

Supporting information:

Short Interfering RNA Guide Strand Modifiers from Computational Screening

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Contents of Supporting Information:

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Supplementary Method

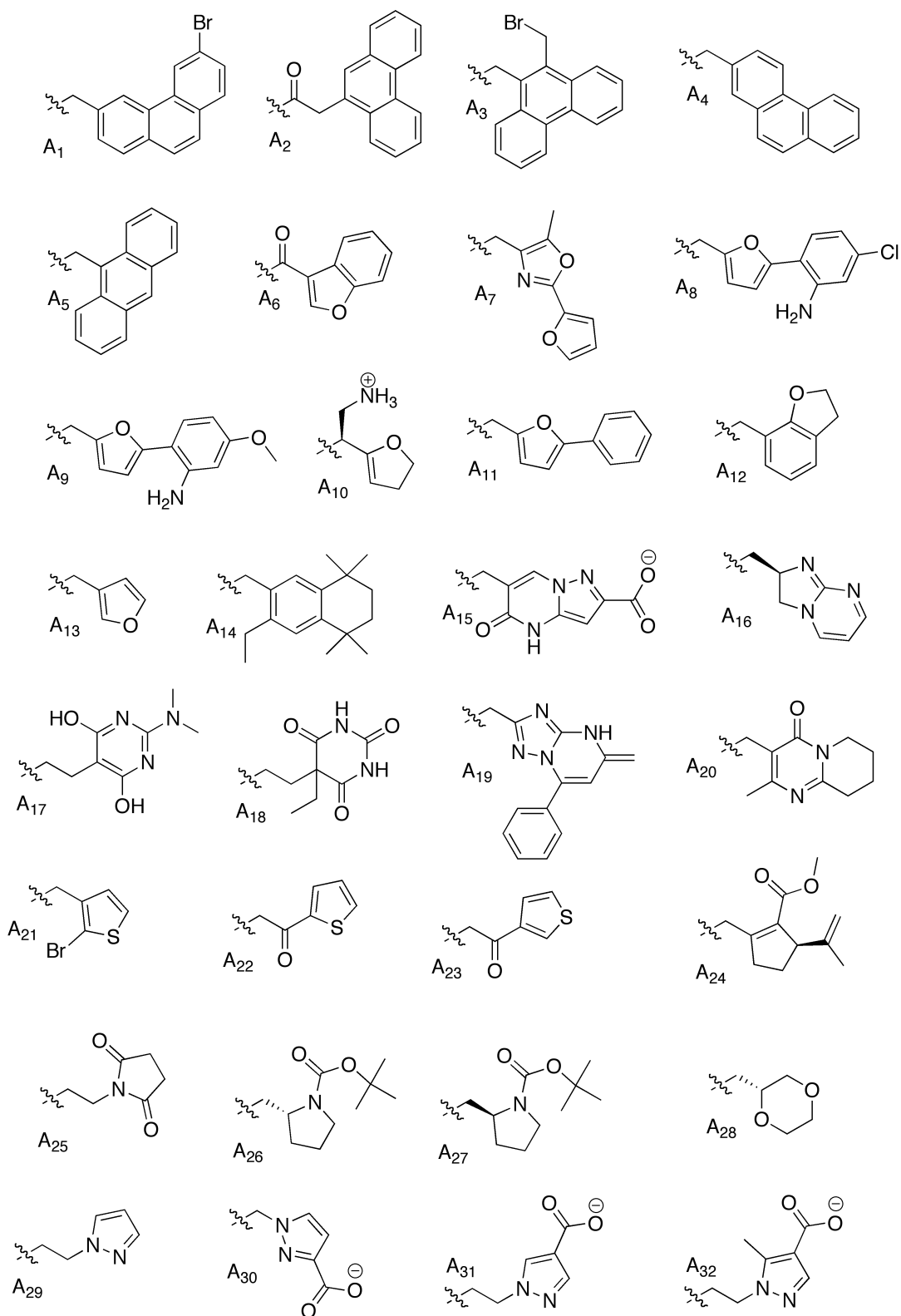
Serum stability assay	S5
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Supplementary Table

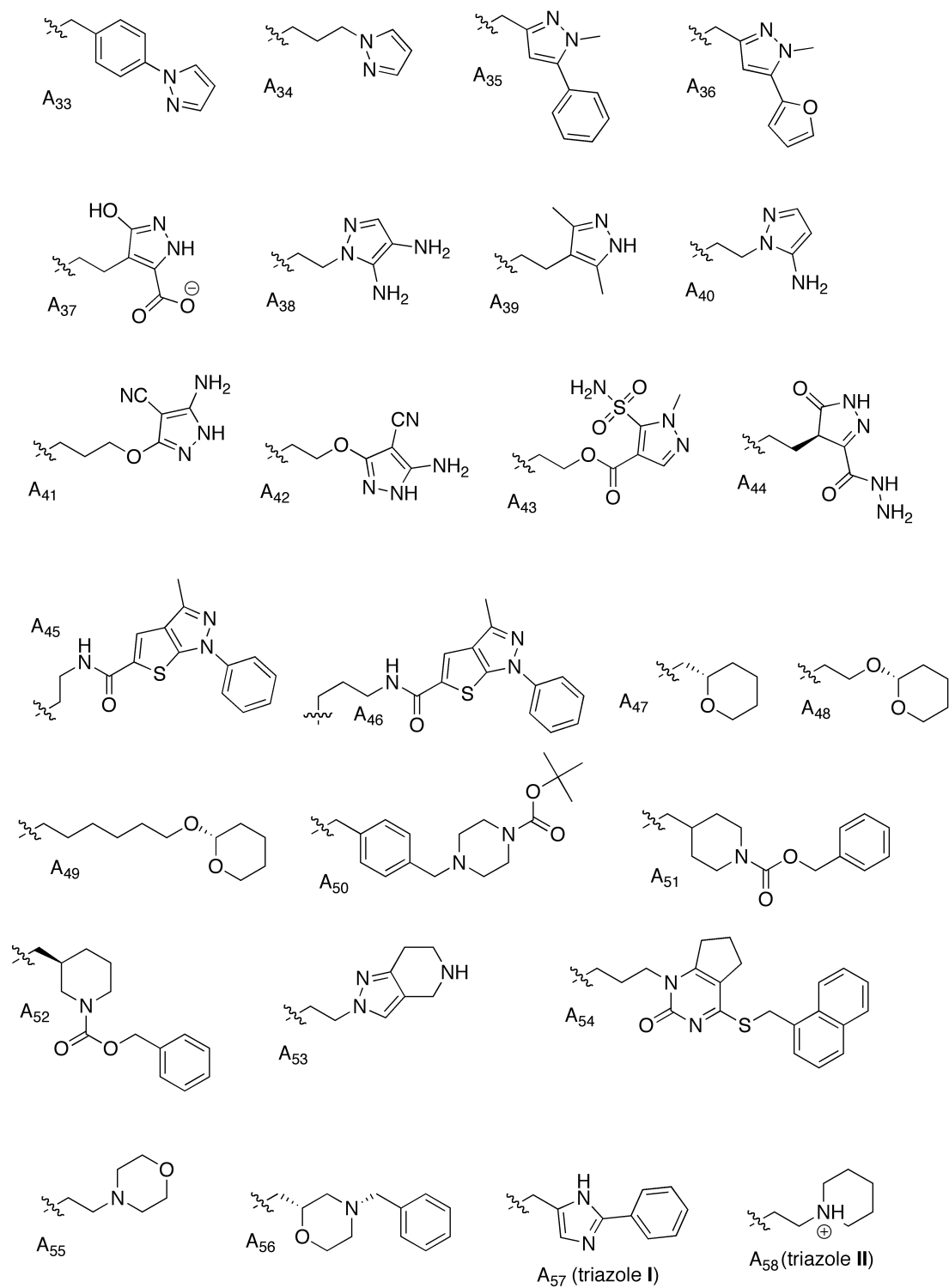
Supplementary Table 1	S6
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NMR data

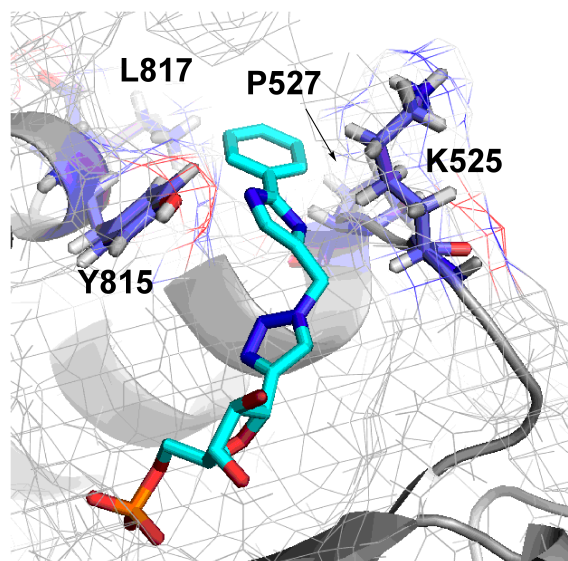
¹ H NMR for compound 2	S7
¹³ C NMR for compound 2	S8
¹ H NMR for compound 3	S9
¹³ C NMR for compound 3	S10
NOE data for compound 3	S11
¹ H NMR for compound 4	S12
¹³ C NMR for compound 4	S13
³¹ P NMR for compound 5	S14



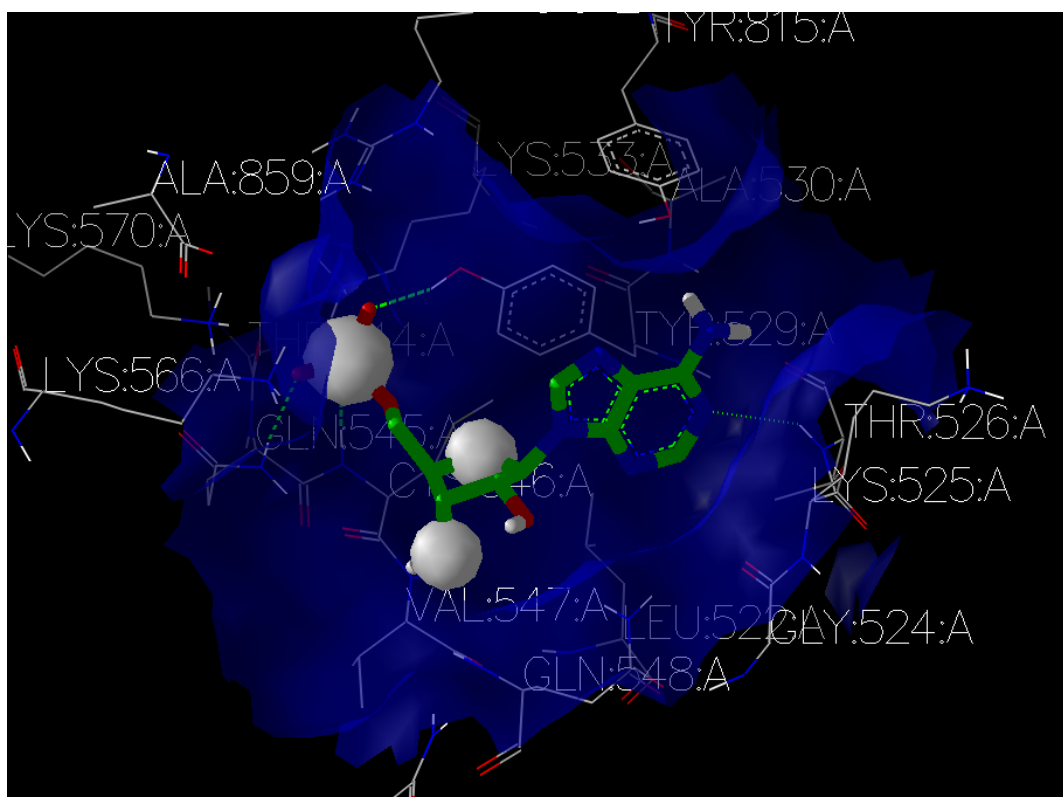
Supplementary Figure 1. Triazole substituents used in the computational screening in this study.



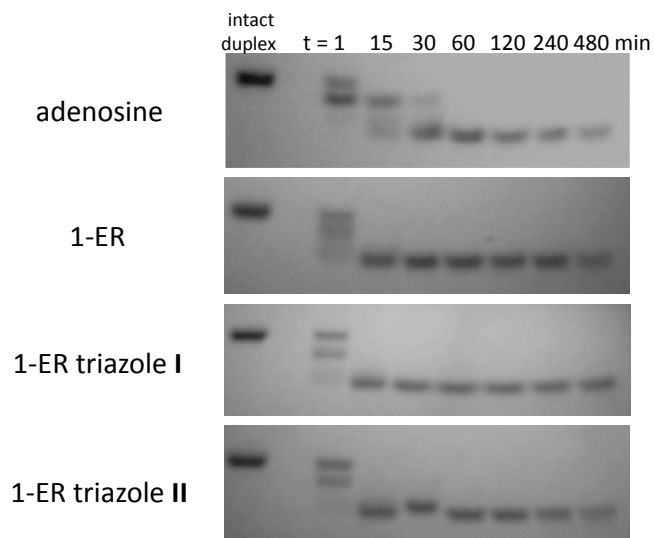
Supplementary Figure 1 (continued).



Supplementary Figure 2. Docking of 1-ER triazole **I** suggests binding in a cleft between the MID (K525, P527) and PIWI (Y815, L817) domains adjacent to the guide strand 5'-end binding site in hAgo2 (Schirle, N.T; MacRae, I.J *Science* **2012**, 336, 1037).



Supplementary Figure 3. Constraints imposed on docking experiment, at the phosphate, ribose 4' oxygen, and 2' oxygen linker (modeled as hydroxyl), shown on crystal structure with adenosine. Visualized with VIDA from OpenEye Suite of programs (OEChem, version 1.7.4, OpenEye Scientific Software, Inc., Santa Fe, NM, USA, www.eyesopen.com, 2010).



Supplementary Figure 4. Serum stability assay data for PIK3CB siRNAs with various guide strand 5' end modifications showing major degradation products formed at the time points specified above. Images above are of 18% native polyacrylamide gels imaged by UV shadow with a F254 TLC plate as a backing and depicted in grayscale for clarity.

Serum stability assay. siRNA duplexes were hybridized in 1x PBS without calcium and magnesium to afford a final reaction concentration of 40 μ M siRNA in 1x PBS with 10% human serum (Sigma Aldrich). Reaction was incubated at 37 $^{\circ}$ C with 5 μ L aliquots removed at desired time lengths (1, 15, 30, 60, 120, 240, and 480 min) and quenched with 5 μ L of quenching solution (10% glycerol in 1x TBE buffer) then immediately stored at -70 $^{\circ}$ C. Reactions were analyzed as previously described¹ and imaged by UV shadow as described for oligonucleotide purifications. Gel images are shown in grayscale for clarity.

1) Hernández, A. R.; Peterson, L. W.; Kool, E. T. *ACS Chem. Biol.* **2012**, 7 (8), pp 1454-1461

		Raw Chemgauss 3 Pose/Score	Raw Conensus Pose/Score	Raw Conensus Pose, Chemgauss3 Score	Raw Chemgauss3 Conensus Score	Raw Chemgauss4 Pose/Score	Normalized Chemgauss3 Pose/Score	Normalized Conensus Pose/Score	Normalized Conensus Pose, Chemgauss3 Score	Raw Chemgauss3 Conensus Score	Normalized Chemgauss4 Pose/Score	Average of Normalized Scores
Supplementary Table 1												
Natural												
	Adenine	-62.90	601	-65.30	556	-13.19		1.05	1.06	1.00	1.08	1.04
	Cytosine	-51.72	1849	-50.46	1940	-10.98		3.09	6.06	3.52	6.41	4.40
	Guanine	-54.31	1975	-52.49	1896	-10.81		2.61	6.57	3.18	6.24	4.34
	Uracil	-62.52	965	-63.49	881	-12.74		1.12	2.52	1.31	2.33	1.73
7-EAA												
	7-EAA	-28.32	2542	-14.22	2612	-3.96	7.34	8.84	9.68	9.00	9.04	8.78
	7-EAA triazole	-13.83	2378	-12.54	2655	-4.14	9.98	8.18	9.97	9.16	8.89	9.24
	A01 7-EAA	-26.68	1957	-26.68	2504	-7.00	7.64	6.49	7.56	8.58	6.40	7.34
	A02 7-EAA	-22.79	2327	-41.46	2081	-7.18	8.35	7.98	5.05	6.95	6.24	6.91
	A03 7-EAA	-24.25	2456	-33.26	2132	-7.37	8.09	8.50	6.45	7.15	6.07	7.25
	A04 7-EAA	-29.29	2528	-39.60	2307	-6.82	7.17	8.78	5.37	7.82	6.55	7.14
	A05 7-EAA	-27.59	2083	-42.10	1775	-7.10	7.48	7.00	4.94	5.78	6.31	6.30
	A06 7-EAA	-45.70	1772	-26.11	1805	-7.21	4.18	5.75	7.66	5.89	6.21	5.94
	A07 7-EAA	-21.96	1878	-15.66	2669	-6.00	8.50	6.18	9.44	9.22	7.27	8.12
	A08 7-EAA	-27.60	2831	-17.02	2622	-4.07	7.48	10.00	9.21	9.04	8.97	8.93
	A09 7-EAA	-28.43	2538	-14.16	2604	-4.16	7.32	8.82	9.69	8.97	8.87	8.73
	A10 7-EAA	-22.68	2510	-30.94	2872	-8.31	8.37	8.71	6.84	10.00	5.25	7.83
	A11 7-EAA	-30.32	1801	-25.22	1955	-7.10	6.98	5.87	7.81	6.47	6.30	6.69
	A12 7-EAA	-30.55	2286	-33.84	2366	-7.92	6.94	7.81	6.35	8.05	5.59	6.95
	A13 7-EAA	-48.02	2109	-40.97	2214	-8.79	3.76	7.10	5.13	7.47	4.83	5.66
	A14 7-EAA	-22.59	1973	-23.48	2396	-6.52	8.39	6.56	8.11	8.17	6.81	7.61
	A15 7-EAA	-41.85	2703	-23.51	2840	-7.32	4.88	9.49	8.10	9.88	6.12	7.69
	A16 7-EAA	-44.22	1996	-44.27	1652	-9.34	4.45	6.65	4.57	5.30	4.36	5.07
	A17 7-EAA	-44.04	2440	-32.42	2588	-8.43	6.07	7.24	7.03	8.10	5.55	6.80
	A18 7-EAA	-32.85	2074	-30.16	1847	-6.81	6.52	6.96	6.97	6.05	6.56	6.61
	A19 7-EAA	-17.98	2415	-20.91	2344	-5.21	9.23	8.33	8.54	7.97	7.95	8.40
	A20 7-EAA	-36.21	1815	-30.80	2156	-8.88	5.91	5.93	6.86	7.24	4.76	6.14
	A21 7-EAA	-53.71	1335	-52.83	1181	-7.83	2.72	4.00	3.12	3.49	5.68	3.80
	A22 7-EAA	-56.95	1430	-56.95	1233	-8.74	2.13	4.38	2.42	3.69	4.88	3.50
	A23 7-EAA	-54.08	1270	-49.79	1348	-8.97	2.65	3.74	3.64	4.13	4.67	3.77
	A24 7-EAA	-19.80	2423	-32.54	2865	-6.64	8.90	8.36	6.57	9.97	6.71	8.10
	A25 7-EAA	-35.30	2143	-29.84	2378	-7.97	6.07	7.24	7.03	8.10	5.55	6.80
	A26 7-EAA	-44.58	2077	-26.82	2063	-8.12	4.38	6.98	7.54	6.88	5.42	6.24
	A27 7-EAA	-40.59	1575	-52.77	1446	-8.15	5.11	4.96	3.13	4.51	5.39	4.62
	A28 7-EAA	-52.22	1294	-52.22	1179	-8.59	2.99	3.84	3.22	3.48	5.01	3.71
	A29 7-EAA	-28.59	2183	-40.30	2089	-6.38	7.29	7.40	5.25	6.98	6.93	6.77
	A30 7-EAA	-32.15	2109	-40.91	1893	-8.56	6.65	7.10	5.15	6.23	5.03	6.03
	A31 7-EAA	-28.04	2094	-39.53	2223	-8.52	7.39	8.65	5.38	7.50	5.07	6.80
	A32 7-EAA	-37.53	2104	-41.14	1832	-7.99	5.67	7.08	5.11	5.99	5.53	5.94
	A33 7-EAA	-34.70	2073	-32.94	2580	-7.23	6.18	6.96	6.50	8.88	6.20	6.94
	A34 7-EAA	-47.25	2230	-32.62	2478	-6.52	3.90	7.59	6.55	8.48	6.81	6.67
	A35 7-EAA	-38.95	1950	-30.06	1878	-8.12	5.41	6.47	6.99	6.17	5.42	6.09
	A36 7-EAA	-43.21	1431	-43.21	1330	-8.36	4.63	4.39	4.75	4.06	5.21	4.61
	A37 7-EAA	-27.90	2321	-28.44	2432	-9.44	7.42	7.95	7.26	8.31	4.27	7.04
	A38 7-EAA	-34.23	2137	-32.21	1916	-7.70	6.27	7.22	6.62	6.32	5.79	6.44
	A39 7-EAA	-39.55	2268	-32.69	1970	-8.98	5.30	7.74	6.54	6.53	4.67	6.16
	A40 7-EAA	-50.08	2065	-50.08	1725	-8.65	3.38	6.93	3.59	5.58	6.70	5.24
	A41 7-EAA	-29.58	1882	-29.58	2035	-6.38	7.12	6.19	7.07	6.78	6.94	6.82
	A42 7-EAA	-29.15	2440	-23.68	2632	-6.73	7.19	8.43	8.07	9.08	6.63	7.88
	A43 7-EAA	-24.14	2347	-36.05	2097	-6.46	8.11	8.06	5.97	7.02	6.86	7.20
	A44 7-EAA	-28.19	2106	-19.52	2162	-5.91	7.37	7.09	8.78	7.27	7.34	7.57
	A45 7-EAA	-22.30	1808	-23.67	2519	-4.95	8.44	5.90	8.08	8.64	8.18	7.85
	A46 7-EAA	-18.38	2733	-13.50	2630	-2.86	9.15	9.61	9.80	9.07	10.00	9.53
	A47 7-EAA	-56.25	1791	-37.19	1423	-8.75	2.26	5.83	5.78	4.42	4.87	4.63
	A48 7-EAA	-47.08	1873	-47.08	1521	-7.26	3.83	6.16	4.10	4.86	6.17	5.04
	A49 7-EAA	-26.34	1877	-26.34	1678	-6.34	7.71	6.17	7.62	5.40	6.97	6.77
	A50 7-EAA	-23.38	1939	-22.43	2202	-3.25	8.24	6.42	8.29	7.42	9.66	8.01
	A51 7-EAA	-37.85	2059	-38.40	2138	-8.30	5.61	6.90	5.57	7.17	5.27	6.10
	A52 7-EAA	-22.96	2045	-17.21	2395	-6.06	8.32	6.85	9.17	8.16	7.21	7.94
	A53 7-EAA	-33.39	1918	-33.39	2303	-6.57	6.42	6.34	6.42	7.81	6.77	6.75
	A54 7-EAA	-13.73	2397	-12.34	2667	-4.07	10.00	8.26	10.00	9.21	8.95	9.28
	A55 7-EAA	-44.54	2233	-42.90	1796	-8.21	4.39	7.60	4.81	5.86	5.34	5.60
	A56 7-EAA	-30.57	2176	-41.19	1882	-7.68	6.93	7.37	5.10	6.19	5.80	6.28
2-AP												
	2-AP triazole	-18.38	2734	-13.49	2642	-2.95	9.15	9.61	9.80	9.11	9.92	9.52
1-ER												
	1-ER	-59.91	900	-59.91	792	-11.02	1.59	2.26	1.92	1.99	2.90	2.13
	A01 1-ER	-59.17	708	-59.17	638	-12.38	1.73	1.49	2.04	1.40	1.70	1.67
	A02 1-ER	-47.60	1806	-36.56	1738	-8.84	3.84	5.89	5.88	5.63	4.79	5.21
	A03 1-ER	-117.01	1092	-49.45	1092	-10.38	3.21	3.37	3.69	3.15	3.45	3.37
	A04 1-ER	-59.91	900	-59.91	792	-11.02	1.59	2.26	1.92	1.99	2.90	2.13
	A05 1-ER	-55.40	1523	-44.43	1446	-11.00	2.41	4.75	4.55	4.51	2.91	3.83
	A06 1-ER	-52.19	1234	-56.33	1129	-10.74	3.00	3.59	2.52	3.29	3.14	3.11
	A07 1-ER	-47.43	1773	-46.41	1530	-9.35	3.87	5.76	4.21	4.83	4.34	4.60
	A08 1-ER	-52.43	1129	-53.26	1081	-9.08	2.96	3.17	3.05	3.10	4.58	3.37
	A09 1-ER	-50.46	1135	-50.26	992	-10.98	3.31	3.20	3.56	2.76	2.93	3.14
	A10 1-ER	-47.42	1491	-52.75	1669	-10.67	3.87	4.63	3.13	5.37	3.19	4.04
	A11 1-ER	-58.59	1120	-51.45	1148	-10.85	1.83	3.14	4.35	4.16	3.36	3.04
	A12 1-ER	-54.04	1314	-48.87	1162	-11.25	2.66	3.92	3.79	3.41	2.69	3.30
	A13 1-ER	-56.86	1731	-44.88	1847	-10.28	2.15	5.59	4.47	6.05	3.53	4.36
	A14 1-ER	-44.30	1355	-40.77	1842	-8.87	4.44	4.08	5.17	6.03	4.76	4.90
	A15 1-ER	-43.74	1621	-39.35	1559	-9.27	4.54	5.15	5.41	4.94	4.41	4.89
	A16 1-ER	-53.18	1370	-51.96	1223	-11.30	2.82	4.14	3.27	3.65	2.65	3.30
	A17 1-ER	-54.89	1694	-50.45	1574	-12.02	2.51	5.44	3.52	5.00	2.02	3.70
	A18 1-ER	-33.89	2457	-31.14	2508	-8.04	6.33	8.50	6.31	8.60	5.49	7.14
	A19 1-ER	-57.23	587	-57.23	535	-10.93	2.08	1.00	2.27	1.00	2.97	1.88
	A20 1-ER	-52.67	1288	-53.67	1116	-11.03	2.91	3.81	2.98	3.24	2.88	3.16
	A21 1-ER	-52.67	1297	-53.67	1125	-11.03	2.91	3.85	2.98	3.27	2.88	3.18
	A22 1-ER	-57.57	1640	-52.93	1423	-10.46	2.02	5.22	3.10	4.42	3.38	3.63
	A23 1-ER	-53.49	1603	-44.30	1725	-11.50	2.76	5.07	4.57	5.58	2.47	4.09
	A24 1-ER	-53.60	1131	-52.62	1389	-10.29	2.74	3.18	3.16	4.29	3.53	3.38
	A25 1-ER	-47.39	1805	-50.79								

KO-369

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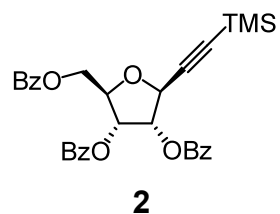
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Solvent: cdcl3

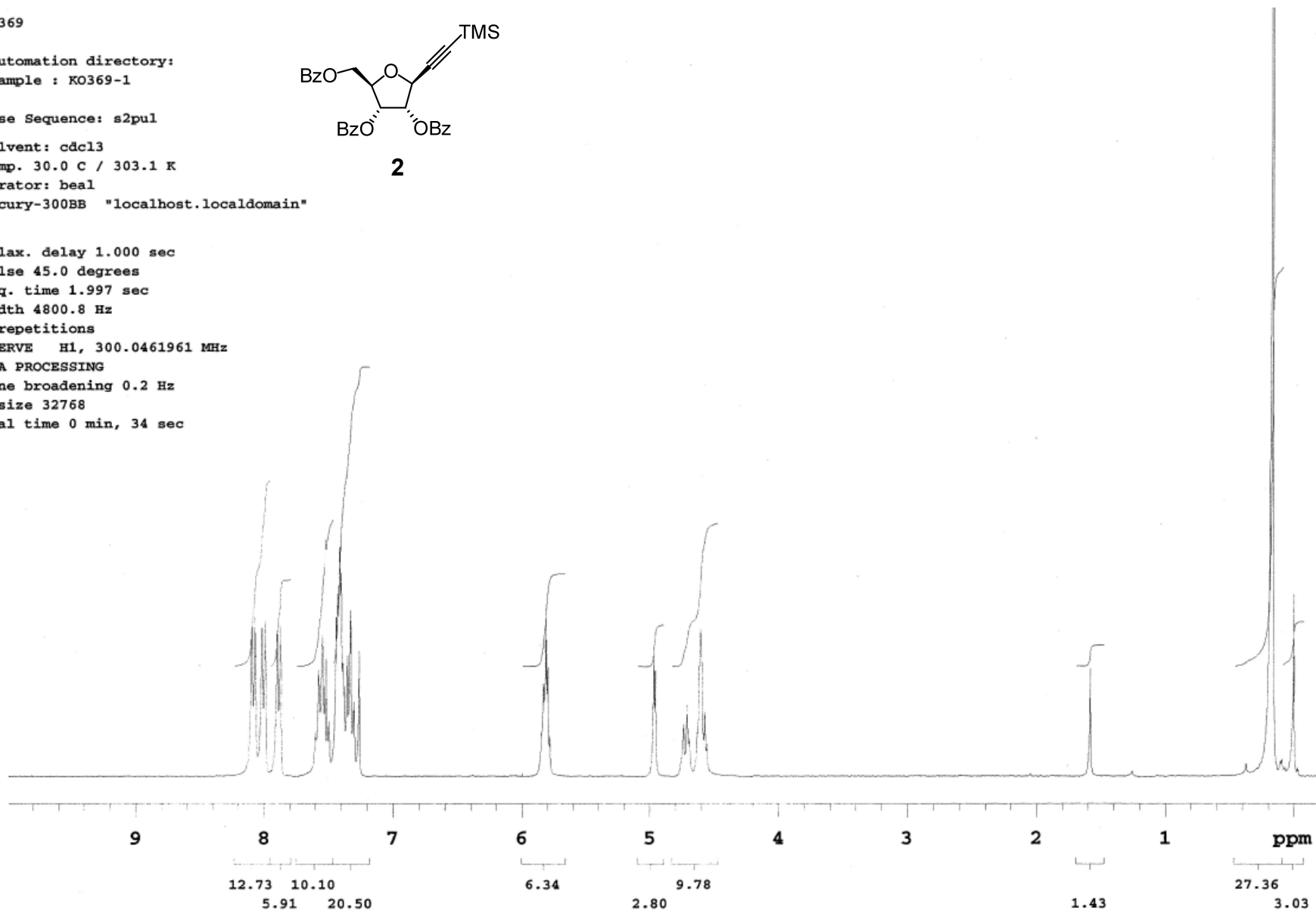
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Operator: beal

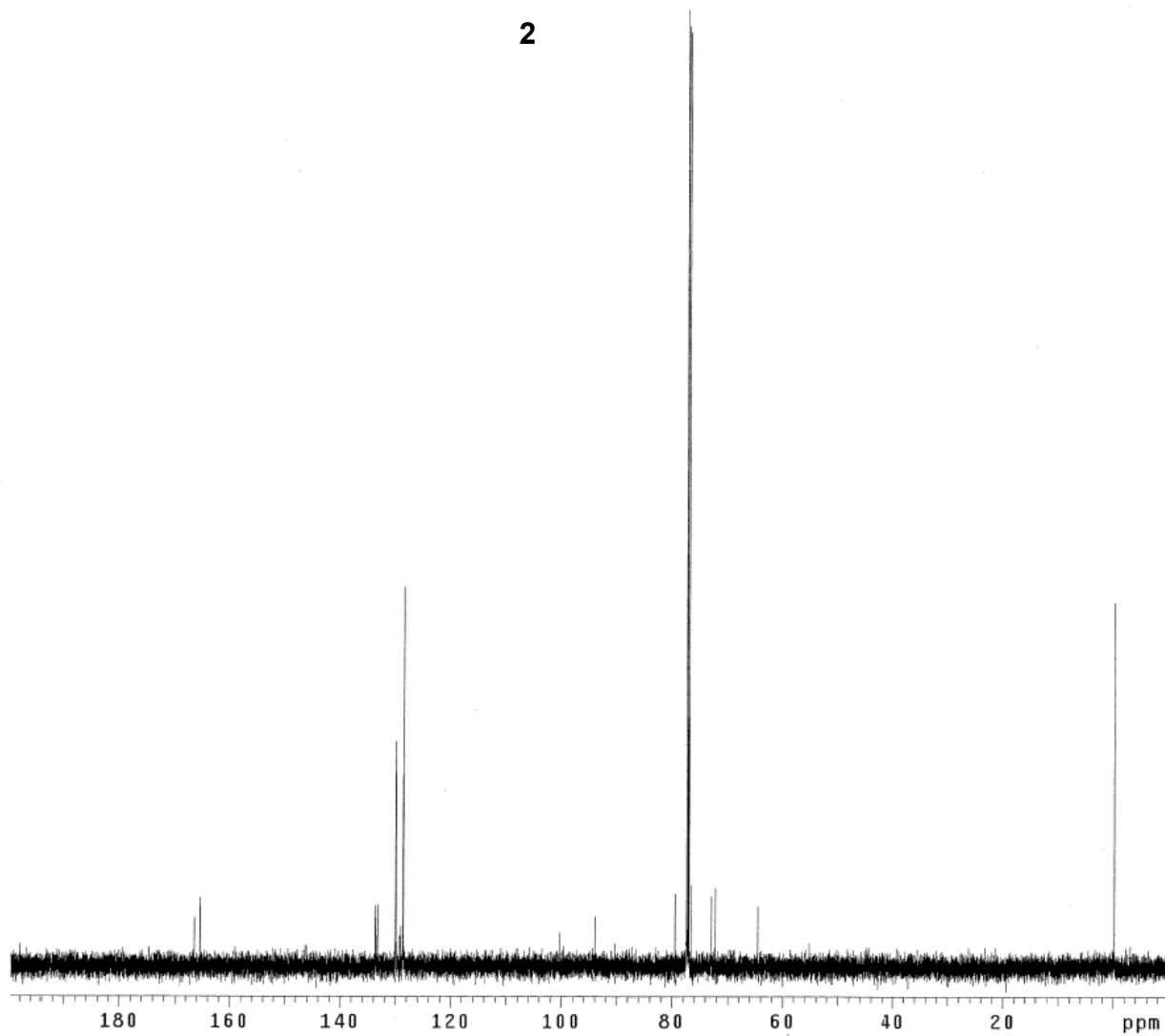
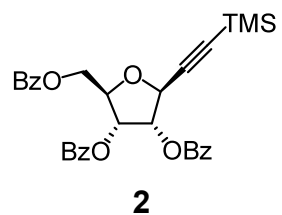
Mercury-300BB "localhost.localdomain"



Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4800.8 Hz
8 repetitions
OBSERVE H1, 300.0461961 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 32768
Total time 0 min, 34 sec

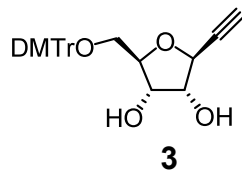


1216 repetitions
OBSERVE C13, 150.7851911 MHz
DECOUPLE H1, 599.6653321 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 3 hr, 12 min, 27 sec



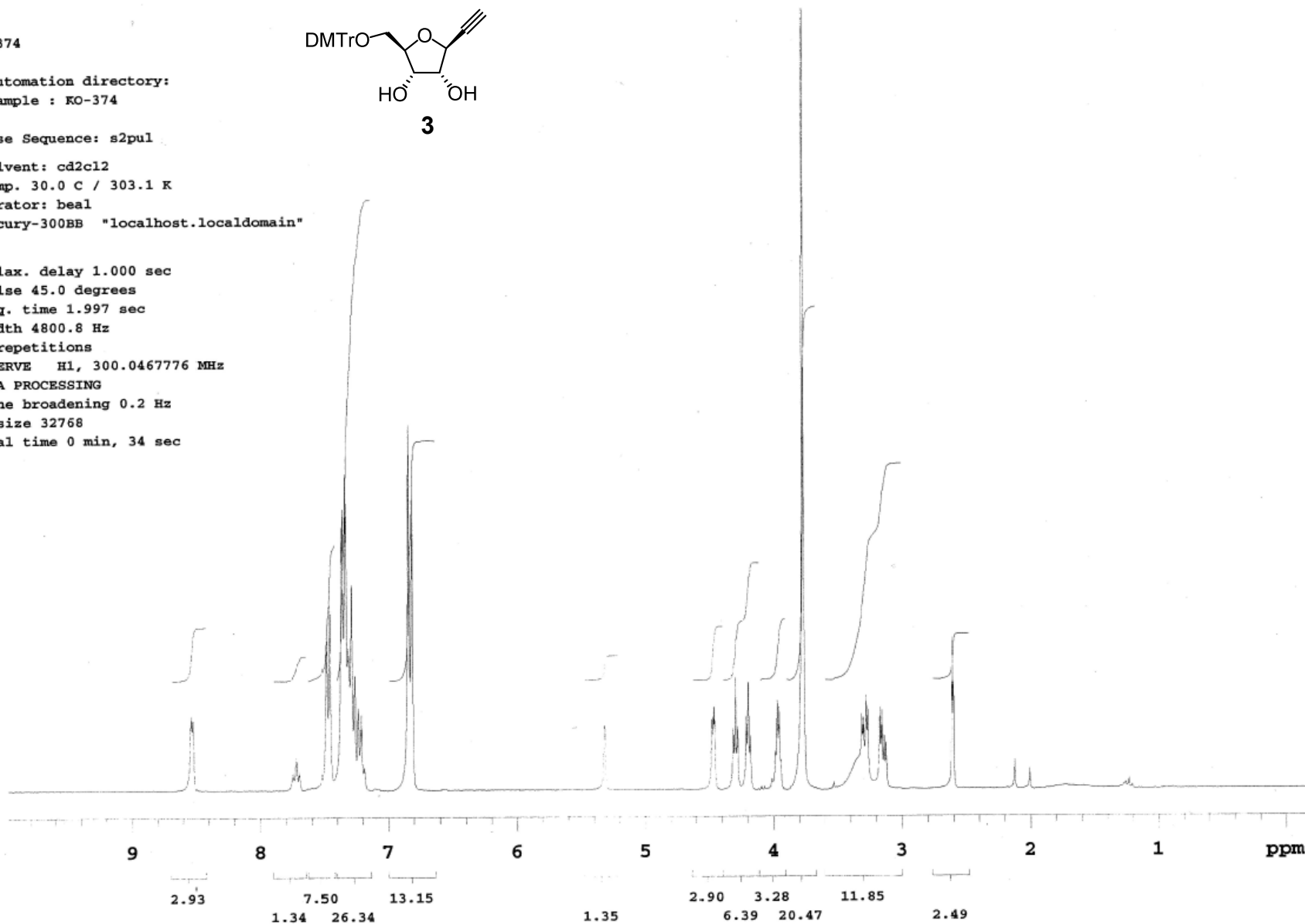
KO-374

Automation directory:
Sample : KO-374

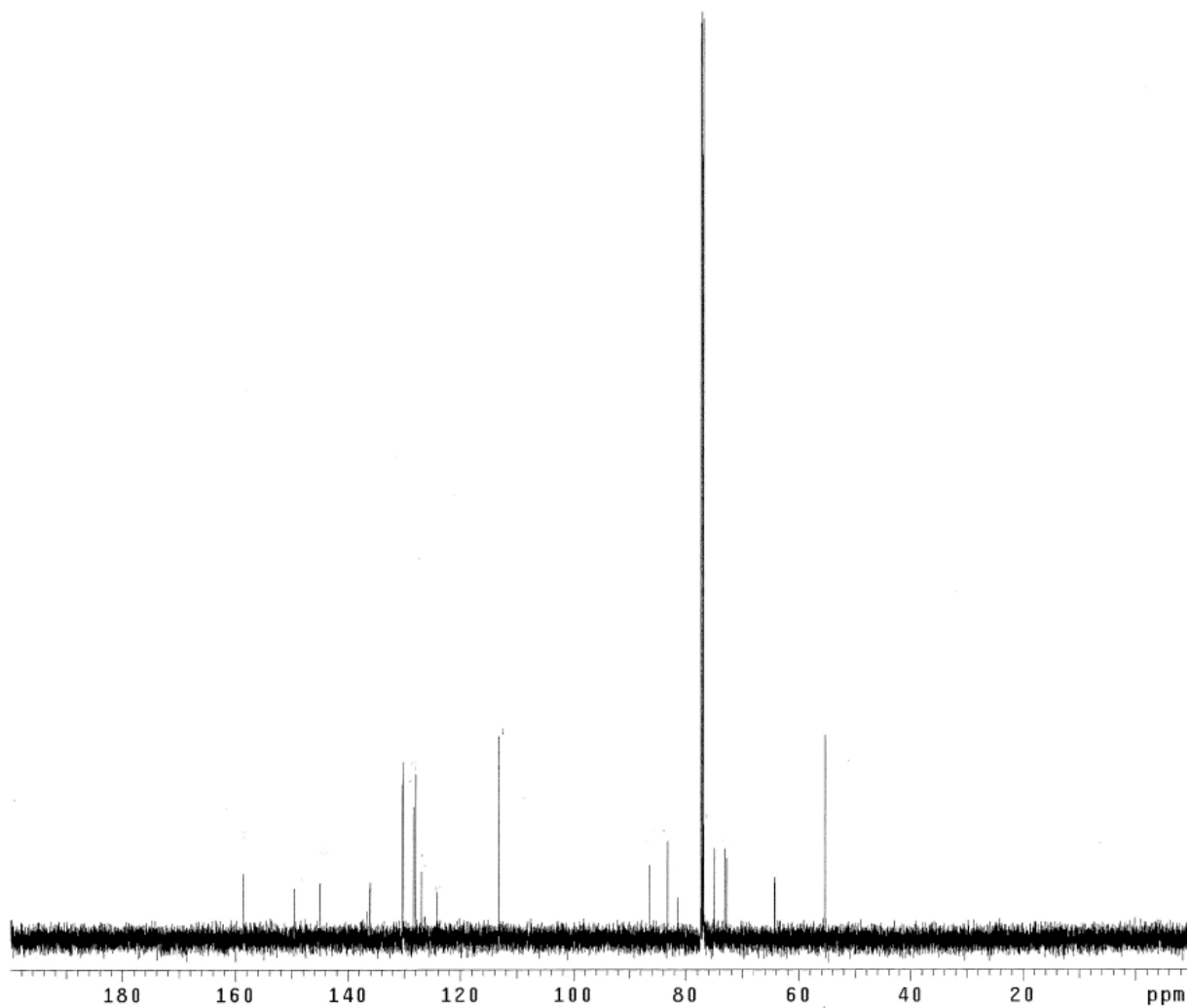
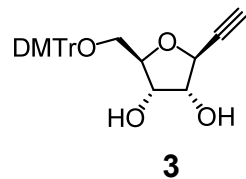


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Solvent: cd2c12
Temp. 30.0 C / 303.1 K
Operator: beal
Mercury-300BB "localhost.localdomain"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.997 sec
Width 4800.8 Hz
8 repetitions
OBSERVE H1, 300.0467776 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 32768
Total time 0 min, 34 sec



896 repetitions
OBSERVE C13, 150.7851925 MHz
DECOUPLE H1, 599.6653321 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 3 hr, 12 min, 27 sec



KO-374

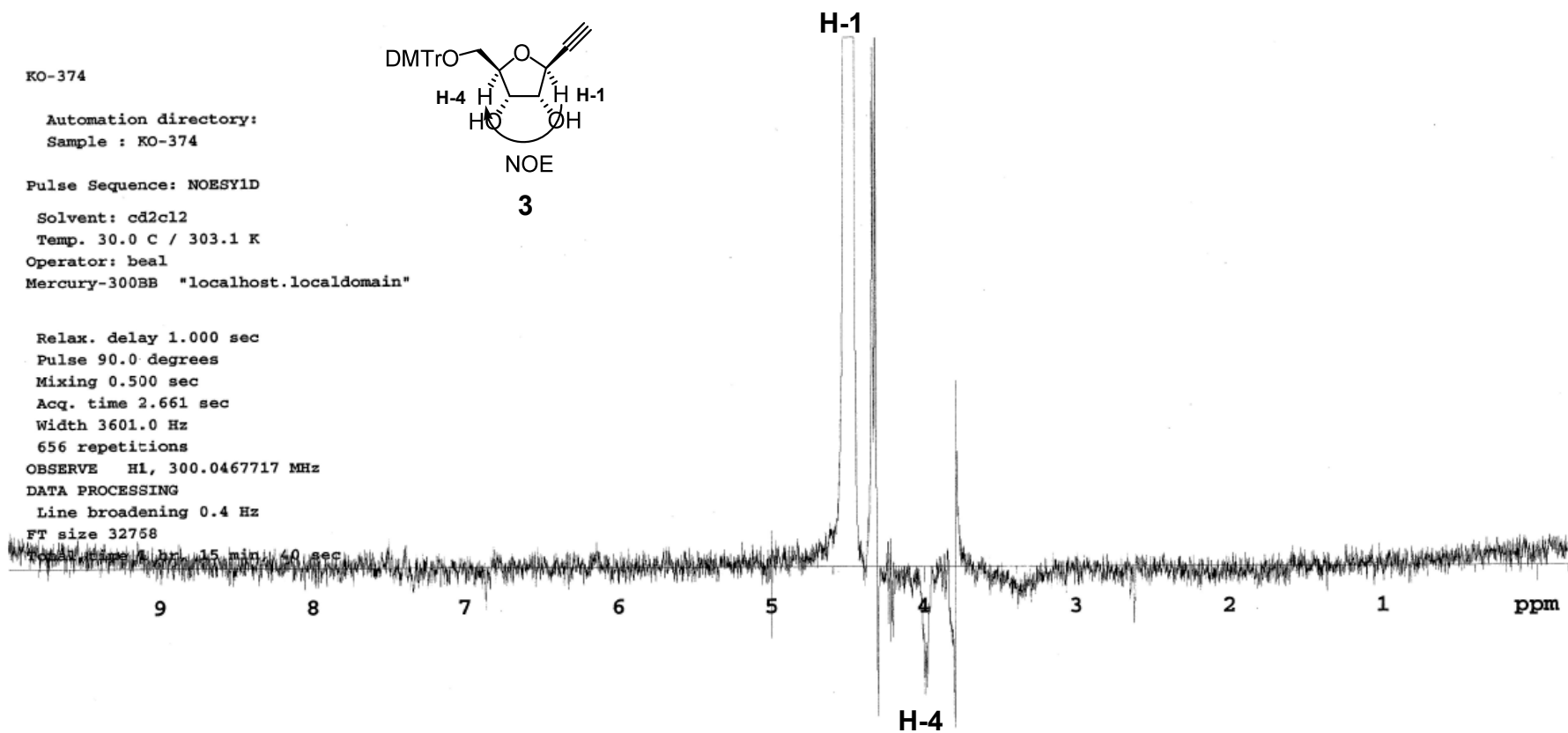
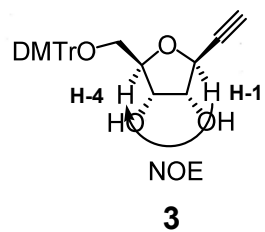
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Sample : KO-374

Pulse Sequence: NOESY1D

Solvent: cd2cl2
Temp. 30.0 C / 303.1 K
Operator: beal
Mercury-300BB "localhost.localdomain"

Relax. delay 1.000 sec
Pulse 90.0 degrees
Mixing 0.500 sec
Acq. time 2.661 sec
Width 3601.0 Hz
656 repetitions
OBSERVE H1, 300.0467717 MHz
DATA PROCESSING
Line broadening 0.4 Hz
FT size 32758

Total time 1 hr 15 min 40 sec



KO412-2

Automation directory:
Sample : KO412-2

Pulse Sequence: s2pul

Solvent: cd2cl2

Temp. 25.0 C / 298.1 K

Operator: beal

Mercury-300BB "localhost.localdomain"

Relax. delay 1.000 sec

Pulse 45.0 degrees

Acq. time 1.997 sec

Width 4800.8 Hz

8 repetitions

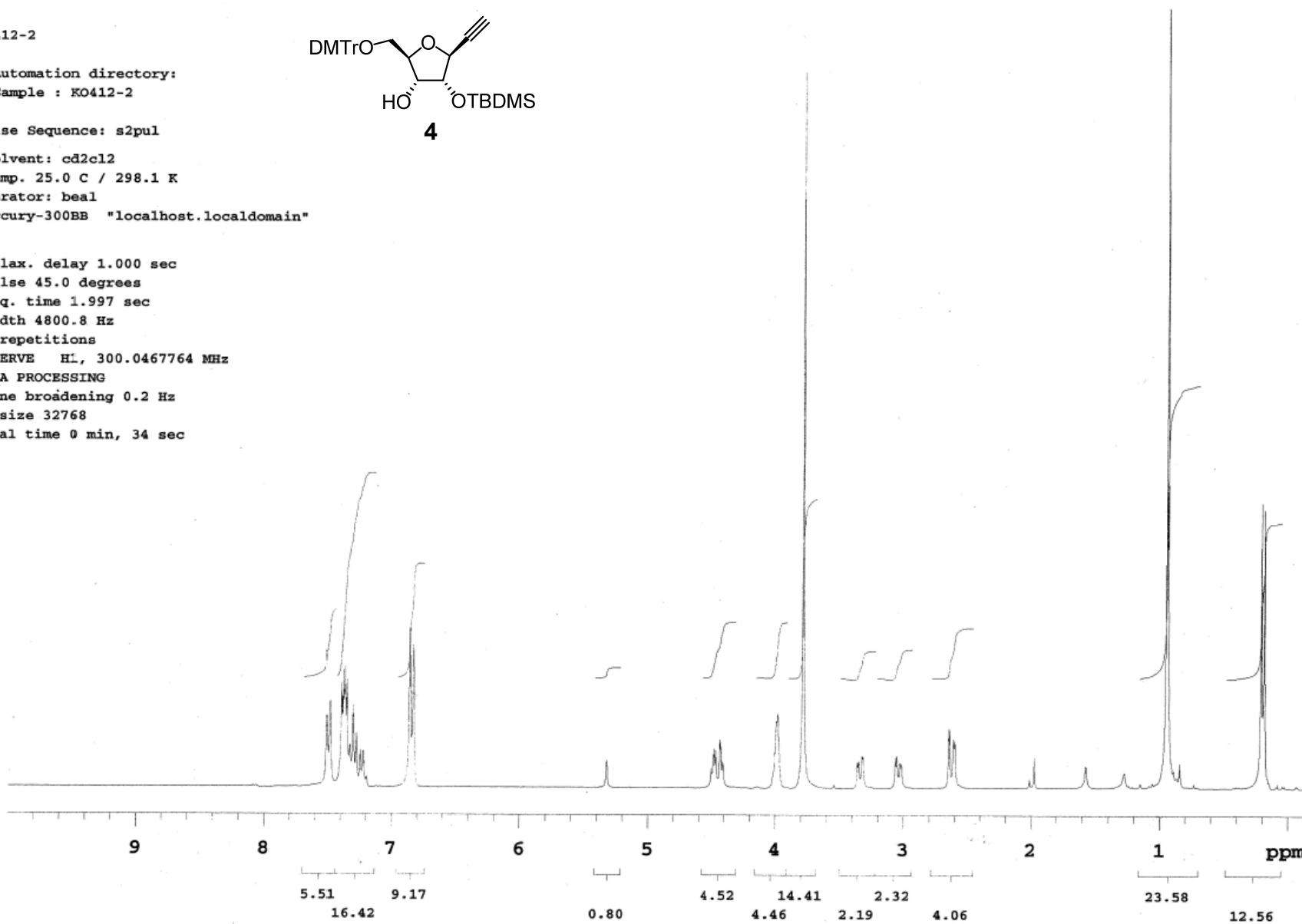
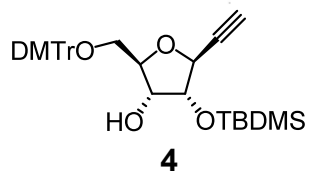
OBSERVE HL, 300.0467764 MHz

DATA PROCESSING

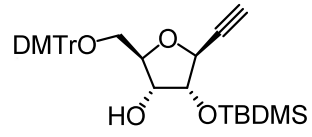
Line broadening 0.2 Hz

FT size 32768

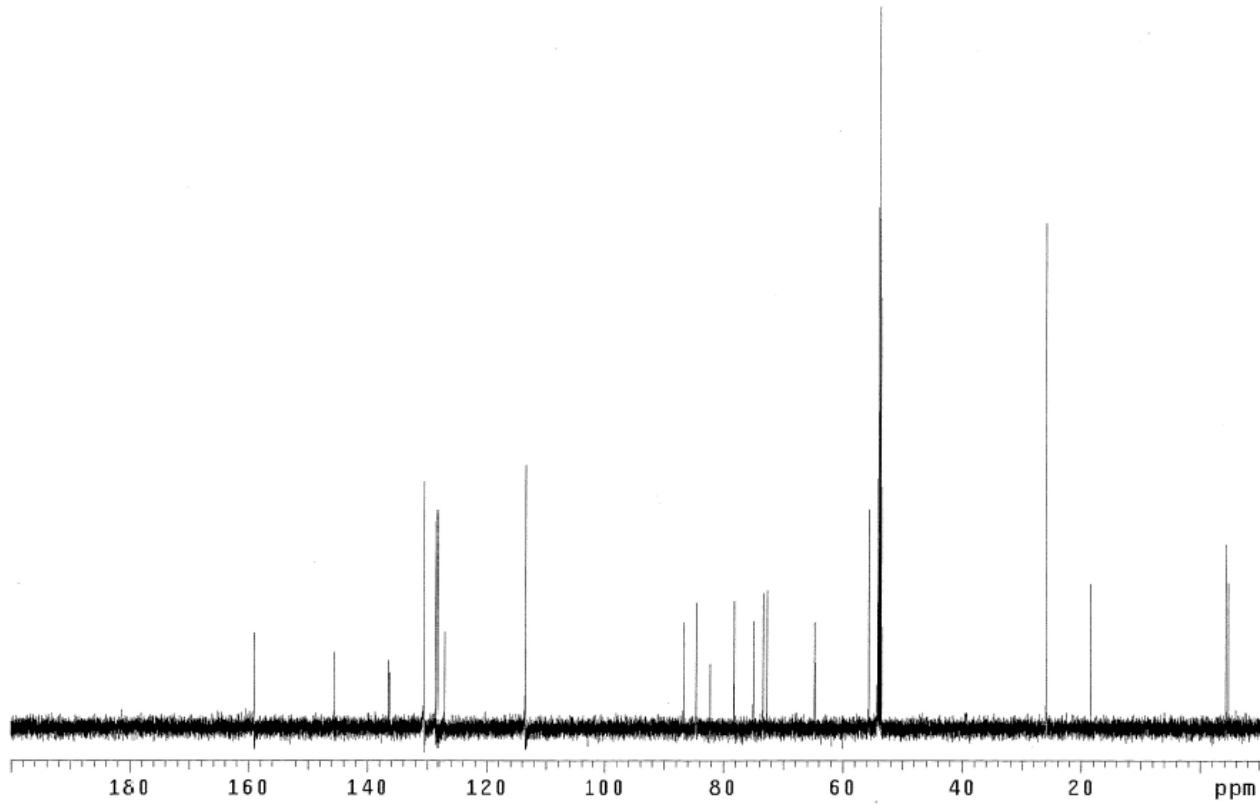
Total time 0 min, 34 sec



OBSERVE C13, 150.7854367 MHz
DECOUPLE H1, 599.6664835 MHz
Power 44 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 3 hr, 12 min, 27 sec



4



KO-380

Automation directory:
Sample : KO-380

Pulse Sequence: s2pul

