Supporting Information for

meCLICK-Seq - a substrate-hijacking and RNA degradation strategy for the study of RNA methylation

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Chemical Synthesis and Characterization

NMR Spectra

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Table S1. Putative mass spectra peaks for the 11-mer after 2 hour degradation. -OPO3 refers to a phosphate at the 3' end, -OPO2 refers to a cyclic phosphate at the 3' end, (Deg-A) regers to click-degrader functionalised adenosine.

Sequence	Lost sequence (end)	Calcd. mass	Observed mass
GGUGG(Deg-A)CUGCC	-	3916	3919
GGUGG(Deg-A)CUGC-OPO_3	C (3')	3691	3691
UGG(Deg-A)CUGCC	GG-OPO_3 (5')	3222	3225
GG(Deg-A)CUGCC	GGU-OPO_3 (5')	2922	2920
GGUGG(Deg-A)C-OPO_2	UGCC-O (3')	2802	2802
GGUGG(Deg-A)-OPO_2	CUGCC-O (3')	2429	2432

Table S2. Putative mass spectra peaks for the functionalised and BCS-quenched 11-mer after 14 hour degradation. -OPO3 refers to a phosphate at the 3' end, -PO4 refers to a phosphate at the 5' end, (Deg-A) refers to click-degrader functionalised adenosine.

Sequence	Lost sequence (end)	Calcd. mass	Observed mass
GGUGG(Deg-A)CUGCC	-	3916	3919
GGUGG(Deg-A)CUGC-OPO_3	C (3')	3691	3691
PO_4-GUGG(Deg-A)CUGCC	G (5')	3651	3649
GGUGG(Deg-A)CU-OPO_3	GCC (3')	3167	3172
GGUGG(Deg-A)CU	PO_4-GCC (3')	3087	3082
GG(Deg-A)CUGCC	GGU-OPO_3 (5')	2922	2920
GGUGG(Deg-A)C	PO_4-UGCC (3')	2740	2737
PO_4-(Deg-A)CUGCC	GGUGG (5')	2384	2388
(Deg-A)CUGCC	GGUGG-OPO_3 (5')	2304	2309

Table S3. METTL3 and METTL16 mRNA substrates determined via meCLICK-Seq. Provided separately as a .txt file.

Table S4. Results of a miCLIP study of m^6A in MOLM13 cells. Provided separately as a .xlsx file.

Table S5. METTL3 and METTL16 lncRNA substrates determined via meCLICK-Seq. Provided separately as a .txt file.

Table S6. List of METTL13 and METTL16-dependent intronic/intergenic peaks determined via meCLICK-Seq. Provided separately as a .xlsx file.

Table S7. List of methylated mRNA species determined via meCLICK-Seq with an overlap with m^7G-Seq study. Provided separately as a .xlsx file.

Table S8. List of intronic and intergenic click-degrader sensitive RNA peaks with an overlap with m7G-Seq study. Provided separately as a .xlsx file.

Table S9. List of detected RNA species.

Label	**Species**	**Calcd. mass**	**Observed mass**
i	Propargylated RNA 11-mer	3559	3560
ii	Non-propargylated RNA 11-mer	3521	3522
iii	Click-degrader 1 functionalised RNA 11-mer	3916	3917
iv	Click-degrader 2 functionalised RNA 11-mer	3828	3830
v	Click-degrader 3 functionalised RNA 11-mer	3740	3742
vi	Non-propargylated RNA 22-mer A	7112	7115
vii	Non-propargylated RNA 22-mer B	7150	7154
viii	Click-degrader 1 functionalised RNA 22-mer A	7507	7510
ix	Click-degrader 1 functionalised RNA 22-mer B	7545	7550

Table S10. List of oligomers used in the *in vitro* study. (A*) indicates Pr^6A for Prop, A for CTRL oligos.

Oligo	Sequence
11-mer	GGUGG(A*)CUGCC
22-mer A	GGUGG(A*)CUGCCUGAGCUCAGGA
22-mer B	AGGCCAAGGCGGGUGG(A*)CUGCC

Table S11. List of primers.

Name	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)
Primers for mRNA exon analysis (Fig. 3e, 3f, S2d)		
SP1	CAGTGGGCTACAGGGGTCT	CTTGCAATGAGCCTCCAGAT
DICER1	CTTAAAGTTGTTAGTGAGTGGAATGAA	CTGTTATCTATCCTGTTATCAACCAAA
BRD4	GACATGAGCACAATCAAGTC	GAACACATCCTGGAGCTTGC
SRGAP2B	CCCTCGAGAGAAGCGGTCTT	GGTCCAGGCATTTCATCTGC
ACTB	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG
FLI1	AACGTGCACAGGGGAGTGAGGG	GTTACAGCCTGACCTCGCAGCC
CADM1	CTGCTGTTGCTCTTCTCCGCCG	TGGTCGCAACCTCTCCCTCGAT
AURKB	GGGAACCCACCCTTTGAGAG	GGGGTTATGCCTGAGCAGTT
CDK6	CACACCGAGTAGTGCATCGCGA	GCAAGGCCGAAGTCAGCGAGTT
ATXN1	AGAAAGAACACGCCTGAGCC	GTACAATCCGCCAACAGCAG
Primers for lncRNA analysis (Fig. S2h)		
NEAT1	GCCTTCTTGTGCGTTTCTCG	TCCCAGCGTTTAGCACAACA
NORAD	GTGACCACTCTGTCGCCATT	AGAATGAAGACCAACCGCCC
TUG1	ACGACTGAGCAAGCACTACC	CTCAGCAATCAGGAGGCACA
MALAT1	CTGAGGAGCAAGCGAGCAAG	AATCGTTAGCGCTCCTTCCTT
Primers for intron analysis (Fig. 4f and 4g)		
SRGAP2B	CCACACCCAGCCAAGCCAACTC	AGGAGGGAAGGTACAGGATGGAGT
HSD17B11	TGCGCTGCACCCACTAATGTGT	ACACTCTGGGGACTGTGGTGGG
CADM1	CCGGAGACAGTGGCATGGAGGA	AGGAGCTCGGCTTGGGAAACCT
RASA3	GCCTGGATGACCTTCCCGTTGC	CCCAGTACCAGAGCACCCCGAT
PSMA1	AGTCAGGCAAGCCTTCTGGAGA	ATGGGGCCTTTGTTCCAGCTGC
ATXN1	GGCAACCCTCACCTCAACCTGT	TGATGCACGTGACCGGGAAGGA
DCP1B	TCAGTCTGGCCAGGTGTTTACCA	TCCAAAGCAAACAGAAGGAAAGAA
FLI1	CCATGCCCTCTGCTTCCTGTGC	ATCTGCAGCCAGGCAACCTGTG
Housekeepers for normalisation		
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
RPL32	GTTCCTGGTCCACAACGTCA	CATTGTGAGCGATCTCGGCA

Table S12. List of gRNAs

Name	Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)
gRNAs used for generation of Δintronic cells (Fig. 4h–j)		
DCP1B	GACAACTCACAATCACCTC	AGAGGTGATTGTGAGTTGTC
ATXN1	GCTTTATCTGGTACTCATAG	CTATGAGTACCAGATAAAGC
RASA3(1)	GAAGCTGTCCGTGTTCACAT	ATGTGAACACGGACAGCTTC
RASA3(2)	GTTGCACAACTCGGTGAACG	CGTTCACCGAGTTGTGCAAC
FLI1	GAAAGGTGAGCGTAAACGTG	CACGTTTACGCTCACCTTTC

Supporting Figures

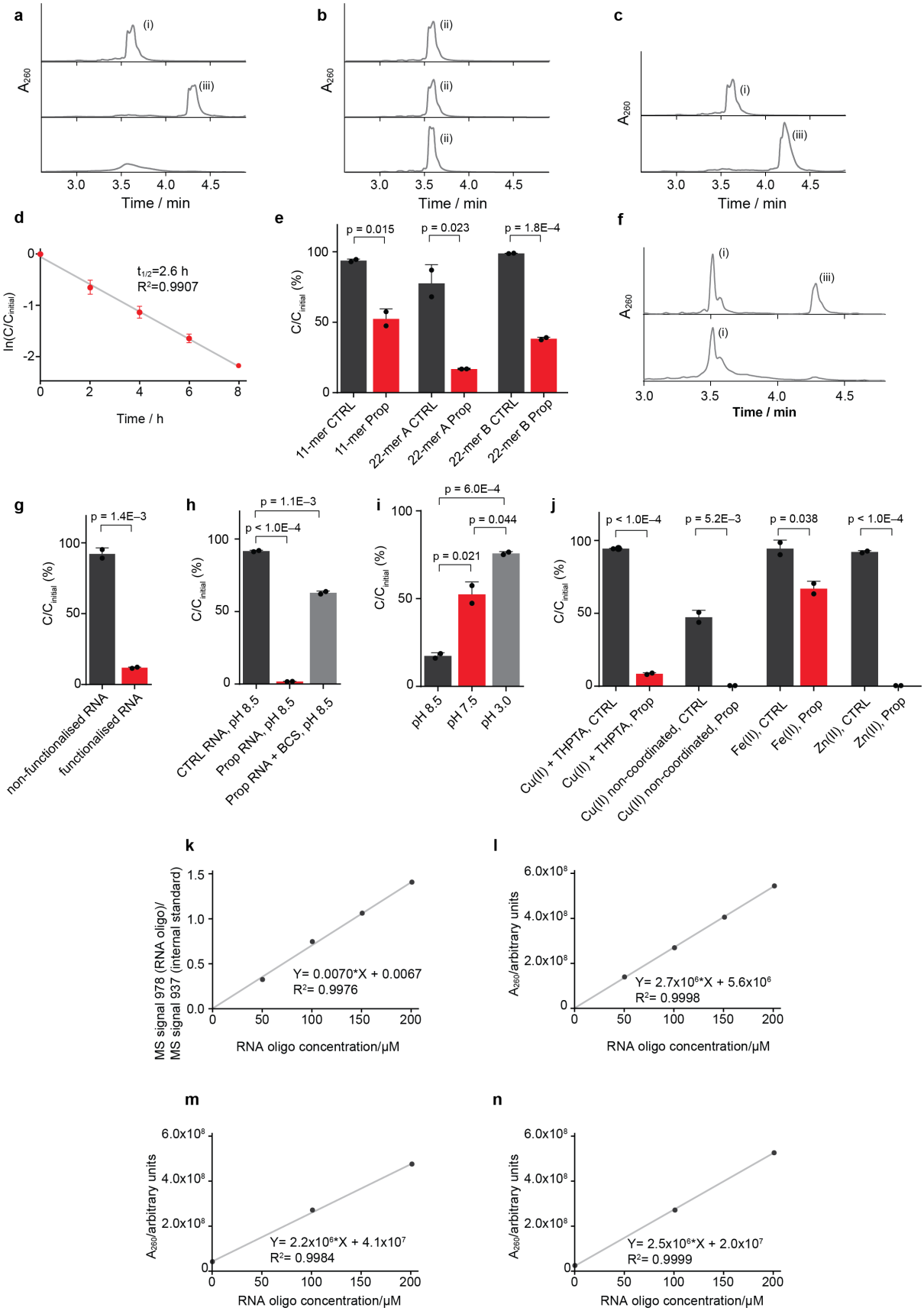


Fig. S1 (**a**) Chromatogram of the propargylated RNA oligo (top), after functionalisation with click-degrader 1 (middle) and 14 h incubation at 37 ºC (bottom). (**b**) Chromatogram of the non-

propargylated RNA oligo (top), after functionalisation with click-degrader 1 (middle) and 14 h incubation at 37 ºC (bottom). (**c**) Chromatogram of the propargylated RNA oligo (top), after functionalisation with click-degrader 1 via treatment with milder CuAAC conditions (100 μM $CuSO_4$, 300 μM THPTA, 400 μM click-degrader, 5 mM NaAsc, 10 min), equivalent to cellular CuAAC conditions. (**d**) Logarithmic (first order) fit of time-dependent RNA degradation. n=2. (**e**) Extent of degradation of three RNA oligomers in 2 h at 37 ºC, pH 7.5, n=2. (**f**) Chromatogram of one-pot CTRL and Prop RNA after functionalisation with click-degrader 1 (top) and 14 hour incubation at 37 ºC (bottom). (**g**) Quantification of the one-pot CTRL and Prop RNA degradation reaction after 14 h. (**h**) Extent of RNA degradation in 14 h at 37 ºC, pH 8.5, n=2. (**i**) Extent of RNA degradation in 2 h at various pH values, n=2. (**j**) Extent of RNA degradation in 14 h at 37 ºC, pH 7.5, in presence of various transition metals, n=2. (**k**) Calibration curve of click-degrader 1-functionalised RNA oligomer (iii). (**l**) Calibration curve of non-functionalised RNA (i). (**m**) Calibration curve of click-degrader 2-functionalised RNA oligomer (iv). (**n**) Calibration curve of click-degrader 3-functionalised RNA oligomer (v). Error bars represent SD.

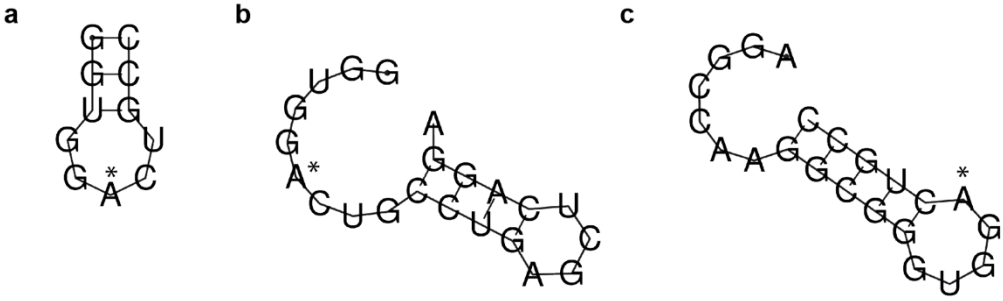


Fig. S2 RNAfold-predicted[1] structures ref of oligomers used in the in vitro study. (**a**) 11-mer. (**b**) 22-mer A. (**c**) 22-mer B. A* indicates Pr^6A for Prop, A for CTRL oligos.

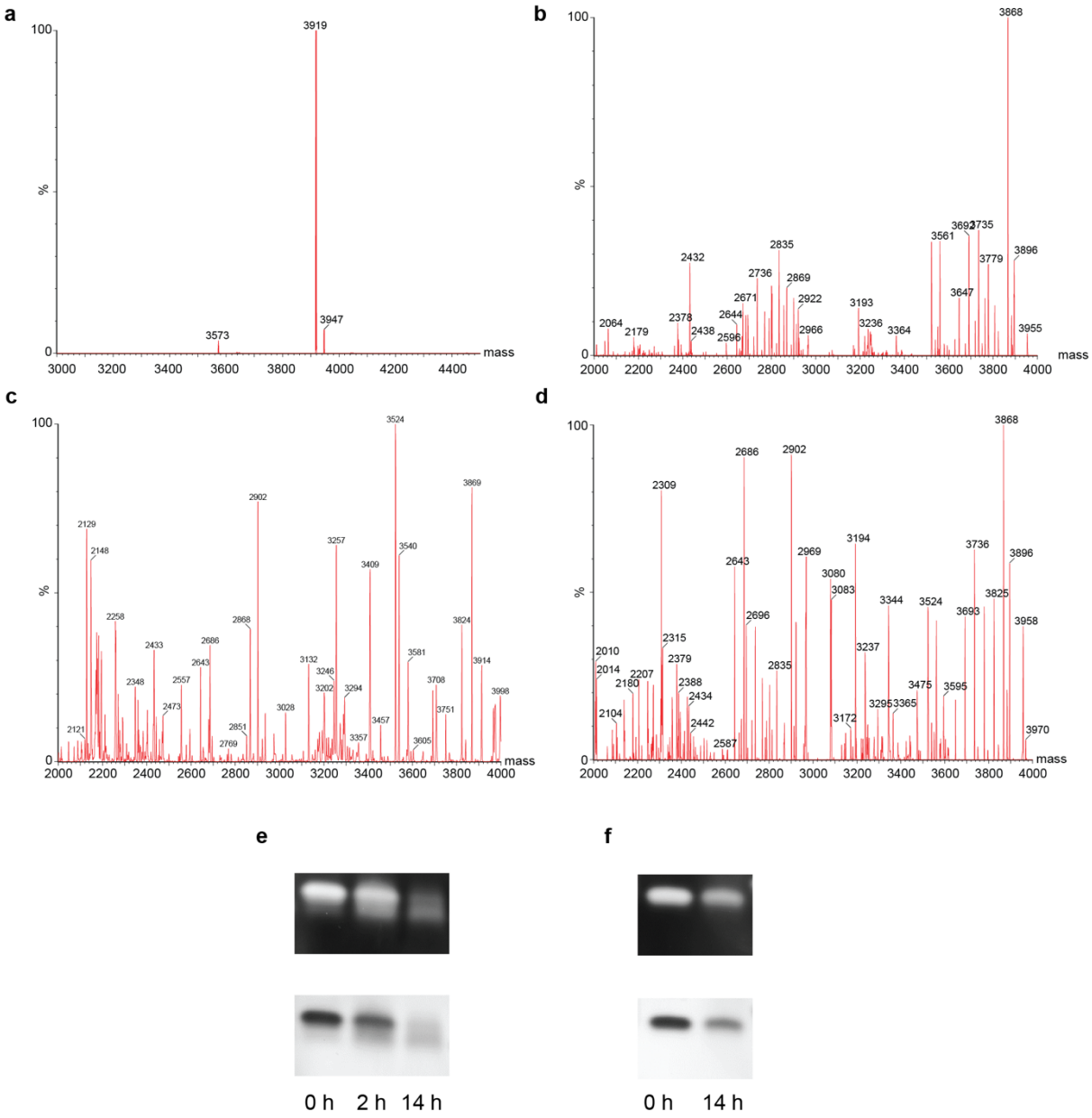


Fig. S3 (**a**) Deconvoluted mass spectrum of click-degrader functionalised 11-mer. (**b**) Deconvoluted mass spectrum of degradation products after 2 h at 37 ºC. (**b**) Deconvoluted mass spectrum of degradation products after 14 h at 37 ºC. (**d**) Deconvoluted mass spectrum of degradation products after quenching copper with BCS followed by 14 h at 37 ºC. (**e**) RNA gels of click-degrader functionalised 11-mer and its degradation products after various incubation times at 37 ºC. (**f**) RNA gels of CTRL 11-mer after various incubation times at 37 ºC.

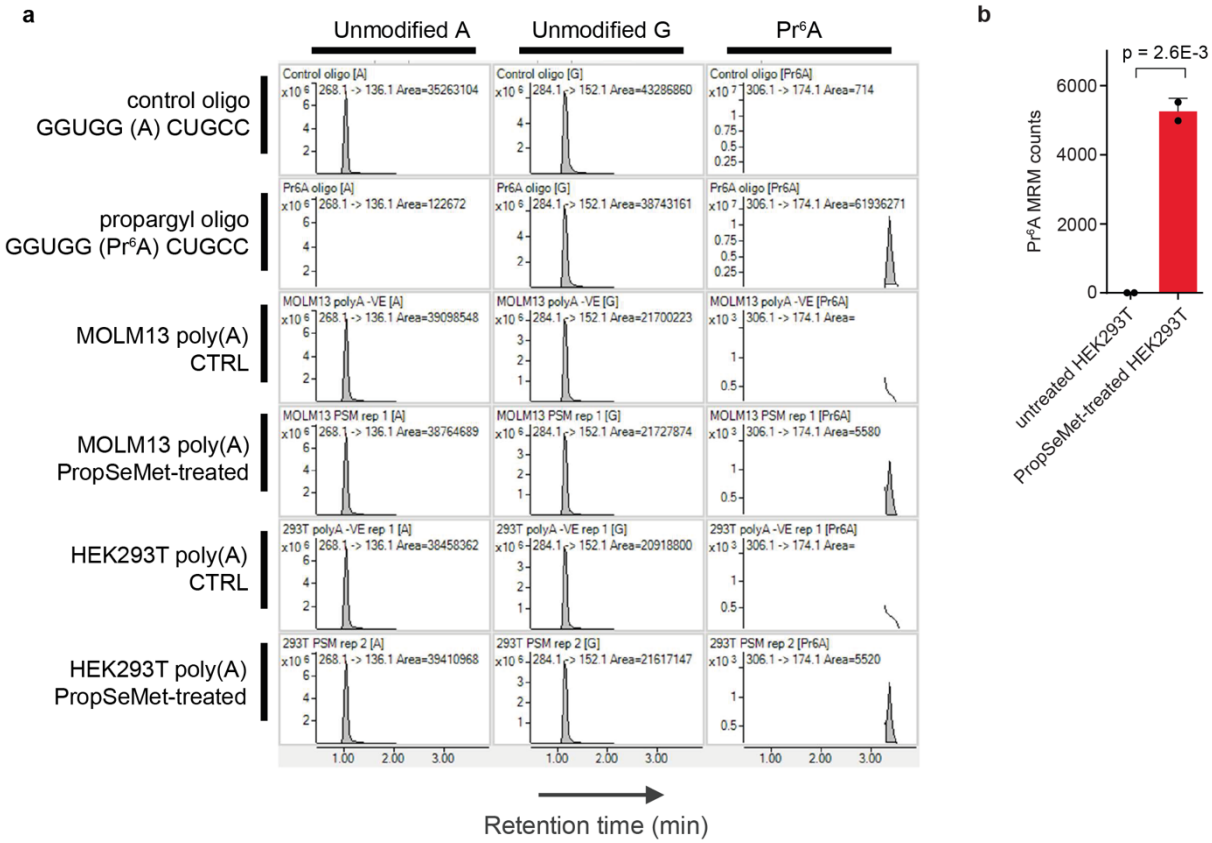


Fig. S4 (**a**) Mass spectrometry analysis of nuclease-digested CTRL and Prop oligomers, CTRL or PropSeMet treated MOLM13 and HEK293T poly(A)-enriched RNA. Pr^6A peak is observed only in the propargyl oligomer and RNA from PropSeMet-treated cells. (**b**) Comparison of size of Pr^6A peak for CTRL and PropSeMet-treated HEK293T cells. Error bars correspond to SD, n=2.

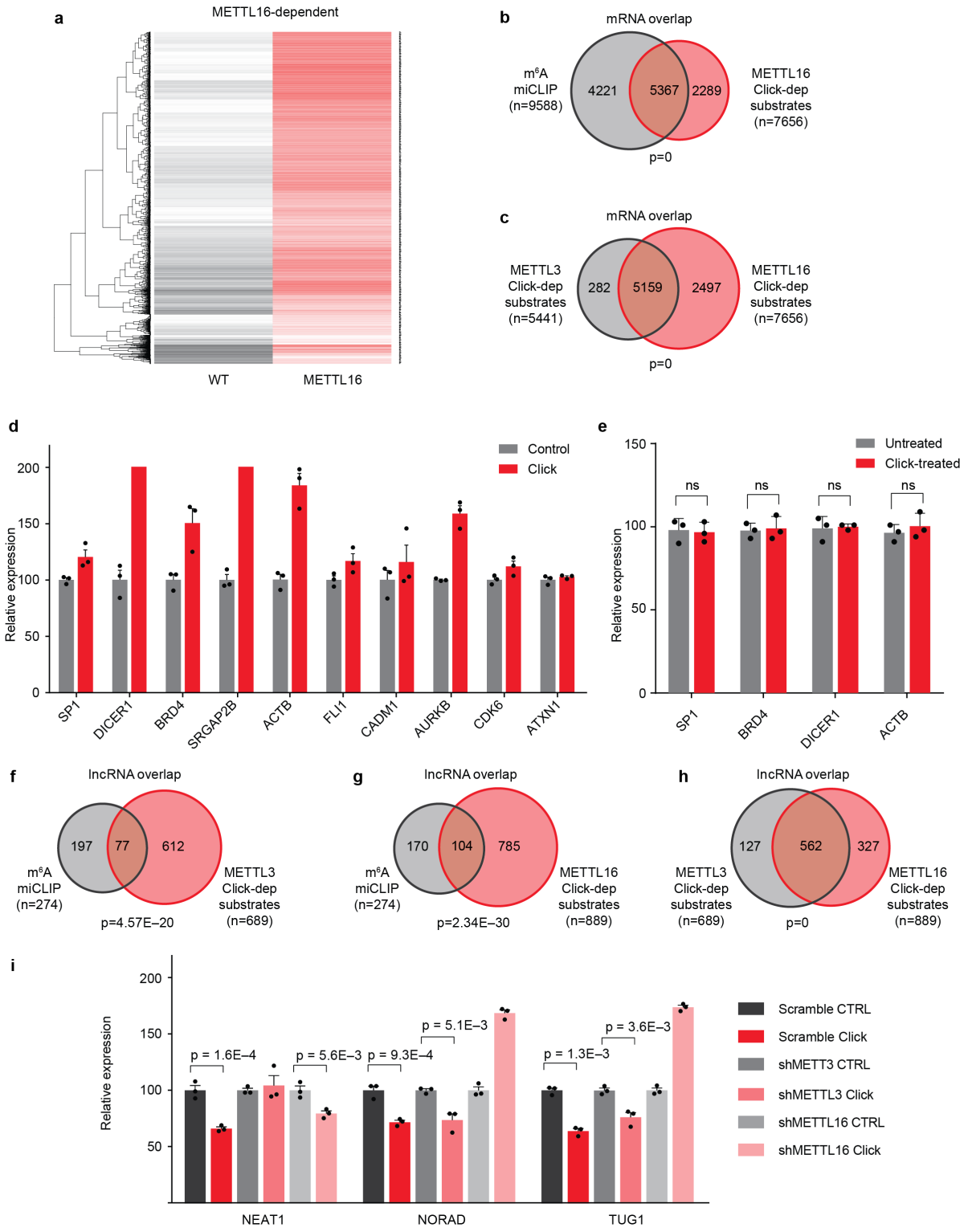


Fig. S5 (**a**) Heatmap showing downregulation of methylated mRNAs and rescue of METTL16-dependent transcripts upon METTL16 depletion. (**b**) Overlap of m^{6}A-containing mRNAs determined via miCLIP and METTL16 mRNA substrates determined via meCLICK-Seq. (**c**) RT-qPCR-based meCLICK-Seq validation of a panel of genes in METTL16 deficient cells, n=3. (**d**) Overlap between METTL3 and METTL16 mRNA substrates determined via meCLICK-Seq. (**e**) RT-qPCR-based evaluation of the effect click-components have on relative levels of RNA species. (**f**) Overlap of m^{6}A-containing lncRNAs determined via miCLIP and

METTL3 lncRNA substrates determined via meCLICK-Seq. (**g**) Overlap of m^{6}A-containing lncRNAs determined via miCLIP and METTL16 lncRNA substrates determined via meCLICK-Seq. (**h**) Overlap between METTL3 and METTL16 lncRNA substrates determined via meCLICK-Seq. (**i**) RT-qPCR-based meCLICK-Seq validation of a panel of lncRNAs in three isogenic cell lines, n=3. ns not significant ($p > 0.05$) Error bars represent SD.

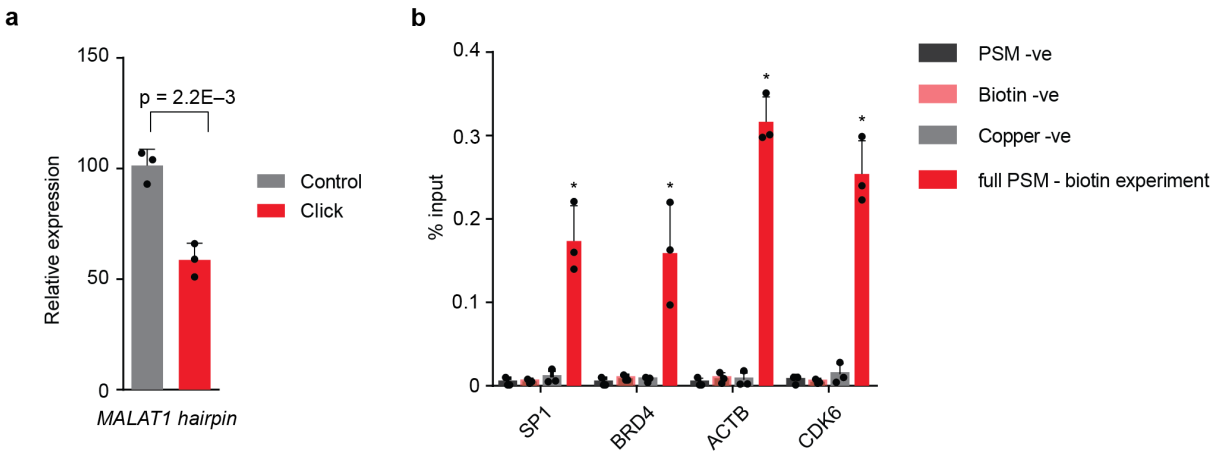


Fig S6 (**a**) RT-qPCR validation of m^{6}A-containing *MALAT1* hairpin degradation. (**b**) RT-qPCR analysis of m^{6}a substrates using propargylated RNA after biotin-streptavidin pulldown. Error bars correspond to SD, n=3. * $p < 0.05$ for all groups compared with full PSM-biotin treatment.

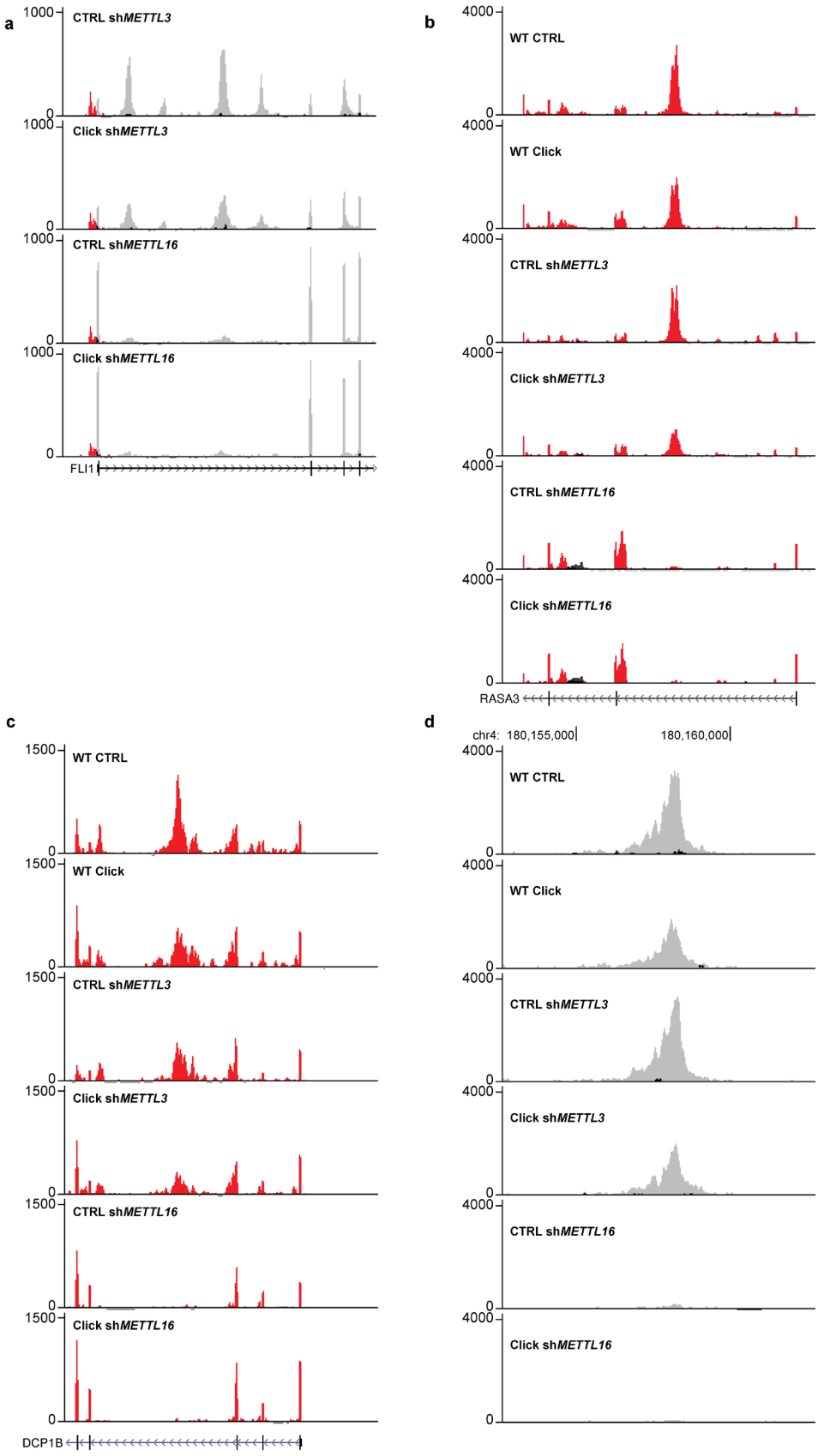


Fig. S7 Examples of intronic peak snapshots. (**a**) Intronic peaks in *FLI1*, METTL16 dependent. (**b**) Intronic peaks in *RASA3*, METTL16 dependent. (**c**) Intronic peaks in *DCP1B*, METTL3 and METTL16 dependent. (**d**) Intronic peaks in an intergenic region in chromosome 4, METTL16 dependent.

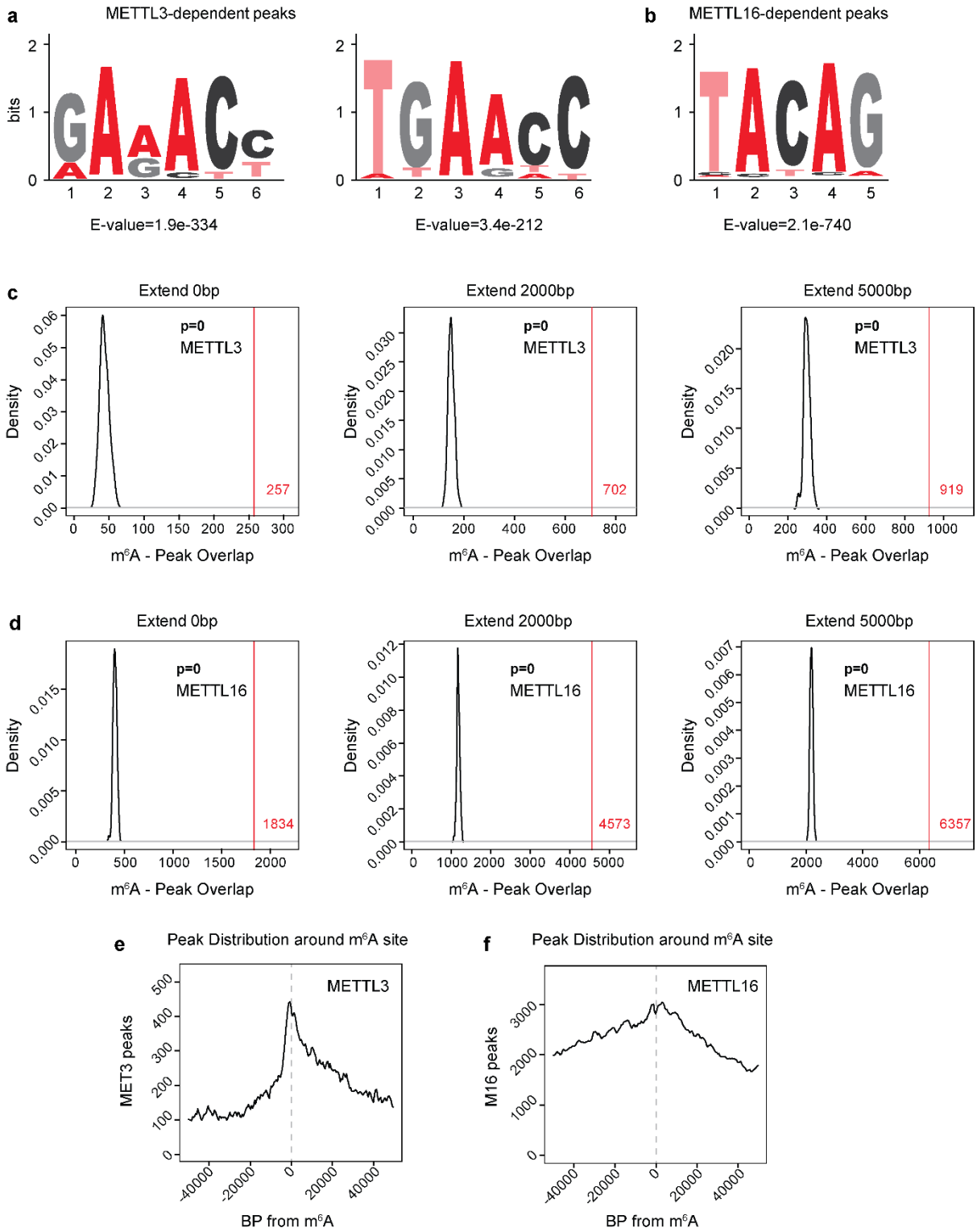


Fig. S8 (**a**) Consensus sequences found in METTL3-dependent peaks. (**b**) Consensus sequence found in METTL16-dependent peaks. (**c**) Overlap between m^{6}A sites determined via miCLIP and METTL3-dependent intronic peaks determined via meCLICK-Seq, by considering miCLIP peaks exact, extended to both directions by 2000 and 5000 base pairs. Red lines indicate experimentally determined overlaps, black curves indicate distributions of 100 simulations of randomly generated m^{6}A sites. (**d**) Overlap of m^{6}A sites determined via miCLIP and METTL16-dependent intronic peaks determined via meCLICK-Seq, similar to (**c**). (**e**)

Distribution of meCLICK-Seq-determined METT3-dependent peaks around m^{6}A sites found via miCLIP. (**f**) Distribution of meCLICK-Seq-determined METT16-dependent peaks around m^{6}A sites found via miCLIP.

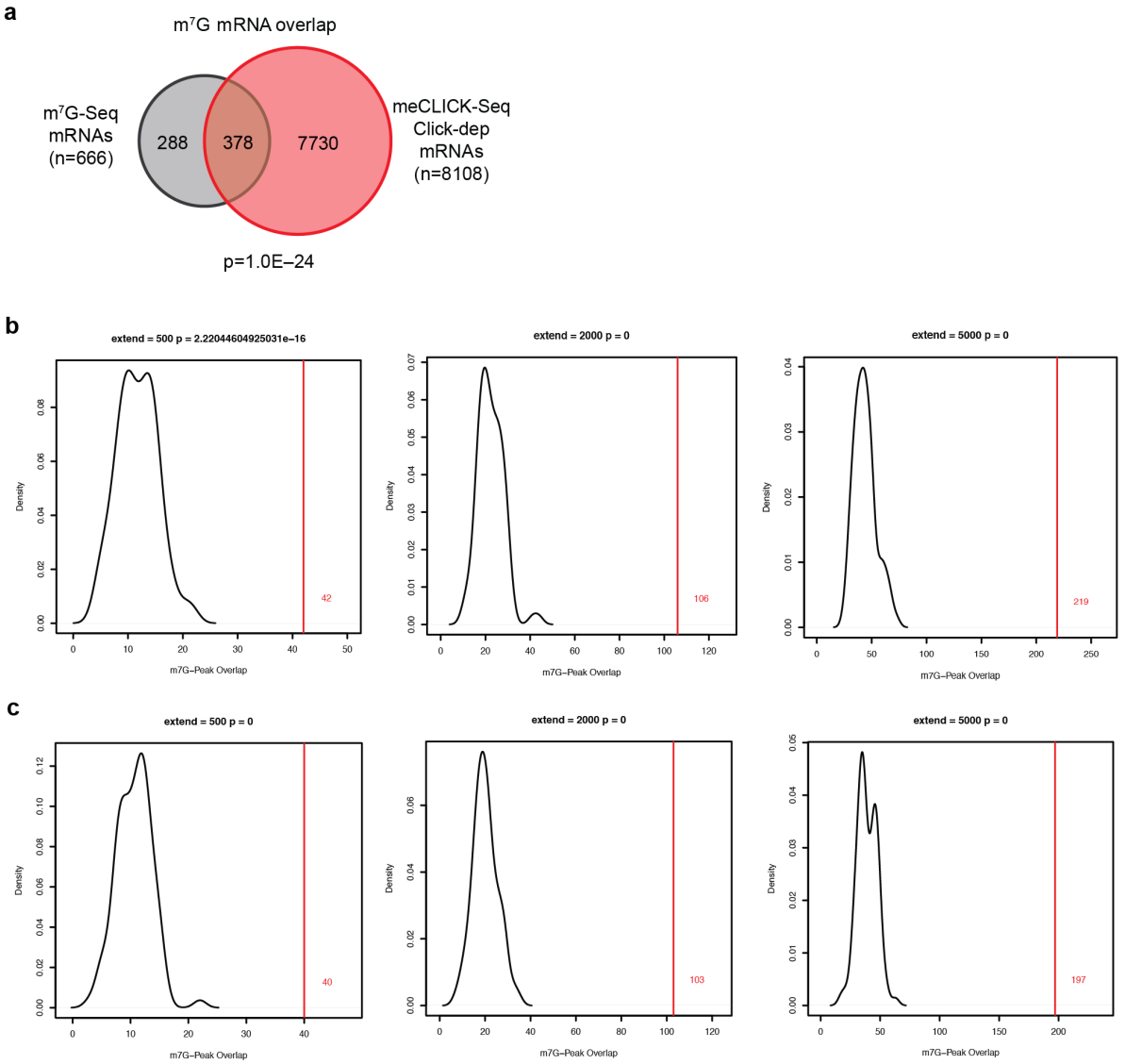


Fig. S9 (**a**) Overlap between all methylated mRNAs determined via meCLICK-Seq and m^{7}G-containing mRNAs determined via m^{7}G-Seq. (**b**) Overlap between m^{7}G sites found via m^{7}G-Seq and click-sensitive intronic peaks determined via meCLICK-Seq, by considering m^{7}G-Seq peaks extended to both directions by 500, 2000 and 5000 base pairs. Red lines indicate experimentally determined overlaps, black curves indicate distributions of 100 simulations of randomly generated m^{7}G sites. (**c**) Overlap between m^{7}G sites found via m^{7}G-Seq and click-sensitive intergenic peaks determined via meCLICK-Seq, similar to (**b**).

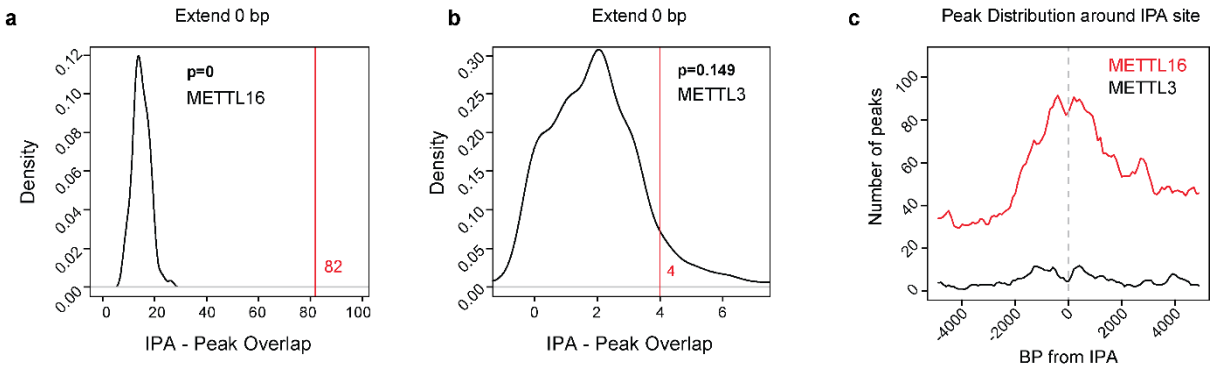


Fig. S10 (**a**) Overlap of intron polyadenylation (IPA) sites determined via 3'-seq in primary CLL cell and METTL16-dependent intronic peaks determined via meCLICK-Seq. Red lines indicate experimentally determined overlaps, black curves indicate distributions of 100 simulations of randomly generated IPA sites. (**b**) Overlap of IPA sites determined via 3'-seq in primary CLL cells and METTL3-dependent intronic peaks determined via meCLICK-Seq. (**c**) Distribution of distances between IPA sites and METTL16- or METTL3- dependent intronic peaks.

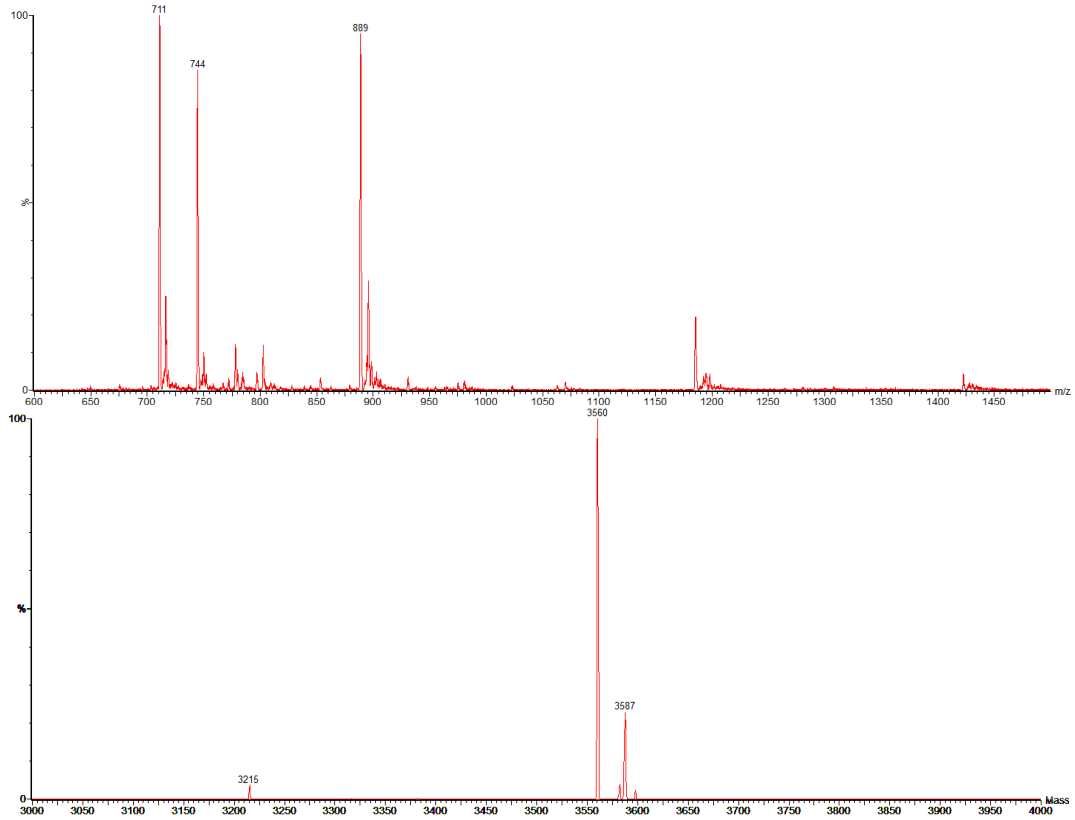


Fig. S11 Negative ion series and deconvoluted mass spectrum of propargylated RNA 11-mer (i).

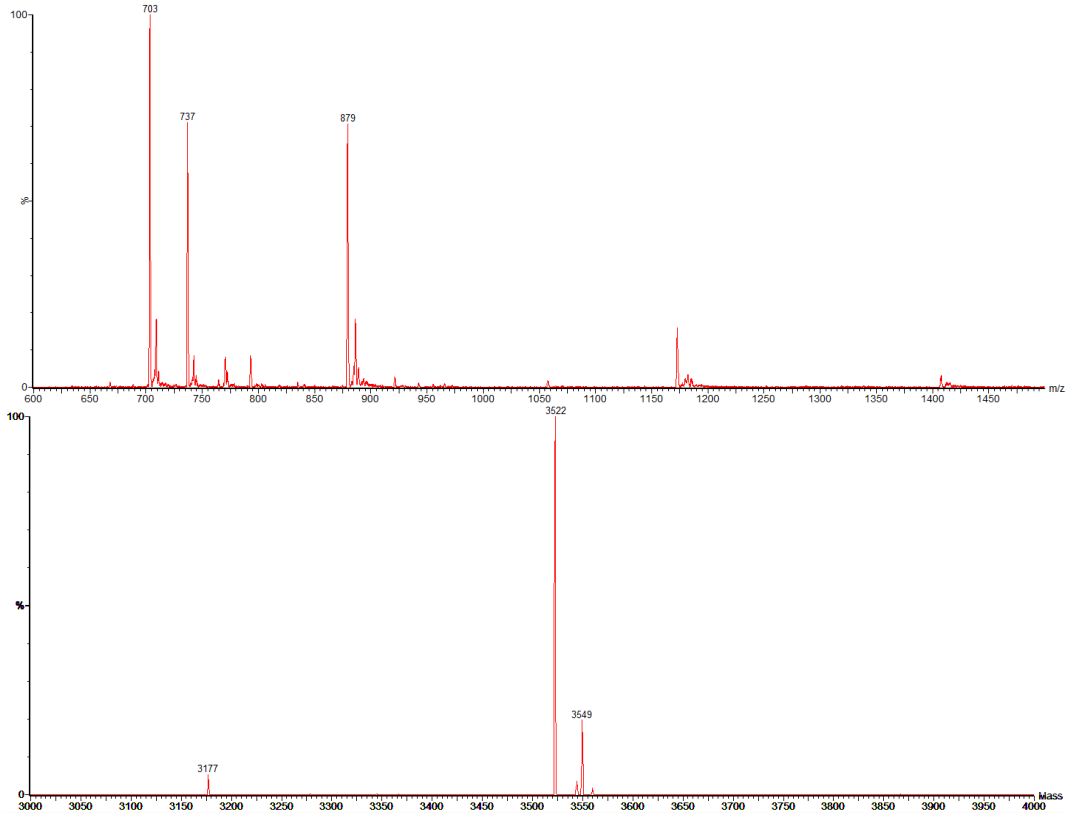


Fig. S12 Negative ion series and deconvoluted mass spectrum of non-propargylated RNA 11-mer (ii).

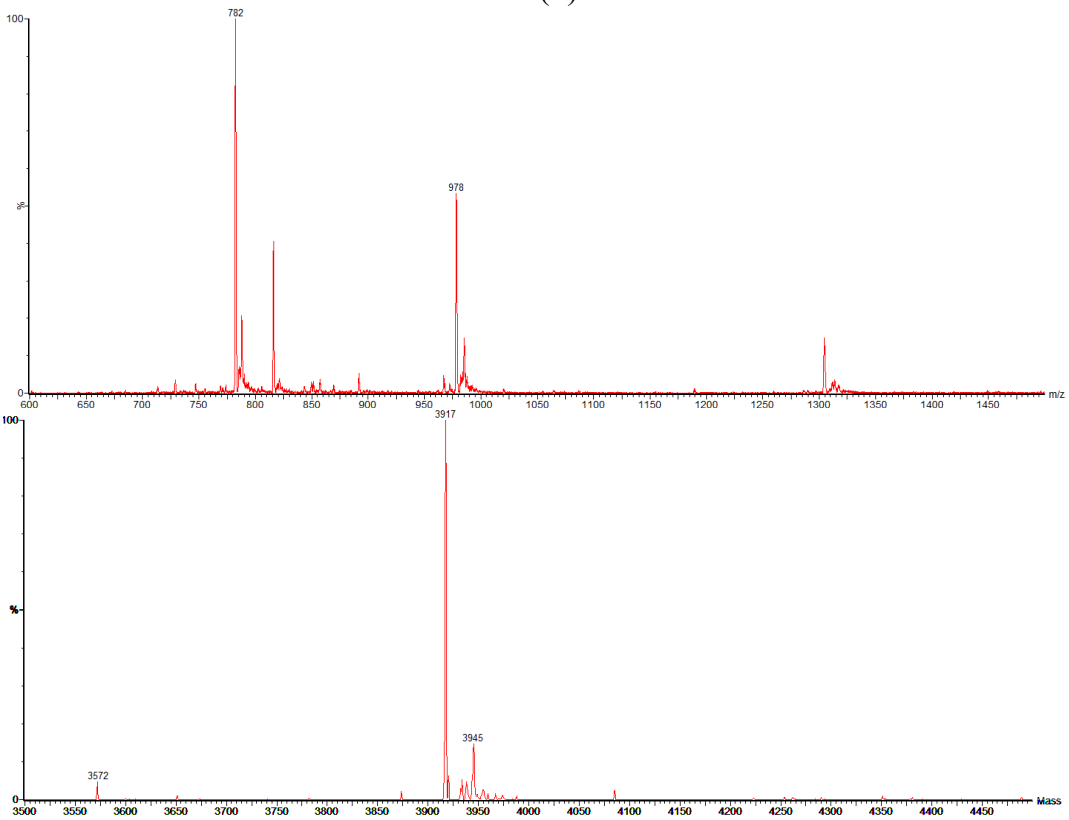


Fig. S13 Negative ion series and deconvoluted mass spectrum of **click-degrader 1**-functionalised RNA 11-mer (iii).

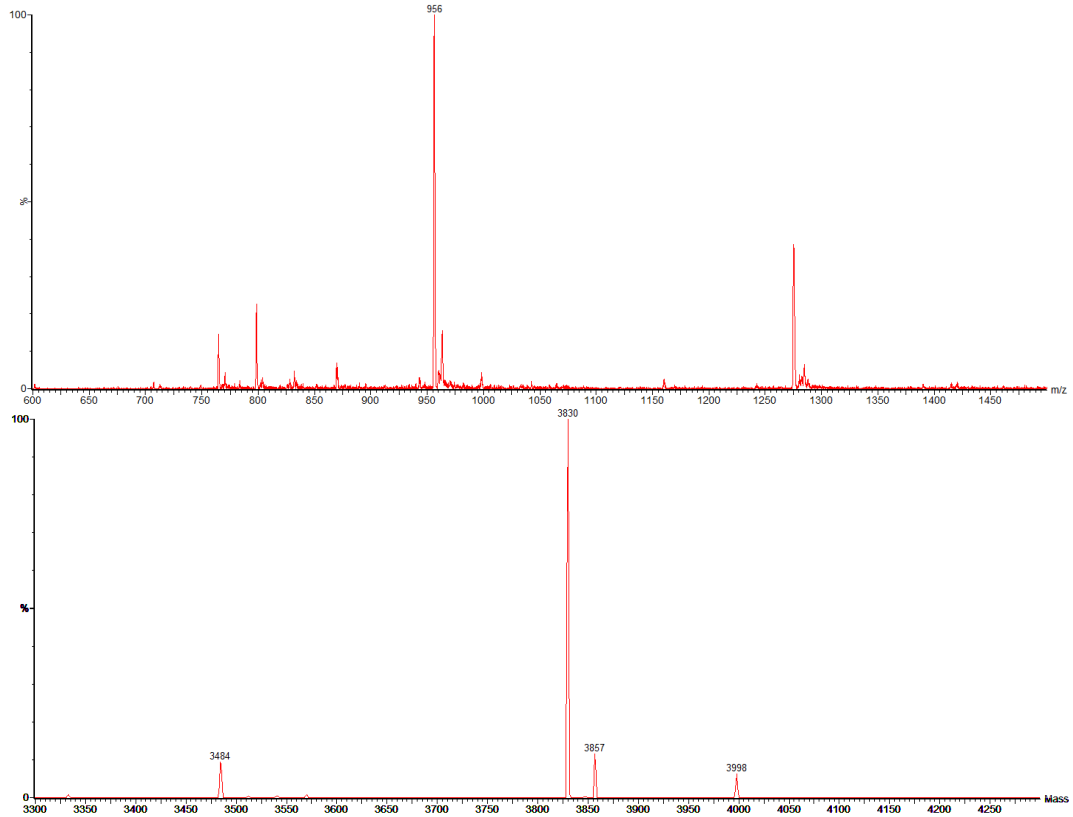


Fig. S14 Negative ion series and deconvoluted mass spectrum of **click-degrader 2**-functionalised RNA 11-mer (iv).

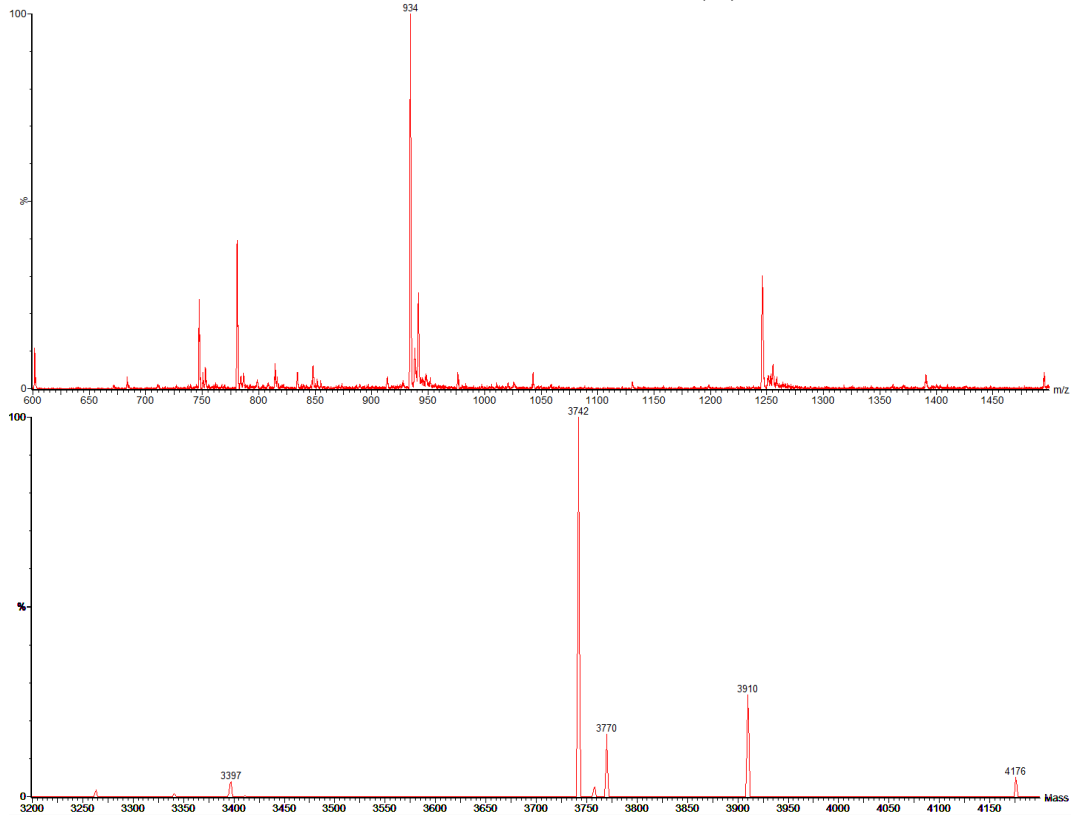


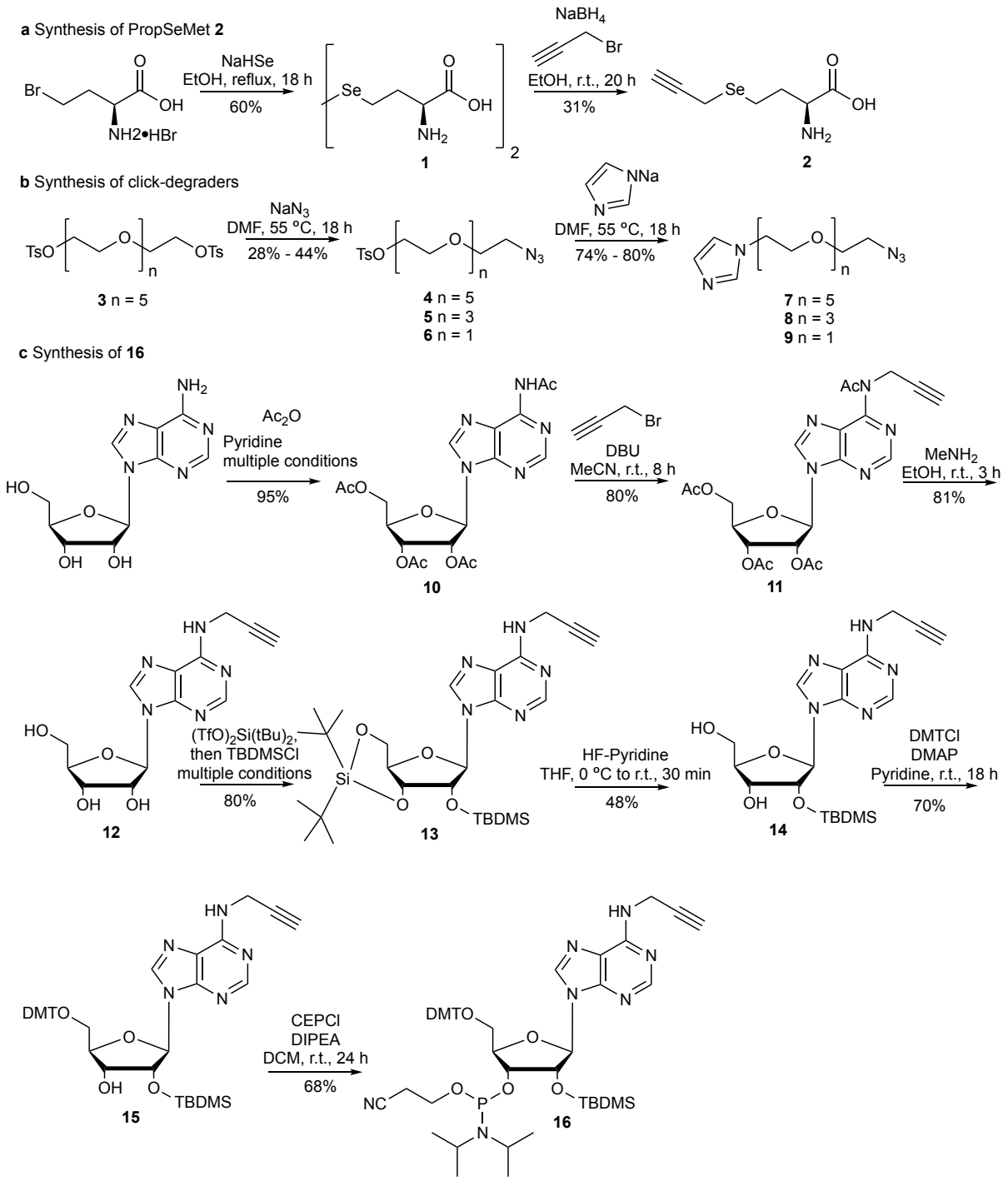
Fig. S15 Negative ion series and deconvoluted mass spectrum of **click-degrader 3**-functionalised RNA 11-mer (v).

Chemical Synthesis

General considerations

Chemicals were purchased from Fisher Chemicals, Sigma-Aldrich, Alfa-Aesar, Fluorochem or BioSynth and used without further purification. All solvents were commercially available grade. All non-aqueous reactions were performed in oven-dried glassware under a nitrogen atmosphere unless otherwise stated. Nitrogen gas was pre-dried via passage through calcium chloride. Reaction vessels were heated using thermostatically controlled DrySyn blocks filled with sand with the liquid level of the ask below that of the heating block. Reaction temperatures refer to the thermostat set point. A reaction temperature of 0 ºC refers to an external ice/water slurry cooling bath. All reagents were purchased from commercial sources and used without further purification unless otherwise stated. DCM, THF and Et_2O were purified either according to the method of Grubbs and Pangborn[2] or by distillation under an inert atmosphere (DCM, MeOH and MeCN were distilled from calcium hydride. THF and Et_2O were pre-dried over sodium wire then distilled from calcium hydride and lithium aluminium hydride). Petroleum ether, n-hexane and EtOAc were distilled on site. 'Petroleum ether' refers to the distillate of petroleum ether collected between 40–60 ºC. Water used experimentally was deionised and prepared on site.

Flash column chromatography was performed using Merck silica gel 60. Analytical thin layer chromatography was performed using Merck Silica gel 60 F254 and visualised by UV (254 nm), by staining with a $KMnO_4$ or $(NH_4)_4Ce(SO_4)_4$ solution. NMR spectra were recorded on a 400 MHz AVIII HD Smart Probe Spectrometer or a 600 MHz Avance 600 BBI Spectrometer. HPLC analysis and purification were carried out on ThermoFisher Scientific Ultimate 3000 HPLC system, using NUCLEOSIL 100-5 C18 semi-preparative column. mqH2O with 0.1 % formic acid (A) and HPLC grade MeCN (B) were used as the mobile phases, with flow rate of 3 mL/min.



Scheme S1. Outline of organic syntheses.

Selenohomocystine (1)

100-Mesh selenium powder (1.8 g, 23 mmol) was suspended in 35 mL absolute ethanol and cooled to 0 ºC. Sodium borohydride (0.87 g, 23 mmol) was added and the mixture was refluxed for 2 h (the solution became deep maroon in colour). Into the reaction vessel was added α-amino-4-bromobutanoic acid hydrobromide (3.00 g, 12 mmol), resulting in a rapid formation of an opaque yellow mixture. The reaction was stirred under reflux for 18 h and then quenched with 5 mL 2 M HCl. After removing bulk solvent by rotary evaporation, the resulting residue was mixed with 15 mL 5% HCl. This aqueous solution was then washed three times with 40

mL Et_2O and subjected to vacuum filtration to remove insoluble materials. A yellow solid was obtained after removing aqueous solvent *in vacuo*. This semi-crude product was then dissolved in 8 mL 1 M HCl and purified over Dowex® 50WX8 ion exchange resin. Activated resin (15 ml) was washed with 100 mL H_2O, followed by the loading of semi-crude product. The resin was then washed with an additional 100 mL H_2O to remove unbound impurities. Product was eluted from the resin using a 5% ammonium hydroxide solution. Fractions containing the desired product (bright yellow solution) were combined. After removing the bulk solvent, the resulting yellow solid was dried *in vacuo* to afford a yellow powder (1.3 g, 3.5 mmol, 60%). Characterisation matched the reported data.[3] ^{1}H NMR (400 MHz, D_2O with 0.1% TFA) δ 4.01 (t, 2H), 2.95 (td, 4H), 2.21 – 2.43 (m, 4H). HRMS [+ scan]: calculated m/z $C_8H_{17}N_2O_4Se_2$ 364.9513; observed 364.9514.

PropSeMet (Propargylic-L-Selenomethionine) (2)

1 (119 mg, 0.33 mmol) was dissolved in EtOH (20 mL) under a N_2 atmosphere, $NaBH_4$ (124 mg, 3.3 mmol) was added and the solution was stirred for 15 min at room temperature. $NaHCO_3$ (150 mg, 1.8 mmol) and 80% propargyl bromide in toluene (245 mg, 2.1 mmol) were added and the mixture was stirred for further 20 h at room temperature. The solvent was removed *in vacuo* and the crude product was dissolved in 5 mL mqH_2O supplemented with 1% TFA. The solution was adjusted to pH = 3 using HCl, the product was purified via HPLC, fractions containing the product were lyophilised resulting in a white powder (45 mg, 0.21 mmol, 31%). ^{1}H NMR (400 MHz, D_2O): δ 3.78 (t, 1H), 3.25 (d, 2H), 2.79 (t, 2H), 2.60 (t, 1H), 2.17 – 2.37 (m, 2H), ^{13}C NMR (100 MHz, D_2O): δ_C 173.1, 81.5, 72.4, 54.2, 31.3, 19.1, 6.4. HRMS [+ scan]: calculated m/z $C_7H_{12}NO_2Se$ 222.0024; observed 222.0028.

Hexaethylene glycol di(p-toluenesulfonate) (3)

Hexaethylene glycol (2.0 g, 7.1 mmol) was dissolved in DCM and cooled to 0 ºC, followed by addition of p-toluenesulfonyl chloride (3.0 g, 14 mmol) and KOH (3.2 g, 57 mmol). The solution was stirred for 3 h at 0 ºC and 30 min at room temperature and quenched with H_2O (20 ml). The product was extracted with DCM (3 x 20 ml), combined organic phases were washed with brine (20 ml) and dried ($MgSO_4$). Organic solvents were removed *in vacuo*, the product was obtained as a colourless oil (3.9 g, 6.5 mmol, 92%). ^{1}H NMR (400MHz, $CDCl_3$): δ 7.78 (d, 4H), 7.33 (d, 4H), 4.14 (t, 4H), 3.67 (br tr, 4H, 3.60 (br s, 8H), 3.57 (br s, 8H), 2.43 (br s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 144.8, 133.0, 129.8, 128.0, 70.5-70.7 (Multiple PEG peaks), 69.3, 68.7, 21.7. HRMS [+ scan]: calculated m/z for $C_{26}H_{39}O_{11}S_2$ 591.1934; observed 591.1931.

Hexaethylene glycol p-toluenesulfonate azide (4)

3 (2.0 g, 3.4 mmol) was dissolved in anhydrous DMF (10 ml). Sodium azide (242 mg, 3.7 mmol) was added, the mixture was placed under N_2 and stirred for 18 h at 55 ºC. Solvent was removed *in vacuo*, products were purified via flash column chromatography (gradient 1:1 Pet. Ether:AcOEt to AcOEt). The product was obtained as a pale-yellow oil (440 mg, 0.95 mmol, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, 2H), 7.36 (d, 2H), 4.18 (t, 2H), 3.59 – 3.73 (20H, PEG), 3.41 (t, 2H), 2.47 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 144.8, 133.0, 129.3, 128.0,

70.5-70.8 (Multiple PEG peaks), 70.0, 69.3, 68.7, 50.7, 21.7. HRMS [+ scan]: calculated m/z for $C_{19}H_{31}N_3NaO_8S$ 484.1730; observed 484.1723.

Tetraethylene glycol p-toluenesulfonate azide (5)

Tetraethylene glycol di(p-toluenesulfonate) (2.7 g, 5.4 mmol) was dissolved in anhydrous DMF (10 ml). Sodium azide (355 mg, 5.4 mmol) was added and the mixture was placed under N_2 and stirred for 18 h at 55 ºC. Solvent was removed *in vacuo*, products were purified via flash column chromatography (3:1 Pet. Ether:AcOEt to 1:1 Pet. Ether:AcOEt). The product was obtained as a colourless oil (798 mg, 2.1 mmol, 39%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, 2H), 7.37 (d, 2H), 4.19 (t, 1H), 3.60 – 3.73 (12H, PEG), 3.40 (t, 2H), 2.47 (s, 3H). HRMS [+ scan]: calculated m/z for $C_{15}H_{23}N_3NaO_6S$ 396.1205; observed 396.1207.

Diethylene glycol p-toluenesulfonate azide (6)

Diethylene glycol di(p-toluenesulfonate) (2.3 g, 5.4 mmol) was dissolved in anhydrous DMF (10 ml). Sodium azide (353 mg, 5.4 mmol) was added and the mixture was placed under N_2 and stirred for 18 h at 55 ºC. Solvent was removed *in vacuo*, products were purified via flash column chromatography (3:1 Pet. Ether:AcOEt to 1:1 Pet. Ether:AcOEt). The product was obtained as a colourless oil (681 mg, 2.4 mmol, 44%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, 2H), 7.35 (d, 2H), 4.17 (t, 2), 3.70 (t, 2H), 3.61 (t, 2H), 3.32 (t, 2H), 2.45 (s, 3H). HRMS [+ scan]: calculated m/z for $C_{11}H_{15}N_3NaO_4S$ 308.0681; observed 308.0670.

Click-degrader 1 (Hexaethylene glycol imidazolate azide) (7)

Imidazole (44 mg, 0.65 mmol) and NaH (60% dispersion in mineral oil, 26 mg, 0.65 mmol) were suspended in anhydrous DMF (2 ml) at 0 ºC. The mixture was placed under N_2 atmosphere, allowed to warm to room temperature and stirred for 30 min. **4** (250 mg, 0.54 mmol) was dissolved in anhydrous DMF (3 ml) and the resulting solution was added to the first mixture. It was then stirred for 20 h at 55 ºC. Solvent was then removed *in vacuo* and the resulting residue was purified via flash chromatography (dry loading, gradient EtOAC to 9:1 EtOAc: MeOH). The product was obtained as a colourless oil (154 mg, 0.43 mmol, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52 (s, 1H), 7.02 (s, 1H), 6.98 (s, 1H), 4.09 (t, 2H), 3.72 (t, 2H), 3.55 – 3.78 (18H, PEG), 3.36 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 137.6, 129.3, 119.4, 70.6-70.7 (Multiple PEG peaks), 70.0, 50.7, 47.0. HRMS [+ scan]: calculated m/z for $C_{15}H_{28}N_5O_5$ 358.2090; observed 358.2084.

Click-degrader 2 (Tetraethylene glycol imidazolate azide) (8)

Imidazole (18 mg, 0.27 mmol) and NaH (60% dispersion in mineral oil, 12 mg, 0.27 mmol) were suspended in anhydrous DMF (1 ml) at 0°C. The mixture was placed under N_2 atmosphere, allowed to warm to room temperature and stirred for 30 min. **5** (100 mg, 0.27 mmol) was dissolved in anhydrous DMF (1 ml) and the resulting solution was added to the first mixture. It was then stirred for 20 h at 55 ºC. Solvent was then removed *in vacuo* and the resulting residue was purified via flash chromatography (dry loading, gradient EtOAC to 9:1 EtOAc: MeOH). The product was obtained as a colourless oil (54 mg, 0.20 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 7.05 (s, 1H), 7.05 (s, 1H), 4.12 (t, 2H), 3.75 (t, 2H), 3.60 – 3.71 (10H, PEG), 3.39 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 137.6, 129.2, 119.4,

70.5-70.7 (multiple PEG peaks), 70.0, 50.7, 47.1. HRMS [+ scan]: calculated m/z for $C_{11}H_{19}N_5O_3$ 270.1566; observed 270.1582.

Click-degrader 3 (Diethylene glycol imidazolate azide) (9)

Imidazole (93 mg, 1.4 mmol) and NaH (60% dispersion in mineral oil, 55.0 mg, 1.4 mmol) were suspended in anhydrous DMF (2 ml) at 0 ºC. The mixture was placed under N_2 atmosphere, allowed to warm to room temperature and stirred for 30 min. **6** (300 mg, 1.1 mmol) was dissolved in anhydrous DMF (3 mL) and the resulting solution was added to the first mixture. It was then stirred for 20 h at 55 ºC. Solvent was then removed *in vacuo* and the resulting residue was purified via flash chromatography (dry loading, gradient EtOAC to 9:1 EtOAc: MeOH). The product was obtained as a colourless oil (139 mg, 0.77 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.06 (s, 1H), 6.99 (s, 1H), 4.14 (t, 2H), 3.75 (t, 2H), 3.60 (t, 2H), 3.36 (t, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 137.5, 129.4, 119.4, 70.6, 70.1, 50.7, 47.1. HRMS [+ scan]: calculated m/z for $C_{11}H_{19}N_5O_3$ 182.1042; observed 182.1041.

N^6 -Acetyl-2´,3´,5´-tri-O-acetyladenosine (10)

To adenosine (2.0 g, 7.5 mmol) were added pyridine (15 mL), and Ac_2O (7.0 mL, 74 mmol), the resulting white cloudy mixture was stirred at room temperature overnight. The resulting solution was heated at 55 ºC overnight. The reaction was cooled down and quenched by addition of excess of EtOH. The solvent was evaporated *in vacuo*. To remove traces of pyridine the residue was co-evaporated successively with portions of EtOH. The resultant foam was dissolved in MeOH (20 mL); imidazole (0.3 g, 4.4 mmol) was added and the solution was stirred at room temperature overnight. The solution was diluted with DCM (150 mL) and washed with brine (4 × 40 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield the product as a white foam (3.1 g, 7.1 mmol, 95%). ^{1}H NMR ($CDCl_3$, 400MHz): δ 9.79 (br s, 1H), 8.66 (s, 1H,), 8.33 (s, 1H), 6.22 (d, 1H), 5.95 (dd, 1H), 5.65 (dd, 1H), 4.45– 4.52 (m, 2H), 4.33 (dd, 1H), 2.59 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.3, 170.4, 169.6, 169.4, 152.5, 151.2, 149.6, 141.9, 122.3, 86.3 80.3, 73.1, 70.6, 63.1, 25.7, 20.7, 20.5, 20.4. HRMS [+ scan]: calculated m/z for $C_{18}H_{22}N_5O_8$ 436.1468; observed 436.1459.

N^6 -Acetyl-2´,3´,5´-tri-O-acetyl-N^6 -propargyladenosine (11)

To a stirred solution of **10** (3.1 g, 7.1 mmol) in anhydrous MeCN under Ar-atmosphere, DBU (3.2 mL, 21.4 mmol) and 80% propargyl bromide in toluene (2.0 mL, 18 mmol) were added at r.t.. The resulting brown mixture was stirred at room temperature for 8 h. The mixture was diluted with 150 mL DCM and extracted with 50 mL 0.5 M HCl and three times with 100 mL brine. The organic layer was dried ($MgSO_4$) and the solvents were removed to yield the product as a brown foam (2.7 g, 5.7 mmol, 80.4%). ^{1}H NMR ($CDCl_3$, 400MHz): δ 8.84 (s, 1H), 8.24 (s, 1H), 6.27 (d, J = 5.1 Hz), 5.99 (dd, 1H), 5.70 (dd, 1H), 5,11 (dd, 2H), 4.45 – 4.54 (m, 2H), 4.37 – 4.45 (m, 1H) 2.40 (s, 3H), 2.18 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 2.02 (s, H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.9, 170.3, 169.6, 169.4, 152.6, 152.5, 152.2, 142.3, 126.9, 86.8, 80.4, 79.2, 73.1, 71.6, 70.5, 63.1, 36.4, 24.4, 20.8, 20.5, 20.4. HRMS [+ scan]: calculated m/z for $C_{19}H_{24}N_5O_8$ 450.1619; observed 450.1626.

N^6 -Propargyladenosine (12)

11 (2.7 g, 5.7 mmol) was dissolved in 25 mL 8M $MeNH_2$ in EtOH under N_2 atmosphere. The solution was stirred for 3 h at room temperature. The solvents were removed, and the residue was dissolved in 180 mL EtOAc and 20 ml EtOH. The organic layer was extracted with 180 mL brine and the aqueous layer was washed with 10x100 mL of EtOAc. The organic layers were dried ($MgSO_4$) and the solvents were removed to yield a yellow solid. 100 mL EtOAc and 100 mL Et_2O were added to the residue and the mixture was kept at 4 ºC for 16 h. The white precipitate was collected by filtration and washed with Et_2O to yield the product as a white powder (1.4 g, 4.6 mmol, 81 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.40 (s, 1H), 8.29 (s, 1H), 8.23 (br s, 1H), 5.91 (d, 1H), 5.49 (d, 1H), 5.40 (dd, 1H), 5.23 (d, 1H), 4.61 (dd, 1H), 4.28 (dd, 1H), 3.98 (dd, 1H), 3.69 (m, 1H), 3.56 (m, 1H), 3.03 (br s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 154.4, 152.7, 149.3, 140.7, 120.4, 88.4, 86.3, 82.3, 74.0, 72.9, 71.0, 62.1, 29.6. HRMS [+ scan]: calculated m/z for $C_{13}H_{15}N_5O_4$ 306.1202; observed 306.1246.

2'-*O*-(*tert*-butyldimethylsilyl)-3',5'-*O*-(di-*tert*-butylsilylene)-N^6-propargyladenosine (13)

12 (1.1 g, 3.7 mmol) was dissolved in anhydrous DMF (20 ml) and cooled to 0 ºC, followed by dropwise addition of di-tert-butylsilyl bis(trifluoromethane sulfonate) (1.4 ml, 4.1 mmol). Mixture was stirred for 30 min at 0 ºC, then 15 min at room temperature. Imidazole (1.0 g, 15 mmol) was then added, followed by TBDMSCl (1.1 g, 7.4 mmol). Mixture was stirred for 1 h at room temperature, then for 3 h at 60 ºC. Then most of the solvent was removed in vacuo and dissolved in 200 mL Et_2O and washed three times with 40 mL H_2O. Organic layer was dried over $MgSO_4$ and filtered; solvent was removed in vacuo. Crude was purified via flash chromatography (3:1 petroleum ether: AcOEt). The product 1.7 g (3.0 mmol) of white powder were obtained, corresponding to yield of 80%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.86 (s, 1H), 6.79 (t, 1H), 5.92 (s, 1H), 4.60 (s, 1H), 4.40 – 4.55 (m, 4H), 4.21 (dt, 1H), 4.03 (m, 1H), 2.24 (t, 1H), 1.65 (s, 3H), 1.07 (s, 9H), 1.03 (s, 9H), 0.92 (s, 9H), 0.16 (s, 3H), 0.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 154.1, 153.2, 148.7, 138.5, 120.6, 92.4, 80.3, 75.8, 75.5, 74.7, 71.3, 67.8, 27.5, 27.0, 25.9, 22.7, 20.3, 18.3, -4.3, -5.0. HRMS [+ scan]: calculated m/z for $C_{27}H_{46}N_5O_4Si_2$ 560.3088; observed 560.3099.

2'-*O*-(*tert*-butyldimethylsilyl)-N^6-propargyladenosine (14)

13 (1.23 g, 2.2 mmol) was dissolved in anhydrous THF (30 mL) and cooled to 0 ºC. HF-pyridine (1.2 mL) was diluted with pyridine (1.2 mL), added to the reaction mixture and allowed to war to room temperature, followed by stirring for 30 min. Reaction was then quenched with pyridine (2 mL) and DCM (60 mL). It was then washed with saturated $NaHCO_3$ solution (40 mL) and brine (2 x 40 mL). Organic layers were dried with $MgSO_4$ and solvents were removed in vacuo. Crude product was purified via column chromatography (dry loading, eluent gradient 5:1 petroleum ether: AcOEt to AcOEt). The product was obtained as a white powder (440 mg, 1.1 mmol, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.77 (s, 1H), 6.62 (d, 1H), 6.11 (br s, 1H), 5,75 (d, 1H), 5.14 (dd, 1H), 4.49 (s, 1H), 4.34 – 4.37 (m, 2H), 3.95 (d, 1H), 3.74 (t, 1H), 2.82 (s, 1H), 2.30 (t, 1H), 0.80 (s, 9H), -0.15, (s, 3H), -0.36 (s, 3H). HRMS [+ scan]: calculated m/z for $C_{19}H_{30}N_5O_4Si$ 420.2067; observed 420.2082.

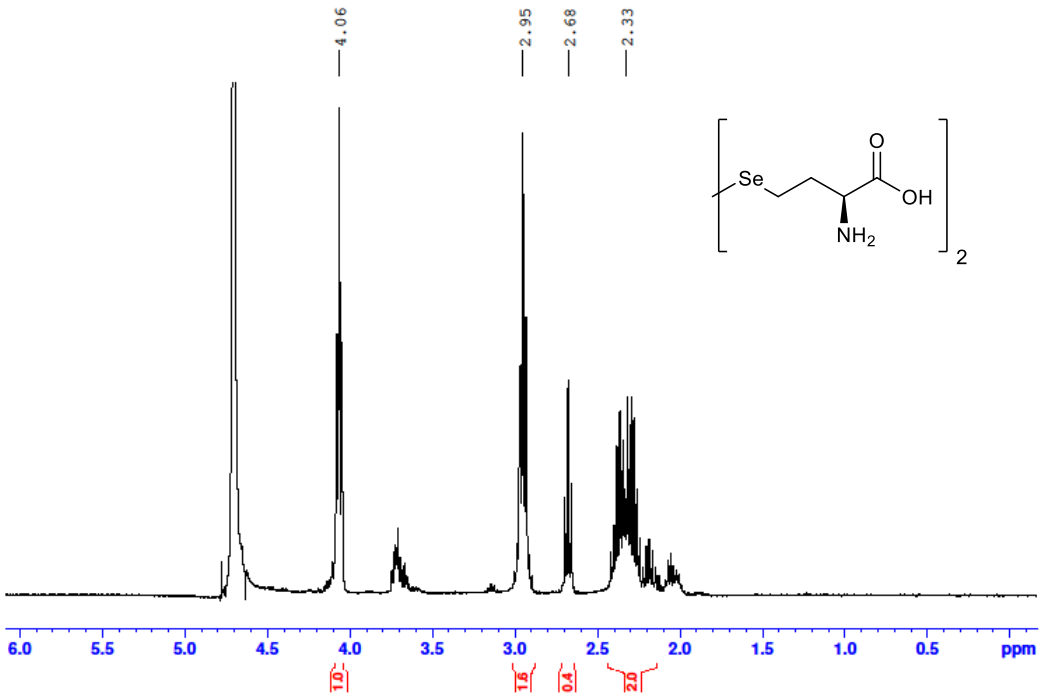
2'-*O*-(*tert*-Butyldimethylsilyl)-5'-*O*-(4,4'-dimethoxytrityl)-N^6-proaprgyladenosine (15)

14 (410 mg, 0.98 mmol) was dissolved in anhydrous pyridine (10 mL), DMTCl (390 mg, 1.1 mmol) and DMAP (23 mg, 0.20 mmol) were added, mixture was placed under N_2 atmosphere and stirred overnight at room temperature for 18 h. The solvent was removed in vacuo and the residue was purified on a column (gradient, DCM + 1% NEt_3 to 4:1 DCM:AcOEt + 1% NEt_3). The product was obtained as a white powder (497 mg, 0.69 mmol, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.99 (s, 1H), 7.45 (d, 2H), 7.34 (d, 4H), 7.18-7.31 (m, 3H), 6.81 (d, 4H) 6.02 (d, 1H), 5.86 (br s, 1H), 4.99 (t, 1H), 4.48 (br s, 2H), 4.34 (m, 1H), 4.25 (m, 1H), 3.79 (s, 6H), 3.52 (dd, 1H), 3.38 (dd, 1H), 2.70 (d, 1H), 2.28 (t, 1H), 0.84 (s, 9H), -0.01, (s, 3H), -0.13 (s, 3H). HRMS [+ scan]: calculated m/z for $C_{40}H_{47}N_5NaO_6Si$ 744.3193; observed 744.3191.

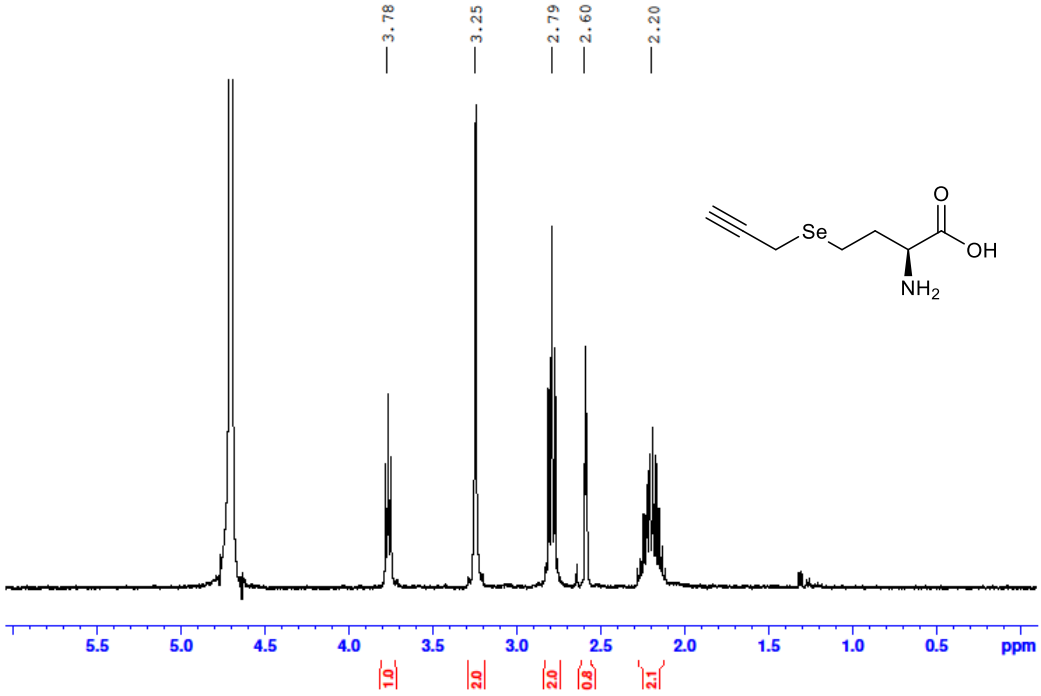
2'-*O*-(*tert*-Butyldimethylsilyl)-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphino)-5'-*O*-(4,4'-dimethoxytrityl)-N^6-propargyladenosine (16)

15 (325 mg, 0.44 mmol) was dissolved in DCM (7 mL) and degassed, DIPEA (140 mL, 1.8 mmol) and CEPCl (140 mg, 1.3 mmol) were added to the mixture and stirred for 24 h at room temperature. The crude mixture was directly purified via a column (8:1 DCM:Acetone + 1% NEt_3). The product was obtained as a white product (284 mg, 0.30 mmol, 68%). ^{1}H NMR (500 MHz, $CDCl_3$, reported for the major of the two diastereomers) δ 8.31 (s, 1H), 7.98 (s, 1H), 7.46 (d, 2H), 7.35 (d, 4H), 7.19-7.29 (m, 3H), 6.81 (d, 4H) 6.01 (d, 1H), 5.84 (br s, 1H), 5.07 (m, 1H), 4.47 (br s, 2H), 4.24 - 4.32 (m, 3H), 3.78 (s, 6H), 3.52 – 3.68 (dd, 5H), 3.31 (m, 1H), 2.65 (m, 2H), 2.29 (m, 2H), 1.15 – 1.21 (m, 12 H), 0.76 (s, 9H), -0.05, (s, 3H), -0.21 (s, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.0, 150.9 HRMS [+ scan]: calculated m/z for $C_{49}H_{64}N_7NaO_7PSi$ 944.4271; observed 944.4258.

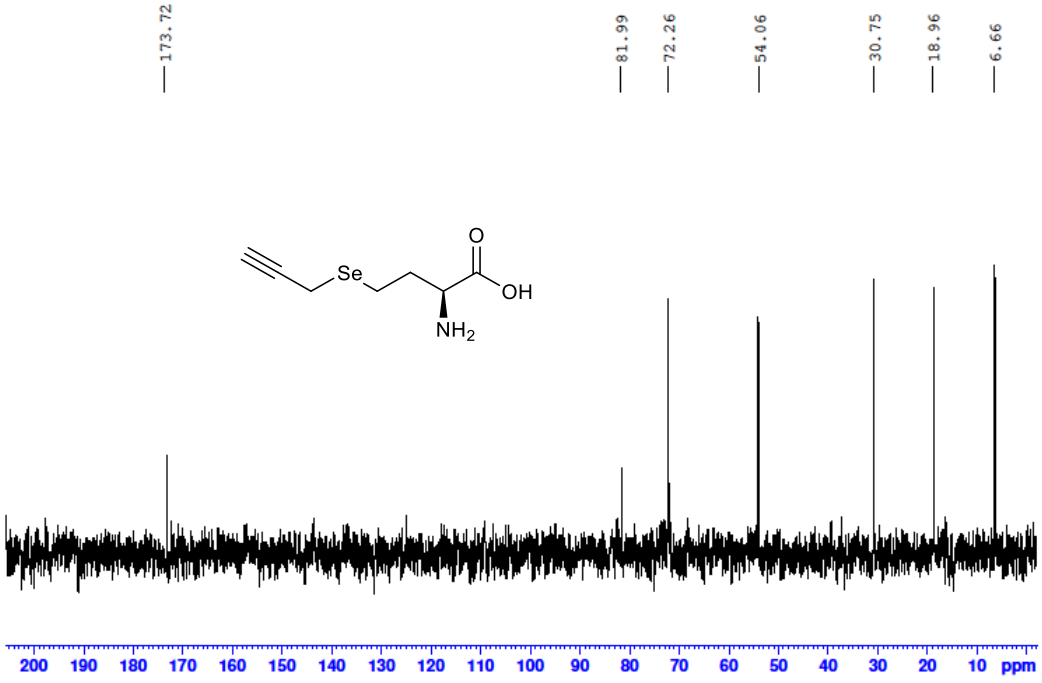
NMR Spectra



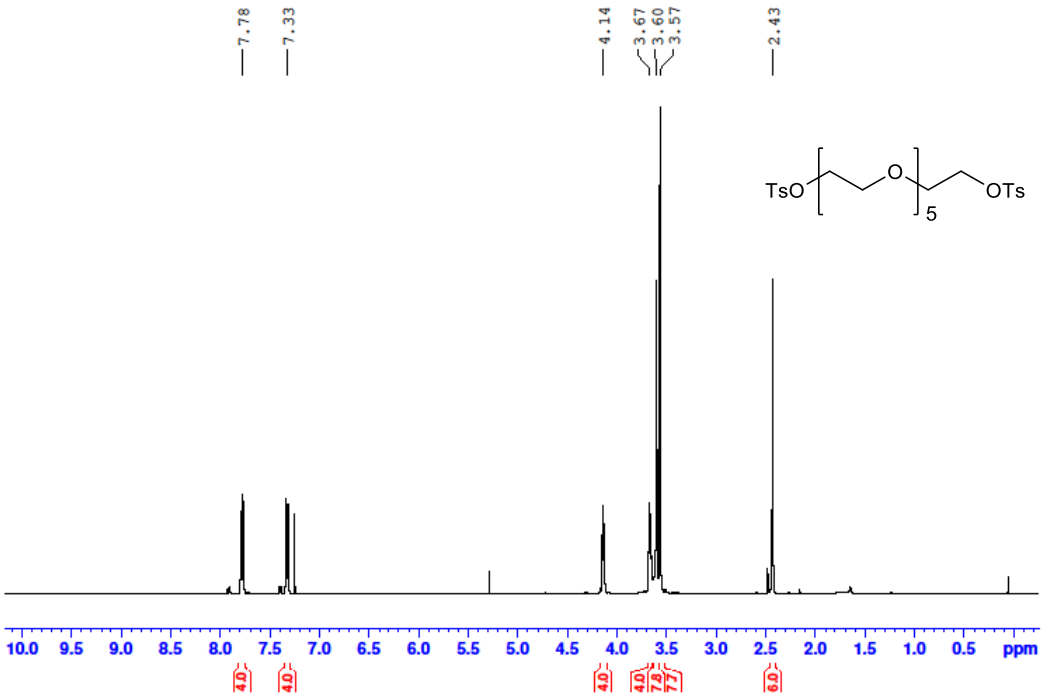
^{1}H NMR (400 MHz, D_2O with 0.1% TFA) spectrum of **1**.



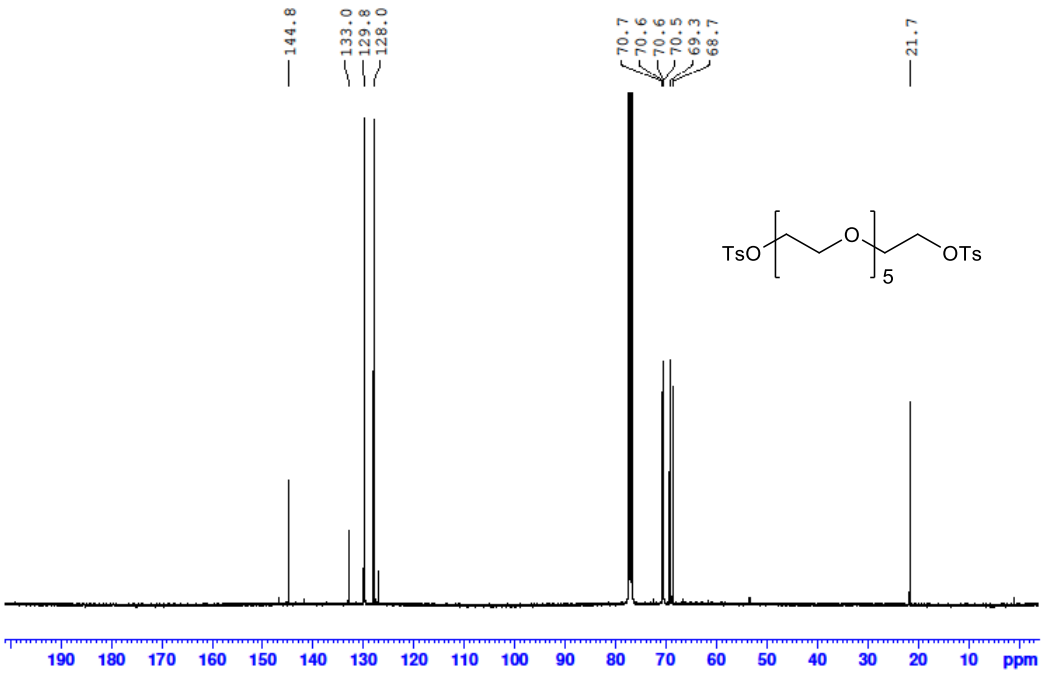
^{1}H NMR (400 MHz, D_2O) spectrum of **2**.



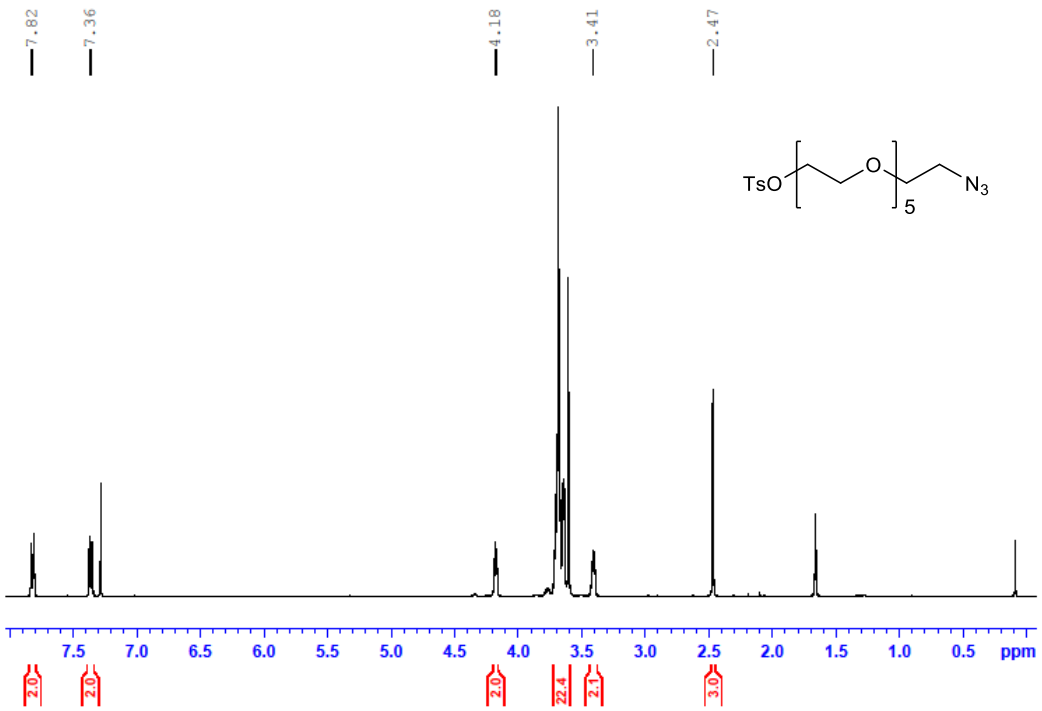
^{13}C NMR (100 MHz, D_2O) spectrum of **2**.



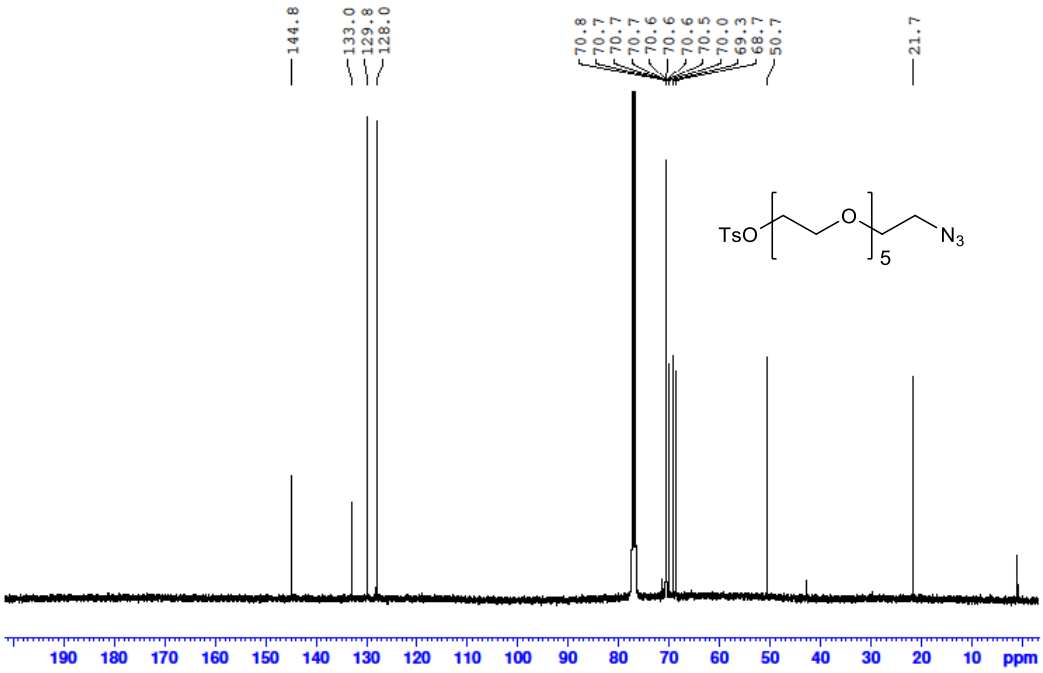
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **3**.



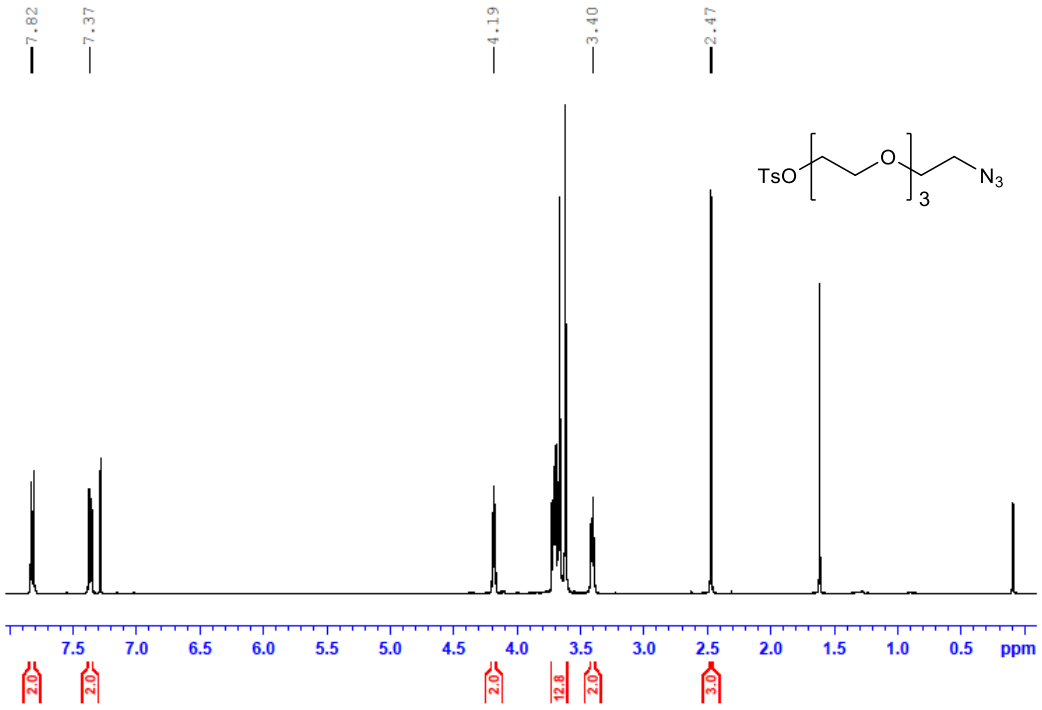
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **3**.



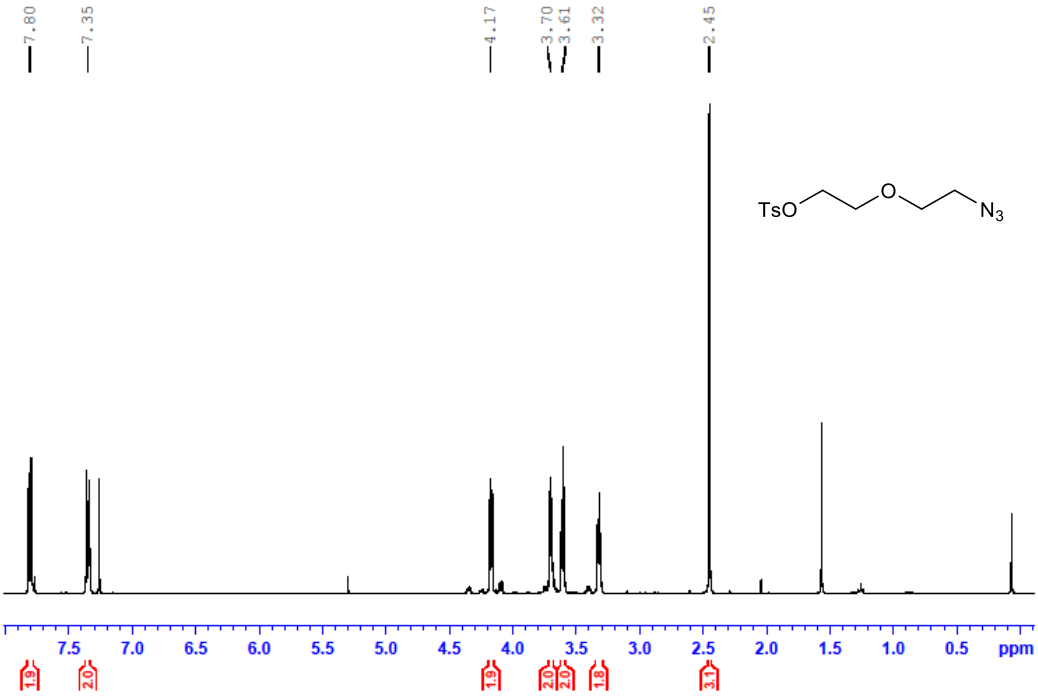
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **4**.



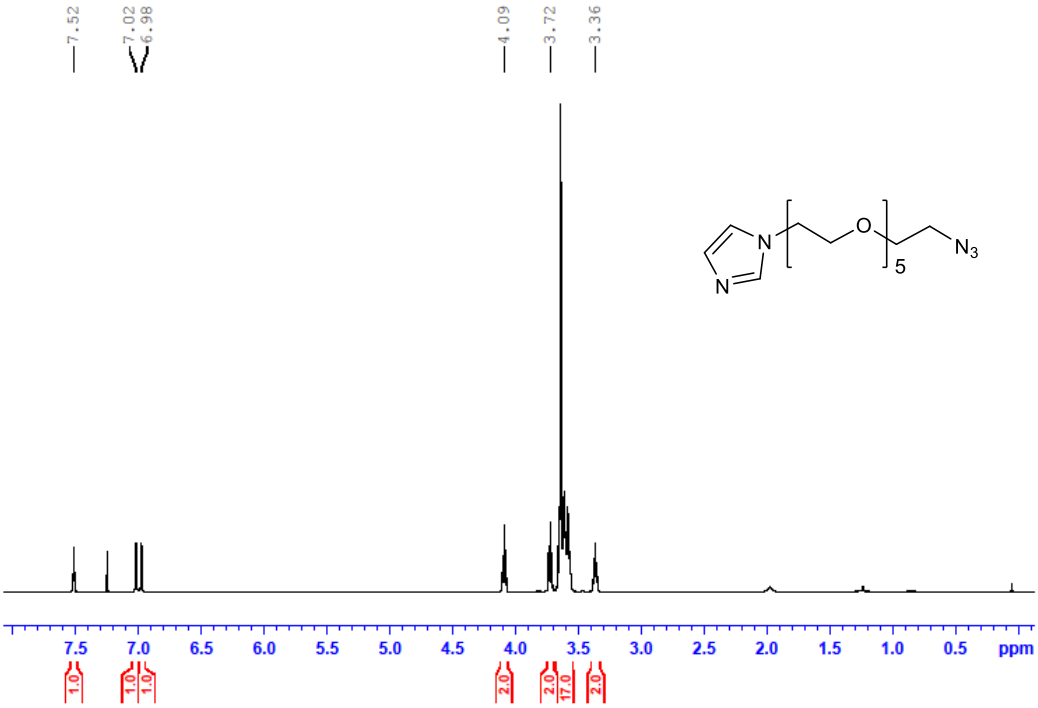
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **4**.



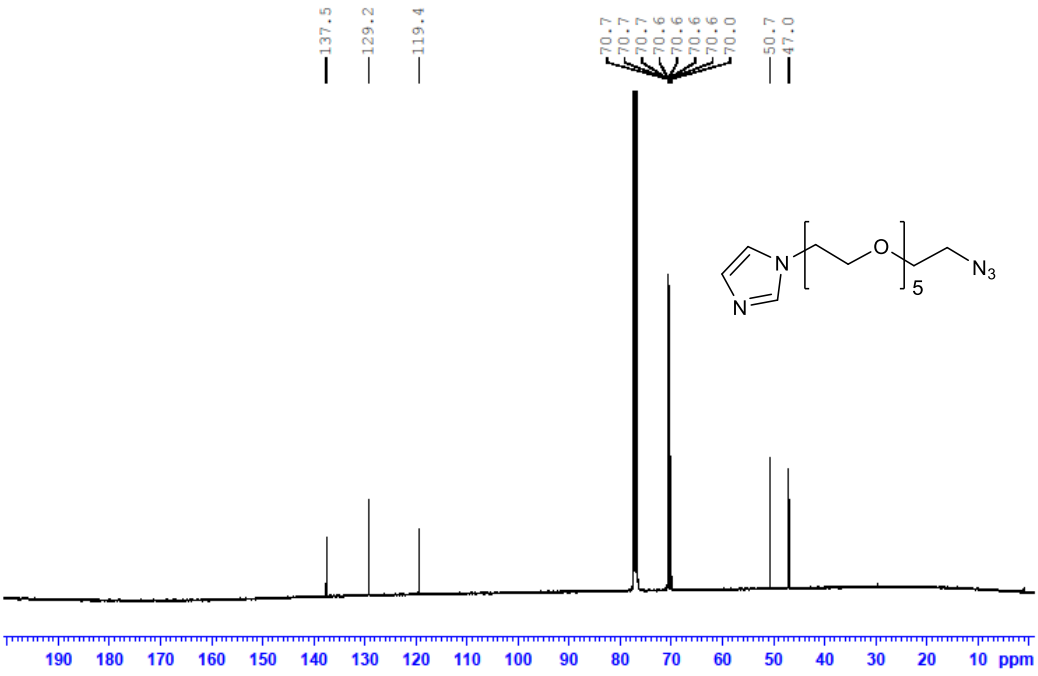
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **5**.



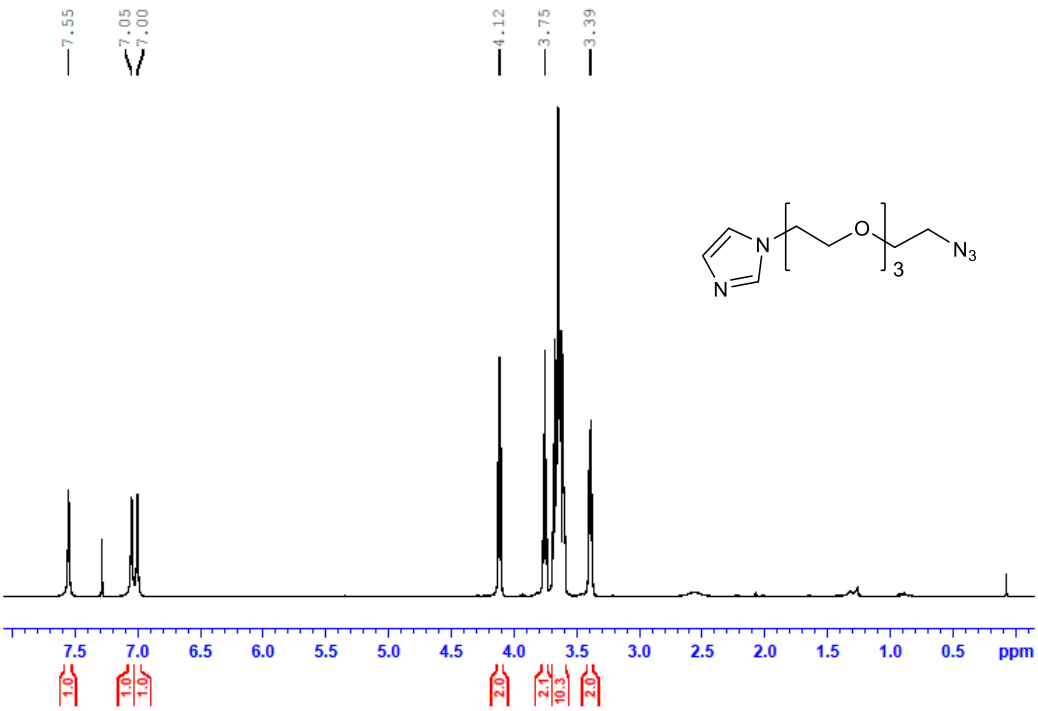
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **6**.



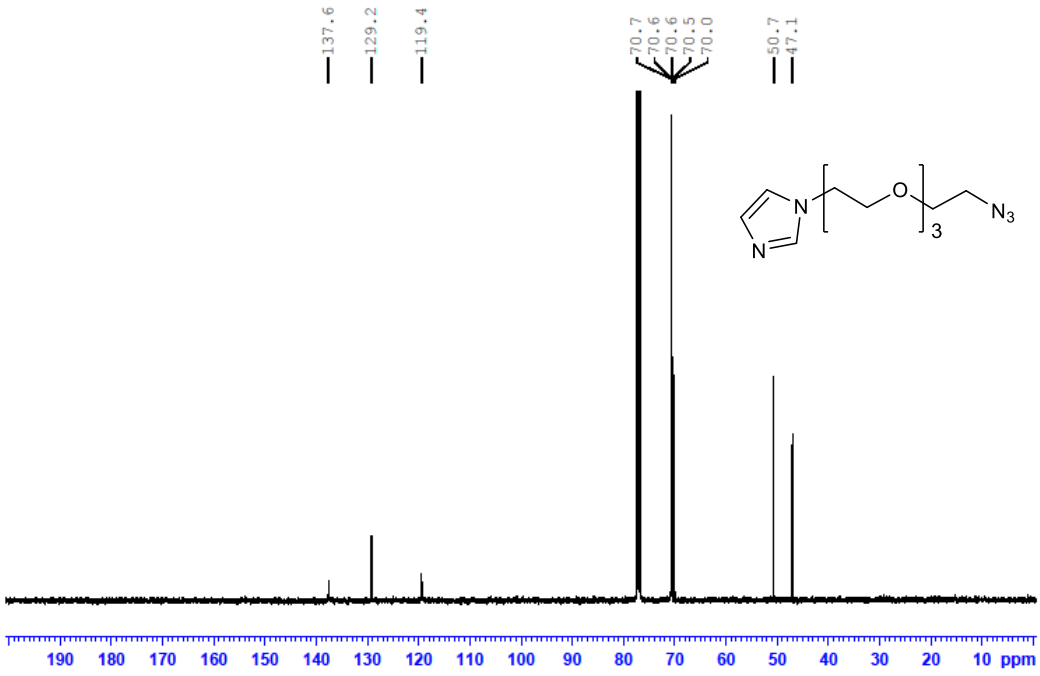
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **7**.



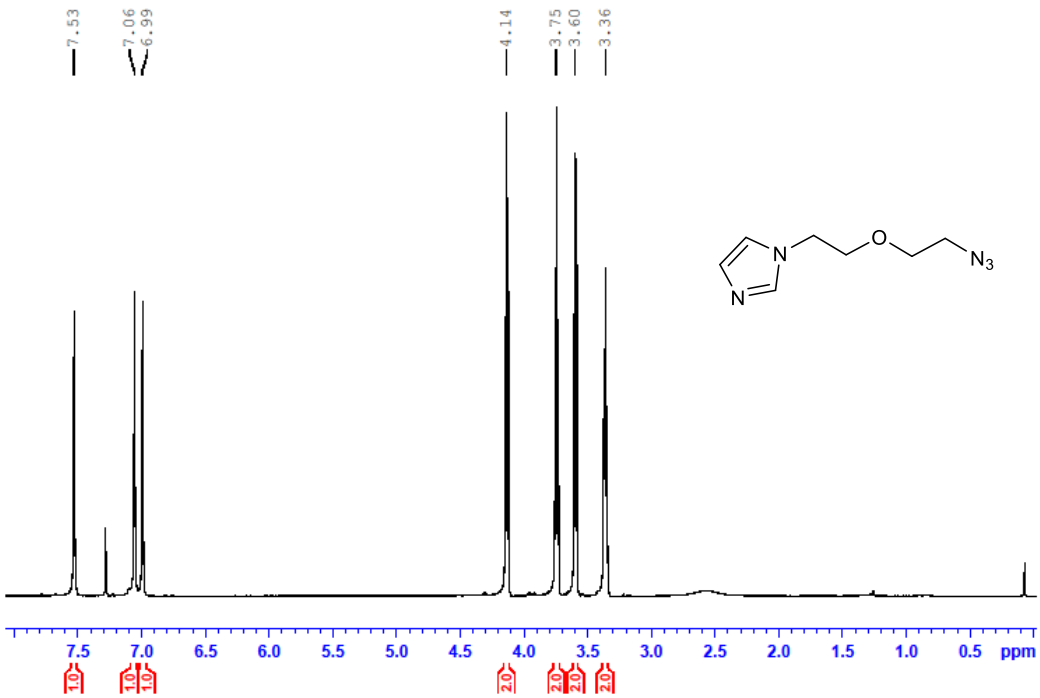
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **7**.



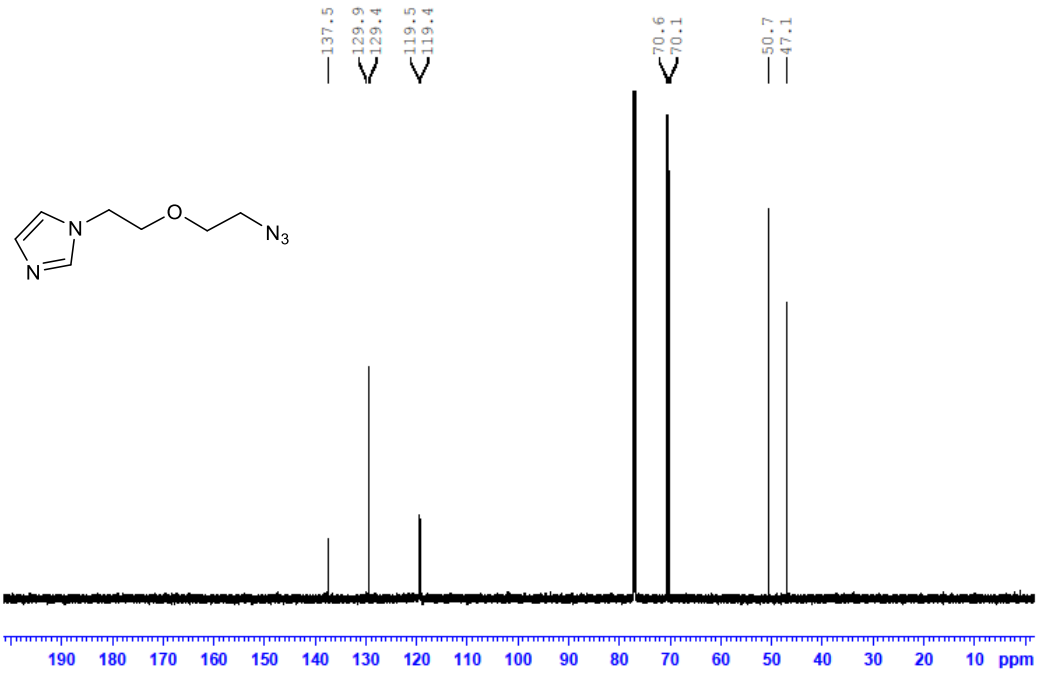
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **8**.



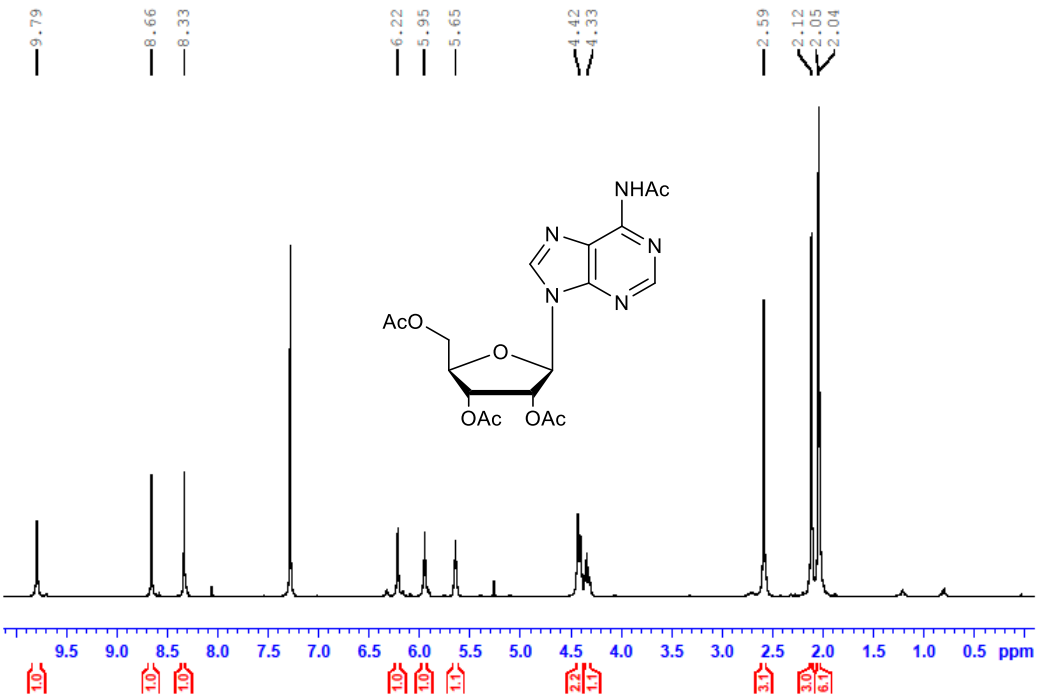
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **8**.



^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **9**.



^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **9**.



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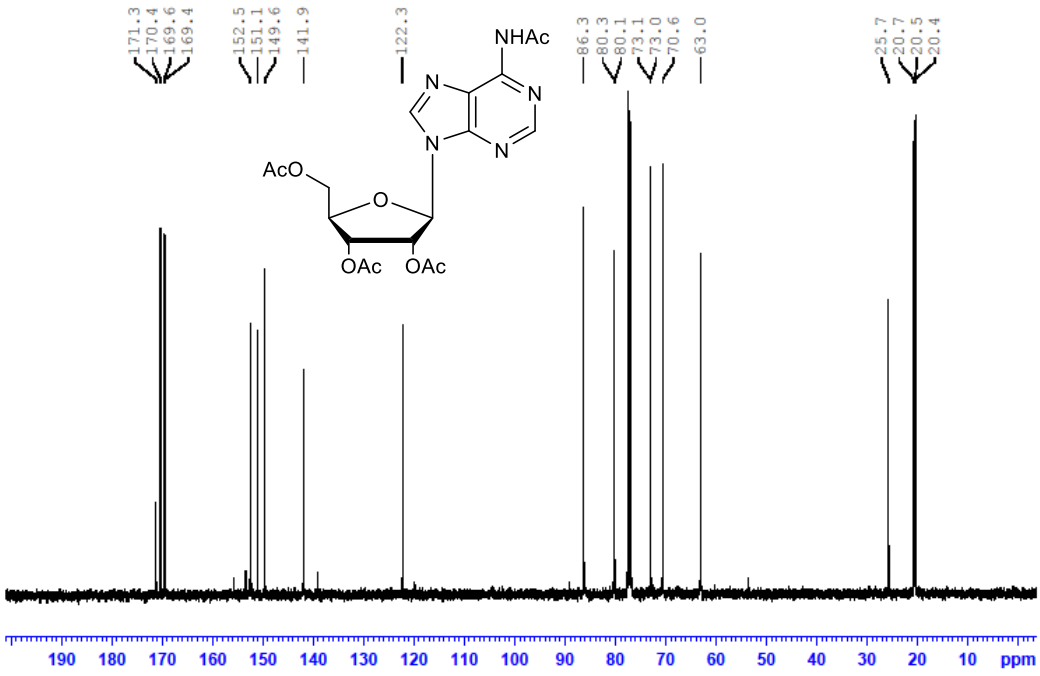
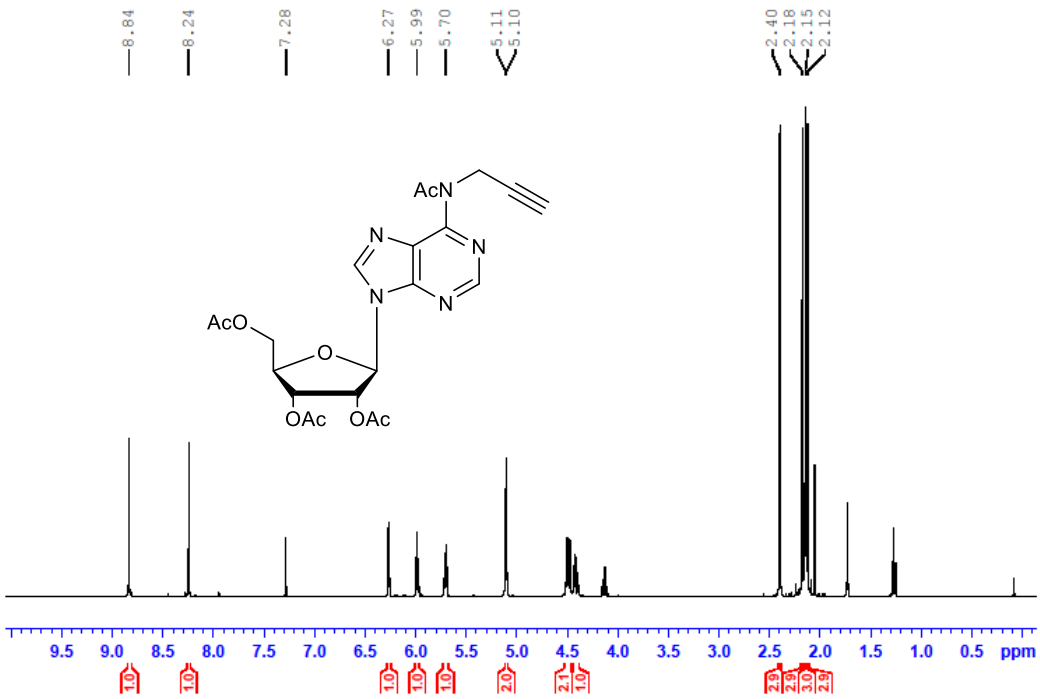
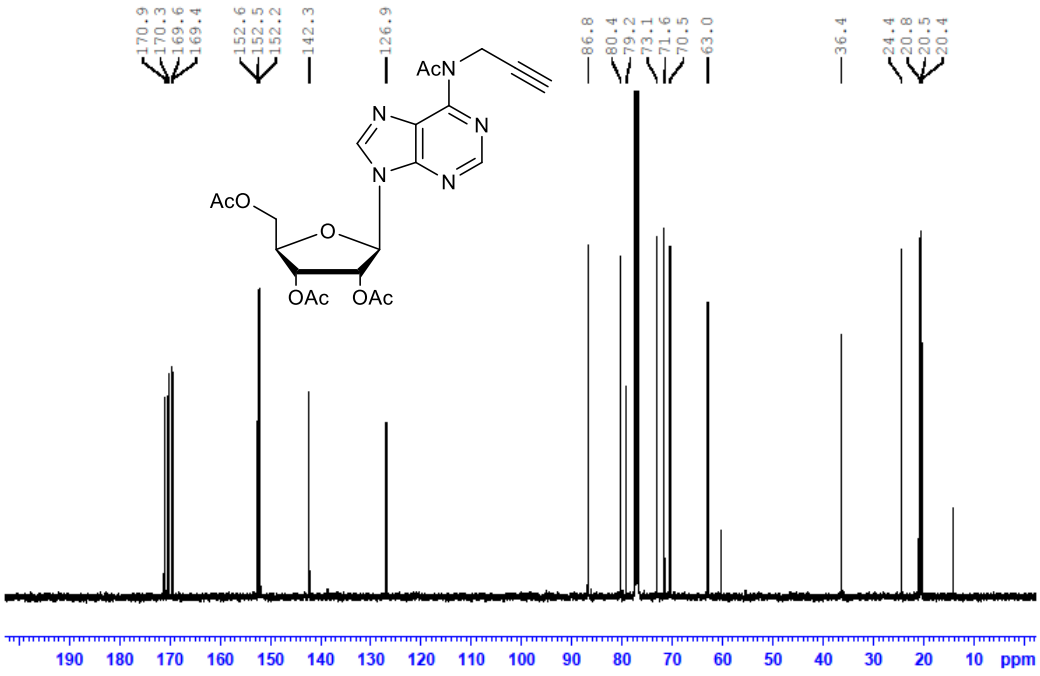


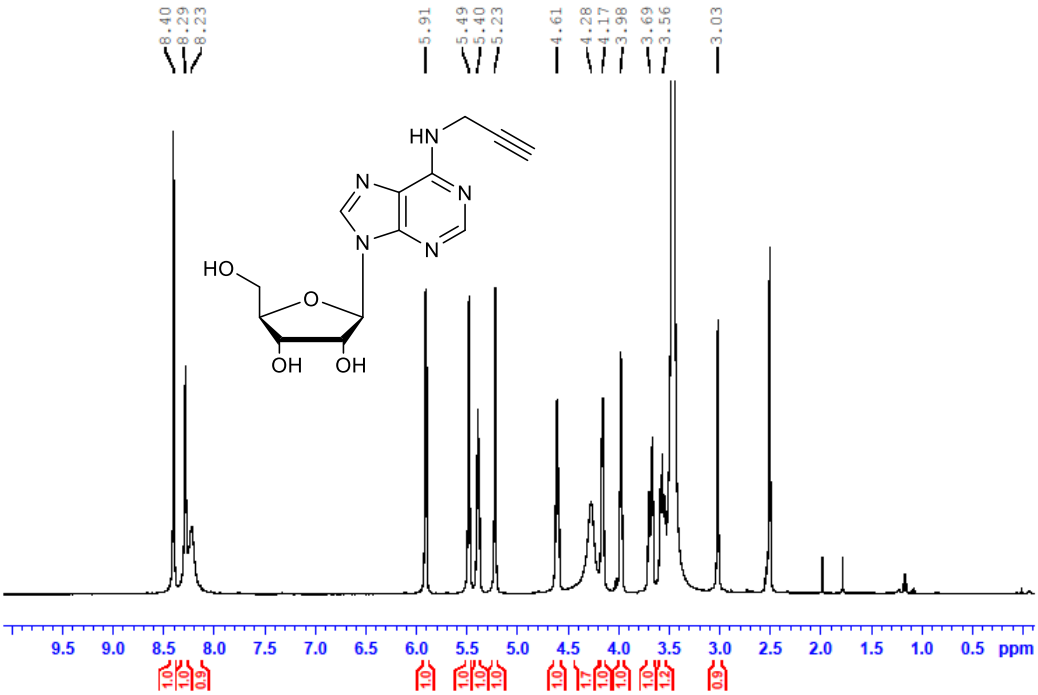
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **10**.



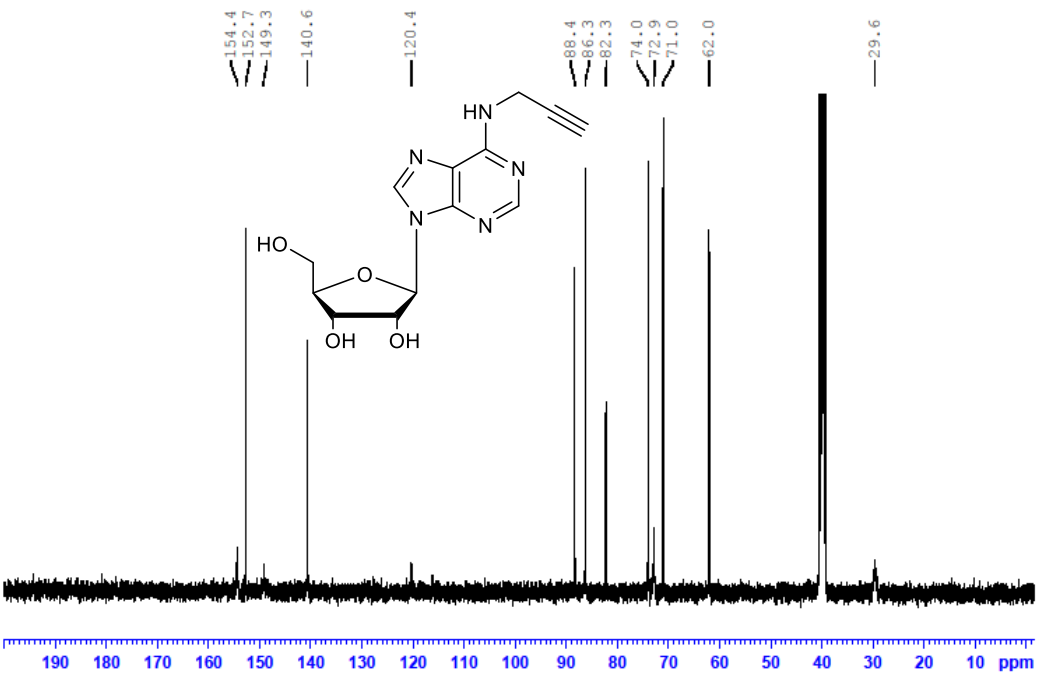
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **11.**



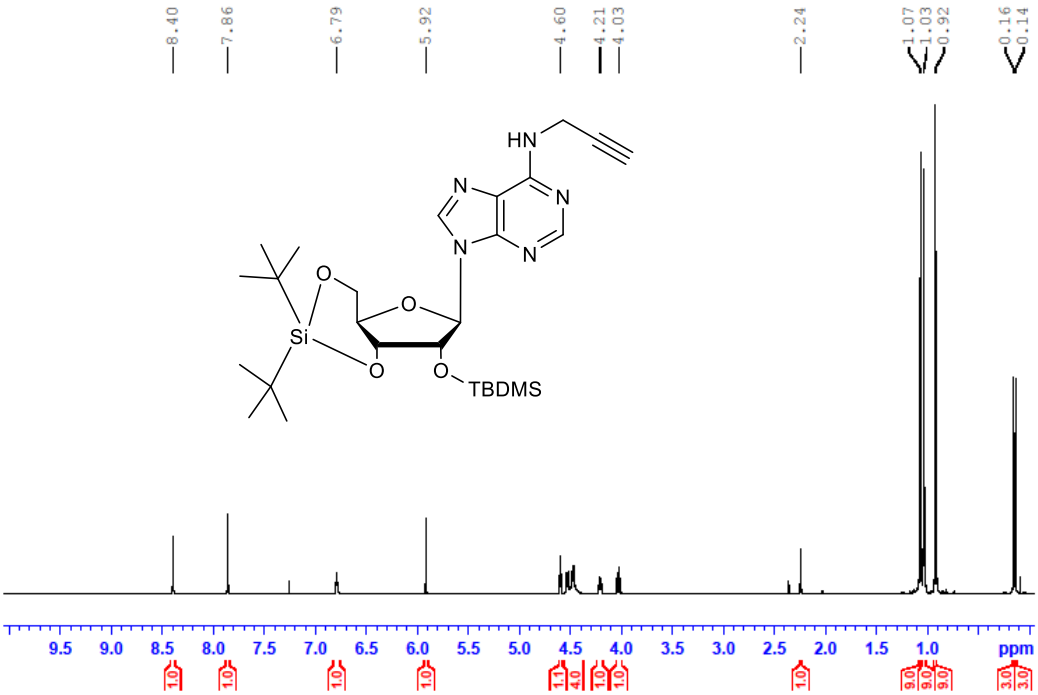
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **11**.



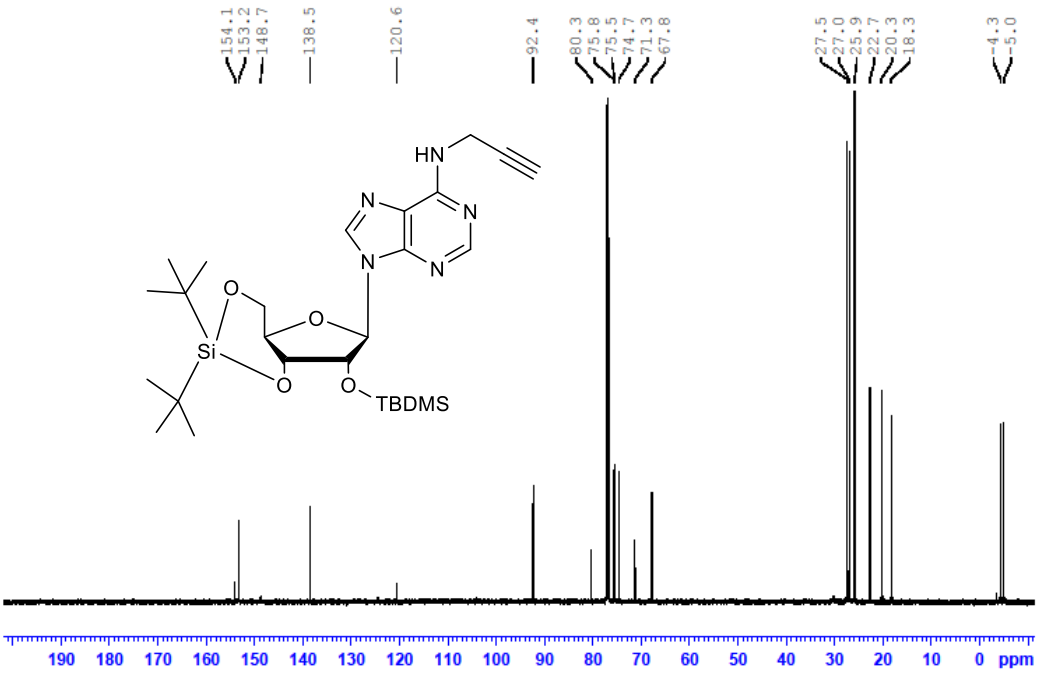
^{1}H NMR (400 MHz, DMSO-d_6) spectrum of **12**.



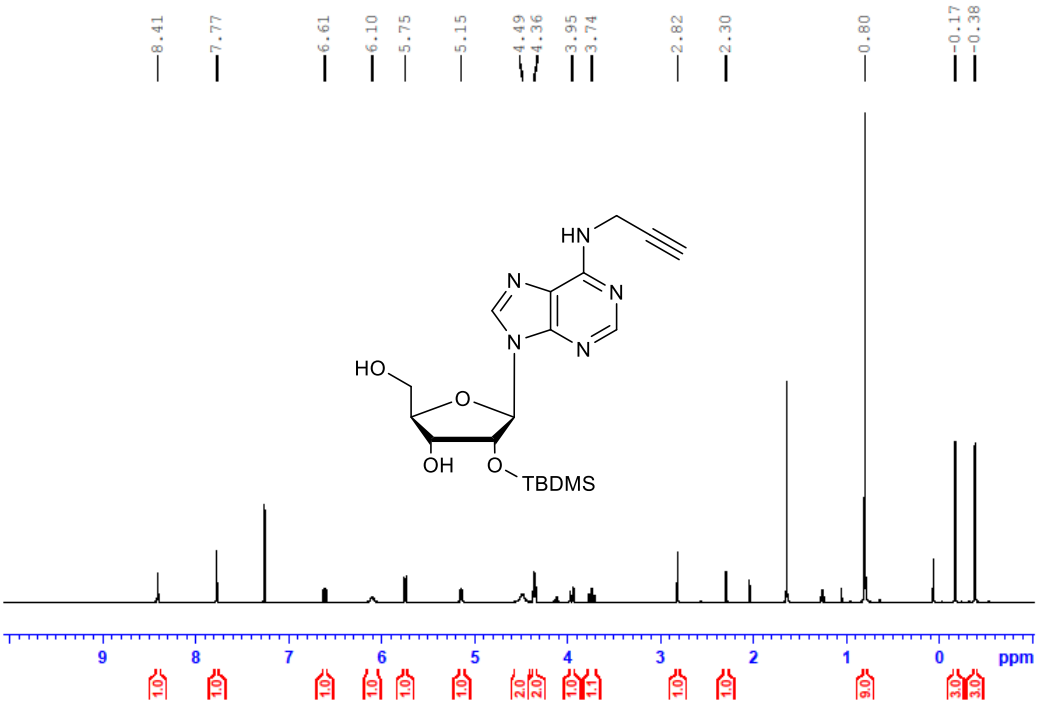
^{13}C NMR (100 MHz, DMSO-d_6) spectrum of **12.**



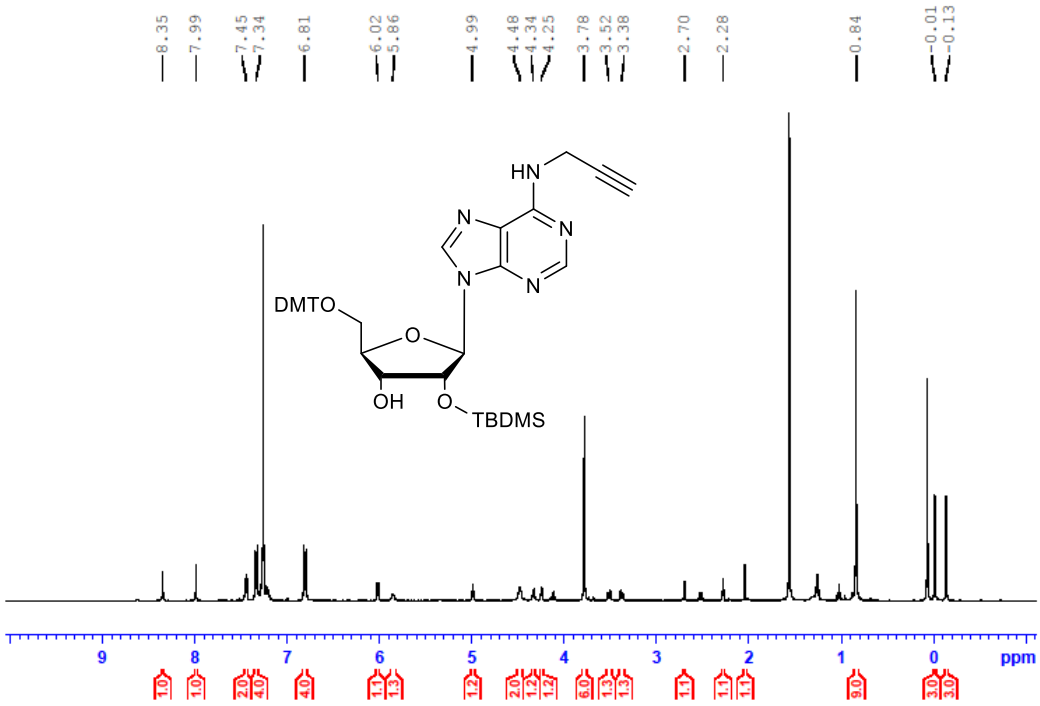
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **13**.



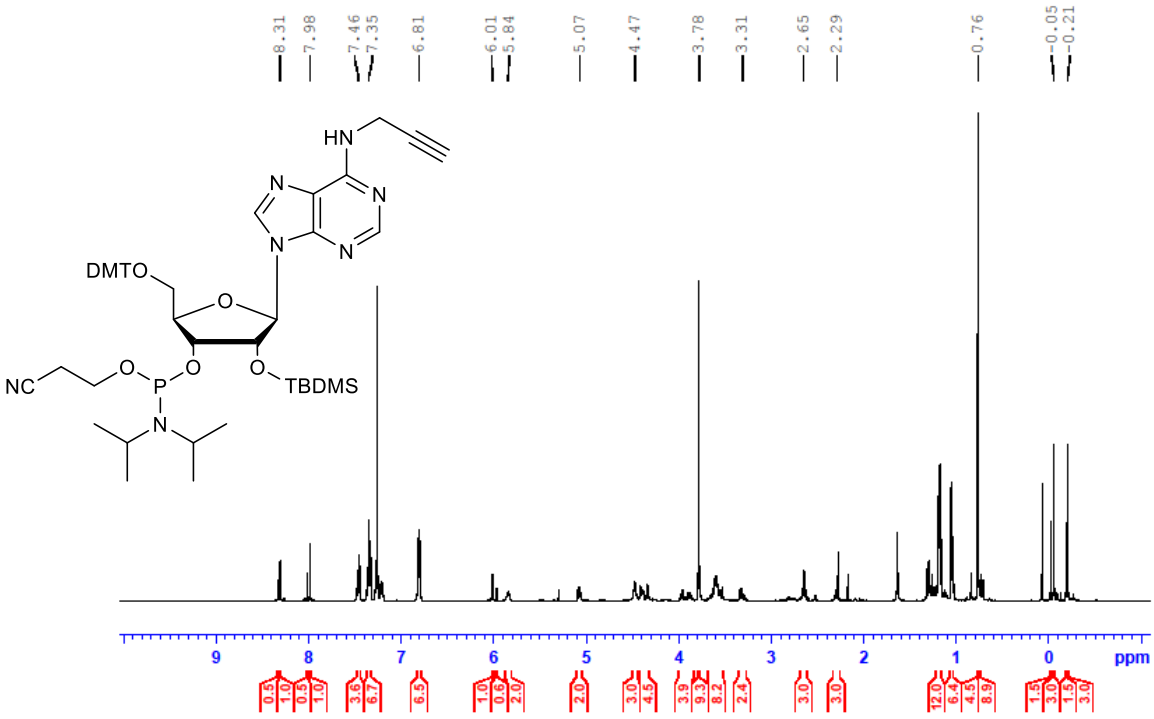
^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **13**.



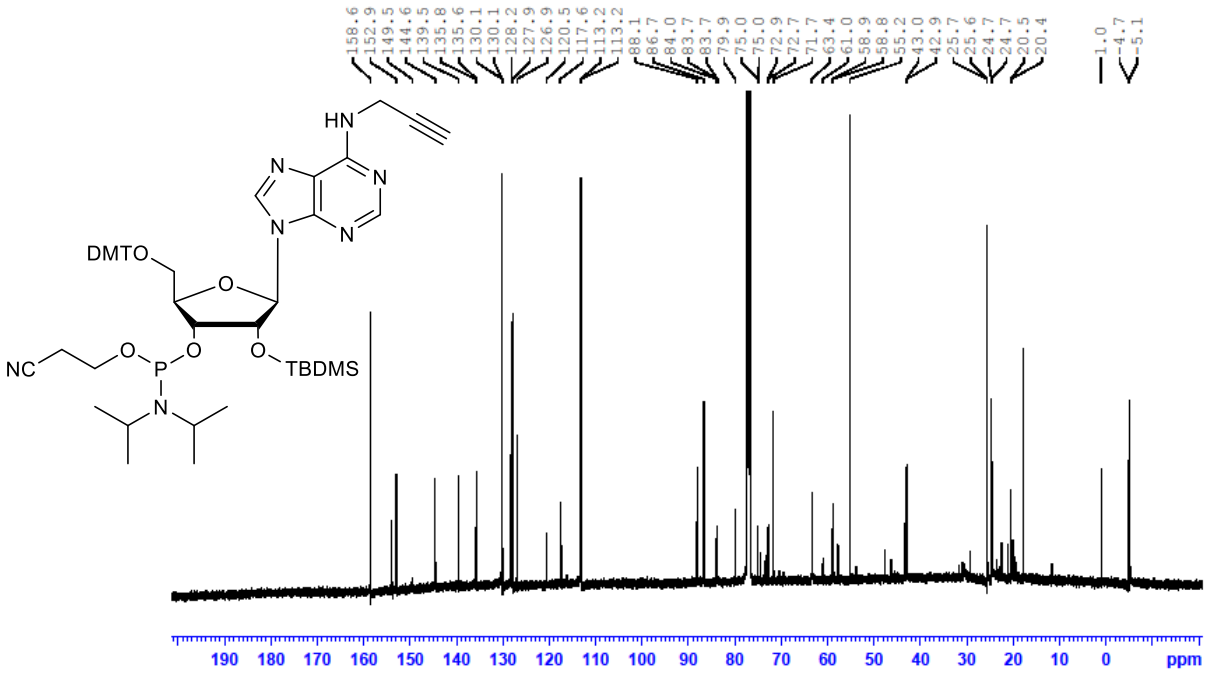
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **14**.



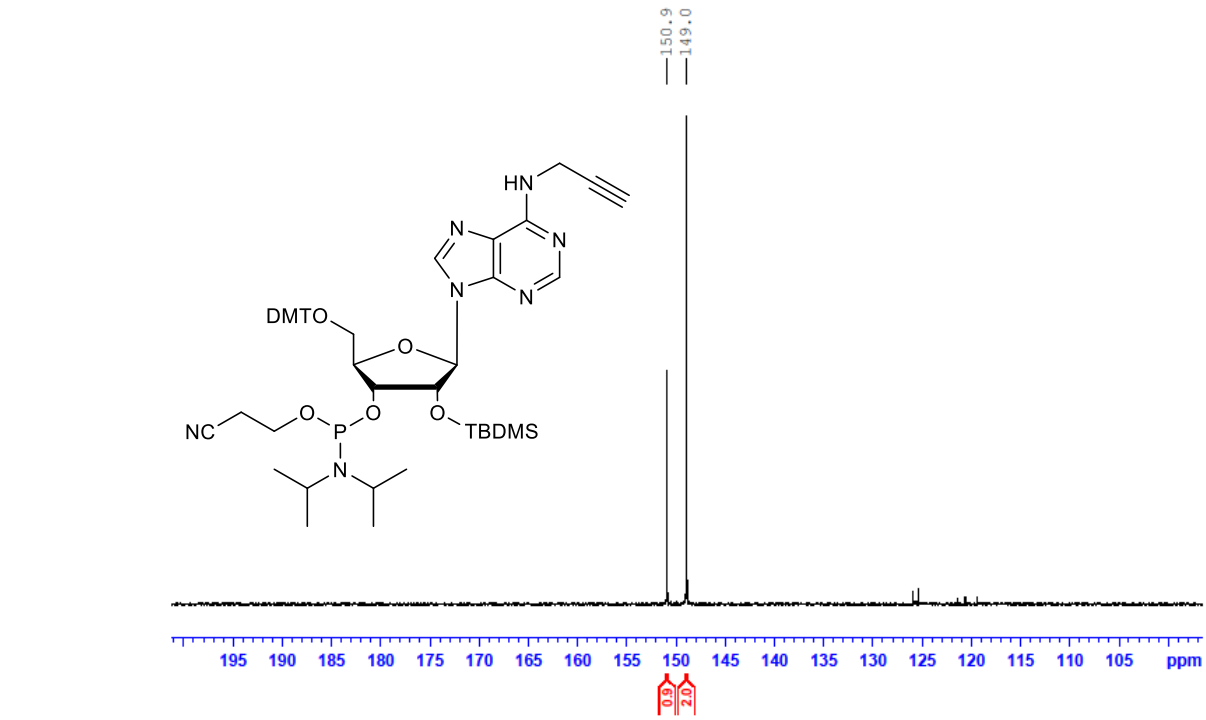
^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **15**.



^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **16**.



^{13}C NMR (100 MHz, $CDCl_3$) spectrum of **16**.



^{31}P NMR (162 MHz, $CDCl_3$) spectrum of **16**.

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