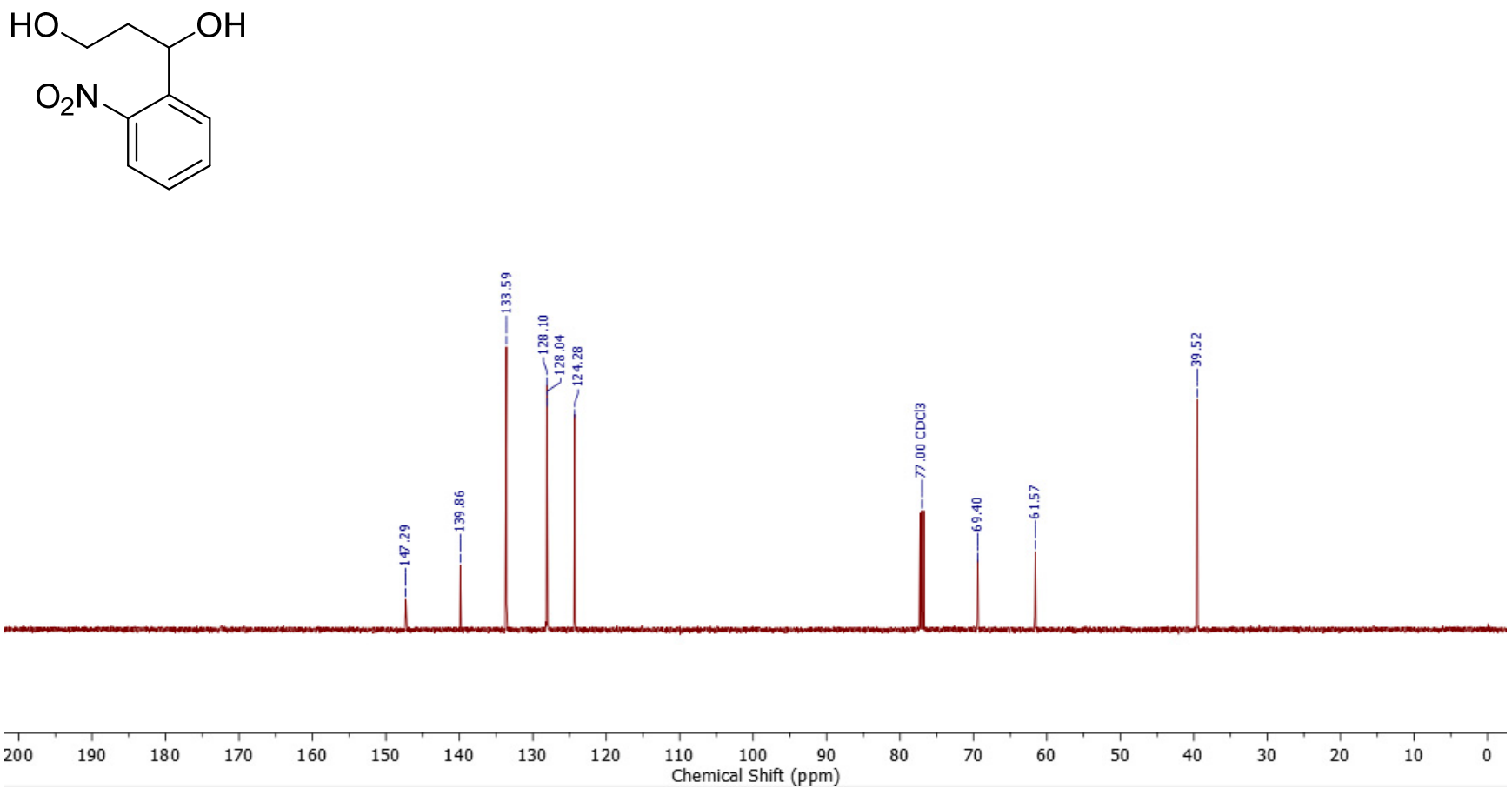
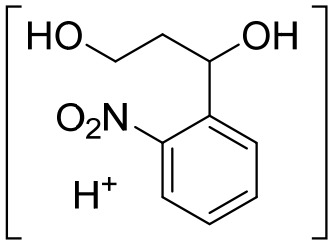


Figure S20: HR ESI-MS spectrum of diol **2** (expected mass: 198.0761)



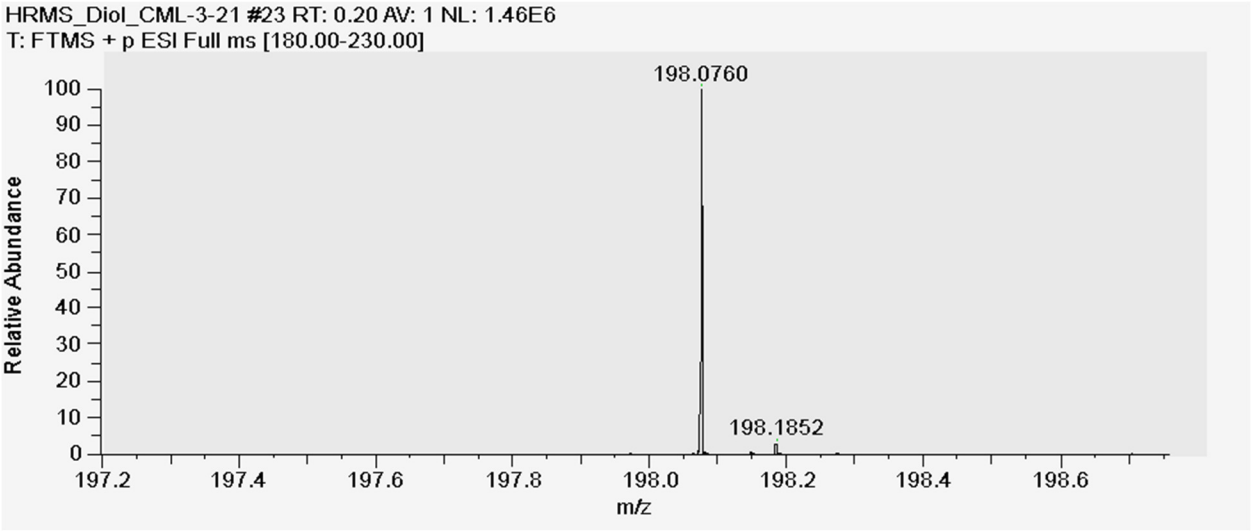


Figure S21: 500 MHz ^{1}H NMR spectrum of pre-amidite **3** (in $CDCl_3$)

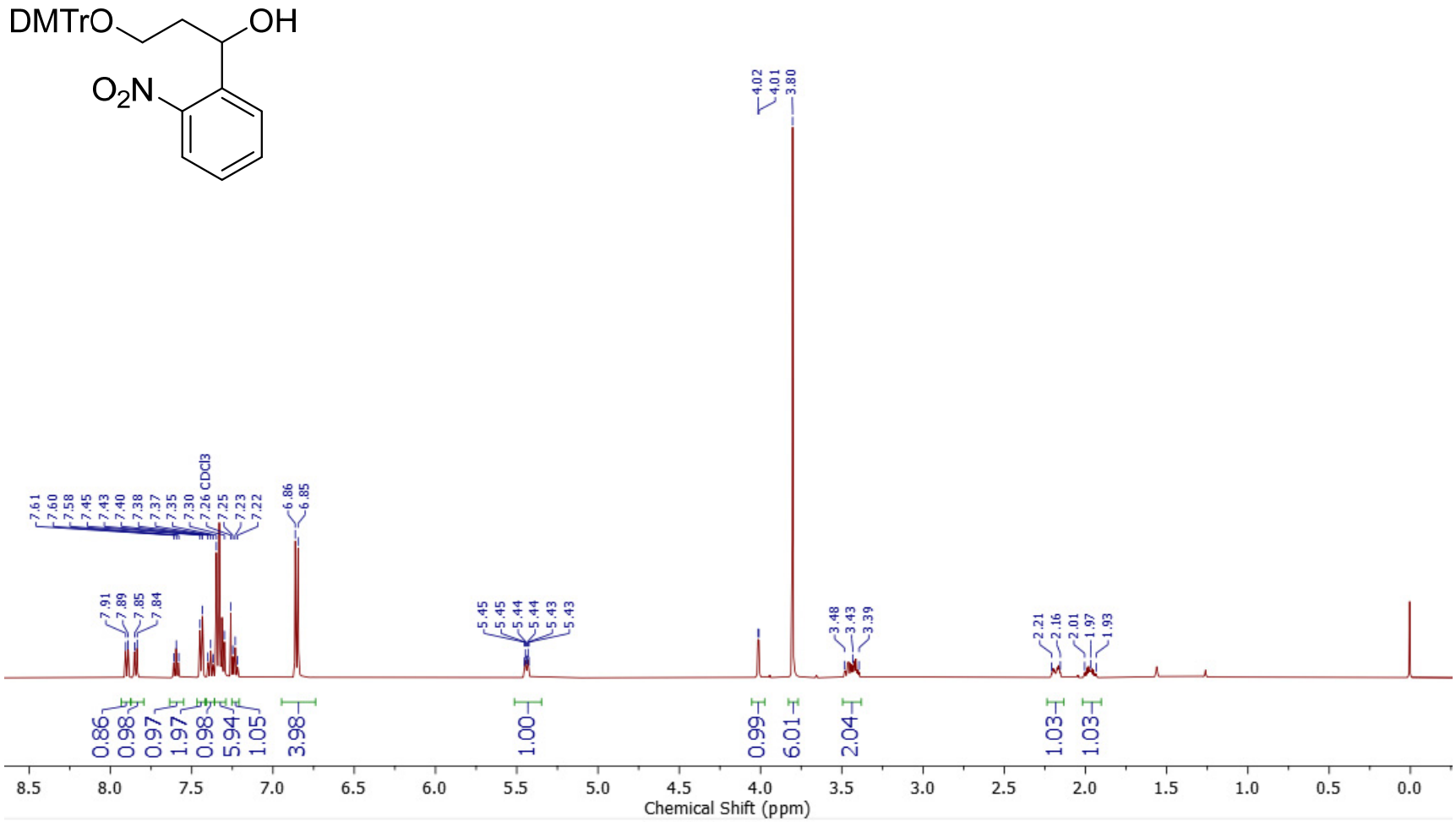


Figure S22: 125.7 MHz ^{13}C NMR spectrum of pre-amidite **3** (in $CDCl_3$)

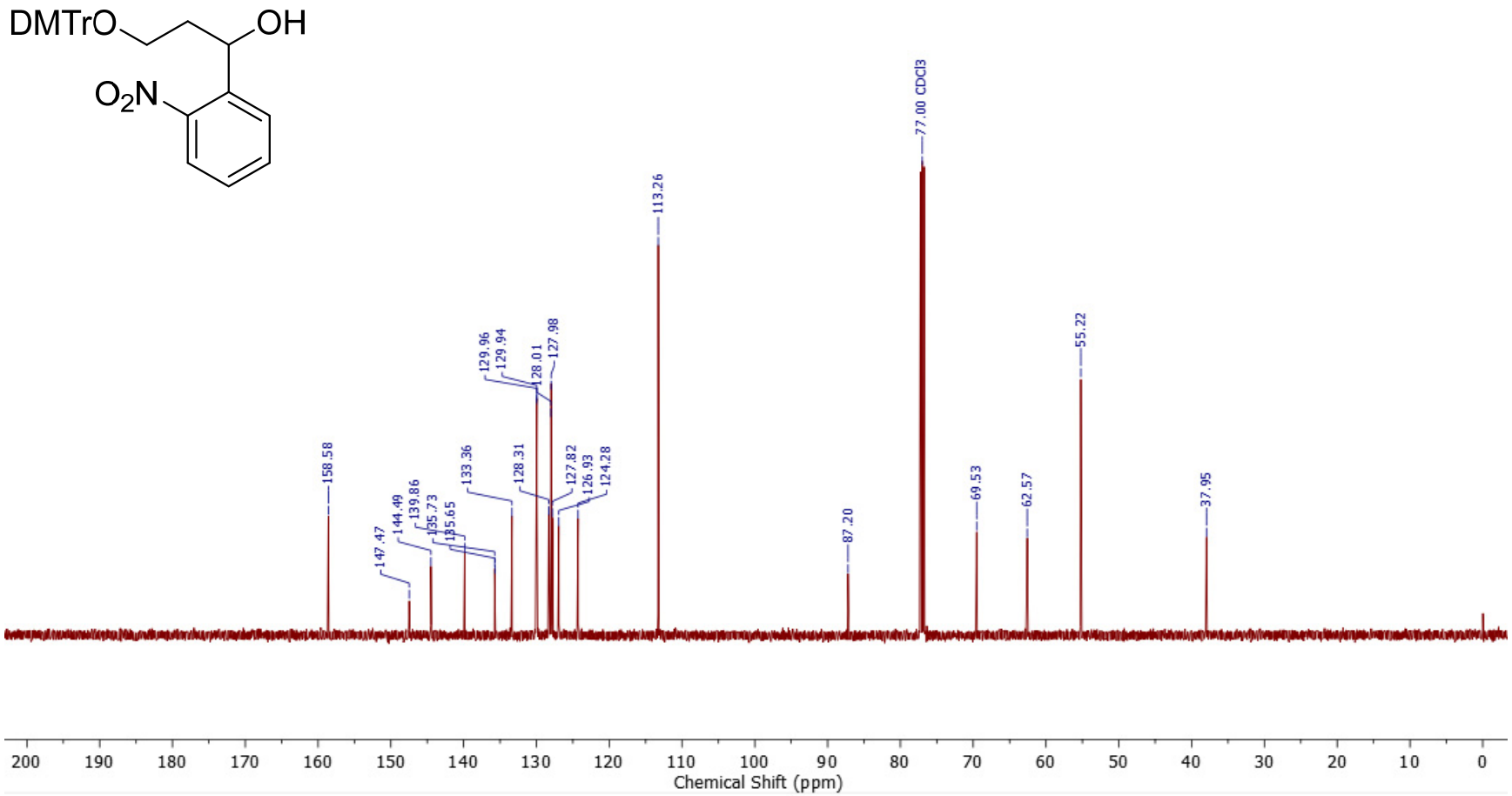
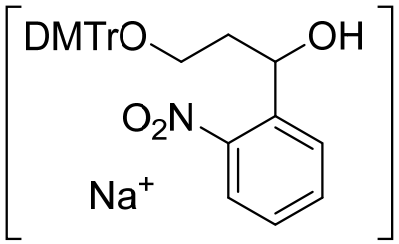


Figure S23: HR ESI-MS spectrum of pre-amidite **3** (expected mass: 522.1887)



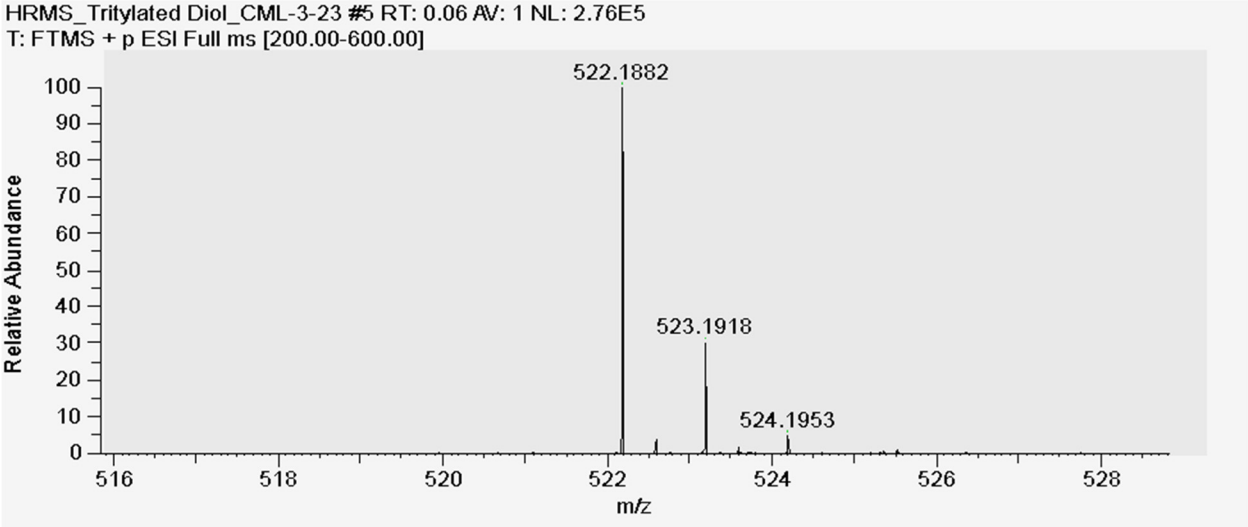


Figure S24: 500 MHz ^{1}H NMR spectrum of photocleavable phosphoramidite **4** (in acetone-d_6)

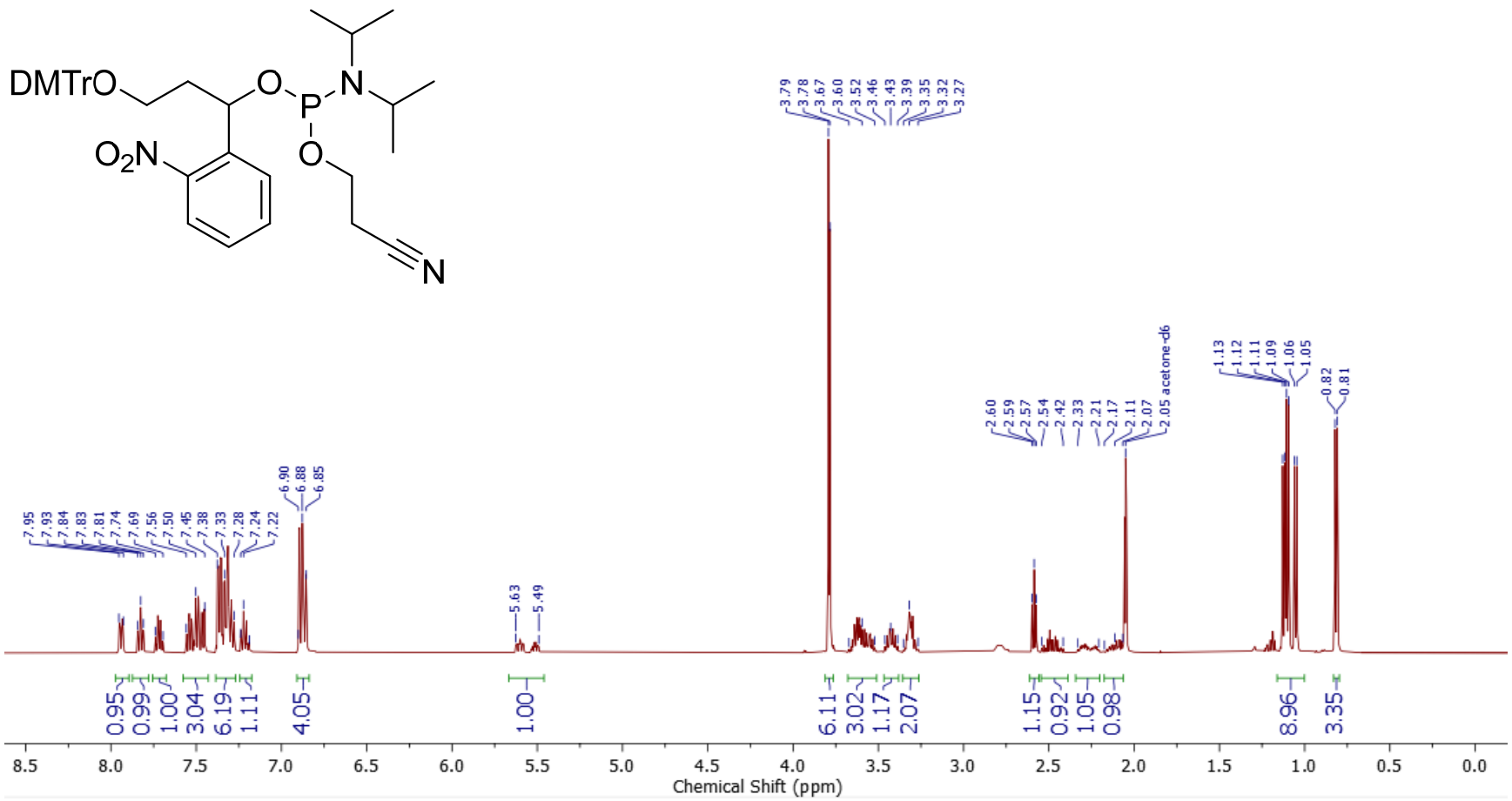


Figure S25: 125.7 MHz ^{13}C NMR spectrum of photocleavable phosphoramidite **4** (in acetone-d_6)

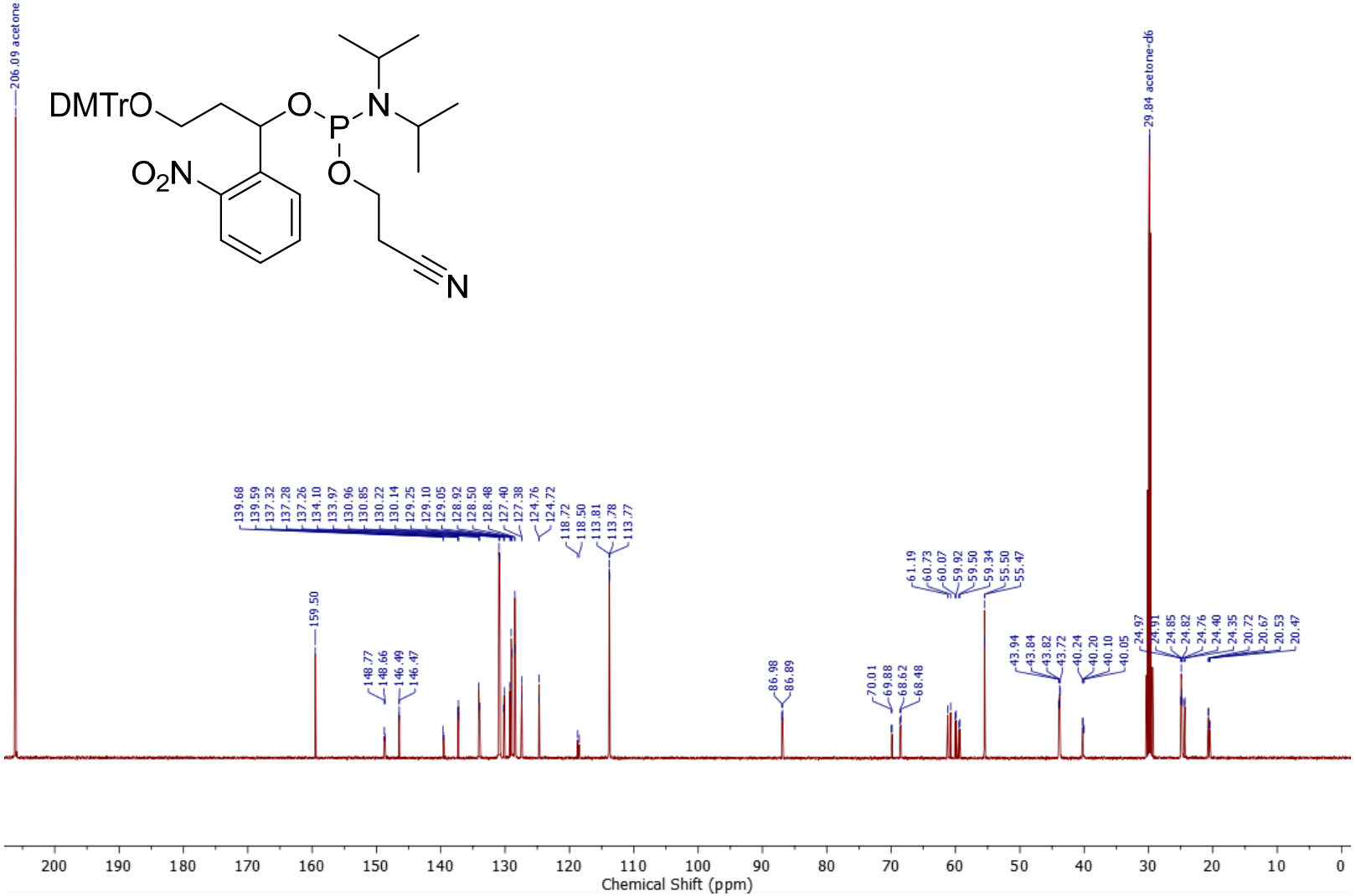


Figure S26: 202.3 MHz ^{31}P NMR spectrum of photocleavable phosphoramidite **4** (in acetone-d_6)

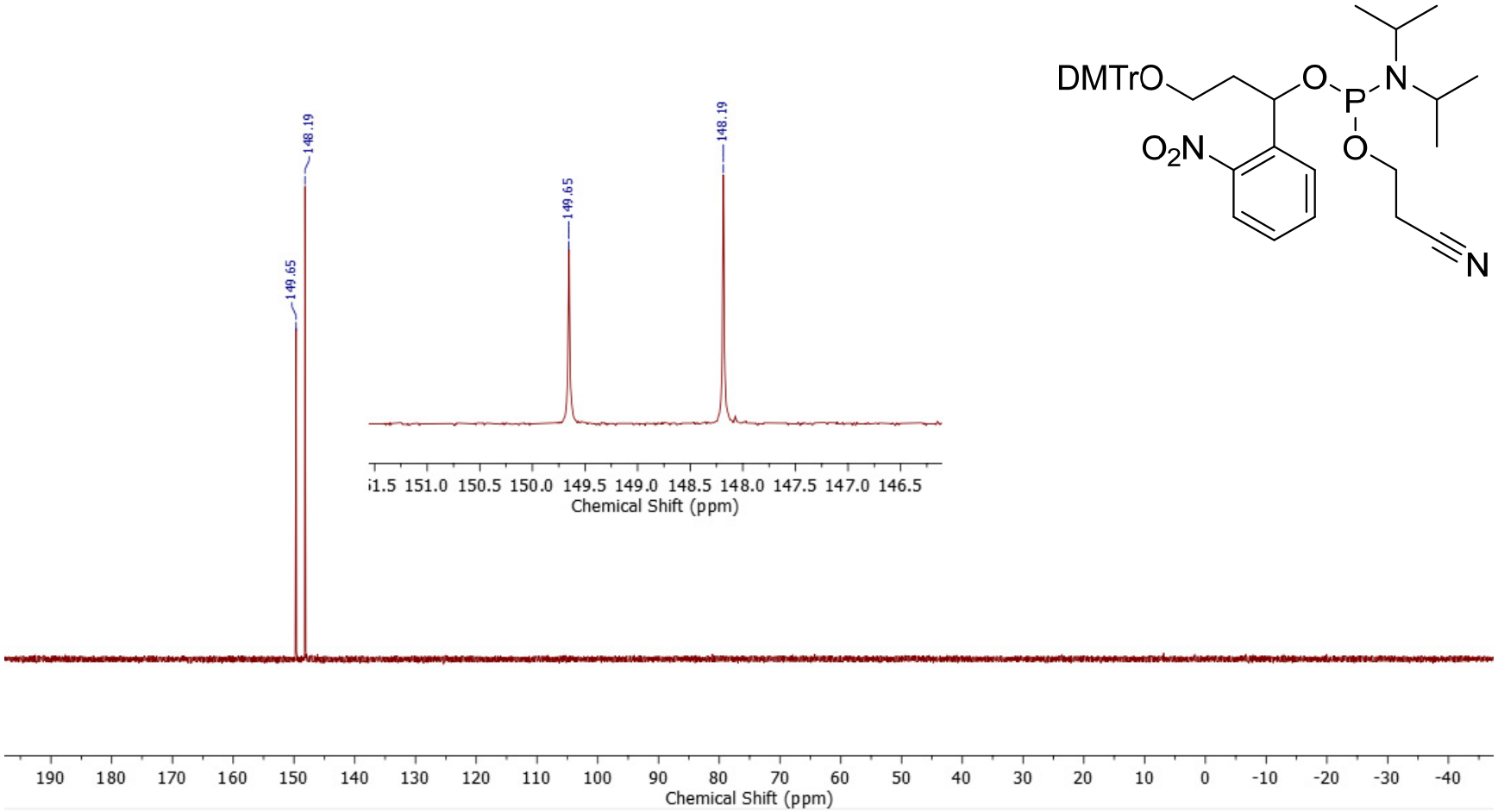
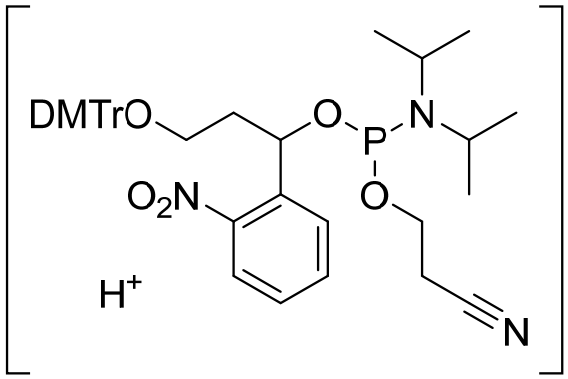


Figure S27: HR ESI-MS spectrum of photocleavable phosphoramidite **4** (expected mass: 700.3146)



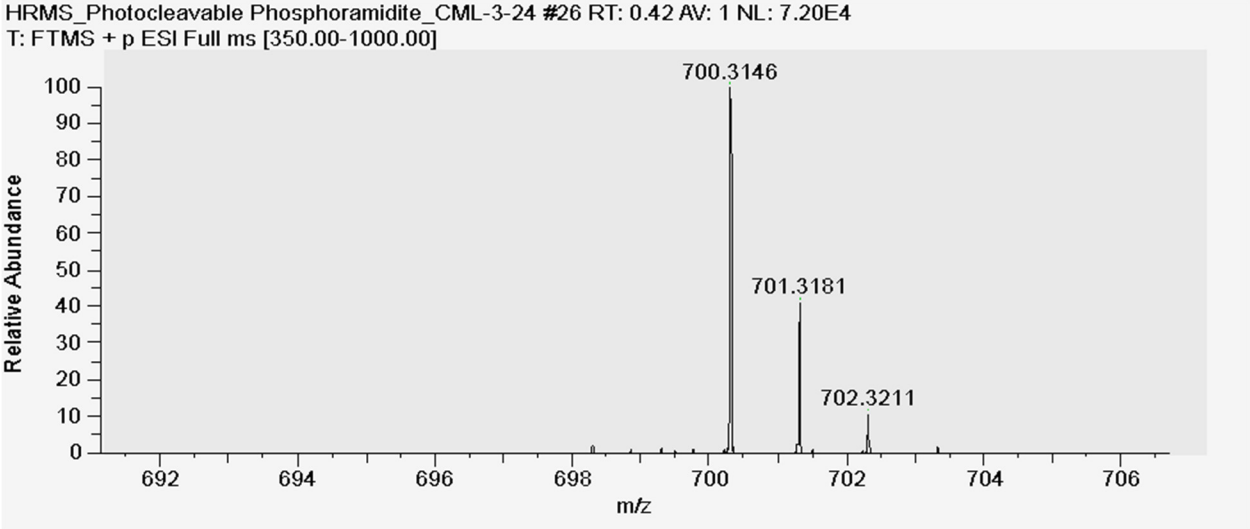


Figure S28: 500 MHz ^{1}H NMR spectrum of amide **5** (in DMSO-d_6)

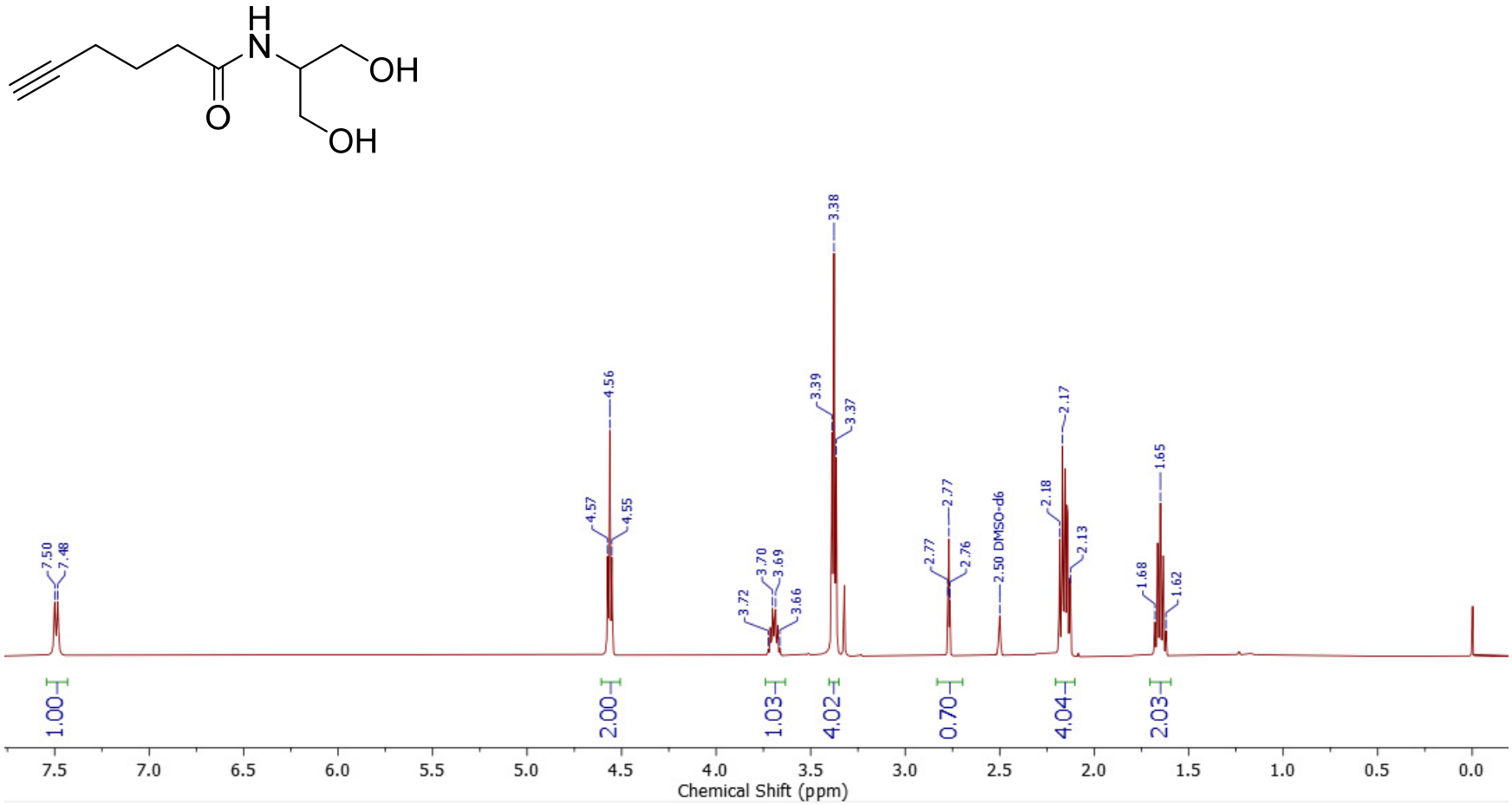


Figure S29: 125.7 MHz ^{13}C NMR spectrum of amide **5** (in DMSO-d_6)

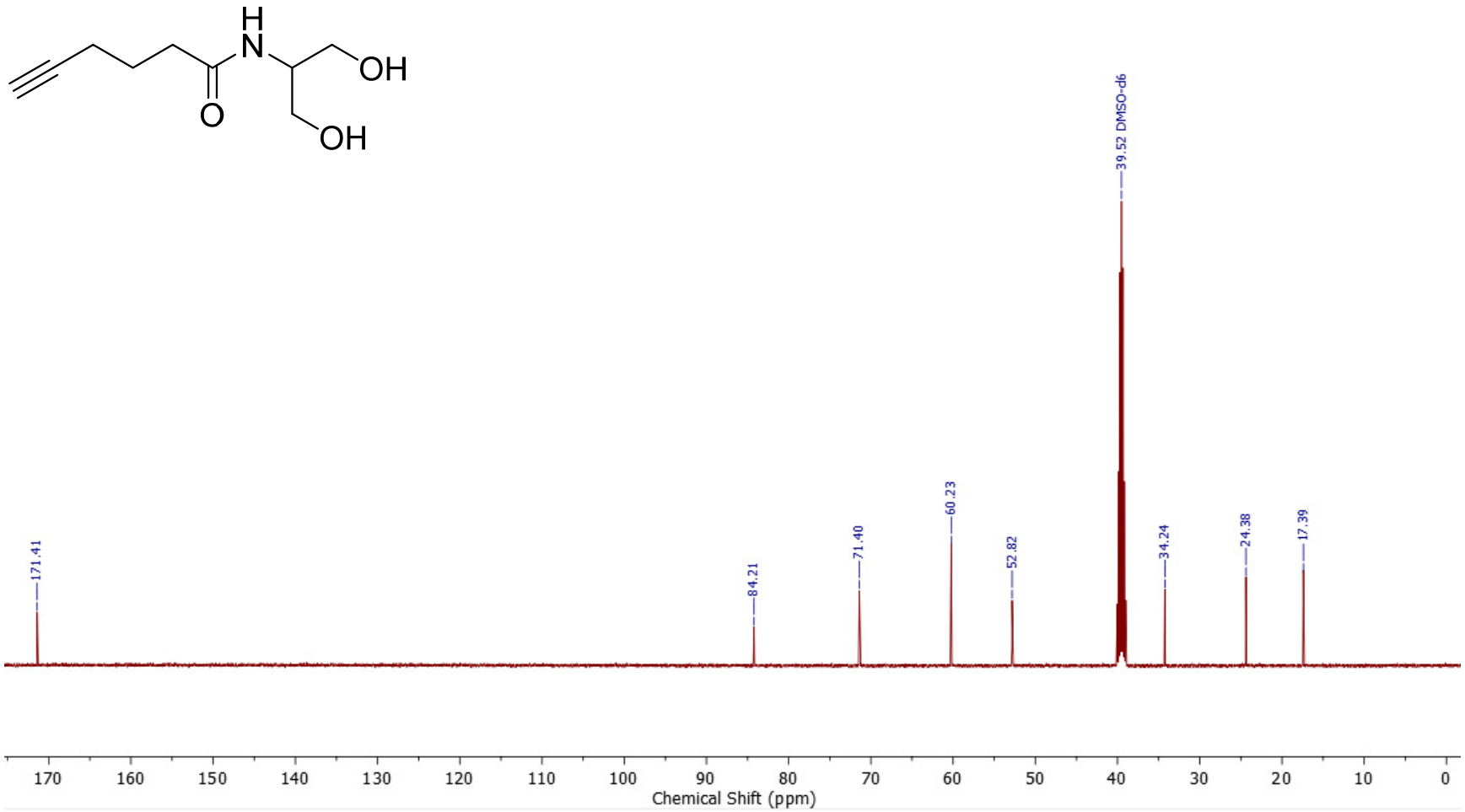
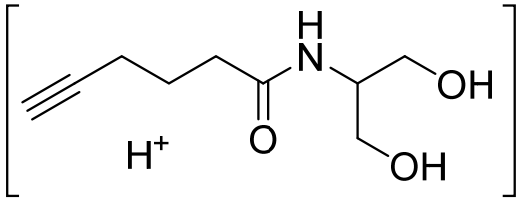


Figure S30: HR ESI-MS spectrum of amide **5** (expected mass: 186.1125)



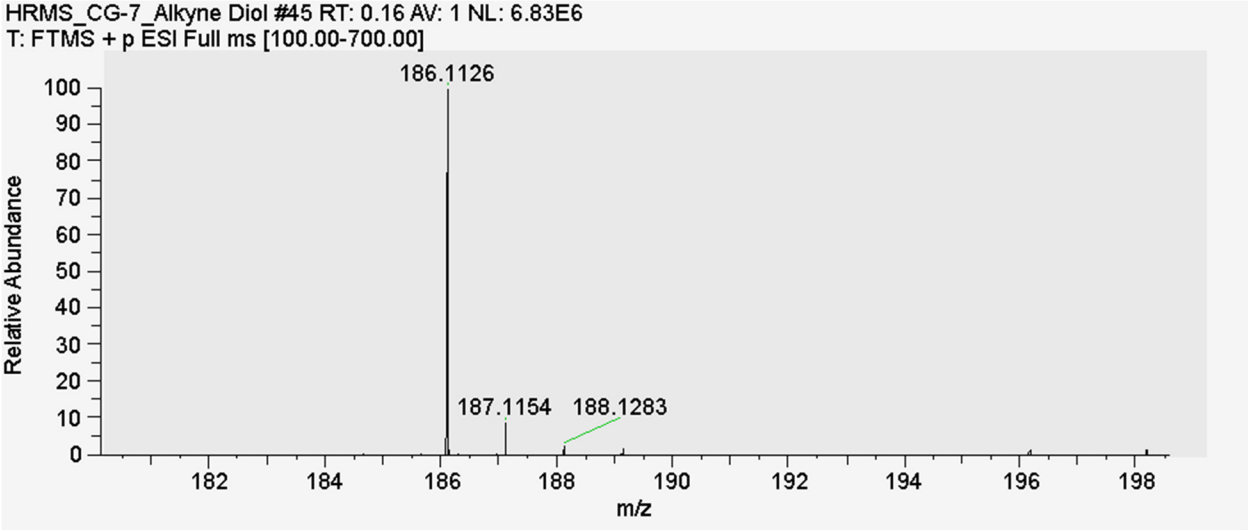


Figure S31: 500 MHz ^{1}H NMR spectrum of pre-amidite **6** (in $CDCl_3$)

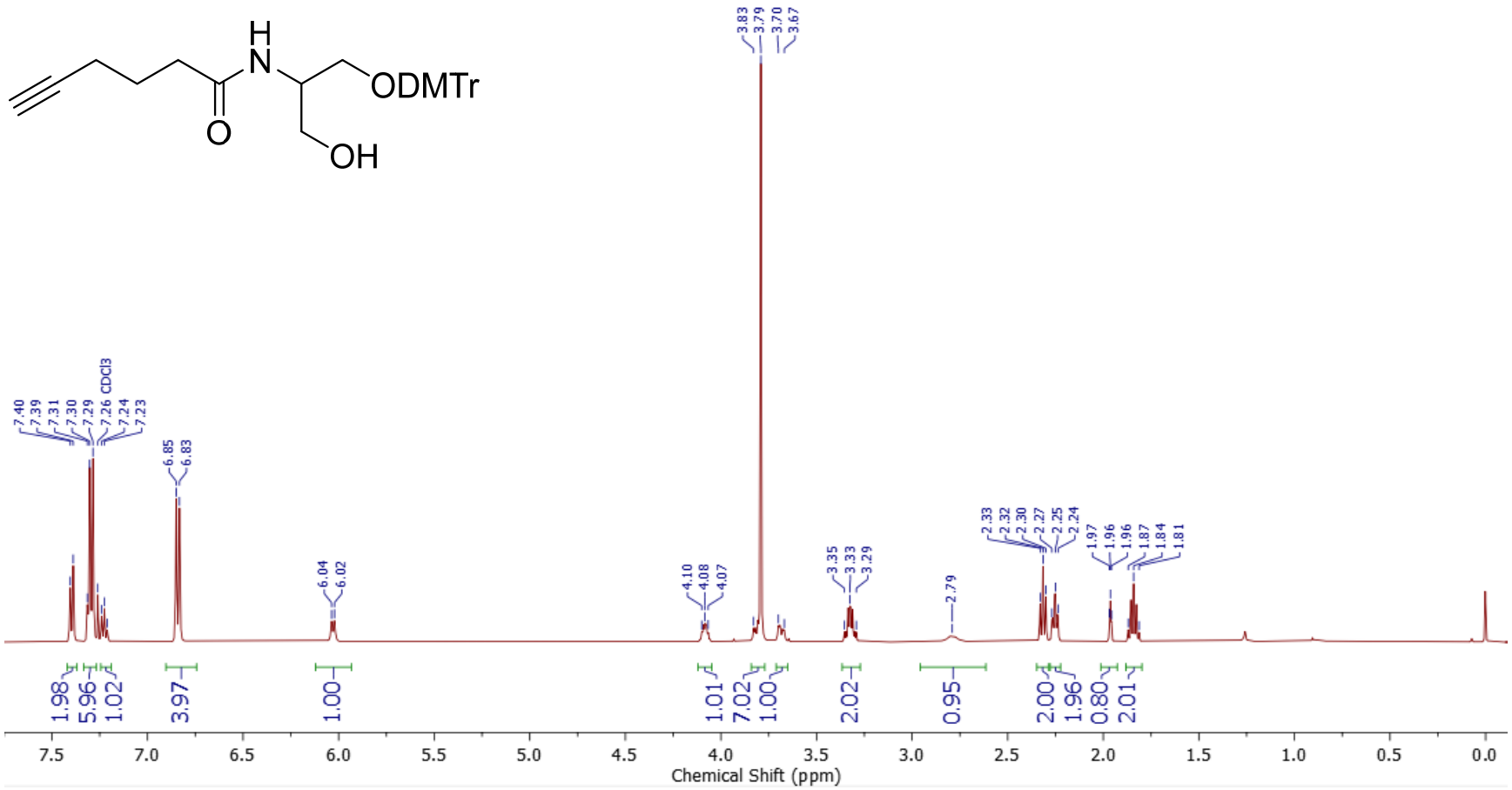


Figure S32: 125.7 MHz ^{13}C NMR spectrum of pre-amidite **6** (in $CDCl_3$)

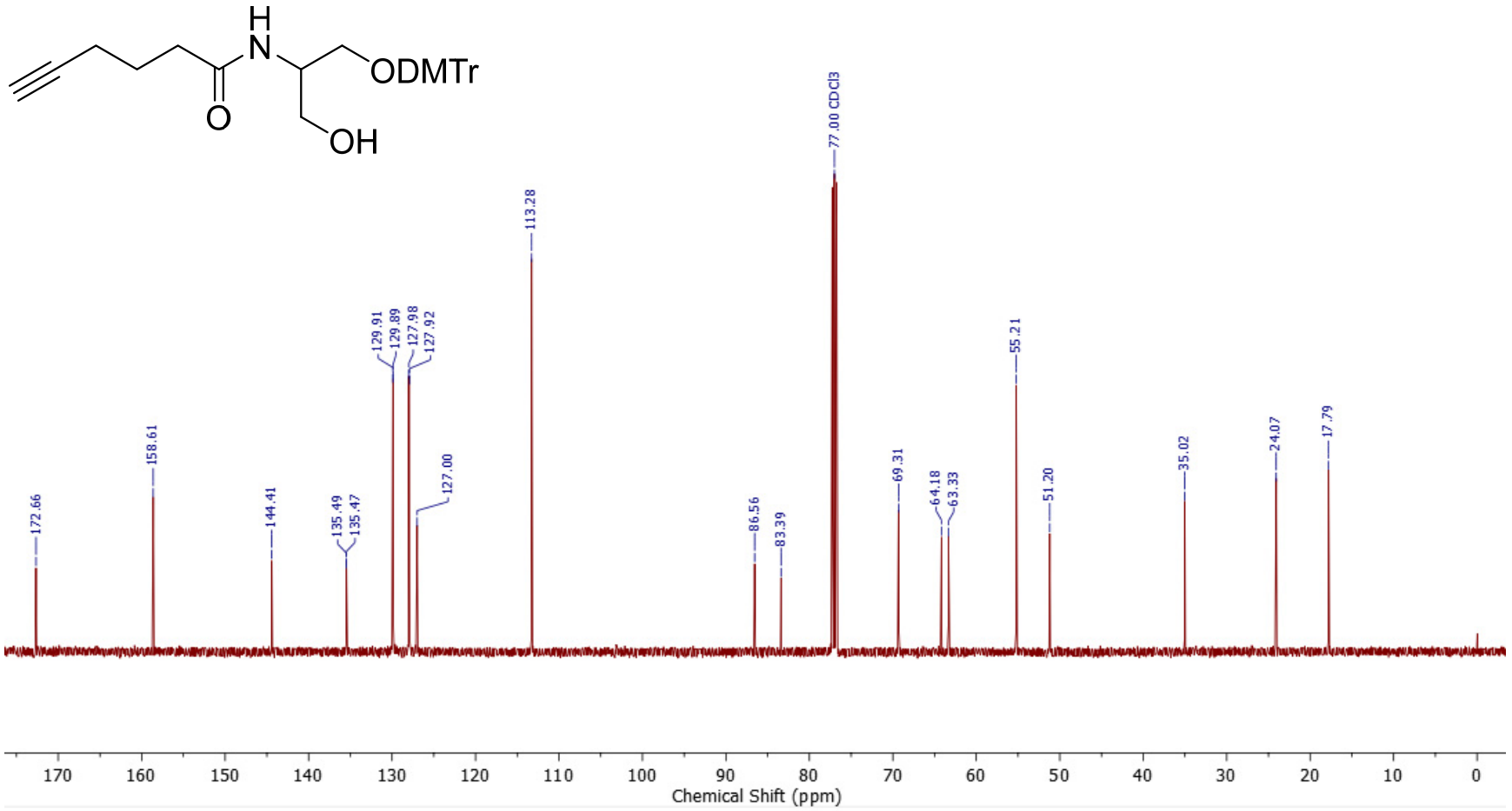
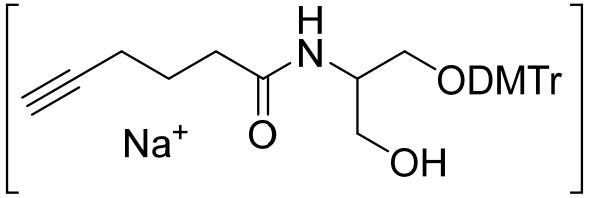


Figure S33: HR ESI-MS spectrum of pre-amidite **6** (expected mass: 510.2251)



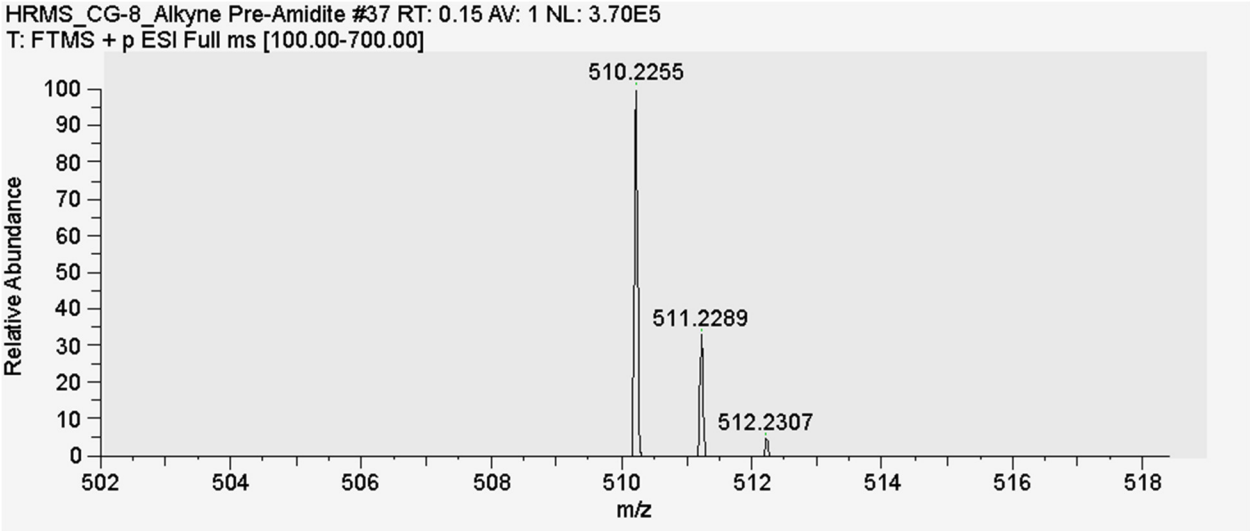


Figure S34: 500 MHz ^{1}H NMR spectrum of alkyne phosphoramidite **7** (in acetone-d_6)

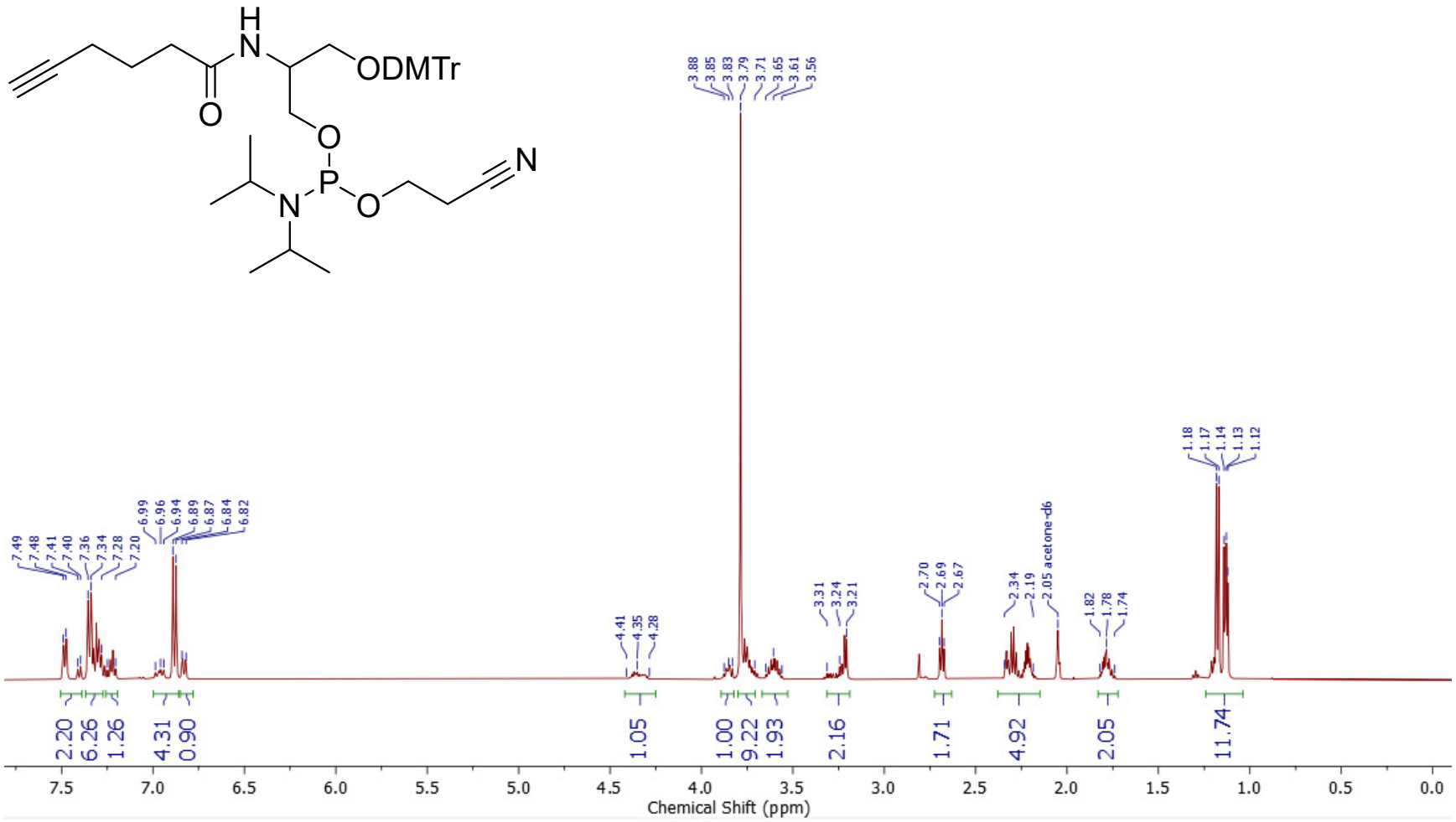


Figure S35: 125.7 MHz ^{13}C NMR spectrum of alkyne phosphoramidite **7** (in acetone-d$_6$)

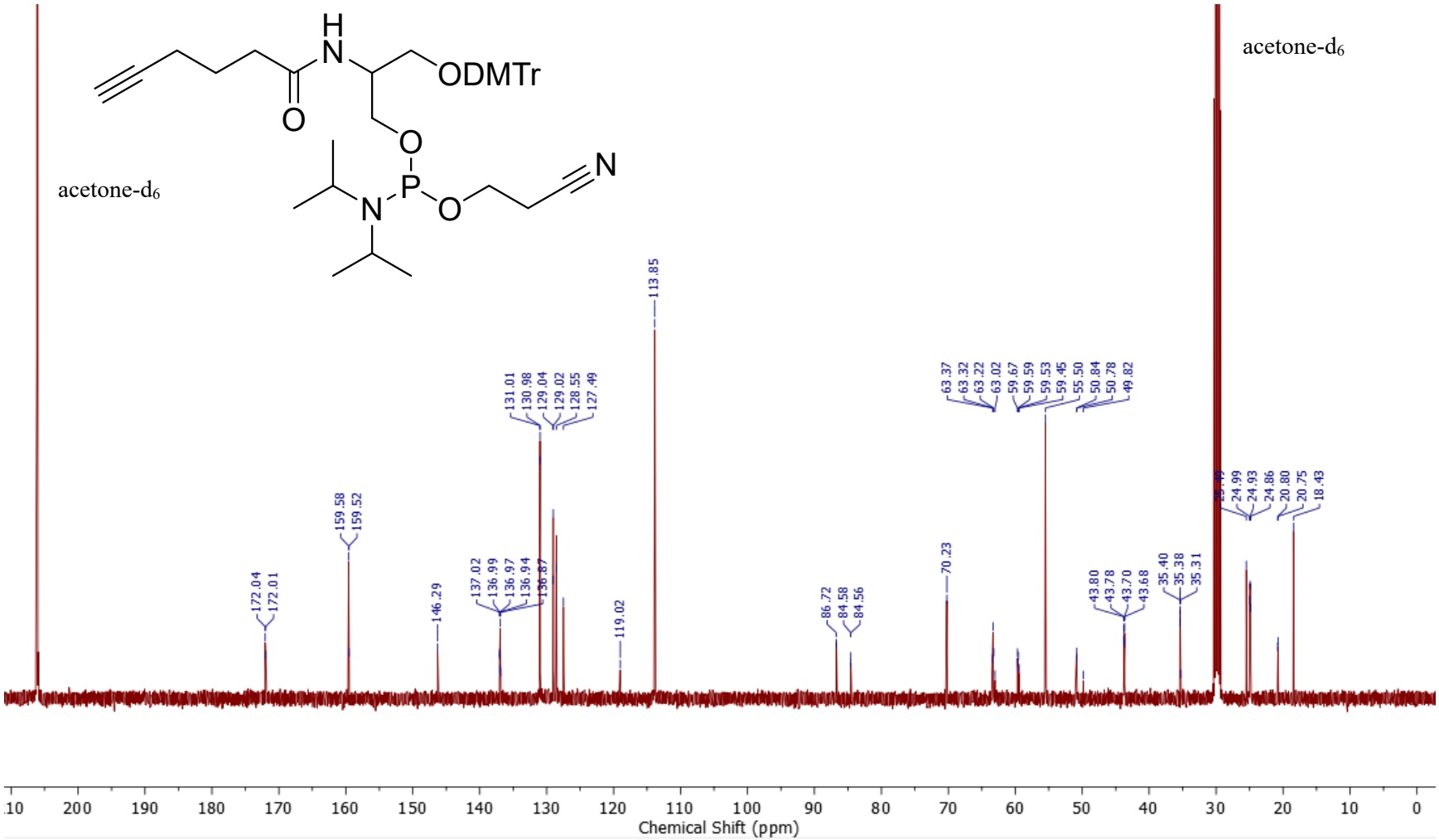


Figure S36: 202.3 MHz ^{31}P NMR spectrum of alkyne phosphoramidite **7** (in acetone-d_6)

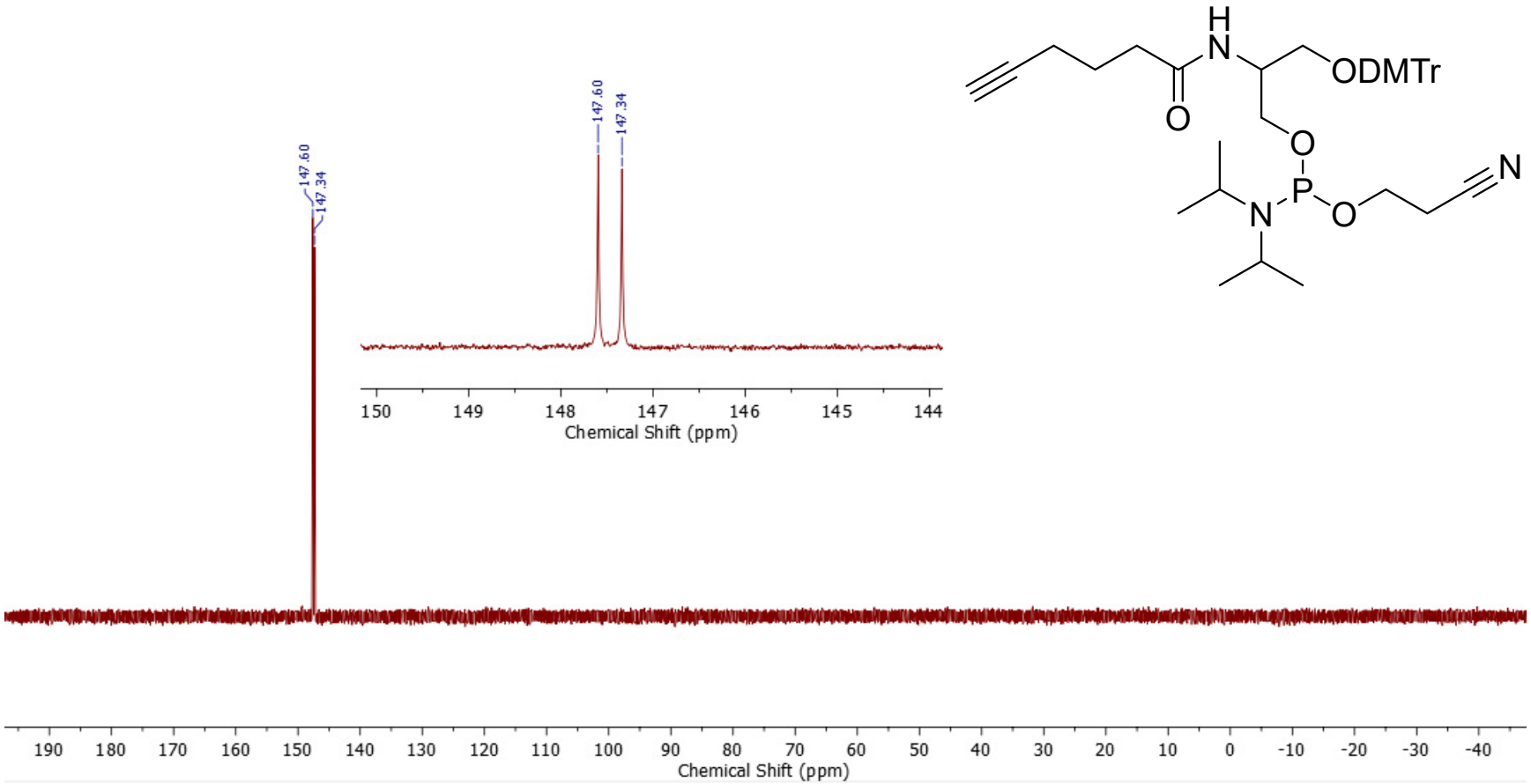
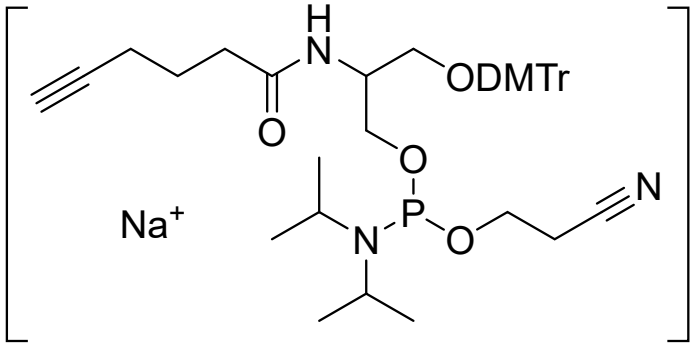


Figure S37: HR ESI-MS spectrum of alkyne phosphoramidite **7** (expected mass: 710.3329)



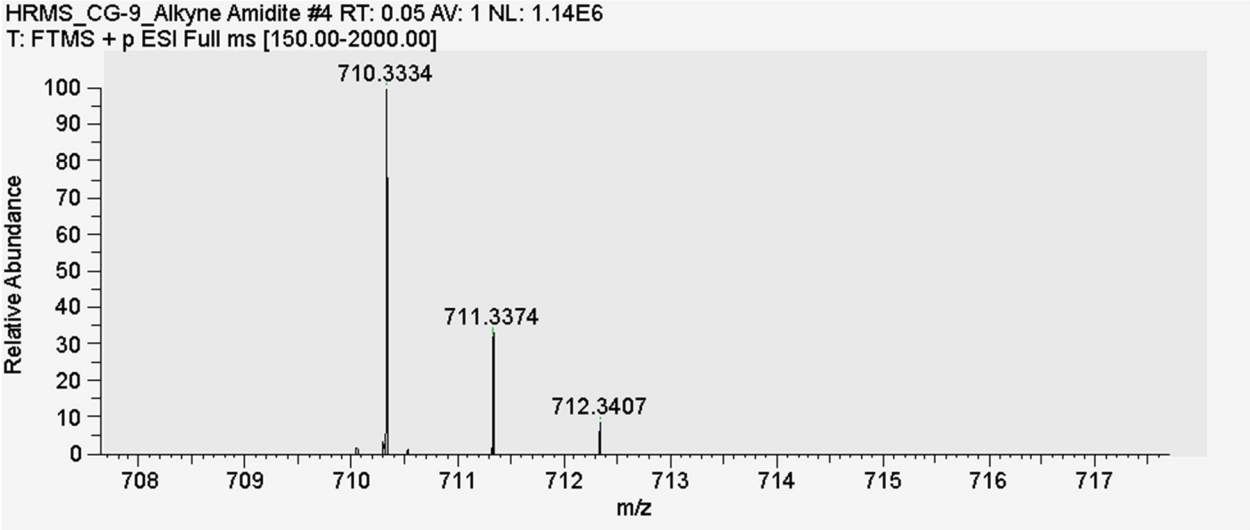


Figure S38: 500 MHz ^{1}H NMR spectrum of control alkyne phosphoramidite **8** (in acetone-d_6)

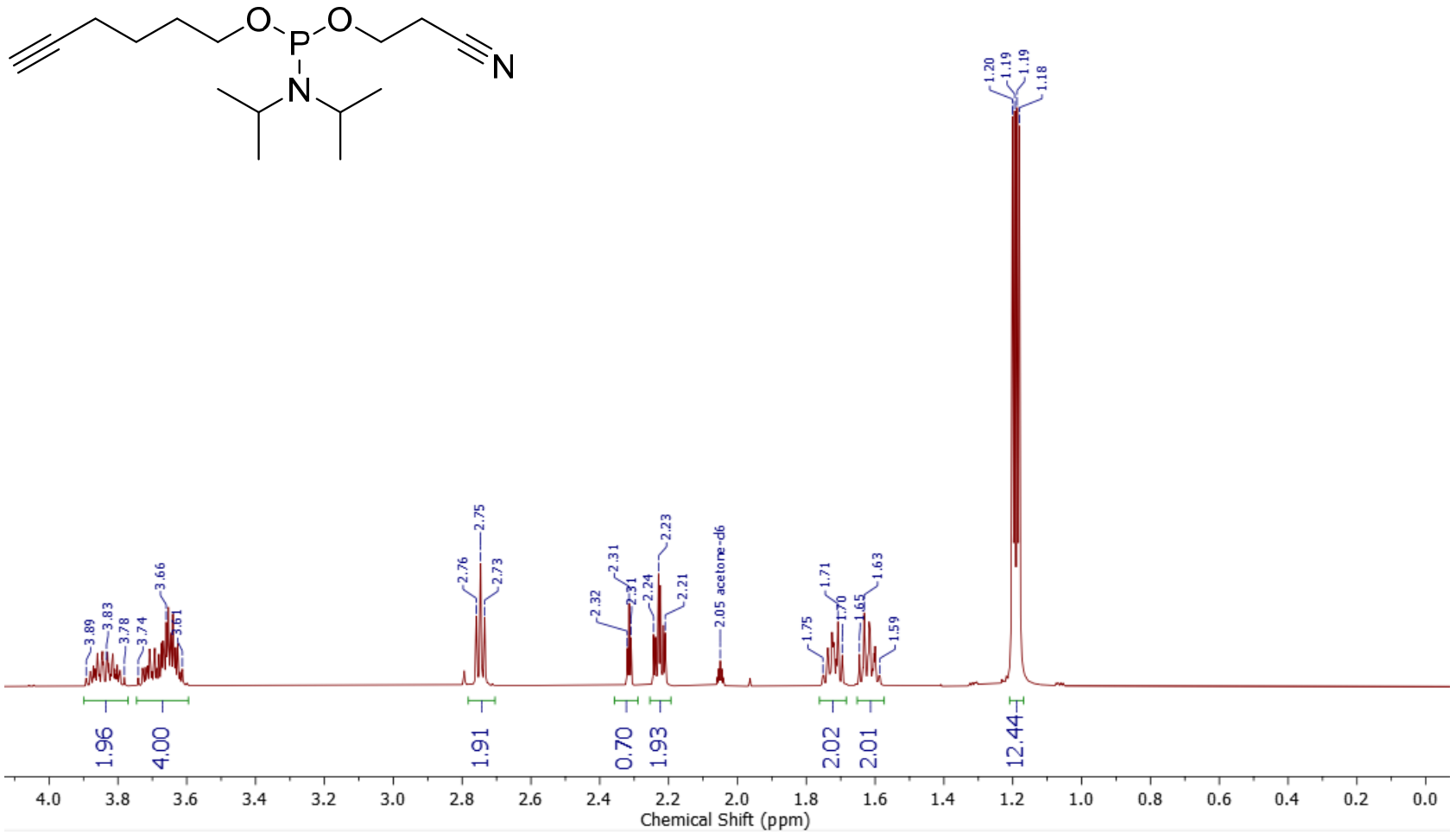


Figure S39: 125.7 MHz ^{13}C NMR spectrum of control alkyne phosphoramidite **8** (in acetone-d_6)

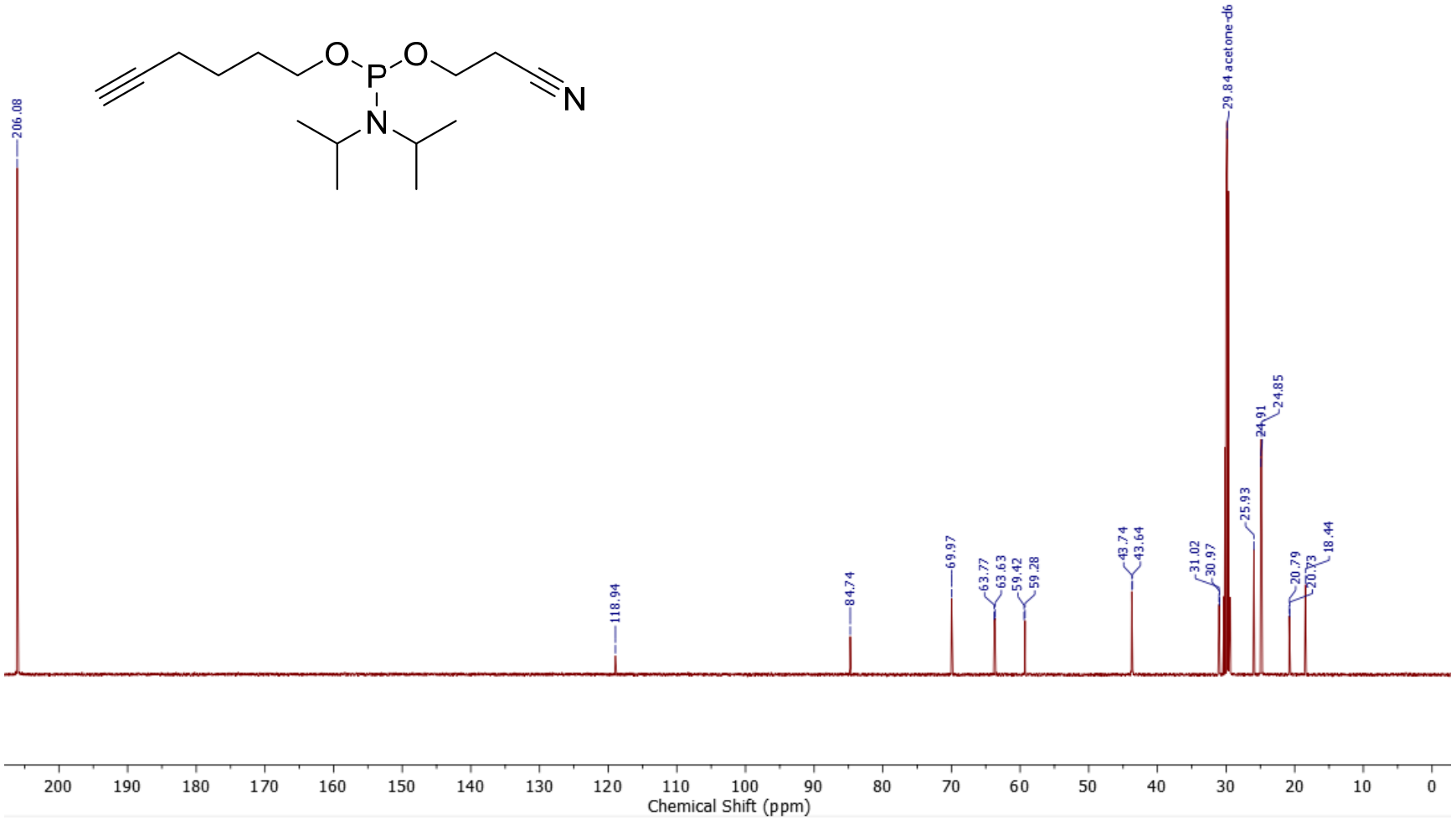


Figure S40: 202.3 MHz ^{31}P NMR spectrum of control alkyne phosphoramidite **8** (in acetone-d_6)

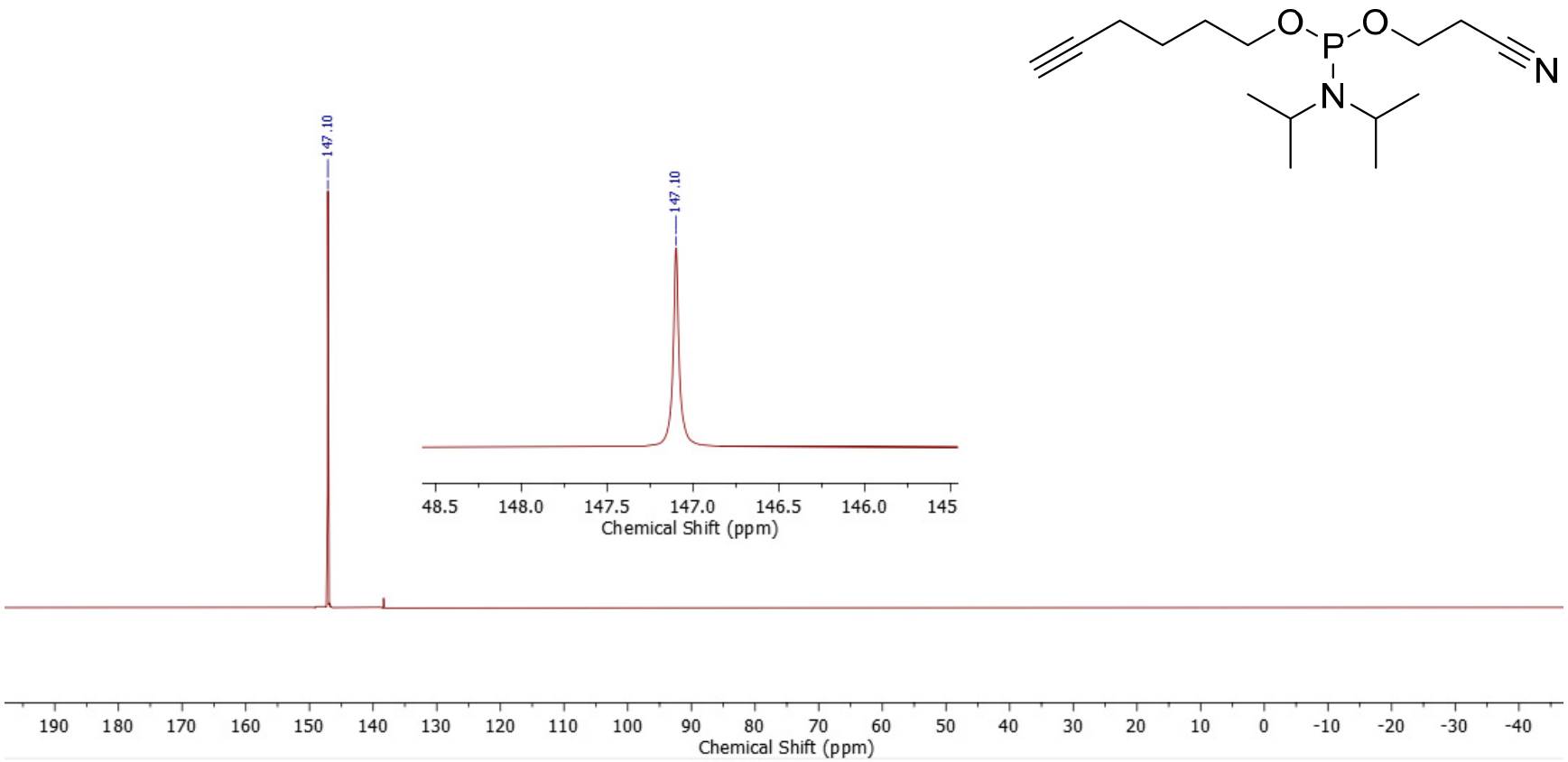
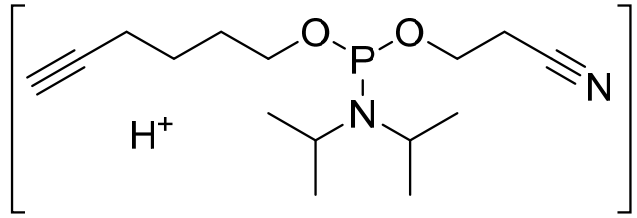


Figure S41: HR ESI-MS spectrum of control alkyne phosphoramidite **8** (expected mass: 299.1883)



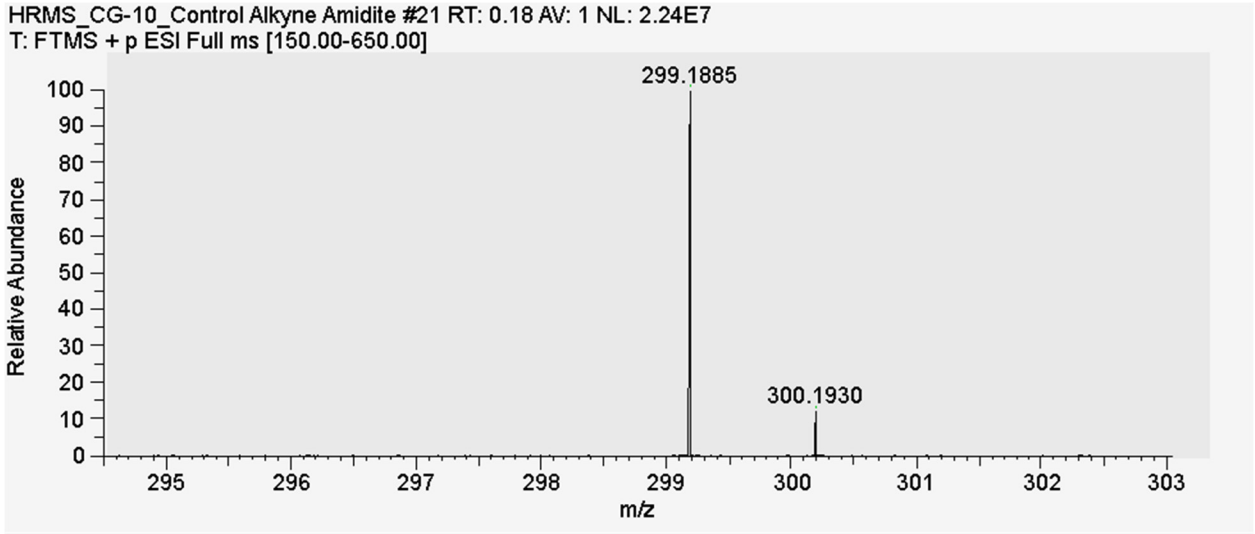
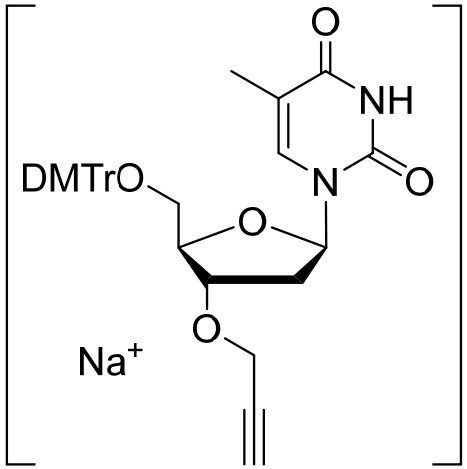
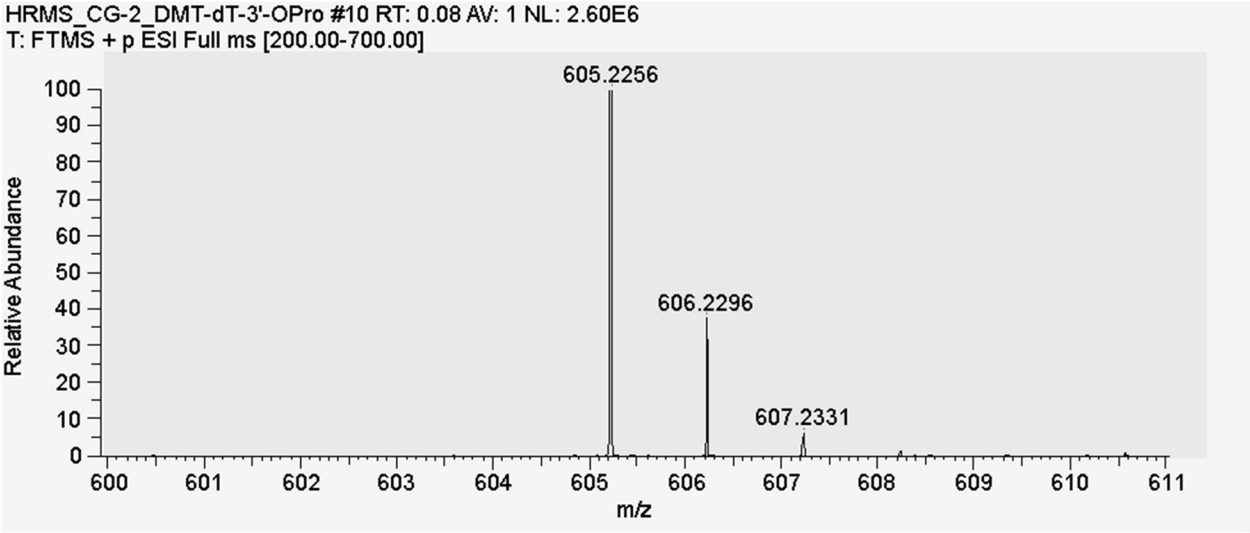


Figure S42: HR ESI-MS spectrum of 5'-*O*-DMTr-3'-*O*-propargyl thymidine **9** (expected mass: 605.2258)





Supporting References

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