

Supporting Information

Donor/Acceptor Chromophores Decorated Triazolyl Unnatural Nucleosides: Synthesis, Photophysical Properties and Study of Interaction with BSA

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1. Summary of UV and fluorescence photophysical properties of various nucleosides

Table S1. Summary of UV and fluorescence photophysical properties of ^{TNDMB}B_{D0}(1β), ^{TDMB}B_{D0}(2β), ^{TPhOB}B_{D0}(3β)

Entry	Solvents	Δf	UV-Vis & Fluorescence		
			λ_{abs} (nm)	ϵ_{max}	λ_{fl} (nm)
^{TNDMB} B _{D0} (1β)	Toluene	0.013	290.5	23900	366
	Dioxane	0.021	287	27400	366
	THF	0.21	283	27300	366
	EtOAc	0.201	281	23140	367
	DMF		289	25450	374
	DMSO	0.265	287	36900	380
	ACN	0.305	288	26560	376
	MeOH	0.309	287	26240	309
^{TDMB} B _{D0} (2β)	Dioxane	0.021	295, 258	1550, 10610	318
	THF	0.21	295, 257	1440, 7370	315
	EtOAc	0.21	295, 257	1670, 9190	317
	CHCl ₃	0.148	295, 257	1380, 8740	320
	DMSO	0.265	295	1620	322
	ACN	0.305	295, 256	1620, 9640	320
	EtOH	0.29	294, 256	1670, 8460	323
	MeOH	0.309	294, 256	1470, 8350	321
^{TPhOB} B _{D0} (3β)	Dioxane	0.021	259	26730	323
	THF	0.21	259	29430	312
	EtOAc	0.201	257	26260	321
	DMF		267	21740	325
	DMSO	0.265	262	25290	329
	ACN	0.305	257	25630	324
	MeOH	0.309	257.5	27320	319
	EtOH	0.29	257.5	25830	329

Table S2. Summary of UV and fluorescence photophysical properties of ^{TMNap}B_{D0}(4 β), ^{TPhen}B_{D0}(5 β) and ^{TPy}B_{D0}(6 β)

Entry	Solvents	Δf	UV-Vis & Fluorescence			
			λ_{abs} (nm)	ϵ_{max}	λ_{fl} (nm)	Φ_f
^{TMNap} B _{D0} (4 β)	Toluene	0.013	301	11232	369	---
	Dioxane	0.021	300	14060	369	---
	THF	0.21	299.5	14920	370	---
	EtOAc	0.201	298.5	14440	370	---
	DMF		300	13180	371	---
	DMSO	0.265	301.5	13340	373	---
	ACN	0.305	298.5	13670	369	---
	EtOH	0.29	299	12930	368	---
^{TPy} B _{D0} (6 β)	Toluene	0.013	353	32610	408	0.129
	Dioxane	0.021	351	41020	407	0.224
	THF	0.21	351	40680	407	0.134
	EtOAc	0.201	349	41220	407	0.084
	CHCl ₃	0.148	351	38590	407	0.122
	DMF		351	38710	407	0.393
	DMSO	0.265	351	39140	408	0.578
	ACN	0.305	348	40810	406	0.093
	EtOH	0.29	347	39610	406	0.103
	MeOH	0.309	346	40790	405	0.089

Table S3. Summary of UV and fluorescence photophysical properties of ^{TBPy}B_{Do}(7 β), ^{TNB}B_{Ac}(8 β) and ^{TCNB}B_{Ac}(9 β)

Entry	Solvents	Δf	UV-Vis & Fluorescence			
			λ_{abs} (nm)	ϵ_{max}	λ_{fl} (nm)	Φ_f
^{TBPy} B _{Do} (7 β)	Toluene	0.013	345	34230	417	0.077
	THF	0.21	344	45200	417	0.093
	EtOAc	0.201	343	42770	417	0.064
	CHCl ₃	0.148	345	38840	417	0.086
	ACN	0.305	343	40300	417	0.078
	EtOH	0.29	342	45650	417	0.087
	MeOH	0.309	342	41350	417	0.078
^{TNB} B _{Ac} (8 β)	Toluene	0.013	312	8620	---	---
	Dioxane	0.021	312	14010	---	---
	CHCl ₃	0.148	311	12220	---	---
	EtOAc	0.201	314	13600	---	---
	THF	0.210	316	13200	---	---
	EtOH	0.290	310	12820	---	---
	ACN	0.307	314	13770	---	---
	MeOH	0.309	308	13350	---	---
^{TCNB} B _{Ac} (9 β)	Dioxane	0.021	270	26300	322	---
	THF	0.21	275	25800	326	---
	EtOAc	0.201	272	26180	324	---
	ACN	0.305	271	26600	330	---
	EtOH	0.29	271	25910	331	---
	MeOH	0.309	273	25710	333	---

Table S4. Summary of time resolved fluorescence photophysical properties of ${}^{\text{TPy}}\text{B}_{\text{D}_0}$ (6β) and ${}^{\text{TBPY}}\text{B}_{\text{D}_0}$ (7β)

Entry	Solvents	Δf	τ	χ^2	K_r/K_{nr}
${}^{\text{TPy}}\text{B}_{\text{D}_0}$ (6β)	EtOAc	0.201	5.941	0.934	0.0917
	ACN	0.305	6.266	0.909	0.1030
	MeOH	0.309	6.314	0.960	0.0979
Entry	Solvents	Δf	τ	χ^2	K_r/K_{nr}
${}^{\text{TBPY}}\text{B}_{\text{D}_0}$ (7β)	EtOAc	0.201	13.101	0.893	0.0684
	ACN	0.305	16.448	0.961	0.0846
	MeOH	0.309	17.612	0.928	0.0846

Table S5. Summary of photophysical properties of ${}^{\text{TPy}}\text{B}_{\text{D}_0}$ (6β) and ${}^{\text{TBPY}}\text{B}_{\text{D}_0}$ (7β) titrated separately with ${}^{\text{TCNB}}\text{B}_{\text{Ac}}$ (9β)

${}^{\text{TPy}}\text{B}_{\text{D}_0}$ (6β) titrated with ${}^{\text{TCNB}}\text{B}_{\text{Ac}}$ (9β) in dioxane									
${}^{\text{TCNB}}\text{B}_{\text{Ac}}$ (9β) added	Conc. of	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm)	Φ	τ	K_r	K_{nr}	K_r/K_{nr}	K_{sv}
0.0 eq.		353	406	0.171	10.0	0.017	0.082	0.206	3.0×10^{-2}
0.2 eq.		353	406	0.145	9.7	0.015	0.088	0.170	
0.4 eq.		353	406	0.126	7.7	0.0164	0.114	0.144	
0.6 eq.		353	406	0.114	8.6	0.013	0.103	0.129	
0.8 eq.		353	406	0.101	9.7	0.010	0.092	0.112	
1.0 eq.		353	406	0.092	8.5	0.011	0.106	0.101	
1.5 eq.		353	406	0.072	11.6	0.006	0.080	0.078	
${}^{\text{TBPY}}\text{B}_{\text{D}_0}$ (7β) titrated with ${}^{\text{TCNB}}\text{B}_{\text{Ac}}$ (9β) in dioxane									
0.0 eq.		344	417	0.30	42.2	0.007	0.012	0.432	5×10^{-2}
0.2 eq.		344	417	0.26	41.7	0.006	0.018	0.351	
0.4 eq.		344	417	0.24	41.9	0.006	0.018	0.314	
0.6 eq.		344	417	0.22	41.6	0.005	0.019	0.279	
0.8 eq.		344	417	0.19	42.0	0.005	0.019	0.241	
1.0 eq.		344	417	0.18	40.8	0.004	0.020	0.212	
1.5 eq.		344	417	0.14	41.1	0.003	0.021	0.164	

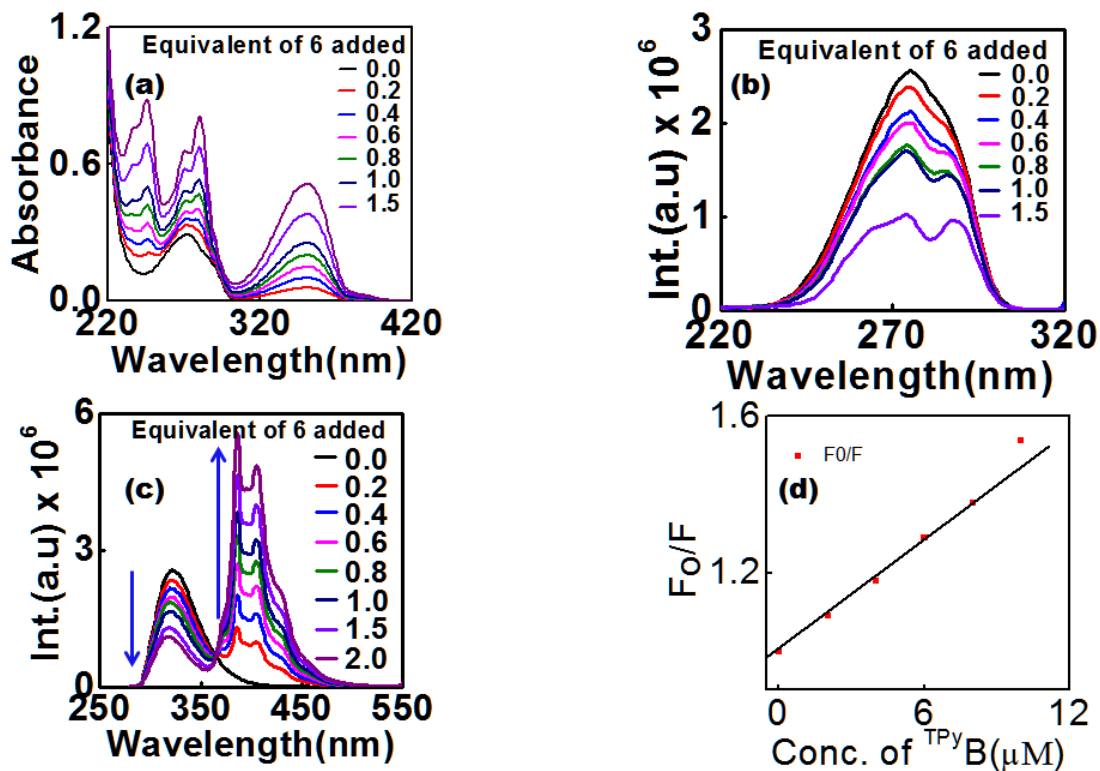


Figure S1. UV-visible (a), excitation (b), fluorescence emission spectra of titration of nucleoside ${}^{\text{TCNB}}\text{B}_{\text{Ac}}$ (9β) with ${}^{\text{TPy}}\text{B}_{\text{Do}}$ (6β) and (d) plot of F_0/F vs. conc. of nucleoside ${}^{\text{TPy}}\text{B}_{\text{Do}}$ (6β).

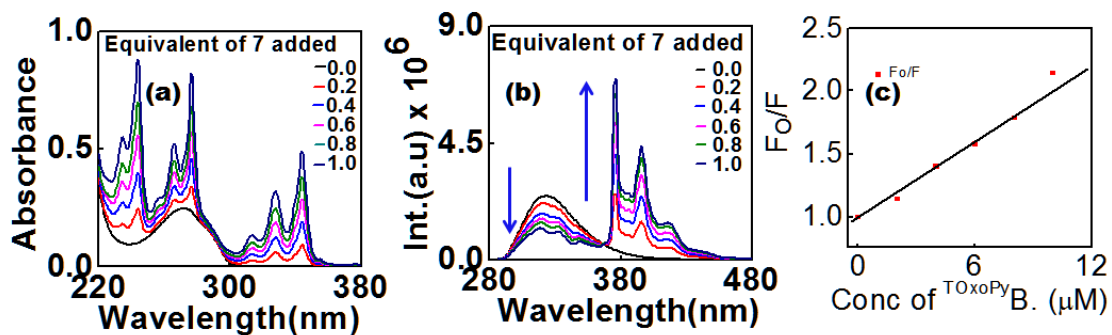


Figure S2A. UV-visible (a), fluorescence emission (b) spectra of titration of nucleoside ${}^{\text{TCNB}}\text{B}_{\text{Ac}}$ (9β) with ${}^{\text{TBPy}}\text{B}_{\text{Do}}$ (7β) and (d) plot of F_0/F vs. conc. of nucleoside ${}^{\text{TBPy}}\text{B}_{\text{Do}}$ (7β).

2. Studies on the interaction of nucleoside ^{TBPy}B_{D0} (7β) with BSA

2.1. Materials

BSA, Na₂HPO₄ and NaH₂PO₄.H₂O (for preparation of phosphate buffer) were purchased from Merck, India and used without further purification. Water was taken from a Milli-Q purification system. All solutions were prepared before 1 hour of experiments done. The probe molecules [nucleoside ^{TBPy}B_{D0} (7β)] was synthesized and purified according to the procedure described.

2.2. Preparation of BSA Solution

Phosphate buffer of pH 7.0 was used to prepare the solution of BSA (Merck). A 250 μM of stock BSA solution was prepared by dissolving 0.0222 gm of BSA in 1.28 mL phosphate buffer (20 mM) of pH 7.0. From that stock solution sub stock of 1000 μM BSA was prepared. The compound stock solution was prepared in DMF because of the poor solubility in water. 0.6 mg of nucleoside ^{TBPy}B_{D0} (7β) was dissolved in 1 mL DMF to make a stock probe solution of concentration 1092.4 μM.

2.3. General experimental on interaction study of BSA by photophysical study:

All the spectral measurements were carried out at room temperature. To study the interaction of compound with BSA, an aqueous solution of nucleoside ^{TBPy}B_{D0} (7β) (10 μM for nucleoside) was titrated with different concentrations of BSA (ranging from 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0 equivalent). The total volume of the final solution for each sample was 3 mL. The % of DMF content was 2%. The presence of 2% DMF does not induce structural changes to biomolecules. Each sample solution was mixed well before spectral measurements.

2.4. UV-Visible Study

The UV-Visible absorbance measurements were performed using Shimadzu UV- 2550 UV Visible spectrophotometer with a cell of 1 cm path length at 298 K. All the UV-Visible studies were carried out in 20 mM phosphate buffer of pH 7.02 containing solution at 298 K. 2 % DMF was used to solubilize the probe. The measurements were taken in absorbance mode and the absorbance values of the sample solutions were measured in the wavelength regime of 200– 700 nm. All the experiments were carried out with freshly prepared sample solutions.

2.5. Fluorescence Study

All fluorescence and steady state anisotropy experiments were performed using a Fluoromax 4 spectrophotometer with a cell of 1 cm path length at 298 K. The excitation wavelength for probe (nucleoside ^{TBPy}B_{D0} (7β)) was set at 280 nm, 350 nm and emission spectra were measured in the wavelength regime of 290–650 nm. Steady state anisotropy of the solutions was measured using Fluoromax 4 spectrophotometer.

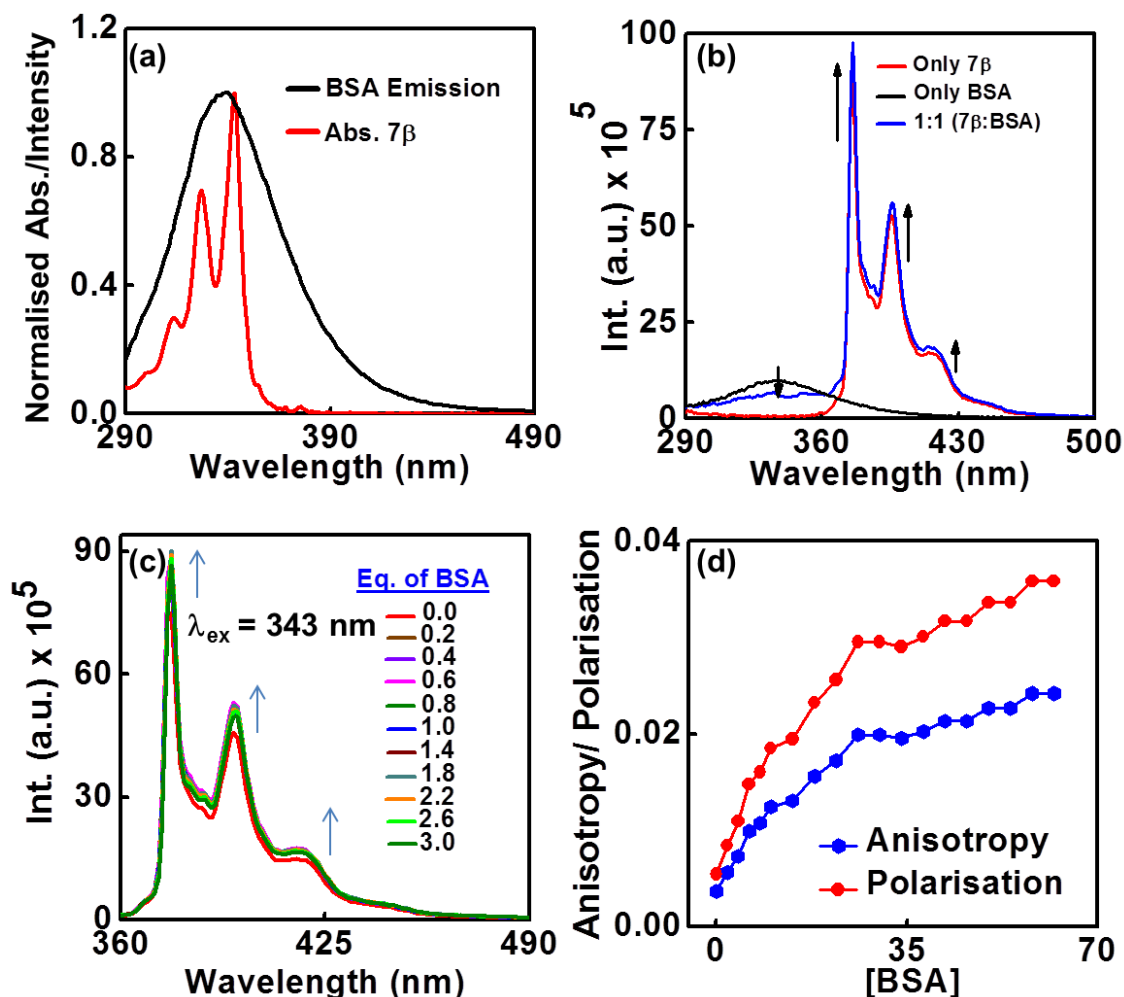
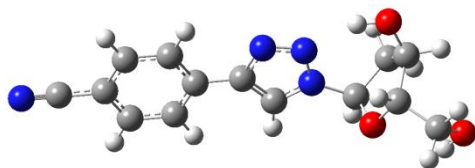


Figure S2B. (a) Normalised overlap of UV-visible spectra of nucleoside ${}^{\text{TBPy}}\mathbf{B}_{\text{D}_0}(7\beta)$ and emission spectra of BSA; (b) Comparison of fluorescence emission spectra of Only BSA, only nucleoside and a 1:1 mixture of BSA: nucleoside showing the FRET from BSA to nucleoside ${}^{\text{TBPy}}\mathbf{B}_{\text{D}_0}(7\beta)$ ($\lambda_{\text{ex}} = 280$ nm); (c) Change in TBPy emission intensity of nucleoside ${}^{\text{TBPy}}\mathbf{B}_{\text{D}_0}(7\beta)$ upon gradual addition of an increasing concentration of BSA when excited at TBPy's absorption at $\lambda_{\text{abs}} = 343$ nm; (d) steady-state anisotropy and polarisation plot of ${}^{\text{TBPy}}\mathbf{B}_{\text{D}_0}(7\beta)$ in presence of increasing BSA concentration. $[{}^{\text{TBPy}}\mathbf{B}_{\text{D}_0}(7\beta)] = 10 \mu\text{M}$.

3. Cartesian Coordinates

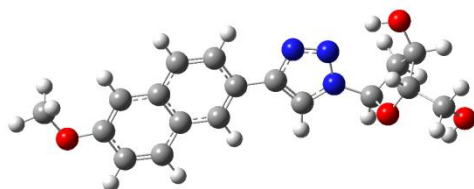


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5	7	0	-0.383839	1.292866	-0.015512
6	7	0	0.885250	1.110358	-0.225146
7	6	0	-4.568122	-1.265881	-0.211521
8	6	0	-3.251849	1.147832	0.293583
9	1	0	-2.730076	2.078640	0.486543
10	6	0	-5.302243	-0.135890	0.186614
11	6	0	-4.630769	1.072990	0.438287
12	1	0	-5.196836	1.946262	0.745842
13	6	0	-3.189224	-1.182324	-0.353811
14	1	0	-2.637808	-2.065821	-0.662613
15	6	0	-1.055094	0.122165	-0.250325
16	6	0	2.419692	-0.738229	-0.818607
17	1	0	2.254366	-1.643748	-1.407632
18	6	0	-2.510400	0.022067	-0.104270
19	6	0	3.392244	0.255009	-1.461541
20	1	0	2.923930	0.957172	-2.153763
21	1	0	4.143226	-0.328840	-2.002262
22	6	0	3.842729	-0.036338	0.909845
23	1	0	3.313416	0.471839	1.725258
24	6	0	4.055844	0.969578	-0.259357
25	1	0	5.123192	1.105474	-0.447767
26	6	0	-0.117768	-0.827259	-0.623739
27	1	0	-0.192901	-1.870052	-0.889833

28	6	0	5.118324	-0.687656	1.429975
29	1	0	5.792973	0.086937	1.807944
30	1	0	4.876176	-1.363626	2.264281
31	8	0	5.812583	-1.370417	0.396815
32	1	0	5.207597	-2.064429	0.088412
33	6	0	-6.725252	-0.218010	0.333910
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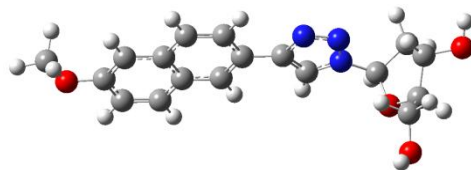


4a

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5	7	0	2.375813	-0.080581	-0.655535
6	7	0	1.048838	1.531995	-0.154854
7	7	0	2.300620	1.234328	-0.347352
8	6	0	-3.355998	-0.672460	-0.206027
9	6	0	-1.825200	1.656973	0.163940
10	1	0	-1.216778	2.544156	0.302368
11	6	0	-4.000266	0.557829	0.139961
12	6	0	-3.189720	1.711566	0.317043
13	1	0	-3.667844	2.652482	0.579398
14	6	0	-1.949674	-0.700208	-0.357145
15	1	0	-1.482376	-1.647347	-0.618767
16	6	0	-5.412424	0.593659	0.296024

17	1	0	-5.878939	1.536875	0.558715
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19	6	0	-6.161415	-0.552743	0.114703
20	6	0	3.671852	-0.767353	-0.815695
21	1	0	3.447779	-1.666796	-1.394232
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23	6	0	-5.524561	-1.777863	-0.230485
24	1	0	-6.148511	-2.655999	-0.364108
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26	1	0	-3.684354	-2.771385	-0.647999
27	6	0	4.755327	0.120879	-1.435017
28	1	0	4.381568	0.846014	-2.160120
29	1	0	5.472775	-0.539699	-1.931509
30	6	0	-8.228652	0.540144	0.587755
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32	1	0	-9.279999	0.251268	0.636462
33	1	0	-8.100974	1.325673	-0.168706
34	6	0	5.065246	-0.146500	0.962682
35	1	0	4.526536	0.428445	1.726176
36	6	0	5.428367	0.802404	-0.218534
37	1	0	6.511642	0.831445	-0.356932
38	6	0	1.138164	-0.631852	-0.643491
39	1	0	0.976599	-1.678087	-0.850830
40	6	0	6.249946	-0.874672	1.585708
41	1	0	6.962532	-0.141701	1.976940
42	1	0	5.903124	-1.494208	2.427277
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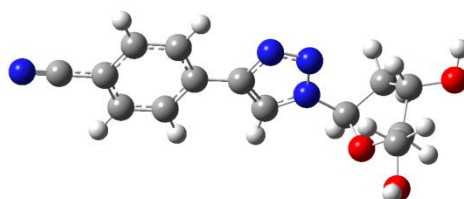
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9	6	0	-5.310683	0.726856	0.303451
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15	1	0	-5.470696	-0.458703	-2.254632
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22	6	0	-0.334357	-0.539488	-0.257677
23	1	0	-0.973892	1.101550	-1.677646
24	6	0	1.120098	-0.509300	-0.067526
25	6	0	1.741964	-1.507971	0.734188
26	6	0	1.913655	0.466929	-0.647560
27	1	0	1.115951	-2.268774	1.187854
28	6	0	3.102283	-1.510171	0.929310
29	6	0	3.316531	0.488473	-0.464184
30	1	0	1.465958	1.247132	-1.259605
31	6	0	3.934862	-0.520424	0.340847
32	1	0	3.560167	-2.281737	1.544151
33	6	0	4.146411	1.484191	-1.054056
34	6	0	5.343839	-0.506629	0.528199
35	6	0	5.503485	1.481574	-0.862346
36	1	0	3.686460	2.256381	-1.666304
37	1	0	5.790353	-1.281409	1.141936
38	6	0	6.114519	0.475359	-0.062868
39	1	0	6.143562	2.236180	-1.308676
40	8	0	7.469838	0.585748	0.045736

41	6	0	8.156746	-0.381791	0.823137
42	1	0	7.824297	-0.366235	1.869567
43	1	0	9.213148	-0.111588	0.774566
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9β

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6	1	0	5.476916	-1.799096	-1.387941
7	6	0	3.841101	1.785166	-0.833702
8	1	0	4.579483	1.659580	-1.643283
9	1	0	2.833833	1.692993	-1.268167
10	6	0	4.105638	-0.733664	-0.499885
11	1	0	3.508588	-0.740537	-1.420774
12	6	0	-0.090192	-0.389622	1.107329
13	1	0	-0.193378	-0.125509	2.148307
14	6	0	-2.436478	-0.240280	0.021155
15	6	0	4.053027	0.656086	0.156920
16	1	0	4.991111	0.829270	0.698956
17	6	0	-3.143916	0.163392	1.166355
18	1	0	-2.620140	0.309980	2.106603
19	6	0	-4.512385	-0.199058	-1.239082
20	1	0	-5.050206	-0.338201	-2.171407
21	6	0	-3.142567	-0.417666	-1.181537
22	1	0	-2.598632	-0.728612	-2.066603

23	6	0	2.431116	-0.679113	1.213967
24	1	0	2.241522	-0.901917	2.267903
25	6	0	-0.990378	-0.473859	0.058899
26	6	0	-4.514094	0.383840	1.117857
27	1	0	-5.051403	0.696077	2.007499
28	6	0	-5.212154	0.203988	-0.088412
29	6	0	3.433739	-1.628628	0.556775
30	1	0	2.970011	-2.529192	0.149944
31	1	0	4.188996	-1.911371	1.297456
32	8	0	4.015423	3.018374	-0.153812
33	1	0	3.810609	3.733091	-0.774835
34	6	0	-6.625870	0.430310	-0.142966
35	7	0	-7.774183	0.614221	-0.186106

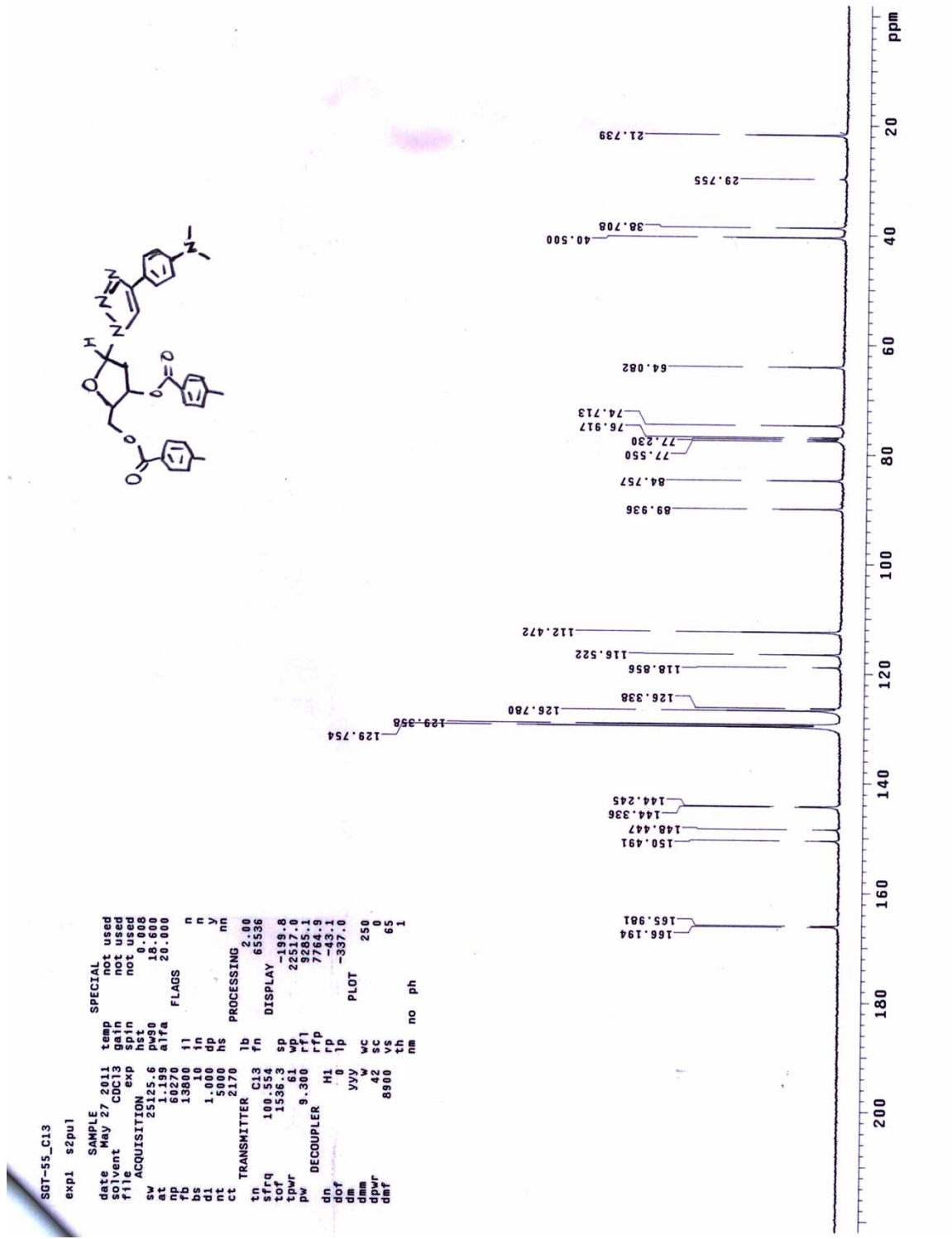


Figure S4. ^{13}C NMR spectra of nucleoside 13 α

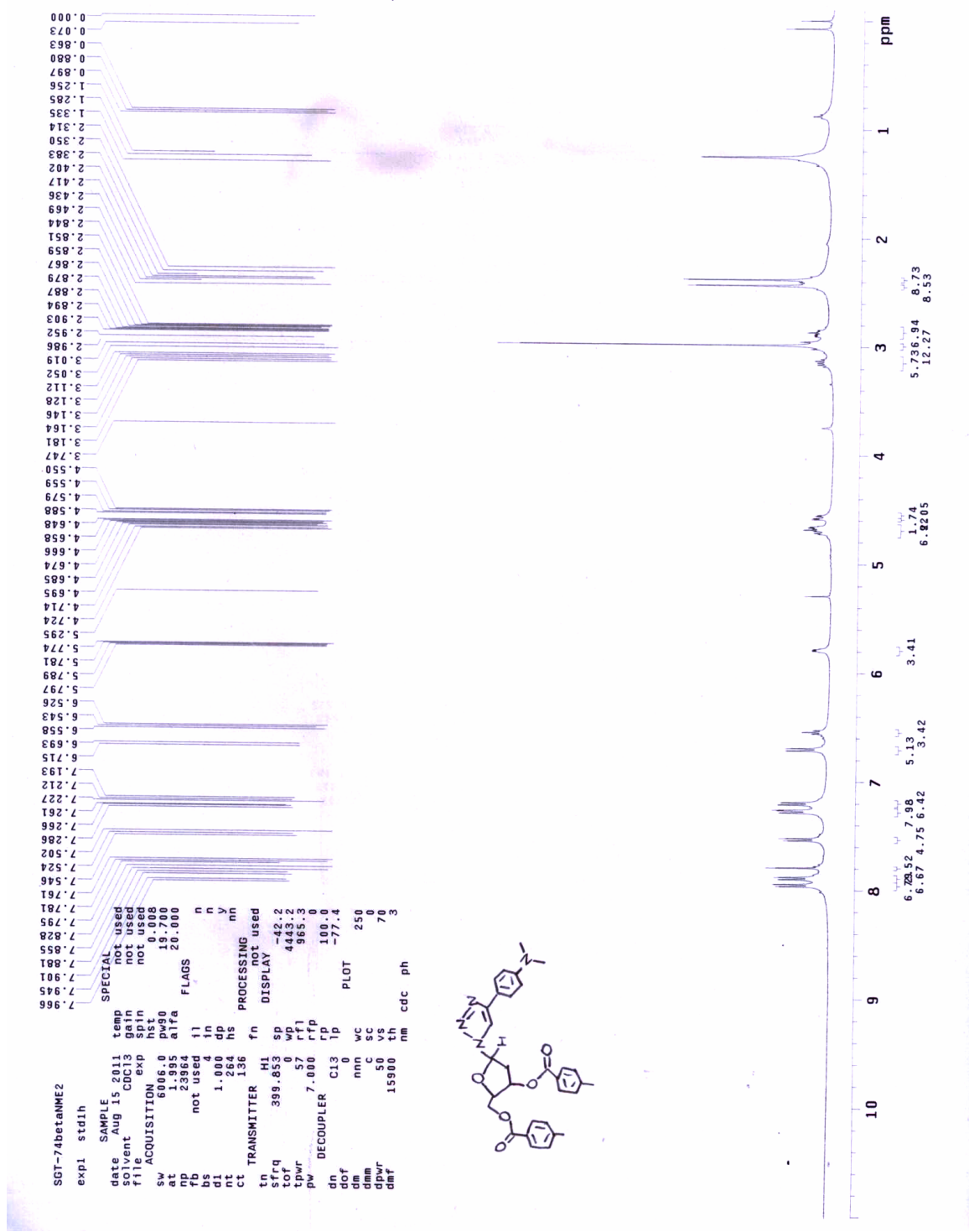


Figure S5. ^1H NMR spectra of nucleoside **13β**

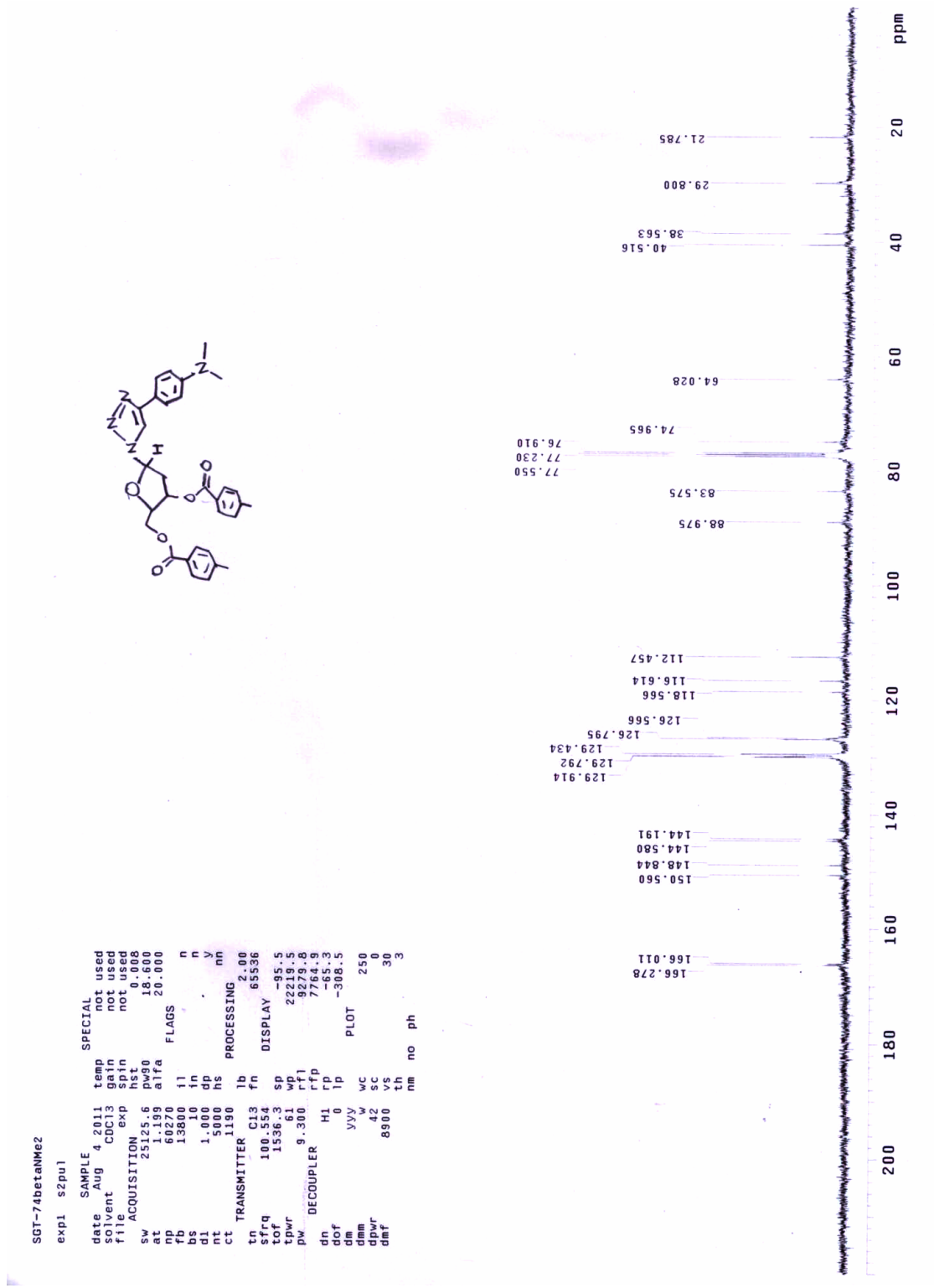


Figure S6. ^{13}C NMR spectra of nucleoside 13B

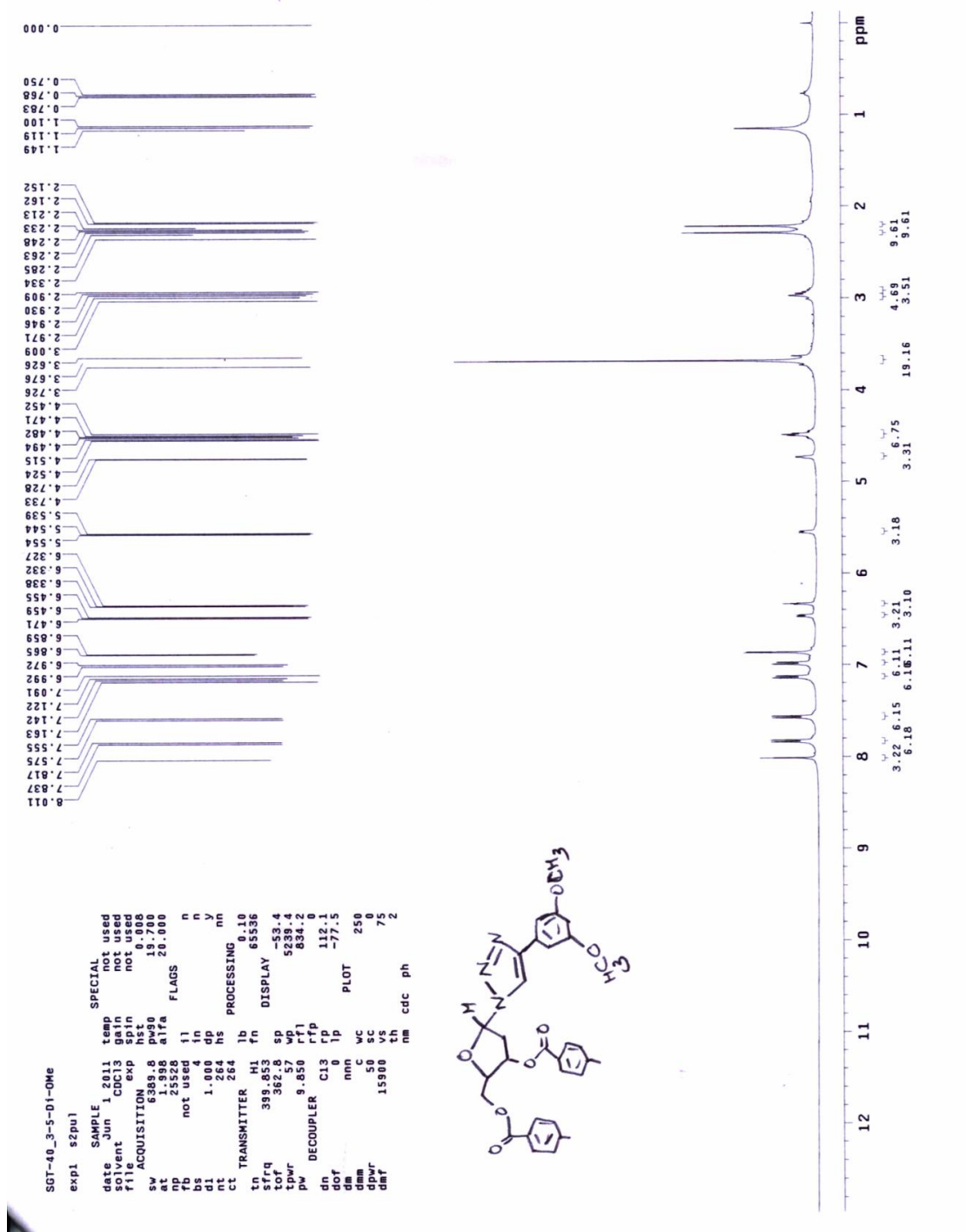


Figure S7. ¹H NMR spectra of nucleoside 14α

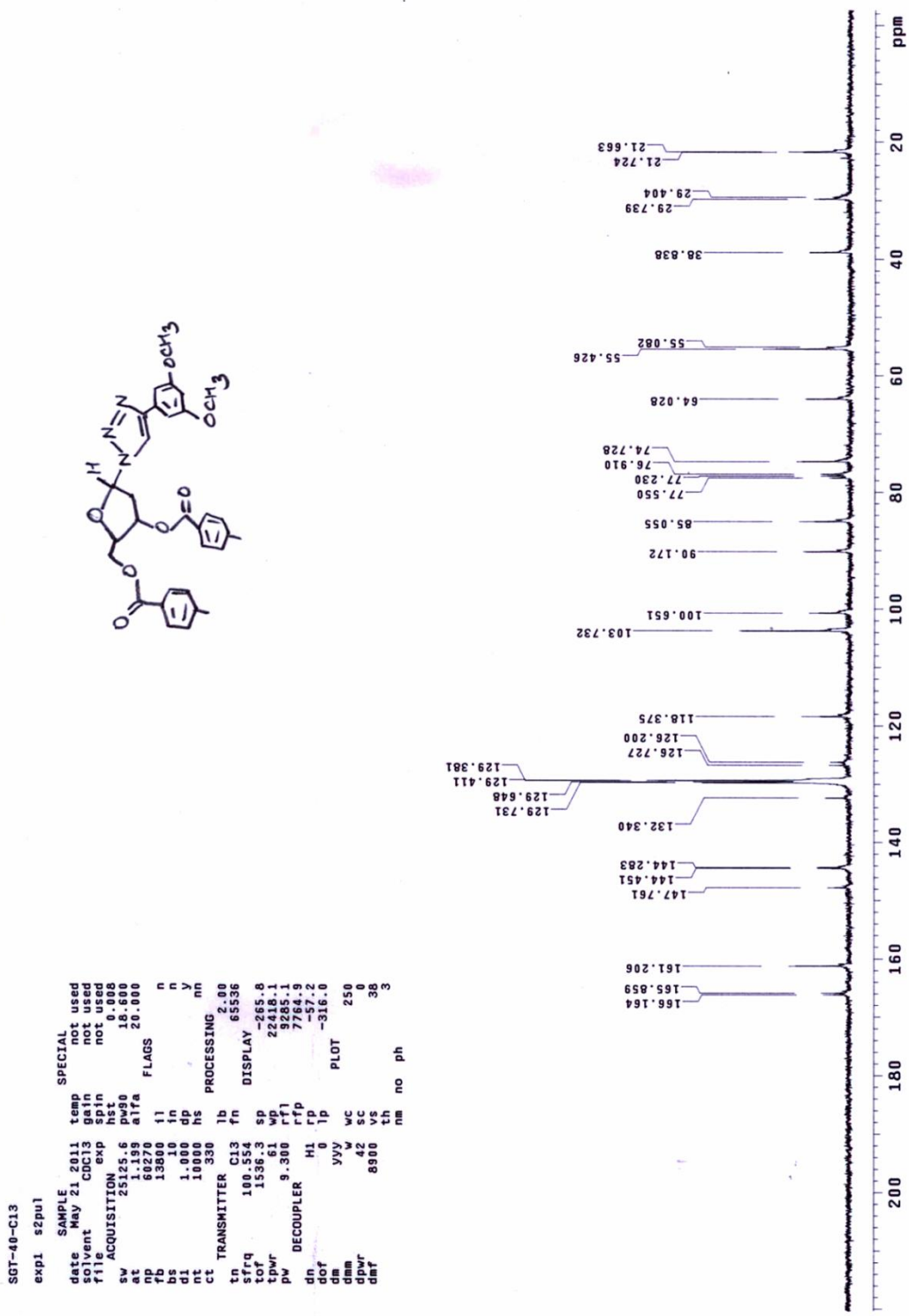


Figure S8. ^{13}C NMR spectra of nucleoside 14α

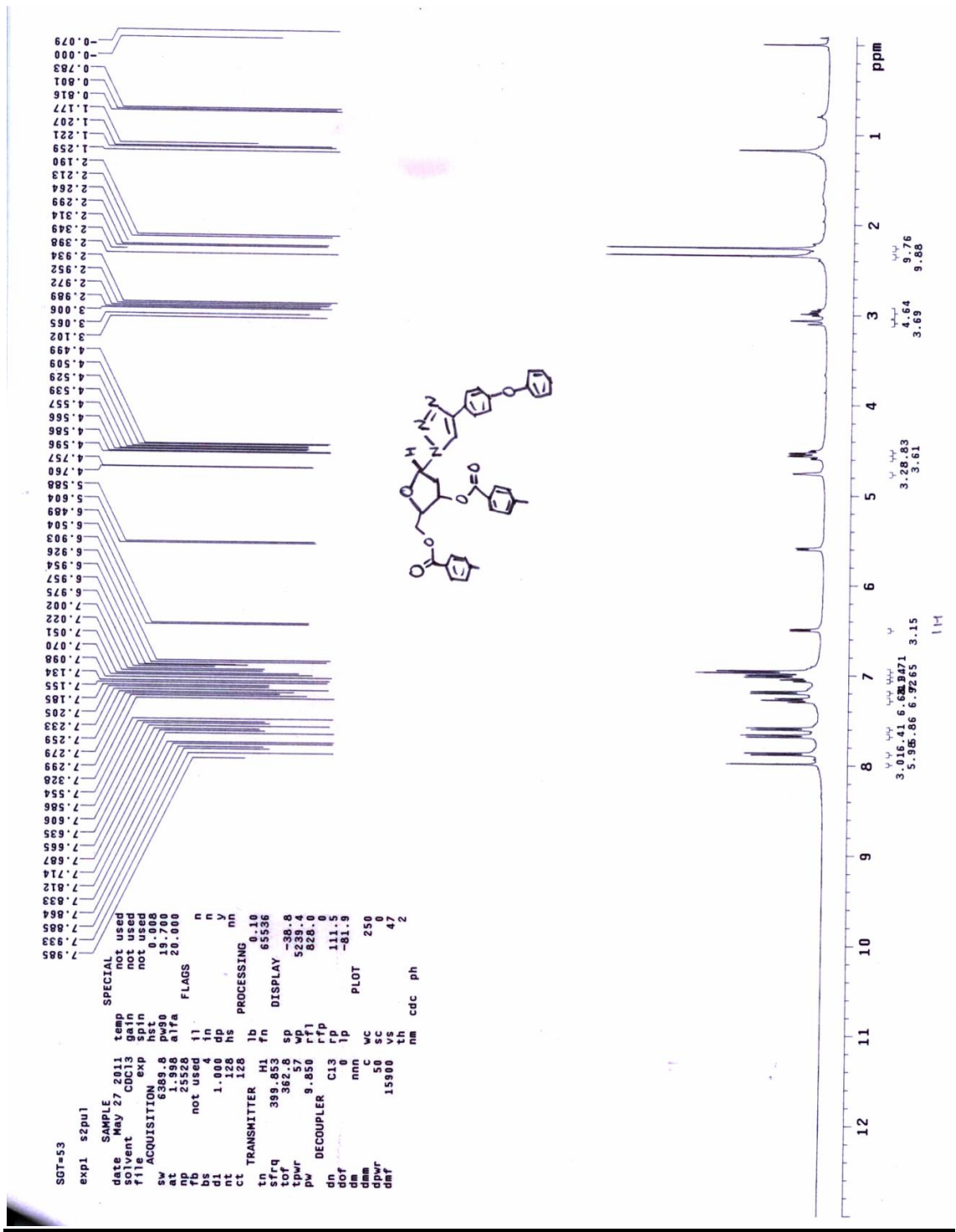


Figure S9. ¹H NMR spectra of nucleoside 15α

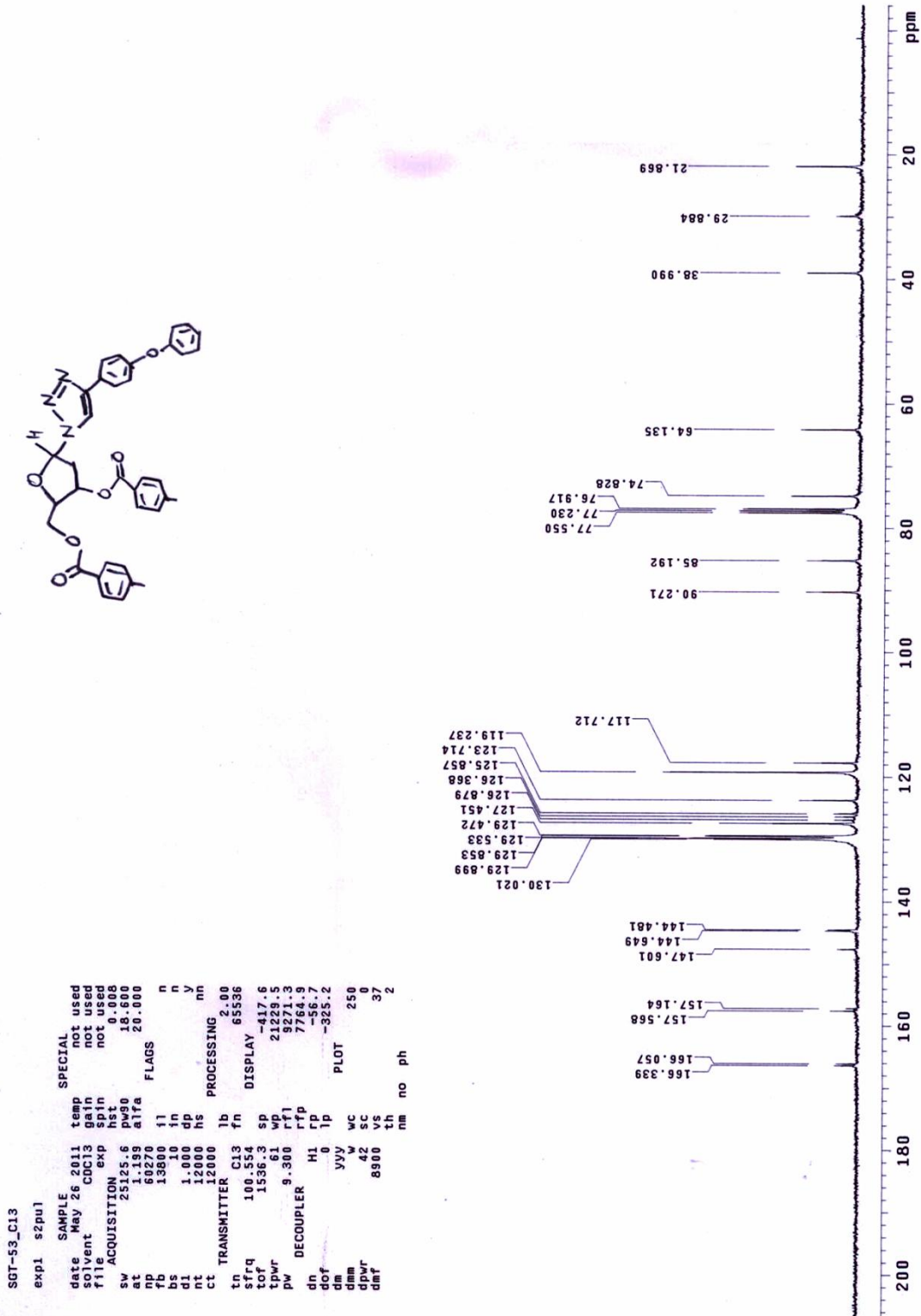


Figure S10. ^{13}C NMR spectra of nucleoside 15α

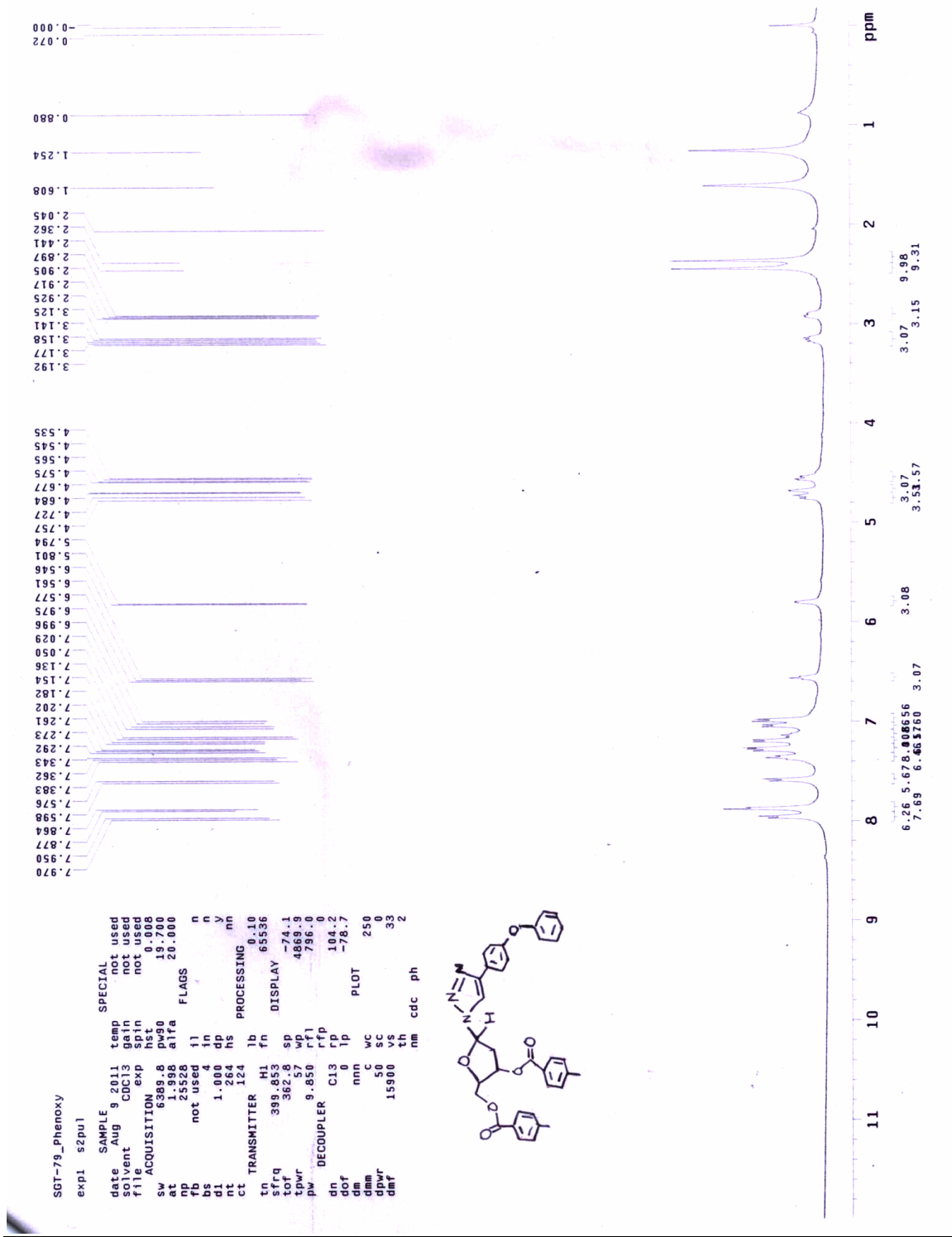


Figure S11. ^1H NMR spectra of nucleoside 15b

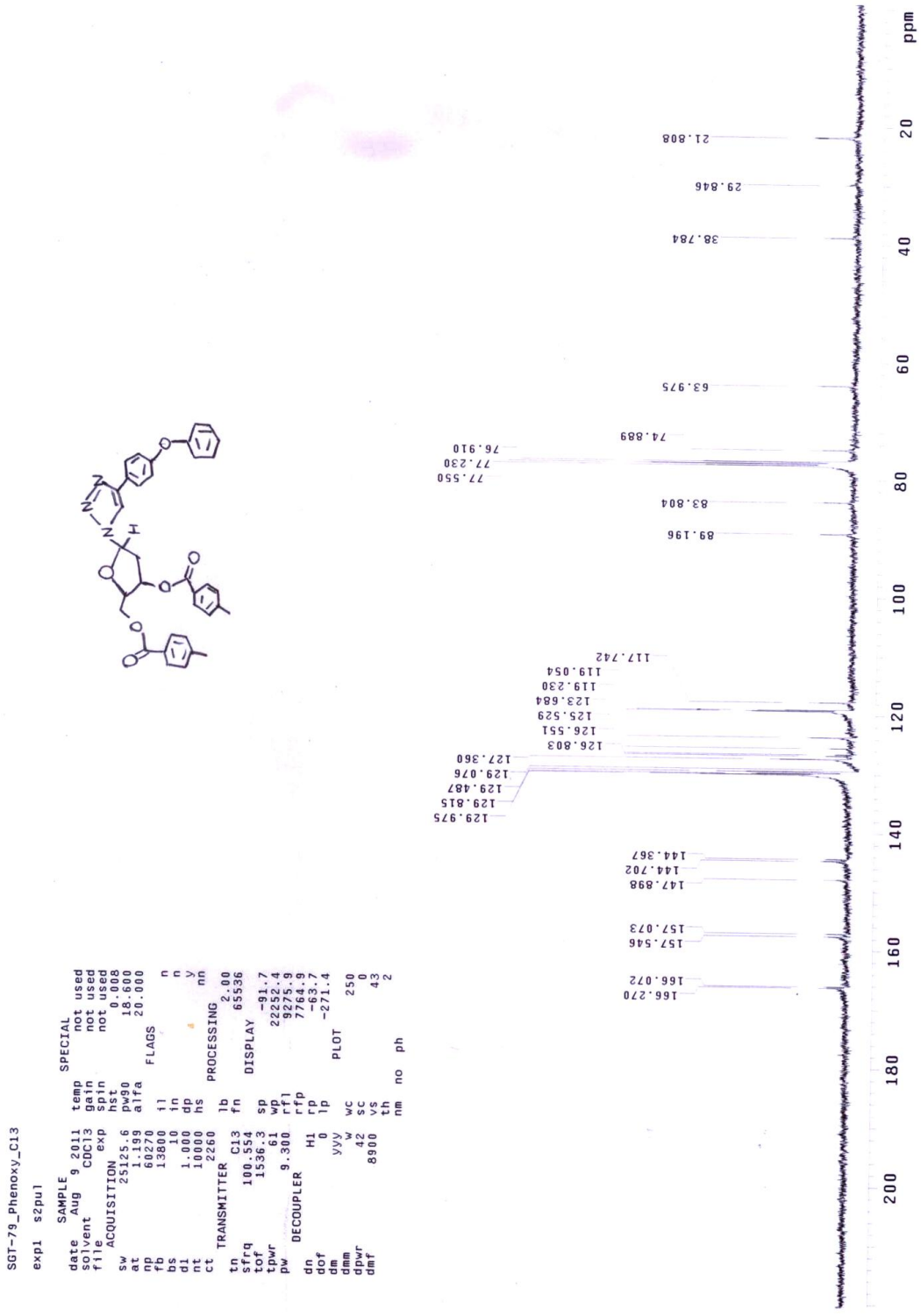


Figure S12. ^{13}C NMR spectra of nucleoside 15 β

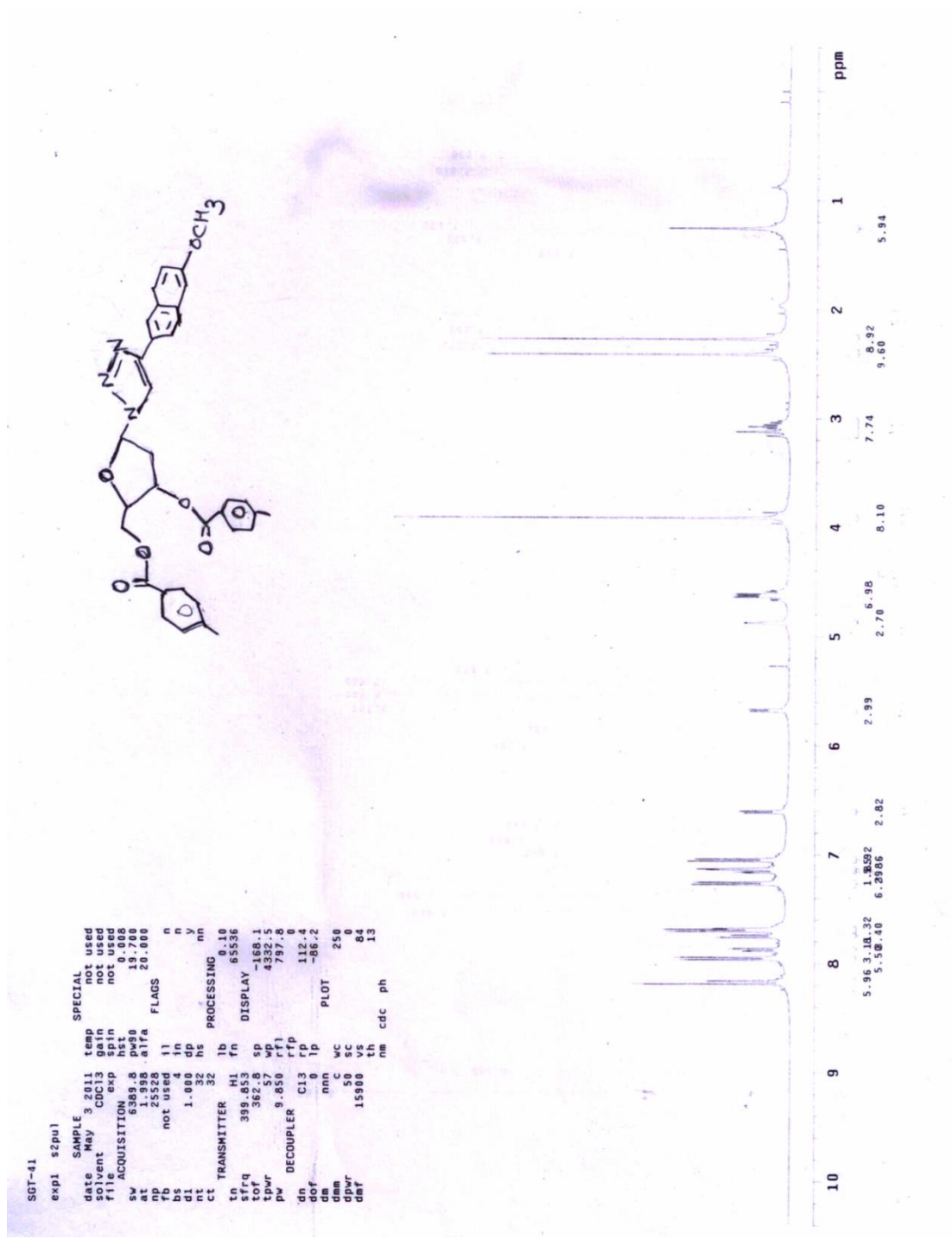


Figure S13. ^1H NMR spectra of nucleoside 16 α

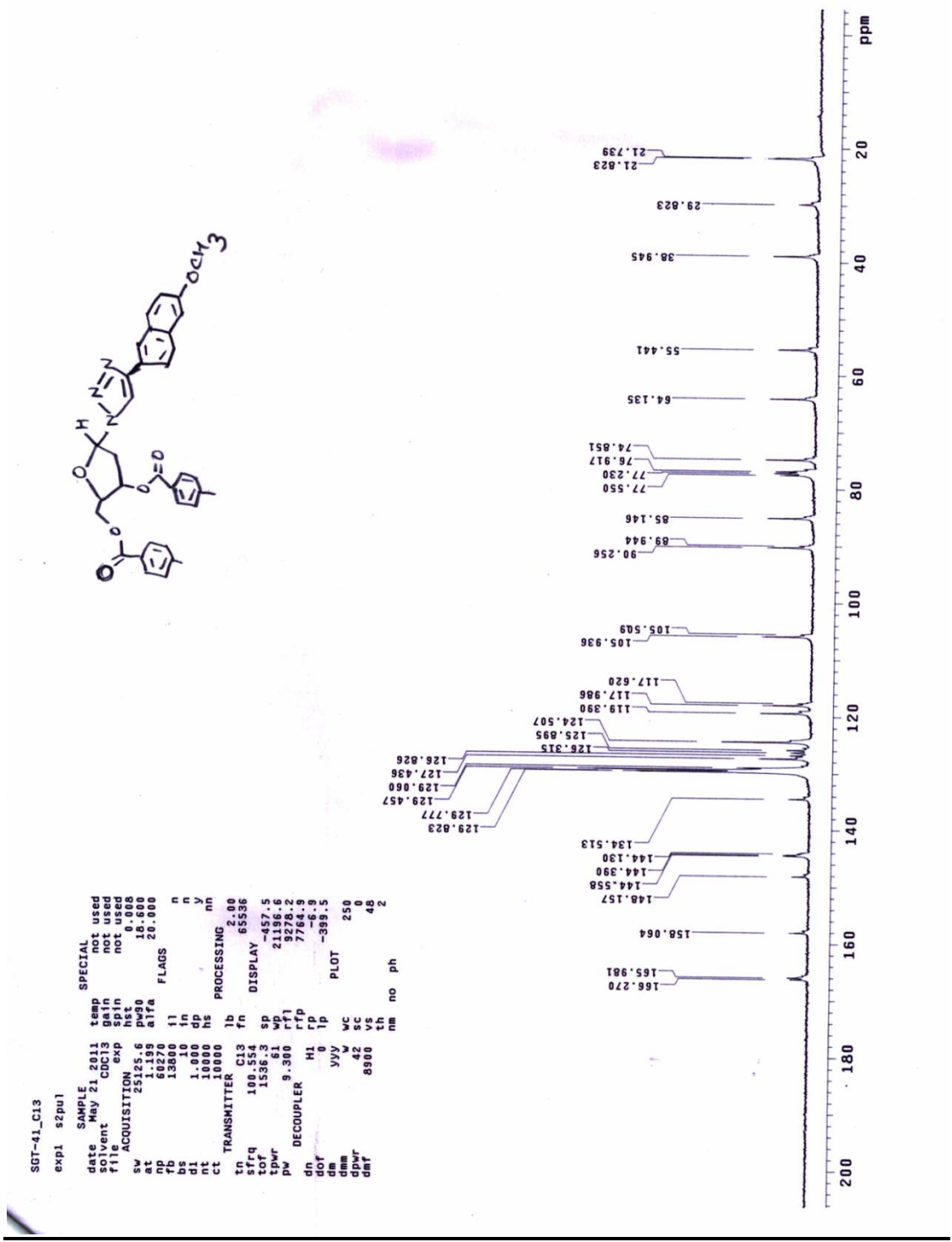


Figure S14. ^{13}C NMR spectra of nucleoside 16α

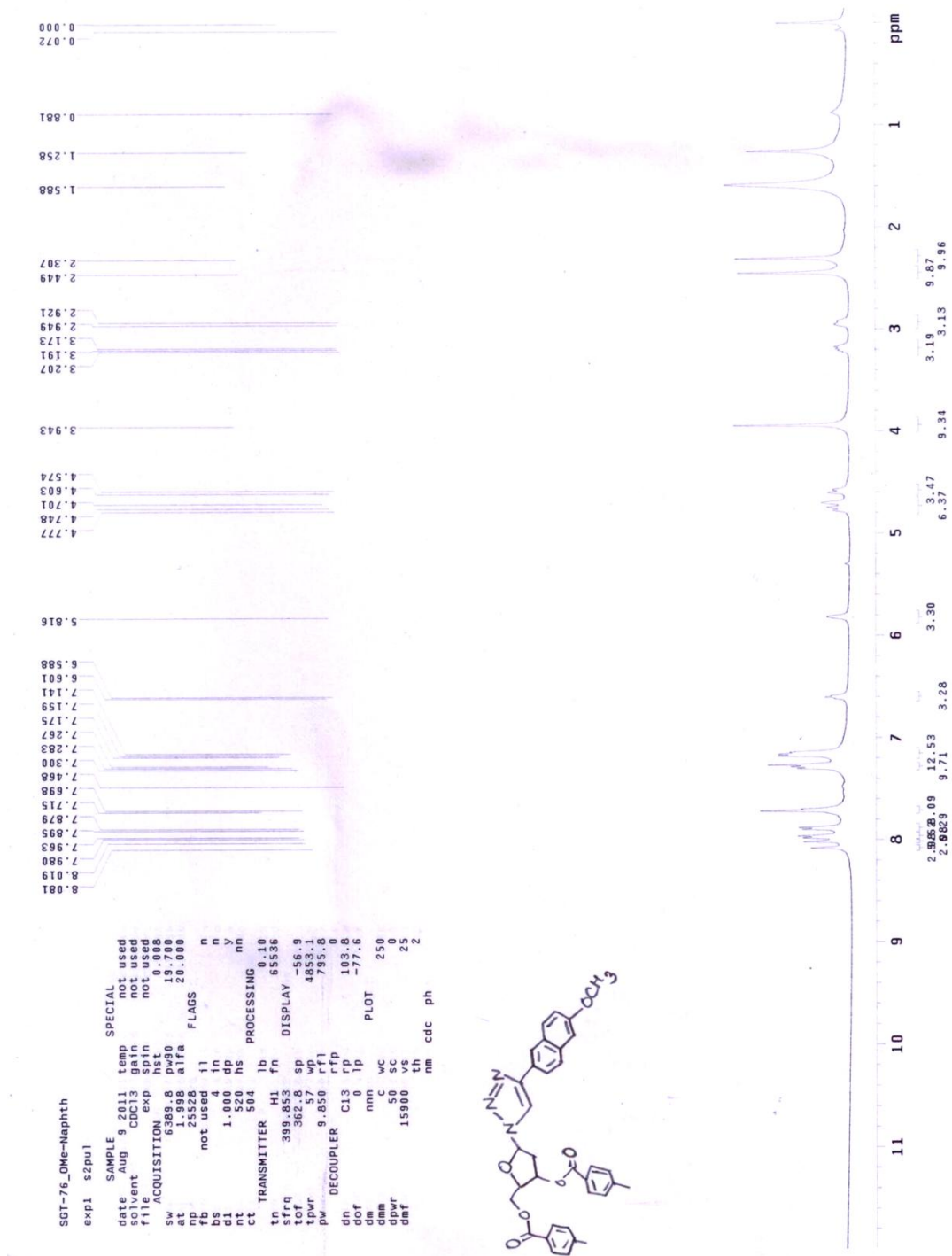


Figure S15. ¹H NMR spectra of nucleoside 16β

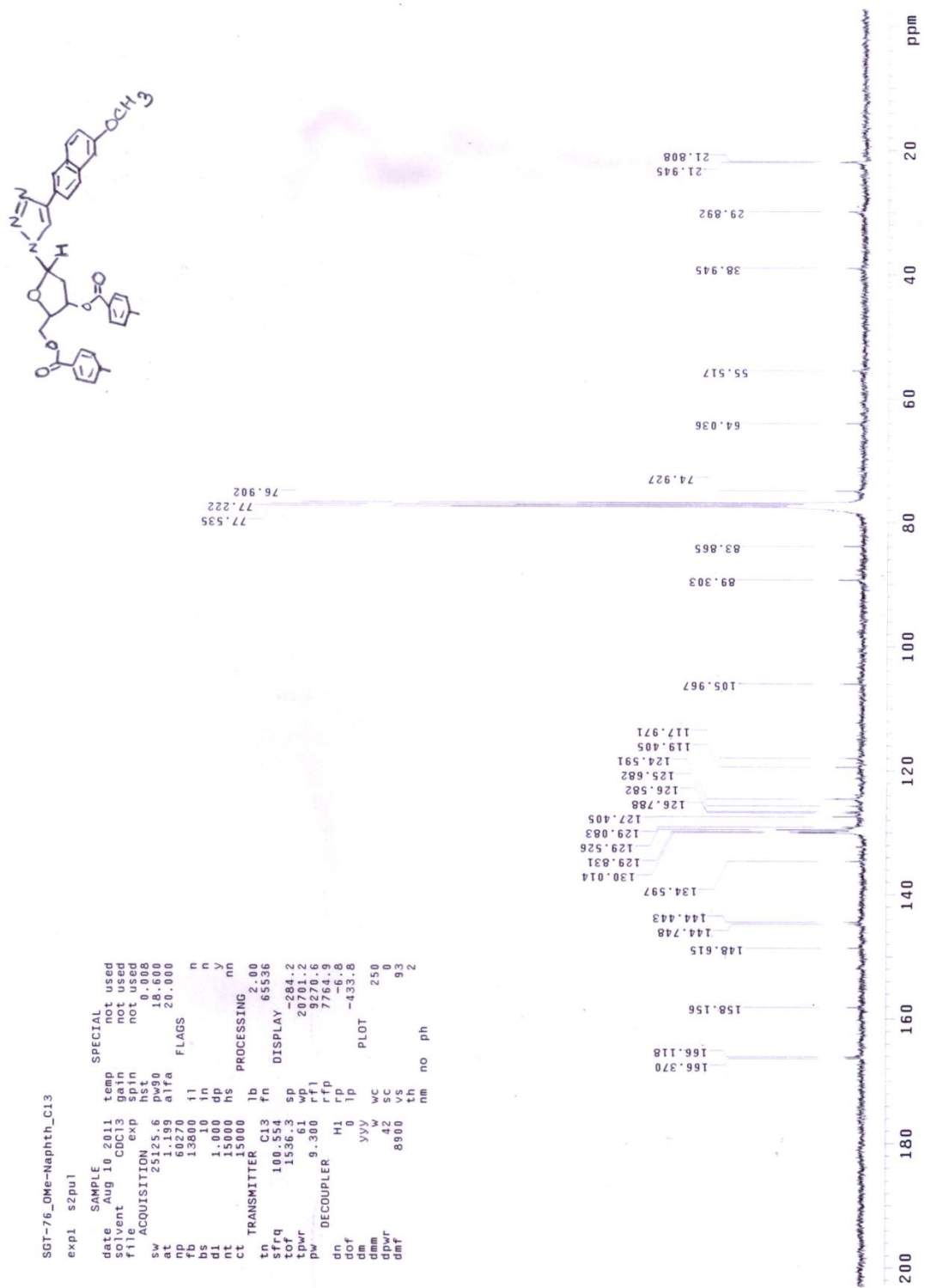


Figure S16. ^{13}C NMR spectra of nucleoside 16 β

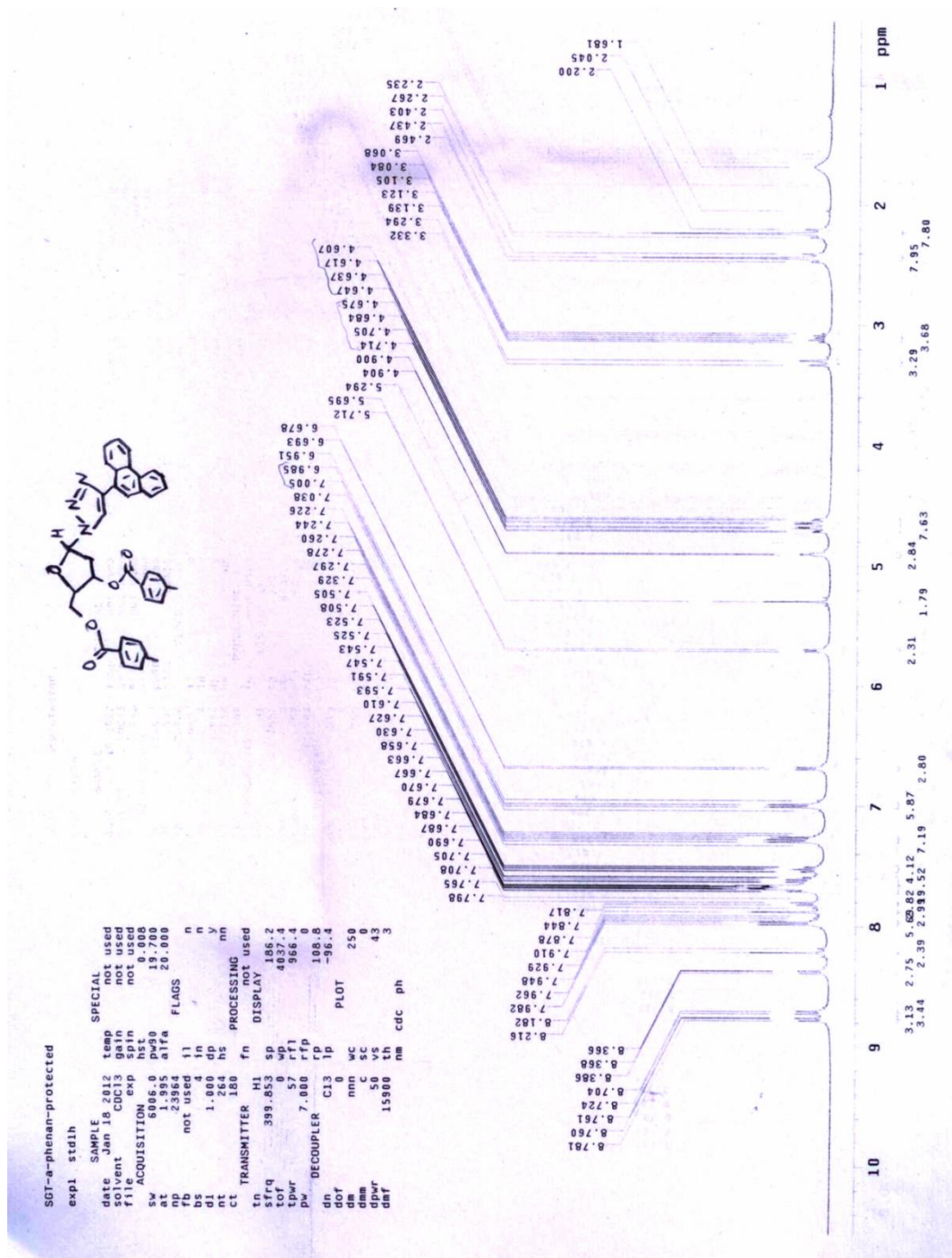


Figure S17. ^1H NMR spectra of nucleoside 17 α

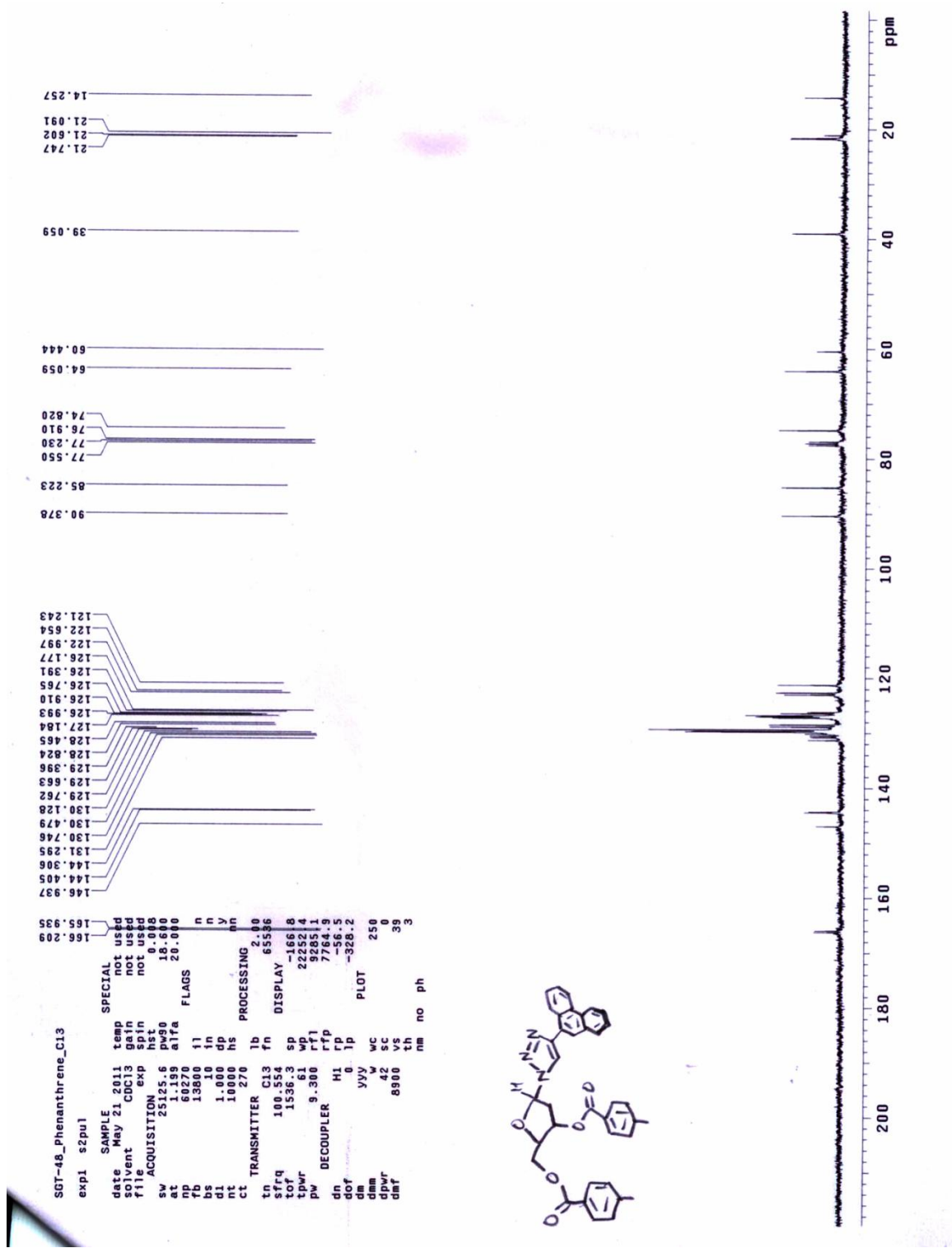


Figure S18. ^{13}C NMR spectra of nucleoside 17 α

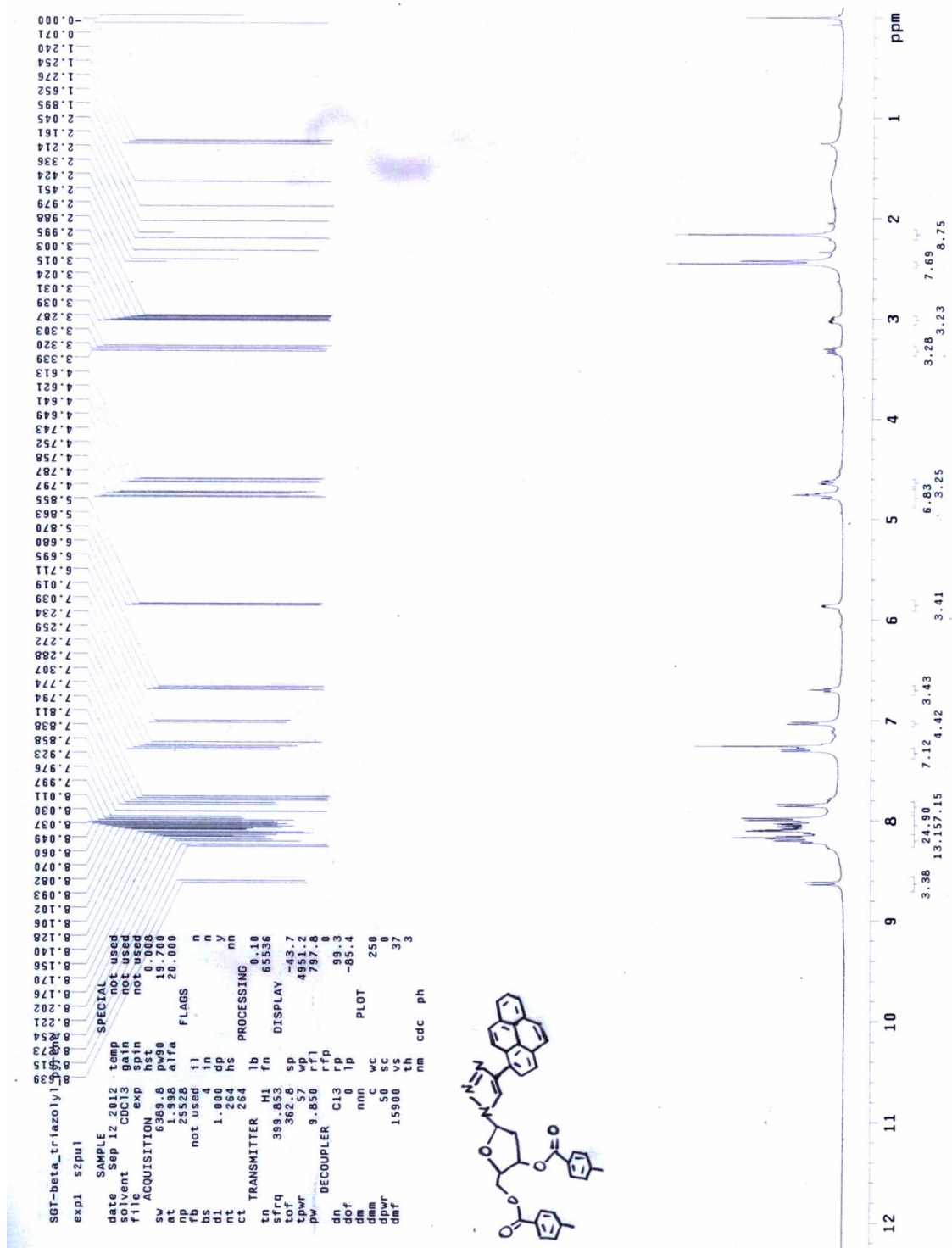


Figure S19. ¹H NMR spectra of nucleoside 18B

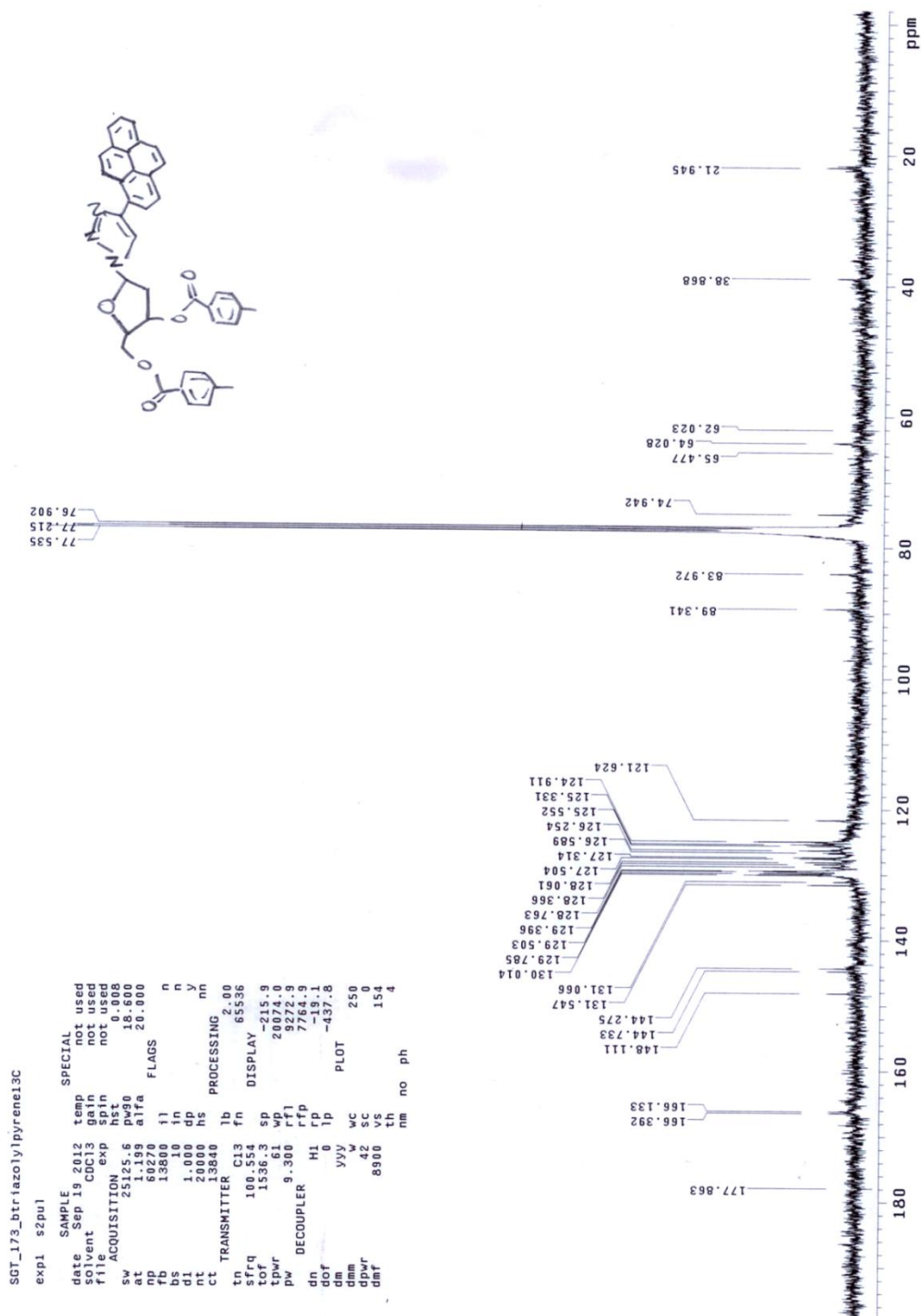


Figure S20. ¹³C NMR spectra of nucleoside 18β

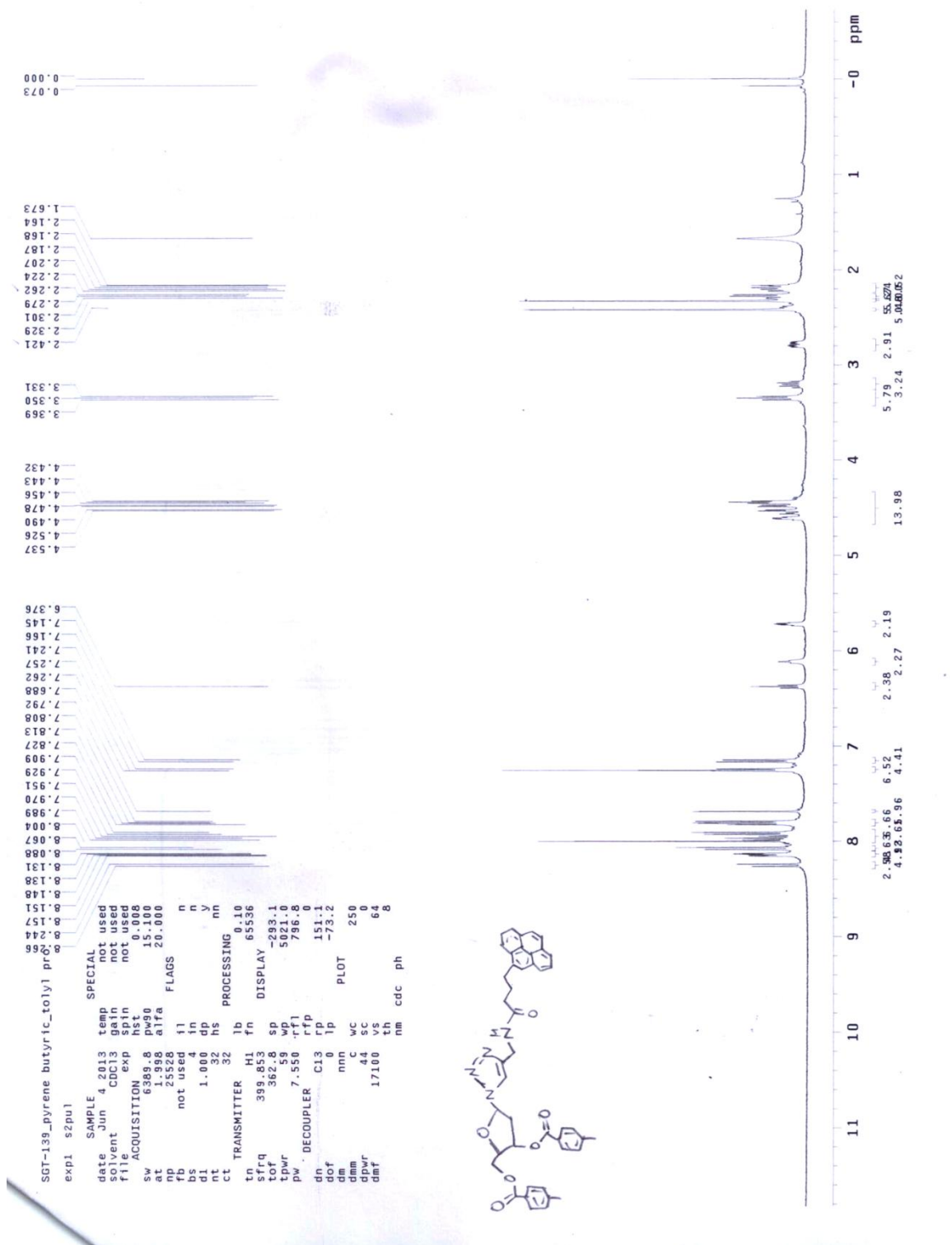


Figure S21. ¹H NMR spectra of nucleoside 19b

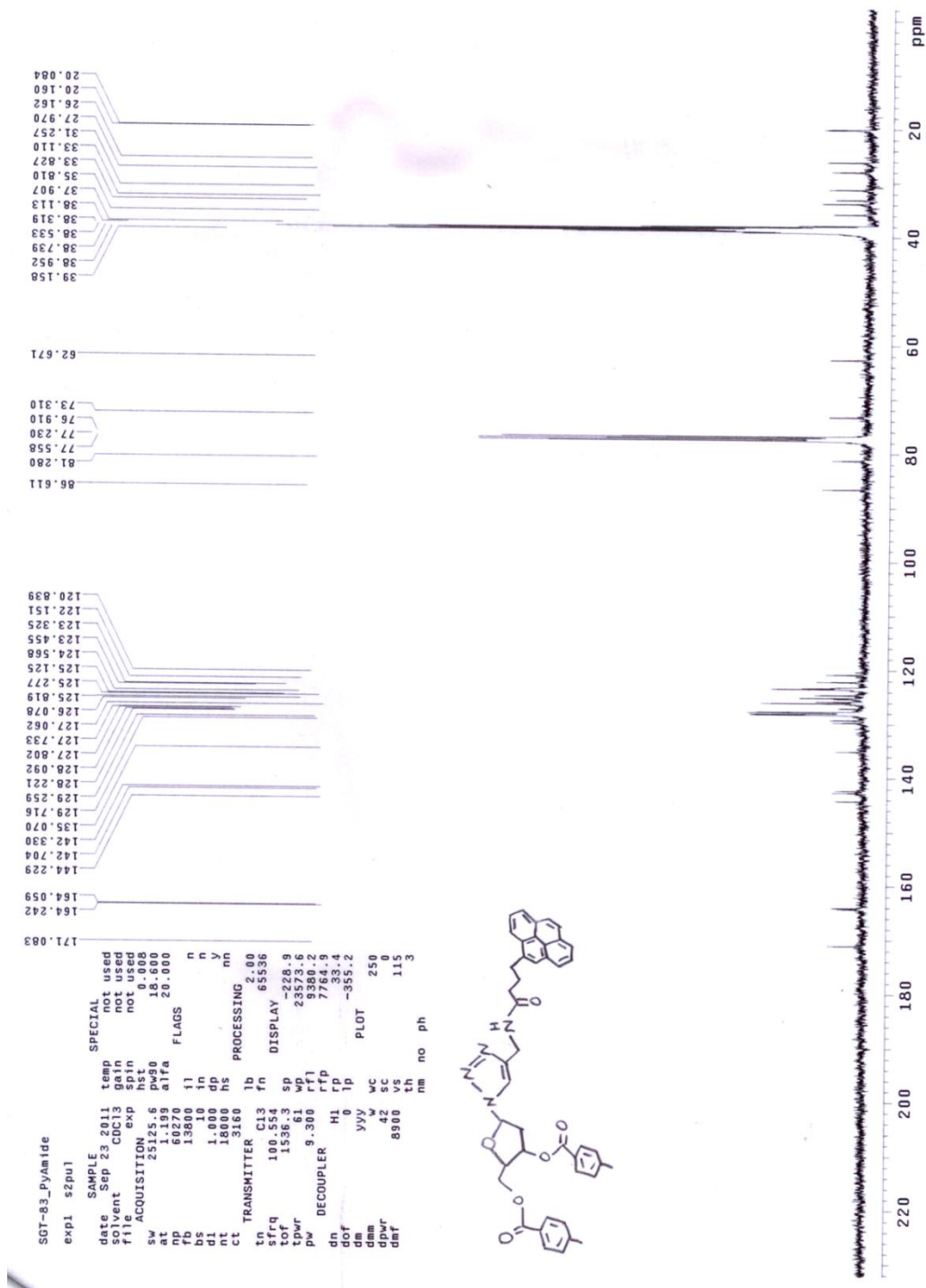


Figure S22. ^{13}C NMR spectra of nucleoside 19b

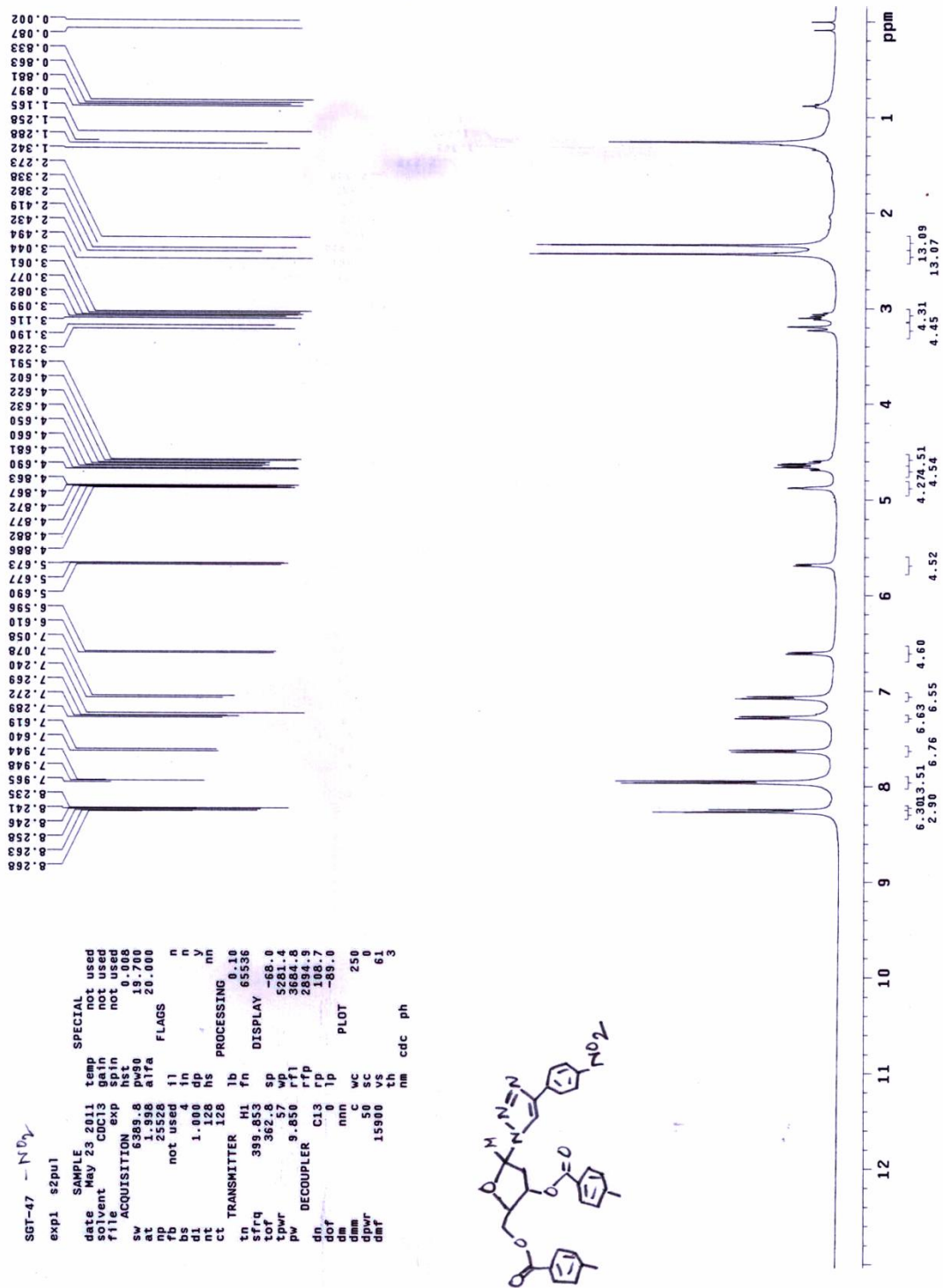


Figure S23. ^1H NMR spectra of nucleoside 20 α

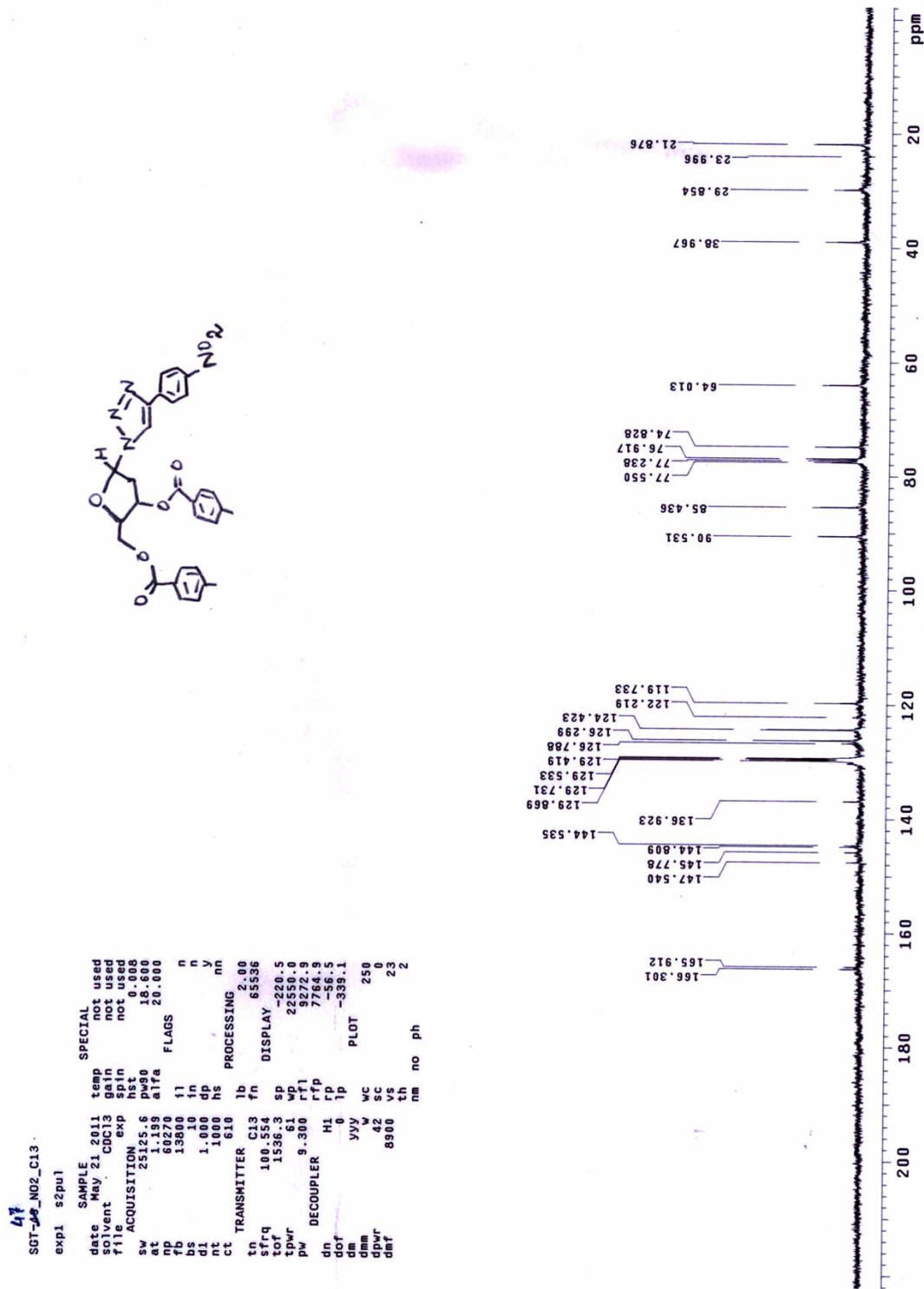


Figure S24. ¹³C NMR spectra of nucleoside 20α

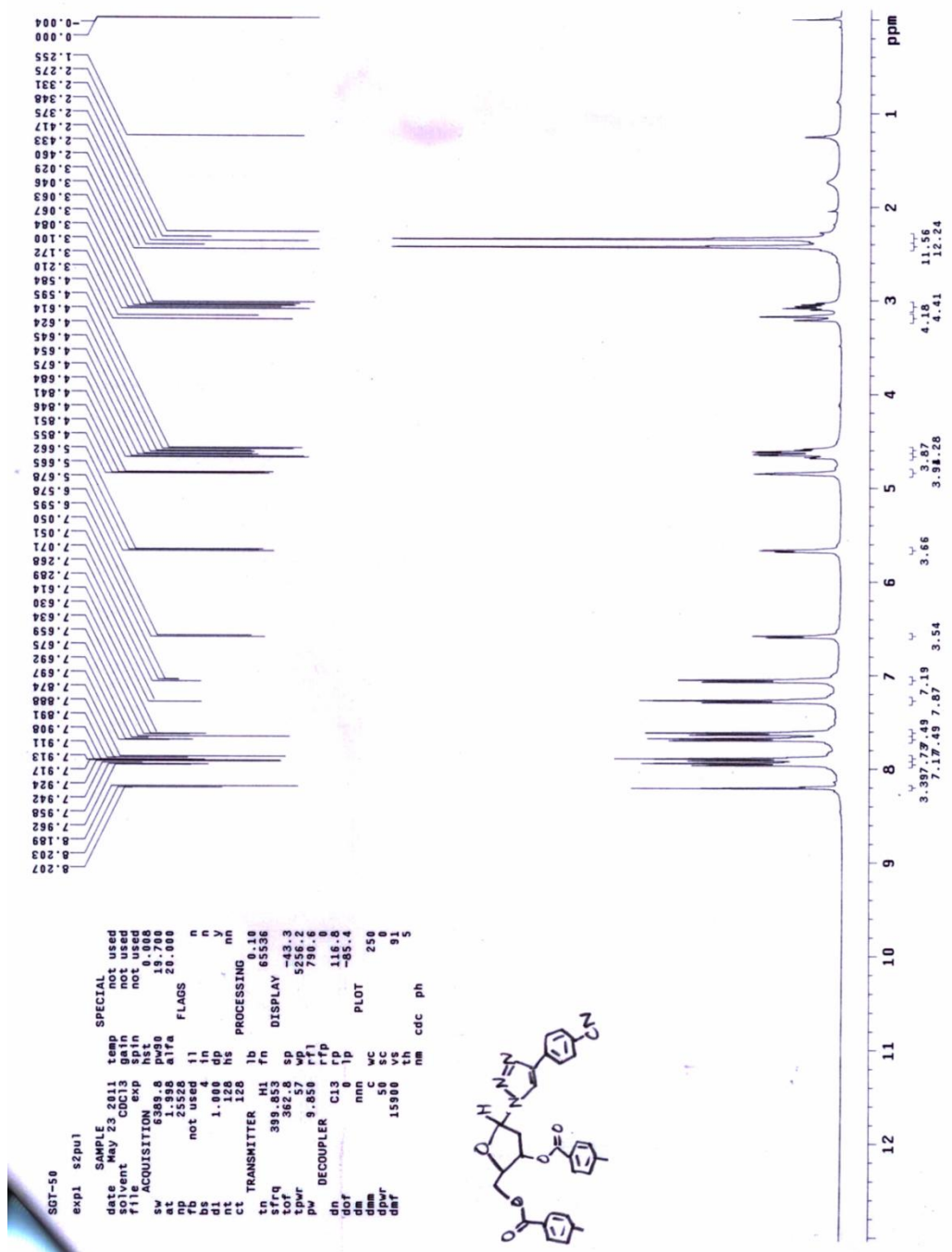


Figure S25. ¹H NMR spectra of nucleoside 21α

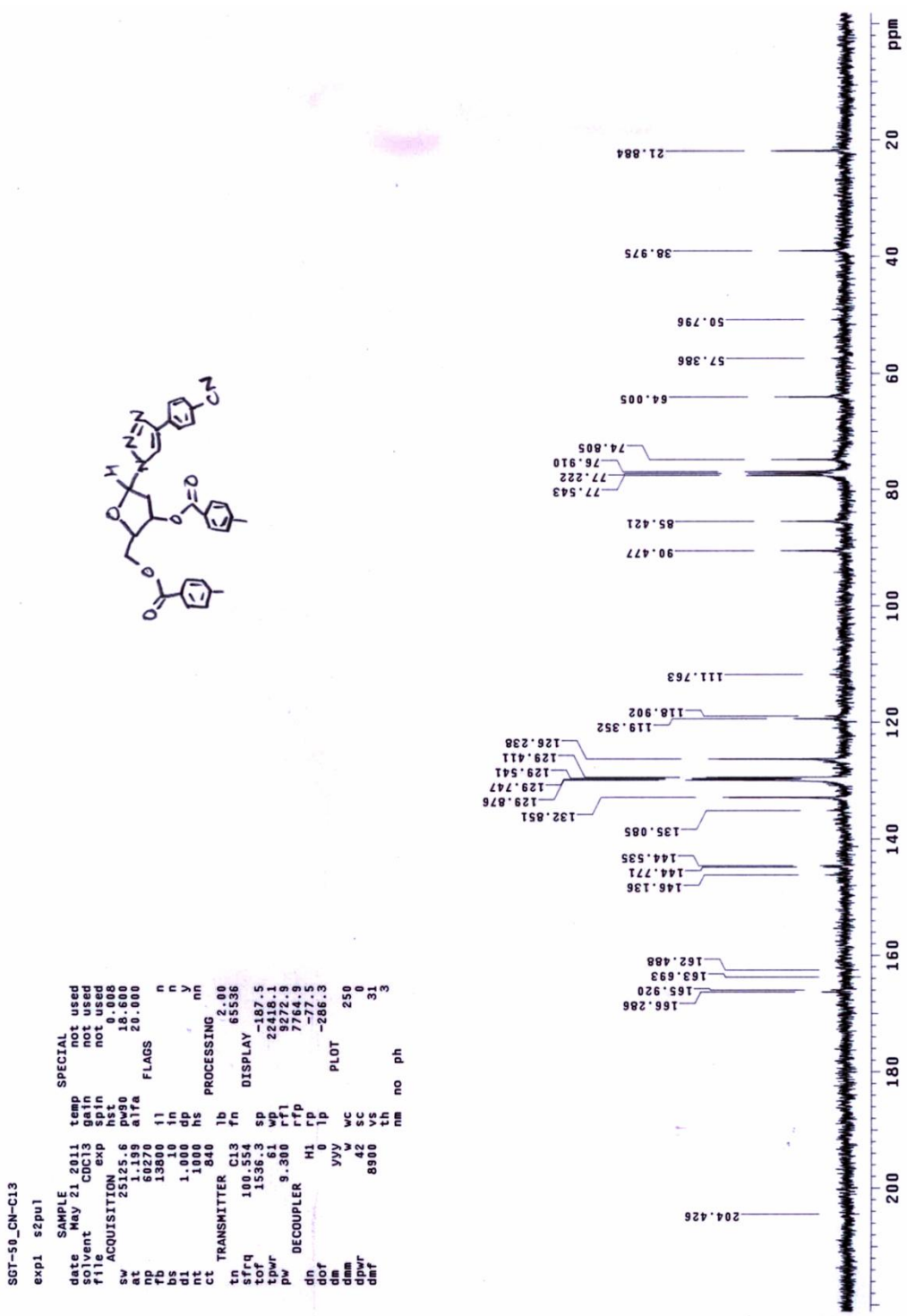


Figure S26. ^{13}C NMR spectra of nucleoside 21α

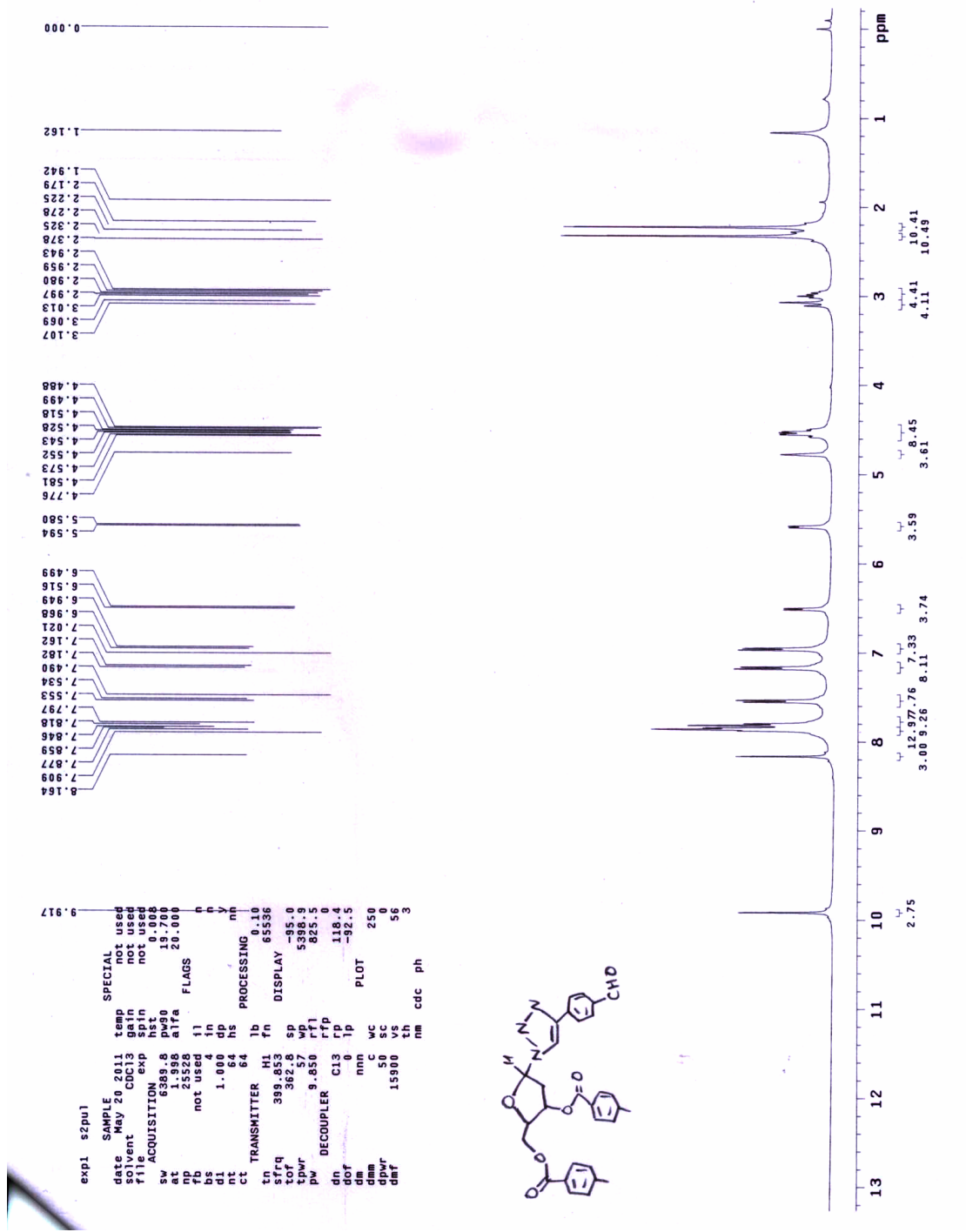


Figure S27. ¹H NMR spectra of nucleoside 22α

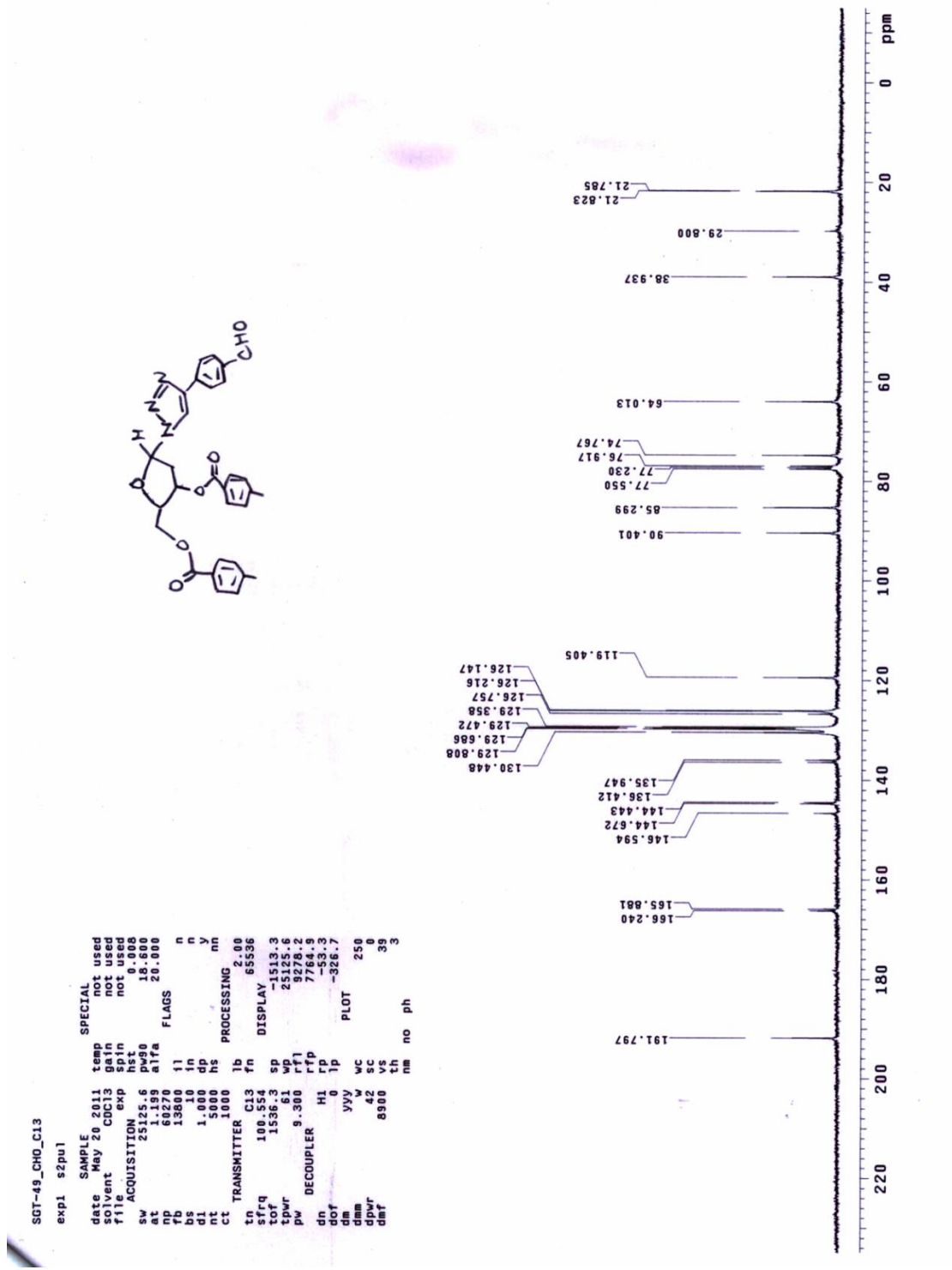


Figure S28. ^{13}C NMR spectra of nucleoside 22α

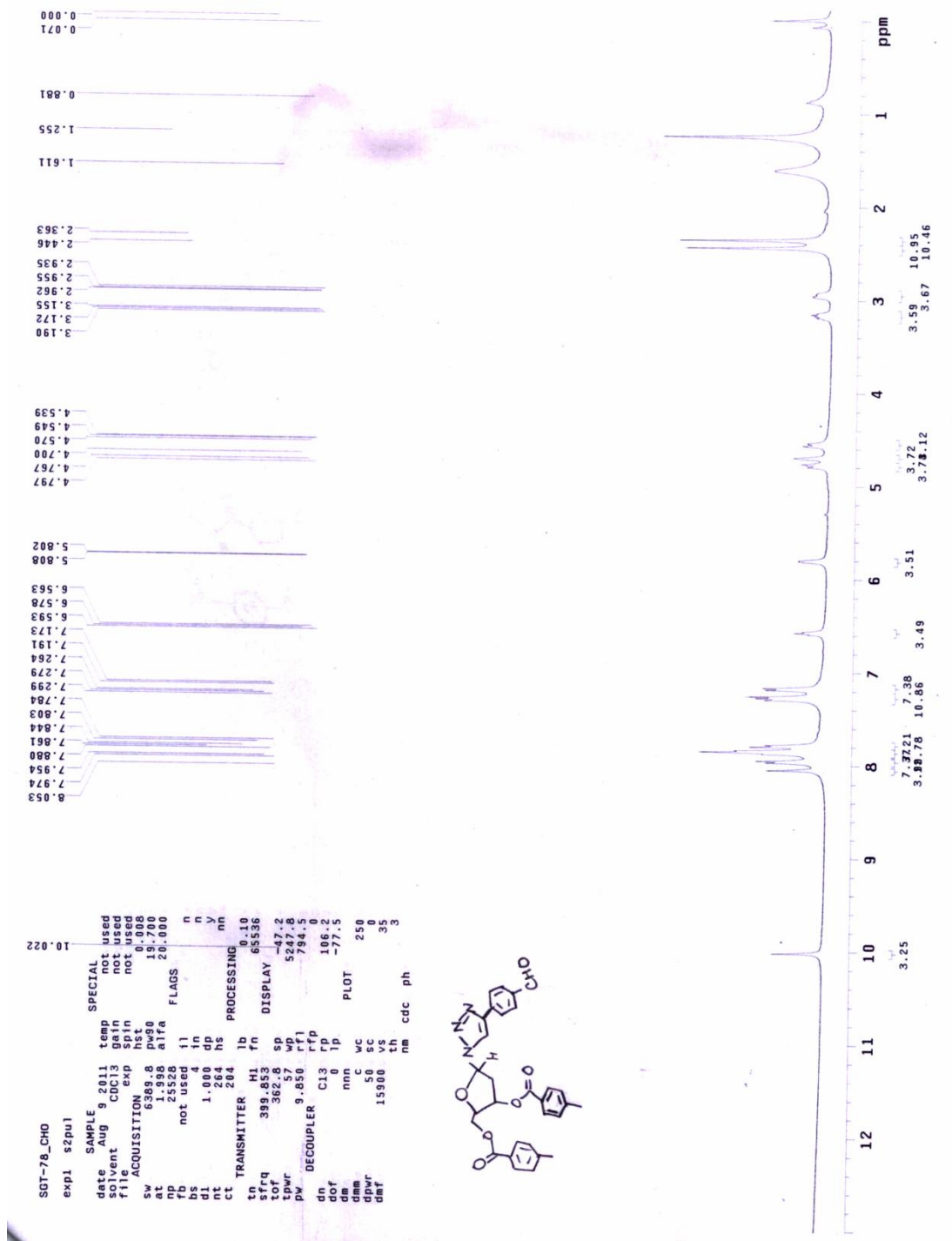


Figure S29. ¹H NMR spectra of nucleoside 22b

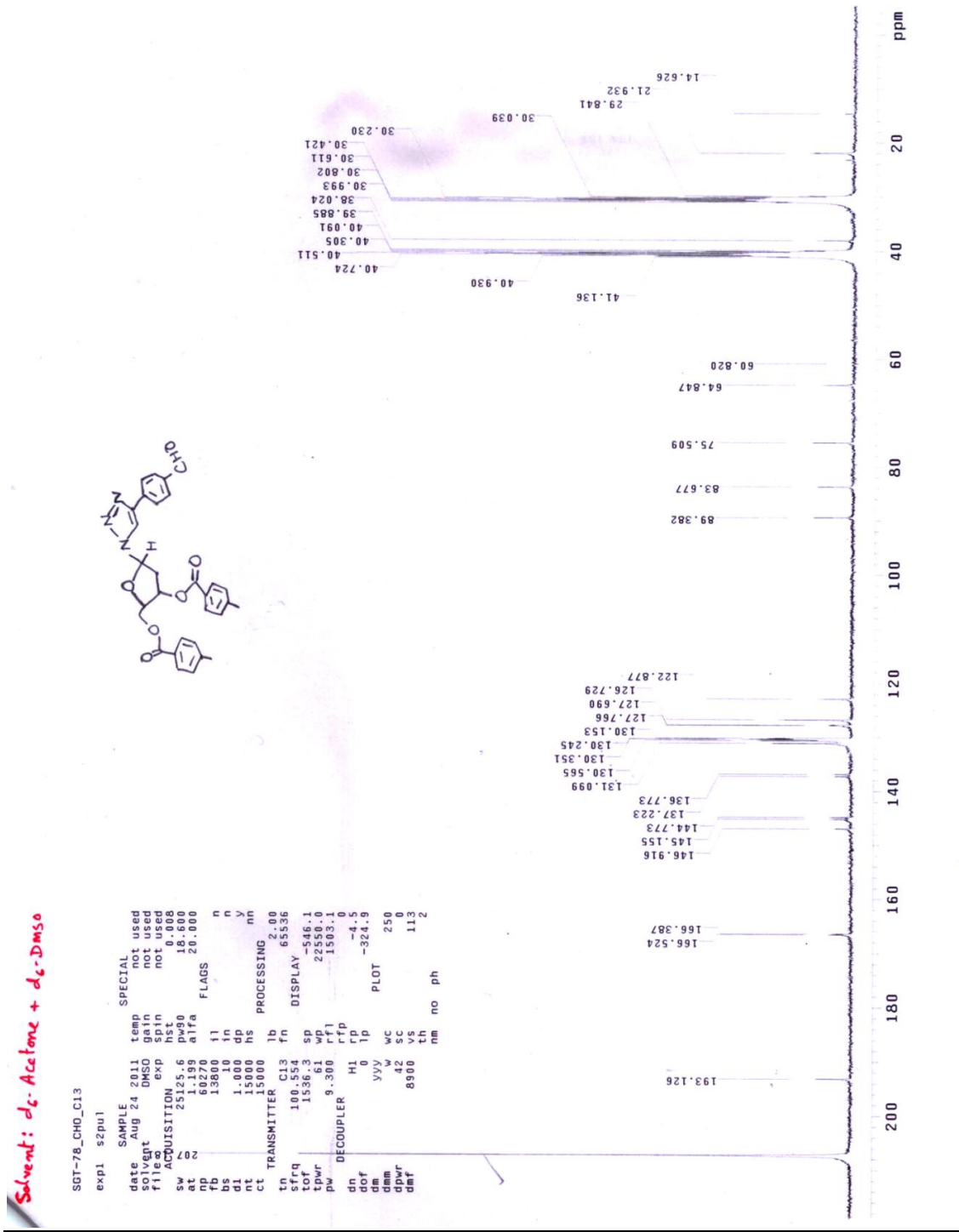


Figure S30. ^{13}C NMR spectra of nucleoside 22 β

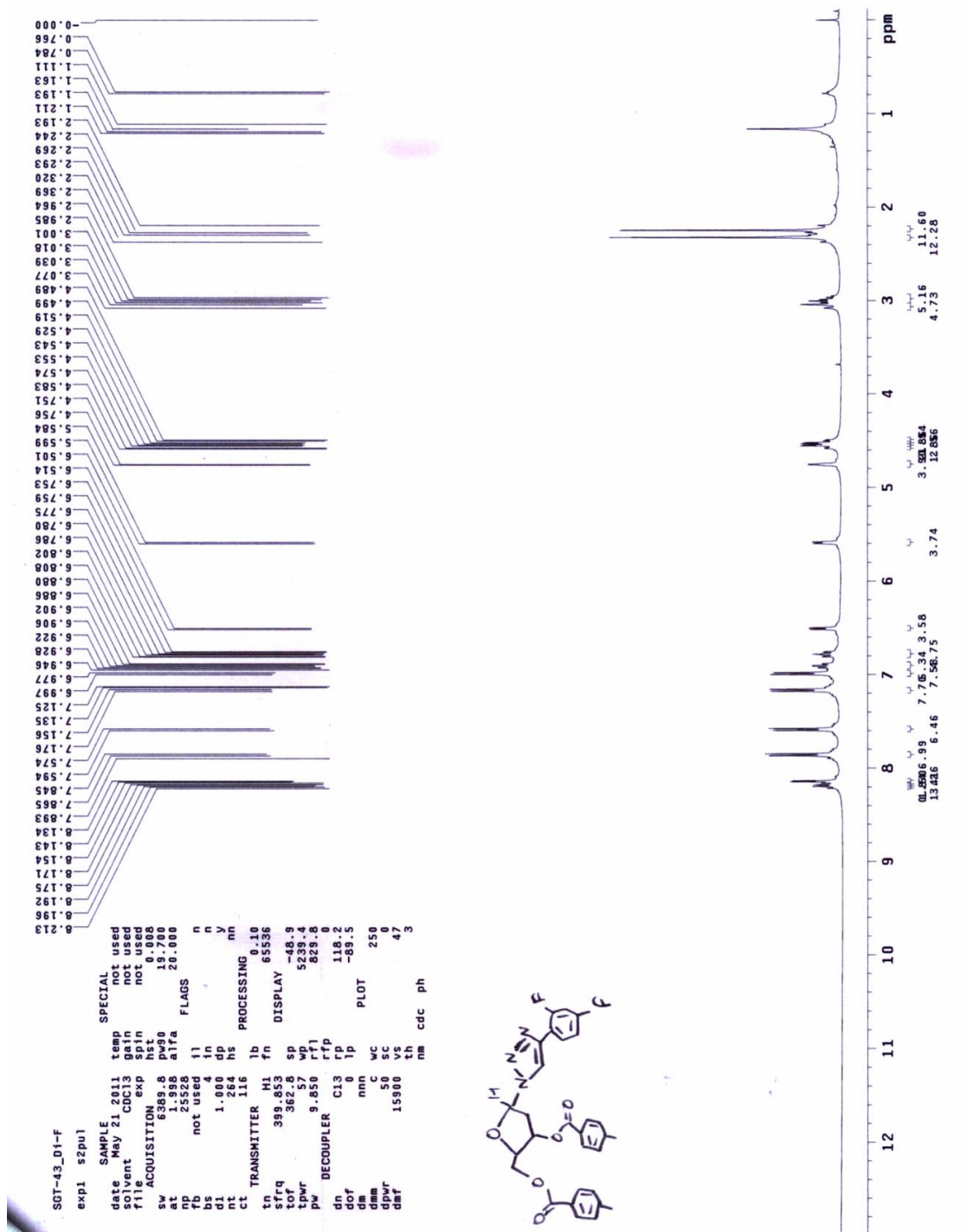


Figure S31. ^1H NMR spectra of nucleoside 23 α

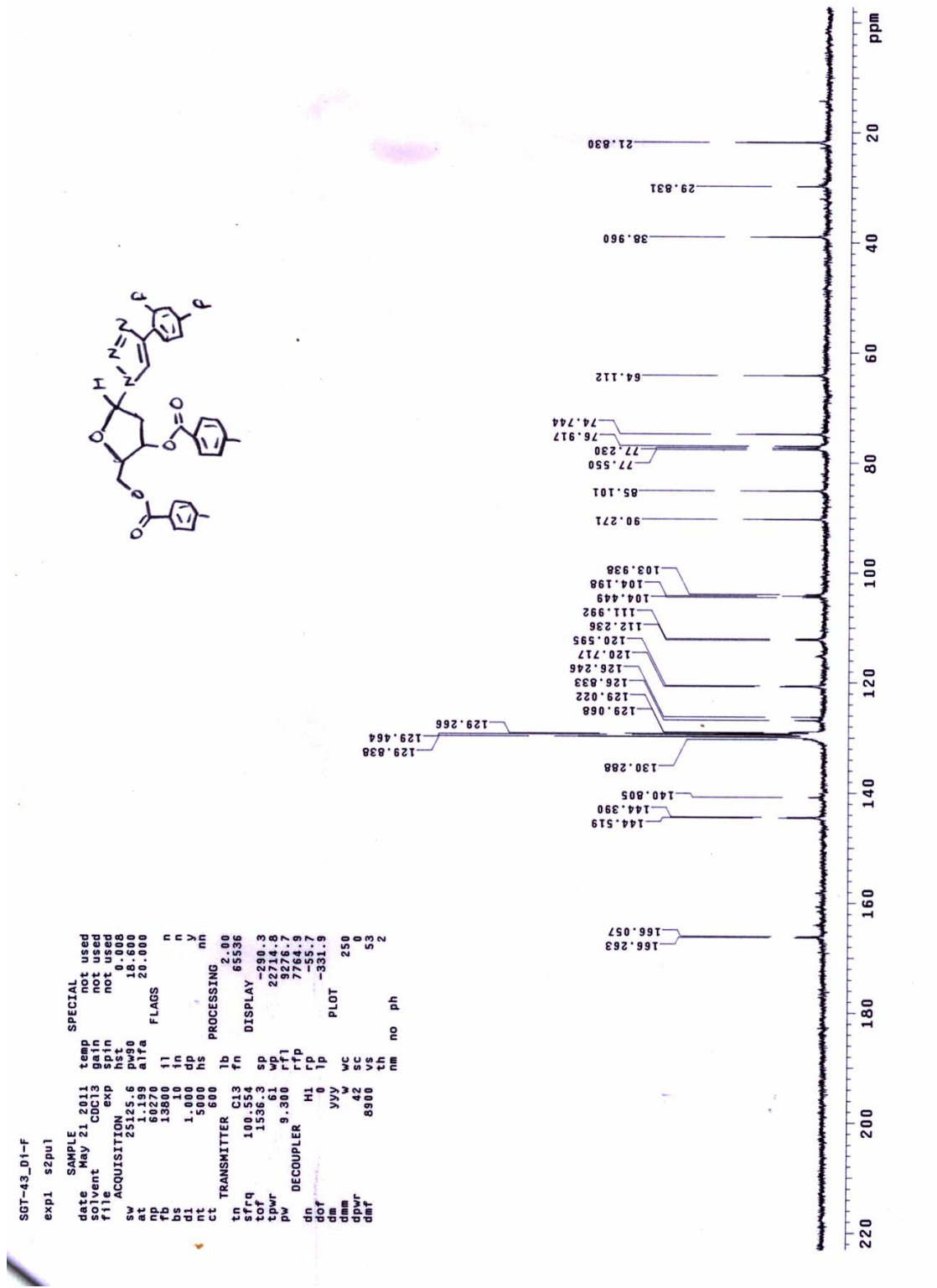


Figure S32. ^{13}C NMR spectra of nucleoside 23 α

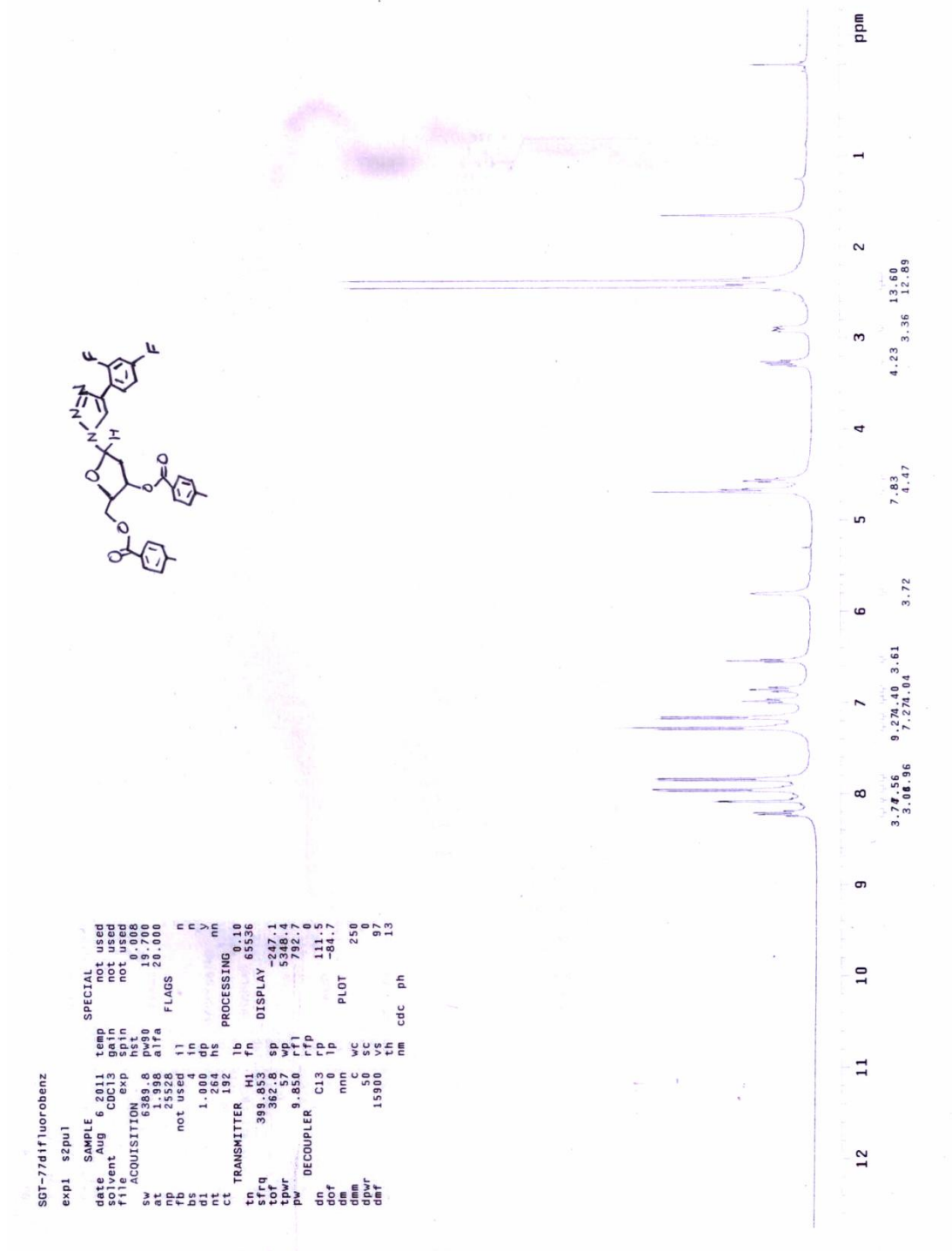


Figure S33. ^1H NMR spectra of nucleoside 23B

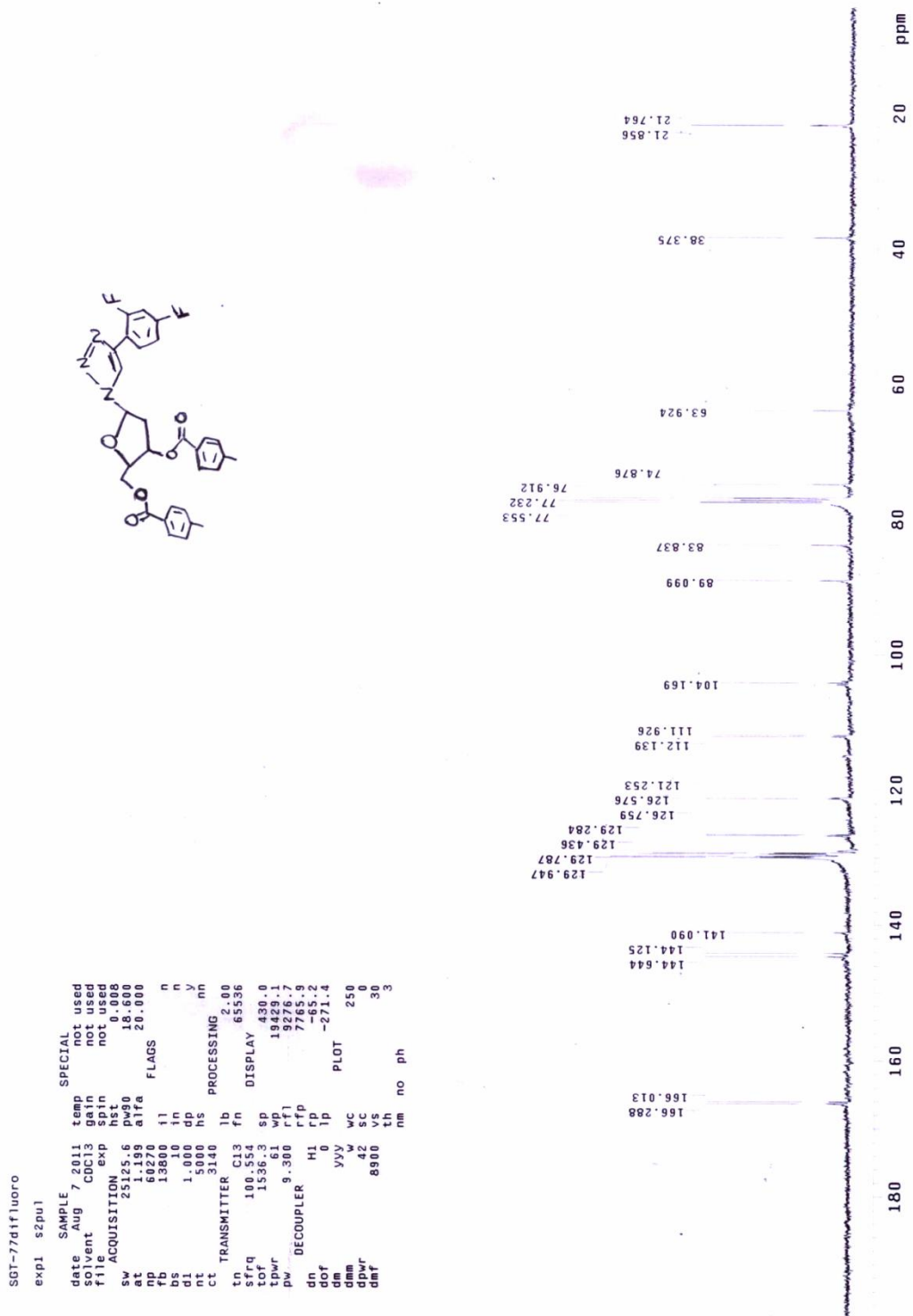


Figure S34. ¹³C NMR spectra of nucleoside 24B

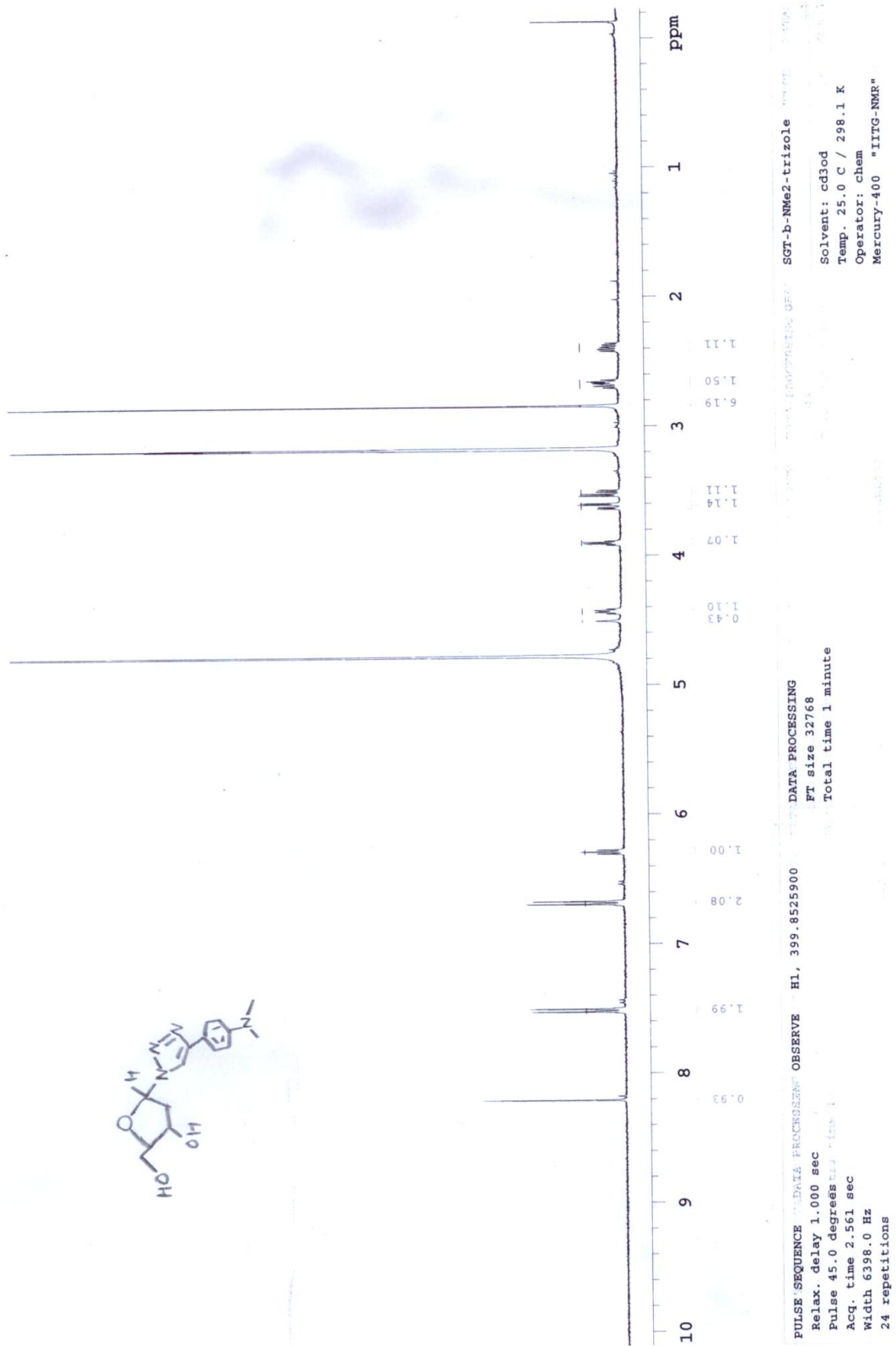


Figure S35. ¹H NMR spectra of nucleoside 1β

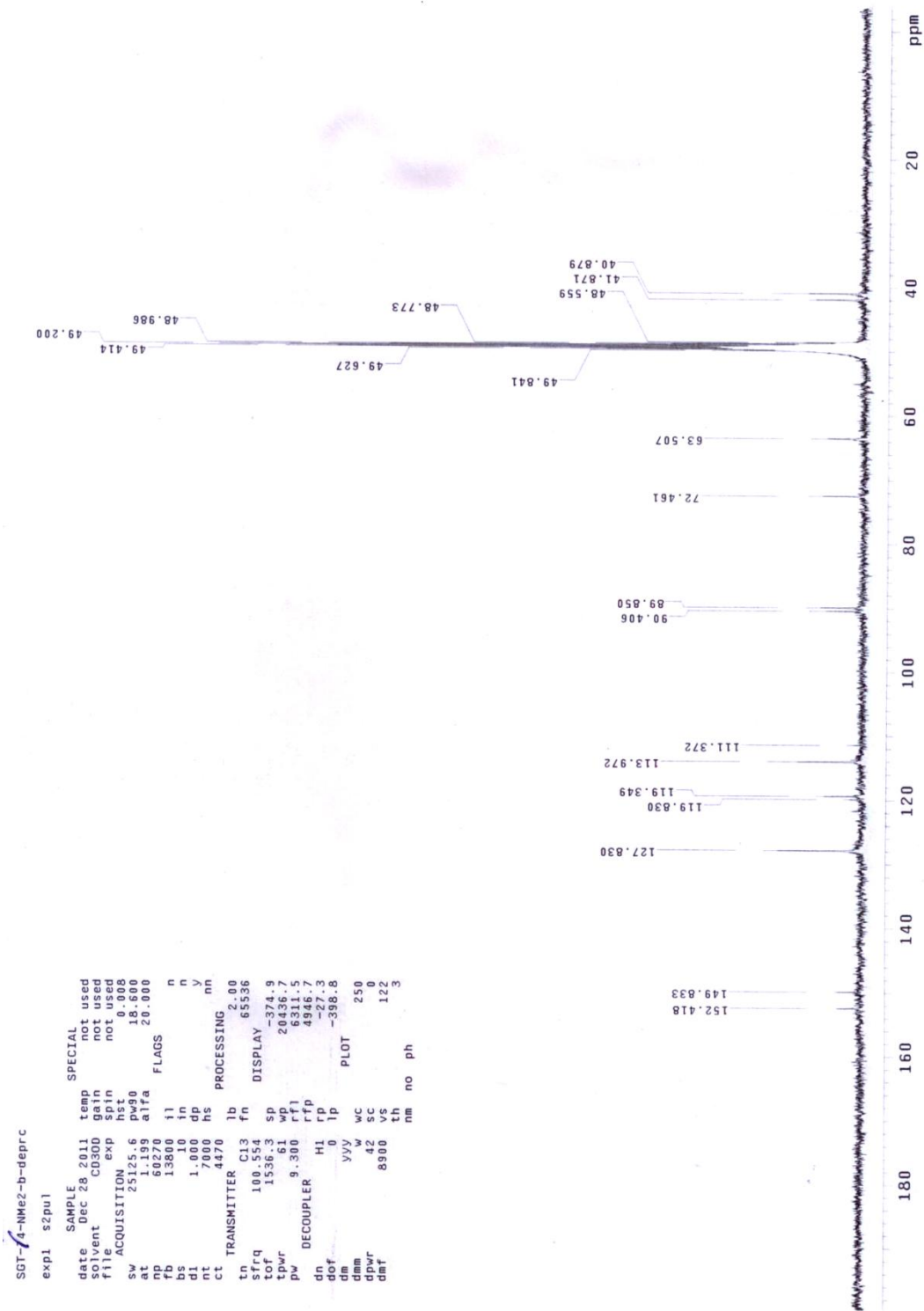


Figure S36. ^{13}C NMR spectra of nucleoside 1B

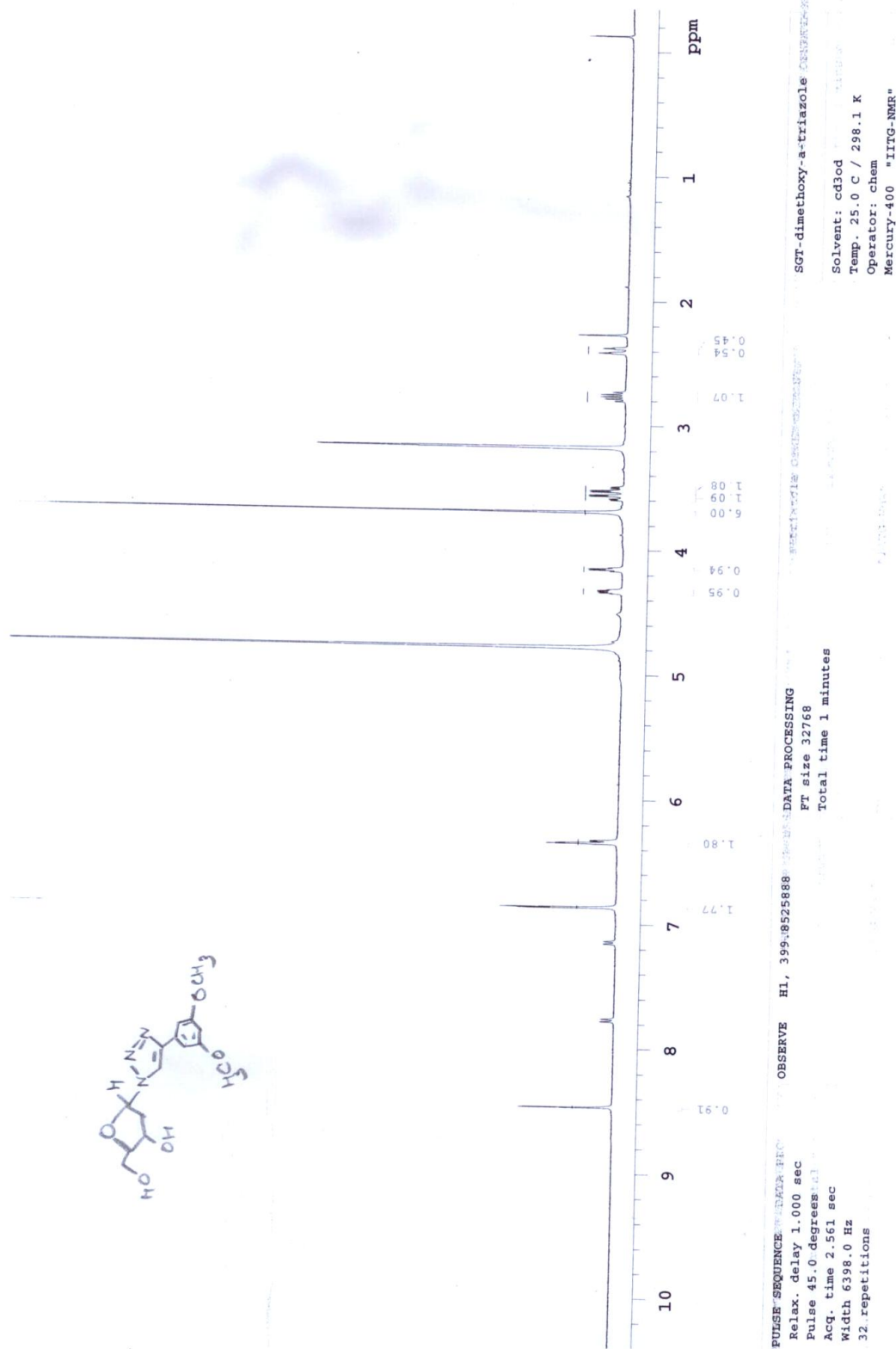


Figure S37. ¹H NMR spectra of nucleoside 2a

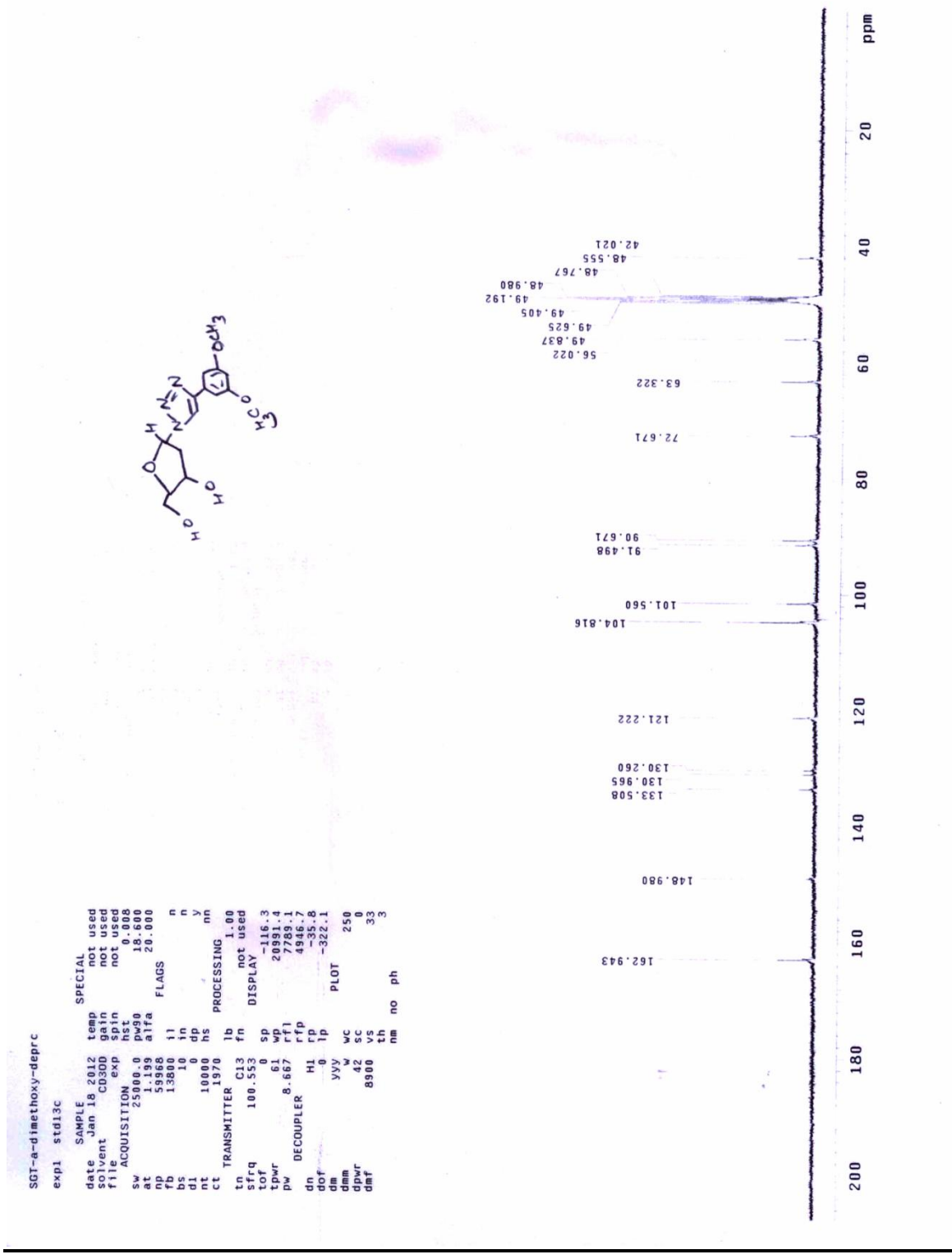


Figure S38. ^{13}C NMR spectra of nucleoside 2 α

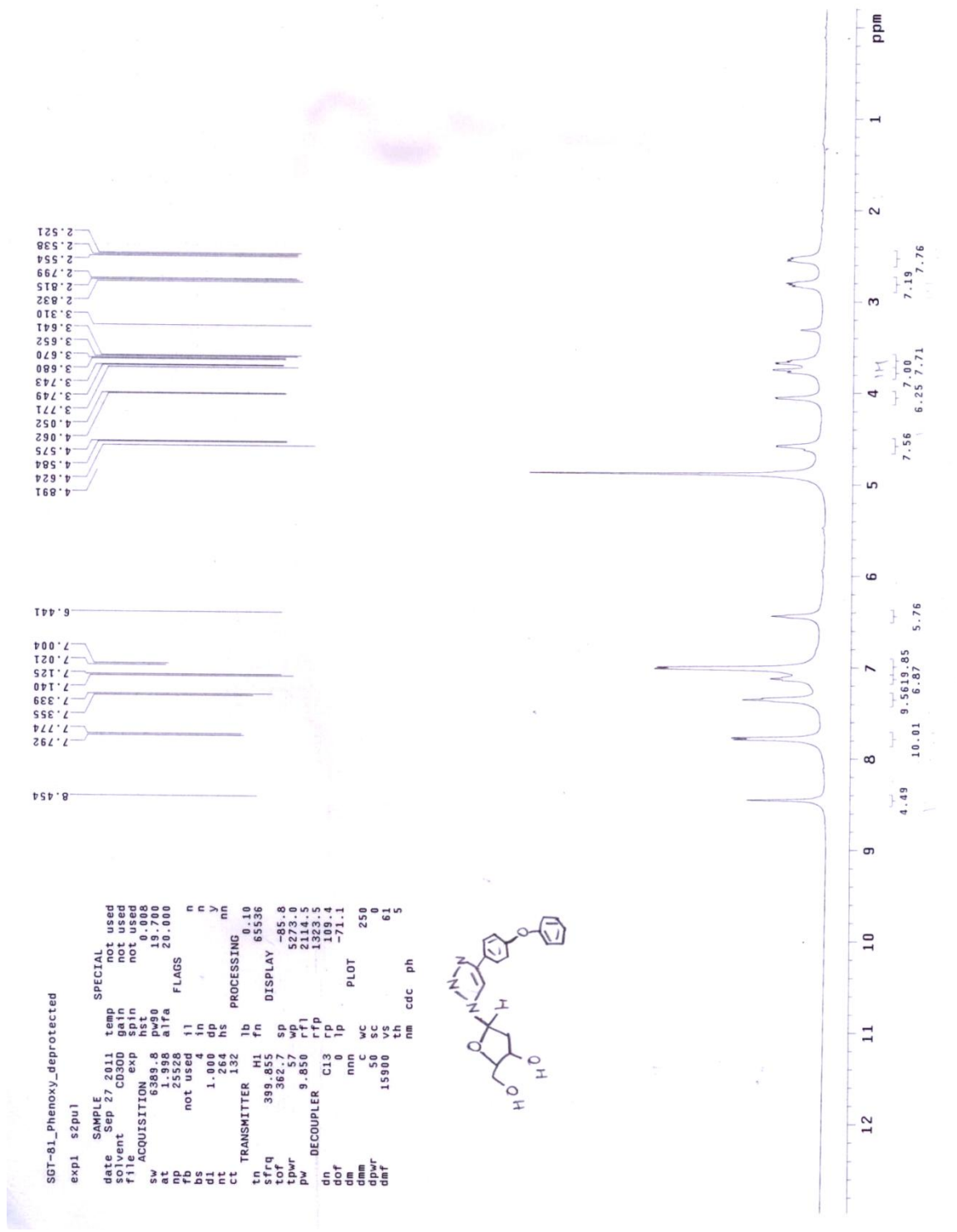


Figure S39. ^1H NMR spectra of nucleoside 3 β

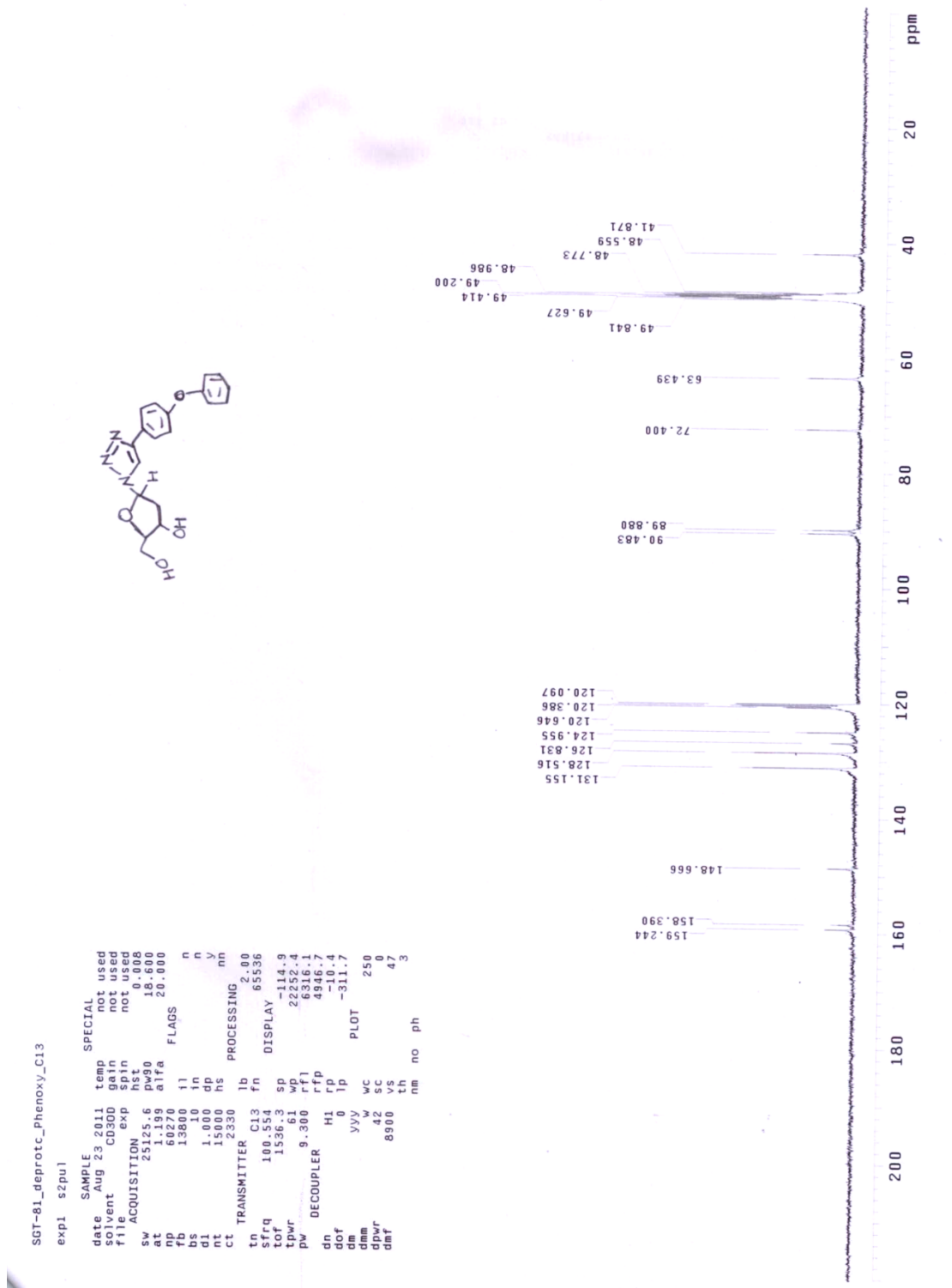


Figure S40. ^{13}C NMR spectra of nucleoside 3B

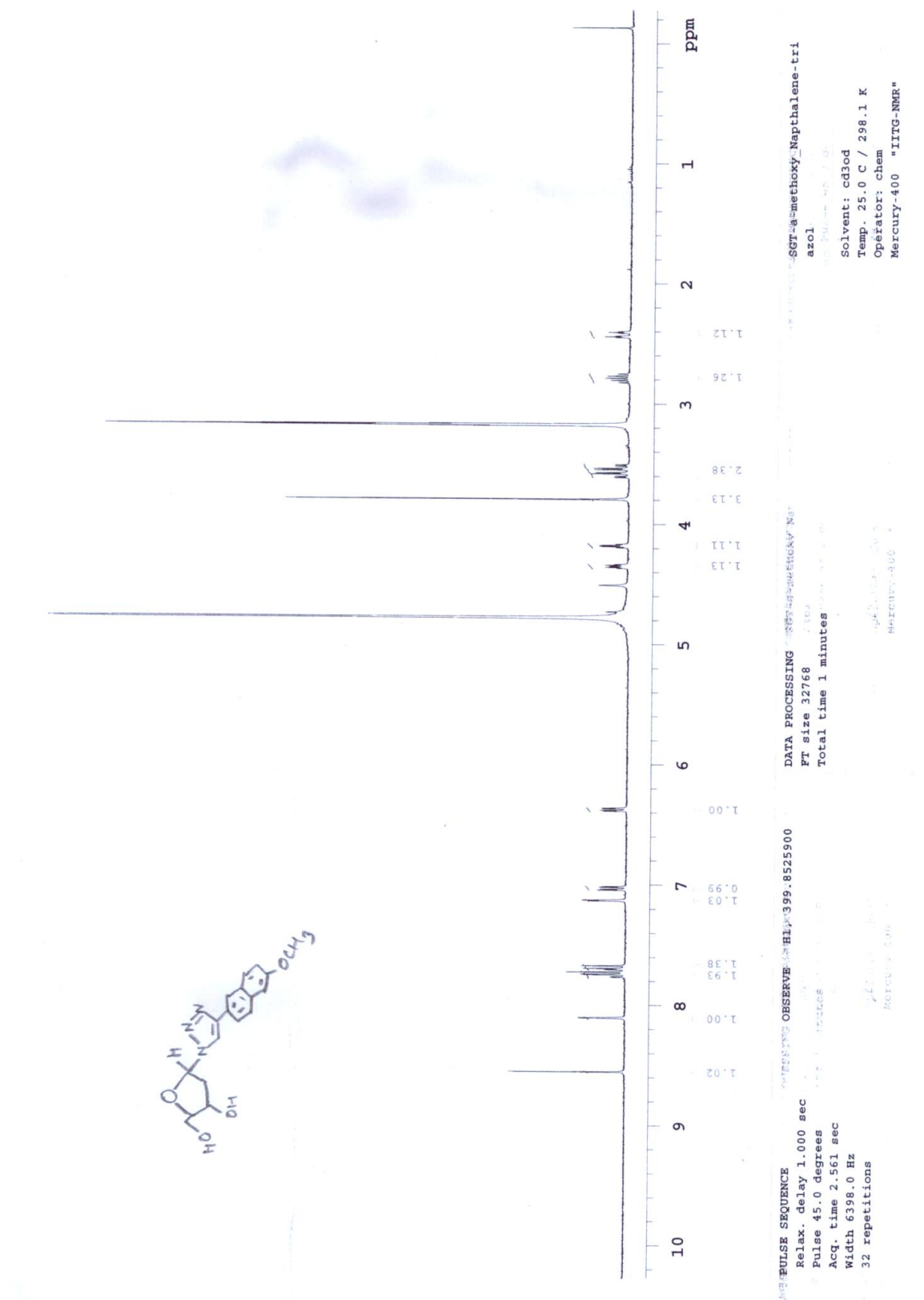


Figure S41. ¹H NMR spectra of nucleoside 4α

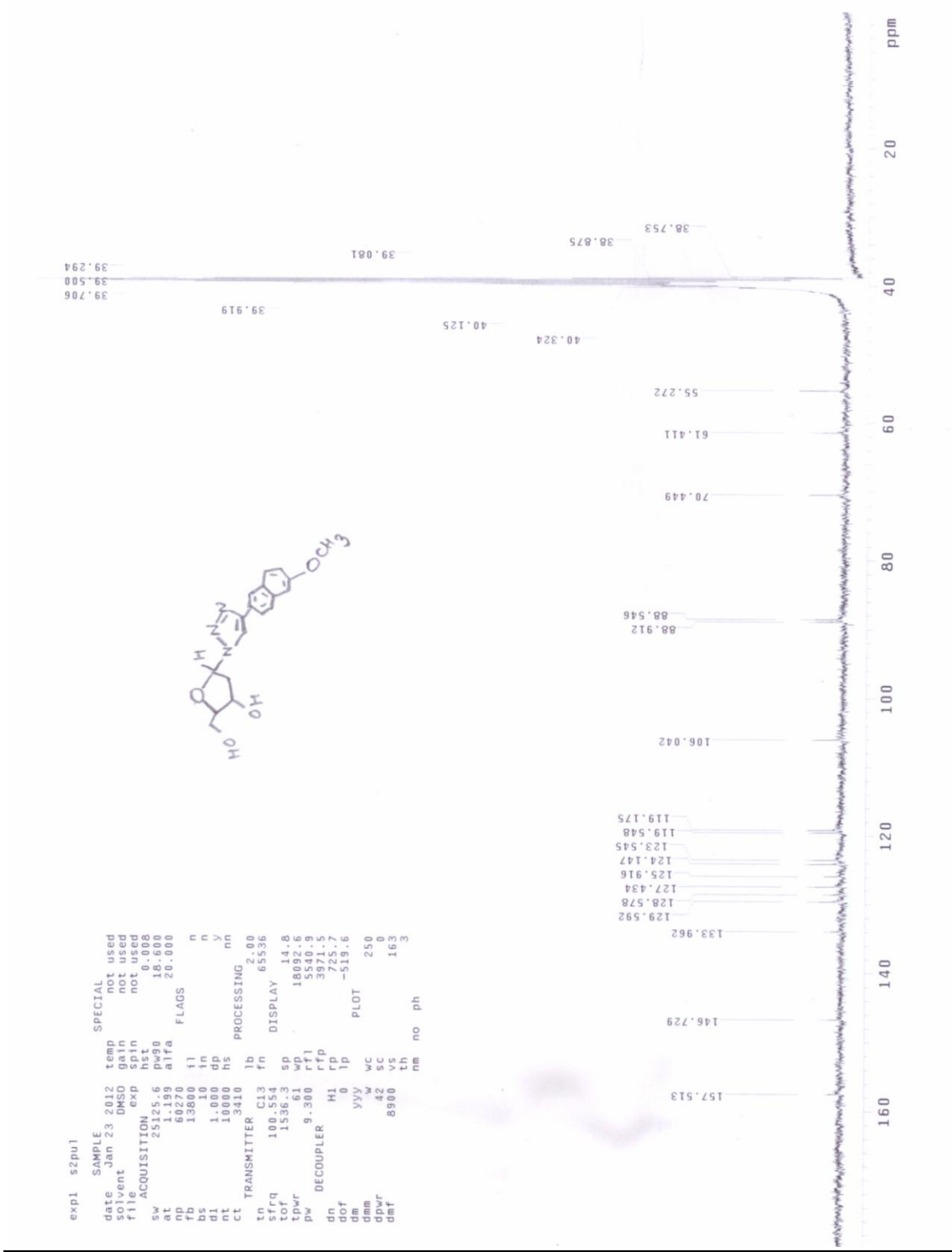


Figure S42. ^{13}C NMR spectra of nucleoside 4 α

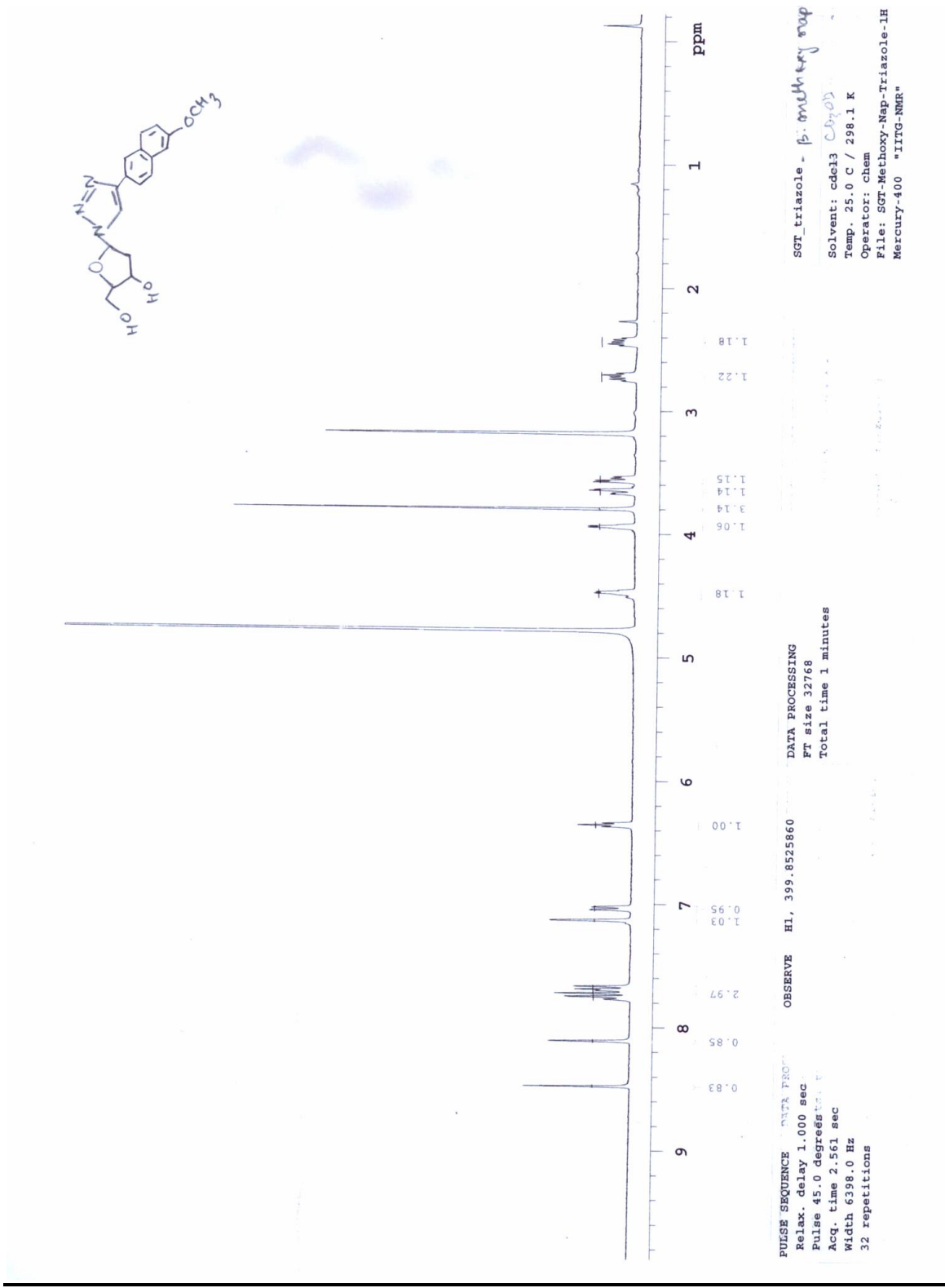


Figure S43. ¹H NMR spectra of nucleoside 4B

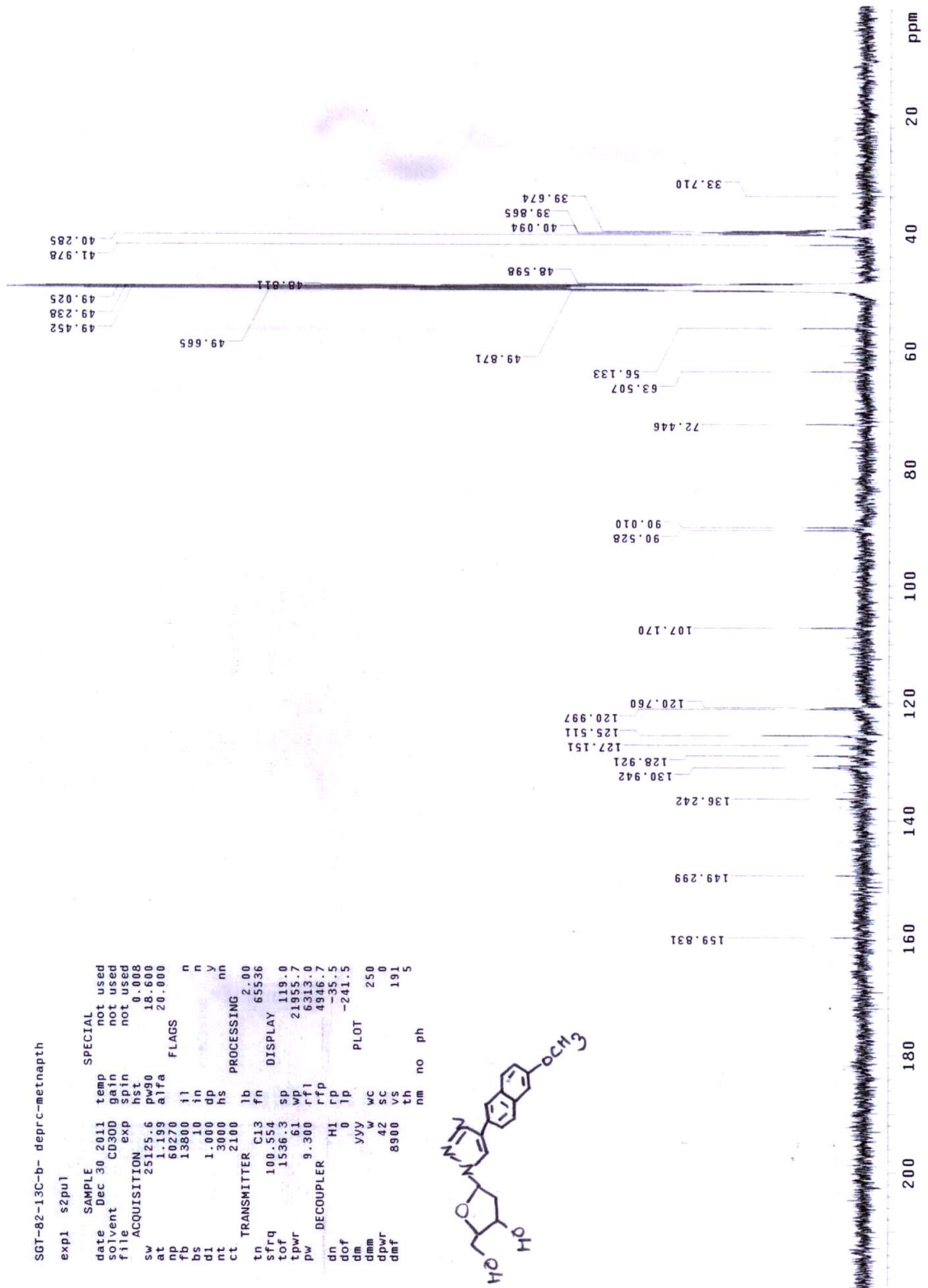


Figure S44. ¹³C NMR spectra of nucleoside 4b

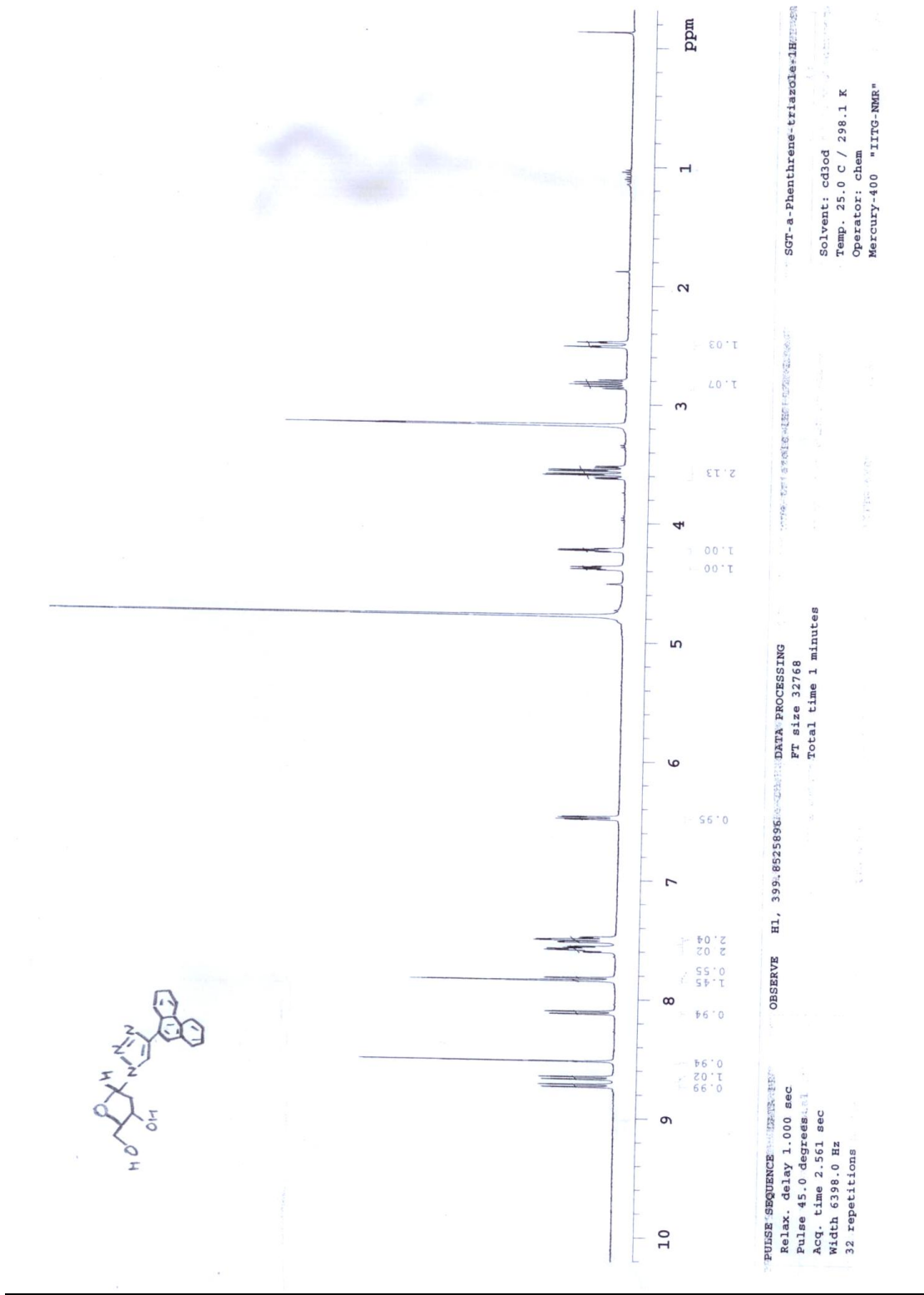


Figure S45. ¹H NMR spectra of nucleoside 5α

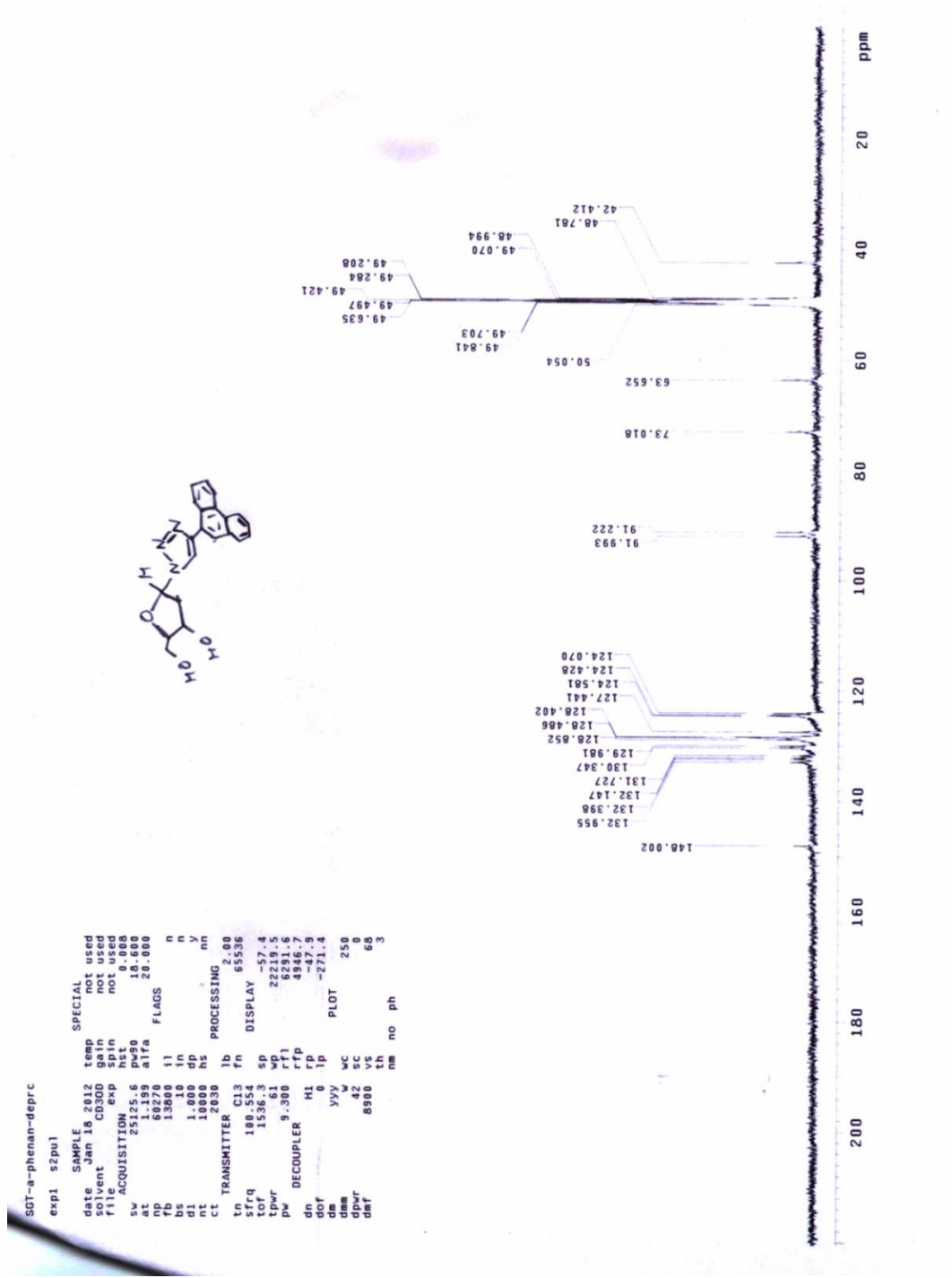


Figure S46. ^{13}C NMR spectra of nucleoside 5 α

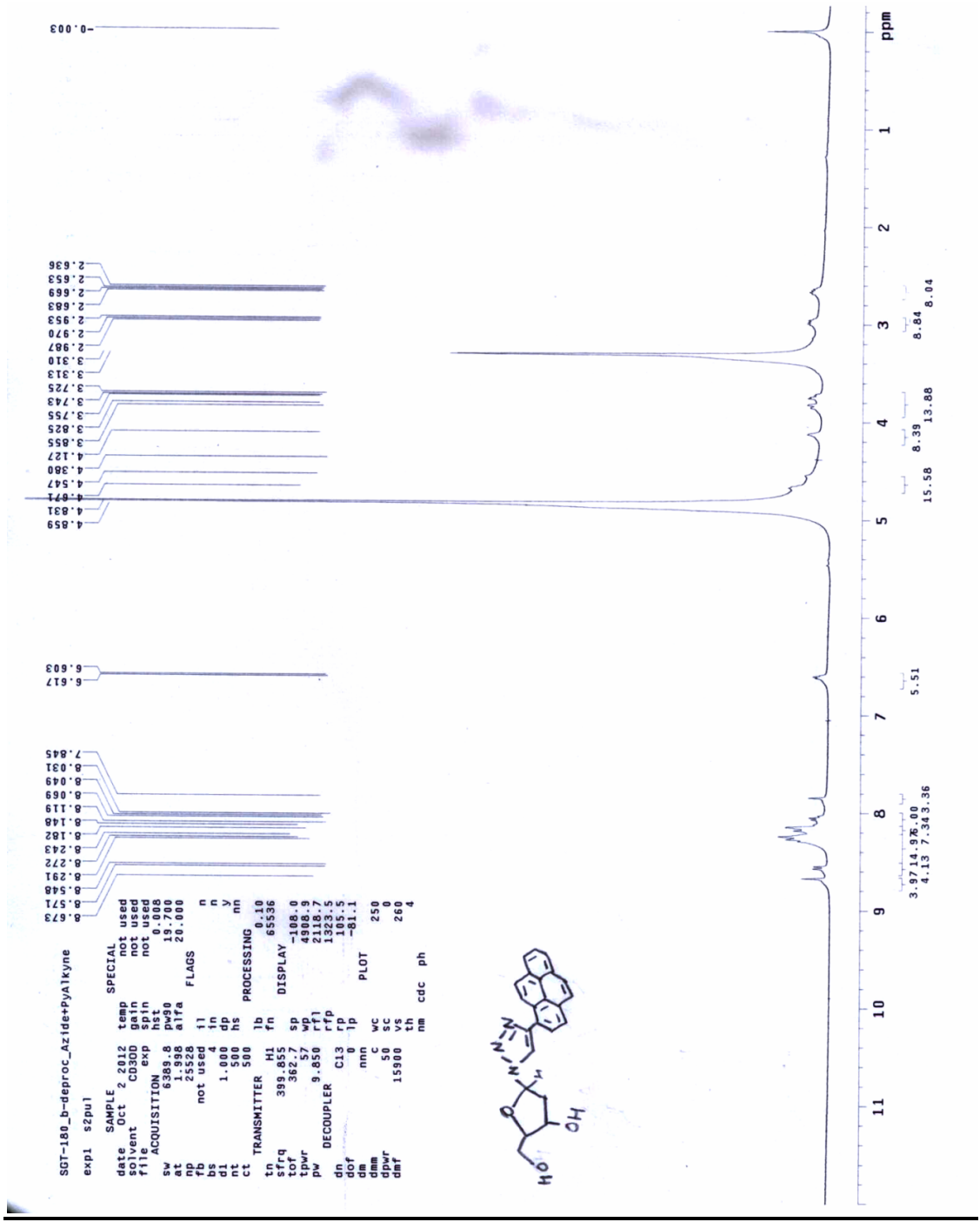


Figure S47. ¹H NMR spectra of nucleoside 6β

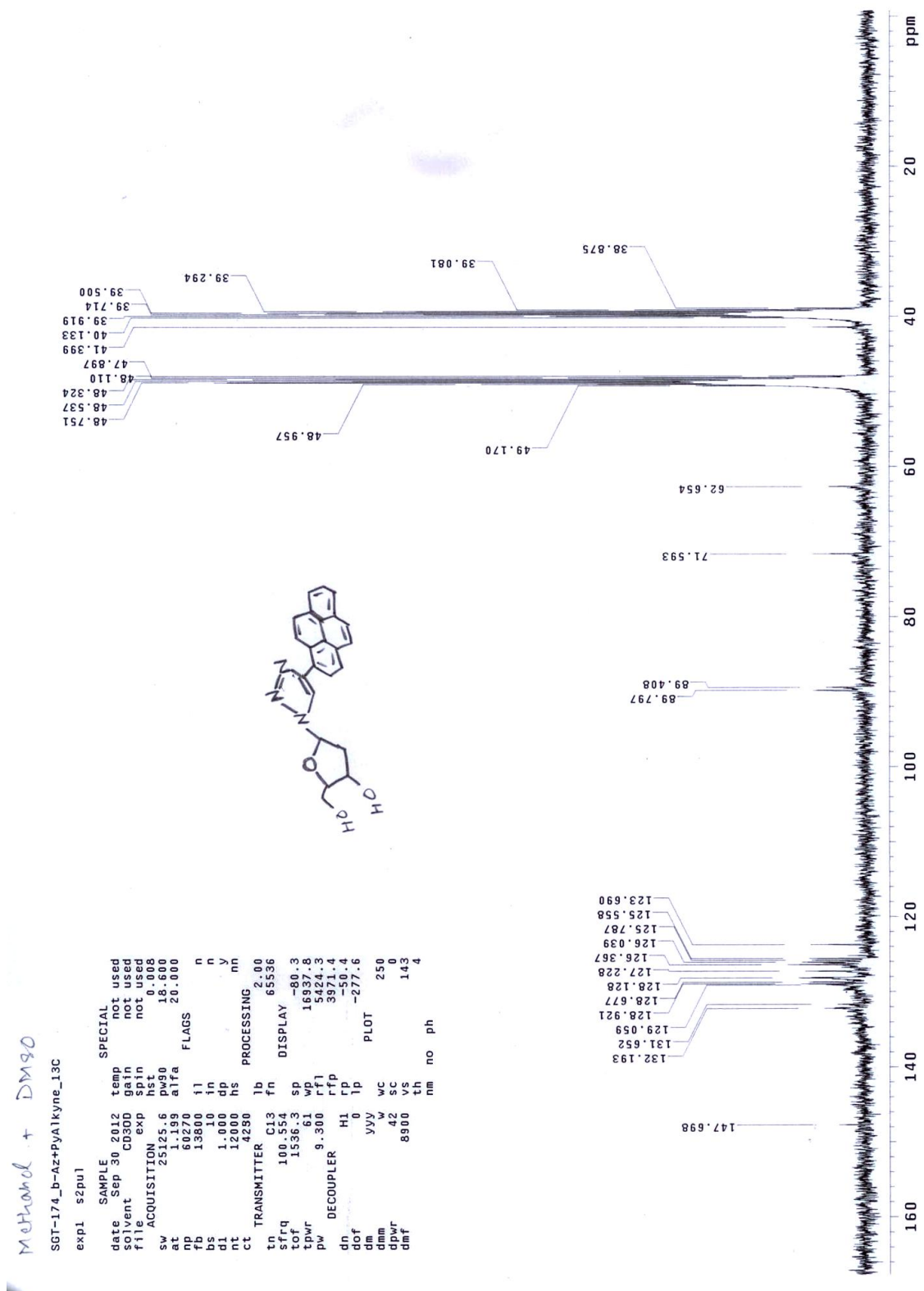


Figure S48. ^{13}C NMR spectra of nucleoside 6B

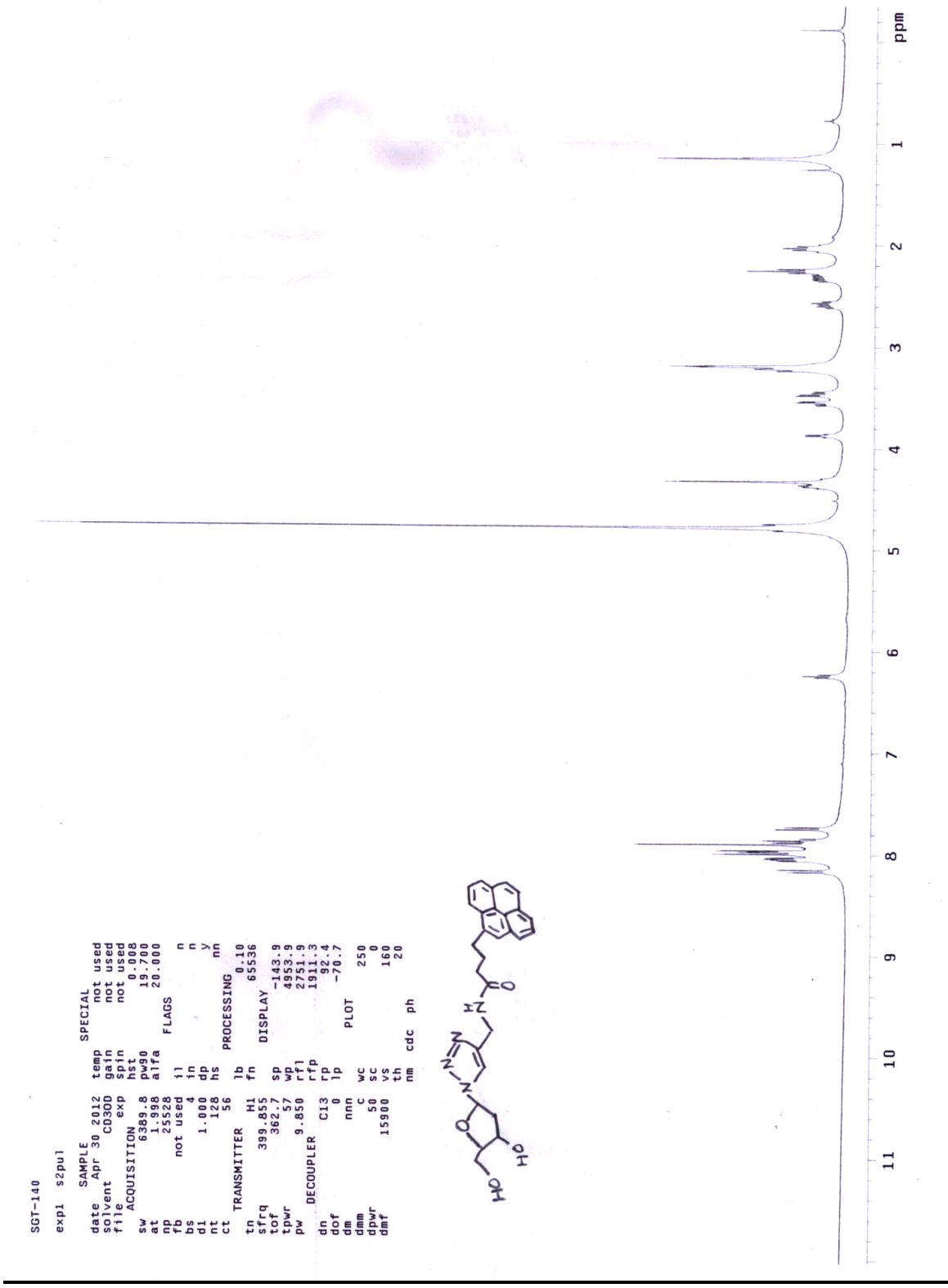


Figure S49. ¹H NMR spectra of nucleoside 7b

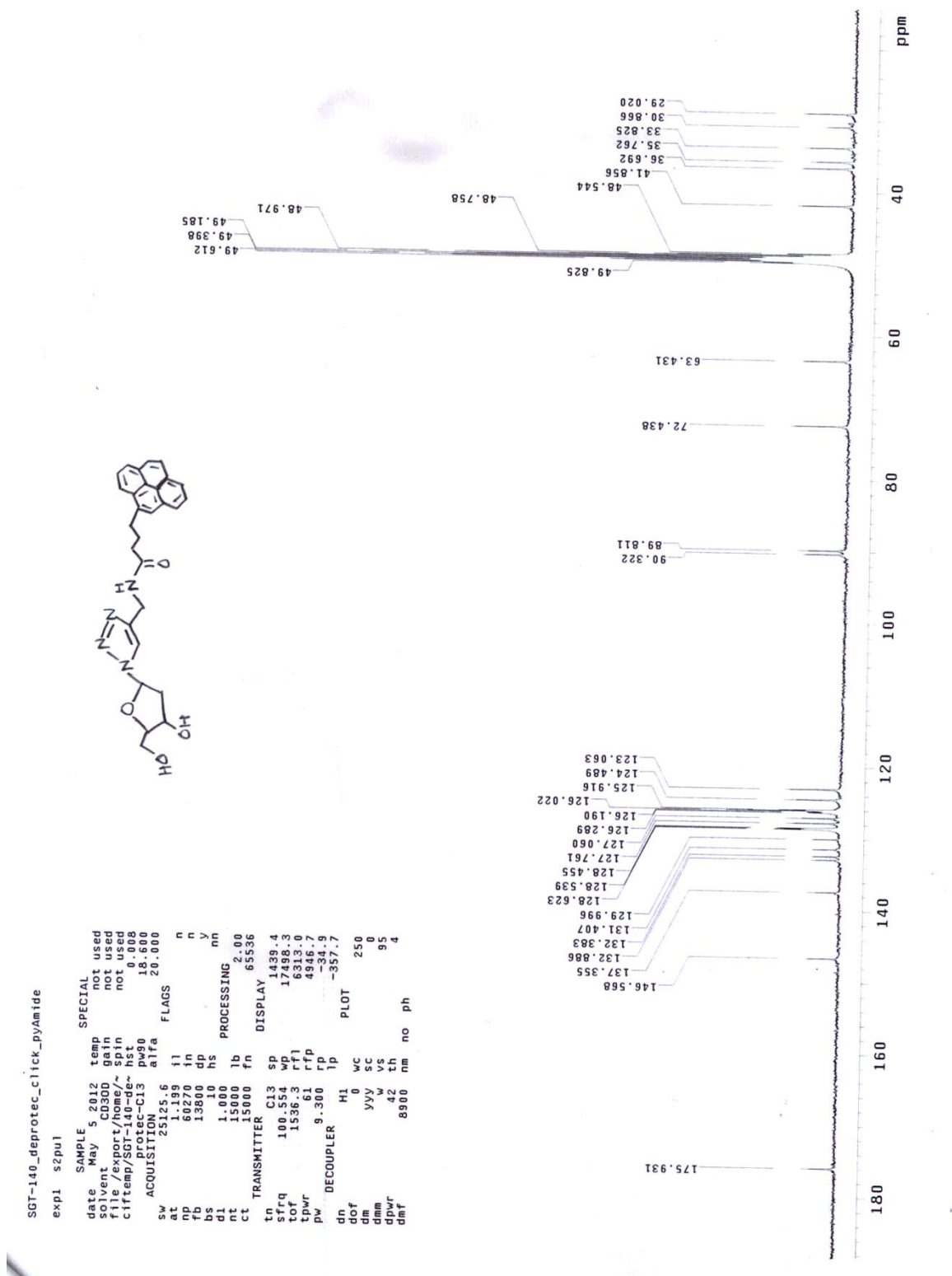


Figure S50. ^{13}C NMR spectra of nucleoside 7B

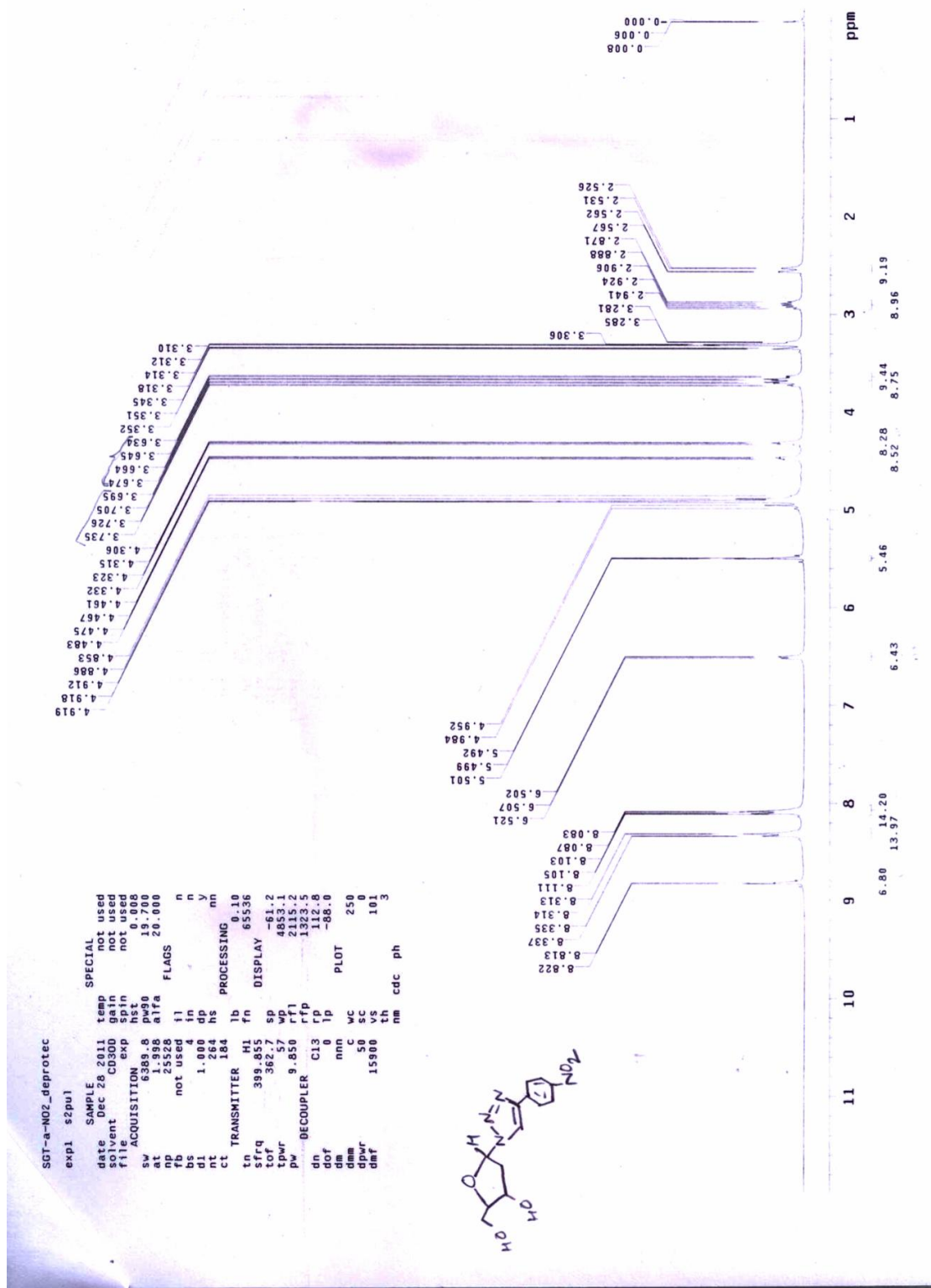


Figure S51. ¹H NMR spectra of nucleoside 8α

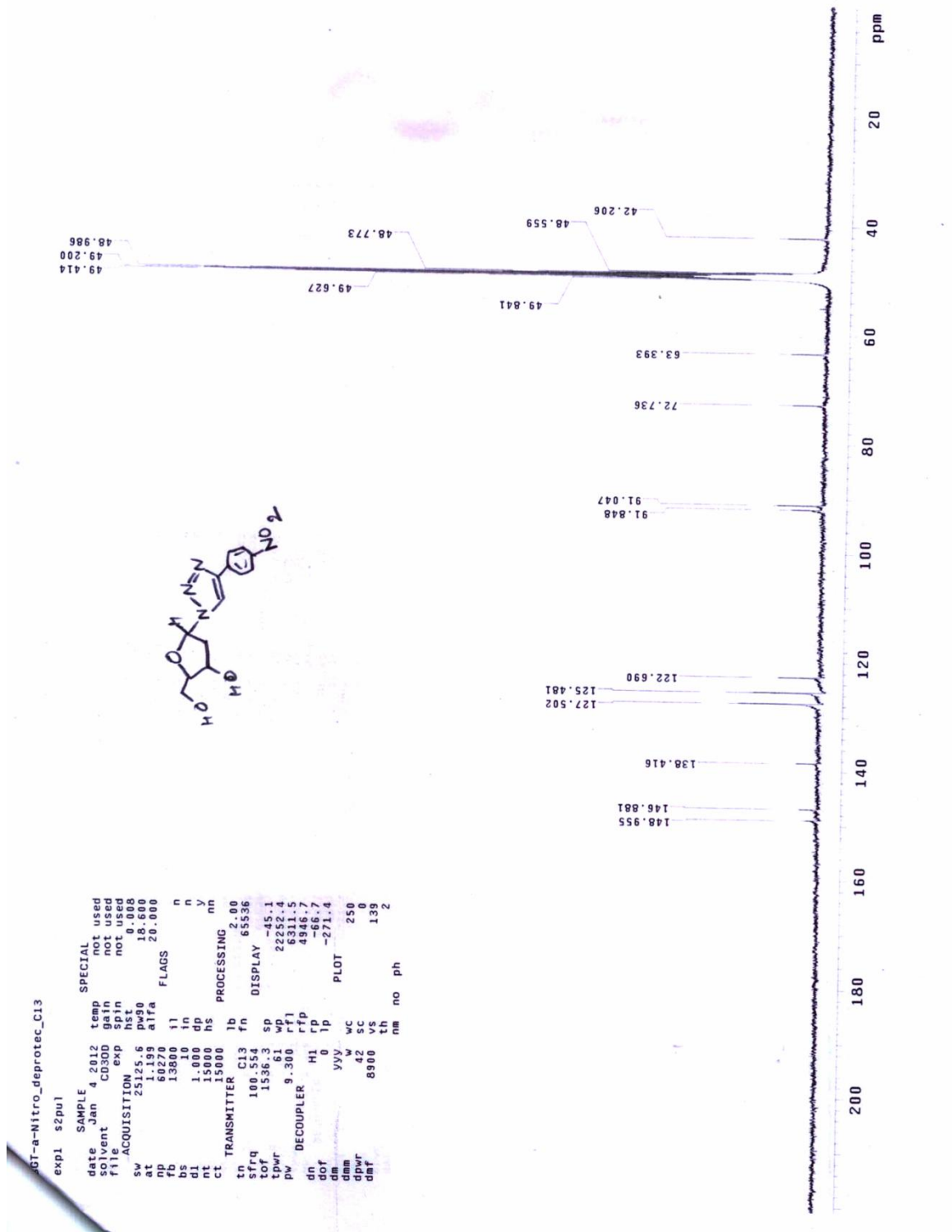


Figure S52. ¹³C NMR spectra of nucleoside 8α

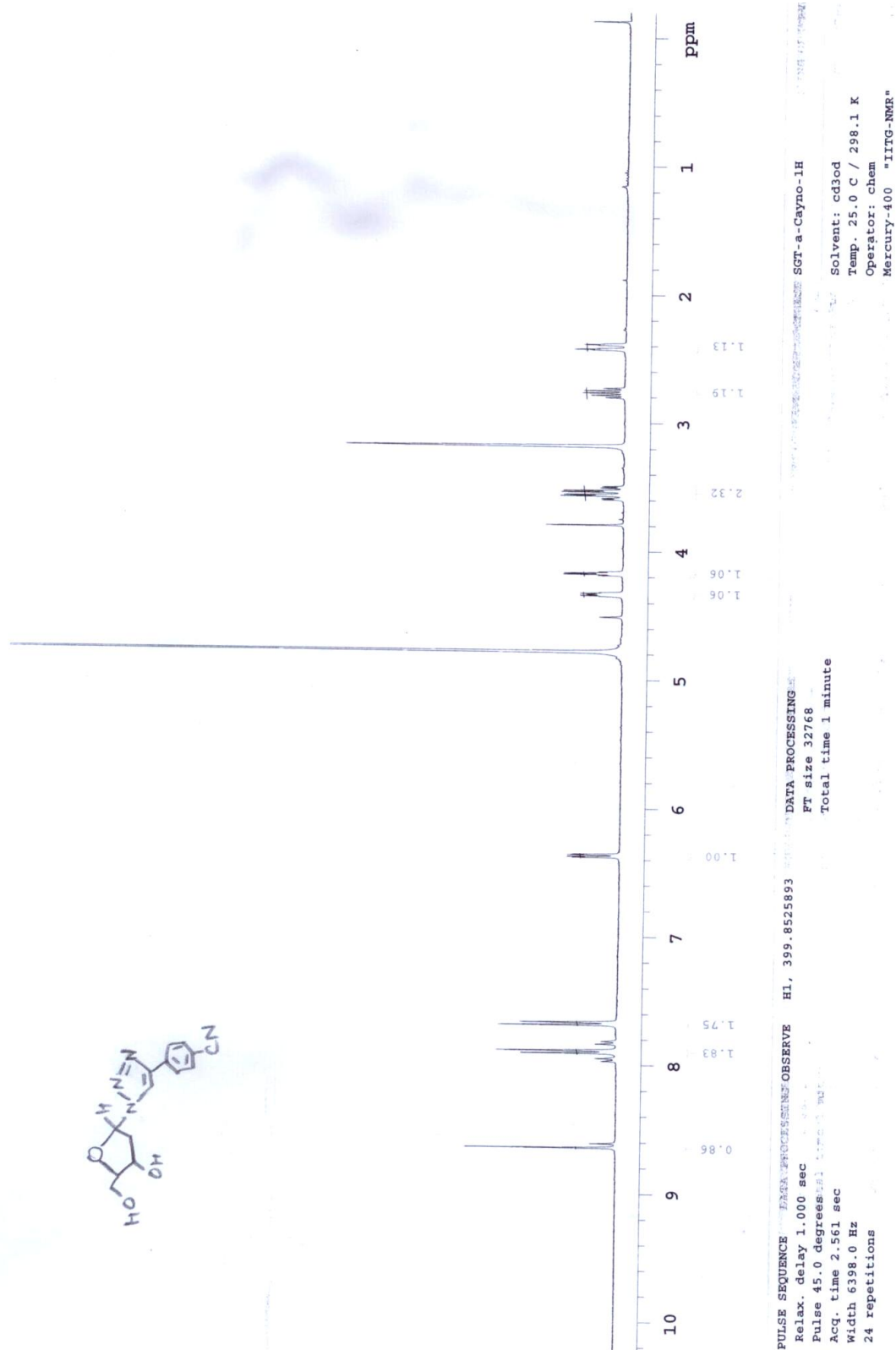


Figure S53. ^1H NMR spectra of nucleoside 9α

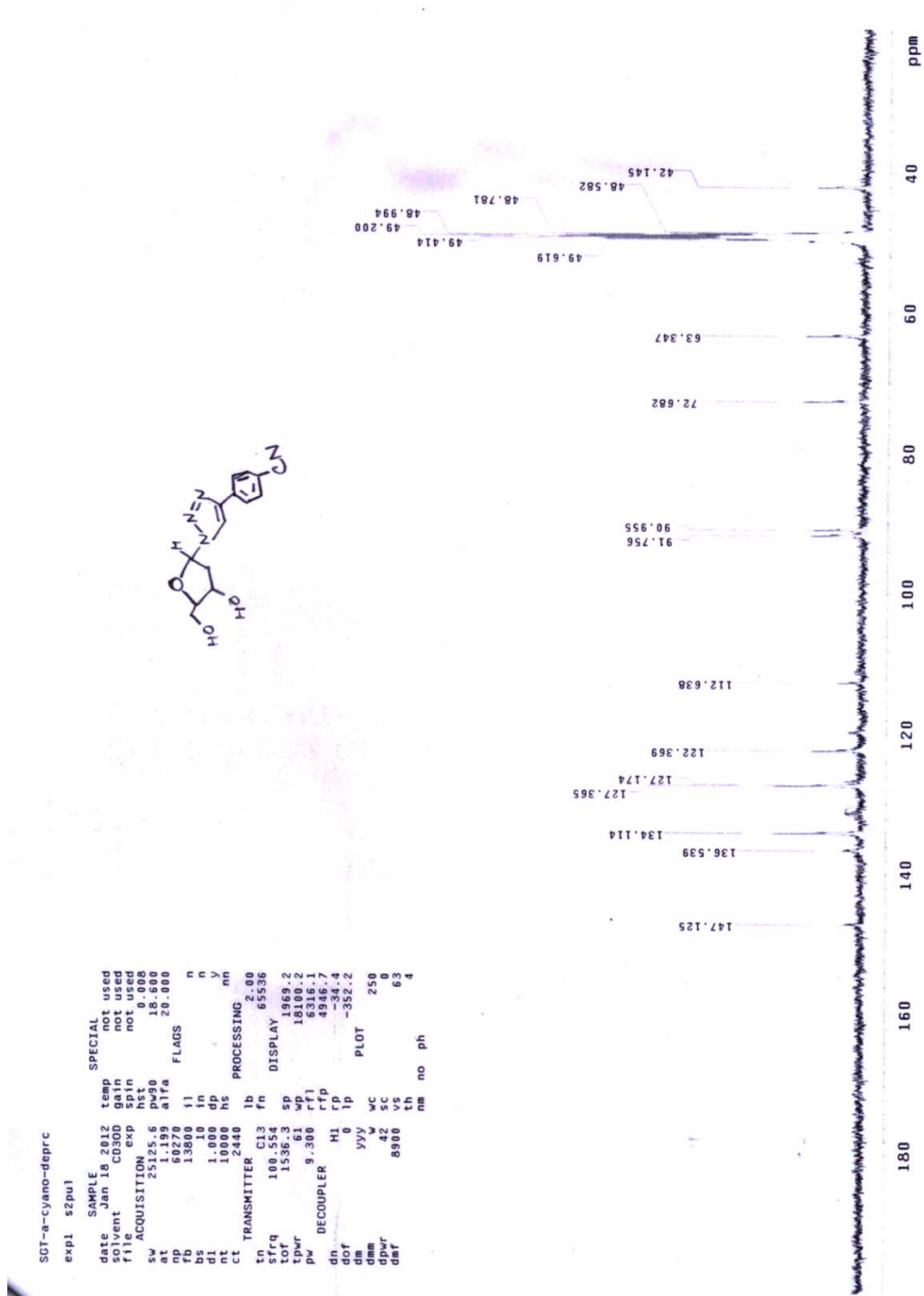


Figure S54. ^1H NMR spectra of nucleoside 9 α