From Chemistry to Targeted Cancer Therapy: Potent Benzimidazole-1,2,3-Triazole Hybrid-Loaded Nanogels Against Breast and Colorectal Malignancies

Supplementary file

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1 Cytotoxicity against MDA-MB-231

Table S1: Cytotoxicity against MDA-MB-231

ID	Conc. ppm/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
MDA	dilution	0.827	0.83	0.821	0.826	0.00216	100	0	
	500	0.042	0.038	0.039	0.039667	0.000981	4.80226	95.19774	
	250	0.063	0.048	0.058	0.056333	0.0036	6.820016	93.17998	
10ng	125	0.101	0.109	0.102	0.104	0.002055	12.5908	87.4092	**31.44**
	62.5	0.142	0.148	0.143	0.144333	0.001515	17.47377	82.52623	
	31.3	0.415	0.416	0.413	0.414667	0.00072	50.20178	49.79822	
	15.6	0.572	0.567	0.563	0.567333	0.002126	68.68442	31.31558	
	7.81	0.697	0.695	0.698	0.696667	0.00072	84.34221	15.65779	
	3.91	0.818	0.814	0.817	0.816333	0.000981	98.8297	1.170299	
	500	0.058	0.055	0.061	0.058	0.001414	7.021792	92.97821	
	250	0.095	0.109	0.102	0.102	0.0033	12.34867	87.65133	
11ng	125	0.174	0.181	0.187	0.180667	0.003067	21.87248	78.12752	**68.35**
	62.5	0.447	0.433	0.431	0.437	0.00411	52.90557	47.09443	
	31.3	0.522	0.526	0.528	0.525333	0.00144	63.59968	36.40032	
	15.6	0.653	0.655	0.659	0.655667	0.00144	79.37853	20.62147	
	7.81	0.748	0.752	0.748	0.749333	0.001089	90.71832	9.281679	
	3.91	0.825	0.817	0.824	0.822	0.002055	99.51574	0.484262	
	500	0.065	0.051	0.052	0.056	0.003682	6.779661	93.22034	
	250	0.084	0.085	0.073	0.080667	0.003139	9.76594	90.23406	
12ng	125	0.109	0.114	0.113	0.112	0.001247	13.55932	86.44068	**16.48**
	62.5	0.189	0.194	0.181	0.188	0.003091	22.76029	77.23971	
	31.3	0.281	0.279	0.284	0.281333	0.001186	34.05973	65.94027	
	15.6	0.436	0.414	0.412	0.420667	0.006278	50.92817	49.07183	
	7.81	0.614	0.605	0.601	0.606667	0.003139	73.44633	26.55367	
	3.91	0.814	0.813	0.82	0.815667	0.001785	98.74899	1.251009	
ID	**Conc. ppm/ml**	**O.D**			**Mean O.D**	**ST.E**	**Viability %**	**Toxicity %**	**IC50**
MDA	dilution	0.833	0.848	0.836	0.839	0.003742	100	0	
	500	0.051	0.059	0.055	0.055	0.001886	6.555423	93.44458	
	250	0.055	0.068	0.063	0.062	0.003091	7.38975	92.61025	
10	125	0.085	0.073	0.075	0.077667	0.003031	9.257052	90.74295	**5.416**
	62.5	0.094	0.105	0.107	0.102	0.0033	12.15733	87.84267	
	31.3	0.12	0.128	0.124	0.124	0.001886	14.7795	85.2205	

ID	Conc. ppm/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
	15.6	0.202	0.21	0.2	0.204	0.002494	24.31466	75.68534	
	7.81	0.324	0.336	0.332	0.330667	0.00288	39.412	60.588	
	3.91	0.508	0.5	0.504	0.504	0.001886	60.07151	39.92849	
	500	0.051	0.056	0.052	0.053	0.001247	6.317044	93.68296	
	250	0.06	0.048	0.055	0.054333	0.002841	6.475963	93.52404	
11	125	0.078	0.062	0.07	0.07	0.003771	8.343266	91.65673	**4.107**
	62.5	0.086	0.085	0.096	0.089	0.002867	10.60787	89.39213	
	31.3	0.102	0.104	0.107	0.104333	0.001186	12.43544	87.56456	
	15.6	0.16	0.154	0.155	0.156333	0.001515	18.63329	81.36671	
	7.81	0.292	0.295	0.289	0.292	0.001414	34.80334	65.19666	
	3.91	0.421	0.431	0.433	0.428333	0.003031	51.05284	48.94716	
	500	0.064	0.057	0.068	0.063	0.002625	7.508939	92.49106	
	250	0.061	0.063	0.051	0.058333	0.003031	6.952721	93.04728	
12	125	0.074	0.062	0.065	0.067	0.002944	7.985697	92.0143	**3.139**
	62.5	0.077	0.082	0.078	0.079	0.001247	9.415971	90.58403	
	31.3	0.091	0.094	0.102	0.095667	0.002681	11.40246	88.59754	
	15.6	0.143	0.149	0.155	0.149	0.002828	17.75924	82.24076	
	7.81	0.27	0.263	0.264	0.265667	0.001785	31.66468	68.33532	
	3.91	0.345	0.354	0.345	0.348	0.002449	41.47795	58.52205	

2 Cytotoxicity against Caco2

Table S2: Cytotoxicity against Caco2

ID	Conc. ppm/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
Caco	dilution	0.61	0.617	0.621	0.616	0.002625	100	0	
	500	0.053	0.068	0.055	0.058667	0.003839	9.52381	90.47619	
	250	0.086	0.082	0.086	0.084667	0.001089	13.74459	86.25541	
10ng	125	0.107	0.109	0.102	0.106	0.0017	17.20779	82.79221	**43.940**
	62.5	0.172	0.171	0.164	0.169	0.002055	27.43506	72.56494	
	31.3	0.405	0.402	0.402	0.403	0.000816	65.42208	34.57792	
	15.6	0.555	0.552	0.561	0.556	0.00216	90.25974	9.74026	
	7.81	0.604	0.603	0.607	0.604667	0.000981	98.16017	1.839827	
	3.91	0.612	0.611	0.619	0.614	0.002055	99.67532	0.324675	
	500	0.063	0.059	0.059	0.060333	0.001089	9.794372	90.20563	
	250	0.075	0.076	0.085	0.078667	0.002596	12.77056	87.22944	
11ng	125	0.124	0.118	0.123	0.121667	0.001515	19.75108	80.24892	**63.810**
	62.5	0.317	0.311	0.308	0.312	0.00216	50.64935	49.35065	
	31.3	0.438	0.447	0.443	0.442667	0.002126	71.86147	28.13853	
	15.6	0.531	0.535	0.533	0.533	0.000943	86.52597	13.47403	
	7.81	0.601	0.591	0.597	0.596333	0.002373	96.80736	3.192641	
	3.91	0.617	0.613	0.613	0.614333	0.001089	99.72944	0.270563	
	500	0.052	0.058	0.047	0.052333	0.002596	8.495671	91.50433	
	250	0.051	0.055	0.056	0.054	0.001247	8.766234	91.23377	
12ng	125	0.058	0.064	0.063	0.061667	0.001515	10.01082	89.98918	**7.904**
	62.5	0.109	0.094	0.108	0.103667	0.003953	16.829	83.171	
	31.3	0.128	0.133	0.135	0.132	0.0017	21.42857	78.57143	
	15.6	0.223	0.228	0.222	0.224333	0.001515	36.41775	63.58225	
	7.81	0.312	0.304	0.311	0.309	0.002055	50.16234	49.83766	
	3.91	0.458	0.453	0.455	0.455333	0.001186	73.91775	26.08225	
ID	**Conc. ppm/ml**	**O.D**			**Mean O.D**	**ST.E**	**Viability %**	**Toxicity %**	**IC50**
Caco	dilution	0.597	0.613	0.608	0.606	0.003859	100	0	
	500	0.053	0.068	0.055	0.058667	0.003839	9.680968	90.31903	
	250	0.056	0.072	0.066	0.064667	0.00381	10.67107	89.32893	
10	125	0.073	0.069	0.082	0.074667	0.003139	12.32123	87.67877	**12.207**
	62.5	0.098	0.091	0.104	0.097667	0.003067	16.11661	83.88339	
	31.3	0.135	0.132	0.142	0.136333	0.002419	22.49725	77.50275	

ID	Conc. ppm/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
	15.6	0.255	0.242	0.251	0.249333	0.003139	41.14411	58.85589	
	7.81	0.364	0.375	0.377	0.372	0.0033	61.38614	38.61386	
	3.91	0.576	0.573	0.569	0.572667	0.001656	94.49945	5.50055	
	500	0.063	0.052	0.051	0.055333	0.003139	9.130913	90.86909	
	250	0.065	0.066	0.058	0.063	0.002055	10.39604	89.60396	
11	125	0.084	0.081	0.073	0.079333	0.002681	13.09131	86.90869	**8.296**
	62.5	0.107	0.121	0.118	0.115333	0.003475	19.0319	80.9681	
	31.3	0.178	0.174	0.163	0.171667	0.003662	28.32783	71.67217	
	15.6	0.239	0.245	0.243	0.242333	0.00144	39.989	60.011	
	7.81	0.312	0.304	0.305	0.307	0.002055	50.66007	49.33993	
	3.91	0.468	0.473	0.485	0.475333	0.004119	78.43784	21.56216	
	500	0.052	0.058	0.047	0.052333	0.002596	8.635864	91.36414	
	250	0.051	0.055	0.056	0.054	0.001247	8.910891	91.08911	
12	125	0.058	0.064	0.063	0.061667	0.001515	10.17602	89.82398	**3.645**
	62.5	0.079	0.074	0.068	0.073667	0.002596	12.15622	87.84378	
	31.3	0.098	0.103	0.105	0.102	0.0017	16.83168	83.16832	
	15.6	0.153	0.148	0.142	0.147667	0.002596	24.36744	75.63256	
	7.81	0.22	0.214	0.211	0.215	0.00216	35.47855	64.52145	
	3.91	0.288	0.283	0.295	0.288667	0.002841	47.63476	52.36524	

3 Cytotoxicity against HEK-293

Table 3: Cytotoxicity against HEK-293

ID	Conc. ppm/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
HEK	dilution	0.722	0.738	0.724	0.728	0.00411	100	0	
	500	0.059	0.059	0.057	0.058333	0.000544	8.012821	91.98718	
	250	0.105	0.098	0.111	0.104667	0.003067	14.37729	85.62271	
10ng	125	0.205	0.204	0.205	0.204667	0.000272	28.11355	71.88645	**77.58**
	62.5	0.414	0.415	0.415	0.414667	0.000272	56.95971	43.04029	
	31.3	0.533	0.528	0.524	0.528333	0.002126	72.57326	27.42674	
	15.6	0.613	0.614	0.618	0.615	0.001247	84.47802	15.52198	
	7.81	0.704	0.706	0.702	0.704	0.000943	96.7033	3.296703	
	3.91	0.718	0.724	0.724	0.722	0.001633	99.17582	0.824176	
	500	0.063	0.055	0.059	0.059	0.001886	8.104396	91.8956	
	250	0.105	0.097	0.096	0.099333	0.002325	13.64469	86.35531	
11ng	125	0.163	0.165	0.158	0.162	0.0017	22.25275	77.74725	**63.41**
	62.5	0.361	0.368	0.372	0.367	0.002625	50.41209	49.58791	
	31.3	0.446	0.455	0.445	0.448667	0.002596	61.63004	38.36996	
	15.6	0.538	0.538	0.533	0.536333	0.001361	73.67216	26.32784	
	7.81	0.659	0.654	0.647	0.653333	0.002841	89.74359	10.25641	
	3.91	0.721	0.725	0.723	0.723	0.000943	99.31319	0.686813	
	500	0.054	0.066	0.051	0.057	0.003742	7.82967	92.17033	
	250	0.071	0.083	0.072	0.075333	0.003139	10.34799	89.65201	
12ng	125	0.094	0.092	0.095	0.093667	0.00072	12.8663	87.1337	**12.48**
	62.5	0.137	0.132	0.138	0.135667	0.001515	18.63553	81.36447	
	31.3	0.229	0.234	0.232	0.231667	0.001186	31.82234	68.17766	
	15.6	0.303	0.307	0.305	0.305	0.000943	41.8956	58.1044	
	7.81	0.457	0.453	0.444	0.451333	0.003139	61.99634	38.00366	
	3.91	0.612	0.624	0.615	0.617	0.002944	84.75275	15.24725	
ID	**Conc. ppm/ml**	**O.D**			**Mean O.D**	**ST.E**	**Viability %**	**Toxicity %**	**IC50**
HEK	dilution	0.712	0.725	0.714	0.717	0.0033	100	0	
	500	0.039	0.054	0.058	0.050333	0.004722	7.019991	92.98001	
	250	0.055	0.078	0.062	0.065	0.005558	9.065551	90.93445	
10	125	0.111	0.123	0.119	0.117667	0.00288	16.41097	83.58903	**30.209**
	62.5	0.211	0.209	0.212	0.210667	0.00072	29.38168	70.61832	
	31.3	0.344	0.348	0.342	0.344667	0.00144	48.07066	51.92934	

ID	Conc. ppm/ml	O.D			Mean O.D	ST.E	Viability %	Toxicity %	IC50
	15.6	0.564	0.551	0.542	0.552333	0.005214	77.03394	22.96606	
	7.81	0.618	0.622	0.614	0.618	0.001886	86.19247	13.80753	
	3.91	0.671	0.676	0.672	0.673	0.001247	93.86332	6.136681	
	500	0.069	0.068	0.059	0.065333	0.002596	9.112041	90.88796	
	250	0.063	0.077	0.072	0.070667	0.003344	9.855881	90.14412	
11	125	0.088	0.063	0.085	0.078667	0.006435	10.97164	89.02836	**17.67**
	62.5	0.096	0.094	0.107	0.099	0.0033	13.80753	86.19247	
	31.3	0.123	0.12	0.127	0.123333	0.001656	17.2013	82.7987	
	15.6	0.203	0.207	0.2	0.203333	0.001656	28.3589	71.6411	
	7.81	0.348	0.347	0.356	0.350333	0.002325	48.86099	51.13901	
	3.91	0.571	0.579	0.574	0.574667	0.001905	80.14877	19.85123	
	500	0.059	0.059	0.077	0.065	0.004899	9.065551	90.93445	
	250	0.098	0.093	0.102	0.097667	0.002126	13.62157	86.37843	
12	125	0.159	0.157	0.164	0.16	0.0017	22.3152	77.6848	**36.86**
	62.5	0.362	0.355	0.36	0.359	0.0017	50.06974	49.93026	
	31.3	0.497	0.503	0.491	0.497	0.002828	69.3166	30.6834	
	15.6	0.694	0.681	0.688	0.687667	0.003067	95.90888	4.09112	
	7.81	0.713	0.718	0.721	0.717333	0.001905	100.0465	-0.04649	
	3.91	0.719	0.712	0.715	0.715333	0.001656	99.76755	0.23245	

4 Enzyme Inhibition Activity on TOP I, TOP II, and Tubulin

Table S4: Enzyme Inhibition Activity on TOP I, TOP II, and Tubulin

TOPO1_%_inhibition					Mean	TOPO2_%_inhibition					Mean	Tubulin % inhibition					Mean
Compound	Conc. ppm	TOPO1 conc. ng/ml	Activity %	Inhibition %		Compound	Conc. ppm	TOPO2 conc. ng/ml	Activity %	Inhibition %		Compound	Conc. ppm	tubulin conc. ng/ml	Activity %	Inhibition %	
	Caco2	0.5324	100	0			Caco2	1.7116	100	0			Caco2	0.5506	100	0	
12ng	500	0.0955	17.93	82.07		12ng	500	0.6286	36.724	63.276		12ng	500	0.3183	57.806	42.194	
	100	0.1235	23.197	76.803	70.45		100	1.047	61.168	38.832	42.07		100	0.5178	94.037	5.963	NA
	50	0.2371	44.534	55.466			50	1.193	69.698	30.302			50	0.5919	107.494	-7.494	
	Caco2	0.5324	100	0			Caco2	1.7116	100	0			Caco2	0.5506	100	0	
11ng	500	0.1284	24.117	75.883		11ng	500	0.613	35.813	64.187		11ng	500	0.3335	60.567	39.433	
	100	0.1475	27.705	72.295	65.06		100	0.8712	50.898	49.102	43.13		100	0.4545	82.541	17.459	17.27
	50	0.2651	49.793	50.207			50	1.276	74.547	25.453			50	0.5094	92.512	7.488	
	Caco2	0.5324	100	0			Caco2	1.7116	100	0			Caco2	0.5506	100	0	
10ng	500	61.833	38.167	61.833		10ng	500	1.302	76.066	23.934		10ng	500	0.5427	98.559	1.441	
	100	63.956	36.044	63.956	63.22		100	1.805	105.453	-5.453	NA		100	0.5243	95.218	4.782	3.43
	50	63.881	36.119	63.881			50	3.43	200.389	-100.389			50	0.519	94.164	5.836	
	Caco2	0.5324	100	0			Caco2	1.7116	100	0			Caco2	0.5506	100	0	
12ng	500	0.1765	33.152	66.848		12ng	500	0.5879	34.347	65.653		10ng	500	0.4029	73.17	26.83	
	100	0.2409	45.248	54.752	52.55		100	0.8244	48.164	51.836	43.97		100	0.5407	98.196	1.804	NA
	50	0.3213	60.349	39.651			50	1.284	75.015	24.985			50	0.5899	107.131	-7.131	
	Caco2	0.5324	100	0			Caco2	1.7116	100	0			Caco2	0.5506	100	0	
11ng	500	0.1819	34.166	65.834		11ng	500	0.7061	41.252	58.748		11ng	500	0.4398	79.872	20.128	
	100	0.2278	42.787	57.213	53.03		100	0.9782	57.149	42.851	40.86		100	0.4887	88.752	11.248	NA
	50	0.3216	60.406	39.594			50	1.248	72.911	27.089			50	0.601	109.147	-9.147	
	Caco2	0.5324	100	0			Caco2	1.7116	100	0			Caco2	0.5506	100	0	
10ng	500	0.3775	70.905	29.095		10ng	500	1.338	78.169	21.831		12ng	500	0.6084	110.491	-10.491	
	100	0.3794	71.262	28.738	28.65		100	1.334	77.936	22.064	20.75		100	0.5878	106.75	-6.75	NA
	50	0.3827	71.882	28.118			50	1.394	81.441	18.559			50	0.5587	101.465	-1.465	

TOPO1_%_inhibition					Mean	TOPO2_%_inhibition					Mean	Tubulin % inhibition					Mean
Compound	Conc. ppm	TOPO1 conc. ng/ml	Activity %	Inhibition %		Compound	Conc. ppm	TOPO2 conc. ng/ml	Activity %	Inhibition %		Compound	Conc. ppm	tubulin conc. ng/ml	Activity %	Inhibition %	
	MDA	0.6309	100	0			MDA	2.5363	100	0			MDA	0.9562	100	0	
12ng	500	0.1419	22.492	77.508		12ng	500	0.5691	22.438	77.562		12ng	500	0.4667	48.808	51.192	
	100	0.2039	32.319	67.681	65.11		100	0.8235	32.468	67.532	63.73		100	0.5857	61.253	38.747	41.82
	50	0.299	47.393	52.607			50	1.283	50.585	49.415			50	0.6037	63.135	36.865	
	MDA	0.6309	100	0			MDA	2.5363	100	0			MDA	0.9562	100	0	
11ng	500	0.1888	29.926	70.074		11ng	500	0.6638	26.172	73.828		11ng	500	0.6763	70.728	29.272	
	100	0.2454	38.897	61.103	53.87		100	1.057	41.674	58.326	59.27		100	0.7491	78.341	21.659	22.81
	50	0.4006	63.497	36.503			50	1.31	51.649	48.351			50	0.7773	81.291	18.709	
	MDA	0.6309	100	0			MDA	2.5363	100	0			MDA	0.9562	100	0	
10ng	500	0.3813	60.437	39.563		10ng	500	1.403	55.316	44.684		10ng	500	0.7977	83.424	16.576	
	100	0.3876	61.436	38.564	39.35		100	1.546	60.954	39.046	7.16		100	0.7866	82.263	17.737	14.12
	50	0.3789	60.057	39.943			50	2.531	99.79	0.21			50	0.8646	90.42	9.58	
	MDA	0.6309	100	0			MDA	2.5363	100	0			MDA	0.9562	100	0	
12ng	500	0.1293	20.495	79.505		12ng	500	0.7748	30.548	69.452		10ng	500	0.4046	42.313	57.687	
	100	0.2156	34.173	65.827	63.32		100	0.9638	38	62	53.09		100	0.8311	86.917	13.083	NA
	50	0.3248	51.482	48.518			50	1.655	65.252	34.748			50	1.081	113.052	-13.052	
	MDA	0.6309	100	0			MDA	2.5363	100	0			MDA	0.9562	100	0	
11ng	500	0.2038	32.303	67.697		11ng	500	0.7883	31.08	68.92		11ng	500	0.644	67.35	32.65	
	100	0.2863	45.38	54.62	51.85		100	1.072	42.266	57.734	48.21		100	0.7321	76.563	23.437	23.71
	50	0.3931	62.308	37.692			50	1.822	71.836	28.164			50	0.7896	82.577	17.423	
	MDA	0.6309	100	0			MDA	2.5363	100	0			MDA	0.9562	100	0	
10ng	500	0.3851	61.04	38.96		10ng	500	1.403	55.316	44.684		10ng	500	0.8146	85.191	14.809	
	100	0.3979	63.069	36.931	38.17		100	1.52	59.929	40.071	28.28		100	0.8623	90.18	9.82	2.96
	50	0.387	61.341	38.659			50	2.216	87.37	12.63			50	0.9545	99.822	0.178	

5 Inhibition activity of target on TOPO I, II and Tubulin

Table S5: Mean % inhibition activity of target on TOPO I, II and tubulin

Compound ID	Mean % inhibition		
	TOPO I	**TOPO II**	**Tubulin**
	Caco2		
10	28.65	20.75	NA
11	53.03	40.86	NA[a]
12	52.55	43.97	NA
10ng	**63.22**	NA	3.43
11ng	65.06	**43.13**	17.27
12ng	**70.45**	**42.07**	NA
Doxorubicin	77.92	58.66	-
Paclitaxel	-	-	63.19
	MDA-MB-231		
10	38.17	28.28	2.96
11	51.85	48.21	23.71
12	63.32	53.09	NA
10ng	39.35	7.16	14.12
11ng	53.87	**59.27**	22.81
12ng	**65.11**	**63.73**	**41.82**
Doxorubicin	75.94	76.29	-
Paclitaxel	-	-	61.72

[a] NA = No significant % inhibition

6 Results of molecular docking experiments

Table S6: parison of 12ng and FDF Ligand Interactions with Topo I

Interaction Type	Ligand	Target Residue	Distance (Å)	Energy (kcal/mol)
H-bond (donor)	12ng	DT116	2.43	-0.5
H-bond (donor)	12ng	MET428	1.94	-0.8
H-bond (acceptor)	12ng	LYS439	2.39	-0.6
H-bond (acceptor)	12ng	LYS587	2.09	-4.6
π–cation	12ng	DA113	2.64	-1.0
π–π stacking	12ng	TGP11	4.09	-1.2
H-bond (donor)	FDF	ASP533	2.64	-4.2
H-bond (acceptor)	FDF	LYS532	3.17	-1.3
H-bond (acceptor)	FDF	ARG364	3.05	-5.6
π–π stacking	FDF	TGP11	4.02	-2.5
π–π stacking	FDF	DA113	2.94	-2.8
π–π stacking	FDF	DA113	1.47	-1.9
π–π stacking	FDF	TGP11	2.59	-2.1
π–π stacking	FDF	DA113	2.63	-1.5
π–π stacking	FDF	DC112	2.76	-1.9

7 Spectral characteristics of target compounds

1-((1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)methyl)-2-((1-*p*-tolyl-1*H*-1,2,3-triazol-4-yl)methylthio)-1*H*-benzo[*d*]imidazole (10)

Yield 82% $_{\text{DMF/ethanol, beige solid}}$, Mp: 186-188 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.36 (s, 6H, Ar-CH_3), 4.77 (s, 2H, SCH_2), 5.53 (s, 2H, CH_2-Imidazole), 7.20-7.22 (m, 2H, Ar), 7.35 , 7.37 (2s, 4H, Ar), 7.62-7.67 (m, 2H, Ar), 7.69-7.72 (m, 4H, Ar) , 8.69 (s, 1H, H-5_{trz}) , 8.77 (s, 1H, H-5_{trz}); ^{13}C NMR (101 MHz, DMSO-d_6) δ 21.01 (CH_3), 27.29 (CH_2S), 110.59, 120.41,120.45, 122.15, 122.35, 130.68, 130.70, 134.58, 134.68, 138.88, 138.97, 143.45 (Ar-C);

Elemental analysis Calcd for [$C_{27}H_{24}N_8S$]: C, 65.83; H, 4.91; N, 22.75; S, 6.51 found C, 65.86; H, 4.98; N, 22.84; S, 6.57.

2-(4-((1*H*-benzo[*d*]imidazol-2-ylthio)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(4-methylbenzyl)acetamide (11)

Yield 80% $_{\text{ethyl acetate, white solid}}$, m.p.: 186-188 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.26 (s, 3H, CH_3), 4.26 (s, 2H, $NHCH_2$), 4.68 (s, 2H, SCH_2), 5.13 (s, 2H, NCH_2CO), 7.12-7.13 (m, 6H, Ar), 7.43 (brs, 1H, Ar), 7.53 (brs, 1H, Ar), 8.10 (s, 1H, H-5_{trz}), 8.79 (s, 1H, CONH), 12.83 (s, 1H, NH).^{13}C NMR (100 MHz, DMSO-d_6):δ_C = 21.13 (CH_3), 26.35 (SCH_2), 42.57 ($NHCH_2$), 52.20 (NCH_2CO), 111.06, 117.99, 121.78, 122.38, 125.87, 127.85, 129.36, 136.07, 136.57, 144.04, 165.69; Elemental analysis Calcd for [$C_{20}H_{20}N_6OS$]: C, 61.20; H, 5.14; N, 21.41; S, 8.17 Found C, 61.23; H, 5.18; N, 21.47; S, 8.20.

(2R,3R,4R,5R,6R)-2-(acetoxymethyl)-6-(4-(((1-((1-((2R,3R,4R,5R,6R)-3,4,5-triacetoxy-6-(acetoxymethyl)tetrahydro-2H-pyran-2-yl)-1H-1,2,3-triazol-4-yl)methyl)-1H-benzo[d]imidazol-2-yl)thio)methyl)-1H-1,2,3-triazol-1-yl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (12)

Yield 78% EtOH, beige solid, Mp: 207-209 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 1.65 (s, 3H, COC**H$_3$**), 1.69 (s, 3H, COC**H$_3$**), 1.95 (s, 12H, 3*COC**H$_3$**), 2.01 (s, 6H, 2*OCOC**H$_3$**), 4.02-4.13 (m, 4H, **H-6**a,**H-6`a, H-6**b,**H-6`b**), 4.32 (s, 2H, **H-5a, H-5b**), 4.65 (s, 2H, SC**H$_2$**), 5.17 (d, *J* = 5.4 Hz, 2H, **H-4a, H-4b**), 5.44 (s, 2H, NC**H$_2$**), 5.51 (dd, *J* = 8.80, 7.72 Hz, 2H, **H-3a, H-3b),** 5,63 (d, *J* = 8.32 Hz, 2H, **H-2a, H-2b**), 6.31 (d, *J* = 8.64 Hz, 2H, **H-1a, H-1b**), 7.18 (s, 2H, Ph-**H**), 7.52, 7.58 (2s, 2H, Ph-**H**), 8.42 (s, 1H, triazolyl-**H**5), 8.47 (s, 1H, triazolyl-**H**5); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 20.21, 20.70, 20.84, 20.93 (***C***H_3CO), 27.17 (***C***H_2S), 39.24 (***C***H_2N), 62.17 (***C***-6), 67.91, 70.39, 70.42, 72.57, 73.73 (***C***-glucose), 84.24 (***C***-1), 110.44, 118.33, 122.25, 122.42, 123.27, 123,40, 143.12, (Ph-***C***), 168.84, 168.88, 169.84,

170.05, 170.49 (CH_3***C***O); Elemental analysis Calcd for [$C_{41}H_{48}N_8O_{18}S$]: C, 50.62; H, 4.97; N, 11.52; S, 3.30 Found C, 50.67; H, 5.07; N, 11.58; S, 3.36.

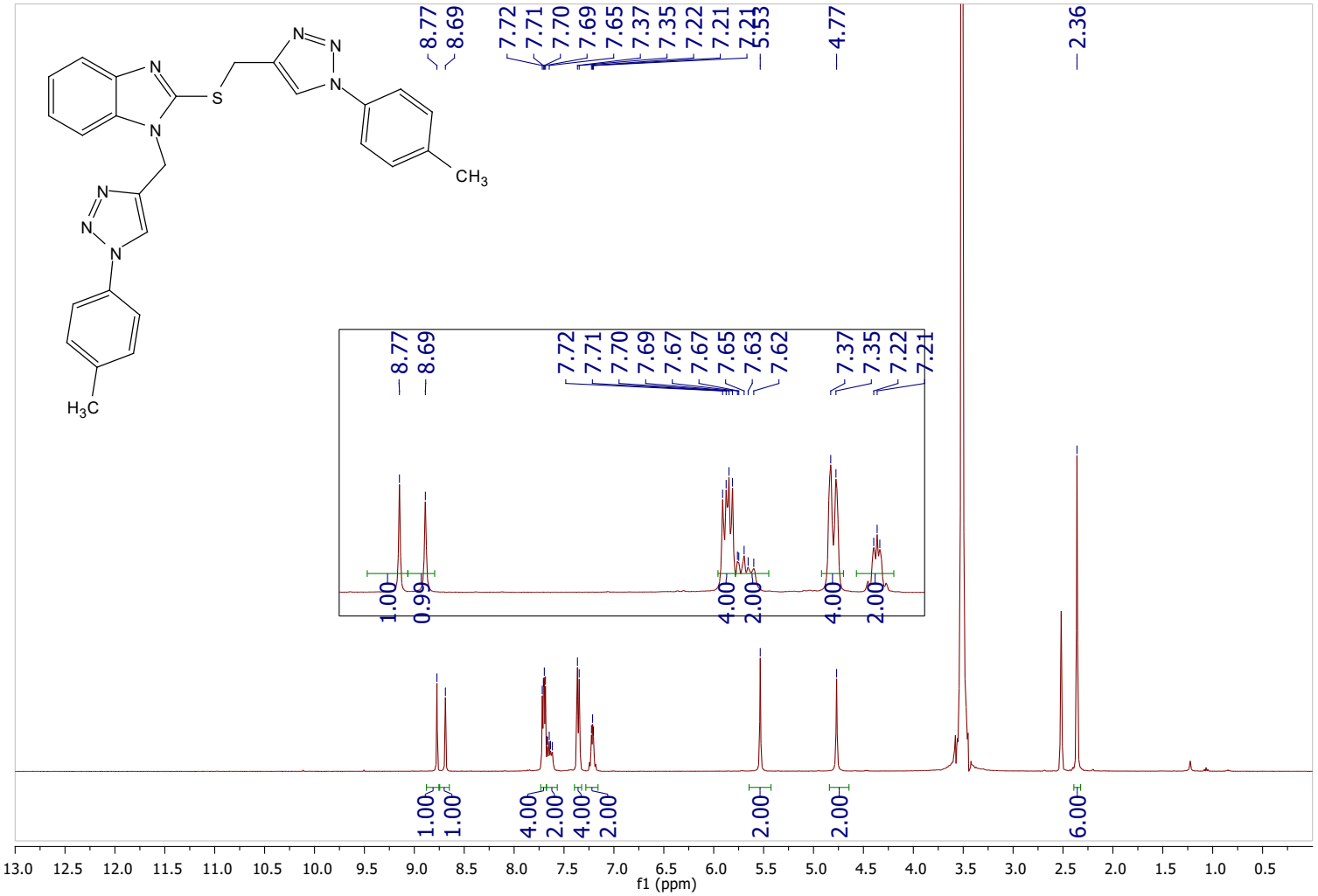

Figure S1: ^{1}H NMR for compound 10

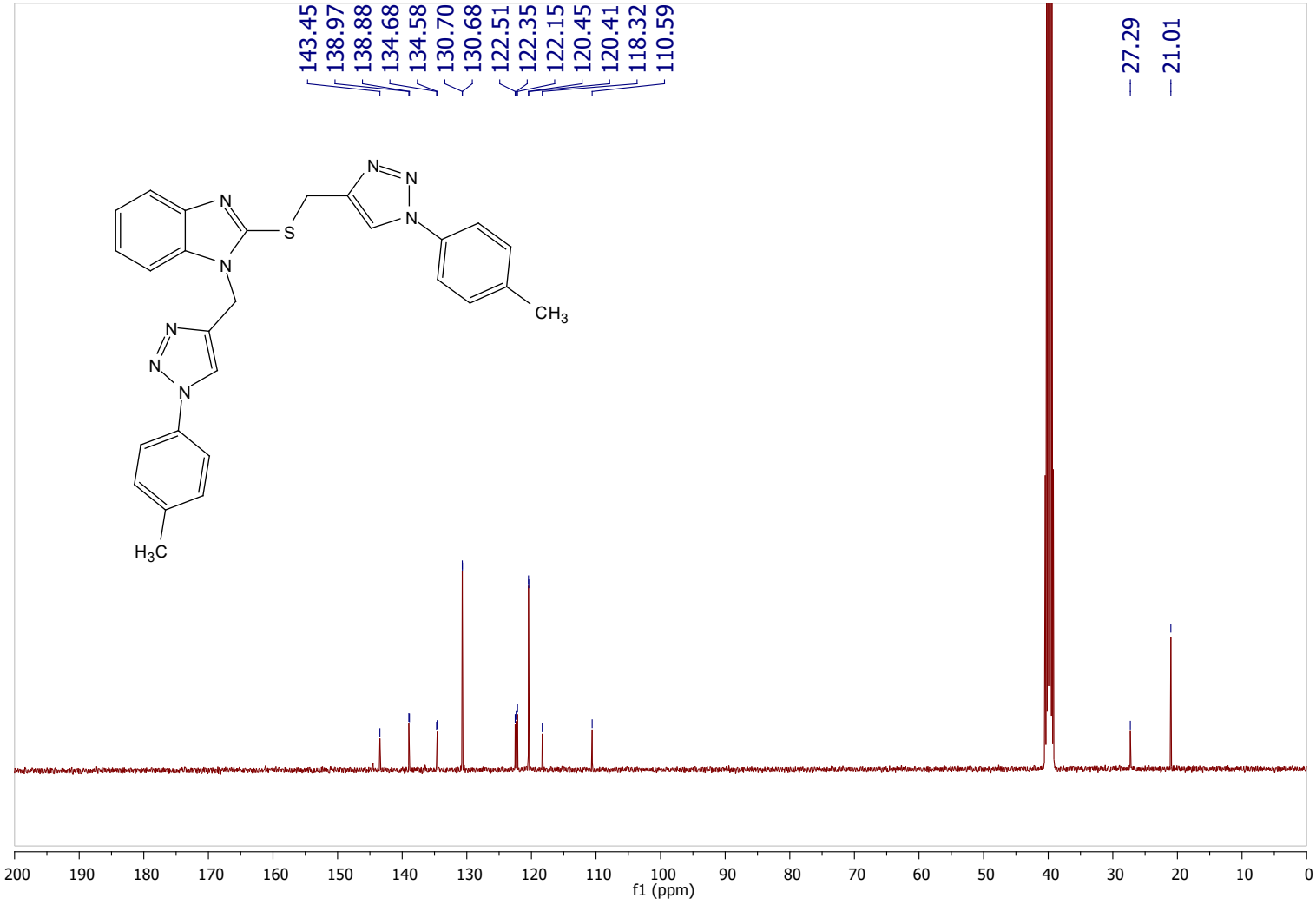

Figure S2: : ^{13}C NMR for compound **10**

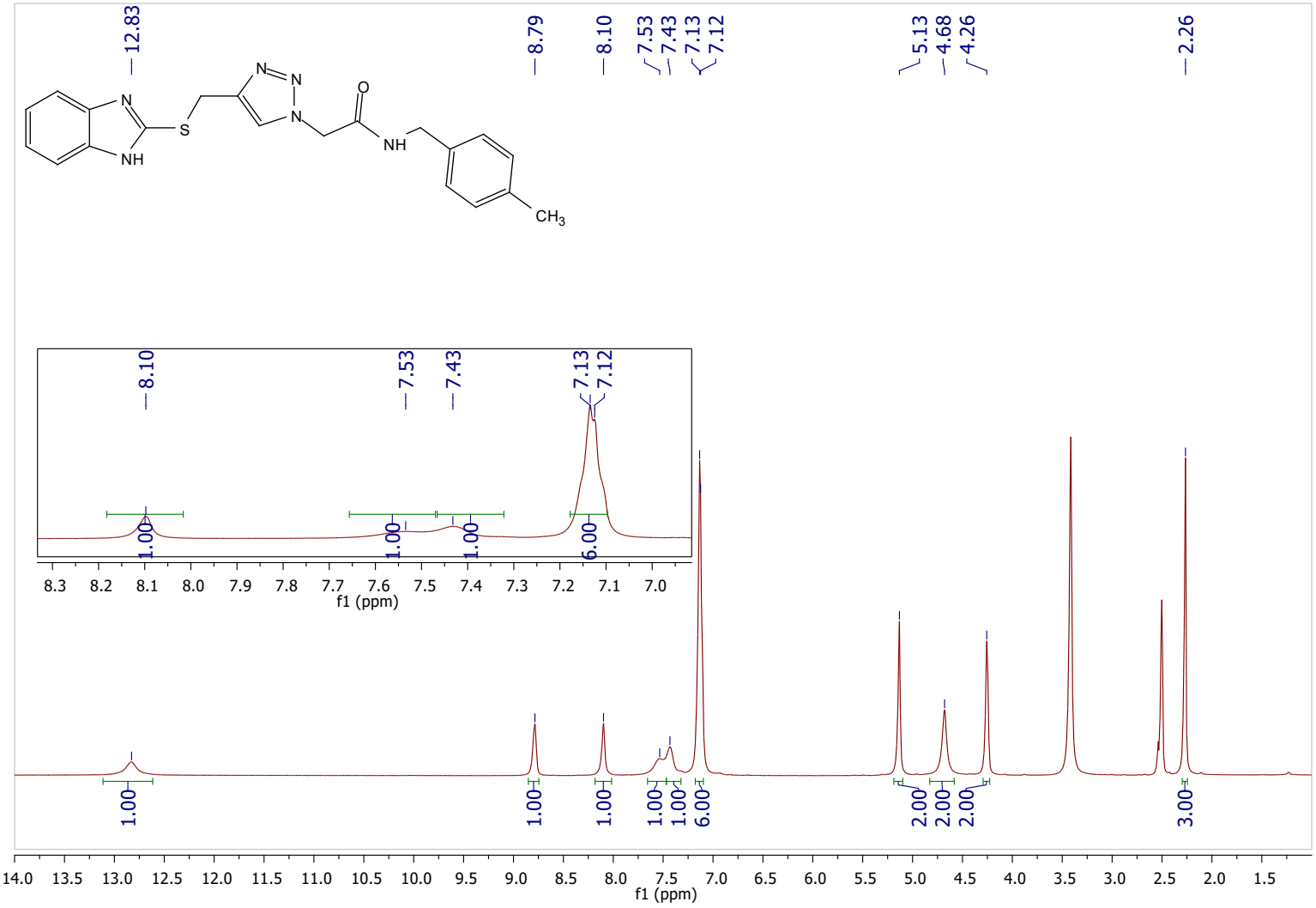

Figure S3: **:** ^{1}H NMR for compound **11**

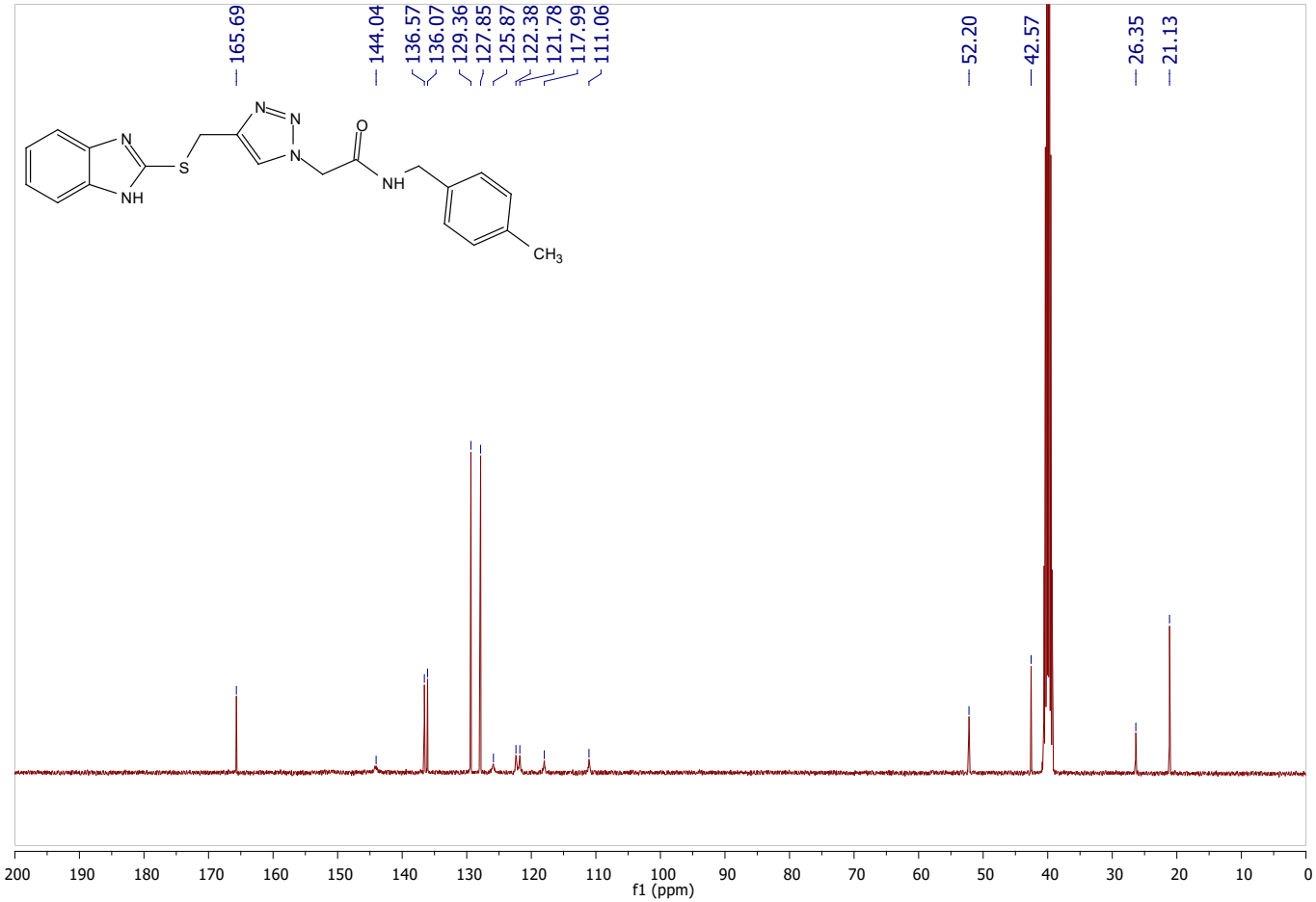

Figure S4: : ^{13}C NMR for compound **11**

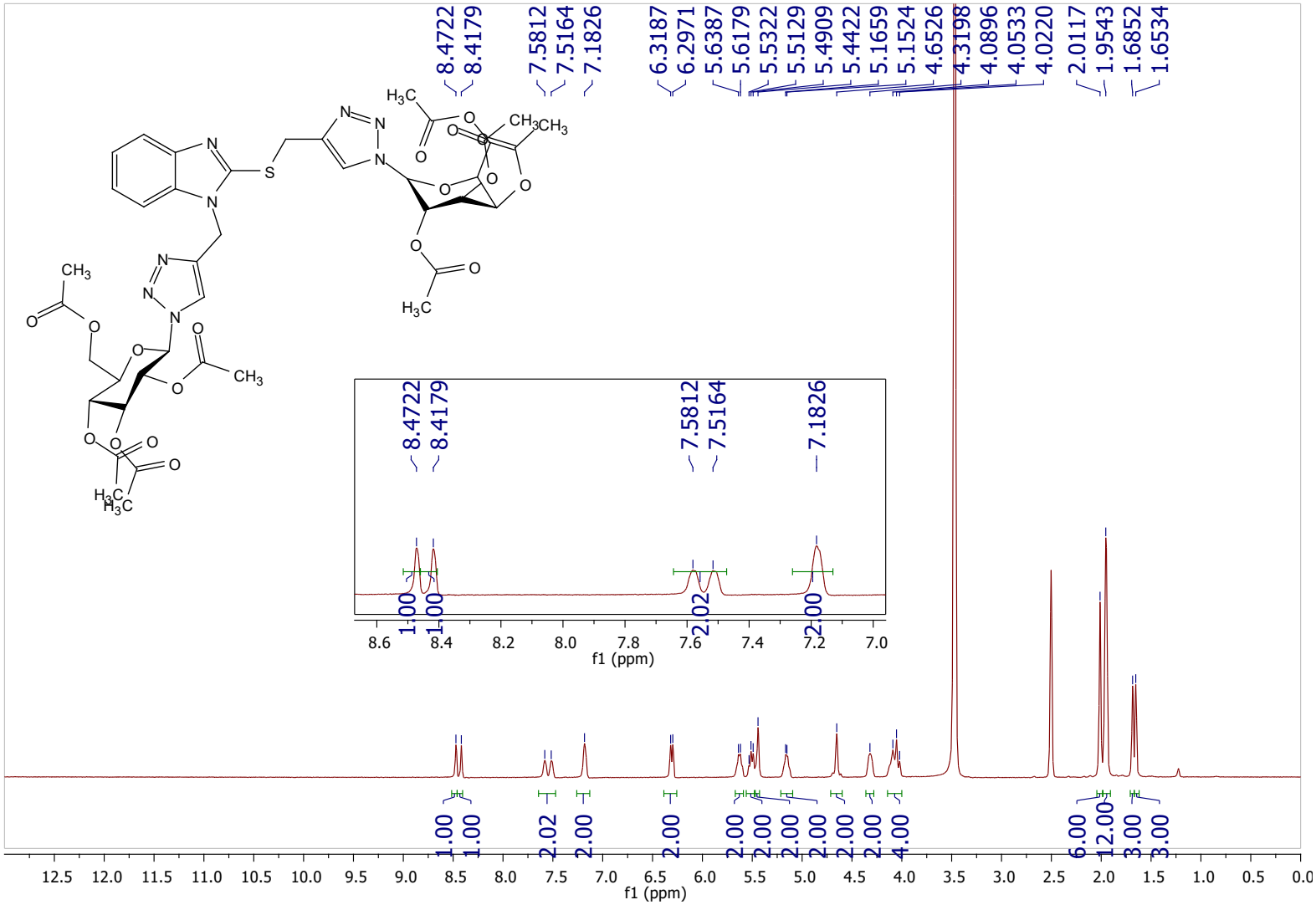

Figure S5 ^{1}H NMR for compound **12**

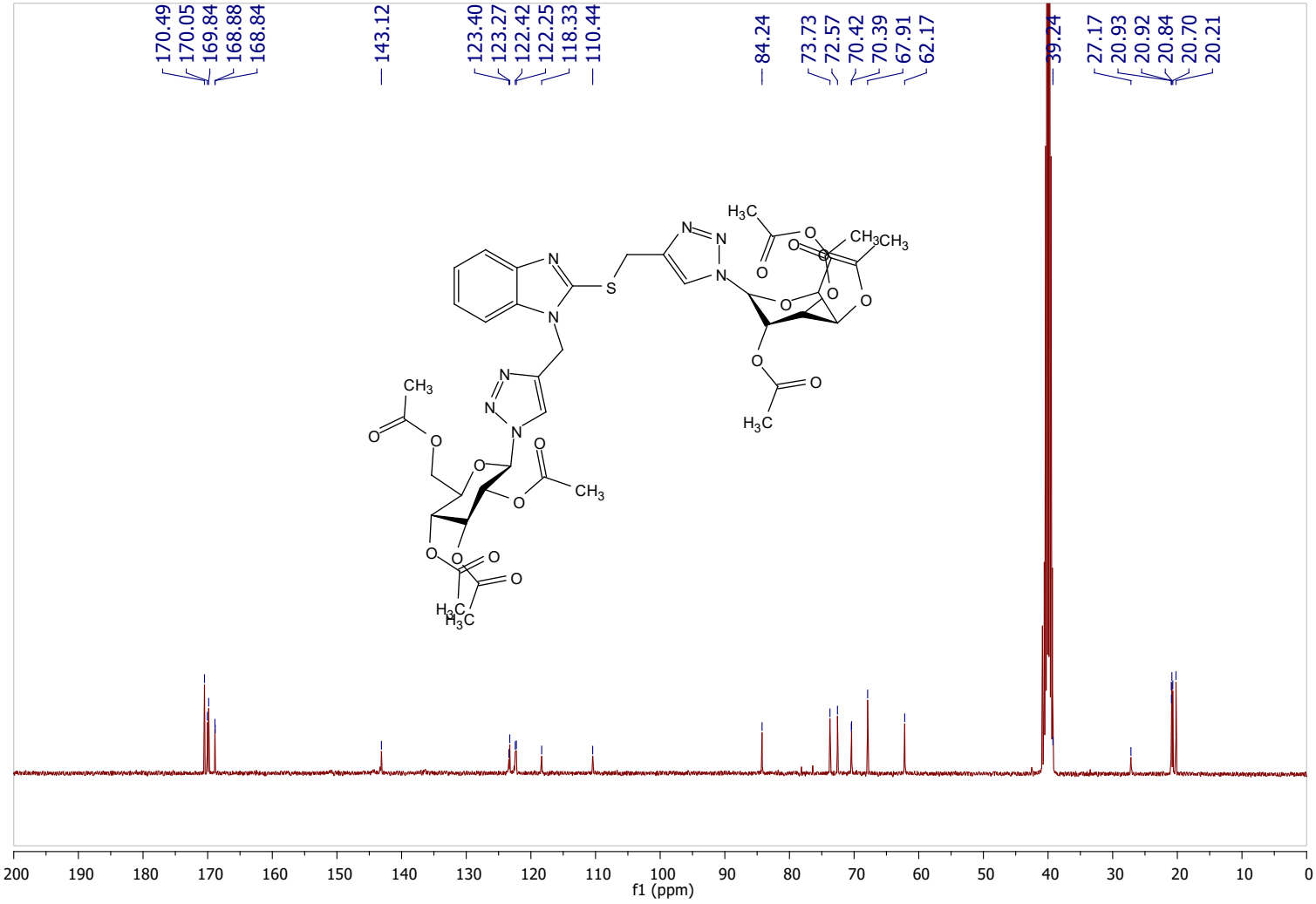

Figure S6: ^{13}C NMR for compound 12

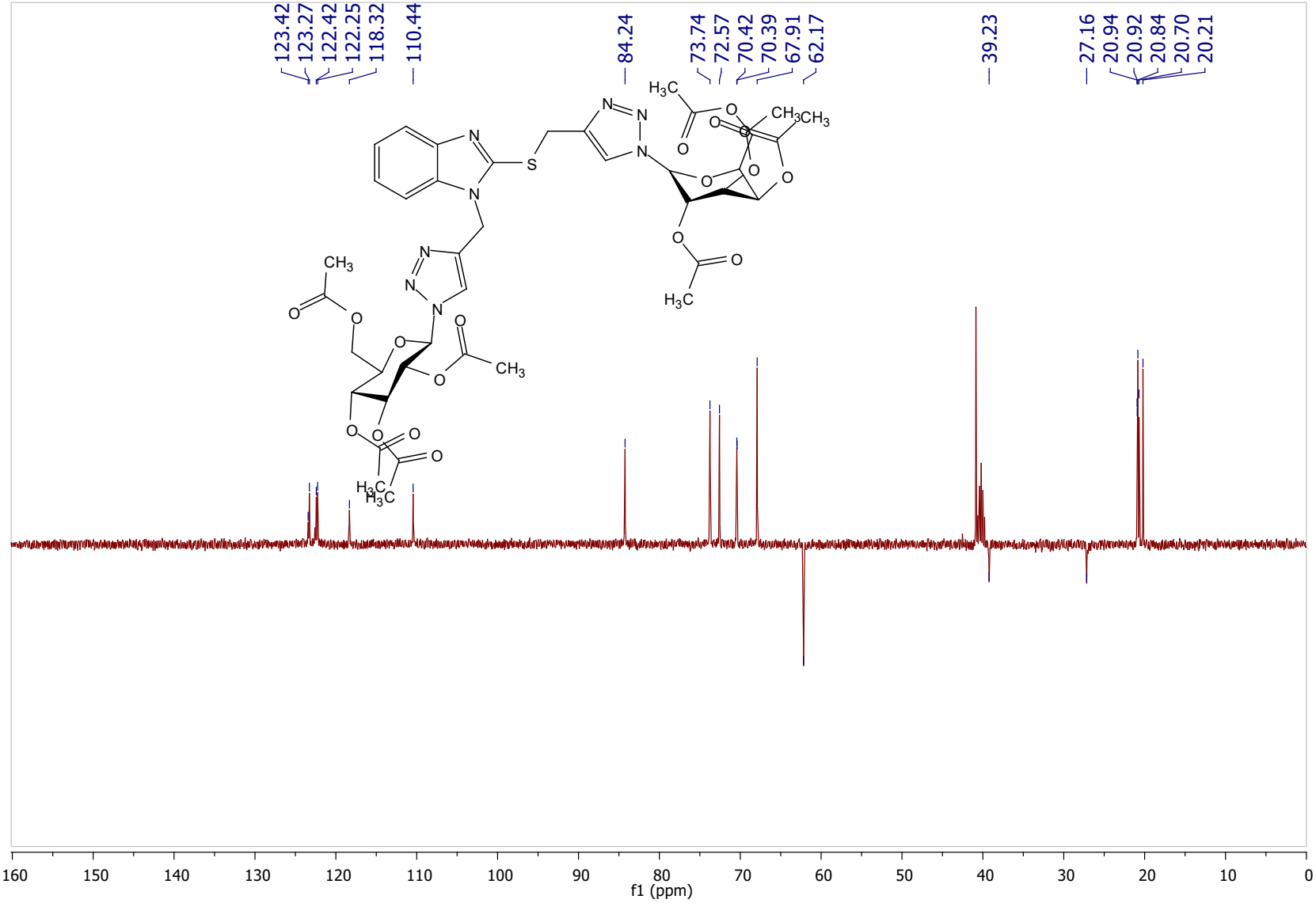

Figure S7: DEPT-^{13}C NMR for compound 12

8 Characterization of Nanogels

The physicochemical and structural properties of the synthesized drug-loaded Cs/PVP nanogel systems were comprehensively evaluated using a complementary suite of analytical techniques. UV-Visible spectrophotometric analysis was conducted on an Agilent Cary 60 spectrophotometer equipped with a xenon flash lamp and dual silicon diode detectors.

Particle size distribution, polydispersity, and surface charge characteristics were meticulously determined using a Malvern Zetasizer Nano ZS90 equipped with a 4 mW He-Ne laser (λ = 633 nm) and an avalanche photodiode detector at a fixed backscattering angle of 173°. Dynamic light scattering measurements were performed in triplicate at 25°C after a 120-second equilibration period, with each measurement consisting of 12 runs of 15-second duration. Samples were prepared by diluting the nanogel suspensions (1:40) in filtered (0.22 μm) ultrapure water, adjusted to pH 5.5, to achieve an optimal attenuator setting of 6-8 and a count rate of 200-300 kcps. A stability study of (Cs/PVP)/TRZS nanogel was conducted using freshly prepared (Cs/PVP)/TRZS nanogel suspensions, which were subjected to accelerated stability studies under physiologically relevant conditions. Aliquots (2 mL) were diluted 1:40 with sterile phosphate-buffered saline (PBS, pH 7.4) and simulated body fluid (SBF, pH 7.4), transferred into sterile Eppendorf tubes, and incubated at 37 °C in a controlled CO_2 incubator. These conditions were selected to mimic the physiological environment and evaluate the colloidal stability of the nanogels. Samples were withdrawn at predetermined intervals of 2, 5, 7, and 10 days for subsequent analysis. Samples were prepared by diluting the nanogel suspensions (1:40) in filtered (0.22 μm) ultrapure water adjusted Aliquots (2 mL) were diluted 1:40 with sterile phosphate-buffered saline (PBS, pH 7.4) and simulated body fluid (SBF, pH 7.4), transferred into sterile Eppendorf tubes, and incubated at 37 °C to

mimic in vivo conditions. The stability of the nanogels was evaluated over predetermined intervals of 2, 5, 7, and 10 days.

High-resolution morphological characterization was performed using a JEOL JEM-2100F transmission electron microscope operating at an accelerating voltage of 200 kV with a point resolution of 0.19 nm. Nanogel suspensions were diluted to 0.01% w/v and deposited onto carbon-coated copper grids (400 mesh).