Supporting Information for

Synthesis of ^{15}N-Enriched Pseudouridine Derivatives

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General:

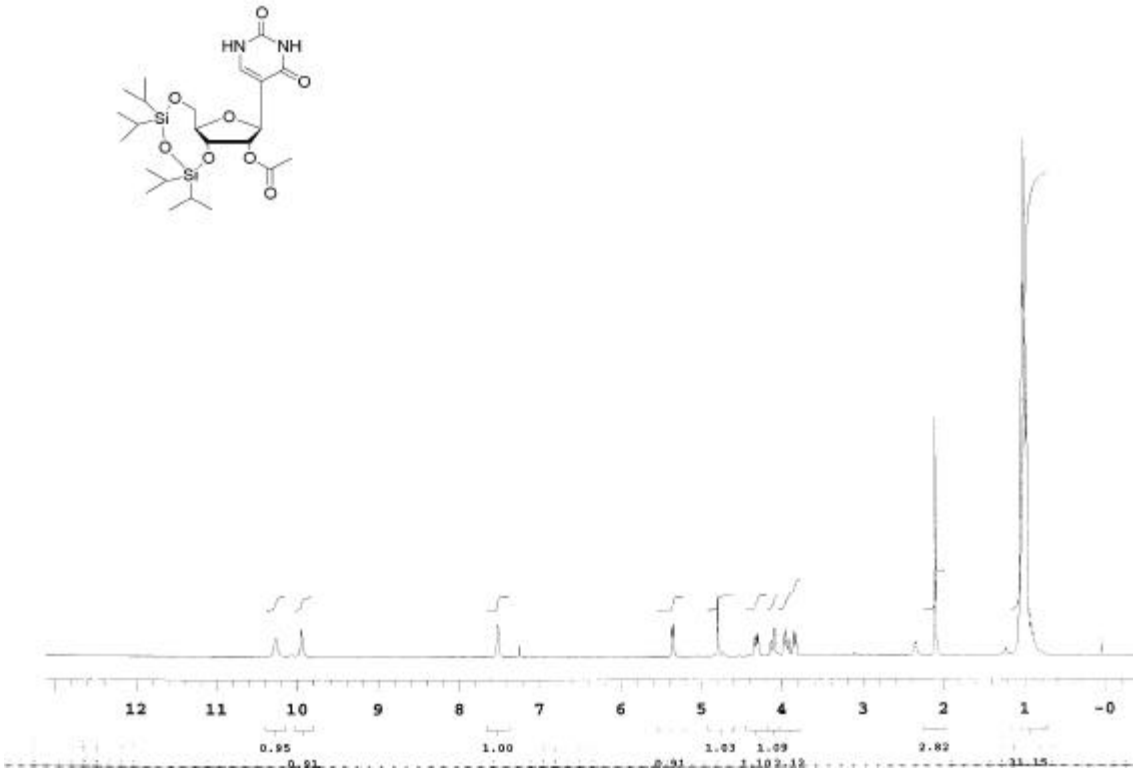
^{1}H NMR and ^{13}C NMR spectra were recorded on either a Varian Unity 300, Mercury 400, or Varian Unity 500 spectrometer and referenced to tetramethylsilane as an internal standard. All ^{15}N NMR spectra were recorded on a Varian Unity 500 spectrometer using concentrated $H^{15}NO_3$ as an external standard in $CDCl_3$. The Larmor frequencies for ^{13}C NMR on the 300, 400, and 500 MHz are 75.6, 100.6, and 125.6; and for ^{15}N NMR at 500 MHz the frequency is 50.6. ESI spectra were recorded on a Quattro LC in positive ion mode.

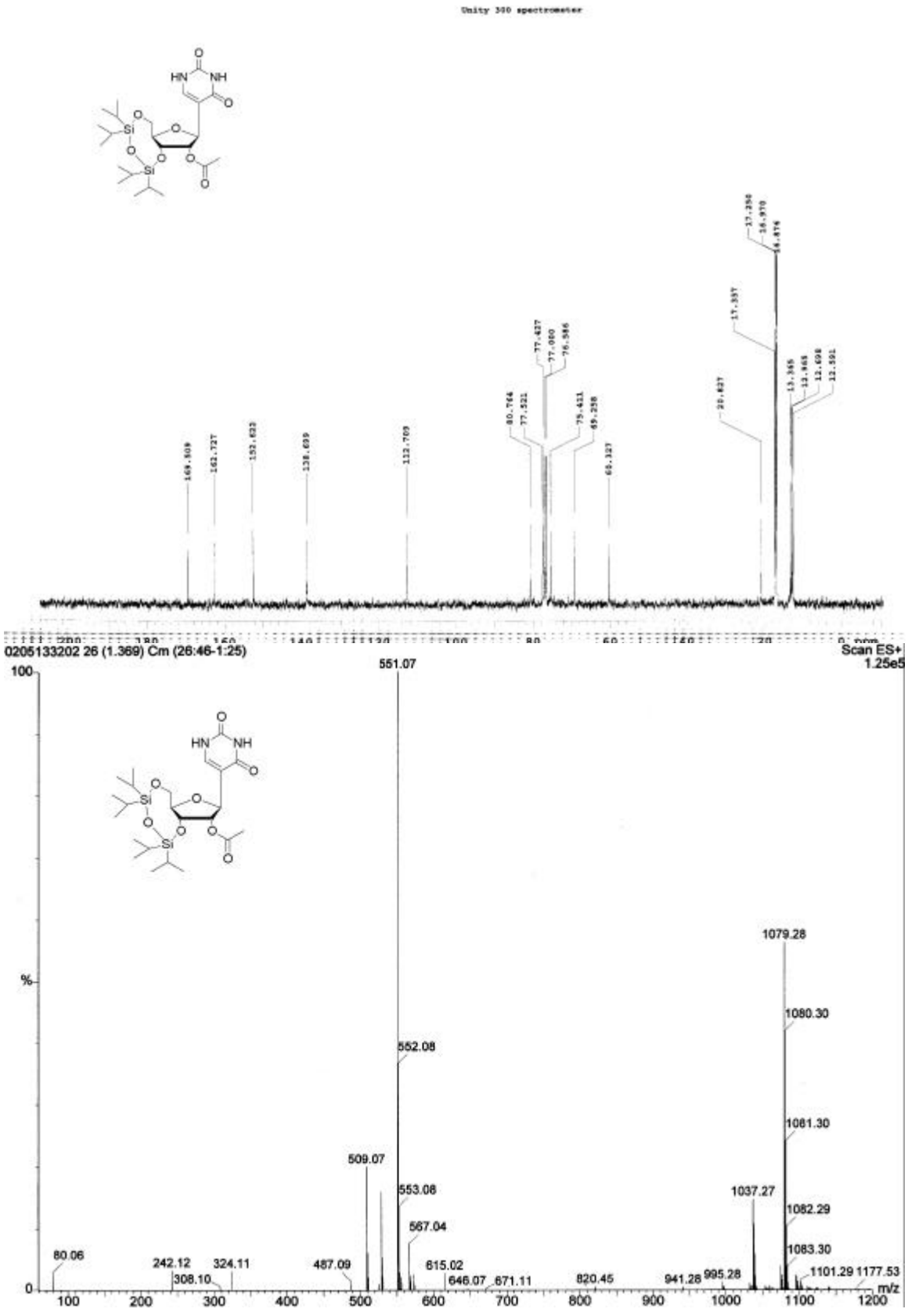
Procedures and Characterizations:

2'-*O*-Acetyl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-pseudouridine (2).

To a solution of **1** (245.2 mg, 0.50 mmol, 1.0 eq) in freshly distilled pyridine was added DMAP (32.5 mg, 0.24 mmol, 0.48 eq). The clear solution was stirred for 30 minutes. Acetic anhydride (50 µl, 53.9 mg, 0.53, 1.1 eq) was added dropwise to the solution and stirred for 6 h. Upon completion, the reaction was quenched with 5% $NaHCO_3$, and extracted with EtOAc. The combined organic layers were washed with brine, and dried over Na_2SO_4, concentrated, and purified by flash column chromatography on silica gel using a 2:1 hexanes:EtOAc gradient. Column purification yielded **2** as an off-white solid in 99% yield (261.4 mg, 0.50 mmol).

TLC (hexanes/EtOAc, 2:1): R_f = 0.16; ^{1}H NMR ($CDCl_3$, 300 MHz) d (ppm) 0.96 - 1.01 (m, 28H), 2.09 (s, 3H), 3.83 (d, 1H, *J* = 8.7 Hz), 3.93 (d, 1H, *J* = 12.9 Hz), 4.10 (d, 1H, *J* = 12.9 Hz), 4.30 (dd, 1H, *J* = 9, 4.8, 5.4 Hz), 4.79 (s, 1H), 5.34 (d, 1H, *J* = 4.8), 7.50 (s, 1H), 9.94 (s, 1H), 10.26 (s, 1H); ^{13}C NMR ($CDCl_3$, 300 MHz) d (ppm) 12.59, 12.70, 12.97, 13.37, 16.88, 16.97, 17.25, 17.36, 20.83, 60.33, 69.26, 75.41, 77.52, 80.76, 112.71, 138.70, 152.62, 162.73, 169.51. ESI-MS (ES^+) calculated for $C_{23}H_{40}O_8N_2Si_2$ 528.2, found 551.1 (M + Na), 1079.3 (2M + Na).

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553.08
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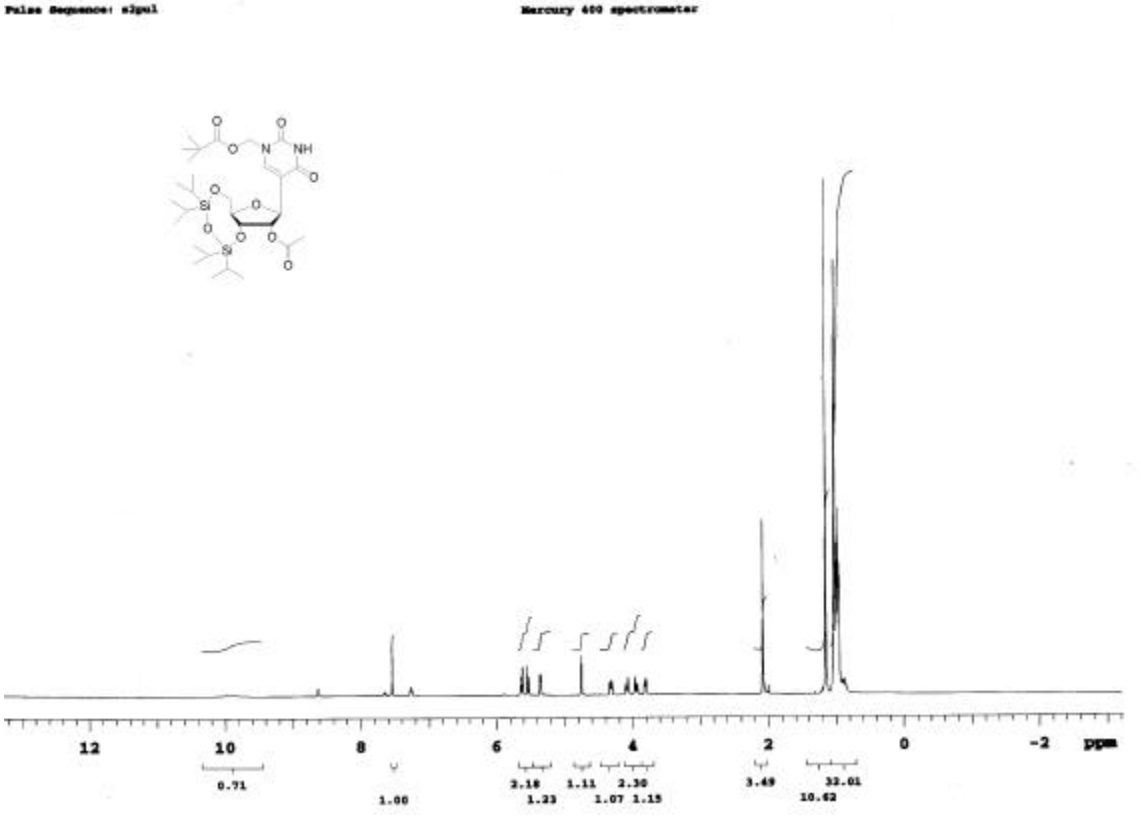
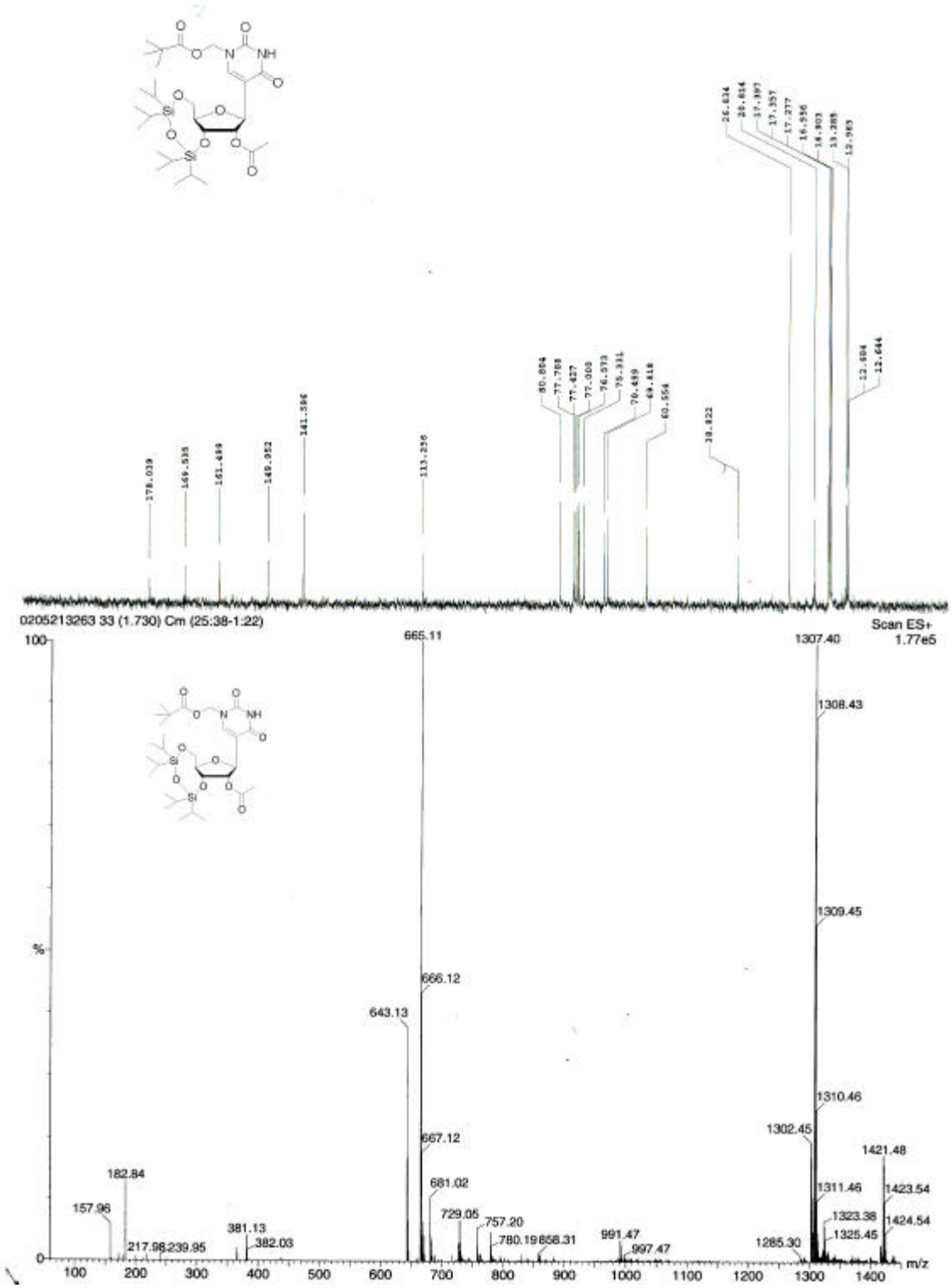
1079.28
1080.30
1081.30
1037.27
1082.29
1083.30
80.06
242.12
308.10
324.11
487.09
615.02
646.07
671.11
820.45
941.28
995.28
1101.29
1177.53
m/z

1-Pivaloyloxymethyl-2'-*O*-acetyl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-pseudouridine (3)

To a clear solution of **2** (251.0 mg, 0.48 mmol, 1.0 eq) in 10 mL of dry triethylamine and 1.7 mL of dry pyridine (6:1) was added chloromethyl pivalate (POMCl) (272 µL, 284 mg, 1.89 mmol, 3.9 eq) dropwise. The reaction was stirred for 72 hours at room temperature. An additional 500 µL of POMCl (522.5 mg, 3.47 mmol, 7.2 eq) was added dropwise and stirred for 24 hours and repeated with a third aliquot of 500 µL POMCl. The reaction was quenched with 5% $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with aqueous brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using a 2:1 hexanes:EtOAc solvent mixture to yield compound **3** as a white crystalline solid in 86% yield (265.5 mg, 0.41 mmol).

TLC (hexanes/EtOAc, 2:1): R_f = 0.32; ^{1}H NMR ($CDCl_3$, 300 MHz) d (ppm) 0.96 – 1.00 (m, 28H), 1.17 (s, 9H), 2.09 (s, 3H), 3.81 (m, 1H), 3.96 (dd, 1H, *J* = 6.5, 2.4 Hz), 4.09 (m, 1H), 4.35 (dd, 1H, *J* = 5.7, 5.4, 9.2 Hz), 4.74 (d, 1H, *J* = 1.2), 5.38 (dd, 1H, *J* = 5.3, 2.1, 1.2 Hz), 5.53 (d, 1H, *J* = 10.5 Hz), 5.63 (d, 1H, *J* = 10.5 Hz), 7.54 (s, 1H), 8.96 (s, 1H); ^{13}C NMR ($CDCl_3$, 300 MHz) d (ppm) 12.64, 12.68, 12.97, 13.29, 16.90, 16.96, 17.28, 17.36, 17.40, 20.81, 26.83, 38.82, 60.55, 69.82, 70.50, 75.33, 77.79, 80.80, 113.26, 141.60, 149.95, 161.50, 169.54, 178.04. ESI-MS (ES^+) calculated for $C_{29}H_{50}O_{10}N_2Si_2$ 642.3, found 643.1 (MH^+), 665.1 (M + Na), 1307.4 (2M + Na).

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666.12
643.13
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780.19
858.31
991.47
997.47
1285.30
1302.45
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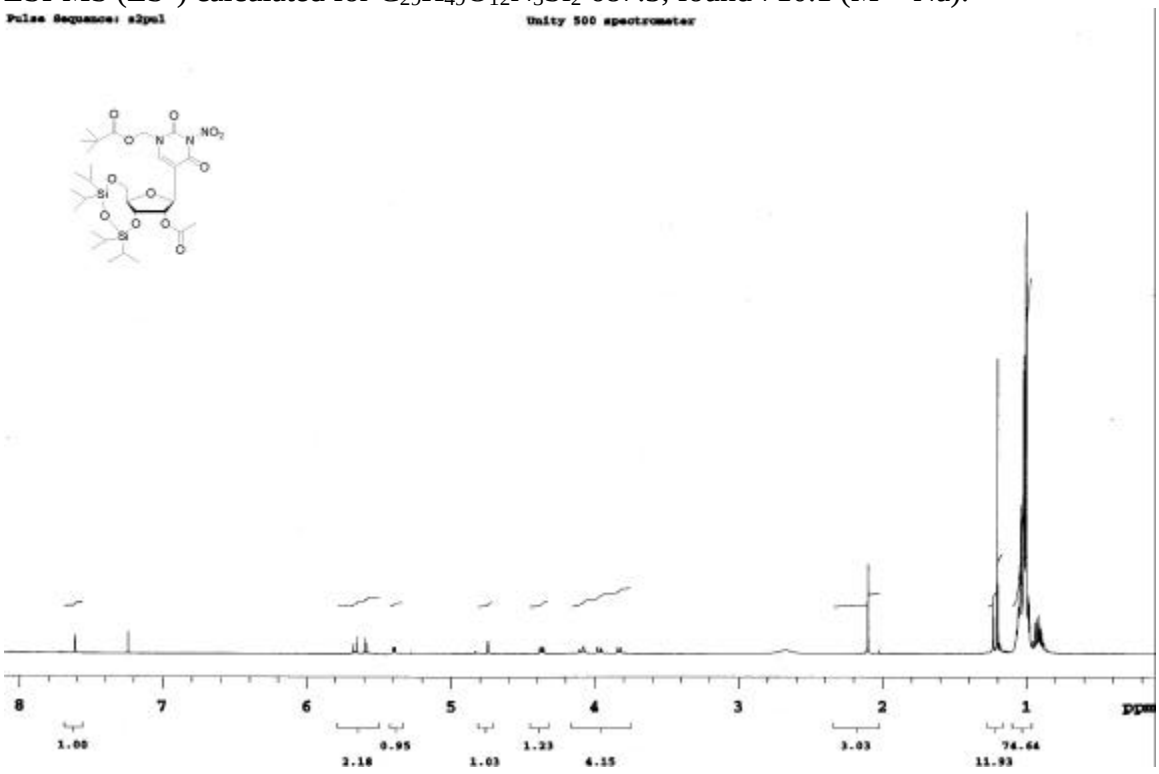
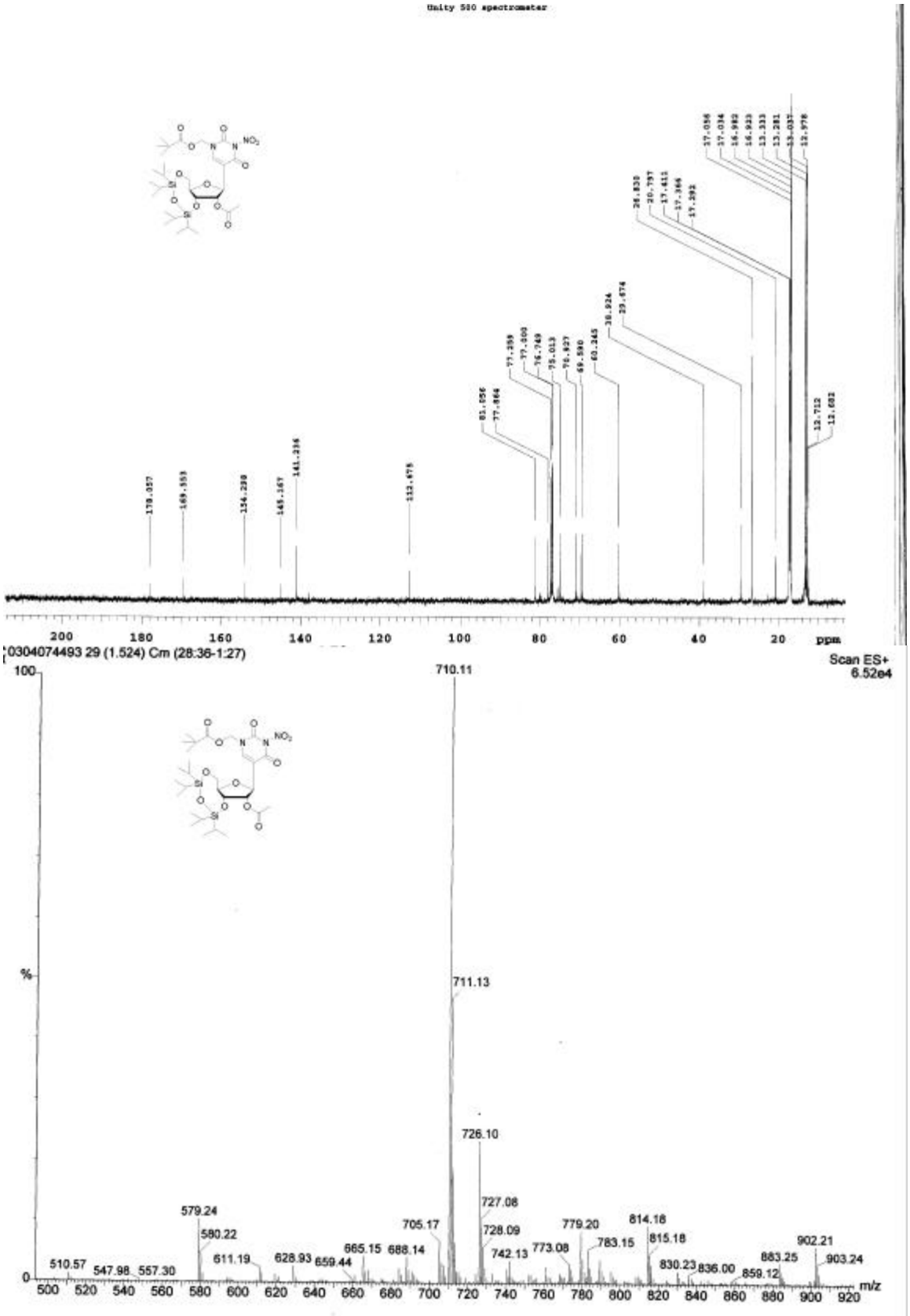
1423.54
1424.54
182.84
157.96
217.98
239.95
381.13
382.03
m/z

3-Nitro-1-pivaloyloxymethyl-2'-*O*-acetyl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-pseudouridine (4)

NH_4NO_3 (155 mg, 1.93 mmol, 6.43 eq) was added to 10 mL of distilled CH_2Cl_2 and stirred vigorously at 0 ºC for 1 hour. Trifluoroacetic acid anhydride (450 µL, 666 mg, 5.84 mmol, 19.5 eq) was added dropwise to the cooled stirring solution to yield a cloudy white solution. In a second flask, 2 mL of CH_2Cl_2 was added to **3** (194 mg, 0.30 mmol, 1.0 eq). This solution was added to the first one dropwise, and stirred for 90 minutes. The reaction was quenched with 5% $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with aqueous brine, and dried over Na_2SO_4, and concentrated under reduced pressure. The crude product was purified using a 3:1 hexanes:EtOAc solvent gradient to yield **4** in 73% yield (151 mg, 0.22 mmol).

TLC (hexanes/EtOAc, 2:1): R_f = 0.65; ^{1}H NMR ($CDCl_3$, 500 MHz) d (ppm) 1.0 – 1.1 (m, 28H), 1.22 (s, 9H), 2.12 (s, 3H), 3.85 (d, 1H, *J* = 9.5 Hz), 3.99 (dd, 1H, *J* = 13, 2.5 Hz), 4.11 (dd, 1H, *J* = 13.3, 2.5, 2 Hz), 4.38 (dd, 1H, *J* = 9, 5.5 Hz), 4.60 (d, 1H, *J* = 2 Hz), 5.41 (dd, 1H, *J* = 5.3, 2.0, 1.5 Hz), 5.60 (d, 1H, *J* = 10.5 Hz), 5.68 (d, 1H, 10.5 Hz), 7.63 (d, 1H, *J* = 1.0 Hz); ^{13}C NMR ($CDCl_3$, 500 MHz) d (ppm) 12.68, 12.71, 12.98, 13.04, 13.28, 13.33, 16.92, 16.98, 17.03, 17.06, 17.29, 17.37, 17.41, 20.76, 26.83, 29.67, 38.92, 60.25, 69.59, 70.93, 75.01, 77.86, 81.06, 112.68, 141.24, 145.17, 154.30, 169.55, 178.06. ESI-MS (ES^+) calculated for $C_{29}H_{49}O_{12}N_3Si_2$ 687.3, found 710.1 (M + Na).

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75.827
69.590
60.345
30.924
29.674
28.830
20.797
17.415
17.366
17.292
17.056
17.034
16.982
16.933
13.333
13.281
13.037
12.978
12.712
12.682
200 180 160 140 120 100 80 60 40 20 ppm
0304074493 29 (1.524) Cm (28:36-1:27)
Scan ES+
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%
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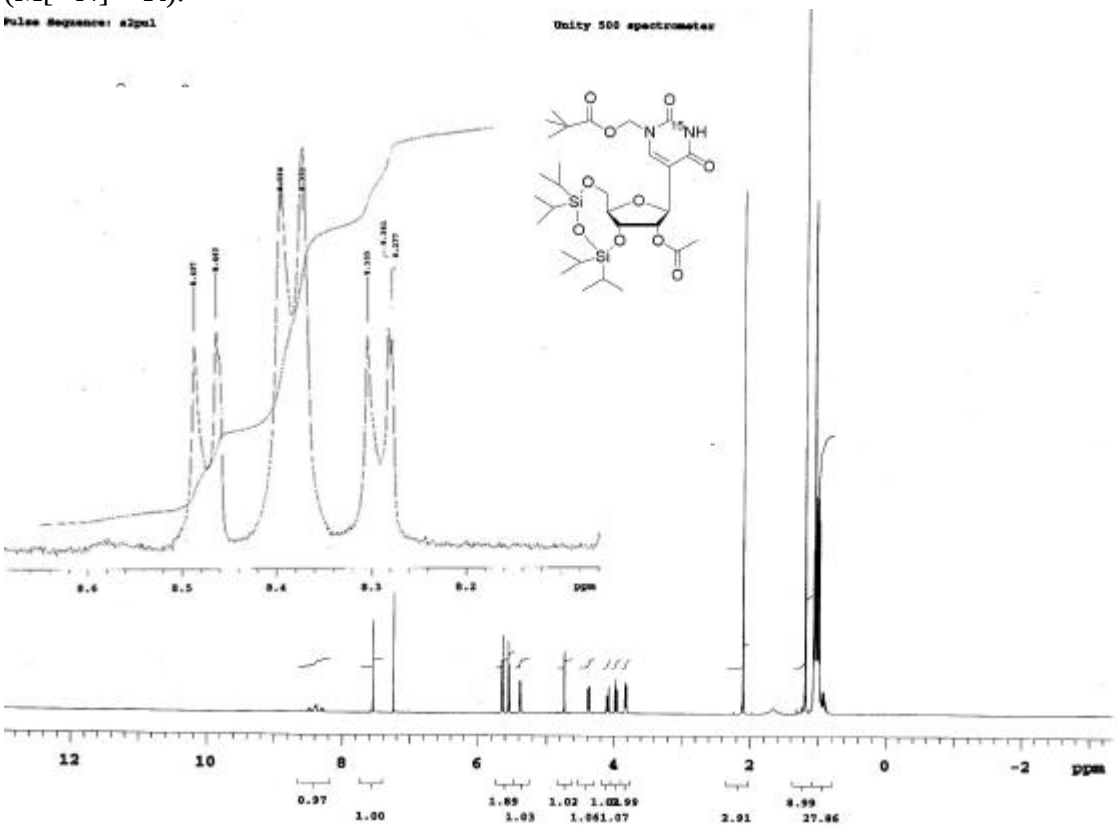
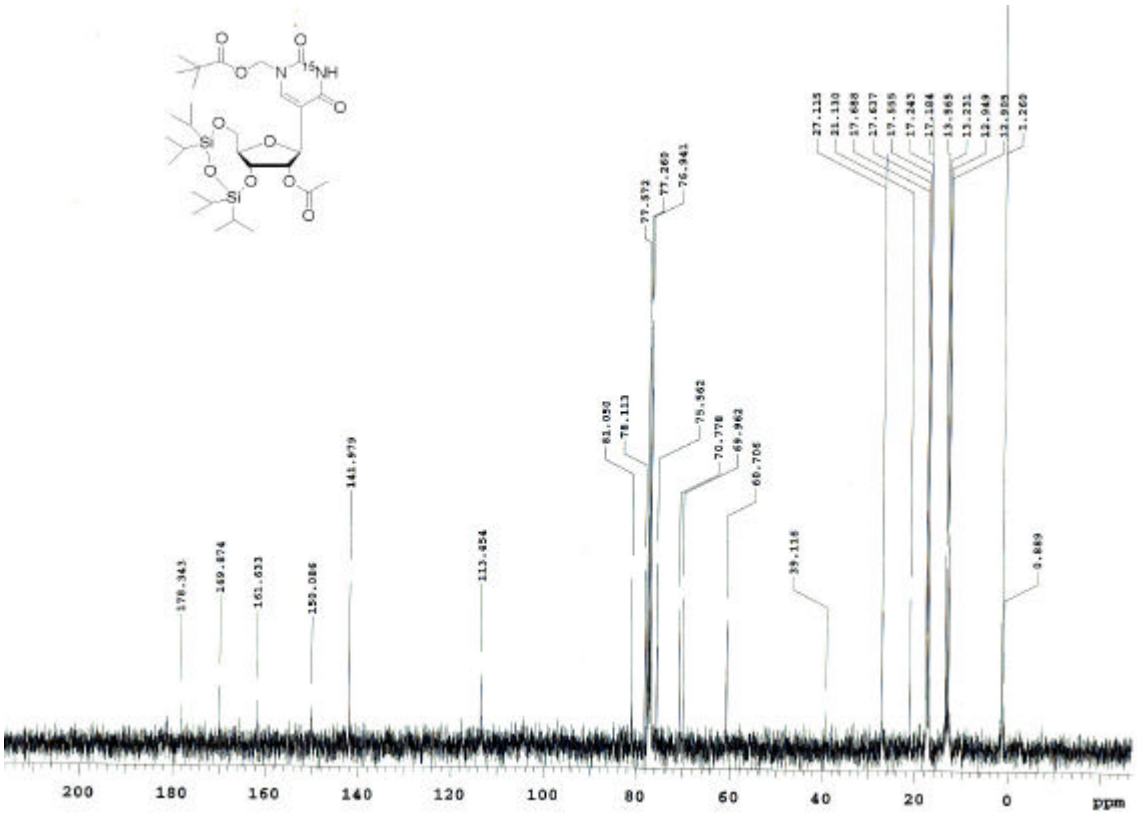
710.11
711.13
726.10
727.08
579.24
580.22
705.17
728.09
779.20
814.18
815.18
783.15
742.13
773.08
688.14
665.15
659.44
628.93
611.19
510.57
547.98
557.30
830.23
836.00
859.12
883.25
902.21
903.24
500 520 540 560 580 600 620 640 660 680 700 720 740 760 780 800 820 840 860 880 900 920 m/z

1-Pivaloyloxymethyl-[3-^{15}N]-2'-*O*-acetyl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-pseudouridine (5)

To a round-bottom flask were added $^{15}NH_4Cl$ (17.6 mg, 0.32 mmol, 1.3 eq) and K_2CO_3 (34.2 mg, 0.25 mmol, 1.0 eq) to a solution containing 620 µL distilled H_2O and 620 µL CH_3CN. The round-bottom flask was sealed with a septum and allowed to stir at room temperature for several minutes. Via syringe, 42 µL of Et_3N (30.1 mg, 0.30 mmol, 1.2 eq), was added dropwise to the stirring solution. A separate solution containing **4** (170 mg, 0.25 mmol, 1.0 eq) in 1.24 mL of CH_3CN was added dropwise to the first solution. The reaction was allowed to stir at room temperature for 24 h, then heated to 55 °C and stirred for another 24 h. The solvent was evaporated, and the crude product was purified by flash column chromatography using a 2:1 hexanes:EtOAc solvent mixture to afford **5** in 53% yield (84.0 mg, 0.13 mmol) at 45% ^{15}N enrichment.

TLC (hexanes/EtOAc, 2:1): R_f = 0.32; ^1H NMR ($CDCl_3$, 500 MHz) d (ppm) 1.00 – 1.06 (m, 28H), 1.20 (s, 9H), 2.12 (s, 3H), 3.82 (m, 1H), 3.96 (dd, 1H, *J* = 3.3, 10.2), 4.095 (dd, 1H, *J* = 9.6, 3.0, 3.3 Hz), 4.74 (s, 1H), 5.38 (d, 1H, *J* = 3.6 Hz), 5.54 (d, 1H, *J* = 9.9), 5.63 (d, 1H, *J* = 10.2), 7.55 (s, 1H), 8.39 (dd, 1H, *J* = 90.9, 11.5 Hz); ^{13}C NMR ($CDCl_3$, 400 MHz) d (ppm) ($CDCl_3$ referenced to 77.260) 12.91, 12.95, 13.23, 13.57, 17.18, 17.24, 17.56, 17.64, 17.69, 21.13, 27.12, 39.12, 60.71, 69.96, 70.78, 75.56, 78.11, 81.05, 113.45, 141.98, 150.09, 161.63, 169.87, 178.34; ^{15}N NMR ($CDCl_3$, 500 MHz) d (ppm) : -215.30 (d, 1N, *J* = 91.6 Hz). ESI-MS (ES^+) calculated for $C_{29}H_{50}O_{10}N_2Si_2$ 642.3, found 643.3 (MH+), 681.3 (M + K), and for $C_{29}H_{50}O_{10}N^{15}NSi_2$ 643.3, found 644.3 (M[^{15}N]H+), 682.3 (M[^{15}N] + K).

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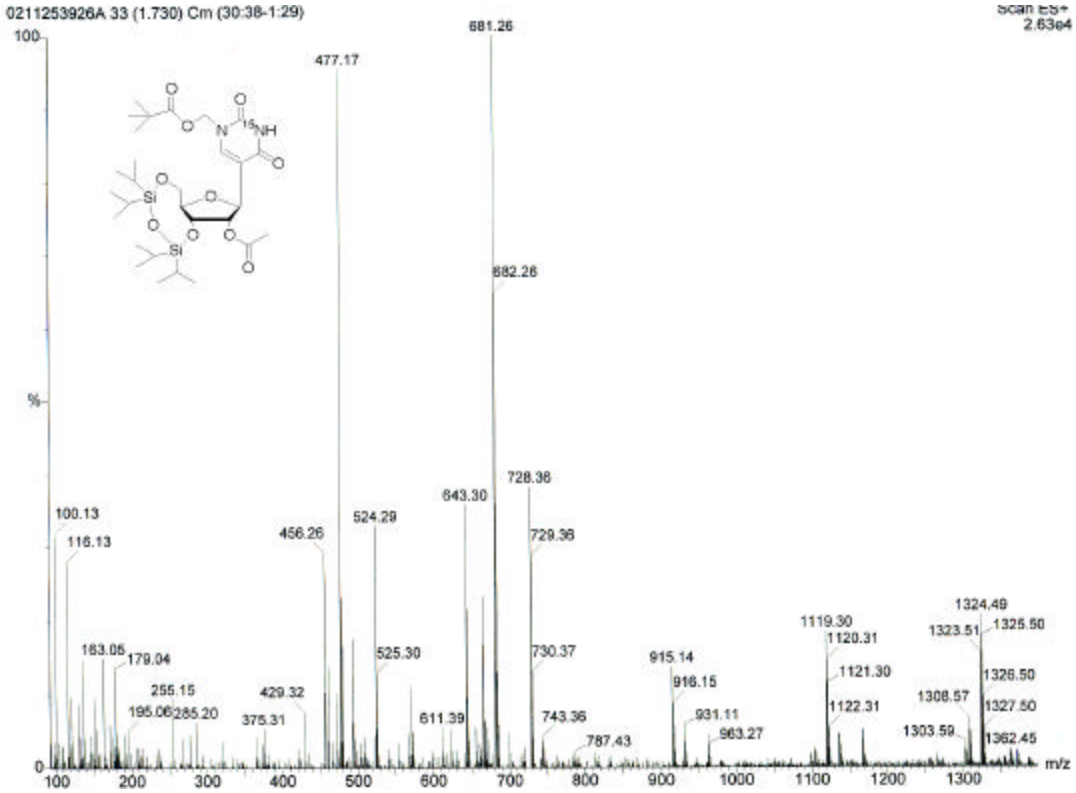
120
100
80
60
40
20
0
ppm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%
0
m/z
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682.26
728.38
643.30
729.38
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456.26
730.37
525.30
611.39
743.36
787.43
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916.15
931.11
963.27
1119.30
1120.31
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1122.31
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1303.59
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285.20
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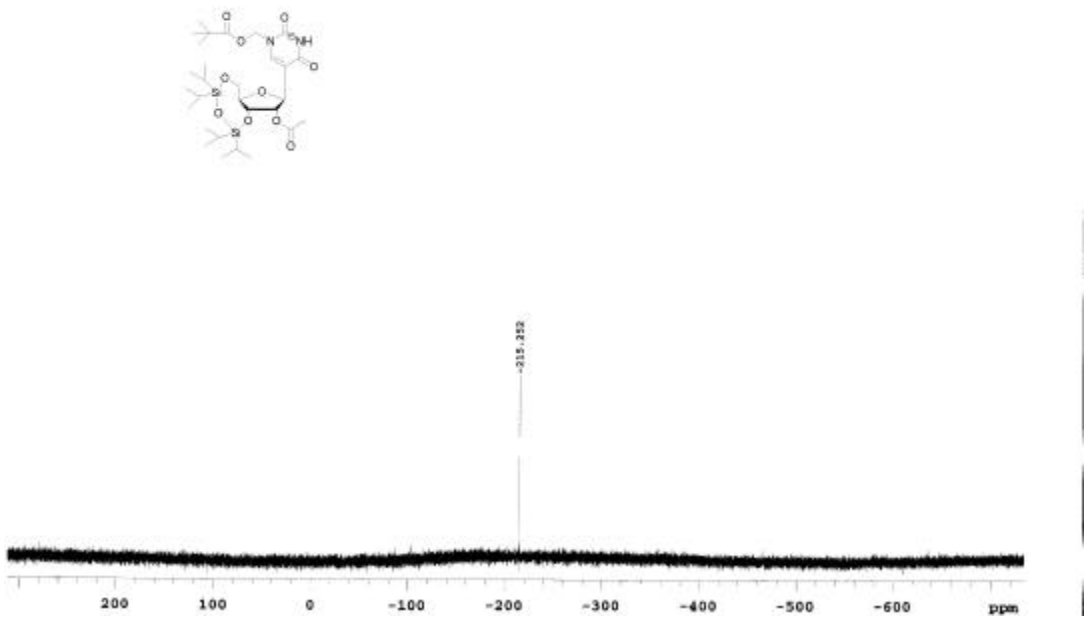
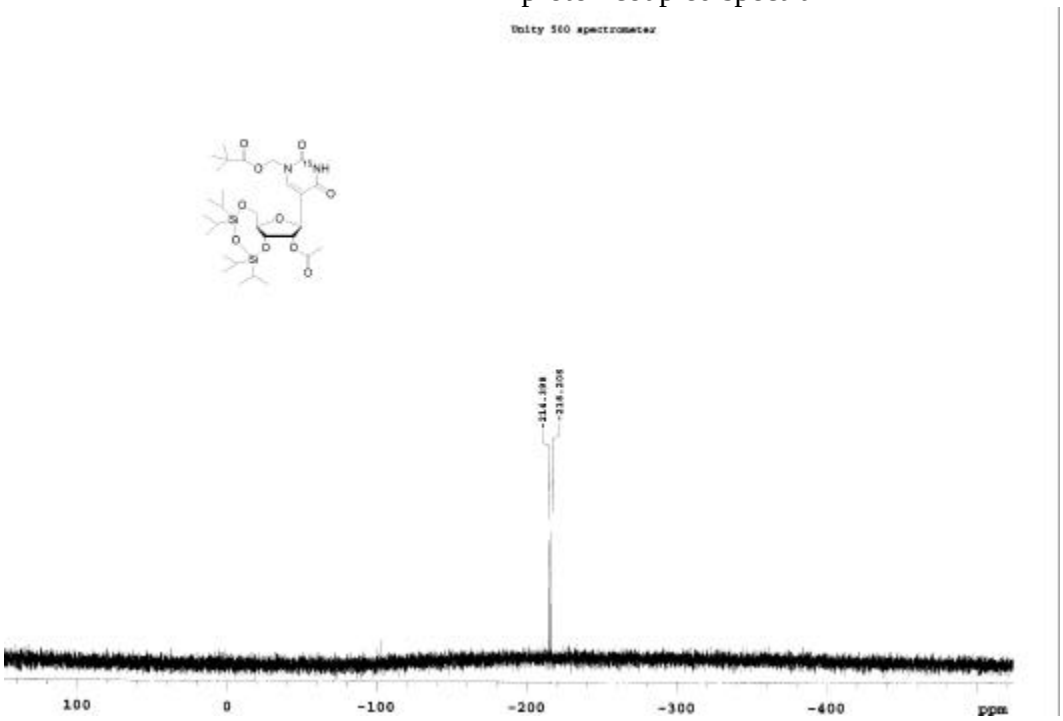
375.31

^{15}N NMR proton decoupled spectrum



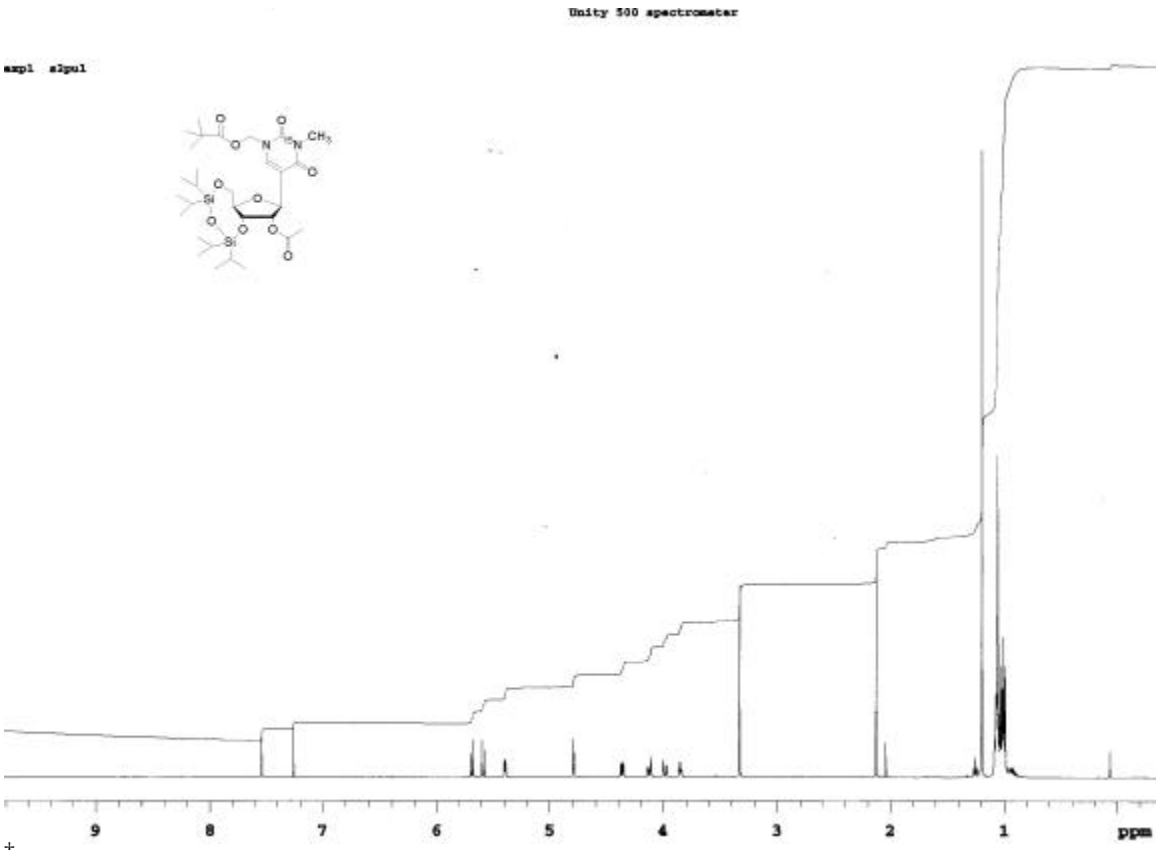
^{15}N NMR proton coupled spectrum



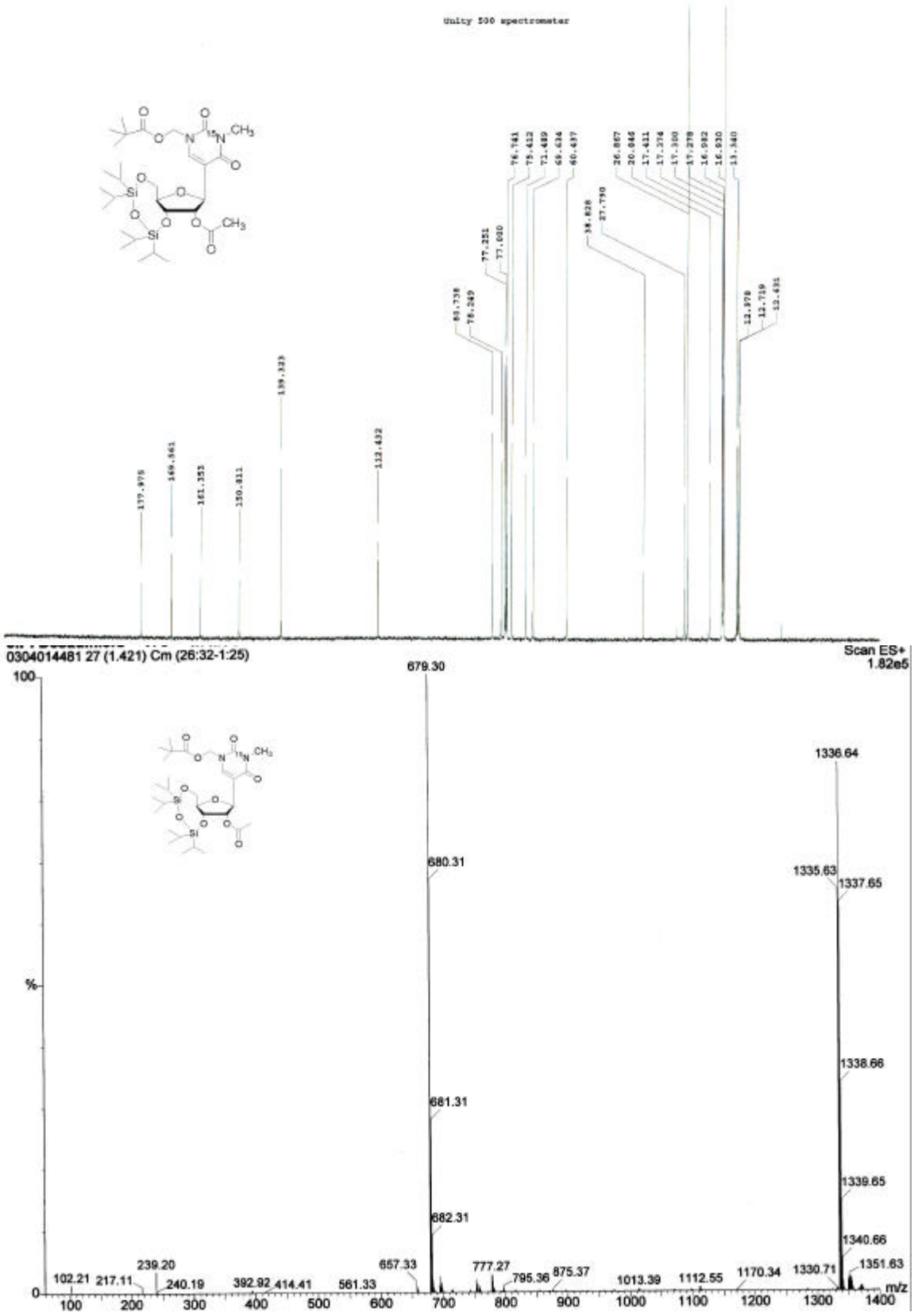
[3-Methyl-^{15}N]-1-pivaloyloxymethyl-2'-*O*-acetyl-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-pseudouridine (6)

To a clear solution of **5** (41 mg, 0.064 mmol) in 1.1 mL of benzene[‡] was added 26 µL of *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) (22.8 mg, 0.19 mmol) dropwise to the stirring solution to yield a light yellow colored solution. The reaction was refluxed for 3 h and the solvent was evaporated. The yellow crude product was purified by flash column chromatography on silica-gel using a 2:1 hexanes:EtOAc solvent mixture to yield compound **6** as a colorless oil in 80% yield (33.4 mg, 0.051 mmol).

TLC (hexanes/EtOAc, 2:1): R_f = 0.42; ^{1}H NMR ($CDCl_3$, 500 MHz) d (ppm) 1.0 – 1.06 (m, 28H), 1.18 (s, 9H), 2.11 (s, 3H), 3.31 (s, 3H), 3.83 (m, 1H), 3.97 (dd, 1H, *J* = 2.5, 3, 13 Hz), 4.11 (dd, 1H, *J* = 13.5, 2.5 Hz), 4.35 (dd, 1H, *J* = 9, 5 Hz), 4.78 (s, 1H), 5.37 (dd, 1H, *J* = 5, 1.5), 5.57 (d, 1H, *J* = 10 Hz), 5.67 (d, 1H, *J* = 10 Hz), 7.52 (s, 1H); ^{13}C NMR ($CDCl_3$, 500 MHz) d (ppm) 12.63, 12.72, 12.98, 13.34, 16.93, 16.98, 17.28, 17.30, 17.37, 17.41, 20.85, 26.87, 27.79, 38.83, 60.44, 69.63, 71.49, 75.41, 78.25, 80.74, 112.43, 139.32, 150.81, 161.35, 169.56, 177.98; ^{15}N NMR ($CDCl_3$, 500 MHz) d (ppm) -215.61. ESI-MS (ES^+) calculated for $C_{30}H_{52}O_{10}N_2Si_2$ 656.3, found 679.3 (M + Na), 1336.6 (2M + Na) and for $C_{30}H_{52}O_{10}N^{15}NSi_2$ 657.3, found 680.3 (M[^{15}N] + Na), 1337.7 (2M[^{15}N] + Na).



[‡]Benzene is a hazardous material and caution should be taken when using this reagent (see MSDS for hazard information)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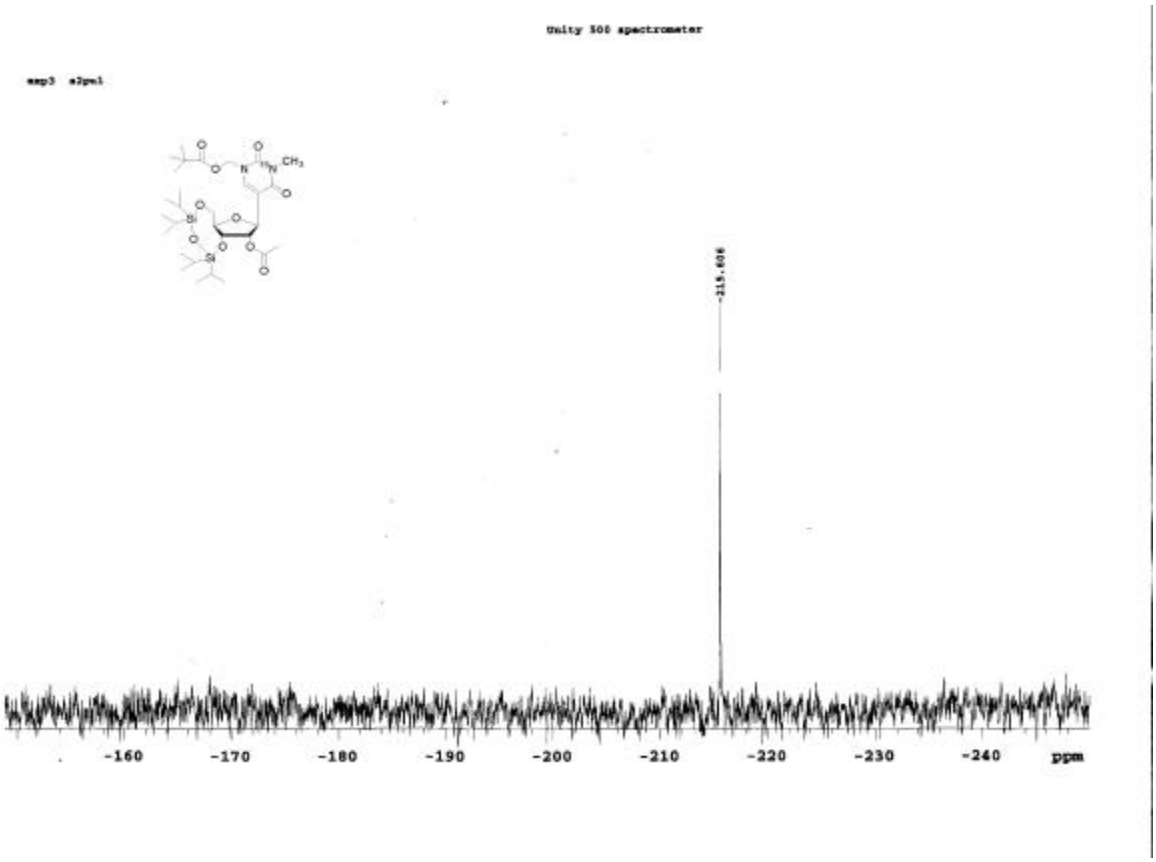
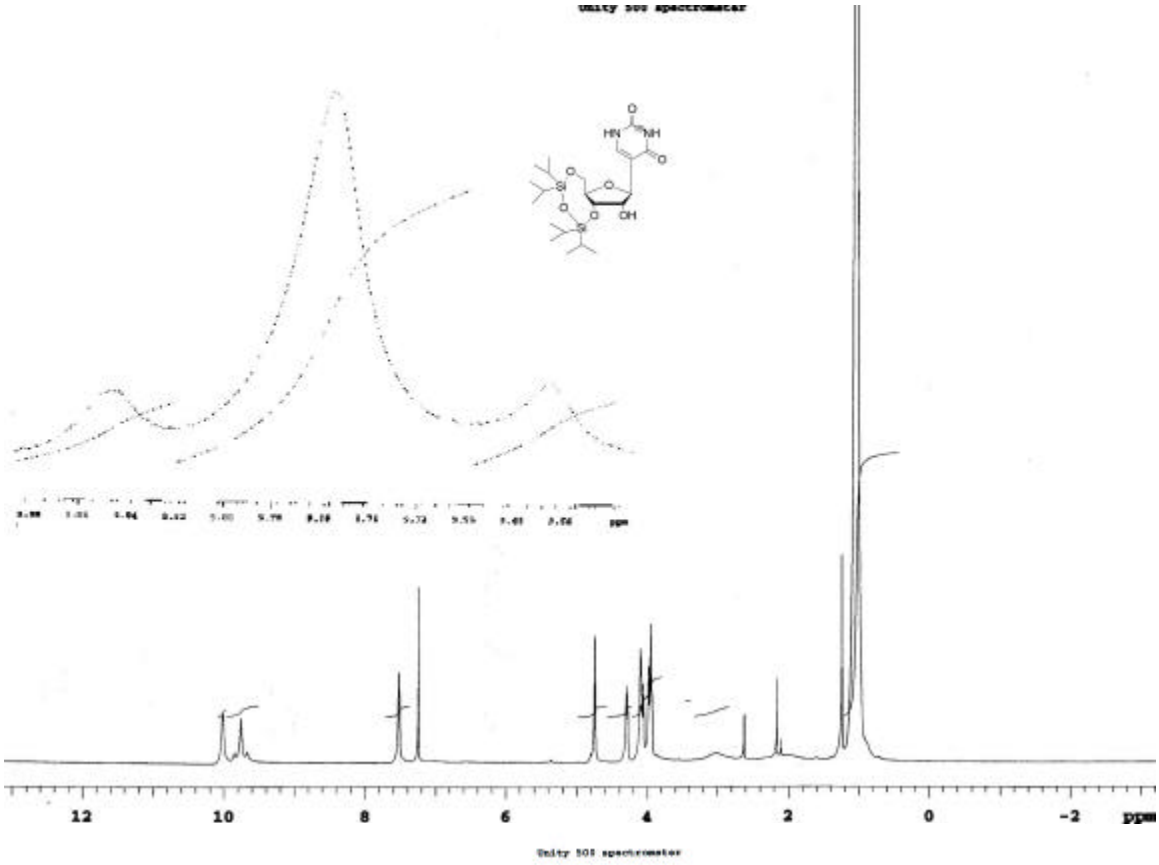
%



[3-^{15}N]-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-pseudouridine (7a):

To compound **5** (200 mg, 0.311 mmol) was added 3 mL of methanolic ammonia (2.0 M in methanol) and stirred for 24 h. The following day, the temperature was increased to 50 °C and stirred for another 24 h. The solvent was evaporated and the product was isolated by flash column chromatography on silica gel using a 4:1 to 2:1 hexanes to EtOAc gradient to afford **7a** as an oil (115 mg, 0.236 mmol) in 76% yield.

TLC (hexanes/EtOAc, 2:1): R_f = 0.03; ^{1}H NMR ($CDCl_3$, 500 MHz) d (ppm) 1.00 – 1.06 (m, 28H), 2.62 (s, 1H), 3.93 – 3.98 (m, 2H), 4.06 – 4.10 (m, 2H), 4.29 (dd, 1H, *J* = 8.5, 5 Hz), 4.74 (s, 1H), 7.52 (s, 1H), 9.76 (d, 1H, *J* = 91.5 Hz), 10.01 (s, 1H); ^{13}C NMR ($CDCl_3$, 500 MHz) d (ppm) 12.53, 12.66, 13.02, 13.36, 16.91, 17.02, 17.12, 17.28, 17.33, 17.40, 29.22, 61.15, 70.71, 74.57, 79.91, 80.79, 112.88, 138.70, 152.40, 162.99; ^{15}N NMR ($CDCl_3$, 500 MHz) d (ppm) -213.34 (d, 1N, *J* = 91.2 Hz). ESI-MS (ES^+) calculated for $C_{21}H_{38}O_7N_2Si_2$ 486.2, found 509.2 (M + Na) and for $C_{21}H_{38}O_7N^{15}NSi_2$ 487.2, found 510.2 (M[^{15}N] + Na).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8
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4
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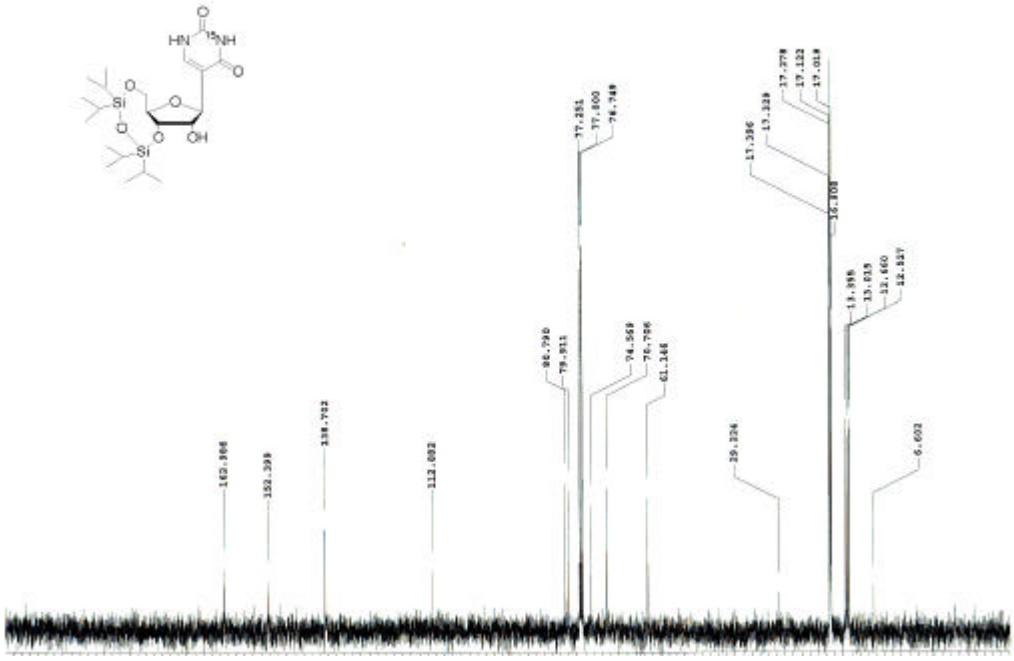
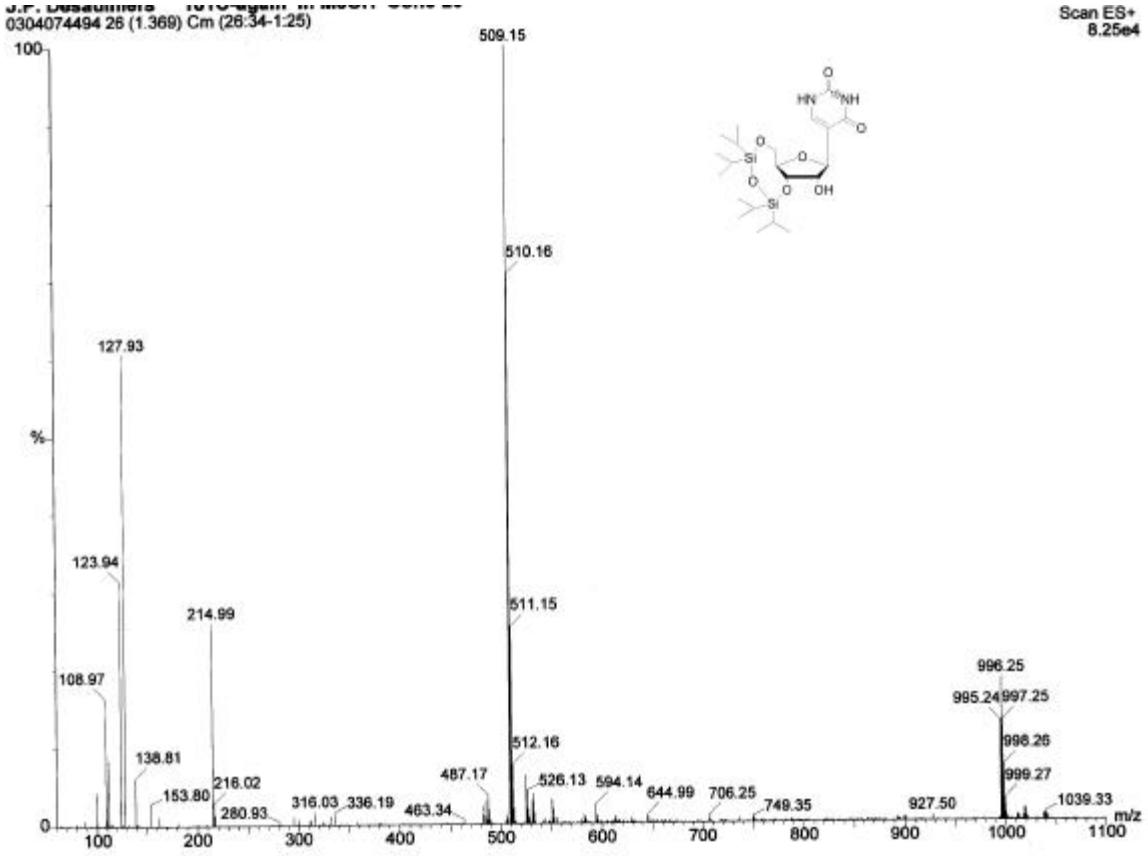
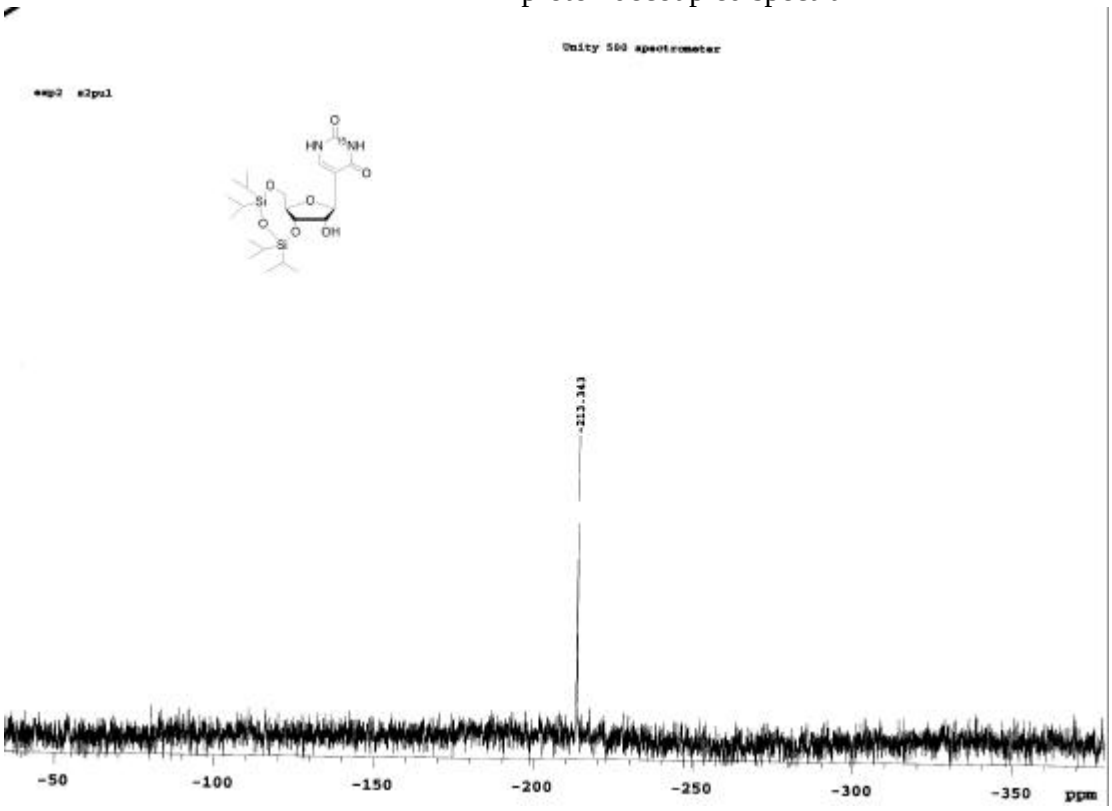
-2
ppm

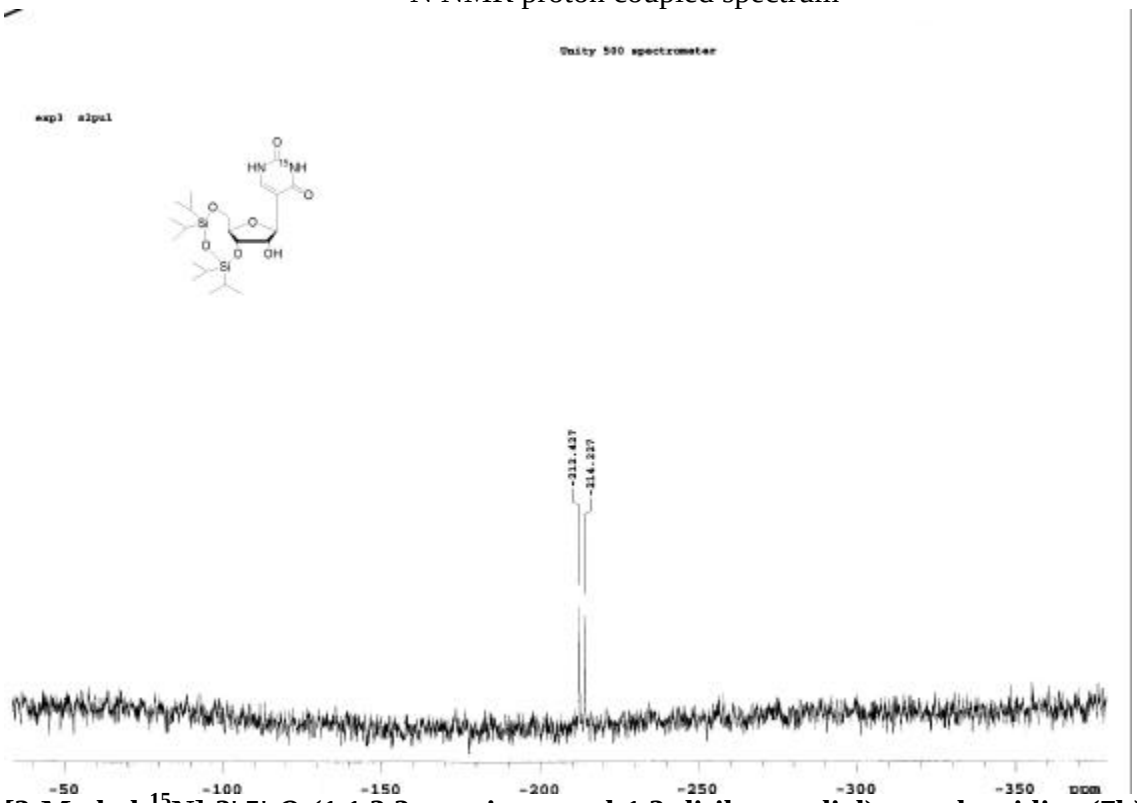




^{15}N NMR proton decoupled spectrum



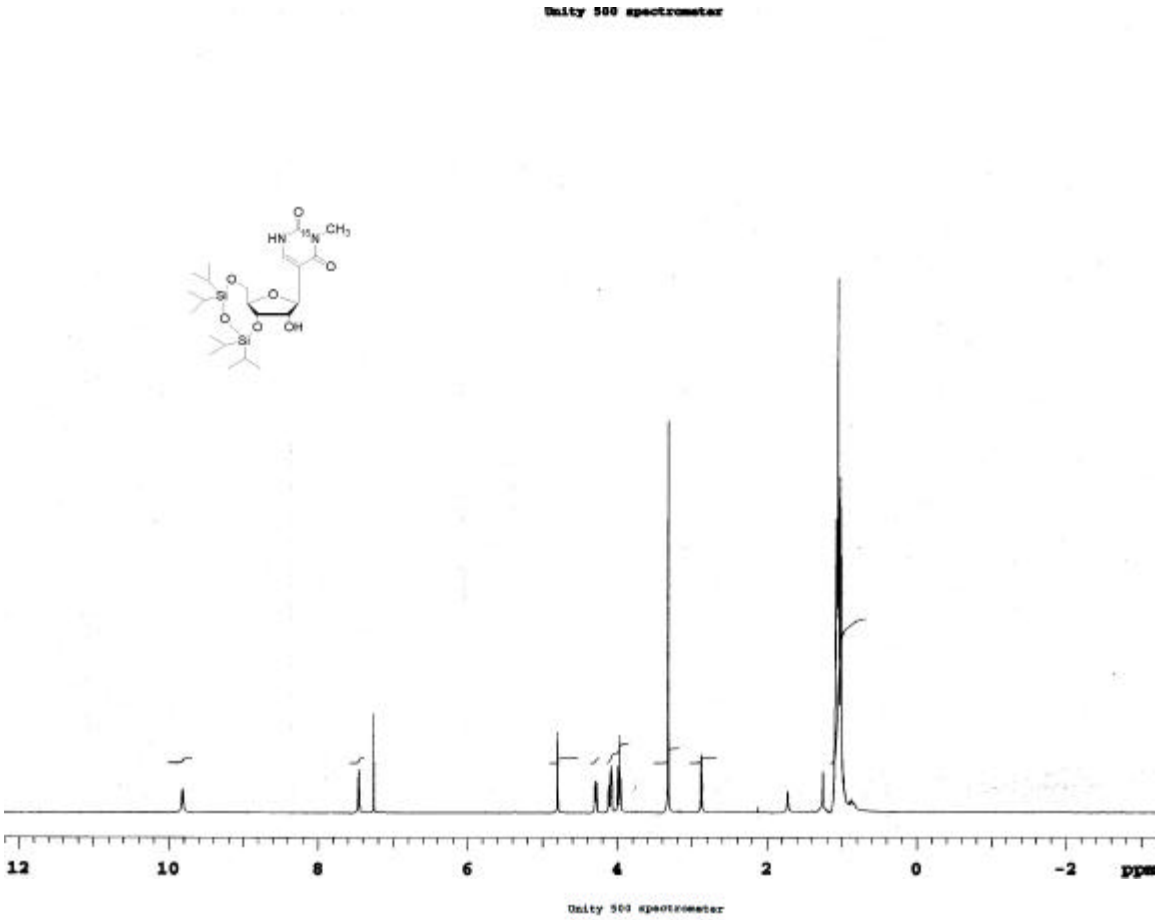
^{15}N NMR proton coupled spectrum



[3-Methyl-^{15}N]-3',5'-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-pseudouridine (7b):

To compound **6** (16 mg, 0.024 mmol) was added 3 mL of methanolic ammonia (2.0 M in methanol) and stirred for 24 h. The following day, the temperature was increased to 50 °C and stirred for another 24 h. The solvent was evaporated and the product was isolated by flash column chromatography on silica gel using a 4:1 to 2:1 hexanes to EtOAc gradient to afford **7b** as an oil (7.7 mg, 0.015 mmol) in 63% yield.

TLC (hexanes/EtOAc, 2:1): R_f = 0.13; ^{1}H NMR ($CDCl_3$, 500 MHz) d (ppm) 0.99 – 1.1 (m, 28H), 2.62 (s, 1H), 3.3 (s, 3H), 3.93 – 3.98 (m, 2H), 4.06 – 4.10 (m, 2H), 4.27 (dd, *J* = 8.5, 5 Hz), 4.78 (s, 1H), 7.44 (s, 1H), 9.80 (s, 1H); ^{13}C NMR ($CDCl_3$, 500 MHz) 12.57, 12.71, 13.03, 13.41, 16.92, 17.00, 17.03, 17.12, 17.28, 17.31, 17.37, 17.42, 27.09, 29.68, 60.98, 70.46, 74.75, 77.40, 80.27, 80.60, 112.54, 135.70, 152.71, 162.20; ^{15}N NMR ($CDCl_3$, 500 MHz) d (ppm) -214.39. ESI-MS (ES^+) calculated for $C_{22}H_{40}O_7N_2Si_2$ 500.2, found 501.2 (MH+) and $C_{22}H_{40}O_7N^{15}NSi_2$ 501.2, found 502.2 (M[^{15}N]H+).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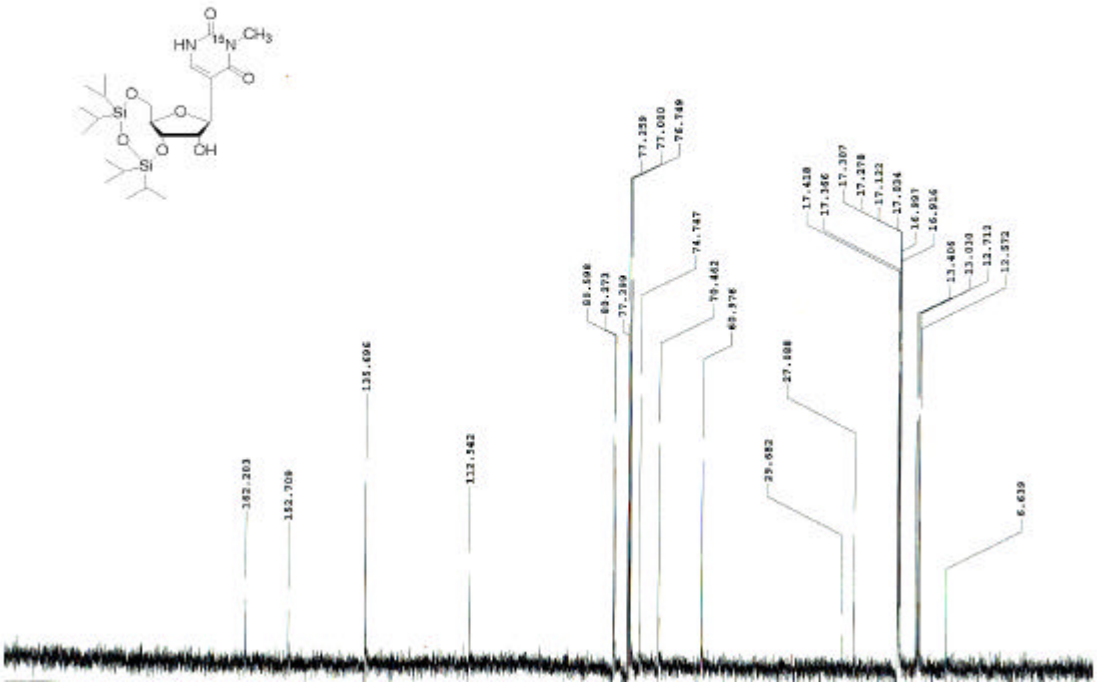
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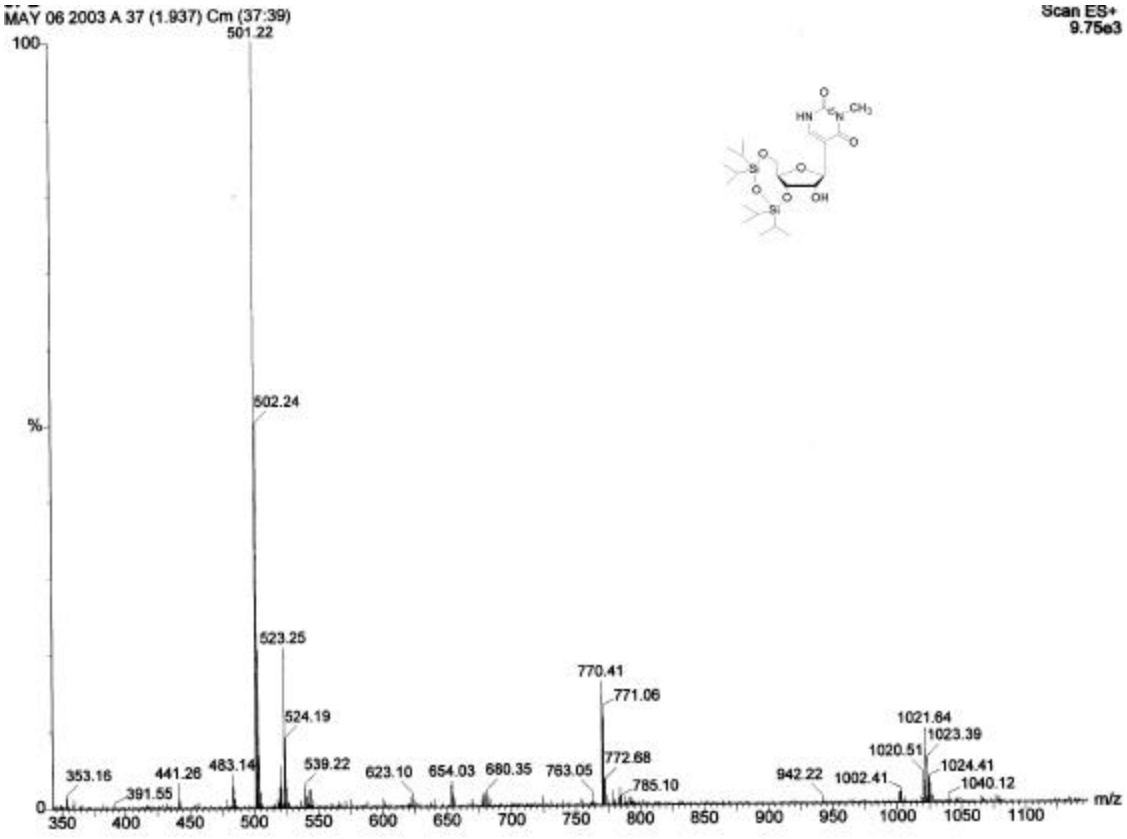
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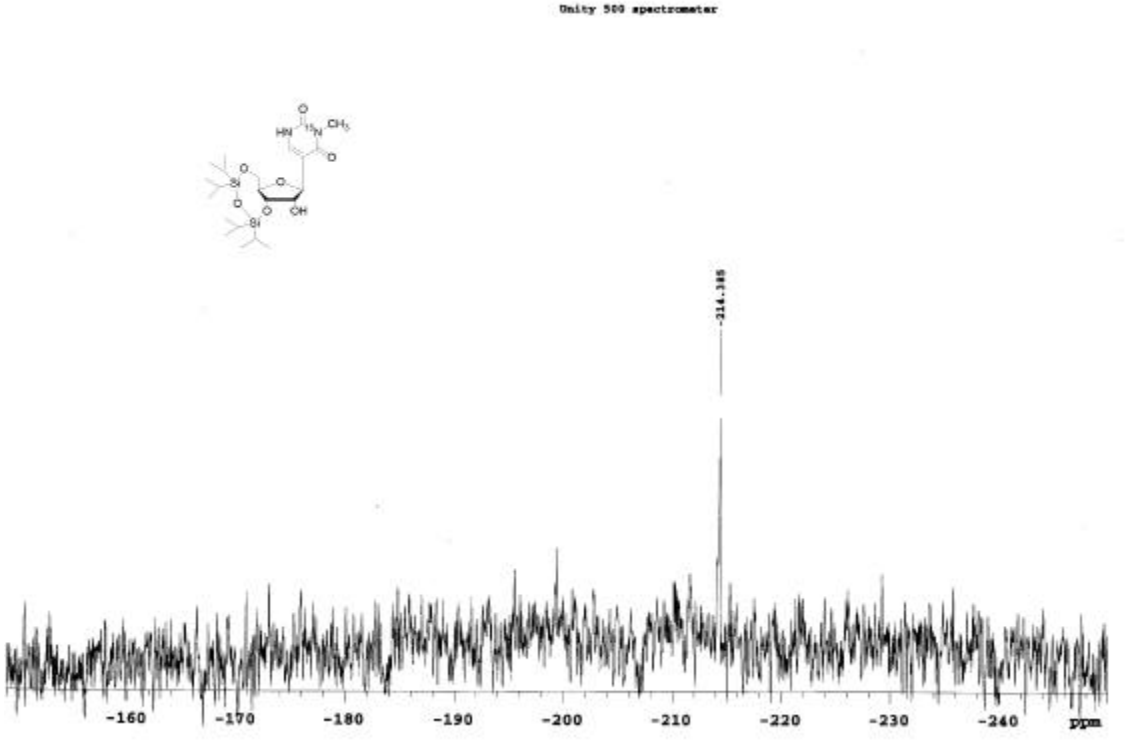
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