Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

Selective functionalization at N^2-position of guanine in oligonucleotides via reductive amination

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General information:

All commercially available chemicals were used without further purification. Custom DNA oligonucleotides were ordered from GCC Biotech or IDT. Dry reactions were performed in oven-dried glassware, and run under argon or nitrogen atmosphere wherever stated. Analytical thin layer chromatography was performed on silica gel 60 F_{254} plates (Merck). Phoshodiesterase I from *Crotalus adamanteus* venom was purchased from Sigma Aldrich and alkaline phosphatase, Calf intestinal (CIP) was purchased from NEB. Column chromatographic purifications were performed on silica gel (230-400 mesh) from Merck. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance III NMR spectrometer. NMR spectra were recorded at 295 K in $CDCl_3$, D_2O or DMSO-d_6 and chemical shifts were calibrated to the residual proton and carbon resonances of the solvent: $CDCl_3$ (^{1}H δ 7.26 ppm; ^{13}C δ 77.16 ppm), D_2O (^{1}H δ 4.79 ppm) and DMSO-d_6 (^{1}H δ 2.50 ppm; ^{13}C δ 39.52 ppm). All chemical shifts are reported in δ ppm downfield of TMS and peak multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), sextet (h), heptet (hept), doublet of doublet (dd) doublet of triplet (dt), doublet of doublet of doublet (ddd), broad (br), and multiplet (m). LC-HRMS spectra were recorded on Bruker Daltonics MicroTOF-Q-II in electrospray ionization (ESI) mode.

Experimental section:

Analytical HPLC: Analytical HPLC was performed on Agilent Tech Series 1260 Infinity II using ZORBAX SB C-18 HPLC column (4.6 mm × 100 mm, 3.5 µm; flow rate 0.5 mL/min). UV detection was performed at 253 nm. Semi-preparative analysis was done on Agilent Tech series 1260 Infinity II using ZORBAX SB-C18 column (9.4 mm x 250 mm, 2 mL/min). Elution was done with a gradient starting with 100% A, and going to 30% A in 15 min. Solvent A = water + 0.1% TFA, and B = acetonitrile.

General procedure 1 (GP1): Synthesis of N^2-modified guanine nucleotide (GMP/dGMP)

The guanine nucleotide monophosphate (245.6 µmol, 1 equiv.) was dissolved in water (1.65 mL, 150 mM), and to this were added 50 equiv. of aldehyde and sodium cyanoborohydride (92.5 mg, 1.47 mmol, 6 equiv.). This solution was stirred vigorously at room temperature in a tightly capped vial for the indicated time and then analyzed by RP-HPLC to monitor consumption of the starting material. The reaction mixture was extracted with ethyl acetate or hexane five times to get rid of excess unreacted aldehyde. The aqueous layer was purified by HPLC to give a white solid.

General procedure 2 (GP2): Reductive alkylation of DNA

DNA oligonucleotides were dissolved in water to make a 5 mM stock solution. To a DNA stock solution (5 mM, 50 µl) in a 500 µl eppendorf tube, 1500 equiv. of aldehyde was added, followed by 150 equiv. of sodium cyanoborohydride. The reaction was incubated on Eppendorf ThermoMixer©C with shaking at 1600 rpm. The progress of the reaction was monitored by taking aliquots from the reaction mixture and analyzing by RP-HPLC. After the indicated time, the reaction mixture was diluted with water (100 µl) and washed with ethyl acetate. This sample was desalted for mass spectrometric analysis.

General procedure 3 (GP3): Synthesis of aldehyde by Swern oxidation

DCM (70 mL) and oxalyl chloride (2.14 g, 1.2 equiv.) were added to an oven dried round bottom flask under nitrogen and the solution was cooled to -78°C. DMSO (2.20 g, 2 equiv.) was added dropwise, and stirred for 30 minutes followed by addition of a solution of alcohol (14 mmol, 1 equiv.) in DCM (30mL). The reaction mixture was stirred at -78°C for 45 minutes followed by addition of triethyamine (5 equiv.). The reaction mixture was stirred for another hour and then slowly warmed to room temperature. The reaction was washed with cold water (3x). The combined organic layers were washed with water, dried over sodium sulphate, and concentrated under reduced pressure. The aldehyde was purified by silica gel column chromatography using 10% ethyl acetate/hexane.

Desalting of oligonucleotides:

Oligonucleotides having a molecular weight > 3 KDa were desalted with a 3 KDa cutoff filter (Amicon) by centrifuging at 10000 rpm until concentrated to ~ 50 µl. The concentrate was washed with water four times. The desalted samples were diluted with water, and analyzed by mass spectrometry.

Digestion of modified oligonucleotides:

Digestion of modified DNA was performed with phosphodiesterase I followed by calf intestinal phosphatase (CIP) following the manufacturer's protocol. The products were analyzed by RP-HPLC,

using a gradient starting with 100% A, and going to 50% A in 25 min (A= 0.1% TFA in water, B= methanol), and compared against previously prepared standards.

Reductive alkylation of RNA:

To an RNA oligonucleotide dissolved in water (~800 µM), excess propanal and sodium cyanoborohydride were added and stirred at 500 RPM at 25°C. After 6h, the reaction mixture was washed with ethyl acetate (3X), and the aqueous layer was used for digestion with phosphodiesterase I and calf intestinal phosphatase (CIP). The digest was analyzed by HPLC to determine the % of guanine in RNA that was converted to N^2-propyl G.

Copper free click reaction of oligonucleotide (ODN 12) with DBCO-TAMRA:

Desalted N^2-5-azidopentyl dG containing oligonucleotide (ODN 12) was diluted with water (67µg, 0.45 mM, 50 µl) and treated with DBCO-TAMRA (7µl, 6 equiv.) at 4°C for 30 min. The progress of the reaction was monitored by HPLC. After 30 min, the reaction was stopped and the excess dye was removed using a 3 KDa filter. The product (ODN 13) was subjected to mass spectrometry. For PAGE analysis, 4 µl of ODN 13 (10 µM) was loaded on 20% urea PAGE. In-gel fluorescence was monitored using Typhoon™ FLA 9000 using a 532 nm exicitation laser.

Calculation of nucleophilicity indices for GMP, AMP and CMP

In order to compare the nucleophilicities of the NMPs in this work, we computed the empirical nucleophilicity index proposed by Jaramillo *et al.* [1]. For a nucleophile A and an electrophile B, the nucleophilicity index is defined as,

$$\omega^{-} = \frac{1}{2}\frac{(\mu_A - \mu_B)^2}{(\eta_A + \eta_B)^2}\eta_A \qquad (S1)$$

where, μ_A and μ_B are the corresponding chemical potentials and η_A and η_B are the respective hardness. Energies of the frontier molecular orbital HOMO (ϵ_H) and LUMO (ϵ_L) were used to compute the chemical potential and hardness as $\mu \approx (\epsilon_H + \epsilon_L)/2$ and $\eta \approx (\epsilon_L - \epsilon_H)$. Note that this nucleophilicity scale is relative, and can only be used for comparison with a fixed electrophile (propanal in this case). This model has been previously rationalized against kinetic data of alkenes, amines, phosphanes and phosphites interacting with corresponding electrophiles [1]. This reactivity descriptor has been used in the literature to explain the reactivity of various systems [2-5].

All calculations were performed at DFT level using ORCA 4.2.1 program package [6] with B3LYP exchange-correlation functional [7] and def2-SVP basis set [8] along with general auxiliary basis set by Weigand [9]. Solvent phase calculations were done using SMD solvation model [10] with the same level of theory. All the geometries were optimized in gas phase and in solvent phase.

The chemical potential, hardness and nucleophilicity index of the different species in gas phase and solvent phase are shown in Tables 1 and 2, respectively. The chemical potential (μ) of nucleophile is always larger than the electrophile (propanal) since the electron flows from the nucleophile to electrophile. We found that the nucleophilicity of GMP was larger than AMP and CMP, which is in agreement with the experimental observation. The order of reactivity becomes even more pronounced in presence of the solvent (water) where the differences in ω^{-} are larger than in the gas phase.

Species	$\boldsymbol{\mu}$ $(\boldsymbol{eV})$	$\boldsymbol{\eta}(\boldsymbol{eV})$	$\boldsymbol{\omega^{-}}(\boldsymbol{eV})$
GMP	-3.1338	5.4348	7.492×10^{-3}
AMP	-3.1970	5.3916	6.038×10^{-3}
CMP	-3.7372	5.3749	5.430×10^{-6}
Propanal	-3.7538	6.3741	

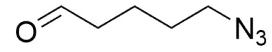
Table 1. Calculated properties of the NMPs in the gas phase, with propanal as the electrophile.

Species	$\boldsymbol{\mu}$ $(\boldsymbol{eV})$	$\boldsymbol{\eta}(\boldsymbol{eV})$	$\boldsymbol{\omega^{-}}(\boldsymbol{eV})$
GMP	-3.1464	5.2963	7.647×10^{-3}
AMP	-3.2954	5.3769	4.476×10^{-3}
CMP	-3.6877	5.5386	1.589×10^{-4}
Propanal	-3.7786	6.4681	

Table 2. Calculated properties of the NMPs in the solvent phase, with propanal as the electrophile.

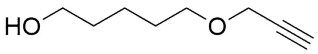
Synthesis and characterization:

5-azidopentanal



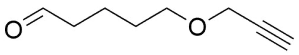
Synthesized by GP3. The aldehyde was isolated as yellow oil in 60% yield.
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 (s, 1H), 3.30 (t, *J* = 6.6 Hz, 2H), 2.49 (t, *J* = 7.1 Hz, 2H), 1.78 – 1.56 (m, 4H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 201.81, 51.23, 43.35, 28.41, 19.35.
LR-MS (ESI, *m/z*): $[M - H]^-$ calculated for $C_5H_8N_3O^-$: 126.1; found: 126.9

Synthesis of 5-(prop-2-yn-1-yloxy) pentan-1-ol



1,5-pentanediol (5 g, 48 mmol, 1 equiv.) was added slowly to a stirred suspension of NaH (1.2 equiv.) in DMF (40 mL) at 0°C. The reaction mixture was brought to room temperature and stirred for 2 hours. The reaction mixture was further cooled to 0^{o} C, and propargyl bromide (1 equiv.) was added slowly with stirring. The reaction was brought to room temperature and stirred for 5 hours. The reaction was worked up by adding cold water and extracting with ethyl acetate (3 times). The combined organic layers were dried with sodium sulphate and concentrated under reduced pressure. The residue was purified by column chromatography to give the title compound as a yellow liquid in 65% yield.
^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (d, *J* = 2.4 Hz, 2H), 3.65 (t, *J* = 6.5 Hz, 2H), 3.53 (t, *J* = 6.5 Hz, 2H), 2.41 (t, *J* = 2.4 Hz, 1H), 1.69 – 1.54 (m, 4H), 1.50 – 1.39 (m, 2H), 1.32 (br, 1H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 80.11, 74.27, 70.20, 62.99, 58.21, 32.62, 29.37, 22.50.
HRMS (ESI, *m/z*): $[M + H]^+$ calculated for $C_8H_{15}O_2^+$: 143.1067; found: 143.1076.

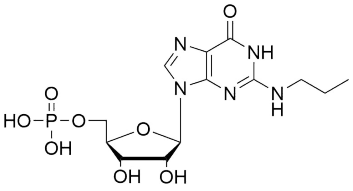
Synthesis of 5-(prop-2-yn-1-yloxy) pentanal



Synthesized by GP3. The aldehyde was isolated as yellow oil in 65% yield.
^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (t, *J* = 1.7 Hz, 1H), 4.13 (d, *J* = 2.3 Hz, 2H), 3.53 (t, *J* = 6.1 Hz, 2H), 2.47 (td, *J* = 7.2, 1.7 Hz, 2H), 2.42 (t, *J* = 2.4 Hz, 1H), 1.79 – 1.69 (m, 2H), 1.68 – 1.59 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$) δ 202.60, 79.97, 74.39, 69.65, 58.23, 43.66, 20.69, 19.00.
HRMS (ESI, *m/z*): $[M + H]^+$ calculated for $C_8H_{13}O_2^+$: 141.0910; found: 141.0920.

N^2-propyl GMP

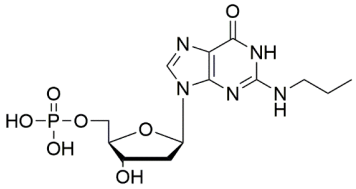
Synthesized by GP1 from GMP and propanal. (Colorless solid, 85% after HPLC purification)



^{1}H NMR (400 MHz, D_2O) δ 8.92 (s, 1H), 6.11 (d, *J* = 3.2 Hz, 1H), 4.76 - 4.73 (m, 1H), 4.51-4.46 (m, 1H), 4.42–4.36 (m, 1H), 4.32–4.23 (m, 1H), 4.19-4.08 (m, 1H), 3.44-3.31 (m, 2H), 1.68 – 1.58 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H).
^{13}C NMR (126 MHz, D_2O) δ 155.24, 154.18, 149.76, 135.34, 107.69, 89.81, 83.62, 74.42, 69.19, 63.71, 42.89, 21.59, 10.49.
^{31}P NMR (162 MHz, D_2O) δ 0.04.
HRMS (ESI, *m/z*): $[M - H]^-$ calculated for $C_{13}H_{19}N_5O_8P^-$: 404.0977; found: 404.0995.

N^2-propyl dGMP

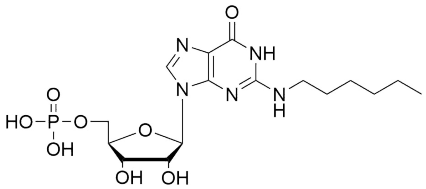
Synthesized by GP1 from dGMP and propanal. (Colorless solid, 85% after HPLC purification)



1H NMR (400 MHz, CDCl3) δ 8.11 (s, 1H), 6.38 (t, J = 6.9 Hz, 1H), 4.21 (dd, *J* = 7.6, 3.9 Hz, 1H), 3.96 (t, J = 4.9 Hz, 2H), 3.34 (t, J = 6.9 Hz, 2H), 2.83 (dt, J = 13.6, 6.7 Hz, 1H), 2.51 (ddd, J = 13.8, 6.5, 3.8 Hz, 1H), 1.76 – 1.51 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). Solvent signal overlaps with a peak at δ ~4.77 (1H).
^{13}C NMR (126 MHz, D_2O) δ 160.67, 154.59, 153.38, 139.31, 117.15, 87.68 (d, *J* = 8.2 Hz), 84.80, 73.11, 65.71, 44.41, 40.20, 23.37, 12.23.
^{31}P NMR (162 MHz, D_2O) δ 0.16.
HRMS (ESI, *m/z*): $[M - H]^-$ calculated for $C_{13}H_{19}N_5O_7P$: 388.1028; found: 388.1038.

N^2-hexyl GMP

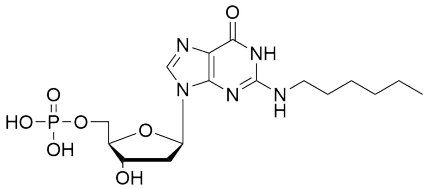
Synthesized by GP1 from GMP and 1-hexanal. (Colorless solid, 70% after HPLC purification)



^{1}H NMR (400 MHz, D_2O) δ 8.16 (s, 1H), 6.00 (d, *J* = 5.6 Hz, 1H), 4.54 – 4.46 (m, 1H), 4.31 (q, *J* = 3.9 Hz, 1H), 4.05 – 3.94 (m, 2H), 3.38-3.24 (m, 2H), 1.55 (p, *J* = 7.1 Hz, 2H), 1.36 – 1.19 (m, 6H), 0.91 – 0.77 (m, 3H). Solvent signal overlaps with a peak at ~4.77 ppm (1H).
^{13}C NMR (126 MHz, D_2O) δ 159.15, 153.03, 152.12, 137.48, 115.45, 86.63, 84.32, 74.06, 70.67, 63.70, 40.96, 30.72, 28.22, 25.69, 21.91, 13.29.
^{31}P NMR (162 MHz, D_2O) δ 3.87.
HRMS (ESI, *m/z*): $[M + Na]^+$ calculated for $C_{16}H_{26}N_5NaO_8P^+$: 470.1411; found: 470.1398.

N^2-hexyl dGMP

Synthesized by GP1 from dGMP and hexanal. (Colorless solid, 85% after HPLC purification)



^{1}H NMR (500 MHz, DMSO-d_6) δ 10.81 (br, 1H), 8.25 (s, 1H), 6.72 - 6.62 (br, 1H), 6.22 (t, *J* = 6.9 Hz, 1H), 4.45 – 4.37 (m, 1H), 4.07 – 3.98 (m, 2H), 3.95 – 3.88 (m, 1H), 3.34 – 3.21 (m, 2H), 2.67 (ddd, *J* = 13.3, 7.4, 6.0 Hz, 1H), 2.30 (ddd, *J* = 13.4, 6.4, 3.4 Hz, 1H), 1.61 – 1.48 (m, 2H), 1.37 – 1.23 (m, 6H), 1.25 – 0.81 (m, 3H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 155.81, 153.05, 150.10, 135.72, 114.40, 85.60 (d, *J* = 7.9 Hz) 83.69, 70.79, 65.61 (d, *J* = 5 Hz), 40.02, 38.94, 30.97, 28.58, 25.97, 22.08, 13.93.
^{31}P NMR (162 MHz, DMSO-d_6) δ -1.24.
HRMS (ESI, *m/z*): $[M - H]^-$ calculated for $C_{16}H_{25}N_5O_7P^-$: 430.1497; found: 430.1503.

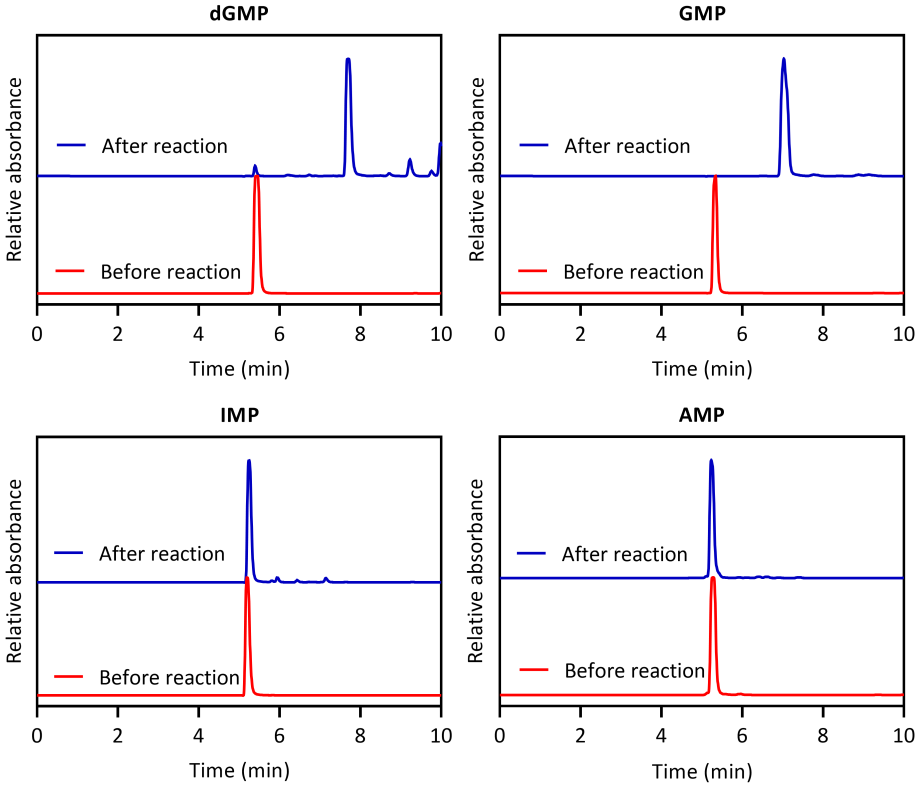


Fig S1: RP-HPLC profiles of dGMP, GMP, IMP and AMP before and after reductive amination with propanal.

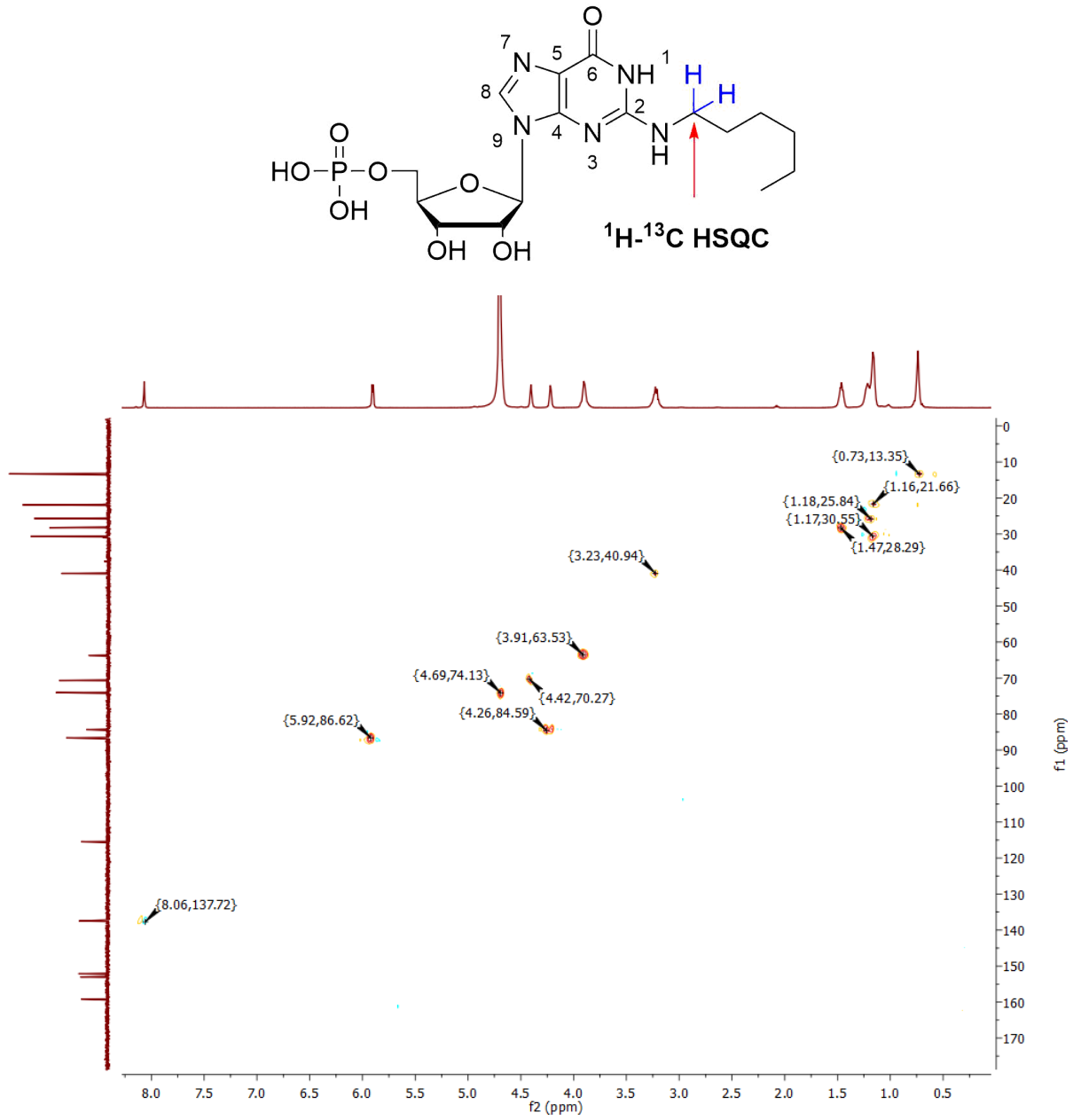


Fig S2a: ^{1}H - ^{13}C HSQC NMR of N^2-hexyl GMP suggests that alkylation occurred adjacent to a 'N' atom, indicated by the cross peak at δ (3.23, 40.94).

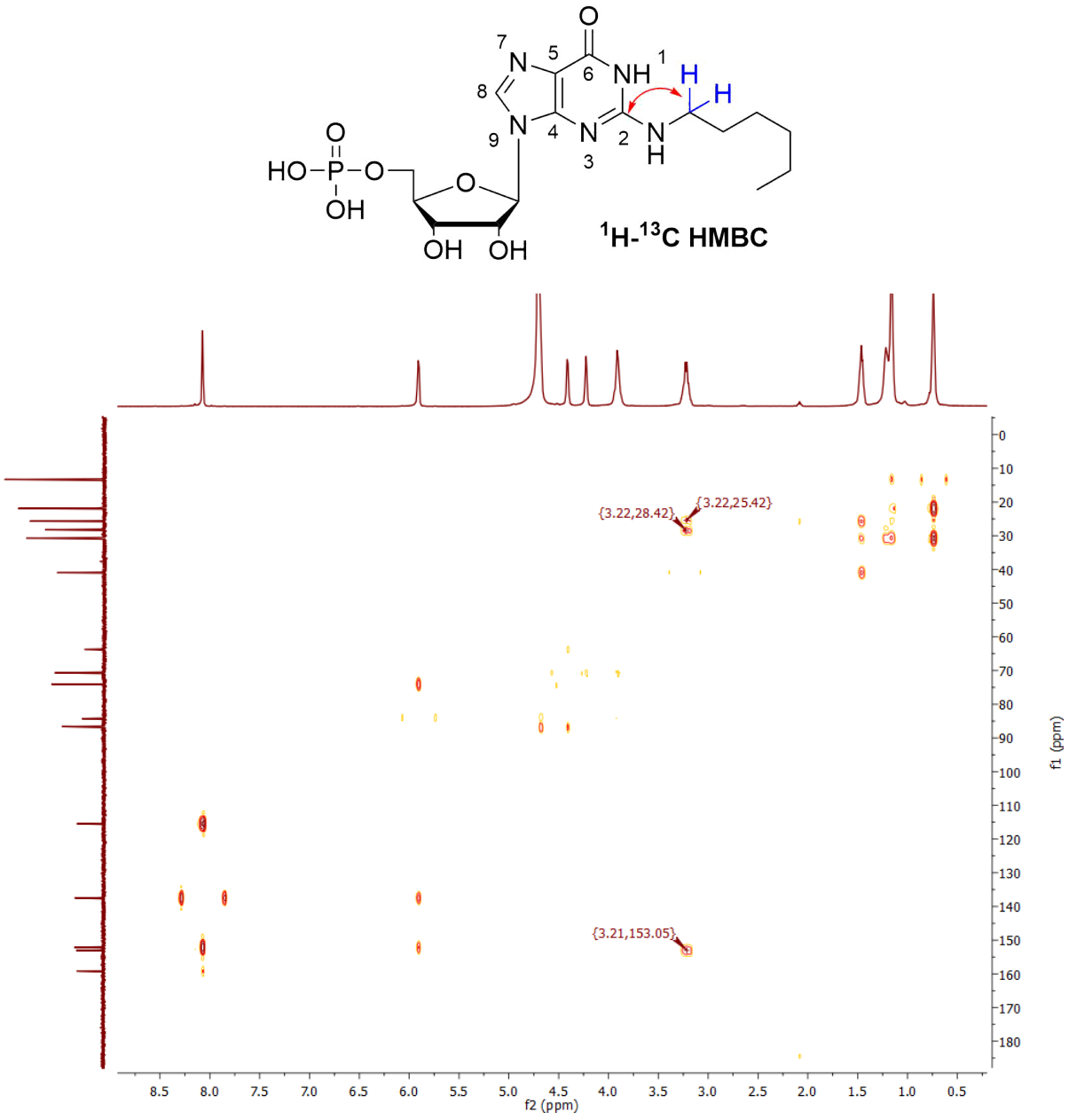


Fig S2b: ^{1}H-^{13}C HMBC NMR of N^2-hexyl GMP. Coupling between C-2 and alkyl H (blue) indicated by cross peak at δ (3.21, 153.05) supports substitution on the N^2-position of guanosine. Substitution at N7- and N1-positions are ruled out due to lack of cross peaks between alkyl H (blue) and C-6 or C-8.

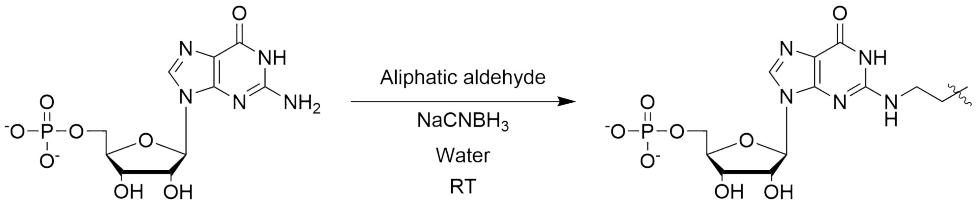
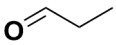
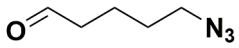
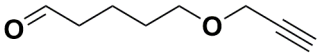
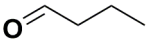
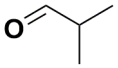
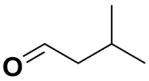
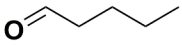
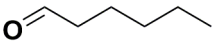
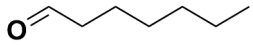


Table S1: Screening of aliphatic aldehydes:

Sr No.	Aldehyde	Log P[a]	Time (hour)	Conversion (%)
1		-0.58	4	95[b]
2		0.05	4	98
3		0.12	4	88
4		0.25	4	90
5		0.44	4	95
6		0.61	4	96
7		0.78	4	95
8		0.84	4	90
9		1.24	4 (50)	8 (97)
10		1.63	4 (50)	2.5 (39)

[a]Log P values were calculated with Marvin Sketch 15.5.18 using default parameters. % conversion was determined by HPLC. [b] one extra peak was also observed in HPLC likely from N^2-ethyl disubstituted GMP.

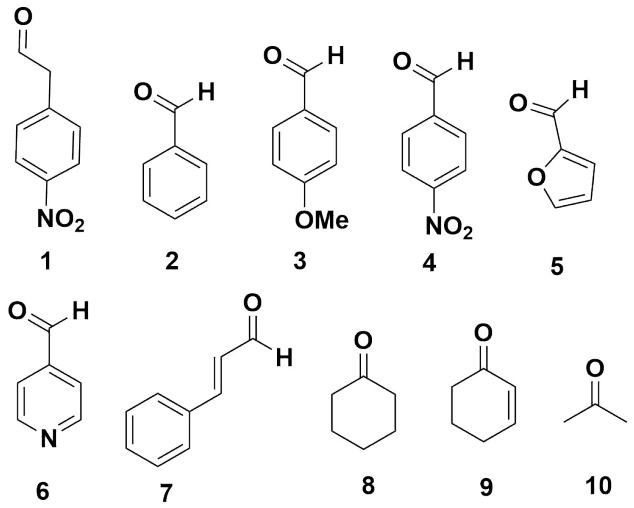


Table S2: Screening of aromatic aldehydes and aliphatic ketones

Sr.No	Reactant	Log P	Time (hour)	Conversion (%)
1	2-(4-nitrophenyl)acetaldehyde	1.22	24	0
2	Benzaldehyde	1.72	24	0
3	p-Methoxy benzaldehyde	1.47	24	0
4	p-Nitro benzaldehyde	1.68	24	0
5	Furfural	0.67	24	0
6	Isonicotinaldehyde	0.41	24	0
7	Trans-cinnamaldehyde	1.99	24	0
8	Cyclohexanone	1.53	24	0
9	Cyclohex-2-en-1-one	1.61	24	0
10	Acetone	0.38	24	2

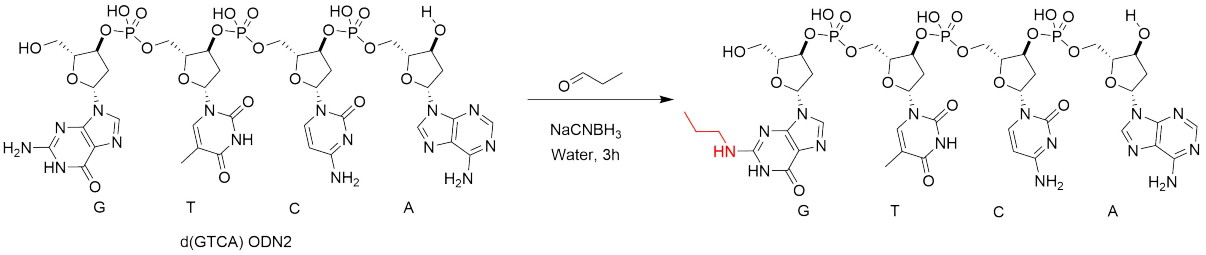
[a]Log P values were calculated with Marvin Sketch 15.5.18 using default parameters. % conversion was determined by HPLC.

Table S3: Reaction optimization with DNA oligonucleotide d(GATC)[a]

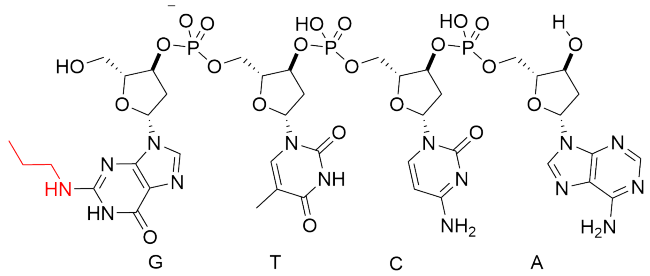
Aldehyde (eq.)	Temperature (°C)	% Conversion[b]	
		3h	24h
300	25	14	35
300	37	43	44
300	45	70	71
300	60	44	39
1500	25	90	95

[a] DNA concentration was 5 mM in water, [b] % conversion was calculated from HPLC.

Reductive amination of oligonucleotide ODN2 with propanal

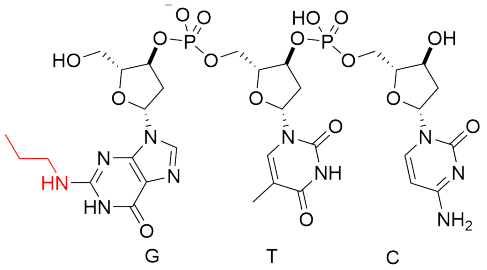


A) Fragment 1 $[M-H]^-$



Chemical Formula: $C_{42}H_{55}N_{15}O_{22}P_3^-$
Expected Mass: 1214.2
Observed Mass: 1214.2

B) Fragment 2 $[M-H]^-$



Chemical Formula: $C_{32}H_{43}N_{10}O_{17}P_2^-$
Expected Mass: 901.2
Observed Mass: 901.2

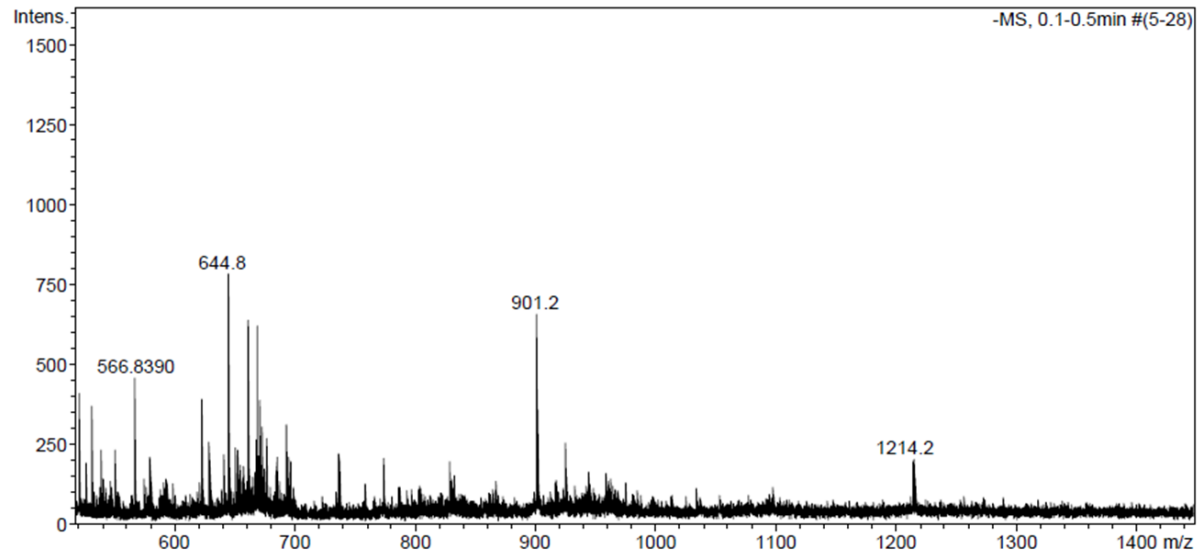
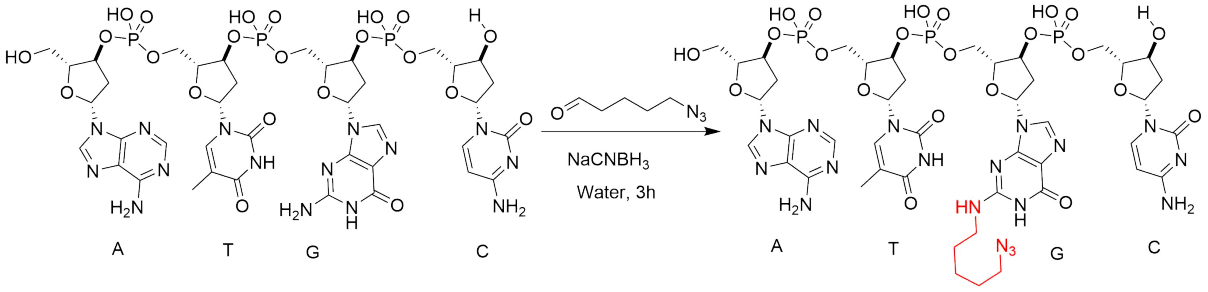
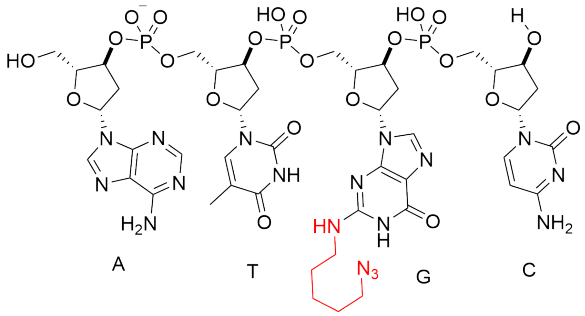


Fig S3: Mass spectrometric analysis of modified oligonucleotide ODN2 after reaction with propanal and sodium cyanoborohydride.

Reductive amination of oligonucleotide ODN3 with 5-azidopentanal

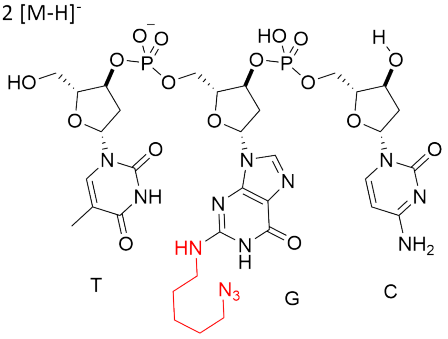


A) Fragment 1 $[M-H]^-$



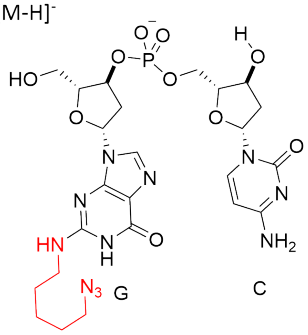
Chemical Formula: $C_{44}H_{58}N_{18}O_{22}P_3^-$
Expected Mass: 1283.3
Observed Mass: 1283.3

B) Fragment 2 $[M-H]^-$



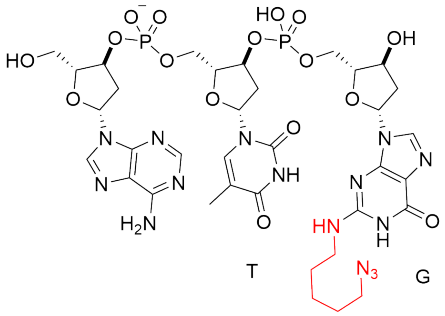
Chemical Formula: $C_{34}H_{46}N_{13}O_{17}P_2^-$
Expected Mass: 970.2
Observed Mass: 970.2

C) Fragment 3 $[M-H]^-$



Chemical Formula: $C_{24}H_{33}N_{11}O_{10}P^-$
Exact Mass: 666.2
Observed Mass: 666.2

D) Fragment 4 $[M-H]^-$



Chemical Formula: $C_{35}H_{46}N_{15}O_{16}P_2^-$
Exact Mass: 994.2
Observed Mass = 994.2

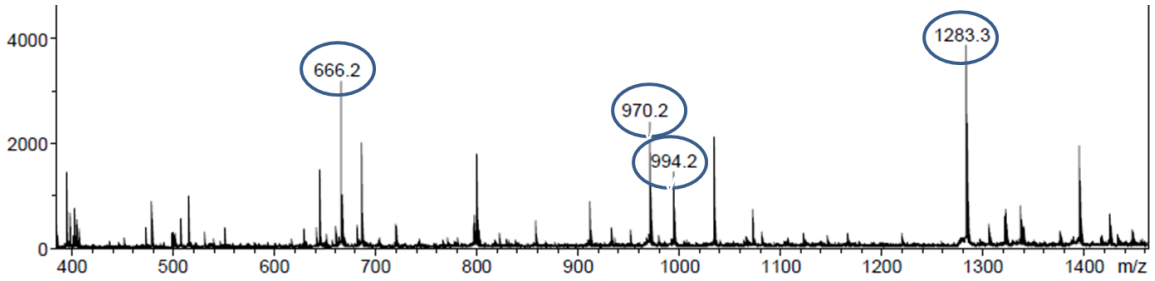


Fig S4: Mass spectrometric analysis of ODN3 after reaction with 5-azidopentanal and sodium cyanoborohydride.

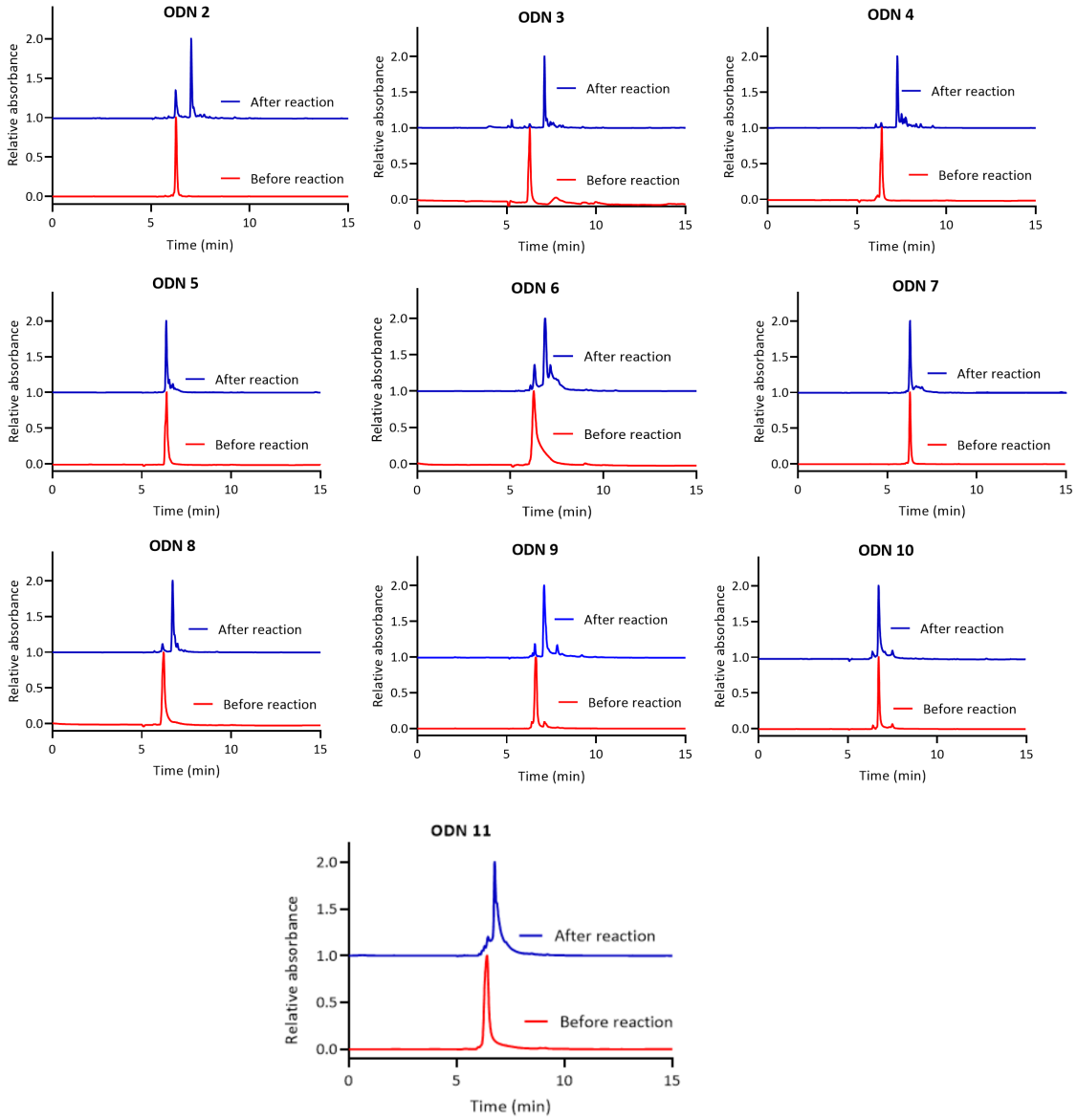


Fig S5: RP-HPLC profiles of single-stranded DNA oligonucleotides before and after reaction with propanal.

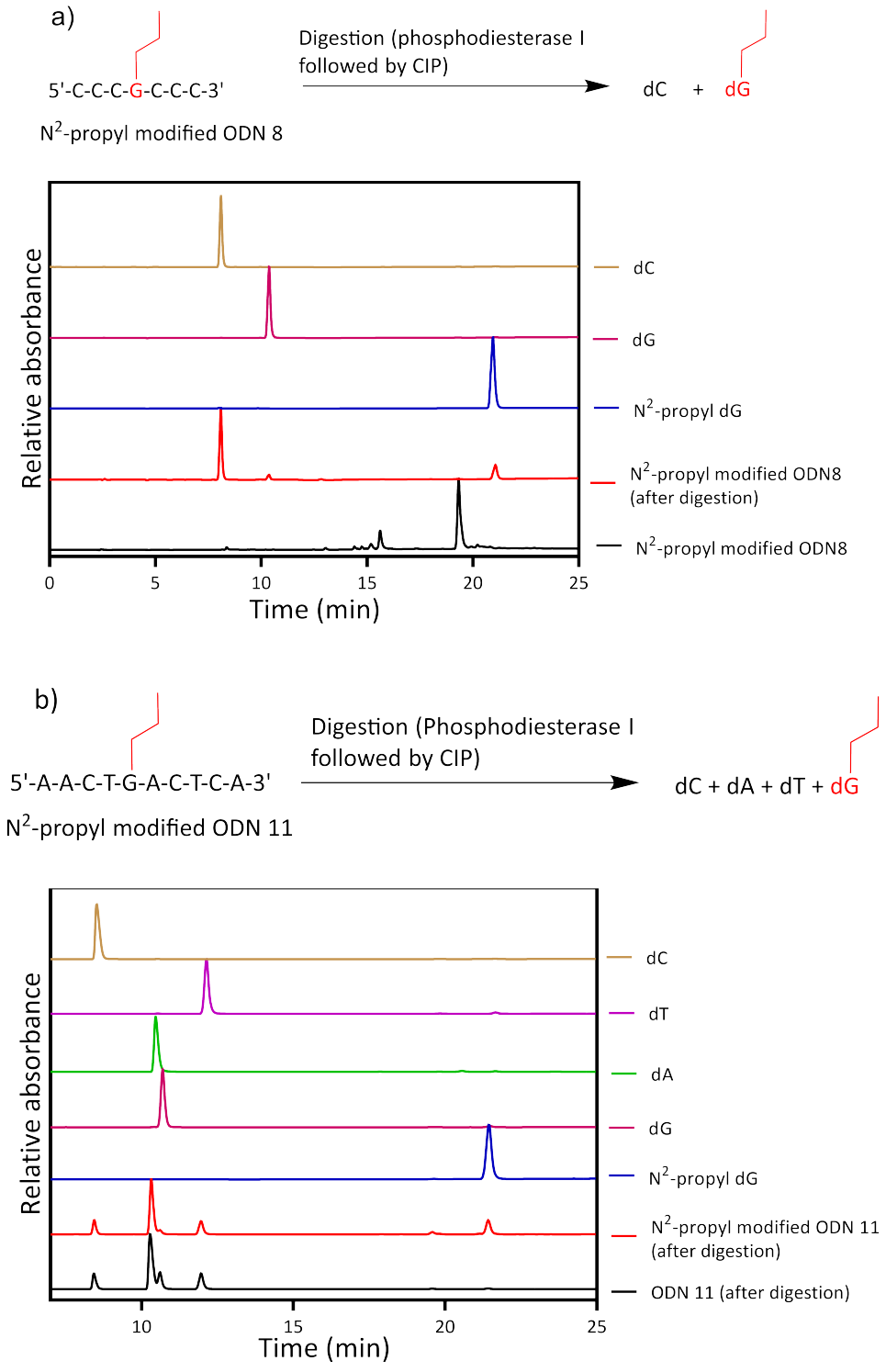


Fig S6: Nuclease digestion of N^2-propyl modified (a) ODN8 and (b) ODN11.

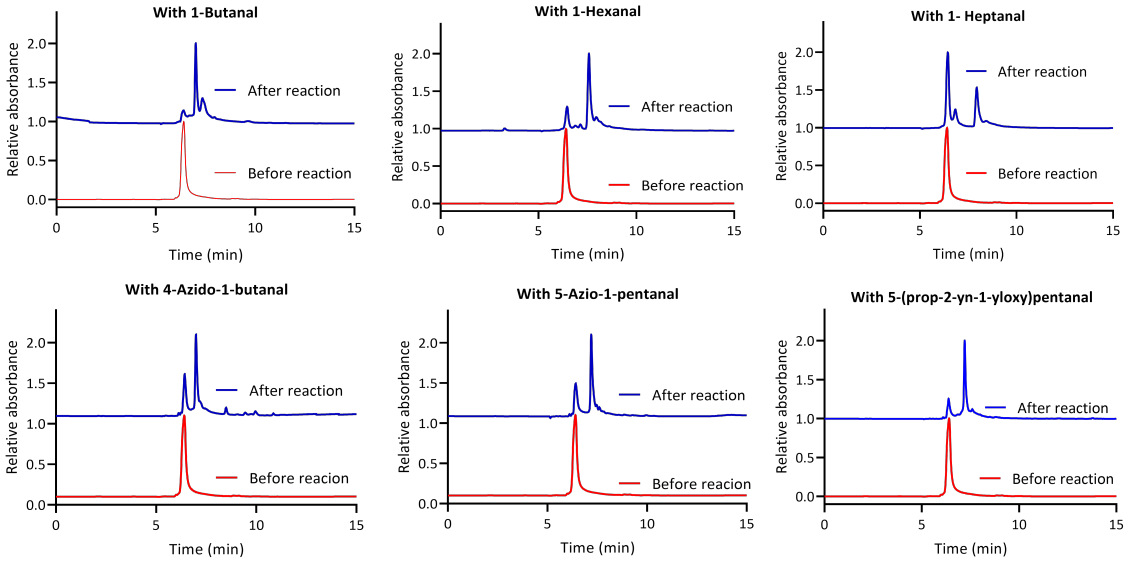
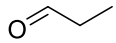
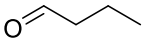
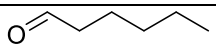
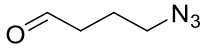
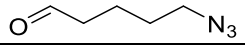
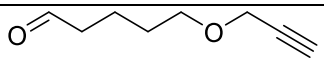


Fig S7: RP-HPLC profiles of ODN11 with different aldehydes before and after reductive amination for 6 hours. Reactions with 1-hexanal and 1-heptanal were carried out in 50% DMF.

Table S4: Mass analysis of ODN11 after reductive amination with various aldehydes. All calculated masses correspond to mono-alkylated products.

Oligonucleotide		Aldehyde	Mass calculated $[M-H]^-$	Mass observed $[M-H]^-$ (Deconvoluted)
ODN11 d(AACTGACTCA)	1		3035.6	3035.5
	2		3049.6	3049.6
	3		3077.6	3077.6
	4		3090.6	3090.5
	5		3104.6*	3105.5*
	6		3117.6	3117.6

* error < 0.03%

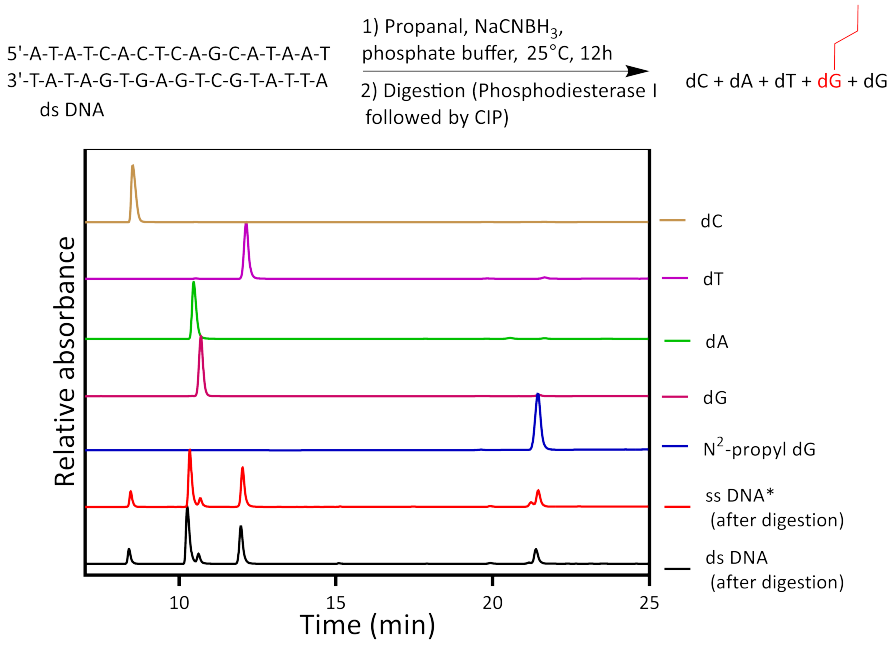


Fig S8: Reductive amination of dsDNA: dsDNA (1 mM) or its constituent single strands were independently subjected to reductive amination in phosphate buffer (100mM NaCl, 20mM phosphate, 0.1mM EDTA) for 12 h. The reaction mixtures (dsDNA, and 1:1 mixture of the two single strand reactions) were digested by nucleases. HPLC analysis of the digest indicated similar guanine reactivity for dsDNA and ssDNA (65% for dsDNA, and 69% for the single strand mixture). % conversion was calculated from area under the peak for G and N^2-propyl G in HPLC.

*The two single strands were reacted separately, and mixed in 1:1 ratio before digestion.

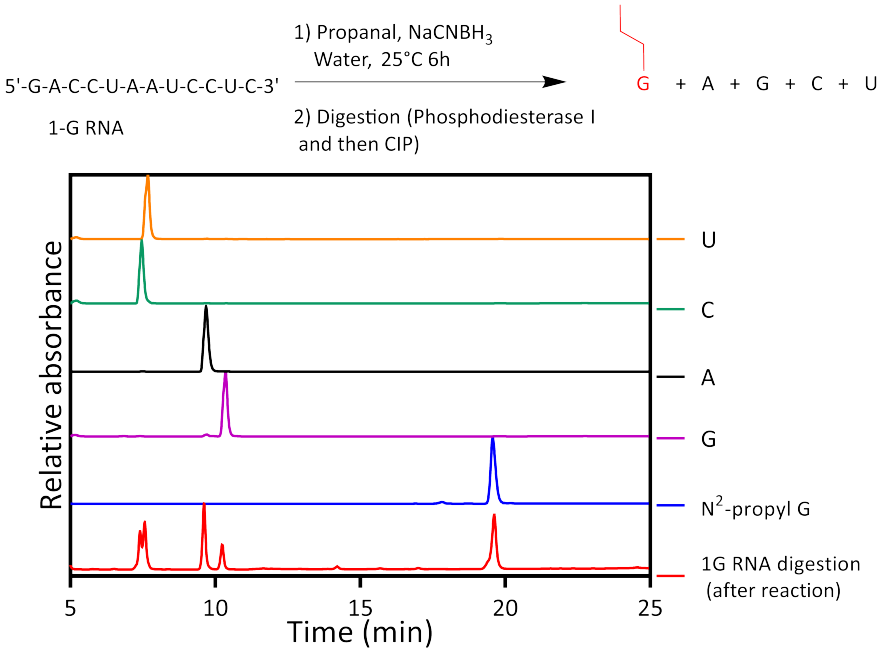


Fig S9: Reductive amination of RNA: 1G-RNA (800 µM) was reacted with propanal and $NaCNBH_3$ for 6h, followed by nuclease digestion. HPLC analysis of the digest showed that 75% of the guanine had converted to N^2-propyl G (% conversion was estimated from the area under the peak for G and N^2-propyl G).

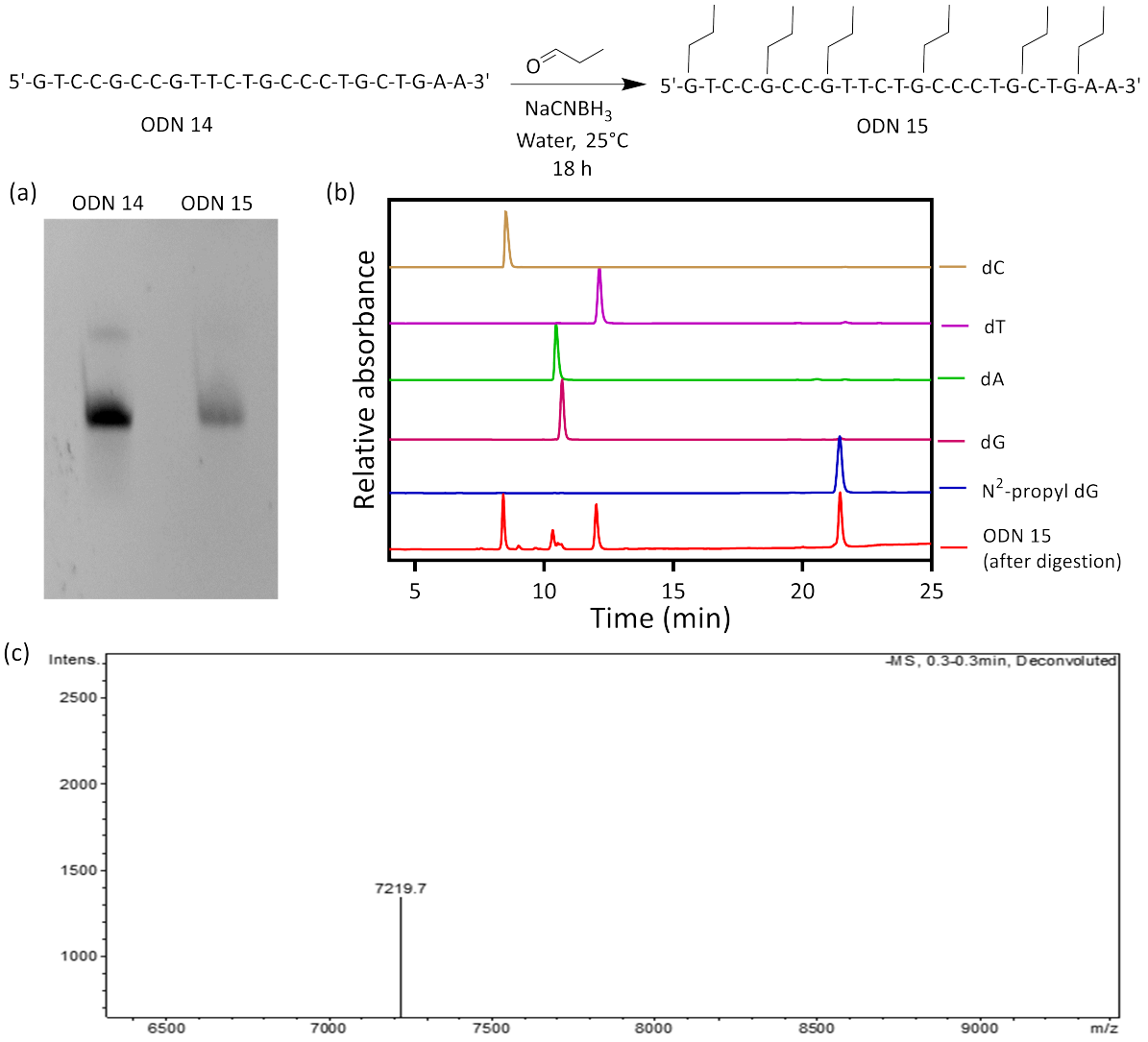


Fig S10: (a) PAGE analysis of ODN14 before and after reductive amination (equal DNA load in both lanes). The DNA bands were stained with ethidium bromide. (b) HPLC analysis of the nuclease digest of ODN15, along with the relevant standards. (c) Deconvoluted mass spectrum of ODN15. The observed mass of 7219.7 corresponds to ODN 15 containing six propyl groups, expected mass for $[M-H]^-$ = 7215.4. The error in mass determination was 0.05%.

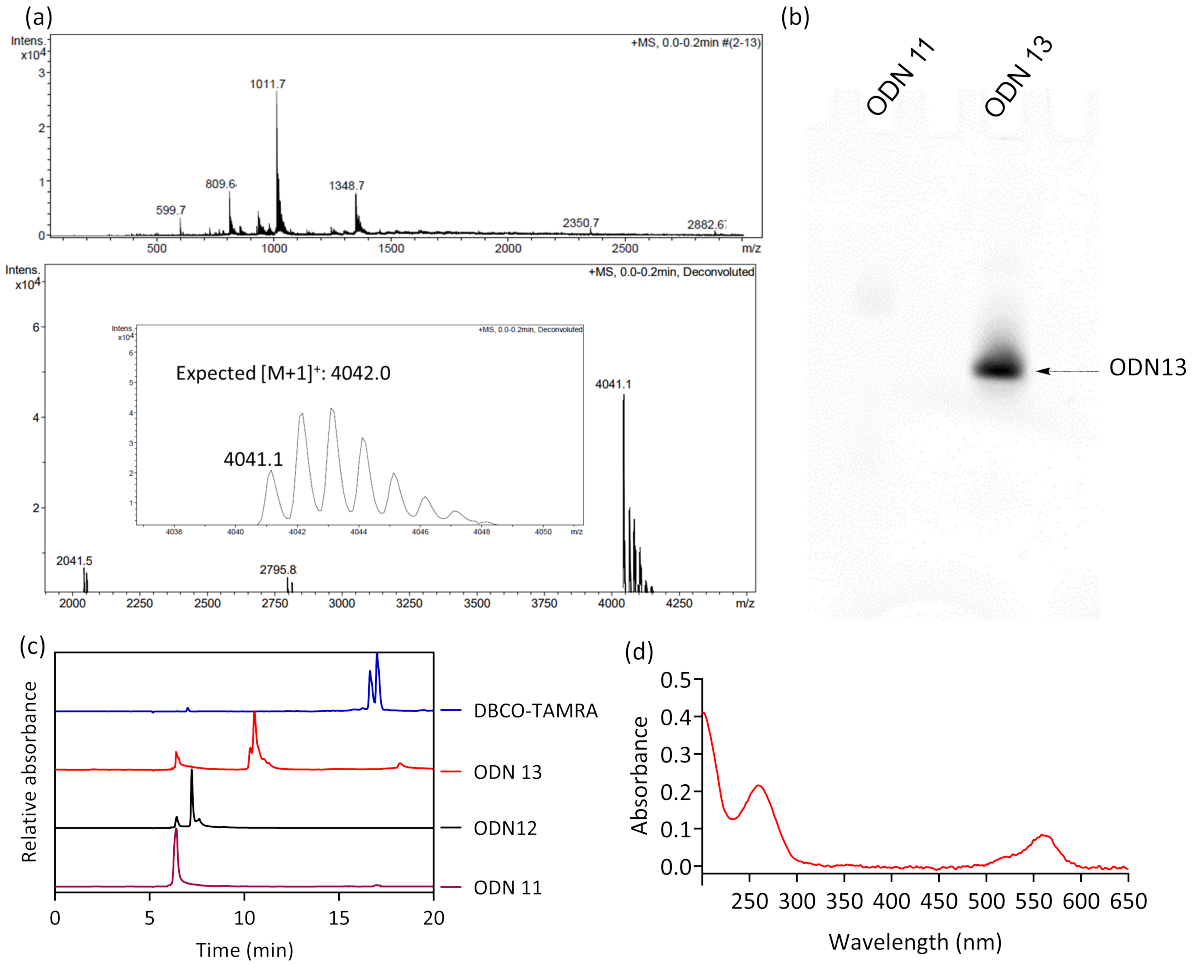
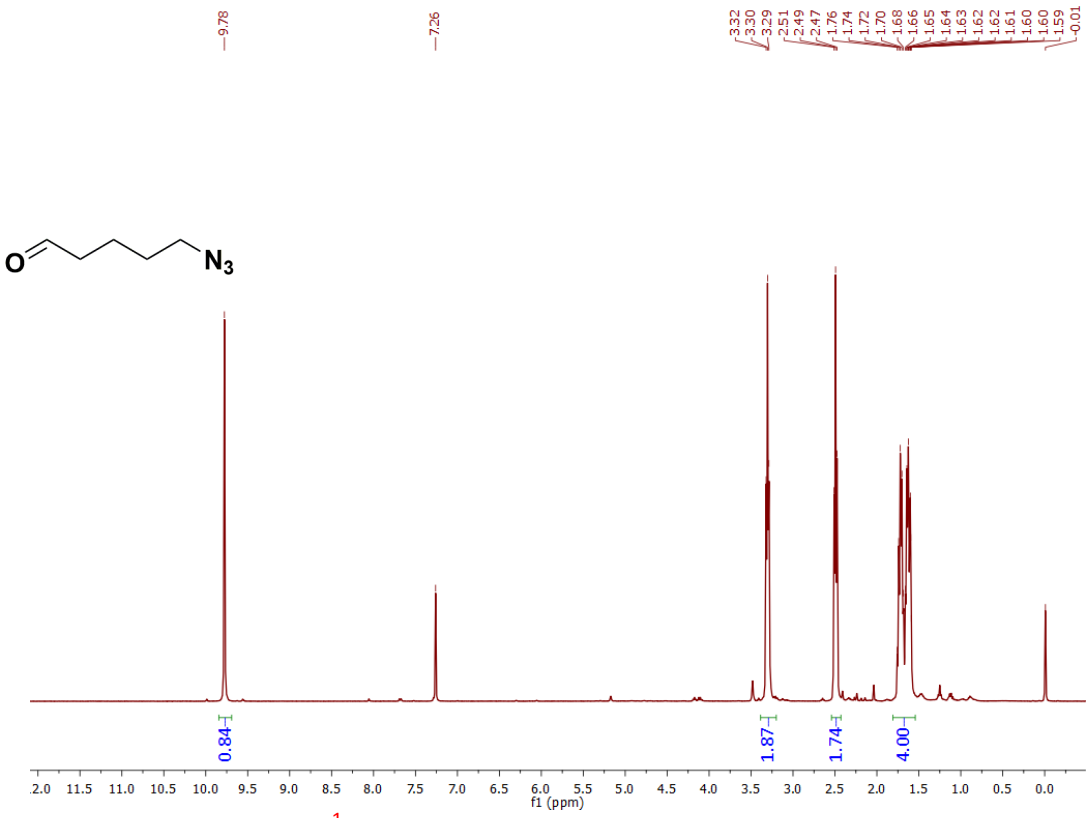
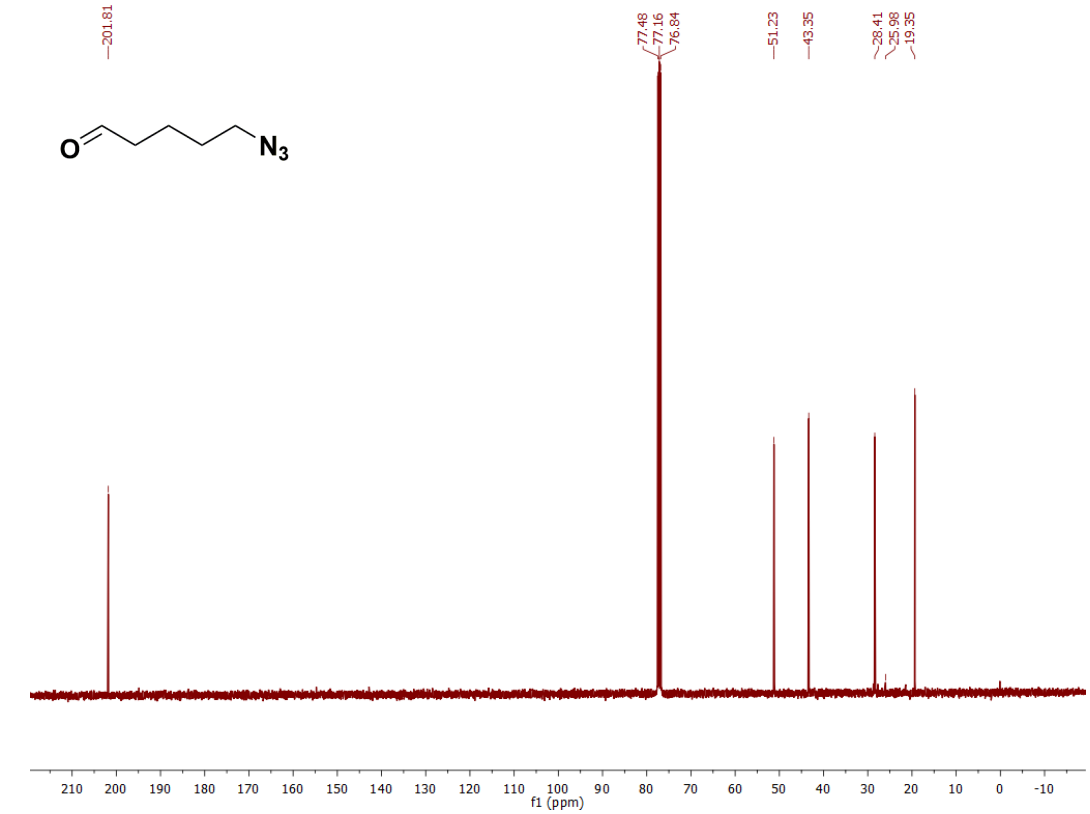


Fig S11: Labeling of ODN11 by copper-free click reaction with DBCO-TAMRA (a) Mass spectrometric analysis for ODN13. The inset shows a zoomed view of the peak at m/z 4041.1. The error in mass determination was ~0.02%. (b) Fluorescent gel image of TAMRA labeled oligonucleotide, ODN13 (Excitation = 532 nm). (c) RP-HPLC profile of ODN13. This is the full profile of the HPLC trace shown in Fig 3b. (d) UV-Visible spectrum of ODN13 showing characteristic peaks at 260 nm and 560 nm for TAMRA labeled DNA.

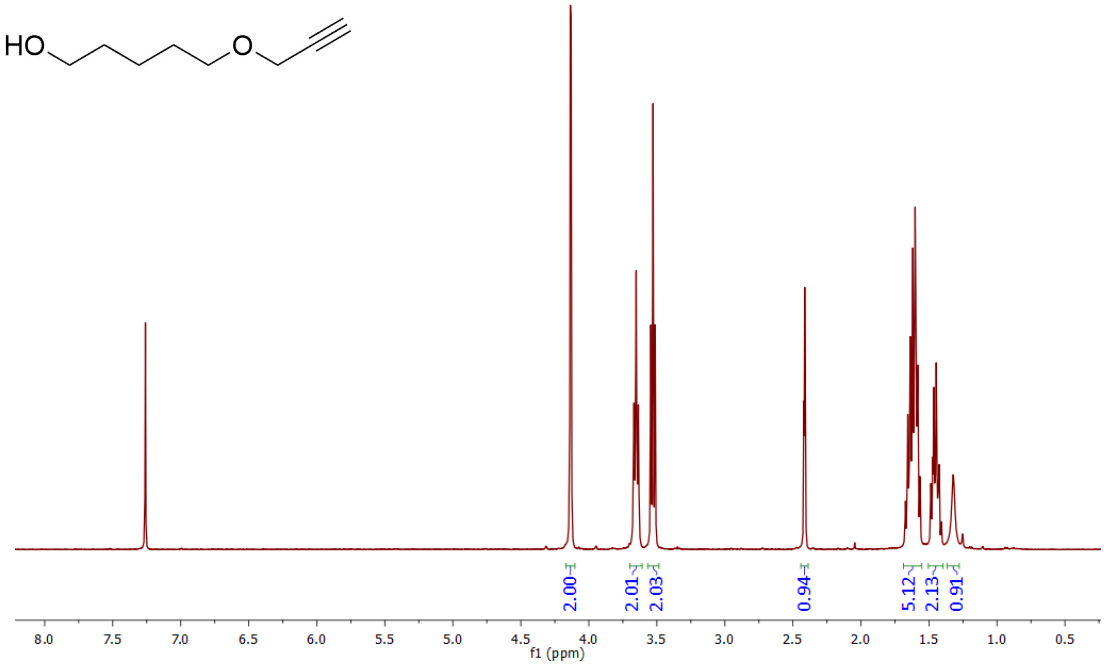
NMR Spectral data:



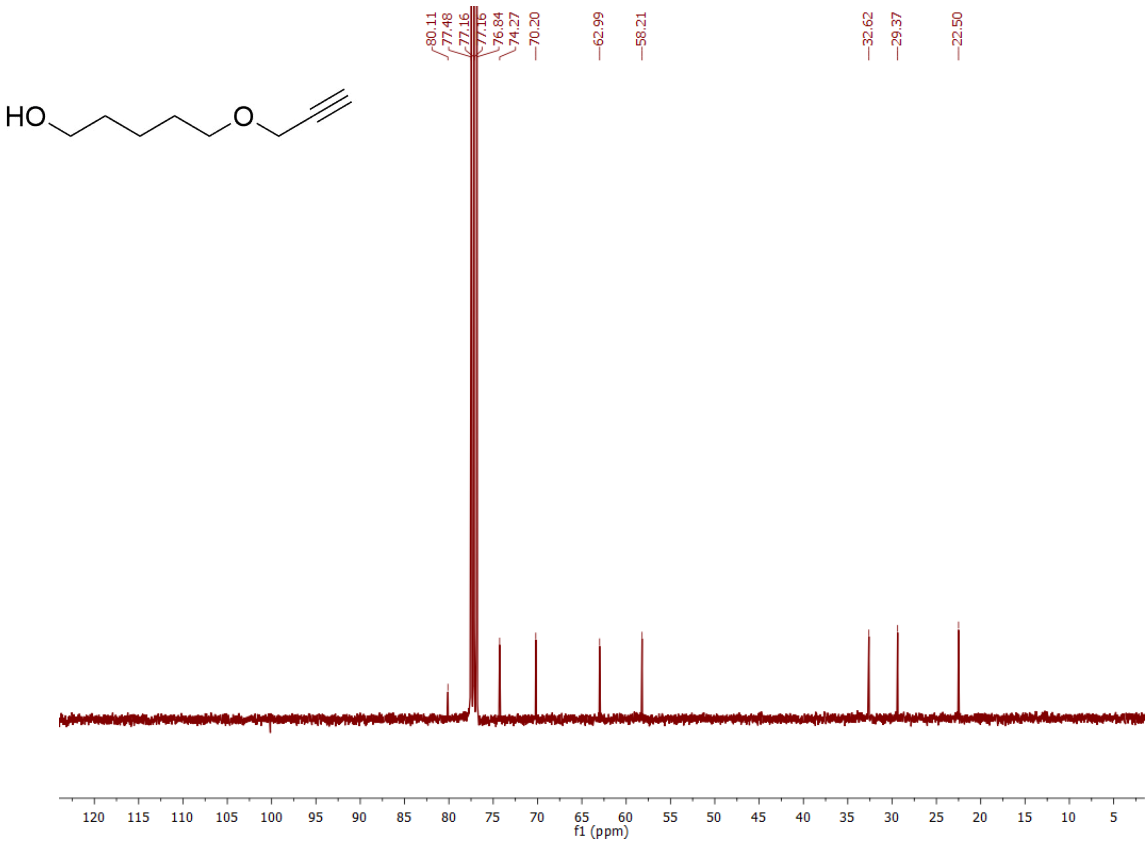
^{1}H NMR of 5-azidopentanal ($CDCl_3$)



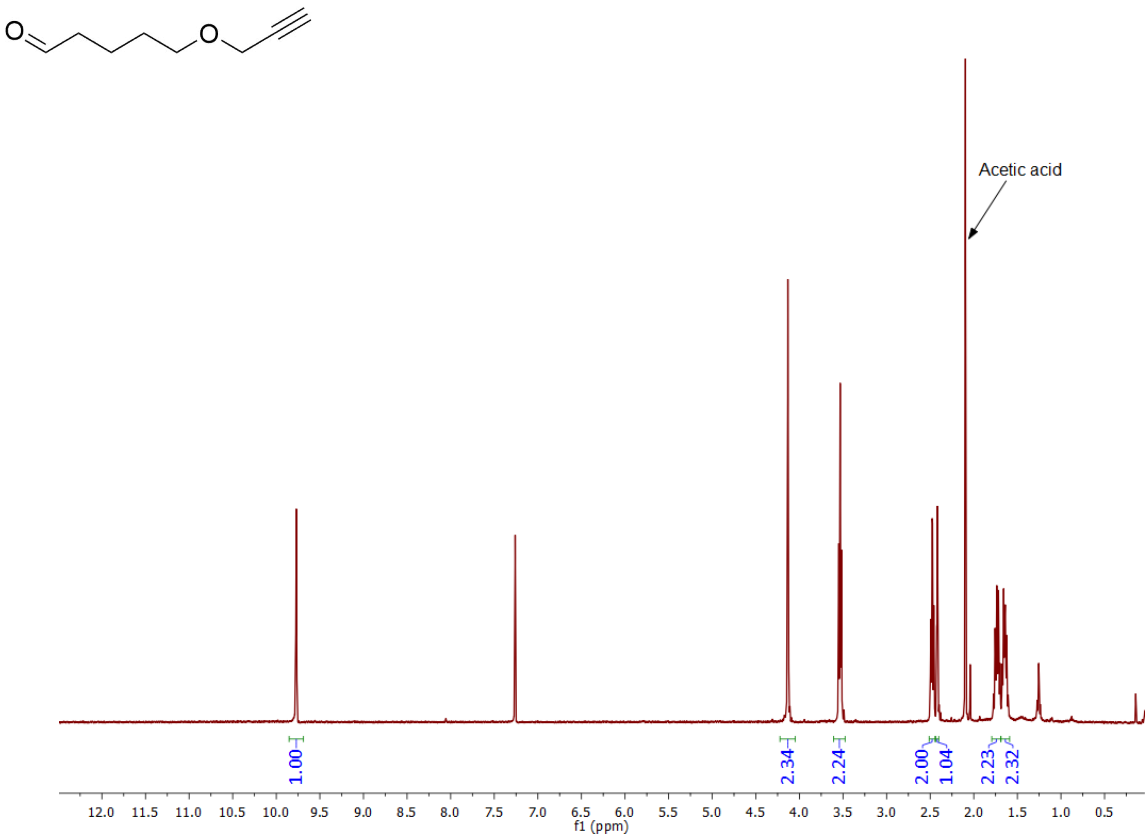
^{13}C NMR of 5-azidopentanal ($CDCl_3$)



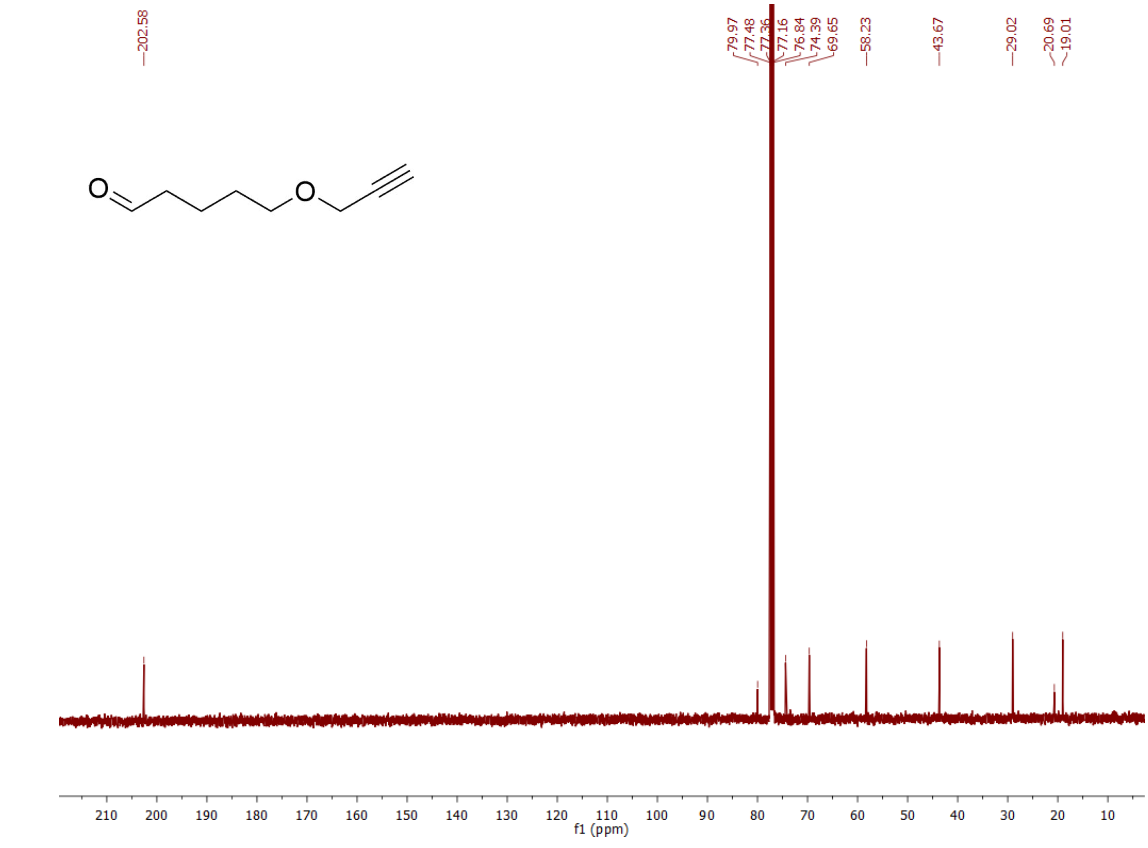
^{1}**H** NMR 5-(prop-2-yn-1-yloxy)pentan-1-ol ($CDCl_3$)



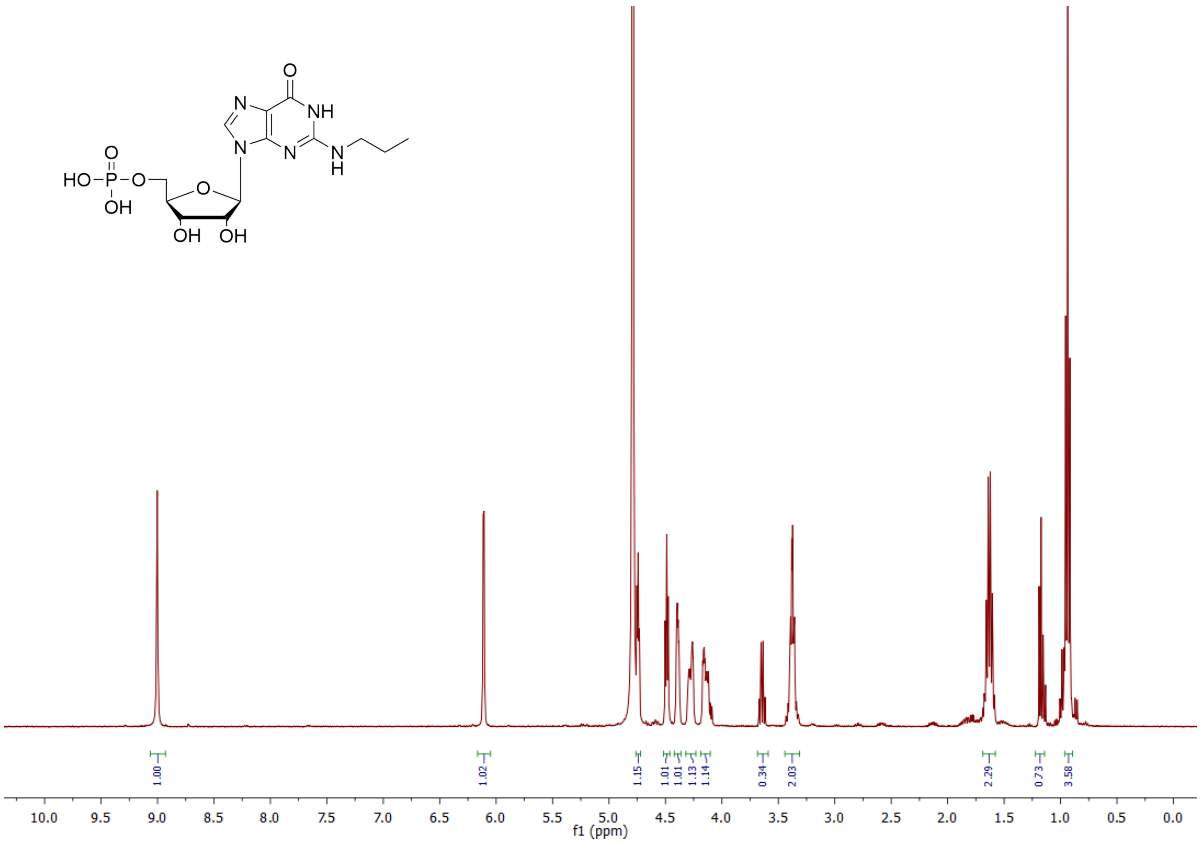
^{13}**C** NMR 5-(prop-2-yn-1-yloxy)pentan-1-ol ($CDCl_3$)



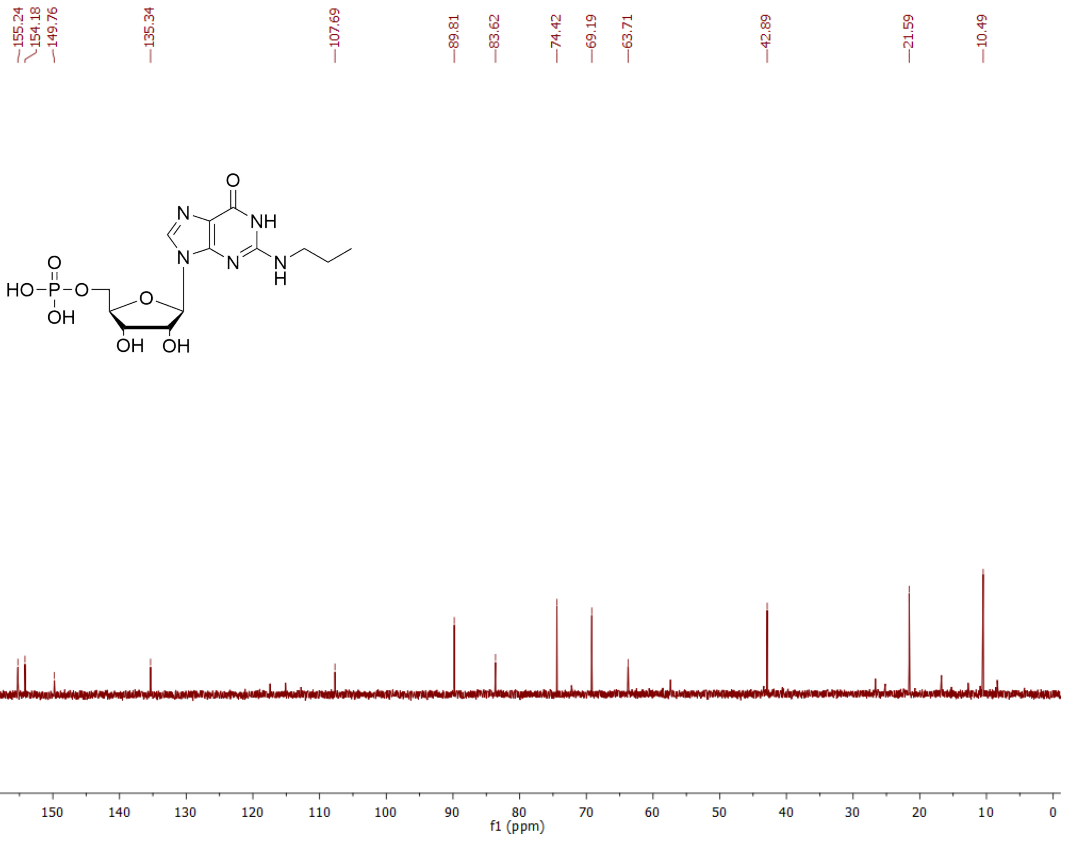
^{1}**H** NMR of 5-(prop-2-yn-1-yloxy)pentanal ($CDCl_3$)



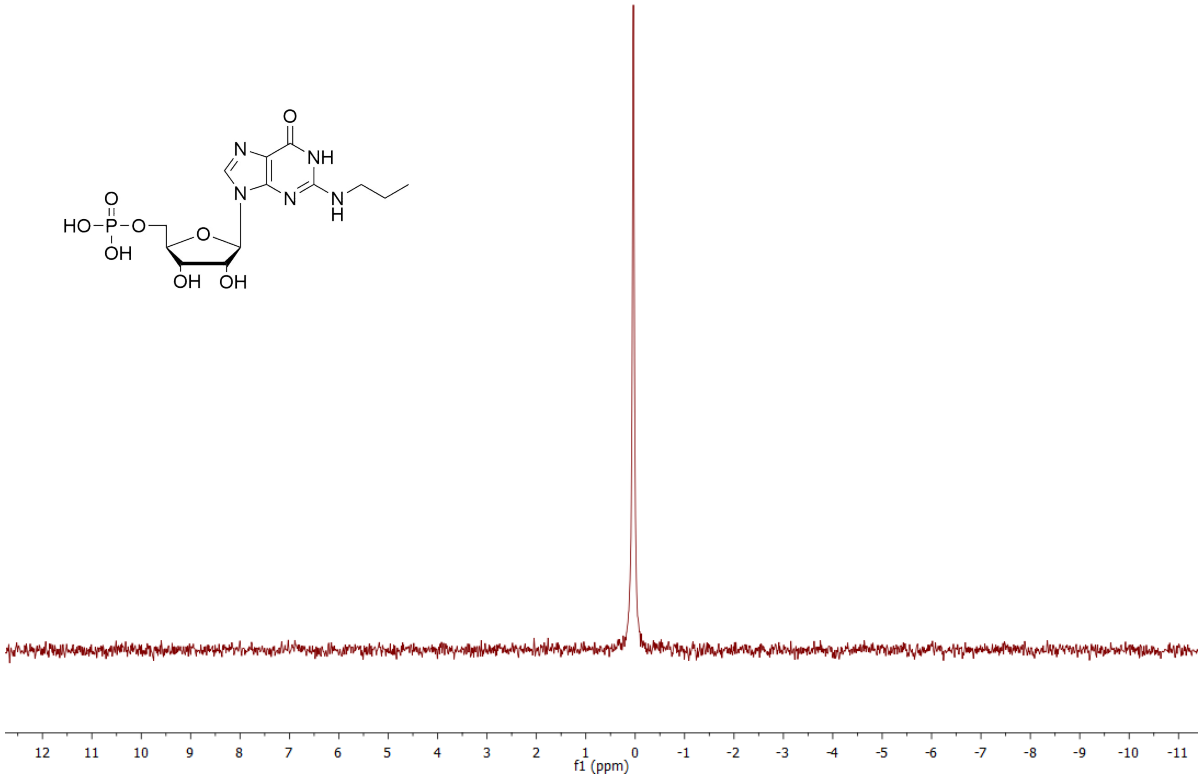
^{13}**C** NMR of 5-(prop-2-yn-1-yloxy)pentanal ($CDCl_3$)



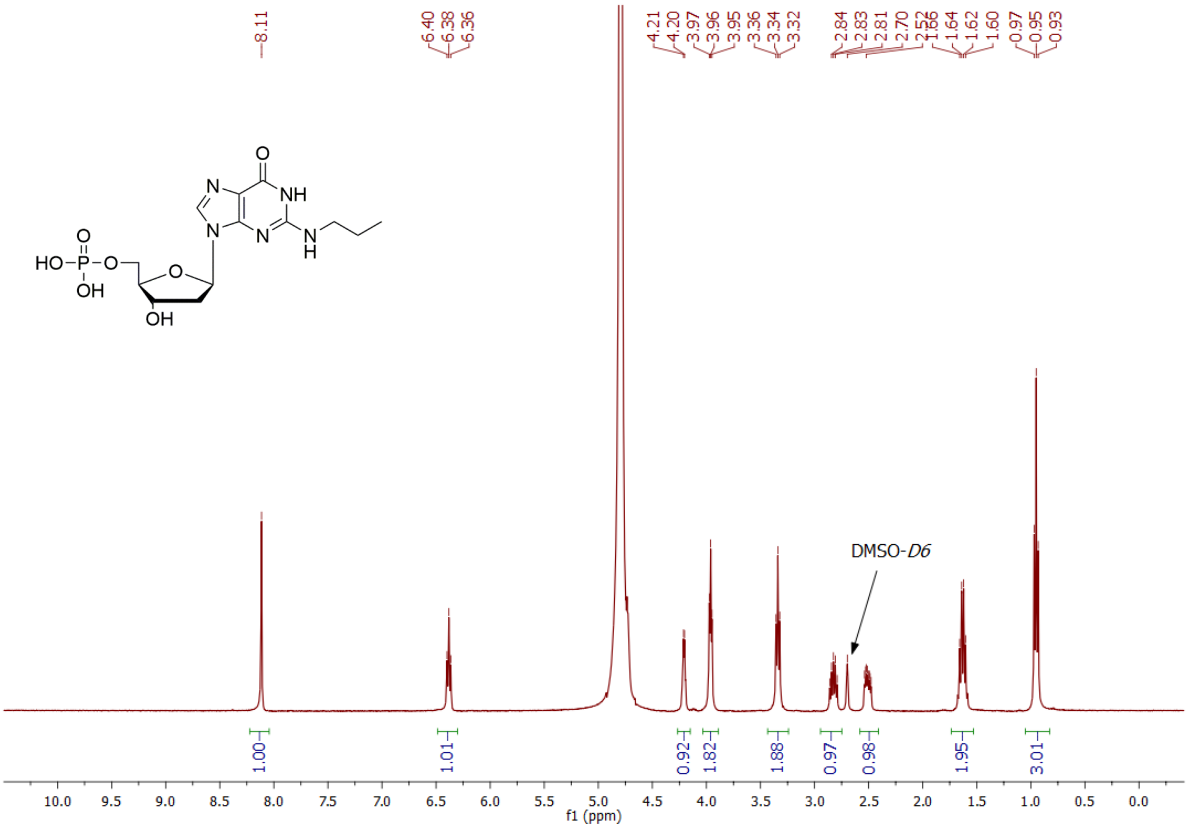
^{1}**H** NMR of N^2-propyl GMP (D_2O)



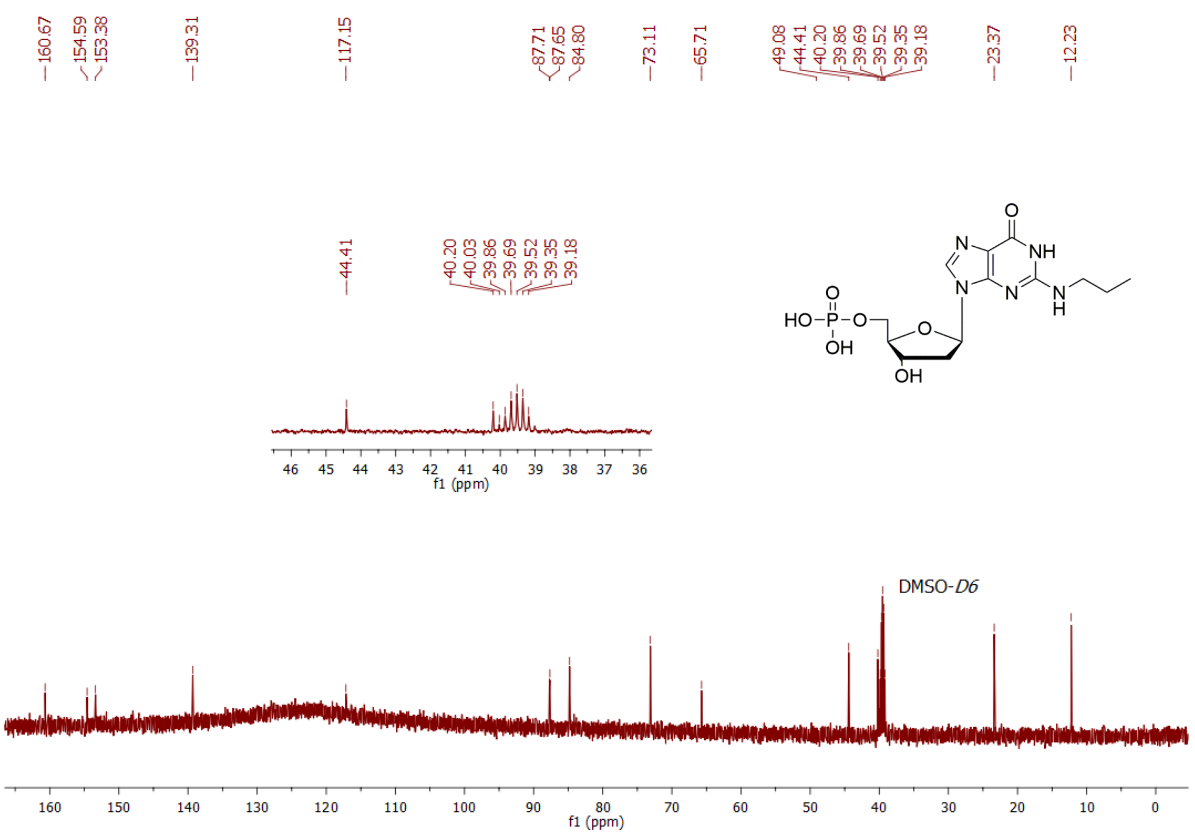
^{13}**C** NMR of N^2-propyl GMP (D_2O)



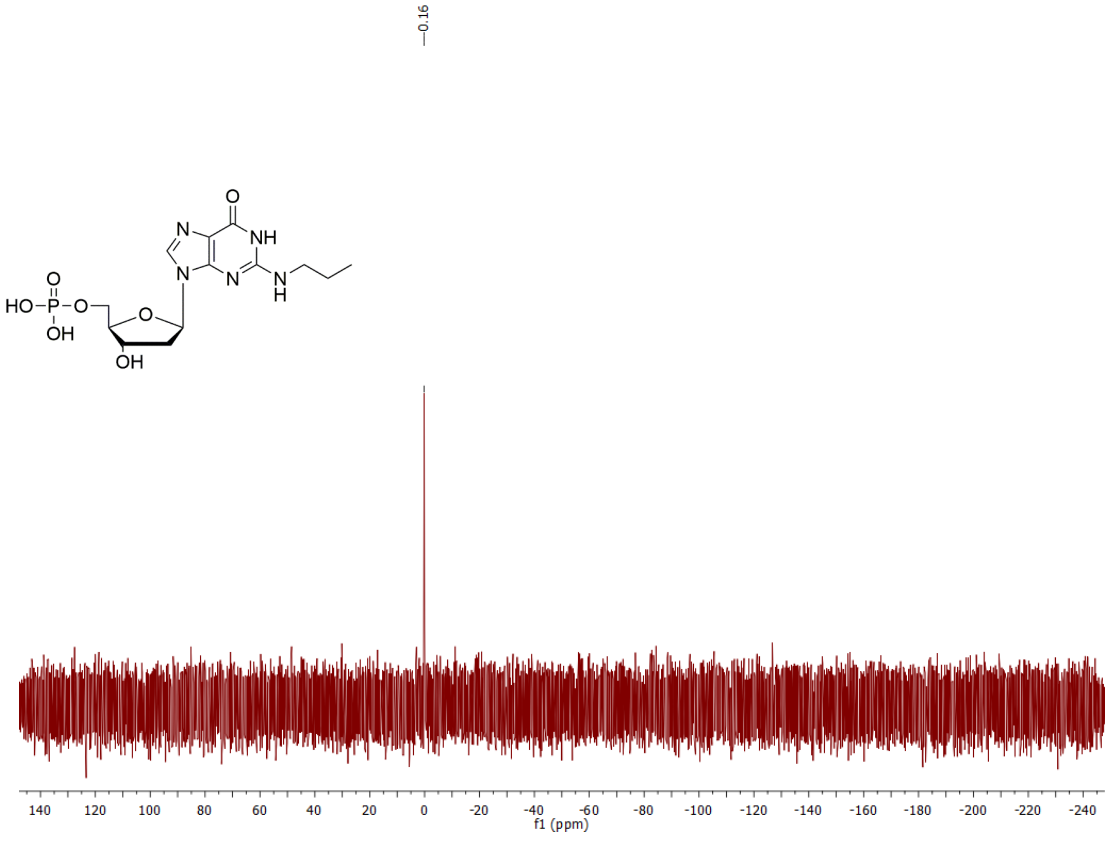
^{31}**P** NMR of N^2-propyl GMP (D_2O)



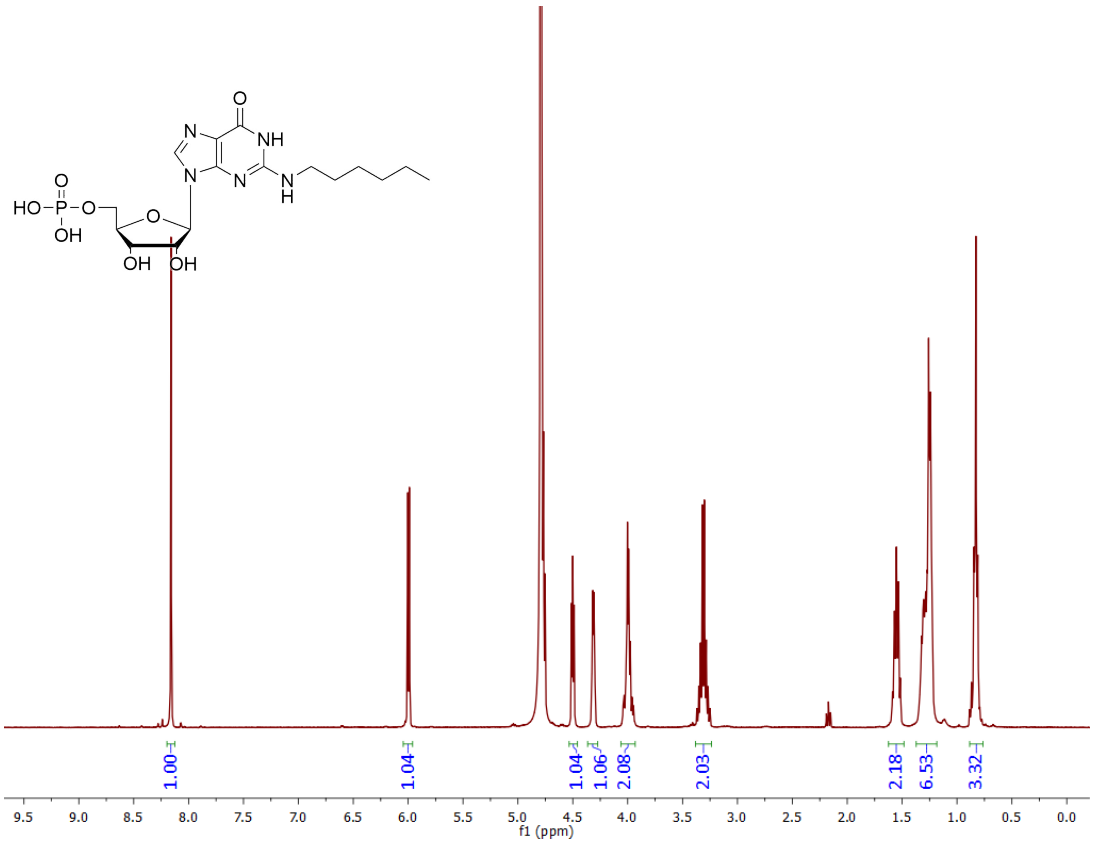
^{1}H NMR of N^2-Propyl dGMP (D_2O/5%DMSO-*D6*)



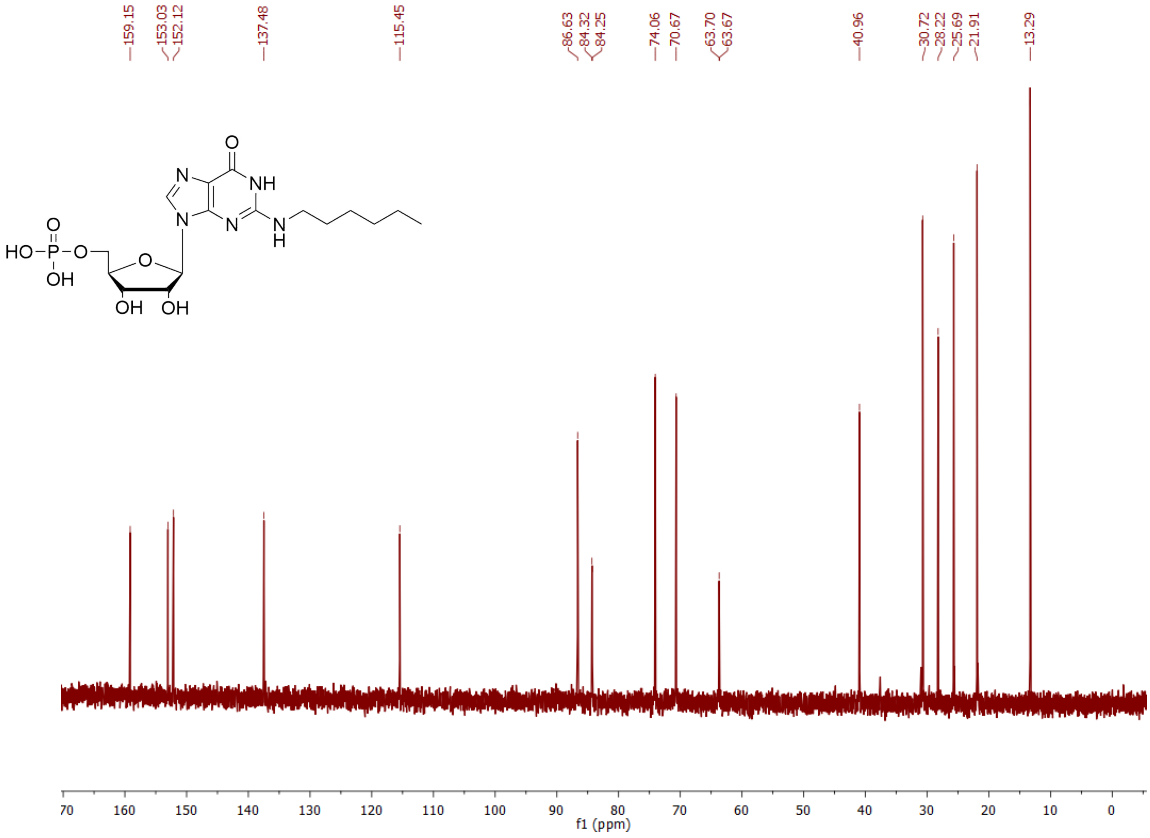
^{13}C NMR of N^2-Propyl dGMP (D_2O/5%DMSO-*D6*)



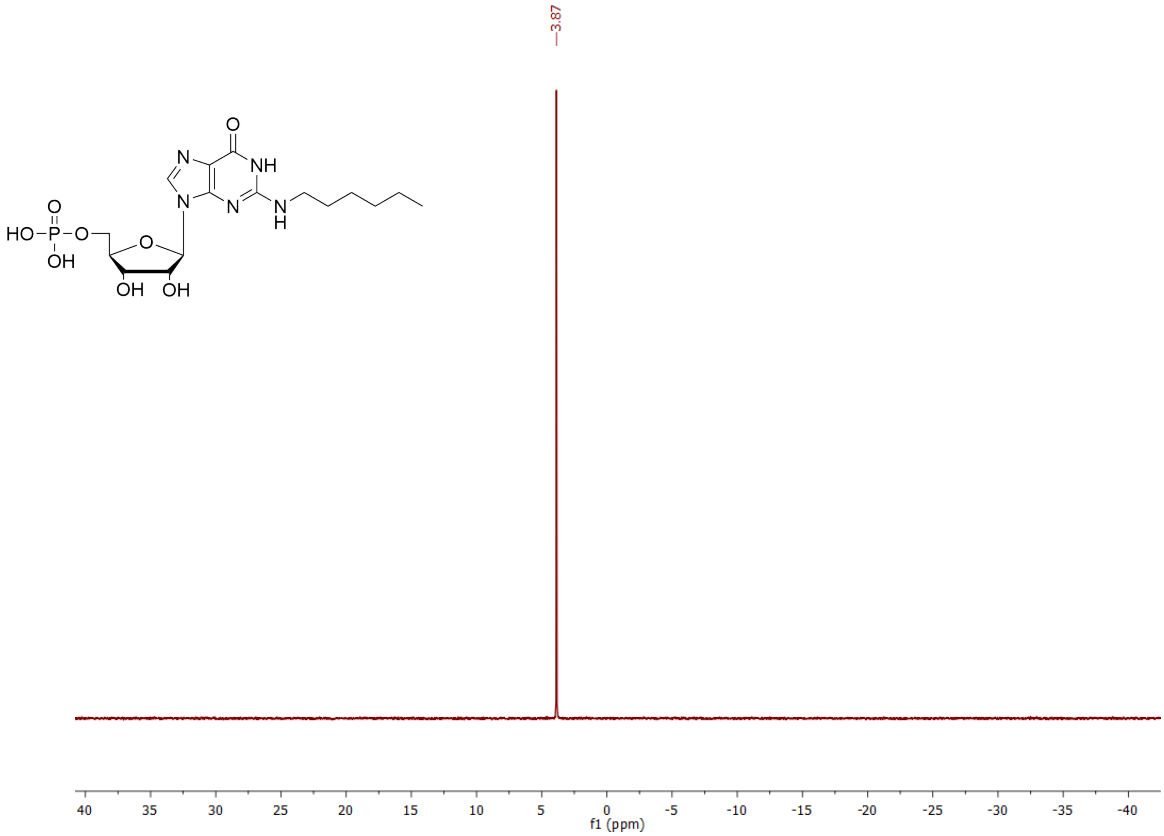
^{31}P NMR of N^2-Propyl dGMP (D_2O)



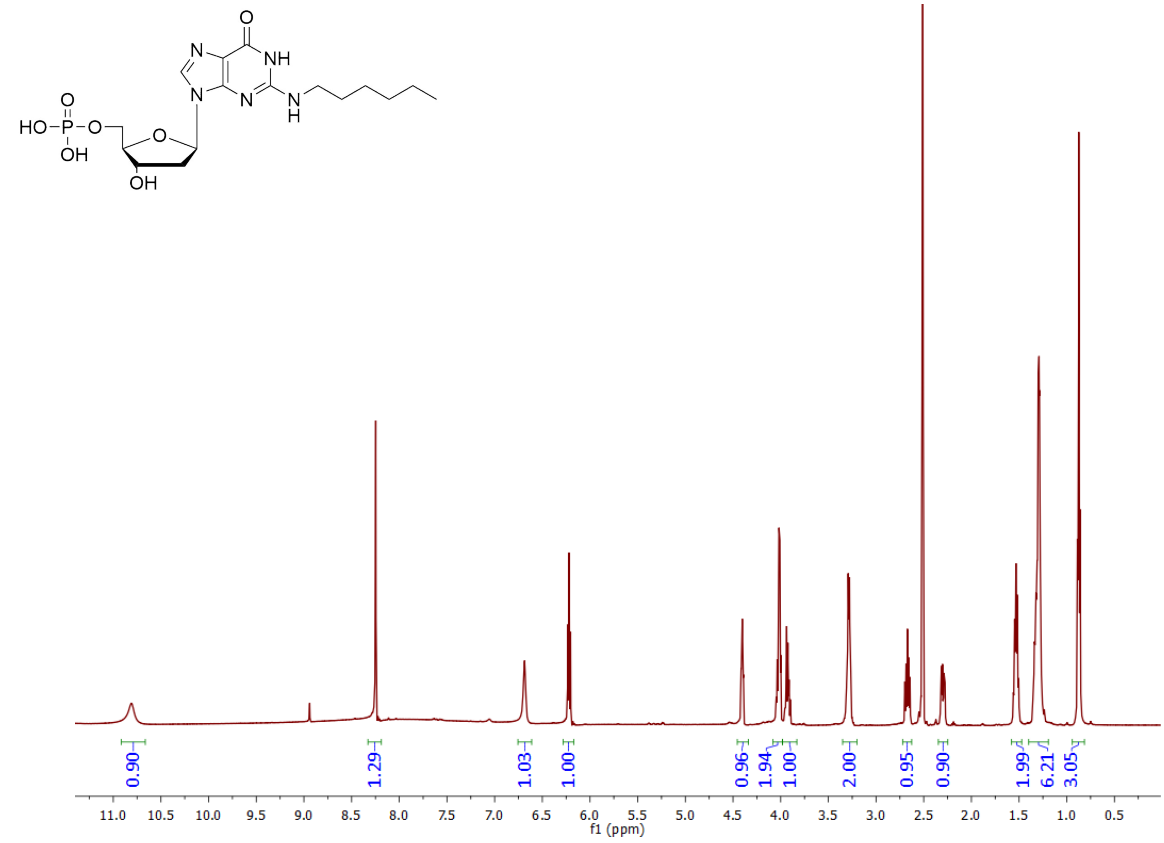
^{1}**H** NMR of N^2-hexyl GMP (D_2O)



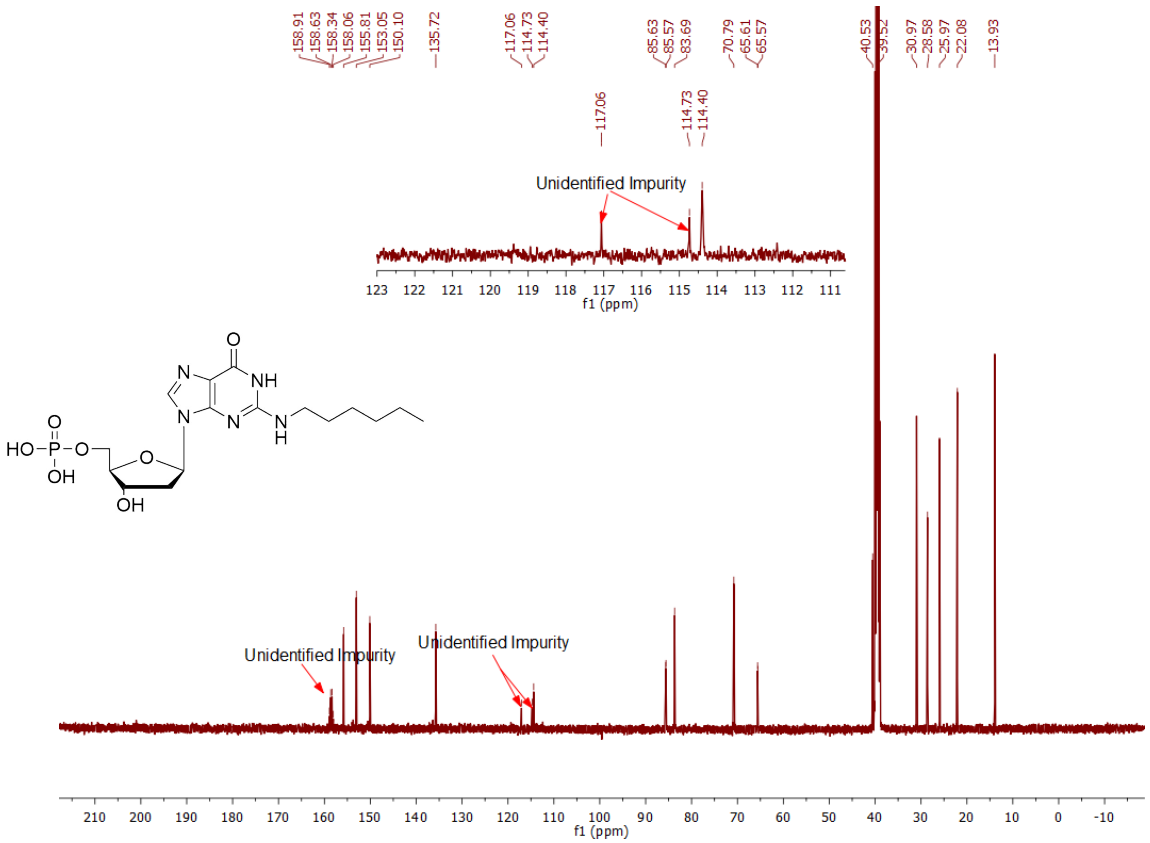
^{13}**C** NMR of N^2-hexyl GMP (D_2O)



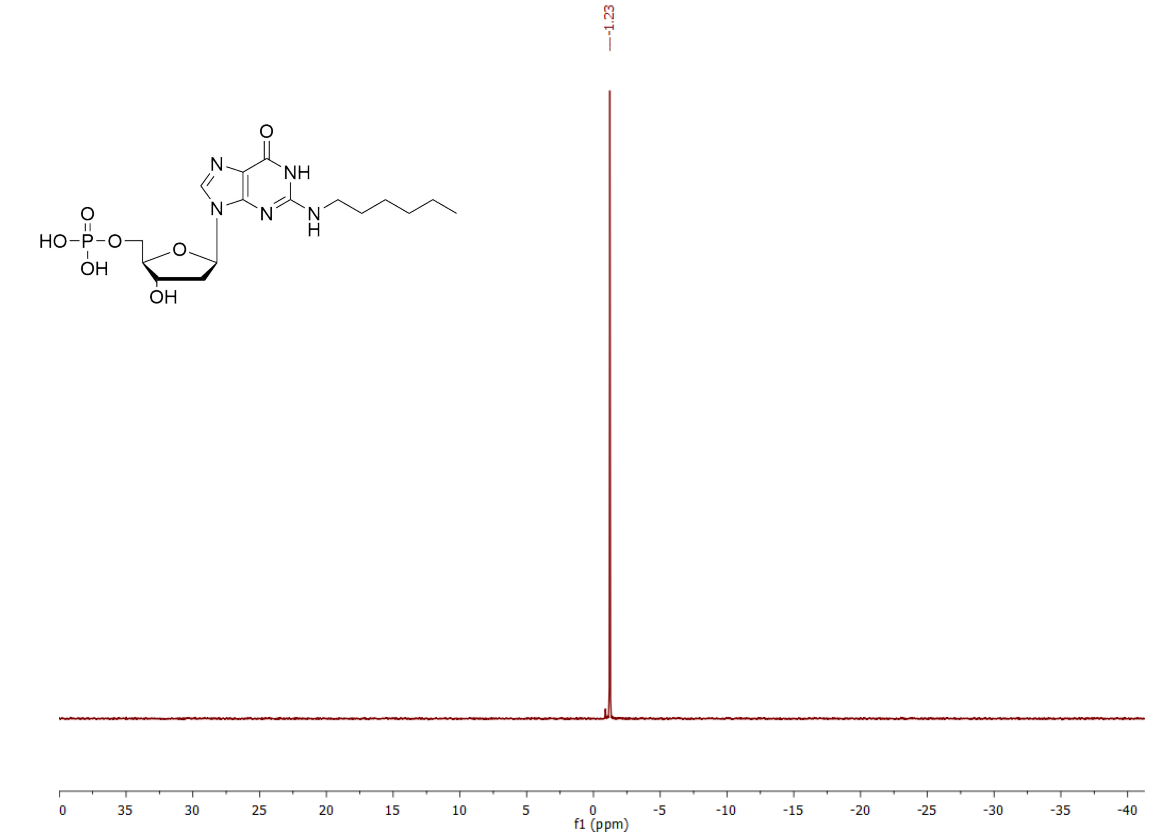
^{31}**P** NMR of N^2-hexyl GMP (D_2O)



^{1}**H** NMR of N^2-hexyl dGMP (D_2O)



^{13}C NMR of N^2-hexyl dGMP (D_2O)



^{31}P NMR of N^2-hexyl dGMP (D_2O)

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