Supporting information for

Synthesis and characterization of modified nucleotides in the 970 hairpin loop of *Escherichia coli* 16S ribosomal RNA

N. Dinuka Abeydeera, Christine S. Chow*

Contents

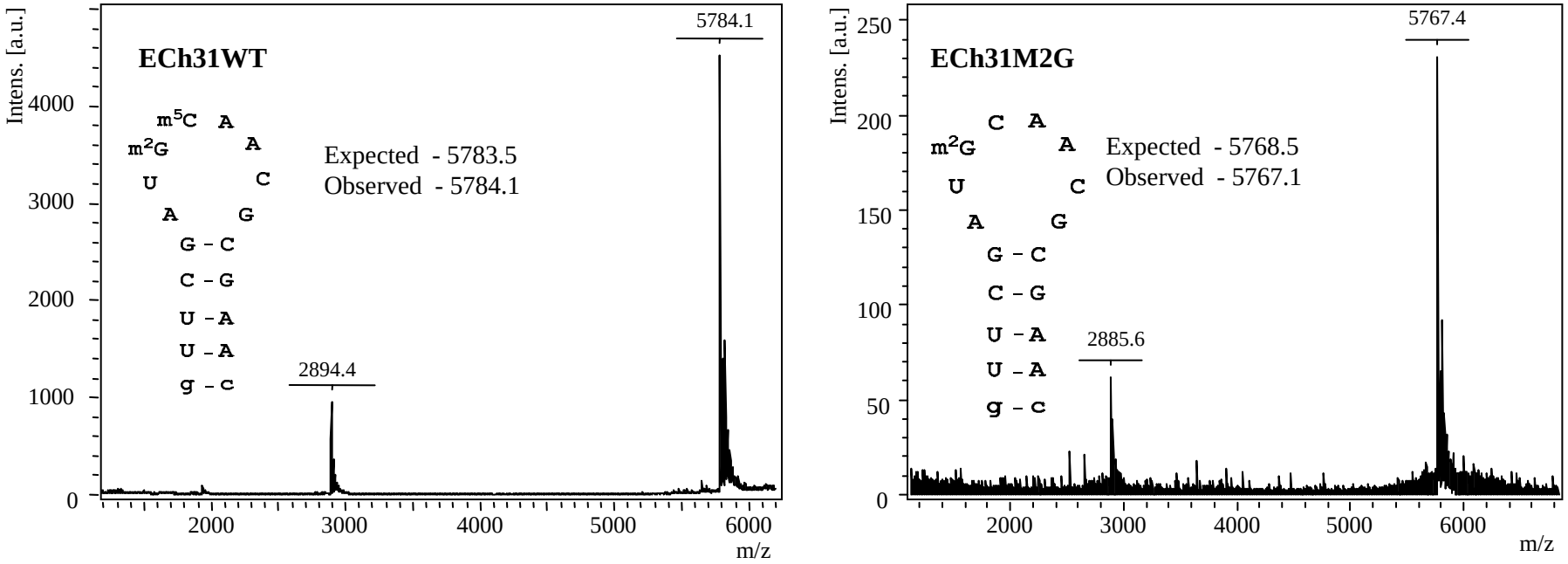


Figure S1. Characterization of helix 31 analogues by MALDI-TOF mass spectrometric analysis.

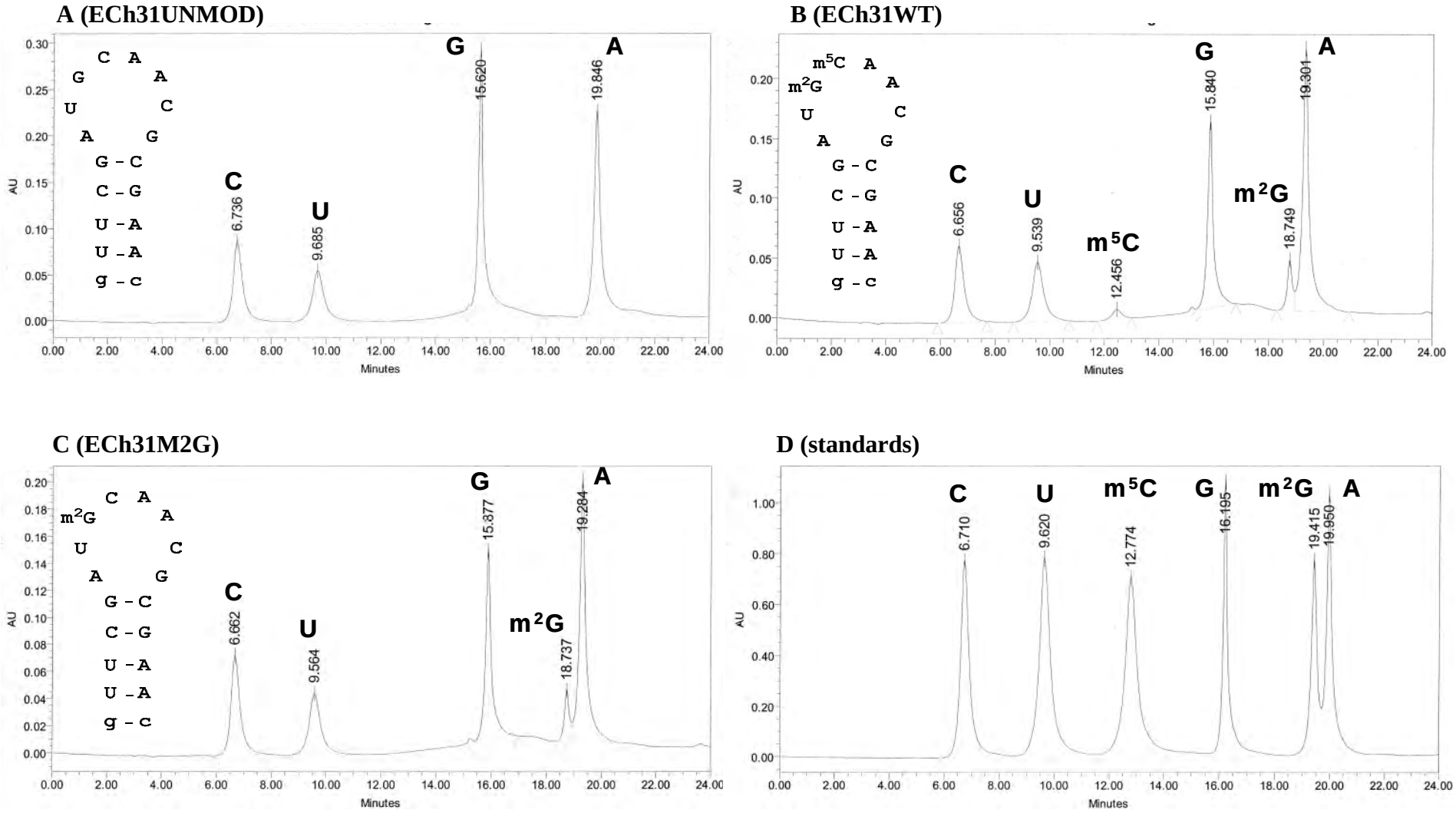


Figure S2. Confirmation of nucleosides present in the synthetic oligos obtained by digestion of ECh31UNMOD (**A**), ECh31WT (**B**) and ECh31M2G (**C**) RNAs with P1 nuclease and treatment of resulting nucleotides with calf intestinal phosphatase were analyzed by reverse-phase HPLC on a Supelco C18 column. Approximately 0.5 OD of RNA were digested and 0.25 OD were injected for each analysis. A linear gradient in 0.1 M TEAA buffer, pH 6.0 from 0 to 30% methanol over 17 min at a flow rate of 1 mL/min was employed. The retention times of the nucleosides were confirmed by injection of authentic standards (D) with a deviation of <0.5% (C = 6.71 min, U = 9.62 min, m^5C = 12.77, G = 16.19 min, m^2G = 19.42 min, A = 19.95 min).

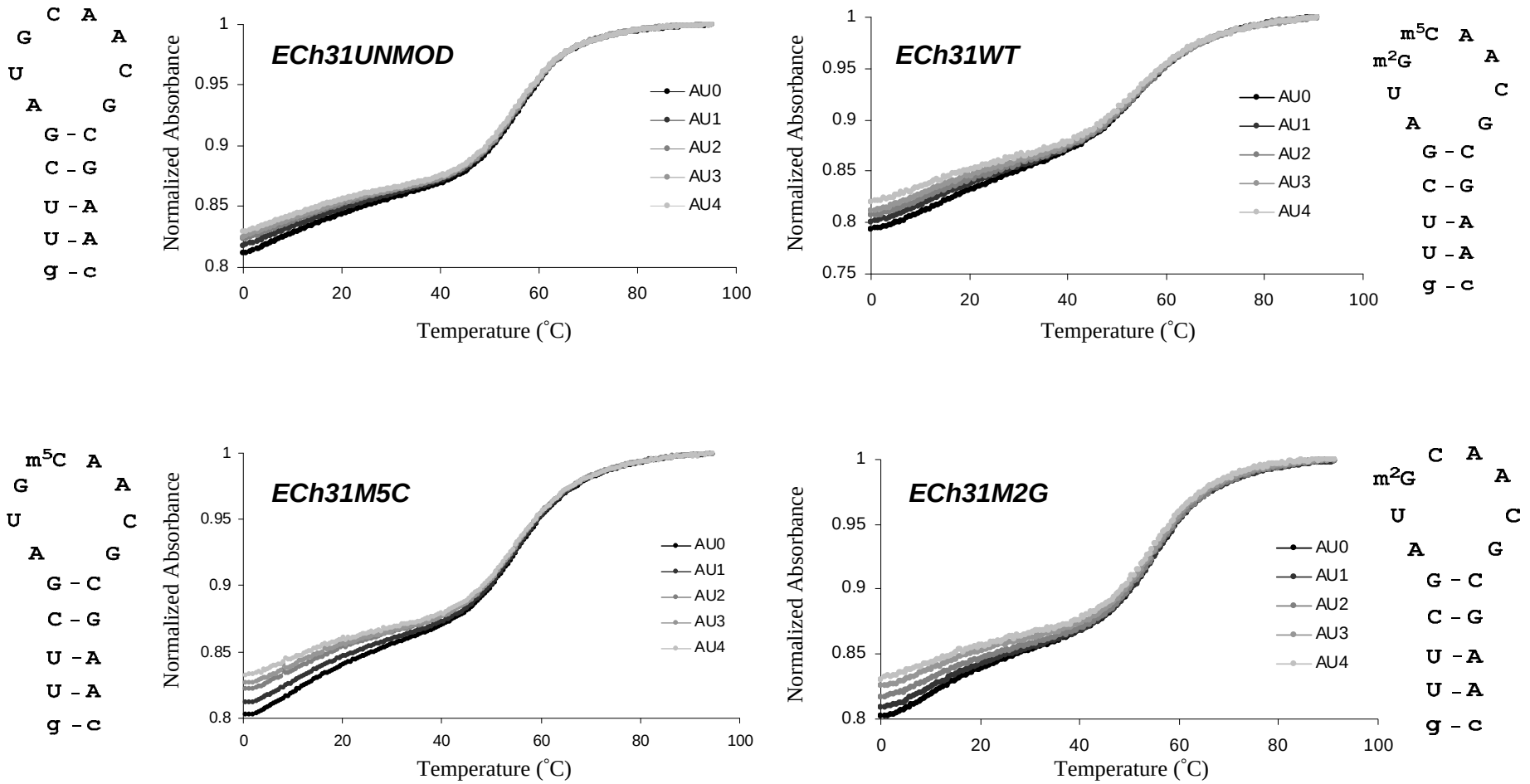
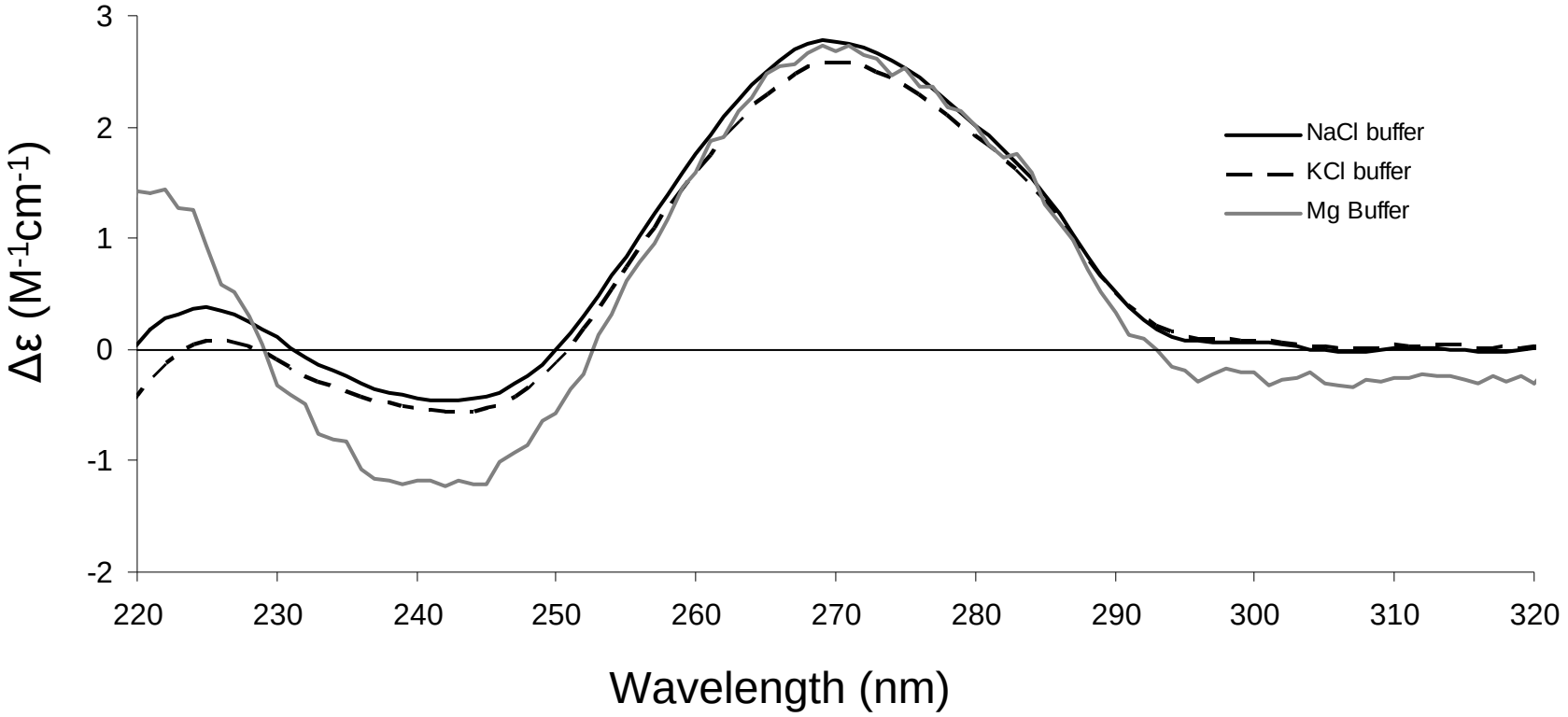


Figure S3. The UV melting profiles representing the melting transitions of four RNAs are shown. AU0-AU4 represent profiles corresponding to different dilutions of each RNA taken in 15 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA at pH 7.0.



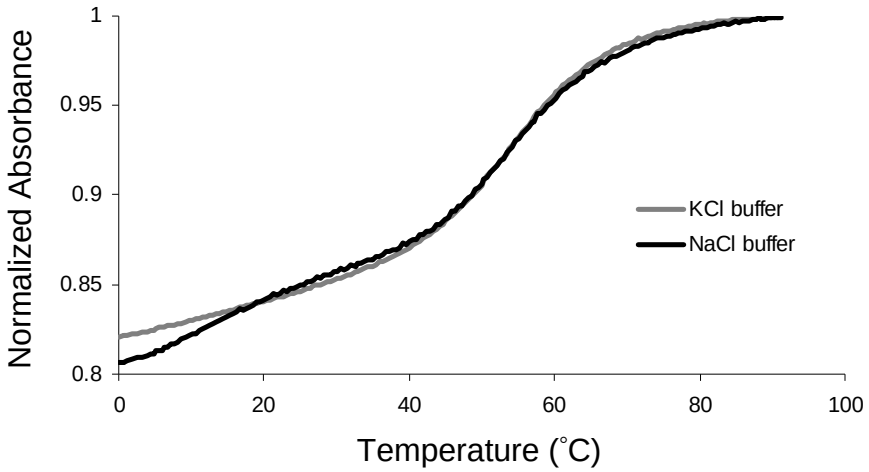
NaCl buffer = 15 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA at pH 7.0

KCl buffer = 15 mM KCl, 20 mM cacodylic acid, 20 mM Tris [basic form], 0.5 mM Na_2EDTA at pH 7.0

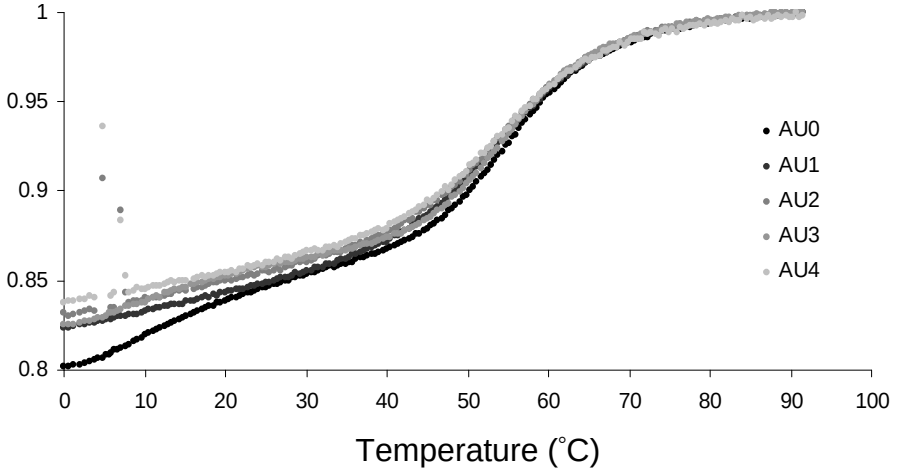
Mg buffer = 25 mM cacodylic acid, 25 mM Tris [basic form], 30 mM KCl, 70 mM NH_4Cl, 3 mM $MgCl_2$ at pH 7.0

Figure S4. CD spectra of the ECh31WT RNA construct acquired in Na^+, K^+, and Mg^{2+} containing buffers.

A



B



C

	ΔG°_{37} (kcal/mol)	ΔH° (kcal/mol)	ΔS° (cal/K.mol)	T_m (°C)
ECh31WT –KCl buffer	**-2.13 ± 0.03**	**-39.37 ± 1.2**	**-120.08 ± 3.8**	**54.7**
ECh31WT –NaCl buffer	**-2.10 ± 0.07**	**-37.26 ± 1.2**	**-113.37 ± 3.6**	**55.5**

Figure S5. Representative normalized UV melting curves of the ECh31WT RNA taken in Na^+ (15 mM NaCl, 20 mM sodium cacodylate, 0.5 mM Na_2EDTA at pH 7.0) and K^+ (15 mM KCl, 20 mM cacodylic acid, 20 mM Tris [basic form], 0.5 mM Na_2EDTA, pH 7.0) buffers (A), AU0-AU4 representing the profiles corresponding to different dilutions of ECh31WT RNA taken in K^+ buffer (B), Thermodynamics of the ECh31WT RNA in Na^+ and K^+ buffers (C).

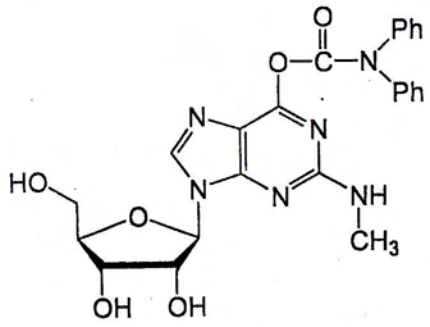
NMR characterization for 2-*N*-Methyl-6-*O*-(diphenylcarbamoyl)guanosine [**5**].

^{1}H NMR (DMSO-d^{6}, 400 MHz) 2.81 (d, 3H), 3.51-3.56 (m, 1H), 3.61-3.66 (m, 1H), 3.90 (m, 1H), 4.02 (q, 1H), 4.15 (br.d, 1H), 4.62 (br.s, 1H), 4.99 (br.s, 1H), 5.21 (d, *J* = 4.8 Hz, 1H), 5.48 (d, *J* = 5.6 Hz, 1H), 5.82 (d, *J* = 5.6 Hz, 1H), 7.28-7.32 (m, 4H), 7.4-7.44 (m, 6H), 8.19 (s, 1H); ^{13}C NMR (DMSO-d^{6}, 400 MHz) 28.3, 61.5, 70.5, 73.1, 85.5, 116.4, 127.1, 129.4, 140.9, 141.8, 150.4, 155.6, 155.9, 159.6

proton spectrum

Pulse Sequence: s2pul

Mercury 400 spectrometer



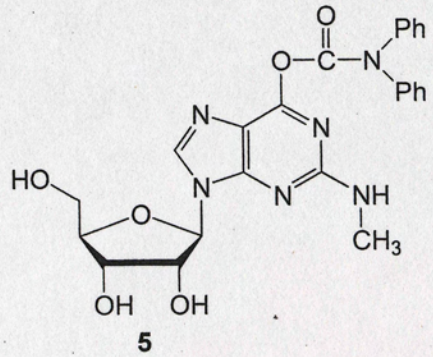
5

2-*N*-Methyl-6-*O*-(diphenylcarbamoyl)guanosine [**5**].

8 7 6 5 4 3 2 1 ppm

Pulse Sequence: s2pul

2-*N*-Methyl-6-*O*-(diphenylcarbamoyl)guanosine [**5**].



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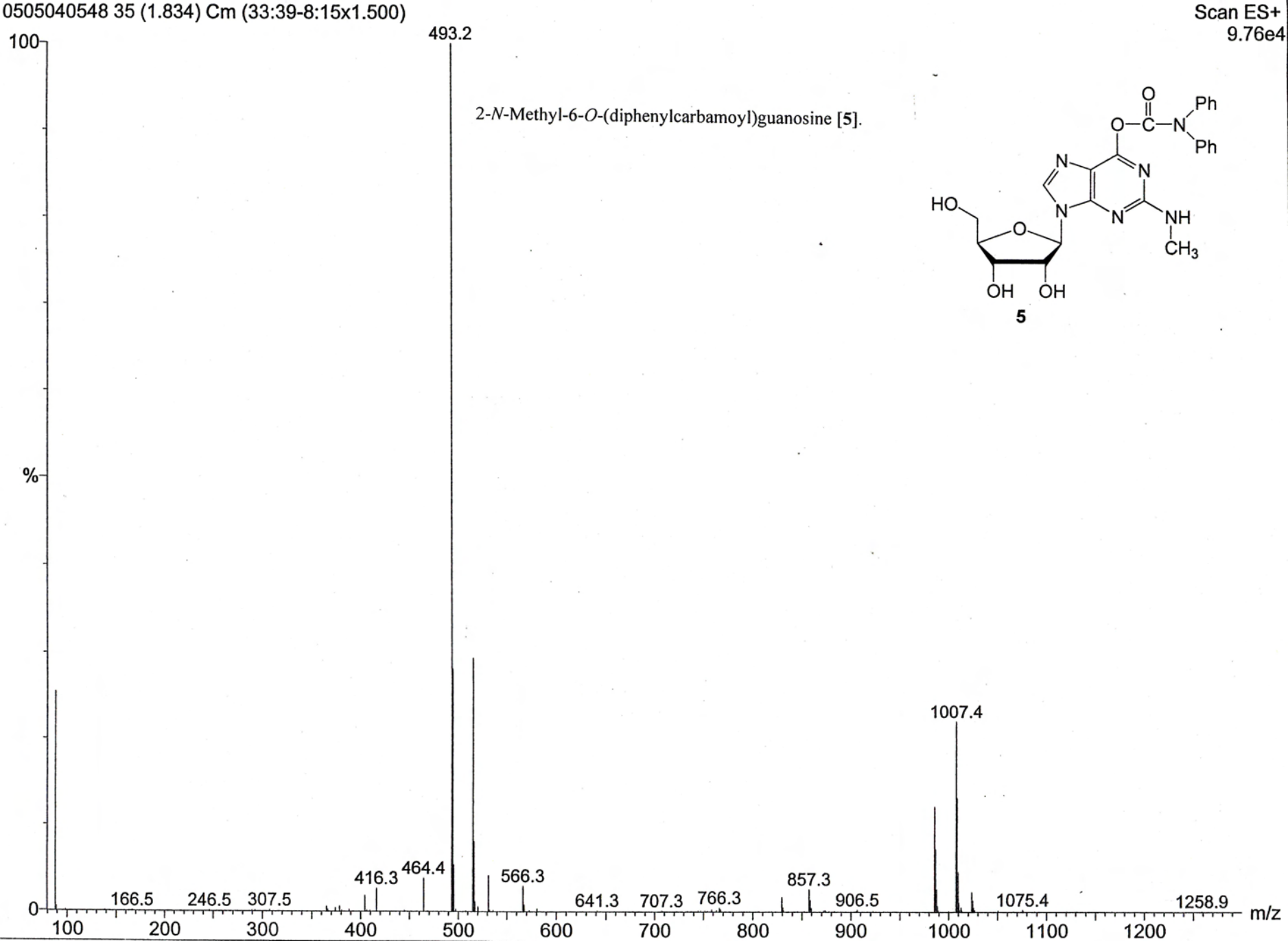
0505040548 35 (1.834) Cm (33:39-8:15x1.500)
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493.2
100
%
0
2-N-Methyl-6-O-(diphenylcarbamoyl)guanosine [5].
5
1007.4
166.5
246.5
307.5
416.3
464.4
566.3
641.3
707.3
766.3
857.3
906.5
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1258.9
m/z
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400
500
600
700
800
900
1000
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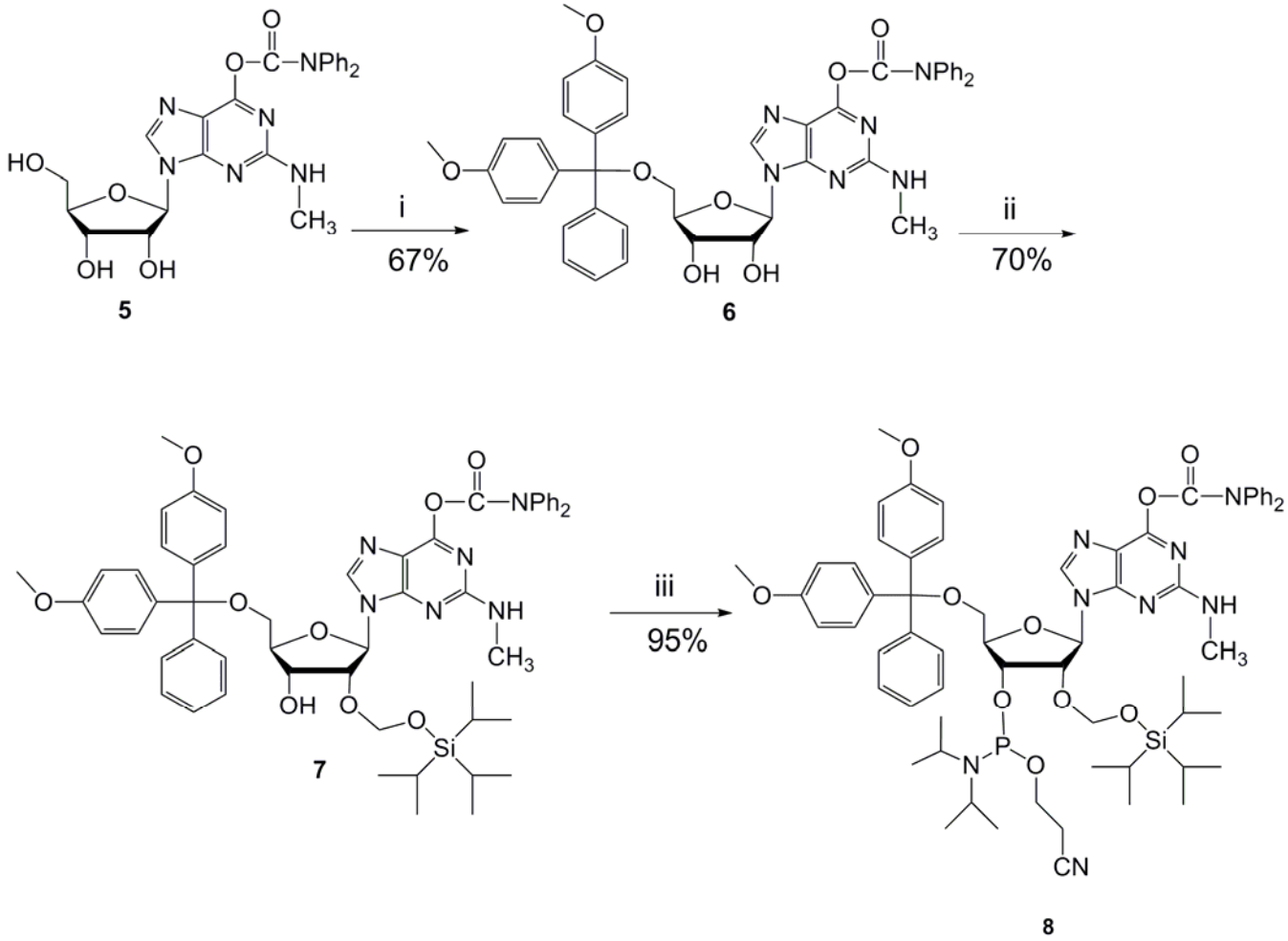
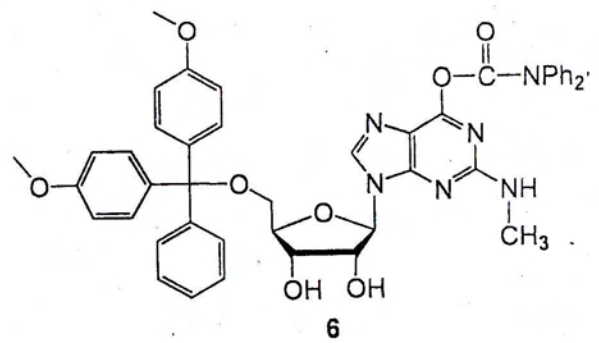


Supplementary scheme. Synthesis of 5′-*O*-DMT-2′-*O*-TOM-6-*O*-DPC-2-*N*-methylguanosine phosphoramidite **8**: (i) DMTCl, DMAP, pyridine, room temperature, 24 h; (ii) a) *tert*-Bu_2SnCl_2, *i*Pr_2NEt, dichloroethane, 70 °C, 15 min; b) TOMCl, room temperature, 3 h; (iii) 2-cyanoethyldiisopropylphosphoramidochloridite, *i*Pr_2NEt, dichloromethane, room temperature, 2 h.

NMR characterization for 5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-methyl-6-*O*-(diphenylcarbamoyl)guanosine [**6**].

^{1}H NMR (CD_3OD, 400 MHz) 2.71 (br.s, 1H), 2.98-3.03 (q, 1H), 3.21 (d, *J* = 3.2 Hz, 3H), 3.25-3.27 (m, 1H), 3.29-3.32 (m, 1H), 3.60 (d, *J* = 4.8 Hz, 6H), 3.66 (m, 1H), 4.07-4.10 (m, 1H), 4.45 (m, 1H), 4.83 (m, 1H), 5.87 (d, *J* = 4.8 Hz, 1H), 6.64-6.69 (m, 5H), 7.05-7.38 (m, 18H), 8.0 (s, 1H); ^{13}C NMR ($(CD_3)_2SO$, 500 MHz) 28.8, 55.6, 72.2, 74.9, 82.3, 85.3, 87.7, 113.8, 114.0, 126.3, 127.8, 128.5, 128.7, 129.1, 129.2, 129.3, 129.9, 130.2, 130.4, 131.2, 141.2, 149.2, 159.9

5'-*O*-(4,4'-Dimethoxytrityl)-2-*N*-methyl-6-*O*-(diphenylcarbamoyl)guanosine [**6**]



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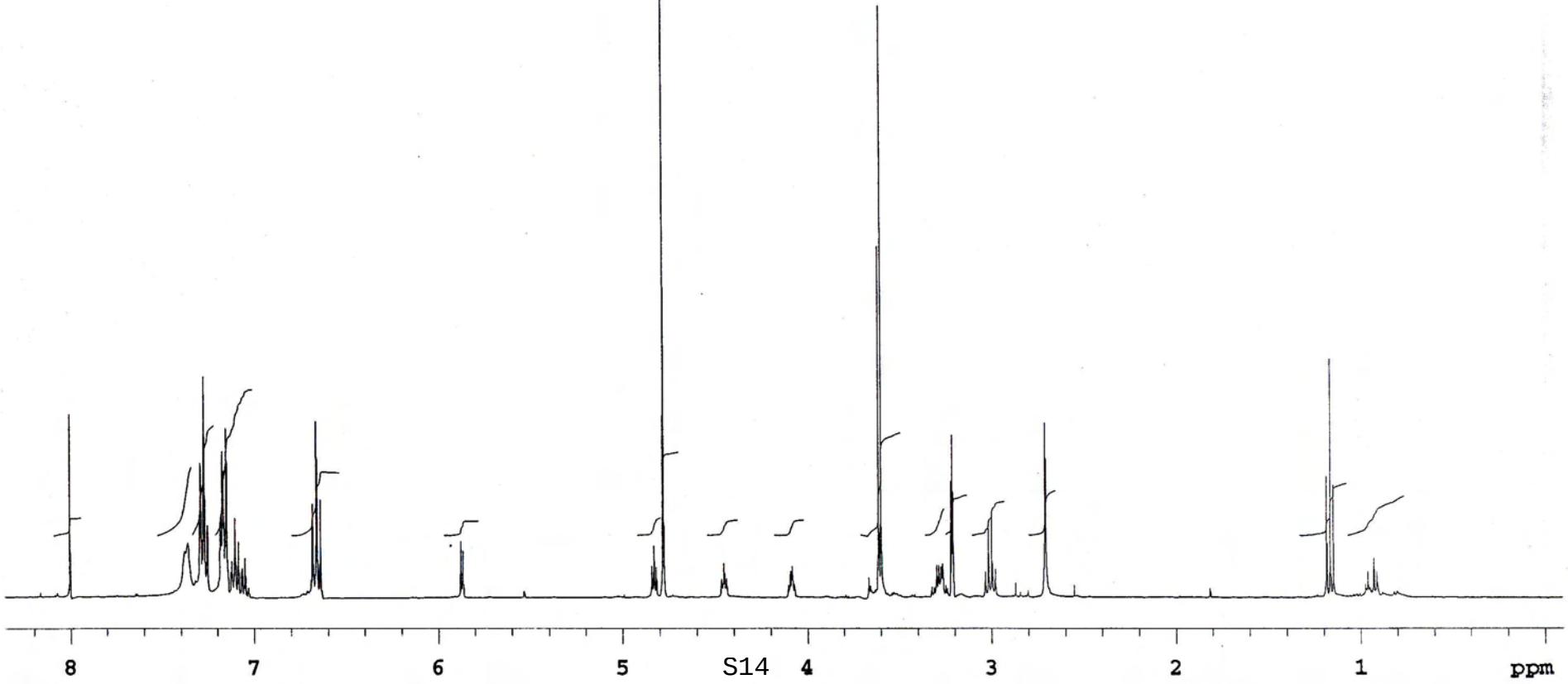
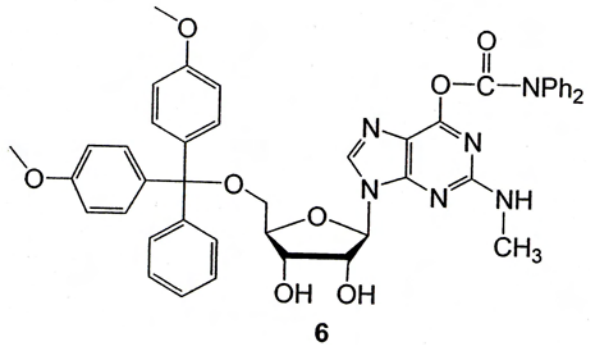


5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-methyl-6-*O*-(diphenylcarbamoyl)guanosine [**6**]



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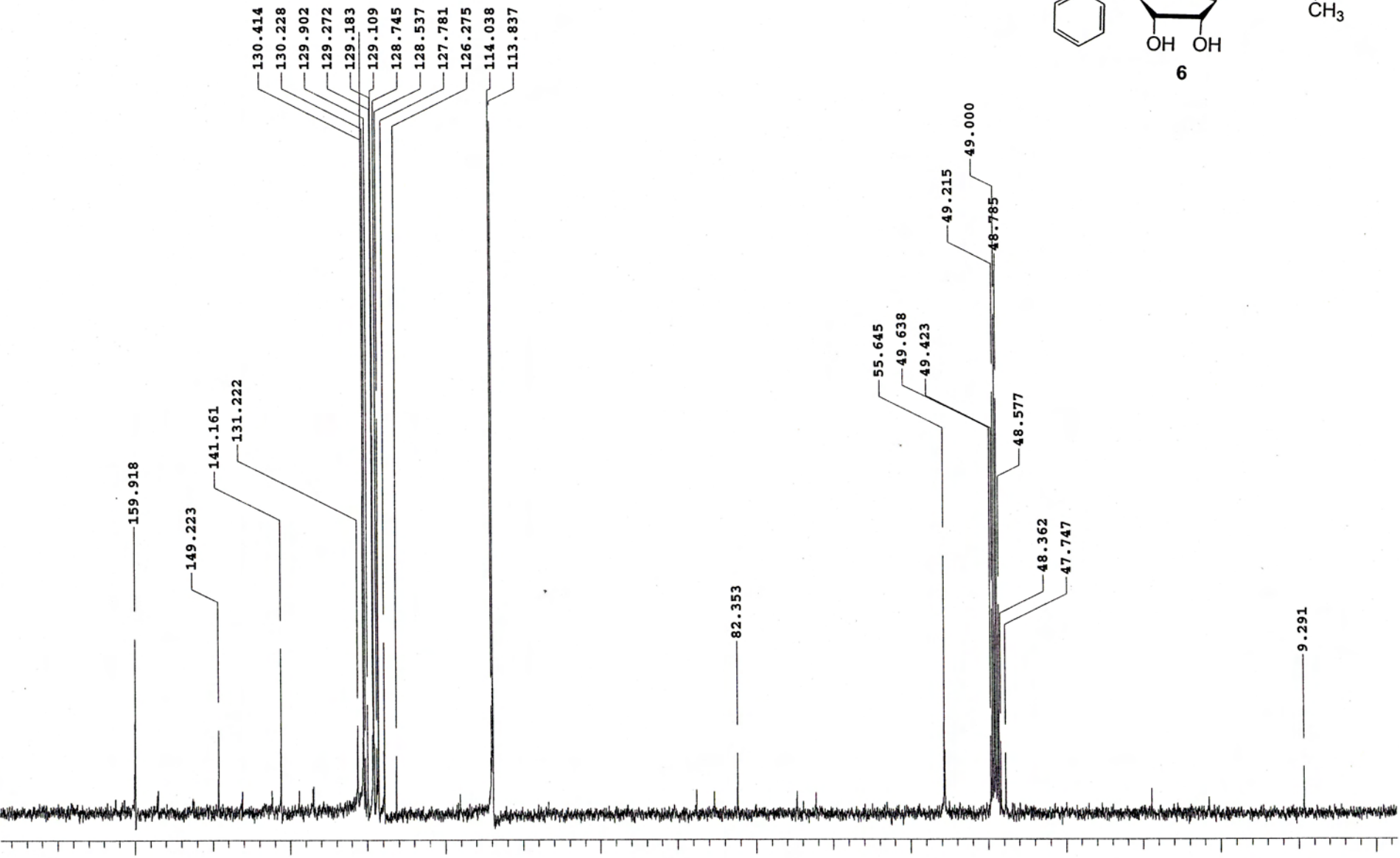

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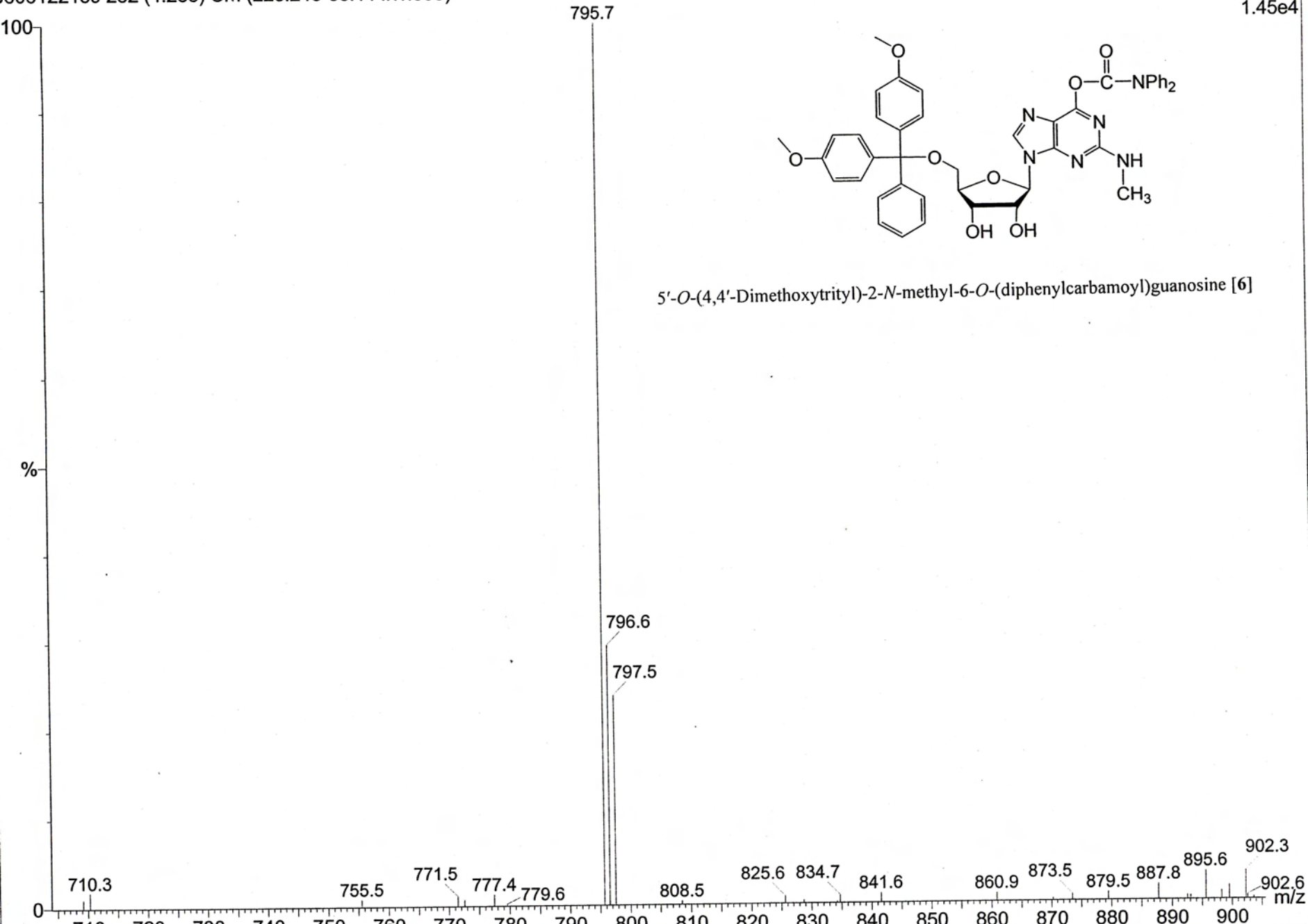


5′-*O*-(4,4′-Dimethoxytrityl)-2-*N*-methyl-6-*O*-(diphenylcarbamoyl)guanosine [**6**]

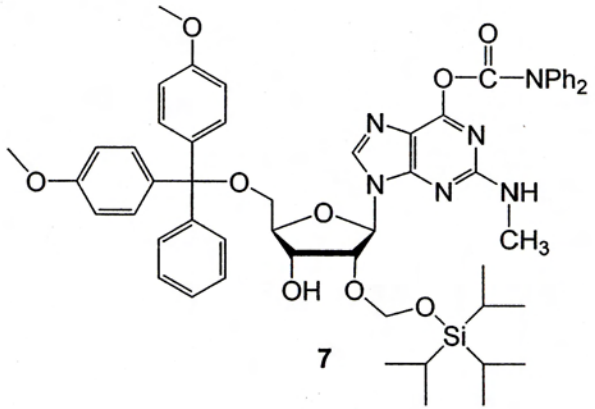
NMR characterization for 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)-oxy]methyl]-2-*N*-methyl-6-*O*-(diphenylcarbamoyl)guanosine [**7**].

^{1}H NMR (CD_3OD, 400 MHz) 0.89-1.05 (m, 18H), 1.25-1.3 (m, 3H), 2.79-2.8 (m, 7H), 3.1 (s, 1H), 3.3-3.5 (m, 2H), 3.70 (d, *J* = 4.8 Hz, 3H), 4.15-4.25 (m, 1H), 4.65-4.7 (m, 1H), 4.85 (s, 2H), 5.05-5.15 (m, 1H), 6.1 (d, *J* = 5 Hz, 1H), 6.79-6.83 (m, 5H), 7.17-7.5 (m, 18H), 8.05 (s, 1H)

proton spectrum

Pulse Sequence: s2pul

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)oxy]methyl]-2-*N*-methyl-6-*O*-(diphenylcarbamoyl) guanosine [7].



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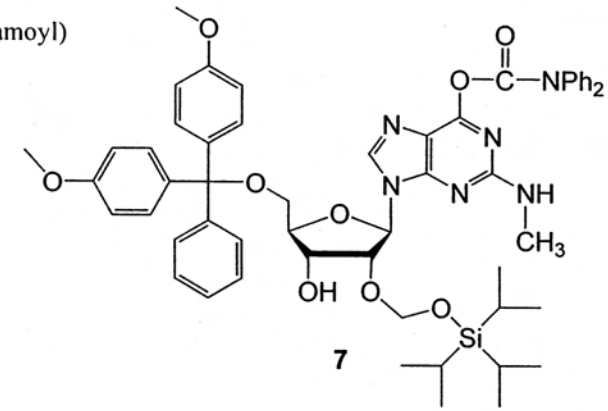
060726313 236 (4.362) Cm (233:250-54:84x1.500)

Scan ES+
1.16e5

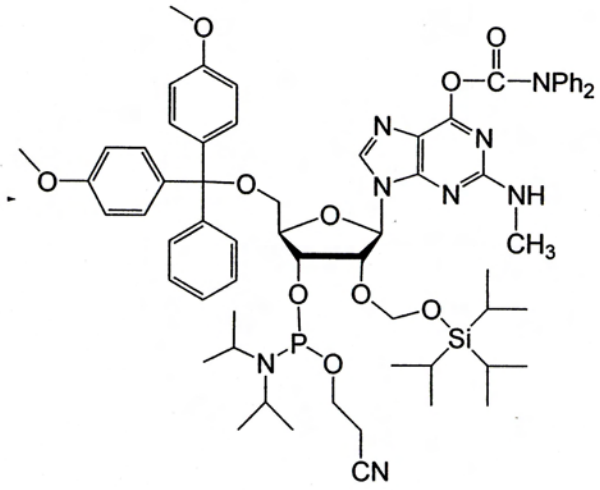
5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)oxy]methyl]-2-*N*-methyl-6-*O*-(diphenylcarbamoyl) guanosine [7].



7

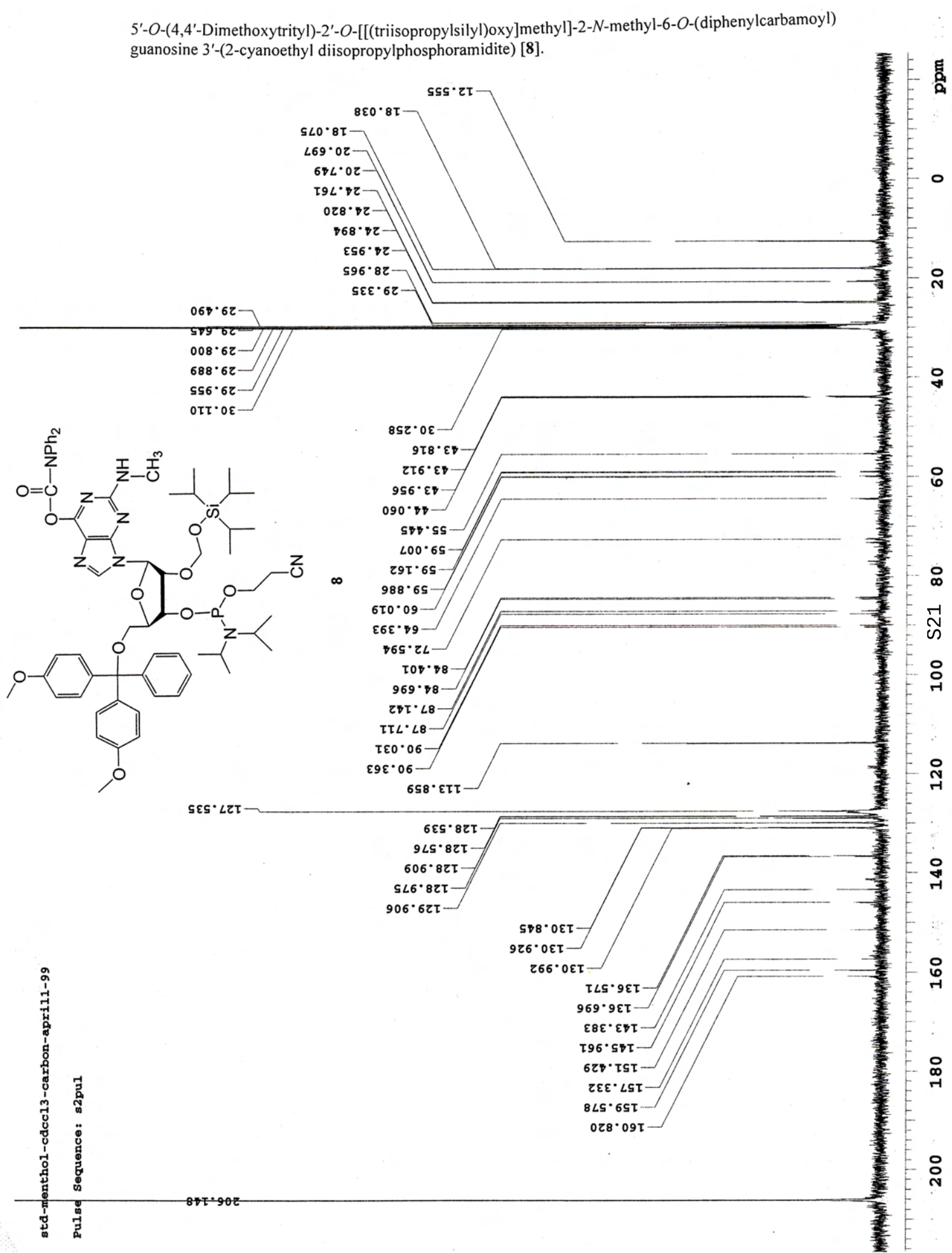
Pulse Sequence: s2pul

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)oxy]methyl]-2-*N*-methyl-6-*O*-(diphenylcarbamoyl) guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) [**8**].



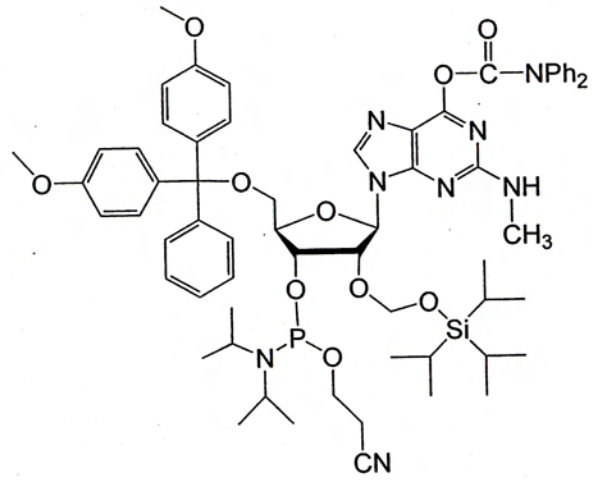
8

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)oxy]methyl]-2-*N*-methyl-6-*O*-(diphenylcarbamoyl) guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) [**8**].



Pulse Sequence: s2pul

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)oxy]methyl]-2-*N*-methyl-6-*O*-(diphenylcarbamoyl) guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) [**8**].



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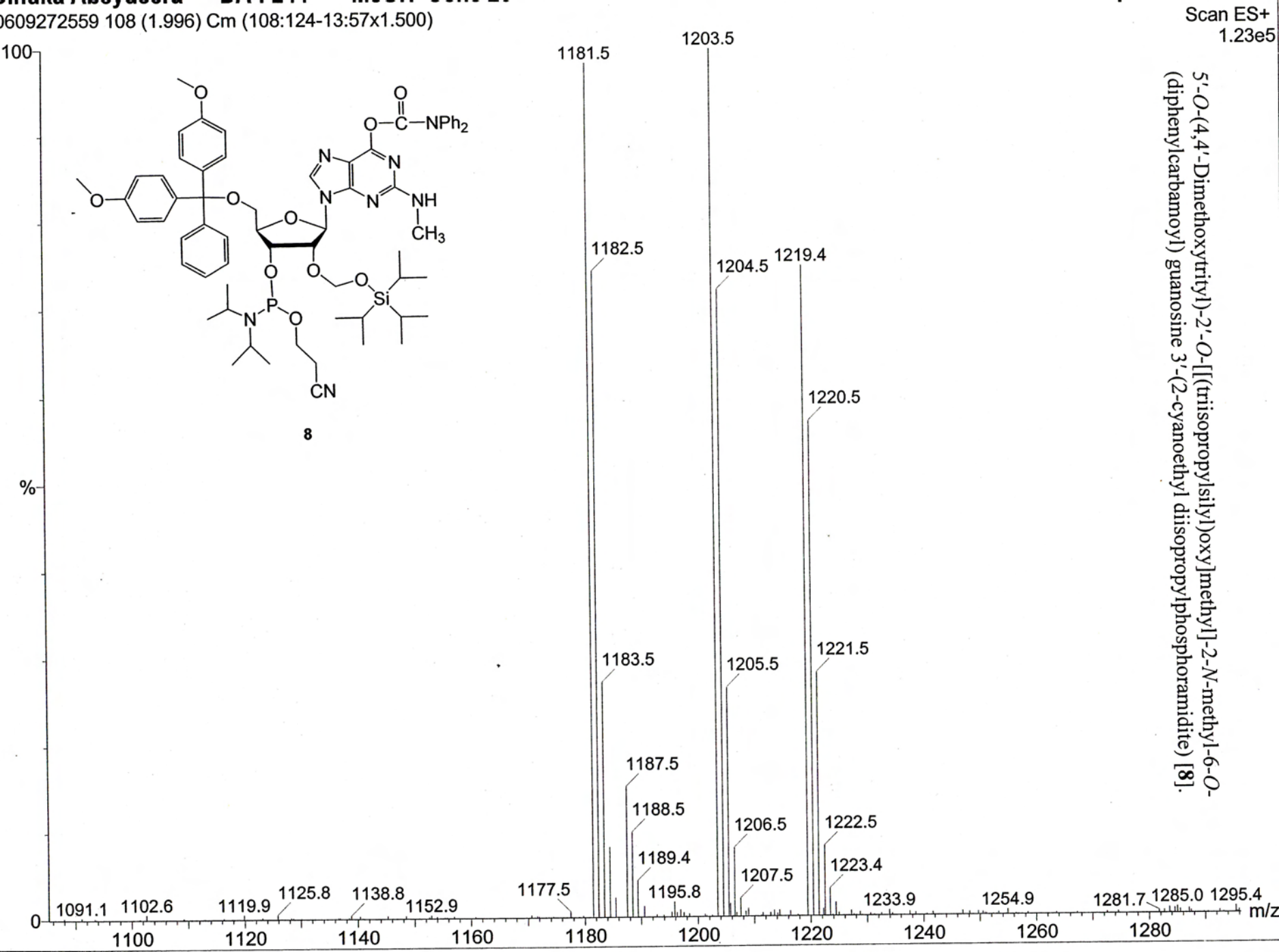
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1204.5
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1195.8
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1140
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1180
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1220
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1280
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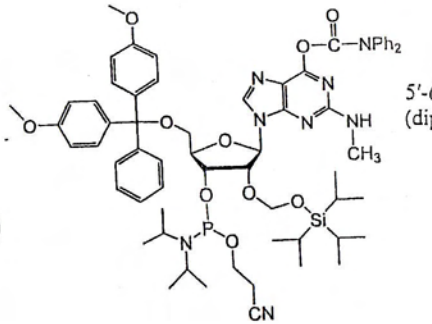
O–C–NPh2
N
N
N
NH
CH3
O
O
O
O
O
O
Si
P
N
O
CN
8
5′-O-(4,4′-Dimethoxytrityl)-2′-O-[[(triisopropylsilyl)oxy]methyl]-2-N-methyl-6-O-(diphenylcarbamoyl) guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) [8].

Elemental Composition Report



8

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-[[(triisopropylsilyl)oxy]methyl]-2-*N*-methyl-6-*O*-(diphenylcarbamoyl) guanosine 3′-(2-cyanoethyl diisopropylphosphoramidite) [8].

Single Mass Analysis

Tolerance = 8.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
1096 formula(e) evaluated with 3 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-66 H: 0-1000 N: 0-8 O: 0-10 23Na: 0-1 Si: 0-1 P: 0-1

Chow- Dinuka Abeydeera DA-i-244 LCT0246 mw1180 4uL meoh
Shay 2008-07b.pro
2008_1212_0246b 14 (0.301) Cm (11:20-(1:6+26:36)x3.000)

LCT Premier 12-Dec-2008 14:48:58
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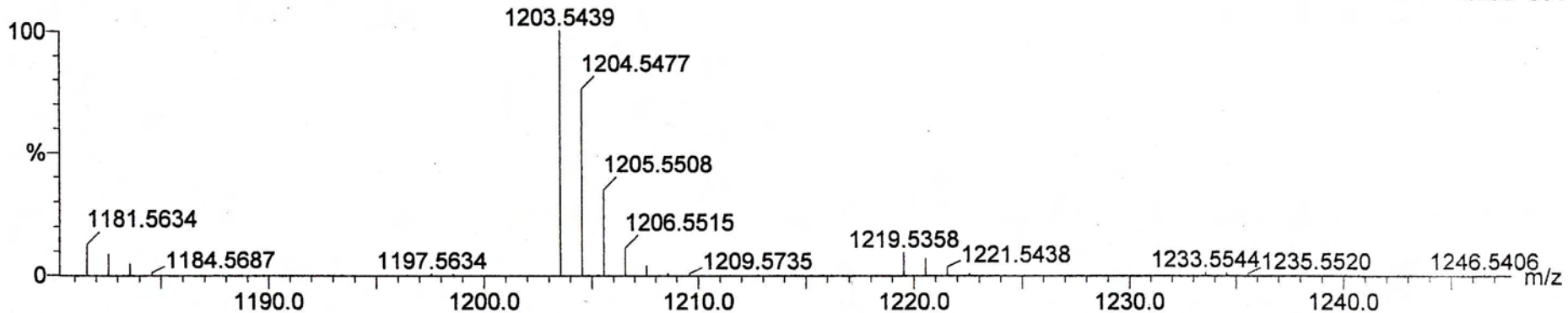
4.25e+004



Minimum:				-1.5			
Maximum:		5.0	8.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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	1181.5661	-2.7	-2.3	29.5	25.7	1.2	C64 H82 N8 O10 Si P ✓
	1181.5605	2.9	2.5	31.5	26.6	2.2	C66 H79 N8 O9 23Na P