Synthesis of novel analogues of dictyoquinazol A for the development of a treatment for stroke

A thesis submitted in fulfilment of the requirements for the degree of

Master of Philosophy in Chemistry

by

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The University of New South Wales

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Stroke is a major cause of mortality and morbidity in Australia, imposing a great social and economic burden. Unfortunately, current treatments for stroke are inadequate, and so there is a high demand for new drugs. Dictyoquinazol A is a mushroom-derived natural product that exhibits neuroprotective activity. It therefore has the potential to be developed into a novel treatment for stroke. The aim of this project was to contribute to this medicinal development process.

The first task in this project was to develop an efficient total synthesis of dictyoquinazol A. Two previous syntheses of this target had been published, but these methods were difficult to reproduce and are not step economical. In this project, a novel three-step synthesis of dictyoquinazol A was successfully developed, representing a substantial improvement over the existing syntheses in terms of yield and step count.

Next, an even more streamlined synthesis of dictyoquinazol A was attempted, based on the insight that this target molecule possesses an element of hidden symmetry. However, this more ambitious synthetic plan was not able to be realised, since the key step in this plan (a Cannizzaro-amidation reaction sequence) did not deliver the desired product in isolable quantity.

The next task in this project was to create a small library of structural analogues of dictyoquinazol A. The target analogues were designed to provide structure-activity data focusing on the primary alcohol and the methoxy groups of dictyoquinazol A. The new three-step synthesis developed above proved to be an effective method for creating these analogues.

The final task in this project was to perform a preliminary biological assessment of dictyoquinazol A and analogues, using a cell-based model of stroke. It was shown that cultured nerve cells could be protected against staurosporine-induced apoptosis by co-treatment with dictyoquinazol A and analogues. This represents a previously unrecognised mode of action of these compounds. Based on this data, a preliminary structure-activity relationship profile of the molecule was created.

Overall, these studies have contributed to the medicinal development of dictyoquinazol A towards a novel treatment for stroke.

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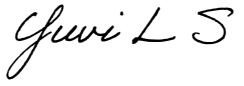
Signed  .....

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Acknowledgements and Statement of Contribution of the Student

This last 18 months during my MPhil project have been really challenging, but I've also found that research is something that I really enjoy. I would not have been able to get through it without the help of all the people who've crossed my path, and I would like to thank them all.

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I greatly appreciate the collaboration of other UNSW academic staff during my MPhil studies, especially the staff of the NMR Facility for their support.

I dedicate this thesis to my family: my parents, my sister, my brother, my sister-in-law, my nephews and my husband Bruno. Especially to my mum, who always motived me in difficult times and who with her love and confidence in me, reminded me to trust myself. And my husband who with his love and company helped me to finish this project.

I certify that this thesis contains work carried out by myself except where otherwise acknowledged.

Abstract

Stroke is a major cause of mortality and morbidity in Australia, imposing a great social and economic burden. Unfortunately, current treatments for stroke are inadequate, and so there is a high demand for new drugs. Dictyoquinazol A is a mushroom-derived natural product that exhibits neuroprotective activity. It therefore has the potential to be developed into a novel treatment for stroke. The aim of this project was to contribute to this medicinal development process.

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new three-step synthesis developed above proved to be an effective method for creating these analogues.

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Overall, these studies have contributed to the medicinal development of dictyoquinazol A towards a novel treatment for stroke.

Abbreviations

aq.	aqueous
bpy	2,2'-bipyridine
COSY	correlation spectroscopy
CuOTf	copper(I) trifluoromethanesulfonate
DCE	1,2-dichloroethane
DCM	dichloromethane
Deoxo-Fluor ™	bis-(2-methoxyethyl)aminosulfur trifluoride
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EDC	*N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide
Et_3N	triethylamine
equiv.	molar equivalents
FBS	Fetal bovine serum
HMBC	heteronuclear multiple-bond correlation
HOBt	1-hydroxybenzotriazole
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single-quantum correlation
IR	infrared
J	coupling constant (NMR)
m.p.	melting point
MEM	minimum essential media
M.S.	molecular sieves

MTT	3-(4,5-dimethyltriazol-2-yl)-2,5-diphenyltetrazolium bromide
m/z	mass-to-charge ratio
NMI	1-methylimidazole
NMR	nuclear magnetic resonance
r.t.	room temperature
STS	1-(5-isoquinolinesulfonyl)-2-methyl-piperazine
TDFH	Tetradecafluorohexane or Perfluorohexane
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
THF	tetrahydrofuran
TLC	thin layer chromatography
TFMS	zinc trifluoromethanesulfinate

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Chapter 1:

Introduction

1.1 Stroke

Stroke occurs when an artery supplying blood to the brain either becomes blocked or begins to bleed[1-3] (Figure 1). Since blood is the sole carrier of oxygen, glucose and other important nutrients to the brain, disruption of the blood supply has catastophic implications. Some of the brain's nerve cells die shortly after the onset of stroke. Other affected cells may survive for a few hours, and may even recover if the blood supply is rapidly restored; but if not, they will also die.

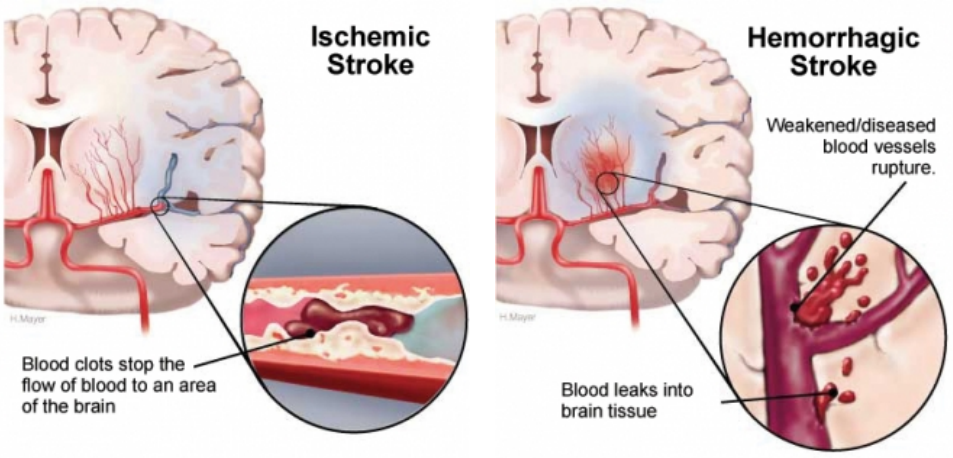


Figure 1: Stroke is caused by arterial blockage or bleeding[4].

Stroke is the second largest cause of mortality in Australia, and even those patients who survive a stroke often suffer temporary or permanent impairment to their speech, mental faculties and/or motor skills depending on which part of the brain was affected. This imposes a great social and economic burden on Australia.

Stroke resulting from an arterial blockage is known as *ischemic stroke*. This represents approximately 80% of cases[2]. The blockage may be caused by a plaque or clot formation (Figure 1), which causes a reduction in the blood flow to the brain. This

leads to a complex cascade of adverse effects. For example, energy failure and ion gradient collapse results in reversal of the reuptake of the excitatory neurotransmitter L-glutamate *via* glutamate transporters, and so the synapses become flooded with excessive L-glutamate. This leads, in turn, to an increase in the uptake of calcium ions *via* NMDA (*N*-methyl-D-aspartate) receptors[5], which promotes cell death by triggering an excitotoxic cascade that involves the activation of calcium ion dependent enzymes, the disruption of mitochondrial function, and cell necrosis or apoptosis[6].

As well as the phenomenon of excitotoxicity described above, ischemic stroke also leads to increased concentrations of superoxide, hydroxyl radicals, hydrogen peroxide and other reactive oxygen species. These species damage important cell components including enzymes, membrane lipids, nucleic acids and mitochondria in a process known as oxidative stress[7].

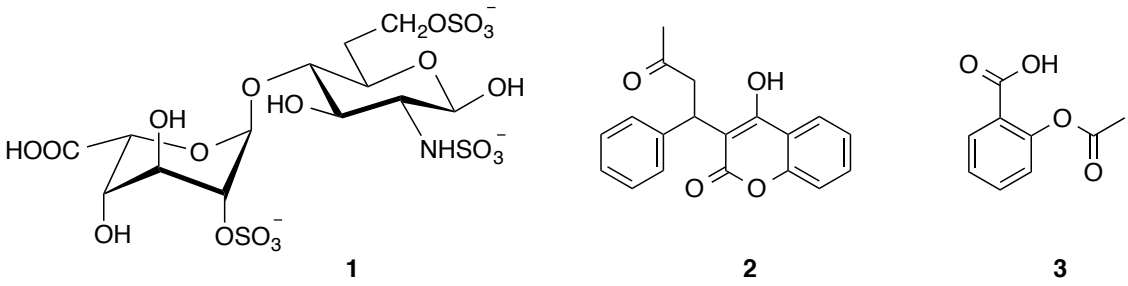
The brain's natural defense against injury and infection is neuroinflammation[8]. Unfortunately however, excessive post-stroke inflammation can result in additional injury. This is caused by the activation of glial cells (astrocytes and microglia), which contribute to neural damage by the release of pro-inflammatory cytokines, reactive oxygen species and excitatory amino acids.

Finally there is apoptosis, or programmed cell death. This is a natural mechanism of cellular self-destruction, and it is an inevitable result of the various types of injury caused by acute stroke.

Hemorrhagic stroke is caused by the rupture of a blood vessel in the brain (Figure 1). The rupture causes bleeding into the surrounding brain tissue[3], which damages the

proximal brain cells as well as reducing the blood flow to cells beyond the leak (which leads to the various adverse effects described above for ischemic stroke). Hemorrhagic stroke is considered to be the most severe type of stroke[9], as patients with intracerebral hemorrhaging require evaluation to detect and treat the cause of the bleeding. In addition, it is critical to keep a hemorrhagic stroke patient's blood pressure, intracranial pressure, sugar levels and oxygen levels at optimum levels to avoid further damage.

There are few available treatments for stroke[1]. Anticoagulants such as heparin (**1**), warfarin (**2**) and aspirin (**3**) are of some use in the prevention and treatment of ischemic stroke (Figure 2), but they would exacerbate bleeding in an intracerebral hemorrhage and hence cannot be used to treat hemorrhagic stroke.



1 **2** **3**

Figure 2: Current drugs for preventing stroke.

The only other approved therapy that can reduce neurological damage in ischemic stroke is a protein-based drug called tissue plasminogen activator (tPA, Figure 3). However this treatment is only effective if administered within 4.5 hours of the onset of stroke[1]; after that time tPA could cause hemorrhage, and it can also worsen neuronal damage caused by L-glutamate. The narrow time window means that,

unfortunately, only a small percentage of stroke patients are currently treated with tPA[10].

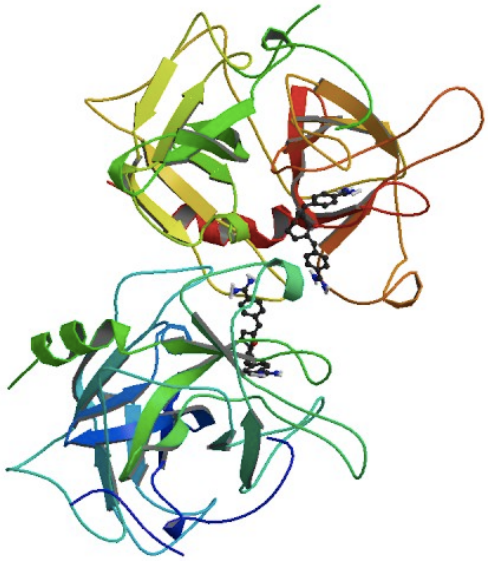


Figure 3: Tissue plasminogen activator is the only approved treatment for ischemic stroke.

[PDB code 1A5H]

Despite intense research efforts, effective pharmacological strategies to protect nerve cells in ischemic tissue remain elusive. This is partly because up to the present, drug development has tended to target a single step of the complex excitotoxic cascade, and it does not distinguish between damaged and healthy tissue. It is clear that there is a great need for new stroke treatments.

1.2 A new lead compound for a neuroprotective drug

Dictyophora indusiata (Figure 4) is an edible mushroom that is found in many tropical locations around the world, including parts of Asia, Africa, the Americas and Australia.

Its distinctive appearance has led to the common name of "the veiled lady"[11]. In addition to its use as a foodstuff, various part of this mushroom have long been used in traditional medicine to treat a wide variety of ailments including inflammatory diseases, neural diseases, hypertension, diarrhea and cancer. It has also been prescribed as an aphrodisiac.

Figure 4: *Dictyophora indusiata* is an edible/medicinal mushroom commonly known as "the veiled lady"[12].

The traditional use of this fungus to treat neural diseases suggests that it may contain novel neuroprotective compounds. Motivated by this possibility, Lee and co-workers in 2002 performed an organic extraction of 1.2 kg of the mushroom[13]. Three novel alkaloids termed dictyoquinazols A–C (**4**–**6**, Figure 5) were subsequently isolated in milligram quantities from the organic extract and characterised by NMR, HRMS, IR and UV spectroscopy.

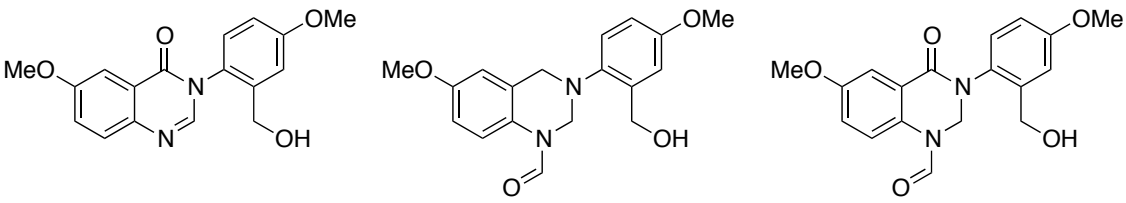


Figure 5: Structures of the novel alkaloids, dictyoquinazols A–C.

Compounds **4–6** contain variations of the quinazolinone moiety[14], a feature that is rare in natural products but that is a privileged structure in CNS-active drugs (Figure 6). For example, the clinical sedative methaqualone (**7**), the diuretic drug quinethazone (**8**) and the sedative/hypnotic/anticancer drug (**9**) are also based on this heterocyclic structure[15,16].

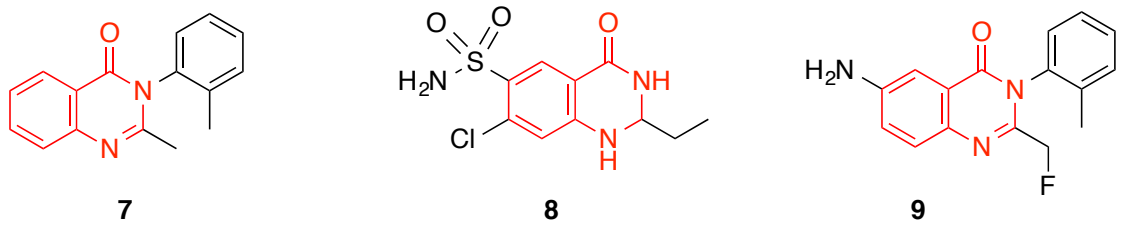


Figure 6: Examples of CNS-active drugs that are based on the quinazolinone structure.

To assess the neuroprotective activity of **4–6** against excitotoxin-induced damage, Lee and co-workers[13] pretreated cell cultures with **4–6** for 1 h before exposing the cultures to toxic concentrations of either L-glutamate (**10**), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, **11**), *N*-methyl-D-aspartate (NMDA, **12**) or kainic acid (kainite, **13**) (Figure 7). The cultures were maintained for an additional 24 h in neurobasal medium, and neuronal viability was then measured by the MTT assay. All three compounds **4–6** were found to protect neurons from glutamate-induced toxicity

to a significant degree at concentrations ranging from 5–10 μM. Also, these compounds protected neurons from NMDA-induced toxicity at concentrations ranging from 10–30 μM.

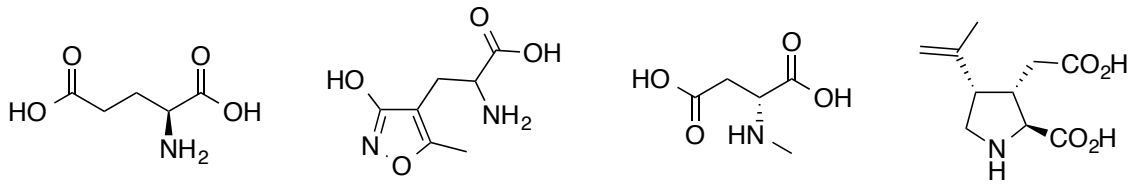


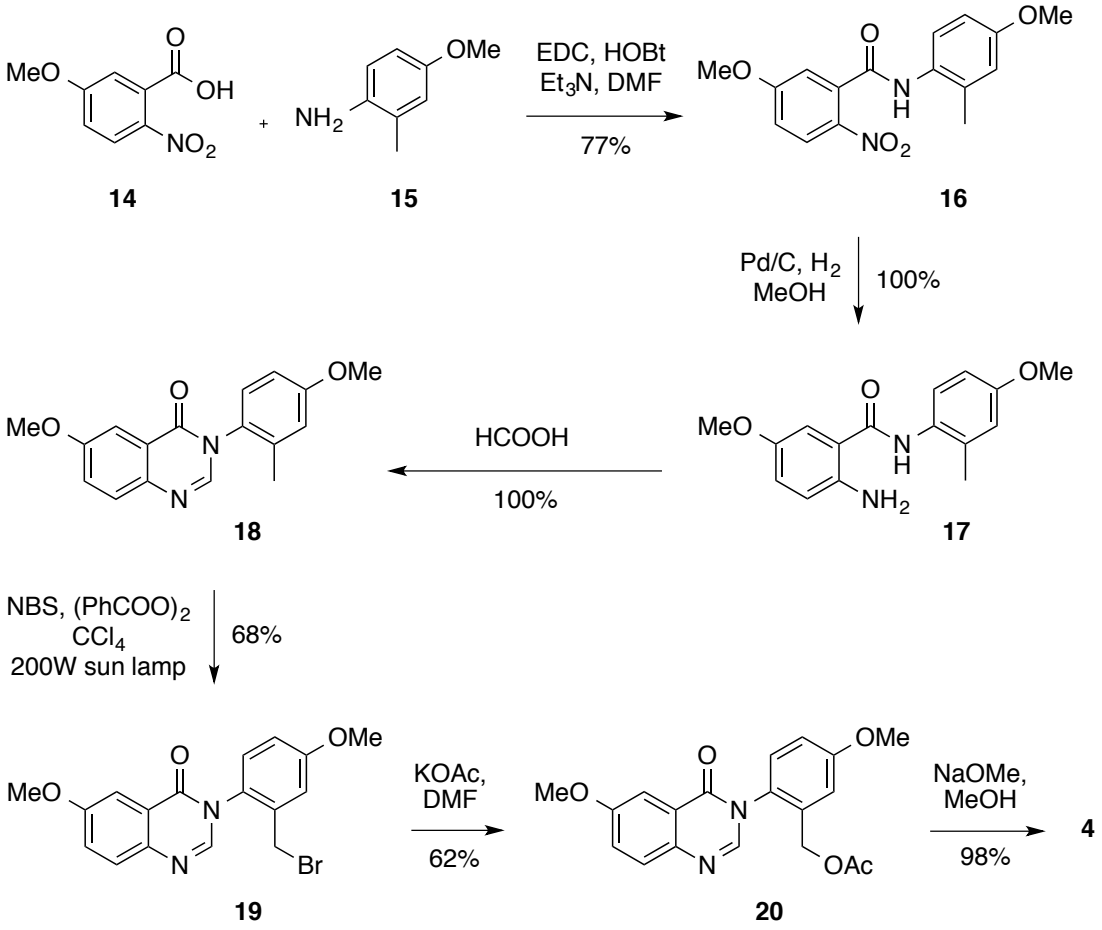
Figure 7: Structures of the excitotoxins used in the biological evaluation of dictyoquinazols A–C.

Of the three natural products investigated by Lee and co-workers (**4**–**6**), dictyoquinazol A (**4**) seems a particularly promising candidate for further development into a possible treatment for stroke. This compound has a relatively low molecular weight (312 g/mol), suggesting that there is ample scope for creating analogues while remaining under the notional molecular weight limit of 500 g/mol recommended by Lipinski[17]. Also, the number of hydrogen bond donor and acceptor groups of **4** are well within the limits recommended by Lipinski. Furthermore, compound **4** has cLogP = 1.95, which is in a range that is typical for existing CNS-active drugs[18].

The first step in the medicinal development of dictyoquinazol A (**4**) would be to achieve an efficient total synthesis of this target molecule. Progress in this area has recently been made by two different research groups.

1.3 Previous syntheses of dictyoquinazol A

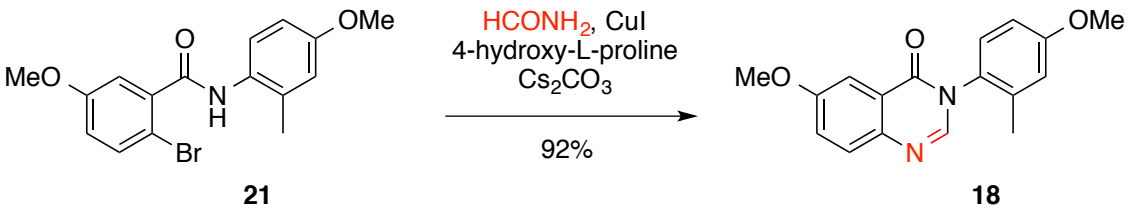
In 2007, Oh and Song reported the first total synthesis of dictyoquinazol A (Scheme 1)[13]. The synthesis strategy required six steps, beginning with the coupling of starting materials **14** and **15** to yield the nitroaryl amide **16**. Intermediate **16** was reduced to the aniline **17**, which in turn underwent cyclisation with formic acid to give the quinazolidinone **18** in quantitative yield. Three subsequent steps were required to transform the methyl group of **18** into the required primary alcohol of **4**, proceeding via the bromomethyl derivative **19** and the acetoxy compound **20**.



Scheme 1: Total synthesis of **4** reported by Oh and Song[15].

Although ultimately successful, this synthesis of **4** (Scheme 1) suffered from a linear stepwise design that was not well suited to the rapid production of analogues. Another undesirable feature of this synthesis was the inclusion of a radical bromination step that required toxic reagents and precise conditions that proved difficult to reproduce (*vide infra*).

In 2012, Ma and coworkers reported a formal total synthesis of **4** via a new route (Scheme 2)[19]. This synthesis used a copper-catalysed cyclisation reaction of an aryl bromide substrate (**21**) to construct the key quinazolidinone moiety (**18**). This synthesis design is potentially better suited to the production of structural analogues of **4**, since it does not include a reduction step (eg. **16**→**17**, Scheme 1) that would be incompatible with certain peripheral groups. However, the cyclisation step (Scheme 2) is lower-yielding than the method of Oh and Song (**17**→**18**, Scheme 1), and the synthesis design still requires the undesirable stepwise method for transforming the aryl methyl group of **18** into the primary alcohol group of **4**.

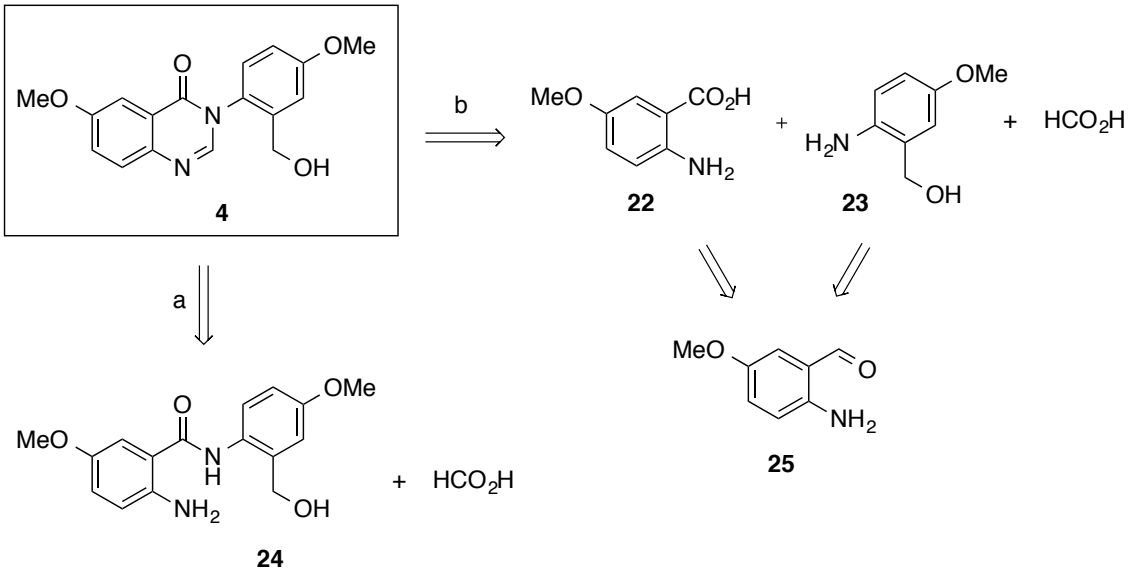


Scheme 2: Formal total synthesis of **4** reported by Ma and coworkers[19]

1.4 Aims of this project

The aim of this project is to investigate the natural product dictyoquinazol A (**4**) as a lead compound for the development of a novel treatment for stroke. In order to achieve this aim, three milestones are envisaged.

First, an improved synthesis of **4** will be developed. Initially, attempts will be made to modify the route of Oh and Song in a way that removes the need for functional group manipulations *en route* to the primary alcohol group of **4** (Scheme 3a).



Scheme 3: New strategies for the synthesis of **4**.

In parallel, a completely new and more ambitious route will also be investigated that exploits the hidden symmetry of **4** (Scheme 3b). In this new proposed route, the target **4** will be derived from a multicomponent reaction of benzoic acid **22**, benzyl alcohol **23** and formic acid. The benzoic acid **22** and benzyl alcohol **23** can be derived, in turn, from the common precursor aldehyde **25** *via* a Cannizzaro reaction. This proposed new

synthesis (Scheme 3b) would require far fewer steps than the existing syntheses, and it would entail no stoichiometric redox reactions.

The second milestone of this project will be to create a small library of structural analogues of **4**. Depending on the outcomes of the synthetic investigations described above, the most efficient route will be employed for analogue synthesis.

The third milestone of this project will be to test the analogues created above, in a variety of cell-based assays that mimic the various deleterious effects of stroke. Cultures of nerve cells will be exposed to various insults, including the addition of L-glutamate (to mimic excitotoxicity) and staurosporine (to induce apoptosis). The protective ability of **4** and its analogues will be measured in each case, and this will form the beginnings of a comprehensive structure-activity relationship profile of the lead compound, **4**.

Chapter 2:

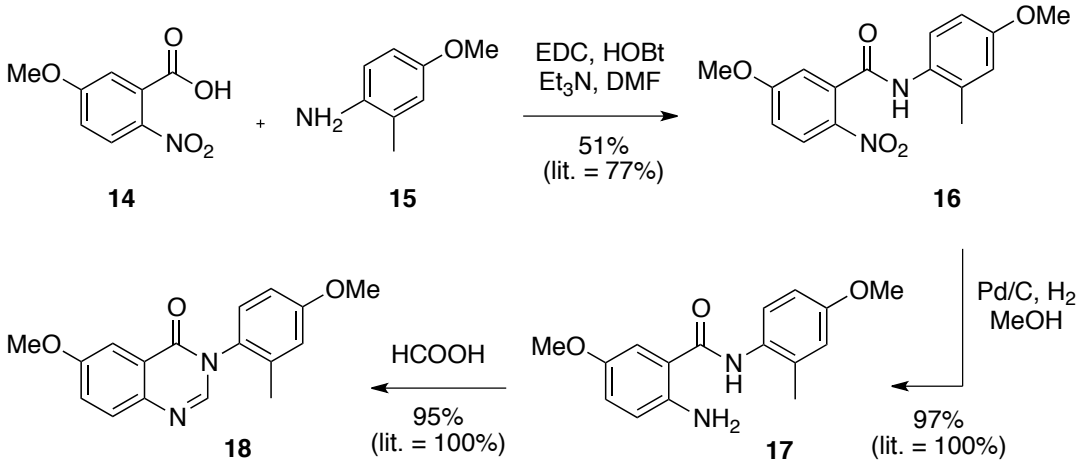
Results and Discussion

2.1 Synthesis

2.1.1 Synthesis of dictyoquinazol A via literature method

Before developing a new synthesis of dictyoquinazol A (**4**), it was decided that the literature method of Oh and Song[13] (Scheme 1) would be reproduced. This would provide a training exercise, and it would allow the literature method to be rigorously analysed in terms of its suitability for the production of analogues. It would also provide authentic samples of some of the key intermediates that were expected to be synthesised later by different routes.

Accordingly, the first step was to condense acid **14** with aniline **15** to produce the amide **16** (Scheme 4). This reaction proved to be straightforward, although the obtained yield of **16** was somewhat lower than the literature figure.



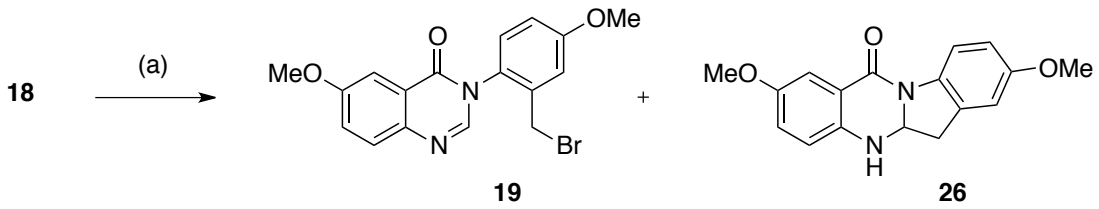
Scheme 4: Repeating the first three steps of the literature synthesis of **4**.

The second step was the reduction of the nitro group of **16**, by hydrogenation over palladium on charcoal (Scheme 4). This reaction delivered a high yield of the expected aniline **17**. The third, key, step was a cyclization reaction using formic acid as the C1

source, and this reaction smoothly delivered the desired quinazolidinone derivative **18** in 95% yield.

After three straightforward steps (Scheme 4), unfortunately difficulties arose in the fourth step. This was the radical bromination of the aryl methyl group of **18** (Table 1). The experimental set-up of this reaction was cumbersome, as it required a sun lamp, a cool bath maintained at 10 °C, and a prolonged reaction time. More seriously, the use of carbon tetrachloride as the solvent was undesirable due to its toxicity and restricted commercial availability. The reaction was found to be unreliable and low-yielding: in several reaction trials, only on one occasion was it possible to isolate the desired product (**19**), and it was obtained in only 9% yield (cf. the literature figure of 68%). The tetracyclic compound **26** was obtained as a byproduct (Table 1). A possible reason why the literature results could not be replicated is that the sun lamp may have had different specifications; these details were not provided in the literature. To make matters worse, the bromomethyl compound **19** seemed to be rather unstable, and started to decompose even upon storage at 4 °C.

Table 1: Attempted bromination reactions



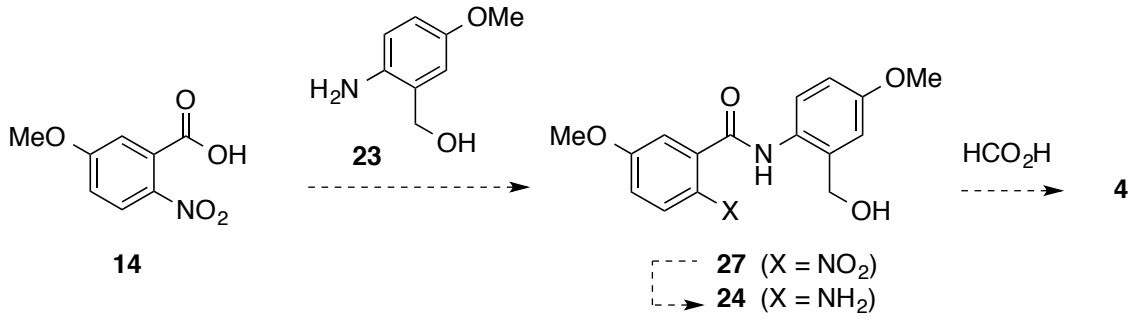
Entry	(a)	Outcome
1	NBS, $(PhCOO)_2$, CCl_4, 200W sun lamp, 24 h	**19** (0–9%) or **26** (10%)
2	NaBr, H_2O_2, H_2SO_4, $CHCl_3$	recovered **18** (24%)

Mestres and co-workers[20] have reported different conditions for the bromination of aryl methyl groups (Table 1, entry 2). Under these conditions, aryl methyl groups are reported to be converted into bromomethyl groups alongside minor quantities of dibromomethyl groups. However, when substrate **18** was exposed to these conditions, unfortunately the desired product **19** was not obtained. Instead, starting material **18** was recovered along with some minor unidentified sideproducts.

The difficulties encountered in the bromination step (**18**→**19**) prompted the conclusion that the literature method for synthesising dictyoquinazol A (Scheme 4) was not a convenient method for the production of analogues. As the problem of this strategy was the transformation of the methyl group of **18** to the primary alcohol of **4**, it was decided that a new strategy should be developed that avoided these steps.

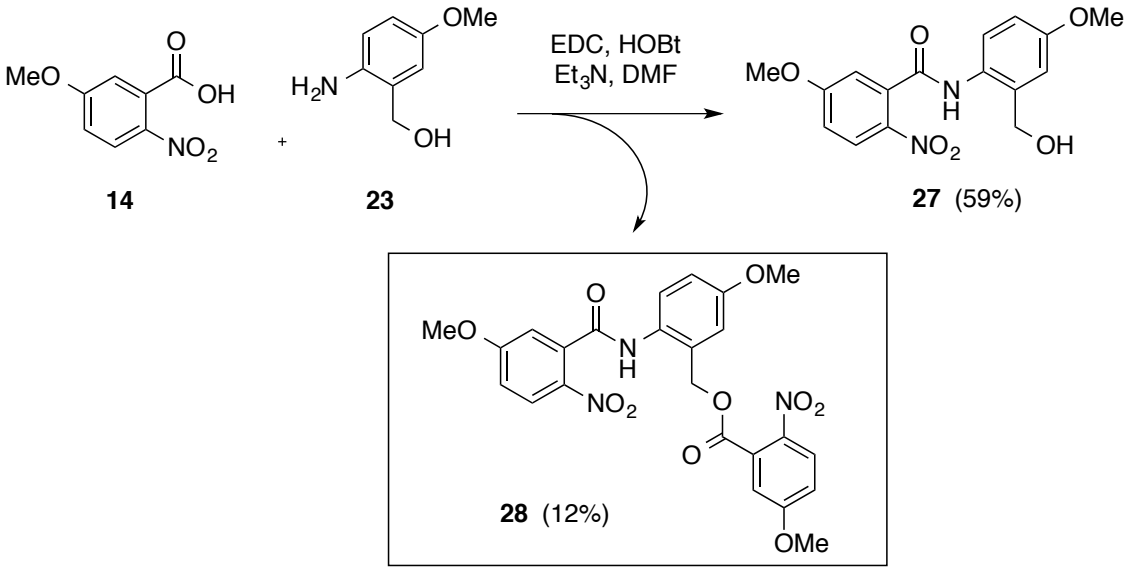
2.1.2 Improving the synthesis of dictyoquinazol A

A new strategy for synthesising **4** was proposed (Scheme 5). This strategy was very similar to the literature method of Oh and Song (Scheme 4), with the modification that the primary alcohol group would be included, unprotected, from the start of the synthesis.



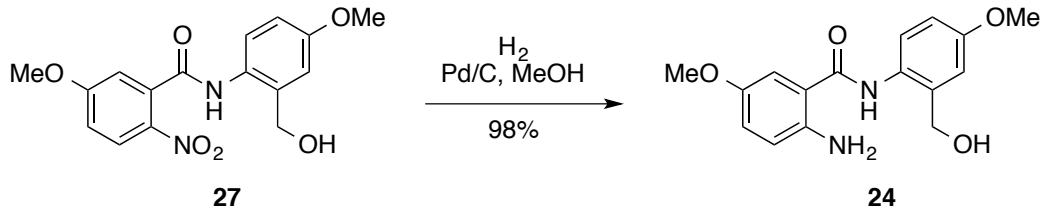
Scheme 5: Synthesis of **4** improving the previous published strategy

The first step of this new strategy was the coupling of **14** and **23** (Scheme 6). This reaction was carried out under similar conditions to those already described (**14**+**15**, Scheme 4), with the desired amide **27** obtained in moderate yield. This reaction however formed a sideproduct **28**, in which ester formation had occurred in addition to amide formation. Attempts were made to limit the formation of **28** by using **23** in excess and keeping the temperature at 0 °C; in this case the byproduct **28** was still produced but in a minor quantity (Scheme 6). Separation of **27** and **28** was readily achieved by column chromatography. It was concluded that the yield of the desired product **27** was high enough to make protection of the alcohol group of **23** unnecessary.



Scheme 6: First step of the new synthesis of dictyoquinazol A.

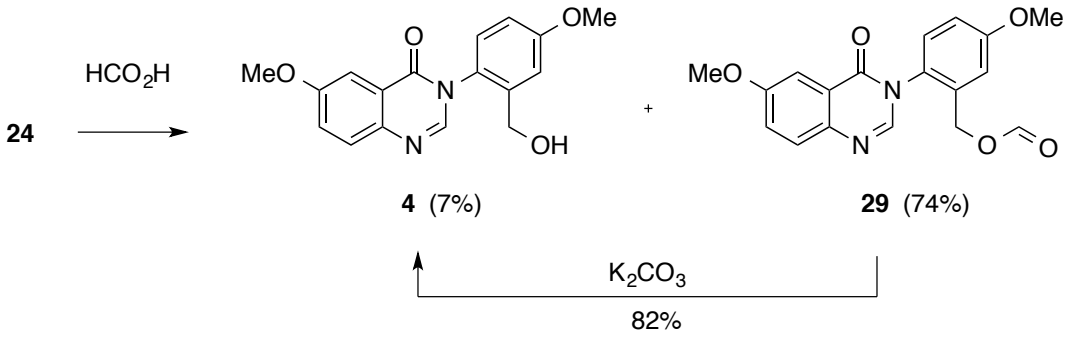
The next step was to reduce the nitro group of **27** (Scheme 7). As expected, this reaction gave a clean and complete conversion of **27** into **24** with a yield of 98%. No chromatographic purification of **24** was required.



27 **24**

Scheme 7: Second step of the new synthesis of dictyoquinazol A.

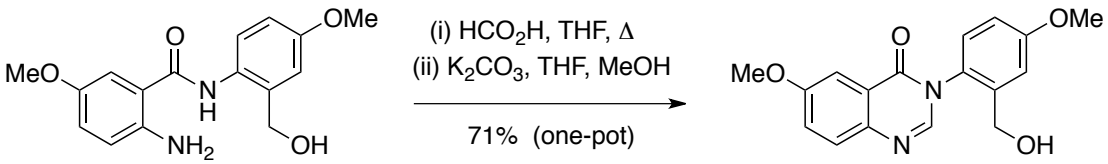
The final step in the new synthesis was the cyclisation of **24** to **4** (Scheme 8). Initially this was attempted using neat formic acid under reflux. These conditions did yield a small quantity of the natural product **4**, but the major product was the formate ester **29** (Scheme 8). Fortunately, this byproduct **29** could be readily hydrolysed back to the natural product (**4**) under basic conditions, giving a combined two-step yield of 68%. The structure of **4** was confirmed by comparing the ^{1}H NMR data with that of the natural sample[13]. It is noteworthy that in contrast with the precursor **24**, two separate ^{1}H NMR signals were observed for the methylene protons of **4**; this implies that the bicyclic ring system of **4** leads to restricted rotation about the C–CH_2OH bond.



Scheme 8: Completing the synthesis with a two-step conversion of **29** into **4**.

However, it was desired to have a high-yielding one-pot conversion of **24** into **4**, without requiring any purification of the side-product **29**. This was achieved by heating a mixture of **24**, formic acid and THF under reflux for 6 h, followed by quenching with

K_2CO_3 in methanol (Scheme 9). This one-pot process delivered the natural product (**4**) directly in 71% yield.



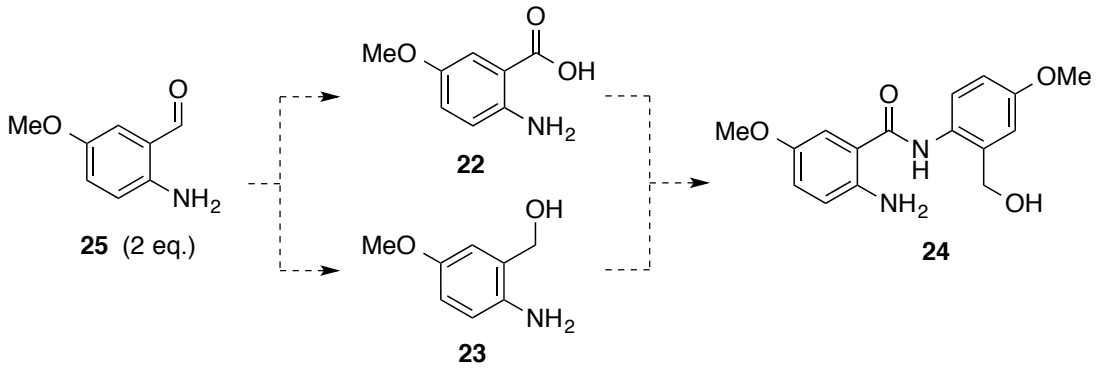
24 **4**

Scheme 9: Completing the synthesis with a one-pot conversion of **24** into **4**.

Thus, the natural product dictyoquinazol A (**4**) was synthesised in three discrete chemical operations, with an overall yield of 43% from **14**. This represents a significant improvement over the previously published synthetic strategy (six steps, 32% overall yield). Despite the formation of two side products, this new synthesis was deemed to be a superior method for producing structural analogues of **4**.

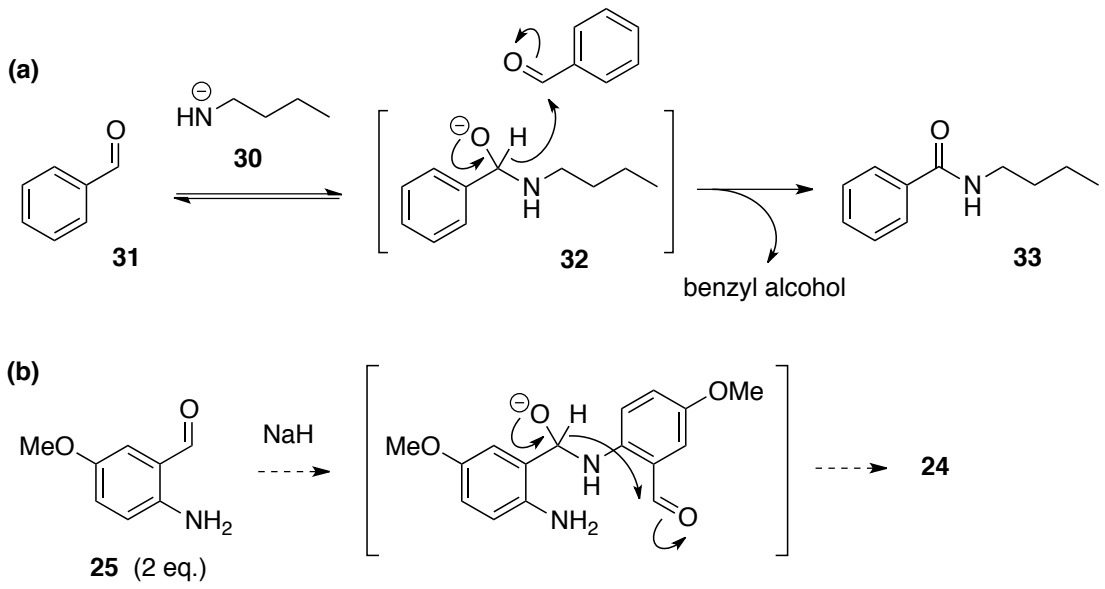
2.1.3 A novel synthesis exploiting the hidden symmetry of 4

Having developed a novel three-step synthesis of dictyoquinazol A (**4**), it was decided to investigate an even more streamlined synthetic strategy (Scheme 10). The new strategy exploited the hidden symmetry of target **4**. It was envisaged that the aldehyde **25** could undergo a Cannizzaro[21,22] reaction to give benzoic acid **22** and benzyl alcohol **23**, and these two molecules could condense to give the amide **24** (Scheme 10). Finally, **24** would be converted into the natural product **4** according to the method that has already been optimised (Scheme 9).



Scheme 10: Cannizzaro approach for a shorter synthesis of **4**.

Furthermore, literature precedent suggested that the Cannizzaro-amidation sequence (**25**→**24**, Scheme 10) could potentially be streamlined into a single operation. Several groups have reported intermolecular variants of this process (Scheme 11a)[21,22]. Typically, a primary amine (eg. **30**) is deprotonated with strong base, and subsequently added to an aldehyde (eg. **31**). This generates an anionic tetrahedral intermediate (**32**), which collapses with transfer of hydride to another molecule of the aldehyde **31** (Scheme 10a).

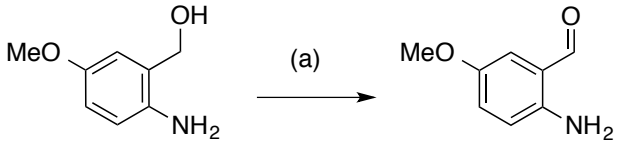


Scheme 11: (a) One-pot intermolecular Cannizzaro-amidation sequence reported by Ishihara and co-workers; (b) Proposed intramolecular variant.

For the planned synthesis of dictyoquinazol A (Scheme 11b), in which both the aldehyde and amino groups are present within the same starting material (**25**), it was proposed that hydride transfer could take place in an intramolecular fashion, via a 6-membered transition state, to give the amide **24** in a single operation. This would set the correct oxidation states at both of the benzylic carbons, without the need for stoichiometric redox reactions.

Precursor **25** at first glance appeared to be quite reactive, as it contains both an aldehyde and an amino group. This compound is listed by several suppliers as being commercially available, but only in small quantities and not deliverable to Australia. Therefore it was decided to prepare **25** in-house, by oxidation of **23** which was previously synthesised in this project (Table 2).

Table 2: Synthesis of aldehyde **25**



23 **25**

Entry	(a)	Outcome
1	DMSO, $(COCl)_2$, Et_3N, DCM	Complex mixture
2	CuOTf, bpy, TEMPO, NMI, MeCN	**25** formed, but impure
3	MnO_2, THF	**25** (quantitative yield)

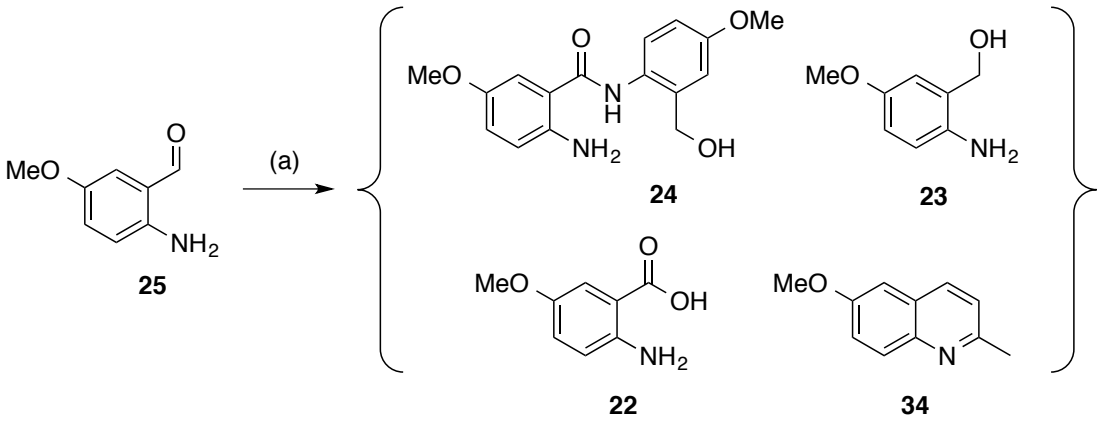
Several attempts were made including Swern oxidation (Table 2, entry 1) and TEMPO oxidation[23] (Table 2, entry 2). In these cases the desired aldehyde **25** was formed, as

shown by the appearance of ^{1}H NMR signals around 9–10 ppm, but the spectra showed the presence of multiple compounds and hence it was believed that self-condensation of the aldehyde **25** may have occurred to give oligomeric or polymeric imines.

Finally, oxidation of **23** was successfully achieved using MnO_2 in THF[24] (Table 2, entry 3). This reaction proceeded with almost 100% yield, giving the crude aldehyde **25** in good purity as judged by ^{1}H NMR. Column chromatography was avoided, in order to prevent decomposition of **25**. It was found that aldehyde **25** could be stored for several days at 4 °C without decomposing, but only if rigorous precautions were taken to exclude air and moisture from the sample.

With the aldehyde **25** in hand, attention was turned to its use in the Cannizzaro-amidation sequence (Table 3). A strong base was required to deprotonate the aniline group of **25**, but the choice of base was constrained by the presence of the aldehyde moiety. Initially, the bases potassium bis(trimethylsilyl)amide (entry 1) and *sec*-BuLi (entry 2) were employed, but in both cases this led to decomposition, giving a complex mixture of unidentifiable products.

Table 3: Attempted Cannizzaro-amidation reaction



Entry	(a)	Outcome
1	potassium bis(trimethylsilyl)amide (0.5–2 eq), THF, 80 mM, 0–45 °C	Decomposition
2	*sec*-BuLi (0.5–2 eq), THF, 80 mM, 0–45 °C	Decomposition
3	NaH (0.3 eq), THF, 13 mM, 0–25 °C	Unreacted **25**, alcohol **23**, target **24** (trace)
4	NaH (0.5–0.8 eq), THF, 80 mM, 0–66 °C	Unreacted **25**
5	NaH (1.15–5 eq), THF, 80 mM, 0–66 °C	Decomposition
6	NaH (1 eq), THF, 53 mM, 0–100 °C	Acid **22**, alcohol **23**, quinoline **34**
7	NaH (1 eq), THF, 80 mM, μW [80 W, 90 °C, 1–7 h]	Decomposition
8	NaH (1 eq), THF, 80 mM, μW [170 W, 100 °C, 0.5 h)	Ester **35** (speculative)
9	NaH (1 eq), toluene, 40 mM, 0–80 °C	Unreacted **25**

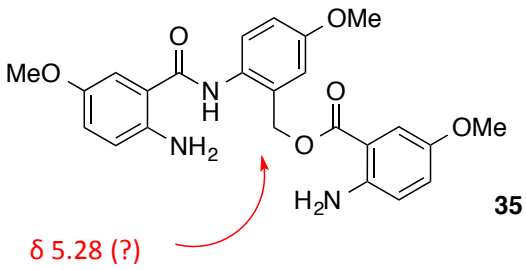
Next, sodium hydride was employed (entries 3–9) with variations in stoichiometry, reaction concentration, solvent, temperature and time. Initially, 0.3 equivalents of NaH were employed (entry 3); this gave mostly unreacted starting material **25**, but small

quantities of the alcohol **23** and (possibly) the desired product **24** were also detected by crude NMR and HRMS respectively ([**24**.Na^+] requires *m/z* 325.1151, found 325.1159). It was considered important to effect complete consumption of the starting material **25**, otherwise it would decompose to a complex mixture during workup and purification, making the isolation of other products impossible. Therefore, the stoichiometry of NaH was gradually increased, over several experiments, up to 0.8 equivalents (entry 4), but this still gave mostly unreacted starting material. When NaH was used in excess, over several experiments (entry 5), decomposition occurred to give a complex mixture of unidentifiable products.

Since increasing the stoichiometry of NaH seemed to lead only to decomposition, the next strategy was to reduce the amount of NaH back to 1 equivalent, and to increase the temperature using a pressure flask (entry 6). Tantalisingly, the alcohol **23** and acid **22** were both formed under these conditions, indicating that some Cannizzaro-type process might be occurring. Unexpectedly however, the quinoline **34** was also formed. Subsequent analysis of the THF solvent, which was sourced from a commercial filtration system, revealed that it was contaminated with a small concentration of acetone, which may explain the formation of **34**. Therefore, for the next experiments (entries 7, 8) a different batch of THF was obtained from a still.

Up to this point, it seemed that excess NaH and/or long reaction times led mostly to decomposition. Accordingly, it was decided to employ microwave irradiation in an attempt to drive the desired reaction to completion. The first microwave reaction (entry 7) was run at 80 W / 90 °C for 1 h, but mostly unreacted **25** was present; after prolonging the reaction for 7 h, decomposition occurred. Next, a higher temperature

and shorter reaction time were employed (entry 8). In this case, a small amount of unreacted **25** remained, but several new products were observed. In particular, one product exhibited a distinctive proton NMR signal at 5.28 ppm, and it was speculated that this product could be the ester **35** (see below). Due to the small quantity of material and the complex mixture of products, it was not possible to fully purify the putative ester **35**. As a final attempt to prove its structure, the crude **35** was treated with K_2CO_3 in MeOH / THF in an attempt to hydrolyse it back to the desired product **24**. TLC and HRMS analysis of this reaction gave some evidence that the desired product **24** was formed, but this conclusion is still tentative.



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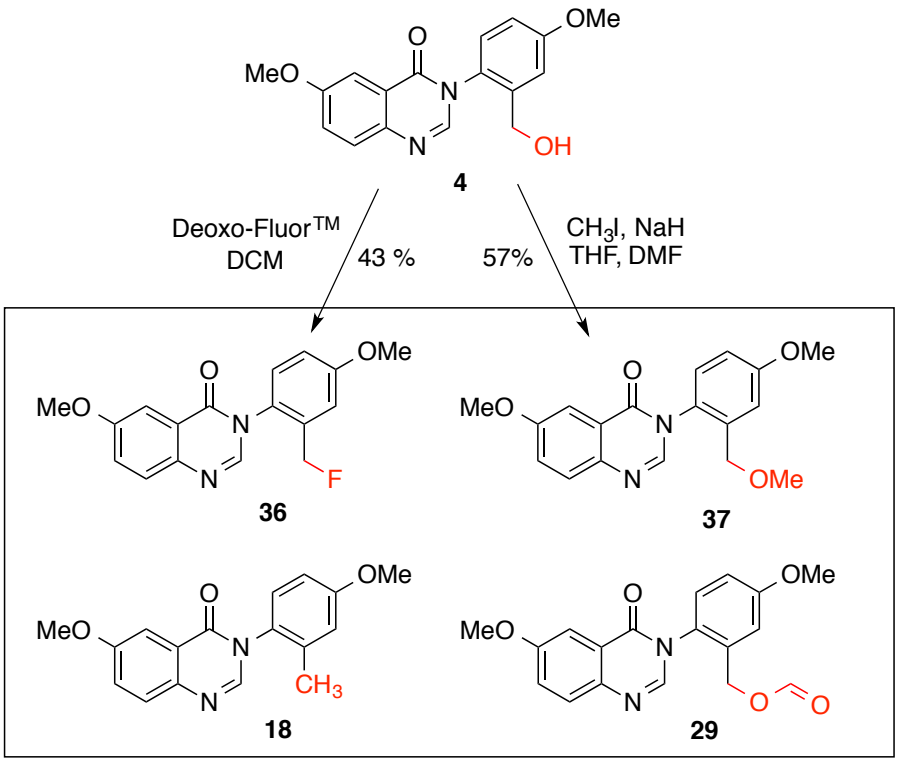
Finally, the Cannizzaro-amidation reaction was performed in toluene in order to investigate the effect of a less polar solvent (Table 3, entry 9). However the starting material was not consumed in this attempt.

Overall, it was concluded that the Cannizzaro-amidation strategy was unsuccessful despite some evidence that alcohol **23**, acid **22** and the desired product **24** were formed in small quantities in some circumstances. Therefore, considering the overall aims of this project, it seemed that the second generation synthesis of dictyoquinazol A (Section 2.2) was the most promising method for producing analogues.

2.1.4 Analogue synthesis

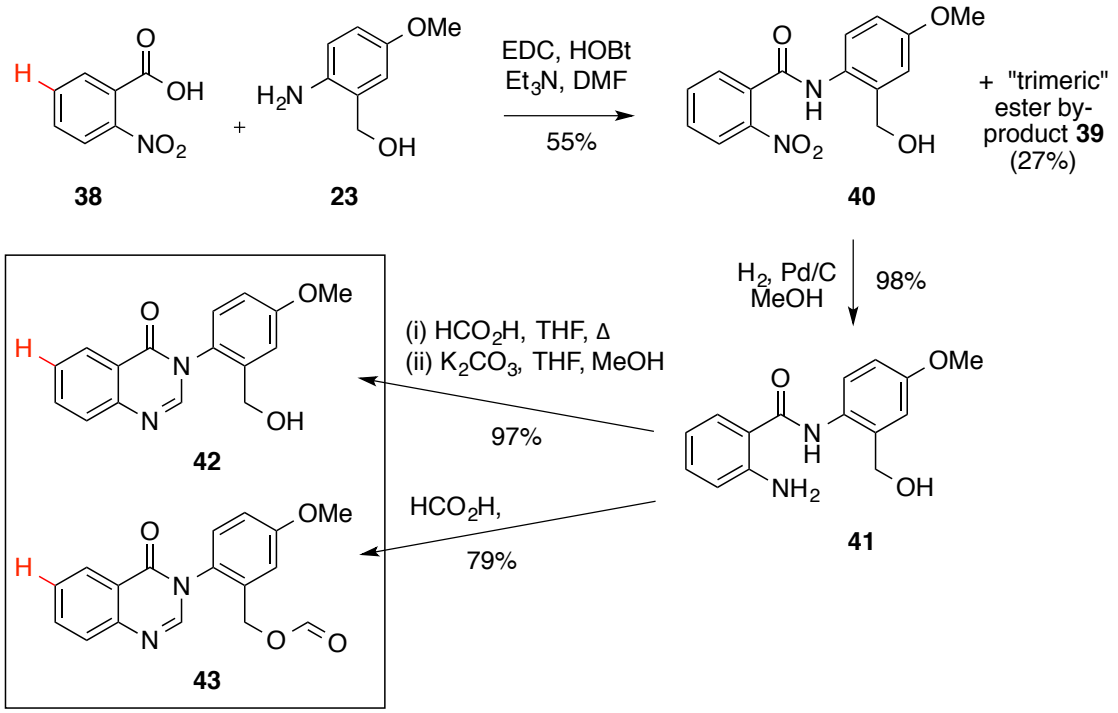
The next goal of this project was to create a small library of structural analogues of dictyoquinazol A (**4**), as a first step towards creating a structure-activity relationship profile of this lead compound.

In order to test whether the primary alcohol group of **4** is important for activity, several modifications at this position were made (Scheme 12). The methyl analogue **18** and the formate ester **29** had already been synthesised (Sections 2.1.1, 2.1.2); to this collection of existing analogues were added the fluoromethyl analogue **36** and the methyl ether **37**, which were obtained by reaction of **4** with Deoxo-Fluor[25] and iodomethane[26] respectively (Scheme 12).



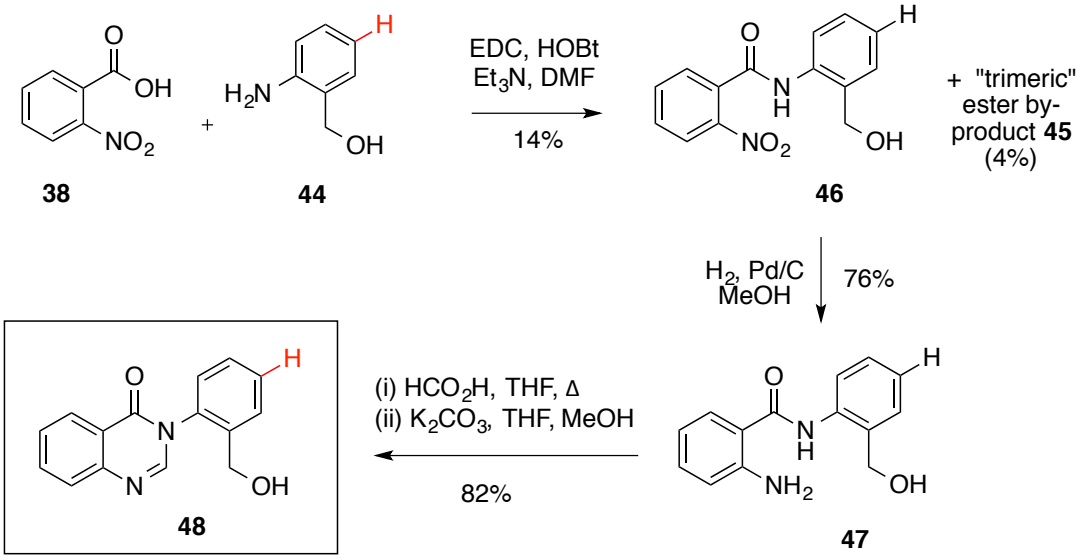
Scheme 12: Analogues **18, 29, 36** and **37** were designed to probe the importance of the primary alcohol group of **4**.

The next analogues (Schemes 13 and 14) were designed to test the importance of the two methoxy groups of **4**. The analogue **42** lacking the western methoxy group was synthesised in an overall yield of 52% according to the methods developed in Section 2.1.2 (Scheme 13). The corresponding formate ester **43** was also prepared separately (Scheme 13).



Scheme 13: Analogues **42** and **43** were designed to probe the importance of the western methoxy group of **4**.

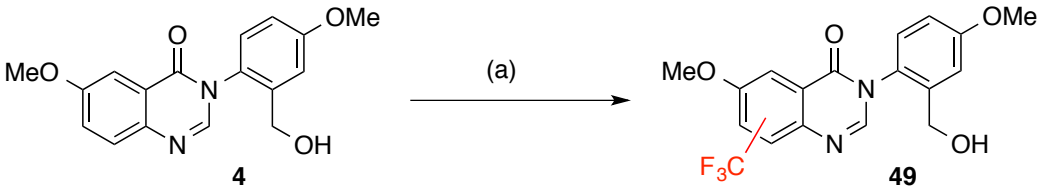
The analogue **48**, lacking both methoxy groups, was synthesised in a similar manner (Scheme 14). The yield in the amide coupling step was very low, but it was not a priority to repeat or optimise this reaction.



Scheme 14: Analogue **48** was designed to probe the importance of the eastern methoxy group of **4**.

Finally, to investigate the importance of the quinazolidione core structure of **4**, a radical trifluoromethylation reaction was attempted (Table 4). Baran and co-workers[27] have shown that treatment of aromatic heterocycles with zinc bis(trifluoromethanesulfinate) (TFMS) leads to direct trifluoromethylation at the inherently most reactive C–H position of the heterocycle. This is a potentially useful strategy for creating analogues that are more stable to metabolism. However, when this procedure was attempted with compound **4**, no reaction was observed under a variety of conditions (Table 4, entries 1–3). A trace quantity of the desired product **49** was tentatively identified by MS when the reaction was warmed in DCM / H_2O (entry 4), but the product was not able to be isolated or further characterised.

Table 4: Attempted trifluoromethylation[27] of dictyoquinazol A.



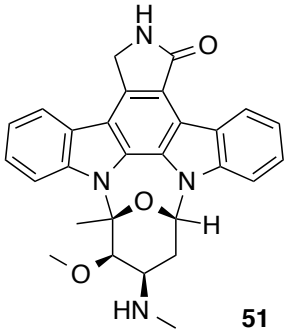
Entry	(a)	Outcome
1	TFMS (2 eq), $(CH_3)_3COOH$, DCM, H_2O, 0–25 °C	Unreacted **4**
2	TFMS (2 eq), $(CH_3)_3COOH$, DCE, H_2O, 0–55 °C	Unreacted **4**
3	TFMS (2 eq), $(CH_3)_3COOH$, TDFH, H_2O, 0–55 °C	Unreacted **4**
4	TFMS (2 eq), $(CH_3)_3COOH$, DCM, H_2O, 0–55 °C	Unreacted **4** + trace **49**

With a first generation of structural analogues in hand, the next goal of the project was to investigate the neuroprotective activity of these analogues using a variety of *in vitro* models of stroke.

2.2 Preliminary biological evaluation

2.2.1 Overview

In order to investigate the neuroprotective activity of dictyoquinazol A (**4**) and analogues, a panel of *in vitro* assays can be used. In these assays, SH-SY5Y neuroblastoma cells are grown in culture, and the cells are then subjected to a variety of "insults" that mimic the different effects of stroke. For example, the cells may be exposed to toxic concentrations of L-glutamate; or they may be induced to undergo apoptosis by the addition of the toxin staurosporine (**51**); or the cells may be exposed to a low oxygen or a low glucose environment. In each case, the experiment can be performed in the presence or absence of dictyoquinazol A (**4**) and analogues, and any improvements in cell survival would be attributed to the neuroprotective activity of the drug molecules.



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Due to time constraints, a limited subset of the available analogues was selected for biological evaluation in this project (Figure 8). These analogues were chosen in order to learn about the importance of the primary alcohol group of **4**, as well as the two methoxy groups. Note that analogue **50** was not synthesised in this project, but was kindly supplied by Gabriella Marcolin (another member of the Hunter group).

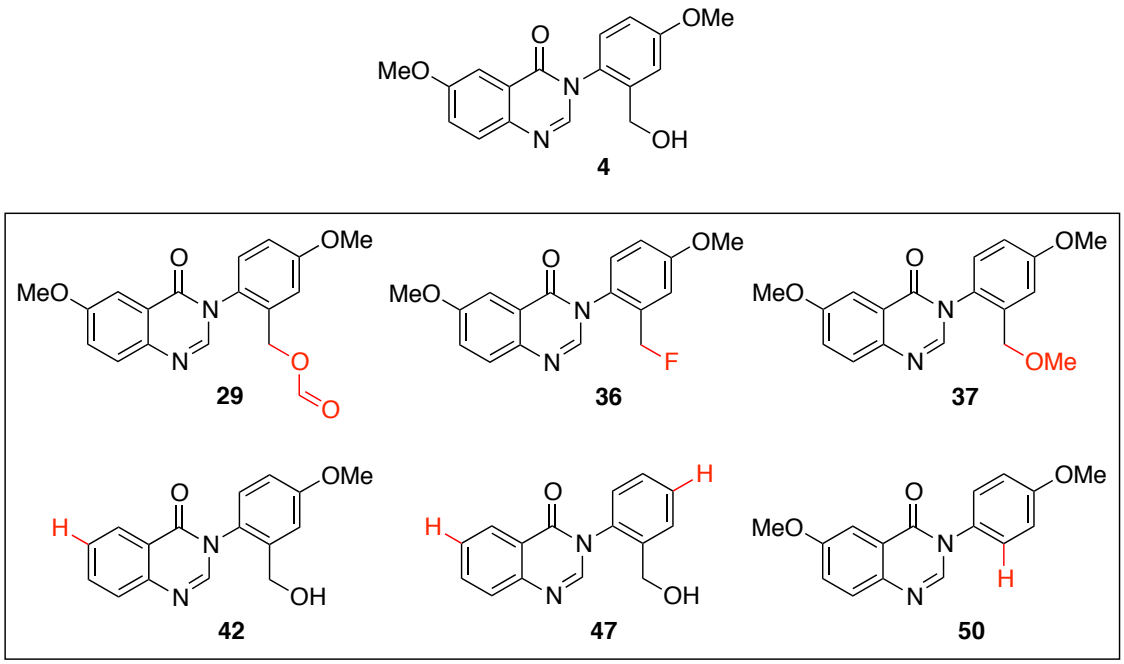


Figure 8: Analogues selected for preliminary biological investigation.

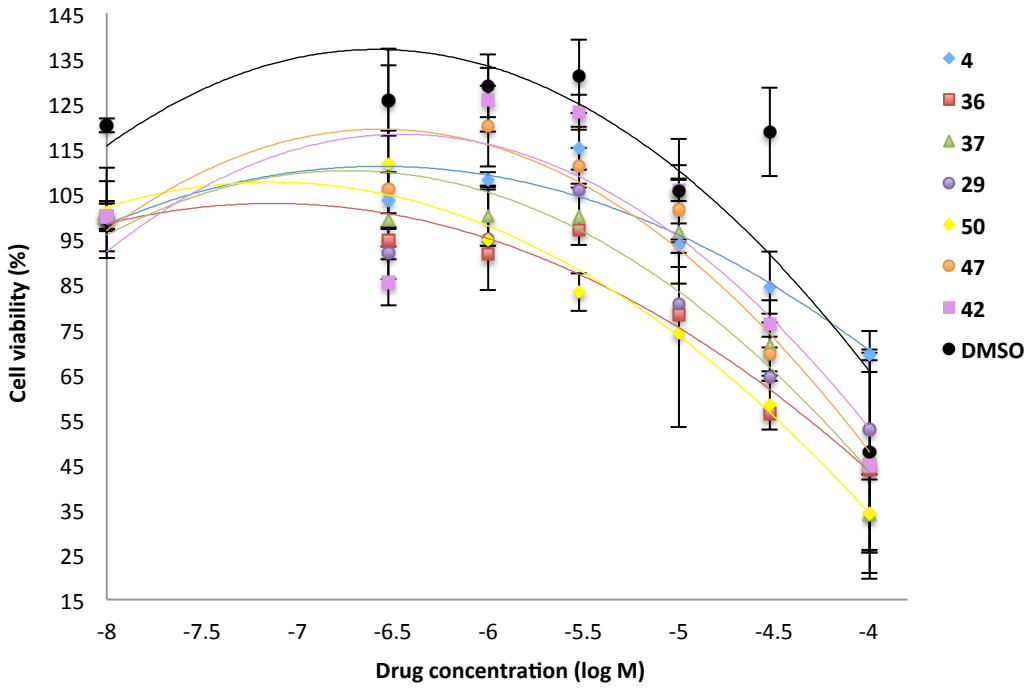
Also due to time constraints, only the assay that mimics apoptosis was performed in this project. It is expected that other assays including L-glutamate toxicity[6,28], hypoxia and hypoglycaemia will be performed in the future. Together, these assays will provide structure-activity relationship information that will serve two purposes. First, more potent analogues may be identified. Second, it may be possible to identify a location on the molecule that may safely be derivatised, in order to create molecular probes for pull-down assays to identify the macromolecular target/s of dictyoquinazol A (**4**).

2.2.2 Neuroprotective activity against staurosporine-induced apoptosis

The first task was to investigate whether compound **4** and its analogues had any inherent toxicity towards nerve cells, in the absence of any stroke-mimicking "insults." Accordingly, neuroblastoma cells were cultured as previously described[29] and a day after plating they were exposed to a range of concentrations of compound **4**, **29**, **36**,

37, **42**, **47** or **50**. Stock solutions of 3 mM in DMSO were used for each compound, and appropriate amounts of these stock solutions were diluted (concentration range of 0.3—100 μM) with media then added to the cell culture (in triplicate). After 24 h, the number of living cells was determined using the MTT cell viability assay[29]. The results were normalised using a positive control of cell medium (=0% cell death when added instead of drug solution), and a negative control of Triton-X100 (=100% cell death when a 0.1% v/v solution was added instead of drug solution). The results of the toxicity experiments are shown in Graph 1.

Graph 1: Investigating the inherent toxicity of compound **4** and analogues towards nerve cells



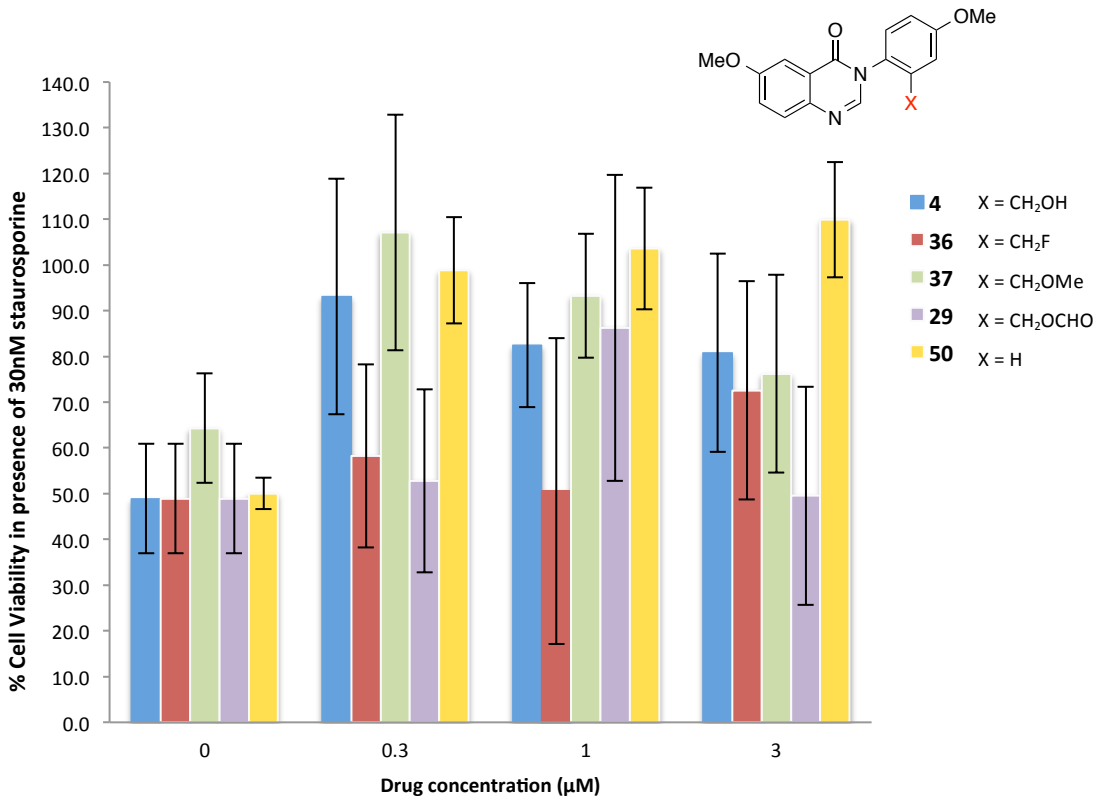
None of the compounds appeared to be toxic at concentrations of up to 3 μM (Graph 1). On the other hand, some toxicity appeared to occur for all compounds at concentrations over 10 μM. However, it was subsequently determined that this

apparent toxicity was likely due to the higher concentrations of DMSO used for the high drug doses. In retrospect it may have been better to employ the same amount of DMSO in every experiment. Nevertheless, two conclusions can be drawn from the data in Graph 1: first, the drugs seem to exhibit no greater toxicity than DMSO itself, which is reassuring; second, the toxicity is negligible in the experiments with drug concentrations up to 3 μM, and so these conditions were appropriate for the subsequent neuroprotective activity assays.

The next task was to determine the appropriate concentration of staurosporine, which was to be used as the "insult" that mimics the apoptotic effect of stroke. It was essential to determine the concentration of staurosporine that killed around 50% of the cells. A similar set of experiments to those outlined above were performed, but since there were unidentifiable problems with the MTT assay, the number of live cells in each well had to be estimated from microscopy images instead (see Appendix). Using this method, it was found that a staurosporine concentration of 30 nM killed approximately 50% of cells.

Subsequent experiments examined whether the synthetic compounds **4**, **29**, **36**, **37**, **42**, **47** and **50** had dose-dependent neuroprotective activity against staurosporine-induced apoptosis (Graphs 2, 3). Neuroblastoma cells were cultured and plated as before, then treated with a combination of staurosporine (30 nM) and a drug candidate (at a range of concentrations, in triplicate). After 24 h, cell viability was determined by the MTT assay[30], and the results were normalised using positive and negative controls of media and Triton-X100 respectively. Any increase in cell viability beyond 50% was attributed to the drug candidate's neuroprotective activity.

Graph 2: Testing the importance of the hydroxyl group of **4** for neuroprotective activity.



It can be seen from Graph 2 that the natural product (**4**) exhibits robust neuroprotective activity at low concentration, giving approximately 93% cell viability (an increase of 44%) at 0.3 μM. However the protective activity of **4** seems to level off at higher concentrations. It should be emphasised that the protective activity of **4** towards staurosporine-induced apoptosis has not previously been investigated, so this is a novel biological action that may now be added to the existing knowledge of the protective activity of **4** against glutamate-induced excitotoxicity. It should be acknowledged, however, that this new data is preliminary in nature, as illustrated by the large error bars. Further replicates of these experiments, using different batches of neuroblastoma cells, are required in the future.

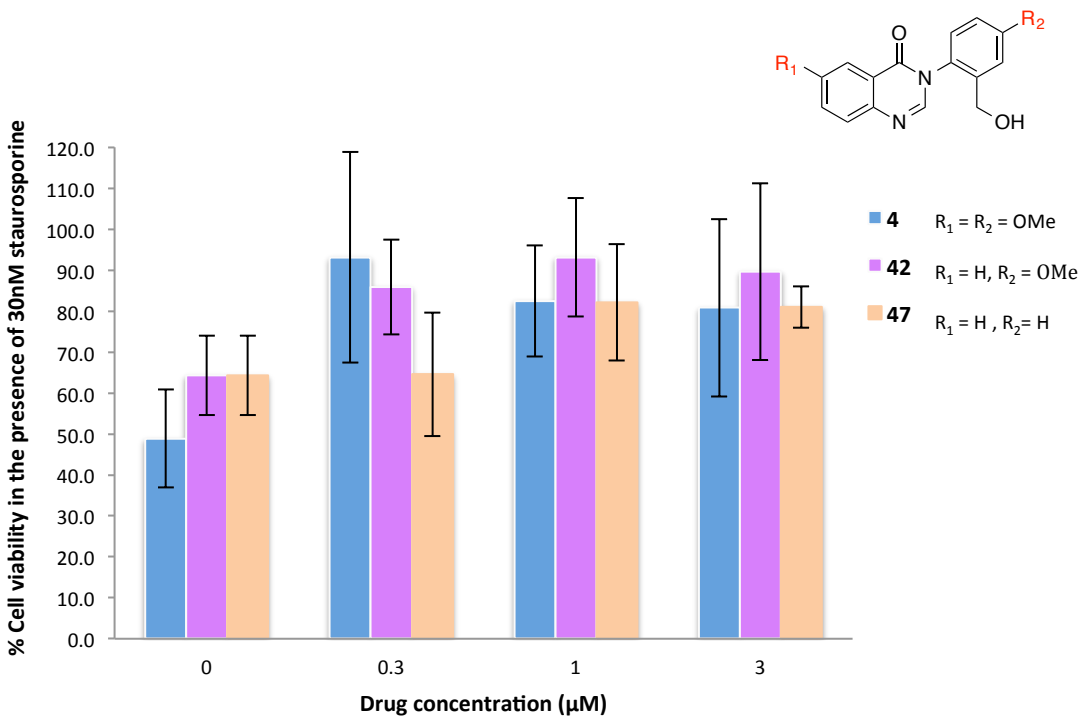
The structural analogues included in Graph 2 were designed to probe the importance of the primary alcohol group of **4**. The first analogue that was considered was the fluoromethyl compound **36**; this compound exhibited substantially lower neuroprotective activity than **4** (eg. giving only 58% cell viability at 0.3 μM). In contrast, the methyl ether **37** was found to exhibit quite similar neuroprotective activity to that of **4**. Together, these results suggest that the hydroxyl group of **4** might be acting as a hydrogen bond acceptor in the interaction of **4** with its putative macromolecular target, since the methyl ether **37** could replicate this interaction well, while the alkyl fluoride **36** would be a weaker H-bond acceptor.

The good activity of methyl ether **37** also suggests that it might be possible to structurally extend the molecule further from this site, which could open up a convenient approach for synthesising further analogues. However, when the formate ester **29** was investigated (Graph 2), the activity was disappointing. Apart from a single high value of neuroprotective activity at one concentration of **29**, which may be an outlier, the cell viability was mostly unchanged at approximately 50%. A possible explanation is that sterically larger groups might not be accommodated at this position of the dictyoquinazol structure.

The final analogue in this series, compound **50**, lacked any substituent at the position of interest (Graph 2). Surprisingly, this analogue was found to exhibit excellent neuroprotective activity (eg. giving 99% cell viability at 0.3 μM). It is difficult to rationalise this activity based on the results described above, but it suggests that the interactions of the hydroxyl group of **4** may not be essential for activity after all.

Having investigated the importance of the hydroxyl group of **4** (Graph 2), the next set of analogues was designed to probe the effect of the two methoxy groups on neuroprotective activity (Graph 3). The analogue **42**, which lacks the western methoxy group, was found to preserve most of the activity of **4**, although it required a slightly higher concentration to reach a similar level of neuroprotection (eg. 93% cell viability at 1 μM). This suggests that the western methoxy group of **4** is not essential for activity.

Graph 3: Testing the importance of the methoxy groups of **4** for neuroprotective activity.



The next analogue, compound **47**, lacked both the western and eastern methoxy groups (Graph 3). In this case a more pronounced reduction in activity was observed, with a maximal cell viability of only 82% achieved. Nevertheless, it is notable that significant activity was still preserved with such a simplified structure.

Chapter 3:

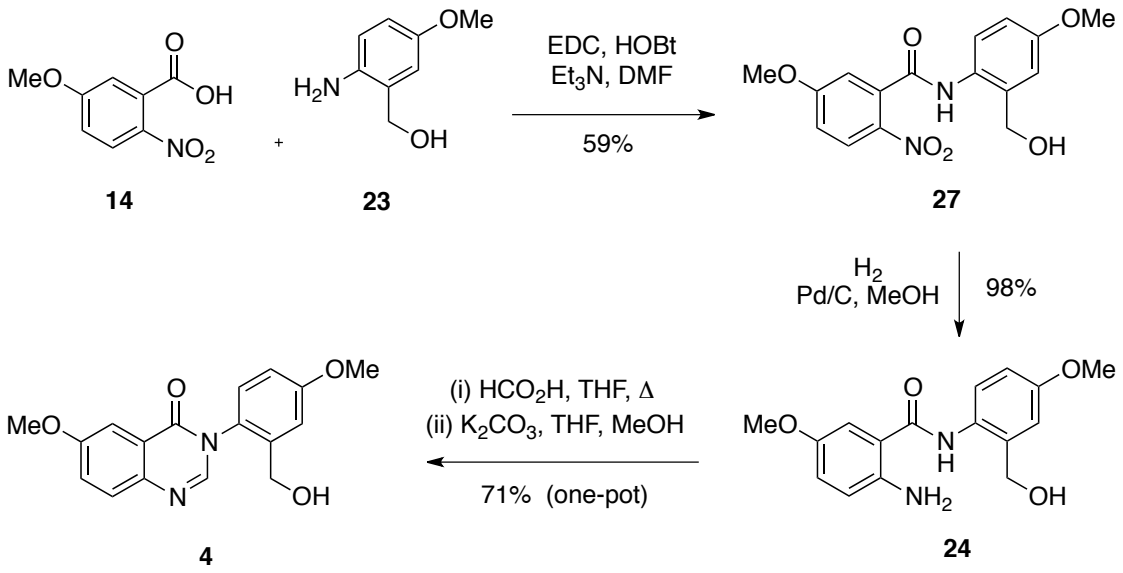
Summary and Future Work

2. Summary and Future Work

The aim of this project was to investigate the mushroom-derived neuroprotective alkaloid, dictyoquinazol A (**4**), as a lead compound for a novel drug to treat stroke. Substantial progress towards this aim has been achieved.

A six-step total synthesis of **4** had previously been published by others[15], and initial work in this project attempted to reproduce the literature synthesis (Section 2.1.1). However, a radical bromination reaction that was part of this synthesis was found to be unreliable and low yielding, so it was decided that the literature method was not suitable for producing analogues of **4**.

Accordingly, efforts were directed to developing a shorter and more efficient synthesis of **4**. This was successfully achieved (Section 2.1.2), with the natural product **4** obtained in just three steps and in 43% overall yield (Scheme 15).



Scheme 15: Three-step total synthesis of dictyoquinazol A (**4**).

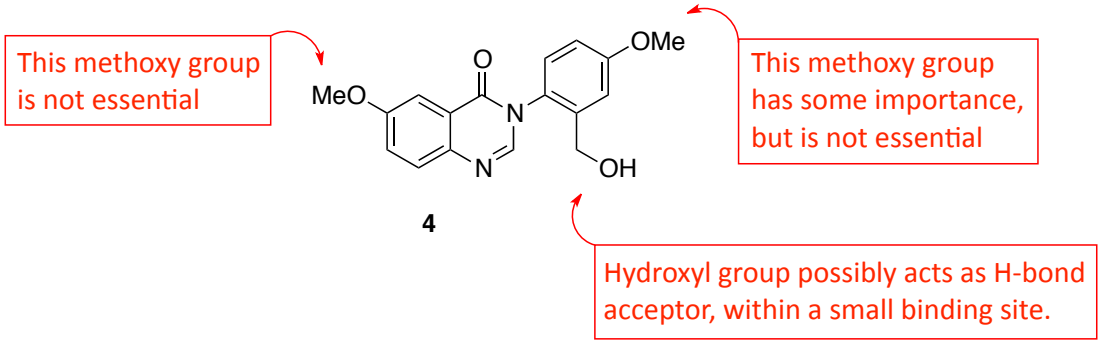
Next, an even more streamlined synthesis of **4** was attempted, which featured a Cannizzaro-amidation sequence as the key step (Section 2.1.3). The new synthetic design exploited the hidden symmetry of **4**, which enabled a route as short as two steps to be envisaged. However, after considerable experimentation, the key Cannizzaro-amidation reaction only delivered the desired product in trace yield. Hence, it was decided that the three-step synthesis (Scheme 15) was the most efficient method.

Accordingly, the three-step method was employed to create several structural analogues of **4** (Section 2.1.4). The analogues were designed to allow some basic questions about the structure-activity relationships of **4** to be answered, such as probing the importance of the primary alcohol group and the two methoxy substituents.

The neuroprotective activity of **4** and its analogues were investigated in a cell-based model of stroke (Section 2.2.2). Compound **4** was shown to exhibit protective activity against staurosporine-induced apoptosis, which is a new mode of action that can now be added to the known protective activity of **4** against glutamate-induced excitotoxicity.

Based on the analogue results, some tentative conclusions about the structure-activity relationships of **4** could also be made (Figure 9). The primary alcohol group might be acting as a H-bond acceptor in the interaction of **4** with its putative receptor, since a methyl ether analogue (**37**) was active while a fluoromethyl analogue (**36**) was only weakly active. Also, the complementary H-bonding site within the putative receptor

seems to be small, since the bulkier formate ester analogue (**29**) was inactive. Finally, a surprising result was obtained with an analogue that completely lacked the hydroxymethyl group (**50**); this analogue was found to be even more potent than the lead compound, suggesting that any H-bonding interactions are not essential for activity.



4

Figure 9: Relationship between the structure of dictyoquinazol A (**4**) and its neuroprotective activity against staurosporine-induced apoptosis.

Next, attention was turned to the importance of the two methoxy groups of **4** (Figure 9). It was found that the western methoxy group was not essential for activity, but the activity dropped somewhat when both methoxy groups were removed. This suggests that the methoxy groups of **4** do play some role, but that there might be more scope for derivatising the molecule at these positions.

It should be emphasised again that the biological data obtained in this project (Section 2.2.2) is preliminary in nature. More experimental replicates are required in order to reduce the error bars and obtain statistically significant results. The data quality may be improved in future if the concentration of DMSO is kept constant across all assays, but this might not be possible for solubility reasons. Also, only one aspect of stroke

(apoptosis) was investigated in this project; assays dealing with other aspects of stroke (eg. glutamate excitotoxicity and oxidative stress) are being concurrently performed within the Hunter group.

Another avenue of future work is to broaden the scope of the analogues synthesised. For example, the methoxy groups could be replaced with other substituents with different electronic character (eg. trifluoromethyl, nitro, chloro, methyl, tert-butyl), or analogues with more than one substituent on each ring could be investigated. It will also be interesting to pursue analogues in which the heterocyclic core structure is modified, in order to assess the importance of the quinazolidinone moiety.

When the structure-activity data from several types of assays and a broader range of analogues are combined, there are two long term goals. The first is to identify a compound that has potent broad-spectrum activity against several of the effects of stroke. The second is to synthesise analogues that can serve as molecular probes, so that pull-down assays can be performed to identify the macromolecular target/s of **4**. Both of these goals will constitute significant progress towards developing a novel treatment for stroke.

Chapter 4:

Experimental

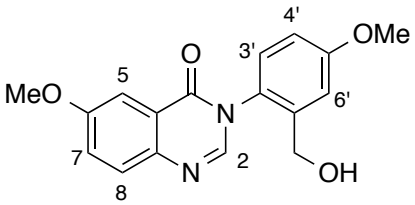
4.1 Synthesis

4.1.1 General methods

Reactions were performed in oven-dried glassware at room temperature and under nitrogen atmosphere with magnetic stirring unless stated otherwise. All commercial reagents were of synthetic grade and were used as received. Reactions were monitored by TLC using Merck aluminium-backed silica gel 60 F_{254} (0.2mm) TLC plates. TLC spots were visualised under short-wave UV light (254 nm). Flash chromatography was performed using Davisil 40-63 mesh silica gel and eluents are stated as volume-to-volume ratios. Melting points were determined using an OptiMelt melting point apparatus MPA100. IR spectra were recorded using a Cary 360 Fourier Transform Infrared (FTIR) spectrometer equipped with attenuated total reflectance (ATR) with a diamond crystal inset. NMR spectra were obtained using Bruker Avance III 300, 400 and 600 MHz instruments at 300 K. Residual solvent peaks were used as an internal reference to calibrate ^{1}H and ^{13}C spectra. The reported peak assignments are supported by data from COSY, HSCQ and/or HMBC experiments. HMRS results were acquired at the UNSW Bioanalytical Mass Spectrometry Facility using an LCQ Deca XP Plus ion trap MS in positive ion mode using electrospray ionization (ESI).

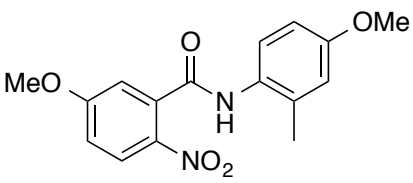
4.1.2 Synthetic procedures and characterization of intermediates

3-(2-(Hydroxymethyl)-4-methoxyphenyl)-6-methoxyquinazolin-4(3*H*)-one (4)



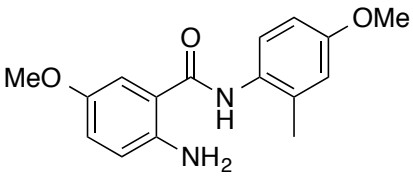
Compound **24** (395 mg, 1.31 mmol) was dissolved in THF (2 mL) and HCOOH (500 µL, 13.3 mmol), the reaction mixture was refluxed at 66°C and stirred for 8 h. The reaction mixture was cooled up to room temperature and diluted with methanol (2 mL) followed by the addition of K_2CO_3 (2.00 g, 14.5 mmol), This new mixture was stirred under N_2 atmosphere at room temperature for 12 h. The reaction was diluted with ethyl acetate (20 mL) and filtered through a silica plug, and washed with ethyl acetate. The filtrate was concentrated onto silica, and the crude product was subjected to flash chromatography eluting with dichloromethane/ethyl acetate to yield the title compound as a pale yellow powder (292 mg, 72%); **m.p.** 191–202 °C; **IR** (neat) ν_{max} (cm^{-1}) 3163, 3058, 2909, 2837, 2685, 2111, 2081, 1684, 1611, 1493; **^{1}H NMR** (400 MHz, MeOD) δ 8.09 (s, 1H, ArH2), 7.72 (d, *J* = 9.0 Hz, ArH8), 7.69 (d, *J* = 3.0 Hz, ArH7), 7.49 (dd, *J* = 9.0, 3.0 Hz, ArH7), 7.29 (d, *J* = 8.6 Hz, ArH6'), 7.21 (d, *J* = 2.9 Hz, ArH3'), 7.03 (dd, *J* = 8.6, 2.9 Hz, ArH4'), 4.44 (d, *J* = 13.4 Hz, C<u>H</u>H), 4.39 (d, *J* = 13.3 Hz, CH<u>H</u>), 3.94 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); **^{13}C{^{1}H} NMR** (101 MHz, MeOD) δ 162.7, 162.2, 160.7, 147.0, 143.7, 141.6, 130.5, 129.8, 129.6, 125.8, 124.1, 115.2, 114.7, 107.6, 61.6, 56.3, 56.1; **HRMS** (ESI, +ve) $C_{17}H_{16}N_2O_4Na^+$ [MNa^+] requires m/z = 363.1002, found 363.0948.

5-Methoxy-*N*-(4-methoxy-2-methylphenyl)-2-nitrobenzamide (16)[15]



Benzoic acid **14** (250 mg, 1.27 mmol) and EDC.HCl (291 mg, 1.52 mmol) were dissolved in DMF (2mL). After stirring for 5 minutes, 1-hydroxybenzotriazole (206 mg, 1.52 mmol), then **15** (191 mg, 1.39 mmol) and triethylamine (300 µL, 2.16 mmol) were added successively to the solution at 0 °C. The reaction mixture was stirred under N_2 atmosphere for 36 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in aq. HCl (10 mL, 1N) and ethyl acetate (20 mL). After partitioning of the two layers, the aqueous layer was extracted with ethyl acetate (4 x 20 mL). All organic layers were combined and washed with water (10 x 20 mL); finally the solvent was dried with $MgSO_4$ and evaporated under reduced pressure. The title compound was obtained as a brown fluffy solid without further purification (206 mg, 51%); **m.p.** 177–185 °C; **IR** (neat) ν_{max} (cm^{-1}) 3223, 3064, 2917, 2840, 2651, 2410, 2307, 2307, 2087, 1924, 1726, 1641, 1577, 1542; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 9.0 Hz, 1H), 7.63 (d, *J* = 8.3 Hz, 1H), 7.12 (s, 1H), 7.06 (d, *J* = 2.7 Hz, 1H), 7.03 (dd, *J* = 9.0, 2.7 Hz, 1H), 6.82–6.78 (m, 2H), 3.94 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3); **^{13}C{^{1}H} NMR** (150 MHz, $CDCl_3$) δ 154.2, 164.0, 158.1, 138.9, 135.8, 133.5, 127.7, 127.5, 126.5, 116.2, 115.0, 114.3, 112.0, 56.4, 55.6, 18.2.

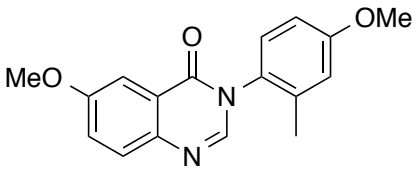
2-Amino-5-methoxy-*N*-(4-methoxy-2-methylphenyl)benzamide (17)[15]



Compound **16** (188 mg, 0.594 mmol) was dissolved in methanol (4mL) and 10% Pd/C (38 mg, 0.036 mmol) was added to this solution. The

reaction mixture was stirred under H_2 atmosphere at room temperature for 4 h. The mixture was filtered through a layer of celite and washed with methanol. The filtrate was concentrated under reduce pressure to produce the title compound as a brown fluffy solid (165 mg, 97%); **m.p.** 168–170 °C; **IR** (neat) ν_{max} (cm^{-1}) 3418, 3273, 3004, 2922, 2834, 2321, 2079, 1877, 1719, 1639, 1584, 1502; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.56 (dd, *J* = 6.8, 2.7 Hz, 1H), 7.08 (d, *J* = 2.9 Hz, 1H), 6.94 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.79–6.76 (m, 2H), 6.73 (d, *J* = 8.9 Hz, 1H), 3.80 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 167.5, 157.6, 151.8, 142.2, 133.1, 128.6, 126.0, 119.6, 119.5, 118.1, 116.2, 112.4, 111.8, 56.2, 55.6, 18.5.

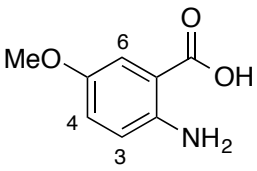
6-Methoxy-3-(4-methoxy-2-methylphenyl)quinazolin-4(3*H*)-one (18)[15]



Compound **17** (152 mg, 0.531 mmol) was dissolved in HCOOH (5.00 mL, 133 mmol), and the reaction mixture was refluxed (oil bath temperature 110 °C) with stirring for 3 h. The reaction mixture was cooled to RT and diluted with chloroform (10 mL) and NaOH (30 mL, 5N). After partitioning of the two layers, the aqueous layer was extracted with chloroform (4 x 40 mL). All organic layers were combined and the solvent was dried with $MgSO_4$ and evaporated under reduced pressure. Compound **18** was collected as a brown solid (148.8 mg, 95%); **m.p.** 122–126 °C; **IR** (neat) ν_{max} (cm^{-1}) 3324, 3073, 2929, 2341, 1896, 1667, 1610, 1484; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.94 (s, 1H), 7.74 (d, *J* = 8.9 Hz, 1H), 7.72 (d, *J* = 3.0 Hz, 1H), 7.40 (dd, *J* = 8.9, 3.0 Hz, 1H), 7.16 (d, *J* = 8.5 Hz, 1H), 6.91 (d, *J* = 2.9 Hz, 1H), 6.87 (dd, *J* = 8.5, 2.9 Hz, 1H), 3.94 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 2.16 (s, 3H, CH_3); **^{13}C{^{1}H} NMR**

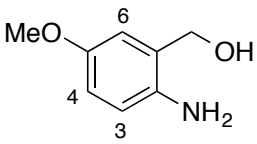
(101 MHz, $CDCl_3$) δ 160.7, 160.4, 159.2, 144.9, 144.2, 137.3, 129.60, 128.9, 124.9, 123.4, 116.6, 112.6, 106.9, 56.1, 55.7, 18.2.

2-Amino-5-methoxybenzoic acid (22)[31]



Compound **14** (3.15 g, 16.0 mmol) was dissolved in methanol (25 mL), and (10%) Pd/C (0.17 g, 0.16 mmol) was added to this solution. The reaction mixture was stirred under H_2 atmosphere at room temperature for 6 h. The mixture was filtered through celite and washed with methanol. The filtrate was concentrated under reduced pressure to yield the title compound as a brown solid (2.62 g, 98%); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.41 (d, *J* = 3.0 Hz, ArH6), 7.01 (dd, *J* = 8.9, 3.0 Hz, ArH4), 6.67 (d, *J* = 8.9 Hz, ArH3), 3.78 (s. 3H, OCH_3); **^{13}C{^{1}H} NMR** (76 MHz, $CDCl_3$) δ 173.0, 150.8, 146.0, 124.9, 118.7, 113.5, 109.6, 56.0. Data in accordance with literature values. (Also commercially available; Sigma-Aldrich catalogue number 665118.)

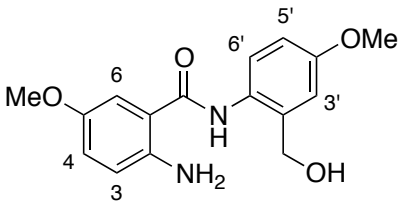
(2-Amino-5-methoxyphenyl)methanol (23)[24]



Compound **22** (2.60 g, 15.6 mmol) was dissolved in dry THF (100 mL) and placed in an ice bath with stirring. To this solution ground $LiAlH_4$ (2.28 g, 53.1 mmol) was carefully added [*caution: $LiAlH_4$ is highly flammable*]. The reaction mixture was warmed to room temperature and stirred under nitrogen atmosphere for 24 h. The mixture was diluted with cool ethyl acetate (50 mL) then quenched with careful addition of cool water until bubbling ceased. The mixture was filtered through a layer of celite and washed several times with ethyl acetate. The filtrate was dried with $MgSO_4$ and evaporated onto silica under

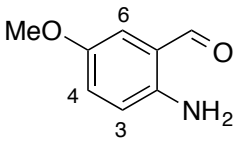
reduced pressure. The residue was subjected to column chromatography eluting with 4:1 dichloromethane/ethyl acetate to produce the title compound as an orange fluffy solid (1.55 g, 65%); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 6.74–6.64 (m, 3H), 4.62 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3), 3.01 (s, 2H, NH_2), **^{13}C{^{1}H} NMR** (76 MHz, $CDCl_3$) δ 152.8, 138.9, 126.9, 117.7, 115.1, 114,6, 64.2, 55.9. (Also commercially available; Atlantic Research Chemicals catalogue number HM00156)

2-Amino-*N*-(2-(hydroxymethyl)-4-methoxyphenyl)-5-methoxybenzamide (24)



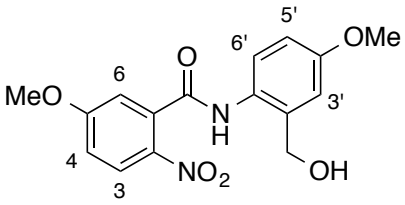
Compound **27** (206 mg, 0.62 mmol) was dissolved in methanol (5 mL), and 10% Pd/C (60 mg, 0.06 mmol) was added to this solution. The reaction mixture was stirred under H_2 atmosphere at room temperature for 4 h. The mixture was filtered through a layer of celite and washed with methanol. The filtrate was concentrated under reduced pressure to produce the title compound as a brown powder (184 mg, 98%); **m.p.** 132–137 °C; **IR** (neat) ν_{max} (cm^{-1}) 3375, 3248, 2830, 2723, 2377, 2098, 1640, 1574; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.96 (s, 1H, NH), 7.90 (d, *J* = 8.8 Hz, ArH6'), 7.07 (d, *J* = 2.8 Hz, ArH6), 6.92 (dd, *J* = 8.8, 2.8 Hz, ArH4), 6.90 (dd, *J* = 8.8, 2.8 Hz, ArH5'), 6.83 (d, *J* = 2.8 Hz, ArH3'), 6.70 (d, *J* = 8.8 Hz, ArH3), 4.79 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 167.7 (C=O), 156.8 (C4'), 151.4 (C5), 143.2 (C2), 132.8 (C1' or C2'), 130.3 (C2' or C1'), 124.8 (C6'), 120.2 (C4), 119.3 (C3), 116.9 (C1), 115.0 (C3'), 113.8 (C5'), 111.7 (C6), 64.5 (CH_2), 56.1 (OCH_3), 55.7 (OCH_3); **HRMS** (ESI, +ve) $C_{16}H_{18}N_2O_4Na^+$ [MNa^+] requires *m/z* 325.1151, found 325.1159.

2-Amino-5-methoxybenzaldehyde (25)[24]



Compound **23** (216 mg, 1.41 mmol) and MnO_2 (900 mg, 10.4 mmol) were dissolved in dry THF (20 mL). The reaction mixture was stirred under nitrogen atmosphere at room temperature for 4 h. After that time another portion of MnO_2 (900 mg, 10.35 mmol) was added, followed by a third portion of MnO_2 (900 mg, 10.35 mmol) in same time interval. The reaction mixture was stirred under N_2 atmosphere at room temperature for a total of 24 h. The mixture was filtered through a layer of celite and washed with small amount of THF. The filtrate was evaporated under reduced pressure to produce the title compound as a brown oil (225 mg, 99%). *This compound is unstable at room temperature and must be kept in a vial filled with nitrogen at –20 °C.* **^{1}H NMR** (400 MHz, $CDCl_3$) δ 9.85 (d, *J* = 0.32 Hz, CHO), 7.01 (dd, *J* = 8.8, 2.9 Hz, ArH4), 6.96 (d, *J* = 2.9 Hz, ArH6), 6.63 (d, *J* = 8.8 Hz, ArH3), 5.83 (s, 2H, NH_2), 3.79 (s, 3H, OCH_3), **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 193.7, 150.9, 144.9, 124.8, 118.6, 117.9, 116.9, 56.0.

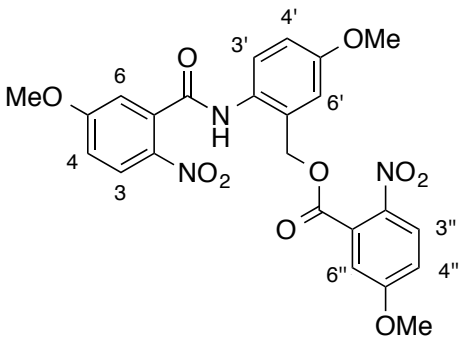
***N*-(2-(Hydroxymethyl)-4-methoxyphenyl)-5-methoxy-2-nitrobenzamide (27)**



A mixture of **14** (240 mg, 1.22 mmol) and EDC.HCl (277 mg, 1.44 mmol) were dissolved in DMF (2 mL) and stirred for 5 minutes. To this solution 1-hydroxybenzotriazole (195 mg, 1.44 mmol), **22** (207 mg, 1.35 mmol) in DMF (2 mL), and triethylamine (0.25 mL, 1.80 mmol) were added successively at 0 °C. The reaction mixture was warmed to room temperature and stirred under N_2 atmosphere for 6 h. The solvent was removed under reduced pressure and the crude product was

dissolved in HCl (10 mL, 1 N) and ethyl acetate (20 mL). After partitioning of the two layers, the aqueous layer was extracted with ethyl acetate (4 x 20 mL). All organic layers were combined and washed with water (10 x 20 mL), then the organic layer was dried with $MgSO_4$ and concentrated onto silica under reduced pressure. The residue was subjected to column chromatography eluting with 1:9→3:7 ethyl acetate/dichloromethane, to afford **28** as a pale orange solid (74 mg, 12%) and **27** as a pale yellow solid (238 mg, 59%). Data for **27**: **m.p.** 168–170 °C; **IR** (neat) ν_{max} (cm^{-1}): 3375, 3257, 3067, 3021, 2946, 2840, 2656, 2321, 2085, 2008, 1926, 1790, 1647, 1578, 1538, 1505; **^{1}H NMR** (400 MHz, CD_3CN) δ 8.64 (s, 1H, NH), 8.14 (d, *J* = 9.1 Hz, ArH3), 7.66 (d, *J* = 8.7 Hz, ArH6'), 7.20 (d, *J* = 2.8 Hz, ArH6), 7.15 (dd, *J* = 9.1, 2.8 Hz, ArH4), 6.98 (d, *J*=3.0 Hz, ArH3'), 6.92 (dd, *J* = 3.0, 8.7 Hz, ArH5'), 4.63 (s, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.45 (s, 1H, OH); **^{13}C {^{1}H} NMR** (101 MHz, CD_3CN) δ 165.8 (C=O), 164.9 (C5), 158.6 (C4'), 140.2 (C1), 137.1 (C1'), 136.6 (C2), 129.1 (C2'), 128.2 (C3), 126.7 (C6'), 115.9 (C4), 115.1 (C6), 114.4 (C3'), 113.7 (C5'), 62.5 (CH_2), 57.2 (OCH_3), 56.1 (OCH_3); **HRMS** (ESI, +ve) $C_{16}H_{16}N_2O_6Na^+$ [MNa^+] requires *m/z* 355.0901, found 355.0894.

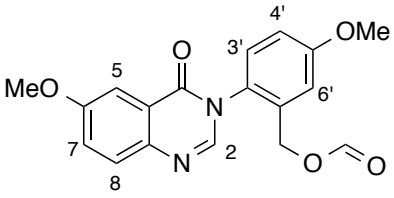
5-Methoxy-2-(5-methoxy-2-nitrobenzamido)benzyl 5-methoxy-2-nitrobenzoate (28)



Data for **28**: **m.p.** 182–184 °C; **IR** (neat) ν_{max} (cm^{-1}): 3206, 2943, 2843, 2649, 2321, 2111, 1896, 1735, 1642, 1579, 1511; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.18 (s, 1H, NH), 8.17 (d, *J* = 9.1 Hz, ArH3), 7.94 (d, *J* = 9.3 Hz, ArH3″) 7.88 (d, *J* = 8.8 Hz, ArH3′), 7.13 (d, *J* = 2.7 Hz, ArH6), 7.03 (d, *J* = 2.6 Hz, ArH6″), 7.00–6.96 (m, 4H,

ArH4, ArH4′, ArH4″, ArH6′), 5.36 (s, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3); **^{13}C {^{1}H} NMR** (76 MHz, $CDCl_3$) 166.7, 165.9, 164.1, 163.8, 157.8, 139.7, 138.9, 135.7, 130.8, 129.0, 128.8, 127.4, 127.3, 126.8, 116.9, 116.3, 115.8, 115.6, 114.2, 113.8, 65.9, 56.5, 56.4, 55.8; **HRMS** (ESI, +ve) $C_{24}H_{21}N_3O_{10}H^+$ [MH^+] requires *m/z* 512.1300, found 512.1291, $C_{24}H_{21}N_3O_{10}Na^+$ [MNa^+] requires *m/z* = 534.1119, found 534.1110.

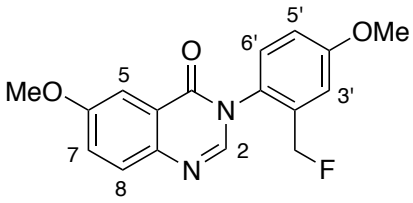
5-Methoxy-2-(6-methoxy-4-oxoquinazolin-3(4*H*)-yl)benzyl formate (29)



Compound **24** (169 mg, 0.600 mmol) was dissolved in HCOOH (300 µL, 7.95 mmol), and the reaction mixture was refluxed (oil bath temperature 105 °C) with stirring for 12 h. The reaction mixture was cooled to room temperature and diluted with chloroform (10 mL) and NaOH (3 mL, 5N). After partitioning of the two layers, the aqueous layer was extracted with chloroform (4 x 10 mL). All organic layers were combined and dried with $MgSO_4$, the solution was concentrated onto silica, and the crude product was subjected to column chromatography eluting with 1:9→3:7 ethyl acetate/dichloromethane. The title compound was produced as a yellow solid (142 mg, 74%); **m.p.** 127–132 °C; **IR** (neat) ν_{max} (cm^{-1}) 3064, 3003, 2919, 2839, 2098, 1712, 1673, 1600, 1482; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.00 (s, 1H, CHO), 7.96 (s, 1H, ArH2), 7.74–7.69 (m, 2H, ArH5, ArH8), 7.40 (dd, *J* = 9.0, 3.0 Hz, ArH7), 7.22 (d, *J* = 8.7 Hz, ArH3'), 7.12 (d, *J* = 2.9 Hz, ArH6'), 7.02 (d, *J* = 8.7, 2.9 Hz, ArH4'), 5.10 (d, *J* = 13.0 Hz, C<u>H</u>H), 5.00 (d, *J* = 13.0 Hz, CH<u>H</u>), 3.93 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3); **^{13}C{^{1}H} NMR** (76 MHz, $CDCl_3$) δ 160.9 (C4), 160.6 (C5'), 160.3 (O-C=O), 159.3 (C6), 144.7 (C2), 142.4 (C9), 134.7 (C2'), 129.6 (C3'), 129.21 (C8 or C5), 129.20 (C1'), 125.0

(C7), 123.1 (C10), 115.8 (C6'), 115.3 (C4'), 106.8 (C5 or C8), 62.0 (CH_2), 56.1 (OCH_3) , 55.8 (OCH_3); **HRMS** (ESI, +ve) $C_{18}H_{16}N_2O_5H^+$ [MH^+] requires *m/z* 341.1132, found 341.1129, $C_{18}H_{16}N_2O_5Na^+$ [MNa^+] requires *m/z* = 363.0951, found 363.0948.

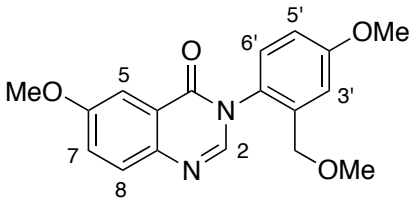
3-(2-(Fluoromethyl)-4-methoxyphenyl)-6-methoxyquinazolin-4(3*H*)-one (36)



Compound **4** (28 mg, 0.09 mmol) was dissolved in anhydrous dichloromethane (2 mL) and cooled to –78 °C. To this solution Deoxo-Fluor™ (25 µL, 1.4 mmol) was also added. The reaction mixture was warmed to room temperature and stirred under N_2 atmosphere for 24 h. The mixture was diluted with dichloromethane (2 mL) and then quenched by dropwise addition of saturated $NaHCO_3$ solution (10mL). After partitioning of the two layers, the aqueous layer was extracted with dichloromethane (4 x 3 mL). All organic layers were combined and the solvent was dried with $MgSO_4$ and evaporated onto silica under reduced pressure. The residue was purified by column chromatography eluting with 1:9 ethyl acetate/dichloromethane to yield the tittle compound as a light yellow solid (12 mg, 43%) and recovered **4** (4 mg, 0.01 mmol). Data for **36**: **m.p.** 164–170 °C; **IR** (neat) ν_{max} (cm^{-1}) 3751, 3328, 3077, 3021, 2922, 2844, 2100, 1983, 1912, 1740, 1667, 1612; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.94 (s, 1H, ArH2), 7.72 (m, 1H, ArH8); 7.70 (m, 1H, ArH5), 7.40 (dd, *J* = 8.9, 3.0 Hz, ArH7), 7.23 (dd, *J* = 8.6, 0.8, Hz, ArH6′), 7.13 (d, *J* = 2.6 Hz, ArH3′), 7.04 (dddd, *J* = 11.5, 8.6, 2.8, 1.2 Hz, ArH5′), 5.28 (dd, *J* = 47.5, 11.5 Hz, C<u>H</u>H), 5.21 (dd, *J* = 47.2, 11.5, Hz, CH<u>H</u>), 3.93 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3); **^{13}C{^{1}H} NMR** (76 MHz, $CDCl_3$) δ 160.9 (C4), 160.6 (C4′), 159.3 (C6), 144.6 (C2), 142.5 (C9), 135.5 (C2′, d, *J* = 16.56 Hz), 129.5 (C6′), 129.3 (C8), 128.5 (C1′, d, *J* = 4.2 Hz), 125.0 (C7), 123.0

(C10), 115.5 (C5′, d, *J* = 2.5 Hz), 114.8 (C3′, d, *J* = 7.76 Hz), 106.7 (C5), 81.2 (CF, d, *J* = 168.0 Hz), 56.0 (OCH_3), 55.8 (OCH_3); **$^{19}F\{^1H\}$ NMR** (76 MHz, $CDCl_3$) δ -212.5 (s, *J* = 56.1, 168.1 Hz); **^{19}F NMR** (76 MHz, $CDCl_3$) δ -212.5 (dd, *J* = 47.4, 94.7 Hz); **HRMS** (ESI, +ve) $C_{17}H_{15}F_1N_2O_4H^+$ [MH^+] requires *m/z* 315.1139, found 315.1134.

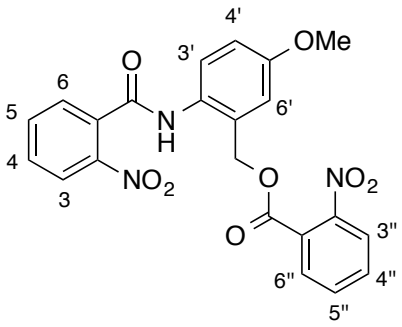
6-Methoxy-3-(4-methoxy-2-(methoxymethyl)phenyl)quinazolin-4(3*H*)-one (37)



A mixture of compound **4** (31 mg, 0.10 mmol), NaH (60% in oil, 12 mg, 0.30 mmol) and THF/DMF (1.5 mL, 1:1) was cooled to 0 °C. After stirring for 10 minutes under N_2 atmosphere, CH_3I (12 µL, 0.20 mmol) was added. The reaction mixture was stirred at room temperature under N_2 atmosphere for 12 h. The reaction mixture was quenched by dropwise addition of methanol (3 mL), and then the solvent was evaporated under reduced pressure. The residue was dissolved in water (5 mL) and ethyl acetate (10 mL). After partitioning of the two layers, the aqueous layer was extracted with ethyl acetate (3 x 10 mL). All organic layers were combined and washed with water (10 x 10 mL); finally the organic solution was dried ($MgSO_4$) and evaporated onto silica. The crude product was purified by column chromatography eluting with 3:7 ethyl acetate/dichloromethane, to produce the title compound as a pale orange oil (27 mg, 82%) and also recovered **4** (4 mg, 10%). Data for **37**: **IR** (neat) ν_{max} (cm^{-1}) 3541, 3164, 3000, 2944, 2626, 2409, 2292, 2064, 1626, 1440; **^1H NMR** (300 MHz, $CDCl_3$) δ 7.95 (s, 1H, ArH2), 7.74 (m, ArH5), 7.71 (m, ArH8), 7.40 (dd, *J* = 8.9, 3.0 Hz, ArH6), 7.19 (d, *J* = 8.6 Hz, ArH6'), 7.12 (d, *J* = 2.9 Hz, ArH3'), 6.97 (dd, *J* = 8.6, 2.9 Hz, ArH5'), 4.30 (d, *J* = 12.3 Hz, C<u>H</u>H), 4.24 (d, *J* = 12.3 Hz, CH<u>H</u>), 3.93 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3) 3.27 (s, 3H, CH_2-OC<u>H</u>$_3$); **$^{13}C\{^1H\}$ NMR** (76 MHz, $CDCl_3$) δ 160.9 (C4), 160.6 (C4'), 159.2 (C6),

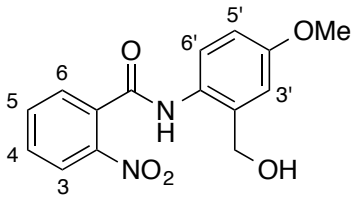
145.1(C2), 142.3 (C9), 137.4 (C1'), 129.4 (C6'), 129.1 (C5), 128.9 (C2'), 124.9 (C7), 123.1 (C10), 114.9 (C3'), 114.6 (C5'), 106.8 (C8), 71.1 (CH_2), 58.8 (CH_2-O$\underline{C}H_3$), 56.1 (OCH_3), 55.8 (OCH_3); **HRMS** (ESI, +ve) $C_{18}H_{18}N_2O_4Na^+$ [MNa^+] requires *m/z* 349.1159, found 349.1139.

5-Methoxy-2-(2-nitrobenzamido)benzyl 2-nitrobenzoate (39)



Data for compound **39**: **m.p.** 135–137 °C; **IR** (neat) ν_{max} (cm^{-1}) 3235, 3069, 2646, 2321, 2103, 1920, 1729, 1655, 1523; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.30 (s, 1H, NH), 8.14 (d, *J* = 8.2 Hz, 1H), 7.84—7.81 (m, 2H), 7.75—7.61 (m, 6H), 7.03—6.98 (m, 2H), 5.36 (s, 2H, CH_2), 3.83 (s, 3H, OCH_3); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 166.0, 165.7, 157.9, 148.0, 146.5, 134.1, 133.3, 133.0, 132.3, 130.8, 130.3, 129.1, 129.0, 128.8, 127.5, 127.0, 124.8, 124.0, 116.8, 115.9, 65.7, 55.7; **HRMS** (ESI, +ve) $C_{22}H_{17}N_3O_8Na^+$ [MNa^+] requires *m/z* 474.0928, found 474.0882.

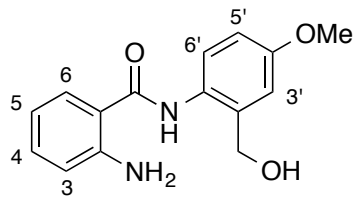
***N*-(2-(Hydroxymethyl)-4-methoxyphenyl)-2-nitrobenzamide (40)**



Compound **38** (120 mg, 0.72 mmol) and EDC.HCl (165 mg, 0.86 mmol) were dissolved in DMF (2 mL) and stirred for 5 minutes. To this solution 1-hydroxybenzotriazole (116 mg, 0.86 mmol), **23** (132 mg, 0.86 mmol) in DMF (1mL) and DIPEA (0.18 mL, 1.08 mmol) were added successively at 0 °C. The reaction mixture was stirred under N_2 atmosphere at room temperature for 24 h. The solvent was evaporated under reduced pressure. The crude product was dissolved in HCl (5 mL,

1N) and ethyl acetate (10 mL). After partitioning of the two layers, the aqueous layer was extracted with ethyl acetate (4 x 15 mL). All organic layers were combined and washed with water (10 x 20 mL). Finally the organic layer was dried with $MgSO_4$ and evaporated onto silica. The crude product was purified by column chromatography, eluting with 1:4 ethyl acetate/dichloromethane, to produce **40** as a moist white solid (118 mg, 55%) and **39** as a yellow solid (50 mg, 27%). Data for **40**: **m.p.** 144–146 °C; **IR** (neat) ν_{max} (cm^{-1}) 3232, 3071, 2918, 2864, 2712, 2450, 2284, 2111, 2069, 1647, 1580, 1521; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.56 (s, 1H, NH), 8.11 (d, *J* = 8.08 Hz, ArH3), 7.97 (d, *J* = 7.72 Hz, ArH6'), 7.68 (m, ArH4, ArH6, ArH5), 6.93 (d, *J* = 8.62 Hz, ArH5'), 6.81 (s, ArH3'), 4.74 (s, 2H, CH_2), 3.82 (s, 3H, OCH_3) 3.96 (s, H, OH); **^{13}C{^{1}H} NMR** (101 MHz, $CDCl_3$) δ 164.9 (C=O), 157.2 (C4'), 146.8 (C2), 134.0 (C4), 133.2 (C1), 132.7 (C1'), 130.9 (C6), 129.5 (C2'), 128.9 (C5), 125.3 (C6'), 124.9 (C3), 113.8 (C5'), 64.5 (CH_2), 55.7 (OCH_3); **HRMS** (ESI, +ve) $C_{15}H_{14}N_2O_5Na^+$ [MNa^+] requires *m/z* 325.0795, found 325.0772.

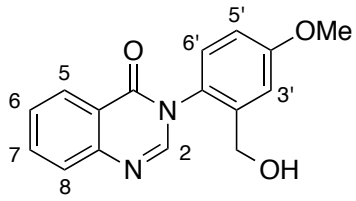
2-Amino-*N*-(2-(hydroxymethyl)-4-methoxyphenyl)benzamide (41)



Compound **40** (200 mg, 0.662 mmol) was dissolved in methanol (5mL), and 10% Pd/C (70 mg, 0.07 mmol) was added to this solution. The reaction mixture was stirred under H_2 atmosphere at room temperature for 3 h. The mixture was filtered through celite and washed with methanol. The filtrate was concentrated under reduced pressure to produce the title compound as sticky green solid (176 mg, 98%); **m.p.** 117–122 °C; **IR** (neat) ν_{max} (cm^{-1}) 3750, 3465, 3353, 3252, 2932, 2833, 2076, 1636, 1611, 1581; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.87 (s, 1H, NH), 7.87 (d, *J* = 8.8 Hz, ArH6'), 7.51 (d,

J = 8.24 Hz, ArH6), 7.25 (m, ArH4), 6.90 (dd, *J* = 8.8, 2.9 Hz, ArH5'), 6.82 (d, *J* = 2.9 Hz, ArH3'), 6.70 (m, 2H, ArH3, ArH5), 5.65 (s, 2H, NH_2), 4.69 (s, 2H, CH_2OH), 3.81 (s, H, OH); **$^{13}C\{^1H\}$ NMR** (101 MHz, $CDCl_3$) δ 168.1 (C=O), 156.8 (C4'), 149.5 (C2), 132.92 (C4 or C2'), 132.87 (C2' or C4), 130.2 (C1'), 127.4 (C6), 125.0 (C6'), 117.7 (C3 or C5) , 116.9 (C5 or C3), 115.7 (C1), 115.0 (C3'), 113.8 (C5'), 64.5 (CH_2), 55.7 (OCH_3); **HRMS** (ESI, +ve) $C_{15}H_{16}N_2O_3Na^+$ [MNa^+] requires *m/z* 295.1053, found 325.1039; $C_{15}H_{16}N_2O_3H^+$ [MH^+] requires *m/z* 273.1234, found 237.1217.

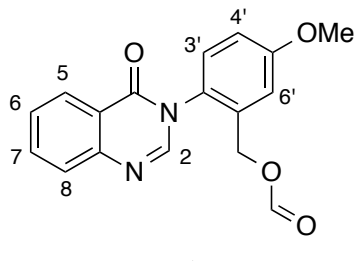
3-(2-(Hydroxymethyl)-4-methoxyphenyl)quinazolin-4(3*H*)-one (42)



Compound **41** (104 mg, 0.382 mmol) was dissolved in HCOOH (0.3 mL, 8 mmol), and refluxed (oil bath temperature 95 ^oC) with stirring for 6 h. The reaction mixture was cooled to room temperature and diluted with MeOH/THF (2 mL, 1:1) followed by the addition of K_2CO_3 (1.15 g, 8.34 mmol). This new reaction mixture was stirred at room temperature under N_2 atmosphere for 12 h. The mixture was diluted with ethyl acetate (20 mL), then filtered through a silica plug and washed with ethyl acetate. The filtrate was evaporated under reduced pressure onto silica, and the crude product was subjected to flash chromatography eluting with 3:1 dichloromethane/ethyl acetate to yield the title compound as a light yellow powder (105.5 mg, 97%); **m.p.** 147–151 °C; **IR** (neat) ν_{max} (cm^{-1}) 3235, 3079, 2964, 2908, 2341, 2110, 2077, 1980, 1890, 1683, 1606, 1500; **^1H NMR** (300 MHz, $CDCl_3$) δ 8.29 (d, *J* = 8.0 Hz, ArH5), 8.01 (s, 1H, ArH2), 7.80 (m, 1H, ArH7), 7.74 (m, 1H, ArH8), 7.54 (ddd, *J* = 8.1, 7.0, 1.3, Hz, ArH6), 7.16 (m, ArH3'), 7.13 (m, ArH6'), 6.94 (dd, *J* = 8.6, 2.9 Hz, ArH5'), 4.43 (d, *J* = 12.8 Hz, C<u>H</u>H), 4.37 (d, *J* = 12.8 Hz, CH<u>H</u>), 3.85 (s, 3H, OCH_3), 3.52 (s,

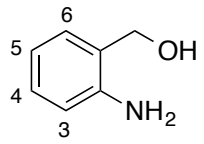
1H, OH); **$^{13}C\{^{1}H\}$ NMR** (76 MHz, CD_3CN) δ 161.9 (C4), 160.6 (C4'), 147.7 (C9), 146.9 (C2), 139.7 (C2'), 135.1 (C7), 128.9 (C6'), 128.01 (C6 or C1'), 127.95 (C1' or C6), 127.5 (C8), 127.3 (C5), 121.8 (C10), 115.0 (C3'), 114.7 (C5'), 61.3 (CH_2), 55.7 (OCH_3); **HRMS** (ESI, +ve) $C_{16}H_{14}N_2O_3Na^+$ [MNa^+] requires *m/z* 305.0897, found 305.0881.

5-Methoxy-2-(4-oxoquinazolin-3(4*H*)-yl)benzyl formate (43)



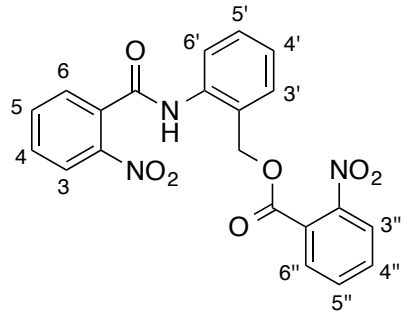
Compound **41** (56 mg, 0.21 mmol) was dissolved in HCOOH (80 µL, 2.1 mmol), and refluxed (oil bath temperature 95 °C) with stirring for 4 h. The mixture was diluted with ethyl acetate (20 mL) and filtered through a silica plug and washed with ethyl acetate. The filtrate was evaporated under reduced pressure onto silica, and the crude product was subjected to flash chromatography eluting with 3:1 dichloromethane/ethyl acetate to yield the title compound as a light yellow powder (50 mg, 79%); **m.p.** 121–125 °C; **IR** (neat) ν_{max} (cm^{-1}) 3205, 3037, 2976, 2839, 2286, 2108, 1868, 1704, 1668, 1606, 1500; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.34 (dd, *J* = 8.0, 1.2 Hz, ArH5), 8.05 (s, 1H, ArH2), 7.99 (s, 1H, CHO), 7.84–7.77 (m, 2H, ArH7, ArH8), 7.55 (dd, *J* = 8.0, 1.2 Hz, ArH5), 7.22 (d, *J* = 8.6 Hz, ArH3′), 7.11 (d, *J* = 2.8 Hz, ArH6′), 7.02 (dd, *J* = 8.6, 2.8 Hz, ArH3′), 5.09 (d, *J* = 13.0 Hz, C<u>H</u>H), 4.99 (d, *J* = 13.0 Hz, CH<u>H</u>), 3.87 (s, 3H, OCH_3); **$^{13}C\{^{1}H\}$ NMR** (101 MHz, $CDCl_3$) δ 161.1 (C4), 160.6 (C5'), 160.3 (O-CO), 148.0 (C9), 146.8 (C2), 134.9 (C7), 134.7 (C2'), 129.6 (C3'), 129.0 (C1'), 127.9 (C6), 127.7 (C8), 127.3 (C5), 122.2 (C10), 115.8 (C6'), 115.2 (C4'), 62.0 (CH_2), 55.8 (OCH_3); **HRMS** (ESI, +ve) $C_{17}H_{14}N_2O_4Na^+$ [MNa^+] requires *m/z* 333.0846, found 333.0822.

(2-Aminophenyl)methanol (44)[32]



2-Aminobenzoic acid (2.14 g, 15.6 mmol) was dissolved in dry THF (100 mL) and placed in an ice bath with stirring. To this solution ground $LiAlH_4$ (2.00 g, 53.1 mmol) was carefully added, and the reaction mixture was warmed up to room temperature and stirred under nitrogen atmosphere for 24 h. The mixture was diluted with cool ethyl acetate (50 mL) then quenched with careful addition of cool water until bubbling ceased. The mixture was filtered through celite and washed several times with ethyl acetate. The filtrate was dried with $MgSO_4$ and evaporated onto silica under reduced pressure. The crude product was subjected to column chromatography eluting with 3:1 dichloromethane/ethyl acetate to produce the title compound as a yellow fluffy solid (1.5 g, 78%); **^{1}H NMR** (300 MHz, $CDCl_3$) δ 7.14 (ddd, *J* = 15.3, 7.7, 1.6 Hz, ArH4), 7.04 (dd, *J* = 7.4, 1.3 Hz, ArH6), 6.75–6.68 (m, 2H, ArH3, ArH5), 4.60 (s, 2H, CH_2), 3.54 (s, 2H, NH_2), **^{13}C{^{1}H} NMR** (76 MHz, $CDCl_3$) δ 145.8, 129.4, 129.3, 125.0, 118.4, 116.2, 64.3.

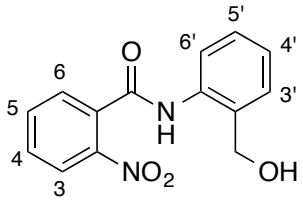
2-(2-Nitrobenzamido)benzyl 2-nitrobenzoate (45)



Data for compound **45**: **m.p.** 121–126 °C; **IR** (neat) ν_{max} (cm^{-1}) 3235, 3069, 2646, 2320, 2103, 1920, 1729, 1655, 1523; **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.64 (s, 1H, NH), 8.10 (d, *J* = 8.1 Hz, ArH6), 8.01 (d, *J* = 7.9 Hz, ArH6') 7.82 (dd, *J* =7.3, 1.7 Hz, ArH3"), 7.74–7.70 (m, 2H, ArH4, ArH5"), 7.67–7.56 (m, 4H, ArH3, ArH5, ArH4", ArH6"), 7,49–7.42 (m, 2H, ArH3', ArH5') 7.24 (dd, *J* = 15.4, 7.9 Hz, 1H, ArH4'), 5.38 (s, 2H, CH_2); **^{13}C{^{1}H} NMR**

(76 MHz, $CDCl_3$) δ 166.1, 164,4, 147.9, 146.4, 136.2, 134.1, 133.3, 132.9, 132.3, 131.9, 130.8, 130.6, 130.1, 128.9, 126.9, 126.6, 126.2, 125.2, 124.7, 124.0, 65.6; **HRMS** (ESI, +ve) $C_{21}H_{15}N_3O_7Na^+$ [MNa^+] requires *m/z* 444.0802, found 444.0771.

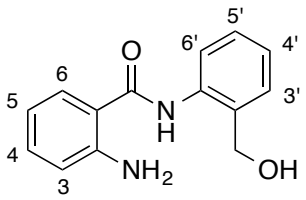
***N*-(2-(Hydroxymethyl)phenyl)-2-nitrobenzamide (46)**



38 (0.34 g, 2.0 mmol) and EDC.HCl (466 mg, 2.40 mmol) were dissolved in DMF (4 mL). After stirring for 5 minutes, 1-hydroxybenzotriazole (301 mg, 2.40 mmol), **44** (0.36 g, 3.0 mmol) in DMF (2 mL) and triethylamine (0.41 mL, 3.0 mmol) were added to this solution. The reaction mixture was stirred at room temperature for 36 h. Then DMF was evaporated under reduced pressure, and the crude product was dissolved in aq. HCl (15 mL, 1N) and ethyl acetate (25 mL). After partitioning of the two layers, the aqueous layer was extracted with ethyl acetate (4 x 25 mL). All organic layers were combined and washed with water (10 x 20 mL). Finally the solvent was dried with $MgSO_4$ and evaporated under reduced pressure onto silica. The crude product was purified by column chromatography eluting with (1:9→3:7) ethyl acetate/hexane (containing 0.5% acetic acid) to produce **45** as a pale orange solid (34.6 mg, 4%), **46** as a white solid (88 mg, 16%), and also recovered **38** (170 mg, 50%). Data for compound **46**: **m.p.** 170–172 °C; **IR** (neat) ν_{max} (cm^{-1}) 3232, 3071, 2918, 2864, 2712, 2284, 2111, 2069, 1647, 1521; **^{1}H NMR** (300 MHz, CD_3CN) δ 9.17 (s, 1H, NH), 8.09 (dd, *J* = 8.0, 0.7 Hz, ArH3), 7.95 (d, *J* = 8.4, 1.3 Hz, ArH6'), 7.83 (m, 2H, ArH5, ArH6), 7.74 (ddd, *J* = 8.0, 7.0, 2.1 Hz, ArH4), 7.40 (m, 2H, ArH5', ArH3'), 7.24 (ddd, *J* = 8.4, 7.6, 1.1 Hz, ArH4'), 4.71 (d, *J* = 3.5, 2H, CH_2), 3.56 (s, 1H, OH); **^{13}C{^{1}H} NMR** (76 MHz, CD_3CN) δ 165.4 (C=O), 148.1 (C2), 137.2 (C1'), 134.9 (C5), 133.7 (C1 or C2'), 133.6 (C2' or C1), 132.1 (C4),

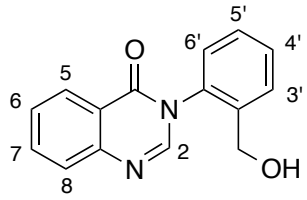
129.6 (C6), 129.4 (C3'), 129.1 (C5'), 126.3 (C4'), 125.5 (C3), 124.1 (C6'), 63.1 (CH_2); **HRMS** (ESI, +ve) $C_{14}H_{12}N_2O_4Na^+$ [MNa^+] requires *m/z* 295.0689, found 295.0672.

2-Amino-*N*-(2-(hydroxymethyl)phenyl)benzamide (47)



Compound **46** (68 mg, 0.25 mmol) was dissolved in methanol (2mL), and (10%) Pd/C (30 mg, 0.03 mmol) was added to this solution. The reaction mixture was stirred under H_2 atmosphere at room temperature for 4 h. The mixture was filtered through a layer of celite and washed with methanol. The filtrate was concentrated under reduced pressure onto silica, and the crude product was subjected to flash chromatography eluting with 3:1 hexane/ethyl acetate to yield the title compound as a white crystalline solid (45 mg, 76%); **m.p.** 140–143 °C; **IR** (neat) ν_{max} (cm^{-1}) 3440, 3278, 3025, 2928, 2877, 2343, 2111, 1997, 1812, 1620, 1617. 1582, 1510; **^{1}H NMR** (300 MHz, CD_3CN) δ 9.45 (s, 1H, NH), 8.04 (dd, *J* = 8.1, 1.2 Hz, ArH6'), 7.57 (dd, *J* = 8.0, 1.5 Hz, ArH6), 7.37–7.22 (m, 3H, ArH5', ArH3', ArH3), 7.13 (ddd, *J* = 14.9, 7.4, 1.2 Hz, ArH4'), 6.77 (dddd, *J* = 9.5, 8.2, 1.2, 0.4, Hz, ArH4), 6.67 (dddd, *J* = 8.2, 8.0, 7.2, 1.2 Hz, ArH5), 5.92 (s, 2H, NH_2) 4.69 (s, *2*H, CH_2), 3.72 (s, 1H, OH); **^{13}C{^{1}H} NMR** (76 MHz, $CDCl_3$) δ 168.6 (C=O), 151.0 (C2), 138.7 (C2'), 133.6 (C3), 132.5 (C1'), 129.4 (C3') , 129.0 (C5'), 128.4 (C6), 125.0 (C4'), 123.5 (C6'), 118.0 (C4); 116.9 (C5), 116.1 (C1), 63.9 (CH_2); **HRMS** (ESI, +ve) $C_{14}H_{14}N_2O_2Na^+$ [MNa^+] requires *m/z* 265.0947, found 265.0944.

3-(2-(Hydroxymethyl)phenyl)quinazolin-4(3*H*)-one (48)



Compound **47** (16 mg, 0.06 mmol) was dissolved in HCOOH (100 µL, 1.86 mmol) and refluxed (oil bath temperature 95 °C) with stirring for 12 h. The reaction mixture was cooled to room temperature and diluted with MeOH/THF (2 mL, 1:1), followed by K_2CO_3 addition (270 mg, 1.95 mmol). This new reaction mixture was stirred at room temperature under N_2 atmosphere for 12 h. The mixture was diluted with ethyl acetate (10 mL) and filtered through a silica plug and washed with ethyl acetate. The filtrate was evaporated under reduced pressure onto silica, and the crude product was subjected to flash chromatography eluting with 1:3 ethyl acetate/hexane, to produce the title compound as a light yellow powder (13 mg, 83%); **m.p.** 154–159 °C; **IR** (neat) ν_{max} (cm^{-1}) 3290, 3042, 2920, 2644, 2320, 2109, 1982, 1942, 1876, 1846, 1672, 1599, 1561; **^{1}H NMR** (600 MHz, CD_3CN) δ 8.26 (dd, *J* = 7.9, 1.5 Hz, ArH5), 8.06 (s, 1H, ArH2), 7.86 (ddd, *J* = 15.3, 7.9, 1.5 Hz, ArH7), 7.77 (dd, *J* = 8.2, 1.2 Hz, ArH8), 7.64 (d, *J* = 7.6 Hz, ArH3′), 7.59 (m, 1H, ArH), 7.56 (ddd, *J* = 15.2, 7.6, 1.3 Hz, ArH4′), 7.49 (ddd, *J* = 15.2, 7.6, 1.5 Hz, ArH5′), 7.37 (dd, *J* = 7.8, 1.3 Hz, ArH6′), 4.44 (d, *J* = 13.9 Hz , C<u>H</u>H) , 4.42 (d, *J* = 13.9 Hz , CH<u>H</u>), 3.24 (s, 1H, OH); **^{13}C{^{1}H} NMR** (150 MHz, CD_3CN) δ 161.8 (C4), 149.3 (C10), 148.1 (C2), 140.3 (C1′), 137.1 (C2′), 135.5 (C7), 130.7 (C4′), 129.9 (C3′), 129.6 (C5′), 129.3 (C6′), 128.5 (C8), 128.4 (C6), 127.5 (C5), 123.4 (C9), 61.2 (CH_2); **HRMS** (ESI, +ve) $C_{15}H_{12}N_2O_2H^+$ [MH^+] requires *m/z* 253.0972, found 253.0968.

4.2 Biological screening

4.2.1 Cell culture protocol for neuroblastoma cells[28]

"Full media" was prepared by mixing FBS (25 mL), F12 growth media (250 mL), DMEM (250 mL), glutamax (5 mL), antibiotic/antimycotic (5 mL) and sodium pyruvate (5 mL). All culture media were obtained from Gibco / Life Sciences and culture procedures were undertaken using aseptic techniques.

SH-SY5Y cells (human, ATCC# CRL-2266) were grown in T-75 flasks at 37 °C and 5% CO_2 (Incubator). Full media was changed every 3–4 days until cells were ready to subculture (around 9–15 days). SHSY5Y cells grow as a mixture of floating and adherent cells.

The media change was performed by transferring the cell suspension from the flask (or flasks) to a centrifuge tube (50 mL), adding 6 mL of full media to each flask and returning to the incubator. The tube with floating cells was centrifuged at 700 rpm for 7 minutes. The supernatant was then discarded, and pellet resuspended in full media (6 mL). Finally 6 mL of cell suspension was added to each flask, so each flask had 12 mL of new full media.

4.2.2 Subculturing protocol

Suspended cells were transferred to a centrifuge tube, then 0.05% triton X100 (3–5 mL) was added and the tube was gently shaken for 2–3 minutes to dissociate any attached cells. Once cells were detached (and checked under microscope), full media (5 mL) was added to each flask. The cellular contents were triturated, transferred to

centrifuge tubes, then centrifuged at 700 rpm for 7 minutes. Finally the supernatant was discarded and the pellet was resuspended in growth media (10 mL). Cells were counted and seeded at 1 x 10^6 cells per flask for subculture, or diluted to ~ 5 x 10^3 cells per mL for plating in 48 well culture dishes.

4.2.3 MTT assay protocol[29]

Stock solutions (3000 μM) of pure synthetic structural analogues of dictyoquinazol A (0.006 mmol) were prepared by dissolving in DMSO (2 mL). These solutions were sterilized by filtration prior to being added to the cells. A series of dilutions was performed from the stock solution in order to obtain final concentrations of 0.3, 1, 3, 10, 30 and 100 μM.

SHSY5Y cells in full media (500 μL) were plated into 48 wells (~5 x 10^3 cells per mL) and kept in the incubator for 24 h prior to experimentation. The cells were treated with the compounds at different concentrations (each compound was tested in triplicate). In each plate there were also reference standards of 0 and 100% live cells, created by replacing the drug dose with fresh media and triton X100 respectively (each also in triplicate). After drug treatment, the plate was returned to the incubator for 24 h. After that time 20 μL of MTT solution was added to each well and returned to the incubator for 30 min. The assay was stopped completely removing the MTT-containing medium from each well and adding DMSO (250 μL). After that plates were covered with foil and kept in the dark for 1 h. Finally reading was performed at 570 nm on a spectrophotometric plate reader; healthy, living cells show an intense purple color

while dead cells do not. Hence, the absorbance of each well is proportional to the number of live cells.

4.2.4 STS assay protocol[33]

SHSY5Y cells in full media (500 µL) were plated into 48 wells (~ 5 x 10^3 cells per mL) and kept in the incubator for 24 h prior to experimentation. The cells were treated with a mixture of STS (4.5 µL from 10 nM stock solution in media) and a varying concentration of drug compounds (each compound was tested in triplicate). On each plate there were also reference standards of 0, 50% and 100% live cells by replacing the drug dose with either fresh media, STS or Triton X100 respectively (each also in triplicate). After dosage, the treatment plate was returned to the incubator for 24 h.

After that time 20 µL of MTT solution was added to each well and returned to the incubator for 30 min. The assay was stopped completely by removing the MTT-containing medium from each well and adding DMSO (250 µL). After that plates were covered with foil and kept in the dark for 1 h. Finally, reading was performed at 570 nm using a spectrophotometric plate reader.

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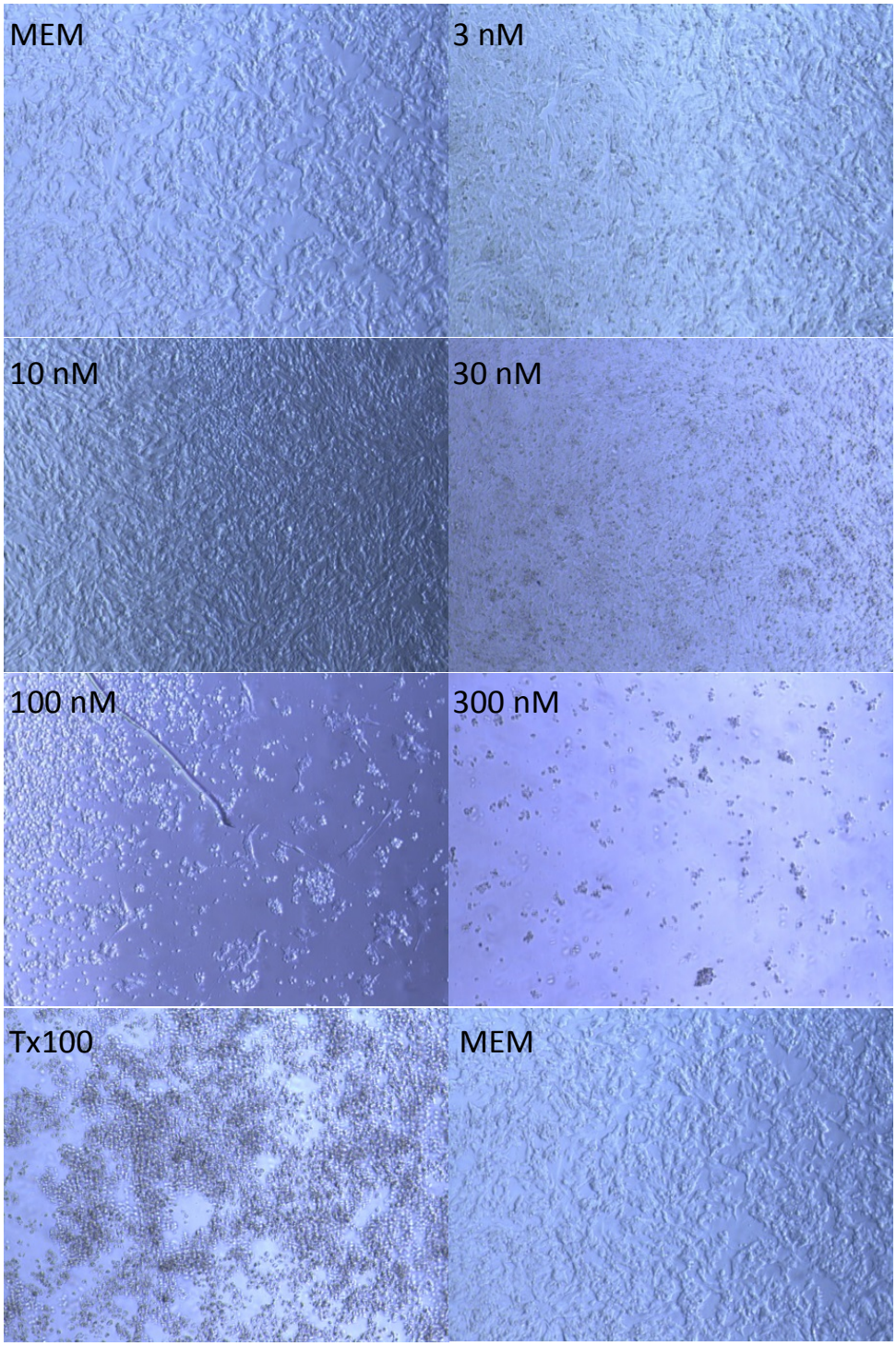
Appendices

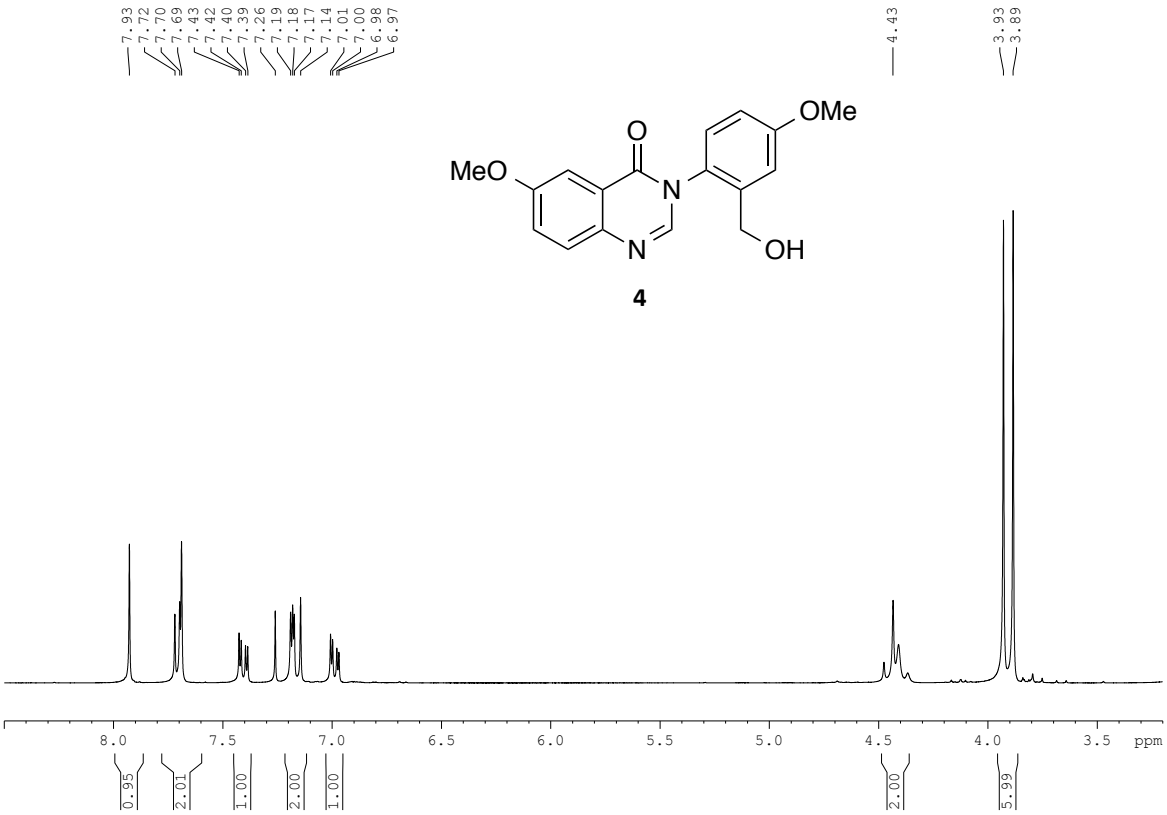
A1 Microscopy images used to determine STS concentration window



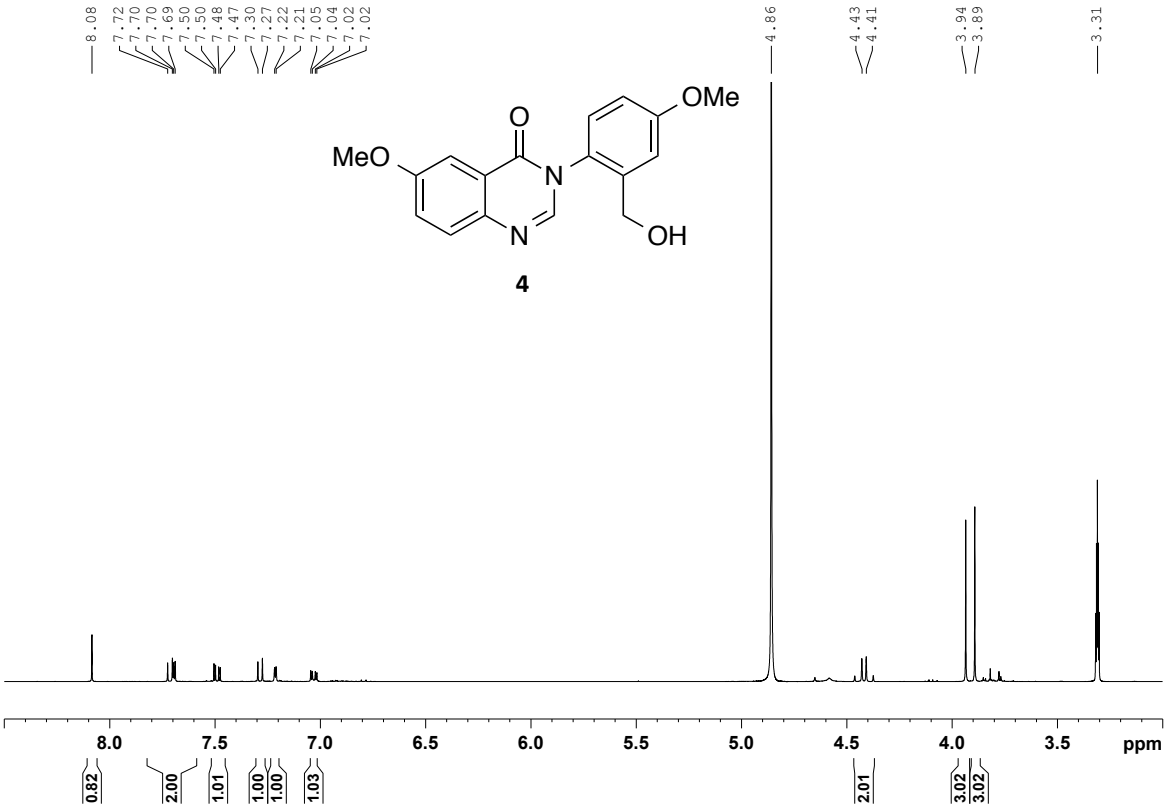
The STS concentration that kills around 50% of cells was determined to be 30nM. “Tx100” and “MEM” represent 0% and 100% cell viability, respectively.

A2 NMR spectra

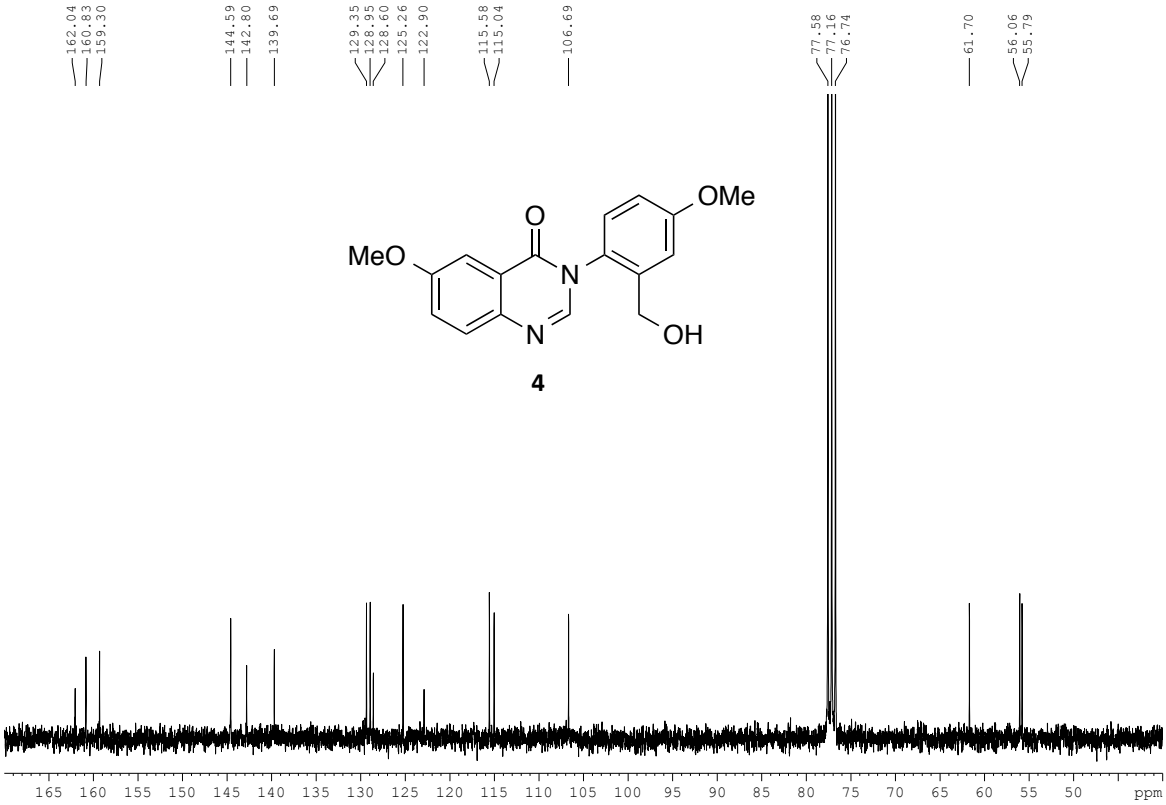
^{1}H NMR (300 MHz, $CDCl_3$)



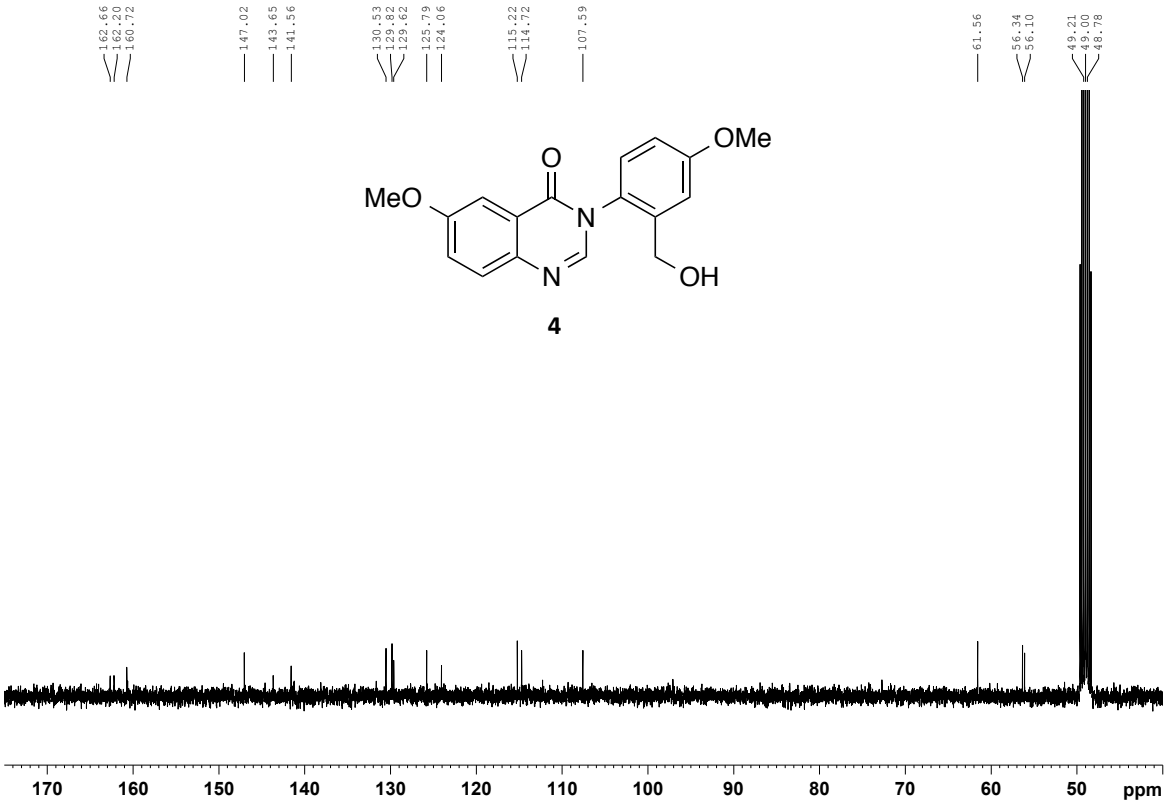
^{1}H NMR (400 MHz, MeOD)



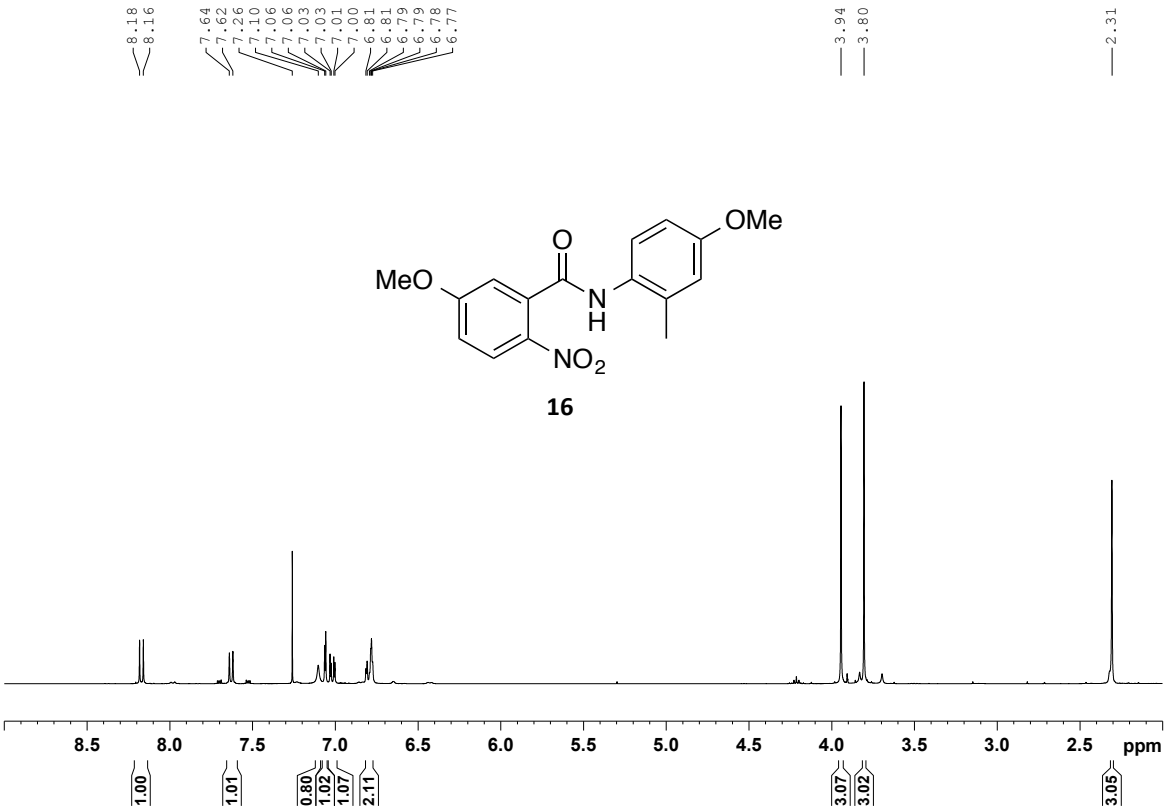
$^{13}C\{^{1}H\}$ NMR (76 MHz, $CDCl_3$)



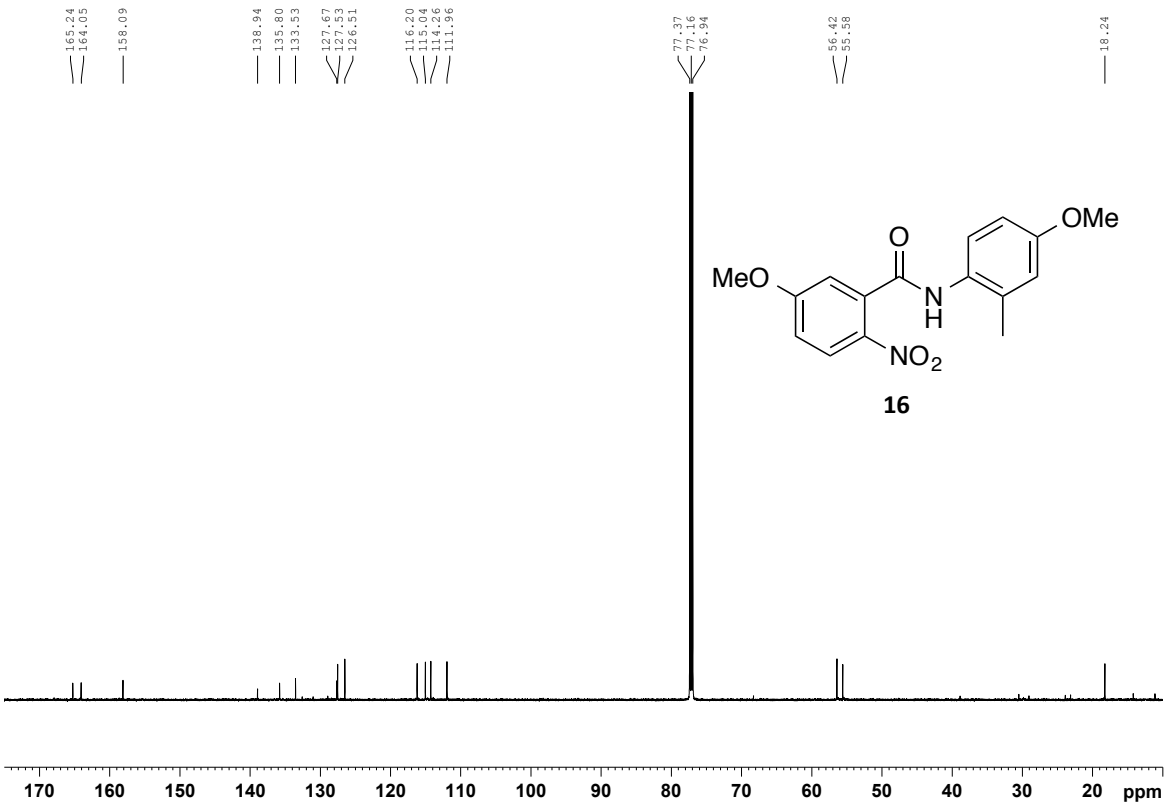
$^{13}C\{^{1}H\}$ NMR (101 MHz, MeOD)



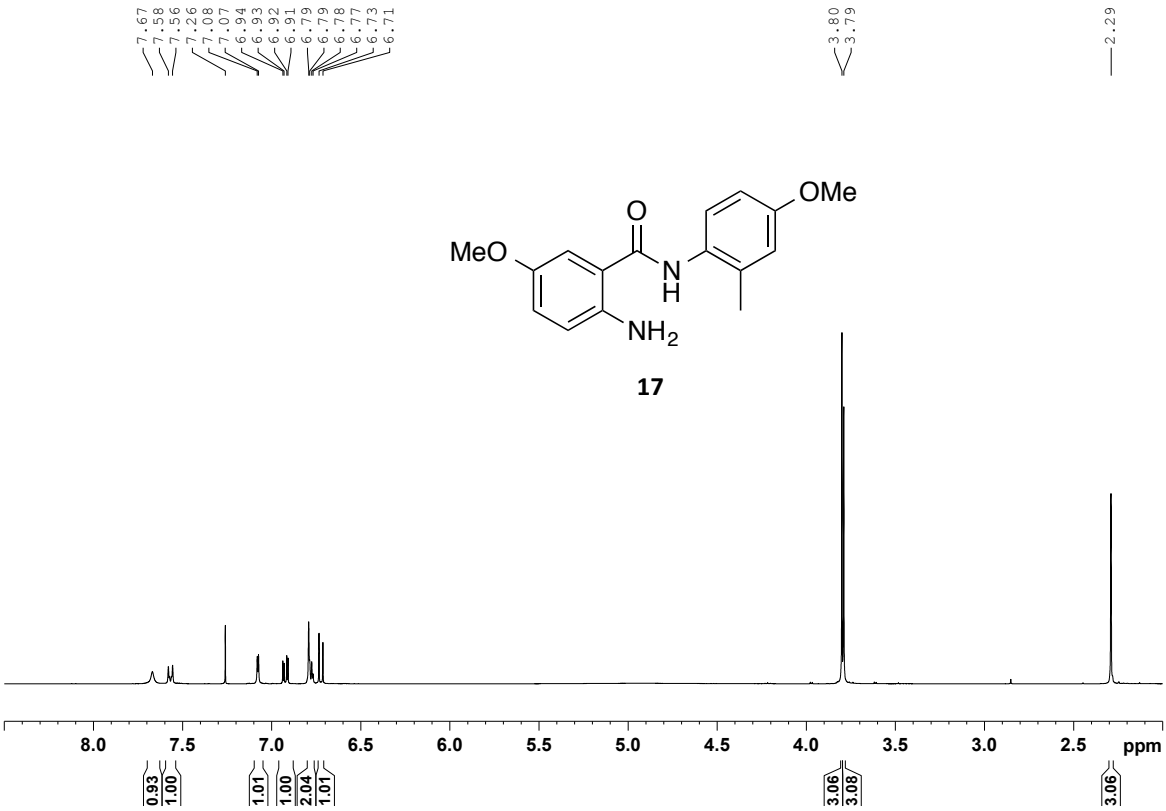
^{1}H NMR (400 MHz, $CDCl_3$)



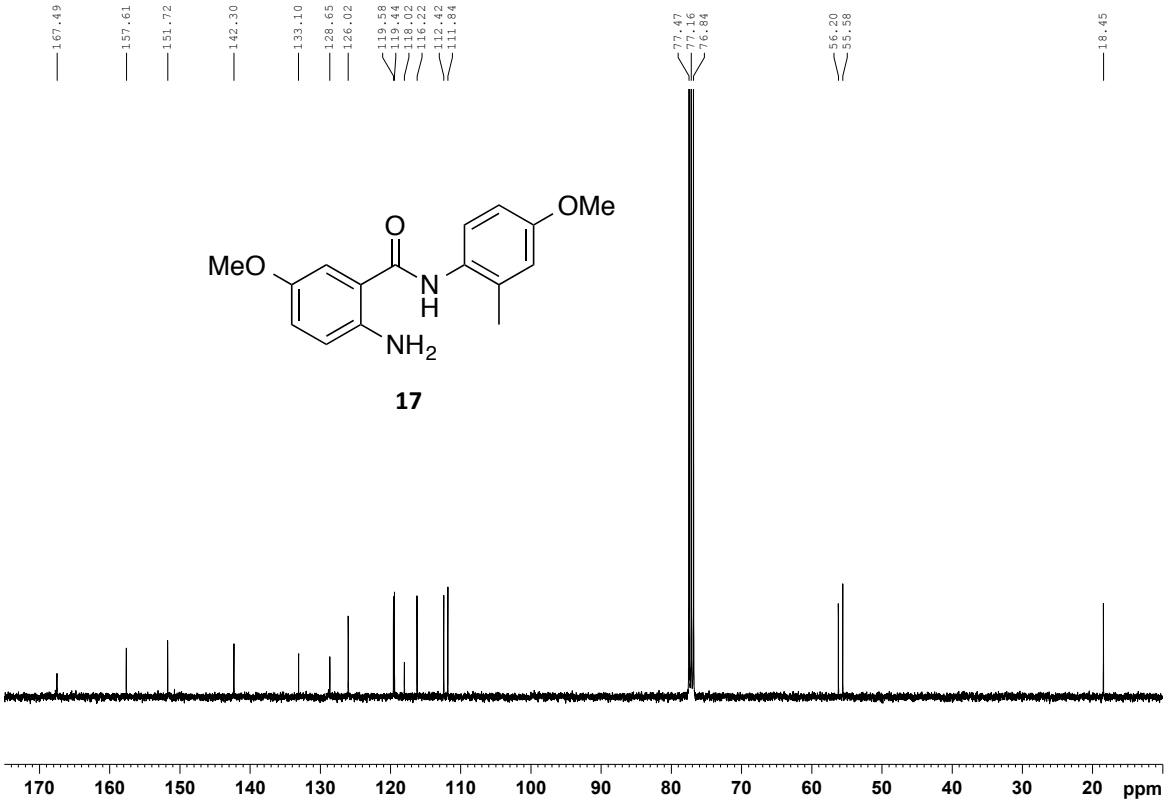
^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$)



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



^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$)



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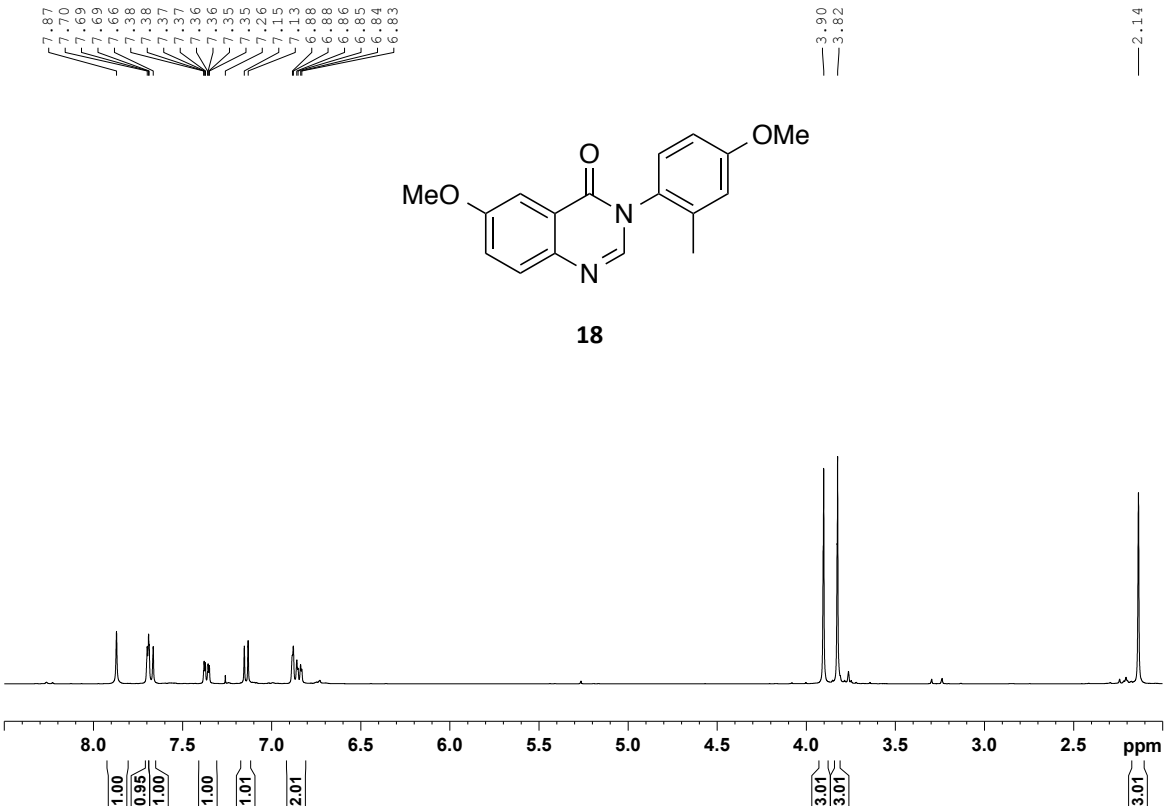
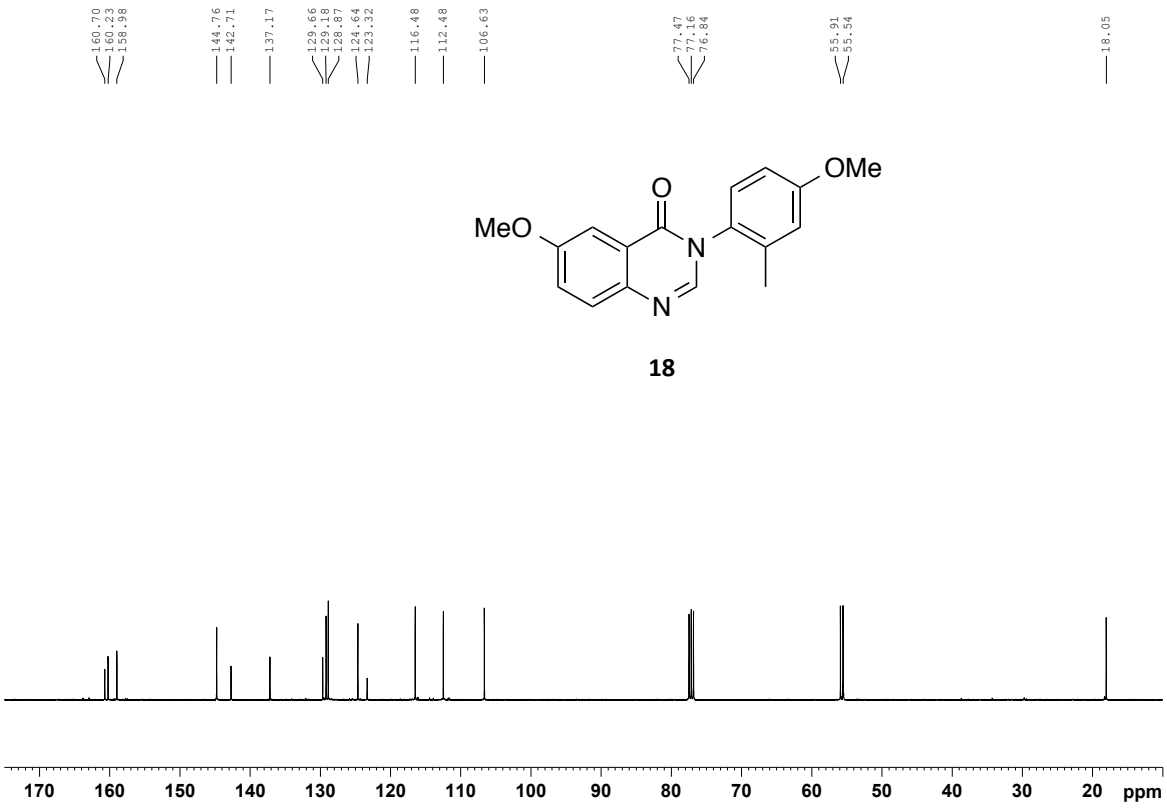


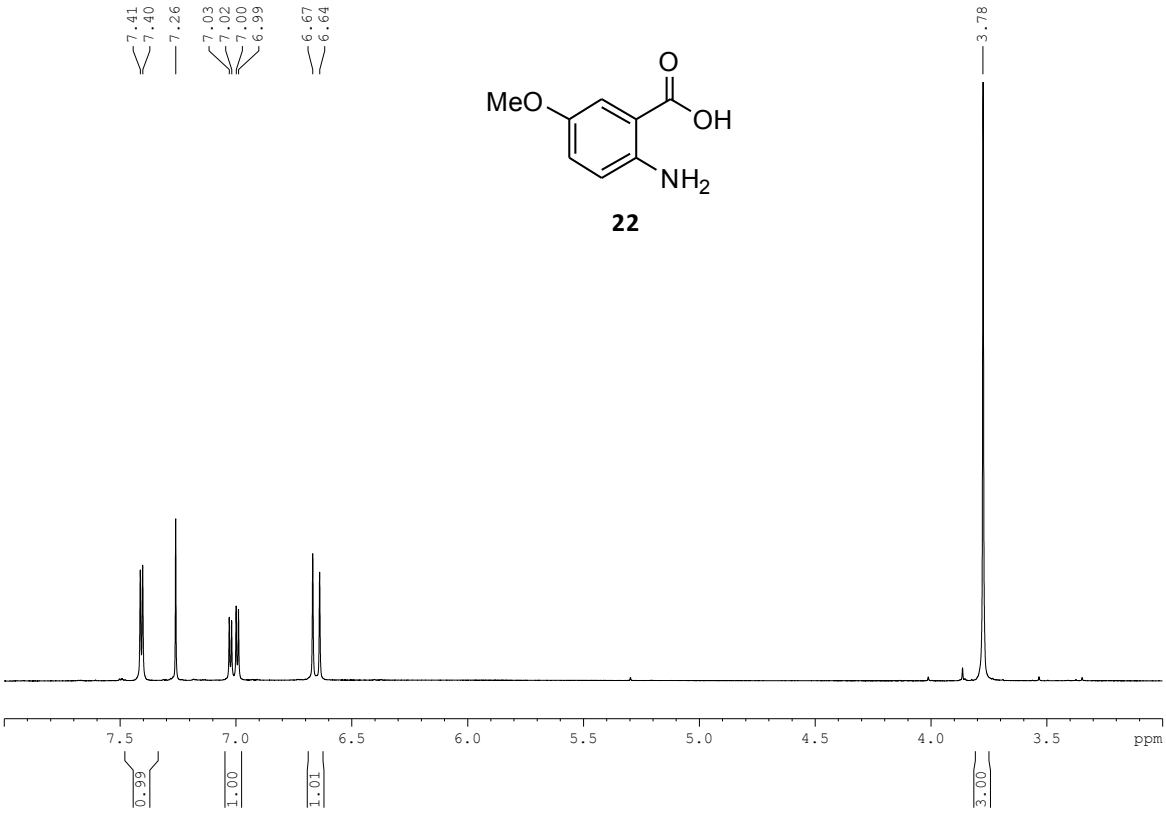
18

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$)

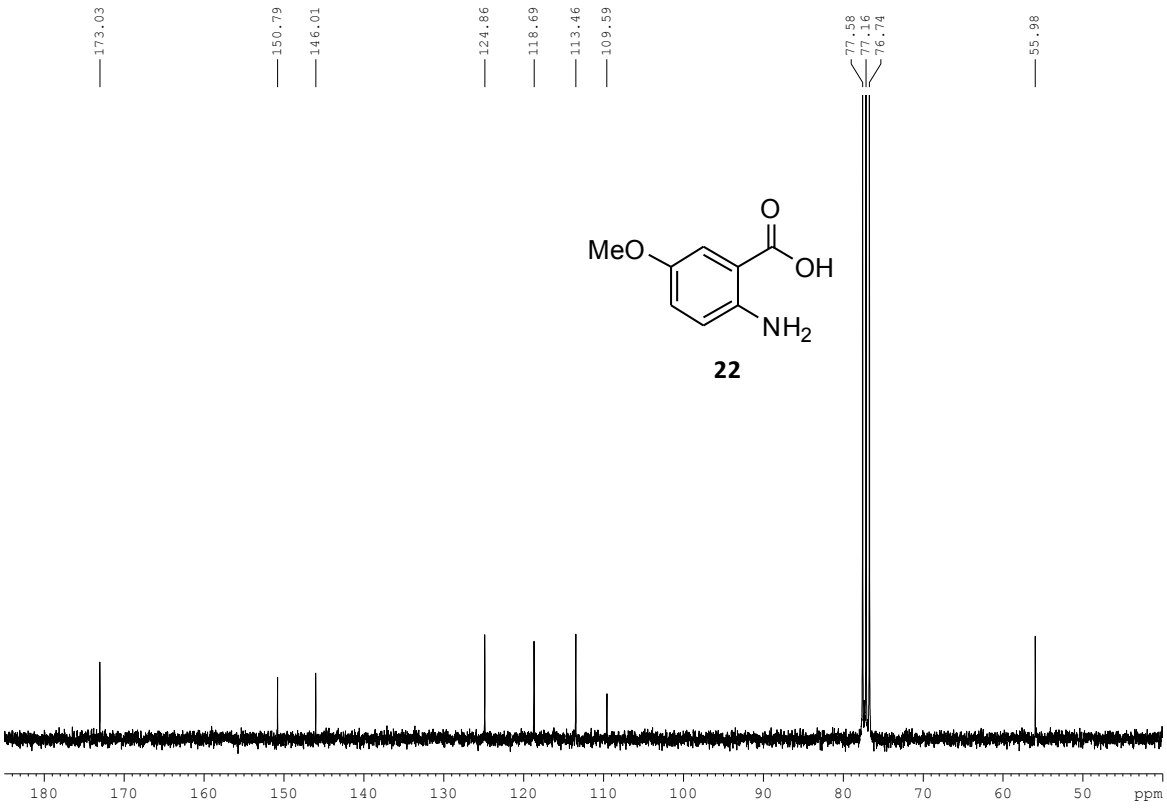


18

^{1}H NMR (300 MHz, $CDCl_3$)



^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$)



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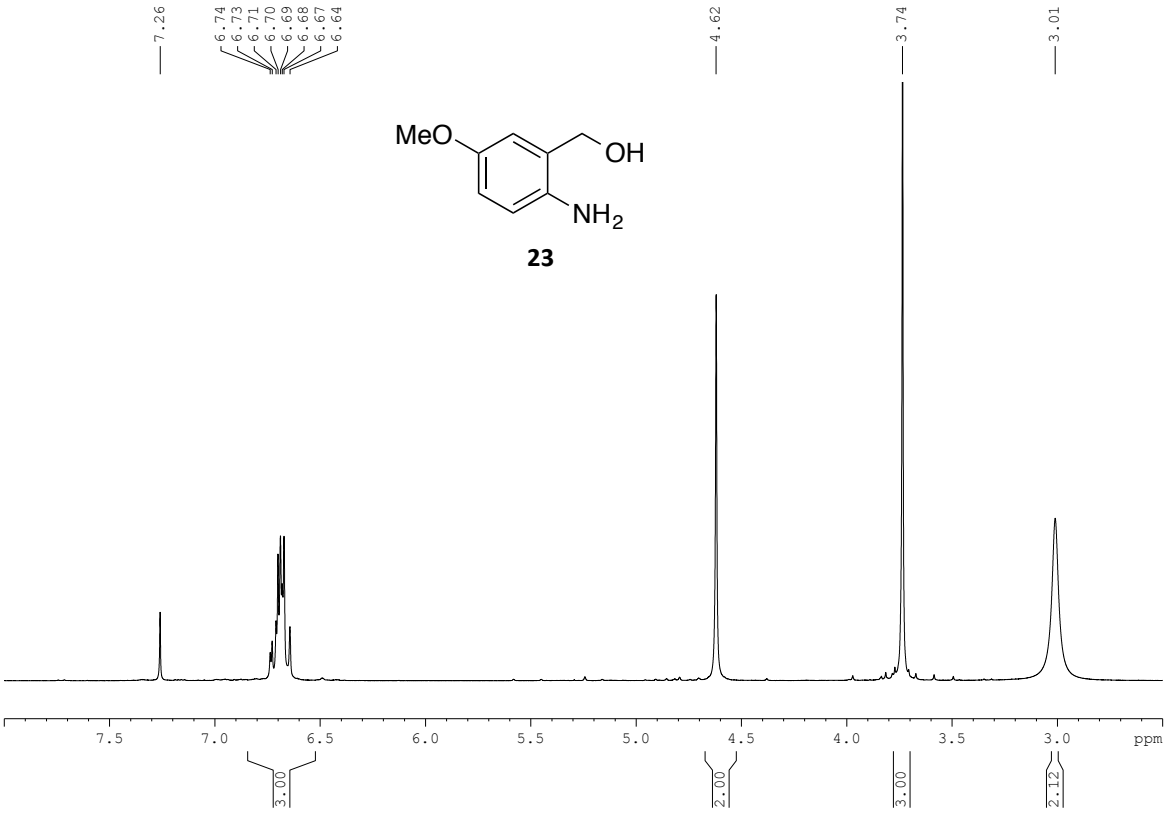

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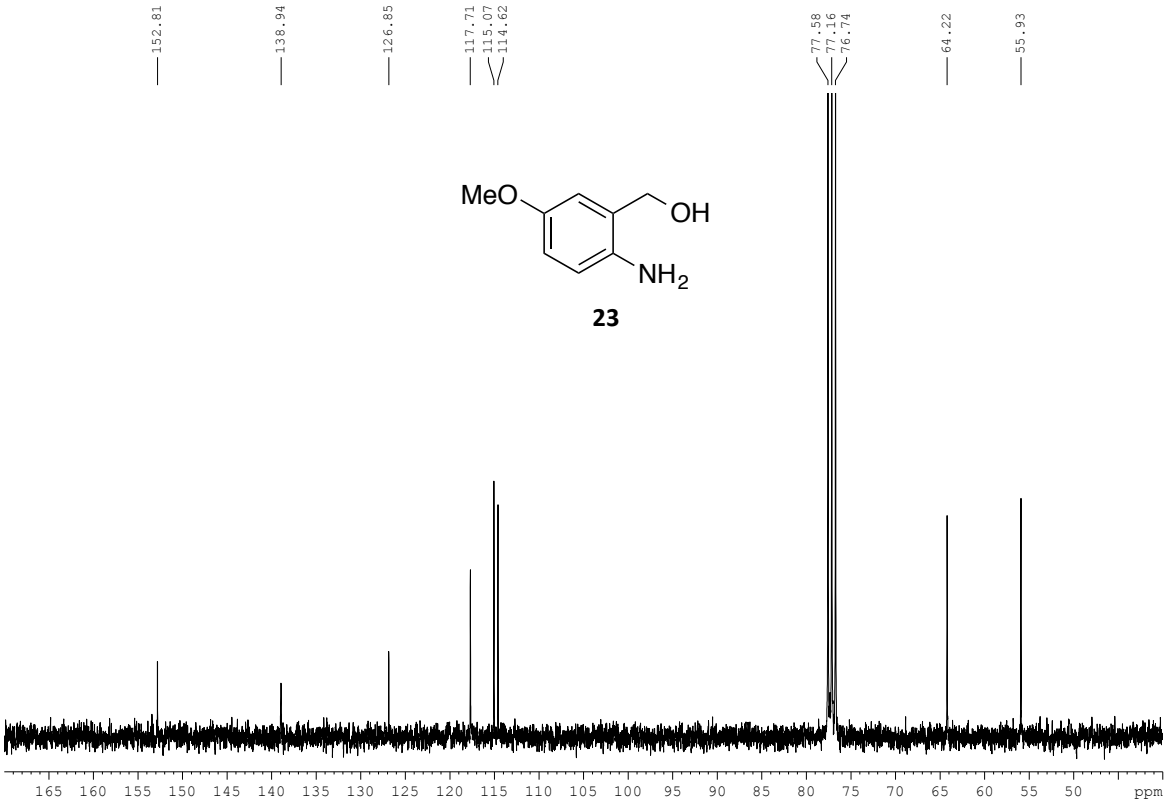
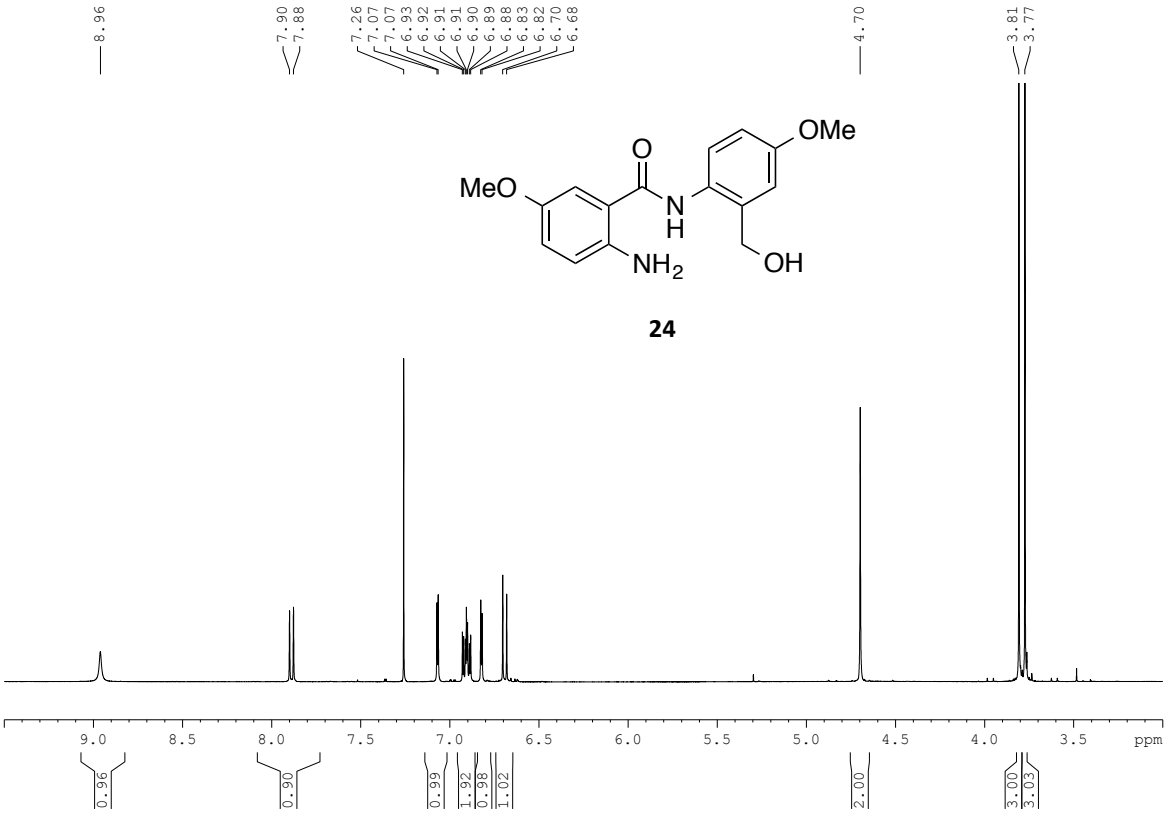
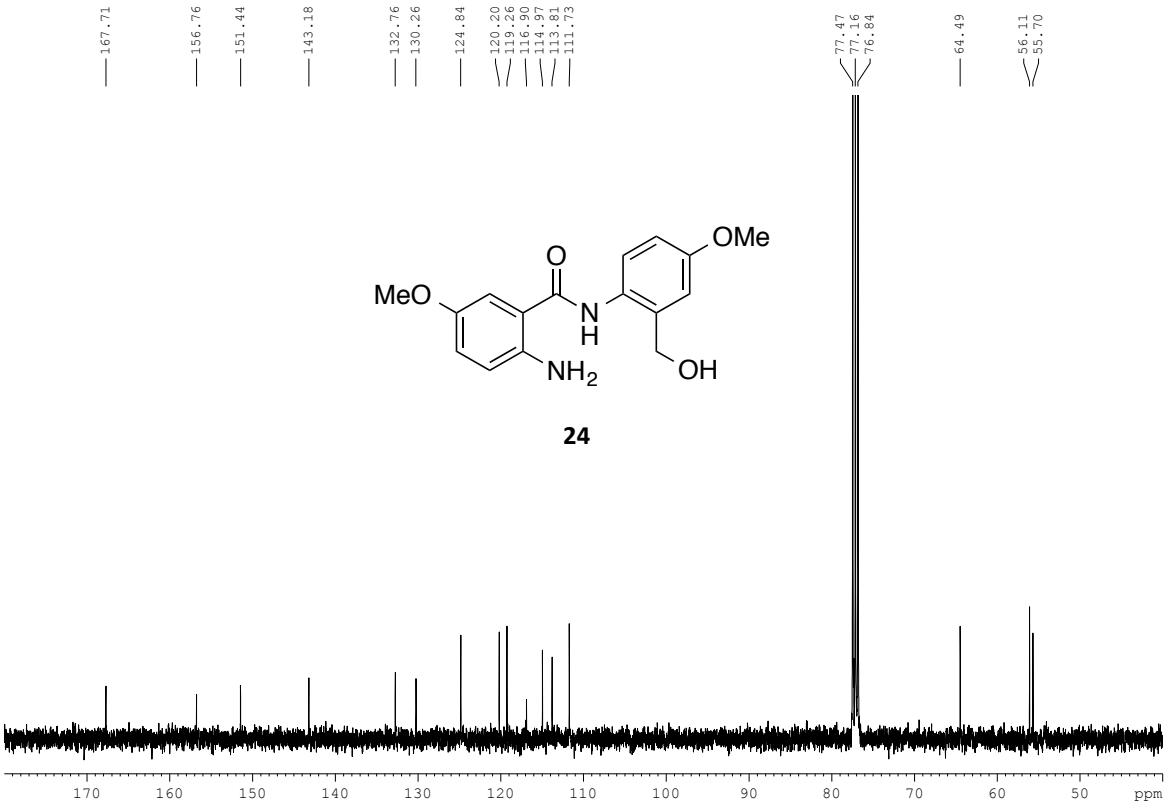


^{1}H NMR (400 MHz, $CDCl_3$)



24

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$)



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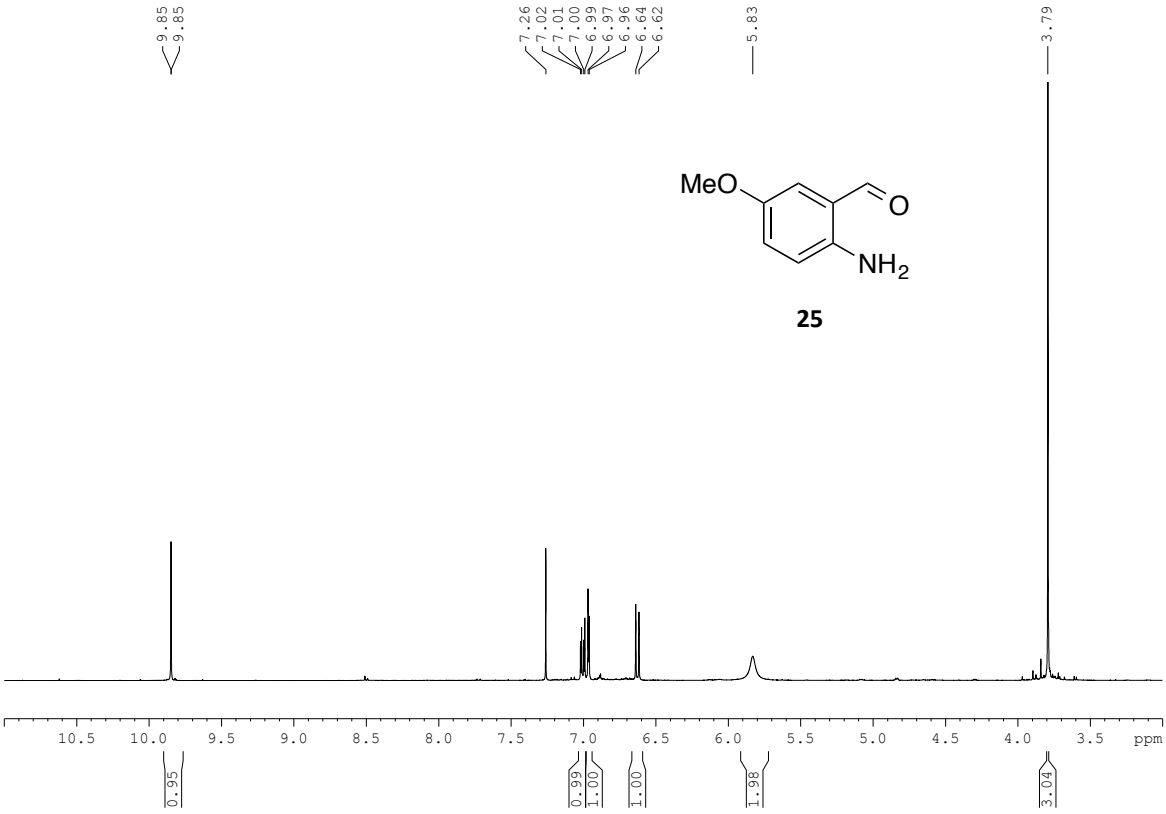

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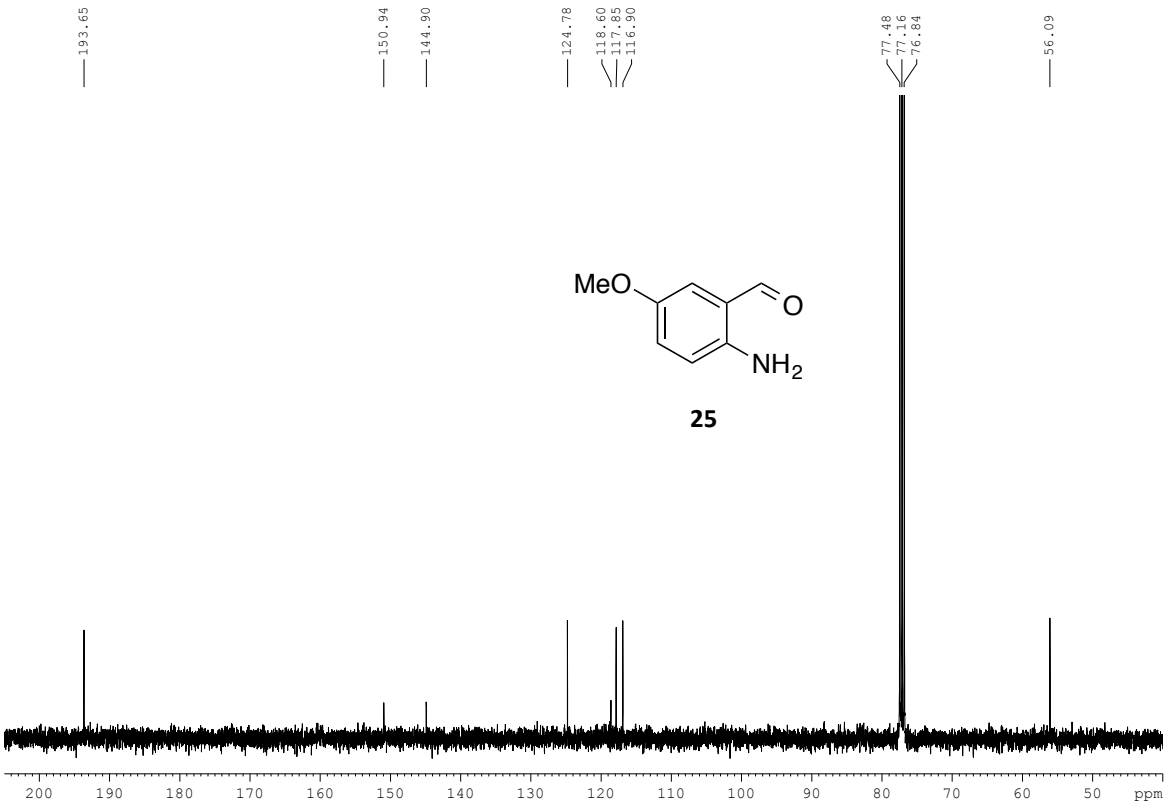

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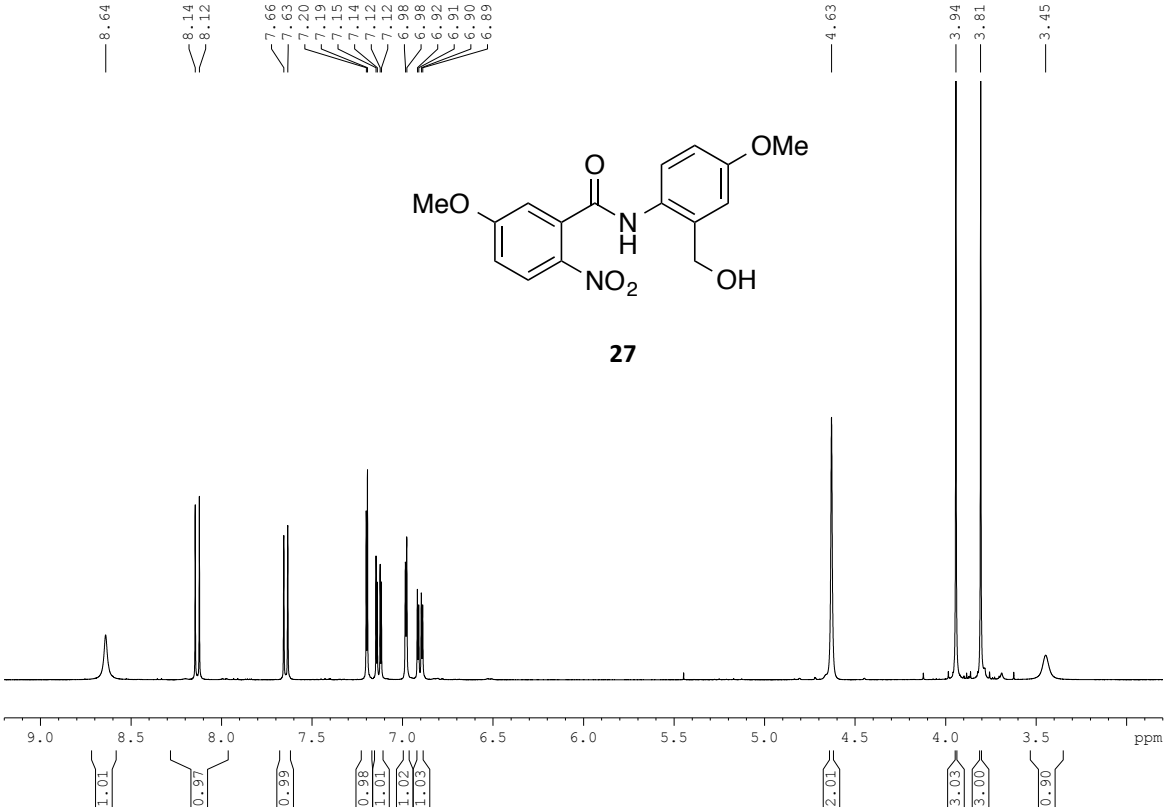
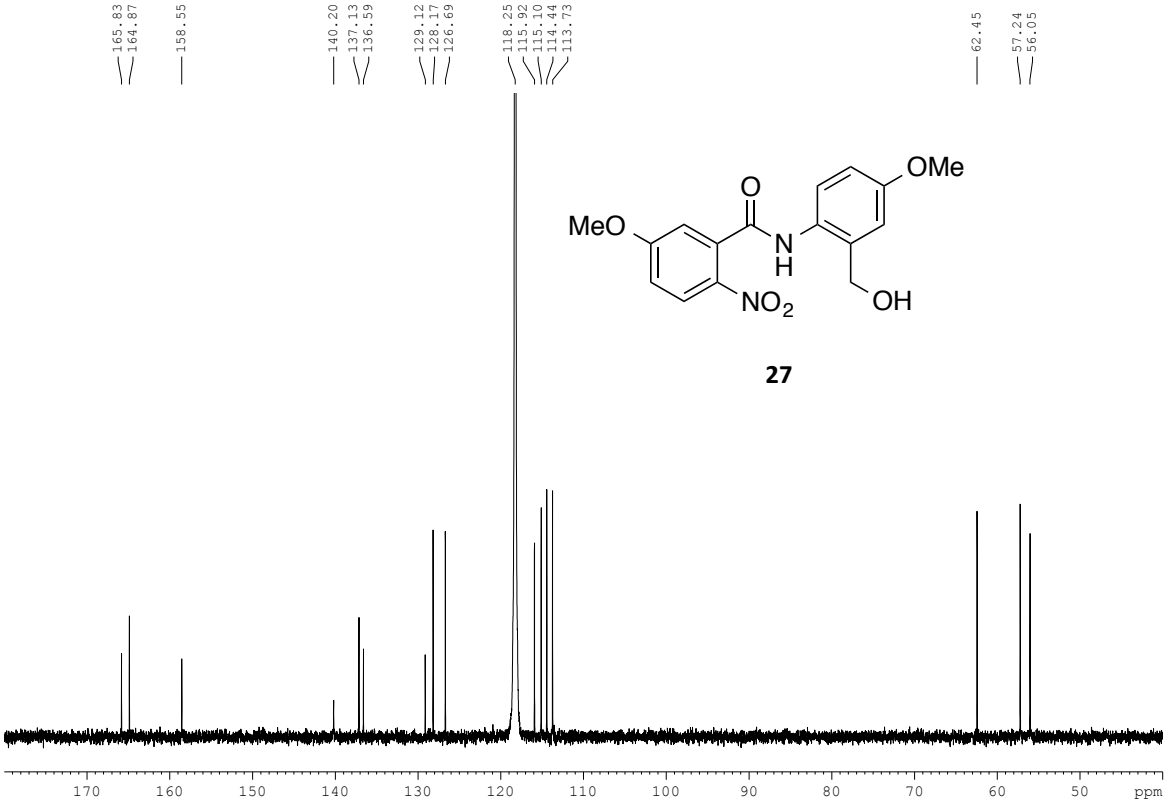
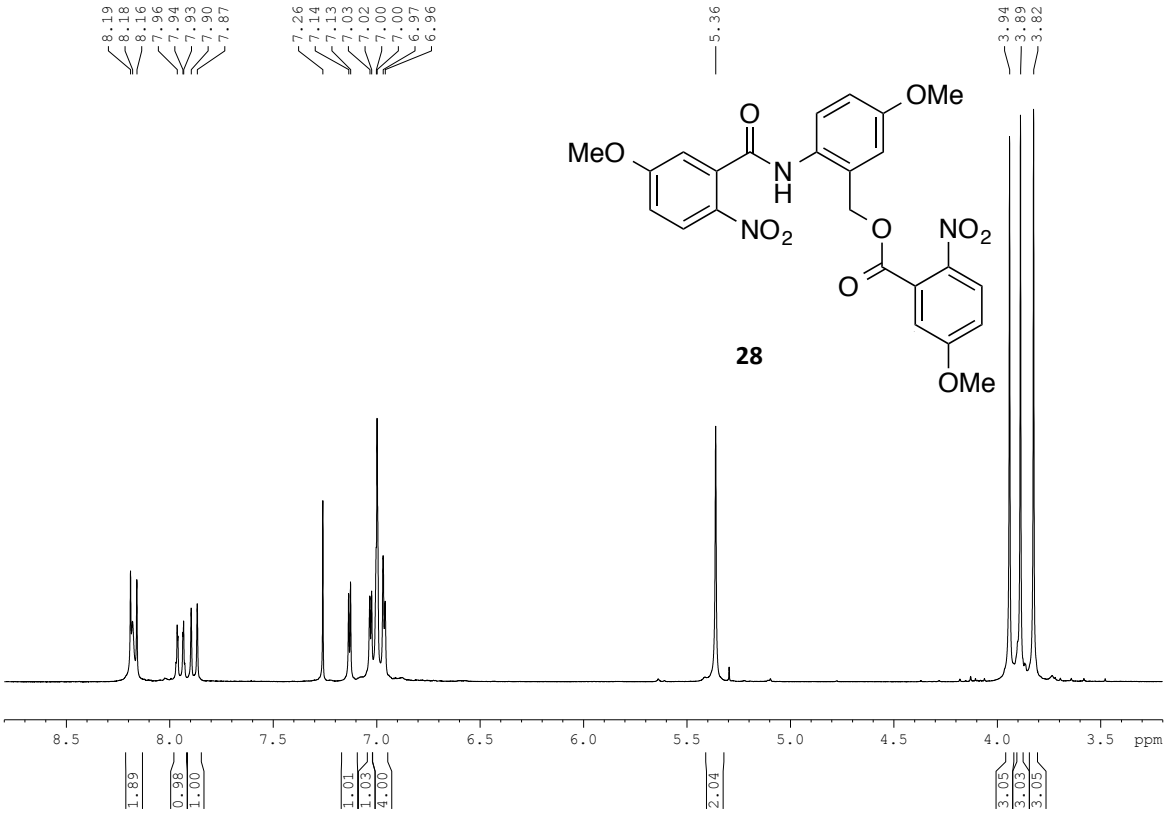


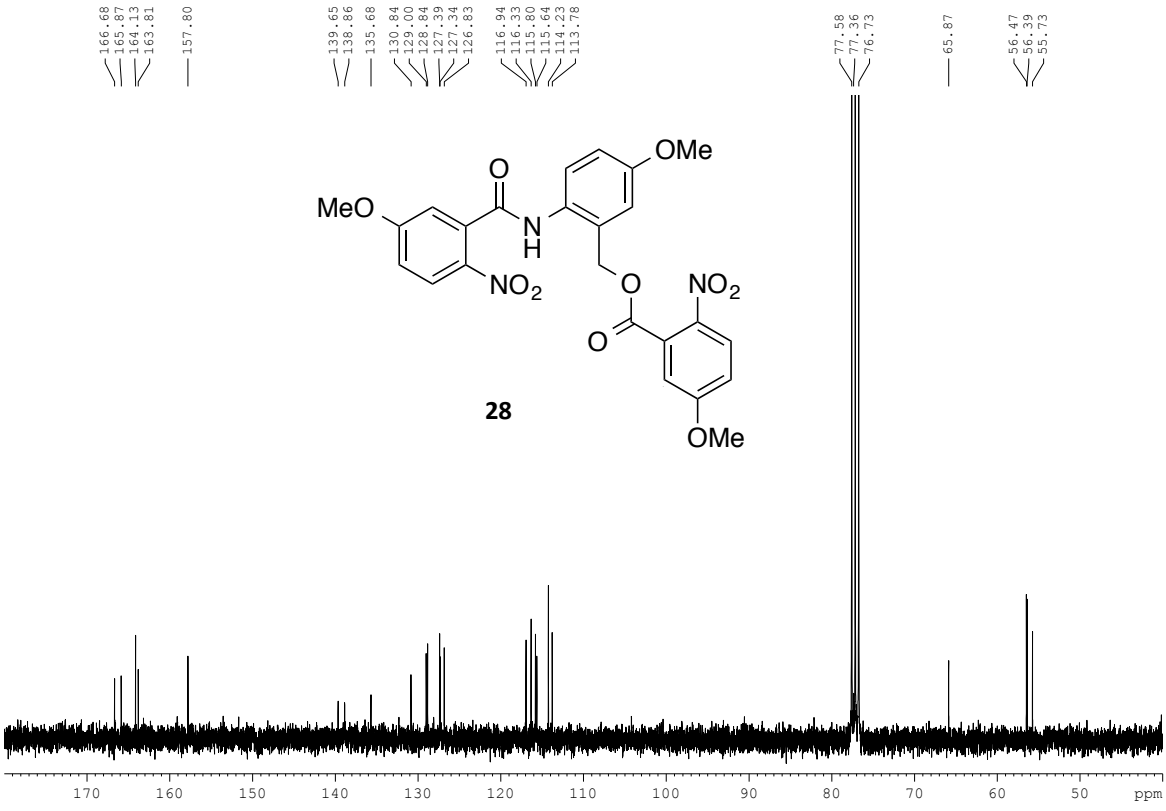
^{13}C {^{1}H} NMR (101 MHz, CD_3CN).



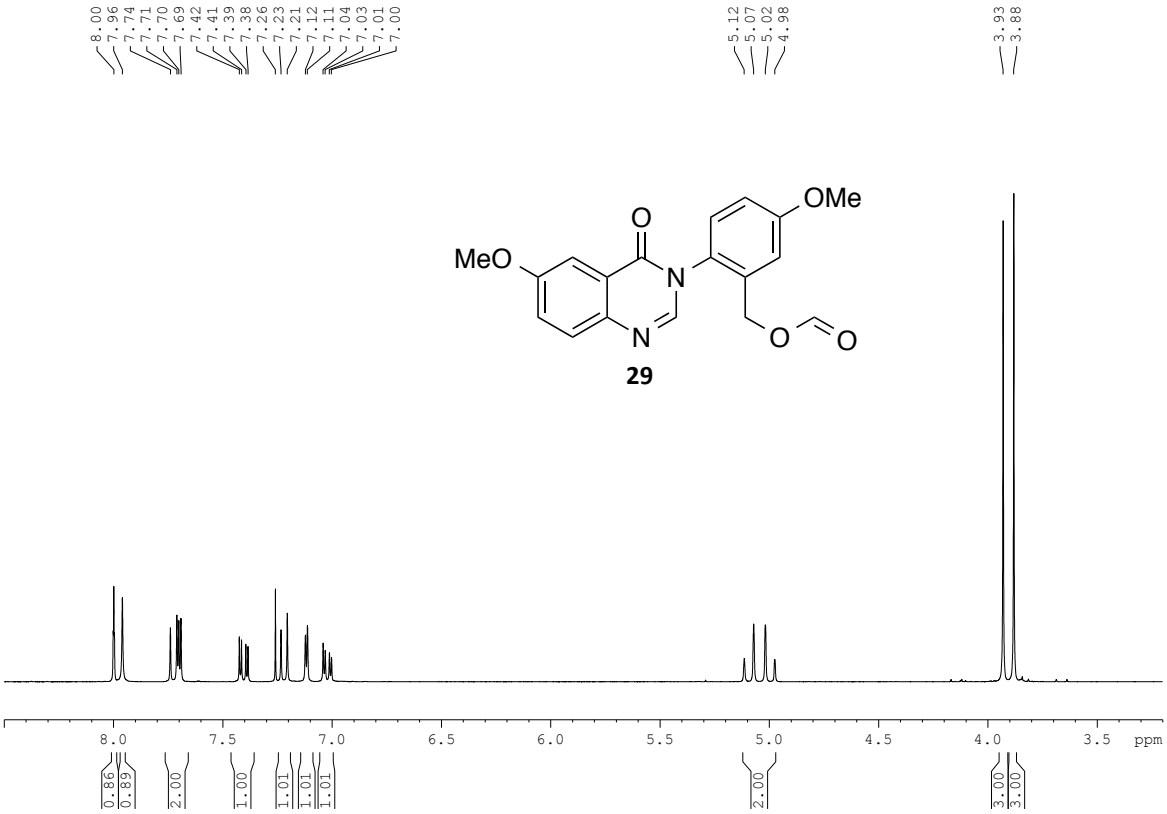
^{1}H NMR (300 MHz, $CDCl_3$)



^{13}C {^{1}H} NMR (76 MHz, $CDCl_3$)

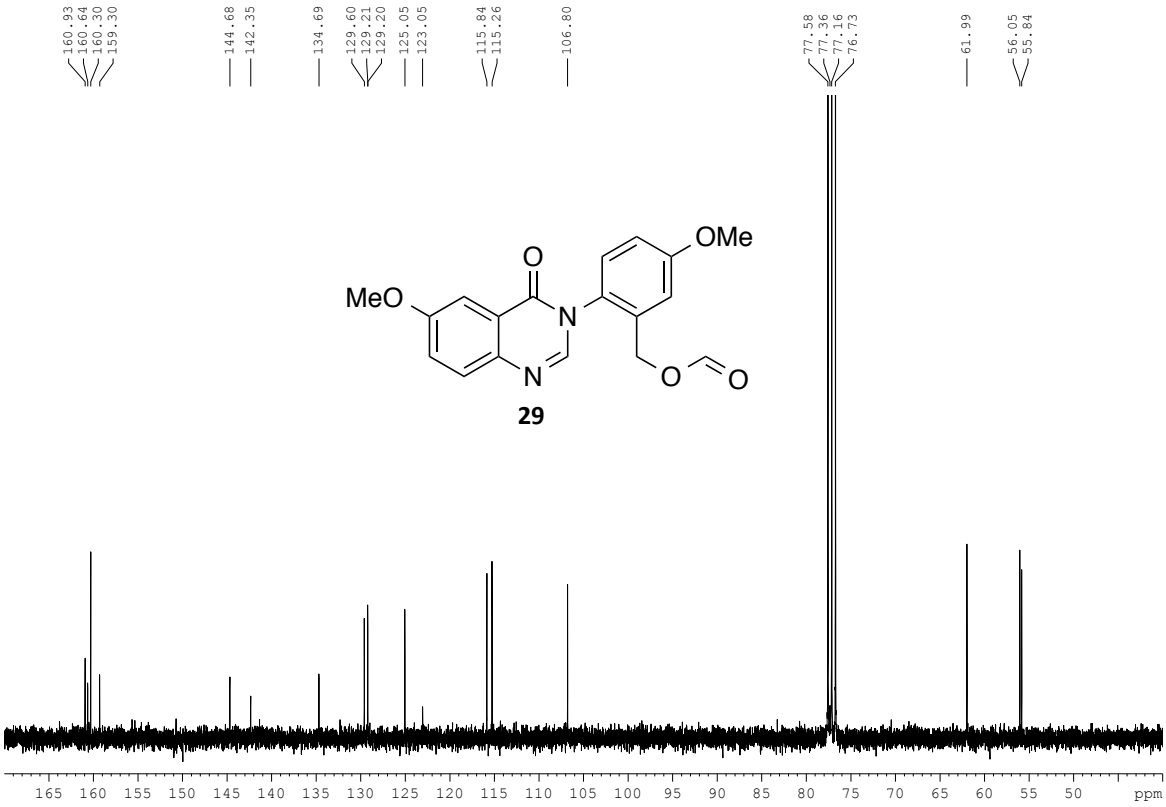


^{1}H NMR (300 MHz, $CDCl_3$)



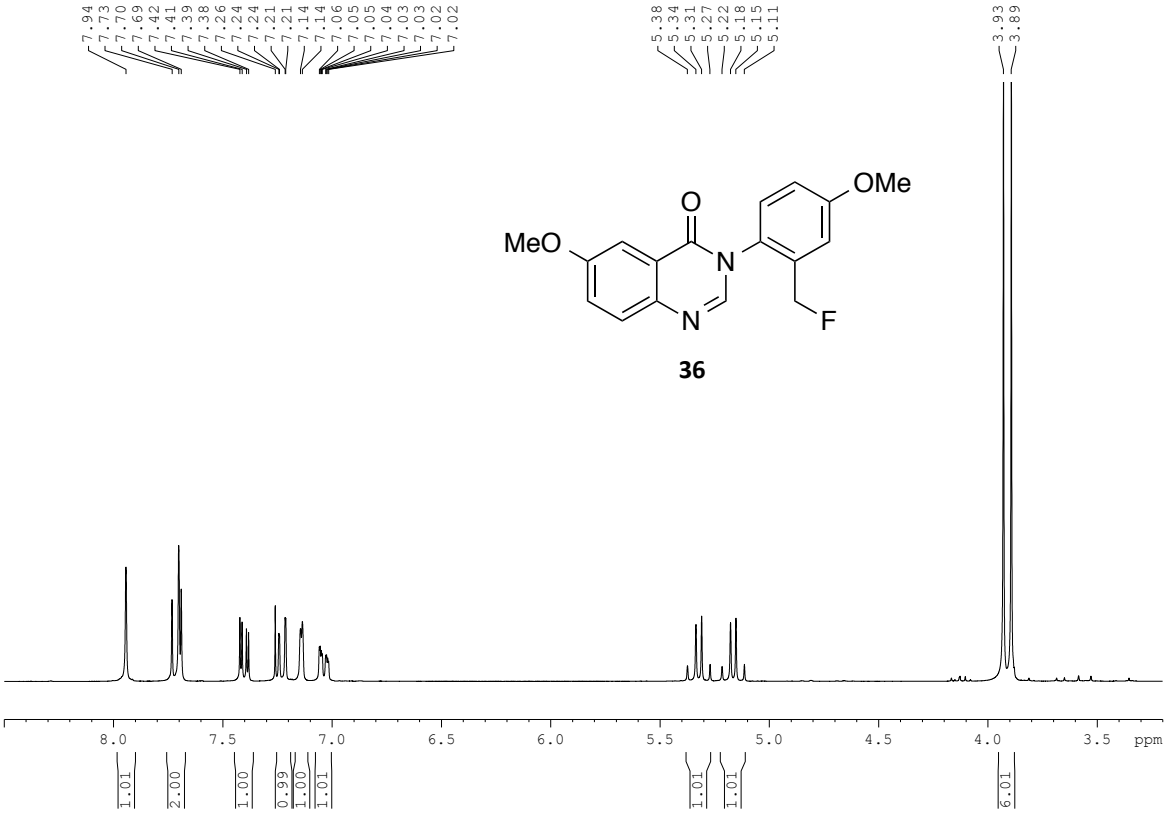
29

^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$)



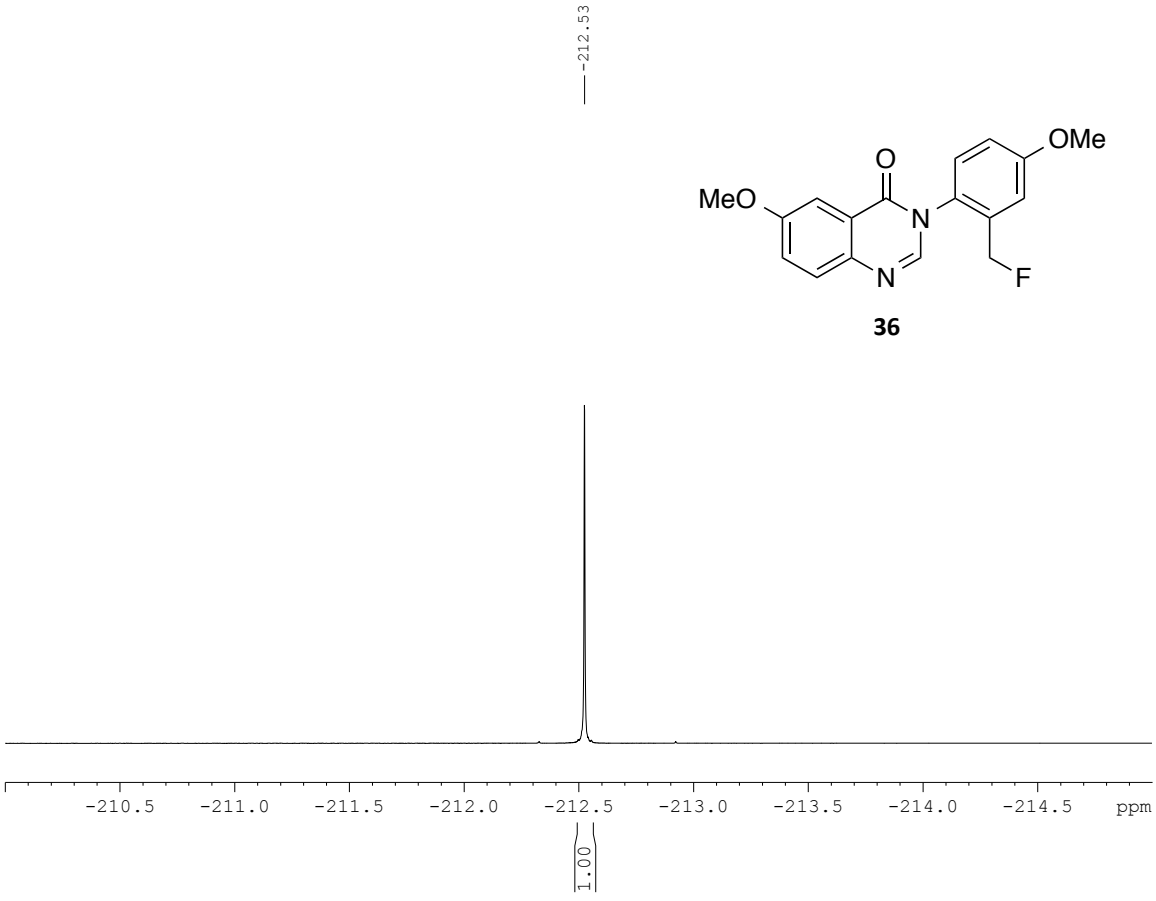
29

^{1}H NMR (300 MHz, $CDCl_3$)



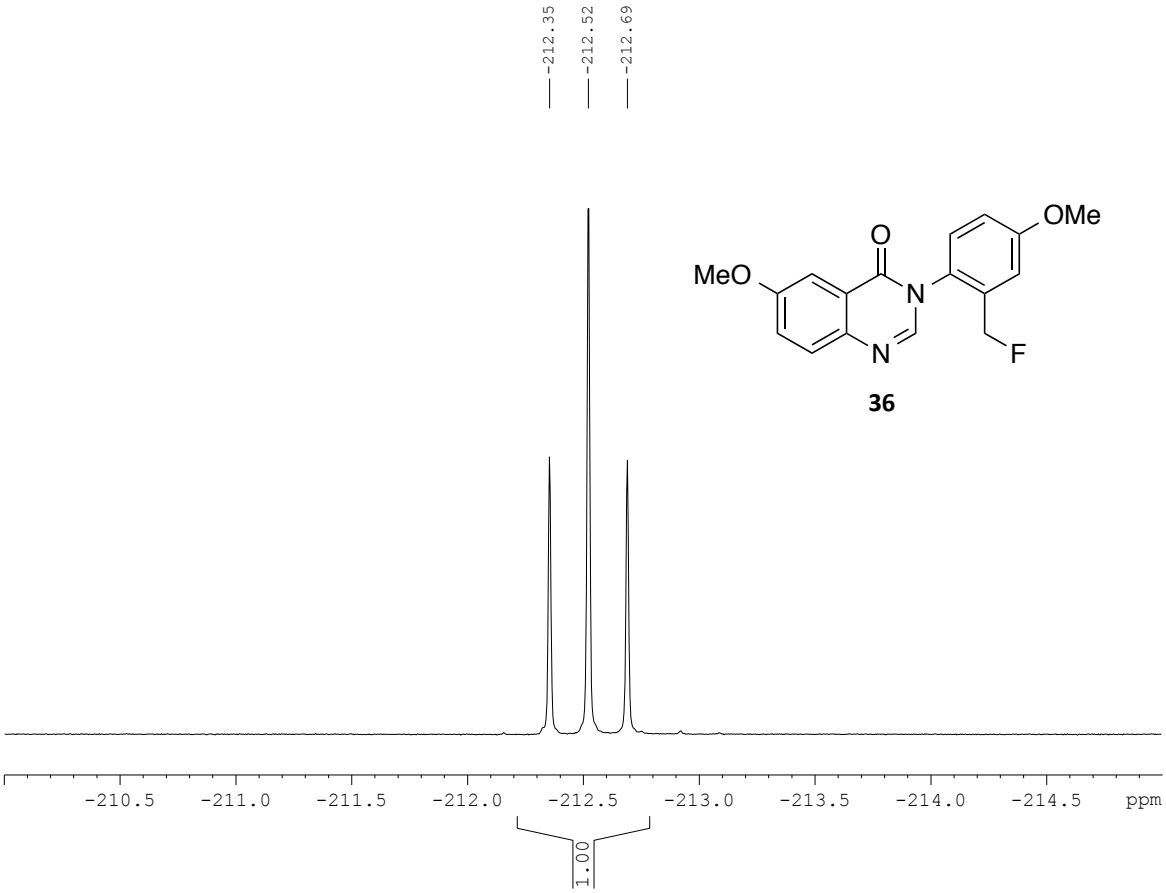
36

^{19}F {^{1}H} NMR (282 MHz, $CDCl_3$)



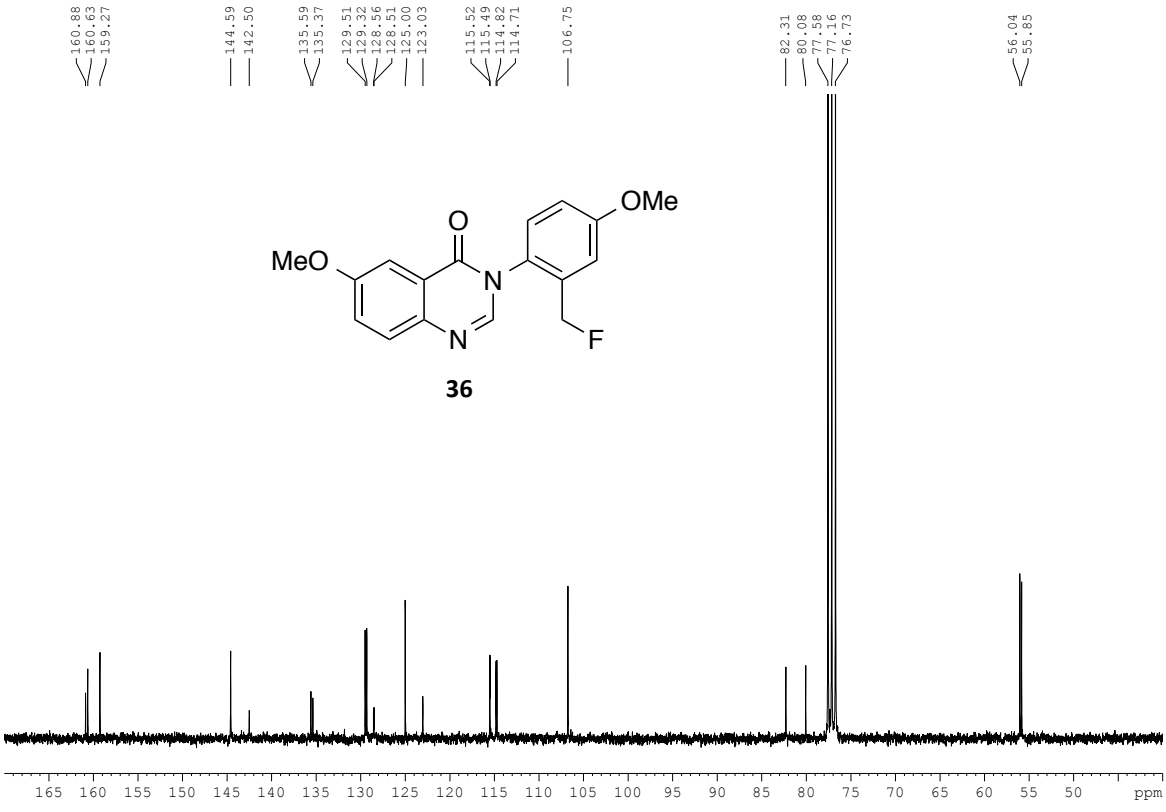
36

^{19}F NMR (282 MHz, $CDCl_3$)



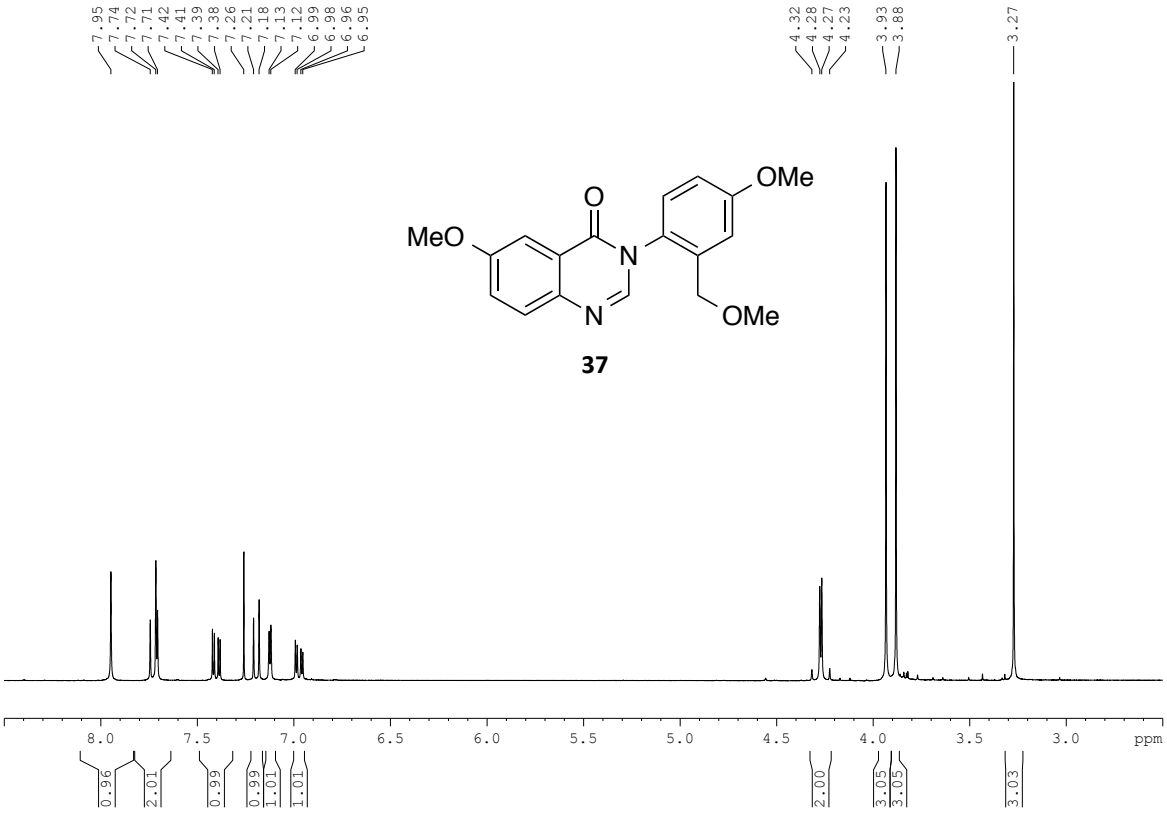
36

^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$)



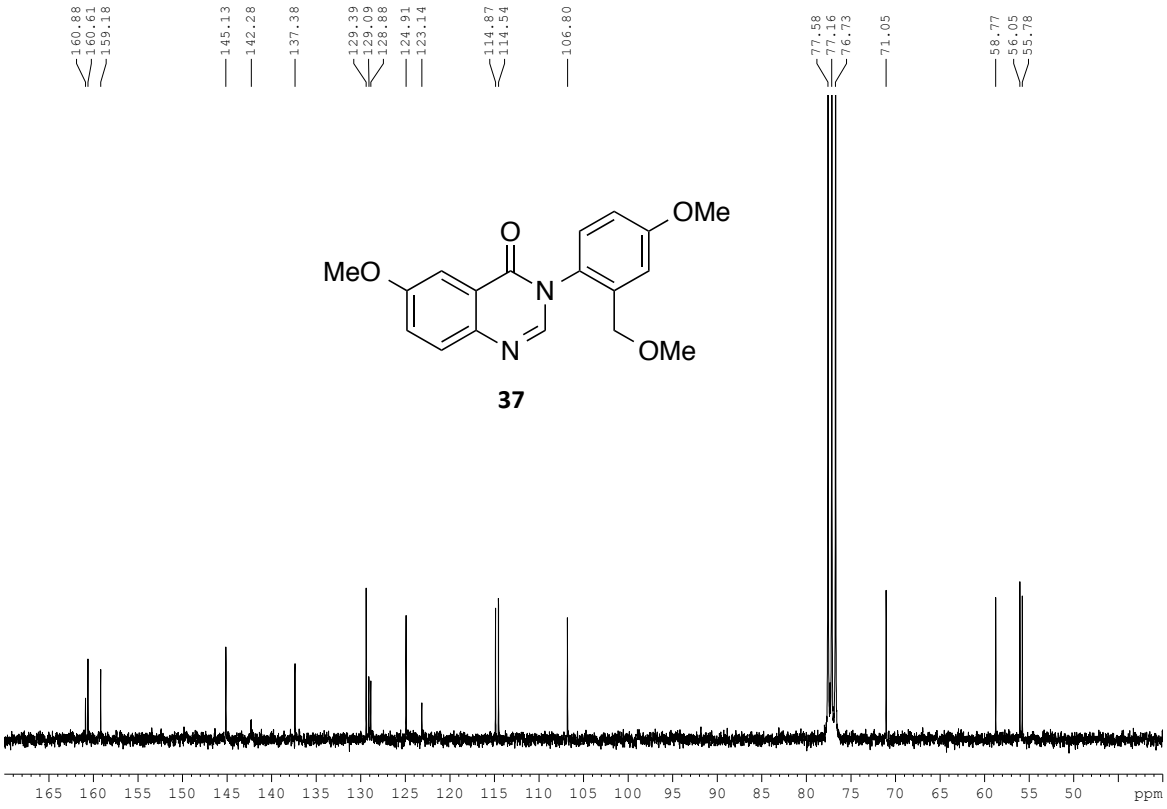
36

^{1}H NMR (300 MHz, $CDCl_3$)



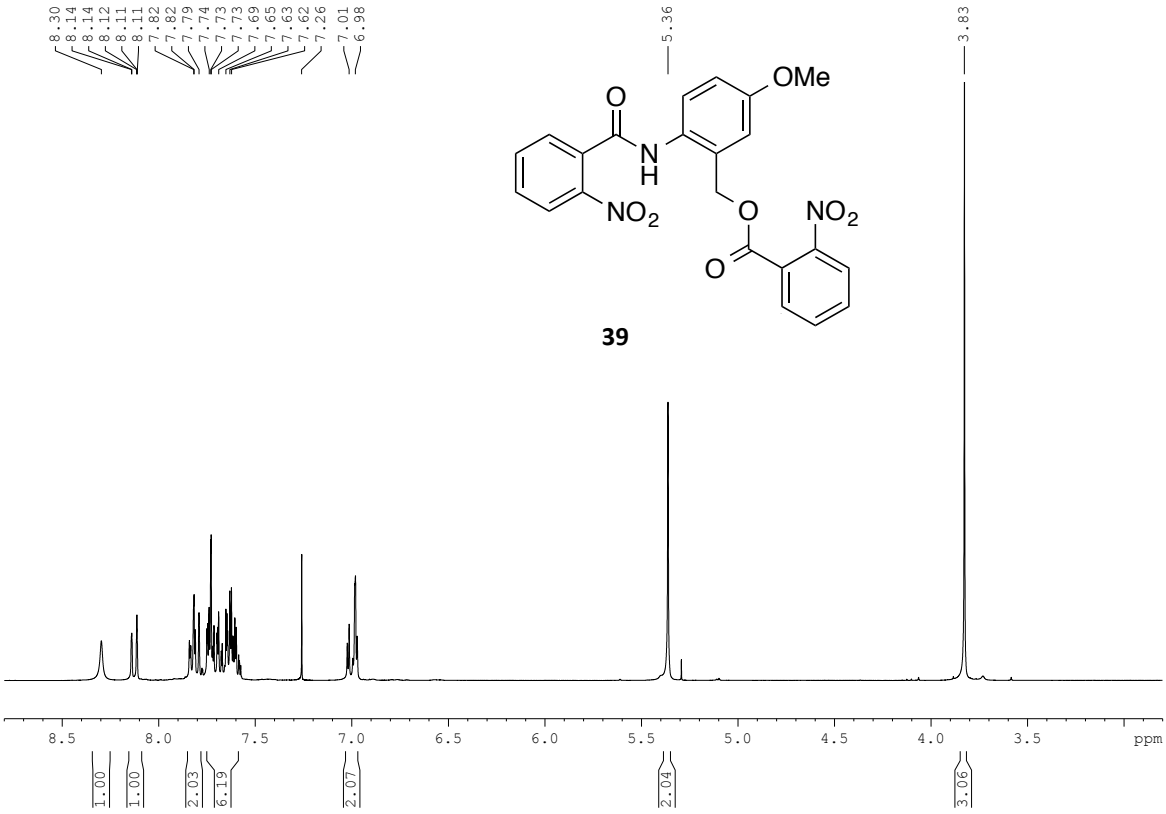
37

^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$)



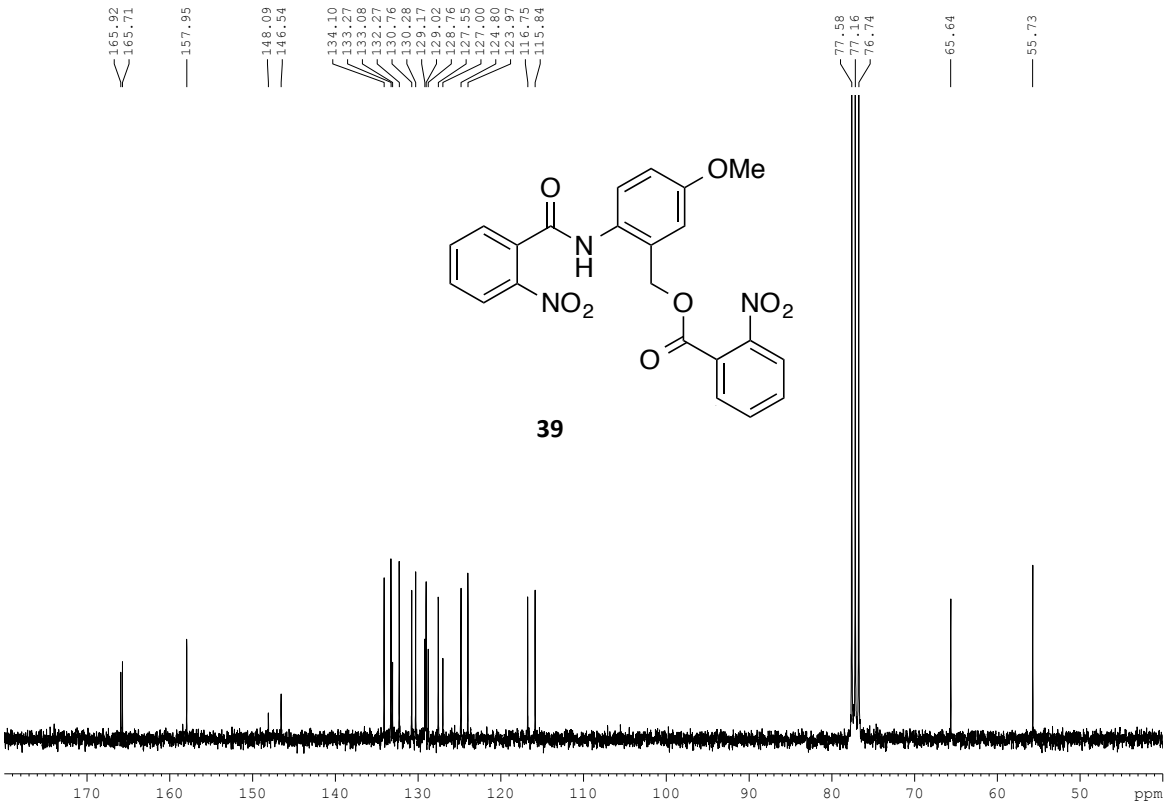
37

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



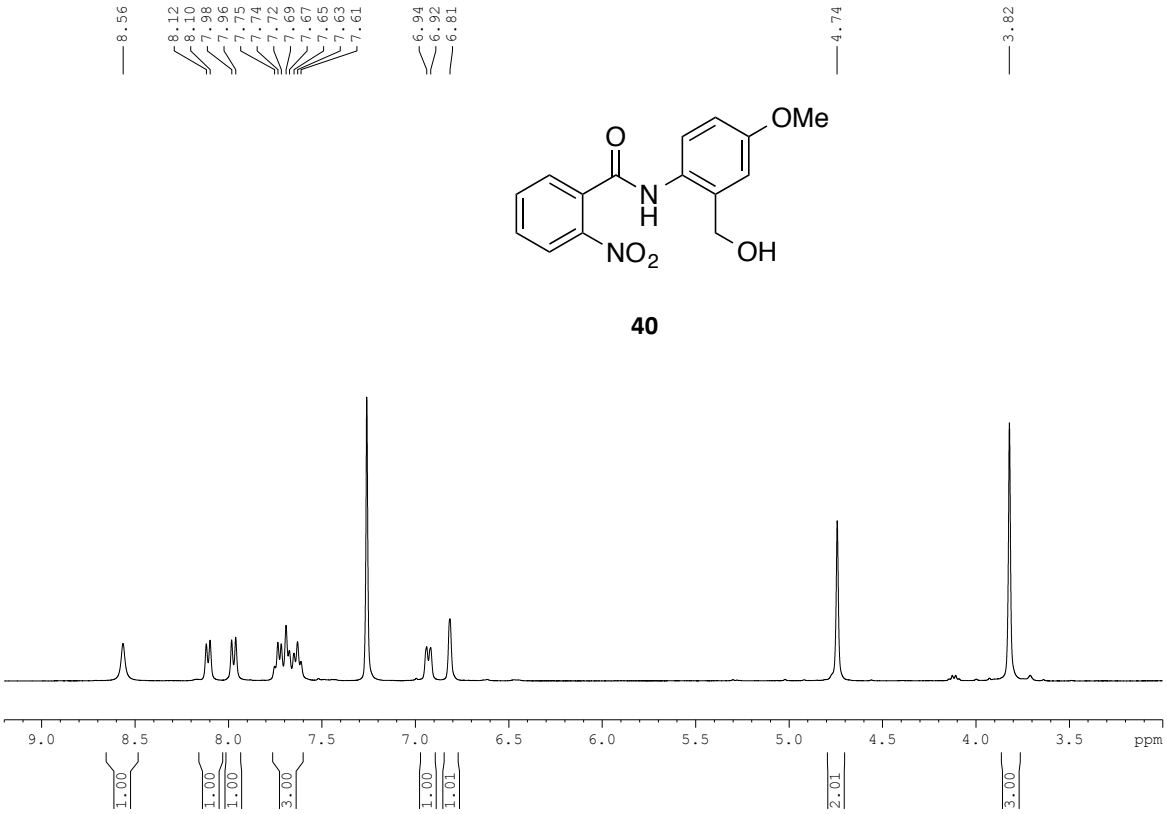
39

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$)



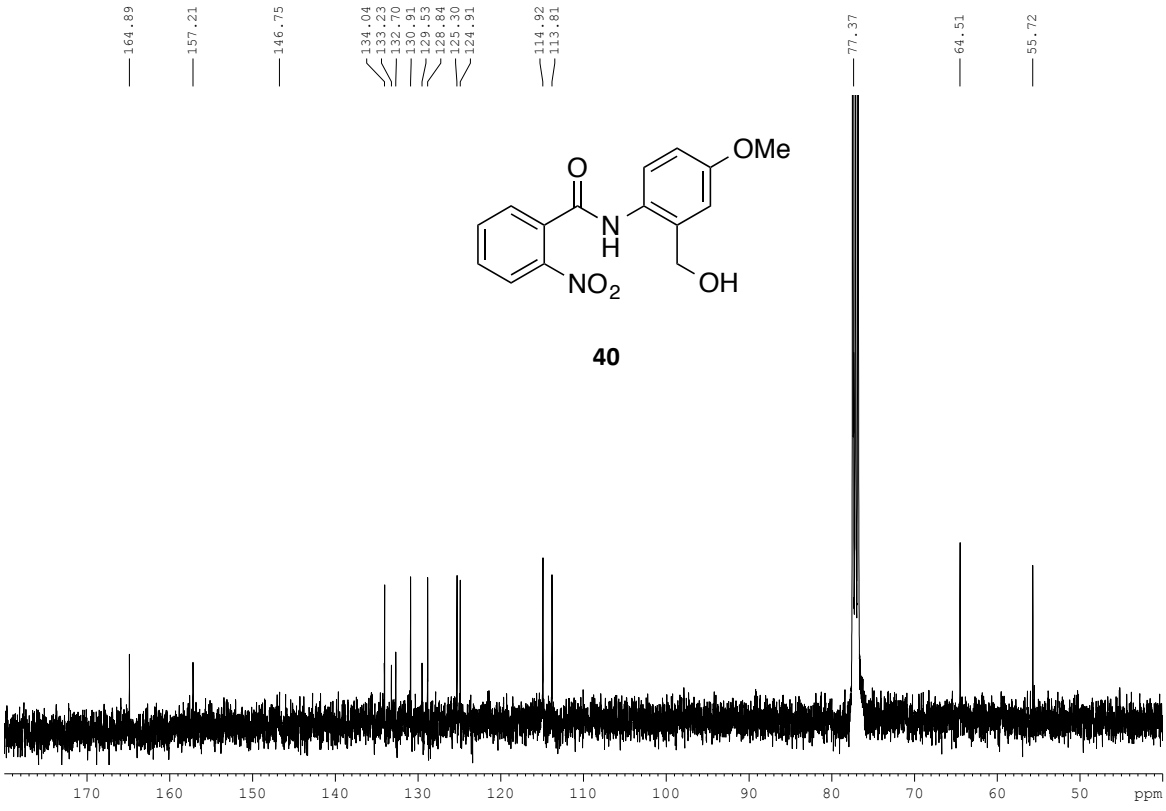
39

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



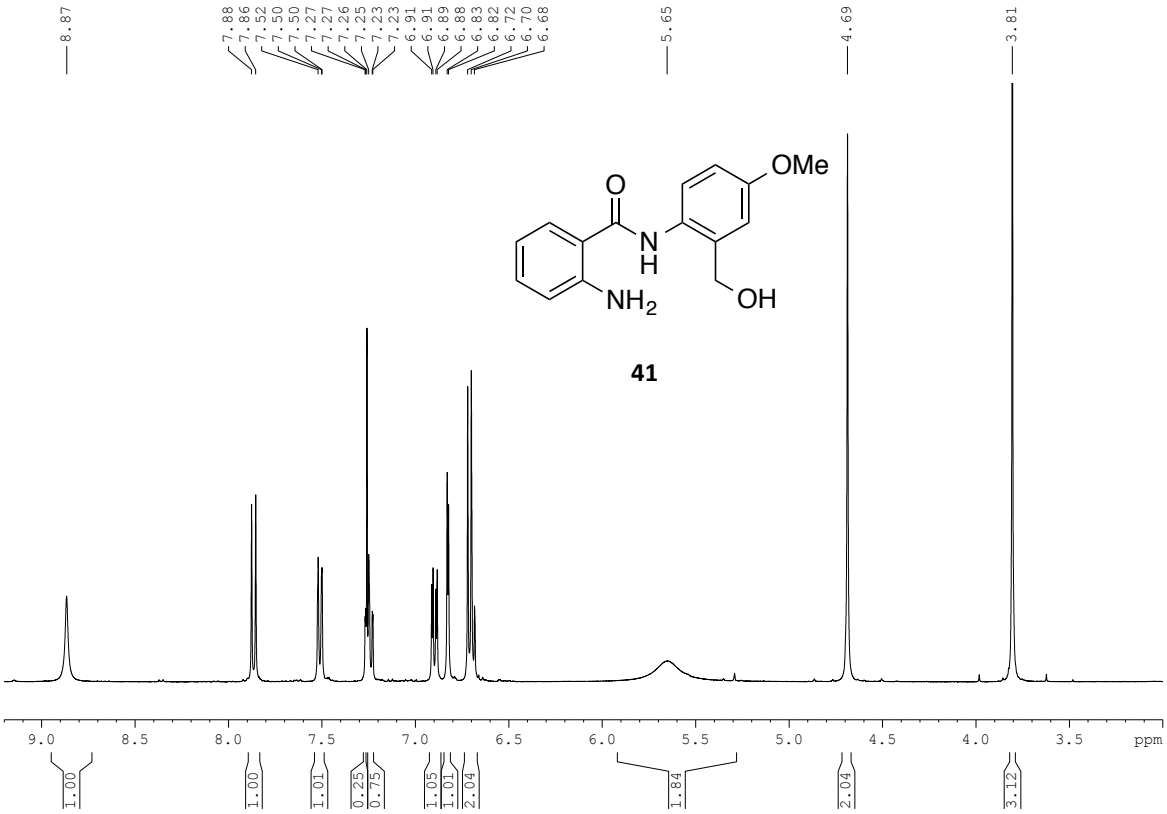
40

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$)

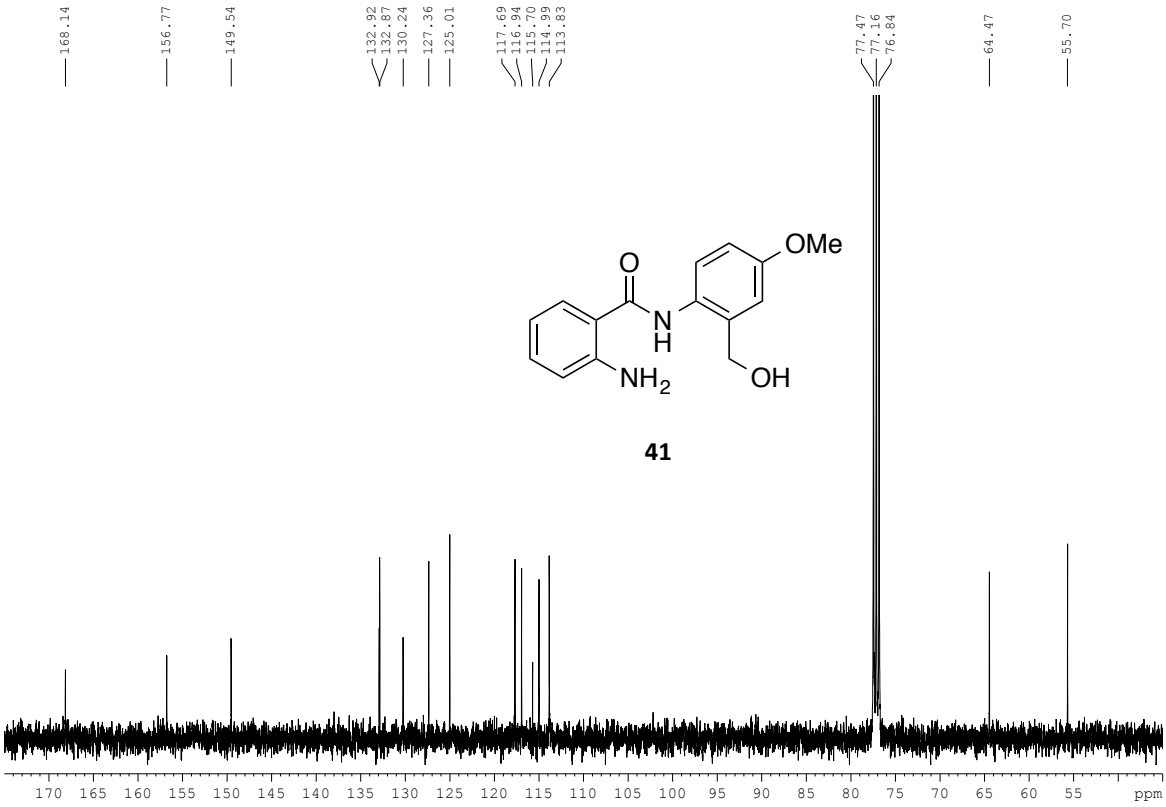


40

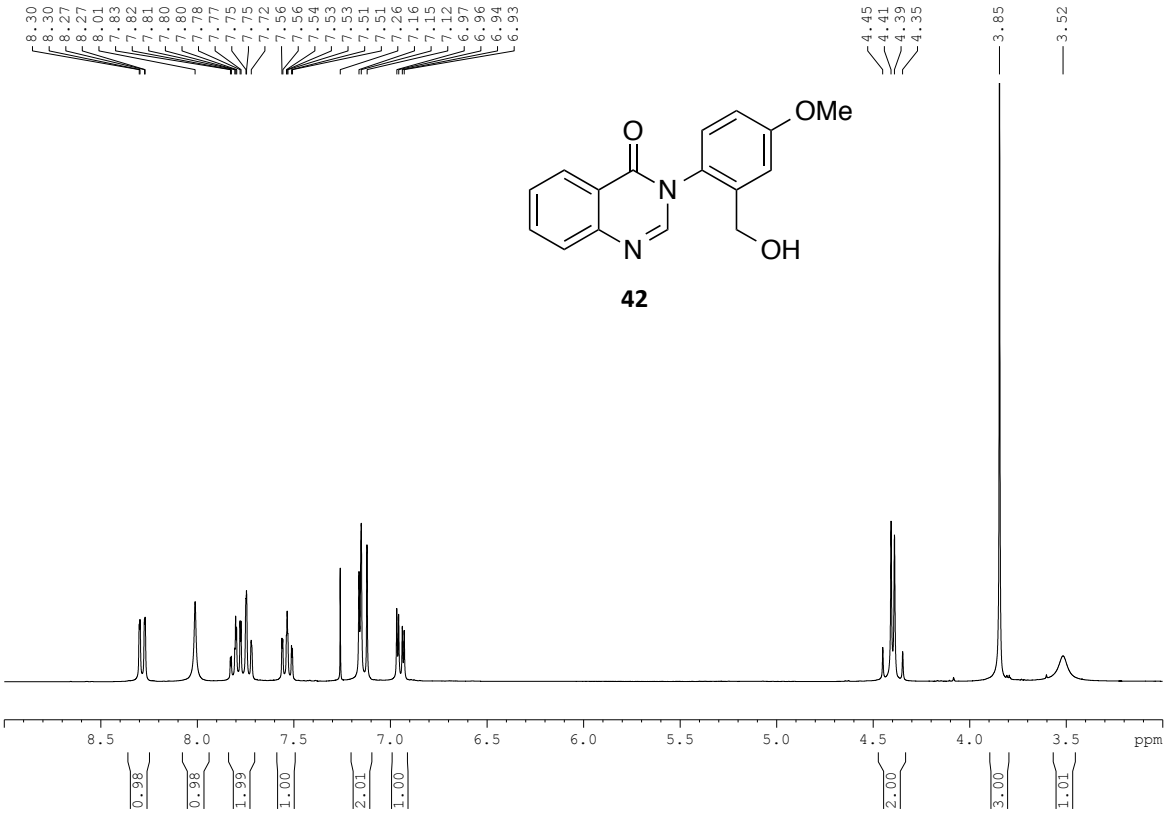
^{1}H NMR (400 MHz, $CDCl_3$)



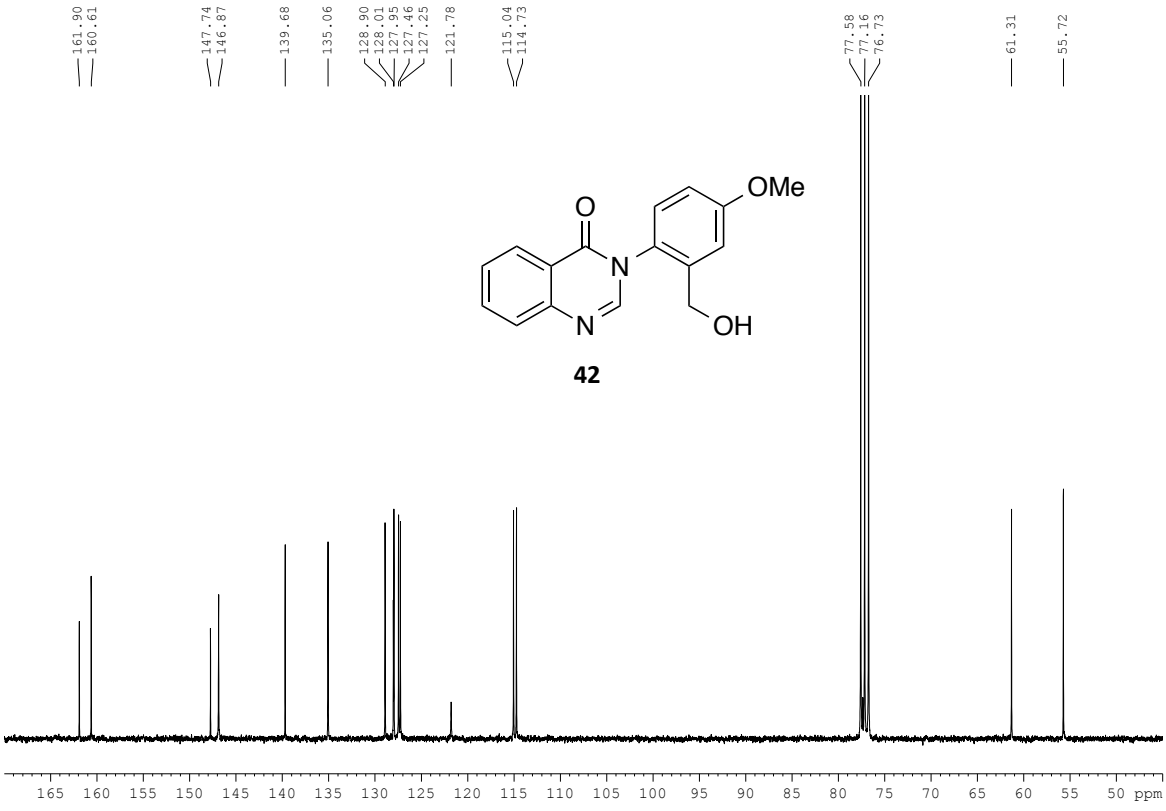
^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$)



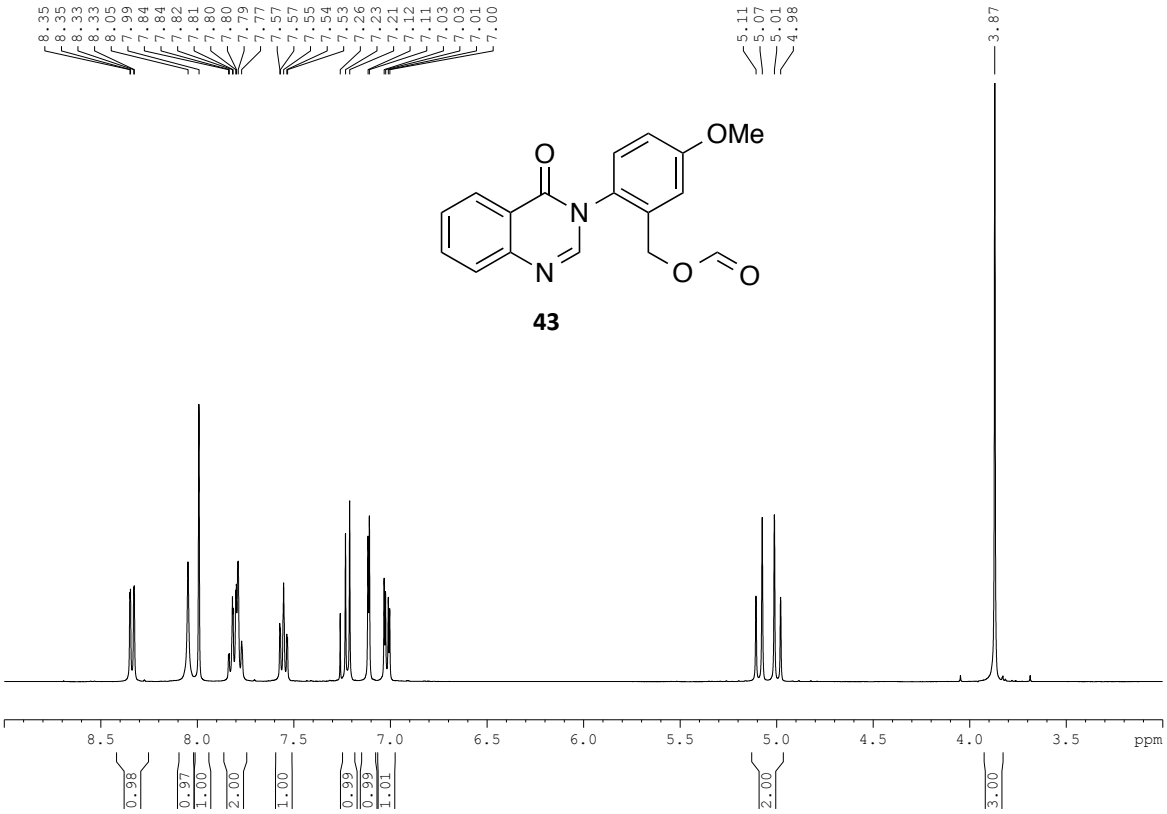
^{1}H NMR (300 MHz, $CDCl_3$)



^{13}C{^{1}H} NMR (76 MHz, CD_3CN)

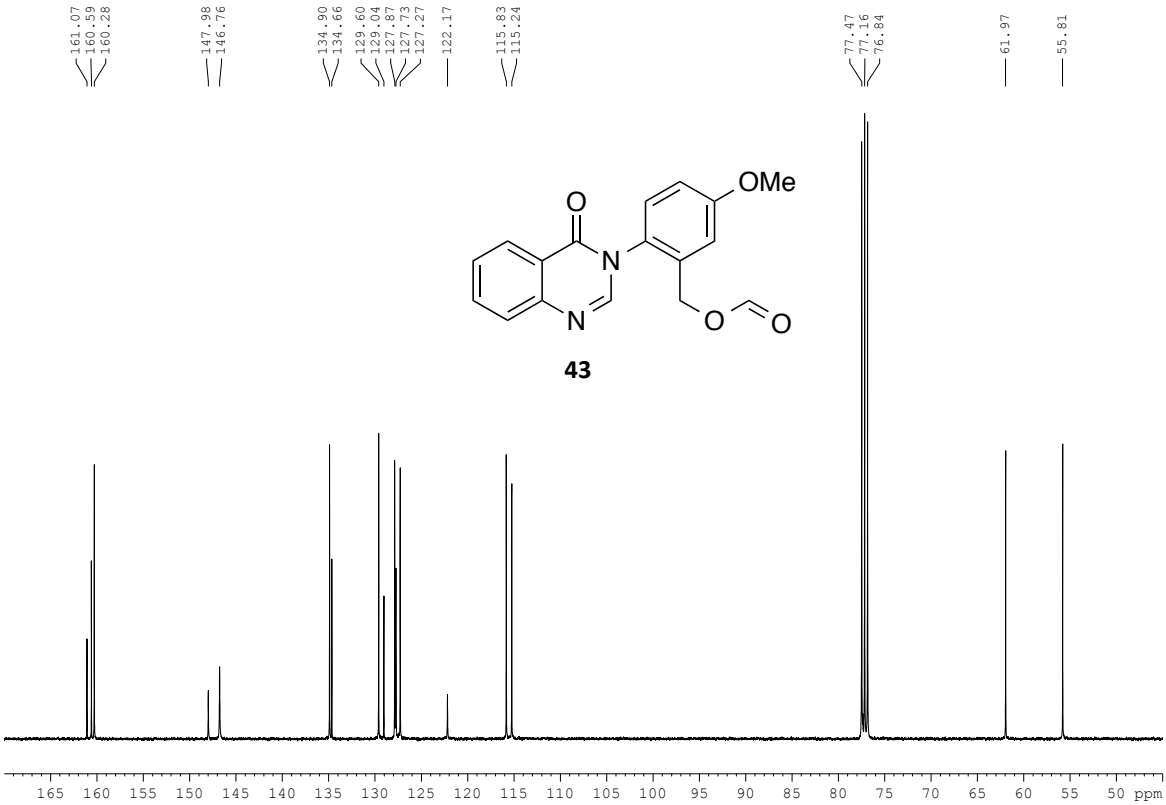


^{1}H NMR (400 MHz, $CDCl_3$)



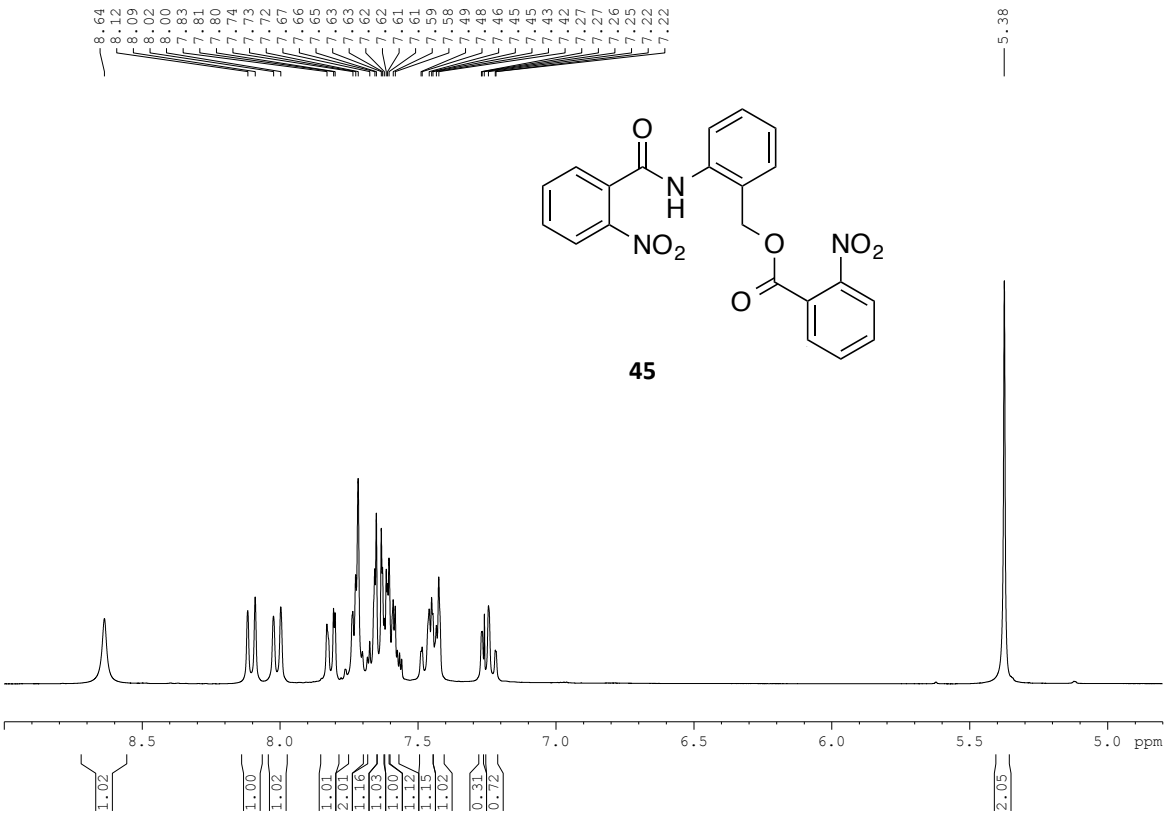
43

^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$)

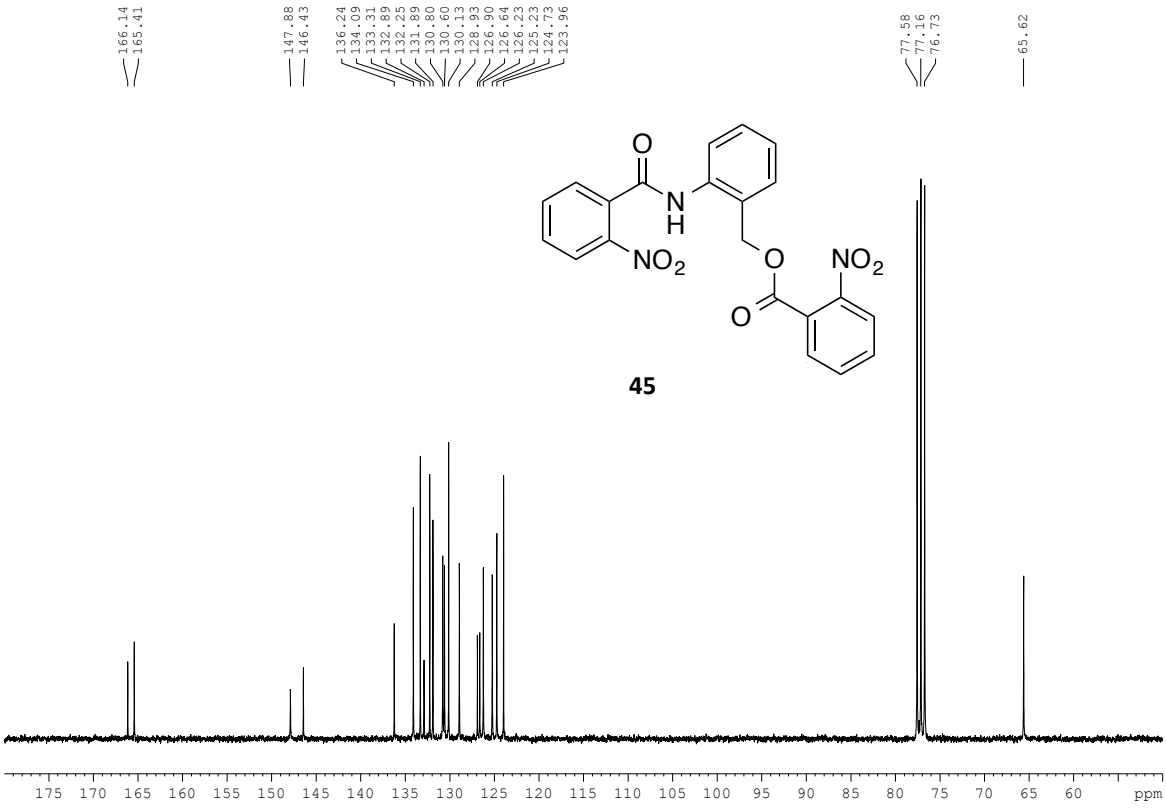


43

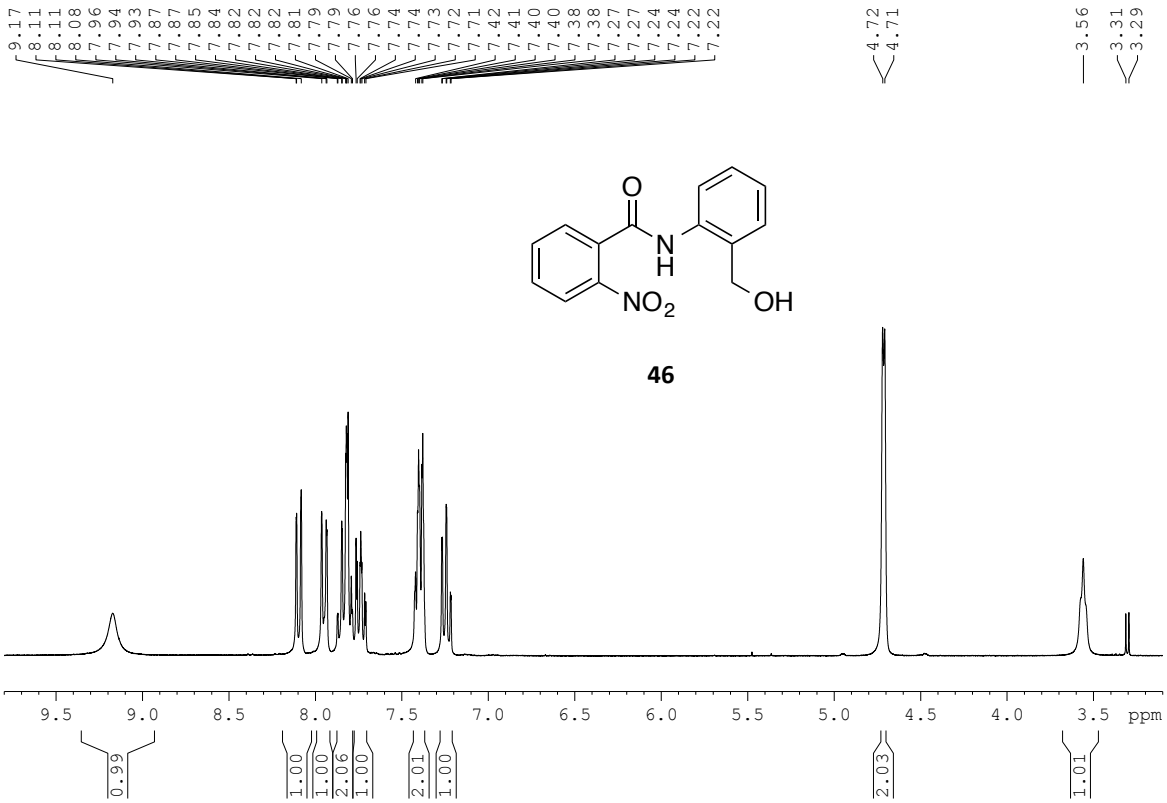
^{1}H NMR (300 MHz, $CDCl_3$)



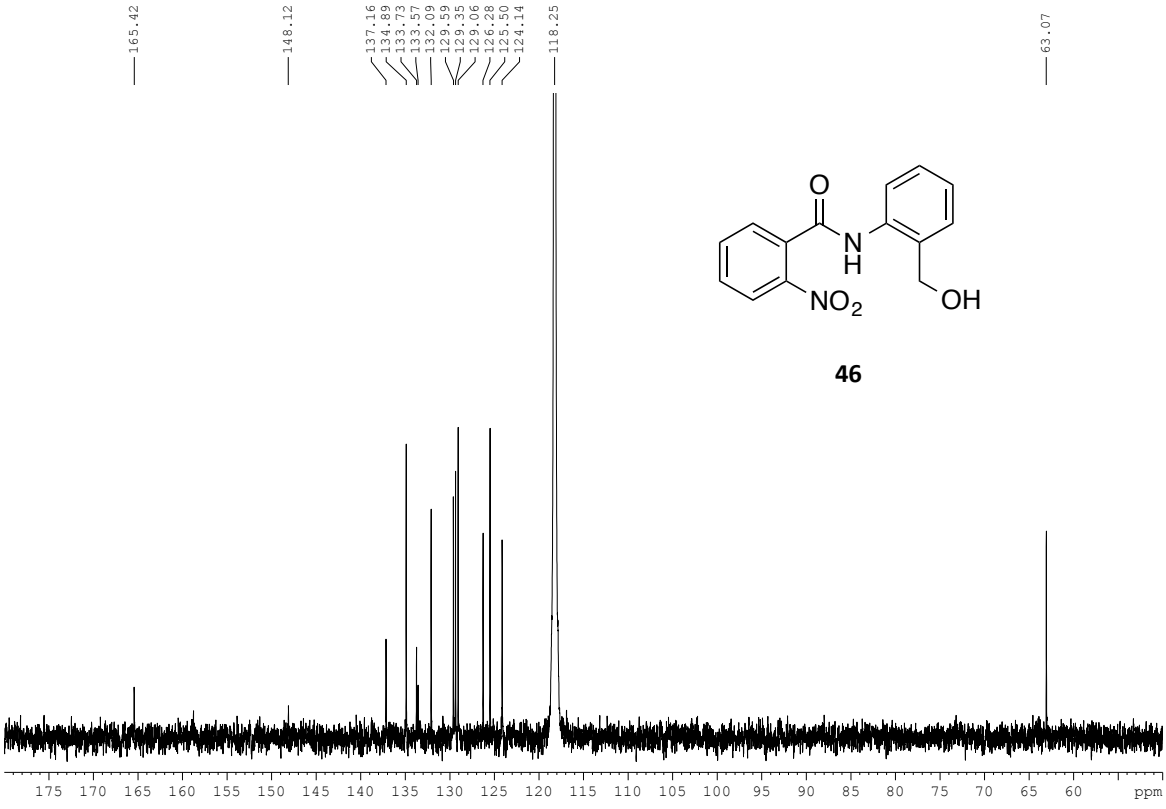
^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$)



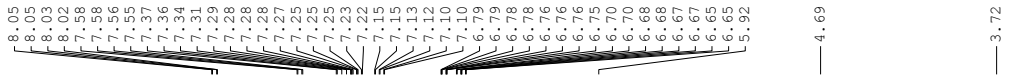
^{1}H NMR (300 MHz, CD_3CN)

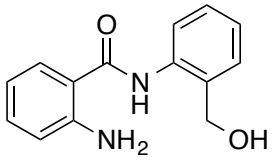


^{13}C{^{1}H} NMR (76 MHz, CD_3CN)

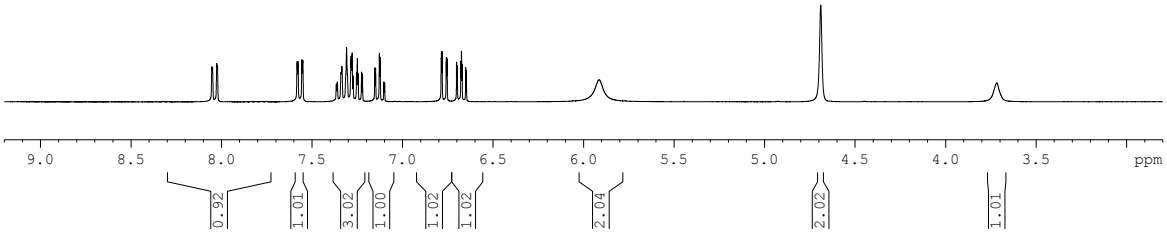


^{1}H NMR (300 MHz, CD_3CN)

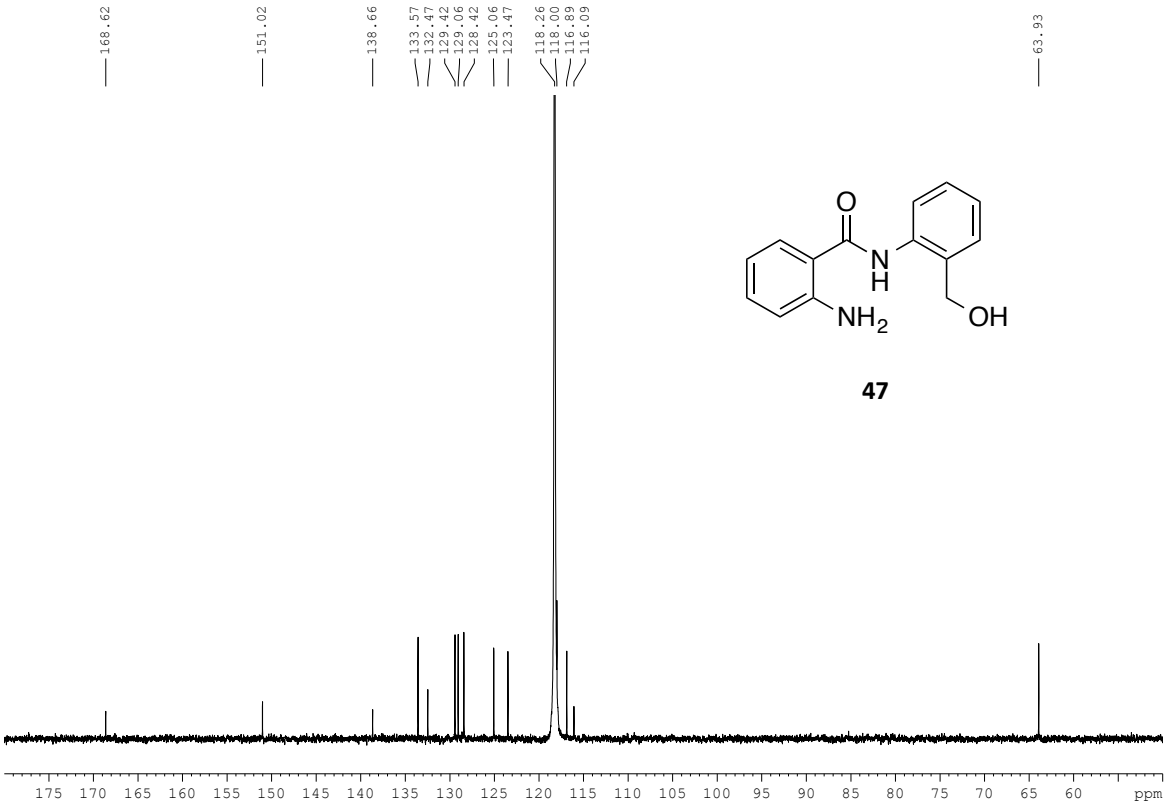




47

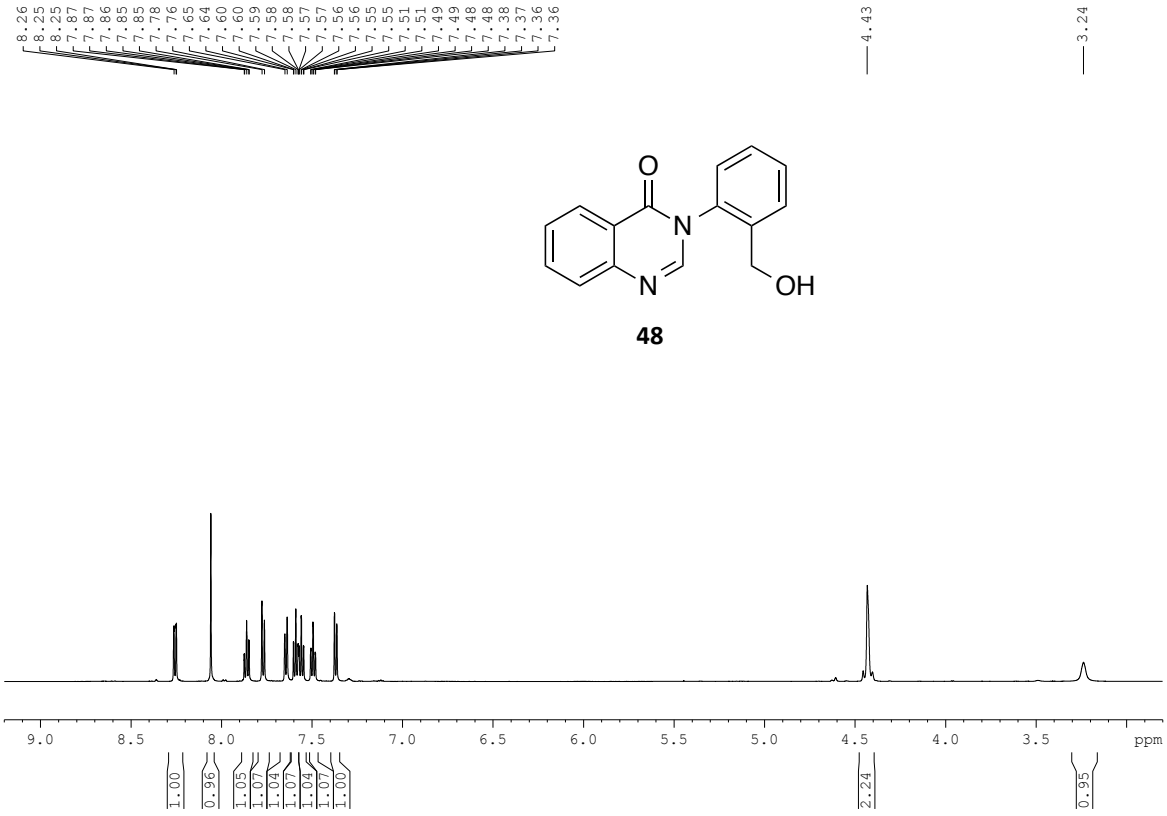


^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$)



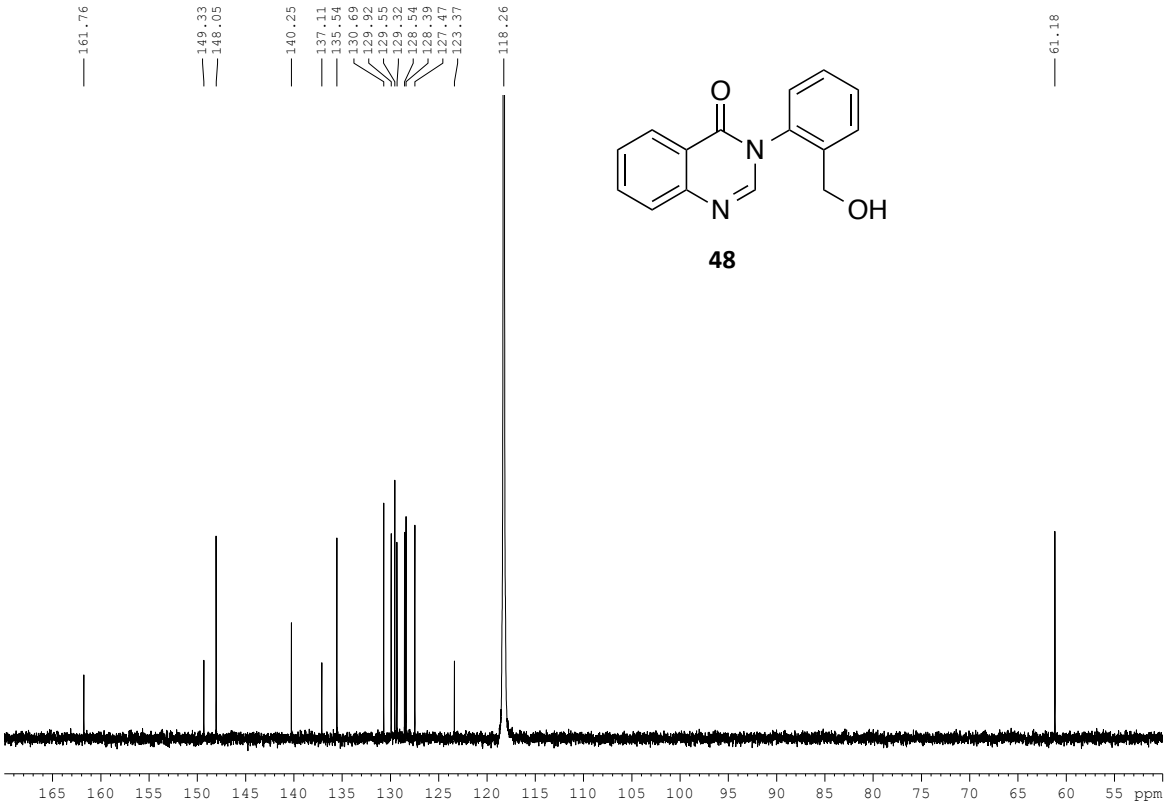
47

^{1}H NMR (600 MHz, CD_3CN)



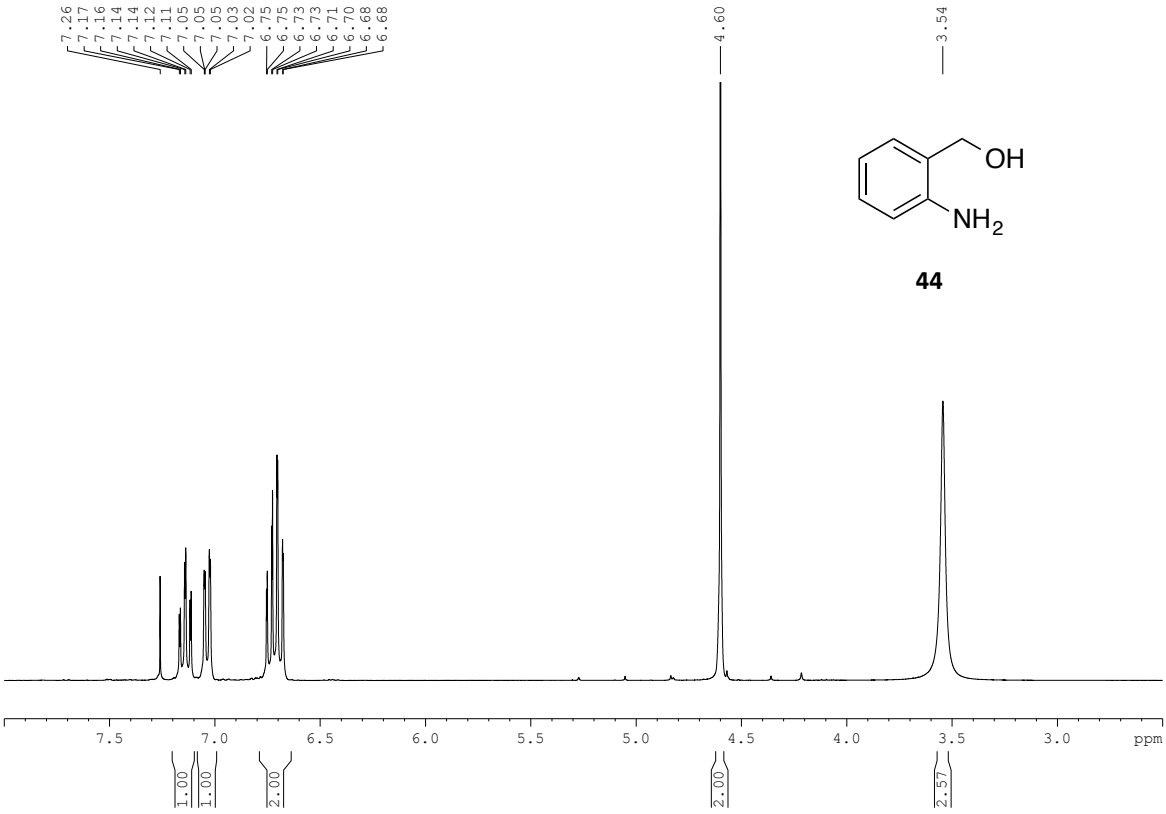
48

^{13}C{^{1}H} NMR (150 MHz, CD_3CN)



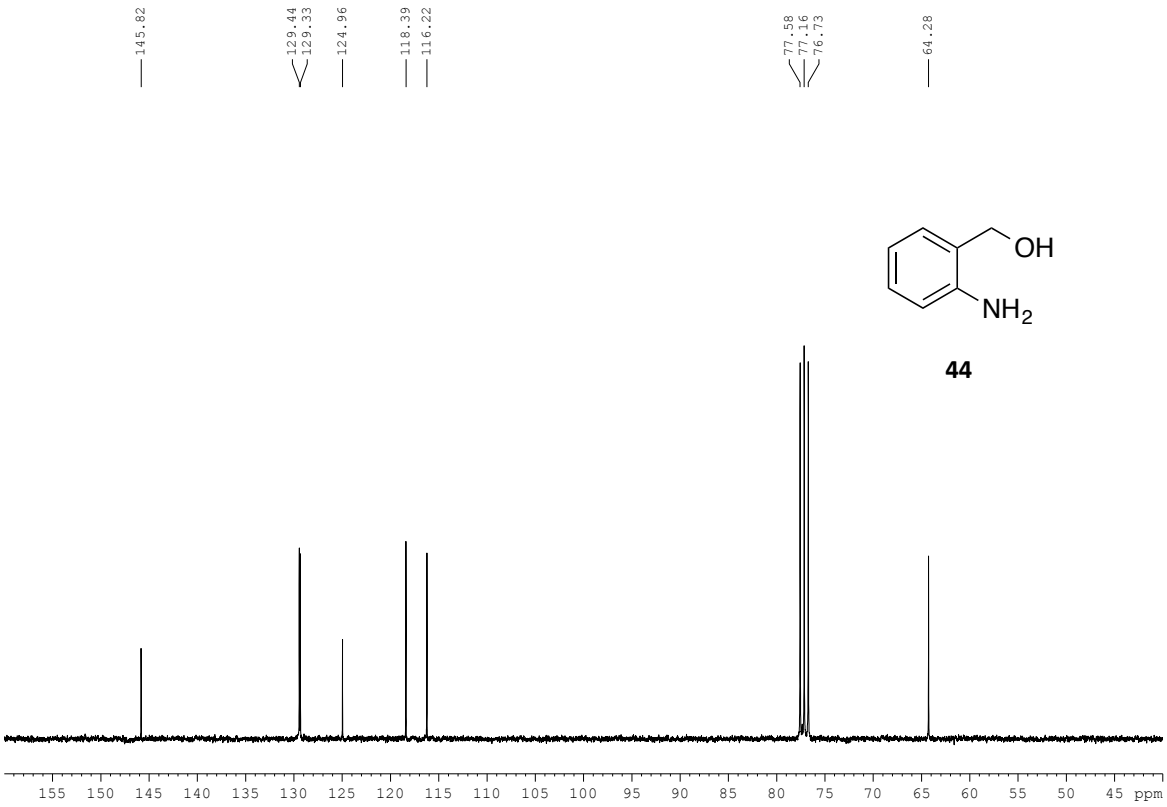
48

^{1}H NMR (300 MHz, $CDCl_3$)



44

^{13}C{^{1}H} NMR (76 MHz, $CDCl_3$)



44