SUPPORTING INFORMATION

Steric Restrictions of RISC in RNA Interference Identified with Size-Expanded RNA Nucleobases

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CONTENTS:

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 251                                A TGGAAGACGC CAAAAACATA
 301  AAGAAAGGCC CGGCGCCATT CTATCCGCTG GAAGATGGAA CCGCTGGAGA
 351  GCAACTGCAT AAGGCTATGA AGAGATACGC CCTGGTTCCT GGAACAATTG
 401  CTTTTACAGA TGCACATATC GAGGTGGACA TCACTTACGC TGAGTACTTC
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1151  TATTCTCCTT CTTCGCCAAA AGCACTCTGA TTGACAAATA CGATTTATCT
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1351  AAACCGGGCG CGGTCGGTAA AGTTGTTCCA TTTTTTGAAG CGAAGGTTGT
1401  GGATCTGGAT ACCGGGAAAA CGCTGGGCGT TAATCAAAGA GGCGAACTGT
1451  GTGTGAGAGG TCCTATGATT ATGTCCGGTT ATGTAAACAA TCCGGAAGCG
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1601  TGATTAAGTA CAAAGGCTAT CAGGTGGCTC CCGCTGAATT GGAATCCATC
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1701  CGATGACGCC GGTGAACTTC CCGCCGCCGT TGTTGTTTTG GAGCACGGAA
1751  AGACGATGAC GGAAAAAGAG ATCGTGGATT ACGTCGCCAG TCAAGTAACA
1801  ACCGCGAAAA AGTTGCGCGG AGGAGTTGTG TTTGTGGACG AAGTACCGAA
1851  AGGTCTTACC GGAAAACTCG ACGCAAGAAA AATCAGAGAG ATCCTCATAA
1901  AGGCCAAGAA GGGCGGAAAG ATCGCCGTGT AA
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Figure S1. Gene sequence of firefly luciferase *luc+* (pGL3 control vector - Promega); nucleotides 280-1932. The 19-mer sequence used to design the antisense strand of the siRNA duplex used in RNAi activity experiments can be found between nucleotides 1203-1221 (underlined, bold-faced and highlighted).

Strand	Sequence	Expected MW	Observed MW	ε_{260} ($M^{-1} \cdot cm^{-1}$)
AS-00	5'-AGAAGCAAUUUCGUGUAAATT-3'	6697.948	6697.422	222,200
AS-01	5'-**A**GAAGCAAUUUCGUGUAAATT-3'	6747.964	6747.966	225,100
AS-03	5'-AG**A**AGCAAUUUCGUGUAAATT-3'	6747.964	6747.962	225,100
AS-04	5'-AGA**A**GCAAUUUCGUGUAAATT-3'	6747.964	6748.278	225,100
AS-07	5'-AGAAGC**A**AUUUCGUGUAAATT-3'	6747.964	6748.351	225,100
AS-08	5'-AGAAGCA**A**UUUCGUGUAAATT-3'	6747.964	6748.358	225,100
AS-09	5'-AGAAGCAA**U**UUCGUGUAAATT-3'	6747.964	6748.614	213,200
AS-10	5'-AGAAGCAAU**U**UCGUGUAAATT-3'	6747.964	6748.238	213,200
AS-11	5'-AGAAGCAAUU**U**CGUGUAAATT-3'	6747.964	6751.651	213,200
AS-14	5'-AGAAGCAAUUUCG**U**GUAAATT-3'	6747.964	6748.367	213,200
AS-16	5'-AGAAGCAAUUUCGUG**U**AAATT-3'	6747.964	6747.856	213,200
AS-17	5'-AGAAGCAAUUUCGUGU**A**AATT-3'	6747.964	6747.538	225,100
AS-18	5'-AGAAGCAAUUUCGUGUA**A**ATT-3'	6747.964	6748.060	225,100
AS-19	5'-AGAAGCAAUUUCGUGUAA**A**TT-3'	6747.964	6747.889	225,100
AS-xA3	5'-AGAAGCAAUUUCGUGU**AAA**TT-3'	6847.995	6847.112	230,900
AS-xU3	5'-AGAAGCAA**UUU**CGUGUAAATT-3'	6847.995	6847.865	195,200
SS-U	5'-UUUACACGAAAUUGCUUCUTT-3'	6548.854	6547.617	203,000
SS-A	5'-UUUACACGAAAUUGCUUCATT-3'	6571.881	6571.755	207,500
SS-xU	5'-UUUACACGAAAUUGCUUC**U**TT-3'	6598.870	6597.295	194,000
SS-xA	5'-UUUACACGAAAUUGCUUC**A**TT-3'	6621.897	6620.700	210,400

Table S1. MALDI mass spectrometry data and calculated molar extinction coefficient values at 260 nm (ε_{260}) of synthesized siRNA strands. xRNA substitutions are displayed as bold and underlined nucleotides. Expected and observed molecular weights (MW) are shown. See Methods section in manuscript for ε_{260} calculation protocol.

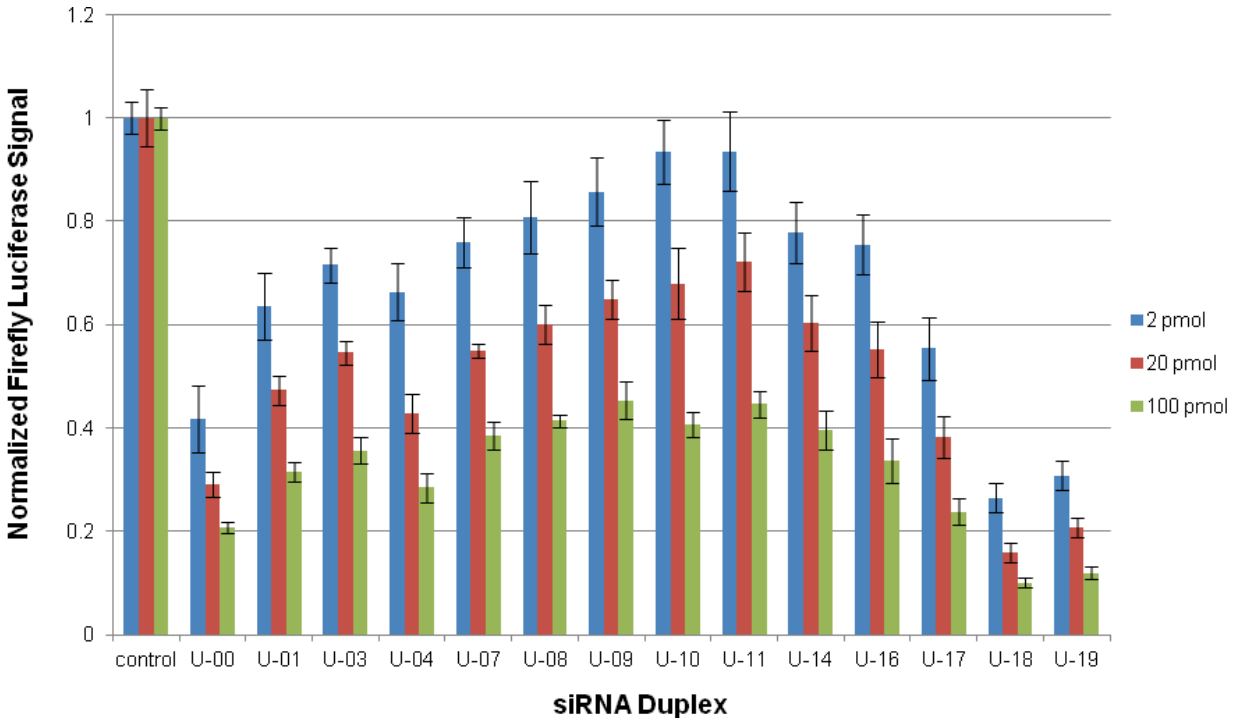


Figure S2. Normalized RNAi activity data set for siR-U series. U-00 corresponds to the unmodified siRNA duplex. Data are averages and s.d. of three independent experiments. HeLa cells were co-transfected with 2 pmol (3.33 nM), 20 pmol (33.3 nM) or 100 pmol (166 nM) of siRNA duplex. Decreased firefly luciferase signal indicates enhanced RNAi activity. Control groups contain HeLa cells treated with luciferase plasmids in the absence of siRNA.

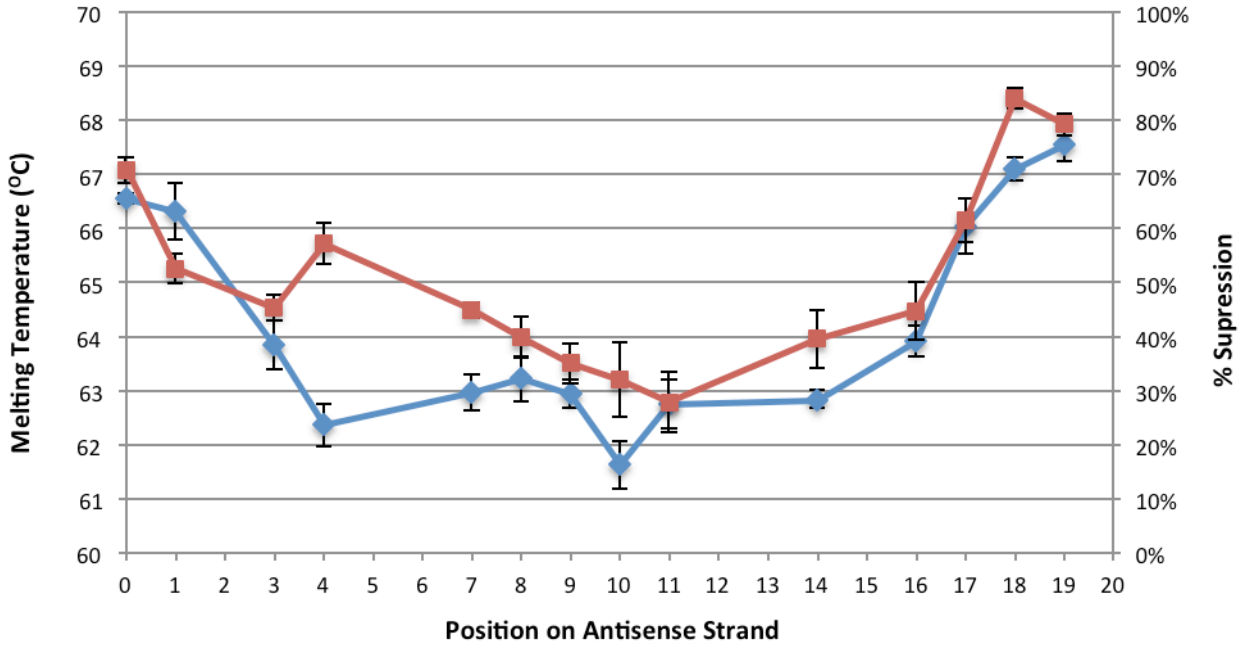


Figure S3. Plot of RNAi activity (red) and melting temperature (T_m, blue) for siR-U series. Data taken from Supplemental Figure S2 at 20 pmol siRNA (33.3 nM) and Figure 2C, respectively. Activity data was inverted and plotted as % suppression. Increased % suppression indicates enhanced RNAi activity. Data for siR-U-00 is shown at position 0. T_m error < ± 0.5 °C.

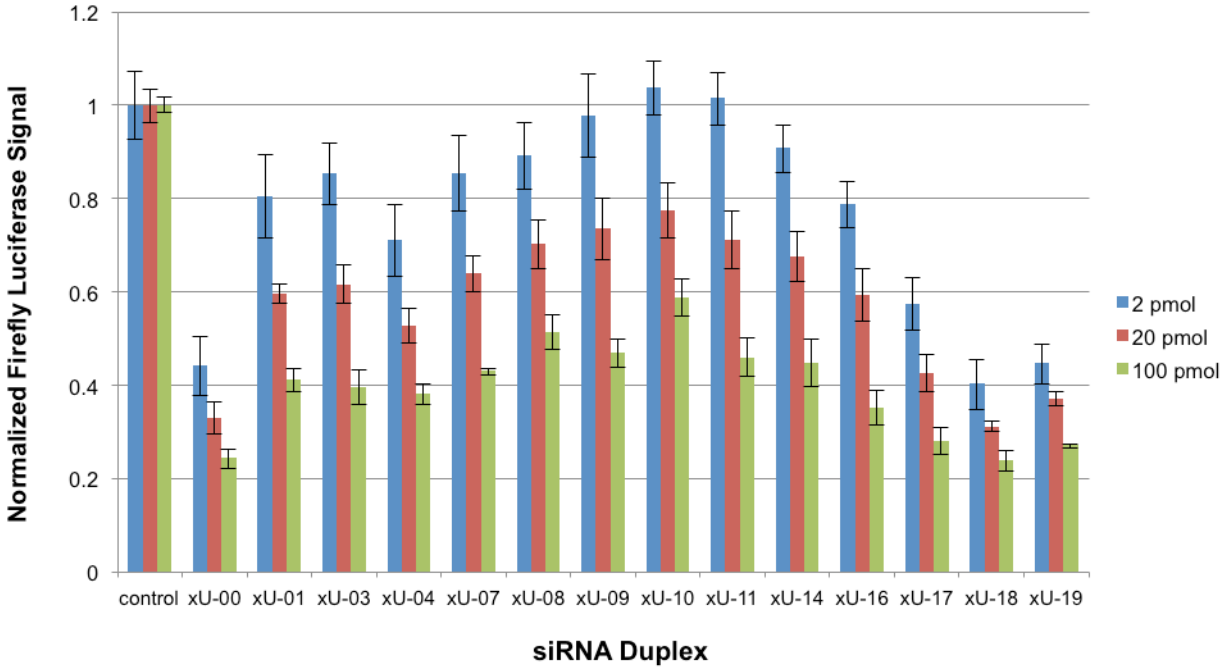


Figure S4. Normalized RNAi activity data set for siR-xU series. xU-00 corresponds to an siRNA duplex containing the unmodified antisense strand, AS-00. Data are averages and s.d. of three independent experiments. HeLa cells were co-transfected with 2 pmol (3.33 nM), 20 pmol (33.3 nM) or 100 pmol (166 nM) of siRNA duplex. Decreased firefly luciferase signal indicates enhanced RNAi activity. Control groups contain HeLa cells treated with luciferase plasmids in the absence of siRNA.

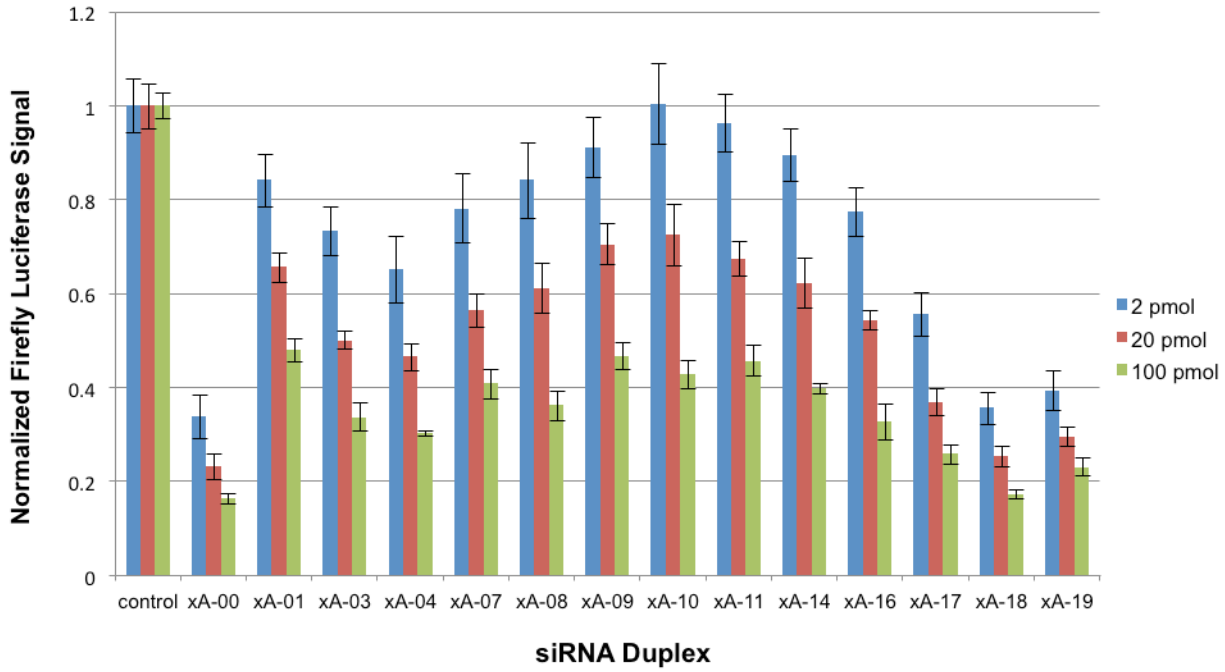


Figure S5. Normalized RNAi activity data set for siR-xA series. xA-00 corresponds to an siRNA duplex containing the unmodified antisense strand, AS-00. Data are averages and s.d. of three independent experiments. HeLa cells were co-transfected with 2 pmol (3.33 nM), 20 pmol (33.3 nM) or 100 pmol (166 nM) of siRNA duplex. Decreased firefly luciferase signal indicates enhanced RNAi activity. Control groups contain HeLa cells treated with luciferase plasmids in the absence of siRNA.

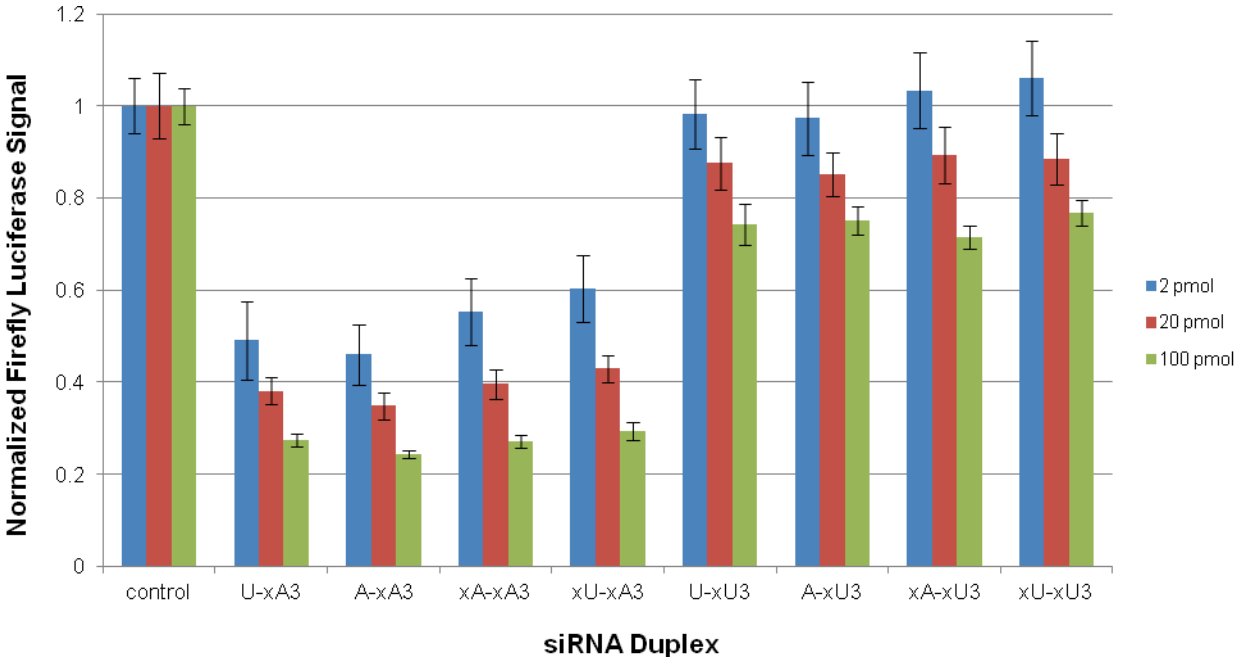


Figure S6. Normalized RNAi activity data set for si-xA3 and siR-xU3 series. Data are averages and s.d. of three independent experiments. HeLa cells were co-transfected with 2 pmol (3.33 nM), 20 pmol (33.3 nM) or 100 pmol (166 nM) of siRNA duplex. Decreased firefly luciferase signal indicates enhanced RNAi activity. Control groups contain HeLa cells treated with luciferase plasmids in the absence of siRNA.

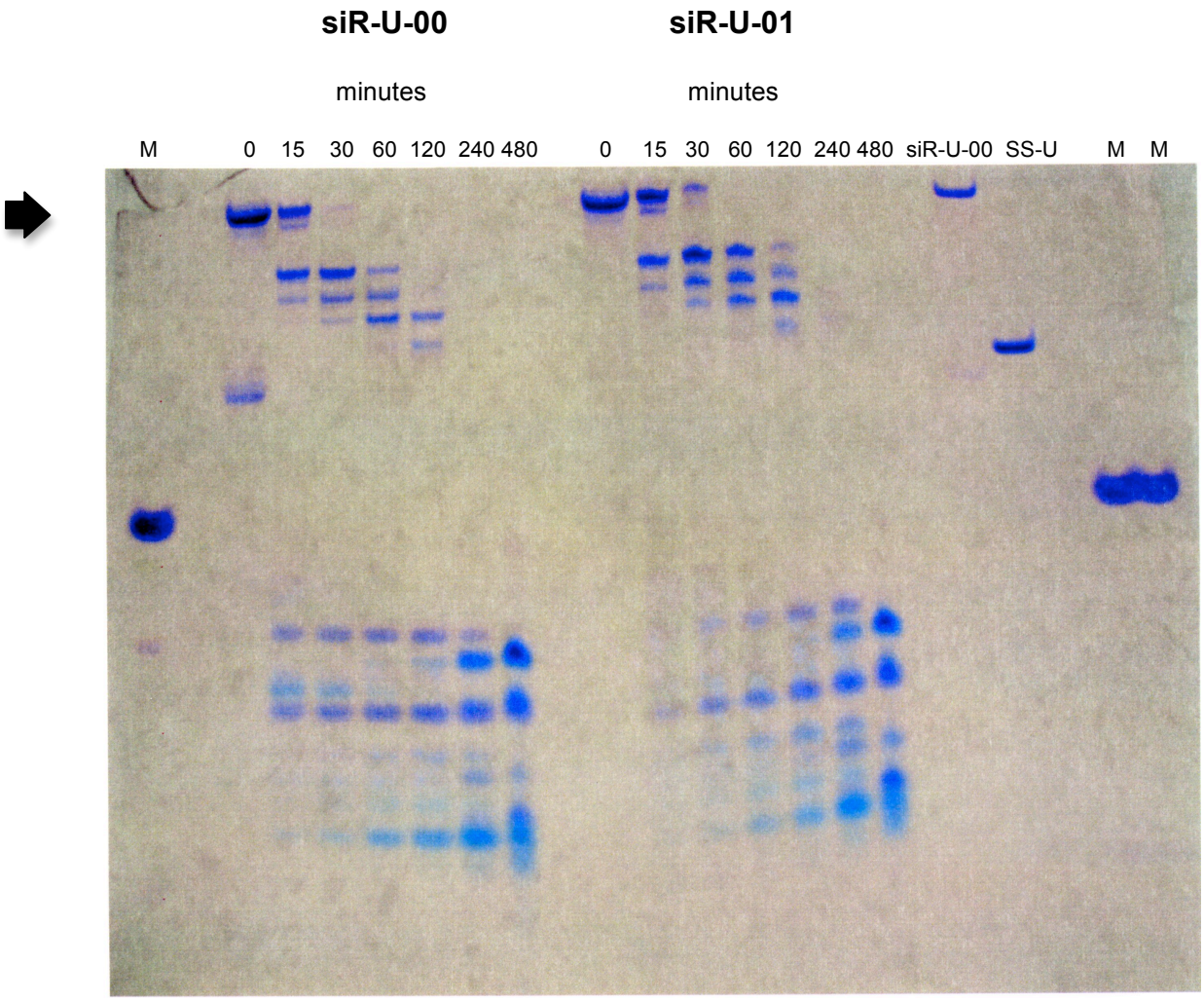


Figure S7. Serum stability data for siR-U-00 and siR-U-01 showing digestion of fully intact siRNA duplexes (black arrow) on a native PAGE gel (visualized by Stains-All staining). Samples of siR-U-00 and SS-U (both in the absence of human serum) were added to the gel to act as standardized markers indicating positions of fully intact siRNA and 21-nt oligoribonucleotide, respectively. "M" represents the marker dye bromophenol blue. Duplex analog sequences shown below (xRNA substitution shown in bold).

AS-00 siR-U-00

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'- AGAAGCAAUUUCGUGUAAATT -3' (antisense)

3'- TTUCUUCGUUAAAGCACAUUU -5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

AS-01 siR-U-01

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'- **A**GAAGCAAUUUCGUGUAAATT -3' (antisense)

3'- TTUCUUCGUUAAAGCACAUUU -5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

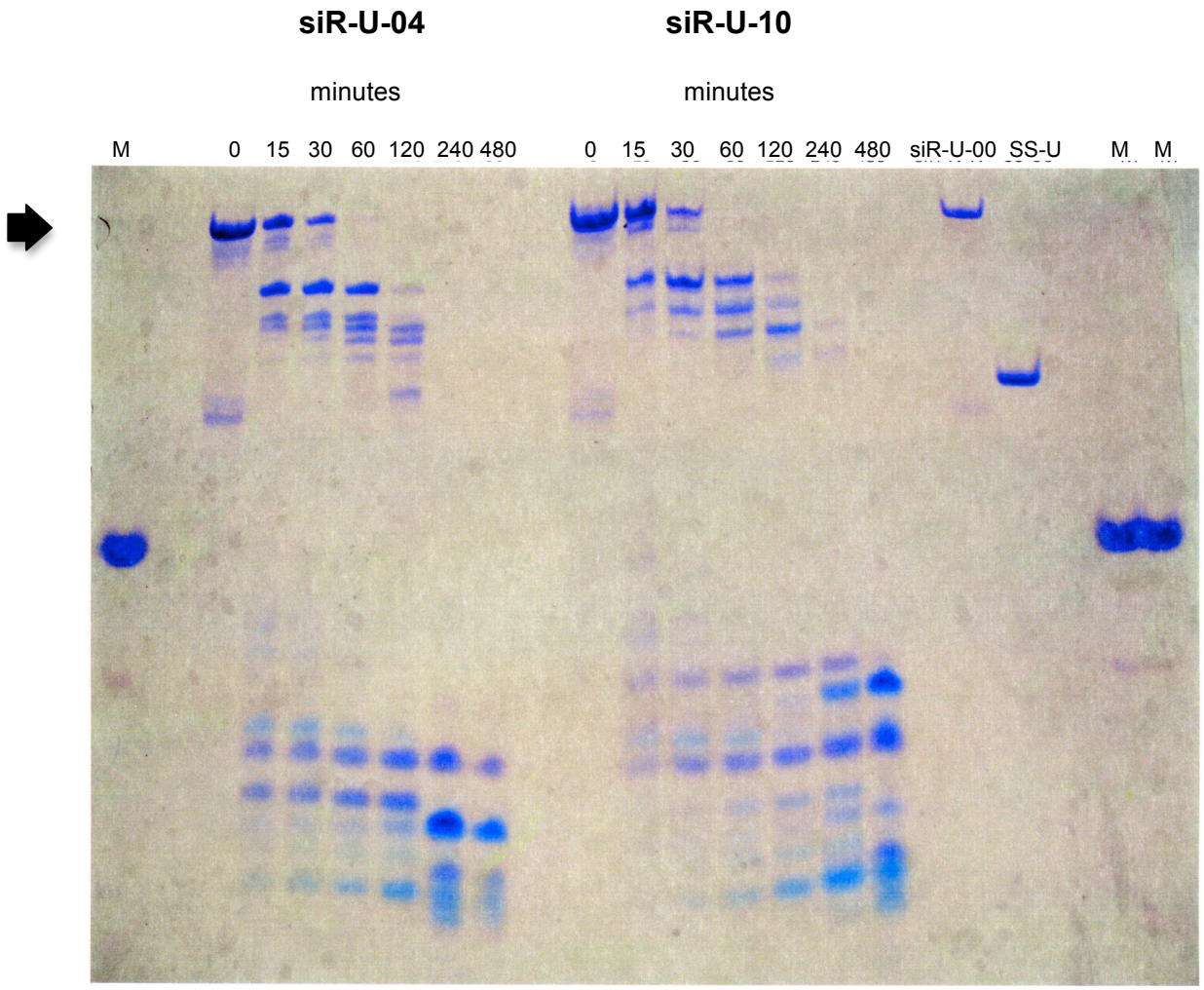


Figure S8. Serum stability data for siR-U-04 and siR-U-10 showing digestion of fully intact siRNA duplexes (black arrow) on a native PAGE gel (visualized by Stains-All staining). Samples of siR-U-00 and SS-U (both in the absence of human serum) were added to the gel to act as standardized markers indicating positions of fully intact siRNA and 21-nt oligoribonucleotide, respectively. "M" represents the marker dye bromophenol blue. Duplex analog sequences shown below (xRNA substitutions shown in bold).

AS-04 siR-U-04

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'– AGA**A**GCAAUUUCGUGUAAATT –3' (antisense)

3'– TTUCUUCGUUAAAGCACAUUU –5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

AS-10 siR-U-10

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'– AGAAGCAAU**U**UCGUGUAAATT –3' (antisense)

3'– TTUCUUCGUUAAAGCACAUUU –5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

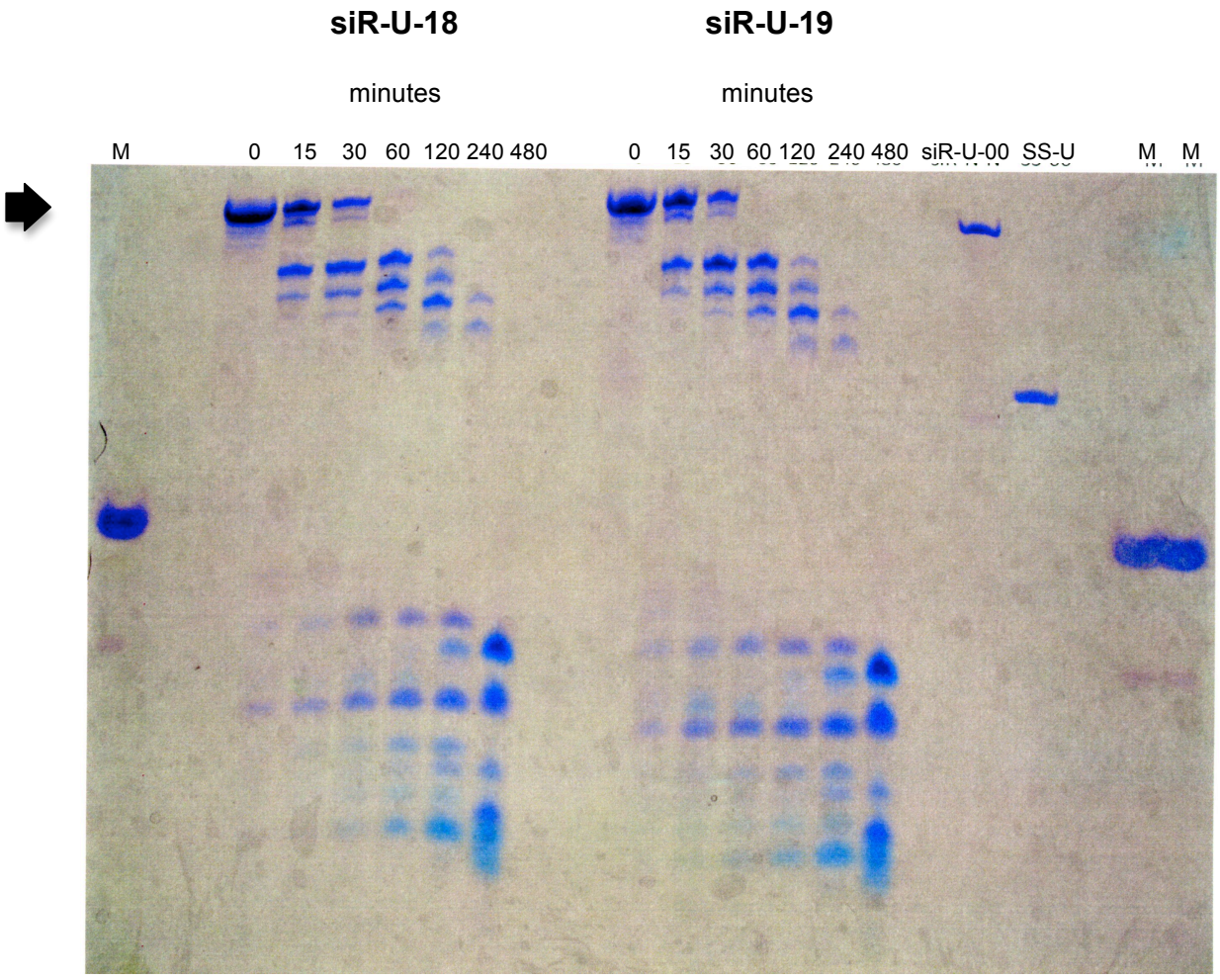


Figure S9. Serum stability data for siR-U-18 and siR-U-19 showing digestion of fully intact siRNA duplexes (black arrow) on a native PAGE gel (visualized by Stains-All staining). Samples of siR-U-00 and SS-U (both in the absence of human serum) were added to the gel to act as standardized markers indicating positions of fully intact siRNA and 21-nt oligoribonucleotide, respectively. "M" represents the marker dye bromophenol blue. Duplex analog sequences shown below (xRNA substitutions shown in bold).

AS-18 siR-U-18

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'– AGAAGCAAUUUCGUGUA**A**ATT –3' (antisense)

3'– TTUCUUCGUUAAAGCACAUUU –5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

AS-19 siR-U-19

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'– AGAAGCAAUUUCGUGUAA**A**TT –3' (antisense)

3'– TTUCUUCGUUAAAGCACAUUU –5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

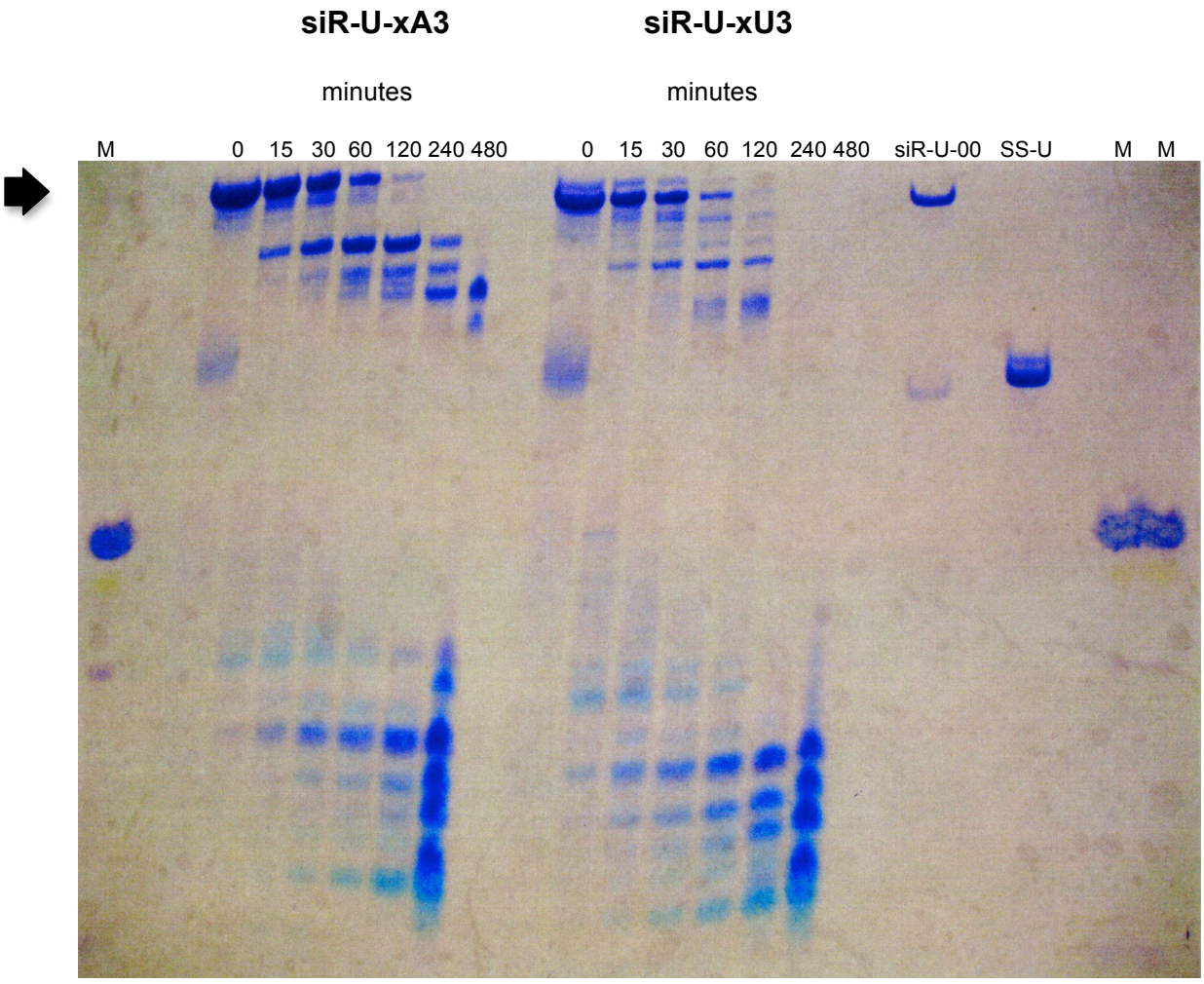


Figure S10. Serum stability data for siR-U-xA3 and siR-U-xU3 showing digestion of fully intact siRNA duplexes (black arrow) on a native PAGE gel (visualized by Stains-All staining). Samples of siR-U-00 and SS-U (both in the absence of human serum) were added to the gel to act as standardized markers indicating positions of fully intact siRNA and 21-nt oligoribonucleotide, respectively. "M" represents the marker dye bromophenol blue. Duplex analog sequences shown below (xRNA substitutions shown in bold).

AS-xA3 siR-U-xA3

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'- AGAAGCAAUUUCGUGU**AAA**TT -3' (antisense)

3'- TTUCUUCGUUAAAGCACAUUU -5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

AS-xU3 siR-U-xU3

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

5'- AGAAGCAA**UUU**CGUGUAAATT -3' (antisense)

3'- TTUCUUCGUUAAAGCACAUUU -5' (sense)

21 20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

SS-U

Materials and Methods

Unless otherwise indicated, all reactions were carried out under an atmosphere of dry argon. Anhydrous solvents were purchased from Acros. Reagents were purchased from Sigma Aldrich and were used as received without further purification. ^{1}H-NMR, ^{13}C-NMR, gCOSY and gHMBC NMR spectra were taken on a 400 MHz Varian Mercury, 500 MHz Varian Inova or 600 MHz Varian Inova spectrophotometer. Chemical shifts are reported in ppm with the solvent resonance as the internal standard. Ribose and nucleobase proton assignments were made using gCOSY data. High-resolution mass spectrometry (HRMS) was performed at the Stanford University Mass Spectrometry Facility and were analyzed by LC/ESI-MS on a Waters Acquity UPLC and Thermo Fisher Exactive mass spectrometer.

8-Amino-3-[3',5'-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-3*H*-imidazo[4,5-g]quinazoline (2). To a solution of dried 8-amino-3-(β-D-ribofuranosyl)-3*H*-imidazo[4,5-g]quinazoline (1.28 g, 4.03 mmol) (**1**)[1] in 40 mL anhy. DMF stirring in ice bath, di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (1.44 mL, 4.43 mmol) was added drop wise and allowed to stir in cold for 30 min., then 15 min. at r.t. Triethylamine (1.69 mL, 12.1 mmol) was then added to the reaction and reaction mixture was allowed to stir for an additional 15 min. Crude product was evaporated *in vacuo* and purified by silica column chromatography (5% methanol in CH_2Cl_2) to give **2** (1.57 g, 85%) as a pale yellow waxy solid. ^{1}H NMR (acetone-d_6, 400 MHz): δ 8.66 (s, 1H, ArH), 8.58 (s, 1H, ArH), 8.55 (s, 1H, ArH), 7.97 (s, 1H, ArH), 7.29 (s br, 2H, $ArNH_2$), 6.02 (d, J = 5.1 Hz, 1H, H(1')), 4.69-4.64 (m, 1H, H(2')), 4.56-4.51 (m, 1H, H(4')), 4.44-4.39 (m, 1H, H(3')), 4.35-4.24 (m, 2H, H_{ab}(5')), 3.77 (s br, 1H, H(2'OH)), 1.10 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 1.07 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$). ^{13}C NMR (acetone-$d_6$, 100 MHz): δ 167.49, 152.24, 147.48, 140.04, 130.94, 129.40, 123.74, 118.75, 116.20, 96.57, 80.17, 79.24, 76.96, 69.01, 27.25, 27.07, 26.93, 25.51, 22.32, 20.03. HRMS (M + H) calculated for $C_{22}H_{32}O_4N_5Si$: 458.2223; found: 458.2207.

8-Amino-3-[2'-*O*-(*tert*-butyldimethylsilyl)-3',5'-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-3*H*-imidazo[4,5-g]quinazoline (3). To a solution of dried **2** (810 mg, 1.77 mmol) in 35 mL anhy. pyridine stirring at r.t., imidazole (1.80 g, 26.5 mmol) was added, followed by *tert*-butyldimethylsilyl chloride (4 g, 26.5 mmol) after imidazole had completely dissolved. A precipitate formed in the reaction mixture about 5 min. after adding TBDMSCl. After stirring overnight at r.t., reaction mixture was evaporated to a syrup, then dissolved in CH_2Cl_2. Organic mixture was washed with dH_2O followed by brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. Crude product was purified by silica column chromatography (3% methanol in CH_2Cl_2) to give **3** (890 mg, 88%) as a pale yellow foamy solid. ^{1}H NMR (acetone-d_6, 400 MHz): δ 8.59 (s, 1H, ArH), 8.54 (s, 1H, ArH), 8.41 (s, 1H, ArH), 7.88 (s, 1H, ArH), 7.11 (s br, 2H, $ArNH_2$), 6.21 (d, J = 5.5 Hz, 1H, H(1')), 4.84-4.81 (m, 1H, H(2')), 4.54-4.44 (m, 2H, H(3'), H(4')), 4.26-4.17 (m, 2H, H_{ab}(5')), 1.12 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 1.09 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 0.95 (s, 9H, $OSi(CH_3)_2(C(\underline{CH_3})_3)$), 0.17 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$), 0.16 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$); ^{13}C NMR (acetone-$d_6$, 100 MHz): δ 166.99, 157.41, 154.23, 147.82, 145.94, 143.70, 138.03, 118.34, 116.50, 105.85, 93.17, 76.87, 75.64, 74.72, 67.69, 40.69, 34.58, 27.43, 27.27, 26.92, 25.73, 22.60, 20.34, 19.98, 18.31, -4.49, -5.43. HRMS (M + H) calculated for $C_{28}H_{46}O_4N_5Si_2$: 572.3088; found: 572.3074.

3-[2'-*O*-(*tert*-Butyldimethylsilyl)-3',5'-*O*-(di-*tert*-butylsilylene)-β-D-ribofuranosyl]-8-*N*-(dimethylacet-amido)-3*H*-imidazo[4,5-g]quinazoline (4). To a solution of dried **3** (1.68 g, 2.94 mmol) in 30 mL anhy. methanol stirring at r.t., *N*,*N*-dimethylacetamide dimethyl acetal (5.16 mL, 35.3 mmol) was added. After stirring at r.t. for 12 h, reaction mixture was evaporated *in vacuo*. Crude product was purified by silica column chromatography (1% methanol in CH_2Cl_2) to give **4** (1.70 g, 90%) as a pale yellow foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.77 (s, 1H, ArH), 8.64 (s, 1H, ArH), 8.11 (s, 1H, ArH), 7.77 (s, 1H, ArH), 5.93 (d, J = 4.3 Hz, 1H, H(1')), 4.51-4.45 (m, 1H, H(4')), 4.41-4.38 (m, 1H, H(2')), 4.23-4.12 (m, 2H, H_a(5'), H(3')), 4.06-4.00 (m, 1H, H_b(5')), 3.22 (s br, 3H, $ArN=C(CH_3)N(\underline{CH_3})_2$), 3.12 (s br, 3H, $ArN=C(CH_3)N(\underline{CH_3})_2$), 2.20 (s, 3H, $ArN=C(\underline{CH_3})N(CH_3)_2$), 1.03 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 1.00

(s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 0.87 (s, 9H, $OSi(CH_3)_2(C(\underline{CH_3})_3)$), 0.067 (s, 6H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.33, 161.33, 154.56, 146.93, 144.48, 143.14, 136.92, 118.49, 117.17, 105.64, 93.12, 76.58, 75.34, 74.30, 67.57, 38.39, 38.14, 27.34, 26.89, 25.72, 22.64, 20.26, 20.24, 18.10, 16.65, -4.22, -5.08. HRMS (M + H) calculated for $C_{32}H_{53}O_4N_6Si_2$: 641.5467; found: 641.3653.

3-[2'-*O*-(*tert*-Butyldimethylsilyl)-*β*-D-ribofuranosyl]-8-*N*-(dimethylacetamido)-3*H*-imidazo[4,5-g] quinazoline (5). To an oven-dried round-bottom flask purged with argon, a solution of 1.6 mL HF-pyridine in 1.8 mL anhy. pyridine was *carefully* made and allowed to stir in ice bath. A solution of dried **4** (1.52 g, 2.38 mmol) in 35 mL anhy. THF was prepared and allowed to stir at 0^OC. Diluted HF-pyridine solution was *carefully* added to the latter solution drop wise and was allowed to stir at r.t. for 15 min., then diluted with 8 ml of pyridine followed by CH_2Cl_2. Organic mixture was washed with 5% aq. $NaHCO_3$ followed by brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. Crude product was purified silica column chromatography (4% methanol in CH_2Cl_2) to give **5** (1.52 g, 87%) as a pale yellow foam. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.72 (s, 1H, ArH), 8.63 (s, 1H, ArH), 8.38 (s, 1H, ArH), 8.33 (s, 1H, ArH), 5.92 (d, *J* = 7.0 Hz, 1H, H(1')), 4.77 (t, *J* = 6.5 Hz, 1H, H(2')), 4.43-4.38 (m, 1H, H(3')), 4.34-4.30 (m, 1H, H(4')), 4.09 (dd, *J* = 8.3, 2.8 Hz, 1H, H_a(5')), 3.95 (dd, *J* = 8.0, 2.8 Hz, 1H, H_b(5')), 3.26 (s br, 3H, $ArN=C(CH_3)N(\underline{CH_3})_2$), 3.16 (s br, 3H, $ArN=C(CH_3)N(\underline{CH_3})_2$), 2.24 (s, 3H, $ArN=C(\underline{CH_3})N(CH_3)_2$), 0.75 (s, 9H, $OSi(CH_3)_2(C(\underline{CH_3})_3)$), -0.22 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$), -0.43 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 167.86, 162.12, 154.27, 146.73, 143.81, 137.39, 118.57, 117.12, 107.38, 90.32, 86.06, 75.05, 71.79, 62.24, 38.63, 25.78, 18.09, 17.19, -5.10, -5.12. HRMS (M + H) calculated for $C_{24}H_{37}O_4N_6Si$: 501.2645; found: 501.2631.

3-[2'-*O*-(*tert*-Butyldimethylsilyl)-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-ribofuranosyl]-8-*N*-(dimethylacetamido)-3*H*-imidazo[4,5-g]quinazoline (6). To a solution of dried **5** (1.17 g, 2.34 mmol) and DMAP (57.2 mg, 0.468 mmol) in 45 mL anhy. pyridine, 4,4'-dimethoxytrityl chloride (2.38 g, 7.02 mmol) was added in one portion and allowed to stir at r.t. At 12 h, TLC indicated that reaction was complete. Reaction mixture was quenched with 0.5 mL methanol and evaporated *in vacuo* to form a gum. Residue was dissolved in EtOAc and washed with 5% aq. $NaHCO_3$, followed by water and brine. Crude product in organic layer was dried over Na_2SO_4, evaporated *in vacuo* and purified by silica column chromatography (0 - 3% methanol in CH_2Cl_2) to give **6** (1.57 g, 86%) as a pale yellow foam. ^1H NMR ($CDCl_3$, 400 MHz): δ 8.78 (s, 1H, ArH), 8.68 (s, 1H, ArH), 8.39 (s, 1H, ArH), 7.89 (s, 1H, ArH), 7.47-7.17 (m, 9H, ArH), 6.86-6.76 (m, 4H, ArH), 6.02 (d, *J* = 6.4 Hz, 1H, H(1')), 4.72 (t, *J* = 5.8 Hz, 1H, H(2')), 4.35-4.31 (m, 1H, H(4')), 4.30-4.26 (m, 1H, H(3')), 3.76 (s, 6H, $R(ArOCH_3)_2$), 3.53 (dd, *J* = 8.2, 3.0 Hz, 1H, H_a(5')), 3.44 (dd, *J* = 7.4, 3.5 Hz, 1H, H_b(5')), 3.28 (s br, 3H, $ArN=C(CH_3)N(\underline{CH_3})_2$), 3.18 (s br, 3H, $ArN=C(CH_3)N(\underline{CH_3})_2$), 2.93 (s br, 1H, OH(3')), 2.28 (s, 3H, $ArN=C(\underline{CH_3})N(CH_3)_2$), 0.79 (s, 9H, $OSi(CH_3)_2(C(\underline{CH_3})_3)$), -0.14 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$), -0.33 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.50, 153.77, 146.51, 144.84, 144.37, 143.05, 137.92, 135.29, 129.99, 129.97, 127.92, 126.93, 118.25, 117.07, 113.21, 105.20, 88.55, 86.76, 84.22, 71.76, 63.63, 55.12, 38.54, 25.43, 17.76, 16.89, -5.25, -5.43. HRMS (M + H) calculated for $C_{45}H_{55}O_6N_6Si$: 803.3952; found: 803.3933.

3-[2'-*O*-(*tert*-Butyldimethylsilyl)-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphino)-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-ribofuranosyl]-8-*N*-(dimethylacetamido)-3*H*-imidazo[4,5-g]quinazoline (7). To a solution of **6** (100 mg, 0.125 mmol), 1-methylimidazole (8 μL, 0.100 mmol) and *N*,*N*-diisopropylethylamine (76 μL, 0.438 mmol) in 5 mL anhy. CH_2Cl_2 stirring at 0°C, 2-cyanoethyl *N*,*N*-diisopropylcholorophosphoramidite (34 μL, 0.150 mmol) was added drop wise and the reaction mixture was allowed to stir at r.t. while monitored by TLC. At 6 h, the reaction was complete by TLC and the mixture was quenched with 5% aq. $NaHCO_3$. Organic layer was dried under $MgSO_4$ and evaporated *in vacuo*. Crude product was purified by silica column chromatography (0 - 2% methanol in CH_2Cl_2 with 1% Et_3N) to give **7** (109 mg, 87%) as a pale yellow foam. ^{31}P NMR ($CDCl_3$, 162 MHz): δ 152.880, 149.550. HRMS (M + H) calculated for $C_{54}H_{72}N_8O_7SiP$: 1003.4953; found: 1003.5052.

8-[3',5'-*O*-(di-*tert*-butylsilylene)-*β*-D-ribofuranosyl]-quinazoline-2,4(1*H*,3*H*)-dione (9). To a solution of dried 8-(*β*-D-ribofuranosyl)-quinazoline-2,4(1*H*,3*H*)-dione (443 mg, 1.50 mmol) (**8**)[1] in 10 mL anhy. DMF stirring in ice bath, di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (535 μL, 1.65 mmol) was added drop wise and allowed to stir in cold for 30 min., then 15 min. at r.t. Triethylamine (0.63 mL, 4.5 mmol) was then added to the mixture and reaction was allowed to stir for an additional 15 min. Crude product was evaporated *in vacuo* and purified by silica column chromatography (5% methanol in CH_2Cl_2) to give **9** (464 mg, 71%) as a pale yellow waxy solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.93 (s br, 1H ArNH), 7.85 (d, *J* = 7.7 Hz, 1H, ArH), 7.62 (d, *J* = 7.7 Hz, 1H, ArH), 7.18 (t, *J* = 7.7 Hz, 1H, ArH), 5.74 (s br, 1H, OH(2')), 5.24 (d, *J* = 6.8 Hz, 1H, H(1')), 4.46-4.42 (m, 1H, H(3')), 4.10-4.01 (m, 3H, H(2'), H(4'), H_a(5')), 3.81-3.76 (m, 1H, H_b(5')), 0.97 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 0.95 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$); ^{13}C NMR (DMSO-$d_6$, 100 MHz): δ 162.63, 150.25, 137.22, 131.53, 126.55, 126.11, 122.27, 114.93, 82.88, 76.67, 73.63, 67.37, 27.34, 27.24, 27.03, 26.77, 22.26, 20.08. HRMS (M + H) calculated for $C_{21}H_{31}O_6N_2Si$: 435.1951; found: 435.1940.

8-[2'-*O*-(*tert*-butyldimethylsilyl)-3',5'-*O*-(di-*tert*-butylsilylene)-*β*-D-ribofuranosyl]-quinazoline-2,4(1*H*,3*H*)-dione (10). To a solution of dried **9** (435 mg, 1.00 mmol) in 10 mL anhy. pyridine stirring at r.t., imidazole (1.02 g, 15.0 mmol) was added, followed by *tert*-butyldimethylsilyl chloride (2.26 g, 15.0 mmol) after imidazole had completely dissolved. A precipitate formed in the reaction mixture about 5 min. after adding TBDMSCl. After stirring overnight at r.t., reaction mixture was evaporated to a syrup, then dissolved in CH_2Cl_2. Organic mixture was washed with dH_2O followed by brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. Crude product was purified by silica column chromatography (0 - 2% methanol in CH_2Cl_2) to give **10** (490 mg, 89%) as a pale yellow foamy solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.98 (s br, 1H, ArNH), 9.31, (s br, 1H, ArNH), 8.12 (d, *J* = 7.8 Hz, 1H, ArH), 7.50 (d, *J* = 7.8 Hz, 1H, ArH), 7.20 (t, *J* = 7.6 Hz, 1H, ArH), 5.13 (d, *J* = 6.3 Hz, 1H, H(1')), 4.55-4.50 (m, 1H, H(4')), 4.40-4.37 (m, 1H, H(2')), 4.12-4.07 (m, 1H, H_a(5')), 4.04-3.98 (m, 1H, H_b(5')), 3.91-3.86 (m, 1H, H(3')), 1.04 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 1.03 (s, 9H, $R_2Si(C(\underline{CH_3})_3)_2$), 0.91 (s, 9H, $OSi(CH_3)_2(C(\underline{CH_3})_3)$), 0.12 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$), 0.087 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.92, 149.88, 138.09, 132.30, 128.20, 123.80, 122.80, 115.73, 88.78, 77.47, 75.86, 74.98, 67.63, 27.40, 26.98, 25.82, 25.60, 22.62, 20.25, 18.09, 17.91, -3.68, -3.95, -5.07. HRMS (M + H) calculated for $C_{27}H_{45}O_6N_2Si_2$: 549.2816; found: 549.2811.

8-[2'-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-quinazoline-2,4(1*H*,3*H*)-dione (11). To an oven-dried round-bottom flask purged with argon, a solution of 0.63 mL HF-pyridine in 0.72 mL anhy. pyridine was *carefully* made and allowed to stir in an ice bath. A solution of dried **10** (500 mg, 0.911 mmol) in 15 mL of anhydrous THF was prepared and allowed to stir at 0°C. Diluted HF-pyridine solution was *carefully* added to the latter solution drop wise and was allowed to stir at r.t. for 15 min., then diluted with 3 ml of pyridine followed by CH_2Cl_2. Organic mixture was washed with 5% aq. $NaHCO_3$ followed by brine, dried over Na_2SO_4, filtered and evaporated *in vacuo*. Crude product was purified on silica column chromatography (3% methanol in CH_2Cl_2) to give **11** (319 mg, 86%) as a pale yellow foam. ^{1}H NMR (acetone-d_6, 400 MHz): δ 10.58 (s br, 1H, ArNH), 10.45 (s br, 1H, ArNH), 8.06 (d, *J* = 7.8 Hz, 1H, ArH), 7.62 (d, *J* = 7.8 Hz, 1H, ArH), 7.23 (d, *J* = 7.8 Hz, 1H, ArH), 4.92 (d, *J* = 7.9 Hz, 1H, H(1')), 4.43-4.39 (m, 1H, H(2')), 4.28-4.35 (m, 1H, H(3')), 4.23-4.21 (m, 1H, H(4')), 3.97-3.93 (m, 2H, H_{ab}(5')), 3.78 (s br, 1H, OH(3')), 0.78 (s, 9H, $OSi(CH_3)_2(C(\underline{CH_3})_3)$), -0.15 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$), -0.34 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$); ^{13}C NMR (acetone-$d_6$, 100 MHz): δ 163.49, 150.94, 139.93, 136.94, 128.59, 124.89, 122.78, 116.54, 87.55, 84.51, 76.44, 73.81, 62.64, 18.51, -5.19, -5.36. HRMS (M + H) calculated for $C_{19}H_{29}O_6N_2Si$: 409.1795; found: 409.1789.

8-[2'-*O*-(*tert*-butyldimethylsilyl)-5'-*O*-(4,4'-dimethoxytrityl)-*β*-D-ribofuranosyl]-quinazoline-2,4(1*H*,3*H*)-dione (12). To a solution of dried **11** (300 mg, 0.734 mmol) and 2,6-di-*tert*-butylpyrdine (810 μL, 3.64 mmol) in 10 mL anhy. CH_3CN, silver nitrate (125 mg, 0.734 mmol) was added in one portion and allowed to stir at r.t. until fully dissolved. To this mixture, 4,4'-dimethoxytrityl chloride (300 mg, 0.881 mmol) was added in one portion and allowed to stir at r.t. for 20 min. Mixture was then filtered into 5 mL of 5% aq. $NaHCO_3$ and partitioned with CH_2Cl_2. Organic layer was dried over Na_2SO_4

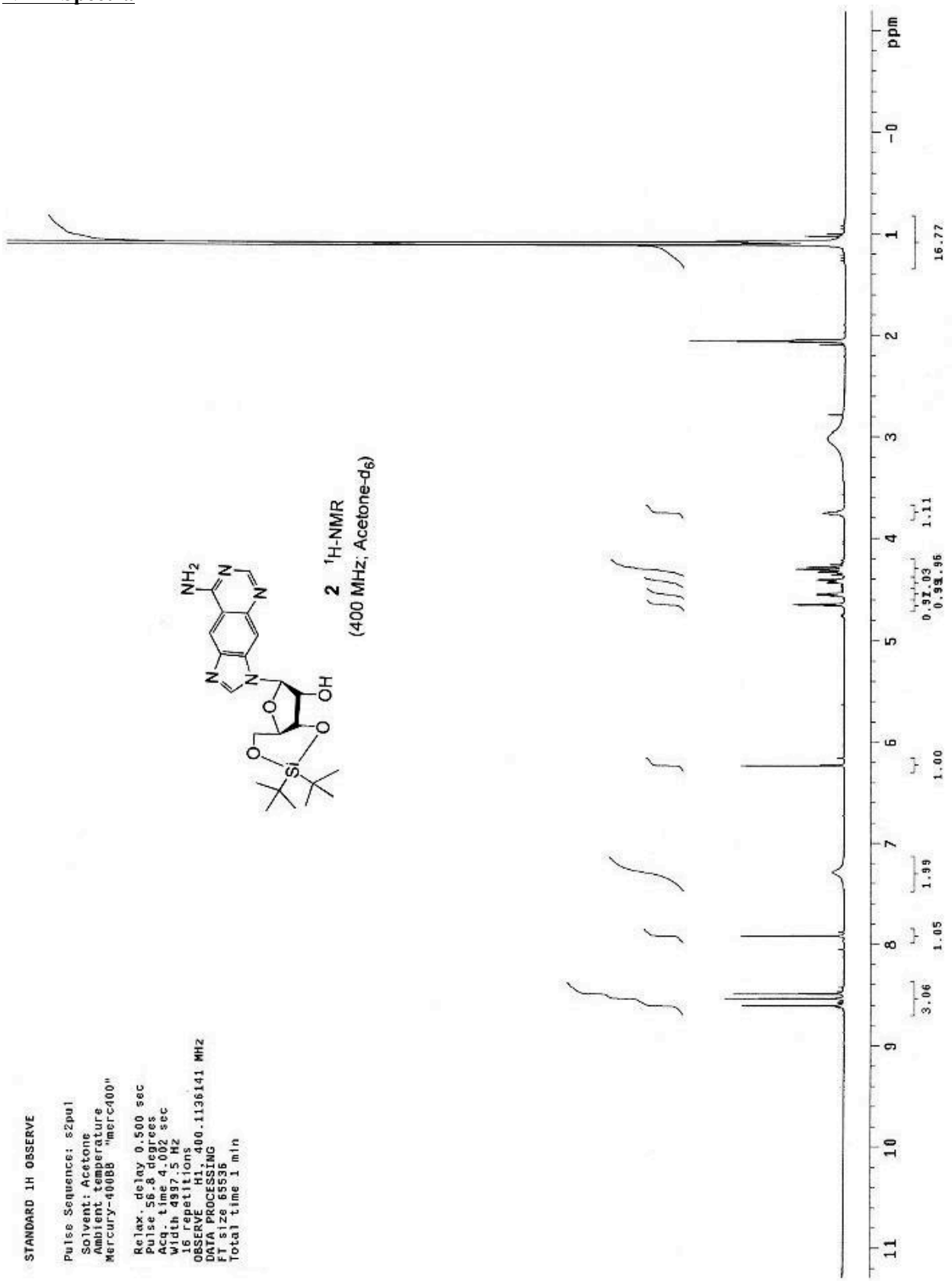
and evaporated *in vacuo*. Crude product was purified by silica column chromatography (0 - 2 % methanol in CH_2Cl_2) to give **12** (428 mg, 82%) as a pale yellow foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.49 (s br, 1H, ArNH), 9.11 (s br, 1H, ArNH), 8.16 (d, *J* = 7.7 Hz, 1H, ArH), 7.74 (d, *J* = 7.7 Hz, 1H, ArH), 7.46-7.16 (m, 9H, ArH), 6.84-6.78 (m, 4H, ArH), 5.06 (d, *J* = 8.5 Hz, 1H, H(1')), 4.35-4.30 (m, 1H, H(2')), 4.29-4.26 (m, 1H, H(4')), 4.02-3.98 (m, 1H, H(3')), 3.78 (s, 6H, R($ArOCH_3$)$_2$), 3.63 (dd, *J* = 8.3, 2.6 Hz, 1H, H_a(5')), 3.37 (dd, *J* = 8.0, 2.5 Hz, 1H, H_b(5')), 2.93 (s br, 1H, OH(3')), 0.83 (s, 9H, $OSi(CH_3)_2(C(\underline{CH_3})_3)$), -0.10 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$), -0.18 (s, 3H, $OSi(\underline{CH_3})_2(C(CH_3)_3)$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.76, 158.52, 149.37, 144.57, 138.31, 135.58, 135.39, 133.55, 130.13, 130.15, 128.04, 127.80, 126.85, 124.00, 122.85, 115.21, 113.12, 86.72, 85.63, 79.81, 78.01, 76.67, 72.75, 63.42, 55.18, 25.57, 17.91, -5.22, -5.25. HRMS (M + Na) calculated for $C_{40}H_{46}O_8N_2NaSi$: 733.2921; found: 733.2907.

8-[2'-*O*-(*tert*-butyldimethylsilyl)-3'-*O*-(2-cyanoethyl-*N*,*N*-diisopropylphosphino)-5'-*O*-(4,4'-dimethoxytrityl)-β-D-ribofuranosyl]-quinazoline-2,4(1*H*,3*H*)-dione (13). To a solution of dried **12** (125 mg, 0.176 mmol) in 5 mL anhy. CH_3CN, pyridinium trifluoroacetate (37.5 mg, 0.194 mmol) was added in one portion and allowed to stir at r.t. until fully dissolved. To this solution, 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphordiamidite (84 μL, 0.264 mmol) was added drop wise and the reaction mixture was allowed to stir at r.t. while monitored by TLC. At 18 h, the reaction was complete by TLC and the mixture was evaporated *in vacuo*. Crude product was purified by silica column chromatography (4:1 hexanes:ethyl acetate with 1% Et_3N) to give **13** (148 mg, 92%) as a white foam. ^{31}P NMR ($CDCl_3$, 162 MHz): δ 151.651, 147.736. HRMS (M + Na) calculated for $C_{49}H_{63}N_4O_9NaSiP$, 933.3994; found: 933.4014.

References

(1) Hernández, A. R. and Kool, E. T. (2011) The components of xRNA: Synthesis and fluorescence of a full genetic set of size-expanded ribonucleosides, *Org. Lett. 13*, 676-679.

NMR Spectra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Width 4997.5 Hz
16 repetitions
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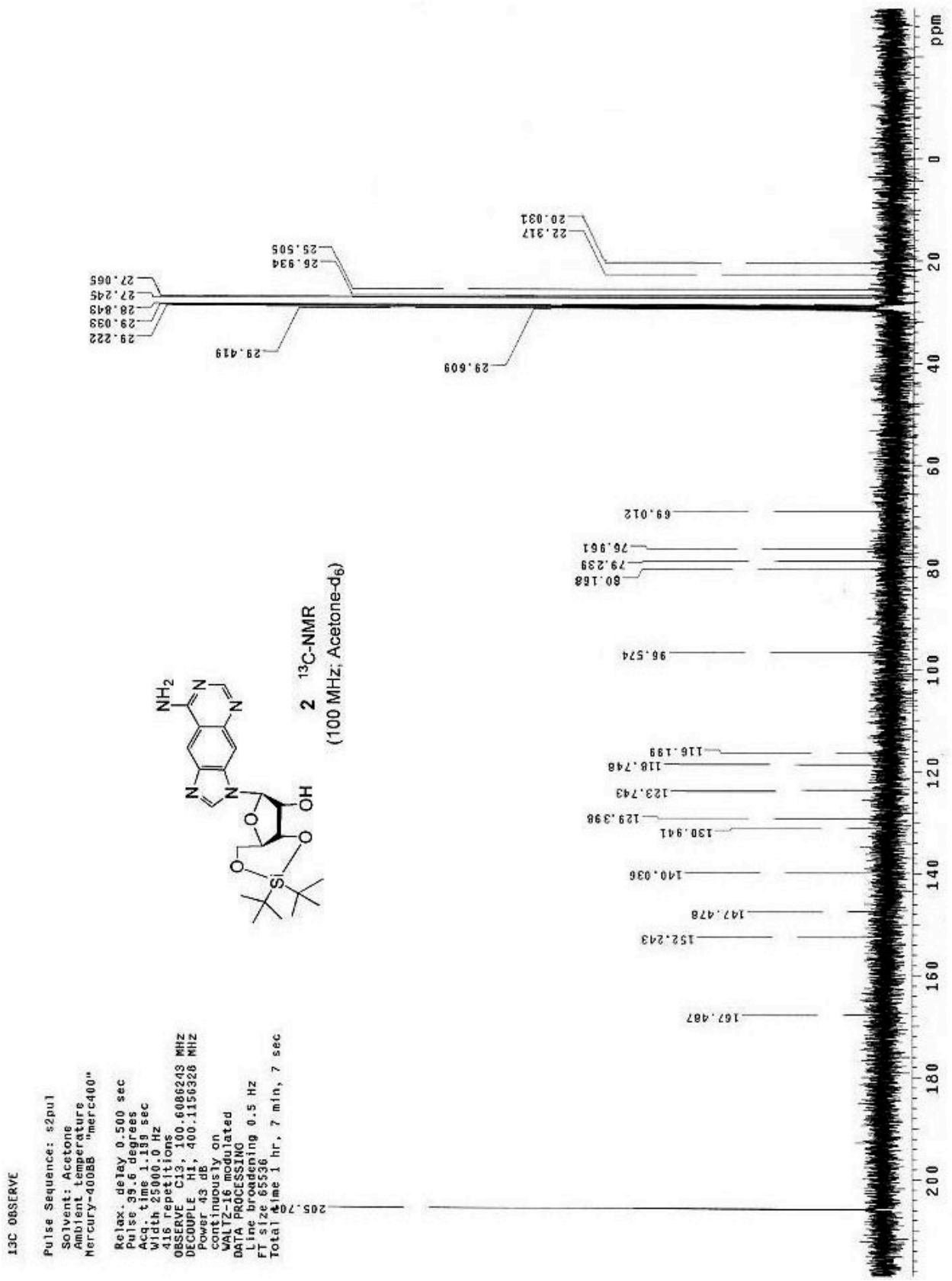
DATA PROCESSING
FT size 65536
Total time 1 min
NH2
N
N
N
N
O
O
OH
O
Si
2 1H-NMR
(400 MHz; Acetone-d6)
ppm
3.06
1.05
1.99
1.00
0.97.03
0.98.96
1.11
16.77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Width 25000.0 Hz
416 repetitions
OBSERVE C13, 100.6086243 MHz
DECOUPLE H1, 400.1156326 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 1 hr, 7 min, 7 sec
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(100 MHz; Acetone-d6)
NH2
N
N
N
N
O
OH
O
O
Si
205.707
167.487
152.243
147.478
140.036
130.941
129.398
123.743
118.748
116.199
96.574
80.168
79.239
76.961
69.012
29.609
29.419
29.222
29.039
28.843
27.245
27.065
25.934
25.505
22.317
20.031
ppm
200
180
160
140
120
100
80
60
40
20
0

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STANDARD 1H OBSERVE

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 Solvent: Acetone
 Ambient temperature
Mercury-400BB  "merc400"

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 Acq. time 0.127 sec
 Width     4032.3 Hz
 2D Width 4032.3 Hz
 Single scan
 128 increments
OBSERVE    H1, 400.1136141 MHz
DATA PROCESSING
 Sq. sine bell 0.063 sec
F1 DATA PROCESSING
 Sq. sine bell 0.016 sec
FT size 1024 x 1024
Total time 3 min, 32 sec
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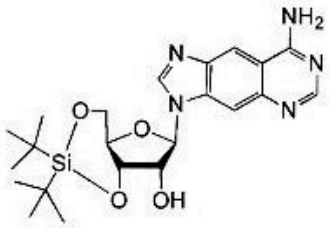
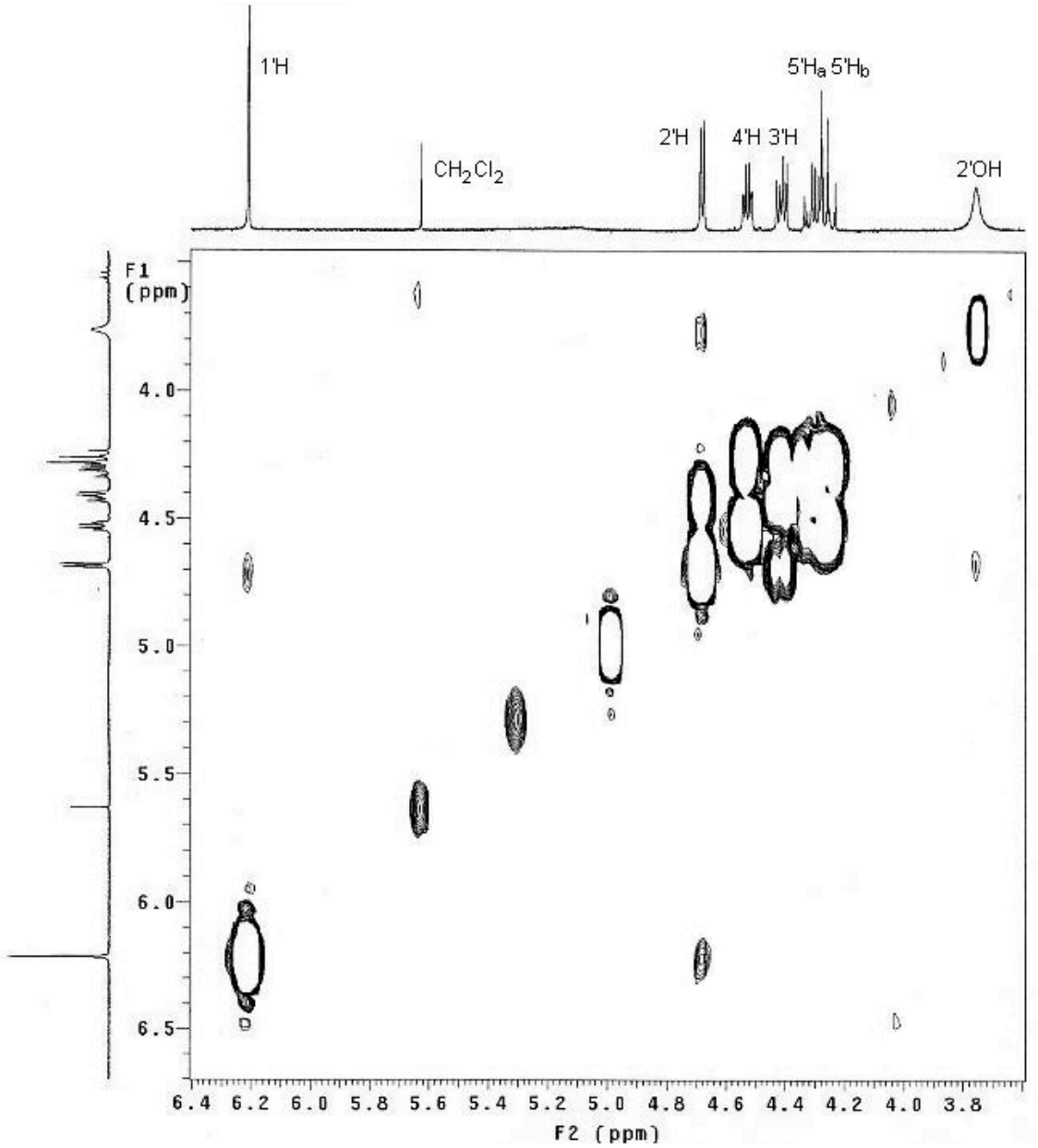
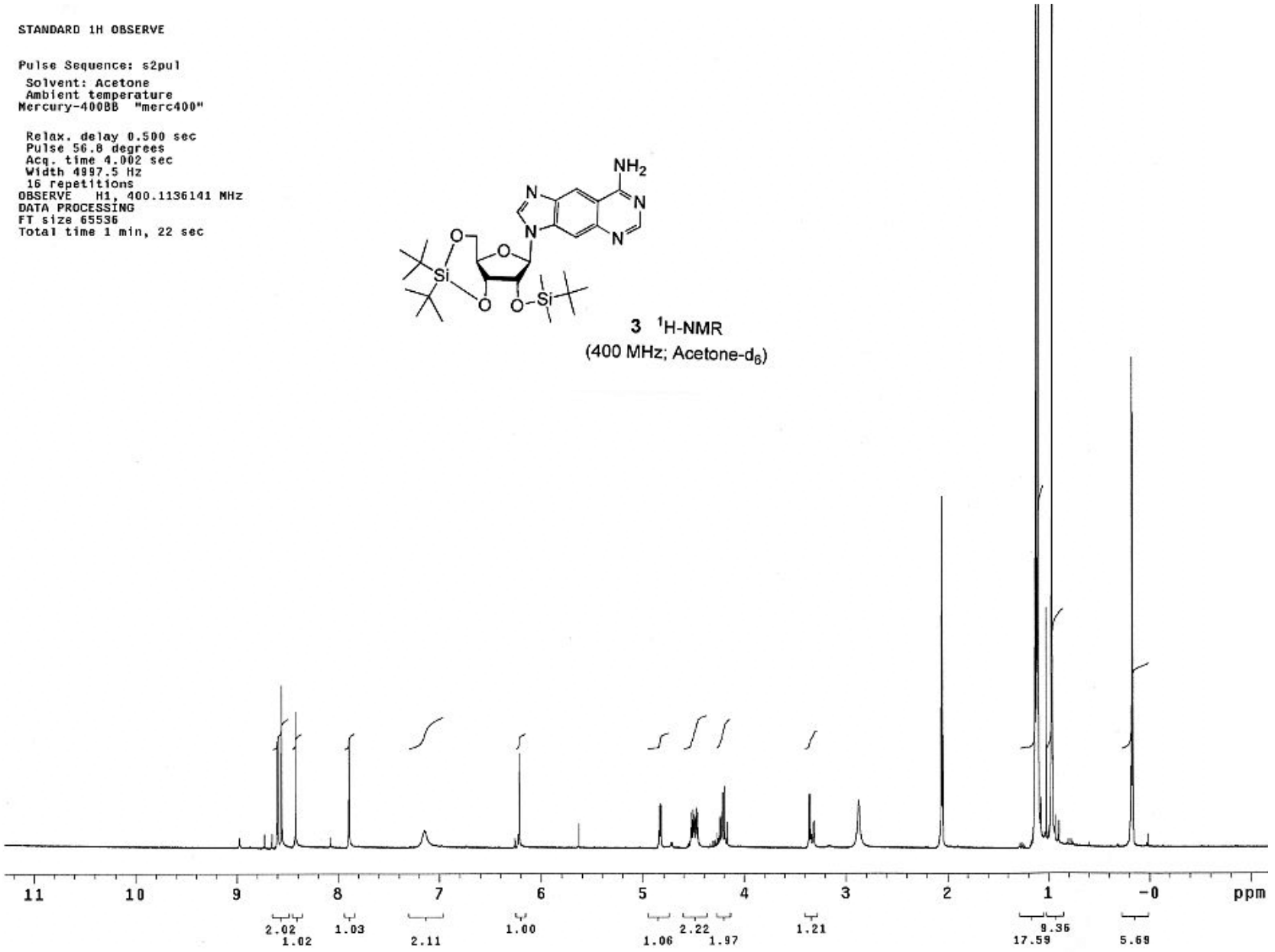


2 gCOSY
(400 MHz; acetone-d_6)

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Pulse 56.8 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
16 repetitions
OBSERVE H1, 400.1136141 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 22 sec
NH2
N
N
N
N
O
O
O
Si
O
O-Si
3 1H-NMR
(400 MHz; Acetone-d6)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
2.02
1.02
1.03
2.11
1.00
1.06
2.22
1.97
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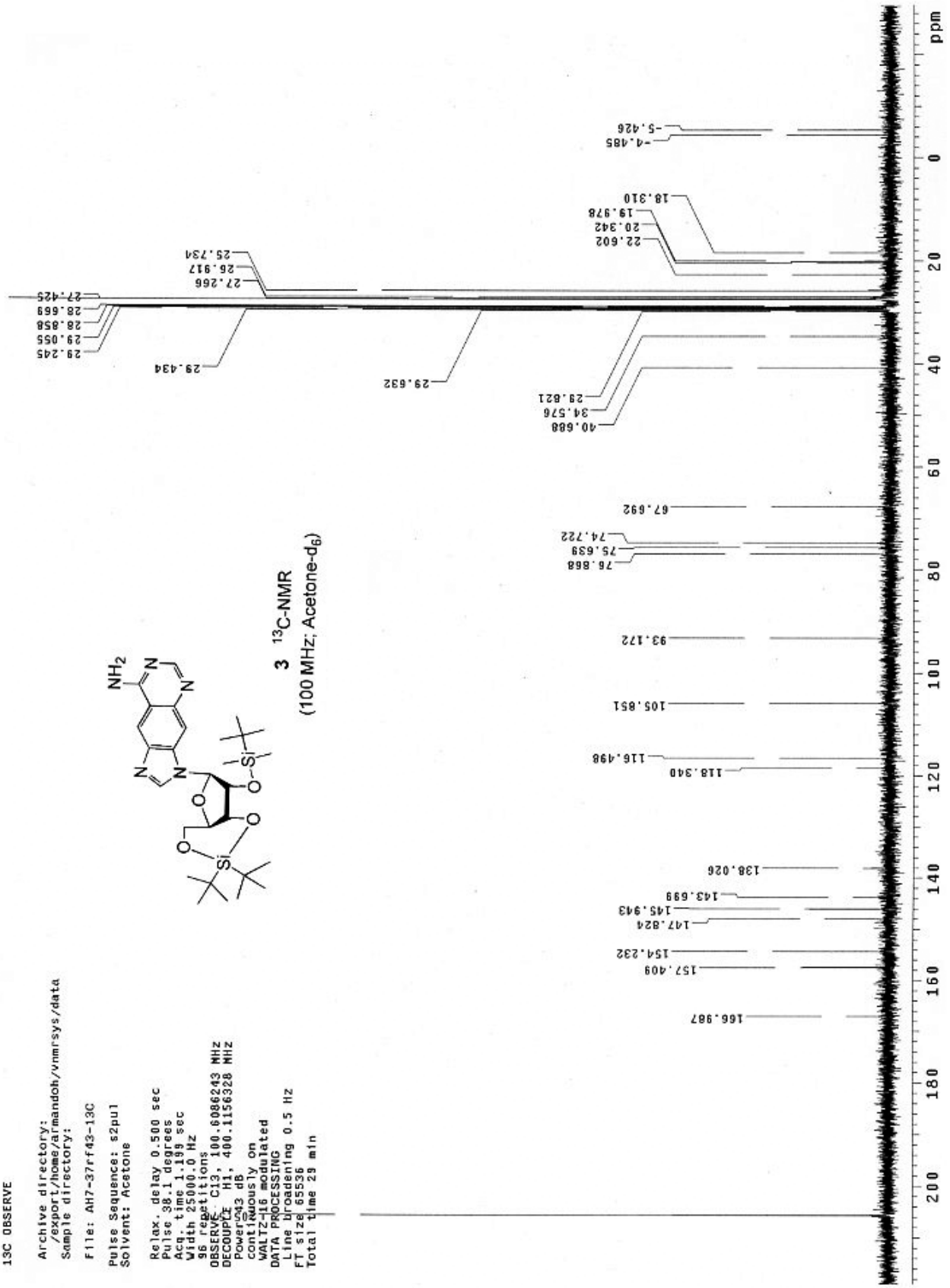
17.59
5.69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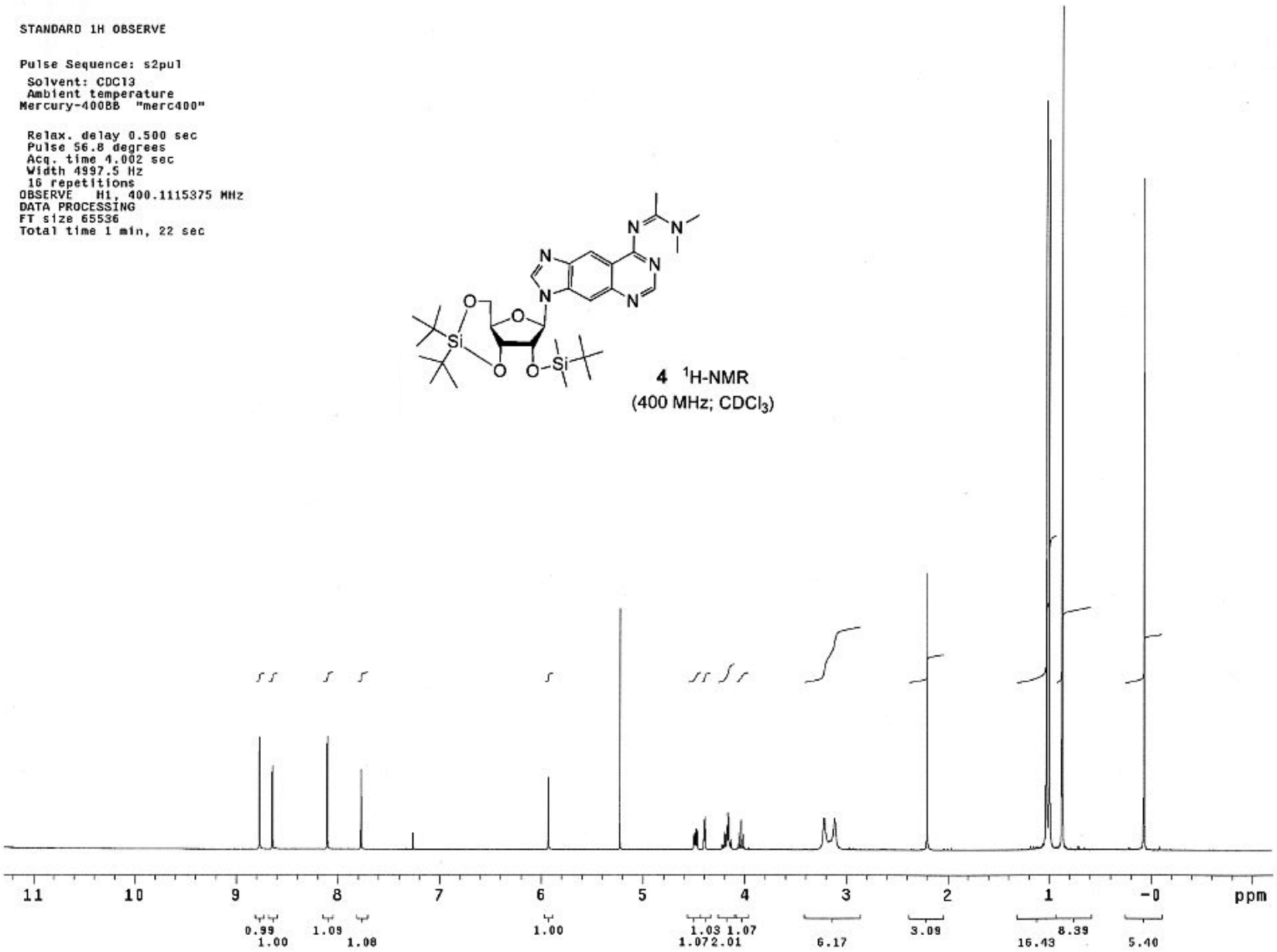
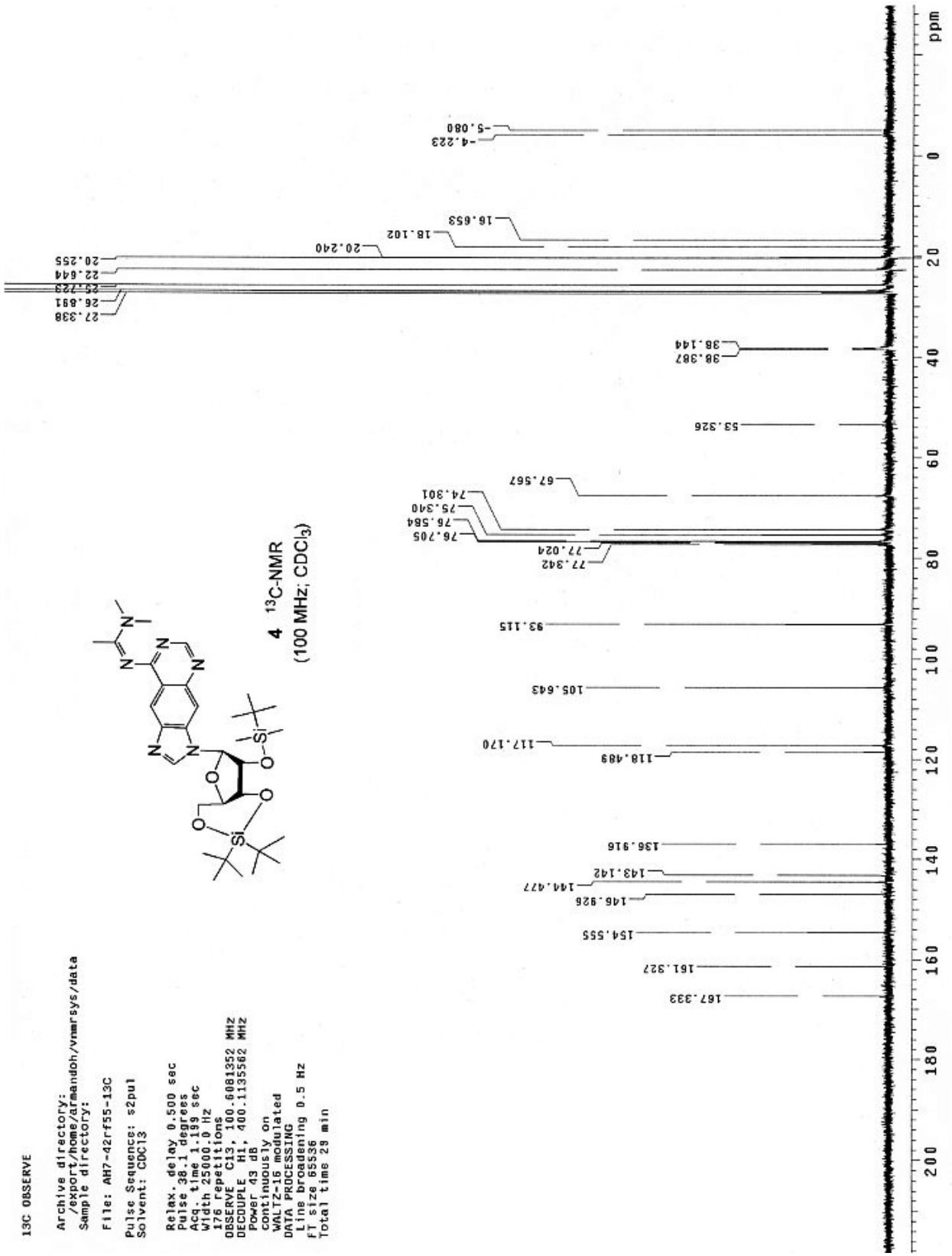
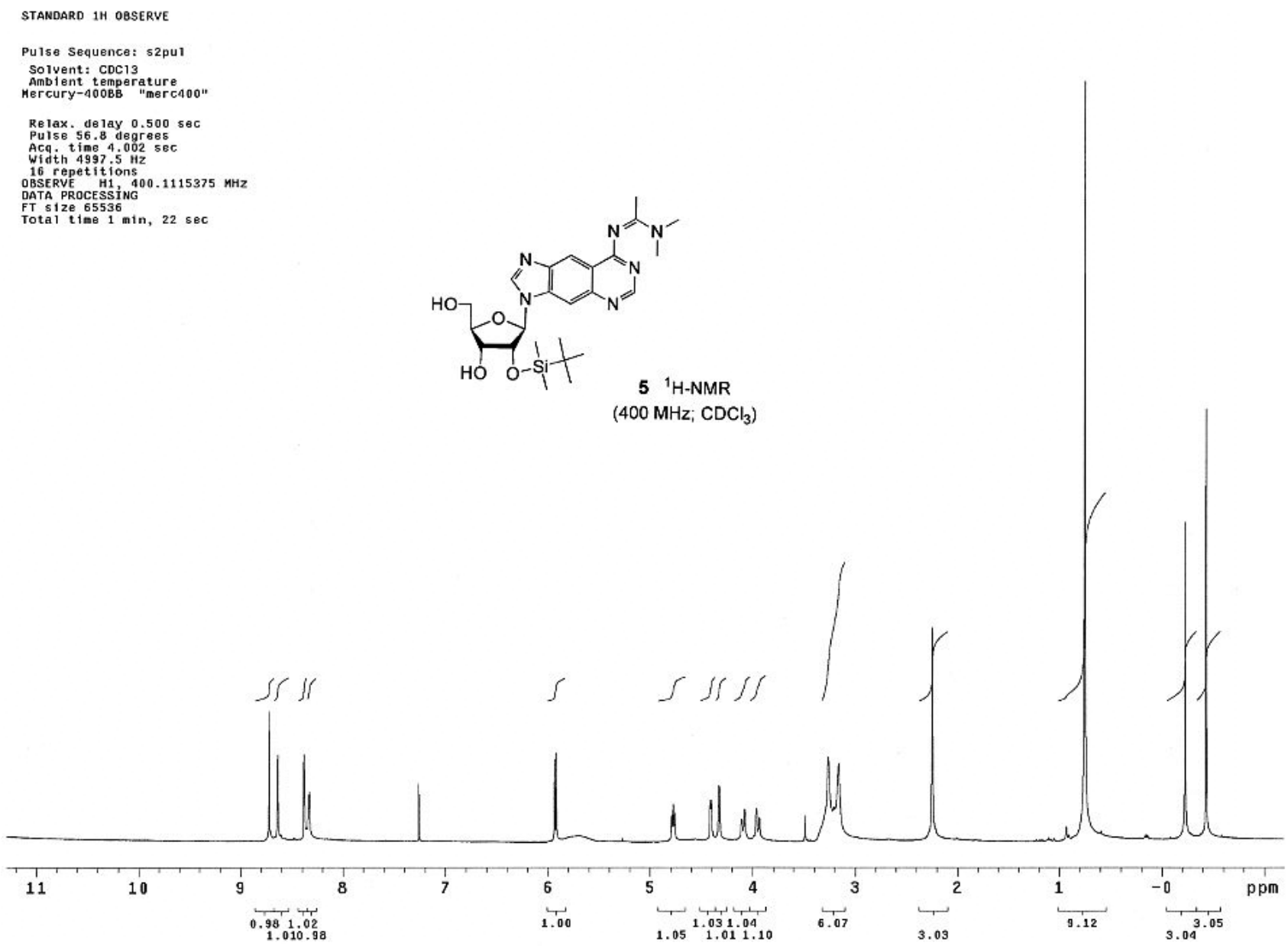
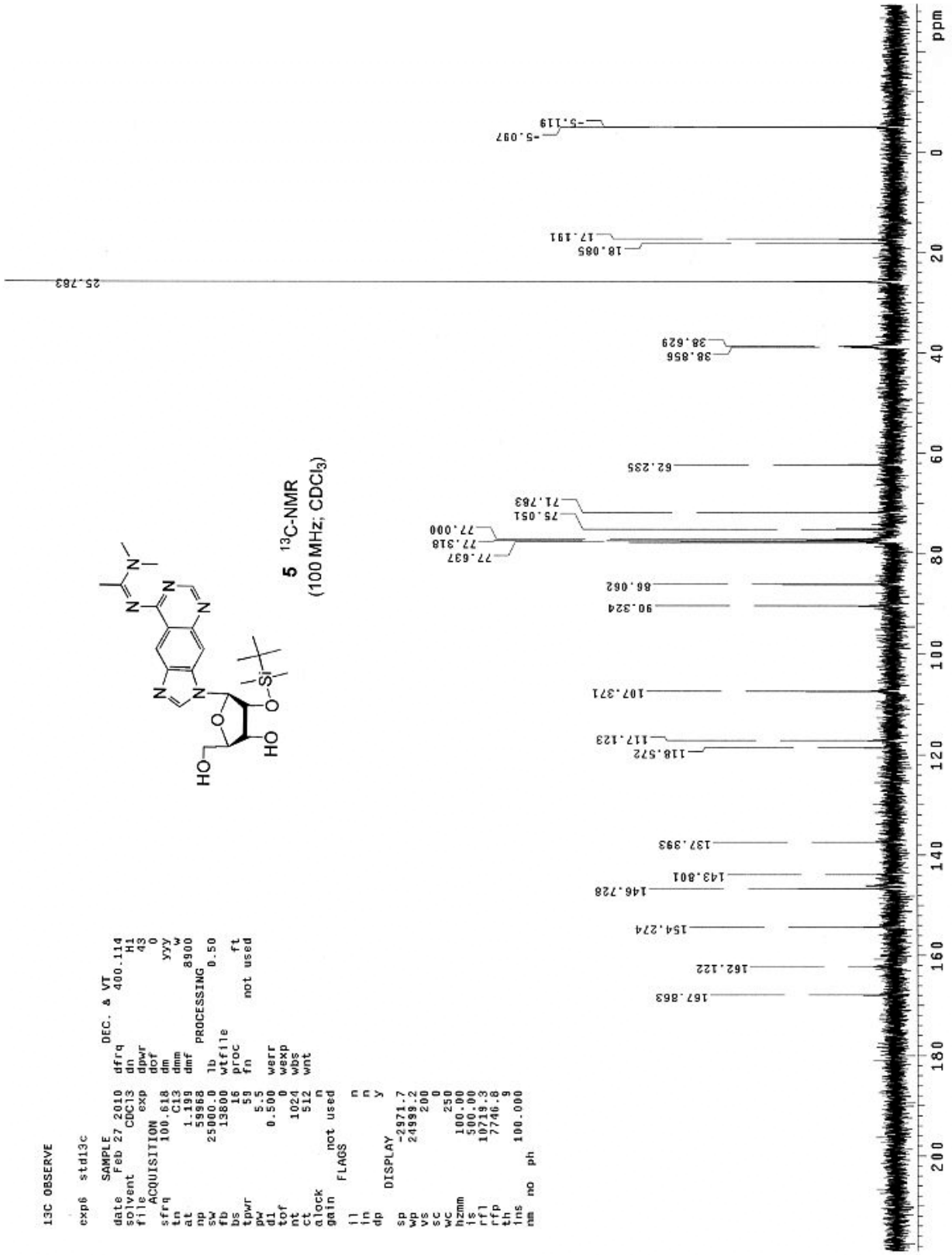
Solvent: Acetone
Relax. delay 0.500 sec
Pulse 38.1 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
96 repetitions
OBSERVE C13, 100.6086243 MHz
DECOUPLE H1, 400.1156328 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 29 min
3 13C-NMR
(100 MHz; Acetone-d6)
NH2
N
N
N
N
O
O
O
Si
Si
-4.485
-5.426
18.310
19.978
20.342
22.602
25.734
26.917
27.266
27.425
28.669
28.858
29.055
29.245
29.434
29.632
23.821
34.576
40.688
67.692
74.722
75.639
76.868
93.172
105.851
116.498
118.340
138.026
143.699
145.943
147.824
154.232
157.409
166.987
ppm
200
180
160
140
120
100
80
60
40
20
0

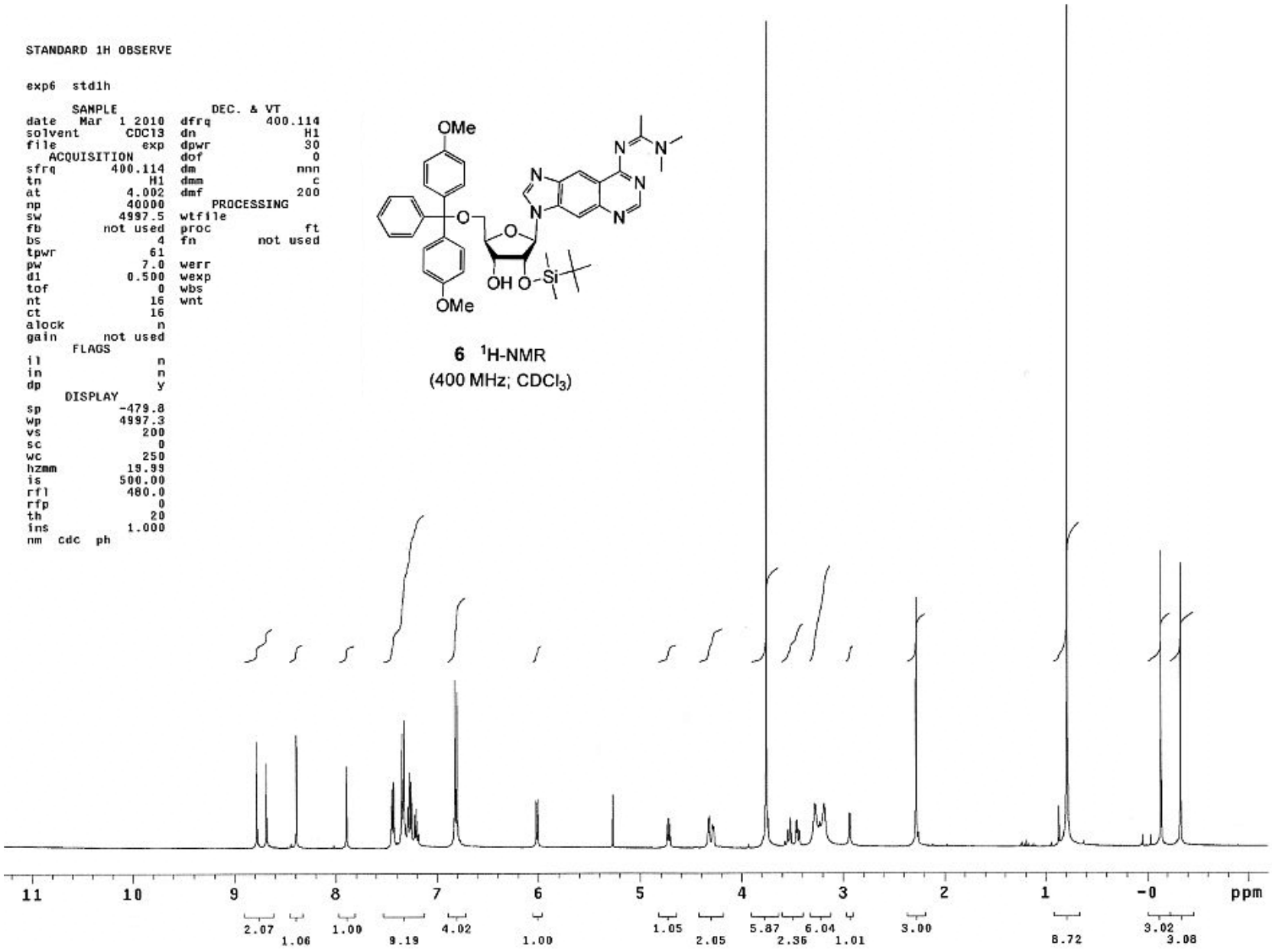

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 56.8 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
16 repetitions
OBSERVE H1, 400.1115375 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 22 sec
4 1H-NMR
(400 MHz; CDCl3)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.99
1.00
1.09
1.08
1.00
1.03
1.07
1.07
2.01
6.17
3.09
16.43
8.39
5.40

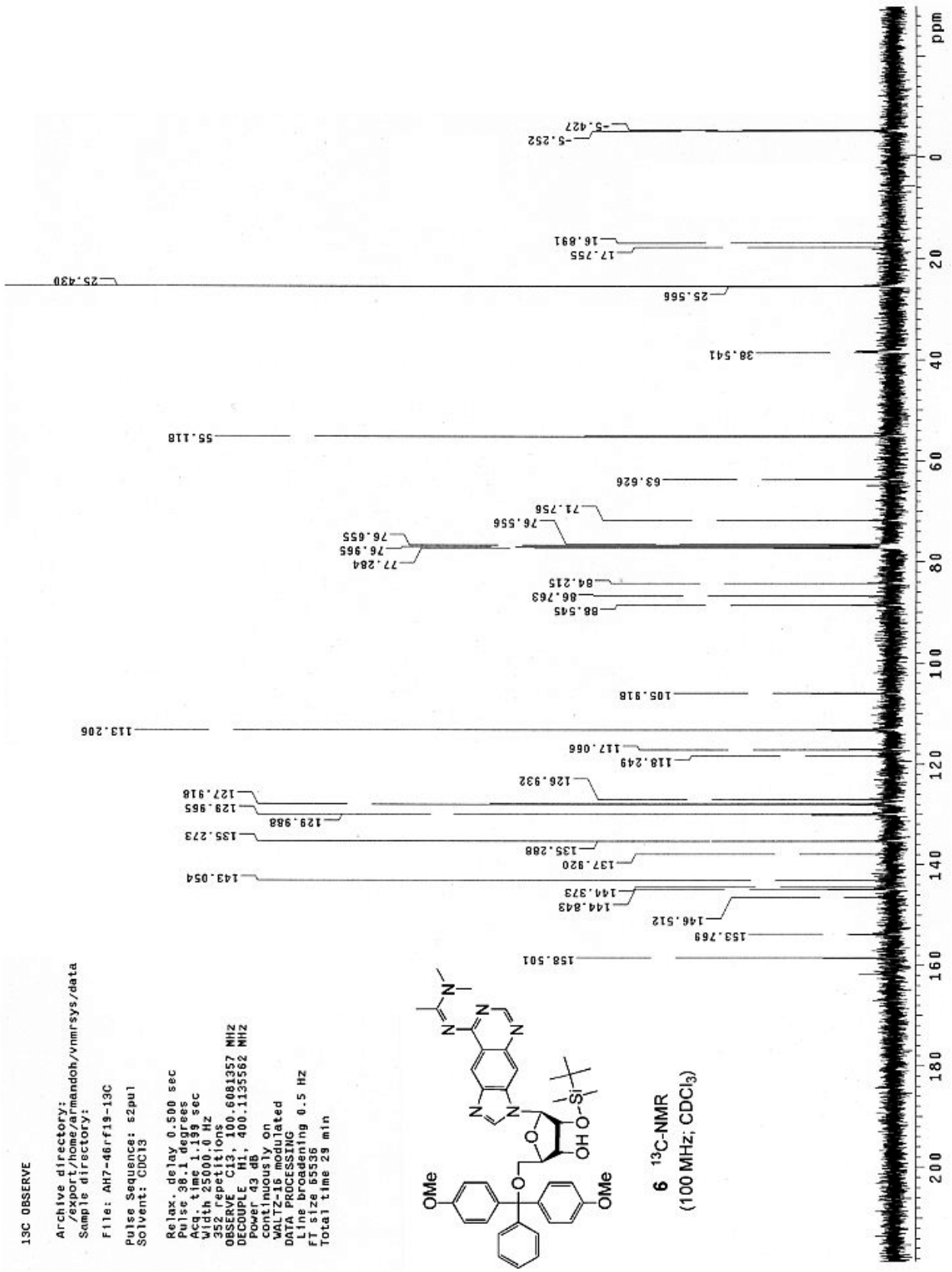

13C OBSERVE
Archive directory:
/export/home/armandoh/vnmrsys/data
Sample directory:
File: AH7-42rf55-13C
Pulse Sequence: s2pul
Solvent: CDCl3
Relax. delay 0.500 sec
Pulse 38.1 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
176 repetitions
OBSERVE C13, 100.6081352 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 29 min
4 13C-NMR
(100 MHz; CDCl3)
-5.080
-4.223
16.653
18.102
20.240
20.255
22.644
25.223
26.691
27.338
38.144
38.387
53.326
67.567
74.301
75.340
76.584
76.705
77.024
77.342
93.115
105.643
117.170
118.489
136.916
143.142
144.477
146.926
154.555
161.327
167.333
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 56.8 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
16 repetitions
OBSERVE H1, 400.1115375 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 22 sec
HO
HO
O-Si
5 1H-NMR
(400 MHz; CDCl3)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.98 1.02
1.01 0.98
1.00
1.05
1.03 1.04
1.01 1.10
6.07
3.03
9.12
3.05
3.04




```
13C OBSERVE
exp6  std13c
                    SAMPLE          DEC. & VT
date    Feb 27 2010 dfrq      400.114
solvent   CDCl3     dn           H1
file        exp     dpwr          43
          ACQUISITION dof          0
sfrq      100.618   dm           yyy
tn            C13   dmm            w
at          1.199   dmf         8900
np          59968   PROCESSING
sw       25000.0    lb         0.50
fb         13600    wtfile       ft
bs            16    proc
tpwr          59    fn        not used
pw           5.5
d1         0.500    werr
tof            0    wexp
nt          1024    wbs
ct           512    wnt
alock          n
gain     not used
          FLAGS
il             n
in             n
dp             y
          DISPLAY
sp       -2971.7
wp       24998.2
vs           200
sc             0
wc           250
hzmm      100.00
is        500.00
rfl      10719.3
rfp       7746.8
th             9
ins       100.000
nm  nO  ph
```

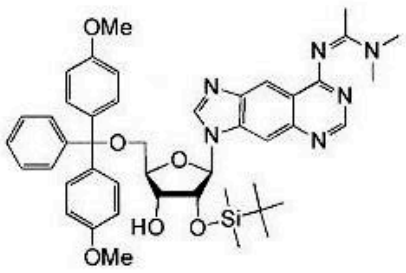

STANDARD 1H OBSERVE
exp6 stdlh
SAMPLE
date Mar 1 2010
solvent CDCl3
file exp
ACQUISITION
sfrq 400.114
tn H1
at 4.002
np 40000
sw 4997.5
fb not used
bs 4
tpwr 61
pw 7.0
d1 0.500
tof 0
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -479.8
wp 4997.3
vs 200
sc 0
wc 250
hzmm 19.99
is 500.00
rfl 480.0
rfp 0
th 20
ins 1.000
nm cdc ph
DEC. & VT
dfrq 400.114
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp
wbs
wnt
OMe
OMe
OH
O-Si
6 1H-NMR
(400 MHz; CDCl3)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
2.07
1.06
1.00
9.19
4.02
1.00
1.05
2.05
5.87
2.36
6.04
1.01
3.00
8.72
3.02
3.08


13C OBSERVE
Archive directory:
/export/home/armandoh/vnmrsys/data
Sample directory:
File: AH7-46rf19-13C
Pulse Sequence: s2pul
Solvent: CDCl3
Relax. delay 0.500 sec
Pulse 38.1 degrees
Acq. time 1.139 sec
Width 25000.0 Hz
352 repetitions
OBSERVE C13, 100.6081357 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 29 min
158.501
153.769
146.512
144.043
144.373
143.054
137.820
135.288
135.273
129.988
129.965
127.918
126.932
118.249
117.066
113.206
105.818
88.545
86.763
84.215
77.284
76.965
76.655
76.556
71.756
63.626
55.118
38.541
25.566
25.430
17.755
16.891
-5.252
-5.427
ppm
OMe
OMe
OH
O
Si
6 13C-NMR
(100 MHz; CDCl3)

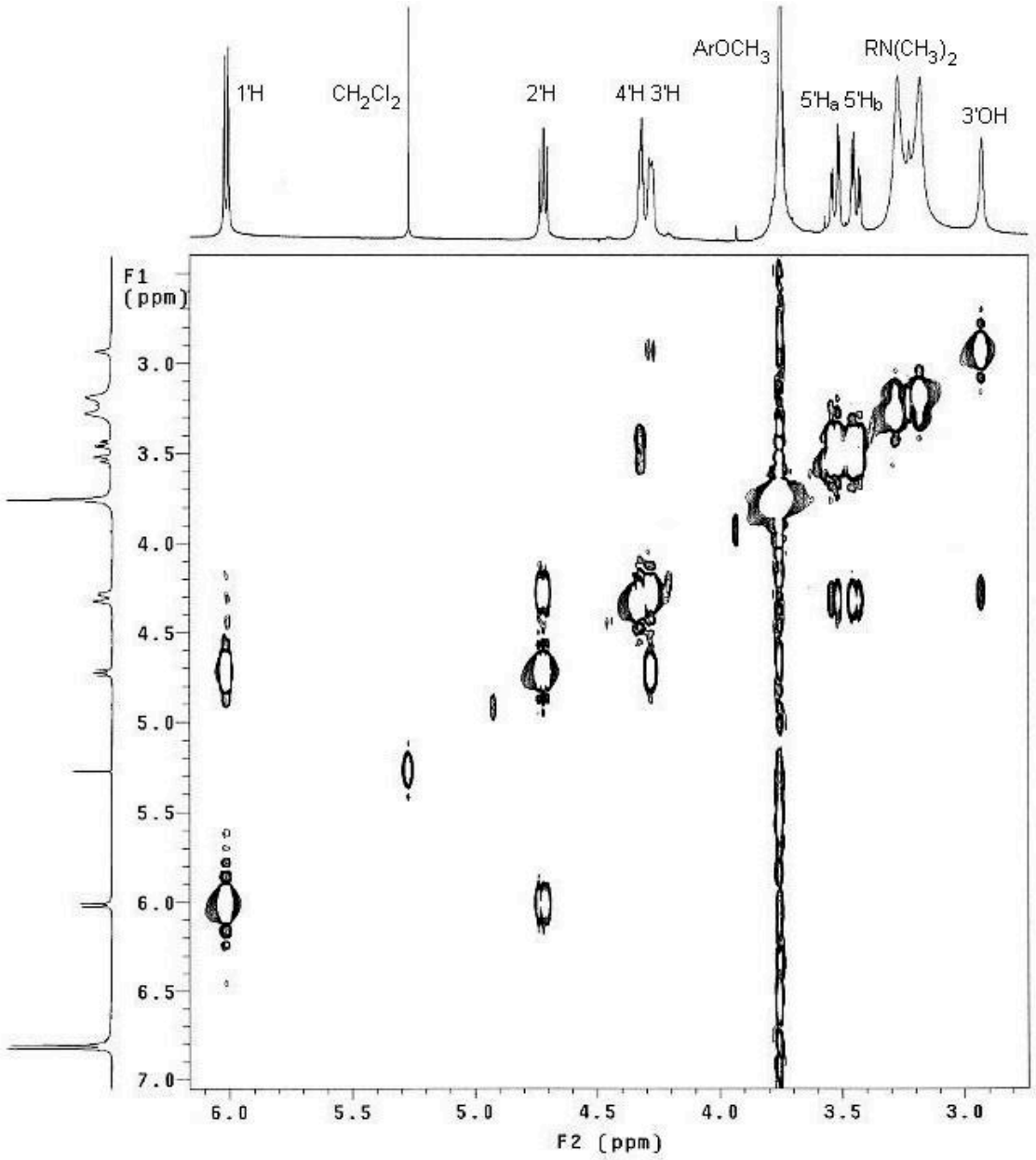
STANDARD 1H OBSERVE

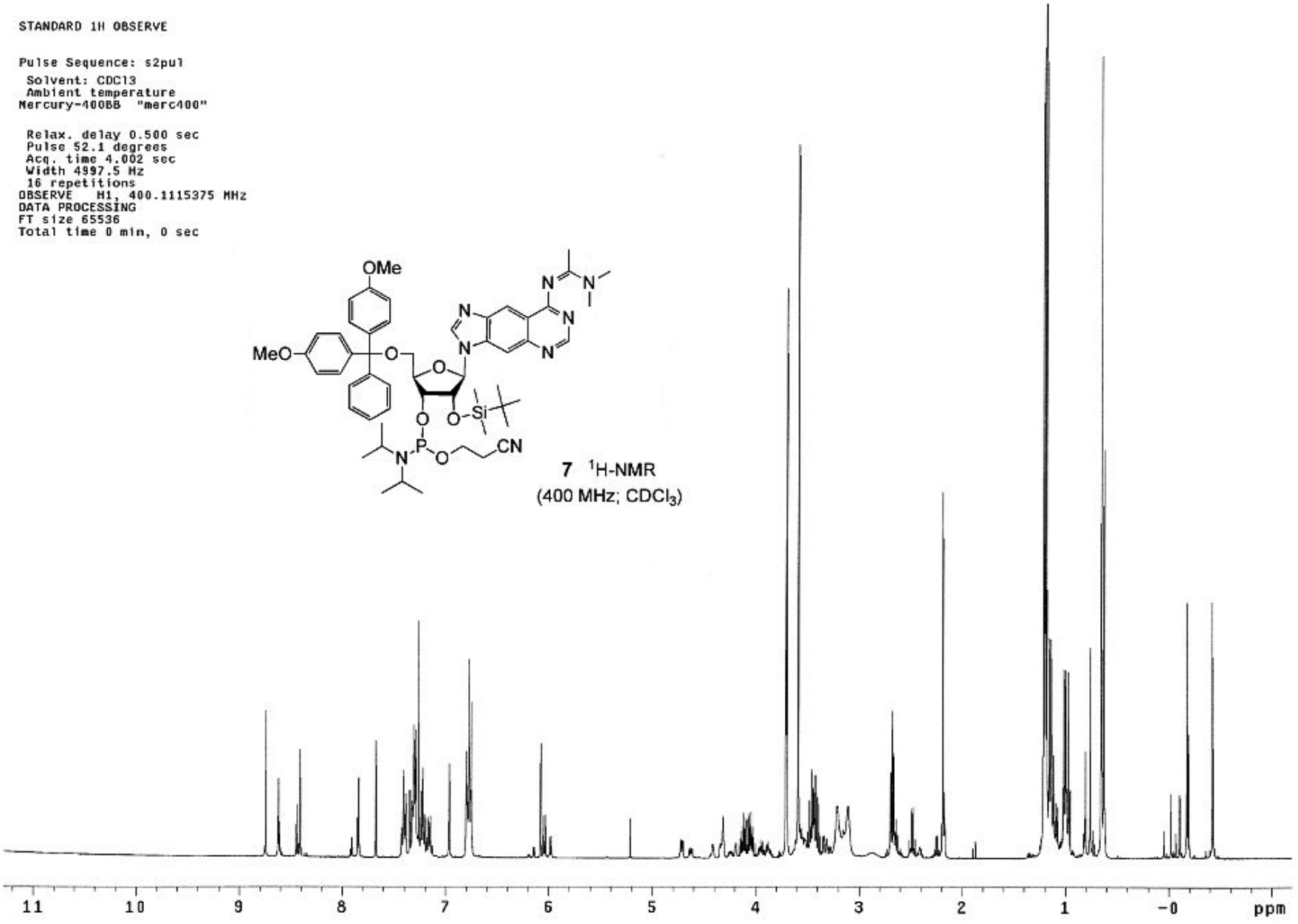
Pulse Sequence: gCOSY
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"

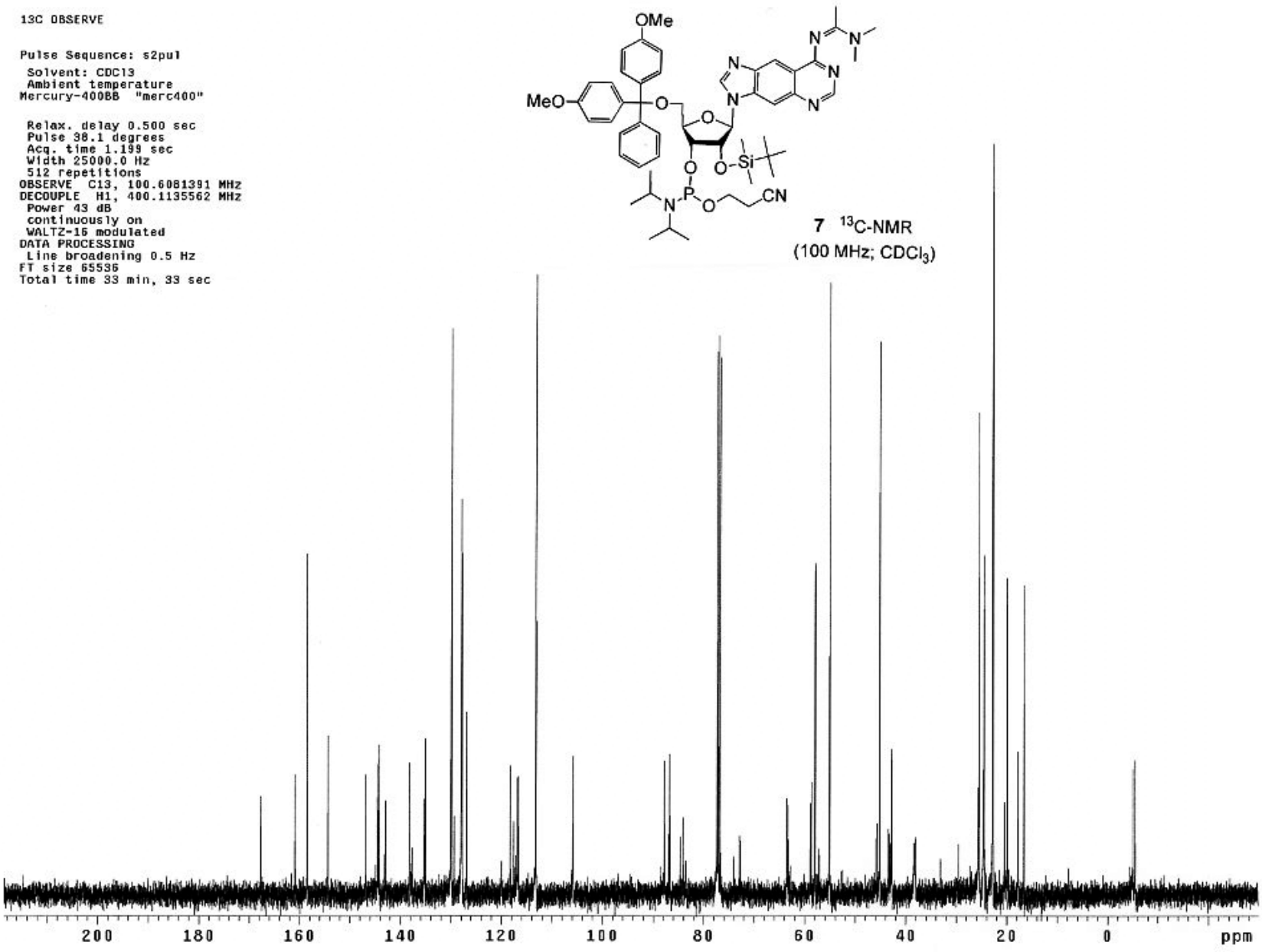
Relax. delay 1.000 sec
Acq. time 0.250 sec
Width 4098.4 Hz
2D Width 4098.4 Hz
Single scan
128 increments
OBSERVE H1, 400.1115375 MHz
DATA PROCESSING
Sine bell 0.125 sec
F1 DATA PROCESSING
Sine bell 0.016 sec
FT size 2048 x 2048
Total time 3 min, 58 sec



6 gCOSY
(400 MHz; $CDCl_3$)



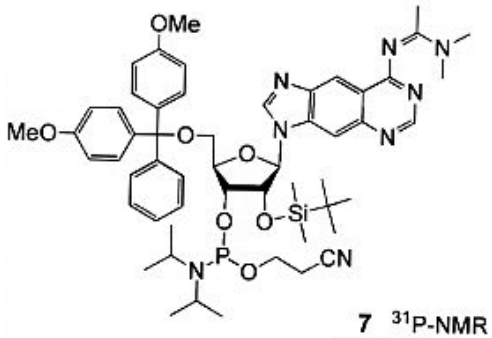

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 52.1 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
16 repetitions
OBSERVE H1, 400.1115375 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
OMe
MeO
N
O
Si
CN
7 ^{1}H-NMR
(400 MHz; $CDCl_3$)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm


13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 38.1 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
512 repetitions
OBSERVE C13, 100.6081391 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 33 min, 33 sec
OMe
MeO
O
N
N
N
N
O
O
O-Si
N
P
O
CN
7 13C-NMR
(100 MHz; CDCl3)
200
180
160
140
120
100
80
60
40
20
0
ppm

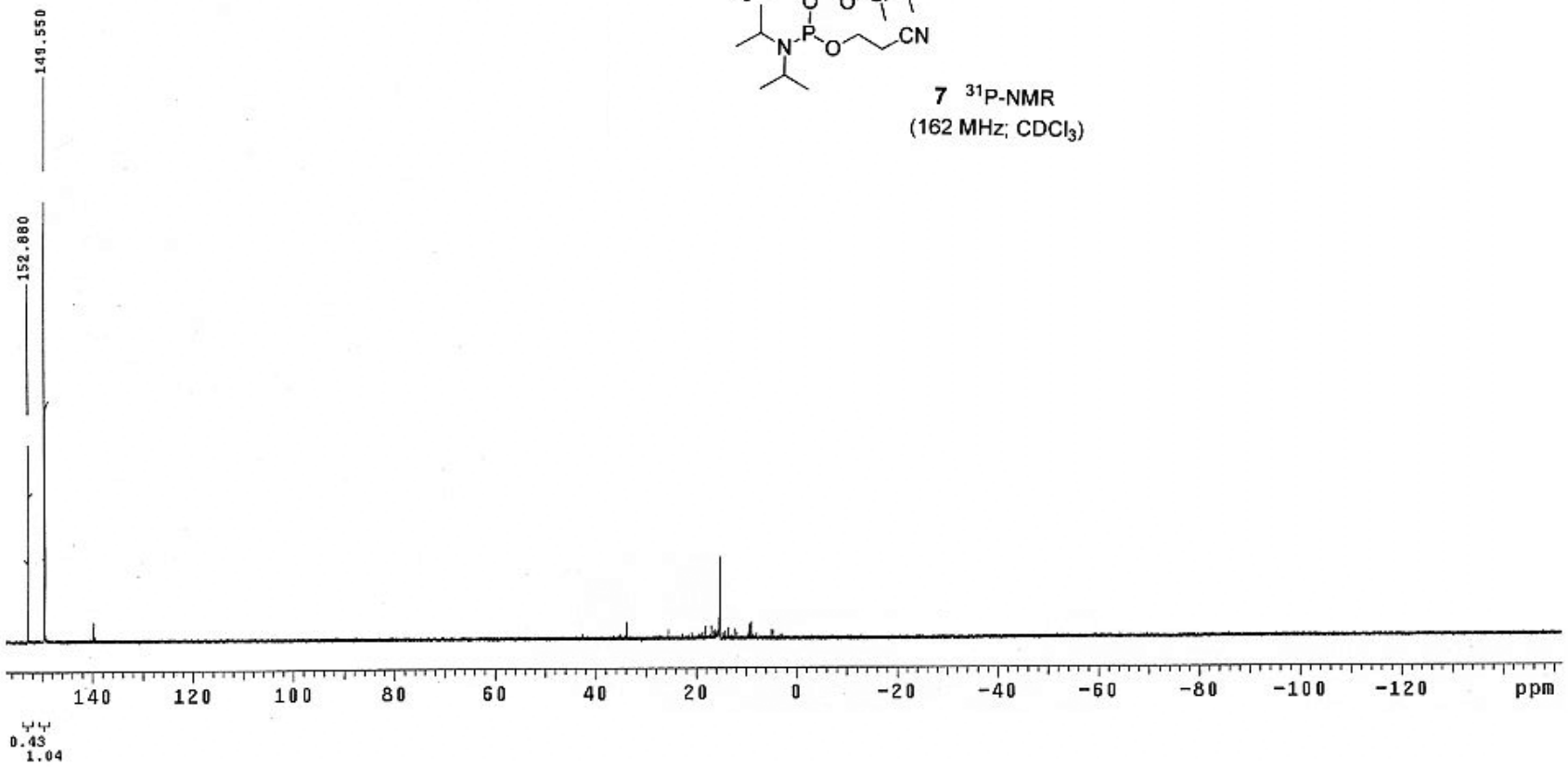
PHOSPHORUS OBSERVE
STANDARD PARAMETERS
PHOSPHATE REGION

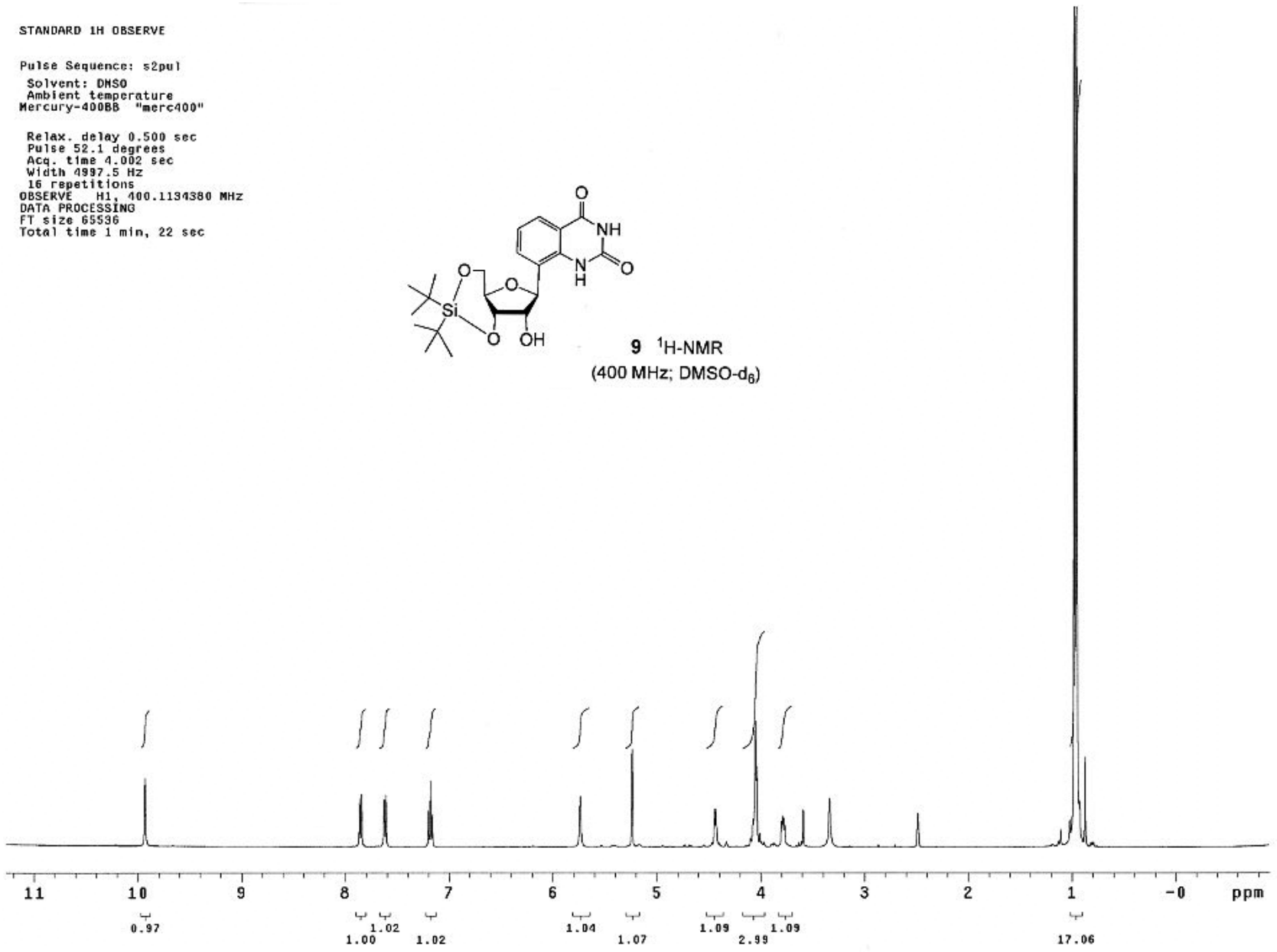
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"

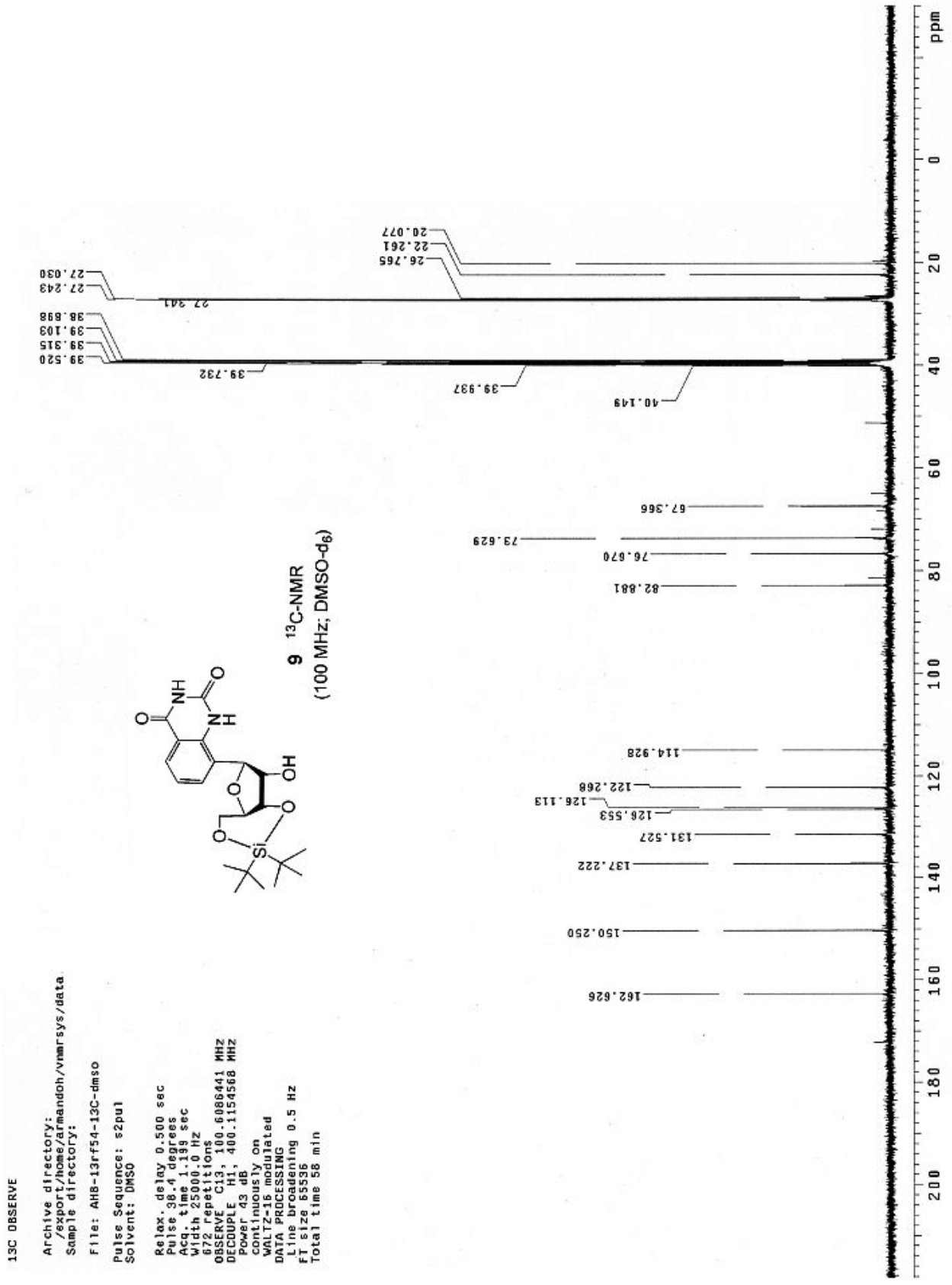
Relax. delay 2.000 sec
Pulse 47.4 degrees
Acq. time 0.800 sec
Width 50000.0 Hz
64 repetitions
OBSERVE P31, 161.9679384 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 8 min, 40 sec



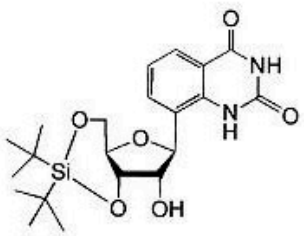
7 ^{31}P-NMR
(162 MHz; $CDCl_3$)



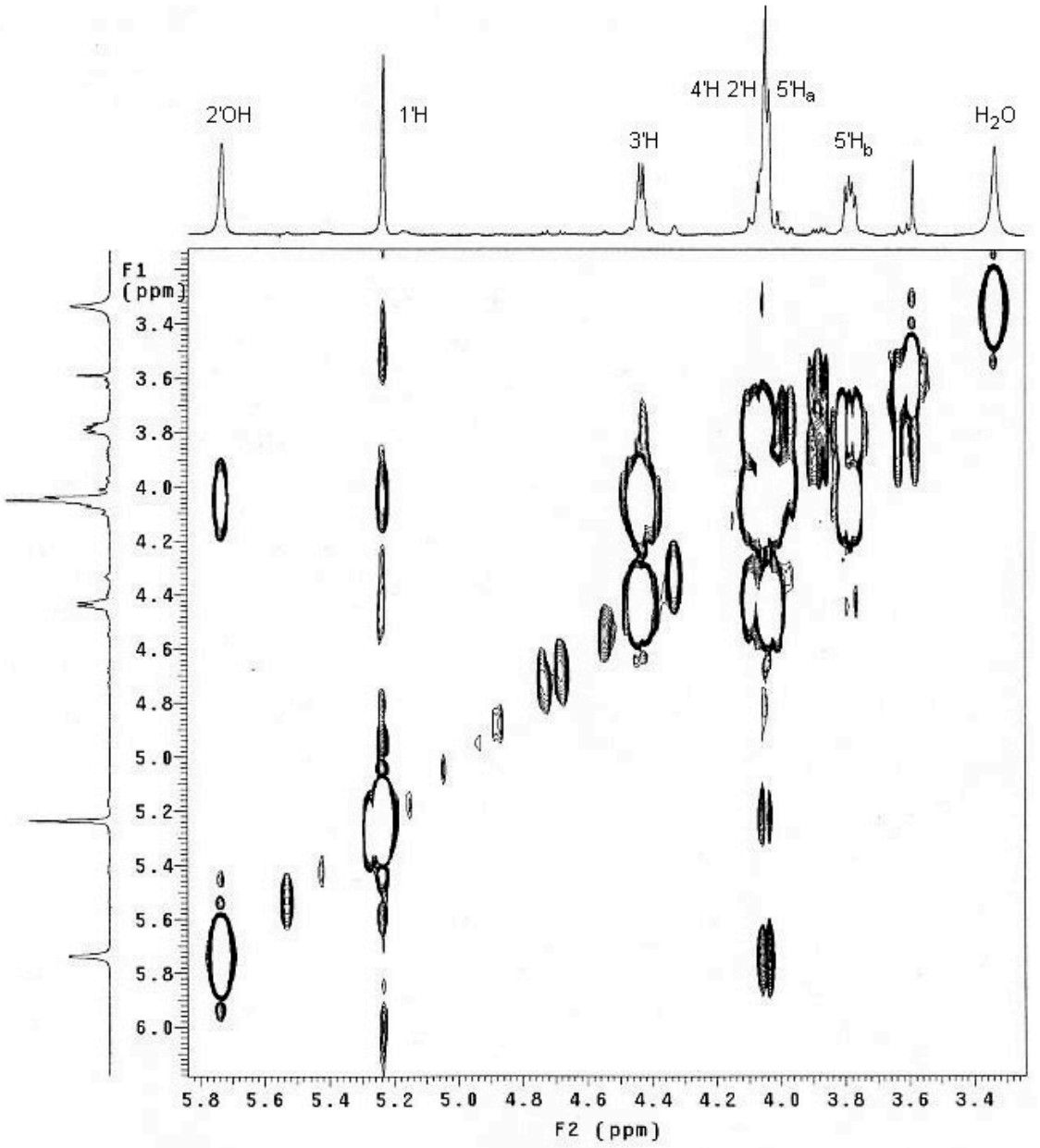

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: DMSO
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 52.1 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
16 repetitions
OBSERVE H1, 400.1134380 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 22 sec
O
NH
N
H
O
O
O
Si
O
OH
9 1H-NMR
(400 MHz; DMSO-d6)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.97
1.00
1.02
1.02
1.04
1.07
1.09
2.99
1.09
17.06

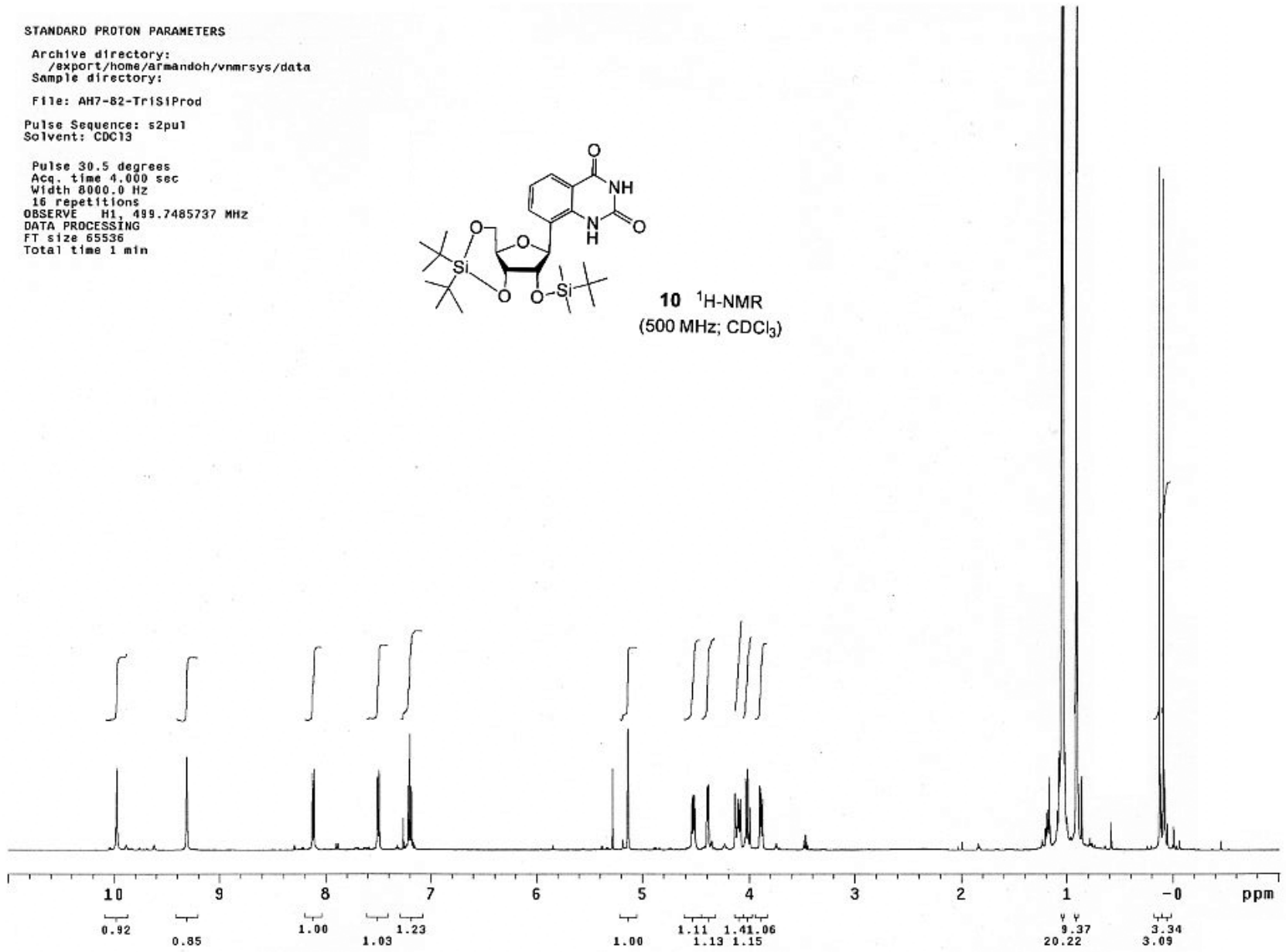

13C OBSERVE
Archive directory:
/export/home/armandoh/vnmrsys/data
Sample directory:
File: AHB-13rf54-13C-dmso
Pulse Sequence: s2pul
Solvent: DMSO
Relax. delay 0.500 sec
Pulse 38.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
672 repetitions
OBSERVE C13, 100.6086441 MHz
DECOUPLE H1, 400.1154568 MHz
Power 43 dB
continuously On
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 58 min
9 13C-NMR
(100 MHz; DMSO-d6)
NH
O
N
H
O
OH
O
O
O
Si
162.626
150.250
137.222
131.527
126.553
126.113
122.268
114.928
82.881
76.670
73.629
67.366
40.149
39.937
39.732
39.520
39.315
39.103
38.898
27.341
27.243
27.030
26.765
22.261
20.077
ppm
0
20
40
60
80
100
120
140
160
180
200

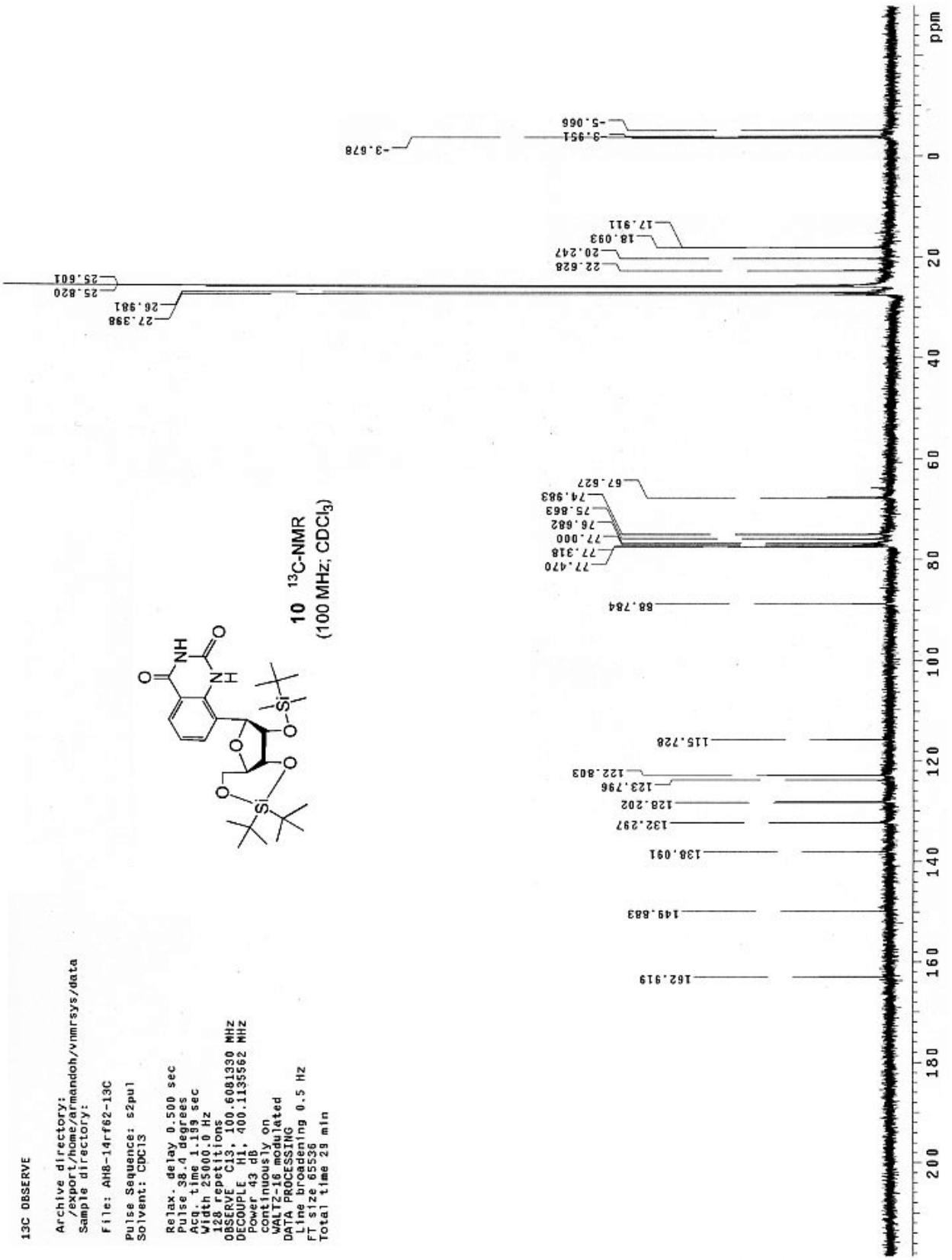
```
STANDARD 1H OBSERVE

 Archive directory:
   /export/home/armandoh/vnmrsys/data
 Sample directory:

 File: AH8-13rf54-gCOSY-dmso

Pulse Sequence: gCOSY
Solvent: DMSO

 Relax. delay 1.000 sec
 Acq. time 0.240 sec
 Width     4273.5 Hz
 2D Width 4273.5 Hz
 Single scan
 128 increments
OBSERVE    H1, 400.1134380 MHz
DATA PROCESSING
 Sq. sine bell 0.120 sec
F1 DATA PROCESSING
 Sq. sine bell 0.015 sec
FT size 2048 x 2048
Total time 3 min
```

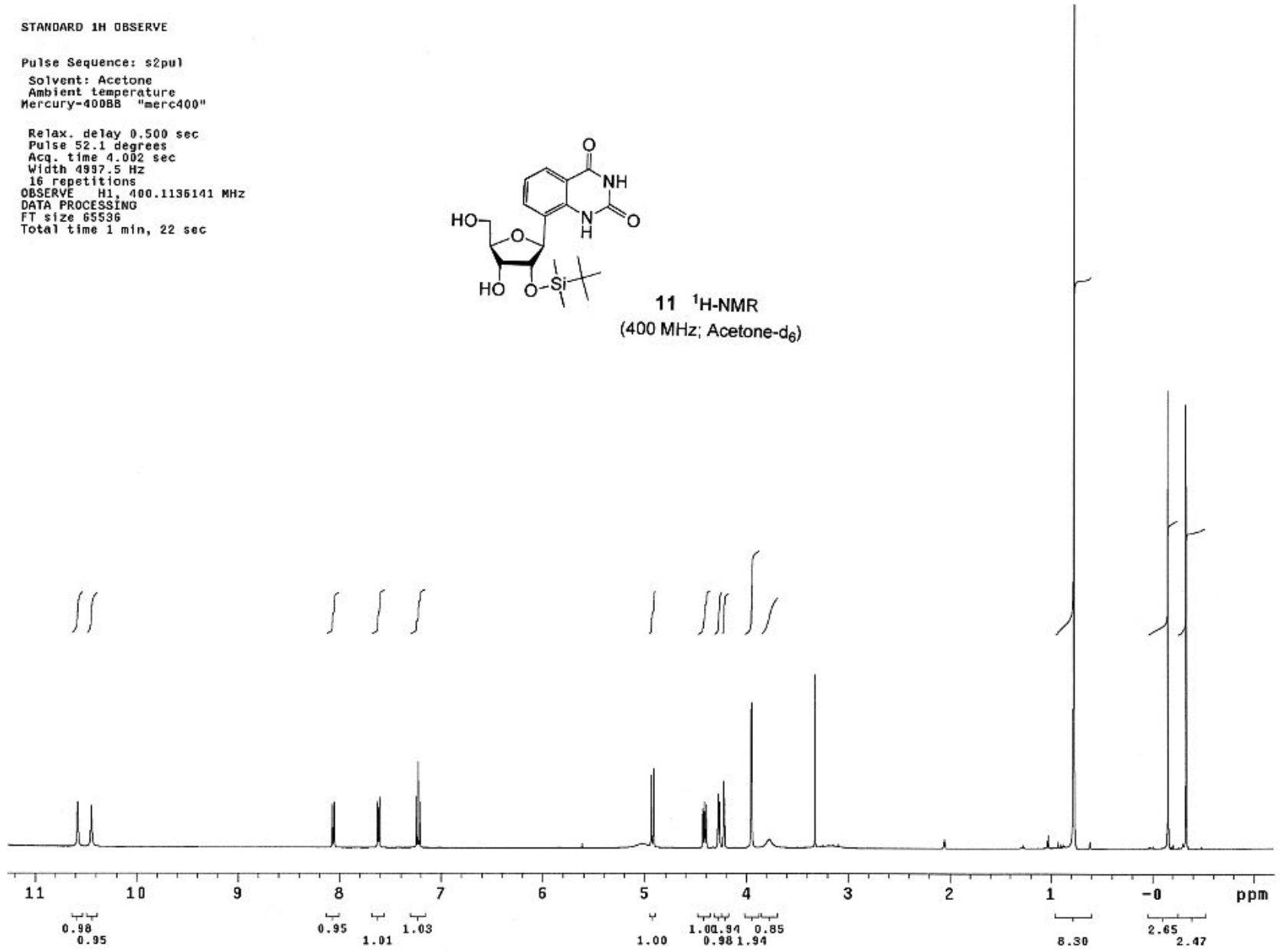


9 gCOSY
(400 MHz; DMSO-d_6)




STANDARD PROTON PARAMETERS
Archive directory:
/export/home/armandoh/vnmrsys/data
Sample directory:
File: AH7-82-TriSiProd
Pulse Sequence: s2pul
Solvent: CDCl3
Pulse 30.5 degrees
Acq. time 4.000 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7485737 MHz
DATA PROCESSING
FT size 65536
Total time 1 min
10 ^{1}H-NMR
(500 MHz; $CDCl_3$)
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.92
0.85
1.00
1.03
1.23
1.00
1.11
1.13
1.41
1.15
1.06
9.37
20.22
3.34
3.09


13C OBSERVE
Archive directory:
/export/home/armandoh/vnmrsys/data
Sample directory:
File: AH8-14rf62-13C
Pulse Sequence: s2pul
Solvent: CDCl3
Relax. delay 0.500 sec
Pulse 38.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
128 repetitions
OBSERVE C13, 100.6081330 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 29 min
10 13C-NMR
(100 MHz; CDCl3)
NH
O
N
H
O
Si
O
O
O
O
Si
-3.678
-5.066
-3.951
17.911
18.093
20.247
22.628
25.601
25.820
26.981
27.398
67.627
74.983
75.863
76.682
77.000
77.318
77.470
88.764
115.728
122.803
123.796
128.202
132.297
138.091
149.683
162.919
ppm
200
180
160
140
120
100
80
60
40
20
0


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 52.1 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
16 repetitions
OBSERVE H1, 400.1136141 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 22 sec
HO
O
NH
N
H
O
HO
O
Si
11 1H-NMR
(400 MHz; Acetone-d6)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.98
0.95
0.95
1.01
1.03
1.00
1.00
0.98
1.94
1.94
0.85
8.30
2.65
2.47

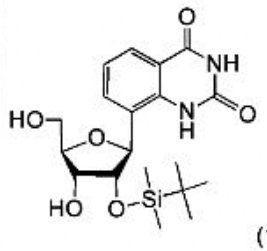
13C OBSERVE

Archive directory:
/export/home/armandoh/vnmrsys/data
Sample directory:

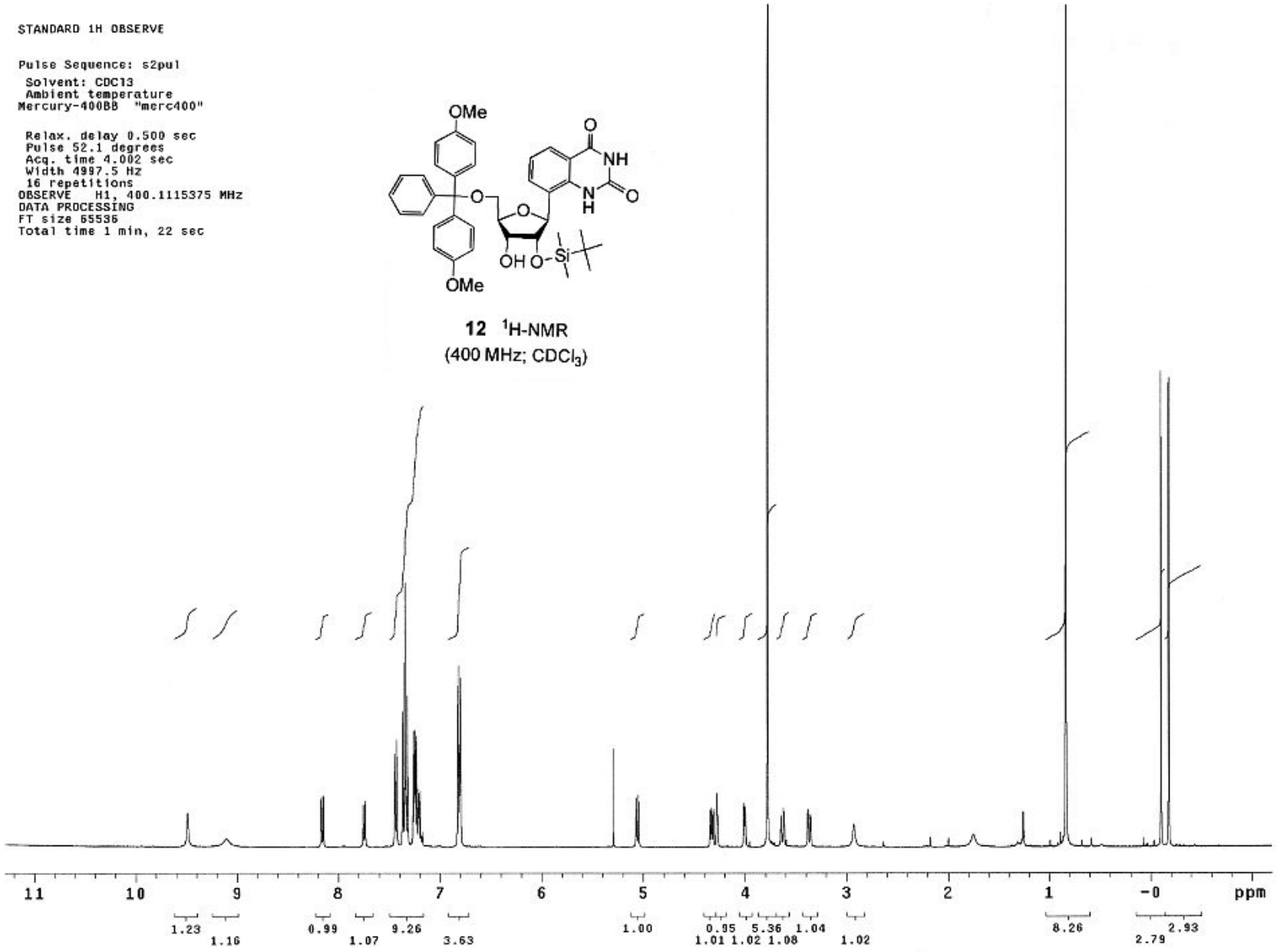
File: AH8-15rf38-13C

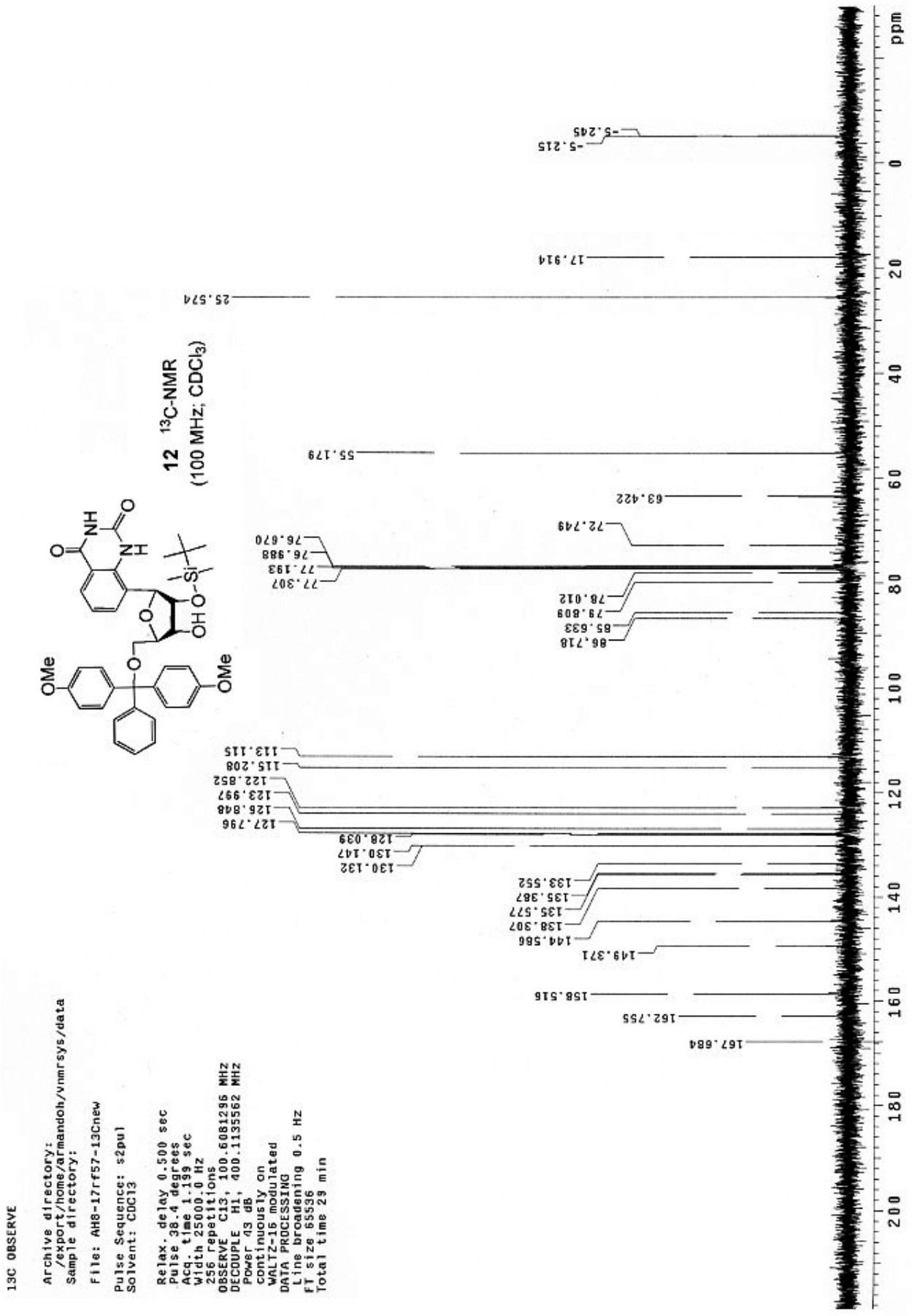
Pulse Sequence: s2pul
Solvent: Acetone

Relax. delay 0.500 sec
Pulse 38.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
288 repetitions
OBSERVE C13, 100.6085692 MHz
DECOUPLE H1, 400.1156328 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 29 min

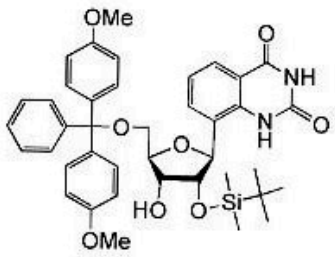


11 ^{13}C-NMR
(100 MHz; Acetone-d_6)

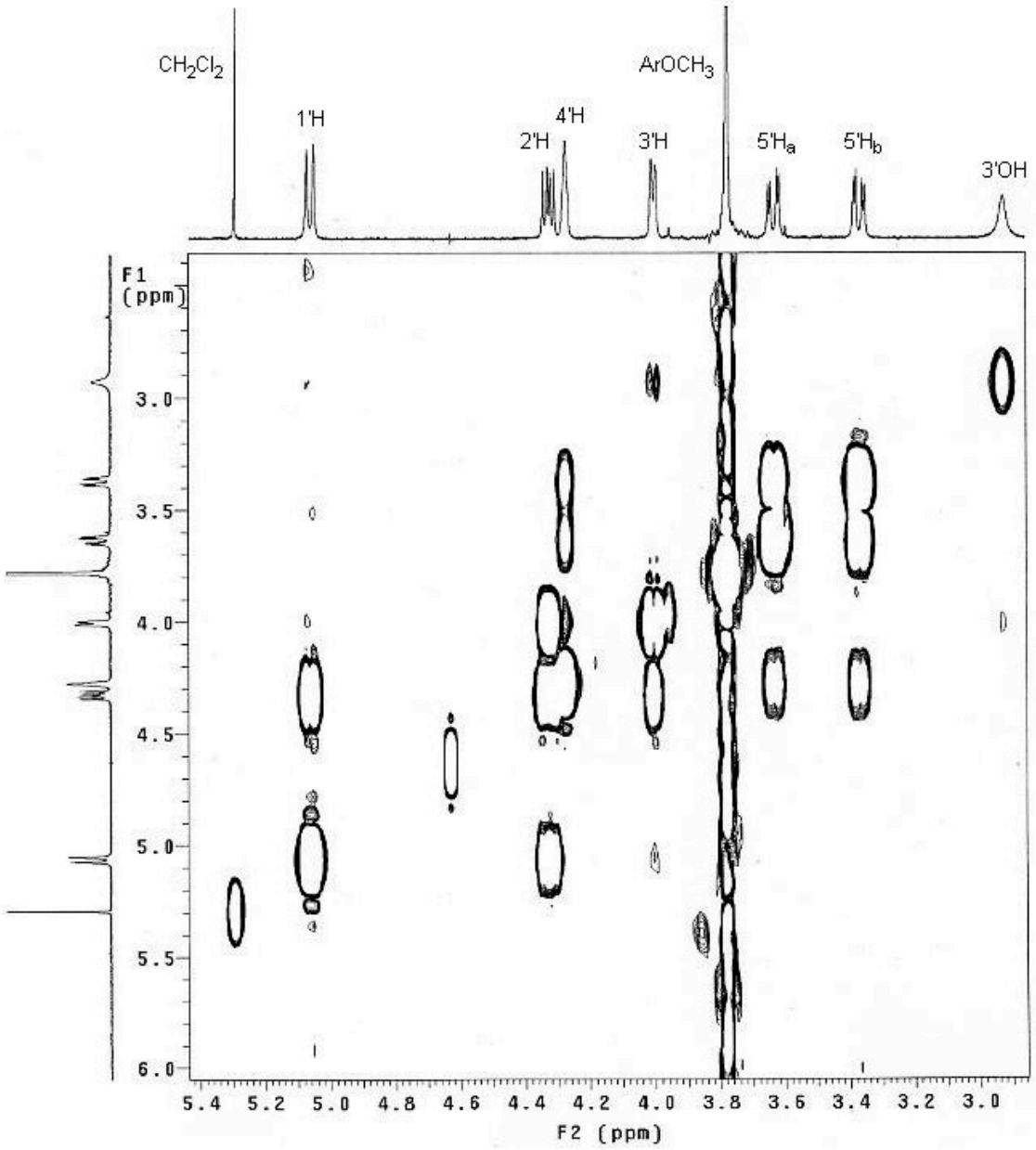

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 52.1 degrees
Acq. time 4.002 sec
Width 4997.5 Hz
16 repetitions
OBSERVE H1, 400.1115375 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 22 sec
OMe
NH
N
H
O
OH
O-Si
OMe
12 1H-NMR
(400 MHz; CDCl3)
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
1.23
1.16
0.99
1.07
9.26
3.63
1.00
0.95
1.01
5.36
1.02
1.08
1.04
1.02
8.26
2.93
2.79

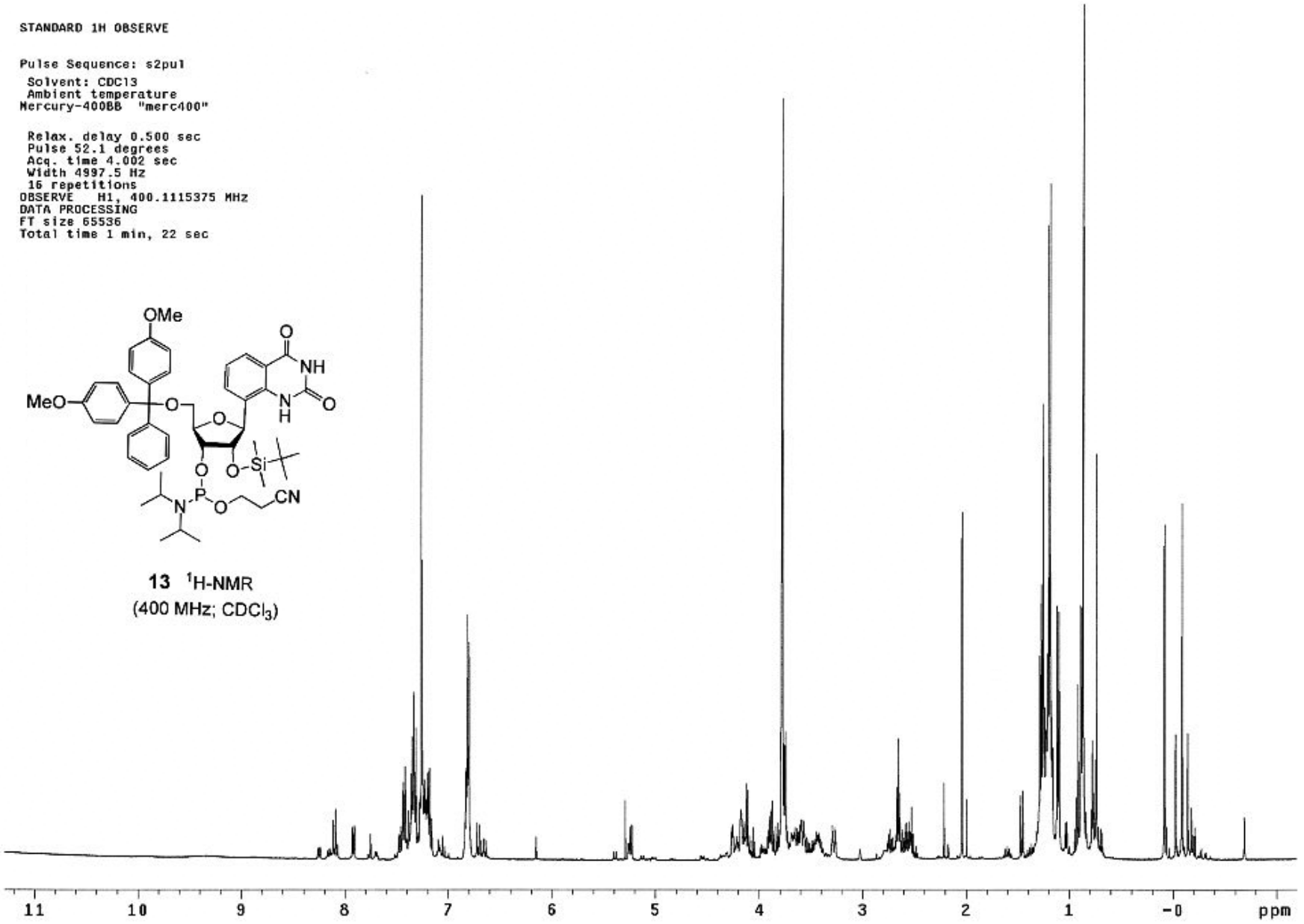

13C OBSERVE
Archive directory:
/export/home/armandoh/vnmrsys/data
Sample directory:
File: AH8-17rf57-13Cnew
Pulse Sequence: s2pul
Solvent: CDCl3
Relax. delay 0.500 sec
Pulse 38.4 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
256 repetitions
OBSERVE C13, 100.6081296 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 29 min
12 13C-NMR
(100 MHz; CDCl3)
-5.245
-5.215
17.914
25.574
55.179
63.422
72.749
76.670
76.988
77.193
77.307
78.012
79.809
85.633
86.718
113.115
115.208
122.852
123.997
126.848
127.796
128.039
130.147
130.132
133.552
135.387
135.577
138.307
144.566
149.371
158.516
162.755
167.684
ppm

```
STANDARD 1H OBSERVE

 Archive directory:
   /export/home/armandoh/vnmrsys/data
 Sample directory:

 File: AH8-17rf57-gCOSY

Pulse Sequence: gCOSY
Solvent: CDCl3

 Relax. delay 1.000 sec
 Acq. time 0.236 sec
 Width    4347.8 Hz
 2D Width 4347.8 Hz
 Single scan
 128 increments
OBSERVE    H1, 400.1115375 MHz
DATA PROCESSING
 Sq. sine bell 0.118 sec
F1 DATA PROCESSING
 Sq. sine bell 0.015 sec
FT size 2048 x 2048
Total time 3 min
```

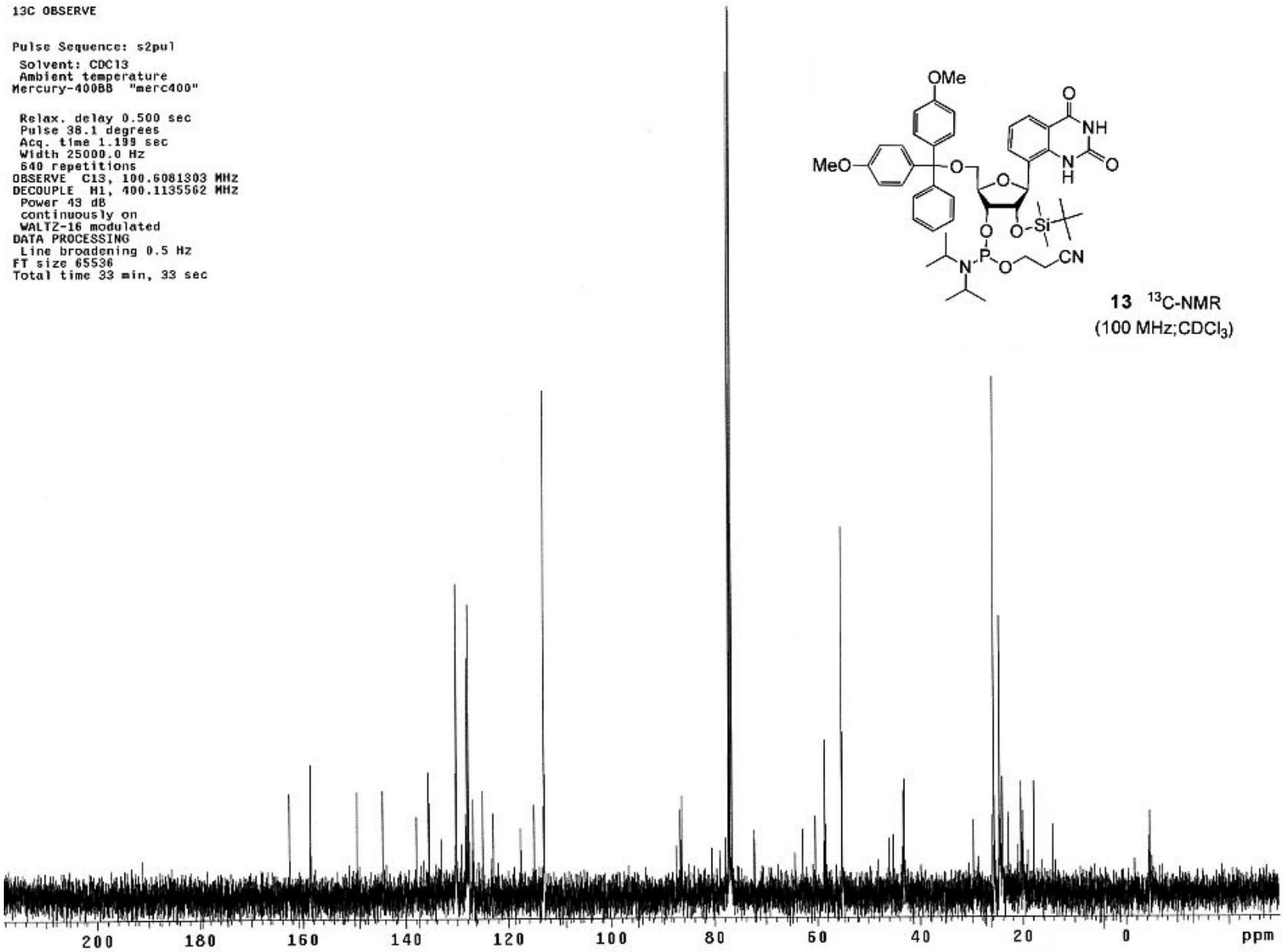


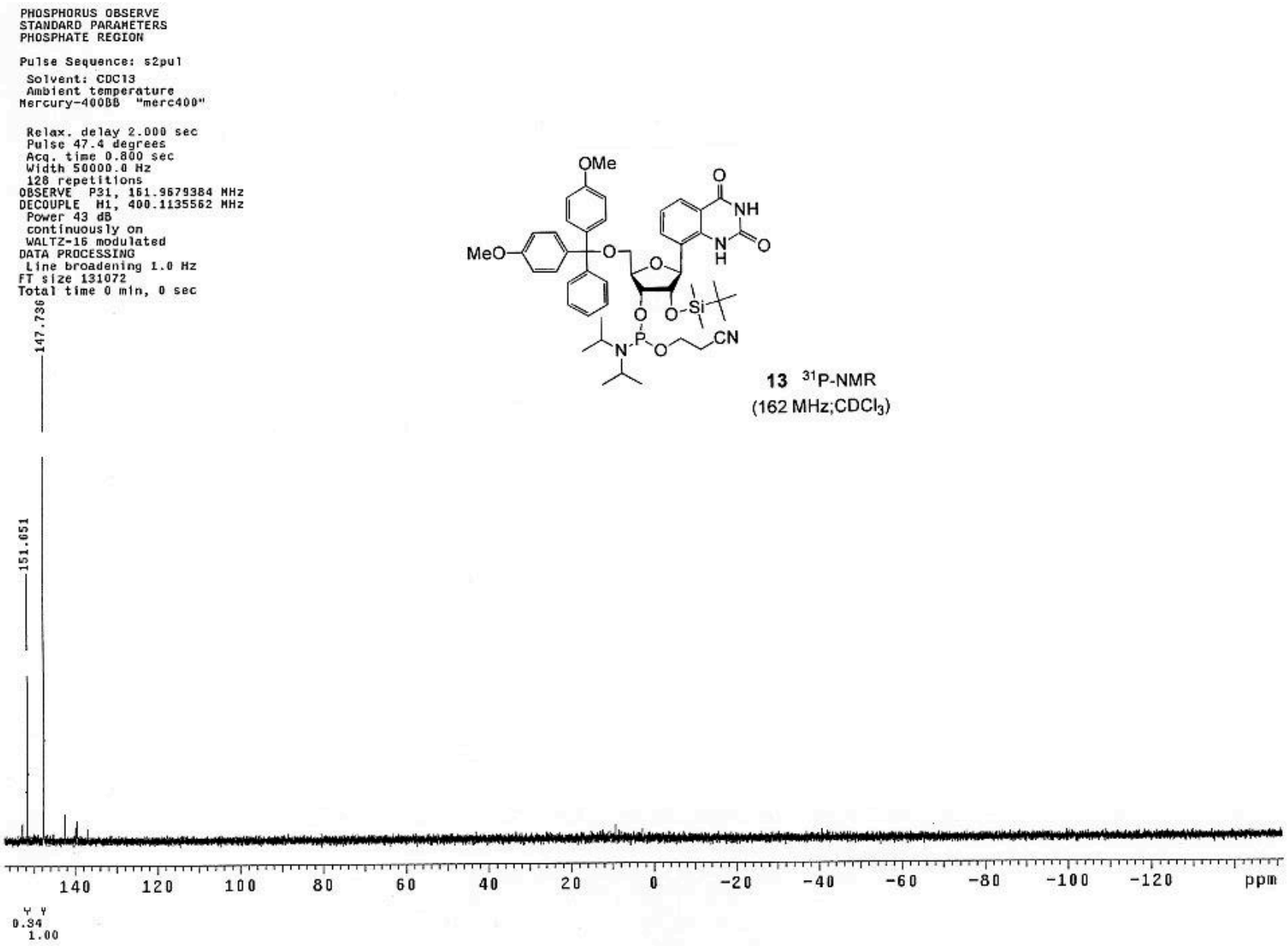
12 g-COSY
(400 MHz; $CDCl_3$)





13 ^{1}H-NMR
(400 MHz; $CDCl_3$)


13C OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Mercury-400BB "merc400"
Relax. delay 0.500 sec
Pulse 38.1 degrees
Acq. time 1.199 sec
Width 25000.0 Hz
640 repetitions
OBSERVE C13, 100.6081303 MHz
DECOUPLE H1, 400.1135562 MHz
Power 43 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 33 min, 33 sec
OMe
MeO
O
NH
N
H
O
O
O
O
Si
O
P
N
O
CN
13 13C-NMR
(100 MHz;CDCl3)
200
180
160
140
120
100
80
60
40
20
0
ppm



13 ^{31}P-NMR
(162 MHz;$CDCl_3$)