Supplementary Information

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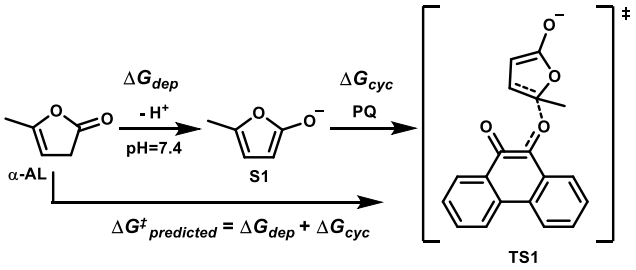
Computational details

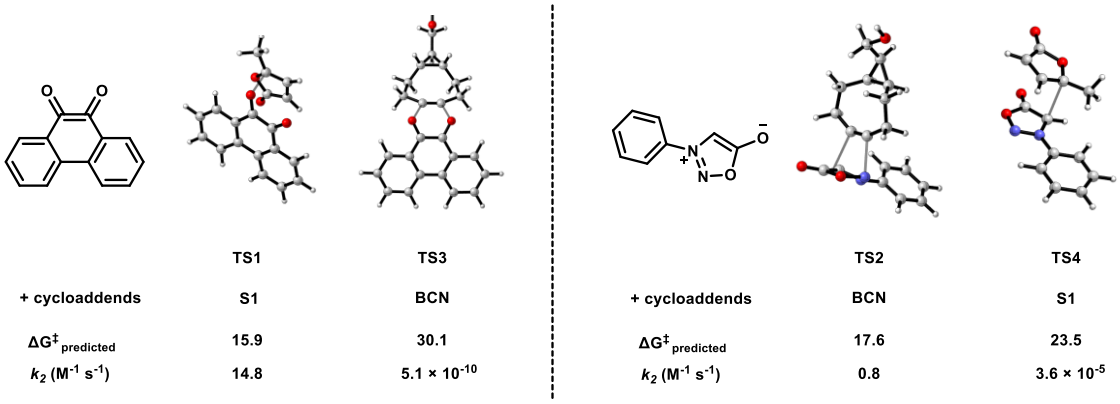
DFT calculations of activation free energy

Geometry optimizations were carried out using density functional theory (DFT) with the M06-2X[1] functional and the 6-31G(d) basis set. Frequency calculations, performed at the same level of theory, were used to confirm the nature of the stationary points as either transition states (one imaginary frequency) or minima (no imaginary frequencies). Thermal corrections to the free energies were obtained at 298.15 K and 1 atm. To improve the accuracy of electronic energies, single-point energy calculations were conducted at the M06-2X level with the 6-311+G(d,p) basis set, using the continuum polarizable continuum model (CPCM)[2] with water as the solvent. Gibbs free energies were computed by combining single-point energies in water with quasi-harmonic entropy corrections.[3] The free energy barriers for all bimolecular addition and cycloaddition reactions were empirically corrected using the formula: $\Delta G^{\ddagger}_{cyc} = (\Delta G^{\ddagger}_{calc} + 8.4)/1.6$.[4] Finally, the second-order reaction rate constants were calculated using the Eyring equation. All computations were performed using the Gaussian 09 software package.[5] The free energy changes for the deprotonation process (ΔG_{dep}) of **α-AL** was predicted based on our previous work,[6] ΔG_{dep} = 6.4 kcal/mol.



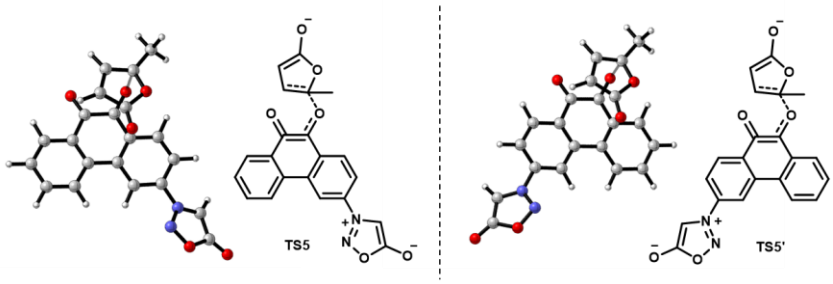
Theoretical calculation results of the cycloaddition between **PQ** with **S1**, **PQ** with **BCN**, sydnone with **BCN**, and sydnone with **S1** were shown below. The predicted second-order rate constant k_2 for the reaction between **PQ** and **BCN** was 5.1 × 10^{-10} M^{-1} s^{-1}, and the predicted k_2 for the sydnone cycloaddition with **α-AL** was 3.6 × 10^{-5} M^{-1} s^{-1}. The extremely small rate constants indicated that

PQ and sydnone should be inert to **BCN** and **α-AL** at room temperature,



	TS1	TS3		TS2	TS4
+ cycloaddends	S1	BCN	+ cycloaddends	BCN	S1
$\Delta G^{\ddagger}_{predicted}$	15.9	30.1	$\Delta G^{\ddagger}_{predicted}$	17.6	23.5
k_2 (M^{-1} s^{-1})	14.8	5.1×10^{-10}	k_2 (M^{-1} s^{-1})	0.8	3.6×10^{-5}

Considering the formation of two regioisomers in the reaction between **PQ-Syd** with **α-AL**, we performed DFT calculations on both structures shown below. The lower $\Delta G^{\ddagger}_{predicted}$ of the reaction between **PQ-Syd** with **α-AL** was 15.2 kcal/mol, the corresponding predicted reaction rate was 44.8 M^{-1} s^{-1}.



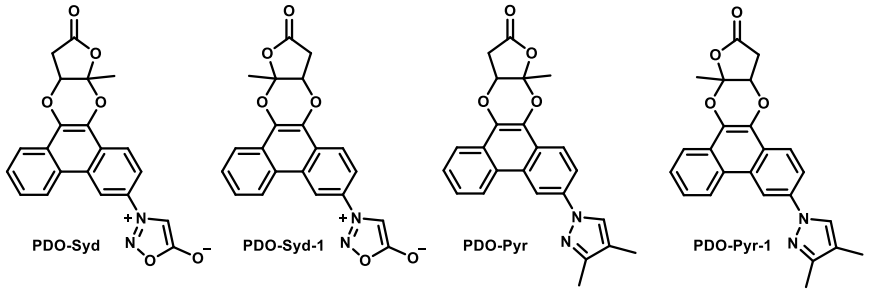
$\Delta G^{\ddagger}_{predicted}$	15.2	$\Delta G^{\ddagger}_{predicted}$	16.7
k_2 (M^{-1} s^{-1})	44.8	k_2 (M^{-1} s^{-1})	4.0

TD-DFT calculations of spectroscopic properties

Based on the stable ground state, the absorption and emission optical properties were simulated using Gaussian 16 program combined with time-dependent density functional theory (TD-DFT). The functional of B3LYP and the basis set of 6-311+g(d,p) were adopted in this study. The self-consistent reaction field (SCRF) approach with CPCM and water was applied to simulate solvent effect here. The views of frontier molecular orbitals were drawn in GaussView.

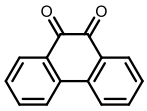
Considering the isomers of **PDO-Syd** and **PDO-Pyr**, we performed TD-DFT calculations on structures shown below. It can be seen that the predicted

optical properties of the corresponding isomers of **PDO-Syd** and **PDO-Pyr** were generally similar.



Molecules	λ_{em}/ nm	*f*	Transition characters
PDO-Syd	526.2	0.5087	$S_1 \rightarrow S_0$, L→H, 98.83%
PDO-Syd-1	529.6	0.4792	$S_1 \rightarrow S_0$, L→H, 98.91%
PDO-Pyr	405.0	0.0655	$S_1 \rightarrow S_0$, L→H, 96.12%
PDO-Pyr1	402.4	0.0628	$S_1 \rightarrow S_0$, L→H, 95.74%

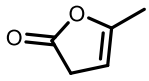
Coordinates and Energies of Computed Stationary Points



PQ

$G_{(water)}$ = -688.526373 Hartree

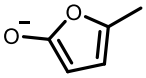
C	0.000000	3.571018	-0.753273
C	0.000000	2.859461	0.436663
C	0.000000	1.463346	0.426333
C	0.000000	0.743889	-0.784122
C	0.000000	1.482524	-1.973455
C	0.000000	2.872353	-1.959087
C	0.000000	0.770385	1.741090
C	-0.000000	-0.743889	-0.784122
C	-0.000000	-1.463346	0.426333
C	-0.000000	-0.770385	1.741090
C	-0.000000	-2.859461	0.436663
H	0.000000	-3.359837	1.399887
C	-0.000000	-3.571018	-0.753273
C	-0.000000	-2.872353	-1.959087
C	-0.000000	-1.482524	-1.973455
H	0.000000	4.655992	-0.745980
H	0.000000	3.359837	1.399887
H	0.000000	0.979741	-2.933261
H	0.000000	3.412918	-2.900722
H	-0.000000	-4.655992	-0.745980
H	-0.000000	-3.412918	-2.900722
H	-0.000000	-0.979741	-2.933261
O	0.000000	-1.358931	2.796897
O	0.000000	1.358931	2.796897



α-AL

$G_{(water)}$ = -344.476932 Hartree

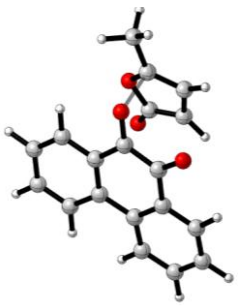
C	-1.192199	-0.176711	-0.024138
C	-0.758174	1.240305	-0.162941
C	0.536157	1.309877	0.130825
C	1.046866	-0.064955	0.467659
O	-0.099451	-0.905834	0.351972
H	1.174007	2.186470	0.128373
O	-2.273171	-0.663887	-0.192278
H	-1.439543	2.025325	-0.459906
C	2.139760	-0.558413	-0.469627
H	1.785123	-0.520718	-1.503099
H	3.032892	0.066429	-0.377694
H	2.404206	-1.589417	-0.224978
H	1.389830	-0.110931	1.509078



S1

$G_{(water)}$ = -344.005159 Hartree

C	-1.247521	0.176555	0.000000
C	-0.922434	-1.190527	0.000000
C	0.501533	-1.305736	0.000000
C	1.016347	-0.048735	0.000000
O	0.000000	0.871252	0.000000
H	1.090771	-2.217921	0.000000
O	-2.291082	0.843016	0.000000
H	-1.652876	-1.986624	0.000000
C	2.406506	0.476713	0.000000
H	2.624453	1.099923	0.881441
H	3.115267	-0.359069	0.000000
H	2.624453	1.099923	-0.881441

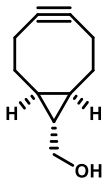


TS1 PQ with S1

$G_{(water)}$ = -1032.521508 Hartree

O	-1.831553	-0.482090	-1.395506
O	0.037815	-2.498480	-1.386142
C	-2.105966	-2.014804	0.668461

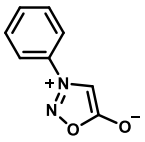
C	-1.082936	-1.492081	1.426264
C	3.844978	-1.924221	0.186076
C	2.574838	-2.118372	-0.324453
C	1.680288	-1.049210	-0.465919
C	2.069033	0.257833	-0.101288
C	3.364658	0.430096	0.423550
C	4.236795	-0.635010	0.566348
C	0.325799	-1.358631	-1.009378
C	1.132386	1.375625	-0.277590
C	-0.185720	1.105203	-0.712437
C	-0.629649	-0.247416	-1.043045
C	-1.093216	2.172405	-0.853665
H	-2.103578	1.926733	-1.163449
C	-0.712411	3.472679	-0.598313
C	0.601518	3.749791	-0.194877
C	1.500338	2.711469	-0.037869
H	4.530502	-2.760308	0.297502
H	2.218668	-3.097022	-0.631615
H	3.691591	1.414460	0.741527
H	5.227473	-0.465680	0.980924
H	-1.429695	4.281705	-0.712000
H	0.913336	4.772208	0.000564
H	2.514033	2.944933	0.272747
C	-1.378703	-0.096991	1.693113
C	-2.890066	-0.922395	0.255699
O	-2.562416	0.181931	0.985327
H	-2.166282	-3.004144	0.237620
O	-0.851074	0.753468	2.373458
H	-0.219934	-1.992944	1.841010
C	-4.226156	-0.907295	-0.395647
H	-4.328741	0.001403	-0.994286
H	-4.307751	-1.770588	-1.060098
H	-5.036641	-0.941444	0.342264



BCN

$G_{(water)}$ = -464.3545023 Hartree

C	-2.418782	0.603908	-0.474622
C	-2.419220	-0.604006	-0.474713
C	-1.853064	1.930277	-0.212649
H	-1.585646	2.436690	-1.147594
H	-2.545200	2.586350	0.325644
C	-1.853173	-1.930263	-0.212568
H	-1.585673	-2.436694	-1.147482
H	-2.545191	-2.586460	0.325733
C	-0.585477	-1.666162	0.639194
H	-0.902363	-1.227523	1.592819
H	-0.099332	-2.622607	0.872795
C	-0.585294	1.666398	0.639113
H	-0.902107	1.227905	1.592828
H	-0.099172	2.622899	0.872515
C	0.421196	0.760342	-0.046069
C	0.421077	-0.760220	-0.046041
C	1.449436	0.000000	0.742622
H	1.301730	0.000031	1.822755
H	0.801371	-1.166710	-0.982214
H	0.801528	1.166755	-0.982258
C	2.902583	-0.000124	0.334074
H	3.401737	0.888842	0.750093
H	3.401574	-0.889186	0.750083
O	2.972285	-0.000122	-1.079750
H	3.902774	-0.000215	-1.337767

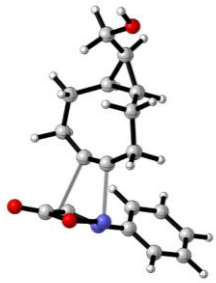


Sydnone

$G_{(water)}$ = -568.1688000 Hartree

C	-2.800583	-0.306451	-0.135121
C	-1.502818	-0.851339	-0.344139
H	-1.199572	-1.781267	-0.789166
O	-2.499772	0.980023	0.390686
N	-1.176889	1.188068	0.492336
N	-0.635213	0.097952	0.044413
O	-3.933737	-0.670607	-0.299558
C	0.796678	0.016113	0.014303
C	1.406183	-1.202606	0.287301
C	1.528619	1.158684	-0.288809
C	2.794359	-1.277128	0.245639
H	0.806193	-2.067695	0.549581
C	2.915670	1.068479	-0.317615

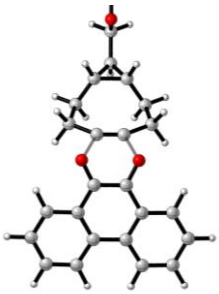
H	1.011181	2.087984	-0.497952
C	3.547895	-0.145210	-0.055907
H	3.285878	-2.220509	0.458309
H	3.502358	1.949517	-0.555037
H	4.630732	-0.208758	-0.085912



TS2 Sydnone with BCN

$G_{(water)}$ = -1032.482276 Hartree

C	3.949836	-2.949105	0.377423
C	4.233973	-2.231799	-0.783585
C	3.705174	-0.960165	-0.968521
C	2.883829	-0.423201	0.019237
C	2.573548	-1.132760	1.175346
C	3.121588	-2.399643	1.352396
H	4.372071	-3.938665	0.519659
H	4.878555	-2.659488	-1.544536
H	3.918313	-0.376949	-1.857591
H	1.891876	-0.708571	1.905206
H	2.886937	-2.962905	2.249634
C	1.861715	1.702970	0.761486
H	2.222899	1.706399	1.778015
N	2.344597	0.876567	-0.180969
N	1.750375	1.100833	-1.351956
O	1.368668	2.423972	-1.285745
C	1.332377	2.843620	0.035306
O	0.929635	3.923506	0.374006
C	-0.204944	0.387380	-0.614384
C	-0.230904	0.824643	0.545385
C	-2.531370	-1.343925	-0.175349
C	-0.995580	1.099907	1.779598
C	-2.621539	-0.763500	1.218951
C	-2.468900	0.707891	1.554809
H	-0.579450	0.535668	2.626021
H	-2.034971	-2.312562	-0.219933
H	-2.188157	-1.402304	1.988215
H	-0.920495	2.162808	2.040279
H	-3.047966	0.939288	2.458257
H	-2.860461	1.340441	0.752130
C	-0.834777	-0.300551	-1.752641
H	-0.325736	-1.256037	-1.934796
H	-0.719219	0.293685	-2.666014
C	-2.325592	-0.532452	-1.439430
H	-2.815696	0.443748	-1.362008
H	-2.805754	-1.054933	-2.274556
C	-3.846977	-1.351854	0.559688
H	-4.177193	-2.318854	0.930942
C	-4.986140	-0.497270	0.073990
H	-5.773453	-0.464301	0.842185
H	-4.659636	0.536948	-0.099861
O	-5.470978	-1.079604	-1.125647
H	-6.237770	-0.569327	-1.419917

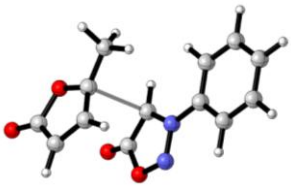


TS3 PQ with BCN

$G_{(water)}$ = -1152.818102 Hartree

C	-1.285371	0.620644	0.306594
C	-1.285277	-0.620497	0.306602
C	-1.915490	1.899089	-0.052047
H	-2.182761	2.437507	0.865345
H	-1.210882	2.535926	-0.598444
C	-1.915349	-1.898921	-0.052247
H	-2.182633	-2.437504	0.865043
H	-1.210685	-2.535631	-0.598723
C	-3.168741	-1.620015	-0.904177
H	-2.857015	-1.145930	-1.842684
H	-3.632367	-2.577082	-1.175915
C	-3.168928	1.620307	-0.903961
H	-2.857265	1.146478	-1.842617
H	-3.632618	2.577425	-1.175417
C	-4.192572	0.758314	-0.194514
C	-4.192478	-0.758234	-0.194603

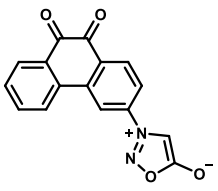
C	-5.232792	0.000022	-0.968068
H	-5.105981	0.000087	-2.050744
H	-4.553835	-1.180688	0.741510
H	-4.553996	1.180608	0.741646
C	-6.677127	-0.000067	-0.529476
H	-7.184555	0.889097	-0.934888
H	-7.184561	-0.889100	-0.935172
O	-6.716945	-0.000302	0.885373
H	-7.641626	0.000286	1.163547
C	3.491700	-3.561333	-0.196275
C	4.598532	-2.874469	-0.704010
C	3.580469	-0.738588	-0.143102
C	2.475350	-1.444914	0.377804
C	2.440734	-2.844889	0.346306
C	3.580544	0.738440	-0.143136
C	4.598819	2.874187	-0.704169
H	5.434283	3.424876	-1.125753
C	3.492085	3.561194	-0.196417
C	2.441056	2.844887	0.346222
C	2.475517	1.444910	0.377770
C	1.360665	0.724010	1.004774
C	1.360592	-0.723868	1.004786
H	3.461539	-4.645996	-0.222209
H	5.433957	-3.425268	-1.125527
H	1.571603	-3.341853	0.765844
H	3.462043	4.645859	-0.222394
H	1.571989	3.341959	0.765763
O	0.306874	-1.294282	1.377130
O	0.307045	1.294559	1.377153
C	4.640298	1.489399	-0.676413
H	5.512630	0.991056	-1.083611
C	4.640161	-1.489684	-0.676306
H	5.512569	-0.991450	-1.083475



TS4 Sydnone with S1

$G_{(water)}$ = -912.141713 Hartree

C	-1.186990	-1.396966	-0.949902
C	-0.135945	-0.419145	-1.074583
H	0.014230	0.259750	-1.896174
O	-0.767179	-2.194800	0.091136
N	0.473215	-1.829842	0.575174
N	0.848951	-0.838406	-0.263291
O	-2.213013	-1.588588	-1.557927
C	2.139486	-0.311029	-0.122871
C	2.556565	0.786304	-0.886138
C	3.024295	-0.905125	0.788011
C	3.847911	1.275096	-0.741828
H	1.870971	1.263610	-1.577077
C	4.309254	-0.396031	0.922847
H	2.684728	-1.754142	1.367513
C	4.734652	0.692117	0.162611
H	4.158695	2.127328	-1.339111
H	4.988380	-0.859060	1.633375
H	5.740178	1.084783	0.276489
C	-1.392866	1.299618	0.259213
O	-2.634185	1.371353	-0.319892
C	-3.523223	0.543441	0.402517
C	-2.755125	-0.016664	1.473928
C	-1.467125	0.505251	1.400609
H	-3.152415	-0.726982	2.182543
H	-0.631114	0.295822	2.058094
O	-4.688817	0.461228	0.054124
C	-0.461437	2.417303	-0.065027
H	0.513093	2.235864	0.400159
H	-0.313956	2.529174	-1.148406
H	-0.839115	3.383021	0.303566

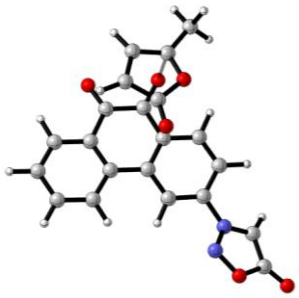


PQ-Syd

$G_{(water)}$ = -1024.559148 Hartree

C	-1.410465	-2.006214	-0.037991
C	-1.681476	-0.642490	0.018439
C	0.660984	-0.064062	0.011890
C	0.941562	-1.441751	-0.049526
C	-0.081043	-2.391863	-0.068281
C	1.757015	0.939727	0.044983
C	2.529786	3.240563	0.142729

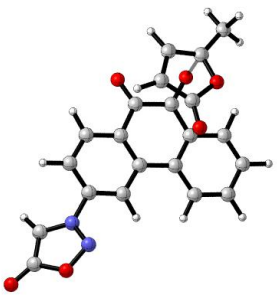
H	2.292604	4.298513	0.194120
C	3.858702	2.823085	0.110540
C	4.139484	1.466382	0.044588
C	3.104262	0.530979	0.011984
C	3.480077	-0.904759	-0.060471
C	2.343405	-1.943697	-0.096404
H	-2.222810	-2.723064	-0.061643
H	0.197915	-3.439341	-0.116425
H	4.663758	3.549903	0.137032
H	5.159525	1.096499	0.016955
O	2.611202	-3.119113	-0.159940
O	4.624919	-1.288310	-0.092387
C	1.495549	2.312518	0.110011
H	0.477225	2.682127	0.136535
C	-0.683562	0.319466	0.050001
H	-0.973300	1.359769	0.130362
C	-3.548638	0.903222	-0.524783
C	-4.939274	0.903853	-0.222746
H	-2.970232	1.563039	-1.145168
N	-3.907029	-0.931922	0.690157
N	-3.046398	-0.205645	0.045219
O	-5.880418	1.610151	-0.459805
O	-5.074316	-0.289506	0.543485



TS5 PQ-Syd with S1

$G_{(water)}$ = -1368.562796 Hartree

C	-1.695657	-1.607317	-0.860826
C	-2.302983	-0.406382	-0.470410
C	-0.183925	0.746126	-0.440807
C	0.446300	-0.455087	-0.847645
C	-0.333600	-1.613797	-1.043942
C	0.630903	1.949033	-0.226078
C	0.871210	4.280039	0.431496
H	0.421405	5.193352	0.811194
C	2.236645	4.242718	0.125414
C	2.792546	3.067079	-0.343834
C	2.010279	1.917795	-0.516391
C	2.701540	0.684832	-0.997938
C	1.885008	-0.540650	-1.060288
H	-2.299765	-2.497165	-0.998121
H	0.188955	-2.520086	-1.328763
H	2.851522	5.128324	0.260943
H	3.846613	2.985717	-0.591022
O	2.446063	-1.650129	-1.302953
O	3.893399	0.700736	-1.301717
C	2.916463	-2.473326	0.551342
O	1.698348	-2.297942	1.125379
C	1.665332	-1.011577	1.706148
C	2.996066	-0.469907	1.524445
C	3.778943	-1.431625	0.925915
H	4.800581	-1.333784	0.587379
O	0.656672	-0.596154	2.232002
C	3.224073	-3.833227	0.039428
H	4.149592	-3.796392	-0.539274
H	3.338856	-4.554815	0.857021
H	2.418120	-4.169186	-0.617341
H	3.277312	0.514361	1.870052
C	0.087233	3.153354	0.257527
H	-0.964857	3.209399	0.517061
C	-1.573378	0.748794	-0.267015
H	-2.099634	1.658493	-0.000397
N	-3.721914	-0.372390	-0.271814
C	-4.382696	0.348516	0.652856
C	-5.758410	0.053816	0.477311
H	-3.878816	0.927270	1.404681
N	-4.479055	-1.092534	-1.038848
O	-5.736753	-0.862711	-0.598651
O	-6.797035	0.382919	1.001062

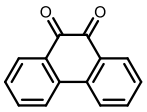


TS5' PQ-Syd with S1

$G_{(water)}$ = -1368.559887 Hartree

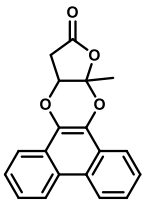
C	-2.463451	3.426819	-0.657662
C	-1.118874	3.764343	-0.438271
C	-0.507008	1.416588	-0.573133
C	-1.857721	1.082812	-0.816407
C	-2.821191	2.109255	-0.842847
C	0.484915	0.340197	-0.516151
C	2.737025	-0.463437	-0.177645
C	2.369366	-1.783398	-0.459351
C	1.048237	-2.024514	-0.775140
C	0.102037	-0.990709	-0.804592
C	-1.298113	-1.366750	-1.153639
C	-2.293679	-0.294115	-1.050378
H	-3.221510	4.205447	-0.681879
H	-3.852760	1.817483	-1.009146
H	3.097754	-2.585572	-0.398003
H	0.688709	-3.022754	-1.003711
O	-3.522729	-0.582100	-1.175061
O	-1.578661	-2.521509	-1.484643
C	-4.226073	-0.995629	0.714951
O	-3.740059	0.113352	1.332358
C	-2.429779	-0.167096	1.772861
C	-2.208150	-1.567284	1.467138
C	-3.369074	-2.086023	0.940536
H	-3.527253	-3.078693	0.543841
O	-1.758301	0.686965	2.303571
C	-5.666116	-0.974573	0.350707
H	-5.889879	-1.841119	-0.275328
H	-6.305096	-0.998334	1.241435
H	-5.886396	-0.068523	-0.218673
H	-1.283243	-2.074780	1.700113
C	1.832959	0.581006	-0.200399
H	2.191041	1.576496	0.031227
C	-0.163157	2.769329	-0.395454
H	0.870662	3.048496	-0.218123
H	-0.829035	4.801144	-0.294530
N	4.113524	-0.185370	0.130369
C	5.184665	-0.810661	-0.392438
C	6.324190	-0.195873	0.185540
H	5.103847	-1.566862	-1.150881
O	5.742333	0.776481	1.033226
O	7.522750	-0.319574	0.099769
N	4.394252	0.747787	0.982566

TD-DFT



PQ

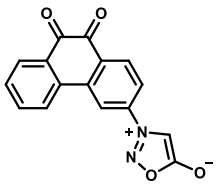
C	-3.562419	-0.740962	-0.000140
C	-2.853492	0.442840	-0.000021
C	-1.437450	0.430326	0.000176
C	-0.730522	-0.795576	0.000139
C	-1.486943	-1.982737	0.000005
C	-2.872715	-1.961785	-0.000129
C	-0.777328	1.729376	0.000383
C	0.730522	-0.795576	0.000099
C	1.437450	0.430326	0.000078
C	0.777327	1.729376	0.000142
C	2.853492	0.442841	0.000046
H	3.365355	1.396316	0.000051
C	3.562419	-0.740961	-0.000018
C	2.872716	-1.961784	-0.000013
C	1.486943	-1.982737	0.000033
H	-4.645886	-0.722902	-0.000202
H	-3.365355	1.396316	-0.000021
H	-0.986852	-2.941225	-0.000056
H	-3.421347	-2.896134	-0.000237
H	4.645886	-0.722902	-0.000110
H	0.986852	-2.941225	0.000042
O	-1.373977	2.804380	0.000283
O	1.373976	2.804380	-0.000795
H	3.421348	-2.896134	-0.000064



PDO

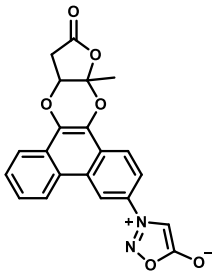
C	-1.586999	3.780343	-0.135110
C	-0.558038	2.835465	0.036513
C	-0.843415	1.466401	0.047128

C	-2.189011	0.965713	-0.109872
C	-3.207236	1.955723	-0.273998
C	-2.899443	3.315143	-0.286696
C	-2.462507	-0.438516	-0.089054
C	-1.400041	-1.402120	0.075122
C	-1.645560	-2.778718	0.088211
H	-0.814348	-3.461017	0.210460
C	-2.956178	-3.273799	-0.052864
C	-4.000786	-2.355449	-0.214070
C	-3.777737	-0.979342	-0.236206
H	-1.366708	4.839790	-0.146763
H	0.466622	3.160648	0.161674
H	-4.240176	1.658225	-0.389516
H	-3.706592	4.029251	-0.416719
H	-3.147914	-4.338778	-0.039057
H	-4.623954	-0.320139	-0.370433
O	1.440210	0.959785	0.403114
O	0.921207	-1.777883	0.336012
C	0.192302	0.498432	0.229864
C	-0.072702	-0.888289	0.208192
H	-5.017427	-2.718759	-0.327345
C	2.276198	-1.333302	0.239574
C	2.812938	-1.192845	-1.184698
C	2.500260	0.066474	0.835270
H	2.851410	-2.063929	0.804531
H	2.041366	-0.856486	-1.883313
H	3.264605	-2.098970	-1.582612
C	2.669515	0.173373	2.330105
H	2.832172	1.215434	2.606890
H	1.775253	-0.193630	2.836477
H	3.525845	-0.423215	2.646856
O	3.658477	0.546408	0.169717
C	3.845437	-0.095122	-1.036524
O	4.727338	0.228463	-1.775899



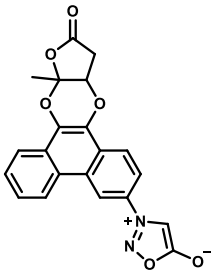
PQ-Syd

C	1.971890	-1.860974	0.219400
C	0.607958	-1.698868	0.304134
C	0.021045	-0.414652	0.229569
C	0.842345	0.732325	0.068785
C	2.228395	0.553211	-0.018088
C	2.798675	-0.728517	0.056185
C	0.214264	2.058897	0.002719
C	-1.209824	2.178410	0.073848
C	-1.825723	3.455990	-0.006601
H	-2.904205	3.520445	0.043619
C	-1.060545	4.594434	-0.144760
C	0.335285	4.484014	-0.208130
C	0.954230	3.236980	-0.137256
H	2.412960	-2.845213	0.277825
H	-0.027245	-2.565200	0.429797
H	2.871580	1.408403	-0.146240
H	-1.534568	5.566094	-0.204340
H	2.033256	3.200960	-0.193693
O	-2.125867	-1.355763	0.510410
O	-3.337865	1.155017	0.301219
C	-1.398865	-0.253674	0.324797
C	-2.001274	1.014632	0.222110
H	0.944760	5.373072	-0.315245
C	-4.147768	-0.007960	0.124898
C	-4.361501	-0.445753	-1.324501
C	-3.558223	-1.267478	0.787102
H	-5.093782	0.235845	0.603651
H	-3.483476	-0.251553	-1.947584
H	-5.226915	0.008254	-1.802475
C	-3.779843	-1.452156	2.266520
H	-3.316023	-2.382909	2.594398
H	-3.341168	-0.620246	2.819808
H	-4.850529	-1.489463	2.471547
O	-4.121409	-2.344048	0.060028
C	-4.541653	-1.942875	-1.193105
O	-4.980529	-2.732395	-1.974966
N	4.180089	-0.898538	-0.029116
C	5.151374	0.078641	-0.167007
C	6.385131	-0.559841	-0.206416
H	4.937680	1.127493	-0.222215
N	4.703661	-2.156862	0.024013
O	6.082903	-1.942925	-0.086414
O	7.562668	-0.208249	-0.312571



PDO-Syd

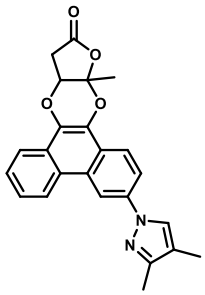
C	1.971890	-1.860974	0.219400
C	0.607958	-1.698868	0.304134
C	0.021045	-0.414652	0.229569
C	0.842345	0.732325	0.068785
C	2.228395	0.553211	-0.018088
C	2.798675	-0.728517	0.056185
C	0.214264	2.058897	0.002719
C	-1.209824	2.178410	0.073848
C	-1.825723	3.455990	-0.006601
H	-2.904205	3.520445	0.043619
C	-1.060545	4.594434	-0.144760
C	0.335285	4.484014	-0.208130
C	0.954230	3.236980	-0.137256
H	2.412960	-2.845213	0.277825
H	-0.027245	-2.565200	0.429797
H	2.871580	1.408403	-0.146240
H	-1.534568	5.566094	-0.204340
H	2.033256	3.200960	-0.193693
O	-2.125867	-1.355763	0.510410
O	-3.337865	1.155017	0.301219
C	-1.398865	-0.253674	0.324797
C	-2.001274	1.014632	0.222110
H	0.944760	5.373072	-0.315245
C	-4.147768	-0.007960	0.124898
C	-4.361501	-0.445753	-1.324501
C	-3.558223	-1.267478	0.787102
H	-5.093782	0.235845	0.603651
H	-3.483476	-0.251553	-1.947584
H	-5.226915	0.008254	-1.802475
C	-3.779843	-1.452156	2.266520
H	-3.316023	-2.382909	2.594398
H	-3.341168	-0.620246	2.819808
H	-4.850529	-1.489463	2.471547
O	-4.121409	-2.344048	0.060028
C	-4.541653	-1.942875	-1.193105
O	-4.980529	-2.732395	-1.974966
N	4.180089	-0.898538	-0.029116
C	5.151374	0.078641	-0.167007
C	6.385131	-0.559841	-0.206416
H	4.937680	1.127493	-0.222215
N	4.703661	-2.156862	0.024013
O	6.082903	-1.942925	-0.086414
O	7.562668	-0.208249	-0.312571



PDO-Syd-1

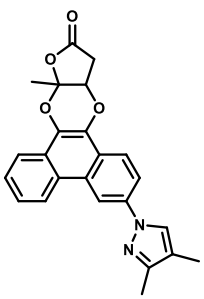
C	-2.145838	-1.849403	-0.382576
C	-0.776719	-1.758483	-0.489438
C	-0.117635	-0.514931	-0.359315
C	-0.868946	0.663918	-0.114129
C	-2.260918	0.557423	-0.004201
C	-2.904463	-0.684631	-0.137027
C	-0.164769	1.946936	0.014346
C	1.262811	1.992053	-0.094673
C	1.952623	3.228206	0.040666
H	3.031042	3.236410	-0.040175
C	1.257506	4.395134	0.271667
C	-0.140406	4.357026	0.375120
C	-0.831357	3.152650	0.249379
H	-2.642343	-2.803170	-0.485331
H	-0.194275	-2.650340	-0.677218
H	-2.851929	1.438276	0.186483
H	1.786774	5.334114	0.374100
H	-1.908441	3.173184	0.339157
O	1.967818	-1.550846	-0.726170
O	3.321373	0.876718	-0.477165
C	1.307837	-0.426186	-0.480439
C	1.978056	0.799627	-0.337886
H	-0.695035	5.269655	0.556389
C	4.098820	-0.300560	-0.397068
C	3.403810	-1.560667	-0.946406
C	4.265257	-1.945312	1.239384

O	4.470696	-2.403930	2.326896
N	-4.290509	-0.783238	-0.029128
C	-5.202140	0.235582	0.191695
C	-6.468608	-0.335394	0.224266
H	-4.928477	1.265573	0.305342
N	-4.885797	-2.005169	-0.144822
O	-6.248175	-1.722625	0.013698
O	-7.621898	0.073377	0.381976
H	3.556881	-1.690703	-2.015157
C	3.967286	-2.662985	-0.064237
H	3.275716	-3.491277	0.079137
H	4.899876	-3.063279	-0.469609
O	4.288979	-0.596179	1.011968
C	5.425310	0.011144	-1.056135
H	5.264156	0.286361	-2.098963
H	5.891850	0.844865	-0.530312
H	6.092893	-0.849833	-1.013774



PDO-Pyr

C	-1.601462	4.639255	-0.042898
C	-2.307464	3.426571	0.058666
C	-1.626540	2.207301	0.094588
C	-0.187467	2.139674	0.023193
C	0.495290	3.391662	-0.086400
C	-0.203262	4.595111	-0.115151
C	0.487193	0.878564	0.054949
C	-0.254449	-0.358232	0.169411
C	0.404082	-1.595460	0.212239
H	-0.183815	-2.499742	0.297176
C	1.798688	-1.683583	0.141609
C	2.536333	-0.488549	0.034087
C	1.903327	0.765062	0.000086
H	-2.130063	5.583445	-0.067912
H	-3.388557	3.428101	0.110846
H	1.574264	3.420176	-0.153540
H	0.357076	5.521083	-0.197899
H	2.304928	-2.635240	0.162842
H	2.503723	1.659249	-0.043212
N	3.937555	-0.553263	-0.036285
C	4.830171	0.458249	-0.309992
C	6.090593	-0.076730	-0.274709
H	4.510617	1.463812	-0.522220
N	4.592155	-1.735643	0.172708
C	5.880380	-1.461924	0.035182
C	7.397209	0.612520	-0.510650
H	7.937748	0.164472	-1.350234
H	7.248378	1.670547	-0.732247
H	8.048560	0.539994	0.366007
C	6.920812	-2.520425	0.200196
H	7.618108	-2.262601	1.003170
H	6.457246	-3.478706	0.436296
H	7.511438	-2.634598	-0.714046
O	-3.676163	1.031165	0.235841
O	-2.358901	-1.427896	0.362183
C	-2.335720	0.965335	0.188296
C	-1.669055	-0.269355	0.250242
C	-4.438031	-0.172273	0.127157
C	-4.711424	-0.641738	-1.301260
C	-3.746496	-1.390915	0.762462
H	-5.367227	0.034498	0.654621
H	-3.868444	-0.435066	-1.967067
H	-5.612110	-0.223639	-1.745708
O	-4.335198	-2.503756	0.099157
C	-3.858800	-1.554161	2.258111
H	-4.908821	-1.612225	2.547230
H	-3.346850	-2.466291	2.566484
H	-3.399594	-0.702090	2.762610
C	-4.838174	-2.141287	-1.129951
O	-5.300862	-2.957122	-1.872798



PDO-Pyr-1

C	1.700132	4.466007	0.149443
C	2.356678	3.234183	-0.014483
C	1.628439	2.043200	-0.092764
C	0.186998	2.028473	-0.005630
C	-0.443767	3.299356	0.166231
C	0.301409	4.472543	0.238223
C	-0.537496	0.797430	-0.084284
C	0.154291	-0.460382	-0.256949
C	-0.550628	-1.668949	-0.342488
H	0.001473	-2.590693	-0.469525
C	-1.948097	-1.706419	-0.261274
C	-2.637378	-0.490615	-0.096375
C	-1.956987	0.736472	-0.018821
H	2.265044	5.387336	0.208434
H	3.436162	3.196808	-0.082638
H	-1.519988	3.366675	0.247955
H	-0.221279	5.414886	0.368194
H	-2.490723	-2.636554	-0.313855
H	-2.523573	1.650042	0.062667
N	-4.039598	-0.504032	-0.010298
C	-4.884291	0.518734	0.356164
C	-6.166821	0.040266	0.301877
H	-4.517877	1.489014	0.643890
N	-4.746009	-1.638775	-0.298370
C	-6.019758	-1.323641	-0.118285
C	-7.439166	0.761826	0.616159
H	-7.982986	0.273691	1.430952
H	-7.242147	1.793110	0.913502
H	-8.108639	0.782696	-0.249528
C	-7.106132	-2.322139	-0.348978
H	-7.797668	-1.977099	-1.123787
H	-6.685971	-3.279297	-0.659263
H	-7.694373	-2.477845	0.560615
O	3.630211	0.801787	-0.315213
O	2.219497	-1.594087	-0.484925
C	2.282720	0.782077	-0.251153
C	1.571521	-0.421848	-0.348932
C	4.377641	-0.397616	-0.380127
C	3.595073	-1.608158	-0.917287
C	4.676046	-2.134554	1.129637
O	4.975665	-2.662190	2.163944
O	4.698152	-0.774923	0.985563
C	4.249015	-2.766474	-0.182604
H	3.573220	-3.605011	-0.024599
H	5.135477	-3.132634	-0.706033
C	5.644540	-0.076381	-1.145268
H	5.394069	0.245094	-2.156896
H	6.171753	0.728774	-0.632166
H	6.298945	-0.946734	-1.199798
H	3.616234	-1.667655	-2.004577

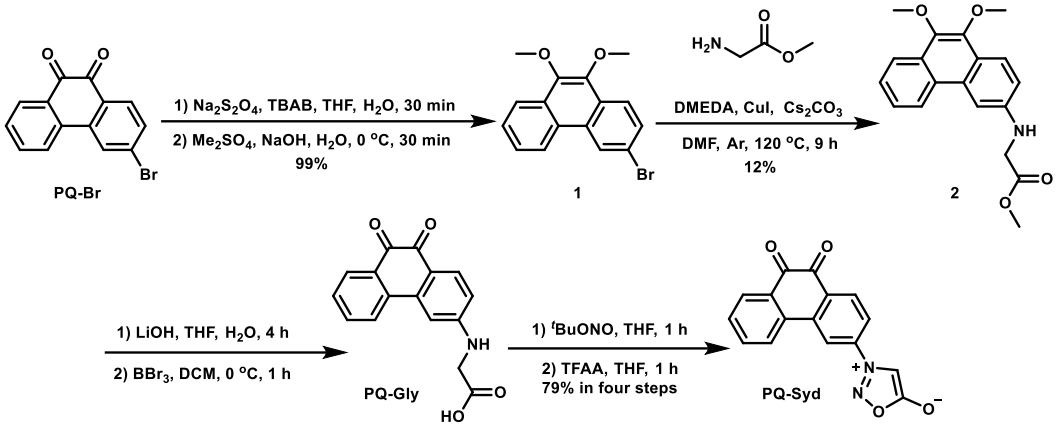
Materials and methods

1. General information

Unless otherwise specified, all reagents were utilized in their as-supplied form from commercial sources without undergoing additional purification. All NMR spectra were recorded on Bruker Avance III HD 500 (500 MHz for ^{1}H and at 125 MHz for ^{13}C) and Bruker Avance III 400 (400 MHz for ^{1}H and at 100 MHz for ^{13}C). Chemical shifts are reported in δ (ppm) relative to the solvent residual peak. Coupling constants are reported in Hz with multiplicities denoted as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). High resolution mass spectrometry (HRMS) analyses were performed on a Thermo Fisher Q Exactive mass spectrometer, and measured values are reported to 4 decimal places. SHIMADZU Prominence LC-20A was employed for high-performance liquid chromatography (HPLC) analysis. Centrifugal separation was conducted using himac CT 15RE centrifuge. Fluorescence spectra were measured using HORIBA Jobin Yvon Fluoromax-4 and PerKinElmer LS 55 fluorescence spectrometer. Absorption spectra were performed on Agilent Technologies Cary Series UV-Vis Spectrophotometer. Light source: a hand-held UV lamp (Taobao, China).

2. Synthetic procedures

Synthesis of 3-(9,10-dioxo-9,10-dihydrophenanthren-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (PQ-Syd)



3-bromo-9,10-dimethoxyphenanthrene (1)

Compound **PQ-Br** (4.45 g, 15.5 mmol, 1.0 eq.), $Na_2S_2O_4$ (11.07 g, 63.6 mmol, 4.0 eq.) and TBAB (2.46 g, 7.6 mmol, 0.4 eq.) were dissolved in H_2O (100 mL) and THF (100 mL). After the mixture stirred for 30 min, NaOH (14.89 g, 372.3 mmol, 24.0 eq.) and Me_2SO_4 (13.68 g, 108.5 mmol, 7.0 eq.) were added and the resulting solution was stirred for 30 min at 0 °C. The reaction was extracted three times with EtOAc, dried through Na_2SO_4 and concentrated. Then the solvent was removed by vacuum and the residue purified by flash column chromatography (PE:EA = 30:1). White solid **1** was then obtained (4.88 g, 99%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.76 (d, *J* = 1.91 Hz, 1H), 8.59 - 8.50 (m, 1H), 8.27 - 8.21 (m, 1H), 8.10 (d, *J* = 8.70 Hz, 1H), 7.70 (dd, *J* = 8.73, 1.90 Hz, 1H), 7.68 - 7.58 (m, 2H), 4.09 (s, 3H), 4.08 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 144.3, 143.8, 130.2, 130.1, 129.7, 127.9, 127.7, 127.6, 126.3, 125.6, 124.1, 122.8, 122.4, 120.3, 61.2, 61.1. HRMS (APCI) calcd. for $[C_{16}H_{14}BrO_2]^+$ m/z: 317.0172, $[M+H]^+$; found 317.0160.

Methyl (9,10-dimethoxyphenanthren-3-yl) glycinate (2)

Under argon atmosphere, compound **1** (4.88 g, 15.4 mmol, 1.0 eq.), CuI (1.20 g, 6.3 mmol, 0.4 eq.), glycinemethylester hydrochloride (5.81 g, 46.2 mmol, 3.0 eq.), Cs_2CO_3 (15.2 g, 46.2 mmol, 3.0 eq.) and DMEDA (1.3 mL, 12.3 mmol, 0.8 eq.) were dissolved in dry DMF (80 mL). The solution was heated to 120 °C

and stirred for 9 h, then it was quenched by pouring into the solution of saturated aqueous NaCl. The quenched reaction was extracted three times with EtOAc, dried through Na_2SO_4 and concentrated. Silica gel chromatography (PE:EA = 8:1) yielded compound **2** as a white solid (0.63g, 12%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 - 8.57 (m, 1H), 8.15 - 7.98 (m, 1H), 7.90 (d, *J* = 8.86 Hz, 1H), 7.68 (d, *J* = 2.28 Hz, 1H), 7.64 - 7.52 (m, 2H), 7.11 (dd, *J* = 8.87, 2.20 Hz, 1H), 6.38 (t, *J* = 6.41 Hz, 1H), 4.18 (d, *J* = 6.39 Hz, 2H), 3.98 (s, 3H), 3.93 (s, 3H), 3.68 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.8, 146.8, 144.1, 139.7, 129.8, 128.9, 127.1, 126.7, 125.1, 123.0, 122.8, 121.5, 119.7, 116.3, 101.8, 60.8, 51.7, 44.6. HRMS (ESI) calcd. For $[C_{19}H_{20}NO_4]^+$ m/z: 326.1387 $[M+H]^+$; found 326.1386.

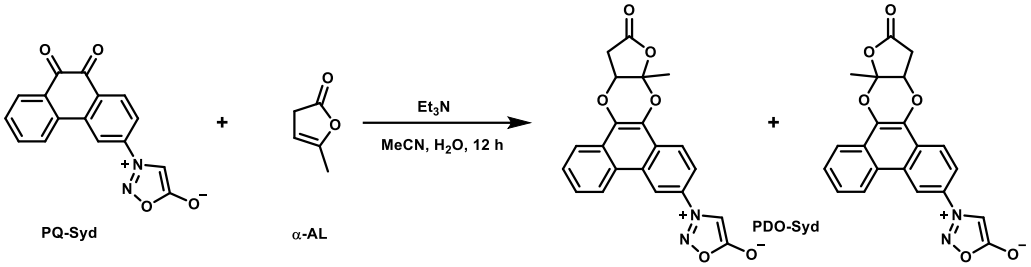
3-(9,10-dioxo-9,10-dihydrophenanthren-3-yl)-1,2,3-oxadiazol-3-ium-5-olate (PQ-Syd)

Compound **2** (0.63 g, 1.9 mmol, 1.0 eq.), LiOH (0.09 g, 3.8 mmol, 2.0 eq.) were dissolved in H_2O (1 mL) and THF (4 mL). After the mixture stirred for 4 h, the pH was adjusted to 5 with 1 M aqueous hydrochloric acid. The solution was extracted three times with EtOAc, dried through Na_2SO_4 and concentrated. Next, the residue was dissolved in DCM and cooled to 0 °C, 1 mol/L BBr_3 in DCM (4 mL) was added dropwise. The reaction was stirred for 1 h, then it was extracted three times with EtOAc, dried through Na_2SO_4 and the solvent was removed by vacuum. Compound **PQ-Gly** without further purification.

Compound **PQ-Gly** were dissolved in THF (20 mL), then isoamyl nitrite (0.30 g, 2.5 mmol, 1.3 eq.) was added dropwise. After the mixture stirred for 1 h, the solvent was removed by vacuum, the residue was dissolved in THF, trifluoroacetic acid anhydride (0.61 g, 2.9 mmol, 1.5 eq.) was added slowly. The reaction was stirred for 1 h, and the solvent was removed by vacuum. Silica gel chromatography (DCM:MeOH = 200:1) yielded compound **PQ-Syd** as a yellow solid (0.45g, 79%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, *J* = 2.08 Hz, 1H), 8.47 (d, *J* = 7.98 Hz, 1H), 8.27 (d, *J* = 8.45 Hz, 1H), 8.17 - 8.00 (m, 3H), 7.85 (td, *J* = 7.68, 1.53 Hz, 1H), 7.63 (td, *J* = 7.51, 0.99 Hz, 1H). ^{13}C

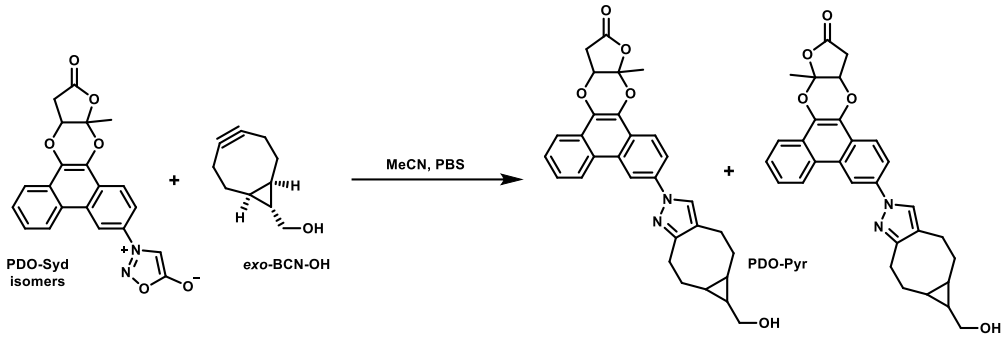
NMR (125 MHz, DMSO-d_6) δ 177.9, 177.5, 168.5, 138.8, 137.2, 135.2, 133.8, 133.7, 131.9, 130.7, 130.3, 129.1, 125.2, 121.6, 117.9, 95.7. HRMS (ESI) calcd. For $[C_{16}H_9N_2O_4]^+$ m/z: 293.0557 $[M+H]^+$; found 293.0549.

3. Preparation of the cycloaddition product PDO-Syd



PQ-Syd (10.0 mg, 0.03 mmol, 1.0 eq.), **α-AL** (14.9 mg, 0.15 mmol, 5.0 eq.) and Et_3N (6 μL, 0.03 mmol, 1.0 eq) were dissolved in CH_3CN/H_2O (2.0 mL/0.5 mL) and the reaction was monitored by TLC. At the end of the reaction, acetonitrile was removed to give light brown solid precipitated from the system. The solid was collected and washed with water and ether, dried in an oven to give the product (7.6 mg, 65%) for further characterization. When zooming in on the 3.5 - 3.6 ppm and 5.2 - 5.4 ppm regions in the ^{1}H NMR spectrum of purified **PDO-Syd**, two sets of protons proximal to the reaction site were observed, indicating the presence of two isomers in an approximate ratio of 3:1. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.32 (d, J = 2.18 Hz, 1H), 9.03 - 8.92 (m, 1H), 8.35 (dd, J = 8.94, 2.20 Hz, 1H), 8.24 - 8.16 (m, 2H), 8.10 (d, J = 1.99 Hz, 1H), 7.88 - 7.68 (m, 2H), 5.38 - 5.30 (m, 1H), 3.60 - 3.49 (m, 1H), 3.07 (dd, J = 17.78, 3.33 Hz, 1H), 1.95 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.6, 168.6, 132.8, 132.1, 131.9, 131.7, 130.0, 128.9, 128.6, 128.5, 126.9, 126.8, 126.6, 126.5, 126.5, 126.5, 126.2, 125.2, 125.1, 123.9, 122.6, 122.2, 120.8, 120.4, 119.5, 119.4, 116.8, 103.4, 103.2, 95.1, 95.0, 74.1, 73.9, 36.6, 36.4, 22.4. HRMS (ESI) calcd. for $[C_{21}H_{15}N_2O_6]^+$ m/z: 391.0925 $[M+H]^+$; found 391.0915.

4. Preparation of the click reaction products PDO-Pyr



PDO-Syd (39.1 mg, 0.10 mmol, 1.0 eq.), ***exo*-BCN-OH** (75.2 mg, 0.50 mmol, 5.0 eq.) were dissolved in CH_3CN/PBS (4.0 mL/1.0 mL) and the reaction was monitored by TLC. At the end of the reaction, acetonitrile was removed to give light solid precipitated from the system. The solid was collected and washed with water and ether, dried in an oven to give the product (34.9 mg, 70%) for further characterization. **PDO-Pyr** as a single isomer was obtained after purification, but it was not possible to determine the exact isomeric structure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.97 (d, J = 1.85 Hz, 1H), 8.92 (d, J = 8.09 Hz, 1H), 8.51 (s, 1H), 8.20 - 8.09 (m, 3H), 7.77 - 7.63 (m, 2H), 5.30 - 5.22 (m, 1H), 4.42 (t, J = 5.50 Hz, 1H), 3.55 - 3.48 (m, 1H), 3.28 (t, J = 5.95 Hz, 2H), 3.17 (d, J = 5.19 Hz, 1H), 3.10 - 2.95 (m, 2H), 2.90 - 2.78 (m, 1H), 2.70 - 2.58 (m, 1H), 2.44 - 2.34 (m, 2H), 1.93 (s, 3H), 1.22 - 1.08 (m, 2H), 0.92 - 0.76 (m, 2H), 0.65 - 0.51 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.8, 154.2, 137.5, 130.1, 129.6, 127.7, 127.4, 127.3, 126.5, 126.0, 125.2, 123.6, 122.6, 121.8, 121.6, 120.6, 118.0, 110.0, 103.4, 73.8, 64.2, 48.6, 36.6, 29.4, 28.3, 27.9, 27.1, 23.2, 22.8, 22.7, 22.5. HRMS (ESI) calcd. for $[C_{30}H_{29}N_2O_5]^+$ m/z: 497.2071 $[M+H]^+$; found 497.2059.

5. Evaluation of the stabilities of PQ-Syd

Method A: a solution containing 0.05 mM **PQ-Syd** and 0.5 mM vitamin C in 1:1 (v/v) DMSO:PBS was prepared. The stability was analyzed by HPLC after

24-hour at 37 ºC.

Method B: a solution containing 0.1 mM **PQ-Syd** and 1 mM glucose in 1:1 (v/v) DMSO:PBS was prepared. The stability was analyzed by HPLC after 24-hour at 37 ºC.

HPLC method: 50% mobile phase A (water) and 50% mobile phase B (MeCN), flow rate: 1 mL/min, running time: 10 min. Detection wavelength: 265 nm. Column: ACE Excel 5 C18 250 × 4.6 mm. Retention time: PDO-Syd, 6.7 min.

Table S1. Stabilities of **PQ-Syd** in DMSO:PBS = 1:1 containing vitamin C or glucose at 37 ºC for 24 h, respectively. Experiments were performed in triplicate. Results are expressed as mean (n = 3).

method	vitamin C		glucose	
PQ-Syd	0 h 100%	24 h 99%	0 h 100%	24 h 98%

6. Optical properties of PQ-Syd, PDO-Syd and PDO-Pyr

Fluorescence spectra and absorption spectra of **PQ-Syd**, **PDO-Syd** and **PDO-Pyr** were measured in DMSO:PBS = 1:1. Fluorescence spectra were performed on a HORIBA Jobin Yvon Fluoromax-4 fluorescence spectrometer (λ_{ex} = 365 nm), the concentrations were: **PQ-Syd** (50 μM), **PDO-Syd** (50 μM) and **PDO-Pyr** (2.5 μM). The standard curves for concentration and emission fluorescence intensity of **PDO-Syd** were measured in 50% DMSO in PBS. Absorption spectra were performed on Agilent Technologies Cary Series UV-Vis Spectrophotometer, the concentrations were: **PQ-Syd** (25 μM), **PDO-Syd** (12.5 μM) and **PDO-Pyr** (10 μM).

Molar absorption coefficients (ε) of **PDO-Syd** and **PDO-Pyr**: solutions of the two fluorophores were prepared in DMSO:PBS = 1:1 at concentrations of 12.5 μM, 25 μM, 50 μM, 75 μM and 100 μM. Absorbance at 311 nm was measured using Agilent Technologies Cary Series UV-Vis Spectrophotometer. Measurements were performed in a cuvette with a 1 cm path length, followed by linear regression analysis between absorbance and concentration. Experiments were performed in triplicate.

Fluorescence quantum yields (Φ) of **PDO-Syd** and **PDO-Pyr**: a standard sample of quinine sulfate dissolved in 0.1 M H_2SO_4 was prepared first. The integrating sphere of the HORIBA Jobin Yvon Fluoromax-4 fluorescence spectrometer was calibrated, and the fluorescence quantum yields of the two fluorophores were subsequently measured via the absolute method using the integrating sphere.

7. Kinetics Studies of the reaction between PQ-Syd and α-AL

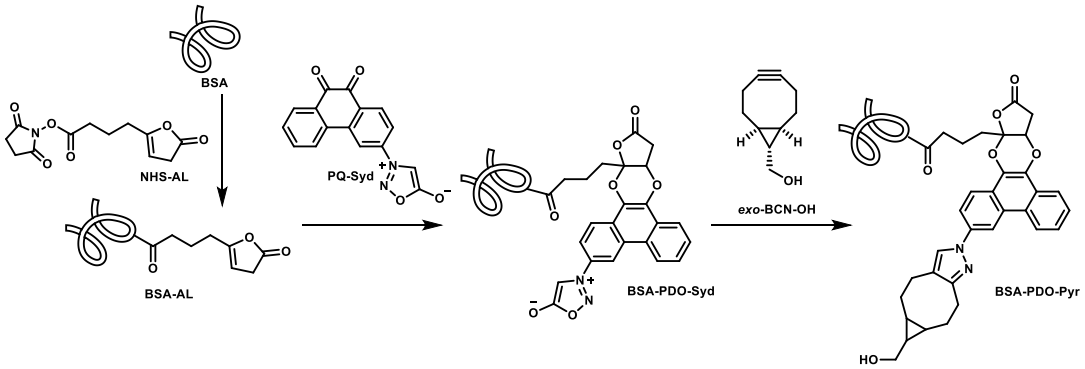
The reaction rate constants of cycloaddition between **PQ-Syd** and **α-AL** were calculated based on fluorescence intensity analysis results. All kinetics experiments were carried out at room temperature in DMSO/PBS = 1:1. Second-order kinetics was performed by combining **PQ-Syd** and **α-AL** in a 1:10 ratio, and the concentration of **PQ-Syd** was 30 μM. The amounts of **PDO-Syd** were monitored by the emission fluorescence intensity at 549 nm (E_X = 365 nm). Percent conversion (x) was calculated by the fluorescence intensity of **PDO-Syd** and the standard curves for concentration and emission fluorescence intensity of **PDO-Syd**. Pseudo-first order rate constant k_{obs} was determined by plotting ln[1/(1-x)] versus time and analysis by linear regression (x represents the percent conversion of **PQ-Syd**). The slope of the linear equation is k_{obs}. The second rate constant k_2 was calculated by k_{obs}/[**α-AL**]. The reported errors for rate constants are based on the standard deviation of the mean for experiments performed in triplicate.

8. Kinetics Studies of the reaction between PDO-Syd and *exo*-BCN-OH

The reaction rate constants of cycloaddition between **PDO-Syd** and ***exo*-BCN-OH** were calculated based on HPLC analysis results. All kinetics experiments were carried out at room temperature in DMSO/PBS = 1:1. Second-order kinetics was performed by combining **PDO-Syd** and

exo*-BCN-OH** in a 1:10 ratio, and the concentration of **PDO-Syd** was 25 μM. The relative amounts of **PDO-Syd** and **PDO-Pyr** were monitored by absorbance at 331 nm and calculated by integration of areas at 331 nm. Instrument specification: SHIMADZU Prominence LC-20A. HPLC method: 25% mobile phase A (water) and 75% mobile phase B (MeCN), flow rate: 1 mL/min, running time: 15 min. Column: ACE Excel 5 C18, 250 × 4.6 mm. Retention time: PDO-Syd, 5.0 min; PDO-Pyr, 8.1 min. Percent conversion (x) was calculated by both disappearance of **PDO-Syd** and appearance of **PDO-Pyr**. Pseudo-first order rate constant k_{obs} was determined by plotting ln[1/(1-x)] versus time and analysis by linear regression (x represents the percent conversion of **PDO-Syd**). The slope of the linear equation is k_{obs}. The second rate constant k_2 was calculated by k_{obs} / [exo*-BCN-OH**]. The reported errors for rate constants are based on the standard deviation of the mean for experiments performed in triplicate.

9. Labeling of proteins with mutually orthogonal reactions



The preparation of the protein BSA-**AL**: 10 mg BSA was mixed with 20 molar equivalents of **NHS-AL** (synthesized according to the literature[6]) in 1 mL PBS (pH = 7.4), after standing at room temperature for 2 hours, the proteins were purified using ultracentrifuge filter (molecular weight cutoff 15 kD, 15,000 g, 5 times), the concentration of BSA-**AL** was quantified with BCA protein assay kit (Pierce) and further stored in PBS at 4 °C.

Labeling of proteins: eight experimental groups containing BSA (2 mg/mL in PBS, 400μL) or BSA-**AL** (2 mg/mL in PBS, 400 μL) along with 20 molar

equivalents of **PQ-Syd** (10 mM in DMSO, 24 μL) , 20 molar equivalents of ***exo*-BCN-OH**(20 mM in DMSO, 12 μL), or PBS, ensuring that the final concentration of BSA or BSA-**AL** was 1.6 mg/mL. The mixture was incubated at room temperature for 12 hours. Protein samples (10 μL) in every lane were further analyzed by gel electrophoresis using 12% polyacrylamide gels. Fluorescence images of the protein gels were taken using a handheld 365nm UV lamp as the light source. After staining with Coomassie brilliant blue, imaged with IVIS Lumina XR III small animal imaging system using corresponding filters.

Supplementary Figures

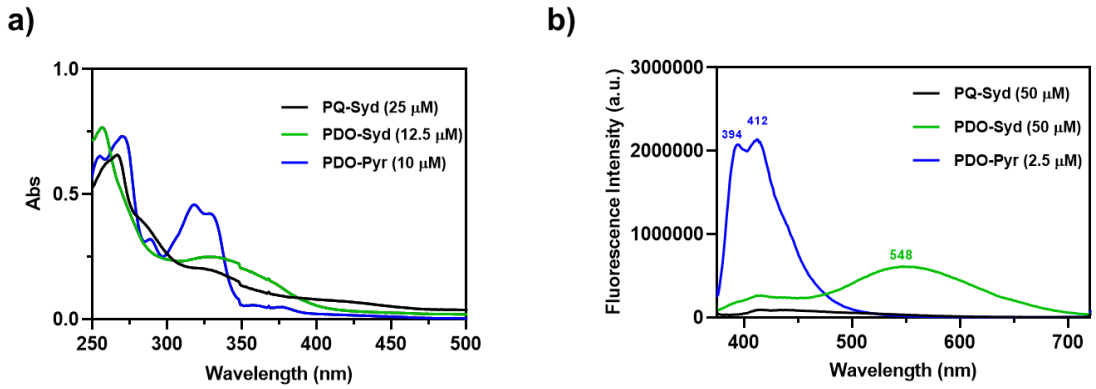


Figure S1. Fluorescence spectra and absorption spectra of **PQ-Syd**, **PDO-Syd** and **PDO-Pyr** were measured in DMSO:PBS = 1:1. a) Absorption spectra were performed on Agilent Technologies Cary Series UV-Vis Spectrophotometer, the concentrations were: **PQ-Syd** (25 μM), **PDO-Syd** (12.5 μM) and **PDO-Pyr** (10 μM). b) Fluorescence spectra were performed on HORIBA Jobin Yvon Fluoromax-4 fluorescence spectrometer (λ_{ex} = 365 nm), the concentrations were: **PQ-Syd** (50 μM), **PDO-Syd** (50 μM) and **PDO-Pyr** (2.5 μM).

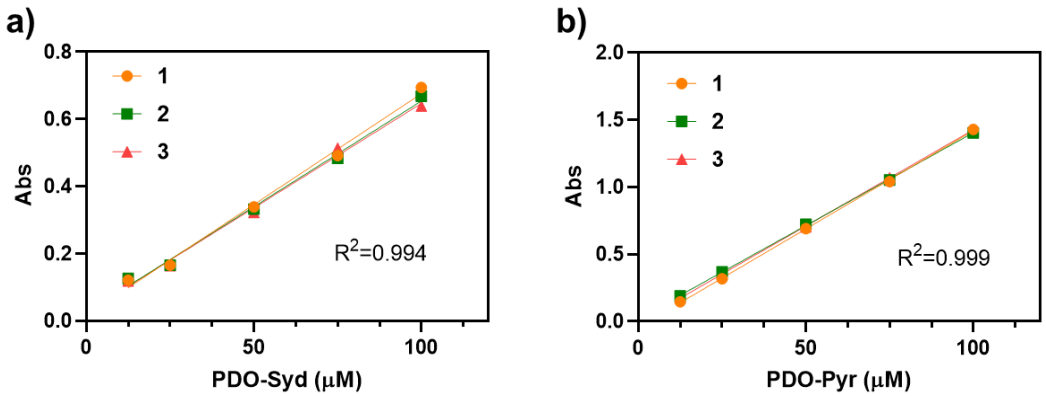


Figure S2. Molar absorption coefficients (ε) of **PDO-Syd** and **PDO-Pyr** were measured in DMSO:PBS = 1:1, performed on Agilent Technologies Cary Series UV-Vis Spectrophotometer. The cuvette has a 1 cm light path. a) ε_{311nm} of **PDO-Syd** is 6367 ± 170 M^{-1} cm^{-1}. b) ε_{318nm} of **PDO-Pyr** is 14233 ± 330 M^{-1} cm^{-1}. Experiments were performed in triplicate. Results are expressed as mean ± SD (n = 3).

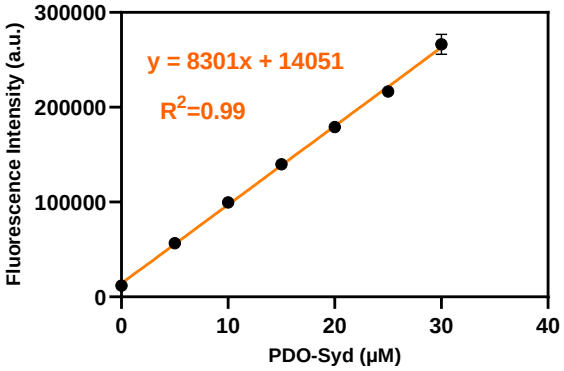


Figure S3. The standard curves for concentration and emission fluorescence intensity of **PDO-Syd** (0 - 30 μM) were measured in 50% DMSO in PBS. Experiments were performed in triplicate. Results are expressed as mean ± SD (n = 3).

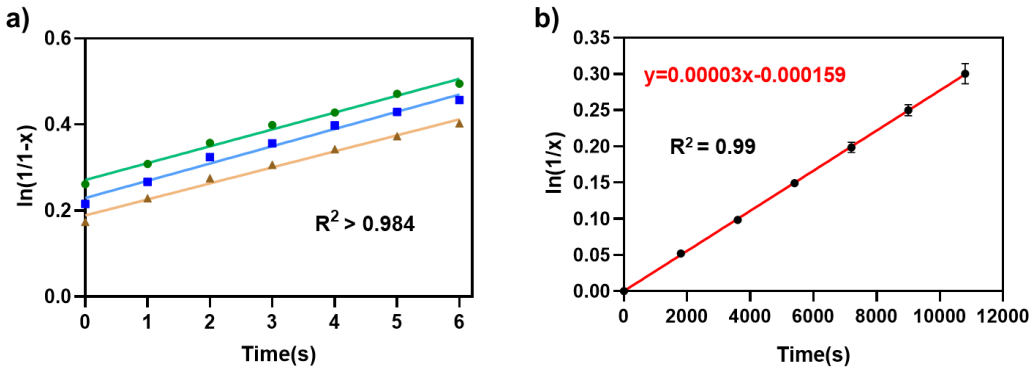
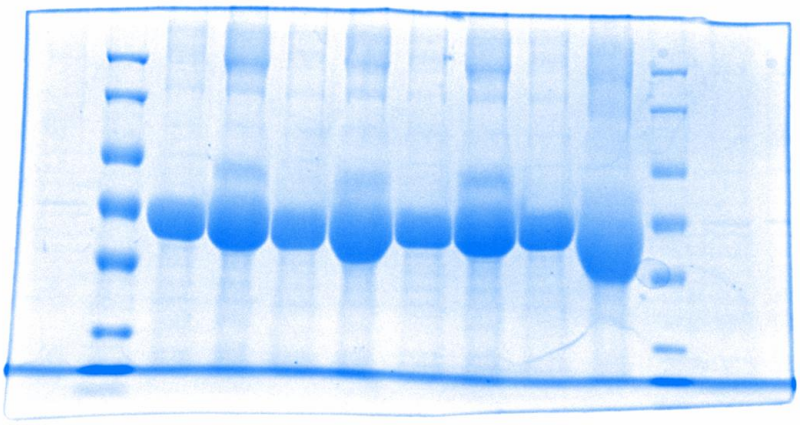


Figure S4. Measurement of second order rate constants. a) reactions of **PQ-Syd** (30 μM) with **α-AL** (300 μM) in DMSO/PBS = 1:1, the rate constant was 97 ± 3 M^{-1} s^{-1}. b) reactions of **PDO-Syd** (25 μM) with ***exo*-BCN-OH** (250 μM) in DMSO/PBS = 1:1, the rate constant was 0.12 ± 0.003 M^{-1} s^{-1}. Experiments were performed in triplicate. Results are expressed as mean ± SD (n = 3).

a)



b)

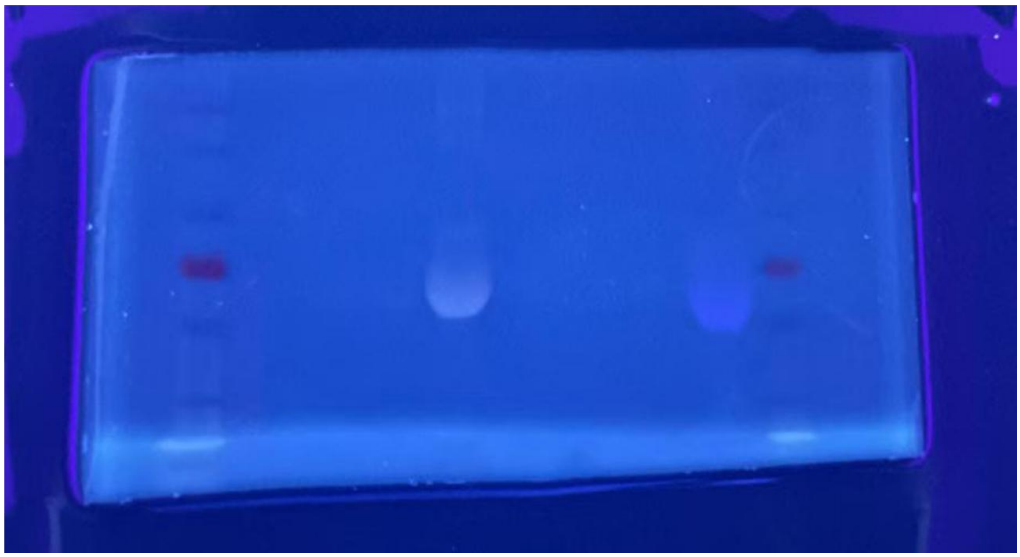


Figure S5. Raw images of the gel electrophoresis for Figure 5. a) Coomassie staining. b) fluorescence image captured by a camera under a handheld 365 nm light source.

References

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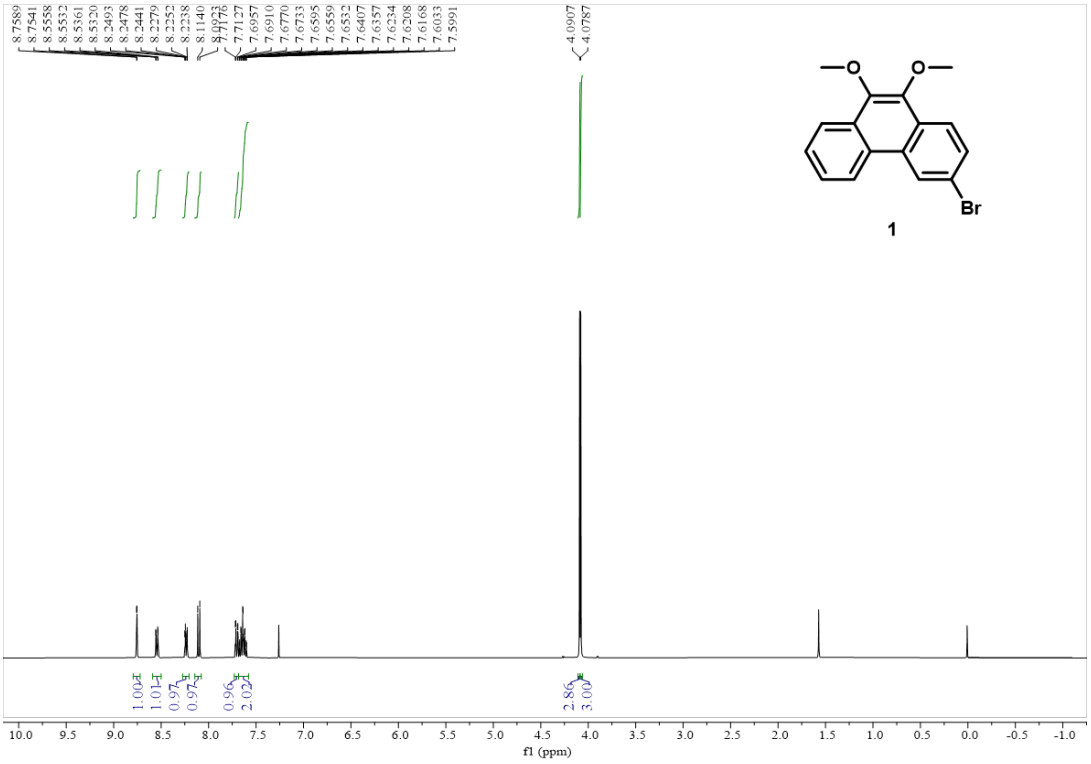
NMR spectra

^{1}H NMR of **1**



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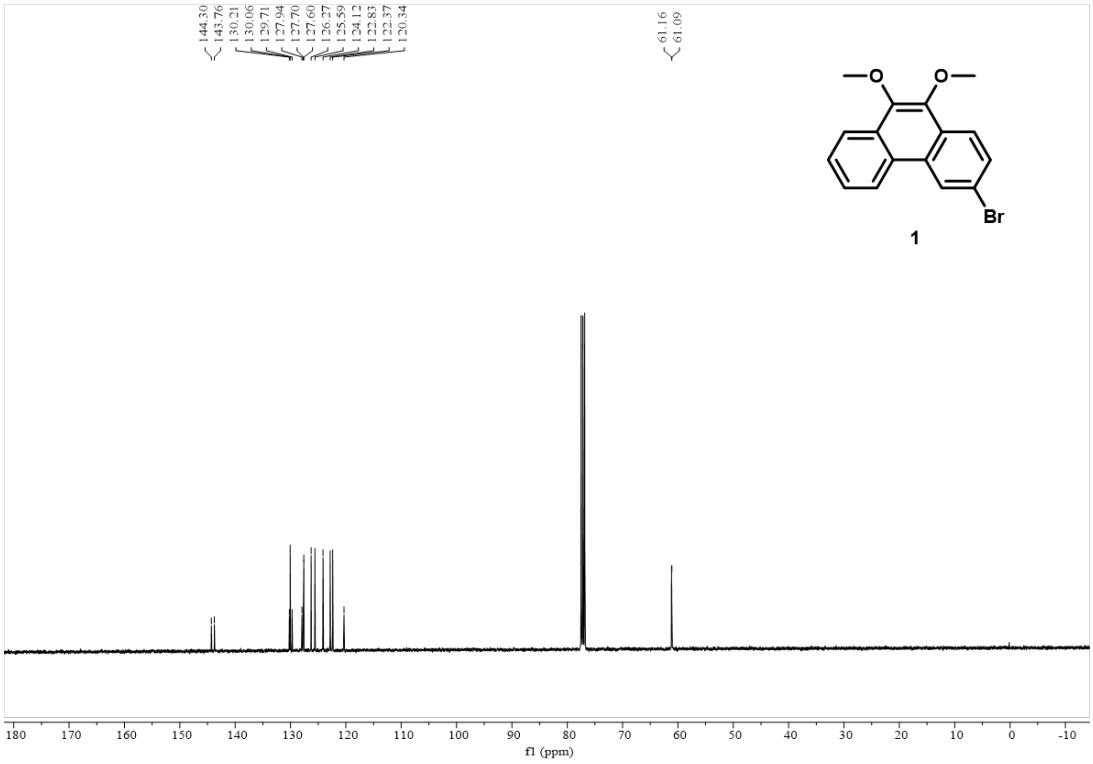

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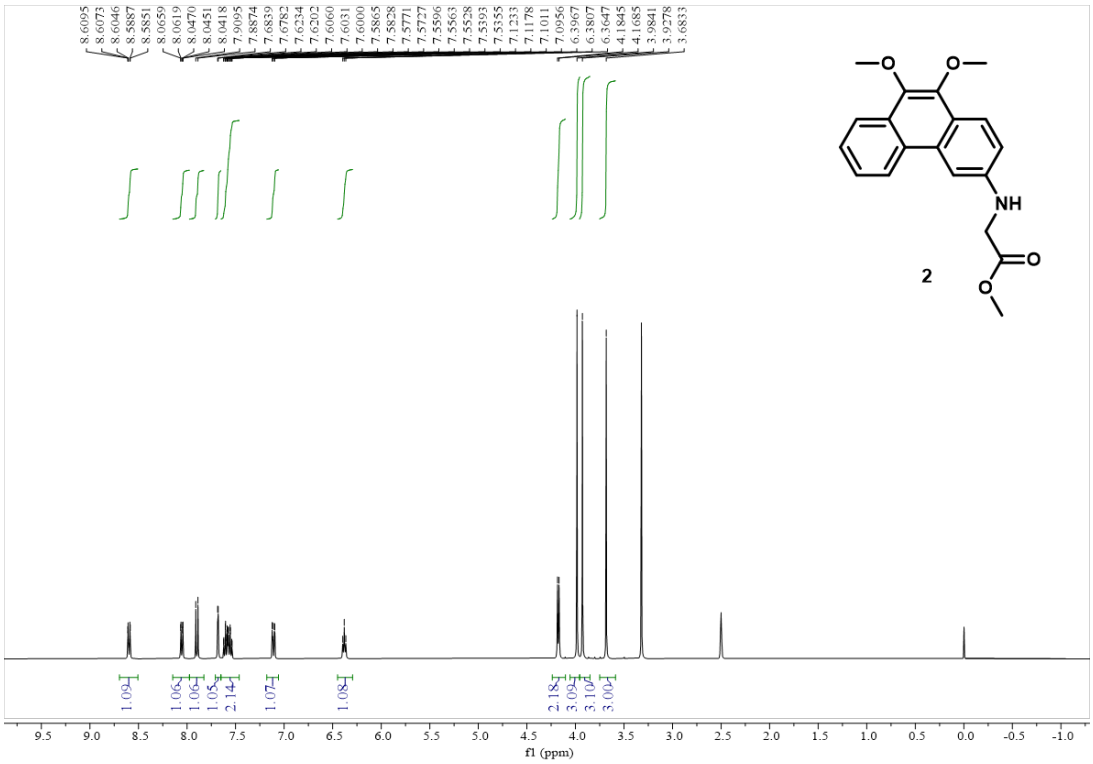

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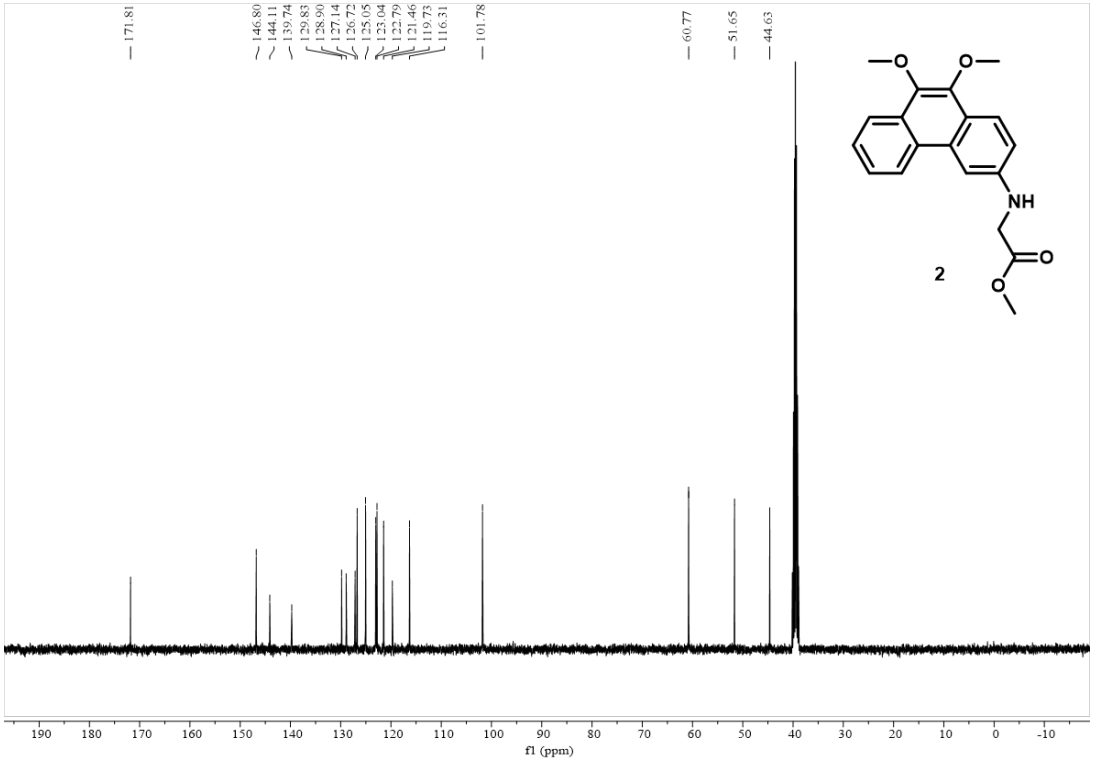

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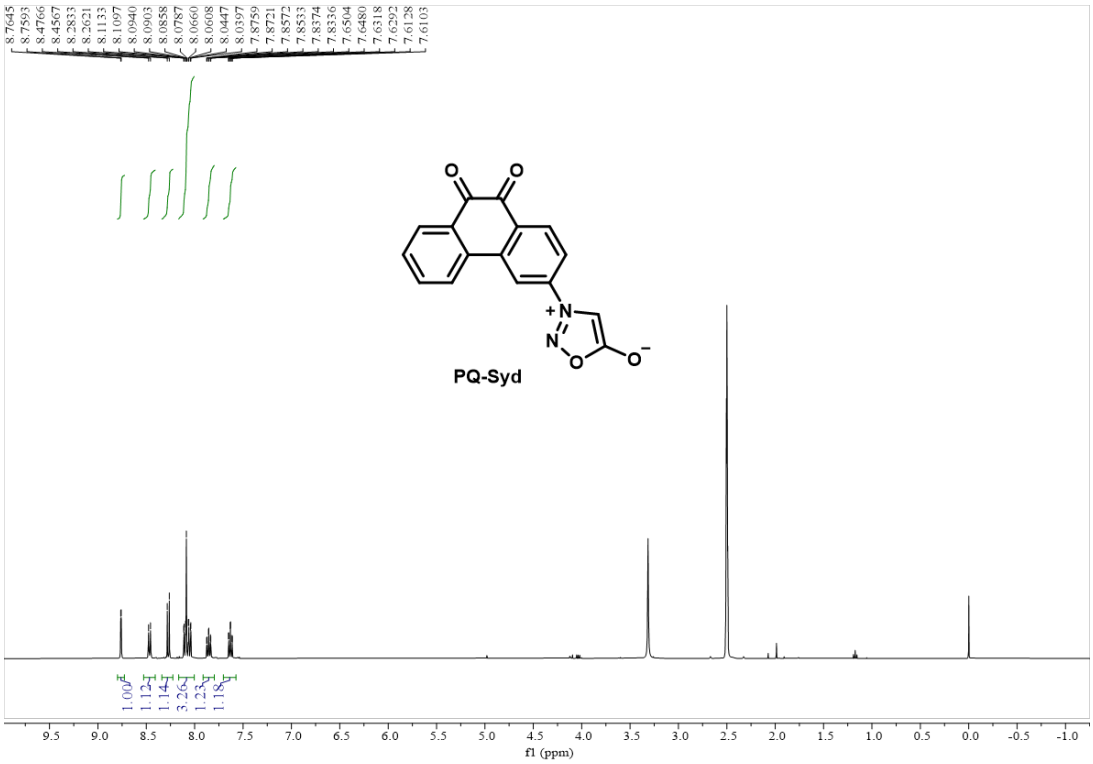

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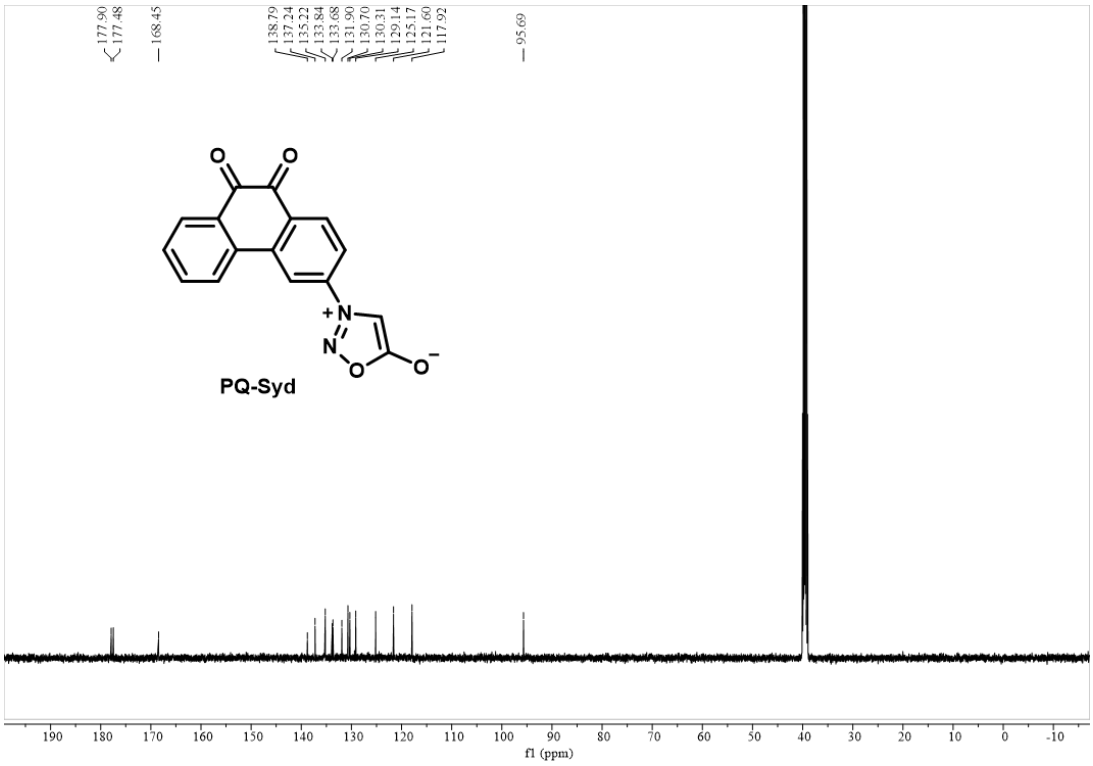

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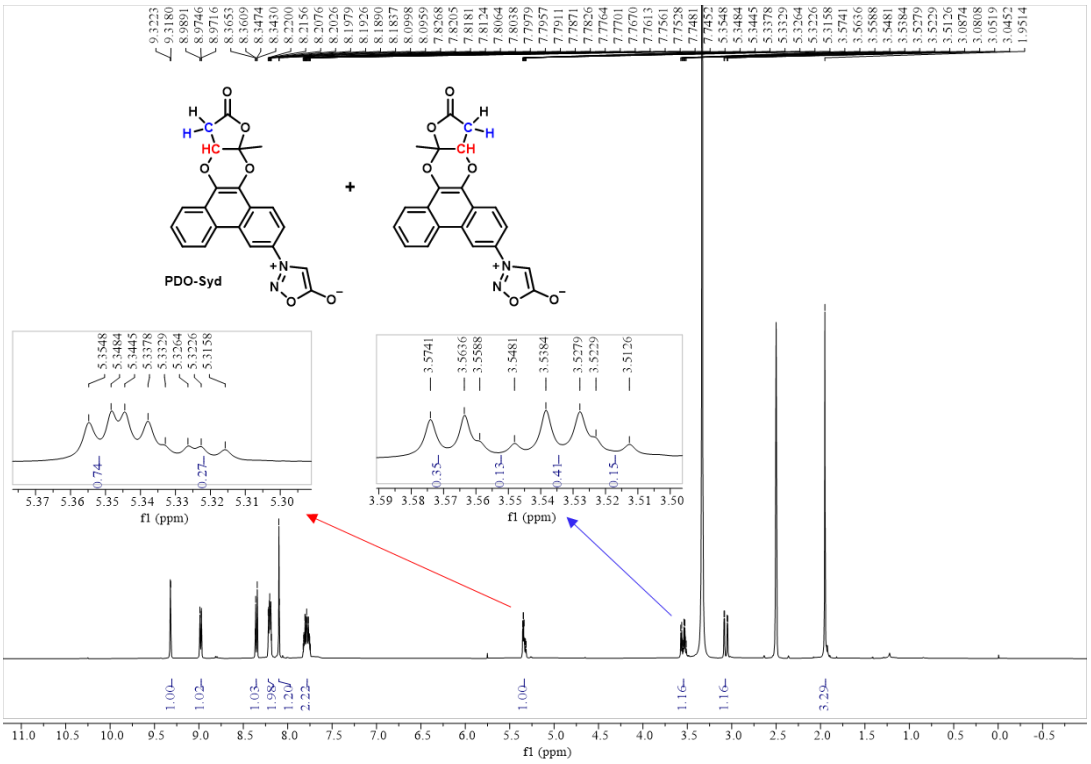

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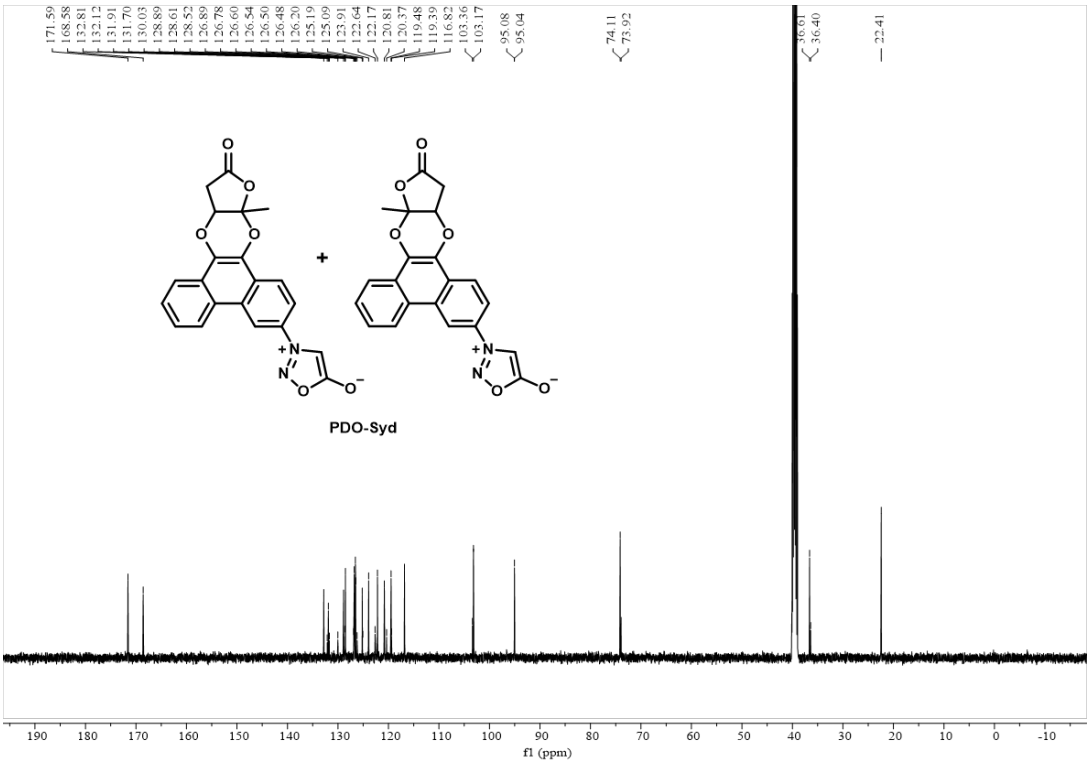

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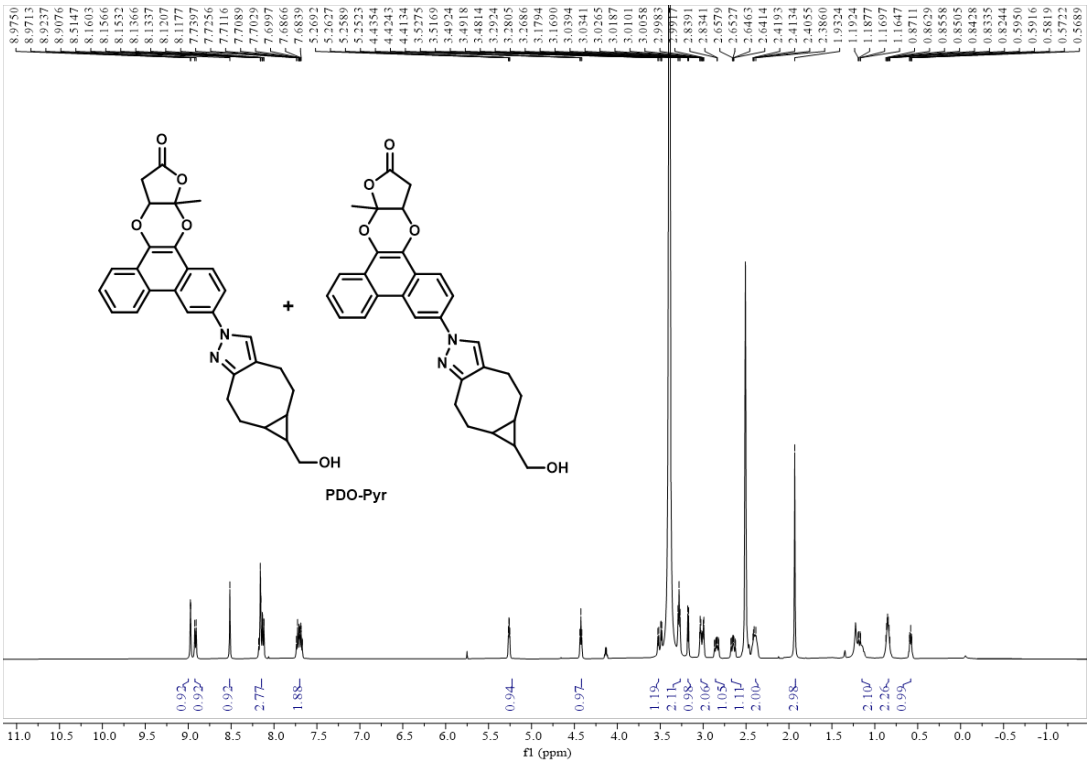

^{13}C NMR of **PDO-Pyr**