A Phthalimidation Protocol That Follows Protein Defined Parameters

Rohith Singudas, Srinivasa Rao Adusumalli, Pralhad Namdev Joshi, Vishal Rai*

Department of Chemistry, Indian Institute of Science Education and Research (IISER) Bhopal, Bhopal, India.

vrai@iiserb.ac.in

Contents

Supplementary methods

General information: The reagents, proteins, and enzymes were purchased from Sigma-Aldrich. Aqueous buffers were prepared freshly using Millipore Grade I water (Resistivity > 5 MΩ cm, Conductivity < 0.2 μS/cm, TOC <30 ppb). The final pH was adjusted using pH meter Mettler Toledo (FE20). All the solvents used were reagent grade. The reaction mixture was stirred for small molecules (Heidolph, 500-600 rpm), whereas it was vortexed in incubator-shaker Thermo Scientific MaxQ 8000 (350 rpm) for proteins. UV spectra were recorded on Shimadzu UV-1800 UV-Vis spectrophotometer. Cellulose membrane (MWCO, 6-8 kD) from Spectrum labs was used for dialysis. Samples were lyophilized using CHRiST ALPHA 2-4 LD plus lyophilizer. Peptide was synthesized by SPPS using Fmoc chemistry on Biotage Syro I parallel peptide synthesis system. HORIBA Scientific, Fluoromax-4 spectrometer was used to record the fluorescence of RNase A, phthalimide labeled RNase A and dimethylaminophthalimide labeled RNase A. Quartz cuvette (10 mm) was used to measure the fluorescence spectra. Samples were excited at 280 nm by keeping excitation and emission slits at 5 nm. Circular Dichroism (CD) measurements were recorded on JASCO J-815 CD spectropolarimeter equipped with peltier temperature controller. All the spectra were measured with a scan speed of 50 nm/min, spectral band width 1 nm using 1 cm path length cuvette at 25 ^{o}C.The CD results were expressed as mean residue ellipticity (MRE) in deg.cm^{2} dmol^{-1}.[1]

Chromatography: Samples were triturated with pentane in most of the cases. For reactions where chromatography was involved, flash column chromatography was carried out on Combiflash Rf 200 using 230-400 mesh silica gel. Thin-layer chromatography (TLC) was performed on Merck (TLC Silica gel 60 F_{254}) and visualized using a UV lamp (254 nm) and stains such as iodine, ninhydrin, cerium sulfate (yellow dip).

Nuclear magnetic resonance spectra: ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on Bruker Avance III 400 MHz NMR spectrometer, Bruker Avance III 500 MHz NMR spectrometer and Bruker Avance III 700 MHz NMR spectrometer. ^{1}H NMR spectra were referenced to TMS (0 ppm), ^{13}C NMR spectra were referenced to $CDCl_3$ (77.16 ppm), D_2O (4.79 ppm) and DMSO-d_6 (39.52 ppm), and ^{31}P spectra was referenced to 85% H_3PO_4 in H_2O (0 ppm). Peak multiplicities are designated by the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; p, pentet; m, multiplet; dd, doublet of doublets. Spectra were recorded at 298 K.

Mass spectrometry: Low resolution mass spectra (ESI) were collected on an Agilent Technologies 1200 series HPLC paired to a 6130 mass spectrometer. Bruker Daltonics MicroTOF-Q-II with electron spray ionization (ESI) was used for HRMS data. Matrix assisted laser desorption/ionisation time of flight mass spectrometry was performed with Bruker Daltonics UltrafleX treme, Software-Flex control version 3.4, Matrix-Sinapic acid and α-Cyano-4-hydroxycinnamic acid (HCCA). Peptide mass[2] and fragment ion calculator (http://db.systemsbiology.net:8080/proteomicsToolkit/FragIonServlet.html) for peptide sequencing were used.

Computational methods:

Transition state and NBO calculations: All computations were performed with the GAUSSIAN 09 Revision A.02 program suite[3] with the DFT method of Becke's three-parameter hybrid Hartree-Fock procedure with the Lee-Yang-Parr correlation function (B3LYP). The geometries of all the substrates in this study were fully optimized by the DFT/B3LYP method with the 6-311++G(d,p) basis set. No symmetry constraints were imposed in the optimizations. Split-valence basis sets, polarized basis sets, diffuse functions, and high-angular-momentum basis sets were used to consider highly delocalized molecules. Charge densities were calculated by NPA with the NBO 3.1 program, which is included in GAUSSIAN 09, with the strongly delocalized NBO set. The synchronous transit-guided quasi-Newton (STQN) method through QST3 option was used for the transition state calculations. The method initially uses a linear or quadratic synchronous transit approach followed by a quasi-Newton or eigenvector-following algorithm in search of the transition state.

Docking studies: For the global search for N-hydroxyphthalimide binding sites on RNase A, Autodock 4.2[4] was used. Protein coordinates were derived from RCSB protein data bank (PDB: 2AAS)[5]. Grid for RNase A was adjusted to consider the whole protein. The ligand probes were then docked with all rotatable torsion angles allowed to rotate freely. The ten lowest energy structures were stored for analysis.

Methods:

Procedure for synthesis of **4b** and **4c**

N-hydroxypthalimide (81.5 mg, 0.5 mmol) was taken in a clean and dry 5 ml round bottom flask charged with magnetic stir bar containing dry dichloromethane. The diphenylphosphorylchloride (104 µl, 0.5 mmol) and triethylamine (69 µl, 0.5 mmol) was added to the reaction mixture through micropipette and was allowed to stir at room temperature for 2 h under nitrogen. Reaction was followed by thin layer chromatography. The product was extracted (3 × 80 ml) using dichloromethane, concentrated and purified by flash column chromatography (silica gel 100-200 mesh, ethyl acetate/hexane 1:9, R_f 0.5).

N-hydroxyphthalimide (16.3 mg, 1 mmol) and allyl halide (14.3 µl, 1.67 mmol) were dissolved in DMSO (5 ml), followed by the addition of anh. K_2CO_3 (91 mg, 0.67 mmol). The reaction mixture was ultrasonicated at 25-35 °C for a period of 3 h. The mixture was poured into ice water. The precipitate of *N*-allyloxyphthalimide was filtered off and dried. The crude product was purified by flash column chromatography (silica gel 100-200 mesh, ethyl acetate/hexane 1:9, R_f 0.64).

Procedure for phthalimidation (**10a-10n**, Scheme 3a, **S1a-S1m**)

N-hydroxypthalimide (16.3 mg, 0.1 mmol) was added to phosphate buffer (1 ml, 0.1 M, pH 7.0) in a 5 ml vial charged with magnetic stir bar. The amine (0.1 mmol) was added to the reaction mixture and was allowed to stir at room temperature. Reaction was followed by thin layer chromatography. In general, the products are insoluble in the reaction mixture and were filtered. For the buffer soluble products, extraction with organic solvent and purification by flash column chromatography yielded analytically pure products.

Procedure for de-phthalimidation (Scheme 3b)

N-benzylpthalimide **7** (23.7 mg, 0.1 mmol) was taken in phosphate buffer (1 ml, 0.1 M, pH 7.0) in a 5 ml vial charged with magnetic stir bar. Hydrazine (31.4 µl, 1 mmol) was added to the reaction mixture followed by stirring at room temperature for 6 h. Reaction was followed by thin layer chromatography (ethylacetate/hexane, 3:7, R_f 0.43). On complete consumption of starting materials, buffer was lyophilized and the product was isolated by flash column chromatography (silica gel 100-200 mesh, ethyl acetate/hexane 100: 0, R_f 0.35).

Procedure for piperidine mediated hydrolysis (Scheme 3c)

N-benzylpthalimide **7** (23.7 mg, 0.1 mmol) was taken in phosphate buffer (1 ml, 0.1 M, pH 7.0) in a 5 ml vial charged with magnetic stir bar. Piperidine (98.7 µl, 1 mmol) or disodium sulphide (78 mg, 1 mmol) was added to the reaction mixture and it was allowed to stir at room temperature. Reaction was followed by thin layer chromatography (ethylacetate/hexane, 3:7, R_f 0.43). On complete consumption of starting materials, buffer was lyophilized and the product was isolated by flash column chromatography (silica gel 100-200 mesh, ethyl acetate/methanol 70:30, R_f 0.48).

Procedure for 4-N,N-dimethylamino-N-hydroxy pthalimide **S4** (Scheme S1)[6]

4-Nitrophthalic anhydride **S2** (2.76 mmol) was dissolved in EtOAc (150 mL). Formalin (15 mL, 36% formaldehyde solution) and Pd/C 10% (200 mg) were added to the reaction mixture

followed by stirring at room temperature under an atmosphere of hydrogen for 3 h. The reaction mixture was filtered through celite, concentrated under reduced pressure and purified by flash column chromatography to isolate **S3**. Pyridine (1 mL) and $NH_2OH{\cdot}HCl$ (1.1 mmol) were stirred in a flask followed by addition of 4-N,N-dimethylaminophthalic anhydride **S3** (1 mmol). The mixture was stirred at 90 °C for 15 h, concentrated under reduced pressure and analytically pure **S4** was isolated after flash column chromatography.

Procedure for protein labeling (Scheme 4)

Under minimized light, RNase A (1 mg, 73 nmol) in phosphate buffer (88 µl, 0.1 M, pH 7.0) was taken into aluminium foil covered eppendorf tube. To this solution, 12 µl of phosphate buffer containing N-hydroxyphthalimide (12 µg, 73 nmol) was added and then the reaction mixture was vortexed at room temperature. Phthalimidation of RNase A was followed by MALDI-ToF-MS using sinapic acid solution as matrix. After 8 h, reaction mixture was further diluted with water (0.4 ml). Unreacted NHP was extracted using ethyl acetate/hexane (6:4, 8×1 ml) and utilized to follow the conversion. The rapid extraction of NHP also enables quenching of the reaction. The reaction mixture was further subjected to dialysis for desalting and concentrated by lyophilisation. The sample was further utilized for digestion and sequencing.

Reactions of RNase A with NHP at various concentrations (0.073, 0.365, 0.73, 2.19 and 3.65 mM, entries 1-5, Table S4), excess of NHP (1, 5, 20, 50 and 100 equiv., entries 1-5, Table S5) and dimethylamino hydroxyl phthalimide (1 equiv., Scheme S2) were performed using similar protocol.

Conversion analysis

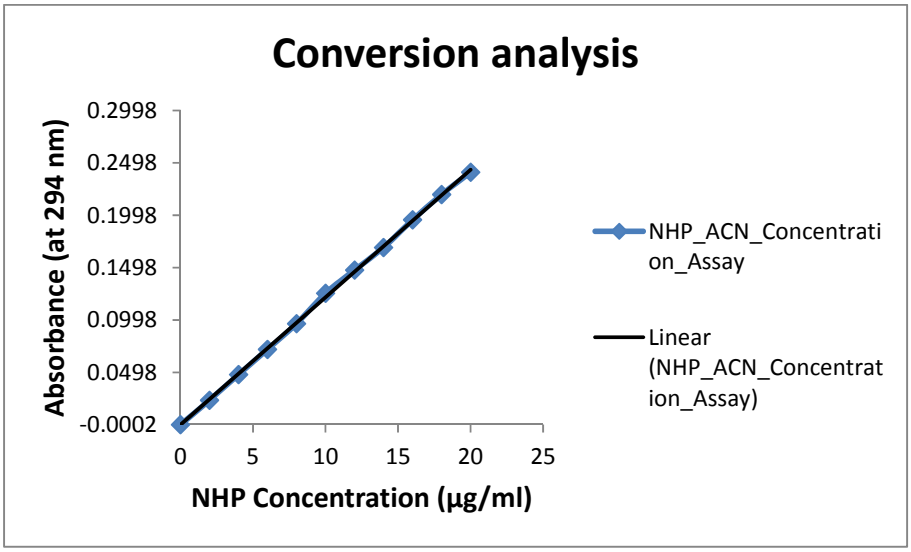


Figure S1: Linear fit to the standard curve was plotted for absorbance of standard NHP solution. Absorbance at 294 nm for NHP standards 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml were recorded in acetonitrile.

Unreacted NHP was isolated from the reaction mixture using liquid-liquid extraction. ***Organic solvent system (ethyl acetate/hexane, 6:4, 8×1 ml) was optimized using standards***

for minimum loss of NHP (±5%) during extraction. Ethyl acetate/hexane was removed in vacuo and recovered NHP was re-dissolved in acetonitrile (ACN, 2 ml). The readings were normalized accordingly and the standard plot (Figure S1) was utilized to calculate the conversions.

Procedure for in-solution digestion of protein

All solutions were made immediately prior to use.[7]

1. 10 µl of RNase A solution containing 0.1 mg of RNase A in 6 M urea, 100 mM tris and 10 mM $CaCl_2$ (pH 7.8), was taken in 1.5 ml eppendorf tube and vortexed for 30 minutes.

2. **Disulfide reduction:** To this solution, 1 µL of reducing agent (0.2 M DTT, 0.1 M tris and 0.01 M $CaCl_2$) was added and sample was vortexed for 1 h at room temperature (25 ˚C).

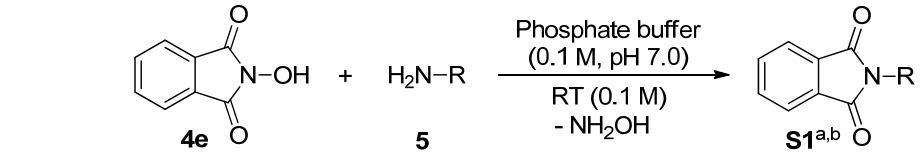
3. **Sulfhydryl alkylation:** To this solution, 4 µL of alkylating agent (0.2 M iodo acetamide, 0.1 M tris and 0.01 M $CaCl_2$) was added and incubated (in dark) for 1 h at room temperature.

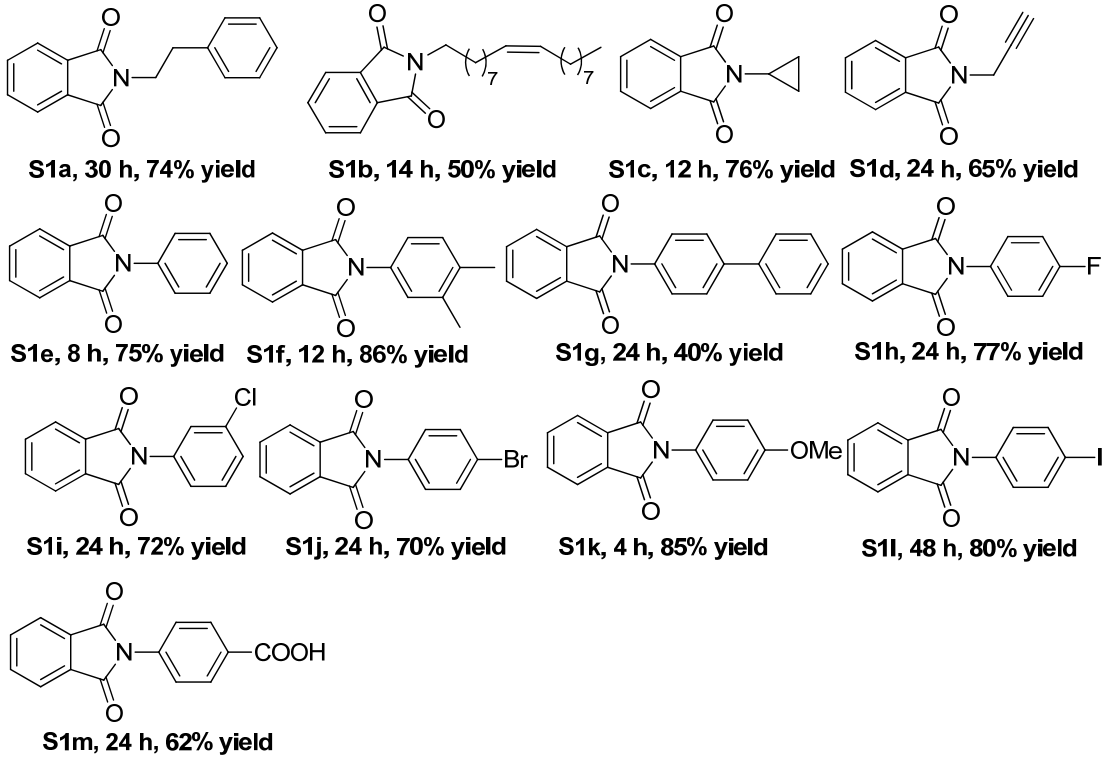
4. **Quenching alkylation:** 4 µL of reducing agent was added again to the mixture to quench remaining iodo acetamide, mixed the sample by vortexing at room temperature for 1 h. Urea concentration was reduced to ~0.6 M by diluting the reaction mixture with grade I water.

5. **α-Chymotrypsin:** 10 µL of chymotrypsin solution (chymotrypsin in 1 mM HCl was dissolved in 0.1 M tris and 0.01 M $CaCl_2$) containing 1 µg of chymotrypsin (based on ratio of chymotrypsin/RNase A (1:100) was added to the reaction mixture. It was mixed by gentle vortex and digested overnight. The pH of digested solution was adjusted to < 6 (verified by pH paper) by adding 0.5% trifluoroacetic acid. [α-Chymotrypsin is a serine endopeptidase that specifically hydrolyzes peptide bonds at the C-termini of Tyr, Phe and Trp at pH 7.0-9.0. Leu and Met are cleaved at a lower rate.] Subsequently, the sample was used for MS investigations.

Supplementary results and discussion

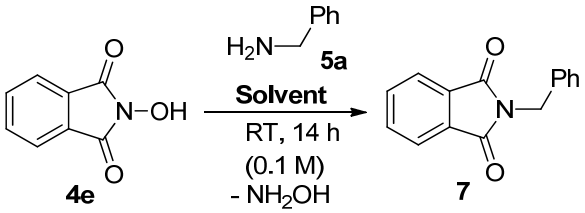
Table S1. Phthalimidation of amines





[a] Isolated yields, [b] Strong electron withdrawing substituents on aryl ring results in <5% conversions.

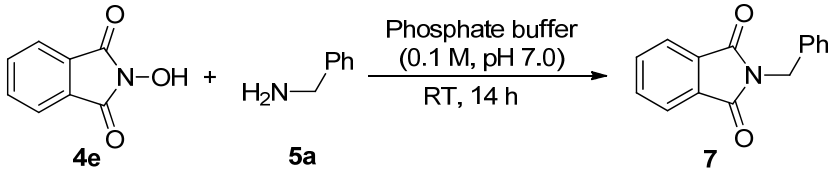
Table S2. Screening of solvents



Solvent	**% Conversion[a]**
H_2O	95 to >99
Phosphate buffer pH: (a) 6.0, (b) 6.5, (c) 7.0, (d) 7.3, (e) 7.6, (f) 7.8	95 to >99

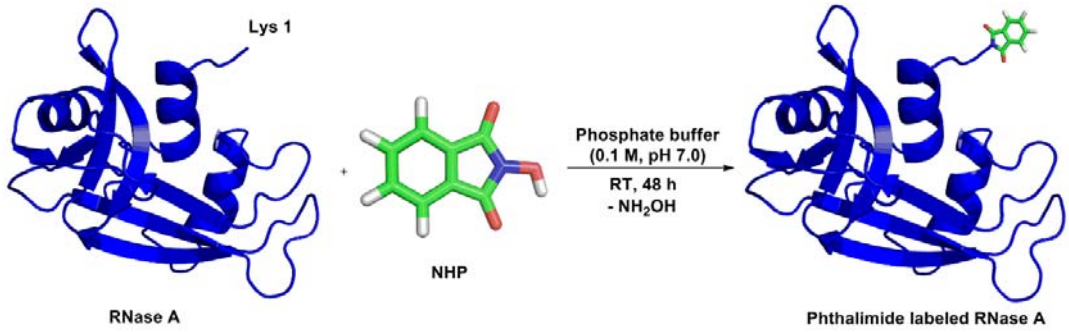
[a] % Conversion is based on ^{1}H NMR of reaction mixture in $CDCl_3$ in four trials.

Table S3. Scalability of reaction



S. No.	Reaction scale	% Yield	Amount of product
1	0.05 mmol	80	10 mg
2	0.5 mmol	70	83 mg
3	5 mmol	72	864 mg
4	50 mmol	70	8.5 g

Table S4. Effect of the reaction concentration and reaction time in phthalimidation of RNase A.



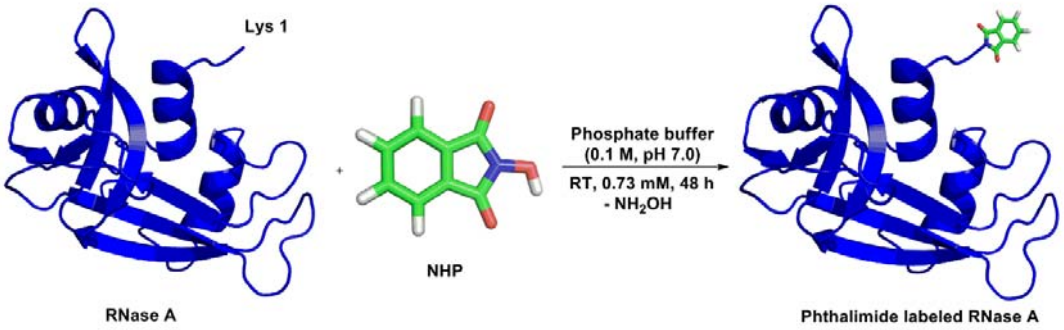
Entry	Reaction concentration (mM)	Time (h)	No. of sites modified on RNase A
1	0.073	1 2 4 8 16 24 30 48	Mono labeled Mono labeled Mono labeled Mono labeled Mono and trace of bis labeled Mono and bis labeled Mono and bis labeled Mono and bis labeled
2	0.365	1 2 4 8 16 24 30 48	Mono labeled Mono labeled Mono labeled Mono labeled Mono and bis labeled Mono and bis labeled Mono and bis labeled Mono and bis labeled

3	0.73	1 2 4 8 16 24 30 48	Mono labeled Mono labeled Mono labeled Mono labeled Mono and bis labeled Mono and bis labeled Mono, bis and tris labeled Mono, bis and tris labeled
4	2.19	1 2 4 8 16 24 30 48	Mono labeled Mono labeled Mono labeled Mono labeled Mono and bis labeled Mono, bis and tris labeled Mono, bis and tris labeled Mono, bis, tris and tetra labeled
5	3.65	1 2 4 8 16 24 30 48	Mono labeled Mono labeled Mono and trace of bis labeled Mono and bis labeled Mono, bis and trace of tris labeled Mono, bis, tris and trace of tetra labeled Mono, bis, tris and tetra labeled Mono, bis, tris and tetra labeled

Discussion: To investigate the effect of reaction concentration on labeling of RNase A using NHP, reaction concentration was varied from 0.073 mM to 3.65 mM. Monolabeled product was formed in 8 h when the reactions were done at 0.073, 0.365, 0.73 and 2.19 mM (entries 1-4, Table S4). At 3.65 mM, bislabeled product was formed along with monolabeled product within 4 h (entry 5, Table S4). Multiple site labeling was found to be dependent on both concentration and reaction time (entries 1-5, Table S4).

Excess of NHP with RNase A resulted in multiple site labeling. When RNase A was treated with equimolar NHP, mono labeled product was formed in 8 h (entry 1, Table S5). Increase in relative concentration of NHP led to modification of two to six sites (entries 2-5, Table S5).

Table S5: Effect of relative ratio of NHP and RNase A on phthalimidation.



Entry	Equiv. of NHP	Time (h)	No. of sites modified on RNase A
1	1	1	Mono labeled
		4	Mono labeled
		8	Mono labeled
		16	Mono and bis labeled
		24	Mono and bis labeled
		30	Mono, bis and tris labeled
		48	Mono, bis and tris labeled
2	5	1	Mono and trace of bis labeled
		4	Mono and bis labeled
		8	Mono and bis labeled
		16	Mono, bis and tris labeled
		24	Mono, bis and tris labeled
		30	Mono, bis and tris labeled
		48	Mono, bis, tris and trace of tetra labeled
3	20	1	Mono, bis, tris and trace of tetra labeled
		4	Mono, bis, tris and tetra labeled
		8	Mono, bis, tris and tetra labeled
		16	Mono, bis, tris and tetra labeled
		24	Mono, bis, tris and tetra labeled
		30	Mono, bis, tris, tetra and penta labeled
		48	Mono, bis, tris, tetra and penta labeled
4	50	1	Mono, bis, tris and tetra labeled
		4	Mono, bis, tris, tetra and trace of penta labeled
		8	Mono, bis, tris, tetra and penta labeled
		16	Mono, bis, tris, tetra and penta labeled
		24	Mono, bis, tris, tetra and penta labeled
		30	Mono, bis, tris, tetra and penta labeled
		48	Mono, bis, tris, tetra and penta labeled
5	100[a]	1	Mono, bis, tris, tetra and penta labeled
		4	Mono, bis, tris, tetra and penta labeled
		8	Mono, bis, tris, tetra and penta labeled
		16	Mono, bis, tris, tetra and penta labeled

		24 30 48	Mono, bis, tris, tetra, penta and hexa labeled Mono, bis, tris, tetra, penta and hexa labeled Mono, bis, tris, tetra, penta and hexa labeled

[a] 10 % DMSO was used to dissolve NHP.

Docking studies: The least energy conformation of N-hydroxyphthalimide (NHP) is stabilized by 4-H bonds involving interaction with Ser 15, Ser 16, and Arg 33 (Figure S2).

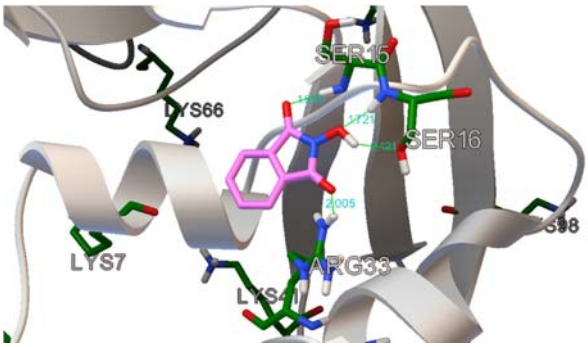


Figure S2. NHP conformation ranked 1 with RNase A (pdb: 2AAS)

Lys 1 is the most solvent exposed lysine and is expected to give kinetically oriented products. However, Lys 41 with several conformations in the vicinity is more likely to react with NHP (Figure S3). Lys 66 ranks second in terms of possible reaction site. Lys 7 also exists in the same domain as Lys 41 and Lys 66 but is less accessible (Figure S4).

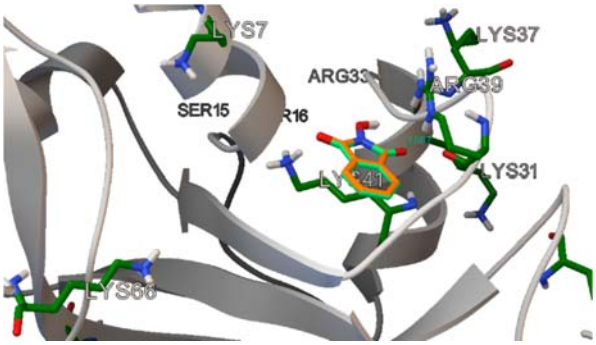


Figure S3. Lys 41 with conformations ranked 6 and 8

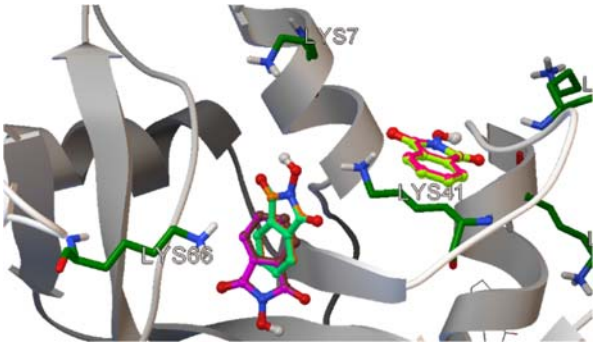
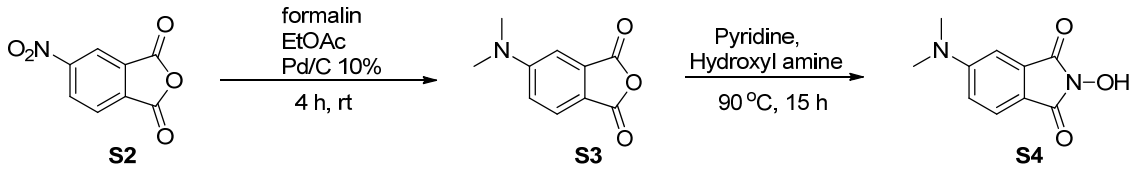
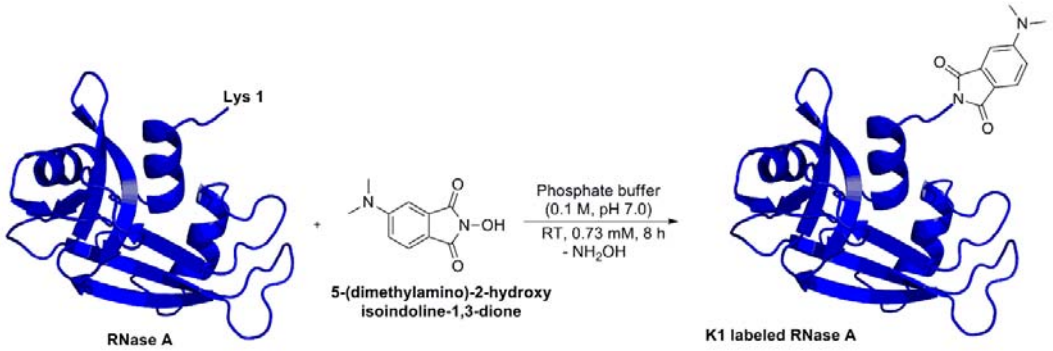


Figure S4. Lys 7, 41 and 66 with conformations ranked 2,3,5,6,7 and 8



Scheme S1. Synthesis of 4-N,N-dimethylamino-N-hydroxypthalimide **S4**



Scheme S2. Synthesis of fluorophore labeled RNase A

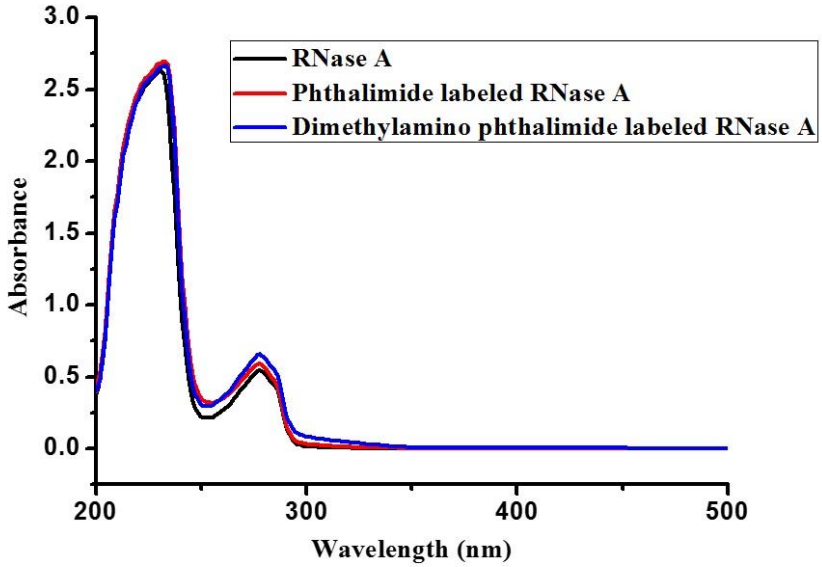


Figure S5: UV spectra of RNase A (black line), phthalimide labeled RNase A (red line) and dimethylaminophthalimide labeled RNase A (blue line) in phosphate buffer (0.1 M, pH 7.0) at concentration 1 mg/ml.

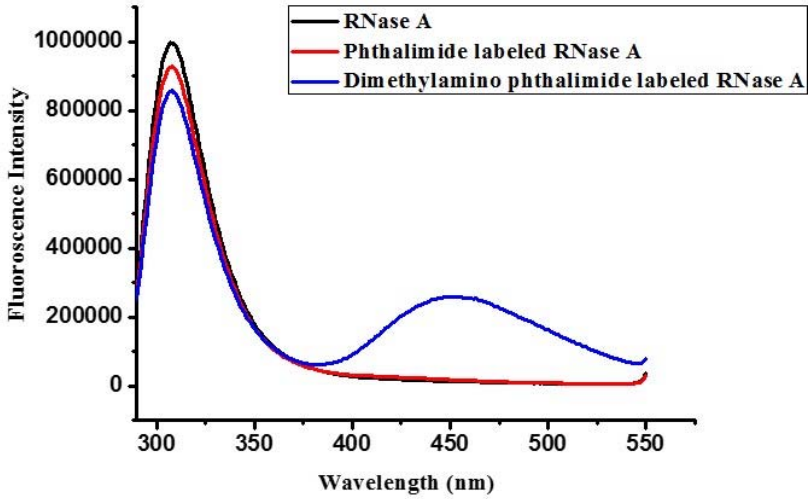


Figure S6: Fluorescence emission spectra of RNase A (black line), phthalimide labeled RNase A (red line) and dimethylaminophthalimide labeled RNase A (blue line) in phosphate buffer (0.1 M, pH 7.0) at concentration 1 mg/ml.

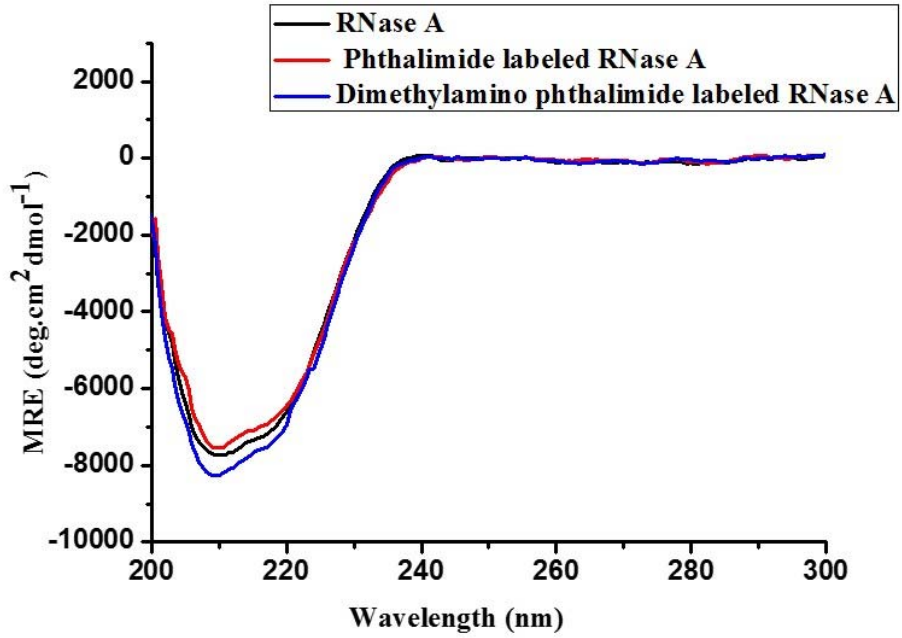
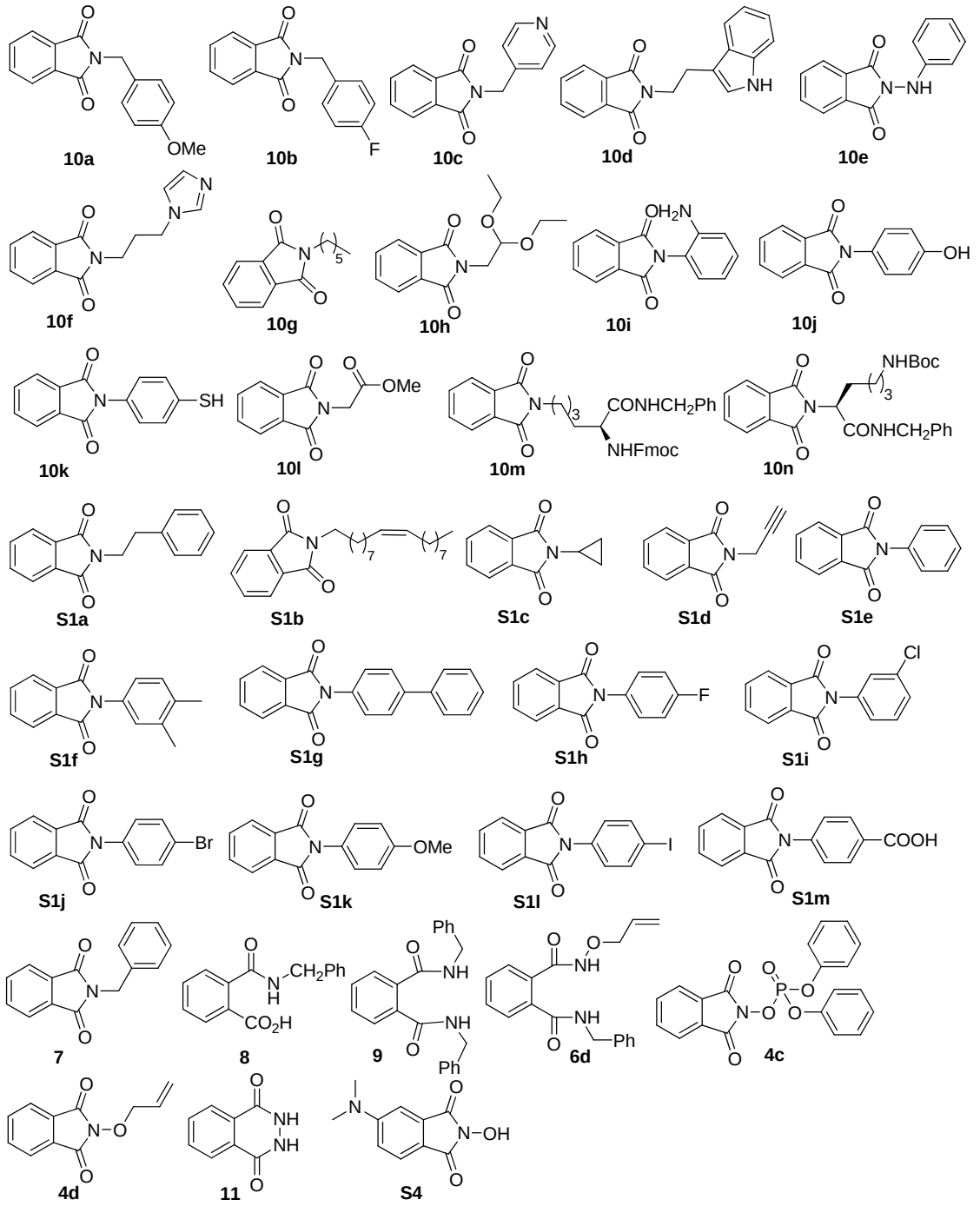


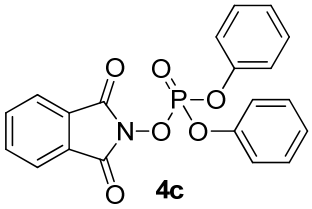
Figure S7: Circular Dichroism (CD) spectra of RNase A (black line), phthalimide labeled RNase A (red line) and dimethylaminophthalimide labeled RNase A (blue line) in phosphate buffer (0.1 M, pH 7.0) at concentration 0.01 mg/ml.

Legend


10a
10b
10c
10d
10e
10f
10g
10h
10i
10j
10k
10l
10m
10n
S1a
S1b
S1c
S1d
S1e
S1f
S1g
S1h
S1i
S1j
S1k
S1l
S1m
7
8
9
6d
4c
4d
11
S4

Compound characterization

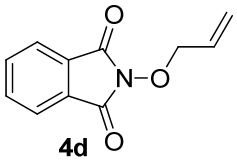
1,3-dioxoisoindolin-2-yl diphenyl phosphate



4c

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.88 (dd, *J*=5.5, 3.1 Hz, 2H), 7.79 (dd, *J*=5.5, 3.1 Hz, 2H), 7.40-7.42 (m, 8H) 7.24-7.27 (m, 2H) ppm. ^{31}P NMR ($CDCl_3$, 161 MHz) δ: -11.72 ppm. MS (ESI) $[MH]^+$ calcd. $C_{20}H_{15}NO_6P$ 396.06, found 396.10

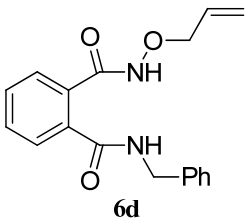
2-(allyloxy)isoindoline-1,3-dione



4d

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (dd, *J*=5.4, 3.1 Hz, 2H), 7.76 (dd, *J*=5.5, 3.1 Hz, 2H), 6.13(ddt, *J*=7.10, 10.1, 6.8 Hz, 1H), 5.38 (dd, *J*=20.7, 5.0 Hz, 2H), 4.71 (d, *J*=6.8 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.7, 134.5, 131.3, 128.8, 123.5, 122.6, 78.8 ppm. HRMS (ESI) $[MH]^+$ calcd. $C_{11}H_{10}NO_3$ 204.0661, found 204.0724

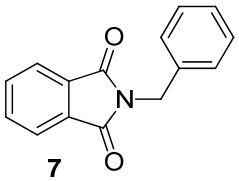
N^1-(allyloxy)-N^2-benzylphthalamide



6d

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.70 (s, 1H), 7.67 (dd, *J*=5.5, 3.4 Hz, 2H), 7.60-7.54 (dd, *J*=5.6, 3.3 Hz, 2H), 7.49 (d, *J*=3.6, 2H), 7.35 (m, 3H), 7.32-7.25 (m, 2H), 6.73 (s, 1H), 6.01 (s, 1H), 4.52 (d, 2H), 5.46-5.26 (m, 2H), 4.59 (d, *J*=10.1 Hz, 2H), 4.6(d, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.1, 166.8, 137.5, 134.4, 132.1, 131.6, 130.7, 130.5, 129.4, 128.8, 128.2, 127.7, 120.7, 44.3 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{18}H_{18}N_2O_3Na$ 333.1215, found 333.1198

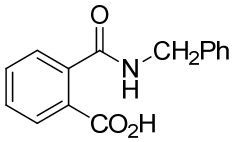
2-benzylisoindoline-1,3-dione :



7

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (dd, *J*=5.4, 3.1 Hz, 2H), 7.71 (dd, *J*=5.4, 3.0 Hz, 2H), 7.43 (dd, *J*=5.4, 3.0 Hz, 2H), 7.35-7.22 (m, 3H), 4.85 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.1, 136.4, 134.0, 132.6, 128.7, 128.6, 127.8, 123.4, 41.6 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{15}H_{11}NO_2Na$ 260.0687, found 260.0698

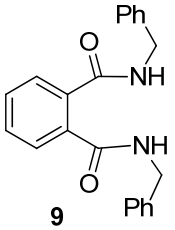
2-(benzylcarbamoyl)benzoic acid



8

^{1}H NMR (D_2O, 400 MHz) δ: 7.58 (d, *J*=7.3 Hz, 1H), 7.54-7.48 (m, 1H), 7.47-7.44 (m, 2H), 7.42 (d, *J*=4.5Hz, 2H), 7.34 (dq, *J*=8.6, 4.2 Hz, 1H), 4.53 (s, 2H) ppm. ^{13}C NMR (D_2O, 100 MHz) δ: 176.0, 172.8, 138.0, 137.7, 134.3, 130.4, 129.4, 128.8, 127.9, 127.5, 127.4, 127.2, 43.5 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{15}H_{14}NO_3$ 256.0960, found 256.0974

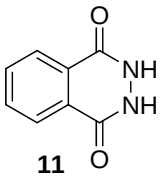
N^1,N^2-dibenzylphthalamide



9

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.60 (dd, *J*=5.5, 3.4 Hz, 2H), 7.46 (dd, *J*=5.6, 3.3 Hz, 2H), 7.39-7.21 (m, 5H), 6.96 (s, 1H), 4.52 (d, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.0, 137.7, 134.5, 130.4, 128.8, 128.4, 127.9, 127.6, 44.2 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{22}H_{20}N_2O_2Na$ 367.1422, found 367.1413

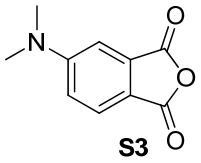
2,3-dihydrophthalazine-1,4-dione



11

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.07 (dd, *J*=5.7, 3.4 Hz, 2H), 7.88 (dd, *J*=5.7, 3.4 Hz, 2H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 155.2, 133.0, 127.7, 125.6 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_8H_7N_2O_2$ 163.0508, found 163.0499

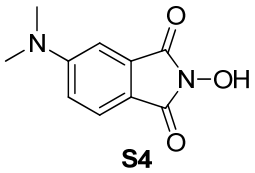
5-(dimethylamino)isobenzofuran-1,3-dione



S3

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.76 (d, *J*=8.7 Hz, 1H), 7.09 (d, *J*=2.4 Hz, 1H), 6.96 (dd, *J*=8.7 Hz, 2.4 Hz, 1H), 3.17 (s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 164.5, 163.2, 155.3, 134.2, 127.0, 117.2, 115.8, 106.4, 40.6 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{10}H_{10}NO_3$ 191.05, found 191.10

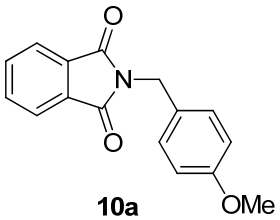
5-(dimethylamino)-2-hydroxyisoindoline-1,3-dione



S4

^{1}H NMR ($CDCl_3$, 500 MHz) δ: 7.63 (d, *J*=8.5 Hz, 1H), 7.08 (d, *J*=2.4 Hz, 1H), 6.78 (dd, *J*=8.6 Hz, 2.5 Hz, 1H), 3.12 (s, 6H) ppm. ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 164.9, 164.8, 154.5, 131.6, 125.4, 114.5, 113.6, 106.3, 40.5 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{10}H_{11}N_2O_3$ 207.0748, found 207.0764

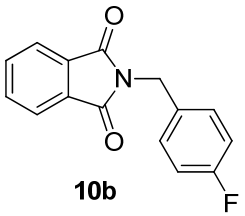
2-(4-methoxybenzyl)isoindoline-1,3-dione



10a

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.82 (dd, *J*=5.4, 3.1 Hz, 2H), 7.68 (dd, *J*=5.4, 3.0 Hz, 2H), 7.38 (d, *J*=8.6 Hz, 2H), 6.83 (d, *J*=8.7 Hz, 2H), 4.78 (s, 2H), 3.76 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.1, 159.2, 133.9, 132.2, 130.1, 128.7, 123.3, 114.0, 55.2, 41.1 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{16}H_{13}NO_3Na$ 290.0793, found 290.0792

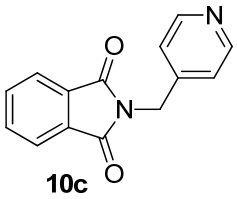
2-(4-fluorobenzyl)isoindoline-1,3-dione



10b

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (dd, *J*=5.4, 3.1 Hz, 2H), 7.70 (dd, *J*=5.4, 3.0 Hz, 2H), 7.42 (dd, *J*= 8.5, 5.4 Hz, 2H), 6.99 (*J*= 8.7 Hz, 2H), 4.81 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.8, 162.2 (d, *J*=246.4 Hz), 133.9, 132.1 (d, *J*=3.3 Hz), 131.9, 130.4 (d, *J*=8.2 Hz), 123.2, 115.4 (d, *J*=21.5 Hz), 40.7 ppm. HRMS (ESI) $[M+MeOH+Na]^+$ calcd. For $C_{16}H_{14}FNO_3Na$ 310.0855, found 310.0856

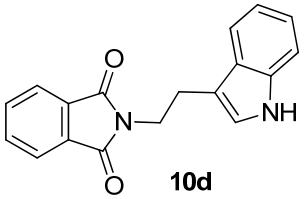
2-(pyridin-4-ylmethyl)isoindoline-1,3-dione



10c

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.57 (d, *J*=3.6, 2H), 7.89 (dd, *J*=5.4, 3.1 Hz, 2H), 7.75 (dd, *J*=5.4, 3.1 Hz, 2H), 7.30 (d, *J*=5.5 Hz, 2H), 4.85 (s, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.7, 150.2, 144.9, 134.3, 131.9, 123.6, 123.0, 40.5 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_{11}N_2O_2$ 239.0821, found 239.0809

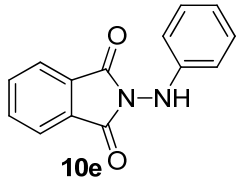
2-(2-(1H-indol-3-yl)ethyl)isoindoline-1,3-dione



10d

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.03 (bs, 1H), 7.83 (dd, *J*=5.4, 3.1 Hz, 2H), 7.73 (d, *J*=7.8 Hz, 1H), 7.69 (dd, *J*=5.4, 3.0 Hz, 2H), 7.34 (d, *J*=8.0 Hz, 1H), 7.18 (t, *J*=7.1 Hz, 1H), 7.11 (m, 2H), 4.01 (t, 2H), 3.16 (t, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.4, 136.2, 133.9, 132.2, 127.4, 123.2, 122.2, 122.0, 119.5, 118.9, 112.50, 111.1, 38.5, 24.5 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{18}H_{14}N_2O_2Na$ 313.0953, found 313.0933

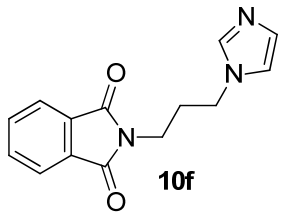
2-(phenylamino)isoindoline-1,3-dione



10e

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.92 (dd, *J*=5.4, 3.1 Hz, 2H), 7.79 (dd, *J*=5.4, 3.1 Hz, 2H), 7.22 (t, *J*=7.9 Hz, 2H), 6.94 (t, *J*=7.4 Hz, 1H), 6.82 (d, *J*=7.9 Hz, 2H), 6.29 (bs, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.4, 145.7, 134.7, 130.0, 129.3, 123.9, 122.4, 114.1 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{14}H_{10}N_2O_2Na$ 261.0640, found 261.0632

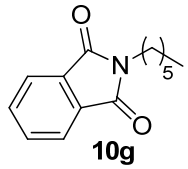
2-(3-(1H-imidazol-1-yl)propyl)isoindoline-1,3-dione



10f

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (dd, *J*=5.4, 3.1 Hz, 2H), 7.75 (dd, *J*=5.4, 3.1 Hz, 2H), 7.59 (bs, 1H), 7.06 (s, 1H), 7.00 (s, 1H), 4.02 (t, *J*=7.0 Hz, 2H), 3.74 (t, *J*=6.6 Hz, 2H), 2.19 (p, *J*=6.8 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 168.3, 137.2, 134.2, 131.8, 129.3, 123.4, 118.8, 44.6, 35.1, 29.9 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_{14}N_3O_2$ 256.1086, found 256.1093

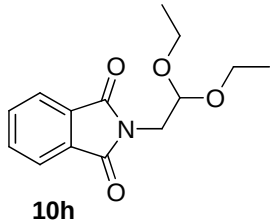
2-hexylisoindoline-1,3-dione



10g

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (dd, *J*=5.4, 3.1 Hz, 2H), 7.71 (dd, *J*=5.4, 3.0 Hz, 2H), 3.68 (t, *J*=6.8 Hz 2H), 1.67 (m, 2H), 1.36-1.26 (m, 6H), 0.88 (t, *J*=6.8 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.5, 133.8, 132.2, 123.1, 38.1, 31.4, 28.6, 26.5, 22.5, 14.0 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_{18}NO_2$ 232.1338, found 232.1313

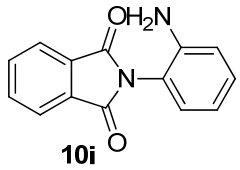
2-(2,2-diethoxyethyl)isoindoline-1,3-dione



10h

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (dd, *J*=5.4, 3.1 Hz, 2H), 7.72 (dd, *J*=5.4, 3.1 Hz, 2H), 4.89 (t, *J*=5.8 Hz, 1H), 3.84 (d, *J*=5.8 Hz, 2H), 3.74 (m, 2H), 3.55 (m, 2H), 1.16 (t, *J*=7.0 Hz, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.1, 134.0, 132.0, 123.3, 98.5, 61.7, 39.8, 15.2 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{14}H_{17}NO_4Na$ 286.1055, found 286.1034

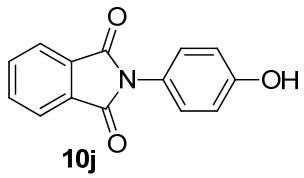
2-(2-aminophenyl)isoindoline-1,3-dione



10i

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.96 (dd, *J*=5.4, 3.1 Hz, 2H), 7.80 (dd, *J*=5.4, 3.1 Hz, 2H), 7.23-7.30 (m, 1H), 7.13 (m, 1H), 6.91 (dd, *J*=11.8, 4.4 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.5, 143.5, 134.5, 132.2, 130.3, 129.3, 124.0, 119.5, 118.2, 118.0 ppm. HRMS (ESI) $[MNa]^+$ calcd. For $C_{14}H_{10}N_2O_2Na$ 261.0640, found 261.0622

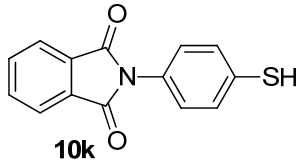
2-(4-hydroxyphenyl)isoindoline-1,3-dione



10j

^{1}H NMR (DMSO-d_6, 400 MHz) δ: 7.94 (m, 2H), 7.89 (m, 2H), 7.21 (d, *J*=8.6 Hz, 2H), 6.87 (d, *J*=8.6 Hz, 2H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 167.4, 157.3, 134.6, 131.6, 128.8, 123.3, 122.8, 115.4 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_{10}NO_3$ 240.0661, found 240.0775

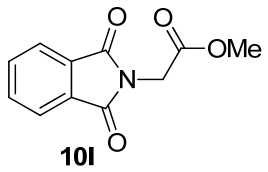
2-(4-mercaptophenyl)isoindoline-1,3-dione



10k

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.95 (dd, *J*=5.4, 3.0 Hz, 2H), 7.79 (dd, *J*=5.4, 3.0 Hz, 2H), 7.40 (d, *J*=8.5 Hz, 2H), 7.33 (d, *J*=8.5 Hz, 2H), 3.54 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.5,134.8, 132.0, 131.6, 130.2, 129.7, 127.4, 124.1 ppm. MS (ESI) $[MH]^+$ calcd. 256. 04, found 256.10

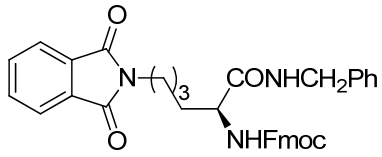
Methyl 2-(1,3-dioxoisoindolin-2-yl)acetate



10l

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.86 (dd, *J*=5.5, 3.1 Hz, 2H), 7.72 (dd, *J*=5.5, 3.1 Hz, 2H), 4.42 (s, 2H), 3.74 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.7, 167.4, 134.3, 132.0, 123.6, 52.7, 38.8 ppm. MS (ESI) $[MH]^+$ calcd. 219.05, found 219.10

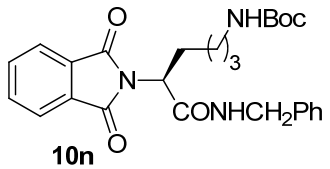
(S)-(9H-fluoren-9-yl)methyl (1-(benzylamino)-6-(1,3-dioxoisoindolin-2-yl)-1-oxohexan-2-yl)carbamate



10m

^{1}H NMR (DMSO-d_6, 700 MHz) δ: 7.89 (m, 2H), 7.83 (dd, *J*=5.4, 3.0 Hz, 2H), 7.80 (dd, *J*=5.4, 3.0 Hz, 2H), 7.72-7.70 (m, 2H), 7.50 (d, *J*=8.1 Hz, 1H), 7.41 (m, 2H), 7.33-7.27 (m, 3H), 7.12-7.19 (m, 3H), 4.26-4.22 (m, 2H), 4.21-4.27 (m, 2H), 4.0-3.96 (m, 1H), 3.55 (t, *J*=7.1 Hz, 1H)1.69-1.64 (m, 1H), 1.62-1.53 (m, 3H), 1.38-1.31 (m, 2H), 1.30-1.24 (m, 2H) ppm. ^{13}C NMR (DMSO-d_6, 175 MHz) δ: 172.0, 168.0, 156.0, 143.8, 140.7, 139.4, 134.4, 131.6, 128.7, 127.7, 127.1, 126.7, 125.4, 123.0, 120.1, 65.6, 54.5, 46.7, 42.0, 37.4, 31.6, 27.7, 23.0 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{36}H_{34}N_3O_5$ 588.2498, found 588.2522

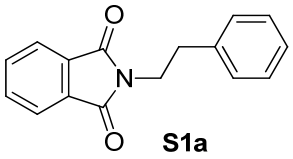
(S)-tert-butyl (6-(benzylamino)-5-(1,3-dioxoisoindolin-2-yl)-6-oxohexyl)carbamate



10n

^{1}H NMR (CD_3OD, 500 MHz) δ: 7.90-7.86 (m, 2H), 7.84-7.81 (m, 2H), 7.30-7.25 (m, 4H), 7.23-7.19 (m, 1H), 4.84-4.81 (m, 1H), 4.43-4.34 (m, 2H), 3.02-2.94 (m, 2H), 2.39-2.31 (m, 1H), 2.23-2.16 (m, 1H), 1.57-1.42 (m, 2H), 1.36 (s, 9H) ppm. ^{13}C NMR (CD_3OD, 125 MHz) δ: 170.2, 168.1, 157.1, 138.5, 134.2, 131.9, 128.0, 127.0, 126.7, 122.9, 78.4, 53.6, 43.0, 39.5, 29.0, 27.9, 27.3, 23.4 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{26}H_{32}N_3O_5$ 466.2342, found 466.2363

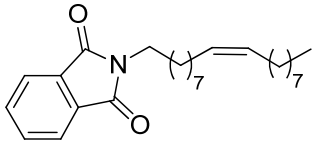
2-phenethylisoindoline-1,3-dione



S1a

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.83 (dd, *J*=5.4, 3.1 Hz, 2H), 7.70 (dd, *J*=5.4, 3.0 Hz, 2H), 7.41-7.13 (m, 5H), 3.93 (t, 2H), 2.99 (t, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.2, 138.0, 133.9, 132.1, 128.9, 128.6, 126.6, 123.2, 39.3, 34.6 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{16}H_{14}NO_2$ 252.1025, found 252.1013

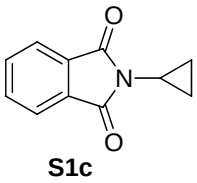
(Z)-2-(octadec-9-en-1-yl)isoindoline-1,3-dione



S1b

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.84 (dd, *J*=7.2, 4.9 Hz, 2H), 7.70 (dd, *J*=5.4, 3.0 Hz, 2H), 5.47-5.21 (m, 1 H), 3.73-3.62 (m, 2H), 1.99 (m, 4H), 1.67 (dd, 2H), 1.29 (m, 24H), 0.87 (t, *J*=6.8 Hz 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.5, 133.8, 132.2, 129.9, 129.8, 123.1, 38.1, 31.9, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2 (d, *J*=4.3 Hz, 1H), 28.6, 27.2 (d, *J*=2.8 Hz, 1H), 26.9, 22.7, 14.1, 1.0 ppm. HRMS (ESI) $[M-H]^+$ calcd. For $C_{26}H_{38}NO_2$ 396.2903, found 396.2890

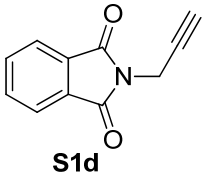
2-cyclopropylisoindoline-1,3-dione



S1c

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.82 (dd, *J*=5.5, 3.0 Hz, 2H), 7.71 (dd, *J*=5.5, 3.0 Hz, 2H), 2.75-2.68 (m, 1H), 1.01 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.9, 134.0, 131.80, 123.1, 20.9, 5.2 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{11}H_{10}NO_2$ 188.0712, found 188.0720

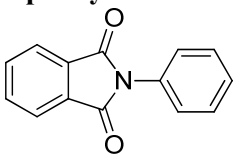
2-(prop-2-yn-1-yl)isoindoline-1,3-dione



S1d

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.89 (dd, *J*=5.3, 3.1 Hz, 2H), 7.75 (dd, *J*=5.3, 3.0 Hz, 2H), 4.46 (d, *J*=2.3 Hz, 2H), 2.23 (t, *J*=2.3 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.0, 134.2, 132.0, 123.6, 77.2, 71.5, 27.0 ppm.

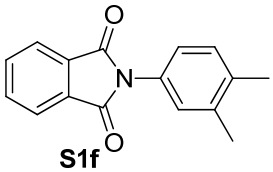
2-phenylisoindoline-1,3-dione



S1e

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.96 (dd, *J*=5.4, 3.0 Hz, 2H), 7.80 (dd, *J*=5.4, 3.0 Hz, 2H), 7.52 (d, *J*=7.0 Hz, 2H), 7.42 (m, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.6, 134.7, 132.1, 132.0, 129.5, 128.5, 126.9, 124.1 ppm. MS (ESI) $[MH]^+$ calcd. 224. 07, found 224.06

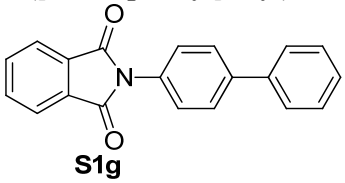
2-(3,4-dimethylphenyl)isoindoline-1,3-dione



S1f

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.92 (dd, *J*=5.3, 3.1 Hz, 2H), 7.76 (dd, *J*=5.3, 3.0 Hz, 2H), 7.25 (d, *J*=7.9 Hz, 1H), 7.18 (s, 1H), 7.14 (d, *J*=7.9 Hz, 1H), 2.30 (bd, 6H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.6, 137.7, 137.1, 134.3, 131.9, 130.3, 129.2, 127.8, 124.2, 123.7, 20.0, 19.6 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{16}H_{14}NO_2$ 252.1025, found 252.1020

2-([1,1'-biphenyl]-4-yl)isoindoline-1,3-dione

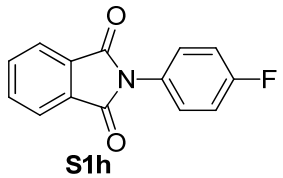


S1g

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.97 (dd, *J*=5.3, 3.0 Hz, 2H), 7.80 (dd, *J*=5.3, 3.0 Hz, 2H), 7.71 (d, *J*=8.4 Hz, 2H), 7.61 (d, *J*=7.4 Hz, 2H), 7.52 (d, *J*=8.4 Hz, 2H), 7.45 (d, *J*=7.5 Hz, 2H), 7.36 (t, *J*=7.3 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.5, 141.3, 140.5, 134.6,

132.0, 131.0, 129.0, 128.0, 127.8, 127.4, 127.0, 124.0 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{20}H_{14}NO_2$ 300.1025, found 300.1028

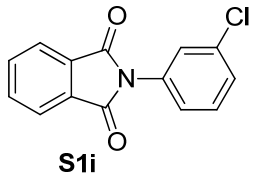
2-(4-fluorophenyl)isoindoline-1,3-dione



S1h

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.95 (dd, *J*=5.3, 3.1 Hz, 2H), 7.80 (dd, *J*=5.3, 3.1 Hz, 2H), 7.42 (m, 2H), 7.19 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.5, 162.3 (d, *J*=248.0 Hz), 134.8, 132.0, 128.7 (d, *J*=8.7 Hz), 127.9 (d, *J*=3.2 Hz), 124.1, 116.4 (d, *J*=22.9 Hz) ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_9FNO_2$ 242.0617, found 242.0618

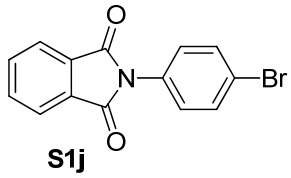
2-(3-chlorophenyl)isoindoline-1,3-dione



S1i

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.96 (dd, *J*=5.3, 3.1 Hz, 2H), 7.81 (dd, *J*=5.3, 3.1 Hz, 2H), 7.50 (t, *J*=1.8 Hz, 1H), 7.44 (dd, *J*=9.1, 6.6 Hz, 1H), 7.38 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.0, 134.8 (2C), 133.0, 131.7, 130.2, 128.3, 126.8, 124.7, 124.0 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_9ClNO_2$ 258.0322, found 258.0308

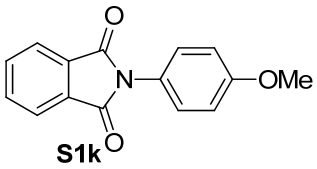
2-(4-bromophenyl)isoindoline-1,3-dione



S1j

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.96 (dd, *J*=5.4, 3.1 Hz, 2H), 7.80 (dd, *J*=5.4, 3.1 Hz, 2H), 7.63 (d, *J*=8.7 Hz, 2H), 7.36 (d, *J*=8.7 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.1, 134.7, 132.4, 131.8, 130.9, 128.1, 124.0, 122.0 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_9BrNO_2$ 301.9817, found 301.9803

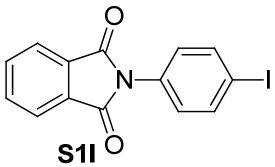
2-(4-methoxyphenyl)isoindoline-1,3-dione



S1k

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.93 (dd, *J*=5.3, 3.0 Hz, 2H), 7.77 (dd, *J*=5.3, 3.0 Hz, 2H), 7.34 (d, *J*=8.9 Hz, 2H), 7.02 (d, *J*=8.9 Hz, 2H), 3.85 (s, 3H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.9, 159.6, 134.7, 132.6, 124.6, 124.0, 114.8, 55.9 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{15}H_{12}NO_3$ 254.0817, found 254.0743

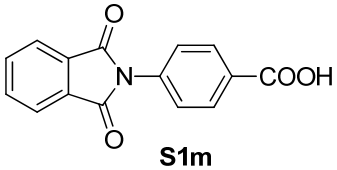
2-(4-iodophenyl)isoindoline-1,3-dione



S1l

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.96 (dd, *J*=5.4, 3.1 Hz, 2H), 7.83 (d, *J*=8.6 Hz, 2H), 7.80 (dd, *J*=5.4, 3.1 Hz, 2H), 7.23 (d, *J*=8.6 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 167.0, 138.4, 134.7, 131.8, 131.6, 128.3, 124.0, 93.4 ppm. HRMS (ESI) $[MH]^+$ calcd. For $C_{14}H_9INO_2$ 349.9678, found 349.9662

4-(1,3-dioxoisoindolin-2-yl)benzoic acid

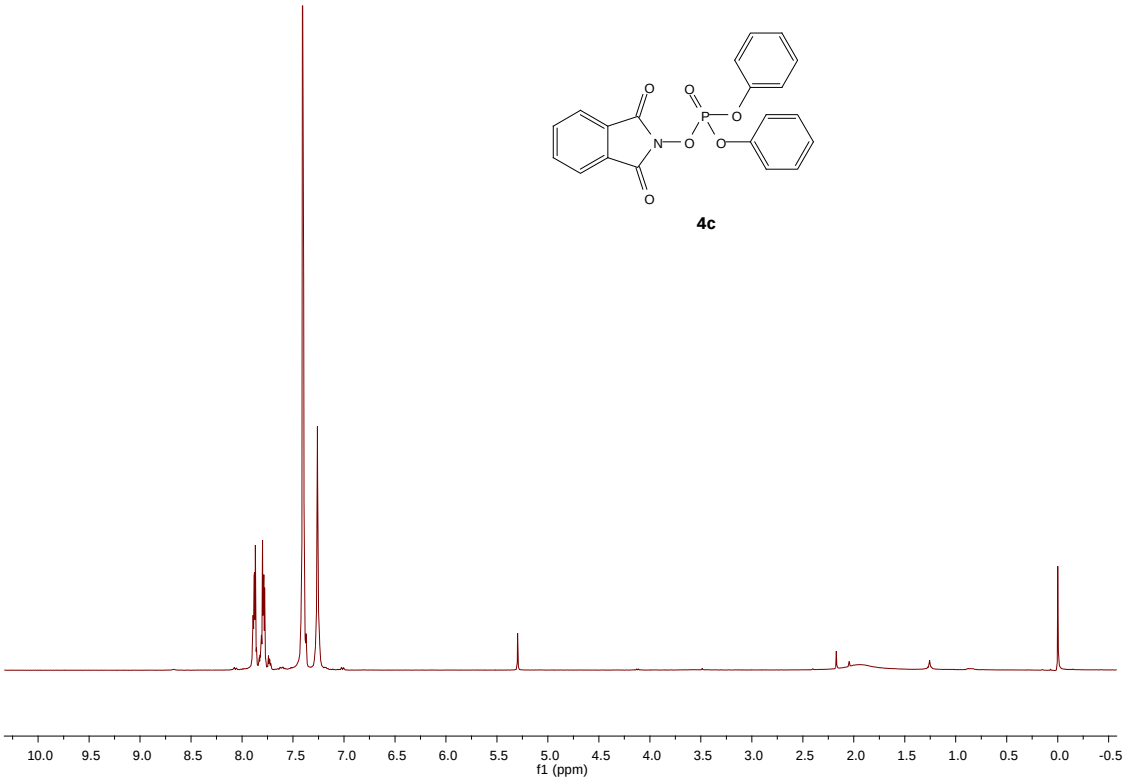


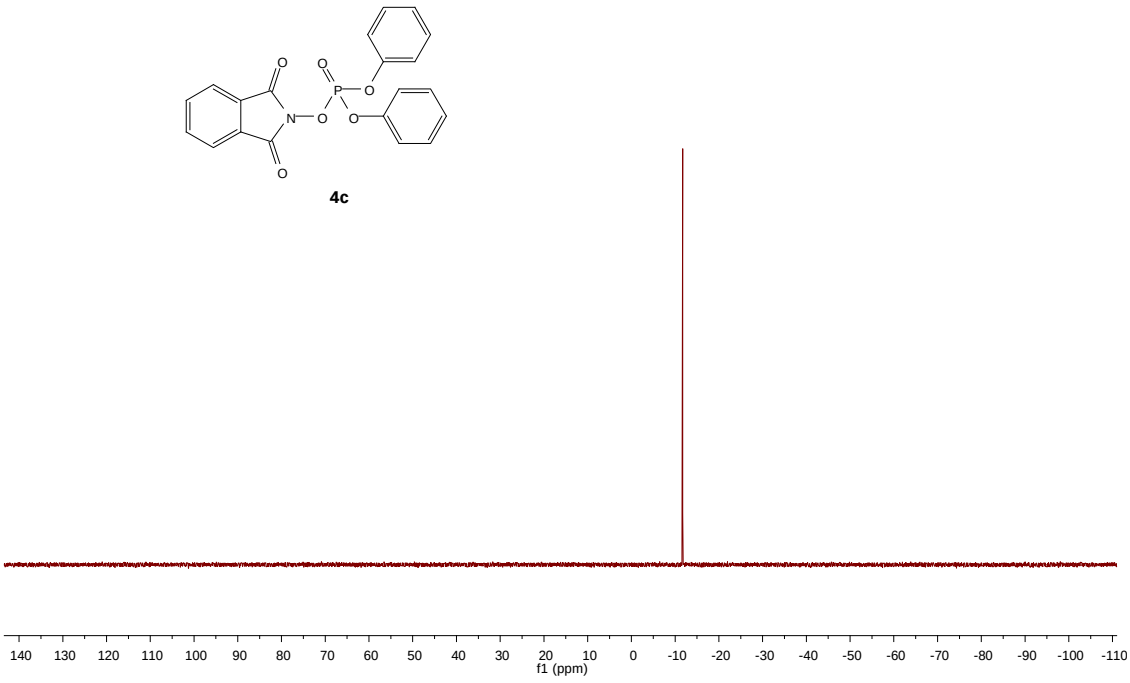
S1m

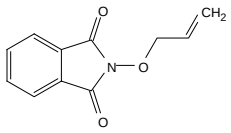
^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.09 (d, *J*=8.5 Hz, 2H), 7.99 (dd, *J*=5.4, 3.1 Hz, 2H), 7.92 (dd, *J*=5.4, 3.1 Hz, 2H), 7.61 (d, *J*=8.5 Hz, 2H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 166.8, 166.7, 135.8, 134.8, 131.5, 130.0, 129.8, 127.0, 123.6 ppm. HRMS (ESI) $[M-H]^+$ calcd. For $C_{15}H_8NO_4$ 266.0453, found 266.0452

Spectral data

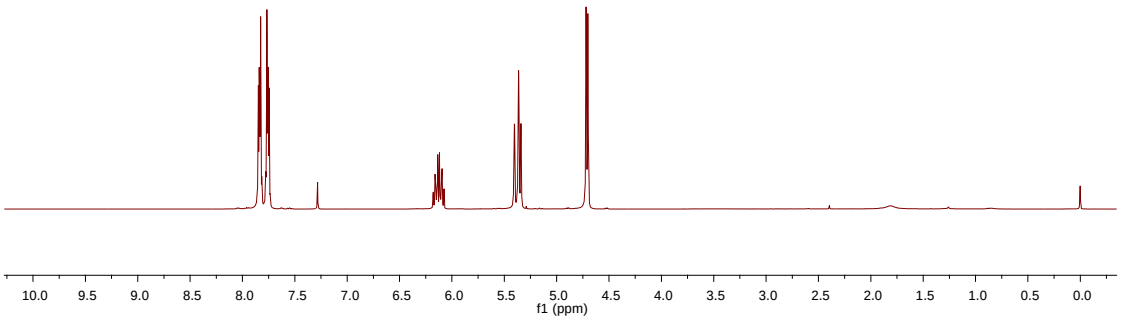
^{1}H, ^{13}C and ^{31}P spectra

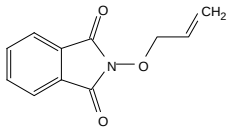





O
CH2
N
O
O

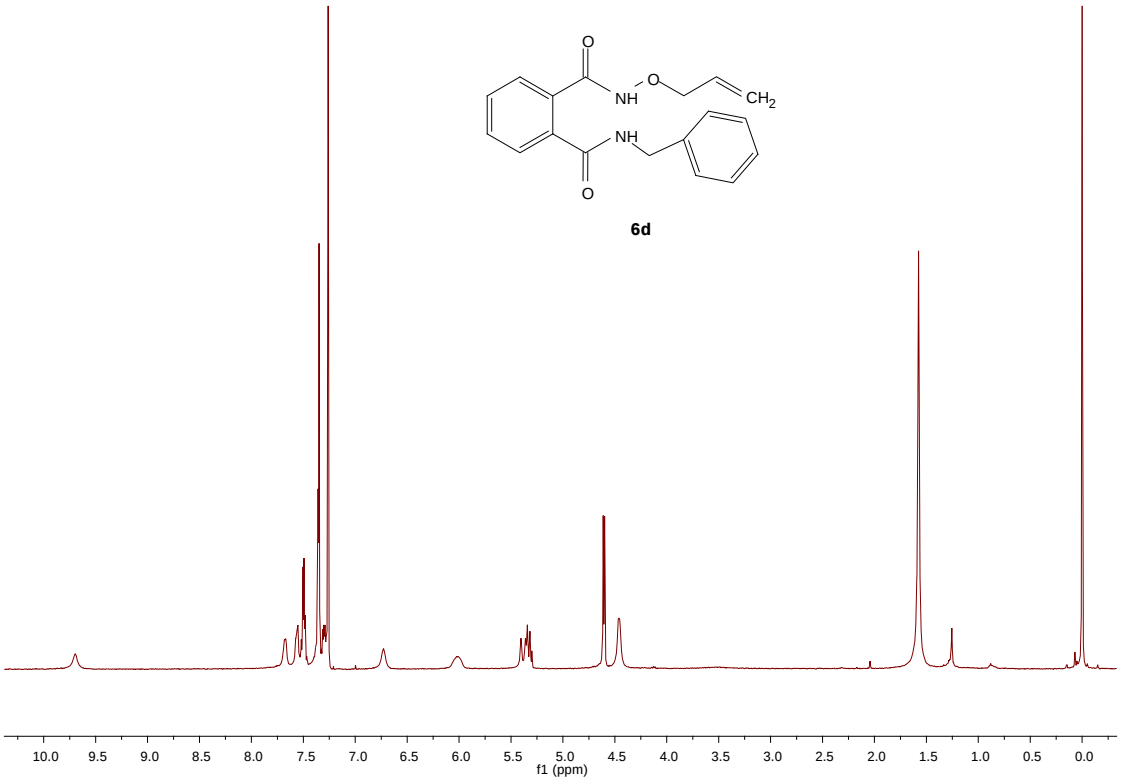
4d


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

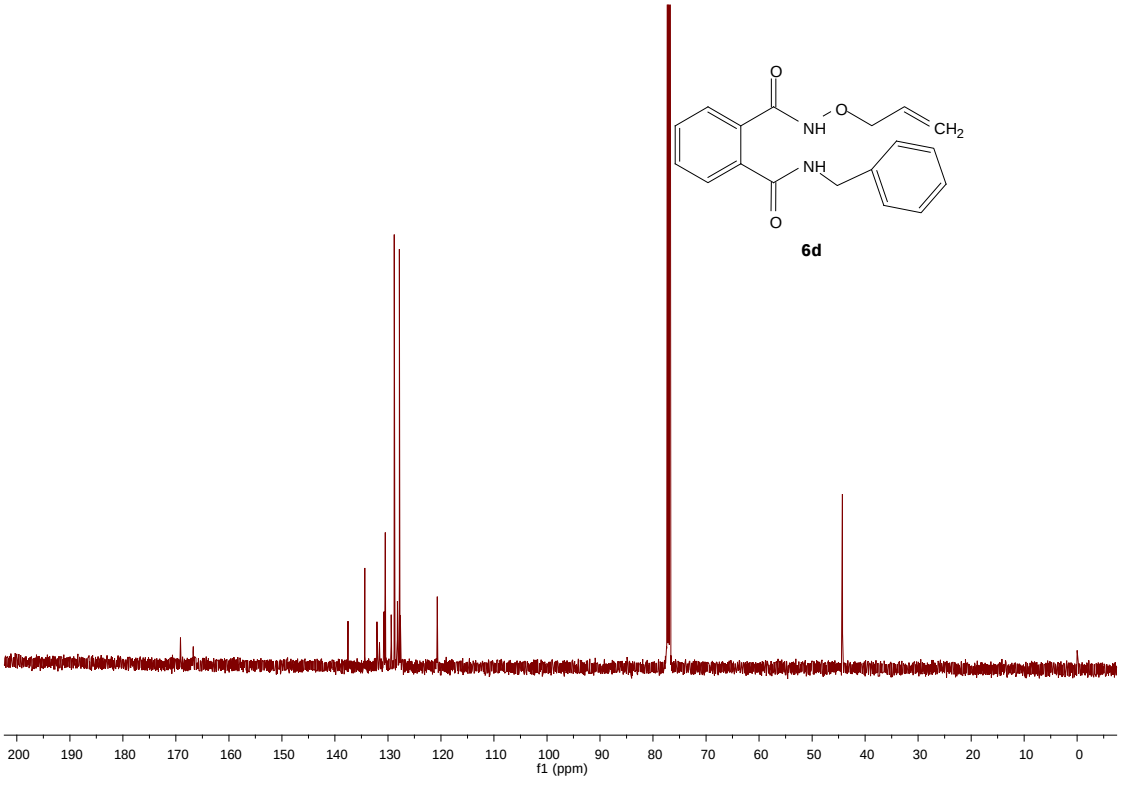

O
CH2
N
O
O

4d

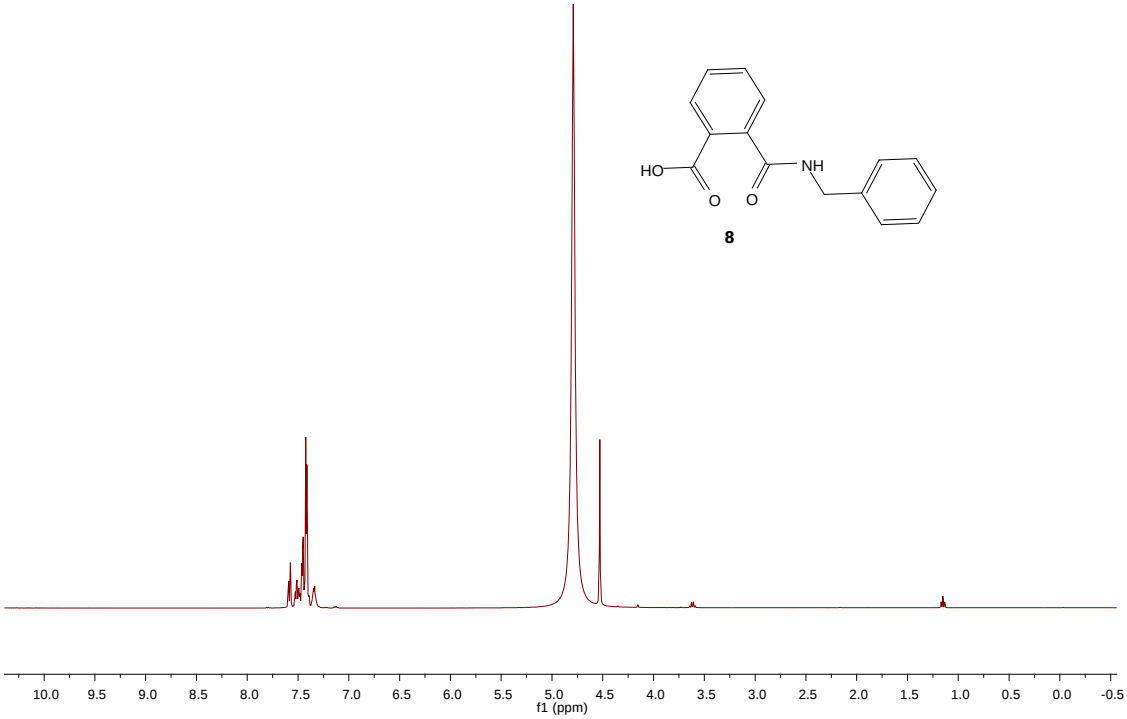
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
NH
O
CH2
NH
O
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

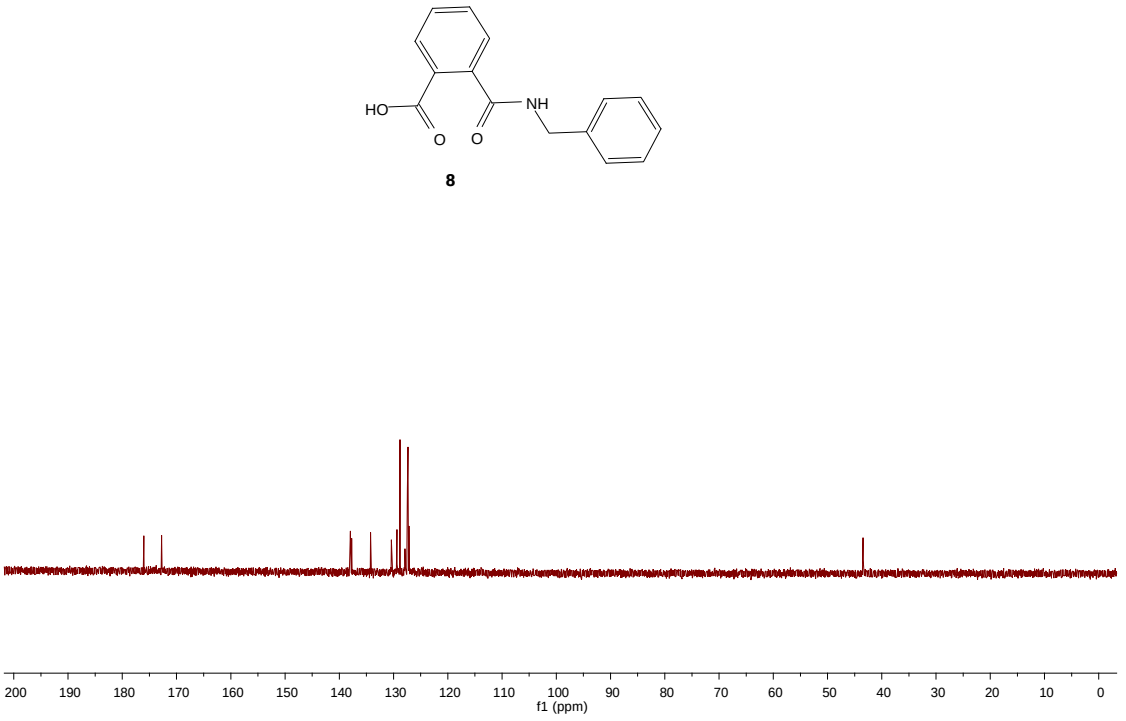
6d


O
NH
O
CH2
NH
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

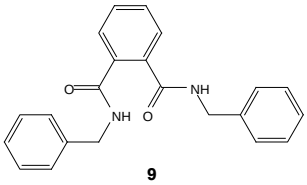
6d


HO
O
O
NH
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

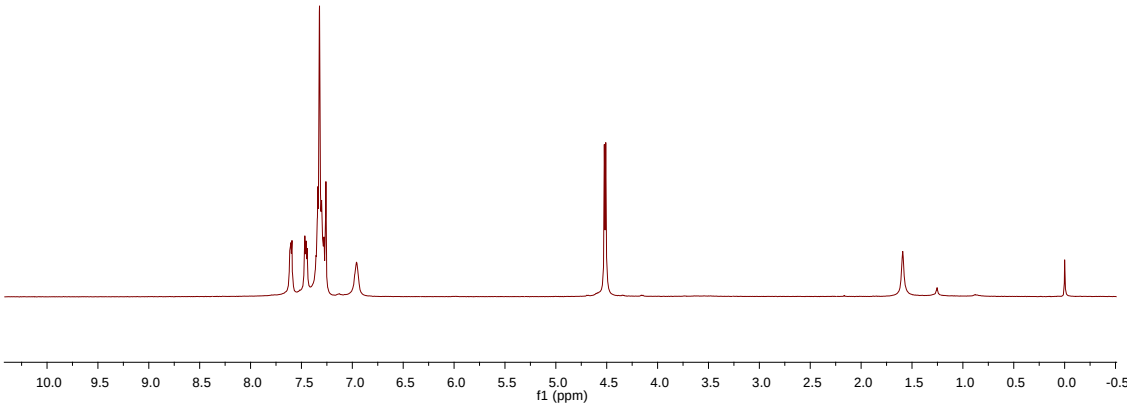
8

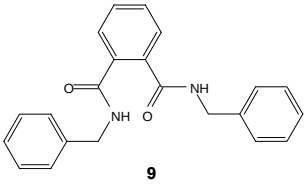

HO
O
O
NH
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

8


O
NH
NH
O

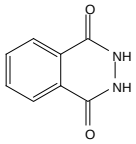
9


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

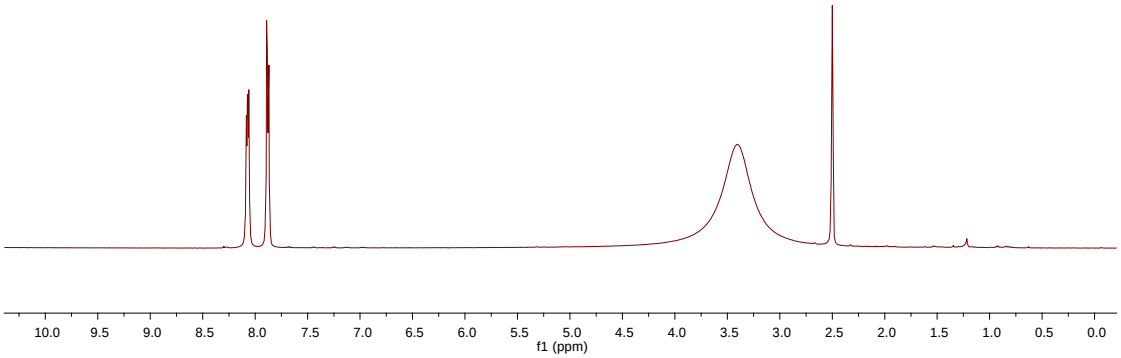

O
NH
NH
O

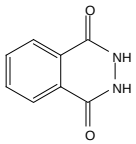
9

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
NH
NH
O

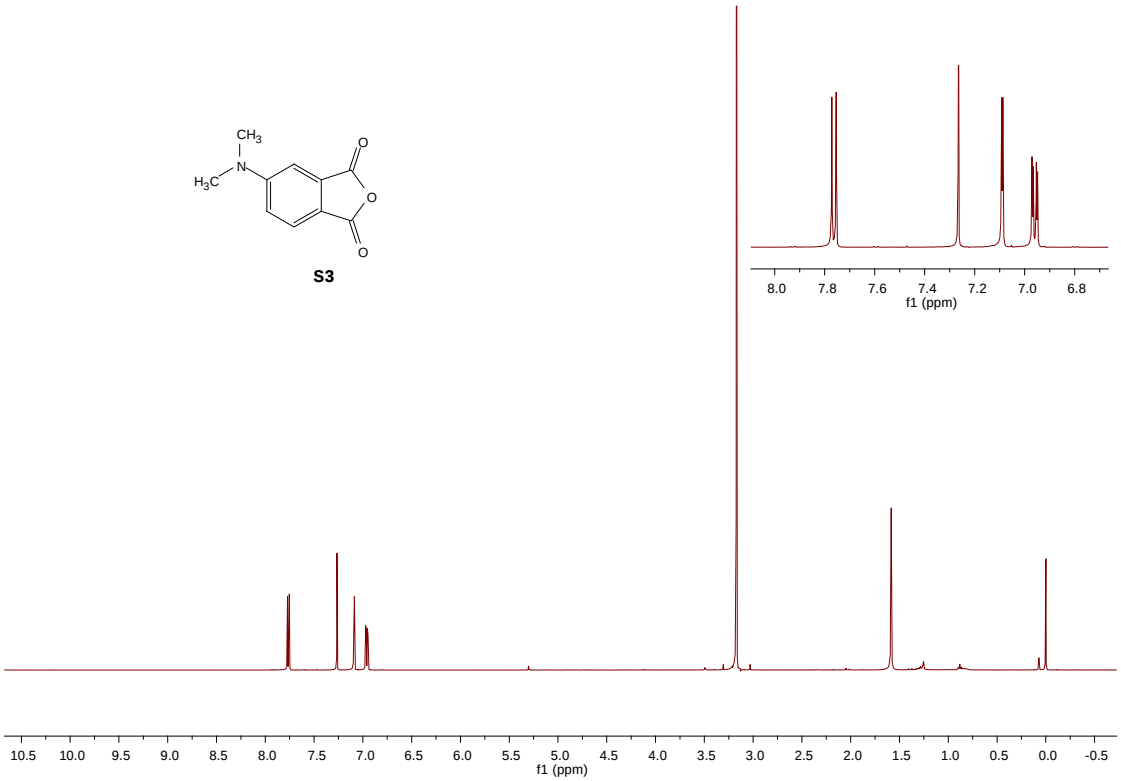
11

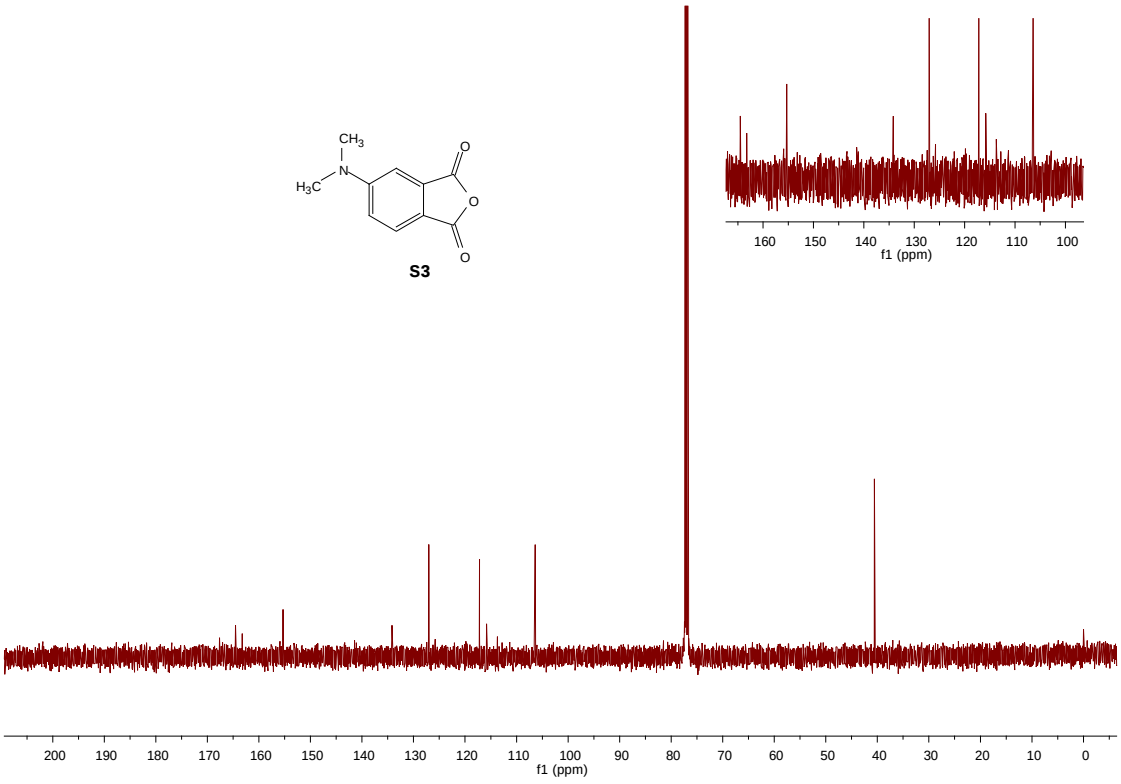

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

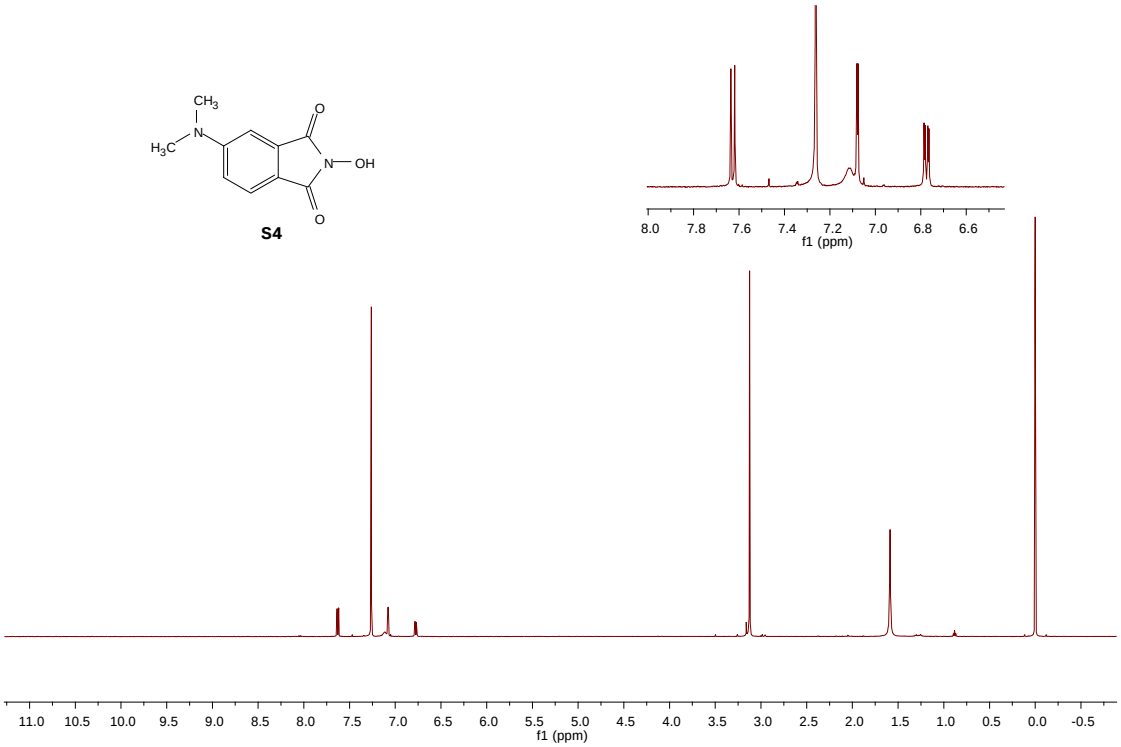

O
NH
NH
O

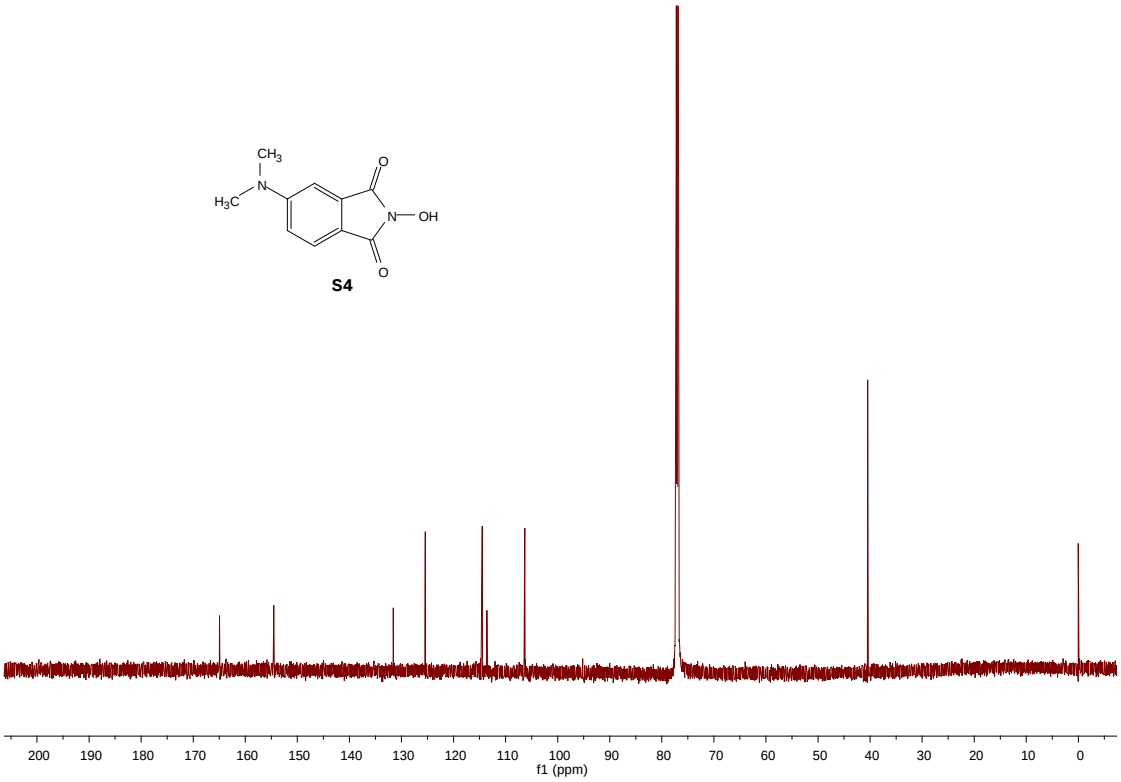
11

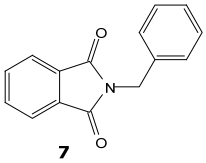
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


CH3
N
H3C
O
O
O
S3
8.0 7.8 7.6 7.4 7.2 7.0 6.8
f1 (ppm)
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

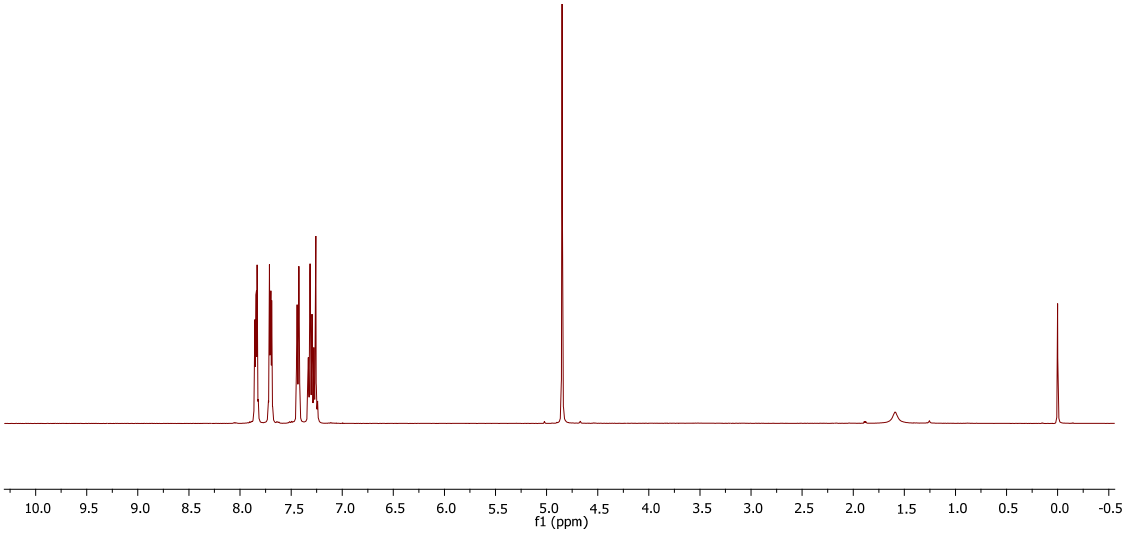

CH3
N
H3C
O
O
O
S3
160 150 140 130 120 110 100
f1 (ppm)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

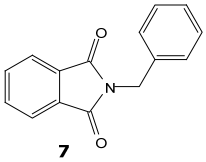

CH3
N
H3C
O
N
OH
O
S4
8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6
f1 (ppm)
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


CH3
N
H3C
O
N
OH
O
S4
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

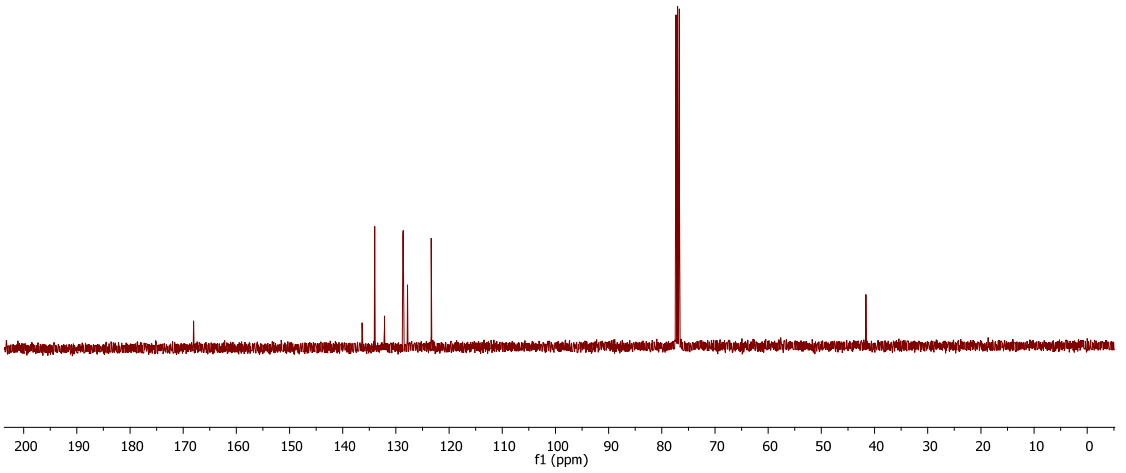

O
N
O

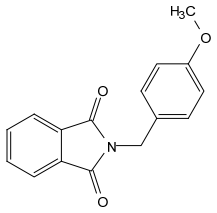
7


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


O
N
O

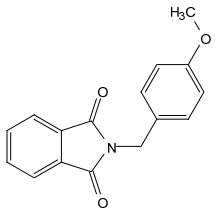
7


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

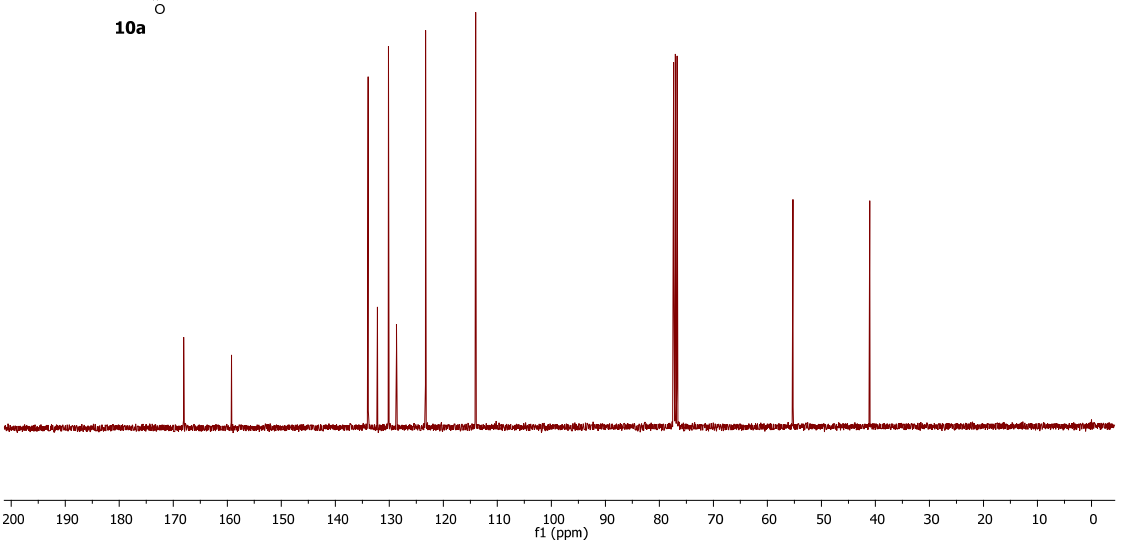

H3C
O
O
N
O

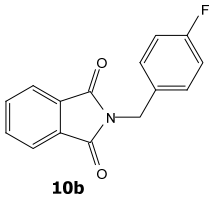
10a

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

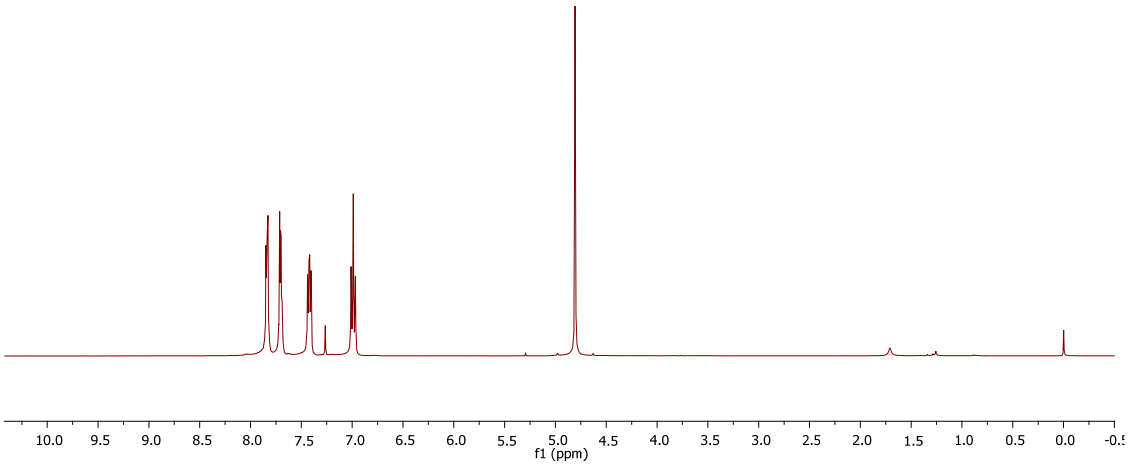

H3C
O
O
N
O

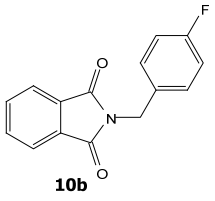
10a


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

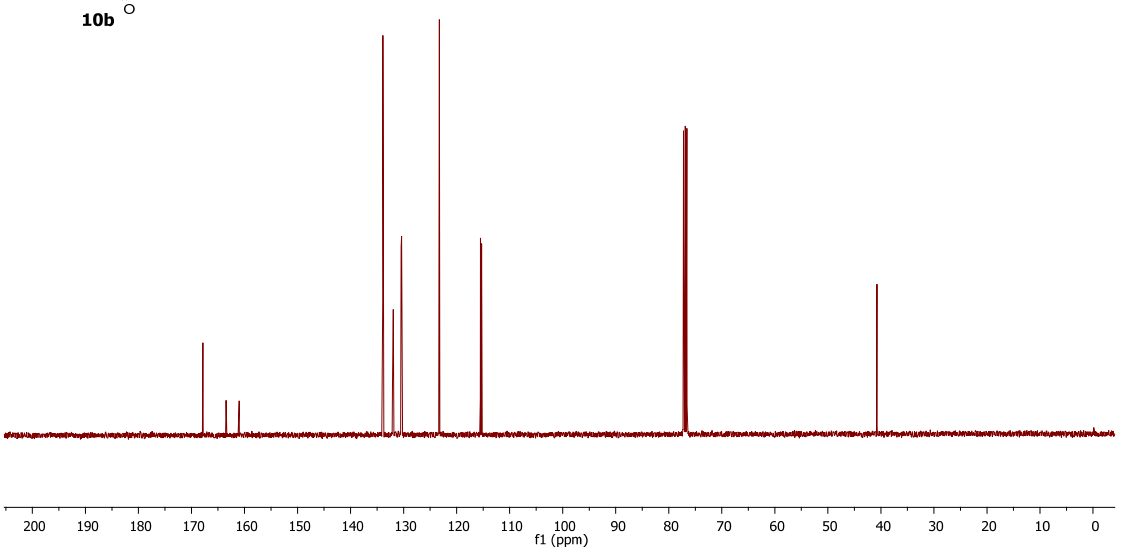

F
O
N
O

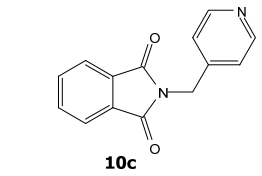
10b


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

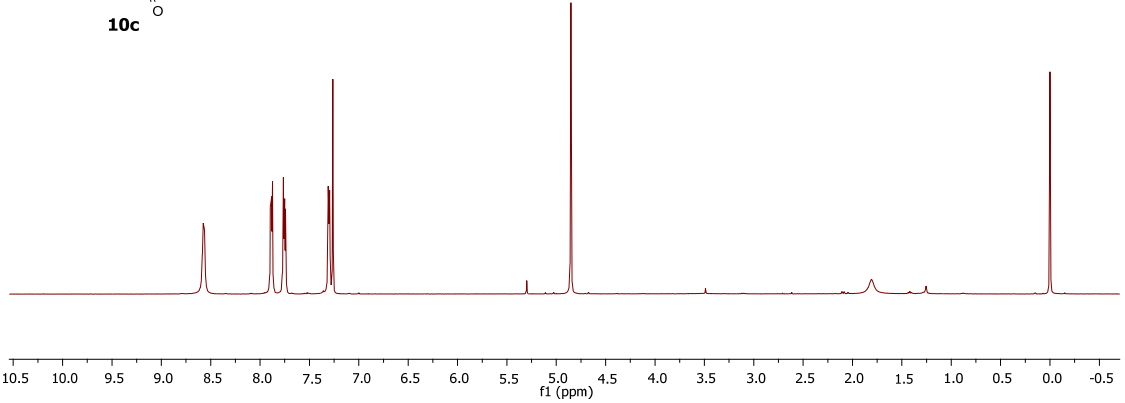

F
O
N
O

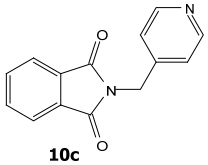
10b


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
N
O
N

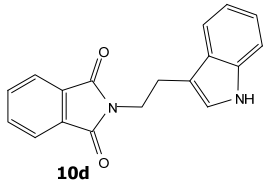
10c


10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

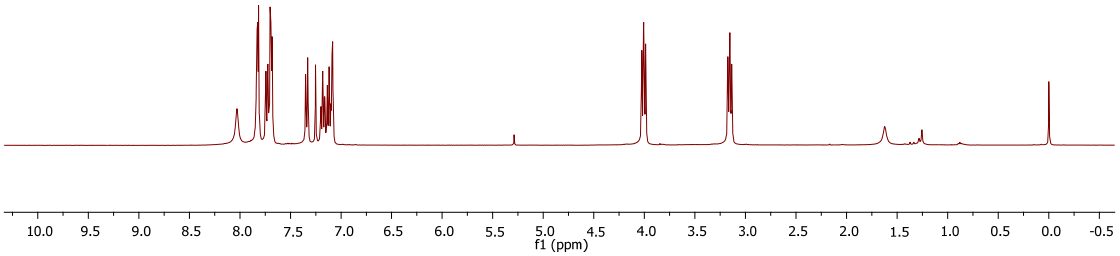

O
N
O
N

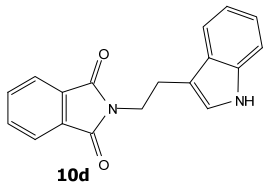
10c

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
N
O
NH

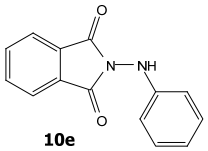
10d


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

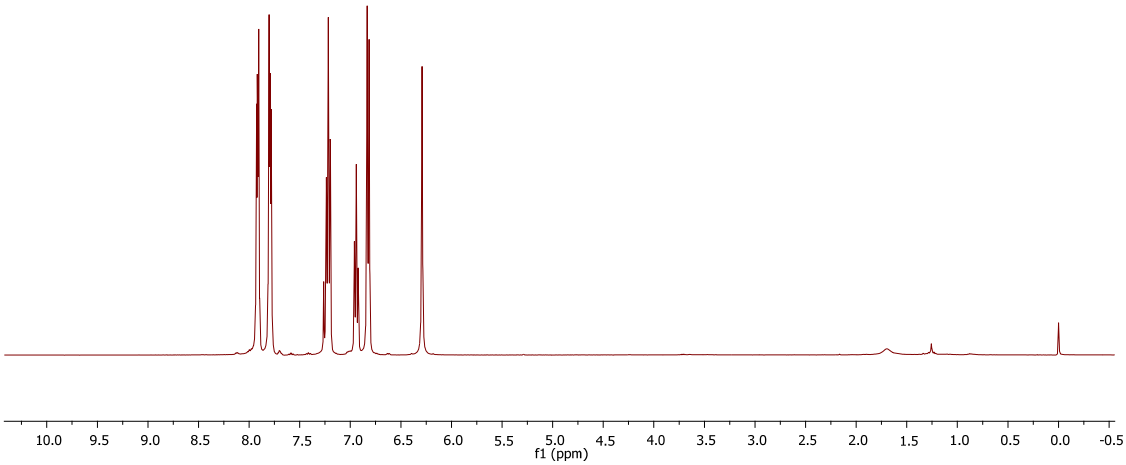

O
N
O
NH

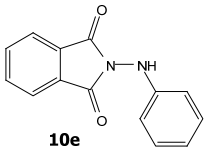
10d

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

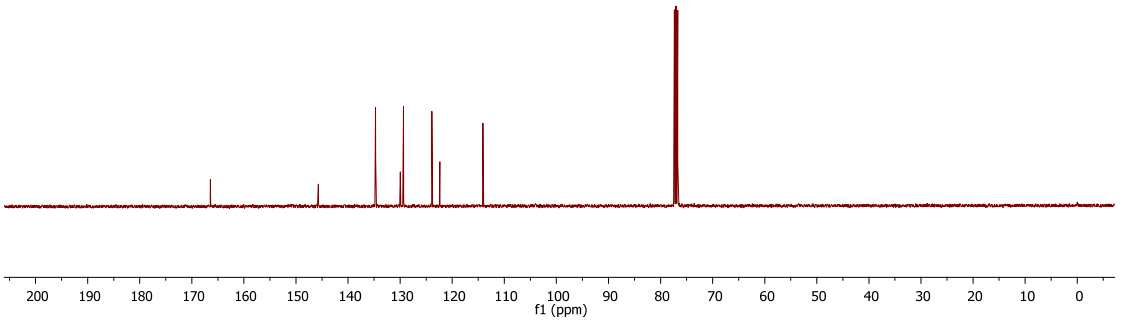

O
N
NH
O

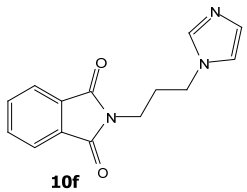
10e


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

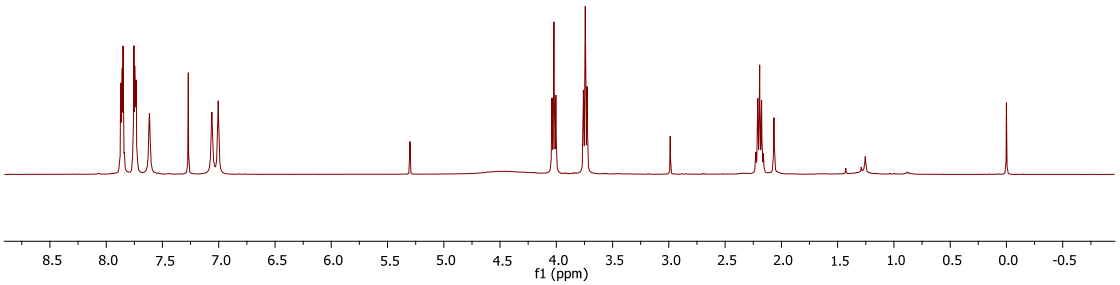

O
N
NH
O

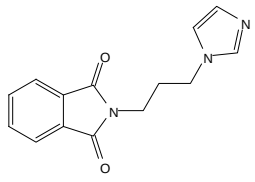
10e


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

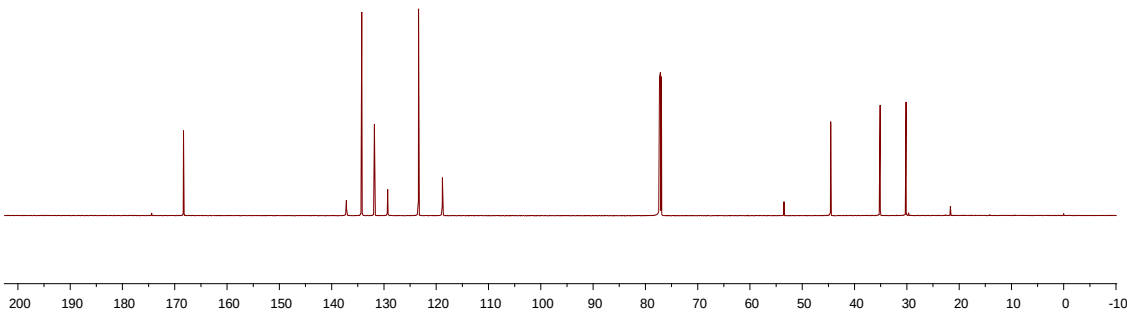


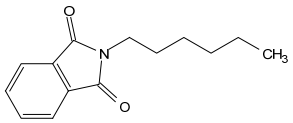
10f


8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

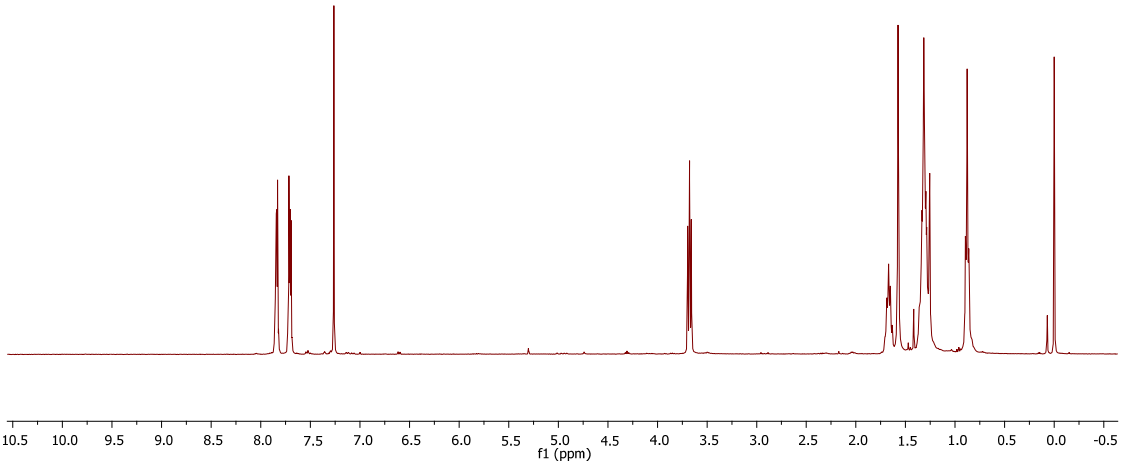


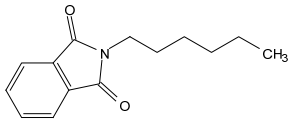
10f


200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10


O
N
CH3
O

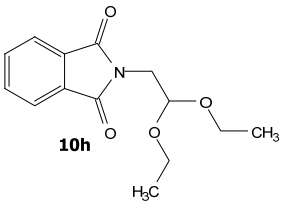
10g


10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

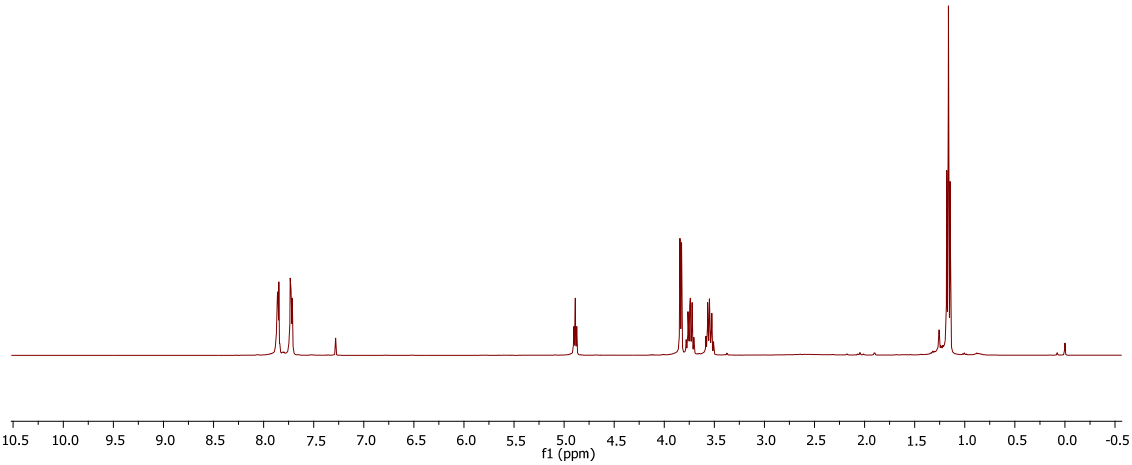

O
N
CH3
O

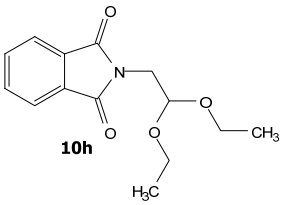
10g

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

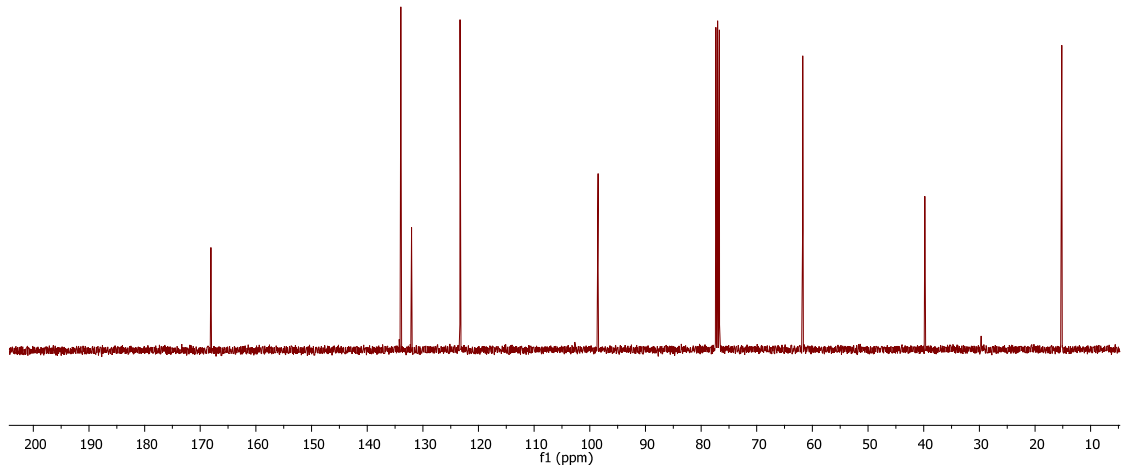

O
N
O
O
O
CH3
O
H3C

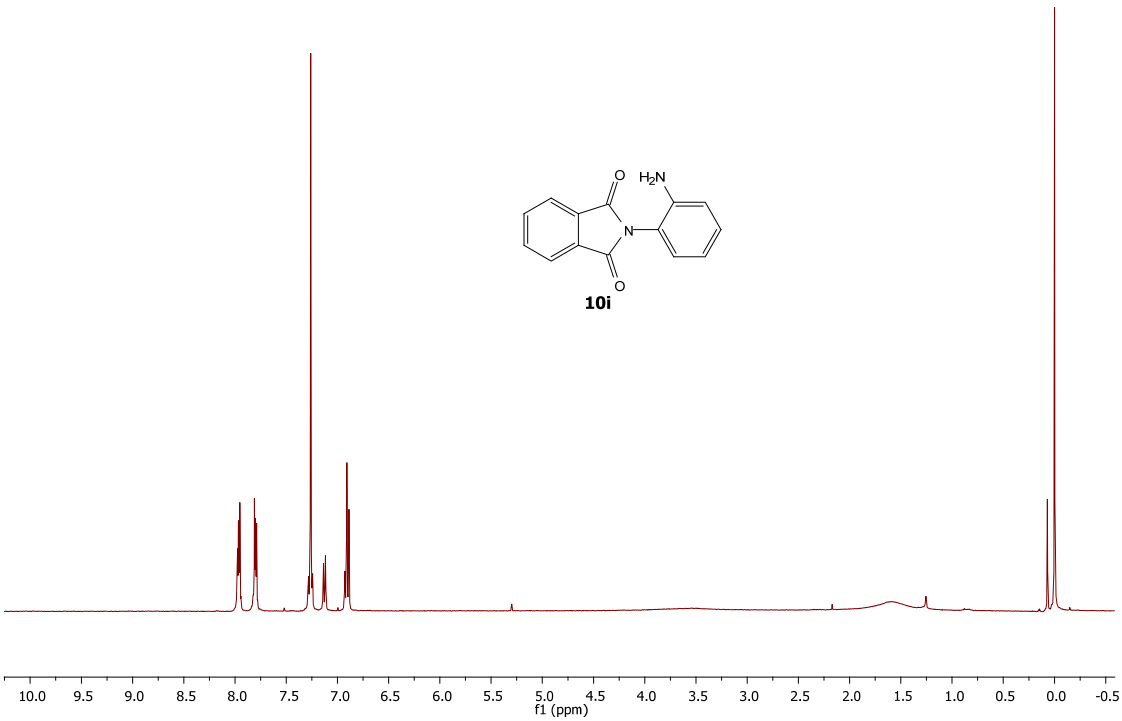
10h

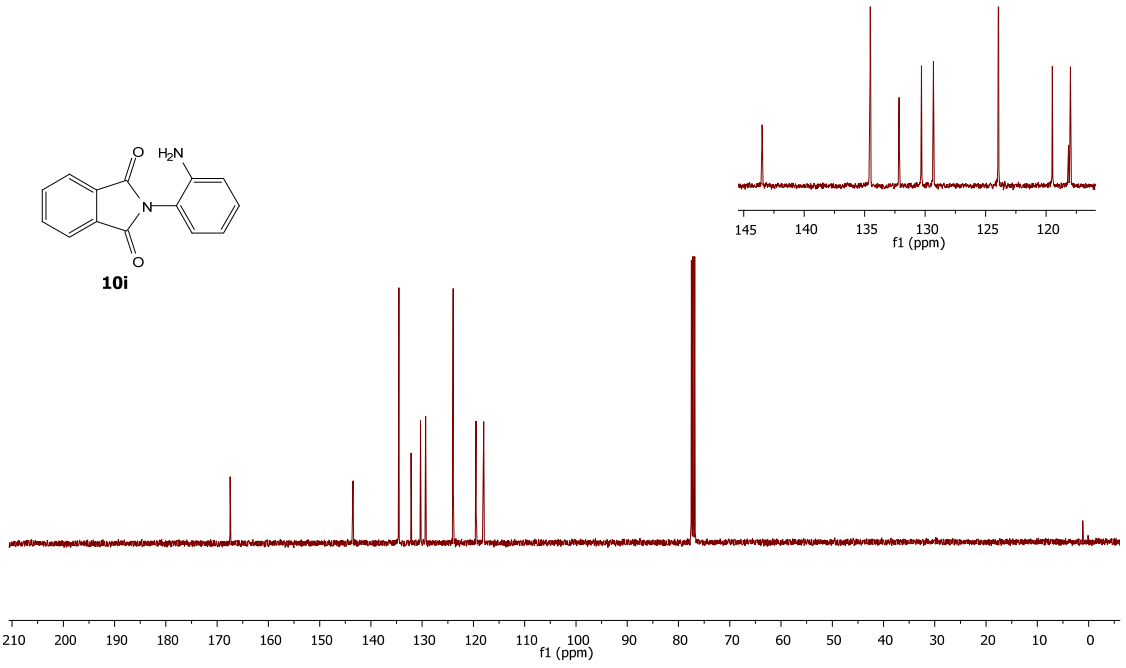

10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

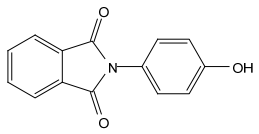

O
N
O
O
O
CH3
O
H3C

10h

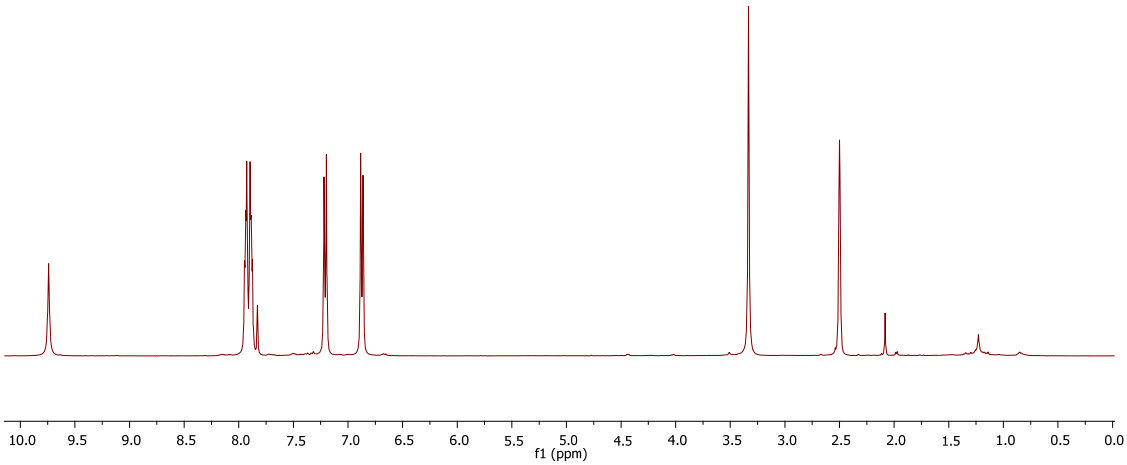

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

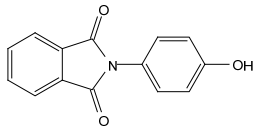

O
H2N
N
O
10i
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)


O
H2N
N
O
10i
145 140 135 130 125 120
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
N
OH
O

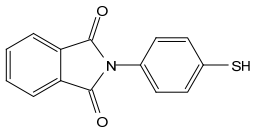
10j


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

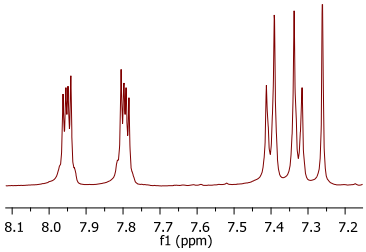

O
N
OH
O

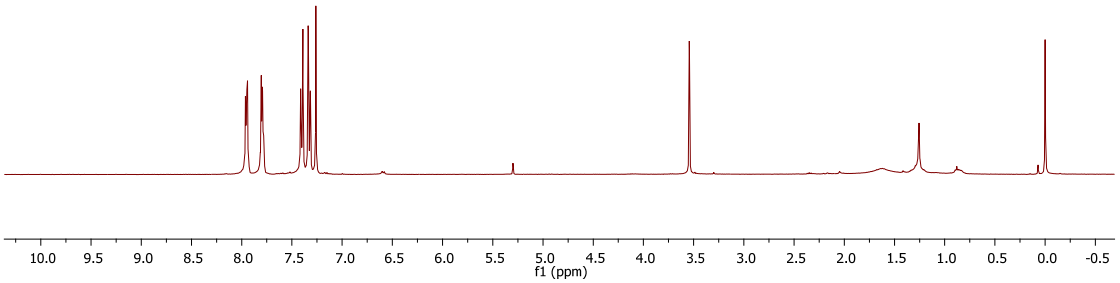
10j

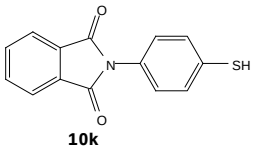
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
N
SH
O

10k

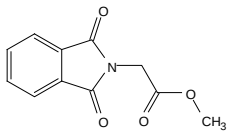

8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2
f1 (ppm)


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

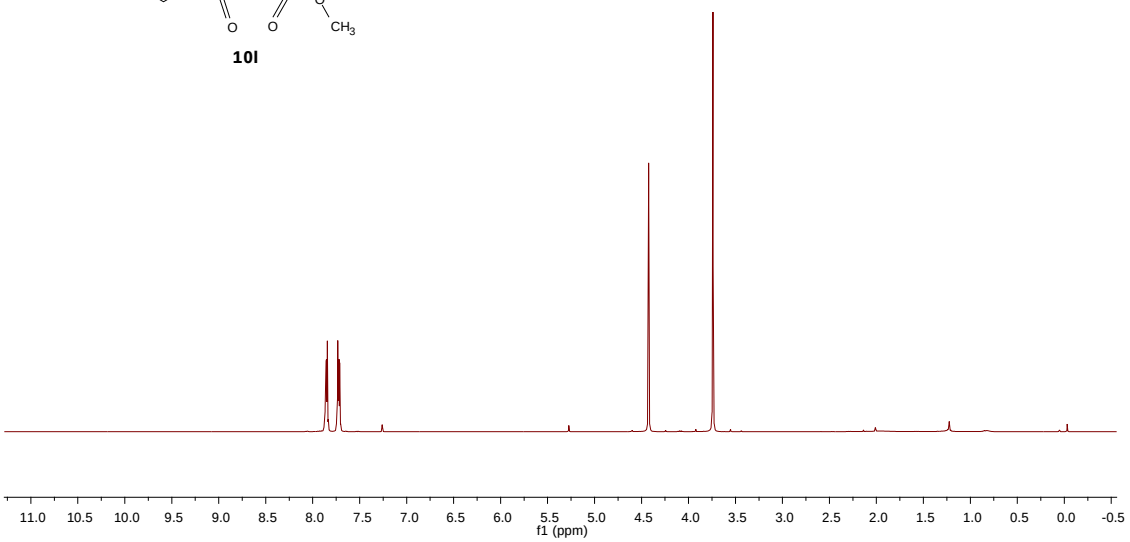

O
N
SH
O

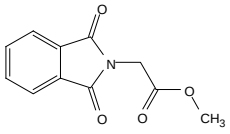
10k

200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



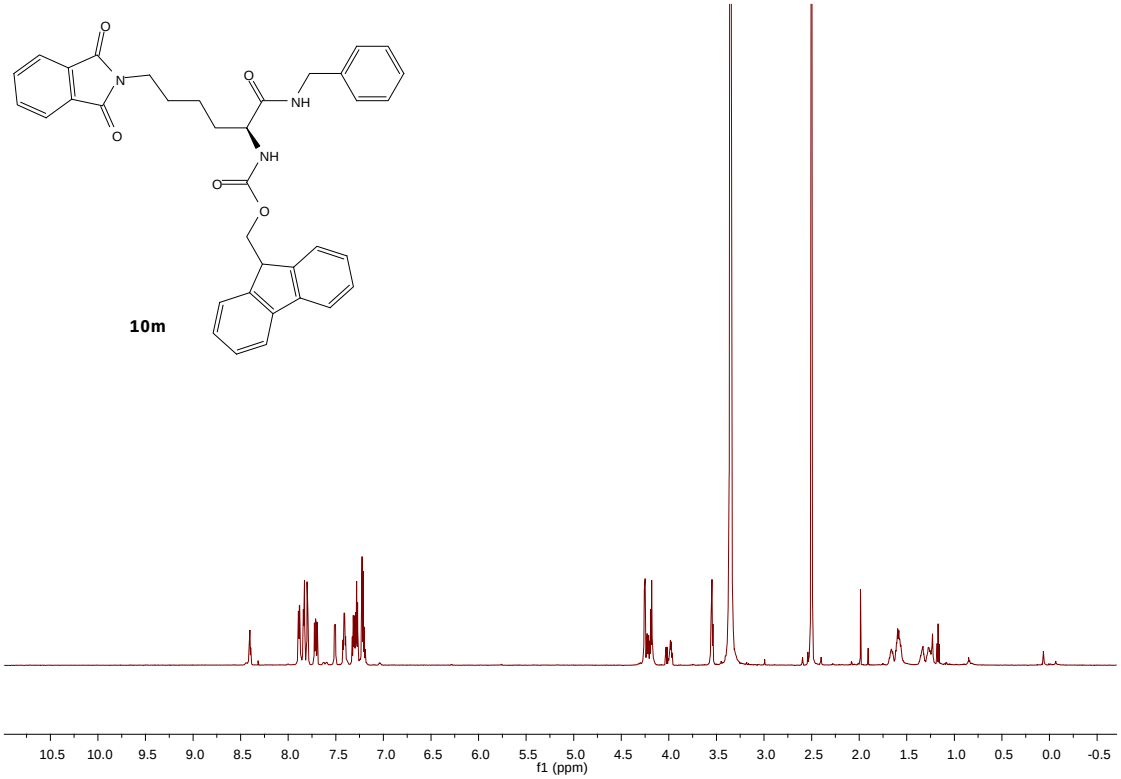
10l

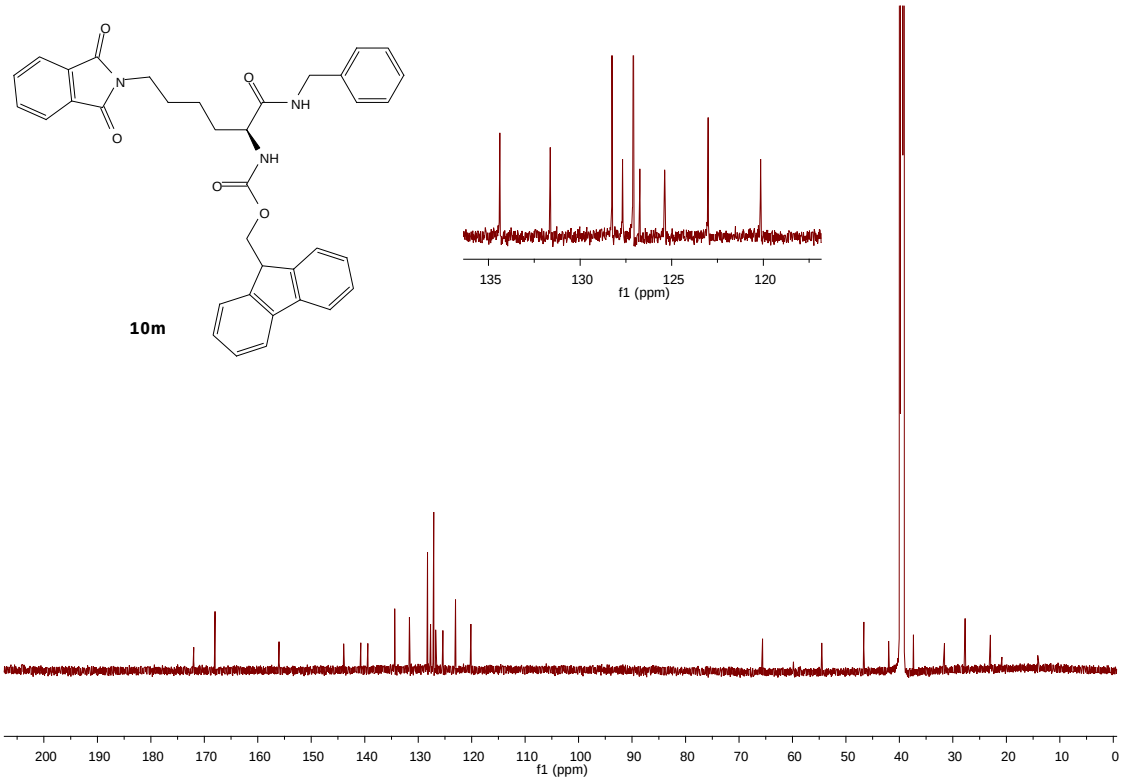

11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

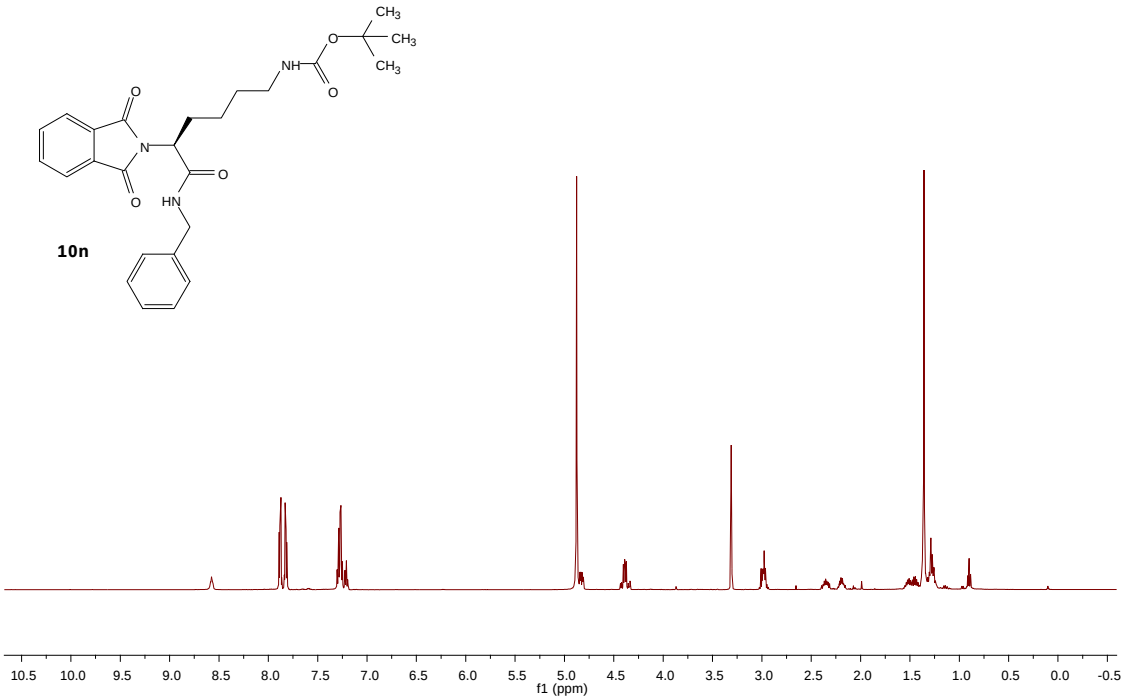


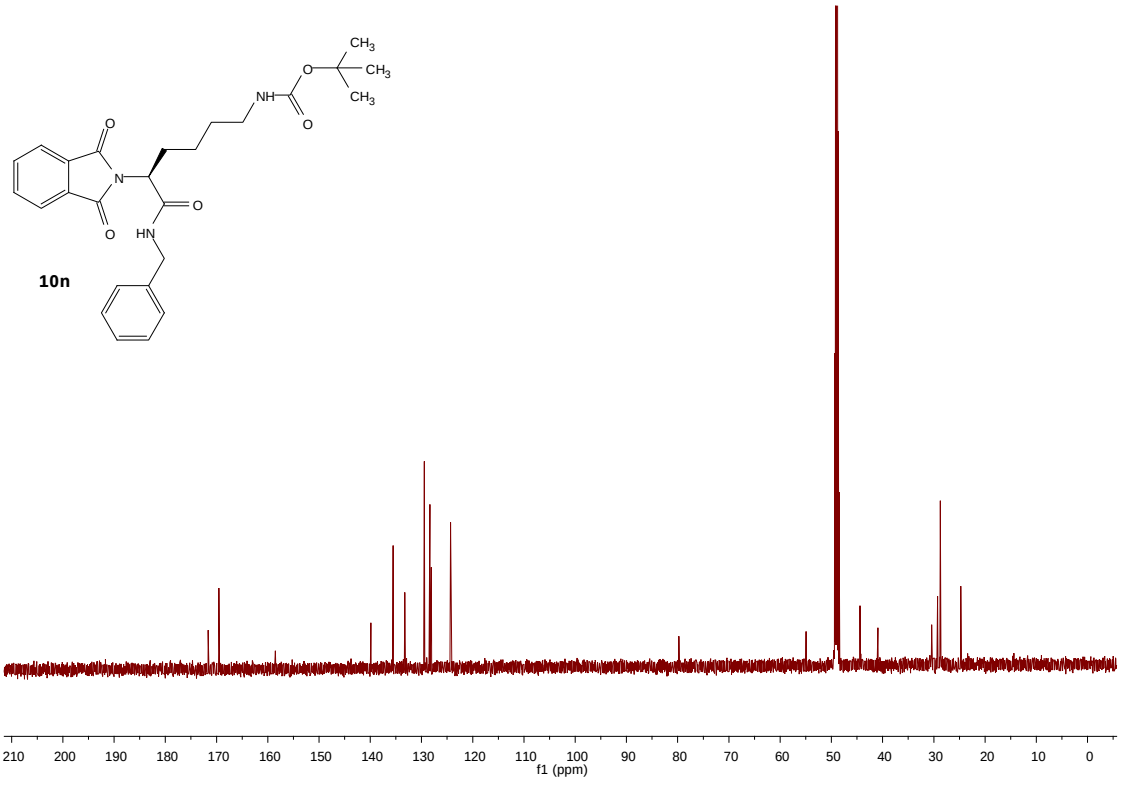
10l

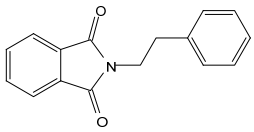
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
N
O
O
NH
O
NH
O
O
10m
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

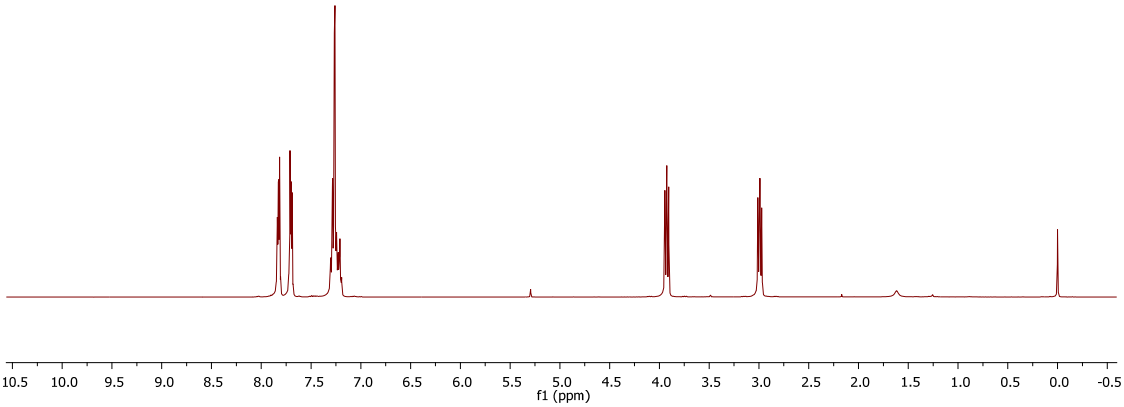

O
N
O
O
NH
O
NH
O
O
10m
135 130 125 120
f1 (ppm)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

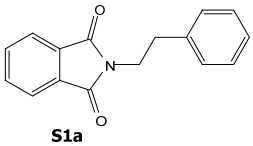

10n
f1 (ppm)


10n
f1 (ppm)

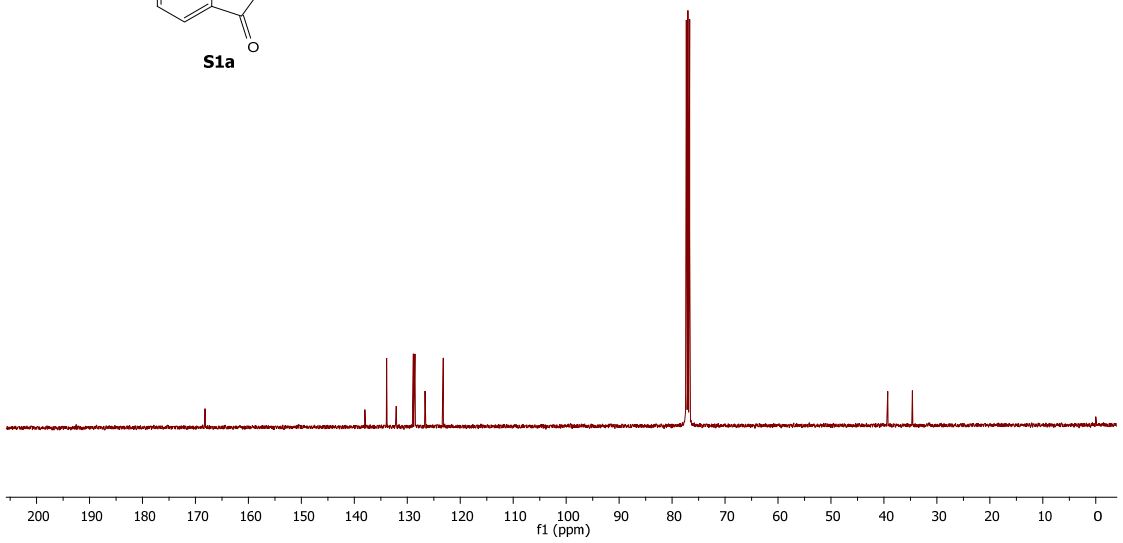


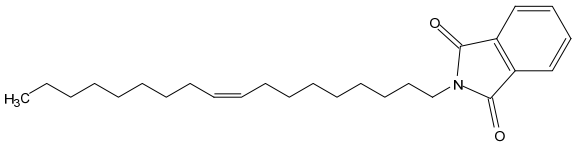
S1a


10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

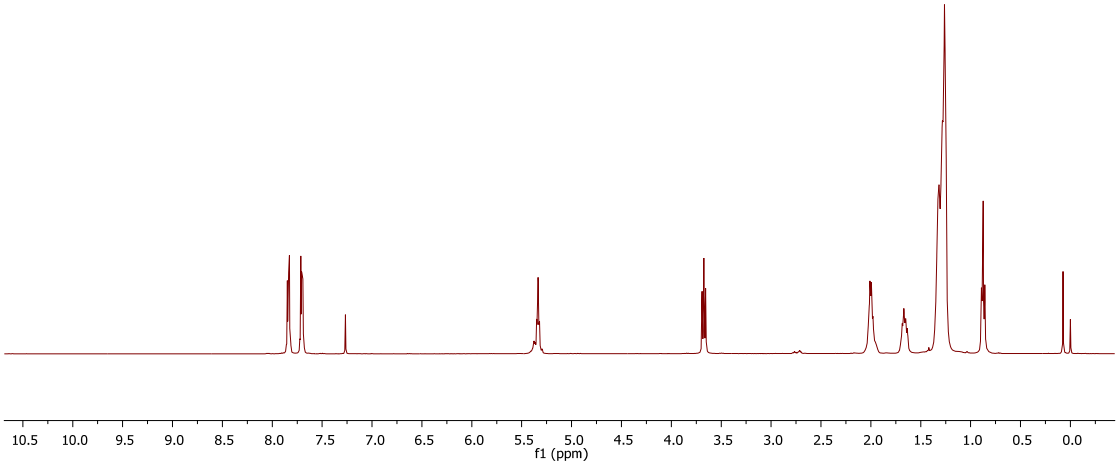


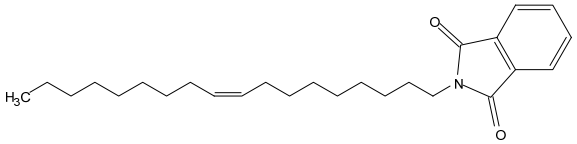
S1a


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

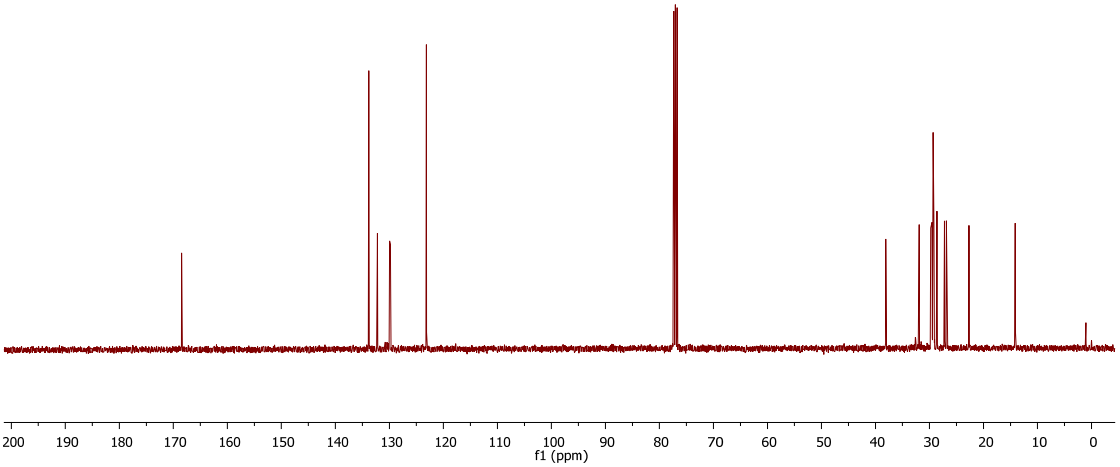

O
N
O
H3C

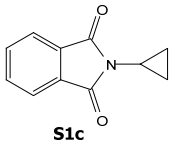
S1b


10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

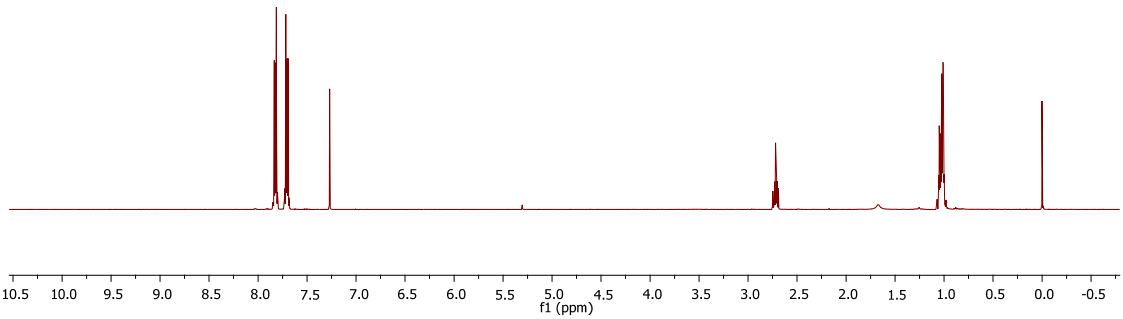

O
N
O
H3C

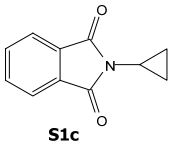
S1b


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

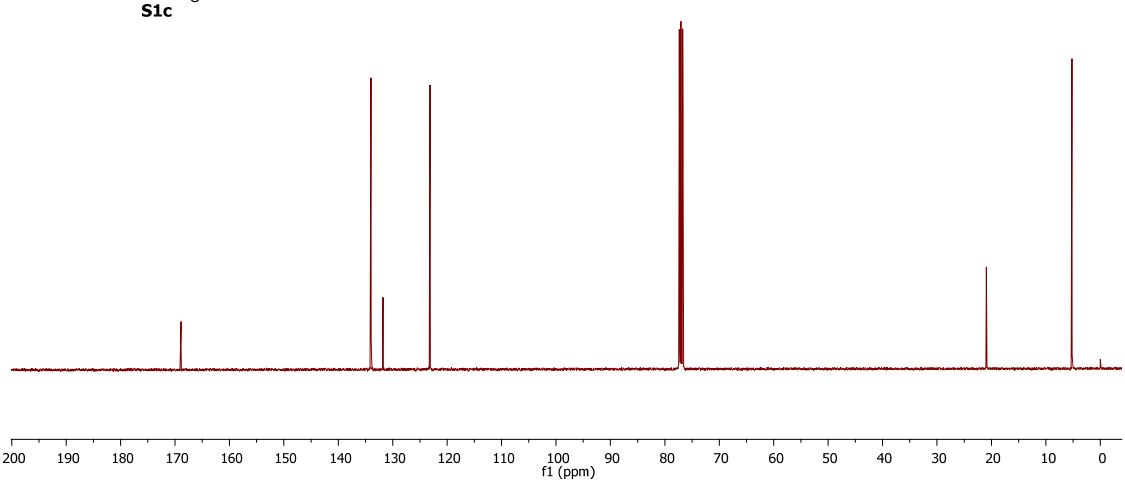

O
N
O

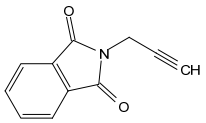
S1c


10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

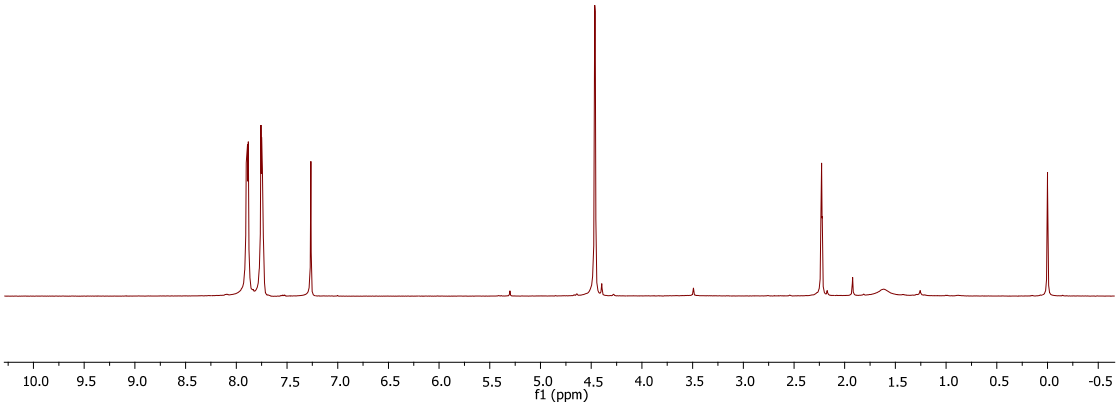

O
N
O

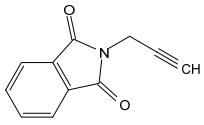
S1c


200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
N
CH
O

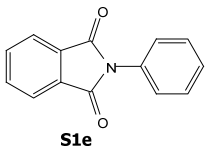
S1d


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

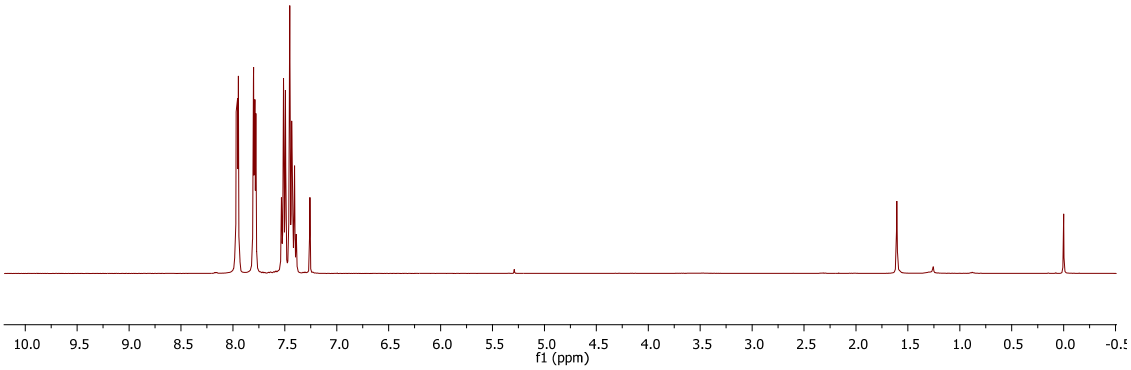

O
N
CH
O

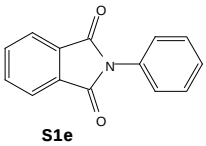
S1d

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

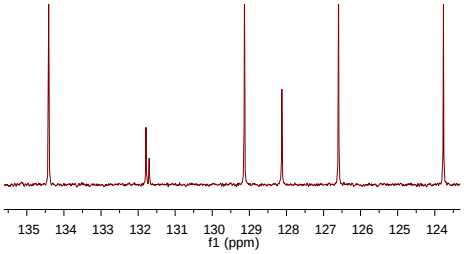


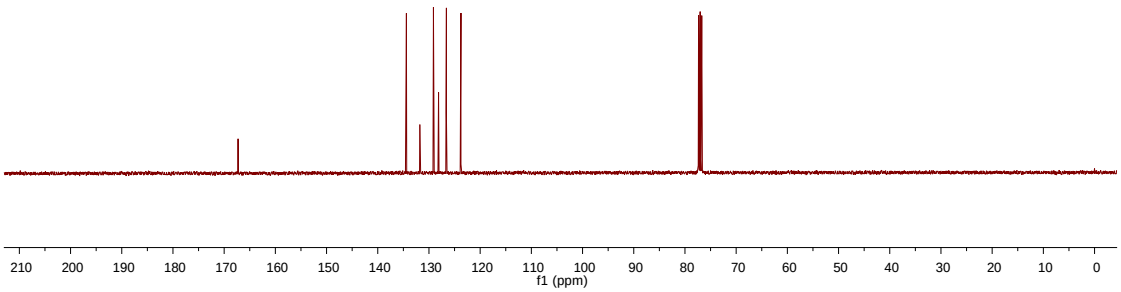
S1e

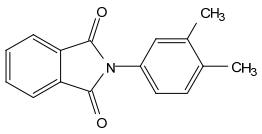

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)



S1e

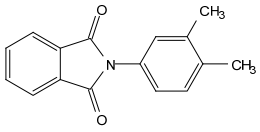

135 134 133 132 131 130 129 128 127 126 125 124
f1 (ppm)


210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

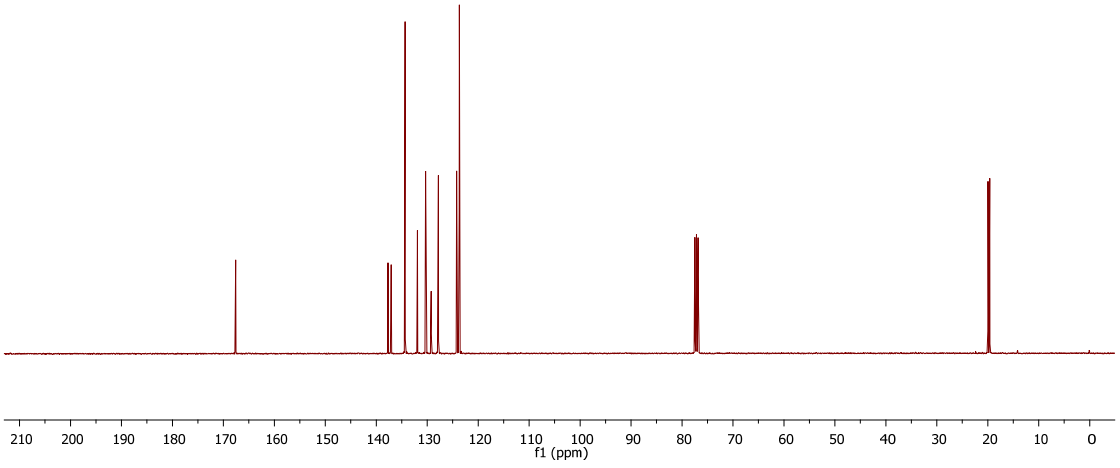

O
CH3
N
CH3
O

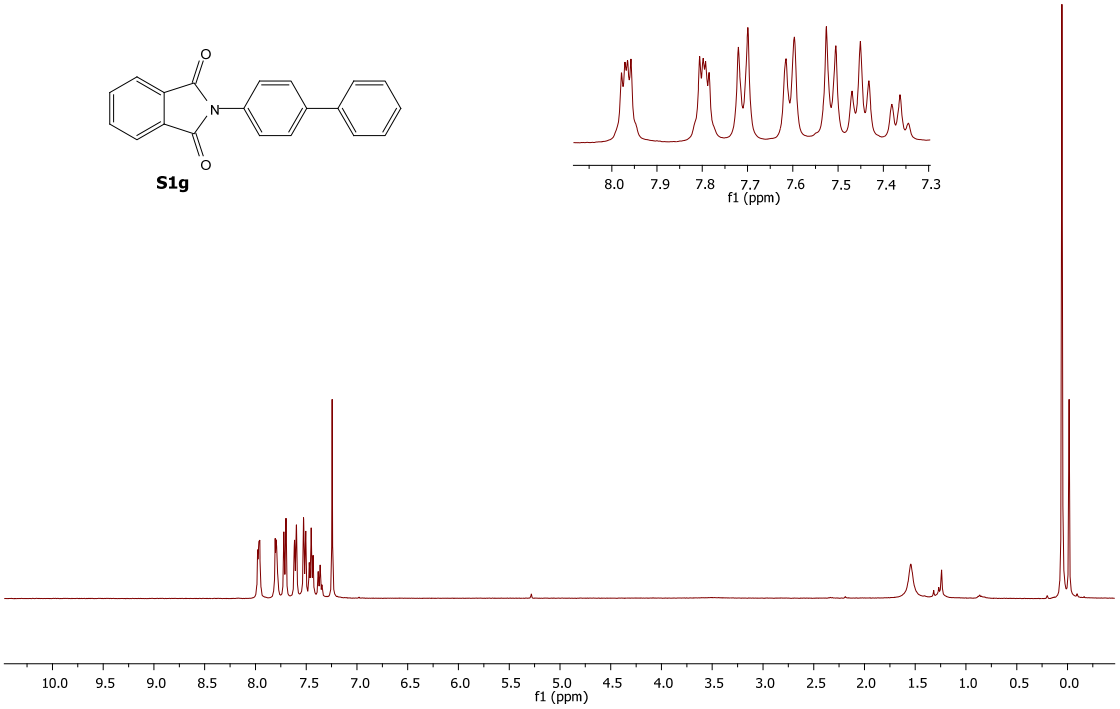
S1f

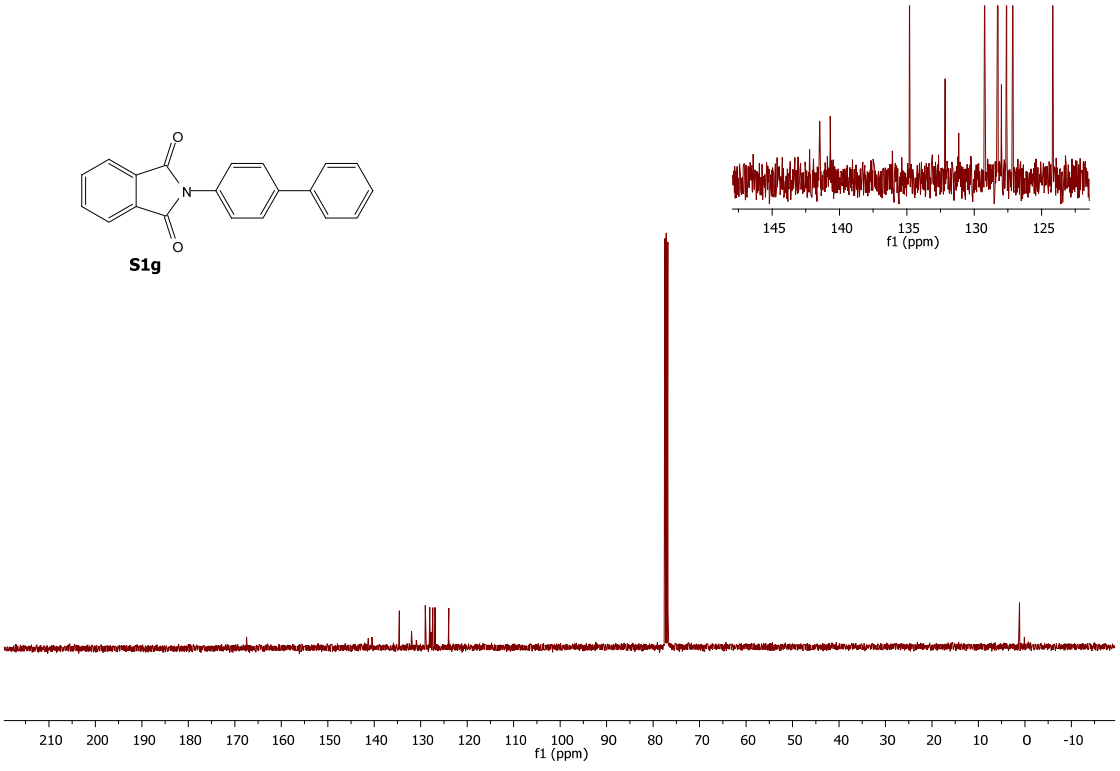
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

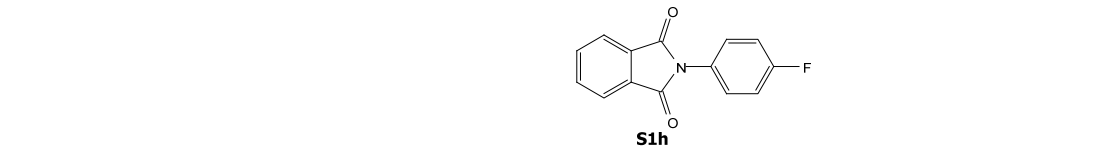

O
CH3
N
CH3
O

S1f

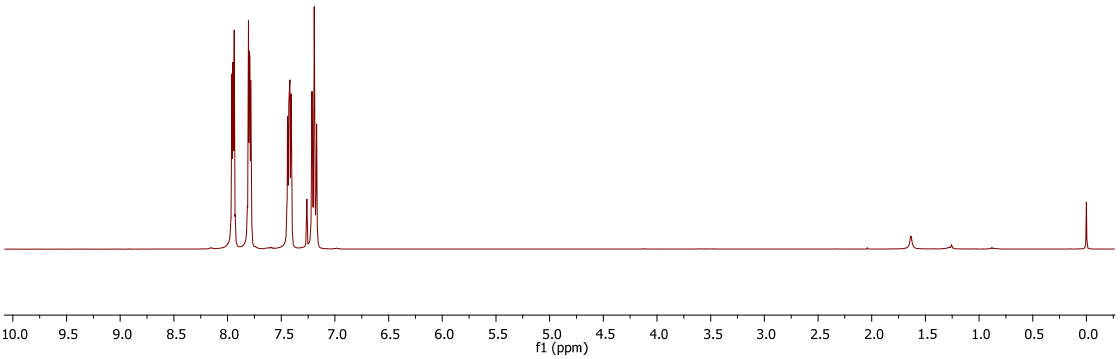

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

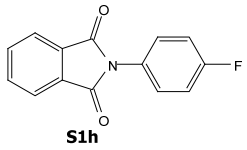

O
N
O
S1g
8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3
f1 (ppm)
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


O
N
O
S1g
145 140 135 130 125
f1 (ppm)
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

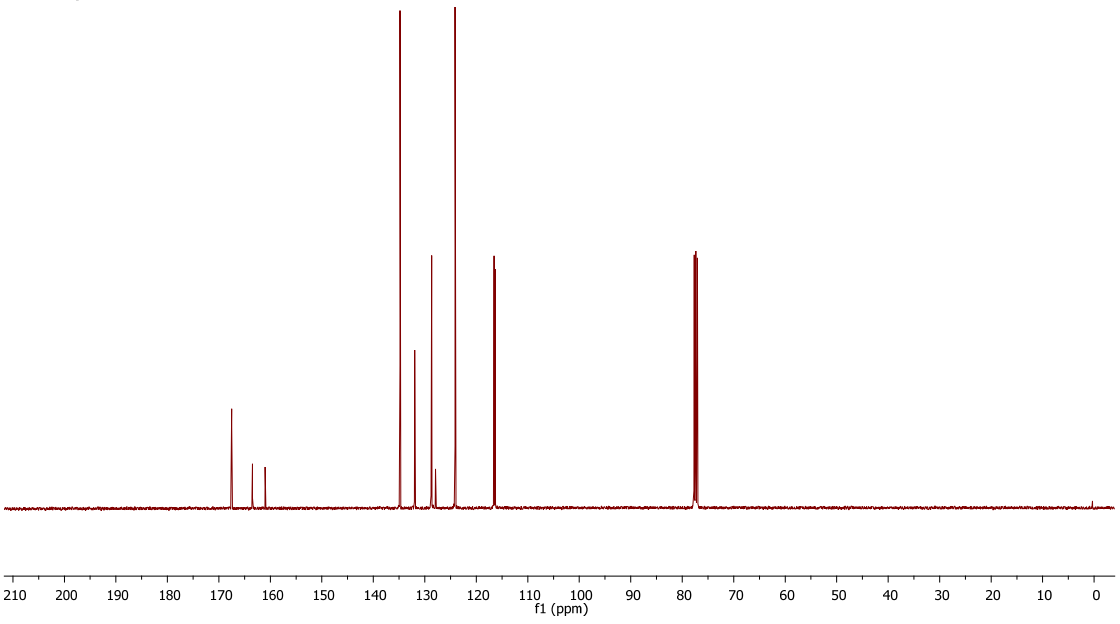

O
N
F
O

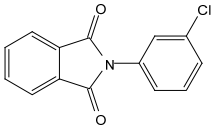
S1h


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

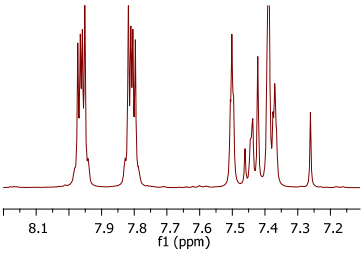

O
N
F
O

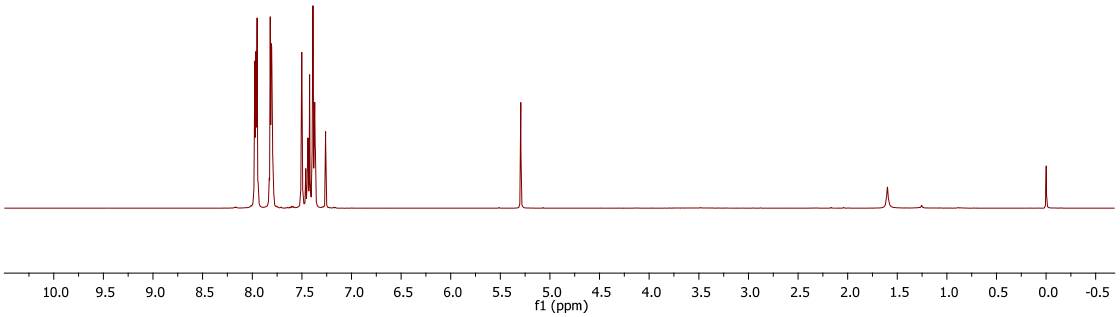
S1h

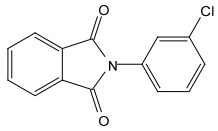

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
Cl
N
O

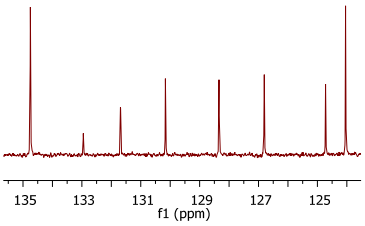
S1i

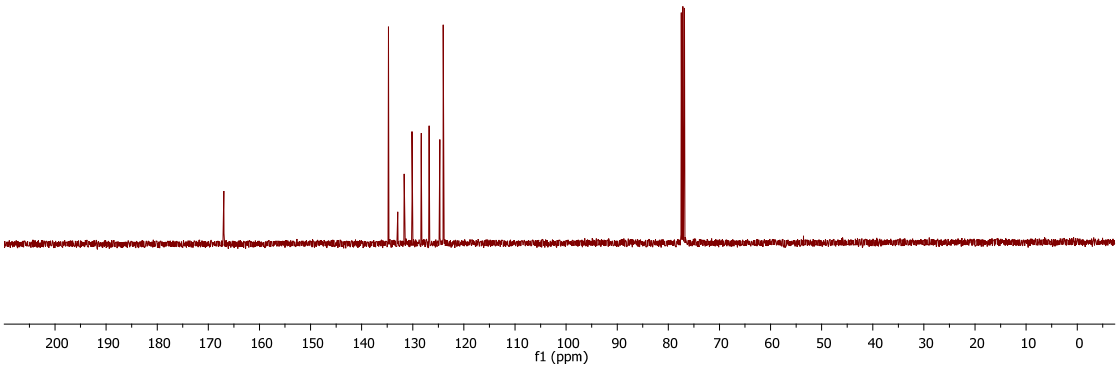

8.1
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
f1 (ppm)

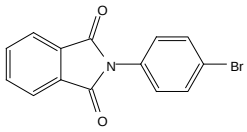

10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


O
Cl
N
O

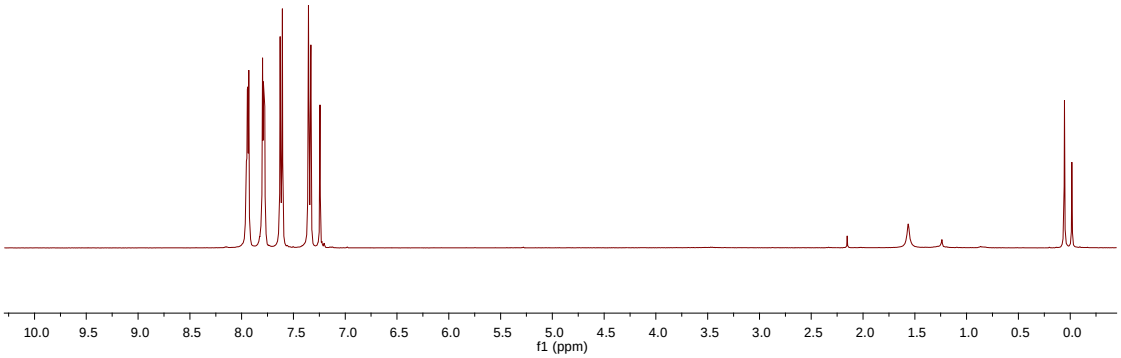
S1i

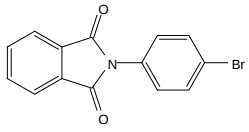

135
133
131
129
127
125
f1 (ppm)


200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

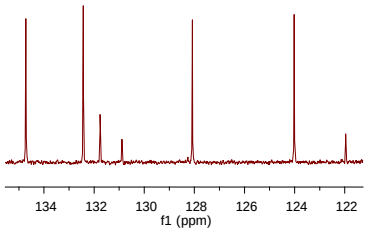

O
N
Br
O

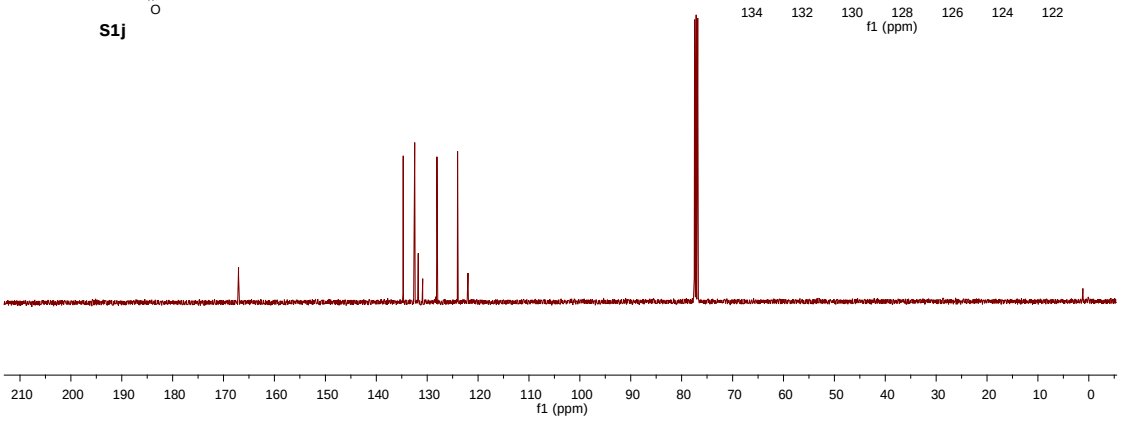
S1j

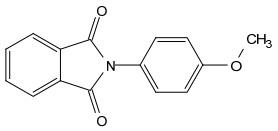

10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


O
N
Br
O

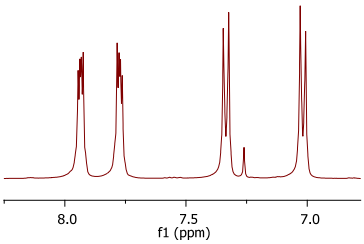
S1j

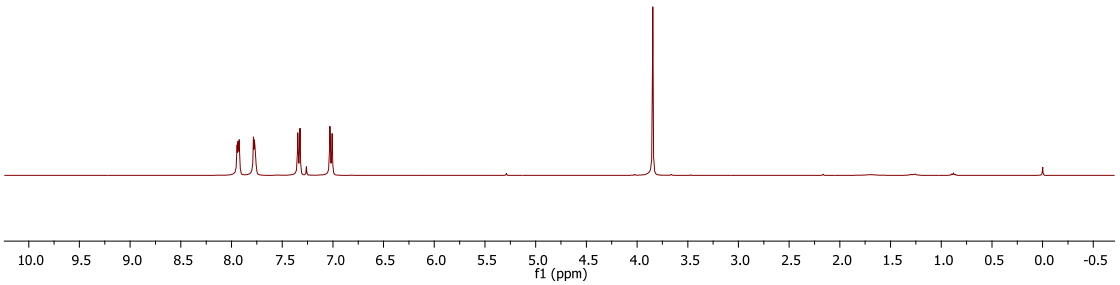

134 132 130 128 126 124 122
f1 (ppm)

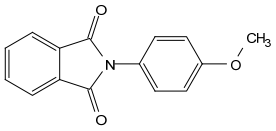

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


O
N
O
CH3
O

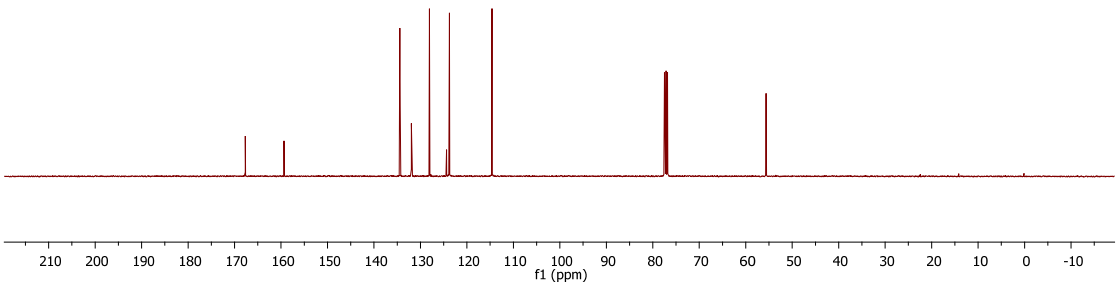
S1k

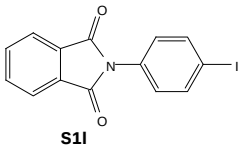

8.0
7.5
7.0
f1 (ppm)


10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

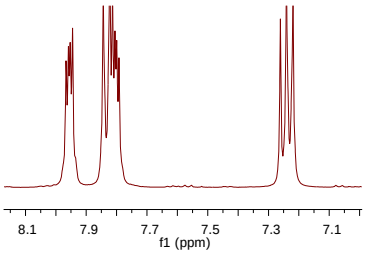

O
N
O
CH3
O

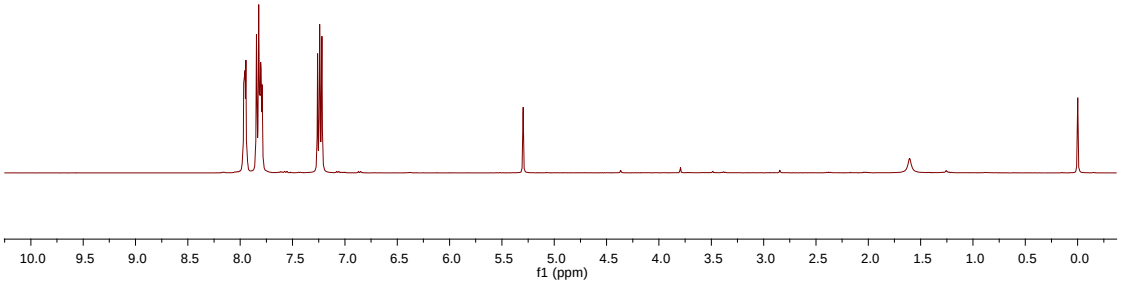
S1k

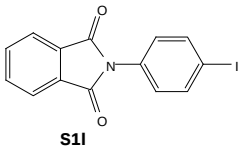

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


O
N
I
O

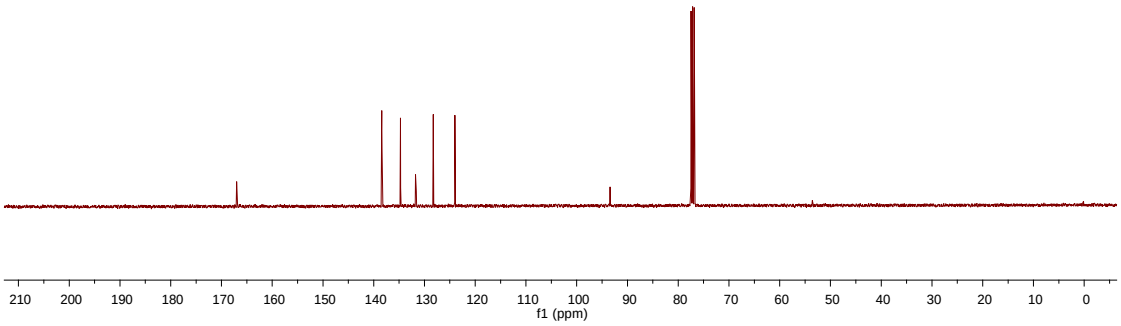
S1l

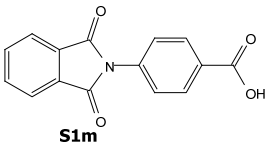

8.1
7.9
7.7
7.5
7.3
7.1
f1 (ppm)


10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

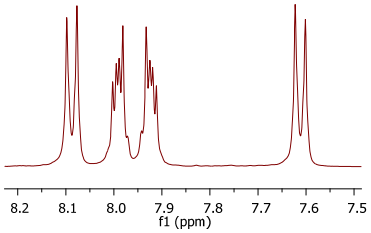

O
N
I
O

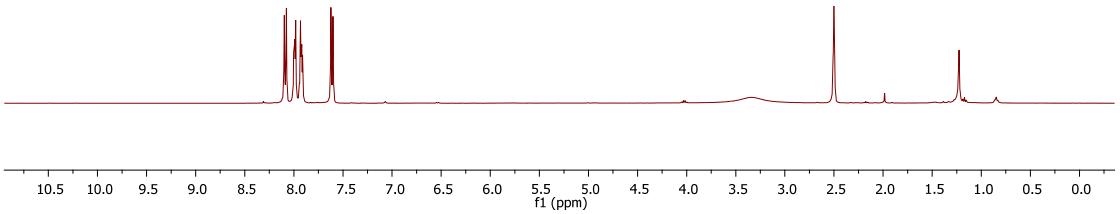
S1l

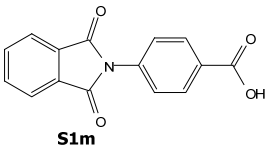

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
N
O
O
OH
O

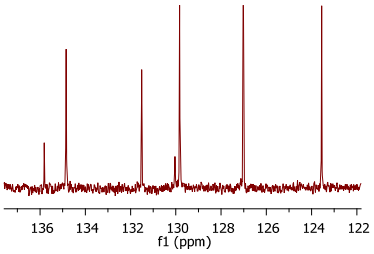
S1m

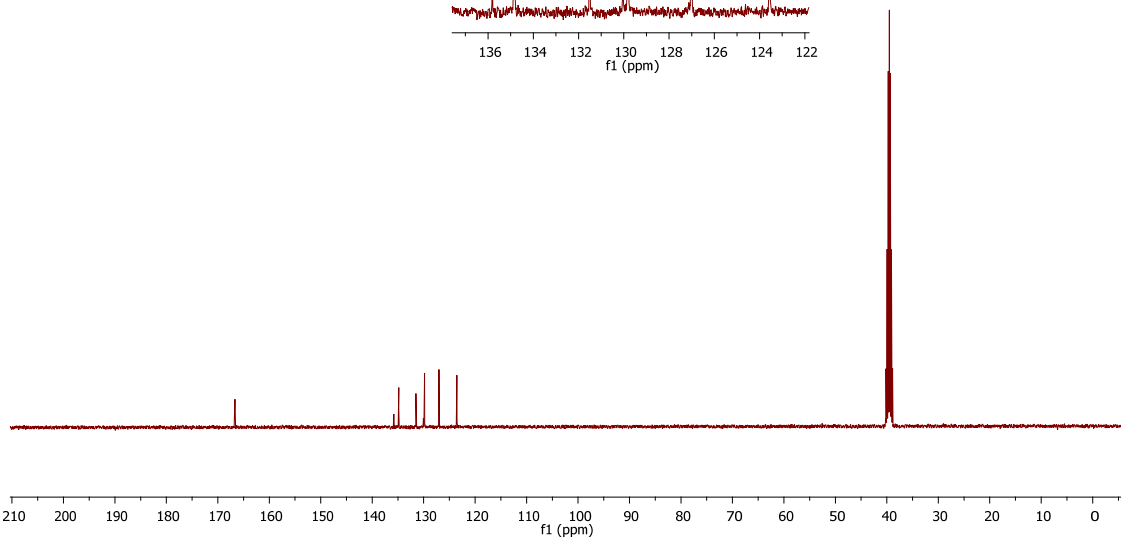

8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
f1 (ppm)


10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


O
N
O
O
OH
O

S1m


136
134
132
130
128
126
124
122
f1 (ppm)


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

MS spectra

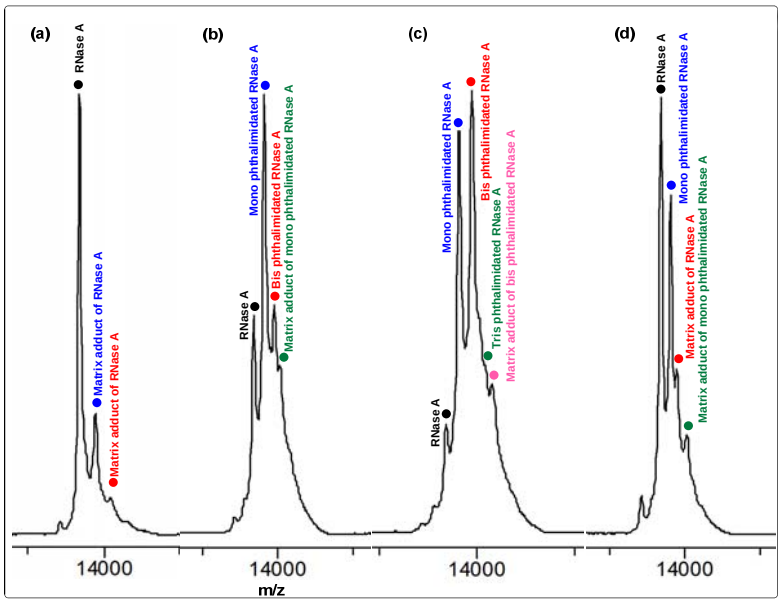


Figure S8: (a) MS spectra of native RNase A (MW 13681 Da). *MS spectra for RNase A (1 equiv.) and NHP (5 equiv.) in* (b) 2 h and (c) 24 h. (d) *MS spectra for RNase A (1 equiv.) and NHP (1 equiv.) in 8 h*: mono phthalimidated RNase A.

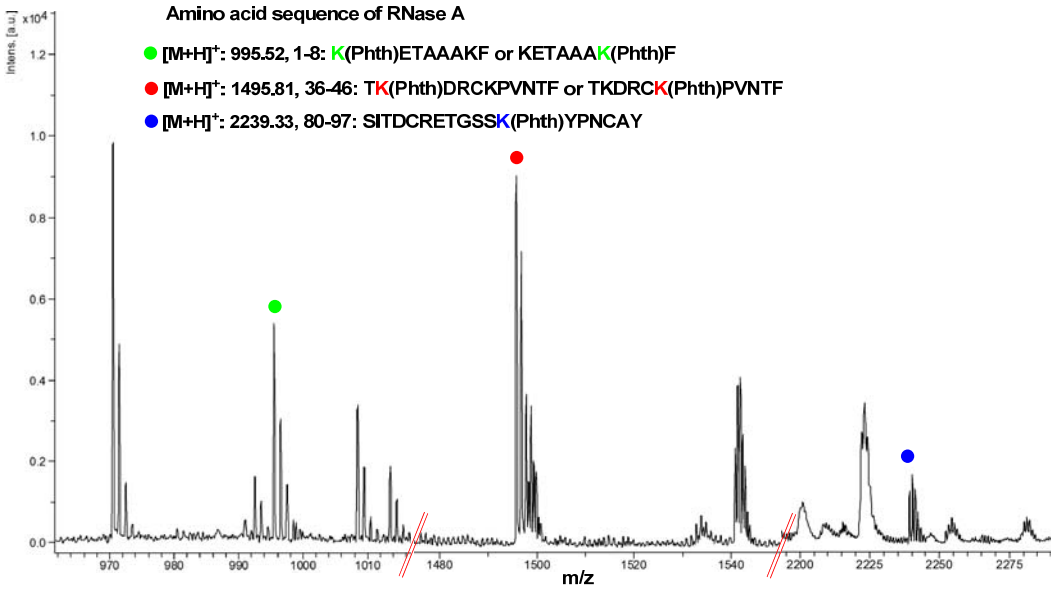


Figure S9: MS spectra of phthalimidated RNase A [*NHP (5 equiv.) in 8 h*, Cysteine residues were alkylated with iodoacetamide followed by digestion with α-chymotrypsin]

The chymotrypsin digest was directly used for MALDI-MS. The peak at m/z 995.55 indicated $[M+H]^+$ of the mono phthalimidation of peptide (sequence: AA 1-8) of RNase A,

KETAAAKF). The modified peptide was further subjected to MALDI-MS/MS to confirm the modified site of the sequence (K1 or K7). The ions, b_2 and y_6 (and others, see Figure S9) confirmed that site of modification is K1.

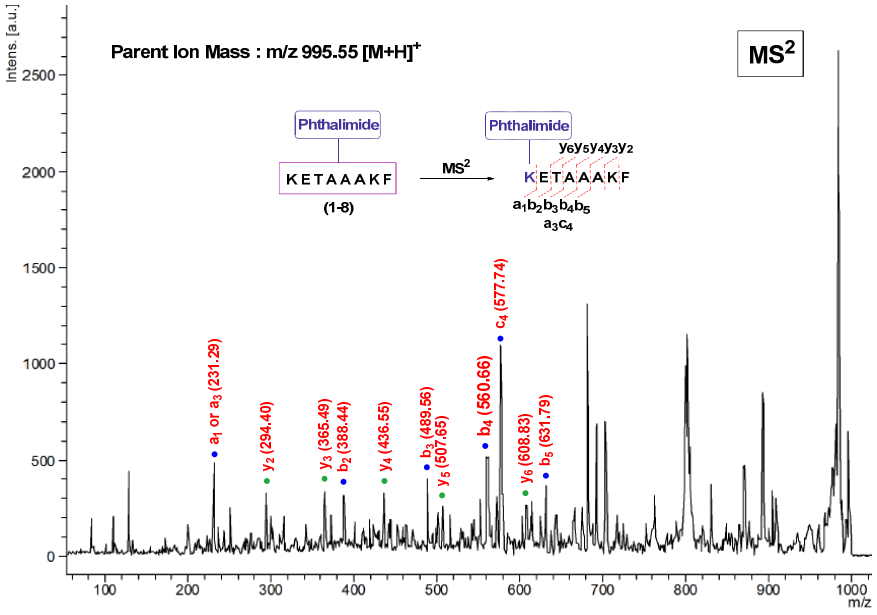


Figure S10: MS^2 spectrum of phthalimidated peptide fragment KETAAAKF (1−8) confirming modification at K1.

Discussion (Figure S11-S15): We designed peptides to investigate relative reactivity of α-amine versus ε-amine. Five peptides, **G**GG**K**-NH_2, **H**GG**K**-NH_2, **N**GG**K**-NH_2, **M**G**K**-NH_2, **S**F**K**-NH_2 with different N-terminus amino acids were synthesized with unprotected Lys residue in the sequence. The peptides were subjected to phthalimidation and site of modification was identified by MS and MS^2 experiments.

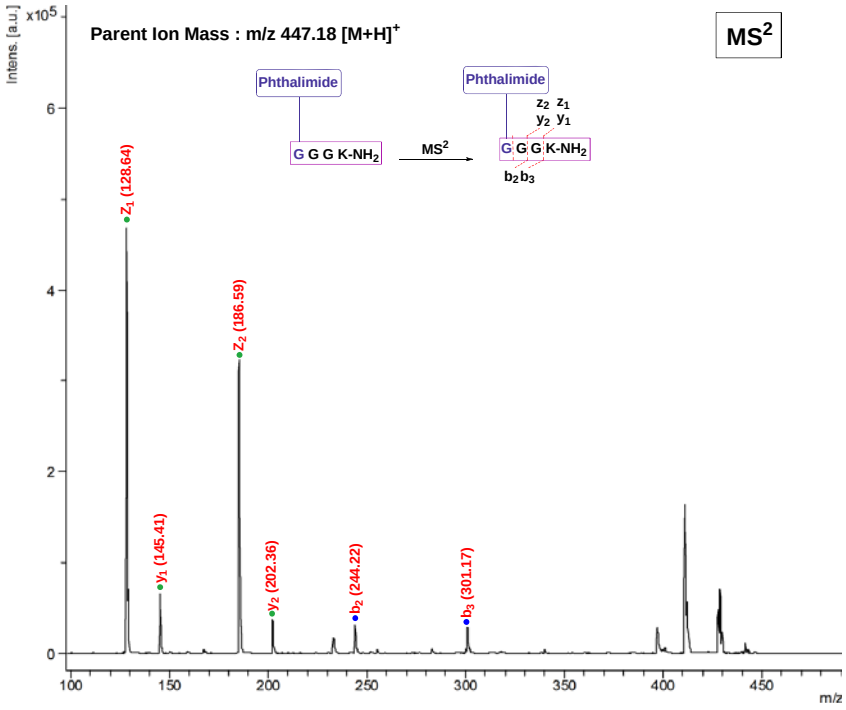


Figure S11: MALDI-MS^2 spectra of phthalimidated GGGK-NH_2

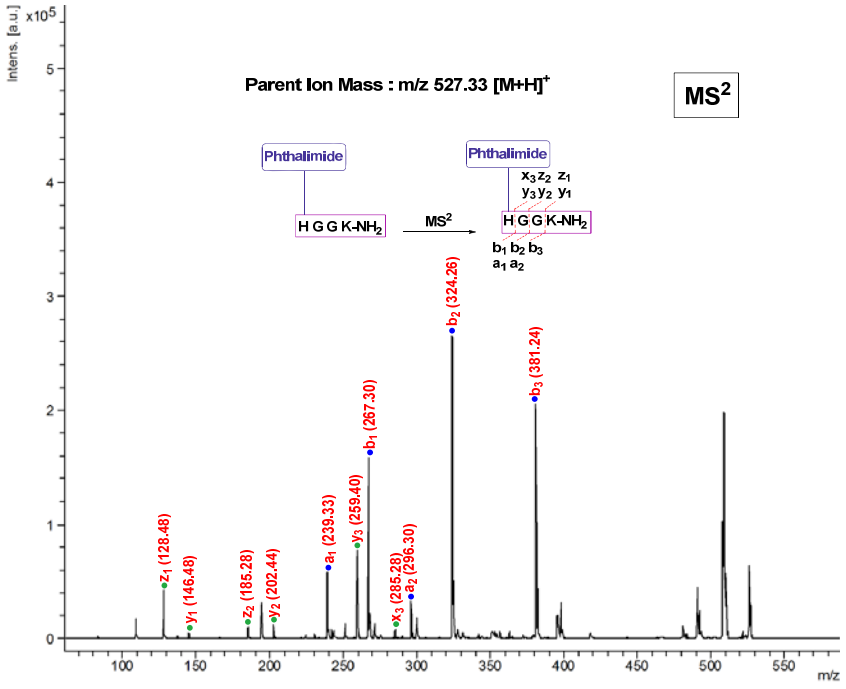


Figure S12: MALDI-MS^2 spectra of phthalimidated HGGK-NH_2

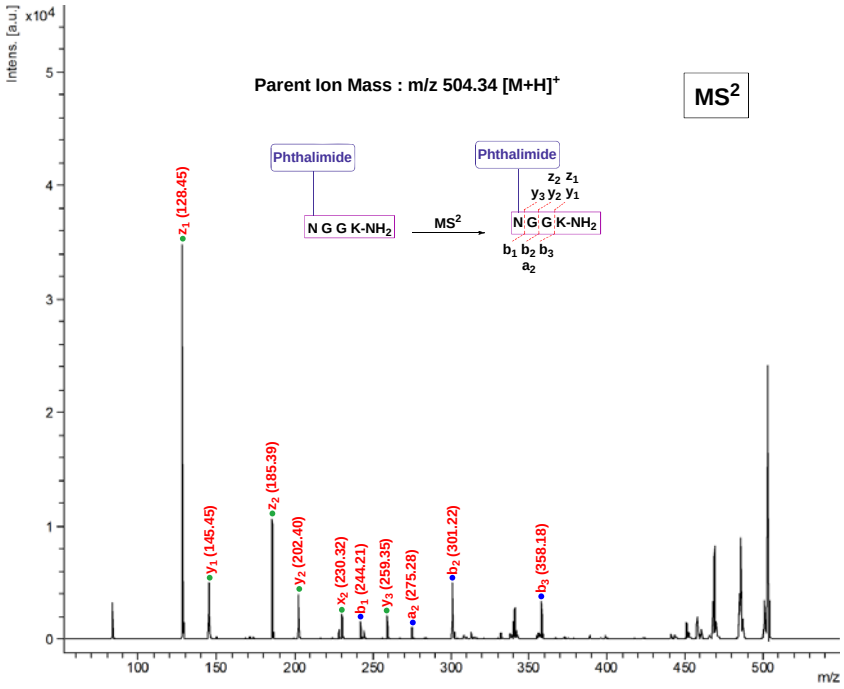


Figure S13: MALDI-MS^2 spectra of phthalimidated NGGK-NH_2

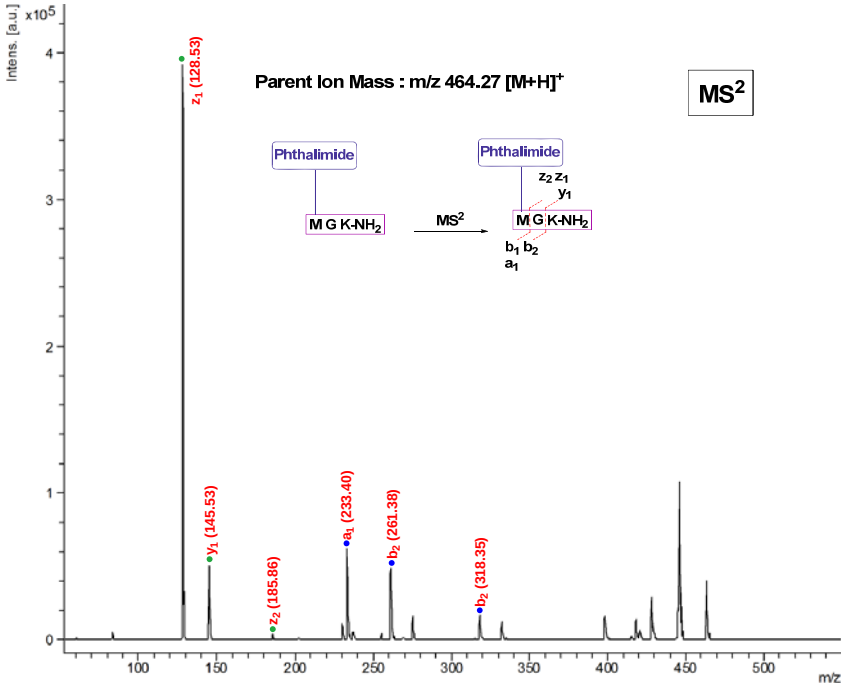


Figure S14: MALDI-MS^2 spectra of phthalimidated MGK-NH_2

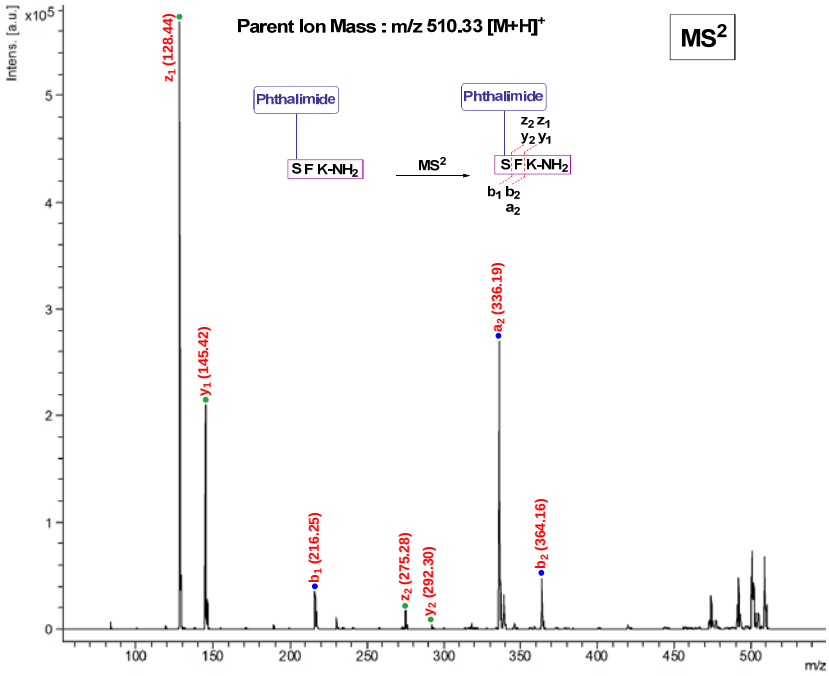


Figure S15: MALDI-MS^2 spectra of phthalimidated SFK-NH$_2$

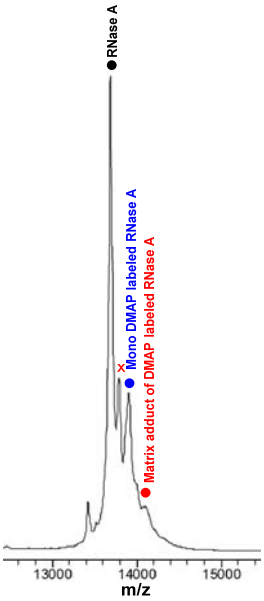


Figure S16: MS spectra for RNase A (1 equiv.) and 4-N,N-dimethylamino-N-hydroxy pthalimide (1 equiv.) in 8 h: mono dimethylamino phthalimide (DMAP) labeled RNase A. (**X** is from RNase A).

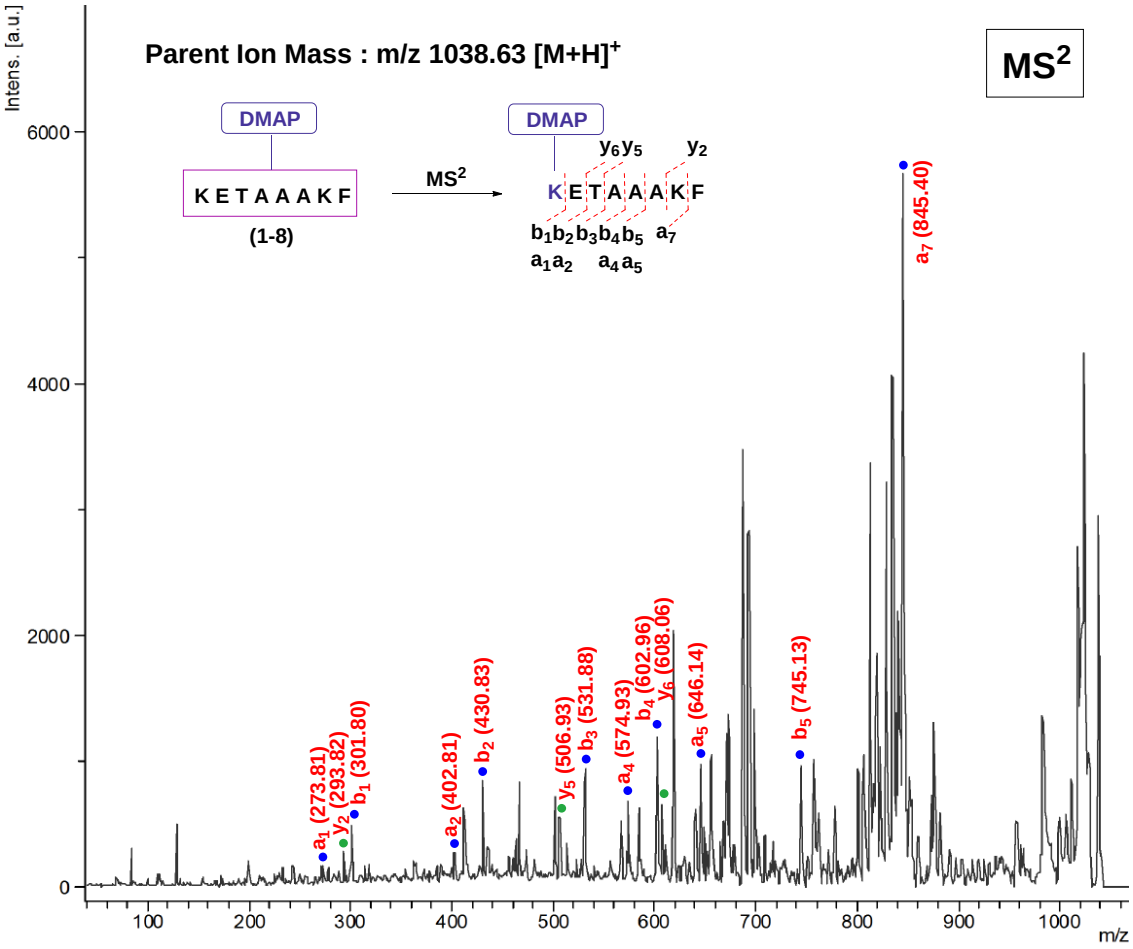
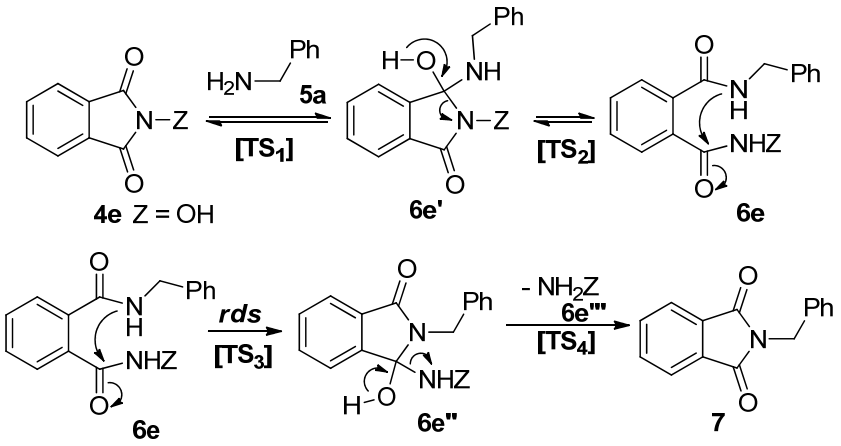


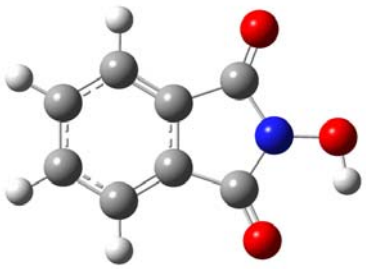
Figure S17: MS^2 spectrum of dimethylamino phthalimide (DMAP) labeled peptide fragment KETAAAKF (1−8) confirming modification at K1.

Calculations: Cartesian coordinates and methods

Method for structure optimization: opt freq rb3lyp/6-311++g(d,p) geom=connectivity.
Method for transition state optimization: opt=qst3 rb3lyp/6-311++g(d,p) geom=connectivity
(frequency and IRC calculations were done to validate the results)

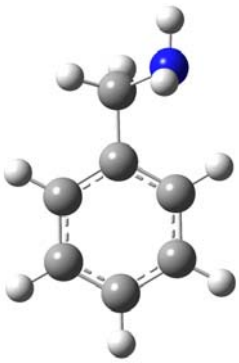


a) 4e



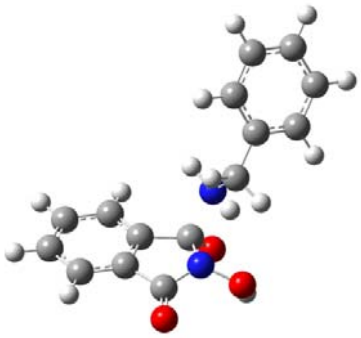
1	6	-2.908665	0.633779	0.000097
2	6	-2.875388	-0.765076	0.000090
3	6	-1.654940	-1.460747	0.000019
4	6	-0.491354	-0.707137	-0.000030
5	6	-0.524063	0.695008	-0.000038
6	6	-1.723279	1.388512	0.000031
7	6	0.935924	-1.132242	-0.000074
8	7	1.658929	0.055298	-0.000350
9	6	0.881527	1.222202	-0.000137
10	8	1.287240	2.362903	0.000092
11	8	1.466722	-2.232003	0.000060
12	8	3.037221	0.058201	0.000166
13	1	-3.866824	1.144331	0.000157
14	1	-3.807652	-1.321359	0.000149
15	1	-1.619475	-2.545219	0.000023
16	1	-1.739407	2.473484	0.000027
17	1	3.252813	-0.896925	-0.000196

b) 5a



1	6	0.478989	1.287865	-0.013619
2	6	1.848794	1.036319	-0.139848
3	6	2.324450	-0.276440	-0.105446
4	6	1.421382	-1.332594	0.055273
5	6	0.054416	-1.078200	0.184838
6	6	-0.432430	0.237281	0.155415
7	6	-1.912212	0.523707	0.336627
8	7	-2.748615	-0.453253	-0.371781
9	1	0.114706	2.312314	-0.048792
10	1	2.540109	1.864130	-0.271567
11	1	3.387444	-0.475923	-0.207272
12	1	1.783165	-2.356987	0.079908
13	1	-0.650943	-1.895210	0.300018
14	1	-2.104764	1.569621	0.046522
15	1	-2.164900	0.446309	1.402805
16	1	-3.731977	-0.310999	-0.160031
17	1	-2.632867	-0.368113	-1.378560

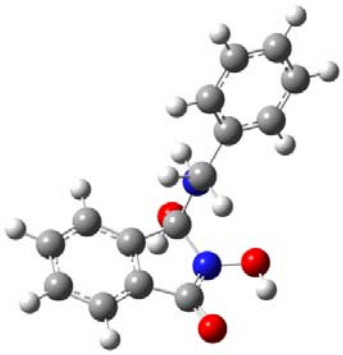
c) TS1



1	6	4.499880	-1.357636	-0.095366
2	6	4.887058	-0.060556	0.244539
3	6	3.949577	0.978870	0.311667
4	6	2.631822	0.667900	0.028635
5	6	2.243154	-0.631962	-0.312147
6	6	3.161691	-1.662642	-0.380464
7	6	1.419171	1.531674	0.013207

8	7	0.382868	0.674367	-0.339645
9	6	0.763600	-0.657578	-0.561174
10	8	-0.025995	-1.658583	-0.903122
11	8	1.254996	2.712261	0.238383
12	8	-0.913679	1.120347	-0.456506
13	1	5.246857	-2.141706	-0.139439
14	1	5.929047	0.145405	0.459844
15	1	4.241296	1.988375	0.574680
16	1	2.852498	-2.666561	-0.645143
17	1	-0.834828	2.068189	-0.241537
18	6	-3.702882	-0.589698	1.273571
19	6	-4.910436	-0.478099	0.584329
20	6	-4.925592	0.006567	-0.721522
21	6	-3.726684	0.377828	-1.330941
22	6	-2.522675	0.269044	-0.639112
23	6	-2.497519	-0.213441	0.674444
24	6	-1.195741	-0.298311	1.448039
25	7	-0.087444	-0.767398	0.607655
26	1	-3.697701	-0.975842	2.288471
27	1	-5.835630	-0.776317	1.065279
28	1	-5.861955	0.090546	-1.261833
29	1	-3.730113	0.753349	-2.348562
30	1	-1.588434	0.546960	-1.112496
31	1	-1.362928	-0.912268	2.345614
32	1	-0.927146	0.703418	1.804199
33	1	0.792444	-0.722739	1.110032
34	1	-0.260482	-2.133556	-0.031447

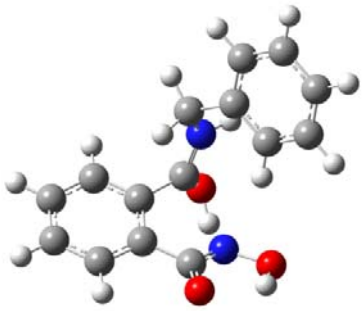
d) 6e'



1	6	2.430578	2.727909	0.427393
2	6	3.263335	2.390434	-0.574590
3	6	3.314864	1.129542	-1.038653
4	6	2.508719	0.222634	-0.471934
5	6	1.676781	0.560648	0.523030
6	6	1.625907	1.812348	0.992947
7	6	2.416254	-1.091591	-0.795704
8	7	1.469416	-1.616945	0.045605

9	6	0.915027	-0.646123	0.973305
10	8	1.259057	-0.918538	2.328667
11	8	2.993067	-1.750159	-1.625502
12	8	1.102903	-2.874185	-0.077022
13	7	-0.531286	-0.482128	0.931275
14	6	-1.086207	-0.038798	-0.346139
15	6	-2.595816	0.103380	-0.282340
16	6	-3.269246	0.404701	-1.408694
17	6	-4.604033	0.543698	-1.410732
18	6	-5.295055	0.381633	-0.273036
19	6	-4.640747	0.081618	0.858585
20	6	-3.305214	-0.055365	0.851153
21	1	2.409071	3.768701	0.792637
22	1	3.916860	3.158935	-1.020902
23	1	4.000902	0.856332	-1.856252
24	1	0.944324	2.074925	1.816719
25	1	2.137427	-0.568761	2.495164
26	1	1.607338	-3.275430	-0.768792
27	1	-0.805231	0.202931	1.677282
28	1	-0.665148	0.956169	-0.620509
29	1	-0.824418	-0.771193	-1.145575
30	1	-2.716604	0.542739	-2.353304
31	1	-5.133461	0.791445	-2.346012
32	1	-6.391871	0.494352	-0.267651
33	1	-5.204230	-0.052309	1.797496
34	1	-2.812958	-0.302280	1.804848

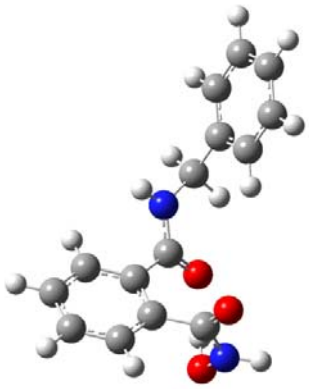
e) TS2



1	6	-3.063665	-2.563604	0.472011
2	6	-3.745489	-2.116150	-0.663196
3	6	-3.505372	-0.841999	-1.182116
4	6	-2.569051	-0.047206	-0.535399
5	6	-1.880742	-0.492180	0.592992
6	6	-2.121940	-1.752834	1.116138
7	6	-2.106815	1.328142	-0.853271
8	7	-1.293996	1.696445	0.173337
9	6	-0.887805	0.577732	1.086293
10	8	-1.057222	0.867672	2.342173
11	8	-2.335440	2.058953	-1.805154
12	8	-0.487259	2.819553	0.041340

13	1	-3.266229	-3.556155	0.858388
14	1	-4.468126	-2.765850	-1.143231
15	1	-4.027369	-0.477573	-2.058856
16	1	-1.597785	-2.106025	1.997232
17	1	-0.828823	3.216441	-0.783033
18	6	3.156202	-1.517308	-0.670318
19	6	4.548094	-1.618397	-0.673723
20	6	5.325648	-0.534649	-0.274642
21	6	4.705099	0.649584	0.126808
22	6	3.316823	0.749827	0.126345
23	6	2.527378	-0.334640	-0.275139
24	6	1.017386	-0.219299	-0.300552
25	7	0.514251	0.289761	0.974723
26	1	2.554532	-2.366527	-0.979166
27	1	5.021021	-2.543736	-0.983612
28	1	6.407291	-0.610442	-0.273768
29	1	5.305529	1.496990	0.439117
30	1	2.831923	1.665866	0.444018
31	1	0.583847	-1.191231	-0.577701
32	1	0.724191	0.493088	-1.079053
33	1	0.814764	-0.263391	1.768804
34	1	-1.851677	1.533553	1.154182

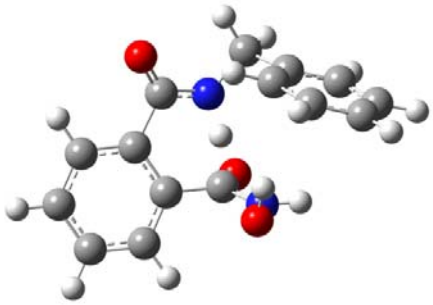
f) 6e



1	6	2.934388	2.729363	-0.533633
2	6	3.790647	1.869661	-1.224389
3	6	3.557461	0.493855	-1.211095
4	6	2.483075	-0.043531	-0.494626
5	6	1.611784	0.822461	0.193585
6	6	1.845573	2.205903	0.163594
7	6	0.458611	0.272839	0.983362
8	6	2.231994	-1.530979	-0.626309
9	7	-0.726814	0.908855	0.820326
10	8	0.586723	-0.699297	1.747312
11	7	2.680965	-2.329562	0.379200
12	8	1.716229	-1.998433	-1.640818

13	6	-1.964250	0.495956	1.483592
14	6	-3.078476	0.198111	0.498226
15	6	-4.327967	0.814985	0.635148
16	6	-5.368603	0.524102	-0.252830
17	6	-5.164994	-0.384382	-1.293214
18	6	-3.918135	-1.001983	-1.439996
19	6	-2.882411	-0.714124	-0.550214
20	8	3.022510	-1.818292	1.628918
21	1	3.113817	3.800095	-0.533220
22	1	4.637945	2.267499	-1.774665
23	1	4.209993	-0.174812	-1.763631
24	1	1.190374	2.872170	0.717568
25	1	-0.791542	1.605291	0.090122
26	1	2.383777	-3.299565	0.383368
27	1	-1.710400	-0.389028	2.073193
28	1	-2.283130	1.278827	2.181733
29	1	-4.491014	1.525446	1.442267
30	1	-6.332377	1.010509	-0.132926
31	1	-5.969962	-0.609901	-1.986554
32	1	-3.752173	-1.709982	-2.246699
33	1	-1.917463	-1.199264	-0.671457
34	1	2.177208	-1.417584	1.953716

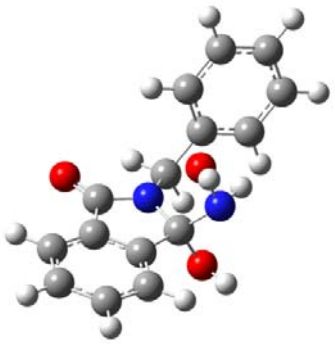
g) TS3



1	6	4.089918	-1.719308	0.352924
2	6	4.530834	-0.442834	0.013014
3	6	3.607833	0.560588	-0.268438
4	6	2.237693	0.299455	-0.234229
5	6	1.790229	-0.984155	0.118447
6	6	2.725107	-1.983664	0.413267
7	6	1.155453	1.332124	-0.414494
8	7	0.567354	2.168514	0.482433
9	6	0.321485	-1.296170	0.138440
10	8	-0.103287	-2.422151	0.419209
11	8	1.504140	2.375321	-1.449026
12	8	0.612697	1.932185	1.852303
13	1	4.803236	-2.506947	0.565691
14	1	5.591903	-0.226172	-0.032170
15	1	3.948601	1.559308	-0.514755

16	1	2.381710	-2.982377	0.659251
17	1	1.575263	1.953852	2.059671
18	6	-3.206909	-1.331369	-0.631119
19	6	-4.495382	-1.321247	-0.096323
20	6	-4.987588	-0.169463	0.511838
21	6	-4.187288	0.971695	0.581243
22	6	-2.903453	0.961276	0.043547
23	6	-2.400736	-0.192381	-0.570154
24	6	-1.013711	-0.202680	-1.180744
25	7	0.016947	0.236037	-0.239849
26	1	-2.826970	-2.232759	-1.101905
27	1	-5.109293	-2.213204	-0.151896
28	1	-5.987231	-0.159189	0.931215
29	1	-4.564053	1.871988	1.053337
30	1	-2.289433	1.853817	0.102051
31	1	-0.774559	-1.207399	-1.542643
32	1	-0.954714	0.476179	-2.033391
33	1	-0.037657	1.864628	-1.158242
34	1	0.463793	3.142364	0.222505

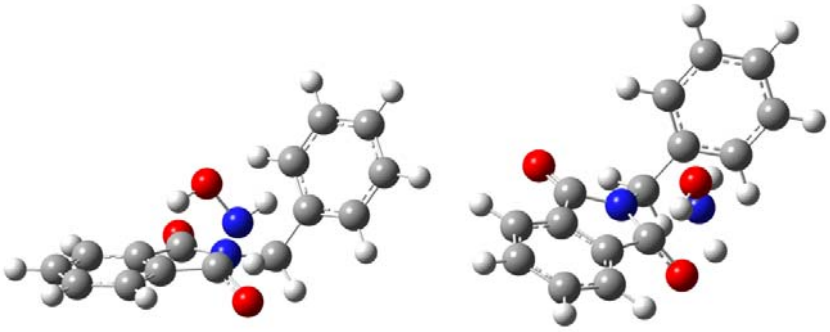
h) 6e''



1	6	4.348980	-0.209967	-0.461632
2	6	3.893033	-1.443275	-0.175920
3	6	2.610458	-1.638862	0.173610
4	6	1.809188	-0.569201	0.230795
5	6	2.257986	0.660532	-0.060091
6	6	3.535964	0.859800	-0.408358
7	6	0.352895	-0.511345	0.569319
8	7	0.172754	0.922503	0.434554
9	6	1.295292	1.612439	0.052419
10	6	-1.081876	1.549961	0.820322
11	8	1.367776	2.805062	-0.115248
12	8	0.120176	-0.996663	1.882983
13	7	-0.417839	-1.367196	-0.320588
14	8	-0.068098	-1.204104	-1.579803
15	6	-2.262294	0.757953	0.307286
16	6	-2.614387	0.828425	-0.988882

17	6	-3.644215	0.111232	-1.463268
18	6	-4.336721	-0.692255	-0.642053
19	6	-3.996490	-0.769883	0.653083
20	6	-2.968802	-0.045936	1.122269
21	1	5.406471	-0.073795	-0.745059
22	1	4.578703	-2.305631	-0.229132
23	1	2.224189	-2.643627	0.404835
24	1	3.916182	1.866599	-0.644382
25	1	-1.108451	1.648264	1.929516
26	1	-1.149314	2.585578	0.413874
27	1	0.306206	-1.936254	1.850360
28	1	-1.429873	-1.181714	-0.222534
29	1	0.821778	-1.485644	-1.715822
30	1	-2.044091	1.475309	-1.676220
31	1	-3.918244	0.177381	-2.529524
32	1	-5.181018	-1.286294	-1.030106
33	1	-4.560929	-1.431302	1.331613
34	1	-2.698891	-0.128079	2.187977

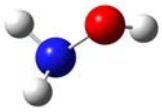
i) TS4



1	6	-4.446202	0.400882	0.553680
2	6	-4.249086	-0.937219	0.197680
3	6	-3.003657	-1.387046	-0.252777
4	6	-1.974660	-0.462177	-0.342728
5	6	-2.171277	0.863939	0.015212
6	6	-3.400797	1.321948	0.467886
7	6	-0.539408	-0.653854	-0.806819
8	7	-0.568865	-1.580060	0.359960
9	6	-0.897303	1.610588	-0.187716
10	8	-0.684801	2.793915	-0.007322
11	8	-0.375472	-1.343364	-1.848597
12	8	-0.782994	-1.411114	1.766431
13	1	-5.421560	0.722244	0.900787
14	1	-5.074652	-1.636062	0.273083
15	1	-2.857131	-2.428212	-0.519219
16	1	-3.533269	2.361888	0.741946
17	1	-1.573322	-1.935280	1.937881
18	6	2.685504	0.757861	0.945849

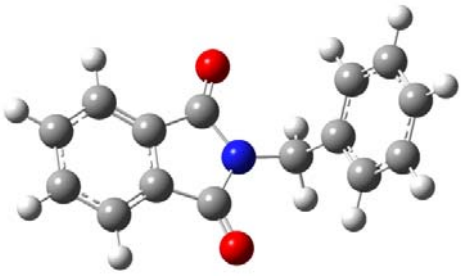
19	6	3.796102	0.263837	1.622996
20	6	4.754595	-0.491910	0.945778
21	6	4.594973	-0.749672	-0.413245
22	6	3.479758	-0.253444	-1.090558
23	6	2.515113	0.503956	-0.419973
24	6	1.325558	1.072382	-1.173121
25	7	0.021453	0.681248	-0.645724
26	1	1.941305	1.340421	1.477281
27	1	3.915546	0.468270	2.681151
28	1	5.619380	-0.875629	1.475434
29	1	5.335254	-1.334462	-0.948026
30	1	3.359752	-0.453656	-2.150902
31	1	1.337972	2.163568	-1.120425
32	1	1.380938	0.785088	-2.226062
33	1	-1.072605	-2.490415	-0.468533
34	1	0.405131	-1.871991	0.288988

j) 6e'''



1	7	-0.691921	-0.000007	0.153472
2	8	0.725982	0.000008	-0.140079
3	1	-1.044573	-0.816561	-0.346870
4	1	-1.044579	0.816586	-0.346799
5	1	1.124740	-0.000044	0.739997

k) 7



1	6	4.268824	0.700058	-0.989756
2	6	4.268838	-0.700250	-0.989538
3	6	3.181789	-1.423579	-0.481066
4	6	2.110467	-0.698300	0.018007
5	6	2.110452	0.698370	0.017780
6	6	3.181759	1.423520	-0.481511
7	6	0.825741	-1.165732	0.620558
8	7	0.121945	0.000186	0.958511
9	6	0.825717	1.165988	0.620133

10	8	0.434913	2.301996	0.806340
11	8	0.434936	-2.301681	0.807117
12	6	-1.214685	0.000273	1.554972
13	6	-2.330112	0.000027	0.527069
14	6	-2.844433	1.208060	0.040278
15	6	-3.860941	1.207625	-0.915313
16	6	-4.371793	-0.000342	-1.393713
17	6	-3.860155	-1.208096	-0.915683
18	6	-2.843631	-1.208167	0.039934
19	1	5.126184	1.231965	-1.390842
20	1	5.126209	-1.232268	-1.390452
21	1	3.171067	-2.508549	-0.474861
22	1	3.170993	2.508491	-0.475645
23	1	-1.273682	-0.888503	2.187435
24	1	-1.273715	0.889383	2.186942
25	1	-2.436941	2.146170	0.406508
26	1	-4.254999	2.150096	-1.284562
27	1	-5.165480	-0.000474	-2.135283
28	1	-4.253544	-2.150726	-1.285234
29	1	-2.435524	-2.146131	0.405852

Second order perturbation theory analysis for intermediates **6e** and **6d**

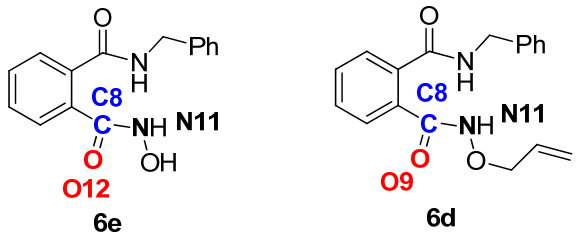


Figure S18. Intermediates **6e** and **6d** involved in rds of phthalimidation

Results: Stabilization energy from n to π* explains the electrophilicity of C8 in the intermediates **6e** and **6d** involved in rate determining step of phthalimidation.

Molecule	**Donor orbital**	**Acceptor orbital**	**Stabilization energy (kcal/mol)**
6e (intermediate from **4e**)	LP (1) N11	BD*(2) C8-O12	28.85
6d (intermediate from **4d**)	LP (1) N11	BD*(2) C8-O9	49.71

Method: B3LYP/6-311G(d,p) guess=save pop=(nbo,savemixed) geom=connectivity test

6e:

1	6	2.936436	2.721403	-0.533813
2	6	3.790350	1.861224	-1.219565
3	6	3.554242	0.489333	-1.206571
4	6	2.478346	-0.042570	-0.495113
5	6	1.609690	0.823560	0.188637

6	6	1.846703	2.203009	0.158995
7	6	0.459200	0.273224	0.981396
8	6	2.220842	-1.528915	-0.626700
9	7	-0.725568	0.907526	0.817095
10	8	0.592160	-0.691981	1.741525
11	7	2.681479	-2.326619	0.373985
12	8	1.691899	-1.991246	-1.627284
13	6	-1.962585	0.494341	1.479725
14	6	-3.077407	0.197445	0.496979
15	6	-4.325847	0.807008	0.641051
16	6	-5.365785	0.517665	-0.242738
17	6	-5.162877	-0.382273	-1.285653
18	6	-3.917322	-0.992494	-1.439808
19	6	-2.881969	-0.705864	-0.554740
20	8	3.039497	-1.818236	1.618099
21	1	3.118961	3.789651	-0.533542
22	1	4.639200	2.256102	-1.765804
23	1	4.206627	-0.179885	-1.755002
24	1	1.192637	2.870687	0.708778
25	1	-0.789475	1.603917	0.089167
26	1	2.384457	-3.295334	0.380338
27	1	-1.710910	-0.390325	2.067402
28	1	-2.281113	1.274088	2.178408
29	1	-4.488489	1.511357	1.450950
30	1	-6.329421	0.998576	-0.117465
31	1	-5.967920	-0.606962	-1.976080
32	1	-3.752253	-1.694181	-2.249585
33	1	-1.917547	-1.186034	-0.682802
34	1	2.203316	-1.422869	1.956462

6d:

1	6	-2.528897	2.924146	1.006472
2	6	-1.665593	3.372709	0.008418
3	6	-0.725804	2.505248	-0.532962
4	6	-0.597633	1.188271	-0.072230
5	6	-1.480049	0.738067	0.927714
6	6	-2.445450	1.608986	1.446856
7	6	0.460911	0.361916	-0.777804
8	6	-1.417984	-0.639375	1.531655
9	8	-0.504838	-1.013096	2.258312
10	8	0.670012	0.538854	-1.975068
11	7	-2.437649	-1.502612	1.235555
12	7	1.159947	-0.523068	-0.026206
13	8	-3.628126	-1.072090	0.659717
14	6	2.213322	-1.367879	-0.589139
15	6	3.612035	-0.824168	-0.364280
16	6	-3.714071	-1.488233	-0.732323
17	6	-4.955730	-0.881376	-1.299695

18	6	-5.941434	-1.589198	-1.843816
19	6	4.062959	0.287896	-1.086394
20	6	5.341868	0.795436	-0.874246
21	6	6.189831	0.197779	0.059431
22	6	5.751023	-0.910806	0.779006
23	6	4.468173	-1.416860	0.566573
24	1	-3.271171	3.589602	1.432163
25	1	-1.729463	4.393091	-0.351954
26	1	-0.070122	2.828134	-1.331589
27	1	-3.135642	1.246976	2.199568
28	1	-2.535621	-2.327608	1.817783
29	1	0.972198	-0.584469	0.967669
30	1	2.125038	-2.359657	-0.138430
31	1	2.011289	-1.458606	-1.657114
32	1	-2.822486	-1.123993	-1.253567
33	1	-3.738613	-2.581485	-0.784265
34	1	-5.008613	0.203449	-1.268284
35	1	-6.810611	-1.109480	-2.278739
36	1	-5.910359	-2.673787	-1.882614
37	1	3.404018	0.748832	-1.814225
38	1	5.680743	1.655459	-1.441505
39	1	7.186447	0.592977	0.221760
40	1	6.404290	-1.382627	1.504717
41	1	4.131346	-2.281746	1.130090

References:

1 B. K. Paul and N. Guchhait, *J. Phys. Chem. B*, 2011, **115**, 10322-10334.

2 M. R. Wilkins, I. Lindskog, E. Gasteiger, A. Bairoch, J. C. Sanchez, D. F. Hochstrasser and R. D. Appel, *Electrophoresis*, 1997, **18**, 403-408.

3 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara,K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski,R. L. Martin, K.Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

4 G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson, *J. Computational Chemistry*, 2009, **16**, 2785-2791.

5 J. Santoro, C. González, M. Bruix, J. L. Neira, J. L. Nieto, J. Herranz and M. Rico, *J. Mol. Biol.*, 1993, **229**, 722-734.

6 N. Sawatari, T. Yokota, S. Sakaguchi, and Y. Ishii, *J. Org. Chem*., 2001, **66**, 7889-7891.

7 M. Kinter and N. E. Sherman, *Wiley Interscience*, 2000.