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Figure S1 Accurate mass measurement of p53 core domain which was treated with hydrogen peroxide followed by alkylation of free thiol groups with NEM. The theoretical isotopologue distributions of p53 core domain with 10 NEM adducts (corresponding to fully reduced p53), 1 disulfide bond and 8 NEM adducts, and 2 disulfide bonds and 6 NEM adducts, are overlaid upon the experimental isotopologue distributions (maximum entropy deconvoluted mass spectra are shown). The observed isotopologue distributions are consistent with the simulated distributions (each with <5 ppm error) clearly demonstrating that 1 and 2 intramolecular disulfide bonds were formed upon addition of H_2O_2, and no free thiol groups were remaining following NEM treatment.

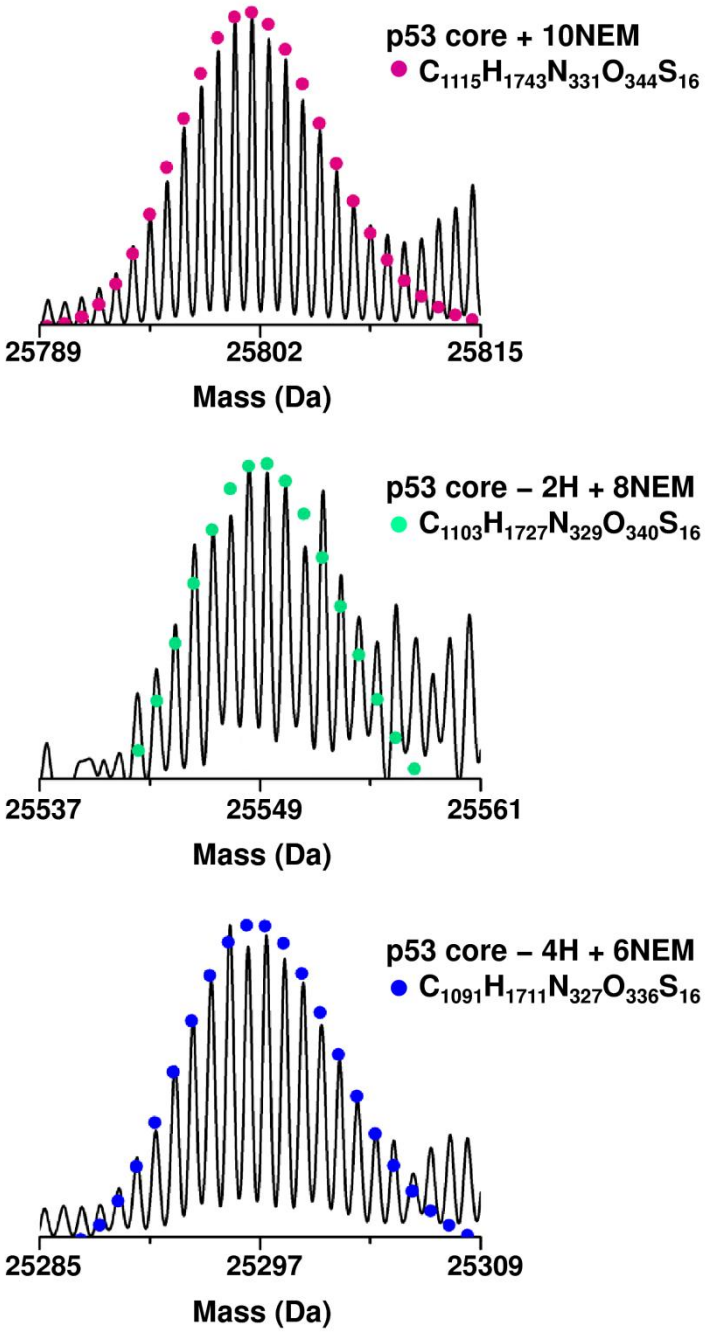

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Table S1. Permutations of 6NEM-p53 that corresponded with the highest peptide bond cleavages resulting from top-down CID FT-ICR MS. The red circles indicate the positions of the NEM adducts.

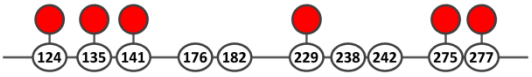
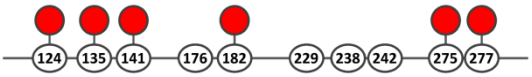
Rank	Permutation	Number of Peptide Bonds Cleaved	Sequence Coverage (%)
1		48	21.9
2		47	21.5
3		44	20.1
3		44	20.1
3		44	20.1
4		39	17.8
4		39	17.8
5		38	17.4
5		38	17.4
6		36	16.4

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Table S2 Product ions assigned in the CID mass spectrum of the 30+ charge state (844 *m/z*) of 6NEM-p53, alkylated at positions 124, 135, 141, 229, 275 and 277.

Product Ion	Measured Molecular Mass (Da)	Theoretical Molecular Mass (Da)	MME (ppm)	Observed *m/z*	*z*
b19	2029.9788	2029.9857	-3.38	1015.9967	2+
b26	2743.3360	2743.3354	0.21	915.4526	3+
b27	2871.4291	2871.4304	-0.46	958.1503	3+
b33	3650.7445	3650.7457	-0.34	1217.9221	3+
	3650.7356	3650.7457	-2.76	731.1544	5+
b34	3737.7787	3737.7777	0.26	1246.9335	3+
	3737.7756	3737.7777	-0.56	748.5624	5+
	3737.7775	3737.7777	-0.04	623.9702	6+
b38	4132.9925	4132.9946	-0.51	1034.2554	4+
b39	4261.0925	4261.0895	0.70	1066.2804	4+
b41	4539.1969	4539.1984	-0.33	1135.8065	4+
b43	4895.3109	4895.3132	-0.47	1224.8350	4+
b44	5008.3945	5008.3973	-0.56	1253.1059	4+
	5008.3906	5008.3973	-1.33	1002.6854	5+
b45	5079.4325	5079.4344	-0.37	1270.8654	4+
	5079.4286	5079.4344	-1.14	1016.8930	5+
b46	5207.5025	5207.5293	-5.15	1302.8829	4+
b48	5536.6306	5536.6332	-0.47	1108.3334	5+
b50	5732.7161	5732.7544	-6.68	1434.1863	4+
	5732.7421	5732.7544	-2.14	1147.5557	5+
	5732.7463	5732.7544	-1.41	956.4650	6+
	5732.7531	5732.7544	-0.23	819.9720	7+
	5732.7538	5732.7544	-0.11	717.6015	8+
b51	5860.8121	5860.8130	-0.15	1173.1697	5+
	5860.8091	5860.8130	-0.66	977.8088	6+
	5860.8076	5860.8130	-0.93	838.2655	7+
b52	5973.8936	5973.8970	-0.57	1195.7860	5+
	5973.8911	5973.8970	-0.98	996.6558	6+
	5973.8926	5973.8970	-0.74	854.4205	7+
	5973.9042	5973.8970	1.20	747.7453	8+
b53	6159.9746	6159.9763	-0.27	1233.0022	5+
	6159.9727	6159.9763	-0.58	1027.6694	6+
	6159.9715	6159.9763	-0.78	881.0032	7+
b54	6259.0501	6259.0447	0.87	1044.1823	6+
	6259.0411	6259.0447	-0.58	895.1560	7+
	6259.0434	6259.0447	-0.21	783.3877	8+
b55	6374.0736	6374.0717	0.30	1275.8220	5+
b114	13088.4027	13088.4157	-0.99	1091.7075	12+
	13088.4003	13088.4157	-1.18	1007.8073	13+
	13088.3777	13088.4157	-2.90	935.8914	14+

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b125	14399.0899	14399.1001	-0.71	960.9466	15+
	14399.0852	14399.1001	-1.04	900.9501	16+
	14399.0922	14399.1001	-0.55	848.0127	17+
b127	14659.1861	14659.2162	-2.05	1048.0920	14+
b128	14788.2395	14788.2588	-1.30	1057.3101	14+
	14788.2394	14788.2588	-1.31	986.8899	15+
	14788.2452	14788.2588	-0.92	925.2726	16+
	14788.2409	14788.2588	-1.21	870.9038	17+
y16	1730.9083	1730.9083	-0.02	577.9767	3+
y18	1965.0184	1965.0200	-0.83	656.0134	3+
y31	3590.8771	3590.8797	-0.72	719.1827	5+
y41	4897.4671	4897.4694	-0.46	817.2518	6+
	4897.4662	4897.4694	-0.66	700.6453	7+
y47	5587.7770	5587.7779	-0.16	699.4794	8+
y48	5700.8298	5700.8620	-5.65	713.6110	8+
y51	5985.0058	5985.0104	-0.77	749.1330	8+
y52	6072.0410	6072.0425	-0.25	760.0124	8+
y54	6274.0970	6274.1014	-0.70	785.2694	8+
	6274.1000	6274.1014	-0.22	698.1295	9+
y55	6403.1402	6403.1440	-0.59	801.3998	8+
y56	6516.2162	6516.2281	-1.82	725.0313	9+
y57	6617.2736	6617.2758	-0.33	736.2599	9+
y71	8173.0892	8173.0907	-0.18	909.1283	9+
	8173.0872	8173.0907	-0.42	818.3160	10+
y73	8347.1522	8347.1547	-0.29	835.7225	10+
y74	8461.1862	8461.1977	-1.35	847.1259	10+
y75	8564.1982	8564.2069	-1.01	857.4271	10+
y87	10074.7703	10074.7919	-2.14	1120.4262	9+
	10074.7782	10074.7919	-1.36	1008.4851	10+
	10074.7694	10074.7919	-2.24	916.8954	11+
y88	10173.8468	10173.8603	-1.33	1131.4347	9+
	10173.8362	10173.8603	-2.36	1018.3909	10+
	10173.8420	10173.8603	-1.80	925.9020	11+
	10173.8199	10173.8603	-3.97	848.8256	12+
y91	10496.9852	10497.0084	-2.21	1050.7058	10+
	10496.9923	10497.0084	-1.54	955.2793	11+
	10496.9931	10497.0084	-1.46	875.7567	12+
y128	14769.2401	14769.1912	3.31	1055.9530	14+
	14769.2299	14769.1912	2.62	985.6226	15+
	14769.2298	14769.1912	2.61	869.7855	17+

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Table S3 Product ions assigned in the CID mass spectrum of the 26+ charge state (975 *m/z*) of 6NEM-p53, alkylated at positions 124, 135, 141, 229, 275 and 277.

Product Ion	Measured Molecular Mass (Da)	Theoretical Molecular Mass (Da)	MME (ppm)	Observed *m/z*	*z*
b22	2427.1951	2427.1971	-0.84	810.0723	3+
b33	3650.7478	3650.7457	0.57	1217.9232	3+
b34	3737.7778	3737.7777	0.02	1246.9332	3+
b37	4018.9534	4018.9516	0.44	1340.6584	3+
b38	4133.0002	4132.9946	1.35	1378.6740	3+
	4132.9813	4132.9946	-3.22	1034.2526	4+
b43	4895.3105	4895.3132	-0.55	1224.8349	4+
b45	5079.4317	5079.4344	-0.53	1270.8652	4+
	5079.4181	5079.4344	-3.21	1016.8909	5+
b46	5207.5017	5207.5293	-5.30	1302.8827	4+
b48	5536.6279	5536.6332	-0.95	923.7786	6+
b50	5732.7169	5732.7544	-6.54	1434.1865	4+
	5732.7396	5732.7544	-2.58	1147.5552	5+
	5732.7487	5732.7544	-0.99	956.4654	6+
b51	5860.8091	5860.8130	-0.66	1173.1691	5+
	5860.8067	5860.8130	-1.07	977.8084	6+
	5860.8097	5860.8130	-0.57	838.2658	7+
b52	5973.8921	5973.8970	-0.82	1195.7857	5+
	5973.8905	5973.8970	-1.08	996.6557	6+
	5973.8982	5973.8970	0.20	854.4213	7+
b53	6159.9751	6159.9763	-0.19	1233.0023	5+
	6159.9715	6159.9763	-0.77	1027.6692	6+
	6159.9708	6159.9763	-0.90	881.0031	7+
b55	6374.0736	6374.0717	0.30	1275.8220	5+
b114	13088.3998	13088.4157	-1.22	1190.8618	11+
	13088.4003	13088.4157	-1.18	1091.7073	12+
	13088.3782	13088.4157	-2.86	1007.8056	13+
b125	14399.0605	14399.1001	-2.75	1029.5116	14+
	14399.0884	14399.1001	-0.82	960.9465	15+
b127	14659.2099	14659.2162	-0.43	1048.0937	14+
b128	14788.2555	14788.2588	-0.22	1233.3619	12+
	14788.2491	14788.2588	-0.66	1138.5649	13+
	14788.2395	14788.2588	-1.30	1057.3101	14+
	14788.2379	14788.2588	-1.42	986.8898	15+
	14788.2228	14788.2588	-2.44	925.2712	16+
b129	14885.2837	14885.3115	-1.87	1146.0291	13+
	14885.2833	14885.3115	-1.89	1064.2418	14+
b131	15111.4001	15111.4069	-0.45	1080.3930	14+
y13	1351.7205	1351.7227	-1.61	1352.7278	1+
	1351.7210	1351.7227	-1.22	676.8678	2+

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y14	1464.8048	1464.8068	-1.33	733.4097	2+
y15	1593.8456	1593.8494	-2.35	797.9301	2+
y16	1730.9052	1730.9083	-1.76	866.4599	2+
y18	1965.0184	1965.0200	-0.83	656.0134	3+
y31	3590.8761	3590.8797	-1.00	719.1825	5+
y41	4897.4647	4897.4694	-0.95	817.2514	6+
y54	6274.0978	6274.1014	-0.58	785.2695	8+
y55	6403.1394	6403.1440	-0.72	801.3997	8+
y87	10074.7858	10074.7919	-0.61	1260.3555	8+
	10074.7730	10074.7919	-1.87	1120.4265	9+
	10074.7772	10074.7919	-1.46	1008.4850	10+
	10074.7727	10074.7919	-1.91	916.8957	11+
y88	10173.8362	10173.8603	-2.37	1272.7368	8+
	10173.8477	10173.8603	-1.24	1131.4348	9+
	10173.8422	10173.8603	-1.78	1018.3915	10+
y90	10399.9378	10399.9556	-1.71	1300.9995	8+
	10399.9457	10399.9556	-0.95	1156.5568	9+
	10399.9382	10399.9556	-1.67	1041.0011	10+
	10399.9371	10399.9556	-1.78	946.4561	11+
y91	10496.9962	10497.0084	-1.16	1313.1318	8+
	10496.9963	10497.0084	-1.15	1167.3402	9+
	10496.9902	10497.0084	-1.73	1050.7063	10+
	10496.9879	10497.0084	-1.96	955.2789	11+
	10496.9931	10497.0084	-1.46	875.7567	12+
y105	12196.8512	12196.8514	-0.01	1220.6924	10+
	12196.8388	12196.8514	-1.04	1109.8108	11+
y165	19026.1787	19026.2225	-2.30	1002.3851	19+
y166	19125.2668	19125.2909	-1.26	1063.5221	18+
y167	19311.3454	19311.3702	-1.28	1073.8598	18+
y168	19424.4111	19424.4542	-2.22	1023.3447	19+

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Table S4. Permutations of 1GSH-9NEM-p53 that corresponded with the highest peptide bond cleavages resulting from top-down ECD FT-ICR MS. The red circles indicate the positions of the NEM adducts and the blue circle indicates the position of the GSH adduct.

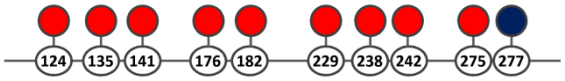
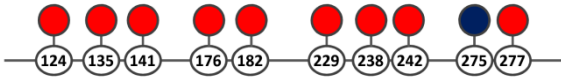
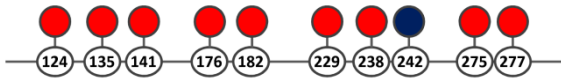
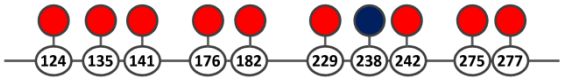
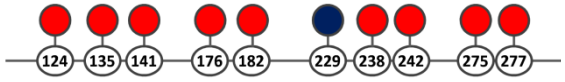
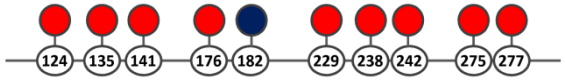
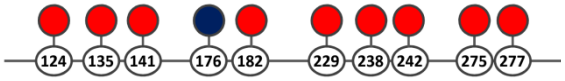
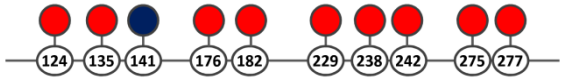
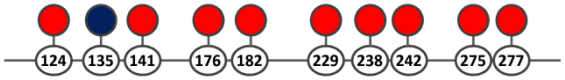
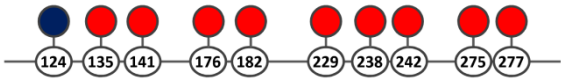
Rank	Permutation	Number of Peptide Bonds Cleaved	Sequence Coverage (%)
1		63	28.8
1		63	28.8
2		57	26.5
2		57	26.5
3		56	26.0
4		53	24.7
4		53	24.7
5		40	18.2
5		40	18.2
6		34	15.5

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Table S5. Product ions assigned in the ECD mass spectrum of the 29+ charge state (897 *m/z*) of 9NEM-p53-SSG, *S*-glutathionylated at position 275 or 277.

Product Ion	Measured Molecular Mass (Da)	Theoretical Molecular Mass (Da)	MME (ppm)	Observed *m/z*	*z*
c6	561.2754	561.2756	-0.31	562.2827	1+
c7	689.3344	689.3342	0.32	690.3417	1+
c8	817.4302	817.4292	1.25	818.4375	1+
c9	918.4785	918.4769	1.77	919.4858	1+
c10	1081.5435	1081.5402	3.07	1082.5508	1+
c12	1266.6248	1266.6202	3.65	1267.6321	1+
c13	1353.6590	1353.6523	4.97	1354.6663	1+
	1353.6532	1353.6523	0.70	677.8339	2+
c15	1573.7380	1573.7370	0.67	787.8763	2+
c17	1876.9090	1876.9066	1.30	939.4618	2+
c19	2047.0184	2047.0121	3.10	1024.5165	2+
	2047.0126	2047.0121	0.23	683.3448	3+
c22	2444.2261	2444.2235	1.05	815.7493	3+
c24	2588.2789	2588.2770	0.72	863.7669	3+
c26	2760.3708	2760.3617	3.31	1381.1927	2+
	2760.3673	2760.3617	2.02	921.1297	3+
c27	2888.4581	2888.4567	0.48	723.1218	4+
c30	3175.6090	3175.6048	1.31	1059.5436	3+
c33	3667.7839	3667.7720	3.24	1223.6019	3+
	3667.7785	3667.7720	1.77	917.9519	4+
c37	4035.9881	4035.9780	2.50	1010.0043	4+
c38	4150.0257	4150.0209	1.16	1038.5137	4+
	4150.0266	4150.0209	1.38	831.0126	5+
c39	4278.1341	4278.1159	4.25	1070.5408	4+
	4278.1246	4278.1159	2.04	856.6322	5+
c51	5877.8626	5877.8393	3.97	1176.5798	5+
c52	5990.9491	5990.9234	4.29	1199.1971	5+
c54	6276.0991	6276.0711	4.46	1256.2271	5+
c56	6478.1566	6478.1300	4.11	1296.6386	5+
	6478.1575	6478.1300	4.25	1080.7002	6+
c61	6927.3710	6927.3575	1.94	990.6317	7+
	6927.3817	6927.3575	3.50	1155.5709	6+
c65	7439.6975	7439.6758	2.91	1063.8212	7+
c67	7641.7756	7641.7534	2.90	1092.6895	7+
c69	7825.8994	7825.8745	3.18	1118.9929	7+
c72	8245.1063	8245.0914	1.81	917.1302	9+
c73	8332.1485	8332.1234	3.01	1191.3142	7+
c77	8829.3586	8829.3291	3.34	1104.6771	8+
	8829.3485	8829.3291	2.20	982.0460	9+
	8829.3412	8829.3291	1.37	883.9414	10+

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c92	10870.2532	10870.2284	2.29	1088.0326	10+
	10870.2603	10870.2284	2.93	906.8623	12+
z7	768.4009	768.4004	0.68	769.4082	1+
z8	896.4942	896.4954	-1.29	449.2544	2+
z10	1084.5740	1084.5751	-0.97	543.2943	2+
z14	1448.7868	1448.7861	0.52	725.4007	2+
z15	1577.8300	1577.8287	0.85	789.9223	2+
z19	2078.0422	2078.0419	0.13	693.6880	3+
z21	2263.1596	2263.1583	0.56	755.3938	3+
z22	2391.2551	2391.2533	0.74	798.0923	3+
z23	2547.3549	2547.3544	0.19	637.8460	4+
z24	2660.4385	2660.4384	0.04	666.1169	4+
z25	2774.4821	2774.4814	0.25	694.6278	4+
z26	2903.5241	2903.5240	0.03	726.8883	4+
z27	3032.5681	3032.5666	0.49	759.1493	4+
z28	3161.6104	3161.6091	0.40	1054.8774	3+
z30	3418.7707	3418.7579	3.74	1140.5975	3+
	3418.7609	3418.7579	0.88	855.6975	4+
z45	5538.6809	5538.6557	4.55	1385.6775	4+
	5538.6731	5538.6557	3.15	1108.7419	5+
z56	6680.2606	6680.2284	4.82	1337.0594	5+
	6680.2519	6680.2284	3.52	1114.3826	6+
z64	7587.8623	7587.8139	6.38	1265.6510	6+
z66	7857.9698	7857.9579	1.51	983.2535	8+
z68	8046.0697	8046.0199	6.19	1342.0189	6+
	8046.0249	8046.0199	0.62	1150.4394	7+
	8046.0410	8046.0199	2.62	1006.7624	8+
z111	13386.3837	13386.3322	3.85	1030.7291	13+
z218	25862.4717	25862.4235	1.87	958.8766	27+

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Table S6 Product ions assigned in the ECD mass spectrum of the 30+ charge state (867 *m/z*) of 9NEM-p53-SSG, *S*-glutathionylated at position 275 or 277.

Product Ion	Measured Molecular Mass (Da)	Theoretical Molecular Mass (Da)	MME (ppm)	Observed *m/z*	*z*
c6	561.2753	561.2756	-0.49	562.2826	1+
c7	689.3339	689.3342	-0.40	690.3412	1+
c10	1081.5433	1081.5402	2.89	1082.5506	1+
c13	1353.6578	1353.6523	4.08	1354.6651	1+
	1353.6524	1353.6523	0.11	677.8335	2+
c15	1573.7372	1573.7370	0.16	787.8759	2+
c18	1989.9962	1989.9906	2.84	996.0054	2+
	1989.9901	1989.9906	-0.27	664.3373	3+
c19	2047.0170	2047.0121	2.42	1024.5158	2+
	2047.0126	2047.0121	0.23	683.3448	3+
c22	2444.2252	2444.2235	0.68	815.7490	3+
c26	2760.3670	2760.3617	1.91	921.1296	3+
c27	2888.4652	2888.4567	2.93	963.8290	3+
	2888.4573	2888.4567	0.21	723.1216	4+
c37	4035.9845	4035.9780	1.61	1010.0034	4+
c38	4150.0301	4150.0209	2.22	1038.5148	4+
	4150.0236	4150.0209	0.66	831.0120	5+
c39	4278.1253	4278.1159	2.20	1070.5386	4+
	4278.1221	4278.1159	1.45	856.6317	5+
c51	5877.8596	5877.8393	3.46	1176.5792	5+
c52	5990.9471	5990.9234	3.96	1199.1967	5+
c56	6478.1546	6478.1300	3.80	1296.6382	5+
	6478.1509	6478.1300	3.23	1080.6991	6+
c61	6927.3829	6927.3575	3.67	1155.5711	6+
	6927.3829	6927.3575	3.66	990.6334	7+
c65	7439.6954	7439.6758	2.63	1063.8209	7+
c67	7641.7777	7641.7534	3.18	1092.6898	7+
c68	7712.8039	7712.7905	1.73	1102.8364	7+
c69	7825.8973	7825.8745	2.91	1118.9926	7+
c71	8117.0616	8117.0328	3.54	1160.5875	7+
	8117.0490	8117.0328	1.99	1015.6384	8+
	8117.0444	8117.0328	1.43	902.9011	9+
	8117.0372	8117.0328	0.55	812.7110	10+
c73	8332.1394	8332.1234	1.92	1042.5247	8+
c74	8460.1874	8460.1820	0.64	1058.5307	8+
c77	8829.3474	8829.3291	2.07	1104.6757	8+
	8829.3440	8829.3291	1.69	982.0455	9+
	8829.3392	8829.3291	1.15	883.9412	10+
c80	9156.5322	9156.5085	2.59	1145.5738	8+
	9156.5219	9156.5085	1.47	1018.3986	9+

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c90	10668.2238	10668.1694	5.10	970.8458	11+
c92	10870.2572	10870.2284	2.65	1088.0330	10+
z8	896.4938	896.4954	-1.73	449.2542	2+
z9	997.5418	997.5431	-1.26	499.7782	2+
z10	1084.5740	1084.5751	-0.97	543.2943	2+
z14	1448.7862	1448.7861	0.10	725.4004	2+
z17	1851.9454	1851.9465	-0.61	618.3224	3+
z19	2078.0419	2078.0419	-0.01	693.6879	3+
z21	2263.1602	2263.1583	0.83	755.3940	3+
z23	2547.3529	2547.3544	-0.59	637.8455	4+
z24	2660.4377	2660.4384	-0.26	666.1167	4+
z25	2774.4813	2774.4814	-0.04	694.6276	4+
z26	2903.5241	2903.5240	0.03	726.8883	4+
z27	3032.5780	3032.5666	3.75	1011.8666	3+
	3032.5677	3032.5666	0.36	759.1492	4+
z30	3418.7668	3418.7579	2.60	1140.5962	3+
	3418.7625	3418.7579	1.34	855.6979	4+
z44	5424.6236	5424.6128	1.99	1085.9320	5+
z45	5538.6686	5538.6557	2.33	1108.7410	5+
z64	7587.8485	7587.8139	4.57	1265.6487	6+
z66	7857.9783	7857.9579	2.59	1123.5756	7+
	7857.9746	7857.9579	2.12	983.2541	8+
	7857.9722	7857.9579	1.82	874.1153	9+
z68	8046.0480	8046.0199	3.49	1150.4427	7+
	8046.0338	8046.0199	1.73	1006.7615	8+
z78	9386.4746	9386.4480	2.84	1043.9489	9+
z218	25862.4527	25862.4235	1.13	924.6663	28+