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Supplementary Information for

Effect of terminal 3′-hydroxymethyl modification of an RNA primer on nonenzymatic primer extension

Ayan Pal,[‡,a,b] Rajat S. Das,[‡,c,d] Weicheng Zhang,[‡,a,e] Megan Lang,[c]
Larry W. McLaughlin,[c] Jack W. Szostak[a,b,e]

[a] Howard Hughes Medical Institute, Department of Molecular Biology and Center for Computational and Integrative Biology, Massachusetts General Hospital, 185 Cambridge Street, Boston, Massachusetts 02114, USA.
[b] Department of Genetics, Harvard Medical School, 77 Avenue Louis Pasteur, Boston, Massachusetts 02115, USA.
[c] Boston College, Department of Chemistry, Merkert Chemistry Center, 2609 Beacon Street, Chestnut Hill, Massachusetts 02467, USA.
[d] Alnylam Pharmaceuticals, 300 Third Street, Cambridge, Massachusetts 02142, USA.
[e] Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford St., Cambridge, Massachusetts 02138, USA.
E-mail: szostak@molbio.mgh.harvard.edu

‡These authors contributed equally to this work.

Table of Contents

1. General Materials and Methods S2

2. Experimental Procedures S2

3. NMR Spectra S9

4. LCMS Analysis of the Oligomers S16

5. Isotopic Mass Distribution of +3 Extension Product of P1 S18

6. Effect of pH and divalent metal ion on primer extension efficiency S19

7. References S20

1. General Materials and Methods

Bulk solvents and chemicals were purchased either from Fisher Scientific (Clifton, NJ) or Sigma Aldrich (St. Louis, MO). Deuterated solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). Water used for this study was obtained from a Barnstead Nanopure System (Thermo Scientific, Dubuque, IA). All reagents used were of highest purity available. The silica gel used for flash chromatography (40-63 μm) and gravitational columns (63-200 μm) were purchased from Silicycle Inc (Québec City, Canada). NMR data were taken on a Varian 500 or 600 MHz spectrometer and analyzed using MestReNova software, referenced against the residual solvent peak. Liquid chromatography followed by high-resolution mass spectroscopy (LC-HRMS) analysis in the ESI mode was carried out on an Agilent 6520 Q-TOF LC/MS-MS instrument. *β*-tributylstannylstyrene was prepared using a method published by Brooke et al.[1] N^2-Isobutyryl-2'-deoxyguanosine was synthesized according to a published procedure.[2]

2. Experimental Procedures

1-(5-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxy-*β*-D-*erythro*-pentofuranosyl)-N^2-isobutyrylguanine (3)

N^2-Isobutyryl-2′-deoxyguanosine **2** (25 gm, 74.2 mmol) and few crystals of DMAP were suspended in 400 ml anhydrous pyridine. TBDPS-Cl (9.9 ml, 38 mmol) was added and the mixture stirred for 8 hours. More TBDPS-Cl (4.9 ml, 18.9 mmol) was then added and stirring continued overnight. The next day another portion of TBDPS-Cl (4.9 ml, 18.9 mmol) was added. After stirring for another 24 hours the solution turned clear and TLC showed complete formation of the product. The solvent was evaporated by rotavap and

the residue re-dissolved in 100 ml methanol. The syrupy liquid was slowly poured into a stirring mix of hexanes (300 ml), diethyl ether (100 ml) and water (800 ml). The product was isolated as white solid via filtration (39.26 gm, 92%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.51 (s, 1H, N_1-H), 11.23 (s, 1H, NH-^{i}Bu), 8.63 (dt, *J* = 4.4, 1.7 Hz, 1H, C_8-H), 7.93 (s, 1H, Ar-H), 7.66 – 7.55 (m, 5H, Ar-H), 7.39 – 7.22 (m, 8H, Ar-H), 6.15 (d, *J* = 6.0 Hz, 1H, $C_{1'}$-H), 5.17 (s, 1H, 3'-OH) 4.78 (q, *J* = 4.7, 4.1 Hz, 1H, $C_{4'}$-H), 4.25 – 4.08 (m, 1H, ^{i}Bu-H), 3.86 (m, 2H, $C_{5'}$-H, $C_{5''}$-H), 2.97 (m, 1H, $C_{3'}$-H), 2.47 (m, 2H, $C_{2'}$-H and $C_{2''}$-H), 1.36 – 1.17 (m, 6H, 2 X ^{i}Bu-CH_3), 1.01 (s, 9H, 3 X TBDPS).

^{13}C NMR (101 MHz, $CDCl_3$) δ 180.68, 156.16, 149.21, 148.65, 148.28, 137.52, 136.74, 135.54, 135.42, 132.96, 132.79, 129.83, 129.79, 127.77, 127.72, 127.69, 124.10, 120.69, 87.91, 84.43, 71.22, 64.49, 60.42, 41.09, 36.01, 26.98, 26.95, 26.93, 26.90, 26.86, 26.80, 26.77, 26.74, 26.72, 21.07, 19.15, 19.09, 19.03, 19.00, 18.97, 14.22.

ESI-MS (positive mode): *m/z* calcd $[M+H]^+$ $C_{30}H_{38}N_5O_5Si$: 576.2642 Da, observed 576.2661 Da $[M + H]^+$.

1-(5-*O*-(*tert*-butyldiphenylsilyl)-3-*O*-((*p*-tolyloxy)thiocarbonyl)-2′-deoxy-*β*-D-*erythro*-pentofuranosyl)-N^2-isobutyrylguanine (4)

Compound **3** (25 gm, 43.5 mmol) and DMAP (10.62 gm, 86.9 mmol) were dissolved in 300 mL anhydrous acetonitrile. *O*-(*p*-Tolyl)-chlorothionoformate (9.3 ml, 60.9 mmol) was dissolved in 50 ml dry acetonitrile and slowly added to the reaction mixture over a period of 15 min under an argon atmosphere. After stirring for 1 hour, product formation was complete by TLC. The solvent was evaporated and the resulting yellow solid was purified by silica gel column chromatography with a step gradient of methanol (0-6%) in DCM. Compound **4** was isolated as a white solid (29.6 gm, 94%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (s, 1H, N_1-H), 11.64 (s, 1H, NH-^{i}Bu), 8.16 (s, 1H, C_8-H), 7.61 (m, 4H, Ar-H), 7.50 – 7.32 (m, 6H, Ar-H), 7.32 – 7.24 (m, 2H, Ar-H), 7.17 – 7.04 (m, 2H, Ar-H), 6.34 (dd, *J* = 8.9, 5.6 Hz, 1H, $C_{1'}$-H), 5.93 (dt, *J* = 6.0, 1.6 Hz, 1H, ^{i}Bu-H), 4.42 (td, *J* = 4.9, 1.7 Hz, 1H, $C_{4'}$-H), 3.95 (qd, *J* = 11.2, 4.9 Hz, 2H, $C_{5'}$-H, $C_{5''}$-H), 3.12 (ddd, *J* = 14.6, 9.1, 5.9 Hz, 1H, $C_{3'}$-H), 2.85 (ddd, *J* = 14.4, 5.6, 1.9 Hz, 1H, $C_{2'}$-H), 2.75 (m, 1H, $C_{2''}$-H), 2.34 (s, 3H, tolyl-CH_3), 1.12 (m, 6H, 2 X ^{i}Bu-CH_3), 0.99 (s, 9H, 3 X TBDPS).

^{13}C NMR (101 MHz, DMSO) δ 194.18, 180.51, 155.23, 151.33, 149.04, 148.67, 137.45, 136.62, 135.53, 135.49, 132.96, 132.82, 130.61, 130.43, 128.37, 128.30, 121.89, 120.91, 84.55, 84.49, 83.55, 64.53, 36.41, 35.27, 27.07, 20.90, 19.31, 19.25, 19.22.

ESI-MS (positive mode): *m/z* calcd $[M+H]^+$ $C_{38}H_{44}N_5O_6SSi$: 726.2782 Da, observed 726.2796 Da $[M + H]^+$.

1-(5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-dideoxy-3′-*C*-formyl-*β*-D-*erythro*-pentofuranosyl)-N^2-isobutyrylguanine (5)

Compound **4** (22 gm, 30.3 mmol), *β*-tributylstannylstyrene (35.9 gm, 91 mmol) and AIBN (2 gm, 12.2 mmol) were dissolved in 250 ml dry toluene and the mixture was degassed by sparging argon through the solution for 30 min. This reaction mix was heated at 80° C for 48 hours and more AIBN (7 gm, 42.6 mmol) was added to the reaction, maintaining the argon atmosphere. After this period, toluene was evaporated and the residue was loaded on a pad of silica and washed with hexanes (500 ml) which eluted unreacted *β*-tributylstannylstyrene. Then it was washed with 800 mL 4:1 DCM : Methanol. The eluent was collected and evaporated on a rotary evaporator. The residue was dissolved in 150

ml dioxane. 4-Methylmorpholine *N*-oxide (3.63 gm, 31 mmol) and osmium tetroxide (160 mg in 0.8 ml water) was added. After stirring for two hours, powdered sodium periodate (11.75 gm, 55 mmol) was slowly added to the reaction mixture over five minutes and stirring continued for another three hours. The resulting aldehyde was far more polar than the starting materials and by-products. The reaction was quenched by adding sodium thiosulfate solution and extracted using dichloromethane. The organic layer was evaporated and purified by purified by silica gel column chromatography with a step gradient of methanol (0-5%) in DCM. Compound **5** was isolated as a white solid (4.97 gm, 28%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 12.24 (s, 1H, N_1-H), 10.08 (s, 1H, NH-^{i}Bu), 9.65 (d, *J* = 1.5 Hz, 1H, aldehyde-H), 7.85 (s, 1H, C_8-H), 7.59 (m, 4H, Ar-H), 7.45 – 7.20 (m, 8H, Ar-H), 6.00 (dd, *J* = 6.9, 4.2 Hz, 1H, $C_{1'}$-H), 4.32 (dt, *J* = 7.4, 4.6 Hz, 1H, $C_{4'}$-H), 3.94 – 3.71 (m, 2H, $C_{5'}$-H, $C_{5''}$-H), 3.58 – 3.42 (m, 1H, $C_{3'}$-H), 2.90 – 2.68 (m, 2H, ^{i}Bu-H and $C_{2'}$-H), 2.65 – 2.49 (m, 1H, $C_{2''}$-H), 1.21 (m, 6H, 2 X ^{i}Bu-CH_3), 1.08 – 0.92 (s, 9H, 3 X TBDPS).

^{13}C NMR (126 MHz, Chloroform-*d*)) δ 198.87, 179.56, 155.71, 148.01, 147.74, 135.82, 135.40, 135.05, 132.52, 132.45, 130.33, 129.75, 128.17, 127.56, 121.33, 84.69, 81.21, 80.84, 77.32, 77.06, 76.81, 64.72, 52.04, 51.68, 36.42, 35.86, 32.43, 26.85, 26.80, 19.14, 19.04, 18.94

ESI-MS (positive mode): *m/z* calcd $[M+H]^+$ $C_{31}H_{38}N_5O_5Si$: 588.2642 Da, observed 588.2666 Da $[M + H]^+$.

1-(5′-*O*-(*tert*-butyldiphenylsilyl)-2′,3′-dideoxy-3′-*C*-hydroxymethyl-*β*-D-*erythro*-pentofuranosyl)-*N*^{2}-isobutyrylguanine (6)

Compound **5** (3.2 gm, 5.45 mmol) was dissolved in 100 ml methanol, cooled in an ice bath and sodium borohydride (416 mg, 11 mmol) was added. The mixture was allowed to warm to room temperature. After 30 min, reduction was complete and the reaction mixture was quenched with ammonium chloride solution. Excess methanol was evaporated and the residue was extracted with brine/dichloromethane. The organic layer was concentrated and the residue was purified via silica gel column chromatography with a step gradient of methanol (0-7%) in DCM. Compound **6** was isolated as a white solid (3.02 gm, 94%).

^{1}H NMR (600 MHz, Chloroform-*d*) δ 12.20 (s, 1H, N_1-H), 9.79 (s, 1H, NH-^{i}Bu), 7.91 (s, 1H, C_8-H), 7.72 – 7.51 (m, 5H, TBDPS-H), 7.47 – 7.18 (m, 8H, TBDPS-H), 6.00 (dd, *J* = 6.8, 3.6 Hz, 1H, $C_{1'}$-H), 4.00 (dt, 1H, $C_{4'}$-H), 3.78 (ddd, *J* = 37.0, 11.2, 4.4 Hz, 2H, $C_{5'}$-H, $C_{5''}$-H), 3.62 (qd, *J* = 10.9, 5.5 Hz, 2H, 2 X $C_{3''}$-H), 2.74 (p, *J* = 15.8, 7.9 Hz, 1H, ^{i}Bu-H), 2.66 (m, 1H, $C_{3'}$-H), 2.46 (m, 1H, $C_{2'}$-H), 2.33 (m, 1H, $C_{2''}$-H), 1.21 (dd, *J* = 6.8, 3.3 Hz, 6H, 2 X ^{i}Bu-CH_3), 1.01 (s, 9H, 3 X TBDPS CH_3).

^{13}C NMR (126 MHz, Chloroform-*d*) δ 180.41, 156.23, 148.20, 148.08, 137.46, 135.53, 135.50, 135.43, 132.84, 132.72, 129.89, 129.82, 127.80, 127.76, 120.75, 84.86, 83.68, 77.37, 77.31, 77.11, 76.86, 64.92, 62.08, 45.29, 41.18, 35.96, 30.93, 29.28, 26.89, 26.86, 26.83, 19.13, 19.11, 19.04, 18.97.

ESI-MS (positive mode): *m/z* calcd $[M+H]^+$ $C_{31}H_{40}N_5O_5Si$: 590.2799, observed 590.2790 Da $[M + H]^+$.

1-(5′-*O*-(*tert*-butyldiphenylsilyl)-(2′,3′-dideoxy-3′-*α*-C-(*O*-dimethoxytrityl)methyl-*β*-D-*erythro*-pentofuranosyl)-N^2-isobutyrylguanine (7)

Thoroughly dried compound **6** (1.35 gm, 2.29 mmol) and a few crystals of DMAP were dissolved in 30 ml dry pyridine under argon atmosphere. 4,4′-dimethoxytrityl chloride (1.55 gm ,4.58 mmol) was added and the reaction mixture was stirred overnight. Next day the flask was cooled in an ice bath and reaction was quenched by adding 5 ml methanol. Solvents were evaporated under reduced pressure and the residue was chromatographed on a silica gel column with a step gradient of methanol (0-4%) in DCM containing 1% triethylamine. Compound **7** was isolated as a white solid (2.39 gm, 93%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 12.21 (s, 1H, N_1-H), 10.87 (s, 1H, NH-^{i}Bu), 8.57 (dt, *J* = 4.3, 1.7 Hz, 6H, Ar-H), 7.93 (s, 1H, C_8-H), 7.75 – 7.51 (m, 7H, Ar-H), 7.46 – 7.04 (m, 22H, Ar-H), 6.82 – 6.64 (m, 4H, Ar-H), 6.01 (dd, *J* = 6.9, 3.0 Hz, 1H, $C_{1'}$-H), 3.96 (ddd, *J* = 8.1, 4.6, 3.3 Hz, 1H, $C_{4'}$-H), 3.87 (dd, *J* = 11.4, 3.3 Hz, 1H, $C_{5'}$-H), 3.72 (s, 6H, 2 X DMTr-CH_3), 3.63 (dd, *J* = 11.4, 4.6 Hz, 1H, $C_{5'}$-H), 3.21 (dd, *J* = 9.4, 5.7 Hz, 1H, $C_{3''}$-H), 3.06 (dd, *J* = 9.5, 5.8 Hz, 1H, $C_{3''}$-H), 2.72 (p, *J* = 6.9 Hz, 1H), 2.67 – 2.58 (m, 1H, $C_{3'}$-H), 2.40 (ddd, *J* = 13.5, 7.7, 3.0 Hz, 1H, $C_{2'}$-H), 2.28 (ddd, *J* = 13.4, 9.7, 7.0 Hz, 1H, $C_{2''}$-H), 1.19 (m, 6H, 2 X ^{i}Bu-CH_3), 1.00 (s, 9H, 3 X TBDPS CH_3).

^{13}C NMR (126 MHz, cdcl$_3$) δ 179.35, 179.32, 158.52, 158.47, 158.43, 155.88, 149.71, 149.69, 149.50, 149.48, 147.93, 147.83, 144.74, 144.72, 144.68, 136.86, 136.52, 136.10, 135.83, 135.76, 135.39, 135.06, 132.91, 132.88, 132.86, 132.82, 132.79, 130.28, 130.11, 129.61, 129.48, 128.32, 128.19, 128.08, 128.05, 127.69, 127.45, 127.41, 127.37, 127.18, 126.49, 124.15, 124.12, 123.46, 123.43, 121.42, 121.41, 113.74, 113.19, 113.08, 112.53, 86.20, 84.21, 77.46, 77.20, 76.95, 64.69, 63.38, 62.96, 56.26, 55.40, 55.25, 55.23, 55.10, 54.95, 54.08, 39.15, 38.68, 36.33, 35.79, 27.89, 26.86, 26.82, 25.83, 19.15, 19.12, 19.05, 19.01, 18.97, 18.94.

ESI-MS (positive mode): *m/z* calcd $[M + H]^+$ $C_{52}H_{58}N_5O_7Si$: 892.4106, observed 892.4098 Da $[M + H]^+$.

1-(2′,3′-dideoxy-3′-*α*-C-(*O*-dimethoxytrityl)methyl-*β*-D-*erythro*-pentofuranosyl)-N^2-isobutyrylguanine phosphoramidite (1)

Compound 7 (1.31 gm, 1.46 mmol) was dissolved in dissolved in 50 mL of dry THF and a solution of 1M TBAF in THF (2.92 mL, 2.92 mmol) was added. The reaction mixture was stirred under argon atmosphere for 8 h and checked for completion. The solvent was removed under reduced pressure and the resulting yellow solid was purified by silica gel column chromatography with a step gradient of methanol (0-6%) in dichloromethane containing 1% TEA. The de-silylated product was dried overnight under high vacuum and dissolved in 20 ml dry acetonitrile under an argon atmosphere. Diisopropylethylamine (0.31 ml, 1.75 mmol), 2-Cyanoethyl *N,N,N',N'*-tetraisopropyl-phosphoramidite (0.72 ml, 2.19 mmol) and tetrazole (0.45 M in ACN, 3.57 ml, 1.61 mmol) were added to the flask and stirred for 2 hours. After all starting material was consumed; the reaction was quenched with 1 ml methanol. Solvents were evaporated under reduced pressure and the residue was dissolved in 100 ml dichloromethane, and washed three times with 100 ml saturated sodium bicarbonate solution. The volume of dichloromethane was reduced to 5 ml and it was added drop wise to 600 ml stirring hexanes. The precipitated solid was collected, dried over high vacuum and used for automated DNA synthesis (1.11 gm, 89%).

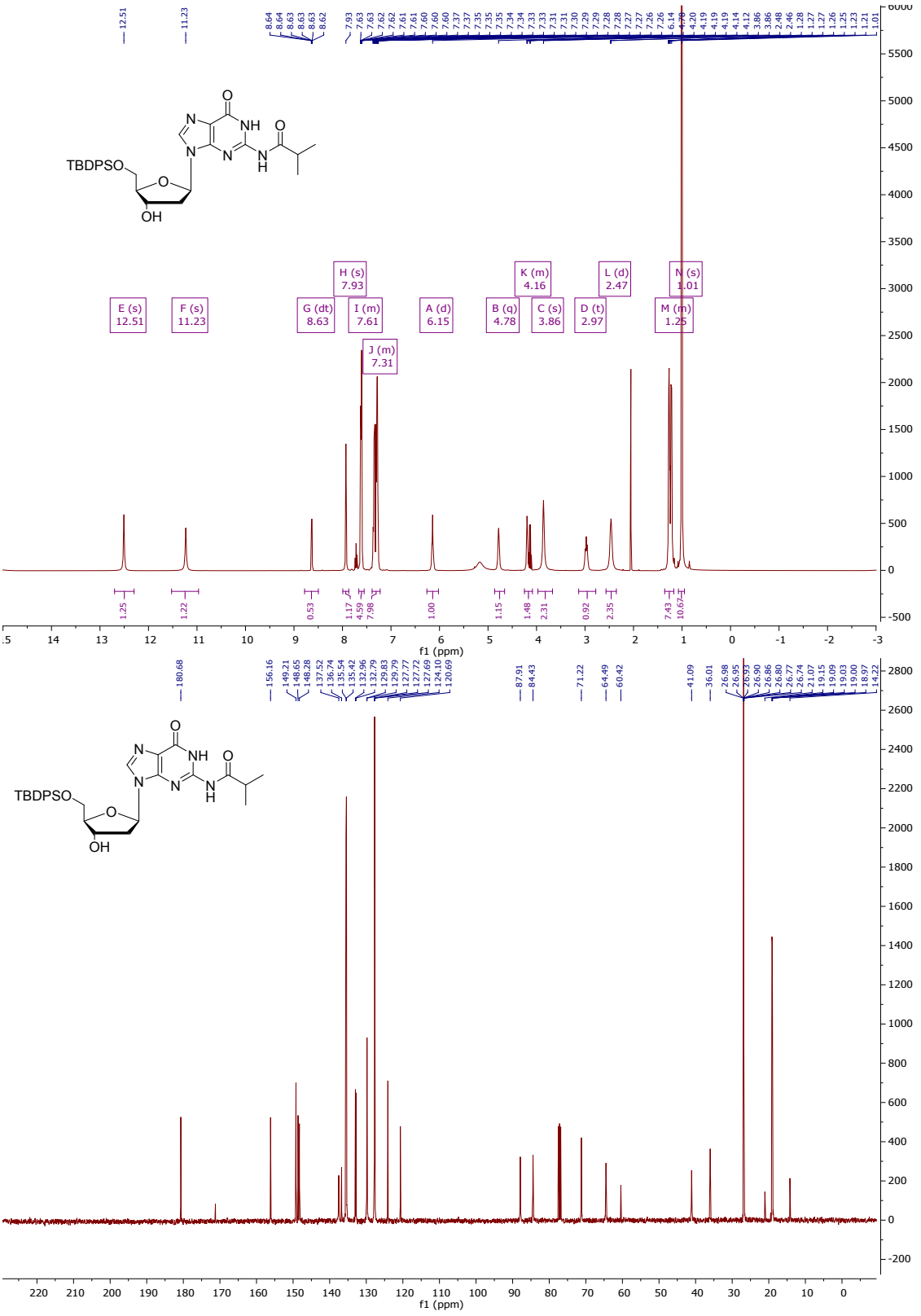
1H NMR (500 MHz, Chloroform-*d*) δ 11.92 (s, 1H, N_1-H), 8.13 – 8.06 (m, 1H, C_8-H), 7.46 – 7.39 (m, 1H, Ar-H), 7.35 – 7.20 (m, 15H, Ar-H), 6.84 (ddt, *J* = 7.4, 2.3, 1.0 Hz, 4H, Ar-

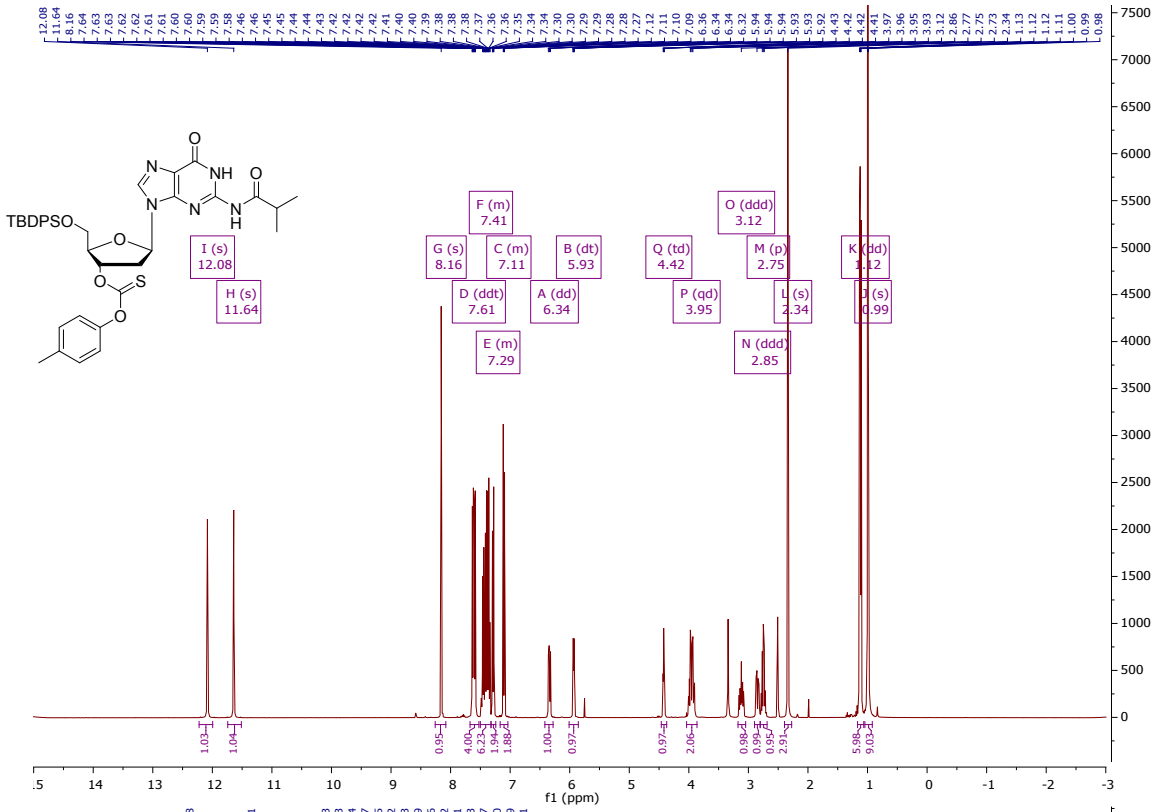
H), 6.06 (dd, *J* = 6.8, 3.9 Hz, 1H, $C_{1'}$-H), 4.10 (dd, *J* = 7.1, 3.6 Hz, 1H, $C_{4'}$-H), 3.95 (m, 1H, ^{i}Bu-H), 3.67 – 3.47 (m, 2H, $C_{5'}$-H), 3.33 – 3.17 (m, 2H, $C_{3''}$-H), 2.82 – 2.70 (m, 1H, $C_{3'}$-H), 2.69 – 2.55 (m, 3H, DMTr-CH_3), 2.53 – 2.42 (m, 1H, $C_{2'}$-H), 2.41 – 2.29 (m, 1H, $C_{2''}$-H), 1.30 – 1.25 (m, 6H, 2 X cyanoethyl-CH_2, 2 X ^{i}Pr-H and), 1.17 (dd, *J* = 6.8, 1.4 Hz, 6H, 2 X ^{i}Pr-CH_3), 1.12 (dd, *J* = 11.8, 6.8 Hz, 6H, 2 X ^{i}Pr-CH_3).

^{31}P NMR (202 MHz, $cdcl_3$) δ 149.16, 148.87

ESI-MS (positive mode): *m/z* calcd $[M+H]^+$ $C_{45}H_{57}N_7O_8P$: 854.4006 Da, observed 854.4024 Da $[M + H]^+$.

3. NMR Spectra

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G (s) 8.16
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B (dt) 5.93
D (ddt) 7.61
A (dd) 6.34
E (m) 7.29
Q (td) 4.42
P (qd) 3.95
O (ddd) 3.12
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N (ddd) 2.85
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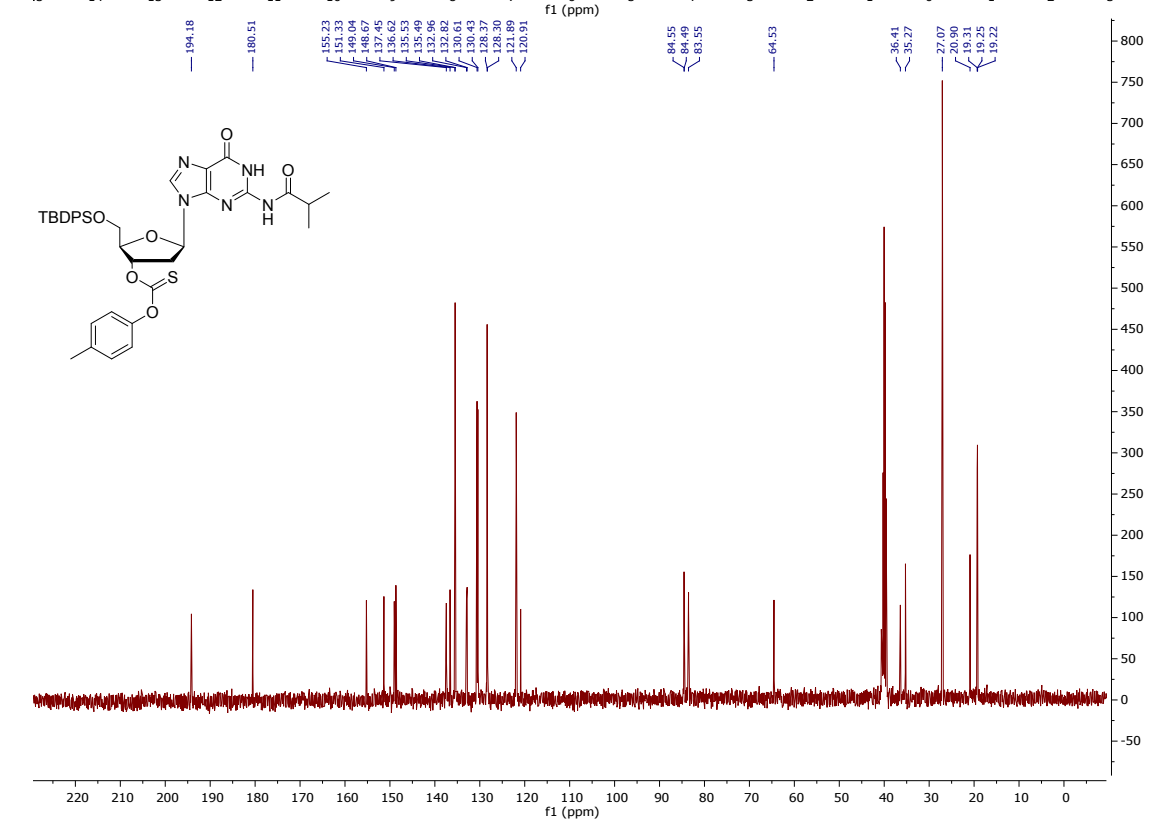
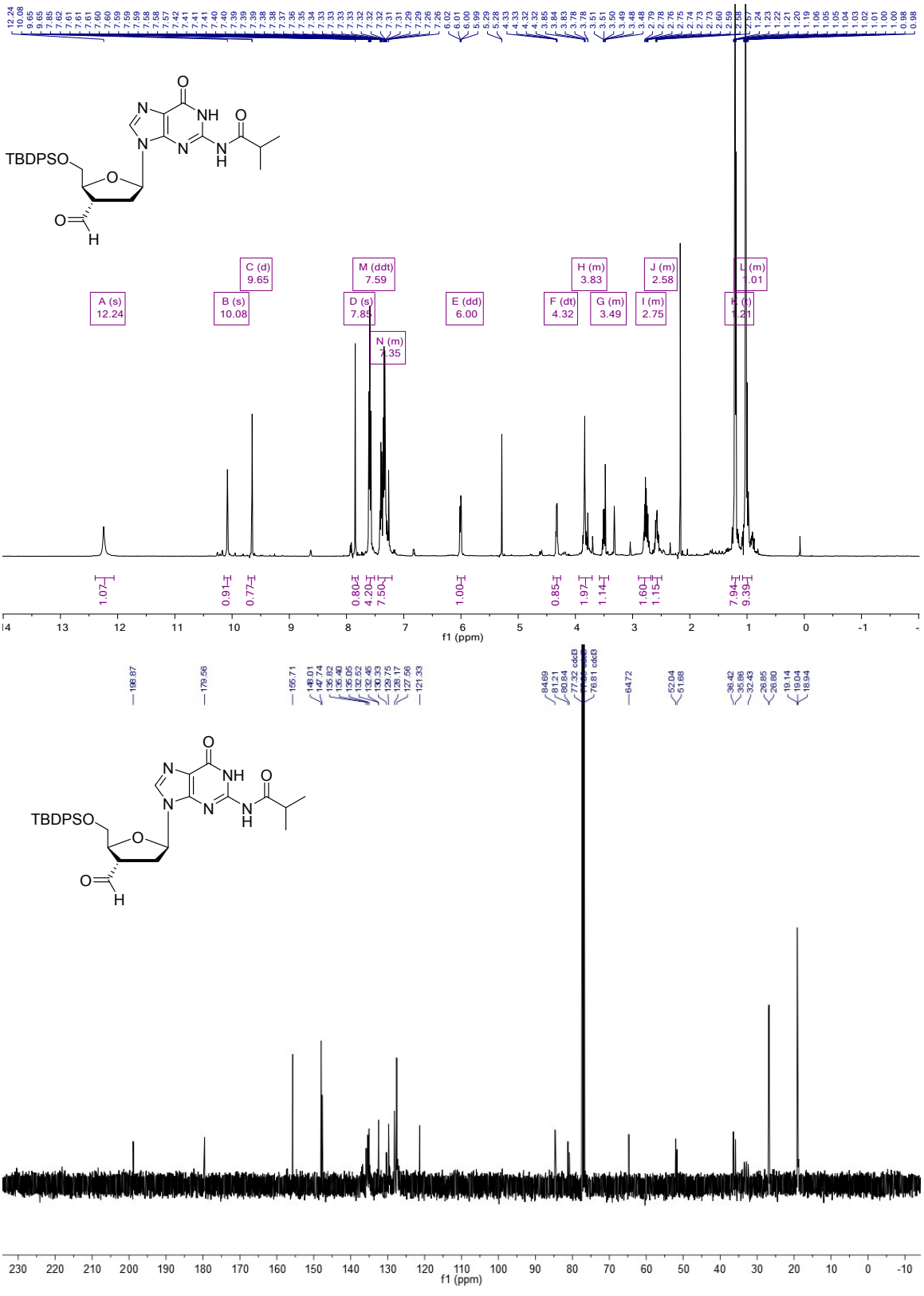
J (s) 0.99
f1 (ppm)


TBDPSO
f1 (ppm)


TBDPSO
O
N
NH
N
N
H
O
H
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B (s) 10.08
C (d) 9.65
D (s) 7.85
M (ddt) 7.59
N (m) 7.35
E (dd) 6.00
F (dt) 4.32
G (m) 3.49
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I (m) 2.75
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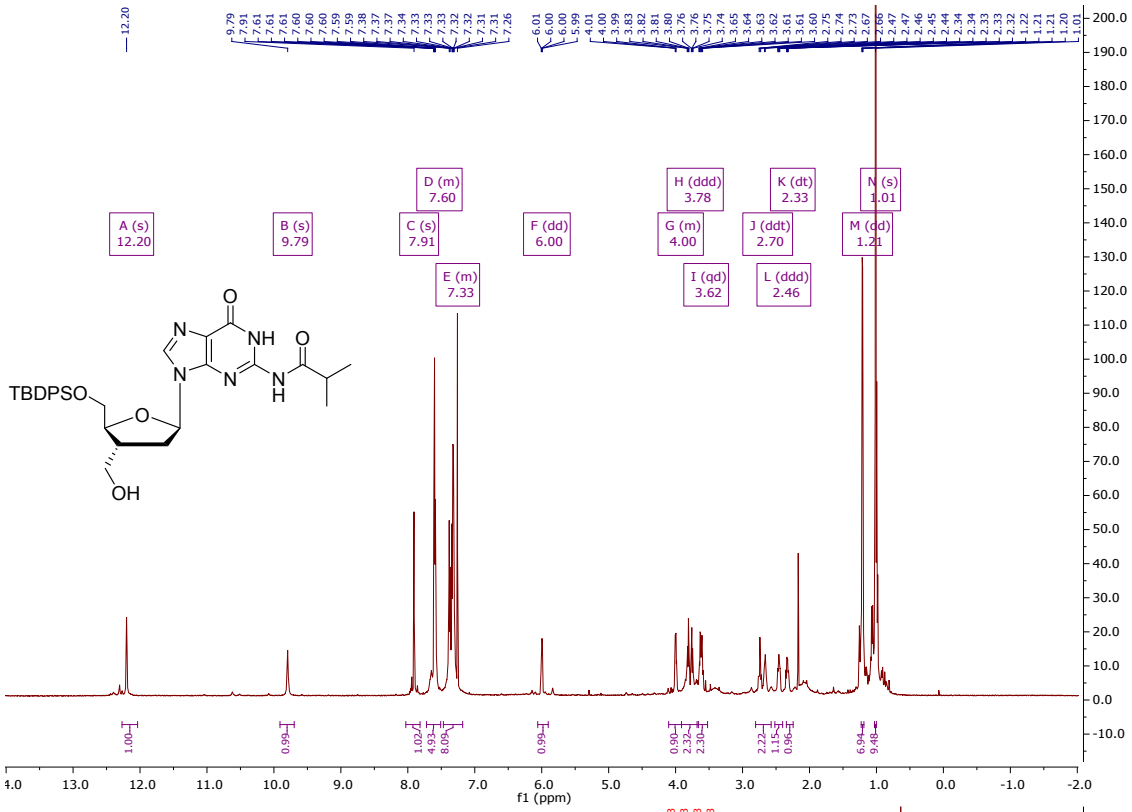
K (t) 1.21
L (m) 1.01
f1 (ppm)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H (ddd) 3.78
I (qd) 3.62
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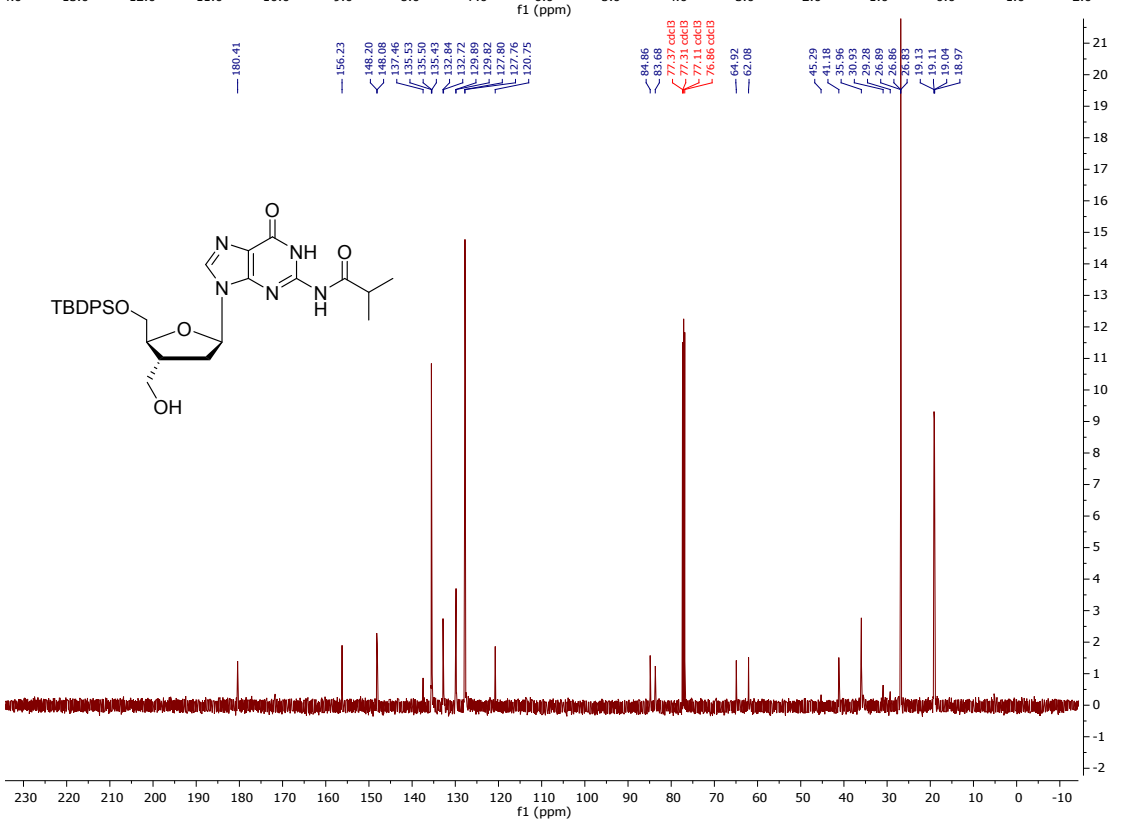
N (s) 1.01
TBDPSO
OH
f1 (ppm)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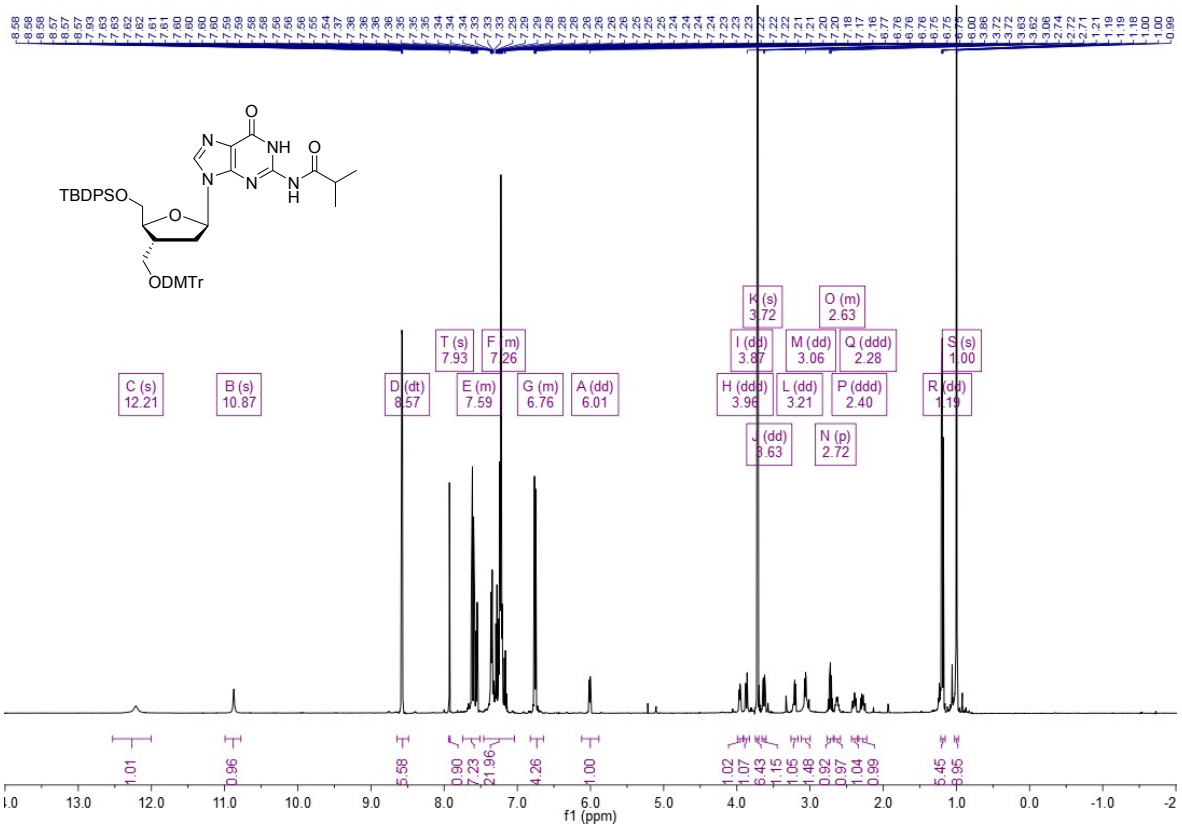
OH
f1 (ppm)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D (dt) 8.57
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G (m) 6.76
A (dd) 6.01
K (s) 3.72
O (m) 2.63
I (dd) 3.87
M (dd) 3.06
Q (ddd) 2.28
S (s) 1.00
H (ddd) 3.96
L (dd) 3.21
P (ddd) 2.40
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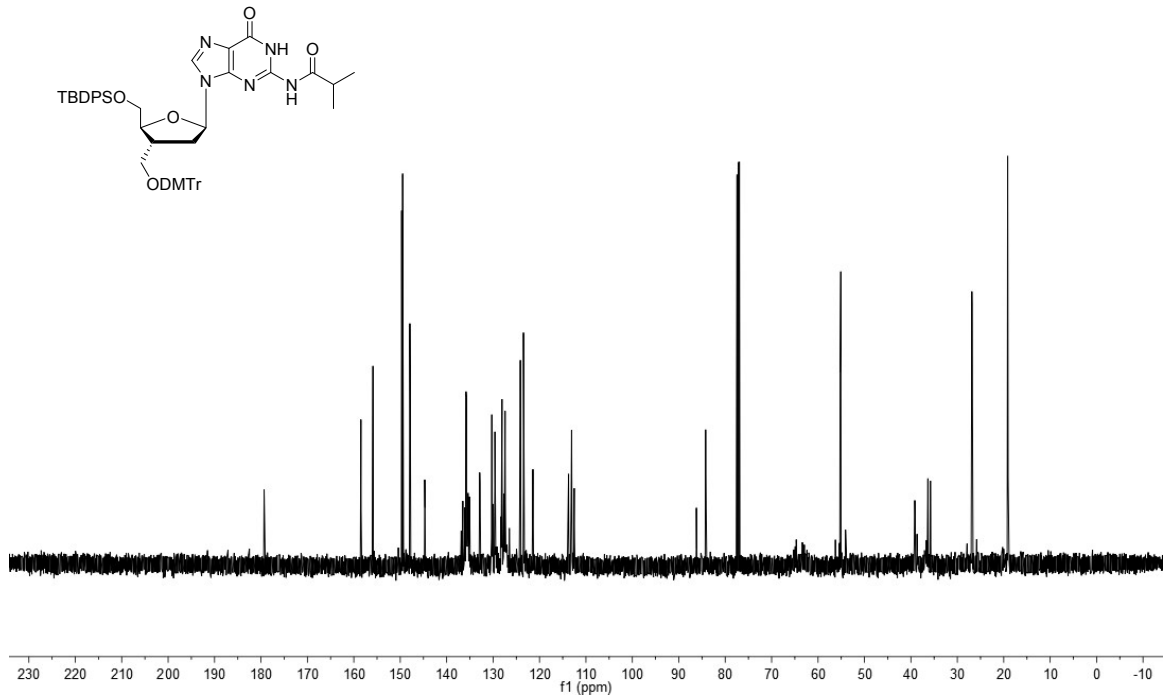
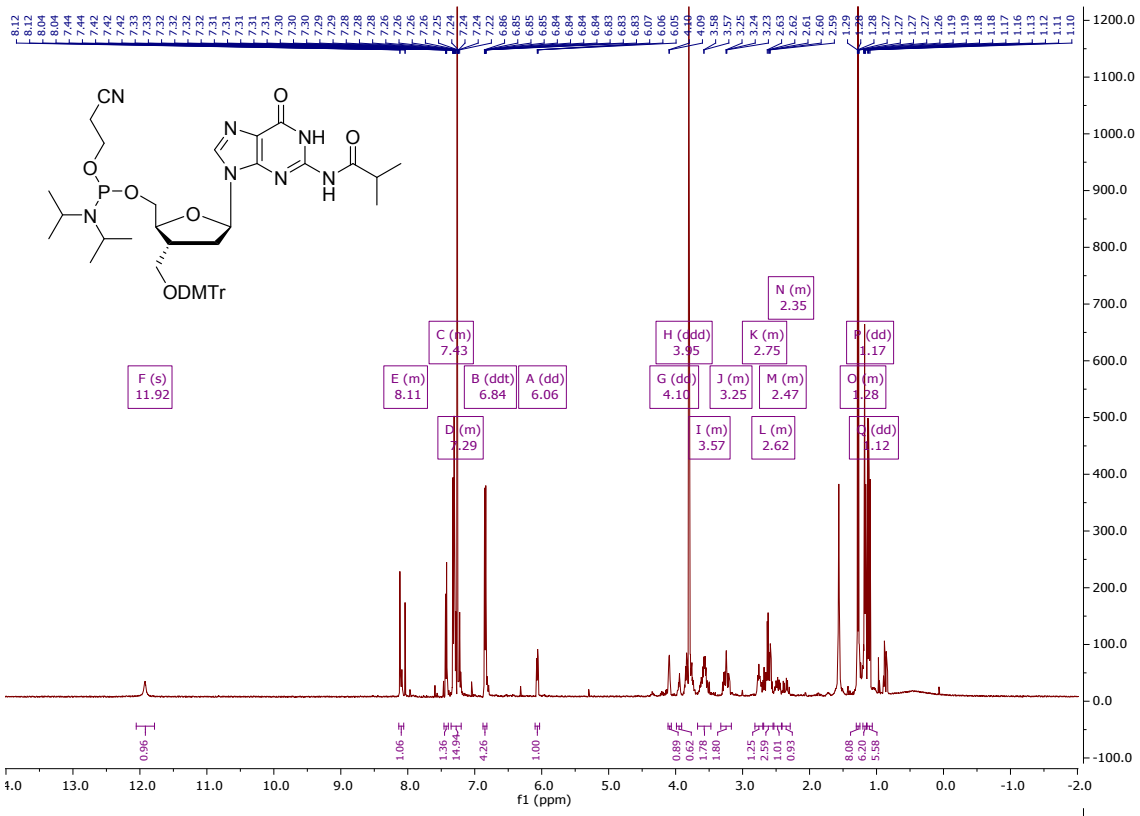
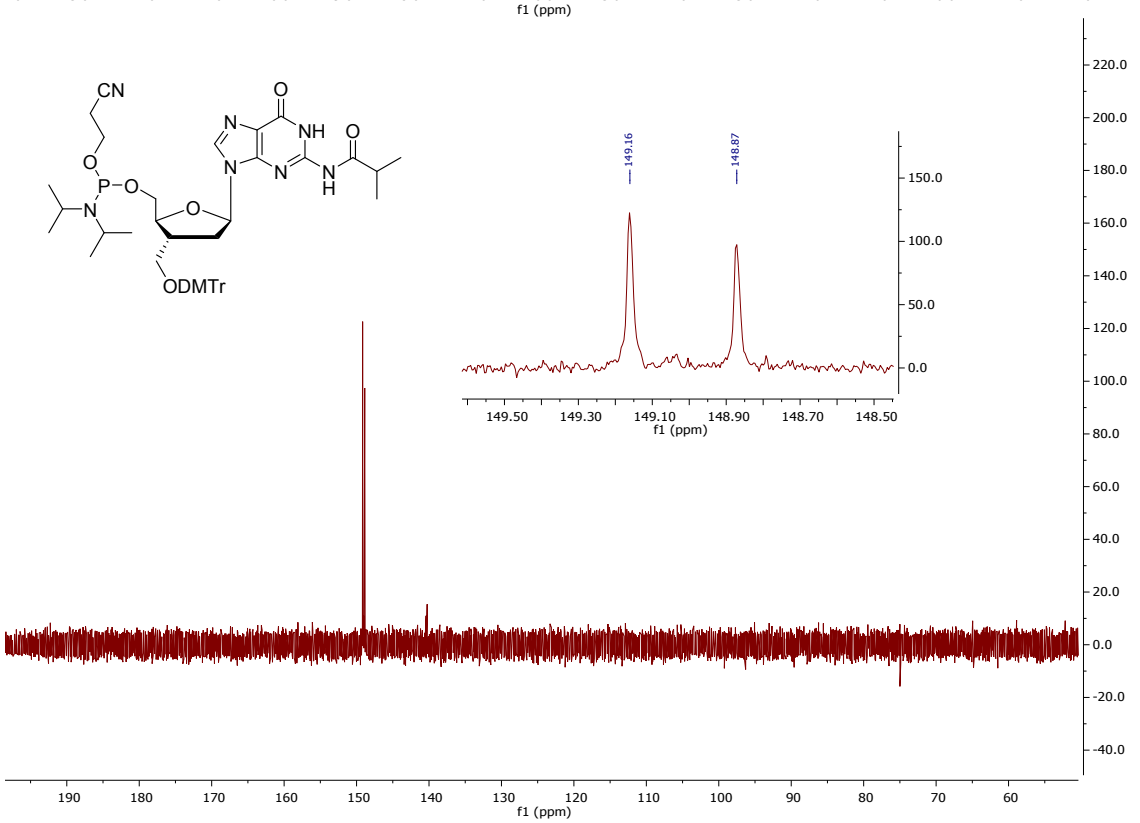
N (p) 2.72
f1 (ppm)


TBDPSO
ODMTr
f1 (ppm)

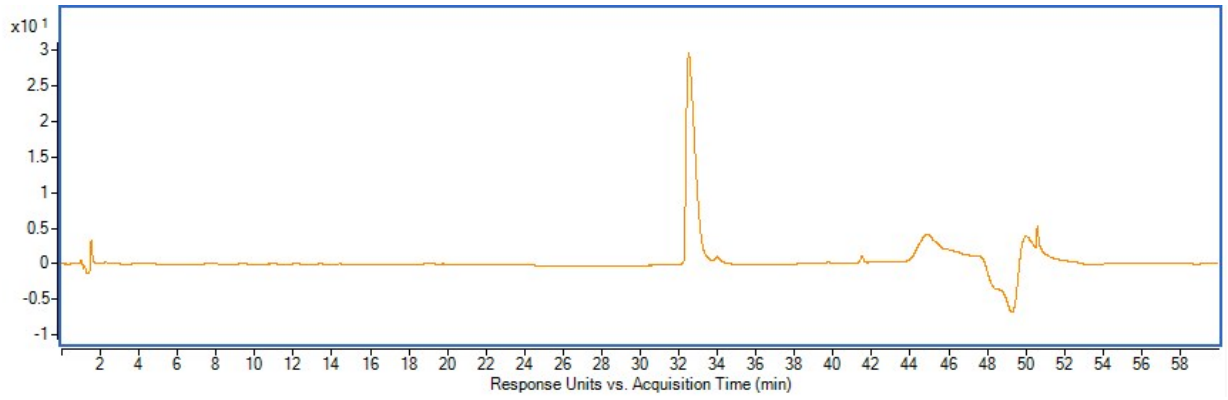

CN
O
N
NH
O
N
N
N
H
P
O
N
O
ODMTr
F (s) 11.92
E (m) 8.11
C (m) 7.43
B (ddt) 6.84
A (dd) 6.06
D (m) 7.29
H (ddd) 3.95
G (dd) 4.10
J (m) 3.25
I (m) 3.57
N (m) 2.35
K (m) 2.75
M (m) 2.47
L (m) 2.62
P (dd) 1.17
O (m) 1.28
Q (dd) 1.12
f1 (ppm)


CN
O
N
NH
O
N
N
N
H
P
O
N
O
ODMTr
149.16
148.87
f1 (ppm)

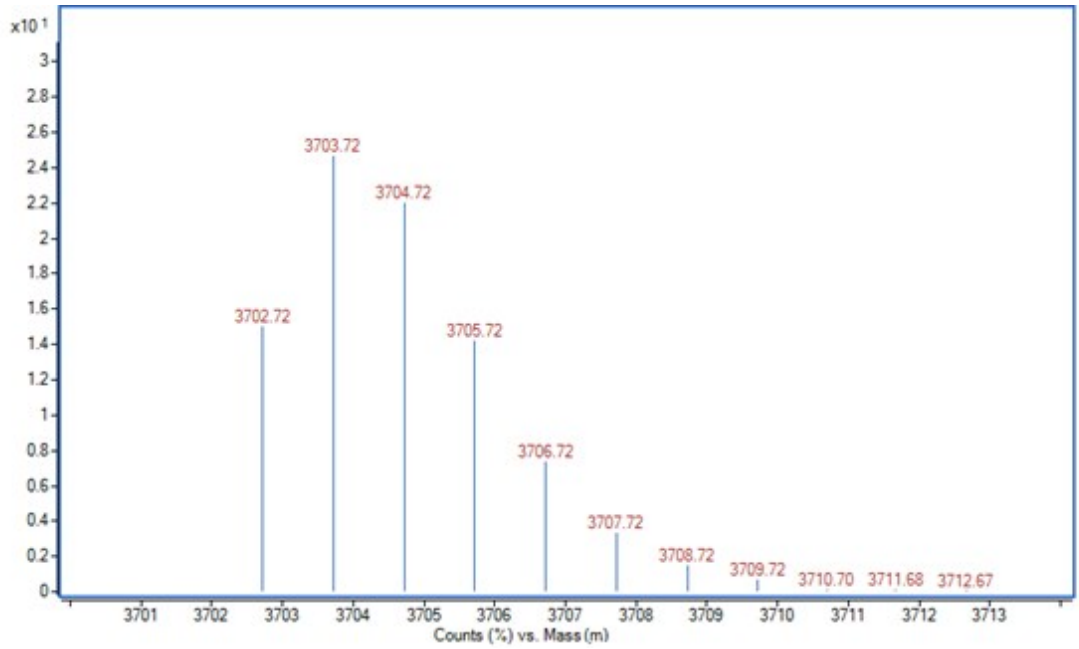
4. LCMS Analysis of the Oligomers

a) 5′-fluorophore tagged dG*-terminated RNA primer (P1, 5′-Cy3-GACUGACUG dG*-3′)

1. LC trace of P1



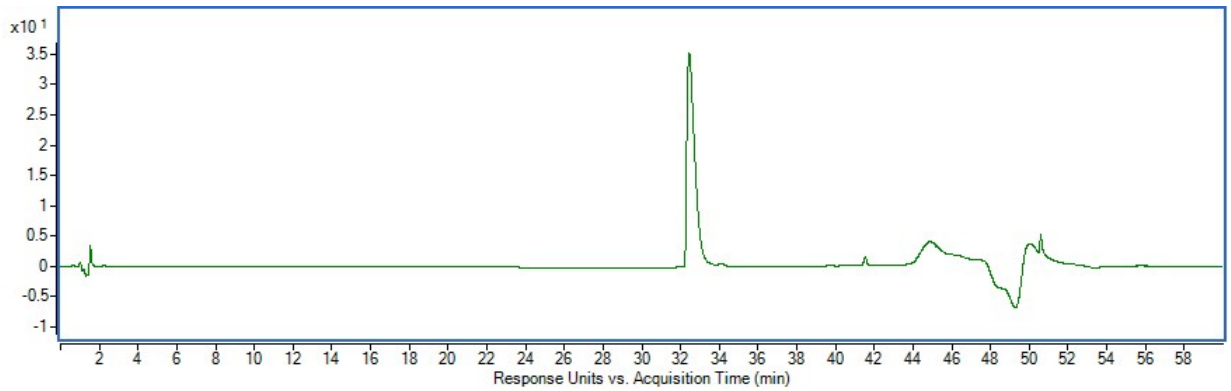
2. MS data



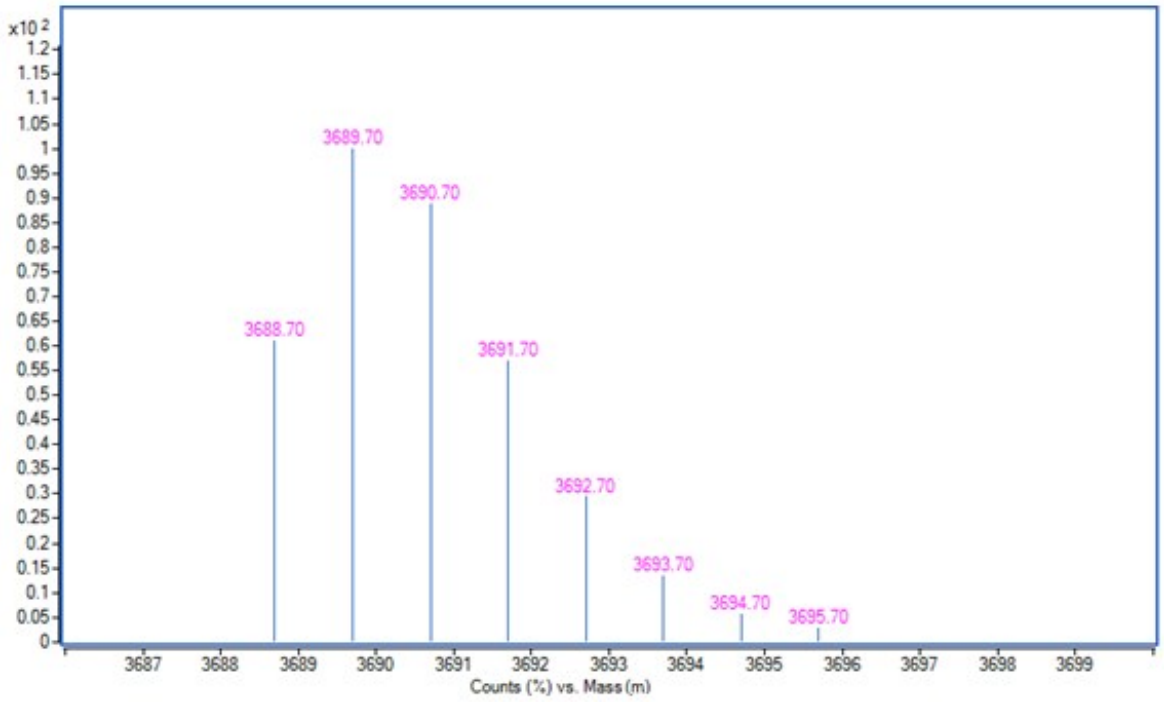
Exact mass: 3702.7264; measured accurate mass: 3702.7182.

b) 5′-fluorophore tagged dG-terminated RNA primer (P2, 5′-Cy3-GACUGACUG dG-3′)

1. LC trace of P2



2. MS data



Exact mass: 3688.7107; measured accurate mass: 3688.6978.

5. Isotopic Mass Distribution of +3 Extension Product of P1

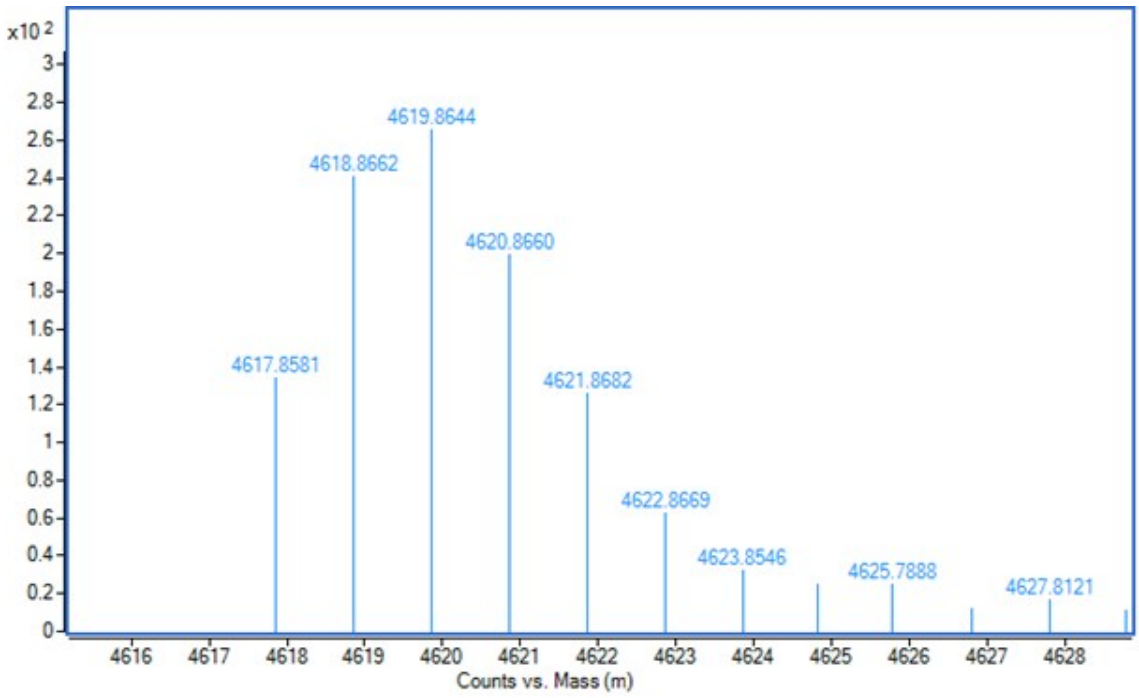


Figure S1. Isotopic mass distribution of the species in the aliquot of primer extension reaction with primer P1 after 24 hours. Exact mass: 4617.8502; measured accurate mass: 4617.8542.

6. Effect of pH and divalent metal ion on primer extension efficiency

a) Using 2-MeImpC to extend on G4 template.

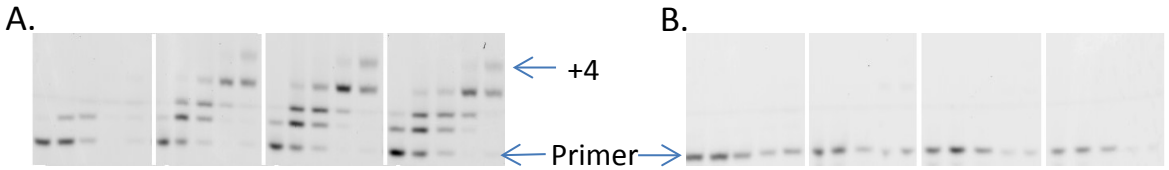
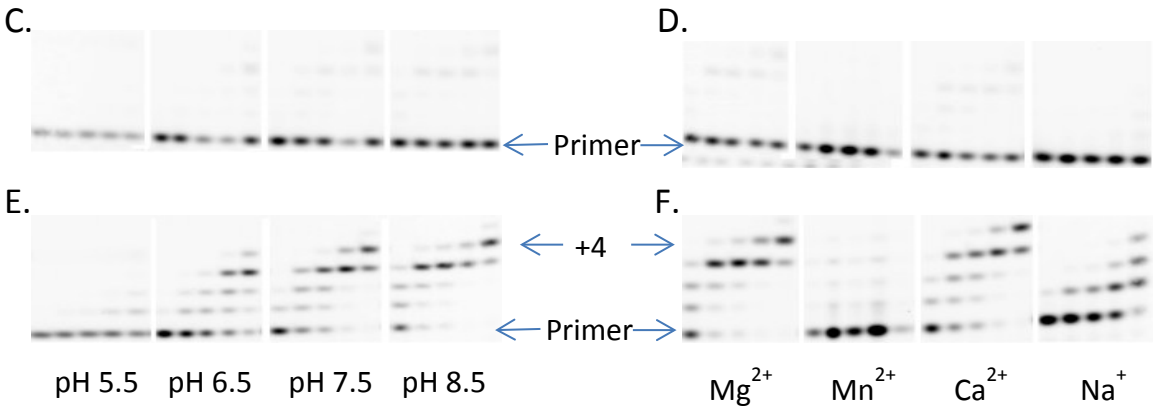


Figure S2. Gel electrophoresis analysis of primer extension reactions. Reaction conditions**:**1 μM primer (P2 (5′-Cy3-GACUGACUG dG-3′) in **A** and P1 (5′-Cy3-GACUGACUG dG*-3′) in **B**), 5 μM template T (3′-CUGACUGACCGGGGAA-5′), 200 mM buffer (MES for pH 5.5, HEPES for pH 6.5, 7.5 and 8.5) 100 mM $MgCl_2$, 50 mM 2-MeImpC. Time points: 5 min, 30 min, 1 hour, 4 hours and 1 day.



b) Using 2-MeImpG to extend on C4 template.

Figure S3. Gel electrophoresis analysis of primer extension reactions. Reaction conditions**:**1 μM primer (P1 (5′-Cy3-GACUGACUG dG*-3′) in **C** and **D**; P2 (5′-Cy3-GACUGACUG dG-3′) in **E** and **F**), 5 μM template (3′-CUGACUGACCCCCCAA-5′), 200 mM buffer (MES for pH 5.5, HEPES for pH 6.5, 7.5 and 8.5) 100 mM metal salt ($MgCl_2$,

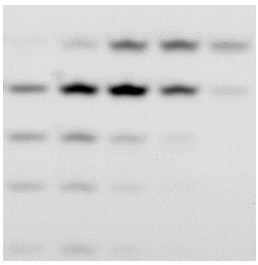
$MnCl_2$, $CaCl_2$ and NaCl), 50 mM 2-MeImpG. Time points: 5 min, 30 min, 1 hour, 4 hours and 1 day. **C** & **E:** pH screen with $MgCl_2$ as metal salt; **D** & **F:** Metal salt screen with HEPES (pH 8.5) as buffer.

Notes (for Section 6b):

1. The very faint extension bands observed for P1 in **C** and **D** could theoretically be due to either very slow extension of P1 from the dG* terminal residue, and/or extension of the primer N-1 impurity, RNA 9-mer P3 (5′-Cy3-GACUGACUG-3′). Our HRMS data shows it is primarily due to latter.

2. In the presence of Mg^{2+}, Mn^{2+}, or Ca^{2+}, P2 extends rapidly and starts to provide some +3 extension product in less than 5 minutes. P2 can also be extended very slowly without the catalytic effect of divalent cations. For example, some +3 product is observed after 4 hours in the presence of monovalent Na^{+}.

3. In **F**, we did not observe any extension in the presence of Mn^{2+}, due to the precipitation of manganese oxyhydroxides observed at pH 8.5. We found Mn^{2+} acts as an effective catalyst at pH 7.5, as seen on the right. The time points are 5 min, 10 min, 30 min, 1 hour and 2 hour.



7. References

1. D. G. Brooke and J. C. Morris, *Tetrahedron Letters*, 2008, **49**, 2414-2417.
2. G. S. Ti, B. L. Gaffney and R. A. Jones, *J. Am. Chem. Soc.*, 1982, **104**, 1316-1319.