

Supporting Information

2-(4-Tolylsulfonyl)ethoxymethyl(TEM) - A New 2'-OH Protecting Group For Solid Support RNA Synthesis

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content

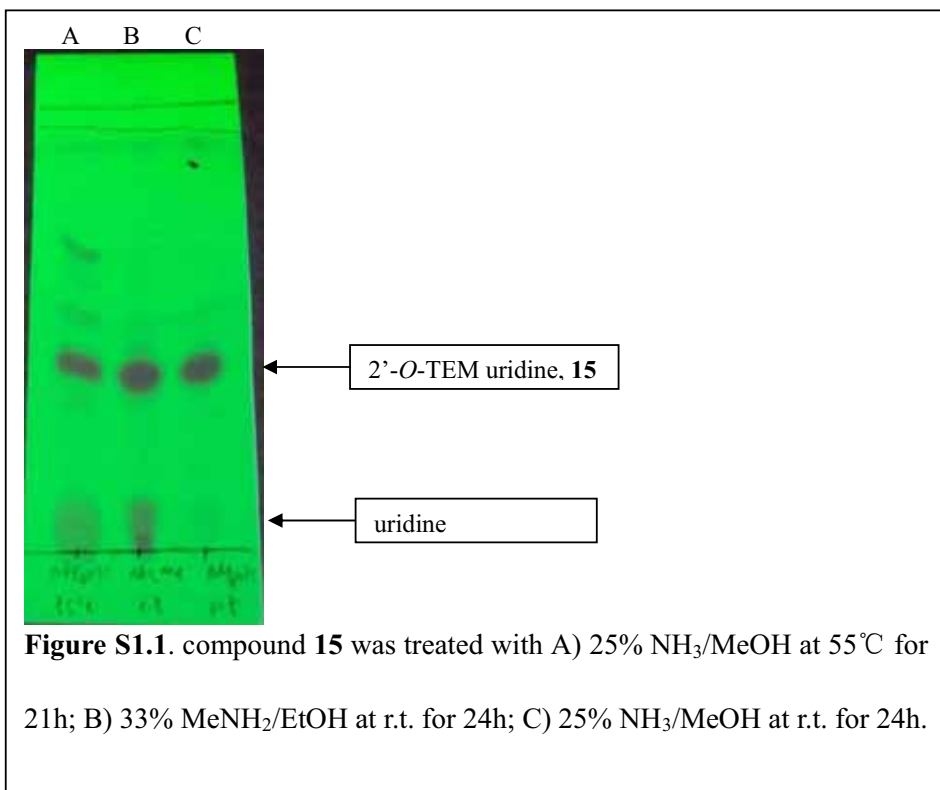
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General Experimental Methods

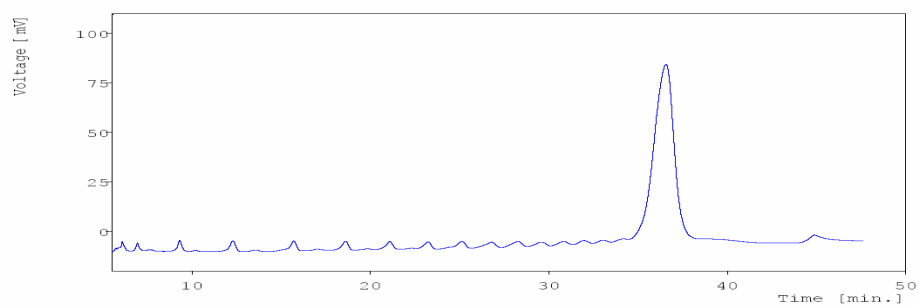
Chromatographic separations were performed on Merck G60 silica gel. Thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F₂₅₄ glass-backed plates. ¹H NMR spectra were recorded at 270 MHz, using TMS (0.0 ppm) as internal standards. ¹³C NMR spectra were recorded at 67.9 MHz, using the central peak of CDCl₃ (76.9 ppm) as an internal standard. ³¹P NMR spectra were recorded at 109.4 MHz using 85% phosphoric acid as external standard. Chemical shifts are reported in ppm (δ scale). The $\delta_{C2'}$, $\delta_{C3'}$, $\delta_{H1'}$, $\delta_{H2'}$, $\delta_{H3'}$ are assigned according to H-H cosy and C-H cosy. MALDI-TOF mass spectra were recorded in positive ion mode. For oligo-RNAs, the mass spectrometer was externally calibrated with standard oligonucleotide using 3-HAP and ammonium citrate as co-matrix. For other compounds, the mass spectrometer was externally calibrated with peptide mixture using THPA and ammonium citrate as matrix.

Table S1. Synthetic cycle and reagents.

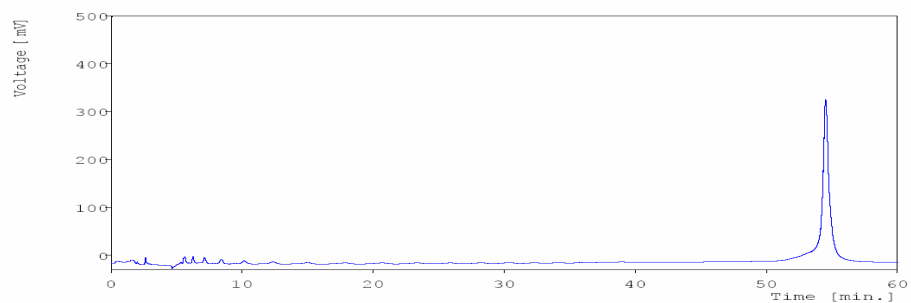
	function	reagents	Time (s)
1	coupling	0.1 M amidite in CH ₃ CN + 0.25M ETT in CH ₃ CN	120
2	capping	0.1 M Ac ₂ O in THF + N-Methylimidazole/THF/Pyridine	15
3	oxidation	0.02 M I ₂ in THF-H ₂ O-Pyridine (7:1:2)	8
4	deblocking	3% DCA in CH ₂ Cl ₂	98



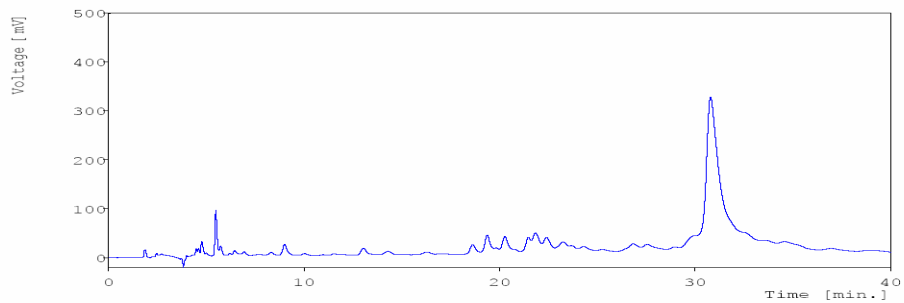
A) ON 1



B) ON 2



C) ON 3



D) ON 4

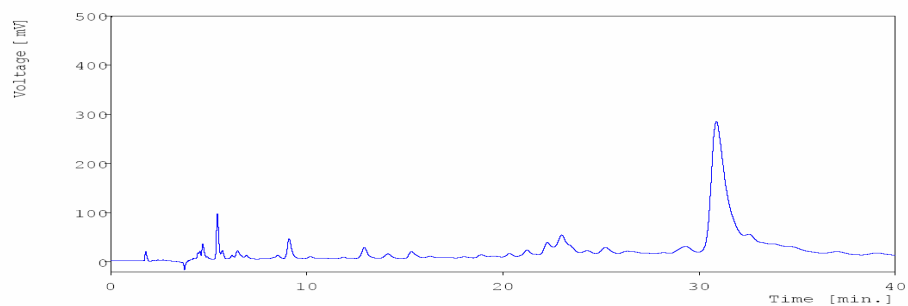


Figure S1.2. HPLC profiles of crude products. HPLC conditions: A) ON1. AE HPLC, 0-40 min, buffer A→A/B 2/8. B) ON 2. anion exchange column, 0-60 min, buffer A/B form 6/4 to 2/8. ON 3 and ON 4 : RP column, 0-40 min, buffer C→C/D 8/2.

MS spectra of synthesized oligo-RNAs.

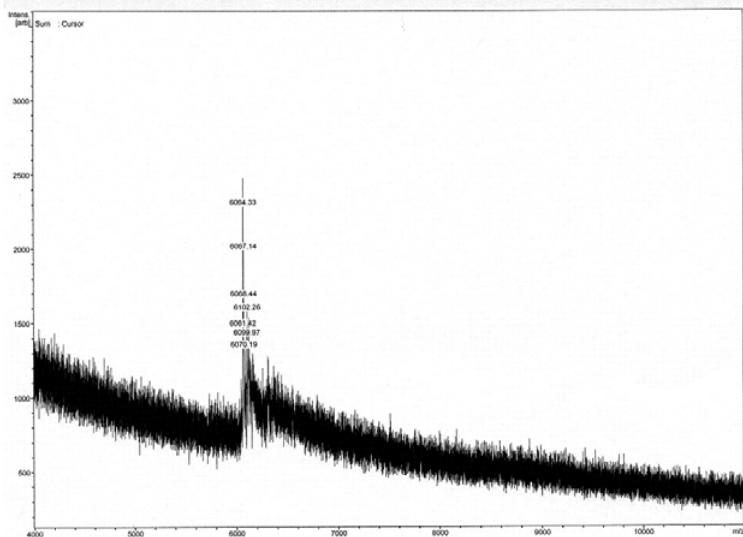


Figure S1.3. MALDI-TOF MS spectrum of ON 1.

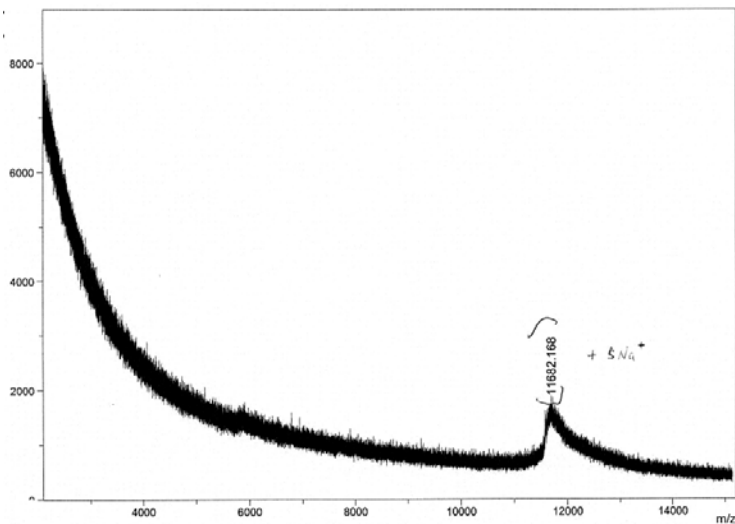


Figure S1.4. MALDI-TOF MS spectrum of ON 2.

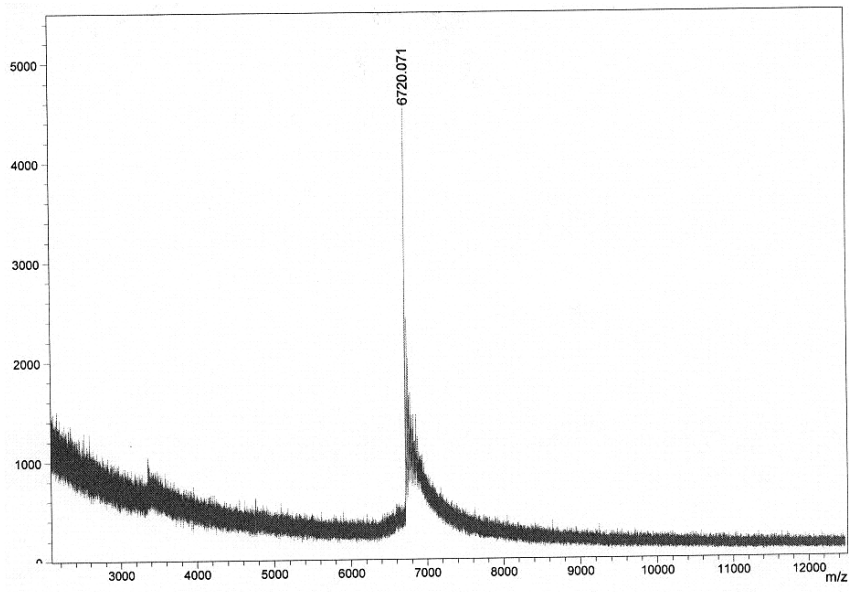


Figure S1.5. MALDI-TOF MS spectrum of ON 3.

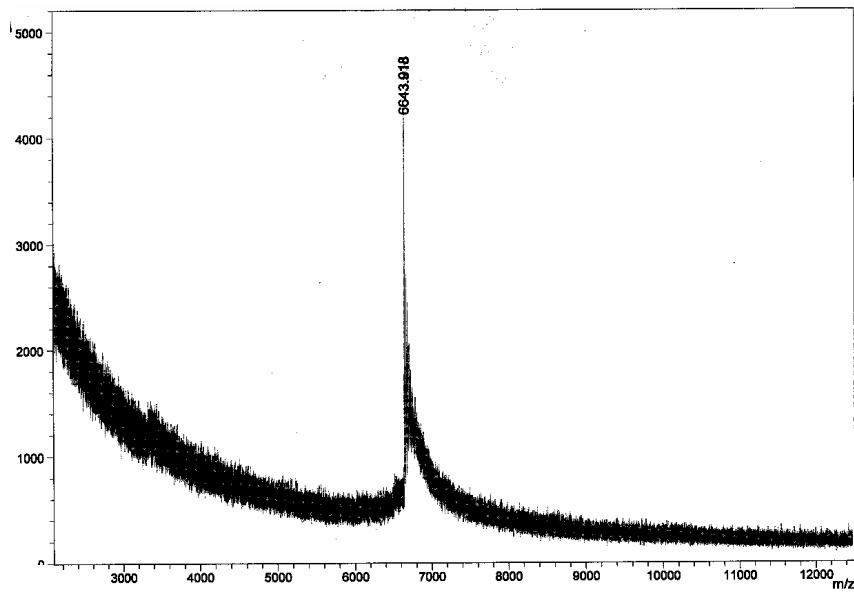


Figure S1.6. MALDI-TOF MS spectrum of ON 4.

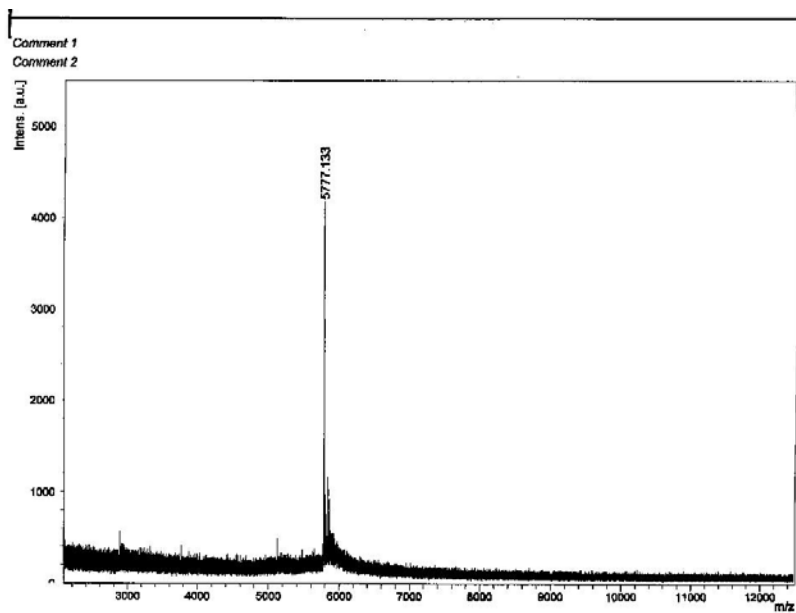


Figure S1.7. MALDI-TOF MS spectrum of ON 5.

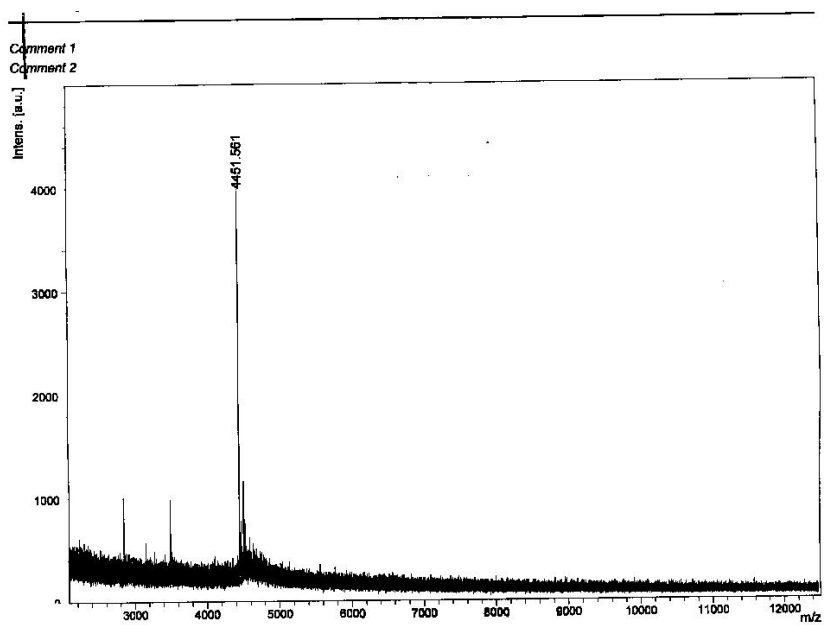


Figure S1.8. MALDI-TOF MS spectrum of ON 6.

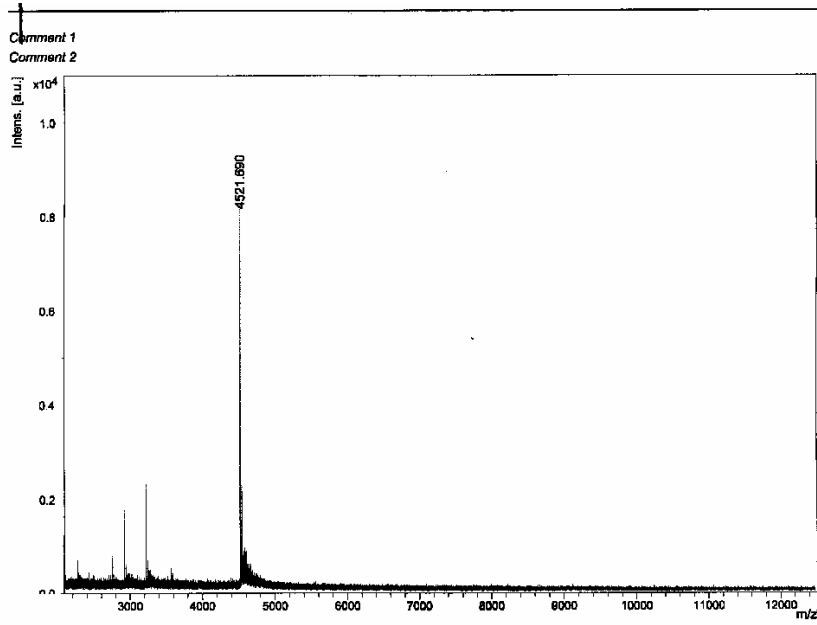


Figure S1.9. MALDI-TOF MS spectrums of ON 7.

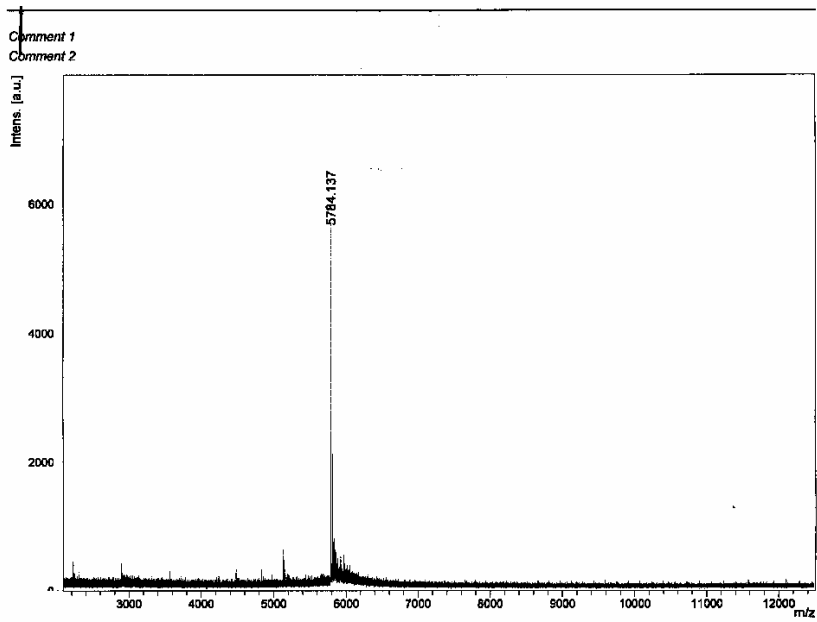


Figure S1.10. MALDI-TOF MS spectrums of ON 8...

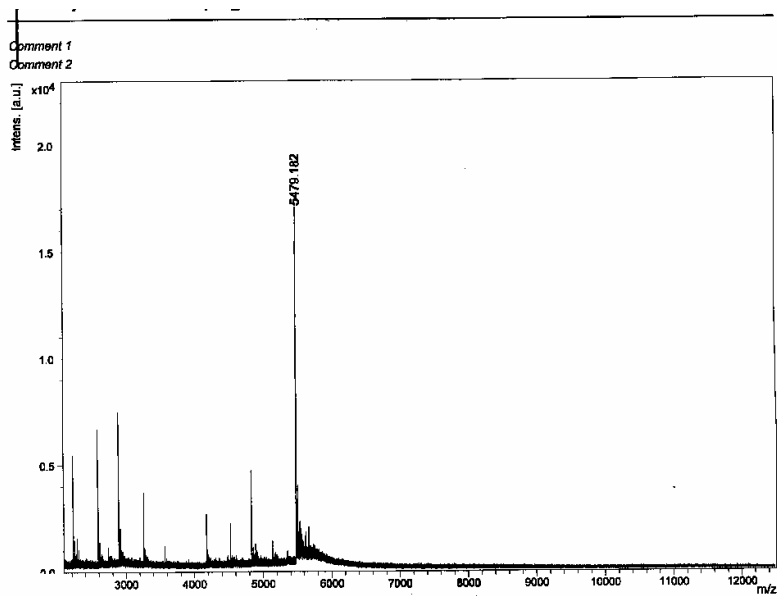


Figure S1.11. MALDI-TOF MS spectrums of ON 9.

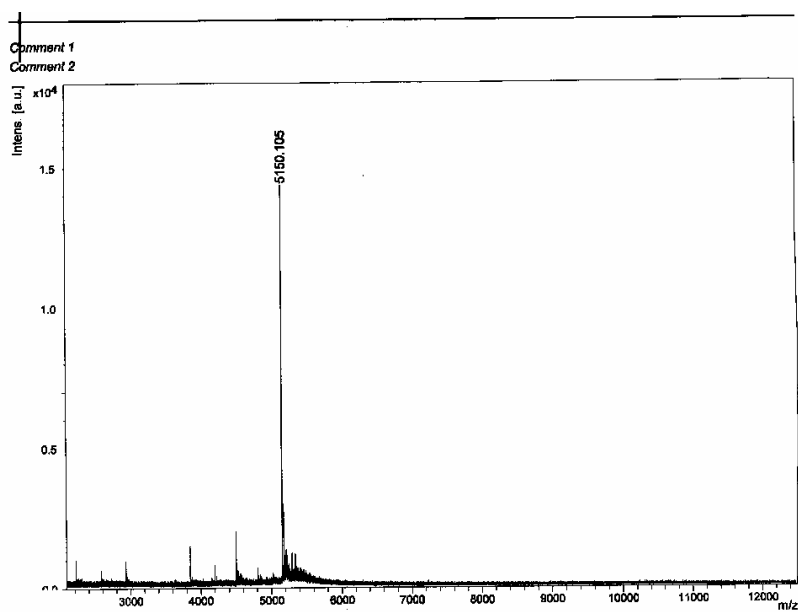


Figure S1.12. MALDI-TOF MS spectrums of ON 10.

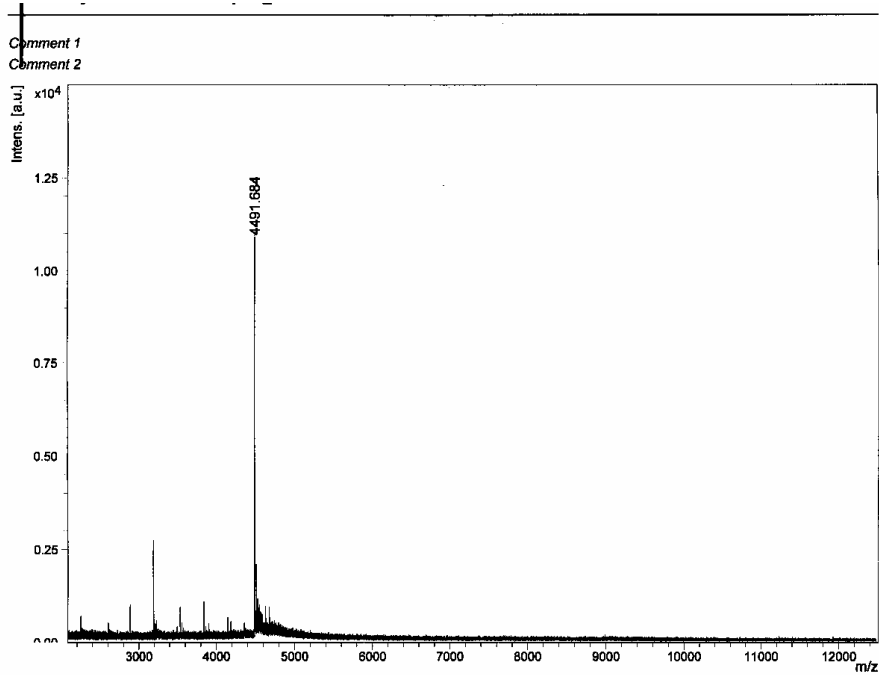


Figure S1.13. MALDI-TOF MS spectrums of ON 11.

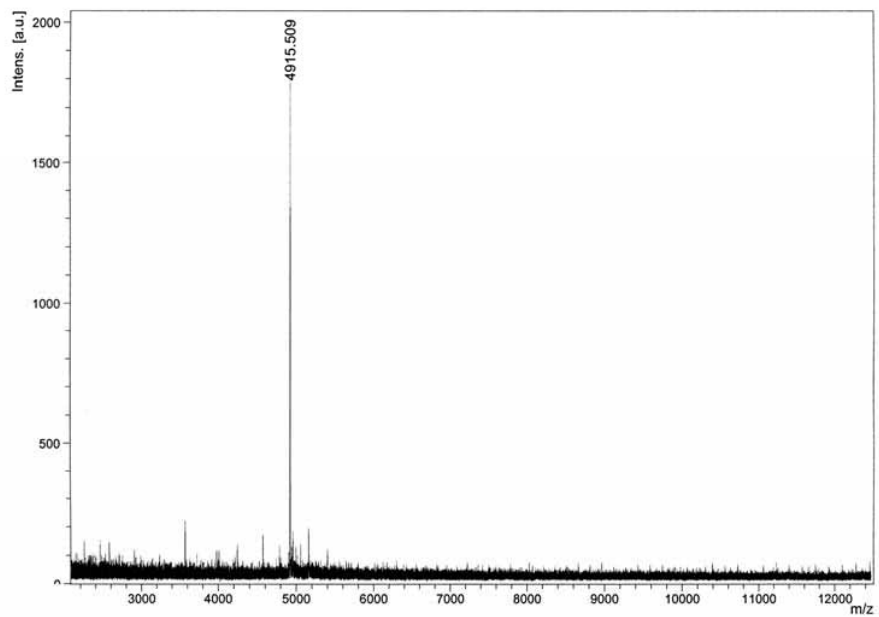


Figure S1.14. MALDI-TOF MS spectrums of ON 12.

^{31}P and ^1H NMR spectra of phosphoramidite.

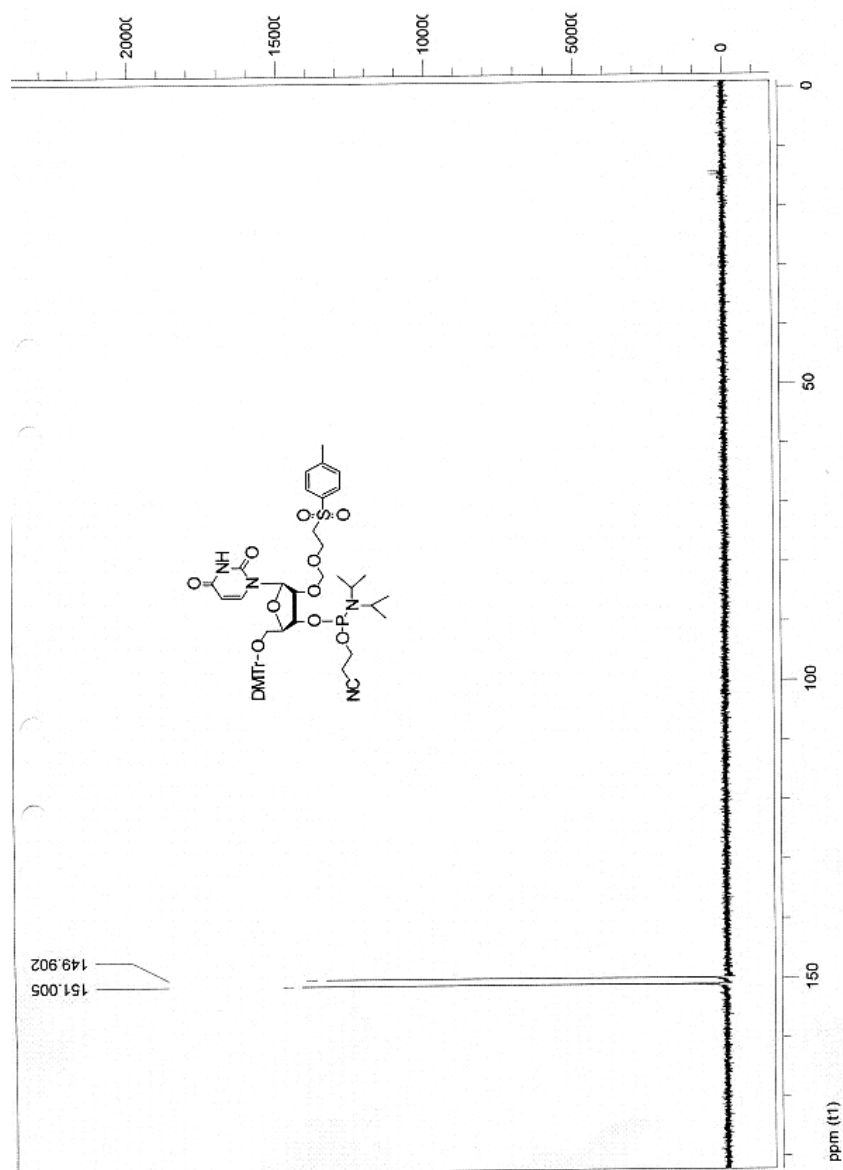


Figure S2.1. ^{31}P NMR spectrum of uridine phosphoramidite.

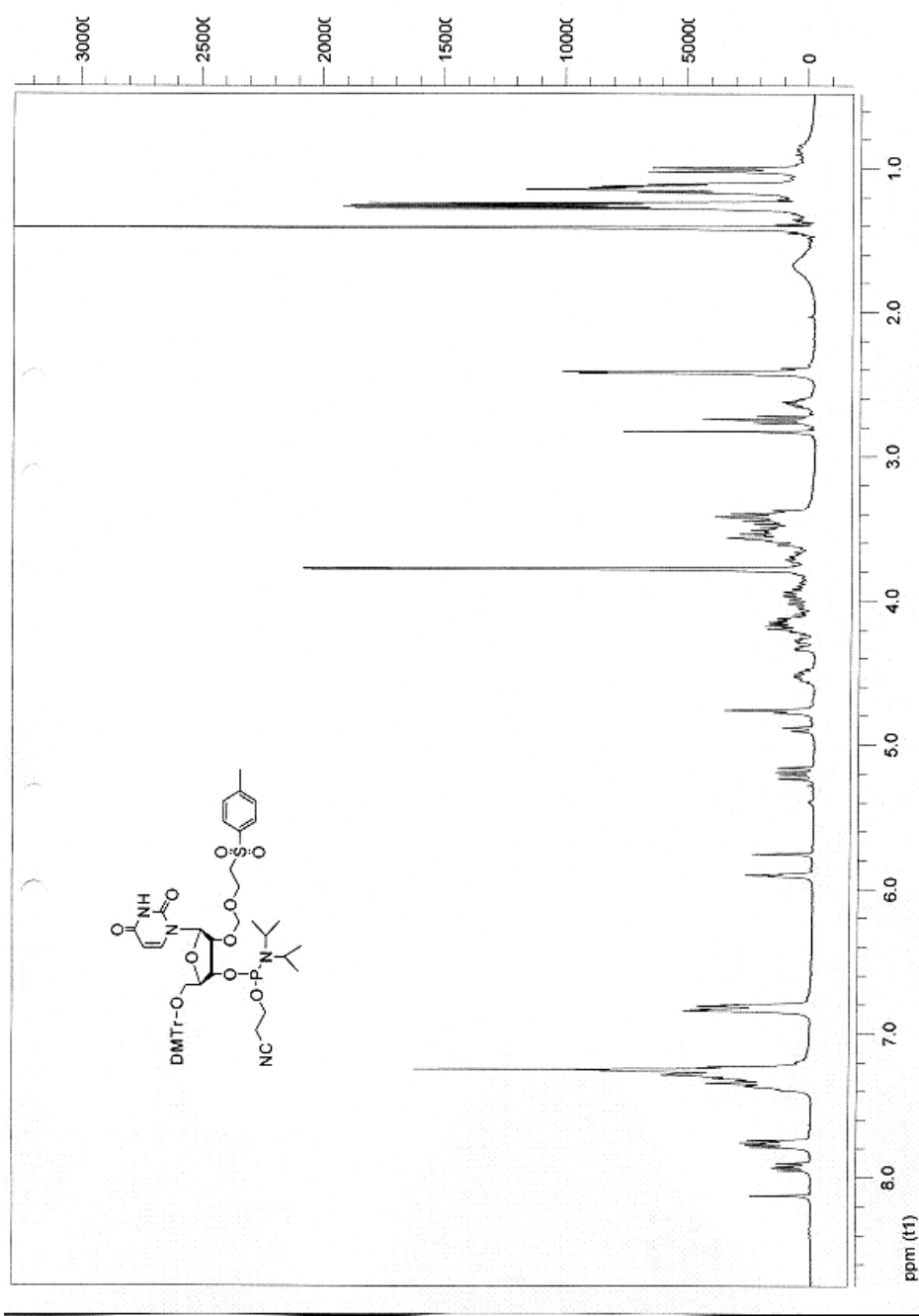


Figure S2.2. ¹H NMR spectrum of uridine phosphoramidite.

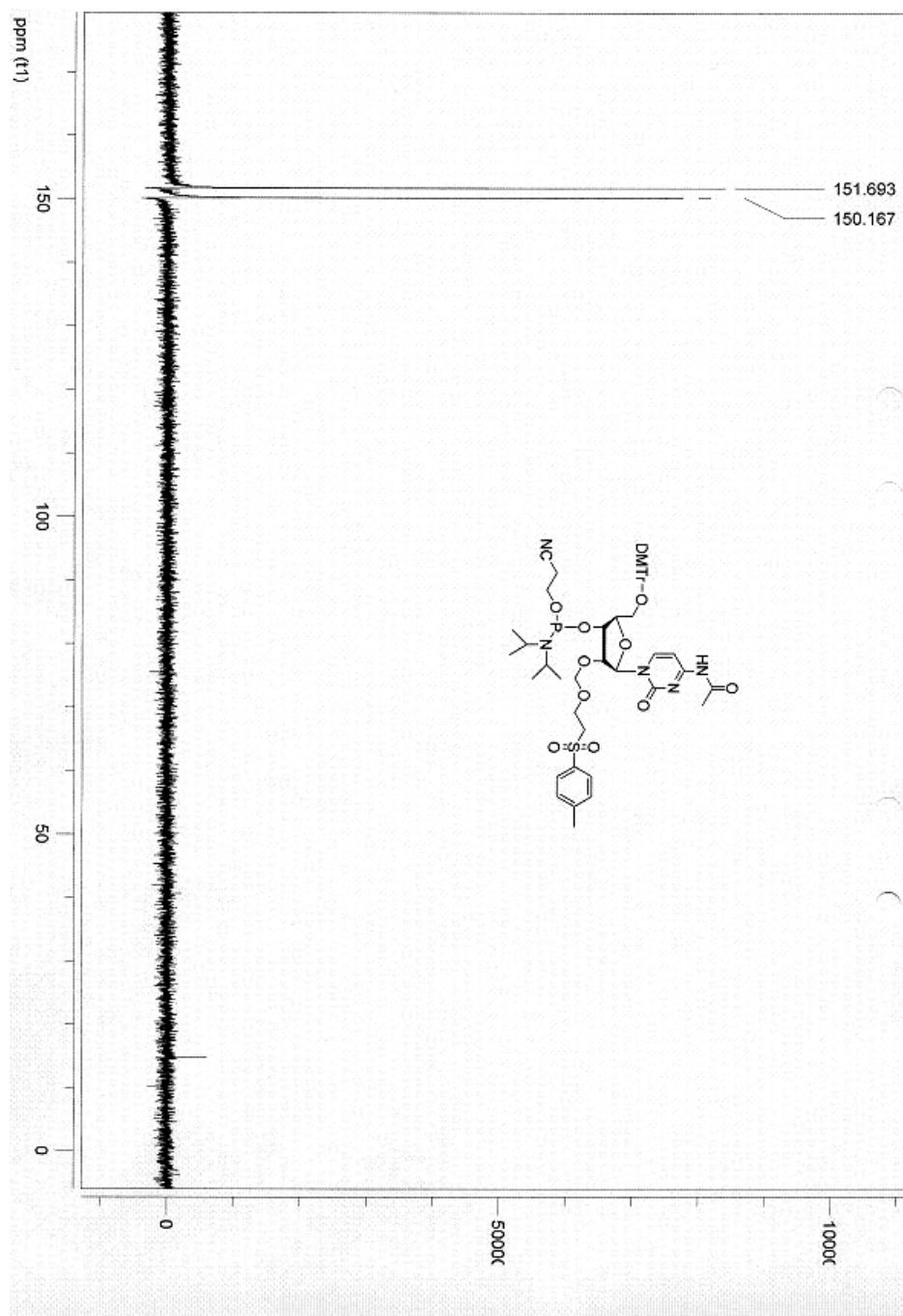


Figure S2.3. ^{31}P NMR spectrum of cytidine phosphoramidite.

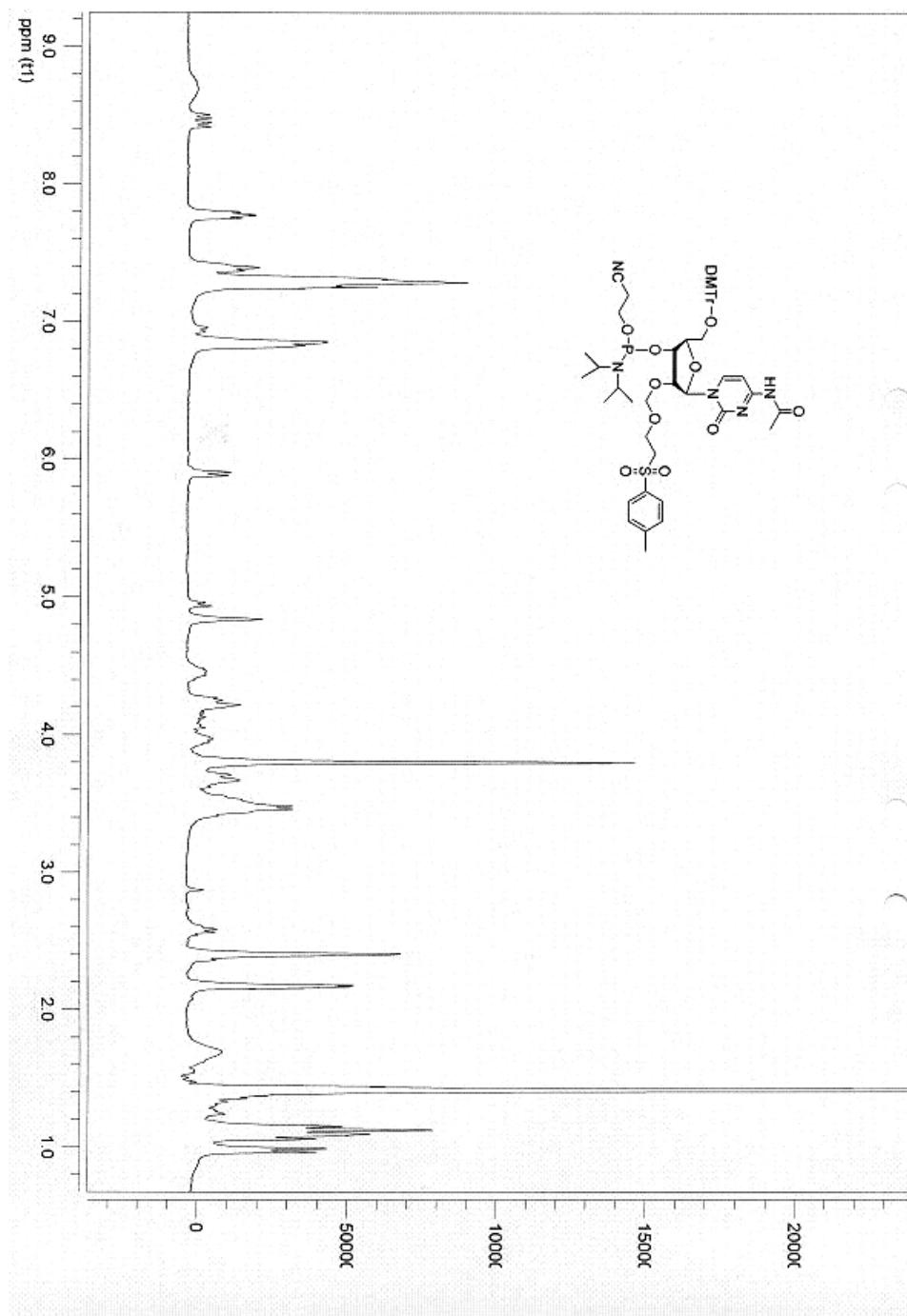


Figure S2.4. ¹H NMR spectrum of cytidine phosphoramidite.

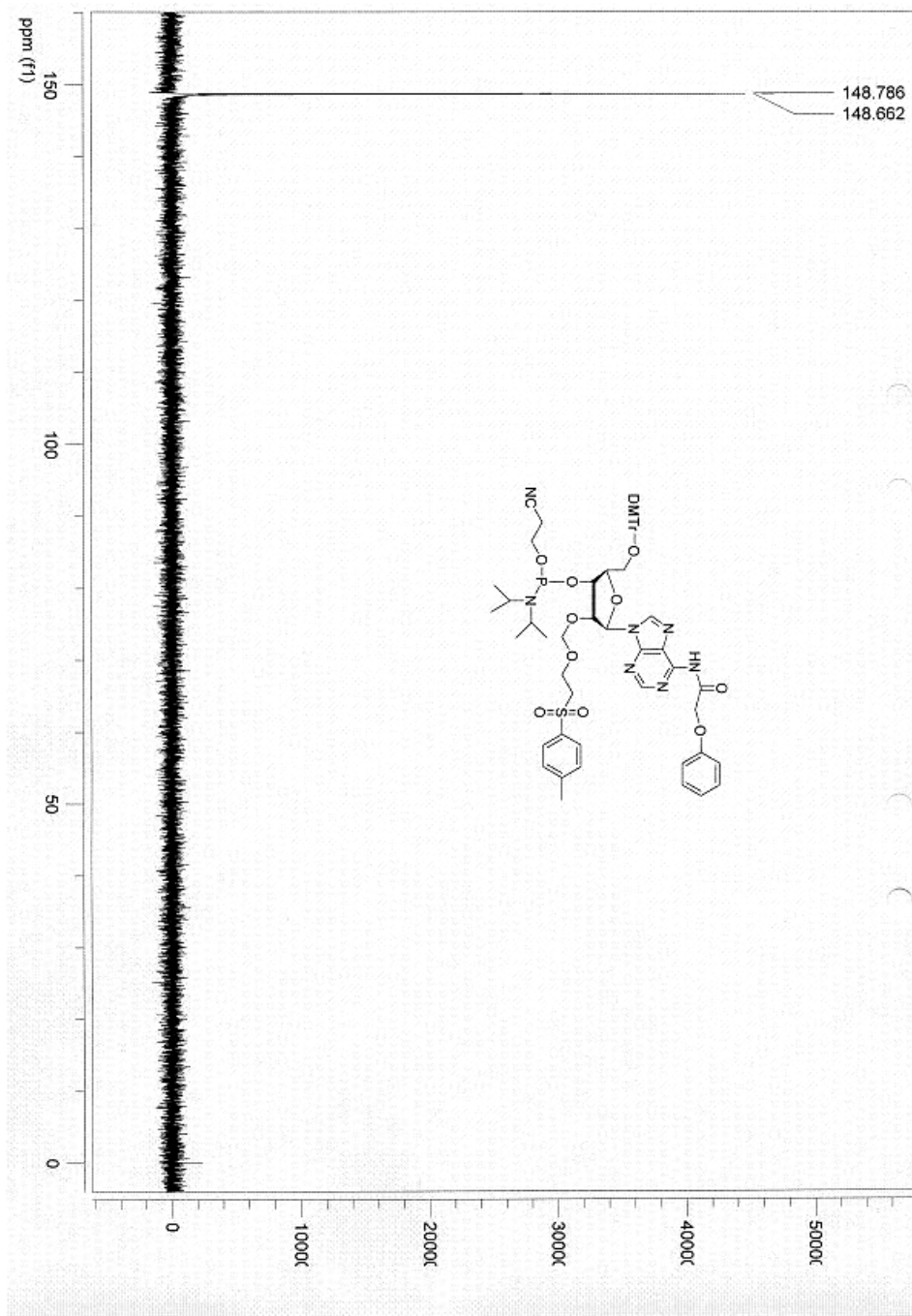


Figure S2.5. ^{31}P NMR spectrum of adenosine phosphoramidite.

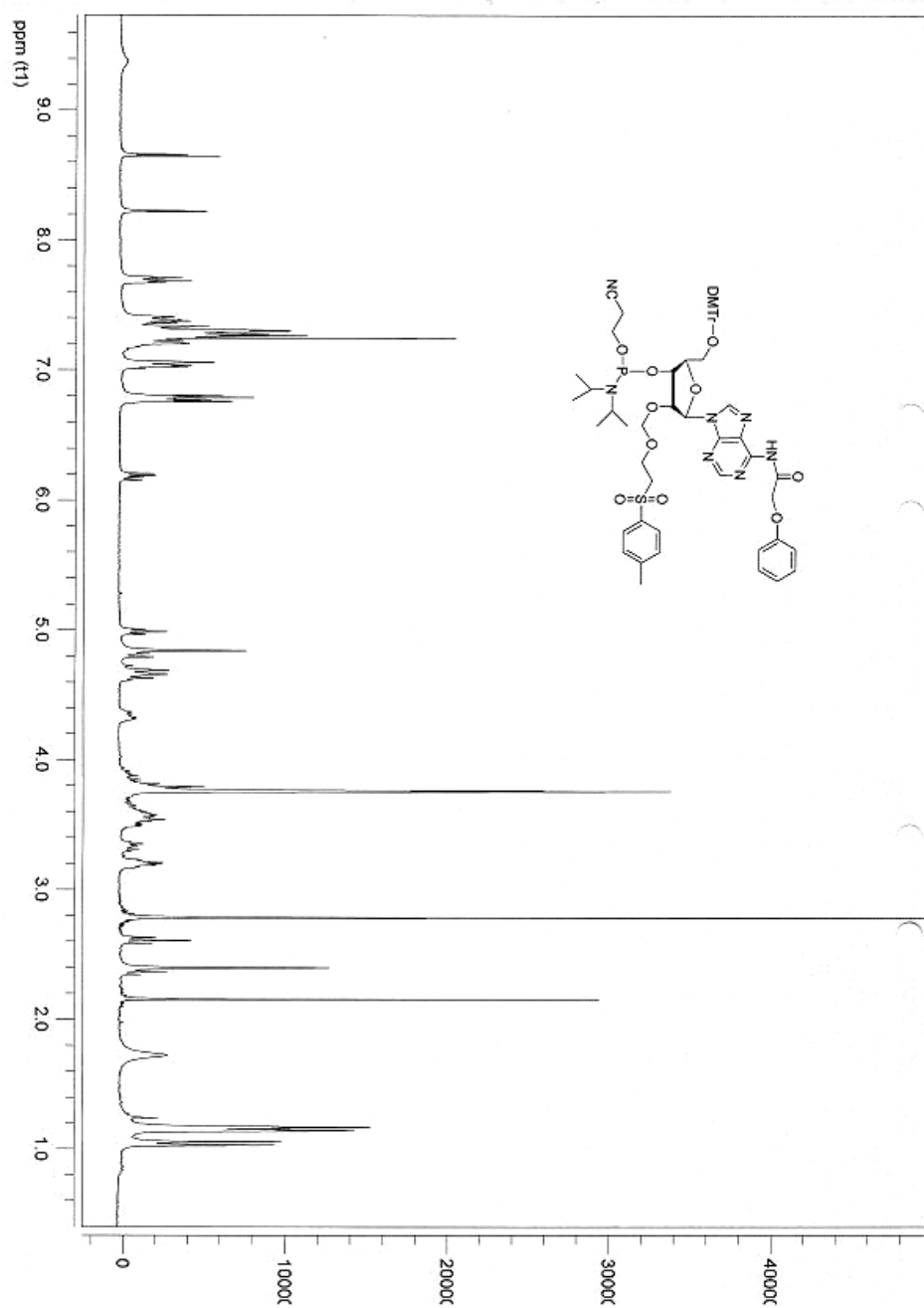


Figure S2.6. ^1H NMR spectrum of adenosine phosphoramidite.

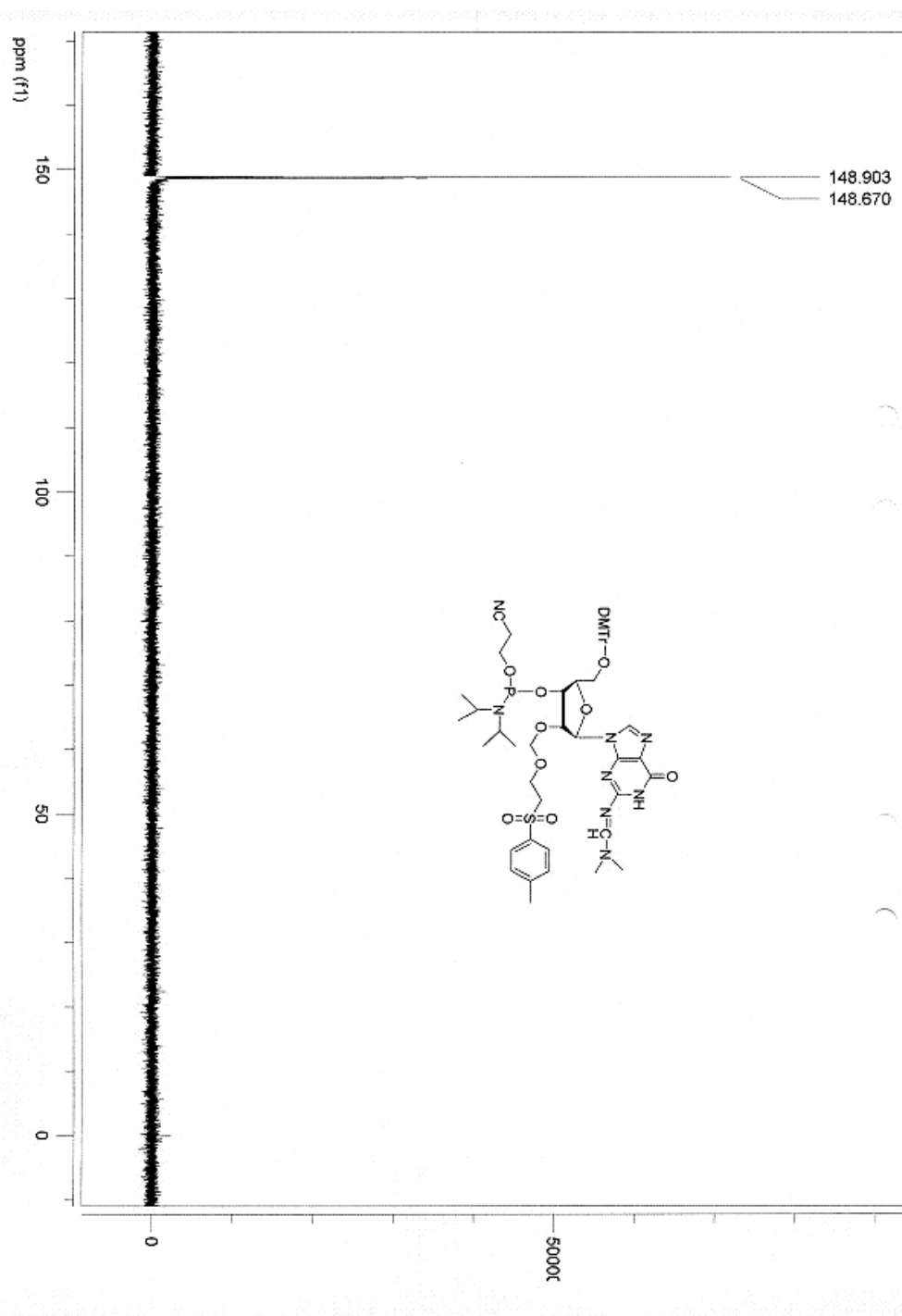


Figure S2.7. ^{31}P NMR spectrum of guanosine phosphoramidite.

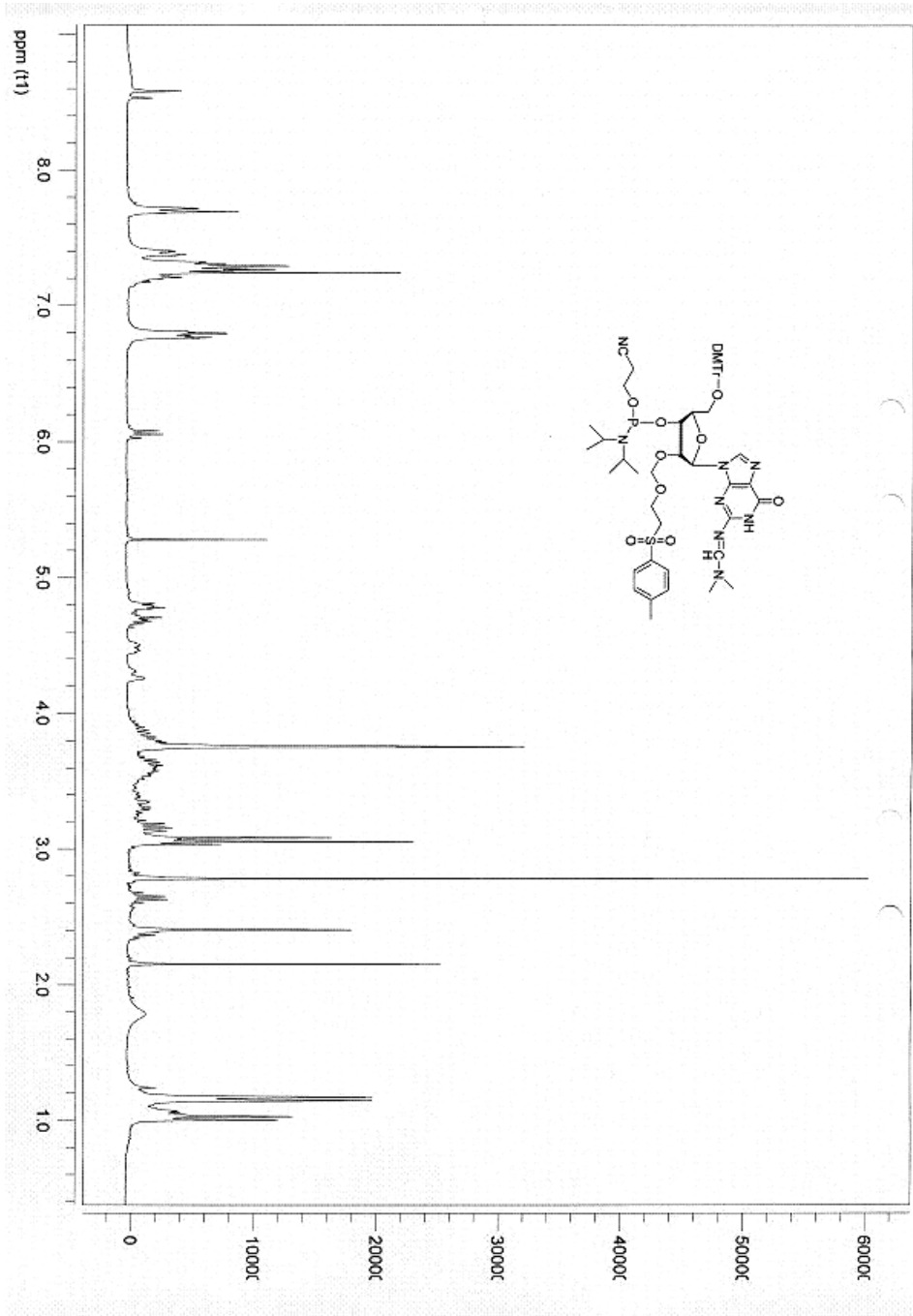


Figure S2.8. ^1H NMR spectrum of guanosine phosphoramidite.

^1H and ^{13}C NMR spectra of 5'-*O*-DMTr-2'-TEM nucleoside.

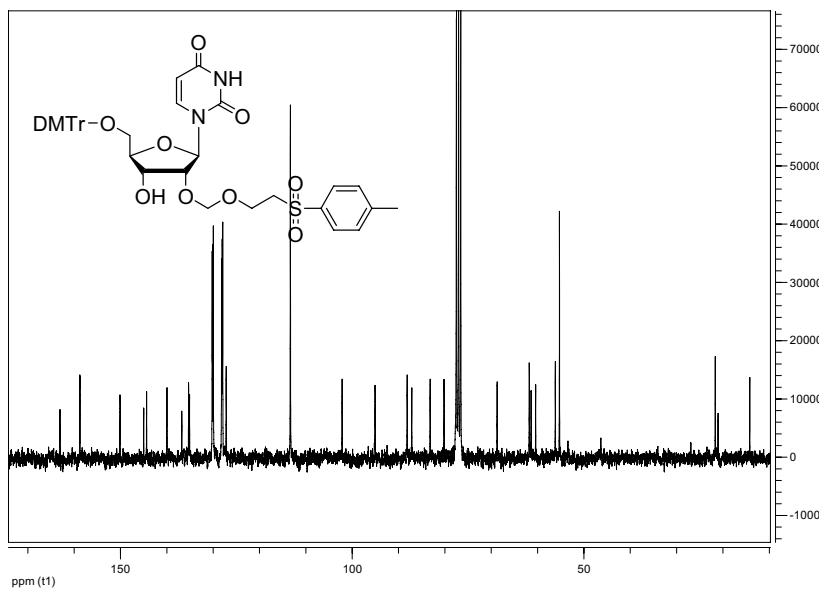
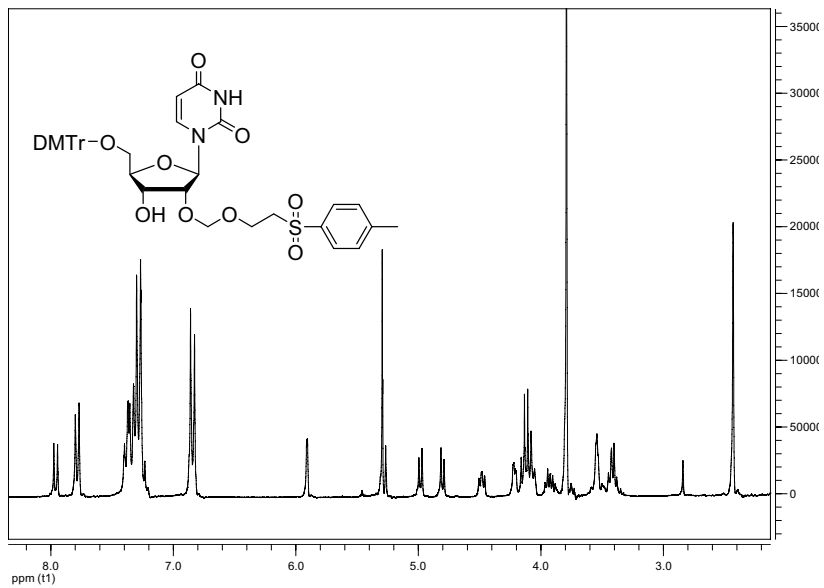


Figure S2.9. ^1H and ^{13}C NMR spectra of 5'-*O*-DMTr-2'-TEM uridine.

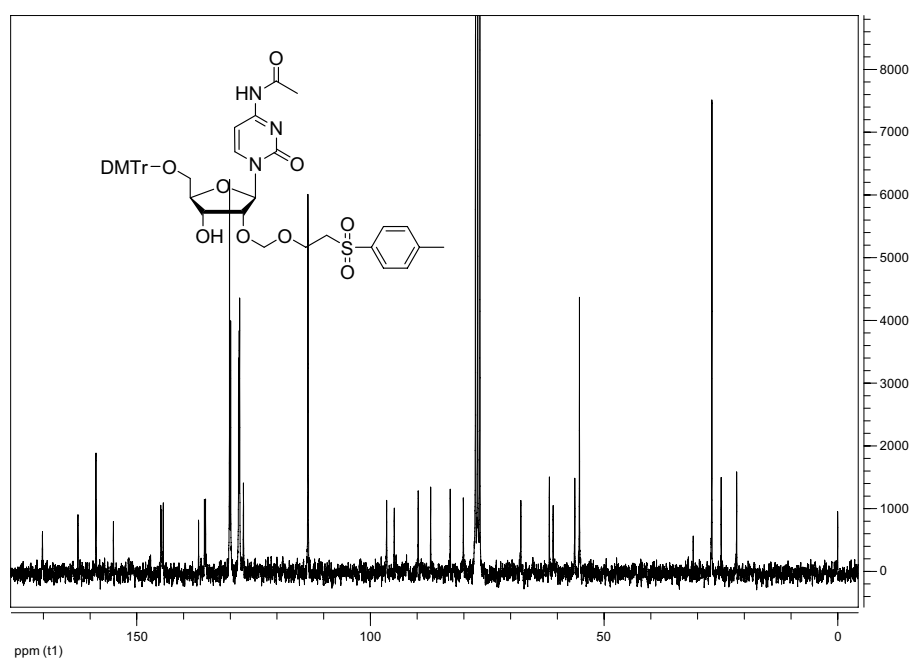
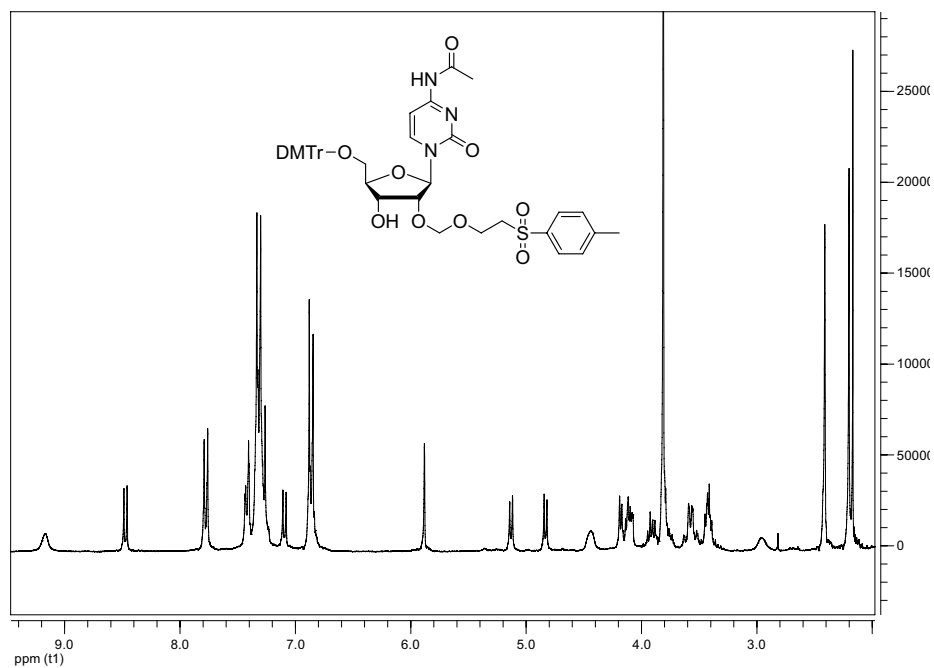


Figure S2.10. ^1H and ^{13}C NMR spectra of 5'-O-DMTr-2'-TEM cytidine.

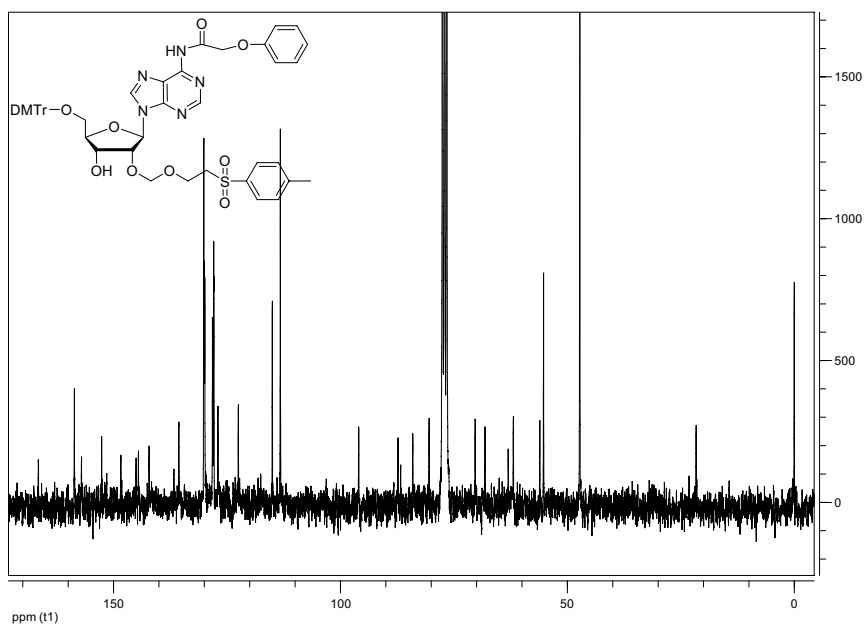
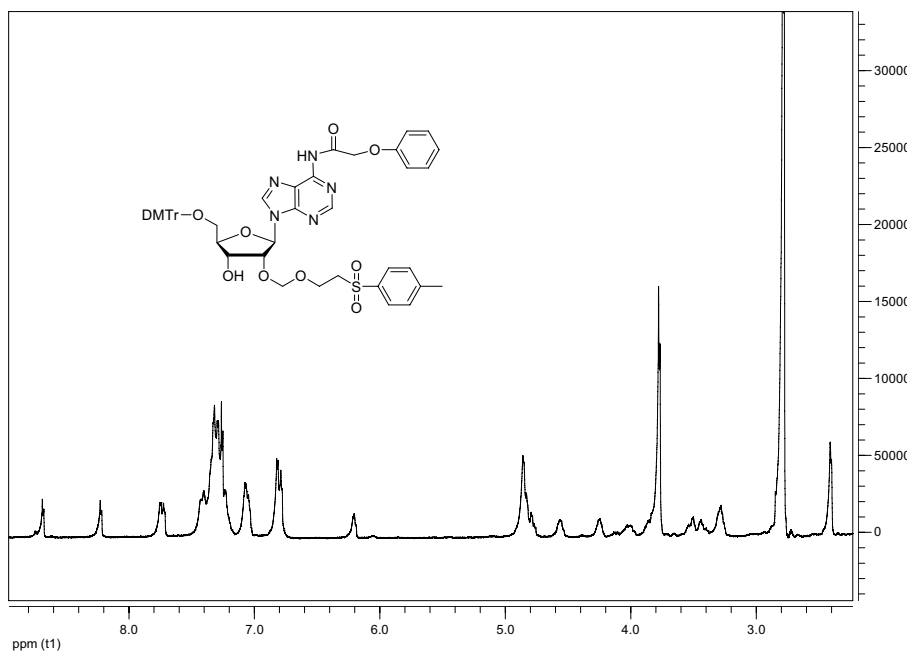


Figure S2.11. ^1H and ^{13}C NMR spectra of 5'-O-DMTr-2'-TEM adenosine.

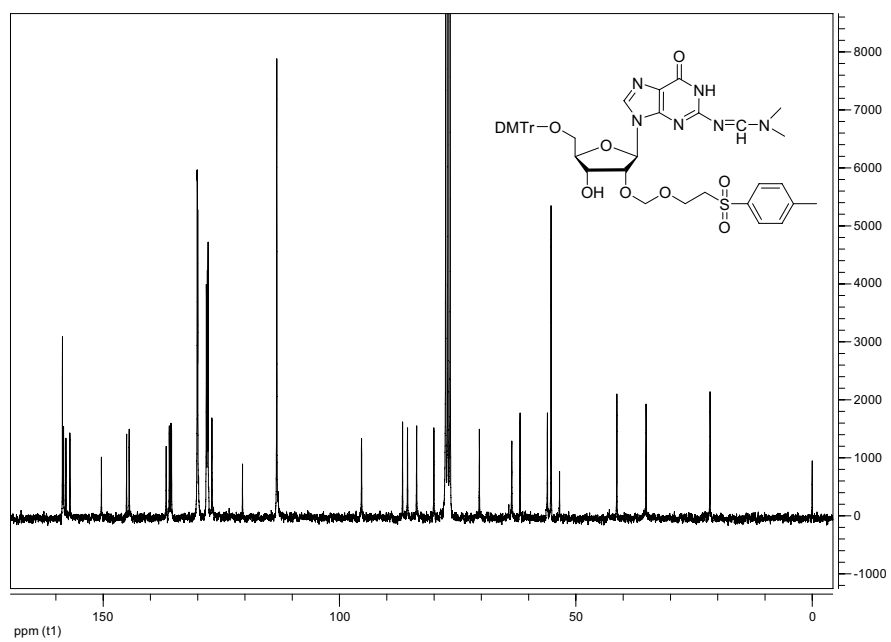
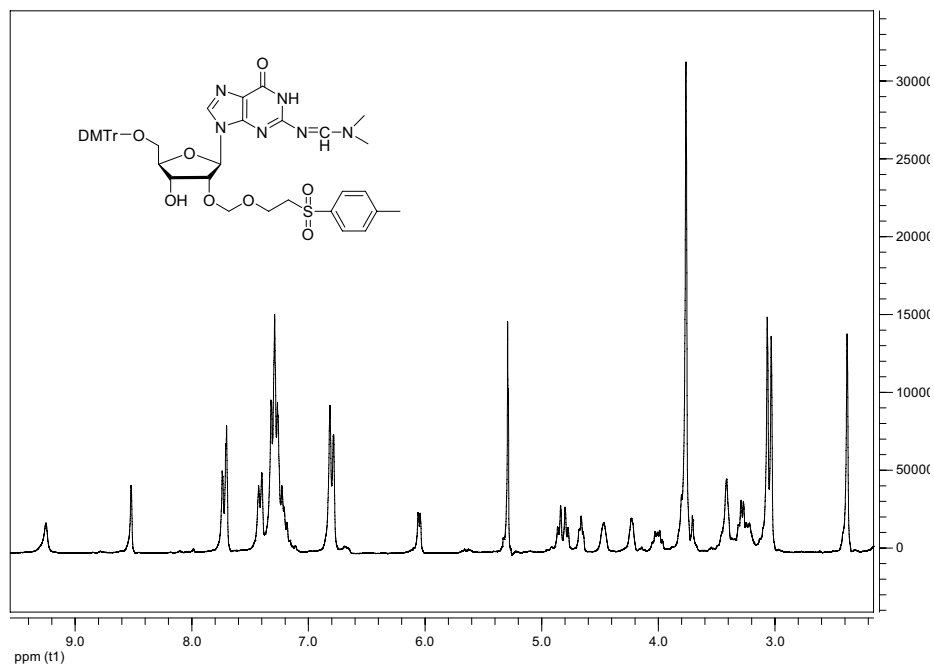


Figure S2.12. ^1H and ^{13}C NMR spectra of 5'-O-DMTr-2'-TEM guanosine.

HPLC profiles of crude ON 3 and 4 under different unblocking conditions.

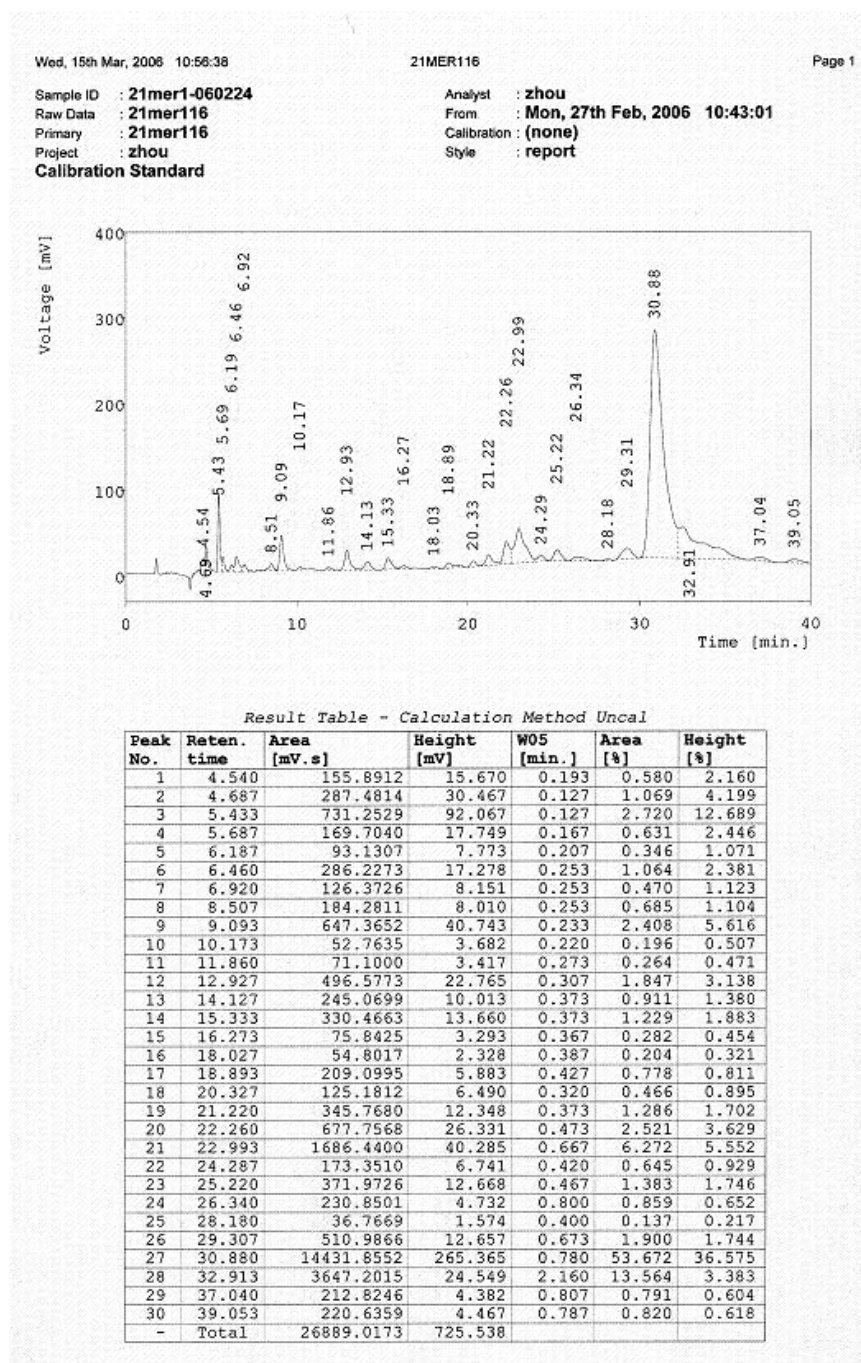
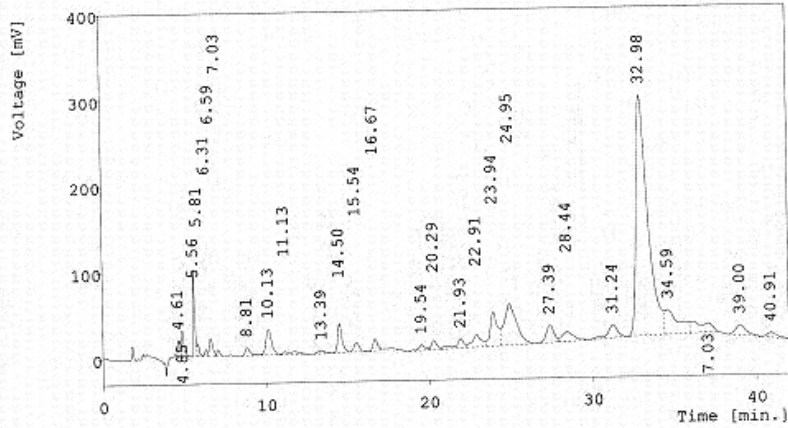


Figure S3.1

Sample ID : 21mer1-060224
 Raw Data : 21mer117
 Primary : 21mer117
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Mon, 27th Feb, 2006 18:26:45
 Calibration : (none)
 Style : report



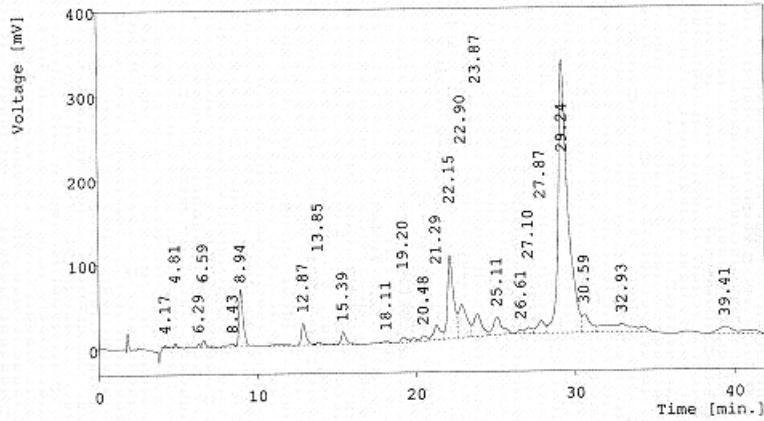
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.607	191.1781	18.272	0.193	0.671	2.236
2	4.853	276.1546	30.350	0.133	0.969	3.714
3	5.560	823.5623	100.948	0.127	2.889	12.352
4	5.807	133.8423	14.279	0.153	0.470	1.747
5	6.307	83.1497	7.603	0.200	0.292	0.930
6	6.587	297.7748	20.489	0.233	1.045	2.507
7	7.033	85.7459	6.946	0.187	0.301	0.850
8	8.813	195.4787	8.913	0.313	0.686	1.091
9	10.133	739.6879	30.317	0.360	2.595	3.710
10	11.133	185.4169	3.371	1.147	0.650	0.413
11	13.393	105.8221	3.484	0.460	0.371	0.426
12	14.500	627.8841	34.111	0.267	2.203	4.174
13	15.540	257.4576	10.975	0.353	0.903	1.343
14	16.673	260.5721	13.820	0.293	0.914	1.691
15	19.540	171.5488	6.547	0.373	0.602	0.801
16	20.287	251.4269	10.935	0.340	0.882	1.338
17	21.933	381.4677	10.857	0.380	1.338	1.328
18	22.907	591.8062	14.839	0.573	2.076	1.816
19	23.940	1211.0865	39.540	0.627	4.249	4.838
20	24.953	2540.6790	48.059	0.847	8.913	5.881
21	27.393	676.7945	20.289	0.520	2.374	2.483
22	28.440	553.6175	10.867	0.820	1.942	1.330
23	31.240	618.4824	15.207	0.553	2.170	1.861
24	32.980	13853.6421	279.492	0.713	48.603	34.199
25	34.593	1664.5863	28.437	0.760	5.840	3.480
26	37.033	932.4753	10.491	1.473	3.271	1.284
27	39.000	531.7612	12.042	0.640	1.866	1.473
28	40.907	260.8151	5.768	0.607	0.915	0.704
-	Total	28503.9167	817.252			

Figure S3.2

Sample ID : 21mer1-0602220
 Raw Data : 21mer118
 Primary : 21mer118
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Tue, 28th Feb, 2006 16:57:29
 Calibration : (none)
 Style : report



Result Table - Calculation Method Uncal

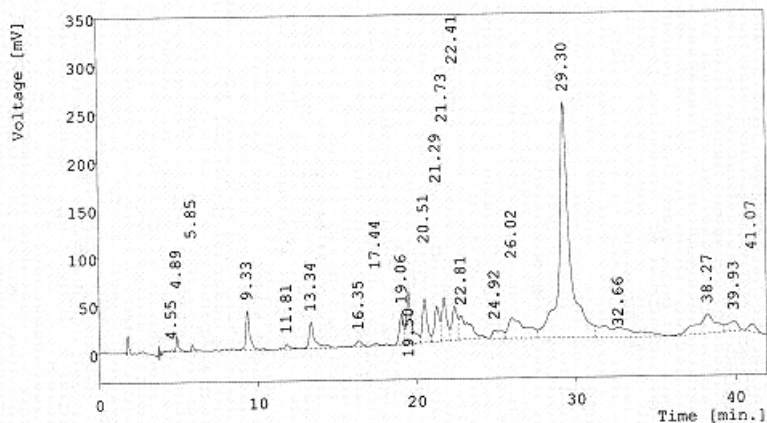
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.173	23.8749	3.811	0.107	0.089	0.515
2	4.813	73.6644	5.212	0.147	0.276	0.704
3	6.293	43.1082	3.995	0.200	0.161	0.540
4	6.593	163.3205	8.804	0.233	0.611	1.189
5	8.433	113.8712	2.841	0.700	0.426	0.384
6	8.940	1176.5041	66.540	0.267	4.403	8.990
7	12.873	614.9826	25.828	0.293	2.301	3.489
8	13.847	78.2672	3.254	0.360	0.293	0.440
9	15.393	342.9642	14.871	0.307	1.283	2.009
10	18.113	89.1900	2.819	0.407	0.334	0.381
11	19.200	159.4787	6.254	0.440	0.597	0.845
12	20.480	247.6766	6.347	0.440	0.927	0.857
13	21.293	536.2703	17.528	0.600	2.007	2.368
14	22.153	2585.9979	98.313	0.393	9.677	13.283
15	22.900	1482.9093	39.223	0.700	5.549	5.299
16	23.867	970.2492	26.299	0.613	3.631	3.553
17	25.107	812.9369	20.670	0.500	3.042	2.793
18	26.607	95.5991	4.071	0.407	0.358	0.550
19	27.100	193.6094	6.799	0.593	0.725	0.919
20	27.873	498.0154	14.767	0.613	1.864	1.995
21	29.240	13472.1677	323.559	0.547	50.414	43.715
22	30.587	759.4316	20.897	0.540	2.842	2.823
23	32.933	1328.3291	9.465	2.480	4.971	1.279
24	39.407	860.6606	7.989	1.067	3.219	1.080
-	Total	26723.0791	740.153			

③

Figure S3.3.

Sample ID : 21mer2-0602224
 Raw Data : 21mer2-2
 Primary : 21mer2-2
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Wed, 1st Mar, 2006 15:51:47
 Calibration : (none)
 Style : report



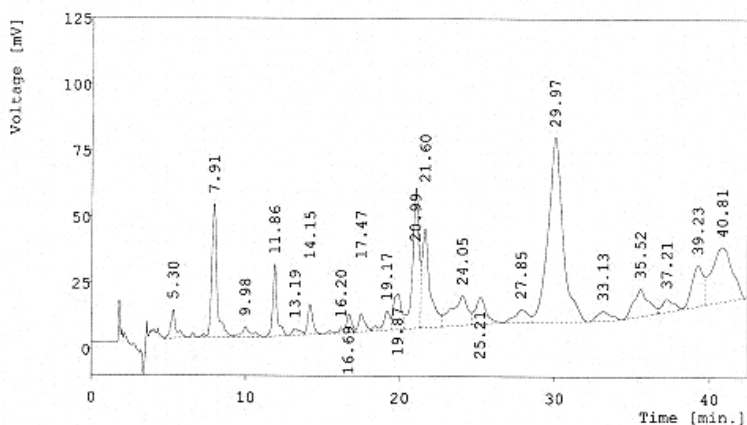
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.547	11.2822	0.927	0.193	0.043	0.133
2	4.887	156.6739	15.580	0.127	0.598	2.230
3	5.853	68.1481	6.254	0.160	0.260	0.895
4	9.327	811.1541	40.421	0.220	3.095	5.785
5	11.807	123.9648	5.346	0.247	0.473	0.765
6	13.340	676.7147	26.856	0.280	2.582	3.844
7	16.347	174.7687	6.070	0.380	0.667	0.869
8	17.440	94.6245	2.866	0.333	0.361	0.410
9	19.060	584.6438	31.029	0.340	2.231	4.441
10	19.500	1234.5845	54.016	0.287	4.710	7.731
11	20.513	940.1233	45.503	0.307	3.587	6.512
12	21.287	776.1521	36.325	0.387	2.961	5.199
13	21.727	978.9635	44.829	0.360	3.735	6.416
14	22.407	824.3803	34.655	0.440	3.145	4.960
15	22.813	1130.5995	24.638	0.880	4.313	3.526
16	24.920	367.1730	8.495	0.820	1.401	1.216
17	26.020	1631.0312	20.856	1.600	6.223	2.985
18	29.300	11447.0512	245.303	0.487	43.673	35.107
19	32.660	1709.7145	10.220	2.327	6.523	1.463
20	38.267	1687.0722	19.888	1.160	6.437	2.846
21	39.933	500.3685	10.376	0.933	1.909	1.485
22	41.067	281.6484	8.278	0.540	1.073	1.182
-	Total	26210.8369	698.729			

Figure S3.4.

Sample ID : 21mer1-0602220
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 Primary : 21mer119
 Project : zhou
Calibration Standard

Analyst : zhou
 From : Fri, 3rd Mar, 2006 11:43:34
 Calibration : (none)
 Style : report



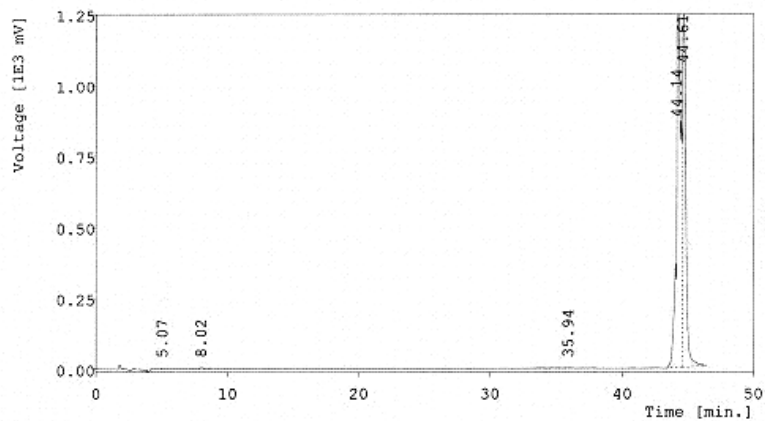
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	5.300	256.5522	10.717	0.293	1.543	2.796
2	7.913	1234.1828	50.494	0.320	7.424	13.172
3	9.980	166.2121	3.894	0.373	1.000	1.016
4	11.860	521.3368	27.197	0.240	3.136	7.095
5	13.187	79.9868	2.484	0.613	0.481	0.648
6	14.147	258.5239	11.434	0.333	1.555	2.983
7	16.200	59.7749	1.872	0.307	0.360	0.488
8	16.687	164.2585	7.021	0.353	0.988	1.831
9	17.473	184.6807	6.721	0.420	1.111	1.753
10	19.167	223.6841	7.214	0.440	1.346	1.882
11	19.873	487.3455	13.348	0.600	2.932	3.482
12	20.993	1502.3377	52.872	0.460	9.037	13.792
13	21.600	1358.7590	37.439	0.513	8.174	9.766
14	24.053	795.0137	11.139	1.307	4.782	2.906
15	25.207	499.0604	10.124	0.733	3.002	2.641
16	27.853	293.8092	4.952	1.027	1.767	1.292
17	29.973	4630.5768	69.620	0.920	27.855	18.161
18	33.127	221.1330	3.510	1.173	1.330	0.916
19	35.520	682.3452	10.232	1.213	4.105	2.669
20	37.213	254.6058	4.749	1.033	1.532	1.239
21	39.233	809.6426	15.604	0.933	4.870	4.071
22	40.813	1940.0089	20.713	1.647	11.670	5.401
-	Total	16623.8304	383.352			

Figure S3.5.

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 Primary : 21mer120
 Project : zhou
Calibration Standard

Analyst : zhou
 From : Fri, 3rd Mar, 2006 16:01:23
 Calibration : (none)
 Style : report



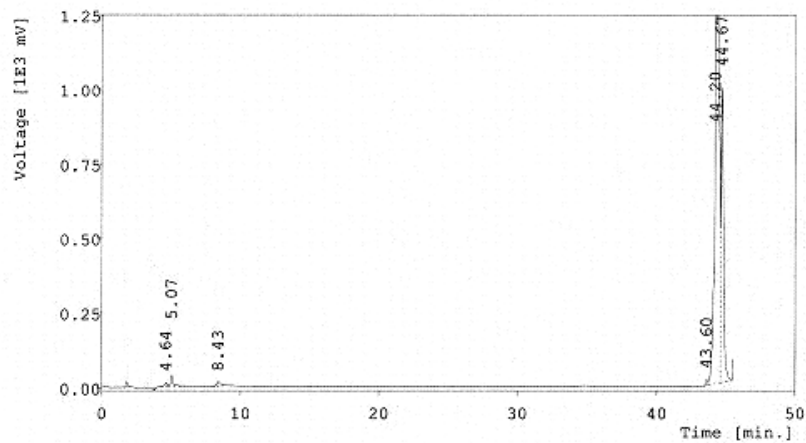
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	5.073	24.7464	2.654	0.140	0.046	0.106
2	8.020	130.7722	4.335	0.287	0.243	0.174
3	35.940	158.1237	1.372	0.440	0.294	0.055
4	44.140	31381.1747	1244.607	0.433	58.310	49.861
5	44.607	22123.3315	1243.167	0.280	41.107	49.804
-	Total	53818.1486	2496.134			

Figure S3.6.

Sample ID : 21mer1-0602224
 Raw Data : 21mer121
 Primary : 21mer121
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Mon, 6th Mar, 2006 16:26:13
 Calibration : (none)
 Style : report



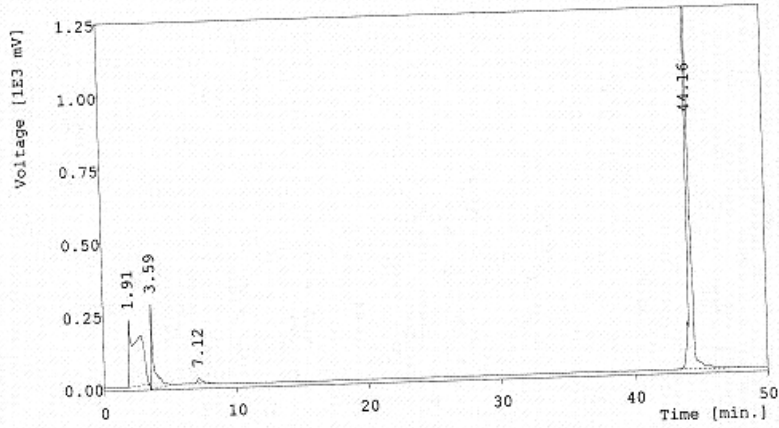
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.640	111.3069	12.776	0.113	0.254	0.554
2	5.067	435.5824	35.129	0.147	0.993	1.523
3	8.427	260.7563	13.332	0.287	0.594	0.578
4	43.600	150.3732	20.127	0.147	0.343	0.873
5	44.200	30176.1408	1237.310	0.407	68.796	53.637
6	44.673	12728.7824	988.148	0.213	29.020	42.835
-	Total	43862.9420	2306.822			

Figure S3.7.

Sample ID : 21mer1-0602224
Raw Data : 21mer122
Primary : 21mer122
Project : zhou
Calibration Standard

Analyst : zhou
From : Tue, 7th Mar, 2006 11:39:40
Calibration : (none)
Style : report



Result Table - Calculation Method Uncal

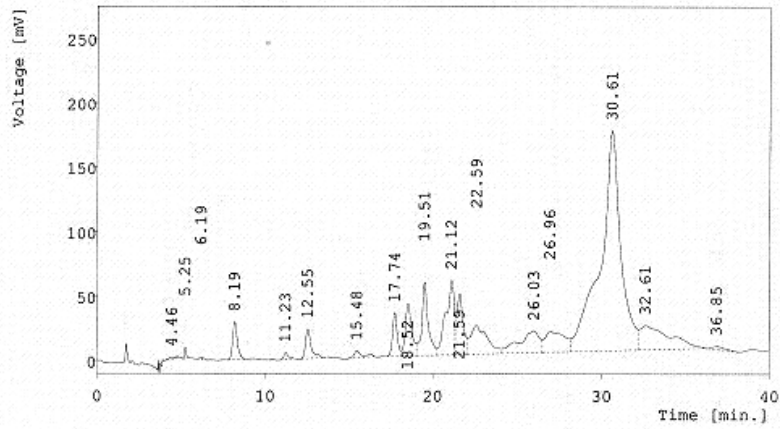
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	1.907	12158.8576	230.570	1.200	31.212	12.887
2	3.593	4111.0340	293.787	0.127	10.553	16.420
3	7.120	604.8310	18.872	0.360	1.553	1.055
4	44.160	22081.0828	1246.000	0.227	56.682	69.638
-	Total	38955.8054	1789.230			

Figure S3.8.

8

Sample ID : 21mer1-0602224
 Raw Data : 21mer123
 Primary : 21mer123
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Tue, 7th Mar, 2006 20:06:34
 Calibration : (none)
 Style : report



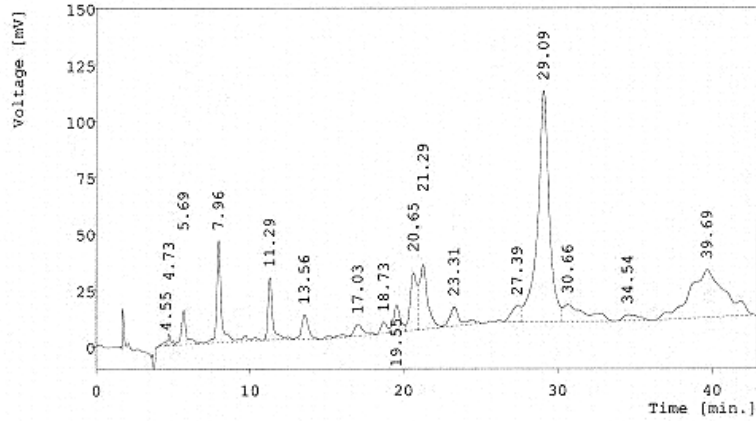
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.460	49.3355	1.687	0.667	0.168	0.299
2	5.247	70.6843	9.199	0.120	0.241	1.633
3	6.193	30.5340	1.813	0.220	0.104	0.322
4	8.187	566.2155	29.502	0.287	1.933	5.238
5	11.227	95.2518	5.294	0.267	0.325	0.940
6	12.553	542.5773	23.343	0.313	1.852	4.144
7	15.480	186.3593	5.769	0.340	0.636	1.024
8	17.740	655.1728	34.227	0.287	2.237	6.077
9	18.520	1098.3316	40.831	0.387	3.749	7.249
10	19.507	1531.9080	57.470	0.320	5.230	10.203
11	21.120	1984.5500	58.380	0.680	6.775	10.365
12	21.593	1317.5156	47.590	0.480	4.498	8.449
13	22.593	1564.6360	22.671	1.260	5.341	4.025
14	26.027	1451.1175	17.059	1.113	4.954	3.029
15	26.960	1323.0817	16.398	1.653	4.517	2.911
16	30.613	14383.5893	170.569	0.880	49.102	30.282
17	32.607	2316.0542	18.949	2.427	7.906	3.364
18	36.847	126.2726	2.516	0.680	0.432	0.446
-	Total	29293.1870	563.268			

Figure S3.9.

Sample ID : 21mer1-0602224
 Raw Data : 21mer124
 Primary : 21mer124
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Wed, 8th Mar, 2006 21:08:21
 Calibration : (none)
 Style : report



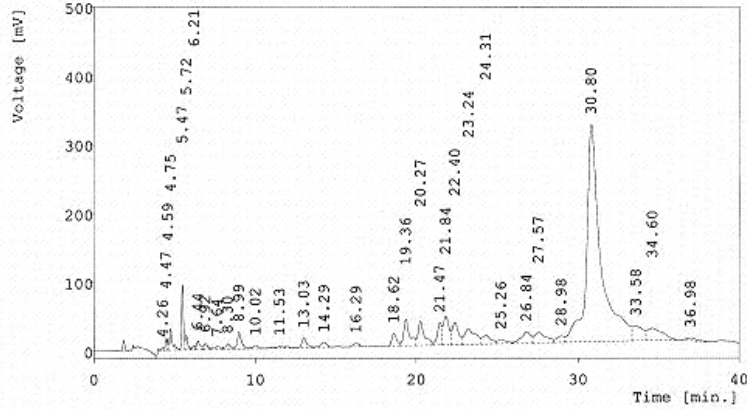
Result Table - Calculation Method Uncal

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.553	36.2000	2.046	0.293	0.236	0.618
2	4.733	70.0691	5.185	0.147	0.456	1.567
3	5.693	342.9990	15.288	0.287	2.233	4.620
4	7.960	1042.2392	45.151	0.253	6.785	13.644
5	11.287	585.0635	27.517	0.273	3.809	8.315
6	13.560	333.5481	10.864	0.447	2.171	3.283
7	17.033	285.5210	5.160	0.573	1.859	1.559
8	18.733	135.9159	4.997	0.467	0.885	1.510
9	19.553	370.2703	12.049	0.433	2.410	3.641
10	20.653	743.6990	25.222	0.507	4.841	7.622
11	21.287	1082.0897	28.510	0.607	7.044	8.615
12	23.313	395.9112	8.477	0.540	2.577	2.562
13	27.393	276.6690	6.951	0.673	1.801	2.100
14	29.093	5356.8608	102.476	0.673	34.871	30.967
15	30.660	763.0301	7.442	1.407	4.967	2.249
16	34.540	177.3239	2.628	1.233	1.154	0.794
17	39.693	3364.6302	20.959	2.593	21.901	6.334
-	Total	15362.0398	330.923			

Figure S3.10.

Sample ID : 21mer2-060224
 Raw Data : 21mer2-1
 Primary : 21mer2-1
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Mon, 27th Feb, 2006 11:47:07
 Calibration : (none)
 Style : report



Result Table - Calculation Method Uncal

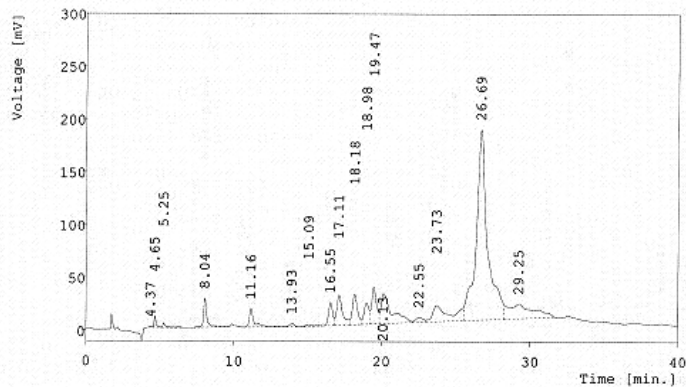
Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.260	32.0300	3.849	0.100	0.095	0.416
2	4.473	119.0869	16.892	0.113	0.354	1.827
3	4.593	107.1956	17.492	0.127	0.318	1.892
4	4.747	350.2760	31.421	0.133	1.041	3.399
5	5.473	733.6216	95.193	0.120	2.179	10.297
6	5.720	218.2045	21.477	0.173	0.648	2.323
7	6.207	86.8179	6.407	0.213	0.258	0.693
8	6.440	202.9084	12.693	0.207	0.603	1.373
9	6.920	146.4063	9.267	0.267	0.435	1.002
10	7.640	126.1506	3.960	0.593	0.375	0.428
11	8.300	166.3989	7.762	0.293	0.494	0.840
12	8.993	457.7123	24.393	0.240	1.360	2.639
13	10.020	93.6310	4.542	0.300	0.278	0.491
14	11.533	145.1511	3.388	0.340	0.431	0.366
15	13.027	296.6766	14.215	0.287	0.881	1.538
16	14.287	178.3365	7.082	0.367	0.530	0.766
17	16.287	123.8551	4.875	0.420	0.368	0.527
18	18.620	392.6208	18.792	0.313	1.166	2.033
19	19.360	994.8430	38.816	0.307	2.955	4.199
20	20.267	972.7175	35.101	0.347	2.889	3.797
21	21.473	609.1061	32.690	0.307	1.809	3.536
22	21.840	1018.0824	41.659	0.487	3.024	4.506
23	22.400	895.8309	32.294	0.500	2.661	3.493
24	23.240	1074.4374	22.574	0.940	3.192	2.442
25	24.313	480.8974	12.412	0.640	1.429	1.343
26	25.260	227.3574	6.408	0.660	0.675	0.693
27	26.840	668.5073	16.664	0.687	1.986	1.803
28	27.567	767.8368	15.372	0.920	2.281	1.663
29	28.980	301.6653	9.205	0.593	0.896	0.996
30	30.800	19336.3928	314.897	0.680	57.439	34.063
31	33.580	963.5682	20.825	0.867	2.862	2.253

(1-1)

Figure S3.11.

Sample ID : 21mer2-0602224
 Raw Data : 21mer2-3
 Primary : 21mer2-3
 Project : zhou
 Calibration Standard

Analyst : zhou
 From : Fri, 10th Mar, 2006 14:03:51
 Calibration : (none)
 Style : report



Result Table - Calculation Method Uncai

Peak No.	Reten. time	Area [mV.s]	Height [mV]	W05 [min.]	Area [%]	Height [%]
1	4.373	5.8963	0.631	0.160	0.032	0.143
2	4.653	102.4957	11.021	0.133	0.553	2.492
3	5.253	80.8893	4.462	0.133	0.437	1.009
4	8.040	440.3505	27.498	0.200	2.378	6.217
5	11.160	390.7956	17.494	0.253	2.110	3.955
6	13.933	104.2970	3.216	0.360	0.563	0.727
7	15.087	75.0544	1.859	0.453	0.405	0.420
8	16.547	418.0514	21.869	0.313	2.257	4.944
9	17.113	716.6348	28.588	0.367	4.193	6.463
10	18.180	689.4104	28.793	0.360	3.723	6.510
11	18.980	476.5012	20.055	0.433	2.573	4.534
12	19.467	790.3087	34.889	0.360	4.267	7.888
13	20.127	1446.1226	28.399	0.553	7.808	6.421
14	22.553	161.3655	4.456	0.660	0.871	1.007
15	23.727	1235.1346	15.313	0.813	6.669	3.462
16	26.693	9648.0064	179.905	0.587	52.095	40.675
17	29.253	1678.6563	13.853	2.553	9.066	3.133
-	Total	18519.9710	442.302			

(2-2)

Figure S3.12

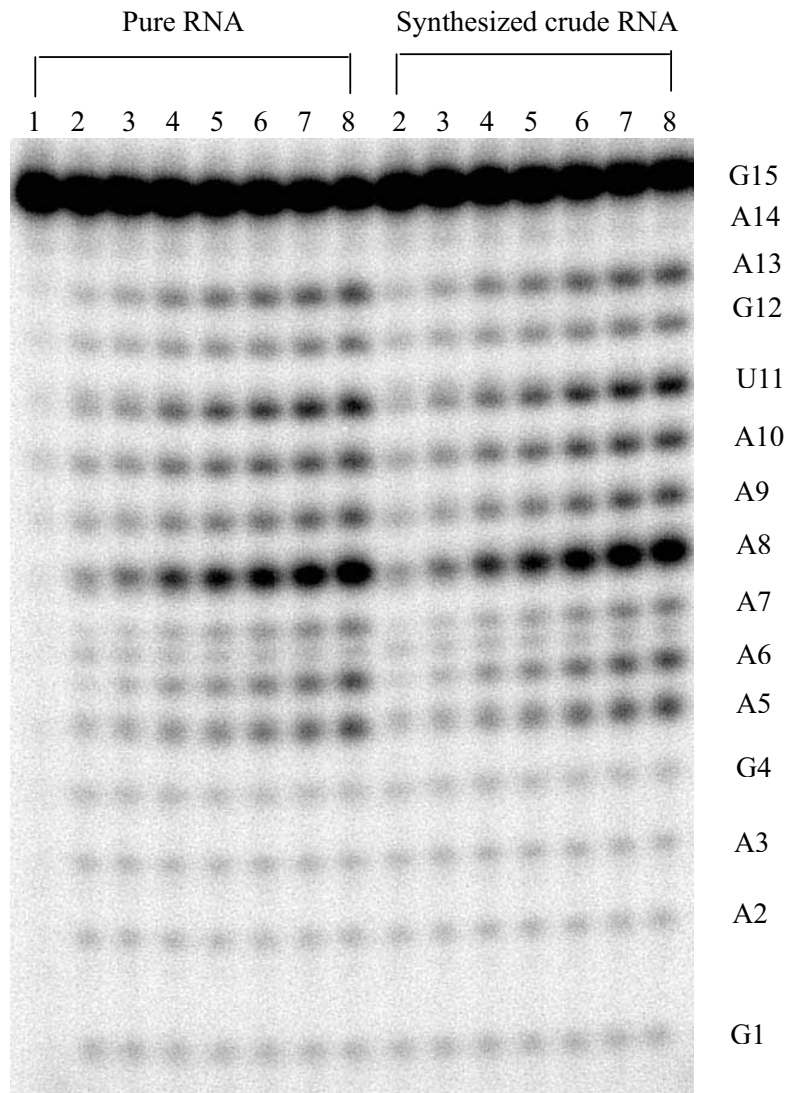


Figure S4. RNase H digestion of 15 mer DNA/RNA duplex. Lanes 1 – 8 represent digestion of RNA after 0, 2, 5, 10, 15, 25, 40, 60 min of incubation with enzyme. Conditions of cleavage reactions: pure RNA ($0.1 \mu\text{M}$) or crude RNA ($0.1 \mu\text{M}$) and complementary DNA ($1 \mu\text{M}$) in buffer containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM MgCl_2 and 0.1 mM DTT at $21 \text{ }^\circ\text{C}$; 0.06 U of RNase H in a total reaction volume of $30 \mu\text{L}$.

