Towards Elucidation of the Reaction Mechanism between Lawsone and Amino Acids

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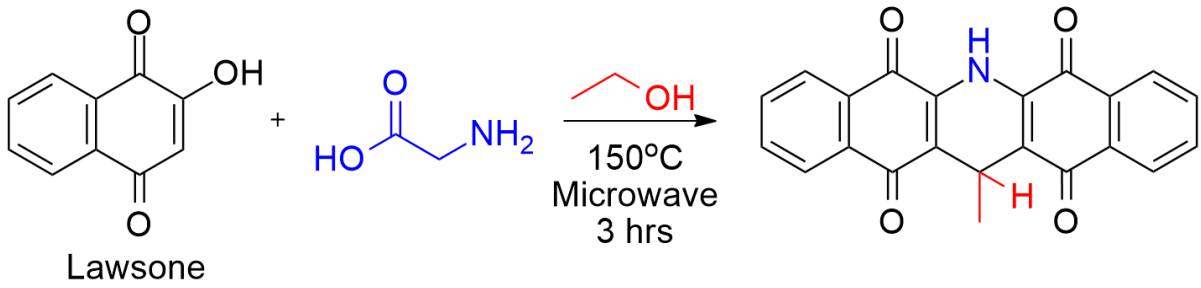
Thesis Summary

Research is constantly ongoing to find new fingerprinting reagents that can be applied to a variety of latent fingermarks and substrates. Particular substrates, such as paper and blood, prove to be challenging due to the high degree of background signals and possibility for reagent interaction with other compounds in the complex chemical matrix. Additionally, commonly used fingermark enhancing reagents such as ninhydrin, 1,2-indandione, and DFO and their formulations have proven toxicity. In 2008, Jelly *et al.* were prompted to investigate lawsone, a primary constituent in henna, as an alternative and safer reagent for the detection of latent fingermarks on porous surfaces. They found that lawsone reacts with amino acids in fingerprint deposits on paper, returning a purple-brown coloured stain with red fluorescent properties. Two contrasting structures have been proposed to be responsible for this red fluorescence, but limited research has been conducted to verify either of these structures. There has also been no investigation into how the reaction of lawsone with amino acids proceeds. Further investigations into lawsone's use have resulted in the inconsistent replication of the observed red fluorescence. This thesis details the identification of the structure responsible for the observed red fluorescence from the reaction between lawsone and glycine, as well as a proposed reaction pathway towards its formation.

The microwave assisted reaction between lawsone and glycine in ethanol was performed in this work, where a red compound exhibiting fluorescence and NMR spectra consistent to that reported by Jelly *et al.* was isolated. Unlike the two previously reported structures, the structure determined in this work consisted of two lawsone molecules connected by an amine and alkyl linker. This structure was consistent with existing literature and therefore the identity of the red fluorescent product was verified. Two additional compounds were isolated from the reaction, where one was identified as an amine intermediate toward red compound formation, and the other was deemed a reaction by-product.



Further investigation into the reaction mixture using different amino acids, deuterated ethanol, and lawsone analogues revealed that the amino acid is incorporated into the red fluorescent

structure at the -OH site of both lawsone molecules to form the amine linker. Surprisingly, the alkyl linker originated from the ethanol solvent and was incorporated at the vinyl position of both lawsone molecules. This alkylation chemistry was unexpected as the traditional conditions for alkylation are seemingly not present in the reaction mixture.

Possible intermediates along the reaction pathway towards the red fluorescent product formation were postulated and synthesised. These proposed intermediates imitated varying potential stages of linker formation. The ability of the reactions between these intermediates with each other, lawsone, glycine, and ethanol to form the fluorescent red product was subsequently used to propose a complete reaction pathway towards dimer formation. It was postulated that the previously isolated amine intermediate is formed first via Strecker degradation between lawsone and the amino acid, followed by condensation with a second lawsone molecule to form a NH-linked dimer intermediate. The alkyl linker is then formed via nucleophilic substitution at one vinyl position of the existing NH-linked dimer intermediate, followed by intramolecular cyclisation to completely form the fluorescent red product. The knowledge obtained from the understanding of this pathway may help to facilitate further optimisation of reagent conditions for use in the reliable and reproducible detection of latent fingermarks using lawsone.

Authorship Declaration

I certify that this thesis:

1. does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and
2. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Simone Madaras

6/11/2020

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Definitions of Abbreviations

[M+H]	Molecular ion
^{1}H	Proton
^{2}H	Deuterium
4-HO-TEMPO	4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl
Ac_2O	Acetic anhydride
acetone-*d*6	Deuterated acetone
ACN	Acetonitrile
APCI	Atmospheric-pressure chemical ionisation
Ar	Aromatic group
ATR	Attenuated total reflectance
$CDCl_3$	Deuterated chloroform
COSY	Homonuclear correlation spectroscopy
DCC	N,N'-Dicyclohexzylcarboiimide
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DSA	Direct sample analysis
EI	Electron ioinisation
ESI	Electrospray ionisation
Et	Ethyl group
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
EtOH	Ethanol
EtOH-*d*6	Deuterated ethanol
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HCl	Hydrochloric acid
HMBC	Heteronuclear multiple bond correlation
HOBt	1-hydroxybenzotriazole
HPLC	High performance liquid chromatography
Hrs	Hours
HSQC	Heteronuclear singular quantum coherence spectroscopy
HX	Hexane
IR	Infrared spectroscopy
J	Coupling constant
KO'Bu	Potassium-*tert*-butoxide
LC	Liquid chromatography
m/z	Mass to charge ratio
Me	Methyl group
MeOH	Methanol
MS	Mass spectroscopy
NADPH	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide

NMR	Nuclear magnetic resonance spectroscopy
Ome	Methoxy group
PDA	Photodiode array
Ph	Phenyl
pK_a	Acid dissociation constant
R	Any moiety with given limitations
R_f	Retention factor
ROS	Reactive oxygen species
RT	Room temperature
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TOF	Time of flight
UPLC	Ultra-high performance liquid chromatography
UV-Vis	Ultraviolet-Visible spectroscopy
δ_C	Chemical shift of carbon nuclei (ppm)
δ_H	Chemical shift of proton nuclei (ppm)
λ_{em}	Wavelength of maximum emission (nm)
λ_{ex}	Wavelength of maximum excitation (nm)
ν_{max}	Wavenumber of maximum absorption (cm^{-1})

1. Introduction

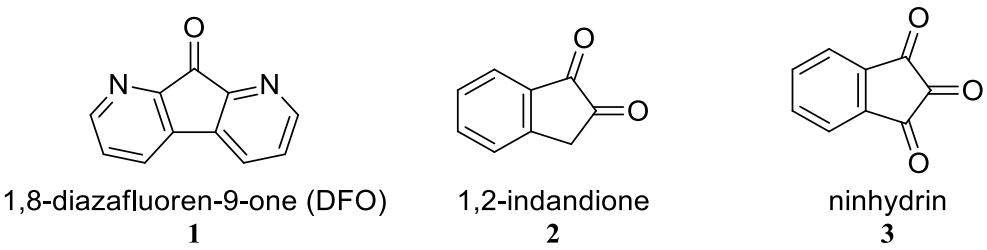
The detection of latent fingermarks at crime scenes provide investigators with vital information regarding the identification of the individual(s) involved.[1,2] This is largely due to the belief that fingerprints, whose patterns are determined by the arrangement of friction ridge skin on the fingertips, are unique to the individual and allows them to be used as personal identification.[1] Fingermarks are composed of secretions from the friction ridge skin, as well as secretions from other parts of the body and exogenous materials.[1,2] Secretions from the friction ridge skin are typically sweat from the eccrine glands that contain compounds such as salts, urea, and amino acids.[1,2] Other secretions include sebum from the sebaceous glands on the face and hair, primarily consisting of fats and oils.[1]

Latent fingermarks are invisible to the naked eye and require visualisation prior to identification of friction ridge skin details. Visualisation techniques include fuming, metal deposition, and the application of powders. Chemical visualisation is another common approach. This involves the application of chemical reagents to the fingermark, where its reaction with components in the fingermark elucidate a visible colour change or form a product that can be viewed either by eye or using high intensity light sources or lasers.[1] Coloured reagents or dyes, such as ninhydrin, 1,8-diazafluoren-9-one (DFO), and 1,2-indandione, are already established choices for chemical visualisation by many law enforcement agencies.[1,3,4] DFO, ninhydrin, and 1,2-indandione, the latter two reagents belonging to the quinone family, react with amino acids in the fingermark to produce a visible colour change. Although popular choices, they come with some disadvantages in their use relating to toxicity and contrast. Consequently, analogues and other quinones are constantly being targeted for research into their effectiveness as fingermark elucidation reagents and whether they perform better than pre-established methods.

Lawsone has been reported as a good reagent candidate for fingermark elucidation.[5–8] It is a naphthoquinone that has been proposed to also react with amino acids in fingerprints to produce a purple-brown colouring that can be used for fingerprint visualisation.[5] Unfortunately, the replication of these results is inconsistent in literature and limited research has been conducted into how the reaction of lawsone with amino acids proceeds.[9–13] This chapter will detail the use and challenges of using quinones as fingermark elucidation reagents and explain the choice of lawsone as an emerging reagent. The reactivity of naphthoquinones, particularly lawsone, will be reviewed in order to understand why inconsistencies in lawsone use were observed.

1.1. Quinones as fingermark elucidation agents

Several compounds contribute to the composition of a fingermark, particularly amino acids, which are not coloured and therefore not easily visualised.[4] The detection of amino acids via colorimetric means has therefore been heavily utilised in the forensic industry to visualise latent fingermarks. These amino acids are commonly targeted for fingermark enhancement to produce visible fingermark ridge detail and minutiae points that can be used for identification. DFO (**1**) (Figure 1.1) is a common fingermark enhancing reagent, utilised for fingermarks on porous surfaces. DFO is highly sensitive and has the added advantage of no post-treatment to produce fluorescent properties.[14] Quinone-like structures such as 1,2-indandione (**2**) and ninhydrin (**3**) are also commonly employed for this purpose (Figure 1.1).

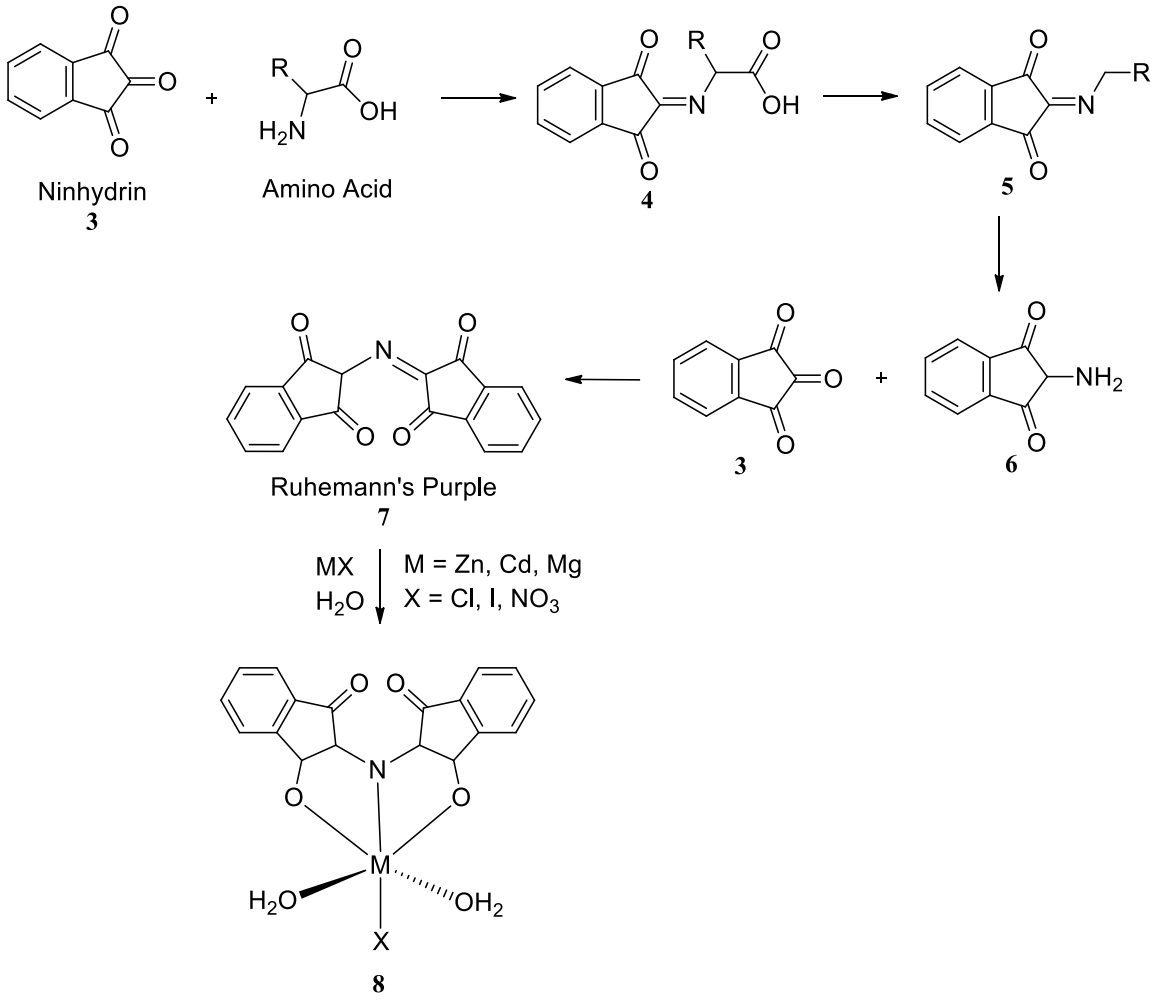


1,8-diazafluoren-9-one (DFO) **1** | 1,2-indandione **2** | ninhydrin **3**

Figure 1.1. The structures of commonly used fingermark enhancement reagents; DFO (**1**), 1,2-indandione (**2**), and ninhydrin (**3**).

Ninhydrin is a quinone that reacts with amino acids in the presence of heat to form a visible purple compound called Ruhemann's purple **7** (Scheme 1.1).[15] Ruhemann[16] first reported this purple compound in 1910, however the mechanism towards its formation was not agreed upon until the 1950's. The formation of Ruhemann's purple starts with the addition of the amino acid at the 2-position, followed by Strecker degradation of the imine **5** to form the amine intermediate **6** (Scheme 1.1). A second ninhydrin molecule then condenses with the amine intermediate **6** to finally form Ruhemann's purple **7**. Due to giving an immediate visible detection and the ability for production of other chromophores, it has since been utilised to visually detect non-coloured primary and secondary amines in addition to α-amino acids for analytical purposes. The spectroscopic properties of Ruhemann's purple can be enhanced by the addition of metal halide salts to form a complex (**8**) with the compound which exhibits fluorescence, luminescence, and phosphorescence (Scheme 1.1).[14,17,18] The enhancement of these fluorescent and luminescent properties requires cooling with liquid nitrogen.[4] A study conducted in 2017 analysed the effectiveness of different ninhydrin applications used by various laboratories on the resulting developed fingerprint.[19] The study found that, despite the wide variety of formulations and

experimental practices recorded, fingerprints were successfully developed in the majority of cases, leading to the conclusion that the use of ninhydrin provided a robust methodology for enhancement.[19] However, there are still issues with ninhydrin use regarding its toxicity and low level of contrast. [20,21]

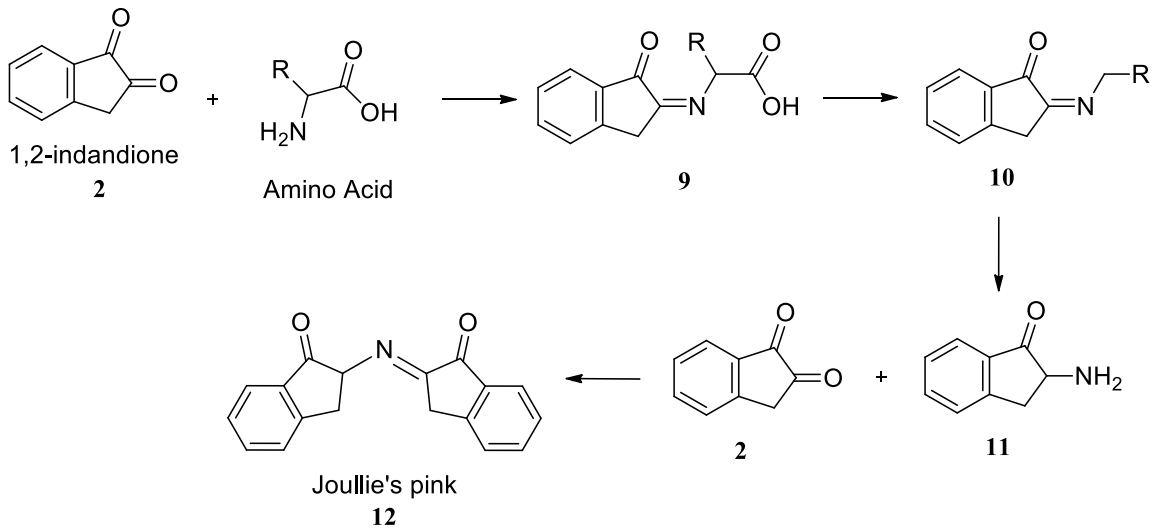


Scheme 1.1. The reaction of ninhydrin (**3**) with amino acids to form a purple compound, known as Ruhemann's Purple (**7**). Complexation with metal ions forms a product **8** with enhanced spectroscopic properties.

Ninhydrin is often the basis of research into whether analogues would be more selective or sensitive for fingermark enhancement. In addition, these analogues have been shown to elicit different spectral properties that can be used for often problematic surfaces.[17] For example, 5-methoxyninhydrin has been reported to exhibit luminescence at a longer wavelength than ninhydrin, and can be employed on cardboard substrates that already possess background luminescence.[17] 5-methylthioninhydrin (5-MTN) is another analogue of ninhydrin and has shown

the most promise at fingermark enhancement out of most analogues synthesised.[22] Porpiglia *et al.*[23] demonstrated the increased sensitivity and fluorescence at room-temperature of 5-MTN in comparison to ninhydrin, however noted that it did not perform better than the already established DFO. Berdejo *et al.*[11] also found no improvement when using 5-MTN in comparison to DFO, however also found no improvement in comparison to ninhydrin. The authors concluded that more investigation is needed to determine whether 5-MTN is a more suitable fingermark enhancing agent.

1,2-indandione reacts with amino acids to form a pink compound, aptly named Joullié's pink (**12**), in a similar manner to ninhydrin (Scheme 1.2). Its advantages over the use of ninhydrin include the production of a fluorescent compound without the need for further treatment with metal salts.[24] Enhancement of latent fingermarks using 1,2-indandione tends to be poorer on most paper types in comparison to DFO, however 1,2-indandione formulations containing zinc chloride were shown to produce brighter fingermarks than those developed using DFO.[24] When applied to fingermarks on brown paper and cardboard, an increased contrast between the resulting stain and substrate background is observed due to Joullié's pink exhibiting a longer fluorescence wavelength.[3,24]



Scheme 1.2. The reaction of 1,2-indandione (**2**) with amino acids to form a pink compound, known as Joullié's Pink (**12**).

While frequently implemented when viewing latent fingermarks, there are still several issues with the use of quinones like ninhydrin and 1,2-indandione. Ninhydrin has been reported to suffer from

poor development due to the low sensitivity of the technique, and a high degree of background staining, meaning the contrast between the background surface and the fingermark is low.[19] There are also safety issues surrounding its use as it has been reported to inhibit aconitase, an important enzyme used for energy production.[20] It has also been shown to promote tumour activity in mice.[21] 1,2-indandione was not as readily accepted by forensic agencies as DFO, as development of prints requires careful environmental control to maintain humidity at an optimum 70%.[25] This was a primary reason why the United Kingdom opted to maintain the use of DFO when it was first reported that other countries such as Israel, who regularly experience high humidity, would implement the change to preferentially use 1,2-indandione.[4,25] The addition of zinc was found to enhance the print when humidity was low, however this meant that there was no international standard for the preparation of the reagent.[25] The overall fingermark enhancement using these methods was initially deemed poorer than pre-established methods using DFO, but there has since been an increase in studies to rectify these issues and provide standard optimised conditions.[3,4,25]

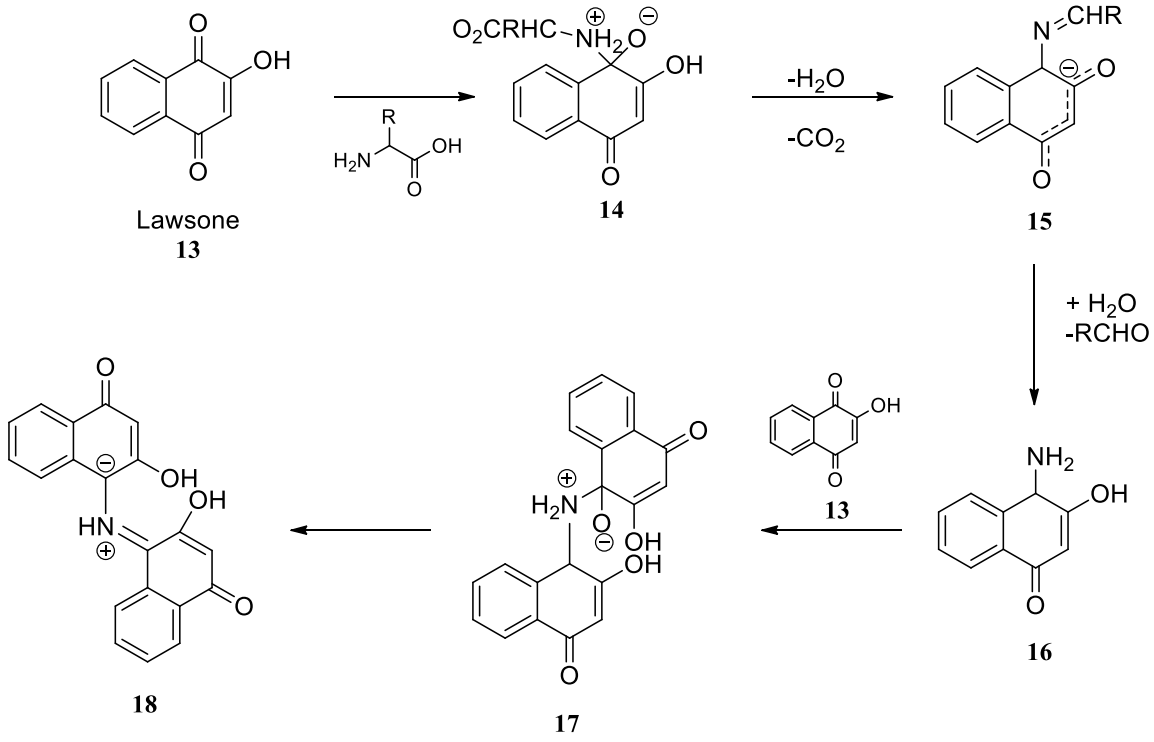
Due to the unsatisfactory fingermark enhancement and safety concerns, there has been a constant interest in whether analogues of these reagents or completely new compounds are more suitable and less toxic.[26,27] This has prompted investigation into the use of naphthoquinones as fingermark enhancement reagents, in particular lawsone.[5] Studies performed by *Jelly et al.*[5,7,8] have shown that lawsone works well to enhance ridge details of latent fingermarks on porous surfaces by producing a purple-brown stain with red luminescent properties, and could potentially be used in future forensic investigations. Following the work of Jelly *et al.*, [5,7,8] several researchers have investigated its use for fingermark enhancement on other substrates.[9–13] A summary of the use of lawsone as a fingermark enhancement reagent is presented in Table 1.1, including methodology and results, and will be discussed in further detail.

Table 1.1. Summary of research investigating the use of lawsone to visualise latent fingermarks on porous substrates

Authors	Substrate	Method	Results
Jelly *et al.* [5,7,8]	Paper	Formulation: Lawsone (50 mg) Ethyl acetate (10 mL) HFE-7100 (40 mL) 140-170°C; 60 min	Bright fluorescence observed at 590 nm
LaFratta *et al.*[9]	Paper	Formulation: Lawsone (50 mg) Ethyl acetate (10 mL) Petroleum ether (40 mL) 150°C; 45 min	Fluorescence observed at 540 nm
Phungyimnoi, Eksinitkun, and Phutdhawong[10]	Thermal paper	Vacuum vaporisation: Lawsone (100 mg) 0.1 Mbar 10-200 A	Clear yellow prints observed 97 minutiae points identified
Berdejo *et al.*[11]	Paper Cardboard Cellophane	Formulation: Lawsone (0.2 g) Ethyl acetate (40 mL) HFE-7100 (160 mL) 80°C; 5 min	Fluorescence observed in some cases Worse than pre-existing DFO and ninhydrin at 473-578 nm
Thomas and Farrugia[12]	Paper Blood	Formulation: Lawsone (0.5 g) Ethyl acetate (100 mL) Petroleum ether (400 mL) 160°C; 50 min	No observable fluorescence or staining at 503-587 nm
Dalrymple and Almog[13]	Brown paper Bond paper	Formulation: Lawsone (50 mg) Ethyl acetate (10 mL) HFE-7100 (40 mL) 140-170°C; 60 min	No fluorescence using lasers set to 532 and 460 nm Very weak fluorescence at 557 nm

Jelly *et al.*[5,7,8] treated latent fingermarks on paper with solutions of various naphthoquinones including lawsone (**13**), 1,2-naphthoquinone-4-sulfonate, 2-methoxy-1,4-naphthoquinone, and 2-methyl-1,4-naphthoquinone. The paper was air-dried and heated to 150°C in an oven for one

hour. The lawsone solution was prepared in a mixture of either ethyl acetate or ethanol, HFE-7100, acetic acid and zinc chloride solution. Fluorescence of the resulting purple-brown stains was observed at a wavelength of 650 nm when excited at 590 nm. To determine whether the naphthoquinones were reacting with the amino acid residues in the fingerprint, a positive control was performed where amino acids deposited on paper were treated with lawsone, to yield the same purple-brown stain and fluorescence. A red compound (**18**) was isolated from a reaction between lawsone and glycine heated to 80°C under reflux for two days, and was postulated to be responsible for the observed fluorescence.[6] The proposed structure and mechanism for this red compound **18** is shown in Scheme 1.3, and was based on the mechanism for ninhydrin.



Scheme 1.3. The proposed reaction mechanism of lawsone with amino acids to form a red fluorescent compound **18**.[5]

The simplicity of the reagent formulation and method, as well as the low cost and perceived low toxicity of lawsone, made it an ideal candidate for further investigation. LaFratta *et al.*[9] investigated visualising fingermarks on paper using lawsone for classroom experiments using a home-made fluorescence microscope. Their experiment involved depositing fingermarks on paper and treating them with a 5 mg/ml lawsone solution in ethyl acetate and hexane. The treated fingermarks were air-dried for one minute, and the paper subsequently heated to 150°C for 45 minutes on a hotplate. The portion of paper exposed to the lawsone turned light brown, while the

fingermark presented as either a faint brown stain or not visible. The authors reported visible fingermarks in 80% of their trials and attributed the invisible prints to a lack of fingermark residue on the paper. The visible fingermarks were successfully viewed using their fluorescence microscope and the appropriate filters.

Vacuum vaporisation techniques for latent fingermark detection using lawsone have also been explored on thermal paper, which are used for credit card receipts and ATM paper.[10] Thermal paper proves difficult to detect fingermarks using formulations of ninhydrin and DFO, as the choice of polar solvent and heat used to develop the fingermark exhibits a black stain on the heat-sensitive side. Vacuum vaporisation provides a solvent free and temperature sensitive alternative to usual reagent treatments. The technique involves using a low-vacuum range system at approximately 0.1 mbar, with a tungsten dimple boat in the vaporisation source that is connected to a 10-200 A power supply to apply heat. The authors placed 100 mg lawsone in the dimple boat and allowed the vacuum to reach 0.1 mbar, where the lawsone was vaporised to the gas phase and deposited on the latent fingermark on paper. The fingermarks were clearly observed under UV light and 97 minutiae points were identified using an Automated Fingerprint Identification System. The chemistry of the reaction was not explored.

Other researchers have attempted to apply lawsone to latent fingermarks but have had mixed success in replicating the staining observed by Jelly *et al*.[5–8] Berdejo *et al.*[11] compared the use of lawsone to pre-existing reagents ninhydrin and DFO for the detection of fingermarks on paper. A 0.1 mg/ml solution of lawsone in ethyl acetate and HFE-7100 (in a ratio of 1:4) was used to treat fingermarks on paper. The paper was air-dried after treatment and heated to 80°C with 70% humidity in an oven for five minutes. The treated fingermarks were visualised using an excitation bandwidth setting of 400-519 nm with a OG593 viewing filter, where it was found that fluorescence was only observed on white substrates. When compared to fingermarks developed using DFO, the results with lawsone were deemed worse in terms of fluorescence intensity and fingermark detail.

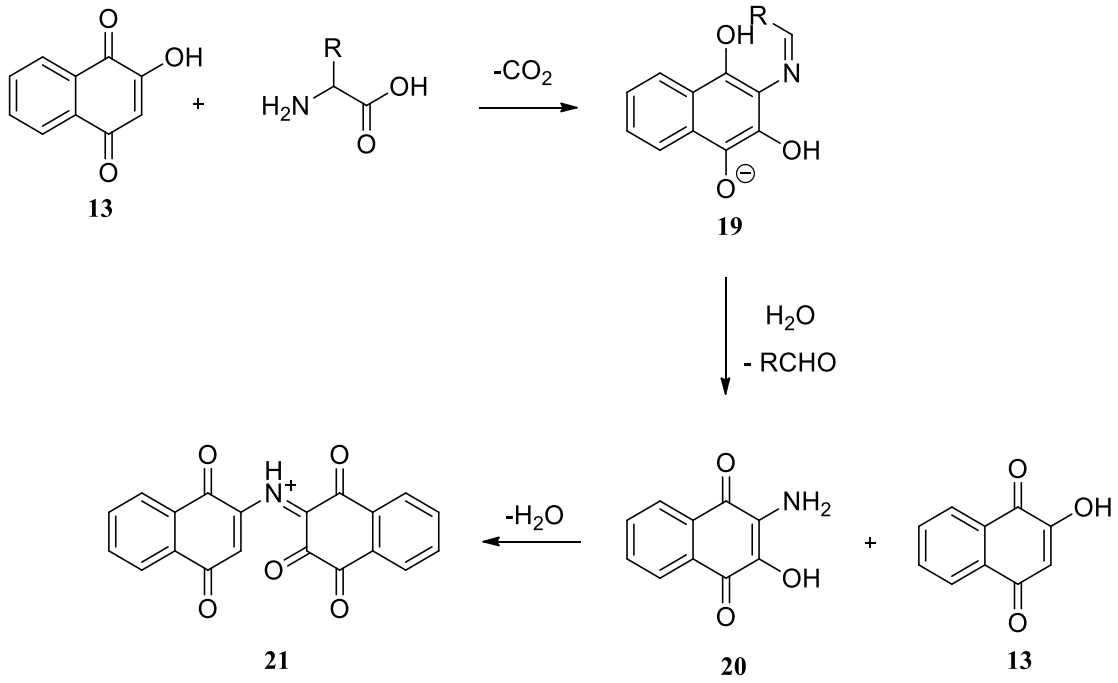
Thomas and Farrugia[12] conducted similar experiments to Berdejo *et al.*, however focused on visualising fingermarks in blood on paper using lawsone when compared to ninhydrin and DFO. Ten diminishing fingermarks in blood were deposited on paper and ranged from very visible to latent residues. The authors included positive and negative controls, which involved latent fingermarks on paper with no blood, and no fingermarks of any kind on the paper respectively. Fingermarks deposited on the paper were treated with a 0.1 mg/ml lawsone solution in ethyl acetate and petroleum ether (in a ratio of 1:4), air-dried, and then heated to 150°C in an oven for

one hour. The lawsone positive control displayed no visible mark on the paper and exhibited no observable fluorescence. The authors reported that 98% of fingermarks in blood demonstrated no observable or fluorescent enhancement, while ridge detail diffusion was observed when a fingermark could be defined.

Attempts have been made to visualise latent fingermarks treated with lawsone using LED light and lasers. Dalrymple and Almog[13] investigated the use of lasers at wavelengths of 460, 532, and 577 nm, and the use of LED lights at a wavelength of 505 nm.[13] The fingermarks were visualised using only lasers and LED lights, as well as with prior treatment with chemical reagents. The lawsone solution used was prepared and introduced to the sample latent fingermarks according to Jelly *et al.*[5] The fingermarks treated with lawsone did not exhibit fluorescence under lasers operating at wavelengths of 532 and 460 nm, as well as under LED lights at 505 nm. Weak fluorescence was observed using a laser set to 557 nm.

There appears to be conflicting reports on the effectiveness of lawsone. Jelly *et al.*[5] and LaFratta *et al.*[9] report visualising defined fingermarks and strong fluorescence, while Berdejo *et al.*[11], Thomas and Farrugia,[12] and Dalrymple and Almog[13] report little to no observable or fluorescent enhancement. The conditions used by these researchers vary, which may impact the reaction mechanism and result in the formation of different products. At present, the compound responsible for the staining, and therefore conditions facilitating its formation, is unknown.

To date, there has been limited work to confirm the structure of the product responsible for the fluorescence. Recently, Chan[28] reproduced the experiments performed under reflux by Jelly[6], albeit in methanol instead of ethanol or ethyl acetate and with serine, alanine, and lysine instead of glycine. The reactions were also only performed over five hours, rather than the two days as outlined by Jelly.[6] A colour change from yellow to red was observed, upon which NMR and LCMS analysis was performed. However, the NMR spectra reported by Chan[28] did not correlate to those reported by Jelly.[6] Chan proposed a new structure (**21**) for the red compound (Scheme 1.4) based on NMR and MS data of compounds separated from the reaction mixture and postulated a mechanism similar to that of ninhydrin. This new compound **21** is unusual in that a charged structure is presented. Chan[28] also proposed some structures for the masses observed from the MS data, however these do not appear to be included in their proposed mechanism.



Scheme 1.4. Postulated structure for the red product **21** and proposed mechanism (simplified), as outlined by Chan.[28]

While two structures and related mechanisms have been proposed by Chan[28] and Jelly[6], there has been no investigation into the feasibility of these mechanisms. Exploration of these mechanisms is of high importance to reproduce consistent fingermarks for identification. For example, research into the reaction between DFO and amino acids proved useful in tailoring a reagent formulation to consistently achieve the required red colouration.[14] Researchers observed that fingermarks were better visualised when methanol was used as the formulation solvent, whereas use of ethanol or *n*-butyl alcohol produced reduced or no visualisation.[14] It was determined that methanol was essential to the reaction, as it formed a hemiketal with the DFO which facilitated further reaction with amino acids.[14] Bulkier alcohols like *n*-butyl alcohol are unable to form this hemiketal, explaining the lack of red product formed. Consequently, the use of methanol was suggested as solvent for DFO formulations.[14] Similarly, investigations into the reaction of 1,2-indandione and amino acids also found hemiketal formation when methanol was used as a solvent.[29] However, hemiketals were found to hinder the formation of Joullié's pink and avoidance of alcohol solvents was recommended for 1,2-indandione formulations.[29]

The determination of reaction mechanisms can be achieved by identifying intermediates within the reaction mixture. Identifying these intermediates may be accomplished through isolation using column chromatography and further spectroscopic analysis with NMR or MS, as was the

case for DFO and 1,2-indandione, or using more recent technological advances by reaction monitoring with analytical techniques such as HPLC-MS[30] or real-time NMR.[31] Similar information about the reaction pathway related to this work may be obtained through similar methods. Alternatively, suspected intermediates may be synthesised and introduced into the reaction mixture, where they might improve the yield of the desired compound if involved in the mechanism of formation. Computational methods may also be used to model intermediates and transition states, where supporting evidence can be obtained about which ones are more energetically favourable and therefore more likely to occur in a reaction. Delarmelina *et al.*[32] was able to use density functional theory calculations to explain why nucleophilic disubstitution of 2,3-dichloro-1,4-naphthoquinone was or was not experimentally observed in numerous reactions by investigating the favourability of intramolecular hydrogen bonding between the naphthoquinone carbonyls and the -OH, -NH, and -SH terminal groups of their chosen nucleophiles.

In addition to isolating or modelling potential intermediates, a knowledge regarding the reactivity of the reagents is essential to understanding the mechanism happening in the reaction mixture. This knowledge assists in determining how identified intermediates are formed and how these can further react to produce the final product. In order to understand what fluorescent product is formed from the reaction between lawsone and amino acids, an understanding of the chemistry of naphthoquinones and lawsone itself is crucial.

1.2. Chemistry of naphthoquinones

Naphthoquinones are highly coloured compounds which have mostly been explored for their medicinal properties. They have been reported for antibacterial, anti-cancer, analgesic, and anti-inflammatory applications.[33] Naphthoquinones are both naturally occurring and synthetically produced derivatives of naphthalene. They consist of a naphthalene skeleton incorporating a quinone in either the ortho (1,2) or para (1,4) position (Figure 1.2).[34] Naphthoquinones make up an important subset of secondary metabolites common to many plants originating from the juglans, plumbago, and lawsonia genera as examples.[35] The roles of naturally occurring naphthoquinones as secondary metabolites along with their biological activities have seen them investigated for a range of medicinal applications, with prominent examples such as atovaquone being utilised as a commercial antibiotic.

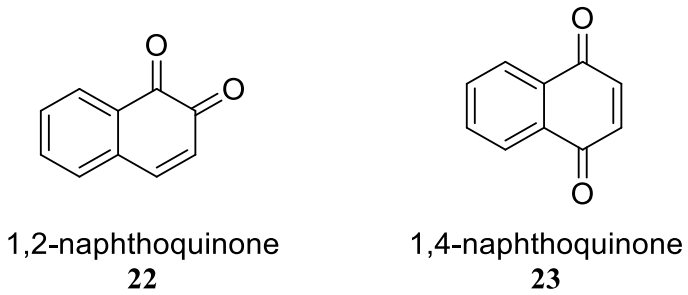


Figure 1.2. Common naphthoquinone configurations, with the quinone in either the ortho (1,2) or para (1,4) conformation.

Naphthoquinones derive their interesting and valuable properties from the chemistry inherent in their structure. Notable interactions involving naphthoquinones commonly involve reaction of a redox nature due to their ability to accept one or two electrons to form semiquinones or hydroquinones.[36] Examples of such chemistry include the one-electron reduction of naphthoquinones by cytochrome P450 to form semiquinones that are toxic to cells.[37] Naphthoquinones can also engage in Michael additions due to the quinone moiety containing an α,β-unsaturated carbonyl system.[36] Michael addition notably occurs between naphthoquinones and keratin in wool for the purposes of fabric dying.[38]

Another important structural consideration is that of the aromatic component of the naphthoquinone structure. Chemical reactions which involve changes in the distribution of electrons from the aromatic structure allow for interactions with the electromagnetic spectrum in the visible light range and result in colour changes. These properties can and have found their utility in industry as dyes and pigments with examples such as lawsone being used to dye hair and

skin.[39,40] The observed colour of naphthoquinones can vary depending on the substitution,[41] pH[42] and interaction with other chemicals such as metals.[43] Additionally, they are used for qualitative visualisation of compounds after chromatographic separation, notably the use of Folin's reagent in the pharmaceutical industry for the detection of amines.[44] Naphthoquinones are known fluorescence switches, where their interaction with other molecules can either induce or quench the observed fluorescence. The variability of naphthoquinones to exhibit fluorescence is dependent on the addition of ions and substituents to the naphthoquinone structure, as well as choice of solvent.[45,46] This fluorescence switching has been utilised to create on-off detection systems for target structures in medical imaging,[47] as well as for the detection of ions in wastewater.[48]

1.2.1. Biological Activity of Naphthoquinones

Plants are well known bio-synthesisers of naphthoquinones for the purposes of self-defence against other plants, bacteria, and insects.[35] The naphthoquinones lawsone and 2-methoxy-1,4-naphthoquinone were present in the nectar of *Impatiens glandulifera*, where it was determined that they inhibit fungal growth and protect the composition of the nectar microbiome.[49] In a similar manner, plumbagin and 7-methoxy juglone were found to inhibit the growth of nutrient-competitive microbes in the digestive juices of *Nepenthes* pitchers.[50] This biological activity of naphthoquinones is commonly attributed to either oxidative stress due to the formation of reactive oxygen species (ROS) from redox cycling, or the arylation of nucleophilic sulfhydryl groups.[51–53]

ROS are molecules containing oxygen that are more reactive than molecular oxygen, for example hydrogen peroxide or hydroxy radicals. They are primarily responsible for oxidative stress experienced by cells and can cause apoptosis or necrosis.[52,54] The ability of these naphthoquinones to produce ROS can be enhanced by the addition of electrophilic functional groups such as hydroxyls, found in naphthoquinones such as plumbagin, lawsone and juglone.[55] ROS are produced by redox cycling, which is the process by which quinones (**22** and **23**) are converted to hydroquinones (**26** and **27**) and vice versa through a semiquinone radical intermediate (**24** and **25**) (Figure 1.3).[54,56] Superoxide anions and hydrogen peroxide are also produced when the semiquinone radical interacts with molecular oxygen.[53,57] Redox cycling of quinones within biological systems is generally triggered by enzymes, for example NADPH-dependant cytochrome P-450 reductase[58] and DT-diaphorase[59].

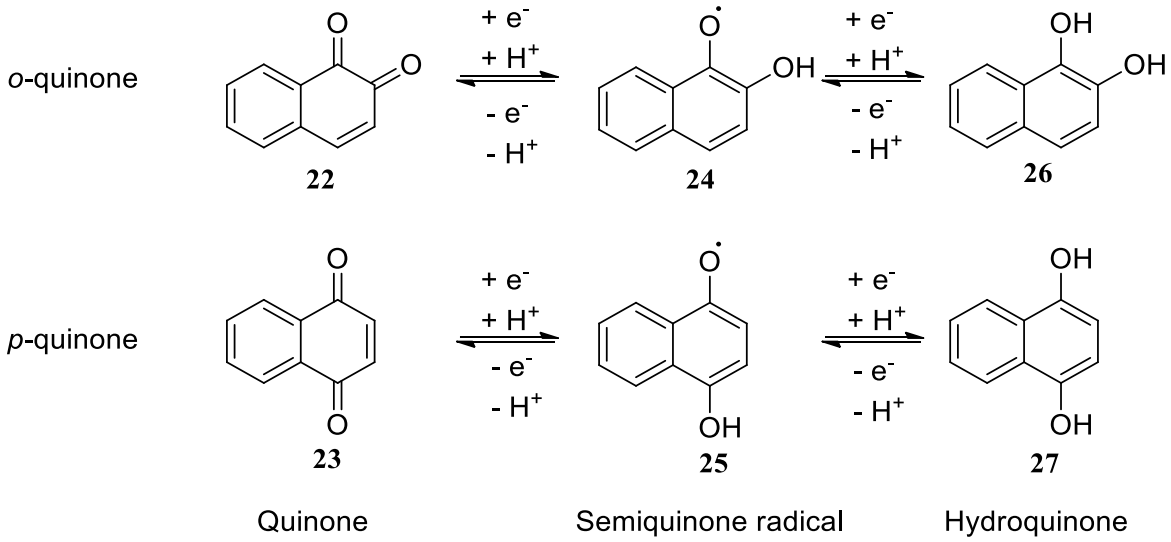


Figure 1.3. Redox cycling of *o*-naphthoquinones (**22**) and *p*-naphthoquinones (**23**), showing the formation of the semiquinone radicals (**24** and **25**) and hydroquinones (**26** and **27**).

Naphthoquinones can undergo either one-electron or two-electron transfer when triggered to undergo redox cycling. Naphthoquinones that undergo two-electron transfer can cycle completely to the hydroquinone, while those that undergo one-electron transfer can only cycle between the quinone and the semiquinone radical.[58] It has been suggested that quinones capable of redox cycling possess one-electron reduction potentials between -270 and -160 mV.[60] A major factor in whether a naphthoquinone is involved in one or two electron transfer is the stability of the form. In the case of lawsone for example, the hydroquinone form is too unstable, which causes an immediate conversion back to the semiquinone radical.[57]

The second known contributor to naphthoquinone toxicity is their ability to react with sulfhydryl groups in proteins. The reaction produces a hydroquinone thioether (Figure 1.4), causing the protein to become denatured and lose its biological activity.[53] Alternatively, reaction at the thiol sites can over-activate certain proteins, causing unwanted effects such as cell damage or death.[61]

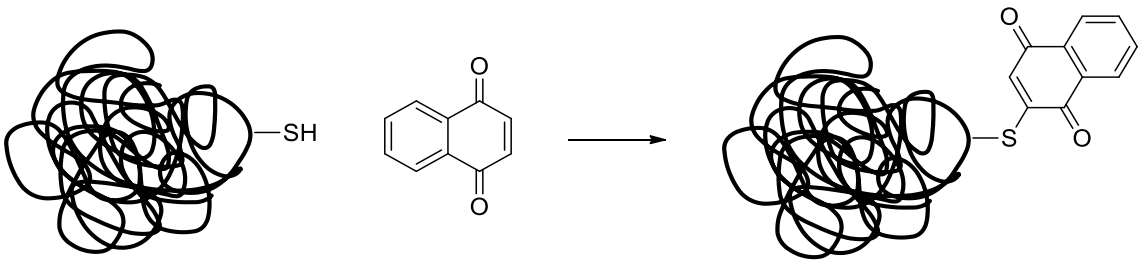


Figure 1.4. The reaction of sulfhydryl groups in proteins with naphthoquinones, forming the hydroquinone thioether.

The toxicity of the naphthoquinone is dependent on the structure and presence of additional functional groups that would allow for more efficient redox cycling or better access to sulfhydryl groups. As such, only one mode of action or a combination of both may be responsible for the toxicity of the naphthoquinone. Gant *et al.*[52] clearly demonstrated this when investigating the reasons for toxicity of menadione and 2,3-dimethoxy-1,4-naphthoquinone towards liver cells. These naphthoquinones provided a useful comparison as menadione is able to redox cycle as well as arylate nucleophiles, while 2,3-dimethoxy-1,4-naphthoquinone is unable to arylate nucleophiles but can redox cycle at a comparative level to menadione. Menadione was found to be significantly more toxic than 2,3-dimethoxy-1,4-naphthoquinone, suggesting that arylation is just as important as redox cycling at exhibiting cytotoxic effects. Consequently, both toxicity mechanisms must therefore be considered when screening naphthoquinones for potential biological effects.

The biological activity of naphthoquinones has propelled their structural motif to prominence in the field of medicinal chemistry research. The redox cycling and arylation chemistry exhibited by naphthoquinones make them excellent candidates for use as anti-cancer, anti-bacterial, anti-fungal and anti-parasitic medicines. Juglone, lawsone, and plumbagin are three naturally occurring naphthoquinones that have been studied extensively for these uses. Lawsone has been reported to exhibit much lower anti-cancer activity than plumbagin and juglone.[62] This has been attributed to lawsone possessing a low redox potential of -415 mV, therefore unable to induce oxidative stress causing cell death, and having very little interaction with protein thiols due to the blockage of the 2- position by the hydroxyl group.[57,60,63] In a separate study by Nasiri *et al.*[64] lawsone derivatives being tested against the bacteria *Helicobacter pyroli* were shown to be effective at inhibiting the activity of quinol/fumarate reductase. Derivatives with long, bulky sidechains at the

3-position, as well as derivatives with bromine at the 2-position and small alkyl chains at the 3-position were found to be most effective. Reactivity at the C-2 and C-3 sites of lawsone are therefore of great interest to researchers in the field to yield lawsone analogues with enhanced medicinal properties. The knowledge gained from their investigations, combined with the known biological activity, can provide great insight into how the lawsone may behave in the reaction mixture with glycine, as described by Jelly *et al.*[5]

1.3. Lawsone

Henna extracts from the *Lawsonia inermis* plant, commonly known as the henna tree, have been used for years as a fabric and skin dye as well as in homeopathic medicine, primarily as a natural antibiotic and anti-oxidant.[62,65] 2-hydroxy-1,4-naphthoquinone, more commonly known as lawsone, is a constituent of henna that is responsible for the orange colour on hair or skin when used as dye. The resultant orange-brown pigment or stain is proposed to be caused by the Michael addition with amino acids on the skin and thio or amine groups in keratin within hair.[48] The exact colour of the resulting stain upon lawsone application can vary between yellow and red depending on the pH of the environment. Lawsone has an experimentally determined pK_a of 3.98 and can exist as the neutral phenol in acidic solutions or as the charged phenolate in more basic solutions (Figure 1.5).[42] In acidic solutions, an absorption maximum around 330 nm is observed in UV-Vis spectra which is reflective of the yellow colour observed.[66] The charged phenolate form causes an increase in absorption maximum to 450 nm, thereby forming a red solution.[38] When dyeing fabrics and skin, however, an acidic pH is required to obtain the strong characteristic orange colour.[38,39] The requirement for a slightly acidic pH was attributed to both allowing enough available lawsone to still exist in solution as the anionic form, while ensuring the availability of complexation sites on the keratin.[39] To mimic this observation, citric acid is commonly applied to henna painted on the skin to extend the wear of the stain.[39]

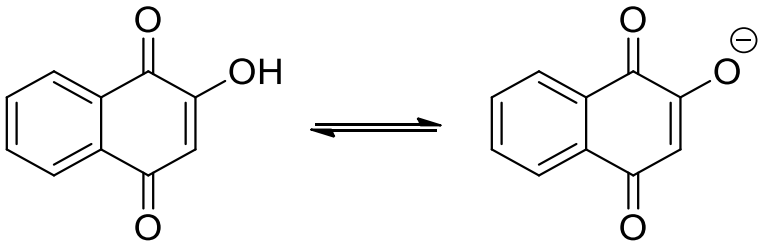


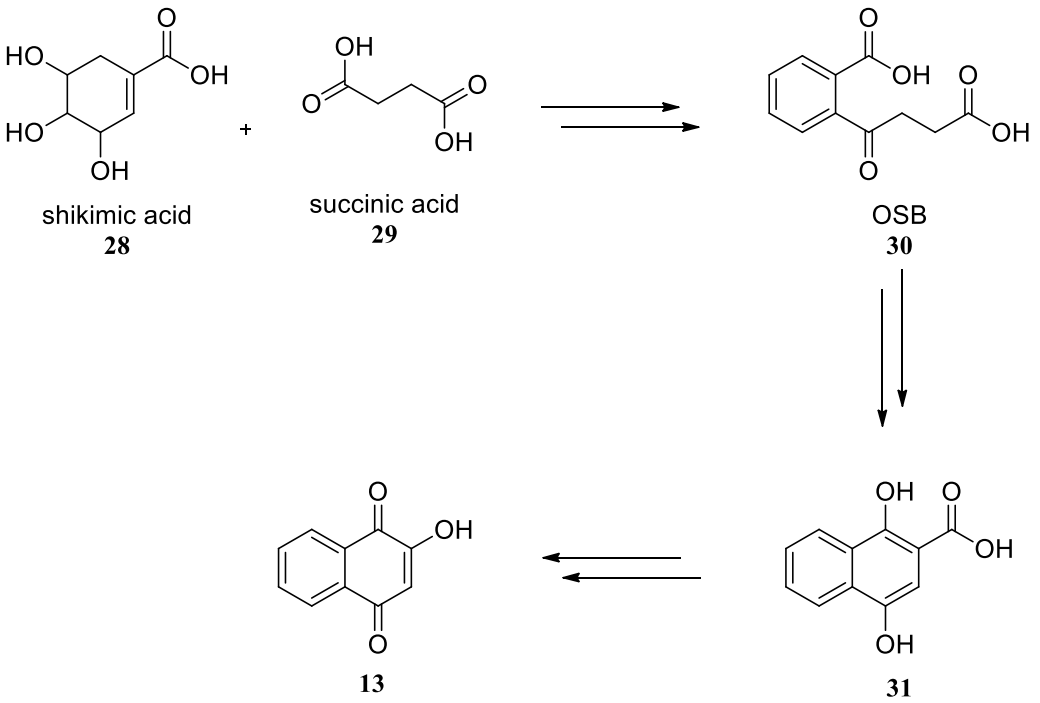
Figure 1.5. The neutral (yellow) and anionic (orange-red) forms of lawsone, with a pK_a of 3.98.

The structure and spectroscopic properties of lawsone allow for it to be a useful electrochemical colourimetric sensor for the detection of anions in aqueous solutions. Hijji, Barare and Zhang[66] were able to qualitatively and quantitatively analyse the presence of cyanide, acetate and fluoride in waste water. Interactions of lawsone with these anions in the way of hydrogen bonding and redox cycling caused colour changes in the solution that could be measured using UV-Vis spectroscopy. Similarly, Kavitha and Stalin[48] used a lawsone – β-cyclodextrin complex that could selectively detect mercury(II) cations in aqueous media. The fluorescence of the solution containing the lawsone – β-cyclodextrin complex was quenched by the addition of mercury(II) cations, due to coordination of the cation with the carbonyl oxygen atoms. The authors also determined fluorescence changes in the lawsone – β-cyclodextrin complex solution that could be used as a pH probe over the pH range of 6-12, whereby fluorescence was exhibited at acidic pH and was quenched at basic pH.

The low redox potential of lawsone has seen its structure constantly modified in order to induce oxidative stress for medicinal uses.[60] However, its low redox potential has made it an ideal candidate for use as a renewable aqueous flow battery. Hu *et al.*[67] successfully used lawsone as an anolyte to induce high operating voltages of 1.30 V and above in their redox-flow battery while using 4-HO-TEMPO as the catholyte. The authors recommended further investigation into lawsone for use in renewable batteries due to its promising energy efficiency and capacity retention rates.

1.3.1. Sources and preparation methods of Lawsone

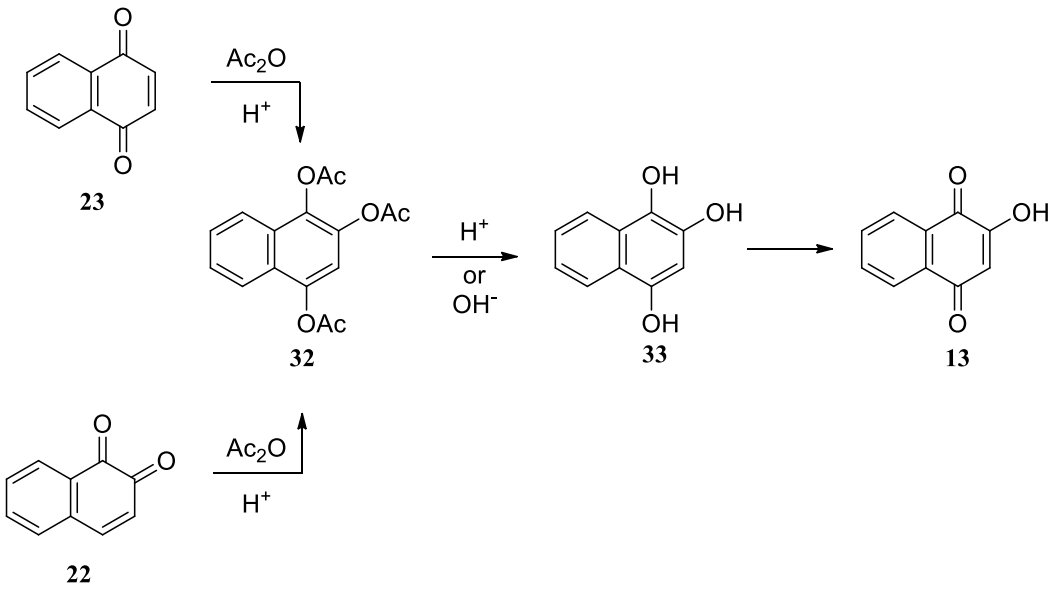
Naphthoquinones can be biosynthesised naturally via at least seven unique pathways, depending on the organism and final naphthoquinone structure.[35] Lawsone has been found to be biosynthesised primarily from the O-succinylbenzoate (OSB) pathway involving shikimic acid (**28**) (Scheme 1.5).[35,68,69] Feeding experiments in *Impatiens balsamina* plants using 2-^{14}C-acetate [70] and shikimic acid-U-^{14}C [71] have confirmed the overall pathway by tracing the inclusion and positions of the ^{14}C atoms. The reaction pathway first involves a condensation reaction with shikimic acid and a succinyl acid molecule **29** to form the title intermediate, OSB (**30**). The OSB intermediate **30** then undergoes intra-molecular cyclisation between the carbonyl originating from the shikimic acid and a methyl originating from the succinyl acid to form the intermediate **31**. Decarboxylation and oxidation of the naphthoic acid finally forms the desired lawsone.



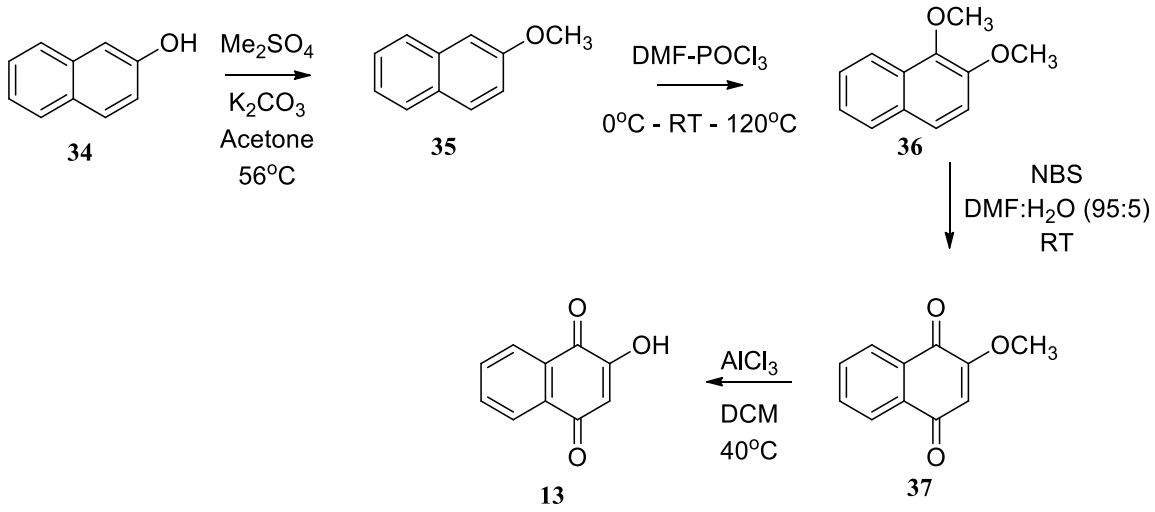
Scheme 1.5. Biosynthesis of lawsone via the OBS pathway.[68,69,71]

Lawsone can be isolated from henna plants using liquid-liquid extraction in chloroform with further purification using column chromatography,[72,73] Soxhlet extraction in ethanol,[74] or by preparative HPLC separation after maceration in water or ethanol.[75] Maximum yields of lawsone from henna plants typically range between 0.5 %w/w and 1.8 %w/w, where the yield has been found to vary according to the geographic origin of the plant.[73,75] Due to the many uses of lawsone and potential to be used for medicinal purposes, several synthesis methods have been created in order to scale up the amount of lawsone produced for these needs. Consequently, only the most common methods will be presented here.

The synthesis of lawsone generally involves the addition of the 1,4-quinone and/or hydroxyl moieties to structures containing a naphthalene-like skeleton[76,77], or complete synthesis of the naphthoquinone ring system[78,79]. A common method of moiety introduction is the Thiele-Winter acetoxylation of **22** or **23** (Scheme 1.6).[76] Alternatively, naphthols such as 2-naphthol (**34**) can be used to synthesise lawsone in four steps via Vilsmeier-Haack formylation followed by oxidation (Scheme 1.7).[77]

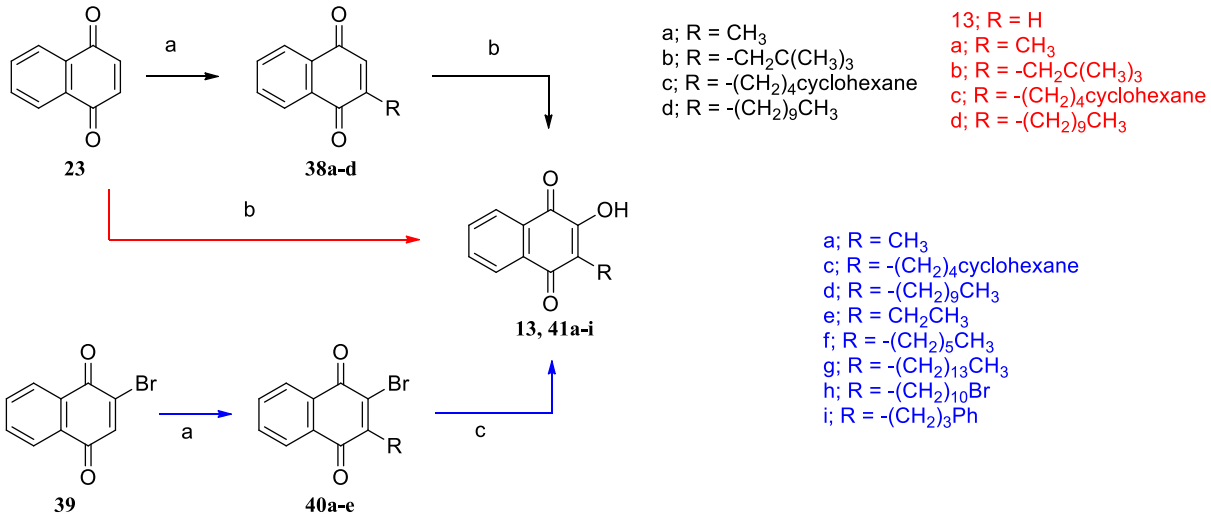


Scheme 1.6. Thiele-Winter acetoxylation of 1,2- and 1,4-naphthoquinone to lawsone.



Scheme 1.7. Synthesis of lawsone via Vilsmeier-Haack formylation of 2-naphthol.[77]

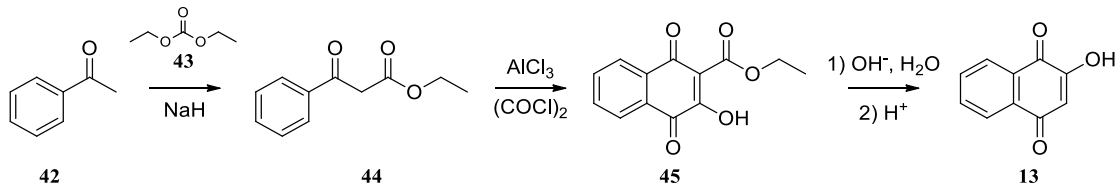
23 can also be subjected to Weitz-Scheffer type epoxidation to form lawsone and derivatives **41a-d** with various alkyl groups at the 3-position (Scheme 1.8, black and red).[64] Alternatively, **23** could be treated directly to nucleophilic substitution with KOH or MeOH to yield the desired lawsone analogues **41a,c,d,e-j** (Scheme 1.8, blue).



(a) Radical Hundsdiecker oxidative decarboxylation/oxa-Michael addition; (i) corresponding acid 1.5 equiv.; (ii) 1.8 equiv. $(NH_4)_2S_2O_8$; (iii) 0.5 equiv. $AgNO_3$,CH_3CN, H_2O, 80 °C, 7 h. (b) Weitz-Scheffer-type epoxidation/epoxide cleavage (i) H_2O_2,Na_2CO_3; (ii) H_2SO_4. (c) Nucleophilic substitution: KOH/MeOH, reflux, 1 h

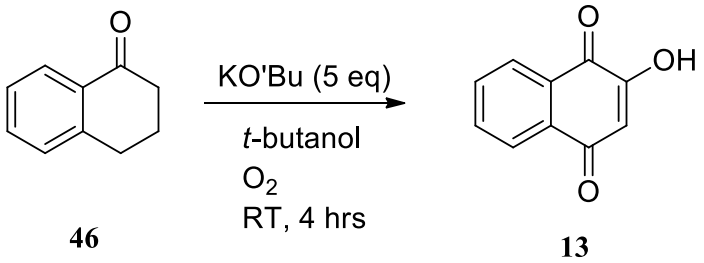
Scheme 1.8. Synthesis of lawsone and derivatives **41a-i** using 1,4-naphthoquinone (**23**) or 2-bromo-1,4-naphthoquinone (**39**) as starting reagents.[64]

Complete construction of the naphthoquinone ring structure and hydroxyl at the 2- position in lawsone can be accomplished in two or three steps via Friedel-Crafts double-acylation of β-keto esters (Scheme 1.9).[78,79] This synthesis method is similar to the proposed biosynthesis of lawsone, outlined in Scheme 1.5. This method has been used to produce isotopically labelled lawsone[79], as well as ring-substituted analogues with substituents in the meta and para positions.[78]



Scheme 1.9. Synthesis of lawsone via intramolecular Friedel-Crafts acylation of ethyl benzoylacetate.[78,79]

A more straightforward approach involves the auto-oxidation of tetralones in the presence of potassium *t*-butoxide and *t*-butanol (Scheme 1.10).[80] This is performed in one step and is a useful method to introduce functional groups on the aromatic ring.[81,82]



Scheme 1.10. Auto-oxidation of 1-tetralone **46** in the presence of potassium *t*-butoxide and *t*-butanol.[81]

1.3.2. Reactivity of lawsone

Lawsone is unique to other similar naphthoquinones such as menadione (**47**), juglone (**48**), or plumbagin (**49**), due to the hydroxyl at the 2- position (Figure 1.6). This allows for enol functionality in addition to enone properties displayed by other naphthoquinones. The hydroxyl also allows for enol-keto tautomerism (Figure 1.7) thereby exposing the 2- and 4- positions as potential reaction sites. Lawsone also contains an enone functional group, allowing for reaction at the 3- position. These functional groups have made lawsone an ideal scaffold for the synthesis of naphthoquinone analogues.

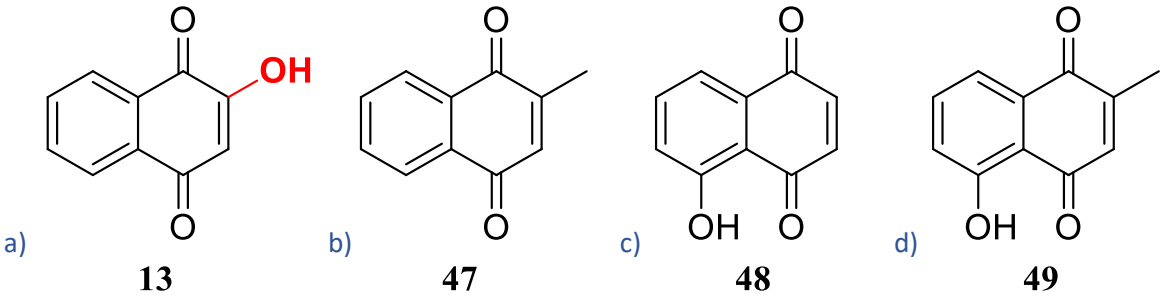


Figure 1.6. Structures of a) lawsone (**13**), b) menadione (**47**), c) juglone (**48**), and d) plumbagin (**49**), illustrating the difference in substituents at the 2-position.

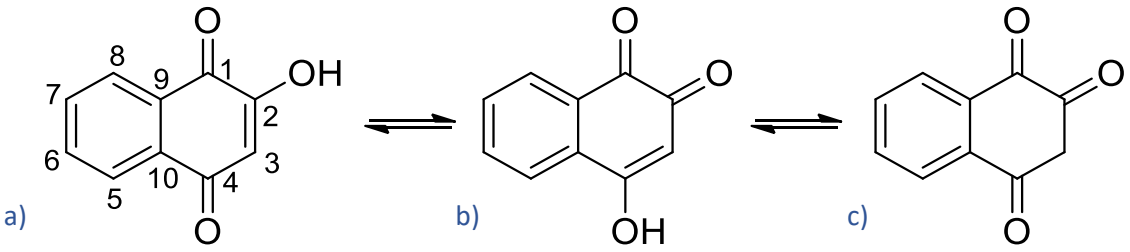


Figure 1.7. Enol-keto tautomerism displayed by lawsone, showing a) 1,4-naphthoquinone; b) 1,2-naphthoquinone; and c) 1,2,4-naphthotrione tautomers.

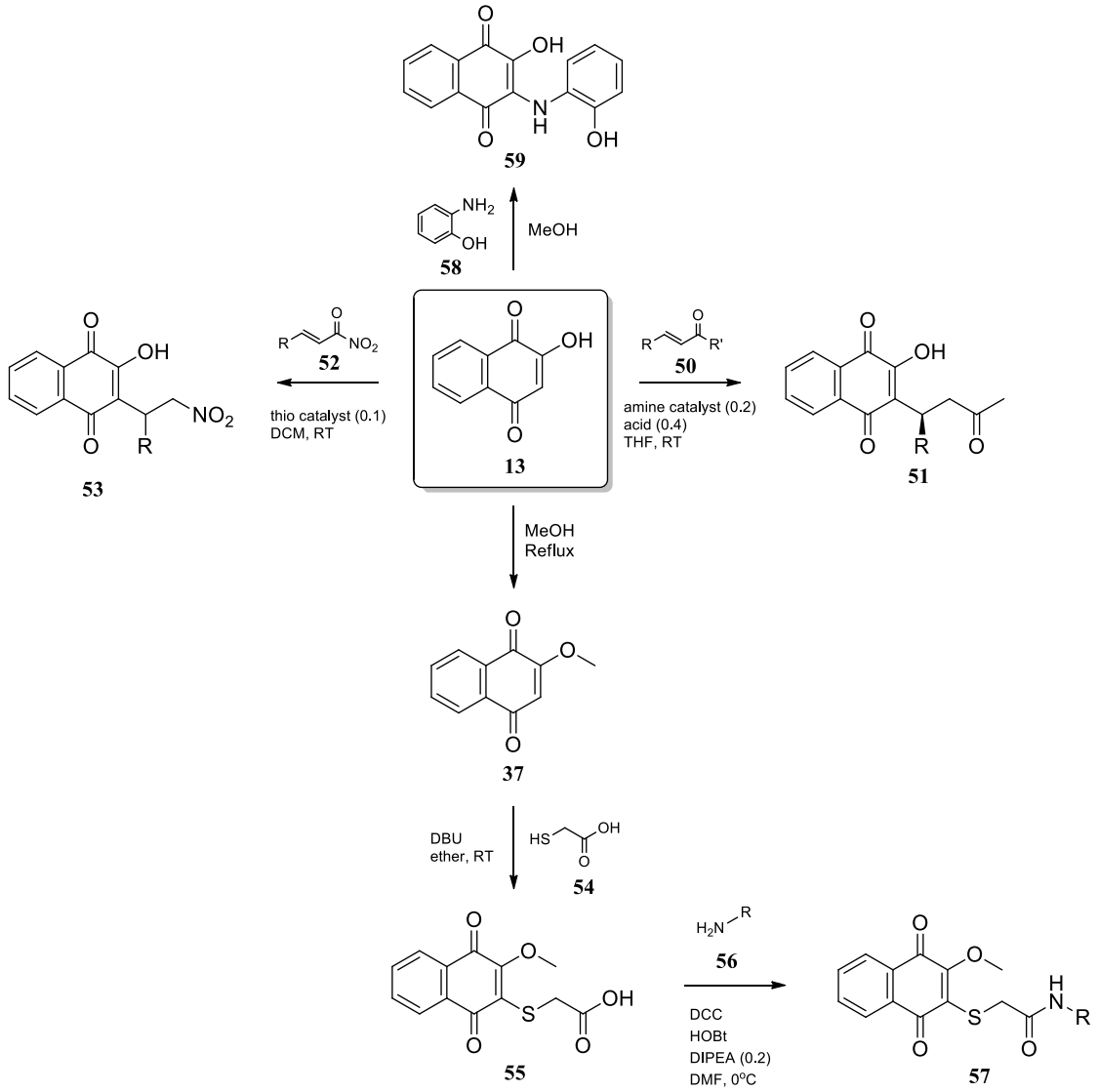
The biological activity and medicinal properties of lawsone have been found to be enhanced through carbon chain extension and the introduction of amines, aromatic and heterocyclic rings, and halides at the 3-position. Annulation at the 2-, 3- and 4- positions, as well as bis-arylation, have also been explored. Alkylation at the 3- position has been achieved through Michael addition, Knoevenagel condensation, and the Mannich reaction. Amination via Strecker degradation has also been reported for quinones. A brief overview of each of these is given below.

Michael addition with lawsone

Lawsone's enone characteristics allow for nucleophilic attack at the 3- position to form single carbon bonds using Michael addition chemistry (Scheme 1.11). The addition of α,β-unsaturated ketones (**50**)[83] and α,β-unsaturated nitroalkenes (**52**)[84] to the lawsone molecule have been reported to introduce extended carbon chain or aromatic functionality. Thio- and amine-based organocatlysts such as thiourea and cinchonidine were used in the activating step of deprotonation of the ketone or nitroalkene. Sreelatha *et al.*[85] and Tandon *et al.*[86] have also reported the synthesis of thio-amide derivatives (**57**) of lawsone using similar methods. The synthesis by Sreelatha *et al.*[85] also involved a protection step to maintain the -OH functionality by first methylating this position through heating under reflux with acidic methanol to form **37**. This alkylation at the 2- or 4- position to form ethers is well reported, often employing alkyl halides in the presence of a base such as potassium carbonate with heating under reflux.[64]

Additionally, lawsone has been used as a model in the presence of aminophenols (**58**)[87] (Scheme 1.11) to mimic the interactions between 2,4,5-trihydroxyphenylalanine quinone (TPQ), an amine oxidase cofactor with significant biological importance,[88] and amines to allow for further understanding of its biological activity. The reactions resulted in the synthesis of 2-(*o*-hydroxy-anilino)-1,4-naphthoquinone derivatives (**59**), whose X-ray crystal structure, ^{1}H NMR spectra, and

cyclic voltammetry spectra were recorded for further consideration of their association with amine oxidases.

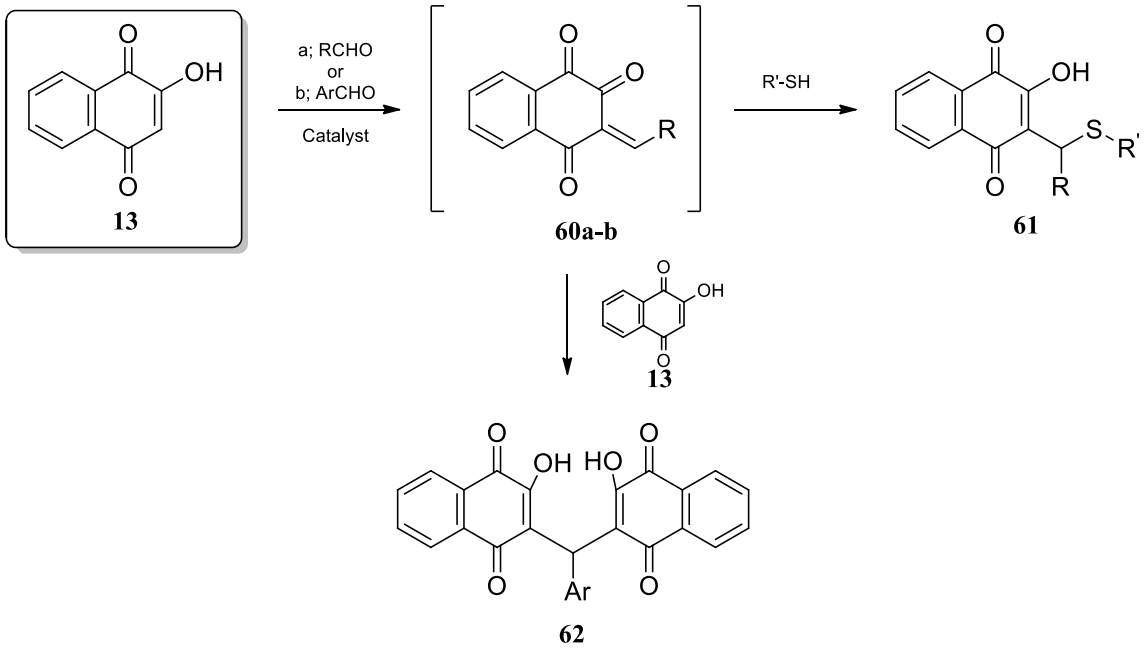


Scheme 1.11. Michael addition of α-β unsaturated and thio reagents to lawsone.[83–85]

Knoevenagel condensation

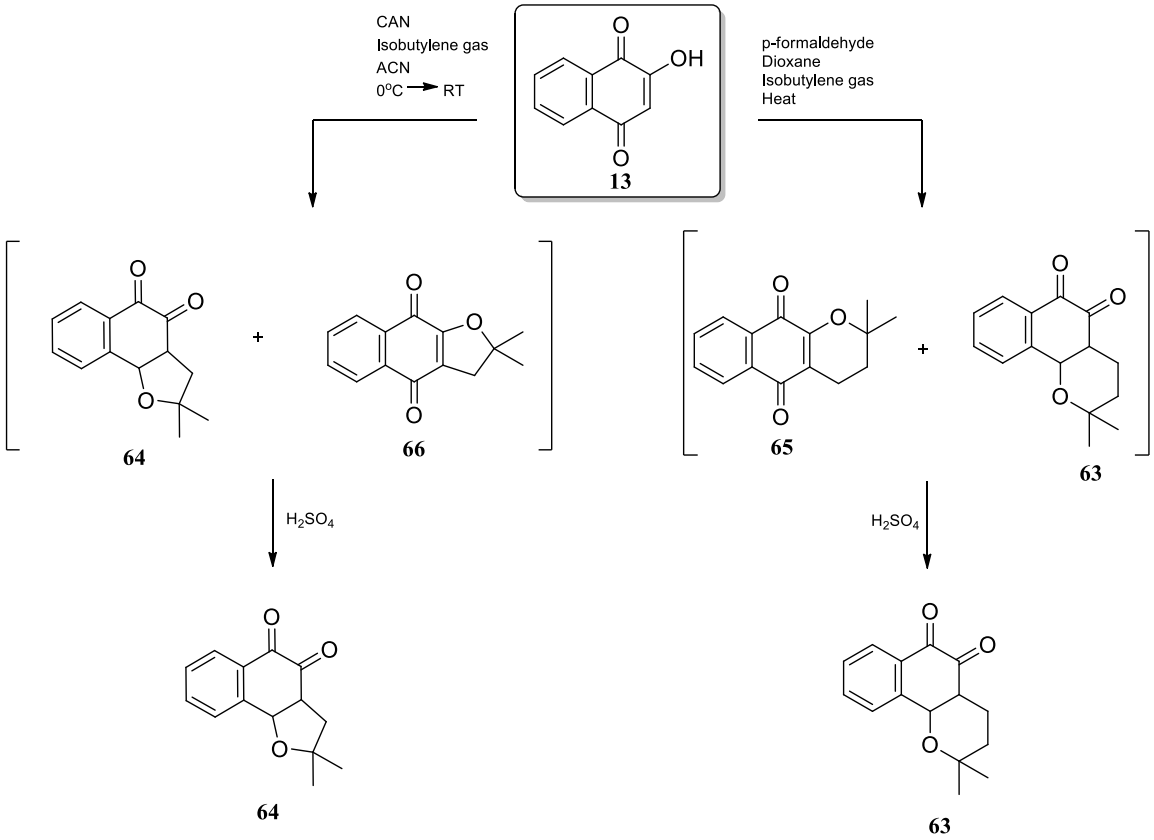
Lawsone in the 1,2,4-naphthotrione tautomer contains an active hydrogen, meaning it can partake in Knoevenagel condensation with other carbonyl-containing compounds (Scheme 1.12). This method, sometimes in combination with Michael addition, has been used to form derivatives with chain extension at the 3- position, dimers, and pyranonaphthoquinones. Sharma *et al.*[89] produced thio-containing lawsone derivatives (**61**) through a combination of Knoevenagel condensation and Michael addition. The synthesis first produced a methide intermediate **60** using an appropriate aldehyde via condensation, followed by Michael addition of the chosen thiol.

Dimeric products (**62**) of lawsone have been synthesised following a similar method via Knoevenagel condensation and subsequent Michael addition between lawsone and aromatic aldehydes.[90,91]



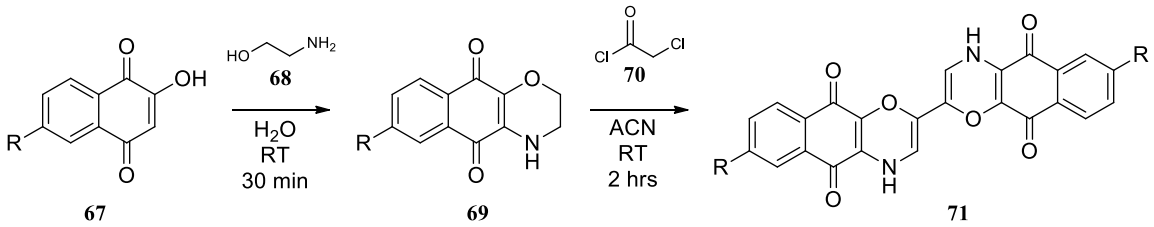
Scheme 1.12. Knoevenagel condensation of lawsone with aldehydes and subsequent Michael addition or dimerization.[89–91]

Pyranonaphthoquinone derivatives involving ring formation at the 3- and OH- position have been successfully synthesised.[92,93] β-lapachone (**63**) and nor-β-lapachone (**64**) have been constant targets using these methods, as well as the α- forms (**65** and **66**). Ferreira *et al.*[94] performed a one-pot synthesis of β-lapachone **63** via Knoevenagel condensation of lawsone with paraformaldehyde and isobutylene, and of nor-β-lapachone **64** via ceric ammonium nitrate (CAN) -induced radical cyclisation of lawsone with isobutylene (Scheme 1.13). Both α- and β- forms were reported as been prepared and present in the crude mixture, however successful treatment of the crude mixture with sulfuric acid allowed for acidic hydrolysis of the α- form and interconversion to the β- form, resulting in the isolation of the β- forms **63** and **64** exclusively in good yield (80%). Similarly, da Rocha *et al.*[95] and Jimenez-Alonso *et al.*[96] have described the microwave-assisted one-pot synthesis of α- and β-lapachone derivatives through Knoevenagel condensation followed by hetero Diels-Alder cycloaddition between lawsone derivatives and unsaturated aldehydes.



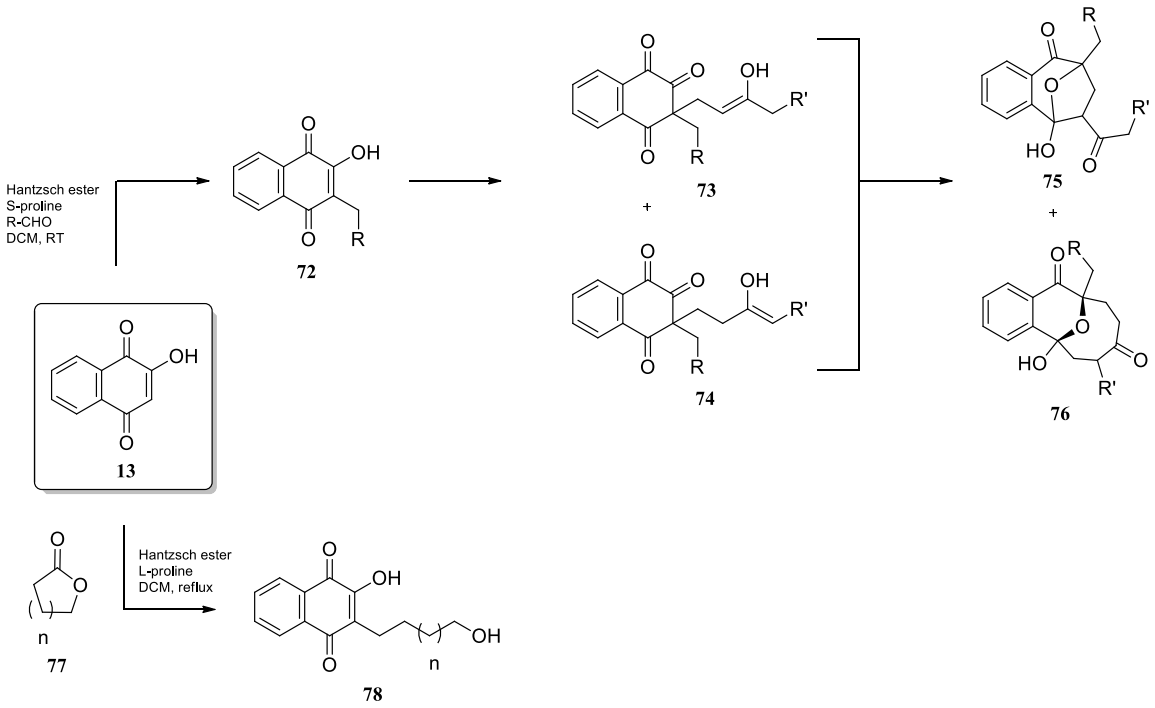
Scheme 1.13. Synthesis of β-lapachone (**63**) and nor-β-lapachone (**64**), as reported by Ferreira *et al.*[94]

Other ring formations including nitrogen have also been reported. For the purposes of creating new lawsone derivatives for exploration into their potential medicinal benefits, Elavarsan *et al.*[97] investigated the reaction between lawsone derivatives **67** and ethanol amine **68** in water. Instead of the expected N-alkylation at the 3-position, the authors observed ring cyclisation of the ethanol amine at the 3- and O- positions of the lawsone (**69**). Further reaction of this cyclised compound with chloroacetylchloride (**70**) in acetonitrile produced a dimeric structure **71** (Scheme 1.14). No reasons or mechanisms were provided for the ring cyclisation or further dimerisation.



Scheme 1.14. Reaction of lawsone derivatives **67** with ethanol amine (**68**) to produce **69**. Further reaction with chloroacetylchloride (**70**) produced the dimeric structure, **71**.

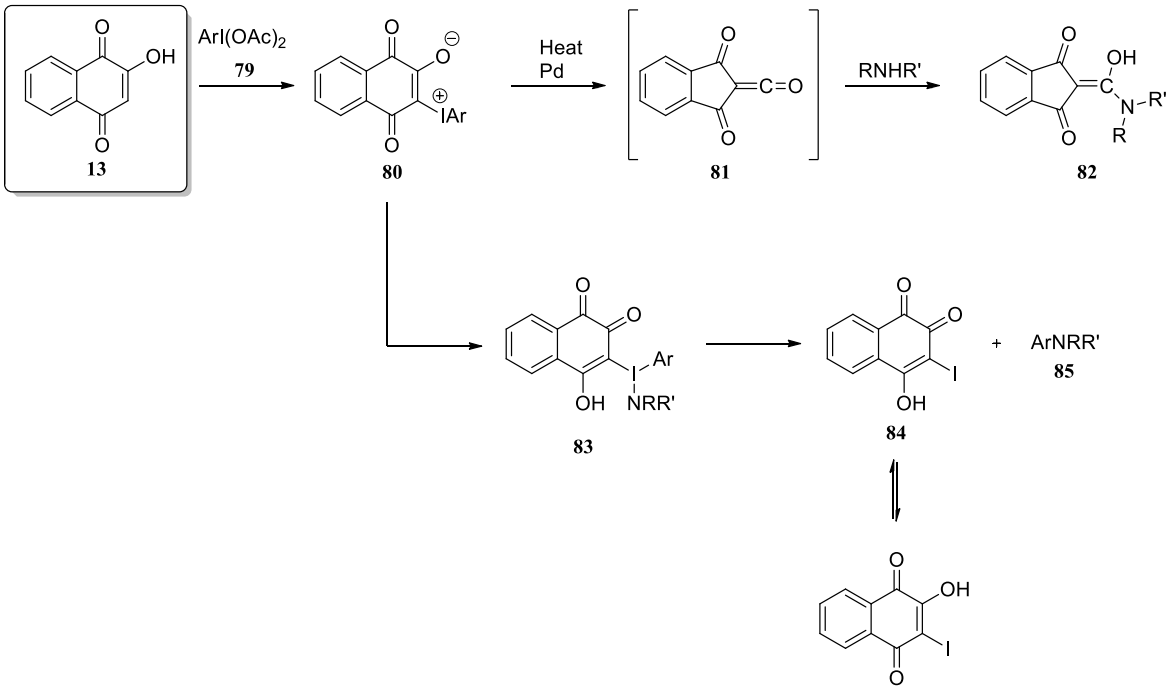
Reductive alkylation of lawsone with aldehydes has also been achieved, primarily through the use of Hantzsch esters and a proline catalyst. Ramachary *et al.*[98] performed this reaction as an initial step (**72**) towards the synthesis of privileged bicyclo[3.2.1]octanes **75** and **76** using S-proline, while Kim *et al.*[99] performed chain extension at the 3-position of lawsone (**78**) via reductive alkylation of ring-opened lactols (**77**) using L-proline (Scheme 1.15).



Scheme 1.15. Reductive alkylation of lawsone with Hantzsch esters and L or S-proline.[98,99]

Ylide formation from lawsone

The hydroxyl group of lawsone makes it a good candidate for conversion to ylides.[76] Iodonium ylide forms of lawsone (**80**) have been prepared via reaction with bis(acetoxy)iodoarenes (**79**), which were then further reacted with amines (Scheme 1.16).[100,101] Under thermal conditions using Pd catalysts, ring contraction was observed to form indandione carboxamides (**82**). Using Cu(II) catalysts with the lawsone ylide offered arylation of the amine (**85**) and **84**.

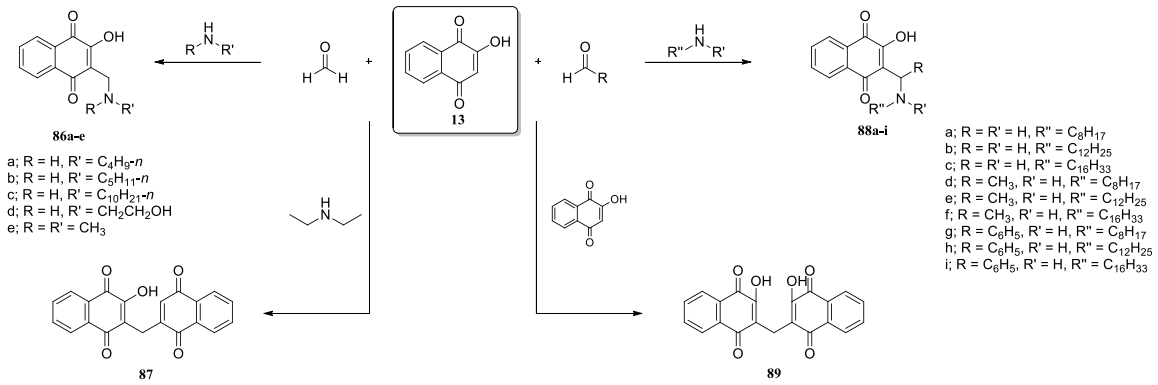


Scheme 1.16. The reaction of lawsone ylides **80** with amines using Pd or Cu(II) catalysts, resulting in indandione carboxamides **82** or amine arylation **85**.[100]

Mannich reactions

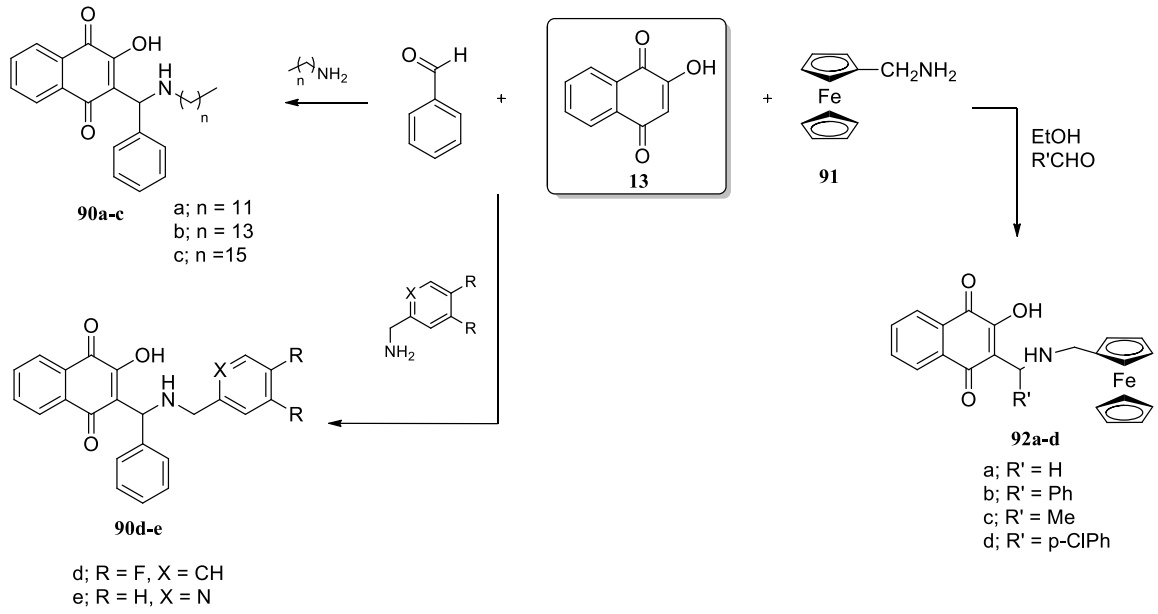
It has been reported that the introduction of nitrogen-containing groups within the lawsone structure at the 2-position enhances its anti-cancer activity.[102] Consequently, researchers have thoroughly investigated the synthesis of nitrogen-containing analogues of lawsone via the Mannich reaction. Classically, an iminium ion is formed via condensation of the aldehyde and amine, which then undergoes nucleophilic attack from the enol form of lawsone. This results in the formation of a β-amino-carbonyl product known as a Mannich base. The formation of a Schiff base from a Mannich base is possible through dehydration.

Leffler and Hathaway[103] were the first to report the use of the Mannich reaction involving lawsone, using formaldehyde and several primary and secondary amines (Scheme 1.17). All primary amines and most secondary amines such as dimethylamine and piperidine gave good yields (87 – 96%) of the associated Mannich base lawsone analogues **86a-e**. However, it was found that diethylamine failed to produce the Mannich base, instead producing methylene-bis derivative **87**. Dalgliesh[104] expanded on the work performed by Leffler and Hathaway, using higher primary and secondary aliphatic amines and aldehydes to include formaldehyde, benzaldehyde and acetaldehyde for the formation of **86a-i** (Scheme 1.17). Methylene-bis-lawsone analogues **89** were also observed when higher secondary amines were used.



Scheme 1.17. Synthesis of Mannich bases **86a-e** and **88a-i** and methylene-bis-lawsone analogues **87** and **89** by Leffler and Hathway[103] (left) and Dalgliesh[104] (right).

Mahal *et al.*[105] and Nariya *et al.*[106] successfully synthesised Mannich bases **90a-e** using a one-pot method with lawsone, substituted aromatic aldehydes, and amines containing phenyl, heterocyclic, short chain alkenes or alkanes, or substituted alkane side chains (Scheme 1.18, left). Baramee *et al.*[107] has reported ferrocenic atovaquone derivatives **92a-d** by employing the Mannich reaction with lawsone, aldehydes and ferrocenyl amines **91** (Scheme 1.18, right).



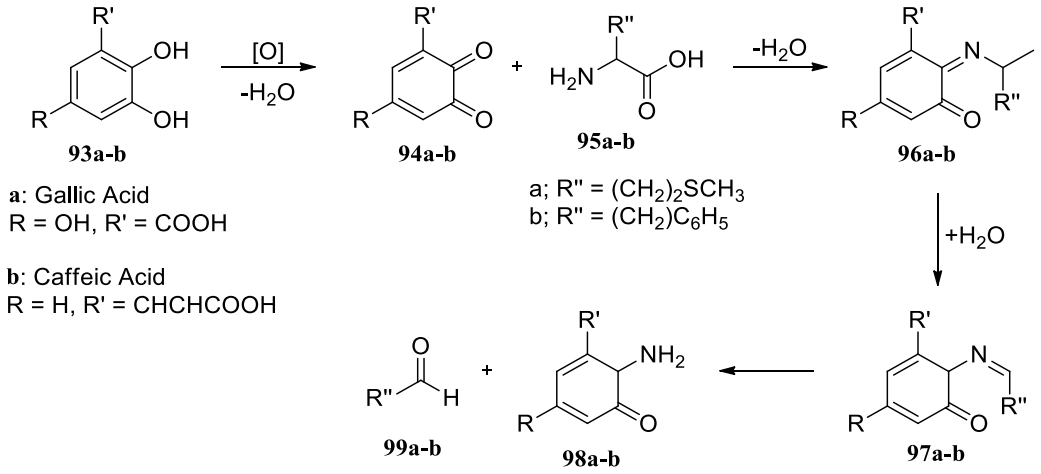
Scheme 1.18. Synthesis of Mannich bases **90a-e** and **92a-d** using amines containing heterocyclic or extended alkane chains (left)[105] and ferrocenyl amines (right).[107]

Strecker degradation

Based on the known ninhydrin reaction chemistry, Jelly *et al.*[5] postulated that a Strecker degradation mechanism may be involved in the formation of the red compound identified in their fingerprint research. However, despite numerous literature search attempts in this research, specific research examining the reaction chemistry of lawsone with amino acids was unable to be found. The general chemistry of the reactions of quinones with amino acids is discussed below.

It was first reported by Schönberg and Moubacher[108] that α-amino acids undergo Strecker degradation in the presence of quinones. The first step of Strecker degradation involves the nucleophilic attack of the -NH_2 from the amino acid at a carbonyl on the quinone to form an imine. This is followed by decarboxylation and subsequent hydrolysis of the imine to produce an aldehyde, known as a Strecker aldehyde, and an α-amino carbonyl.

The interaction of quinones in general with amino acids has been studied extensively in the food industry, as these reactions can influence characteristics such as the smell and taste of the product.[109] The importance of the Strecker degradation between α-amino acids and *o*-quinones in wine was confirmed by Oliveira *et al.*[110] The authors determined that polyphenols with galloyl (**93a**) or catechol (**93b**) groups were readily oxidised to *o*-quinones **94a-b**, which then reacted with methionine **95a** and phenylalanine **95b** to produce the associated Strecker aldehydes; methional **99a** and phenylacetaldehyde **99b** (Scheme 1.19). The production of these Strecker aldehydes has been attributed to the off-flavour and therefore spoilage of wines. It has been found that thiol components can assist to prevent the production of these aldehydes by preferentially reacting with the *o*-quinones **94** instead.[111]



Scheme 1.19. Oxidation and subsequent Strecker degradation of gallic (**93a**) and caffeic (**93b**) acid to produce the associated amine (**98a-b**) and Strecker aldehyde products (**99a-b**).

1.4. Project Aims

Since initial research in 2008, lawsone has scarcely been successfully implemented as a useful reagent for latent fingermark elucidation. The reaction chemistry is poorly understood, thus impacting the poor reproducibility of the observed fluorescence by Jelly *et al.* [5–8], as well as its effectiveness compared to other, already established reagents. The structure of the red compound proposed to be responsible for the observed fluorescence has been investigated, however two contrasting structures have been reported.[6,28] The reaction between lawsone and amino acids to form this red compound has also not been elucidated, nor fully optimised. In order to determine lawsone's use as an effective fingermark reagent, the fluorescent compound needs to be identified and the reaction conditions optimised for reliability. The aim of the following experiments were to:

1) Unambiguously determine the structure of the red product that was identified by Jelly as being the cause of the required red fluorescence
2) Identify the role of the reagents in the formation of the red fluorescent product
3) Propose a reaction pathway towards the formation of the red fluorescent product

Chapter 2 details the replication of Jelly's initial work to address aim 1) through the isolation and identification of the red product using NMR and MS analysis. Chapter 3 will address aim 2) by using analogues of glycine and ethanol to determine their role in the reaction. Chapter 4 will also address aim 2) through the use of a lawsone analogue to identify the reaction sites of the involved reagents. Chapter 5 details the synthesis and reaction of intermediates proposed in earlier chapters, therefore addressing aim 3).

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2. Elucidation of Fluorescent Compounds

2.1. Introduction

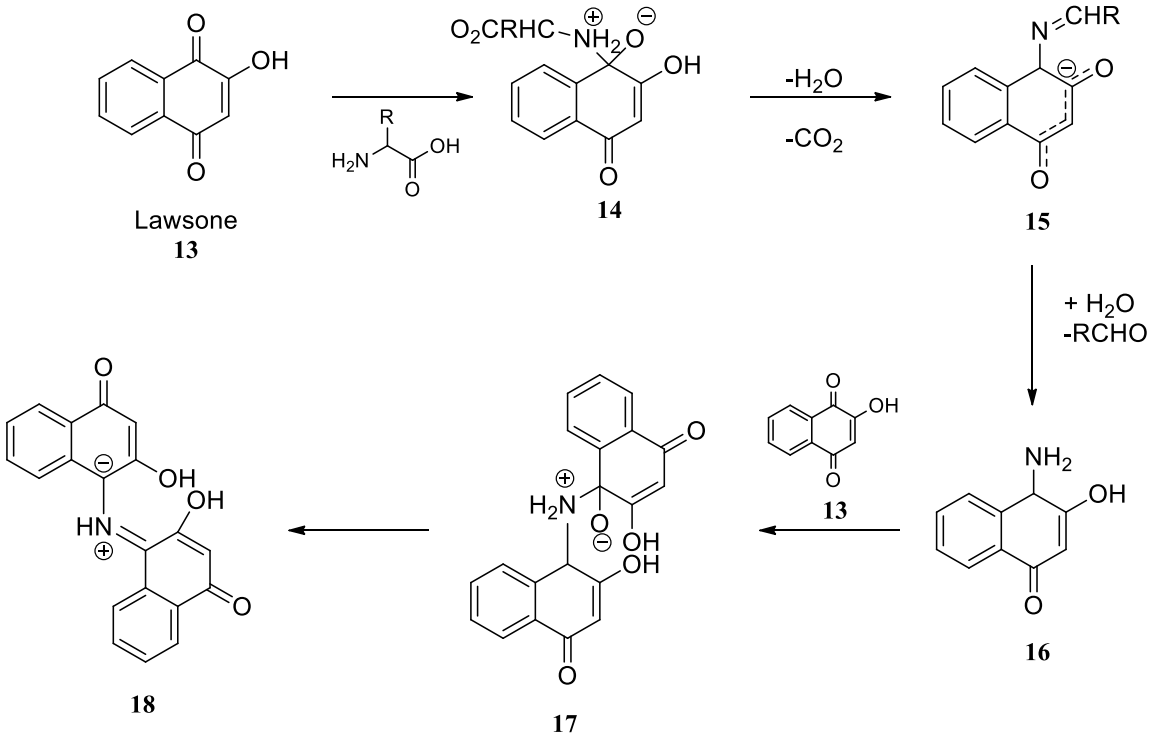
Research is ongoing to find new fingerprinting reagents that can be applied to a variety of latent fingermarks and substrates. Particular substrates, such as paper and blood, prove to be challenging due to the high degree of background signals and possibility for reagent interaction with other compounds in the complex chemical matrix.[1] In 2008, Jelly *et al.*[2,3] were prompted to investigate lawsone as an alternative and novel reagent for the detection of latent fingermarks on porous surfaces as a result of research conducted by Almog *et al.*[4,5] into the use of genipin. Almog *et al.*[4,5] found that genipin had advantages over the commonly used ninhydrin, as the resulting blue stain upon reaction of genipin with amino acids was more stable, safer to the user and environmentally friendlier, and produced photoluminescent emissions that did not overlap with background signals on certain substrates.

Jelly *et al.*[2,3] showed that lawsone reacts with fingerprint deposits on paper, turning the fingermark a purple-brown colour with red luminescent properties. Since this report, several attempts have been made to replicate these experiments and the reported fluorescence results on porous surfaces, however they have mostly been unsuccessful. Berdejo *et al.*[6] reported observing very weak fluorescence of lawsone treated latent fingermarks on paper, and determined the fluorescence was weaker than pre-existing methods utilising 1,8-diazofluoren. The researchers also claim that fluorescence peaked after five minutes at 170°C and was not improved with longer heat exposure times. Thomas and Farrugia[7] attempted to apply lawsone detection to fingermarks in blood, however they could not obtain a fluorescent or even visual latent fingermarks while following methods reported by Jelly *et al.*[2,3] to be used as a positive control without blood. Dalrymple and Almog[8] reported very weak fluorescence using a 557 nm laser when investigating the application of lawsone to latent fingermarks on bond and brown paper. These contrasting results show that more investigation is required into whether lawsone can be reliably used as a fingermark elucidating agent.

By reacting solutions of lawsone and amino acids on filter paper, Jelly *et al.*[2,3] showed that the fluorescent product was likely to result from amino acids in the fingerprints. In an attempt to identify the luminophore, synthetic studies were undertaken where lawsone and glycine (as a model amino acid) were reacted in a two-to-one ratio in ethanol and heated under reflux for two days. The resulting purple precipitate was separated using column chromatography (no reported R_f values) and two products isolated; a yellow and red product. Fluorescence measurements indicated that the red product was the likely source of the observed fluorescence on paper.

Attempts at characterisation of the isolated products were reported by Jelly,[9] but the results were inconclusive. The isolated yellow product was analysed by GCMS, and the resulting MS spectrum was compared to a library where it was identified as 2-amino-1,4-naphthoquinone (**102**) based on a [M+H] of 173. However, no other evidence was presented to support this structure. The isolated red product was analysed by ATR-FTIR, NMR, and GCMS. They noted two peaks from the IR spectra, at 2900 and 1650 cm^{-1} pertaining to an aliphatic alkyl chain and a C=O stretch respectively, suggesting that some of the lawsone had been retained in the product structure. Analysis of the ^{1}H and ^{13}C NMR spectra revealed that there appeared to be the presence of a secondary ethyl group due to the coupling of a -CH group at 4.65 ppm to a $-CH_3$ group at 1.31 ppm, however could not rationalise its presence in the final structure nor identify the source of this group in the reaction mixture. They also observed that the lawsone sub-structure was present, with the peak corresponding to the vinyl proton now absent in their red product. It was also observed from ^{13}C NMR that the structure had at least twelve carbon environments. Upon closer examination of the ^{1}H NMR spectra they concluded that the structure must consist of two lawsone fragments due to the doubled integration of the aromatic protons in relation to the ethyl protons. They reported that no suitable MS data could be obtained.

Jelly *et al.* hypothesised a structure and mechanism of formation for this red product **18** as shown in Scheme 2.1,[2] and is based on the reaction between ninhydrin and amino acids.[10] The authors proposed that lawsone undergoes a Strecker degradation to form a Schiff base intermediate at the 1-position (**15**). This subsequently forms an amine intermediate **16**, which undergoes condensation at the -N position with a second molecule of lawsone to form the hypothesised dimer **18**.



Scheme 2.1 Hypothesised mechanism and structure for the compound **18** responsible for fluorescence, as proposed by Jelly *et al.* and reported in Chemical Communications, 2008. [2]

Interestingly, the proposed dimer **18** in Scheme 2.1 seems to contradict observations made from their NMR data, as this structure does not incorporate the ethyl group that was observed in the ^{1}H NMR spectrum. The structure also retains the vinyl position of the lawsone fragments despite the reported absence of the associated peaks in the ^{1}H NMR spectrum. The structure also does not account for the 12 carbon environments as reported in their ^{13}C NMR spectrum. Thus, the hypothesised structure and mechanism appears to be incorrect.

This chapter describes experiments undertaken to elucidate the structures of three distinctly coloured compounds isolated from the reaction between lawsone and glycine, and resolves discrepancies reported by Jelly *et al.*[2,3] regarding the identity of the red compound. The red, yellow, and orange compounds that were isolated were each purified and characterised by IR, UV-Vis, MS, and NMR spectroscopy, and new structures proposed.

2.2. Results and Discussion

Preliminary experiments with the view of using lawsone for the detection of amino acids and biogenic amines were performed according to Jelly *et al.*[2,3], where aqueous solutions of various amino acids or biogenic amines were first pipetted onto filter paper followed by lawsone solution in either ethyl acetate or ethanol and placed in an oven at 170°C for 1-2 hours. The resulting light brown stains were visualised through orange goggles using a polilight at excitation wavelengths of 490-555 nm, however very-weak-to-no fluorescence was observed for the biogenic amines and amino acids used. The experimental for these preliminary experiments can be found in Appendix 1. Consequently, it was decided to investigate the reaction on a larger scale with the aim of confirming the identity of the products and improving their yields.

Initial synthesis was undertaken as described by Jelly[9], where lawsone and glycine were reacted in a 2:1 ratio in ethanol with heating under reflux for between two days to a week. A purple precipitate was obtained in accordance with Jelly.[9] Analysis using HPLC-MS was performed on the crude purple precipitate (Appendix 1), which showed a wide range of peaks, indicating a complex mixture of products. The identity of any of these products could not be confirmed from the MS data available.

Several solvent systems were trialled to appropriately separate the components of the reaction mixture by silica gel column chromatography in order to obtain pure samples for further characterisation. A variety of systems comprising one or more of hexane, dichloromethane, ethyl acetate, ether, toluene, and further additives were tested and are summarised in Table 2.1. TEA was used as it can interact with the silica, which causes less interaction between the silica and the mixture components, allowing for slightly more movement of these compounds along the TLC plate or the column. As most of the compounds in the reaction mixture were highly polar, the TEA was added in small amounts to the solvent system and all silica was pre-treated with this mixture in order to further move these compounds from the baseline. The majority of solvent systems outlined in Table 2.1 were not suitable for purification due to a high level of co-elution and streaking of compounds along the TLC plate. 2D TLC separation was also attempted using a combination of these systems, however did not improve the separation of compounds. As a result, the separation of each compound was difficult due to the polarity similarities of compounds in the reaction mixture, as evidenced by impurities noticed in the NMR spectra. The best separations were achieved using a combination of hexane and ethyl acetate in a 4:1 ratio, with 0.1% TEA added.

The separation system employing a 4:1 mix of HX:EtOAc with 1% TEA on silica gel yielded distinct red (**100**, Figure 2.9) yellow (**101**, Figure 2.15), and orange (**102**, Figure 2.22) products. Compounds **100** and **102** were reported by Jelly[9], while **101** is a newly reported compound. The red compound **100**

was identified by Jelly *et al.* as being the dimer responsible for the observed fluorescence, and consequently, the research was mainly targeted towards purifying and identifying this product. The spectral analysis and structure elucidation of these products are discussed in the following sections.

Table 2.1 Solvent systems trialled for the appropriate separation of the reaction mixture resulting from the reaction between lawsone and glycine.

HX (%)	DCM (%)	Et_2O (%)	EtOAc (%)	Toluene (%)	TEA (%)	MeOH (%)
100						
	100				0; 0.1	
		100				
			100			
80			20		0; 0.1	
80	20					
70	30					
	50	50			0; 0.1	
	20	80			0; 0.1	
90			10		0; 0.1	
80		20				
90		10				
			12.5	87.5		
	90				0.1	10
	97.5				0.1	2.5
	95		5			

Compound **100** was isolated from the purple precipitate in yields below 1% after long reaction times of a week, and it was thought the use of a microwave may be useful to obtain higher yields in a shorter time period. Microwave synthesis also produced a purple precipitate and compound **100** was isolated in similar yields, however microwave synthesis was employed in favour of a standard reflux set-up. Higher temperatures of 130°C as opposed to 80°C could be reached due to the high-pressure environment, and the same precipitate can be obtained in a much shorter time period of only a few hours. Temperature could therefore be considered as a potentially important factor in the formation of compound **100**, and could be investigated in future work. It is worth noting that previous research using lawsone was conducted at 150°C or higher on a hot plate or in an oven[2,6,7,11], and so using a microwave would better replicate these temperature conditions instead of a standard reflux setting.

2.2.1. Spectral analysis and structure elucidation of the red product (100)

The isolated red compound **100** was characterised using UV-vis, fluorescence, IR, NMR, and mass spectrometry. The UV-vis spectrum, displayed in Figure 2.1, exhibits peaks at 275.6, 330.5 (shoulder), and 515.5 nm. The strong absorbance at 275.6 nm indicates that the structure is likely to contain some aromatic functionality. The absorbances in the visible region indicate that there may be some level of conjugation in the structure. Solution based fluorescence spectrophotometry performed in chloroform resulted in excitation and emission maxima of 520 nm and 615 nm respectively (Figure 2.2). These values were lower than those reported by Jelly *et al.* who reported that the red product responsible for the observed fingermark fluorescence had excitation and emission wavelengths of 590 nm and 645 nm respectively. It is likely that the lower experimental values found in this study were collected as a result of differing experimental protocols. In this work the compound was dissolved in chloroform, however Jelly *et al.* obtained their fluorescence values from the dried compound on a paper substrate. Chloroform is a polar solvent that can lower the energy difference between the ground and excited state of the analyte, leading to a lower emission wavelength. Additionally, Jelly *et al.* reported their fluorescence values from a crude complex reaction mixture, whereas the experimental values obtained were from the isolated and relatively pure sample of compound **100**. Other compounds in the crude mixture may have also contributed to the overall fluorescence activity.

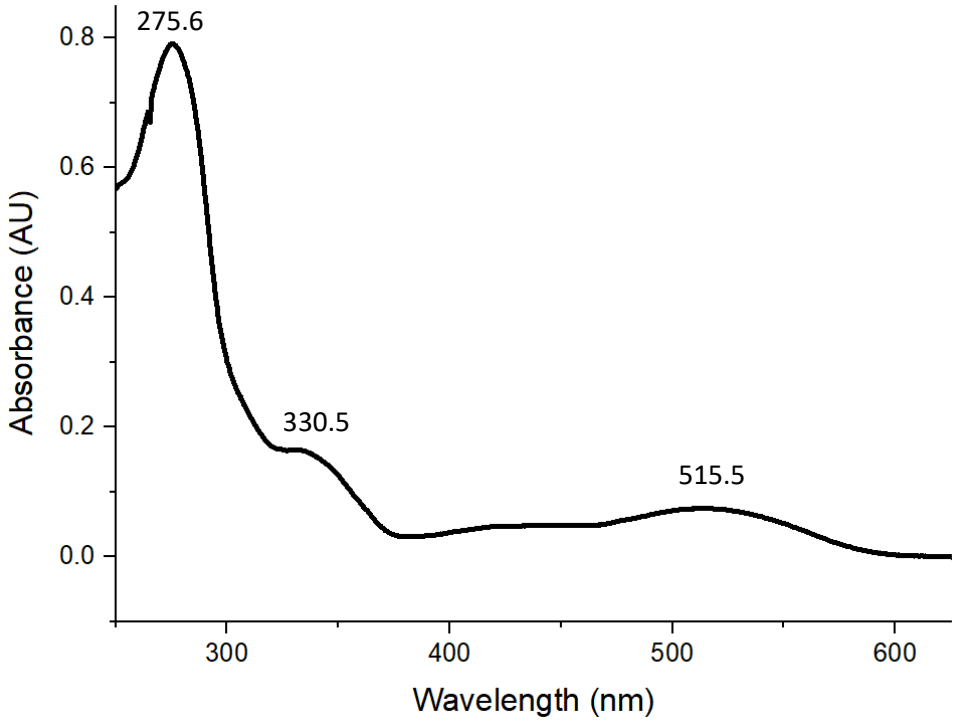


Figure 2.1 UV-Vis spectrum of compound **100**, showing the absorption wavelengths of 275.6, 330.5 (shoulder), and 515.5 nm.

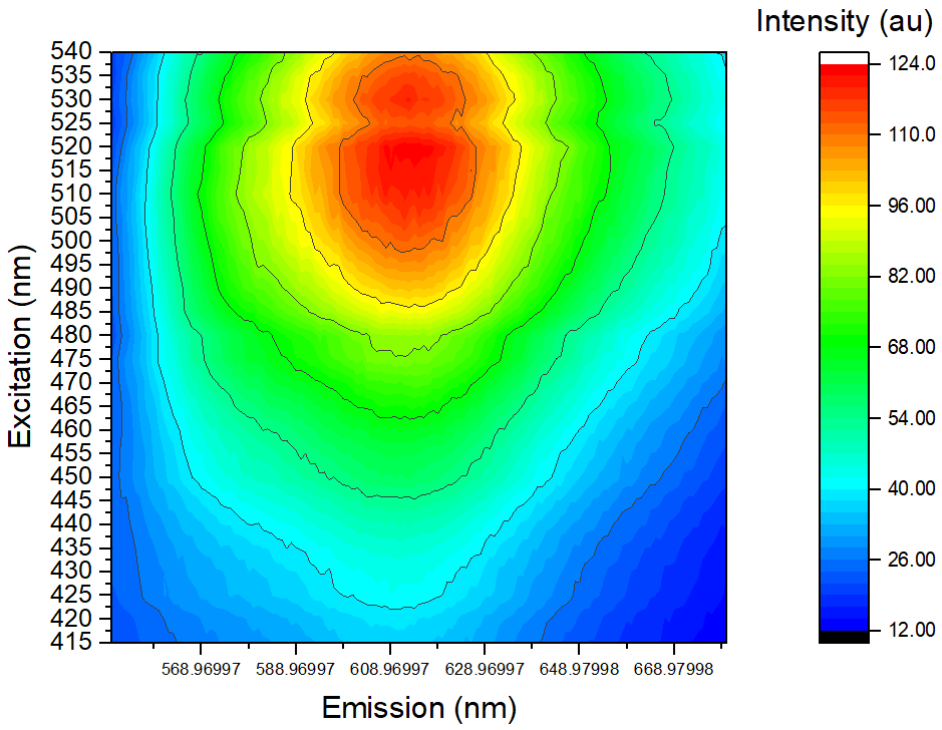


Figure 2.2 3D fluorescence spectrum of compound **100**, showing the excitation and emission wavelengths of 520 nm and 615 nm.

The IR spectrum for compound **100**, as seen in Figure 2.3, exhibited peaks at 3370 and 1681 cm^{-1}, most likely correlating to an -NH stretch and a -C=O stretch respectively. The other peaks were attributed to various C-H and C-C stretching that could be present in the molecule.

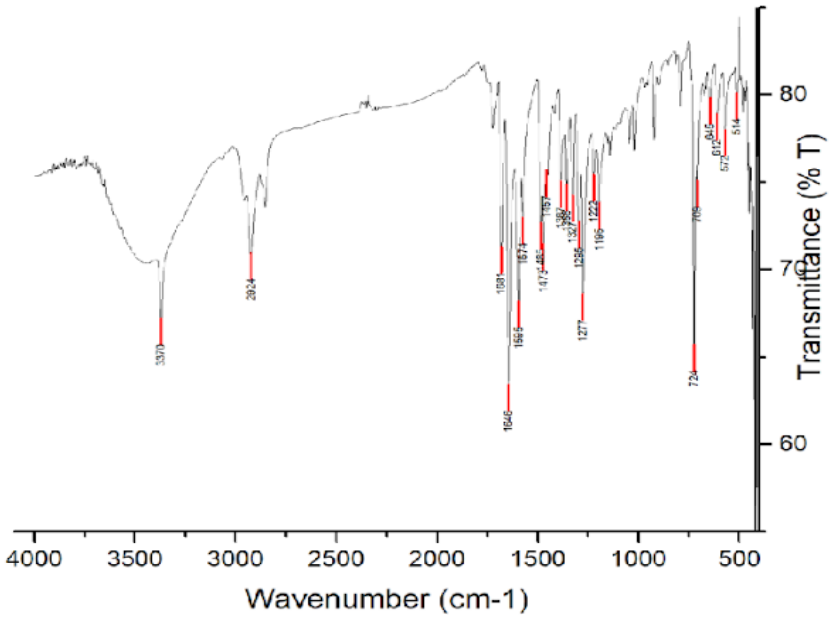


Figure 2.3 IR spectrum of compound **100.**

^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR spectra were obtained from compound **100** and can be viewed in full in Appendix 1. The ^{1}H and ^{13}C NMR shifts for this red product are reported in Table 2.2, however these can be visualised in Figure 2.4 and Figure 2.7 respectively. These NMR spectra are consistent with the spectra reported by Jelly *et al.* for their isolated red product and suggests that the same compound was isolated.

The proton NMR spectrum revealed the compound to have at least seven different proton environments. Peaks at 8.14 ppm, 8.11 ppm, 7.77 ppm, and 7.70 ppm most likely correspond to protons existing in an aromatic environment, and each of these peaks integrates for two equivalent protons. The two peaks at 8.14 ppm and 8.11 ppm are doublets, indicating that these protons are next to a carbon environment containing only one proton, while the peaks at 7.77 ppm and 7.70 ppm are both triplets which indicate these protons are next to a carbon environment containing two protons.

In order to determine more about the product structure, the ^{1}H and ^{13}C NMR spectra of Compound **100** was compared to that of the starting reagent, lawsone (Figure 2.4 and Figure 2.7 respectively). The NMR spectra and peak assignment for lawsone can be found in Appendix 1. The aromatic peaks observed in the lawsone spectrum between 8.12 ppm and 7.33 ppm were also observed in the red product, but their position had slightly shifted. This suggests that the aromatic structure from lawsone has been retained in the product but that the surrounding environments have changed. Comparison of the integration of the aromatic peaks revealed an integration of one in lawsone and two in

compound **100**. This suggests that there are two similar aromatic environments in the red compound **100**.

There is also a singlet peak at 6.37 ppm observed in the lawsone spectrum that was attributed to the proton at the H-3 (vinyl) position, that is now absent in the product spectrum. This suggests that a reaction has taken place at this vinyl- position. Similarly, the peak at 7.34 ppm correlating to the OH group in lawsone is now absent in the product NMR. This suggests structural fragment as seen in Figure 2.5, where a lawsone fragment is present with other substituents at the C-2 and C-3 positions.

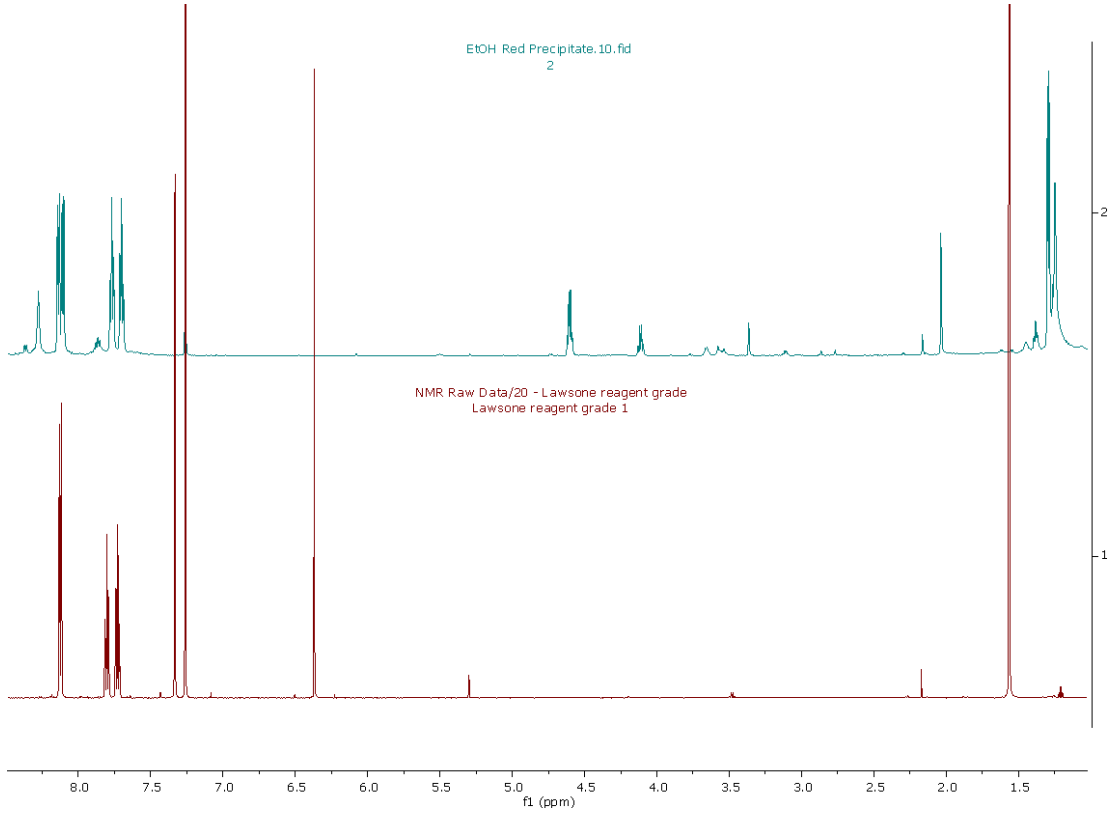


Figure 2.4 ^{1}H NMR spectrum of the obtained compound **100** (top, green) and lawsone (bottom, red).

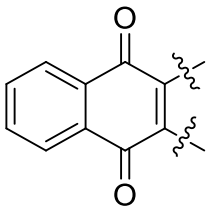


Figure 2.5 Possible structural fragment originating from the lawsone reagent present in Compound **100.**

There is a broad singlet at 8.28 ppm that integrates for one proton, with the broad shape suggesting that this most likely originates from a -NH environment, and the singlet pattern suggests that there are no protons directly adjacent to this environment. A peak at 4.60 ppm with a quartet splitting pattern most likely pertains to a highly deshielded methyl group that is next to a highly electronegative atom. The integration indicates that there is only one proton in this environment, therefore this peak was attributed to a -CH group. A smaller quartet is observed at 4.12 ppm that was unable to be removed after further purification, and was attributed to a product with similar moieties and physical properties to the major compound. There is an additional doublet at 1.29 ppm corresponding to a methyl group which integrates for three protons, indicating that it is not surrounded by electronegative atoms. This is most likely a $-CH_3$ group in an alkyl chain. These proton signals are observed by Jelly *et al.* but were not accounted for in their proposed structure. The peaks at 4.60 ppm and 1.29 ppm in the spectrum for compound **100** were not observed in the lawsone spectrum, indicating that these are new additions to the naphthoquinone structure. It is therefore suggested that other structural fragments within Compound **100** include a secondary amine, as well as a secondary ethyl group, seen in Figure 2.6.

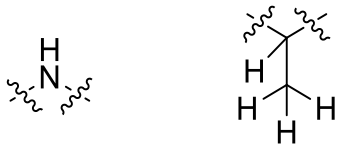


Figure 2.6 Possible secondary amine and ethyl group fragments present in Compound **100.**

The ^{13}C NMR reveals that the red compound **100** has at least twelve equivalent carbon environments. Two of the carbon signals are within the alkyl region, at 22.06 ppm and 24.92 ppm, while the rest are within the aromatic/allylic region. Peaks at 185.06 ppm and 182.09 ppm in the lawsone NMR correlate to the carbons at the C-1 and C-4 positions, and are observed slightly downshifted in the product NMR at 182.37 ppm and 179.24 ppm. This indicates that these carbons from the lawsone molecule have been retained in the product structure, however a reaction has occurred at a position near them to cause the downshift.

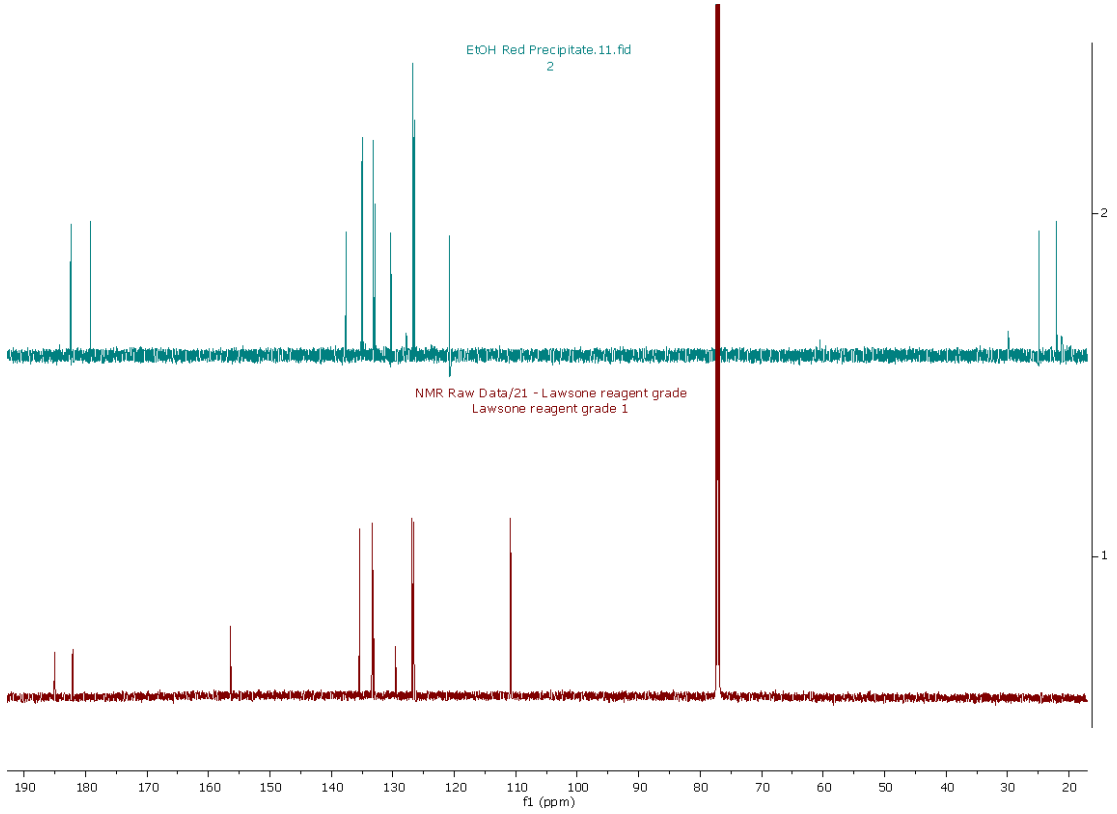


Figure 2.7 ^{13}C NMR spectrum of compound **100** (top, green) and lawsone (bottom, red).

COSY, HSQC, and HMBC spectral data is summarised in Table 2.2. The relevant HMBC and COSY data and the position assignment is also visually represented in Figure 2.9. The most important piece of information that is obtained from the COSY NMR is that the doublet at 1.29 ppm (attributed to a -CH_3 moiety) couples to the quartet at 4.60 ppm (attributed to a -CH group). It is most likely that these methyl groups are adjacent to each other in the product structure, and are not directly adjacent to the aromatic structure retained from the lawsone starting material. This was confirmed using HSQC, where it was observed that the proton signal at 4.60 ppm correlates to the carbon signal at 24.92 ppm, which both pertain to a CH group. The proton signal at 1.29 ppm pertaining to the CH_3 group correlates to the carbon signal at 24.92 ppm.

The HMBC spectrum showed that there is a long-range correlation between the peak at 8.28 ppm, pertaining to a potential NH, and the allylic peaks at 182.37 ppm and 179.24 ppm, suggesting that the NH substituent has been inserted next to these carbonyls in the final structure. There is also a correlation between the peak at 1.29 ppm, pertaining to a CH_3, and peaks in the allylic region at 24.92 and 120.73 ppm. The proton peak at 4.60 ppm has multiple long-range correlations to 22.06, 120.73, 137.64, and 182.37 ppm, suggesting proximity.

Table 2.2 ^{1}H, ^{13}C, HSQC, COSY, and HMBC NMR data for compound **100**, refer to Figure 2.9 for position assignment.

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$) [a]	COSY	HMBC (H→C)
1	22.06	1.29, d (6.8)	4.60	24.92, 120.73
2	24.92	4.60, q (6.8)	1.29	22.06, 120.73, 137.64, 182.37
12	120.73			
9	126.46	8.11, d (7.6)	7.7	126.46, 130.36, 132.91, 179.24
6	126.71	8.14, d (7.6)	7.77	126.46, 133.19, 182.37, 182.37
5	130.36			
10	132.91			
7	133.19	7.70, t (7.5)	8.11	130.36, 133.19
8	135.00	7.77, t (7.5)	8.14	126.47, 130.36, 132.91
3	137.64			
11	179.24			
4	182.37			
13		8.28, bs		120.73, 126.46, 179.17

[a] δ_H, multiplicity (*J* in Hz)

A mass spectrum was obtained using a DSA TOF MS in positive mode. A base peak at m/z=356.086 was observed in the resulting spectrum (Figure 2.8). Since APCI is considered a 'soft' ionisation technique, meaning only a small amount of energy is applied to the molecule, it is expected that very little fragmentation would be observed of the compound of interest as evidenced in the spectrum. It was concluded that the peak at m/z=356.086 was the molecular ion of compound **100** due to its intensity in the spectrum. This is indicative that the compound has a molecular mass of approximately 355 $gmol^{-1}$, suggesting a molecular formula of $C_{22}H_{13}NO_4$. It would be expected that a compound with this formula would have a [M+H] of 356.092. As the observed [M+H] was 356.086, a mass error of -16.8 ppm was calculated, which is within an acceptable error range.

Another peak of interest was observed at m/z = 711.173. It is suspected that this may be an impurity in the sample, or possibly a dimer of the desired compound as the mass of the impurity is almost exactly twice the product's mass.

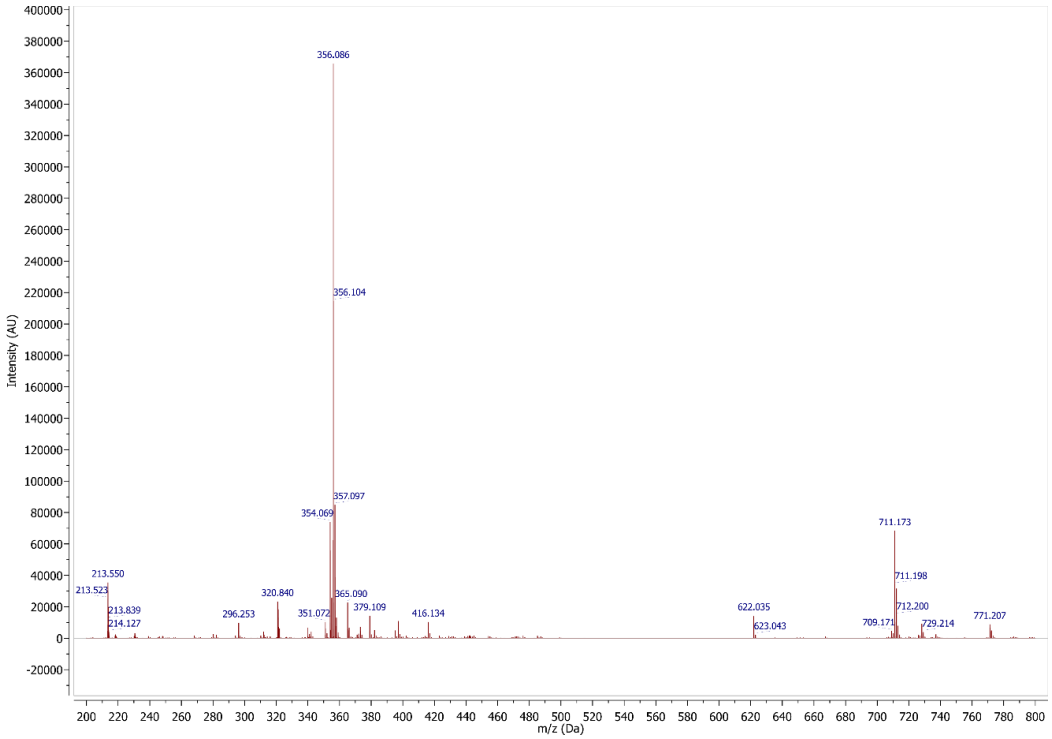
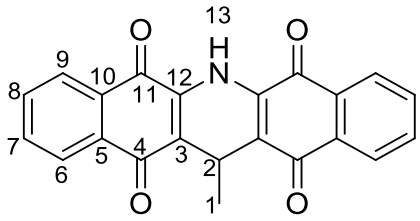


Figure 2.8 Mass spectrum of compound **100**, showing a possible molecular ion of m/z = 356.086.

On the basis of this evidence, the structure of compound **100** was determined to be that as shown in Figure 2.9, which is a dimer of two naphthoquinone moieties connected at the vinyl positions by -NH and ethyl groups. This structure, 13-methyl-6,13-dihydro-6-azapentacene-5,7,12,14-tetraone, incorporates the ethyl group observed by Jelly *et al.* in the ^{1}H NMR and accounts for the missing vinyl protons.

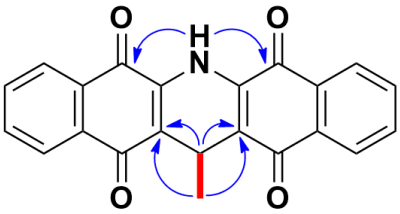
A literature search for the proposed structure in Figure 2.9 indicated that this compound had previously been synthesised by Marcos *et al*.[12] The physical characteristics and ^{1}H and ^{13}C NMR shifts reported by the authors strongly correlate to the experimentally obtained data in this study (Table 2.3), suggesting the compound has been successfully identified. The IR values reported by Marcos *et al.*[12] regarding the structure displayed in Figure 2.9 differ slightly from the experimental values obtained in this study (Figure 2.3). It should be noted that the raw data or signal intensities were not included by Marcos *et al.* and were performed on the product compressed in a KBR disc, therefore comparisons to these reported values may not be accurate. The peaks at 3440 (N-H), 1670 (C=O), 1600, 1495, and 1300 cm^{-1} were observed with some shifting in the experimentally obtained data. However, peaks at 1625, 1620, 1505, and 750 cm^{-1} were absent in the experimental data for this study. There are some additional peaks in the experimental data not reported in literature, including 2924, 1646, 1574, 1473, 1387, 1356, 1327, 1277, 1222, 1195, 724, 709, 645, 612, 572, 514 cm^{-1}, which may

be attributed to slight impurities in the sample (as observed in MS and NMR spectra), or more sensitive IR parameters or equipment.



Chemical Formula: $C_{22}H_{13}NO_4$
Exact Mass: 355.08
Molecular Weight: 355.34

a)



b)

Figure 2.9 a) Proposed structure of compound **100** from the reaction between amino acids and lawsone; b) Structure depicting the COSY and HMBC correlations of compound **100.**

Table 2.3 Comparison of observed NMR values and those reported by Marcos *et al.*[12] for Compound **100.** All δ are reported in ppm.

δ_C ($CDCl_3$) Observed	δ_C ($CDCl_3$) Reported	$\Delta\delta_C$	δ_H ($CDCl_3$) [a] Observed	δ_H ($CDCl_3$) [a] Reported	$\Delta\delta_H$
22.06	21.9	-0.16	1.29, d (6.8); 3H	1.31, d (6.6); 3H	0.02
24.92	29.7	4.78	4.60, q (6.8); 1H	4.63, q (6.6); 1H	0.03
120.73	120.6	-0.13			
126.46	126.3	-0.16	8.11, d (7.6); 2H		
126.71	126.6	-0.11	8.14, d (7.6); 2H	8.15, two overlapped d; 4H	0.01
130.36	130.2	-0.16			
132.91	132.7	-0.21			
133.19	133	-0.19	7.70, t (7.5); 2H		
135.00	134.8	-0.2	7.77, t (7.5); 2H	7.75, m; 4H	-0.02
137.64	137.5	-0.14			
179.24	179.1	-0.14			
182.37	182.2	-0.17			
			8.28, bs; 1H	8.31, s; 1H	0.03

[a] δ_H, multiplicity (*J* in Hz); integration

2.2.2. Spectra analysis and structure elucidation of the yellow product (101)

The isolated yellow compound **101** was characterised using UV-vis, fluorescence, IR, NMR, and mass spectrometry. The UV-vis spectrum, displayed in Figure 2.10, exhibits peaks at 268.9, 335.9 (shoulder), and 423.4 nm. These signals indicate that the structure is likely to contain some aromatic functionality and that there may be some level of conjugation in the structure. Solution based fluorescence spectrophotometry performed in chloroform resulted in very little fluorescence, with possible excitation and emission maxima of 364 nm and 439 nm respectively (Figure 2.11). Fluorescence was also attempted in ACN and DCM to combat possible solvent quenching, however similar results were obtained.

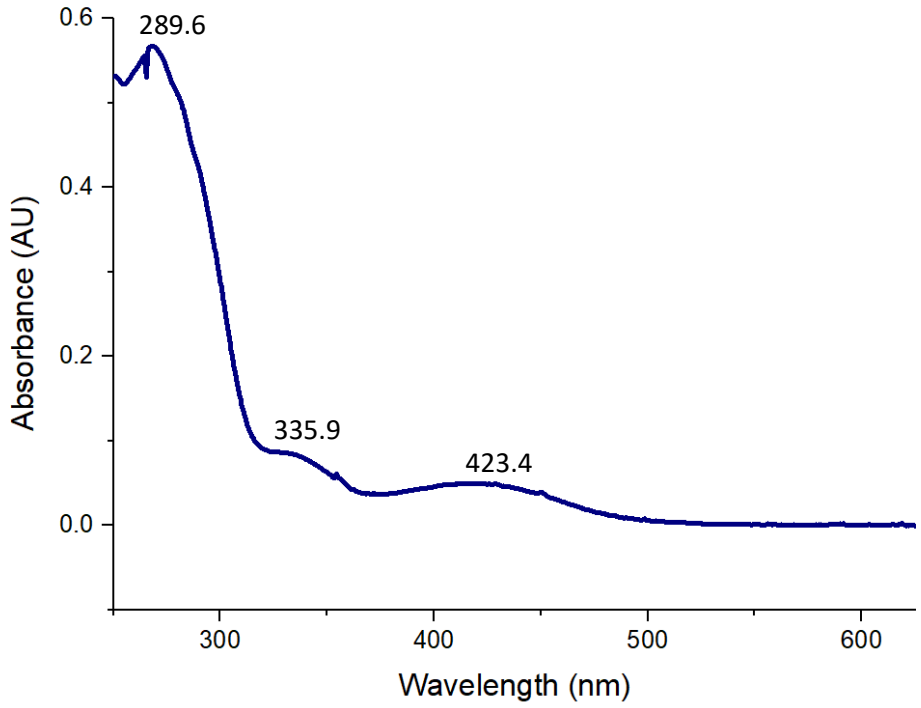


Figure 2.10 UV-Vis spectrum of compound **101**, showing the absorption wavelengths of 268.9, 335.9 (shoulder), 423.4 nm.

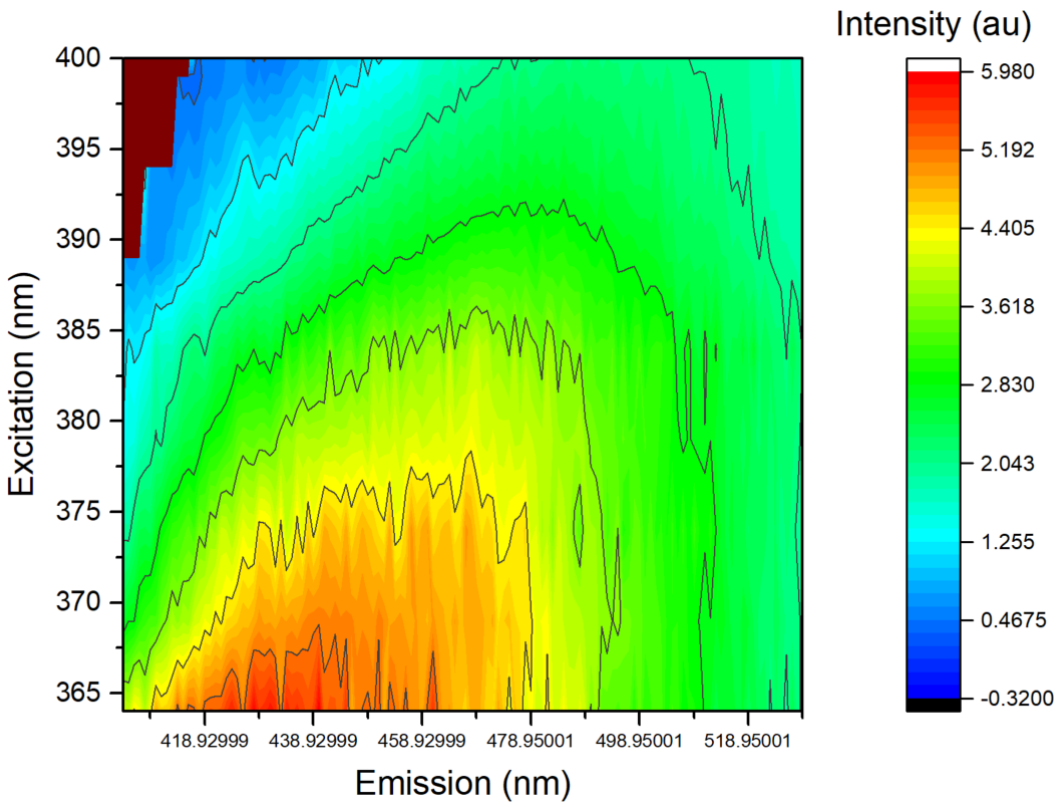


Figure 2.11 3D Fluorescence spectrum of compound **101**, showing the excitation and emission wavelengths of 364 nm and 439 nm respectively.

The IR spectrum for compound **101**, as seen in Figure 2.12, exhibited peaks at 1708, 1620, and 2924 and 2851 cm^{-1}, most likely correlating to an C=O stretch, a C=C stretch, and aromatic C-H stretches respectively. The other peaks were attributed to various C-H and C-C stretching that could be present in the molecule.

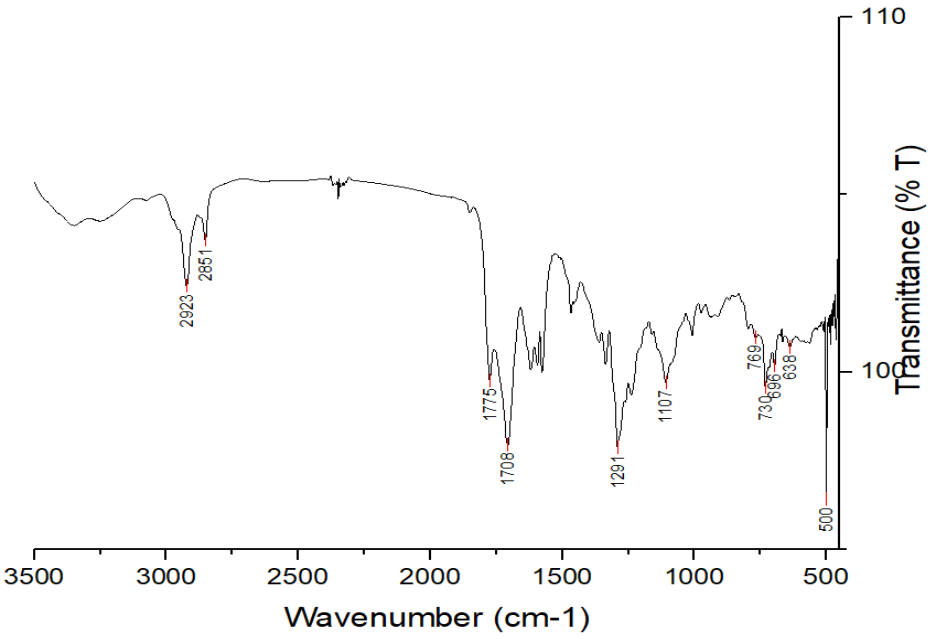


Figure 2.12 IR spectrum of compound **101.**

Compound **101** was found to have eight unique proton environments and fourteen unique carbon environments. The ^{1}H, ^{13}C, COSY, HSQC, and HMBC data are summarised in Table 2.4, and can be observed in full in Appendix 1. The aromatic peaks between 7.60 and 8.07 ppm and the peaks at 5.08 ppm and 1.42 ppm have the same splitting pattern observed for peaks in a similar region in the ^{1}H NMR for compound **100**, as observed in Figure 2.13 comparing these spectra. This suggests that these moieties have been retained in the final structure of compound **101**. In particular, the shifts at 5.08 ppm (72.02 ppm) and 1.42 ppm (18.94 ppm) indicate that an ethyl group is still present in the structure in a similar manner to Compound **100**. However, the significant upshift of the quartet at 5.08 ppm and associated ^{13}C shift of 72.02 ppm (from 4.60 ppm and 24.92 ppm respectively in Compound **100**) suggests a highly electronegative environment is adjacent. The absence of the NH peak at 8.28 ppm would suggest that either an -N-R group is not present in the structure of compound **101**, or a reaction has occurred at this position.

Table 2.4 ^{1}H, ^{13}C, HSQC, COSY, and HMBC NMR data for compound **101**, refer to Figure 2.15 for position assignment.

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$)[a]	COSY	HMBC (H→C)
1	15.56	1.22, t (7.0)	3.48	64.65
14	18.94	1.42, d (6.7)	5.08	72.02, 115.26
13	64.65	3.48, qd (7.0, 1.3)	1.22, 7.60	15.56, 72.02
2	72.02	5.08, q (6.7)	1.42	18.94, 64.65, 115.26, 145.37, 182.25
3	115.26			
9	126.04	8.03, dd (7.7, 1.3)	7.60	134.67, 182.05
6	126.32	8.07, dd (7.7, 1.2)	7.70	132.12, 182.25
5	130.62			
7	132.12	7.60, td (7.5, 1.2)	3.48, 8.03	126.32, 130.62
10	133.30			
8	134.67	7.70, td (7.6, 1.3)	8.07	126.04, 133.30
12	145.37			
11	182.05			
4	182.25			

[a] δ_H, multiplicity (*J* in Hz)

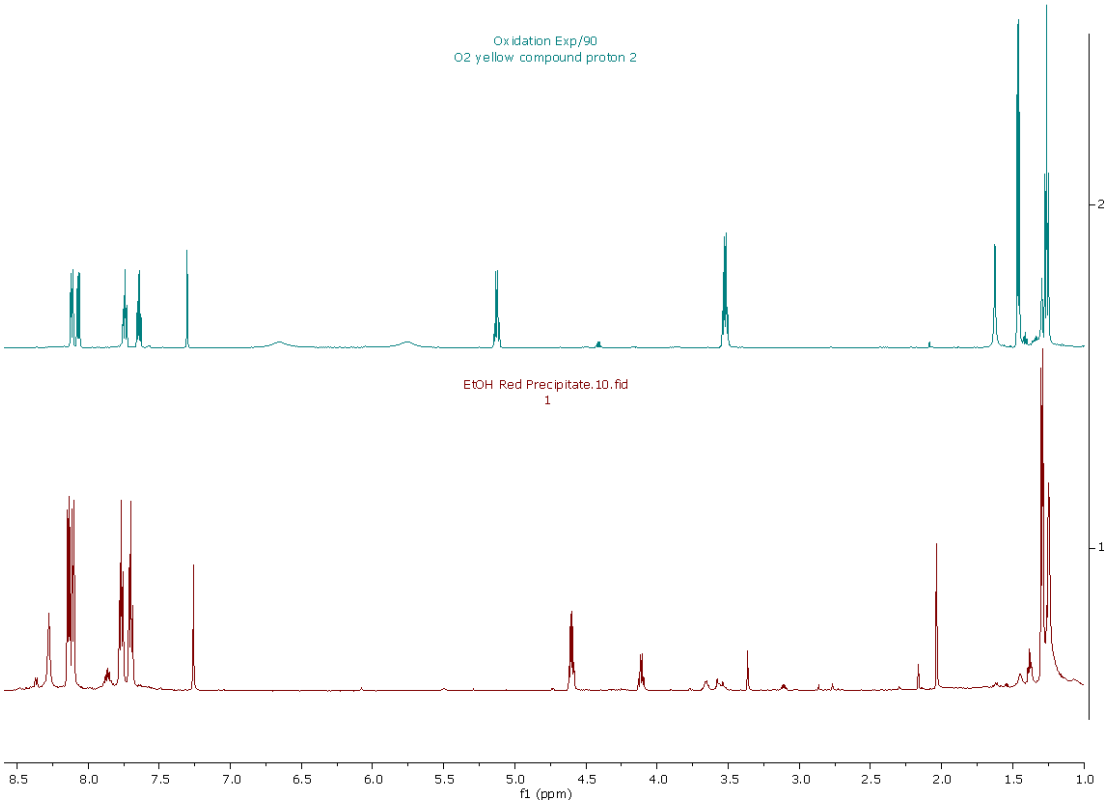


Figure 2.13 ^{1}H NMR spectrum of compound **101** (top, green) and compound **100** (bottom, red).

Although not supported by the shift integrations observed in the NMR, it was originally proposed that the compound **101** was similar in structure to the compound **100**, however was ethylated at the N-position due to the up-shift of the quartet at 5.08 ppm, and the addition of a quartet and a triplet at 3.48 ppm and 1.22 ppm respectively. The formation of a similar compound for comparison was attempted by reacting the red dimer with ethyl iodide in DCM with K_2CO_3 under a nitrogen atmosphere, however the formation of a yellow product was not observed using this method.

It would be expected that the integration observed for this proposed N-alkylated structure at the -CH and -CH_3 positions of the ethyl bridge would be one and three respectively, and the integration for the -CH_2 and -CH_3 positions of the N-alkyl chain would be two and three respectively. Accordingly, the integration for each aromatic environment would be expected as two due to the symmetrical dimeric structure. While these -CH integrations are observed, the integrations for the aromatic proton environments are one, not two, thereby suggesting the proposed structure may not be a dimer.

A mass spectrum was obtained using a DSA TOF MS in positive mode. A molecular ion at m/z=244.0969 was observed in the resulting spectrum (Figure 2.14), indicating the compound to have a molecular mass of approximately 243 $gmol^{-1}$, suggesting a molecular formula of $C_{14}H_{13}NO_3$. It would be expected that a compound with this formula would have a [M+H] of 244.097. As the observed [M+H] was 244.0969, a mass error of 0.33 ppm was calculated, which is within an acceptable error range. A molecular formula of $C_{14}H_{13}NO_3$ is consistent with the hypothesis that compound **101** is not a dimer analogue of lawsone, as there are only fourteen carbons where at least 20 is needed. Additionally, the fourteen carbons is in agreement with the NMR data, that determined there were only fourteen carbon environments.

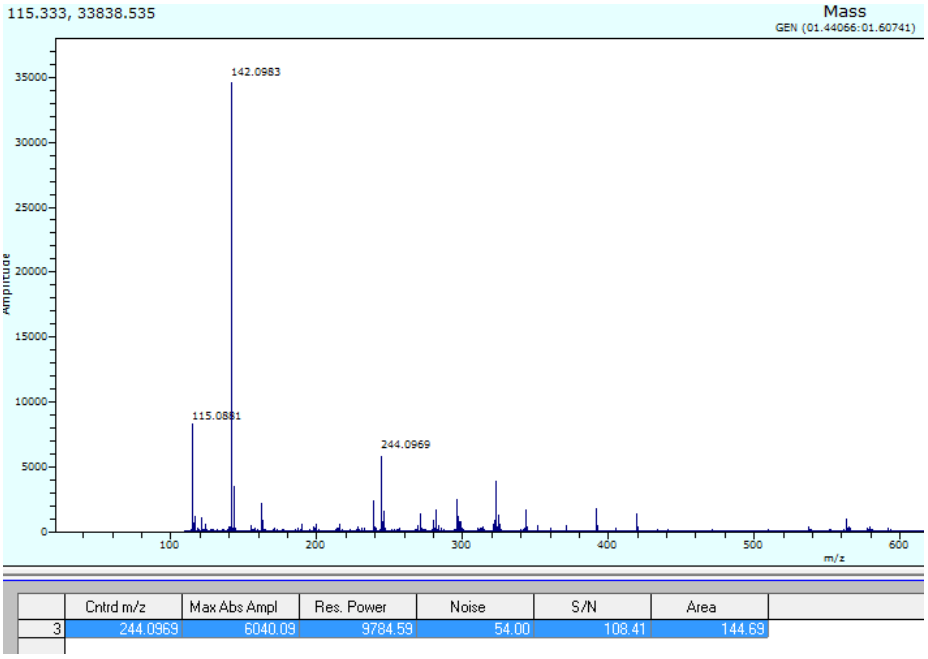


Figure 2.14 . Mass spectrum of compound **101**, showing a possible molecular ion of m/z=244.0969.

A new structure was proposed that aligns with the previously mentioned requirements, MS, and NMR data, as seen in Figure 2.15. This compound, 1-ethyl-3-methylnaphtho[2,3-c]isoxazole-4,9(1H,3H)-dione, has not previously been reported in literature. The reported structure retains the naphthoquinone moiety as well as the ethyl bridge from compound **100**, and incorporates the ethyl at the N- position. However, this structure is not a dimer, but instead the ethyl bridge is bonded to a 2,5-dihydroisoxazole moiety. This bonding would explain the absence of the -NH peak, observed at 8.28 ppm in the NMR spectrum for compound **100**, as the amine is now tertiary and incorporated into a heterocyclic ring, and would explain the retention but slight shifting of the peaks pertaining to the ethyl bridge. The integration for the aromatic proton environments is now one, while the integration for the N-alkyl chain and the ethyl bridge remain in accordance with the NMR data. The observed MS data is also in agreement with the theoretical mass of 244.0969 amu, with a small error of only 0.41 ppm calculated.

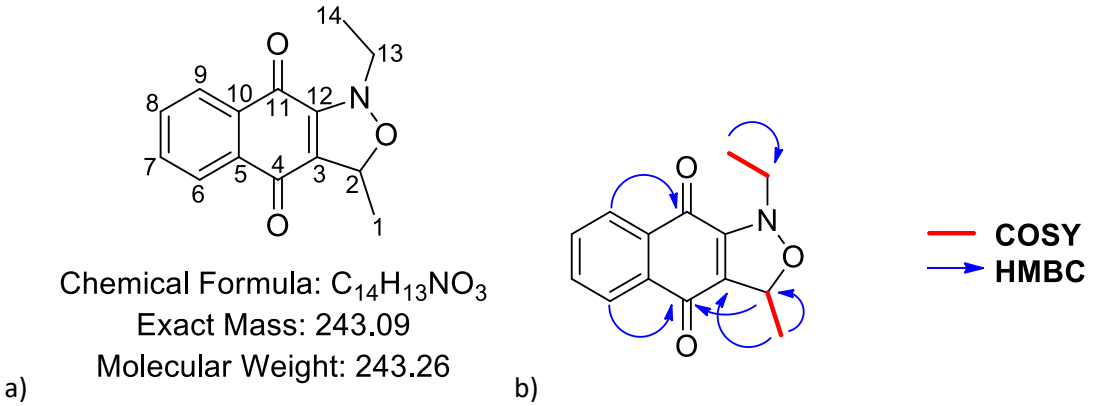


Figure 2.15 a) Proposed structure for compound **101**; b) Structure depicting the COSY and HMBC correlations of compound **101.**

2.2.3. Spectra analysis and structure elucidation of the orange product (102)

An orange compound **102** was isolated and characterised by UV-vis, fluorescence, IR, NMR, and mass spectrometry. The UV-vis spectrum, displayed in Figure 2.16, exhibits peaks at 285.47, 332.06, and 425 nm. These signals indicate that the structure is likely to contain some aromatic functionality and that there may be some level of conjugation in the structure. Solution based fluorescence spectrophotometry performed in chloroform resulted in very little fluorescence, with potential excitation and emission maxima of 334 nm and 566 nm respectively (Figure 2.17).

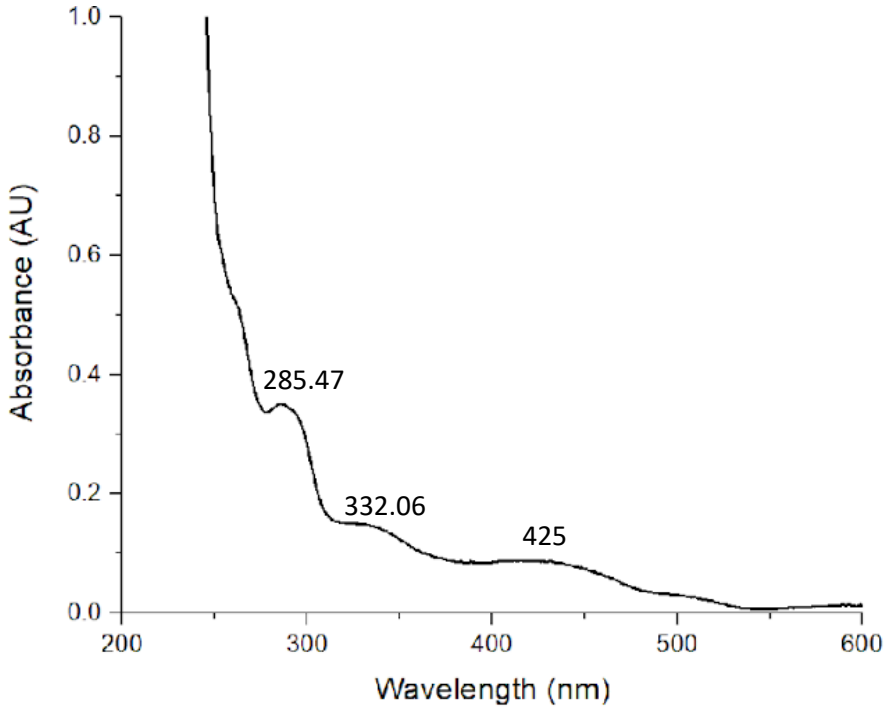


Figure 2.16 UV-Vis spectrum of compound **102**, showing the absorption wavelengths of 285.47, 332.06, and 425 nm.

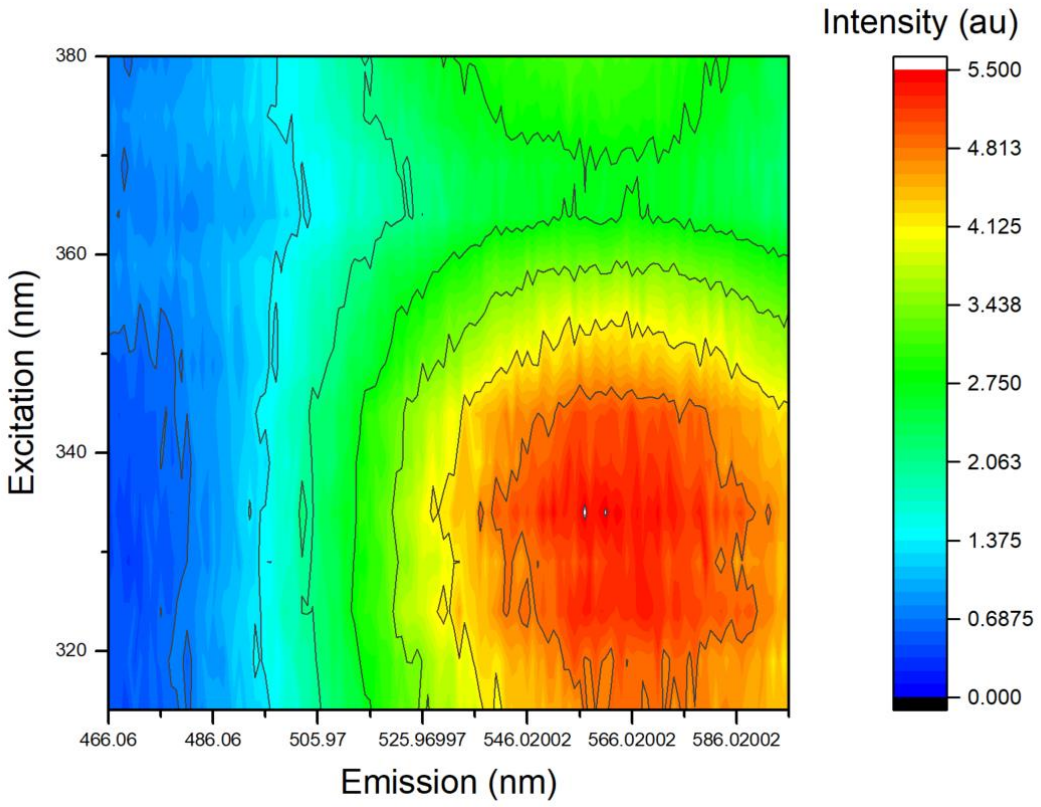


Figure 2.17 3D Fluorescence spectrum of compound **102**, showing the excitation and emission wavelengths of 334 nm and 566 nm respectively.

The IR spectrum for compound **102**, as seen in Figure 2.18, exhibited peaks between 3408-3324 cm^{-1} and 1618-1551 cm^{-1}, most likely correlating to N-H stretches and bends. A peak at 1688 cm^{-1} was observed, correlating to C=O stretches. The other peaks were attributed to various aromatic C-H stretching that could be present in the molecule.

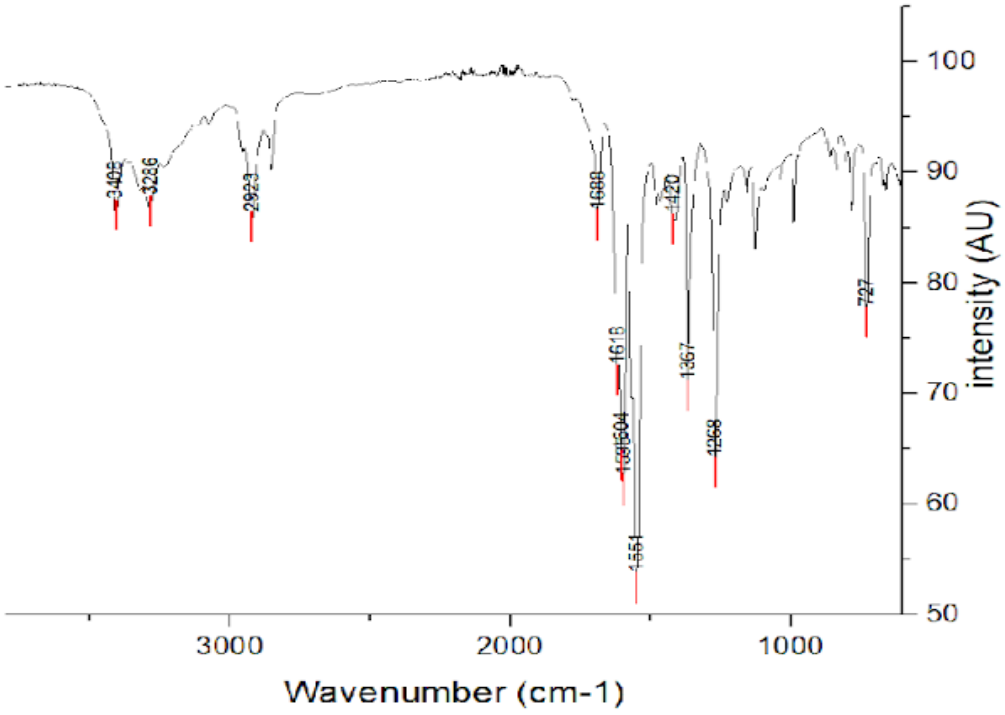


Figure 2.18 IR spectrum of compound **102.**

Compound **102** was found to have six unique proton environments and ten unique carbon environments. The ^{1}H, ^{13}C, COSY, HSQC, and HMBC data are summarised in Table 2.5, but can be seen in full in Appendix 1. The aromatic peaks between 7.73 and 8.08 ppm have similar splitting patterns observed for peaks in a similar region in the ^{1}H NMR for lawsone, as observed in Figure 2.19 comparing these spectra. The peaks between 8.08 ppm and 8.06 ppm in the ^{1}H NMR spectrum for compound **102** have a wider splitting pattern than those observed in the same region for lawsone, however these regions in each spectrum still integrate for two protons. The singlet at 6.37 ppm pertaining to the vinyl proton in the ^{1}H NMR for lawsone (H-3) is slightly downshifted in the compound **102** spectrum, where it now appears at 6.00 ppm. The carbonyl peaks at 182.09 ppm and 185.06 ppm in the lawsone ^{13}C NMR (C-1 and C-4 respectively) have also been retained in the ^{13}C NMR for compound 3, now observed at 183.88 ppm and 181.98 ppm (Figure 2.20). This information suggests that the naphthoquinone structure has been retained in the final structure of compound **102**. Comparing the ^{1}H NMR spectra

for lawsone and compound **102**, the sharp singlet at 7.33 ppm pertaining to the -OH group in the lawsone molecule is now absent in the spectrum for compound **102**. Instead, a broad singlet at 5.15 ppm, integrating for two protons, can be observed in the spectrum for compound **102**. This peak is characteristic of a $-NH_2$ group. As a known compound, a pure sample was subsequently obtained from Accela ChemBio where spectroscopic data was in agreement with the data obtained for Compound **102**, thus confirming its identity.

Table 2.5 ^{1}H, ^{13}C, HSQC, COSY, and HMBC NMR data for compound **102**, refer to Figure 2.22 for position assignment.

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$)[a]	COSY	HMBC (H→C)
9	183.88			
2	181.98			
10	148.37			
5	134.74	7.73, t (7.5)	8.08	126.35, 133.46
3	133.46			
6	132.40	7.64, t (7.5)	8.06	126.35, 130.66
8	130.66			
4	126.35	8.06, d (7.7)	7.64	126.28, 133.46, 181.98
7	126.28	8.08, d (7.8)	7.73	130.66, 183.88
1	105.31	6.00, s	8.06	126.35, 133.46, 148.37, 181.98
11		5.15, bs		

[a] δ_H, multiplicity (*J* in Hz)

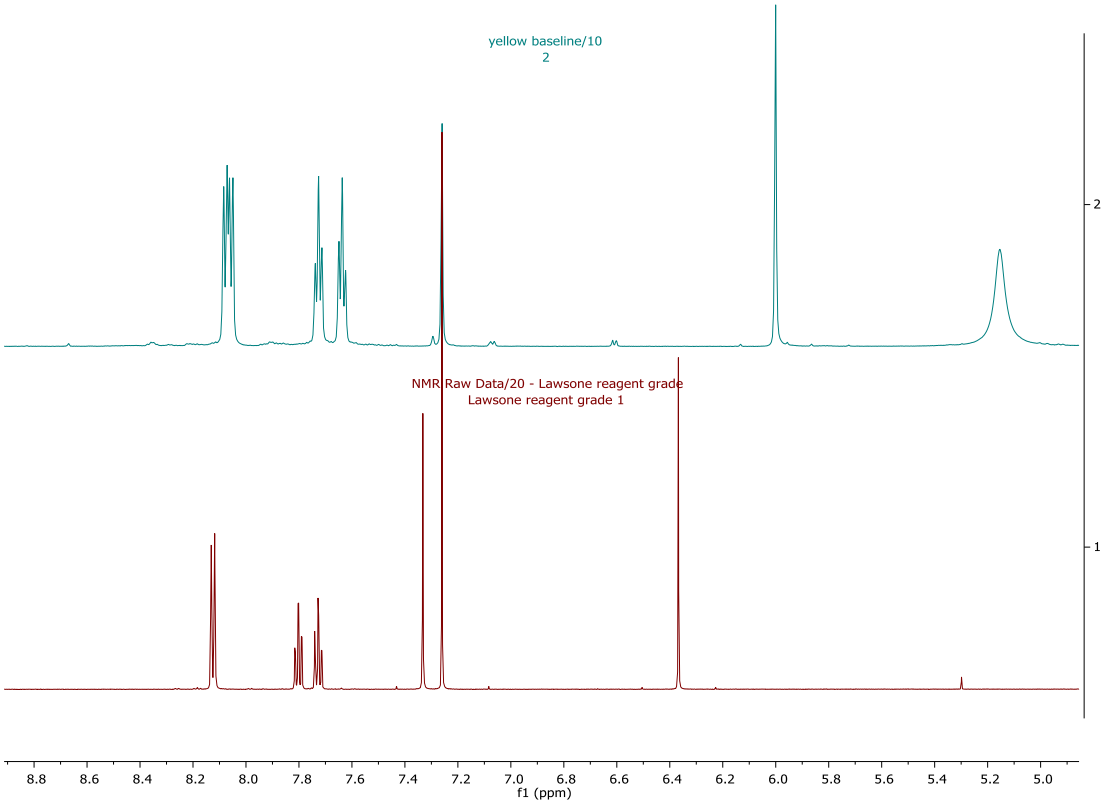


Figure 2.19 ^{1}H NMR spectrum of compound **102** (top, green) and lawsone (bottom, red).

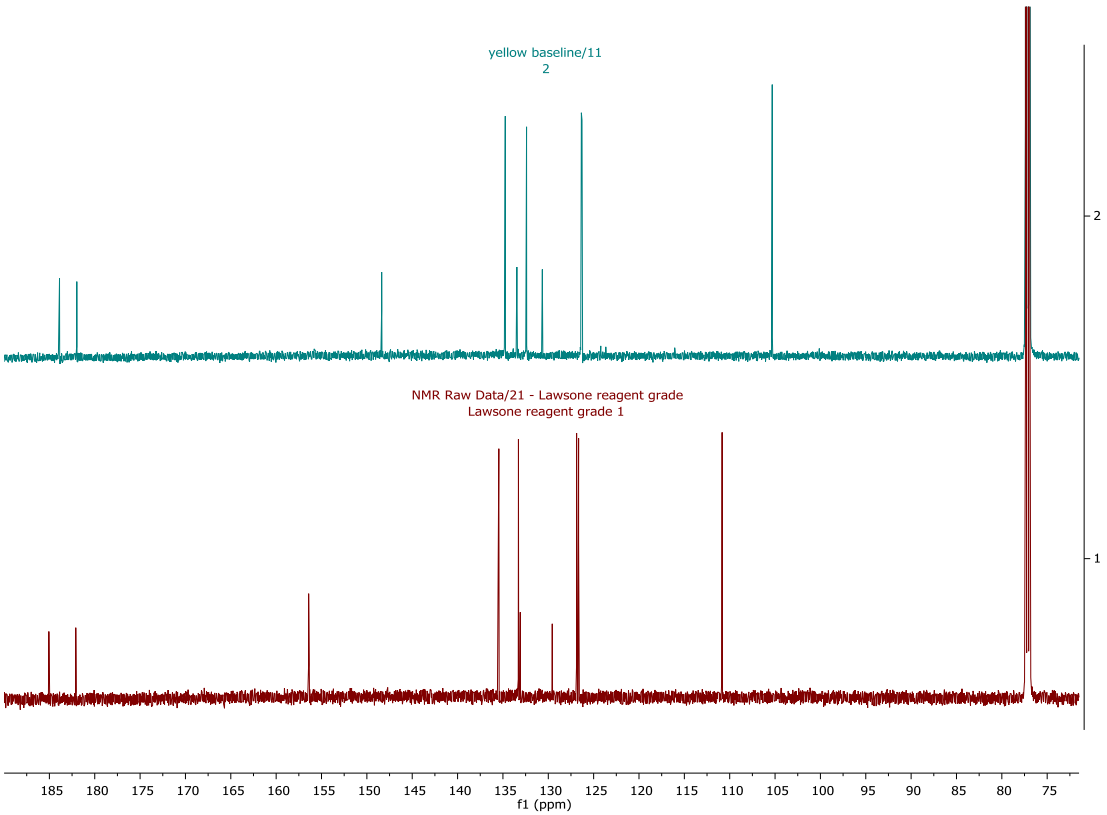
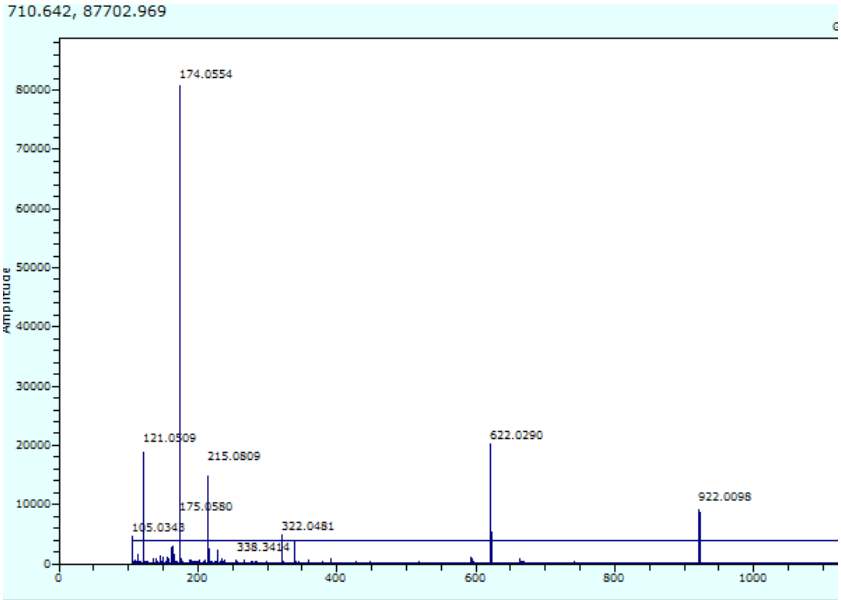


Figure 2.20 ^{13}C NMR spectrum of compound **102** (top, green) and lawsone (bottom, red).

A mass spectrum was obtained using a DSA TOF MS in positive mode. A molecular ion at m/z=174.0554 was observed in the resulting spectrum (Figure 2.21), indicating the compound to have a molecular mass of approximately 173 $gmol^{-1}$, suggesting a molecular formula of $C_{10}H_7NO_2$. It would be expected that a compound with this formula would have a [M+H] of 174.055. As the observed [M+H] was 174.0554, a mass error of 2.30 ppm was calculated, which is within an acceptable error range.



	Cntrd m/z	Max Abs Ampl	Res. Power	Noise	S/N	Area
1	105.0343	4636.81	7195.93	3827.00	1.18	65.93
2	121.0509	19833.55	6827.62	3827.00	5.13	331.32
3	174.0554	81294.42	9393.81	3827.00	21.08	1443.17

Figure 2.21 Mass spectrum of compound **102**, showing a possible molecular ion of m/z=174.0554.

Based on this evidence, the structure of compound **102** was determined to be 2-amino-1,4-naphthoquinone, as shown in Figure 2.22. The NMR spectra correlate strongly to the values reported by Zhang and Chang[13,] while the experimentally obtained absorption wavelength of 425 nm in $CHCl_3$ is shifted from the literature value of 329 nm, potentially due to the MeOH solvent used.[14] Comparison of the experimentally obtained IR spectrum values to a spectrum available from the Spectral Database for Organic Compounds[15] show some correlation. Peaks at 3383 and 3323 cm^{-1} (N-H), 1687 and 1619 cm^{-1} (C=O), 1366, 1272, 1166, 986, 789, and 725 cm^{-1} were observed with some shifting in the experimentally obtained data, however peaks at 3289, 3192, 1568, 1420, 1220, 1061, 831, 657, 604, and 533 cm^{-1} were absent in the experimental data for this study. There are some additional peaks in the experimental data not observed in literature, including 3287, 3368, 2955, 2923, 2853, 1604, 1595,

and 1551 cm^{-1}, which may be attributed to slight impurities in the sample (as observed in MS and NMR spectra), or different IR parameters or equipment.

Jelly *et al.* claimed to have isolated a yellow product pertaining to the structure of compound **102**, however very little supporting evidence was given. It was originally thought that compound **101** was isolated by Jelly, however upon analysis using GCMS was degraded to compound **102** and therefore misidentified. Compound **101** was subjected to GCMS analysis according to parameters outlined by Jelly[9] where they identified compound **102** at 35 minutes, however no evidence of compound **102** could be found at this time. It is unclear whether compound **101** was previously isolated but misidentified, or whether the physical properties of compound **102** were improperly reported.

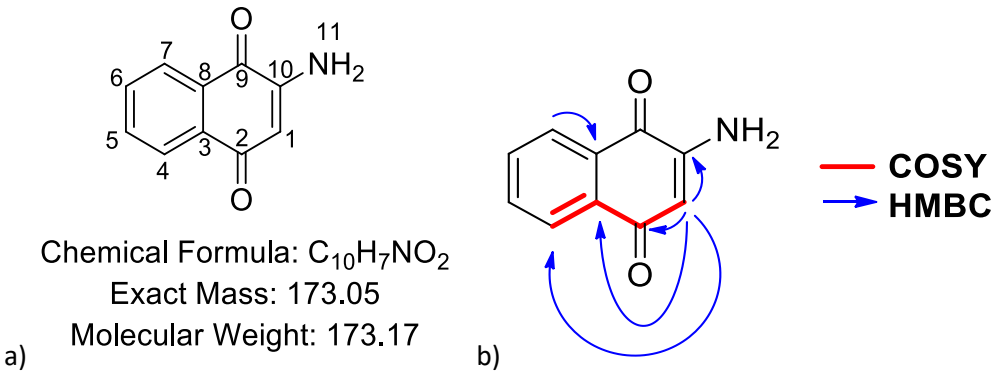


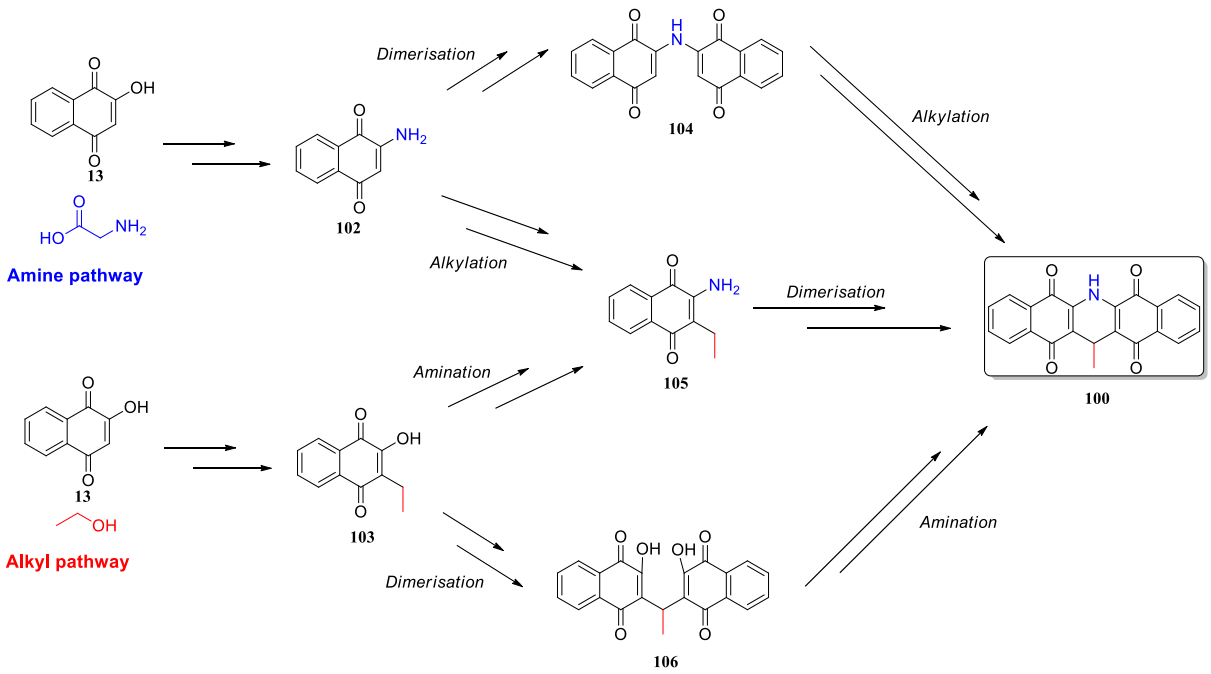
Figure 2.22 a) Proposed structure for compound **3**; b) Structure depicting the COSY and HMBC correlations of compound **102.**

2.2.4. Postulation of reaction mechanism

Due to the extremely low yields obtained, it is evident that the reaction does not favour the formation of compound **100**. The dimer consists of two lawsone molecules connected via two 'linkers' – an amine linker, and an alkyl linker. It is proposed that the reaction pathway for the formation of Compound **100** begins with the addition of one of these linkers, before subsequent addition of the other. It is currently unknown whether the amine linker forms before or after the alkyl linker, or whether they are formed simultaneously, and what effect the order of these events have on the mechanism. Understanding how these moieties are included in the structure is crucial to choosing conditions that favour the formation of the red dimer for forensic fingerprinting purposes, as well as displaying novel chemistry that allows for the easy synthesis of similar analogues.

Scheme 2.2 depicts two general pathways that could lead to the formation of Compound **100**. The amine pathway involves the inclusion of the amine linker first to form **102**, followed by dimerization to **104**, prior to insertion of the alkyl linker. The alkyl pathway involves the inclusion of the alkyl linker

first in the form of **103**. It is also feasible that each of these pathways may have consecutive addition of both linkers before dimerization to Compound **100**. All pathways involve amination, alkylation, and dimerization requiring the lawsone, alcohol, and amino acid reagents, as well as possible intermediates.

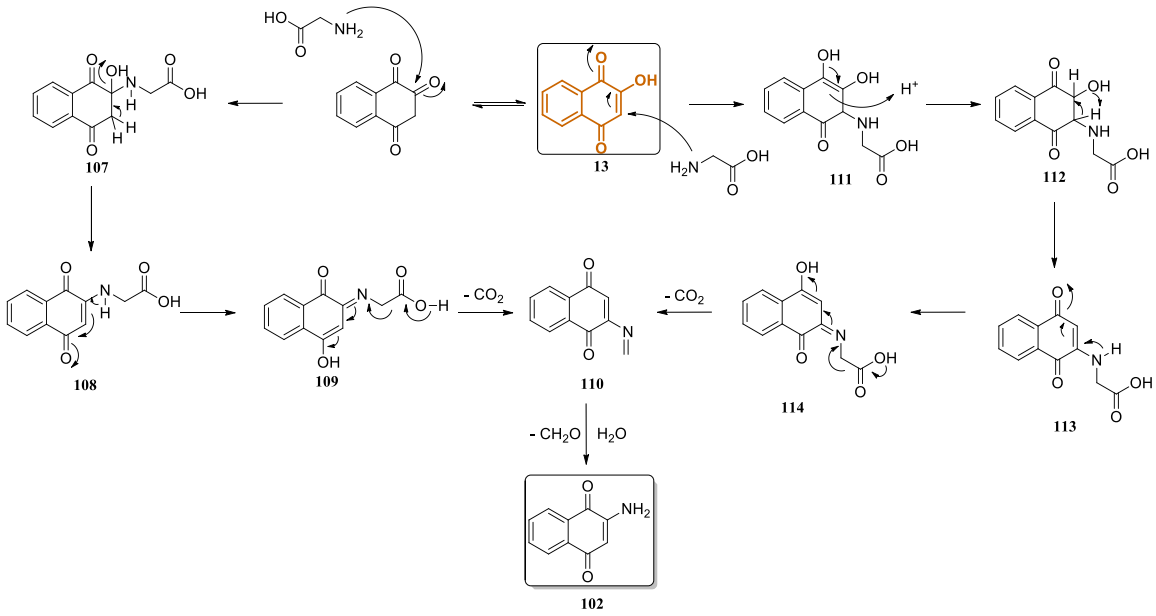


Scheme 2.2 Proposed reaction pathways for the formation of Compound **100.**

Quinone interactions with amino acids have previously been reported in literature and can result in direct substitution or conjugate addition of the amino acid at varying positions around the quinone moiety.[16] It is likely that other quinones such as lawsone can also undergo a similar reaction with amino acids, shown in Scheme 2.3, involving direct substitution at the 2- carbonyl position. Lawsone can tautomerize to the keto form a carbonyl also now at the 2-position, leading to this position being open to nucleophilic attack by the nitrogen on the amino acid (**107**). The resulting Schiff base **109** undergoes decarboxylation of the amino acid and further hydrolysis to release the corresponding aldehyde, followed by the oxidation of the formed amine **110** to form **102**.

Alternatively, it is documented that 1,2-quinones can undergo conjugate addition at the 3-position.[17] It is therefore equally as likely that the amino acid can attach at the 3-position to give **102**, also seen in Scheme 2.3. Here, lawsone retains its 1,4-quinone structure while the nitrogen from the amino acid attacks the 3-position carbon (**111**). Proton abstraction and rearrangement to **114** is followed by decarboxylation to **110.** Hydrolysis of **110** forms **102**. It is therefore of interest that **102** was isolated

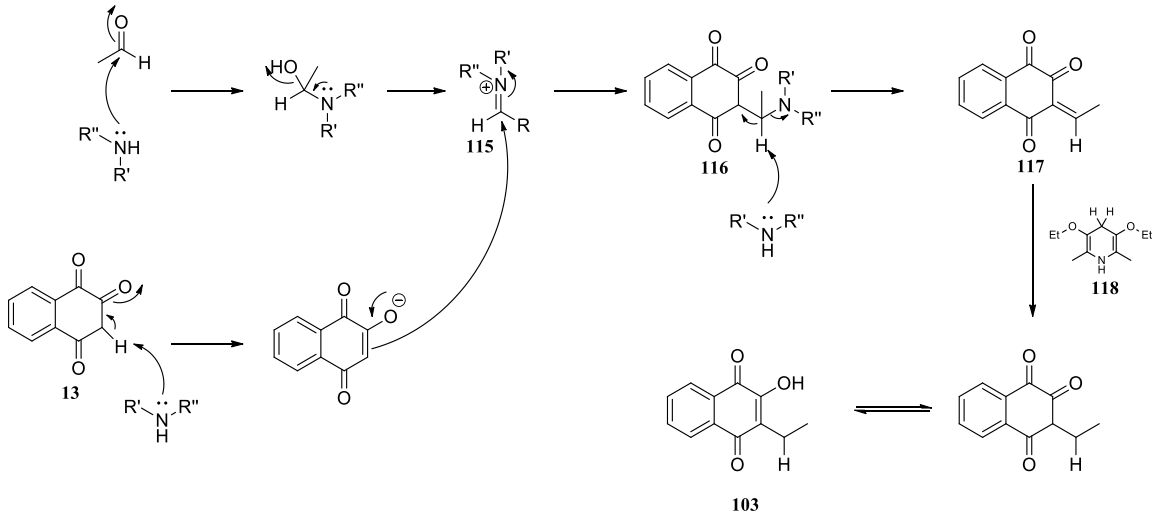
from the reaction mixture. The presence of this compound would suggest that it is possible for the reaction mechanism to incorporate **102** as an intermediate, thereby suggesting this may form part of the mechanistic pathway. As **102** is a common intermediate that can be obtained, reactions were performed using it in place of or in addition to lawsone in the reaction mixture with glycine and ethanol. These results are explored further in Chapter 5.



Scheme 2.3 Proposed mechanisms of direct substitution (left) and conjugate addition (right) between amino acids and lawsone, arriving at the common intermediate, **102**.

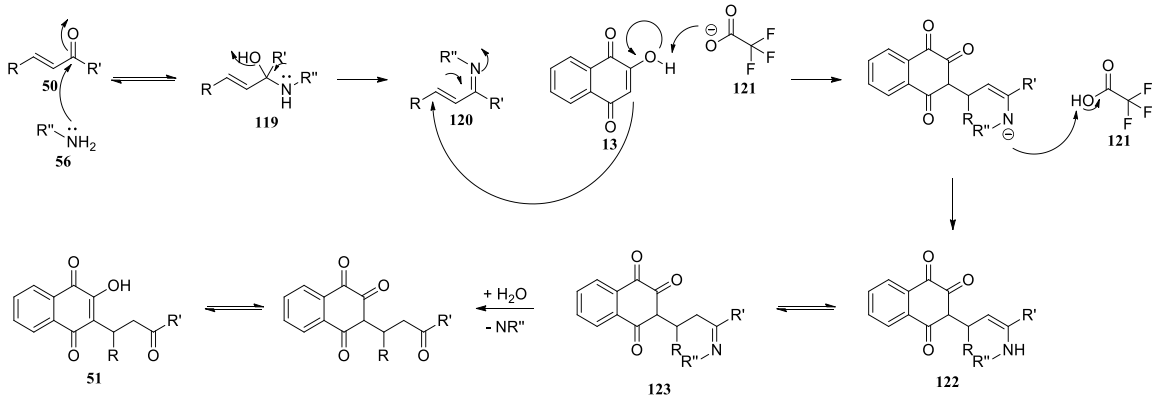
The formation of the linked dimer is more complex however, as further bonds need to be formed at this 2-position to form the amine linker, as well as at the 3-position of the resulting naphthoquinone to form the carbon linker. The two linkers could be formed simultaneously or one at a time, and sequential formation of the linkers could occur in either order. Formation of a linker through the nitrogen can be rationalised through a substitution reaction of **102** with a second tautomerized lawsone molecule to form a product bridged with the amine. The introduction of an ethyl group into the structure of compound **100** is of particular interest, as usually alkylating agents are required to be present for the attachment of such a group to an enol group, as is present in lawsone. Alkylating agents that are used to form carbon-carbon bonds include aldehydes, olefins, and alkyl halides, and can be used in the presence of acid or base catalysts or organocatalysts. Many examples of carbon-carbon alkylation at the 3-position of lawsone exist in literature, and a sample is outlined in detail.

Ramachary *et al.*[18] alkylated lawsone at the 3- position to form **103** using proline as a catalyst, acetaldehyde, and a Hantzsch ester (Scheme 2.4). Acetaldehyde and lawsone partake in a Knoevenagel condensation, with proline acting as a catalyst. Upon the conversion of the acetaldehyde to an iminium ion **115**, it is subjected to nucleophilic attack by an activated lawsone molecule to form an α,β-unsaturated intermediate **117**. The introduction of a Hantzsch ester **118** allows for the alkene reduction of **117** to form the final product **103**.



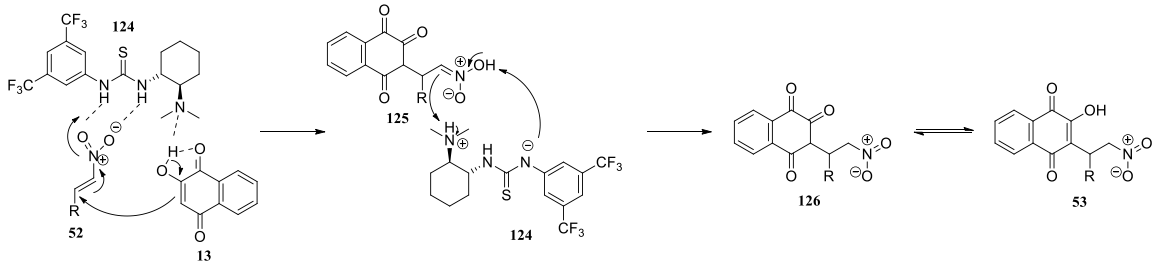
Scheme 2.4 The alkylation of lawsone to **103** using proline, acetaldehyde, and Hantzsch ester **118**.

Zhang *et al.*[19] reported the acid-catalysed Michael addition of α,β-unsaturated ketones **50** to lawsone using a primary amine organocatalyst derived from cinchonidine **56**, and TFA (**121**) (Scheme 2.5) to form lapachol analogues. The organocatalyst **56**, lawsone **13**, and TFA **121** undergo Michael addition where the 3-position of **13** attacks the β-position of intermediate **120** to form the imine **122**. This is followed by hydrolysis of intermediate **123** to form the final product **51**.



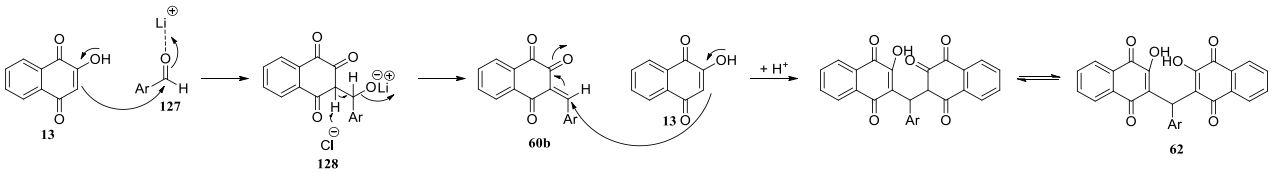
Scheme 2.5 The acid-catalysed Michael addition of α,β-unsaturated ketones **50** to lawsone, using a primary amine organocatalyst **56** and TFA (**121**).

Zhou *et al.*[20] used thiourea and cinchona alkaloid organocatalysts to achieve the Michael addition of various nitroalkenes to lawsone (Scheme 2.6). Organocatalysts containing a thiourea group such as **124** form hydrogen bonds with the oxygen atoms of the nitro moiety in the nitroalkene **52** and the oxygen atom of the hydroxy moiety in the lawsone **13**, thereby presenting favourable electronics for Michael addition of the vinyl electrons of **13** at the β-position of **52**. Oxidation of the resulting intermediate **125**, resulting in the reformation of the catalyst, forms the final product **53**.



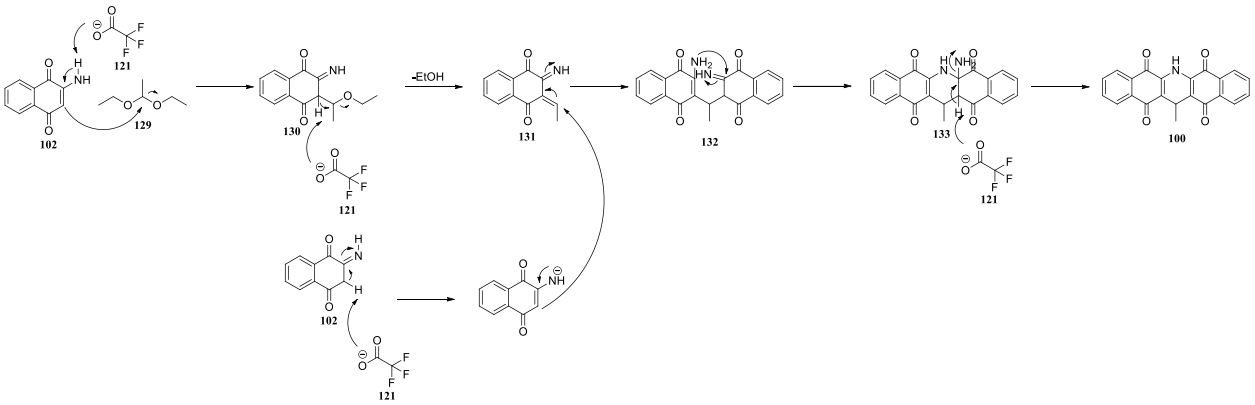
Scheme 2.6 The Michael addition of α,β-unsaturated nitroalkenes **52** to lawsone, using a thiourea organocatalyst **124**.

Tisseh and Bazgir[21] formed a dimeric product **62** consisting of two lawsone molecules joined by an aromatic linker at the vinyl position (Scheme 2.7). The reaction involved the Knoevenagel condensation of one lawsone molecule at the 3- position to the lithium chloride activated aromatic aldehyde **128**. The alkene intermediate **60b** then undergoes Michael addition with a second molecule of **13** to form the final product **62**.



Scheme 2.7 The lithium chloride catalysed addition of aromatic aldehydes **127** to lawsone to produce a dimeric final product **62**.

Compound **100** has previously been synthesised via a different route using **102** and 1,1-diethoxyethane (**129**) as starting materials (Scheme 2.8).[12] According to the methodology used by Marcos *et al.*,[12] alkylation occurs at the 3-position of **102**, followed by rearrangement and ethanol elimination to form the intermediate **131**. **131** then reacts with a second molecule of **102** via conjugate addition and the resulting intermediate **132** cyclises at the 2-position of each respective molecule to form the final product **100**.



Scheme 2.8 The previously reported synthesis of compound **100** using **102** and 1,1-dioxyethane (**129**) as reagents.[12]

While the previously mentioned reaction conditions involve alkylating agents such as aldehydes and olefins to achieve alkylation at the 3-position of lawsone, these reagents are absent in the reaction conditions used in this research despite a similar final product obtained. It is therefore necessary to propose a different mechanism for the formation of this ethyl linker in the dimeric structure of compound **100.**

As there are limited reagents used in the reaction, the origin of this ethyl insertion was hypothesised to either be from the amino acid itself, or the ethanol solvent used. These hypotheses are covered in more detail in Chapter 3, however it should be noted that it is unusual to see an alcohol or amino acid react in this way. The reactions of amines/amino acids and alcohols/aldehydes in relation to lawsone are also further discussed in Chapter 3.

2.3. Conclusions

Three products were isolated from the high pressure and high temperature reaction between lawsone and glycine in the presence of ethanol. Spectroscopic analyses were performed using UV-Vis, fluorescence, IR, NMR, and mass spectroscopy, where these compounds were identified as 13-methyl-6,13-dihydro-6-azapentacene-5,7,12,14-tetraone (**100**), 1-ethyl-3-methylnaphtho[2,3-c]isoxazole-4,9(1H,3H)-dione (**101**), and 2-amino-1,4-naphthoquinone (**102**). Compound **101** is a newly isolated compound that has not been previously reported in literature, while compound **100** is structurally different to what has been previously postulated. As the final structure of compound **100** presents the possibility of an interesting and novel alkylation method, a mechanism for the formation of compound **100** was proposed and is to be investigated in future chapters.

2.4. Experimental

2.4.1. Analysis Parameters

The analysis parameters outlined in Section 2.4.1. were applied to all experiments throughout this thesis unless stated otherwise.

All reagents were used neat from the source unless specified. The following reagents were sourced from Sigma Aldrich: 1,4-naphthoquinone; 1-propanol; 2'-methyl acetophenone; 4'-methoxy acetophenone; 6'-methoxy-1-tetralone; acetaldehyde; aluminium chloride; ammonium acetate; benzaldehyde; benzyl alcohol; calcium hydride; chloroform-*d*; diethyl carbonate; diglyme; DMSO; ethanol-*d*6; ethyl iodide; ethylacetoacetate; formaldehyde; lawsone; N-butyl amine; oxygen gas; oxalic acid; phenethylamine; potassium *t*-butoxide; propionaldehyde; sodium hydride; *t*-butanol. The following reagents were sourced from Chem Supply: 1-butanol; acetic acid; DCM; dichloroethane, diethyl ether; DMF; ethanol; ethyl acetate; formic acid; glycine; hydrochloric acid; hexane; isoleucine; L-proline; methanol; oxalyl chloride; sodium bicarbonate; sodium carbonate; sodium sulfate; toluene; triethylamine. 2-amino-1,4-naphthoquinone (**102**) was sourced from Accela ChemBio. HPLC grade acetonitrile was sourced from Rowe Scientific. Gamma aminobutyric acid was sourced from TCI.

Microwave assisted reactions were performed using a CEM Discover S-class microwave in sealed pyrex reaction vessels of 10 or 35 ml (no more than 2 or 8 mL in each vessel respectively), operated in dynamic mode with a power of 300 W, pressure maximum at 17 bar, temperatures between 130-150^{o}C according to the solvent used (internal probe), high stirring, and air cooling on. Microwaves generated from this system are focused at the sample. Microwave assisted reactions were also performed using a Milestone StartSYNTH Microwave Labstation (MA156-001) in sealed pyrex reaction vessels of 40 mL (no more than 16 mL and no less than 4 mL) with a power of 200-500 W, pressure maximum of 14 bar, temperatures between 130-150^{o}C according to the solvent used (internal probe), with stirring. Microwaves generated from this system are diffused across the microwave chamber.

Reactions that were required to be dry were performed under a nitrogen environment, where all glassware was flame-dried previously. Solvents were dried accordingly: DCM and diglyme were distilled over calcium hydride, and ethanol was dried over 4 Å molecular sieves. Reactions performed under an oxygen environment required a needle bubbling oxygen directly into the reaction solution, or an oxygen atmosphere was created via a stream above a reflux condenser.

Analytical TLC were performed on DC Kieselgel 60 F254 silica alumina backed sheets and visualised under UV light and/or developed using ninhydrin or potassium permanganate solution. Column chromatography was performed using Sanpont 230-400 mesh silica gel.

Mass spectra were obtained using two mass spectrometers. A Perkin Elmer AxION DSA TOF MS was used in positive mode using APCI, scanning between 100-1000 amu. A Waters Synapt High Definition TOF MS using ESI was also employed, used by itself or in tandem with HPLC. Samples were analysed in positive and negative mode with a cone voltage at 40 V over a mass range of 50-1000 amu and a desolvation temperature of 350°C. Scans were collected in centroid mode at 1 $scans^{-1}$.

Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence Spectrometer equipped with a Xenon flash lamp. A scan speed of 120 nm/min and a slit width opening of 5 nm was used. Spectra for compound **100** were acquired in the excitation wavelength range of 550-680 nm and the emission wavelength range of 350-540 nm. Spectra for compound **101** were acquired in the excitation wavelength range of 364-414 nm and the emission wavelength range of 381-550 nm. Spectra for compound **102** were acquired in the excitation wavelength range of 314-444 nm and the emission wavelength range of 460-600 nm. A quartz fluorescence cell and $CHCl_3$ as solvent were used.

UV-Vis spectra were recorded on a Varian Cary-50 UV-Vis spectrophotometer equipped with a xenon pulse light source. Spectra were acquired between the wavelength range of 250-800 nm. A quartz cuvette was used, as well as $CHCl_3$ and DCM as solvents.

IR spectra were recorded on a Perkin-Elmer Frontier FTIR, from 4000 to 300 cm^{-1} over 12 scans. Samples were prepared as a thin film on NaCl discs.

^{1}H and ^{13}C NMR spectra were measured with a Bruker Ultrashield 600 (600 MHz and 150 MHz respectively). The instrument was also used to obtain COSY, HMBC and HSQC spectra. All samples were analysed in $CDCl_3$ unless otherwise stated and calibrated from residual $CDCl_3$ signals (7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C). For each compound synthesised, the chemical shift, multiplicity, J-coupling constants, and integration are reported where appropriate. The multiplicities are stated as follows: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, dt=doublet of triplets, br=broad.

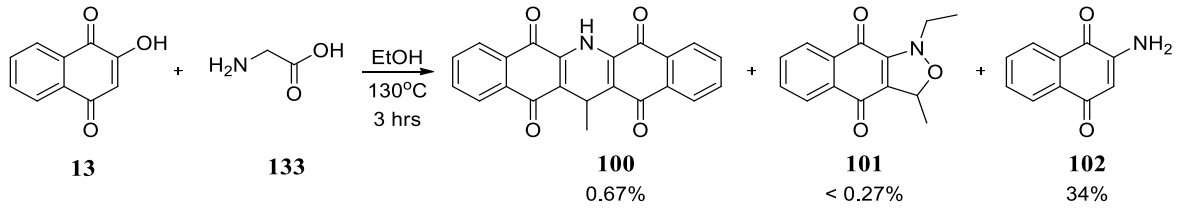
HPLC, by itself or in tandem with MS, was performed using an Agilent 1200 HPLC, or a Waters Aquity UPLC coupled to a Waters Synapt high definition TOF MS. Separation was performed using a Zorbax Eclipse XDB-C18 (4.6 mm x 150 mm x 5 µm) column. The solvent system consisted of water with 0.1% formic acid (A) and acetonitrile (B), with the following gradient used: 0 min 90% A : 10% B, 0-25 min 40% A : 60% B, 25-28 min 10% A : 90% B, 28-33 min 10% A : 90% B, 33-37 min 90% A : 10% B. The system was run with a flow rate of 1 $mlmin^{-1}$ and an oven temperature at 25°C. An injection volume of 10 µL was used. A diode array detector was employed, scanning between 190-400 nm or 380-500 nm.

GCMS was performed using an Agilent 7890 gas chromatograph with an Agilent 7693 autosampler coupled to an Agilent 5975 quadrupole mass spectrometer. Separation was performed using a HP-5MS (5%-phenyl)-methylpolysiloxane (30m x 250 µm x 0.25 µm) column. A helium carrier gas at a flow rate of 1.3 mL/min with a 5 minute solvent delay was employed. A splitless injection with an injection temperature of 280°C was used. The column temperature was initially 40°C for 1 minute, followed by ramping at 10°C/min to 260°C and held for a further 25 minutes. The mass detection range was between 50-500 amu using electron impact ionisation at 70 eV.

Modelling was performed using Gaussian 09W software with a GaussView 5.0 interface. The parameters used to model each structure are outlined in their respective tables, which can be found in the Appendices.

2.4.2. The reaction of lawsone with glycine in ethanol

Recorded spectra not included in this chapter can be found in Appendix 1.



Scheme 2.9 Microwave reaction of lawsone and glycine, that produces **100**, **101**, and **102**

Ethanol (5.6 mL) was added to a mixture of lawsone (0.5050 g, 2.9 mmol) and glycine (0.1126 g, 1.5 mmol) and heated with stirring under reflux to 80°C for a week. Alternatively, ethanol (2 mL) was added to a mixture of lawsone (0.3308 g, 1.9 mmol) and glycine (0.0825 g, 1.1 mmol) and heated to between 130°C for three hours in a microwave (300 W). The resulting purple precipitates were dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using silica gel pre-treated with 1% triethylamine.

A red compound (**100**) (0.0026 g, 0.67% yield) was isolated: R_f (80:20 HX:EtOAc) 0.3; Fluorescence ($CHCl_3$) λ_{ex} 520 nm, λ_{em} 615 nm; UV-vis (DCM) 275.6, 330.5 (shoulder), 515.5 nm; ν_{max} (thin film) 3370, 2924, 1681, 1646, 1595, 1574, 1485, 1473, 1387, 1356, 1327, 1295, 1277, 1222, 1195, 724, 709, 645, 612, 572, 514 cm^{-1}; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.28 (s, 1H), 8.14 (d, *J* = 7.6 Hz, 2H), 8.11 (d, *J* = 7.6 Hz, 2H), 7.77 (t, *J* = 7.5 Hz, 2H), 7.70 (t, *J* = 7.5 Hz, 2H), 4.60 (q, *J* = 6.8 Hz, 1H), 1.29 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 182.37, 179.24, 137.64, 135.00, 133.19, 132.91, 130.36, 126.71, 126.46, 120.73, 24.92, 22.06; MS (APCI) [M+H]: 356.086.

A yellow compound (**101**) (< 10 mg) was isolated: R_f (80:20 HX:EtOAC) 0.45; Fluorescence ($CHCl_3$) λ_{ex} 364 nm, λ_{em} 439 nm; UV-vis (DCM) 268.9, 335.9 (shoulder), 423.4 nm; ν_{max} (thin film) 3351, 3248, 2924, 2851, 1775, 1706, 1620, 1594, 1577, 1467, 1336, 1291, 1239, 1107, 1007, 730, 696 cm^{-1}; ^1H NMR (600 MHz, Chloroform-*d*) δ 8.07 (dd, *J* = 7.7, 1.2 Hz, 1H), 8.03 (dd, *J* = 7.7, 1.3 Hz, 1H), 7.70 (td, *J* = 7.6, 1.3 Hz, 1H), 7.60 (td, *J* = 7.5, 1.2 Hz, 1H), 5.08 (q, *J* = 6.7 Hz, 1H), 3.48 (qd, *J* = 7.0, 1.3 Hz, 2H), 1.42 (d, *J* = 6.7 Hz, 3H), 1.22 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 182.25, 182.05, 145.37, 134.67, 133.30, 132.12, 130.62, 126.32, 126.04, 115.26, 72.02, 64.65, 18.94, 15.56; MS (APCI) [M+H]: 244.0969.

An orange compound (**102**) (0.0902 g, 34% yield) was isolated: R_f (80:20 HX:EtOAc) 0.14; Fluorescence ($CHCl_3$) λ_{ex} 334 nm, λ_{em} 566 nm; UV-vis ($CHCl_3$) 285.47, 332.06, and 425 nm; ν_{max} (thin film) 3408, 3368, 3324, 3287, 2955, 2923, 2853, 1688, 1618, 1604, 1595, 1551, 1367, 1268, 1125, 988, 781, 727 cm^{-1}; ^1H NMR (600 MHz, Chloroform-*d*) δ 8.08 (d, *J* = 7.8 Hz, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 7.73 (t, *J* = 7.5 Hz, 1H), 7.64 (t, *J* = 7.5 Hz, 1H), 6.00 (s, 1H), 5.15 (s, 2H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 183.88, 181.98, 148.37, 134.74, 133.46, 132.40, 130.66, 126.35, 126.28, 105.31; MS (APCI) [M+H]: 174.0554.

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3. Investigations into the Origin of Dimeric Linkers

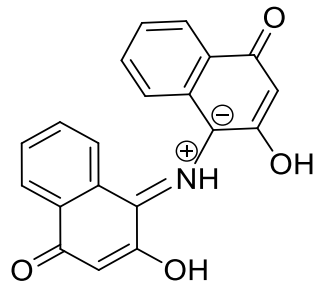
3.0. Overview

Compound 100 was isolated in the work of Jelly *et al.*[1,2] however the structure was misidentified. A primary reason for this misidentification was the observation of a secondary ethyl group in its ^{1}H NMR spectrum. The incorporation of such an ethyl group could not be explained at the time, as Jelly *et al.* could not identify reagents within the reaction mixture that could contribute this moiety. Consequently, this ethyl group was not included in their proposed structure. With the confirmation in Chapter 2 that an ethyl group does indeed exist in the structure for Compound **100**, referred to as the alkyl linker, this chapter investigated the postulation that the alkyl linker may originate from the amino acid or the ethanol solvent. Results confirmed that the ethanol solvent was the source of the alkyl linker. Upon this confirmation, proposed reaction requirements or conditions including nucleophilic substitution and the presence of aldehydes and oxidising agents, such as H_2O_2, were investigated.

3.1. Introduction

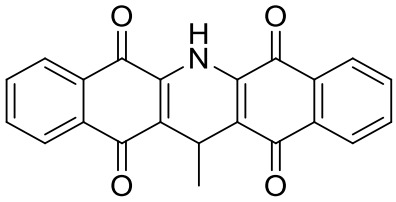
Lawsone was first investigated as a possible reagent for the detection of latent fingermarks by Jelly *et al.*[1,2] Their research showed that the application of lawsone to fingermark deposits resulted in a purple-brown stain with red luminescent properties. Jelly *et al.* isolated a red compound from the purple-brown mixture and attempted to identify its structure through NMR analysis. The ^{1}H NMR spectrum for this compound exhibited two key signals that indicated the presence of a secondary ethyl group in the molecule: a doublet at 1.29 ppm and a quartet at 4.60 ppm. Although Jelly *et al.*[3] observed these signals and postulated the existence of the ethyl group, they could not identify a reagent with appropriate reactivity to provide such a moiety to the structure of Compound 100. It was therefore not included in their proposed structure **18** (Figure 3.1), nor was it investigated further.

a)



18

b)



100

Figure 3.1 a) Structure of the red compound (**18**) proposed by Jelly *et al.*[1,2] b) Revised structure **100** proposed in Chapter 2.

In this thesis (Chapter 2), the red Compound **100** was isolated from the reaction between lawsone and glycine in ethanol. The fluorescence, ^{13}C, and ^{1}H NMR spectra for this compound were consistent with that reported by Jelly *et al*.[3] from their investigations into the structure elucidation. A new structure was proposed (Figure 3.1) that incorporated the secondary ethyl group as a linker between two lawsone molecules. A closer examination of the reaction mixture in Chapter 2 identified two possible reagents that could contribute such an ethyl group: glycine and ethanol. These reagents contain the needed ethyl chain backbone, while only ethanol has the appropriate configuration of hydrogens. A mechanism to explain the insertion of the chosen amino acid into the structure of Compound 100 was proposed in Chapter 2 (Figure 3.2). However, the addition of alkyl chains from such functional groups was not postulated as this usually requires the presence of additional reagents which are absent in this reaction mixture.

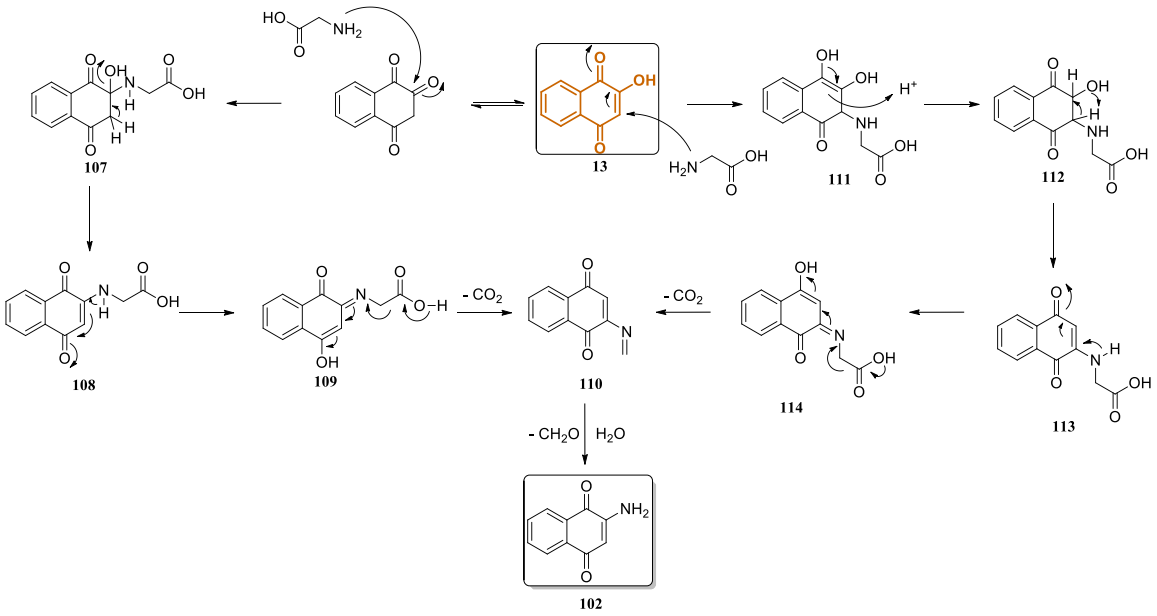


Figure 3.2 Proposed mechanisms of direct substitution (left) and conjugate addition (right) between glycine and lawsone.

The feasibility of either the glycine or the ethanol contributing the ethyl linker to Compound 100 was examined in this chapter. Glycine was replaced with different amino acids and amines to identify the contribution of glycine to Compound **100**, as well as investigate the importance of decarboxylation in dimer formation. The contribution of the ethanol solvent was explored using deuterated ethanol, while the synthesis of potential analogues was attempted by replacing the ethanol with alcohols of differing chain length. Three possible reaction requirements for alcohol incorporation with only reagents that may be found in the reaction were proposed and tested. These include the possibility of nucleophilic substitution to introduce the alkyl linker, the presence of aldehyde forms of the alcohol solvent, and the requirement of oxidative conditions to drive product formation.

3.2. Results and Discussion

3.2.1. Investigations into the role of the amino acid

The mechanism in Figure 3.2 describes the formation of an imine **110** as a result of decarboxylation of the amino acid, which can be further hydrolysed to form the suspected 2-amino-1,4-naphthoquinone (**102**) intermediate. Investigations were undertaken to determine the importance of using an amino acid, and whether it contributes to the Compound **100** structure in other ways besides forming the amine linker. If the amino acid is important for driving the reaction towards the intermediate **102**, it was theorised then that amines would not form the dimer when used in the reaction. Primary amines N-butylamine and phenethylamine were used instead of glycine in the reaction under microwave conditions described in Chapter 2, using two equivalents of lawsone to the amine.

The reaction of N-butylamine with lawsone in ethanol produced an orange compound (R_f = 0.37). Analysis of the acquired ^{1}H NMR spectrum indicated the structure to be that of **134** (Figure 3.3). The triplet at 0.96 ppm, multiplets at 1.43 and 1.66 ppm, and quartet at 3.16 ppm originate from the butyl chain in the structure and integrate for three, two, two, and one proton(s) respectively. The aromatic signals between 7.59 and 8.10 ppm and vinylic signal at 5.72 ppm, originating from the lawsone, integrate for one proton each as expected. Importantly, these lawsone signals integrate in a 1:1 ratio for the expected number of protons in comparison to the butyl chain signals and suggests that dimerization has not occurred. **134** has previously been synthesised by Liu *et al.*[4], and the ^{1}H NMR data presented in their work strongly correlate to that presented here.

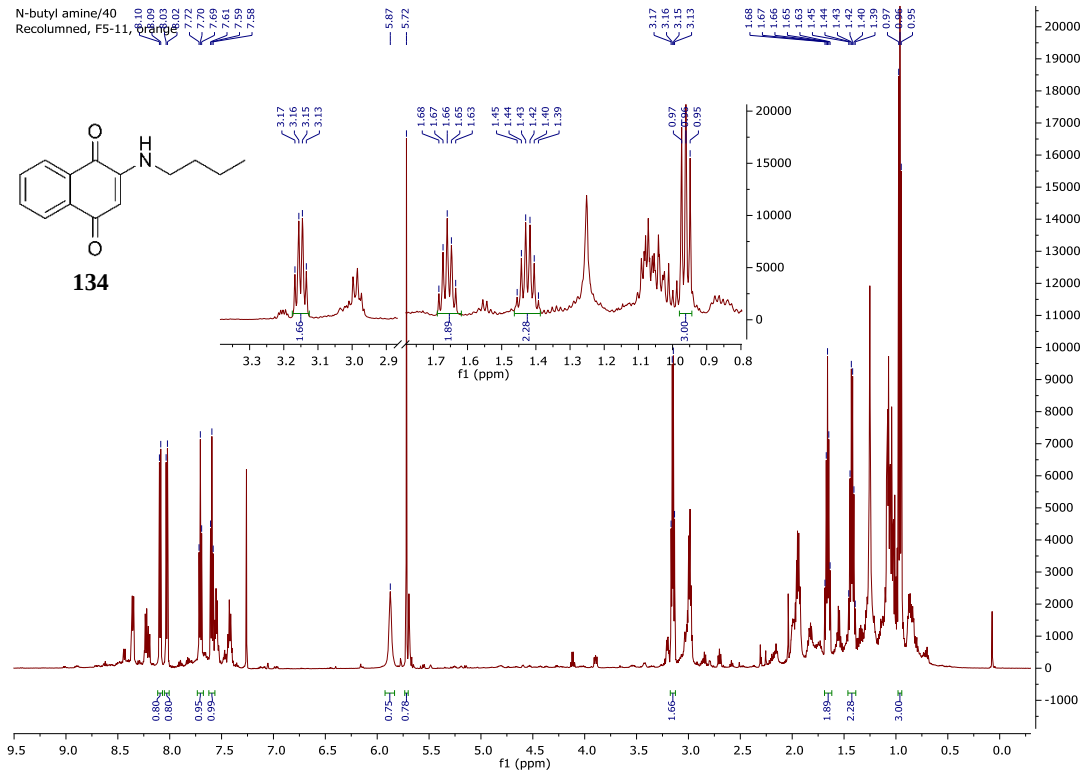


Figure 3.3 ^{1}H NMR spectrum of **134**, resulting from the reaction between N-butylamine and lawsone in ethanol.

The reaction using phenethylamine produced an orange compound (R^f = 0.15). The ^{1}H NMR spectrum of this compound (Figure 3.4) exhibited a triplet and quartet at 2.98 and 3.47 ppm respectively. These integrated for two protons each, and correlate to the ethyl chain originating from the phenethylamine. Aromatic triplets at 7.26 and 7.34 ppm integrating for one and two protons respectively, as well as a doublet at 7.23 ppm integrating for two protons, were present. These correlate to signals from the phenethylamine benzene ring. Aromatic peaks originating from the lawsone molecule are present at 7.61, 7.73, 8.03, and 8.10 ppm, integrating for one proton each. The vinylic proton at 5.78 ppm was also observed, integrating for one proton. The integrations of the phenethylamine moiety in relation to the lawsone moiety are to be expected if a 1:1 reaction has occurred, similar to that observed for the reaction using N-butylamine. This information suggests the structure **135** (Figure 3.4). A Scifinder structure search (May 2020) for **135** returned only one study that reported ^{1}H NMR spectra. Josey *et al.*[5] reported to have synthesised **135** via a similar reaction between 2-bromo-1,4-naphthoquinone and phenethylamine in ethanol, however the reported NMR spectra does not correlate to the data reported in this work. Closer examination of their published ^{1}H NMR spectra shows evidence of an additional proton environment, as well as four less carbon environments, than would be expected and

are therefore inconsistent with **135**. Given these inconsistencies, ^{13}C and 2D NMR spectra were also collected (shift assignments have been included in Appendix 2). In combination with the ^{1}H NMR shifts, the sixteen ^{13}C shifts observed and 2D correlations are consistent with the proposed structure of **135**.

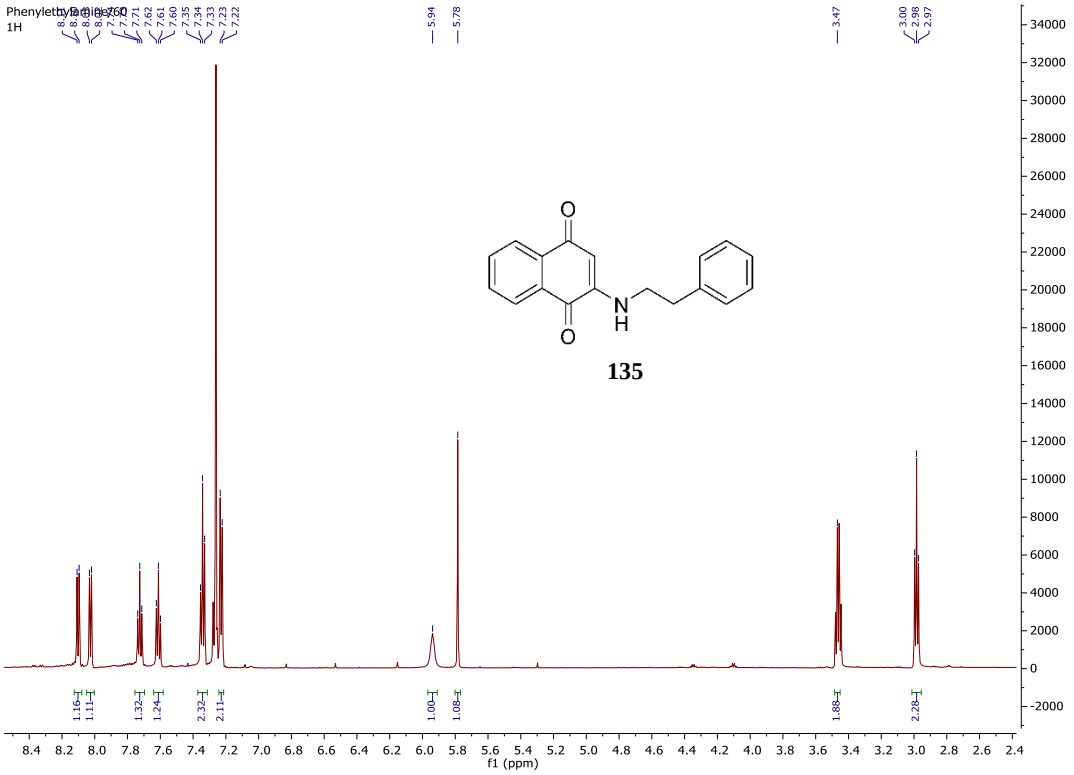
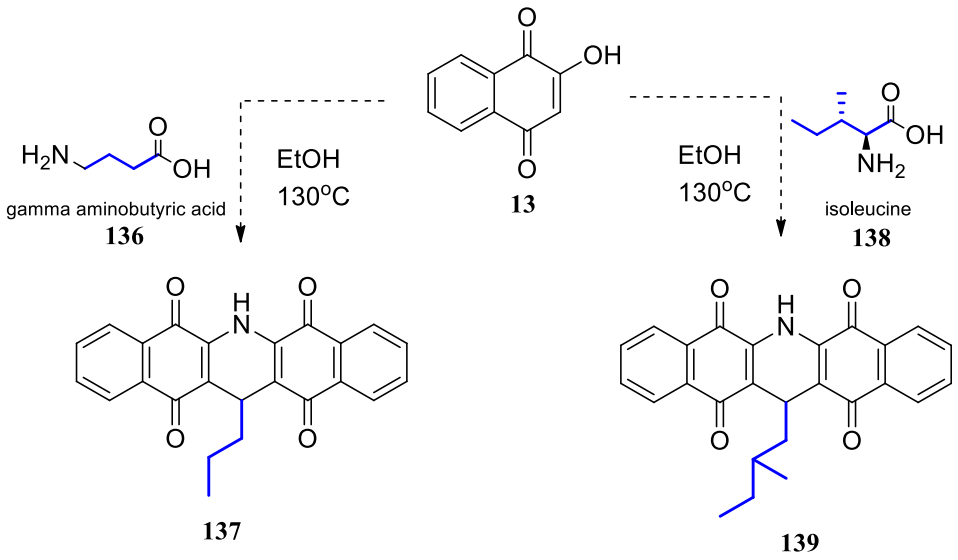


Figure 3.4 ^{1}H NMR spectrum of **135**, isolated from the reaction between phenethylamine and lawsone in ethanol.

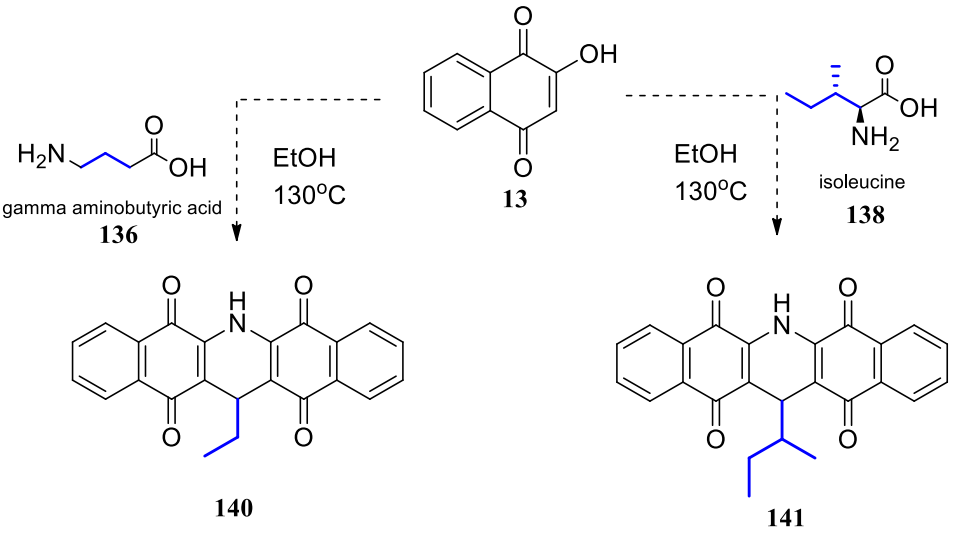
The reactions with primary amines did not yield dimers analogous to Compound **100**, which indicates that the inclusion of the amino acid is required for dimer formation. The Strecker degradation outlined in Figure 3.2 was similarly postulated by Jelly *et al.*,[3] albeit at a C-1 reaction site on the lawsone. Lysine, serine, proline, and glycine were all reacted with lawsone on filter paper by Jelly *et al.*,[3] where they reported that all amino acids except proline produced a purple stain. They proposed that the reaction did not proceed with proline due to its nature as a secondary amine. All amino acids used in their experiments were α-amino acids, however the effect of other amino acid configurations, such as γ, was not tested.

The importance of using an α-amino acid in the reaction, in addition to whether the amino acid could be the source of the alkyl linker, was investigated by using isoleucine **138** and γ-Aminobutyric acid (GABA) **136** (Figure 3.5) in place of glycine. It is plausible to consider that the alkyl linker may originate from the amino acid, as both glycine and ethanol contain the correct number of carbons required for

the formation of the alkyl linker in Compound **100**. In this case, the glycine must remain carboxylated prior to incorporation to yield the correct number of carbons, however it is unclear how the amine and carboxylic acid are removed to leave only the ethyl linker. Alternatively, as shown in Figure 3.2, the decarboxylation and hydrolysis of the imine intermediate forms the associated Strecker aldehyde. It is also plausible that this Strecker aldehyde may react with the lawsone to form Compound **100**. The incorporation of either the amino acid itself or the Strecker aldehyde were considered (Figure 3.5).



a)



b)

Figure 3.5 Proposed products from the reaction of isoleucine or GABA with lawsone. a) The proposed products **137** and **139** if the amino acid is incorporated entirely as the alkyl linker in the dimer; b) the proposed products **140** and **141** if the associated Strecker aldehyde is incorporated as the alkyl linker in the dimer.

The reaction between isoleucine and lawsone in ethanol returned a red compound, with an R_f value of 0.26. ^{1}H NMR analysis (Figure 3.6) showed a quartet and doublet at 4.65 and 1.32 ppm, as well as two triplets at 7.72 and 7.78 ppm and two doublets at 8.14 and 8.17 ppm. The quartet and doublet at 4.65 and 1.32 ppm, integrating for one and three protons respectively, are characteristic of the vinyl ethyl group also observed in Compound **100**. If the amino acid itself or the Strecker aldehyde is incorporated at the vinyl position, a doublet or triplet respectively around 4.5 ppm would be observed instead of the quartet, as seen in Figure 3.6, due to the unique spin-spin coupling of their alkyl linkers. Additional multiplets integrating for two or three protons would also be seen. The absence of these expected shifts, and the presence of those consistent with Compound **100**, would suggest that Compound **100** was also returned when using isoleucine. The aromatic signal shifts and their integration of two protons each are also consistent with patterns observed for the naphthoquinone moiety in Compound **100**.

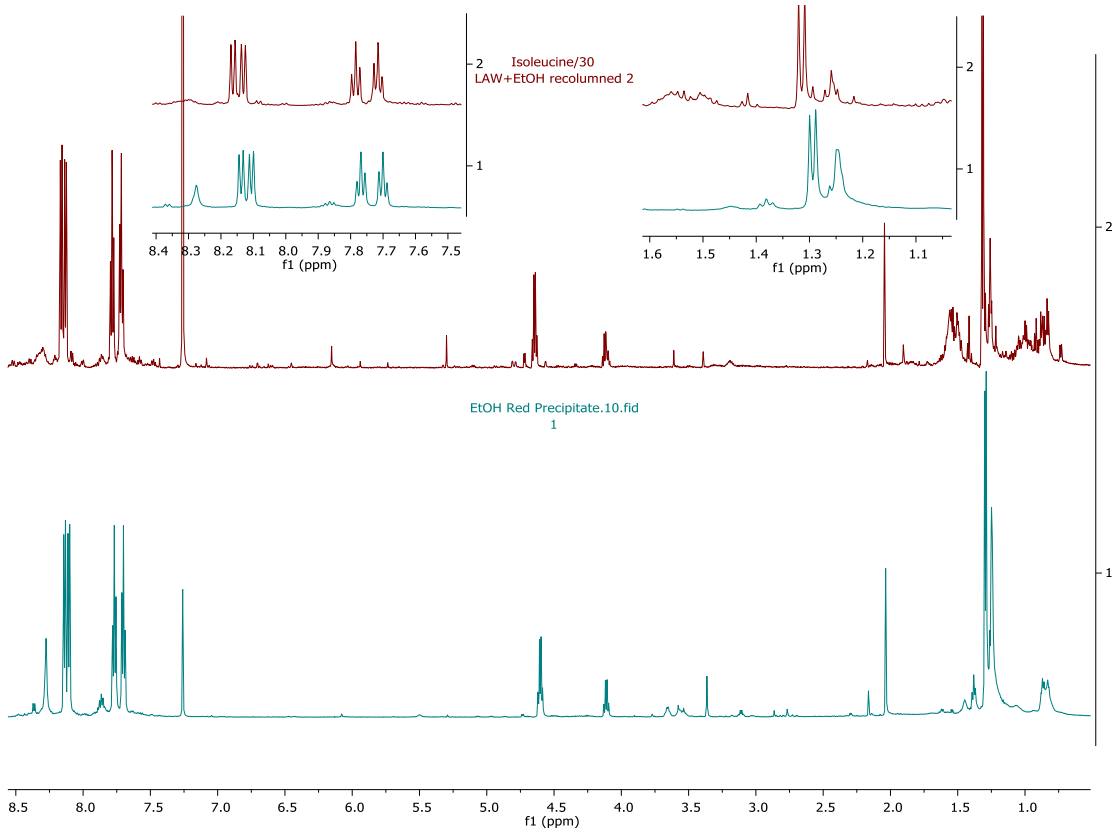


Figure 3.6 ^{1}H NMR spectrum of the isolated red product from the reaction with isoleucine (top, red), and known Compound **100** (bottom, green).

Replacing glycine with GABA also returned a red compound (R_f = 0.25). It would be predicted that the structures outlined in Figure 3.5 would display a triplet around 4.5 ppm and several multiplets between 1-3 ppm corresponding to a propyl or butyl chain. The aromatic region of the ^{1}H NMR spectrum (Figure 3.7) for this compound remains consistent with that seen for Compound 100. Crucially, a quartet at 4.60 ppm and a doublet at 1.32 ppm are present, with no triplets at approximately 4.5 ppm able to be identified. Similar to the outcome with isoleucine, the quartet and doublet are suggestive of a vinyl ethyl alkyl inker, and indicative that this red compound is also that of Compound **100**.

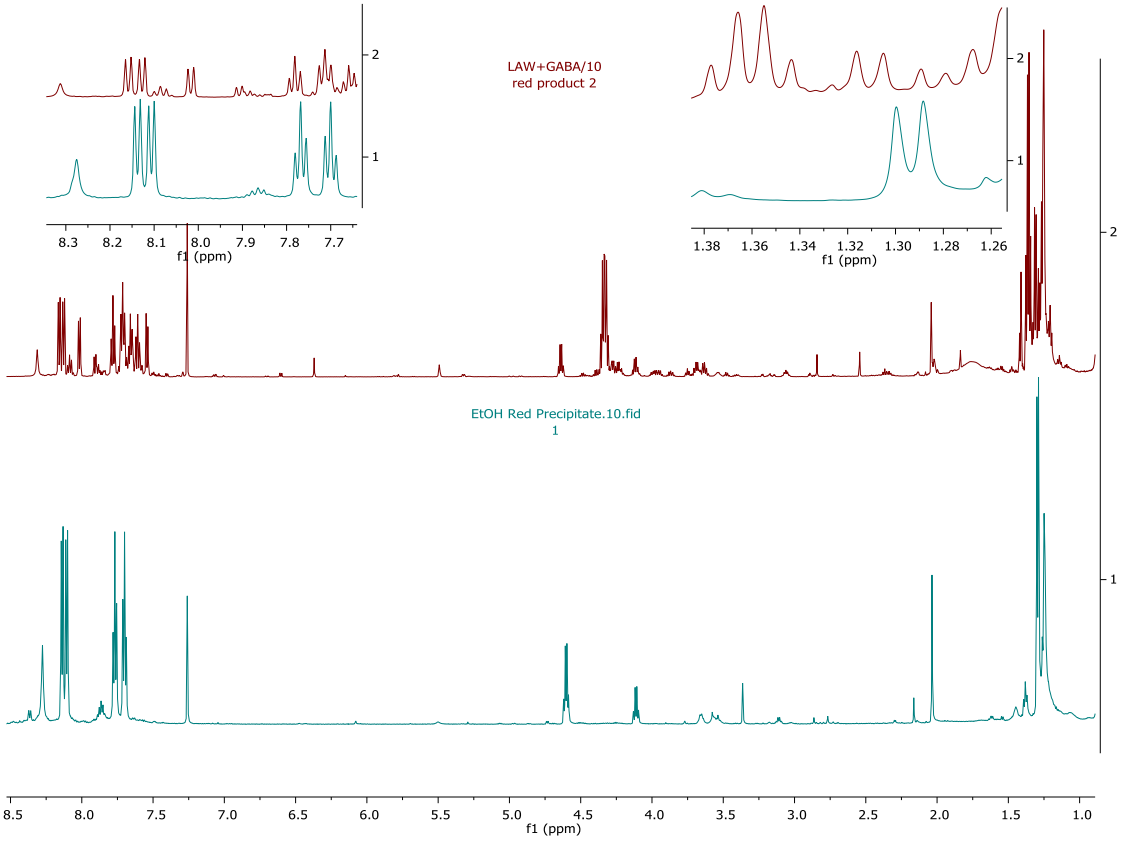


Figure 3.7 ^{1}H NMR spectrum of the isolated red product from the reaction with GABA (top, red), and known Compound **100** (bottom, green).

The ^{1}H NMR spectra of the microwave reaction with isoleucine and GABA confirm that Compound **100** was reproduced, regardless of the amino acid chain length or side chains present. This is consistent with the mechanisms proposed for the incorporation of the amino acid into the dimer, outlined in Figure 3.2. The mechanism outlined in Figure 3.2 considers α-amino acids, where the charged

intermediates resulting from decarboxylation are stabilised and enabled by the formation of the imine.[6] Initially, it would appear that the mechanism requires an α-amino acid to obtain facilitative electron stabilisation of the intermediates. However, GABA has been reported to undergo an analogous reaction with the quinone ninhydrin to produce the familiar Ruhemann's purple via Strecker degradation.[7] β-amino acids were not trialled in this work with lawsone as they were not readily available, but there are varying reports of reactivity of with ninhydrin for comparison. Several studies have reported reactivity based on colour change. These ranged from very low detection[8] to slightly higher detection[9–11] with basic or acidic conditions, or longer heating times.

3.2.2. Investigations into the role of the alcohol solvent

Given that the evidence suggests that the amino acid is not the source of the alkyl linker in Compound **100**, it was decided to investigate other reagents in the reaction mixture, particularly the alcohol solvent. Ethanol provides the required number of carbons seen in the alkyl linker for Compound **100**, and no other reagent in the reaction mixture could provide this. Reaction conditions were repeated using deuterated ethanol (EtOH-*d*6), as the incorporation of deuteriums into the dimeric structure can be detected using MS and NMR spectroscopy.

It was postulated that if the alkyl chain of EtOH-*d*6 was incorporated into Compound **100**, MS could be used to observe an increase in mass to accommodate for the four deuterium atoms. As Compound 100 was found to have a reported [M+H] = 356.086 and exact mass of 355.086 amu, it would therefore be expected that the deuterated Compound 100b would have a heavier molecular weight of 359.37 gmol^{-1} (Figure 3.8).

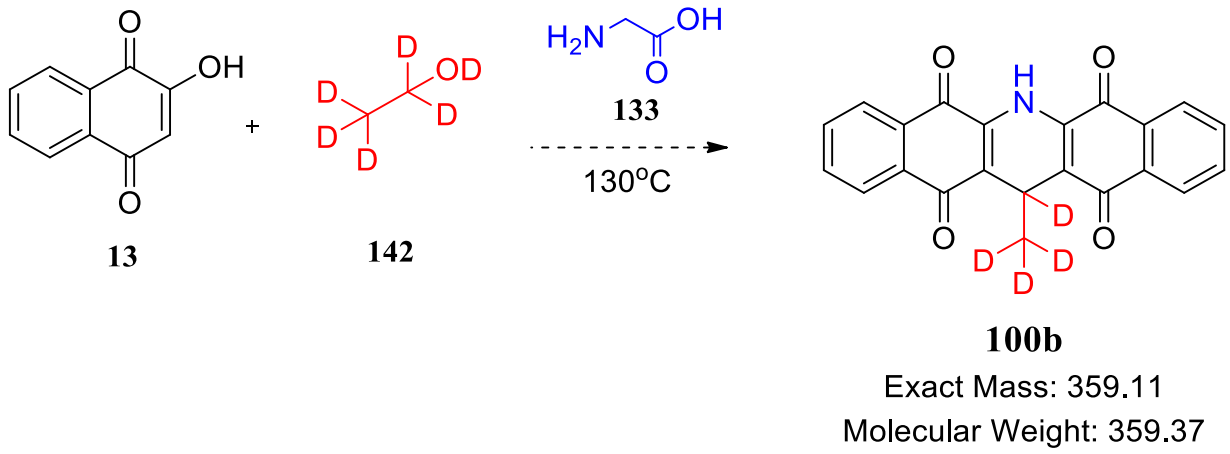


Figure 3.8 Expected deuterated product (**100b**) from the reaction between lawsone and glycine in EtOH-*d*6.

The mass spectrum obtained for this deuterated red compound **100b** (Figure 3.9) displayed a [M+H] = 360.1167, suggesting a molecular weight of approximately 359 $gmol^{-1}$. The molecular formula of **100b**, $C_{22}H_9D_4NO_4$, is plausible for this mass. The mass error was calculated to be -0.277 ppm, which suggests that the compound is that of **100b**, as this is within an acceptable error range.

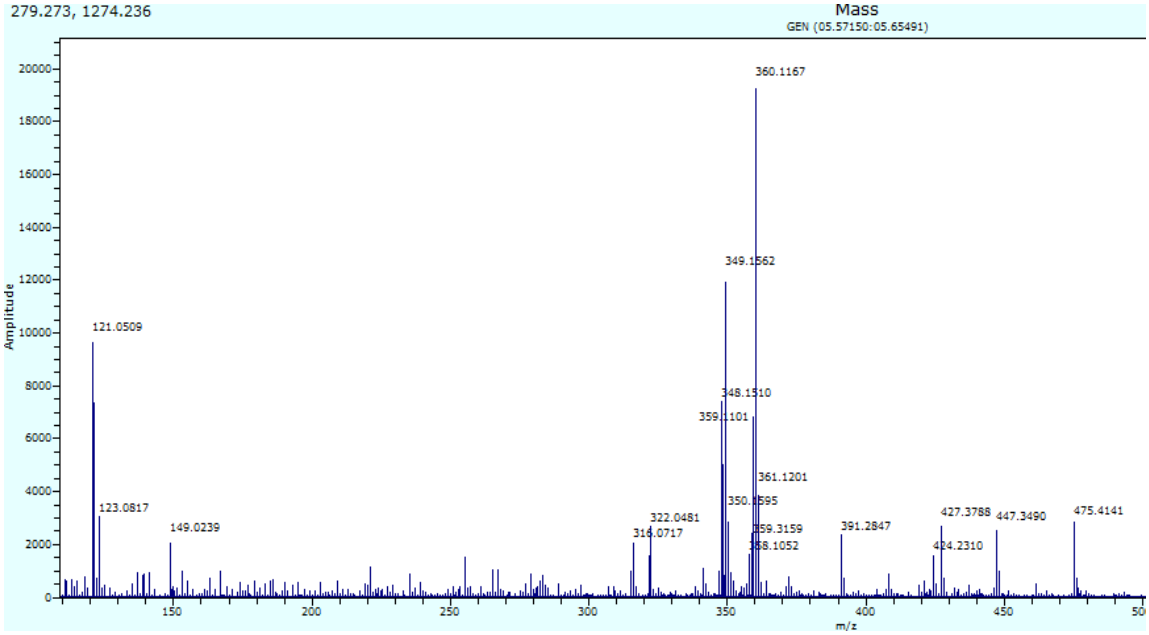


Figure 3.9 Mass spectrum of deuterated compound **100b**, showing a possible molecular ion of m/z = 360.1167.

The ^{1}H NMR and ^{13}C NMR signals exhibited by **100b** are in agreement with those stated in Chapter 2 for Compound **100** (Table 3.1). However, it is noticeable that the quartet at 4.60 ppm and doublet at 1.29 ppm of the Compound **100** ^{1}H NMR spectrum are absent in the ^{1}H NMR spectrum for **100b** (Figure 3.10). Similarly, the ^{13}C NMR spectrum for **100b** returned ten signals in close agreement with the aromatic signals of Compound **100** (Figure 3.11), however the shifts pertaining to the ethyl linker are absent.

The inclusion of deuterium into the compound is likely to impact the information obtained from NMR spectra. A primary reason for this is the difference in gyromagnetic ratios between hydrogen and deuterium, which ultimately affects nucleus interaction with the magnetic field and therefore signal sensitivity. The gyromagnetic moment is a constant that is unique to each isotope. The magnetic moment (μ) of a nucleus is defined by equation 1, where it is proportional to its nucleus spin (I) and gyromagnetic ratio (γ).

$$\mu = l\gamma \quad (1)$$

Hydrogen atoms exhibit higher sensitivity in their NMR spectra due to having a high gyromagnetic ratio of 42.58 MHz/T, and why protons are normally used for NMR purposes.[12,13] Deuterium atoms are usually absent in ^{1}H NMR spectra due to having a much lower gyromagentic ratio of 6.54 MHz/T, causing a severe decrease in sensitivity.[12,13] Therefore, if the deuteriums were incorporated to form Compound **100b**, it would be expected that the signals pertaining to the ethyl linker observed in the spectra for Compound **100** would not be easily observed. As all ^{1}H NMR signals for Compound **100** were observed except for those pertaining to the ethyl linker, there was no evidence to suggest that deuteration had occurred at any other position of Compound **100** other than the ethyl linker. The presence of deuterium can also affect how signals appear in ^{13}C NMR spectra due to its spin of 1 (as opposed to ½ for hydrogen) and the decoupling methods used. In typical ^{13}C NMR spectra where ^{1}H are present, the ^{13}C signals are decoupled from the ^{1}H signals to result in singlets pertaining to a unique carbon environment. Decoupling does not occur for coupling between ^{13}C and ^{2}H, and therefore the signal will present as multiplets dictated by equation 2.

Splitting patterns of specific environments can be predicted using the following equation 2, considering the number of neighbouring nuclei the atom couples to (n) and the atom nuclei spin (I):

$$2nl + 1 \quad (2)$$

In the absence of decoupling methods between ^{13}C and ^{2}H, the splitting patterns of the ^{13}C signals would be expected to appear as multiplets according to equation 2. Decoupling methods involving ^{13}C and ^{1}H take advantage of the Overhauser effect (cross-relaxation between ^{13}C and ^{1}H) to efficiently remove coupling effects so that one singlet is observed, thereby amplifying the intensity of the signal. As a high degree of splitting is expected due to coupling, particularly for the terminal C-$^{2}H_3$ group, it is not unreasonable to suspect that the intensity of these signals is low and may be hidden amongst baseline noise.[14] Additionally, the relaxation of magnetic moments in nuclei back to equilibrium of a ^{13}C atom bonded to ^{2}H atom is much longer than that bonded to ^{1}H atom.[13–15] Consequently, if not enough time is given between magnetic pulses to allow for the establishment of equilibrium states of ^{13}C nuclei, signals pertaining to these nuclei are decreased.[14]

Table 3.1 Comparison of observed ^{1}H and ^{13}C NMR values for Compound **100b** and Compound **100**.

All δ are reported in ppm.

δ_C (100b)	δC (100)	$\Delta\delta_C$	δ_H (100b) [a]	δ_H (100) [a]	$\Delta\delta_H$
	22.06			1.29, d (6.8); 3H	
	24.92			4.60, q (6.8); 1H	
120.72	120.73	0.01			
126.49	126.46	-0.03	8.13, d (7.6); 2H	8.11, d (7.6); 2H	-0.02
126.75	126.71	-0.04	8.16, d (7.7); 2H	8.14, d (7.6); 2H	-0.02
130.41	130.36	-0.05			
132.96	132.91	-0.05			
133.22	133.19	-0.03	7.72, t (7.4); 2H	7.70, t (7.5); 2H	-0.02
135.03	135.00	-0.03	7.78, t (7.6); 2H	7.77, t (7.5); 2H	-0.01
137.73	137.64	-0.09			
179.31	179.24	-0.07			
182.45	182.37	-0.08			
			8.31, bs; 1H	8.28, bs; 1H	-0.03

[a] δ_H, multiplicity (J in Hz); integration

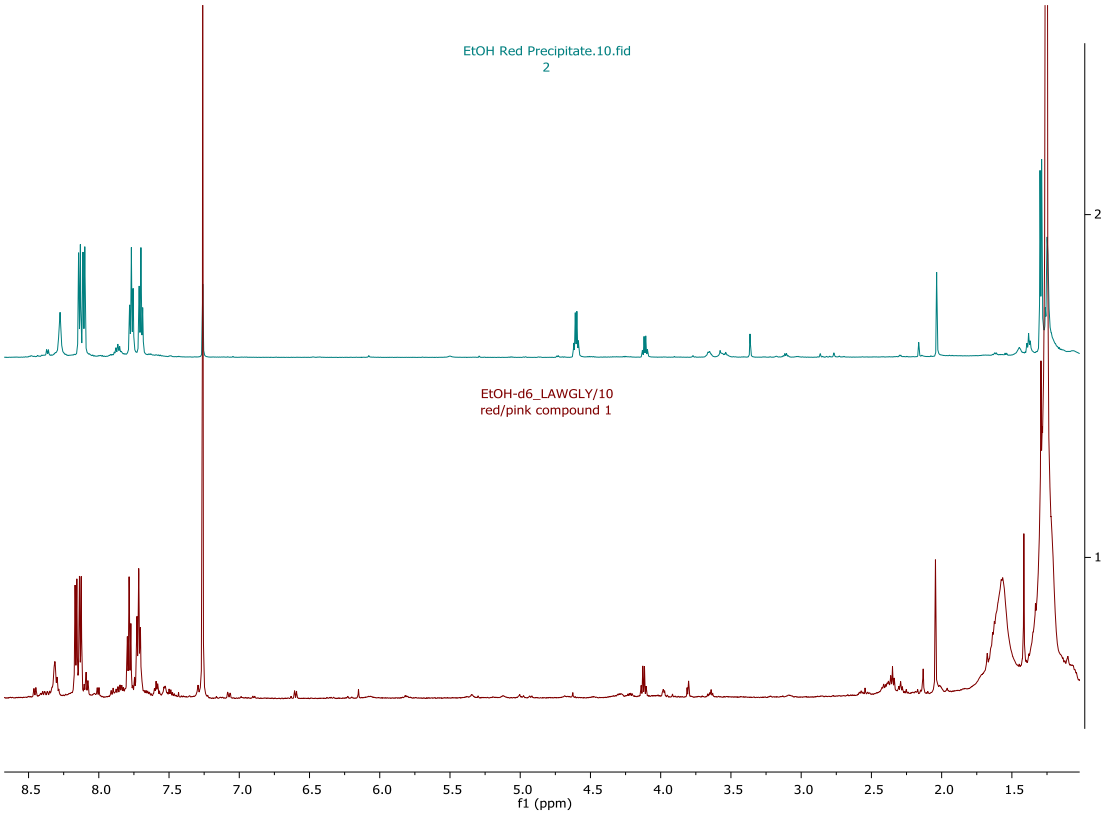


Figure 3.10 ^{1}H NMR spectrum of the red dimer **100** (top, green) and the deuterated dimer **100b** (bottom, red).

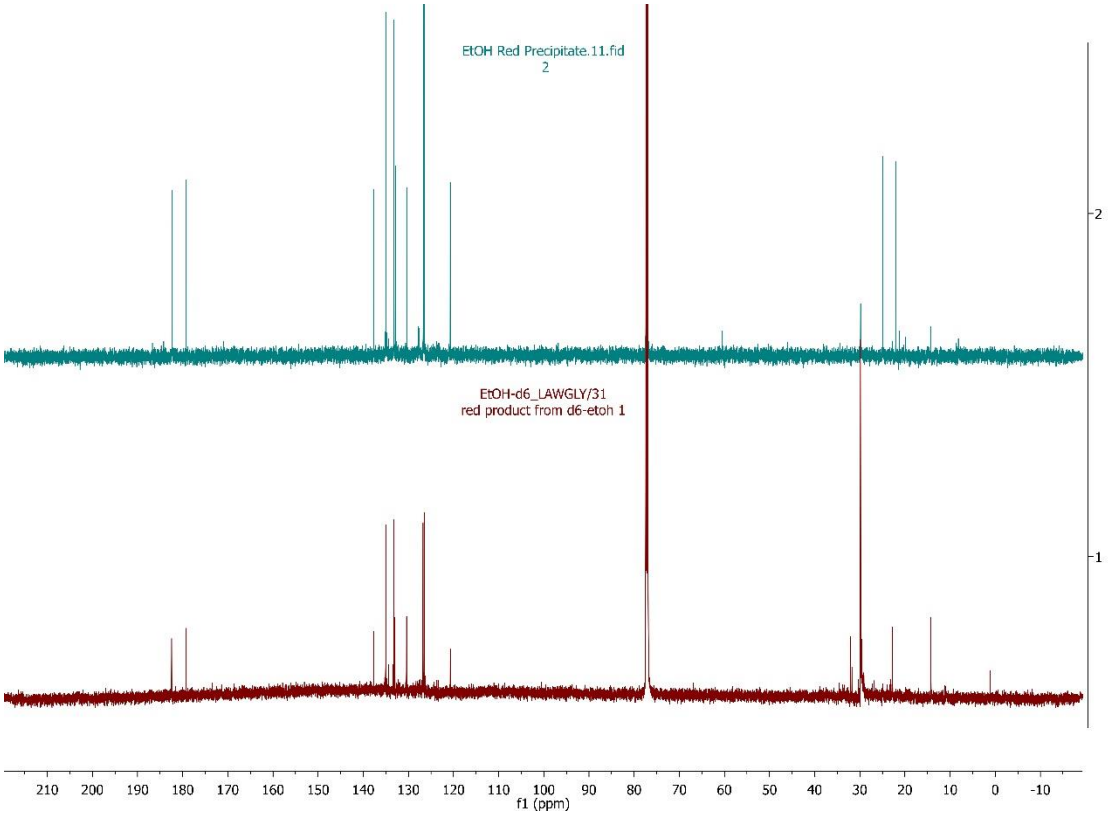


Figure 3.11 ^{13}C NMR spectrum of the red dimer **100** (top, green) and the deuterated dimer **100b** (bottom, red).

The returned [M+H] of 360.1167 and agreement of ^{1}H and ^{13}C NMR data to that of Compound **100** suggest that **100b** was successfully synthesised. The formation of **100b** supports the proposal that the alcohol is incorporated into the dimeric structure of Compound **100**, and that the alkyl chain does not come from other reagents such as the amino acid.

The incorporation of the alcohol allows for investigation into a straight-forward method to synthesise potential analogues. These analogues could be subjected to further investigation into the effects of the carbon chain length on the spectral properties of the compound, and may be used in fingermark elucidation to shift the absorbance or fluorescence signals from the fingermark away from substrate

signals. To investigate whether other alcohols could also be incorporated to form a dimer with a longer alkyl chain linker, a series of reactions were performed where ethanol was replaced with methanol, 1-propanol, 1-butanol, and benzyl alcohol to form analogues **140, 143-145**. The interpretation of the resulting ^{1}H NMR spectra was difficult due to the small amount of product that was able to be isolated, as well as the large amount of impurities that were unable to be removed after repeated attempts. These difficulties prevented the collection of MS and 2D NMR spectra, despite theoretically being able to provide more insight into the product structures, as the information they provide is more accurate when performed on pure samples. Only ^{1}H NMR spectra were collected for the reactions described in Table 3.2, and signals suspected to be from these desired products are presented in Table 3.3.

Table 3.2 Expected dimers from the use of methanol, 1-propanol, 1-butanol, and benzyl alcohol as solvent in the reaction of lawsone and glycine

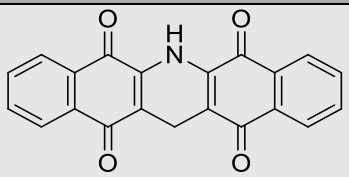
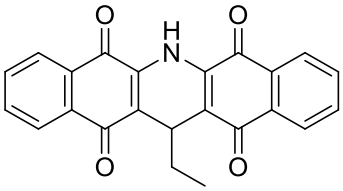
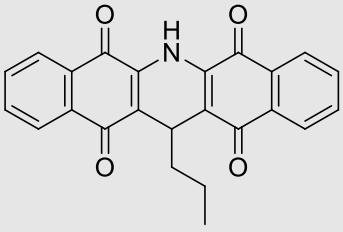
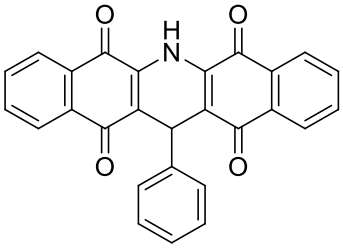
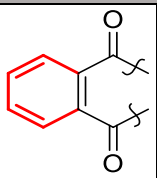
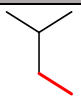
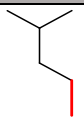
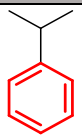
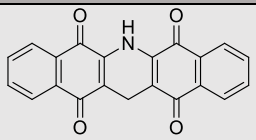
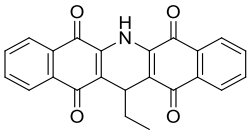
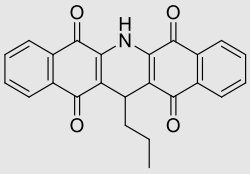
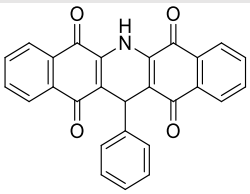
Solvent	Expected Product
Methanol	 **143**
1-propanol	 **140**
1-butanol	 **144**
Benzyl alcohol	

Table 3.3 Suspected ^{1}H NMR signals and their associated product moieties that could originate from structures **140, 143-145**

	Moiety						
Product	NH						
 **143**	9.58, s; 1H	8.48, m; 2H 8.40, m; 2H 7.93, m; 4H	5.30, s; 1H or 3.82, s; 1H or 3.11, s; 1H	-	-	-	-
 **140b**	8.31, s; 1H	8.15, dd (15.1, 7.6); 4H 7.78, t (7.5); 2H 7.72, t (6.9); 2H	4.85, t (9.7); 1H or 4.70, t (5.4); 1H	4.35, q (9.3); 2H or 1.73, q (7.6); 2H	0.84, t (7.6); 3H	-	-
 **144**	8.34, s; 1H	8.17, d (7.4); 2H 8.14, d (7.6); 2H 7.80, t (7.5); 2H 7.72, t (7.5); 2H	4.7, t (5.7); 1H	NA	1.94, m (6.7); 2H	1.04, t (7.5); 3H	-
 **145**	8.46, s; 1H	8.14, dd (12.3, 7.6); 3H 8.07. dd (12.9, 7.6); 3H 7.74, t (7.6); 4H 7.69, t (7.5); 2H 7.47, d (7.7); 2H	5.73, s; 1H or 3.94, s; 1H	-	-	-	8.14, dd (12.3, 7.6); 3H 8.07. dd (12.9, 7.6); 3H 7.74, t (7.6); 4H 7.69, t (7.5); 2H 7.47, d (7.7); 2H

a δH, multiplicity (J in Hz); integration

Based on the structures for the expected products in Table 3.2, it would be expected that the ^{1}H NMR signals in the aromatic region would not greatly differ, as they share structural similarities through the amine linker and the aromatic moiety originating from the lawsone molecule. The ^{1}H NMR spectra should contain two triplets and two doublets integrating for two protons each in the aromatic region. Additionally, a singlet at approximately 8.3 ppm correlating to the NH should be observed for all products. The ^{1}H NMR spectrum of each analogue **140, 143-145** (Figure 3.12) in Table 3.2 show clear singlets integrating for the expected one proton correlating to the amine linker. The analogues produced using 1-propanol in the presence of H_2O_2 as an oxidising agent (**140b**) and 1-butanol (**144**) exhibited aromatic signals in the ^{1}H NMR spectrum with the expected doublet and triplet splitting patterns and integrating for the expected total of eight protons (two protons for each doublet and triplet observed). The analogue (**143**) produced using methanol exhibited aromatic signals in the ^{1}H NMR spectrum as multiplets instead of the expected doublet and triplet splitting pattern, however integrated appropriately for eight protons total across this region. The ^{1}H NMR spectrum of the analogue (**145**) produced using benzyl alcohol produced several overlapping signals in the aromatic region, resulting in difficulty assigning peaks to the correct moieties of **145**.

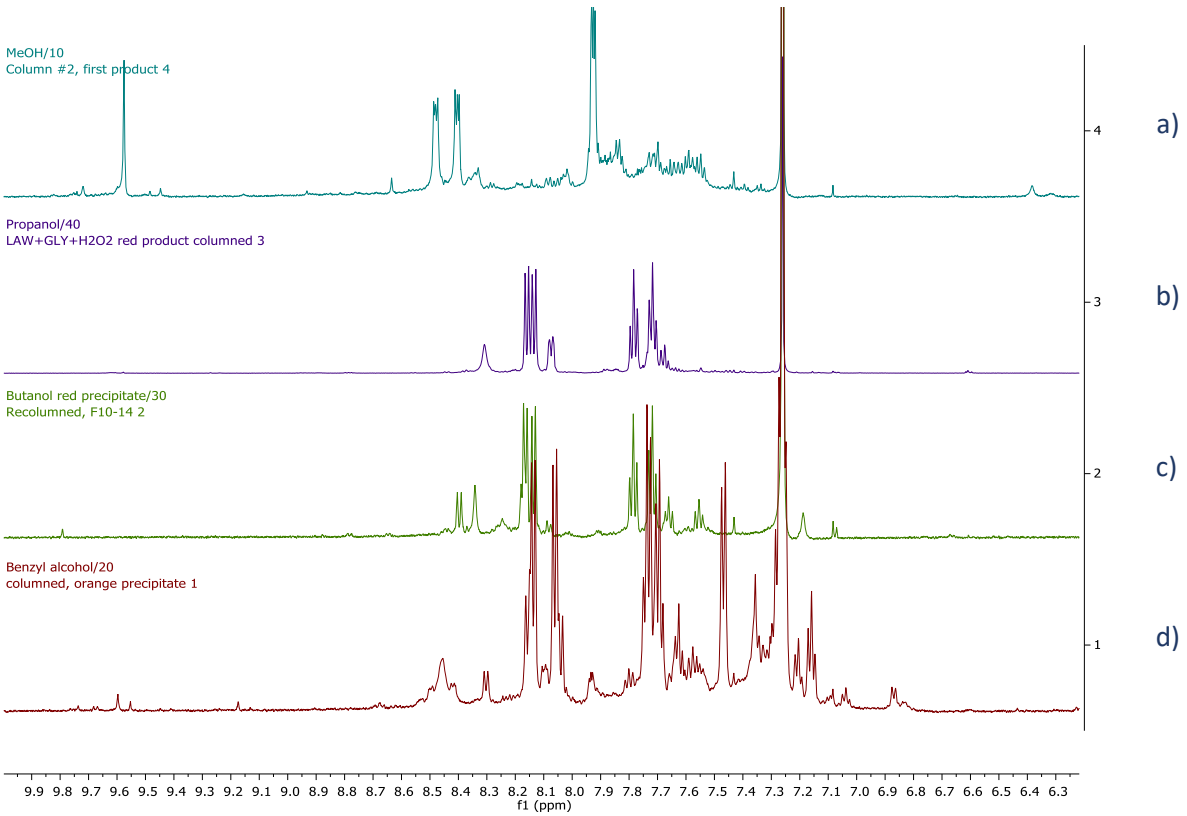


Figure 3.12 Comparison of the ^{1}H NMR spectra of a) **143**, from the reaction in methanol; b) **140**, from the reaction in 1-propanol; c) **144**, from the reaction in butanol; d) **145**, from the reaction in benzyl alcohol.

As the aromatic region of the ^{1}H NMR spectra was similar for analogues **140, 143-145**, alkyl signals between 1-5 ppm and their correlating integrations were used to determine if the analogues had been synthesised. A red-orange compound with a R_f value of 0.08 was isolated from the reaction using methanol, performed under reflux instead of the microwave due to pressure instabilities within the reaction vessel. It would be expected for **143** that a singlet at approximately 4.5 ppm integrating for two protons corresponding to this methyl group would be observed in addition to the expected aromatic and NH signals in the ^{1}H NMR spectrum. Examination of the alkyl region of the ^{1}H NMR spectrum showed possible alkyl linker singlets at 5.30 ppm, 3.82 ppm, and 3.11 ppm, however they only integrate for one proton each instead of the expected two. As can be observed in Figure 3.13, there are many signals in the spectrum that do not correspond to the expected structure or overlap with existing signals. These indicate that the sample was a complex mixture of products and prevents the conclusive assignment of a structure to this compound using ^{1}H NMR. The reaction was repeated in an attempt to improve the purity, however similar results were observed.

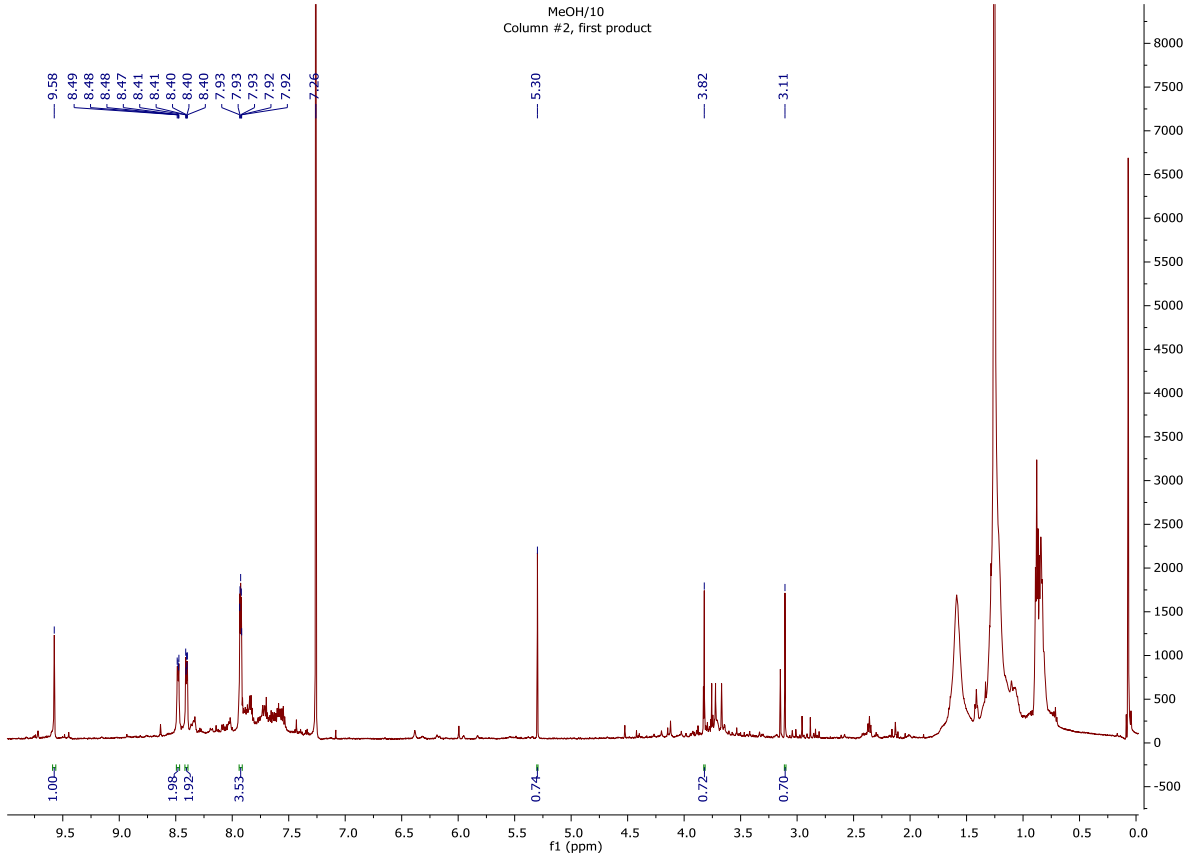


Figure 3.13 ^{1}H NMR spectrum of the isolated product, expected to be **143**, from the reaction between lawsone and glycine using a methanol solvent.

Reactions in the presence of 1-propanol were expected to produce **140** (Table 3.2). The ^{1}H NMR signals for **140** are expected to include a triplet corresponding to the -CH environment at approximately 4-5 ppm, as well as a multiplet and another triplet between 1-2 ppm corresponding to the -CH_2 and -CH_3 environments respectively. The ^{1}H NMR spectrum (Figure 3.14) of the purified red product (R_f = 0.29) (**140a**) showed two doublets at 8.13 and 8.09 ppm, integrating for two protons each (expected integration of two each), and a multiplet at 7.72 ppm integrating for six protons (expected integration of four). These signal positions are consistent with aromatic signals observed for Compound **100**, however the integration for the multiplet is two protons higher than expected. Similarly, the positions of the signals in the alkyl region indicate the potential formation of the desired **140**, however the integrations of these are inconsistent. A triplet can be observed at 3.98 ppm integrating for five protons (expected integration of one), as well as a multiplet at 1.93 ppm integrating for four protons (expected integration of two). Another triplet at 1.07 ppm, possibly correlating to the terminal -CH environment, integrating for seven protons (expected integration of three) is also present. In addition, the NH signal that should appear at approximately 8.3 ppm is unable to be

assigned. There are several doublets and triplets around 8.3 ppm, however these splitting patterns are inconsistent with what would be expected. The ^{1}H NMR spectrum of **140a** exhibits several signals not expected for this compound, likely impurities, and variation in observed integrations are most likely caused by interference by signals from these impurities.

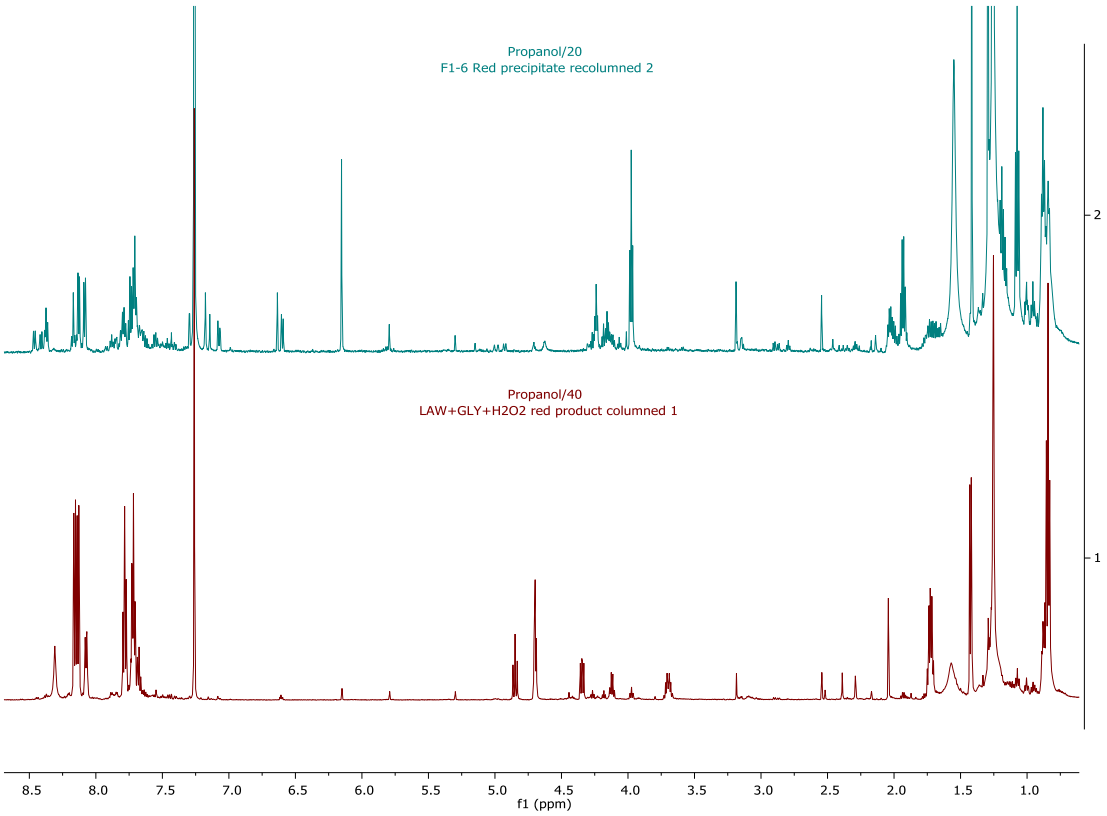


Figure 3.14 ^{1}H NMR spectrum of expected product **140**, from the reaction between lawsone and glycine in 1-propanol (**140a**) (top, green); and the same reaction with H_2O_2 (**140b**) (bottom, red).

Section 3.2.5 proposes that oxidative conditions may help to improve the yield of the dimer **100** and its analogues by driving the production of required intermediates. Therefore, it was considered that the addition of hydrogen peroxide may improve their synthesis. The reaction in 1-propanol with the addition of hydrogen peroxide yielded a product (**140b**) with a much cleaner ^{1}H NMR spectrum (Table 3.3, Figure 3.14). Within the alkyl region, two triplets are present at 4.85 and 4.70 ppm that each integrate for one proton (expected integration of one each). Either of these signals may correlate to the -CH environment at the vinyl positions of the lawsone molecules, but there is insufficient data to determine which one. A similar pattern was observed in the ^{1}H NMR spectrum for Compound **100**, where two quartets were present at approximately 4.6 ppm that would correlate to the -CH moiety.

This may indicate that the alcohol is involved in forming another product with similar structural characteristics as the dimer. There are two quartets at 4.35 and 1.73 ppm, each integrating for one proton (expected integration of two each). It is not unreasonable to consider either of these to be the -CH_2 linker environment, despite the large difference in peak position, as a large difference of 4 ppm between the -CH and -CH_3 environments was also observed in the spectrum for Compound **100**. The triplet at 0.84 ppm likely corresponds to the -CH_3 environment and integrates appropriately (expected integration of three). Despite overlapping and extra signals in the ^{1}H NMR spectrum, it is strongly suspected that analogue **140b** was successfully isolated.

The reaction using 1-butanol as the solvent was expected to form **144** (Table 3.2) and resulted in the isolated of a red product (R_f = 0.4). The butyl chain alkyl linker would be expected to yield a triplet, a quartet, a multiplet, and another triplet in descending order between 4.5 and 1 ppm that integrate for one, two, two, and three protons respectively. Analysis of the ^{1}H NMR spectrum (Table 3.3, Figure 3.15) reveals a triplet at 4.70 ppm integrating for the expected one proton, a multiplet at 1.94 ppm integrating for the expected two protons, and another triplet at 1.04 ppm integrating for the expected three protons. This suggests they could result from the vinylic -CH, terminal -CH_2, and terminal -CH_3 environments. A second set of each of these signals with similar splitting patterns at 4.18, 1.62, and 0.84 ppm are also observed, however integrate for one more proton than expected, and could originate from a similarly structured compound that was unable to be separated from the major product. The expected quartet pertaining to the -CH_2 adjacent to the -CH could not be assigned from the spectrum. The positions and splitting patterns of the observed signals suggest that the ^{1}H NMR spectrum could result from **144**, however the additional peaks and absence of the expected quartet mean a structure cannot be conclusively assigned.

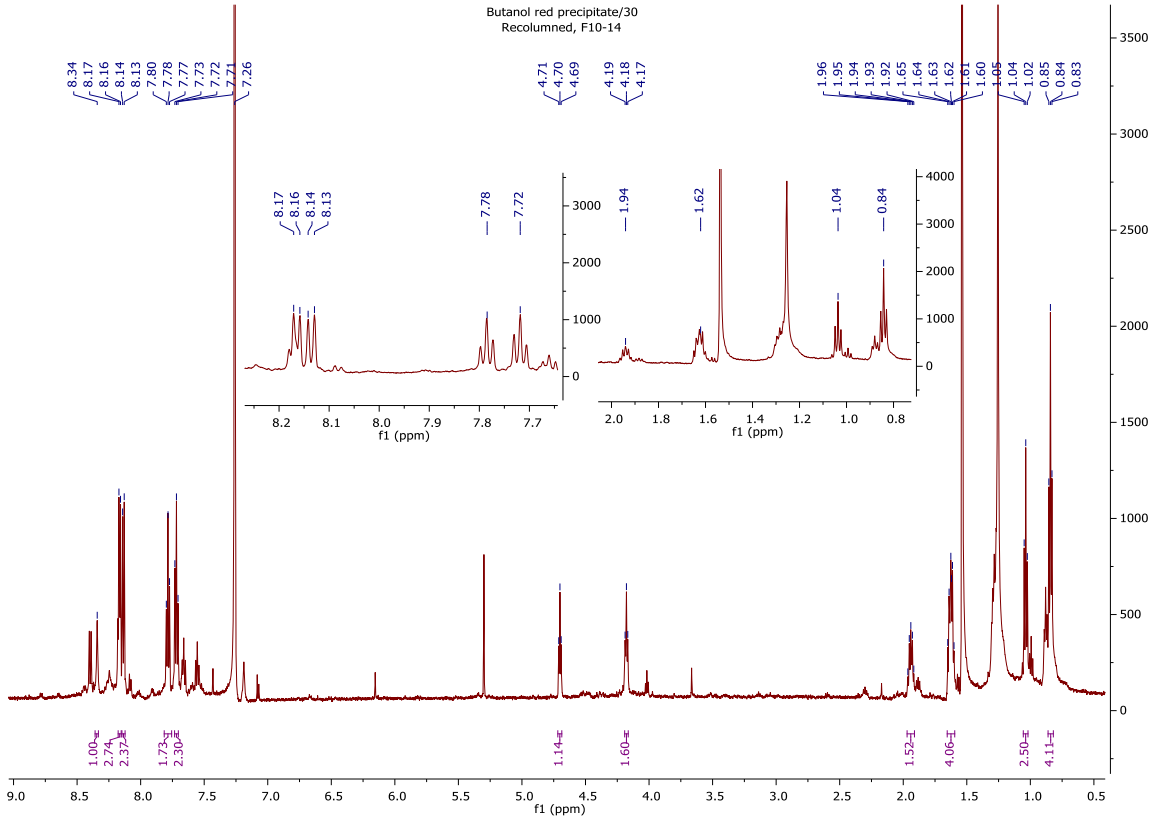


Figure 3.15 ^{1}H NMR spectrum of expected product **144**, from the reaction between lawsone and glycine in 1-butanol.

The ^{1}H NMR spectrum resulting from compound **145** (Table 3.2, Figure 3.16), from the reaction in benzyl alcohol, would be expected to contain mostly aromatic signals from the lawsone fragments and the benzene ring from the alkyl linker, as well as a singlet pertaining to a -CH_2. There are several aromatic signals between 7.47 and 8.14 ppm, however due to their overlapping and signals from impurities, it is difficult to distinguish which aromatic signal could correlate to which group in the expected structure. These signals integrate for a total of fourteen aromatic protons, which is one more than the expected total of thirteen protons for **145**. The -NH singlet at 8.46 ppm, integrating correctly for one proton, is visible. Two singlets at 3.94 and 5.73 ppm that integrate for one proton each are observed. While both of these singlets have the correct splitting pattern to be the -CH group of the alkyl linker, neither integrate for the expected two protons and therefore it cannot be determined which, if any, of these singlets could be from the expected product **145**.

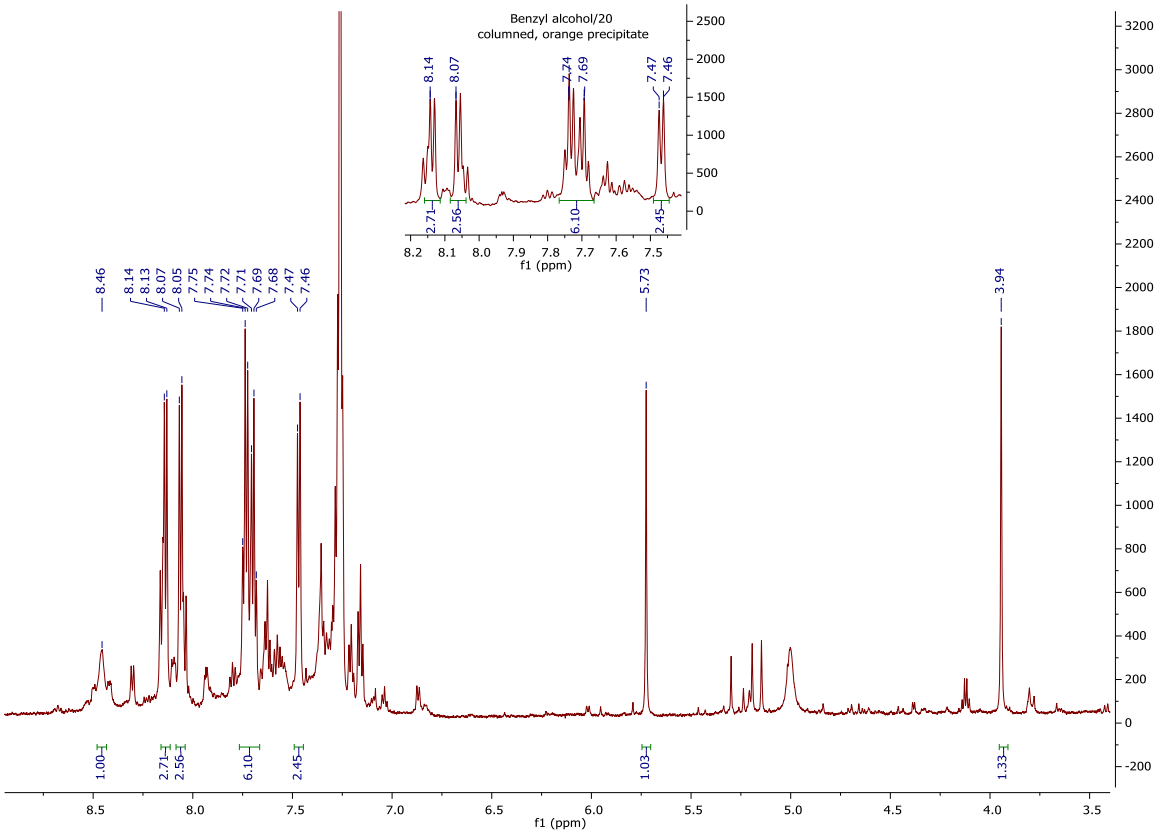


Figure 3.16 ^{1}H NMR spectrum of expected product **145**, from the reaction between lawsone and glycine in benzyl alcohol

Although the deuterated experiments confirm that the alcohol solvent is incorporated into the dimer to form the alkyl linker, it appears as though ethanol is the optimal alcohol and favours dimer formation more than other alcohols trialled in this work. 1-propanol was able to produce the corresponding analogue **140**, however required the use of hydrogen peroxide. Alkylation using alcohols is usually performed under basic or acidic conditions and requires specific alkylating agents such as alkyl halides and aldehydes (as discussed in detail in Chapter 2). The requirement of these conditions in relation to the formation of Compound **100** were investigated by including ethyl iodide and aldehydes in the reaction in place of ethanol.

3.2.3. Nucleophilic substitution with ethyl iodide

The simplest proposal for the incorporation of the alcohol solvent into the dimer of Compound **100** is that the alkylation occurs at the vinyl carbon via a nucleophilic substitution reaction. Alkylation using alcohols usually requires some type of activating reagent, such as a lewis acid or organocatalyst as outlined in Chapter 2, since -OH is typically a poor leaving group. However, neither of these conditions are present in this reaction mixture. Despite this, the proposal that the alcohol may directly react in this manner was still tested, albeit with the use of a strong alkylating agent in ethyl iodide.

In order to test this theory, a reaction was performed where the ethanol was replaced with ethyl iodide. It was expected that Compound **100** would form if the pathway were to proceed following this reaction mechanism. TLC of the crude mixture did not show a red compound with a comparable Rf value to Compound **100**. Despite observing streaking of compounds using TLC, the reaction mixture was separated by column chromatography where two red fractions were isolated. The ^{1}H NMR for both fractions did not contain the major expected aliphatic doublet at 1.3 ppm or the quartet at 4.6 ppm (Appendix 2). This indicated that neither fraction contained Compound **100**. Compounds considered to have formed instead of **100** included dimers incorporating the addition of the ethyl iodide at one vinyl carbon position or N-position (**146** and **147**), as well as structures where the lawsone molecule or 2-amino equivalent were alkylated at the C-2 (**103**), O- (**148**), or N- (**149**) position respectively (Figure 3.17). In all cases, a quartet relating to the -CH_2 of the ethyl chain would be observed around 3-5 ppm, which was not present in the obtained ^{1}H NMR. As iodide is a better leaving group than OH, the observation that the reaction does not proceed using ethyl iodide under these conditions would suggest that it is highly unlikely an alcohol would also favourably undergo nucleophilic substitution in a similar manner to form Compound **100**. Consequently, other mechanisms must be considered when determining reaction pathways involving the addition of the alkyl linker.

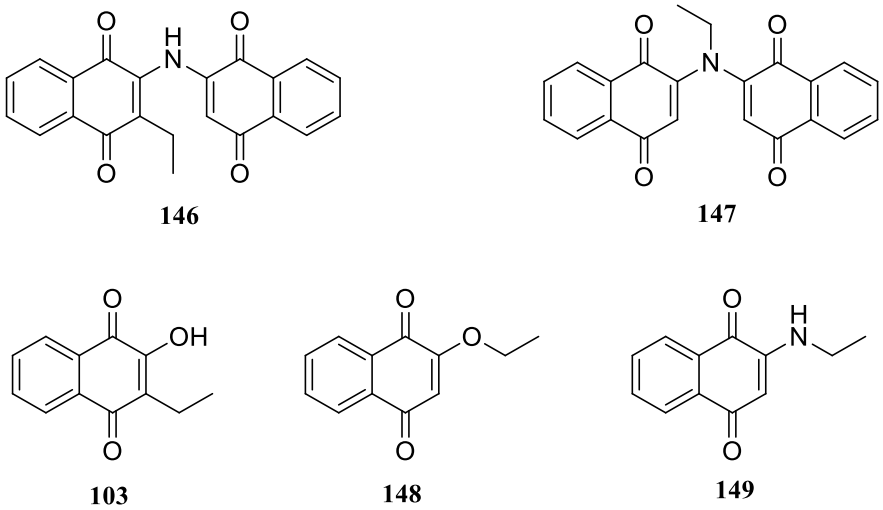


Figure 3.17 Other structures considered for the identity of the isolated red compound from the reaction between lawsone and glycine in ethyl iodide.

3.2.4. Investigations into the role of aldehydes

Chapter 2 outlined several pathways that could be followed to produce Compound **100**. In all of the proposed pathways, there is an increase in oxidation state of the vinylic carbon from -1 to 0. This suggests that, if any of these pathways were followed, one of the intermediates would be required to undergo oxidation prior to the addition of the alkyl linker. Upon reviewing the reagents in the reaction, no obvious oxidant was identified. Thus, it was suggested that one possible reagent in the reaction capable of being an oxidising agent was the aldehyde form of the alcohol. Typically, for an alcohol to be converted to the aldehyde, a very strong oxidising agent must be present. It might be unlikely that this initial oxidation from alcohol to aldehyde is needed to oxidise the intermediate, as the oxidising agent responsible for this conversion could also oxidise the intermediate itself. The proposed involvement of an aldehyde was tested despite this, as there were no other obvious alkylation-facilitating reagents in the initial reaction mixture, and it is possible that the alcohols used contained small amounts of aldehyde impurities already.

It has been previously reported that aldehydes can undergo Michael addition at the C-3 position of lawsone to create a diene, which can then undergo Michael addition at the C-3 position on another lawsone molecule.[16] The previous work reported the formation of the dimeric **89** (Figure 3.18), which is similar in structure to the carbon-link contained within Compound **100**. Therefore, a series of experiments were undertaken similar to reactions outlined in Chapter 2, where the alcohol solvent was replaced with the analogous aldehydes. Acetaldehyde, propionaldehyde, and benzaldehyde were

attempted, however the volatile nature of acetaldehyde was incompatible with microwave parameters and therefore not reported.

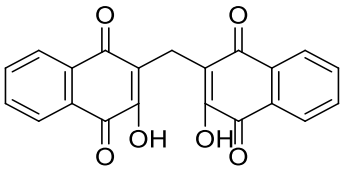


Figure 3.18 Structure of **89**, synthesised from the Michael addition of formaldehyde to lawsone.[16]

A red compound similar in appearance to Compound **100** was not produced from any of the reactions involving aldehydes. Compounds that appeared reddish-brown using TLC were targeted for isolation instead, while other compounds that showed any affinity to the mobile phase were difficult to separate due to smearing. Reactions with propionaldehyde were compared with the resulting products from the 1-propanol reaction, as they should both form compound **140**. The ^{1}H NMR spectrum of the crude reaction material did not exhibit the expected NH shift around 8.5 ppm, nor did it exhibit a triplet at around 4.5 ppm correlating to the carbon chain. Similarly, the reaction in benzaldehyde also did not seem to proceed, as the ^{1}H NMR spectrum of the crude material exhibits very little signal with several overlapping aromatic peaks. The observation that the reaction does not proceed using aldehydes suggests that the proposed requirement of an oxidising agent is not the oxidised form of the alcohol, and the oxidised alcohol is not the form participating in the alkyl linker insertion.

Notably, Jelly *et al*.[1–3] reported observation of the same fluorescent compound using both ethanol and ethyl acetate on filter paper using higher temperatures of 140-170^{o}C using an oven or linen press. However, repeating the microwave synthesis in ethyl acetate yielded no Compound **100** despite being heated in intervals for twice as long at the same temperature. Ethyl acetate is not readily reduced to ethanol under these conditions and therefore it is reasonable that this reaction would not proceed well and would require harsher conditions to occur.

3.2.5. Investigations into the requirement of oxidative conditions

It was proposed that, whilst the aldehydes did not assist in the production of the dimer, oxidative conditions would be required to obtain the favourable change in oxidation state to facilitate alkyl linker addition at the vinyl position of lawsone. Consequently, the reaction was repeated in a reflux setting where hydrogen peroxide or oxygen was added to force potential oxidation, as well as under nitrogen to minimise the influence of atmospheric oxidants.

A series of reactions were performed where lawsone and glycine in ethanol were heated under reflux at 80°C for five days. These were performed under atmospheric conditions (1), with the addition of hydrogen peroxide (2), with the addition of oxygen (3), and under a nitrogen atmosphere (4) (Table 3.4). These reactions were conducted at the same time with the same reagents and in the same quantities. Conditions (5) and (6) outlined in Table 3.4 were analogous to conditions (1) and (2), with microwave heating instead of under reflux.

Table 3.4 Conditions for the reaction between lawsone and glycine in ethanol to produce **100**, to investigate the influence of oxidising agents.

Conditions	Additional Reagents	Set up	Time	Yield of Compound 100
(1)	-	Reflux	5 days	4%
(2)	Hydrogen Peroxide	Reflux	5 days	3%
(3)	Oxygen	Reflux	5 days; Initial heating time of 24 hours, followed by oxygen bubbled through reaction for 8 hours Subsequent heating for 3.5 days	2%
(4)	Nitrogen	Reflux	5 days	4%
(5)	-	Microwave	3 hours	0.67%
(6)	Hydrogen Peroxide	Microwave	3 hours	3%

Under normal atmospheric conditions (1) Compound **100** was isolated in a 4% yield, and its identity confirmed through ^{1}H NMR analysis. The addition of hydrogen peroxide (2) resulted in a 3% yield of Compound **100**, also confirmed through ^{1}H NMR analysis. While there was no improvement to the

yield, the presence of hydrogen peroxide appeared to reduce the impurities observed in the ^{1}H NMR spectrum of Compound **100** (Figure 3.19). This observation was consistent when repeating the reaction using conditions (5) and (6).

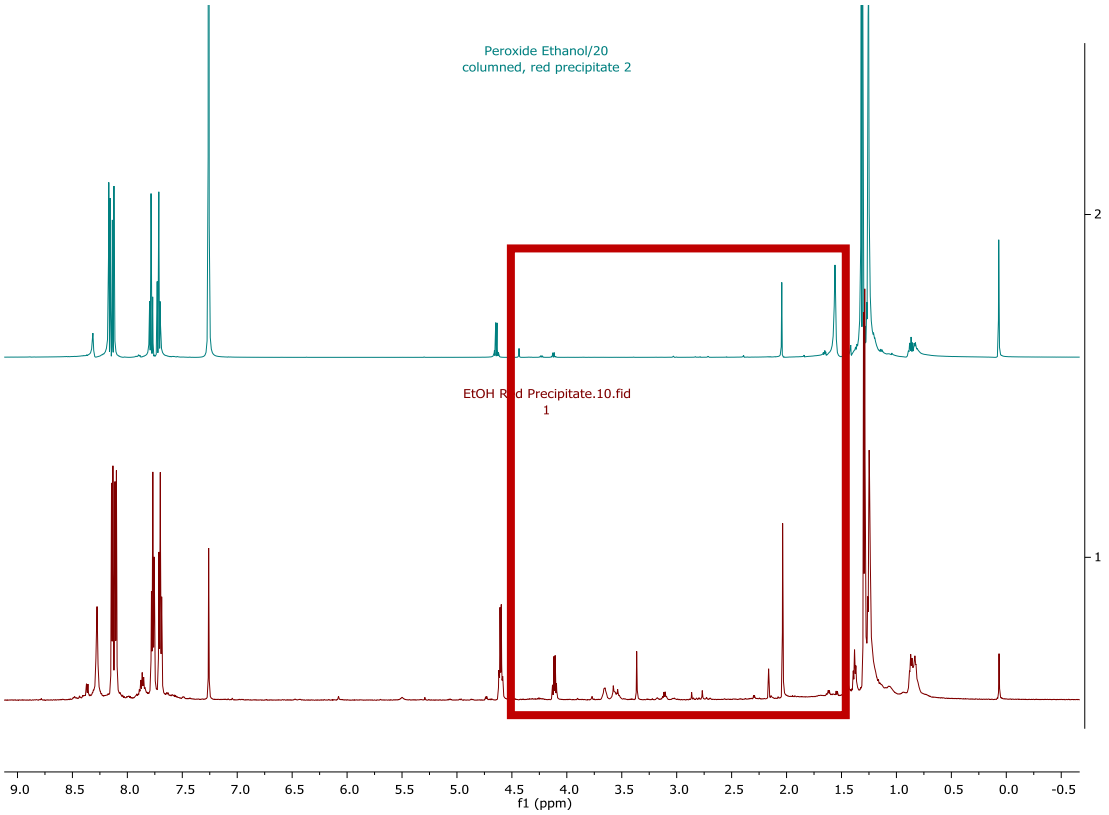


Figure 3.19 ^{1}H NMR spectrum of the red dimer **100** from the reaction using conditions (6) (top, green) and using conditions (5) (bottom, red). The area where the biggest reductions of purity were observed is boxed.

The addition of oxygen for 8 hours after an initial heating period of 24 hours, condition (3), resulted in the isolation of Compound **100**, confirmed through ^{1}H NMR analysis, in a 2% yield. This product exhibited slightly more impurities in the ^{1}H NMR spectrum compared to the compound isolated from condition (1) (Figure 3.20). Performing the reaction under nitrogen (4) also resulted in the isolation of Compound **100**, in a 4% yield that is comparable to the yield from atmospheric conditions (1). The ^{1}H NMR spectrum of Compound **100** from (4) is also of similar purity to that isolated from (1) (Figure 3.21).

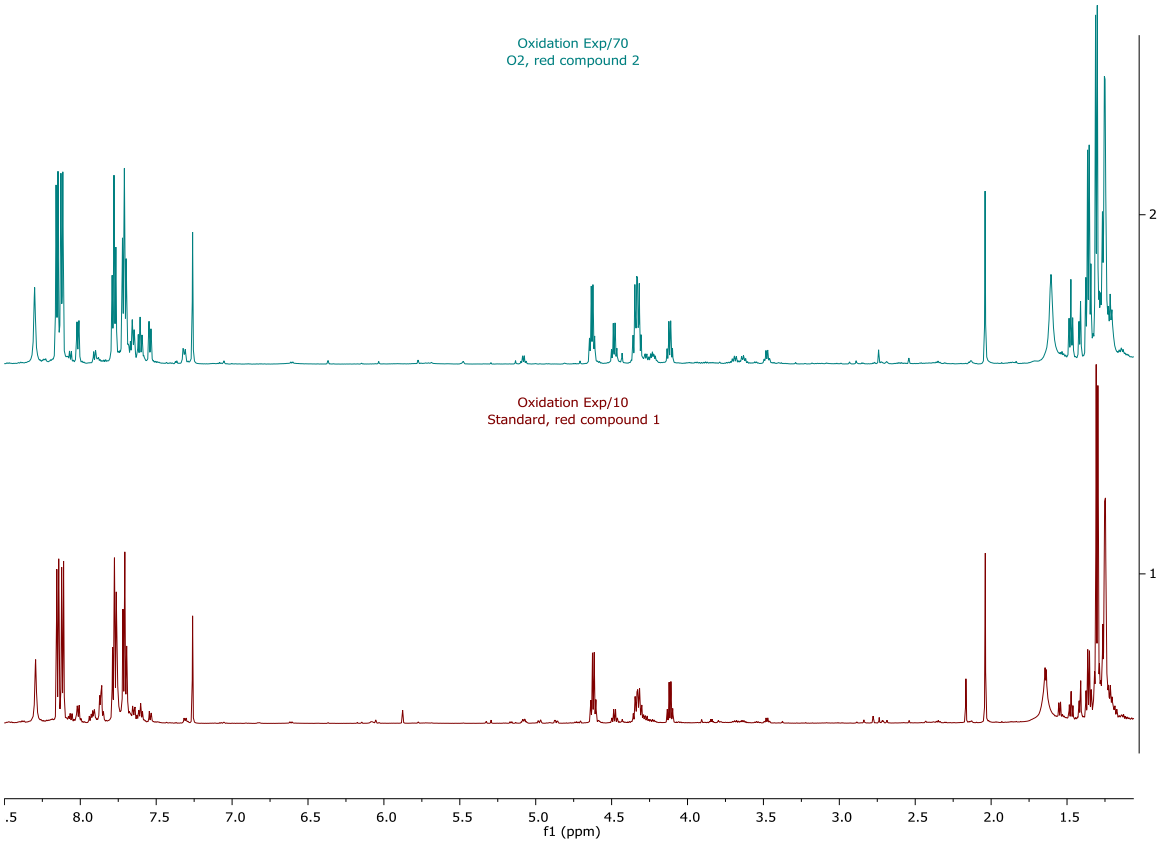


Figure 3.20 ^{1}H NMR spectrum of the red dimer **100** from the reaction using conditions (3) (top, green) and using conditions (1) (bottom, red).

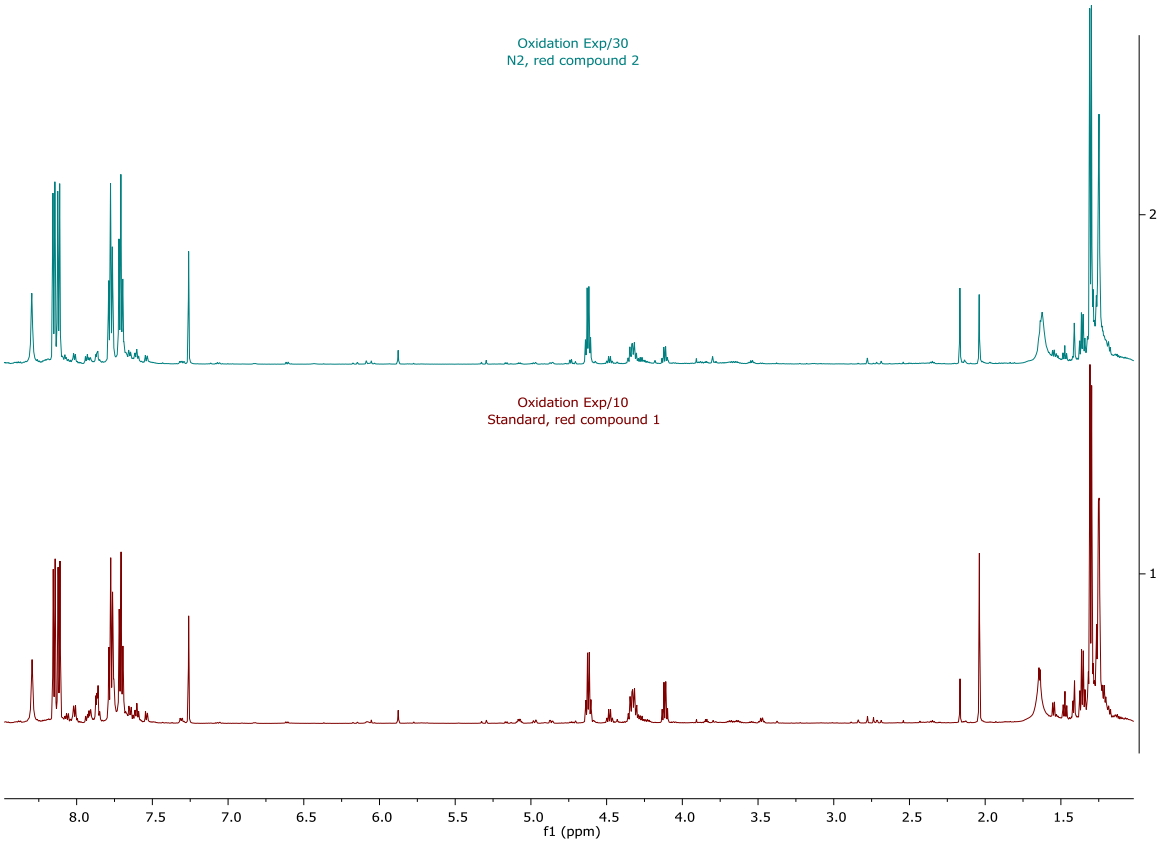


Figure 3.21 ^{1}H NMR spectrum of the red dimer **100** from the reaction using conditions (4) (top, green) and using conditions (1) (bottom, red).

Given that the nitrogen conditions (3) gave comparable results to that under atmospheric conditions (1), and condition (3) seemed to perform worse in terms of yield, it is proposed that atmospheric or dissolved oxygen may not be a principal source of oxidation, if even required. It is apparent that the reproducibility of the successful formation of Compound **100** from the reaction between glycine and lawsone in ethanol is inconsistent, as previous in-situ set-ups and microwave conditions have returned the compound in yields of between 1-4%. The experiments with hydrogen peroxide and oxygen suggest that the reaction pathway is not enhanced by the addition oxidants. However, the addition of hydrogen peroxide to the reaction is beneficial as it reduces the number of compounds that are co-eluted with Compound **100** under the separation system used. This is potentially a result of a reduction in the number of side reactions occurring. It is possible that this difference in yield results from different reaction parameters surrounding the temperature and pressure. These oxidation studies were required to be carried out under reflux at atmospheric pressure, where a temperature of only 80oC could be obtained. Experiments performed in a microwave are exposed to much higher temperatures of 130^{o}C and pressures of 17 bar. The variability in yield and purity is also susceptible to

changes in separation procedures. Small amounts of product lost due to co-elution or transfer can have an amplified effect on the calculated yield as very little product is available to begin with.

It is still highly possible that an oxidising agent is required for the addition of the alkyl linker. A candidate that was not explored in this work was the naphthoquinone, lawsone itself. Quinones are largely documented to undergo redox cycling (Figure 3.22) in numerous biological systems,[17–19] and could act as an oxidising agent in this reaction. Lawsone has been shown to undergo reduction in the presence of reductases in biological systems, consequently forming a semiquinone radical.[18,20] This semiquinone radical then reacts with oxygen to generate a superoxide anion and hydrogen peroxide.[18,20] A similar redox cycling may be occurring in this reaction, however further investigation is required to determine the exact mechanism.

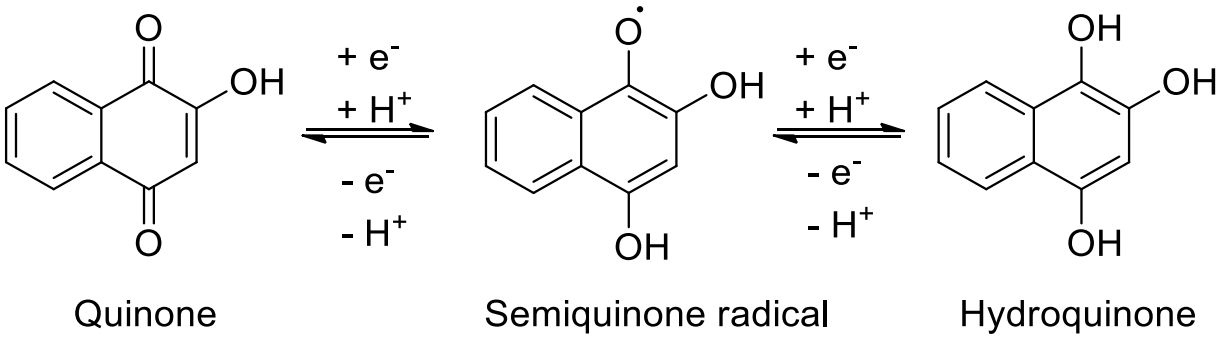


Figure 3.22 Redox cycling of lawsone, showing the quinone, semiquinone radical, and hydroquinone forms.

3.3. Conclusions

It was determined through studies using deuterated ethanol that the formation of the alkyl linker in the structure of Compound **100** most likely originated from the alcohol used as the solvent, and not from the amino acids present. Reactions between lawsone and phenethylamine or N-butyl amine in the presence of ethanol did not produce the expected dimers. This suggests that the presence of the decarboxylation step of the postulated Strecker degradation is important for the synthesis of Compound **100** and analogues. There appears to be some hindrance in the formation of analogues **140, 143-145** as the alcohol chain increases, where ethanol was found to be the most favourable alcohol for dimer formation. Proposed methods of alkylation using ethyl iodide and aldehydes did not yield the expected dimers and were therefore dismissed as possibilities. The requirement of an oxidising agent to facilitate the reaction pathway was also investigated. Hydrogen peroxide assisted

in isolating a purer sample, however oxidative conditions had very little impact on the yield of Compound **100**.

3.4. Experimental

General experimental details regarding analytical protocol and equipment, as well as preparation of common reagents, can be found in Section 2.4.1. NMR spectra for the compounds listed in this Chapter not shown in Section 3.2 can be seen in Appendix 2.

3.4.1. Procedures for the investigations into the role of amino acids

Ethanol (4 mL) was added to a mixture of lawsone (0.5026 g, 2.9 mmol) and N-butyl amine (0.4218 g, 3.4 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitates were dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 90:10 hexane:ethyl acetate solvent system respectively. **134** was isolated as an orange solid in 0.0112 g (1.7 %) yield: R_f (8:2 HX:EtOAc) 0.37; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.09 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 7.7 Hz, 1H), 7.70 (t, J = 7.5 Hz, 1H), 7.59 (t, J = 7.5 Hz, 1H), 5.87 (s, 1H), 5.72 (s, 1H), 3.15 (q, J = 6.9 Hz, 2H), 1.66 (p, J = 7.4 Hz, 2H), 1.42 (dq, J = 14.8, 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H).

Ethanol (2 mL) was added to a mixture of lawsone (0.3336 g, 1.9 mmol) and phenylethylamine (0.1372 g, 0.87 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitates were dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. **135** was isolated as an orange solid in 0.0031 g (1.3%): R_f (8:2 HX:EtOAc) 0.15; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.09 (dd, J = 7.7, 1.3 Hz, 1H), 8.01 (dd, J = 7.7, 1.3 Hz, 1H), 7.71 (td, J = 7.5, 1.3 Hz, 1H), 7.60 (td, J = 7.6, 1.3 Hz, 1H), 7.34 (t, J = 7.5 Hz, 2H), 7.26 (t, 1H), 7.23 (d, 6.8 Hz, 2H), 5.94 (d, J = 6.0 Hz, 1H), 5.78 (s, 1H), 3.45 (q, J = 6.8 Hz, 2H), 2.98 (t, J = 7.1 Hz, 2H); ^{13}C NMR (151 MHz, Chloroform-d) δ 182.93, 181.73, 147.66, 137.74, 134.70, 133.55, 131.93, 130.44, 128.83, 128.56, 126.90, 126.21, 126.15, 100.97, 43.58, 34.29.

Ethanol (8 mL) was added to a mixture of lawsone (0.8090 g, 5.7 mmol) and isoleucine (0.3420 g, 2.9 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitates were dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system, followed

by further purification using a 90:10 hexane:ethyl acetate system. Compound **100** was isolated as a red solid in 0.0083 g (0.9 %) yield.

Ethanol (8 mL) was added to a mixture of lawsone (0.5502 g, 3.2 mmol) and GABA (0.1635 g, 1.6 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitates were dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. Compound **100** was isolated as a red solid in a 0.0060 g (1%) yield.

3.4.2. Procedures for the investigations into the alcohol solvent

EtOH-*d*6 (2 mL) was added to a mixture of lawsone (0.3339 g, 1.92 mmol) and glycine (0.0649 g, 0.86 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system to yield 0.0047 g (1.5%) of red solid **100b**: R_f (8:2) 0.45; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.31 (s, 1H), 8.16 (d, J = 7.7 Hz, 2H), 8.13 (d, J = 7.6 Hz, 2H), 7.78 (t, J = 7.6 Hz, 2H), 7.72 (t, J = 7.4 Hz, 2H); ^{13}C NMR (151 MHz, Chloroform-d) δ 182.45, 179.31, 137.73, 135.03, 133.22, 132.96, 130.41, 126.75, 126.49, 120.72; MS (APCI) [M+H]: 360.1167.

Methanol (15 mL) was added to a mixture of lawsone (0.9443 g, 5.42 mmol) and glycine (0.316 g, 4.21 mmol) and heated to 80°C under reflux for a week. The resulting purple precipitate was dried under vacuum or N2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system to yield <10 mg of reddish-orange solid: R_f (8:2 HX:EtOAc) 0.05; ^{1}H NMR (600 MHz, Chloroform-d) δ 9.58 (s, 1H), 8.50 – 8.47 (m, 2H), 8.42 – 8.39 (m, 2H), 7.93 (dd, J = 6.1, 2.6 Hz, 4H), 5.30 (s, 1H), 3.82 (s, 1H), 3.11 (s, 1H).

1-propanol (2 mL) was added to a mixture of hydrogen peroxide (30%, 0.05 mL), lawsone (0.4259 g, 2.4 mmol) and glycine (0.0783 g, 1 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system to yield <10 mg of red solid **140**: R_f (8:2 HX:EtOAc) 0.38; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.31 (s, 1H), 8.15 (dd, J = 15.1, 7.6 Hz, 4H), 7.78 (t, J = 7.5 Hz, 2H), 7.72 (t, J = 6.9 Hz, 2H), 4.85 (t, J = 9.7 Hz, 1H), 4.70 (t, J = 5.4 Hz, 1H), 4.35 (dd, J = 9.3, 6.4 Hz, 1H), 1.73 (dd, J = 7.6, 5.4 Hz, 1H), 0.84 (t, J = 7.6 Hz, 3H).

Butanol (8 mL) was added to a mixture of lawsone (1.021 g, 5.8 mmol) and glycine (0.3081 g, 4.1 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under

vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. Further purification by silica gel chromatography was performed using DCM as eluent to yield <10 mg of red solid: R_f (8:2 HX:EtOAc) 0.40; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.34 (s, 1H), 8.17 (d, J = 7.4 Hz, 2H), 8.14 (d, J = 7.6 Hz, 2H), 7.80 (t, J = 7.5 Hz, 2H), 7.72 (t, J = 7.5 Hz, 2H), 4.70 (t, J = 5.7 Hz, 1H), 1.94 (m, J = 6.7 Hz, 2H), 1.04 (t, J = 7.5 Hz, 3H).

Benzyl alcohol (2 mL) was added to a mixture of lawsone (0.3463 g, 1.99 mmol) and glycine (0.0727 g, 0.97 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system to yield <10 mg of red solid: R_f (8:2 HX:EtOAc) 0.37; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.46 (s, 1H), 8.14 (dd, J = 12.3, 7.6 Hz, 3H), 8.07 (dd, J = 12.9, 7.6 Hz, 3H), 7.74 (t, J = 7.6 Hz, 4H), 7.69 (t, J = 7.5 Hz, 2H), 7.47 (d, J = 7.7 Hz, 2H), 5.73 (s, 1H), 3.94 (s, 1H).

Ethyl acetate (8 mL) was added to a mixture of lawsone (0.3205 g, 1.84 mmol) and glycine (0.0723 g, 0.96 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum or N_2 stream, and the crude mixture analysed using ^{1}H NMR. No discernible peaks were observed.

3.4.3. Procedures for the inclusion of ethyl iodide

Ethyl iodide (2 mL, 25 mmol) was added to a mixture of lawsone (0.3595 g, 2.1 mmol) and glycine (0.0731 g, 0.97 mmol) and heated to 130°C in a microwave for three hours. The crude material was dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system to yield two red fractions in < 10 mg yield each.

Fraction 1: ^{1}H NMR (600 MHz, Chloroform-d) δ 8.15 – 8.11 (m, 2H), 8.08 (d, J = 7.5 Hz, 1H), 7.79 – 7.69 (m, 4H), 6.15 (s, 1H), 4.10 (q, J = 7.0 Hz, 2H), 1.53 (t, J = 7.1 Hz, 4H).

Fraction 2: ^{1}H NMR (600 MHz, Chloroform-d) δ 8.99 (d, J = 8.3 Hz, 0H), 8.93 (d, J = 7.9 Hz, 0H), 8.37 (d, J = 7.7 Hz, 0H), 8.32 (d, J = 8.2 Hz, 0H), 8.10 (dd, J = 7.7, 1.3 Hz, 0H), 8.05 (dd, J = 7.7, 1.3 Hz, 0H), 7.81 (ddd, J = 7.1, 5.6, 1.8 Hz, 1H), 5.82 (s, 0H), 5.73 (s, 0H), 3.26 – 3.21 (m, 1H), 0.88 (t, J = 6.9 Hz, 1H).

3.4.4. Procedures for the investigation into the role of aldehydes

Propionaldehyde (7 mL) was added to a mixture of lawsone (0.7718 g, 6.3 mmol) and glycine (0.2287 g, 3.3 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 DCM:hexane solvent system to yield <10 mg of red-orange solid: R_f (8:2 HX:DCM) 0.26; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.12 (d, J = 7.5 Hz, 1H), 8.08 – 8.06 (m, 1H), 7.76 – 7.73 (m, 1H), 7.67 (t, J = 7.6 Hz, 1H), 5.44 (s, 1H), 3.52 (d, J = 7.1 Hz, 2H), 2.62 – 2.56 (m, 2H), 1.40 (t, J = 7.1 Hz, 5H), 1.00 – 0.96 (m, 4H).

Benzaldehyde (2 mL) was added to a mixture of lawsone (0.4259 g, 2.4 mmol) and glycine (0.0783 g, 1 mmol) and heated to 170°C under reflux with stirring for three days in a microwave. The resulting purple precipitate was dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system to yield <10 mg of red solid; R^f (8:2 HX:DCM) 0.16; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.11 (d, J = 7.7 Hz, 1H), 8.01 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.73 – 7.68 (m, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.39 (d, J = 7.6 Hz, 2H), 7.23 (t, J = 7.6 Hz, 1H), 5.50 (s, 1H), 3.95 (s, 2H).

3.4.5. Procedures for the investigation into oxidative conditions

General procedure for conditions (1) – (4): Ethanol (10 mL) was added to a mixture of lawsone (2.9 mmol) and glycine (1.4 mmol) and heated with stirring under reflux to 80°C over a period of five days. The resulting purple precipitates were dried under vacuum or N_2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system.

Condition (1): Performed exactly as described in general procedure above. Compound **100** was isolated in a 0.0213 g (4%) yield.

Condition (2): Procedure performed with the addition of hydrogen peroxide (30%, 0.2 mL). Compound **100** was isolated in a 0.0171 g (3%) yield.

Condition (3): The reaction was initially heated at 80°C under reflux with no additional oxygen for 24 hours. Oxygen was then gently bubbled through with supervision for eight continuous hours, after which the oxygen source was removed, and the reaction continued to heat under reflux for another three days. Compound **100** was isolated in a 0.0094 g (2%) yield.

Condition (4): The ethanol was dried over molecular sieves prior to addition into the reaction mixture. All glassware was flame dried prior to use and the reaction performed under a stream of N_2 in a sealed set-up. Compound **100** was isolated in a 0.0186 g (4%) yield.

General procedure for conditions (5) and (6): Ethanol (2 mL) was added to a mixture of lawsone (2 eq.) and glycine (1 eq.) and heated to 130°C in a microwave for three hours. The resulting purple precipitates were dried under vacuum or N2 stream, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system.

Condition (5): Performed exactly as described in general procedure above, with addition of ethanol to lawsone (0.3327 g, 1.9 mmol) and glycine (0.0814 g, 1.1 mmol) to yield Compound **100** in 0.0026 g (0.67%) yield.

Condition (6): Procedure performed with the addition of ethanol to hydrogen peroxide (30%, 0.2 mL), lawsone (0.3471 g, 2.0 mmol) and glycine (0.0800 g, 1.1 mmol) to yield Compound **100** in 0.0111 g (2.9%) yield.

3.4. References

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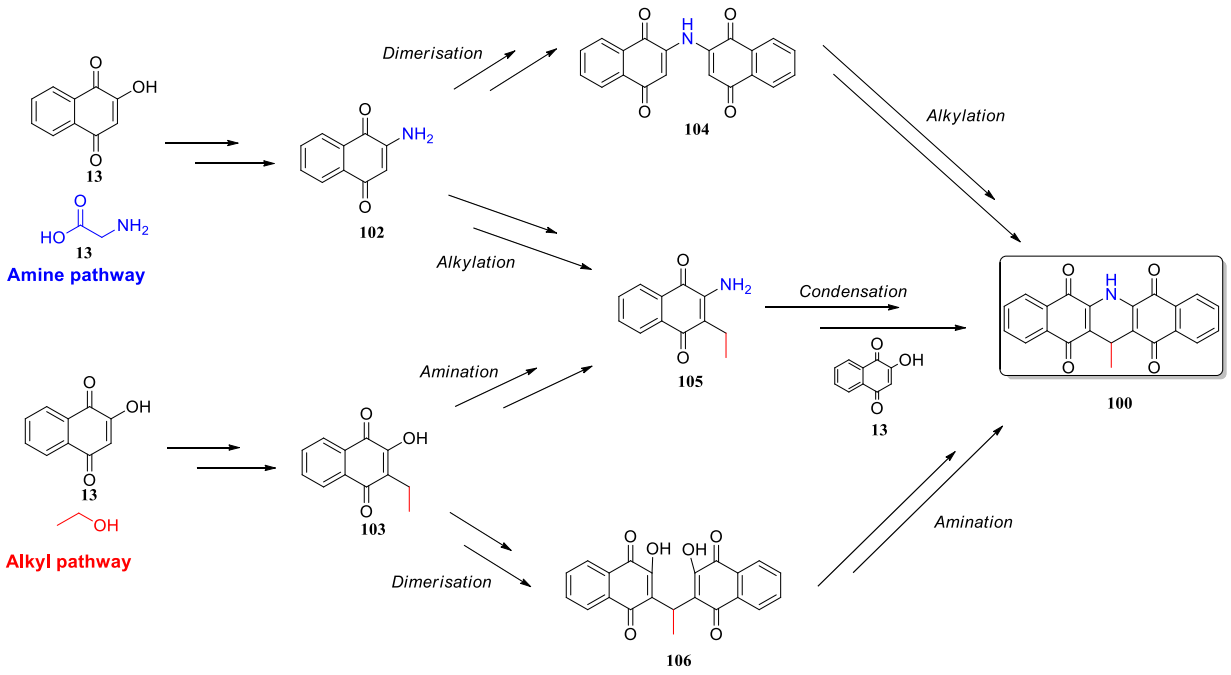
4. Amino Acid Reaction with Lawsone Analogues

4.0. Overview

Chapter 2 discussed the isolation and identification of the dimeric Compound **100** from a complex reaction mixture resulting from the reaction between lawsone and glycine. The structure consisted of two lawsone molecules connected by an amine and alkyl linker. Chapter 3 determined that the amine linker originated from the amino acid used and the alkyl linker originated from the alcohol solvent, but provided no insight into the reaction sites of these reagents. This chapter introduces the use of a lawsone analogue to be able to track the spatial orientation of the lawsone molecule in relation to the insertion of the amine and alkyl linkers, and provide information about the possible reactions taking place at each position.

4.1. Introduction

Chapter 2 proposed four alternative reaction pathways in which lawsone and glycine could react to form compound **100**. These were categorised into two broader pathways depending on whether the amine or alkyl linker was inserted first, namely the amine pathway and the alkyl pathway (Scheme 4.1). Each of these pathways involved the dimerization of two lawsone molecules through the stepwise introduction of i) an amine and ii) an alkyl group.



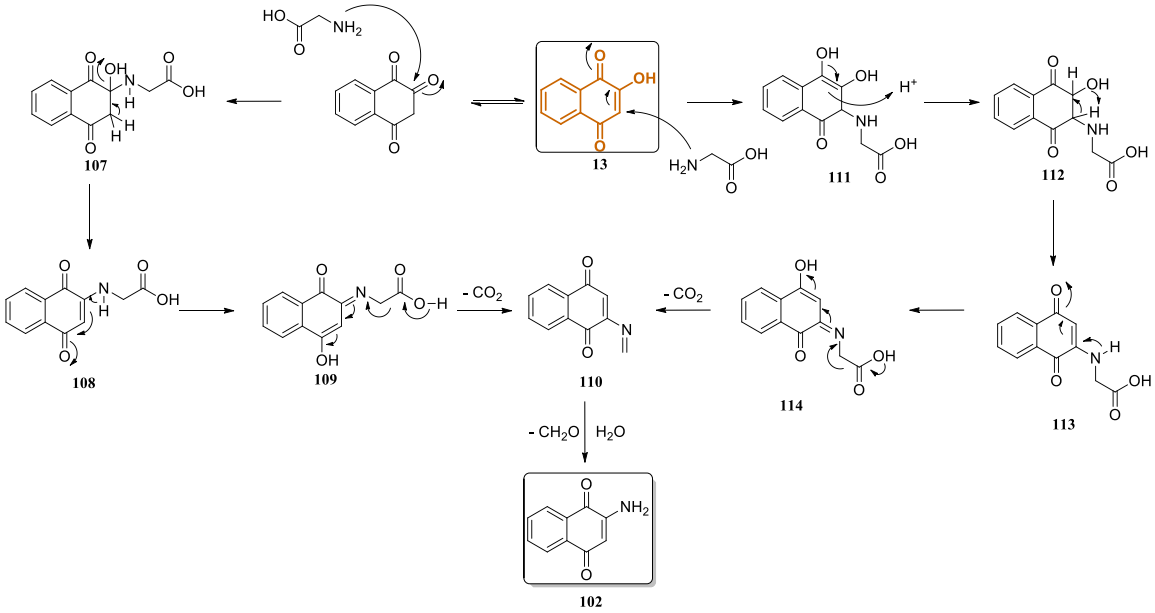
Scheme 4.1 The formation of compound **100** from the reaction between lawsone and glycine in ethanol, showing a) initial insertion of the amine linker using glycine (amine pathway), and b) initial insertion of the alkyl linker using ethanol (alkyl pathway).

The amine pathway involves the addition of the amino acid to the lawsone molecule prior to dimerisation. This was proposed to proceed via the formation of intermediate **102** which could then react either through i) alkylation with the alcohol (**105**) or ii) dimerization with itself or a lawsone molecule (**104**) to further react to produce Compound **100**. The alkyl pathway involves the addition of the alkyl linker to the lawsone molecule prior to dimerisation, to form intermediate **103** which subsequently reacts through i) amination with the amino acid (**105**) or ii) dimerization with itself or a lawsone molecule (**106**) to further react to produce Compound **100**. Chapter 3 showed that the alcohol solvent was the source of the alkyl linker. These results raise several questions:

1) Does the amine or alkyl linker form first?
2) How does the alkylation reaction proceed?
3) How does the amination reaction proceed?
4) At what position on the lawsone molecule (C-2 or C-3) do the amine and alkyl linkers insert?

Regarding the third question posed, the amine pathway involves an initial addition of the amino acid to the lawsone and can proceed via an addition or substitution mechanism (Scheme 4.2). The addition

mechanism may occur similar to that of a Michael addition, via initial attachment of the amino acid at the C-3 position of lawsone (**111**), followed by proton abstraction and elimination of the OH at the C-2 position (**112**). The substitution mechanism may occur via Strecker degradation of the amino acid at the C-2 position (**107**). It is unknown by which mechanism attachment of the amino acid occurs, as the resulting intermediate from both mechanisms is **102**.



Scheme 4.2 Proposed mechanisms of direct substitution at the C-2 position via Strecker degradation (left) and conjugate addition (right) between amino acids and lawsone, arriving at the common intermediate, **102**.

Michael additions have been reported for 1,4-naphthoquinones with no substituent at the C-2 or C-3 position,[1,2] however substitution has been observed when a good leaving group such as chloride is present.[2,3] Strecker degradation of amino acids, as present in this work, is also possible in the presence of ortho and para quinones.[4] It is therefore important to distinguish which reaction pathway is occurring in this work. Traditional methods to differentiate reaction pathways and mechanisms use isotopic labelling to track the movement of atoms within a reaction. This has been successfully employed to determine information such as the inclusion of specific reagents within a final structure or to support intramolecular rearrangements.[5,6] Isotopic labelling was also utilised in Chapter 3 to support the theory that the alkyl linker originated from the alcohol solvent. However, this chapter will use a chemical label such as a methoxy substituent due to availability and ease of analysis.

In this chapter, a lawsone analogue with a functional group at a position of the naphthoquinone ring, where reaction is not expected, was synthesised to act as an orientational tag. In this instance, a tagged molecule contains a traceable substituent that acts as an orientational marker where its position can be determined through analytical methods such as NMR. Upon reaction of the tagged lawsone with amino acids, three possible end products were proposed based on whether the amine is incorporated at C-2 only (substitution, **156**), C-3 only (Michael addition, **157**), or at C-2 and C-3 of each lawsone molecule (**158**) (Figure 4.1). These result in the tag on both lawsone molecules of the dimer aligning with either the amine (**156**) or alkyl linker (**157**), or one tag aligning with the amine linker and the other tag aligning with the alkyl linker (**158**) (Figure 4.1).

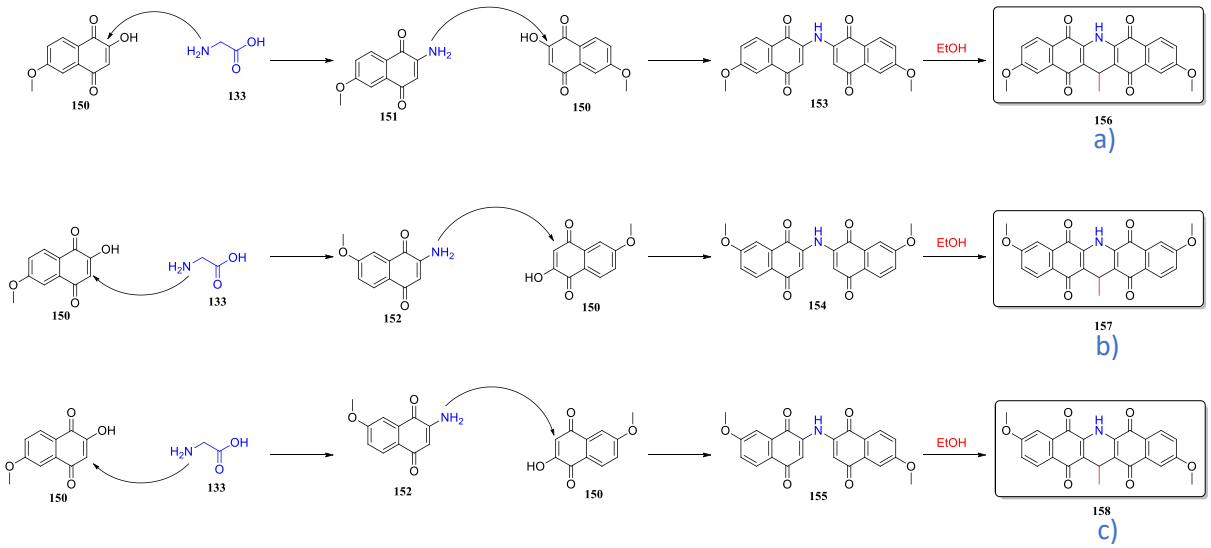


Figure 4.1 Three proposed products resulting from the reaction of the tagged lawsone molecule with glycine to produce a) the substituent on both lawsone molecules aligning with the carbon linker (**156**); b) the substituent on both lawsone molecules aligning with the amine linker (**157**); or c) the amine and carbon linkers aligning with a substituent from each lawsone molecule (**158**).

The proximity of the tag to specific environments in the resulting product can therefore be traced to determine where the amino acid and ethanol molecules react on the lawsone molecule. Methyl and methoxy functional groups were incorporated at the ortho or meta positions on the lawsone molecule based on already reported syntheses of similar analogues, and were chosen based on simplicity, perceived limited reactivity with reagents used in the microwave experiments of Chapter 2, and availability of established methods in literature. The inclusion of these functional groups into the structure may also provide useful structural analogues to Compound **100** with more desired fluorescent, UV, or luminescent properties.

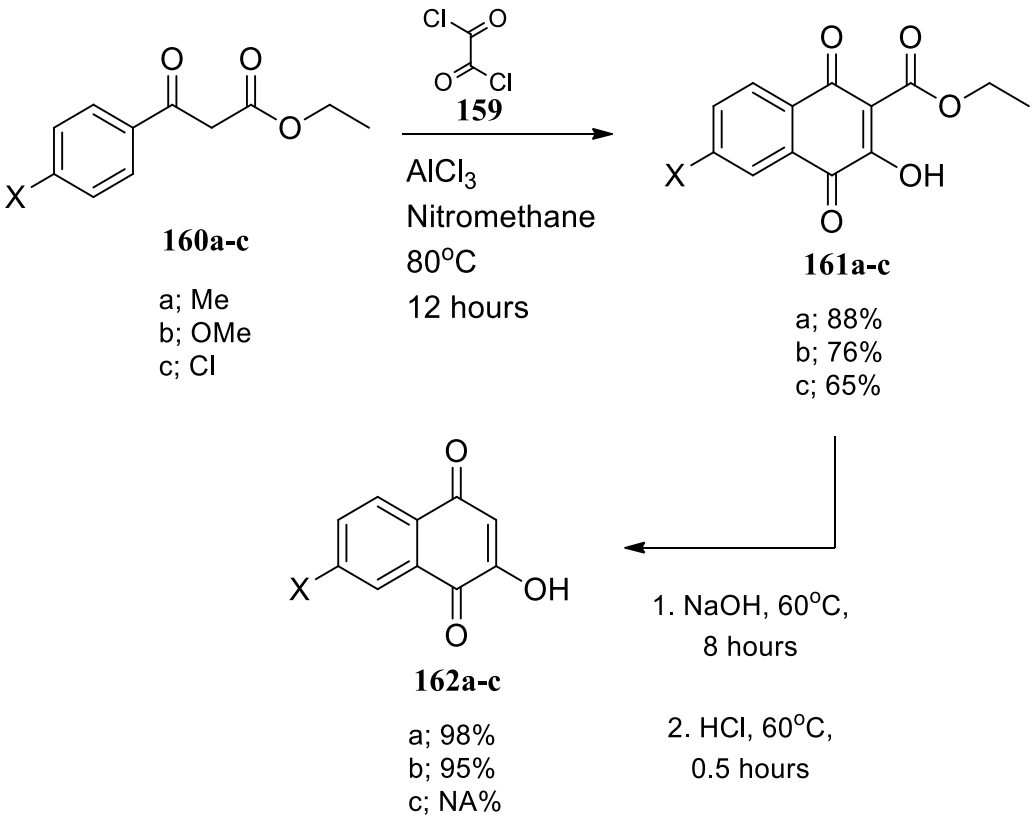
4.2. Results and Discussion

Lawsone analogues with various substituents on the benzene ring have been synthesised and reported in literature, and two methods were adapted in this chapter. The first was based on the work of Sartori *et al.*[7] who reported the synthesis of lawsone analogues with methyl and methoxy groups in the para (C-6) position (Method A). The second method (Method B) was reported by Inagaki *et al.*[8] where more preferable lawsone analogues with a methoxy substituent in the C-6 position were synthesised. Upon successful synthesis of this lawsone analogue, it was further reacted with glycine in the presence of ethanol in a microwave (see Chapter 2 for parameters) and analysed using 2D NMR to determine the reaction sites of glycine and ethanol.

4.2.1. Synthesis of tagged lawsone molecules

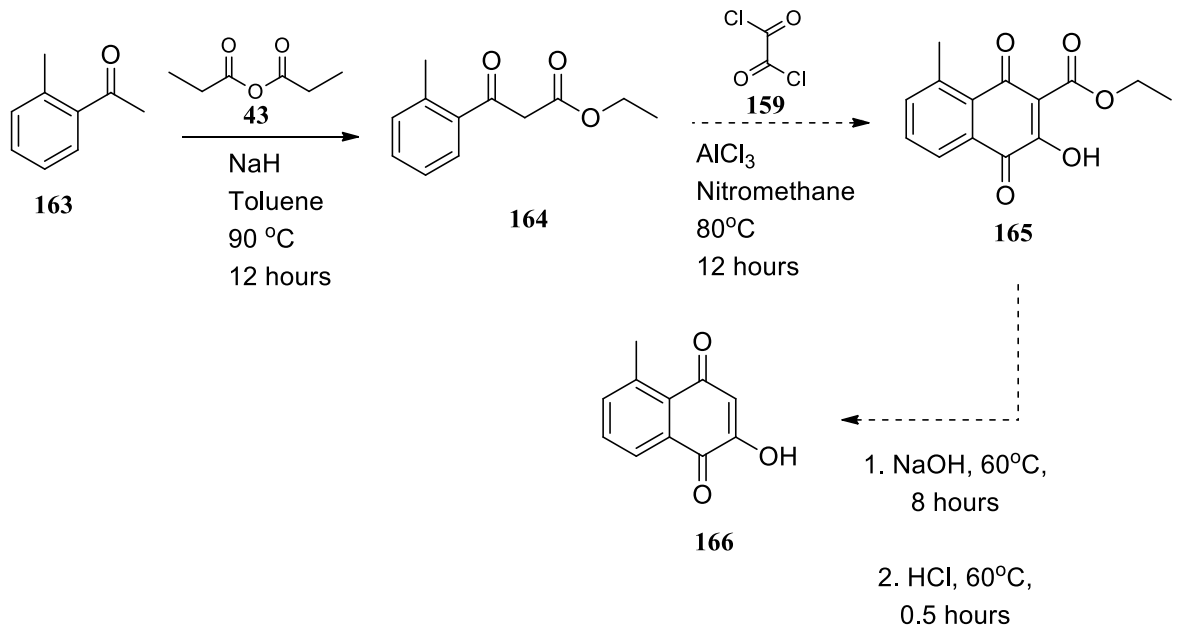
Method A

Insertion of a methyl group in the ortho position of the lawsone molecule was attempted using a method adjusted from that reported by Sartori *et al.*,[7] who synthesised analogues with methyl, methoxy, or chloro substituents in the ortho and meta positions, as well as in the para position only (Scheme 4.3, showing para position only). The first step along the synthesis pathway begins with the successive Friedel-Crafts acylation of the β-keto ester **160a-c** with **159** to form **161a-c**. **161a-c** then undergoes a decarboxylative step to form the desired **163a-c** in yields of 65-88%.



Scheme 4.3 The synthesis pathway used by Sartori *et al.*[7] to produce various analogues of hydroxynaphthoquinones **163a-c**.

For the purposes of this research, 2'-methyl acetophenone (**163**) was used as the starting reagent to form the required β-keto ester **164**, adapted from methodology outlined by Wang, Zhang, Lang, and Wang.[9] **164** was then used in a similar synthesis pathway outlined in Scheme 4.3 in an attempt to synthesise lawsone analogue **166** with the substituent in the meta (C-5) position only (Scheme 4.4).



Scheme 4.4 Proposed synthesis pathway for the formation of lawsone analogue **166**.

The starting reagent **163** was first subjected to reaction with sodium hydride and diethyl carbonate to form a β-keto ester **164** with a methyl at the meta position, according to conditions outlined in Scheme 4.4. A yellow oil was isolated from the reaction mixture, where ^{1}H NMR analysis (Figure 4.2) showed signals consistent with the formation of both keto and enol forms of **164**. The relative peak integration for shifts at 1.24 ppm (H-12), 4.2 ppm (H-11), and 3.95 ppm (H-8), corresponding to the keto form of the β-keto tail, are larger than those at 1.34 ppm (H-12), 4.27 ppm (H-11), and 5.28 ppm (H-8) corresponding to the enol form, suggesting the keto form is in higher abundance (Figure 4.2, inserts). The peak ratios obtained from the integration of the peaks from the β-keto ester moiety and the methyl moiety of the product were consistent with the expected number of protons in each moiety. However, the integration value of four for the aromatic peaks between 7.41 and 7.66 ppm was lower than the expected five protons when considering both keto and enol integration ratios of the product. Despite this, the product was used in the next step, as the shifts and integrations for all other proton environments were consistent with expected values. The yellow oil **164** was isolated in a 40% yield, which was considerably lower than the yields of at least 65% obtained by Sartori *et al.*[7] for other analogues.

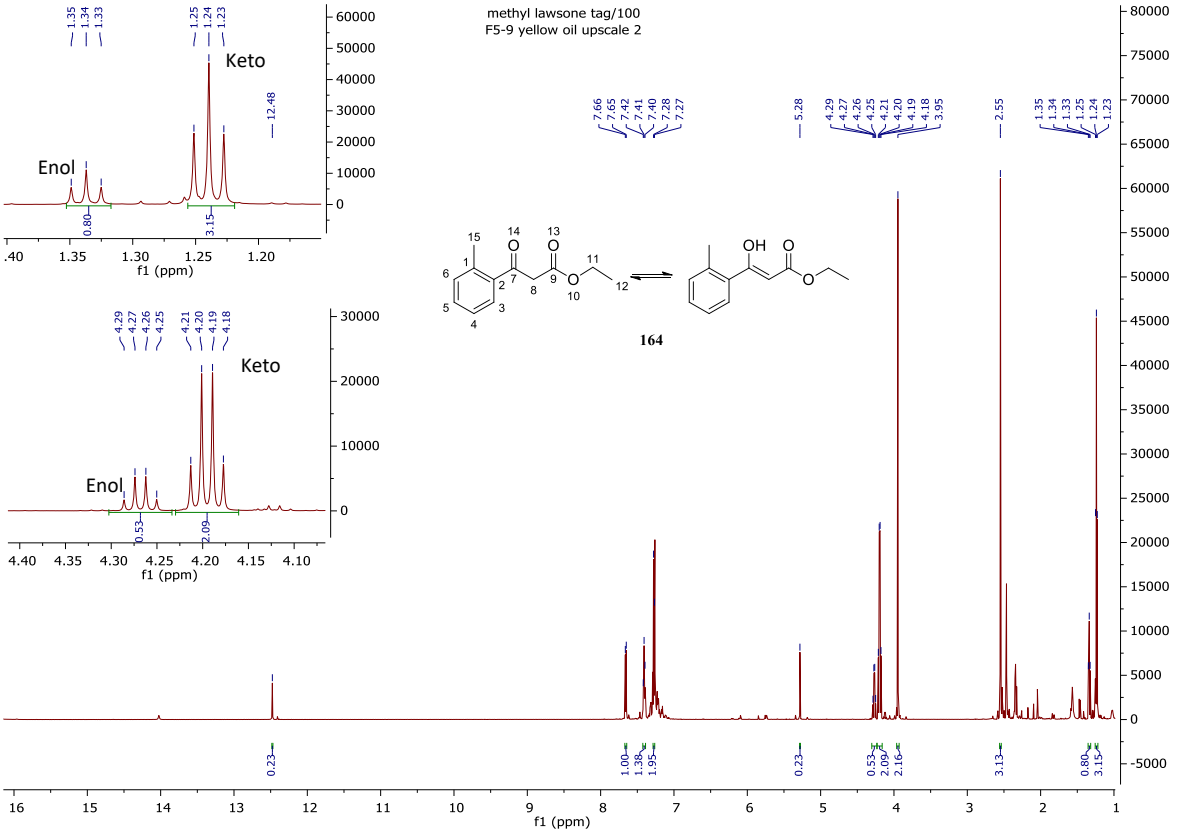


Figure 4.2 ^{1}H NMR of **164**, exhibiting signals for both the keto and enol tautomers.

The β-keto ester **164** was reacted with oxalyl chloride in the presence of aluminium chloride as described by Sartori *et al.*[7] (Scheme 4.3) in an attempt to form the carbethoxynaphthoquinone **165**. Three products were observed using TLC of the reaction mixture after work-up, and were subsequently separated on silica gel using a solvent system of 4:1 HX:EtOAc. It would be expected that **166** would contain seven proton environments, integrating for twelve protons in total, when analysed using ^{1}H NMR.

The first compound isolated (R_f = 0.59) exhibited ^{1}H NMR shifts at 2.67 ppm (s, 3H); 7.29 ppm (t, 2H); 7.46 ppm (t, 1H); and 8.07 ppm (d, 1H). These shifts are similar to those at 2.55, 7.41, and 7.65 ppm found in the β-keto ester **164** (Figure 4.2), however upshifted by 0.05-0.5 ppm, which correspond to the methyl substituent and the aromatic ring. There were no peaks at 4.20 and 1.24 ppm or 4.27 and 1.34 ppm pertaining to the keto and enol forms of the β-keto ester moiety respectively, which would be expected if the β-keto ester portion of the starting molecule **164** is no longer present or has been replaced. It was therefore considered that this compound was not that of **165**, as the appropriate number of proton environments and protons were not observed. The second compound isolated (R_f = 0.24) exhibited ^{1}H NMR shifts at 1.32 ppm (t, 8H); 2.70 (s, 2H); 4.26 ppm (q, 5H); 5.86 ppm (s, 2H);

7.32 ppm (dd, 3H); 7.51 ppm (t, 1H); 8.05 ppm (d, 1H); and 11.72 ppm (s, 2H). When compared to the ^{1}H NMR of the starting β-keto ester **164** (Figure 4.3), peaks at 1.24, 2.55, 4.2, 7.41, 7.65, and 12.48 ppm have now shifted in the product ^{1}H NMR to 1.32, 2.70, 4.26, 7.51, 8.05, and 11.72 ppm respectively. These shifts are suggestive of tautomerism of **164** to the enol form. The peak at 5.28 ppm, corresponding to the proton at the H-8 position in the β-keto ester, is absent in the product. This suggests that a reaction may have occurred at the C-8 position of the β-keto ester starting material, but not cyclisation to the aromatic ring to form **165**, as all aromatic environments were still observed. The last product (R_f = 0) was determined to be an inseparable mixture of side-products. The ^{1}H NMR shifts observed from the crude mixture did not contain peaks that were consistent with the expected **165**. No further characterisation was performed as it was determined that neither of these products contained the expected ^{1}H NMR shifts and integrations for the desired **165**.

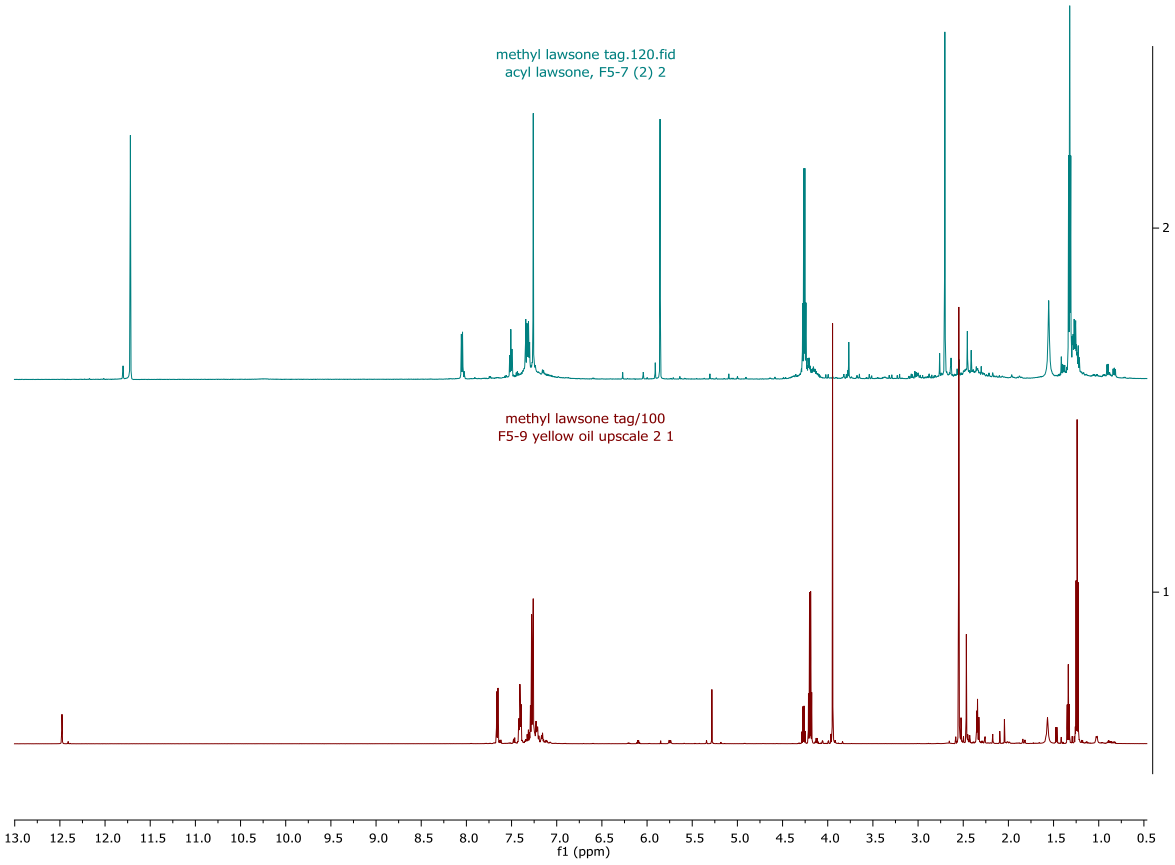
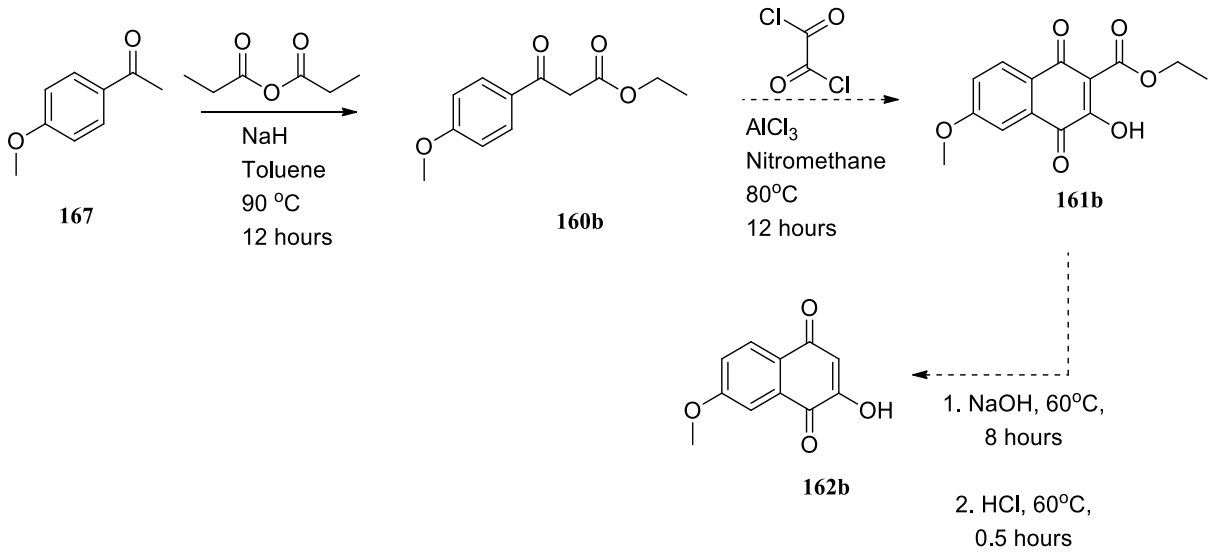


Figure 4.3 ^{1}H NMR spectra comparison of the ortho-methyl β-keto ester **164** (top, green) and the compound at R_f = 0.24 from the attempted synthesis of the ortho-methyl carbethoxynaphthoquinone **165** (bottom, red).

Numerous attempts at preparing the desired **165** were unsuccessful. Initially, dichloroethane was used instead of nitromethane as a safer solvent alternative. However, as this method failed to return the desired **165**, nitromethane was subsequently used to obtain higher temperatures more similar to literature. Despite this, in addition to extending the reaction time from four hours up to 36 hours and increasing the temperature further from 90°C to 160°C (through use of higher temperature solvents such as diglyme), the reaction was not successful. The use of heat to dry the glassware, nitrogen streams to remove atmospheric oxygen, distillation, and silica and molecular sieves to purify all solvents and reagents were also ineffective. An acetophenone with a methoxy substituent in the para position was consequently trialled which was more in-line with what Sartori *et al.*[7] reported.

Reaction of 4'-methoxy acetophenone (**167**) with sodium hydride and diethyl carbonate as set out in Scheme 4.5 resulted in the corresponding para-methoxy β-keto ester **160b** as a yellow oil in a yield of 80%, confirmed through ^{1}H NMR analysis (Figure 4.4). However, the para-methoxy β-keto ester **160b** was unable to be converted to the carbethoxynaphthoquinone **161b** using the oxalyl chloride/aluminium chloride conditions as described by Sartori *et al.*[7] One fraction was isolated from the reaction mixture (R_f = 0.26), however it exhibited ^{1}H NMR shifts indicative of a mixture of products. Due to the low trace yield obtained, this was not further purified. Repeated attempts varying the reaction conditions and stoichiometry of the oxalyl chloride and aluminium chloride proved unsuccessful.



Scheme 4.5 Proposed synthesis pathway for the formation of lawsone analogue **162b**.

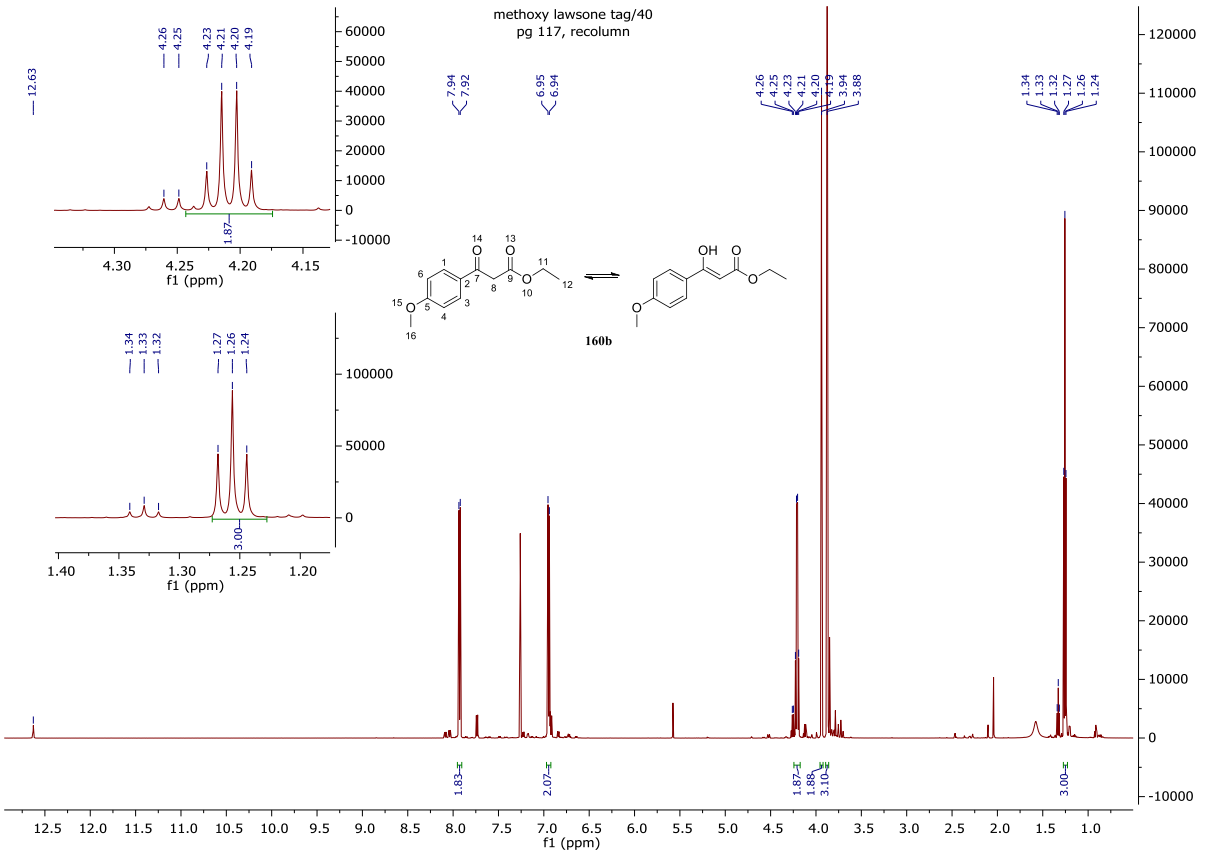


Figure 4.4 ^{1}H NMR of **160b**, exhibiting signals for both the keto and enol tautomers.

The lack of success of the reaction using **163** is likely due to the presence of the methyl group at the C-1 in the ortho position relative to the β-keto ester (refer to Figure 4.5 for position assignments around the aromatic ring). Prior literature[7,9] employing this synthetic strategy has only been reported where substituents are in the meta (C-4) or para (C-5) positions. The only report of a successful ring closure using an ortho substituent was achieved when a second substituent was attached in the meta position (therefore C-1 and C-4), where both substituents were methoxy groups.[7] It is likely that this reaction was able to proceed as a methoxy or methyl group in the C-4 position are ortho or para directing groups, and could activate the ring closure at the C-3 position (Figure 4.5, d).[10] Furthermore, rotation of the ring around the C-2 position could place a substituent at the C-3 position and therefore block ring closure from occurring (Figure 4.5, d). An ortho substituent, employed in this work, could possibly either activate addition on the benzene ring in an unfavourable position, or block the desired reaction occurring at the correct position (Figure 4.5, a). A para substituent also employed in this work in the form of **167** would result in deactivation at the C-3 position (Figure 4.5, c). Although ring closure at the C-3 is not favoured, this does not mean that reaction would never proceed, as Sartori *et al.*[7] were able to produce **161b** in 76% yield. Due to the apparent stabilising effects and less hinderance

at the C-3 position, it was considered that the reaction may require the substituent to be in the meta (C-4) position to allow for the desired ring closure to the carbethoxynaphthoquinone (Figure 4.5, b). An acetophenone with a methoxy substituent in the meta position was not readily available for repetition of this synthesis pathway. Consequently, it was decided to trial a different synthesis method altogether.

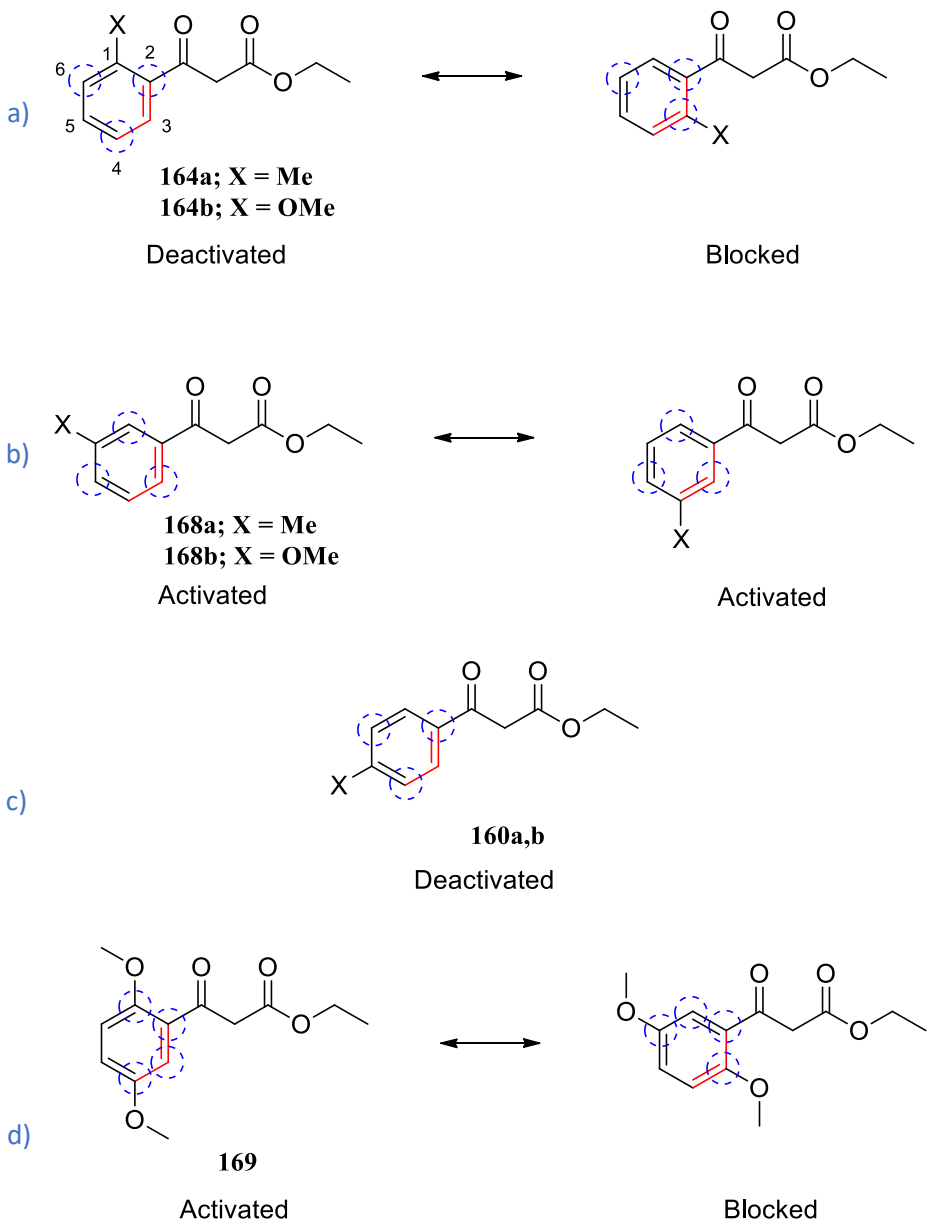
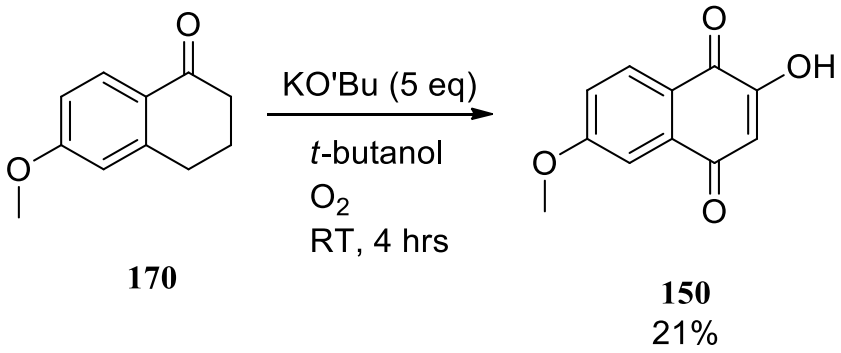


Figure 4.5 Rotation about the C-2 position of alkyl or methoxy substituted β-keto esters could activate or block the desired reaction position, or direct more favourable reactions at positions elsewhere in the molecule. The red C position indicates the desired reaction position, while the blue circles indicate where reaction is favourable as brought about by electronic effects of the chosen substituent. a) The deactivated and blocked reaction positions of **164a,b**, as attempted herein; b); the activated reaction positions of **168a,b**; c) the deactivated reaction positions of **160a,b**, as used by Sartori *et al.*[7]; d) the activated and blocked reaction positions of **169** as used by Sartori *et al.*[7]

Method B

Due to the several unsuccessful attempts at forming a methyl or methoxy lawsone analogue via Method A, a second pathway was attempted where the methoxy-substituted lawsone was synthesised in one step in according to Inagaki *et al.*[8] Although a more straightforward procedure than Method A, it was unable to be attempted until reagents became available.

The starting material, 6'-methoxy-1-tetralone (**170**), was oxidised in the presence of pure oxygen and potassium *tert*-butoxide while stirring in *t*-butanol at 30°C for 4-12 hours (Scheme 4.6). The resulting yellow precipitate was isolated in a 21% yield. Analysis using NMR, MS, and IR spectroscopy confirmed that the identity of this precipitate was the desired lawsone analogue **150**. The ^{1}H, ^{13}C, COSY, HSQC, and HMBC data for this compound are summarised in Table 4.1 and represented in Figure 4.6. Associated spectra are presented in full in Appendix 3. Prior to this research, only IR, ^{1}H and ^{13}C NMR spectra have been recorded for this compound. The ^{1}H and ^{13}C NMR shifts correlate closely to those reported by Malerich *et al.*[11], although slightly shifted due to the different NMR solvents used (Table 4.2).



170 **150** 21%

Scheme 4.6 The synthesis pathway used by Inagaki *et al.*[8] to produce **150.**

Table 4.1 ^{1}H, ^{13}C, HSQC, COSY, and HMBC NMR data for compound **150**, refer to Figure 4.6 for position assignment.

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$) [a]	COSY	HMBC (H→C)
11	56.23	3.97, s; 3H		165.69
3	110.08	6.31, s; 1H		156.87, 180.59
5	110.78	7.57, sd (2.6); 1H	7.16	165.69, 185.02
7	119.58	7.16, dd (8.6, 2.6); 1H	7.57, 8.06	122.77
10	122.77			
8	129.35	8.06, d (8.6); 1H	7.16	135.75, 165.69, 180.59
9	135.75			
2	156.87			
6	165.69			
1	180.59			
4	185.02			
OH		7.43, s		156.87

[a] δ_H, multiplicity (*J* in Hz); integration

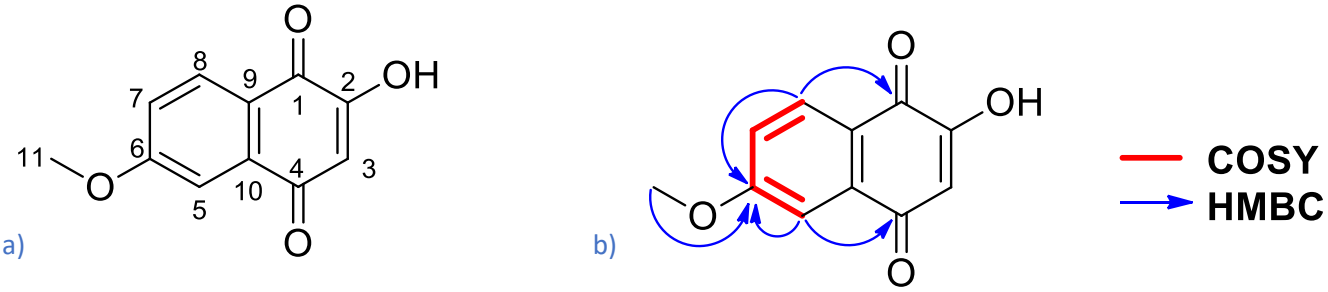


Figure 4.6 a) Structural assignment of 2-hydroxy-6'-methoxy-1,4-naphthoquinone **150**; b) Structure depicting selected COSY and HMBC correlations of **150**.

Table 4.2 Comparison of observed NMR values and those reported by Malerich *et al*.[11] for Compound **150**. All δ are reported in ppm.

Position	δ_C ($CDCl_3$) Observed	δ_C (DMSO-d_6) Reported	$\Delta\delta_C$	δ_H ($CDCl_3$) [a] Observed	δ_H (Acetone-d_6) [a] Reported	$\Delta\delta_H$
11	56.23	56.3	0.07	3.97, s; 3H	3.98, s; 3H	0.01
3	110.08	110.1	0.02	6.31, s; 1H	6.17, s; 1H	-0.14
5	110.78	110.9	0.12	7.57, sd (2.6); 1H	7.46, d (2.6); 1H	-0.11
7	119.58	119.1	-0.48	7.16, dd (8.6, 2.6); 1H	7.28, dd (2.6, 8.6); 1H	0.12
10	122.77	124	1.23			
8	129.35	129.1	-0.25	8.06, d (8.6); 1H	8.01, d (8.6); 1H	-0.05
9	135.75	134.6	-1.15			
2	156.87	160.3	3.43			
6	165.69	164.5	-1.19			
1	180.59	180.3	-0.29			
4	185.02	184.7	-0.32			
OH				7.43, s; 1H	-	-

[a] δ_H, multiplicity (*J* in Hz); integration

^{1}H and HMBC NMR spectral data were carefully assigned to establish a reference for the planned determination of the position of reactivity in the final dimeric structure. Consistent with Malerich *et al.*,[11] the ^{1}H NMR spectrum of the yellow precipitate **150** exhibited three aromatic signals at 7.16, 7.58, and 8.07 ppm, and two aliphatic signals at 3.97 and 6.31 ppm. The aliphatic signals correspond to the methoxy (H-11) and vinyl (H-3) positions respectively. A sharp singlet at 7.43 ppm is also observed, correlating to the -OH. The aromatic doublets can be distinguished by using the coupling constants of these signals in relation to each other. A coupling constant of 2-3 Hz is indicative of meta coupling, while a constant of 7-10 Hz is indicative of ortho coupling. The ^{1}H NMR signal at 7.58 ppm indicates that this proton is meta coupled due to exhibiting a coupling constant of 2.6 Hz, whilst the signal at 8.07 ppm displayed a coupling constant of 8.59 Hz, indicative of ortho coupling. The signal at 7.16 ppm exhibits both coupling constants of 2.6 and 8.59 Hz. Therefore, the signals at 7.16 and 7.58 ppm couple together and are arranged in a meta position in relation to each other, while the signals at 7.16 and 8.07 ppm also couple together and are arranged in an ortho position in relation to each other. Therefore, proton environments corresponding to 7.16, 7.58, and 8.07 ppm directly correlate to the environments at positions C-7, C-5, and C-8 respectively (Figure 4.6).

HMBC correlations between the carbon at C-6 and the -OH and vinyl reaction sites at C-2 and C-3, respectively, can be used to understand the spatial relationship (Figure 4.6). A correlation between the meta-coupled H-5 and carbonyl C-4 can be observed, while a correlation between the ortho-coupled H-8 and carbonyl C-1 is present. Changes in these correlations can be monitored for evidence of future reactions at C-2 or C-3. These correlations can then be traced back to the appropriate aromatic signals, and therefore the tag, to determine site reactivity.

An IR spectrum and a MS spectrum (obtained using a DSA TOF MS in positive mode) were obtained for **150** (Appendix 3). The signals observed in the IR spectrum are in close agreement with previously reported values by Kasturi and Arunachalam[12], while the MS exhibits a [M+H] of 205.0491 (mass error of -1.95 ppm). This information confirms the structure of **150** to be that outlined in Figure 4.6.

4.2.2. Reaction of methoxy lawsone analogue 150 with glycine

Lawsone analogue **150** was reacted with glycine in a microwave under the previous conditions outlined in Chapter 2. TLC using the same conditions as outlined in Chapter 2 resulted in a red compound with an R_f value of 0.28. The colour and R_f value were similar to that of Compound **100** (obtained from the reaction between lawsone and glycine) and consequently this compound was targeted for isolation. This red product was isolated using column chromatography (conditions as described in chapter 2), and its MS, IR, and ^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR were obtained. There were no other red compounds observed using TLC, and the only other visible compound observed was yellow (R_f = 0.42). A small quantity of this product was isolated but the identity of this compound was unable to be confirmed (Appendix 3 for ^{1}H NMR), however the R_f value is quite different to that of the red compound **100** and therefore unlikely to be the methoxy dimer. The remaining compounds within the mixture remained at the baseline on the TLC plate and were not eluted under the conditions used.

All NMR data for the isolated red compound is summarised in Table 4.3, and select COSY and HMBC correlations are represented in Figure 4.7. The ^{1}H NMR still exhibits three aromatic signals at 7.15 ppm, 7.61 ppm, and 8.06 ppm, and a singlet at 3.97 ppm, indicating that the aromatic portion of the naphthoquinone ring containing the methoxy substituent has remained in the final structure. However, the -OH singlet and the C-3 singlet originally in **150** at 7.43 ppm and 6.31 ppm respectively are not observed. Two new peaks appear at 1.29 ppm and 4.59 ppm, with splitting patterns that suggest a coupled -CH_3 and -CH group respectively. A singlet at 8.37 ppm also appears, which is indicative of an -NH shift. The shifts, splitting patterns, and integrations of these peaks relating to the alkyl and amine linkers are consistent with those seen for similar regions in compound **100**, suggesting that the equivalent dimer incorporating the methoxy substituent has been successfully made. Notably,

the integrations for the aromatic and methoxy signals, in relation to the integrations for the amine and alkyl linkers, are in a ratio of two-to-one, which is indicative of a symmetrical dimer. This information immediately eliminates the possibility of the asymmetrical structure **158** in Figure 4.1, as it would be expected that all integrations would be in a ratio of one-to-one for this structure. The ^{13}C NMR exhibits thirteen unique carbon environments, also to be expected from such a product.

Table 4.3 ^{1}H, ^{13}C, HSQC, COSY, and HMBC NMR data for compound **156**, refer to Figure 4.7 for position assignment.

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$) [a]	COSY	HMBC (H→C)
12	24.98	4.59, q (6.6); 1H	1.29	119.41, 137.91, 182.43
13	29.88	1.29, d (6.4); 6H	4.59	24.98, 120.28
11	56.18	3.97, s; 6H		165.26
5	110.62	7.61, s; 2H		119.41, 123.73, 135.39, 165.26, 182.43
7	119.41	7.15, dd (8.6, 2.5); 2H	8.06	110.62, 123.73, 165.26
3	120.28			
10	123.73			
8	129.11	8.06, d (8.6); 2H	7.15	135.39, 165.26, 178.10
9	135.39			
2	137.91			
6	165.26			
1	178.10			
4	182.43			
NH		8.37, s		119.41, 178.10

[a] δ_H, multiplicity (*J* in Hz); integration

In order to determine whether structure **156** or **157** (Figure 4.1) was the symmetrical structure, HSQC, HMBC and COSY NMR experiments were performed. The key signals for the determination of reactivity sites involve the aromatic signals and their long-range coupling to the amine and alkyl linker in the dimeric product (**156** or **157**). Signals that exhibit strong coupling to the C-1 carbonyl are located at the original site of the -OH group (C-2), while signals exhibiting large coupling to the C-4 carbonyl are now located at the original site of the vinyl group (C-3) (refer to Figure 4.7 for position assignments). The following observed correlations suggest the structure outlined in Figure 4.7. Aromatic environments at ortho-coupled H-8 (8.06 ppm) and meta-coupled H-5 (7.61 ppm) were observed to couple to the carbonyls at C-1 (178.10 ppm) and C-2 (182.43 ppm) respectively. Strong correlations were observed between the NH peak at 8.37 ppm and the carbonyl at C-1 (178.10 ppm). This suggests that the NH is located adjacent to the C-1 carbonyl. The proton environments at H-12

(4.59 ppm) and H-13 (1.29 ppm) correlated strongly with the signals at C-4 (182.43 ppm) and C-3 (120.28 ppm) respectively. This suggests that the ethyl group is adjacent to C-4, and is consistent with the structure of **156** presented in Figure 4.7. Importantly, the methoxy group appears in the same position on either side of the dimer due to its symmetrical NMR patterns. This therefore suggests that initial reaction of the amino acid occurs at the C-2 position for both lawsone molecules. Similarly, the ethanol reaction occurs at the C-3 position of both lawsone molecules. These observations indicate a direct substitution rather than an addition mechanism for the incorporation of the amino acid into the dimer.

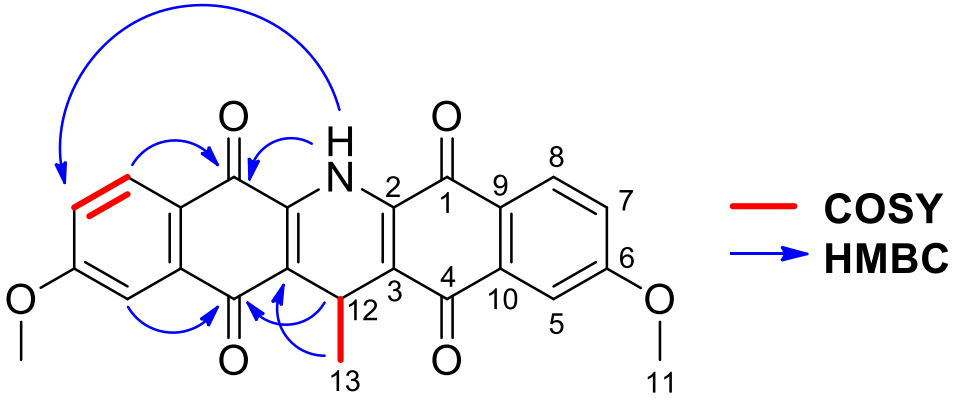


Figure 4.7 Structural assignment and selected COSY and HMBC correlations of dimeric product **156** from the reaction of **150** and glycine.

A mass spectrum of **156** was obtained using a DSA TOF MS in positive mode. The compound in Figure 4.7 with a molecular formula of $C_{24}H_{17}NO_6$ would be expected to have a [M+H] of 416.1128. As the molecular ion at m/z=416.1132 was observed in the resulting spectrum (Figure 4.8), a mass error of 0.961 ppm was calculated, which is within an acceptable error range. Results from the mass spectrum are therefore consistent with the structure of **156** displayed in Figure 4.7.

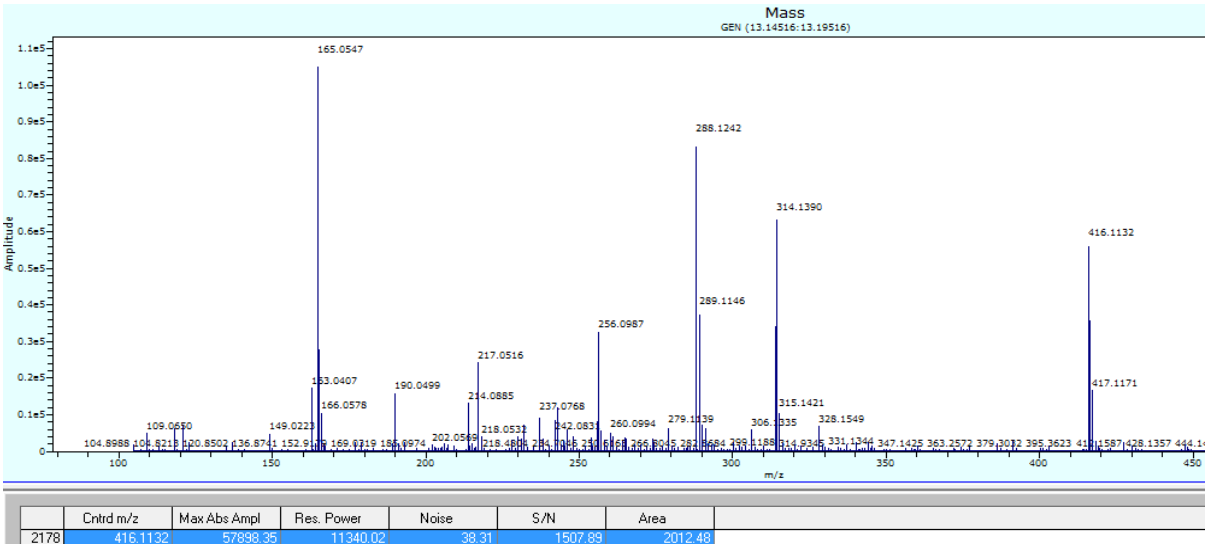


Figure 4.8 Mass spectrum of compound **156**, showing a molecular ion of m/z = 416.1132.

The IR spectrum for **156** (Figure 4.9) exhibited peaks at 3354 cm^{-1}, attributed to the -NH, and 1763 and 1719 cm^{-1}, corresponding to the quinone moiety present. These defining NH and C=O stretches are also present in Compound **100** at 3370, 1681, and 1646 cm^{-1}, however compound **156** exhibits an additional peak at 1281 cm^{-1}, as expected due to its additional methoxy groups. Signals at 2924, 2853, 1670, 1647, 1589, 1495, 1467, 1050, 1024, 774, 751 cm^{-1} were attributed to various C-C, C-H, or N-H stretching and bending in the aromatic and aliphatic regions of the molecule.

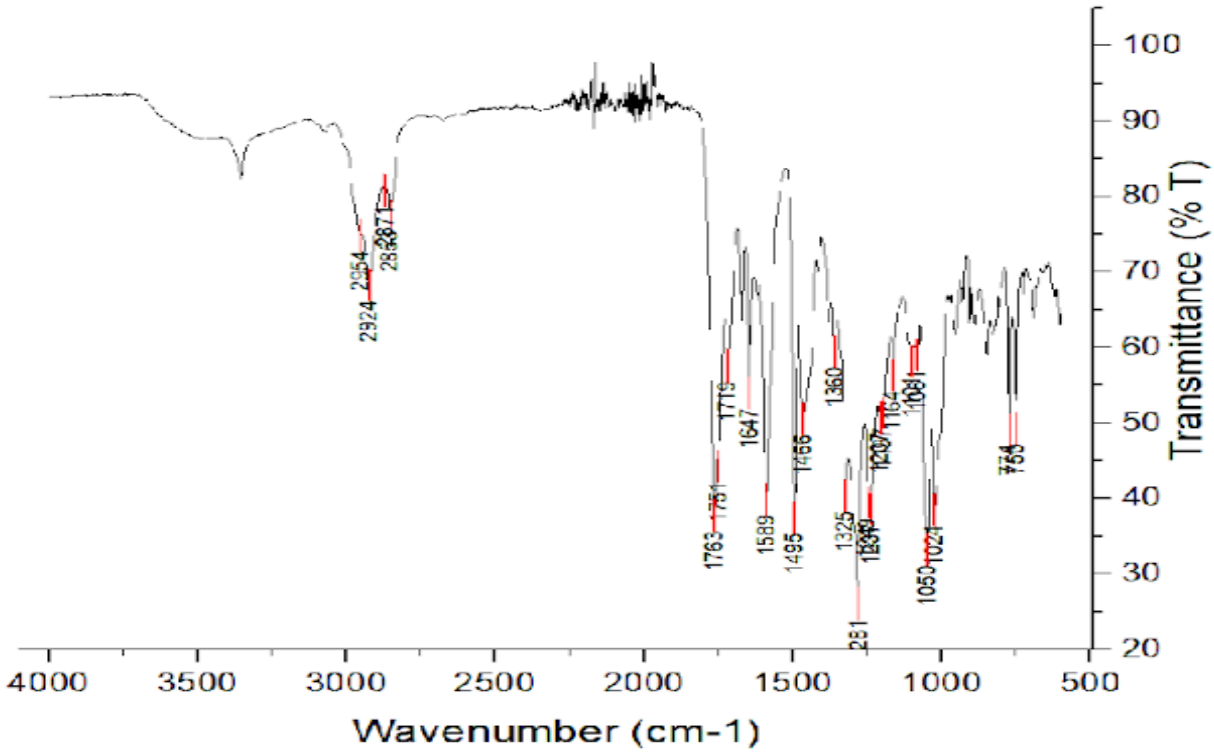


Figure 4.9 IR spectrum of Compound **156**.

The substituted dimeric analogue **156** was successfully synthesised in this work. This potentially opens opportunities for more analogues to be synthesised with unique spectral properties. Chapter 3 detailed the successful synthesis of analogue **140b** from the reaction between lawsone and glycine in 1-propanol with hydrogen peroxide. It was proposed that analogue **171** (Figure 4.10) could be synthesised from the reaction of methoxy lawsone **150** and glycine in the presence of 1-propanol and hydrogen peroxide. Purification of the resulting reaction mixture resulted in the isolation of a red compound with a R_f value of 0.49. ^{1}H and ^{13}C NMR data were collected of this red compound. The ^{1}H NMR spectrum (Figure 4.11) exhibited a potential -NH signal at 8.34 ppm, integrating for the expected one proton. The three expected aromatic signals were difficult to assign due to the number of signals in the aromatic region with the expected doublet and singlet splitting patterns. A triplet at 4.83 ppm was observed that could potentially correspond to the -CH of the alkyl linker, however the expected multiplet and triplet corresponding to the $-CH_2$ and $-CH_3$ groups of the linker were not observed. Analysis of the ^{13}C NMR spectrum (Figure 4.12) revealed 46 possible carbon environments, which is too many signals to possibly originate from the structure of **171**. The amount of ^{1}H and ^{13}C NMR signals indicates that a complex mixture of products was isolated, unlike the isolation of **156** which yielded

relatively clean spectra. Consequently, it was concluded that **171** was not formed from the reaction of **150** and glycine in 1-propanol with hydrogen peroxide.

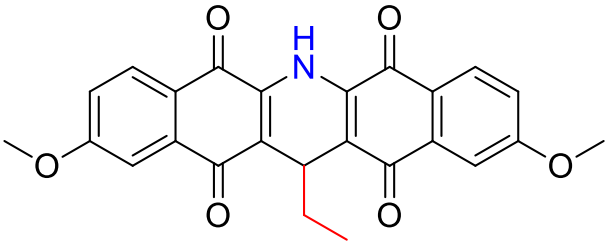


Figure 4.10 Expected product **171** from the reaction between **150** and glycine in 1-propanol with hydrogen peroxide.

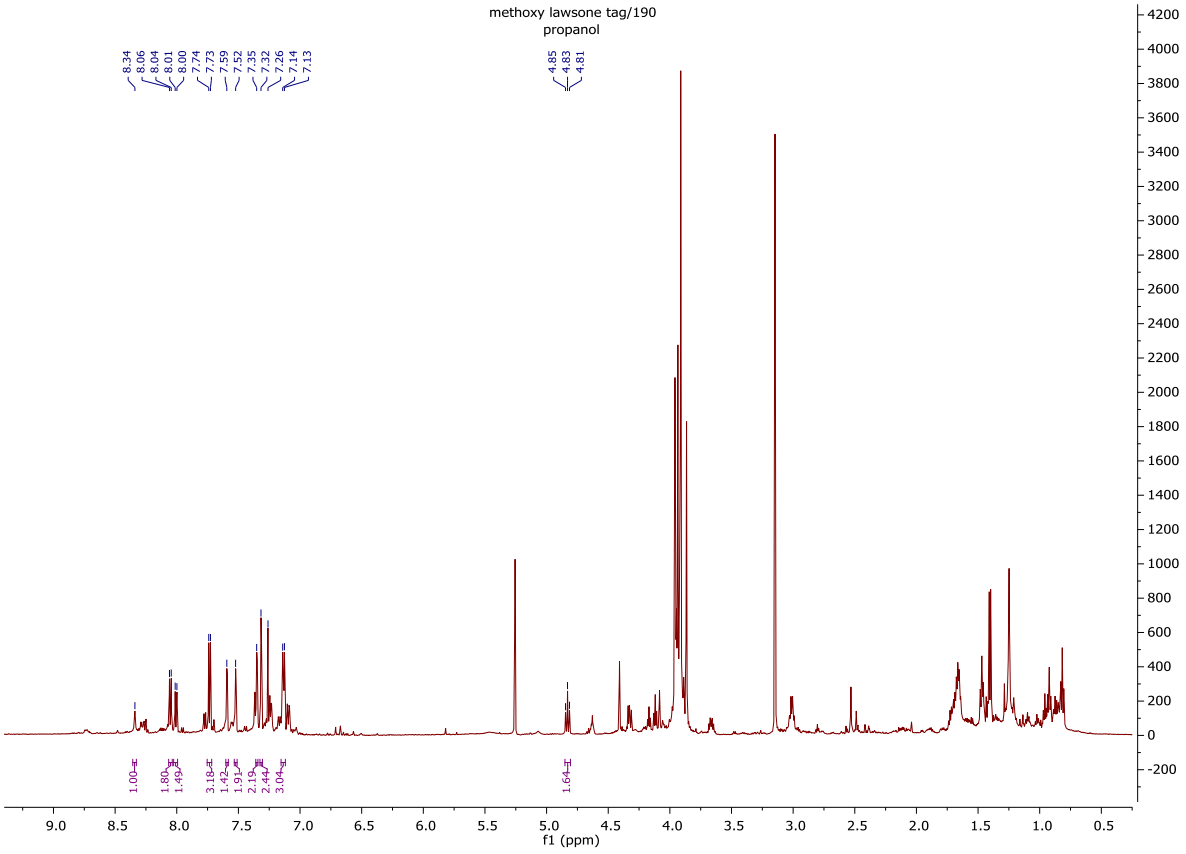


Figure 4.11 ^{1}H NMR spectrum of red compound isolated from the reaction between **150** and glycine in 1-propanol with H_2O_2.

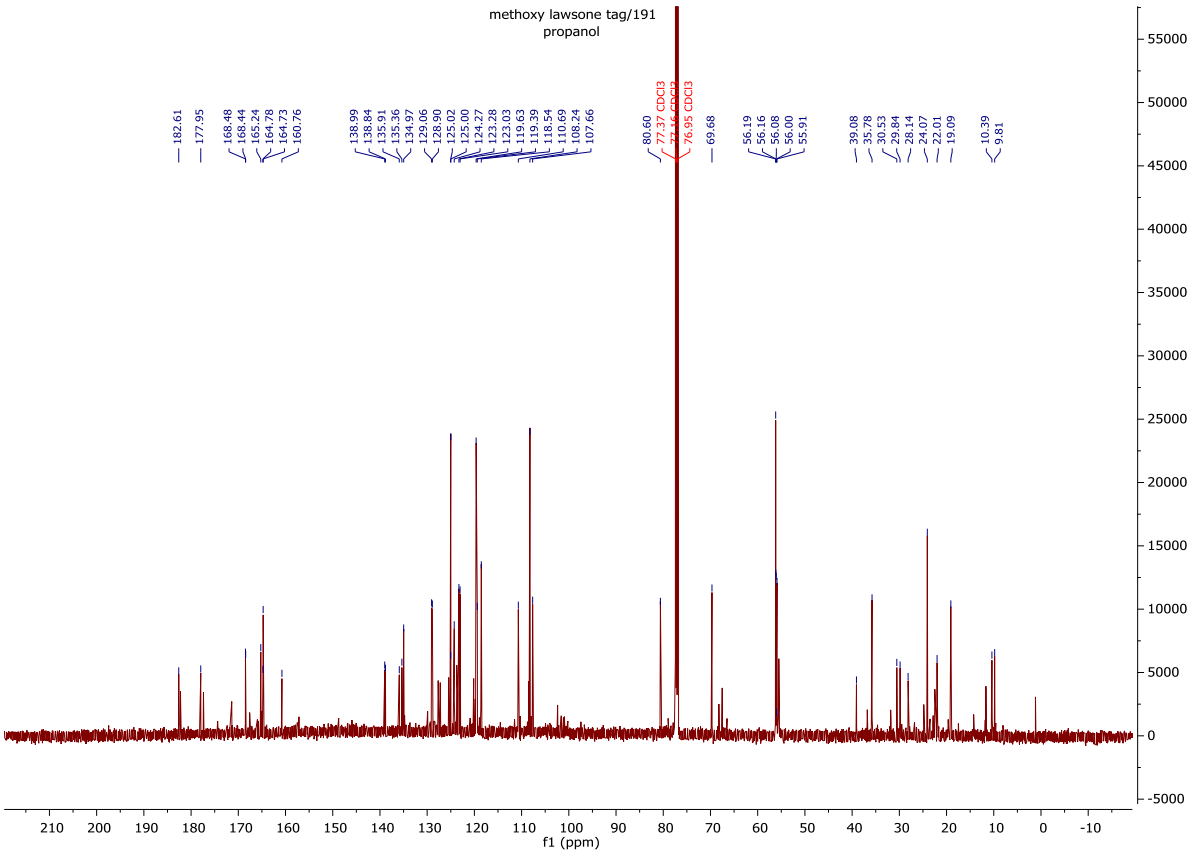
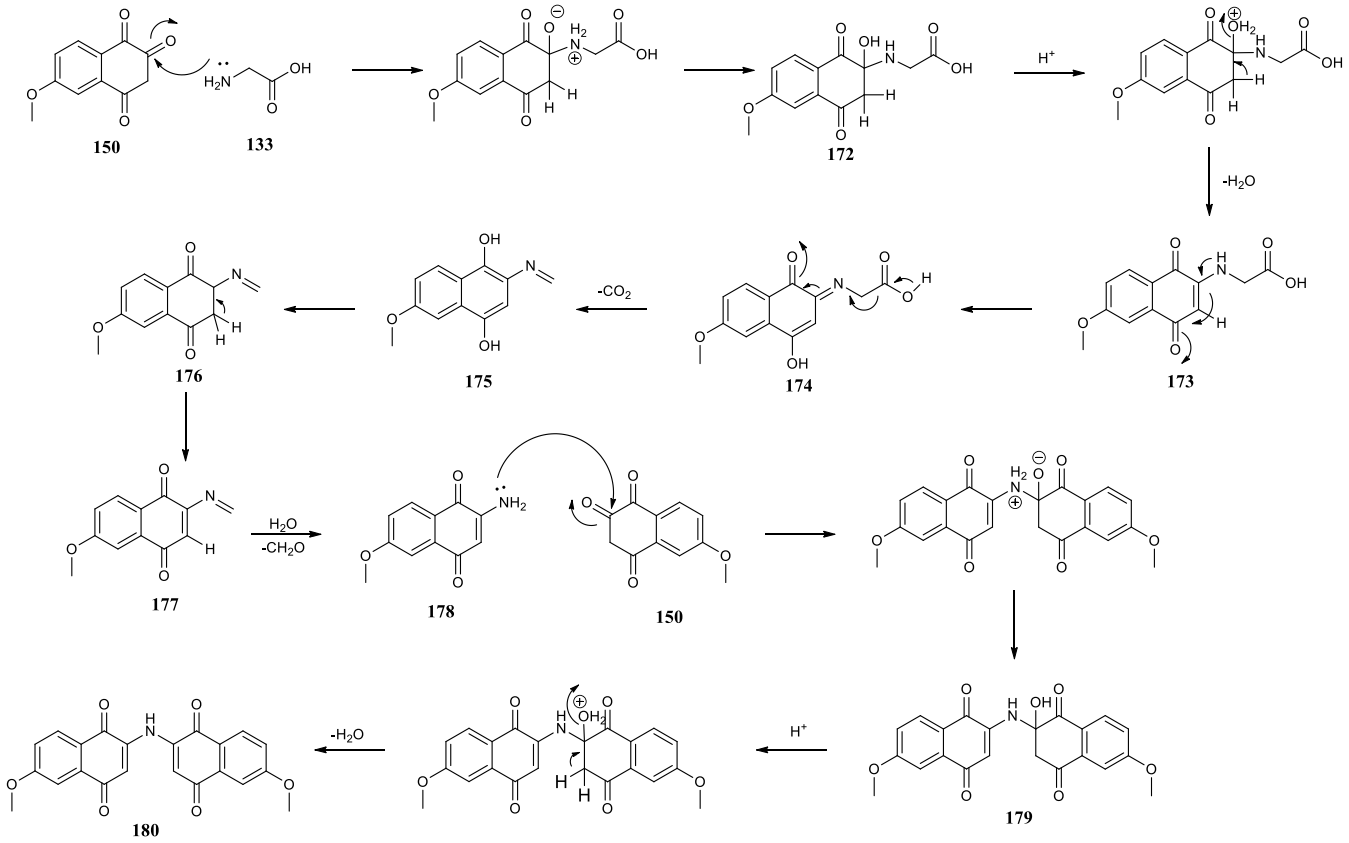


Figure 4.12 ^{13}C NMR spectrum of red compound isolated from the reaction between **150** and glycine in 1-propanol with H_2O_2.

The results from this study can be used to begin forming a reaction pathway towards formation of Compound **100**. It is likely that a substitution mechanism involving the formation of an imine and subsequent hydrolysis is responsible for the initial insertion of the amino acid at the C-2 position and potential subsequent dimerization, as seen in Scheme 4.7. This finding supports the postulation made in Chapter 2 that Compound **102**, isolated from the reaction mixture, is an intermediate. Modelling of the intermediates (Appendix 3) resulting from a substitution or addition mechanism suggested that the substitution intermediate **107** was lower in energy than the addition intermediate **111** (Scheme 4.2). This further supports the idea that the substitution mechanism is preferable. However, the ethylation mechanism cannot be elucidated from these results alone; only that ethylation occurs at each C-3 position of the lawsone that allows for its 1,4-quinone structure to be maintained. These results also do not provide insight into which pathway is more likely from Scheme 4.1, however Scheme 4.7 shows how the complete amine pathway may be possible.



Scheme 4.7 Proposed substitution mechanism for the insertion of the amine linker, showing the formation of an imine **177** followed by hydrolysis to produce a compound **178** (analogous to **102**, as postulated in Chapter 2). Immediate dimerisation of **178** with a second lawsone analogue molecule **150** to intermediate **180** is also shown.

Consequently, whether the alkyl pathway or amine pathway is followed is still unknown. However, potential intermediates can now be postulated and targeted for future intermediate studies. Chapter 5 will detail investigations into the synthesis of the postulated intermediates outlined in Scheme 4.1 and their introduction into the reaction mixture to determine the viability of different pathways.

4.3. Conclusions

The insertion sites of the amino acid and the alcohol in relation to lawsone were determined using a tagged lawsone analogue. The amino acid undergoes reaction at the C-2 site of both lawsone molecules following a substitution mechanism, while the alcohol undergoes reaction at the C-3 site of both lawsone molecules. These results provide the opportunity to explore potential intermediates for introduction into the reaction, where it would be expected that the red product **100** would form more easily if key intermediates were already present.

4.4. Experimental

General experimental details regarding analytical protocol and equipment, as well as preparation of common reagents, can be found in Section 2.4.1. NMR spectra for the compounds listed in this Chapter not shown in Section 4.2 can be seen in Appendix 3.

4.4.1. Proposed Pathway A.1

*Formation of ethyl 3-(2-methylphenyl)-3-oxopropanoate (**164**)*

2'-methyl acetophenone (0.5 mL, 3.73 mmol) was added to a stirring solution of NaH (0.3101 g, 7.45 mmol) in toluene under N_2. After 15 minutes, diethyl carbonate (0.45 mL, 3.73 mmol) was added. The solution was stirred and heated under reflux at 90°C overnight. The solution was quenched with ice water and acidified to pH 4 with 10% acetic acid. The aqueous layer was washed with ethyl acetate and the organic layers combined and dried under vacuum.[7] The crude material was purified on silica gel, first using a 95:5 HX:EtOAc solvent system, followed by 90:10 HX:EtOAc after elution of the first compound. The β-keto ester (**164**) was obtained as an orange oil in 0.3101 g (40%) yield: R_f (95:5 HX:EtOAc) 0.35; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 12.48 (s, 1H), 7.66 (d, *J* = 7.6 Hz, 1H), 7.41 (t, *J* = 6.9 Hz, 1H), 7.27 (d, *J* = 7.5 Hz, 2H), 5.28 (s, 0H), 4.27 (q, *J* = 7.1 Hz, 0H), 4.20 (q, *J* = 7.1 Hz, 2H), 3.95 (s, 2H), 2.55 (s, 3H), 1.34 (t, *J* = 7.1 Hz, 1H), 1.24 (t, *J* = 7.1 Hz, 3H).

*Attempted formation of ethyl 8-methyl-3-hydroxy-1,4-dioxo-1,4-dihydronaphthalene-2-carboxylate (**165**)*

164 (0.3101 g, 1.5 mmol) dissolved in dichloroethane was added to $AlCl_3$ (0.4013 g, 3.0 mmol) with stirring under N_2. After 15 minutes, oxalyl chloride in DCM (2M, 0.75 mL, 1.5 mmol) was added with stirring. After another 15 minutes, the solution was heated to 80°C under reflux for three hours. The reaction was cooled to room temperature and quenched with a solution of 10% oxalic acid.[7] The solution was extracted with diethyl ether, and the organic layers washed with 5% $NaHCO_3$ solution. The aqueous layer was washed with diethyl ether, and the organic layers collected. The remaining

aqueous layers were acidified with 10% HCl and extracted with diethyl ether. The ether layers were dried with sodium sulfate, and the crude mixture purified on silica gel pre-treated with 1% triethylamine using a solvent system of 80:20 hexane:ethyl acetate.[7] Two fractions were isolated where signals were decipherable, while another fraction on the baseline was isolated as a complex mixture of products.

Fraction 1: R_f (80:20 HX:EtOAc) 0.59; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.07 (d, *J* = 8.3 Hz, 1H), 7.46 (t, *J* = 6.9 Hz, 1H), 7.29 (t, *J* = 7.2 Hz, 2H), 2.67 (s, 3H).

Fraction 2: R_f (80:20 HX:EtOAc) 0.24; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 11.72 (s, 2H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.32 (dd, *J* = 17.7, 7.8 Hz, 3H, 5.86 (s, 2H), 4.26 (q, *J* = 7.1 Hz, 5H), 2.70 (s, 2H), 1.32 (t, *J* = 7.1 Hz, 8H).

4.4.2. Proposed Pathway A.2

*Formation of ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (**160b**)*

4'-methoxy acetophenone (0.5978 g, 3.98 mmol) was added to a stirring solution of NaH (0.4204 g, 17.5 mmol) in toluene (15 mL) under N_2. After 15 minutes, diethyl carbonate (0.81 mL, 6.79 mmol) was added. The solution was stirred and heated under reflux at 90°C overnight. The solution was quenched with ice water and acidified to pH 4 with 10% acetic acid. The aqueous layer was washed with ethyl acetate and the organic layers combined. The crude material was purified using silica gel chromatography.[9] The β-keto ester (**160b**) was obtained as a yellow oil in 0.5888 g (79.6%) yield: R_f (80:20 HX:EtOAc) 0.42; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.93 (d, *J* = 9.0 Hz, 2H), 6.95 (d, *J* = 8.9 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 3.94 (s, 2H), 3.88 (s, 3H), 1.26 (t, *J* = 7.1 Hz, 3H).

*Attempted formation of ethyl 3-hydroxy-6-methoxy-1,4-dioxo-1,4-dihydronaphthalene-2-carboxylate (**161b**)*

160b (0.5832 g, 2.62 mmol) dissolved in nitromethane (15 mL) was added to $AlCl_3$ (1.0324g, 7.74 mmol) with stirring under N_2. After 15 minutes, 0.2M oxalyl chloride in DCM (1.3 mL, 2.62 mmol) was added with stirring. After another 15 minutes, the solution was heated to 80°C under reflux for three hours. The reaction was cooled to room temperature and quenched with a solution of 10% oxalic acid (10 mL).[9] The solution was extracted with ethyl acetate (50 mL x 3) and the organic layers combined. The organic layers were dried with sodium sulfate, to yield a crude mixture. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 11.72 (s, 1H), 8.01 (d, *J* = 8.8 Hz, 1H), 6.88 (d, *J* = 8.8 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 2.12 (s, 2H), 1.26 (t, *J* = 7.1 Hz, 5H).

4.4.3. Proposed Pathway B

*Formation of 2-hydroxy-6-methoxy-1,4-naphthoquinone (**150**)*

Potassium tert-butoxide (1.0823 g, 1.77 mmol) was dissolved in *t*-butanol (10 ml) in a dry flask. 6'-methoxy-1-tetralone (0.3117 g, 9.65 mmol) was slowly added to the mixture with stirring. Oxygen was bubbled through the reaction for 4-12 hours, where a colour change from brown to pink was observed. Concentrated hydrochloric acid was added until all solution had changed from pink to yellow. The product was extracted in ether (50 ml x 3) and basified with saturated sodium bicarbonate solution (pH 9). The aqueous layer was collected and re-acidified with concentrated hydrochloric acid (pH 4).[8] The resulting brown precipitate was filtered from solution and re-crystalised in ethanol to give a yellow solid (**150**) in 0.0731 g (21%) yield: R_f (80:20 HX:EtOAc) 0; ν_{max} (thin film) 3185, 1672, 1651, 1591, 1571, 1494, 1435, 1380, 1336, 1327, 1268, 1240, 1127, 1067, 983, 870, 792, 763, 728, 687, 665, 618 cm^{-1}; ^1H NMR (600 MHz, Chloroform-*d*) δ 8.06 (d, *J* = 8.6 Hz, 1H), 7.57 (sd, *J* = 2.6 Hz, 1H), 7.43 (s, 1H), 7.16 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.31 (s, 1H), 3.97 (s, 3H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 185.02, 180.59, 165.69, 156.87, 129.35, 119.58, 110.78, 110.08, 56.23; MS (APCI) [M+H]: 205.0491.

4.4.4. Reaction of 150 with glycine

150 (0.4908 g, 2.4 mmol) was added to glycine (0.1307 g, 1.74 mmol) in ethanol (8 ml) and heated to 130°C in a microwave for 3 hours. The resulting crude purple precipitate from the reaction in ethanol was separated using column chromatography using silica gel pre-treated with 1% triethylamine. A red compound (**156**) was isolated from the reaction in ethanol in 0.0138 g (1.4%) yield: R_f (80:20 HX:EtOAc) 0.28; ν_{max} (thin film) 3354, 2924, 2853, 1763, 1719, 1670, 1647, 1589, 1495, 1467, 1281, 1050, 1024, 774, 751 cm^{-1}; ^1H NMR (600 MHz, Chloroform-*d*) δ 8.37 (s, 1H), 8.06 (d, *J* = 8.6 Hz, 2H), 7.61 (s, 2H), 7.15 (dd, *J* = 8.6, 2.5 Hz, 2H), 4.59 (q, *J* = 6.6 Hz, 1H), 3.97 (s, 6H), 1.29 (d, *J* = 6.4 Hz, 11H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 182.43, 178.10, 165.26, 137.91, 135.39, 129.11, 123.73, 120.28, 119.41, 110.62, 77.16, 56.18, 29.88, 24.98; MS (APCI) [M+H]: 416.1132.

150 (0.2433 g, 1.33 mmol) was added to glycine (0.0470 g, 0.66 mmol) in 1-propanol (2 ml) and heated to 130°C in a microwave for 3 hours. The resulting crude purple precipitate from the reaction in ethanol was separated using column chromatography using silica gel pre-treated with 1% triethylamine A red compound was isolated in 0.0421 g yield: R_f (8:2 HX:EtOAc) 0.49; ^1H NMR (600 MHz, Chloroform-*d*) δ 8.34 (s, 1H), 8.05 (d, *J* = 8.5 Hz, 2H), 8.01 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 8.3 Hz, 3H), 7.59 (s, 1H), 7.52 (s, 2H), 7.35 (s, 2H), 7.32 (s, 2H), 7.13 (d, *J* = 8.4 Hz, 3H), 4.83 (t, *J* = 9.7 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 182.61, 177.95, 168.48, 168.44, 165.24, 164.78, 164.73, 160.76, 138.99, 138.84, 135.91, 135.36, 134.97, 129.06, 128.90, 125.02, 125.00, 124.27, 123.28, 123.03, 119.63,

119.39, 118.54, 110.69, 108.24, 107.66, 80.60, 77.37, 77.16, 76.95, 69.68, 56.19, 56.16, 56.08, 56.00, 55.91, 39.08, 35.78, 30.53, 29.84, 28.14, 24.07, 22.01, 19.09, 10.39, 9.81.

4.5. References

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5. Intermediate Studies Towards Reaction Pathway Elucidation

5.0. Overview

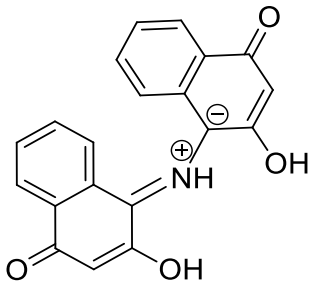
Potential intermediate Compound **102** was isolated, in addition to Compound **100**, from the reaction between lawsone and glycine in ethanol under microwave conditions in Chapter 2. Its identity was confirmed to be that of 2-amino-1,4-naphthoquinone through NMR and MS analysis. The reaction pathway put forward for the formation of Compound **100** in Chapter 2 saw Compound **102** as an initial intermediate upon Strecker degradation of glycine with lawsone. Chapter 3 used deuterated ethanol to confirm that the ethanol solvent was incorporated into the structure of Compound **100** as the alkyl linker. The specific mechanism of ethanol inclusion and its reactivity with lawsone was not elucidated. A lawsone analogue **150** was employed in Chapter 4 to confirm that the amine linker was formed at the -OH position of each lawsone molecule, while the alkyl linker was incorporated at the vinyl positions of each lawsone molecule.

Although reagents and reaction sites have been determined, a reaction pathway is yet to be established. From the four reaction pathways proposed in Chapter 2, four potential intermediates, including Compound **102**, were identified that could determine which reaction pathway is most likely towards the formation of Compound **100**. This chapter details their synthesis and reactivity with lawsone, glycine, ethanol, and other intermediates under microwave conditions. Finally, it proposes the pathway most likely to be occurring within the reaction.

5.1. Introduction

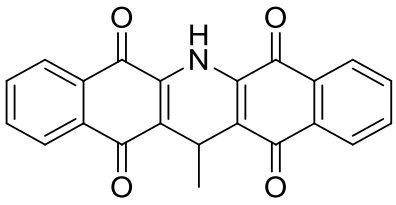
Research performed by Jelly *et al.*[1,2] showed that a lawsone solution could be applied to fingermark deposits to result in a purple-brown stain that exhibited red luminescent properties. In order to determine the cause of the red luminescence, Jelly[3] performed *in-situ* experiments involving the reaction of lawsone and glycine in ethanol, where subsequent silica-gel chromatography of the crude mixture isolated a red compound. Its identity was investigated through NMR analysis, where structure **18** in Figure 5.1 a) was proposed.

a)



18

b)



100

Figure 5.1 a) Structure of red compound **18** proposed by Jelly *et al.*[1,2] b) Revised structure **100** proposed in Chapter 2.

The reaction between lawsone and glycine in ethanol was investigated in Chapter 2, where a red compound with consistent fluorescence, ^{13}C, and ^{1}H NMR spectra to that reported by Jelly[3] was isolated. A new structure **100** was proposed that better reflected the NMR spectra (Figure 5.2 b), particularly with the inclusion of a secondary ethyl group that Jelly noted in their NMR spectra but did not include in their structure. In addition to Compound **100**, Compounds **101** and **102** (Figure 5.2) were also isolated from the reaction mixture and their structures confirmed through NMR and MS analysis. It was suspected that Compound **102** may be an intermediate along the pathway to Compound **100** formation, while Compound **101** was a by-product that does not react further.

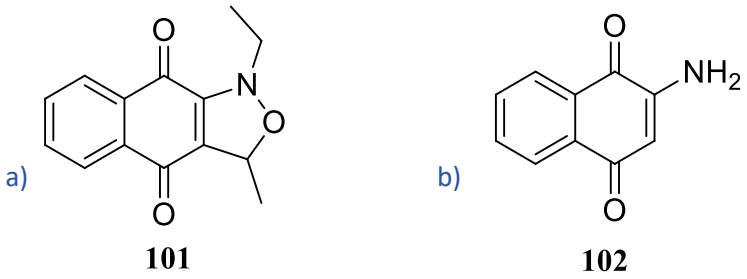
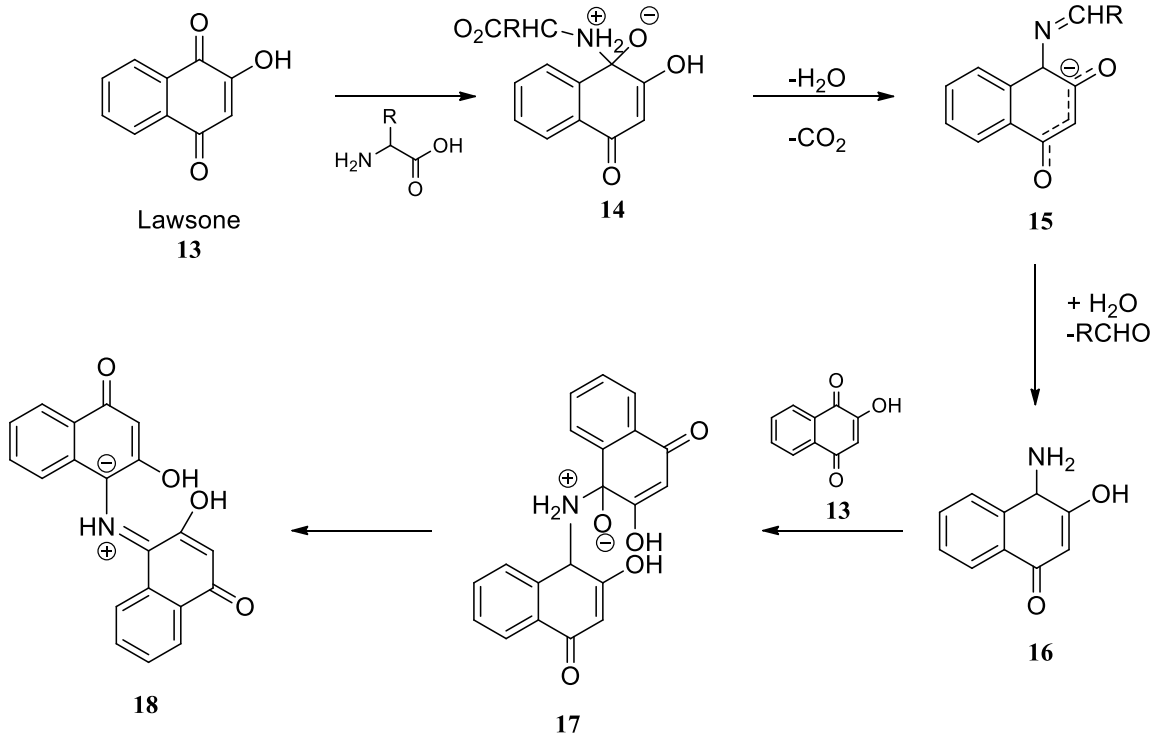


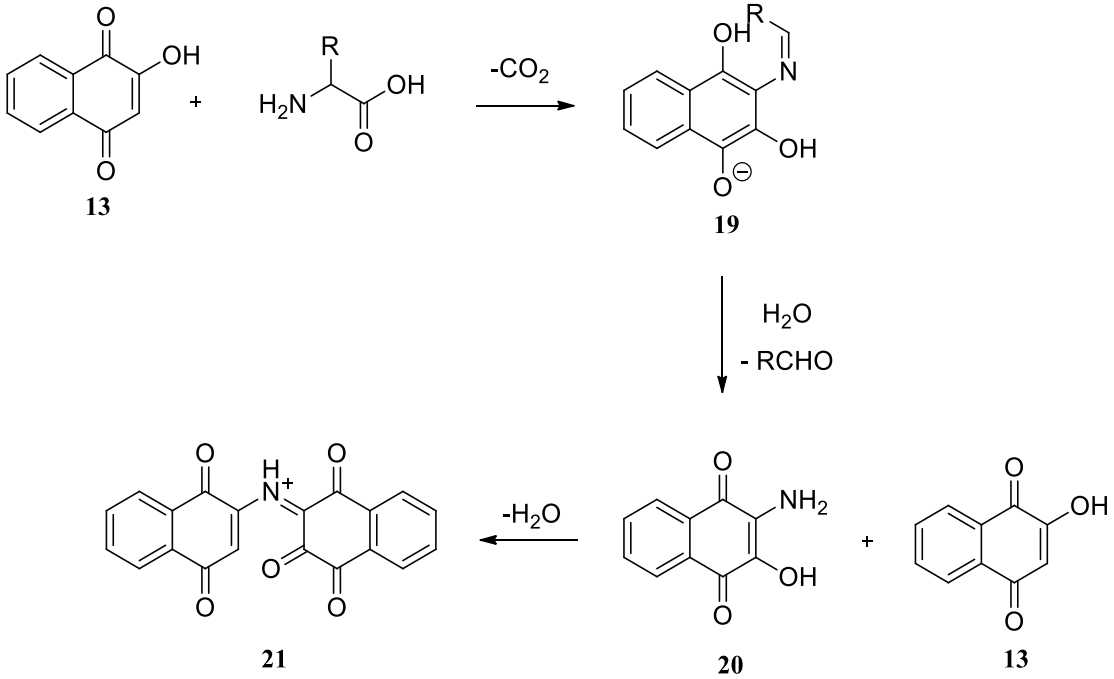
Figure 5.2 Structures of isolated products from the reaction between lawsone and glycine in ethanol a) Compound **101**; and b) Compound **102**.

To date, there have been two previous attempts at elucidating a reaction pathway towards a red dimer, albeit with different final structures. Jelly *et al.*[1] were the first to propose a reaction pathway (Scheme 5.1) using the reaction of amino acids with ninhydrin as a reference. The authors proposed that Strecker degradation occurs between lawsone and the amino acid to form a Schiff base at the 1-position of lawsone. The resulting amine intermediate undergoes a condensation reaction with a second molecule of lawsone, resulting in their proposed dimer.

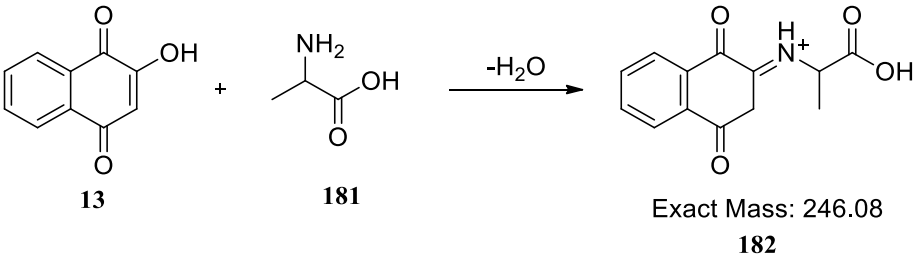


Scheme 5.1 The proposed reaction mechanism of lawsone with amino acids to form a coloured compound **18**.[1]

In 2019, Chan[4] reported their investigations into the reaction between lawsone and amino acids. Chan[4] proposed a new structure **21** using ^{1}H, ^{13}C, and 2D NMR spectroscopy on their isolated red product, although their spectra did not correlate to those presented by Jelly[3] or those reported in this work. They conducted LCMS experiments on the reaction mixture to identify any possible intermediates using only the determined masses. Also using the reaction between amino acids and ninhydrin as a reference, Chan proposed a reaction pathway based on these postulated intermediates (Scheme 5.2). Strecker degradation was proposed to occur between lawsone and the amino acid at the vinyl position, resulting in a 2-hydroxy-3-amino-1,4-naphthoquinone intermediate **20**. A final condensation between the hydroxyl of the lawsone and the amine of the intermediate formed their proposed red dimer structure **21**. This structure proposed by Chan is unusual, due to its charged nature. Their proposed pathway also does not seem to correlate with their proposed intermediates from the MS data. For example, Chan observed a compound with a m/z = 246.0748 and suggested a structure of **182** (Scheme 5.3), proposing that this could be an intermediate from the Strecker degradation of lawsone and the amino acid but prior to decarboxylation. Structure **182** proposes addition of the amino acid at the C-2 position, however their proposed mechanism has an initial amino acid introduction at the C-3 position to instead form **20**.



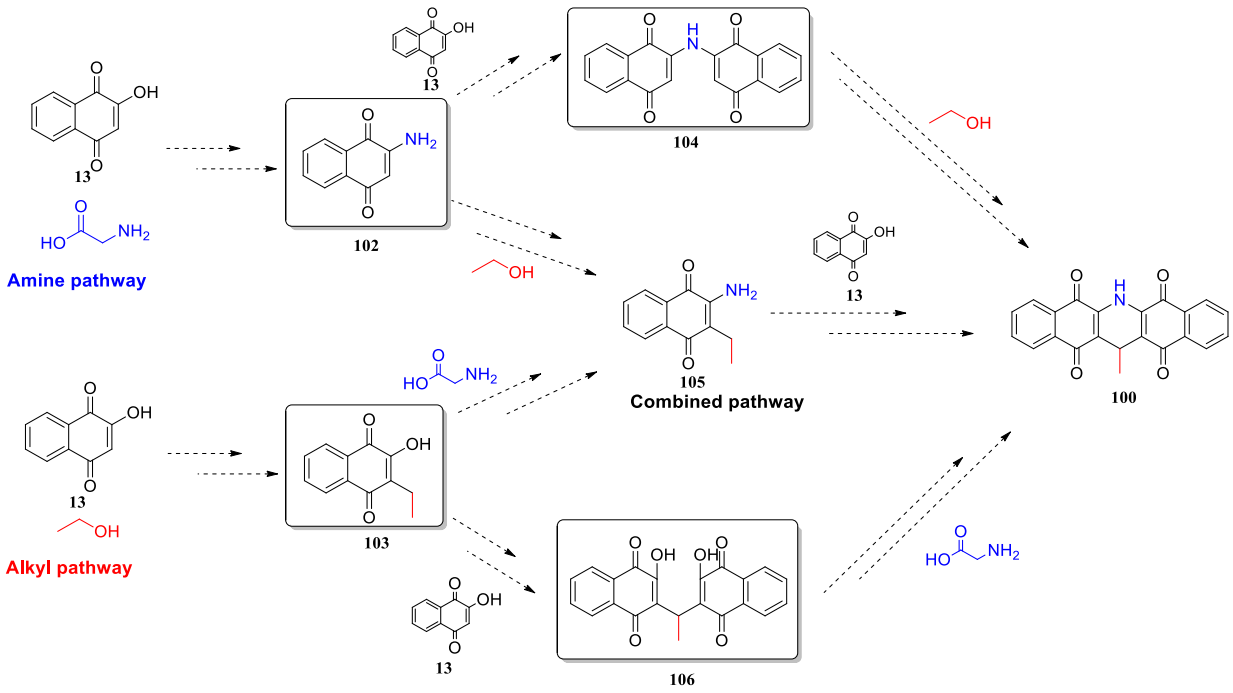
Scheme 5.2 Postulated structure for the red product and proposed mechanism (simplified) with initial amino acid introduction at the C-3 position, as outlined by Chan.[4]



Scheme 5.3 Proposed intermediate **182** from the reaction between lawsone and alanine using MS data, as reported by Chan.[4]

In each proposed pathway, the reaction begins with Strecker degradation between the lawsone and amino acid, followed by a condensation reaction between the amino group of the resulting intermediate and the hydroxyl or carbonyl of a second lawsone molecule. However, both Chan[4] and Jelly[1] did not account for the ethyl linker in their reaction schemes and so provided no suggestion into its inclusion. However, their schemes support the hypothesis that an amine structure similar to Compound **102** is a plausible intermediate, as it is analogous to structure **16** in Scheme 5.1 and structure **20** in Scheme 5.2. Both authors also propose a final condensation step to complete the amine linker in their proposed structures for Compound **100**.

This work proposes a new pathway that incorporates the Strecker degradation and final condensation proposed by Jelly[1] and Chan[4] (Scheme 5.4). Compound **102** was suspected to form from the Strecker degradation of glycine with the -OH group on the lawsone, where it could condense with a second lawsone molecule to form the amine linker by following the amine pathway (Scheme 5.4). Chapter 3 used deuterated ethanol to show that the alkyl linker was formed via the addition of the ethanol solvent at the vinyl positions of each lawsone molecule (Scheme 5.4). The specific sites of the amine and alkyl linker additions in relation to each lawsone molecule were confirmed in Chapter 4 by using a lawsone analogue with a substituent on the benzene ring, where structural correlations between the substituent and the linkers were determined using 2D NMR.



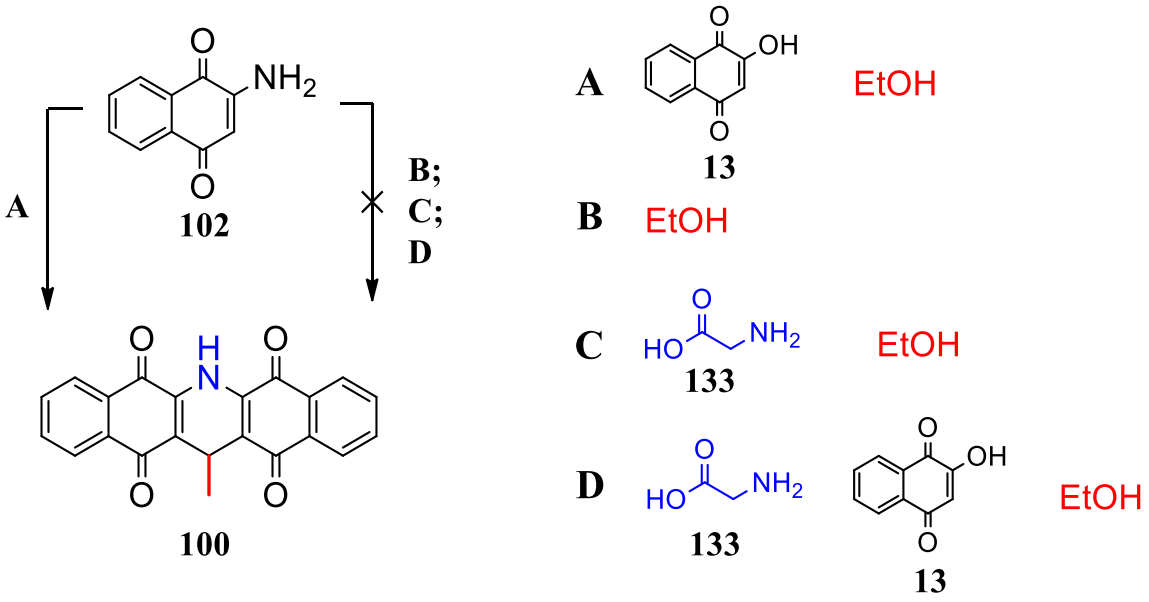
Scheme 5.4 Three proposed reaction pathways towards the formation of Compound **100**, where; the amine linker could fully form first (amine pathway); the alkyl linker could fully form first (alkyl pathway); or both linkers are introduced prior to dimerization (combined pathway).

Chapters 3 and 4 confirmed the role of each reagent in the reaction mixture and their reaction sites, however they do not indicate the order of steps towards the formation of Compound **100**. Four possible pathways are proposed in Scheme 5.4, which can be categorised into three pathways: i) the amine pathway; ii) the alkyl pathway; and iii) the combined pathway. The amine pathway and the alkyl pathway completely form the amine and alkyl linker respectively prior to addition of the other linker. The combined pathway considers that either the amino acid or ethanol reagent may be incorporated first, followed by incorporation of the other reagent, where these pathways merge to form **100** via intermediate **105**. This chapter details the synthesis and/or use of four intermediates proposed in the reaction pathway to Compound **100** (Scheme 5.4, **102**, **103**, **104**, **106**), and the conditions under which they can be formed using only lawsone, glycine, and ethanol. Finally, a reaction pathway towards the formation of Compound **100** will be proposed.

5.2. Results and Discussion

5.2.1. Investigation into 102 as an intermediate

In Chapter 2, compound **102** was isolated from the crude reaction mixture between glycine and lawsone. Compound **102** was proposed to be formed from the Strecker degradation between lawsone and glycine (Scheme 5.4). It was speculated that this may be an initial intermediate in the reaction mechanism, and the first intermediate produced if the amine linker was to be formed first. Four experiments were performed in order to test this theory, using identical microwave conditions that involved heating to 130°C for three hours (Scheme 5.5). Condition A replicated some conversion of lawsone to Compound **102**, followed by subsequent reaction with lawsone. Condition B replicated complete conversion of lawsone to Compound **102**, followed by dimerization. Conditions C and D were designed to determine if the inclusion of glycine facilitates the formation of Compound **100**.



Scheme 5.5 Experiments performed using varying stoichiometry of **102**, lawsone, glycine, and ethanol to determine whether **102** is a potential intermediate.

Initially, **102** was reacted with lawsone in ethanol in a 1:1 stoichiometric ratio in the absence of glycine (Scheme 5.5, Condition A) to determine if **102** was an intermediate along the reaction pathway towards formation of Compound **100**. Chromatographic separation of the crude reaction mixture resulted in the isolation of a red product that was identified by ^{1}H NMR to be the desired Compound **100**. The compound was isolated in a 2.8% yield, which is comparable to the yield of 1% from the reaction using lawsone and glycine (2:1 stoichiometry) in ethanol. The comparable yields suggest that

neither condition is more favourable than the other, however are still feasible reaction pathways. Consequently, this supports the postulation that **102** is a likely intermediate.

The remaining three experiments were undertaken to provide more insight into the exact steps along the reaction pathway. Firstly, **102** was heated in ethanol (Scheme 5.5, Condition B). Analysis of the reaction mixture using TLC showed only starting material. This suggests that, if **102** was part of the reaction pathway, dimerization with itself does not occur to form Compound **100**, and that the presence of lawsone and/or the glycine is still required. Secondly, glycine and **102** were reacted in a 1:2 stoichiometric ratio in ethanol (Scheme 5.5, Condition C). A yellow compound was isolated from the reaction, that exhibited very similar ^{1}H NMR shifts to **102** but missing the vinyl proton at 6.0 ppm, suggesting a substituent at this position (Figure 5.3). The only logical reaction would be the addition of a -NH_2 to give 2,3-diamino-1,4-naphthoquinone (**183**, Figure 5.4), however the singlet at 4.96 ppm pertaining to the -NH_2 at the 2- position integrates for two protons instead of four in relation to the aromatic signals integrating for one proton each. Importantly, a red compound could not be isolated from the reaction mixture, indicating that the addition of lawsone is fundamental to forming Compound **100**.

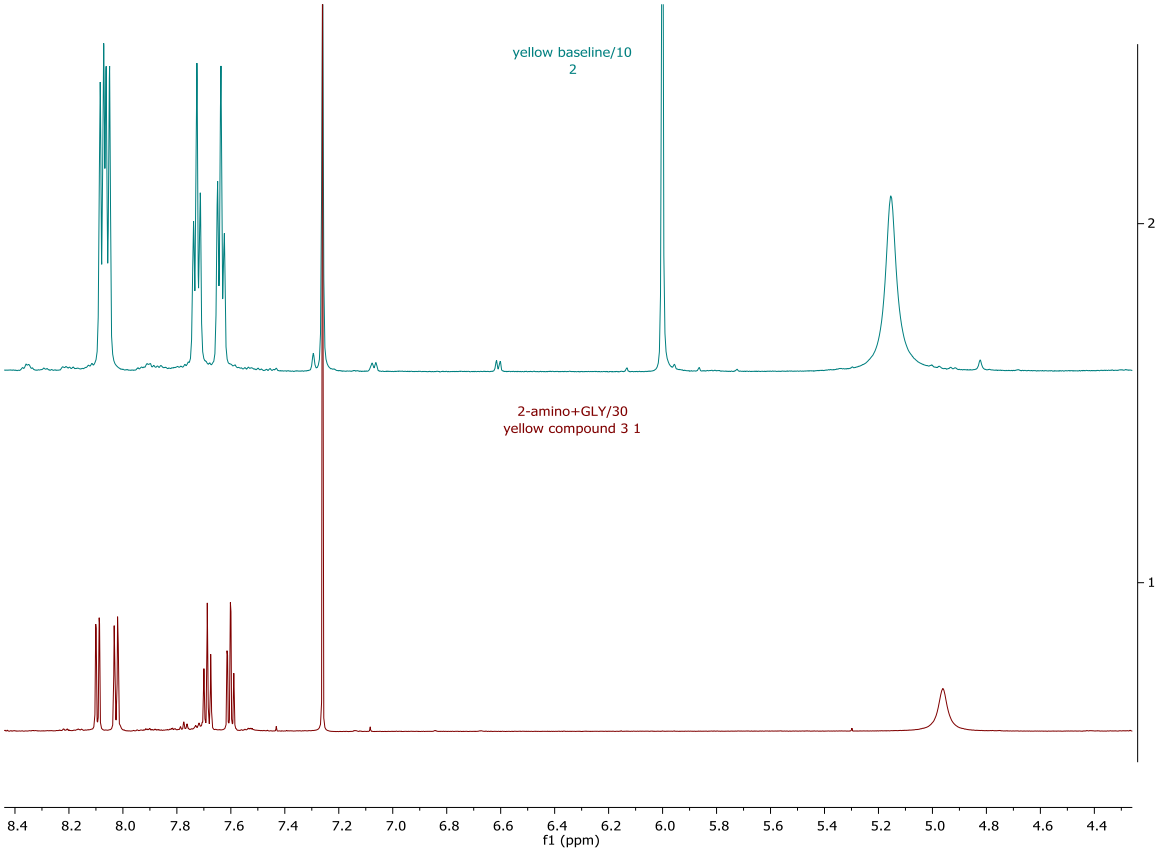
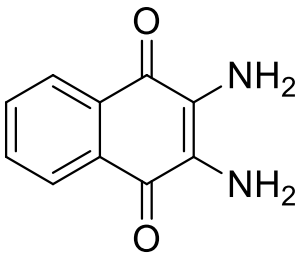


Figure 5.3 ^{1}H NMR comparison **102** (top, green) and the yellow product from the reaction between **102** and glycine in ethanol (bottom, red).

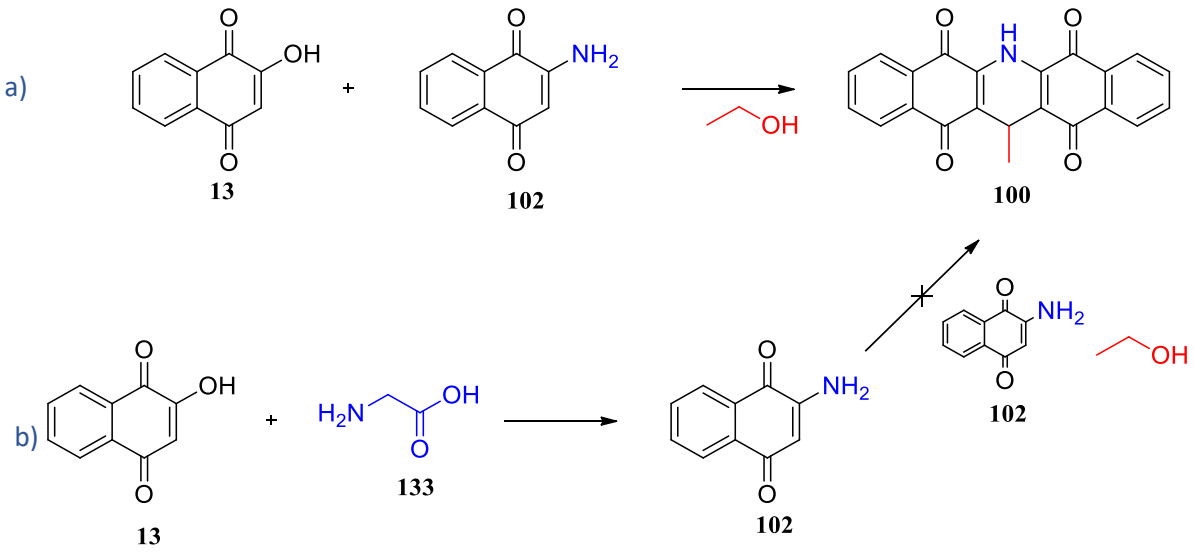


183

Figure 5.4 Structure of 2,3-diamino-1,4-naphthoquinone, suspected but not confirmed to have been isolated from the reaction between **102** and glycine

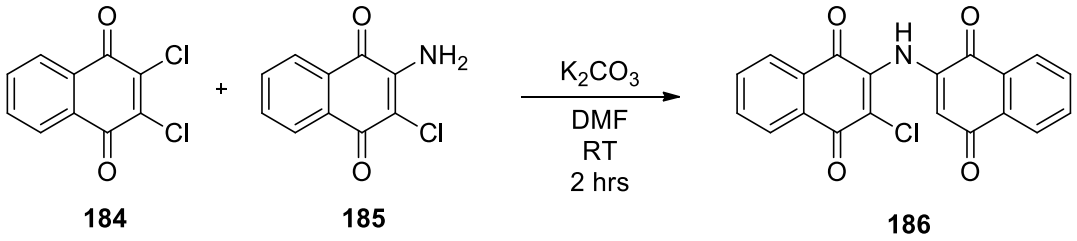
Finally, a similar reaction with glycine, lawsone, and **102** was performed in a 1:1:1 stoichiometric ratio (Scheme 5.5, Condition D), where a red fraction was isolated. Due to the small amount of product and significant presence of impurities in the ^{1}H NMR spectrum obtained, Compound **100** could not be confirmed to be present. It is likely that, if Compound **100** did form, it formed in yields difficult to

detect due to hinderance from possible competing reactions (Scheme 5.6). From previous experiments performed under Condition A, one molecule of lawsone must condense with one molecule of **102** to produce Compound **100**. However, the lawsone is also reacting with the glycine to form more **102**. Conditions B and C suggest that **102** cannot dimerise to form Compound **100**, and therefore if the lawsone was being used up by reacting with glycine, there would not be available lawsone to condense with the existing **102**.



Scheme 5.6 Potential competing reactions occurring under Condition D. a) Lawsone and **102** can condense to form Compound **100**, however b) lawsone is also being consumed to form **102** which cannot dimerise to form Compound **100**.

The results from experiments performed under Conditions A-D provide supporting evidence for the formation of **102** as an intermediate. They also suggest that the mechanism requires condensation of a lawsone molecule with this **102** intermediate, and Compound **100** cannot form via dimerization between two of the same molecule. Revisiting the amine pathway in Scheme 5.4, a condensation between lawsone and **102** is likely to form the NH-linked intermediate **104**, possibly prior to any alkyl insertion at the vinyl position. An attempt was made to synthesise such an intermediate, adapted from the work of Bittner *et al.*[5] In their work, Bittner *et al.*[5] synthesised N,N-Bis(3-chloro-1,4-naphthoquinonyl)amine (**186**) (Scheme 5.7) using 2,3-dichloro-1,4-naphthoquinone (**184**) and 2-amino-3-chloro-1,4-naphthoquinone (**185**) in the presence of potassium carbonate in DMF.



Scheme 5.7 Synthesis of **186** via a substitution reaction between **184** and **185**.

The above synthesis was repeated in this work using 1,4-naphthoquinone and **102** in order to synthesise suspected intermediate **104**. The desired **104** was not isolated from the reaction mixture, however a red compound was. ^{1}H, ^{13}C, and 2D NMR was performed on this red compound, where it was determined from the ^{1}H NMR spectrum (Figure 5.5) that the structure of this compound contained a possible fourteen proton environments. A broad singlet at 8.68 ppm integrating for one proton is observed, with a similar shift and peak shape indicative of a secondary -NH environment. A singlet at 5.30 ppm integrating for one proton is present, postulated to be a vinyl proton. The ^{1}H NMR spectrum also contains two sets of triplets and two sets of doublets in the aromatic region, assumed to be signals from the naphthoquinone skeleton. The doublets at 8.14 and 8.61 ppm integrate for one proton each and appear to be structurally related to the triplets at 7.84 and 7.96 ppm, also integrating for one proton, due to their similar peak height and one-to-one integration ratios. The doublets at 7.88 and 8.18 ppm integrate for one proton each and appear to be structurally related to the overlapping triplets at 7.75 and 7.72 ppm, integrating for two protons each. These sets appear in the spectrum with similar peak heights to each other with a one-to-two integration ratio, however the peak heights are slightly larger and the integration ratios slightly higher than the previous sets. This pattern may indicate the presence of two structurally similar compounds in the isolated fraction that were unable to be separated using a combination of recrystallisation and silica-gel chromatography. It is also possible that this is indicative of a complex polymeric structure consisting of similar proton environments, where assigning any peak in the spectrum an integration of one proton will vastly underestimate the true number of protons in the structure. Singlets at 2.63, 2.92, and 3.42 ppm, as well as a doublet at 2.18 ppm, may also be signals originating from a more complex structure. Due to the integration of these peaks being significantly lower than those previously mentioned, this supports the proposal of a much larger polymeric structure if these signals originate from the one compound.

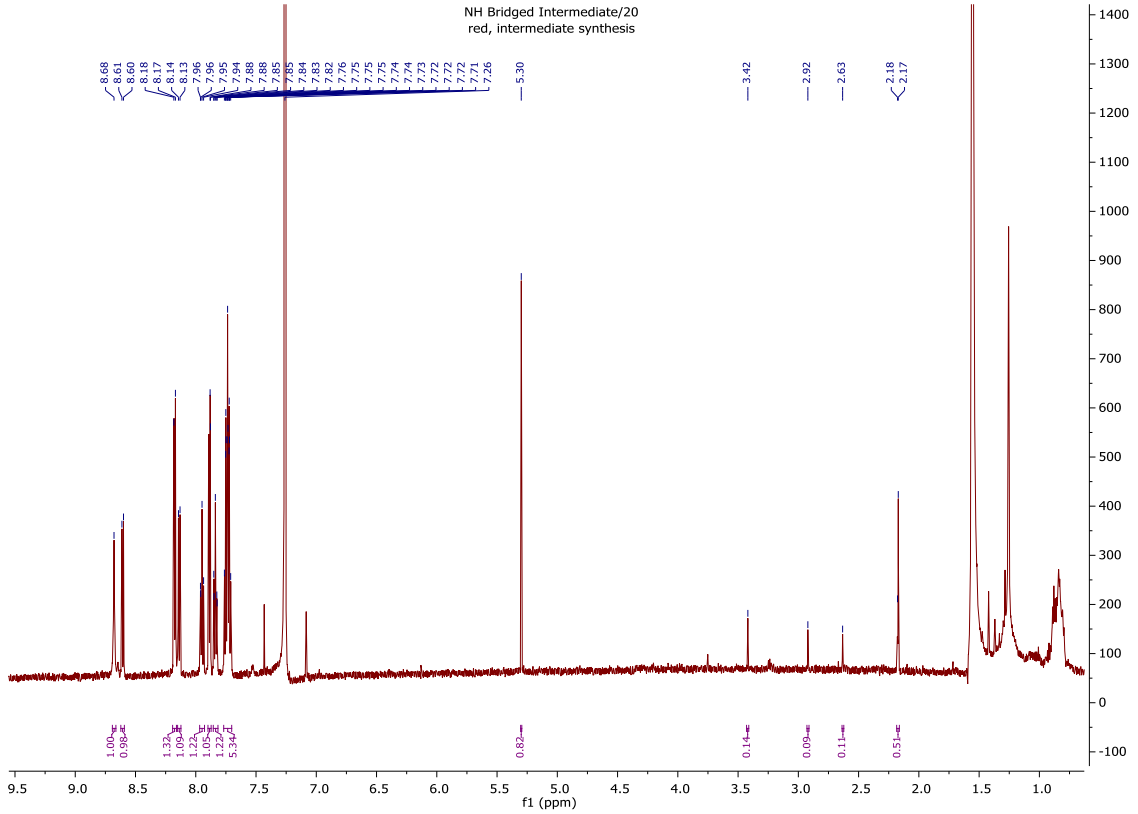


Figure 5.5 ^{1}H NMR spectrum of the red compound isolated from the attempted synthesis of **104**.

The ^{13}C NMR spectrum (Figure 5.6) indicates that there are a possible 53 unique carbon environments in the sample. Given that there were only fourteen proton environments observed, significantly lower than the number of carbon environments observed, this further suggests the presence of a structurally complex polymer, or the presence of a mixture of compounds in the sample. COSY, HSQC, and HMBC spectra were collected (Appendix 4), however provided little insight into the structural identity. This was due to broad peaks that overlapped several ^{1}H and ^{13}C signals and made it difficult to assign specific correlations.

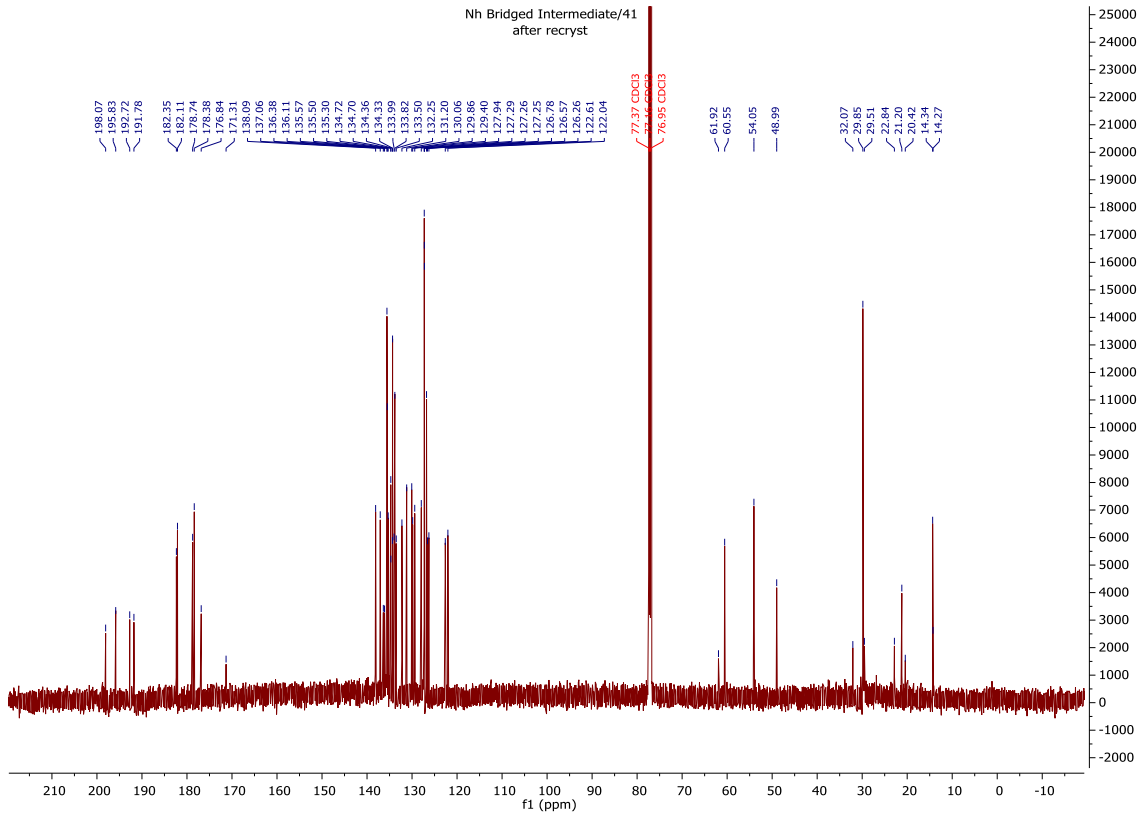


Figure 5.6 ^{13}C NMR spectrum of the red compound isolated from the attempted synthesis of **104**.

To clarify whether the structure was polymeric in nature, a mass spectrum was obtained using a DSA TOF MS in positive mode, scanning between 90 and 1000 amu. Five peaks of interest at m/z = 123.08, 139.07, 227.32, 427.38, ad 663.45 were observed in the resulting spectrum (Figure 5.7) and are summarised, along with potential molecular formulas, in Table 5.1. It is assumed from the ^{1}H NMR data that a -NH environment, naphthoquinone skeleton, and a vinyl proton environment are present. Therefore, only formulas containing carbon, hydrogen, nitrogen, and oxygen atoms were considered. The possibility of common adducts such as Na+ or MeOH+ were not considered as adducts are difficult to assign to unknown structures with certainty. The assumed peak with the largest m/z = 663.4541 returned two formulas within an appropriate error range, however neither formula is reflective of the 53 carbon environments observed in the ^{13}C NMR spectrum. This would suggest that, if the sample is a pure compound, then the true mass of the compound is well above 1000 amu and the masses that are present in Figure 5.7 are only fragments of a larger structure. As a result, a [M+H] could not be assigned to the red compound.

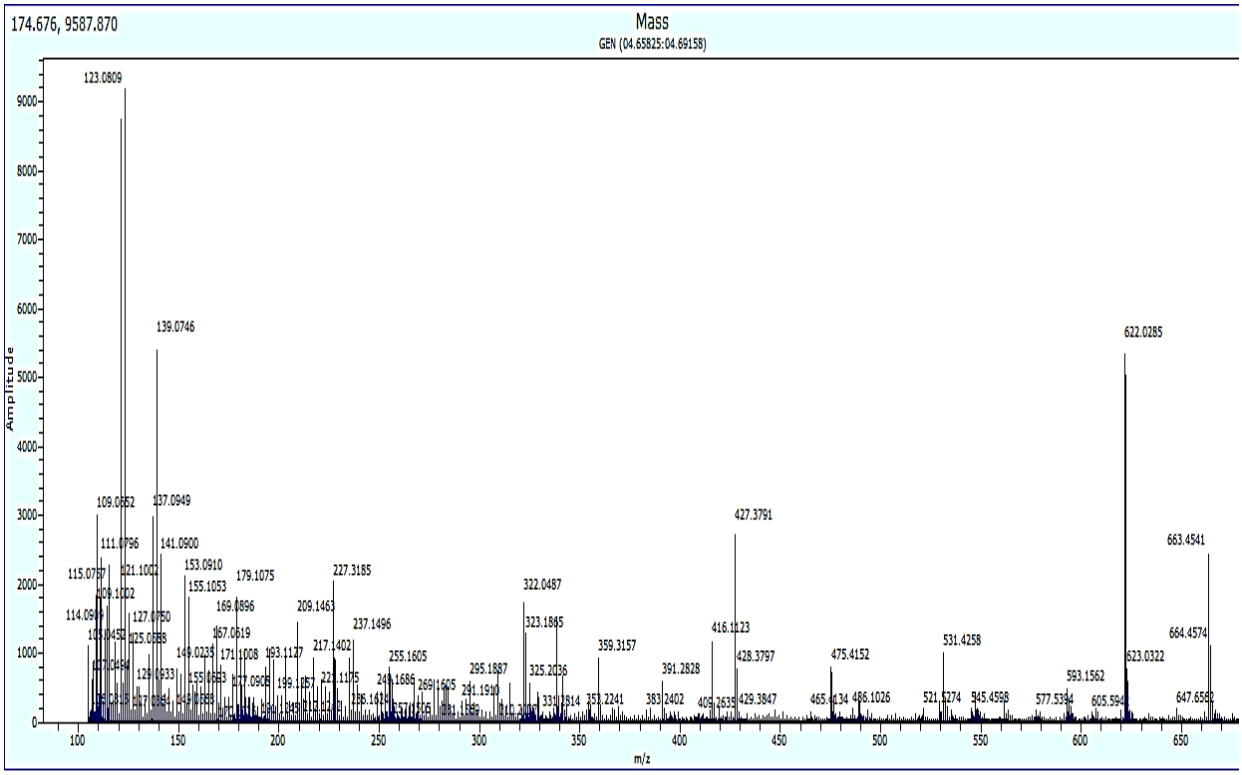


Figure 5.7 Mass spectrum of the red compound isolated from the attempted synthesis of **104**.

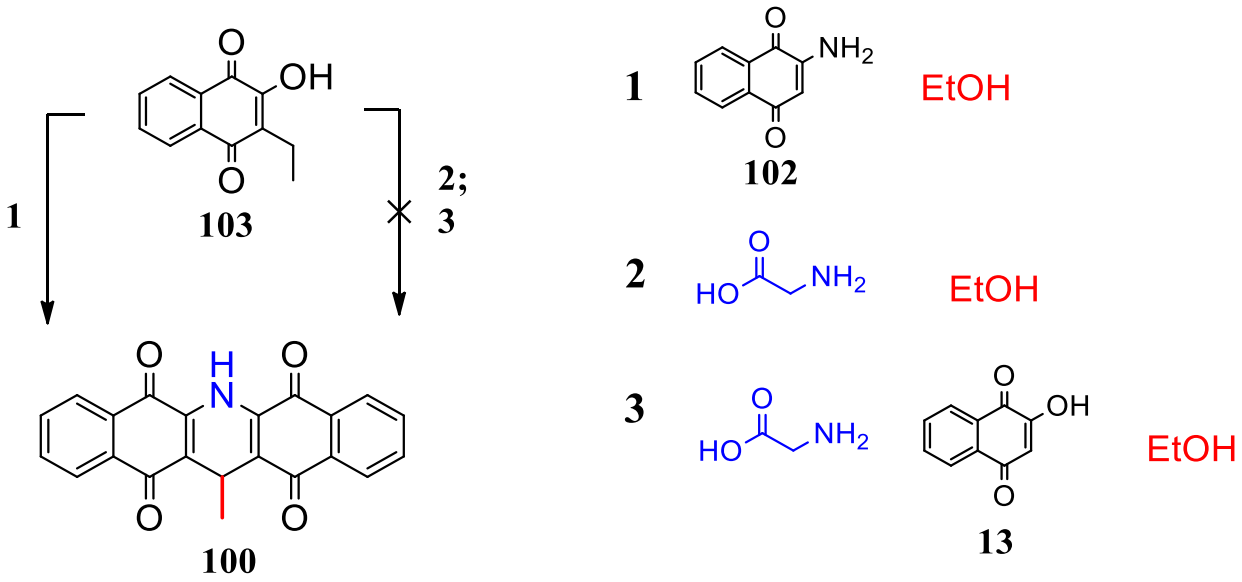
Table 5.1 Potential [M+H] or fragments originating from the attempted synthesis of **104**.

m/z (amu)	Molecular Formula	Error (ppm)
123.0809	$C_8H_{10}O$	-0.73
139.0746	$C_6H_8N_3O$	0.27
227.3185	$C_{13}H_{26}N_2O$	467.23
427.3791	$C_{24}H_{48}N_3O_3$	4
663.4541	$C_{31}H_{56}N_{11}O_5$	-0.47
	$C_{32}H_{62}N_4O_{10}$	-0.48

A structure could not be assigned to the red compound isolated from the attempted synthesis of **104**. However, this reaction provides opportunities for future investigations into the identity of the red product, as well as research into a synthesis pathway towards the desired amine linked intermediate. Due to the unsuccessful synthesis of **104**, the feasibility of this compound being an intermediate along the reaction pathway towards Compound **100** could not be verified.

5.2.2. Investigation into 103 as an intermediate

In Scheme 5.4, the alkyl pathway starts with the attachment of the ethanol at the 3-position of lawsone to form intermediate **103**. Intermediate **103** can either react with lawsone to form the alkyl linked dimer **106**, or could undergo amination with glycine to form the 2-amino-3-ethyl-1,4-naphthoquinone intermediate **105**. These possibilities involving the alkyl pathway were investigated in a similar manner to the amine pathway, where intermediate **103** was synthesised according to established methods[10,11] and subjected to Conditions 1-3 (Scheme 5.8). Condition 1 was performed to determine if condensation between lawsone and **102** was still possible with ethyl attachment at the 3-position. Condition 2 replicated the possibility of some conversion of **103** to the ethylated Compound **102**, followed by condensation with ethyl attachment at the 3-position on both molecules. Condition 3 tested conversion of either lawsone or **103** to Compound **102** or the analogous ethylated intermediate, followed by condensation.



Scheme 5.8 Experiments performed using varying stoichiometry of **103**, **102**, lawsone, glycine, and ethanol to determine whether **103** is a potential intermediate.

103 was initially reacted with **102** in a 1:1 stoichiometry using microwave conditions (Scheme 5.8, Condition 1). The aim of this reaction was to determine if the alkyl linker could be inserted prior to dimerization, or whether the dimer was needed to form first which then facilitated alkylation at the 3- position. Analysis of the crude mixture using TLC showed mostly starting material and evidence of a red compound. This red compound was isolated in a less than 1% yield from the crude mixture and confirmed to be Compound **100** using ^{1}H NMR. This indicates that the alkylation of the lawsone prior

to condensation is plausible but does not eliminate any reaction pathways outlined in Scheme 5.4. This reaction using Condition 1 (Scheme 5.8) appeared to produce a cleaner reaction, unlike other reactions involving lawsone and glycine that produced a purple precipitate containing Compound **100** and a variety of other compounds. This may be indicative that this is a more favoured pathway, although all suggested in Scheme 5.4 are possible.

To more closely mimic the original reaction conditions outlined in Chapter 2, **103** was reacted with glycine in a 2:1 stoichiometry in ethanol (Scheme 5.8, Condition 2). A yellow and red compound were isolated from the reaction mixture using DCM with 1% TEA on silica gel. ^{1}H NMR analysis of the yellow compound suggested it to be starting material (Figure 5.8). ^{1}H NMR analysis of the red compound showed that Compound **100** was not present, however it appeared to indicate a mixture of the starting material and **105** (Figure 5.9). **105** has been synthesised in literature by Ashok and Ilangovin[6] and while the splitting patterns and coupling of the signals are consistent, the shifts reported in this work are 0.2-0.6 ppm upshifted in comparison to literature (Table 5.2). The presence of the **105** suggests that amination is able to occur, however subsequent condensation is prevented. In combination with the successful production of Compound **100** from the reaction under Condition 1, these results suggest that **102** must be formed prior to addition of the alkyl linker, otherwise condensation to Compound **100** cannot occur.

Table 5.2 Comparison of observed ^{1}H NMR values and those reported by Ashok and Ilangovin[6] for **105**. All δ are reported in ppm.

δ_H ($CDCl_3$) [a] Observed	δ_H ($CDCl_3$) [a] Reported	$\Delta\delta_H$ (ppm)
8.45, d (8.2); 1H	8.02, d (7.6); 1H	-0.43
8.2, d (8.5); 1H	8.01, d (8); 1H	-0.19
7.63, t (7.5); 1H	7.70, t (7.6); 1H	0.07
7.54, t (7.8); 1H	7.60, t (7.6); 1H	0.06
5.6, bs; 1H	5.0, bs; 2H	-0.6
3.06, q (7.7); 2H	2.51, q (7.6); 2H	-0.55
1.37, t (7.6); 4H	1.12, t (7.6); 3H	-0.25

[a] δ_H, multiplicity (*J* in Hz); integration

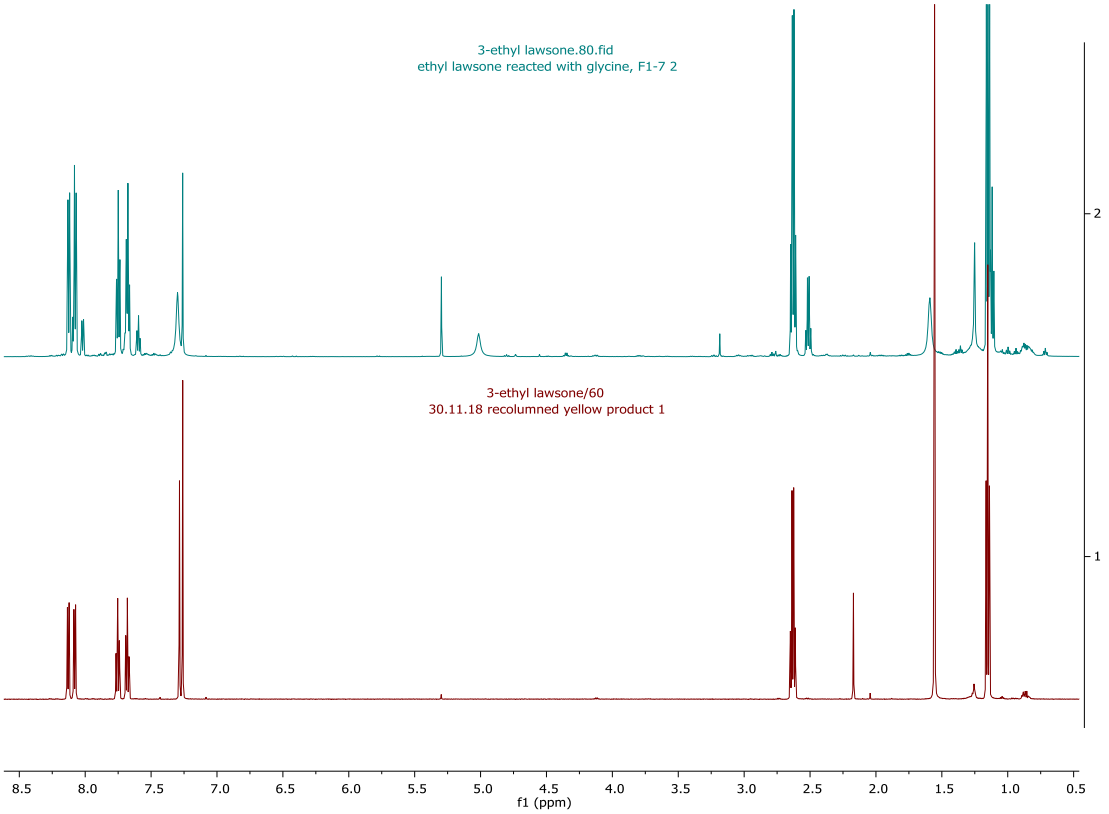


Figure 5.8 ^{1}H NMR comparison of the yellow product isolated at R_f = 0.34 in DCM (1% TEA) and **103**, confirming the return of starting material.

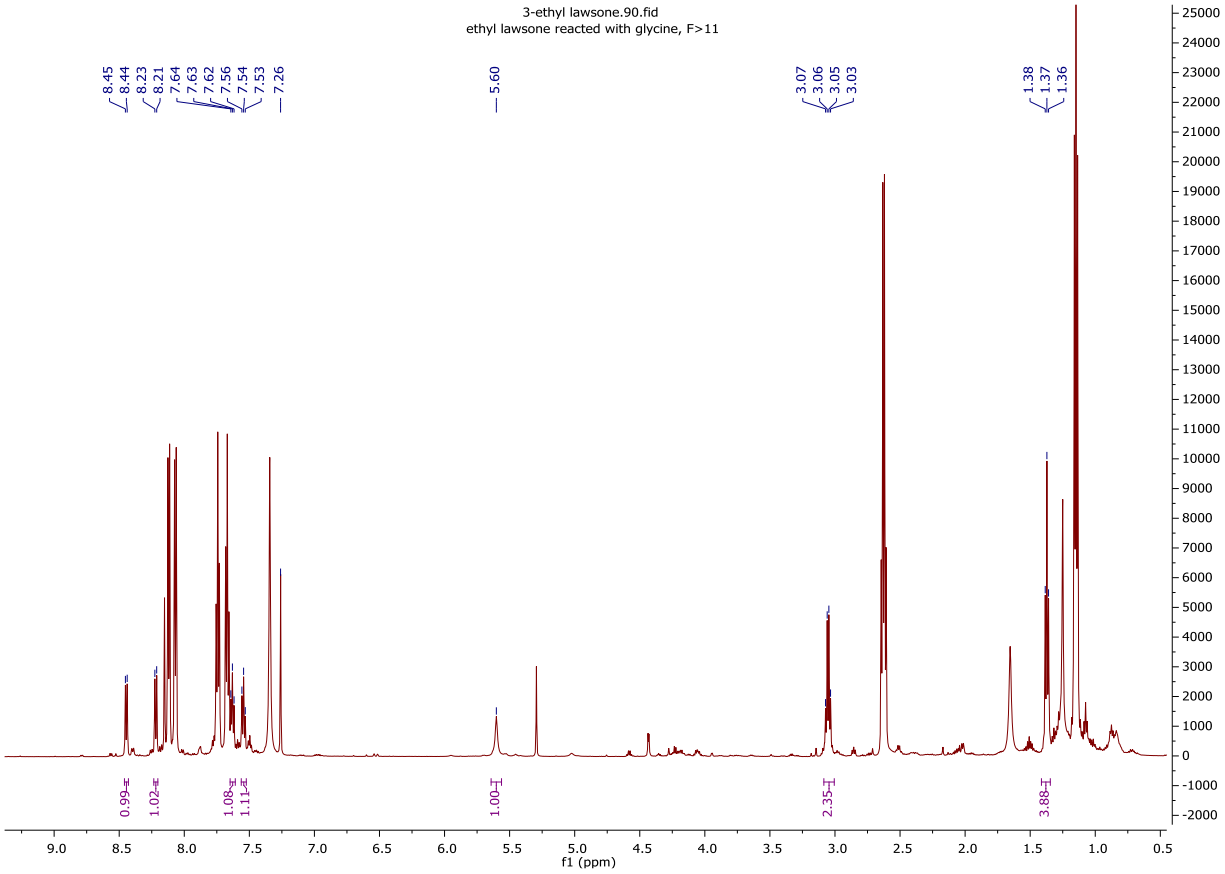


Figure 5.9 ^{1}H NMR spectrum of the red compound isolated at R_f = 0.34 from the reaction between **103** and glycine. Signals believed to originate from **105** are labelled.

The theory that condensation cannot occur between **103** and **105** was investigated by reacting **103** and lawsone with glycine in a 1:1:1 stoichiometry in ethanol (Scheme 5.8, Condition 3) using microwave conditions. Separation of the resulting crude precipitate was attempted, however proved unsuccessful due to co-elution or poor retention factors. ^{1}H NMR analysis of the crude precipitate (Figure 5.10) showed mostly starting material and no evidence of signals originating from Compound **100**. Signals believed to originate from **105**, identified from the reaction under Condition 2 (Scheme 5.8), are observable. It is suspected that, in a similar scenario to the reaction under Condition D (Scheme 5.5), competing reactions may have prevented the formation of Compound **100** in detectable amounts or entirely. Addition of the amino acid could occur on either the lawsone or **103**, leading to the production of both **102** and **105**. This presents the opportunity for four competing reactions to occur, which may facilitate or prevent the formation of Compound **100** (Figure 5.11).

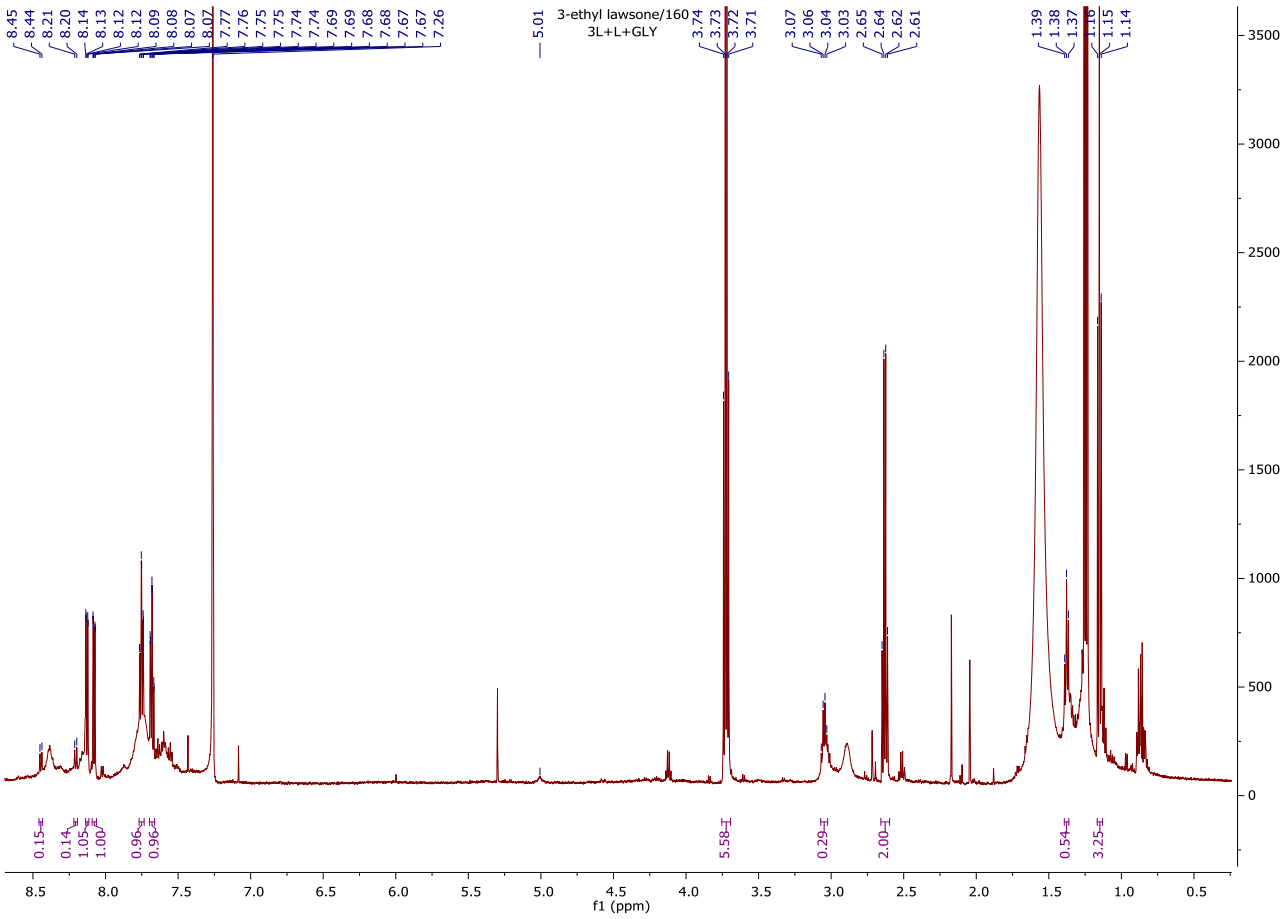


Figure 5.10 ^{1}H NMR of crude mixture from the reaction between **103**, lawsone, and glycine.

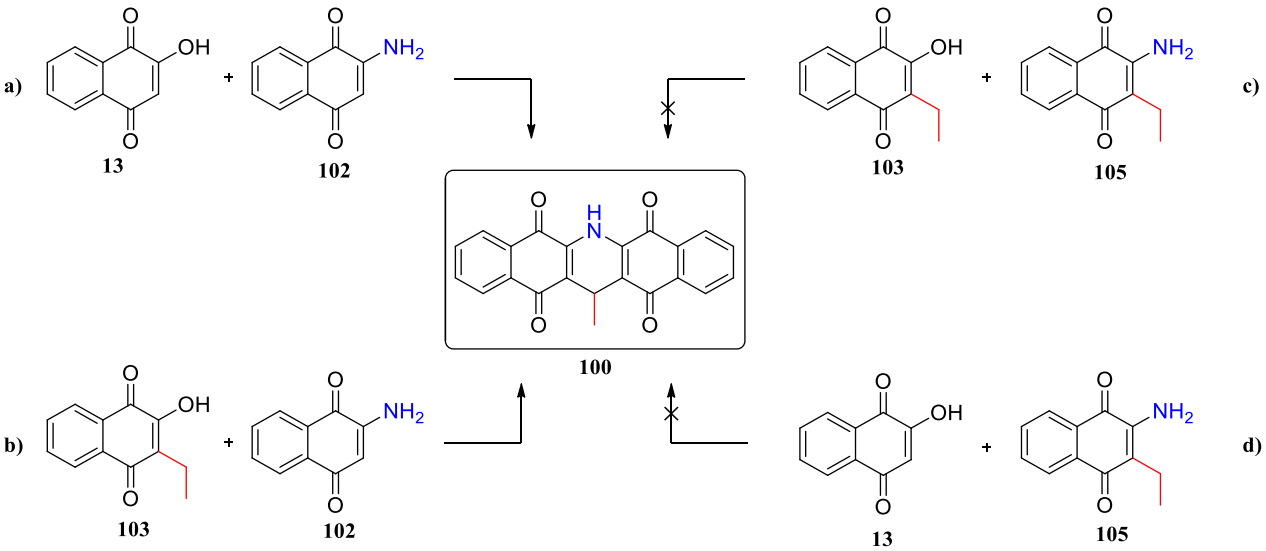
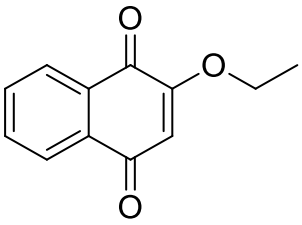


Figure 5.11 Possible competing reactions a) – d) in the reaction mixture between **103**, lawsone, and glycine in ethanol.

It is now known that reactions a) and b) in Figure 5.11 can form Compound **100** and are possible in this reaction mixture. However, it was also postulated that reactions c) and d) could occur in this reaction mixture. The reaction under Condition 2 (Scheme 5.8) suggested that reaction c) (Figure 5.11) is unable to produce Compound **100**, and therefore if this reaction was favoured, it is one explanation as to why Compound **100** was not observed. Additionally, it was postulated that reaction d) (Figure 5.11) was possible due to the detection of **105** in the crude ^{1}H NMR. As Compound **100** was not made, it can be inferred that this intermediate cannot react further with lawsone to produce Compound **100**. If the reaction under Condition 3 was capable of producing four competing reactions with only two likely to proceed towards Compound **100** (reactions a) and b), Figure 5.11), and given that Compound **100** did not seem to form, it is proposed that reactions c) and d) (Figure 5.11) were more favoured under these conditions. Modelling of **105** indicated that this intermediate is slightly more energetically stable with a lower calculated Gibbs free energy of -669 au when compared to the **102** intermediate with a Gibbs free energy calculated to be -590 au (Appendix 4). This is a possible reason as to why **105** was preferentially formed, however more investigation into the activation energy and transition states of either intermediate formation is required.

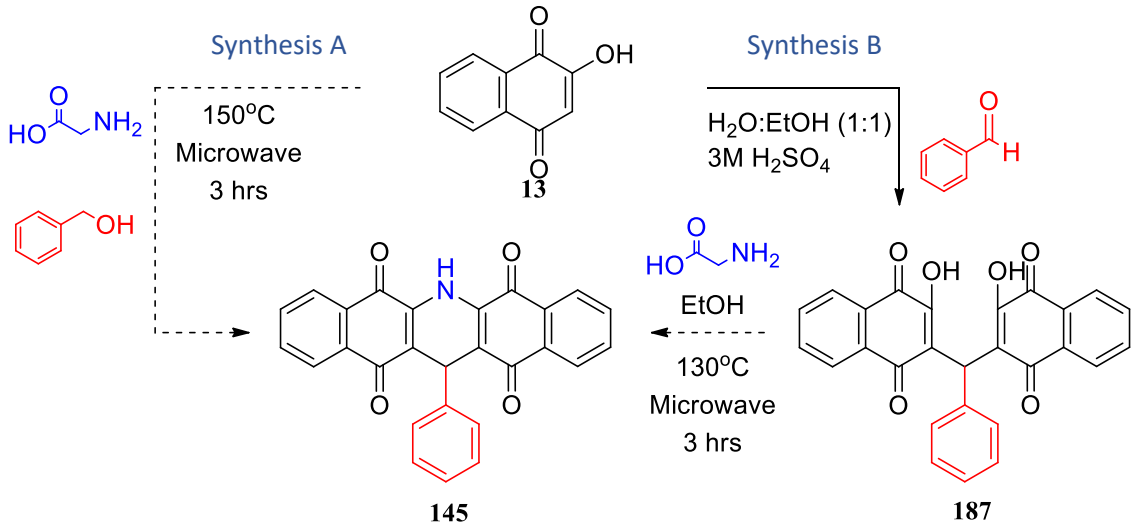
A reaction under microwave conditions between lawsone and ethanol was performed to determine how the postulated **103** intermediate is formed. The crude mixture was separated using silica gel chromatography, where three fractions were isolated. Two yellow fractions were obtained as inseparable mixtures in less than 10 mg yield and could not be identified using ^{1}H NMR. However, no shifts were observed corresponding to **103**. A red compound **148** was isolated, where signals at 8.13, 8.08, 7.76-7.69, 6.15, 4.32, and 1.53 ppm were observed in the ^{1}H NMR spectrum (Appendix 4). The aromatic signals at 8.13, 8.08, and between 7.69 and 7.76 ppm, integrating for a total of four protons, are consistent with those observed in the ^{1}H NMR spectrum for lawsone. Additionally, a singlet integrating for one proton at 6.15 ppm is observed in the ^{1}H NMR spectrum for **148** and is consistent with a vinylic proton. A triplet integrating for three protons is present at 1.53 ppm, while a quartet integrating for two protons is observed at 4.61 ppm. These signals are indicative of a primary ethyl chain. The ^{1}H NMR spectrum for **148** suggests a structure shown in Figure 5.12, where an ethoxy is now present at the 2- position. This data is consistent with that previously reported by Valente *et al*.[7]



148

Figure 5.12 Postulated structure of **148**, isolated from the reaction of lawsone with ethanol.

The reaction of a lawsone analogue with glycine in Chapter 4 showed that the amine linker is inserted at the -OH sites of both lawsone molecules, while the alkyl linker is inserted at the vinyl position of both lawsone molecules. While the reaction sites have been confirmed, this does not eliminate either of the three possible pathways in Scheme 5.4. The alkyl pathway proposes complete formation of the alkyl linker prior to insertion of the amine linker, where alkyl linked intermediate **106** was suggested as an intermediate. To investigate the possibility of completely forming the alkyl linker prior to amination at the OH position, an alkyl linked intermediate similar to **106** was synthesised that could subsequently be reacted with glycine. Khurana *et al.*[8] and Tisseh and Bazgir[9] have reported synthesising such 3,3-(Arylmethylene)bis(2-hydroxynaphthalene-1,4-diones) by heating lawsone with the appropriate aromatic aldehyde in the presence of H_2SO_4 under reflux until reaction completion. The synthesis of **187** was reported by Khurana *et al.*[8] and repeated in this work, using benzaldehyde and heated under reflux at 80^{o}C for one hour (Scheme 5.9). A yellow precipitate was filtered and washed with ethanol, where comparison of ^{1}H NMR spectra to that reported by Tisseh and Bazgir[9] confirmed it to be **187**.



Scheme 5.9 Two proposed synthesis methods for the formation of **145**. Synthesis A forms **188** from the microwave reaction between lawsone and glycine in benzyl alcohol. Synthesis B forms **188** via intermediate **187**, followed by reaction with glycine in ethanol under microwave conditions.

Postulated intermediate **187** was reacted with glycine in ethanol using microwave conditions in a 1:1 stoichiometry (Scheme 5.9). A red compound (R_f = 0.37) was isolated from the reaction mixture using silica gel chromatography with a 80:20 hexane:ethyl acetate solvent system. The ^{1}H NMR spectrum of this compound exhibited shifts at 8.46 ppm, 8.13 ppm, 8.06 ppm, 7.7-7.67 ppm, 7.48-7.44 ppm, and 5.72 ppm, where it was expected that **145** was formed (Figure 5.13). The broad singlet at 8.45 ppm integrating for one proton likely correlates to the secondary -NH (expected integration of one), while the aromatic signals integrating for a total of thirteen protons (expected integration of thirteen) likely correlate to the benzyl linker and aromatic protons of the naphthoquinone ring. The singlet at 5.72 ppm integrating for one proton (expected integration of one) is suspected to originate from the -CH group of the alkyl linker. **145** was postulated to be formed from a reaction between lawsone and glycine in benzyl alcohol in Chapter 3, but its structure could not be conclusively confirmed using ^{1}H NMR. A comparison between the ^{1}H NMR spectra of the products isolated are shown in Figure 5.13 and Table 5.3.

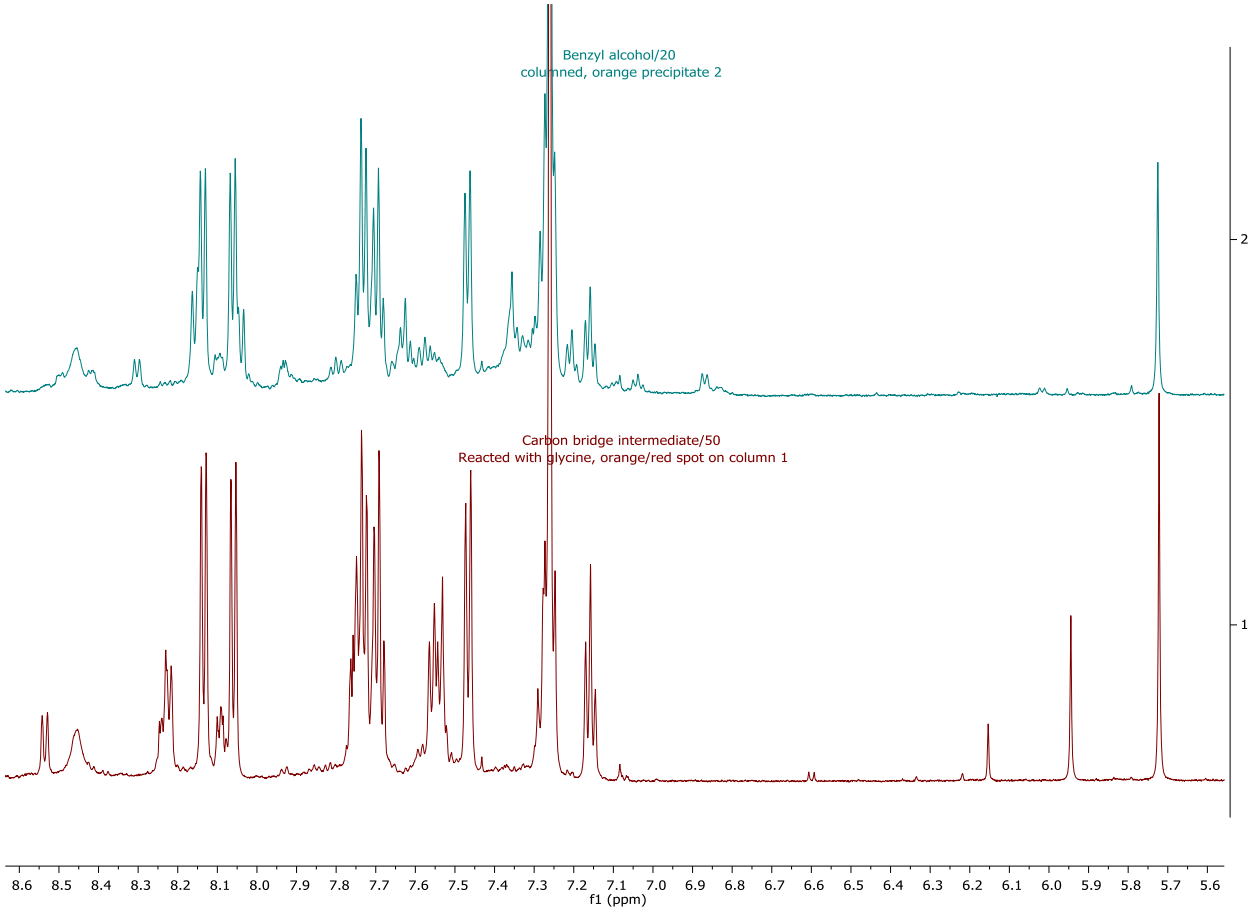


Figure 5.13 ^{1}H NMR spectrum of the red compound isolated from the reaction between **187** and glycine in ethanol, expected to be **145**.

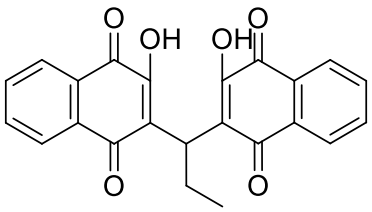
Table 5.3 Comparison of observed ^{1}H NMR values for Compound **145**, formed using Synthesis A or Synthesis B in Scheme 5.9. All δ are reported in ppm.

δ_H ($CDCl_3$) [a] Synthesis A	**δ_H ($CDCl_3$) [a] Synthesis B**	**$\Delta\delta_H$**
8.46, s; 1H	8.46, s; 1H	0
8.13, dd (7.6, 1.4); 2H	8.14, dd (12.3, 7.6); 3H	0.01
8.06, dd (7.6, 1.3); 2H	8.07, dd (12.9, 7.6); 3H	0.01
7.77 – 7.67, m; 7H	7.74, t (7.6); 4H 7.69, t, (7.5); 2H	-
7.48 – 7.44 (m, 2H)	7.47, d (7.7); 2H	-
5.72, s; 1H	5.73, s; 1H	0.01

[a] δ_H, multiplicity (*J* in Hz); integration

The ^{1}H NMR spectra (Figure 5.13) of **145** produced from both methods are very similar, differing by presence of impurities and the integration and J-coupling of the aromatic region (Table 5.3). The aromatic region for **145** from Synthesis B exhibits integration for thirteen protons (expected integration of thirteen protons), while the aromatic region for **145** from Synthesis A exhibits integration for fourteen protons. The ^{1}H NMR spectrum for **145** using Synthesis A also contained a signal at 3.94 ppm, and it was postulated in Chapter 3 that either this shift or the shift at 5.73 ppm could be the -CH group. This experiment seems to suggest that the shift at 5.73 ppm pertains to this -CH, and the shift at 3.94 ppm originates from an impurity.

The ^{1}H NMR spectra of suspected **145** resulting from both synthesis pathways appear consistent with each other, suggesting that the same structure has been formed in each pathway. However, the overlapping signals, impurities, and small inconsistencies in integration prevent the unambiguous assignment of a structure from these ^{1}H NMR spectra. A more straight-forward structure with a straight-chain alkyl linker was proposed to eliminate ambiguity from overlapping ^{1}H NMR signals. There was evidence in Chapter 3 to suggest that structure **140** was successfully isolated by reacting lawsone and glycine in the presence of 1-propanol. The synthesis method B for the formation of **187** was attempted to be adapted to form **188** (Figure 5.14) using propionaldehyde instead of benzaldehyde, however the desired product was not made and this experiment could not be repeated for structure **140**.



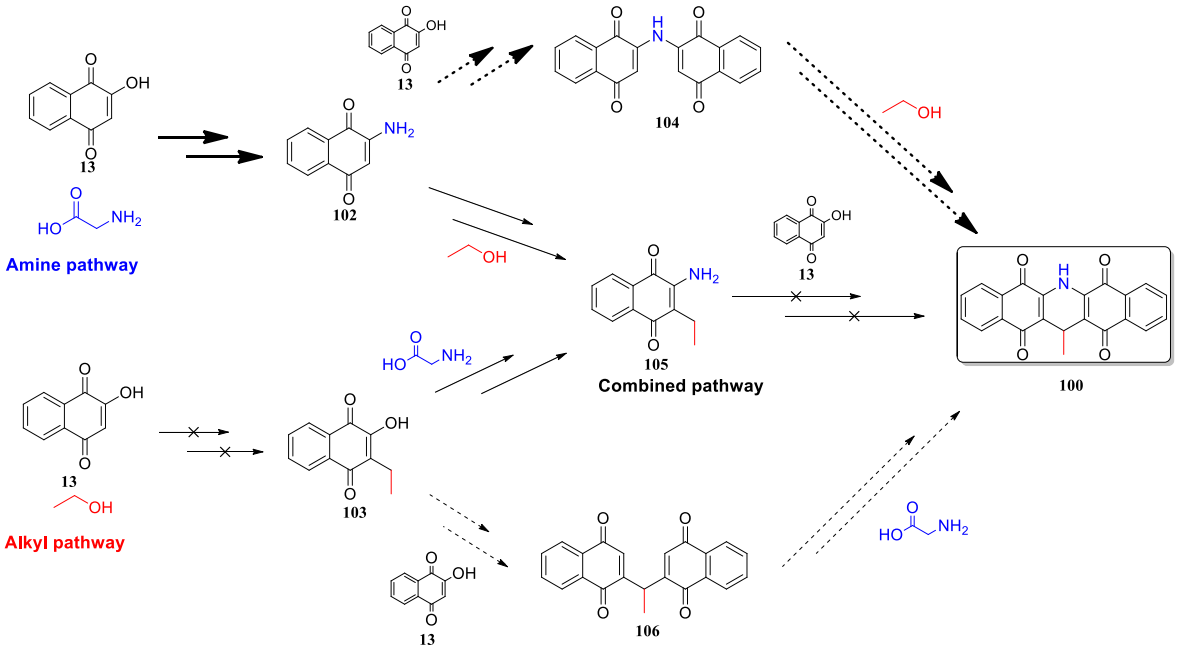
188

Figure 5.14 Structure of **188**, attempted to be synthesised from the reaction using propionaldehyde adapted from methods reported by Khurana *et al.*[8]

5.2.3. Reaction mechanism postulation

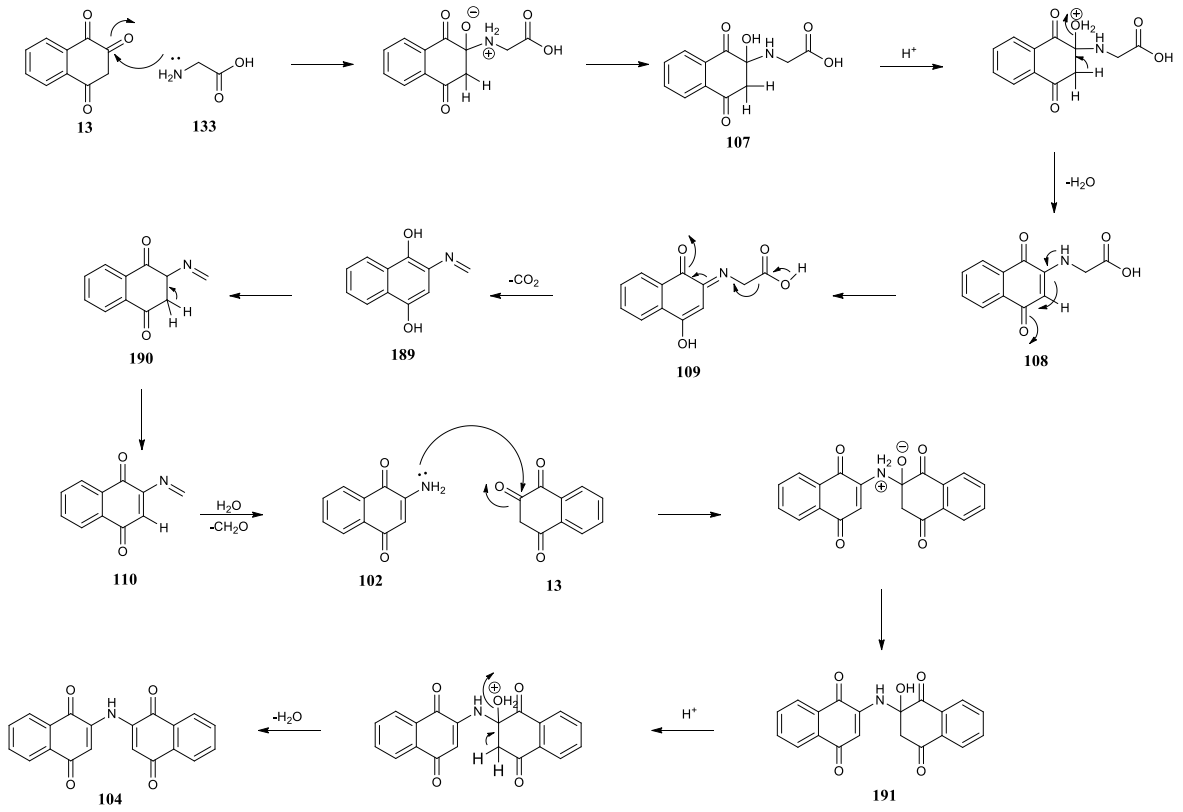
The outcomes from the reactions involving postulated intermediates **102** and **103** (Scheme 5.5 and Scheme 5.8) can be used to propose a likely reaction pathway towards the formation of Compound **100** (Scheme 5.10). Postulated intermediate **102** could be reacted with lawsone as well as **103** to form Compound **100**. **102** has already been isolated from the reaction mixture in Chapter 2, thereby providing evidence for its involvement along the reaction pathway. Intermediate **103**, however, has

not been isolated and could not be formed from the reaction between lawsone and ethanol. **103** also could not form Compound **100** in the presence of lawsone and glycine in ethanol. The possibility of reaction progression through an alkyl linked intermediate (**106**, Scheme 5.10) was also tested but no conclusive evidence was obtained. All steps proposed along the alkyl pathway were therefore eliminated as possibilities towards Compound **100** formation. The proposed combined pathway suggests an intermediate **105** (Scheme 5.10), which was postulated to be present in the crude reaction mixture of the reaction between **103** and glycine. However, Compound **100** was not formed from this reaction, nor could **105** be isolated from the reaction between **102** and ethanol. Subsequently, this pathway was also eliminated as a possibility.



Scheme 5.10 Revised proposed pathways, showing that reactions along the amine pathway are most likely to occur.

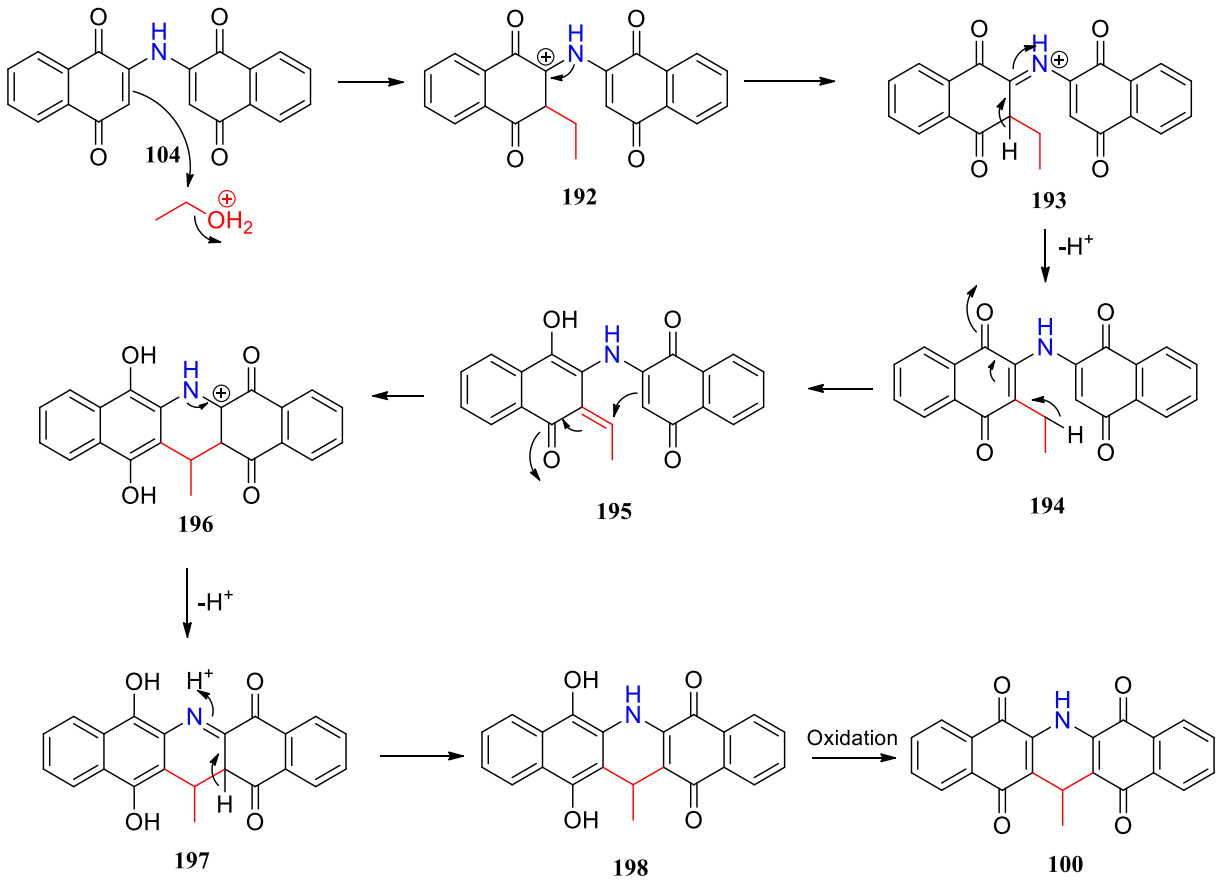
This research proposes that the amine pathway is the most likely reaction pathway towards Compound **100**. The first intermediate along this pathway, Compound **102**, is suspected to form via Strecker degradation between glycine and the 2-position of lawsone (Scheme 5.11). It is also proposed that intermediate **104** may be formed via condensation between **102** and lawsone (Scheme 5.11). These steps were initially proposed in Chapter 4 for the reaction of **150** with glycine upon the finding that the amine reacts at the C-2 position of both lawsone molecules.



Scheme 5.11 Proposed mechanism showing formation of **104**. Firstly, **102** is formed via Strecker degradation between lawsone and glycine. Finally, condensation occurs between **102** and another molecule of lawsone.

The final introduction of the alkyl linker is not so straight-forward, however a mechanism will be proposed. It is postulated the formation of the alkyl linker is driven by the conformation of the amine linked intermediate **104**, and why Compound **100** was not formed via reactions with **103**. A proposed mechanism for the incorporation of the ethanol is shown in Scheme 5.12, where the electrons across the C-2 and C-3 double bond initially partake in nucleophilic substitution with the δ-positive carbon of protonated ethanol to form the carbocation intermediate **192**. Stabilisation and proton abstraction of the carbocation intermediate **192** allows for the formation of intermediate **195**, which may then undergo intramolecular ring cyclisation to form the alkyl linker in **196**. It is proposed that this intramolecular step is highly favourable for formation of Compound **100**, and occurs much faster and easier than a reaction between intermediate **105** and lawsone, hence why this intermediate was observed but did not form Compound **100**. A second proton abstraction, followed by an oxidation step of intermediate **198**, allows for eventual formation of Compound **100**. It should be noted that the mechanism shown in Scheme 5.12 is merely a proposal, and requires further investigation into its validity. A mechanism similar to this was tested in Chapter 3 through the reaction of ethyl iodide with

glycine and lawsone, where it failed to produce **100**. It is theorised that the ethyl iodide reacted too quickly before **104** could be formed, therefore the pathway towards **100** formation could not occur.



Scheme 5.12 Postulated mechanism for the inclusion of ethanol to form the alkyl linker of Compound **100**, from intermediate **104**.

5.3. Conclusions

This chapter detailed the use of proposed intermediate **102**, and successful synthesis and use of proposed intermediate **103**. It also details the attempted synthesis of proposed intermediates **104** and **106**.It was determined that **102**, originally isolated from the reaction mixture in Chapter 2, was a likely intermediate along the reaction pathway. **102** was able to undergo a condensation reaction with lawsone to successfully produce Compound **100** in yields comparative to those found using lawsone and glycine in a microwave. The attempted synthesis of the amine linked dimer **104** was unsuccessful, and therefore further investigation into its feasibility as an intermediate is required. Reactions using the synthesised **103** could form Compound **100** when **102** was present. However, it is considered an unlikely intermediate due to its failure to be produced upon the reaction of lawsone with ethanol only. The alkyl linked intermediate **145** was also synthesised, however did not provide conclusive evidence for Compound **100** formation. Evidence for a combined pathway was observed through the

identification of **105**. Compound **100** was not formed when this intermediate was observed, therefore suggesting that the inclusion of each linker to one lawsone molecule as an initial step prevents dimerization. The alkyl and combined pathways were eliminated based on this evidence, subsequently leaving the amine pathway as the most likely reaction pathway. It was also postulated that the poor yield of Compound **100** may be due to several competing side reactions that deplete the reagents required for formation.

A reaction mechanism was proposed for the formation of Compound **100**, where **102** is formed by Strecker degradation between lawsone and glycine, followed by condensation between **102** and lawsone to form the intermediate **104**. The alkyl linker is suspected to be inserted via nucleophilic substitution at one of the vinyl positions in **104**, followed by intramolecular cyclisation to produce Compound **100**.

5.4. Experimental

General experimental details regarding analytical protocol and equipment, as well as preparation of common reagents, can be found in Section 2.4.1. NMR spectra for the compounds listed in this Chapter not shown in Section 5.2 can be seen in Appendix 4.

5.4.1. Condition A

Ethanol (2 mL) was added to a mixture of **102** (0.1520 g, 0.85 mmol) and lawsone (0.1468 g, 0.87 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. A red solid **100** was isolated in 0.0084 g (2.8%) yield: R_f (8:2 HX:EtOAc) 0.36; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.29 (s, 1H), 8.15 (d, *J* = 7.7 Hz, 1H), 8.08 (d, *J* = 7.5 Hz, 2H), 7.78 – 7.75 (m, 2H), 7.70 (t, *J* = 7.5 Hz, 3H), 4.62 (q, *J* = 6.7 Hz, 1H), 1.29 (d, *J* = 2.9 Hz, 3H).

5.4.2. Condition B

Ethanol (2 mL) was added to **102** (0.0909 g, 0.52 mmol) and heated to 130°C for three hours in a microwave. The crude reaction mixture was analysed using TLC on silica pre-treated pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. Starting material was returned.

5.4.3. Condition C

Ethanol (2 mL) was added to a mixture of **102** (0.1925 g, 1.1 mmol) and glycine (0.0428 g, 0.57 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. An orange solid was isolated in 0.0059 g yield: R_f (8:2 HX:EtOAc) 0.19; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.09 (d, *J* = 8.1 Hz, 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 7.69 (t, *J* = 7.6 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 4.96 (s, 2H).

5.4.4. Condition D

Ethanol (2 mL) was added to a mixture of **102** (0.1023 g, 0.59 mmol), lawsone (0.1076 g, 0.62 mmol) and glycine (0.0428 g, 0.57 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. A red amorphous solid was isolated in 0.0029 g (1.4%) yield as a mixture of compounds: R_f (8:2 HX:EtOAc) 0.39.

5.4.5. Attempted synthesis of 104

DMF (5 mL) was added to a mixture of naphthoquinone (0.2372 g, 1.50 mmol), **102** (0.2903 g, 1.68 mmol), and potassium carbonate (0.1598 g, 1.16 mmol) and stirred at room temperature for three

hours. Cold hydrochloric acid (2%, 10 mL) was added to form a red precipitate. The precipitate was filtered and washed with cold water. Upon recrystallisation in glacial acetic acid, the precipitate was separated using silica gel chromatography using DCM as the solvent system to yield red amorphous solid in 0.2144 g yield: R_f (DCM) 0.70; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.68 (s, 1H), 8.61 (d, *J* = 7.8 Hz, 1H), 8.18 (d, *J* = 7.9 Hz, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.95 (t, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 1.6 Hz, 1H), 7.84 (t, *J* = 7.5 Hz, 1H), 7.77 – 7.70 (m, 5H), 5.30 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 198.07, 195.83, 192.72, 191.78, 182.35, 182.11, 178.74, 178.38, 176.84, 171.31, 138.09, 137.06, 136.38, 136.11, 135.57, 135.50, 135.30, 134.72, 134.70, 134.36, 134.33, 133.99, 133.82, 133.50, 132.25, 131.20, 130.06, 129.86, 129.40, 127.94, 127.29, 127.26, 127.25, 126.78, 126.57, 126.26, 122.61, 122.04, 77.37, 77.16, 76.95, 61.92, 60.55, 54.05, 48.99, 32.07, 29.85, 29.51, 22.84, 21.20, 20.42, 14.34, 14.27.

5.4.6. Condition 1

Ethanol (2 mL) was added to a mixture of **102** (0.0431 g, 0.27 mmol) and **103** (0.0539 g, 0.25 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. A red solid **100** was isolated in < 10 mg yield: R_f (8:2 HX:EtOAc) 0.42; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.32 (s, 1H), 8.17 (d, *J* = 7.7 Hz, 2H), 8.13 (d, *J* = 7.6 Hz, 2H), 7.79 (dd, *J* = 8.1, 6.8 Hz, 2H), 7.72 (t, *J* = 7.5 Hz, 3H), 4.65 (q, *J* = 6.8 Hz, 1H), 1.32 (d, *J* = 6.8 Hz, 4H).

5.4.7. Condition 2

Ethanol (8 mL) was added to a mixture of **103** (0.8029 g, 4.0 mmol) and glycine (0.1515 g, 2.0 mmol) and heated to 130°C for three hours in a microwave. The resulting purple precipitate was dried under vacuum, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and DCM as eluent. A red fraction was isolated as a mixture of **105** and unknown compounds in a < 10 mg yield: R_f (DCM) 0.21; ^{1}H NMR (600 MHz, Chloroform-d) δ 8.45 (d, J = 8.2 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 8.12 (d, *J* = 7.7 Hz, 4H), 8.07 (d, *J* = 7.6 Hz, 4H), 7.63 (t, J = 7.5 Hz, 1H), 7.54 (t, J = 7.8 Hz, 1H), 5.60 (s, 1H), 3.05 (q, J = 7.7 Hz, 2H), 1.37 (t, J = 7.6 Hz, 3H).

5.4.8. Condition 3

Ethanol (8 mL) was added to a mixture of **103** (0.2618 g, 1.3 mmol), lawsone (0.2490 g, 1.4 mmol) and glycine (0.1147 g, 1.5 mmol) and heated to 130°C for three hours in a microwave. The crude reaction mixture was dried under vacuum and analysed using ^{1}H NMR. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.13 (d, *J* = 7.8 Hz, 4H), 8.08 (d, *J* = 7.6 Hz, 3H), 7.75 (t, *J* = 7.6 Hz, 4H), 7.68 (t, *J* = 7.5 Hz, 4H), 5.30 (s, 1H), 5.01 (s, 1H), 3.08 – 3.01 (m, 4H), 1.38 (t, *J* = 7.7 Hz, 4H).

5.4.9. Attempted synthesis of 103 using ethanol and lawsone

Ethanol (2 mL) was added to lawsone (0.1573 g, 1.1 mmol) and heated to 130°C for three hours in a microwave. The crude reaction mixture was dried under vacuum, and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system. A red solid (**148**) was isolated as a mixture in 0.0089 g yield: R_f (8:2 HX:EtOAC) 0.42; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.13 (d, *J* = 7.6 Hz, 1H), 8.08 (d, *J* = 7.5 Hz, 1H), 7.76 – 7.69 (m, 2H), 6.15 (s, 1H), 4.10 (q, *J* = 7.0 Hz, 2H), 1.53 (t, *J* = 7.0 Hz, 3H).

5.4.10. Preparation of Hantzsch ester, diethyl 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (118)

Water (30 mL) was added to mixture of formaldehyde 40% solution (0.61 mL, 6.7 mmol), ethylacetoacetate (3.4 mL, 27 mmol) and ammonium acetate (1.4358 g, 18 mmol). The solution was heated to approximately 80°C under reflux for 3 hours, where a yellow precipitate was formed. The reaction was cooled to room temperature and the yellow precipitate was filtered and washed with cold water and acetone. **118** was isolated as a yellow solid in a 1.523 g (90.3%) yield.[10] ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.12 (s, 1H), 4.16 (q, 4H), 3.26 (s, 2H), 2.19 (s, 6H) , 1.28 (t, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.17, 144.84, 99.75, 59.80, 24.94, 19.35, 14.62.

5.4.11. Synthesis of 2-hydroxy-3-ethyl-1,4-naphthoquinone (103)

Dichloromethane (15 mL) was added to a mixture of lawsone (1.0063 g, 5.7 mmol), acetaldehyde (0.64 mL, 11.4 mmol), and Hantzsch ester **118** (1.4968 g, 6.3 mmol). L-proline (0.1519 g, 1.1 mmol) was added with stirring to the mixture, and the resulting solution was stirred at room temperature overnight.[11] The mixture was separated using silica gel chromatography using gel pre-treated with 1% triethylamine and a 60:40 hexane:ethyl acetate solvent system to isolate yellow solid **103** in a 0.9664 g (83%) yield: R_f (6:4 Hx:EtOAc) 0.49; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.13 (d, *J* = 7.7 Hz, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.75 (t, *J* = 7.6 Hz, 1H), 7.68 (t, *J* = 7.5 Hz, 1H), 7.29 (s, 1H), 2.63 (q, *J* = 7.6 Hz, 2H), 1.15 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 184.71, 181.72, 152.89, 135.00, 133.01, 129.60, 126.92, 126.22, 77.37, 77.16, 16.92, 12.84.

5.4.12. Synthesis of 3,3'-(phenylmethylene)bis(2-hydroxynaphthalene-1,4-dione) (187)

A 50:50 mixture of ethanol and water (10 mL) was added to lawsone (0.3815 g, 2 mmol), benzaldehyde (0.1182 g, 1 mmol), and sulfuric acid (3M, 0.8 mL, 0.2 mmol) and heated to 80°C with stirring under reflux for an hour. The reaction was monitored by TLC using 60:40 ethyl acetate:hexane.[8] The resulting yellow precipitate (0.3270 g, 74.9 %) was filtered and dried under vacuum: ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.01 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.97 (d, *J* = 7.6 Hz, 4H), 7.93 (dd, *J* = 7.7, 1.3 Hz, 2H), 7.87 – 7.79 (m, 3H), 7.76 (t, *J* = 7.5 Hz, 2H), 7.22 – 7.16 (m, 2H), 7.11 (t, *J* = 7.0 Hz, 1H), 6.17 (s, 1H).

5.4.13. Attempted synthesis of 13-phenyldibenzo[b,i]acridine-5,7,12,14(6H,13H)-tetraone (145)

187 (0.1995 g, 0.46 mmol) was added to glycine (0.0229 g, 0.31 mmol) in ethanol (2 mL) and heated to 130°C in a microwave for three hours. The crude reaction mixture was dried under vacuum and compounds separated using silica gel column chromatography using gel pre-treated with 1% triethylamine and a 80:20 hexane:ethyl acetate solvent system to yield a red solid as a mixture of compounds in < 10 mg yield: R_f (8:2 HX:EtOAc) 0.37; ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.46 (s, 1H), 8.14 (dd, *J* = 12.3, 7.6 Hz, 3H), 8.07 (dd, *J* = 12.9, 7.6 Hz, 3H), 7.74 (t, *J* = 7.6 Hz, 4H), 7.69 (t, *J* = 7.5 Hz, 2H), 7.47 (d, *J* = 7.7 Hz, 2H), 5.73 (s, 1H), 3.94 (s, 1H).

5.5. References

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6. Conclusions and Future Directions

6.1. Conclusions

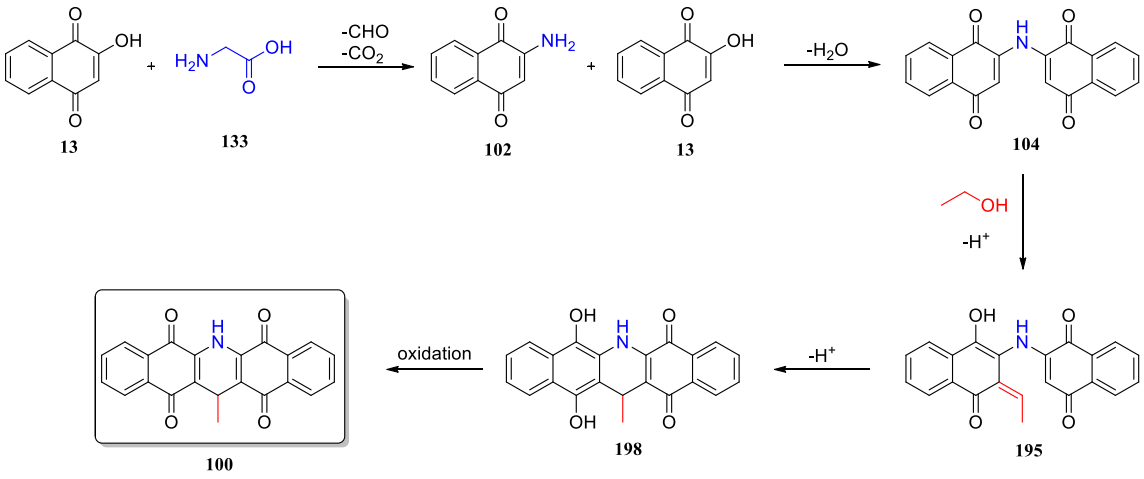
Two contrasting structures have been proposed to be responsible for the red fluorescence of fingermarks which was observed from the reaction of amino acids with lawsone.[1,2] Chapter 2 outlines the microwave assisted reaction between lawsone and glycine in ethanol and the isolation of the red fluorescent product **100**, newly reported potential by-product **101**, and postulated intermediate **102**. This work showed that both of the proposed structures given by Jelly *et al.*[1] (**18**) and Chan[2] (**21**) were incorrect. In contrast to the proposed structures, the red product **100** is composed of two lawsone molecules connected via an amine and ethyl linker. This structure was verified using existing literature spectra.[3]

Chapter 3 confirmed the involvement of glycine and ethanol in the reaction and that they are incorporated into the structure of **100** as the amine and alkyl linker respectively. Chapter 4 detailed the synthesis of a methoxy-substituted lawsone analogue **150**, which was reacted with glycine and ethanol to identify the reaction sites of these reagents. 2D NMR confirmed that the amino acid was incorporated at the 2- position of both lawsone molecules and the accompanying alcohol was incorporated at the 3- position of both lawsone molecules.

Finally, Chapter 5 detailed the reaction of isolated intermediate **102** and synthesised intermediate **103** with varying combinations of each other, lawsone, glycine and ethanol. The ability of these reactions to form compound **100** were subsequently used to propose a reaction pathway towards dimer formation (Scheme 6.1). It is proposed that glycine initially undergoes condensation with the lawsone at the 2-position, followed by Strecker degradation to give the intermediate **102**. **102** then undergoes condensation with a second lawsone molecule to completely form the amine linker **104**. Nucleophilic substitution of ethanol at the C-3 position of lawsone forms **195,** followed by intramolecular cyclisation to complete the formation of the alkyl linker in **198.** This then oxidises to form Compound **100**.

Currently, the reaction conditions between lawsone and amino acids produce a product **100** that is responsible for the observed fluorescence, but is not the favoured reaction product. Future lawsone formulations and associated fingermark development methods would therefore need to contain reagents and conditions that are conducive to the production of **100** for more replicable results. As summarised in Chapter 1 (Table 1.1), previous attempts at using lawsone formulations in ethyl acetate to elucidate latent fingermarks has resulted in very weak fluorescence or none at all. That **100** is not a favourable reaction product may explain these observations.

Understanding of this pathway may help to facilitate further optimisation of reagent conditions for use in the reliable and reproducible detection of latent fingermarks. In particular, the use of ethanol in the lawsone formulation to be used on latent fingermarks is crucial in order to obtain the fluorescent product **100**. The red compound **100** does not seem to be a favourable product of the reaction, and therefore reagents such as metal salts or lewis acids may need to be added to the final formulation to facilitate its formation. Harsh conditions, such as the high temperatures and pressures used in this work, may also need to be incorporated into the optimised method for fingermark development to facilitate the formation of **100**. In addition to reaction condition optimisation and reduction of side products, there are several opportunities for future directions to better understand the pathway.



Scheme 6.1 Simplified reaction pathway toward formation of Compound **100**, showing complete formation of the amine linker via Strecker degradation, followed by nucleophilic substitution and intramolecular cyclisation with ethanol at the 3-position

6.2. Future directions into optimal reaction conditions

More investigation into the reaction conditions and factors influencing the formation of Compound **100** is required. In Chapter 2, a microwave reaction vessel was used as opposed to *in-situ* experiments under reflux. This allowed for higher temperatures to be reached which produced Compound **100** in a shorter time period, with yields comparable to the traditional set-up. These conditions also more closely resembled the higher temperatures reported in literature for techniques elucidating fingermarks using lawsone. However, not much research has been done in the way of determining optimal temperatures to perform the reaction at, as variances in literature ranging from 80 – 170°C

(dependant on heat source) were reported with varying success. The influence of the pressure of the reaction vessel has also never been investigated. While high pressures may only be useful to allow for higher than normal temperatures when using low boiling-point solvents, it may also increase interactions between the lawsone and amino acid molecules, thereby increasing the yield of Compound **100** and ultimately increasing its detection when trying to visualise latent fingermarks.

Other optimisation factors, such as the addition of metal salts or acids, could also be trialled when determining the best lawsone formulation. Metal halides such as zinc chloride have been found to enhance the observed fluorescence when using ninhydrin or 1,2-indandione through complexation with the reaction product.[4] The metal halide can either be added to the formulation prior to development or introduced to the reaction product after development. The addition of metal halides to the lawsone reaction mixture may have a similar complexation interaction with Compound **100**. The addition of Lewis acids such as aluminium chloride to the reaction mixture and potential lawsone formulations could also be trialled to create conditions more favourable for the formation of **100**, as they are traditionally used to activate nucleophilic attack and therefore may facilitate the addition of the ethyl linker at the vinyl positions of intermediate **104** (Scheme 6.1).

Multiple analogues of the red dimer **100** were synthesised in Chapters 3 and 4, which could be further investigated for unique or advantageous spectral properties. These analogues included chain extension of the alkyl linker (compounds **140**, **143**, **144**, and **145**) or a methoxy substituent on the aromatic ring (**150**). The synthesis of compounds **140**, **143**, **144**, and **145**, as outlined in Chapter 3, may require optimisation of the current method (perhaps informed by investigations into the optimisation of the synthesis of Compound **100**) or use of an entirely new method in order to obtain cleaner analogues in enough yield to accurately obtain fluorescence, UV, IR, or other spectroscopic technique profiles. These profiles may be promising to allow for the shift of signals originating from the reaction product between the fingermark and visualising reagent away from background signals due to the substrate.

The purity of the compounds isolated from the reaction mixture was a consistent issue faced throughout this work. This was particularly problematic for determining their structural identity as signals from impurities could not be distinguished from compound signals. Changes to the isolation technique may prove useful to resolve these issues and obtain cleaner samples. For example, preparative HPLC methods for compound isolation could be developed. Preliminary studies into preparative HPLC were trialled for use in this work, using either the detection of fluorescence or specific retention times as collection triggers. However, issues with drifting retention times and

inconsistencies in fluorescence detection meant that this method was not suitable with the equipment available.

Additionally, major peaks were obstructed by signals originating from other moieties within the compound and impurities in the sample. Since the primary analysis technique was NMR, emerging methods in this technique could be adapted to the naphthoquinone structures in this work. For example, the use of pure shift NMR experiments such as Diffusion Ordered Spectroscopy (DOSY) and Pure Shift Yielded by Chirp Excitation (PSYCHE) NMR may produce cleaner spectra that are more interpretable.[5] DOSY NMR experiments can separate and group ^{1}H signals according to the diffusion coefficients of the originating compound, thereby assigning signals in the spectrum to a compound in the mixture.[5] PSYCHE NMR experiments decouple homonuclear coupling between ^{1}H atoms which results in a single peak in the obtained spectrum, therefore reducing signal overlap caused by peak splitting.[5] These experiments require significant method development depending on the contents of the sample which could be explored for future reaction mixture investigation.

6.3. Future directions for proposed mechanism validation

Chapter 5 proposed a reaction pathway towards Compound **100**. An amine-linked intermediate **104** (Scheme 6.1) was proposed as the final step towards the formation of Compound **100** and required an alkylation mechanism which was not elucidated. The formation of this proposed intermediate and whether it can be converted to Compound **100** requires further investigation. An attempt was made in Chapter 5 at the synthesis of this intermediate by Michael addition using **102** and 1,4-naphthoquinone, which was adapted from a previous method used to synthesise **186**.[6] While the attempt did not make the desired intermediate **104**, it did return another red compound with interesting 2D NMR spectra which could be analysed further to identify its structure. Furthermore, the synthesis of the desired intermediate **104** requires exploration, as its synthesis has never been reported to date.

The pathway in Scheme 6.1 postulates a conversion from **197** to **100** as a result of an oxidation step. Chapter 3 discussed the requirement of oxidative conditions to facilitate the formation of Compound **100** where hydrogen peroxide and neat oxygen were added to the reaction mixture. These only improved the purity of the sample via NMR analysis and did not seem to increase the yield of Compound **100**. As strong oxidants such as hydrogen peroxide are not present in large quantities in the original reaction mixture, other potential oxidants may be investigated. Primarily, the ability of naphthoquinone species such as lawsone and **102** to undergo redox cycling in the reaction mixture to facilitate the postulated oxidation step should be explored. Redox cycling of naphthoquinones has

been previously observed through monitoring of oxygen or hydrogen peroxide production, which could be adapted to monitor redox cycling occurring in this reaction mixture.[7–9]

Computational molecular modelling may provide further insight into the proposed reaction pathway in Scheme 6.1. The energies of any proposed intermediates and transition states at each step along the pathway can be calculated to determine if the overall reaction is energetically favourable, and therefore if the pathway proposed in this work is feasible.

6.4. References

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Appendix 1: Elucidation of Compound Structure

Preliminary Reaction on Filter Paper

1 $mgml^{-1}$ aqueous solutions of glycine, histamine, histidine, spermine, spermidine, tyramine, and phenylethylamine were prepared as per *Jelly et. al.*, where 900 $ugml^{-1}$ solutions were used. 1 $mgml^{-1}$ solutions of lawsone in ethanol and in ethyl acetate were also prepared. 10 uL of aqueous amine/amino acid solution was spotted onto filter paper and allowed to air dry before 10 uL of the lawsone solution in ethanol or ethyl acetate was spotted on top. A water blank treated with lawsone and lawsone by itself were also spotted onto filter paper as controls. The filter paper was placed in an oven at 170°C for 1-2 hours. The resulting spots on filter paper were visualised using a polilight between 490-555 nm and orange goggles.

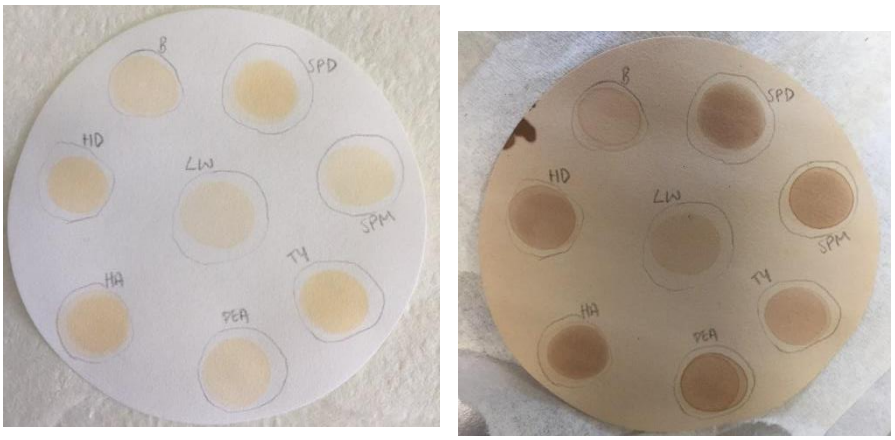


Figure 1. Appearance of filter paper spotted with amines and lawsone in ethanol solution prior to and after treatment with heat.

HPLC-MS

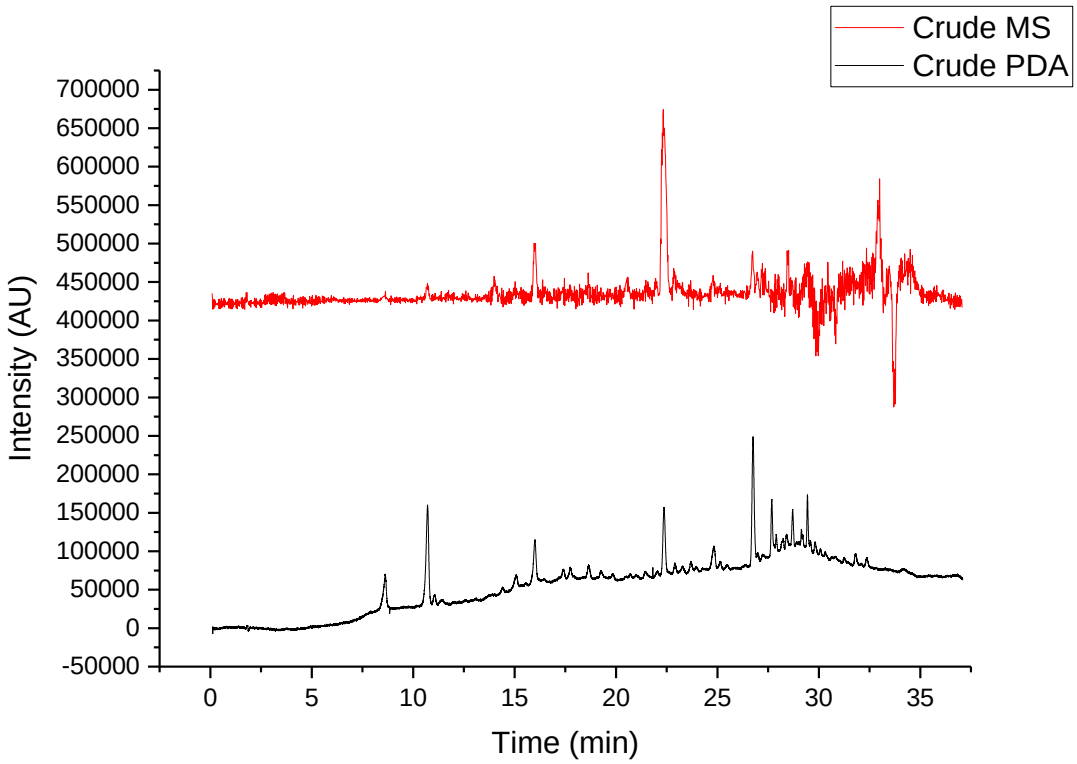


Figure 2. The MS (top, red) and PDA (bottom, black) traces resulting from the LCMS separation of the crude purple precipitate from the reaction between lawsone and glycine, showing a complex mixture.

GCMS

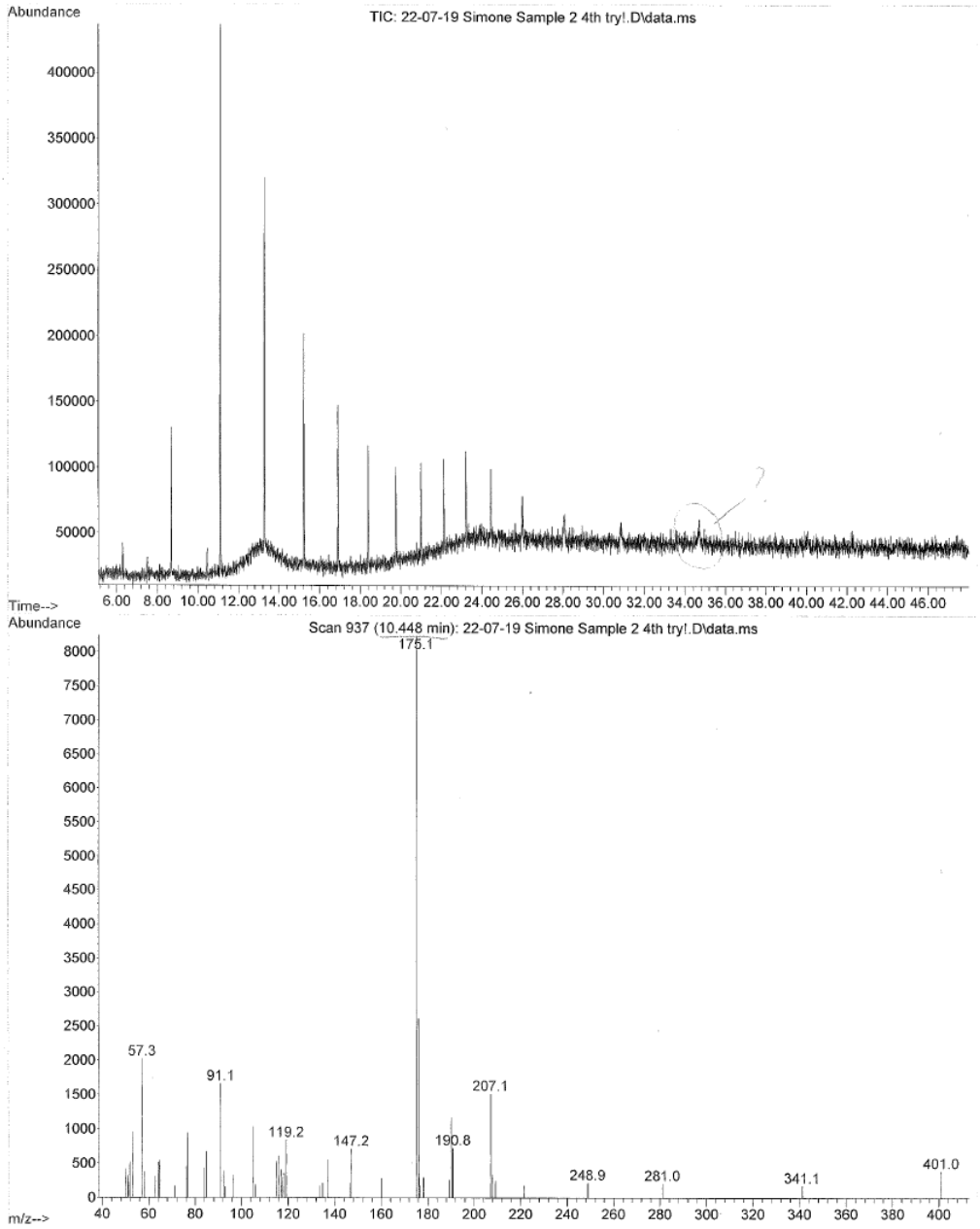


Figure 3.1. GCMS trace of Compound **102**, and EIC at 10.448 and minutes showing a base peak of m/z = 175.1

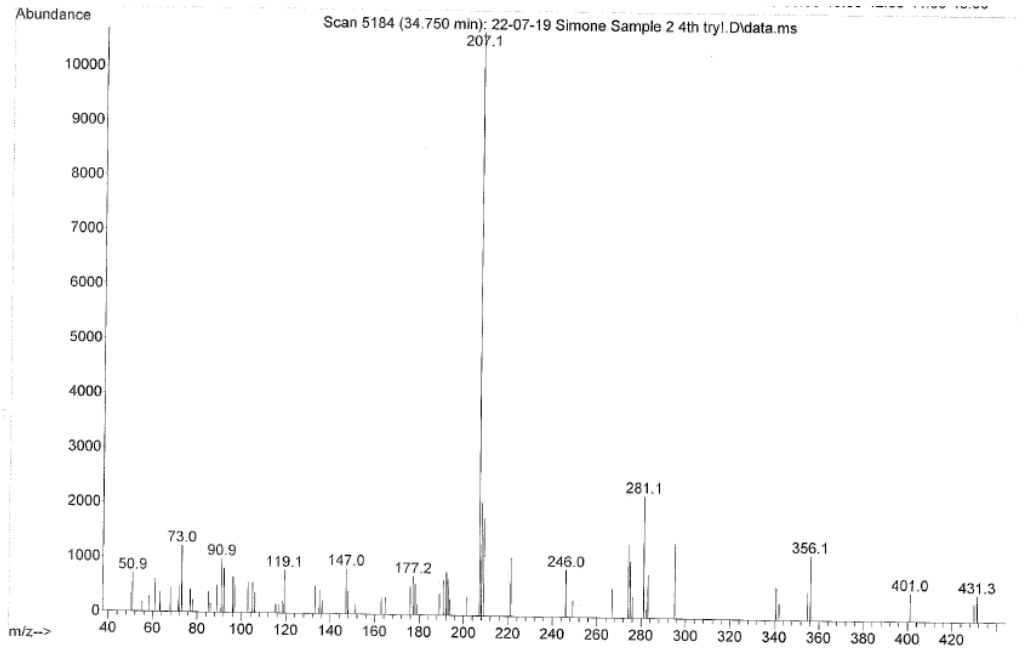


Figure 3.2. EIC at 34.755 minutes, showing a base peak of m/z = 207.1

Compound **100** NMR Spectra

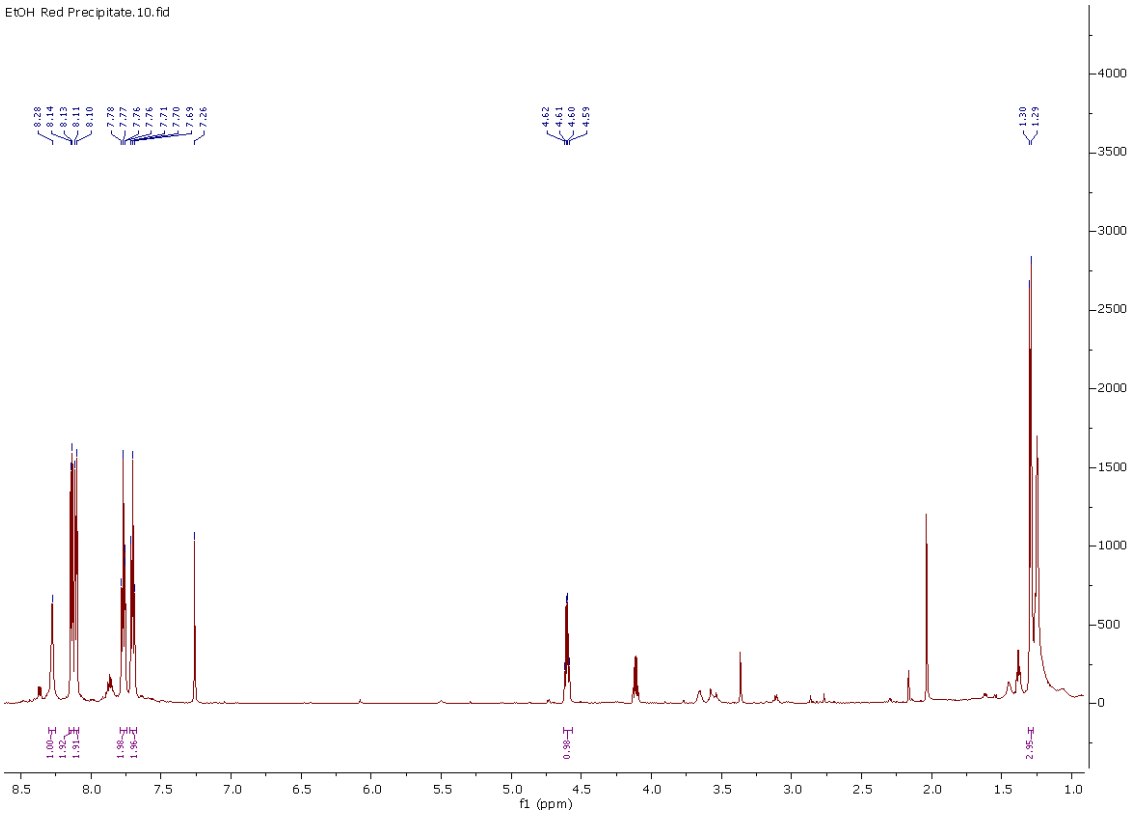


Figure 4. ^{1}H NMR spectrum of compound **100**

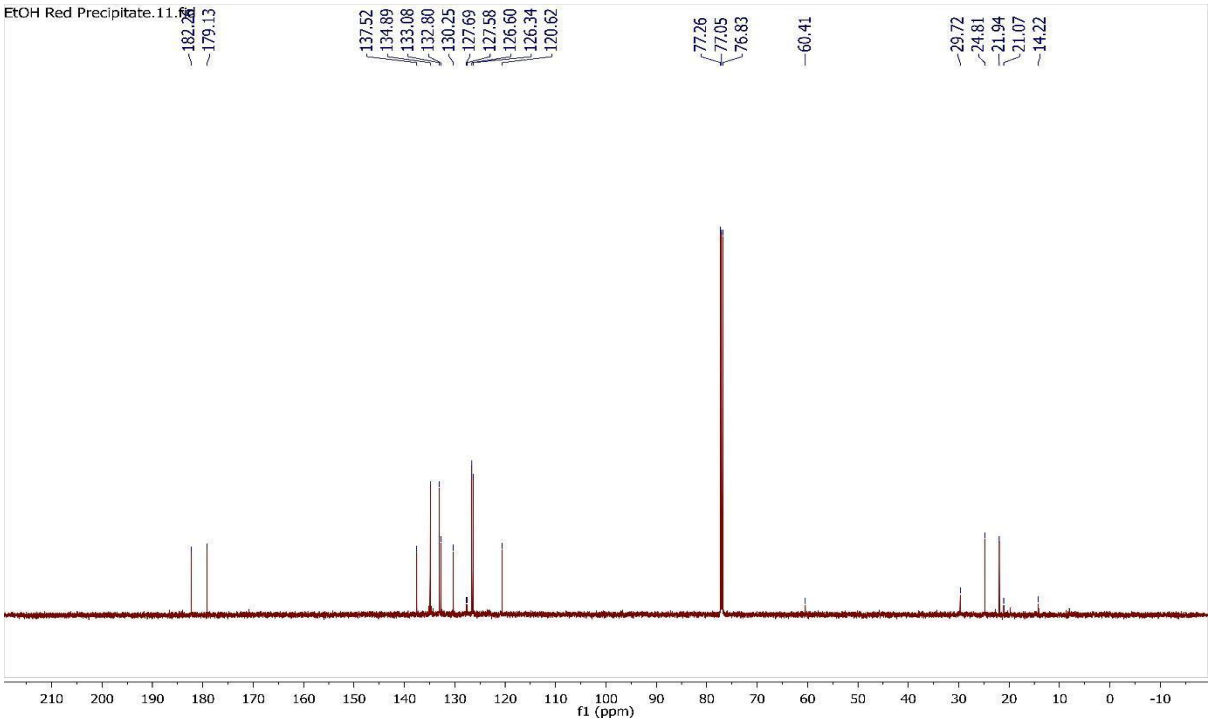


Figure 5. ^{13}C NMR spectrum of compound **100**

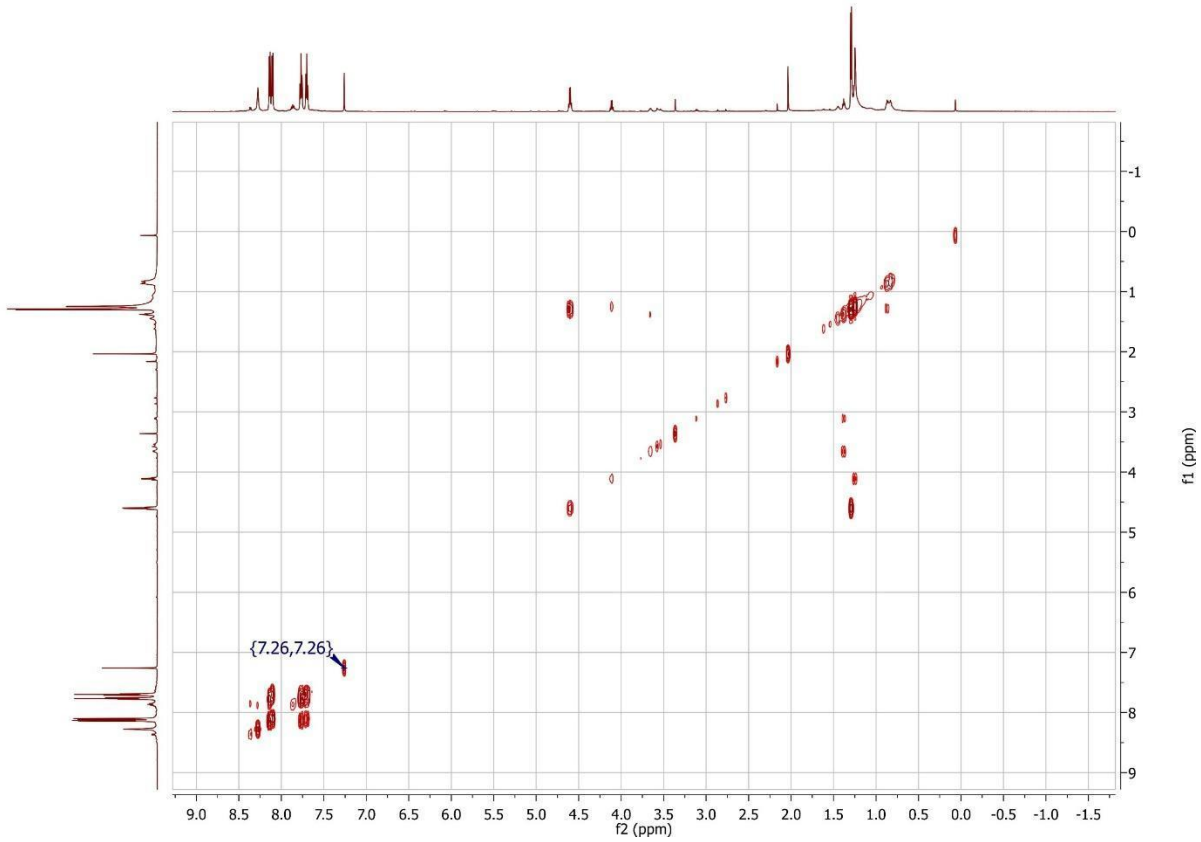


Figure 6. COSY NMR of compound **100**

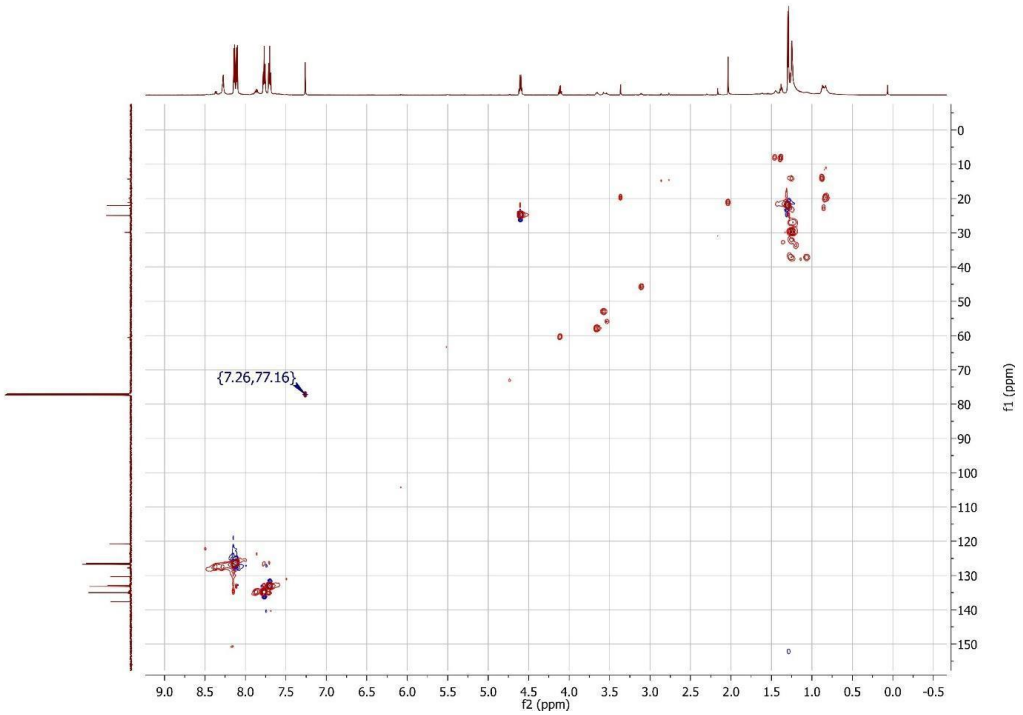


Figure 7. HSQC NMR spectrum of compound **100**

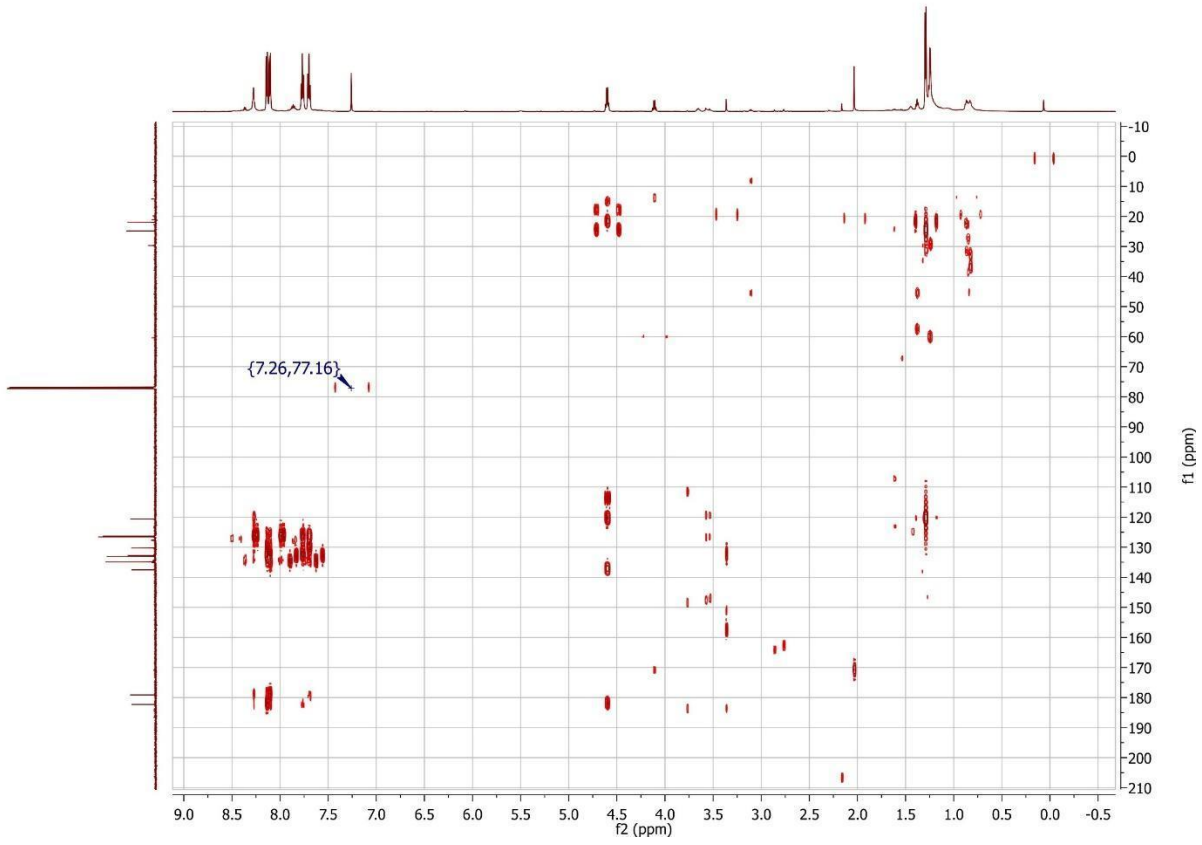


Figure 8. HMBC NMR spectrum of compound **100**

Compound **101** NMR Spectra

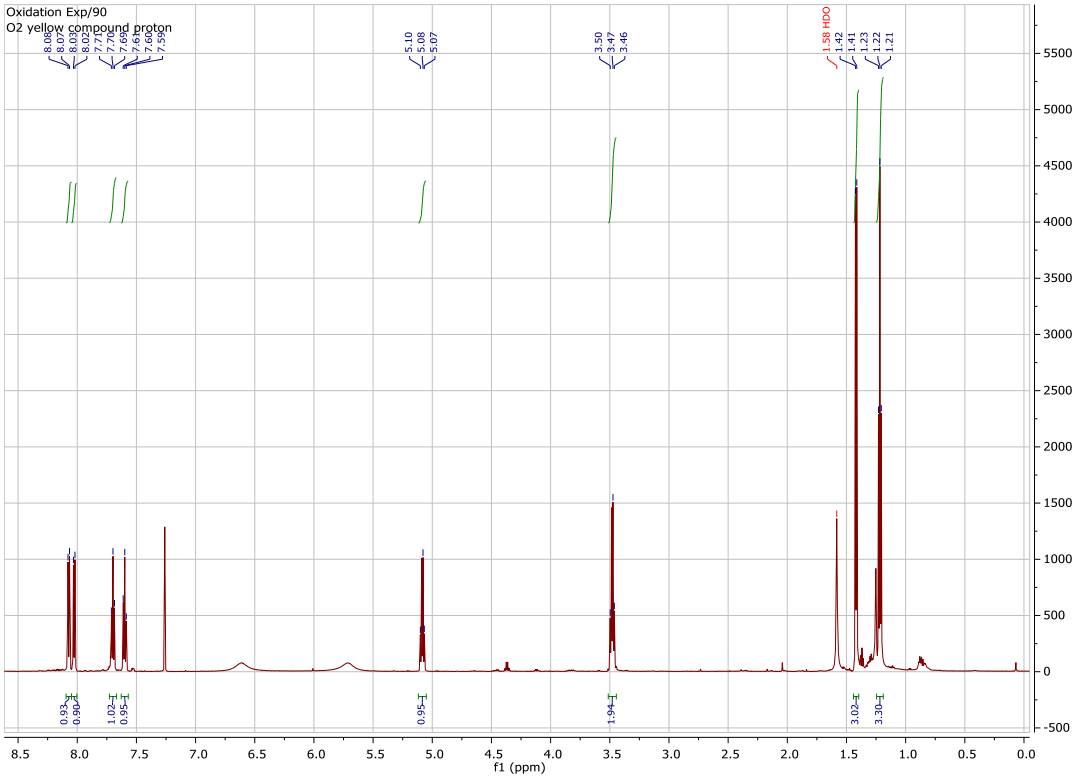


Figure 9. ^{1}H NMR spectrum for compound **101**

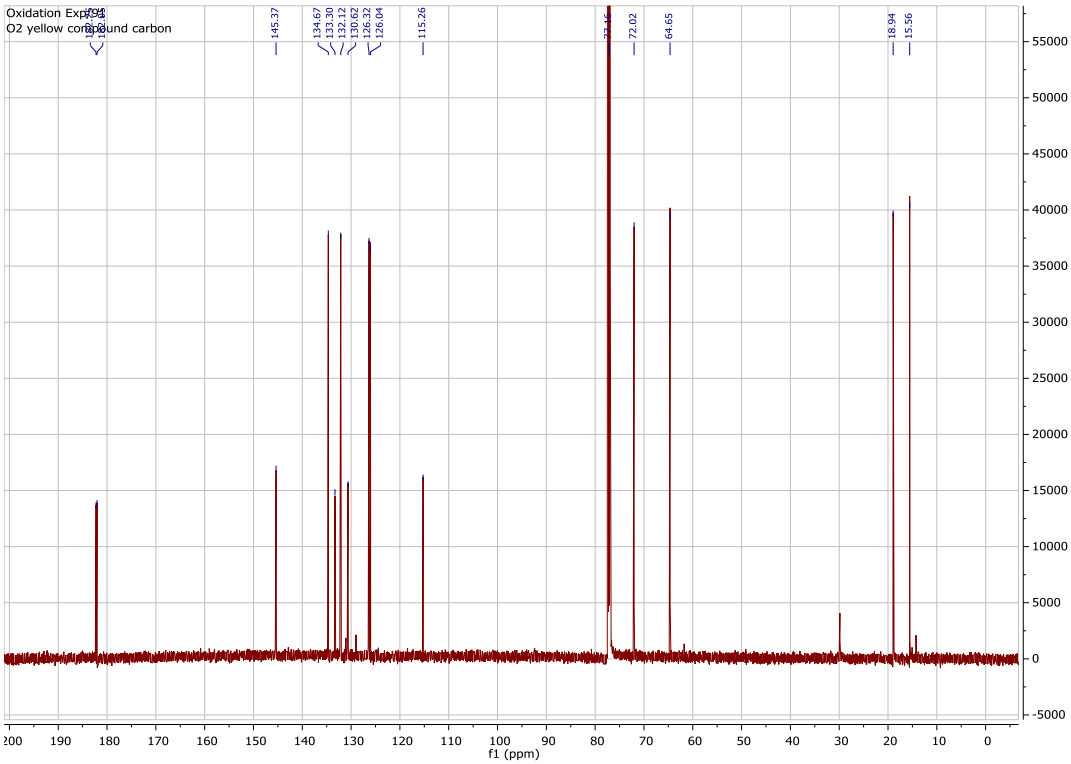


Figure 10. ^{13}C NMR spectrum of compound **101**

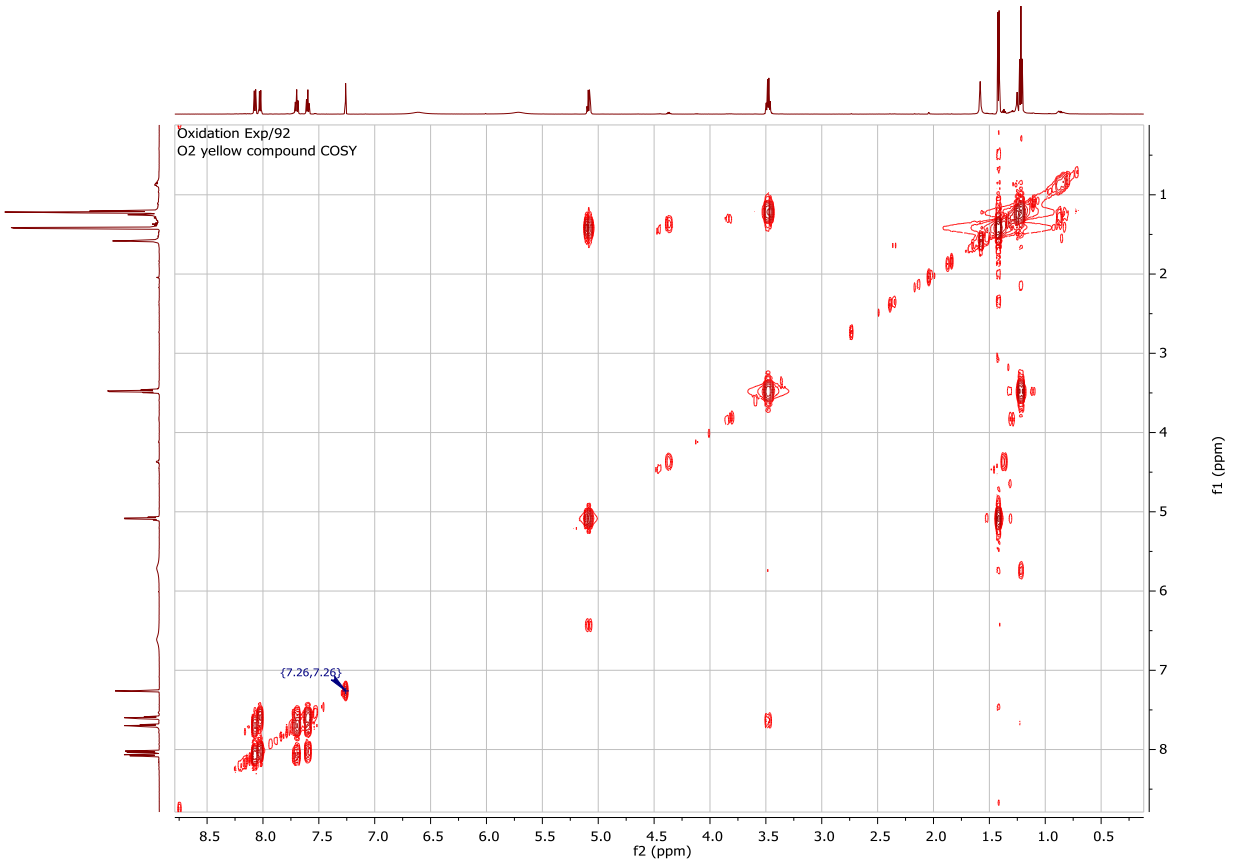


Figure 11. COSY NMR spectrum of compound **101**

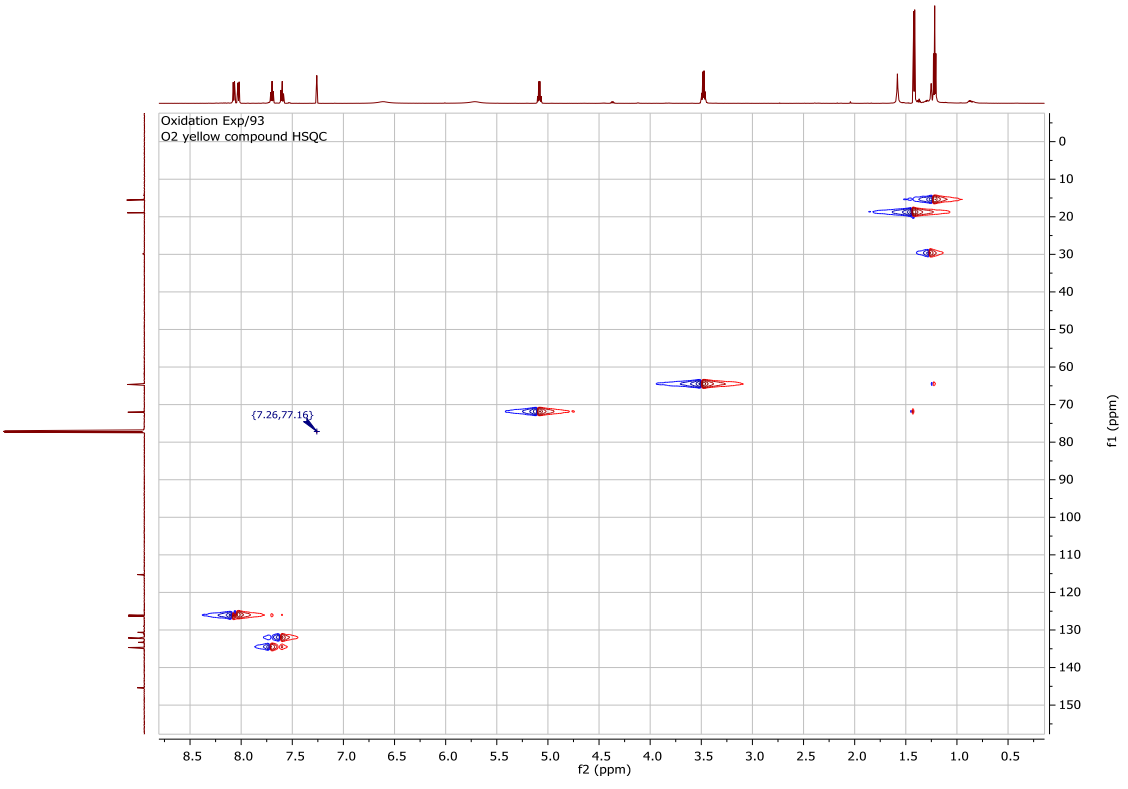


Figure 12. HSQC NMR spectrum for compound **101**

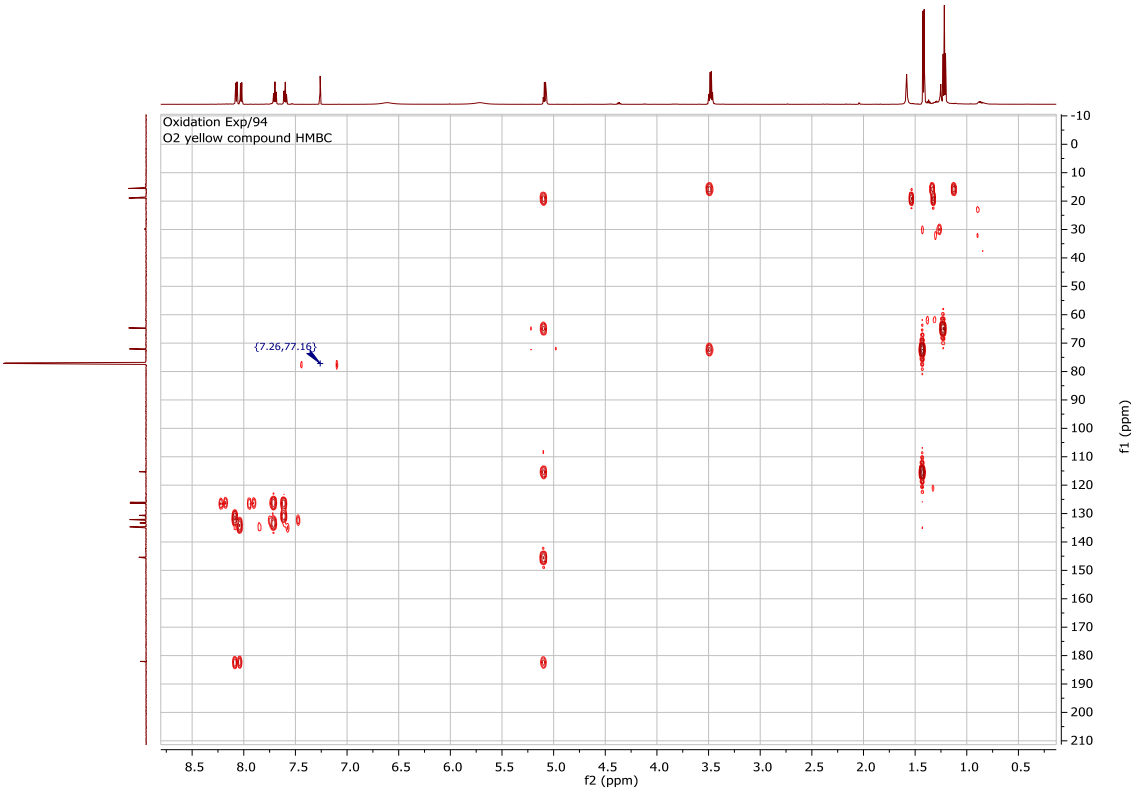


Figure 13. HMBC NMR spectrum for compound **101**

Compound **102** NMR Spectra

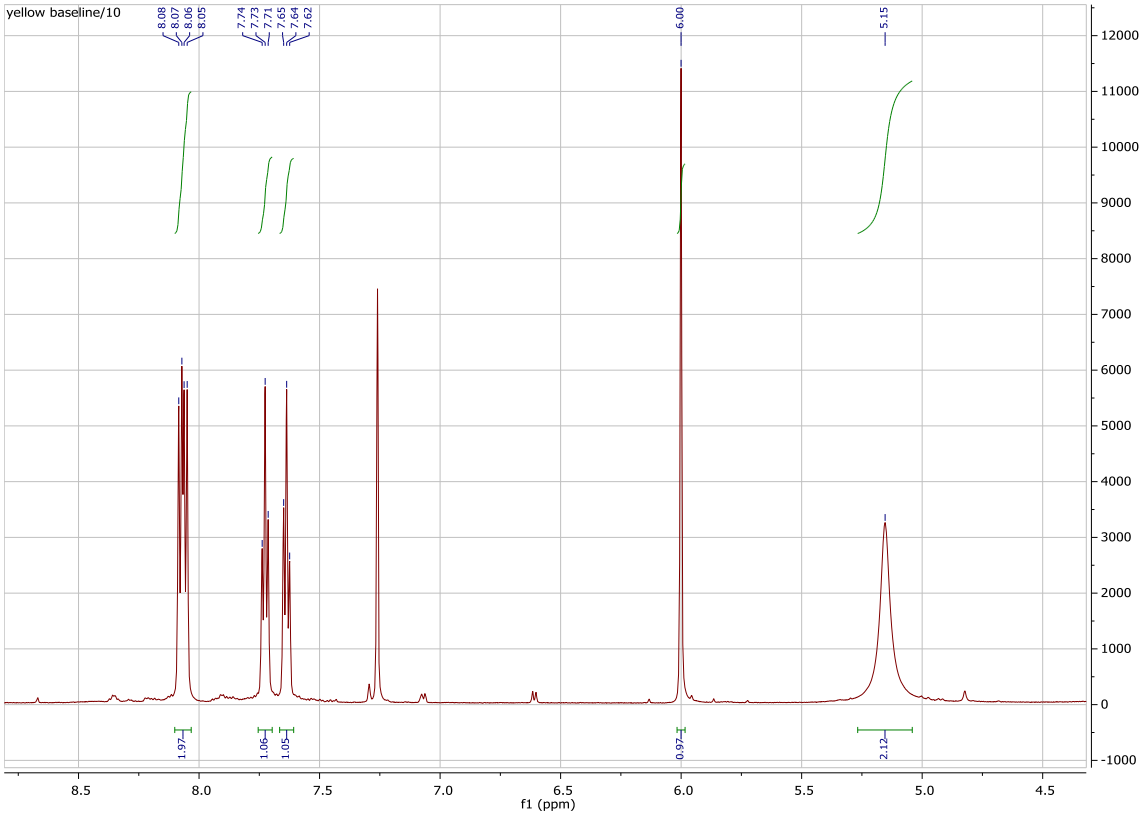


Figure 14. ^{1}H NMR spectrum of compound **3**

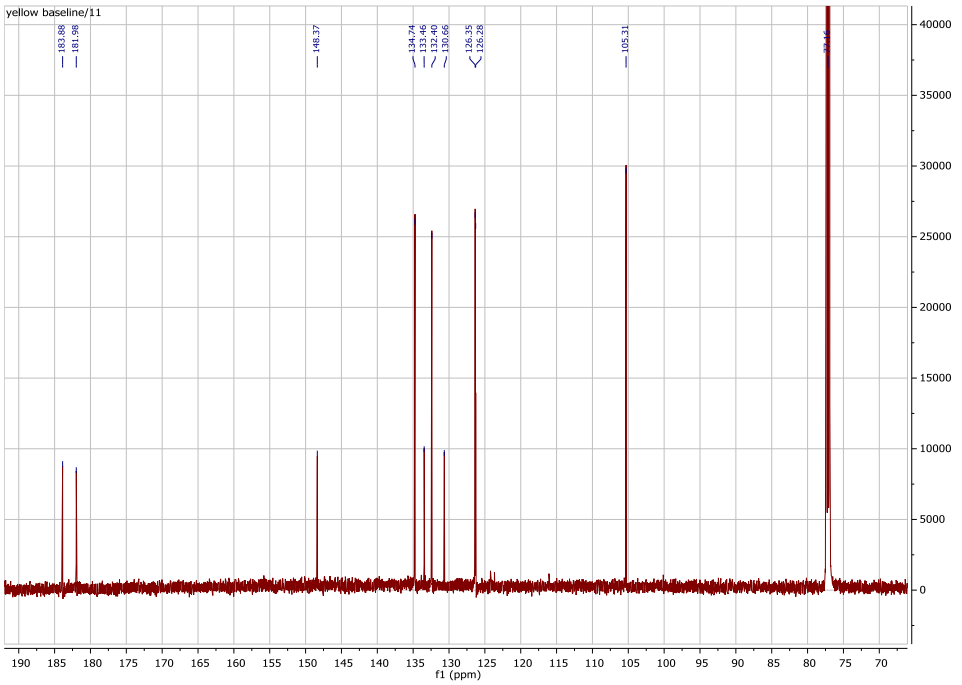


Figure 15. ^{13}C NMR spectrum of compound **102**

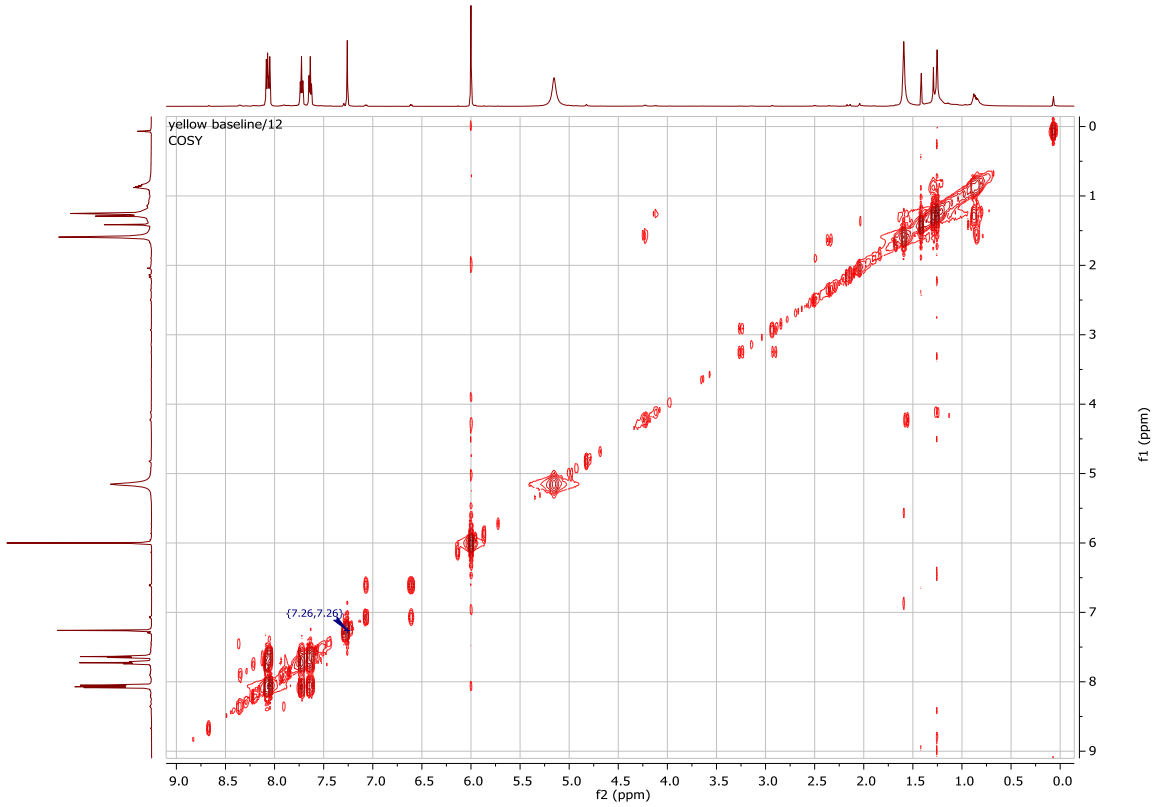


Figure 16. COSY NMR spectrum of compound **102**

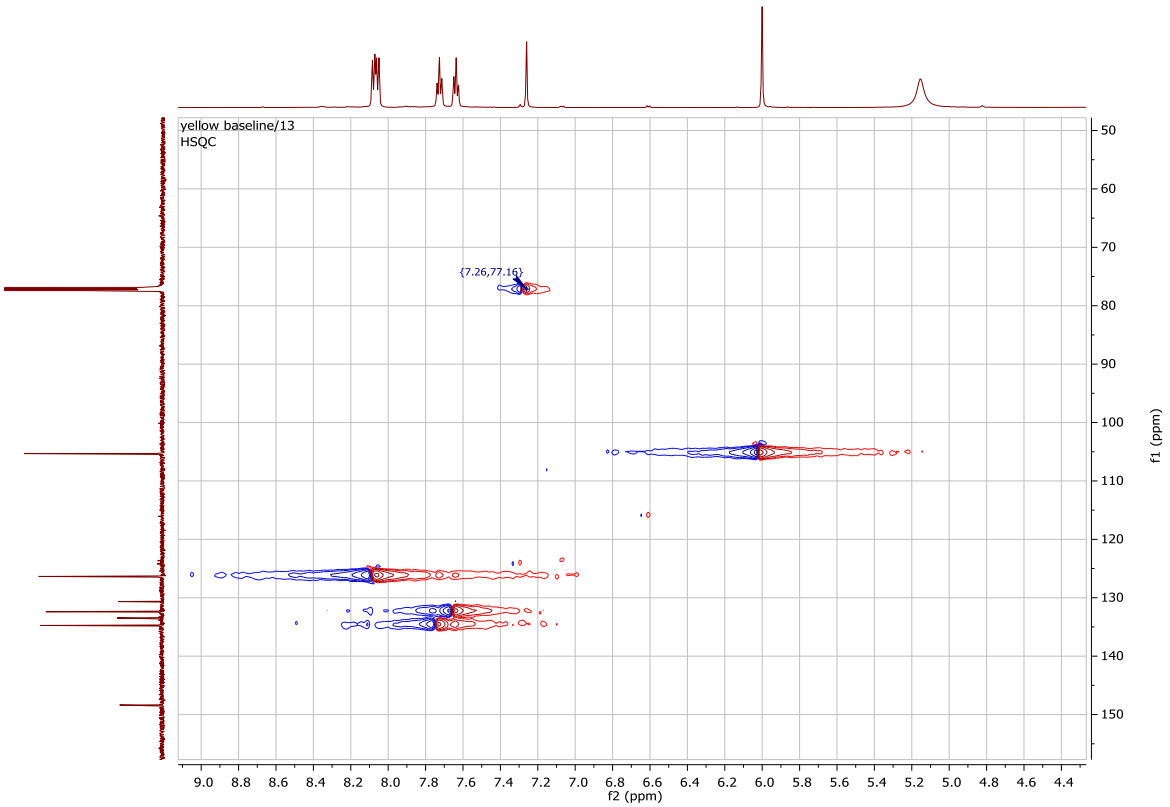


Figure 17. HSQC NMR spectrum of compound **102**

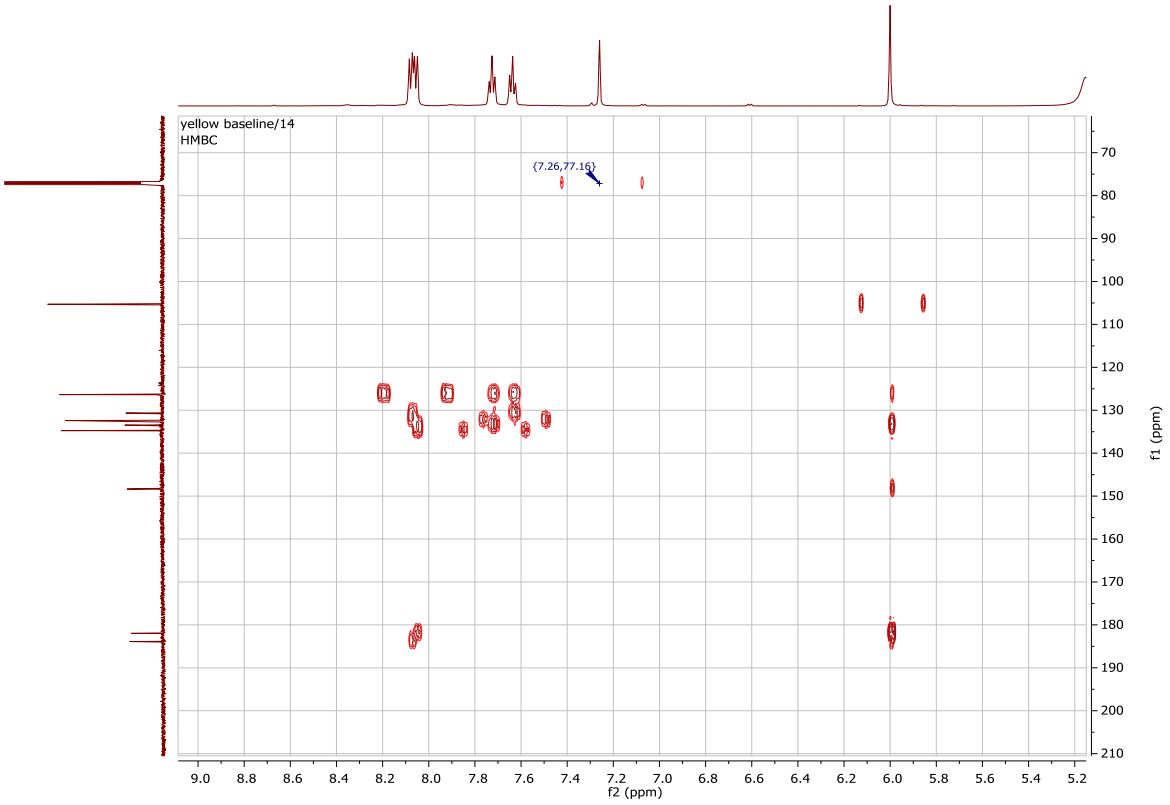


Figure 18. HMBC NMR spectrum of compound **102**

Lawsone (**13**) NMR Spectra

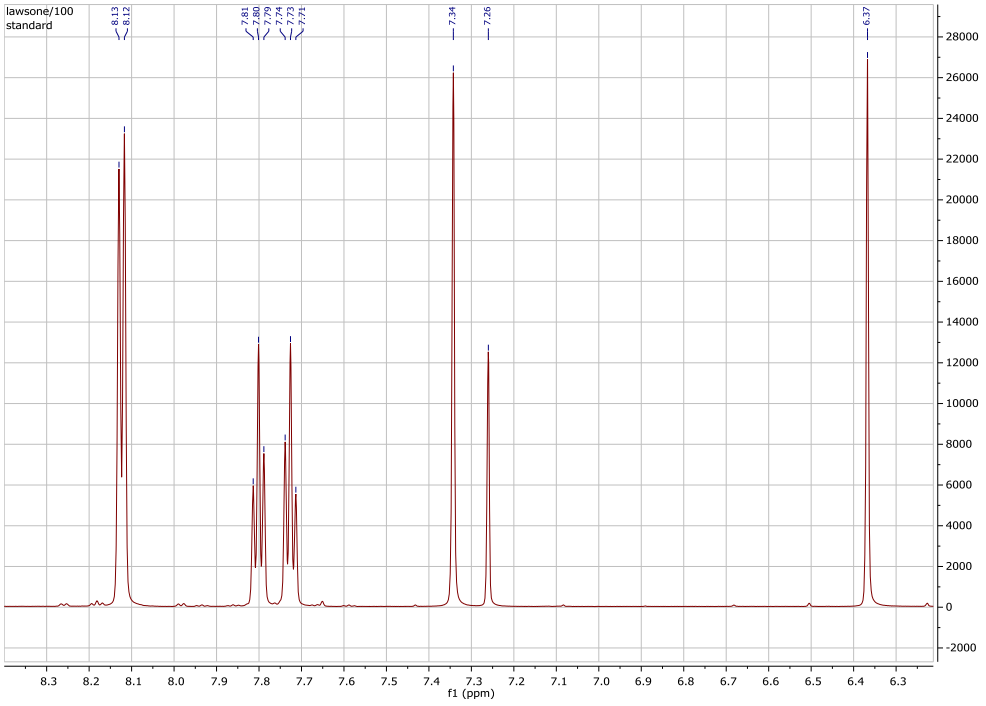


Figure 19. ^{1}H NMR spectrum for lawsone standard

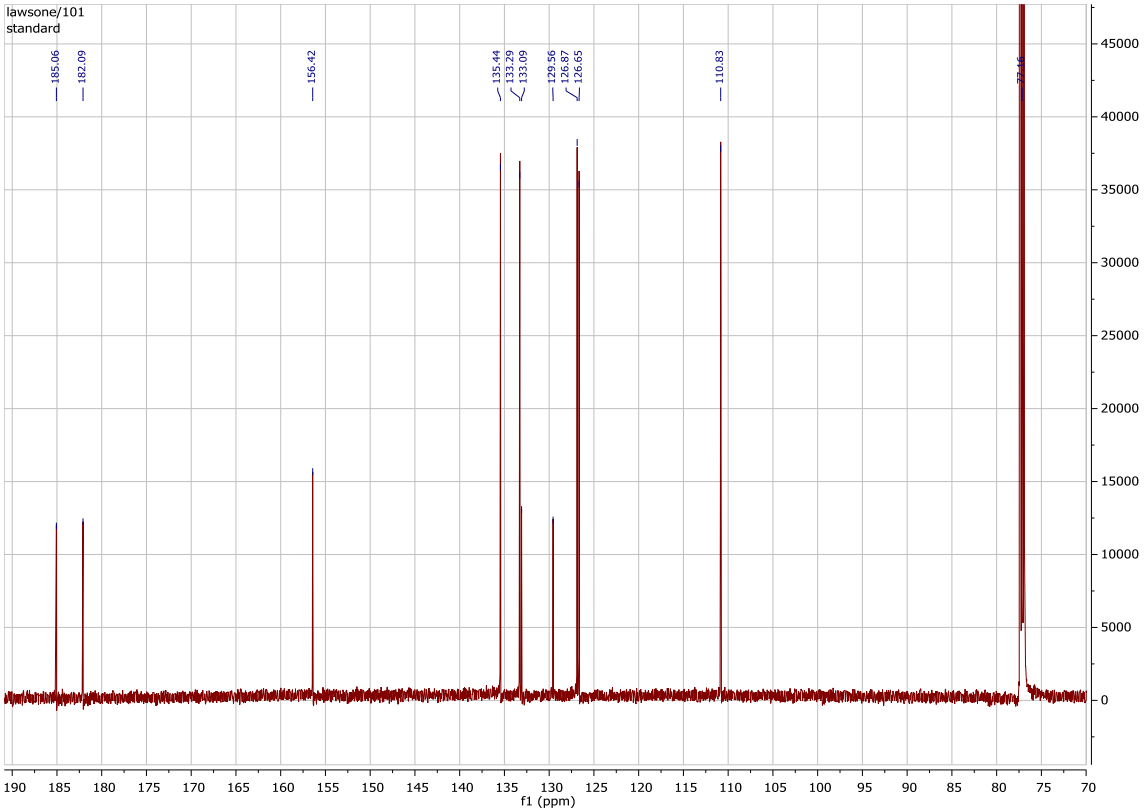


Figure 20. ^{13}C NMR spectrum for lawsone standard

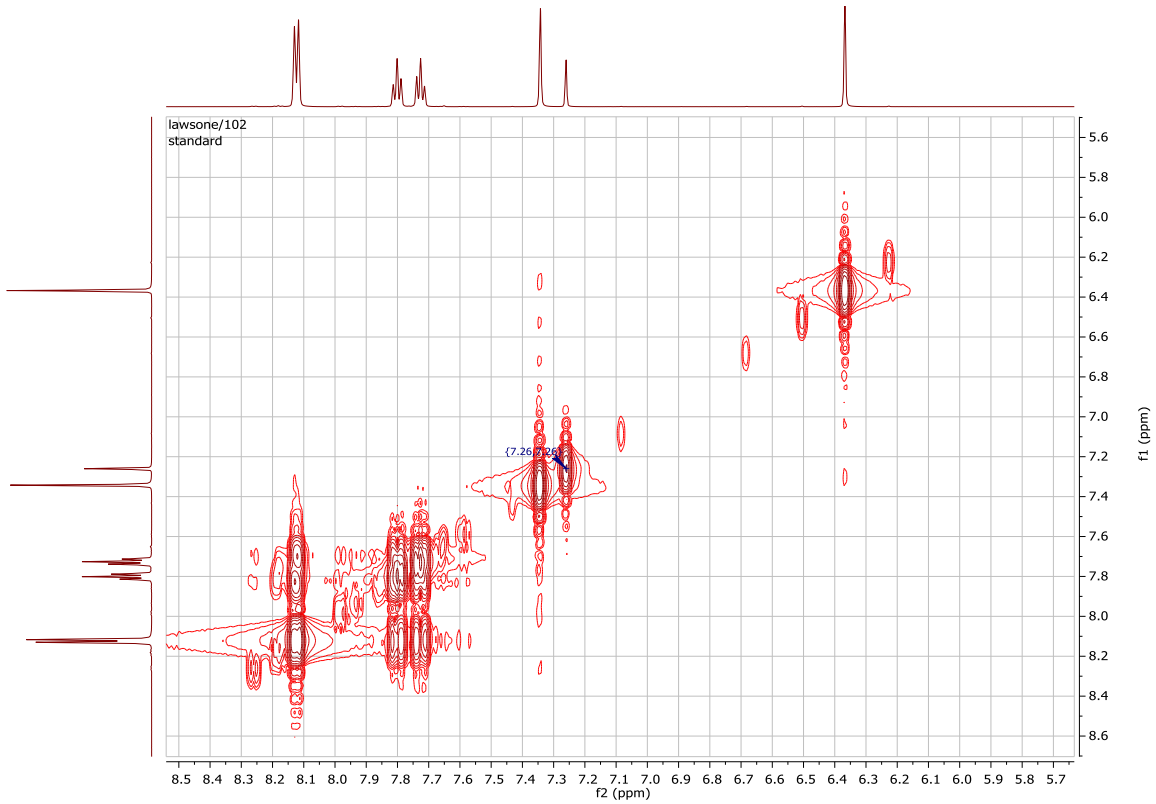


Figure 21. COSY NMR spectrum for lawsone standard

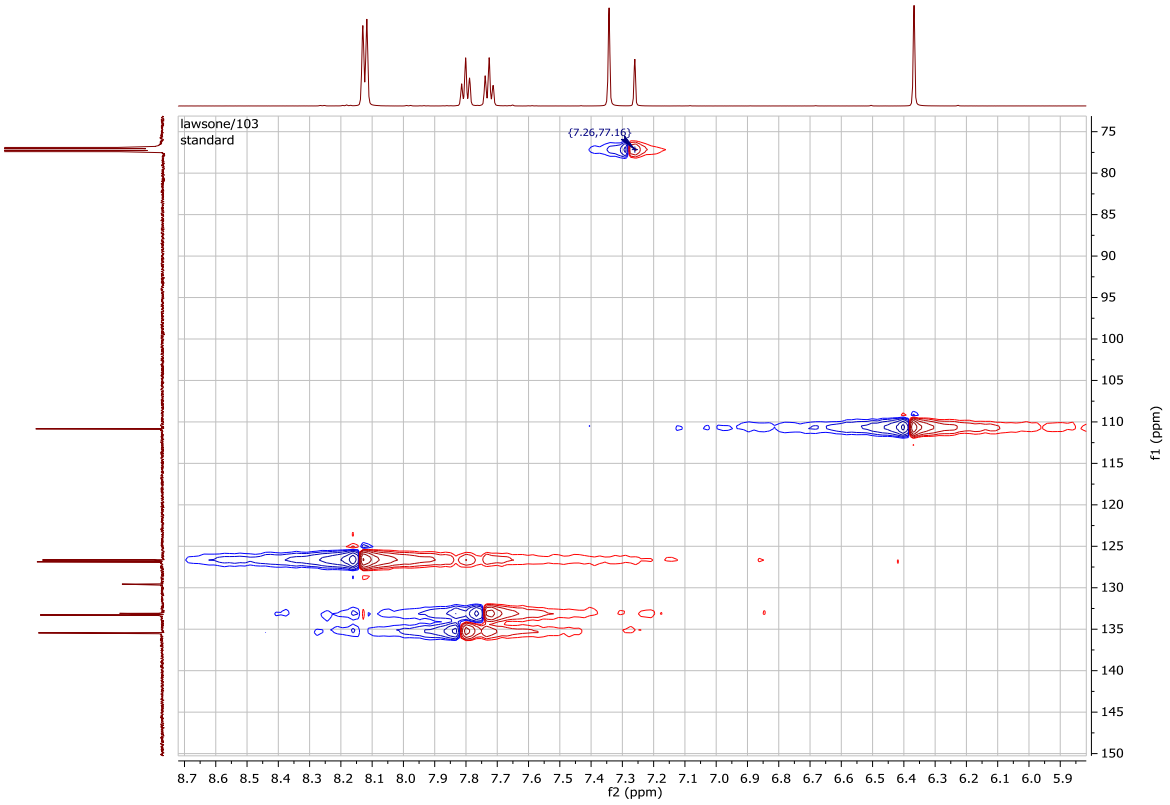


Figure 22. HSQC NMR spectrum for lawsone standard

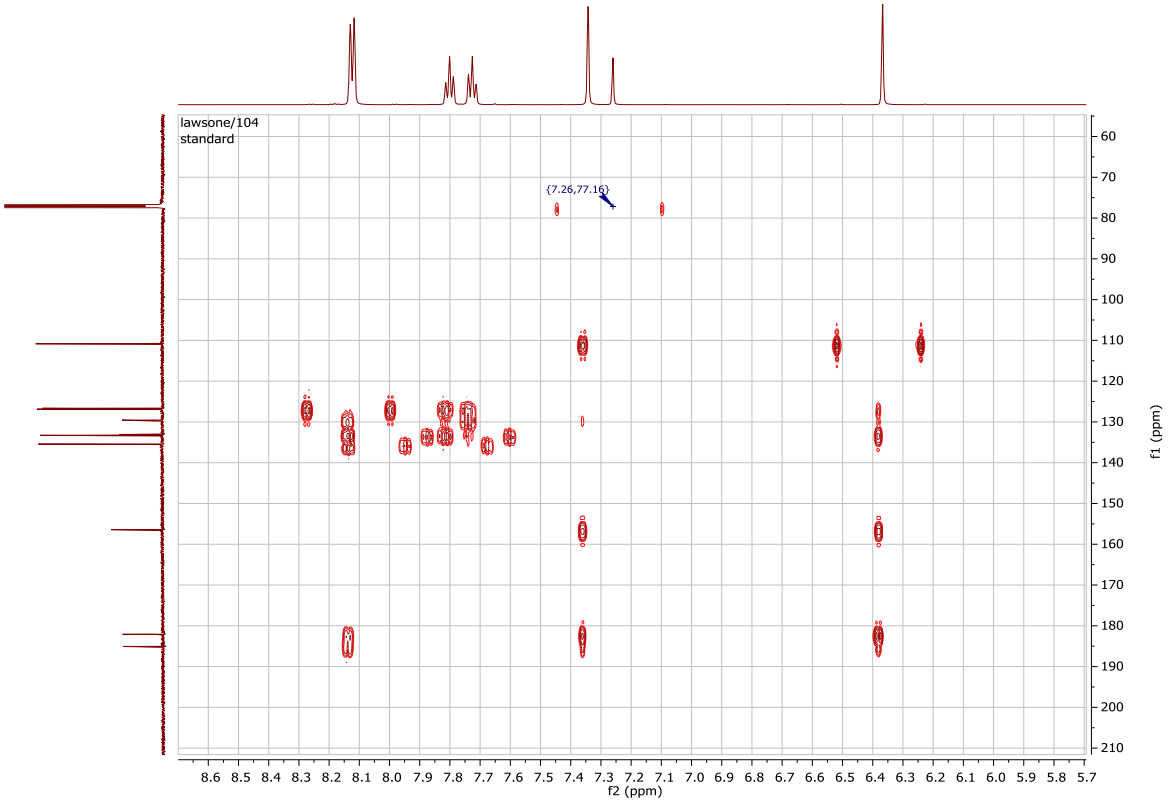


Figure 24. HMBC NMR spectrum for lawsone standard

Table 1. ^{1}H, ^{13}C, HSQC, COSY, and HMBC NMR data for lawsone, refer to Figure 25 for position assignment

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$) [a]	COSY	HMBC (H→C)
3	110.83	6.37		110.83, 126.88, 133.29, 156.42, 182.09
9	126.66	8.12	7.80, 7.73	126.66, 129.56, 133.09, 135.45, 182.09, 185.06
6	126.88	8.12	7.80, 7.73	126.66, 129.56, 133.09, 135.45, 182.09, 185.06
5	129.56			
10	133.09			
7	133.29	7.73		126.88, 133.29
8	135.45	7.80		126.66, 133.09, 133.29, 135.45
2	156.42			
4	182.09			
1	185.06			
OH		7.33		110.83, 156.42, 182.09

[a] δ_H, multiplicity (*J* in Hz)

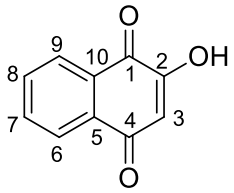


Figure 25. Structure of lawsone, showing position assignment as referred to in Table 1

Appendix 2: Investigations into the Origin of Dimeric Linkers

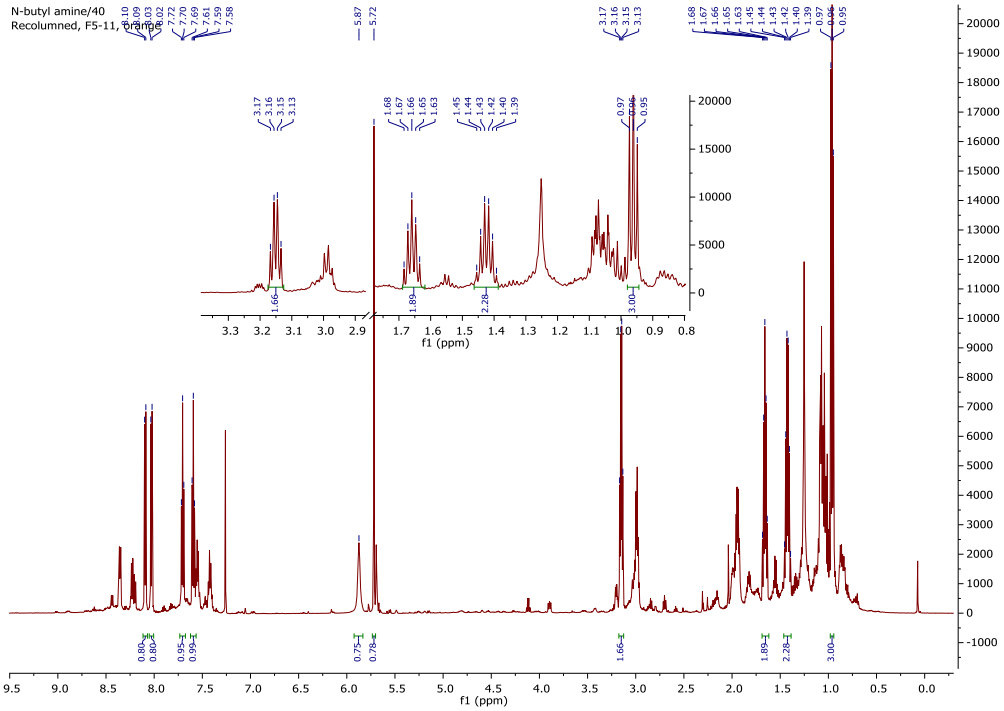


Figure 26: ^{1}H NMR of the orange compound **134** formed from the reaction of N-butyl amine and lawsone in ethanol

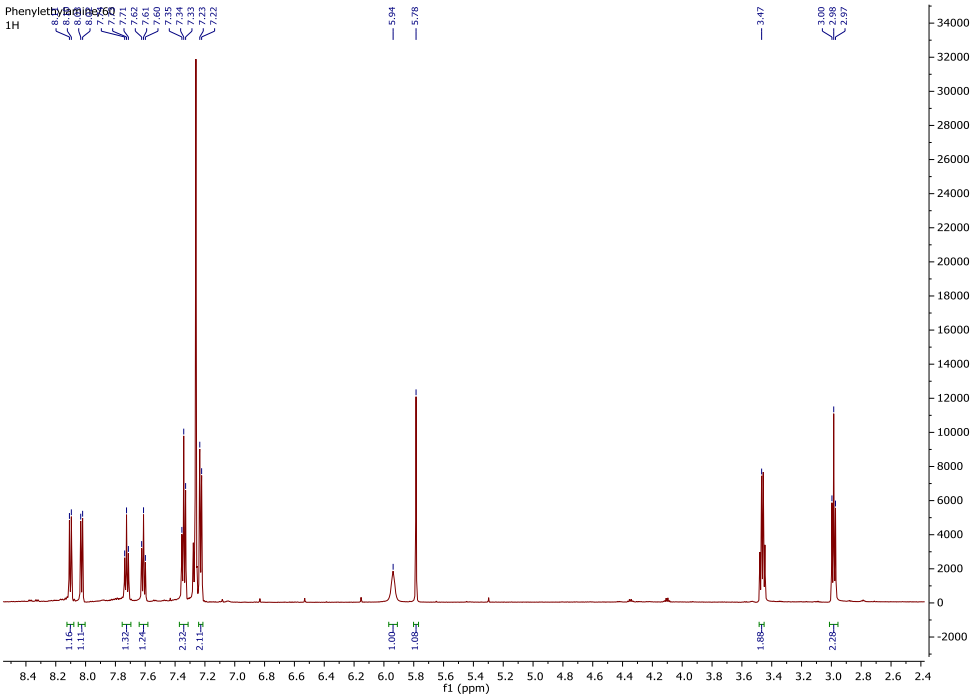


Figure 27: ^{1}H NMR of the orange compound **135** formed from the reaction of phenylethylamine and lawsone in ethanol

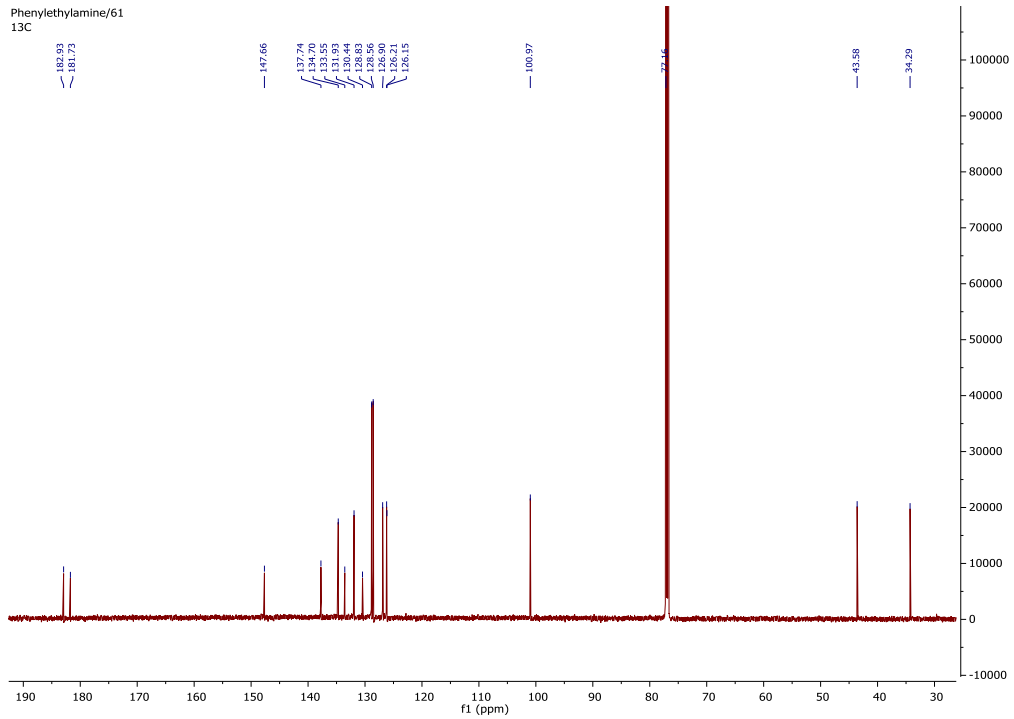


Figure 28: ^{13}C NMR of the orange compound **135** formed from the reaction of phenylethylamine and lawsone in ethanol

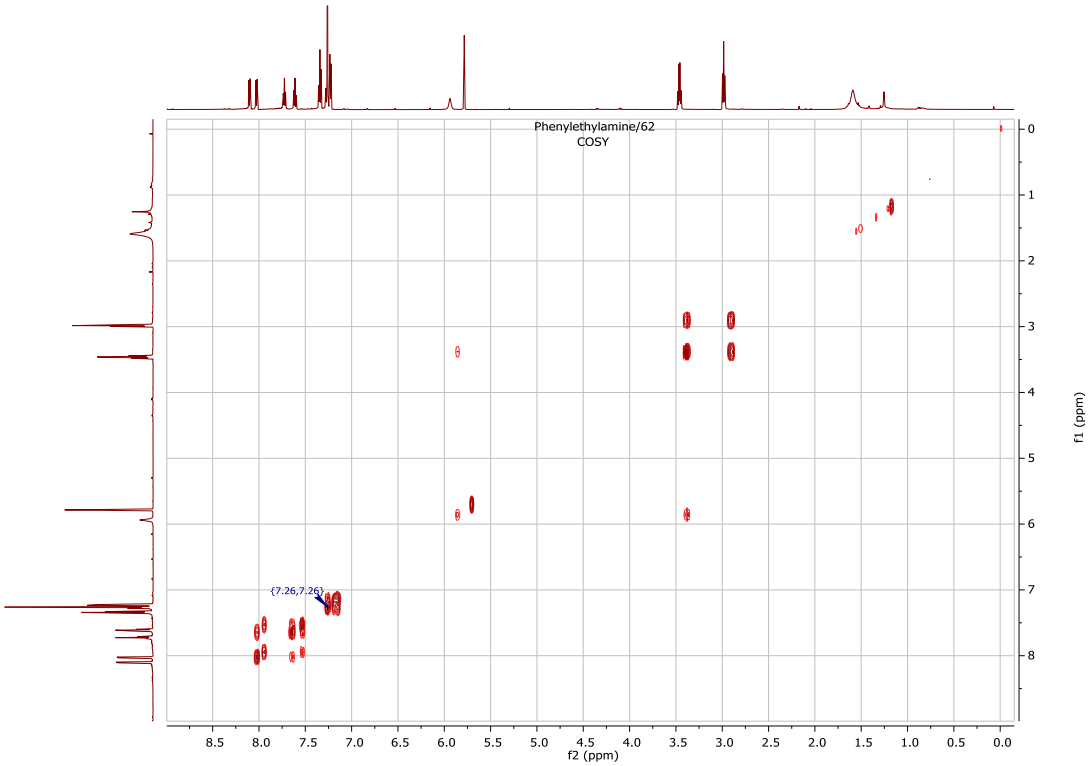


Figure 29: COSY NMR of the orange compound **135** formed from the reaction of phenylethylamine and lawsone in ethanol

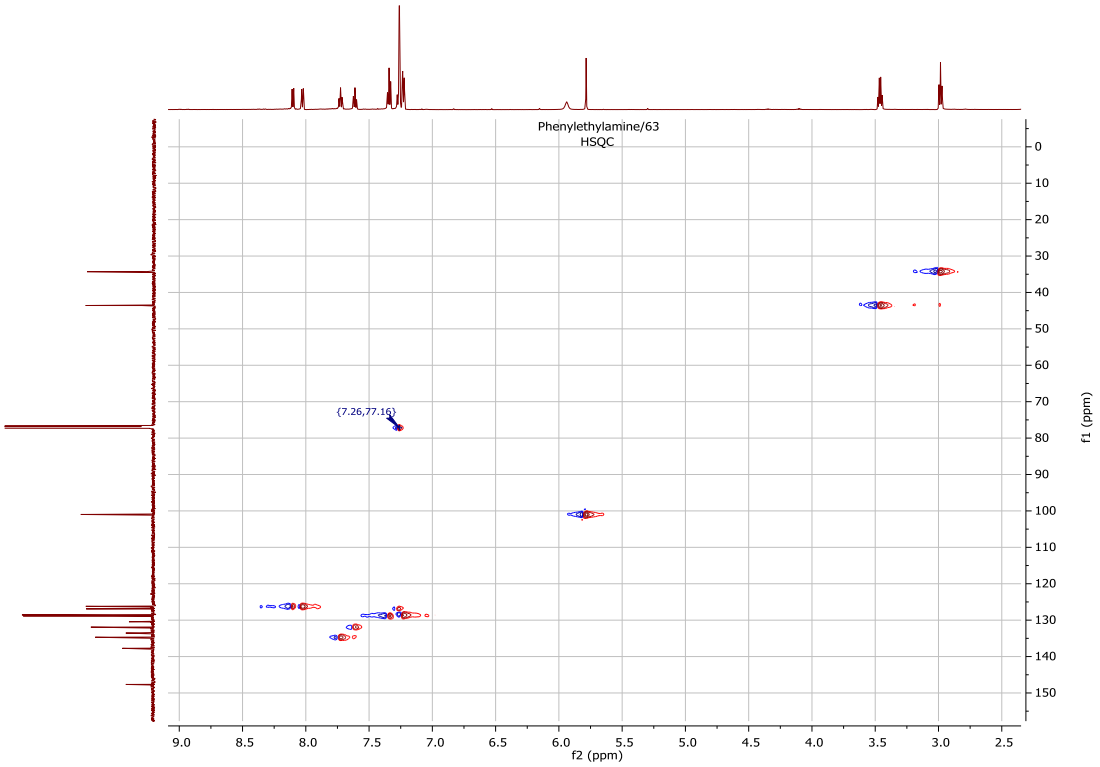


Figure 30: HMBC NMR of the orange compound **135** formed from the reaction of phenylethylamine and lawsone in ethanol

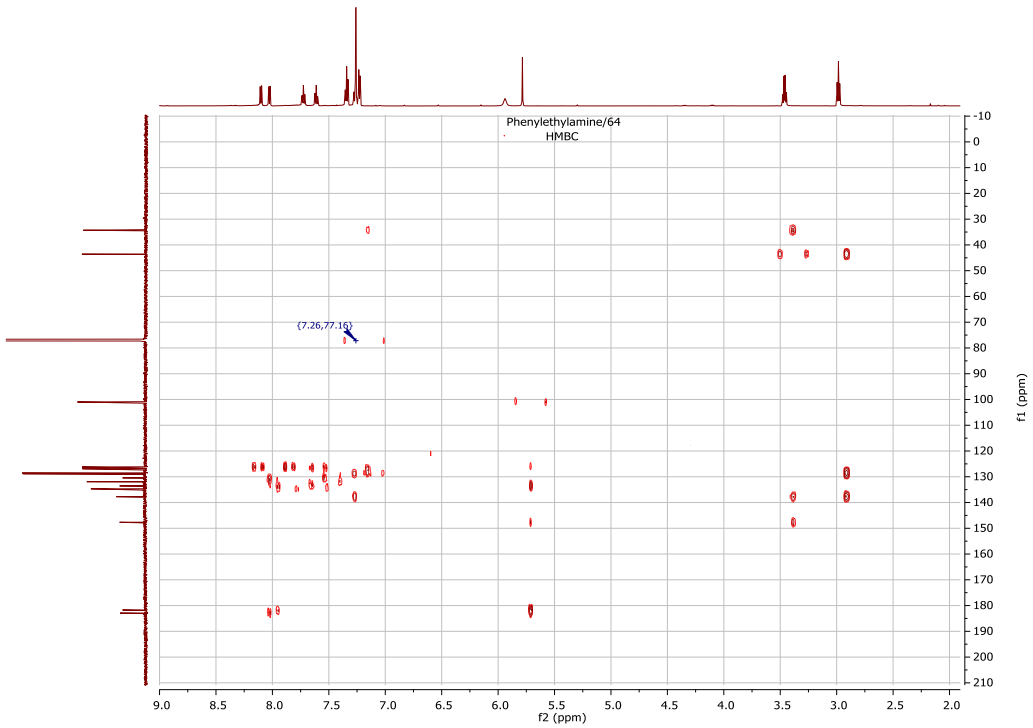


Figure 31: HMBC NMR of the orange compound **135** formed from the reaction of phenylethylamine and lawsone in ethanol

Table 2: Summary of ^{1}H, ^{13}C, and 2D NMR, assigning observed shifts to the phenylethylamine structure **135**

Position	δ_C ($CDCl_3$)	δ_H ($CDCl_3$) [a]	COSY	HMBC (H→C)
13	34.29	2.98, t (7.1)	3.47	43.58, 128.56, 137.74
12	43.58	3.47, q (6.8)	2.98	34.29, 137.74, 147.66
3	100.97	5.78, s		126.15, 133.55, 147.66, 182.93, 181.73
9	126.15	8.10, d (7.7, 1.3)	7.73	182.93, 126.15, 130.44
6	126.21	8.03, d (7.7, 1.3)	7.61	181.73, 126.21, 133.55
17	126.90	7.26, t	7.23, 7.24	126.90
15, 19	128.56	7.23, d (6.8)	7.34, 7.26	128.56, 34.29
16, 18	128.83	7.34, t (7.5)	7.26, 7.23	128.83, 137.74
10	130.44			
7	131.93	7.61, t (7.6, 1.3)	8.03	126.15, 130.44, 133.55
5	133.55			
8	134.70	7.73, t (7.5, 1.3)	8.19	126.21
14	137.74			
2	147.66			
4	181.73			
1	182.93			
11		5.94, bs		

[a] δ_H, multiplicity (*J* in Hz)

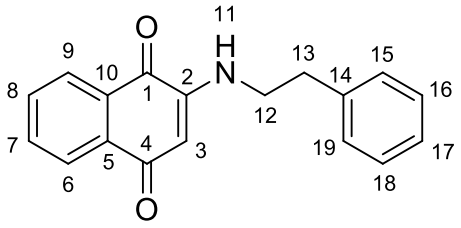


Figure 32: Structure of **135**, showing position assignment as referred to in Table 2

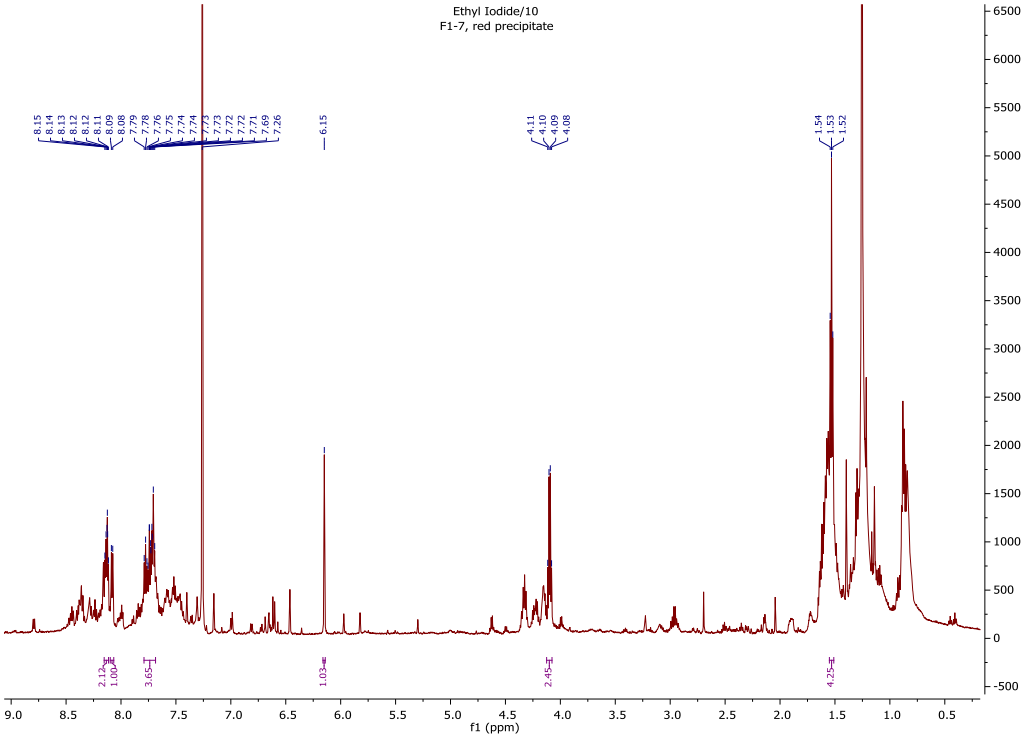


Figure 33: ^{1}H NMR of Fraction 1 isolated from the reaction between lawsone and glycine using ethyl iodide as a solvent

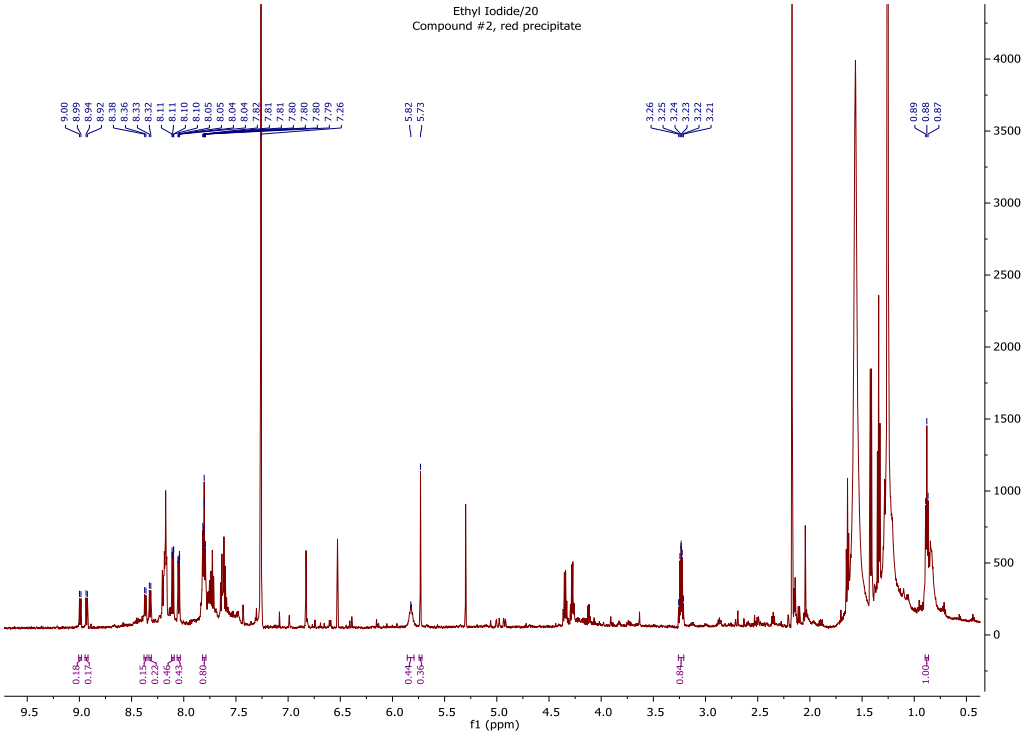


Figure 34: ^{1}H NMR of Fraction 2 isolated from the reaction between lawsone and glycine using ethyl iodide as a solvent

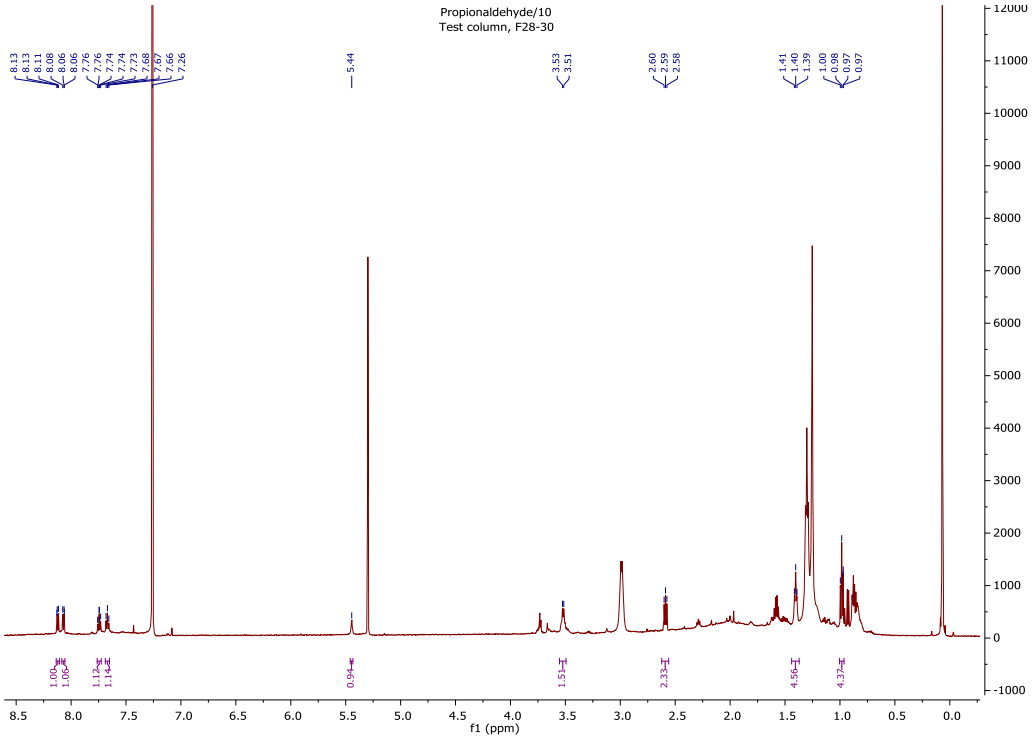


Figure 35: ^{1}H NMR of the reaction between lawsone and glycine in propionaldehyde

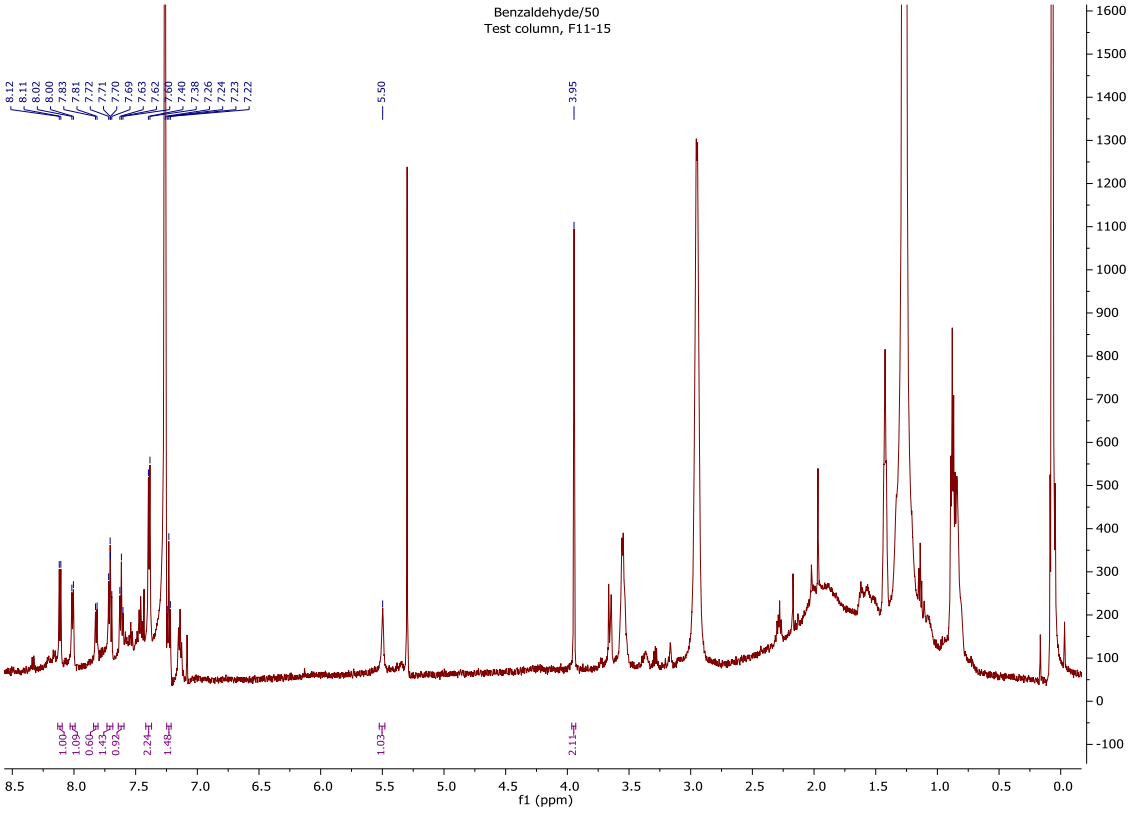


Figure 36: ^{1}H NMR of the reaction between lawsone and glycine in benzaldehyde

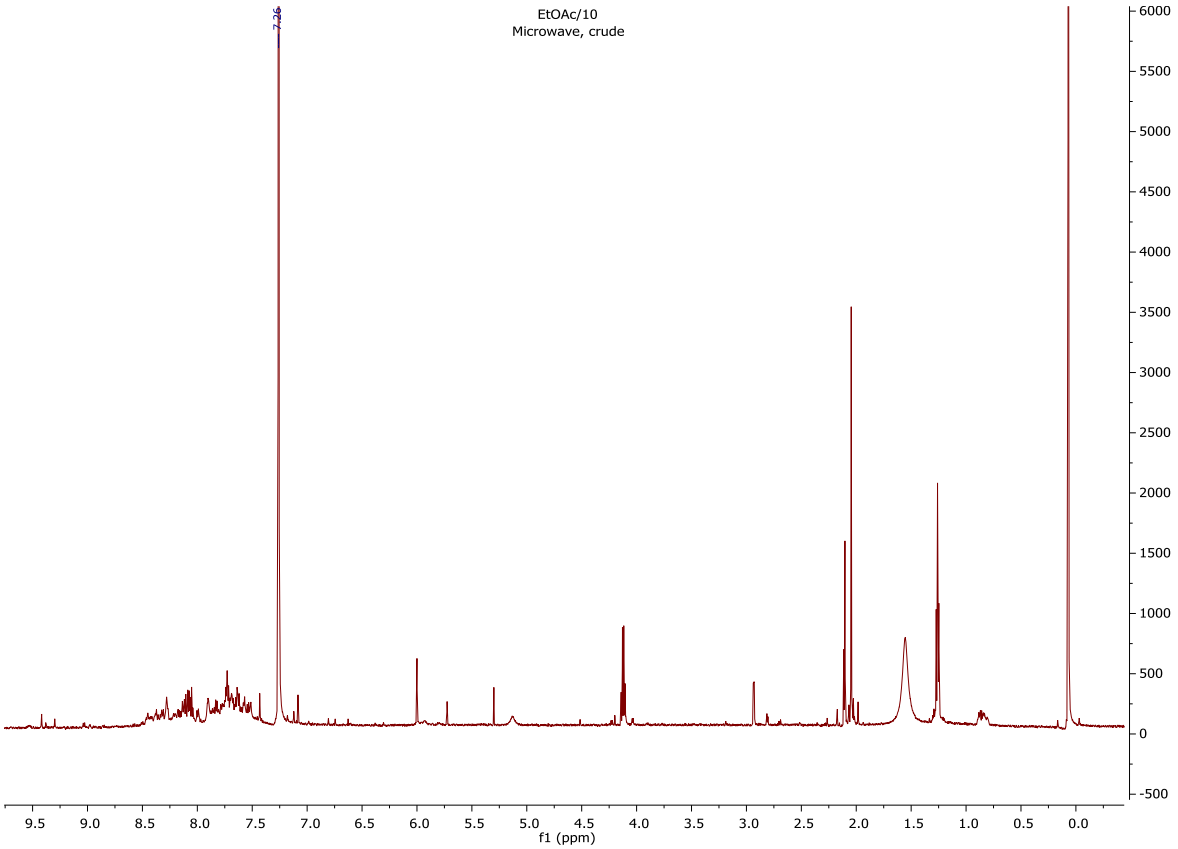


Figure 37: ^{1}H NMR of the crude reaction mixture between lawsone and glycine in ethyl acetate.

Appendix 3: Amino Acid Reaction with Lawsone Analogues

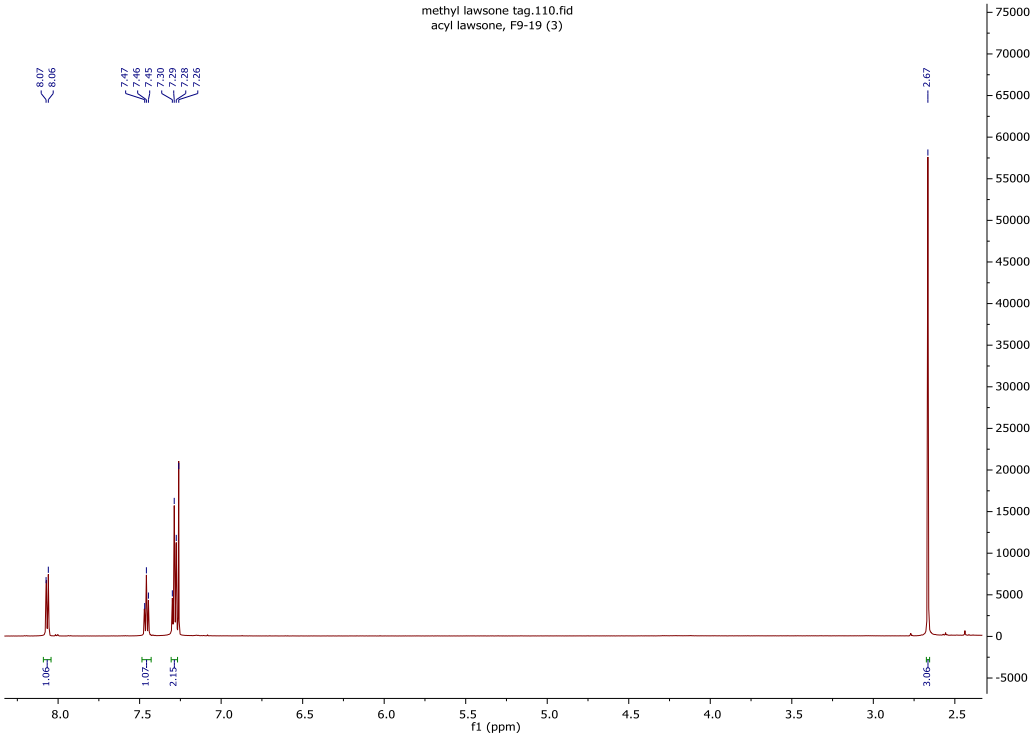


Figure 38: ^{1}H NMR spectrum of Fraction 1, isolated from the reaction of **164** with oxaloyl chloride and aluminium chloride

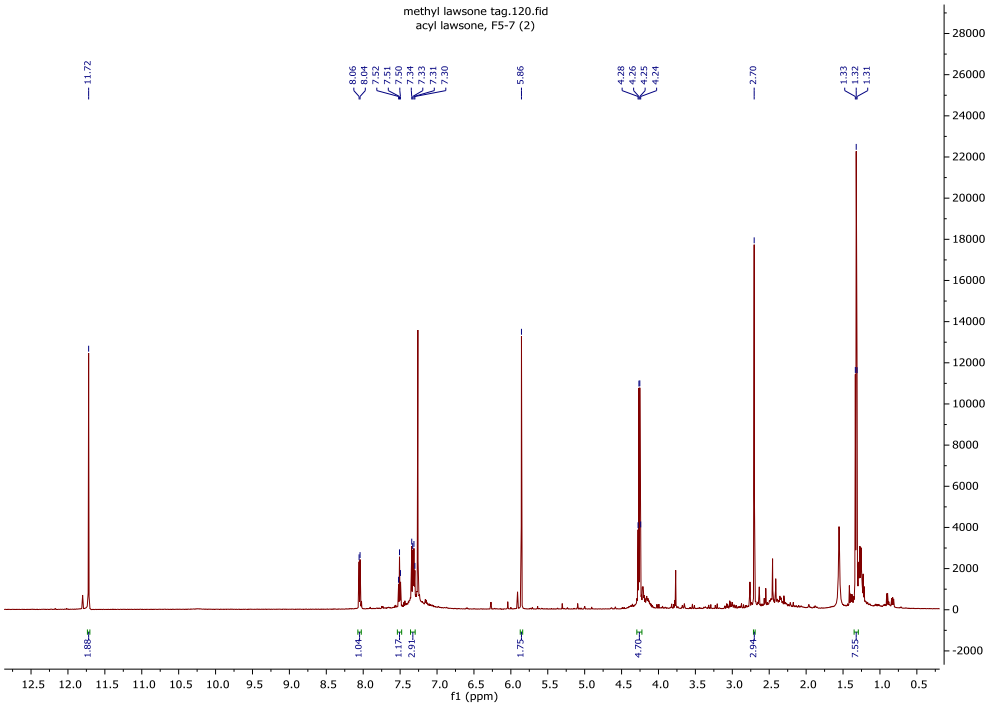


Figure 39: ^{1}H NMR spectrum of Fraction 2, isolated from the reaction of **164** with oxaloyl chloride and aluminium chloride

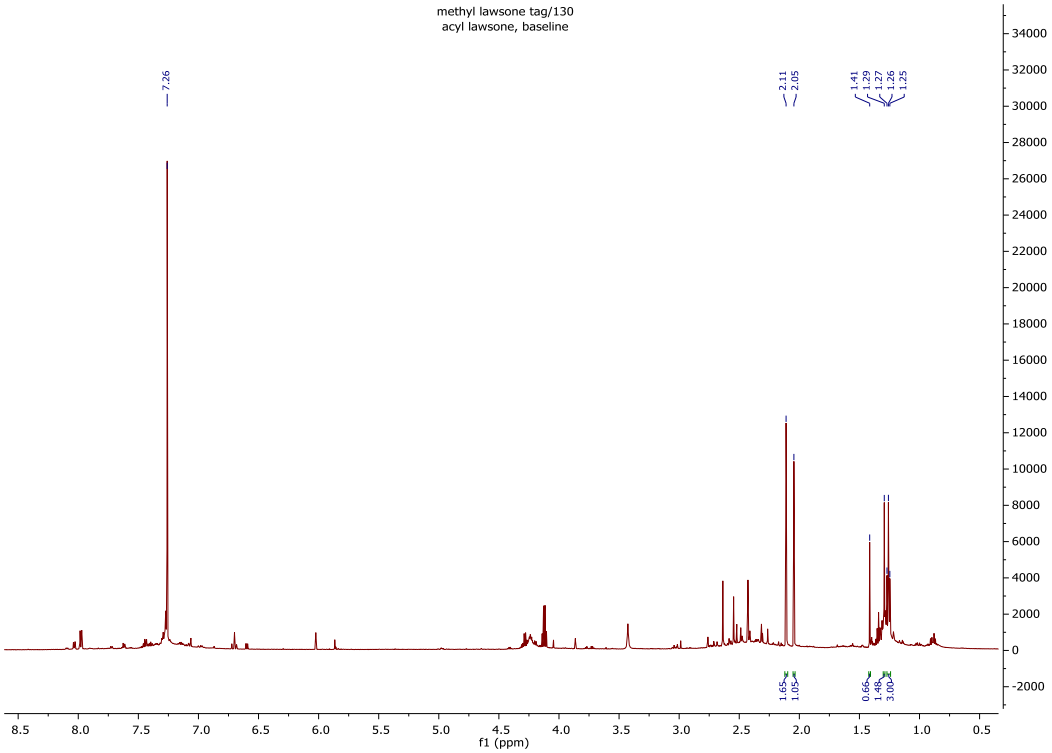


Figure 40: ^{1}H NMR spectrum of Fraction 3, the baseline isolated from the reaction of **164** with oxaloyl chloride and aluminium chloride

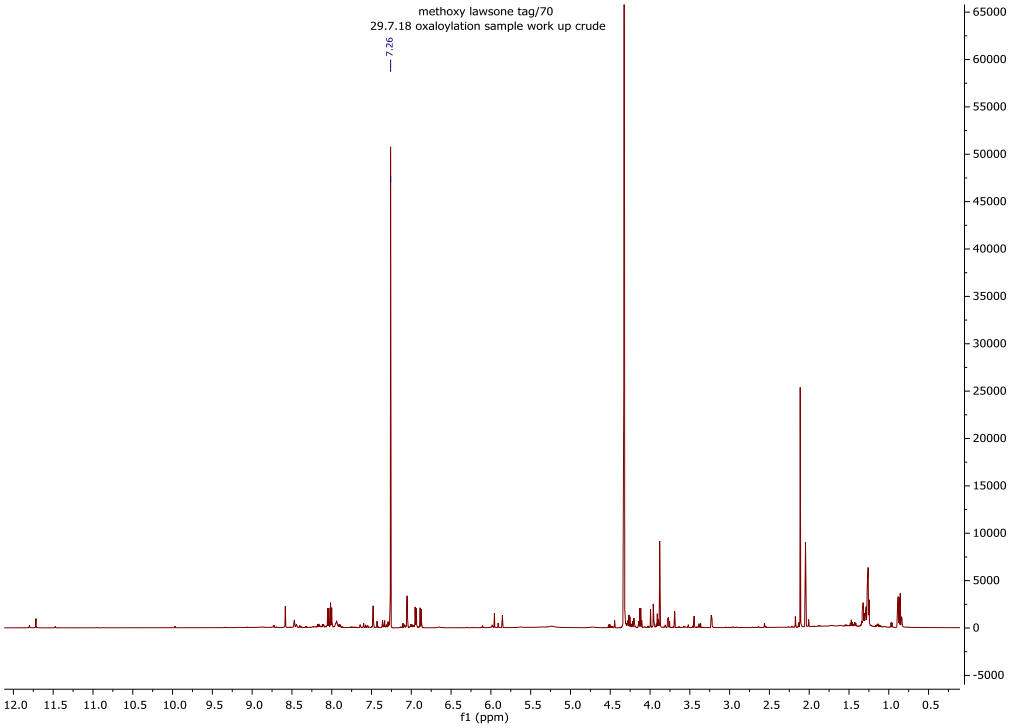


Figure 41: ^{1}H NMR spectrum of the isolated product from the reaction of **160b** with oxaloyl chloride and aluminium chloride

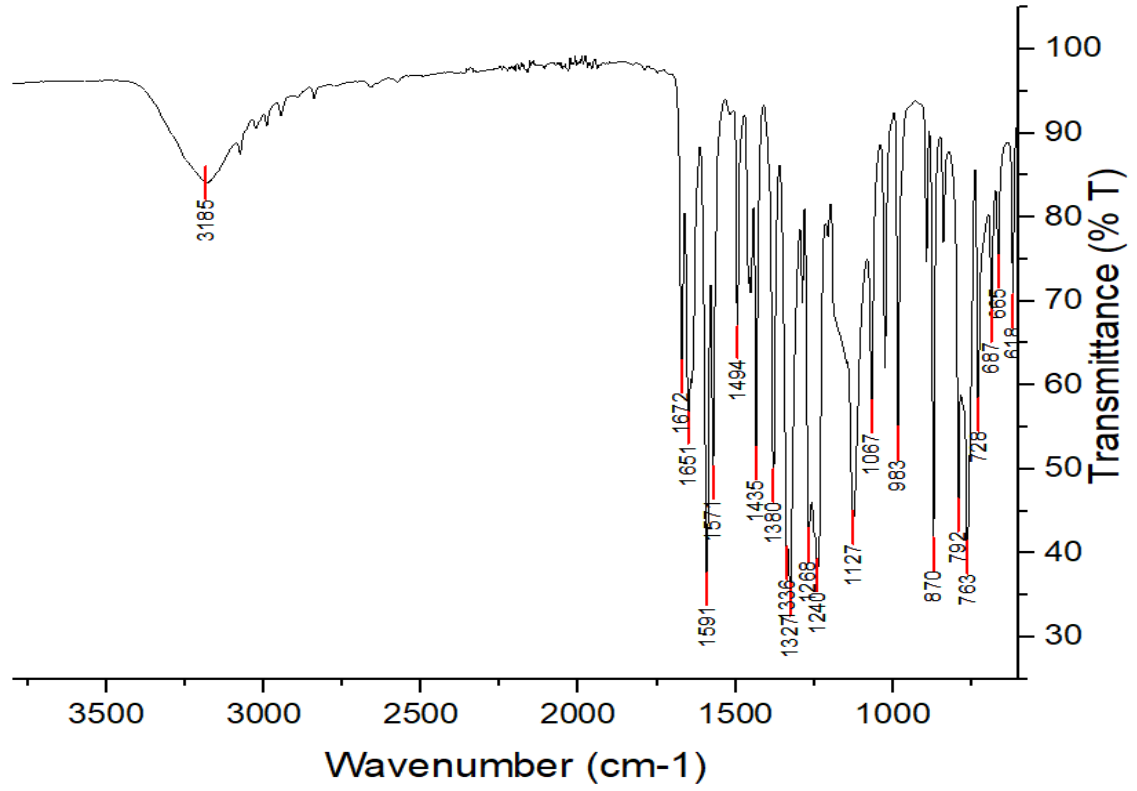


Figure 42: IR spectrum of 2-hydroxy-6-methoxy-1,4-naphthoquinone (**150**)

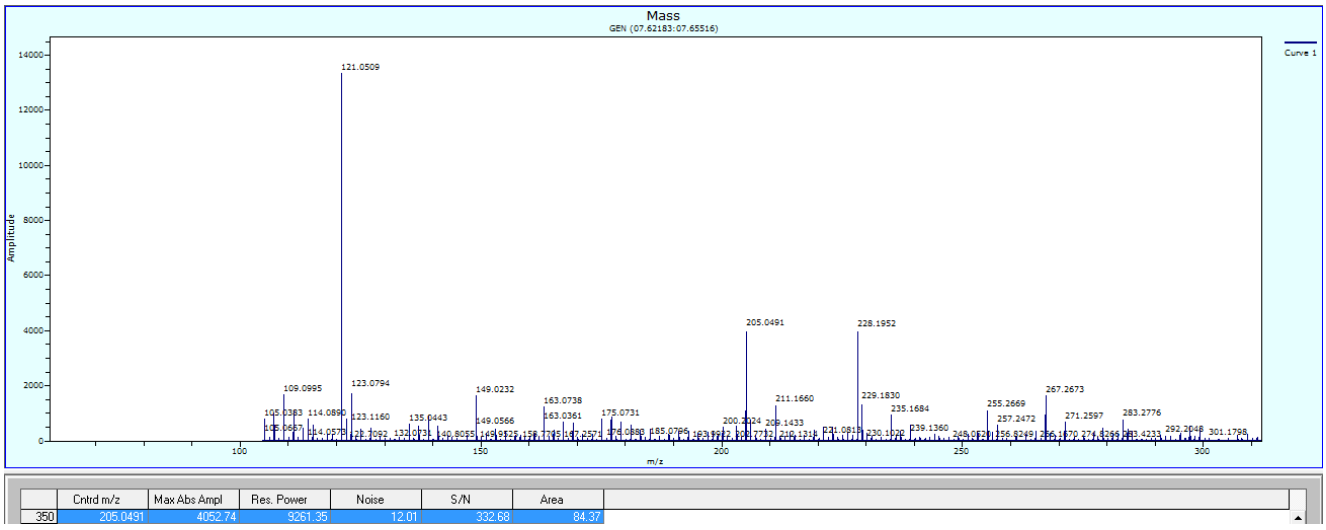


Figure 43: Mass spectrum of compound **150** showing a possible molecular ion of m/z=205.0491

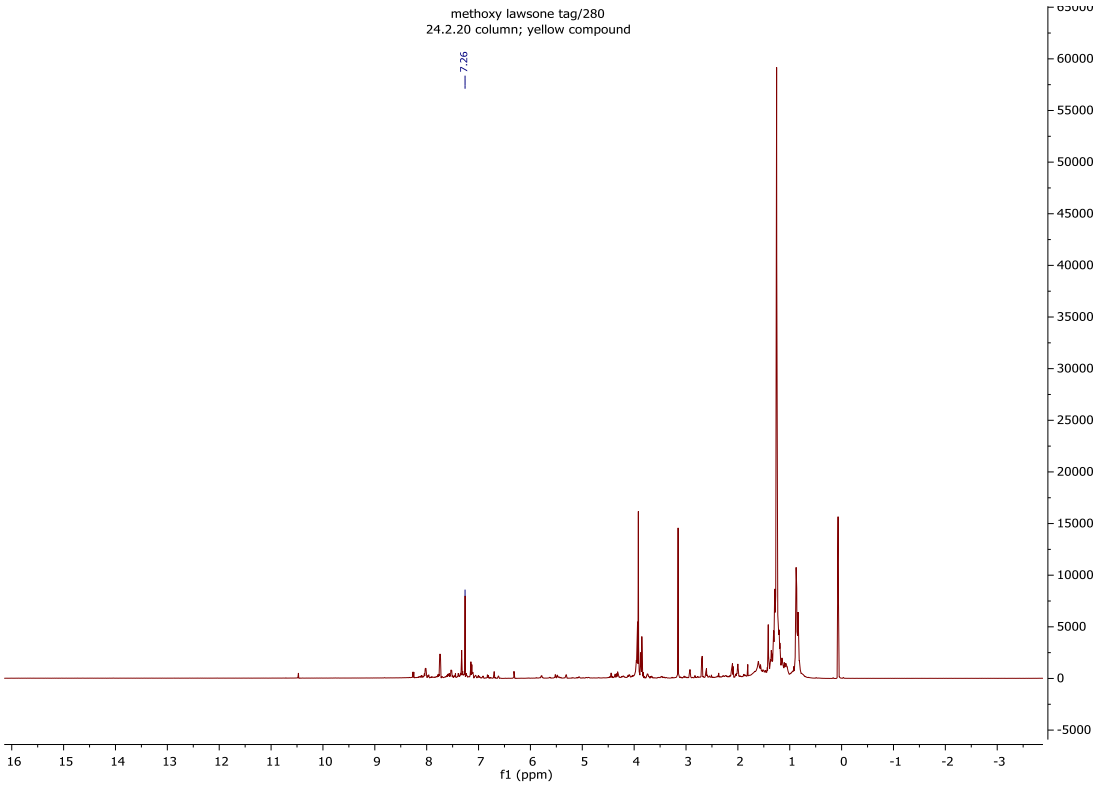


Figure 44: ^{1}H NMR spectrum of the isolated yellow product from the reaction of **150** with glycine in ethanol

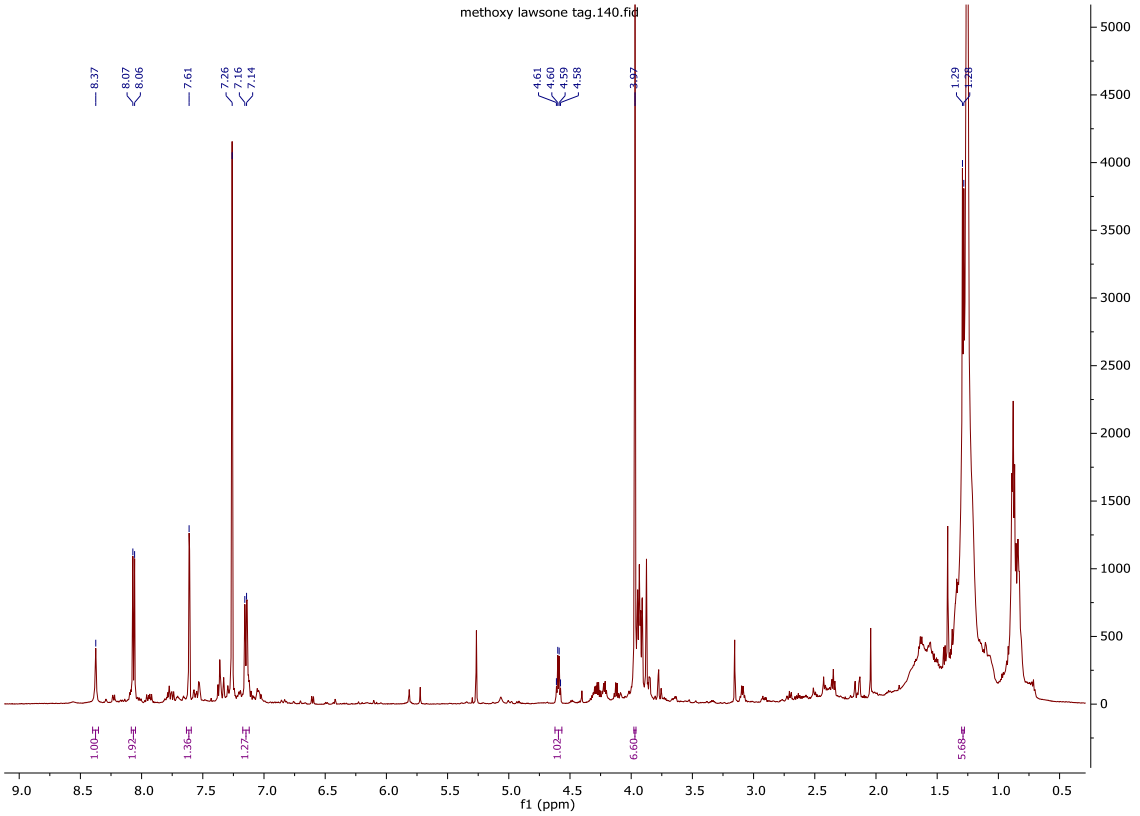


Figure 45: ^{1}H NMR spectrum of red dimer **156**, from the reaction between glycine and **150** in ethanol and H_2O_2

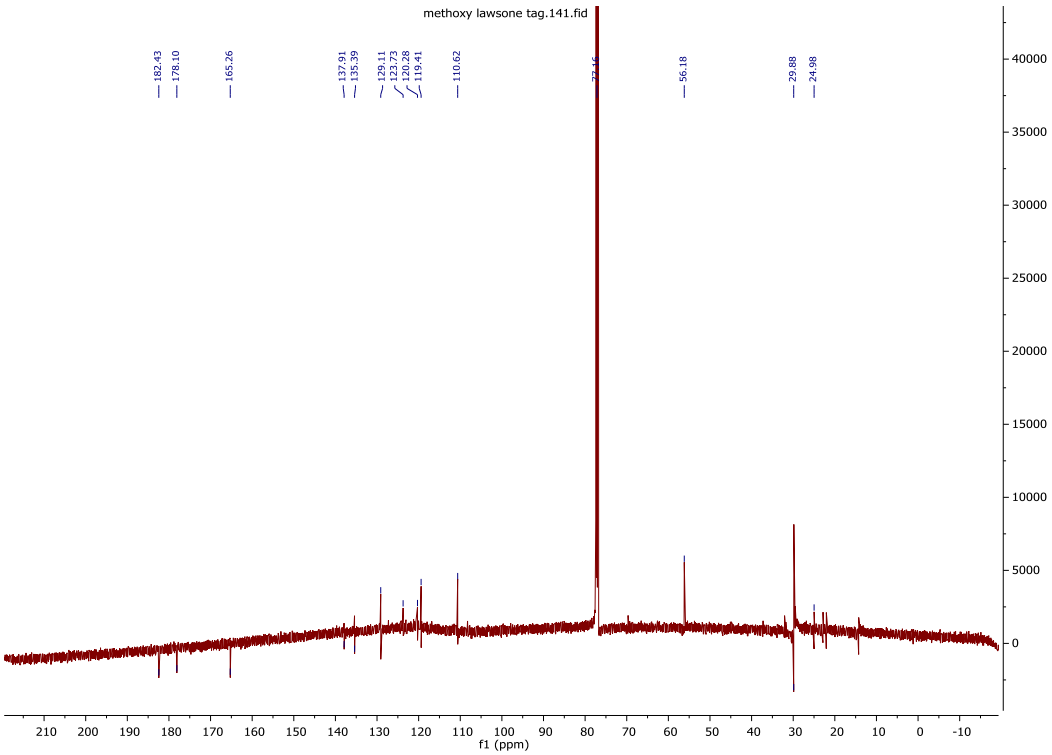


Figure 46: ^{13}C NMR spectrum of red dimer **156**, from the reaction between glycine and **150** in ethanol and H_2O_2

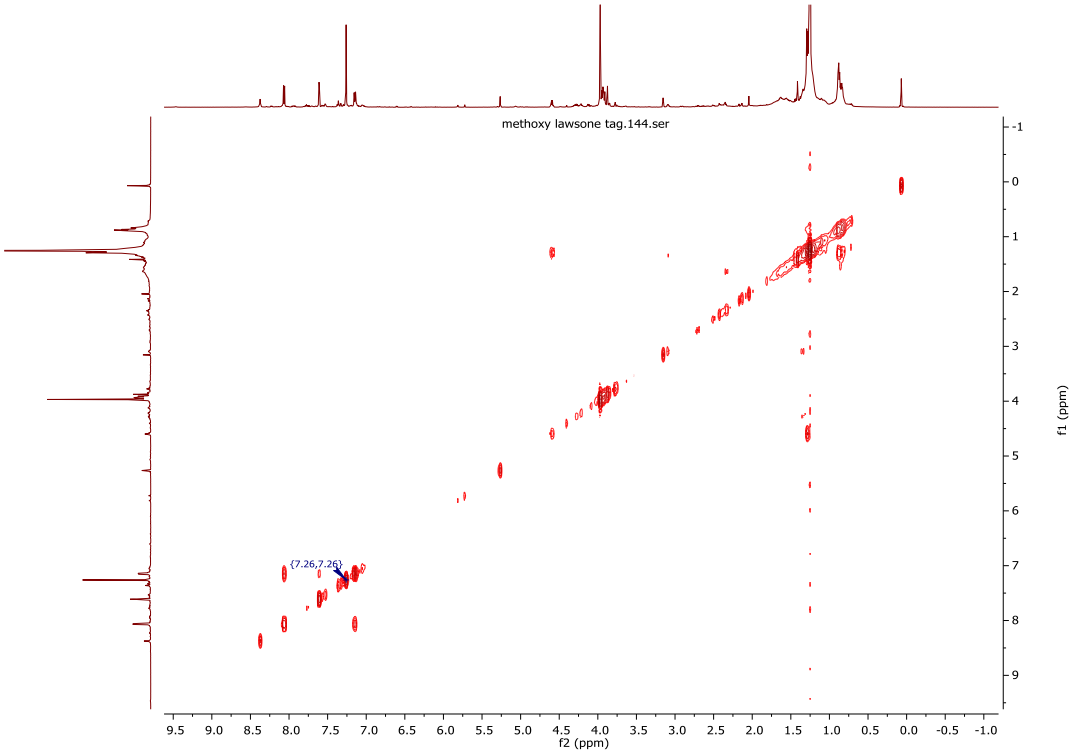


Figure 47: COSY 2D NMR spectrum of red dimer **156**, from the reaction between glycine and the **150** in ethanol and H_2O_2

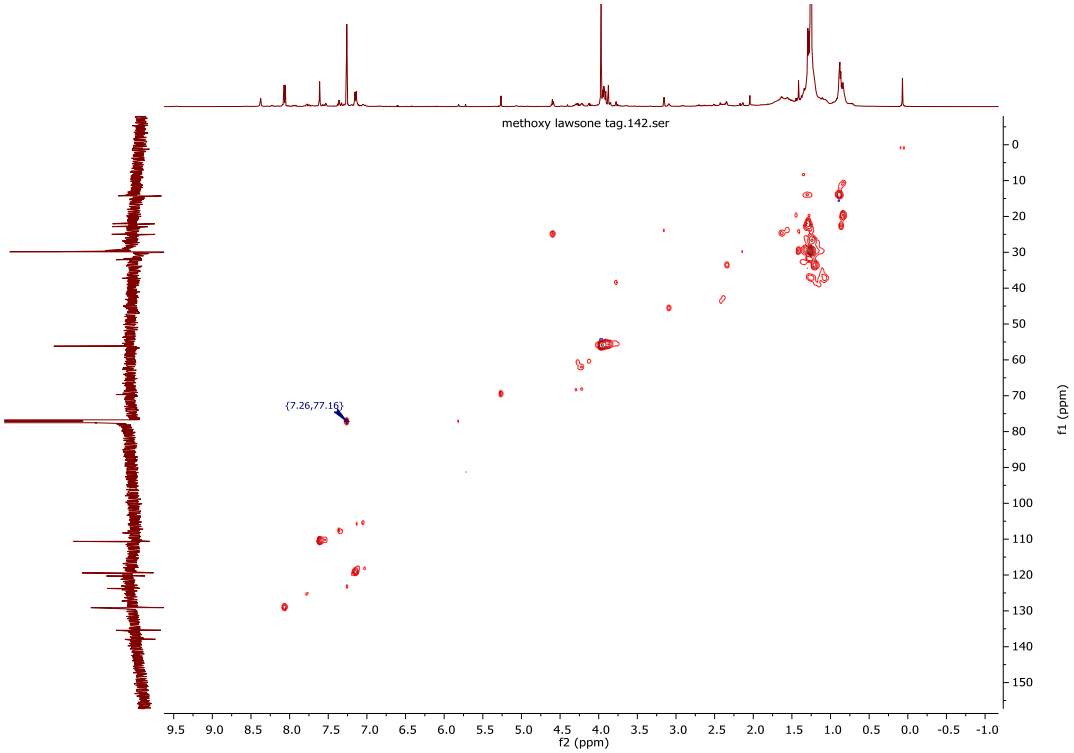


Figure 48: HSQC 2D NMR spectrum of red dimer **156**, from the reaction between glycine and **150** in ethanol and H_2O_2

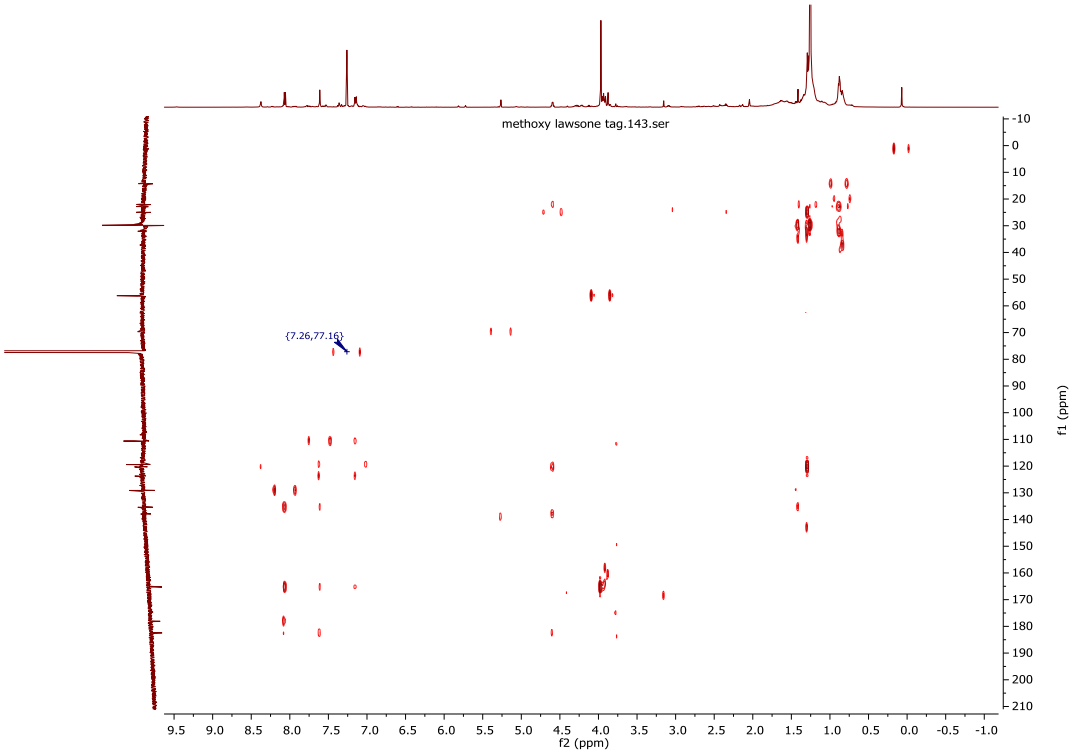
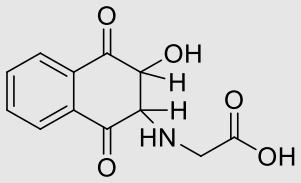
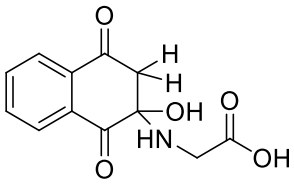


Figure 49: HMBC 2D NMR spectrum of red dimer **156**, from the reaction between glycine and **150** in ethanol and H_2O_2

Table 3: Modelling of intermediates via addition (**122**) and substitution mechanism (**107**), showing the Gibbs Free Energy of the optimised structures

Intermediate	Parameters	Gibbs Free Energy (au)
 **112** Addition mechanism	Optimisation, min B3LYP 6-31 G (d)	-894.76615544 au
 **107** Substitution mechanism	Opt, min B3LYP 6-31 G (d)	-889.60757935 au

Appendix 4: Intermediate Studies Towards Reaction Pathway Elucidation

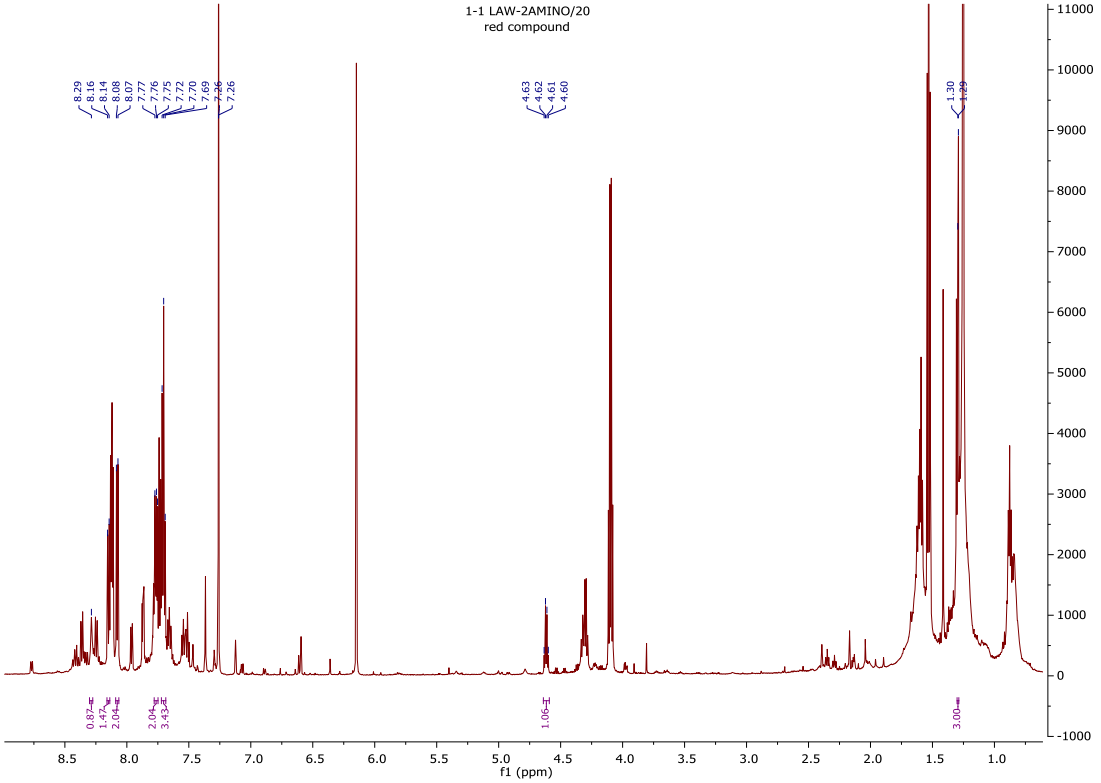


Figure 50. ^{1}H NMR spectrum of Compound **100** isolated from the reaction between lawsone and **102** (Condition A)

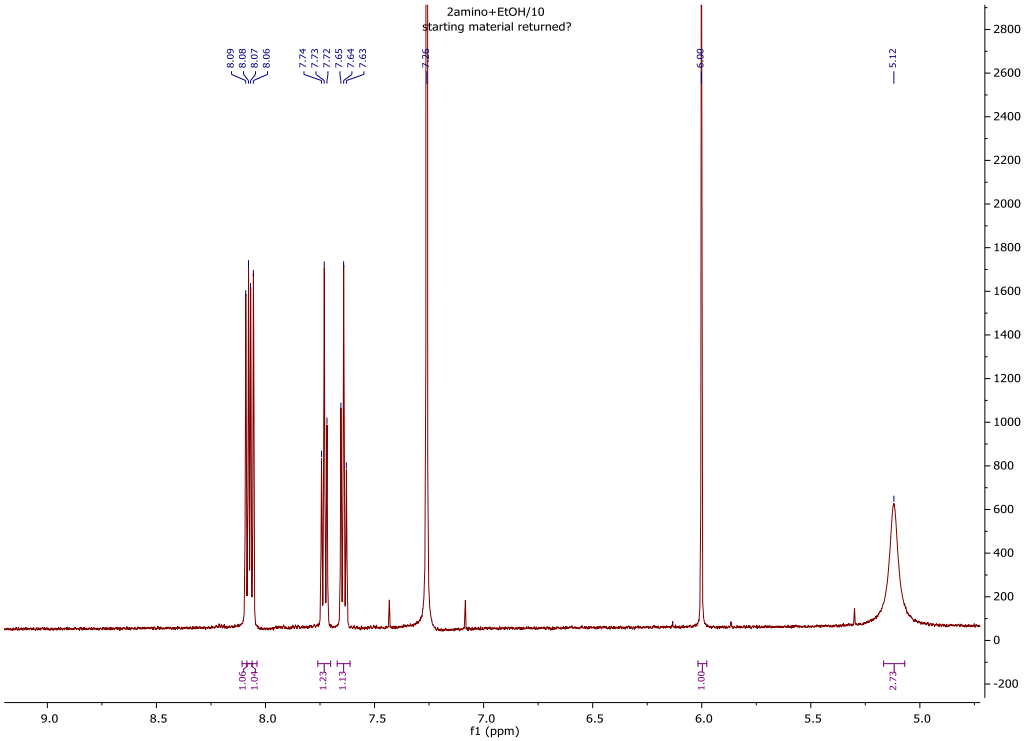


Figure 51. ^{1}H NMR spectrum of starting material returned from the reaction of **102** in ethanol (Condition B)

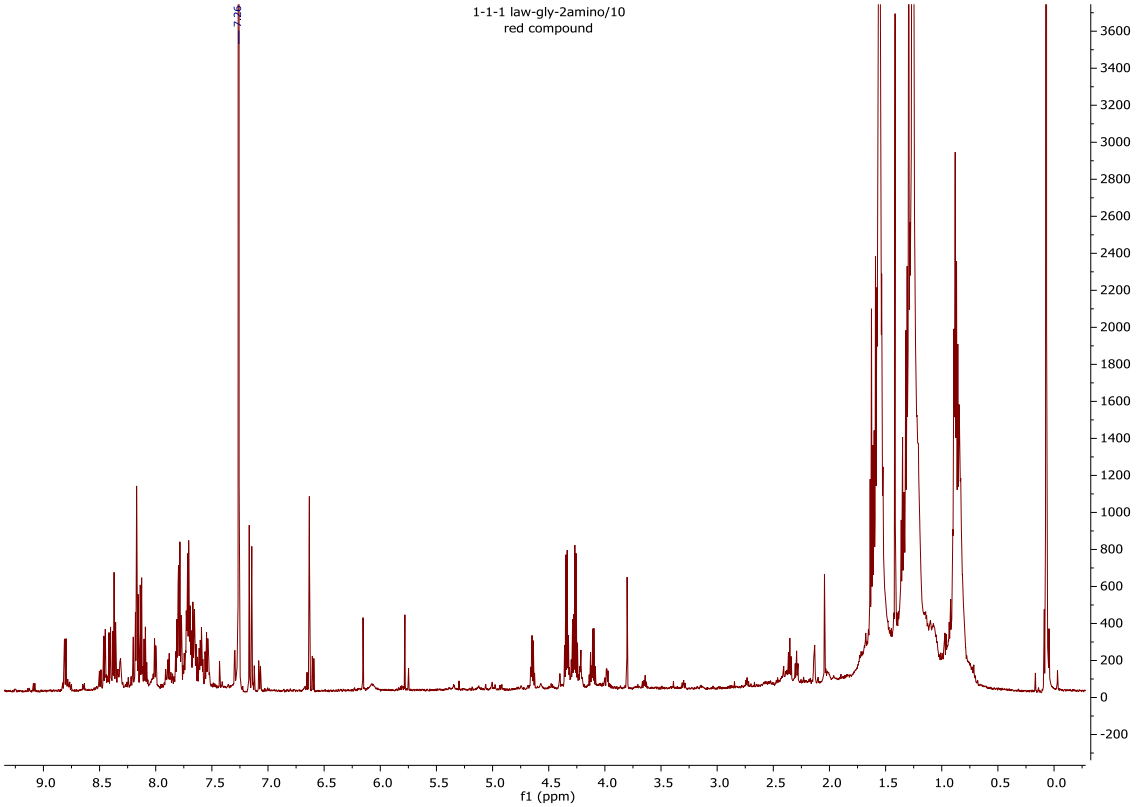


Figure 52. ^{1}H NMR spectrum of the crude reaction mixture from the reaction between glycine, lawsone and **102** in ethanol (Condition D)

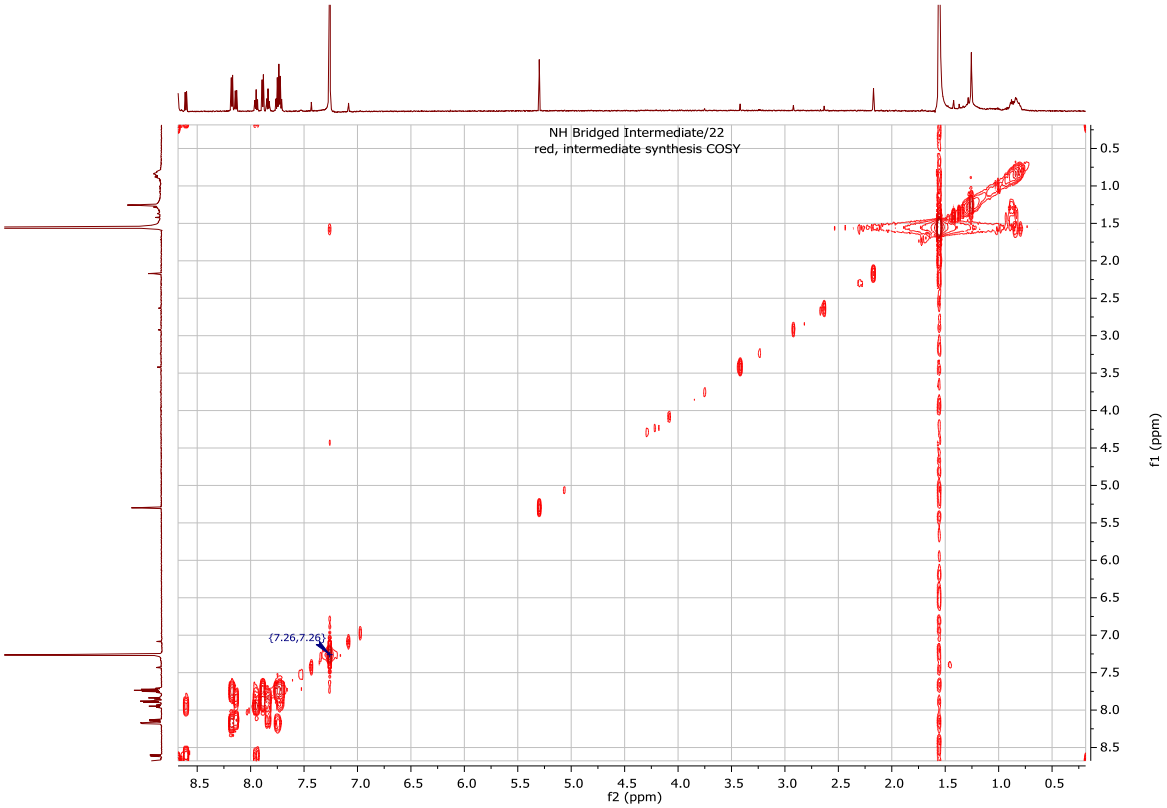


Figure 53. COSY NMR spectrum of red compound isolated from the attempted synthesis of **104**

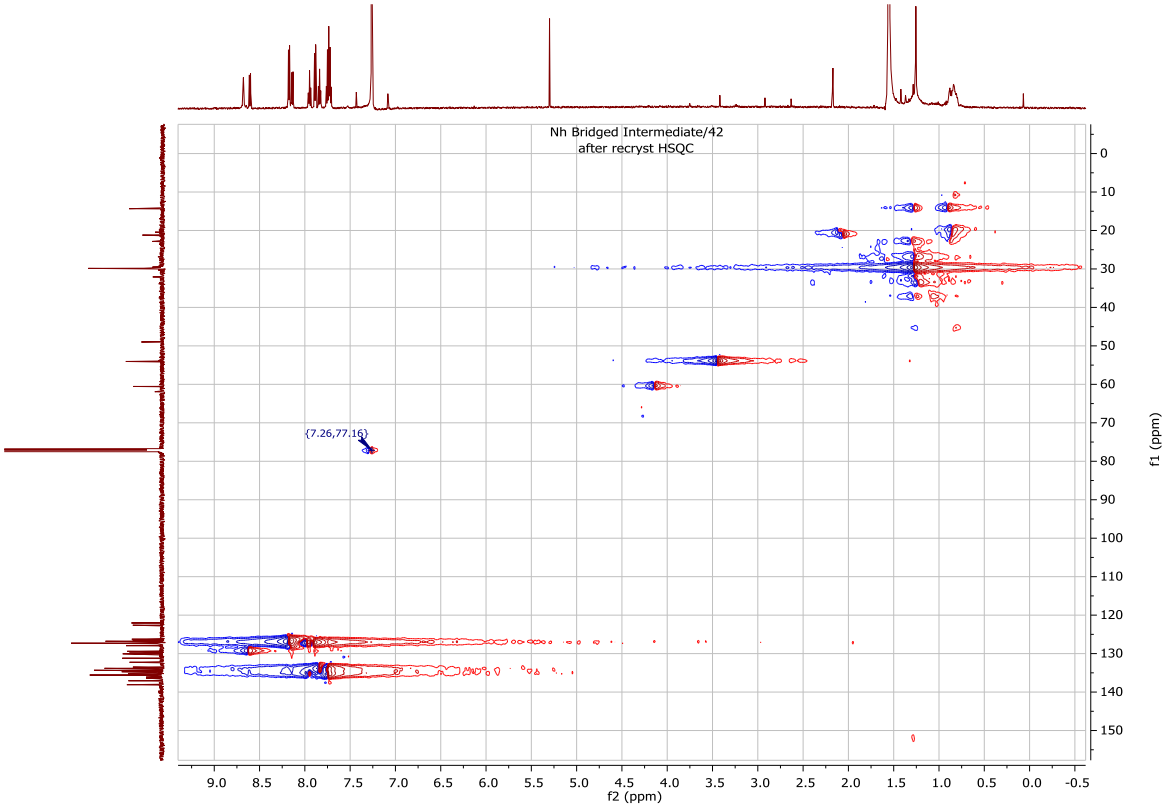


Figure 54. HSQC NMR spectrum of red compound isolated from the attempted synthesis of **104**

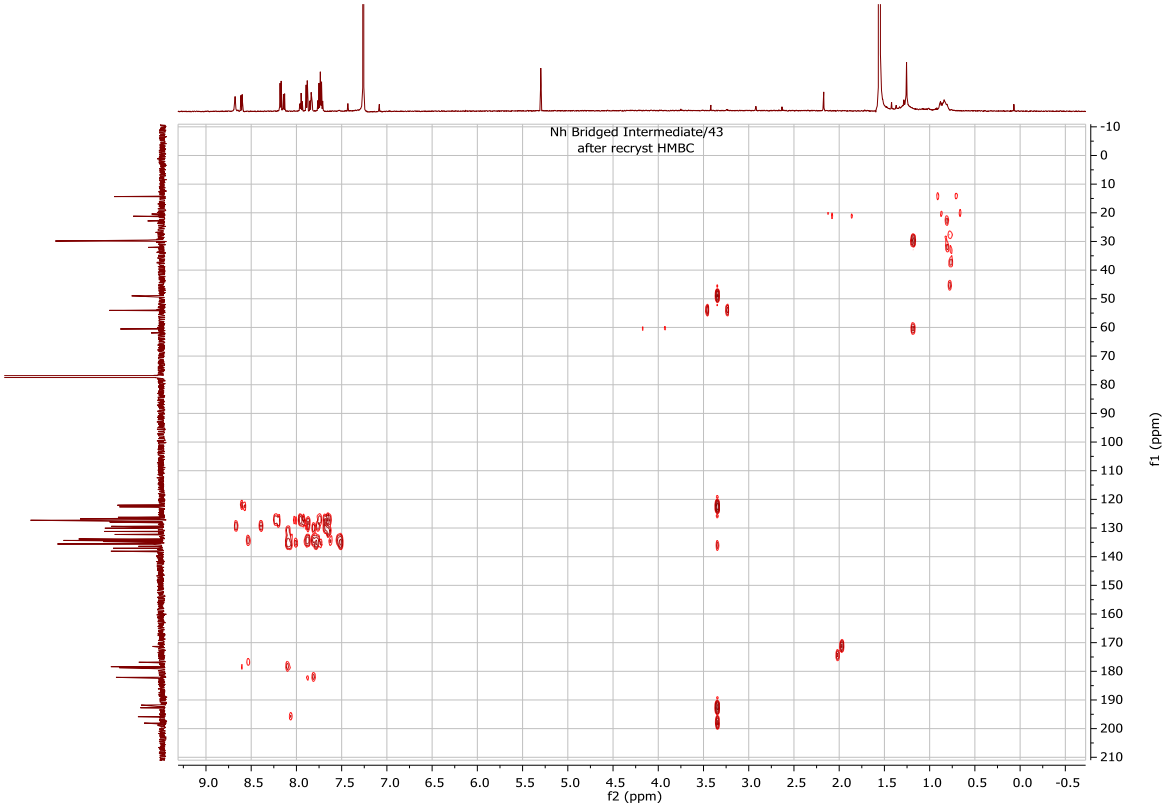


Figure 55. HMBC NMR spectrum of red compound isolated from the attempted synthesis of **104**

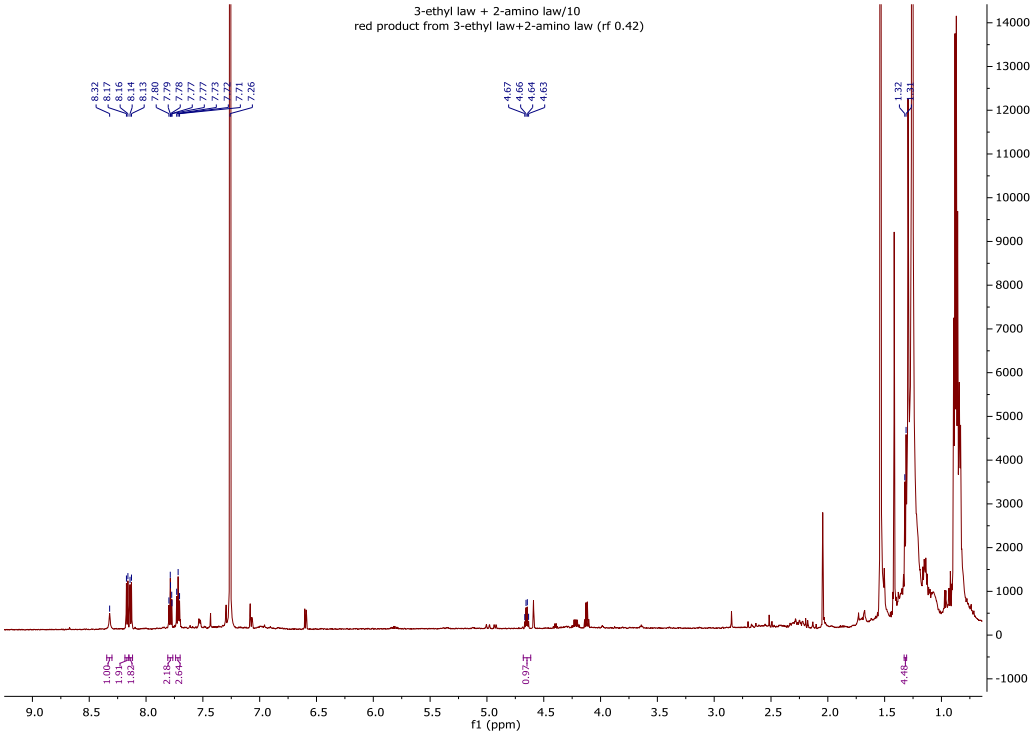


Figure 56. ^{1}H NMR of Compound **100** isolated from the reaction between **102** and **103** in ethanol (Condition 1)

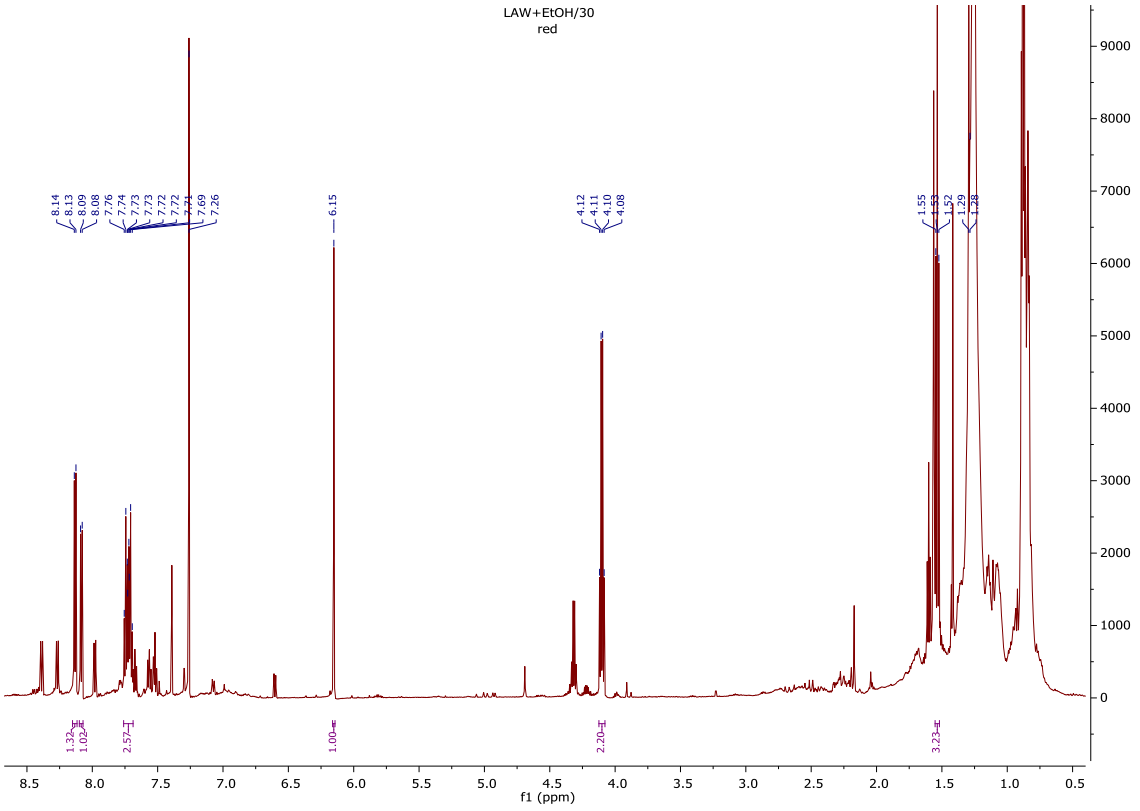


Figure 57. ^{1}H NMR of red compound **148** isolated from the reaction of lawsone in ethanol

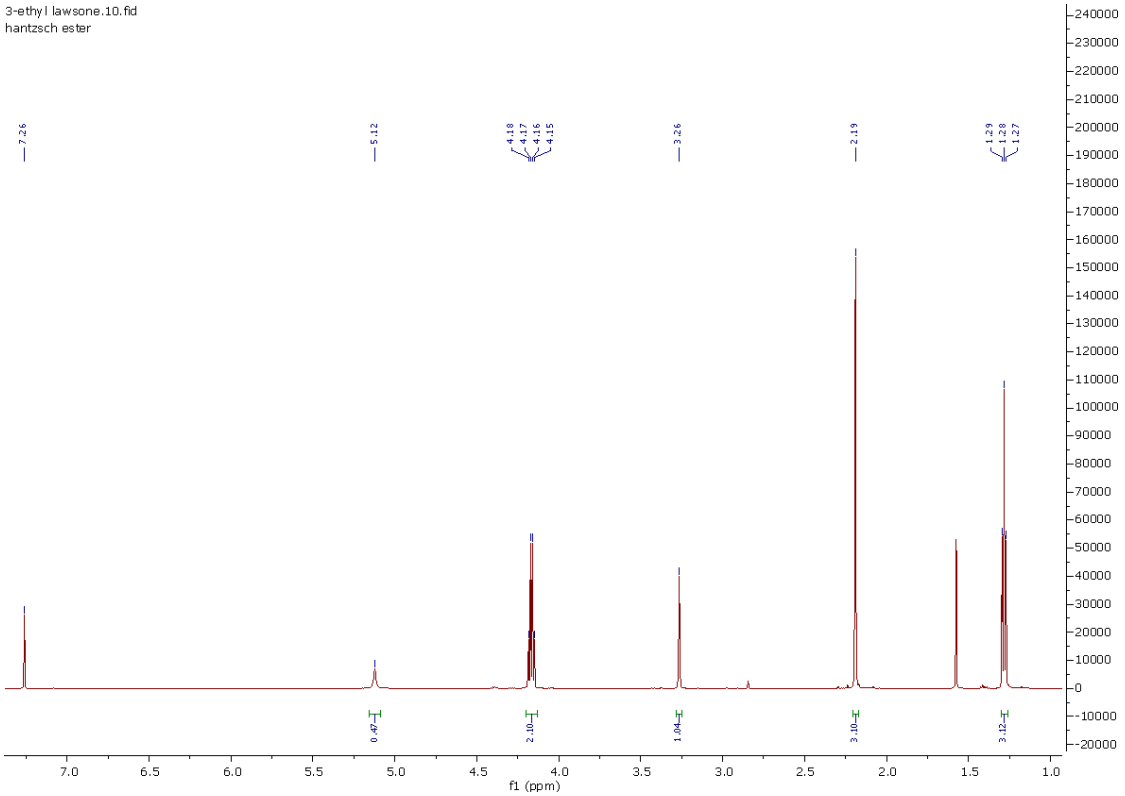


Figure 58. ^{1}H NMR for the synthesised Hantzsch ester (**118**)

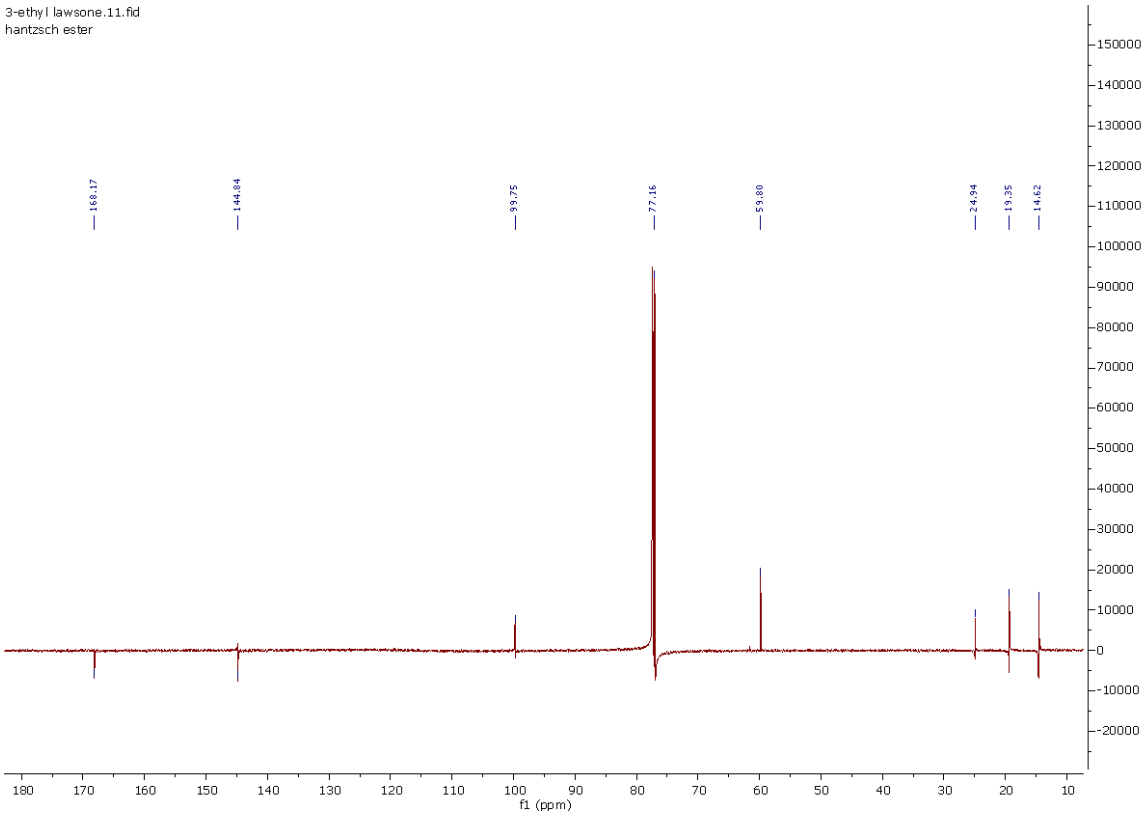


Figure 59. ^{13}C NMR for the synthesised Hantzsch ester (**118**)

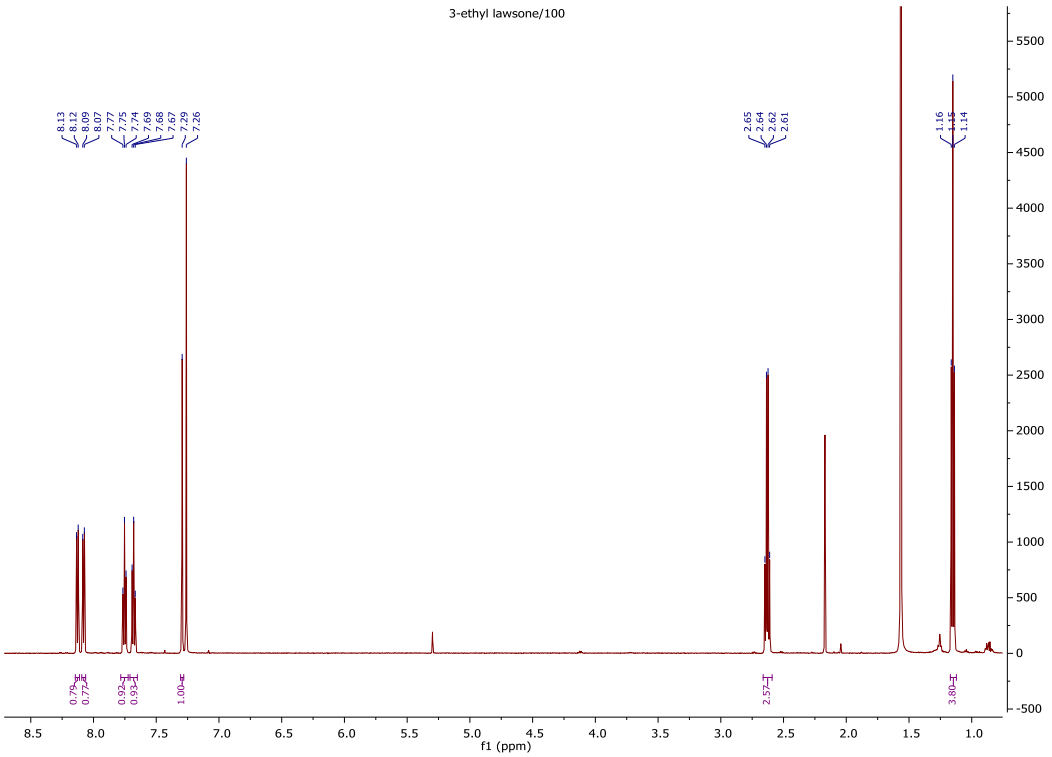


Figure 60. ^{1}H NMR for the synthesised 2-hydroxy-3-ethyl-1,4-naphthoquinone (**103**)

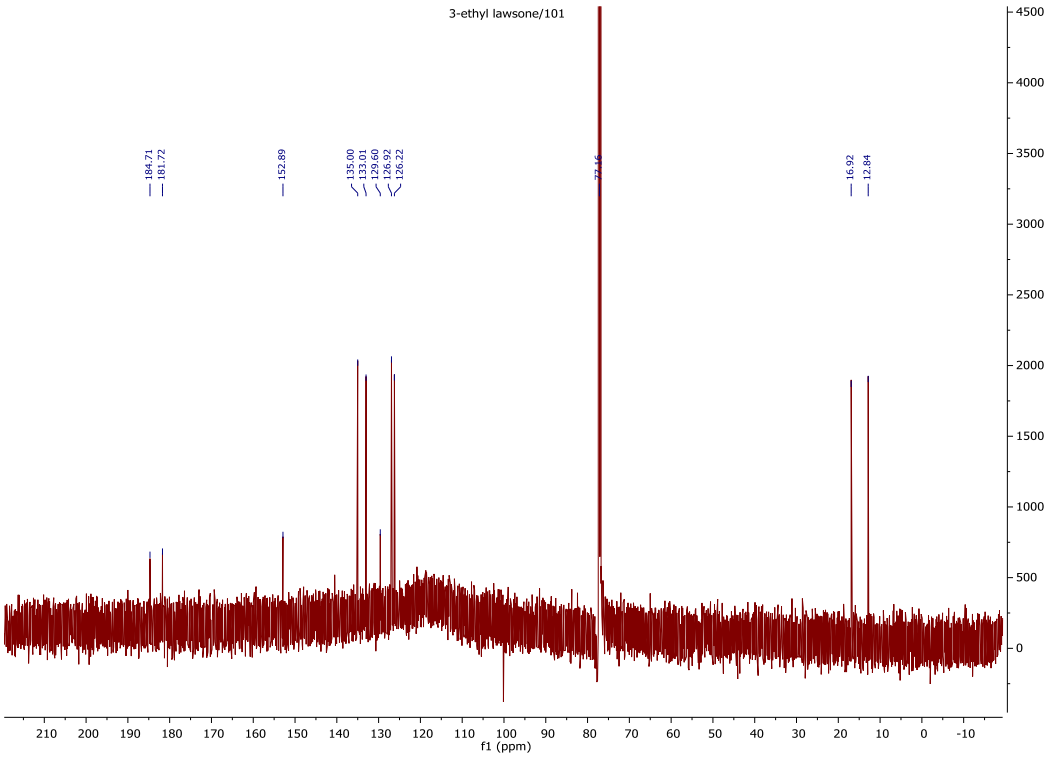


Figure 61. ^{13}C NMR for the synthesised 2-hydroxy-3-ethyl-1,4-naphthoquinone (**103**)

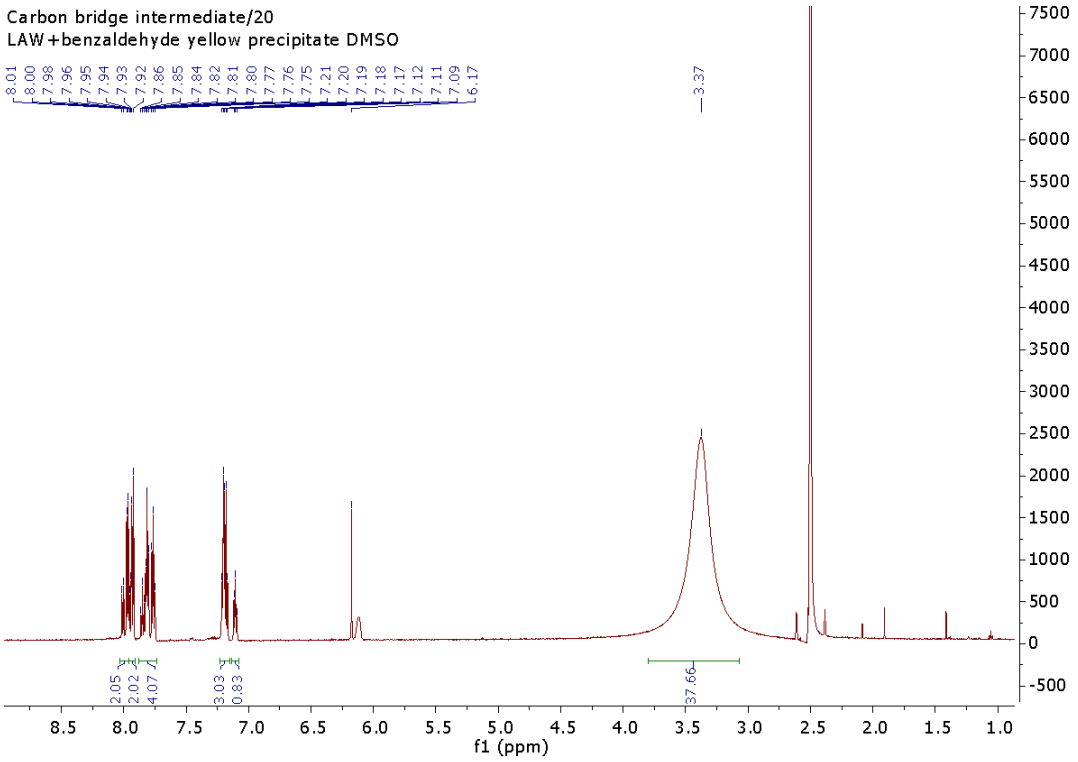


Figure 62. ^{1}H NMR of the yellow compound **187** formed from the reaction of benzaldehyde and lawsone in water:ethanol (50:50) and sulfuric acid

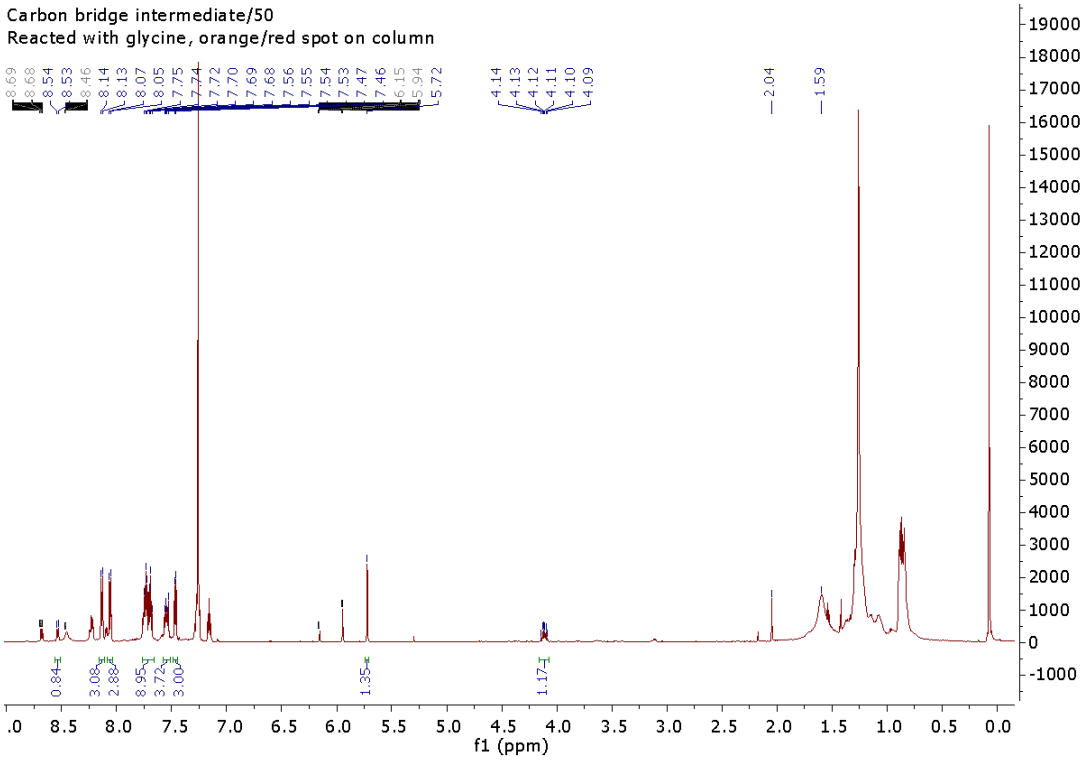


Figure 63. ^{1}H NMR of the red solid **145**, isolated from the reaction between **187** and glycine

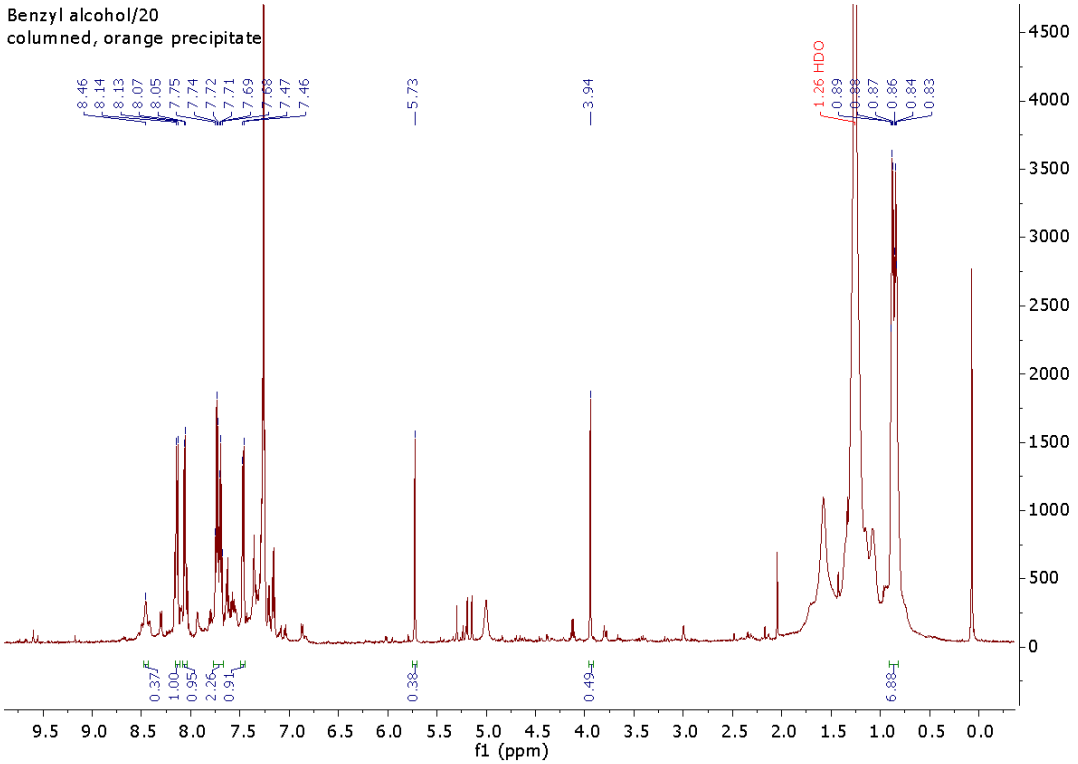
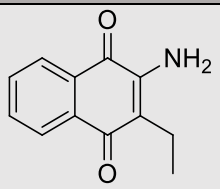
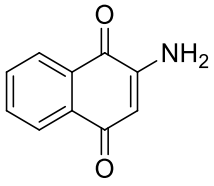


Figure 64. ^{1}H NMR of the orange/red compound **145** formed from the reaction between lawsone and glycine in benzyl alcohol

Table 4. Modelling of intermediates **105** and **102**, showing the Gibbs Free Energy of the optimised structures

Intermediate	Parameters	Gibbs Free Energy (au)
 2-amino-3-ethyl-,1,4-naphthoquinone (**105**)	Optimisation, min B3LYP 6-31 G (d)	-669.11686300 au
 2-amino-1,4-napthoquinone (**102**)	Opt, min B3LYP 6-31 G (d)	-590.48440527 au