Synthesis and characterisation of α-carboxynitrobenzyl photocaged L-aspartates for applications in time-resolved structural biology

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Contents

Dark (aqueous) Stability Studies – NMR

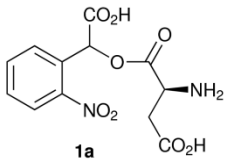
In order to assess the aqueous stability of the αCNB caged aspartates (**1**), samples were dissolved in aqueous buffer with 12.5% DMSO-d_6, and monitored by ^{1}H NMR with a water suppression pulse sequence. The residual DMSO signal was used as a reference for chemical shift (2.50 ppm) and integration (set arbitrarily to 10 for the range 2.53 – 2.47 ppm). The concentrations reported here are those of the aqueous buffer before addition of DMSO-d_6. The buffers were either monosodium phosphate (50 mM) or ammonium sulfate (400 mM) with monosodium citrate (50 mM), and were adjusted to the required pH with conc. HCl or NaOH (12 M) and tested with a calibrated pH meter. Each sample (ca. 20 mg) was dissolved in a buffer/DMSO-d_6 mixture (0.7 mL) and transferred to an amber NMR tube. Spectra were recorded once per hour for 24 h at ambient temperature using an Avance III 400 NMR spectrometer. Afterwards, the samples were diluted with 1 M HCl and extracted to afford the hydroxyacids (**4**), identical to those obtained previously.

The benzylic proton signal in each spectrum was integrated for each diastereomer (denoted the A and B isomer respectively for the downfield and upfield shift) separately. This allowed the kinetics of the diastereoisomers to be monitored independently, albeit without any assignment of the stereochemistry possible at this time, however in every case the A isomer hydrolysed more rapidly (Table S1). For a given time series, the same chemical shift regions were integrated across all spectra.

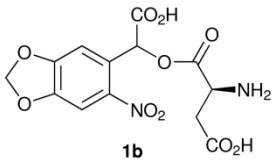
Compound **1a** exhibits a sharp change in hydrolysis rate between pH 4.2 and pH 5.8, whereas the other pH conditions give kinetics indistinguishable within the error bounds. This is consistent with the neighbouring group participation mechanism discussed in the main text. Compound **1b** also exhibits this behaviour, but the transition is between pH 5.8 and pH 7.0 – indicating the pK_a (benzylic carboxylic acid) of **1b** lies between 5.8 and 7.0, whereas for compound **1b** the pK_a is lower, between 4.2 and 5.8. Compound **1c**, interestingly, shows only a slight rate difference between pH 4.2 and 5.8, very close to the error bounds, but the rate is also substantially slower at pH 9 than all other pH conditions tested. This may suggest that the pK_a of the benzylic carboxylic acid is lower than 4.2, while at pH 9 the amine is no longer protonated, reducing the electrophilicity of the ester carbonyl.

Table S1: Hydrolysis half-lives of photocaged L-aspartates **1a – 1c**. ^{1}H NMR integrals of the benzylic CH signals of each diastereoisomer were measured once per hour for 24 h at r.t. in aqueous buffers with a water suppression pulse sequence. [a]50 mM sodium citrate, 400 mM ammonium sulfate, 12.5% DMSO-d_6. [b]50 mM sodium phosphate, 12.5% DMSO-d_6. The uncertainties quoted are derived from the 2σ confidence intervals of the fitted parameters (vide infra, Figures S13 – S15).

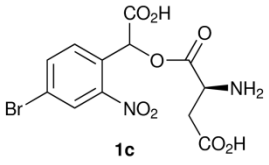
	half-life / h					
pH	**1a** (**A** / **B** isomer)		**1b** (**A** / **B** isomer)		**1c** (**A** / **B** isomer)	
4.2[a]	16.9 ± 0.4	29 ± 2	15 ± 2	24 ± 4	11.2 ± 0.2	19.3 ± 0.6
5.8[b]	13 ± 1	23 ± 2	16 ± 1	26 ± 2	10.3 ± 0.4	18 ± 1
7.0[b]	13.9 ± 0.2	25.7 ± 0.6	13 ± 1	22 ± 2	10.2 ± 0.1	18.5 ± 0.2
9.0[b]	14.6 ± 0.2	26.2 ± 0.4	13 ± 1	21 ± 4	17.2 ± 0.6	28 ± 2



1a



1b



1c

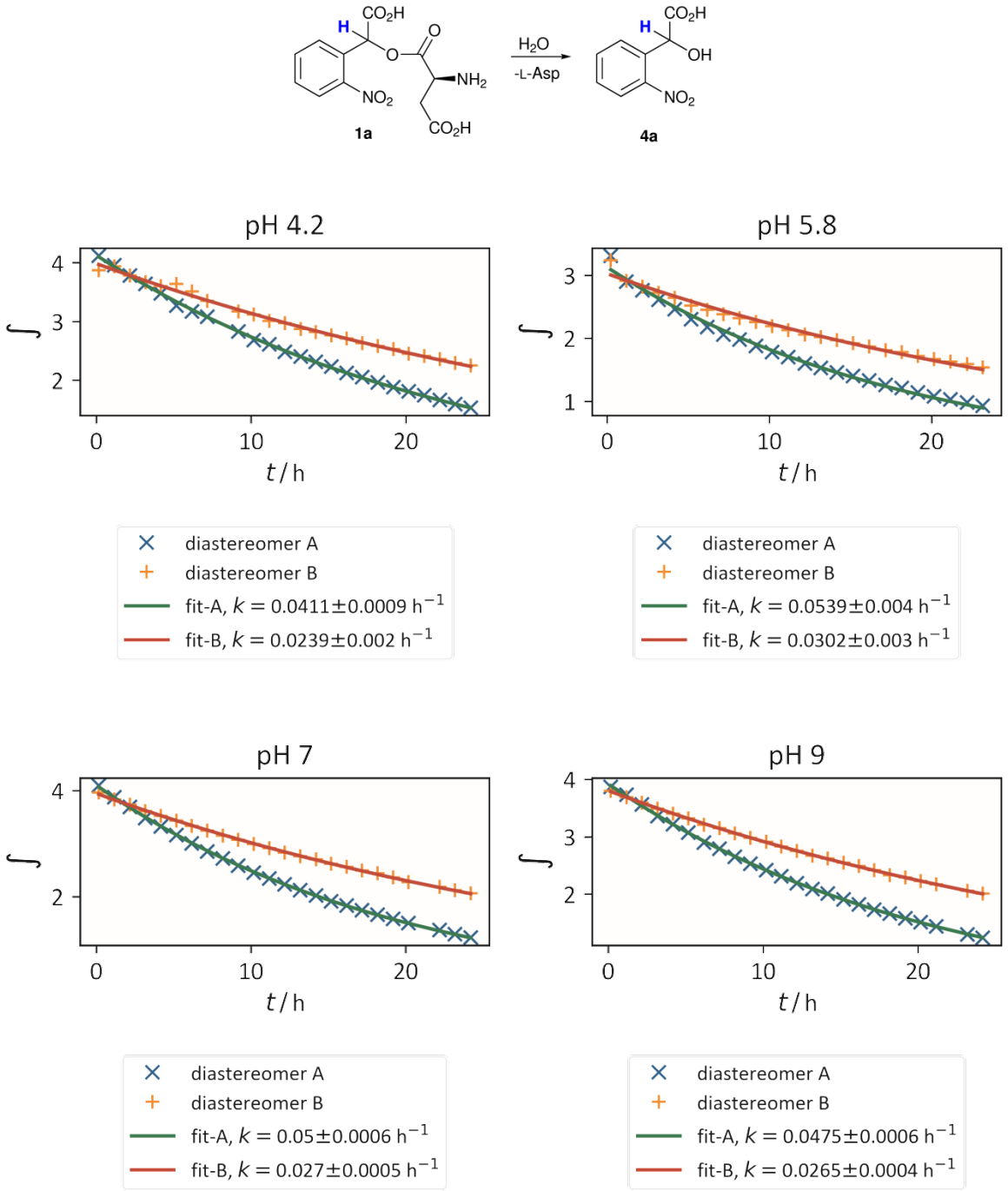


Figure S1: Plots of ^{1}H NMR integrals (benzylic CH signals, see Figures S1 – S4) over time at various pHs, showing the hydrolysis of **1a** under these conditions. x and + denote the experimental data and the exponential fits are plotted as lines. The decays obeyed the first-order exponential decay function $f(t) = Ae^{-kt}$. Least-squares curve fitting was carried out using the SciPy[1] python library, the data were plotted using Matplotlib[2] and the quoted errors are the 1σ confidence interval of *k* estimated by the fitting algorithm scipy.optimize.curve_fit(). Note that the pH 4.2 sample was dissolved in 50 mM citrate buffer with 400 mM ammonium sulfate, the other samples were dissolved in 50 mM phosphate buffer. Therefore, the pH 4.2 data are not directly comparable with the others. DMSO-d_6 was added to each sample (12.5% of total volume), and the integral of the residual DMSO signal was set to 10 in each spectrum. In this case the rate is fastest at pH 5.8, slowest at pH 4.2 and decreases with increasing pH in the range measured. We propose two effects to explain this – one, that at lower pH, the α-carboxyl group is protonated and cannot participate in a neighbouring-group mechanism,[3] whereas at higher pH the primary amine is not protonated and therefore the neighbouring carbonyl is less electrophilic.

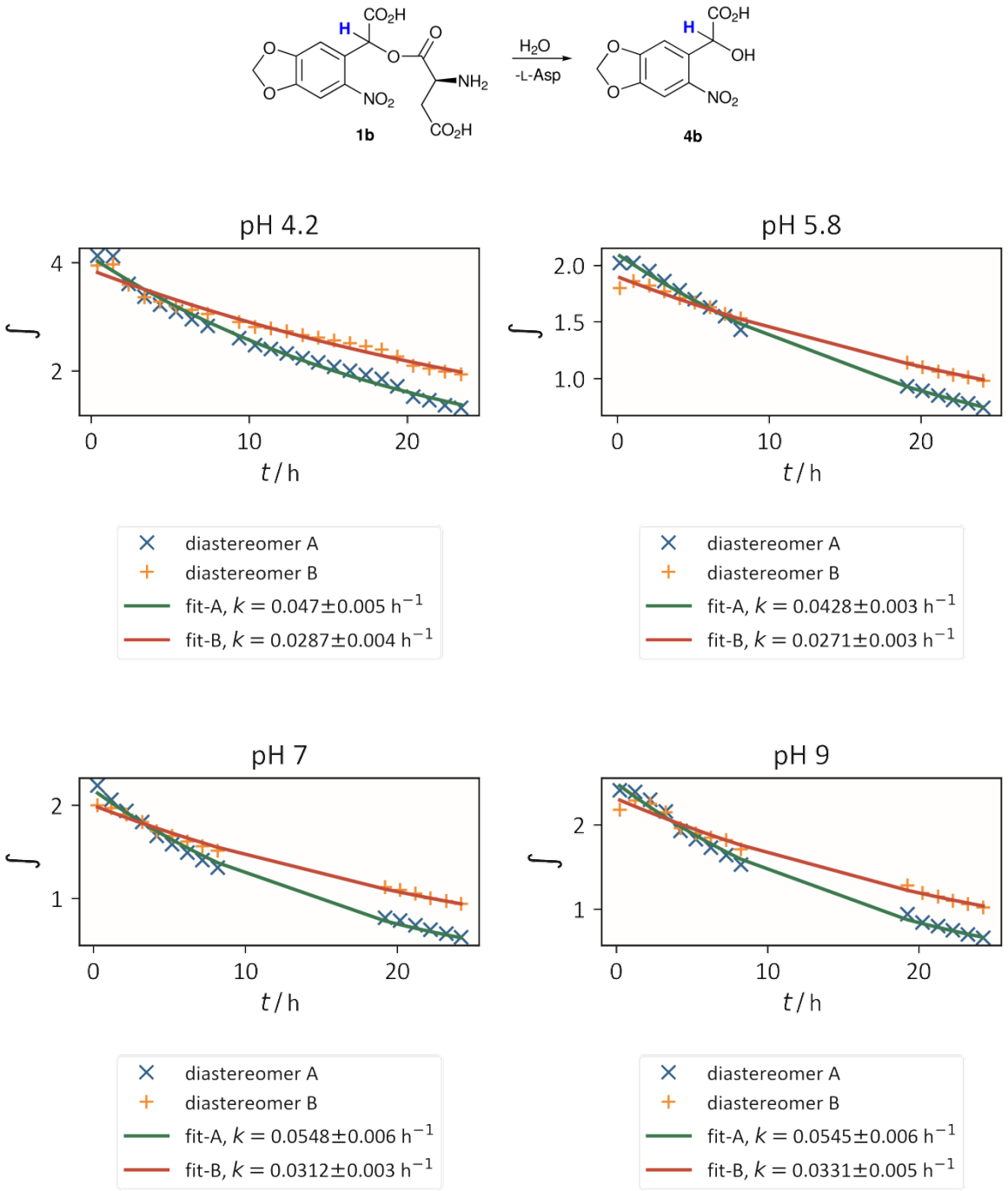


Figure S2: Plots of ^{1}H NMR integrals (benzylic CH signals, see Figures S5 – S8) over time at various pHs, showing the hydrolysis of **1b** under these conditions. x and + denote the experimental data and the exponential fits are plotted as lines. The decays obeyed the first-order exponential decay function $f(t) = Ae^{-kt}$. Least-squares curve fitting was carried out using the SciPy[1] python library, the data were ploted using Matplotlib[2] and the quoted errors are the 1σ confidence interval of k estimated by the fitting algorithm scipy.optimize.curve_fit(). Note that the pH 4.2 sample was dissolved in 50 mM citrate buffer with 400 mM ammonium sulfate, the other samples were dissolved in 50 mM phosphate buffer. Therefore, the pH 4.2 data are not directly comparable with the others. DMSO-d_6 was added to each sample (12.5% of total volume), and the integral of the residual DMSO signal was set to 10 in each spectrum. In some samples, several data points are missing due to an error that occurred with the automatic sample changer during the night, which was not remedied until morning. In the case of compound **1b**, the maximum rate is at pH 7 (isomer A) or pH 9 (isomer B), whereas the lowest rates are at pH 5.8.

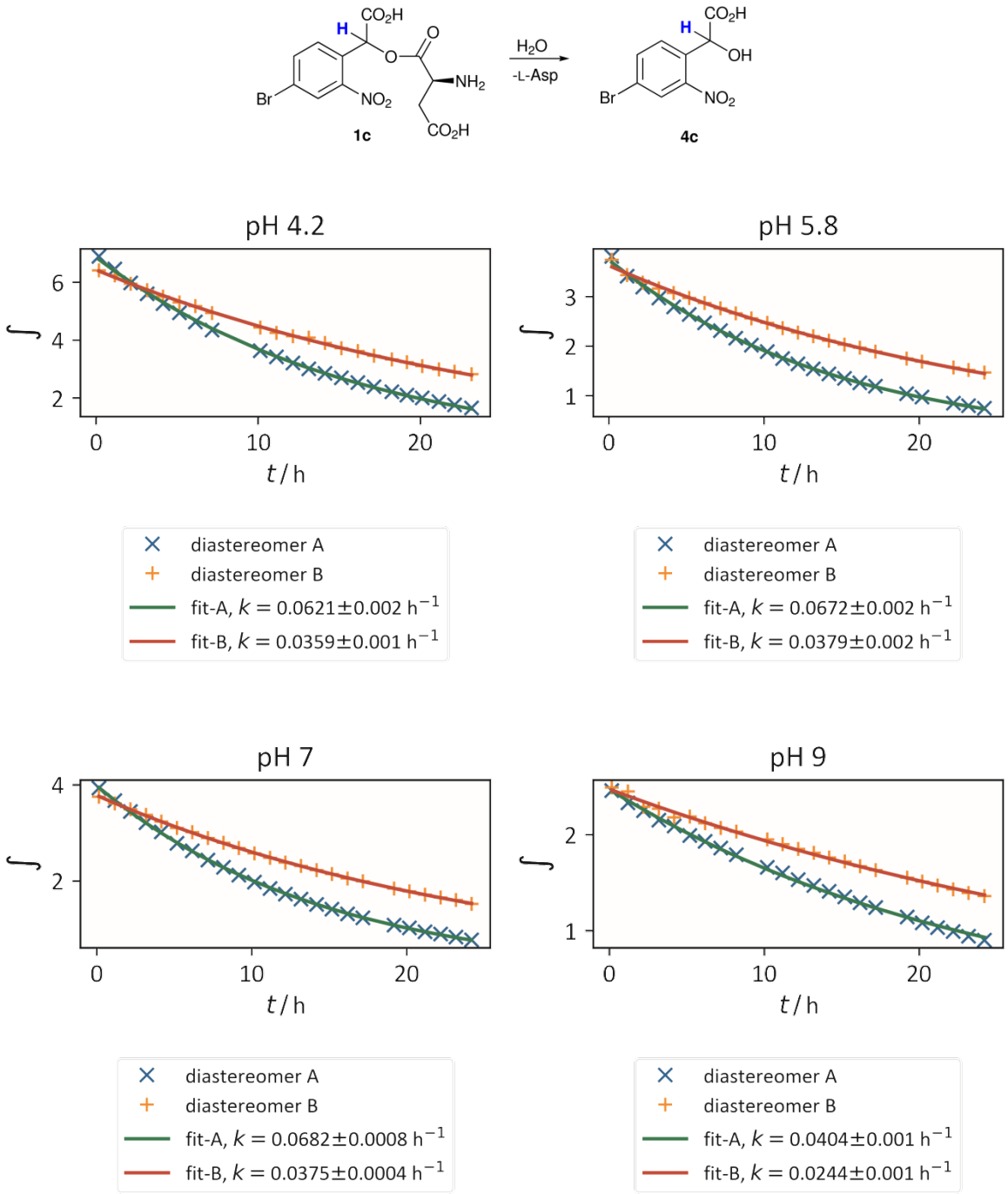


Figure S3: Plots of ^{1}H NMR integrals (benzylic CH signals, see Figures S9 – S12) over time at various pHs, showing the hydrolysis of **1c** under these conditions. x and + denote the experimental data and the exponential fits are plotted as lines. The decays obeyed the first-order exponential decay function $f(t) = Ae^{-kt}$. Least-squares curve fitting was carried out using the SciPy[1] python library, the data were ploted using Matplotlib[2] and the quoted errors are the 1σ confidence interval of *k* estimated by the fitting algorithm scipy.optimize.curve_fit. Note that the pH 4.2 sample was dissolved in 50 mM citrate buffer with 400 mM ammonium sulfate, the other samples were dissolved in 50 mM phosphate buffer. Therefore, the pH 4.2 data are not directly comparable with the others. DMSO-d_6 was added to each sample (12.5% of total volume), and the integral of the residual DMSO signal was set to 10 in each spectrum. In the case of compound **1c**, hydrolysis rates were highest at pH 5.8 and 7, with the lowest rate at pH 9. We propose that the rate is fastest when both the α-carboxyl and the amine are ionised.

Steady State Absorption Spectra

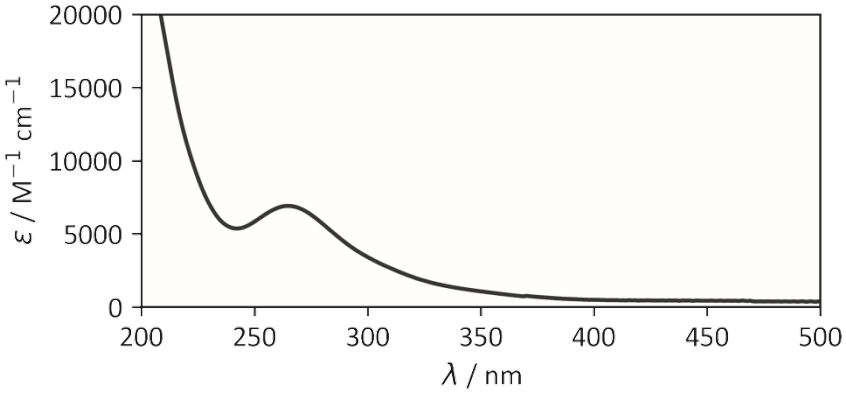


Figure S4: UV absorption spectrum of 1-(CNB)-Asp (**1a**), 0.1 mM in 50 mM sodium phosphate, pH 7.

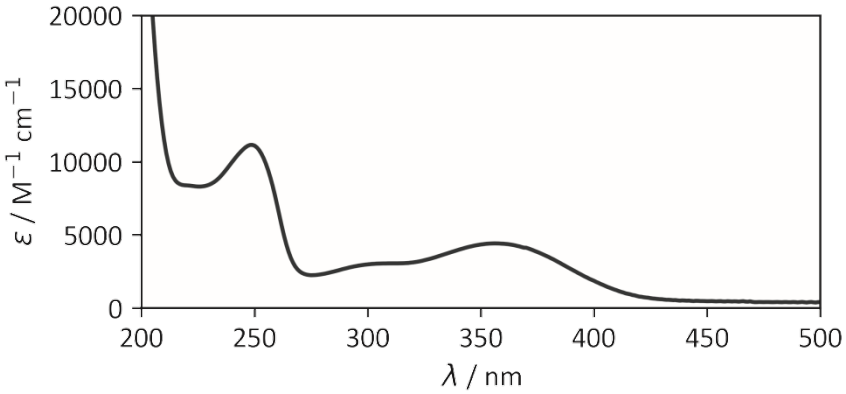


Figure S5: UV absorption spectrum of 1-(CNP)-Asp (**1b**), 0.1 mM in 50 mM sodium phosphate, pH 7.

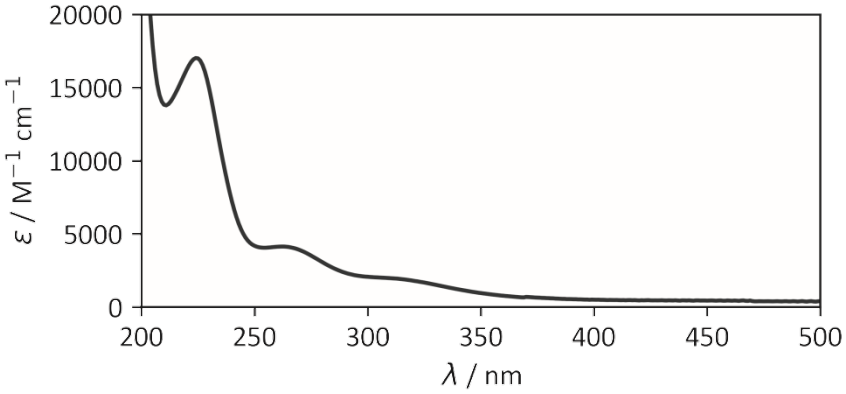


Figure S6: UV absorption spectrum of 1-(*p*BrCNB)-Asp (**1c**), 0.1 mM in 50 mM sodium phosphate, pH 7.

Laser Flash Photolysis

The photoreactions were investigated by two separate different laser flash photolysis (LFP) setups. One was an Applied Photophysics LKS80 instrument with a Surelite™ SL I-10 Nd:YAG pump laser (355 nm third harmonic, 10 Hz repetition rate, 4 – 6 ns pulse width, 6 mm FWHM diameter, 26 mJ pulse energy). The other was an Edinburgh Instruments LP920 setup with an Andor technology DH720 iCCD and a Spectra Physics Quanta Ray Nd:YAG Lab170 pump laser (355 nm, 10 Hz, 2 – 3 ns, < 8 mm, 30 mJ). A 1 cm x 1 cm quartz cuvette was used with a cross-beam configuration for both setups. A pulsed xenon lamp and a photomultiplier coupled to a monochromator were used to record the transient absorption signals. The photocages were supplied as solutions in 50 mM phosphate buffer at pH 7. Due to the irreversible nature of the reaction, the cuvette was refreshed with new solution for each measurement (3.0 mL of fresh sample for every laser shot). Results were typically taken as the average of several such measurements. Unless otherwise stated, the absorbance trace of each measurement was calculated with one pumped and one unpumped set, and the absorbance values were averaged after computing log10(unpumped/pumped). In addition, the pump pulse and all measured pre- and post-pump data were collected within the plateau region of a pulsed xenon arc lamp. To simplify the analysis, pre-pump, fluorescence and rise of signal were omitted from the fitting by cutting the data to be fitted to start with the maximum absorbance value recorded in each set.

1-(α-carboxy-2-nitrobenzyl)-L-Aspartate (**1a**)

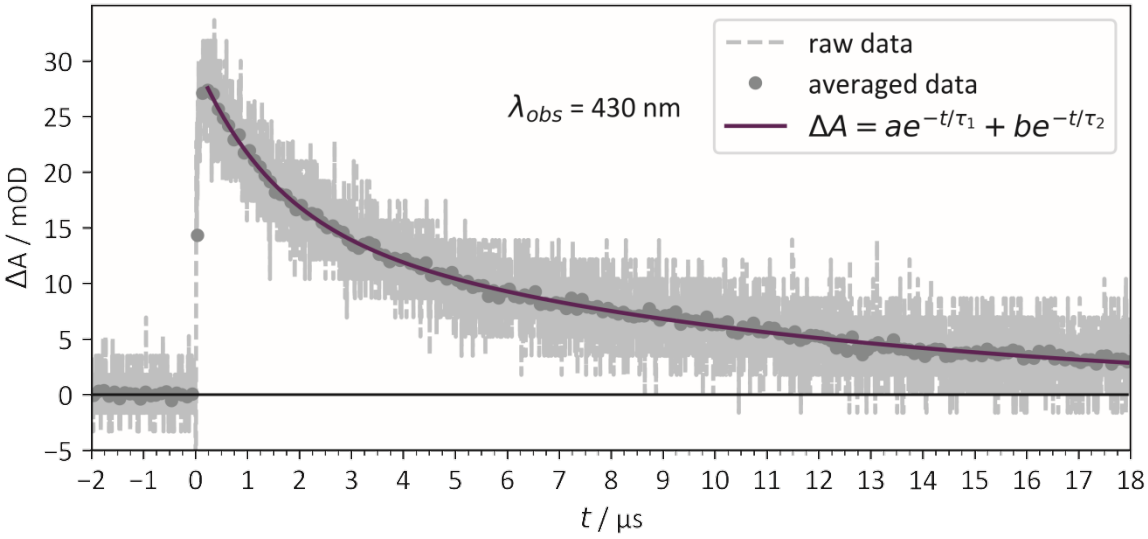


Figure S7: Decay of nitronate intermediates generated by a pulse of a Surelite™ SL I-10 Nd:YAG laser frequency tripled to 355 nm. Pump occurs at t = 0 µs. The original data sampling rate was 0.5 GHz and the data were averaged (adjacent averaging, bin size = 50). A 5 kΩ resistor was used. The photocaged aspartate (**1a**) was supplied as a 0.4 mM solution in sodium phosphate, 50 mM, pH 7. The decay constants obtained from non-linear least-squares fitting were τ_1 = 1.5 ± 0.1 µs (46% ± 2% of signal) and τ_2 = 10.4 ± 0.4 µs (54% ± 2% of signal). Errors given are ± 2σ. Average of four measurements.

1-(*α*-carboxy-6-nitropiperonyl)-L-Aspartate (**1b**)

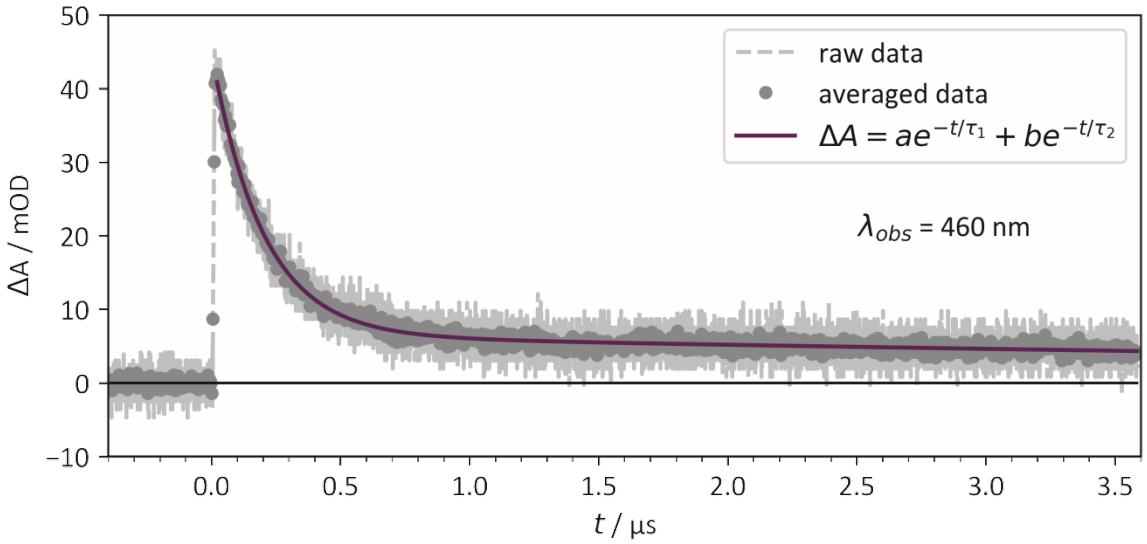


Figure S8: Decay of nitronic acid intermediates generated by a pulse of a Surelite™ SL I-10 Nd:YAG laser frequency tripled to 355 nm. Pump occurs at *t* = 0 µs. The digital sampling rate was 1 GHz, the data were then averaged (adjacent averaging, bin size = 10) to give 100 MHz. The photocaged aspartate (**1b**) was supplied as a 0.4 mM solution in sodium phosphate, 50 mM, pH 7. The decay constants obtained from non-linear least-squares fitting were τ_1 = 199 ± 4 ns (86% ± 1% of signal) and τ_2 = 9 ± 1 µs (14.6% ± 5%). Errors given are ± 2σ. Average of four measurements.

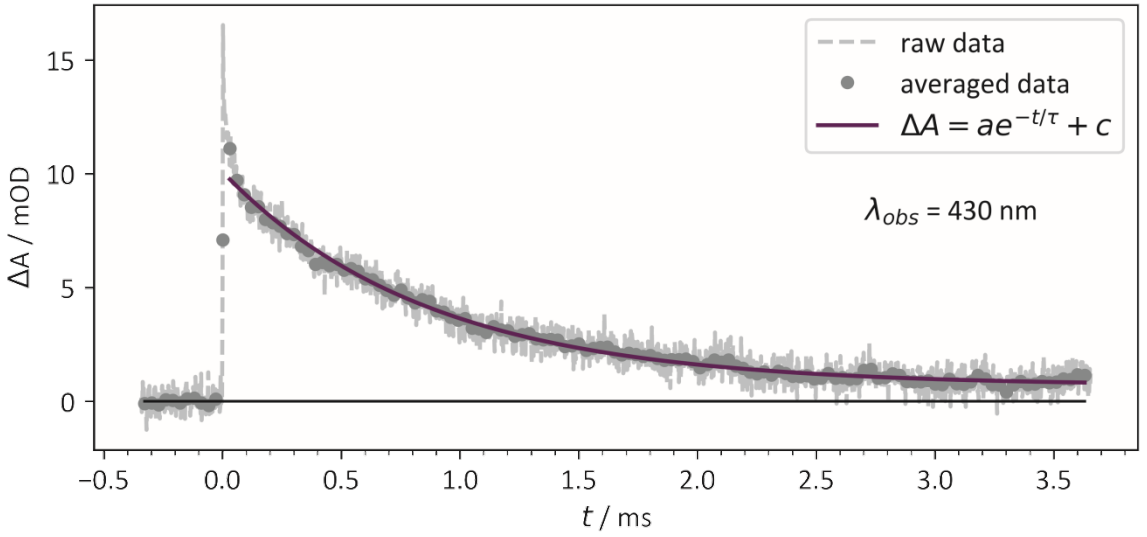


Figure S9: Decay of nitronate intermediates generated by a pulse of a Spectra Physics Quanta Ray Lab170 Nd:YAG laser frequency tripled to 355 nm. Pump occurs at *t* = 0 µs. The data collection rate was 200 kHz. The photocaged aspartate (**1b**) was supplied as a 0.4 mM solution in 5xPBS buffer, pH 7. The decay constant obtained was 0.87 ± 0.04 ms (93% ± 2%), with a steady state contribution of 6.8% ± 0.9%. Average of three pumped and five unpumped measurements. The sharp feature just after the pump time is an artefact. 5xPBS buffer = 685 mM NaCl, 13.5 mM KCl, 50 mM Na_2HPO_4, 9 mM KH_2PO_4, pH was adjusted by addition of 0.1 M HCl (1% of total volume).

p-Br-*α*-carboxynitrobenzyl L-Aspartate (**1c**)

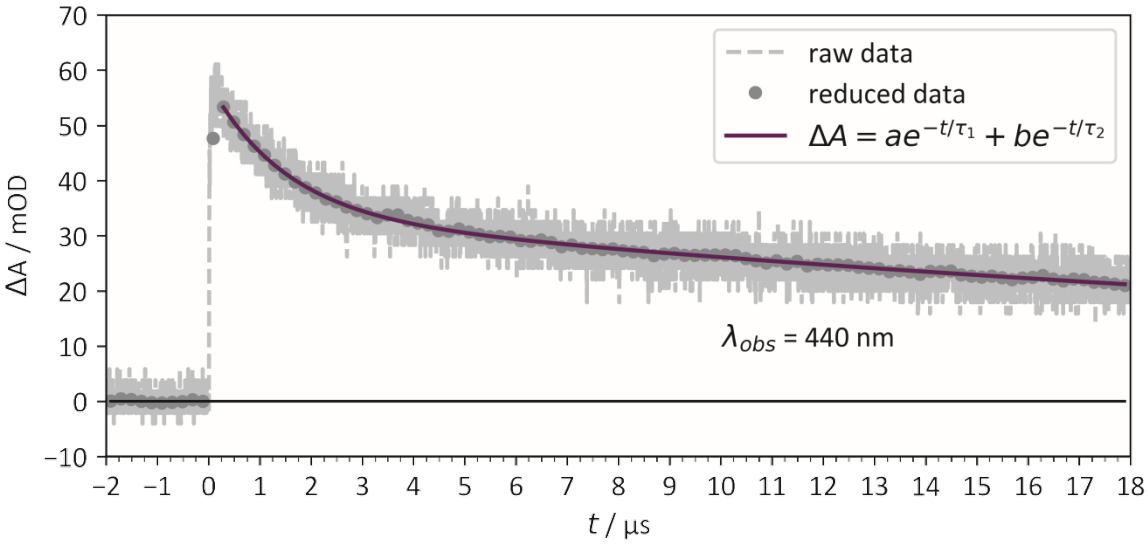


Figure S10: Decay of nitronate intermediates generated by a pulse of a Surelite™ SL I-10 Nd:YAG laser frequency tripled to 355 nm. Pump occurs at t = 0 µs. 10 000 data points over 20 µs (oversampled), the data were then averaged (adjacent averaging, bin size = 50). A 5 kΩ resistor was used. The photocaged aspartate (**1c**) was supplied as a 0.5 mM solution in sodium phosphate, 50 mM, pH 7. The decay constants obtained from non-linear least-squares fitting were τ_1 = 1.48 ± 0.08 µs (41% ± 1% of the signal) and 38 ± 1 µs (58.7% ± 0.7% of the signal). Average of three measurements.

Quantum Yield Determination

LED Characterisation

Irradiation was carried out using a M365LP1-C1 collimated LED from Thorlabs with a variable power supply (set to 200 mA at one controller and division 3 out of 7 on an upper dial), λ_{max} = 365 nm. The beam diameter was 50 mm, which was focused with an additional lens (fused silica UV anti-reflective coating, 100 mm focal length) onto a cuvette placed in a holder. Cuvettes were 1 cm x 1 cm (with two frosted sides), Quartzglass Suprasil® from Hellma Analytics, with a small stir bar in the base. According to a literature protocol,[4] phenylglyoxylic acid (50 mM in 25% H_2O-MeCN) was used as a chemical actinometer to determine the irradiant power in terms of einsteins (moles of photons) incident on the cuvette per minute. The LED was allowed to warm up for 30 minutes before each experiment. Two identical cuvettes were prepared with 3.0 mL of actinometer solution, and one was kept in the dark, so that $\Delta A = A_{dark} - A_{light}$. Absorbance changes were monitored at 380 (Φ_{app} = 0.735) and 390 nm (Φ_{app} = 0.728), and converted to Δc by $\Delta c = \Delta A \div \Delta\varepsilon$ (where $\Delta\varepsilon$ = 29 and 16 at 380 and 390 nm, respectively). Δn/einsteins was then given by $\Delta n = \Delta c \times V \div \Phi_{app}$. Δn/min was then obtained for both series together by linear least-squares regression to the equation $\Delta n = \Delta n/\text{min} \times t$, and the uncertainty in Δn/min was given by the 2σ error in the fitted parameter. These were also corrected for the absorption spectrum of the actinometer solution and the LED spectrum (see below for details, Figure S11). The procedure was repeated twice measuring once per minute for 25 minutes (total

irradiation time), and twice every 5 minutes for 25 minutes. The mean result was 0.59 ± 0.01 µeinstein (n = 4) per minute.

In order to determine the incident light on the cuvette from the amount absorbed, spectra were recorded of the light source (intensity spectrum, recorded with an OceanOptics USB2000+ series spectrometer) and of each actinometer solution (absorbance spectra, measured with a Cary 50 Bio UV-Visible spectrophotometer), each with a spectral resolution of 1 nm in the range 315 – 415 nm. The LED spectrum was normalised to a sum of 1. Then, the overall absorption (as a fraction, F_A) of LED light by the solution was given by:

$$F_A = \sum_{\lambda=315\text{ nm}}^{\lambda=415\text{ nm}} I_\lambda(1 - 10^{-A_\lambda})$$

where I_λ is the normalised intensity at wavelength λ, and A_λ is the absorbance of the actinometer solution at that wavelength. Incident photon amount per minute, Δn_I, is then given by $\Delta n_A \div F_A$.

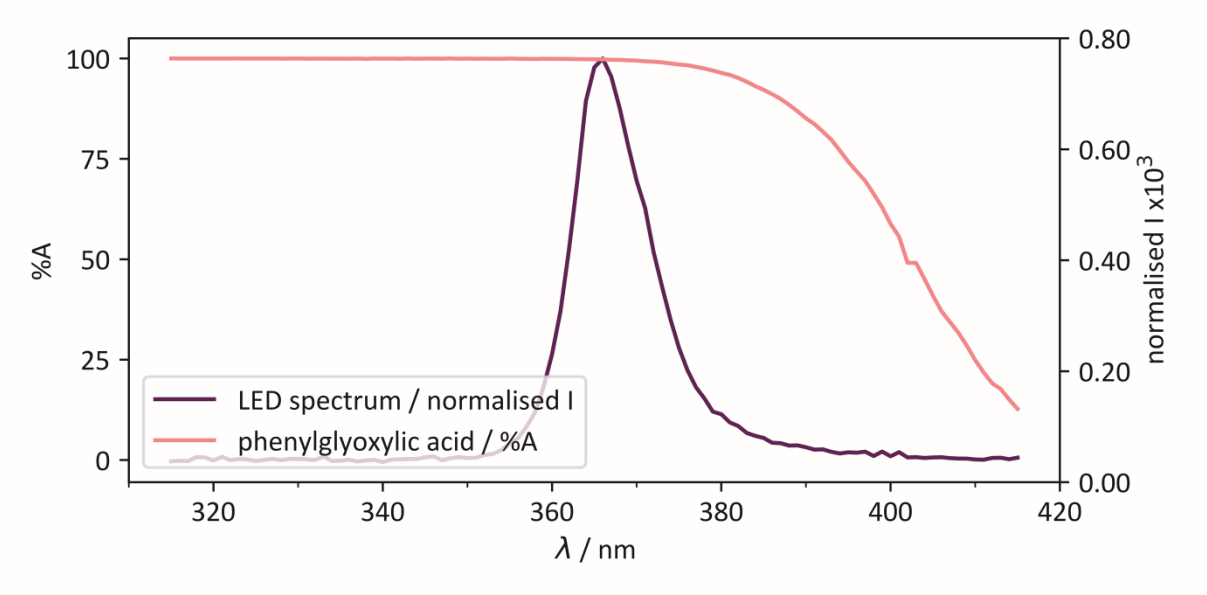


Figure S11: %A (100 - %T) of a cuvette containing 50 mM phenylglyoxylic acid in 25% H_2O – MeCN, and a spectrum of the LED light source with the intensity normalised to sum to 1. The sum of intensities at each point multiplied by %A gives a total spectral absorption of 98% (including a correction for approximately 4% of unabsorbed light reflecting back into the cuvette).

HPLC Standards

Standards of pure hydroxyacid (**4**) were prepared volumetrically by dissolving 0.1 mmol in 100 mL of sodium phosphate buffer (12.5 mM, pH 7) and dilutions were carried out with a burette (see Syntheses for synthesis and purification of hydroxyacids **4**). Standards were then analysed by HPLC (Waters Nova-Pak® C18 column, 3.9 x 150 mm, 4 µm particle size), with isocratic elution (9% MeOH-12.5 mM sodium phosphate, pH 7). The analysing wavelength was 280 nm and 0.3 mM L-Tyrosine

was used as an internal standard (ISTD). The HPLC instrument was an Agilent Technologies 1260 Infinity, with a G1311B Quaternary Pump, G1329B Sample Holder with programmable injection robot and a G1365C multiple wavelength detector for UV/Vis analysis.

Photocage Irradiation and HPLC Analysis

For each experiment, a 10 ml, 1 mM solution of **1** in sodium phosphate buffer (50 mM, pH 7) was prepared volumetrically. Then, 3.0 mL of photocage (**1**) was transferred to the cuvette and irradiated by the UV LED for a fixed time *t*. Next, aliquots were taken from both the irradiated and non-irradiated samples, which were filtered through a 0.22 µm PVDF syringe filter. The aliquots were allowed to stand for ca. 3 days to allow complete hydrolysis of the remaining starting material (**1**), in order to simplify HPLC analysis (hydroxyacids **4** were used as a proxy for remaining **1**, due to the hydrolysis of **1** over time and the ease of preparation of pure standards of **4**). The hydrolysis products (**3**) were analysed by HPLC with reference to the standard calibration described above, and using the same method and equipment. Conversion in the below graphs, was obtained by the difference in concentration between irradiated and non-irradiated aliquots of the same sample, multiplied by the volume. In each of the below figures (S12 – S14), maximum possible conversion is 3 µmol, the x-axis is given in terms of total photons absorbed in µeinsteins (µmols of photons), after correcting for the absorption of incident photons per minute (determinted by phenylglyoxylic acid actinometry). This was necessary due to slight differences in the absorbance of each solution and allows the determination of Φ_{app} (the apparent quantum yield) by orthogonal diagonal regression (linear fit) of the graph of amount conversion vs photons absorbed, taking into account errors in both the independent and dependent variables.

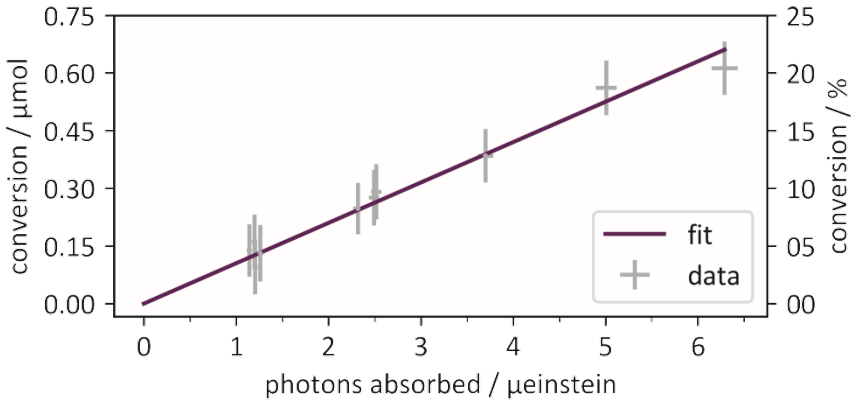


Figure S12: Amount conversion of CNB-Asp (**1a**) vs photons absorbed. Φ_{app} = 0.105 ± 0.006. Error bars and error in ϕ are 2σ. Orthogonal distance regression (ODR) – implemented by the Python package scipy.odr – was used as a fitting algorithm. A linear function was used for fitting, with an explicit intercept at (0, 0) and a gradient of Φ_{app}.

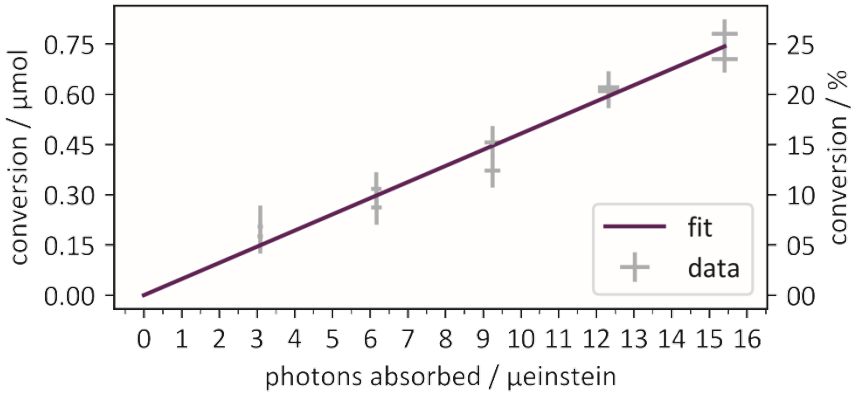


Figure S13: Amount conversion of CNP-Asp (**1b**) vs photons absorbed. Φ_{app} = 0.048 ± 0.002. Error bars and error in ϕ are 2σ. ODR was used as a fitting algorithm. A linear function was used for fitting, with an explicit intercept at (0, 0) and a gradient of Φ_{app}.

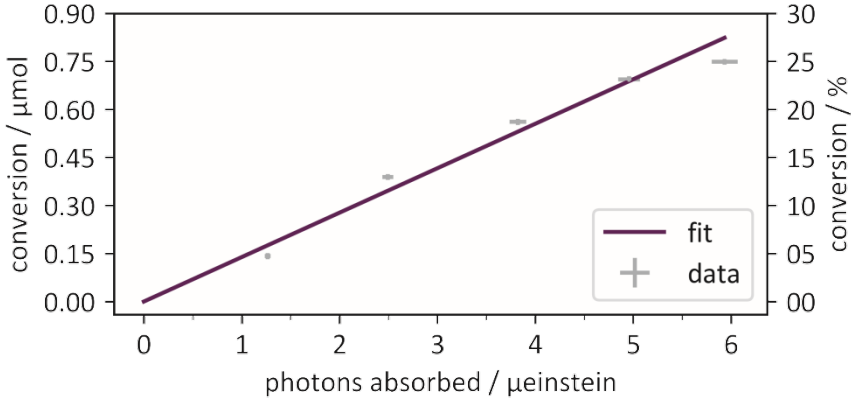


Figure S14: Amount conversion of *p*BrCNB-Asp (**1c**) vs photons absorbed. Φ_{app} = 0.14 ± 0.01. Error bars and error in ϕ are 2σ. ODR was used as a fitting algorithm. A linear function was used for fitting, with an explicit intercept at (0, 0) and a gradient of Φ_{app}. NB: these results appear to show some non-linearity. A more accurate Φ_{app} may be obtained if a larger number of 5 and 10 minute determinations are taken, and the longer experiments discarded.

Syntheses

General Methods

Chemicals (reagent grade) were purchased from Sigma-Aldrich or TCI Europe, solvents (reagent or HPLC grade) were purchased from Thermo-Fisher and all were used as received unless otherwise stated. Trifluoroacetic acid (reagent grade) was purchased from Thermo-Fisher and fractionally distilled immediately prior to use. Anhydrous acetonitrile (SureSeal™, with 3 Å molecular sieves) was purchased from Sigma-Aldrich and used as received. Where specified as 'anhydrous', dichloromethane was dried over 4 Å molecular sieves (10% w/v) for at least 48 h before use.[5] All reactions and purification procedures were conducted under red light, unless otherwise stated. The following abbreviations are used: DMAP = 4-(dimethylamino)pyridine, EDC = *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide, TBTA = *tert*-butyl 2,2,2-trichloroacetimidate, TFA = trifluoroacetic acid, TMSCN = trimethylsilyl cyanide, hexanes = hexane fraction from petroleum, mixture of isomers.

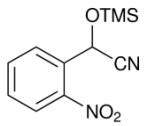
^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE I/II/III 400, DRX 500 or AVANCEIII HD 600 MHz instrument (^{1}H frequencies). Chemical shifts (^{1}H and ^{13}C respectively) are reported in ppm and were referenced to the residual solvent peaks at 7.26 and 77.16 ppm for $CDCl_3$, 2.50 and 39.52 ppm for DMSO-d_6, or 2.05 and 29.84 ppm for acetone-d_6.[6] Coupling constants are given to the nearest 0.1 Hz and multiplicities are reported as: s (singlet), d (doublet), t (triplet), m (multiplet), app stands for apparent and br for broad. Where chemical shift assignments (numbered as in the name of each compound) were given these were determined by a combination of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC NMR experiments. UV/Vis measurements were performed with a Cary 50 Bio UV-Visible spectrophotometer and are reported with λ in units of nm and ε in units of mol^{-1} dm^3 cm^{-1}. HRMS experiments were carried out with an Agilent 6224 ESI-ToF spectrometer coupled to an Agilent HPLC 1200 series. MS measurements were performed using a Bruker UltrafleXtreme MALDI ToF-ToF device equipped with a Smartbeam II laser (matrix: 2,5-dihydroxybenzoic acid) or an ESI-ToF LCT Premier (Waters/Micromass – used for the ionic compounds **1a** – **1c**, which were dissolved in water for sample delivery). Calculated is abbreviated as calcd. MALDI MS or ESI-ToF MS were used for some compounds where the accurate mass could not detected by HRMS (ESI-ToF). Elemental Analyses (Elem. Anal.) were performed with a EuroEA Elemental Analyser equipped with a HEKAtech HT Oxygen Analyser, bromine was determined using ion chromatography following digestion with a START-1500 T-280 microwave pressure digestion apparatus. Melting points were recorded on an LLG Labware MPM-H2 apparatus and are uncorrected. Thin-layer chromatography was performed with ALUGRAM® plates (silica gel 60 with fluorescent indicator UV_{254}) from Machery-Nagel and visualised using UV_{254} illumination. Flash chromatography was carried out using ACROS Organics silica gel pore size 60 Å, particle size 0.030 – 0.200 mm.

TMSCN Addition to Aldehydes

General procedure

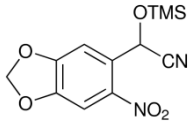
Based on a literature procedure,[7] (substituted) 2-nitrobenzaldehyde (10 mmol) was dissolved in anhydrous acetonitrile (20 ml) under an inert atmosphere in oven-dried glassware. This was treated with trimethylsilyl cyanide (1.31 ml, 10.5 mmol) and stirred for 18 – 20 h at r.t. After this time, TLC analysis (20% EtOAc-hexanes) indicated complete consumption of the starting material. The solvents were removed under reduced pressure to afford the product which was used without purification in the next step.

2-Nitrobenzaldehyde cyanohydrin trimethylsilyl ether (3a)



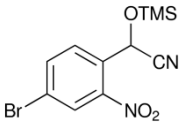
The reaction time was 20 h and the title compound was obtained as an orange oil (2.45 g, quant.). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.17 (1H, dd, *J* = 8.2, 1.0 Hz, H3), 8.02 (1H, app. d, *J* = 7.9 Hz, H6), 7.77 (1H, td, *J* = 7.7, 1.0 Hz, H5), 7.60 (1H, td, *J* = 7.8, 1.2 Hz, H4), 6.22 (1H, s, CH), 0.28 (9H, s, TMS). ^{13}C NMR (125 MHz, $CDCl_3$): δ 146.5 (C2), 134.6 (C5), 132.2 (C1), 130.3 (C4), 128.6 (C6), 125.4 (C3), 118.0 (CN), 60.3 (CH), -0.4 (TMS).

4,5-Methylenedioxy-2-nitrobenzaldehyde cyanohydrin trimethylsilyl ether (3b)



The reaction time was 18 h and the title compound was obtained as a yellow oil (2.96 g, quant.), which solidified on storage at 4 °C, mp 65.9 – 69.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66 (1H, s, H3), 7.42 (1H, s, H6), 6.19 (2H, s, CH_2), 6.15 (1H, s, CH), 0.28 (9H, s, TMS). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.2 (C5), 148.7 (C4), 140.7 (C2), 130.0 (C1), 118.0 (CN), 107.4 (C6), 105.9 (C3), 103.7 (CH_2), 60.2 (CH), -0.3 (TMS). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{12}H_{14}N_2O_5SiNa$ 317.0564; found 317.0513.

4-Bromo-2-nitrobenzaldehyde cyanohydrin trimethylsilyl ether (3c)



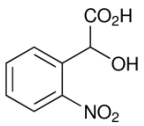
The reaction time was 18 h and the title compound was obtained as a yellow oil (3.10 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 – 8.30 (1H, m, H3), 7.91 – 7.88 (2H, m, H5/H6), 6.14 (1H, s, CH), 0.29 (9H, s, TMS). ^{13}C NMR (125 MHz, $CDCl_3$): δ 146.8 (C2), 137.5 (C5), 131.3 (C1), 130.2 (C6), 128.4 (C3), 123.9 (C4), 117.5 (CN), 60.0 (CH), -0.4 (TMS).

Hydrolysis of Trimethylsilyl Cyanohydrins

General Procedure

Based on a literature procedure,[8] (substituted) 2-nitrobenzaldehyde cyanohydrin trimethylsilyl ether (ca. 10 mmol) was cooled in an ice bath and suspended in conc. HCl (20 ml), then stirred for 1 h at 0 °C, 18 – 22 h at r.t., and 1 h at reflux (bath temp 100 °C). Reaction progress was monitored by TLC. After cooling to r.t., the reaction mixture was diluted with a mixture of water (30 ml) and brine (30 ml), then extracted with EtOAc (3x 80 ml). The combined extracts were dried over $MgSO_4$, filtered and concentrated in vacuo to afford the *hydroxyacid*, which was used without purification in the next step.

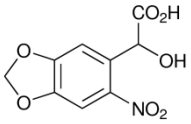
2-Nitromandelic acid (4a)[8,9]



2-Nitrobenzaldehyde trimethylsilyl cyanohydrin (2.45 g, 9.79 mmol) was treated according to the general procedure (the reaction mixture was stirred for 22 h at r.t. after the first stage). The title compound was obtained as a yellow solid (1.92 g, 97%). An analytical sample was obtained by flash chromatography (SiO_2, CH_2Cl_2-MeOH-FA 97:2:1, R_f 0.24), colourless crystals, mp 139.7 – 142.0 °C (AcOH/hexanes, lit.[8] 139 °C from EtOAc/hexanes). ^{1}H NMR (400 MHz, acetone-d_6): δ 8.02 (1H, dd, *J* = 8.0, 1.2 Hz, H3), 7.91 (1H, dd, *J* = 7.6, 1.2 Hz, H6), 7.77 (td, *J* = 7.8, 1.2 Hz, H5), 7.61 (1H, td, *J* = 7.8, 1.6 Hz, H4), 5.92 (s, 1H, CH), 5.72 – 5.00 (br s, 1H, OH). ^{13}C NMR (100 MHz, acetone-d_6): δ 172.6 (CO_2H), 149.2 (C2), 135.3 (Ar), 134.1 (Ar), 130.0 (Ar), 129.9 (Ar), 125.4 (Ar), 70.2 (CH) ppm. MS (MALDI-ToF)

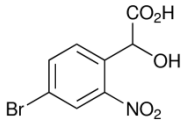
m/z: $[M + Na]^+$ calcd. for $C_8H_7NO_5Na$, 220.022; found, 220.190. Elem. Anal. calcd. for $C_8H_7NO_5$: C, 48.74; H, 3.58; N, 7.10; O, 40.58; found: C, 48.83; H, 3.58; N, 7.03; O, 40.63.

4,5-Methylenedioxy-2-nitromandelic acid (4b)



6-Nitropiperonal cyanohydrin trimethylsilyl ether (2.93 g, 9.95 mmol) was treated according to the general procedure (the reaction mixture was stirred for 20 h at r.t. after the first stage). The title compound was obtained as an ochre solid (2.23 g, 93%). An analytical sample was obtained by flash chromatography (SiO_2, CH_2Cl_2-MeOH-FA 975:15:10, R_f 0.28), yellow needles, 176.1 °C (dec., AcOH/hexanes). ^{1}H NMR (400 MHz, acetone-d_6): δ 7.57 (1H, s, H3), 7.33 (1H, s, H6), 6.26 (1H, d, J = 0.9 Hz, CH_2), 6.25 (1H, d, J = 0.9 Hz, CH_2), 5.89 (1H, s, CH), 5.57 – 5.31 (1H, br s, CH-OH). ^{13}C NMR (150 MHz, acetone-d_6): δ 172.3 (CO_2H), 153.1 (C5), 148.5 (C4), 142.9 (C2), 133.0 (C1), 108.3 (C6), 105.9 (C3), 104.5 (CH_2), 70.4 (CH). MS (MALDI-ToF) m/z: $[M + Na]^+$ calcd for $C_9H_7NO_7Na$, 264.012; found, 264.160. Elem. Anal. calcd. for $C_9H_7NO_7$: C, 44.83; H, 2.93; N, 5.81; O, 46.44; found: C, 44.95; H, 2.97; N, 5.74; O, 46.32.

4-Bromo-2-nitromandelic acid (4c)



4-Bromo-2-nitrobenzaldehyde cyanohydrin trimethylsilyl ether (3.10 g, 9.42 mmol) was treated according to the general procedure (the reaction mixture was stirred for 18 h at r.t. after the first stage). The title compound was obtained as a pale yellow solid (2.64 g, quant.). An analytical sample was obtained by flash chromatography (SiO_2, CH_2Cl_2-MeOH-FA 975:15:10, R_f 0.21), colourless crystals, mp 146.1 – 148.6 °C (AcOH/hexanes). ^{1}H NMR (400 MHz, acetone-d_6): δ 8.19 (1H, d, J = 2.0 Hz, H3), 7.96 (1H, dd, J = 8.4, 2.0 Hz, H5), 7.87 (1H, d, J = 8.4 Hz, H6), 5.90 (1H, s, CH). ^{13}C NMR (100 MHz, acetone-d_6): δ 172.1 (CO_2H), 149.7 (C2), 137.0 (C5), 134.9 (C1), 131.7 (C6), 128.1 (C3), 122.1 (C4), 69.9 (CH). MS (MALDI-ToF) m/z: $[M + Na]^+$ calcd for $C_8H_6BrNO_6Na$ 297.933; found 298.075. Elem. Anal. calcd. for $C_8H_6BrNO_6$: C, 34.81; H, 2.19; Br, 28.95; N, 5.07; O, 28.98; found: C, 34.46; H, 2.19; Br, 27.72; N, 4.84; O, 28.83.

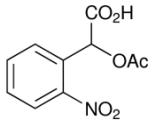
Acetylation of Hydroxyacids

General procedure

Based on a literature procedure,[10] (substituted) 2-nitromandelic acids (4 – 10 mmol) were suspended in neat Ac_2O (1.8 ml / mmol) and stirred for 18 – 20 h at r.t., affording a clear solution. THF (1.5 ml / mmol) and water (1.5 ml / mmol) were added, producing a cloudy mixture which turned clear again while stirring for 2 h at 45 °C. The product mixture was cooled to r.t., toluene (3 ml / mmol) was added and the layers were separated. The aqueous phase was extracted again with 60% EtOAc-toluene (2.5 ml / mmol) followed by EtOAc (2x 1 ml / mmol). The combined organic layers were washed with brine (1 ml / mmol), dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was further concentrated azeotropically with toluene (3x 3 ml / mmol) and dried under high

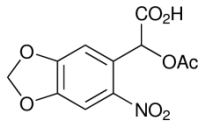
vacuum to afford the *acetate*. This procedure did not remove all traces of AcOH but provided sufficiently pure product to continue to the next step.[a]

_O_-Ac-2-Nitromandelic acid (5a)[10]



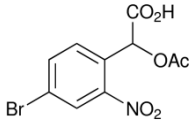
2-Nitromandelic acid (1.91 g, 9.69 mmol) was treated according to the general procedure (the reaction time was 18 h) to afford the title compound as an oil (2.44 g, nominally 105%). ^{1}H NMR (400 MHz, acetone-d_6): δ 8.53 – 8.15 (1H, br s, CO_2H), 8.07 (1H, app d, J = 8.4, H3), 7.80 – 7.85 (1H, m, Ar), 7.70 – 7.64 (2H, m, Ar), 6.84 (1H, s, CH), 2.17 (3H, s, OAc). ^{13}C NMR (100 MHz, acetone-d_6): δ 170.0 ($COCH_3$), 169.0 (CO_2H), 149.3 (C2), 134.5 (Ar), 130.9 (Ar), 130.3 (C1), 130.2 (Ar), 125.7 (C3), 70.3 (CH), 20.4 (CH_3). MS (MALDI-ToF) m/z: $[M + Na]^+$ calcd for $C_{10}H_9NO_6Na$ 262.033; found 262.187. Elem. Anal. calcd. for $C_{10}H_9NO_6$: C, 50.22; H, 3.79; N, 5.86; O, 40.13; found: C, 51.01; H, 3.73; N, 4.42; O, 41.23.

_O_-Ac-4,5-Methylenedioxy-2-nitromandelic acid (5b)



4,5-Methylenedioxy-2-nitromandelic acid (1.00 g, 4.15 mmol) was treated according to the general procedure, except the acetylation reaction was carried out for 4 h at 60 °C, then allowed to cool slightly before adding THF and water. Also, the second extraction failed to separate so the solvents were all combined before washing with brine. The title compound was obtained as a pale yellow solid (1.29 g, nominally 110%), mp 144.3 – 145.5 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ 8.88 – 8.18 (1H, br s, CO_2H), 7.59 (1H, s, H3), 7.13 (1H, s, H6), 6.82 (1H, s, CH), 6.27 (1H, s, CH_2), 2.18 (3H, s, Ac). ^{13}C NMR (100 MHz, acetone-d_6): δ 170.0 ($COCH_3$), 168.9 (CO_2H), 153.2 (C5), 149.3 (C4), 143.6 (C2), 127.2 (C1), 108.4 (C6), 106.2 (C3), 104.8 (CH_2), 70.3 (CH), 20.5 (CH_3). Elem. Anal. calcd. for $C_{11}H_9NO_8$: C, 46.65; H, 3.20; N, 4.95; O, 45.20; found: C, 46.54; H, 3.19; N, 4.92; O, 44.33.

_O_-Ac-4-Bromo-2-nitromandelic acid (5c)



4-Bromo-2-nitromandelic acid (2.64 g, 9.56 mmol) was treated according to the general procedure to afford title compound as a yellow oil (3.46 g, nominally 92%). ^{1}H NMR (400 MHz, acetone-d_6): δ 8.25 (1H, d, J = 2.0 Hz, H3), 7.98 (1H, dd, J = 8.4, 2.0 Hz, H5), 7.73 (1H, d, J = 8.4 Hz, H6), 6.80 (s, 1H, CH), 2.18 (3H, s, Ac), 1.96. ^{13}C NMR (100 MHz, acetone-d_6): δ 169.8 ($COCH_3$), 168.4 (CO_2H), 149.8 (C2), 137.4 (C5), 132.0 (C6), 129.8 (C1), 128.6 (C3), 123.4 (C4), 69.9 (CH), 20.4 (CH_3).

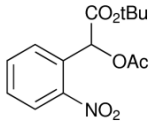
[a]An alternative workup is possible: extraction and washing steps can be skipped and the product mixture simply evaporated azeotropically with portions of toluene (e.g. 3x 3 ml / mmol).

tert-Butyl Ester Protection

General procedure

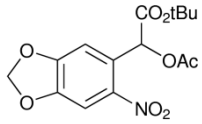
Based on a literature procedure,[10] (substituted) *O*-acetyl-2-nitromandelic acid (ca. 4 – 10 mmol) was dissolved in anhydrous benzene (1.8 ml / mmol) under an inert atmosphere, treated with TBTA (1.5 eq.) and stirred for 2 – 3 d at r.t. After this time, the product mixture was filtered, the filtrate concentrated in vacuo and the crude product was purified by flash chromatography to give the tert-*butyl ester*.

***tert*-Butyl *O*-Ac-2-nitromandelate (6a)**[10]



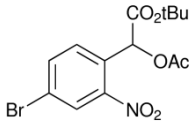
O-Ac-2-nitromandelic acid (2.44 g, nominally 10.2 mmol) was reacted for 66 h according to the general procedure. Flash chromatography (150 g SiO_2, 10% EtOAc-hexanes, R_f 0.19) gave the title compound as a clear oil (2.19 g, 75% over 4 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.98 (1H, app d, *J* = 8.1 Hz, H3), 7.63 (2H, app d, *J* = 4.0 Hz, H4/H5), 7.54 – 7.48 (1H, m, H6), 6.73 (1H, s, CH), 2.18 (3H, s, Ac), 1.37 (9H, s, tBu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5 ($COCH_3$), 166.5 (CO_2tBu), 148.3 (C2), 133.5 (C4), 129.8 (C1), 129.7 (C6), 129.2 (C5), 125.1 (C3), 83.6 ($C(CH_3)_3$), 70.3 (CH), 27.8 ($COC(CH_3)_3$), 20.7 ($COCH_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{14}H_{17}NO_6Na$, 318.0948; found, 318.0905.

***tert*-Butyl *O*-Ac-4,5-methylenedioxy-2-nitromandelate (6b)**



O-Ac-4,5-methylenedioxy-2-nitromandelic acid (1.08 g, nominally 3.81 mmol) was reacted for 48 h according to the general procedure. Flash chromatography (100 g SiO_2, 15% EtOAc-hexanes, R_f 0.22) afforded the title compound as a yellow oil (1.04 g, 83% over 4 steps), which slowly crystallised, mp 72.2 – 74.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (1H, s, H3), 7.02 (1H, s, H6), 6.73 (1H, s, CH), 6.14 (2H, s, CH_2), 2.19 (3H, s, Ac), 1.41 (9H, s, tBu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5 ($COCH_3$), 166.7 (CO_2tBu), 152.1 (C5), 148.2 (C4), 142.7 (C2), 126.8 (C1), 107.9 (C6), 106.1 (C3), 103.4 (CH_2), 83.5 ($C(CH_3)_3$), 70.6 (CH), 27.9 ($C(CH_3)_3$), 20.8 ($COCH_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{15}H_{17}NNaO_8$, 362.0852; found, 362.0884.

***tert*-Butyl *O*-Ac-4-bromo-2-nitromandelate (6c)**



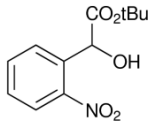
O-Ac-4-Bromo-2-nitromandelic acid (3.46 g, 9.25 mmol) was reacted for 44 h according to the general procedure, except the product mixture was not filtered before concentrating. Flash chromatography (150 g SiO_2, 6% EtOAc-hexanes, R_f 0.26) afforded the title compound as a colourless oil (2.46 g, 71%, 66% over 4 steps). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.15 (1H, d, J = 2.0 Hz, H3), 7.77 (1H, dd, J = 8.3, 1.9 Hz, H5), 7.53 (1H, d, J = 8.4 Hz, H6), 6.69 (1H, s, CH), 2.20 (3H, s, Ac), 1.40 (9H, s, tBu).

^{13}C NMR (125 MHz, $CDCl_3$): δ 169.4 ($COCH_3$), 166.1 (CO_2tBu), 148.7 (C2), 136.5 (C5), 130.6 (C6), 129.0 (C1), 128.2 (C3), 123.2 (C4), 84.0 ($C(CH_3)_3$), 70.0 (CH), 27.9 ($C(CH_3)_3$), 20.7 ($COCH_3$). HRMS (ESI-TOF) m/z: $[M + Na]^+$ calcd. for $C_{14}H_{16}BrNO_6Na$, 396.0053; found 396.0139.

Deacetylation

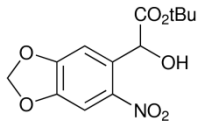
Cs_2CO_3 (5 mol %) was added to a stirred solution of (substituted) *tert*-butyl *O*-Ac-2-nitromandelate **5** (0.20 M) in methanol. After stirring for 1 h at r.t., the reaction mixture was diluted to triple volume with EtOAc, filtered over a short silica plug and concentrated in vacuo to afford the crude *alcohol*, which was used without purification in the next step.

tert-Butyl 2-nitromandelate (7a)



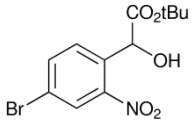
tert-Butyl *O*-Ac-2-nitromandelate (0.78 g, 2.64 mmol) was treated according to the general procedure to afford the title compound (0.67 g, quant.). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (1H, dd, *J* = 8.1, 0.8 Hz, H3), 7.67 (1H, app d, *J* = 7.6 Hz, H6), 7.59 (1H, td, *J* = 7.6, 0.8 Hz, H5), 7.44 (1H, td, *J* = 7.8, 1.2 Hz, H4), 5.80 (1H, s, CH), 3.91 – 3.75 (1H, br s, OH), 1.34 (9H, s, tBu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.0 (CO_2tBu), 148.2 (C2), 133.6 (C1), 133.2 (C5), 129.1 (C6), 129.0 (C4), 125.0 (C3), 84.1 ($C(CH_3)_3$), 69.8 (CH), 27.7 ($C(CH_3)_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{12}H_{15}NO_5Na$, 276.0848; found, 276.0808.

tert-Butyl 4,5-methylenedioxy-2-nitromandelate (7b)



tert-Butyl *O*-Ac-4,5-methylenedioxy-2-nitromandelate (0.97 g, 2.86 mmol) was treated according to the general procedure to afford the crude *alcohol* as a yellow oil (0.81 g, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (1H, s, H3), 7.12 (1H, s, H6), 6.18 – 6.06 (2H, m, CH_2), 5.81 (1H, s, CH), 4.06 – 3.86 (1H, br s, OH), 1.38 (9H, s, tBu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8 (CO_2tBu), 151.9 (C5), 147.5 (C4), 142.1 (C2), 130.9 (C1), 107.7 (C6), 105.8 (C3), 103.2 (CH_2), 83.7 ($C(CH_3)_3$), 69.9 (CH), 27.7 ($C(CH_3)_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{13}H_{15}NNaO_7$, 320.0764; found, 320.0753.

tert-Butyl 4-bromo-2-nitromandelate (7c)



tert-Butyl *O*-Ac-4-bromo-2-nitromandelate (0.58 g, 1.55 mmol) was treated according to the general procedure to afford the crude *alcohol* as a yellow solid (0.47 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (1H, d, *J* = 2.0 Hz, H3), 7.74 (1H, dd, *J* = 8.4, 2.0 Hz, H5), 7.61 (1H, d, *J* = 8.4 Hz, H6), 5.82 (1H, d, *J* = 2.6 Hz, CH), 3.69 (1H, d, *J* = 2.0 Hz, OH), 1.37 (9H, s, tBu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7 (CO_2tBu), 148.7 (C2), 136.2 (C5), 132.6 (C1), 130.4 (C6), 128.0 (C3), 122.3 (C4), 84.9 ($C(CH_3)_3$),

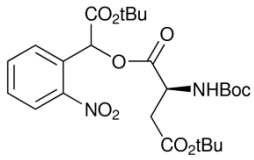
69.3 (CH), 27.8 ($C(CH_3)_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{12}H_{14}BrNO_5Na$, 353.9948; found, 353.9959.

Coupling to L-Aspartate

General procedure

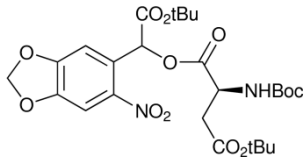
Based on a literature protocol,[11] (substituted) *tert*-butyl 2-nitromandelate (1 – 2 mmol) was dissolved in anhydrous CH_2Cl_2 (10 ml / mmol) under an inert atmosphere, 4-*tert*-butyl *N*-Boc L-aspartate (1.1 eq.) was added and the mixture was cooled in an ice bath for 15 min. Then, DMAP (0.9 eq.) and EDC·HCl (1.5 eq.) were added and the mixture was stirred for 1 h at 0 °C. The ice bath was removed and stirring continued for 24 h at r.t. After this time, the reaction mixture was placed again in an ice bath for 15 min, quenched with brine (10 ml / mmol), allowed to warm to r.t. and extracted with CH_2Cl_2 (10 ml / mmol). The combined extracts were washed sequentially with 0.5 M HCl (10 ml / mmol), water (10 ml / mmol) and $NaHCO_3$ (10 ml / mmol), dried over $MgSO_4$, filtered and concentrated in vacuo to afford the crude *ester*. The compounds were purified by flash chromatography.

***tert*-Butyl *N*-Boc-1-(*tert*-butyloxycarbonyl-(2-nitrophenyl)methyl) *L*-aspartate (8a)**



tert-Butyl 2-nitromandelate (0.67 g, 2.65 mmol) was treated according to the general procedure to afford the crude *ester*. Flash chromatography (SiO_2 126 g, 18% EtOAc-hexanes, R_f 0.29) afforded the title compound as a clear, sticky oil (1.12 g, 81%), ca 1:1 mixture of diastereoisomers (based on ArCH NMR integrals). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 – 7.98 (1H, m, H3'), 7.69 – 7.59 (2H, m, H5'/H6'), 7.55 – 7.48 (1H, m, H4'), 6.81, 6.79 (1H, 2 s, ArCH), 5.59 – 5.50 (1H, m, NH), 4.76 – 4.66 (1H, m, H2), 3.00 – 2.89 (1H, m, H3), 2.87 – 2.71 (1H, m, H3), 1.46 – 1.32 (27H, m, 3x tBu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.1, 170.0, 169.9 (4x CO_2tBu), 165.9, 165.7 (2x C1), 155.5, 155.4 (2x NCO_2tBu), 148.1 (C2'), 133.59, 133.57 (2x C5'/C6'), 129.75, 129.72 (2x C4'), 129.68, 129.66 (2x C1'), 129.4, 129.3 (2x C5'/C6'), 125.2 (C3'), 83.8, 83.7, 81.94, 81.92, 80.2 (5x $C(CH_3)_3$), 71.4, 71.3 (2x ArCH), 50.2 (C2), 37.9, 37.7 (2x C3), 28.4, 28.1, 28.0, 27.8 (4x $C(CH_3)_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{25}H_{36}N_2O_{10}Na$ 547.2262; found 547.2209.

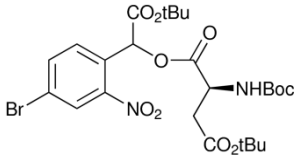
***tert*-Butyl *N*-Boc-1-(*tert*-butyloxycarbonyl-(4,5-methylenedioxy-2-nitrophenyl)methyl) *L*-aspartate (8b)**



tert-Butyl 4,5-methylenedioxy-2-nitromandelate (0.47 g, 1.39 mmol) was treated according to the general procedure. Flash chromatography (SiO_2 89 g, 20% EtOAc-hexanes, R_f 0.30) afforded the title compound as a colourless foam (0.70 g, 87% over two steps), ca 1:1 mixture of diastereoisomers (estimated based on H6' NMR integrals). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55, 7.54 (1H, 2 s, H3'), 7.06, 7.04 (1H, 2 s, H6'), 6.79, 6.77 (1H, 2 s, ArCH), 6.17 – 6.11 (2H, m, OCH_2), 5.57 –

5.49 (1H, m, NH), 4.73 – 4.65 (1H, m, H2), 2.98 – 2.88 (1H, m, H3), 2.84 – 2.72 (1H, m, H3), 1.46 – 1.36 (27H, m, 3x tBu). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.2, 170.1, 170.0, 169.9 (4x CCO_2tBu), 166.0, 165.8 (2x C1), 155.6, 155.4 (2x NCO_2tBu), 152.2 (C5'), 148.2 (C4'), 142.5 (C2'), 126.8, 126.7 (2x C1'), 108.1, 108.0 (2x C6'), 106.1 (C3'), 103.4 (OCH_2), 83.7, 83.6, 82.0, 80.3 (4x $C(CH_3)_3$), 71.8, 71.6 (2x ArCH), 50.3 (C2), 38.0, 37.7 (2x C3), 28.4, 28.2, 28.1, 27.9 (4x $C(CH_3)_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{26}H_{36}N_2NaO_{12}$, 591.2166; found 591.2213.

***tert*-Butyl *N*-Boc-1-(*tert*-butyloxycarbonyl-(4-bromo-2-nitrophenyl)methyl) *L*-Aspartate (8c)**



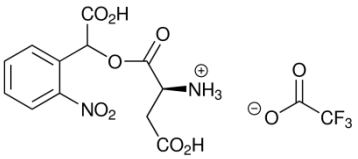
tert-Butyl 4-bromo-2-nitromandelate (0.47 g, 1.42 mmol) was treated according to the general procedure, except reagent loadings were relative to the amount of compound **6c** used in the previous step (1 equiv = 1.55 mmol). Flash chromatography (SiO_2 87 g, 14% EtOAc-hexanes, R_f 0.28) afforded the title compound as a clear oil (0.68 g, 73% over two steps), ca 1:1 mixture of diastereoisomers (estimated based on ArCH NMR integrals). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 – 8.16 (1H, m, H3'), 7.78 – 7.73 (1H, m, H5'), 7.58 – 7.51 (1H, m, H6'), 6.75, 6.74 (1H, 2 s, ArCH), 5.58 – 5.48 (1H, m, NH), 4.75 – 4.65 (1H, m, H2), 2.98 – 2.89 (1H, m, H3), 2.85 – 2.72 (1H, m, H3), 1.48 – 1.35 (27H, 3x tBu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.1, 170.04, 169.97 (4x CCO_2tBu), 165.4, 165.3 (2x C1), 155.6, 155.4 (2x NCO_2tBu), 148.4 (C2'), 136.6 (C5'), 130.7, 130.6 (2x C6'), 128.84, 128.79 (2x C1'), 128.2 (C3'), 123.2, 123.1 (2x C4'), 84.2, 84.1, 82.1, 80.3 (4x $C(CH_3)_3$), 71.1, 71.0 (2x ArCH), 50.2 (C2), 37.8, 37.6 (2x C3), 28.4, 28.2, 28.0, 27.8 (4x $C(CH_3)_3$). HRMS (ESI-ToF) m/z: $[M + Na]^+$ calcd. for $C_{25}H_{35}BrN_2NaO_{10}$, 627.1347; found 627.1353.

Deprotection of N-Boc and tert-Butyl Esters

General procedure

In an oven dried flask, protected photocage (**8**) (0.2 – 0.3 mmol) was dissolved in anhydrous CH_2Cl_2 (5 ml / mmol), to which was added freshly distilled TFA (5 ml / mmol). The reaction mixture was placed under a $CaCl_2$ drying tube and stirred for 24 h. The product mixture was concentrated in vacuo and then co-evaporated with dry CH_2Cl_2 (3x 2 ml) to afford the photocaged L-aspartate trifluoroacetate salt (**1**), which was sufficiently pure for further experiments.

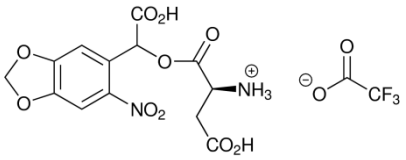
1-(carboxy-(2-nitrophenyl)methyl) *L*-aspartate trifluoroacetate salt (1a)



Compound **8a** (0.15 g, 0.29 mmol) was treated according to the general procedure to afford the title compound as a white solid (0.12 g, 97%). UV/Vis (50 mM phosphate buffer, pH 7) λ_{max} (ε): 265 (5160). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.10 – 8.22 (3H, br s, NH_3^+), 8.15 – 8.06 (1H, m, H3'), 7.86 – 7.68 (3H, m, Ar), 6.78 (1H, s, ArCH), 4.57 – 4.48 (1H, m, H2), 3.07 – 2.82 (2H, m, H3). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.64, 170.60 (2x C4), 167.8, 167.70, 167.66, 167.6 (4x C1/α-carboxy), 158.5 (q, *J* = 35.0 Hz, TFA CO), 147.83, 147.79 (2x C2'), 134.11, 134.06, 130.64, 130.58, 129.8, 129.7 (6x Ar),

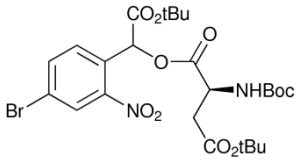
128.22, 128.18 (2x C1'), 125.1 (C3'), 118.2 (app t, *J* = 294.5 Hz, CF_3), 70.8, 70.7 (2x ArCH), 48.6 (C2), 34.3, 34.1 (2x C3). MS (ESI-ToF) m/z: $[M + H]^+$ calcd. for $C_{12}H_{13}N_2O_8$ 313.0666; found 313.21.

1-(carboxy-(4,5-methylenedioxy-2-nitrophenyl)methyl) *L*-aspartate trifluoroacetate salt (1b)



Compound **8b** (0.26 g, 0.23 mmol) was treated according to the general procedure to afford the title compound as a yellow solid (0.21 g, 98%), 1:1 mixture of diastereoisomers (estimated based on H6' NMR integrals). UV/Vis (50 mM phosphate buffer, pH 7) λ_{max} (ε): 248 (10300), 312 (2680), 357 (4060). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.96 – 8.25 (3H, br s, NH_3^+), 7.73, 7.71 (1H, 2 s, H3'), 7.31, 7.24 (1H, 2 s, H6'), 6.76, 6.74 (1H, 2 s, ArCH), 6.30 – 6.26 (2H, m, OCH_2), 4.54, 4.49 (1H, 2 t, *J* = 5.4, 4.9 Hz, H2), 3.05 – 2.82 (2H, m, H3). ^{13}C NMR (150 MHz, DMSO-d_6): δ 170.7, 170.6 (2x C4), 167.7, 167.62, 167.61, 167.5 (4x C1/α-carboxy), 158.3 (q, *J* = 33.0 Hz, TFA CO), 152.1, 151.9 (2x C5'), 148.2 (C4'), 142.1 (C2'), 125.04, 125.01 (2x C1'), 116.7 (app d, *J* = 295.5 Hz, CF_3), 108.2, 108.0 (2x C6'), 105.6 (C3'), 103.94, 103.93 (2x OCH_2), 70.9, 70.8 (2x ArCH), 48.6 (C2), 34.3, 34.1 (2x C3). MS (ESI-ToF) m/z: $[M + H]^+$ calcd. for $C_{13}H_{13}N_2O_{10}$ 357.06; found 357.16.

1-(carboxy-(4-bromo-2-nitrophenyl)methyl) *L*-Aspartate trifluoroacetate salt (1c)



Compound **8c** (0.16 g, 0.27 mmol) was treated according to the general procedure to afford the title compound as a white crystalline solid (0.13 g, 96%), 1:1 mixture of diastereoisomers (estimated based on H6' NMR integrals). UV/Vis (50 mM phosphate buffer, pH 7) λ_{max} (ε): 224 (16000), 262 (3640), 307 (sh, 1600). ^{1}H NMR (400 MHz, DMSO-d_6): 14.33 – 11.99 (2H, br s, 2x CO_2H), 8.85 – 8.40 (3H, br s, NH_3^+), 8.33, 8.31 (1H, 2 d, *J* = 1.8, 1.8 Hz, H3'), 8.08 – 7.98 (1H, m, H5'), 7.77, 7.71 (1H, 2 d, *J* = 8.4, 8.4 Hz, H6'), 6.72, 6.71 (1H, 2 s, ArCH), 4.55 – 4.46 (1H, m, H2), 3.06 – 2.79 (2H, m, H3). ^{13}C NMR (100 MHz, DMSO-d_6): 170.62, 170.58 (2x C4), 167.64, 167.59, 167.3, 167.2 (4x C1/α-carboxy), 158.4 (app d, *J* = 33.0 Hz, TFA CO), 148.4, 148.3 (2x C2'), 136.72, 136.69 (2x C5'), 131.8, 131.5 (2x C6'), 127.8 (C1'), 127.6, 127.5 (2x C3'), 122.74, 122.72 (2x C4'), 70.5, 70.3 (2x ArCH), 48.6, 48.5 (2x C2), 34.3, 34.1 (2x C3). MS (ESI-ToF) m/z: $[M + H]^+$ calcd. for $C_{12}H_{12}BrN_2O_8$ 390.98, 392.98; found 391.11, 393.11 (1:1 isotopic ratio).

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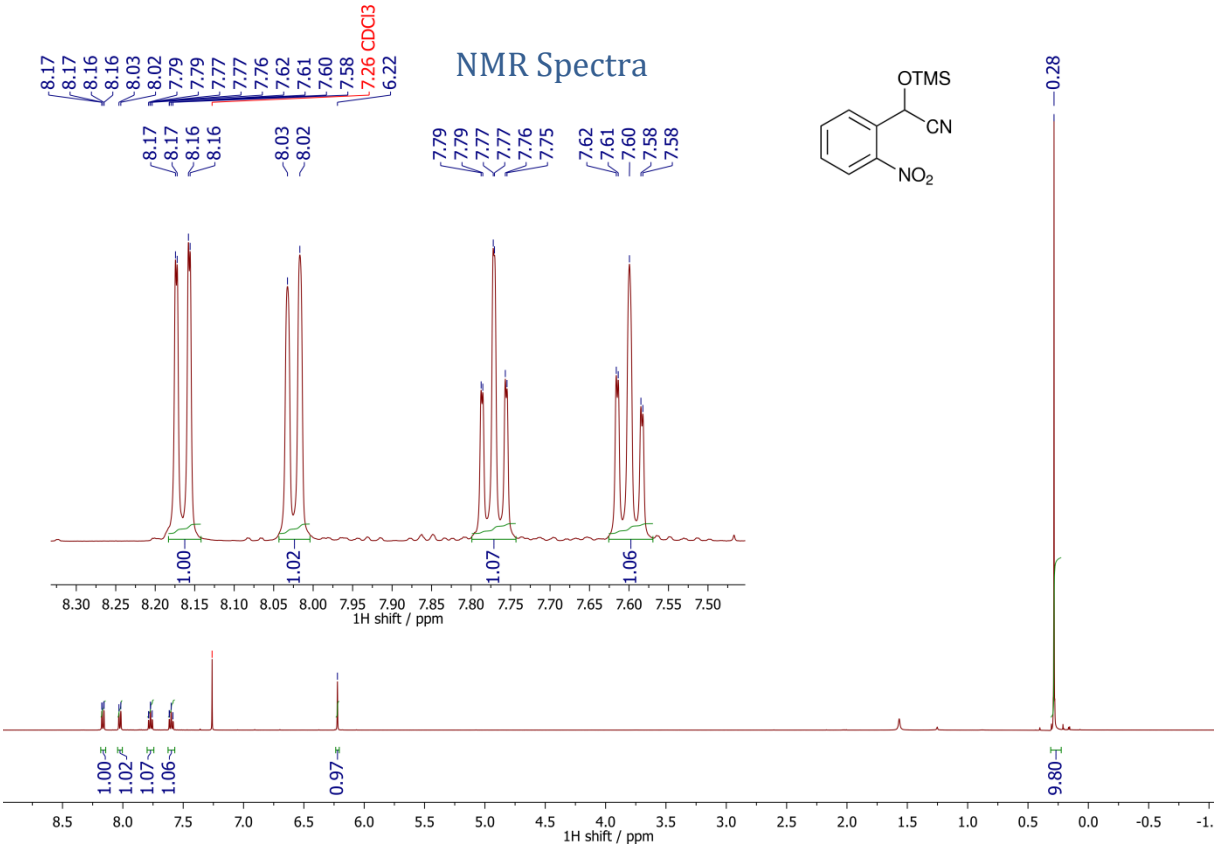

Figure S15: ^{1}H NMR spectrum of compound **3a** (500 MHz, $CDCl_3$)

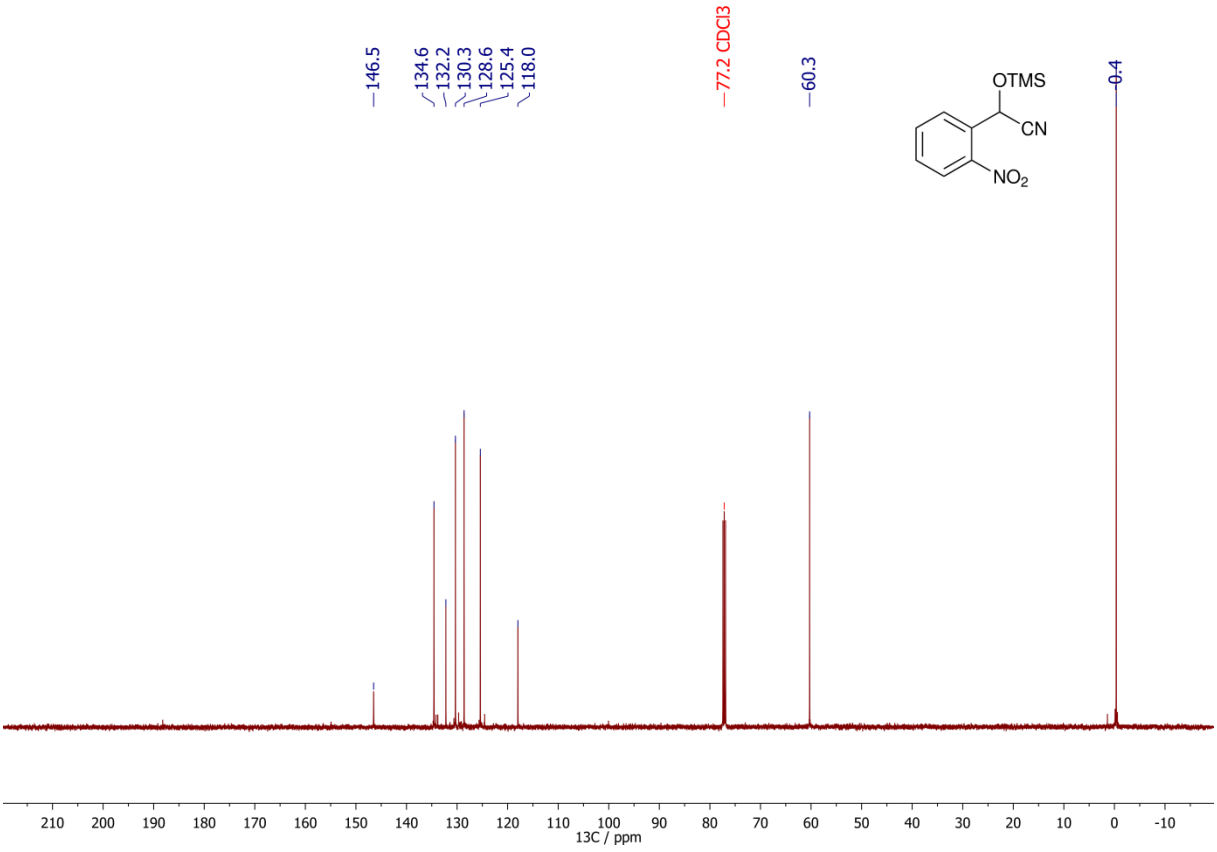

Figure S16: ^{13}C NMR spectrum of compound **3a** (125 MHz, $CDCl_3$)

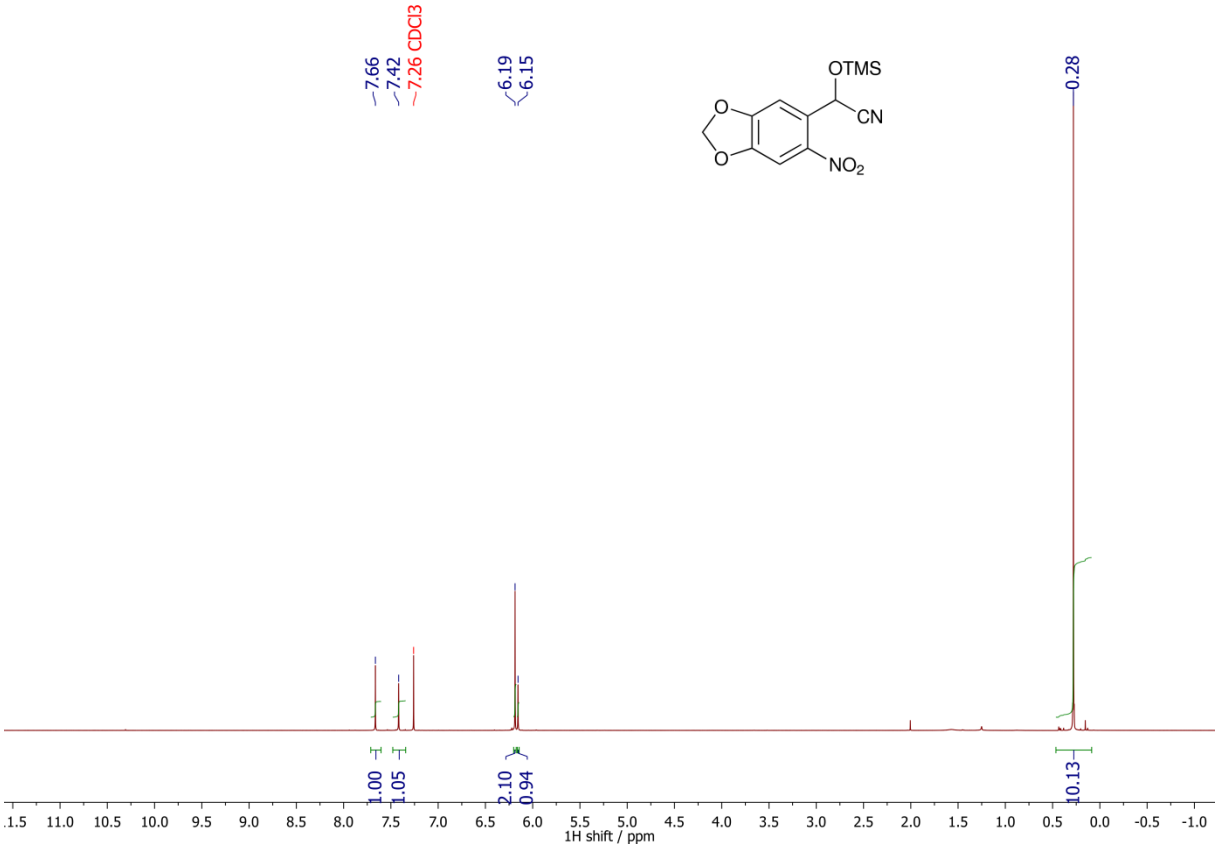

Figure S17: ^{1}H NMR spectrum of compound **3b** (400 MHz, $CDCl_3$)

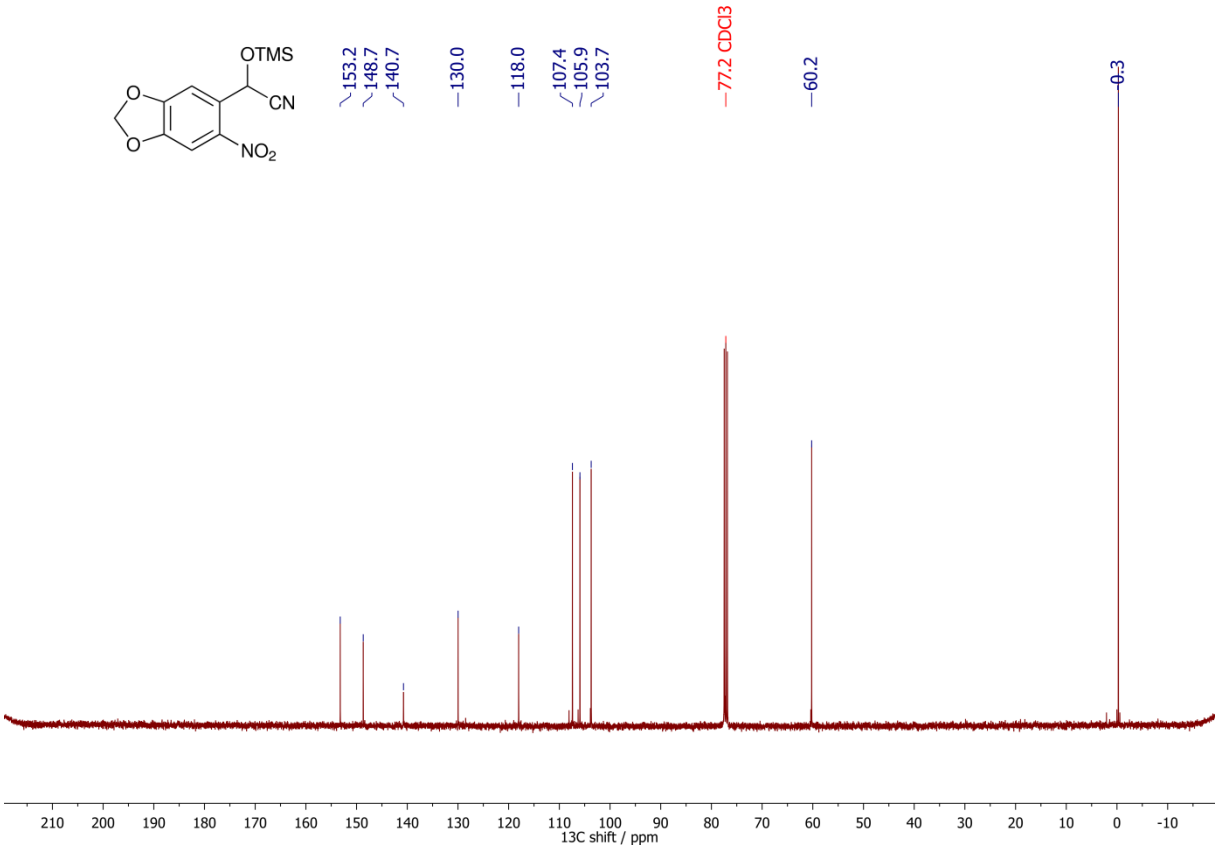

Figure S18: ^{13}C NMR spectrum of compound **3b** (100 MHz, $CDCl_3$)

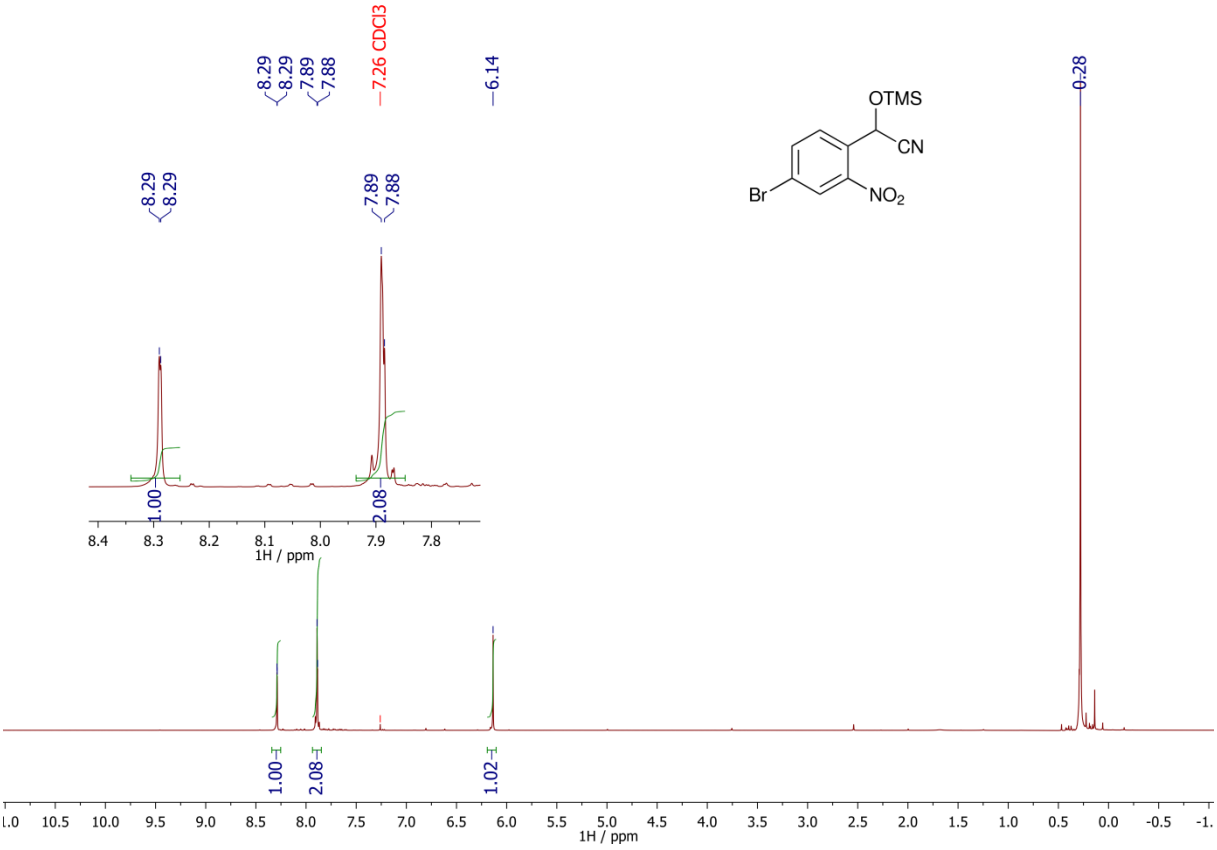

Figure S19: ^{1}H NMR spectrum of compound **3c** (500 MHz, $CDCl_3$)

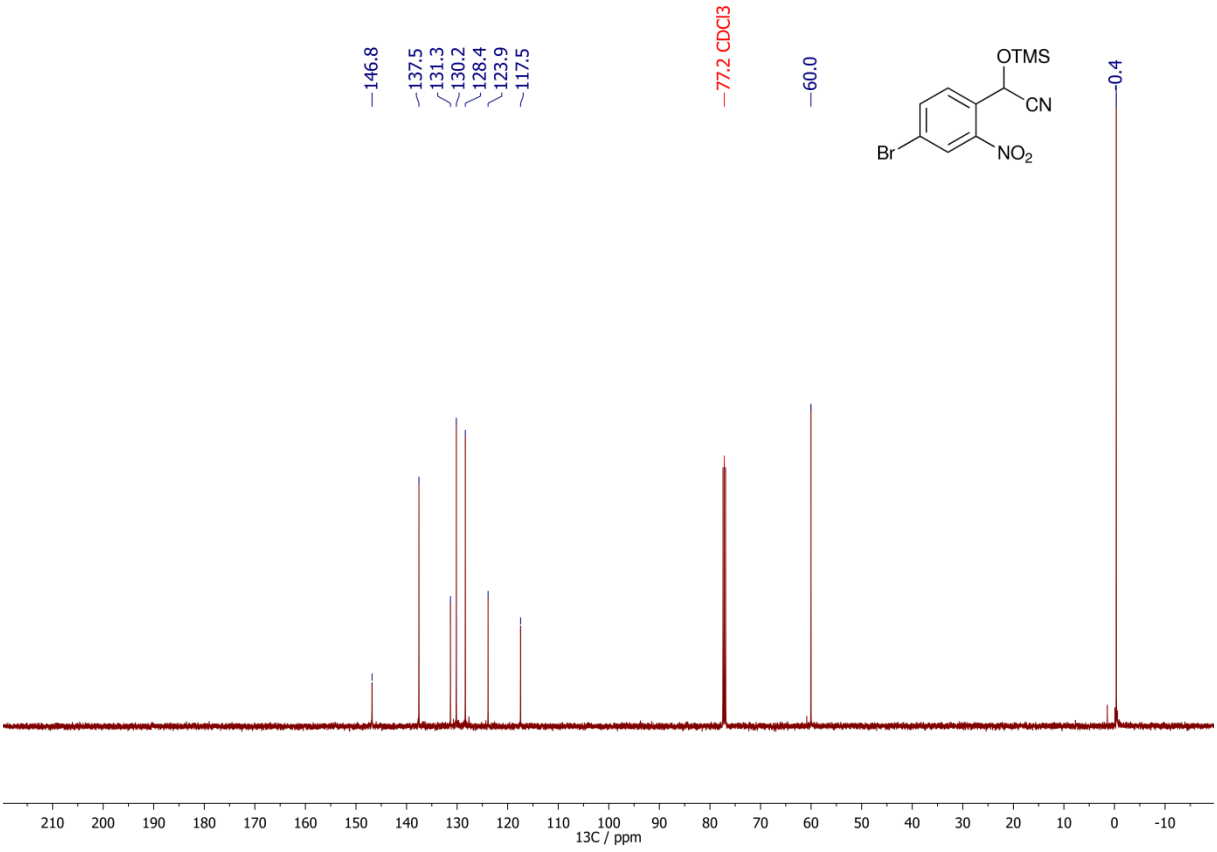

Figure S20: ^{13}C NMR spectrum of compound **3c** (125 MHz, $CDCl_3$)

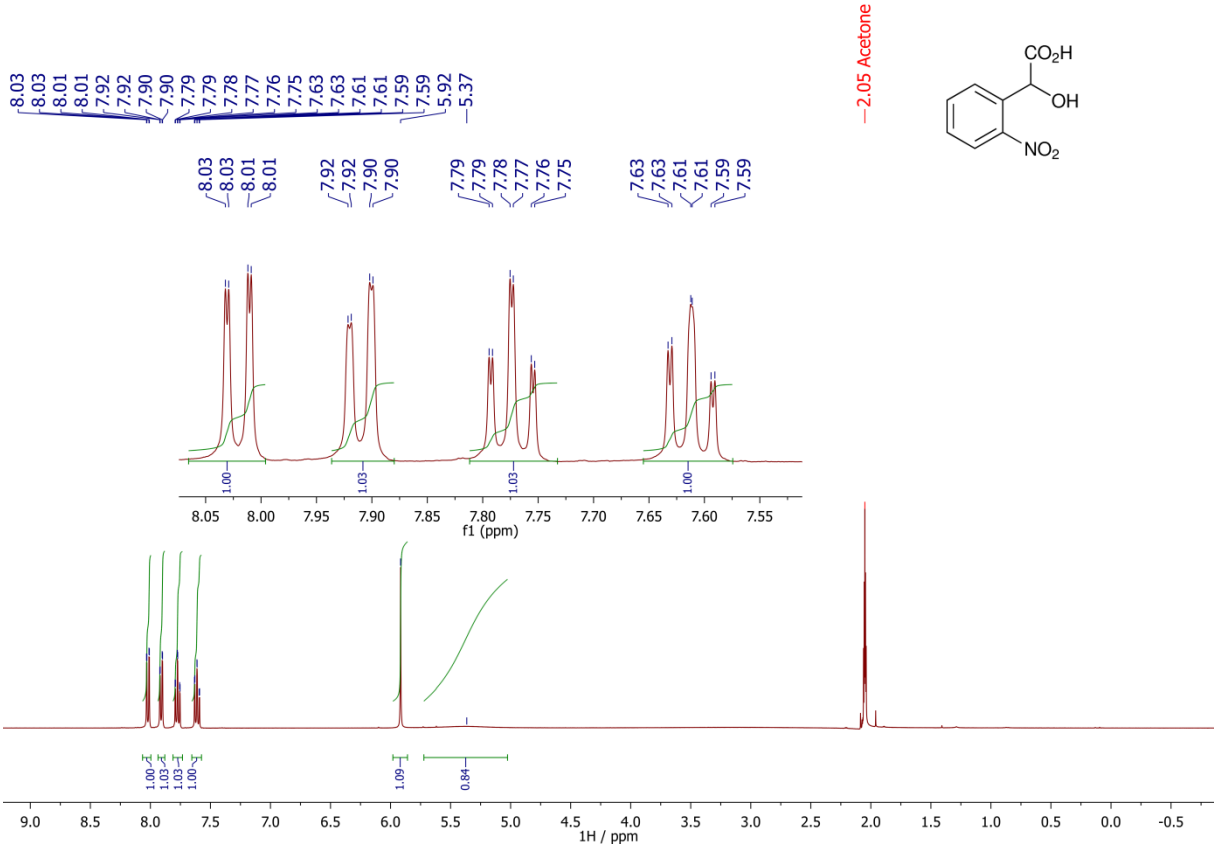

Figure S21: ^{1}H NMR spectrum of compound **4a** (400 MHz, acetone-d_6)

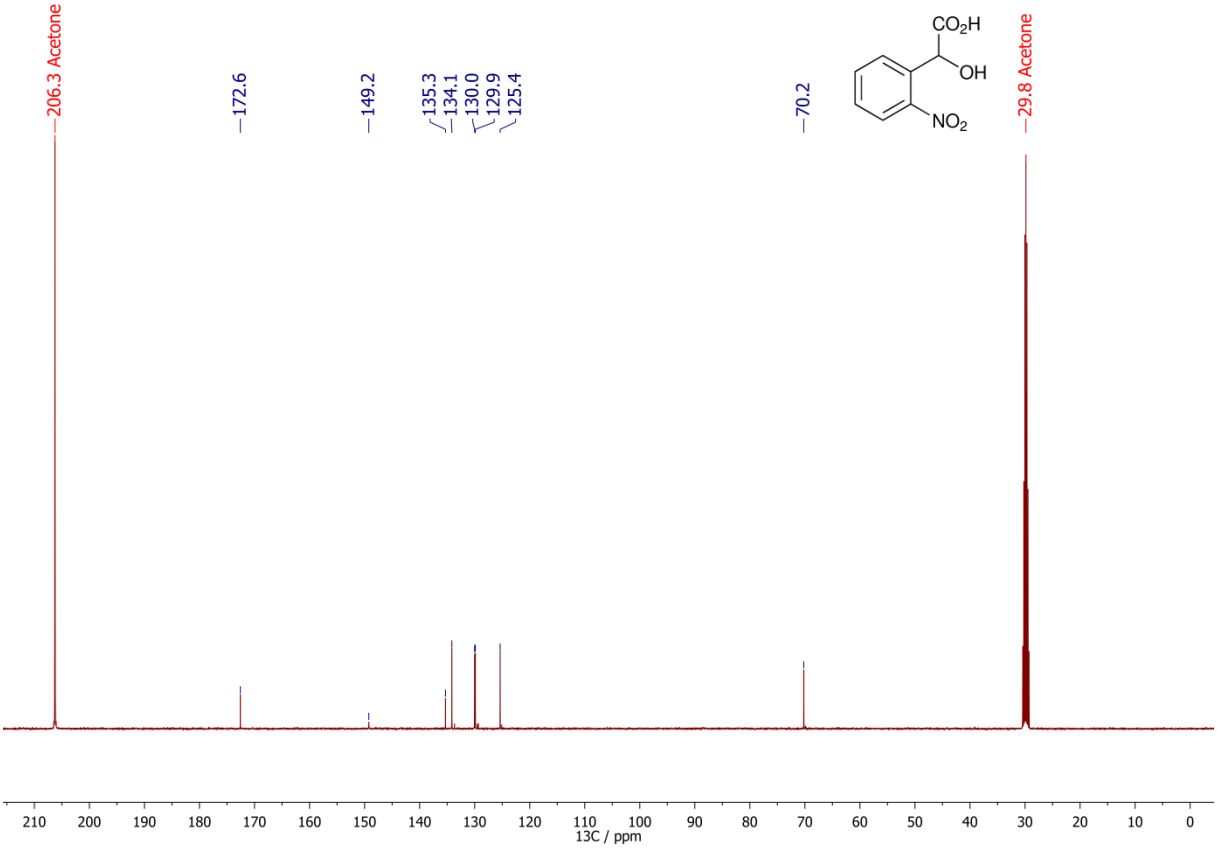

Figure S22: ^{13}C NMR spectrum of compound **4a** (100 MHz, acetone-d_6)

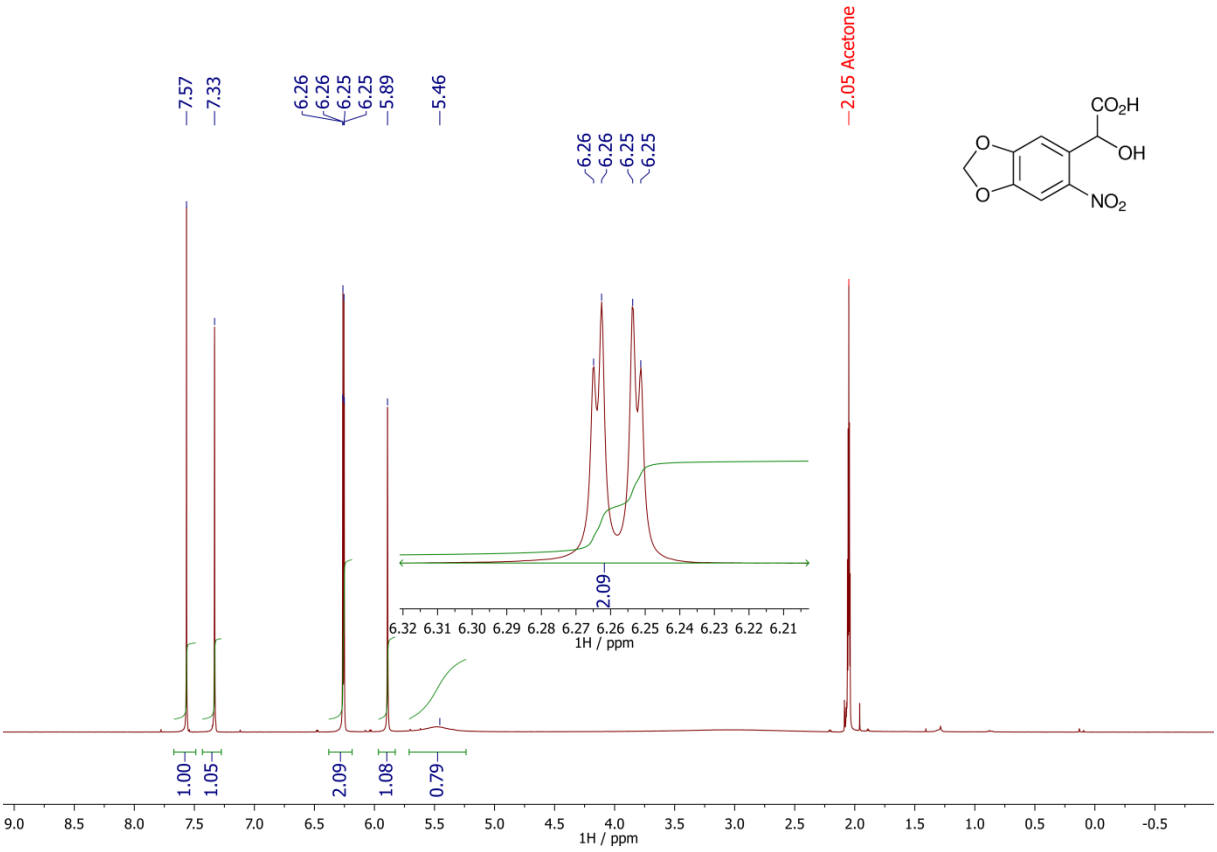

Figure S23: ^{1}H NMR spectrum of compound **4b** (400 MHz, acetone-d_6)

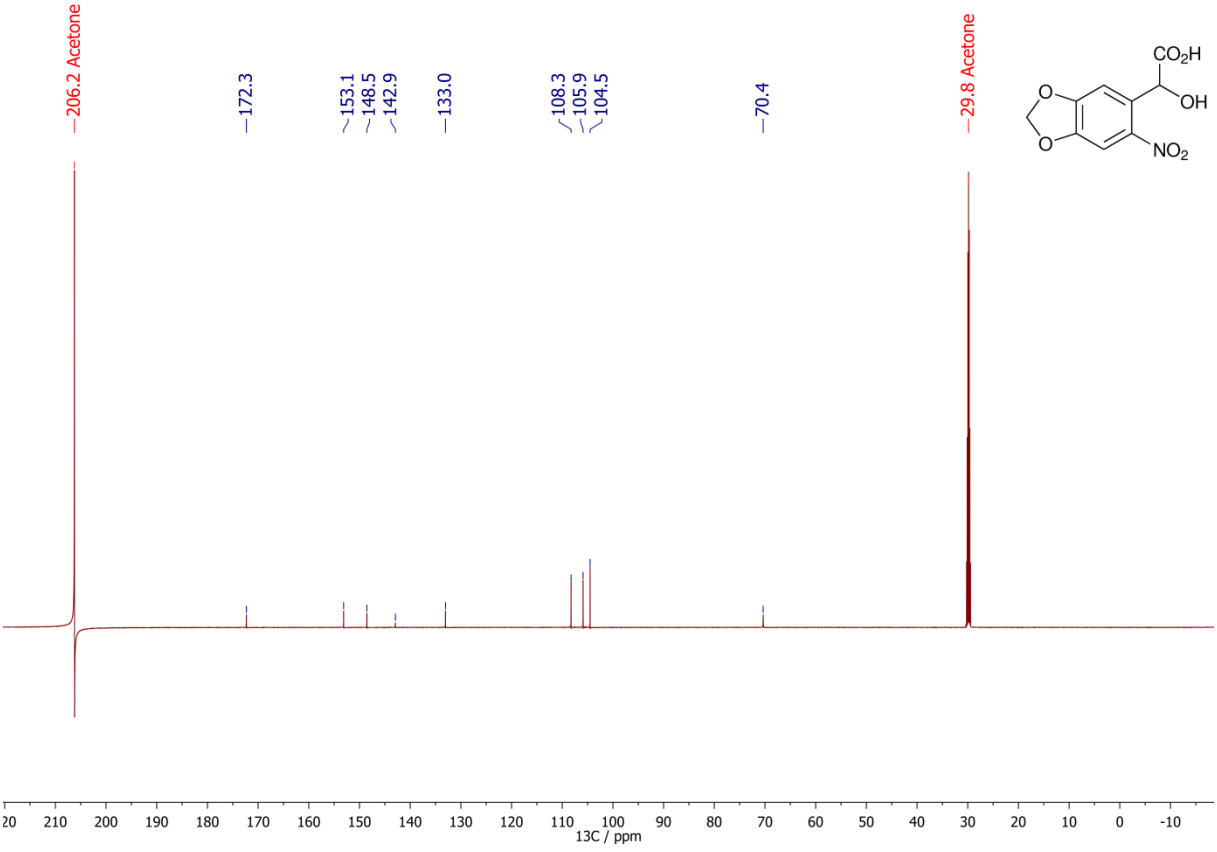

Figure S24: ^{13}C NMR spectrum of compound **4b** (150 MHz, acetone-d_6)

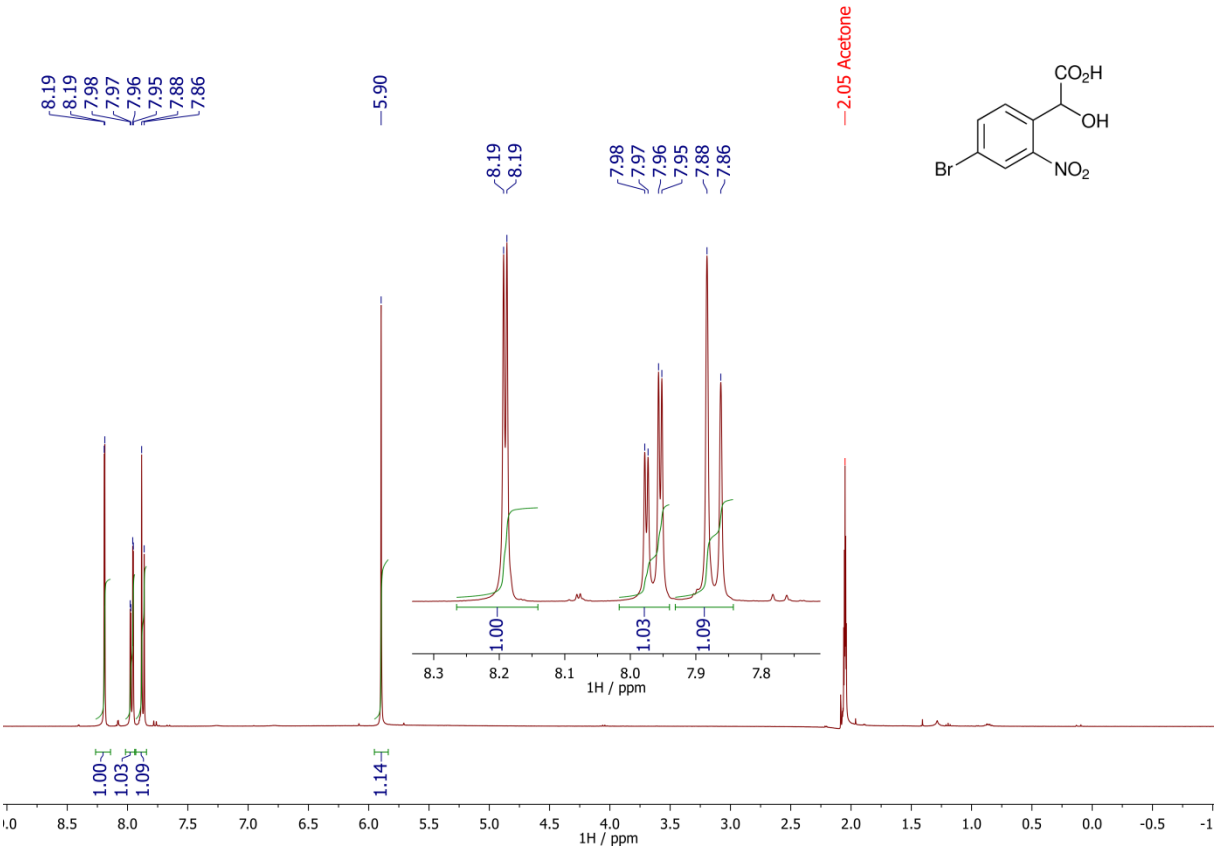

Figure S25: ^{1}H NMR spectrum of compound **4c** (400 MHz, acetone-d_6)

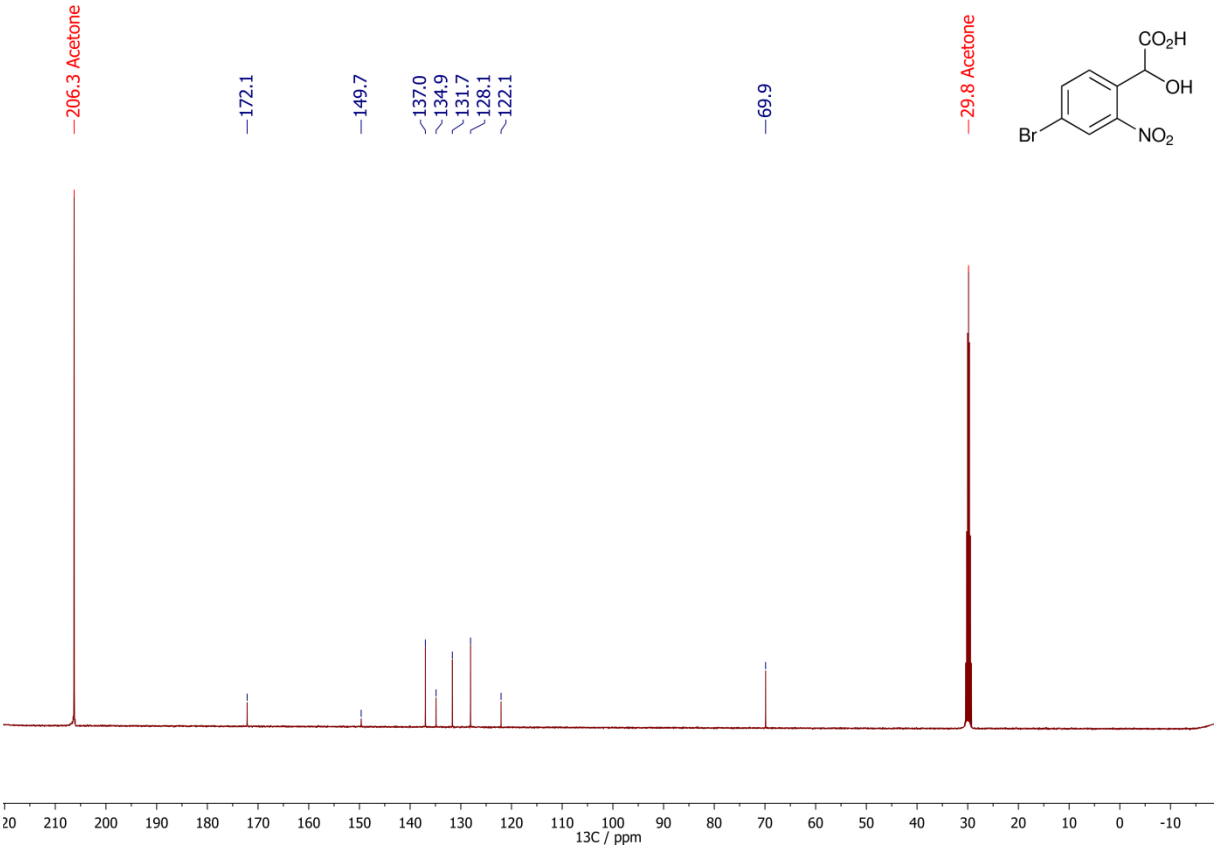

Figure S26: ^{13}C NMR spectrum of compound **4c** (100 MHz, acetone-d_6)

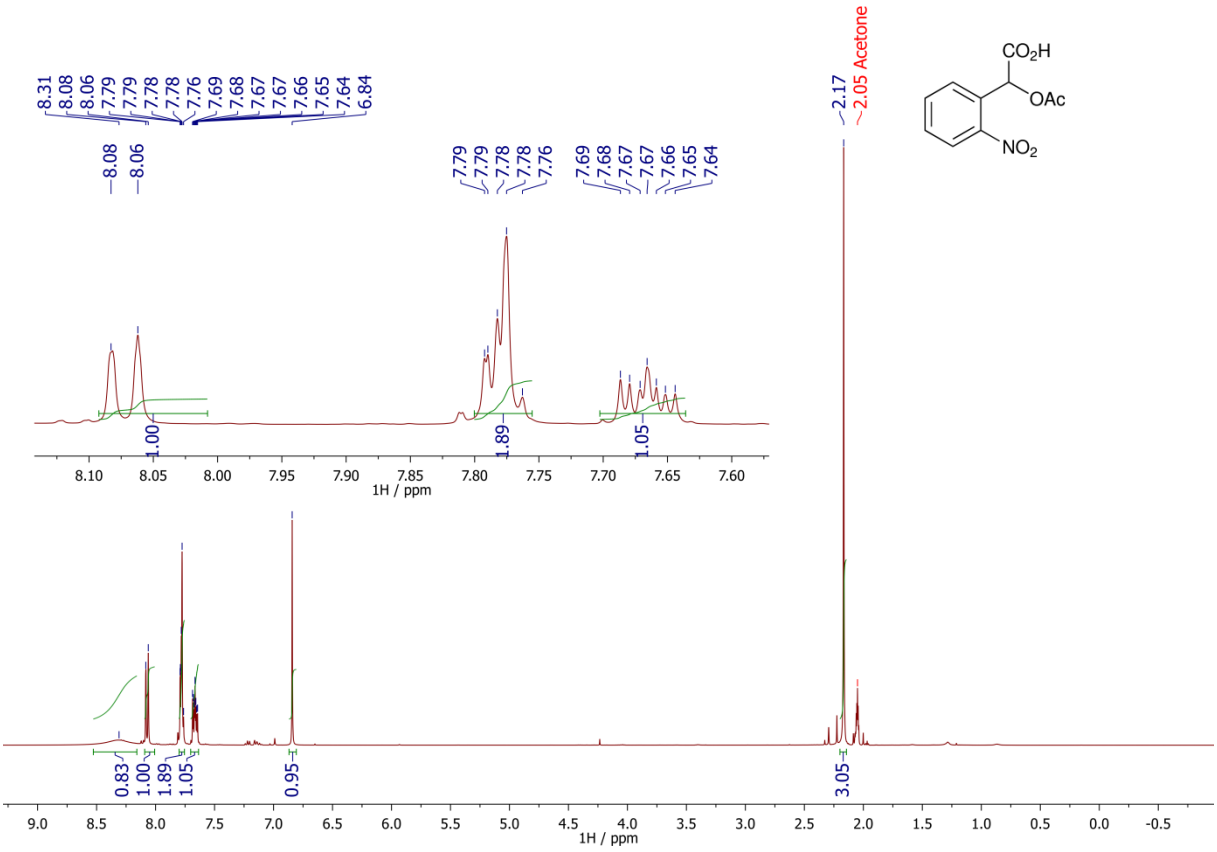

Figure S27: ^{1}H NMR spectrum of compound **5a** (400 MHz, DMSO-d_6)

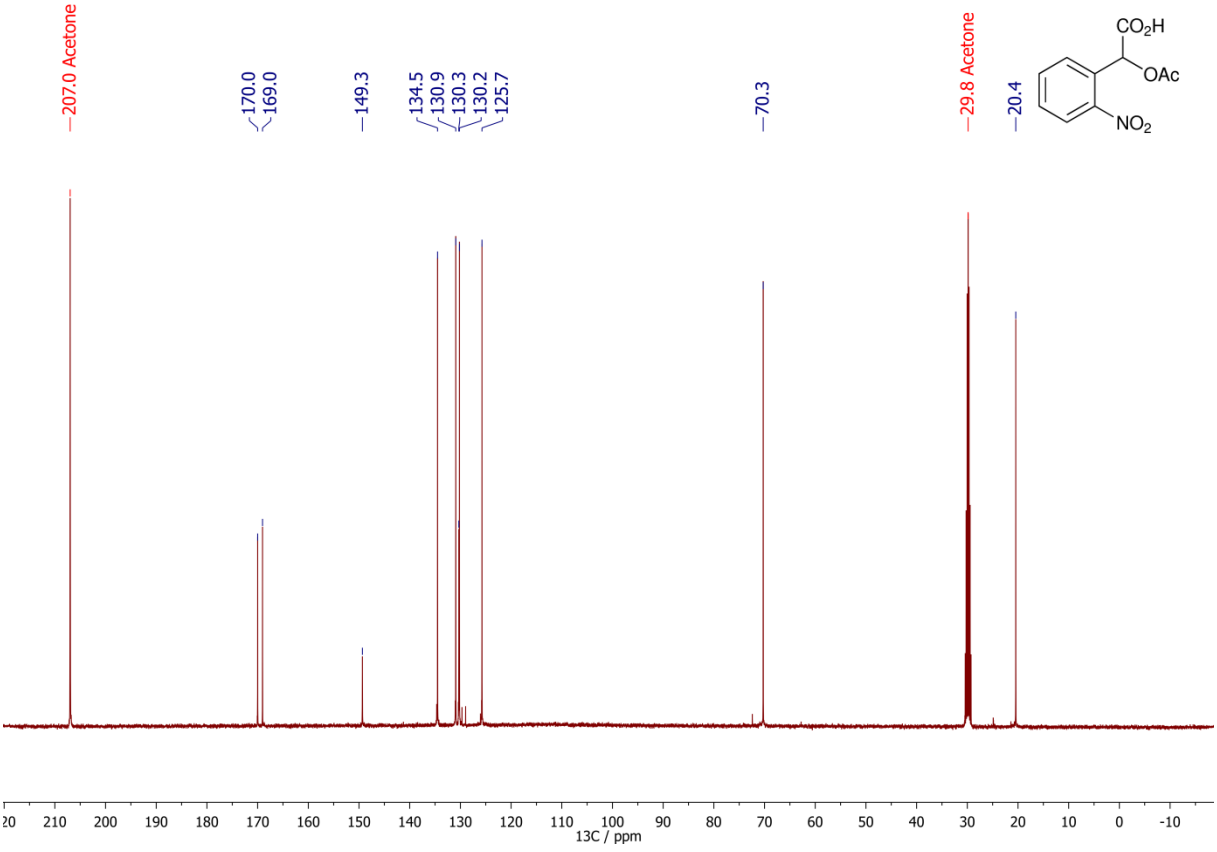

Figure S28: ^{13}C NMR spectrum of compound **5a** (100 MHz, acetone-d_6)

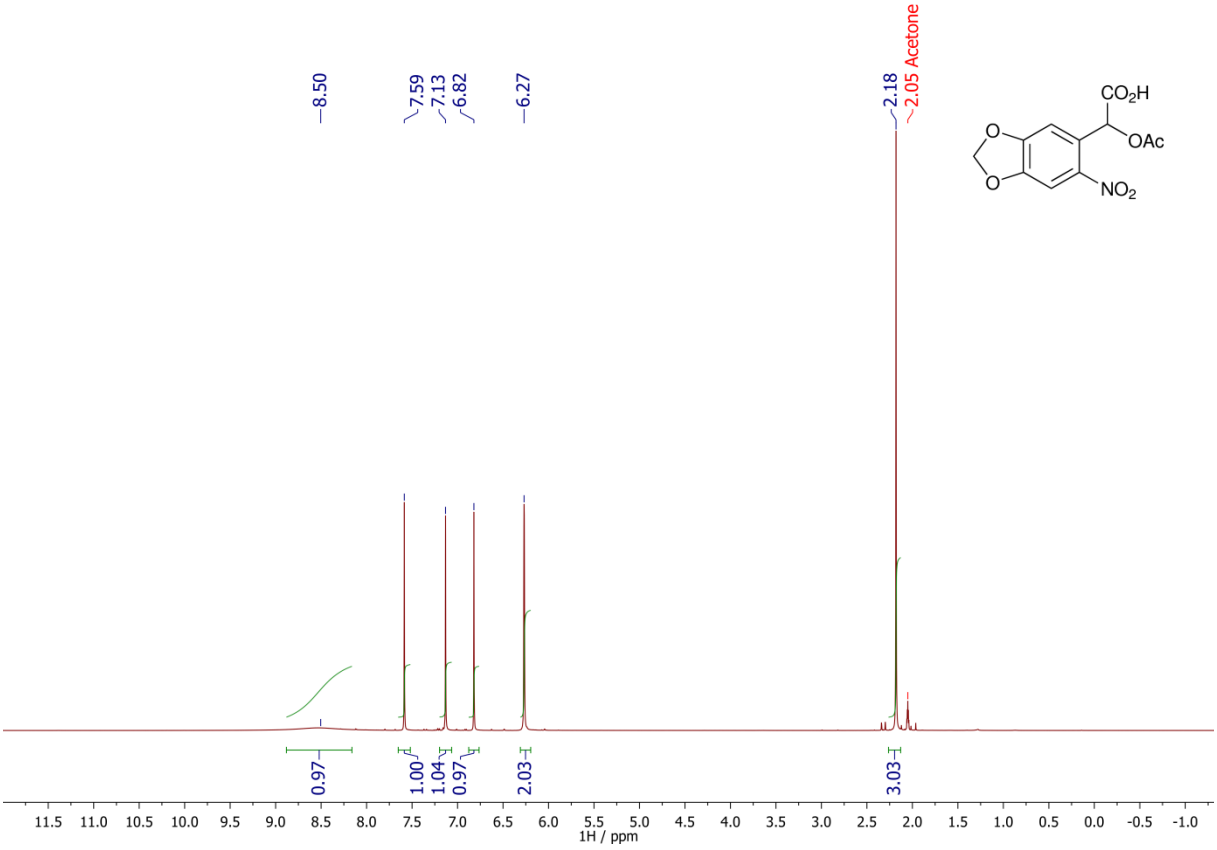

Figure S29: ^{1}H NMR spectrum of compound **5b** (400 MHz, acetone-d_6)

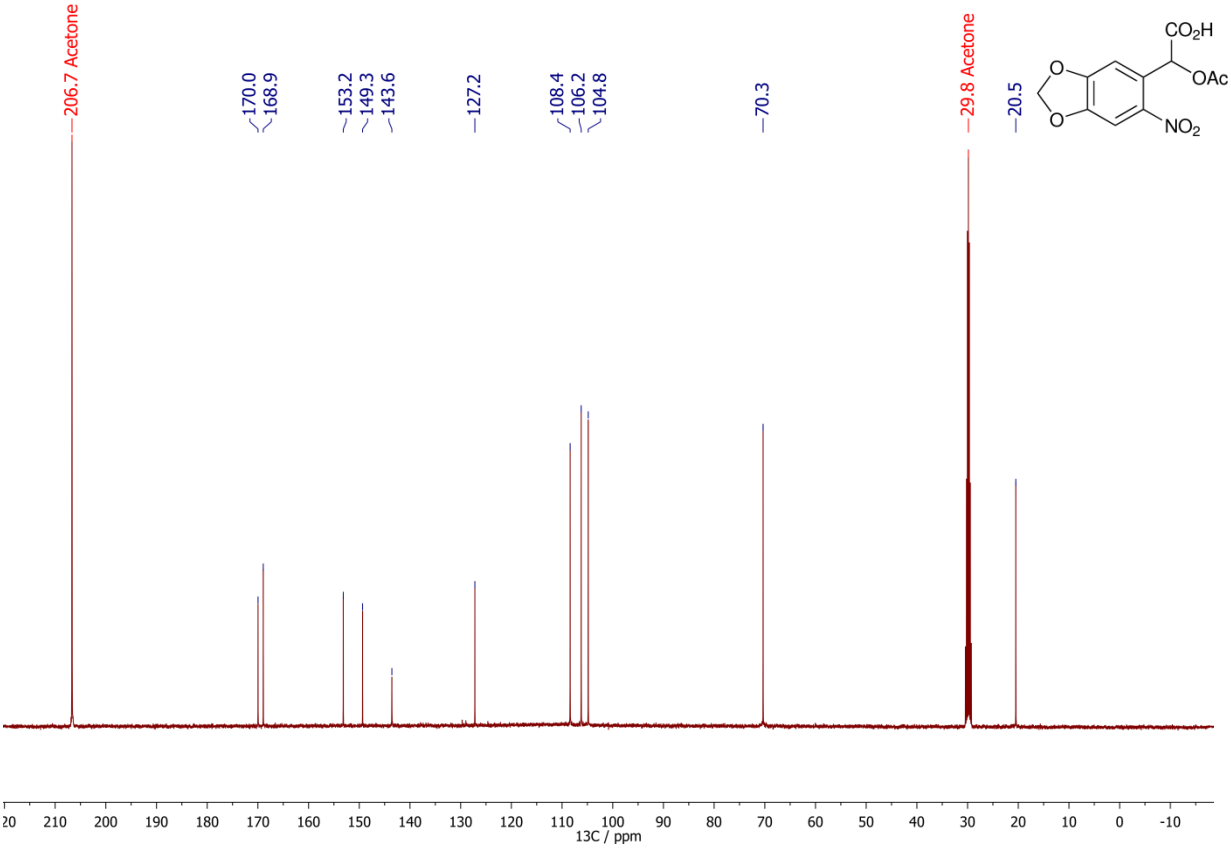

Figure S30: ^{13}C NMR spectrum of compound **5b** (100 MHz, acetone-d_6)

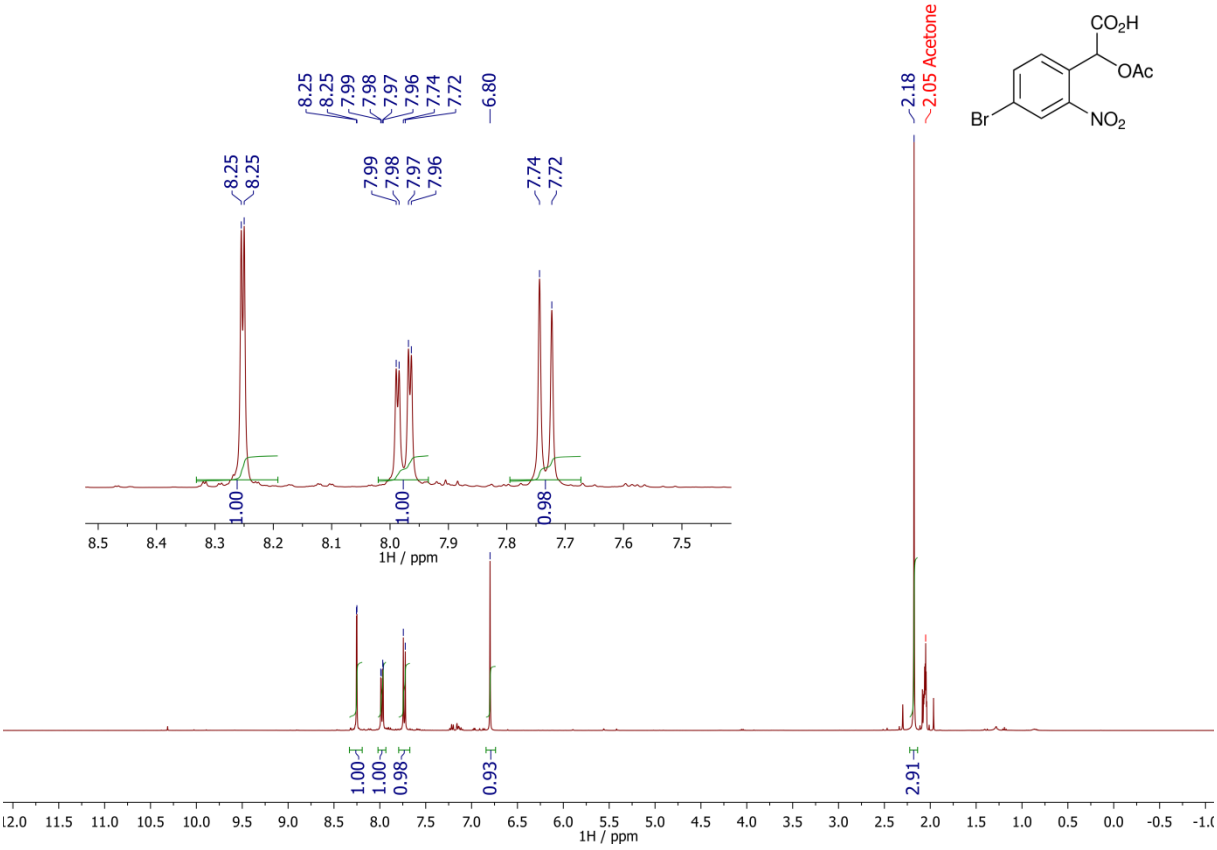

Figure S31: ^{1}H NMR spectrum of compound **5c** (400 MHz, acetone-d_6)

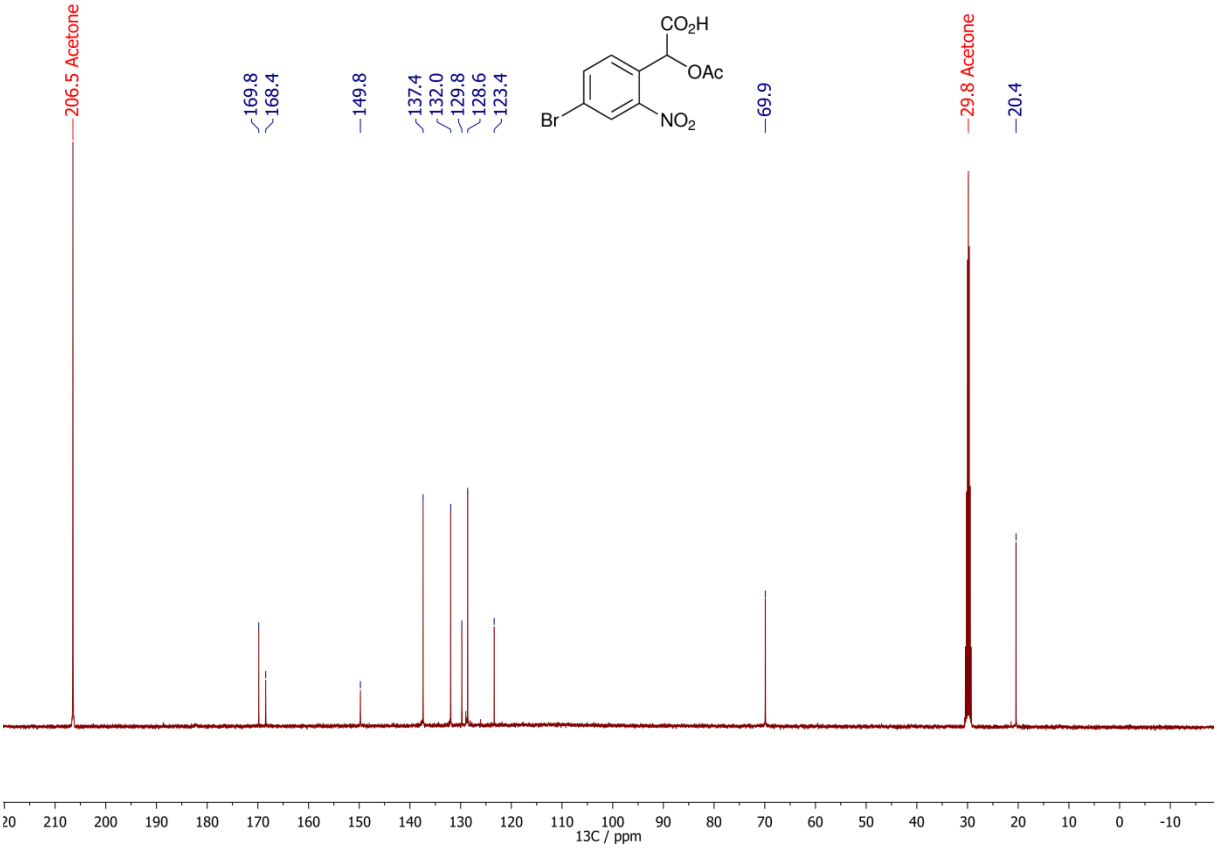

Figure S32: ^{13}C NMR spectrum of compound **5c** (100 MHz, acetone-d_6)

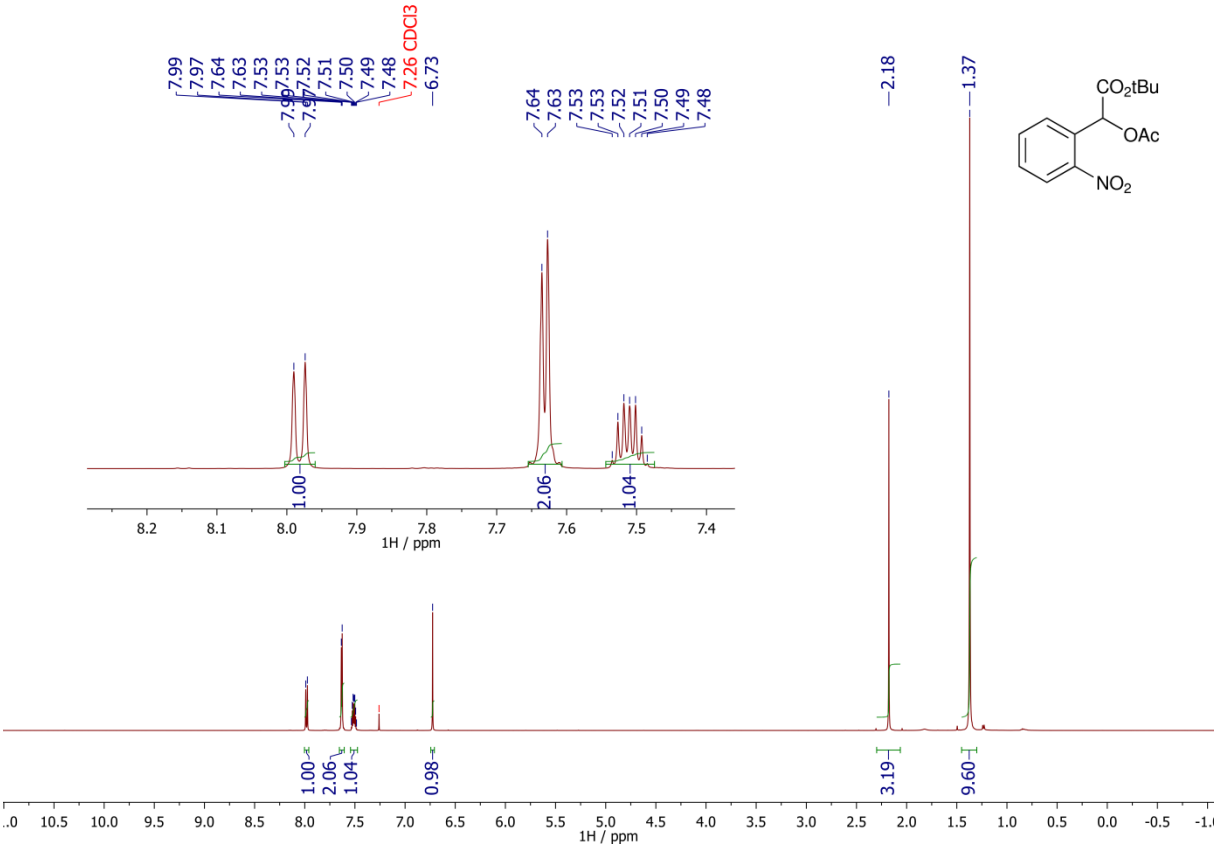

Figure S33: ^{1}H NMR spectrum of compound **6a** (500 MHz, $CDCl_3$)

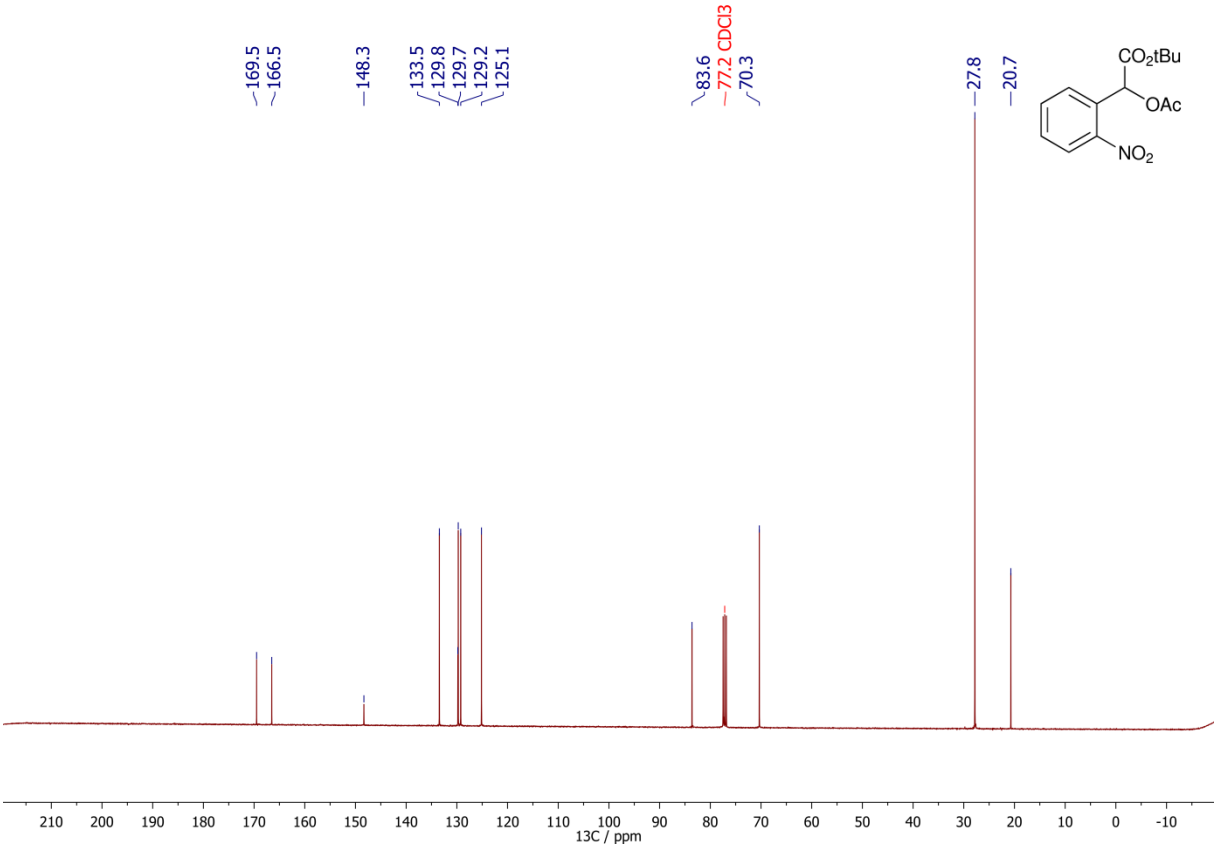

Figure S34: ^{13}C NMR spectrum of compound **6a** (100 MHz, $CDCl_3$)

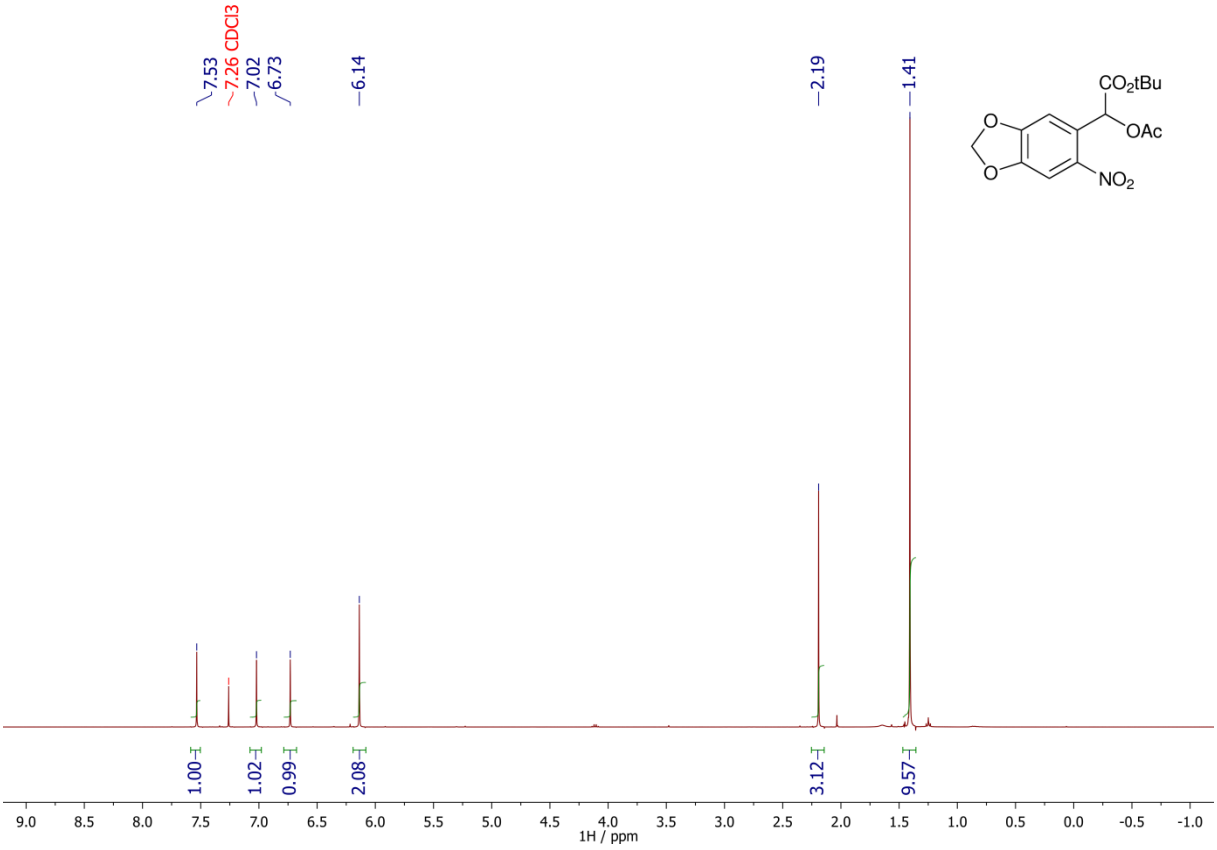

Figure S35: ^{1}H NMR spectrum of compound **6b** (400 MHz, $CDCl_3$)

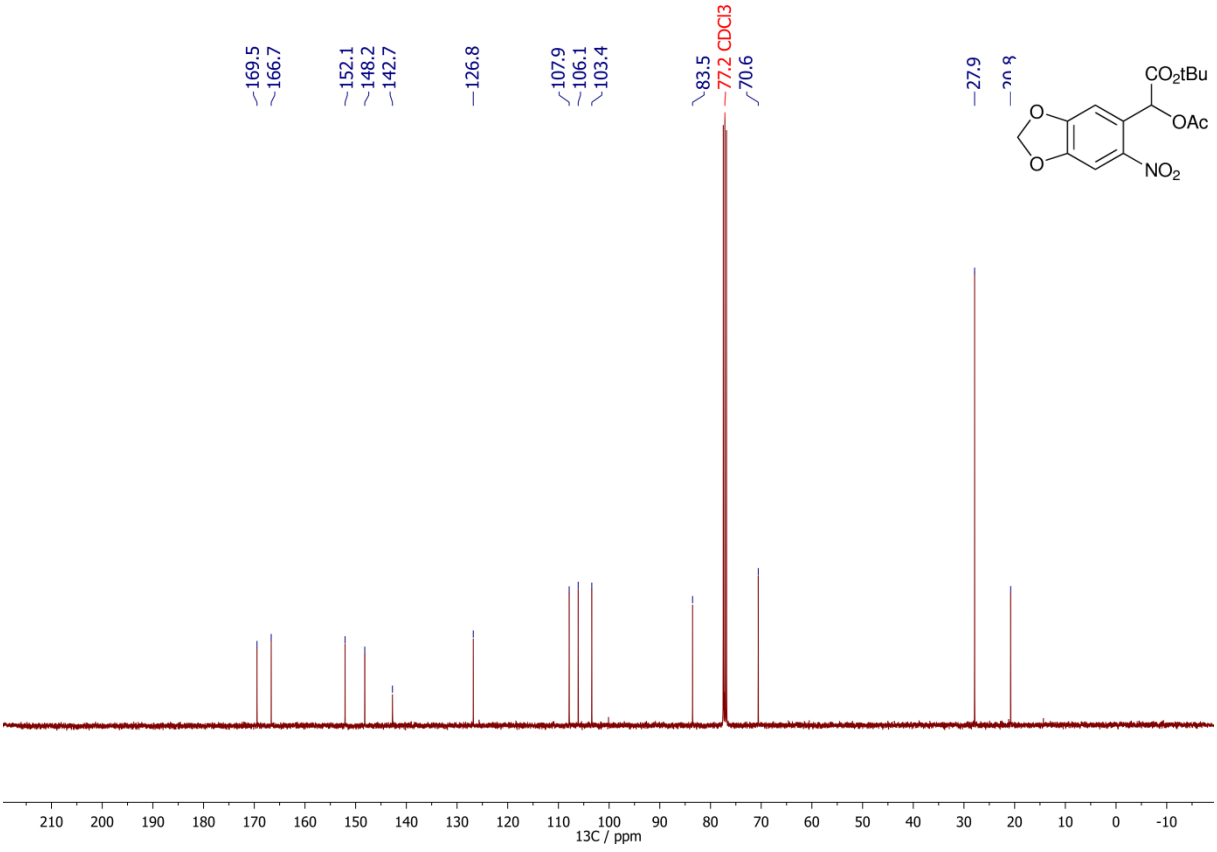

Figure S36: ^{13}C NMR spectrum of compound **6b** (100 MHz, $CDCl_3$)

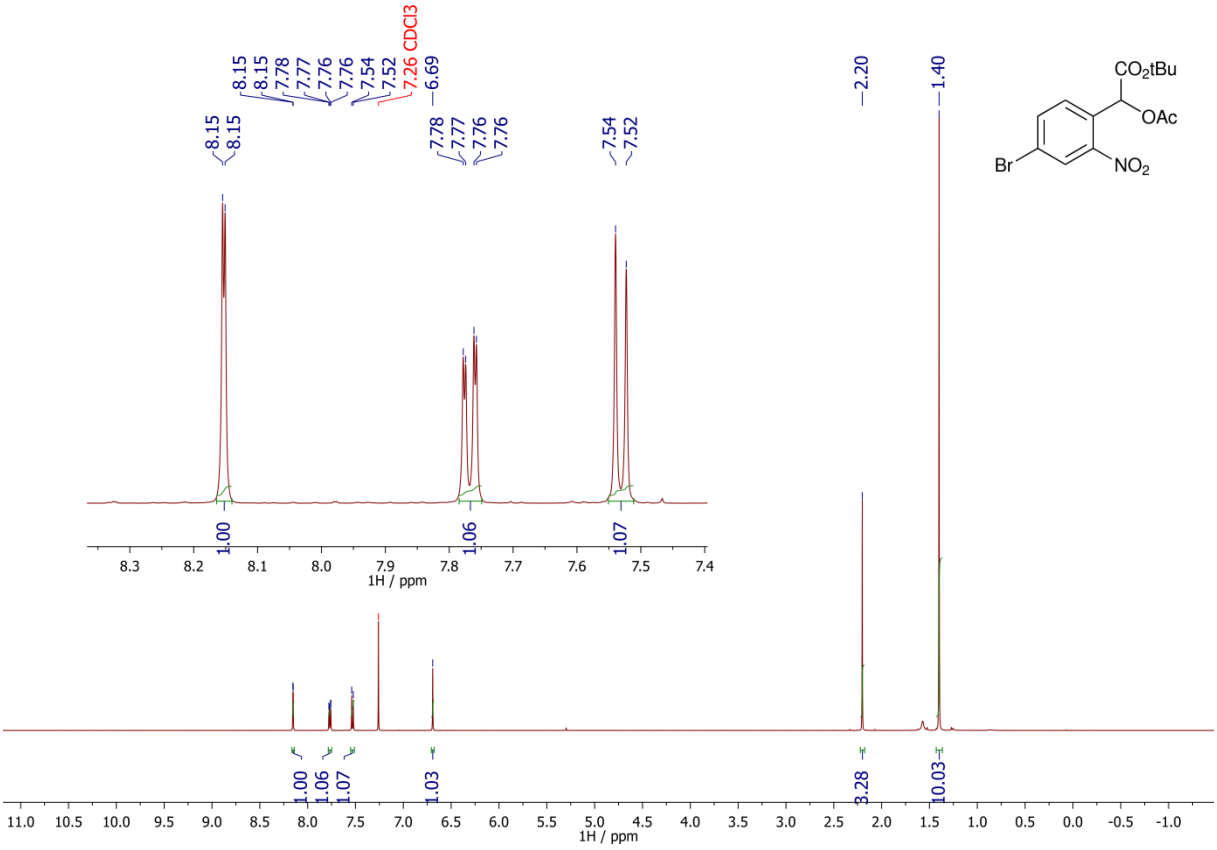

Figure S37: ^{1}H NMR spectrum of compound **6c** (500 MHz, $CDCl_3$)

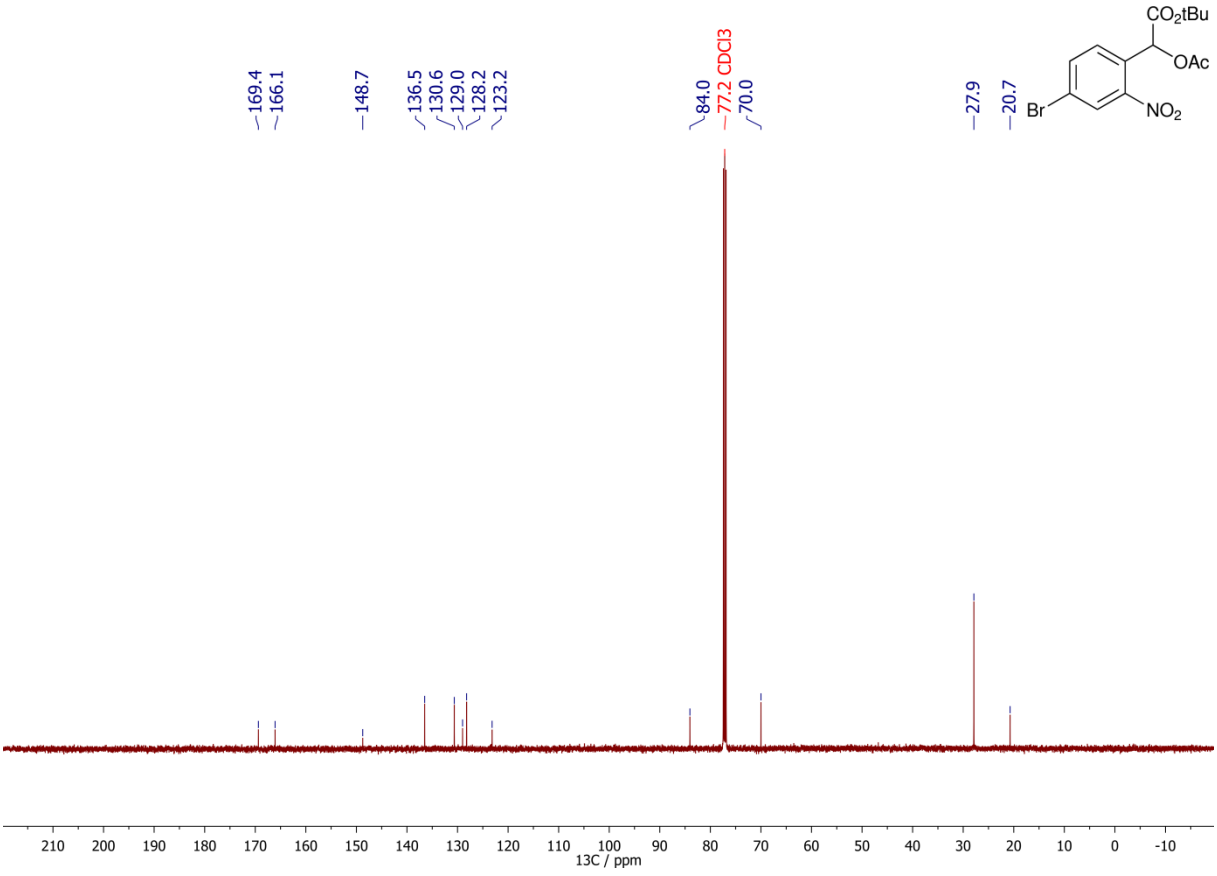

Figure S38: ^{13}C NMR spectrum of compound **6c** (125 MHz, $CDCl_3$)

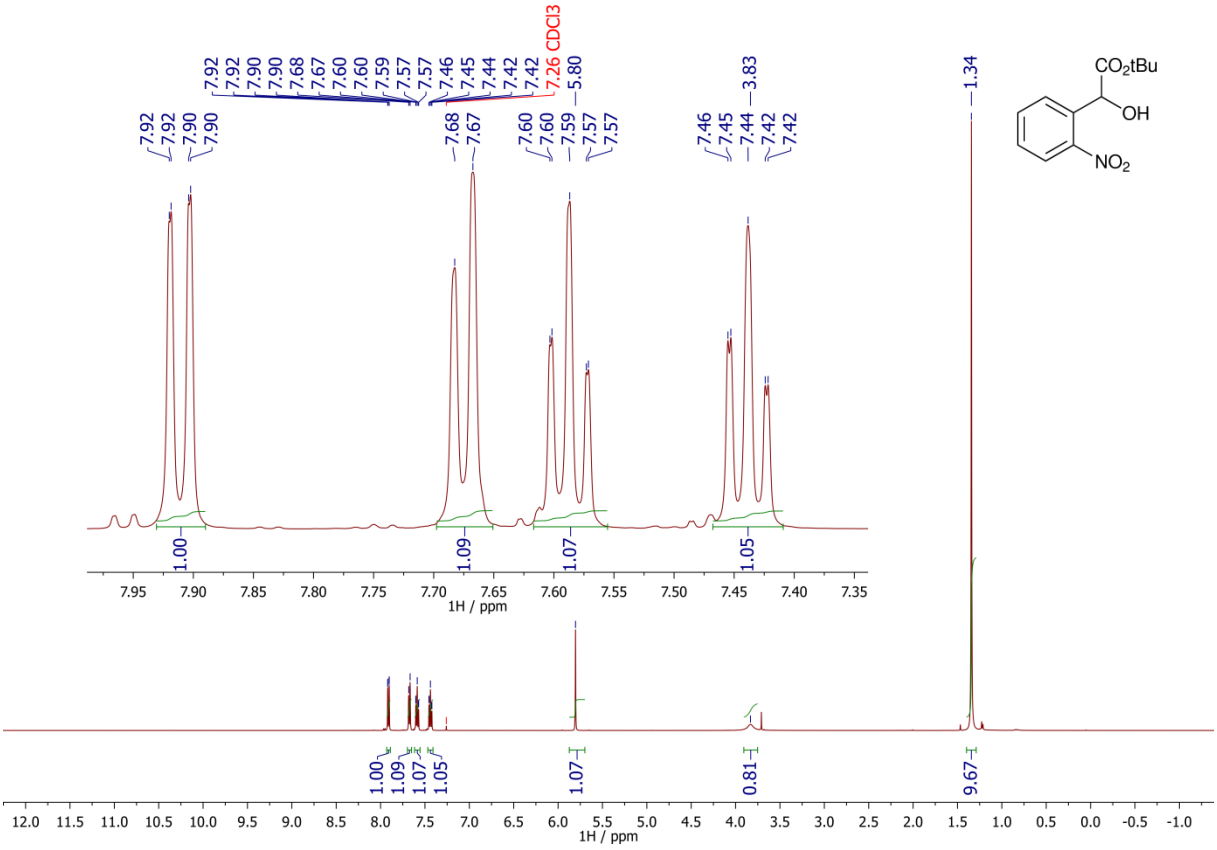

Figure S39: ^{1}H NMR spectrum of compound **7a** (500 MHz, $CDCl_3$)

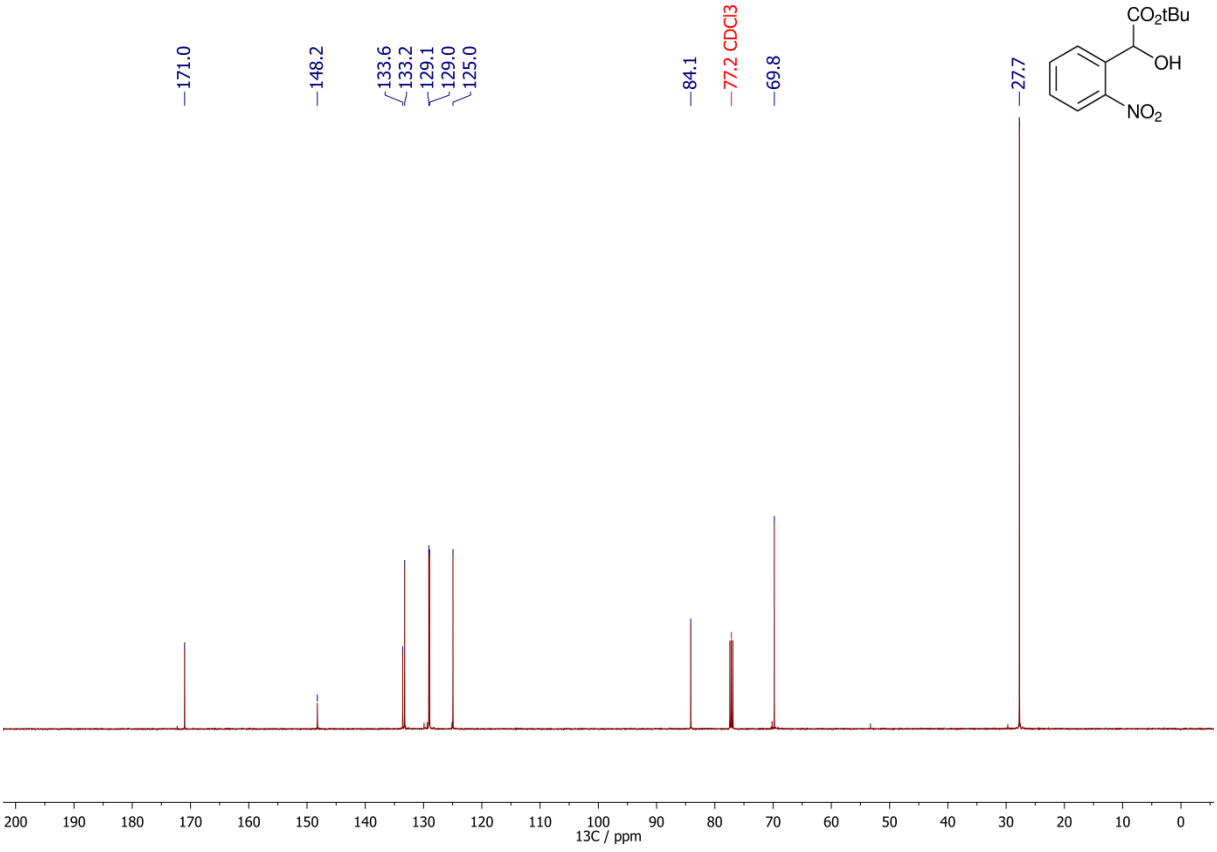

Figure S40: ^{13}C NMR spectrum of compound **7a** (125 MHz, $CDCl_3$)

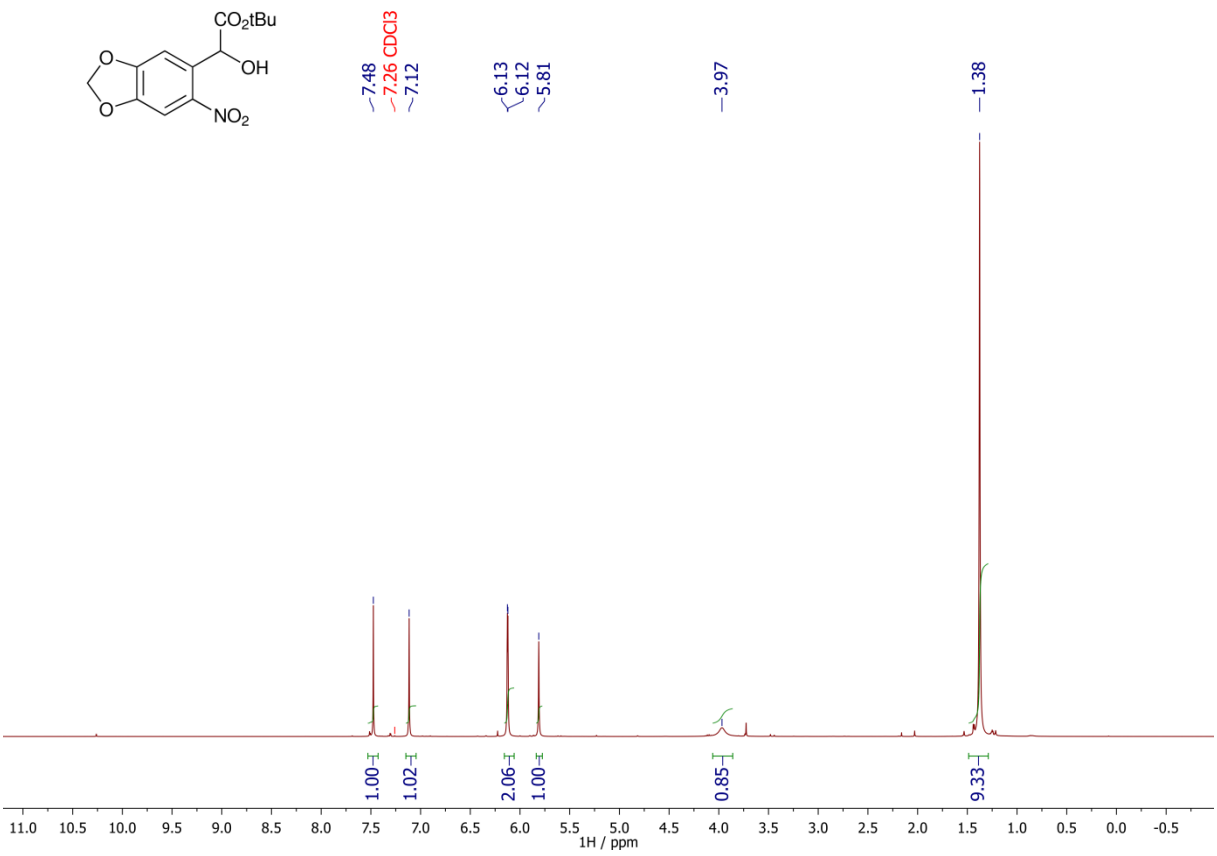

Figure S41: ^{1}H NMR spectrum of compound **7b** (400 MHz, $CDCl_3$)

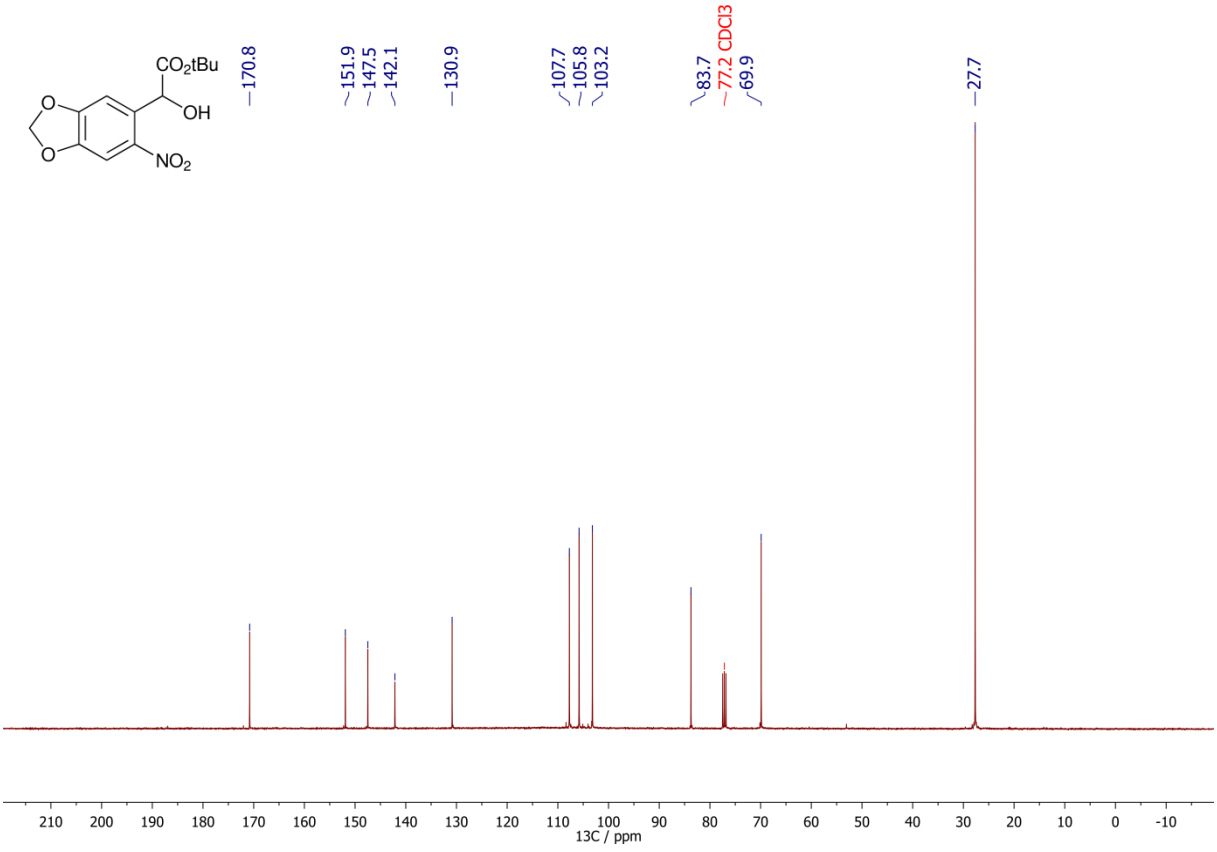

Figure S42: ^{13}C NMR spectrum of compound **7b** (100 MHz, $CDCl_3$)

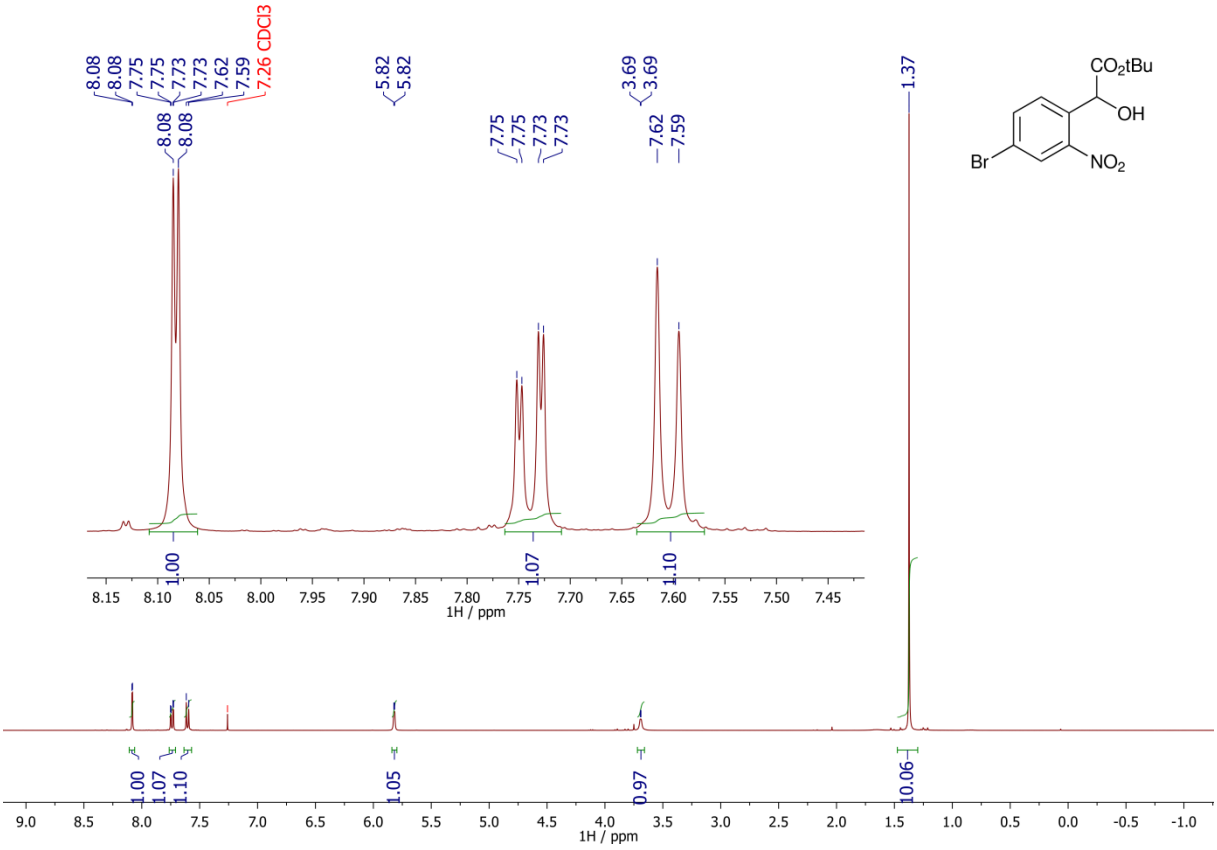

Figure S43: ^{1}H NMR spectrum of compound **7c** (400 MHz, $CDCl_3$)

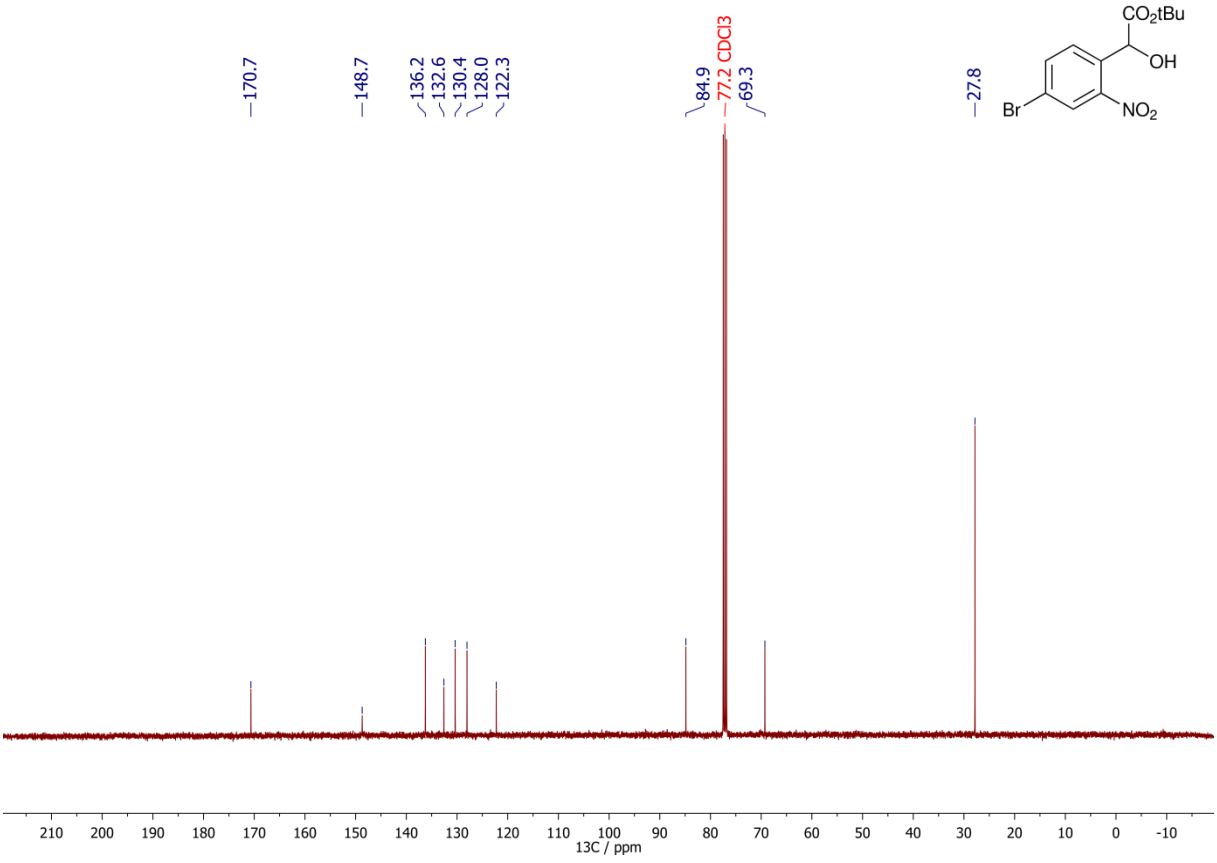

Figure S44: ^{13}C NMR spectrum of compound **7c** (100 MHz, $CDCl_3$)

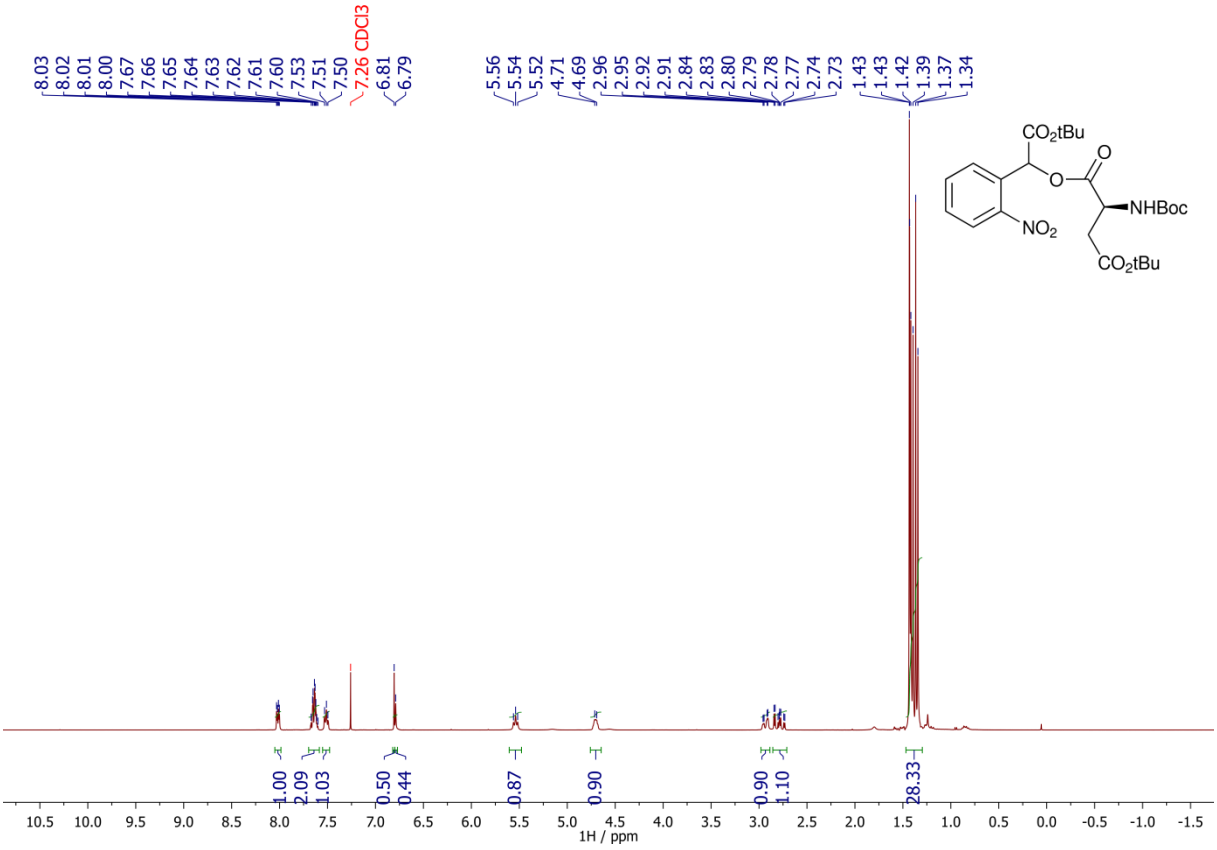

Figure S45: ^{1}H NMR spectrum of compound **8a** (400 MHz, $CDCl_3$)

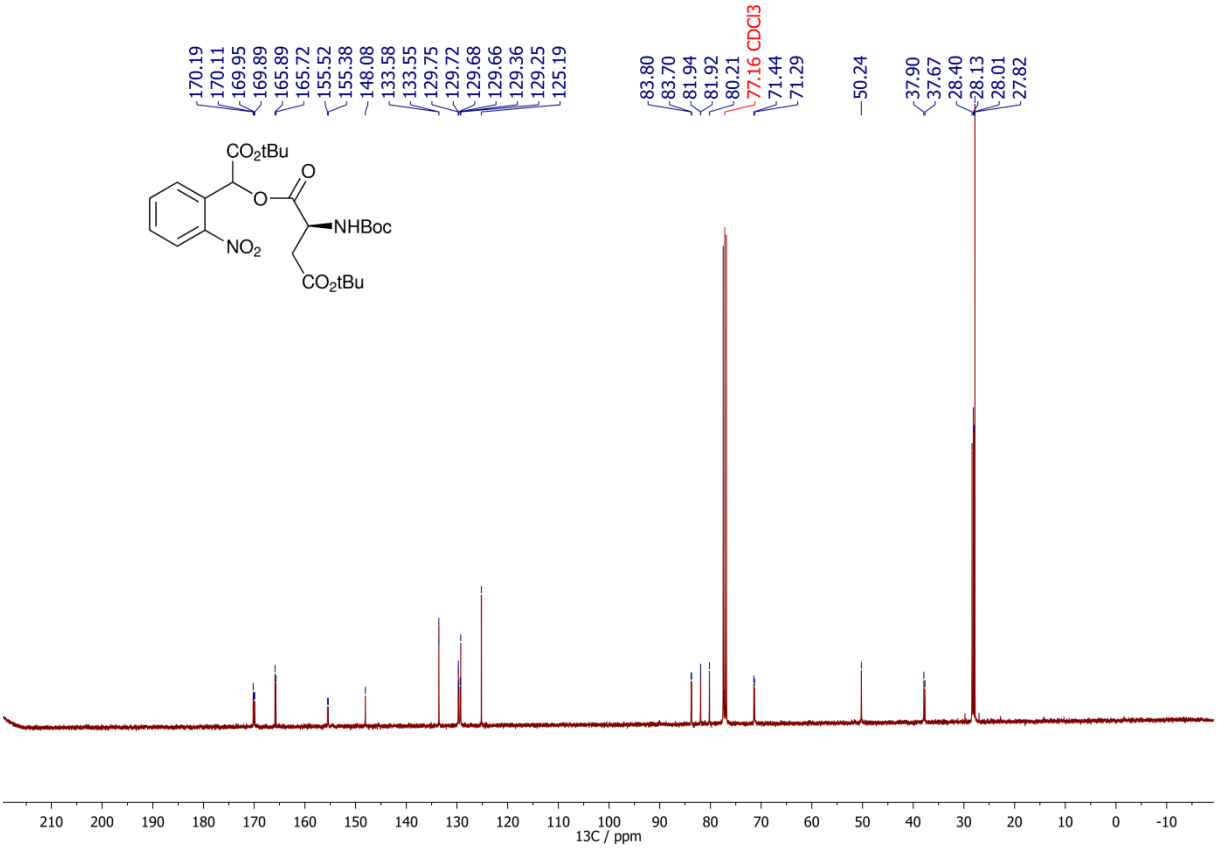

Figure S46: ^{13}C NMR spectrum of compound **8a** (100 MHz, $CDCl_3$)

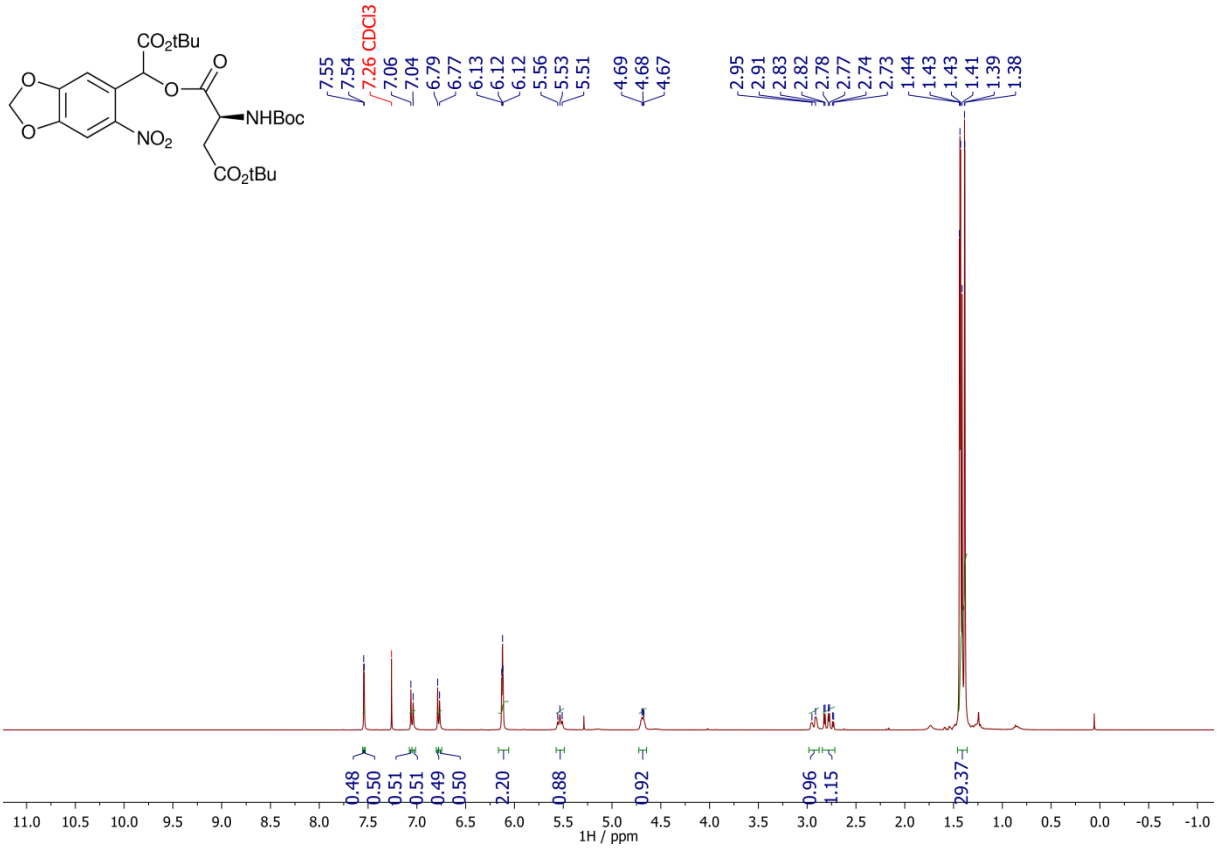

Figure S47: ^{1}H NMR spectrum of compound **8b** (400 MHz, $CDCl_3$)

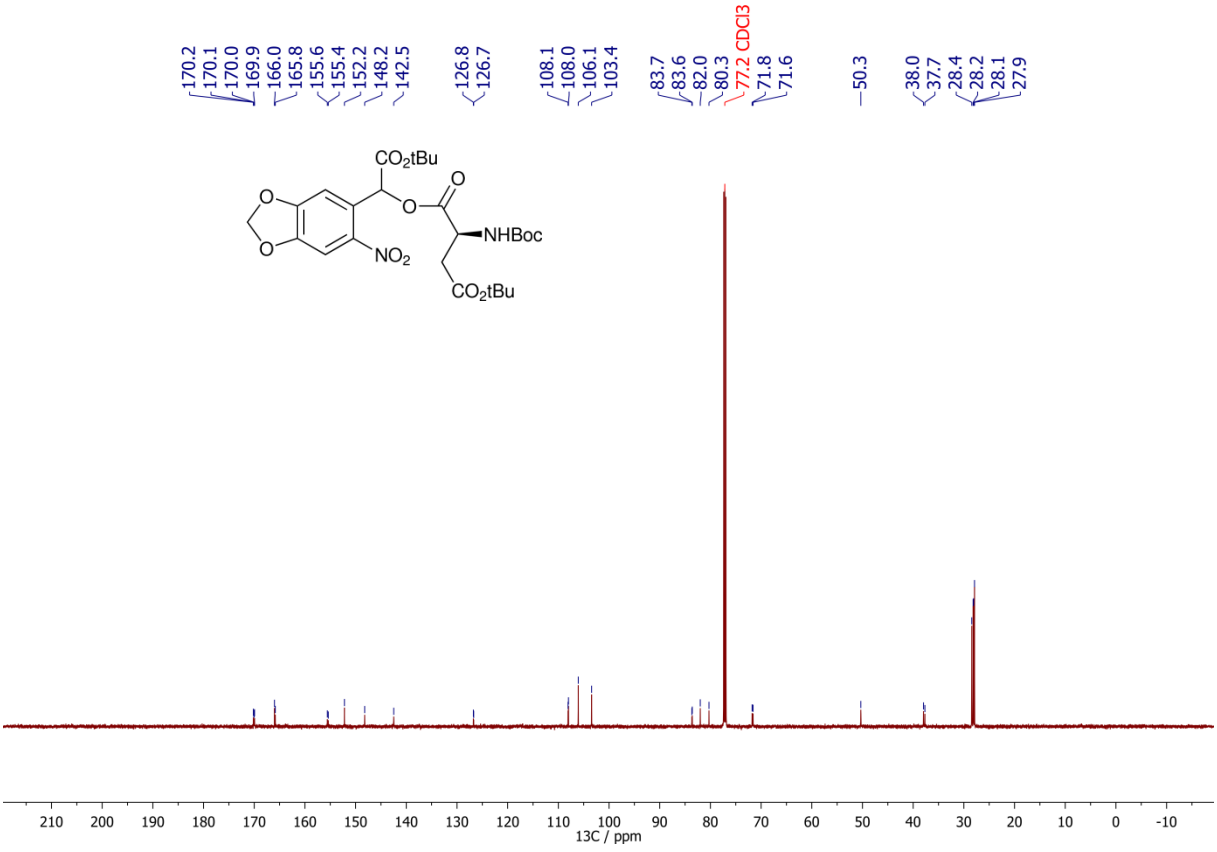

Figure S48: ^{13}C NMR spectrum of compound **8b** (150 MHz, $CDCl_3$)

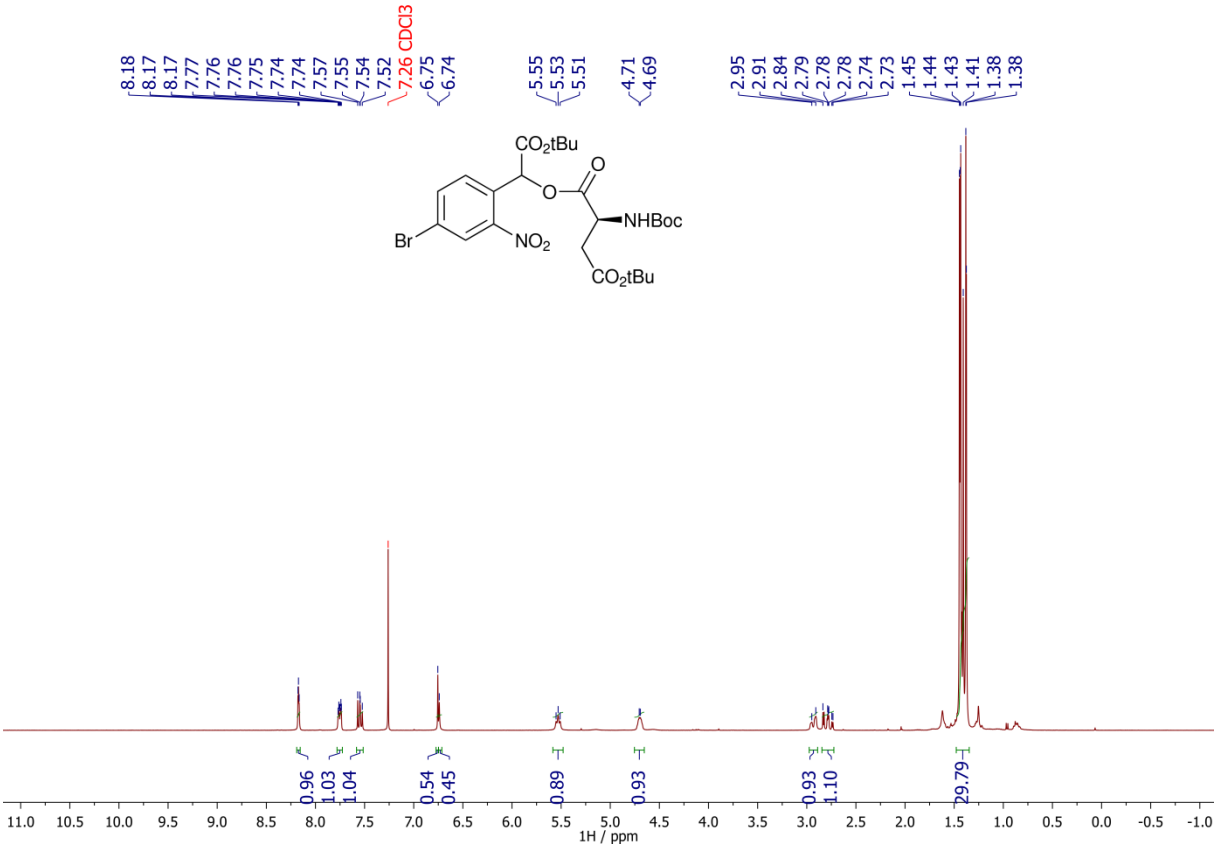

Figure S49: ^{1}H NMR spectrum of compound **8c** (400 MHz, $CDCl_3$)

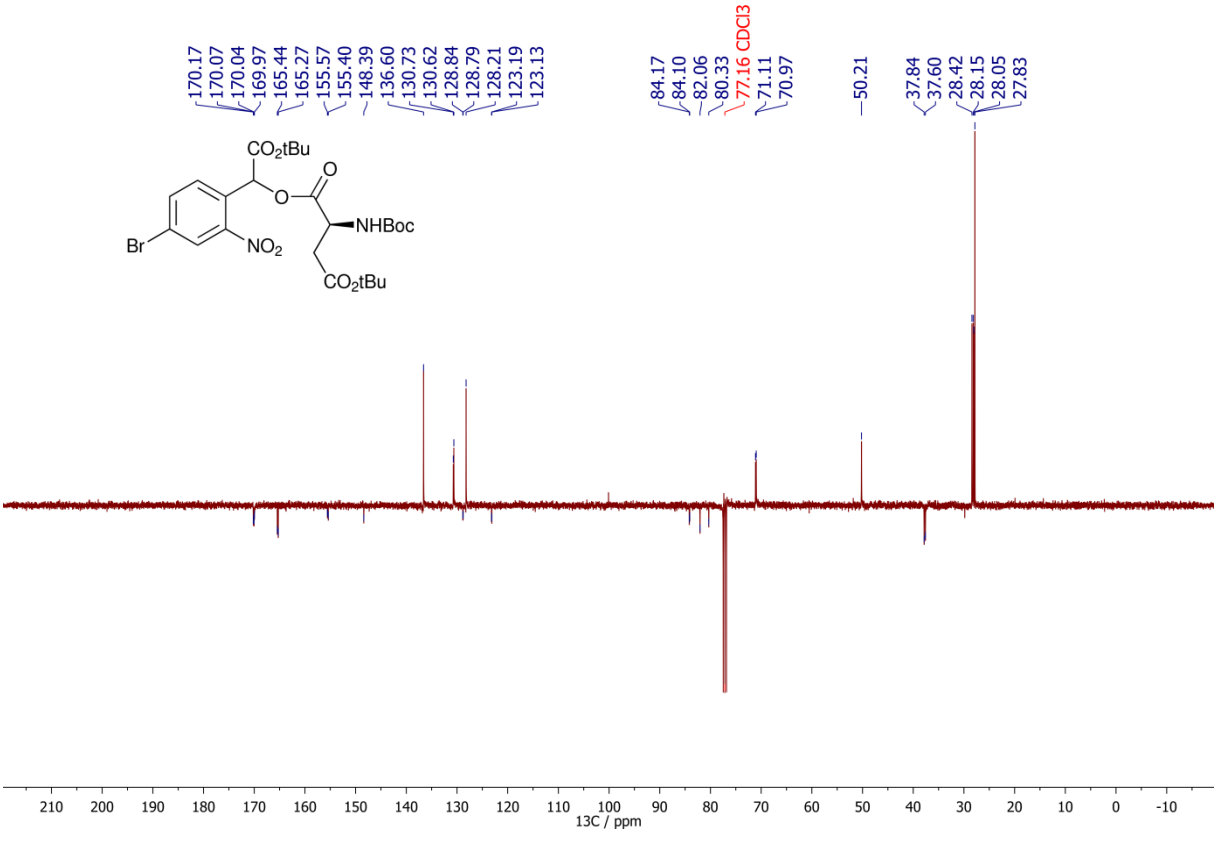

Figure S50: ^{13}C NMR spectrum of compound **8c** (DEPTQ-BBO, 100 MHz, $CDCl_3$)

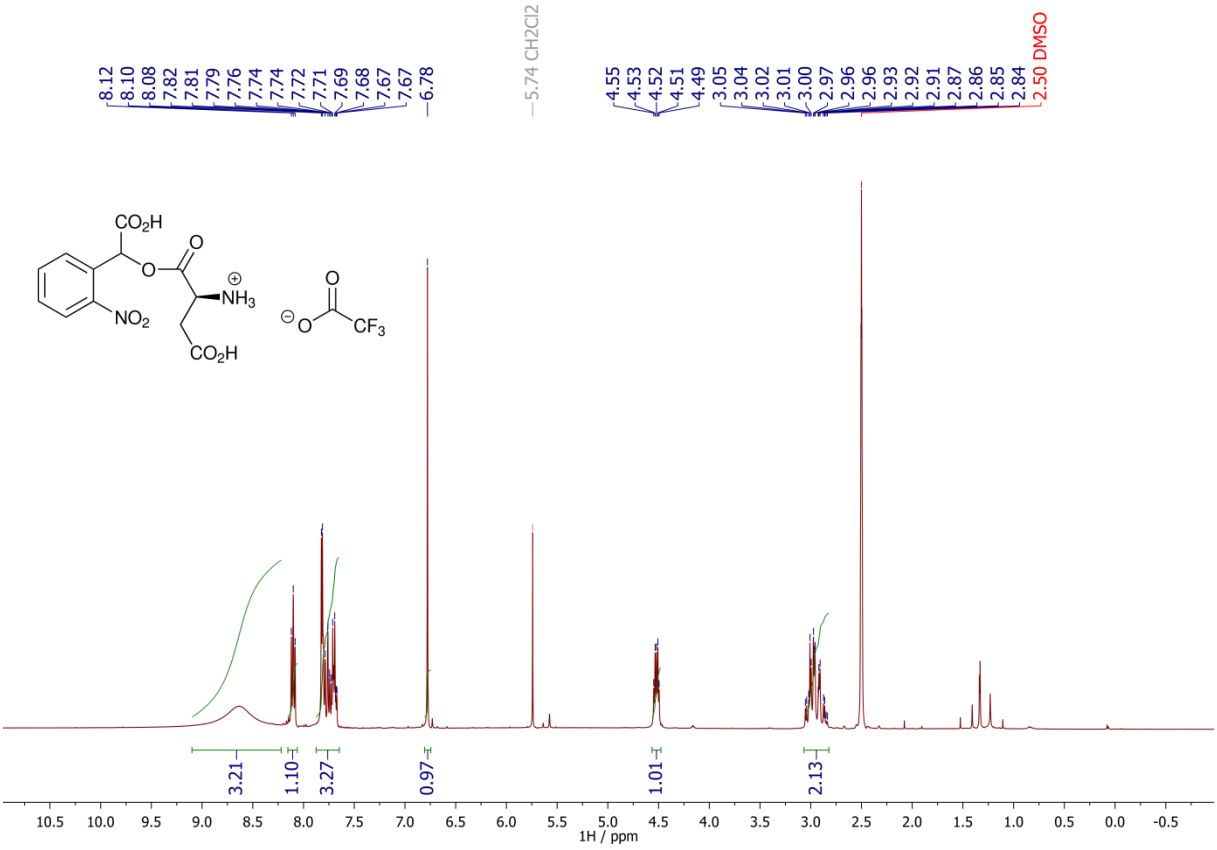

Figure S51: ^{1}H NMR spectrum of compound **1a** (400 MHz, DMSO-d_6)

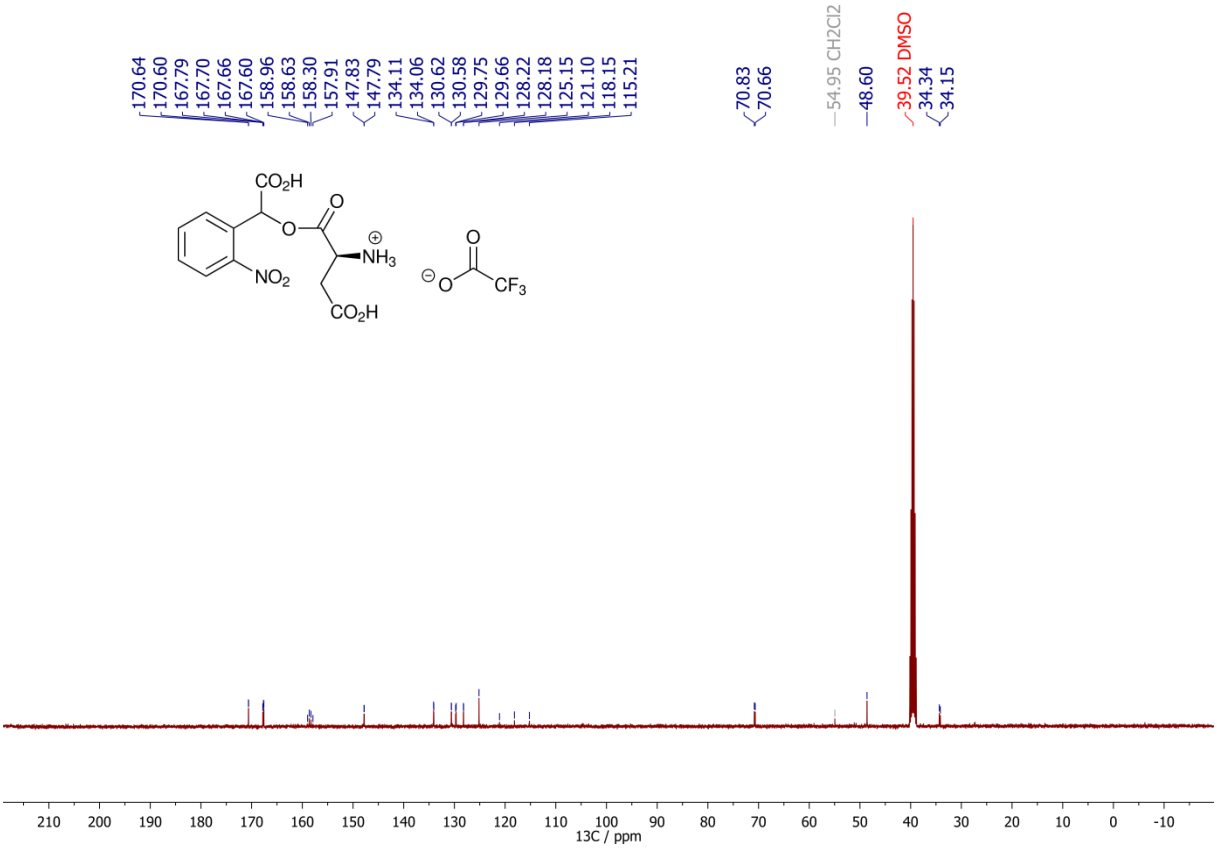

Figure S52: ^{13}C NMR spectrum of compound **1a** (100 MHz, DMSO-d_6)

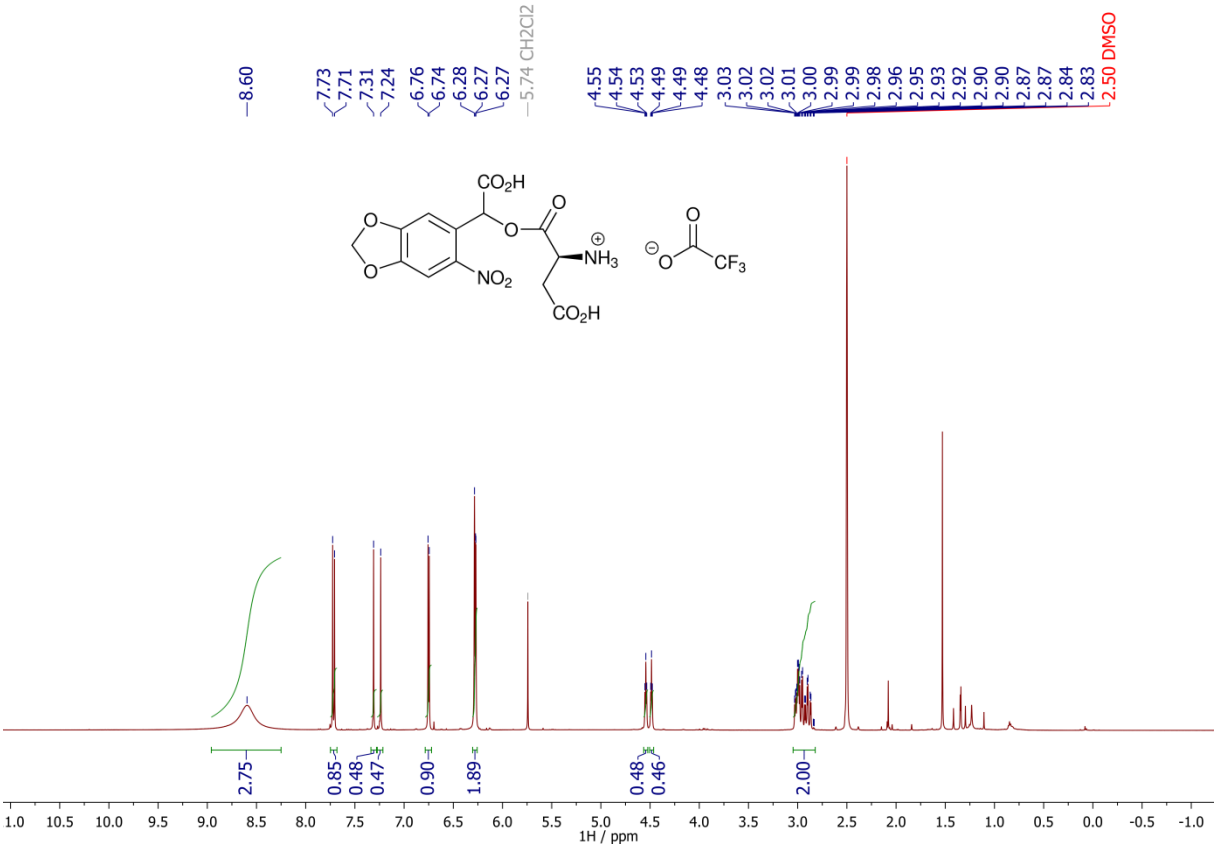

Figure S53: ^{1}H NMR spectrum of compound **1b** (600 MHz, DMSO-d_6)

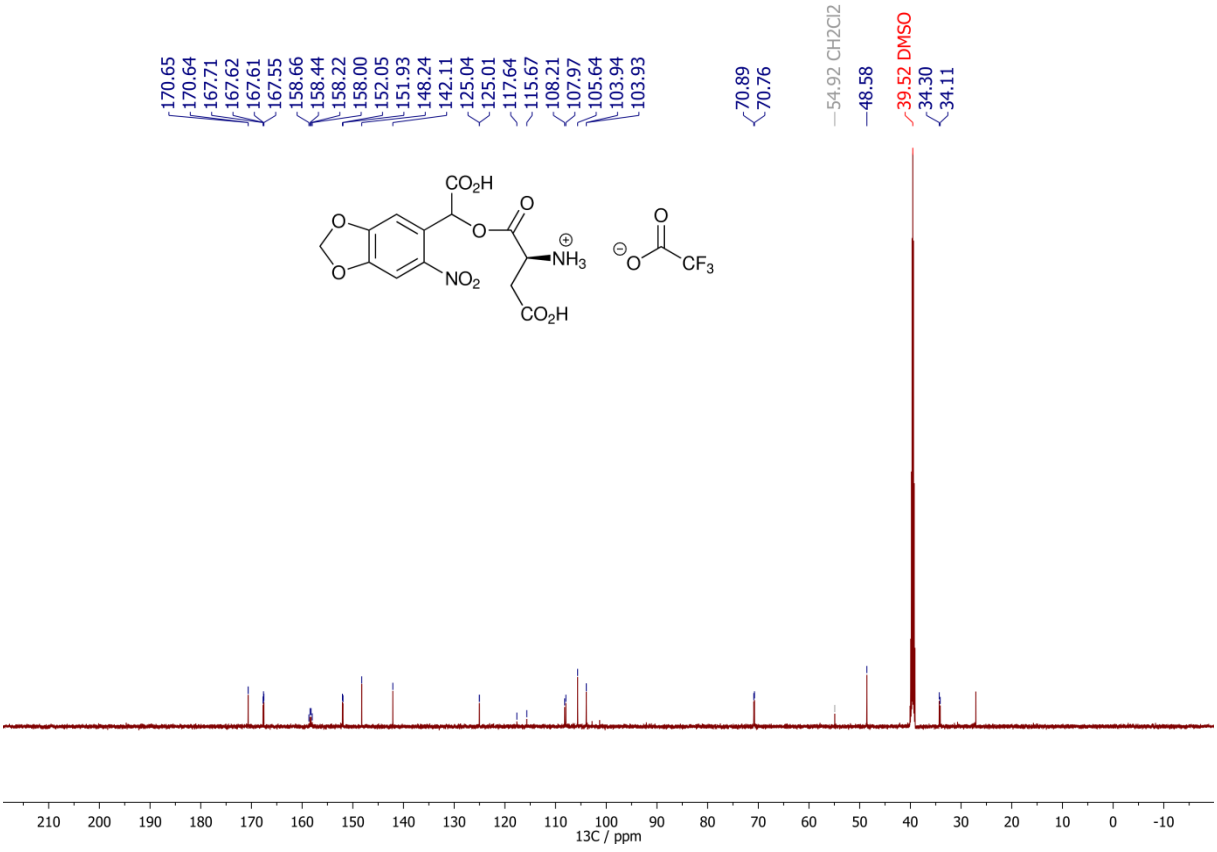

Figure S54: ^{13}C NMR spectrum of compound **1b** (150 MHz, DMSO-d_6)

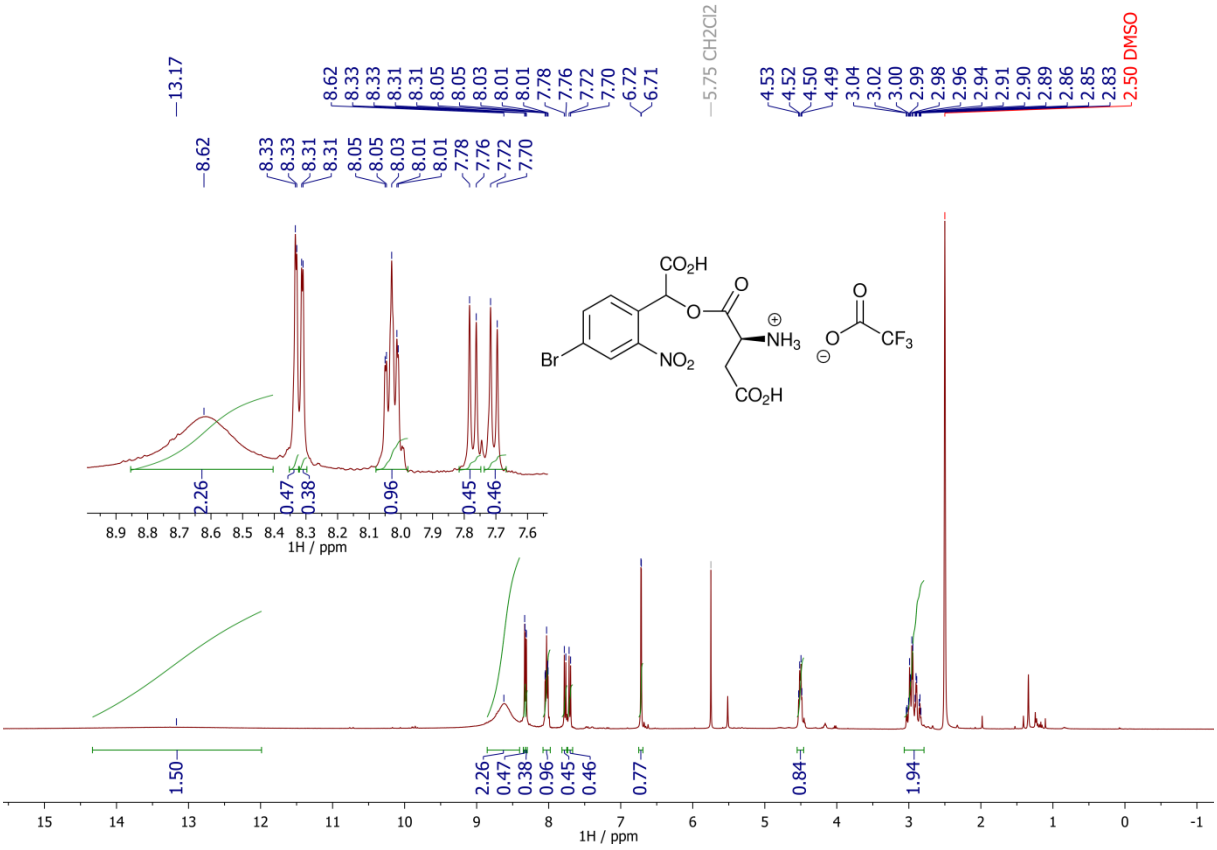

Figure S55: ^{1}H NMR spectrum of compound **1c** (400 MHz, DMSO-d_6)

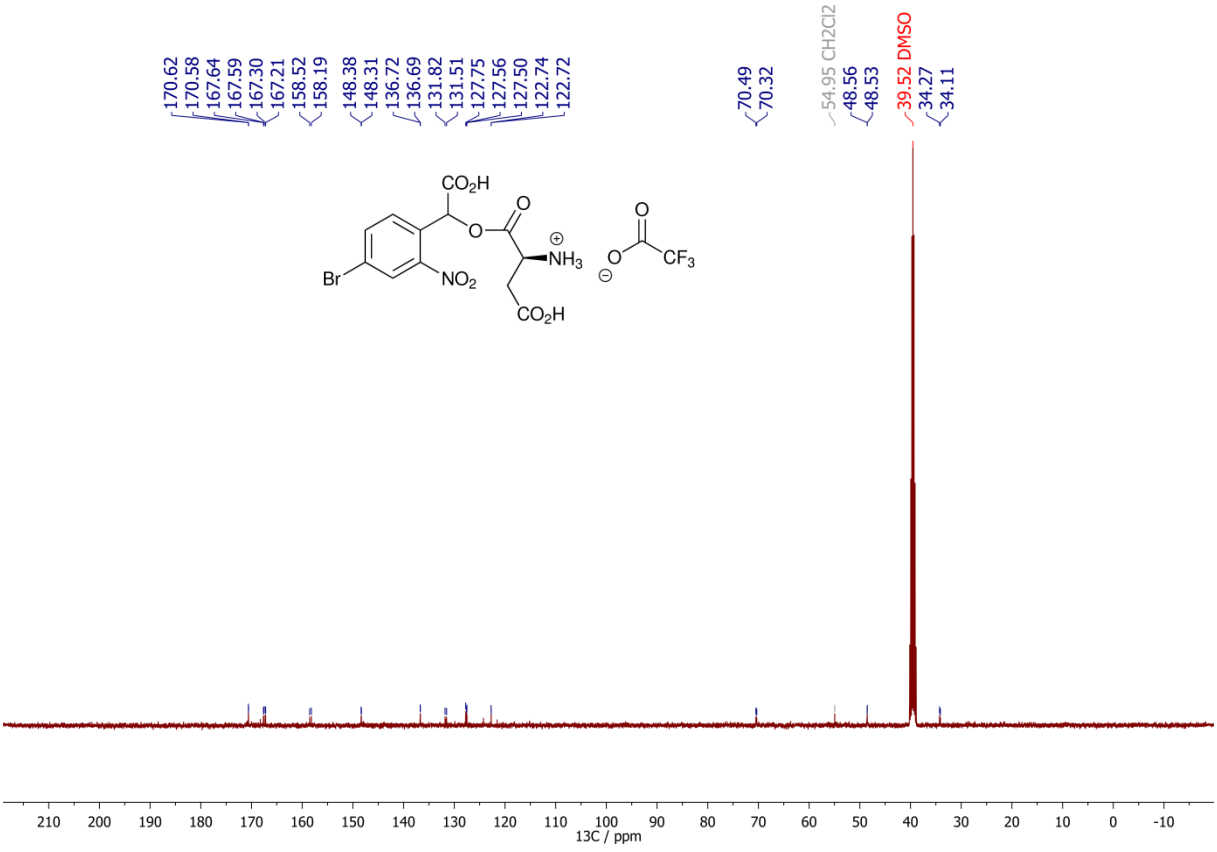

Figure S56: ^{13}C NMR spectrum of compound **1c** (100 MHz, DMSO-d_6)