Supporting Information

for

A rhodium-catalyzed [4 + 2] annulation strategy to synthesize pyranone-mounted tyrosines with anti-neuroinflammatory activity

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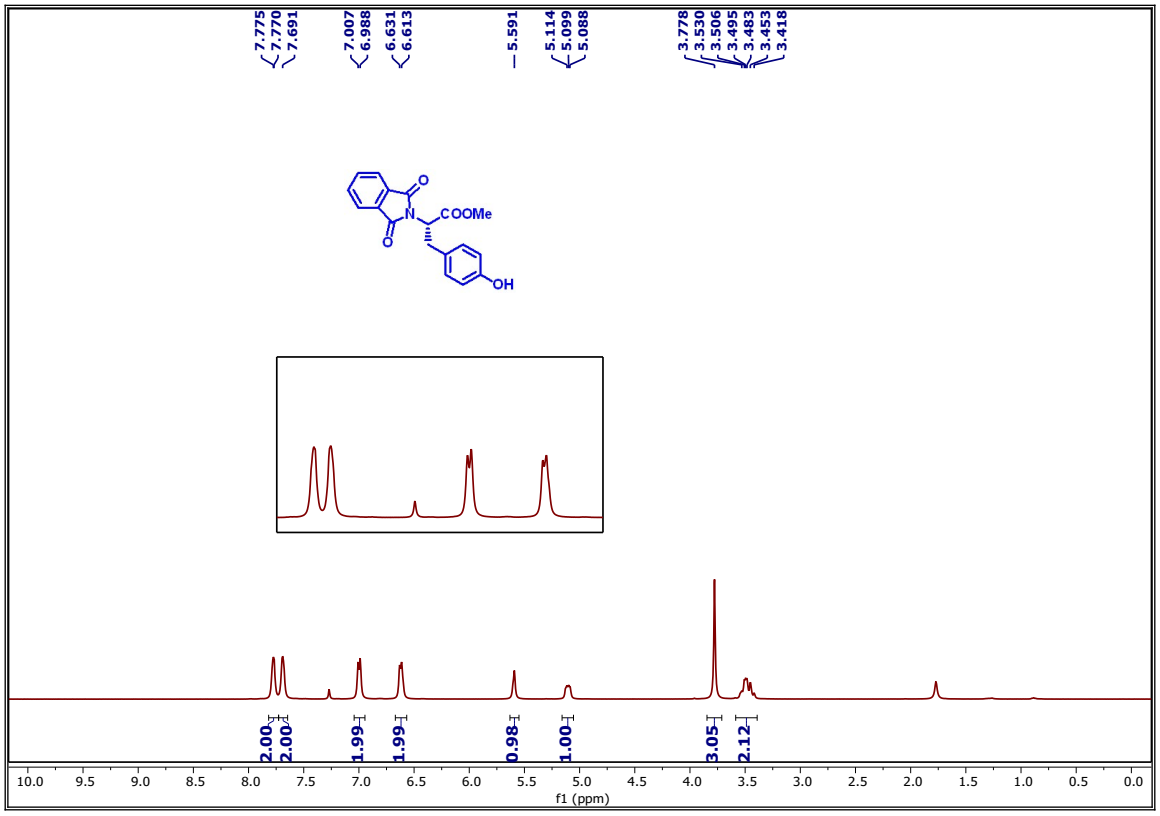
Contents

1. Preparation of unsymmetrical internal alkynes

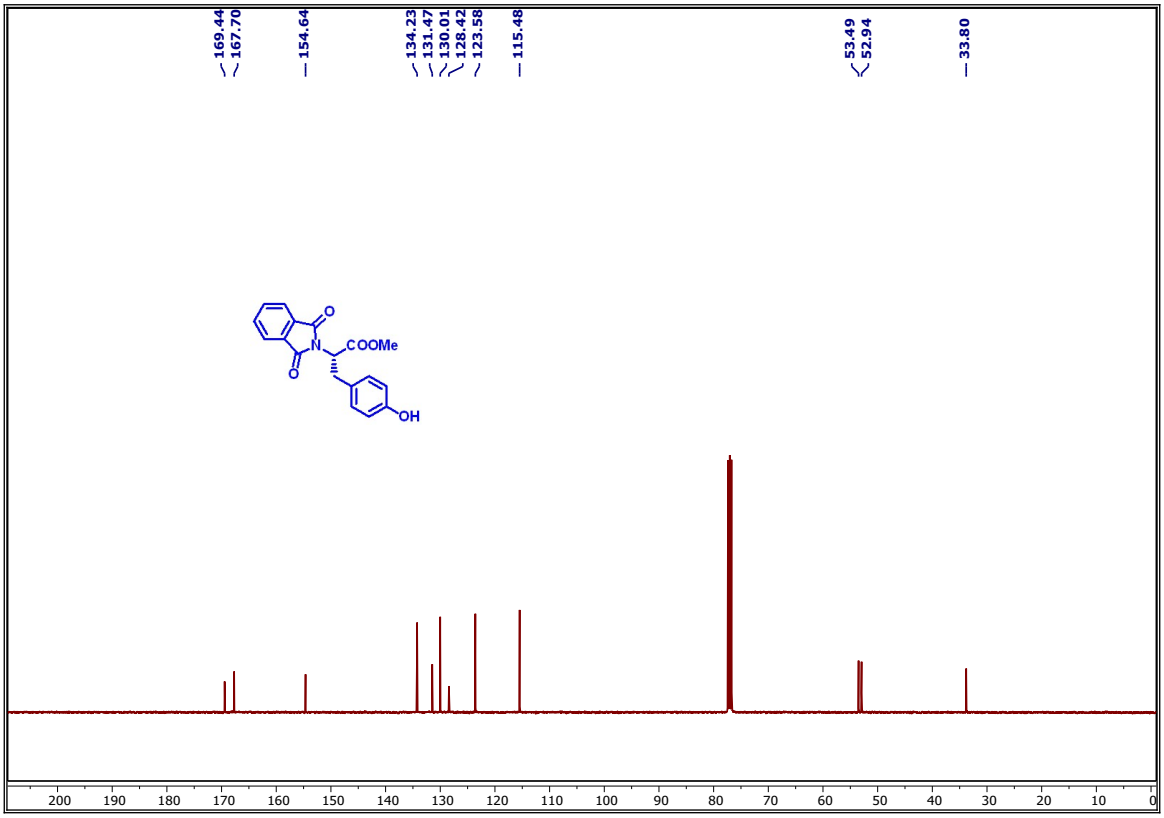
Internal alkynes (**2j**),[1] (**2l-o**),[2] (**2p-q**)[3-4] and (**2r-s**)[5] were prepared by the reported procedures.

2. Original NMR spectra of *N*-phthalimido tyrosine methyl ester

^{1}H NMR of *N*-Phthalimido tyrosine methyl ester (400 MHz, $CDCl_3$)

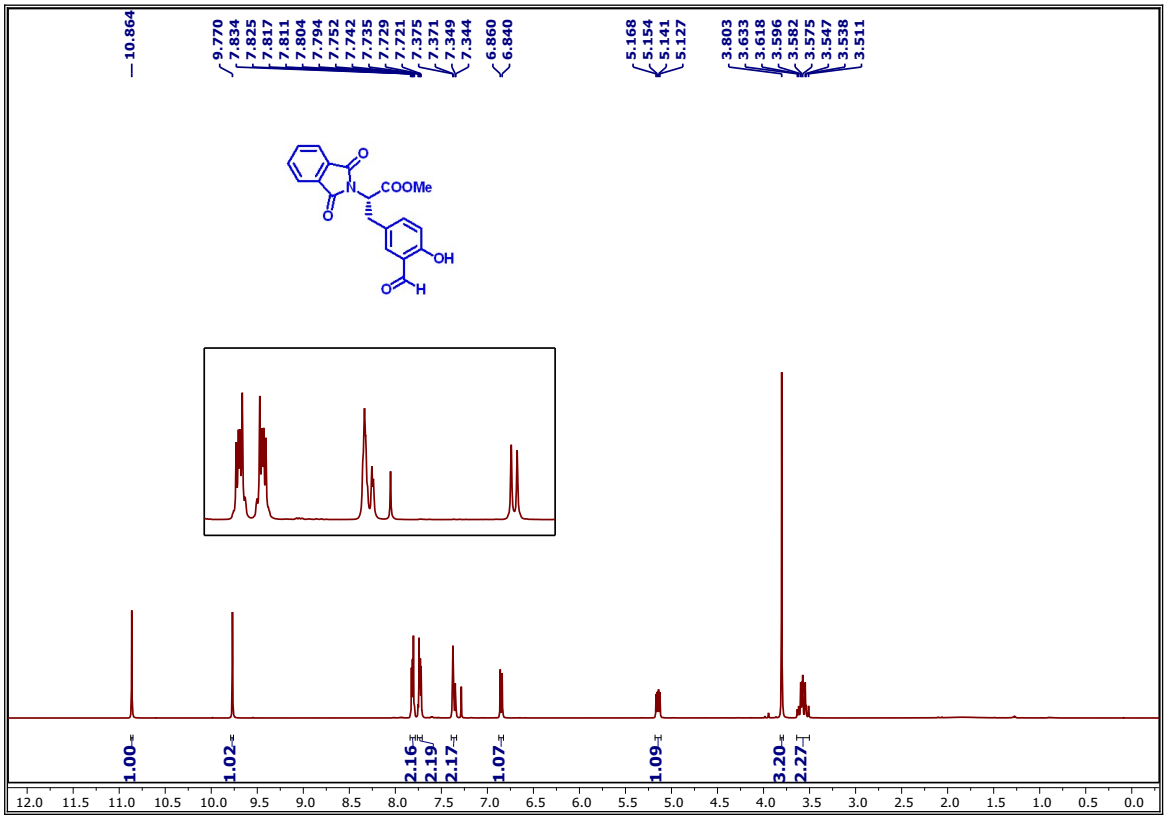


^{13}C NMR of N-Phthalimido tyrosine methyl ester (100 MHz, $CDCl_3$)

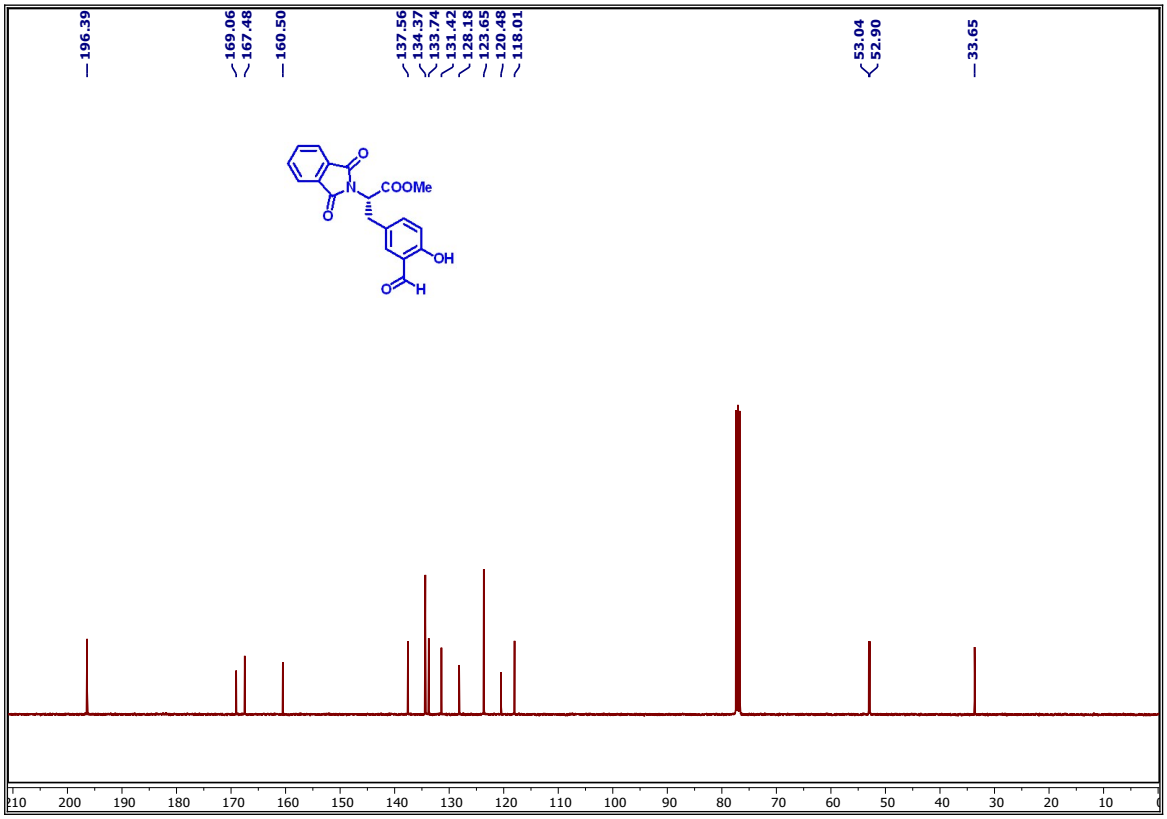


3. Original NMR spectra of *N*-phthalimido C3$_{Ar}$-formyl tyrosine methyl ester (1a)

^{1}H NMR of 1a (400 MHz, $CDCl_3$)

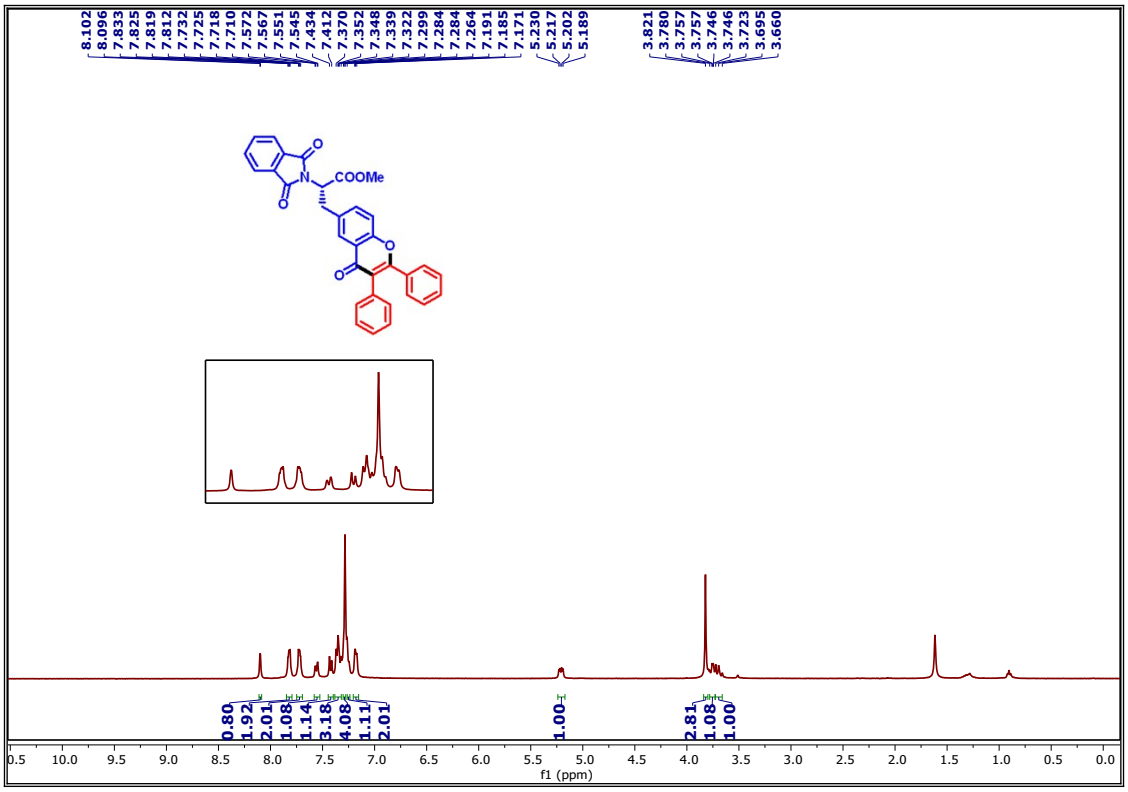


^{13}C NMR of 1a (100 MHz, $CDCl_3$)

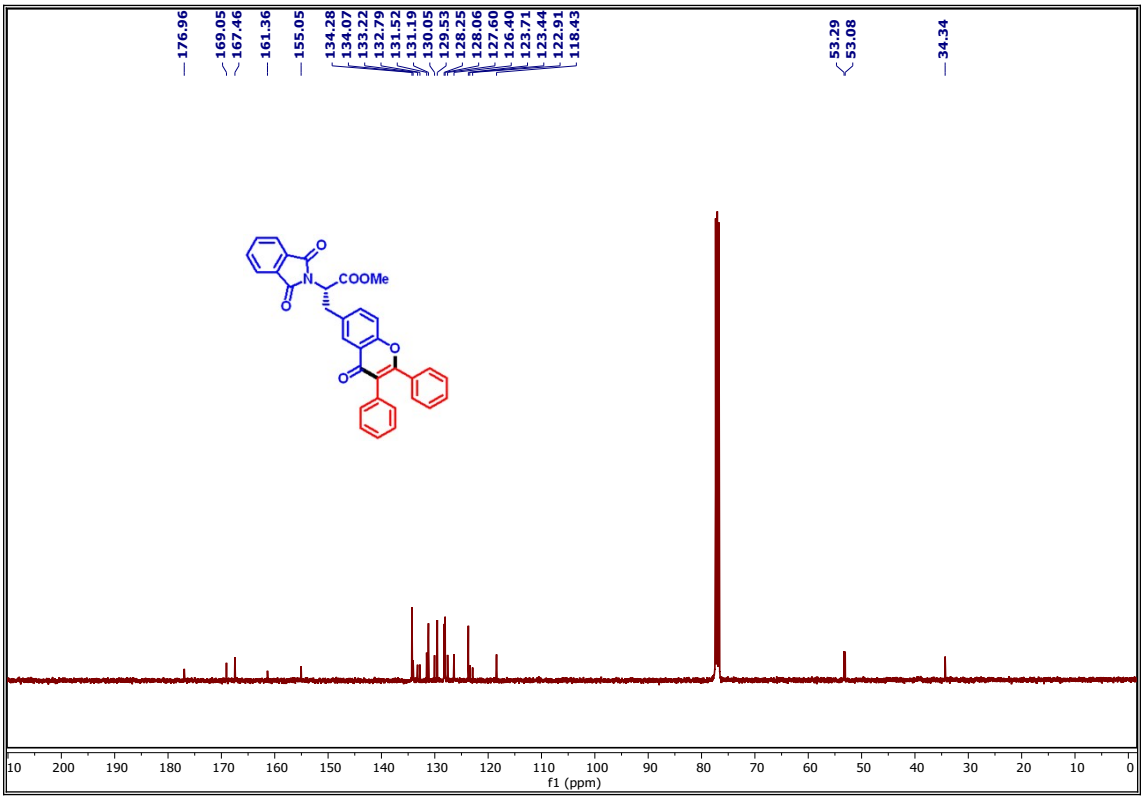


4. Original NMR spectra of 3

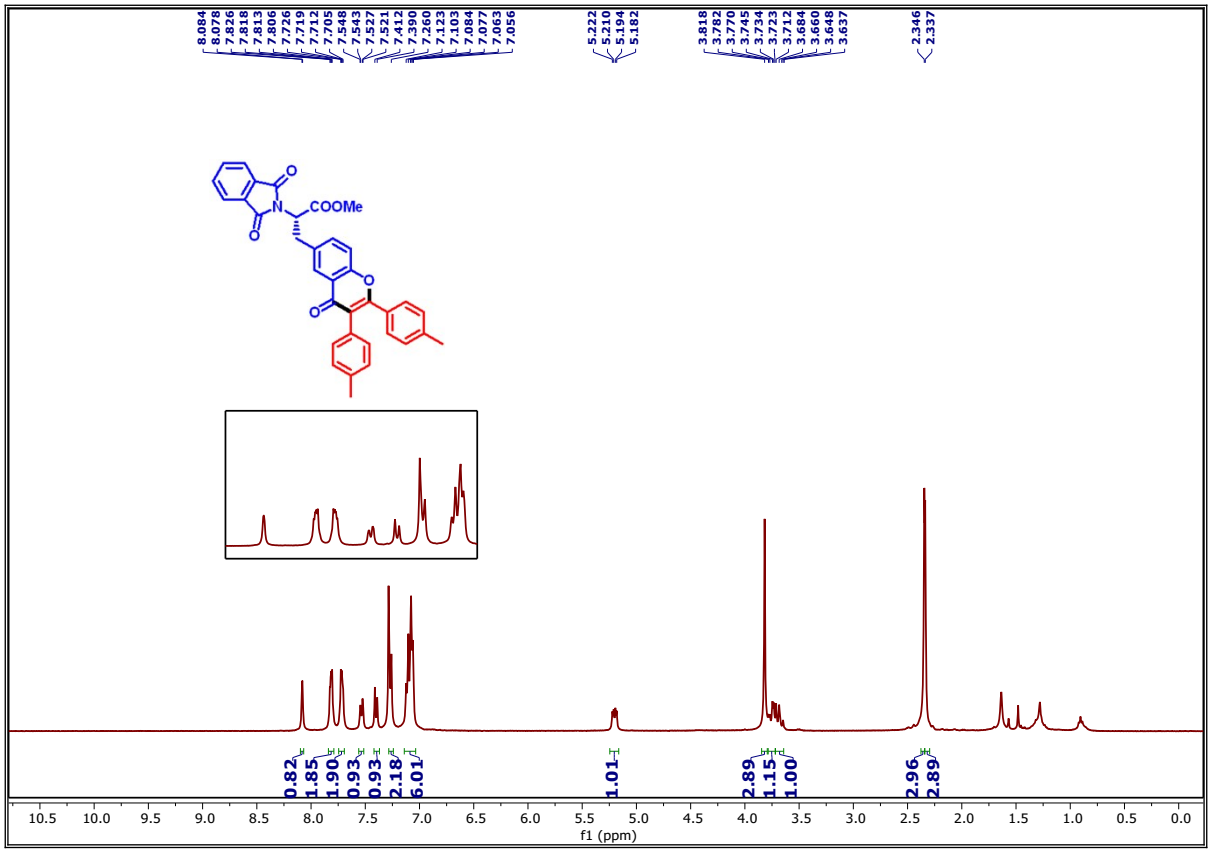
^{1}H NMR of 3aa (400 MHz, $CDCl_3$)



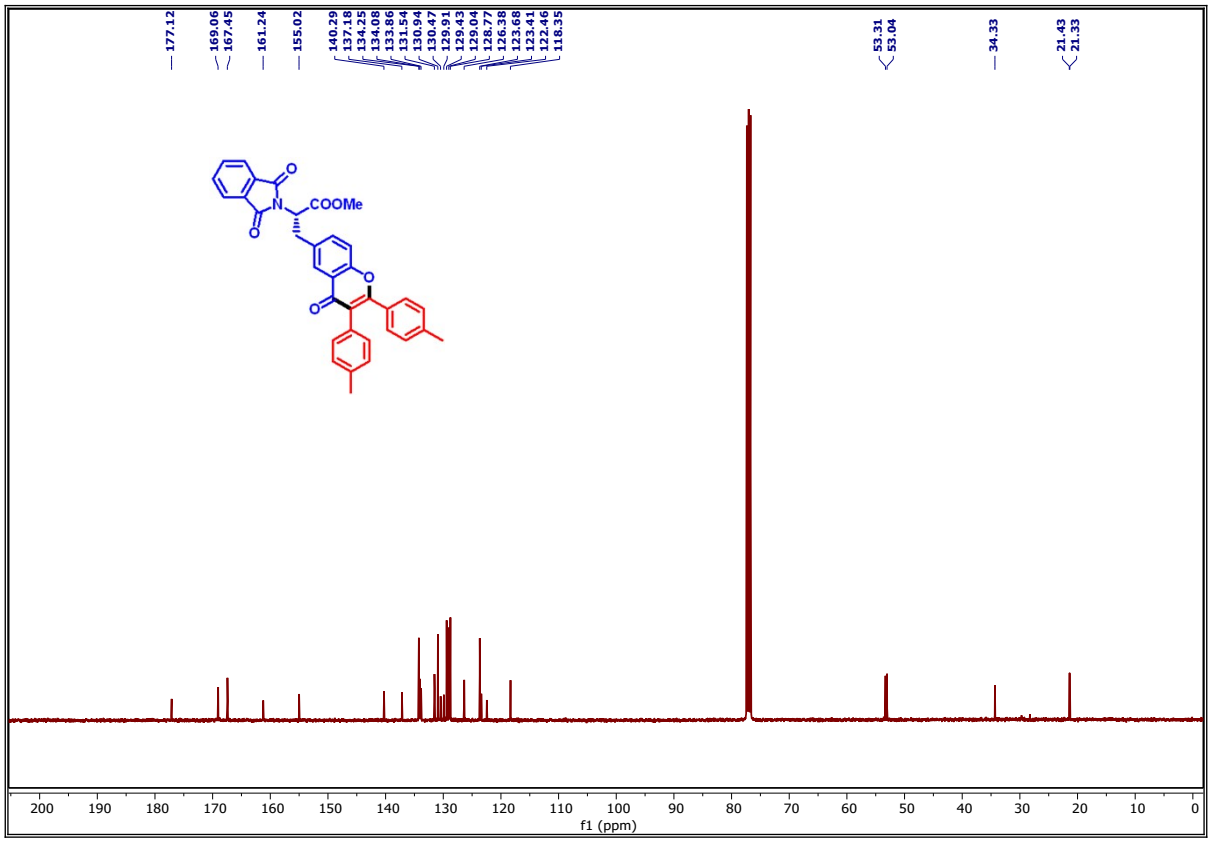
^{13}C NMR of 3aa (100 MHz, $CDCl_3$)



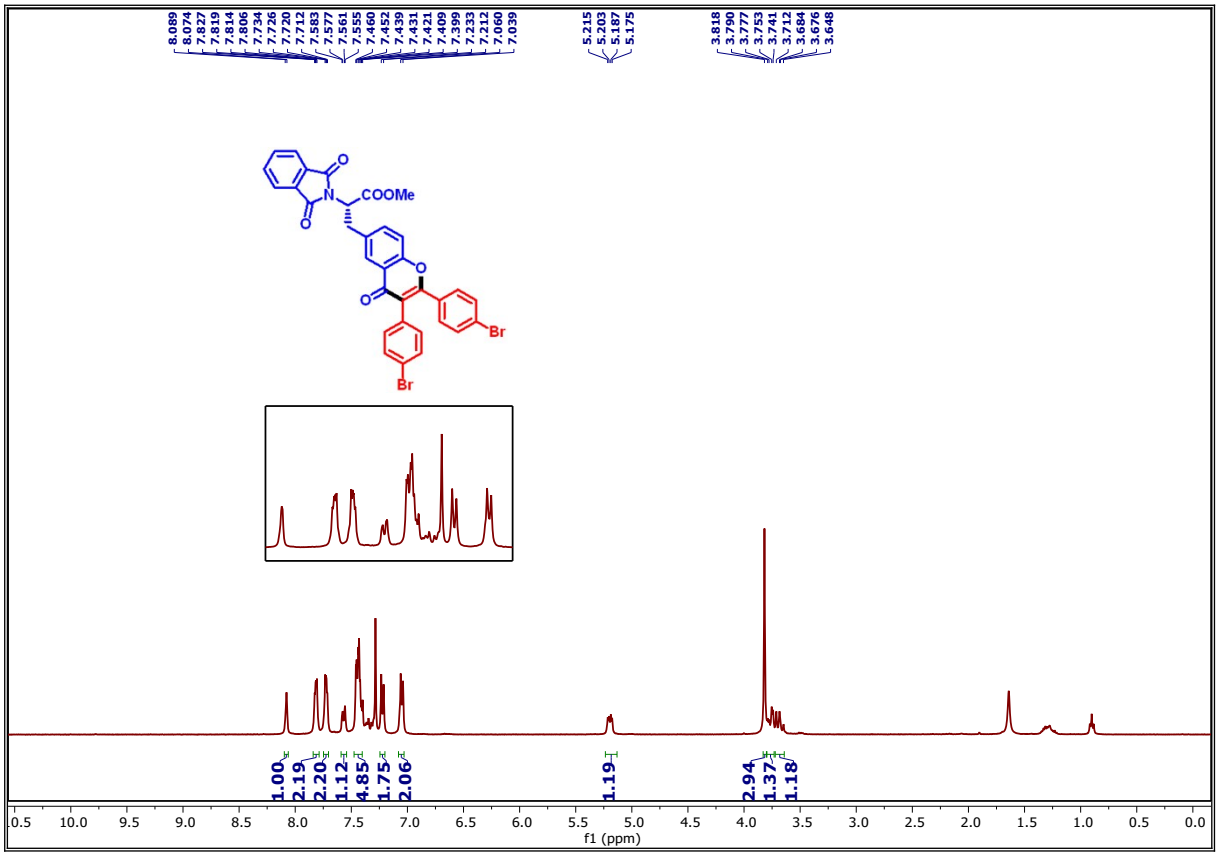
^{1}H NMR of 3ab (400 MHz, $CDCl_3$)



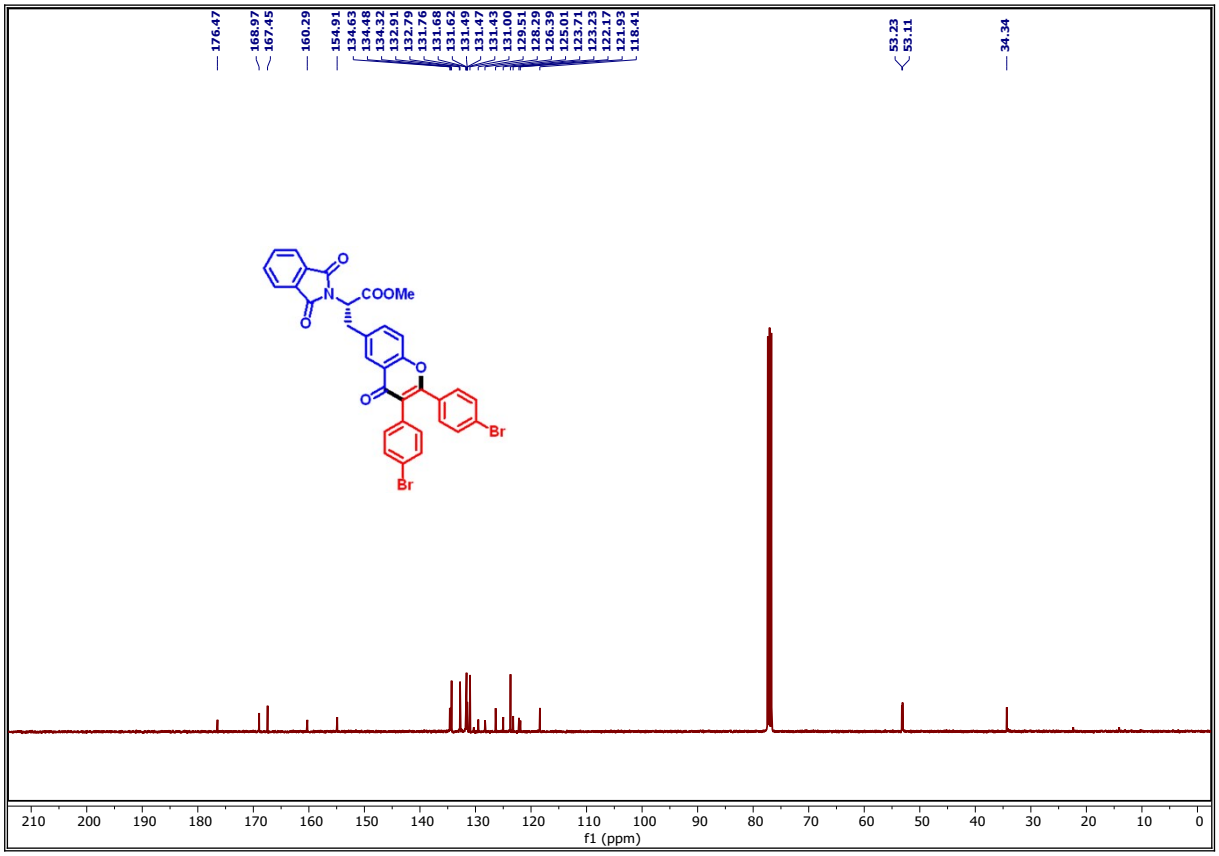
^{13}C NMR of 3ab (100 MHz, $CDCl_3$)



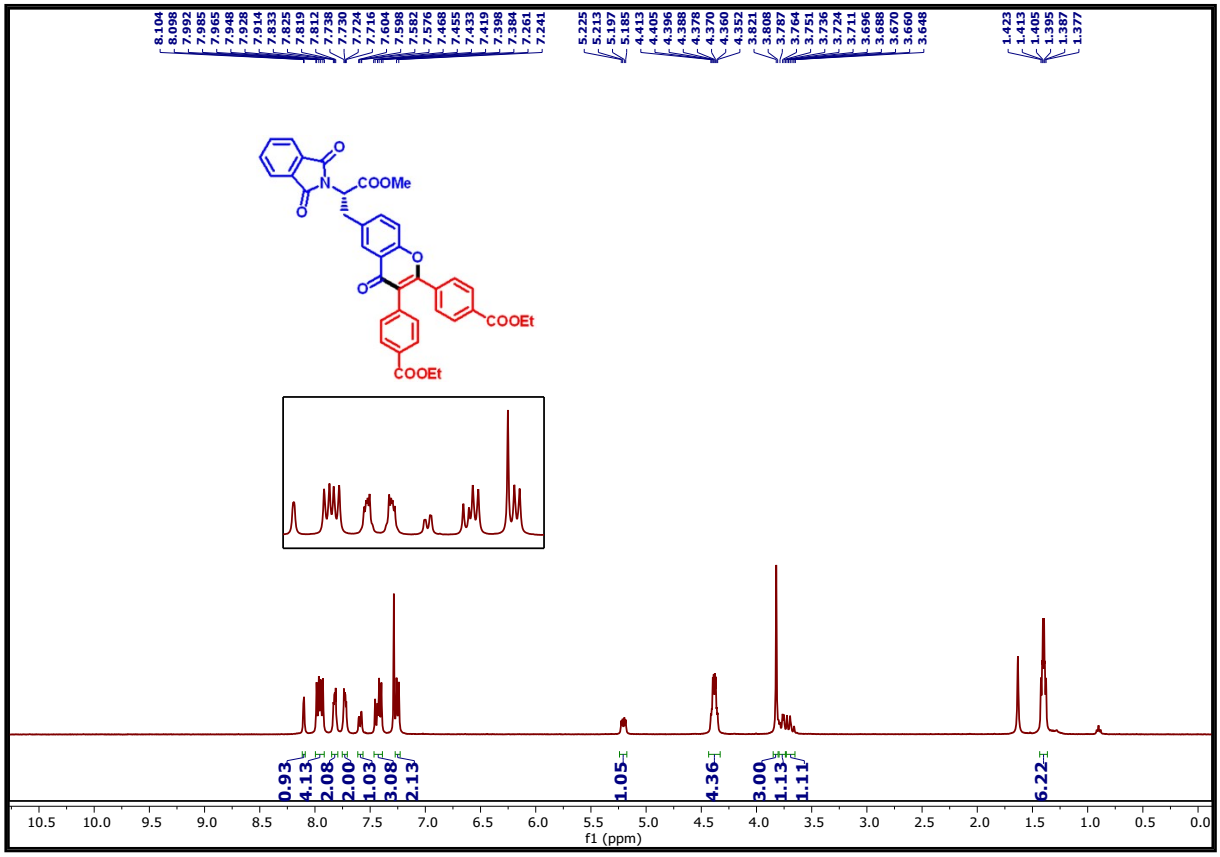
^{1}H NMR of 3ac (400 MHz, $CDCl_3$)



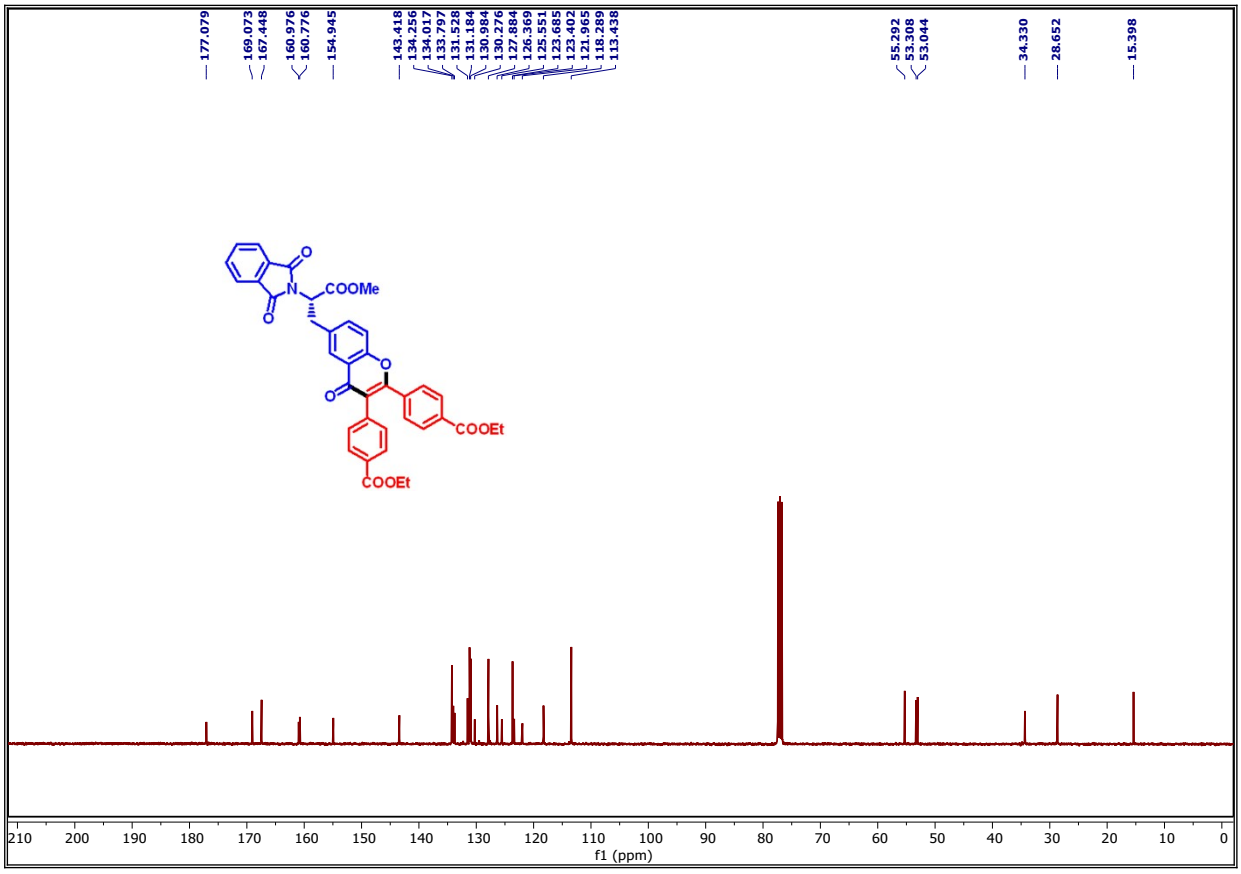
^{13}C NMR of 3ac (100 MHz, $CDCl_3$)



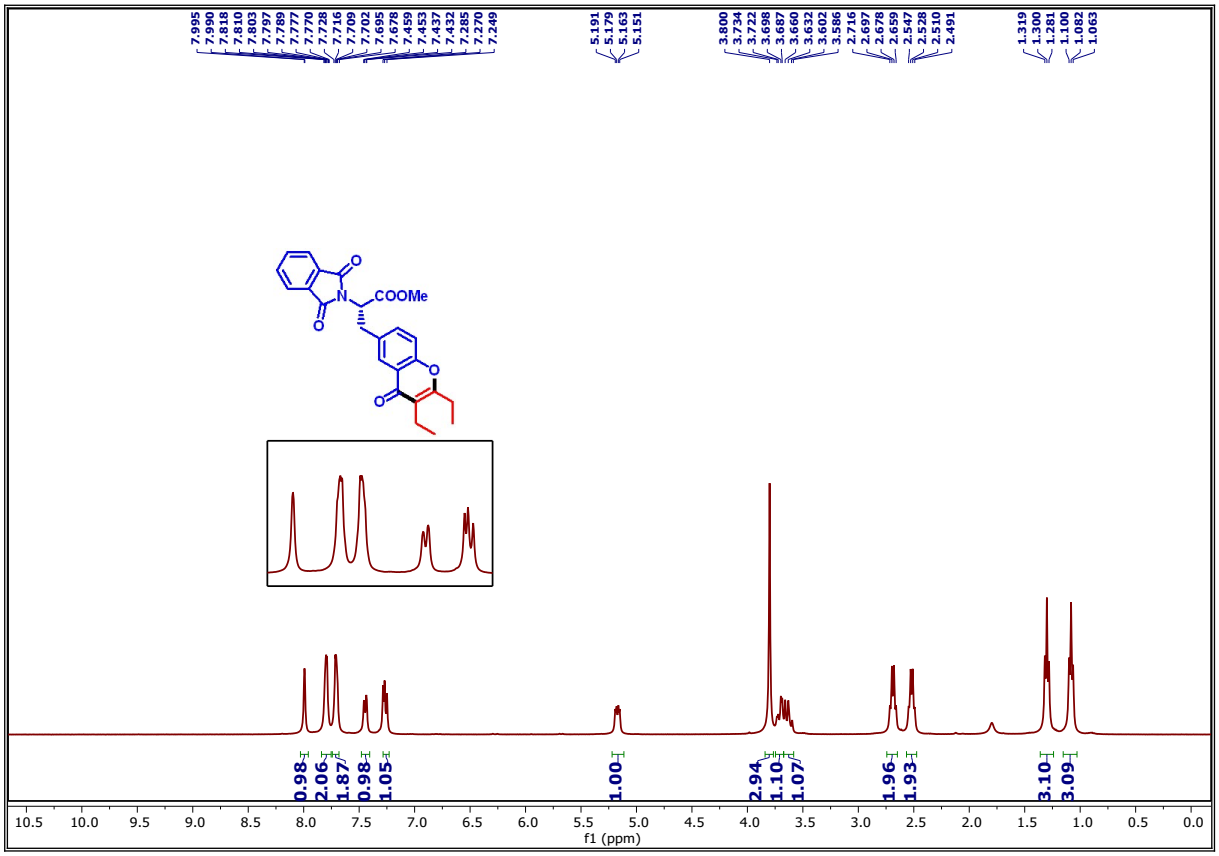
^{1}H NMR of 3ad (400 MHz, $CDCl_3$)



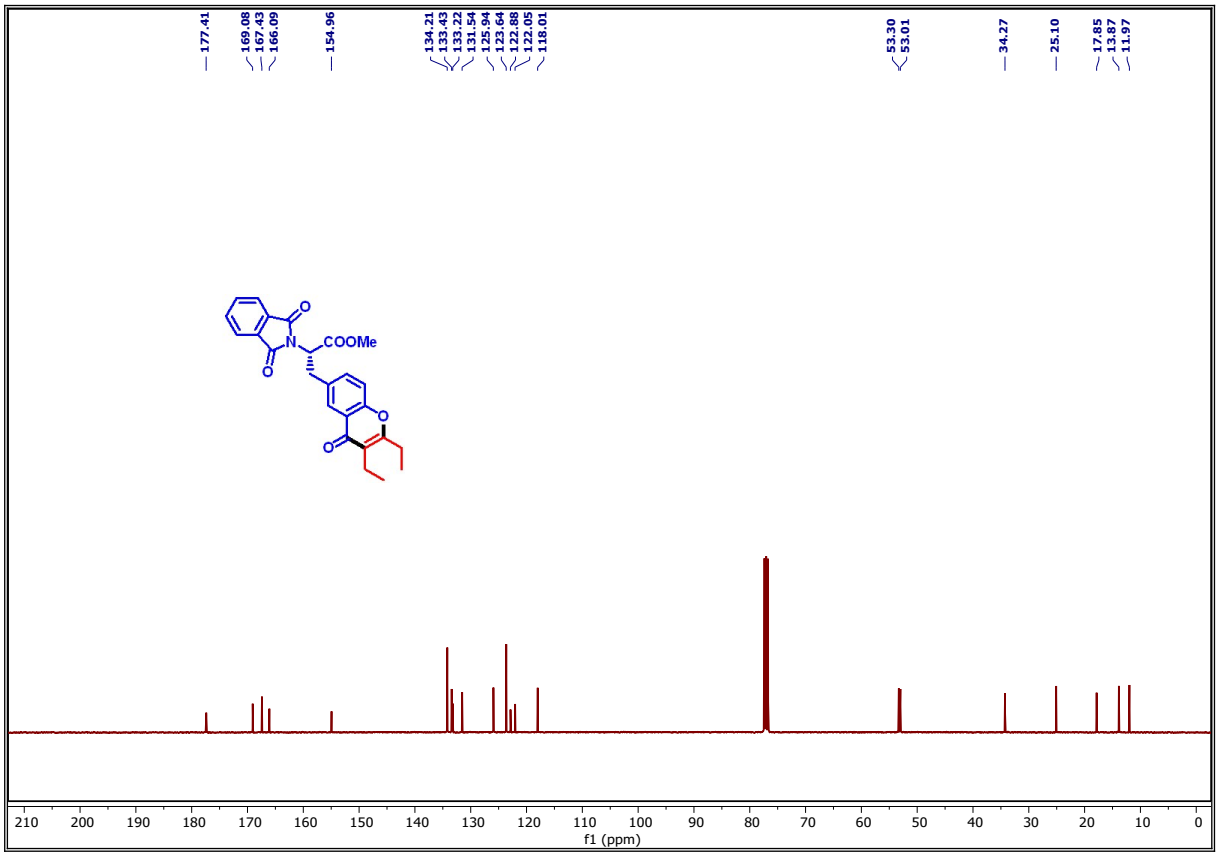
^{13}C NMR of 3ad (100 MHz, $CDCl_3$)



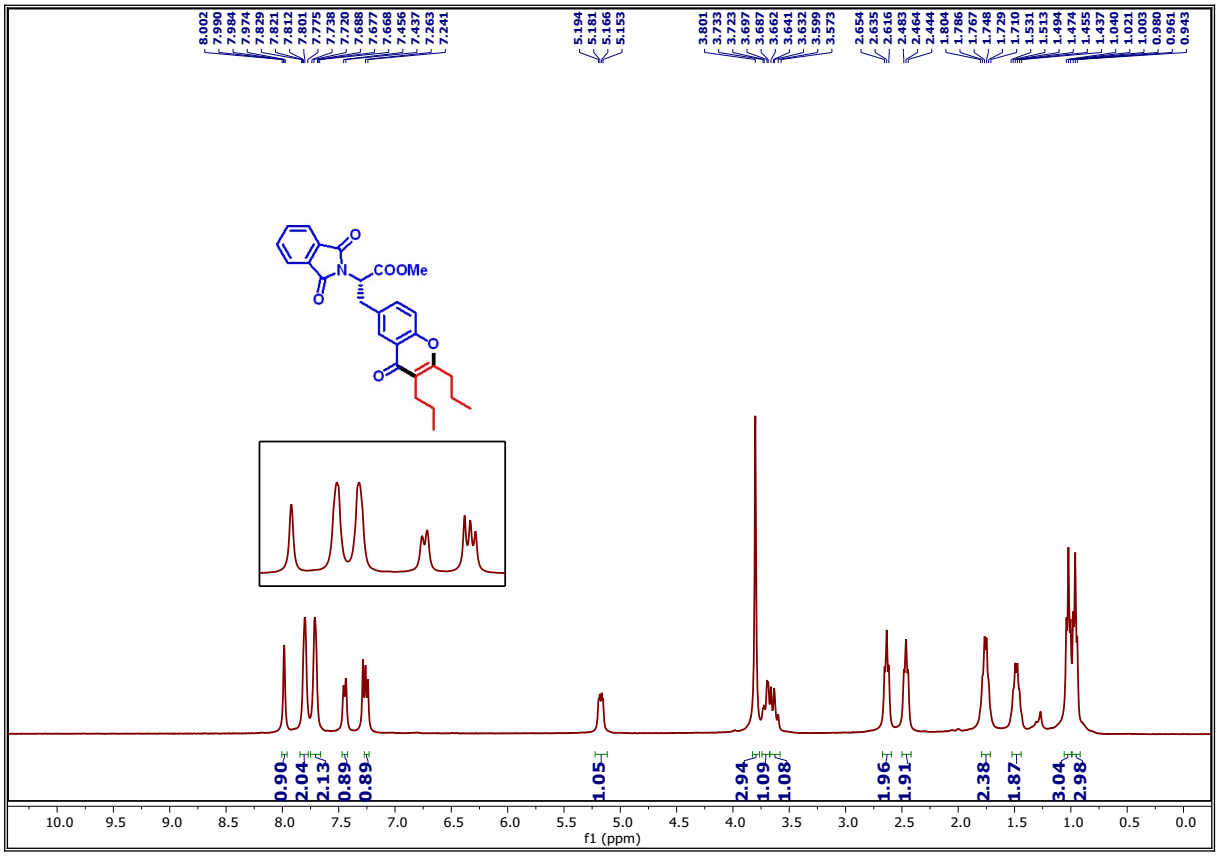
^{1}H NMR of 3ae (400 MHz, $CDCl_3$)



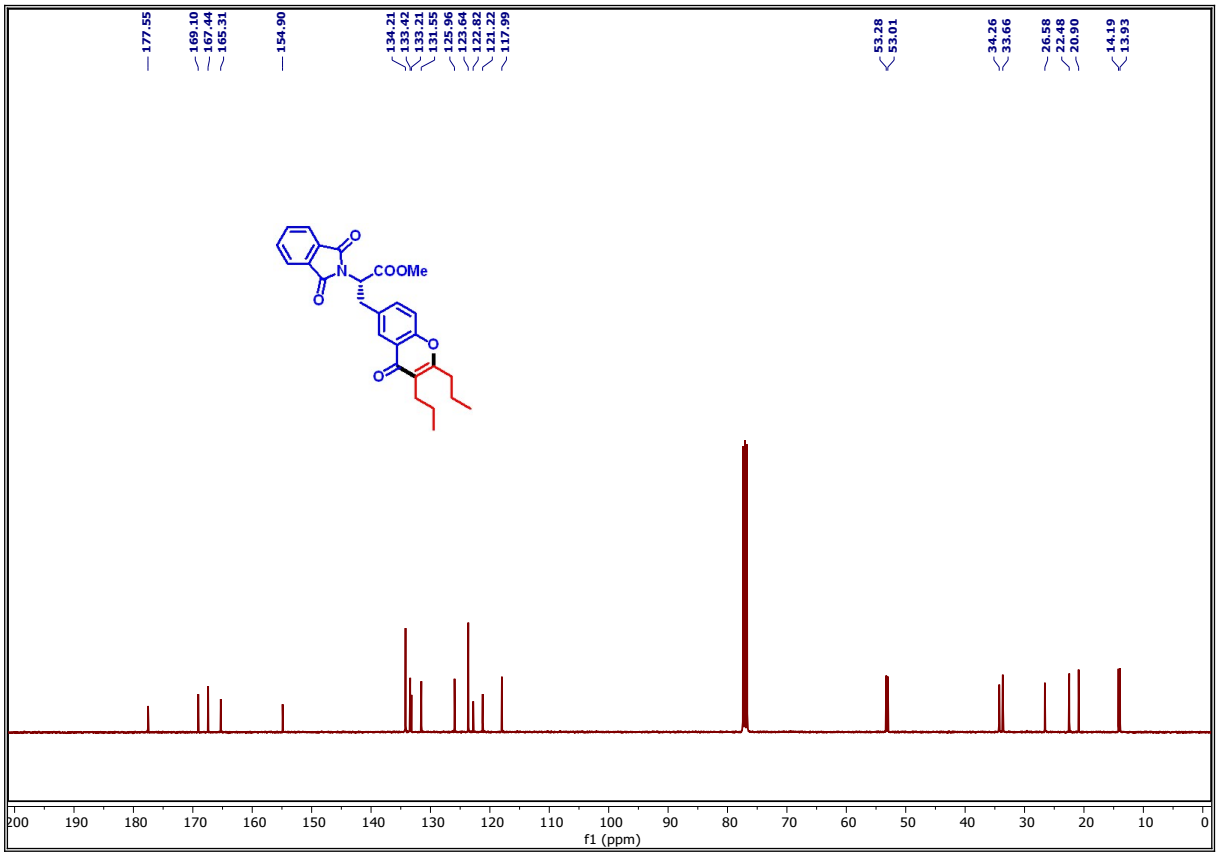
^{13}C NMR of 3ae (100 MHz, $CDCl_3$)



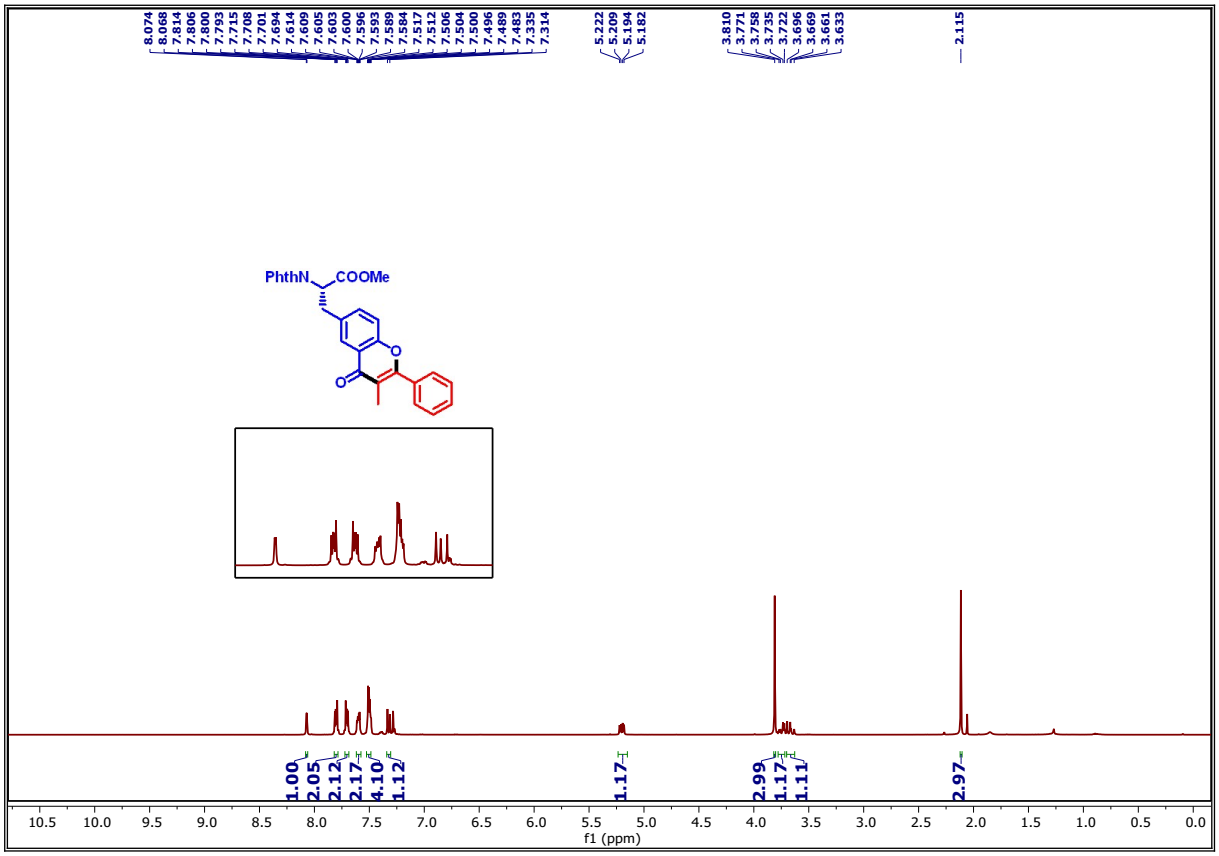
^{1}H NMR of 3af (400 MHz, $CDCl_3$)



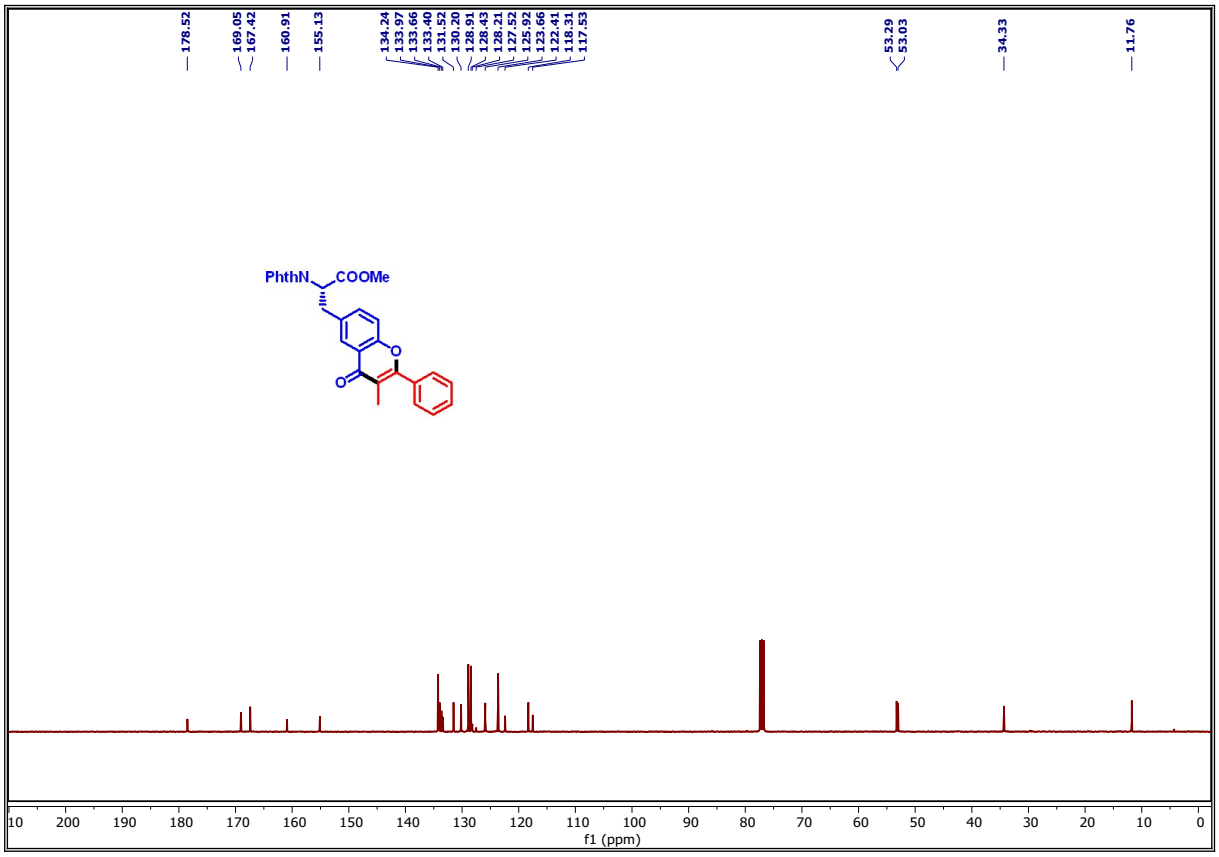
^{13}C NMR of 3af (100 MHz, $CDCl_3$)



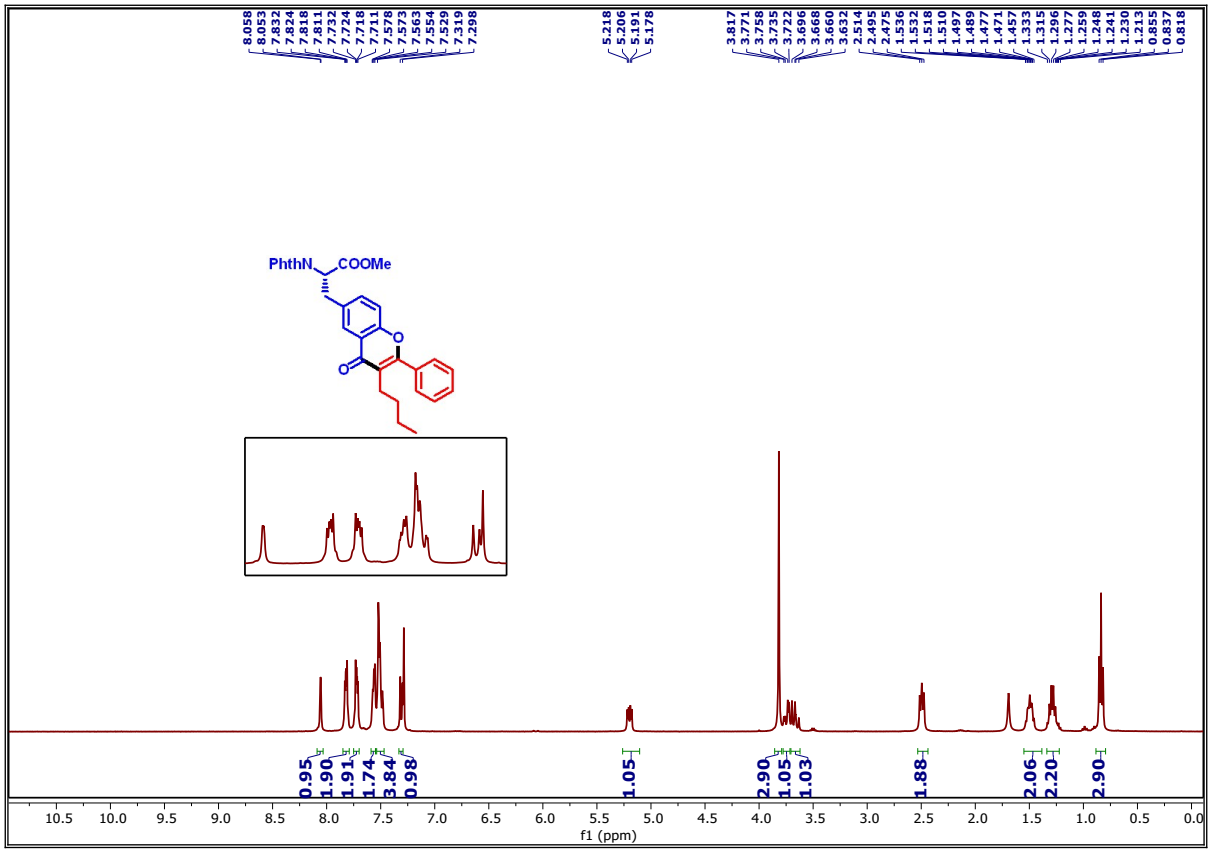
^{1}H NMR of 3ag (400 MHz, $CDCl_3$)



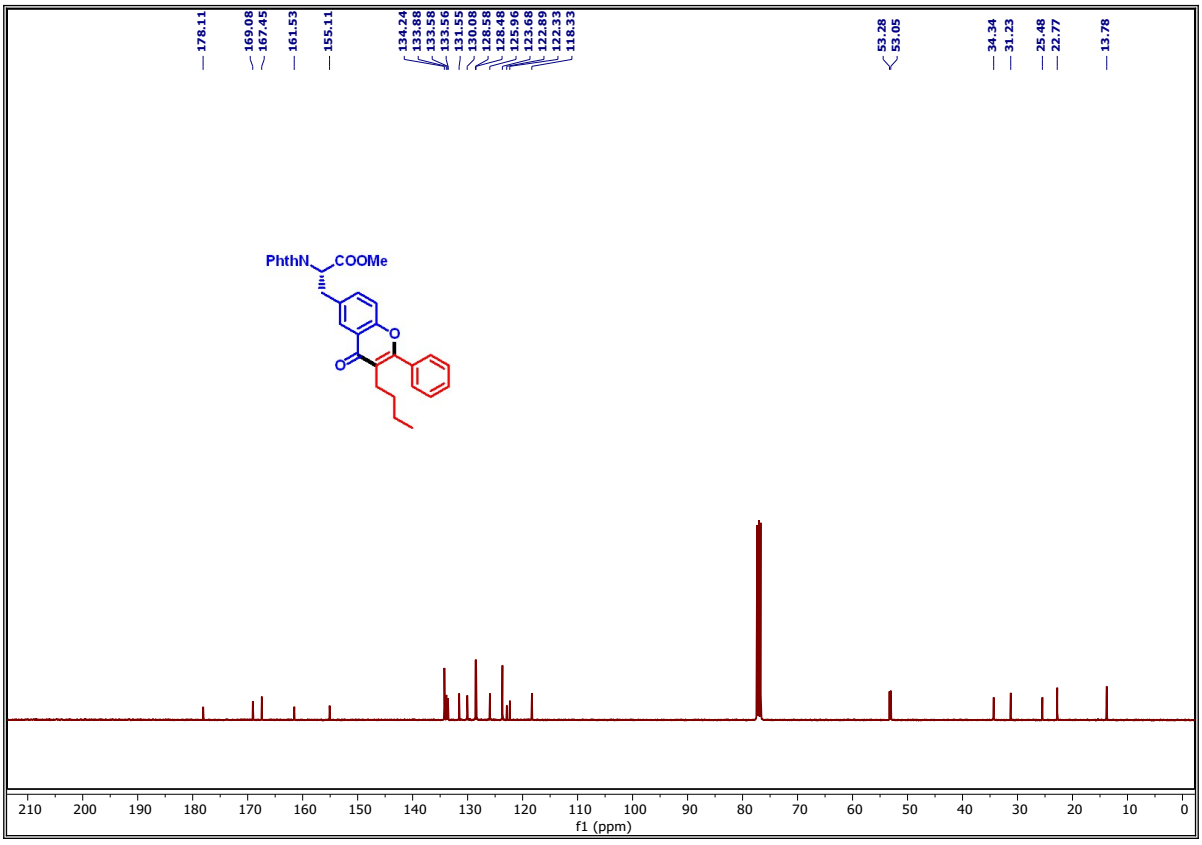
^{13}C NMR of 3ag (100 MHz, $CDCl_3$)



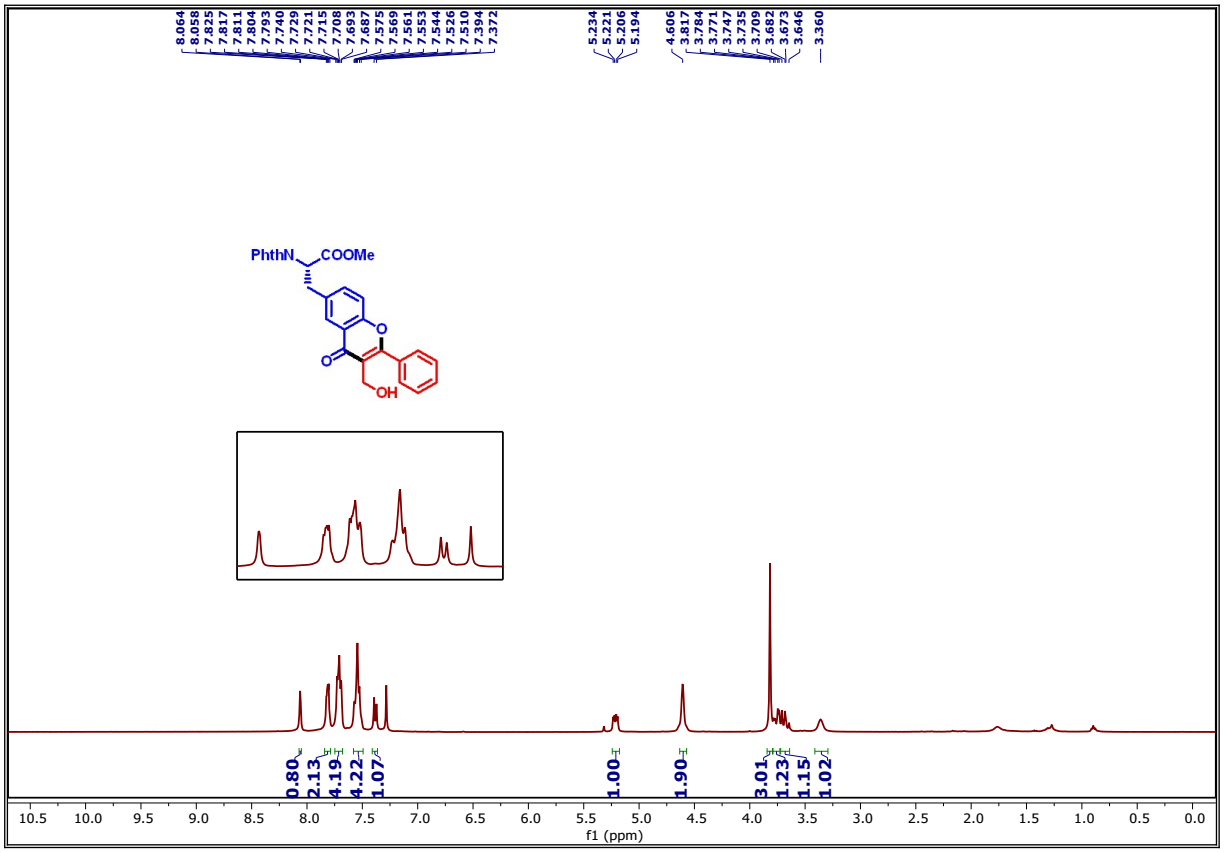
^{1}H NMR of 3ah (400 MHz, $CDCl_3$)



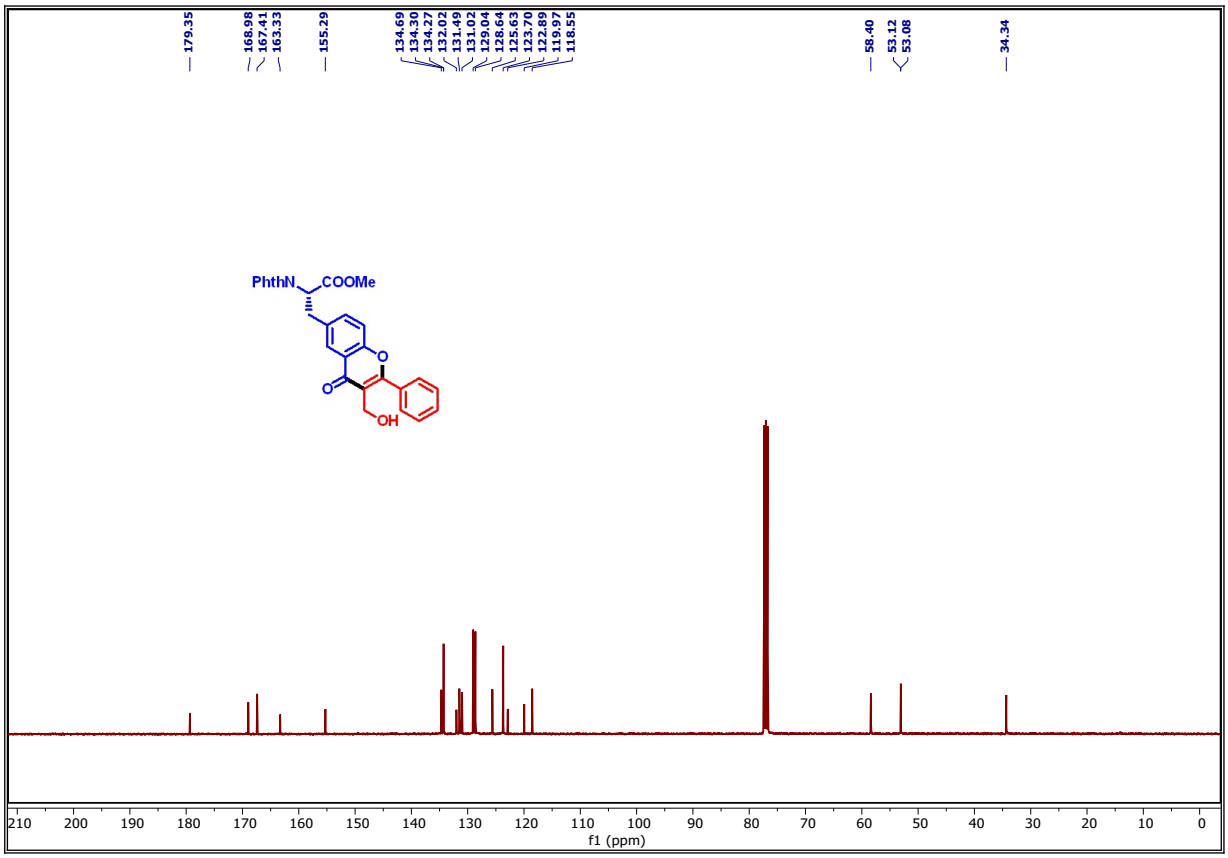
^{13}C NMR of 3ah (100 MHz, $CDCl_3$)



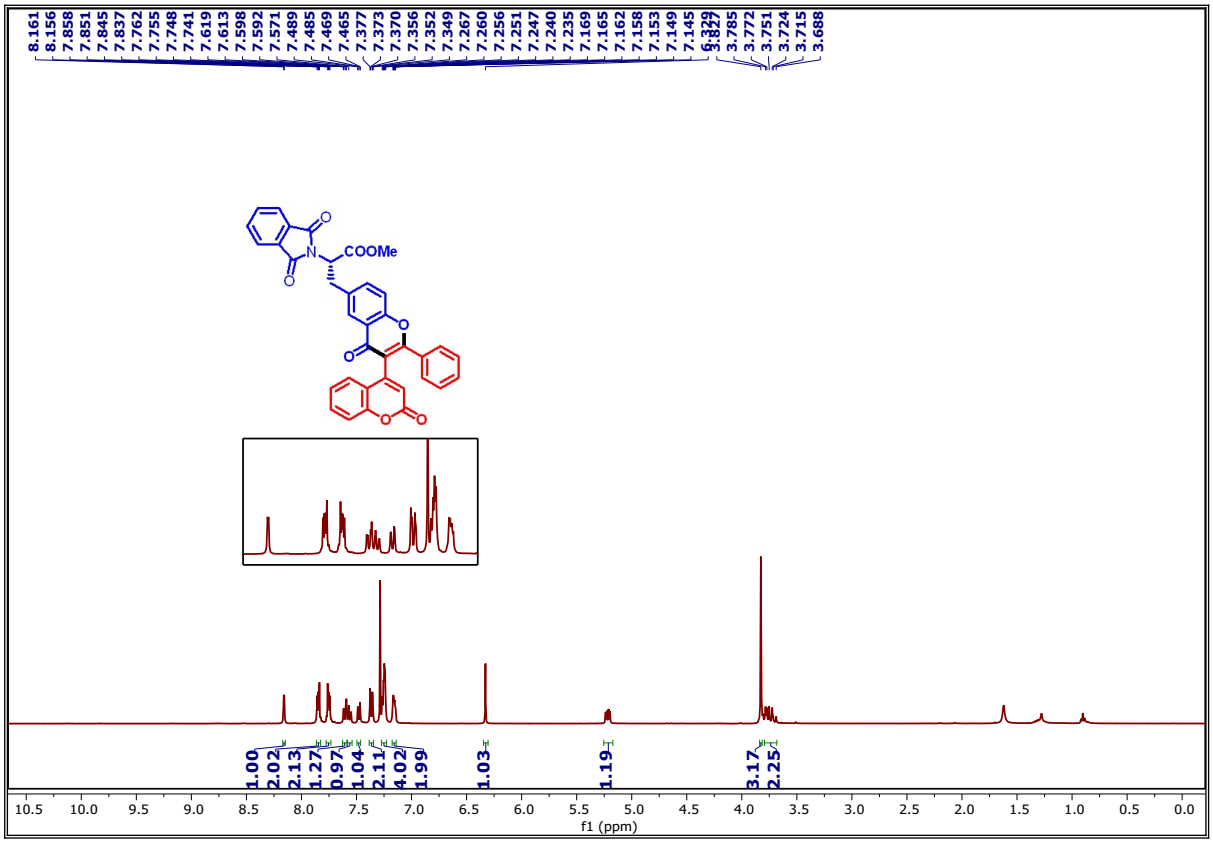
^{1}H NMR of 3ai (400 MHz, $CDCl_3$)



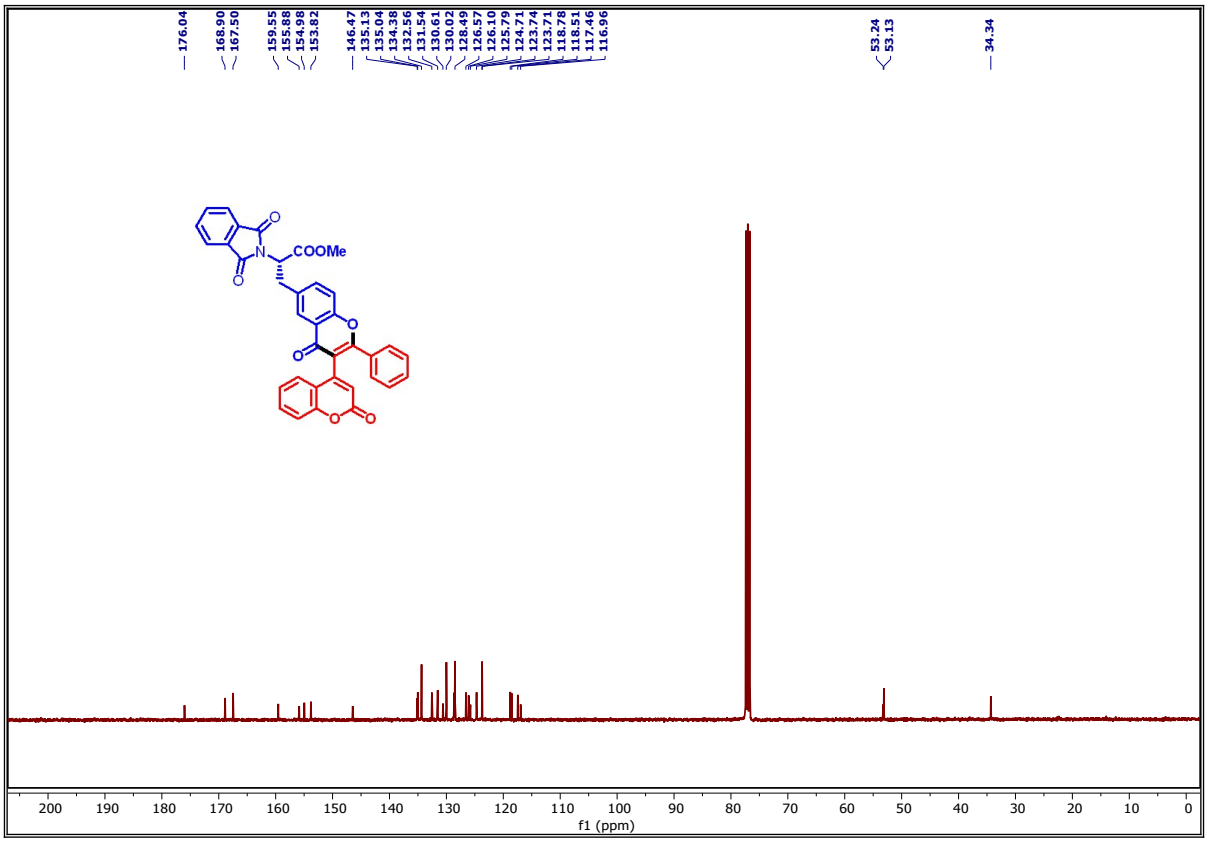
^{13}C NMR of 3ai (100 MHz, $CDCl_3$)



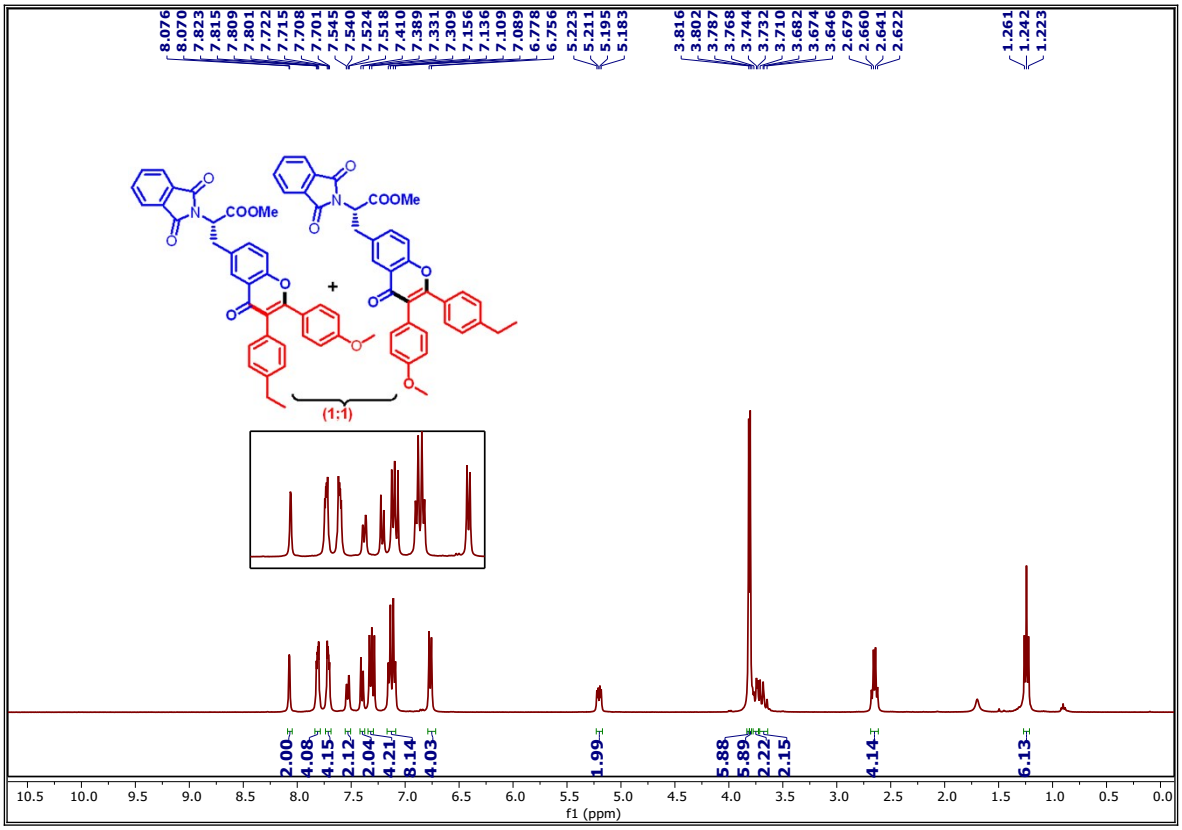
^{1}H NMR of 3aj (400 MHz, $CDCl_3$)



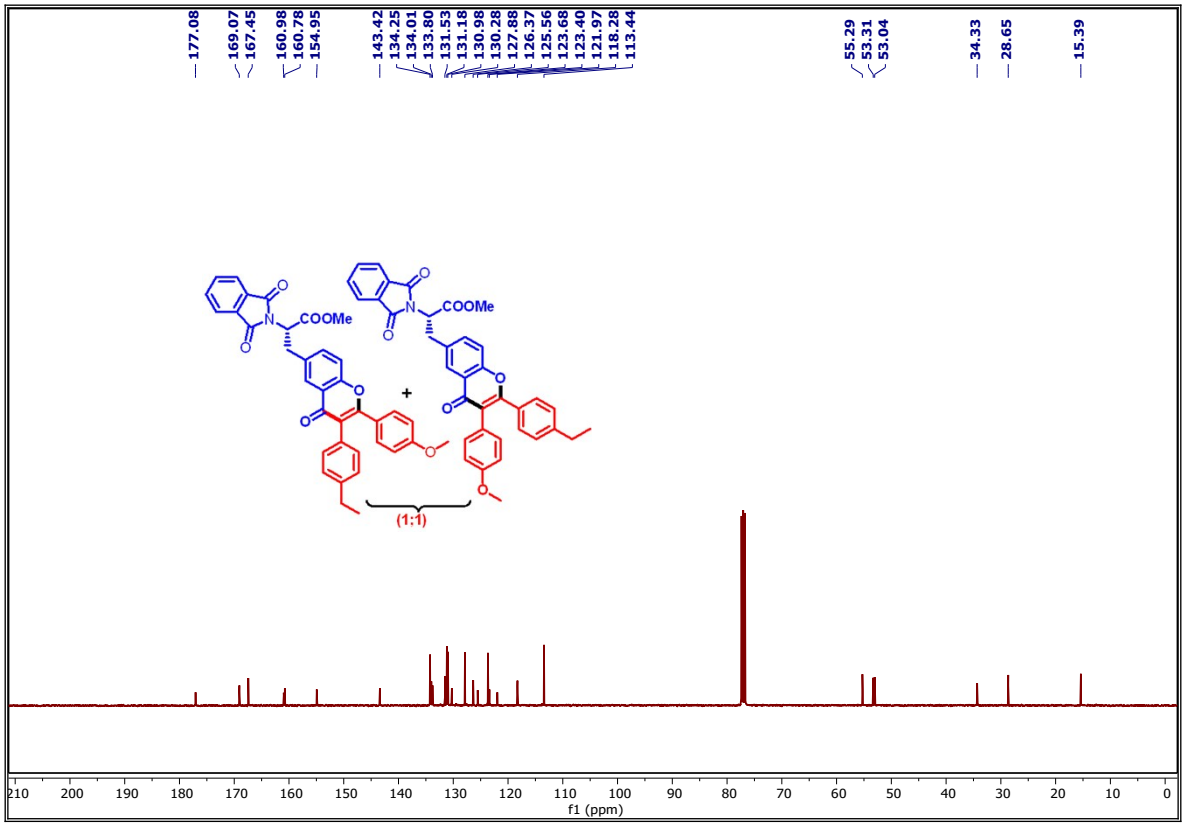
^{13}C NMR of 3aj (100 MHz, $CDCl_3$)



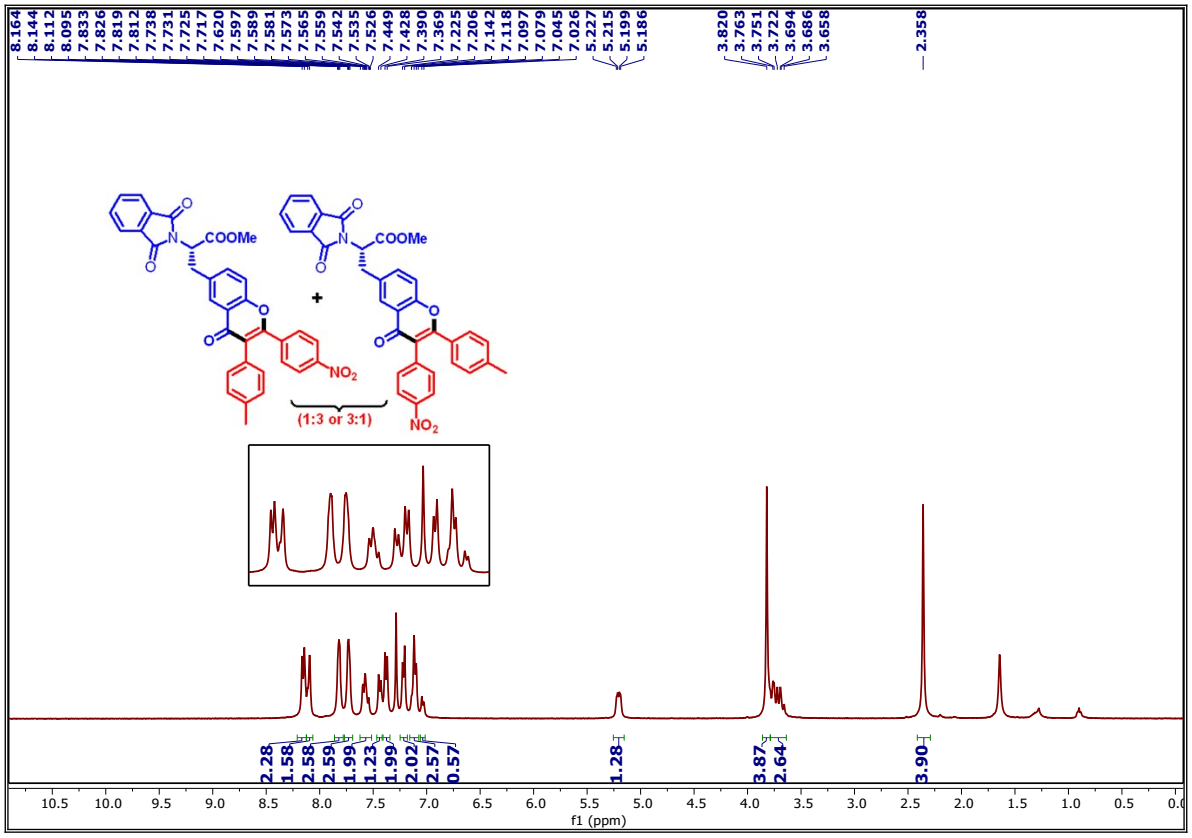
^{1}H NMR of 3ak (400 MHz, $CDCl_3$)



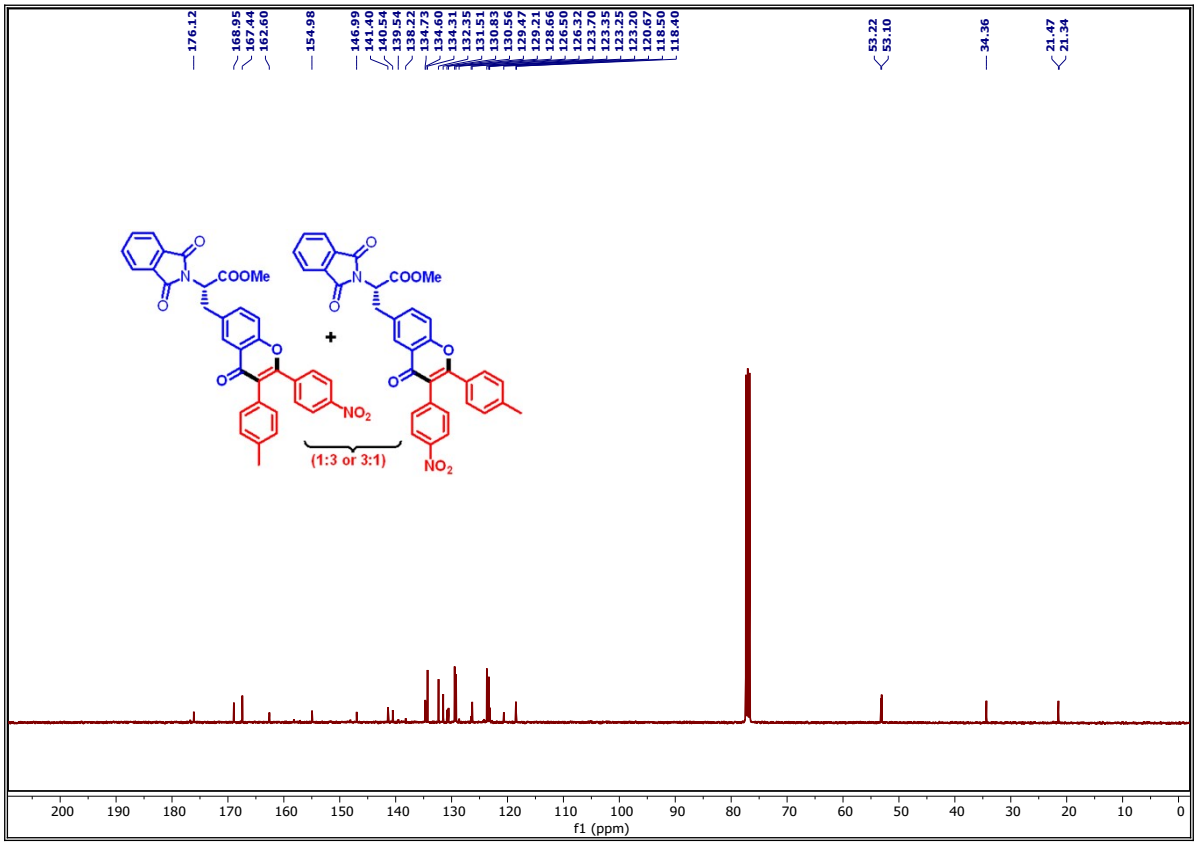
^{13}C NMR of 3ak (100 MHz, $CDCl_3$)



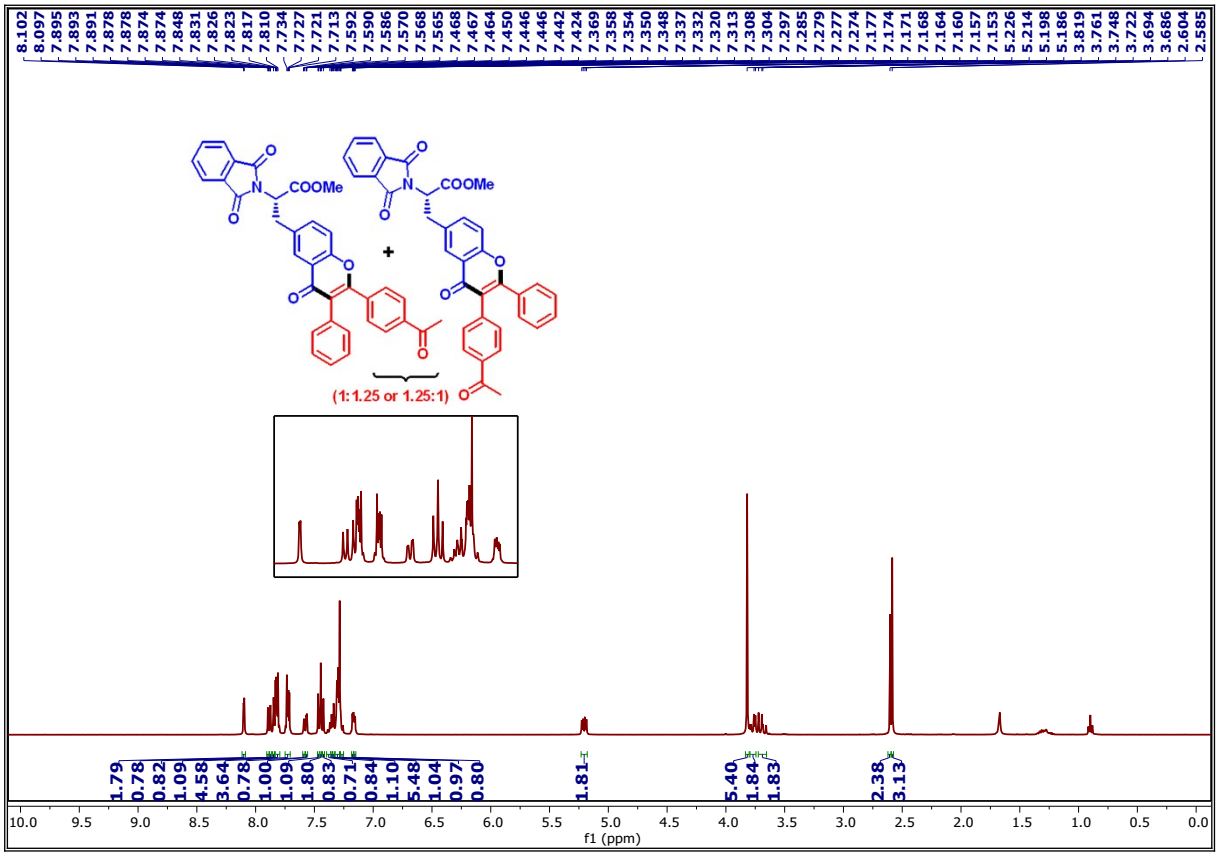
^{1}H NMR of 3al (400 MHz, $CDCl_3$)



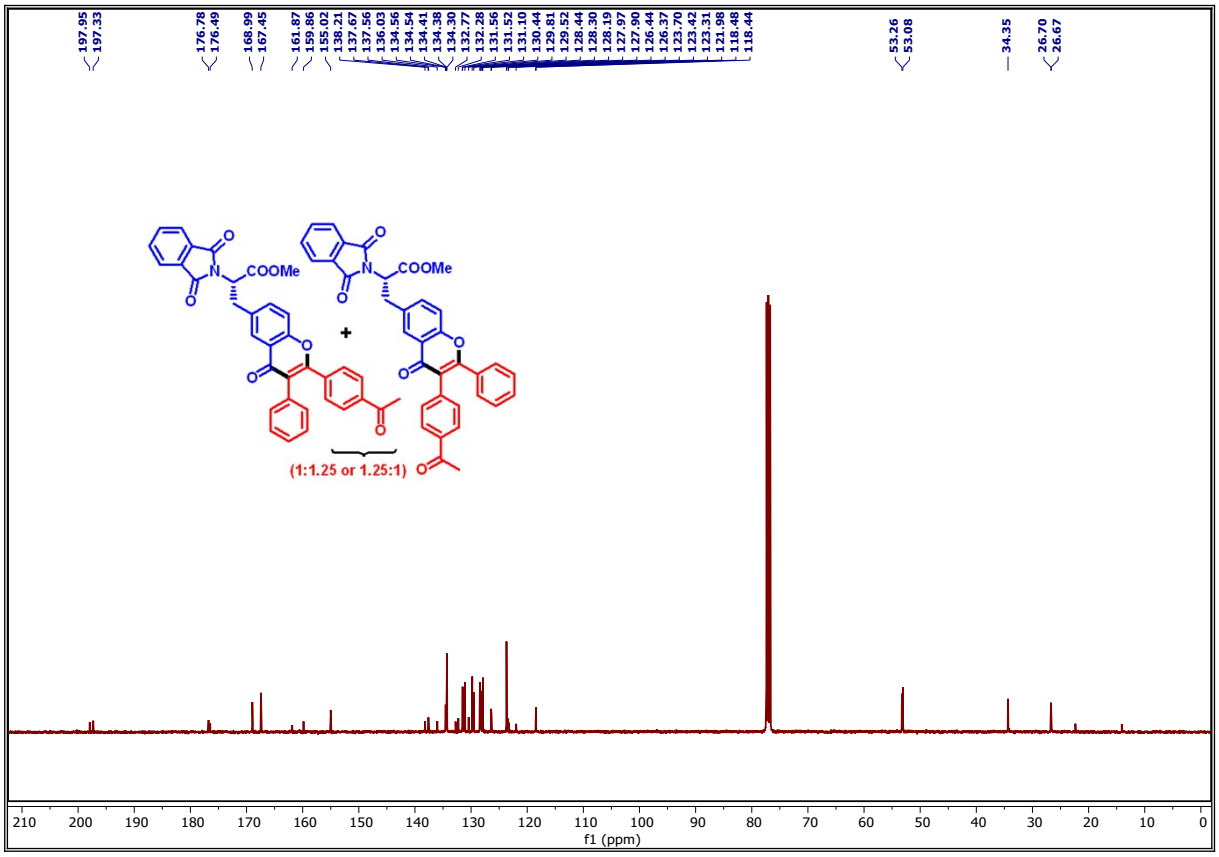
^{13}C NMR of 3al (100 MHz, $CDCl_3$)



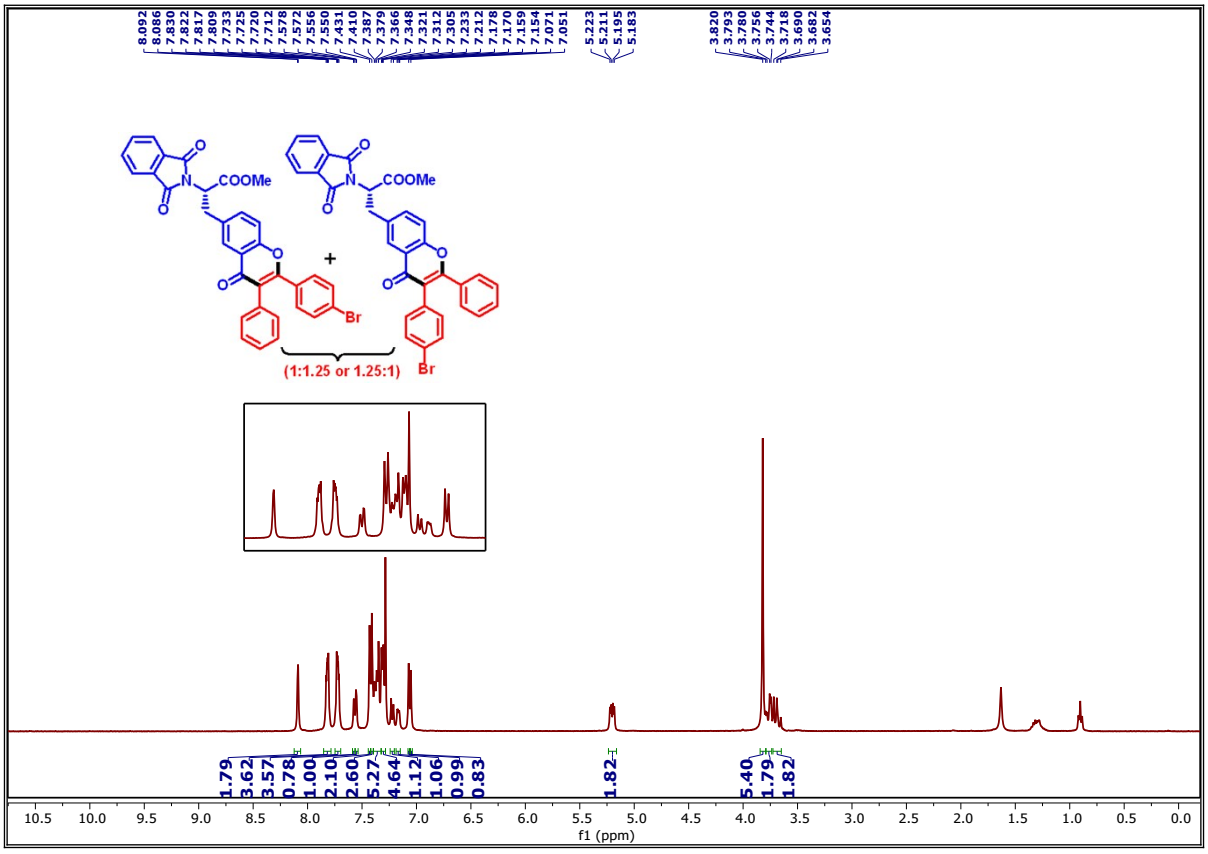
^{1}H NMR of 3am (400 MHz, $CDCl_3$)



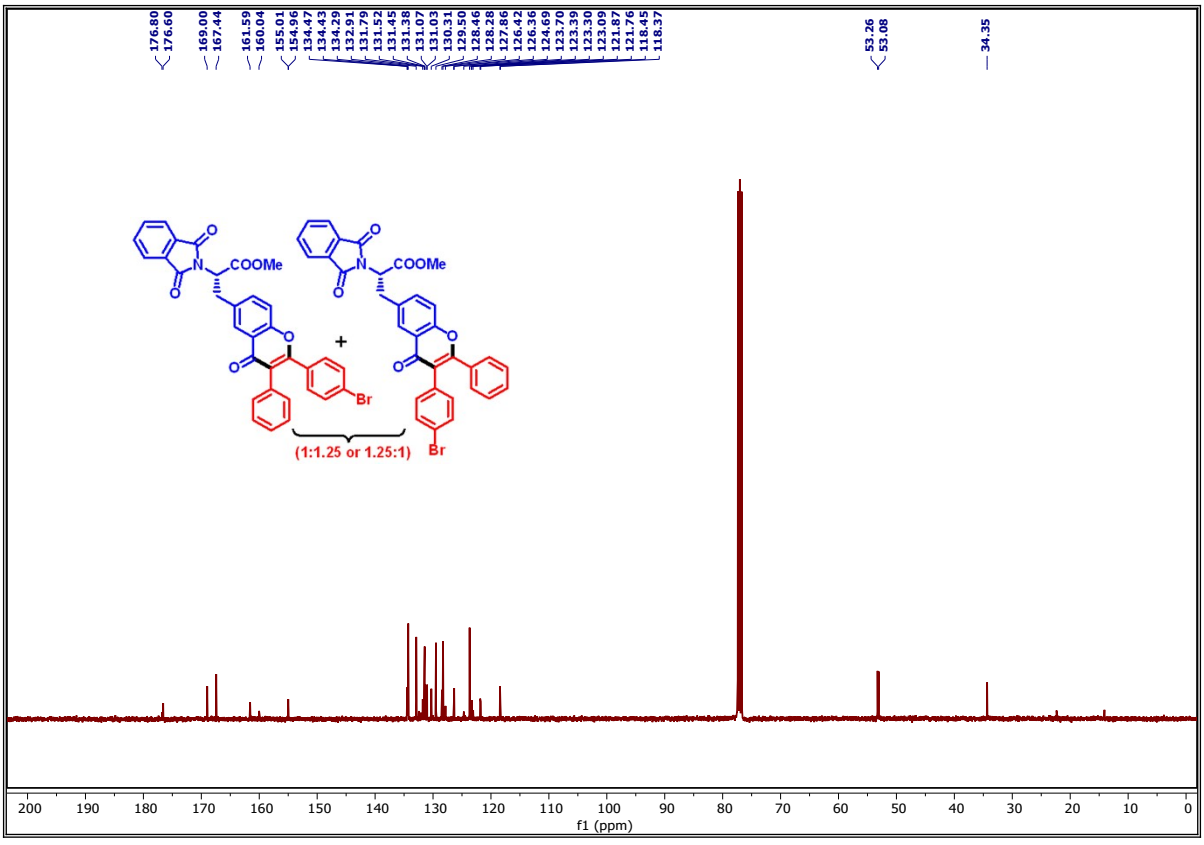
^{13}C NMR of 3am (100 MHz, $CDCl_3$)



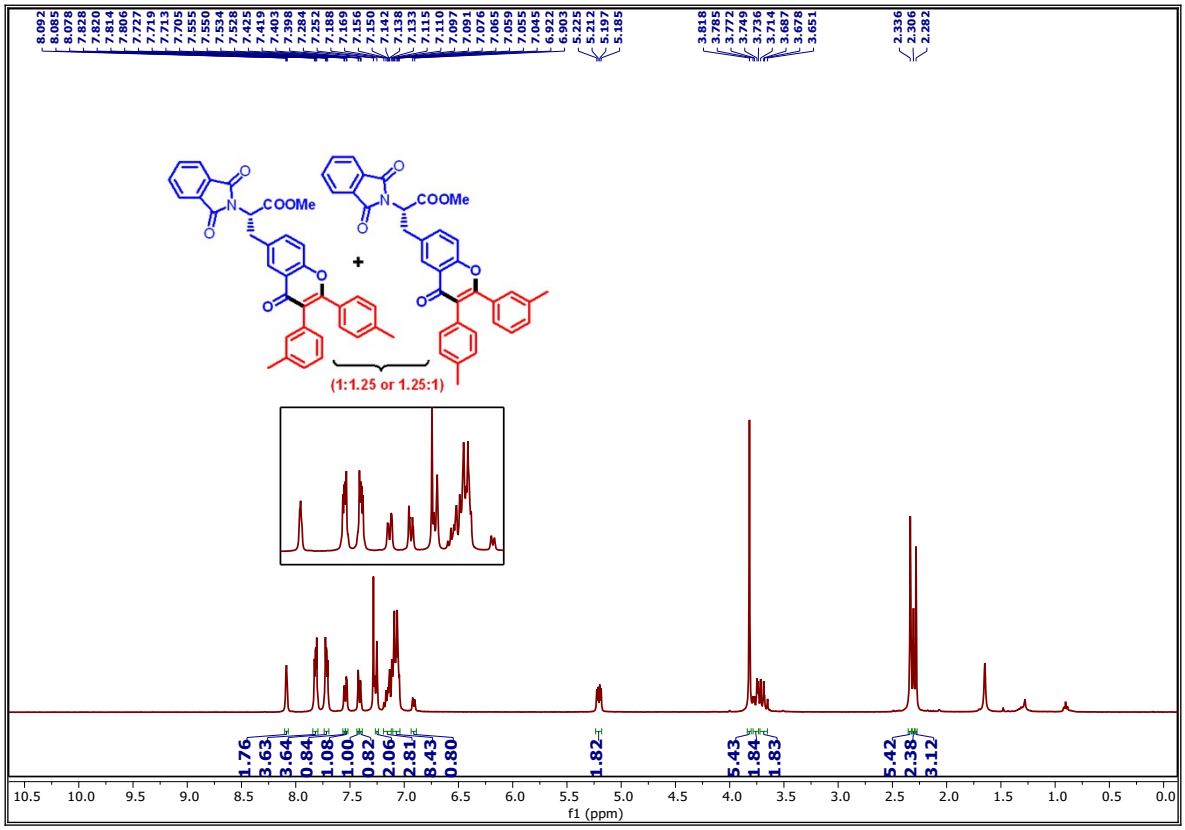
^{1}H NMR of 3an (400 MHz, $CDCl_3$)



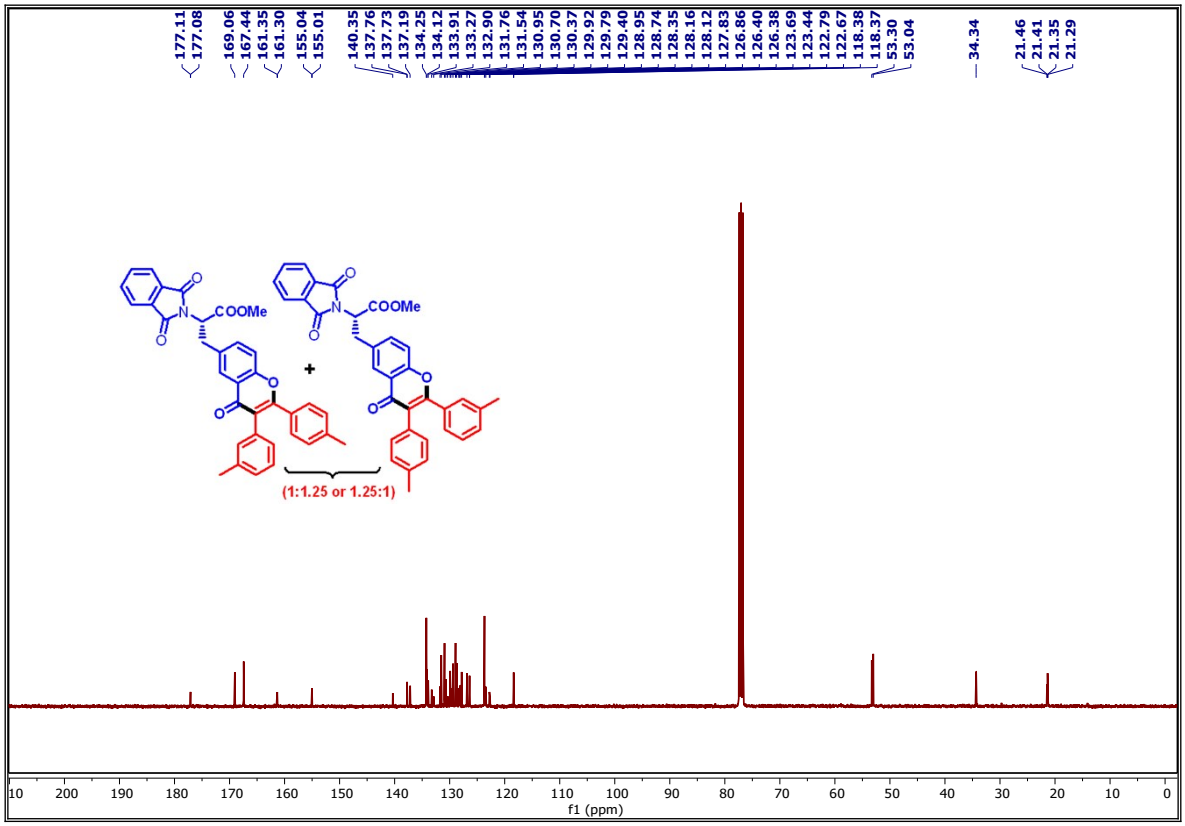
^{13}C NMR of 3an (100 MHz, $CDCl_3$)



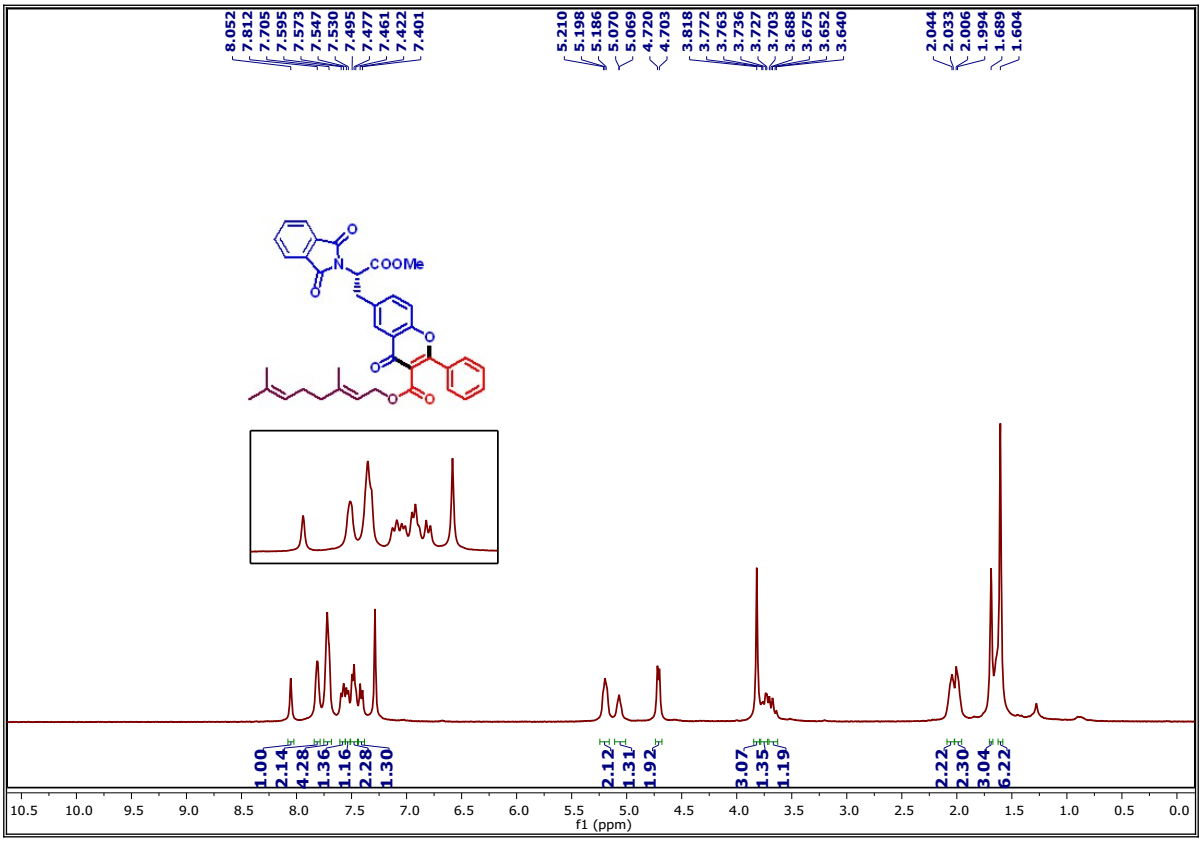
^{1}H NMR of 3ao (400 MHz, $CDCl_3$)



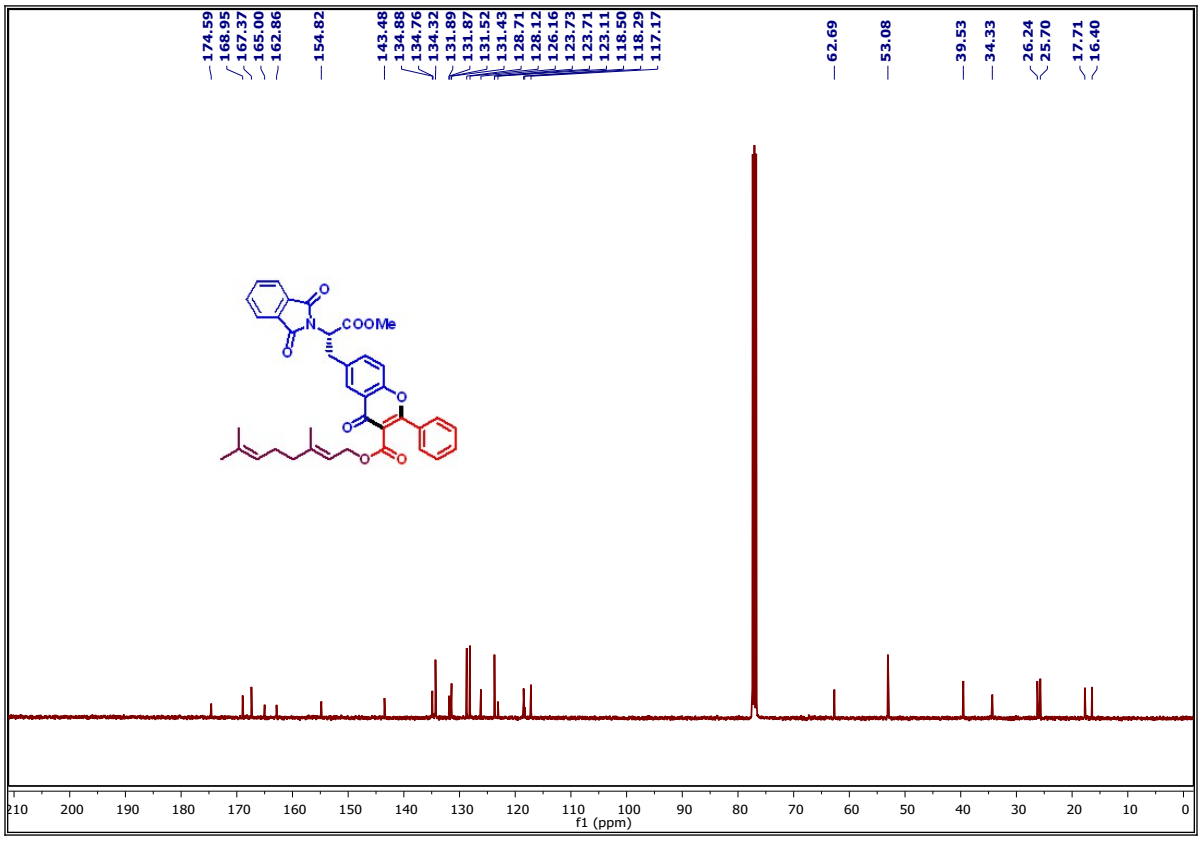
^{13}C NMR of 3ao (100 MHz, $CDCl_3$)



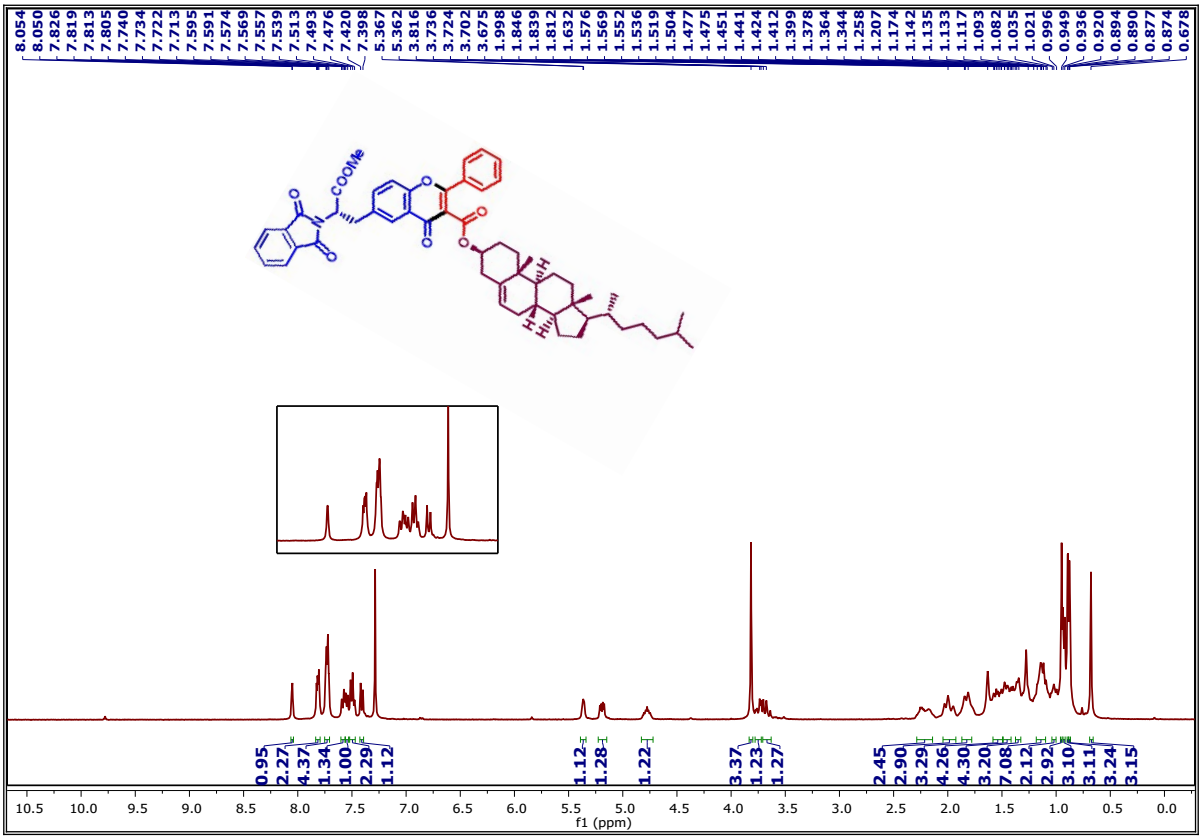
^{1}H NMR of 3ap (400 MHz, $CDCl_3$)



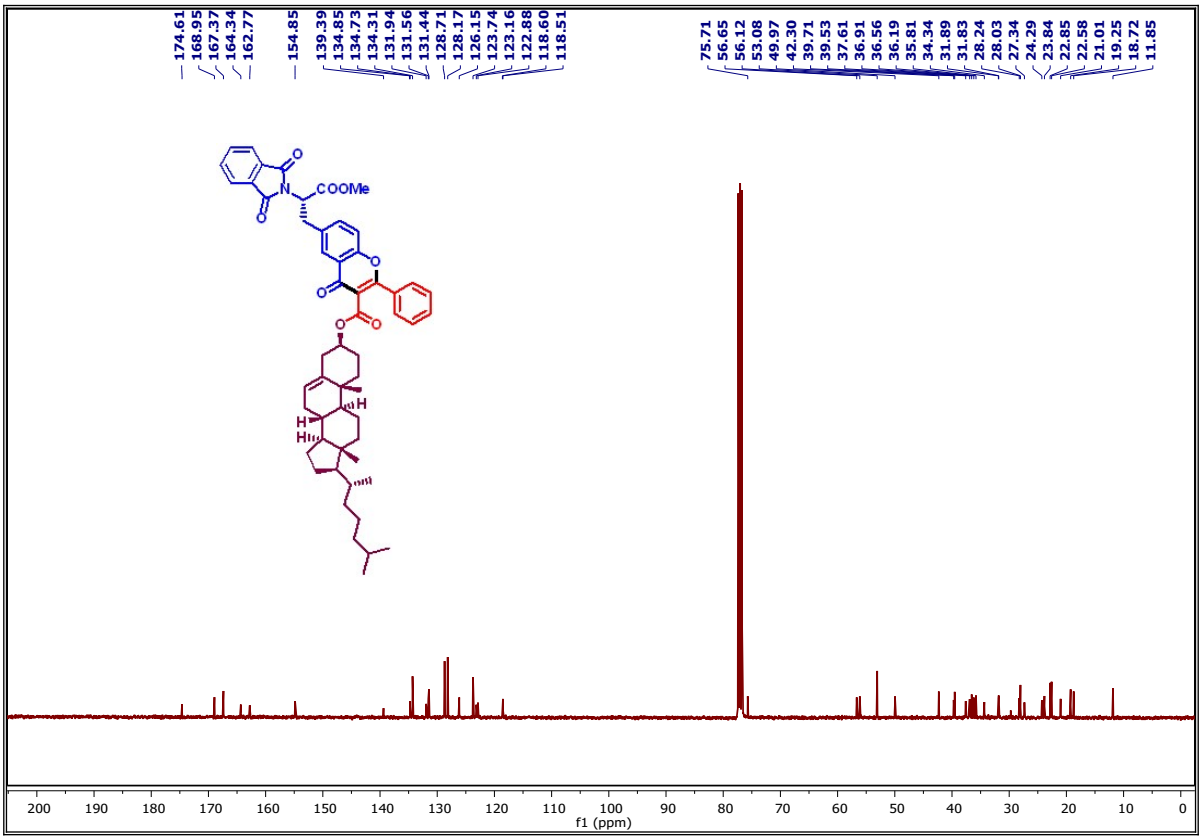
^{13}C NMR of 3ap (100 MHz, $CDCl_3$)



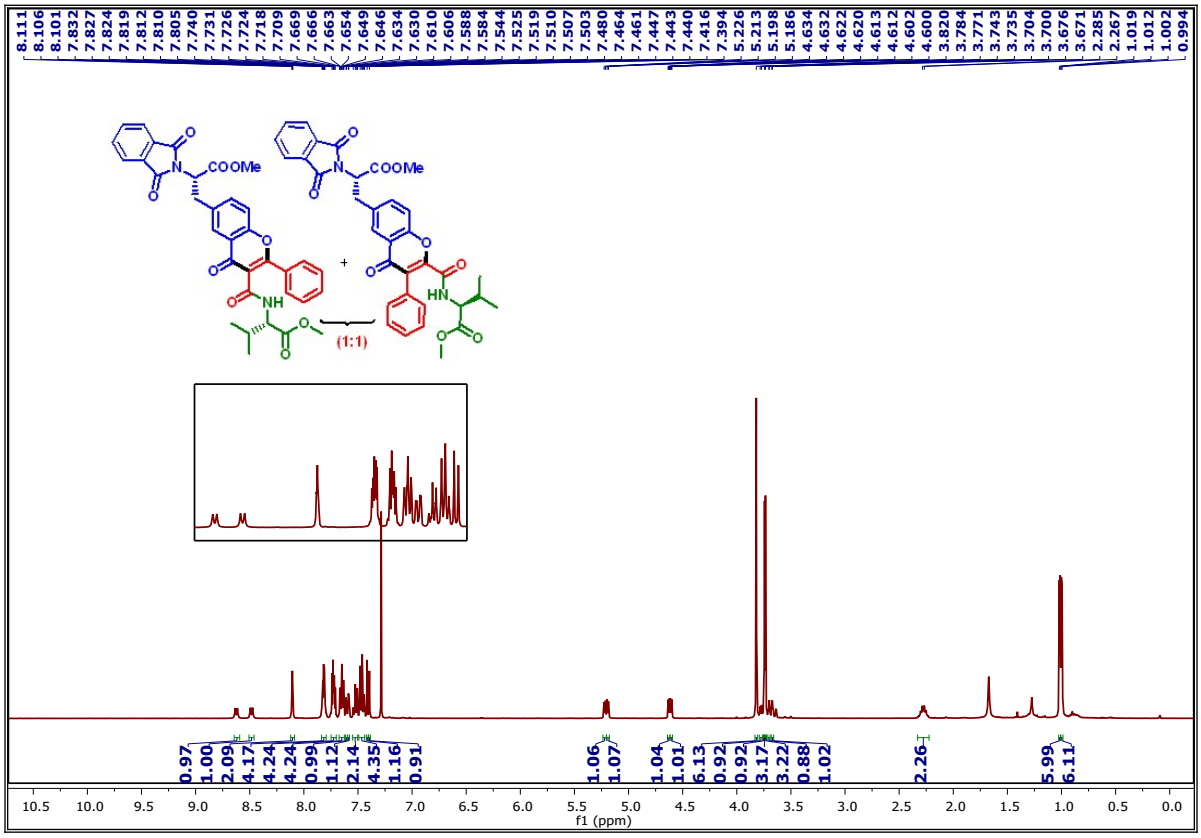
^{1}H NMR of 3aq (400 MHz, $CDCl_3$)



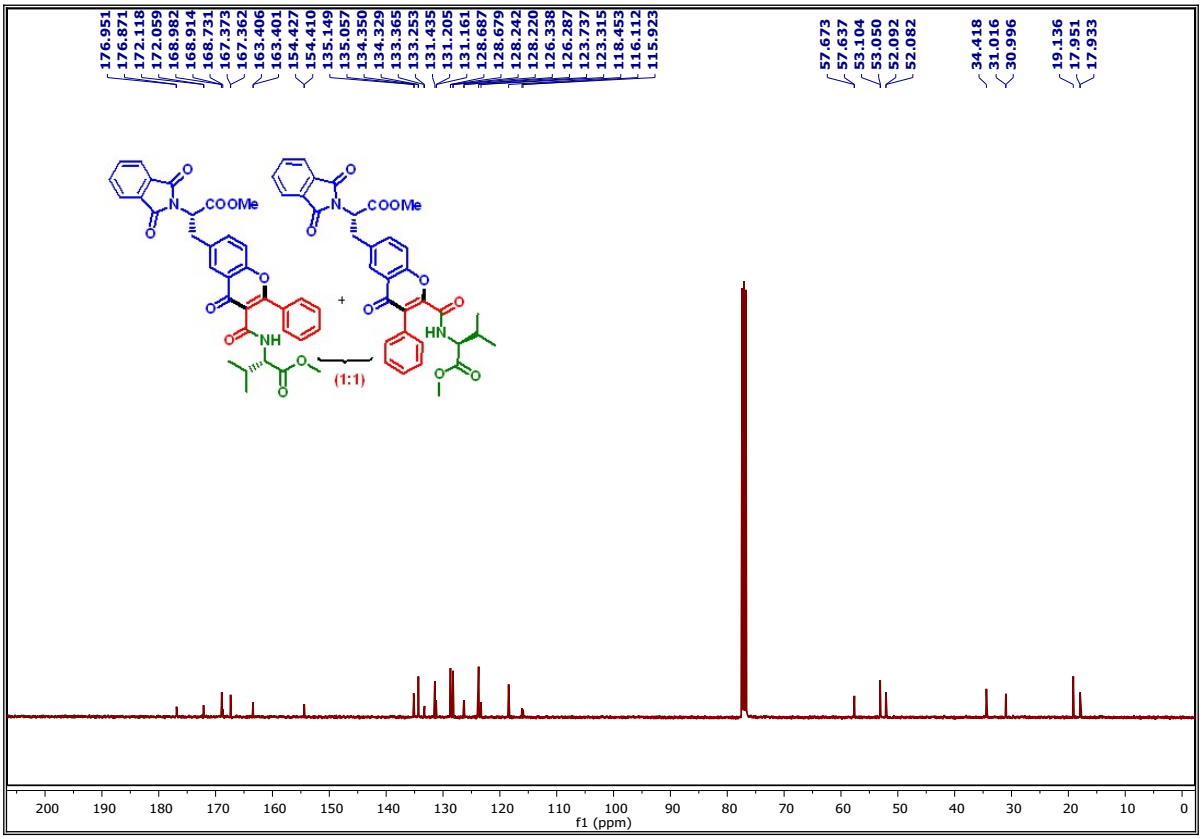
^{13}C NMR of 3aq (100 MHz, $CDCl_3$)



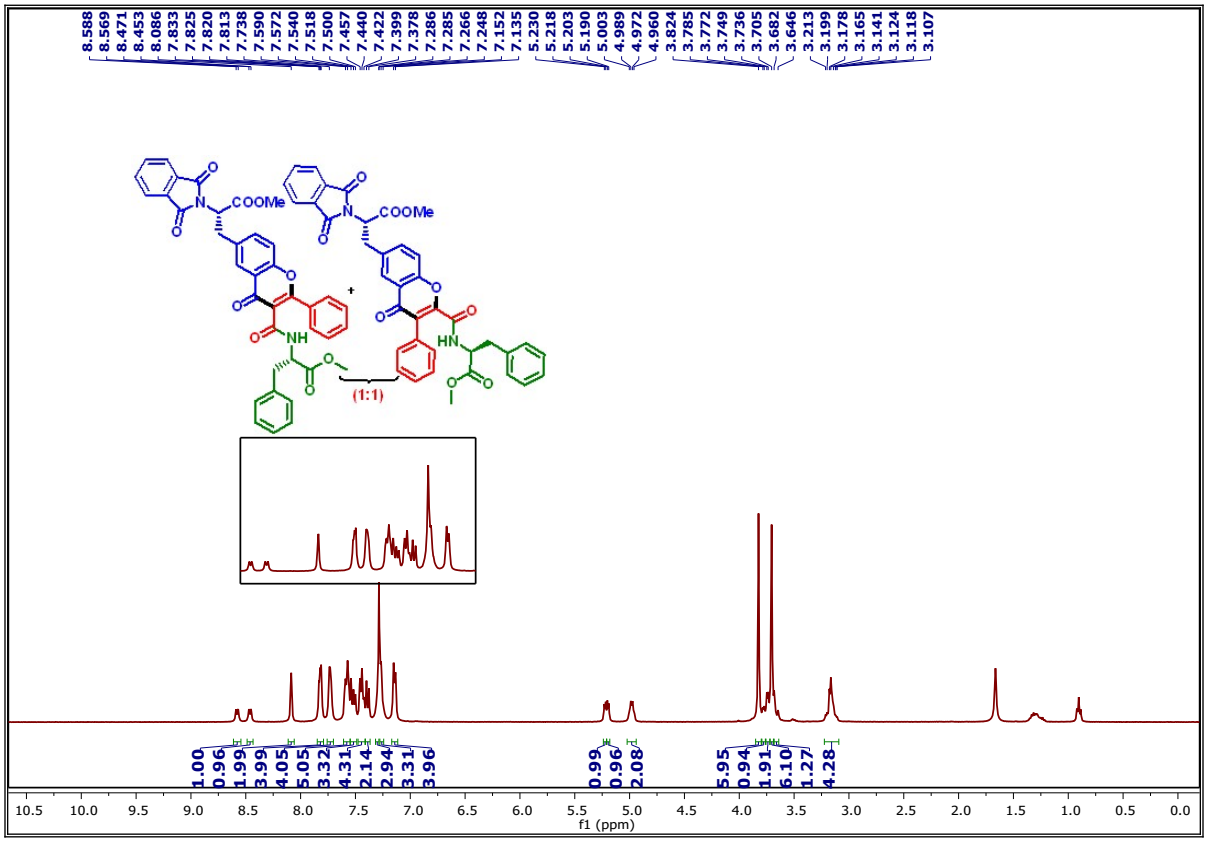
^{1}H NMR of 3ar (400 MHz, $CDCl_3$)



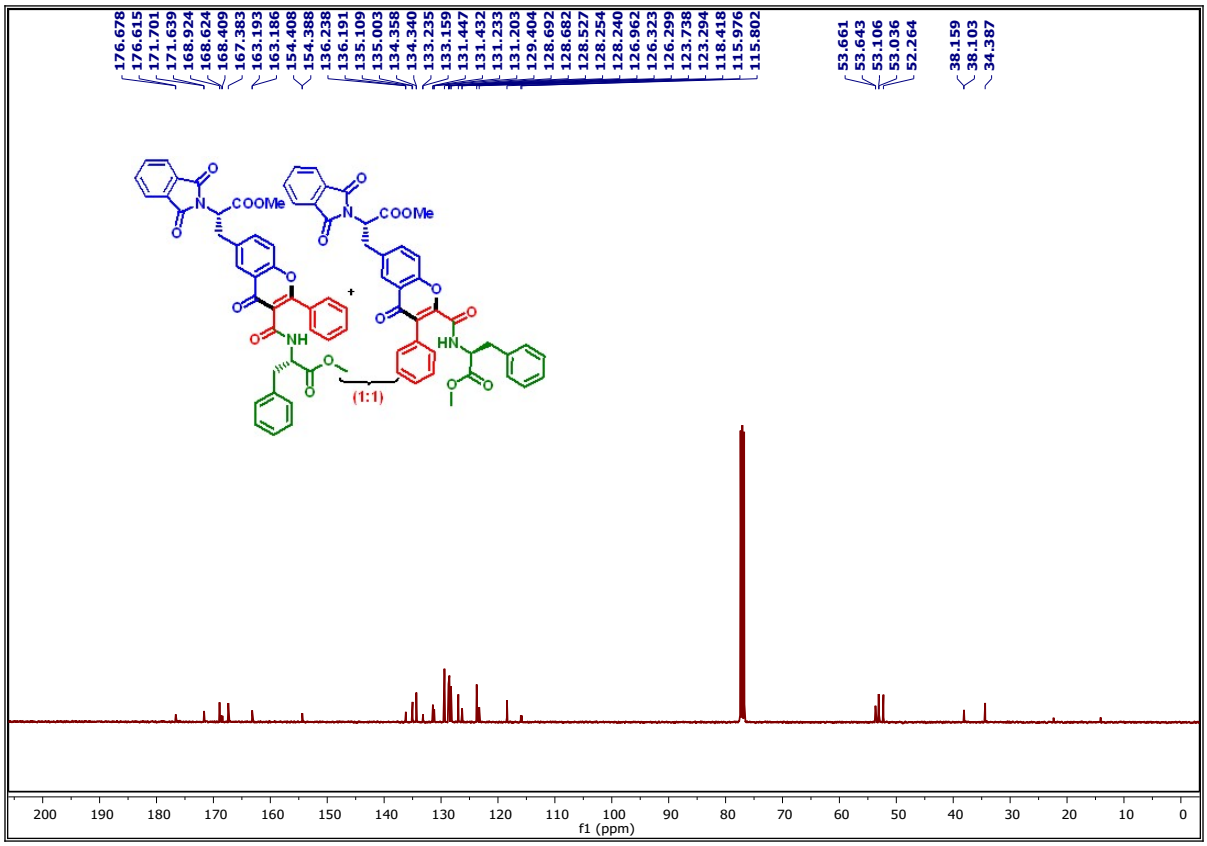
^{13}C NMR of 3ar (100 MHz, $CDCl_3$)



^{1}H NMR of 3as (400 MHz, $CDCl_3$)

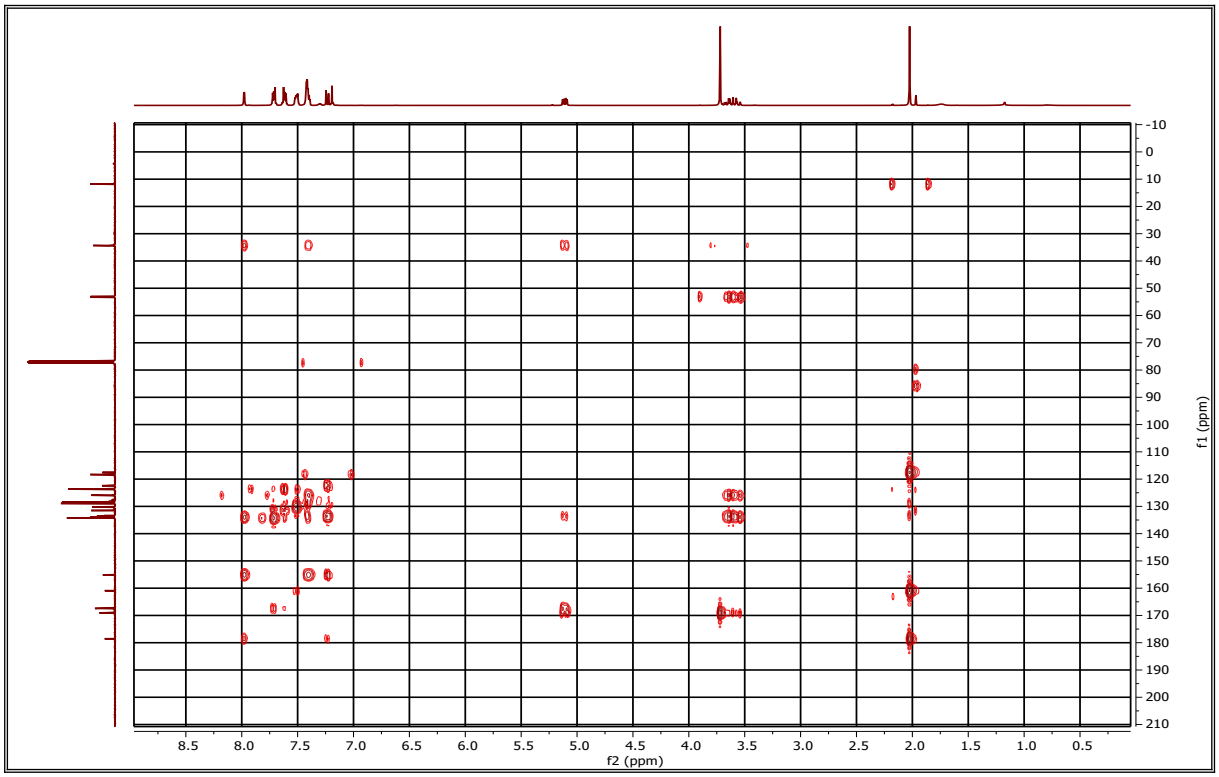


^{13}C NMR of 3as (100 MHz, $CDCl_3$)

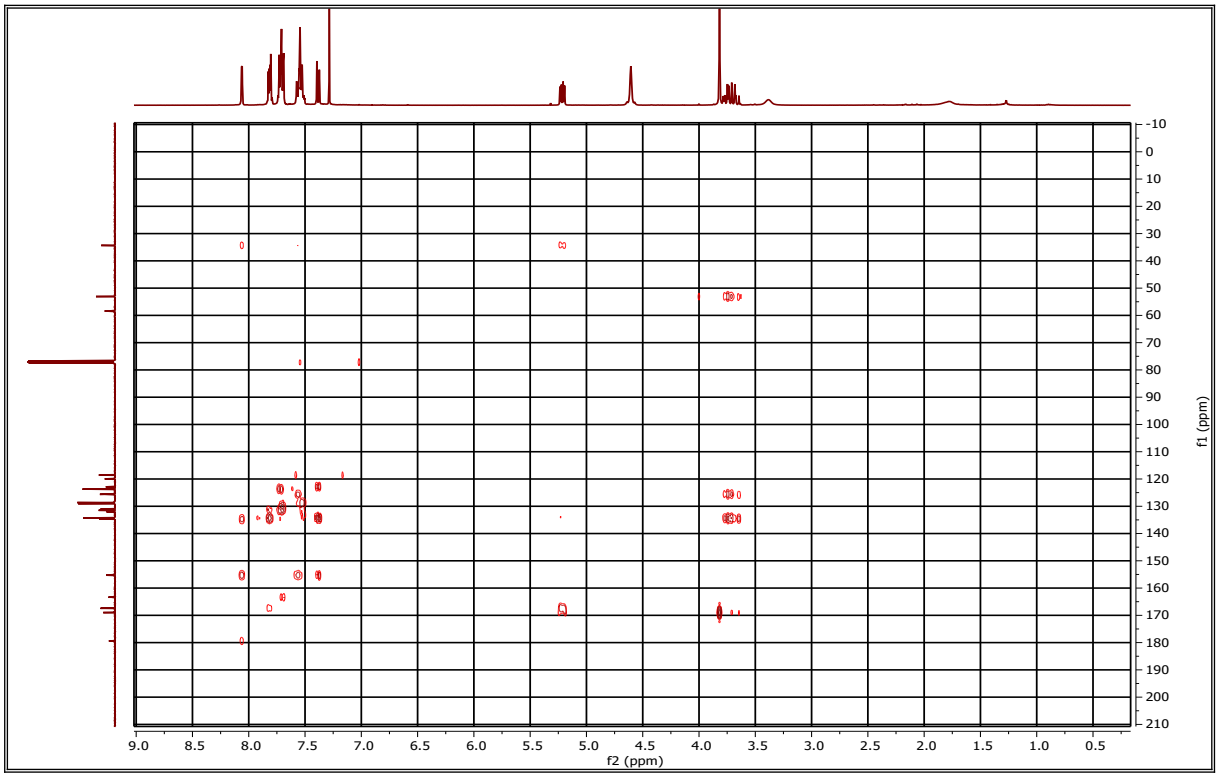


5. HMBC of 3ag, 3ai,3aj

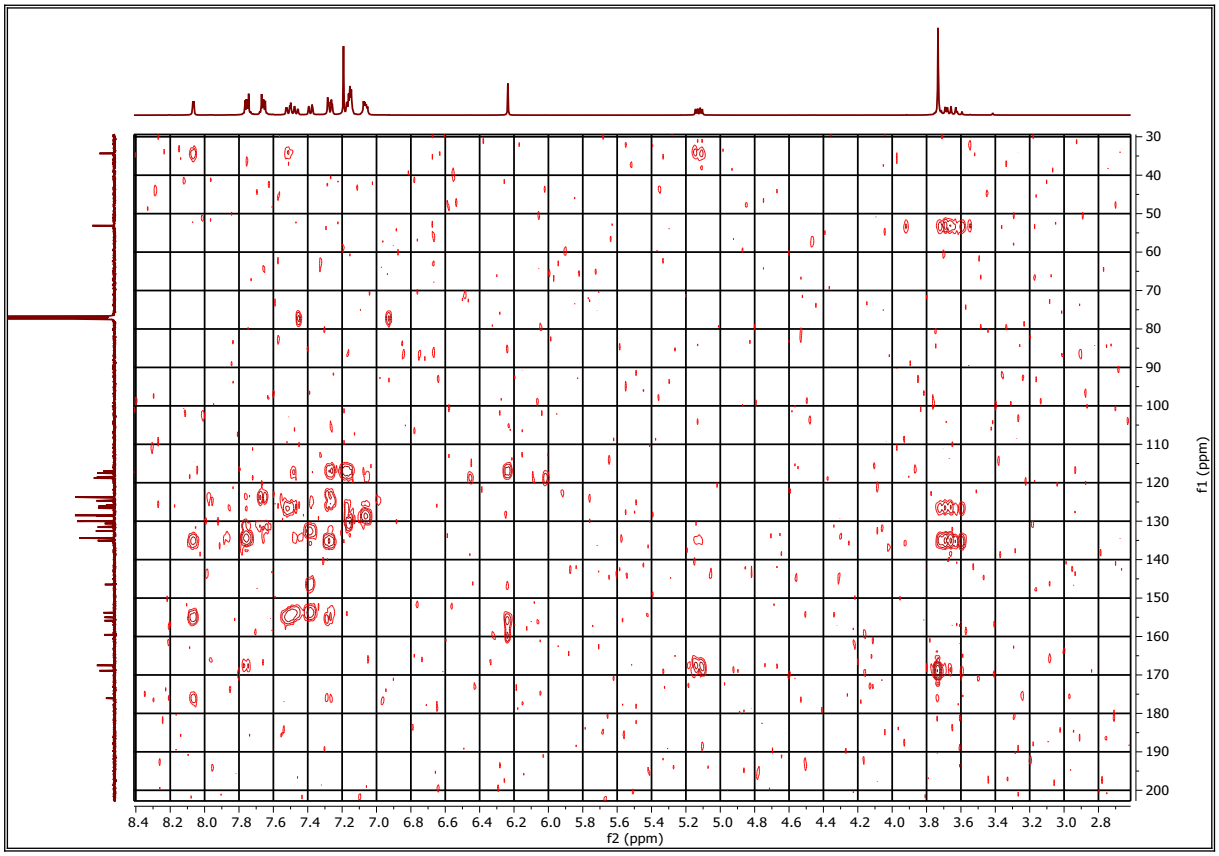
^{1}H-^{13}C correlation NMR of 3ag (HMBC)



^{1}H-^{13}C correlation NMR of 3ai (HMBC)



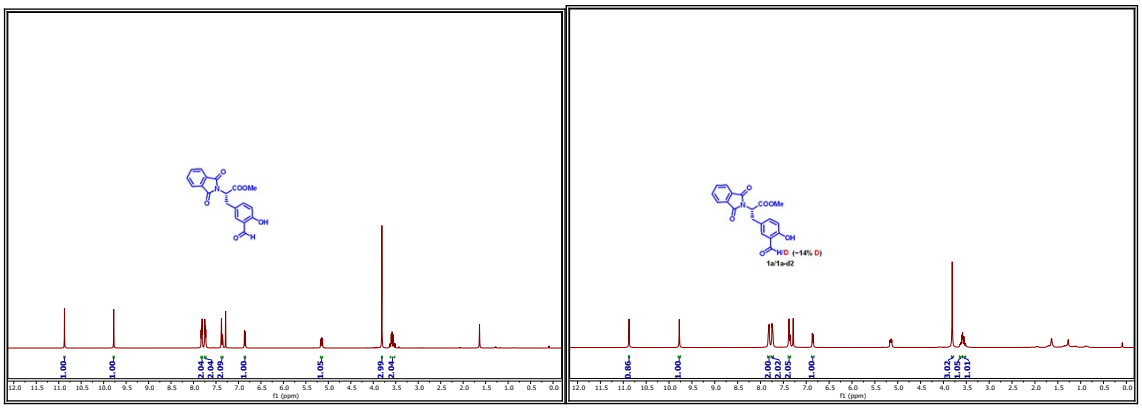
^{1}H-^{13}C correlation NMR of 3aj (HMBC)



6. Deuterium labelling study [Refer Scheme 4i]

To an oven-dried 10 mL round bottom flask charged with 3 mL of ACN : D_2O (2:1), **1a** (1 equiv), $[Cp^*RhCl_2]_2$ (2.5 mol %), $Cu(OAc)_2{\cdot}H_2O$ (0.5 equiv) were added. The reaction was allowed to stir at 80 ºC for 4 h. The reaction was cooled to room temperature, quenched with water and extracted with DCM (2 × 15 mL). The organic layers were combined, dried over anhydrous sodium sulphate and concentrated under reduced pressure. Purification by column chromatography using ethyl acetate/hexanes (2:8) as eluent afforded the desired product (**1a/1a-*d*$_2$**); ^{1}H NMR of this product indicated approximately 14% deuteration scrambling on $C3_{Ar}$-formly tyrosine proton when compared with the ^{1}H NMR of **1a**.

^{1}H NMR of 1a and 1a/1a-*d*$_2$(400 MHz, $CDCl_3$)



7. HRMS Analysis of crude reaction mixture[Refer Scheme 4ii]

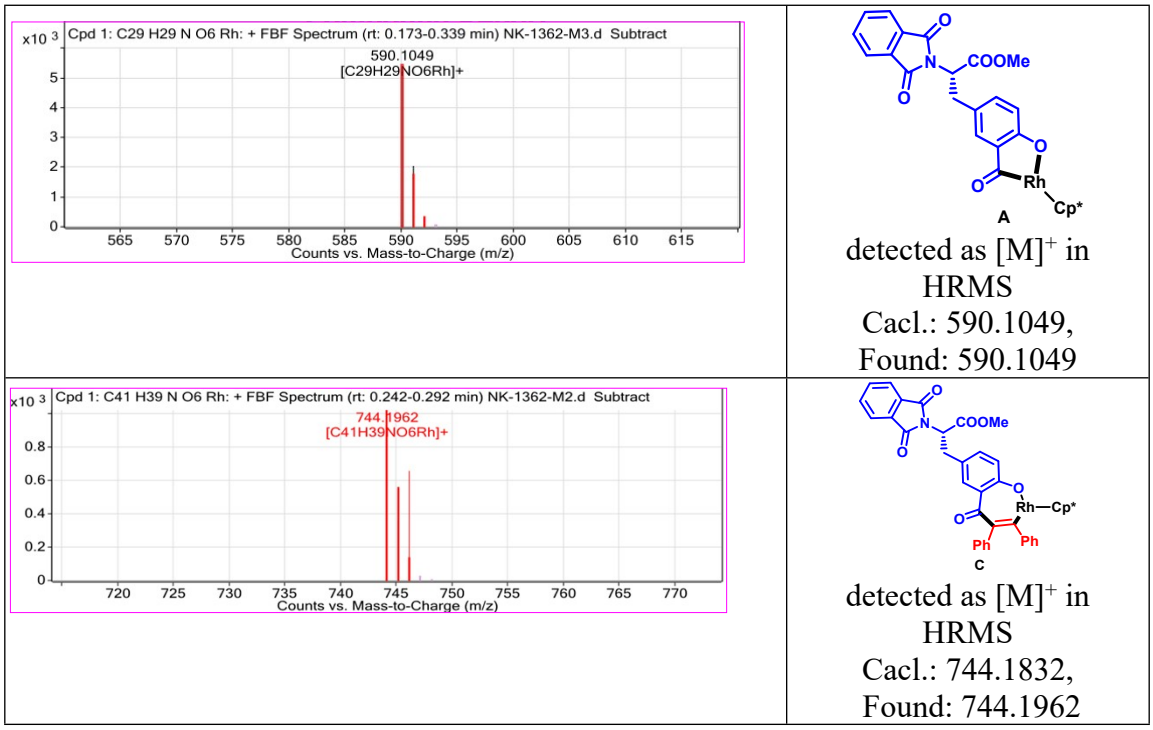
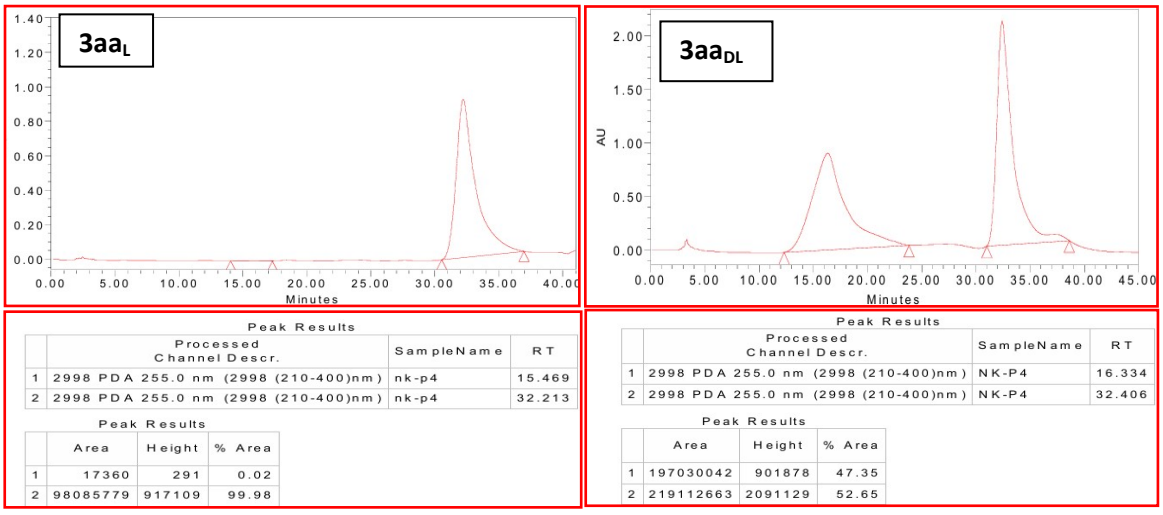


Figure 1. HRMS Data of crude reaction mixture between 1a and 2a

8. HPLC chromatograms of $3aa_L$ and $3aac_{DL}$

HPLC separation of **$3aa_L$** (Chiralpak® IA-3, ^{n}hexane/^{i}PrOH: 85:15, v/v, Detection wavelength: -255 nm), 1.0 mL/min.): t_r (major) = 15.5 min, t_r (minor) = 32.2 min, >99% ee. HPLC separation of **$3aa_{DL}$** (Chiralpak® IA-3, ^{n}hexane/^{i}PrOH: 85:15, v/v), 1.0 mL/min., Detection wavelength: 255 nm): t_r = 16.3 min, t_r = 32.4 min.



9. In cellulo studies of the selected compounds.

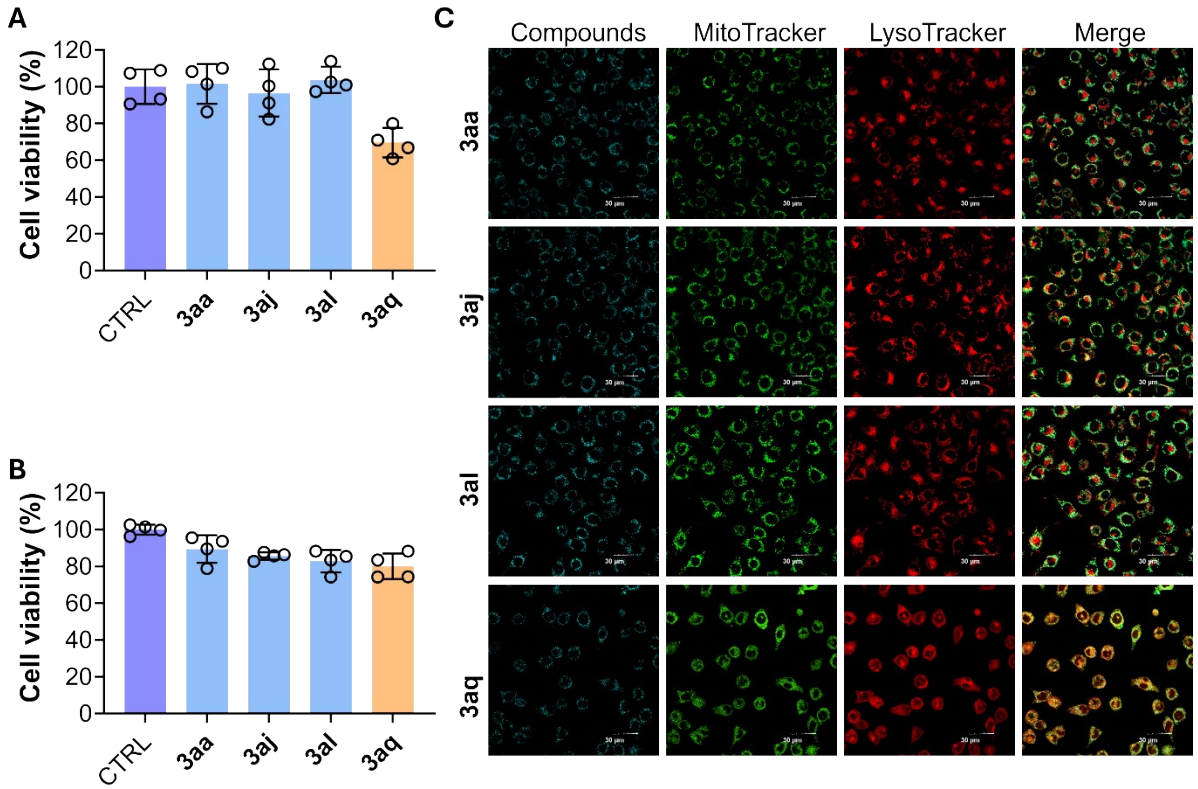


Figure S1. *In cellulo* studies of the tested compounds. The cell viability assay of the molecules in (A) SH-SY5Y human neuroblastoma cells and (B) N9 murine microglial cells. (C) The cellular uptake study of the molecules (Cyan= molecules, Green= MitoTracker green and Red= LysoTracker red). (The experiment was repeated with n = 3).

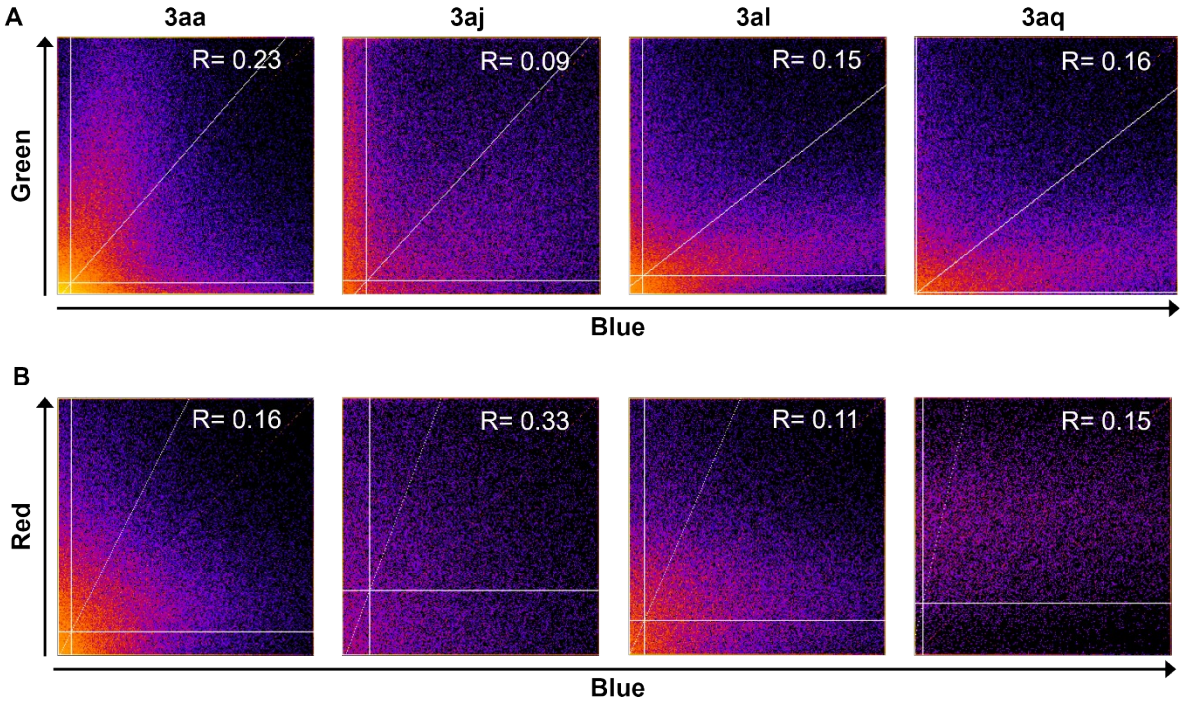


Figure S2. Colocalization of the molecules with mitochondria (A) and lysosome (B). Pearson coefficient is given in each image.

10. References

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