Diels-Alder / Ene Reactions of 3-Vinyl-1*H*-indoles: Rapid Synthesis of Unsaturated Carbazoles and Pyridazino[3,4-*b*]indoles

by

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Abstract

Unsaturated carbazole frameworks are found in several important naturally occurring and synthetic biologically active compounds (Figure 1)[1,2]

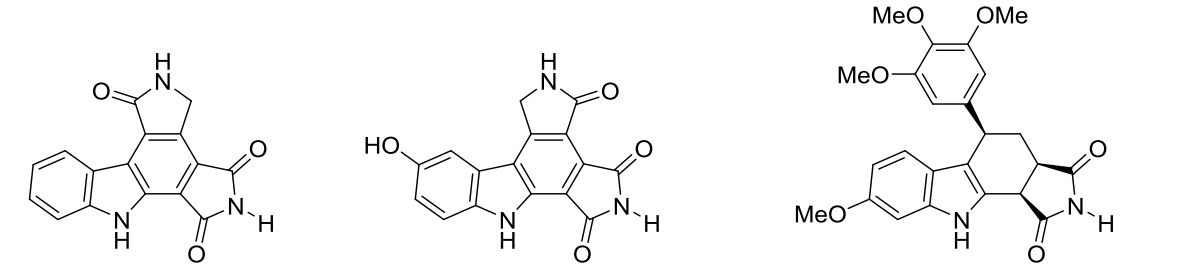
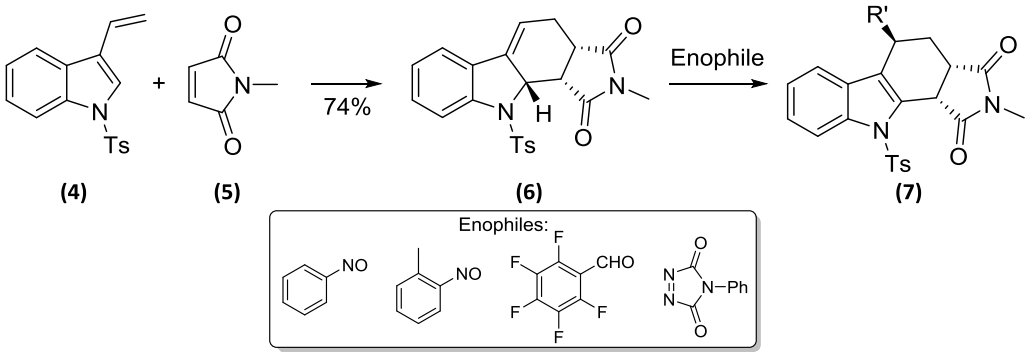


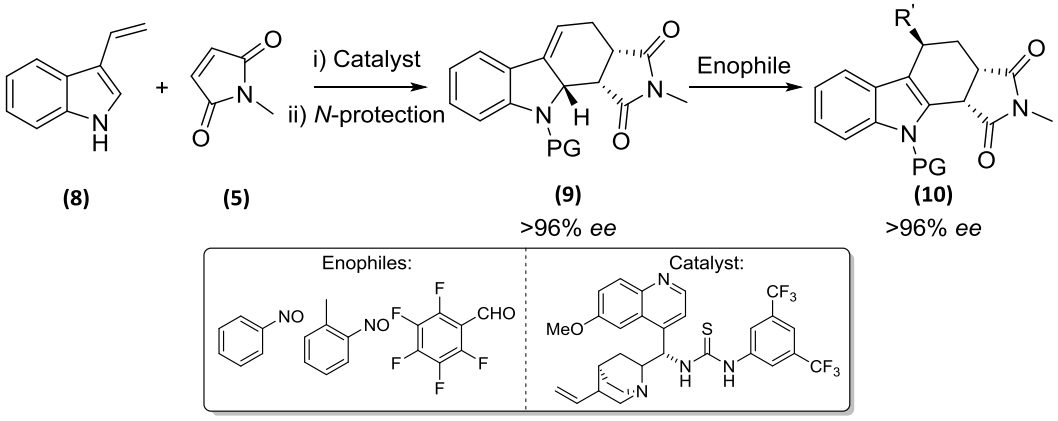
Figure 1 – Biologically active compounds based on carbazoles.

We envisaged synthesising compounds of this type using an intermolecular Diels-Alder reaction between 3-vinyl-1*H*-indole and a dienophile, followed by an intermolecular ene reaction between the resultant Diels-Alder adduct (**6**) and an enophile. This route was tested using tosyl protected 3-vinyl-1*H*-indole (**4**) and *N*-methylmaleimide (**5**) in a Diels-Alder reaction, followed by ene reactions with a range of enophiles to give unsaturated carbazoles (**7**) in yields of 50-60% over the two steps (Scheme 1).



Scheme 1 – Two-step Diels-Alder/ ene reaction sequence.

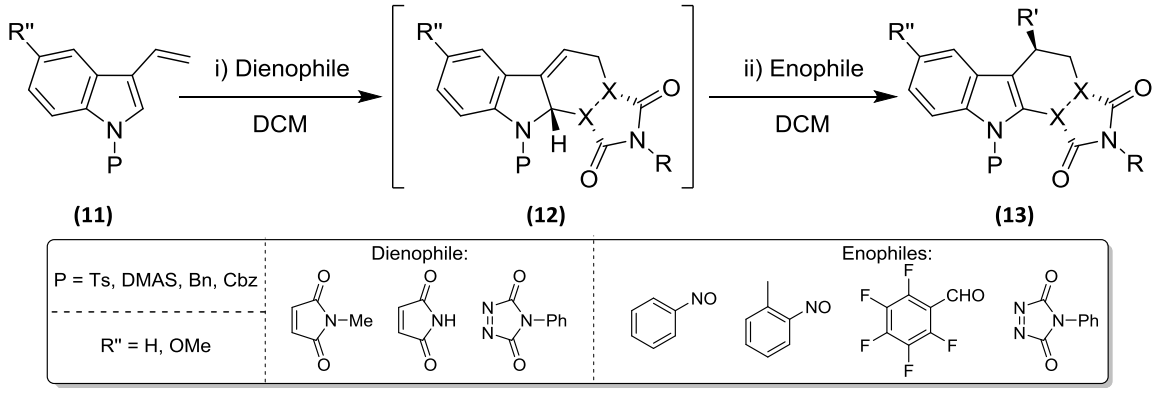
Through utilisation of Soos's organo-catalyst[3], we have been able to control the Diels-Alder reaction between 3-vinyl-1*H*-indole (**8**) and *N*-methylmaleimide (**5**), to form the Diels-Alder adduct (**9**) with high enantiomeric excess (Scheme 2).[4]



Scheme 2 – Enantioselective Diels-Alder, protection, enantiospecific ene reaction sequence.

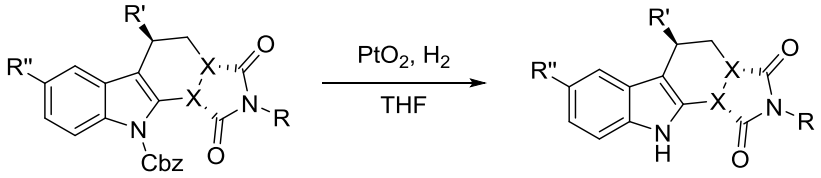
The subsequent stereospecific ene reaction then gives unsaturated carbazoles (**10**) in >96% *ee*.

We then developed a sequential "one-pot" Diels-Alder / ene methodology with an *N*-protected 3-vinyl-1*H*-indole (**11**), a dienophile and an enophile to make unsaturated carbazoles and pyridazino[3,4-*b*]indoles (**13**) (Scheme 3).[3] This methodological approach gave increased overall yields and reduced purification steps.



Scheme 3 – One-pot Diels-Alder / ene reaction.

When Cbz *N*-protected 3-vinyl-1*H*-indoles were used the Diels-Alder / ene products (**14**) could then be deprotected through a PtO_2 catalysed hydrogenation reaction to give molecules (**15**) (Scheme 4). Several of the synthesised compounds (**15**) showed moderate biological activity against *Escherichia coli, Staphylococcus aureus* and *Schizosaccharomyces pombe*. Compounds (**15**) are also moderate (μM) inhibitors of several important kinases, including Chk2, Aurora B, Src and JAK2, which are potential targets for cancer treatment.



Scheme 4 – Removal of Cbz group by hydrogenation.

1) Conchon, E.; Anizon, F.; Aboab, B.; Golsteyn, R. M.; Léonce, S.; Pfeiffer, B.; Prudhomme, M. *Eur. J. Med. Chem.* **2008**, *43*, 282.
2) Tya, N.; Dupeyrea, G.; Chabotb, G.; Seguinb, J.; Quentinb, L.; Chiaronic, A.; Tillequina, F.; Schermanb, D.; Michela, S.; Cacheta, X. *Eur. J. Med. Chem.* **2010**, *45*, 3726.
3) Vakulya, B.; Varga, S.; Csampai, A.; Soos, T. *Org. Lett.* **2005**, *7*, 1967.
4) Gioia, C.; Bernardi, L.; Ricci, A; Hauville, A.; Fini, F.; *Angew. Chem. Int. Ed.*, **2008**, *47*, 9236.

Abbreviations

Å	Angstrom
Aq	Aqueous
Ar	Aromatic
Bn	Benzyl
br	Broad
calcd	Calculated
Cbz	Carboxybenzyl
d	Days
DMAP	4-(Dimethylamino)pyridine
DMAS	*N,N*-dimethylaminosulfonyl
DMSO	Dimethyl sulfoxide
eq	Equivalent
Et	Ethyl
g	Grams
h	Hours
HRMS	High resolution mass spectroscopy
Hz	Hertz
IR	Infrared
M	Molar
MP	Melting point
Me	Methyl
mg	Milligram
min	Minutes
mL	Millilitre
NBS	*N*-bromosuccinimide
NMM	*N*-methylmaleimide
NMR	Nuclear magnetic resonance
NPM	*N*-phenylmaleimide
Ph	Phenyl
ppm	Parts per million
PTAD	4-phenyl-1,2,4-triazoline-3,5-dione
rt	Room temperature
R_f	Retention factor
SOMO	Singly occupied molecular orbital
TLC	Thin layer chromatography
THF	Tetrahydrofuran
Ts	Tosyl

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Chapter 1 – Introduction-

The main focus of this thesis is to look at the synthesis of complex indole containing compounds with potential biological activity. We believe this can be achieved through the use of multiple pericyclic reactions in a single reaction process which will allow the rapid synthesis of a diverse range of compounds, whilst also allowing close control of the stereochemistry.

This introduction chapter will be split into four sections covering the key background areas of the thesis:

1.0 – Pericyclic reactions

2.0 – Pericyclic reactions in multi-component reactions

3.0 – Indoles in biologically active compounds

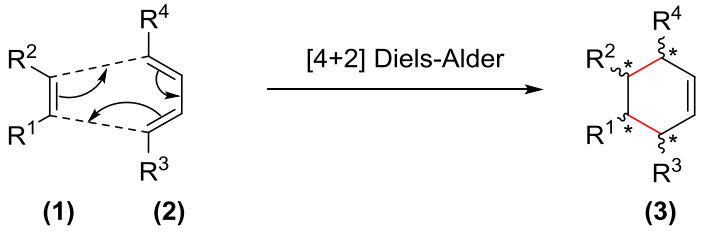
4.0 – Diels-Alder / ene reactions with vinyl-heteroaromatics

1.1.0 Pericyclic reactions-

As we propose to use Diels-Alder and ene reactions in our synthesis, we will review general Diels-Alder and ene reactions from the literature, to fully understand the pericyclic reactions and the products formed from these reactions.

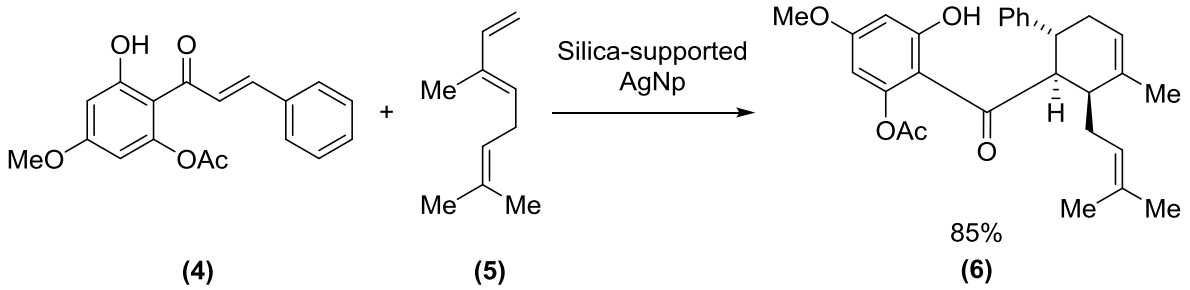
1.1.1 The Diels-Alder reaction-

Since its discovery in 1928[1], the Diels-Alder reaction has been widely used in organic synthesis and is one of the most well understood reactions.[2,3] The Diels-Alder reaction is a [4+2] cycloaddition which allows the simultaneous, stereocontrolled formation of two C-C or C-X bonds and the creation of up to four new stereocentres (Scheme 1.1). These qualities are highly desirable for organic chemists and led to its pioneers, Otto Diels and Kurt Alder, winning the Nobel Prize for Chemistry in 1950.



Scheme 1.1 – General [4+2] Diels-Alder reaction.

As the Diels-Alder reaction forms two new bonds and leads to the generation of multiple new stereocentres, complex molecules can be rapidly synthesised from relatively simple starting materials (Scheme 1.2).[4]



Scheme 1.2 - Diels-Alder reaction in the synthesis of the drug panduratin (6).

Conventional Diels-Alder reactions use an electron deficient dienophile and an electron rich *cis*-diene. Simple dienes like 1,3-butadiene can adopt two conformers, *s-cis* (**7**) and *s-trans* (**8**), though only the *s-cis* stereoisomer is in the correct orientation to allow a Diels-Alder reaction to occur (Figure 1.1). This problem can be overcome through the use of cyclic dienes, such as cyclopentadiene (**10**), where the two double bonds are fixed in an *s-cis* orientation.

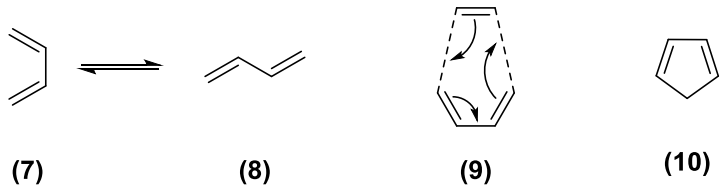
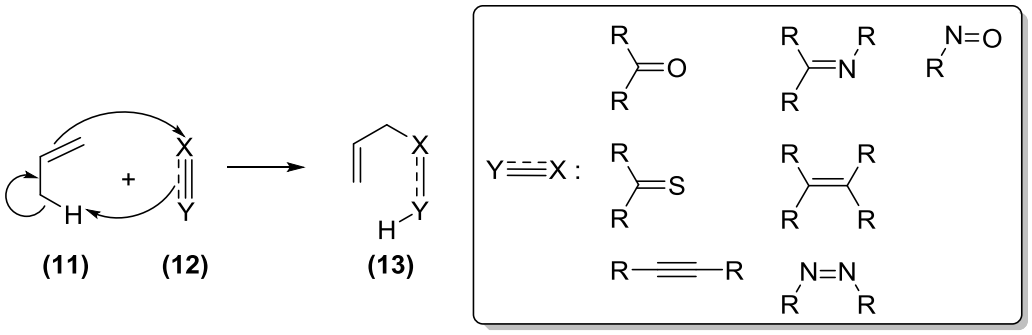


Figure 1.1 – Cis-(7) and trans-(8) 1,3-butadiene and cyclopentadiene (10).

1.1.2 The ene reaction-

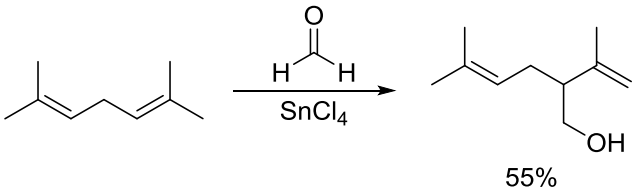
The ene reaction is an electrocyclic reaction involving an allylic system (ene component) and a molecule that contains a π-bond (enophile) (Scheme 1.3).



Scheme 1.3 - Example of a generic ene reaction.[5]

1.1.3 Lewis acid catalysed carbonyl ene reactions-

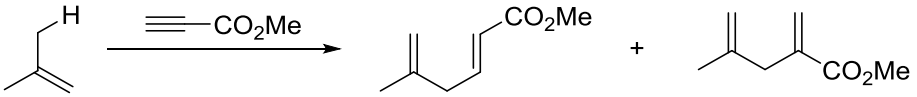
There are a wide range of different ene components that can be used in ene reactions, although the exact reaction mechanism is dependent upon the nature of the enophile. Ene reactions generally require higher temperatures than Diels-Alder reactions, therefore Lewis acids have been used extensively to catalyse the reaction (Scheme 1.4).[5]



(14) **(15)**

Scheme 1.4 - Ene reaction employing a Lewis acid catalyst.

The use of Lewis acid catalysts can lead to changes in product distribution when compared to thermal reactions that do not use Lewis acid catalysts (Scheme 1.5).[6]



(16) **(17)** **(18)**

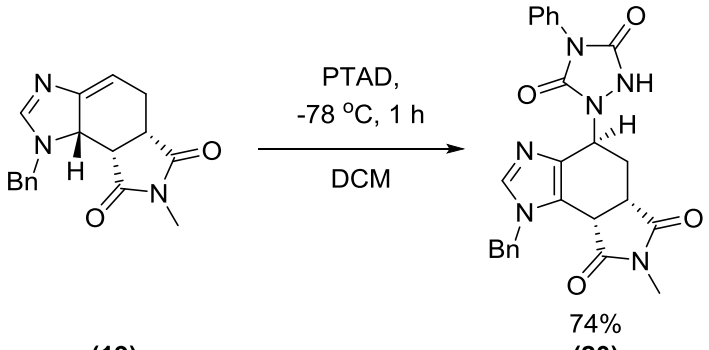
220 °C	93	:	7	45 %
$AlCl_3$ 25 °C	100	:	0	61 %

Scheme 1.5 - Ene reaction under thermal conditions or Lewis acid catalysis.

In the case of carbonyl ene reactions, Lewis acids are used to coordinate to the carbonyl oxygen of the ester, making the C=O double bond more electron deficient thus increasing the regiospecificity of the reaction. The use of Lewis acid catalysts also allows the ene reaction to proceed under relatively mild reaction conditions.

1.1.4 Aza ene chemistry-

Previous work in the group has demonstrated that aza ene reactions can be performed with 4-phenyl-1,2,4-triazole-3,5-dione (PTAD) acting as the enophile (Scheme 1.6).[7] The N=N bond is relatively weak and therefore the reaction rapidly goes to completion at a low temperature and short reaction time of one hour.

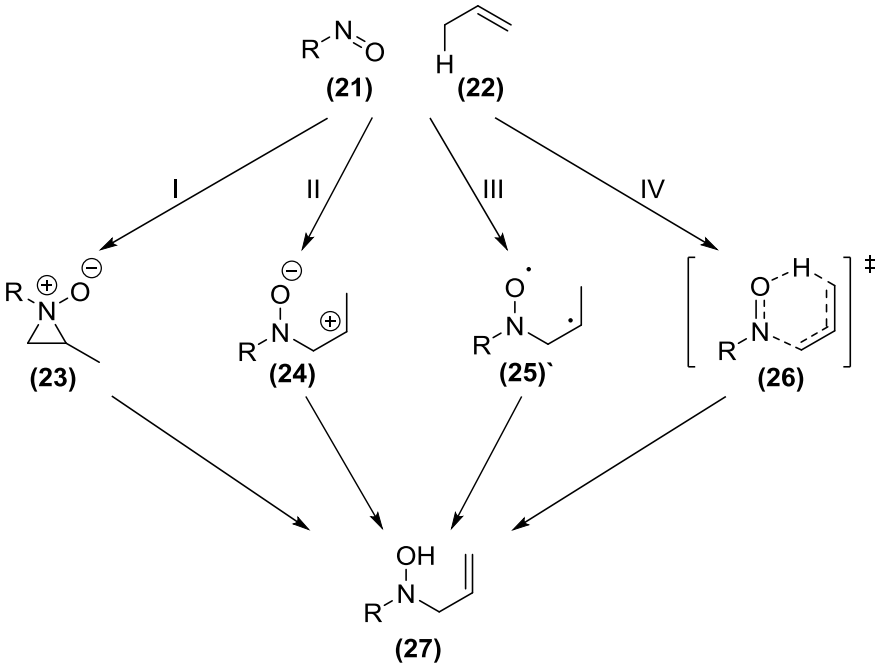


(19) **(20)**

Scheme 1.6 - Example of an aza-ene reaction on imidazole compound (20).

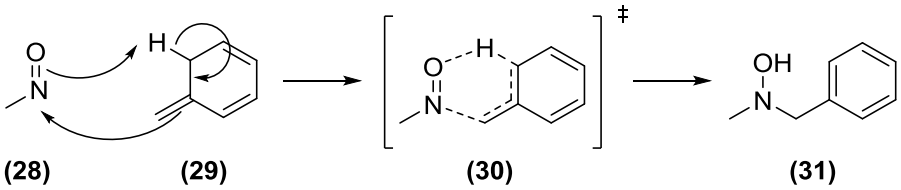
1.1.5 Nitroso ene chemistry-

Niroso containing molecules can also undergo ene reactions. There is some debate as to the precise mechanism of the nitroso ene reaction *i.e.* whether it is a radical, ionic, pericyclic or stepwise process. Scheme 1.7 shows the different proposed mechanisms for an ene reaction involving a simple ene component (**22**) and a nitroso enophile (**21**).



Scheme 1.7 - The four possible reaction mechanisms proposed for the ene reaction I = via a three membered ring intermediate. II = via a zwitterion intermediate. III = via a radical pathway. IV = via a concerted, pericyclic pathway.[8]

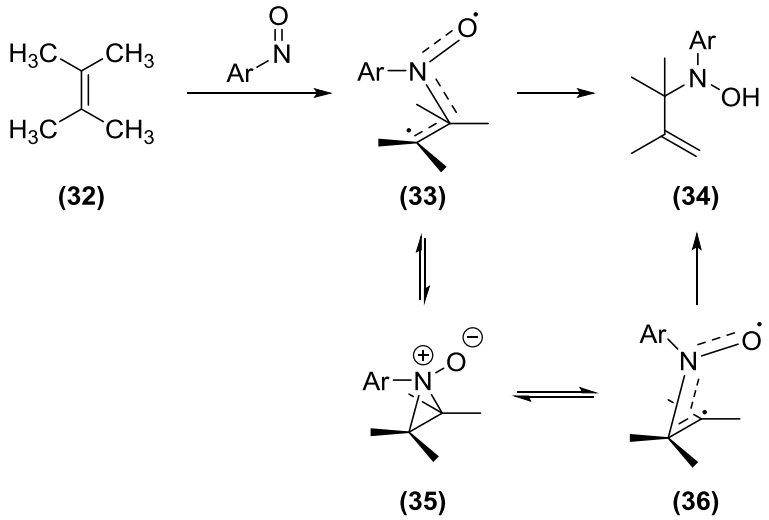
It has previously been shown that the reaction between nitroso-methane (**28**) and *o*-isotoluene (**29**) occurs via a concerted pericyclic mechanism (Scheme 1.8).[8] Density functional calculations have demonstrated that transition state (**30**) does not contain either a diradical or a zwitterionic species suggesting that the reaction proceeds via a fully concerted, pericyclic pathway.



Scheme 1.8 - Concerted nitroso-ene reaction mechanism.

Although there is evidence from computational studies to support the theory that the ene reaction proceeds via a fully concerted mechanism as in the instance above, there is evidence to suggest a different mechanistic pathway is prominent in other ene reactions.

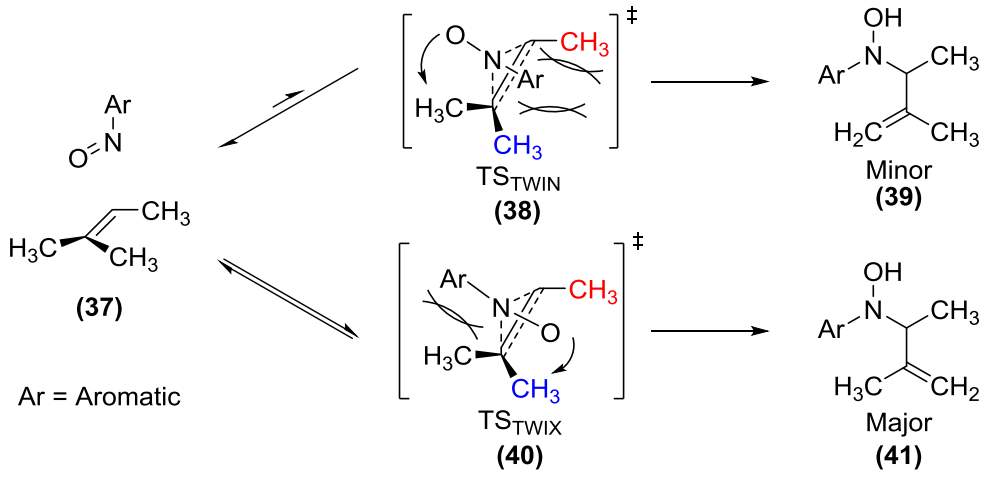
It has been reported that a biradical / zwitterionic mechanism may be favoured by molecules that are too hindered for the concerted mechanism to occur, and that have the ability to stabilise a radical / charged intermediate.[5,9] This is supported by the fact that ene reactions using cyclopentene and cyclohexene can be initiated with a free radical initiator.[5] The proposed mechanism for the radical reaction involves a three membered ring intermediate (Scheme 1.9).



Scheme 1.9 – Proposed radical nitroso-ene pathway.[10]

1.1.6 Ene regioselectivity-

The final step in the ene reaction, which is common to all of the mechanisms proposed, is abstraction of a hydrogen. In an unsymmetrical alkene there can be several possible hydrogens that may be abstracted during the ene reaction. The hydrogen which is abstracted depends upon the sterics of the surrounding groups (Scheme 1.10).[10]



Scheme 1.10 - Transition state (38) will be more favoured than the bottom reaction pathway (40) due to the steric effects between the aromatic ring and the methyl groups.

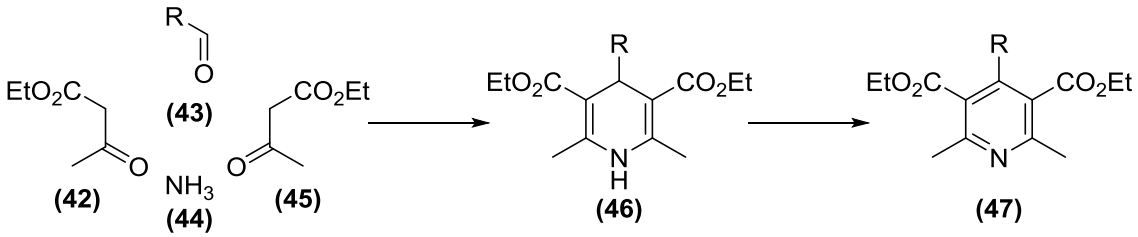
In the TS_{TWIN} structure (**38**) the aromatic group (Ar) is pointing towards the more sterically hindered side of the alkene, which makes this transition state high in energy and therefore unfavourable. In the TS_{TWIX} orientation (**40**), the aromatic group is pointing towards the unsubstituted region of the alkene where there are less steric interactions. This makes the TS_{TWIX} orientation lower in energy and therefore kinetically favourable than the TS_{TWIN} orientation.

In the favoured TS_{TWIX} orientation (**40**) there are two CH_3 groups from which a proton can be abstracted, the top red CH_3 group or the bottom blue CH_3 group. However, the aromatic group on the nitroso compound experiences a steric interaction with the single CH_3 group on the opposite side of the molecule. This steric interaction causes the nitroso compound to rotate, moving the oxygen towards the bottom blue CH_3 group. Therefore the proton abstraction will occur from the blue CH_3 group leading to the formation of (**41**) as the major product.

1.2.0 Multi-component reactions-

Our plan is to utilise two pericyclic reactions in a multi-component process to allow the rapid synthesis of complex compounds with high control of the stereochemistry. Therefore we will review literature work which has used multi-component reactions in organic synthesis.

Multi-component reaction processes involve a single chemical operation in which the majority of the atoms from three or more components are combined to form a new target molecule. Multi-component reactions are not a new idea in organic chemistry and have been utilised in many famous synthetic reactions. One of the first, the Strecker synthesis of amino acids, was reported in 1850[11] and still remains a popular reaction for organic chemists today.[12] The great appeal of multi-component reactions is the ability to start from very simple starting materials and rapidly generate highly complex molecules in an efficient, atom economical manner. For example, the Hantzsch synthesis involves the creation of complex substituted pyridine systems simply starting from ammonia, an aldehyde and a β-keto-ester (Scheme 1.11).



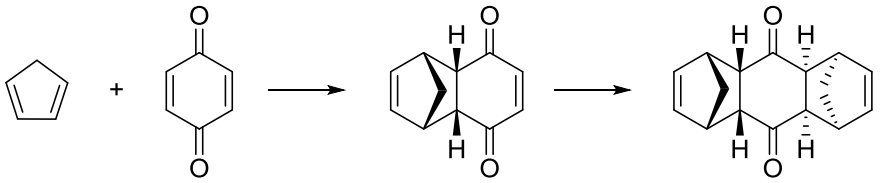
Scheme 1.11 - Hantzsch pyridine synthesis.

Pericyclic reactions, such as Diels-Alder and ene reactions, can form multiple C-C / C-X bonds and new stereocentres. However, despite the advantage of forming highly complex

compounds in a relatively simple reaction sequence, there are few examples of tandem pericyclic reaction processes being used in the literature.

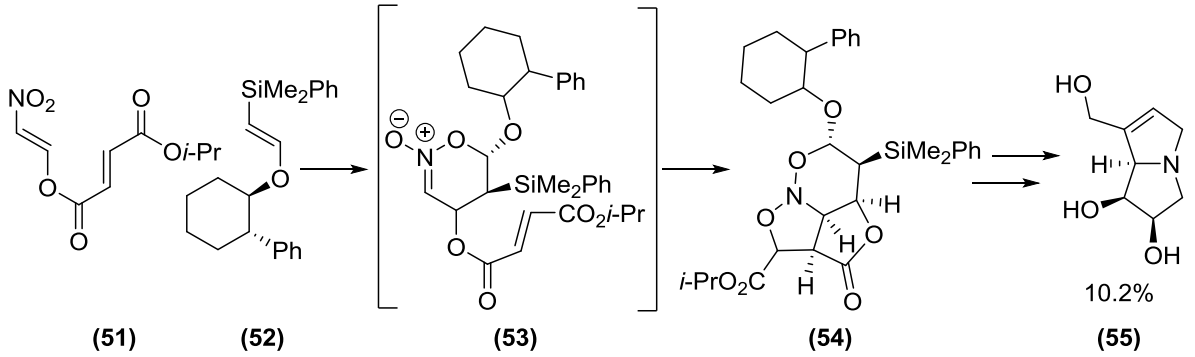
1.2.1 Diels-Alder / Diels-Alder reactions-

There are a large number of tandem, pericyclic reaction sequences that involve the use of multiple Diels-Alder reaction steps. In Diels and Alder's original paper from 1928[1], they identified the products formed from a single Diels-Alder reaction (**49**) and a tandem Diels-Alder / Diels-Alder reaction (**50**) process (Scheme 1.12).



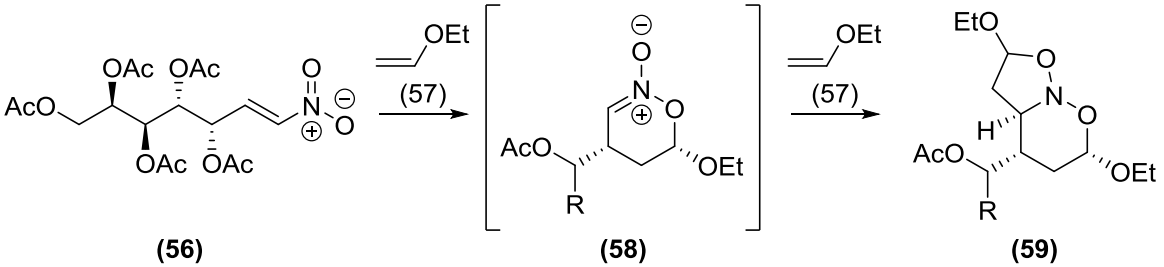
Scheme 1.12 - Products from single Diels-Alder (49) and tandem Diels-Alder reactions (50).

Denmark *et. al.*[13-16] have carried out extensive work in developing tandem intermolecular Diels-Alder / intramolecular cycloaddition reaction sequences involving nitroalkenes. A nitroalkene is used as a dienophile which undergoes a [4+2] Diels-Alder reaction, followed immediately by a further [3+2] cycloaddition reaction. This has allowed the researchers to utilise this tandem Diels-Alder reaction sequence to great effect in the synthesis of many natural products including (+)-crotanecine (**55**), a base component in a range of pyrrolizidine alkaloids (Scheme 1.13).[13]



Scheme 1.13 - Tandem Diels-Alder reactions used in the synthesis of (+)-crotanecine (55).

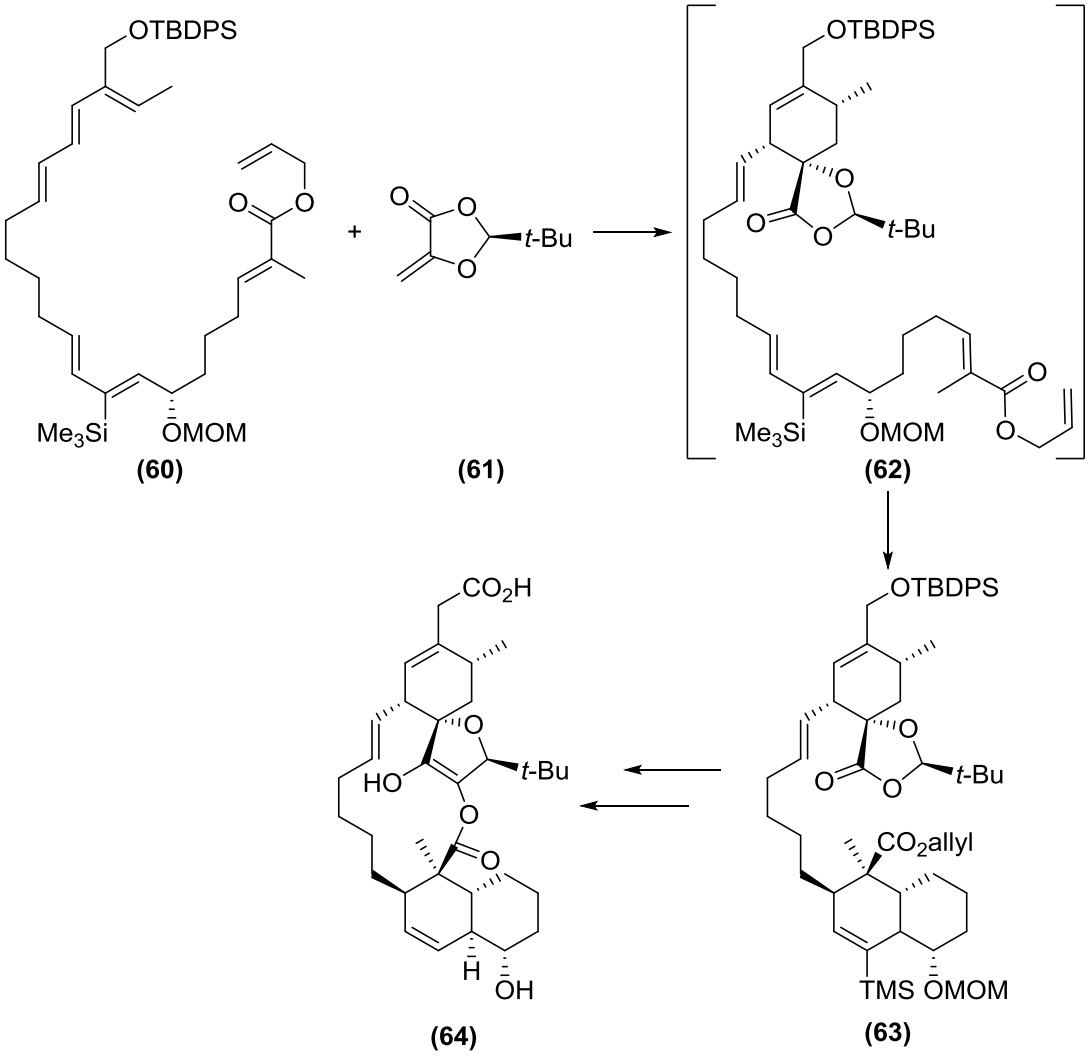
Many people have built upon the work by Denmark's group including Avalos *et al.*[17], who have adapted the tandem [4+2] Diels-Alder / [3+2] cycloaddition reactions in their synthesis of carbohydrates (Scheme 1.14). However, whilst Denmark's work involved an intermolecular Diels-Alder / intramolecular Diels-Alder reaction sequence, Avalos *et al*. have developed an intermolecular Diels-Alder / intermolecular Diels-Alder reaction sequence using nitroalkene (**56**) as the diene and ethyl vinyl ether (**57**) as the dienophile.



Scheme 1.14 - [4+2] Diels-Alder / [3+2] cycloaddition reaction sequence

Through consideration of frontier molecular orbital theory, it is possible to predict the stereo- and regio-selectivity of the Diels-Alder reaction and therefore the stereochemistry of the resultant Diels-Alder adduct.

Roush *et al*.[18] have exploited these properties in their total synthesis of (-)-chlorothricolide (**64**), through a domino intermolecular [4+2] Diels-Alder / intramolecular [4+2] Diels-Alder reaction sequence to form (**64**) in a yield of 55%. Product (**64**) is the major product formed in preference to 96 other compounds that could be formed from two sequential Diels-Alder reactions when the endo, exo, stereo-, and regio- selectivity possibilities are considered (Scheme 1.15).

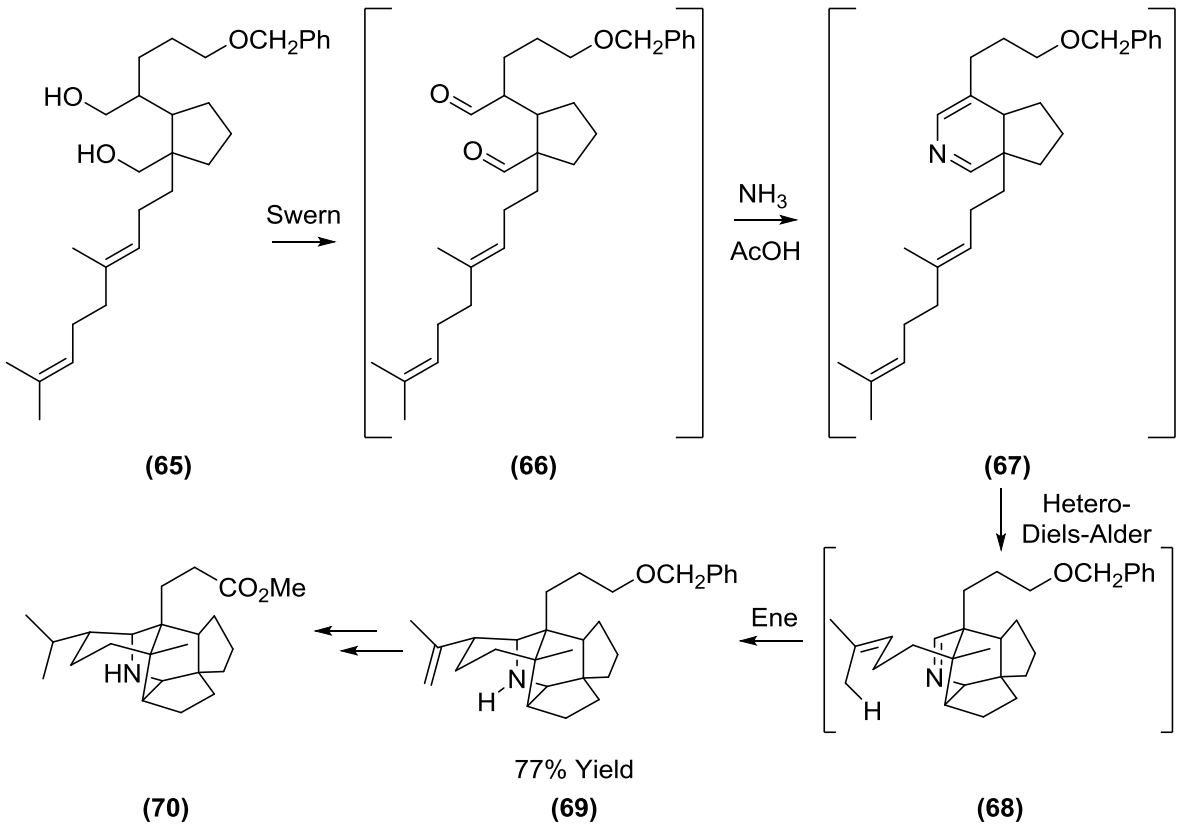


Scheme 1.15 - Intermolecular Diels-Alder / intramolecular Diels-Alder reaction sequence in the synthesis of (-)-chlorothricolide (64).

1.2.2 Diels-Alder / ene reactions-

All Diels-Alder reactions generate a new double bond, therefore it could be envisaged that this double bond could be reacted further in an ene reaction, leading to the formation of new C-C or C-X bonds. There are however, surprisingly few examples of this reaction sequence in the literature.

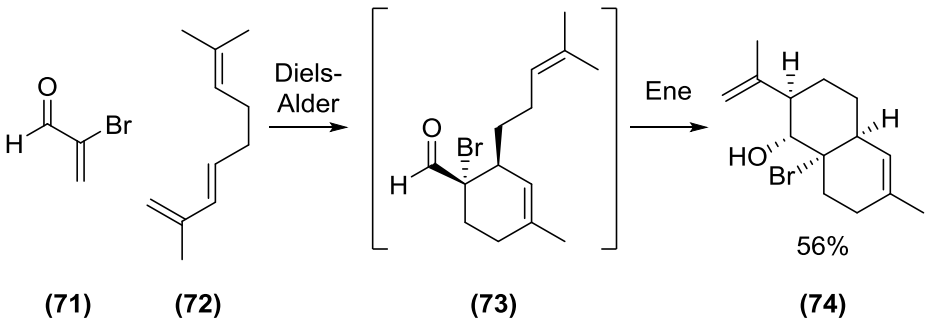
Heathcock *et al*.[19] demonstrated in their synthesis of Daphniphyllum alkaloid (±)-methyl homosecodaphniphyllate (**70**), how a one-pot intra-Diels-Alder / intra-ene reaction could be used to rapidly generate complex compounds (Scheme 1.16).



Scheme 1.16 - One-pot Diels-Alder / ene reaction in the synthesis of (±)-methyl homosecodaphniphyllate (70).

Diol (**65**) is subjected to a Swern reaction followed by cyclisation to form diene (**67**). Diene (**67**) is then able to undergo an intramolecular hetero-Diels-Alder reaction and subsequent intramolecular ene reaction, to give compound (**69**) in an overall yield of 77%. This elegant synthesis exemplifies the great utility in combining one-pot Diels-Alder / ene reactions to generate high molecular complexity.

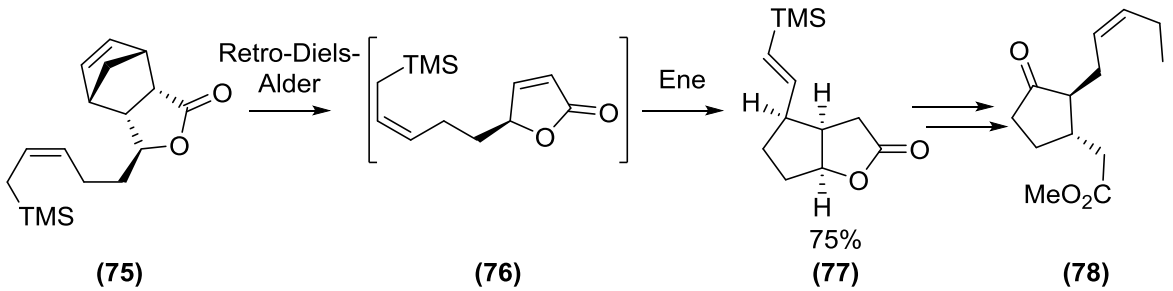
Despite Heathcock demonstrating the power of one-pot Diels-Alder / ene reactions, the next example of an intermolecular-Diels-Alder / intramolecular ene reaction was not reported until 2004 by Kraus and Kim.[20] Whilst trying to synthesise bromo-aldehyde (**73**) via a Diels-Alder reaction, they discovered that the major product (**74**) isolated resulted from a Diels-Alder / ene reaction sequence (Scheme 1.17)



Scheme 1.17 - Unexpected ene adduct (74) formed from a Diels-Alder / ene reaction.

The initial Diels-Alder reaction between aldehyde (**71**) and diene (**72**) leads to the formation of Diels-Alder adduct (**73**) which then undergoes a carbonyl ene reaction with the aldehyde group to form (**74**). This one-pot Diels-Alder / ene reaction leads to the formation of four new stereocentres.

In the same year as Kraus and Kim's one-pot Diels-Alder / ene reaction, Inomata *et al.* published a paper[21] on the total synthesis of plant extract (+)-methyl jasmonate (**78**). The formation of (+)-methyl jasmonate (**78**) was achieved with the use of a one-pot retro-Diels-Alder / intramolecular ene reaction sequence (Scheme 1.18).



Scheme 1.18 - retro-Diels-Alder / ene reaction used in the synthesis of (+)-methyl jasmonate (78).

Inomata's group discovered that, as the ene reaction was the rate determining step for the retro-Diels-Alder / ene reaction, the rate of the ene reaction could be greatly improved with a trimethylsilyl group adjacent to the enophile. The trimethylsilyl group donates electron density into the alkene which raises the energy of the HOMO making it more reactive towards enophiles. The same reaction, with a less reactive H group instead of a TMS group, ran for the same amount of time, gave a 2: 1 mixture of unsaturated lactone (**76**) and ene adduct (**77**).

1.3.0 Indole in biologically active compounds-

Our target is to synthesise bioactive indole containing compounds using multi-component pericyclic reactions. Therefore we need to consider why compounds based upon indole are of interest. We will look at a range of biologically active indole compounds from the literature. This will allow us to tailor our reactions towards specific molecular frameworks and biological properties.

1.3.1 Common indole-containing compounds-

The heteroaromatic indole is found in a large number of naturally occurring compounds. It is most abundant in nature as tryptophan (**79**), one of the naturally occurring amino acids and it is also found in many important alkaloids such as tryptamine (**80**) and serotonin (**81**) (Figure 1.2).

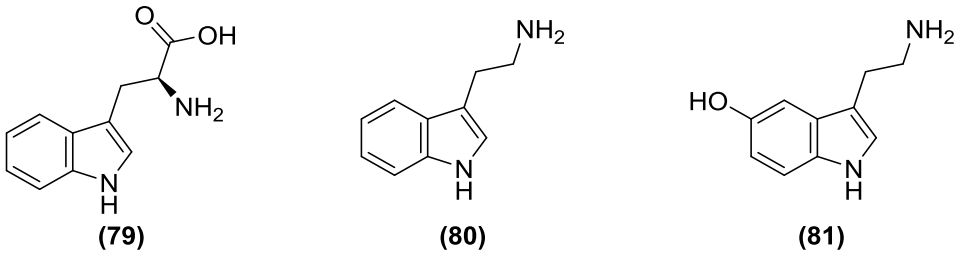


Figure 1.2 - Tryptophan (79), tryptamine (80) and serotonin (81).

Many indoles are also used clinically in drugs. The migraine drug sumatriptan (**84**), hallucinogenic lysergic acid diethylamide (LSD) (**83**) and the anti-emetic ondansetron (**82**), all contain an indole ring and often contain tryptophan-like structures (Figure 1.3). These drugs all have similar structural motifs and are all known to bind to serotonin receptors in the brain.[22-24]

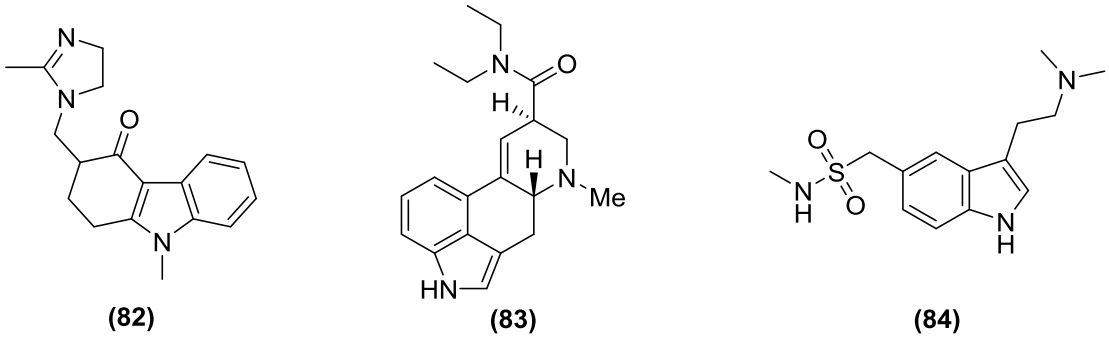


Figure 1.3 - Ondansetron (82), LSD (83) and sumatriptan (84).

In addition to these tryptophan-based bioactive compounds (Figure 1.3), many alkaloids including rebeccamycin (**85**) and staurosporine (**86**), have a *bis*-indole ring structure (Figure 1.4). Compounds in this class are produced by bacteria, such as *Staphomycetes*, and have a range of biological effects.[25]

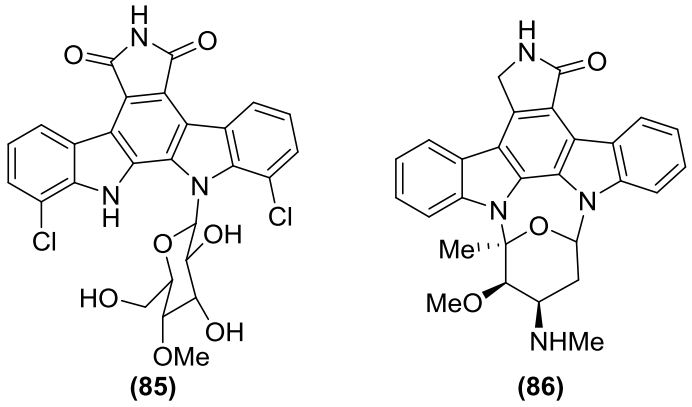


Figure 1.4 - Rebeccamycin (85) and staurosporine (86).

Although rebeccamycin (**85**) and staurosporine (**86**) have very similar structures, their biological activity is due to inhibition of two different biological targets. Rebeccamycin (**85**)

works by inhibiting topoisomerase I which is an important enzyme involved in the replication and transcription of DNA.[26] Structure activity relationship studies of synthetic analogues of rebeccamycin, such as (**87**) and (**88**) (Figure 1.5), have shown that a sugar moiety on the indolic nitrogen must be present for the compound to be an effective topoisomerase I inhibitor.[27,28]

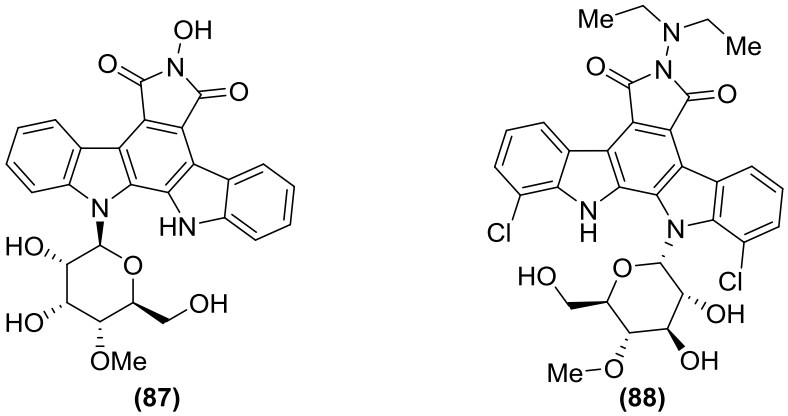


Figure 1.5 - Semi-synthetic analogues of rebeccamycin.

Staurosporine (**86**), on the other hand, is a kinase inhibitor (IC_{50} value of 2.7 nM against protein kinase C)[29] and works by binding to the ATP site on the kinase.[30] However, due to the lack of selectivity of staurosporine (**86**), it is unsuitable as a clinical kinase inhibitor. This has led to the development of a number of semi-synthetic analogues which show more selectivity, such as midostaurin (**89**) and lestaurtinib (**90**) (Figure 1.6).[28]

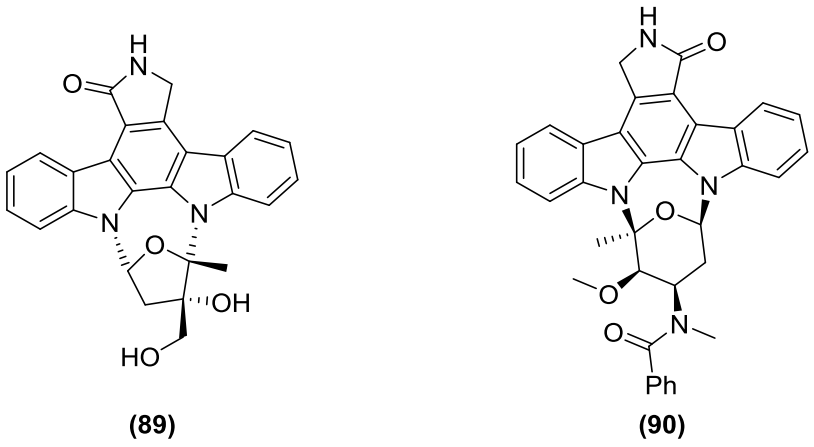


Figure 1.6 - Midostaurin (89) and lestaurtinib (90).

1.3.2 Granulatimide (91) and isogranulatimide (92) compounds-

Another set of compounds which have shown a lot of promise as anti-cancer drugs, is granulatimide (**91**) and isogranulatimide (**92**). Originally isolated from the sea squirt *Didemnum granulatum*, granulatimide (**91**) and isogranulatimide (**92**) have shown to be potent inhibitors of Chk1 kinase (Figure 1.7).[31] Many current cancer treatments, such as

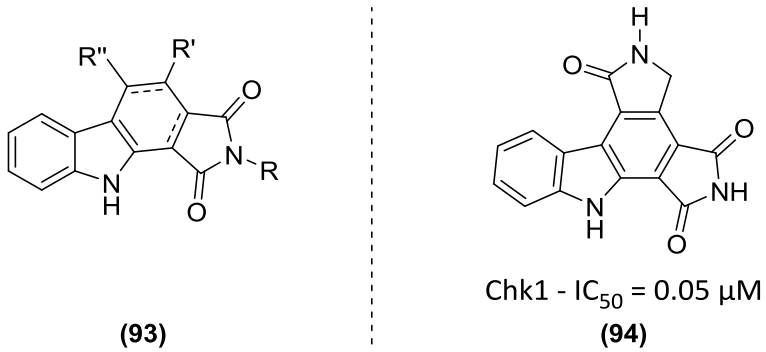
radiation or chemotherapy, are designed to damage the DNA of cancer cells leading to cell death. The Chk1 kinase plays a vital role in the G2 cell cycle checkpoint, which delays the cell cycle to allow for DNA repair to occur. Therefore Chk1 kinase inhibitors prevent the repair of damaged DNA in cells, which leads to death of the cell.[32]

Chk1 - IC_{50} = 0.25 µM
(91)

Chk1 - IC_{50} = 0.10 µM
(92)

Figure 1.7 - Structure of granulatimide (91) and isogranulatimide (92)

Both granulatimide (**91**) and isogranulatimide (**92**) contain a pyrrolo[3,4-*a*]carbazole-1,3-dione framework (**93**) shown below (Figure 1.8).



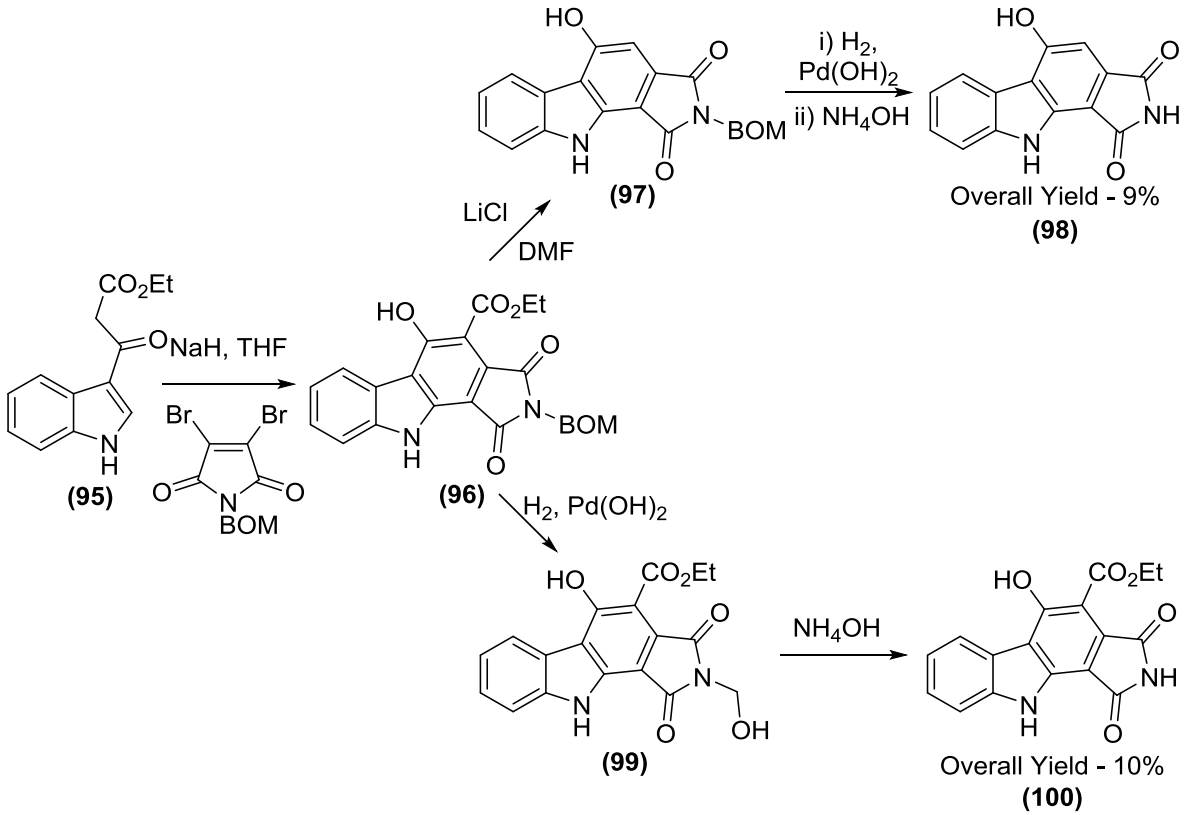
(93)

Chk1 - IC_{50} = 0.05 µM
(94)

Figure 1.8 - The pyrrolo[3,4-a]carbazole-1,3-dione framework

1.3.3 Pyrrolo[3,4-a]carbazole-1,3-dione compounds-

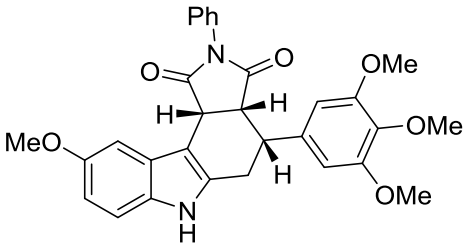
The promising bioactivity shown by granulatimide (**91**) and isogranulatimide (**92**) has led to much work being carried out in the synthesis of compounds containing a pyrrolo[3,4-*a*]carbazole-1,3-dione framework, such as compound (**94**) (Figure 1.8).[33] Structure activity relationship (SAR) studies have been conducted on compounds containing this framework, to try and identify the pharmacaphore (Scheme 1.19).[33]



Scheme 1.19 - Synthesis of granulatimide analogues (98) and (100).

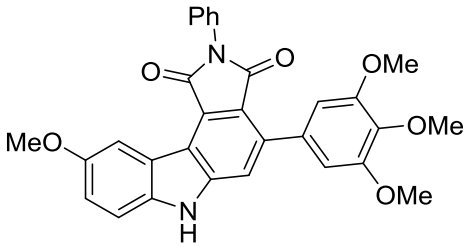
The synthesis of (**98**) and (**100**) is achieved in three steps with an overall yield of 9 and 10% respectively and both compounds were shown not to inhibit Chk1 kinase. This suggested that the succinimide group in the top region of granulatimide (**91**) is important for biological activity.

Building upon this work by Prudhomme *et al.* led to Caballero *et al*.[34] synthesising compounds such as (**101**) and (**102**) (Figure 1.9) and testing them for activity against a range of cancer cell lines.



Mouse lymphoma P-388 IC_{50} = 0.5 µM

(101)



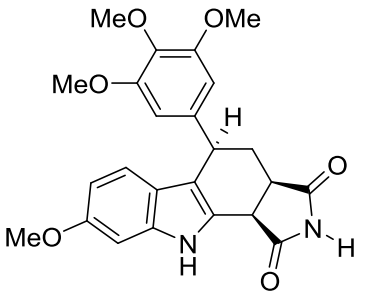
Mouse lymphoma P-388 IC_{50} = 9.8 µM

(102)

Figure 1.9 - Synthetic biologically active indole compounds.

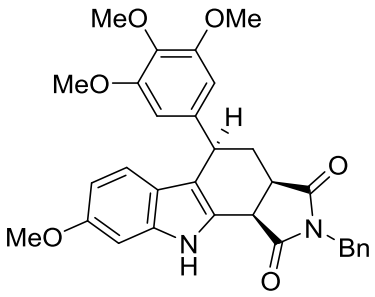
Caballero *et al*. observed that tetrahydrocarbazole compounds which are not fully aromatic such as (**101**), showed better bioactivity than carbazole compounds like (**102**).

This has led to the development of similar compounds such as (**103**) and (**104**), which have been shown to have good biological activity against B16 melanoma cells [35] (Figure 1.10).



B16 melanoma cells - IC_{50} = 2.6 μM

(103)



B16 melanoma cells - IC_{50} = 3.7 μM

(104)

Figure 1.10 - Synthetic bioactive indoles.

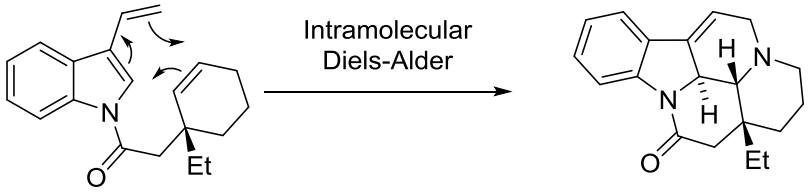
1.4.0 Diels-Alder reactions with vinyl-heteroaromatics-

We intend to use vinyl-indole compounds as the start point in our multi-component reactions and therefore we should review general and related work that use vinyl-indole compounds in pericyclic reactions.

1.4.1 Vinyl-heteroaromatic Diels-Alder reactions in synthesis-

There have been a considerable number of studies into Diels-Alder reactions that use vinyl-heteroaromatics as the diene, including vinyl-indoles[36-40], vinyl-imidazoles[41-46], vinyl-pyrazole[47,48] and vinyl-imidazolone.[49,50] Although vinyl-heteroaromatics are not traditional dienes in Diels-Alder reactions due to the involvement of aromatic π-bonds, they are electron rich and therefore can be excellent dienes.

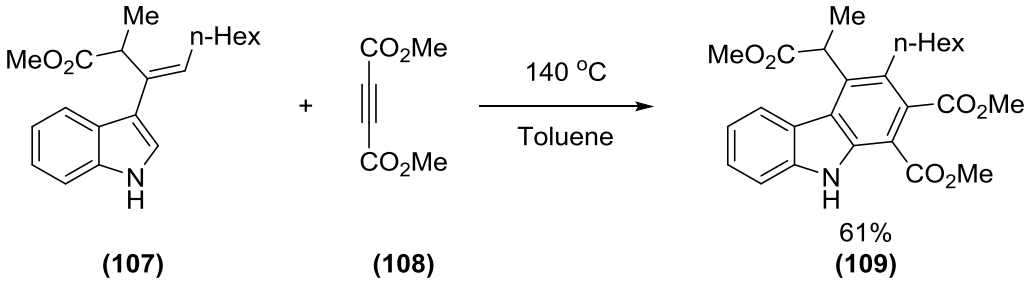
Diels-Alder reactions of vinyl-heteroaromatics have been used in the synthesis of useful biological targets.[38] In the total synthesis of the alkaloid eburnamonine (**106**), an intramolecular aza-Diels-Alder reaction is used to simultaneously form two rings of the penta-cyclic ring system (Scheme 1.20).



(105) **(106)**

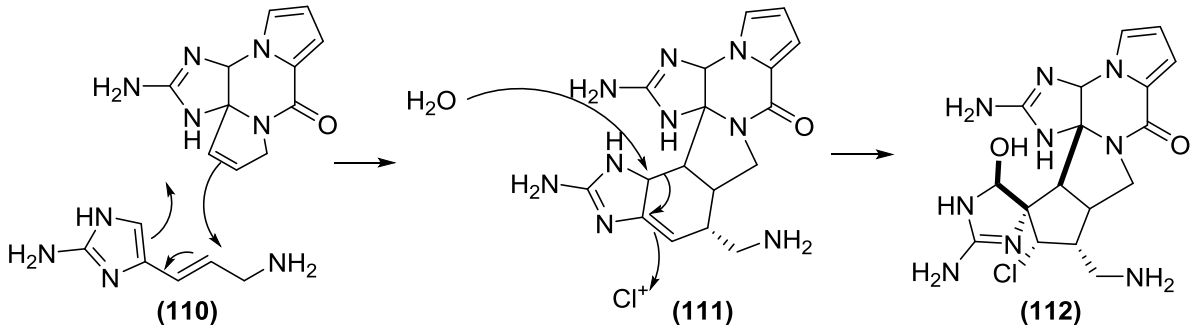
Scheme 1.20 - An intramolecular aza-Diels-Alder reaction in the synthesis of eburnamonine (106).

Intermolecular Diels-Alder reactions with vinyl-indoles have also been reported.[51-53] For example, the reaction shown in Scheme 1.21 shows the Diels-Alder reaction between a substituted vinyl-indole (**107**) and substituted acetylene (**108**).



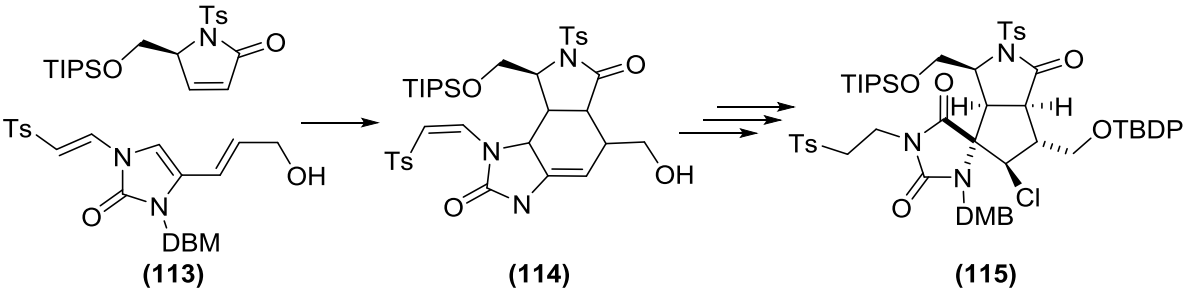
Scheme 1.21 - Intermolecular Diels-Alder reaction of vinyl-indole (107) with substituted acetylene (108).

Much of the Diels-Alder chemistry of vinyl-imidazoles has centred on the synthesis of oroidin alkaloids.[41,42] Scheme 1.22 below shows the Diels-Alder step used in the synthesis of Palau'amine (112).



Scheme 1.22 - Vinyl-imidazole Diels-Alder reaction in the formation of Palau'amine (112).[42]

In addition, vinyl-imidazolones have been used in the total synthesis of many naturally occurring compounds[50], including the complex ring structures found in oroidin alkaloids (Scheme 1.23) and the related bisguanidine marine alkaloids.[49]

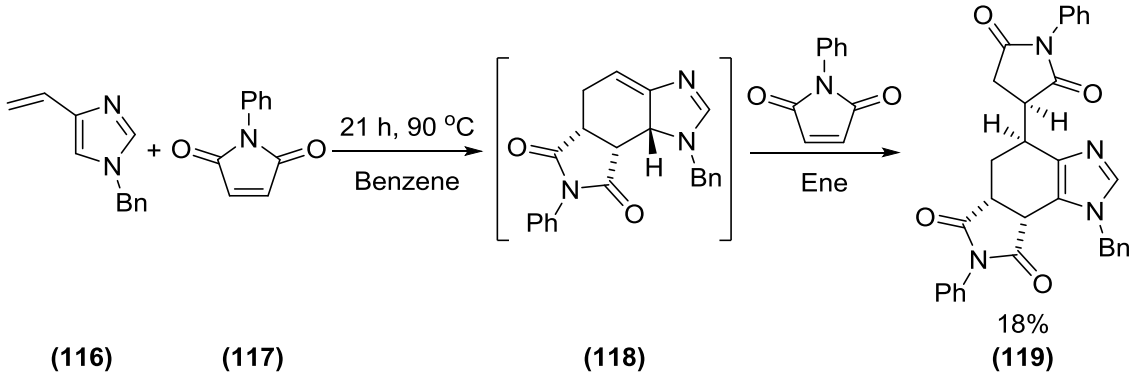


Scheme 1.23 – Diels-Alder reaction in the synthesis of complex ring structures found in oroidin alkaloids.

1.4.2 Diels-Alder / ene reactions with vinyl-heteroaromatics-

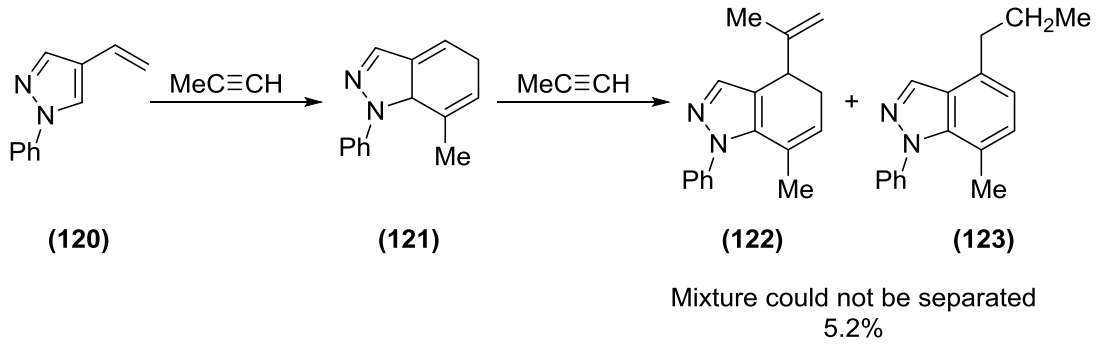
In a few cases, Diels-Alder reactions with vinyl-heteroaromatics have led to an interesting side reaction occurring between the Diels-Alder product and excess dienophile in the reaction mixture. The Diels-Alder product of 3-vinyl-imidazole (**116**) and *N*-phenylmaleimide

(NPM) (**117**) as shown below (Scheme 1.24) can undergo an ene reaction with excess NPM (**117**) in the reaction mixture to give product (**119**).



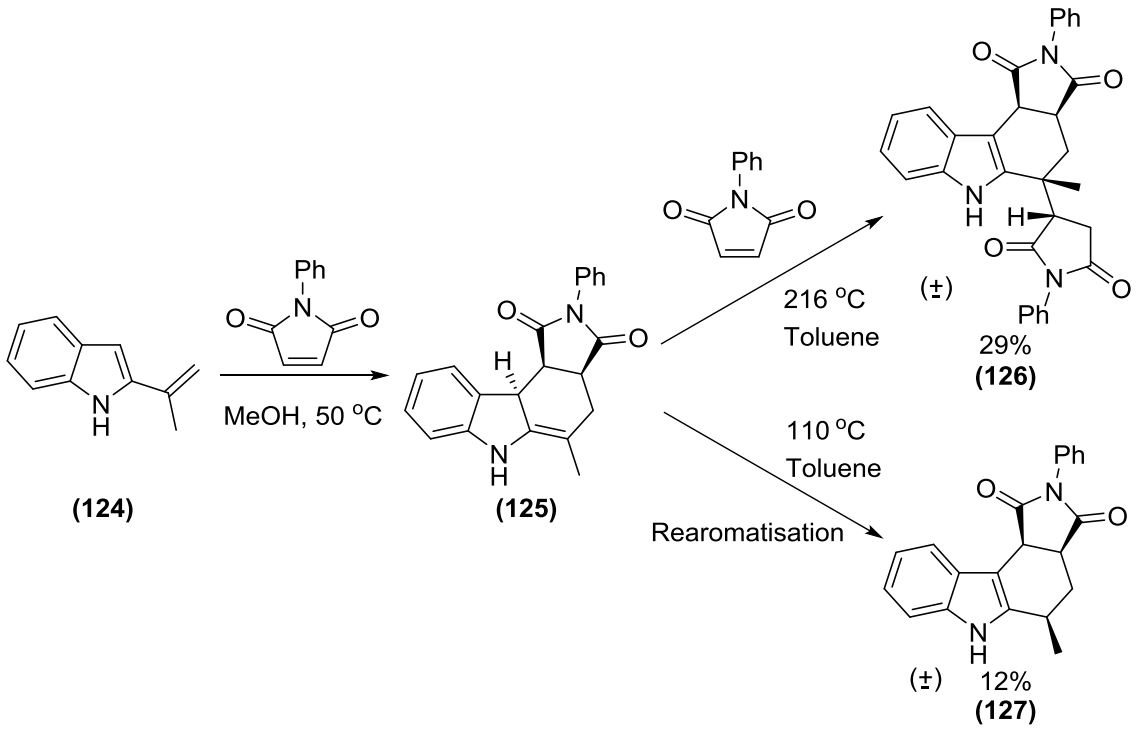
Scheme 1.24 – Diels-Alder/ene reaction of 1-benzyl-4-vinyl-1H-imidazole (116) and NPM (117).[42]

A similar reaction has also been observed in the Diels-Alder reactions of vinyl-pyrazole, whereby initial Diels-Alder product (**121**) reacts with excess dienophile in an ene reaction to give (**122**) and (**123**) (Scheme 1.25).



Scheme 1.25 - Diels-Alder / ene reaction of 1-phenyl-4-vinyl-1H-pyrazole (120) and prop-1-yne.[48]

Sequential Diels-Alder / ene reactions have also been noted with 2-vinyl-indoles. Here the ene reaction is in competition with the rearomatisation of Diels-Alder adduct (**125**) (Scheme 1.26).[36]



Scheme 1.26 – Diels-Alder / ene versus Diels-Alder / rearomatisation reactions of 2-(prop-1-en-2-yl)-1H-indole (124).

1.5.0 Conclusions-

This introduction chapter has served to be an overall guide through the important chemistry that will be discussed in this thesis. We have shown the great synthetic utility of pericyclic Diels-Alder and ene reactions that allow the formation of multiple new bonds and stereocentres. We have also seen how combining two or more Diels-Alder / ene reactions into one multi-component reaction process, allows the synthesis of highly complex compounds from relatively simple starting materials.

The literature on biologically active indole molecules offers insight into the structural features necessary for a compound to show biological activity. With this information from the literature, we can develop a synthetic plan that will allow us to use this chemistry in a novel synthesis of biologically active indole-containing compounds.

Chapter 2- Synthesis and Diels-Alder Chemistry of Vinyl-Indoles

2.1.0 Synthetic route to pyrrolo[3,4-a]carbazole-1,3-dione framework-

We envisaged synthesising compounds with a pyrrolo[3,4-*a*]carbazole-1,3-dione framework, such as (**103**), using an intermolecular Diels-Alder reaction between 3-vinyl-1*H*-indole and a dienophile, followed by an intermolecular ene reaction with the resultant Diels-Alder adduct (**129**) formed (Figure 2.1).

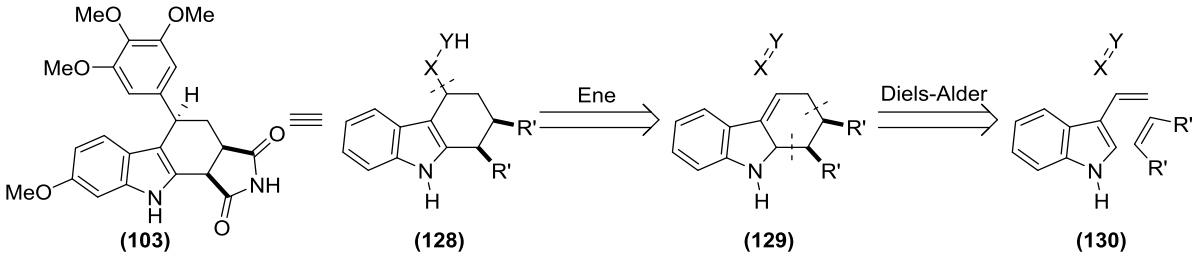


Figure 2.1 - Retrosynthetic approach to biologically active compounds.

Given the wide range of dienophiles and enophiles available, we reasoned that this synthetic route could rapidly lead to a diverse series of potentially biologically active compounds.

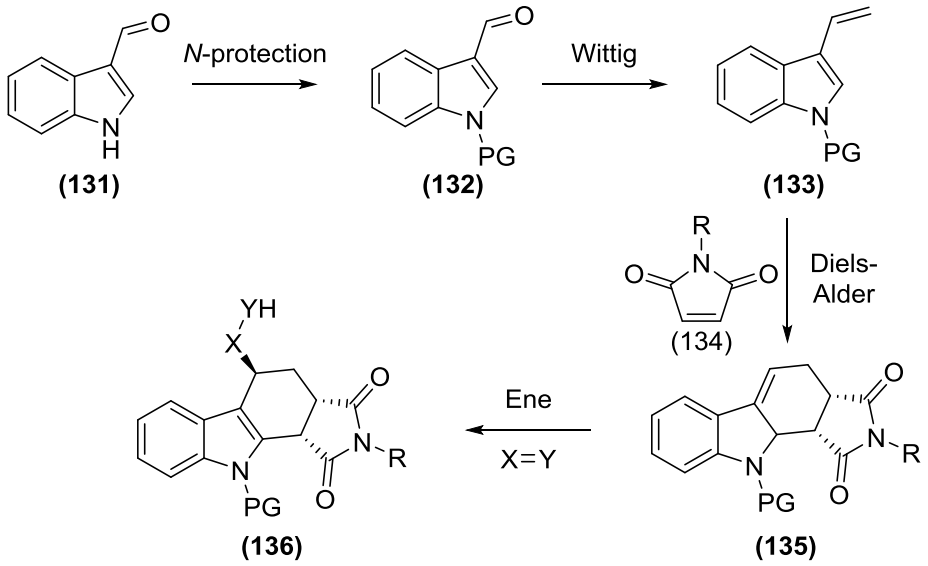
2.1.1 Synthetic plan-

The initial aim of the project is to synthesise *N*-protected 3-vinyl-1*H*-indoles (**133**) from commercially available 3-carboxaldehyde-1*H*-indole (**131**). The nitrogen protecting group has a large effect upon the electronics of the indole ring system and consequently a large effect upon the Diels-Alder/ ene reactions. As normal electron demand Diels-Alder reactions require an electron rich dienophile, a variety of *N*-protecting groups will be screened to determine the most suitable system for the planned Diels-Alder/ ene reaction.

Following this, the *N*-protected indoles (**132**) will be subjected to a Wittig reaction to give *N*-protected 3-vinyl-1*H*-indole compounds (**133**). Once a range of *N*-protected 3-vinyl-1*H*-indoles (**133**) are obtained, the Diels-Alder chemistry with a range of dienophiles will be investigated.

We intend to predominantly use maleimide based dienophiles (**134**). Maleimides are excellent examples of electron poor dienophiles, which are required in normal electron demand Diels-Alder chemistry. In addition, maleimides (**134**) are a common feature in a range of biologically active unsaturated carbazole compounds (Chapter 1.3).

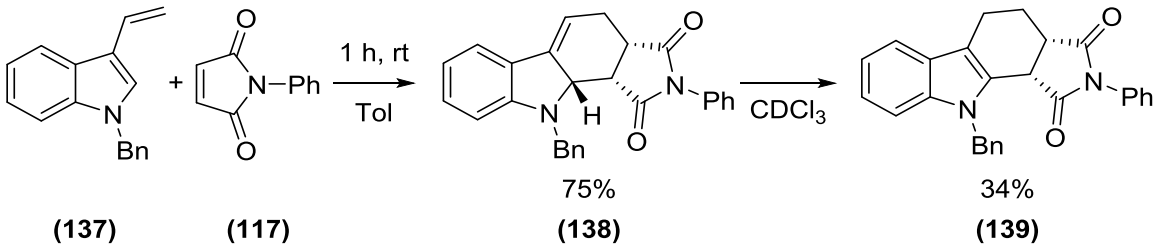
Once Diels-Alder cycloadducts (**135**) are obtained, they will be reacted with a range of enophiles, forming compounds (**136**) with a pyrrolo[3,4-*a*]carbazole-1,3-dione framework (Scheme 2.1).



Scheme 2.1 - Planned synthetic route to carbazole compounds.

2.2.0 Synthesis of N-protected 3-vinyl-indoles and investigation of their Diels-Alder / ene chemistry-

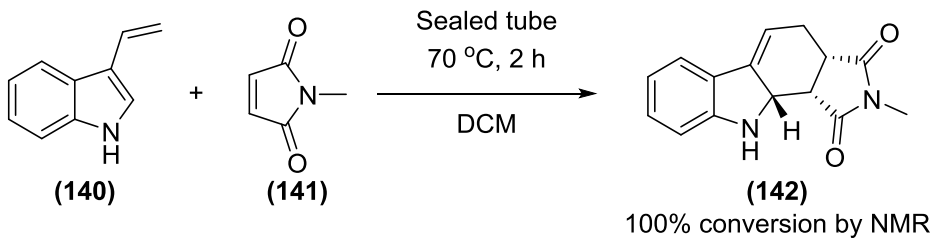
The initial step in the synthetic route (Scheme 2.1) was to synthesise *N*-protected 3-vinyl-1*H*-indoles. Previous work by Porter *et al*. has shown that protection of the indolic nitrogen plays a vital role in controlling the rate of Diels-Alder reactions and the stability of Diels-Alder adducts (**135**) formed.[54] Porter *et al*. showed that electron rich dienes, such as 1-benzyl-3-vinyl-1*H*-indole (**137**), readily react with *N*-phenylmaleimide (NPM) (**117**) to generate Diels-Alder adduct (**138**). Diels-Alder adduct (**138**) can be isolated by recrystallisation, however rapidly undergoes rearomatisation to form compound (**139**) in polar solvents (Scheme 2.2). The reactivity of Diels-Alder adduct (**138**) makes isolation and further chemistry difficult.



Scheme 2.2 - Formation of rearomatised Diels-Alder adduct (139).

2.2.1 Preliminary Diels-Alder reactions-

To examine the effect of having no protecting group on the indolic nitrogen, a Diels-Alder reaction was carried out between 3-vinyl-1*H*-indole (**140**) and *N*-methylmaleimide (NMM) (**141**). The reaction was carried out in a sealed tube at 70 ^{o}C for two hours in DCM with clean conversion of 3-vinyl-1*H*-indole (**140**) to Diels-Alder adduct (**142**) being observed (Scheme 2.3).



Scheme 2.3 - Diels-Alder reaction with 3-vinyl-1H-indole (140) and NMM (141).

However, attempts at purifying the crude reaction mixture using column chromatography or recrystallisation led to rearomatisation of the indole ring to form rearomatised Diels-Alder adduct (**143**) (Figure 2.2).

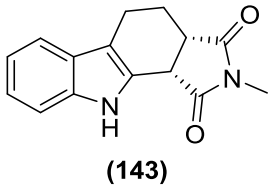


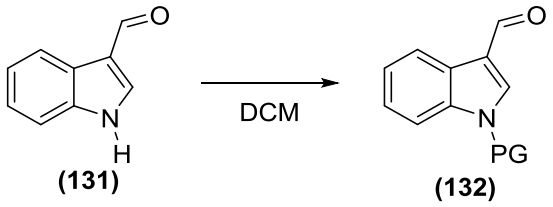
Figure 2.2 - Rearomatised Diels-Alder adduct (143).

Although the Diels-Alder reaction between 3-vinyl-1*H*-indole (**140**) and NMM (**141**) offered good conversion to Diels-Alder adduct (**142**), the rapid rearomatisation of the indole ring made further chemistry difficult.

2.2.2 N-Protection of 3-carboxaldehyde-1H-indole-

As Porter's research[54] and preliminary reactions had shown that no protecting group and electron donating *N*-protecting groups led to rapid rearomatisation, we rationalised that electron withdrawing groups might stabilise Diels-Alder adduct (**135**) and allow further chemistry to be performed. Therefore we proposed using tosyl and Boc as *N*-protecting groups for our initial investigations. The use of two protecting groups with different electron withdrawing capabilities allows a comparison to be made between the relative rate of Diels-Alder reactions with maleimides and the stability of resultant Diels-Alder adducts formed.

Boc and tosyl protection of the indolic nitrogen of 1*H*-indole-3-carbaldehyde (**131**) was attempted using mild, basic conditions based on literature procedure.[55] The reactions worked well and the desired compounds were isolated in excellent yields.



(131) (132)

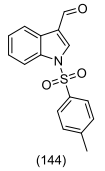
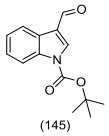
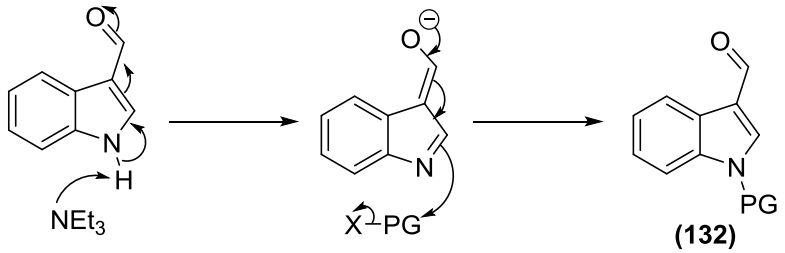
Conditions	Yield	Product
i) NEt_3, 0 °C, 1h ii) TsCl, rt, 18 h	90%	 (144)
i) NEt_3, 0 °C, 1h ii) $(Boc)_2O$, rt, 18 h	87%	 (145)

Table 2.1 - N-Protection of 3-carboxaldehyde-1H-indole (131).

Generally, the deprotonation of an indolic NH requires the use of a strong base.[56] However, in the case of 3-carboxaldehyde-1*H*-indole (**131**), the nitrogen lone pair is conjugated with the electron withdrawing carbonyl group which makes the NH more acidic. As a result of this, weak bases such as triethylamine, are sufficient to carry out the protection (Scheme 2.4).

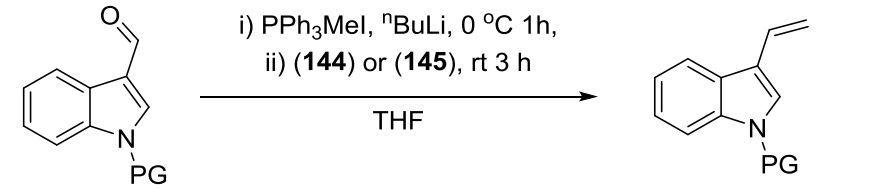


Scheme 2.4 - N-protection of 3-carboxaldehyde-1H-indole.

2.2.3 Wittig reactions of N-protected-3-carboxaldehyde-indoles-

With 1-tosyl-1*H*-indole-3-carbaldehyde (**144**) and *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate (**145**) in hand, the next step was to convert them into the corresponding *N*-protected 3-vinyl-indole compounds (**146**) and (**147**). We did this through a Wittig reaction between *N*-protected 3-carboxaldehyde-1*H*-indoles (**144**) and (**145**), and methylenetriphenyl-λ^5-phosphane.

The desired *1*-tosyl-3-vinyl-1*H*-indole (**146**) was obtained in good yield. However, after several attempts, *tert*-butyl 3-vinyl-1*H*-indole-1-carboxylate (**147**) could only be obtained in a poor yield of 13% in addition to 10% of 3-vinyl-1*H*-indole (**140**) (Scheme 2.5).



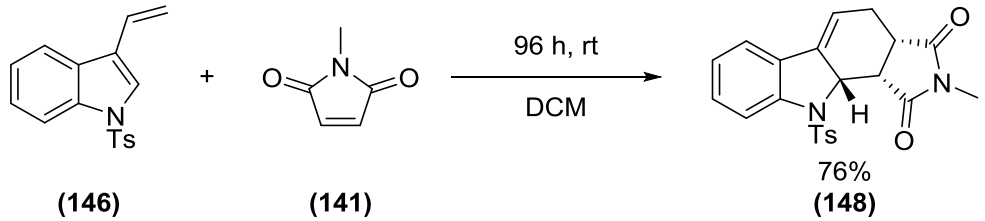
Scheme 2.5 - Synthesis of N-protected 3-vinyl-indoles (146) and (147).

The formation of 3-vinyl-1*H*-indole (**140**) suggested that the Wittig reaction on *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate (**145**) was proceeding, but *tert*-butyl 3-vinyl-1*H*-indole-1-carboxylate (**147**) formed was not stable to Wittig reaction conditions. It was therefore decided to proceed to the Diels-Alder chemistry using *1*-tosyl-3-vinyl-1*H*-indole (**146**).

2.2.4 Diels-Alder chemistry of 1-tosyl-3-vinyl-1H-indole (146)-

With the Wittig reaction giving 1-tosyl-3-vinyl-1*H*-indole (**146**) in a good yield, the ability of (**146**) to undergo Diels-Alder chemistry was investigated.

A 1: 1 mixture of 1-tosyl-3-vinyl-1*H*-indole (**146**) and NMM (**141**) was dissolved in DCM and the solution was stirred at room temperature (Scheme 2.6).



Scheme 2.6 - Initial Diels-Alder reaction between 1-tosyl-3-vinyl-1H-indole (146) and NMM (141).

The initial reaction gave the desired Diels-Alder adduct (**148**) in a good yield of 76%, after stirring for 96 hours at room temperature.

Optimisation of the reaction was performed in different solvents and at different temperatures to try and reduce the Diels-Alder reaction time. We used ^{1}H NMR to monitor the reaction. A typical example is shown below (Figure 2.3).

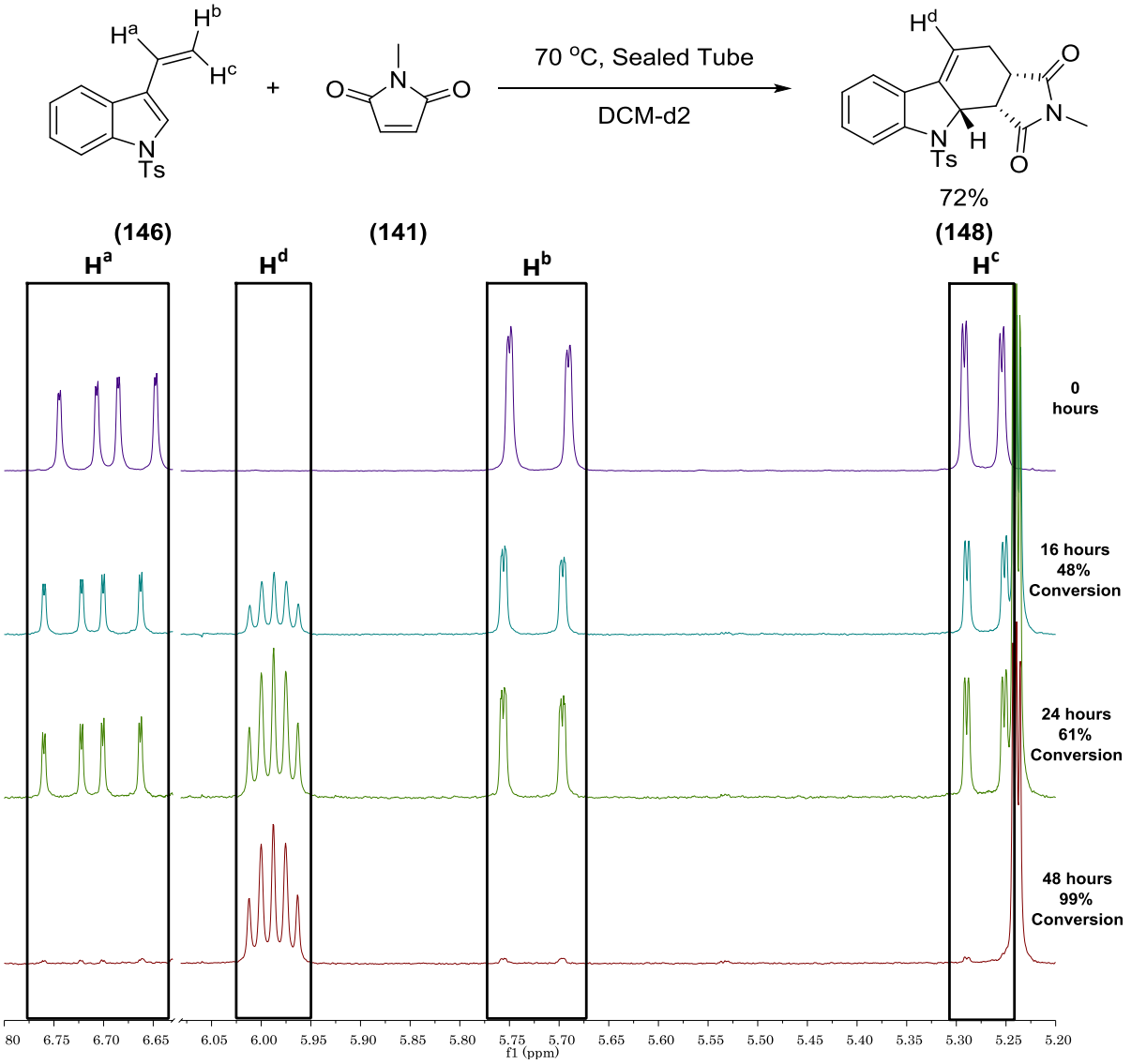
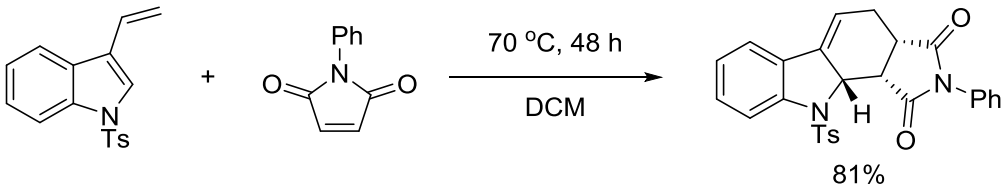


Figure 2.3 - ^{1}H NMR of Diels-Alder reaction performed at 70 ^{o}C in DCM-d_2 at 0, 16, 24 and 48 hours.

The best conditions in terms of reaction time were obtained when the Diels-Alder reaction was performed in a sealed tube, in DCM at 70 ^{o}C. This lead to a substantial reduction in the reaction time, from 96 hours at room temperature, to 48 hours using the new conditions.

2.2.5 N-phenylmaleimide Diels-Alder chemistry-

After optimising the Diels-Alder reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and NMM (**141**), the next step was to investigate the scope of the Diels-Alder reaction with further dienophiles. The Diels-Alder reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and NPM (**117**) was performed using identical conditions to the previous Diels-Alder reaction with NMM (**141**) (Scheme 2.7).

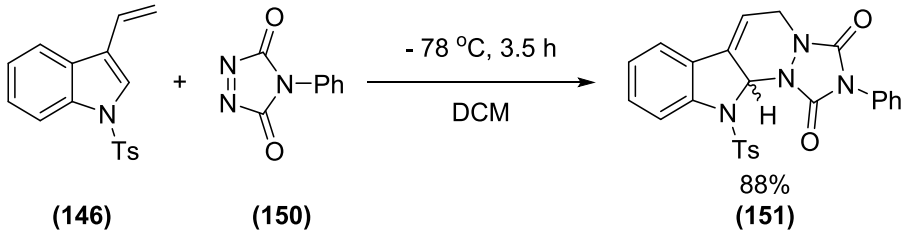


Scheme 2.7 - Diels-Alder reaction between 1-tosyl-3-vinyl-1H-indole (146) and NPM (117).

The Diels-Alder reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and NPM (**117**) gave Diels-Alder adduct (**149**) in a good yield of 81%.

2.2.6 PTAD Diels-Alder chemistry-

4-Phenyl-1,2,4-triazole-3,5-dione (PTAD) (**150**) is a potent dienophile and is known to readily undergo Diels-Alder reactions with indole based compounds.[57-59] Due to the reactivity of PTAD (**150**), the Diels-Alder reaction with 1-tosyl-3-vinyl-1*H*-indole (**146**) was carried out at -78 °C to give desired Diels-Alder adduct (**151**) in an excellent yield of 88% (Scheme 2.8).



Scheme 2.8 - Diels-Alder reaction between 1-tosyl-3-vinyl-1H-indole (146) and PTAD (150).

2.3.0 Relative stereochemistry of Diels-Alder adducts (148), (149) and (151)-

Diels-Alder adducts (**148**) and (**149**), generated from the reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and NMM (**141**) or NPM (**117**), can form two possible diastereomers (Figure 2.4). The Diels-Alder adduct formed is dependent upon the geometry of the transition state. The substituents can arrange themselves in either an *endo* or *exo* transition state which leads to the *endo*-Diels-Alder adduct (**153**) or *exo*-Diels-Alder adduct (**152**) being formed. In our Diels-Alder reactions, only one diastereomer is observed in the ^{1}H NMR.

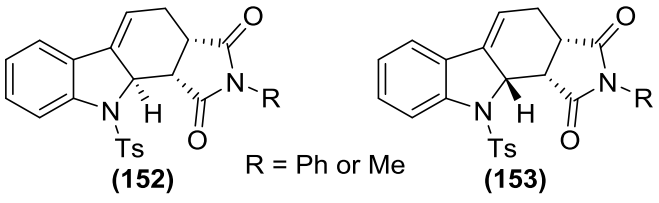
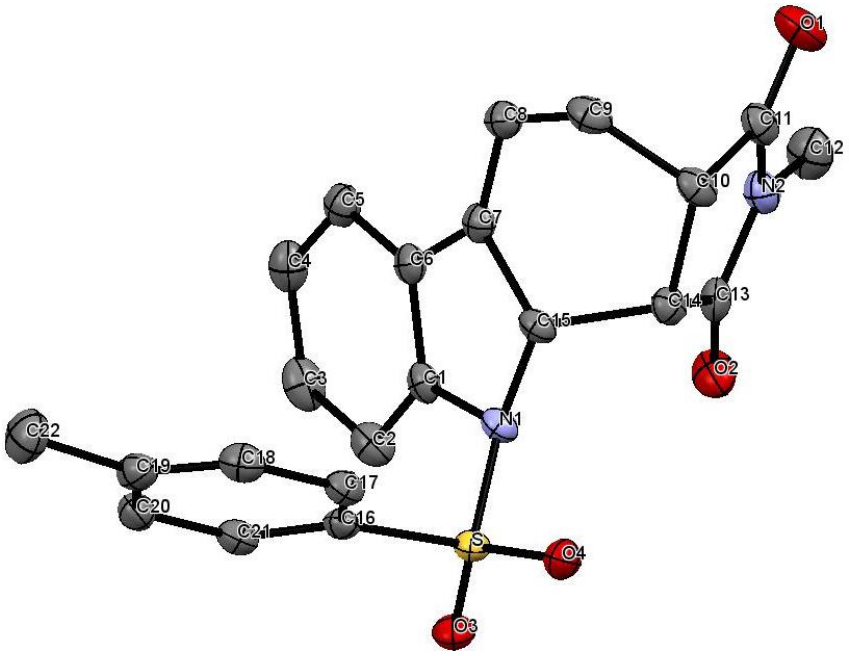
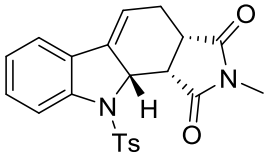


Figure 2.4 - The two possible Diels-Alder products, exo (152) and endo (153).

In order to confirm whether the *endo*- or *exo*- Diels-Alder adduct was formed, crystals were grown of Diels-Alder adduct (**148**) for X-ray crystallography, by slow evaporation from DCM.

The X-ray crystal structure showed the formation of Diels-Alder adduct (**148**) proceeded through an *endo* transition state (Figure 2.5).





(148)

Figure 2.5 - X-ray structure of tosyl protected Diels-Alder adduct.

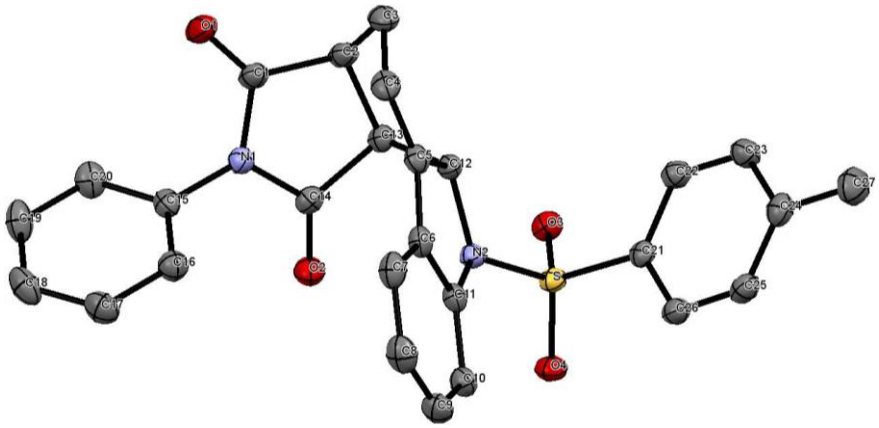
This suggests that the reaction is under kinetic control as the highest energy Diels-Alder adduct (**148**) is formed preferentially and it also demonstrates that the reaction is not reversible. A reversible Diels-Alder reaction would have led to the formation of the more thermodynamically stable *exo*-Diels-Alder adduct.

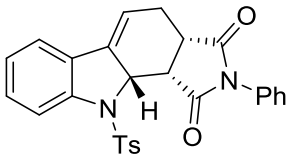
The crystal structure also confirms that Diels-Alder adduct (**148**) is formed in the reaction. The bond length between C(15) and C(7) is 1.510 Å, which is characteristic for a C-C single bond. The bond length between C(7) and C(8) on the other hand is 1.334 Å, which is typical for a C-C double bond. This suggests that the double bond is between the C(7) and C(8) bond and Diels-Alder adduct (**148**) has not rearomatised.

This is supported by the hybridisation of C(15) compared to C(8). The bond angle at C(7)-C(15)-C(14) is 109.10^{o} which suggests that C(15) is an sp^{3} hybridised carbon atom. In comparison, the bond angle at C(7)-C(8)-C(9) is 116.98^{o} which suggests C(8) is an sp^{2} hybridised carbon.

The formation of *endo*-Diels-Alder adduct (**148**) is further supported by the ^{1}H NMR spectrum of (**148**). The coupling constant between H(14) and H(15) is 7.2 Hz, which is typical for coupling between an axial and equatorial proton. If the *exo*-Diels-Alder adduct had been formed then the dihedral angle would be approximately 180^{o}, which would give a coupling constant of around 10 Hz due to axial-axial coupling.

The same relative stereochemistry is observed in the X-ray crystal structure of Diels-Alder adduct (**149**) (Figure 2.6).



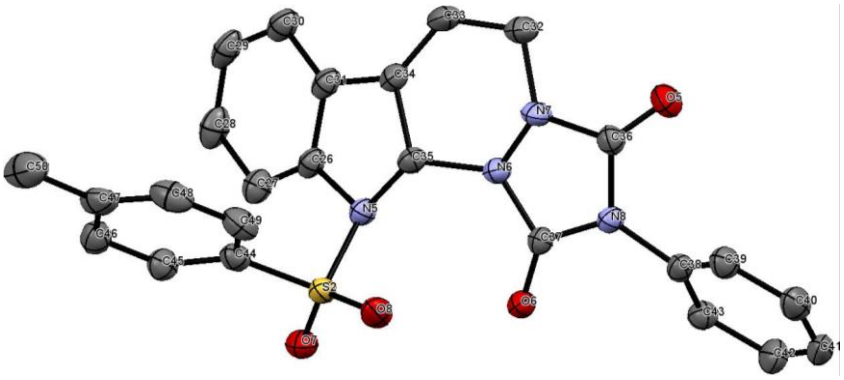


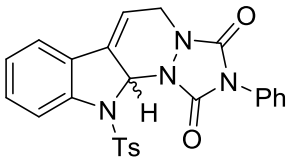
(149)

Figure 2.6 - X-ray structure of Diels-Alder adduct (149).

The bond lengths between C(5)-C(12) is 1.507 Å and C(4)-C(5) is 1.335 Å which are comparable to the equivalent bond lengths seen in Diels-Alder adduct (**148**).

Diels-Alder adduct (**151**) generated from the reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and PTAD (**150**) was crystallised from DCM by slow evaporation. The X-ray structure confirmed that the expected Diels-Alder adduct (**151**) had indeed been formed (Figure 2.7).





(151)

Figure 2.7 - X-ray structure of PTAD Diels-Alder adduct (151).

Although the Diels-Alder reaction proceeds through either an *endo* or *exo* transition state, the nature of the dienophile makes it impossible to assign whether Diels-Alder adduct (**151**) is formed from an *endo* or *exo* transition state.

2.4.0 Carbonyl ene reactions with Diels-Alder adduct (148)-

With an efficient method for the synthesis and purification of Diels-Alder adduct (**148**), the next step was to investigate the ene chemistry of adduct (**148**). Carbonyl compounds have long been used as enophiles in ene reactions and there are many examples in the literature[60-62] demonstrating their readiness to undergo ene reactions. We therefore proposed reacting a range of carbonyl enophiles with Diels-Alder adduct (**148**).

Lewis acids have previously been shown to catalyse carbonyl ene reactions by coordinating to the oxygen of the carbonyl bond (Figure 2.8).[63] The Lewis acid draws electron density

away from the carbonyl bond thus making it more reactive towards nucleophilic attack and therefore ene chemistry.

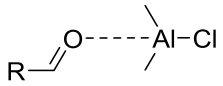
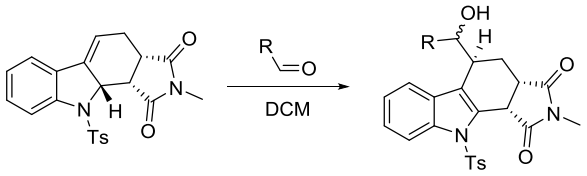
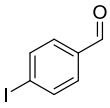
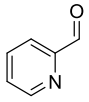
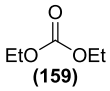
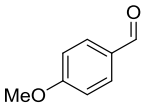
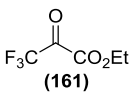


Figure 2.8 - Lewis acid DMAC binding to carbonyl compound.

We proposed reacting Diels-Alder adduct (**148**) with a range of carbonyl enophiles, using dimethylaluminium chloride (DMAC) as the Lewis acid.

Due to the reactive nature of the DMAC, Diels-Alder adduct (**148**) and carbonyl enophiles (**155**) – (**164**) were first dissolved in DCM before the solution was cooled to -78 ^{o}C for the addition of DMAC. The reaction was then allowed to warm to room temperature overnight. The results are summarised in Table 2.2.

Carbonyl Ene Reactions				
 (148) (154)				
Entry	**R-CHO**	**Conditions**	**Product**	**Yield**
1	 (155)	i) Me_2AlCl, -78 ^{o}C, 15 min ii) rt, 5 days	No Reaction	N/A
2	 (156)	i) Me_2AlCl, -78 ^{o}C, 15 min ii) rt, 5 days	No Reaction	N/A
3	 (157)	i) Me_2AlCl, -78 ^{o}C, 15 min ii) rt, 5 days	No Reaction	N/A
4	 (158)	i) Me_2AlCl, -78 ^{o}C, 15 min ii) rt, 5 days	No Reaction	N/A
5	 (159)	i) Me_2AlCl, -78 ^{o}C, 15 min ii) rt, 5 days	No Reaction	N/A
6	 (160)	i) Me_2AlCl, -78 ^{o}C, 15 min ii) rt, 5 days	No Reaction	N/A
7	 (161)	i) Me_2AlCl, -78 ^{o}C, 15 min ii) rt, 5 days	No Reaction	N/A

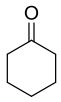
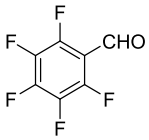
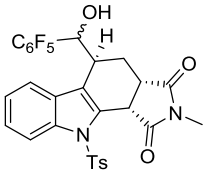
8	 (162)	i) Me_2AlCl, -78 °C, 15 min ii) rt, 5 days	No Reaction	N/A
9	 (163)	i) Me_2AlCl, -78 °C, 15 min ii) rt, 5 days	No Reaction	N/A
10	 (164)	i) Me_2AlCl, -78 °C, 15 min ii) rt, 18 h	 (165)	82%

Table 2.2 - Results of the reaction between Diels-Alder adduct (148) and a range of carbonyl enophiles (155) – (164).

The carbonyl ene reactions with the aldehydes and ketones (Table 2.2, entries 1-9) saw no reaction occur after 5 days of stirring, and Diels-Alder adduct (**148**) was recovered from the reactions.

The reaction between 2,3,4,5,6-pentafluorobenzaldehyde (**164**) and Diels-Alder adduct (**148**) (Table 2.2, entry 10) afforded ene adduct (**165**) as a 6:1 mixture of diastereomers. The crude product was purified by column chromatography to give an excellent yield of 82%, although the mixture of diastereomers could not be separated.

2.5.0 Aza-ene reactions with Diels-Alder adduct 148-

Our investigations had shown that the carbonyl ene reaction required a very electron deficient enophile before the reaction could occur. Therefore we decided to move away from carbonyl compounds and instead look at more reactive enophiles. Previous work in the literature[7,64] has shown that 4-phenyl-1,2,4-triazole-3,5-dione (PTAD) (**150**) is an excellent enophile as a result of the N=N bond being adjacent to two electron withdrawing carbonyl groups. We therefore examined the Diels-Alder reaction between Diels-Alder adduct (**148**) and PTAD (**150**).

Due to the reactivity of PTAD (**150**), the ene reaction between Diels-Alder adduct (**148**) and PTAD (**150**) was carried out at 0 °C and led to the formation of desired ene adduct (**167**) as a single diastereomer in a good yield (Table 2.3).

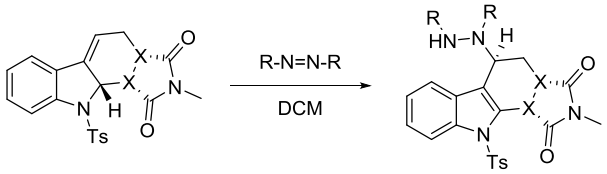
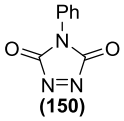
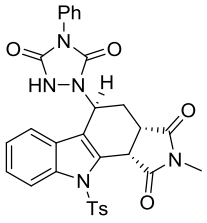
Aza Ene Reactions						
 (148) (166)						
Entry	**X**	**R'**	**R-N=N-R**	**Conditions**	**Product**	**Yield**
1	CH	Me	 (150)	0 °C, 2.5 h	 (167)	73%

Table 2.3 - Ene reaction between Diels-Alder (148) and PTAD (150).

2.6.0 Nitroso-ene reactions with Diels-Alder adduct (148)-

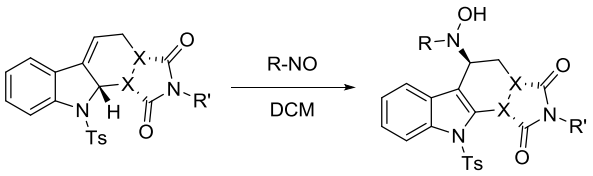
Nitroso compounds are well known in the literature[10] for their ability to undergo ene reactions. Previous work[7] in the MJH group has shown that nitrosobenzene readily undergoes ene reactions with appropriate imidazole compounds. We therefore proposed to examine the nitroso-ene chemistry of Diels-Alder adduct (**148**).

Our initial investigations were carried out by dissolving Diels-Alder adduct (**148**) in DCM at room temperature and adding nitrosobenzene to the solution. This led to the formation of the desired ene adduct (**171**) in a good yield (Table 2.4, Entry 1). The ^{1}H NMR of ene adduct (**171**) was carried out at 30 °C as the spectra obtained at room temperature (21 °C) showed a series of broad peaks instead of well-defined peaks. However upon running the ^{1}H NMR at 30 °C, the broad peaks were resolved into sharp peaks.

With a working method for the ene reaction between Diels-Alder adduct (**148**) and nitrosobenzene (**170**) in hand, the next step was to test the scope of this reaction with other enophiles.

We did this by examining the ene reaction between a range of commercially available nitroso compounds and Diels-Alder adduct (**148**). The results are summarised in Table 2.4, entries 1-5.

Nitroso-ene Reactions



(168) (169)

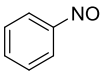
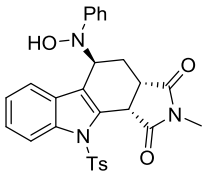
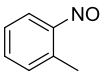
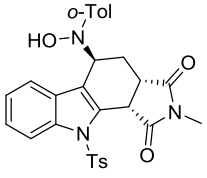
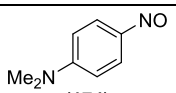
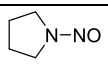
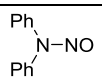
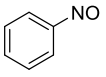
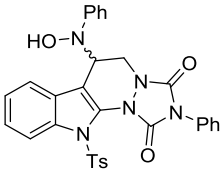
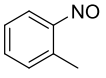
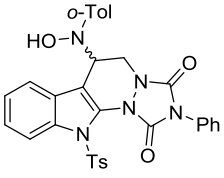
Entry	X	R'	R-NO	Conditions	Product	Yield
1	CH	Me	 (170)	rt, 18 h	 (171)	68%
2	CH	Me	 (172)	rt, 18 h	 (173)	72%
3	CH	Me	 (174)	rt, 7 days	No Reaction	N/A
4	CH	Me	 (175)	rt, 7 days	No Reaction	N/A
5	CH	Me	 (176)	rt, 7 days	No Reaction	N/A
6	N	Ph	 (170)	rt, 18 h	 (177)	74%
7	N	Ph	 (172)	rt, 18 h	 (178)	72%

Table 2.4 – Nitroso-ene reactions with Diels-Alder adduct (148).

The two successful ene reactions between nitrosobenzene (**170**) / 1-methyl-2-nitrosobenzene (**172**) and Diels-Alder adduct (**148**) (Table 2.4, entries 1 and 2) gave the desired ene adducts (**171**) and (**173**) in good yields of 68 and 72%.

There was no reaction between the nitroso compounds with adjacent electron donating groups (Table 2.4, entries 3-5) and Diels-Alder adduct (**148**). The ^{1}H NMR spectra showed no reaction had occurred after seven days at room temperature and Diels-Alder adduct (**148**) starting material could be recovered from the reaction.

The electron donating groups adjacent to the nitroso group can donate electron density into the nitroso group. This raises the energy of the LUMO of the enophile and thus raises the activation energy of the ene reaction (Figure 2.9).

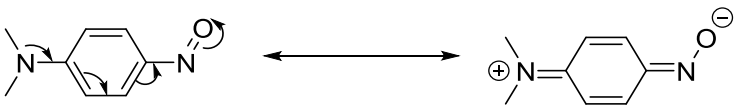
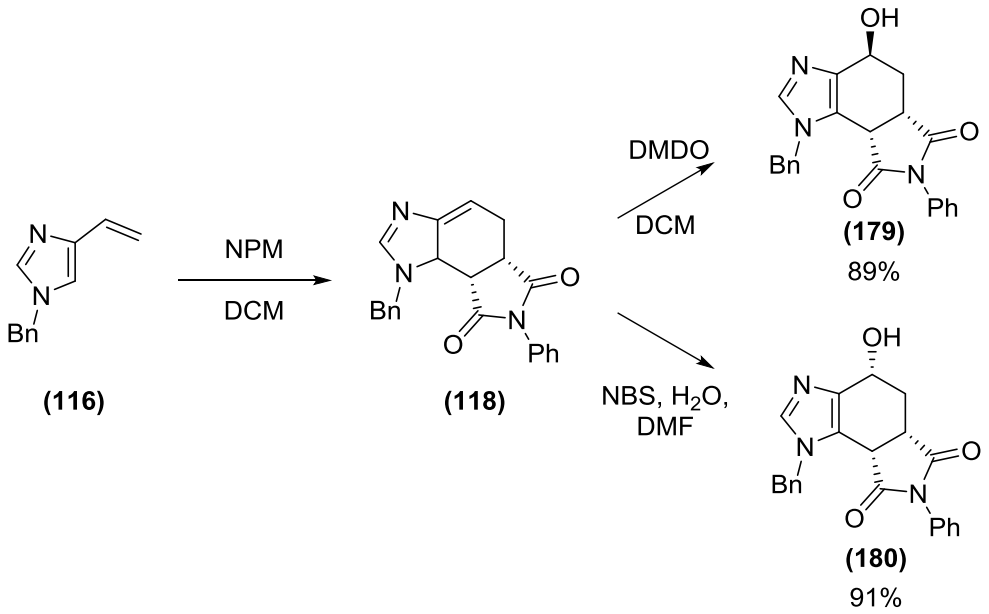


Figure 2.9 - Resonance form of N,N-dimethyl-4-nitrosoaniline (174).

The successful ene reactions were repeated using PTAD Diels-Alder adduct (**151**) and the same reaction conditions (Table 2.4, entries 6-7). The desired ene adducts (**177**) and (**178**) were obtained in excellent yields, comparable to the previous nitroso-ene reactions with Diels-Alder adduct (**148**) (Table 2.4, entries 1-2).

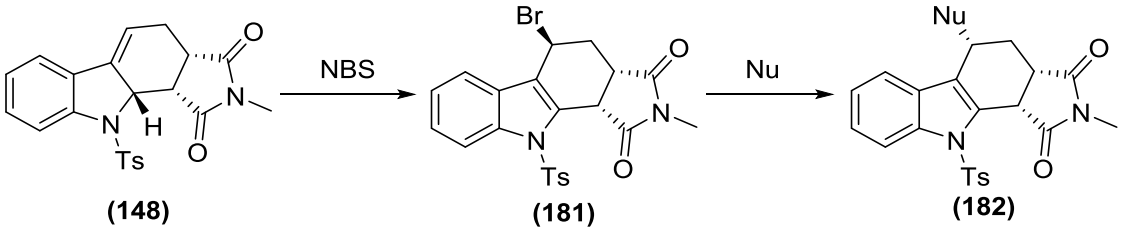
2.7.0 Halogenation reactions-

Having examined a range of different conditions and a variety of enophiles, we next investigated what other chemistry could be performed on the double bond of Diels-Alder adduct (**148**). Previous work by Lovely *et al*.[65] has shown that the double bond formed from a Diels-Alder reaction between vinyl-imidazole (**116**) and NPM (**117**), can react with both dimethyldioxirane (DMDO) and *N*-bromosuccinimide (NBS) (Scheme 2.9).



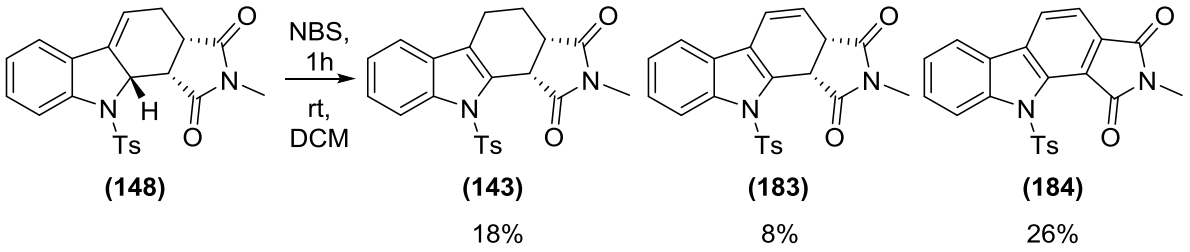
Scheme 2.9 - Lovely's reaction using DMDO or NBS to create alcohols (179) and (180).

We proposed using *N*-bromosuccinimide (NBS) to brominate Diels-Alder adduct (**148**), and then displace the bromine with a nucleophile through a nucleophilic substitution reaction (Scheme 2.10).



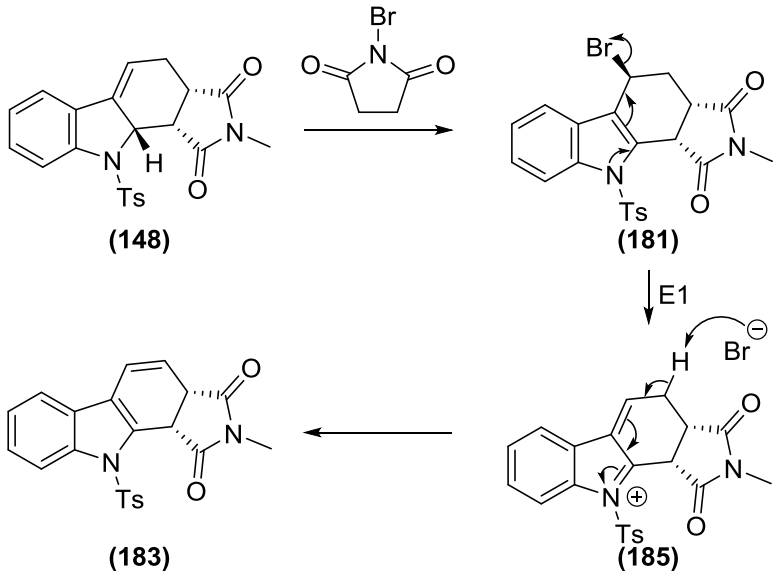
Scheme 2.10 - General scheme for bromination / substitution reactions.

The first step in investigating this route was the bromination of Diels-Alder adduct (**148**) to form compound (**181**). The bromination reaction was initially performed at room temperature using one equivalent of NBS, for one hour. Three major products were isolated from the crude mixture using column chromatography (Scheme 2.11).



Scheme 2.11 - Products formed from the reaction with NBS.

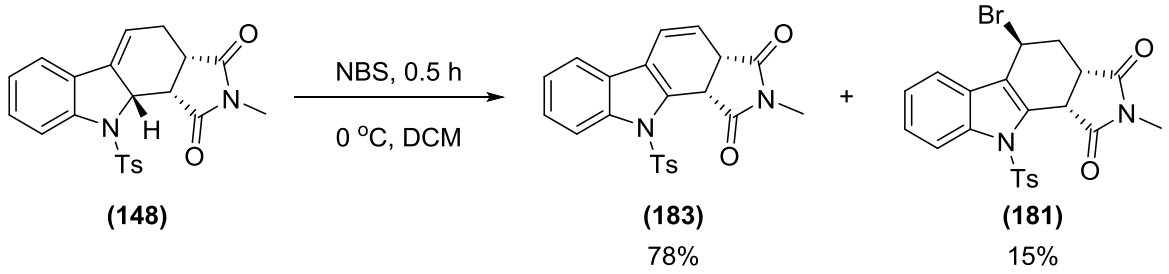
Although the bromination reaction (Scheme 2.11) did not give desired product (**181**), carbazole compounds (**183**) and (**184**) suggested that brominated product (**181**) was being formed as an intermediate. We propose that carbazole (**183**) is generated from the E1 elimination reaction of brominated compound (**181**) (Scheme 2.12).



Scheme 2.12 - Proposed reaction to form carbazole (183) via brominated intermediate (181).

In an attempt to isolate brominated product (**181**), the reaction was repeated but the time was reduced from one hour to thirty minutes and the reaction temperature was dropped

from room temperature to 0 °C (Scheme 2.13). It was hoped that this would allow isolation of brominated product (**181**) before the elimination to carbazole (**183**) could occur.



Scheme 2.13 - Bromination reaction performed at 0 °C for 0.5 hours.

The major product isolated from the reaction was eliminated carbazole product (**183**), in a higher yield than was achieved in the previous reaction. The other major products isolated from the reaction were a mixture of desired brominated compound (**181**) and another unidentified compound with an identical R_f value. The mixture of two compounds could not be separated by column chromatography so instead a recrystallisation by slow evaporation was attempted to determine if one product crystallised preferentially to the other. One product did crystallise preferentially and therefore it was possible to get an X-ray crystal structure (Figure 2.10).

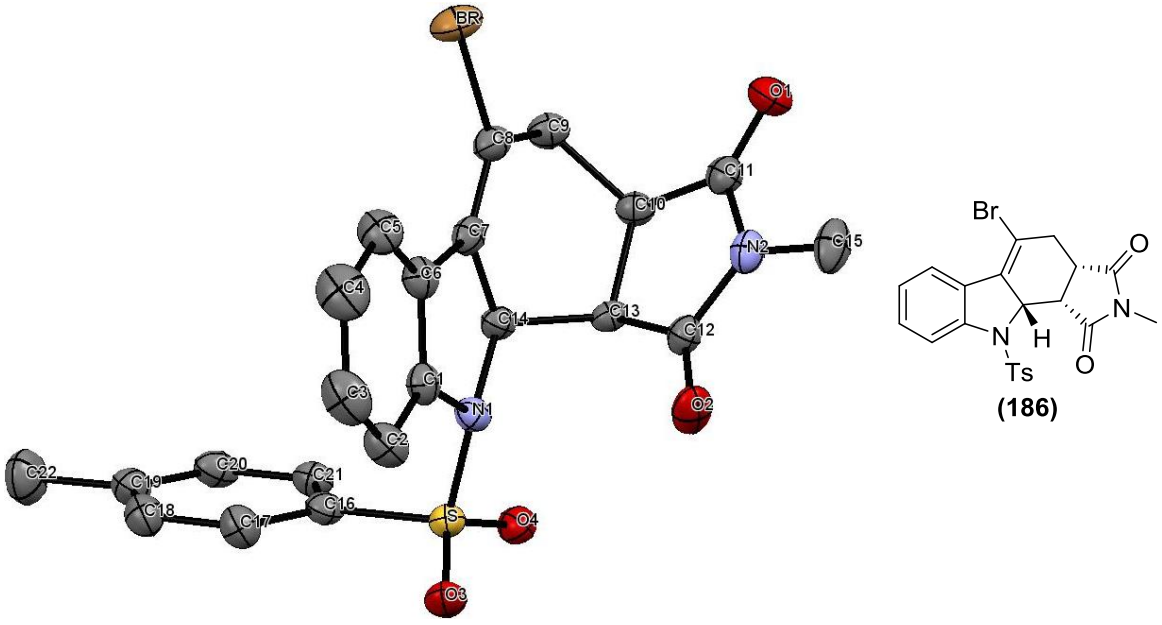
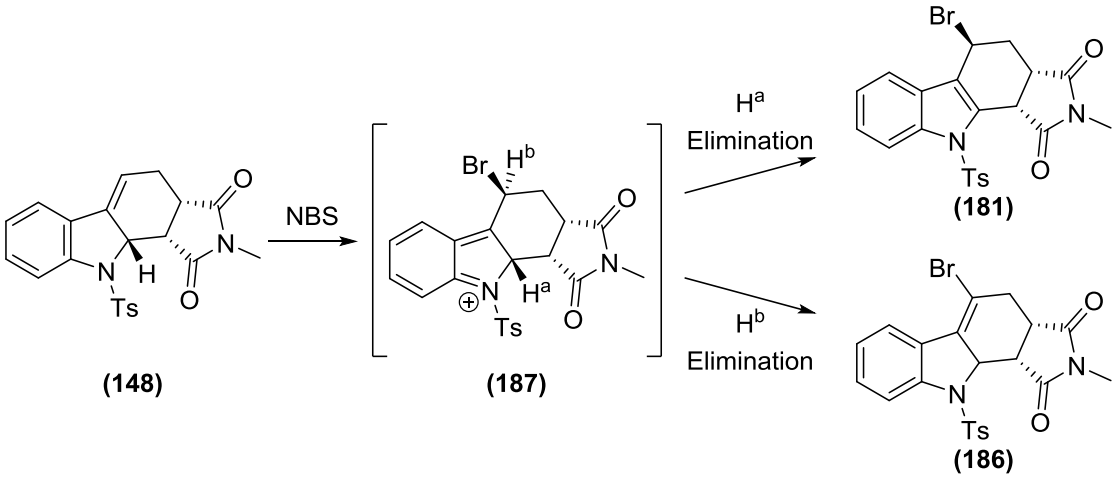


Figure 2.10 - X-ray structure of brominated Diels-Alder product (186).

The X-ray structure obtained was of a new compound (**186**) which we had not previously identified. The X-ray crystal structure indicated that the double bond was between C(7) and C(8) which is backed up by examination of the bond lengths and bond angles. The C(7)-C(8) bond length is 1.328 Å, typical of a C-C double bond, while the C(14)-C(7) bond length is 1.516, typical for a C-C single bond. This is confirmed by examining the hybridisation of C(7)

and C(14). The bond angle between C(7)-C(14)-C(13) is 111.6 ^{o} which suggests C(14) is an sp^3 hybridised carbon. The bond angle between C(7)-C(8)-C(9) is 120.0 ^{o} which would be expected if C(8) is an sp^2 hybridised carbon.

We propose that product (**186**) arises from the final elimination step, after the initial bromination step has occurred (Scheme 2.14). There are two possible hydrogens that can be eliminated (H^a or H^b) and hence a mixture of products is formed. Elimination of H^a leads to the thermodynamically favourable rearomatised indole ring (**181**), while elimination of H^b leads to the formation of minor product (**186**), which crystallises preferentially.



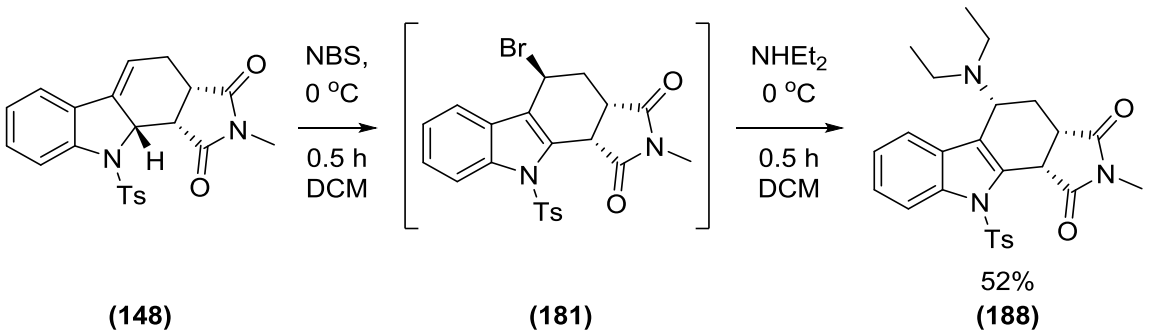
Scheme 2.14 - Products formed in the bromination reaction.

Although brominated compound (**186**) crystallised preferentially to compound (**181**), it was still not possible to get a pure sample of either brominated products (**181**) or (**186**). This was due to the brominated products (**181**) and (**186**) rapidly decomposing in solution to elimination product (**183**) (Scheme 2.12).

2.7.1 One-pot bromination / substitution-

Although brominated product (**181**) could not be isolated, it could be formed *in situ*. Therefore another approach had to be developed for the bromination / substitution reaction. A one-pot bromination / substitution reaction was attempted as this negated the need to isolate brominated product (**181**) (Scheme 2.15).

It was reasoned that substituted product (**188**) would be more stable towards isolation. Diethylamine was chosen as a simple nucleophile to be used in the reaction.



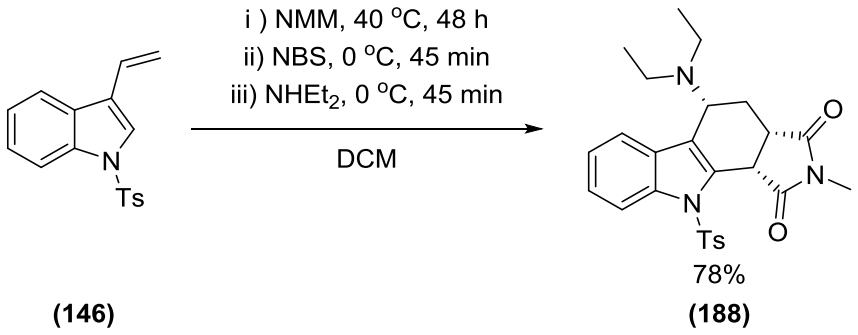
Scheme 2.15 - One-pot bromination / substitution.

The one-pot bromination / substitution reaction led to the isolation of diethylamine substituted product (**188**) in a moderate yield of 52%, along with 16% of unreacted Diels-Alder adduct (**148**). The isolation of 16% of Diels-Alder adduct (**148**) suggested that the initial bromination reaction was not going to completion before addition of diethylamine. It was therefore reasoned that the reaction time should be increased to allow the Diels-Alder adduct (**148**) to react fully.

2.7.2 One-pot Diels-Alder / bromination / substitution-

As the Diels-Alder reaction to form Diels-Alder adduct (**148**) had already been optimised, it allowed a total one-pot Diels-Alder / bromination / substitution reaction to be attempted.

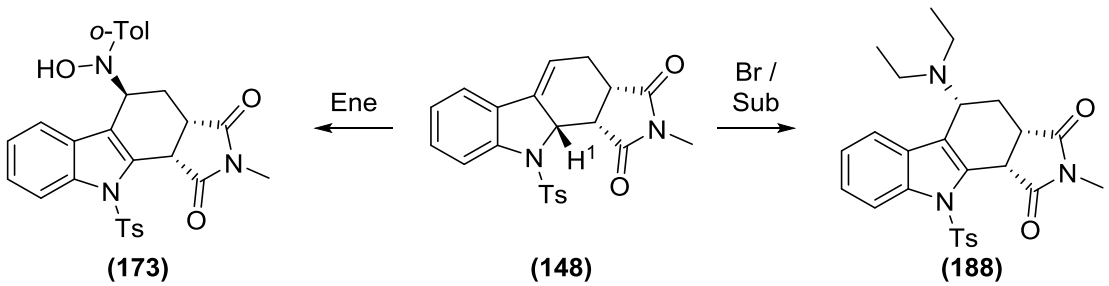
Starting from 1-tosyl-3-vinyl-1*H*-indole (**146**), the Diels-Alder reaction with NMM (**141**) was carried out as before to give Diels-Alder adduct (**148**). The reaction mixture was then cooled to 0 °C for the bromination reaction, before diethylamine was added to form compound (**188**) (Scheme 2.16).



Scheme 2.16 - One-pot Diels-Alder / bromination / substitution.

Column chromatography led to the isolation of compound (**188**) in an excellent yield of 78% (Scheme 2.16).

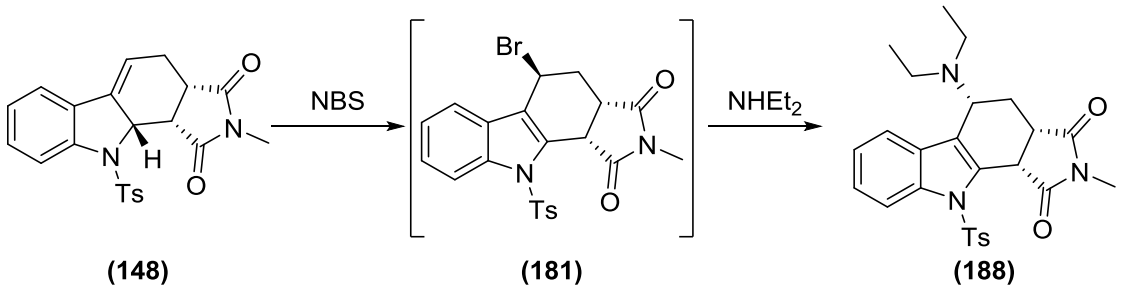
We propose the relative stereochemistry of the final substituted product (**188**) is different to that of the ene products formed previously (Chapter 2.6.0) (Scheme 2.17).



Scheme 2.17 – Comparison of the relative stereochemistry of ene product (173) and substituted product (188).

The ene reaction proceeds through a concerted mechanism whereby H^1, adjacent to the indolic nitrogen, is abstracted in concurrence with formation of the new C-N bond. This ensures that the nitroso group is added to the same face as H^1. The Diels-Alder reaction proceeds via an *endo*-transition state, which results in the succinimide group of Diels-Alder adduct (**148**) being on the opposite face to H^1. Therefore the nitroso group and the succinimide group are on opposite faces in ene adduct (**173**).

We propose that the bromination / substitution reaction results in the diethylamine group occupying the same face of compound (**188**) as the succinimide group (Scheme 2.18).



Scheme 2.18 - Proposed stereochemistry of diethylamine substituted product (188).

The succinimide group sterically blocks one face of Diels-Alder product (**148**), forcing bromine to add to the opposite face, which is less sterically hindered. This leads to the formation of compound (**181**). Substitution of bromine by diethylamine occurs via an S_N2 mechanism, leading to an inversion of stereochemistry and product (**188**) being formed.

This theory is supported by comparison of the *J*-coupling between axial (H^a) and equatorial (H^e) protons, in the ^{1}H NMR spectrum of substituted compound (**188**) and ene compound (**173**).

Firstly the conformation of the six-membered cyclohexene ring on which the hydroxylamine group sits must be considered. The cyclohexene adopts a half chair conformation, which is the lowest possible energy conformation for the compound. This leads to the six-membered ring shown in Figure 2.11, with H^1 sitting equal distance between H^a and H^e.

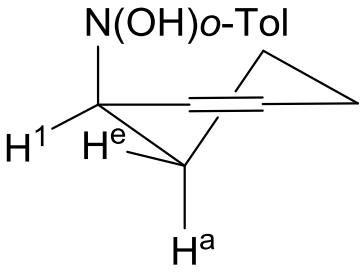


Figure 2.11 – Conformatio adopted by the six-membered cyclohexene ring in (173).

In the ^{1}H NMR of ene adduct (**173**), the signal corresponding to proton H^{1} is split into an apparent triplet (Figure 2.12). This can only occur if proton H^{1} is coupled to the adjacent axial proton H^{a} and equatorial proton H^{e}, with the same or very similar coupling constants.

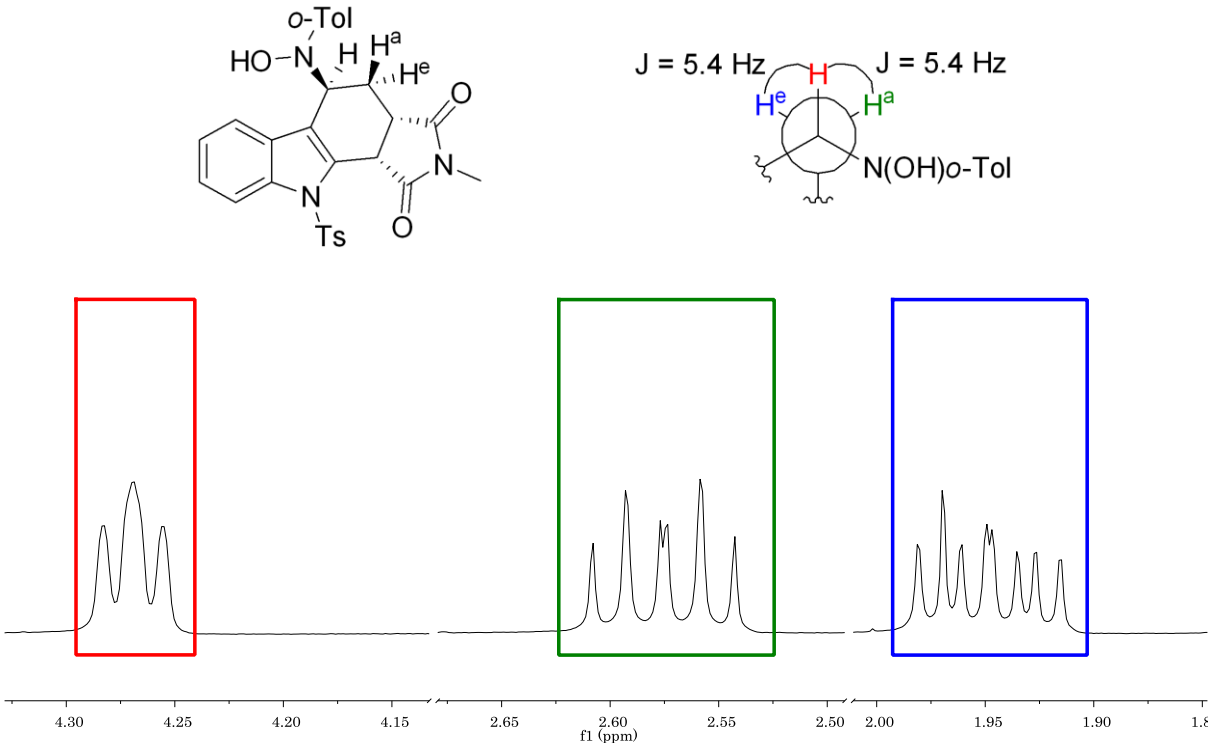


Figure 2.12 - ^{1}H NMR of ene adduct (173).

In the ^{1}H NMR spectrum of substituted product (**188**), the coupling constant between protons H^{1} and H^{e} is 4.5 Hz, which indicates an equatorial-axial coupling, whereas the coupling constant between protons H^{1} and H^{a} is 9.2 Hz. The size of this coupling is typical for an axial-axial coupling. This suggests the dihedral angle between protons H^{1} and H^{a} is approximately 180 ^{o} and the dihedral angle between protons H^{1} and H^{e} is around 60 ^{o}. This indicates that compound (**188**) has the structure shown below (Figure 2.13).

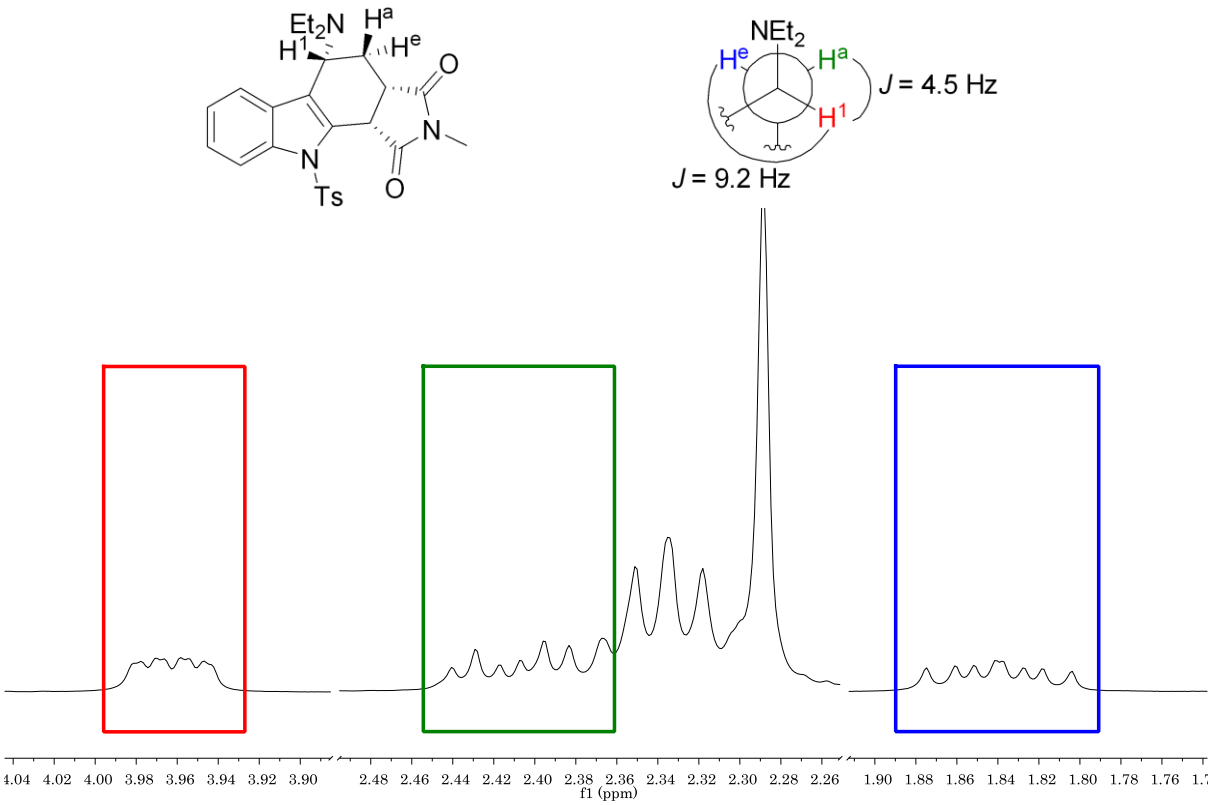


Figure 2.13 - ^{1}H NMR of substituted product (188).

2.8.0 Conclusions-

The main aim of this chapter was to synthesise *N*-protected 3-vinyl-1*H*-indole compounds and examine their subsequent reaction in Diels-Alder / ene reactions with a range of dienophiles and enophiles. We have shown that 1-tosyl-3-vinyl-1*H*-indole (**146**) can be synthesised in a quick and high yielding reaction sequence.

The same reaction sequence to form *tert*-butyl 3-vinyl-1*H*-indole-1-carboxylate (**147**) led to a mixture of 3-vinyl-1*H*-indole (**140**) and *tert*-butyl 3-vinyl-1*H*-indole-1-carboxylate (**147**) in a poor yield of 13%. We propose the mixture of products formed from the Wittig reaction is a result of the instability of *tert*-butyl 3-vinyl-1*H*-indole-1-carboxylate (**147**) to Wittig reaction conditions.

The synthesised 1-tosyl-3-vinyl-1*H*-indole (**146**) readily undergoes Diels-Alder reactions with a range of dienophiles including NMM (**141**), NPM (**117**) and PTAD (**150**). The single diastereomer Diels-Alder adducts formed from these reactions readily crystallise and allow the relative stereochemistry of the Diels-Alder adducts to be determined. The Diels-Alder reactions proceed through an *endo*-transition state which indicates that the Diels-Alder reactions are under kinetic control and are not reversible.

Screening Diels-Alder adducts (**148**) and (**151**) has shown they readily undergo ene reactions with reactive nitroso compounds, such as nitrosobenzene (**170**) and 1-methyl-2-nitrosobenzene (**172**). However the ene reaction is very dependent upon the structure of

the nitroso compound; electron donating groups adjacent to the nitroso group prevent the ene reaction from occurring. We believe this is due to the electron donating groups raising the energy of the LUMO of the enophile, which raises the activation energy of the ene reaction, preventing any reaction from occurring.

Further ene reactions with carbonyl compounds demonstrated that an ene reaction would only occur with the addition of a Lewis acid to catalyse the reaction. However even with the Lewis acid, only the very electron deficient 2,3,4,5,6-pentafluorobenzaldehdye (**164**) would react with Diels-Alder adduct (**148**).

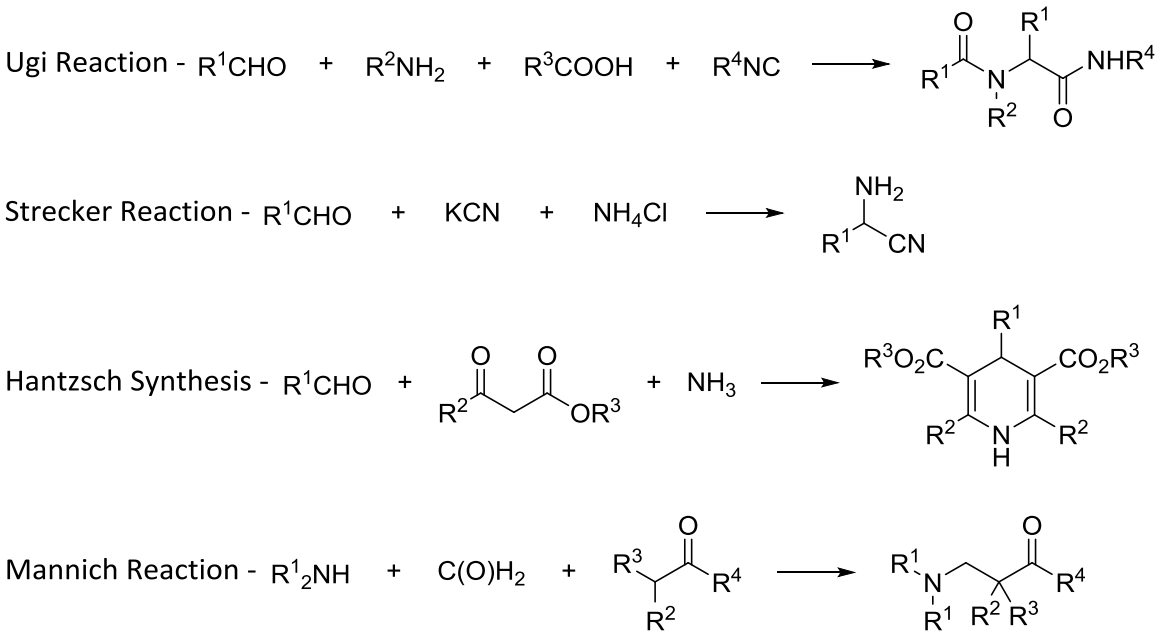
Attempts to isolate the product formed from the reaction between NBS and Diels-Alder adduct (**148**) led to the isolation of E1 elimination products (**183**) and (**184**). Although the bromination reaction led to the formation of (**181**), (**181**) rapidly underwent elimination reactions before isolation of brominated compound (**181**) could be achieved. This was in part due to the ability of the lone pair of electrons on the indolic nitrogen to eliminate the bromine via an E1 reaction mechanism.

Diels-Alder adduct (**148**) could be subjected to a one-pot bromination / substitution reaction with NBS and diethylamine to give substituted indole compound (**188**), in a moderate yield of 52%. However the yield could be greatly improved through a one-pot Diels-Alder / bromination / substitution reaction to give the same substituted Diels-Alder adduct in an overall yield of 78%.

Chapter 3 – One-pot Diels-Alder / Ene Chemistry of Vinyl-indoles

3.1.0 One-pot multi-component reactions-

One-pot multi-component reactions (MCR) are a popular tool in organic chemistry as a means of improving the efficiency of a chemical process, by having several sequential reactions occur in one reaction vessel without the need for isolation of intermediates. This allows a reduction of waste as it negates the need for any isolation / purification steps. Examples of one-pot multi-component reactions which have been widely utilised in organic synthesis include the Strecker[66], Hantzsch[67], Mannich[68] and Ugi[69] reactions (Scheme 3.1).



Scheme 3.1 – Example of Ugi, Strecker, Hantzsch and Mannich MCR.

The reactions shown above (Scheme 3.1) are described under the all-encompassing term as one-pot reactions, whereby multiple chemical processes occur in one lab process. In order to more specifically describe a one-pot reaction process, the terms domino, tandem, cascade and sequential are all widely used. Several groups have tried to define the range of terminology used in one-pot MCR chemistry:

One-pot – Any reaction where multiple chemical processes are occurring in the same reaction vessel.

Multi-component reaction – A reaction where three or more reagents react to form a single product and most of the atoms from the starting material contribute to the product.

MCRs can then be further referred to as domino, tandem, cascade or sequential:

Domino – Tietze has defined a domino reaction as "*a process involving two or more bond-forming transformations (usually C-C bonds) which take place under the same reaction*

conditions without adding additional reagents and catalysts, and in which the subsequent reactions result as a consequence of the functionality formed in the previous step".[70]

Tandem – Denmark and Thorarensen define a tandem reaction as any reactions which occur one after the other and then qualifies this term with either cascade or sequential to describe how the reactions occur.[71]

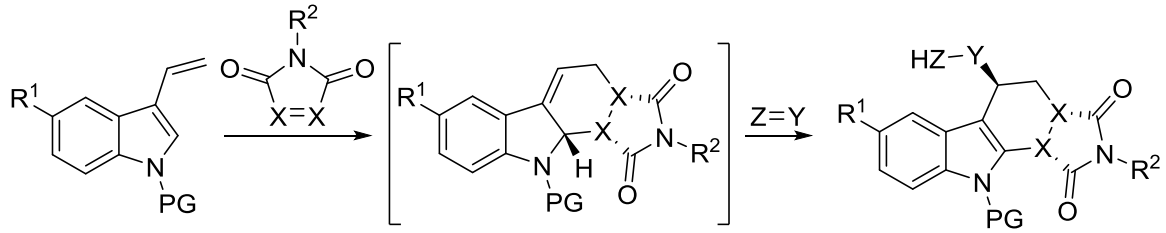
Cascade – A cascade reaction is one in which the next reaction can only occur due to the structural change brought on by the previous step.[71]

Sequential – The sequential reaction is defined as one which requires the addition of a reagent in the second stage of the reaction.[71]

One-pot MCRs offer several advantages over traditional linear synthetic routes including maximising atom economy, reducing purification steps (and therefore waste), and the opportunity for rapid complexity generation.[72,73] In the previous chapter we demonstrated the synthesis of tetrahydracarbazoles in a Diels-Alder / ene reaction sequence. We believed that this reaction sequence could be improved through the use of a one-pot MCR sequence.

3.1.1 Aims-

The Diels-Alder / ene reactions with *N*-protected vinyl-indole compounds in the previous chapter gave the desired tetrahydrocarbazole products in reasonable overall yields (50-60% over two steps). We wanted to develop this Diels-Alder / ene chemistry into a one-pot reaction process, whereby Diels-Alder adduct (**190**) would not be isolated (Scheme 3.2). We believed this would lead to better yields of ene adduct (**191**), reducing waste and making the process operationally simple.

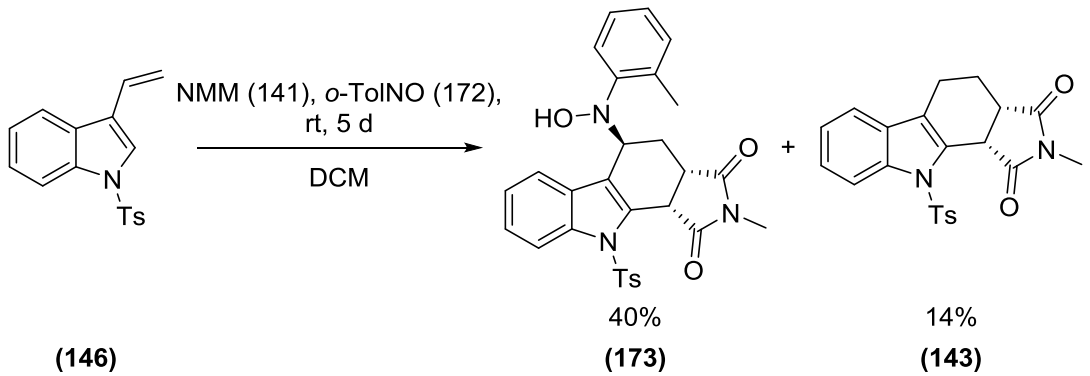


(189) **(190)** **(191)**

Scheme 3.2 - Proposed one-pot Diels-Alder / ene reaction sequence.

3.2.0 Domino One-pot Diels-Alder / ene reaction-

First we looked at domino Diels-Alder / ene conditions, as this would be practically the most simple one-pot MCR. 1-Tosyl-3-vinyl-1*H*-indole (**146**), NMM (**141**) and 1-methyl-2-nitrosobenzene (**172**) were all added to the reaction vessel at the beginning of the reaction and stirred for five days (Scheme 3.3).



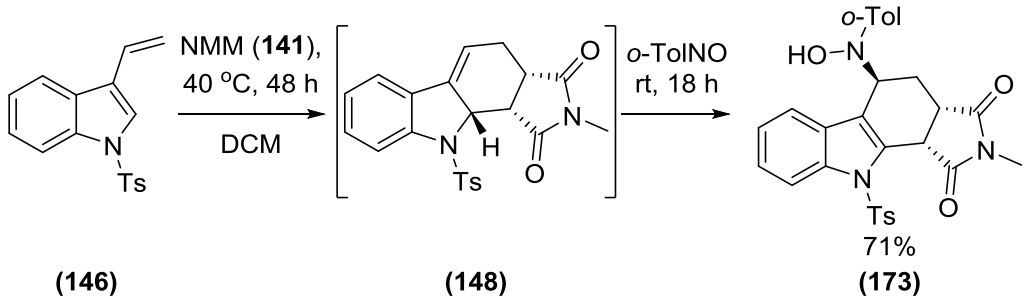
Scheme 3.3 - "Domino" Diels-Alder / ene reaction.

After five days, no 1-tosyl-3-vinyl-1*H*-indole (**146**) could be observed by TLC. The crude ^{1}H NMR showed that several products had been formed. The two products isolated were rearomatised Diels-Alder adduct (**143**) and desired ene product (**173**). Although the desired ene adduct (**173**) was obtained from the reaction, it was only isolated in a moderate yield of 40% and there were several unidentified side products from the reaction.

Therefore to try and improve the yield of the reaction, a sequential one-pot process was attempted to avoid the formation of unwanted side products.

3.3.0 Sequential One-pot NMM Diels-Alder / ene-

The Diels-Alder reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and NMM (**141**) was repeated, followed by addition of 1-methyl-2-nitrosobenzene (**172**) to the reaction mixture with no purification, isolation or solvent swaps (Scheme 3.4).



Scheme 3.4 - One-pot Diels-Alder / ene reaction.

Diels-Alder/ ene adduct (**173**) was isolated in a 71% yield as a single diastereomer.

Due to the success of the sequential one-pot reaction to form ene adduct (**173**), we expanded the range of enophiles used to test the scope of the reaction (Table 3.1). Two aryl-nitroso compounds (**170**) and (**172**), PTAD (**150**) and a carbonyl compound (**164**) were utilised as enophiles in the reaction as they had proven to be good enophiles previously (Chapter 2). This allowed a direct comparison to be made between the yields of the two-step Diels-Alder / ene and the sequential one-pot MCR Diels-Alder / ene reactions.

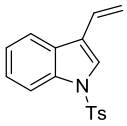
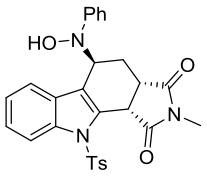
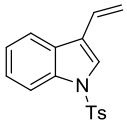
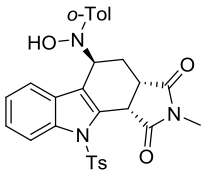
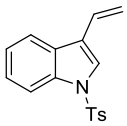
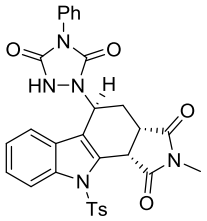
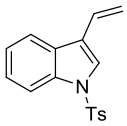
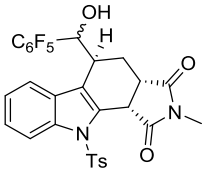
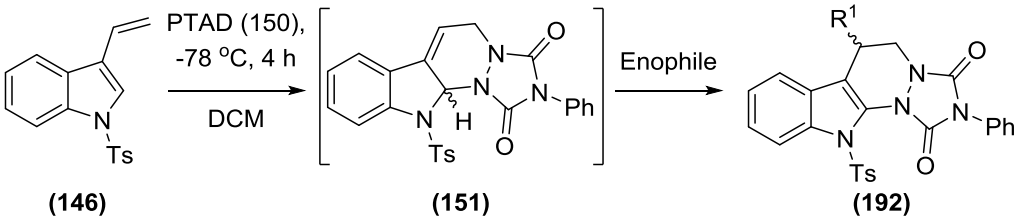
Starting Material	Diels-Alder Conditions	Ene Conditions	Ene Adduct	MCR Yield
 (146)	NMM (**141**), 40 °C, 48 h	PhNO (**170**), rt, 18 h	 (171)	71%
 (146)	NMM (**141**), 40 °C, 48 h	*o*-TolNO (**172**), rt, 18 h	 (173)	71%
 (146)	NMM (**141**), 40 °C, 48 h	PTAD (**150**), 0 °C, 4 h	 (167)	76%
 (146)	NMM (**141**), 40 °C, 48 h	C_6F_5CHO (**164**), DMAC, -78 °C to rt, 18 h	 (165)	72%

Table 3.1 - Sequential one-pot Diels-Alder / ene reaction conditions and yields.

Nitroso and aza ene products (**171**), (**173**) and (**167**) were isolated as single diastereomers in excellent yields ranging from 71-76%. Diels-Alder/ ene adduct (**165**) was isolated as a 6: 1 mixture of diastereomers. The MCR process led to a reduction in waste and an increase in the overall % yield of the ene adducts when compared to the two-step approach seen in Chapter 2.

3.3.1 One-pot Diels-Alder / ene (PTAD / nitroso-ene)-

In Chapter 2.2.6 we demonstrated that the Diels-Alder reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and PTAD (**150**) led to Diels-Alder adduct (**151**) in a good yield. Diels-Alder adduct (**151**) could then be reacted in an ene reaction with nitrosobenze (**170**) and 1-methyl-2-nitrosobenzene (**172**), leading to the formation of ene adducts (**177**) and (**178**). We wanted to see if these reactions could be improved through a one-pot MCR process. Therefore the Diels-Alder / ene reactions from 1-tosyl-3-vinyl-1*H*-indole (**146**) using PTAD (**150**) as the dienophile and nitroso compounds (**170**) and (**172**) as the enophile, were repeated using our one-pot MCR conditions (Table 3.2).



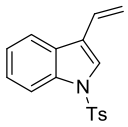
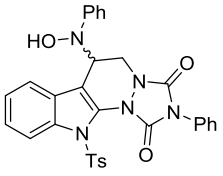
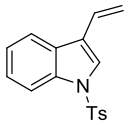
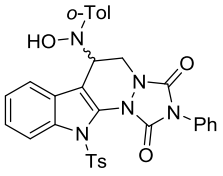
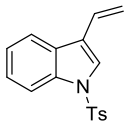
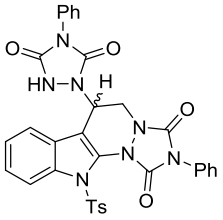
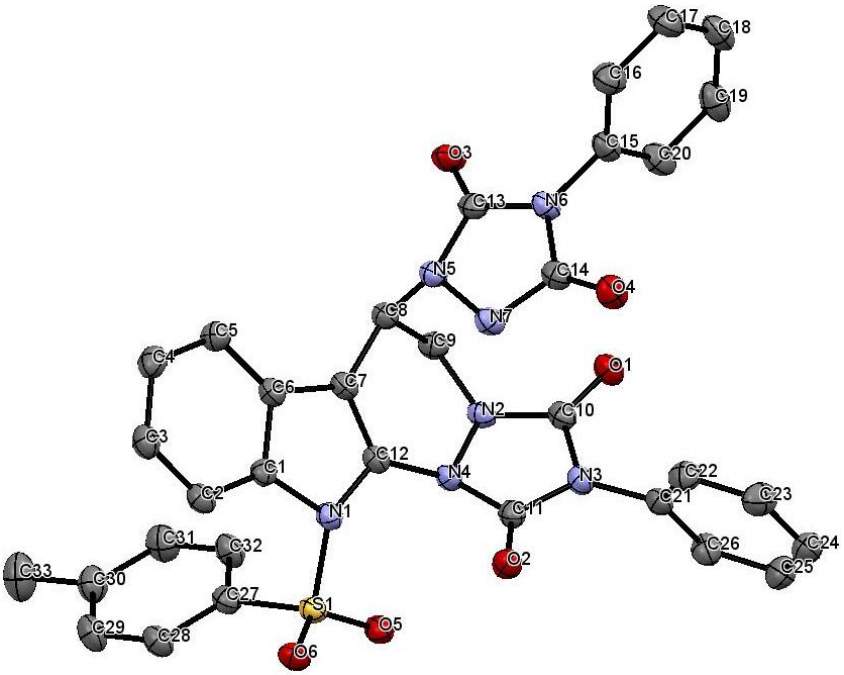
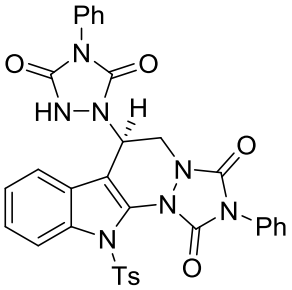
Starting Material	Diels-Alder Conditions	Ene Conditions	Ene Adduct	MCR Yield
 (146)	PTAD (**150**), -78 °C, 4 h	PhNO (**170**), rt, 18 h	 (177)	66%
 (146)	PTAD (**150**), -78 °C, 4 h	*o*-TolNO (**172**), rt, 18 h	 (178)	66%
 (146)	PTAD (**150**), -78 °C, 4 h	PTAD (**150**), 0 °C, 4 h	 (193)	65%

Table 3.2 - Sequential one-pot Diels-Alder / ene reaction conditions and yields.

The one-pot MCR led to the formation of desired nitroso ene adducts (**177**) and (**178**) in good yields of 66%. As the reaction using PTAD as both the dienophile and enophile progressed, a precipitate formed, suggesting ene adduct (**193**) was only partially soluble. This allowed ene adduct (**193**) to be purified by trituration leading to a reduction in waste as no column chromatography had to be performed.

Confirming the structure of ene adduct (**193**) was difficult to do with ^{1}H NMR analysis, as PTAD does not contain any protons in the five-membered ring. Therefore an X-ray crystal of ene adduct (**193**) was grown by slow evaporation from DCM, as a way of confirming the structure (Figure 3.1).





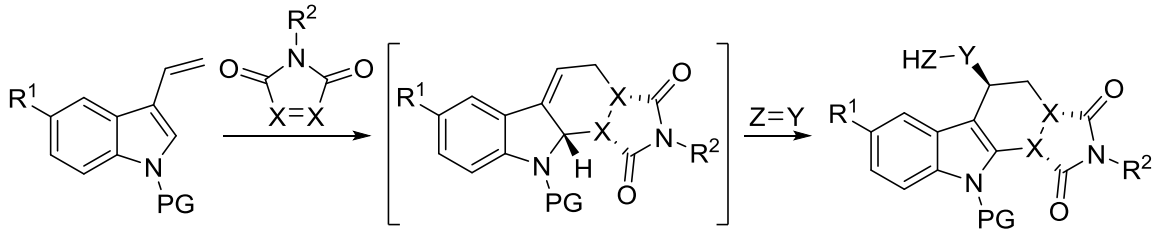
(193)

Figure 3.1 - X-ray structure of ene adduct (193).

The X-ray crystal structure showed that Diels-Alder / ene adduct (**193**), crystallised in the monoclinic space group $P2_1/c$, with four molecules in the unit cell. Due to Diels-Alder product (**151**) being racemic, the ene reaction leads to a racemic mixture of (*R*)-(**193**) and (*S*)-(**193**) being formed. The unit cell contains two molecules of (*R*)-(**193**) and two molecules of (*S*)-(**193**). The X-ray crystal structure shows that there is a H-bonding interaction between N^7H on one molecule and O^1 on the adjacent molecule.

3.4.0 Maleimide Diels-Alder chemistry-

With our sequential one-pot MCR operating successfully, we turned our attention to applying this technique to synthesising biologically active targets. The Diels-Alder / ene sequence we have developed leads to the formation of tetrahydrocarbazole compounds (**191**) as single diastereomers (Scheme 3.5).



PG = Ts; R^1 = H, OMe; X = C, N; R^2 = Me, Ph; X=Y = N(OH)Ph, N(OH)o-Tol, $C(OH)C_6F_5$, PTAD

(189) **(190)** **(191)**

Scheme 3.5 - One-pot MCR Diels-Alder / ene reaction sequence with N-protected vinyl-indoles.

The tetrahydrocarbazole compounds (**191**) synthesised from the Diels-Alder / ene reaction (Scheme 3.5) have many structural similarities to biologically active cabazole and

tetrahydrocarbazole compounds (**194**), (**195**) and (**196**) described in the literature (Figure 3.2).[33,35,74]

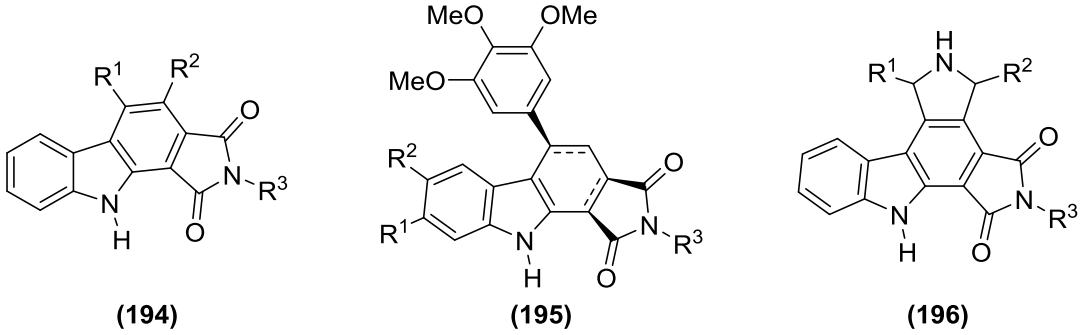


Figure 3.2 - Range of biologically active indole containing compounds from the literature.

We therefore reasoned that our one-pot MCR Diels-Alder / ene sequence could allow the rapid synthesis of a library of potentially biologically active compounds.

Ty *et al*.[35] have undertaken a structural activity relationship (SAR) study on compounds of class (**195**) (Figure 3.3). The compounds in the SAR study were judged for their cytotoxicity against B16 melanoma cells and inhibition of tubulin polymerisation (ITP).

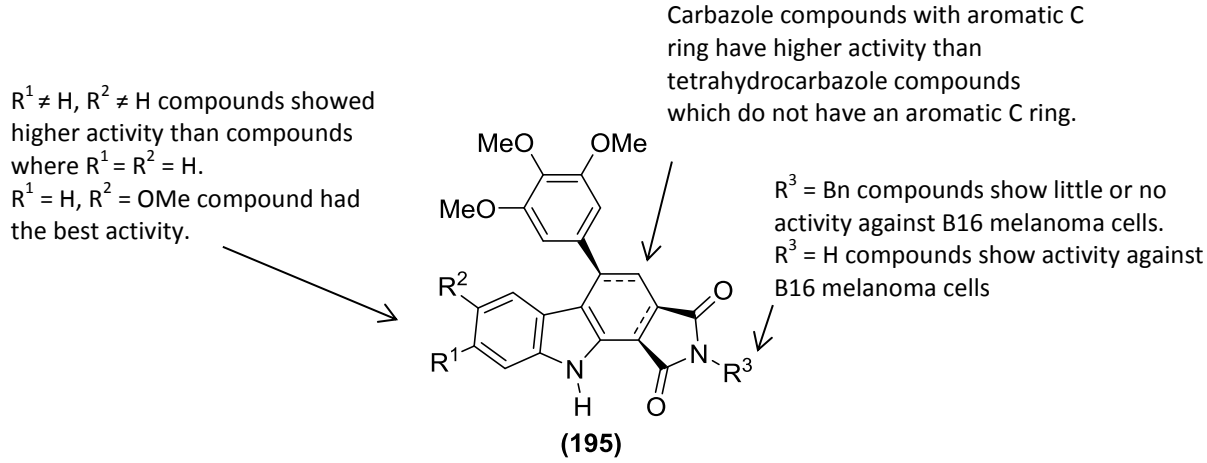
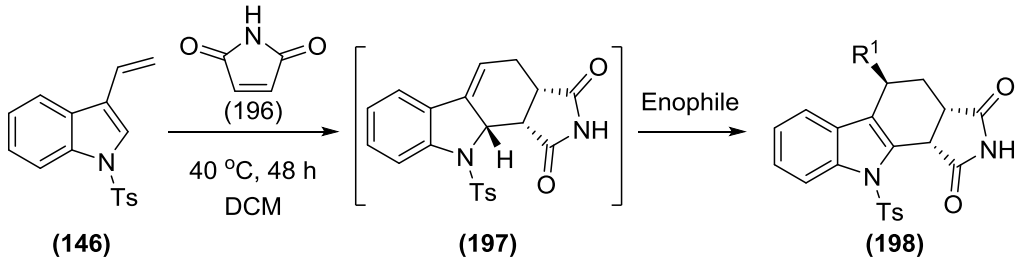


Figure 3.3 - SAR study of compounds of the class (195).

Tetrahydrocarbazole compounds from our one-pot Diels-Alder / ene reactions (**191**) have either a methyl or phenyl group on the succinimidyl nitrogen. Therefore to synthesise compounds with a free NH on the succinimide moiety, which would more closely match the structure Ty *et al*. proposed in their SAR study, a series of one-pot MCRs were attempted with maleimide (**196**) employed as the dienophile (Scheme 3.6).



Scheme 3.6 - One-pot maleimide Diels-Alder/ ene reactions between 1-tosyl-3-vinyl-1H-indole (146) and maleimide (196).

The Diels-Alder reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and maleimide (**196**) proceeded in the same time as the reaction between 1-tosyl-3-vinyl-1*H*-indole (**146**) and NMM (**141**). To allow comparison with the previous MCR one-pot Diels-Alder/ ene reactions (Chapter 2), nitrosobenzene (**170**), 1-methyl-2-nitrosobenzene (**172**), PTAD (**150**) and pentafluorobenzaldehyde (**164**) were employed as enophiles (Table 3.3).

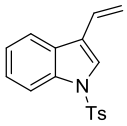
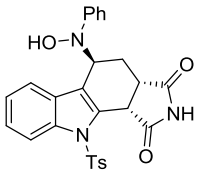
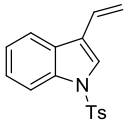
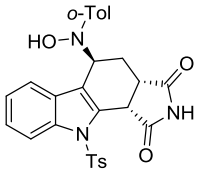
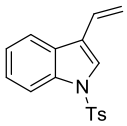
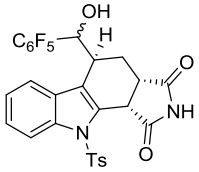
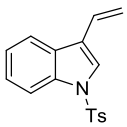
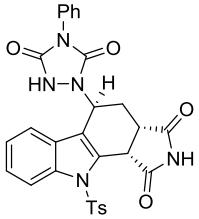
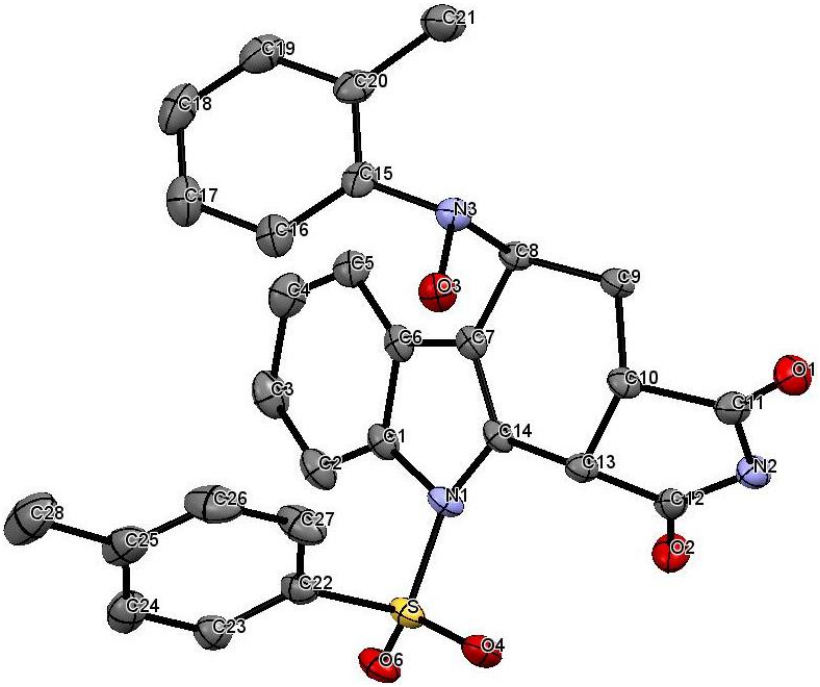
Starting Material	Diels-Alder Conditions	Ene Conditions	Ene Adduct	MCR Yield
 (146)	Maleimide (**196**), 40 °C, 48 h	PhNO (**170**), rt, 4 h	 (199)	89%
 (146)	Maleimide (**196**), 40 °C, 48 h	*o*-TolNO (**172**), rt, 4 h	 (200)	82%
 (146)	Maleimide (**196**), 40 °C, 48 h	C_6F_5CHO (**164**), DMAC, -78 °C to rt, 18 h	 (201)	71%
 (146)	Maleimide (**196**), 40 °C, 48 h	PTAD (**150**), 0 °C, 4 h	 (202)	75%

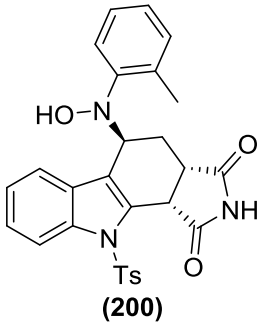
Table 3.3 - Diels-Alder / ene between 1-tosyl-3-vinyl-1H-indole (146) and maleimide (196).

The one-pot MCR with maleimide (**196**) as the dienophile gave the desired ene adducts as single diastereomers in excellent yields ranging from 71-89%.

3.4.1 Relative stereochemistry of Diels-Alder/ ene adduct (200)-

Confirming the relative stereochemistry of ene adduct (**200**) is difficult to accomplish using NMR techniques. Therefore a single crystal was grown of ene adduct (**200**) for X-ray crystallography by slow evaporation from DCM. This allowed the relative stereochemistry of the ene reaction to be confirmed and shows that the hydroxylamine group and the succinimide group are on opposite faces of the compound to each other (Figure 3.4).



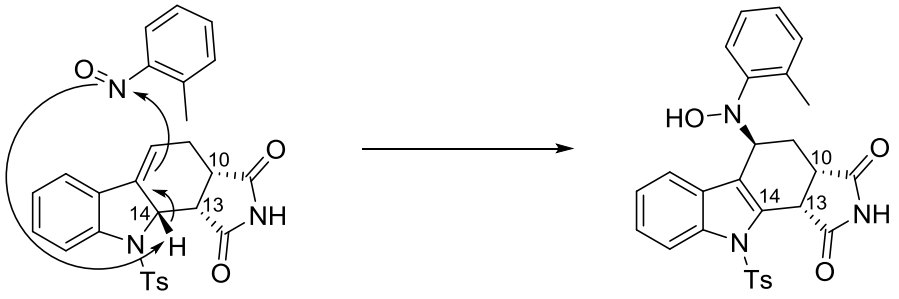


(200)

Figure 3.4 - X-ray crystal structure of Diels-Alder/ ene adduct (200) with hydrogens removed for clarity.

The X-ray crystal structure showed that, in ene adduct (**200**), the enophile adds to the *si*-face of the alkene which results in the hydroxylamine group being on the opposite face of Diels-Alder adduct (**197**) relative to the dienophile (Figure 3.4).

This stereochemistry is dictated by the initial *endo*-Diels-Alder reaction which sets the relative stereochemistry at positions C^{10}, C^{13} and C^{14}. As the ene reaction is a concerted process, the nitroso compound is directed on to the *si*-face by the *twix*-proton at C^{13} (Scheme 3.7).



Scheme 3.7 - Proposed reaction mechanism for nitroso-ene reaction

This results in 1-methyl-2-nitrosobenzene (**172**) adding to the *si*-face of the alkene in Diels-Alder adduct (**148**), which is the opposite face relative to the succinimide moiety.

3.5.0 Functionality on the indole ring-

Many of the examples of biologically active carbazole compounds have functionality around the indole ring system (R^1 and R^2 positions). Ty *et al*. have demonstrated using SAR studies that compounds where $R^1 \neq H$ and $R^2 \neq H$, show better biological activity against B16 melanoma cells (Figure 3.5) than compounds where $R^1 = R^2 = H$.

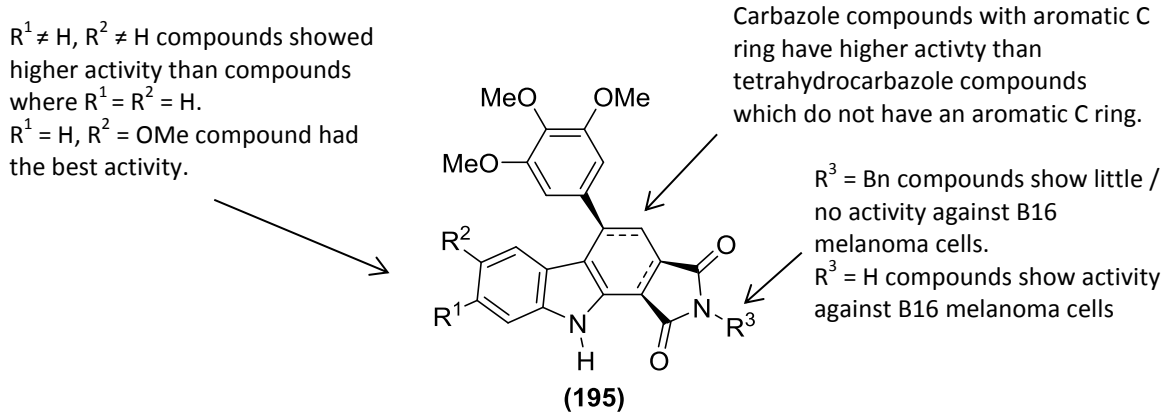
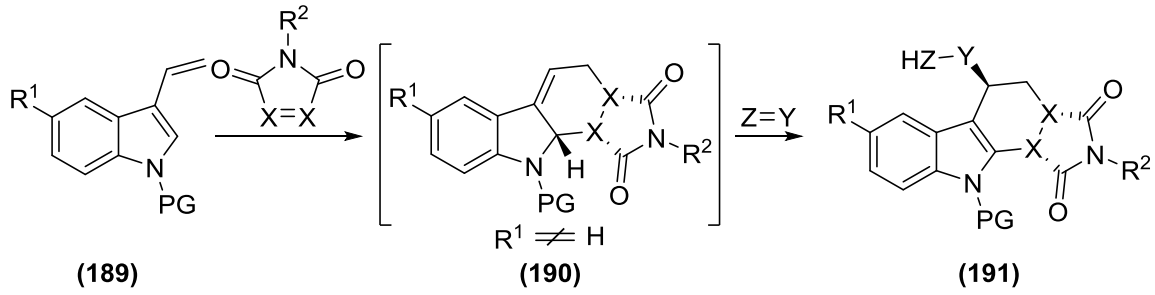


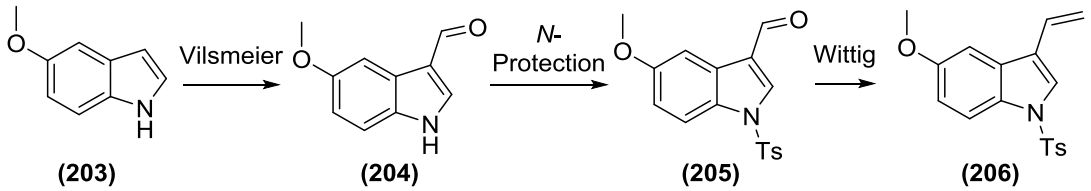
Figure 3.5 - Biologically active carbazole compounds with functionality around the indole ring.

We wanted to introduce the same functionality in to our one-pot Diels-Alder / ene reactions by synthesising compounds of the type shown below (**191**) (Scheme 3.8).



Scheme 3.8 - One-pot Diels-Alder / ene reaction with 5-substituted N-protected vinyl-indole.

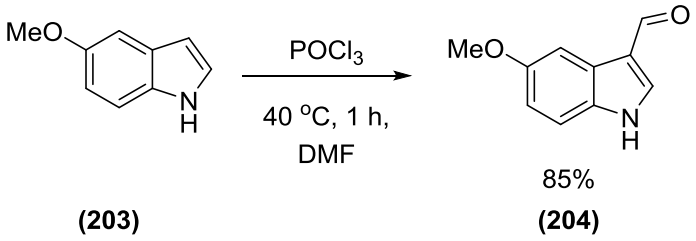
We started with the addition of a simple methoxy group to the 5-position of the indole ring, using 5-methoxy-1*H*-indole (**203**) which was commercially available. We proposed a Vilsmeier / *N*-protection / Wittig reaction sequence to synthesise desired 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (**206**) (Scheme 3.9).



Scheme 3.9 - Proposed route to synthesise 5-methoxy-1-tosyl-3-vinyl-1H-indole (206).

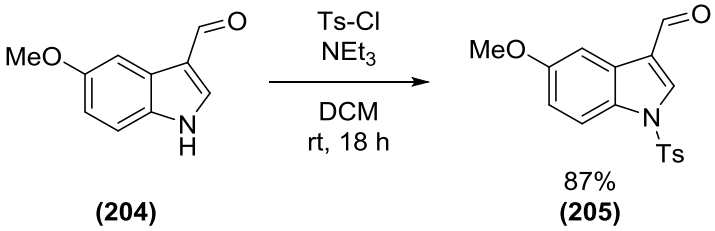
We expected the electron donating properties of the methoxy group of 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (**206**) to increase the electron density of the diene, which would lower the activation energy for the Diels-Alder reaction with maleimide compounds.

Starting from 5-methoxy-1*H*-indole (**203**), 5-methoxy-1*H*-indole-3-carbaldehyde (**204**) was synthesised in an excellent yield via a Vilsmeier reaction (Scheme 3.10).



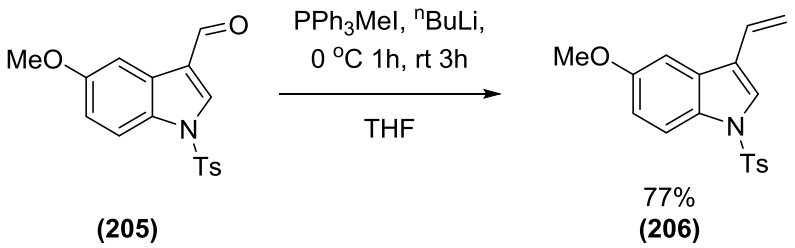
Scheme 3.10 - Vilsmeier reaction to form 5-methoxy-1H-indole-3-carbaldehyde (204).

Following on from the Vilsmeier reaction, the next step was the tosyl protection of the indolic NH group. The NH was protected using tosyl chloride and triethylamine to give 5-methoxy-1-tosyl-1*H*-indole-3-carbaldehyde (**205**) in an excellent yield (Scheme 3.11).



Scheme 3.11 - Tosyl protection of 5-methoxy-1H-indole-3-carbaldehyde (204).

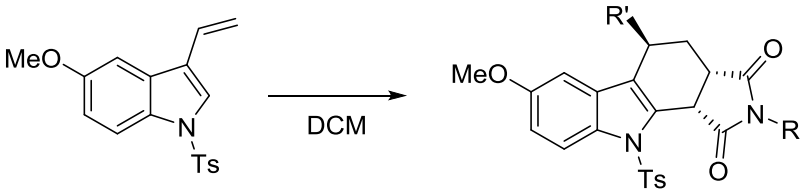
The final step was to carry out the Wittig reaction to synthesise 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (**206**) (Scheme 3.12).



(205) **(206)**

Scheme 3.12 - Wittig reaction to form 5-methoxy-1-tosyl-3-vinyl-1H-indole (206)

The Wittig reaction formed the desired 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (**206**) in a 77% yield. With an efficient method to synthesise of 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (**206**) in hand, several one-pot Diels-Alder / ene reactions were attempted between (**206**), NMM (**141**) and 2,3,4,5,6-pentafluorobenzaldehyde (**164**) and between (**206**), maleimide (**196**) and PTAD (**150**) (Scheme 3.13).



(206) **(207)**

Scheme 3.13 - One pot Diels-Alder / ene.

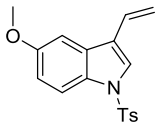
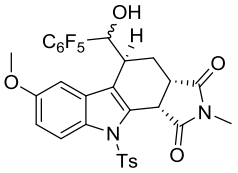
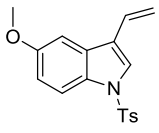
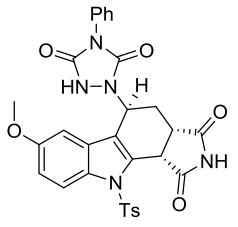
Starting Material	Diels-Alder Conditions	Ene Conditions	Ene Adduct	MCR Yield
 **(206)**	NMM (**141**), 40 °C, 48 h	C_6F_5CHO (**164**), DMAC, -78 °C to rt, 18 h	 **(208)**	70%
 **(206)**	Maleimide (**196**), 40 °C, 48 h	PTAD (**150**), 0 °C, 4 h	 **(209)**	75%

Table 3.4 - One-pot Diels-Alder / ene reaction with 5-methoxy-1-tosyl-3-vinyl-1H-indole (206).

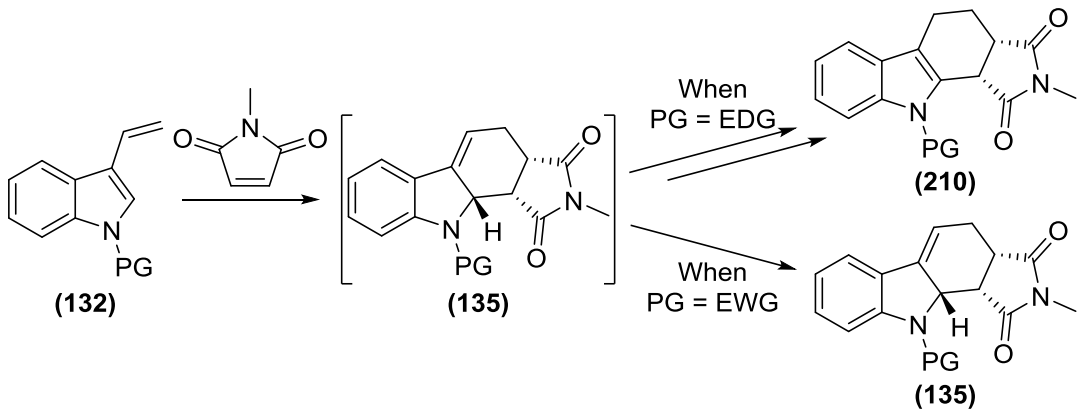
The one-pot MCRs with 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (**206**), resulted in the formation of (**208**) and (**209**) in good yields with (**208**) produced as a single diastereomer. Ene adduct (**209**) was isolated as an inseparable mixture of diastereomers in an 8: 1 ratio. We expected the electron donating methoxy group to increase the electron density of the indole ring and

therefore increase the rate of the Diels-Alder/ ene reactions. However, we discovered that the added electron density did not have a significant effect on the overall reaction time of the Diels-Alder/ ene reaction (Table 3.4). The success of these reactions suggested that our one-pot MCR could tolerate simple electron donating groups on the indole ring.

3.6.0 Alternative N-protecting groups-

The previous examples of one-pot Diels-Alder / ene reaction sequences gave an operationally simple, highly efficient method to quickly synthesise tetrahydrocarbazoles and pyridazino[3,4-*b*]indoles.

In Chapter 2, we discovered that an electron donating *N*-protecting group on the indolic nitrogen increased the rate of the Diels-Alder reaction and caused the Diels-Alder adduct formed to rapidly rearomatise to (**210**). Conversely, electron withdrawing *N*-protecting groups were found to slow the Diels-Alder reaction and stabilise the Diels-Alder adduct (**135**) generated (Scheme 3.14).

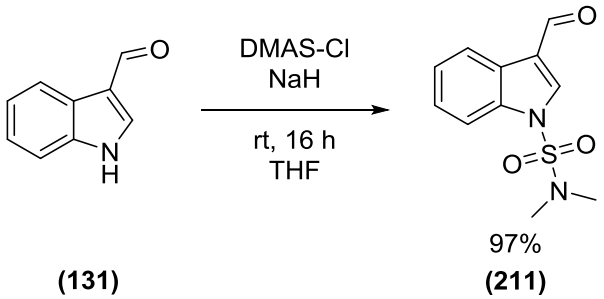


Scheme 3.14 - Effect of N-protecting group upon the stability of Diels-Alder adduct (135).

We wanted to examine the scope of the one-pot Diels-Alder / ene reactions with a range of different *N*-protecting groups. It was hoped that changing the *N*-protecting group would expand the range of enophiles that we could use in the one-pot MCR.

The one-pot Diels-Alder / ene reaction sequences were very successful with a tosyl protecting group, so the next protecting group examined was *N,N*-dimethylaminosulfonyl (DMAS), which is another sulfonyl based group similar to tosyl.

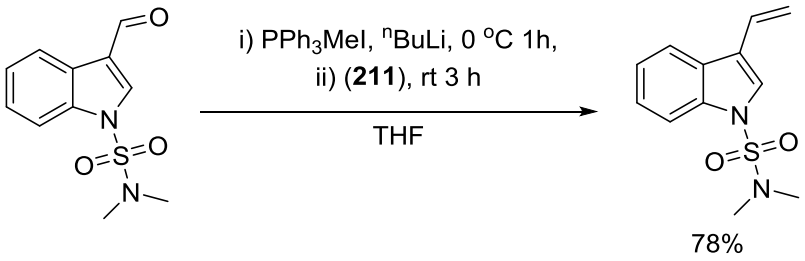
To synthesise DMAS protected indole, sodium hydride was added to 1*H*-indole-3-carbaldehyde (**131**) followed by the addition of *N,N*-dimethylsulfamoyl chloride, to give 3-formyl-*N*,*N*-dimethyl-1*H*-indole-1-sulfonamide (**211**) (Scheme 3.15).



(131) **(211)**

Scheme 3.15 - DMAS protection of 1H-indole-3-carboxaldehyde (131).

N,*N*-dimethyl-1*H*-indole-1-sulfonamide (**211**) was then reacted in a Wittig reaction with methylenetriphenyl-λ^5-phosphane to form *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (**212**) in a good yield (Scheme 3.16).

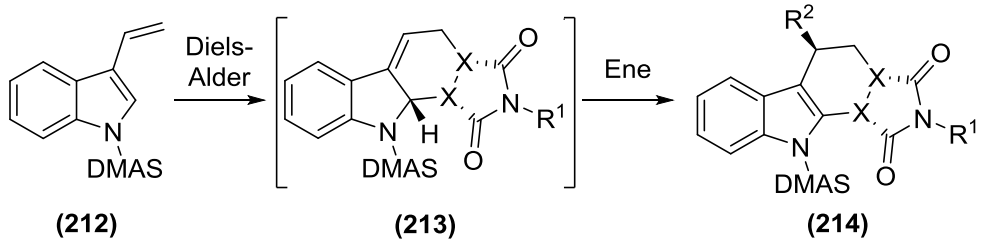


(211) **(212)**

Scheme 3.16 - Wittig reaction to form N,N-dimethyl-3-vinyl-1H-indole-1-sulfonamide (212).

3.7.0 DMAS protected Diels-Alder / ene chemistry-

With *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (**212**) in hand, the next step was to screen it against a range of dienophiles and enophiles in a one-pot Diels-Alder / ene reaction sequence (Scheme 3.17).



Scheme 3.17 - One-pot DMAS Diels-Alder / ene reaction.

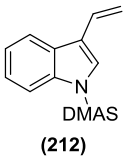
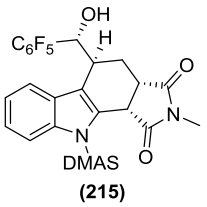
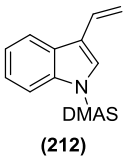
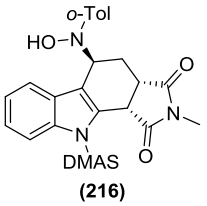
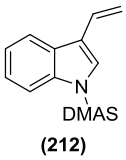
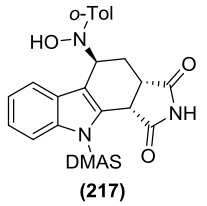
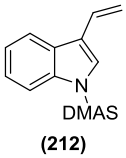
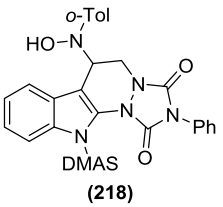
Starting Material	Diels-Alder Conditions	Ene Conditions	Ene Adduct	MCR Yield
 (212)	NMM (**141**), 40°C, 48 h	C_6F_5CHO (**164**), DMAC, -78 °C , 1 h	 (215)	77%
 (212)	NMM (**141**), 40°C, 48 h	*o*-TolNO (**172**), rt, 3 h	 (216)	74%
 (212)	Maleimide (**196**), 40 °C, 48 h	*o*-TolNO (**172**), rt, 3 h	 (217)	77%
 (212)	PTAD (**150**), -78 °C, 1 h	*o*-TolNO (**172**), rt, 4 h	 (218)	76%

Table 3.5 – DMAS Diels-Alder / ene reaction conditions.

The one-pot MCR with *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (**212**) as starting material gave the desired ene adducts as single diastereomers in good overall yields. We expected *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (**212**) to have similar reactivity to 1-tosyl-3-vinyl-1*H*-indole (**146**) as both are sulfonyl based protecting groups. This was confirmed as the Diels-Alder reaction between *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (**212**) and NMM (**141**) took the same amount of time to go to completion as the Diels-Alder reactions between tosyl-3-vinyl-1*H*-indole (**146**) and NMM (**141**) (Chapter 2.2.4).

The nitroso-ene reactions with DMAS protected Diels-Alder adduct, went to completion at a moderately improved rate relative to the tosyl protected nitroso-ene reactions. The nitroso-ene reactions with tosyl protected Diels-Alder adduct (**148**) took 18 hours to go to completion however with DMAS protecting group, that time was reduced to 3-4 hours. The same trend was observed when 2,3,4,5,6-pentafluorobenzaldehyde (**164**) was used as the enophile, with the reaction taking 1 hour with DMAS protected Diels-Alder adduct (**213**) to go to completion, compared to 18 hours with tosyl protected Diels-Alder adduct (**148**).

This change in the reactivity of the ene reaction suggested the electronics of the Diels-Alder adducts (**148**) and (**213**) are dependent upon the protecting group. It also suggests that the DMAS protecting group is less electron withdrawing than the tosyl protecting group. This means DMAS protected Diels-Alder adduct (**213**) is more electron rich compared to (**148**) making it more reactive towards enophiles.

3.7.1 Stereochemistry of Diels-Alder / ene adduct (215)-

The one-pot Diels-Alder / ene adduct from the reaction between *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (**212**), NMM (**141**), and 2,3,4,5,6-pentafluorobenzaldehyde (**164**) has the potential to form two diastereomers (Figure 3.6).

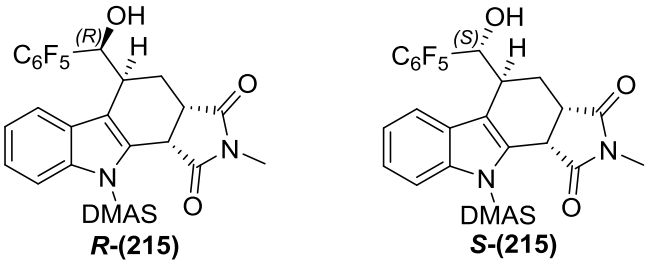
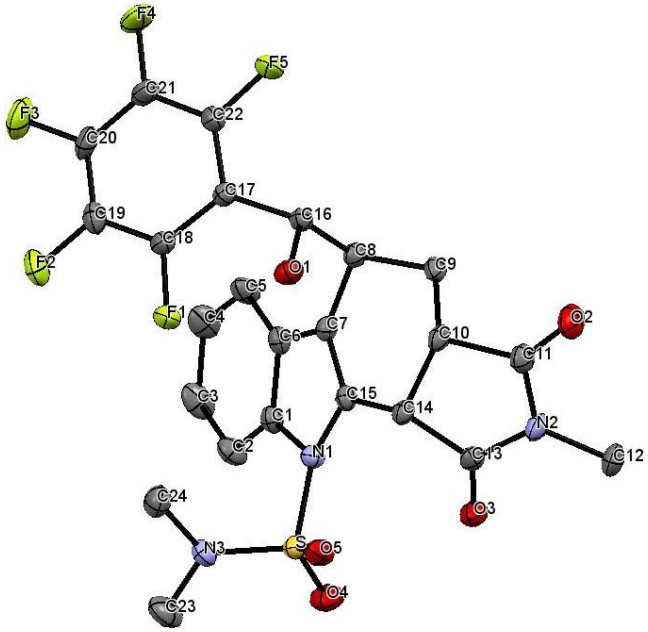
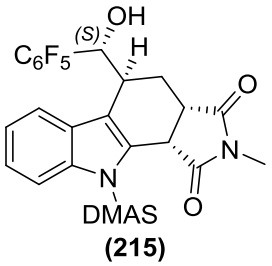


Figure 3.6 - Two possible diastereomers that can be formed in the reaction between N,N-dimethyl-3-vinyl-1H-indole-1-sulfonamide (212), N-methylmaleimide (141), and 2,3,4,5,6-pentafluorobenzaldehyde (164).

Only one diastereomer is seen in the ^{1}H NMR of Diels-Alder / ene adduct (**215**). It was difficult to elucidate which diastereomer is formed using NMR studies, therefore (**215**) was crystallised from DCM using slow evaporation to give single crystals suitable for X-ray analysis. The X-ray crystal structure showed that Diels-Alder / ene adduct (**215**) had an (*S*) stereocentre at the C^{16} position (Figure 3.7).

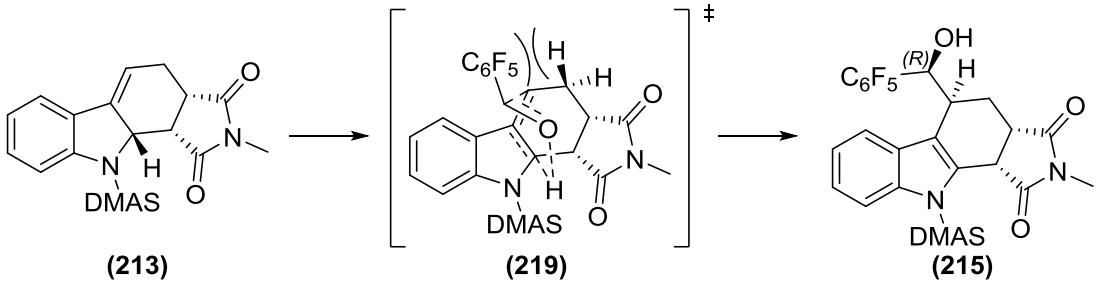




(215)

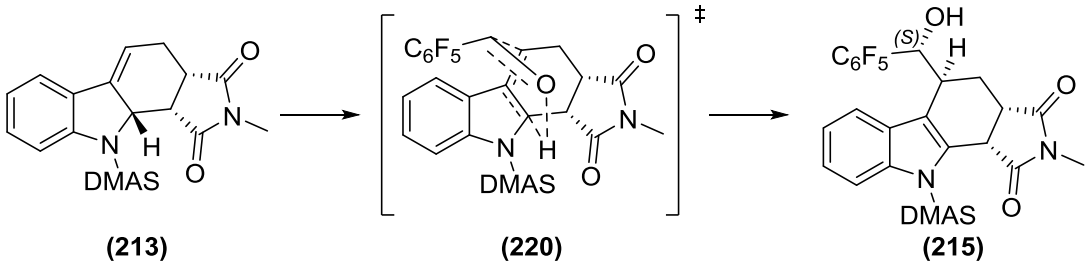
Figure 3.7 - X-ray crystal structure of Diels-Alder / ene adduct (215).

The possibility of two diastereomers being formed arises from two possible transition states in the ene reaction between Diels-Alder adduct (**213**) and 2,3,4,5,6-pentafluorobenzaldehyde (**164**). However, only (*S*)-diastereomer (**215**) is generated due to one of the two possible transition states being higher energy and therefore unfavourable (Scheme 3.18).



Scheme 3.18 - Ene reaction with unfavourable transition state (219) due to steric clash between C_6F_6 group and H^9.

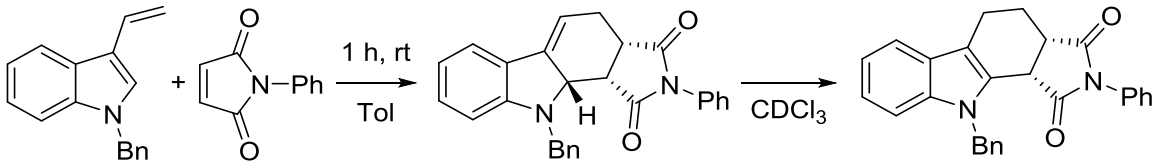
In transition state (**219**) there is an unfavourable steric interaction between the C_6F_5 group of 2,3,4,5,6-pentafluorobenzaldehyde (**164**) and the two protons at the C^9 position. On the other hand transition state (**220**) has the C_6F_5 ring pointing away from any groups so that there are no unfavourable steric interactions. This means that transition state (**220**) is lower in energy, relative to (**219**), making ene adduct (**215**), formed as a result of this transition state, the major diastereomer (Scheme 3.19).



Scheme 3.19 - Ene reaction with favoured transition state (220).

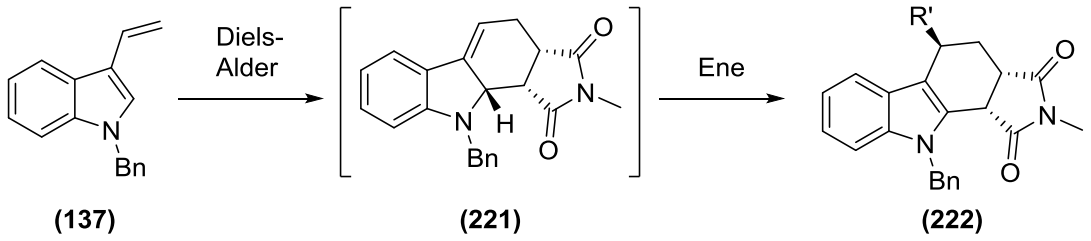
3.8.0 One-pot Diels-Alder / ene reactions of Bn protected indoles-

Previous work by Porter *et al*.[54] has shown that the Diels-Alder reaction between 1-benzyl-3-vinyl-1*H*-indole (**137**) and NPM (**117**) rapidly goes to completion. However, Diels-Alder adduct (**138**) is difficult to handle and readily rearomatises to form (**139**) due to the electron donating properties of the benzyl protecting group (Scheme 3.20).



Scheme 3.20 - Diels-Alder / rearomatisation reaction leading to formation of (139).[54]

It was because of this difficulty in purifying and handling Diels-Alder adduct (**221**) that we had focused on more electron withdrawing groups, such as tosyl and DMAS. However, our new one-pot Diels-Alder / ene reaction sequence means that benzyl Diels-Alder adduct (**221**) only has to be formed *in situ* and therefore the problems with handling and isolating it could be negated (Scheme 3.21).

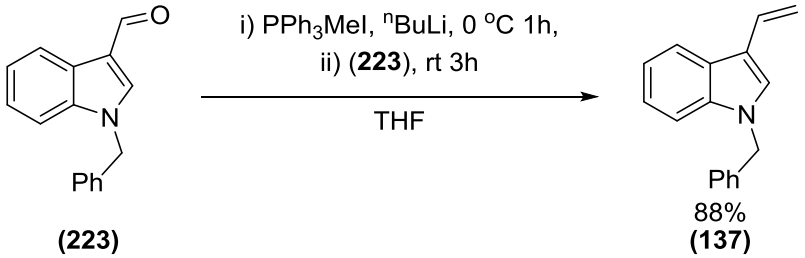


Scheme 3.21 - One-pot Diels-Alder / ene reactions of Bn protected indoles.

We anticipated that as the ene reaction of Diels-Alder adduct (**221**) leads to concurrent rearomatisation of the indole ring system, this would make ene adduct (**222**) more stable to purification. We proposed exploiting the electron donating benzyl protecting group by employing a wider range of less reactive enophiles.

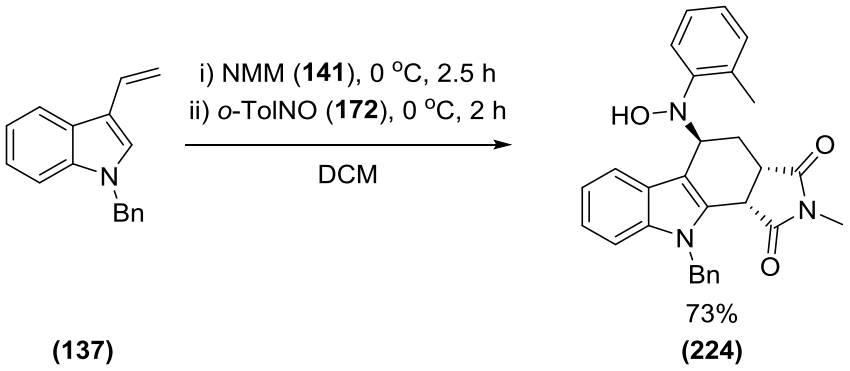
To test this theory, 1-benzyl-3-vinyl-1*H*-indole (**137**) first had to be synthesised. Starting from 1-benzyl-1*H*-indole-3-carbaldehyde (**223**), a Wittig reaction was performed with

methylenetriphenyl-λ^5-phosphane to give 1-benzyl-3-vinyl-1*H*-indole (**137**) in an excellent yield (Scheme 3.22).



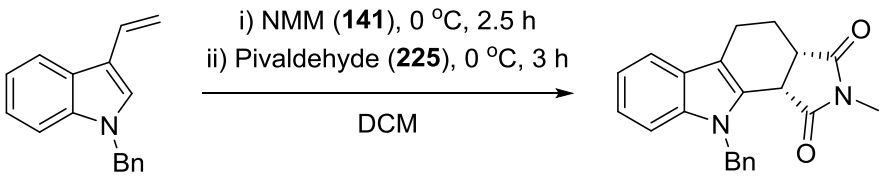
Scheme 3.22 - Wittig reaction to form 1-benzyl-3-vinyl-1H-indole (137).

1-Benzyl-3-vinyl-1*H*-indole (**137**) was then subjected to a one-pot Diels-Alder / ene reaction with NMM (**141**) and 1-methyl-2-nitrosobenzene (**172**). As the benzyl protecting group increased the reactivity of the indole towards dienophiles, the Diels-Alder reaction was carried out at 0 °C. After two and a half hours, 1-methyl-2-nitrosobenzene (**172**) was added and the reaction was maintained at 0 °C (Scheme 3.23). The ene reaction went to completion after two hours and afforded ene adduct (**224**), which could be readily purified in an excellent overall yield of 73%.



Scheme 3.23 - One-pot benzyl Diels-Alder / ene to form (224).

The previous one-pot Diels-Alder / ene reaction had shown that 1-benzyl-3-vinyl-1*H*-indole (**137**) was more reactive towards dienophiles and enophiles than the corresponding 1-tosyl-3-vinyl-1*H*-indole (**146**) or *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (**212**). It was thought that a less reactive enophile could be used in the ene reaction. Therefore the Diels-Alder reaction between 1-benzyl-3-vinyl-1*H*-indole (**137**) and NMM (**141**) was repeated before pivaldehyde (**225**) was added to the reaction as the enophile (Scheme 3.24). Pivaldehyde was chosen as an example of a very simple aldehyde that could be easily observed in the ^{1}H NMR spectrum.



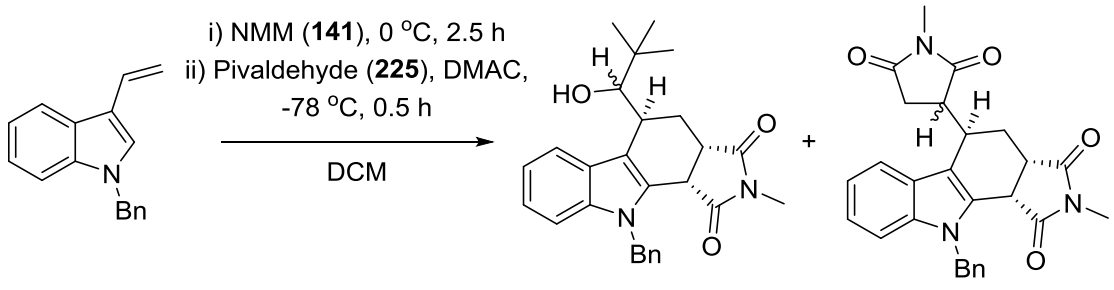
(137) **(226)**

Scheme 3.24 - One-pot MCR with 1-benzyl-3-vinyl-1H-indole (137), NMM (141) and pivaldehyde (225).

The ene reaction between Diels-Alder adduct (**138**) and pivaldehyde (**225**) was allowed to stir at 0 °C for three hours, however in this time no ene reaction occurred and rearomatised Diels-Alder adduct (**226**) was the only product isolated from the reaction. This suggested that the relative rate of the rearomatisation reaction was much greater than the rate of the ene reaction with pivaldehyde (**225**) under these conditions.

DMAC had previously been used to catalyse the ene reaction between 2,3,4,5,6-pentafluorobenzaldehyde (**164**) and tosyl protected Diels-Alder adduct (**148**) (Chapter 2.4.0). Therefore DMAC was used to catalyse the ene reaction between Diels-Alder adduct (**138**) and pivaldehyde (**225**). The one-pot Diels-Alder ene reaction was repeated with the Lewis acid DMAC used to catalyse the ene reaction (Scheme 3.25). The crude ^{1}H NMR of the reaction showed that no starting material remained, however the reaction had formed two major compounds as well as other minor products.

The two products could be separated using column chromatography. The major product isolated was the expected pivaldehyde ene product (**227**), in a modest yield of 29% (Scheme 3.25).



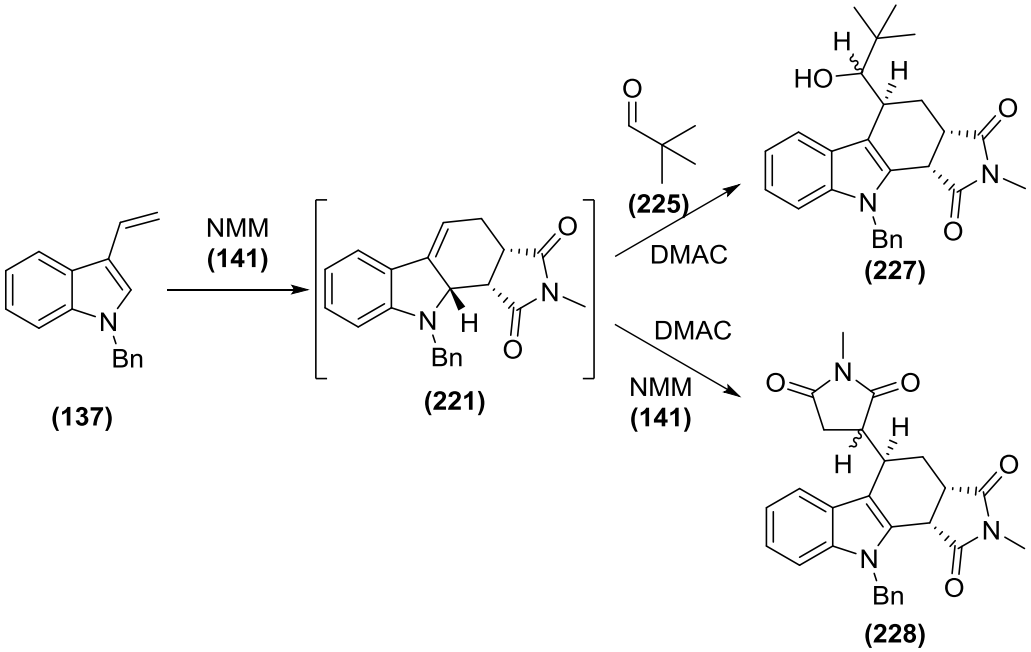
(137) **(227)** **(228)**

Scheme 3.25 - DMAC catalysed Diels-Alder / ene reaction with pivaldehyde.

The minor product from the reaction was the result of two NMM (**228**) molecules undergoing a Diels-Alder and ene reaction with 1-benzyl-3-vinyl-1*H*-indole (**137**) (Scheme 3.25).

The formation of compound (**228**) suggested that, as only one equivalent of NMM (**141**) was added initially, the Diels-Alder reaction was not going to completion in the initial 2.5 hours.

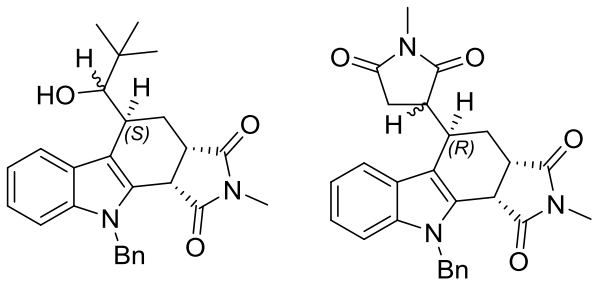
That meant that when the reaction was cooled to -78 ^{o}C and DMAC added, the DMAC was catalysing the ene reaction between Diels-Alder adduct (**221**) and another NMM (**141**) molecule as well as the ene reaction with pivaldehyde (**225**) (Scheme 3.26). The yields obtained for (**227**) and (**228**) only account for 59% of the total NMM (**141**) added to the reaction. This suggested that NMM (**141**) was reacting to form other products which were unidentified besides the two major products we isolated.



Scheme 3.26 - Diels-Alder / ene reaction leading to the formation of (227) and (228).

The formation of (**228**) indicated that the Diels-Alder reaction of 1-benzyl-3-vinyl-1*H*-indole (**137**) and NMM (**141**) was not going to completion before the addition of DMAC and pivaldehyde (**225**). The same reaction was attempted again with the Diels-Alder step reaction time being increased to four hours. It was hoped that this would allow the Diels-Alder reaction to go to completion so no NMM (**141**) remained when DMAC was added. However, this led to decomposition of Diels-Alder adduct (**221**) to the rearomatised Diels-Alder adduct (**222**) before the ene reaction could occur and therefore no ene adduct could be isolated. Diels-Alder adduct (**221**) was found to be unstable in solution and readily rearomatised unless it was reacted further to form the more stable ene adduct (**227**).

The Diels-Alder / ene reaction led to the formation of two new stereocentres in products (**227**) and (**228**) (Figure 3.8).



(227) **(228)**

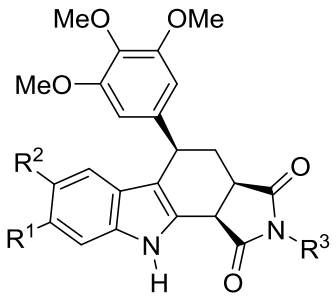
Figure 3.8 - Products (227) and (228) formed in the one-pot Diels-Alder / ene reaction.

The stereocentres on the six membered ring can be assigned either (*R*) or (*S*) using the concerted ene mechanism which we postulated in Chapter 3.4.1, which predicts that the enophiles attack from the *si* face of the Diels-Alder adduct. This leads to an (*S*) stereocentre in (**227**) and an (*R*) stereocentre in (**228**). Attempts were made to grow single crystals of both (**227**) and (**228**) in order to confirm the stereochemistry of both stereocentres adjacent to the alcohol group, however this was not successful.

Due to the capricious nature of the benzyl protected indoles in the Diels-Alder / ene reaction sequence, they were not studied any further.

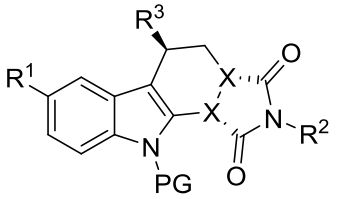
3.9.0 Bioassay of Ts, DMAS and Bn protected Diels-Alder / ene adducts-

Using our one-pot MCR process, a range of tosyl, DMAS and benzyl protected ene adducts were synthesised, analogous to a range of known biologically active compounds (Figure 3.9). Compounds of the class (**195**) have been shown in the literature to have good activity against B16 melanoma cells with an IC_{50} of 2.6 µM +/- 1.5 µM (R_1 = OMe, R_2 =H, R_3 = H).[35]



(195)

R^1/R^2 = H, OMe, Br, NO_2, NH_2, $NHCOCF_3$;
R^3 = H, Bn



(191)

R^1 = OMe, H; X = C, N; R^2 = H, Me, Ph;
R^3 = -N(OH)Ph, -N(OH)*o*-Tol, PTAD, -C(OH)C_6F_5;
PG = Ts, DMAS, Bn

Figure 3.9 - Compound class (195) active against B16 melanoma cells and general structure of synthesised compounds from Diels-Alder / ene reactions (191).

The synthesised tosyl, DMAS and benzyl Diels-Alder / ene compounds were run in a Kirby-Bauer disk diffusion assay, as a test for biological activity.

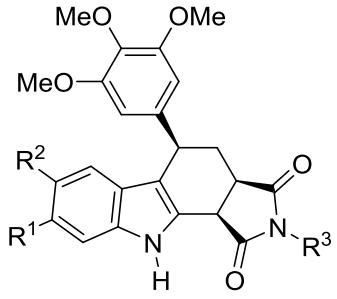
We chose to screen the compounds against two bacteria and a yeast - *Staphylococcus aureus* (*S. auereus*), *Escherichia coli* (*E. coli*) and *Schizosaccharomyces pombe* (*S. pombe*). *S. pombe* is used as a toxicity model for eukaryotic organisms. *S. pombe* is a yeast and therefore has cell walls so will have a different uptake mechanism of compounds compared to human cells. However, it is still relevant as a fast and simple method to gain insight into toxicity towards eukaryotic organisms.

S. aureus is used as a representation of Gram-positive bacteria. This species of bacteria include several pathogenic strains including methicillin resistant *S. aureus* (MRSA). MRSA is immune to many common antibiotics and the increasing number of MRSA cases, especially in hospitals, is of great concern. Therefore many efforts are being made to find novel antibiotics that kill the bacteria.[75]

E. coli is used as an example of Gram-negative bacteria and also contains many pathogenic strains including New Delhi metallo-β-lactamase (NDM1). NDM1 is a relatively newly discovered antibiotic resistant bacteria and therefore, as with MRSA, new compounds which can kill the bacteria are of great importance.[76]

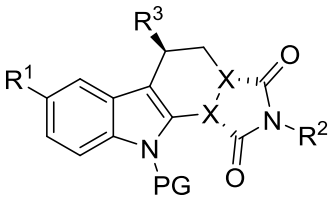
Each Diels-Alder / ene compound was dissolved in DCM at a concentration of 10 mg/ mL and was tested for activity against *S. aureus*, *S. pombe* and *E. coli*. The plates were left to incubate at 30 ^{o}C overnight after which time no zone of inhibition could be observed on the plates.

A comparison between the compounds that are known to be active against B16 melanoma cells (**195**) and the general structure of Diels-Alder / ene compounds (**191**) we had synthesised, suggested that a free NH on the indole moiety of the compound was important for biological activity (Figure 3.10).



(195)

R^1/R^2 = H, OMe, Br, NO_2, NH_2, $NHCOCF_3$;
R^3 = H, Bn



(191)

R^1 = OMe, H; X = C, N; R^2 = H, Me, Ph;
R^3 = -N(OH)Ph, -N(OH)*o*-Tol, PTAD, -C(OH)C_6F_5;
PG = Ts, DMAS, Bn

Figure 3.10 - Biologically active compound (195) and general structure of synthesised compounds from Diels-Alder / ene reaction (191).

We hypothesised that a lack of a free NH on the Diels-Alder / ene compounds that we synthesised prevented them from being biologically active. Therefore to increase the likelihood that our compounds would be biologically active, attempts were made to remove the protecting groups.

3.9.1 Ts and DMAS protecting group removal-

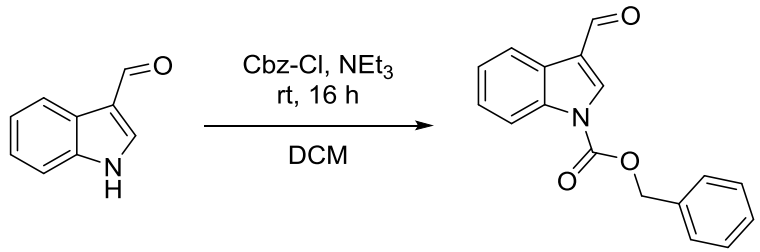
The benzyl compounds (**224**), (**227**) and (**228**) were very reactive and could only be isolated in low yields. We thought that they would not be stable to deprotection under hydrogenation conditions and therefore no attempt was made to remove the benzyl group.

Previous work in the group had found difficulties in removing the DMAS and tosyl protected Diels-Alder / ene compounds.[77] The tosyl and DMAS protected compounds were subjected to either basic deprotection conditions using potassium or sodium hydroxide, or single electron transfer conditions using sodium or magnesium in mercury amalgams.[78-80] It was found that neither the tosyl or DMAS protecting groups could be removed using these conditions without leading to decomposition of the molecule. Therefore an alternate protecting group had to be found that was compatible with our one-pot Diels-Alder / ene conditions and that would readily undergo deprotection.

3.10.0 Cbz protected indole-

Previous work (Chapter 2) had shown that an electron withdrawing protecting group was required for the Diels-Alder / ene chemistry to occur. We required a protecting group that would allow the one-pot MCR to occur and could be easily removed from the resulting Diels-Alder / ene adducts. The ideal protecting group would have to be sufficiently electron withdrawing to stabilise the Diels-Alder adduct formed from the Diels-Alder reaction with a range of dienophiles. However, if the protecting group were too electron withdrawing, it would prohibit further chemistry from being carried on the Diels-Alder adduct. The protecting group would also need to be easily removed under mild conditions so as not to cause other functional groups on the molecule to decompose.

Our choice of protecting group was the carboxybenzyl (Cbz) protecting group as it was electron withdrawing and we believed it could be readily removed under hydrogenation conditions. The first step in the synthesis of benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) was the synthesis of benzyl 3-formyl-1*H*-indole-1-carboxylate (**229**) using triethylamine, benzyl chloroformate and 1*H*-indole-3-carboxylate (**131**) (Scheme 3.27).

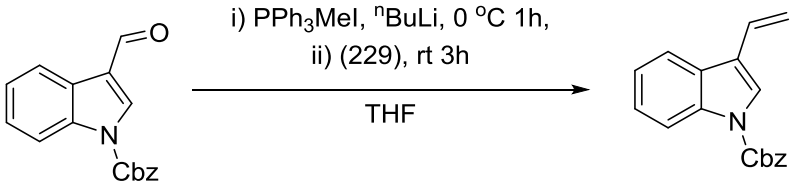


(131) **(229)**

Scheme 3.27 - Cbz protection of 1H-indole-3-carboxylate (131).

The *N*-protection reaction gave the desired benzyl 3-formyl-1*H*-indole-1-carboxylate (**229**) in an excellent yield of 92%.

Benzyl 3-formyl-1*H*-indole-1-carboxylate (**229**) was then subjected to a Wittig reaction with methylenetriphenyl-λ^5-phosphane to form benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) (Scheme 3.28).



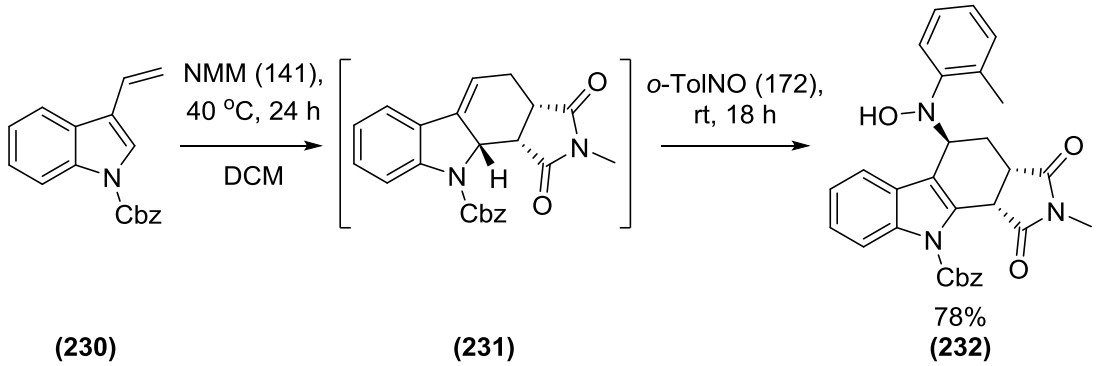
(229) **(230)**

Scheme 3.28 - Synthesis of benzyl 3-vinyl-1H-indole-1-carboxylate (230).

The Wittig reaction gave benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) in a good yield of 76%.

Once a reliable method to synthesise benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) was established, we planned to first test the one-pot Diels-Alder / ene chemistry with NMM (**141**) as the dienophile and 1-methyl-2-nitrosobenzene (**172**) as the enophile. This allowed us to determine if the Cbz protected Diels-Alder / ene adducts could be readily synthesised as single diastereomers in good yields.

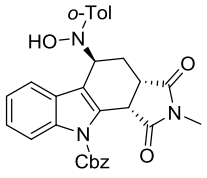
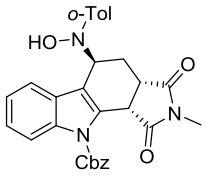
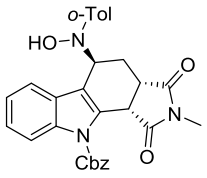
The Diels-Alder reaction between benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) and NMM (**141**) was closely monitored by TLC and showed clean conversion to Diels-Alder adduct (**231**) after stirring at 40 ^{o}C for 24 hours. The enophile 1-methyl-2-nitrosobenzene (**172**) was then added to the solution and the ene reaction went to completion after 18 hours (Scheme 3.29).



Scheme 3.29 - One-pot Cbz protected Diels-Alder / ene reaction.

Ene adduct (**232**) could be isolated using column chromatography in a good yield of 78%. This initial test reaction suggested that benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) readily underwent Diels-Alder / ene reactions and the ene product formed was stable to isolation and purification. We then moved on to investigate if the Cbz protecting group could be readily removed.

We attempted to remove the Cbz group in a series of hydrogenation reactions using 5% or 10% palladium on carbon as the catalyst, and either atmospheric pressure or 5 atmospheres of hydrogen gas. The hydrogenation deprotection reactions were unsuccessful and Diels-Alder / ene adduct (**232**) was recovered from the reaction in each case (Table 3.6, entries 1-3).

Entry	Ene Adduct	Deprotection Conditions	Product	Yield
1	 (232)	5% Pd/C, 1 atm H_2, rt, 8 h	No Reaction	NA
2	 (232)	10% Pd/C, 1 atm H_2, rt, 8 h	No Reaction	NA
3	 (232)	10% Pd/C, 5 atm H_2, rt, 18 h	No Reaction	NA

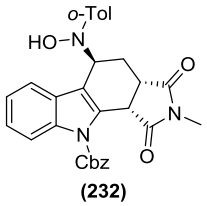
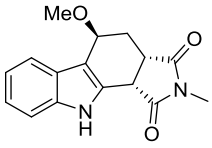
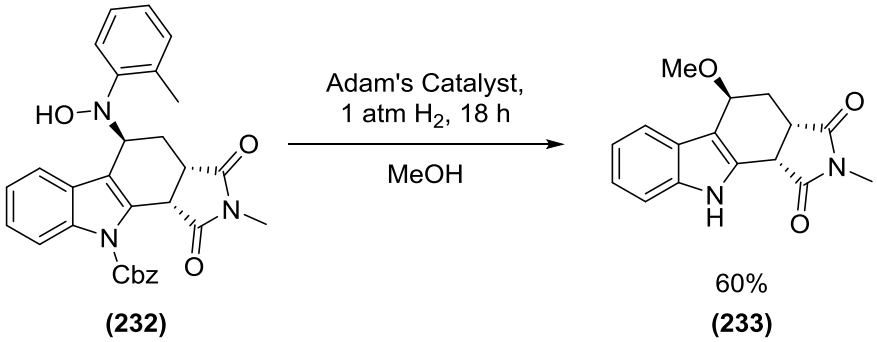
4	 (232)	PtO_2, rt, 18 h	 (233)	60%

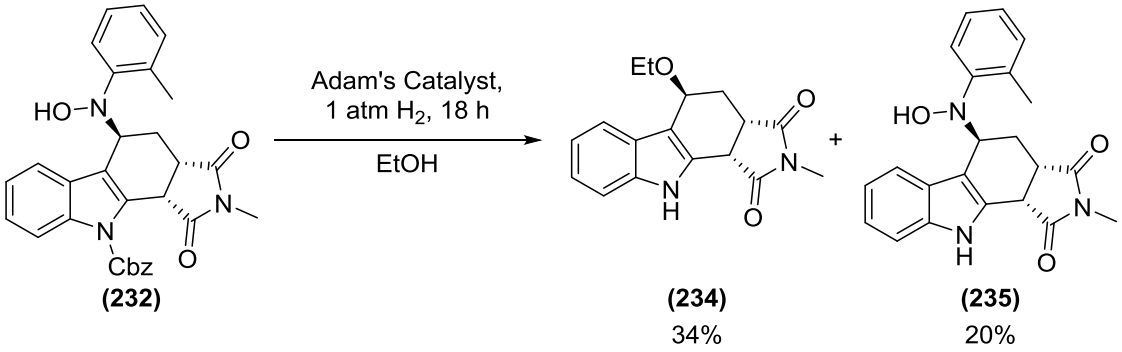
Table 3.6 - Reaction conditions attempted for Cbz deprotection.

As the hydrogenation deprotection reactions with palladium on carbon were having no success, the hydrogenation catalyst was changed to platinum (IV) oxide (Adam's catalyst). The hydrogenation was attempted with platinum (IV) oxide and an atmospheric pressure of hydrogen for 18 hours. The crude ^{1}H NMR showed loss of the distinctive benzylic Cbz peaks, indicating that the deprotection had been successful. However, upon purification of the crude reaction mixture by column chromatography, the isolated product was not the expected deprotected ene adduct (Table 3.6, Entry 4). Instead, methoxy substituted product (**233**) was isolated in a 60% yield (Scheme 3.30).



Scheme 3.30 - Unexpected methoxy product (233) isolated from the hydrogenation reaction.

We believed that the methoxy group originated from the reaction solvent and hoped that changing the solvent to a more sterically bulky, less polar solvent would circumvent the substitution. Therefore the reaction was repeated with platinum (IV) oxide and an atmospheric pressure of hydrogen, with ethanol as the solvent (Scheme 3.31).



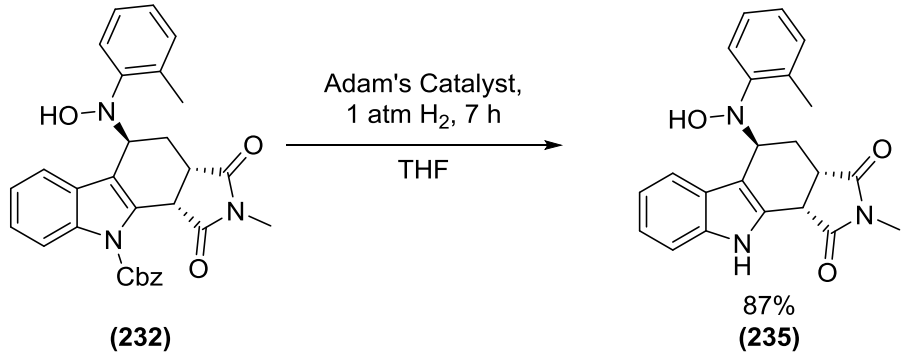
Scheme 3.31 - Deprotection reaction with ethanol solvent.

The crude ^{1}H NMR showed the reaction had formed two major products; the expected ethoxy substituted compound (**234**) and a small amount of deprotected ene adduct (**235**).

As 20% of deprotected product (**235**) was isolated from the reaction, the change in solvent from methanol to ethanol clearly has an effect upon the reaction.

We proposed that changing the solvent to a non-nucleophilic solvent would allow the formation of the desired deprotected Diels-Alder / ene product (**235**).

The reaction was therefore repeated with THF as the solvent (Scheme 3.32).



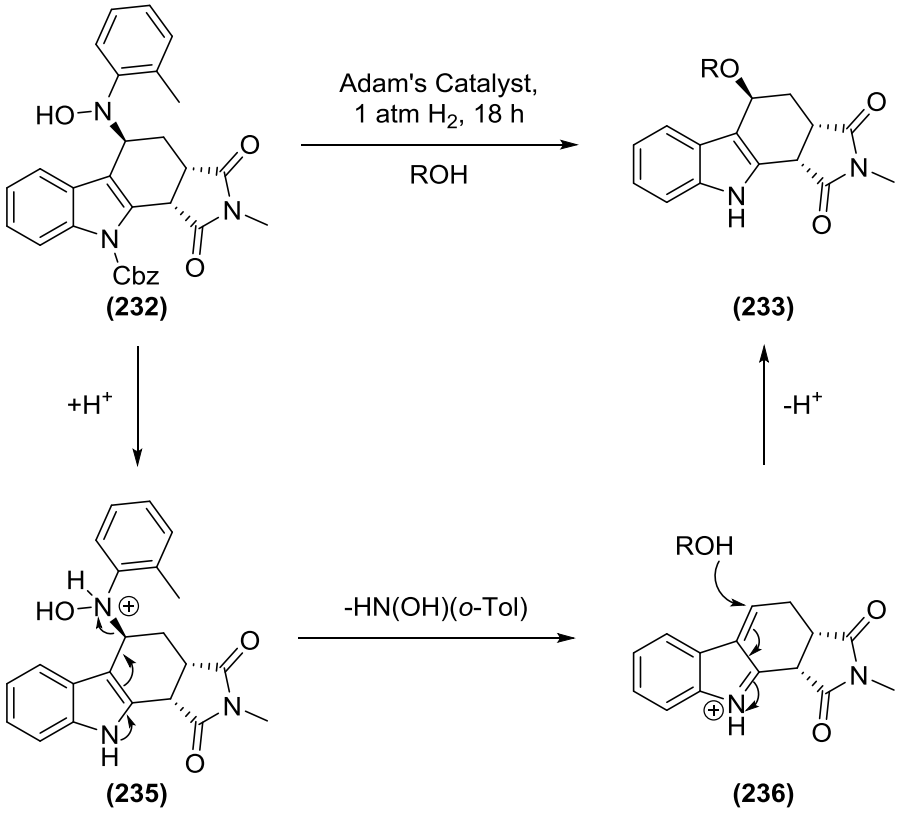
(232) **(235)**

Scheme 3.32 - Cbz deprotection hydrogenation reaction.

The change to a non-nucelophilic solvent meant no substitution reaction could occur and the desired deprotected ene adduct (**235**) was isolated as a single diastereomer in an excellent yield of 87%.

3.10.1 Mechanism for the formation of (233) and (234)-

We believed the methoxy and ethoxy substituted products (**233**) and (**234**) could be formed via two possible reaction pathways; S_N1 or S_N2. The S_N1 mechanism could arise from the hydrogenation reaction firstly removing the Cbz group from the indolic nitrogen to form (**235**). This increases the electron density throughout the indole ring system allowing the indolic nitrogen to eliminate the hydroxylamine group leading to the formation of (**236**). After the hydroxylamine group has been eliminated, methanol can attack the unsaturated iminium group via a Michael addition, forming (**233**) (Scheme 3.33).



Scheme 3.33 - Proposed S_N1 mechanism by which compound (233) is formed.

The change in solvent from MeOH to EtOH shows a decrease in the amount of substituted product (**234**) being formed. This can be rationalised in the S_N1 reaction as the formation of the charged unsaturated iminum compound (**236**) would be favoured by a more polar solvent which can delocalise the charge. Therefore the more polar MeOH solvent stabilises the formation of (**236**). The EtOH on the other hand, does not favour the elimination of the hydroxylamine group and therefore there is a reduction in the amount of unsaturated iminium (**236**) and consequently ethoxy substituted product (**234**) formed in the reaction.

All of the one-pot Diels-Alder / ene adducts synthesised were subjected to high resolution mass spectroscopy (HRMS) to confirm the expected compounds had indeed been synthesised. The HRMS data showed that one of the major peaks for all of the ene adducts formed, regardless of protecting group, was the hydroxylamine eliminated product (**237**) (Figure 3.11).

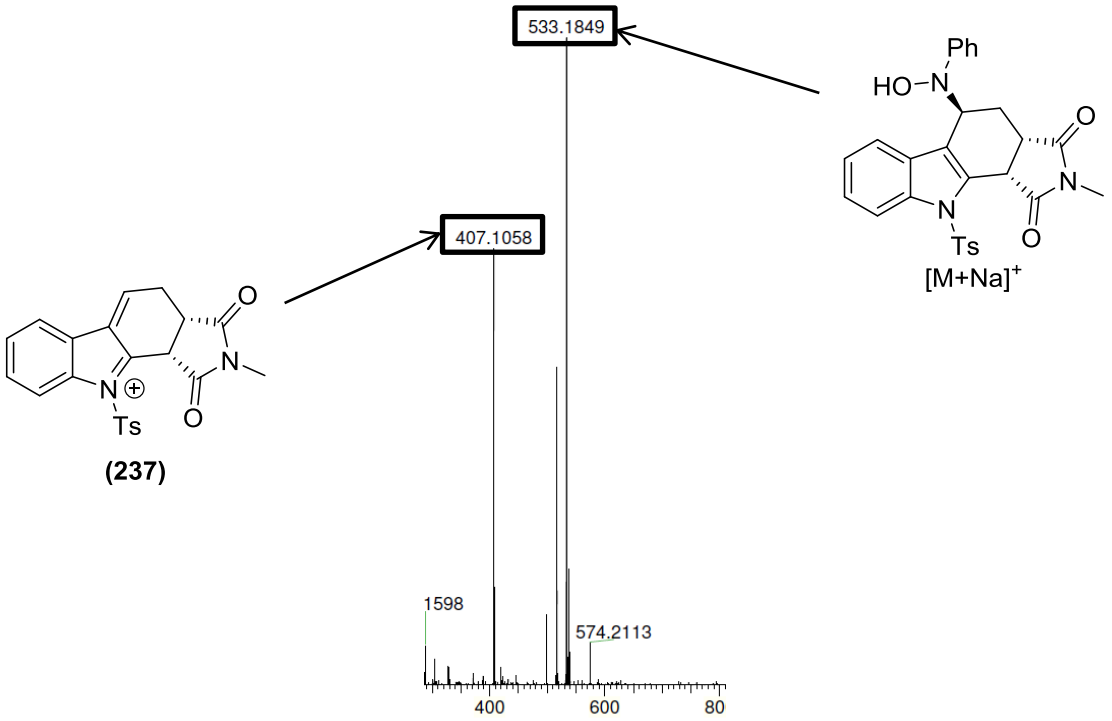
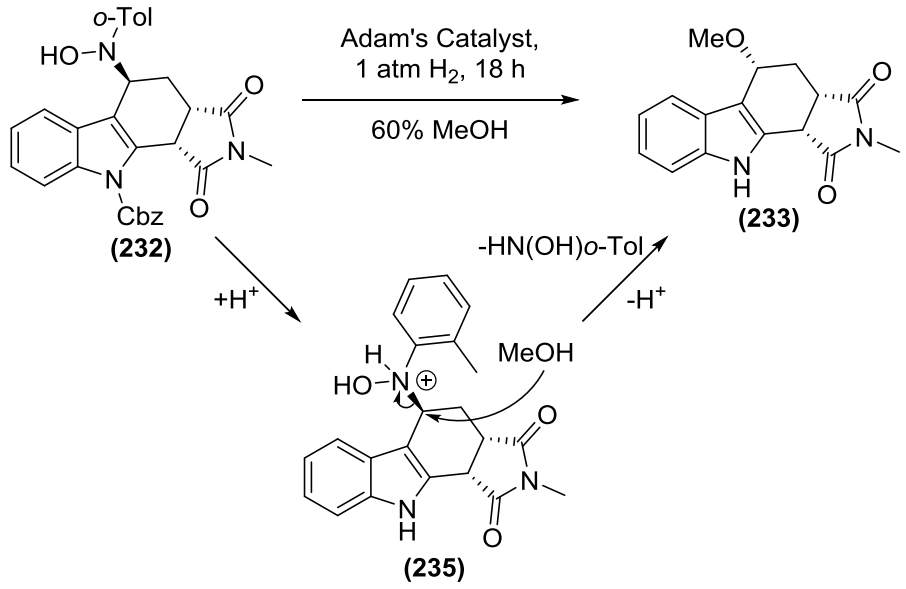


Figure 3.11 - HRMS of tosyl protected ene adduct (171).

This provides evidence for the possibility of (**236**) being generated from the elimination of the hydroxylamine group from (**235**), as it is readily formed under ionising conditions.

There is, however, also the possibility that (**233**) is formed through an S_N2 reaction mechanism. The S_N2 mechanism could arise from the removal of the Cbz group from the indolic nitrogen to generate (**235**). This is followed by the methanol attacking (**235**) in an S_N2 mechanism, with hydroxylamine acting as the leaving group, leading to the formation of (**233**) (Scheme 3.34).

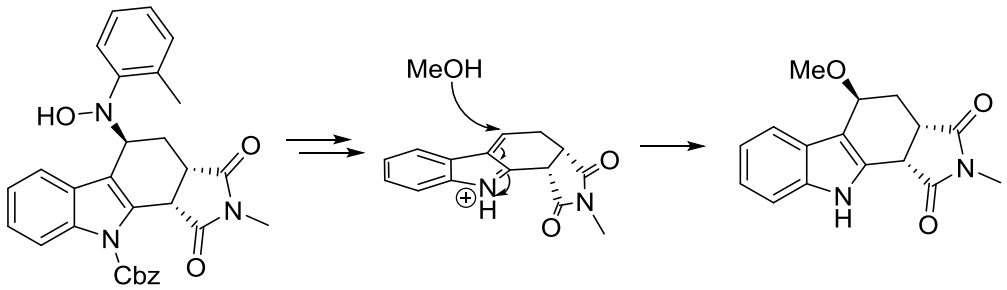


Scheme 3.34 - Proposed S_N2 reaction pathway for the formation of (233).

The difference in the amount of substituted product formed, depending on which solvent is used, can also be explained in the S_N2 mechanism. The S_N2 reaction is dependent upon the rate the solvent attacks the tertiary carbon centre on (**235**). The smaller methanol molecule can more easily do this compared to the more sterically hindered ethanol. Therefore more methoxy substituted product (**233**) is formed compared the ethoxy product (**234**) under the same reaction conditions.

As both of mechanisms can be used to explain the change in products generated depending on the solvent used, another method had to be found to deduce the reaction mechanism. We thought we could do this by studying the relative stereochemistry of products (**233**) and (**234**).

We propose that the relative stereochemistry of methoxy product (**233**), formed from the S_N1 reaction pathway, is determined by which face (*re*- or *si*-) of compound (**236**) the methanol attacks from. We believe the succinimide moiety of compound (**236**) will block one face of the compound leading to the methanol attacking from the least hindered, *si*-face of compound (**236**) (Scheme 3.35).

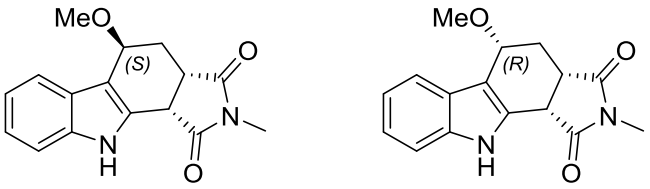


(232) **(236)** **(233)**

Scheme 3.35 - Methanol attack leading to the relative stereochemistry seen in compound (233).

This leads to the methoxy group being on the opposite face of compound (**233**) relative to the succinimide moiety. Overall the substitution is an S_N1 reaction with retention of stereochemistry.

In the S_N2 reaction mechanism, the final step is nucleophilic attack 180^o to the hydroxylamine leaving group. This would result in an inversion of stereochemistry at the reaction centre leading to the formation of (**233**) (Figure 3.12).

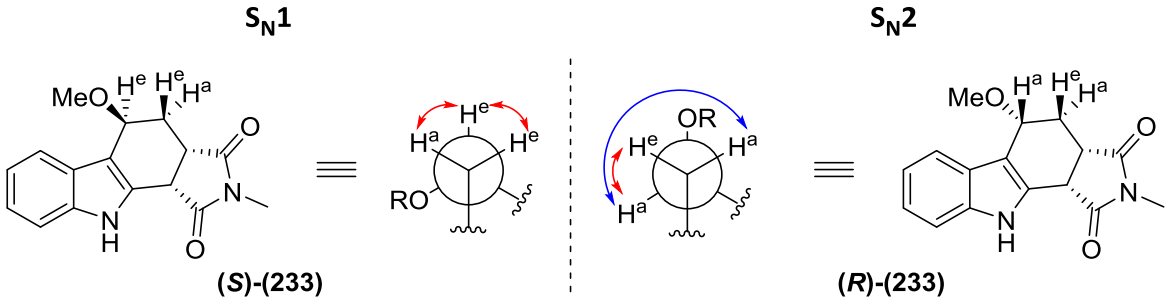


(*S*)-(233) **(*R*)-(233)**

Figure 3.12 - S_N1 reaction would lead to the formation of (S)-(233) while S_N2 reaction would lead to the formation of (R)-(233).

By considering the coupling constants for proton (H^a) (Figure 3.13) attached to the same carbon as the methoxy group, we are able to deduce which mechanism occurs. In the product generated via an S_N1 mechanism, the equatorial proton (H^e) is coupled to both the equatorial proton (H^e) and axial proton (H^a) on the adjacent carbon. The bond angles are similar for H^e-H^e and H^e-H^a and therefore you would expect the coupling constants to be similar for both (Figure 3.13).

Axial proton (H^a) on product (*R*)-(**233**) from the S_N2 reaction, would couple to the equatorial proton H^e and axial proton H^a on the adjacent carbon. As there is a large difference between the bond angles of the axial-axial proton and axial-equatorial protons you would expect to see one large coupling constant for the axial-axial (H^a-H^a) interaction and one much smaller coupling constant for the axial-equatorial (H^a-H^e) interaction.



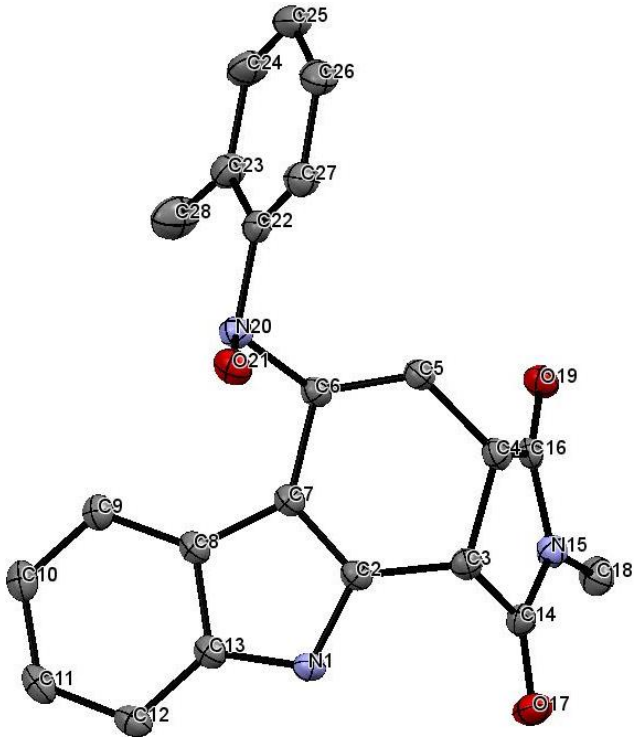
(*S*)-(233) **(*R*)-(233)**

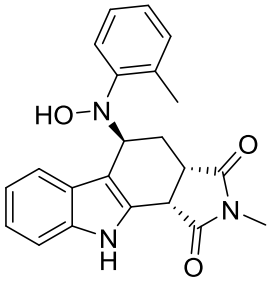
Figure 3.13 - The two diastereomers possible as a result of an S_N1 or S_N2 reaction.

The ^{1}H NMR shows that proton H^a, in both (**233**) and (**234**), is an apparent triplet with a coupling constant of 2.7 Hz for methoxy product (**233**) and 2.8 Hz for ethoxy product (**234**). This suggests that the substitution reactions proceed via an S_N1 reaction mechanism to give the stereochemistry shown in (*S*)-(**233**) (Figure 3.12). If the products were formed through an S_N2 reaction mechanism, the coupling between H^a-H^e and H^a-H^a would be sufficiently different to observe a clear doublet of doublets peak.

3.10.2 X-ray structure of (235)-

To confirm the structure and stereochemistry of deprotected Diels-Alder / ene adduct (**235**), a single crystal was grown by slow evaporation from DCM and was then subjected to X-ray diffraction (Figure 3.14).





(235)

Figure 3.14 - X-ray crystal structure of deprotected ene product (235).

The structure generated from the X-ray diffraction confirmed that the Cbz group had been lost from the indolic nitrogen. It also allowed us to prove the relative stereochemistry of deprotected product (**235**) had not changed from Cbz protected Diels-Alder/ ene adduct (**232**), with the succinimide moiety remaining on the opposite face of the molecule relative to the hydroxylamine group.

3.10.3 Cbz one-pot Diels-Alder / ene chemistry-

With an efficient method for the removal of the Cbz protecting group, the scope of the Cbz Diels-Alder / ene reaction and subsequent Cbz deprotection could be investigated. To allow for comparison with the previous tosyl protected Diels-Alder / ene adducts (**208**) and (**209**), functionality was added to the indole ring with the introduction of a methoxy group in the five position.

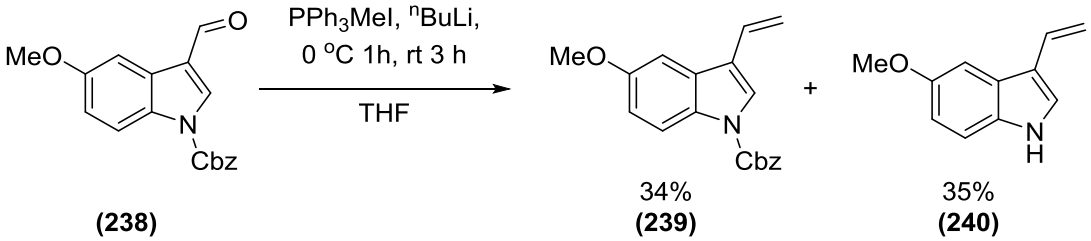
Therefore, benzyl 5-methoxy-3-vinyl-1H-indole-1-carboxylate (**239**) needed to be synthesised. Starting from 5-methoxy-1*H*-indole-3-carbaldehyde (**237**), the Cbz protection could be carried out using benzyl chloroformate and triethylamine (Scheme 3.36).

(237) 98% **(238)**

Scheme 3.36 - Cbz protection of 5-methoxy-1H-indole-3-carbaldehyde (238).

The reaction gave the desired product (**238**) in an excellent yield of 98%.

The next step was the Wittig reaction with methylenetriphenyl-λ^5-phosphane to form benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) (Scheme 3.37).



(238) 34% **(239)** 35% **(240)**

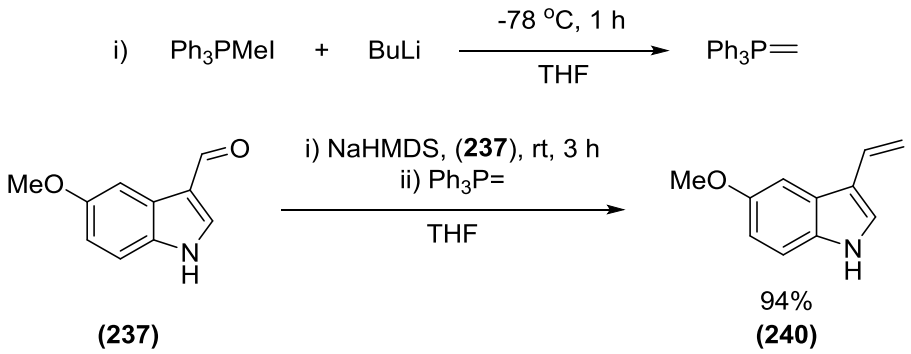
Scheme 3.37 - Cbz protected Wittig reaction.

The crude ^{1}H NMR showed a mixture of two compounds was generated in a 1: 1 ratio. Separation by silica gel chromatography gave the desired benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) and 5-methoxy-3-vinyl-1*H*-indole (**240**). This suggested that, although the Wittig reaction was giving desired product (**239**), the conditions used in the Wittig reaction were leading to decomposition of benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) to form 5-methoxy-3-vinyl-1*H*-indole (**240**).

The isolation of both (**239**) and (**240**) from the Wittig reaction did not occur when the methoxy group was not on the indole ring. The previous Wittig reaction to form benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) gave a good yield of 76% with no deprotected product being observed in the reaction. The fact that the Cbz group is only removed when there is a methoxy group at the 5 position on the indole ring suggests that the electron donating properties of the methoxy group is stabilising a positive charge build up in the decomposition mechanism.

As the Wittig reaction conditions were leading to loss of the Cbz group and therefore only modest yields of benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**), a more reliable synthesis had to be developed. We proposed doing the Wittig reaction first to form (**240**), before carrying out the protection step to form (**239**). That required deprotonating the indolic NH as a means of temporarily protecting the indolic nitrogen. This would stop the ylide formed in the Wittig reaction from deprotonating the acidic NH instead of attacking the carbonyl group. If the NH is already deprotonated, it forces the ylide to react with the aldehyde and undergo a Wittig reaction.

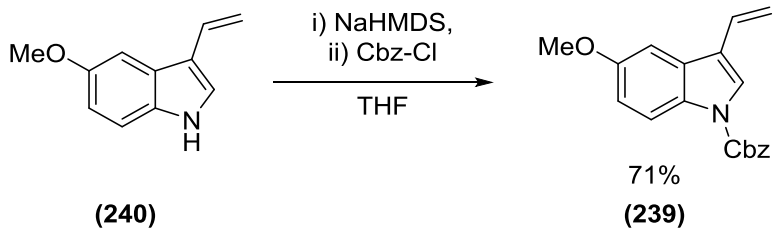
The first step in the new reaction sequence was the Wittig reaction of 5-methoxy-1*H*-indole-3-carbaldehyde (**237**) to give 5-methoxy-3-vinyl-1*H*-indole (**240**). This was achieved by first deprotonating 5-methoxy-1*H*-indole-3-carbaldehyde (**237**) with sodium bis(trimethylsilyl)amide (NaHMDS), before performing the Wittig reaction using Ph_3PMeI and ^{n}butyllithium (Scheme 3.38).



Scheme 3.38 - Wittig reaction to form 5-methoxy-3-vinyl-1H-indole (240).

The Wittig reaction gave 5-methoxy-3-vinyl-1*H*-indole (**240**) in an excellent yield of 94%.

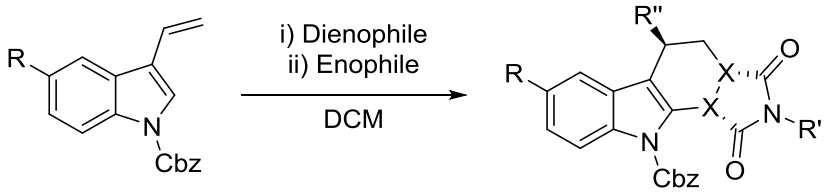
The final step in the synthesis of benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) was the Cbz protection of the indolic nitrogen using NaHMDS and benzyl chloroformate (Scheme 3.39).



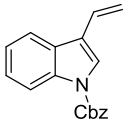
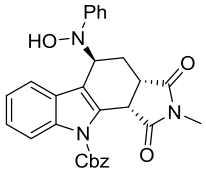
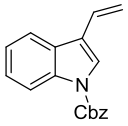
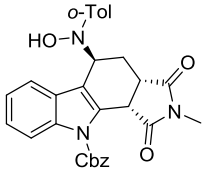
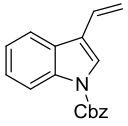
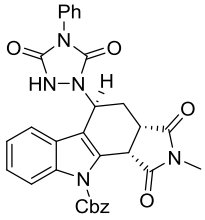
Scheme 3.39 - Cbz protection of 5-methoxy-3-vinyl-1H-indole (240).

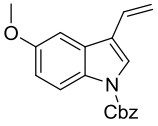
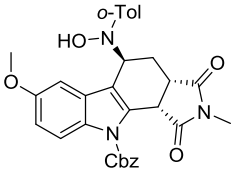
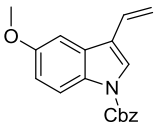
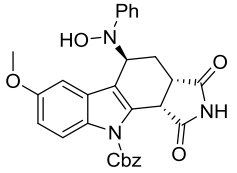
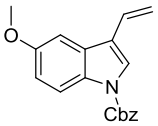
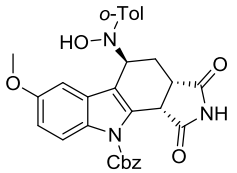
The Cbz protection led to benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) being isolated in a good yield of 71%. The deprotonation step requires the use of the strong base NaHMDS compared to the protection step with benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**237**), which used triethylamine. The NH in benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**237**) is conjugated into the aldehyde double bond making the NH more acidic compared to benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**240**). The lack of aldehyde for the NH to conjugate into makes the NH a lot less acidic and therefore a stronger base is required.

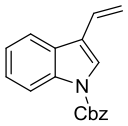
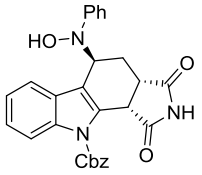
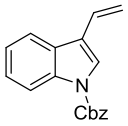
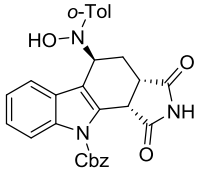
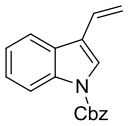
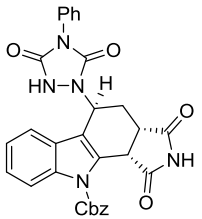
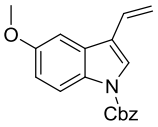
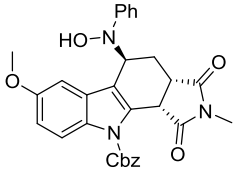
With an efficient method for the synthesis of benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) and benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**), the one-pot Diels-Alder / ene reactions could be attempted with NMM (**141**), maleimide (**196**), PTAD (**150**) as dienophiles and nitrosobenzene (**170**), 1-methyl-2-nitrosobenzene (**172**), and PTAD (**150**) as enophiles (Scheme 3.40). The one-pot chemistry developed in the synthesis of ene adduct (**232**) could be used to quickly synthesise a range of Cbz protected ene adducts (Table 3.7).



Scheme 3.40 - General scheme for Cbz protected one-pot Diels-Alder / ene reaction.

Starting Material	D-A / Ene Conditions	Ene Adduct	MCR Yield
 **(230)**	i) NMM (**141**), 40 °C, 24 h ii) PhNO (**170**), rt, 18 h	 **(241)**	74%
 **(230)**	i) NMM (**141**), 40 °C, 24 h ii) *o*-TolNO (**172**), rt, 18 h	 **(242)**	78%
 **(230)**	i) NMM (**141**), 40 °C, 24 h ii) PTAD (**150**), 0 °C 1 h, rt 18 h	 **(243)**	54%

Starting Material	D-A / Ene Conditions	Ene Adduct	MCR Yield
 **(239)**	i) NMM (**141**), 40 °C, 18 h ii) o-TolNO (**172**), rt, 3 h	 **(244)**	74%
 **(239)**	i) Maleimide (**196**), 40 °C, 18 h ii) PhNO (**170**), rt, 2.5 h	 **(245)**	79%
 **(239)**	i) Maleimide (**196**), 40 °C, 18 h ii) o-TolNO (**172**), rt, 3.5 h	 **(246)**	76%

 **(230)**	i) Maleimide (**196**), 40 °C, 24 h ii) PhNO (**170**), rt, 18 h	 **(247)**	70%
 **(230)**	i) Maleimide (**196**), 40 °C, 24 h ii) *o*-TolNO (**172**), rt, 4 h	 **(248)**	83%
 **(230)**	i) Maleimide (**196**), 40 °C, 24 h ii) PTAD (**150**), 0 °C, 1 h	 **(249)**	58%
 **(239)**	i) PTAD (**150**), -78 °C, 1.5 h ii) PhNO (**170**), rt,3 h	 **(250)**	73%

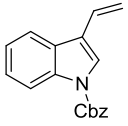
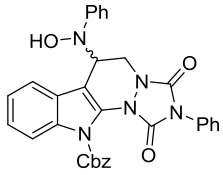
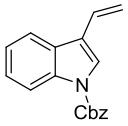
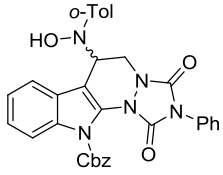
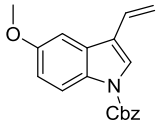
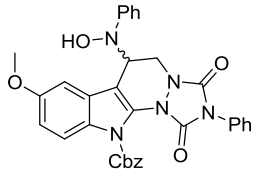
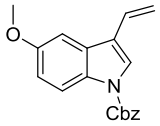
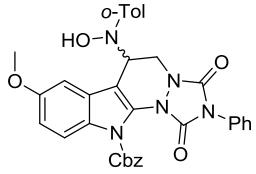
 **(230)**	i), PTAD (**150**), -78 °C, 5 h ii) PhNO (**170**), rt, 3 h	 **(251)**	72%
 **(230)**	i) PTAD (**150**), -78 °C, 5 h ii) *o*-TolNO (**172**), rt, 18 h	 **(252)**	68%
 **(239)**	i) PTAD (**150**), -78 °C, 1.5 h ii) PhNO (**170**), rt, 20 h	 **(253)**	78%
 **(239)**	i) PTAD (**150**), -78 °C, 1.5 h ii) *o*-TolNO (**172**), rt, 24 h	 **(254)**	82%

Table 3.7 - One-pot Cbz protected Diels-Alder / ene reaction conditions.

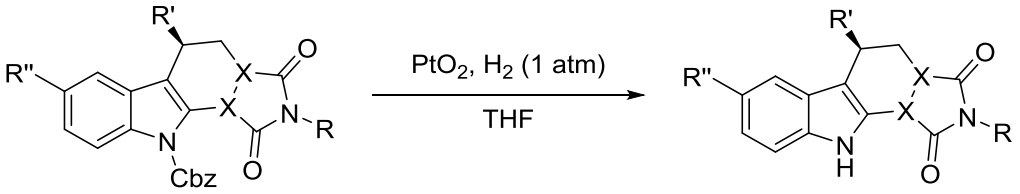
The one-pot MCRs allowed us to synthesise fourteen novel Cbz protected Diels-Alder / ene adducts as single diastereomers. The nitroso-ene reactions gave yields ranging from 68-83% whilst the aza-ene reactions, which used PTAD as an enophile, gave slightly lower yields of 54-58%. This slightly lower yield was due to several unidentified side products that were also formed in the reaction.

The Diels-Alder reactions between benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**) and NMM (**141**) or maleimide (**196**), all went to completion in the same time of 24 hours, with no significant difference between the NMM Diels-Alder reactions and the maleimide Diels-Alder reactions. The tosyl and DMAS protected Diels-Alder reactions took 48 hours to go to completion.

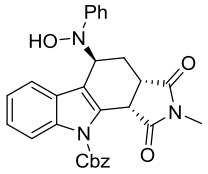
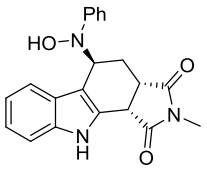
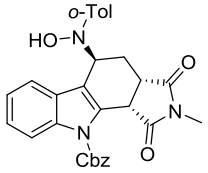
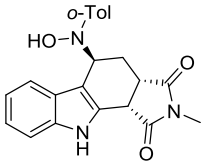
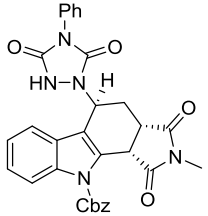
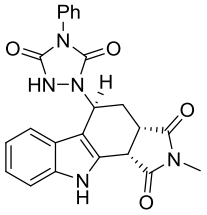
The NMM (**141**) or maleimide (**196**) Diels-Alder reactions with benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) went to completion after just 18 hours, which we propose is due to the electron donating methoxy group making the diene more electron rich. The same trend is observed with the PTAD Diels-Alder reactions. The PTAD Diels-Alder reaction with benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (**239**) goes to completion after 1.5 hours compared to 5 hours with benzyl 3-vinyl-1*H*-indole-1-carboxylate (**230**).

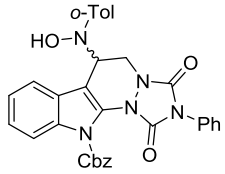
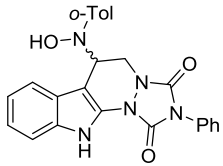
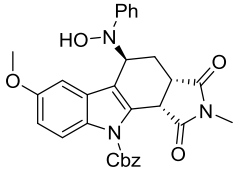
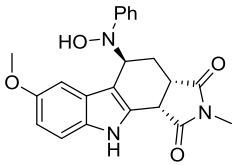
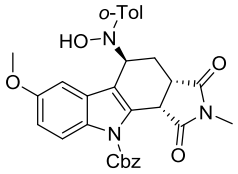
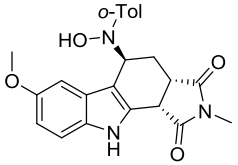
3.10.4 Cbz protecting group removal-

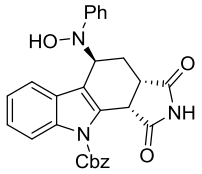
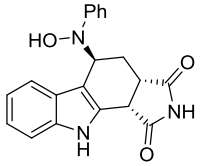
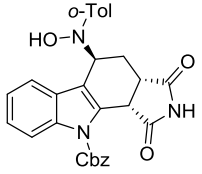
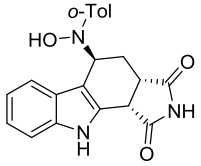
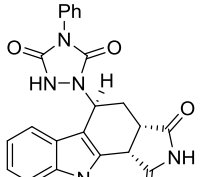
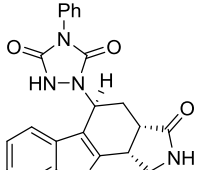
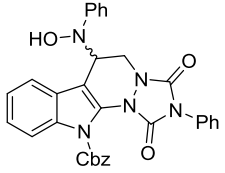
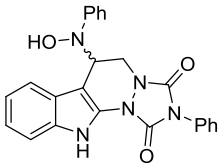
With a range of Cbz protected ene adducts, the next step in the synthesis of biologically active analogues was the removal of the Cbz protecting group in a hydrogenation reaction. As seen previously (Chapter 3.10.0), this could be done using Adam's catalyst and an atmospheric pressure of hydrogen gas (Scheme 3.41).



Scheme 3.41 - Cbz protecting group removal.

Starting Material	Hydrogenation Conditions	Product	Yield
 **(241)**	PtO_2, H_2 (1 atm), 5 h	 **(255)**	75%
 **(242)**	PtO_2, H_2 (1 atm), 7 h	 **(256)**	87%
 **(243)**	PtO_2, H_2 (1 atm), 5 h	 **(257)**	91%

Starting Material	Hydrogenation Conditions	Product	Yield
 **(252)**	PtO_2, H_2 (1 atm), 5 h	 **(258)**	44%
 **(250)**	PtO_2, H_2 (1 atm), 5 h	 **(259)**	70%
 **(244)**	PtO_2, H_2 (1 atm), 5 h	 **(260)**	70%

 (247)	PtO_2, H_2 (1 atm), 10 h	 (261)	41%
 (248)	PtO_2, H_2 (1 atm), 6 h	 (262)	70%
 (249)	PtO_2, H_2 (1 atm), 5 h	 (263)	64%
 (251)	PtO_2, H_2 (1 atm), 5 h	 (264)	65%

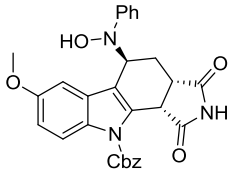
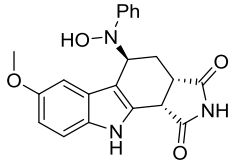
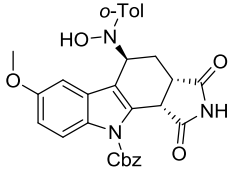
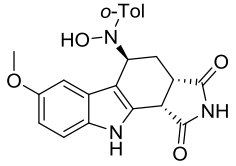
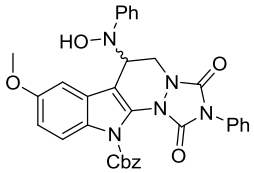
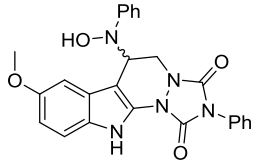
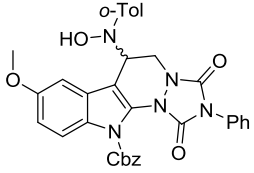
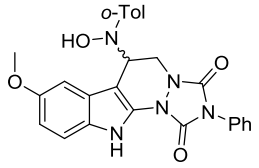
 (245)	PtO_2, H_2 (1 atm), 5 h	 (265)	60%
 (246)	PtO_2, H_2 (1 atm), 5 h	 (266)	85%
 (253)	PtO_2, H_2 (1 atm), 5 h	 (267)	38%[81]
 (254)	PtO_2, H_2 (1 atm), 5 h	 (268)	64%

Table 3.8 - Hydrogenation reaction conditions.

The hydrogenation reactions all gave the desired deprotected ene adducts as single diastereomers.

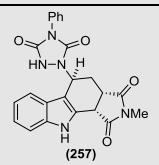
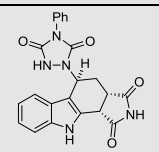
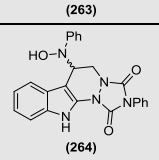
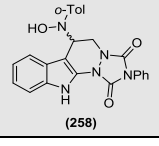
3.11.0 Biological Activity-

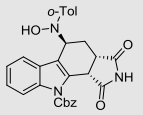
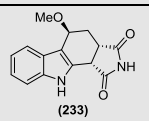
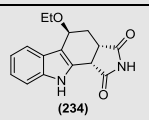
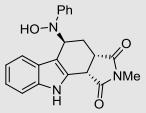
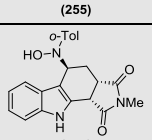
With fourteen deprotected ene adducts, plus methoxy (**233**) and ethoxy (**234**) side products in hand, we tested the compounds for biological activity. The compounds were subjected to a Kirby-Bauer disk diffusion assay against *Staphylococcus aureus* (*S. aureus*), *Schizosaccharomyces pombe* (*S. pombe*) and *Escherichia coli* (*E. coli*) at a concentration of 10 mg/ mL.

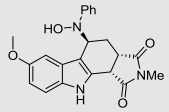
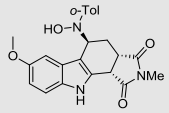
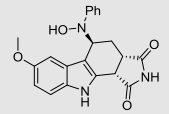
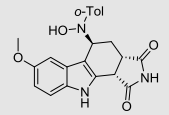
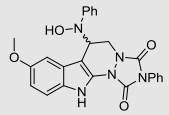
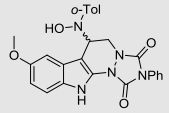
The deprotected compounds, along with Cbz protected compound (**232**), were dissolved in DMSO, spotted on filter paper disks and placed on Agar plates inoculated with *S. aureus*, *S. pombe* or *E.Coli* Each plate was also spotted with an appropriate positive control for comparison (nystatin for *S. pombe*, Kanamycin for *S. aureus* and Ampicillin for *E. coli*) and incubated overnight at 30 ^{o}C. The zones of inhibition could then be measured for each compound giving an indication of their relative biological activity.

As expected, compound (**232**) which was Cbz protected, showed no activity against any of the bacteria and yeast. This backed up the data from the previous bio-assay of the tosyl, benzyl and DMAS protected compounds, which suggested the indolic NH must be deprotected for the compound to show biological activity (Chapter 3.9.0).

The deprotected samples showed moderate biological activity against the two bacteria and yeast (Table 3.9).

Compound	Zone of inhibition/ cm		
	S. pombe	***S. aureus***	***E. coli***
 (257)	-	1.2	-
 (263)	-	-	-
 (264)	-	1.4	-
 (258)	-	1.4	-

Compound	Zone of inhibition/ cm		
	S. pombe	***S. aureus***	***E. coli***
 (232)	-	-	-
 (233)	-	0.7	0.6
 (234)	-	0.6	-
 (255)	-	0.7	-
 (256)	1.1	-	-

 (259)	0.9	1.1	-
 (260)	0.7	1.2	-
 (265)	0.8	1.5	-
 (266)	1.2	2.0	-
 (267)	-	1.1	-
 (268)	-	1.2	-
Positive control zone of inhibition/ cm			
Nystatin	3.5	-	-
Kanamycin	-	3.0	-
Ampicillin	-	-	2.3

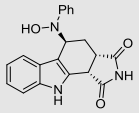
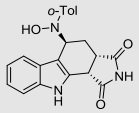
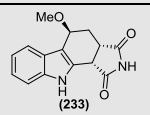
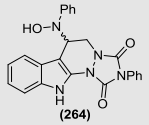
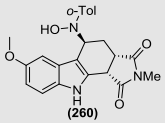
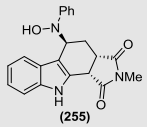
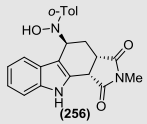
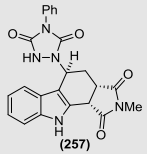
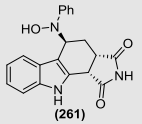
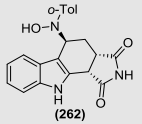
 (261)	-	0.6	-
 (262)	1.4	1.6	0.6
Positive control zone of inhibition/ cm			
Nystatin	0.8	-	-
Kanamycin	-	2.1	-
Ampicillin	-	-	4.0

Table 3.9 - Results from disk diffusion assay.

The results from the disk diffusion assay gave a good indication of which compounds were the most biologically active. Following on from the disk diffusion assay, the minimum inhibitory concentration (MIC) of the tested compounds was determined against *Staphylococcus aureus* and *Escherichia coli* (Table 3.10).[82]

Compound	MIC in µg/ mL	MIC in µg/ mL
	S. aureus	***E. coli***
 (233)	>125	>125
 (264)	25	-
 (260)	100	-
 (255)	125	>125
 (256)	62.5	>125
 (257)	>100	-
 (261)	125	>125
 (262)	15.6	125

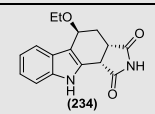
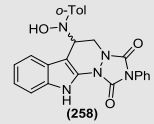
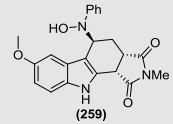
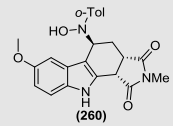
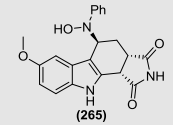
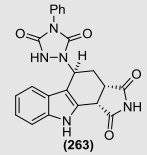
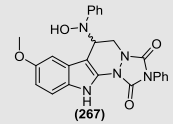
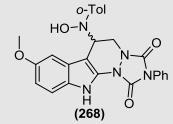
Compound	MIC in µg/ mL	MIC in µg/ mL
	S. aureus	***E. coli***
 (234)	>125	>125
 (258)	25	-
 (259)	100	-
 (260)	100	-
 (265)	100	-
 (263)	>100	-
 (267)	50	-
 (268)	50	-

Table 3.10 - MIC of tested compounds against S. aureus and E. Coli.

The results from the MIC experiment suggest that the methoxy group in the 5 position of the indole ring has a detrimental effect upon the biological activity of the compounds. Compounds (**258**) and (**264**), deriving from the Diels-Alder reaction with PTAD, have a MIC of 25 µg/ mL against *S. aureus* without the methoxy group. However, when the 5-methoxy group is added (compounds (**267**) and (**268**)) the MIC rises to 50 µg/ mL against *S. aureus*. The same trend is observed when comparing hydroxylamine compounds (**255**), (**256**), (**261**) and (**262**) with 5-methoxy substituted compounds (**259**), (**260**), (**265**) and (**266**).

Another trend that can be observed from the MIC results is that compounds (**257**) and (**263**), derived from the ene reaction with PTAD, are not as active as the compounds derived from the ene reaction with the nitroso compounds. The hydroxylamine compounds have an average MIC of 63 µg/ mL, which is significantly better than the >100 µg/ mL that is seen from the PTAD derived compounds.

From these results it is possible to build an initial SAR diagram of the tetrahydrocarbazole framework with the various groups we have tested (Figure 3.15).

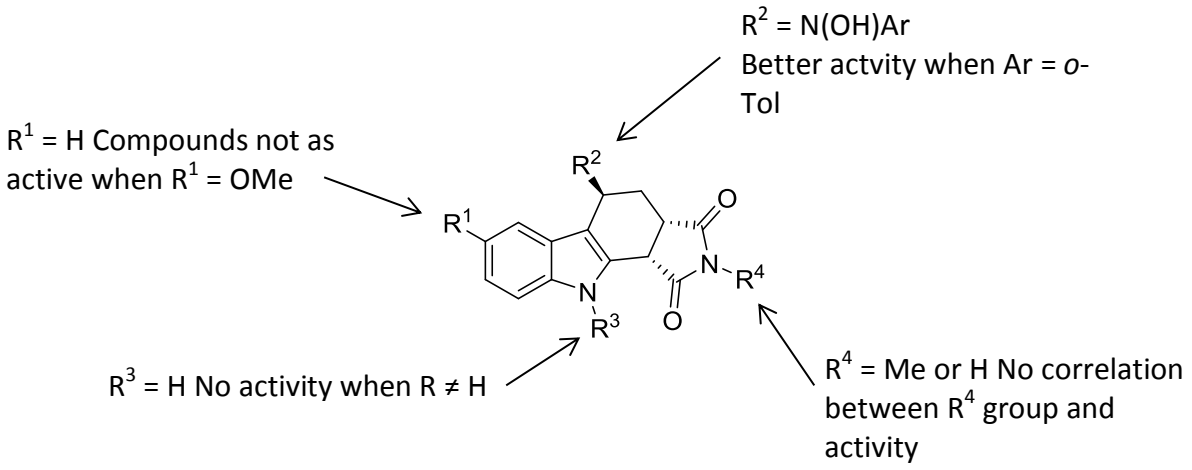
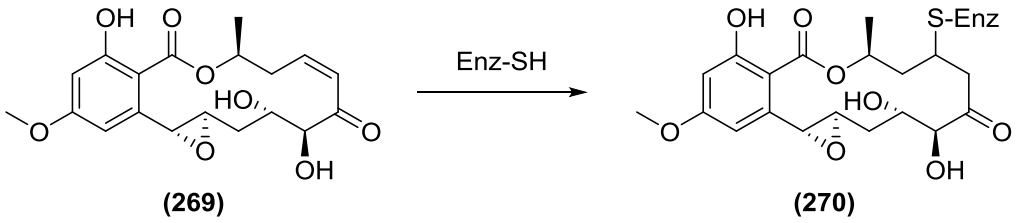


Figure 3.15 - SAR diagram of tetrahydrocarbazole framework.

3.11.1 Proposed mode of action-

We suggest the difference in biological activity between the PTAD and hydroxylamine ene adducts can be explained through a difference in the mode of action of the compounds. It is possible that the mode of action of compounds (**255**) – (**268**) that we synthesised is a conjugate addition mechanism. There are many compounds in clinical trials that use an electrophilic site on the drug molecule to react with nucleophiles at the target binding site.[83-87] These compounds, such as the kinase inhibitor hypothemycin (**269**) shown below, bind irreversibly to protein kinases through a Michael addition on a 1,4-unsaturated carbonyl system, which leads to inhibition of the kinase (Scheme 3.42).[84] Hypothemycin (**269**) inhibits a range of human kinases including MEK and ERK.

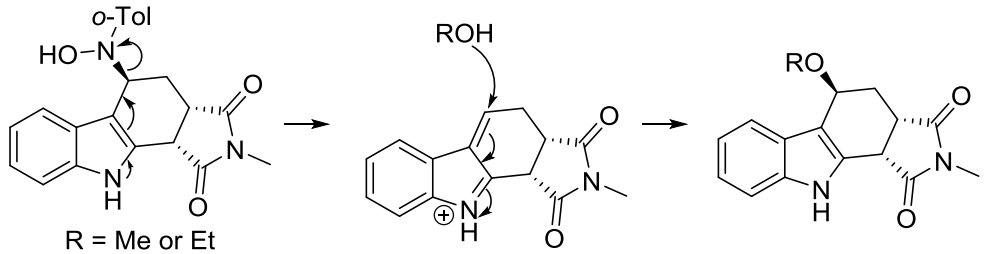


(269) **(270)**

Scheme 3.42 - Mode of action for hypothemycin (269).

Molecules such as hypothemycin (**269**) are competitive inhibitors of ATP, binding to the protein kinases through a nucleophilic cysteine. This blocks any ATP from binding to the kinase, which has an inhibitory effect.

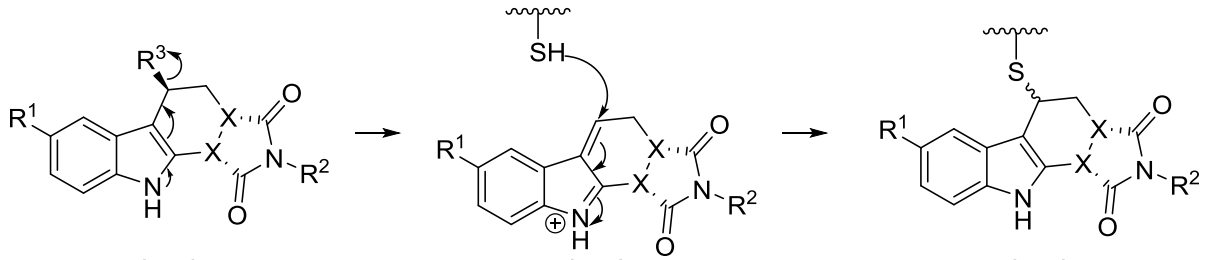
We propose that our compounds synthesised *via* a one-pot Diels-Alder / ene reaction are active through a similar pathway. We have shown previously, with the synthesis of methoxy and ethoxy substituted products (**233**) and (**234**), that the hydroxylamine groups are labile (Scheme 3.43).



(232) **(236)**

Scheme 3.43 - S_N1 reaction to form compounds (233) and (234).

We believe that compounds such as (**232**) can undergo the same substitution reaction in a biological system and competitively bind to the ATP binding site on kinases (Scheme 3.44).



(271) **(272)** **(273)**

R^1 = H, OMe; R^2 = H, Me; R^3 = N(OH)Ph, N(OH)*o*-Tol, PTAD; X = C, N

Scheme 3.44 - Proposed mode of action for compounds (255) – (268).

If our proposed mode of action was correct, it would explain why compounds (**257**) and (**263**), where R^3 = PTAD (Scheme 3.44), show less activity than those where R^3 = hydroxylamine. The activity is dependent upon the elimination of R^3 (Scheme 3.44) before the resulting compound (**272**) can covalently bind to the ATP binding site in the biological

system through a nucleophilic sulphur from a cysteine amino acid. The hydroxylamine groups are better leaving groups than PTAD and this would explain why the PTAD compounds (**257**) and (**263**) are not as active as the hydroxylamine compounds.

3.11.2 Human kinase screening-

As several tetrahydrocarbazoles are known to be kinase inhibitors[74,88], four of our deprotected Diels-Alder / ene adducts were submitted for screening against 51 human kinases at a 10 µM concentration. Many kinases are known to have an important role in the growth and survival of tumours therefore there are many kinase inhibitors currently in clinical trials as anti-cancer drugs.[89]

Compounds (**256**) and (**262**), where the enophile used was 1-methyl-2-nitrosobenzene, showed little or no activity against any of the kinases. However compounds (**255**) and (**261**), where the enophile used was nitrosobenzene, both showed modest activity against MLK3 and Src kinases. These results indicate that changing the aromatic ring from a phenyl to an *o*-tolyl has a large effect upon the activity of the compounds.

Additionally, compound (**255**) showed activity against JAK2 kinase and compound (**261**) showed activity against Aurora B kinase (Table 3.11).

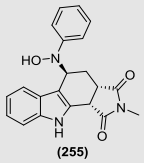
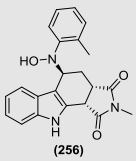
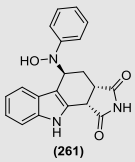
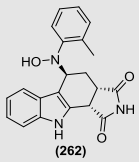
	% Activity Remaining (10 µM)			
	 (255)	 (256)	 (261)	 (262)
Aurora B	32±2	56±2	21±1	67±2
JAK2	28±13	81±8	68±10	98±15
MLK3	28±6	64±2	17±3	73±7
Src	15±6	57±5	17±1	76±0

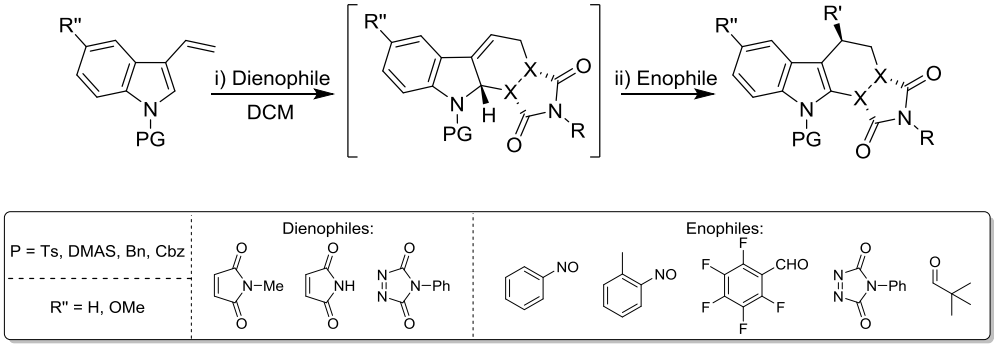
Table 3.11 - Table showing activity against a range of kinases.

Although the human kinome contains over 500 kinases, identifying selective kinase inhibitors is not trivial and remains a very active area of research.[90] Compound (**261**) showed good activity against Aurora B, MLK3 and Src but limited activity against JAK2. This selectivity demonstates that compounds (**255**), (**256**), (**261**) and (**262**) do not intrinsically inhibit all kinases. This makes the compoundssuitable as potential drug compounds as targeting of specific kinases is required for drug compounds. In addition, by inhibiting certain kinases and monitoring the changes in the biological system when compared to a control, allows a greater understanding of the function the inhibited kinase has in the organism.[91,92]

Although (**255**), (**256**), (**261**) and (**262**) showed selective inhibition of several kinases, there are other properties that need to be addressed before the compounds can be considered as potential drug molecules. The Diels-Alder / ene adducts would need to be developed further so that they are active at nM concentrations, for them to be considered as viable drug compounds.

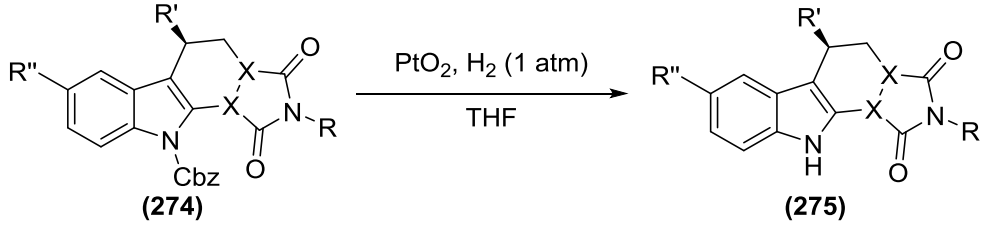
3.12.0 Conclusion-

Using the same Diels-Alder / ene chemistry that was developed in the previous chapter, we have built upon the chemistry to make the reaction sequence a one-pot procedure. The one-pot procedure led to an increase in the overall yields, from around 50% for the two step procedure to 75%, as well as a reduction in waste and operational complexity. Using the one-pot procedure, a range of Diels-Alder / ene compounds were rapidly synthesised in yields ranging from 29-89% (Scheme 3.45).



Scheme 3.45 - General scheme for one-pot Diels-Alder / ene reaction.

Of the Diels-Alder / ene compounds synthesised the Ts, DMAS and Bn were tested for biological activity against *S. Aureus*, *S. Pombe* and *E. Coli* but were found to show no activity. We reasoned this was due to the protecting groups on the indolic nitrogen of the compounds. Attempts to remove the Ts and DMAS protecting groups were unsuccessful. Therefore a new protecting group had to be found that could be easily removed, whilst still allowing the one-pot Diels-Alder / ene reactions to occur. Cbz was used to protect the indolic NH during the Diels-Alder / ene reactions and could be easily removed using hydrogenation chemistry (Scheme 3.46).



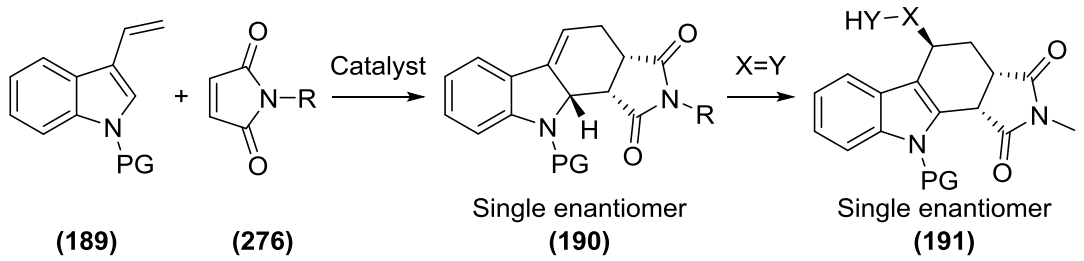
Scheme 3.46 - General scheme for Cbz protecting group removal.

The fourteen deprotected compounds formed from the hydrogenation reaction, were then tested for biological activity in a disk diffusion assay. The compounds showed promising biological activity against *S. Pombe* and *S. Aureus* with compound (**262**) having the best MIC of 15.6 µg/ mL. This confirmed our theory that for the Diels-Alder / ene adducts to be biologically active, a free indolic NH is essential. Four of the compounds were then submitted for screening against 51 human kinases; (**255**) and (**261**) showed moderate activity against Aurora B, JAK2, MLK3 and Src kinases which are important targets in anti-cancer research.

Chapter 4 - Enantioselective Diels-Alder / Ene Chemistry

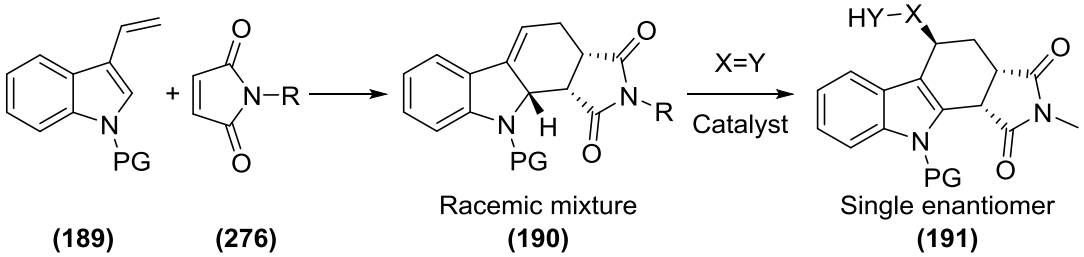
4.1.0 Enantioselective Diels-Alder / ene reaction-

In Chapters 1 and 2, we developed an efficient one-pot Diels-Alder / ene reaction process that led to the formation of a range of tetrahydrocarbazole compounds which showed moderate biological activity. We next want to investigate if we can make this process enantioselective, so that only one enantiomer of the tetrahydrocarbazole compound is formed in the reaction. We hope this will lead to an improvement in the biological activity of the compounds. This can be done by two different methods. The first method is making the Diels-Alder reaction an enantioselective process so that only one enantiomer (**190**) is formed. Then, as the ene reaction is stereospecific, ene adduct (**191**) will be produced as a single enantiomer (Scheme 4.1).



Scheme 4.1 - Catalysed Diels-Alder reaction followed by ene reaction to form enantiopure tetrahydrocarbazole (191).

The second method involves carrying out the Diels-Alder reaction with no catalyst to form racemic Diels-Alder product (**190**). Then an enantioselective catalyst can be used in the ene step leading to the formation of enantiopure product (**191**) (Scheme 4.2).



Scheme 4.2 - Racemic Diels-Alder reaction followed by catalysed ene reaction to form enantiopure tetrahydrocarbazole (191).

However, one disadvantage of making the ene reaction the enantioselective step is the fact that only half of racemic Diels-Alder adduct (**190**) will react in the ene reaction. Due to this disadvantage in making the ene reaction the enantioselective step, we chose to concentrate on making the initial Diels-Alder reaction the enantioselective process.

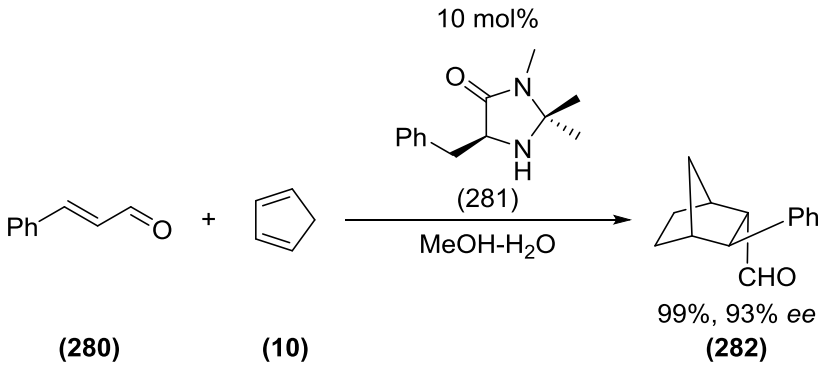
4.2.0 Enantioselective organocatalysts-

Enantioselective organocatalysts have many advantages over traditional metallic or enzymatic catalysts including their lack of toxicity, ease of availability, ease of synthesis and their tolerance of many functional groups.[93] We proposed using an organocatalyst due to these reasons as well as the large amount of literature available on using enantioselective organocatalysts in Diels-Alder reactions.[94-97] Therefore we needed to identify an enantioselective organocatalyst that would allow the enantioselective synthesis of tetrahydrocarbazoles.

There are three main classes of enantioselective organocatalysts that each use different methods to make a reaction enantioselective; singly occupied molecular orbital (SOMO) catalysts, hydrogen bonding catalysts and covalent catalysts.

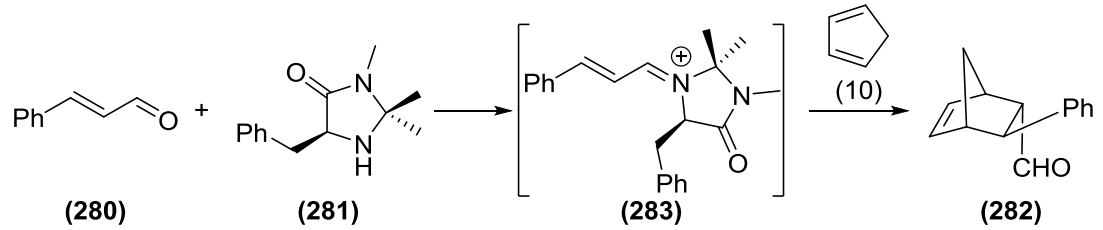
4.2.1 Covalent catalysts-

Natural products such as proline have become popular tools in organocatalysis as they are produced enantiomerically pure in nature. Proline and organocatalysts derived from proline, work through the formation of covalent bonds with the substituents. This then forces the reaction to occur through a particular geometry leading to enantiopure products being formed. The first example of this type of organocatalysed process utilised in Diels-Alder chemistry was by MacMillan *et al*., in what they described as "the first highly enantioselective organocatalytic Diels-Alder reaction" (Scheme 4.4).[98]



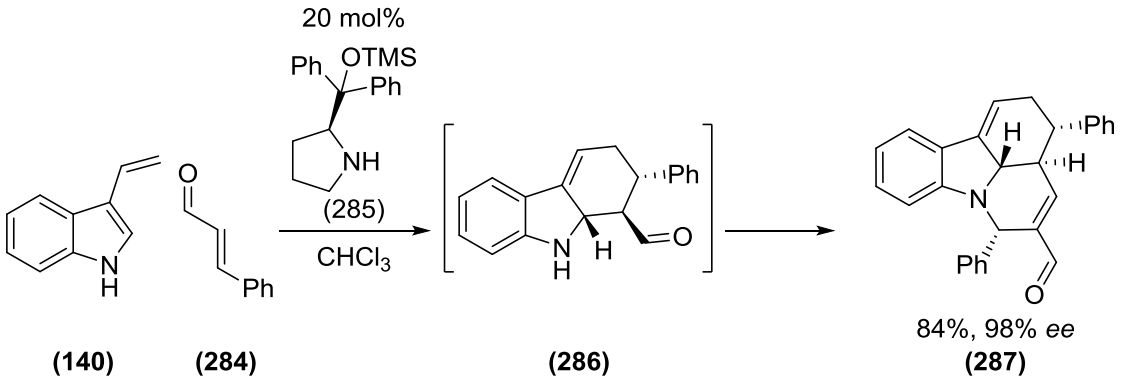
Scheme 4.4 - MacMillan et al. first example of highly enantioselective Diels-Alder reaction.

Organocatalyst (**281**) works through a reaction with (**280**), forming iminium ion (**283**) (Scheme 4.5). The formation of iminium (**283**) activates the dienophile towards the Diels-Alder reaction by lowering the energy level of the LUMO, as well as dictating the orientation of attack by blocking one face of iminum (**283**). This leads to the generation of (**282**) as a single enantiomer.



Scheme 4.5 - The mechanism by which catalyst (281) catalyses the Diels-Alder reaction.

There have also been examples of enantioselective Diels-Alder reactions with 3-vinyl-indole compounds similar to the ones which were used in the Chapters 1 and 2. Enders *et al*.[99] used a proline derived organocatalyst in the reaction between 3-vinyl-1*H*-indole (**140**) and cinnamaldehyde (**284**) (Scheme 4.6).

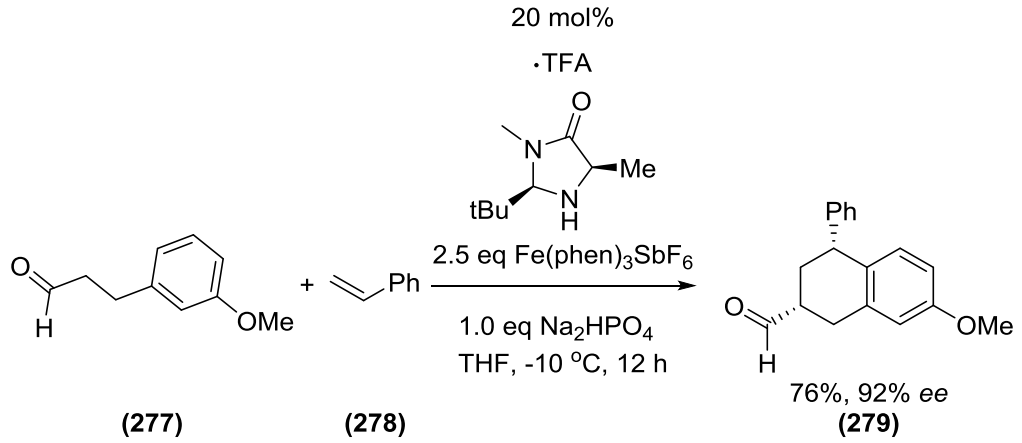


Scheme 4.6 - Organocatalysed Diels-Alder/aza-Michael/aldol condensation reaction.

In the reaction developed by Enders *et al*., organocatalyst (**285**) does not just catalyse the Diels-Alder reaction, but also catalyses the Michael addition and aldol condensation reactions through iminium activation and enamine activation. This leads to the formation of product (**287**), which has four new stereocentres, in an excellent *ee*.

4.2.2 SOMO-organocatalysis-

SOMO-catalysis is based upon a one electron oxidation of an enamine system to form a highly reactive radical cation. This process activates a singly occupied molecular orbital which can rapidly react with a range of weak nucleophiles. There are few examples of SOMO-catalysed Diels-Alder reactions in the literature. The few that are available in the literature are not traditional concerted Diels-Alder reactions between a dienophile and a diene. Instead, an alkene and an aldehyde react through a step-wise manner (Scheme 4.3).[100,101]



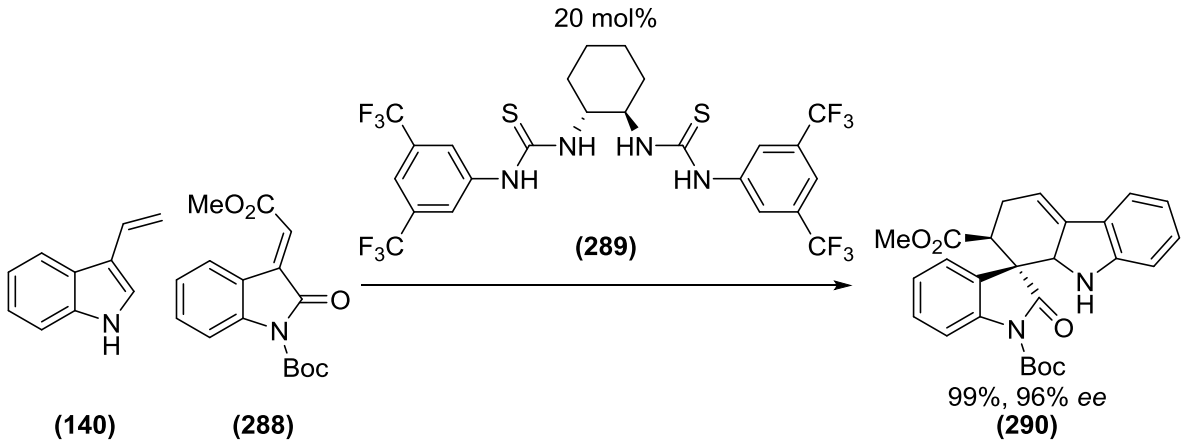
Scheme 4.3 - SOMO-catalysed cycloaddition between aldehyde (277) and alkene (278).

The enantioselectivity in SOMO catalysis derives from the organocatalyst either hindering one face of the substrate or directing a substrate on to one face preferentially.

4.2.3 Hydrogen bonding catalysts-

The final major class of organocatalyst is one that uses hydrogen bonding to hold one or both of the reactants in a particular geometry. A lot of hydrogen bonding organocatalysts are based upon a thiourea framework, such as catalyst (**289**) developed by Barbas *et al.*[102], which has four NH units that can form hydrogen bonds.

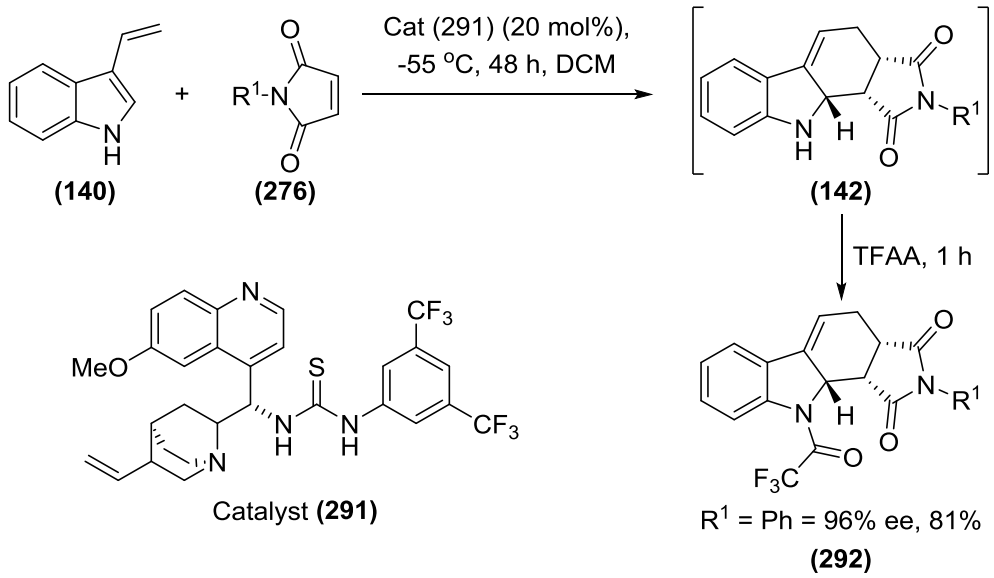
Barbas *et al*.[102] have utilised organocatalyst (**289**) in the Diels-Alder reaction between 3-vinyl-1*H*-indole (**140**) and methyleneindolinone (**288**) to form a range of compounds, such as (**290**), containing a carbazolespirooxindole framework (Scheme 4.7).



Scheme 4.7 - Barbas et al. organocatalysed Diels-Alder reaction to form carbazolespirooxindoles (290).

Catalyst (**289**) works by forming hydrogen bonds with the reactants which fixes the orientation of the Diels-Alder reaction, leading to the formation of (**290**) as a single enantiomer.

Work by Ricci *et al.*[103,104] has demonstrated, through use of organocatalyst (**291**) that was developed by Soos *et al.*[105], a method for the stereocontrolled Diels-Alder reaction between 3-vinyl-1*H*-indole (**140**) and a range of maleimide based dienophiles (Scheme 4.8). Diels-Alder adduct (**142**), initially formed in the reaction, is then subjected to an indolic *N*-protection with trifluoroacetic anhydride (TFAA) to give product (**292**).



Scheme 4.8 - Ricci's reaction to form enantiomerically pure compounds of type (292).

Ricci *et al*.[103] postulated that catalyst (**291**) works through hydrogen bonding both the 3-vinyl-1*H*-indole (**140**) and maleimide compound (**276**) in the transition state, which fixes the orientation of the Diels-Alder reaction, resulting in a single enantiomer being formed (Figure 4.1).

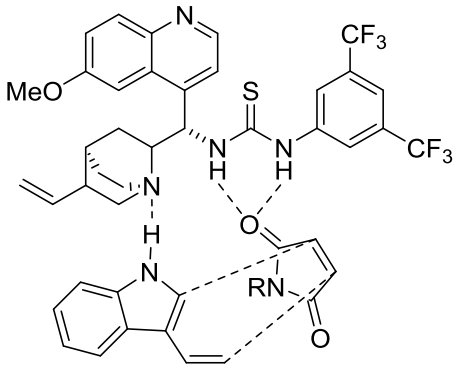


Figure 4.1 - Ricci's proposed transition state for the Diels-Alder reaction between 3-vinyl-1H-indole (140) and maleimide (276).

4.3.0 Enantioselective organocatalysed Diels-Alder / ene plan-

Previous work[81] in the group has attempted to use the work by Barbas *et al*. to develop enantioselective Diels-Alder / ene chemistry. However this was not successful due to the capricious nature of the Diels-Alder reaction, and unwanted rearomatisation of the initial

Diels-Alder adduct formed before the ene reaction could occur. Also, the carbazolespirooxindoles generated in Barbas's catalytic procedure do not have many structural similarities with the tetrahydrocarbazoles (**190**) we synthesised in Chapters 1 and 2 (Figure 4.2).

The work by Enders *et al*. was also not compatible with our Diels-Alder / ene approach as the proline derived catalyst used requires a free aldehyde group to attack. Our Diels-Alder reactions between *N*-protected 3-vinyl-indoles and maleimide compounds means there is no free carbonyl group for the catalyst to attack.

Ricci's procedure led to the formation of single enantiomer tetrahydrocarbazole compounds (**292**) which are similar to the compounds (**190**) we had been synthesising using our one-pot Diels-Alder / ene approach (Figure 4.2). We therefore decided to use Ricci's catalytic procedure as a means of synthesising single enantiomer Diels-Alder adducts (**292**).

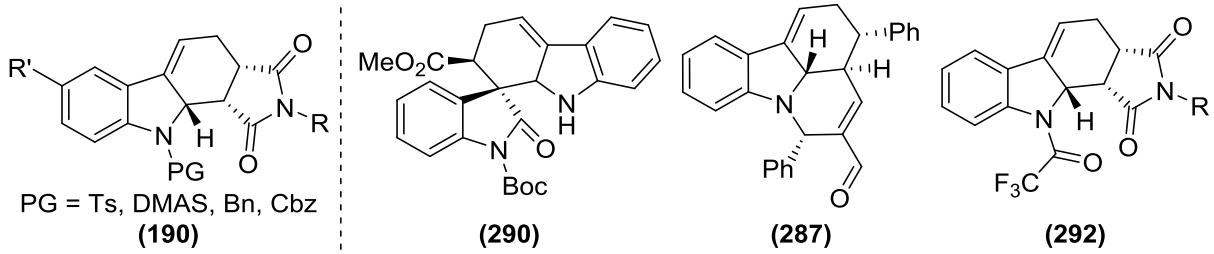
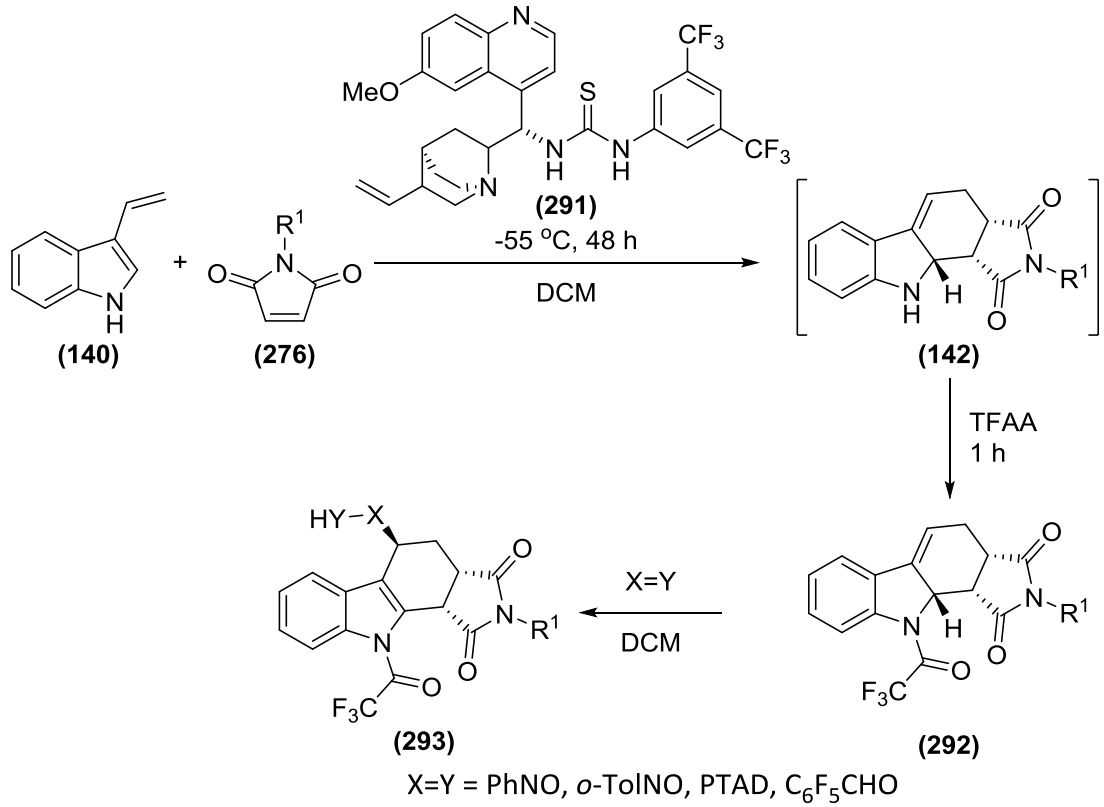


Figure 4.2 - Compounds (190) synthesised through our one-pot Diels-Alder ene procedure and compound (290) synthesised by Barbas, compound (287) synthesised by Enders and compounds (292) synthesised by Ricci.

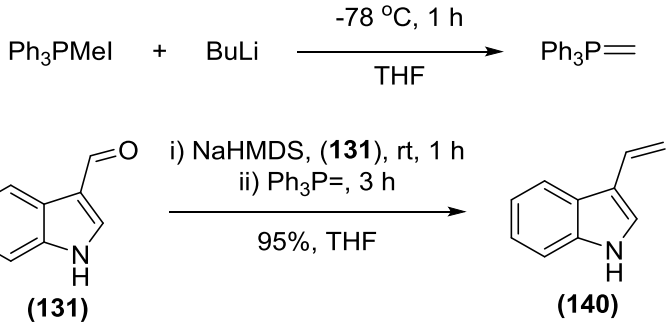
We believed we could use Ricci's organocatalytic procedure to synthesise enantiopure compounds such as (**190**). With single enantiomer Diels-Alder adducts (**190**) in hand, we could then subject them to ene reactions with the same nitroso, aza, and carbonyl enophiles used in Chapters 2 and 3, to produce a range of single enantiomer Diels-Alder / ene adducts (**293**) (Scheme 4.9).



Scheme 4.9 - Proposed route to single enantiomer Diels-Alder / ene adducts (293).

4.4.0 Enantioselective organocatalysed Diels-Alder chemistry-

We needed to synthesise 3-vinyl-1*H*-indole (**140**) in a good yield. Ricci *et al*. has shown that deprotonating 1*H*-indole-3-carbaldehyde (**131**) and reacting the ion formed with methylenetriphenyl-λ^5-phosphane, leads to the formation of 3-vinyl-1*H*-indole (**140**) in an excellent yield (Scheme 4.10).

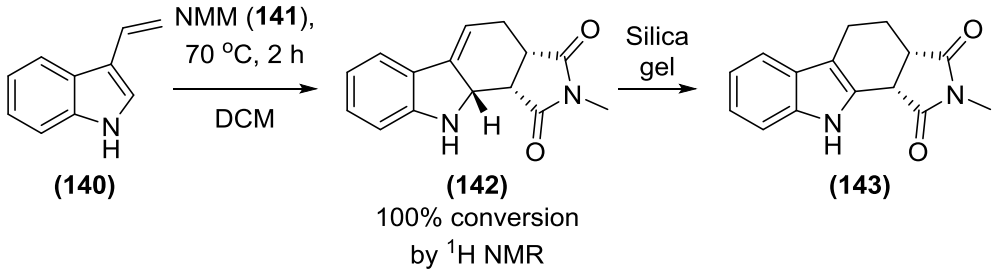


Scheme 4.10 - Wittig reaction to form 3-vinyl-1H-indole (140) from 1H-indole-3-carbaldehyde (131).

The deprotonation is a means of temporarily protecting the NH and stopping it from being deprotonated by methylenetriphenyl-λ^5-phosphane. After the Wittig reaction has occurred, the indolic nitrogen is protonated during the work up.

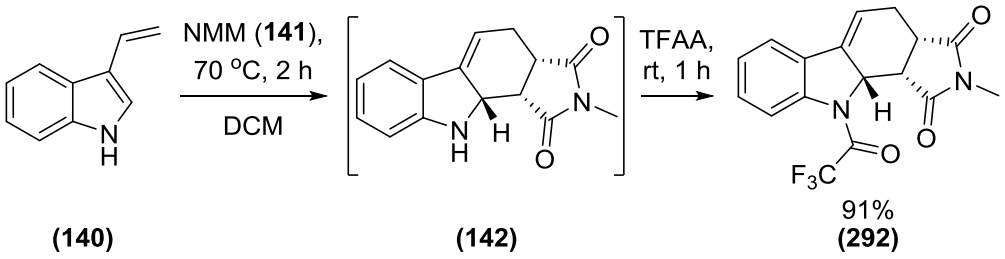
With 3-vinyl-1*H*-indole (**140**) in hand, we first decided to test the Diels-Alder / ene chemistry as a non-enantioselective process, before using catalyst (**291**) to make the process enantioselective. This would allow us to optimise the Diels-Alder / *N*-protection / ene chemistry before using the catalyst.

In Chapter 2 we discussed the Diels-Alder reaction between 3-vinyl-1*H*-indole (**140**) and NMM (**141**) (Scheme 4.11). Diels-Alder adduct (**142**) could be observed by ^{1}H NMR however attempts to isolate it led to rearomatisation of the indole ring to form (**143**).



Scheme 4.11 - Diels-Alder reaction between 3-vinyl-1H-indole (140) and NMM (141).

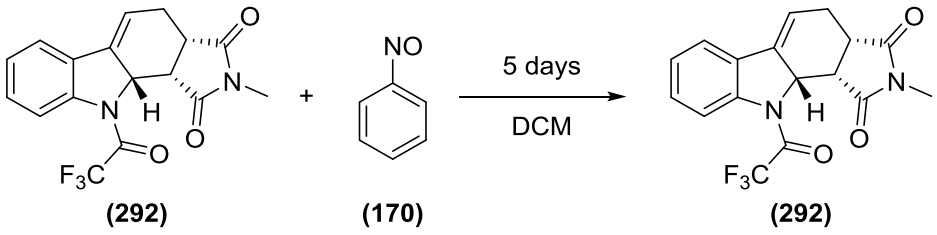
The Diels-Alder reaction between 3-vinyl-1*H*-indole (**140**) and NMM (**141**) was repeated and after two hours the reaction was cooled to room temperature before TFAA was added (Scheme 4.12).



Scheme 4.12 – One-pot Diels-Alder / N-protection reaction to form (292).

The reaction gave the desired *N*-protected Diels-Alder adduct (**292**) as a racemic mixture in an excellent yield of 91%.

With Diels-Alder adduct (**292**) in hand, we then attempted an ene reaction between Diels-Alder adduct (**292**) and nitrosobenzene (**170**) (Scheme 4.13).



Scheme 4.13 - Ene reaction between Diels-Alder adduct (292) and nitrosobenzene (170).

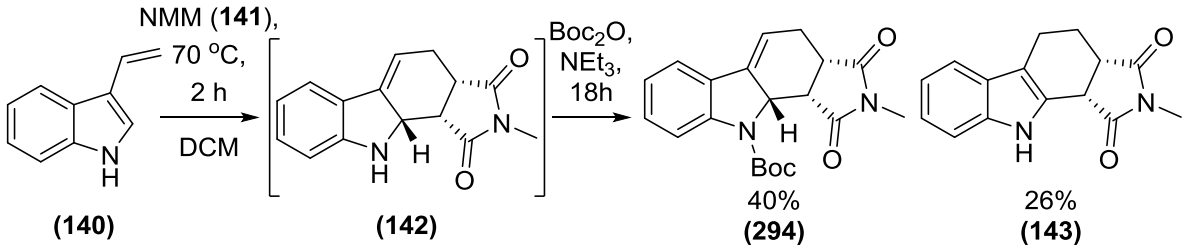
The ene reaction was stirred at room temperature for five days, however no reaction occurred and Diels-Alder adduct (**292**) was recovered. We propose this is due to the electron withdrawing nature of the trifluoroacetyl protecting group on the indolic nitrogen. Previous work (Chapters 1 and 2) has shown the significant effect the electronics of the *N*-protecting group have upon the rate of both the Diels-Alder and ene reactions. The electron withdrawing nature of the trifluoroacetyl group pulls electron density away from the indole ring which prohibits the ene reaction from occurring within a reasonable timeframe.

As Diels-Alder adduct (**292**) would not undergo an ene reaction, an alternative, less electron withdrawing protecting group had to be found.

4.4.1 Diels-Alder / Boc protection of 3-vinyl-1H-indole (140)-

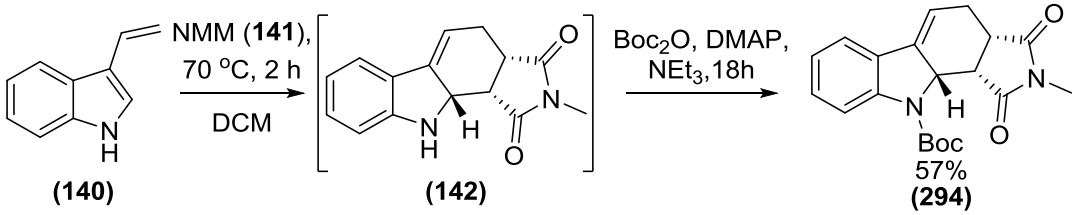
We needed a protecting group that had similar chemistry to the trifluoroacetyl group, however it needed to be less electron withdrawing to allow further ene reactions to occur. We therefore investigated the Boc protecting group. The Boc group has similar chemistry to TFAA in that it is also an anhydride, therefore we hoped that this would make it compatible with organocatalyst (**291**).

The Diels-Alder reaction between 3-vinyl-1*H*-indole (**140**) and NMM (**141**) was repeated. Upon conversion to Diels-Alder adduct (**142**), di-*tert*-butyl dicarbonate and triethylamine were added (Scheme 4.14).



Scheme 4.14 - Diels-Alder / N-protection reaction between 3-vinyl-1H-indole (140) and NMM (141) to give (294).

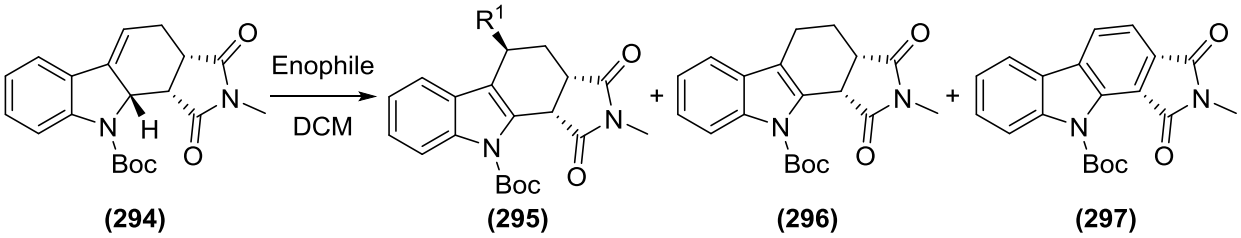
The reaction did form desired Diels-Alder adduct (**294**) in a poor yield of 40%. Rearomatised Diels-Alder adduct (**143**) was also isolated in a 26% yield which suggested that the final Boc protection step was not occurring before Diels-Alder adduct (**142**) rearomatised to (**143**). Therefore, to try to increase the rate of the protection step, the reaction was attempted again and a catalytic amount of DMAP was added to the final protection step (Scheme 4.15).



Scheme 4.15 - Diels-Alder / protection reaction with Boc anhydride and catalytic DMAP.

The addition of DMAP to the reaction led to an increase in the yield of Diels-Alder adduct (**294**) by 17% on the previous attempt. There was a reduction of rearomatised Diels-Alder adduct (**143**) isolated from the reaction to just 8%.

With a method for the synthesis of Diels-Alder adduct (**294**) in a moderate yield developed, we next investigated the ene reaction between Diels-Alder adduct (**294**) and a range of enophiles (Scheme 4.16). The enophiles chosen were nitrosobenzene (**170**), 1-methyl-2-nitrosobenzene (**171**) and PTAD (**150**) which we had previously used successfully (Chapters 2 and 3).



Scheme 4.16 - Proposed ene reaction with Diels-Alder adduct (294).

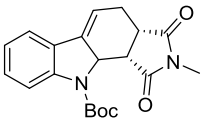
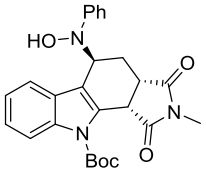
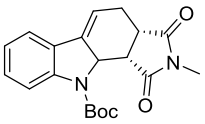
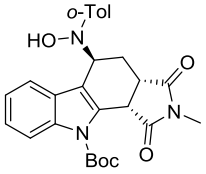
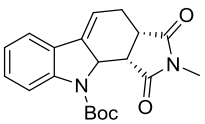
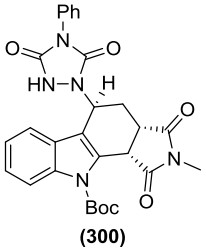
Starting Material	Ene Conditions	Ene Adduct	Ene Adduct yield	Yield (296)	Yield (297)
 (294)	PhNO (**170**), rt, 4 h	 (298)	47%	10%	23%
 (294)	*o*-TolNO (**171**), rt, 3 h	 (299)	37%	5%	14%
 (294)	PTAD (**150**), 0 °C, 4 h	 (300)	46%	9%	11%

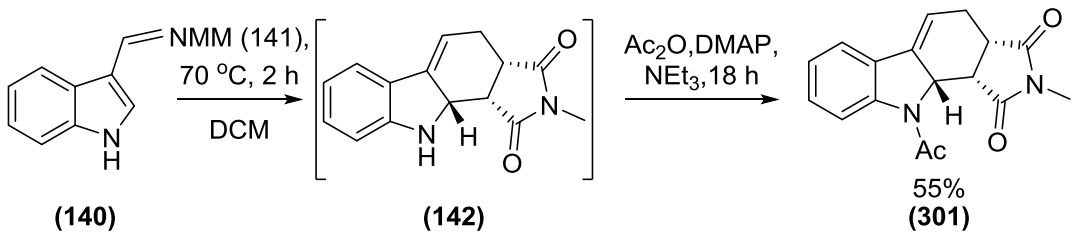
Table 4.1 - Reaction conditions for ene reactions with Diels-Alder adduct (294).

The ene reactions gave desired products (**298**) – (**300**) in generally poor yields of between 37 – 47%. The low yields for the ene reactions are partially a result of unwanted side products (**296**) and (**297**) being formed as well as further unidentified products. The formation of these side products suggested that Diels-Alder adduct (**294**) was not stable in solution or when exposed to enophiles.

Due to the moderate yields of the Diels-Alder reaction to form (**294**) and the poor yields for the ene reactions, work on the Boc protecting group was stopped. We chose instead to investigate the acetyl protecting group.

4.4.2 Diels-Alder / acetyl protection of 3-vinyl-1H-indole (140)-

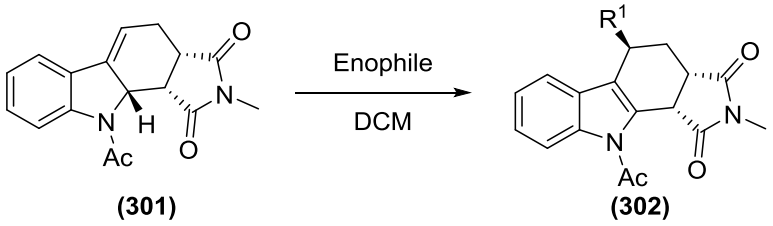
To test the suitability of an acetyl protecting group in the Diels-Alder / *N*-protection and ene chemistry, the Diels-Alder reaction between 3-vinyl-1*H*-indole (**140**) and NMM (**141**) was repeated as previously, before acetic anhydride, triethylamine and DMAP were added (Scheme 4.17).



Scheme 4.17 - Diels-Alder / acetyl protection reaction between 3-vinyl-1H-indole (140) and NMM (141).

The reaction gave the desired Diels-Alder adduct (**301**) in a moderate yield of 55%. Unprotected Diels-Alder adduct (**143**) was also isolated in a 6% yield, which suggested the protection reaction was again not going to completion. The reaction was attempted once more, with a longer reaction time for the final protection step of 24 hours, however the overall yield remained unchanged.

We then moved to screening compound (**301**) in a range of ene reactions. The same enophiles as previously used with the Boc protected Diels-Alder adduct (**294**) were again utilised. The results are summarised in Table 4.2.



Scheme 4.18 - Proposed ene reaction with Diels-Alder adduct (302).

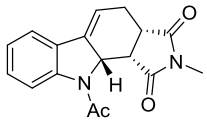
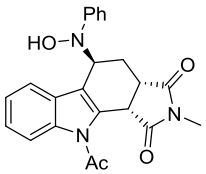
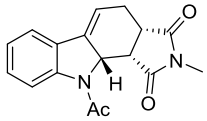
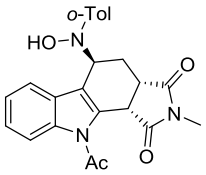
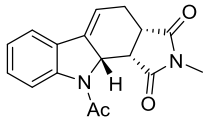
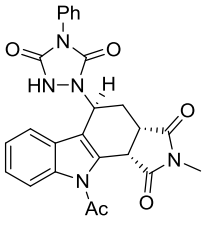
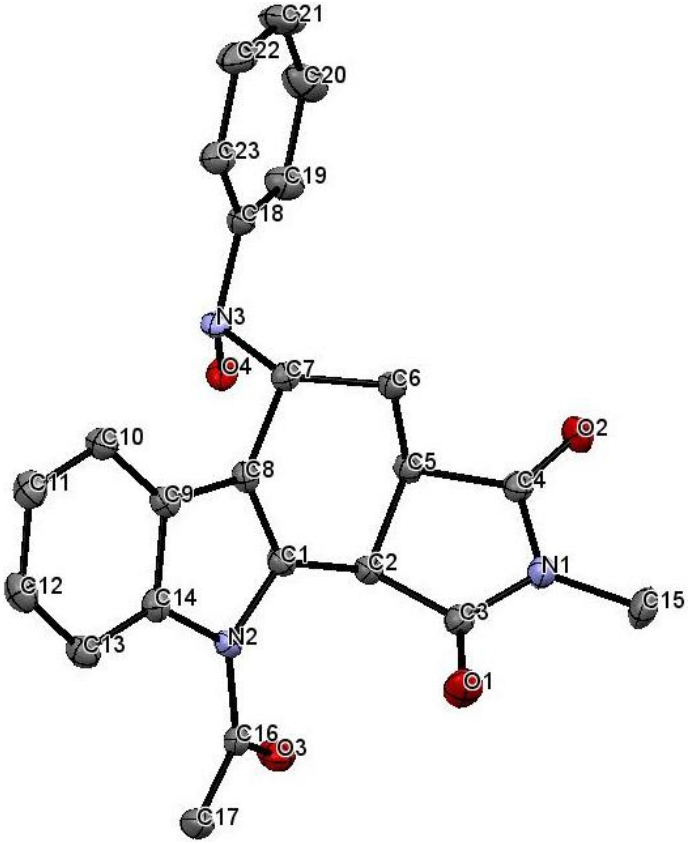
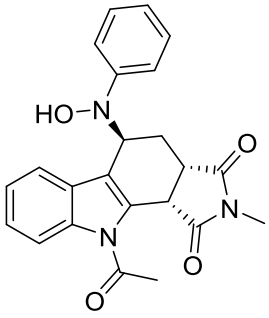
Starting Material	Ene Conditions	Ene Adduct	Yield %
 **(301)**	PhNO (**170**), rt, 18 h	 **(303)**	64%
 **(301)**	*o*-TolNO (**172**), rt, 18 h	 **(304)**	75%
 **(301)**	PTAD (**150**), 0 °C, 5 h	 **(305)**	57%

Table 4.2 - Reaction conditions for ene reactions with Diels-Alder adduct (301).

The ene reactions formed the desired ene adducts (**303**) – (**305**) as single diastereomers in yields ranging from 57 – 75%. Many attempts were made to improve the yields of the ene adducts formed, however the ene reactions were found to be variable and the yields were difficult to replicate.

To confirm the structure and relative stereochemistry of ene adduct (**303**), a single crystal was grown for analysis by X-ray diffraction, by slow evaporation from chloroform (Figure 4.3).





(303)

Figure 4.3 - X-ray crystal structure of ene adduct (303).

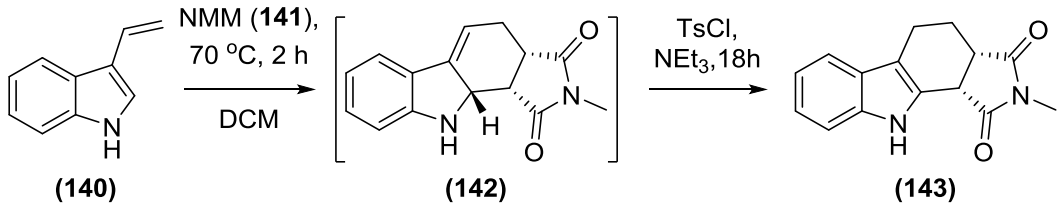
Ene adduct (**303**) crystallises in the triclinic crystal system with a $P\bar{1}$ space group, containing two molecules in the asymmetric unit. The X-ray crystal structure showed that ene adduct (**303**) has the hydroxylamine group on the opposite face of the molecule relative to the succinimide group. This is consistent with the previous X-ray crystal structures obtained from one-pot Diels-Alder / ene chemistry (Chapter 2). Interestingly the X-ray crystal structure indicates that there is hydrogen bonding between the N3 on one molecule and the O4H of the adjacent molecule. This H-bond interaction has a length of 2.793 Å.

The *N*-protection, step after the Diels-Alder reaction, had given a moderate yield of Diels-Alder adduct (**301**) of 55%. We wanted a procedure that would lead to a higher yield of Diels-Alder compound, therefore a new protecting group had to be found that would allow the reliable synthesis of the Diels-Alder and ene adducts.

4.4.3 Diels-Alder / tosyl protection of 3-vinyl-1H-indole (140)-

In previous chapters, we had a lot of success with the tosyl protecting group and had already optimised the ene reactions with tosyl protected Diels-Alder adduct (**148**). Therefore we attempted to synthesise tosyl protected Diels-Alder adduct (**148**) from 3-vinyl-1*H*-indole (**140**). The *N*-protection step had to be optimised as most tosyl protections are performed using *p*-toluenesulfonyl chloride, which is not as reactive as the anhydrides we had previously tried. We attempted to perform a Diels-Alder reaction between 3-vinyl-1*H*-

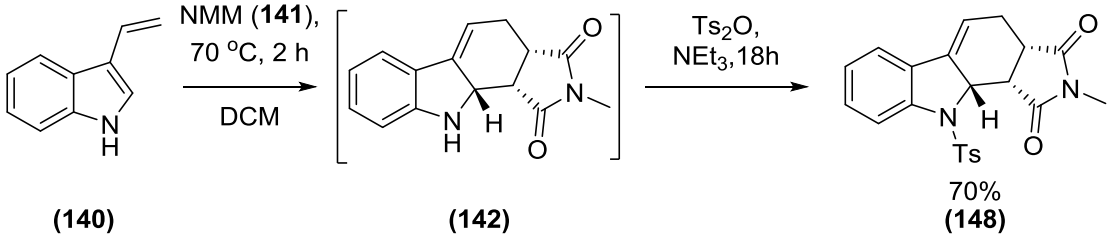
indole (**140**) and NMM (**141**), followed by tosyl protection with *p*-toluenesulfonyl chloride (Scheme 4.19).



Scheme 4.19 - Diels-Alder / N-protection between 3-vinyl-1H-indole (140) and NMM (141).

The Diels-Alder reaction was carried out as previous (Chapter 2) and after two hours *p*-toluenesulfonyl chloride and triethylamine were added. However, the ^{1}H NMR of the crude reaction mix showed that the *N*-protection reaction had not occurred and instead Diels-Alder adduct (**142**) had rearomatised to form (**143**). This suggested that *p*-toluenesulfonyl chloride was not reactive enough to perform the *N*-protection step before the Diels-Alder adduct (**142**) rearomatised to (**143**).

We therefore used a more reactive source of the tosyl protecting group in the form of *p*-toluenesulfonic anhydride. The reaction was repeated as above but in the *N*-protection step *p*-toluenesulfonic anhydride, triethylamine and DMAP were added (Scheme 4.20).



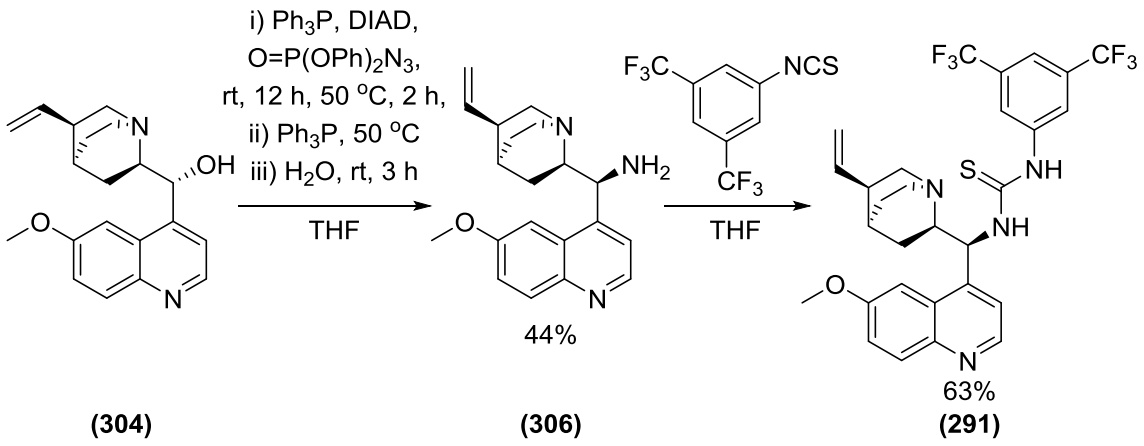
Scheme 4.20 - Diels-Alder / N-protection to form Diels-Alder adduct (148).

The Diels-Alder / *N*-protection reaction gave the desired Diels-Alder adduct (**148**) as a single diastereomer in a good yield of 70%.

With an efficient method for the synthesis of Diels-Alder adduct (**148**) starting from 3-vinyl-1*H*-indole (**140**) developed, we next wanted to see if this reaction could be performed as an enantioselective process using Ricci's procedure.[103]

4.5.0 Synthesis of Soos's organocatalyst (291)-

Firstly, we needed to synthesise Soos's organocatalyst (**291**). We employed the same method as Soos had used in his original paper[105] (Scheme 4.21). Starting from quinine (**304**), a one-pot Mitsunobu / Staudinger reduction lead to compound (**306**), which was then reacted with 3,5-bis(trifluoromethyl)phenyl isothiocyanate to give catalyst (**291**).

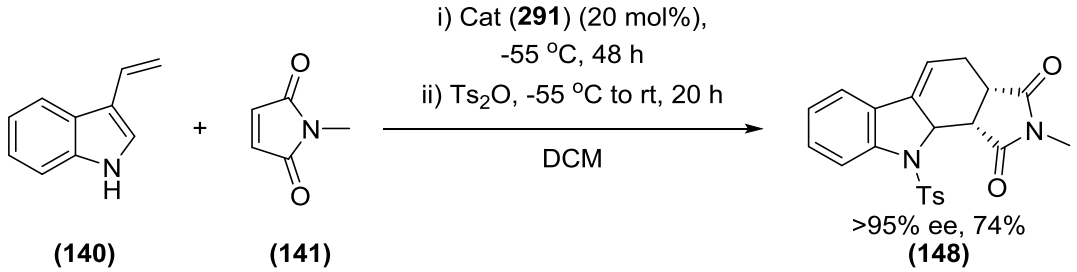


Scheme 4.21 - Synthesis of Soos's organocatalyst (291).

With catalyst (**291**) synthesised, we could then investigate the catalysed Diels-Alder / tosyl protection reaction.

4.6.0 Organocatalysed Diels-Alder chemistry-

We attempted a Diels-Alder / *N*-protection reaction using the same conditions Ricci *et al*. had optimised. 3-Vinyl-1*H*-indole (**140**), catalyst (**291**) and NMM (**141**) were stirred at -55 °C for 48 h before *p*-toluenesulfonic anhydride was added to the reaction (Scheme 4.22).

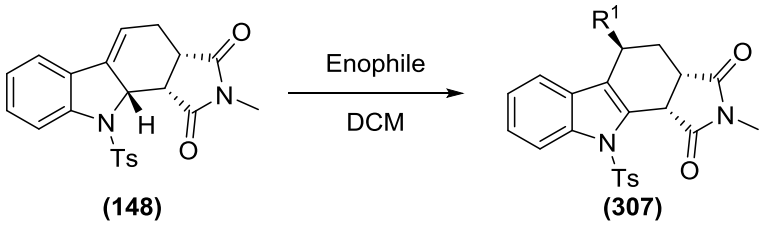


Scheme 4.22 - Organocatalysed Diels-Alder / N-protection reaction to form Diels-Alder adduct (148).

The organocatalysed Diels-Alder reaction led to the formation of desired Diels-Alder adduct (**148**) in a good yield of 74%.

Having previously synthesised Diels-Alder adduct (**148**) as a racemic product (Scheme 4.20), it allowed us to compare the chiral HPLC traces of the racemic adduct to the single enantiomer. The racemic mixture was run through an AD-H chiral column with a hexane and isopropanol solvent system. The HPLC trace clearly shows the two enantiomers at 35 and 77 minutes. We then ran the single enantiomer Diels-Alder adduct using the same column and conditions. The peak at 35 minutes remains unchanged however the second peak at 77 minutes has drastically reduced. This indicates the formation of a single enantiomer from the reaction with an *ee* of >95%. This is confirmed by the specific rotation, which was calculated be $[\alpha]_D$= +180.

With an efficient method for the synthesis of Diels-Alder adduct (**148**) as a single enantiomer in a good yield and good *ee*, we could investigate whether the single enantiomer Diels-Alder adduct (**148**) lead to the formation of single enantiomer ene adducts. We planned to react Diels-Alder adduct (**148**) with nitrosobenzene (**170**), 1-methyl-2-nitrosobenze (**172**), and 2,3,4,5,6-pentafluorobenzaldehyde (**164**) catalysed by DMAC (Scheme 4.23).



Scheme 4.23 - Proposed ene reaction with Diels-Alder adduct (148).

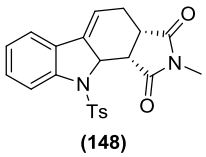
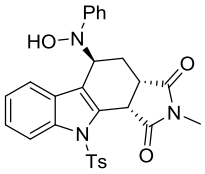
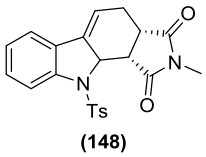
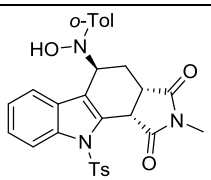
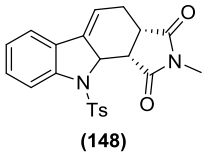
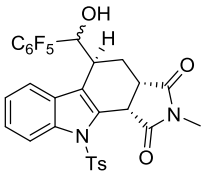
Starting Material	Ene Conditions	Ene Adduct	Yield	*ee*
 (148)	PhNO (**170**), rt, 18 h	 (171)	73%	>95%
 (148)	*o*-TolNO (**172**), rt, 18 h	 (173)	70%	>95%
 (148)	C_6F_5CHO (**164**), DMAC, -78 °C 45 min, rt 18 h	 (165)	70%	>95%

Table 4.3 – Ene reactions between enantiomerically pure Diels-Alder adduct (148) and a range of enophiles.

The ene reactions between the enantiomerically pure Diels-Alder adduct (**148**) and enophiles (**170**), (**172**) and (**164**) gave the desired ene adducts as single diastereomers in good yields. We had previously synthesised compounds (**171**), (**173**) and (**165**) as racemic products and this allowed us to optimise the chiral HPLC conditions. As with Diels-Alder adduct (**148**), the traces for the racemic ene adducts were compared to the traces for the organocatalysed ene adducts. This allowed us to calculate the enantiomeric excess for (**171**), (**173**) and (**165**). This showed that the enantiomeric excess from the enantioselective

organocatalysed Diels-Alder reaction was retained through the ene reaction, leading to the formation of ene adducts (**171**), (**173**) and (**165**) with an *ee* of >95%.

Conclusions-

We have demonstrated a simple procedure for the synthesis of complex tetrahydrocarbazole compounds such as (**171**), (**173**) and (**165**) with excellent enantiomeric excess. The ene reactions with acetyl and Boc protected Diels-Alder adducts (**301**) and (**294**) were found to be capricious and the ene adducts could not be isolated in good yields. We propose this is due to the reactivity of the acetyl and Boc protected Diels-Alder adducts, whereby several undesired side reactions, including rearomatisation, were competing with the ene reaction. Exchanging the acetyl and Boc protecting groups for a tosyl protecting group allowed the reliable synthesis of Diels-Alder adduct (**148**) in high enantiomeric excess, and the subsequent ene reactions formed the desired ene adducts in high yields. Thus far, only compounds with a tosyl protecting group have been reliably synthesised, however further work would involve expanding the range of protecting groups.

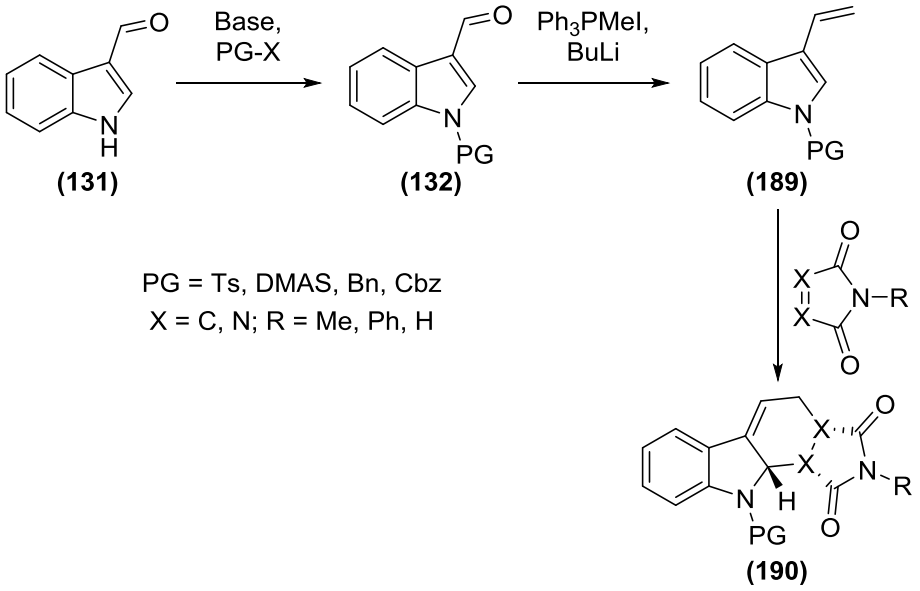
In Chapter 2 we demonstrated how tetrahydrocarbazole compounds such as (**171**), (**173**) and (**165**) without an *N*-protecting group, show moderate biological activity. A synthetic route that allows the synthesis of these compounds as single enantiomers could greatly improve the biological activity that these compounds show. Therefore developing the organocatalysed Diels-Alder / *N*-protection to allow for the addition of a Cbz protecting group, which we have shown can be easily removed (Chapter 3.10.4), would be of great interest.

Chapter 5 – Conclusions and Future Work

5.0 Conclusions-

The aim of this project was to examine the Diels-Alder / ene chemistry of *N*-protected 3-vinyl-1*H*-indole compounds. The tetrahydrocarbazole compounds formed would be analogues of known biologically active compounds in the literature. We hoped this would give us a means of synthesising biologically active compounds using a novel Diels-Alder / ene route.

We developed a procedure that allowed the synthesis of Diels-Alder adducts (**190**) from the reaction between *N*-protected 3-vinyl-indoles (**189**) and maleimide compounds (Scheme 5.1).



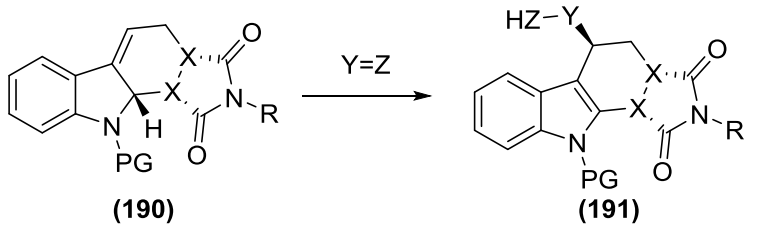
Scheme 5.1 - Route to the synthesis of Diels-Alder adducts (190).

We discovered that the more electron donating the protecting group, the quicker the Diels-Alder reaction went to completion. We propose that electron donating groups increase the electron density of the indole diene. This raises the energy level of the diene HOMO and therefore increases the rate of the Diels-Alder reaction.

Using X-ray crystallography we were able to deduce the relative stereochemistry of several Diels-Alder adducts (**190**). The X-ray crystal structures revealed that the Diels-Alder reaction goes through an *endo* transition state leading to the *endo*-Diels-Alder adduct (**190**). This means the hydrogen adjacent to the indolic nitrogen is on the opposite face of the molecule relative to the succinimide moiety.

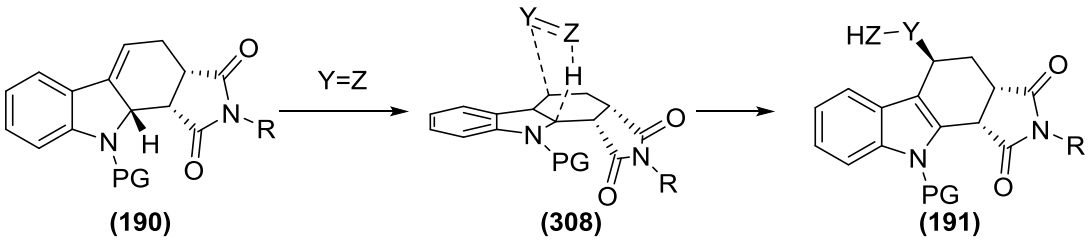
After devising a reliable method for the synthesis of Diels-Alder adducts (**190**), we then examined their ability to undergo ene chemistry with a range of nitroso, aza and carbonyl enophiles. All of the ene reactions were found to be very dependent upon the structure of

the enophile. Enophiles with an electron donating group adjacent to the nitroso or carbonyl group did not react. We believe that the electron donating groups raises the energy of the enophile LUMO, which prevents the reaction from occurring within a reasonable time frame. We discovered that Diels-Alder adducts (**190**) readily undergo ene reactions with nitrosobenzene (**170**), 1-methyl-2-nitrosobenzene (**172**), PTAD (**150**) and 2,3,4,5,6-pentafluorobenzaldehyde (**164**) (Scheme 5.2).



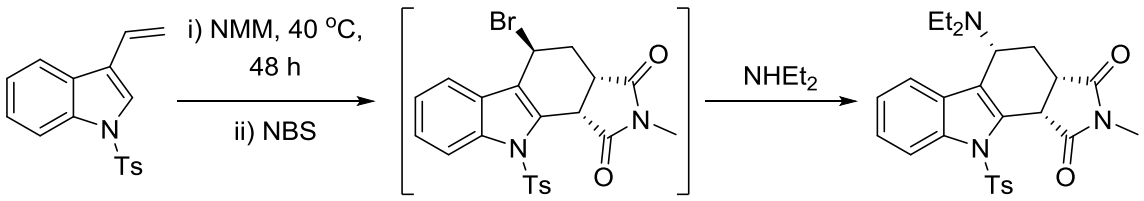
Scheme 5.2 - General scheme for the ene reactions with Diels-Alder adduct (190).

The success of the ene reactions demonstrated that it was possible to perform chemistry on the double bond formed in the Diels-Alder reaction. We were able to use X-ray crystallography to deduce the relative stereochemistry of several of the ene adducts (**191**). They showed that the enophile adds to the opposite face of the molecule relative to the succinimide moiety. This suggests that the ene reaction is undergoing a concerted mechanism whereby the new bond between the enophile and Diels-Alder adduct is being formed at the same time as the hydrogen adjacent to the indolic nitrogen is being abstracted (Scheme 5.3).



Scheme 5.3 - Proposed mechanism for ene reaction with Diels-Alder adduct (190).

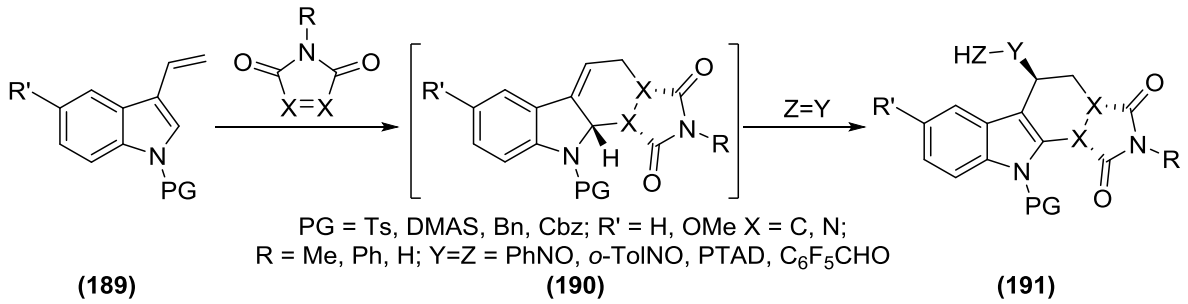
The readiness of Diels-Alder adduct (**190**) to react with electrophilic enophiles suggested that the nucleophilic double bond in Diels-Alder adduct (**190**) could react with further electrophiles. Therefore we developed a one-pot Diels-Alder/ bromination / nucleophilic substitution reaction starting from (**146**) (Scheme 5.4).



Scheme 5.4 - One-pot Diels-Alder/ bromination/ nucleophilic substitution reaction.

Although the one-pot Diels-Alder/ bromination / nucleophilic substitution reaction gave the desired product (**188**), the reaction was far more capricious than the ene reactions and bromintated product (**181**) rapidly decomposed to form eliminated products.

Optimisation of the Diels-Alder and ene reactions meant we could develop a one-pot Diels-Alder / ene approach, which allowed us to synthesise ene adducts of the type (**191**) in one step without the need to isolate Diels-Alder adduct (**190**) (Scheme 5.5).

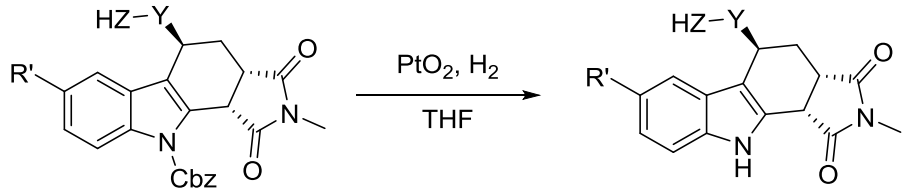


Scheme 5.5 - General one-pot Diels-Alder / ene reaction sequence.

Using this method we were able to synthesise over thirty different Diels-Alder / ene adducts in excellent yields, in what is the first example of a one-pot intermolecular Diels-Alder / intermolecular ene reaction sequence.

The compounds synthesised from the one-pot Diels-Alder / ene reaction contained the same pyrrolo[3,4-*a*]carbazole-1,3-dione framework seen in a range of biologically active compounds from the literature[33,34]. Therefore the compounds we synthesised were tested for biological activity in a disk diffusion assay against *S. Aureus*, *E. coli* and *S. Pombe.* However, the disk diffusion assay showed no halo of inhibition for any of the compounds. Comparison of our adducts with known biologically active compounds suggested that the protecting groups on the indolic nitrogen were prohibiting our molecules from being active. Therefore we attempted to remove the protecting groups.

Previous work within the group had found difficulties in removing the tosyl and DMAS protecting groups without decomposition of the Diels-Alder / ene compound (**190**). However, we found the Cbz protecting group could be readily removed using Adam's catalyst and an atmospheric pressure of hydrogen gas. This allowed the synthesis of fourteen novel deprotected compounds (**275**) (Scheme 5.6).



R' = H, OMe X = C, N; R = Me, Ph, H; Y=Z = PhNO, *o*-TolNO, PTAD

(274) **(275)**

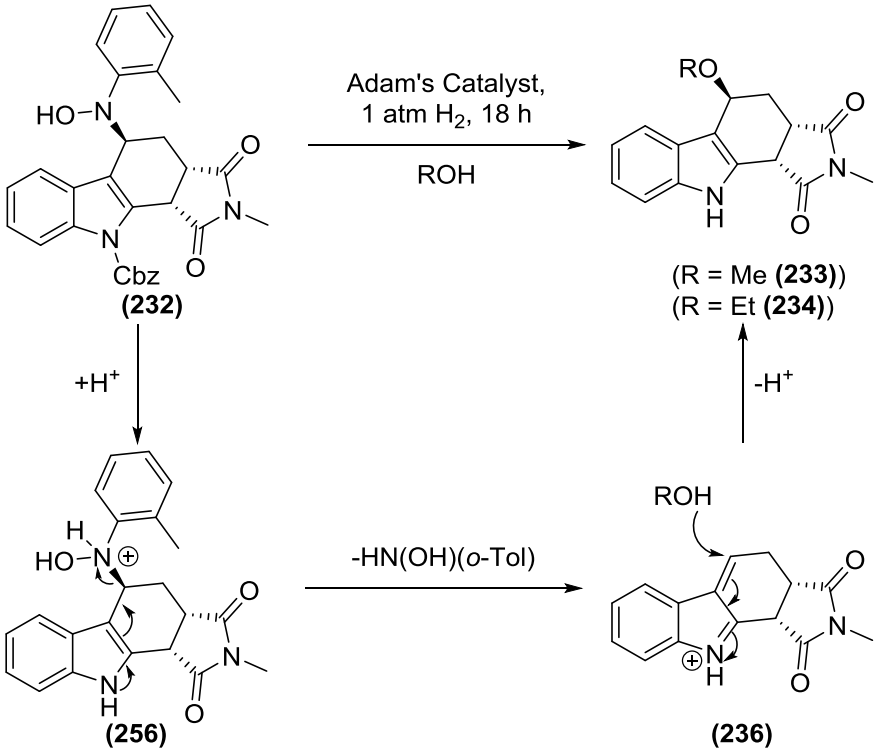
Scheme 5.6 - Cbz deprotection using Adam's catalyst and an atmospheric pressure of hydrogen gas.

A disk diffusion assay on deprotected compounds (**275**) showed that they had moderate activity against *S. Aureus, E. coli* and *S. Pombe* with the most active compound having a MIC of 16 µg/ mL. Four of these compounds were chosen for screening against fifty one human kinases, of which two compounds showed moderate activity.

The activity of the deprotected adducts (**275**) suggests that a free indolic NH is essential for the compounds to be active. We believe there are several possible reasons that the free NH is essential for activity. The free NH could be forming a hydrogen bonding interaction between the compound and the active site, which would greatly improve the overall binding interaction between the two. This would not be possible with a protecting group on the nitrogen.

It is also possible that removing the large lipophilic protecting group leads to a decrease in the partition coefficient (log P) value of the compounds, bringing them more in line with Lipinski's rule of five.[106] The rule of five states that a compound with a high log P factor is more likely to suffer from poor absorption into biological systems, making it less likely to be a successful drug compound.

The final possibility is that removal of the protecting group has a large effect on the electronics of the indole ring system which, in turn, has an effect on the mode of action of the compound. With no protecting group on the indolic nitrogen, the lone pair of electrons on the nitrogen can eliminate the hydroxylamine group from the Diels-Alder / ene compounds. This was observed when the deprotection reactions were carried out in methanol or ethanol, with the hydroxylamine group being substituted by a methoxy or ethoxy group in an S_N1 reaction (Scheme 5.7).



Scheme 5.7 - Proposed S_N1 mechanism by which compounds (233) and (234) are formed.

We believe it is possible that unsaturated iminium compound (**236**) is the compound responsible for biological activity. As the double formed in the elimination is highly electrophilic, it could readily be attacked by a nucleophilic group at an active site. This would lead to formation of a covalent bond between the active site and compound (**236**), and therefore a strong binding interaction. If our proposed mode of action is correct, the ability of (**256**) to eliminate the hydroxylamine group and form unsaturated iminium compound (**236**) would be vital for the compound to be biologically active. A free NH group would allow the elimination of the hydroxylamine to occur rapidly, leading to the active unsaturated iminium (**236**).

Future SAR studies on Diels-Alder / ene adducts of the type (**256**), could test the theory that the unsaturated iminium compound (**236**) is the active compound. If the elimination step is necessary for the molecules to become active, the addition of electron withdrawing groups (such as a nitro group) at the 5- and 7- position of the indole ring would severely hinder the elimination step (Figure 5.1).

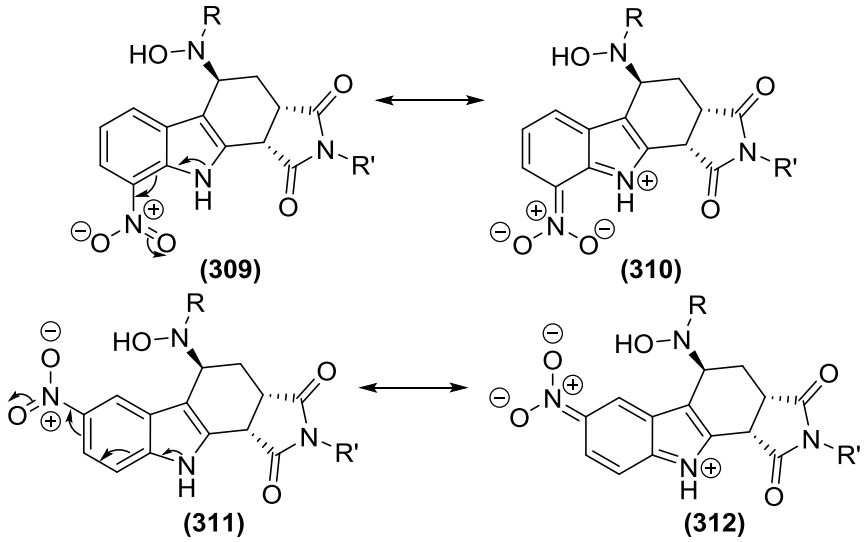


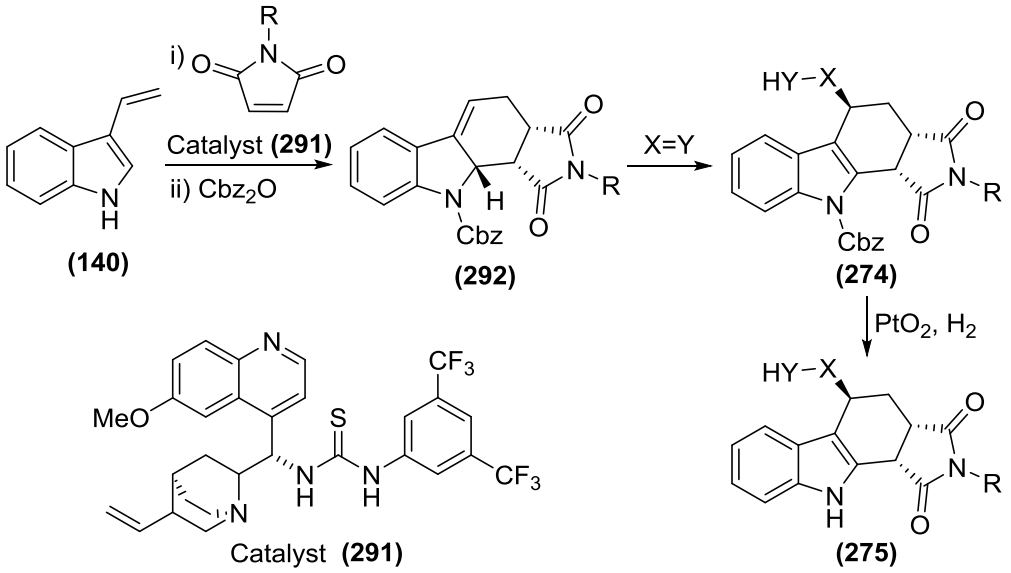
Figure 5.1 - Resonance forms of 5- and 7- nitro substituted Diels-Alder / ene compounds.

The lone pair of electrons on the nitrogen would be conjugated into the electron withdrawing nitro group on the indole ring. This would reduce the rate of elimination to form unsaturated iminium (**236**) and, if our proposed mode of action for this class of compounds is correct, the biological activity would drop. Conversely, the introduction of electron donating groups on to the indole ring should promote the elimination to unsaturated iminium (**236**) and thus increase the biological activity of the compounds.

5.1 Future Work-

All of the Diels-Alder / ene adducts we have tested for biological activity have been racemic. Synthesising the same compounds as single enantiomers could lead to a substantial increase in their activity. The binding sites in biological systems are often chiral and therefore a single enantiomer compound will show a significantly better binding affinity than a racemic mixture of compounds.

Forming the single enantiomer compounds could be achieved using the same catalytic Diels-Alder approach seen in Chapter 3, followed by removal of the protecting group (Scheme 5.8).



Scheme 5.8 – Catalysed Diels-Alder / N-protection / ene / N-deprotection leading to single enantiomer compound (275).

The reaction would have to be modified from the method used in Chapter 3, as previous work has shown that it is difficult to remove the tosyl protecting group from tetrahydrocarbazoles. That would mean having to use dibenzyl dicarbonate in the protection step, leading to the formation of Cbz protected tetrahydrocarbazoles (**275**).

Catalyst (**291**) will generate one enantiomer of Diels-Alder adduct (**274**), however it is unknown which enantiomer is the most active. Therefore it may be necessary to synthesise both enantiomers of tetrahydrocarbazole (**275**) for comparison, and so the opposite enantiomer of catalyst (**291**) would need to be synthesised. This could be achieved using quinidine, the opposite enantiomer to quinine, as the starting material.

Further future work could adapt our Diels-Alder / ene reaction process to try and synthesise compounds that more closely resemble (**195**)[35], which is already known in the literature to show good biological activity (Figure 5.2).

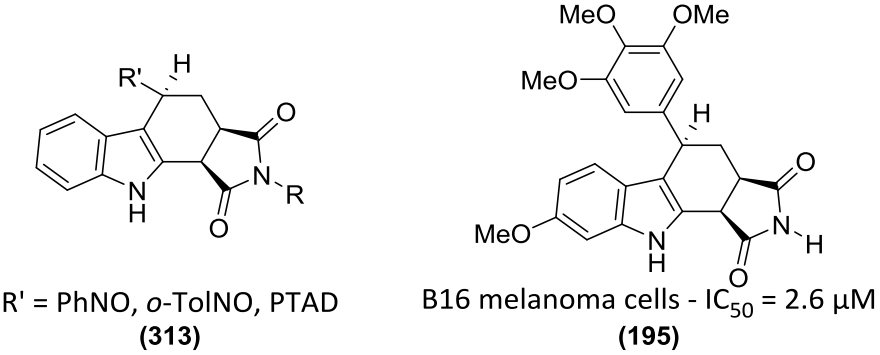
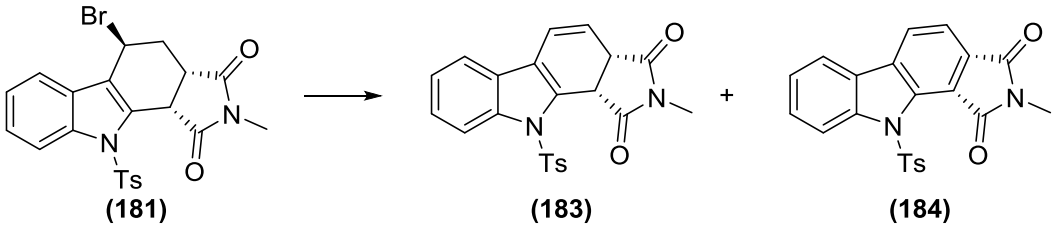


Figure 5.2 - Product from Diels-Alder / ene reaction (313) and known biologically active compound (195).

However, to synthesise molecules like (**195**) would require our compounds (**313**) to have an aromatic group rather than a hydroxylamine, aza or carbonyl group, on the non-aromatic six membered ring. We believe this could be achieved by adapting the bromination chemistry seen in Chapter 1. We developed a one-pot procedure whereby Diels-Alder adduct (**148**) underwent bromination to generate (**181**), followed by a substitution reaction with a nucleophile such as diethylamine. One of the problems we faced with this reaction was bromination product (**181**) rapidly decomposed via an elimination reaction to form compounds (**183**) and (**184**) (Scheme 5.9). This elimination occurs via the lone pair of electrons on nitrogen eliminating bromine which leads to the formation of an unsaturated iminium compound. This unsaturated iminium compound then rapidly decomposes to eliminated products (**183**) and (**184**).



Scheme 5.9 - Products (183) and (184) formed as a result of an elimination reaction.

We believe we could reduce the formation of eliminated products (**183**) and (**184**) in two ways. Firstly, the addition of electron withdrawing groups on the indole ring system would prevent the lone pair of electrons on the indolic nitrogen from eliminating bromine, as the electron density would be delocalised into the electron withdrawing group (Figure 5.1). A more electron withdrawing protecting group could also be used to achieve the same result (Figure 5.3).

Alternatively, the addition of R' groups to the carbon adjacent to the C-Br bond would prevent any elimination from occurring, as there would not be any hydrogens available for elimination (Figure 5.3).

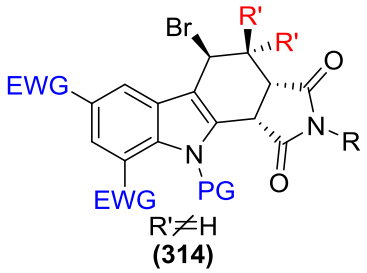
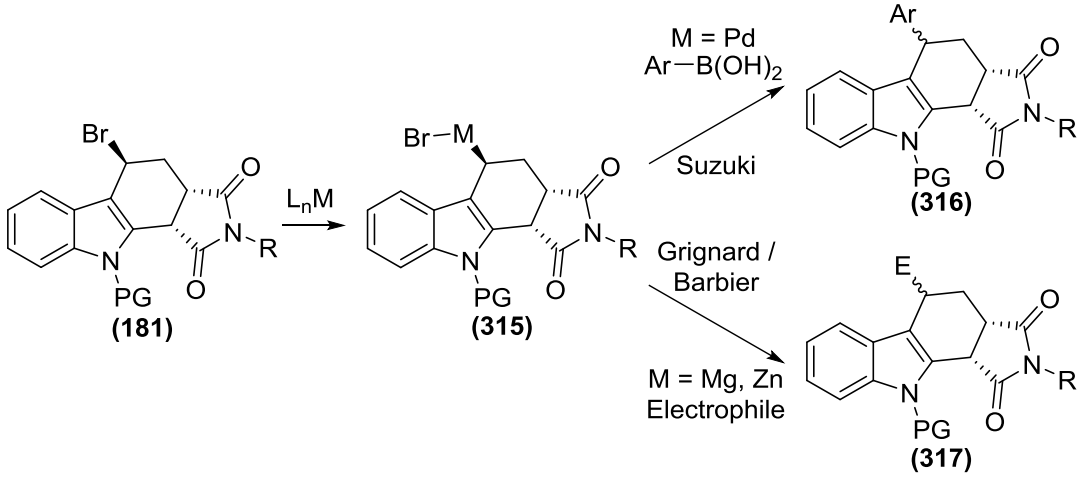


Figure 5.3 - Two possible methods for preventing the elimination reactions from occurring.

These methods would make the brominated product more stable in solution and enable us to perform further chemistry on the compound. Starting from brominated product (**181**),

there are several reactions that would allow the synthesis of molecules such as (**195**) as well as further substituted compounds.

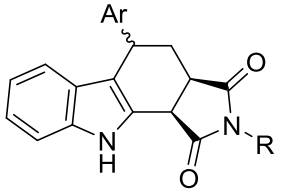
The addition of a metal catalyst would lead to the formation of organometallic compound (**315**) (Scheme 5.10). Depending upon the metal used, a wide range of organometallic chemistry would then be available. For example, a zinc catalyst would allow a Barbier reaction to be performed; magnesium would lead to the formation of a Grignard reagent and a palladium catalyst would allow a Suzuki reaction to occur (Scheme 5.10).



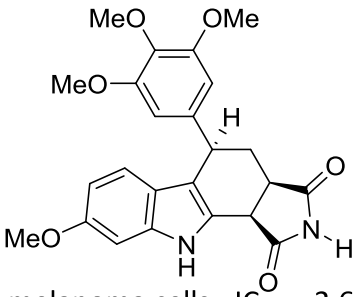
Scheme 5.10 - Reaction pathway for a Suzuki reaction and a Grignard / Barbier reaction.

There are a large number of electrophiles that are commercially available, which means that the Grignard / Barbier reaction pathway could be a quick and simple method for the synthesis of a range of compounds such as (**317**).

Due to the many commercially available boronic acids and the relative ease by which boronic acids can be synthesised, an sp^2-sp^3 Suzuki coupling could rapidly lead to the formation of a wide range of compounds. This would allow the insertion of a variety of molecules with the general structure (**313**) that show a great resemblance to compounds such as (**195**)[35], which are known to have very good biological activity against B16 melanoma cells.



(313)



B16 melanoma cells - IC_{50} = 2.6 µM

(195)

Figure 5.4 - Product from Suzuki reaction (313) and known biologically active compound (195).

Chapter 6 – Experimental-

6.1 General experimental information

^{1}H and ^{13}C NMR spectra were recorded directly with a Jeol Lambda 500 MHz, Jeol ECS-400 MHz or Bruker Avance 300 MHz. HRMS data were provided by the EPSRC National Mass Spectrometry Service (University of Swansea). X-ray diffraction data was obtained on an Oxford Diffraction Gemini. IR spectra were obtained as neat samples using a Varian 800 FT-IR Scimitar Series spectrometer scanning from 4000-600 cm^{-1}. Melting points were obtained using a Stuart SMP3 melting point machine. THF was distilled from sodium/benzophenone and used directly. DCM was distilled from calcium hydride and used directly.

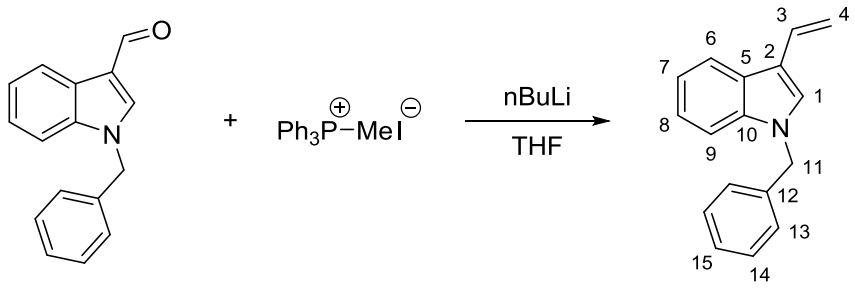
Kirby-Bauer Disk-Diffusion Assay

A 10mg/mL solution in DMSO of each test compound was prepared, from which 10µL of each was added onto discs of filter paper (6 mm diameter). Agar plates prepared from a 1:1 mixture of Nutrient Agar and LB, were inoculated with *S. aureus* or *E.Coli*., whilst YPD agar plates were inoculated with *S. pombe.* The disks containing test compounds were placed on the agar plates along with an appropriate positive control (nystatin, kanamycin or ampicillin) and the agar plates were incubated over night at 30 ^{o}C. Following this, the plates were photographed and zones of inhibition measured in mm.

Minimum Inhibitory Concentration

For determining the minimum inhibitory concentrations (MIC), the tested compounds were examined via the micro dilution method using Mueller Hinton Broth 2 (Casein hydrolysate 17.5g/L, Beef extract 3 g/L, Starch 1.5g/L, pH 7.3). *Staphylococcus aureus* and *Escherichia coli* NCTC 10418. The compounds were dissolved in DMSO. The final DMSO concentration in the cultures did not exceed 5%. Growth inhibition was evaluated after incubation for 18h at 37^{o}C in a FluoroStar optima plate reader, determining the optical density at 600 nm.

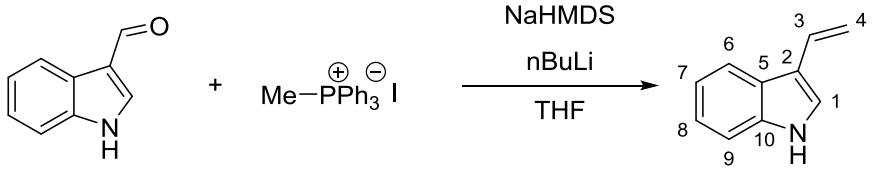
1-benzyl-3-vinyl-1*H*-indole – 137



In a Schlenk flask, methyltriphenylphosphonium iodide (9.50 g, 23.4 mmol) was dissolved in dry THF (100 mL). The solution was cooled to -78 ^{o}C and ^{n}BuLi (8.5 mL, 21.2 mmol) was added over 15 minutes. The yellow solution was warmed to 0 ^{o}C and was left to stir for 1 hour before being cooled to -78 ^{o}C. To the stirred solution, 1-benzyl-1*H*-indole-3-carbaldehyde (5.00 g, 21.2 mmol) was added and the solution was stirred at room temperature for 3 hours. The reaction was poured into water (80 mL) and extracted with ethyl acetate (3 x 60 mL). The combined organic layers were dried with $MgSO_4$, filtered and the solvent removed under pressure to leave the crude product as yellow oil. The product was purified using column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 3 cm, silica = 15 cm) to give 1-benzyl-3-vinyl-1*H*-indole (4.36 g, 18.7 mmol, 88 %) as a yellow powder.

MP: 77.1 – 78.9 ^{o}C; R_f: 0.94 (Pet:EA 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.01 – 7.89 (1H, m), 7.38 – 7.29 (4H, m), 7.28 – 7.21 (2H, m), 7.21 – 7.14 (2H, m), 6.93 (1H, dd, *J* = 17.8, 11.3 Hz, H^3), 5.74 (1H, dd, *J* = 17.8, 1.5 Hz, H^4-trans), 5.32 (2H, s, H^{11}), 5.20 (1H, dd, *J* = 11.3, 1.5 Hz, H^4-cis); ^{13}C NMR (101 MHz, DMSO-d_6): δ_C 138.4, 137.2, 130.2, 129.4, 129.1, 128.0, 127.6, 126.4, 122.4, 120.5, 120.4, 114.2, 111.1, 110.2, 49.6; IR (cm^{-1}): *v* 3135 (CH), 2829 (CH); Anal. Calcd for $C_{17}H_{15}N_2$: C, 87.52; H, 6.48; N, 6.00. Found: C, 87.39; H, 6.67; N, 5.95.

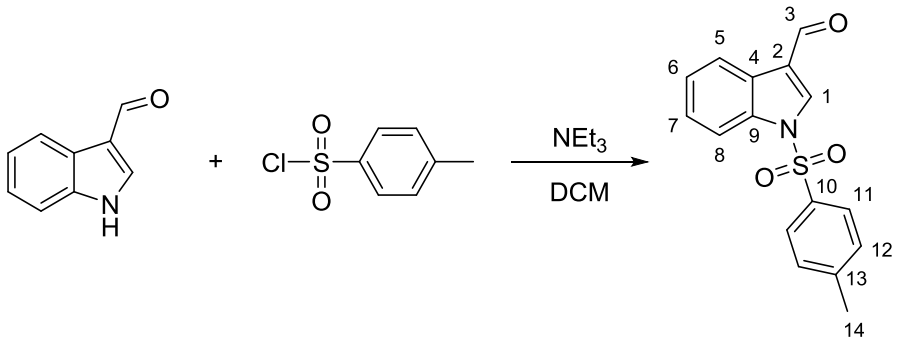
Synthesis of 3-vinyl-1*H*-indole – 140



In a Schlenk flask, methyltriphenylphosphonium iodide (2.14g, 5.3 mmol) was dissolved in dry THF (13 mL). The solution was cooled to -78 ^{o}C and ^{n}butyllithium (2.9 mL, 4.6mmol) was added over 10 minutes. The yellow solution was warmed to 0 ^{o}C and was left to stir for 1 hour before being cooled to -78 ^{o}C. In a separate Schlenk flask, 1*H*-indole-3-carboxylate (0.67 g, 4.6 mmol) was dissolved in THF (7 mL) and to the solution sodium bis(trimethylsilyl)amide (2.3 mL, 4.6 mmol) was added. This solution was transferred into the first Schlenk flask and the red solution was allowed to stir at room temperature for 1 hour. The reaction was poured into water (30 mL) and extracted with ethyl acetate (2 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under pressure to leave the crude product as yellow oil. The product was purified using column chromatography (Petrol: Diethyl ether 7: 3, column diameter = 4 cm, silica = 20 cm) to give 3-vinyl-1*H*-indole (0.636 g, 4.4 mmol, 95%) as a yellow powder.

MP: 78.4-80.7 ^{o}C (literature – 80-81 ^{o}C); Rf = 0.76 (Pet:EA, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.02 (1H, br s, NH), 7.85 – 7.80 (1H, m), 7.32 – 7.28 (1H, m), 7.18 (1H, s, H^1), 7.18 – 7.09 (2H, m), 6.83 (1H, ddd, *J* = 17.7, 11.2, 0.5 Hz, H^3), 5.65 (1H, dd, *J* = 17.7, 1.5 Hz, H^4-trans), 5.11 (1H, dd, *J* = 11.2, 1.5 Hz, H^4-cis); ^{13}C NMR (400 MHz, $CDCl_3$) δ 136.8 (C^{10}), 129.5, 125.7 (C^5), 123.6, 122.6, 120.4, 120.2 C^9), 115.9 (C^2), 111.4 (C^6), 110.9 (C^4); IR (cm^{-1}): *v* 3660 (NH), 2981 (CH sp^2).

1-tosyl-1*H*-indole-3-carbaldehyde – 144

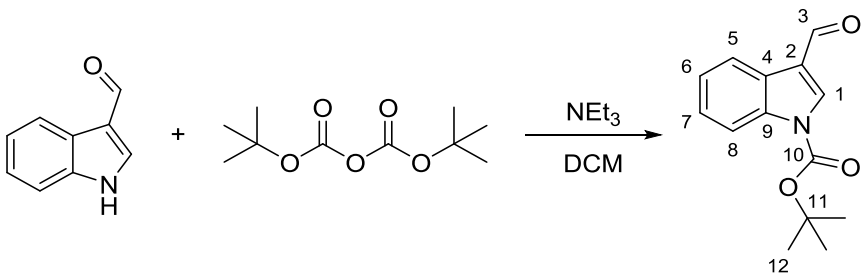


Into a Schlenk flask, was placed 1*H*-indole-3-carbaldehyde (5.0 g, 34.5 mmol) and DCM (100 mL). The resulting stirred solution was cooled to 0 ^{o}C before triethylamine (12 mL, 86.2 mmol) was added dropwise via syringe. To the stirred solution, *p*-toluenesulfonyl chloride (7.23 g, 37.9 mmol) in DCM was added dropwise over a period of 20 minutes. The solution was stirred at 0 ^{o}C for a further one hour before warming to room temperature over 18 hours. The solution was washed into a separating funnel with DCM (20 mL) and washed with water (2 x 100 mL) and brine (100 mL). The organic extracts were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give the crude product as a pale orange oil. The product was purified by recrystallisation from hot ethyl acetate (150 mL) to give 1-tosyl-1*H*-indole-3-carbaldehyde (8.38 g, 28 mmol, 82%) as orange crystals.

MP: 145.3 – 148.8 ^{o}C; ^{o}C; R_f: 0.83 (EA:Petrol, 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 10.11 (1H, s, H^3), 8.29-8.26 (1H, m), 8.19 – 8.16 (1H, m), 8.16 (1H, s, H^1), 7.89 – 7.85 (1H, m), 7.78 (2H, d, *J* = 8.4 Hz, H^{11}), 7.36 – 7.25 (2H, m), 7.22 (2H, d, *J* = 8.4 Hz, H^{12}), 2.29 (3H, s, H^{14}); ^{13}C NMR (400 MHz, CDCl3) δ 185.3 C^3, 146.1, 136.2, 135.4, 134.1, 130.3, 130.2, 127.2, 127.1, 126.2, 124.9, 122.5, 122.2, 113.1, 21.5 C^{14}; IR (cm^{-1}): *v* 3140 (CH), 1663 (C=O)**;** Anal. Calcd for $C_{16}H_{13}NO_3S$: C, 64.20; H, 4.38; N, 4.68. Found: C, 63.97; H, 4.52; N, 4.72.

.

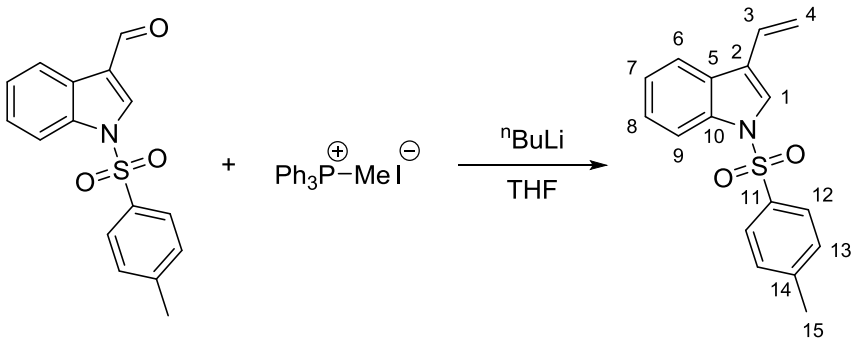
tert-butyl 3-formyl-1*H*-indole-1-carboxylate – 145



In a Schlenk flask, 1*H*-indole-3-carbaldehyde (4.0 g, 27.4 mmol) was dissolved in DCM (120 mL). The stirred solution was cooled to 0 ^{o}C before triethylamine (5.8 mL, 41.1 mmol) was added dropwise via a syringe. To the solution, di-*tert*-butyl dicarbonate (6.92 mL, 30.14 mmol) was added via syringe over 10 minutes. The solution was stirred at 0 ^{o}C for one hour before warming to room temperature for 18 hours. The solution was washed into a separating funnel with DCM (20 mL) and washed with water (2 x 100 mL) and brine (100 mL). The organic extracts were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to give the crude product as a white solid. The product was purified by recrysatllisation from refluxing ethyl acetate (120 mL) to give *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate (5.87 g, 21.9 mmol, 87%) as a white powder.

MP: 126.2-129.0 ^{o}C; R_f: 0.75 (Pet:EA, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 10.04 (1H, s, H^3), 8.24 – 8.21 (1H, m, H^8), 8.17 (1H, s, H^1), 8.13 (1H, d, *J* = 8.1 Hz, H^5), 7.43 – 7.32 (2H, m, $H^{6/7}$), 1.64 (9H, s, H^{12}); ^{13}C NMR (400 MHz, $CDCl_3$) δ_C 185.9 (C^3), 148.9 (C^{10}), 136.6 (C^1), 136.1 (C^9), 126.2 (C^6), 126.2 (C^2), 124.7 (C^7), 122.2 (C^8), 121.7 (C^4), 115.2 (C^5), 85.8 (C^{11}), 28.2 (C^{12}); IR: v 2980 (CH sp^2), 2884 (CH sp^3), 1740 (CO); Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.56; H, 6.15; N, 5.87.

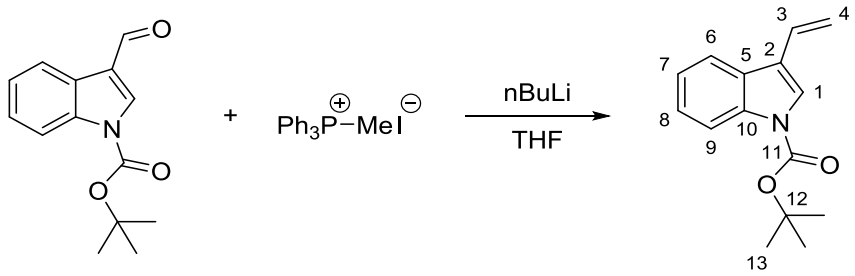
1-tosyl-3-vinyl-1*H*-indole – 146



A Schlenk flask was charged with methyltriphenylphosphonium iodide (1.29 g, 3.2 mmol) dissolved in dry THF (25 mL) under a nitrogen atmosphere. The solution was cooled to -78 ^{o}C and ^{n}butyllithium (1.81 mL, 2.97 mmol) was added dropwise via syringe over 10 minutes. The solution was warmed to 0 ^{o}C and left to stir for 2 hours. In a separate Schlenk flask, 1-tosylindoline-3-carbaldehyde (0.8 g, 2.7 mmol) was dissolved in THF (5 mL). The indole solution was transferred via cannula to the Schlenk flask containing the solution of methyltriphenylphosphonium iodide and the solution was stirred for 18 hours. The reaction poured into water (50 mL) and extracted with ether (3 x 40 mL). The organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to leave the crude product as an orange oil. The crude product was purified by column chromatography (Petrol: Ethyl acetate; 10: 1, 2 cm diameter column) to give 1-tosyl-3-vinyl-1*H*-indole (0.56 g, 3.9 mmol, 70%) as a pale yellow powder.

MP: 90.3 – 94.6 ^{o}C; R_f: 0.86 (Pet:EA, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.93 – 7.90 (1H, m), 7.69 (2H, d, *J* = 8.4 Hz, H^{12}), 7.70-7.65 (1H, m), 7.53 (1H, s, H^{1}), 7.29-7.19 (2H, m), 7.29 – 7.17 (2H, m), 7.15 (2H, d, *J* = 8.4 Hz, H^{13}), 6.70 (1H, app ddd, *J* =17.9, 11.3, 0.7 Hz), 5.72 (dd, 1H, *J* = 17.9, 1.2 Hz, H^{4}-trans), 5.28 (1H, dd, *J* = 11.3, 1.2 Hz, H^{4}-cis), 2.26 (3H, s, H^{15}); ^{13}C NMR (400 MHz, CDCl3) δ_C 145.1, 135.6, 135.2, 130.0 (C^{12}), 129.1, 127.6, 126.9 (C^{13}), 125.0, 124.2, 123.6, 121.0, 120.5, 115.4, 113.8, 21.7 (C^{15}); IR (cm^{-1}): *v* 3119 (CH-sp^2), 3072 (CH-sp^3); Anal. Calcd for $C_{17}H_{15}NO_2S$: C, 68.66; H, 5.08; N, 4.71. Found: C, 68.70; H, 5.21; N, 4.61.

tert-butyl 3-vinyl-1*H*-indole-1-carboxylate – 147

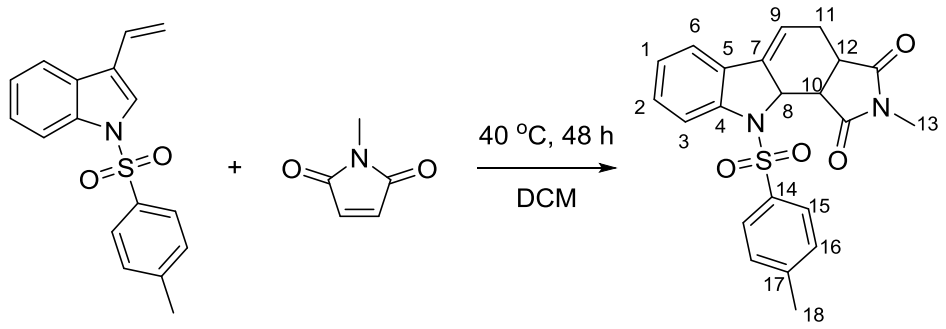


A Schlenk flask was charged with methyltriphenylphosphonium iodide (3.0 g, 7.43 mmol) and put under a nitrogen atmosphere before being dissolved in dry THF (25 mL). The solution was cooled to -78 ^{o}C and ^{n}butyllithium (4.3 mL, 6.81 mmol) was slowly added dropwise via syringe over 10 minutes. The solution was warmed to 0 ^{o}C and left to stir for 2 hours. In a separate Schlenk flask, *tert*-butyl 3-formyl-1*H*-indole-1-carboxylate (1.52 g, 6.19 mmol) was dissolved in THF (15 mL). The indole solution was transferred, via cannula, to the flask containing the methyltriphenylphosphonium iodide solution and allowed to stir for 18 hours. The reaction was quenched by pouring into water (50 mL) and extracted with ether (3 x 60 mL). The organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced atmosphere to leave the crude product as a pale yellow oil. The crude product was purified by column chromatography (Petrol: Ethyl acetate, 10: 1, column diameter = 3 cm, silica = 20 cm) to give *tert*-butyl 3-vinyl-1*H*-indole-1-carboxylate (0.195 g, 0.8 mmol, 13%) as a pale oil.

MP: Oil at room temperature; R_f: 0.90 (Pet:EA, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.08 (1H, d, *J* = 8.1 Hz, H^6), 7.72 – 7.68 (1H, m, H^9), 7.53 (1H, s, H^1), 7.28 – 7.15 (2H, m $H^{7/8}$), 6.72 (1H, dd, *J* = 17.8, 11.3 Hz, H^3), 5.72 (d, *J* = 17.8 Hz, H^4-trans), 5.23 (1H, d, *J* = 11.3 Hz, H^4-cis), 1.58 (9H, s, H^{13}); ^{13}C NMR (400 MHz, $CDCl_3$) δ 149.2 (**C**O), 136.0, 128.8, 128.3, 124.7, 124.1, 123.0, 120.1, 119.3, 115.5, 114.5, 83.9 (C^{12}), 28.3 (C^{13}); IR (cm^{-1}): *v* 3119 (CH sp^2), 2974 (CH sp^3), 1730 (CO); MS (pAPCI): 188.1 (100%, $(M-^{t}Bu+H)^+$), 244.1 (93%, $(M+H)^+$), 487.3 (4%, $(2M+H)^+$); HRMS (pAPCI): calcd $C_{15}H_{18}NO_2$ $[M+H]^+$: 244.1332; observed: 244.1334.

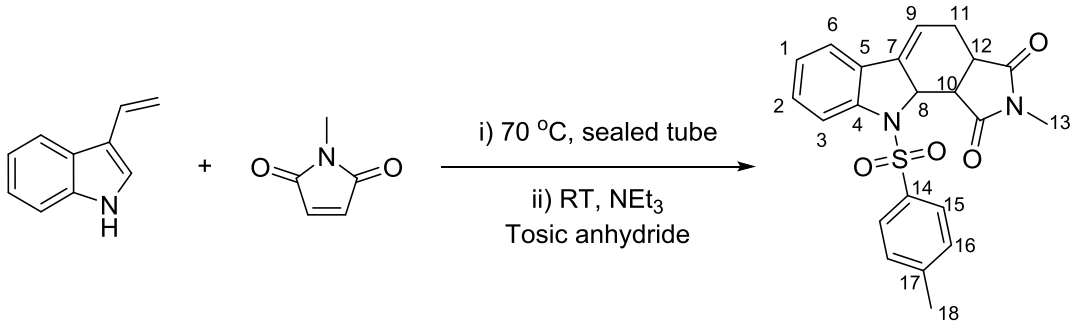
(3a*S,10b*S**)-2-methyl-10-tosyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 148**

Method A:



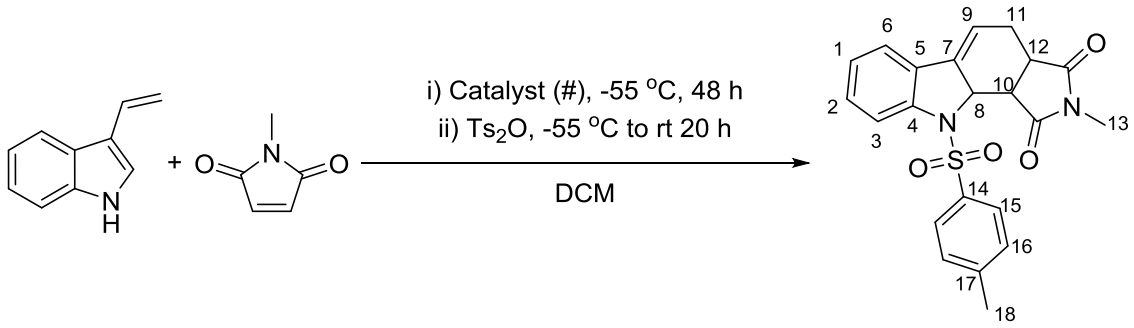
Into a round bottomed flask, 1-tosyl-3-vinyl-1*H*-indole (2.0 g, 6.7 mmol) and DCM (10 mL) was added. To the stirred solution, *N*-methylmalemide (0.75 g, 6.7 mmol) was added and the solution was stirred at 40 °C for 48 hours. The solvent was removed under reduced pressure to leave the crude product as orange oil. The product was purified by column chromatography (Petrol : Ethyl acetate, 4 : 1, column diameter = 4 cm, silica = 15 cm) to give (3a*S**,10b*S**)-2-methyl-10-tosyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (2.01 g, 5.0 mmol, 76%) as a white powder.

Method B:



In a boiling tube, 3-vinyl-1*H*-indole (0.2 g, 1.4 mmol) and DCM (7 mL) was added. To the stirred solution, *N*-methylmaleimide (0.16 g, 1.4 mmol) was added and the sealed tube was heated at 70 °C for 3 hours. The solution was cooled to room temperature and triethylamine (0.48 mL, 3.5 mmol) and *p*- toluenesulfonic anhydride (0.64 g, 1.96 mmol) were added to the reaction. The reaction was stirred at room temperature for 2 hours before the solution was poured into water (20 mL) and extracted with DCM (2 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to leave the crude product as an orange oil. The product was purified by column chromatography (Petrol: Ethyl acetate, 4: 1, column diameter = 2 cm, silica = 15 cm) to give (3a*S**,10b*S**)-2-methyl-10-tosyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (0.405 g, 0.99 mmol, 70%) as a white powder.

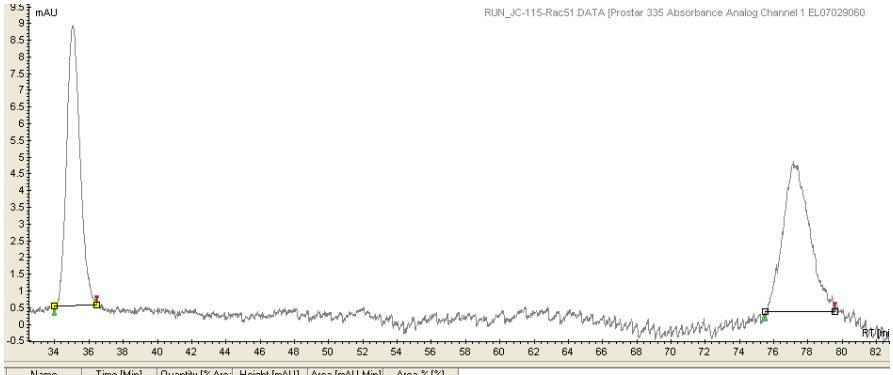
Method C:



Into a Schlenk flask was added NMM (17 mg, 0.15 mmol), 1-(3,5-bis(trifluoromethyl)phenyl)-3-((1*R*)-(6-methoxyquinolin-4-yl)((2*R*,4*R*,5*S*)-5-vinylquinuclidin-2-yl)methyl)thiourea (18 mg, 20 mol%) and DCM (1 mL), The solution was cooled to -55 °C before a pre-cooled solution of 3-vinyl-1*H*-indole (25 mg, 0.18 mmol) in DCM (0.5 mL) was added. The solution was stirred at -55 °C for 48 h before a solution of *p*-toluenesulfonic anhydride (245 mg, 0.75 mmol) in DCM (1.5 mL) was added. The solution was warmed to room temperature and stirred for 20 hours. The solution was poured into $NaHCO_3$ and extracted with DCM (3 x 5 mL). The combined organic extracts were dried over MgSO4, filtered and the solvent removed under reduced pressure to leave the crude product as an orange oil. The product was purified by column chromatography (Petrol: DCM: Et_2O, 2: 1: 1, column diameter = 1 cm, silica = 14 cm) to give (3a*S**,10b*S**)-2-methyl-10-tosyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (74%, 45 mg, 0.11 mmol) as a white solid.

MP: 204.2 – 208.0 °C; R_f: 0.09 (Pet:EA, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 7.72 (2H, d, *J* = 8.0 Hz, H^{15}), 7.61 (1H, d, *J* = 8.5 Hz, H^6), 7.21 – 7.19 (2H, d, J = 8.0, H^{16}), 7.21-7.16 (1H, m), 6.92 (1H, app t, *J* = 7.5 Hz), 6.01 – 5.96 (1H, m, H^9), 4.47 (1H, dd, *J* = 7.0, 3.3 Hz, H^8), 3.99 (1H, app t, *J* = 8.1 Hz, H^{10}), 3.12 (1H, app t, *J* =8.1 Hz, H^{12}), 2.99-2.92 (1H, m, H^{11}), 2.76 (3H, s, H^{13}), 2.30 (3H, s, H^{18}), 2.11 (1H, ddd, *J* = 18.0, 6.4, 2.4 Hz, H^{11}); ^{13}C NMR (400 MHz, $CDCl_3$) δ_C 178.9 C=O, 174.2 C=O, 144.7, 144.6, 137.4, 134.3, 130.4, 129.9, 127.5, 126.4, 123.9, 121.0, 115.4, 112.9, 61.6 C^8, 43.3 C^{10}, 37.2 C^{12}, 25.3 C^{11}, 25.1 C^{13}, 21.7 C^{18}; IR (cm^{-1}): *v* 2981 (CH- sp^2), 2889 (CH-sp3), 1694 (CO); MS (pNSI): 409.2 (61%, $(M+H)^+$), 426.1 (100%, $(M+(NH_4))^+$), 834.3 (52%, $(2M+(NH_4))^+$); HRMS (pNSI): calcd $C_{22}H_{21}N_2O_4S$ $[M+H]^+$: 409.1217; observed: 409.1218.

The *ee* of the product was determined by HPLC using an AD-H column (*n*-hexane/*i*PrOH 80:20, flow rate 0.75 mL/min, t_{maj} = 35.1 min, t_{min} = 77.2 min, >95% *ee*). $[\alpha]_D^{25}$ = +180 (*c* = 0.16 in $CHCl_3$).



Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
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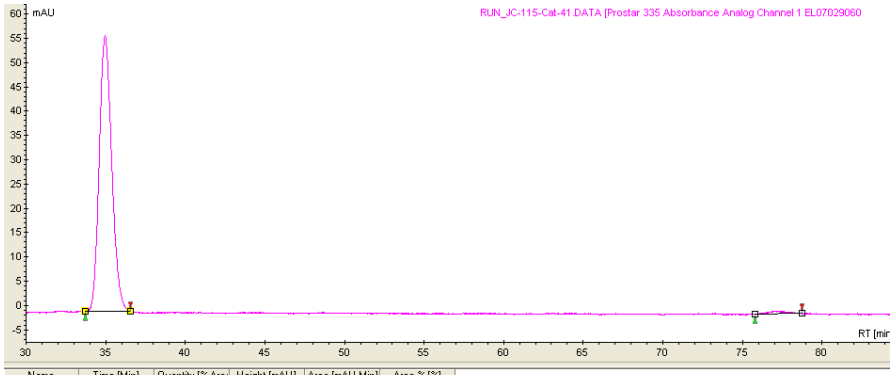
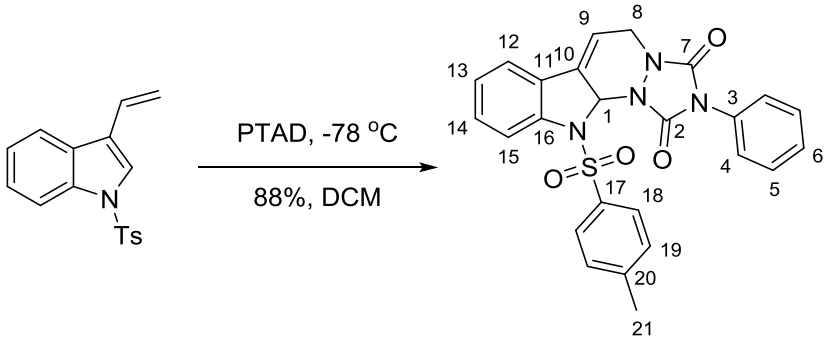
UNKNOWN	77.15	51.45	4.5	8.0	51.445
		100.00	12.9	15.6	100.000



Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
UNKNOWN	34.95	98.39	56.7	51.6	98.395
UNKNOWN	77.03	1.61	0.6	0.8	1.605
		100.00	57.3	52.5	100.000

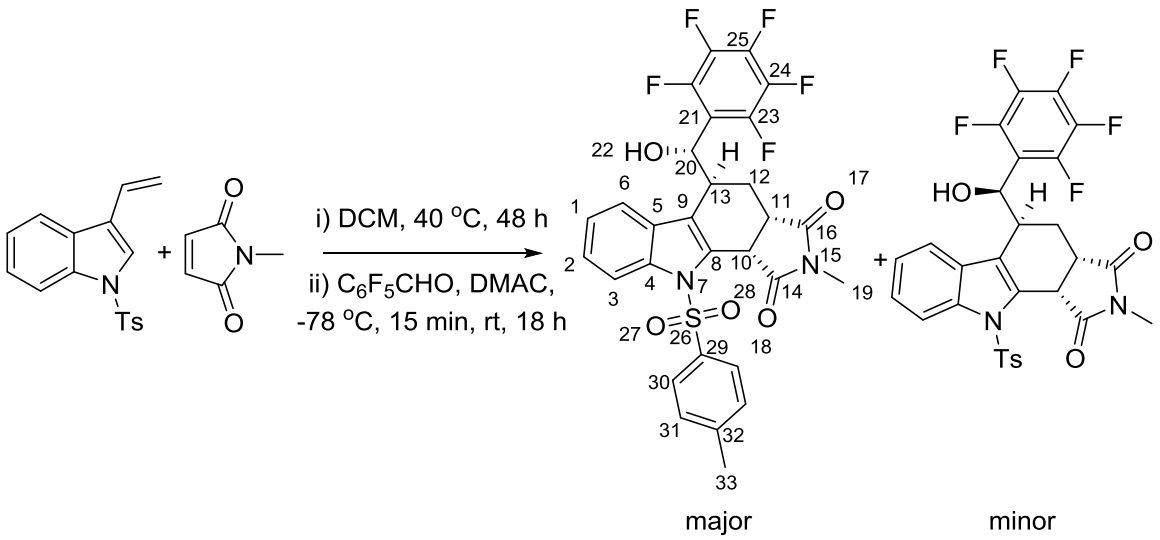
2-phenyl-11-tosyl-11,11a-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione – 151



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol) and DCM (7 mL) and the solution was cooled to -78 °C. To the stirred solution was added 4-phenyl-3H-1,2,4-triazole-3,5(4*H*)-dione (60 mg, 0.34 mmol) and the resulting solution was stirred at -78 °C for 3.5 hours before the solvent was removed under reduced pressure to leave the crude product as a pale red solid. The product was purified by column chromatography (Petrol : Ether: DCM 2: 1: 1, column diameter = 1 cm, silica = 20 cm) to give 2-phenyl-11-tosyl-11,11a-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione (88%, 140 mg, 0.30 mmol) as a pale red powder.

Mp: 160.1 – 162.8 °C; R_f: 0.14 (Pet:Et_2O:DCM 2:1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.86 (2H, d, J = 8.4 Hz), 7.63 – 7.55 (2H, m), 7.51 – 7.46 (2H, m), 7.46 – 7.42 (1H, m), 7.42 – 7.39 (1H, m), 7.38 – 7.34 (2H, m), 7.26 – 7.22 (2H, m), 7.09 (1H, app td, J = 7.5, 1.0 Hz).6.26 (1H, td, *J* = 2.6, 1.8 Hz, H^1), 6.18 (1H, app dt, *J* = 5.3, 2.7 Hz, H^9),4.56 – 4.46 (1H, app td, *J* = 17.6, 2.8 Hz, H^8), 4.39 (1H, ddd, *J* = 17.6, 5.2, 1.9 Hz, H^8), 2.37 (3H, s, H^{21}); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 152.7 (CO), 150.8 (CO), 144.7, 143.7, 135.6, 134.8, 131.5, 130.4, 129.6, 128.9, 128.5, 128.1, 126.8, 125.7, 125.2, 120.9, 117.4, 113.8, 74.8 (C^1), 44.6 (C^8), 21.4 (C^{21}); IR (cm^{-1}): *v* 3070 (CH), 2926 (CH), 1719 (CO); MS (pNSI): 473.1 (100%, $(M+H)^+$), 522.2 (30%); HRMS (pNSI): calcd for $C_{25}H_{21}N_4O_4S$ $[M+H]^+$: 473.1278; observed: 473.1277.

(3a*S,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione and (3a*S**,5*S**,10b*S**)-5-((*R**)-hydroxy(perfluorophenyl)methyl)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 165**

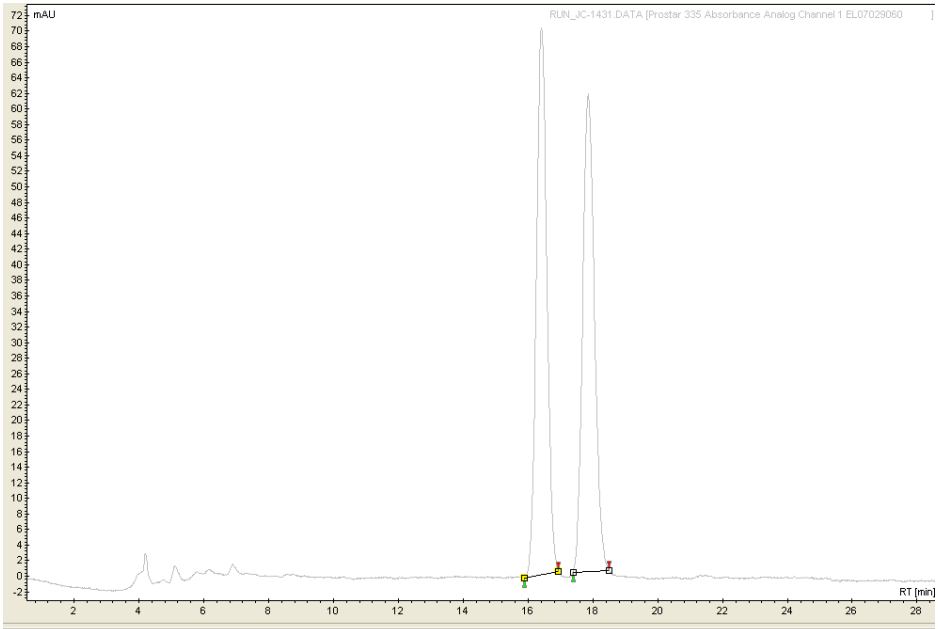


To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol) DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (38 mg, 0.34 mmol) and the resulting solution was heated at reflux for 48 hours. The reaction was cooled to -78 °C and 2,3,4,5,6-pentafluorobenzaldehyde (0.04 mL, 0.34 mmol) was added followed by DMAC (1M in hexane, 0.34 mL, 0.34 mmol). The reaction was stirred at -78 °C for 15 minutes before being allowed to warm to room temperature. The reaction was stirred at room temperature for 18 hours. The reaction was poured into saturated sodium bicarbonate solution (10 mL) and extracted with DCM (2 x 10mL). The combined organic layers were dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure to give the crude product as a pale brown solid. The product was purified by column chromatography (Petrol : Ethyl acetate 3 : 1, column diameter = 2 cm, silica = 15 cm) to give a separable 5:1 mixture of (3a*S**,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione and (3a*S**,5*S**,10b*S**)-5-((*R**)-hydroxy(perfluorophenyl)methyl)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (72 %, 149 mg, 0.25 mmol).

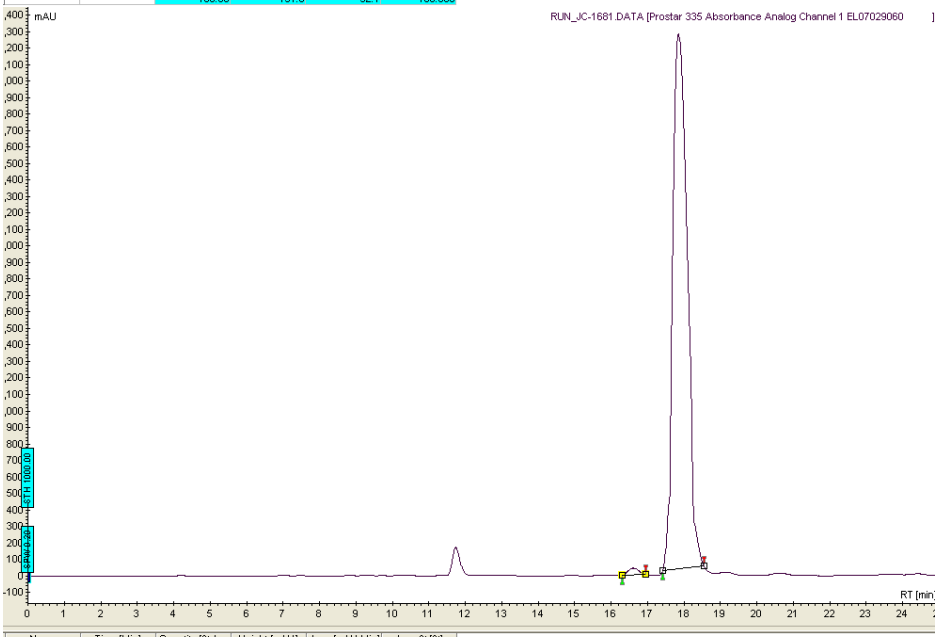
Major Diastereomer: Mp: 120.4-121.7 °C; R_f: 0.24 (Pet:EA 3:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H δ 7.85 (3H, app d, *J* = 8.4 Hz), 7.31 – 7.22 (4H, m), 7.22 – 7.14 (1H, m), 5.13 (1H, d, *J* = 8.1Hz, H^{20}), 4.99 (1H, d, *J* = 7.5 Hz, H^{10}), 3.71 – 3.52 (2H, m, H^{13} + H^{11}), 3.01 (3H, s, H^{19}), 2.38 (3H, s, H^{33}), 2.20 – 2.10 (1H, m, H^{12}), 1.67 (1H, app td, *J* = 13.8, 5.3 Hz, H^{12}); ^{13}C NMR (400 MHz, $CDCl_3$): δ_C 177.7 C=O, 173.3 C=O, 145.3, 137.4, 135.4, 129.8, 129.7, 129.6, 127.2, 125.3, 123.9, 120.1, 119.9, 115.2, 70.2 C^{20}, 41.5 C^{10}, 39.1 C^{11}, 36.9 C^{13}, 28.7 C^{19}, 25.2 C^{12}, 21.7 C^{33}; IR(neat): υ_{max}/cm^{-1} 3371, 2981, 2889, 1690;MS (pNSI): 605.1 (40%, $(M+H)^+$), 622.1 (88%, $(M+NH_4)^+$), 627.1 (100%, $(M+Na)^+$), 643.1 (17%), 709.1 (15%); HRMS (pNSI): calcd for

$C_{29}H_{21}F_5N_2NaO_5S$ $[M+Na]^+$: 627.0984; observed: 627.0968. *Note: ^{13}C NMR missing peaks due to C-F coupling.*

The *ee* of the product was determined by HPLC using an AD-H column (*n*-hexane/*i*PrOH 80:20, flow rate 0.75 mL/min, t_{maj} = 16.4 min, t_{min} = 17.9 min, >95% *ee*). $[\alpha]_D^{25}$ = +56 (*c* = 0.60 in $CHCl_3$).

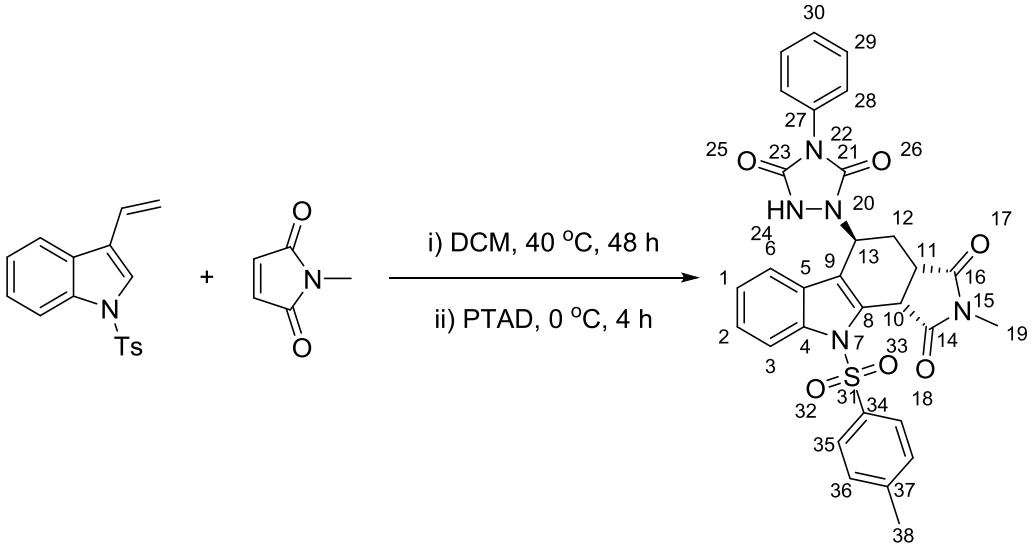


Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
UNKNOWN	16.43	50.55	70.2	26.3	50.550
UNKNOWN	17.87	49.45	61.3	25.7	49.450
		100.00	131.6	52.1	100.000



Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
UNKNOWN	16.61	0.89	40.3	13.5	0.895
UNKNOWN	17.85	99.11	3244.4	1497.2	99.105
		100.00	3284.7	1510.7	100.000

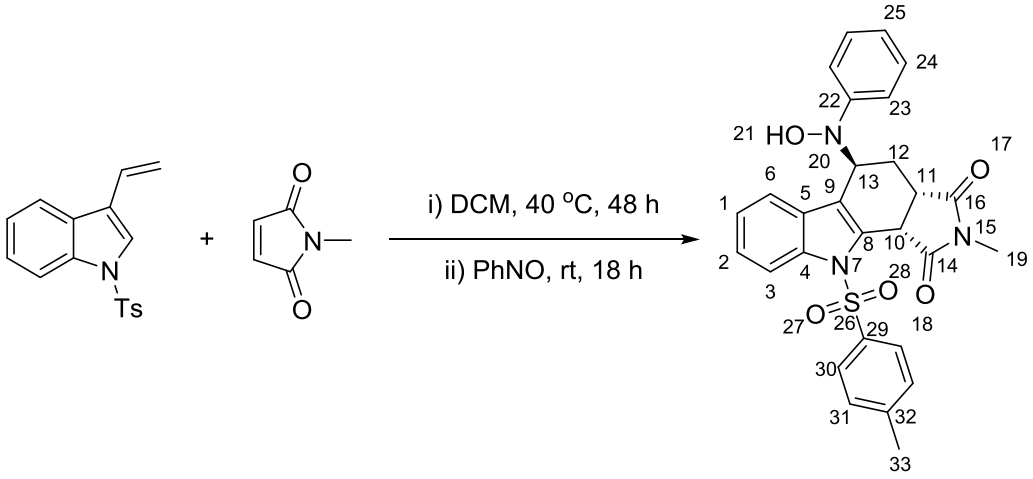
(3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 167**



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol), DCM (5 mL) and and 1-methyl-1*H*-pyrrole-2,5-dione (38 mg, 0.34 mmol) and the resulting solution was heated at reflux for 48 hours. The reaction was cooled to 0 °C before PTAD (60 mg, 0.34 mmol) was added. The reaction was stirred at 0 °C for 4 hours. The solvent was removed under reduced pressure to leave the crude product as a pale red powder. The product was purified by column chromatography (Petrol : Ethyl acetate 4 : 1, column diameter = 1 cm, silica = 14 cm) to give (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (76%, 150 mg, 0.26 mmol) as a white powder.

Mp: 183.4 – 187.7 °C; R_f: 0.05 (Pet:EA 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.84 (2H, d, *J* =8.4 Hz), 7.67 (1H, d, *J* = 8.2 Hz), 7.52 (1H, d, *J* = 7.6 Hz), 7.46-7.31 (5H, m), 7.29-7.19 (2H, m), 7.17 (2H, d, *J* = 8.3 Hz), 5.55 (1H, app t, *J* = 4.7 Hz, H^{13}), 5.08 (1H, d, *J* = 7.7 Hz, H^{10}), 3.66 (1H, ddd, *J* = 10.5, 7.7, 5.8 Hz, H^{11}), 2.96 (3H, s, H^{19}), 2.49 (1H, app dt, *J* = 14.8, 5.3 Hz, H^{12}), 2.28 (3H, s, H^{38}), 2.14 (1H, ddd, *J* = 14.8, 10.5, 5.5 Hz, H^{12}); ^{13}C NMR (400 MHz, $CDCl_3$): δ_C 177.4 C=O, 173.4 C=O, 153.6, 152.8, 145.5, 137.0, 135.3, 132.3, 130.8, 130.0, 129.3, 128.5, 127.5, 126.9, 125.7, 125.6, 124.3, 119.4, 115.4, 114.9, 47.8 C^{13}, 40.3 C^{10}, 39.0 C^{11}, 28.4 C^{19}, 25.4 C^{12}, 21.7 C^{38}; IR(neat): υ_{max}/cm^{-1} 3665, 2984, 2884, 1699; MS (pNSI): 601.2 (100%, $(M+NH_4)^+$), 1184.3 (13%, $(2M+NH_4)^+$); HRMS (pNSI): calcd for $C_{30}H_{29}N_6O_6S$ $[M+NH_4]^+$: 601.1864; observed: 601.1861.

(3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 171**

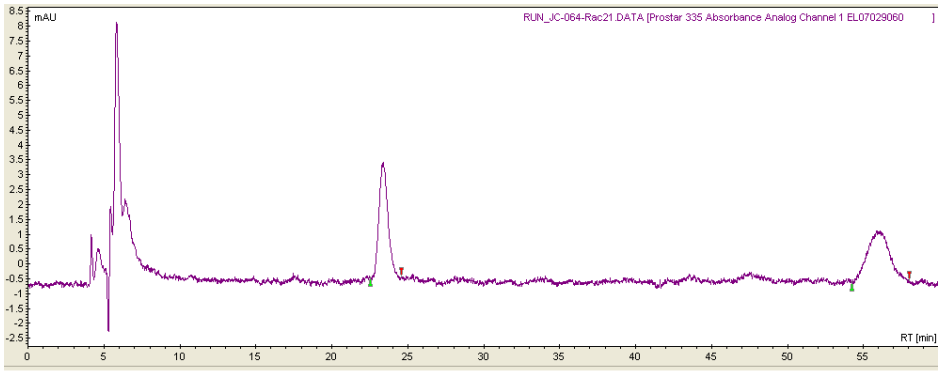


To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol), DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (38 mg, 0.34 mmol). The resulting solution was heated at reflux for 48 hours. The reaction was allowed to cool to room temperature, nitrosobenzene (40mg, 0.34 mmol) was added, and the solution was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid which was purified by column chromatography (Petrol : Ethyl acetate 4 : 1, column diameter = 1 cm, silica = 16 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione as a yellow powder (71%, 128 mg, 0.24 mmol).

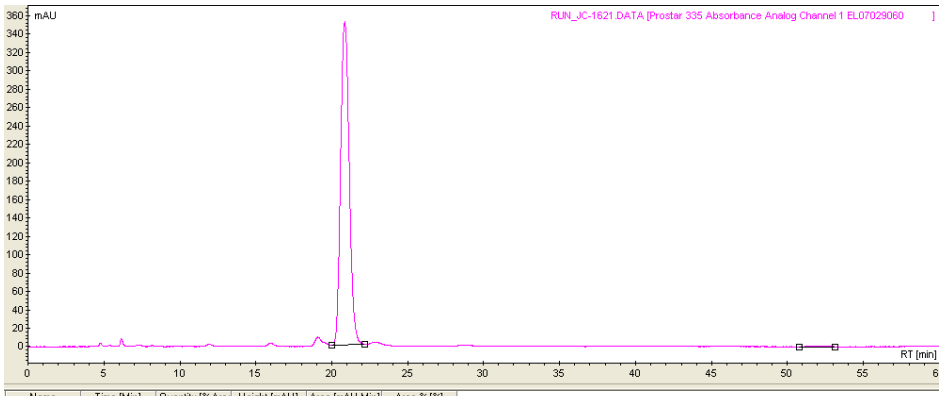
Mp: 196.8-199.5 ^{o}C; R_f: 0.64 (Pet:EA, 1:1); ^{1}H NMR (500 MHz, DCM-d2): δ_H 7.94 (1H, d, *J* = 8.4 Hz), 7.69 (2H, d, *J* = 8.2 Hz), 7.60 (1H, d, *J* = 7.9 Hz), 7.32-7.25 (3H, m) , 7.23 (2H, d, *J* = 8.2 Hz), 7.18-7.13 (3H, m), 7.02 (1H, t, *J* = 7.3 Hz), 5.06 (1H, d, *J* = 8.0 Hz, H^{10}), 4.75 (1H, app. t, *J* = 5.9 Hz, H^{13}), 4.72 (1H, s, OH), 3.64 (1H, app. q, *J* = 7.2 Hz, H^{11}), 2.95 (3H, s, H^{19}), 2.43 (1H, app dt, *J* = 13.6, 6.4 Hz, H^{12}), 2.35 (3H, s, H^{33}), 2.06 (1H, ddd, *J* = 13.6, 7.2, 4.9, H^{12}); ^{13}C NMR (400 MHz, DCM-d2): δ_C 178.1 C=O, 173.6 C=O, 150.7, 145.3, 137.5, 134.9, 131.4, 129.7, 128.9, 128.9, 126.8, 125.2, 124.2, 122.6, 121.7, 120.2, 117.2, 115.4, 58.0 C^{13}, 40.5 C^{10}, 39.5 C^{11}, 25.0 C^{19}, 23.3 C^{12}, 21.4 C^{33}; IR(neat): υ_{max}/cm^{-1} 3661, 2990, 2886, 1690; MS (pNSI): 407.1 (66%, $(M-(C_6H_5NOH))^+$), 516.2 (49%, $(M+H)^+$), 533.2 (100%, $(M+NH_4)^+$), 1031.3 (57%, $(2M+H)^+$), 1053.3 (13%, $(2M+Na)^+$); HRMS (pNSI): calcd for $C_{28}H_{26}N_3O_5S$ $[M+H]^+$: 516.1588; observed: 516.1584.

Note: ^{1}H NMR run at 35 ^{o}C, broad signals observed at room temperature.

The *ee* of the product was determined by HPLC using an AD-H column (*n*-hexane/*i*PrOH 80:20, flow rate 0.75 mL/min, t_{maj} = 23.4 min, t_{min} = 56.1 min, >95% *ee*). $[\alpha]_D^{25}$ = +42 (*c* = 0.50 in $CHCl_3$).

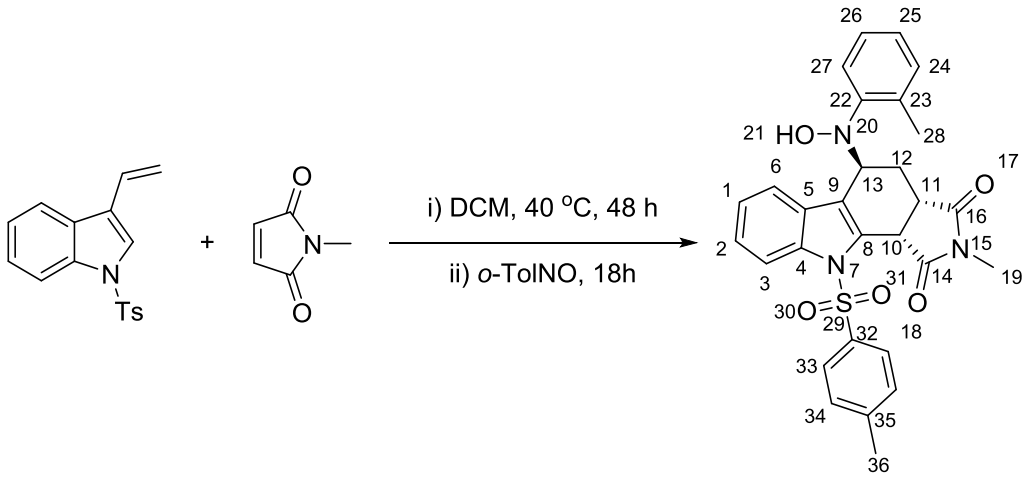


Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
UNKNOWN	23.37	48.26	3.9	2.6	48.257
UNKNOWN	56.07	51.74	1.7	2.8	51.743
		100.00	5.6	5.4	100.000



Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
UNKNOWN	20.85	99.75	351.9	231.2	99.751
UNKNOWN	51.72	0.25	0.5	0.6	0.249
		100.00	352.5	231.8	100.000

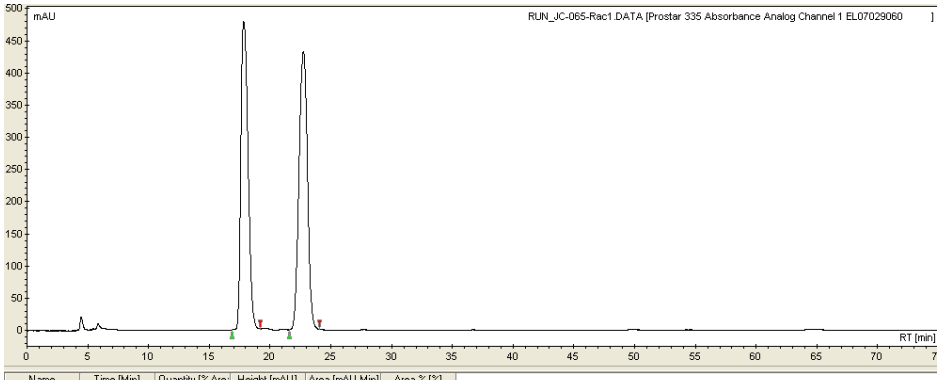
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 173**



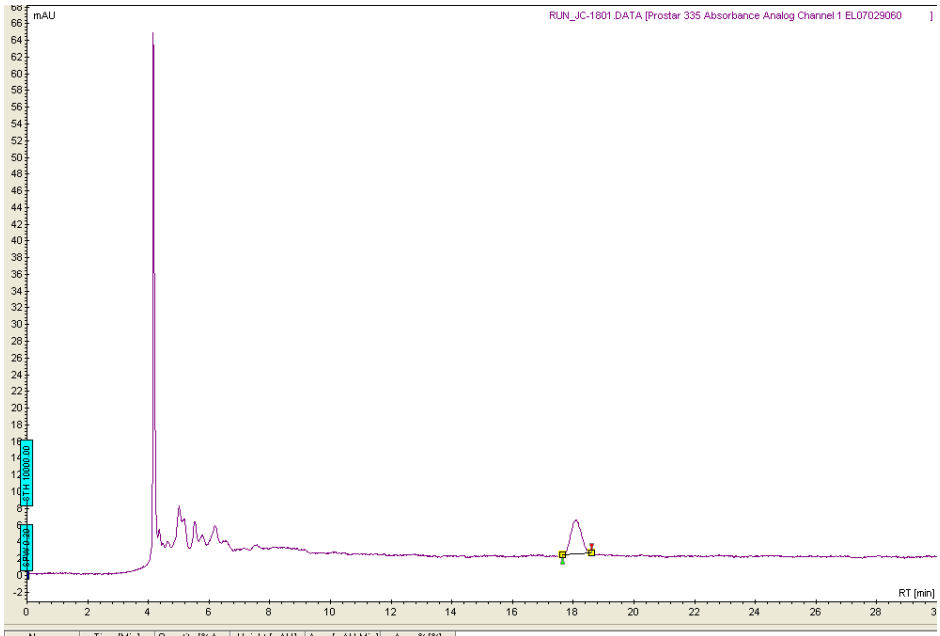
To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol), DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (38 mg, 0.34 mmol). The resulting solution was heated at reflux for 48 hours. The reaction was allowed to cool to room temperature, 1-methyl-2-nitrosobenzene (42 mg, 0.17 mmol) was added and the solution was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol : Ethyl acetate 4 : 1, column diameter = 1 cm, silica = 14 cm) to give **(**3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (71%, 128 mg, 0.24 mmol) as a yellow powder.

Mp: 193.0-196.7 ^{o}C; R_f: 0.59 (Pet:EA 1:1); ^{1}H NMR (400 MHz, DCM-d2): δ_H 7.89 (1H, d, *J* = 8.3 Hz), 7.65 (2H, d, *J* = 8.4 Hz), 7.40 (1H, d, *J* = 7.9 Hz), 7.28 (1H, d, *J* = 7.7 Hz) 7.21-7.18 (3H, m), 7.13-6.99 (4H, m), 5.04 (1H, d, *J* = 8.1 Hz, H^{10}), 4.87 (1H, s, OH), 4.27 (1H, app. t, *J* = 5.4 Hz, H^{12}), 3.73 (1H, app td, *J* = 8.0, 6.1 Hz, H^{11}), 2.91 (3H, s), 2.58 (1H, app dt, *J* = 12.9, 6.2 Hz, H^{12}), 2.32 (3H, s, H^{28}), 2.25 (3H, s, H^{36}), 1.95 (1H, ddd, *J* = 12.9, 7.8, 4.5 Hz, H^{12}); ^{13}C NMR (400 MHz, DCM-d2): δ_C 178.2 C=O, 173.6 C=O, 149.3, 145.3, 137.3, 134.9, 131.6, 130.9, 129.7, 129.7, 129.2, 126.8, 126.2, 125.0, 124.9, 124.1, 121.4, 120.5, 115.3, 57.3 C^{13}, 40.6 C^{10}, 39.4 C^{11}, 25.0 C^{19}, 24.6 C^{28}, 21.4 C^{12}, 18.3 C^{36}; IR(neat): υ_{max}/cm^{-1} 3662, 2990, 2886, 1701; MS (pNSI): 407.1 (98%, (M-((*o*-CH_3)-C_6H_4NOH))$^+$), 530.2 (52%, (M+H)$^+$), 547.2 (65%, (M+NH_4)$^+$), 1059.3 (100%, (2M+H)$^+$); HRMS (pNSI): calcd for $C_{29}H_{28}N_3O_5S$ [M+H]$^+$: 530.1744; observed: 530.1743.

The *ee* of the product was determined by HPLC using an AD-H column (*n*-hexane/*i*PrOH 80:20, flow rate 0.75 mL/min, t_{maj} = 17.8 min, t_{min} = 22.8 min, >95% *ee*). $[\alpha]_D^{25}$ = +44 (*c* = 0.50 in $CHCl_3$).

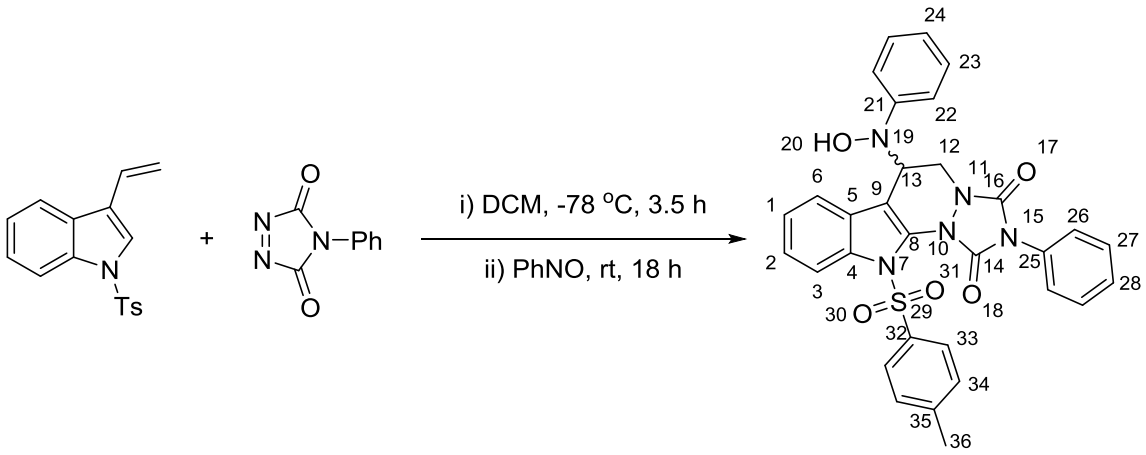


Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
UNKNOWN	17.84	48.45	478.0	329.2	48.454
UNKNOWN	22.75	51.55	432.4	350.3	51.546
		100.00	910.4	679.5	100.000



Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
UNKNOWN	18.08	100.00	4.1	1.7	100.000
		100.00	4.1	1.7	100.000

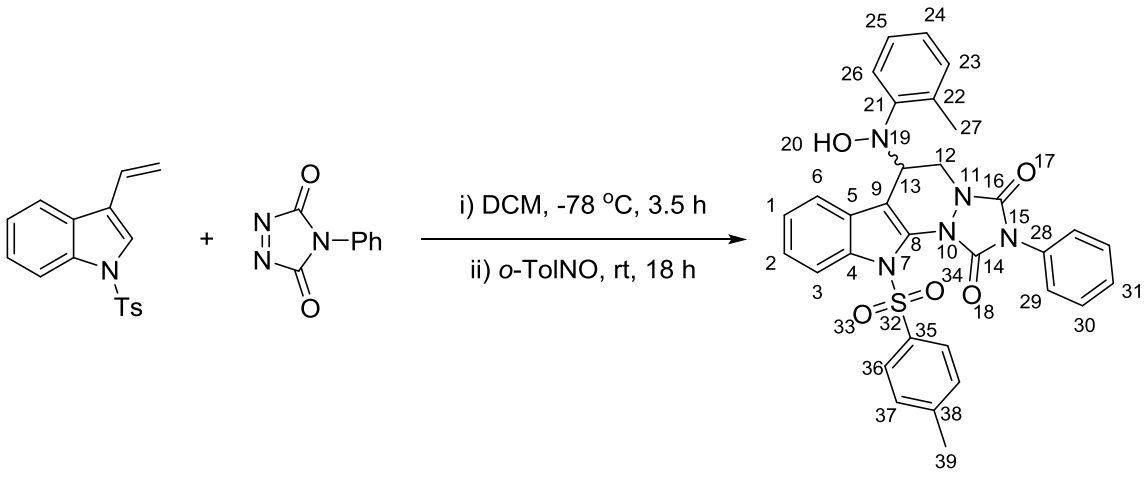
6-(hydroxy(phenyl)amino)-2-phenyl-11-tosyl-5,6-dihydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*,11*H*)-dione – 177



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol) and DCM (5 mL) and the solution was cooled to -78 °C. To this solution PTAD (70 mg, 0.34 mmol) was added and the reaction was stirred at -78 °C for 3.5 hours. The reaction was warmed to room temperature, nitrosobenzene (44 mg, 0.34 mmol) was added and the reaction was stirred for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow oil. The product was purified by column chromatography (column diameter = 1 cm, silica = 16 cm, eluent = Petrol: Ether: DCM 2: 1: 1) to give 6-(hydroxy(phenyl)amino)-2-phenyl-11-tosyl-5,6-dihydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*,11*H*)-dione (72%, 54 mg, 0.09 mmol) as a white powder.

Mp: 176.1 – 180.0 °C; R_f: 0.48 (Pet:Et_2O 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.03 (1H, d, *J* = 8.3 Hz), 7.62 (2H, d, *J* = 8.1 Hz), 7.56 (2H, d, *J* = 7.6 Hz), 7.46 (2H, app t, *J* = 7.7 Hz), 7.42-7.36 (1H, m), 7.23-7.07 (7 H, m), 7.05-6.99 (2H, m), 6.59 (1H, d, *J* = 7.4 Hz), 5.81 (1H, br s, OH), 5.18 (1H, d, *J* = 13.5 Hz, H^{12}), 4.59 (1H, s, H^{13}), 3.23 (1H, d, *J* = 13.5 Hz, H^{12}), 2.30 (3H, s, H^{36}); ^{13}C NMR (101 MHz, DMSO-d_6): δ_C 152.7 C=O, 152.2, 150.5 C=O, 146.0, 134.9, 132.9, 132.5, 131.8, 130.4, 129.8, 129.3, 129.2, 128.5, 127.3, 127.3, 125.5, 125.4, 122.5, 119.8, 117.6, 116.6, 108.6, 55.9 C^{13}, 44.6 C^{12}, 21.6 C^{36}; IR(neat): υ_{max}/cm^{-1} 2981, 2884, 1714; MS (pAPCI): 138.1 (100%), 157.0 (95%), 213.1 (50%), 248.1 (86%), 279.1 (62%), 317.1 (33%), 333.1 (29%), 471.1 (31%), 564.2 (11%), 580.2 (10%, $(M+H)^+$); HRMS (pAPCI): calcd for $C_{31}H_{26}N_5O_5S$ $[M+H]^+$: 580.1649; observed: 580.1640.

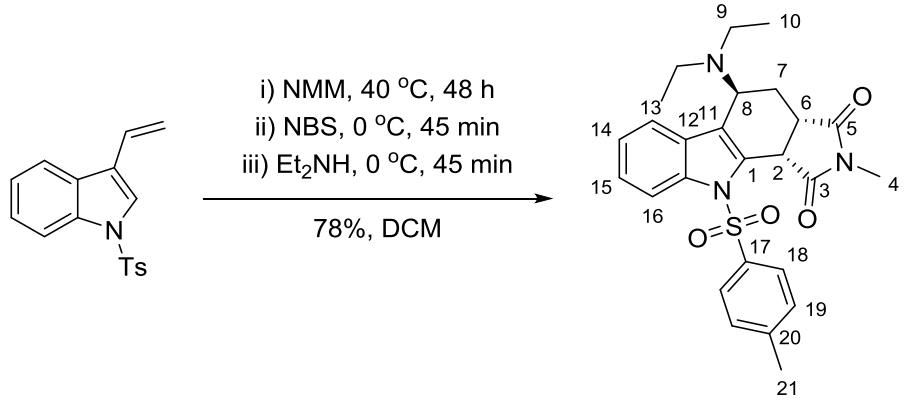
6-(hydroxy(*o*-tolyl)amino)-2-phenyl-11-tosyl-5,6-dihydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*,11*H*)-dione – 178



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol) and DCM (5 mL) and the solution cooled to -78 °C. To this solution PTAD (70 mg, 0.34 mmol) was added and the reaction was stirred at -78 °C for 3.5 hours. The reaction was warmed to room temperature and 1-methyl-2-nitrosobenzene (42 mg, 0.34 mmol) was added and the reaction was stirred for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow oil. The product was purified by column chromatography (column diameter = 1 cm, silica = 14 cm, eluent = Petrol: Ether: DCM 2: 1: 1) to give 6-(hydroxy(*o*-tolyl)amino)-2-phenyl-11-tosyl-5,6-dihydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*,11*H*)-dione as a white powder (78%, 60 mg, 0.10 mmol).

Mp: 149.7 – 153.1 °C; R_f: 0.32 (Pet:Et_2O:DCM 2:1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.99 (d, 1H, *J* = 8.3 Hz), 7.61-7.58 (m, 5H), 7.43-7.40 (m, 1H), 7.47 (2H, app t, *J* = 7.7 Hz), 7.40-7.37 (m, 1H), 7.18 (2H, app t, *J* = 7.8 Hz), 7.11 (d, 2H, *J* = 8.2 Hz), 6.97 (2H, app q, *J* = 7.2 Hz), 6.85 (d, 1H, *J* =7.5 Hz), 6.50 (d, 1H, *J* = 7.8 Hz), 5.93 (s, 1H, OH), 5.24 (d, 1H, *J* = 13.5 Hz, H^{12}), 4.44 (s, 1H, H^{13}), 3.11 (d, 1H, *J* = 13.5 Hz, H^{12}), 2.29 (s, 3H, H^{27}), 1.91 (s, 3H, H^{39}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 153.3 C=O, 150.3 C=O, 148.7, 145.4, 135.1, 133.7, 132.0, 131.7, 131.3, 130.6, 129.6, 129.3, 128.7, 127.9, 127.2, 127.0, 126.8, 125.9, 124.9, 124.8, 122.6, 118.0, 116.8, 107.0, 59.1 C^{13}, 44.5 C^{12}, 21.7 C^{27}, 17.8 C^{39}; IR(neat): υ_{max}/cm^{-1} 3068, 2981, 1713; MS (pAPCI): 138.1 (100%), 157.0 (82%), 262.1 (55%), 279.1 (76%), 317.1 (50%), 391.3 (37%), 471.1 (21%), 594.2 (10%, $(M+H)^+$); HRMS (pAPCI): calcd for $C_{32}H_{28}N_5O_5S$ $[M+H]^+$: 594.1806; observed: 594.1801.

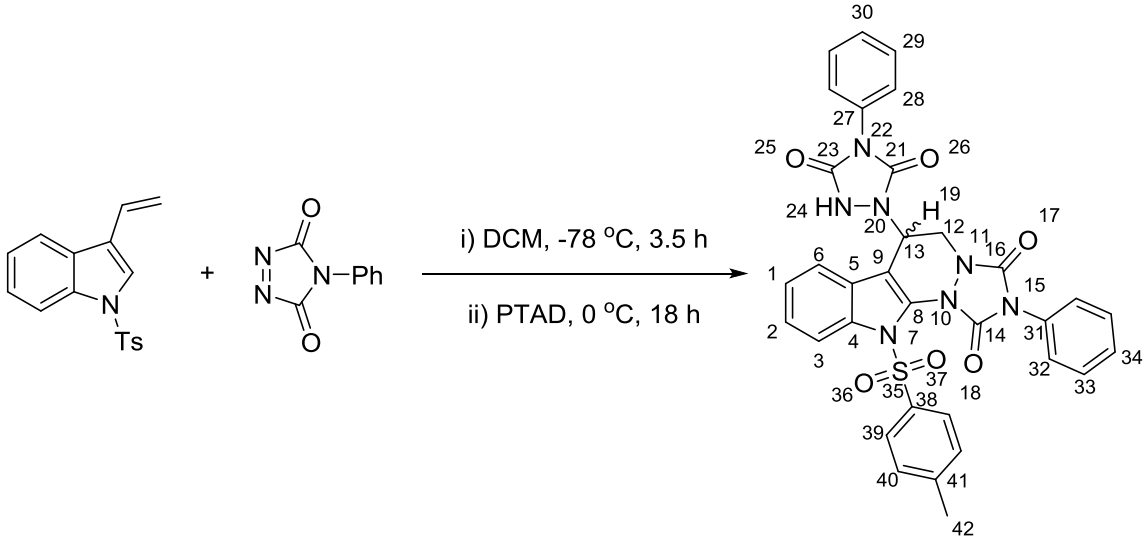
(3a*S,5*S**,10b*S**)-5-(diethylamino)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 188**



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (60 mg, 0.20 mmol), DCM (3 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (22 mg, 0.20 mmol) was added and the resulting solution was heated at reflux for 48 hours. The reaction was cooled to 0 °C and *N*-bromosuccinimide (36 mg, 0.20 mmol) was added. The reaction was stirred at 0 °C for 45 minutes before diethylamine (50 µL, 0.40 mmol) was added and the reaction was stirred at 0 °C for 45 minutes before the solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol: Ether: DCM 2: 1: 1, column diameter = 1.5 cm, silica = 17 cm) to give (3a*S**,5*S**,10b*S**)-5-(diethylamino)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (75 mg, 0.16 mmol, 78%) as a yellow powder.

Mp: 168.8 – 171.5 °C; R_f: 0.37 (Pet:Et_2O:DCM 2:1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.99 (1H, d, *J* = 7.7 Hz), 7.92 (1H, d, *J* = 8.3 Hz), 7.58 (2H, d, *J* = 8.2 Hz, H^{18}), 7.24 (1H, t, *J* = 7.7 Hz), 7.15 (1H, t, *J* = 7.6 Hz), 7.11 (2H, d, *J* = 8.2 Hz, H^{19}), 5.02 – 4.98 (1H, m, H^2), 3.99 – 3.93 (1H, m, H^8), 3.50 (1H, app dt, *J* = 8.2, 5.1 Hz, H^6), 2.93 (3H, s, H^{21}), 2.45 – 2.31 (3H, m), 2.29 (3H, s, H^4), 1.88 – 1.80 (1H, m, H^7), 0.94 (3H, t, *J* = 7.1 Hz, H^{10}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 178.4 (CO), 173.9 (CO), 144.7, 138.4, 134.6, 130.1, 129.9, 129.4 (C^{18}), 126.9, 126.8 (C^{19}), 125.2, 123.8, 122.3, 116.0, 51.5, 43.4 (C^9), 40.4, 39.4, 25.3, 21.6, 21.2, 14.1 (C^{10}); IR (cm^{-1}): *v* 2919 (CH), 2850 (CH), 1703 (CO); MS (pAPCI): 407.1 (100%, $(M-(N(Et_2))^+$), 480.2 (63%, $(M+H)^+$), 959.4 (21%, $(2M+H)^+$); HRMS (pAPCI): calcd for $C_{26}H_{30}N_3O_4S$ $[M+H]^+$: 480.1952; observed: 480.1946.

6-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-phenyl-11-tosyl-5,6-dihydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*,11*H*)-dione - 193

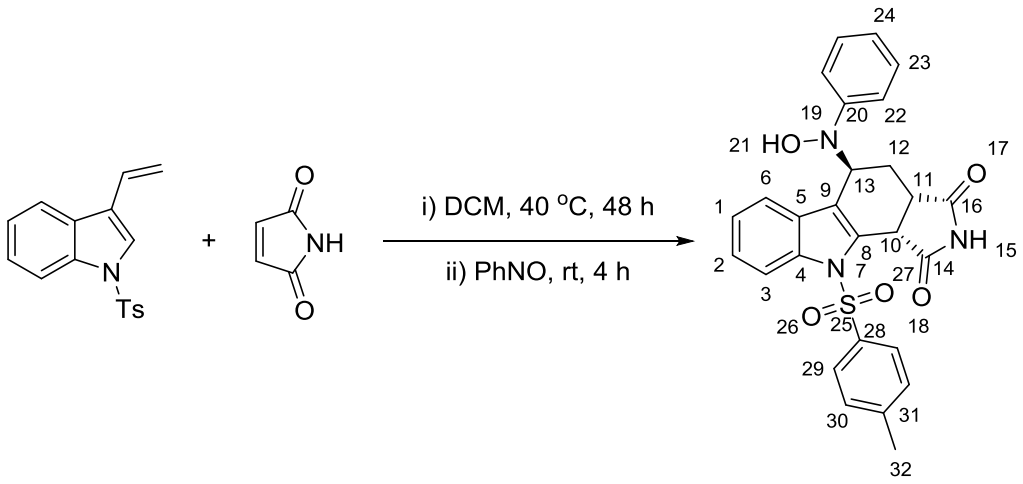


To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol) and DCM (5 mL).he resulting solution was cooled to -78 °C and then PTAD (70 mg, 0.34 mmol) was added. The reaction was stirred at -78 °C for 3.5 hours. The reaction was warmed to 0 °C and a further equivalent of PTAD (60 mg, 0.34 mmol) was added.The reaction was stirred 0 °C for 4 hours, resulting in the formation of a white precipitate. The reaction mixture was filtered and 6-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-phenyl-11-tosyl-5,6-dihydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*,11*H*)-dione (29%, 61 mg, 0.094 mmol) was recovered as a white powder.

Mp: 227.8-230.6 °C; ^{1}H NMR (400 MHz, DMSO-d6): δ_H 10.76 (s, 1H, NH), 7.91 (d, 1H, *J* = 7.8 Hz), 7.61 (d, 2H, *J* = 8.3 Hz), 7.48-7.24 (m, 15H), 5.52 (s, 1H, H^{19}), 4.76 (d, 1H, *J* = 13.8 Hz, H^{12}), 3.86 (d, 1H, *J* = 13.8 Hz, H^{12}), 2.24 (s, 3H, H^{42}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 154.3, 153.5, 150.6, 149.5, 146.0, 135.1, 133.3, 133.0, 131.7, 131.5, 130.4, 129.6, 129.5, 129.4, 128.8, 128.1, 127.6, 127.5, 126.9, 125.9, 125.4, 119.3, 116.8, 104.1, 48.8 C^{13}, 43.3 C^{12}, 21.6 C^{42}; IR(neat): υ_{max}/cm^{-1} 2971, 2883, 1714; MS (pNSI): 263.0 (36%), 345.0 (51%), 371.1 (42%), 665.2 (89%, $(M+NH_4)^+$), 670.1 (100%, $(M+Na)^+$); HRMS (pNSI): calcd for $C_{33}H_{25}N_7NaO_6S$ $[M+Na]^+$: 670.1479; observed: 670.1475.

Note – No R_f data due to poor solubility in DCM.

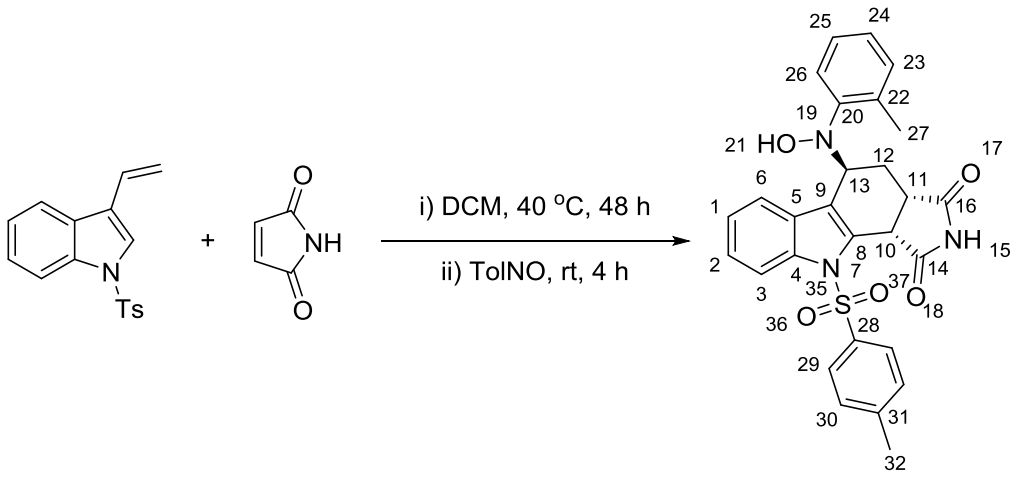
(3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 199**



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol), DCM (5 mL) and 1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol) and the resulting solution was heated at reflux for 48 hours. The reaction was cooled to room temperature and nitrosobenzene (36 mg, 0.34 mmol) was added. The reaction was stirred at room temperature for 4 hours before the solvent was removed under reduced pressure to leave the crude product as a white solid. The product was purified by tritriation from DCM to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione as a white powder (151 mg, 0.30 mmol) in a 89% yield.

Mp: 203.7-206.9 °C; R_f: 0.15 (Pet:EA 2:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 11.26 (1H, s), 8.45 (1H, s), 7.88 (1H, d, *J* = 8.3 Hz). 7.76 (2H, d, *J* = 8.2 Hz), 7.42 (1H, d, *J* = 7.8 Hz), 7.33 (2H, d, *J* = 8.2 Hz), 7.29-7.04 (5H, m), 6.89 (1H, t, *J* = 7.2 Hz), 5.17 (1H, d, *J* = 7.8 Hz, H^{10}), 4.88 (1H, app t, *J* = 4.4 Hz, H^{13}), 3.65 (1H, app td, *J* = 8.8, 5.9 Hz, H^{11}), 2.32 (3H, s, H^{32}), 2.36-2.27 (1H, m, H^{12}), 1.81 (1H, ddd, *J* = 14.0, 9.4, 5.0 Hz, H^{12}); ^{13}C NMR (400 MHz, DMSO-d6): δ_C 179.4 C=O, 174.7 C=O, 152.0, 144.7, 136.4, 135.0, 131.5, 129.8, 128.9, 128.4, 126.6, 124.4, 123.4, 121.2, 120.7, 120.6, 117.0, 114.4, 56.8 C^{13}, 41.5 C^{10}, 40.6 C^{11}, 25.2 C^{12}, 20.9 C^{32}; IR(neat): υ_{max}/cm^{-1} 3452, 2981, 1715; MS (pNSI): 393.1 (100%, (M-(C_6H_5NOH)$^+$), 502.1 (14%, (M+H)$^+$), 519.2 (96%, (M+NH_4)$^+$), 524.1 (17%, (M+Na)$^+$), 1003.3 (40%, (2M+H)$^+$), 1025.3 (15%, (2M+Na)$^+$); HRMS (pNSI): calcd for $C_{27}H_{24}N_3O_5S$ [M+H]$^+$: 502.1431; observed: 502.1428.

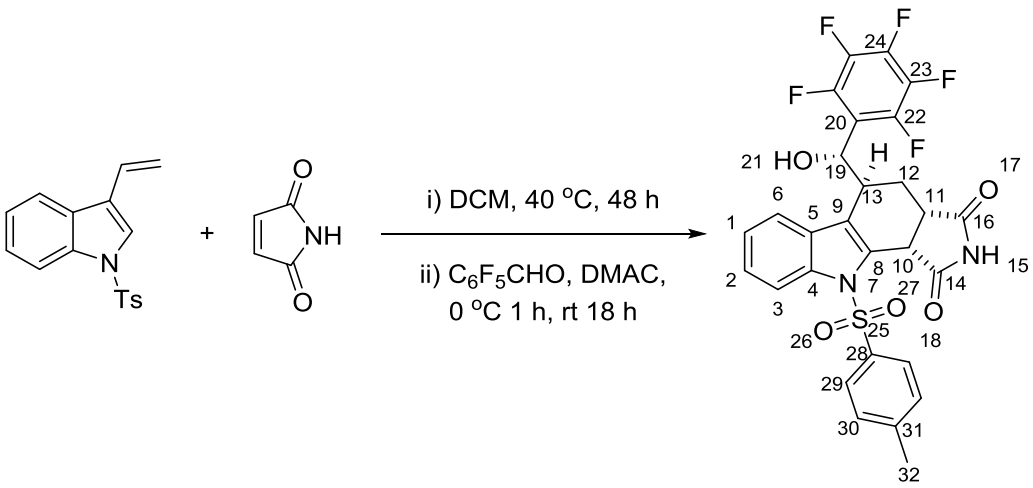
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 200**



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol), DCM (5 mL) and 1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol) was added and the resulting solution was heated at reflux for 48 hours. The reaction was cooled to room temperature and 1-methyl-2-nitrosobenzene (41 mg, 0.34 mmol) was added. The reaction was stirred at room temperature for 4 hours before the solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol : Ether: DCM 2 : 1 : 1, column diameter = 2 cm, silica = 14 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (82%, 143 mg, 0.28 mmol) as a yellow powder.

Mp: 171.1-174.0 °C; R_f: 0.13 (Pet:EA 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (1H, d, *J* = 8.4 Hz), 7.73 (2H, d, *J* = 8.2 Hz), 7.77 (1H, s), 7.35 (2H, dd, *J* = 15.7, 7.9 Hz), 7.20 (3H, app d, *J* = 8.2 Hz), 7.09 (2H, d, *J* = 7.5 Hz), 7.07 – 7.00 (2H, m), 5.17 (1H, d, *J* = 8.1 Hz, H^{10}), 4.75 (1H, s, OH), 4.39 (1H, app t, *J* = 5.1 Hz, H^{13}), 3.80 (1H, app q, *J* = 8.3, 5.7 Hz, H^{11}), 2.64 (1H, app dt, *J* = 13.1, 5.5 Hz, H^{12}), 2.34 (3H, s, H^{32}), 2.26 (3H, s, H^{27}), 1.96 (1H, ddd, *J* = 15.9, 7.9, 3.7 Hz, H^{12}); ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 178.3 C=O, 173.4 C=O, 149.3, 144.7, 137.4, 135.8, 131.3, 130.9, 129.8, 129.6, 129.5, 129.0, 127.0, 126.3, 125.1, 124.7, 123.7, 121.5, 120.1, 115.3, 57.3 C^{13}, 42.0 C^{10}, 40.8 C^{11}, 26.0 C^{12}, 21.4 C^{32}, 18.3 C^{27}; IR(neat): υ_{max}/cm^{-1} 3294, 2981, 1713; MS (pAPCI): 293.1 (16%), 332.1 (13%), 342.1 (16%), 393.1 (100%, $(M-(o-(CH_3)C_6H_4NOH)^+)$), 489.1 (54%, $(M-H_2O)^+$), 516.2 (26%, $(M+H)^+$); HRMS (pAPCI): calcd for $C_{28}H_{26}N_3O_5S$ $[M+H]^+$: 516.1588; observed: 516.1576.

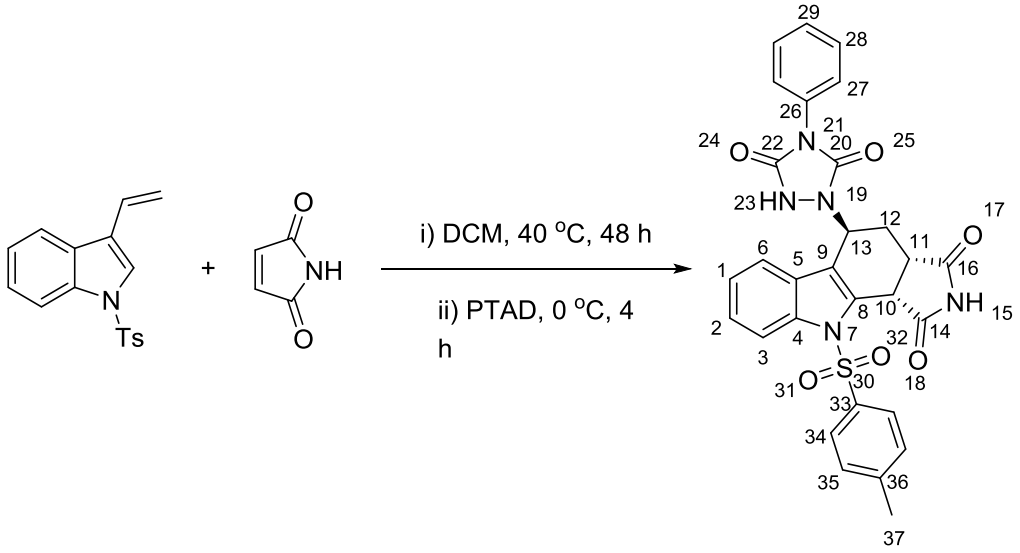
(3a*S,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 201**



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol), DCM (5 mL) and 1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol). The resulting solution was heated at reflux for 48 hours. The reaction was cooled to 0 °C and pentafluorobenzaldehyde (67 mg, 0.34 mmol) and DMAC (1M in hexane, 0.34 mL, 0.34 mmol) were added. The solution was stirred at 0 °C for 1 hour and then warmed to room temperature for 18 hours. The reaction was poured into sodium bicarbonate (15 mL) and extracted with DCM. The combined organic layers were dried with MgSO4, filtered and the solvent was removed under reduced pressure to give the crude product as an off white solid. The product was purified by column chromatography (diameter = 1.5 cm, silica = 15 cm, eluent = Pet: EA 2: 1) to give (3a*S**,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (71 %, 0.1427 g, 0.24 mmol) as an off white solid.

Mp: 181.3-185.1 °C; R_f: 0.22 (Pet:EA 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.03 (1H, br, NH), 7.81 (3H, app d, *J* = 7.9 Hz), 7.28 (1H, d, *J* = 8.0 Hz), 7.25 – 7.22 (3 H, m), 7.16 (1H, app t, J = 7.6 Hz), 5.08 (1H, d, *J* = 8.3 Hz, H^{19}), 5.05 (1H, d, *J* = 7.4 Hz, H^{10}), 3.61 – 3.66 (1H, m, H^{13}), 3.49 – 3.57 (1H, m, H^{11}), 2.34 (3H, s, H^{32}), 2.09 (1H, dd, *J* = 14.0, 4.5 Hz, H^{12}), 1.74 (1H, app td, *J* = 13.9, 5.3 Hz, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 177.4 C=O, 173.0 C=O, 145.3, 137.4, 135.5, 129.8, 129.7, 129.1, 127.1, 125.5, 123.9, 120.2, 120.0, 115.3, 70.2 C^{19}, 42.5 C^{10}, 40.3 C^{11}, 36.8 C^{13}, 28.5 C^{12}, 21.7 C^{32}; IR(neat): υ_{max}/cm^{-1} 3240, 2981, 1717; MS (pAPCI): 157.0 (79%), 221.1 (61%), 393.1 (15%, $(M-(C_6F_5COH)^+)$, 443.1 (51%), 573.1 (8%, $(M-H_2O)^+$), 591.1 (100%, $(M+H)^+$); HRMS (pAPCI): calcd for $C_{28}H_{20}F_5N_2O_5S$ $[M+H]^+$: 591.1008; observed: 591.1001. *Note: ^{13}C NMR missing peaks due to C-F coupling.*

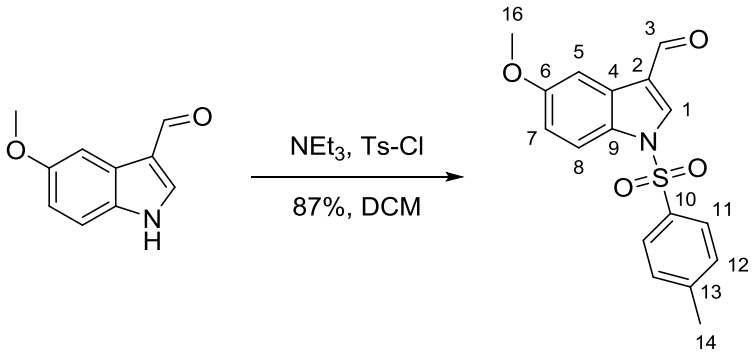
(3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 202**



To a stirred round bottomed flask was added 1-tosyl-3-vinyl-1*H*-indole (100 mg, 0.34 mmol), DCM (5 mL) and 1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol). The resulting solution was heated at reflux for 48 hours. The reaction was cooled to 0 °C and PTAD was added. The solution was stirred at 0 °C for 4 hours and the solvent was removed under reduced pressure to give the crude product as pale red. The product was purified by column chromatography (diameter = 1.5 cm, silica = 17 cm, eluent = Pet: EA 1: 1) to give (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (23 %, 0.044 g, 0.08 mmol) as an off white solid.

Mp: 206.4-209.7 °C; R_f = 0.10 (Pet:EA 2:1); ^{1}H NMR (400 MHz, DMSO-d6): δ_C 11.37 (1H, s, NH), 10.77 (1H, s, NH), 7.82 (2H, d, *J* = 8.3 Hz), 7.72 (1H, d, *J* = 7.9 Hz), 7.48 – 7.43 (2H, m), 7.42 – 7.35 (4H, m), 7.28 (2H, d, *J* = 8.5 Hz), 7.25 – 7.18 (2H, m), 5.46 (1H, app t, *J* = 4.9 Hz, H^{13}), 5.19 (1H, d, *J* = 7.7 Hz, H^{10}), 3.77 – 3.65 (1H, m, H^{11}), 2.41 (1H, app dt, *J* = 9.8, 5.1 Hz, H^{12}), 2.26 (3H, s, H^{37}), 2.24 – 2.16 (1H, m, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_H 178.8 C=O, 173.6 C=O, 153.6, 152.4, 145.4, 136.9, 135.3, 132.2, 130.9, 129.9, 129.2, 128.4, 127.5, 126.9, 125.7, 125.6, 124.2, 119.3, 115.1, 114.8, 47.3 C^{13}, 41.5 C^{10}, 40.2 C^{11}, 28.8 C^{12}, 21.7 C^{37}; IR(neat): υ_{max}/cm^{-1} 3194, 2981, 2980, 1699; MS (pNSI): 587.2 (100% $(M+NH_4)^+$), 592.1 (30% $(M+Na)^+$); HRMS (pNSI): calcd for $C_{29}H_{27}N_6O_6S$ $[M+NH_4]^+$: 587.1707; observed: 587.1706.

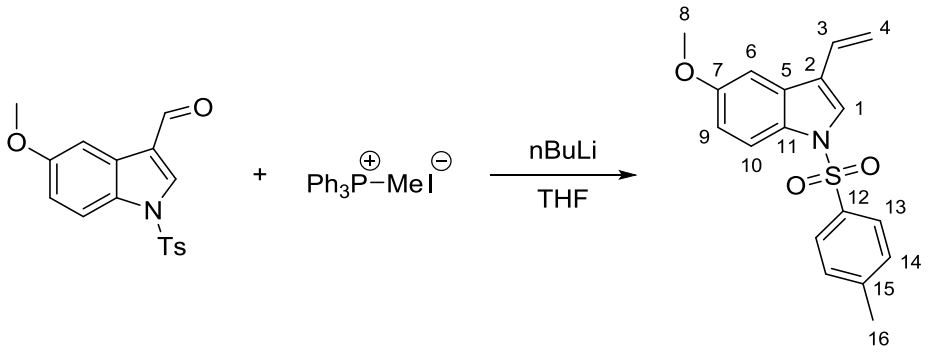
5-methoxy-1-tosyl-1*H*-indole-3-carbaldehyde – 205



To a stirred round bottomed flask was added 5-methoxy-1*H*-indole-3-carbaldehyde (0.70 g, 4.00 mmol) and DCM (20 mL) and the solution was cooled to 0 ^{o}C. To the stirred solution was added triethylamine (1.40 mL, 10.0 mmol) and the resulting solution was stirred at 0 ^{o}C for 1 hour. To the stirred solution was added *p*-toluenesulfonyl chloride (0.84 g, 4.40 mmol) in DCM (10 mL) and the solution was stirred at room temperature for 18 hours. The reaction was poured into water (50 mL) and extracted with DCM (3 x 20 mL). The combined organic extracts were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to leave the crude product as a pale orange solid. The product was purified using column chromatography (Petrol: Ether: DCM 2: 1: 1, column diameter = 2 cm, silica = 15 cm) to give 5-methoxy-1-tosyl-1*H*-indole-3-carbaldehyde (1.14 g, 3.48 mmol, 87 %) as a pale brown powder.

Mp: 126.1 – 128.4 ^{o}C; R_f: 0.71 (Pet:Et_2O:DCM 2:1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 10.07 (1H, s, H^3), 8.19 (1H, s, H^1), 7.86 – 7.83 (1H, m, H^8), 7.84 (2H, d, *J* = 8.5 Hz, H^{11}), 7.72 (1H, d, *J* = 2.6 Hz, H^5), 7.29 (2H, d, *J* = 8.5 Hz, H^{12}), 7.02 (1H, dd, *J* = 9.1, 2.6 Hz, H^7), 3.86 (3H, s, H^{16}), 2.38 (3H, s, H^{14}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 185.6 (C^3), 157.8 (C^6), 146.2, 136.8, 134.4, 130.4, 129.8, 127.4, 127.2, 122.3, 116.2, 114.2, 104.1, 55.8 (C^{16}), 21.8 (C^{14}); IR (cm^{-1}): *v* 3128 (CH), 2832 (CH), 1671 (CO); Anal. Calcd for $C_{17}H_{15}NO_4S$: C, 61.99; H, 4.59; N, 4.25. Found: C, 61.77; H, 4.70; N, 4.29.

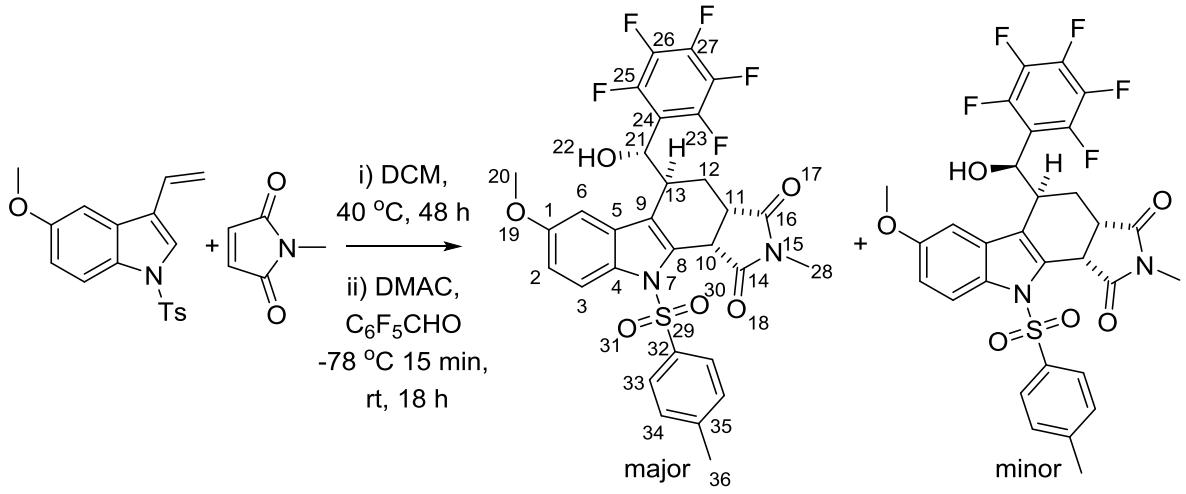
5-methoxy-1-tosyl-3-vinyl-1*H*-indole – 206



In a Schlenk flask, methyltriphenylphosphonium iodide (1.35 g, 3.34 mmol) was dissolved in dry THF (30 mL). The solution was cooled to -78 ^{o}C and ^{n}BuLi (1.2 mL, 3.03 mmol) was added over 5 minutes. The yellow solution was warmed to 0 ^{o}C and was allowed to stir for 1 hour before being cooled to -78 ^{o}C. To the stirred solution, 5-methoxy-1-tosyl-1*H*-indole-3-carbaldehyde (1.00 g, 3.03 mmol) in DCM (10 mL) was added and the solution was stirred at room temperature for 3 hours. The reaction was poured into water (40 mL) and extracted with ethyl acetate (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under pressure to leave the crude product an orange oil. The product was purified using column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 2 cm, silica = 16 cm) to give 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (0.79 g, 2.42 mmol, 80 %) as a brown powder.

Mp: 101.4 – 103.9 ^{o}C; R_f: 0.66 (Pet:EA 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.87 (1H, d, *J* = 9.0 Hz, H^{10}), 7.73 (2H, d, *J* = 8.4 Hz, H^{13}), 7.55 (1H, s, H^{1}), 7.19 (2H, d, *J* = 8.3 Hz, H^{14}), 7.14 (1H, d, *J* = 2.5 Hz, H^{6}), 6.93 (1H, dd, *J* = 9.0, 2.5 Hz, H^{9}), 6.72 (1H, dd, *J* = 17.9, 11.3 Hz, H^{3}), 5.73 (1H, dd, *J* = 17.9, 1.1 Hz, H^{4}-trans), 5.32 (dd, *J* = 11.3, 1.1 Hz, H^{4}-trans), 3.82 (3H, s, H^{8}), 2.31 (3H, s, H^{16}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 156.7 (C^{7}), 145.0, 135.1, 130.3, 130.1, 130.0, 127.6, 126.9, 124.9, 121.1, 115.2, 114.7, 113.7, 103.2, 55.8 (C^{8}), 21.7 (C^{16}); IR (cm^{-1}): *v* 3128 (CH), 2832 (CH), 1671 (CO); MS (pNSI): 328.1 (100%, $(M+H)^+$), 350.1 (15%, $(M+Na)^+$), 672.2 $(2M+NH_4)^+$); HRMS (pNSI): calcd for $C_{18}H_{18}NO_3S$ $[M+H]^+$: 328.1002; observed: 328.1007.

(3a*S,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-7-methoxy-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione and (3a*S**,5*S**,10b*S**)-5-((*R**)-hydroxy(perfluorophenyl)methyl)-7-methoxy-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 208**

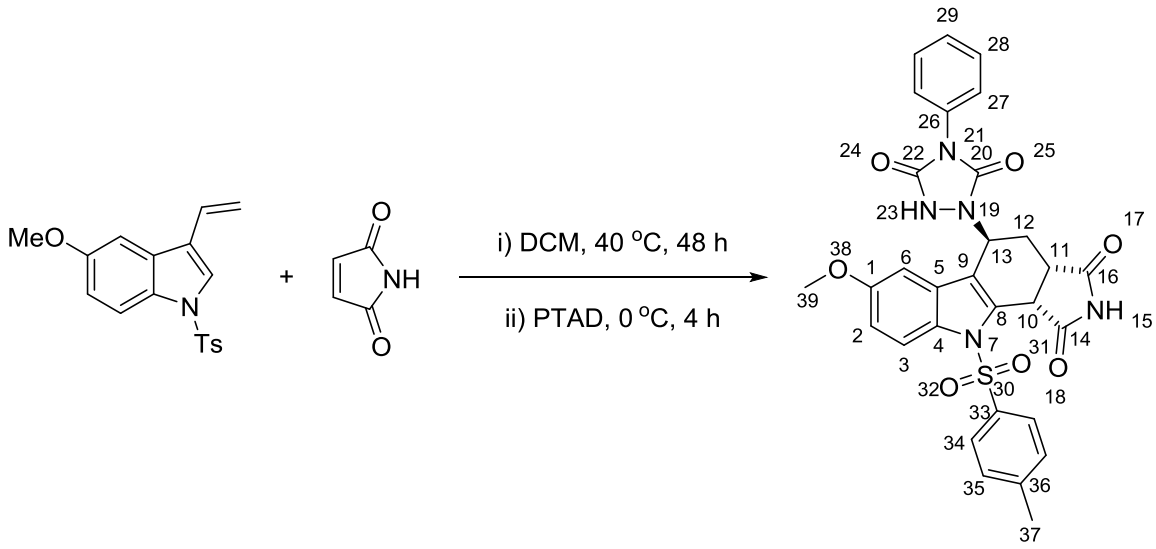


To a stirred round bottomed flask was added 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (112 mg, 0.34 mmol), DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (38 mg, 0.34 mmol). The reaction was heated at reflux for 48 hours. The reaction was cooled to -78 °C and pentafluorobenzaldehyde (0.04 mL, 0.34 mmol) and DMAC (1M in hexane, 0.34 mL, 0.34 mmol) were added. The solution was stirred at -78 °C for 15 minutes and then at room temperature for 18 hours. The solvent was removed under reduced pressure to give the crude product as an off white solid. The product was purified by column chromatography (column diameter = 2 cm, silica = 15 cm, eluent = Petrol: Ethyl acetate 3: 1) to give a 8:1 mixture of (3a*S**,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-7-methoxy-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione and (3a*S**,5*S**,10b*S**)-5-((*R**)-hydroxy(perfluorophenyl)methyl)-7-methoxy-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (70%, 0.151 mg) as a white powder.

Major Diastereomer: Mp: 136.7-139.0 °C; R_f: 0.40 (Pet:EA 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.76 (2H, d, *J* = 8.3 Hz), 7.69 (1H, d, *J* = 9.1 Hz), 7.22 (2H, d, *J* = 8.3 Hz), 6.83 (1H, dd, *J* = 9.1, 2.5 Hz), 6.72 (1H, d, *J* = 2.5 Hz), 5.08 (1H, d, *J* = 8.3 Hz, H^{23}), 4.87 (1H, d, *J* = 7.4 Hz, H^{10}), 3.71 (3H, s, H^{20}), 3.58 – 3.47 (2H, m, H^{23} + H^{11}), 2.94 (3H, s, H^{28}), 2.34 (3H, s, H^{36}), 2.11 – 2.04 (1H, m, H^{12}), 1.60 (1H, app td, *J* = 13.9, 5.3 Hz, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 177.7 C=O, 173.2 C=O, 156.8, 145.2, 135.2, 132.0, 130.9, 130.3, 129.8, 127.0, 120.6, 116.2, 114.0, 102.6, 70.2 C^{21}, 55.6 C^{20}, 41.5 C^{10}, 39.0 C^{11}, 36.9 C^{13}, 28.6 C^{12}, 25.3 C^{28}, 21.7 C^{36}; IR(neat): υ_{max}/cm^{-1} 2981, 2884, 1709; ; MS (pAPCI): 157.0 (80%), 221.1 (92%), 281.1 (49%) 475.1 (94%), 635.1 (100%, $(M+H)^+$); HRMS (pAPCI): calcd for $C_{30}H_{24}F_5N_2O_6S$ $[M+H]^+$: 635.1270; observed: 635.1266.

Note: ^{13}C NMR missing peaks due to C-F coupling.

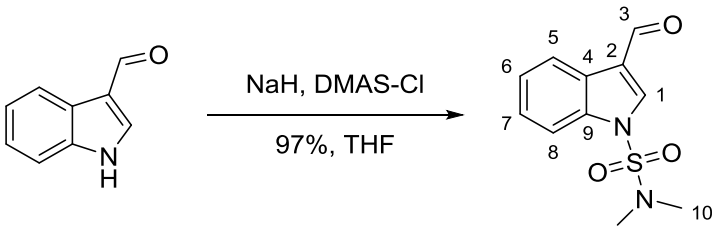
(3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-7-methoxy-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 209**



To a stirred round bottomed flask was added 5-methoxy-1-tosyl-3-vinyl-1*H*-indole (112 mg, 0.34 mmol), DCM (5 mL) and 1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol). The reaction was heated at reflux for 48 hours. The reaction was cooled to 0 °C and PTAD (60 mg, 0.34 mmol) was added. The reaction was stirred at 0 °C for 4 hours before the solvent was removed under reduced pressure to give the crude product as a pale red solid. The product was purified by column chromatography (column diameter = 2 cm, silica = 16 cm, eluent = Pet: EA 1: 1) to give (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-7-methoxy-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (75%, 0.152 mg, 0.25 mmol) as a pale yellow powder.

Mp: 189.9-193.3 °C; R_f: 0.07 (Pet:EA 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 9.31 (1H, br s, NH), 8.60 (1H, br s, NH), 7.67 (2H, d, *J* = 7.5 Hz), 7.55 (1H, d, *J* = 8.8 Hz), 7.38-7.23 (5H, m), 7.00 (2H, d, *J* = 7.8 Hz), 6.90 (1H, s), 6.80 (1H, d, *J* = 8.9 Hz), 5.53 (1H, br s, H^{13}), 5.18 (1H, d, *J* = 6.7 Hz, H^{10}), 3.74-3.69 (1H, m, H^{11}), 3.64 (3H, s, H^{39}), 2.48-2.55 (1H, m, H^{12}), 2.17 (3H, s, H^{37}), 2.12-2.02 (1H, m, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 179.0 C=O, 173.9 C=O, 156.9, 153.7, 152.7, 145.2, 135.1, 132.7, 131.5, 131.0, 129.8, 129.2, 128.4, 128.0, 127.2, 125.6, 115.9, 115.5, 114.3, 101.9, 55.7 C^{39}, 47.6 C^{13}, 41.6 C^{10}, 40.1 C^{11}, 28.8 C^{12}, 21.6 C^{37}; IR(neat): υ_{max}/cm^{-1} 2972, 2885, 1781, 1709; MS (pNSI): 617.2 (69%, $(M+NH_4)^+$), 622.1 (100%, $(M+Na)^+$), 644.1 (48%); HRMS (pNSI): calcd for $C_{30}H_{29}N_6O_7S$ $[M+NH_4]^+$: 617.1813; observed: 617.1817.

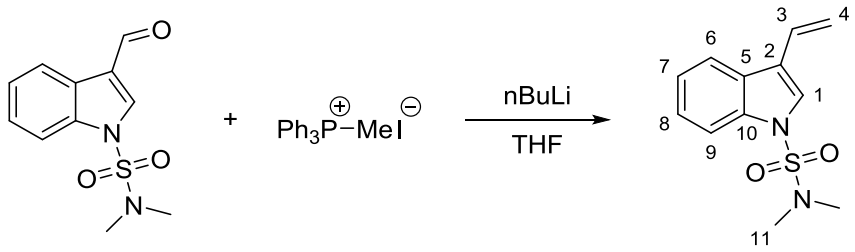
3-Formyl-*N*,*N*-dimethyl-1*H*-indole-1-sulfonamide – 211



To a stirred round bottomed flask was added 1*H*-indole-3-carbaldehyde (3.0 g, 20.7 mmol) and THF (70 mL) and the solution was cooled to 0 ^{o}C. To the stirred solution was added sodium hydride (1.7 g, 41.4 mmol) in THF (30 mL) and the resulting solution was stirred at 0 ^{o}C for 1 hour. To the stirred solution was added dimethylsulfamoyl chloride (2.4 mL, 20.7 mmol) and the solution was stirred at room temperature for 18 hours. The reaction was poured into water (100 mL) and extracted with DCM (3 x 60 mL). The combined organic extracts were dried over MgSO4, filtered and the solvent was removed under reduced pressure to leave the crude product as a pale red pink solid. The product was purified by recrystallization from ethyl acetate to give 3-formyl-*N*,*N*-dimethyl-1*H*-indole-1-sulfonamide (97%, 5.07 g, 20.1 mmol) as a pink powder.

Mp: 149.0 – 150.9 ^{o}C; R_f: 0.63 (Pet:EA, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 10.08 (1H, s, H^3), 8.31 (1H, app dd, *J* = 7.2, 1.4 Hz, H^5), 8.09 (1H, s, H^1), 7.94 – 7.88 (1H, m), 7.40 (2H, app ddd, *J* = 5.9, 3.3, 1.6 Hz, $H^{6/7}$), 2.91 (6H, s, H^{10}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 185.5 (C^3), 137.3, 136.0 (C^9), 126.1, 125.9 (C^4), 124.9, 122.6, 120.8 (C^2), 113.6, 38.6 (C^{10}); IR (cm^{-1}): *v* 3124 (CH), 2945 (CH), 1662 (CO); Anal. Calcd for $C_{11}H_{12}N_2O_3S$: C, 52.37; H, 4.79; N, 11.10. Found: C, 52.23; H, 4.91; N, 10.92.

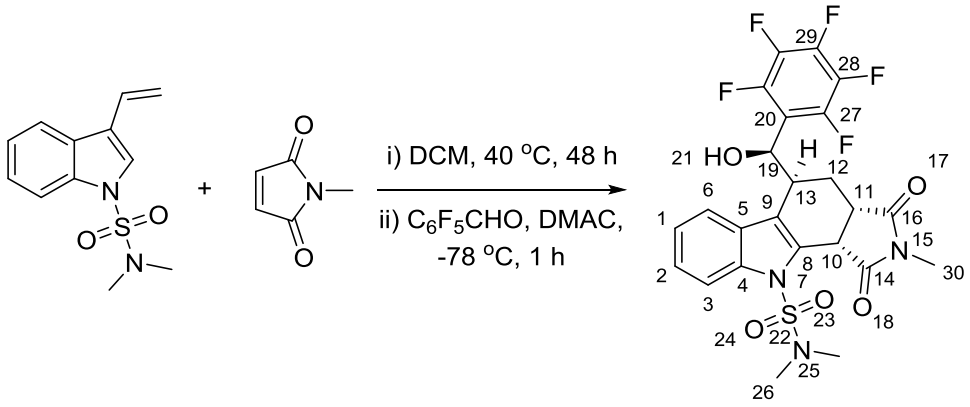
_N_,_N_-dimethyl-3-vinyl-1_H_-indole-1-sulfonamide – 212



In a Schlenk flask, methyltriphenylphosphonium iodide (7.00 g, 17.4 mmol) was dissolved in dry THF (75 mL). The solution was cooled to -78 ^{o}C and ^{n}BuLi (6.4 mL, 15.9 mmol) was added over 10 minutes. The yellow solution was warmed to 0 ^{o}C and was left to stir for 1 hour before being cooled to -78 ^{o}C. To the stirred solution, 3-formyl-*N*,*N*-dimethyl-1*H*-indole-1-sulfonamide (4.00 g, 15.9 mmol) was added and the solution was stirred at room temperature for 3 hours. The reaction was poured into water (70 mL) and extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under pressure to leave the crude product as yellow oil. The product was purified using column chromatography (Petrol: Diethyl ether 4: 1, column diameter = 3 cm, silica = 14 cm) to give *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (3.11 g, 12.4 mmol, 78 %) as a pale orange powder.

Mp: 68.7 – 67.8 ^{o}C; R_f: 0.63 (Pet:EA 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.98 (1H, dd, *J* = 8.0, 1.4 Hz), 7.85 (1H, dd, *J* = 7.7, 1.5 Hz), 7.35 (2H, app ddd, *J* = 7.0, 5.3, 1.6 Hz), 6.83 (1H, dd, *J* = 17.8, 11.2 Hz, H^4-cis), 5.84 (1H, dd, *J* = 17.8, 1.2 Hz, H^4-trans), 5.38 (1H, dd, *J* = 11.3, 1.2 Hz, H^4-cis), 2.86 (6H, s, H^{11}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 136.1 (C^{10}), 128.2, 127.8, 125.2, 124.7, 123.1, 120.4, 118.8, 114.9, 114.0, 38.6 (C^4); IR (cm^{-1}): *v* 3123 (CH), 2945 (CH); Anal. Calcd for $C_{12}H_{14}N_2O_2S$: C, 57.58; H, 5.64; N, 11.19. Found: C, 57.68; H, 5.75; N, 11.05.

(3a*S,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-*N*,*N*-dimethyl-1,3-dioxo-1,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(2*H*)-sulfonamide – 215**

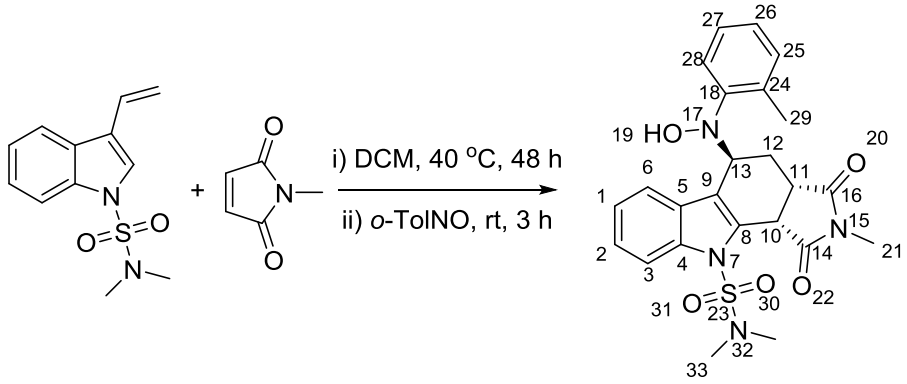


To a stirred round bottomed flask was added *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (85 mg, 0.34 mmol), DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (38 mg, 0.34 mmol). The reaction was heated at reflux for 48 hours. The reaction was cooled to -78 °C and pentafluorobenzaldehyde (66 mg, 0.34 mmol) and DMAC (1M in hexane, 0.34 mL, 0.34 mmol) were added and the reaction was stirred for 1 hour. The reaction was poured into a solution of sodium bicarbonate (10 mL) and extracted with DCM (3 x 10 mL). The combined organic extracts were dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure to leave the crude product as a pale pink solid. The product was purified by column chromatography (diameter = 2cm, silica = 17 cm, eluent = Pet: EA 2:1) to give (3a*S**,5*S**,10b*S**)-5-((*S**)-hydroxy(perfluorophenyl)methyl)-*N*,*N*-dimethyl-1,3-dioxo-1,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(2*H*)-sulfonamide (77%, 0.145 g, 0.26 mmol) as a pale pink solid.

Mp: 255.8-257.2 °C; R_f: 0.30 (Pet:EA 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.77 (1H, d, *J* = 8.3 Hz), 7.39 (1H, d, *J* = 7.8 Hz), 7.33-7.28 (1H, m), 7.21 (1H, app t, *J* = 7.8 Hz), 5.14 (1H, d, *J* = 8.0 Hz, H^{19}), 4.80 (1H, d, *J* = 7.3 Hz, H^{10}), 3.67-3.62 (1H, m, H^{13}), 3.48 (1H, ddd, *J* = 12.8, 7.2, 5.0 Hz, H^{11}), 2.98 (6H, s, H^{26}), 2.93 (3H, s, H^{30}), 2.49 (1H, br s, OH), 2.06 (1H, app dd, *J* = 14.9, 4.1 Hz, H^{12}), 1.59 (1H, app dt, *J* = 13.8, 6.9 Hz, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 177.8, 173.4, 137.2, 130.0, 129.3, 125.0, 123.5, 120.0, 118.2, 114.6, 70.3 C^{19}, 41.5 C^{10}, 39.2 C^{13}, 38.2 C^{26}, 36.7 C^{11}, 28.6 C^{12}, 25.2 C^{30}; IR(neat): υ_{max}/cm^{-1} 3415, 2972, 2884, 1713; MS (pNSI): 371.1 (22%), 558.1 (81% $(M+H)^+$), 580.1 (100%, $(M+Na)^+$), HRMS (pNSI): calcd $C_{24}H_{20}O_5N_3F_5S$ $[M+H]^+$: 558.1117; observed: 558.1118.

Note: ^{13}C NMR missing peaks due to C-F coupling.

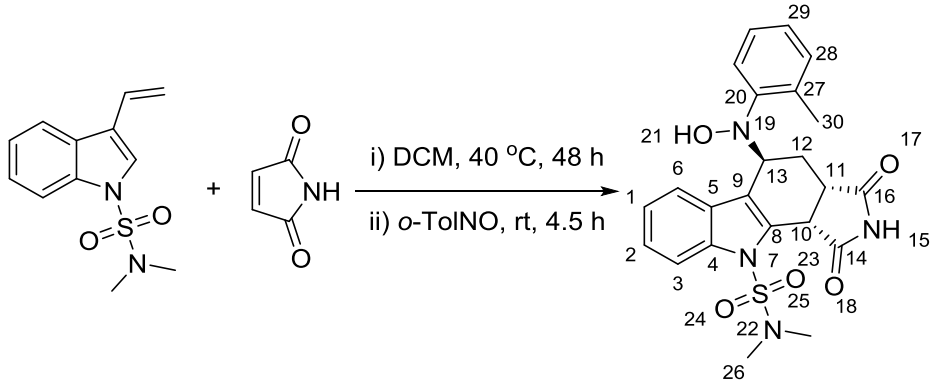
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-*N*,*N*,2-trimethyl-1,3-dioxo-1,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(2*H*)-sulfonamide – 216**



To a stirred bottomed flask was added *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (85 mg, 0.34 mmol), DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (38 mg, 0.34 mmol). The solution was heated at reflux for 48 hours. The reaction was cooled to room temperature and 1-methyl-2-nitrosobenzene (41 mg, 0.34 mmol) was added. The reaction was stirred for 3 hours at room temperature before the solvent was removed under reduced pressure to give the crude product. The crude product was purified by column chromatography (column diameter = 2 cm, silica = 16 cm, eluent = Petrol : Ethyl Acetate 2 : 1) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-*N*,*N*,2-trimethyl-1,3-dioxo-1,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(2*H*)-sulfonamide (74%, 0.122 g, 0.25 mmol) as an off white solid.

Mp: 169.3 – 171.9 °C; R_f: 0.32 (Pet:Ea 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.84 (1 H, d, *J* = 8.4 Hz), 7.60 (1H, d, *J* = 7.9 Hz), 7.50 (1H, d, *J* = 8.0 Hz), 7.28 (1H, app t, *J* = 7.8 Hz), 7.21-7.16 (2H, m), 7.13 (1H, d, *J* = 8.1 Hz), 7.07-7.03 (1H, m), 4.98-4.96 (2H, m, H^{10} + OH), 4.35-4.31 (1H, m, H^{13}), 3.69 (1H, app t, *J* = 7.7 Hz, H^{11}) , 2.93 (9H, s, H^{33} + H^{21}), 2.60 (1H, app dt, *J* = 13.1, 6.2 Hz, H^{12}), 2.33 (3H, s, H^{29}), 1.97 (1H, ddd, *J* = 13.1, 7.7, 4.6 Hz, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 178.4 C=O, 173.8 C=O, 149.2, 137.4, 131.9, 131.1, 129.6, 128.4, 126.6, 125.3, 124.7, 123.7, 121.5, 120.4, 118.8, 115.0, 57.5 C^{13}, 40.5 C^{10}, 39.4 C^{11}, 38.4 C^{33}, 25.2 C^{21}, 24.7 C^{12}, 18.7 C^{29}; IR(neat): υ_{max}/cm^{-1} 3426, 2981, 1712; MS (pAPCI): 221.1 (9%), 251.1 (13%), 360.1 (100%, (M-$MeC_6H_4NOH)^+$), 465.2 (15%, (M-$H_2O)^+$), 483.2 (15%, (M+H)$^+$); HRMS (pAPCI): calcd for $C_{24}H_{27}N_4O_5S_1$ [M+H]$^+$: 483.1697; observed: 483.1685.

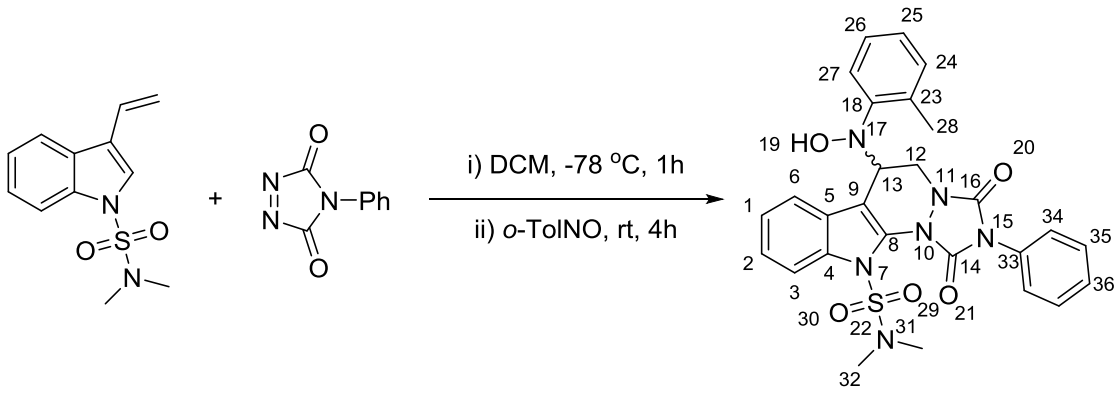
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-*N*,*N*-dimethyl-1,3-dioxo-1,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(2*H*)-sulfonamide – 217**



To a round bottomed flask was added *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (85 mg, 0.34 mmol), DCM (5 mL) and 1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol). The reaction was heated at reflux for 48 hours and then cooled to room temperature. 1Methyl-2-nitrosobenzene (41 mg, 0.34 mmol) was added and the reaction is stirred for 4.5 hours. The solvent was removed to give the crude product as pale yellow solid. The crude product was purified by trituration from DCM to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-*N*,*N*-dimethyl-1,3-dioxo-1,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(2*H*)-sulfonamide (45%, 72 mg, 0.15 mmol) as a white solid.

Mp: 199.9-201.0 °C; R_f: 0.64 (Pet:EA 3:1); ^{1}H NMR (300 MHz, DMSO-d6): δ_H 11.15 (1H, s, NH), 8.38 (1H, s, OH), 7.88 (1H, d, *J* = 8.4 Hz), 7.36 (1H, d, *J* = 7.9 Hz), 7.31-7.20 (2H, m), 7.13 (3H, app dt, *J* = 14.3, 7.3 Hz), 7.01 (d, *J* = 7.3 Hz, 1H), 4.98 (1H, d, *J* = 7.8 Hz, H^{10}), 4.38 (1H, app t, *J* = 4.8 Hz, H^{13}), 3.73 (1H, app q, *J* = 7.8 Hz, H^{11}), 2.70 (6H, s, H^{26}), 2.45 – 2.37 (1H, m, H^{12})2.36 (s, 3H, H^{30}), 1.77-1.65 1.77 (1H, m, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 180.4 C=O, 175.5 C=O, 151.6, 137.2, 133.2, 131.1, 129.3, 129.1, 126.6, 124.4, 124.3, 123.4, 121.8, 121.1, 118.6, 115.3, 56.5 C^{13}, 42.2 C^{10}, 41.1 C^{11}, 38.7 C^{26}, 26.6 C^{12}, 18.9 C^{30}; IR(neat): υ_{max}/cm^{-1} 3426, 2981, 1712; MS (pAPCI): 237.1 (60%), 346.1 (100%, $(M\text{-}MeC_6H_4NOH)^+$), 451.1 (25%, $(M\text{-}H_2O)^+$), 469.2 (22%, $(M+H)^+$); HRMS (pAPCI): calcd $C_{23}H_{25}N_4O_5S_1$ $[M+H]^+$: 469.1540; observed: 469.1537.

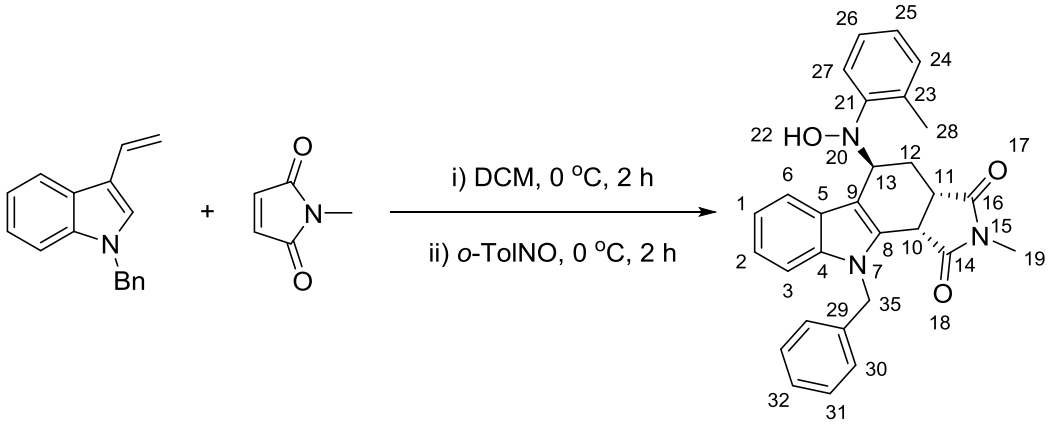
6-(hydroxy(*o*-tolyl)amino)-*N*,*N*-dimethyl-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11(1*H*)-sulfonamide – 218



To a stirred round bottomed flask was added *N*,*N*-dimethyl-3-vinyl-1*H*-indole-1-sulfonamide (85 mg, 0.34 mmol), DCM (5 mL) and cooled to -78 °C. To this solution PTAD (60 mg, 0.34 mmol) was added and the reaction stirred at -78 °C for 1 hour. 1-Methyl-2-nitrosobenzene (41 mg, 0.34 mmol) was added and the reaction was stirred 4 hours before the solvent was removed under reduced pressure to give the crude product. The crude product was purified by column chromatography (column diameter = 2 cm, silica = 16 cm, eluent = Petrol : Ethyl Acetate 2 : 1) to give 6-(hydroxy(*o*-tolyl)amino)-*N*,*N*-dimethyl-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11(1*H*)-sulfonamide (76%, 0.141 g) as an off white solid.

Mp: 168.5 – 172.8 °C; R_f: 0.25 (Pet:EA 2:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.87 (1H, d, *J* = 8.3 Hz), 7.73 (1H, d, *J* = 8.1 Hz), 7.60 (2H, d, *J* =7.4 Hz), 7.51 (2H, t, *J* = 7.6 Hz), 7.46-7.38 (1H, m), 7.30-7.18 (2H, m), 7.04 (2H, t, *J* = 7.5 Hz), 6.90 (1H, d, *J* = 7.6 Hz), 6.71 (1H, d, *J* = 7.8 Hz), 5.76 (1H, s, H^{13}), 5.37 (1H, d, *J* = 14.1 Hz, H^{12}), 4.66 (1H, s, OH), 3.46 (1H, d, *J* = 14.1 Hz, H^{12}), 2.90 (6H, s, H^{32}), 1.94 (3H, s, H^{28}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 153.5 C=O, 150.5 C=O, 148.7, 135.1, 132.1, 131.9, 131.2, 130.6 129.4, 128.7, 127.1, 126.9, 126.8, 125.9, 124.4, 124.2, 122.7, 118.1, 115.9, 104.7, 59.4 C^{13}, 44.7 C^{12}, 38.7 C^{32}, 17.9 C^{28}; IR(neat): υ_{max}/cm^{-1} 3322, 2971, 1707; MS (pNSI): 339.1 (33%), 424.1 (94%, $(M\text{-}MeC_6H_4NOH)^+$), 547.2 (72%, $(M+H)^+$), 569.2 (100%, $(M+Na)^+$); HRMS (pNSI): calcd $C_{27}H_{27}N_6O_5S_1$ $[M+H]^+$: 547.1758; observed: 547.1761.

(3a*S,5*S**,10b*S**)-10-benzyl-5-(hydroxy(*o*-tolyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 224**



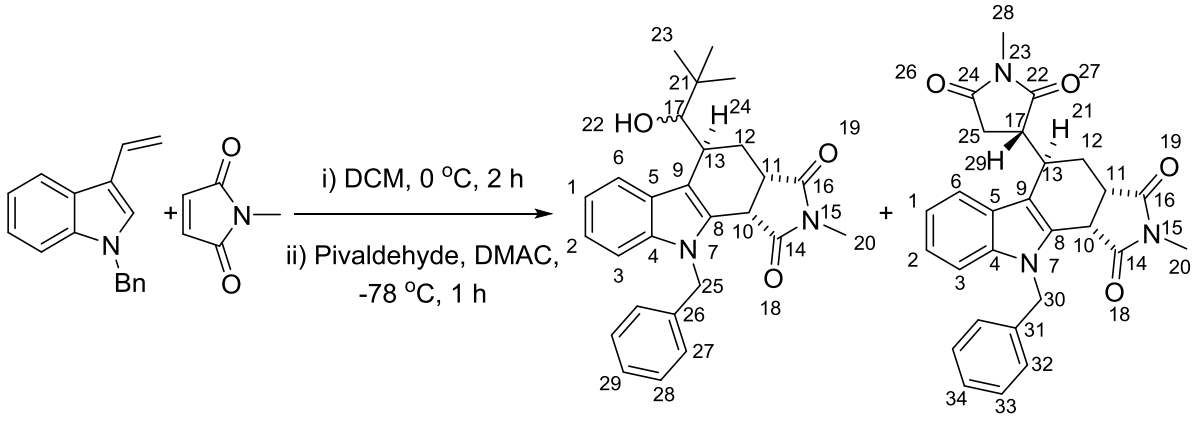
Into a round bottomed flask, 1-benzyl-3-vinyl-1*H*-indole (100 mg, 0.43 mmol) and DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (48 mg, 0.43 mmol) were added. The reaction was cooled to 0 °C and stirred for 2 hours. To the stirred reaction 2-nitrosotoluene (52 mg, 0.43 mmol) was added and the reaction was stirred for a further 2 hours. The solvent was removed and to give the crude product as a yellow solid. The reaction was purified by column chromatography (column diameter – 2cm, silica – 14cm, eluent – Petrol: Ethyl Acetate 3: 1) to give (3a*S**,5*S**,10b*S**)-10-benzyl-5-(hydroxy(*o*-tolyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (73%, 0.1411 g, 0.30 mmol) as a pale yellow solid.

Mp: 114.7-116.9 °C; R_f: 0.32 (Pet:EA 2:1); ^{1}H NMR (400 MHz, C_6D_6): δ_H 7.74-7.69 (1H, m), 7.66 (1H, d, *J* = 7.9 Hz), 7.07-6.86 (9H, m), 6.78 (2H, d, *J* = 8.1 Hz), 6.13 (1H, d, *J* = 17.4 Hz, H^{35}), 5.28 (1H, d, *J* = 17.4 Hz, H^{35}), 4.51 (1H, s, OH), 4.40 (1H, dd, *J* = 6.6, 4.4 Hz, H^{13}), 3.34 (1H, d, *J* = 7.9 Hz, H^{10}), 2.80 (1H, td, *J* = 7.7, 5.3 Hz, H^{11}), 2.43 (3H, s, H^{19}), 2.35 (1H, ddd, *J* = 13.3, 7.0, 5.4 Hz, H^{12}), 2.14 (3H, s, H^{28}), 1.74 (1H, ddd, *J* = 13.3, 7.5, 4.3 Hz, H^{12}); ^{13}C NMR (101 MHz,C_6D_6-d_6): δ_C 177.4 C=O, 174.7 C=O, 150.1, 138.5, 138.0, 130.7, 130.4, 129.6, 128.7,, 127.2, 126.4, 126.3, 126.1, 124.7, 122.4, 121.7, 120.3, 120.3, 111.9, 110.1, 58.2 C^{13}, 47.4 C^{35}, 39.2 C^{10}, 39.1 C^{11}, 24.3 C^{19}, 24.0 C^{12}, 18.4 C^{28}; IR(neat): υ_{max}/cm^{-1} 3439, 2981, 1704; MS (pAPCI): 343.1 (100%, $(M\text{-}MeC_6H_4NOH)^+$), 359.1 (18%), 448.2 (18%, $(M\text{-}H_2O)^+$), 466.2 (4%, $(M+H)^+$); HRMS (pAPCI): calcd $C_{29}H_{28}N_3O_3$ $[M+H]^+$: 466.2125; observed: 466.2115.

(3a*S,5*S**,10b*S**)-10-benzyl-5-((*S**)-1-hydroxy-2,2-dimethylpropyl)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 227**

and

(3a*S,5*R**,10b*S**)-10-benzyl-2-methyl-5-((*S**)-1-methyl-2,5-dioxopyrrolidin-3-yl)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 228**



To a stirred round bottomed flask was added 1-benzyl-3-vinyl-1*H*-indole (100 mg, 0.43 mmol) and DCM (5 mL). The reaction was cooled to 0 ^{o}C, 1-methyl-1*H*-pyrrole-2,5-dione (48 mg, 0.43 mmol) was added and the reaction was stirred at 0 ^{o}C for 2 hours. The reaction was cooled to -78 ^{0}C and pivaldehyde (0.05 mL, 0.43 mmol) and DMAC (1M in hexane, 0.43 mL, 0.43 mmol) were added and the solution stirred for 1 hour. The solution was poured into a solution of $NaHCO_3$ (10 mL) and extracted with DCM (3 x 10 mL). The combined organic layers were dried with $MgSO_4$, filtered and the solvent was removed to give the crude product as a pale brown solid. The reaction was purified by column chromatography (column diameter – 2cm, silica – 15cm, eluent – Petrol: Ethyl Acetate 3: 1) to give (3aS*,5S*,10bS*)-10-benzyl-5-((S*)-1-hydroxy-2,2-dimethylpropyl)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione (29%, 54 mg, 0.13 mmol) as an off-white solid and (3a*S**,5*R**,10b*S**)-10-benzyl-2-methyl-5-((*S**)-1-methyl-2,5-dioxopyrrolidin-3-yl)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (15%, 28 mg, 0.06 mmol) as a brown solid.

(3a*S,5*S**,10b*S**)-10-benzyl-5-((*S**)-1-hydroxy-2,2-dimethylpropyl)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione**

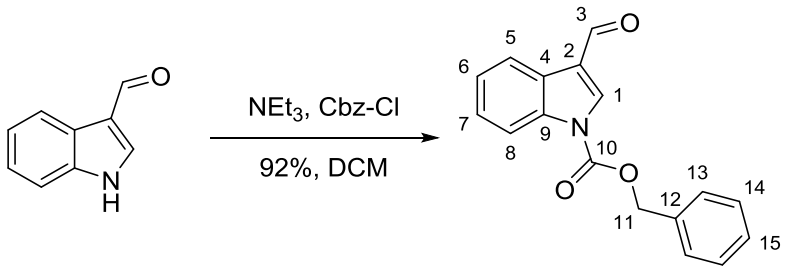
Mp: 99.4 – 101.9 ^{o}C; R_f: 0.63 (Pet: EA 1: 1); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.58 (1H, d, *J* = 7.8 Hz), 7.31 (1H, d, *J* = 8.2 Hz), 7.24-7.17 (4H, m), 7.14-7.09 (1H, m), 6.91-6.87 (2H, m), 6.06 (1H, d, *J* = 17.3 Hz, H^{25}), 5.60 (1H, d, *J* = 17.3 Hz, H^{25}), 4.00 (1H, d, *J* = 1.5 Hz, H^{17}), 3.97 (1H, d, *J* = 7.7 Hz, H^{10}), 3.68 (1H, ddd, *J* = 10.7, 7.7, 5.2 Hz, H^{24}), 3.45-3.43 (1H, m, H^{11}), 2.97 (3H, s, H^{20}), 2.61-2.55 (1H, m, H^{12}), 1.89 (1H, ddd, *J* = 13.8, 10.7, 5.3 Hz, H^{12}), 1.16 (9H, s, H^{23}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C δ 179.3 C=O, 175.6 C=O, 138.2, 138.1, 129.6, 129.0, 127.4, 126.0, 125.1, 122.5, 119.8, 118.9, 114.0, 110.5, 80.7 C^{17}, 47.6 C^{25}, 40.4 C^{13}, 39.3 C^{10}, 35.9 C^{21}, 33.4

C^{11}, 27.5 C^{23}, 26.5 C^{12}, 25.1 C^{20}; IR(neat): υ_{max}/cm^{-1} 3440, 2971, 1707; MS (pNSI): 431.2 (100%, $(M+H)^+$); HRMS (pNSI): calcd $C_{27}H_{31}N_2O_3$ $[M+H]^+$: 431.2329; observed: 431.2328.

(3a*S,5*R**,10b*S**)-10-benzyl-2-methyl-5-((*S**)-1-methyl-2,5-dioxopyrrolidin-3-yl)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione**

Mp: 126.2 – 130.0 ^oC; R_f:0.21 (Pet: EA 1: 1); ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.50 (1H, d, *J* = 8.0 Hz), 7.32-7.21 (4H, m), 7.20-7.16 (1H, m), 7.11-7.08 (1H, m), 6.86 (2H, d, *J* = 7.9 Hz), 6.12 (1H, d, *J* = 17.4 Hz, H^{30}), 5.55 (1H, d, *J* = 17.4 Hz, H^{30}), 4.11 (1H, d, *J* = 8.5 Hz, H^{10}), 4.05 (1H, dt, *J* = 9.1, 4.4 Hz, H^{29}), 3.68 (1H, dd, *J* = 9.9, 4.6 Hz, H^{25}), 3.37 (1H, dt, *J* = 9.1, 4.9 Hz, H^{11}), 3.01 (3H, s, H^{28}), 2.94 (3H, s, H^{20}), 2.69 (1H, dd, *J* = 18.9, 9.5 Hz, H^{21}), 2.31-2.25 (2H, m, H^{12} + H^{25}), 1.46 (1H, ddd, *J* = 13.0, 10.7, 5.8 Hz, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$): δ_C 178.3, 177.5, 176.7, 175.3, 138.1, 137.8, 129.6, 129.0, 127.6, 125.9, 124.9, 122.7, 120.4, 119.3, 111.4, 110.6, 47.3 C^{30}, 42.8 C^{17}, 39.5 C^{11}, 38.9 C^{10}, 31.4 C^{25}, 31.1 C^{13}, 25.4 C^{20}, 25.0 C^{28}, 23.9 C^{12}; IR(neat): υ_{max}/cm^{-1} 3029, 2928, 1693; MS (pNSI): 456.2 (66%, $(M+H)^+$), 478.2 (73%, $(M+Na)^+$), 933.4 (100%, $(2M+Na)^+$); HRMS (pNSI): calcd $C_{27}H_{26}N_3O_4$ $[M+H]^+$: 456.1918; observed: 4561.1914.

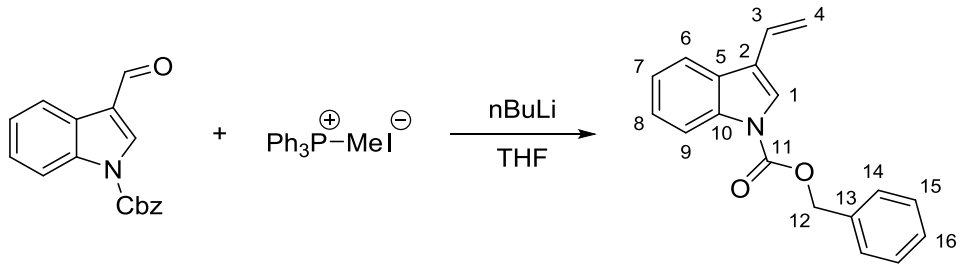
Benzyl 3-formyl-1*H*-indole-1-carboxylate – 229



To a solution of 1*H*-indole-3-carbaldehyde (1.0 g, 6.9 mmol) in DCM (20 mL) at 0 ^{o}C was added triethylamine (1.8 mL, 17.3 mmol) dropwise. The solution was stirred at room temperature for 1 hour before benzyl chloroformate (1.4 mL, 8.3 mmol) was added. The solution was stirred for 18 hours after which it was poured into water and extracted with DCM (3 x 20 mL). The organic fractions were combined, dried over MgSO4, filtered and the solvent was removed under reduced pressure to give the crude product as an orange powder. The crude product was purified by column chromatography (column diameter = 2.5 cm, eluent = Petrol: Ethyl acetate 2: 1) to give benzyl 3-formyl-1*H*-indole-1-carboxylate (1.74 g, 6.35 mmol, 92%) as a pale orange powder.

MP: 90.6 – 91.9 ^{o}C; $R_{f:}$ 0.68 (Pet:EA, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 10.06 (1H, s, H^3), 8.30 – 8.25 (1H, m), 8.23 (1H, s, H^1), 8.17 (1H, d, *J* = 8.0 Hz), 7.50 (2H, app dd, *J* = 7.7, 1.8 Hz), 7.42 (3H, app ddd, *J* = 6.6, 5.1, 1.5 Hz), 7.38 (1H, app t, *J* = 1.6 Hz), 7.37 – 7.34 (1H, m), 5.49 (2H, s, H^{11}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 185.8 C^3, 150.2 C^{10}, 136.1, 136.1, 134.3, 129.3, 129.0, 128.9, 126.5, 126.1, 125.0, 122.3, 122.3, 115.2, 69.9 C^{11}; IR (cm^{-1}): *v* 3127, 3008, 1733; MS (pNSI): 280.1 (100%, $(M+H)^+$), 302.1 (96%, $(M+Na)^+$), 581.2 (25%, $(2M+Na)^+$); HRMS (pNSI): calcd for $C_{17}H_{14}NO_3$ $[M+H]^+$: 280.0968; observed: 280.0970.

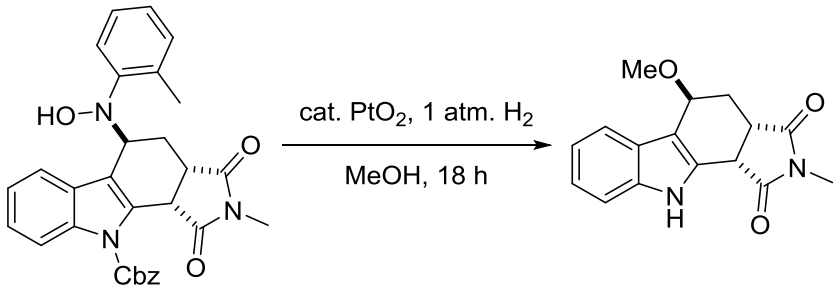
Benzyl 3-vinyl-1*H*-indole-1-carboxylate – 230



In a Schlenk flask, methyltriphenylphosphonium iodide (2.38 g, 5.90 mmol) was dissolved in dry THF (30 mL). The solution was cooled to -78 ^{o}C and ^{n}BuLi (2.15 mL, 5.35 mmol) was added over 10 minutes. The yellow solution was warmed to 0 ^{o}C and was left to stir for 1 hour before being cooled to -78 ^{o}C. To the stirred solution, benzyl 3-formyl-1*H*-indole-1-carboxylate (1.50 g, 5.35 mmol) was added and the solution was stirred at room temperature for 3 hours. The reaction was poured into water (50 mL) and extracted with ethyl acetate (3 x 40 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under pressure to leave the crude product as yellow oil. The product was purified using column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 2 cm, silica = 15 cm) to give benzyl 3-vinyl-1*H*-indole-1-carboxylate (1.14 g, 4.07 mmol, 76 %) as a yellow powder.

MP: 42.6 – 44.9 ^{o}C; R_f: 0.73 (Pet:EA, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.33 (1H, d, *J* = 7.1 Hz), 7.91 – 7.86 (1H, m), 7.84 (1H, s, H^1), 7.58- 7.55 (2H, m), 7.52 – 7.44 (m, 4H), 7.43 – 7.36 (1H, m), 6.87 (1H, dd, *J* = 17.8, 11.3 Hz, H^3), 5.92 (1H, d, *J* = 17.8 Hz, H^4-trans), 5.51 (2H, s, H^{12}), 5.44 (1H, d, *J* = 11.3 Hz, H^4-cis); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 150.8 C^{11}, 135.1, 128. 9, 128.9, 128.8, 128.6, 128.4, 128.0, 125.1, 123.6, 123.4, 120.2, 120.2, 115.5, 115.0, 68.9 C^{12}; IR (cm^{-1}): *v* 3153, 2962, 1729; MS (pAPCI): 181.1 (50%), 260.1 (100%), 278.1 (25%, $(M+H)^+$); HRMS (pAPCI): calcd for $C_{18}H_{16}NO_2$ $[M+H]^+$: 278.1176; observed: 278.1173.

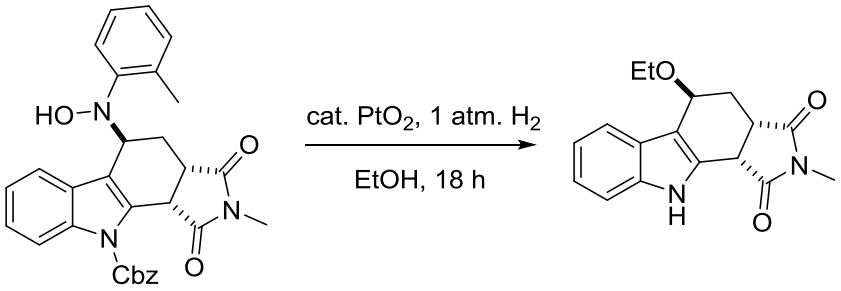
(3a*S,5*S**,10b*S**)-5-methoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 233**



To a stirred Schlenk flask was added benzyl (3aS*,5S*,10bS*)-5-(hydroxy(o-tolyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-a]carbazole-10(1H)-carboxylate (120 mg, 0.24 mmol), platinum (IV) oxide (53 mg, 0.24 mmol) and methanol (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 18 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as an orange solid, The crude product was purified by column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2cm, silica = 13 cm) to give (3a*S**,5*S**,10b*S**)-5-methoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (60%, 41 mg, 0.14 mmol) as a pale yellow powder.

Mp: 139.4 – 142.7 ^{o}C; R*f*: 0.25 (Pet:EA 1:1); ^{1}H NMR (400 MHz, DCM-d2): δ_H 8.91 (1H, s), 7.56 (1H, d, *J* = 7.8 Hz), 7.34 (1H, d, *J* = 7.7 Hz), 7.14 (1H, app t, *J* = 7.5 Hz), 7.09 (1H, app t, *J* = 7.4 Hz), 4.73 (1H, app t, *J* = 2.7 Hz), 4.14 (1H, d, *J* = 8.8 Hz), 3.31 – 3.25 (1H, m), 3.22 (3H, s), 2.94 (1H, app t, *J* = 2.4 Hz), 2.90 (3H, s), 1.88 (1H, ddd, *J* = 14.2, 6.9, 2.2 Hz);^{13}C NMR (101 MHz, DCM-d2): δ_C 178.9, 176.1, 136.4, 128.8, 126.6, 122.3, 120.1, 118.2, 111.9, 111.3, 69.4, 56.1, 39.,5 37.3, 27.4, 25.1; IR(neat): υ_{max}/cm^{-1} 3398, 2931, 2870, 1689; MS (pNSI): 285.0 (29%), 355.1 (100%), 371.1 (57%), 560.0 (21%);HRMS (pNSI): calcd $C_{15}H_{13}N_2O_2$ $[M\text{-}OMe]^+$: 253.0972; observed: 253.0974.

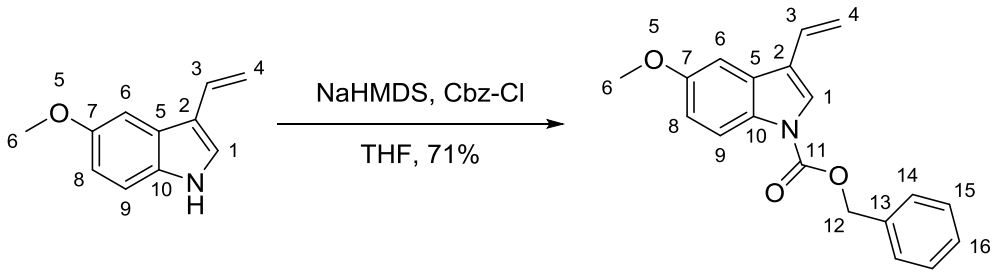
(3a*S,5*S**,10b*S**)-5-ethoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 234**



To a stirred Schlenk flask was added benzyl (3aS*,5S*,10bS*)-5-(hydroxy(o-tolyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-a]carbazole-10(1H)-carboxylate (100 mg, 0.20 mmol), platinum (IV) oxide (45 mg, 0.20 mmol) and ethanol (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 18 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as an yellow solid, The crude product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 2cm, silica = 15 cm) to give (3a*S**,5*S**,10b*S**)-5-ethoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (24%, 20 mg, 0.04 mmol) as a brown powder.

Mp: 100.6 – 102.8 ^{o}C; R*f*: 0.16 (Pet:EA 2:1); ^{1}H NMR (400 MHz, DCM-d2): δ_H 8.78 (1H, s), 7.54 (1H, d, *J* = 7.7 Hz), 7.35 (1H, d, *J* = 7.7 Hz), 7.17 – 7.12 (1H, m), 7.09 (1H, app t, *J* = 7.0 Hz), 4.83 (1H, app t, *J* = 2.8 Hz), 4.16 (1H, d, *J* = 8.8 Hz), 3.55 (1H, app td, *J* = 6.9, 1.8 Hz), 3.32 – 3.26 (2H, m), 2.93 – 2.91 (1H, m), 2.90 (3H, s), 1.88 (1H, ddd, *J* = 14.3, 7.1, 2.7 Hz), 0.97 (1H, t, *J* = 7.0 Hz); ^{13}C NMR (101 MHz, DCM-d2): δ_C 178.9, 176.0, 136.3, 128.8, 126.5, 122.3, 120.1, 118.1, 112.5, 111.3, 67.4, 63.5, 39.5, 37.3, 27.9, 25.0, 15.2; IR(neat): υ_{max}/cm^{-1} 3300, 2969, 1690;MS (pAPCI): 108.1 (24%), 298.1 (6%, $(M)^+$), 299.1 (5%, $(M+H)^+$); HRMS (pAPCI): calcd $C_{17}H_{19}N_2O_3$ $[M+H]^+$: 299.1390; observed: 299.1387.

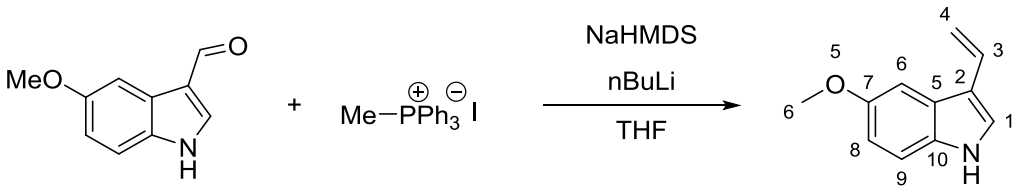
Benzyl-5-methoxy-3-vinyl-1*H*-indole-1-carboxylate – 239



In a Schlenk flask, 5-methoxy-3-vinyl-1*H*-indole (1.15 g, 6.61 mmol) was dissolved in THF (30 mL) and the solution was cooled to 0 ^{o}C. To the stirred solution, sodium bis(trimethylsilyl)amide (7.27 mL, 7.27 mmol) was added and the solution was stirred for 30 minutes before benzyl chloroformate (0.90 mL, 6.61 mmol) was added. The solution was stirred for 30 minutes at room temperature before being added to water (50 mL) and extracted with ethyl acetate (3 x 30 mL). The combined organic washings were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to give the crude product as an orange oil. The product was purified using column chromatography (Petrol: Ethyl acetate 5: 1, column diameter = 2.0 cm, silica = 15 cm) to give benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (1.78 g, 4.7 mmol, 71%) as a yellow powder.

MP: Oil at room temperature; R_f: 0.82 (Pet:EA, 5:1); ^{1}H NMR (400 MHz, δ_H 8.09 (1H, s), 7.66 (1H, s, H^{1}), 7.50 (2H, d, *J* = 7.2 Hz), 7.45 – 7.37 (1H, m), 7.25 (1H, d, *J* = 2.5 Hz), 6.96 (1H, dd, *J* = 9.0, 2.2 Hz), 6.79 (1H, dd, *J* = 17.8, 11.4 Hz, H^{3}), 5.80 (1H, d, *J* = 17.8 Hz, H^{4}-trans), 5.42 (2H, s, H^{12}), 5.34 (1H, d, *J* = 11.4 Hz, H^{4}-cis), 3.85 (3H, s, H^{6}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 156.4 C^{11}, 135.2, 129.7, 128.9, 128.9, 128.7, 128.6, 128.0, 124.1, 124.1, 120.0, 116.1, 114.8, 113.3, 103.3, 68.8 C^{12}, 55.8 C^{6}; IR (cm^{-1}): *v* 2955, 2834, 1726; MS (pAPCI): 181.1 (32%), 260.1 (100%), 308.1 (28%, $(M+H)^{+}$); HRMS (pAPCI): calcd for $C_{19}H_{18}NO_3$ $[M+H]^{+}$: 308.1281; observed: 308.1277.

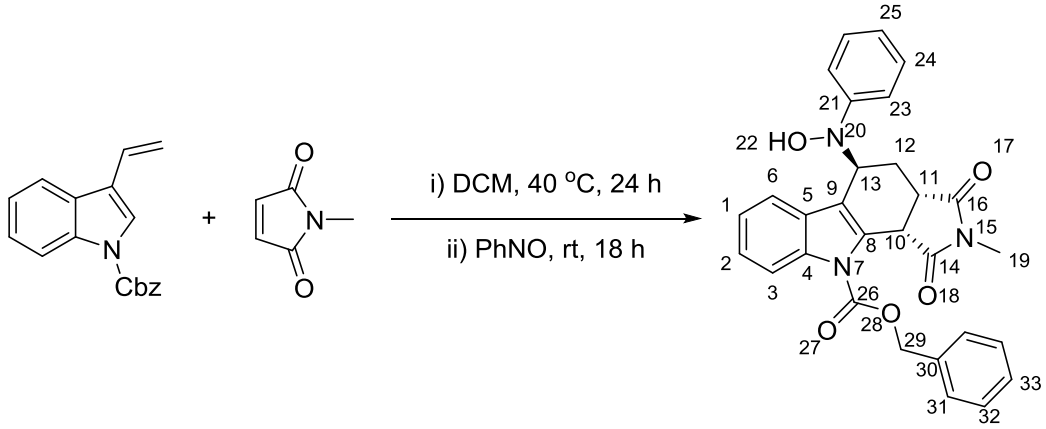
5-methoxy-3-vinyl-1*H*-indole – 240



In a Schlenk flask, methyltriphenylphosphonium iodide (3.54 g, 8.70 mmol) was dissolved in dry THF (34 mL). The solution was cooled to -78 ^{o}C and ^{n}BuLi (3.1 mL, 7.87 mmol) was added over 10 minutes. The yellow solution was warmed to 0 ^{o}C and was left to stir for 1 hour before being cooled to -78 ^{o}C. In a separate Schlenk flask, 5-methoxy-1*H*-indole-3-carbaldehyde (1.38 g, 7.87 mmol) was dissolved in THF (10 mL) and to the solution sodium bis(trimethylsilyl)amide (7.87 mL, 7.87 mmol) was added. This solution was transferred into the first Schlenk flask and the red solution was allowed to stir at room temperature for 1 hour. The reaction was poured into water (50 mL) and extracted with ethyl acetate (2 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under pressure to leave the crude product as yellow oil. The product was purified using column chromatography (Petrol: Diethyl ether 2: 1, column diameter = 2.5 cm, silica = 16 cm) to give 5-methoxy-3-vinyl-1*H*-indole (1.38 g, 7.6 mmol, 97%) as a yellow powder.

MP: 190.0 – 192.8 ^{o}C; R_f: 0.49 (Pet:Et_2O, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.28 (1H, s), 7.66 (1H, d, *J* = 2.4 Hz), 7.29 (1H, d, *J* = 8.8 Hz), 7.24 (1H, d, *J* = 2.7 Hz), 7.20 – 7.10 (2H, m), 7.18 (d, *J* = 2.5 Hz, 1H), 7.15 (dd, *J* = 4.5, 2.1 Hz, 2H), 7.10 (s, 1H), 5.95 (1H, dd, *J* = 17.8, 1.5 Hz), 5.46 (1H, dd, *J* = 11.2, 1.5 Hz), 4.07 (3H, s); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 154.4, 132.5, 130.8, 126.4, 126.1, 114.2, 113.0, 112.0, 109.1, 102.0, 55.9 C^6; IR (cm^{-1}): *v* 3410, 2925, 2836; MS (pNSI): 174.1 (100%, $(M+H)^+$), 520.3 (100%, $(3M+H)^+$); HRMS (pNSI): calcd for $C_{11}H_{12}NO$ $[M+H]^+$: 174.0913; observed: 174.0912

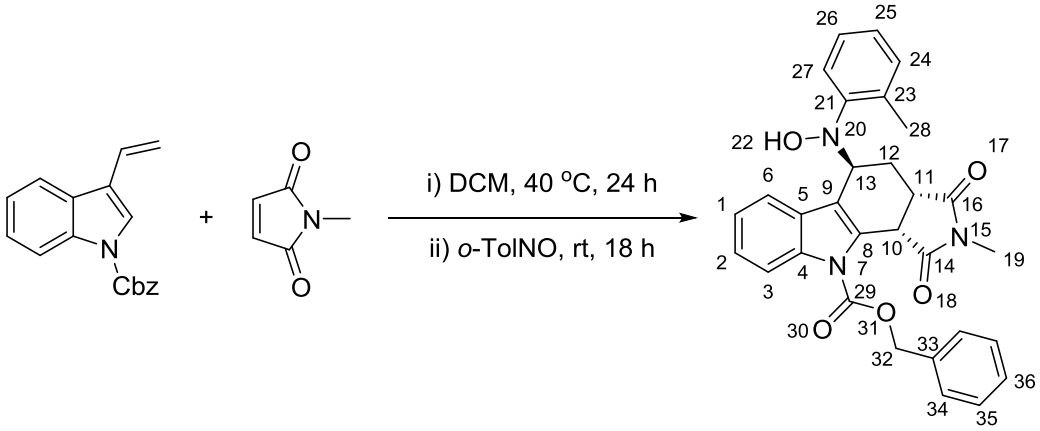
benzyl (3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 241**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (94 mg, 0.34 mmol), DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol). The resulting solution was heated at reflux for 24 hours. The reaction was allowed to cool to room temperature and to the stirred solution nitrosobenzene (36 mg, 0.34 mmol) was added and the solution was stirred for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol: Ethyl acetate 3: 1, column diameter = 2 cm, silica = 14 cm) to give benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (74%, 124 mg, 0.25 mmol) as a yellow powder.

Mp: 105.7 – 109.2 °C; R_f: 0.34 (Pet:EA 2:1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 8.10 (1H, br d, *J* = 8.1 Hz), 7.70 (1H, br d, *J* = 7.3 Hz), 7.49 (2H, br d, *J* = 6.6 Hz), 7.39 (3H, br app q, *J* = 6.9, 6.4 Hz), 7.35 – 7.25 (3H, br m), 7.22 – 7.13 (3H, br m), 7.01 (1H, br app t, *J* = 6.7 Hz), 5.55 (1H, d, *J* = 11.8 Hz, H^{29}), 5.41 (1H, d, *J* = 11.8 Hz, H^{29}), 4.88 (1H, br d, *J* = 6.8 Hz, H^{10}), 4.87 (1H, br s, OH), 4.80 – 4.77 (1H, br m, H^{13}), 3.54 – 3.41 (1H, br m, H^{11}), 2.85 (3H, s, H^{19}), 2.40 – 2.25 (1H, br m, H^{12}), 2.08 – 1.93 (1H, br m, H^{12}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.4 C=O, 174.6 C=O, 151.6, 151.0, 137.0, 135.0, 130.0, 129.0, 128.9, 128.8, 128.8, 127.6, 125.0, 123.3, 122.3, 120.0, 118.3, 117.1, 115.1, 69.5 C^{29}, 57.7 C^{13}, 40.2 C^{10}, 39.2 C^{11}, 24.9 C^{12}, 22.6 C^{19}; IR(neat): υ_{max}/cm^{-1} 3433, 2953, 1699; MS (pNSI): 387.1 (97%, $(M\text{-}N(OH)Ph)^+$), 494.2 (100%, $(M\text{-}H)^+$), 518.2 (30%, $(M+Na)^+$), 991.4 (15%, $(2M+H)^+$), 1013.3 (10%, $(2M+Na)^+$); HRMS (pNSI): calcd $C_{29}H_{25}N_3O_5Na$ $[M+Na]^+$: 518.1686; observed: 518.1676.

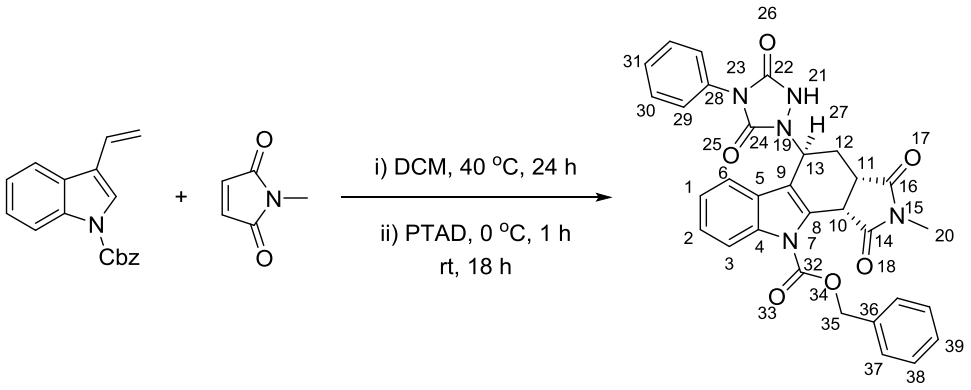
benzyl (3a*S*,5*S***,10b*S***)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 242**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (94 mg, 0.34 mmol), DCM (5 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol). The resulting solution was heated at reflux for 24 hours. The reaction was allowed to cool to room temperature and to the stirred solution 1-methyl-2-nitrosobenzene (41 mg, 0.34 mmol) was added and the solution was stirred for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol: Ethyl acetate 3 : 1, column diameter = 2 cm, silica = 16 cm) to give benzyl (3a*S***,5*S***,10b*S***)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylateas (78%, 135 mg, 0.26 mmol) as a yellow powder.

Mp: 128.4 – 131.5 °C; R_f: 0.45 (Pet:EA 3:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.11 (1H, d, *J* = 8.3 Hz), 7.59 (1H, d, *J* = 7.8 Hz), 7.55 (1H, d, *J* = 8.1 Hz), 7.50-7.48 (2H, m), 7.45-7.38 (3H, m), 7.29-7.23 (1H, m), 7.20 (1H, app t, *J* = 7.6 Hz), 7.14 (1H, d, *J* = 7.6 Hz), 7.10 (1H, d, *J* = 7.8 Hz), 7.05 (1H, d, *J* = 7.3 Hz), 5.56 (1H, d, *J* = 11.8 Hz, H^{32}), 5.46 (1H, d, *J* = 11.8 Hz, H^{32}), 5.02 (1H, s, OH), 4.93 (1H, d, *J* = 7.6 Hz, H^{10}), 4.38 (1H, t, *J* = 5.2 Hz, H^{13}), 3.63 (1H, app q, *J* = 6.9 Hz, H^{11}), 2.91 (3H, s, H^{19}), 2.59 (1H, app dt, *J* = 12.3, 6.0 Hz, H^{12}), 2.29 (3H, s, H^{28}), 1.92 (1H, ddd, *J* = 12.5, 7.5, 4.6 Hz, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 178.5 C=O, 174.6 C=O, 151.7 C^{21}, 149.2 C^{29}, 136.8, 134.8, 131.1, 129.9, 129.0, 129.0, 128.9, 128.9, 127.7, 126.6, 125.3, 125.0, 123.3, 121.5, 120.1, 118.1, 115.3, 69.6 C^{32}, 57.4 C C^{13}, 40.4 C^{10}, 39.1 C^{11}, 25.2 C^{19}, 24.1 C^{12}, 18.6 C^{28}; IR(neat): υ_{max}/cm^{-1} 3450, 2954, 1699; MS (pNSI): 343.1 (40%), 387.1 (82%, (M-(N(OH)2-Tol))$^+$), 508.2 (100%, (M-(H_2)+H)$^+$), 532.2 (59%, (M+Na)$^+$); HRMS (pNSI): calcd $C_{30}H_{27}N_3O_5Na$ [M+Na]$^+$: 532.1843; observed: 532.1834.

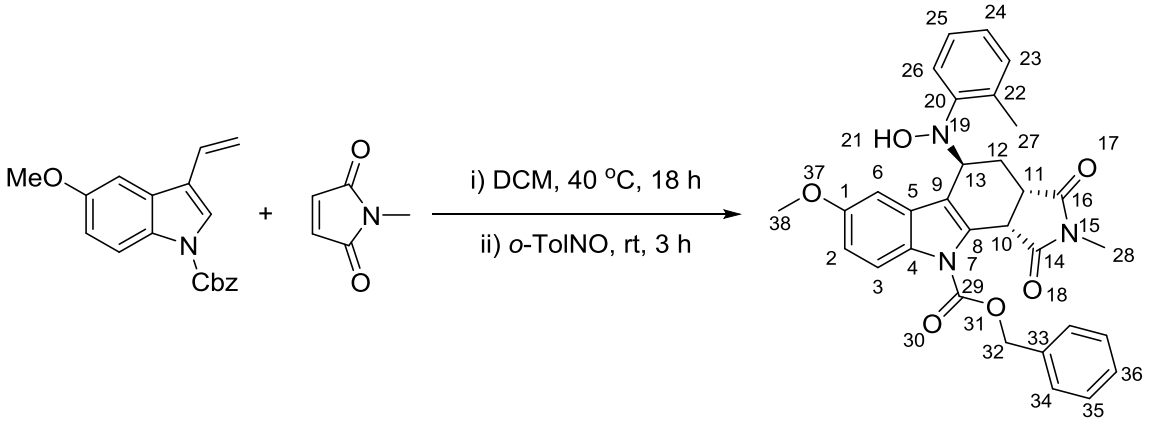
(3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 243**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (189 mg, 0.68 mmol), 1-methyl-1*H*-pyrrole-2,5-dione (76 mg, 0.68 mmol) and DCM (10 mL). The reaction mixture was heated at reflux for 24 hours. The reaction was cooled to 0 °C and then 4-phenyl-1,2,4-triazolidine-3,5-dione (120 mg, 0.68 mmol) was added. The reaction was stirred at 0 °C for 1 hour then at room temperature for 18 hours. The solvent was removed under reduced pressure to leave the crude product as an orange powder. The product was purified by column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2 cm, silica = 20 cm) to give (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (54%, 207 mg, 0.37 mmol) as an off-white powder and (3aS*,5S*,10bS*)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (27%, 76 mg, 0.19 mmol) as a white powder.

Mp: 202.3-203.9 °C; R_f: 0.15 (Pet: EA 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.38 (1H, s, NH), 8.07 (1H, d, *J* = 8.4 Hz), 7.51 (1H, app dt, *J* = 7.6, 0.9 Hz), 7.49 – 7.37 (4H, m), 7.39 – 7.33 (6H, m), 7.30 (1H, ddd, *J* = 8.6, 7.3, 1.3 Hz), 7.21 (1H, app td, *J* = 7.5, 1.0 Hz), 5.53 (1H, app t, *J* = 5.2 Hz, H^{27}), 5.44 (1H, d, *J* = 11.8 Hz, H^{35}), 5.38 (1H, d, *J* = 11.8 Hz, H^{35}), 4.89 (1H, d, *J* = 8.0 Hz, H^{11}), 3.50 (1H, ddd, *J* = 9.6, 8.0, 5.5 Hz, H^{11}), 2.87 (3H, s, H^{20}) 2.41 (1H, app dt, *J* = 14.2, 5.5 Hz, H^{12}), 2.14 (1H, ddd, *J* = 14.2, 9.5, 5.5 Hz, H^{12}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 177.4 C=O, 173.6 C=O, 153.6 C=O, 152.2 C=O, 151.3 C=O, 136.9, 134.5, 130.9, 130.8, 129.3, 129.1, 128.9, 128.9, 128.4, 126.1, 125.8, 125.3, 123.8, 118.8, 115.7, 113.8, 69.8 C^{35}, 47.5 C^{13}, 40.0 C^{10}, 38.4 C^{11}, 27.5 C^{12}, 25.2 C^{20}; IR (neat): ν_{max} cm^{-1} 3462, 2969, 1699; MS (pNSI): 199.2 (16%), 387.1 (19%, $(M\text{-PTAD})^+$), 564.2 (59%, $(M+H)^+$), 581.2 (100%, $(M+NH_4)^+$), 643.2 (15%), 1144.4 (39%, $(2M+NH_4)^+$); HRMS (pNSI): calcd $C_{31}H_{26}N_5O_6$ $[M+H]^+$: 564.1878; observed: 564.1873.

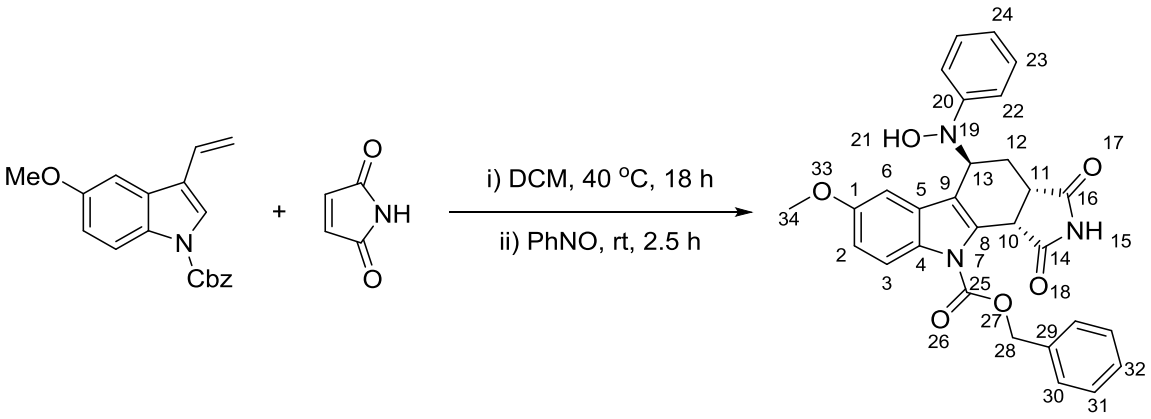
benzyl (3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 244**



To a stirred Schlenk flask was added benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (209 mg, 0.68 mmol), DCM (10 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (76 mg, 0.68 mmol). The resulting solution was heated at reflux for 18 hours. The reaction was cooled to room temperature and 1-methyl-2-nitrosobenzene (82 mg, 0.68 mmol) was added. The solution was stirred at room temperature for 3 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 2 cm, silica = 15 cm) to give benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (74%, 279 mg, 0.50 mmol) as a pale yellow powder.

Mp: 107.6 – 110.1°C; R_f: 0.29 (Pet:EA 2:1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 7.93 (1H, d, *J* = 9.1 Hz), 7.54 (1H, dd, *J* = 8.1, 1.3 Hz), 7.49 – 7.45 (2H, m), 7.43 – 7.35 (3H, m), 7.19 (1H, ddd, *J* = 7.6, 6.9, 1.9 Hz), 7.10 – 7.01 (2H, m), 6.88 (1H, d, *J* = 2.6 Hz), 6.80 (1H, dd, *J* = 9.1, 2.6 Hz), 5.52 (1H, d, *J* = 11.9 Hz, H^{32}), 5.37 (1H, d, *J* = 11.9 Hz, H^{32}), 5.18 (1H, s, OH), 4.87 (1H, d, *J* = 8.1 Hz, H^{10}), 4.33 – 4.29 (1H, m, H^{13}), 3.65 (3H, s, H^{37}), 3.67 – 3.61 (1H, m, H^{11}), 2.86 (3H, s, H^{28}), 2.61 (1H, app dt, *J* = 13.4, 5.9 Hz, H^{12}), 2.22 (3H, s, H^{27}), 1.86 (1H, ddd, *J* = 13.4, 8.5, 4.3 Hz, H^{12}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.4 C=O, 174.4 C=O, 156.1 C^1, 151.6 C^{29}, 149.8 C^{20}, 135.0, 134.9, 131.2, 130.8, 130.6, 130.4, 128.8, 128.8, 128.5, 126.4, 125.3, 121.7, 117.5, 115.7, 113.6, 102.0, 69.3 C^{32}, 57.8 C^{13}, 55.4 C^{38}, 40.5 C^{10}, 38.9 H^{11}, 25.0 C^{28}, 24.9 C^{12}, 18.2 C^{27}; IR(neat): υ_{max}/cm^{-1} = 3370, 2965, 2887, 1699; MS (pNSI): 207.1 (39%), 417.1 (34%, (M-(N(OH)2-Tol))$^+$), 438.2 (100%, (M-(H_2)+H)$^+$), 1075.4 (15%, (2(M-(H_2)+H)$^+$); HRMS (pNSI): calcd $C_{31}H_{28}N_3O_6$ [M-(H_2)+H]$^+$:538.1976; observed: 538.1973.

benzyl (3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 245**

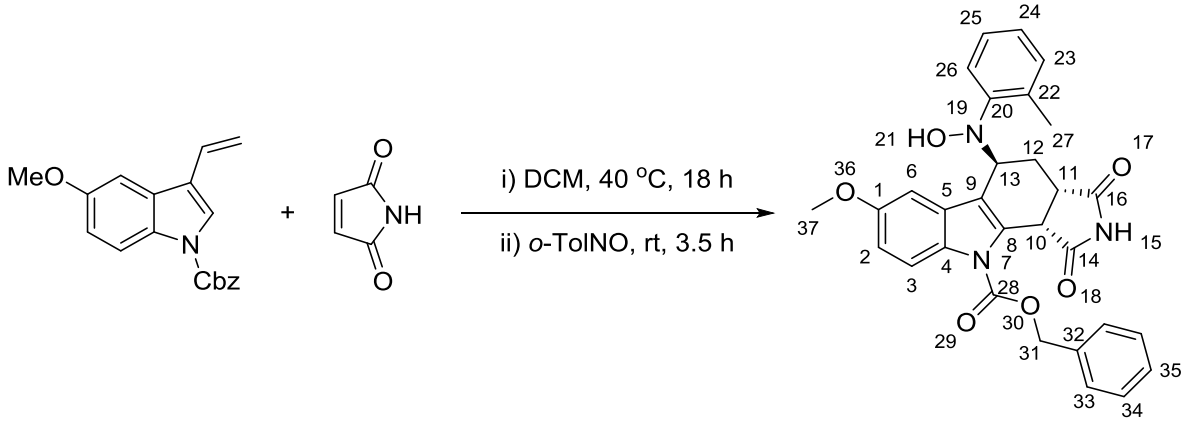


To a stirred Schlenk flask was added benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (209 mg, 0.68 mmol), DCM (10 mL) and 1*H*-pyrrole-2,5-dione (66 mg, 0.68 mmol). The resulting solution was heated at reflux for 18 hours. The reaction was cooled to room temperature and nitrosobenzene (72 mg, 0.68 mmol) was added and the reaction was stirred for 2.5 hours. The solvent was removed under reduced pressure to leave the crude product as a pale orange oil. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 1 cm, silica = 16 cm,) to give benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (79%, 317 mg, 0.54 mmol) as a pale orange powder.

Mp: 134.2 – 136.9 °C; R_f: 0.34 (Pet:EA 3:2);

^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 8.42 (1H, br s, NH), 7.94 (1H, d, *J* = 9.0 Hz), 7.47 – 7.44 (2H, m), 7.40 – 7.34 (3H, m), 7.30 – 7.27 (2H, m), 7.19 – 7.17 (2H, m), 7.01 – 6.98 (1H, m), 6.93 (1H, br s), 6.81 (1H, d, *J* = 9.0 Hz), 5.49 (1H, d, *J* = 11.9 Hz, H^{28}), 5.39 (1H, br s, OH), 5.34 (1H, d, *J* = 11.9 Hz, H^{28}), 4.92 (1H, br d, *J* = 6.2 Hz, H^{10}), 4.74 (1H, br s, H^{13}), 3.64 (3H, s, H^{34}), 3.57 – 3.51 (1H, br m, H^{11}), 2.39 – 2.35 (1H, br m, H^{12}), 1.91 – 1.89 (1H, br m, H^{12}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.6 C=O, 174.4 C=O, 156.2 C^1, 151.5, 151.0, 135.0, 131.4, 130.3, 129.0, 128.9, 128.8, 128.8, 128.4, 122.6, 117.5, 117.4, 116.0, 113.5, 102.3, 69.4 C^{28}, 57.9 C^{13}, 55.6 C^{34}, 41.5 C^{10}, 40.5 C^{11}, 24.0 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3233, 2952, 1708; MS (pNSI): 403.1 (37%, $(M-(N(OH)Ph))^+$), 510.2 (100%, $(M-(H_2)+H)^+$), 532.1 (26%, $(M-(H_2)+Na)^+$); HRMS (pNSI): calcd $C_{29}H_{24}N_3O_6$ $[M-(H_2)+H]^+$:510.1654; observed: 510.1660.

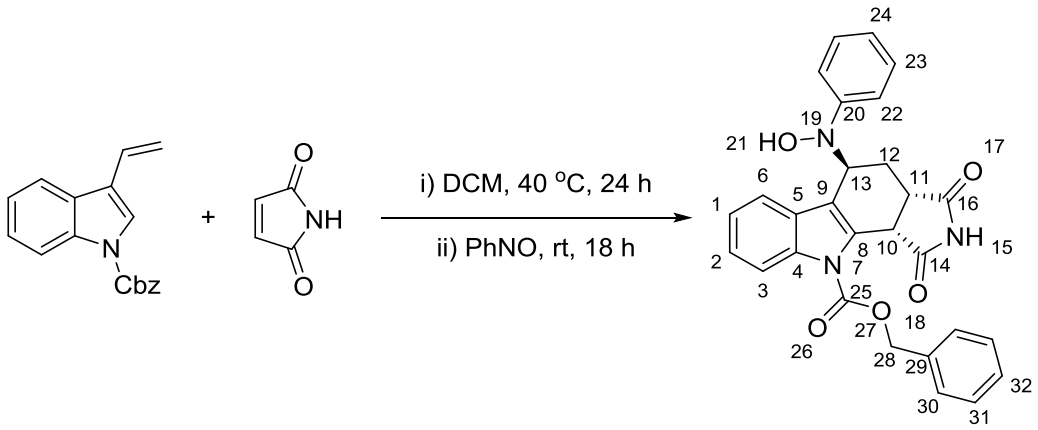
benzyl (3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 246**



To a stirred Schlenk flask was added benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (209 mg, 0.68 mmol), DCM (10 mL) and 1*H*-pyrrole-2,5-dione (66 mg, 0.68 mmol). The resulting solution was heated at reflux for 18 hours. The reaction was cooled to room temperature and 1-methyl-2-nitrosobenzene (82 mg, 0.68 mmol) was added. The solution was stirred at room temperature for 3.5 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 2 cm, silica = 14 cm) to give benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (76%, 255 mg, 0.52 mmol) as a pale yellow powder.

Mp: 193.0 – 195.6 °C; R_f: 0.20 (Pet:EA 2:1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 7.92 (1H, d, *J* = 9.7 Hz), 7.81 (1H, s, NH), 7.53 (1H, d, *J* = 7.8 Hz), 7.46 (2H, dd, *J* = 7.8, 1.6 Hz), 7.42 – 7.33 (3H, m), 7.21 – 7.15 (1H, m), 7.09 – 7.00 (2H, m), 6.84 – 6.75 (2H, m). 5.50 (1H, d, *J* = 11.9 Hz, H^{31}), 5.35 (1H, d, *J* = 11.9 Hz, H^{31}), 5.16 (1H, s, OH), 4.96 (1H, d, *J* = 7.6 Hz, H^{10}), 4.37 (1H, app t, *J* = 4.9 Hz, H^{13}), 3.71 (1H, app td, *J* = 8.7, 5.6 Hz, H^{11}), 3.64 (3H, s, H^{37}), 2.64 (1H, app dt, *J* = 13.6, 5.6 Hz, H^{12}), 2.21 (3H, s, H^{27}), 1.88 (1H, ddd, *J* = 13.6, 9.3, 4.3 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 180.6 C=O, 176.4 C=O, 155.6, 151.8, 151.7, 135.8, 131.6, 130.8, 130.7, 130.2, 129.1, 129.1, 129.0, 126.7, 124.8, 122.3, 117.2, 115.2, 113.5, 102.7, 69.1 C^{31}, 57.3 C^{13}, 55.5 C^{37}, 41.9 C^{10}, 40.3 C^{11}, 27.2 C^{12}, 18.5 C^{27}; IR(neat): υ_{max}/cm^{-1} = 3457, 3367, 2981, 2886, 1712; MS (pNSI): 403.1 (100%, (M-(N(OH)-2-Tol))$^+$), 524.1 (75%, (M-(H_2)+H)$^+$), 548.2 (16%, (M+Na)$^+$), 1073.4 (5%, (2M+Na)$^+$); HRMS (pNSI): calcd $C_{30}H_{27}N_3O_6Na$ [M+Na]$^+$: 548.1792; observed: 548.1785.

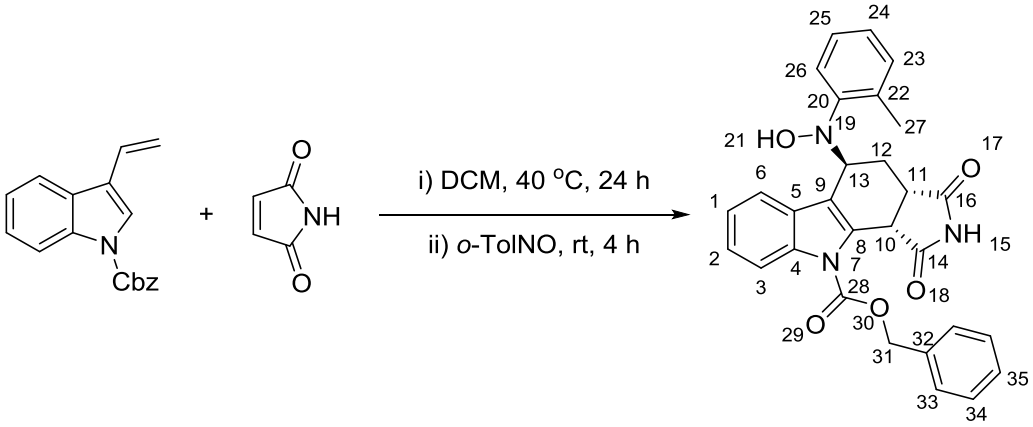
benzyl (3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 247**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (189 mg, 0.68 mmol), 1*H*-pyrrole-2,5-dione (66 mg, 0.68 mmol) and DCM (10 mL). The resulting solution was heated at reflux for 24 hours. The reaction was allowed to cool to room temperature and to the stirred solution nitrosobenzene (73 mg, 0.68 mmol) was added and the solution was stirred for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a yellow powder. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 2 cm, silica = 20 cm) to give benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (70%, 230 mg, 0.48 mmol) as a yellow powder.

Mp: 177.1-177.8 °C; R_f: 0.38 (Pet:EA 2:1); ^{1}H NMR (500 MHz, CD_2Cl_2) δ_H 8.20 (1H, s, NH), 8.11 (1H, d, *J* = 8.3 Hz), 7.66 (1H, d, *J* = 7.8 Hz), 7.50 (2H, dd, *J* = 7.9, 1.6 Hz), 7.43-7.36 (3H, m), 7.33 (2H, app t, *J* = 7.8 Hz), 7.28 (1H, app t, *J* = 7.8 Hz), 7.22 (2H, d, *J* = 8.0 Hz), 7.16 (1H, app t, *J* = 7.5 Hz), 7.02 (1H, app t, *J* = 7.3 Hz), 5.55 (1H, d, *J* = 11.9 Hz, H^{28}), 5.40 (1H, d, *J* = 11.9 Hz, H^{28}), 5.19 (1H, s, OH), 4.96 (1H, br d, *J* = 8.1 Hz, H^{10}), 4.82 (1H, app t, *J* = 5.7 Hz, H^{13}), 3.56 (1H, br app q, *J* = 7.4 Hz, H^{11}), 2.35 (1H, br app dd, *J* = 13.4, 6.7 Hz, H^{12}), 2.00 – 1.92 (1H, br m, H^{12}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.5 C=O, 174.5 C=O, 151.6 C^{25}, 150.7, 136.9, 134.9, 129.7, 129.0, 128.9, 128.9, 128.8, 127.6, 125.1, 123.3, 122.6, 119.8, 118.0, 117.3, 115.2, 69.5 C^{28}, 57.7 C^{13}, 41.4 C^{10}, 40.6 C^{11}, 23.0 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3418, 3329, 2970, 1705; MS (pNSI): 199.2 (87%), 373.1 (68%, (M-(N(OH)Ph))$^+$), 480.2 (100%, (M-(H_2)+H)$^+$); HRMS (pNSI): calcd $C_{28}H_{23}N_3O_5Na$ [M+Na]$^+$: 504.1530; observed: 504.1522.

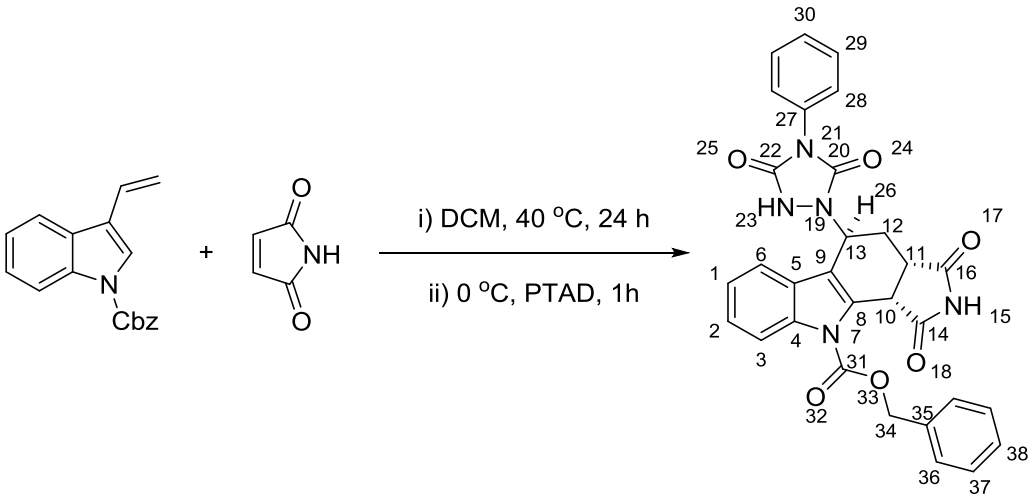
benzyl (3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 248**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (94 mg, 0.34 mmol), DCM (5 mL) and 1*H*-pyrrole-2,5-dione (33 mg, 0.34 mmol). The resulting solution was heated at reflux for 24 hours. The reaction was allowed to cool to room temperature and to the stirred solution 1-methyl-2-nitrosobenzene (41 mg, 0.34 mmol) was added and the solution was stirred for 4 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The product was purified by column chromatography (Petrol: Ethyl acetate 3: 2, column diameter = 2 cm, silica = 16 cm) to give benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (83%, 140 mg, 0.28 mmol) as a yellow powder.

Mp: 181.4 – 183.9 °C; R_f: 0.48 (Pet:EA 3:2); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.26 (1H, s, NH), 8.41 (1H, s, OH), 7.93 (1H, d, *J* = 8.3 Hz), 7.51 (2H, d, *J* = 7.0 Hz), 7.45-7.33 (4H, m), 7.18-7.08 (3H, m), 7.03-6.91 (3H, m), 5.56 (1H, d, *J* = 12.1 Hz, H^{31}), 5.32 (1H, d, *J* = 12.1 Hz, H^{31}), 4.96 (1H, d, *J* = 8.0 Hz, H^{10}), 4.34 (1H, app t, *J* = 3.7 Hz, H^{13}), 3.73 (1H, app q, *J* = 8.7 Hz, H^{11}), 2.50-2.44 (1H, m, H^{12}), 2.17 (3H, s, H^{27}), 1.74-1.64 (1H, m, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 180.6 C=O, 176.5 C=O, 151.6, 136.2, 135.7, 131.1, 130.8, 130.0, 129.2, 129.1, 129.1, 128.2, 126.6, 124.7, 124.5, 122.9, 122.2, 120.6, 117.5, 114.4, 69.2 C^{31}, 57.1 C^{13}, 41.8 C^{10}, 40.2 C^{11}, 26.7 C^{12}, 18.6 C^{27}; IR(neat): υ_{max}/cm^{-1} 3495, 3325, 2953, 1711; MS (pNSI): 373.1 (51%, (M-(N(OH)2-Tol))$^+$), 494.2 (16%, (M-(H_2)+H)$^+$), 518.2 (21%, (M+Na)$^+$); HRMS (pNSI): calcd $C_{29}H_{25}N_3O_5Na$ [M+Na]$^+$: 518.1686; observed: 518.1681.

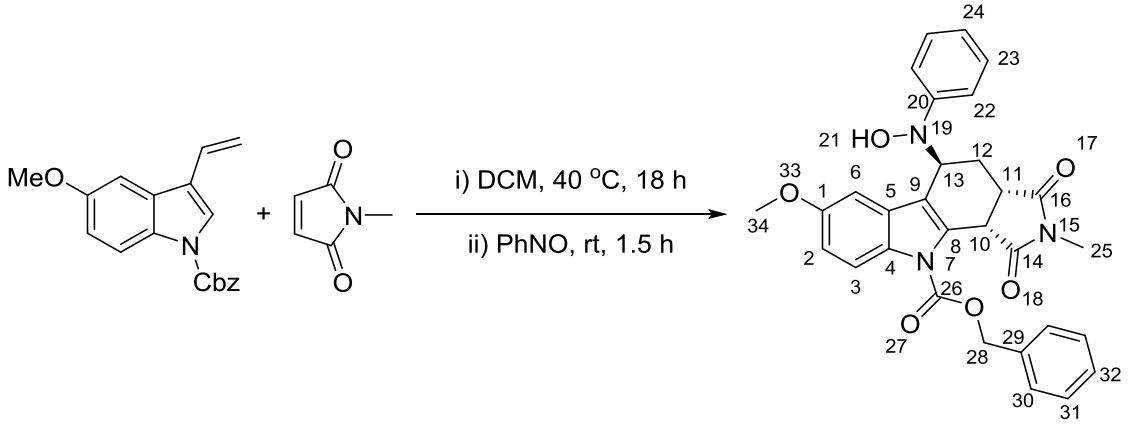
benzyl (3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 249**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (189 mg, 0.68 mmol), DCM (10 mL) and 1*H*-pyrrole-2,5-dione (66 mg, 0.68 mmol). The resulting solution was heated at reflux for 24 hours. The reaction was cooled to 0 °C and 4-phenyl-1,2,4-triazolidine-3,5-dione (120 mg, 0.68 mmol) was added. The solution was stirred at 0 °C for 1 hour. The solvent was removed under reduced pressure to leave the crude product as an off white solid. The product was purified by column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2 cm, silica = 13 cm) to give benzyl (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (58%, 216 mg, 0.39 mmol) as a pale pink powder.

Mp: 174.6 – 177.1 °C; R_f: 0.06 (Pet:EA 1:1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 8.78 (1H, s, NH), 8.05 (1H, d, *J* = 8.3 Hz), 7.54 (1H, d, *J* = 7.7 Hz), 7.44 – 7.38 (6H, m), 7.38 – 7.30 (4H, m), 7.30 – 7.24 (1H, m), 7.20 (1H, app t, *J* = 7.5 Hz), 5.51 (1H, app t, *J* = 5.3 Hz, H^{26}), 5.45 (1H, d, *J* = 11.9 Hz, H^{34}), 5.31 (1H, d, *J* = 11.9 Hz, H^{34}), 4.98 (1H, d, *J* = 7.9 Hz, H^{10}), 3.46 (1H, app q, *J* = 8.1 Hz, H^{11}), 2.36 – 2.30 (1H, m, H^{12}), 2.18 (1H, ddd, *J* = 13.9, 8.6, 5.3 Hz,H^{12}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.1, 173.8, 153.7, 152.4, 151.4, 136.8, 134.7, 131.1, 130.6, 129.1, 128.9, 128.8, 128.8, 128.4, 126.2, 125.7, 125.6, 123.7, 119.0, 115.5, 114.2, 69.7 C^{34}, 47.7 C^{13}, 41.0 C^{10}, 39.5 C^{11}, 26.8 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3169, 2975, 1699; MS (pNSI): 279.1 (38%), 373.1 (13%, $(M\text{-PTAD})^+$), 550.2 (21%, $(M+H)^+$), 567.2 (100% $(M+NH_4)^+$), 1116.4 (39%, $(2M+NH_4)^+$), 1666.5 (6%, $(3M+NH_4)^+$); HRMS (pNSI): calcd $C_{30}H_{24}N_5O_6$ $[M+H]^+$: 550.1721; observed: 550.1719.

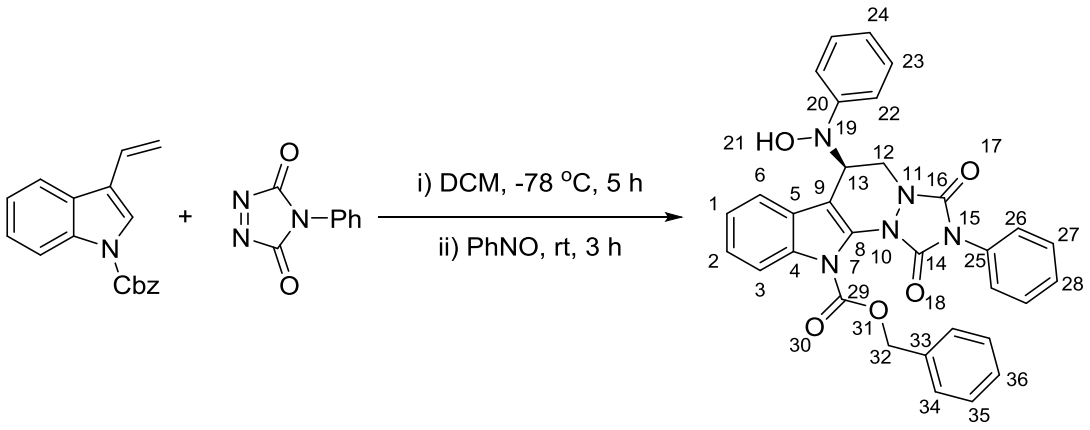
benzyl (3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 250**



To a stirred Schlenk flask was added benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (209 mg, 0.68 mmol), DCM (10 mL) and 1-methyl-1*H*-pyrrole-2,5-dione (76 mg, 0.68 mmol). The resulting solution was heated at reflux for 18 hours. The reaction was cooled to room temperature and nitrosobenzene (72 mg, 0.68 mmol) was added and the reaction was stirred for 1.5 hours. The solvent was removed under reduced pressure to leave the crude product as a pale orange oil. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 1 cm, silica = 16 cm,) to give benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (73%, 218 mg, 0.44 mmol) as an orange powder.

Mp: 106.8 – 110.2 °C; R_f: 0.65 (Pet:EA 2:1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 7.97 (1H, br d, *J* = 8.3 Hz), 7.49 – 7.47 (2H, m), 7.41 – 7.37 (3H, m), 7.33 – 7.30 (2H, m), 7.21 – 7.19 (2H, m), 7.05 – 7.00 (2H, m), 6.85 (1H, br d, *J* = 8.4 Hz), 5.53 (1H, d, *J* = 11.9 Hz, H^{28}), 5.39 (1H, d, *J* = 11.9 Hz, H^{28}), 5.02 (1H, br s, OH), 4.91 – 4.87 (1H, m, H^{10}), 4.75 (1H, br s, H^{13}), 3.68 (3H, s, H^{34}), 3.50 (1H, br s, H^{11}), 2.86 (3H, s, H^{25}), 2.36 (1H, br s, H^{12}), 2.01 (1H, br s, H^{12}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.3 C=O, 174.5 C=O, 156.3 C^1, 151.5 C^{26}, 151.1, 135.0, 131.5, 130.7, 129.0, 128.9, 128.8, 128.8, 128.4, 122.4, 117.7, 117.2, 115.9, 113.4, 102.4, 69.4 C^{28}, 57.9 C^{13}, 55.6 C^{34}, 40.3 C^{10}, 39.2 C^{11}, 24.9 C^{25}, 23.4 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3408, 2969, 2890, 1699; MS (pNSI): 417.1 (100%, (M-(N(OH)Ph))$^+$), 524.2 (68%, (M-(H_2)+H)$^+$), 548.2 (16%, (M+Na)$^+$), 1073.4 (4%, (2M+Na)$^+$); HRMS (pNSI): calcd $C_{30}H_{27}N_3O_6Na$ [M+Na]$^+$: 548.1792; observed: 548.1785.

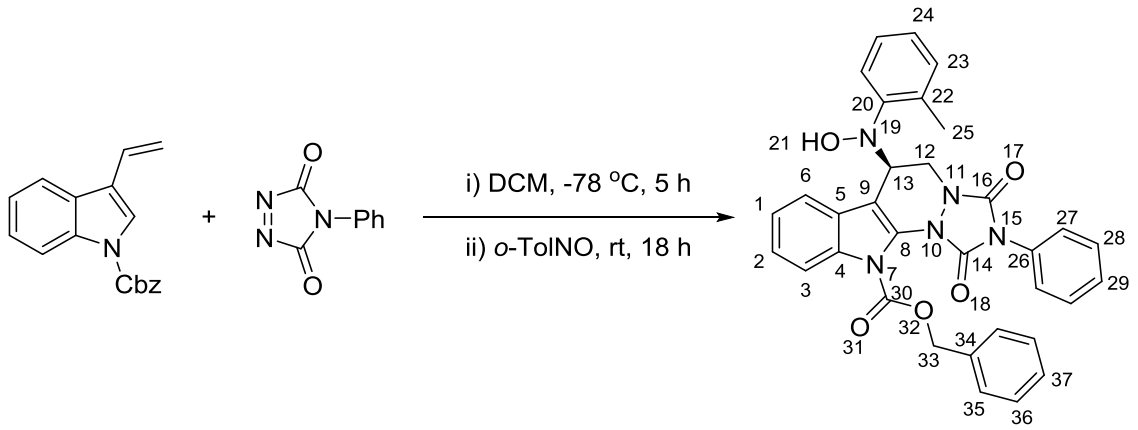
benzyl (*R)-6-(hydroxy(phenyl)amino)-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate – 251**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (189 mg, 0.68 mmol) and DCM (10 mL). The solution was cooled to -78 °C and 4-phenyl-1,2,4-triazolidine-3,5-dione (120 mg, 0.68 mmol) was added. The reaction was stirred at -78 °C for 5 hours. The reaction was warmed to room temperature, nitrosobenzene (73 mg, 0.68 mmol) was added and the reaction was stirred for 3 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow oil. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 1 cm, silica = 16 cm,) to give benzyl (*R**)-6-(hydroxy(phenyl)amino)-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (72%, 274 mg, 0.49 mmol) as a white powder.

Mp: 101.2 – 103.1°C; R_f: 0.53 (Pet:EA 2:1; ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 8.09 (1H, d, *J* = 8.2 Hz), 7.51 – 7.38 (8H, m), 7.38 – 7.24 (5H, m), 7.23 – 7.07 (4H, m), 6.87 (1H, d, *J* = 7.9 Hz), 6.36 (1H, s, OH), 5.52 (1H, d, *J* = 12.1 Hz, H^{32}), 5.39 (1H, d, *J* = 12.1 Hz, H^{32}), 5.22 (1H, dd, *J* = 14.0, 1.7 Hz, H^{12}), 4.97 – 4.89 (1H, m, H^{13}), 3.66 (1H, dd, *J* = 14.0, 3.4 Hz, H^{12}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 147.2, 147.0, 146.6, 145.6, 131.0, 130.1, 127.3, 126.2, 125.4, 125.2, 124.9, 124.9, 124.9, 124.8, 124.8, 122.4, 122.3, 120.7, 120.5 119.7, 115.8, 114.5, 110.5, 97.2, 66.2 C^{32}, 55.1 C^{13}, 39.6 C^{12}**;** IR(neat): υ_{max}/cm^{-1} = 3337, 3063, 1716; MS (pAPCI): 395.1 (100%), 451.1 (59%, (M-(N(OH)Ph))$^+$), 542.2 (5%, (M-(H_2O)+H)$^+$), 558.2 (1%, (M-H)$^+$), 560.2 (1%, (M+H)$^+$); HRMS (pAPCI): calcd $C_{32}H_{26}N_5O_5$ [M+H]$^+$: 560.1928; observed: 560.1913.

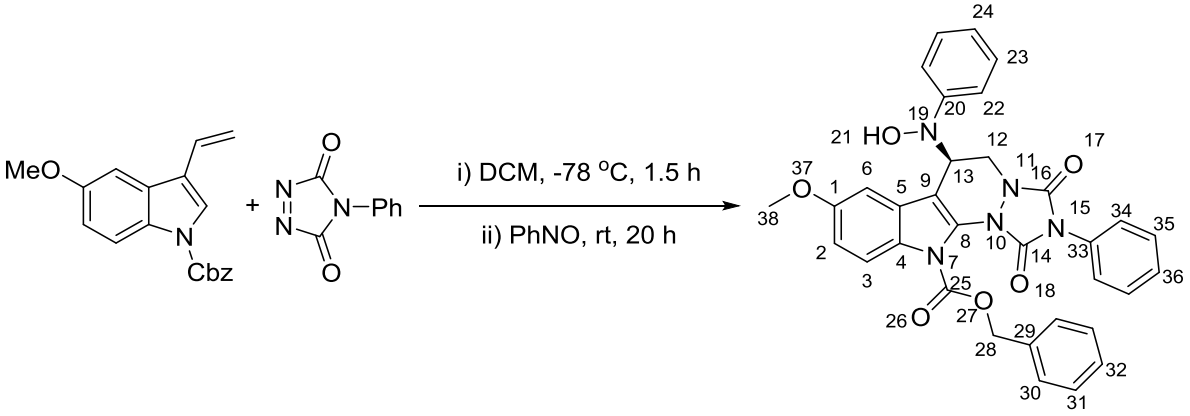
benzyl (*R)-6-(hydroxy(*o*-tolyl)amino)-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate – 252**



To a stirred Schlenk flask was added benzyl 3-vinyl-1*H*-indole-1-carboxylate (94 mg, 0.34 mmol) and DCM (10 ml). The reaction mixture was cooled to -78 °C and 4-phenyl-1,2,4-triazolidine-3,5-dione (60 mg, 0.34 mmol) was added. The reaction mixture was stirred at -78 °C for 5 hours, 1-methyl-2-nitrosobenzene was added (41 mg, 0.34 mmol) and the reaction stirred at room temperature for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a yellow powder. The product was purified by column chromatography (Petrol: Ethyl acetate 3: 1, column diameter = 2 cm, silica = 20 cm) to give benzyl (*R**)-6-(hydroxy(*o*-tolyl)amino)-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (68%, 132 mg, 0.23 mmol) as an off white powder.

Mp: 163.8-165.1 °C; R_f: 0.49 (Pet:EA 3:1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 8.01 (1H, d, *J* = 8.3 Hz), 7.65 (1H, dd, *J* = 8.1, 1.3 Hz), 7.54 – 7.38 (7H, m), 7.35 (3H, dd, *J* = 5.0, 2.1 Hz), 7.19 (2H, app dtd, *J* = 8.5, 7.2, 6.7, 1.4 Hz), 6.99 (2H, app tdd, *J* = 7.5, 3.4, 1.2 Hz), 6.90 (1H, dd, *J* = 7.7, 1.4 Hz), 6.75 (1H, d, *J* = 7.8 Hz), 5.87 (1H, s, OH), 5.48 (1H, d, *J* = 11.9 Hz, H^{33}), 5.34 (1H, d, *J* = 11.9 Hz, H^{33}), 5.19 (1H, dd, *J* = 14.1, 2.3 Hz, H^{12}), 4.63 (1H, app t, *J* = 2.3 Hz, H^{13}), 3.52 (1H, dd, *J* = 14.1, 2.3 Hz, H^{12}), 1.94 (3H, s, H^{25}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 151.9, 150.3, 150.0, 148.8, 134.6, 133.6, 131.6, 131.1, 130.6, 130.0, 129.4, 128.9, 128.9, 128.8, 128.8, 127.0, 126.6, 126.0, 125.7, 124.4, 123.6, 122.7, 117.9, 114.6, 101.7, 70.1 C^{33}, 58.6 C^{13}, 43.5 C^{12}, 17.7 C^{25}; IR(neat): υ_{max}/cm^{-1} = 3291, 2981, 1782, 1737, 1699; MS (pNSI): 199.2 (100%), 407.2 (79%), 451.1 (81%, (M-(N(OH)2-Tol))$^+$), 572.2 (25%, (M-H)$^+$), 596.2 (65%, (M+Na)$^+$); HRMS (pNSI): calcd $C_{33}H_{27}N_5O_5Na$ [M+Na]$^+$:596.1904; observed: 596.1898.

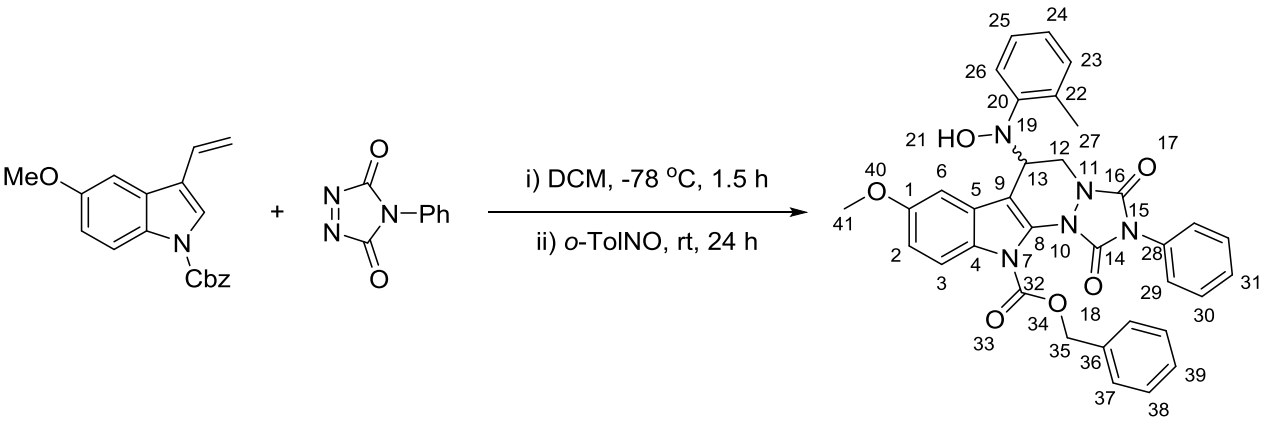
benzyl (*R)-6-(hydroxy(phenyl)amino)-8-methoxy-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate – 253**



To a stirred Schlenk flask was added benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (209 mg, 0.68 mmol) and DCM (10 mL). The solution was cooled to -78 °C and 4-phenyl-1,2,4-triazolidine-3,5-dione (120 mg, 0.68 mmol) was added. The reaction was stirred at -78 °C for 1.5 hours. The reaction was warmed to room temperature, nitrosobenzene (73 mg, 0.68 mmol) was added and the reaction was stirred for 20 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow oil. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 1 cm, silica = 16 cm) to give benzyl (*R**)-6-(hydroxy(phenyl)amino)-8-methoxy-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (78%, 312 mg, 0.53 mmol) as a white powder.

Mp: 110.4 – 113.2 °C; R_f: 0.20 (Pet:EA 2: 1); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 7.88 (1H, d, *J* = 9.1 Hz), 7.42 – 7.38 (5H, m), 7.36 – 7.32 (3H, m), 7.29 – 7.26 (2H, m), 7.24 – 7.20 (2H, m), 7.13 (2H, d, *J* = 8.1 Hz), 7.05 (1H, app t, *J* = 7.3 Hz). 6.76 (1H, dd, *J* = 9.1, 2.6 Hz), 6.24 (1H, s, OH), 6.10 (1H, d, *J* = 2.5 Hz), 5.43 (1H, d, *J* = 12.0 Hz, H^{28}), 5.32 – 5.29 (1H, m, H^{28}), 5.25 – 5.18 (1H, m, H^{12}), 4.84 – 4.80 (1H, m, H^{13}), 3.64 (1H, dd, *J* = 14.0, 3.3 Hz, H^{12}), 3.55 (3H, s, H^{38}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 156.5, 151.1, 150.3, 149.4, 134.8, 131.2, 130.3, 129.2, 129.0, 129.0, 128.70, 128.7, 128.7, 128.6, 128.2, 126.9, 126.2, 124.6, 119.7, 115.3, 113.2, 101.0, 100.5, 69.9 C^{28}, 59.1 C^{13}, 55.5 C^{38}, 44.0 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3336, 2935, 1716; MS (pNSI): 481.1 (17%, $(M-(N(OH)Ph))^+$), 588.2 (100%, $(M-(H_2)+H)^+$), 612.2 (15%, $(M+Na)^+$); HRMS (pNSI): calcd $C_{33}H_{27}N_5O_6Na$ $[M+Na]^+$: 612.1854; observed: 612.1838.

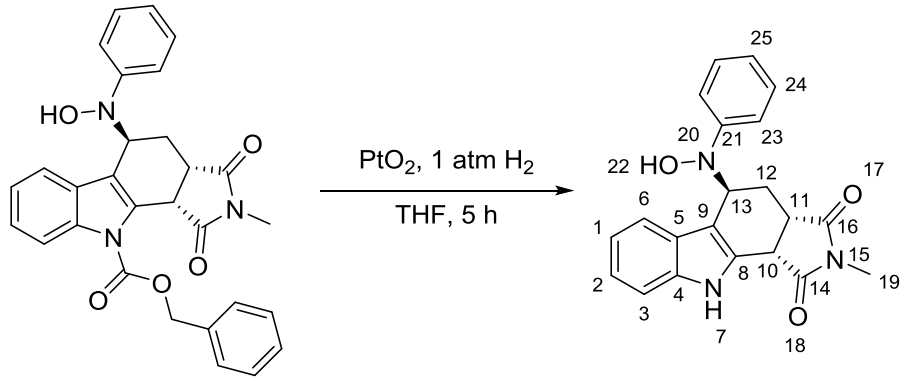
benzyl (*R)-6-(hydroxy(*o*-tolyl)amino)-8-methoxy-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate – 254**



To a stirred Schlenk flask was added benzyl 5-methoxy-3-vinyl-1*H*-indole-1-carboxylate (209 mg, 0.68 mmol) and DCM (10 mL). The solution was cooled to -78 °C and 4-phenyl-1,2,4-triazolidine-3,5-dione (120 mg, 0.68 mmol) was added. The reaction was stirred at -78 °C for 1.5 hours. The reaction was warmed to room temperature, 1-methyl-2-nitrosobenzene (73 mg, 0.68 mmol) was added and the reaction was stirred for 24 hours. The solvent was removed under reduced pressure to leave the crude product as a pale orange oil. The product was purified by column chromatography (Petrol: Ethyl acetate 2: 1, column diameter = 2 cm, silica = 17 cm) to give benzyl (*R**)-6-(hydroxy(*o*-tolyl)amino)-8-methoxy-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (82%, 338 mg, 0.56 mmol) as an off white powder.

Mp: 181.1 – 183.0 °C; R_f: 0.55 (Pet:EA 2:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.70 (1H, s), 7.84 (1H, d, *J* = 9.0 Hz), 7.55 – 7.48 (2H, m), 7.46 – 7.41 (3H, m), 7.41 – 7.33 (4H m), 7.33 – 7.29 (2H, m), 7.13 – 7.03 (2H, m), 6.97 (1H, app td, *J* = 7.4, 1.3 Hz), 6.83 (1H, dd, *J* = 9.1, 2.6 Hz). 6.38 (1H, s, OH), 5.43 (1H, d, *J* = 12.1 Hz, H^{35}), 5.33 (1H, d, *J* = 12.1 Hz, H^{35}), 4.80 (1H, dd, *J* = 13.7, 1.8 Hz, H^{12}), 4.71 (1H, dd, *J* = 3.3, 1.8 Hz, H^{13}), 3.68 – 3.61 (1H, dd, *J* = 13.7, 3.3 Hz, H^{12}), 3.58 (s, 3H, H^{41}), 2.30 (s, 3H, H^{27}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 156.3, 151.6, 150.8, 150.3, 149.8, 135.3, 131.6, 130.9, 130.3, 130.0, 129.7, 129.2, 129.1, 129.1, 128.9, 128.0, 127.4, 127.1, 126.8, 125.2, 121.8, 115.3, 113.2, 103.1, 101.9, 69.9 C^{35}, 55.9 C^{13}, 55.6 C^{41}, 43.9 C^{12}, 18.2 C^{27}; IR(neat): υ_{max}/cm^{-1} = 3212, 2939, 1720; MS (pNSI): 481.2 (100%, (M-N(OH)2-Tol)$^+$), 602.2 (34%, (M-(H_2)+H)$^+$), 626.2 (100%, (M+Na)$^+$); HRMS (pNSI): calcd $C_{34}H_{29}N_5O_6Na$ $[M+Na]^+$: 626.2010; observed: 626.2006.

(3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 255**



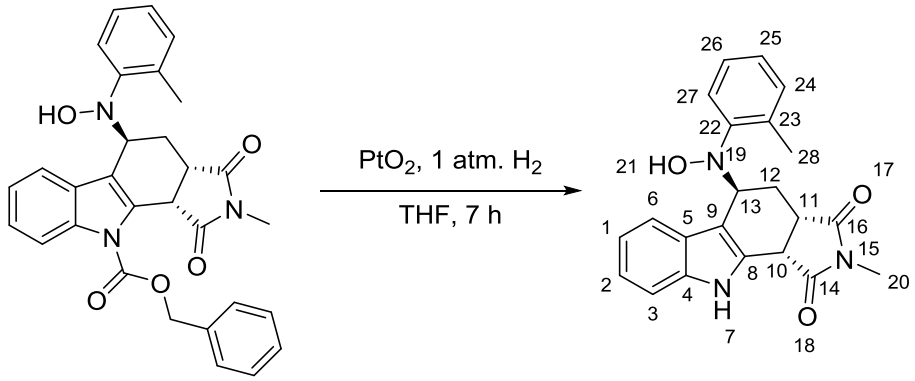
To a stirred Schlenk flask was added benzyl (3aS*,5S*,10bS*)-5-(hydroxy(phenyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (120 mg, 0.25 mmol), platinum (IV) oxide (57 mg, 0.25 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a yellow solid, The crude product was purified by trituration from DCM to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (75%, 68 mg, 0.19 mmol) as a pale yellow powder.

Mp: 157.2 – 161.9 ^{o}C; R_f: 0.17 (Pet:EA 1:1);

^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.06 (1H, s), 8.20 (1H, s), 7.35 (1H, d, *J* = 8.0 Hz), 7.26 (1H, d, *J* = 8.0 Hz), 7.24 – 7.19 (2H, m), 7.17 – 7.08 (2H, m), 6.98 (1H, app ddd, *J* = 8.2, 7.0, 1.2 Hz), 6.91 – 6.81 (1H, m), 6.81 (1H, app ddd, *J* = 8.0, 6.9, 1.0 Hz), 4.88 (1H app t, *J* = 4.9 Hz, H^{13}), 4.27 (1H, d, *J* = 8.8 Hz, H^{10}), 3.64 (1H, app td, *J* = 8.8, 6.1 Hz, H^{11}), 2.81 (3H, s, H^{19}), 2.33 (1H, app dt, *J* = 13.7, 6.1 Hz, H^{12}), 1.84 (1H, ddd, *J* = 13.7, 8.8, 4.9 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 179.8 C=O, 176.3 C=O, 153.3, 137.0, 130.0, 129.0, 126.3, 121.5, 121.2, 119.9, 119.0, 117.2, 111.7, 110.0, 57.4 C^{13}, 39.6 C^{10}, 39.3 C^{11}, 25.8 C^{19}, 25.1 C^{12}; IR(neat): υ_{max}/cm^{-1} 3374, 3306, 2919, 1683; MS (pAPCI): 108.0 (28%), 251.1 (100%), 253.1 (68%, (M-(N(OH)Ph)+H)$^{+}$), 344.1 (13%, (M-(OH)+H)$^{+}$), 361.1 (3%, (M+H)$^{+}$); HRMS (pAPCI): calcd $C_{21}H_{20}N_3O_3$ [M+H]$^{+}$: 362.1499; observed: 362.1501.

Note H^1 NMR ran at 40 ^{o}C

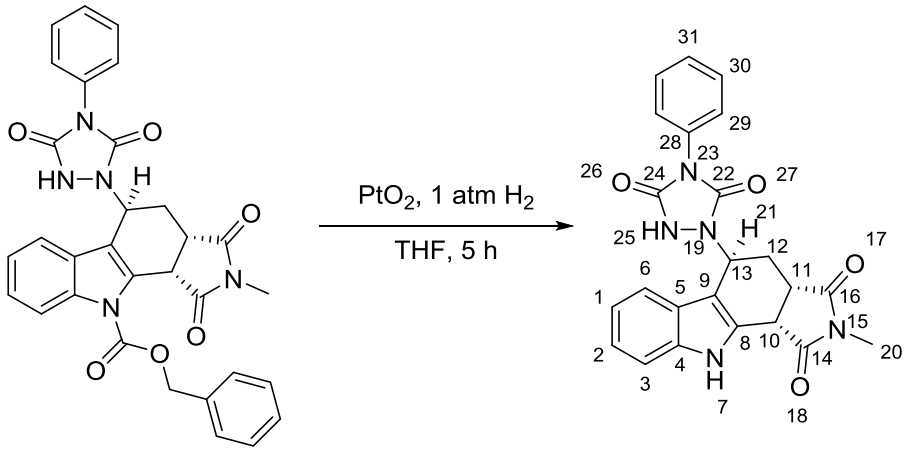
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 256**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (100 mg, 0.20 mmol), platinum (IV) oxide (45 mg, 0.20 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 7 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a yellow solid. The crude product was purified by column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2cm, silica = 16 cm) to give (3aS*,5S*,10bS*)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (87%, 65 mg, 0.17 mmol) as a pale orange powder.

Mp: 179.3 – 181.0 ^{o}C; R_f: 0.60 (Pet:EA 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.08 (1H, s, NH), 8.28 (1H, s, OH), 7.50 (1H, d, *J* = 8.0 Hz), 7.29 (1H, d, *J* = 8.1 Hz), 7.14 – 7.10 (1H, m), 6.91 – 6.87 (4H, m), 6.67 (1H, app t, *J* = 7.4 Hz), 4.29 (1H, d, *J* = 8.2 Hz, H^{10}), 4.27 (1H, app t, *J* = 3.9 Hz, H^{13}), 3.83 – 3.74 (1H, m, H^{11}), 2.81 (3H, s, H^{20}), 2.65 – 2.56 (1H, m, H^{12}), 1.98 (3H, s, H^{28}), 1.67 (1H, ddd, *J* = 13.6, 10.5, 3.9 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 180.0 C=O, 176.3 C=O, 152.2, 136.7, 130.7, 130.5, 130.3, 126.5, 126.5, 124.5, 122.4, 121.2, 119.3, 118.9, 111.7, 109.7, 58.3 C^{13}, 40.0 C^{10}, 38.6 C^{11}, 27.3 C^{12}, 25.1 C^{20}, 18.3 C^{28}; IR(neat): υ_{max}/cm^{-1} 3379, 2955, 2873, 1691; MS (pAPCI): 108.1 (98%), 251.1 (100%), 253.1 (61%, (M-(N(OH)2-Tol)+H)$^+$), 271.1 (6%, (M-(N(OH)-2-Tol)+OH_2)$^+$), 358.2 (13%, (M-OH)$^+$); HRMS (pAPCI): calcd $C_{22}H_{22}N_3O_3$ $[M+H]^+$: 376.1656; observed: 376.1658.

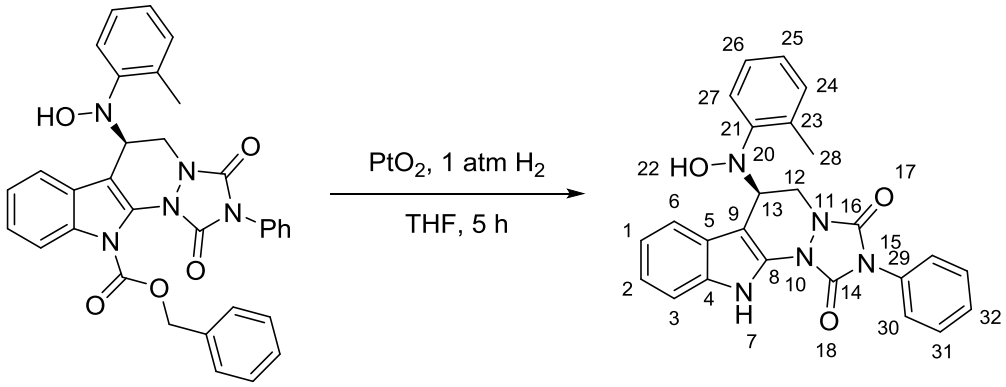
(3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3*aH*)-dione – 257**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (110 mg, 0.20 mmol), platinum (IV) oxide (46 mg, 0.20 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a yellow solid. The product was purified by trituration from DCM to give (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3*aH*)-dione (91 %, 79 mg, 0.18 mmol) as an off-white solid.

Mp: 262.4 – 264.0 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.43 (1H, s, NH), 10.67 (1H, br s, NH), 7.52 – 7.42 (4H, m), 7.39-7.37 (2H, m), 7.21 (1H , d, *J* = 7.9 Hz), 7.06 (1H, app t, *J* = 7.6 Hz), 6.94 (1H, app t, *J* = 7.5 Hz), 5.39 (1H, app t, *J* = 6.1 Hz, H^{21}), 4.33 (1H, d, *J* = 8.0 Hz, H^{10}), 3.72 (1H, app q, *J* = 6.8 Hz, H^{11}), 2.81 (3H, s, H^{20}), 2.35 – 2.31 (2H, m, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 178.9 C=O, 175.8 C=O, 152.7 C=O, 152.7 C=O, 137.2, 132.3, 130.7, 129.5, 128.5, 126.7, 125.3, 122.2, 119.9, 118.4, 112.3, 107.3, 48.1 C^{13}, 39.5 C^{10}, 38.3 C^{11}, 27.7 C^{12}, 25.3 C^{20}; IR(neat): υ_{max}/cm^{-1} 3229, 1693; MS (pAPCI): 178.1 (35%), 253.1 (100%, (M-(PTAD)+H)$^{+}$); HRMS (ASAP) calcd $C_{15}H_{13}N_2O_2$ [M-PTAD+H]$^{+}$: 253.0972; observed: 253.0969.

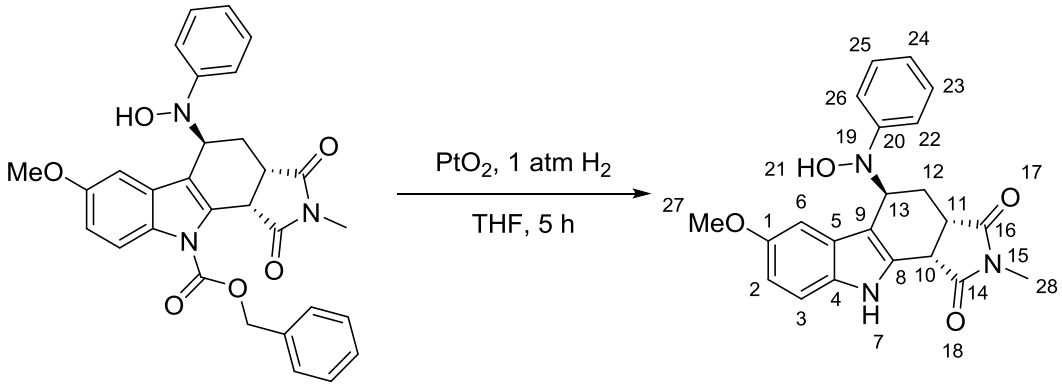
(*R)-6-(hydroxy(*o*-tolyl)amino)-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione – 258**



To a stirred Schlenk flask was added benzyl (*R**)-6-(hydroxy(*o*-tolyl)amino)-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (140 mg, 0.24 mmol), platinum (IV) oxide (55 mg, 0.24 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a white solid. The crude product was purified by trituration from DCM to give (*R**)-6-(hydroxy(*o*-tolyl)amino)-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione (44 %, 46 mg, 0.11 mmol) as a white powder.

Mp: 188.1 – 189.6 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.65 (1H, s, NH), 8.58 (1H, s, OH), 7.59 – 7.51 (5H, m), 7.47 – 7.42 (1H, m), 7.36 (1H, d, *J* = 8.0 Hz), 7.17 – 7.13 (1H, m), 6.96 – 6.91 (3H, m), 6.79 – 6.69 (2H, m), 4.69 (1H, d, *J* = 12.8 Hz, H^{12}), 4.58 (1H, br s, H^{13}), 3.62 (1H, dd, *J* = 12.8, 3.3 Hz, H^{12}), 2.06 (3H, s, H^{28}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 151.4 C^{20}, 150.4 C=O, 146.8 C=O, 134.0, 131.8, 131.2, 130.6, 129.8, 129.7, 128.9, 127.0, 126.7, 126.0, 125.1, 122.6, 121.0, 120.1, 118.1, 112.2, 92.3, 57.8 C^{13}, 43.5 C^{12}, 18.2 C^{28}; IR(neat): υ_{max}/cm^{-1} = 3426, 3380, 2950, 1712; MS (pAPCI): 108.1 (100%), 317.1 (37%, $(M-(N(OH)2-Tol)+H)^+$), 422.2 (4%, $(M-(H_2O)+H)^+$), 438.2 (6%, $(M-H)^+$); HRMS (pAPCI): calcd $C_{25}H_{20}N_5O_3$ $[M-H]^+$: 438.1561; observed: 438.1553.

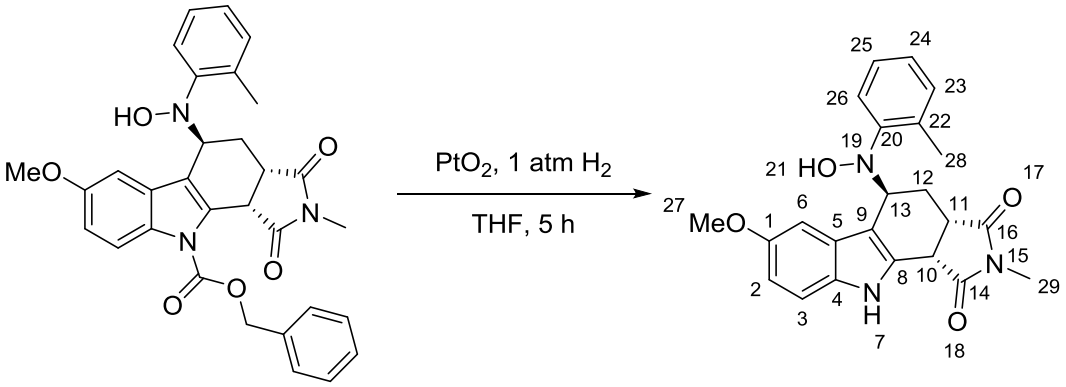
(3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 259**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (130 mg, 0.25 mmol), platinum (IV) oxide (57 mg, 0.25 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as an orange solid. The crude product was purified by column chromatography (Petrol: Ethyl acetate 3: 2, column diameter = 2cm, silica = 14 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (70%, 69 mg, 0.18 mmol) as a pale yellow powder.

Mp: 149.1 – 151.2 °C; R_f: 0.19 (Pet:EA 3:2); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 10.95 (1H, s, NH), 8.29 (1H, s, OH), 7.23 – 7.19 (2H, m), 7.18 (1H, d, *J* = 2.8 Hz), 7.14 – 7.10 (2H, m), 6.87 – 6.83 (1H, m), 6.57 (1H, dd, *J* = 8.7, 2.5 Hz), 6.51 (1H, d, *J* = 2.5 Hz), 4.83 (1H, app t, *J* = 4.8 Hz, H^{13}), 4.24 (1H, d, *J* = 8.2 Hz, H^{10}), 3.64 (1H, app td, *J* = 9.1, 6.1 Hz, H^{11}), 3.48 (3H, s, H^{27}), 2.80 (3H, s, H^{28}), 2.35 (1H, ddd, *J* = 13.7, 6.1, 4.8 Hz, H^{12}), 1.83 (1H, ddd, *J* = 13.7, 9.1, 4.8 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 179.9 C=O, 176.3 C=O, 153.6 C^1, 153.4, 132.0, 130.6, 129.0, 126.6, 121.2, 117.4, 112.3, 111.5, 109.5, 101.7, 57.7 C^{13}, 55.5 C^{27}, 39.5 C^{10}, 39.1 C^{11}, 27.1 C^{28}, 25.1 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3394, 2937, 2833, 1690; MS (pAPCI): 283.1 (100%, (M-(N(OH)Ph)+H)$^+$), 374.1 (18%, (M-(H_2O)+H)$^+$), 390.1 (2%, (M-H)$^+$), 392.2 (1%, (M+H)$^+$); HRMS (pAPCI): calcd $C_{22}H_{22}N_3O_4$ [M+H]$^+$: 392.1605; observed: 392.1597.

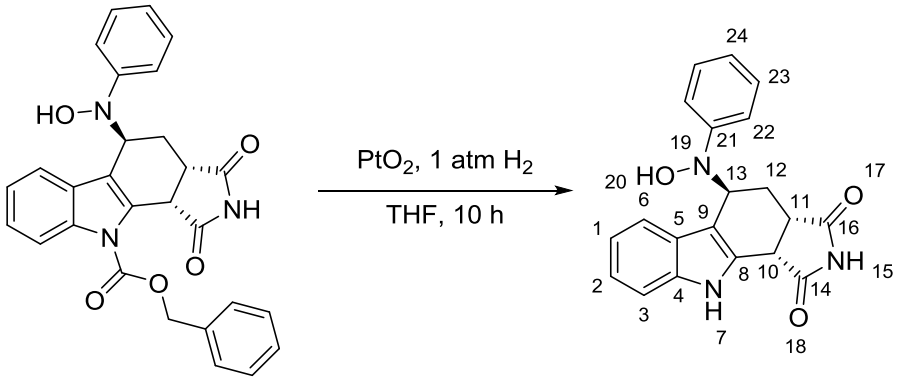
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 260**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (108 mg, 0.20 mmol), platinum (IV) oxide (46 mg, 0.20 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a white solid. The crude product was purified by column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2 cm, silica = 15 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (70%, 57 mg, 0.14 mmol) as an off white powder.

Mp: 139.7 – 142.5 °C; R_f: 0.36 (Pet:EA 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 10.89 (1H, s, NH), 8.36 (1H, s, OH), 7.52 (1H, d, *J* = 8.0 Hz), 7.15 – 7.11 (2H, d, *J* = 8.7 Hz), 6.93 – 6.85 (2H, m), 6.49 (1H, dd, *J* = 8.7, 2.4 Hz), 6.23 – 6.17 (1H, m), 4.28 (1H, d, *J* = 8.2 Hz, H^{10}), 4.22 (1H, app t, *J* = 3.8 Hz, H^{13}), 3.78 (1H, ddd, *J* = 10.8, 8.2, 6.1 Hz, H^{11}), 3.45 (3H, s, H^{27}), 2.81 (3H, s, H^{29}), 2.70 – 2.63 (1H, m, H^{12}), 1.89 (3H, s, H^{28}), 1.66 (1H, ddd, *J* = 14.1, 10.8, 3.8 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 180.1 C=O, 176.3 C=O, 153.2 C^1, 152.5 C^{20}, 131.6, 131.1, 130.7, 130.4, 126.8, 126.5, 124.7, 122.7, 112.2, 111.4, 109.2, 100.6, 58.8 C^{13}, 55.3 C^{27}, 39.6 C^{10}, 38.4 C^{11}, 28.3 C^{12}, 25.1 C^{29}, 18.1 C^{28}; IR(neat): υ_{max}/cm^{-1} = 3384, 2954, 2866, 1693; MS (pAPCI): 283.1 (100%, (M-(N(OH)2-Tol)+H)$^+$), 388.2 (34%, (M-(H_2O)+H)$^+$), 404.2 (13%, (M-H)$^+$), 406.2 (11%, (M+H)$^+$); HRMS (pAPCI): calcd $C_{23}H_{24}N_3O_4$ [M+H]$^+$: 406.1761; observed: 406.1750.

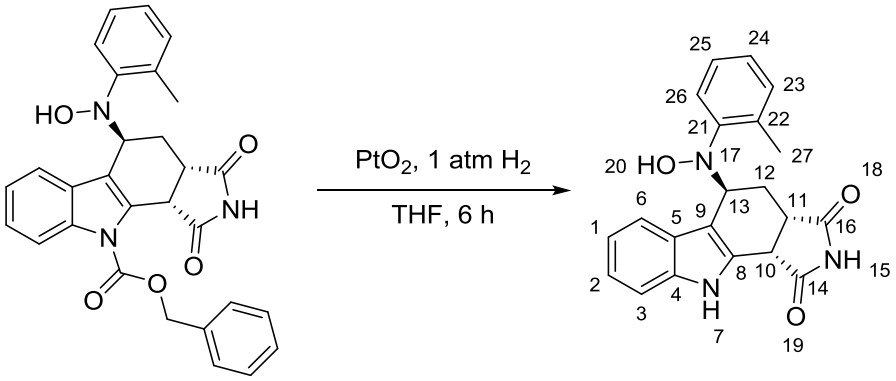
(3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 261**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (90 mg, 0.18 mmol), platinum (IV) oxide (41 mg, 0.18 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 10 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as an yellow solid, The crude product was purified by column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2 cm, silica = 14 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (41%, 26 mg, 0.07 mmol) as a yellow powder.

Mp: 150.0 – 153.1 ^{o}C; R_f: 0.33 (Pet:EA 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.22 (1H, s, NH), 11.09 (1H, s, NH), 8.24 (1H, s, OH), 7.33 (1H, d, *J* = 8.1 Hz), 7.27 – 7.23 (1H, m), 7.20 (2H, app d, *J* = 7.3 Hz), 7.12 (2H, d, *J* = 7.7 Hz), 7.00 – 6.95 (1H, m), 6.85 (1H, app t, *J* = 7.2 Hz), 6.80 (1H app t, *J* = 7.3 Hz),4.89 (1H, app t, *J* = 4.9 Hz, H^{13}), 4.25 (1H, d, *J* = 8.1 Hz, H^{10}), 3.63 – 3.52 (1H, m, H^{11}), 2.27 (1H, app dt, *J* = 13.6, 5.6 Hz, H^{12}), 1.80 (1H, ddd, *J* = 13.6, 9.2, 4.8 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 181.2 C=O, 177.6 C=O, 153.3, 137.0, 130.3, 129.0, 126.3, 121.4, 121.1, 119.9, 118.9, 117.2, 111.7, 109.9, 57.4 C^{13}, 40.9 C^{10}, 40.6 C^{11}, 25.7 C^{12}; IR(neat): υ_{max}/cm^{-1} 3302, 2924, 1706; MS (pAPCI): 108.1 (18%), 237.1 (100), 239.1 (46%, (M-(N(OH)Ph)+H)$^{+}$); HRMS (pAPCI): calcd $C_{14}H_{11}N_2O_2$ [M-(N(OH)Ph)+H]$^{+}$: 239.0815; observed: 239.0810.

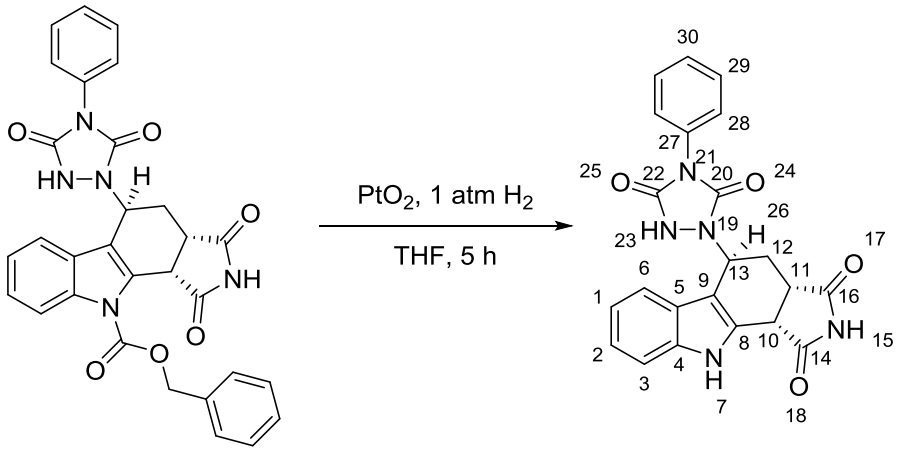
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 262**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (120 mg, 0.24 mmol), platinum (IV) oxide (54 mg, 0.24 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 6 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a yellow solid, The crude product was purified by column chromatography (Petrol: Ethyl acetate 2: 3, column diameter = 2cm, silica = 17 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (70%, 61 mg, 0.17 mmol) as an off white solid.

Mp: 149.9 – 153.2 ^{o}C; R_f: 0.52 (Pet:EA 2:3); ^{1}H NMR (400 MHz, CD_2Cl_2) δ_H 8.81 (1H, s, NH), 8.21 (1H, s, NH), 7.56 (1H, d, *J* = 8.0 Hz), 7.30 (1H, d, *J* = 7.9 Hz), 7.18 (2H, d, *J* = 6.9 Hz), 7.06 (1H, app t, *J* = 7.6 Hz), 7.03 – 6.99 (2H, m), 6.91 – 6.85 (1H, m),5.36 (1H, s, OH), 4.51 (1H, app t, *J* = 4.5 Hz, H^{13}), 4.19 (1H, d, *J* = 8.6 Hz, H^{10}), 3.80 (1H, app td, *J* = 9.3, 6.4 Hz, H^{11}), 2.74 (1H, app dt, *J* = 13.6, 5.5 Hz, H^{12}), 2.15 (3H, s, H^{27}), 1.83 (1H, ddd, *J* = 13.6, 10.1, 4.1 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 181.5 C=O, 177.6 C=O, 152.2, 136.7, 130.6, 130.5, 130.5, 126.5, 126.5, 124.5, 122.4, 121.1, 119.3, 118.9, 111.6, 109.5, 58.3 C^{13}, 55.5 C^{10}, 40.9 C^{11}, 27.3 C^{12}, 18.3 C^{27}; IR(neat): υ_{max}/cm^{-1} 3372, 3298, 1683; MS (nNSI) = 186.0 (100%), 237.1 (97%, (M-(N(OH)-2-Tol)-H)$^-$), 358.1 (35%, (M-H_2)$^-$), 394.1 (23%); HRMS (nNSI): calcd $C_{21}H_{18}N_3O_3$ [M-H]$^-$: 360.1354; observed: 360.1348.

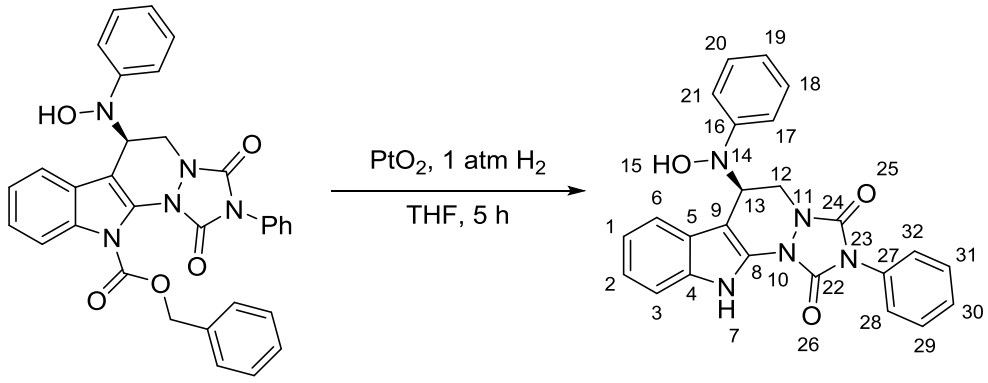
(3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 263**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (110 mg, 0.20 mmol), platinum (IV) oxide (46 mg, 0.20 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a white solid. The crude product was purified by trituration from DCM to give (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (64%, 53 mg, 0.13 mmol) as a white powder.

Mp: 212.6 – 213.9 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.39 (1H, s, NH), 11.36 (1H, s, NH), 10.66 (1H, s, OH), 7.51 – 7.44 (4H, m), 7.41 – 7.37 (2H, m), 7.23 (1H, d, *J* = 7.8 Hz), 7.07 (1H, app t, *J* = 7.5 Hz), 6.96 (1H, app t, *J* = 7.5 Hz), 5.42 (1H, app t, *J* = 6.0 Hz, H^{26}), 4.29 (1H, d, *J* = 8.0 Hz, H^{10}), 3.69 (1H, app q, *J* = 6.8 Hz, H^{11}), 2.37 – 2.21 (2H, m, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 180.3 C=O, 177.1 C=O, 152.7 C=O, 152.6 C=O, 137.2, 132.3, 131.0, 129.5, 128.5, 126.7, 125.3, 122.2, 119.9, 118.4, 112.3, 107.1, 55.5 C^{13}, 48.1 C^{10}, 40.9 C^{11}, 27.5 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3310, 3155, 3077, 1719, 1674; MS (pAPCI): 239.1 (100%, (M-(PTAD)+H)$^+$), 414.1 (2%, (M-H)$^+$); HRMS (pAPCI): calcd $C_{22}H_{16}N_5O_4$ [M-H]$^+$: 414.1197; observed: 414.1185.

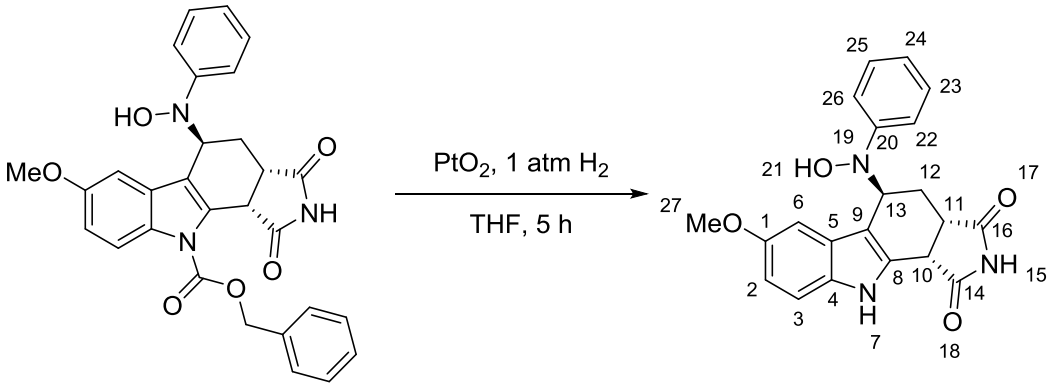
(*R)-6-(hydroxy(phenyl)amino)-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione – 264**



To a stirred Schlenk flask was added benzyl (*R**)-6-(hydroxy(phenyl)amino)-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (110 mg, 0.20 mmol), platinum (IV) oxide (46 mg, 0.20 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a yellow solid. The product was purified by trituration from DCM to give (*R**)-6-(hydroxy(phenyl)amino)-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione (65%, 55 mg, 0.13 mmol) as an off-white solid.

Mp: 174.3 – 175.2 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.63 (1H, s, NH), 8.61 (1H, s, OH), 7.54 – 7.38 (6H, m), 7.18 (2H, app t, *J* = 7.8 Hz), 7.10 (2H, app d, *J* = 7.8 Hz), 7.00 – 6.96 (2H, m), 6.91 – 6.83 (2H, m), 5.18 – 5.15 (1H, m, H^{13}), 4.48 (1H, dd, *J* = 13.0, 2.0 Hz, H^{12}), 3.77 (1H, dd, *J* = 13.0, 4.2 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 152.6 C^{16}, 149.6 C=O, 146.6 C=O, 134.2, 131.7, 129.8, 129.6, 128.9, 128.9, 127.0, 125.8, 122.3, 121.1, 120.2, 118.5, 118.3, 112.3, 92.4, 57.4 C^{13}, 43.4 C^{12}; IR(neat): υ_{max}/cm^{-1} 3431, 3054, 1698;MS (pAPCI): 317.1 (100%, (M-(N(OH)Ph)+H)$^+$), 407.1 (5%, (M-H_2O)$^+$); HRMS (pAPCI) calcd $C_{24}H_{19}N_5O_3$ [M-H]$^+$: 424.1404; observed: 424.1398.

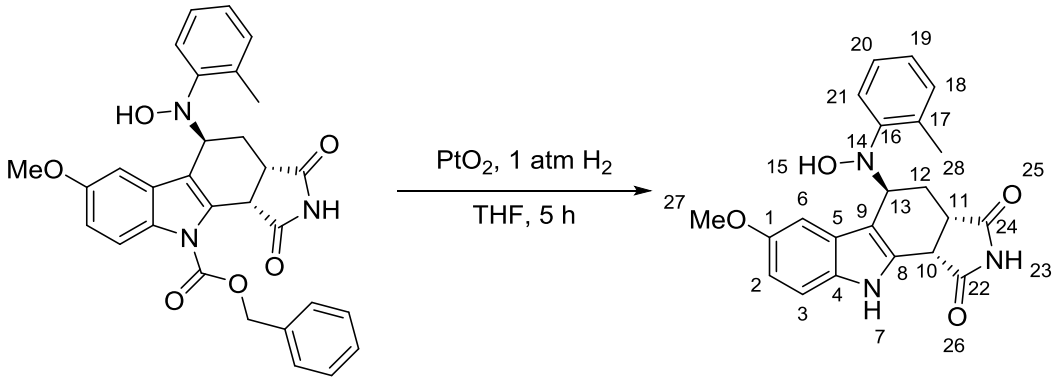
(3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 265**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (131 mg, 0.25 mmol), platinum (IV) oxide (57 mg, 0.25 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as an orange solid. The crude product was purified by column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2 cm, silica = 15 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-7-methoxy-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (60%, 57 mg, 0.15 mmol) as a pale orange powder.

Mp: 146.2 – 147.9 °C; $R_{f.}$ 0.22 (Pet:EA 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.21 (1H, s, NH), 10.89 (1H, s, NH), 8.26 (1H, s, OH), 7.23 – 7.16 (3H, m), 7.11 (1H, d, *J* = 7.8 Hz), 6.85 (1H, app t, *J* = 7.2 Hz), 6.57 (1H, dd, *J* = 8.7, 2.4 Hz), 6.52 (1H, d, *J* = 2.3 Hz), 4.84 (1H, app t, *J* = 5.2 Hz, H^{13}), 4.23 (1H, d, *J* = 9.6 Hz, H^{10}), 3.58 (1H, ddd, *J* = 14.3, 9.6, 7.5 Hz, H^{11}), 3.49 (3H, s, H^{27}), 2.33 (1H, app dt, *J* = 14.3, 5.2 Hz, H^{12}), 1.82 (1H, ddd, *J* = 14.3, 9.6, 5.2 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 181.3 C=O, 177.6 C=O, 153.6 C^1, 153.4 C^{20}, 132.0, 130.9, 129.0, 126.6, 121.2, 117.4, 112.3, 111.4, 109.4, 101.7, 57.7 C^{13}, 55.6 C^{27}, 41.0 C^{10}, 40.5 C^{11}, 27.0 C^{12}; IR(neat): υ_{max}/cm^{-1} = 3328, 3070, 2944, 1704; MS (pAPCI): 269.1 (100%, (M-(N(OH)Ph)+H)$^+$), 360.1 (3%, (M-(H_2O)+H)$^+$); HRMS (pAPCI): calcd $C_{21}H18N_3O3$ [M-(H_2O)+H]$^+$: 360.1343; observed: 360.1334.

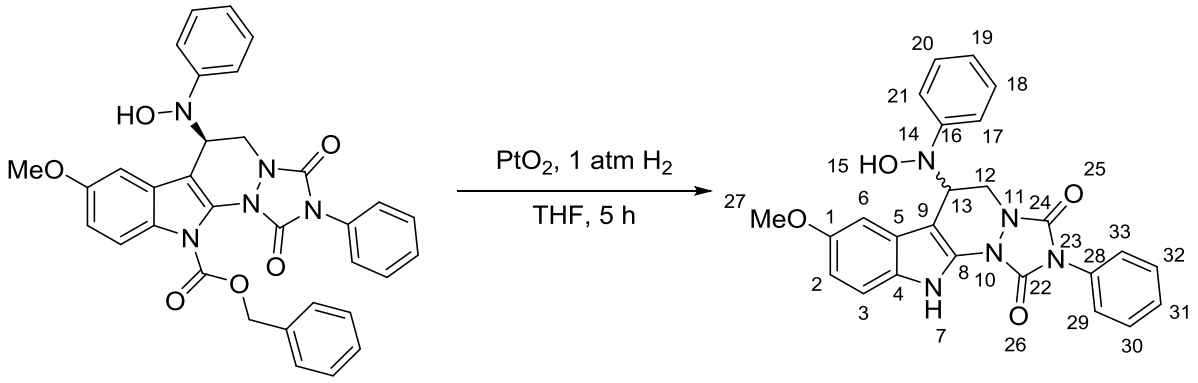
(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 266**



To a stirred Schlenk flask was added benzyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (98 mg, 0.19 mmol), platinum (IV) oxide (43 mg, 0.19 mmol) and THF (4.2 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a yellow solid. The product was purified by column chromatography (Petrol: Ethyl Acetate 1: 1, column diameter = 2 cm, silica = 20 cm) to give (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-7-methoxy-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (85%, 63 mg, 0.16 mmol) as a yellow solid.

Mp: 336.8 – 339.0 ^{o}C; R_f: 0.28 (Pet:EA 1:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.24 (1H, s, NH), 10.85 (1H, s, OH), 8.34 (1H, s), 7.52 (1H, d, *J* = 8.0 Hz), 7.15 – 7.11 (2H, m), 6. 90 – 6.88 (2H, m), 6.49 (1H, dd, *J* = 8.7, 2.4 Hz), 6.22 (1H, s), 4.27 (1H, d, *J* = 8.3 Hz, H^{10}), 4.23 (1H, app t, *J* = 4.0 Hz, H^{13}), 3.76 – 3.68 (1H, m, H^{11}), 3.46 (3H, s, H^{27}), 2.65 (1H, app dt, *J* = 13.0, 5.2 Hz, H^{12}), 1.90 (3H, s, H^{17}), 1.66 (1H, app td, *J* = 13.9, 12.5, 3.8 Hz, H^{12}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 181.6 C=O, 177.6 C=O, 153.2 C^{1}, 152.5 C^{16}, 131.6, 131.1, 130.4, 126.8, 126.5, 124.7, 122.7, 122.6, 112.2, 111.4, 109.1, 100.6, 58.8 C^{27}, 55.3 C^{13}, 40.9 C^{10}, 39.7 C^{11}, 28.2 C^{12}, 18.1 C^{28}; IR(neat): υ_{max}/cm^{-1} 3450, 3367, 1782, 1714; MS (pAPCI): 108.1 (100%), 374.1 (12%, $(M-(H_2O)+H)^+$), 390.1 (3%, $(M-H)^+$), 392.2 (2%, $(M+H)^+$; HRMS (pAPCI) calcd $C_{22}H_{22}N_3O_4$ $[M+H]^+$: 392.1605; observed: 392.1596

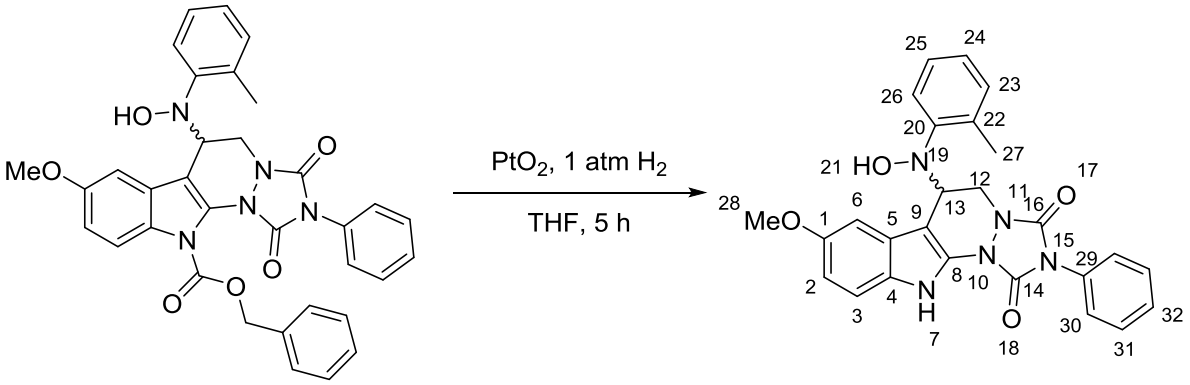
(*R)-6-(hydroxy(phenyl)amino)-8-methoxy-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione – 267**



To a stirred Schlenk flask was added benzyl (*R**)-6-(hydroxy(phenyl)amino)-8-methoxy-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (118 mg, 0.20 mmol), platinum (IV) oxide (46 mg, 0.20 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a yellow solid. The product was purified by trituration from DCM to give (*R**)-6-(hydroxy(phenyl)amino)-8-methoxy-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione (38%, 35 mg, 0.08 mmol) as an off-white solid.

Mp: 173.7 – 176.4 ^{o}C; R_f: 0.18 (Pet:EA 3:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.44 (1H, s, NH) 8.62 (1H, s, OH), 7.54 – 7.47 (4H, m), 7.44 – 7.41 (1H, m) 7.25 – 7.18 (3H, m), 7.12 (2H, d, *J* = 7.7 Hz), 6.90 (1H, app t, *J* = 6.8 Hz), 6.56 (1H, d, *J* = 8.7 Hz), 6.26 (1H, s), 5.12 (1H, br s, H^{12}), 4.51 (1H, d, *J* = 13.0 Hz, H^{12}), 3.81 (1H, dd, *J* = 13.0, 3.3 Hz, H^{12}), 3.49 (3H, s, H^{27}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 154.3 C^{1}, 152.9, 149.7, 146.4, 131.8, 130.1, 129.6, 129.6, 128.9, 128.9, 127.0, 126.3, 122.3, 118.4, 112.9, 110.7, 100.8, 92.3, 57.7 C^{13}, 55.5 C^{27}, 44.3 C^{12}; IR(neat): υ_{max}/cm^{-1} 3362, 3000, 1758, 1700; MS (pAPCI): 213.1 (70%), 347.1 (100%,(M-(N(OH)Ph)+H)$^{+}$); HRMS (pAPCI): calcd $C_{25}H_{20}N_5O_4$ [M-H]$^{+}$: 454.1510; observed: 454.1502.

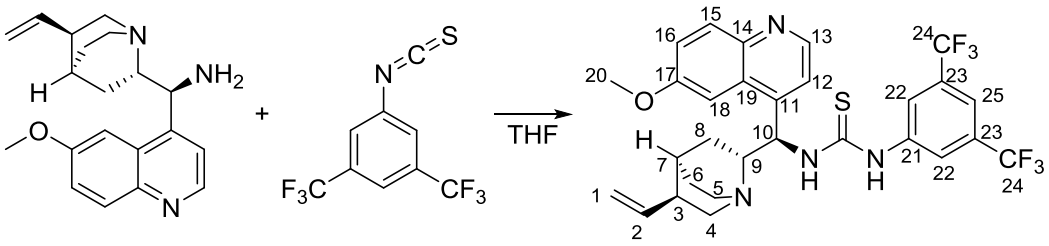
(*R)-6-(hydroxy(*o*-tolyl)amino)-8-methoxy-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione – 268**



To a stirred Schlenk flask was added benzyl (*R**)-6-(hydroxy(*o*-tolyl)amino)-8-methoxy-1,3-dioxo-2-phenyl-2,3,5,6-tetrahydro-1*H*,11*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-11-carboxylate (120 mg, 0.20 mmol), platinum (IV) oxide (46 mg, 0.20 mmol) and THF (5 mL). The resulting suspension was placed under an atmosphere of H_2 and stirred at room temperature for 5 hours. The suspension was filtered through celite and the solvent was removed under reduced pressure to leave the crude product as a white solid. The crude product was purified by trituration from DCM to (*R**)-6-(hydroxy(*o*-tolyl)amino)-8-methoxy-2-phenyl-6,11-dihydro-1*H*,5*H*-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione (64%, 53 mg, 0.13 mmol) as a white powder.

Mp: 171.9 – 173.8 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 11.44 (1H, s, NH), 8.64 (1H, s, OH), 7.59 (1H, d, *J* = 8.1 Hz), 7.54 – 7.50 (4H, m), 7.46 – 7.43 (1H, m), 7.19 (1H, d, *J* = 8.5 Hz), 7.15 (1H, d, *J* = 7.5 Hz), 6.97 – 6.91 (2H, m), 6.50 (1H, dd, *J* = 8.7, 2.2 Hz), 6.01 (1H, s), 4.73 (1H, d, *J* = 12.8 Hz, H^{13}), 4.52 (1H, s, H^{12}), 3.67 – 3.61 (1H, m, H^{12}), 3.47 (3H, s, H^{28}), 1.96 (3H, s, H^{27}); ^{13}C NMR (101 MHz, DMSO-d_6) δ_C 154.1 C^1, 151.8 C^{20}, 150.5 C=O, 146.4 C=O, 131.8, 131.6, 130.6, 130.0, 129.7, 128.9, 128.7, 127.0, 126.7, 126.5, 125.3, 122.8, 112.7, 110.7, 100.0, 91.8, 58.3 C^{12}, 55.4 C^{28}, 44.3 C^{13}, 18.0 C^{27}; IR(neat): υ_{max}/cm^{-1} = 3442, 3394, 2939 (CH), 1699 (CO); MS (pAPCI): 347.1 (68%, (M-(N(OH)2-Tol)+H)$^+$), 391.1 (100%), 452.2 (4%, (M-(H_2O)+H)$^+$), 468.2 (2%, (M-H)$^+$); HRMS (pAPCI): calcd $C_{26}H_{22}N_5O_4$ [M-H]$^+$: 468.1666; observed: 468.1658.

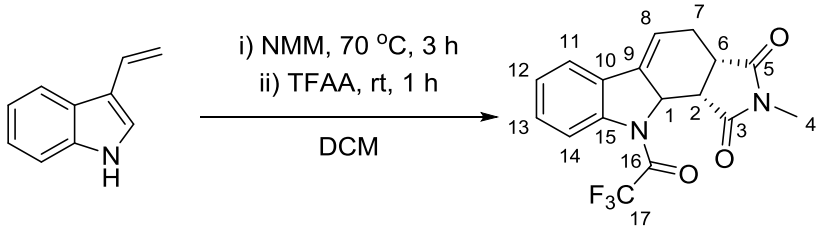
1-(3,5-bis(trifluoromethyl)phenyl)-3-((1*R*)-(6-methoxyquinolin-4-yl)((2*R*,4*R*,5*S*)-5-vinylquinuclidin-2-yl)methyl)thiourea – 291



Into a Schlenk flask was added, (1*S*)-(6-methoxyquinolin-4-yl)((2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl)methanamine (180 mg, 0.56 mmol) and THF (5 mL). To the stirred solution 3,5-bis(trifluoromethyl)phenyl isothiocyanate (0.1 mL, 0.56 mmol) was added and the solution was stirred for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow oil. The product was purified using column chromatography (Ethyl acetate : Methanol, 300 : 5, column diameter = 3 cm, silica = 25 cm) to give 1-(3,5-bis(trifluoromethyl)phenyl)-3-((1*R*)-(6-methoxyquinolin-4-yl)((2*R*,4*R*,5*S*)-5-vinylquinuclidin-2-yl)methyl)thiourea (63%, 210 mg, 0.35 mmol) as a white powder.

R_f: 0.07 (EA:MeOH 300:5); ^{1}H NMR (400 MHz, MeOD-d_4) δ_H 8.65 (1H, d, *J* = 6.0 Hz, H^{13}), 8.09 (s, 2H, H^{22}), 8.01 (1H, br, H^{18}), 7.91 (1H, d, *J* = 11.5 Hz, H^{15}), 7.57 (1H, br, H^{25}), 7.55 (1H, d, *J* = 6.0 Hz, H^{12}), 7.39 (1H, dd, *J* = 11.6, 3.3 Hz, H^{16}), 6.34 (1H, d, *J* = 13.3 Hz, H^{10}), 5.73 – 5.65 (1H, m, H^2), 5.01 – 4.97 (2H, m, H^1), 4.97 (2H, m, NH), 3.99 (3H, s, H^{20}), 3.64 – 3.60 (1H, m), 3.50 – 3.40 (1H, m), 3.33 – 3.27 (1H, m), 3.27 – 3.21 (1H, m), 2.82 – 2.72 (2H, m), 2.39 – 2.32 (1H, m), 1.72 – 1.58 (3H, m), 1.42 – 1.34 (1H, m), 0.94 – 0.85 (1H, m); ^{13}C NMR (101 MHz, MeOH-d4) δ_C 181.2 (C=S), 158.4 (C^{17}), 146.9 (C^{13}), 146.0 (C^{11}), 143.8 (C^{14}), 141.7 (C^{21}), 140.9 (C^2), 131.4 (q, C^{23}), 129.9 (C^{15}), 128.8 (C^{19}), 124.7 (q, C^{24}), 122.2 (C^{16}), 122.0 (C^{22}), 119.87 (C^{12}), 116.5 (C^{25}), 113.9 (C^1), 102.8 (C^{18}), 60.2 (C^9), 55.3 (C^4), 55.2 (C^{20}), 53.5 (C^{10}), 41.6 (C^5), 39.2 (C^3), 27.4 (C^7), 27.1 (C^8), 25.5 (C^6); IR (cm^{-1}): *v* 3200 (NH), 2976 (CH sp^2), 2890 (CH sp^3), MS (pNSI): 595.2 (100%, $(M+H)^+$), 617.2 (3%, $(M+Na)^+$); HRMS (pNSI): calcd for $C_{29}H_{29}F_6N_4OS$ $[M+H]^+$: 595.1961; observed: 595.1949.

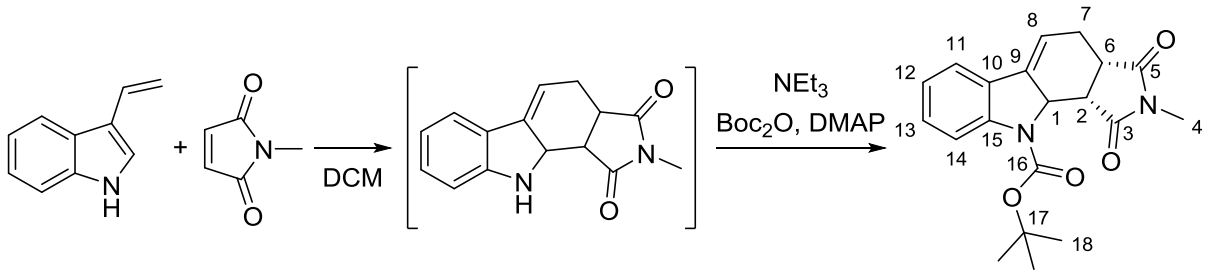
(3a*S,10b*S**)-2-methyl-10-(2,2,2-trifluoroacetyl)-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 292**



Into a Schlenk flask was added, 3-vinyl-1H-indole (200 mg, 1.40 mmol), 1-methyl-1H-pyrrole-2,5-dione (155 mg, 1.40 mmol) and DCM (5 mL). The solution was stirred at 70 °C for 3 hours before the reaction was cooled to room temperature and trifluoroacetic anhydride (1.47 mL, 7.0 mmol) was added. The solution was stirred at room temperature for 1 hour before the solution was poured into water and extracted with DCM (3 x 10 mL). The combined organic extracts were dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure to leave the crude product as a yellow oil. The crude product was purified using column chromatography (Petrol: Ethyl acetate 1: 1, column diameter = 2 cm, silica = 14 cm) to give (3a*S**,10b*S**)-2-methyl-10-(2,2,2-trifluoroacetyl)-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (92%, 452 mg, 1.29 mmol) as an off white solid.

MP: 214.3 – 216.3 °C; R_f: 0.20(Pet:EA, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.32 (d, *J* = 8.4 Hz, 0.5H), 7.44 – 7.35 (1.5H, m), 7.32 – 7.20 (1H, m), 7.15 – 7.02 (1H, m), 6.17 (1H, dd, *J* = 6.9, 3.5 Hz, H^8), 5.02 – 4.89 (1H, m, H^1), 4.30 (0.5H, app t, *J* = 8.1 Hz, H^2), 3.80 (0.5H, app t, *J* = 8.1 Hz, H^2), 3.19 (1H, app t, *J* = 7.6 Hz, H^6), 3.09 – 2.97 (1H, m, H^7), 2.71 (3H, s, H^4), 2.29 – 2.15 (1H, m, H^7); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C δ 178.8 C=O, 178.3, 174.7, 173.9, 144.9, 140.9, 137.1, 135.2, 130.5, 130.2, 127.7, 126.5, 126.0, 125.4, 121.3, 120.3, 119.1, 115.0 (C^{17}, q), 114.1, 113.5, 111.2, 77.5, 77.3, 77.1, 76.6, 61.4, 59.7, 42.0, 39.0, 38.2, 37.8, 25.1 25.1, 24.9; IR (cm^{-1}): *v* 3010 (CH), 2941 (CH), 1652 (CO); MS (pNSI): 297.1 (100%, $(M+H)^+$), 615.2 (10%, $(2M+Na)^+$); HRMS (pNSI): calcd for $C_{17}H_{17}N_2O_3$ $[M+H]^+$: 297.1234; observed: 297.1237.

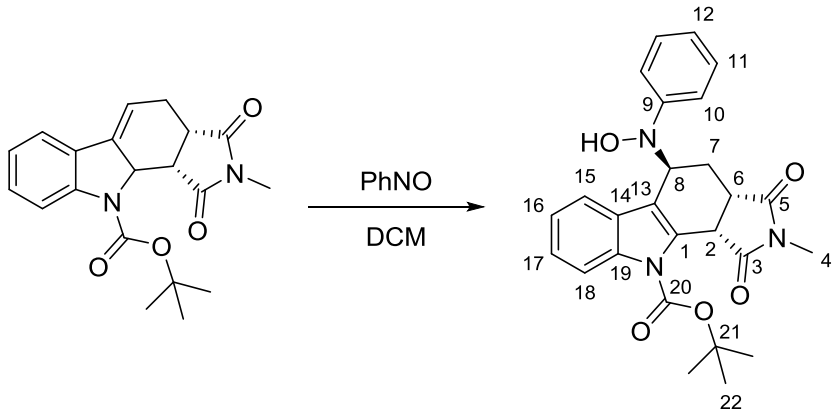
tert-butyl (3a*S,10b*S**)-2-methyl-1,3-dioxo-2,3,3a,4,10a,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 294**



In a boiling tube, 3-vinyl-1*H*-indole (100 mg, 0.70 mmol) was dissolved in DCM (3 mL). Into the stirred solution, *N*-methylmaleimide (78 mg, 0.70 mmol) was added and the sealed tube was heated at 70 ^{o}C for 2.5 hours. The solution was cooled to room temperature and di-*tert*-butyl dicarbonate (0.64 mL, 2.8 mmol), triethylamine (0.39 mL, 2.8 mmol) and DMAP (17 mg, 0.14 mmol) were added to the reaction. The reaction was stirred room temperature for 48 hours before the solution was poured into water (20 mL), and extracted with DCM (2 x 15 mL). The combined organic extracts were dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure to leave the crude product as an orange oil. The crude product was purified by column chromatography (Petrol: Diethyl ether: DCM 2: 1: 1), column diameter = 2 cm, silica = 19 cm) to give *tert*-butyl (3a*S**,10b*S**)-2-methyl-1,3-dioxo-2,3,3a,4,10a,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (57%, 142 mg, 0.40 mmol) as a yellow powder.

MP: 97.2 - 100.1 ^{o}C; R_f: 0.39 (Pet:Et_2O:DCM, 2:1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 6.14 (1H, dd, *J* = 7.5, 3.7 Hz, H^8), 4.74 (0.6H, app dt, *J* = 7.6, 3.1 Hz, H), 4.66 (0.4H, app dt, *J* = 7.8, 3.0 Hz, H^1), 4.33 (0.6H, app t, *J* = 8.2 Hz, H^2), 4.04 (0.4 H, t, *J* = 8.4 Hz, H^2), 3.22 – 3.02 (2H, m, H^7 + H^6), 2.80 (1.4H, s, H^4), 2.79 (1.6H, s, H^4), 2.27 – 2.14 (1H, m, H^7), 1.70 (5H, s, H^{18}), 1.64 (4H, s, H^{18}); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 178.9 C=O, 174.6 C=O, 137.8, 130.0, 125.9, 122.3, 120.4, 120.2, 115.9, 115.7, 111.8, 82.1 C^{17}, 59.5 C^1, 41.0 C^2, 37.8 C^6, 28.5 C^{18}, 24.9 C^4, 24.7 C^7; IR (cm^{-1}): *v* 2975 (CH), 2930 (CH), 1695 (CO); MS (pNSI): 255.1 (100%, $(M-(Boc)+H))^+$), 299.1 (47%, $(M-(C(Me_3))+H)^+$), 355.2 (20%, $(M+H)^+$), 372.2 (57%, $(M+(NH_4)^+)$), 726.4 (5%, $(2M+(NH_4)^+)$); HRMS (pNSI): calcd for $C_{20}H_{26}N_3O_4$ $[M+NH_4]^+$: 372.1918; observed: 372.1919.

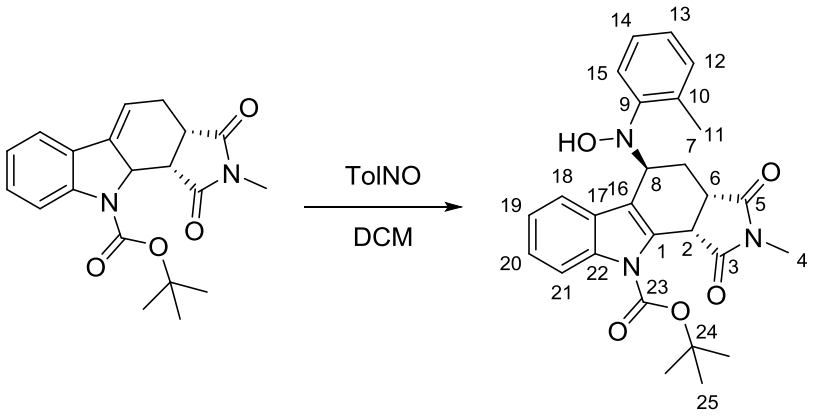
tert-butyl (3a*S,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 298**



To a stirred Schlenk flask was added *tert*-butyl (3a*S**,10b*S**)-2-methyl-1,3-dioxo-2,3,3a,4,10a,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (60 mg, 0.17 mmol), nitrosobenzene (18 mg, 0.17 mmol) and DCM (4 mL). The solution was stirred at room temperature for 4 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The crude product was purified by column chromatography (Petrol: Diethyl ether: DCM 2: 1: 1, column diameter = 1 cm, silica = 15 cm) to give tert-butyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(phenyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (47%, 37 mg, 0.08 mmol) as a yellow powder.

MP: 152.3 - 155.0 ^{o}C; R_f: 0.61 (Pet:Et_2O:DCM, 2:1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.12 (d, *J* = 8.0 Hz), 7.73 (1H, d, *J* = 7.1 Hz), 7.33 – 7.27 (3H, m), 7.22 (2H, d, *J* = 6.9 Hz), 7.18 – 7.14 (1H, m), 7.01 (1H, app t, *J* = 6.4 Hz), 5.02 (1H, d, *J* = 7.1 Hz, H^2), 4.96 (1H, br s, OH), 4.80 (1H, app t, *J* = 4.7 Hz H^8), 3.61 – 3.48 (1H, m, H^6), 2.87 (3H, s, H^4), 2.38 – 2.31 (1H, m, H^7), 2.09 – 2.03 (1H, m, H^7), 1.69 (9H, s, H^{22}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 178.3, 174.5, 150.8, 150.1, 137.1, 130.0, 129.0, 127.2, 124.7, 122.9, 122.2, 119.7, 117.6, 116.9, 115.2, 84.7 C^{21}, 57.7 C^8, 40.0 H^2, 39.2 H^6, 27.8 C^4, 24.9 C^{22}, 22.3 C^7; IR (cm^{-1}): *v* 3384 (OH), 2981 (CH), 1699 (CO); MS (pNSI): 253.1 (100%, $(M-((N(OH)Ph)(Boc))+H)^+$), 353.1 (60%, $(M-(N(OH)Ph))^+$), 360.1 (20%, $(M-(Boc)+H)^+$), 460.2 (60%, $(M-H)^+$), 462.2 (5%, $(M+H)^+$), 923.4 (4%, $(2M+H)^+$); HRMS (pNSI): calcd for $C_{26}H_{28}N_3O_5$ $[M+H]^+$: 462.2023; observed: 462.2020.

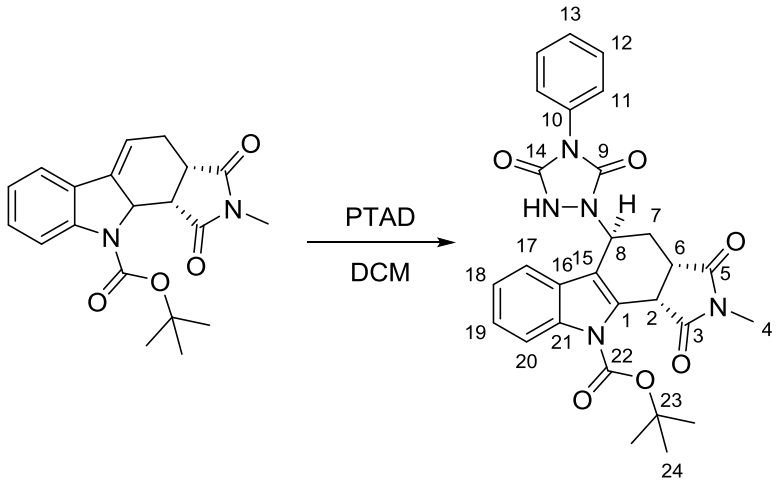
tert-butyl (3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 299**



To a stirred Schlenk flask was added *tert*-butyl (3a*S**,10b*S**)-2-methyl-1,3-dioxo-2,3,3a,4,10a,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (100 mg, 0.28 mmol), 2-methyl-1-nitrosobenzene (34 mg, 0.17 mmol) and DCM (4 mL). The solution was stirred at room temperature for 3 hours. The solvent was removed under reduced pressure to leave the crude product as a yellow solid. The crude product was purified by column chromatography (Petrol: Diethyl ether: DCM 2: 1: 1, column diameter = 1 cm, silica = 16 cm) to give *tert*-butyl (3a*S**,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (37%, 49 mg, 0.10 mmol) as an off white powder.

MP: 157.9 - 159.6 ^{o}C; R_f: 0.50 (Pet:Et_2O:DCM, 2:1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.11 (1H, d, *J* = 8.6 Hz), 7.66 (1H, d, *J* = 7.9 Hz), 7.57 (1H, d, *J* = 8.1 Hz), 7.30 – 7.24 (1H, m), 7.21 (1H, app t, *J* = 7.7 Hz), 7.16 – 7.09 (2H, m), 7.05 (1H, app t, *J* = 7.4 Hz), 5.08 (1H, d, *J* = 8.1 Hz, H^2), 4.97 (1H, br s, OH), 4.41 – 4.34 (1H, app t, *J* =5.7 Hz, H^8), 3.67 (1H, app q, *J* = 7.4 Hz, H^6), 2.91 (3H, s, H^4), 2.31 (3H, s, H^{11}), 2.59 (1H, app dt, *J* = 12.9, 6.2 Hz, H^7), 1.98 (1H, ddd, *J* = 12.9, 7.6, 4.6 Hz, H^7), 1.73 (9H, s, H^{25}); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 178.6, 174.5, 150.3, 149.2, 137.0, 131.1, 130.0, 129.8, 127.6, 126.5, 125.2, 124.7, 122.9, 121.5, 120.0, 117.6, 115.3, 84.7 C^{24}, 57.4 C^8, 40.3 C^2, 39.1 C^6, 28.2 C^{25}, 25.2 C^4, 23.9 C^7, 18.6 C^{11}; IR (cm^{-1}): *v* 3474 (OH), 2981 (CH), 2929 (CH), 1696 (CO); MS (pNSI): 253.1 (100%, (M-((N(OH)Ph)(Boc))+H)$^+$), 353.1 (72%, (M-(N(OH)2-Tol))$^+$), 374.2 (38%, (M-(Boc)+H)$^+$), 458.2 (32%, (M-(H_2O)+H)$^+$), 474.2 (62%, (M-H)$^+$), 475.2 (18%, (M+H)$^+$), 498.2 (30%, (M+Na)$^+$), 973.4 (15%, 2M+Na)$^+$); HRMS (pNSI): calcd for $C_{27}H_{30}N_3O_5$ $[M+H]^+$: 476.2180; observed: 476.2173.

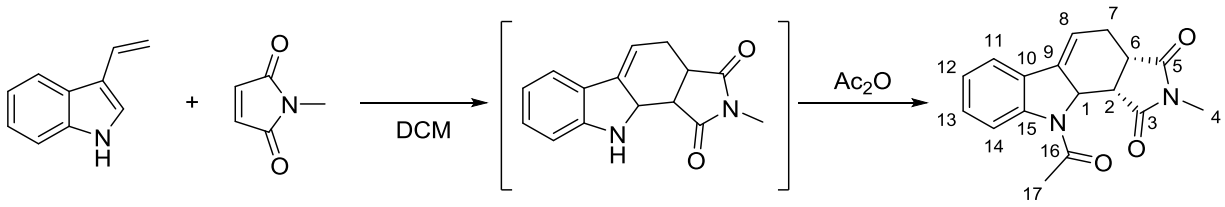
tert-butyl (3a*S,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate – 300**



To a stirred Schlenk flask was added *tert*-butyl (3a*S**,10b*S**)-2-methyl-1,3-dioxo-2,3,3a,4,10a,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (57 mg, 0.16 mmol) and DCM (3 mL). The solution was cooled to 0 ^{o}C before 4-phenyl-3*H*-1,2,4-triazole-3,5(4*H*)-dione (35 mg, 0.20 mmol) was added. The solution was stirred at 0 ^{o}C for 4 hour before the solvent was removed under reduced pressure to leave the crude product as an orange oil. The crude product was purified by column chromatography (Petrol: Diethyl ether: DCM 2: 1: 1, column diameter = 1 cm, silica = 14 cm) to *tert*-butyl (3a*S**,5*S**,10b*S**)-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-1,3-dioxo-2,3,3a,4,5,10b-hexahydropyrrolo[3,4-*a*]carbazole-10(1*H*)-carboxylate (46%, 39 mg, 0.07 mmol) as a yellow powder.

MP: 142.5 – 144.7 ^{o}C; R_f: 0.06 (Pet:Et_2O:DCM, 2:1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.08 (1H, d, *J* = 8.4 Hz), 7.52 (1H, d, *J* = 7.7 Hz), 7.43 (4H, app dt, *J* = 13.0, 7.3 Hz), 7.38 – 7.28 (2H, m), 7.21 (1H, app t, *J* = 7.5 Hz), 5.55 (1H, app t, *J* = 5.3 Hz, H^8), 5.07 (1H, d, *J* = 8.0 Hz, H^2), 3.61 – 3.55 (1H, m, H^6), 2.92 (3H, s, H^4), 2.45 (1H, dt, *J* = 13.8, 5.7 Hz, H^7), 2.24 (1H, ddd, *J* = 14.1, 8.9, 4.3 Hz, H^7), 1.68 (9H, s, H^{24}); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 177.6 C=O, 173.6 C=O, 153.7, 152.5, 150.0, 131.0, 130.8, 129.3, 128.4, 126.1, 126.0, 125.5, 125.4, 123.5, 118.8, 115.7, 113.2, 85.3 C^{23}, 47.9 C^8, 39.9 C^2, 38.4 C^6, 28.2 C^{24}, 27.4 C^4, 25.3 C^7; IR (cm^{-1}): *v* 3251 (NH), 3075 (CH), 2942 (CH), 1691 (CO); MS (pNSI): 253.1 (100%, $(M-((PTAD)(Boc))+H)^+$), 474.1 (29%, $(M-(C(Me)_3+H)^+$), 530.2 (30%, $(M+H)^+$), 547.2 (100%, $(M+NH_4)^+$), 1076.4 (14%, $(2M+(NH_4)^+$); HRMS (pNSI): calcd for $C_{28}H_{28}N_5O_6$ $[M+H]^+$: 530.2034; observed: 530.2031.

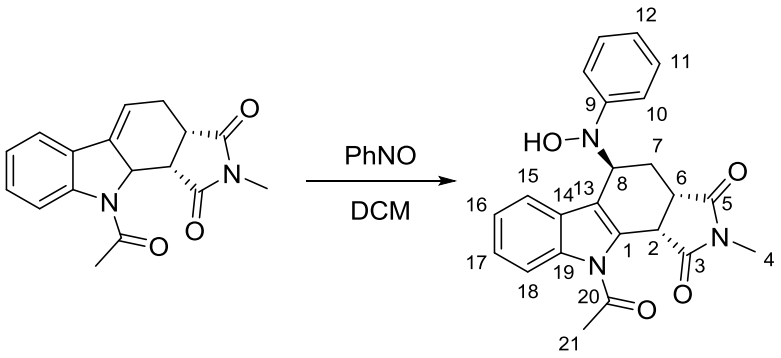
(3aS*,10bS*)-10-acetyl-2-methyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 301



In a boiling tube, 3-vinyl-1*H*-indole (0.20 g, 1.4 mmol) was dissolved in DCM (4 mL). Into the stirred solution, *N*-methylmaleimide (0.16 g, 1.4 mmol) was added and the sealed tube was heated at 70 ^{o}C for 2.5 hours. The solution was cooled to room temperature and acetic anhydride (0.66 mL, 7.0 mmol) was added to the reaction. The reaction was stirred at room temperature for 48 hours before the solution was poured into a solution of sodium bicarbonate (30 mL) and extracted with DCM (2 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to leave the crude product as a pale orange yellow solid. The crude product was purified by column chromatography (Petrol: Ethyl Acetate 3: 1 (300 mL) Petrol : Ethyl Acetate 1 : 1 (200 mL), column diameter = 2 cm, silica = 18 cm) to give (3aS*,10bS*)-10-acetyl-2-methyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (56%, 0.23 g, 0.77 mmol) as a white powder.

MP: 186.6 - 189.0 ^{o}C; R_f: 0.10 (Pet:EA, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.40 (1H, d, J = 7.6 Hz), 7.27 – 7.22 (2H, m), 7.02 – 6.98 (1H, m), 6.13 (1H, app dt, J = 7.3, 3.6 Hz, H^8), 4.86 (1H, dd, J = 6.7, 3.3 Hz, H^1), 4.37 (1H, app t, *J* = 7.9 Hz, H^2), 3.17 (1H, t, J = 7.9 Hz, H^6), 3.03 (1H, app dd, J = 14.3, 7.4 Hz, H^7), 2.73 (3H, s, H^4), 2.57 (3H, s, H^{17}), 2.21 (1H, app ddq, J = 14.3, 6.8, 3.4 Hz, H^7); ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 179.3 C=O, 175.4 C=O, 169.0 C=O, 144.0, 136.9, 130.0, 127.4, 123.2, 121.5, 114.3, 112.7, 112.7, 59.8 C^1, 40.1 C^2, 38.0 C^6, 25.8C^4, 25.2 C^7, 25.0 C^{17}; IR (cm^{-1}): *v* 3051 (CH), 2930 (CH), 1696 (CO); MS (pNSI): 297.1 (100%, $(M+H))^+$), 615.2 (10%, $(2M+Na)^+$); HRMS (pNSI): calcd for $C_{17}H_{17}N_2O_3$ $[M+H]^+$: 297.1234; observed: 297.1237.

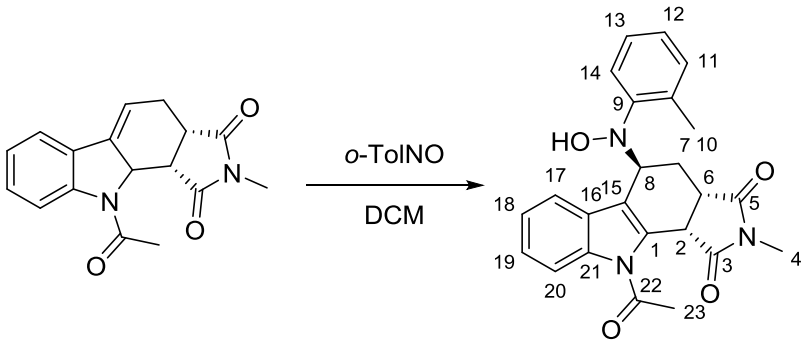
(3aS*,5S*,10bS*)-10-acetyl-5-(hydroxy(phenyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 303



To a stirred Schlenk flask was added (3aS*,10bS*)-10-acetyl-2-methyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (60 mg, 0.20 mmol), nitrosobenzene (22 mg, 0.20 mmol) and DCM (3 mL). The solution was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale red solid. The crude product was purified by column chromatography (Petrol: Diethyl ether 2: 1, column diameter = 1 cm, silica = 16 cm) to give (3aS*,5S*,10bS*)-10-acetyl-5-(hydroxy(phenyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (64%, 52 mg, 0.13 mmol) as an off white powder.

MP: 88.7 - 90.3 ^{o}C; R_f: 0.06 (Pet:Et_2O, 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.81 – 7.64 (1H, m), 7.37 – 7.25 (2H, m), 7.25 – 7.12 (2H, m), 7.04 – 7.00 (1H, m), 5.61 (1H, br s, OH), 5.03 – 4.99 (1H, m), 4.80 – 4.75 (1H, m), 3.54 – 3.42 (1H, m), 2.80 (6H, s, H^4 + H^{21}), 2.39 – 2.30 (1H, s), 2.01 – 1.92 (1H, m); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.4 (C=O), 174.7 (C=O), 171.1 (C=O), 151.0, 136.5, 130.6, 129.0, 128.0, 124.8, 123.0, 122.3, 120.5, 118.7, 117.1, 114.1, 57.8 C^8, 39.9 C^2, 39.2 C^6, 27.3 C^{21}, 24.9 C4, 22.6 C^7; IR (cm^{-1}): *v* 3359 (OH), 2956 (CH), 2859 (CH), 1695 (CO); MS (pNSI): 295.1 (34%, $(M-(N(OH)Ph))^+$), 426.1 (100%, $(M+Na)^+$), 807.3 (16%, $(2M+H)^+$), 829.3 (36%, $(2M+Na)^+$); HRMS (pNSI): calcd for $C_{23}H_{21}N_3O_4Na$ $[M+Na]^+$: 426.1424; observed: 426.1424.

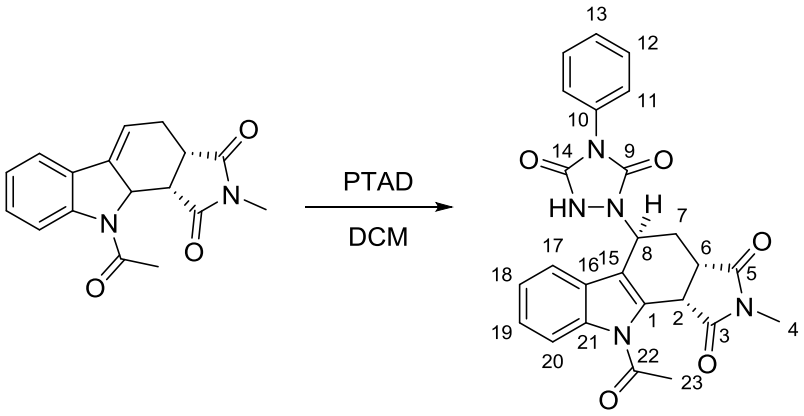
(3a*S,5*S**,10b*S**)-10-acetyl-5-(hydroxy(*o*-tolyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 304**



To a stirred Schlenk flask was added (3aS*,10bS*)-10-acetyl-2-methyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (60 mg, 0.20 mmol), 2-mehtyl-1-nitrosobenzene (25 mg, 0.20 mmol) and DCM (3 mL). The solution was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure to leave the crude product as a pale yellow solid. The crude product was purified by column chromatography (Petrol: Ethyl Acetate 3: 1, column diameter = 1 cm, silica = 14 cm) to give (3a*S**,5*S**,10b*S**)-10-acetyl-5-(hydroxy(*o*-tolyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (75%, 63 mg, 0.15 mmol) as a pale yellow powder.

MP: 104.7 - 106.5 ^{o}C; R_f: 0.50 (Pet:EA, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.68 (1H, d, *J* = 8.4 Hz), 7.57 (1H, d, *J* = 8.1 Hz), 7.54 (1H, d, *J* = 7.7 Hz), 7.27 (1H, d, *J* = 7.7 Hz), 7.20 (1H, app td, *J* = 8.1, 7.5, 2.1 Hz), 7.11 (1H, app t, *J* = 7.6 Hz), 7.08 – 7.01 (2H, m), 5.28 (1H, br s, OH), 5.18 (1H, d, *J* = 8.1 Hz, H^2), 4.38 (1H, dd, *J* = 6.0, 4.5 Hz, H^8), 3.70 (1H, app td, *J* = 8.1, 5.6 Hz, H^2), 2.65 (1H, app dt, *J* = 13.5, 5.9 Hz, H^7), 2.25 (3H, s, H^{10}), 1.88 (1H, ddd, *J* = 13.5, 8.4, 4.4 Hz, H^7); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 178.7 C=O, 174.8 C=O, 171.3 C=O, 149.5, 136.0, 130.9, 130.9, 130.1, 128.2, 126.6, 125.3, 124.6, 122.9, 121.7, 120.6, 118.1, 113.6, 57.5 C^8, 40.2 C^2, 38.9 C^6, 29.8 C^7, 27.4 C^{23}, 25.1 C^{10}; IR (cm^{-1}): *v* 3379 (OH), 3008 (CH), 2922 (CH), 1691 (CO); MS (pNSI): 295.1 (21%, (M-(N(OH)*o*-Tol))$^+$), 440.2 (100%, (M+Na)$^+$), 857.3 (16%, (2M+Na)$^+$); HRMS (pNSI): calcd for $C_{24}H_{23}N_3O_4Na$ [M+Na]$^+$: 440.1581; observed: 440.1581.

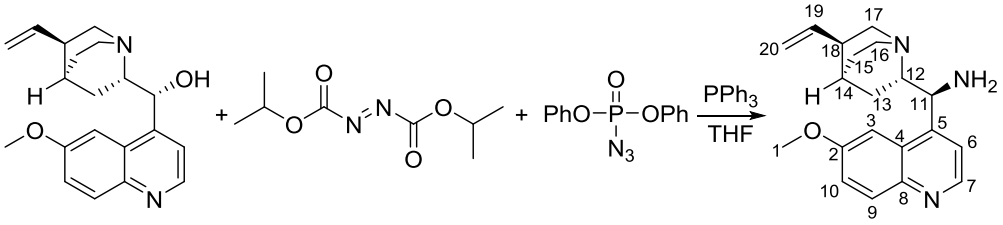
(3a*S,5*S**,10b*S**)-10-acetyl-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 305**



To a stirred Schlenk flask was added (3aS*,10bS*)-10-acetyl-2-methyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (60 mg, 0.20 mmol) and DCM (3 mL). The solution was cooled to 0 oC before 4-phenyl-3H-1,2,4-triazole-3,5(4H)-dione (35 mg, 0.20 mmol) was added. The solution was stirred at 0 oC for 1 hour before the solvent was removed under reduced pressure to leave the crude product as an orange oil. The crude product was purified by column chromatography (Petrol: Ethyl Acetate 1: 1, column diameter = 1 cm, silica = 15 cm) to give (3a*S**,5*S**,10b*S**)-10-acetyl-5-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione (57%, 54 mg, 0.11 mmol) as an orange powder.

MP: 238.4 - 240.1 ^{o}C; R_f: 0.07 (Pet:EA, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.80 (1H, br s, NH), 7.62 (1H, d, *J* = 8.4 Hz), 7.54 (1H, d, *J* = 7.7 Hz), 7.46 – 7.41 (2H, m), 7.41 – 7.36 (2H, m), 7.33 – 7.28 (2H, m), 7.29 – 7.24 (1H, m), 5.57 (1H, app t, *J* = 4.8 Hz, H^8), 5.07 (1H, d, *J* = 7.9 Hz, H^2), 3.62 – 3.49 (1H, m, H^2), 2.88 (3H, s, H^4), 2.69 (3H, s, H^{23}), 2.53 – 2.43 (1H, m, H^7), 2.09 (1H, ddd, *J* = 14.7, 10.4, 5.4 Hz, H^7); ^{13}C NMR (101 MHz, CD_2Cl_2) δ_C 177.6, 173.9, 154.5, 153.6, 152.1, 136.2, 131.6, 131.0, 129.3, 128.5, 126.7, 125.7, 125.6, 125.4, 123.5, 119.4, 113.9, 47.2, 39.9, 38.3, 28.0, 27.1, 25.2; IR (cm^{-1}): *v* 3255 (NH), 3080 (CH), 2932 (CH), 1693 (CO); MS (pNSI): 295.1 (44%, $(M-(PTAD)^+)$), 472.2 (100%, $(M+H)^+$), 489.2 (81%, $(M+(NH_4))^+$), 960.3 (35%, $(2M+(NH_4)^+)$); HRMS (pNSI): calcd for $C_{25}H_{22}N_5O_5$ $[M+H]^+$: 472.1615; observed: 472.1606.

(1*S*)-(6-methoxyquinolin-4-yl)((2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl)methanamine – 306



Into a Schlenk flask, quinine (0.50 g, 1.54 mmol), triphenylphoshine (0.49 g, 1.84 mmol) and THF (30 mL) were added. The solution was cooled to 0 ^{o}C and diisopropyl azodicarboxylate (0.36 mL, 1.84 mmol) was added. In a separate flask, a solution of diphenyl phosphoryl azide (0.39 mL, 1.84 mmol) in THF (10 mL) was prepared. The solution was added to the Schlenk flask dropwise and the resulting solution was warmed to room temperature and stirred for 18 hours. The reaction was heated to 55 ^{o}C for 2 hours before triphenylphosphine (0.49 g, 1.84 mmol) was added. Heating was maintained until gas evolution had ceased (2 hours). The reaction was cooled to room temperature and water (1 mL) was added and the solution stirred for a further 3 hours. The solvent was removed under reduced pressure and the residue was dissolved in DCM:HCl (20 mL). The aqueous phase was washed with DCM and then made alkaline with NaOH (10 mL) and washed with DCM (20 mL). The organic layer was dried with $NaSO_4$, filtered, and the solvent was removed under reduced pressure to leave the crude product as a white powder. The crude product was purified using column chromatography (Methanol: Ethyl acetate: Ammonium hydroxide, 50: 50: 1, column diameter = 3 cm, silica = 20 cm) to give (1*S*)-(6-methoxyquinolin-4-yl)((2*S*,4*S*,5*R*)-5-vinylquinuclidin-2-yl)methanamine (44%, 220 mg, 0.668 mmol) as a pale orange oil.

R_f: 0.08 (MeOH:EA, 1:1); ^{1}H NMR (400 MHz, MeOD-d_4) δ_H 8.69 (1H, d, *J* = 4.7 Hz, H^{7}), 7.97 (1H, d, *J* = 9.2 Hz, H^{9}), 7.68 (1H, br, H^{3}), 7.61 (1H, d, *J* = 4.7 Hz, H^{6}), 7.44 (1H, dd, *J* = 9.2, 2.7 Hz, H^{10}), 5.92 - 5.90 (1H, m, H^{19}), 5.06 - 4.96 (2H, m, H^{20}), 4.92 (2H, br, NH_2), 4.74 (1H, d, *J* = 10.1 Hz, H^{11}), 4.00 (3H, s, H^{1}), 3.39 - 3.14 (3H, m), 2.87 - 2.76 (2H, m), 2.38 - 2.24 (1H, m), 1.61 - 1.56 (3H, m), 1.48 - 1.40 (1H, m), 0.75 - 0.68 (1H, m); ^{13}C NMR (101 MHz, MeOD-d_4) δ_C 158.4 (C^{2}), 147.9 (C^{5}), 147.1 (C^{7}), 143.8 (C^{8}), 141.6 (C^{19}), 130.2 (C^{9}), 129.0 (C^{4}), 122.1 (C^{10}), 119.8 (C^{6}), 113.6 (C^{20}), 101.5 (C^{3}), 62.0 (C^{12}), 55.8 (C^{17}), 55.0 (C^{1}), 51.1 (C^{11}), 40.4 (C^{16}), 39.7 (C^{18}), 27.7 (C^{15}), 27.6 (C^{13}), 25.7 (C^{14}); IR (cm^{-1}): *v* 3620 (NH), 2937 (CH), 2866 (CH), 1228 (CO).

References

(1) Diels, O.; Alder, K. *Justus Liebigs Ann. Chem.* **1928**, *460*, 98.

(2) Nicolaou, K. C.; Snyder, S. A.; Montagnon, T.; Vassilikogiannakis, G. *Angew. Chem. Int. Ed* **2002**, *41*, 1668.

(3) Takao, K.; Munakata, R.; Tadano, K. *Chem. Rev.* **2005**, *105*, 4779.

(4) Cong, H.; Becker, C. F.; Elliot, S. J.; Grinstaff, M. W.; Porco Jr, J. A. *J. Am. Chem. Soc.* **2010**, *132*, 7514.

(5) Mikami, K.; Shimizu, M. *Chem. Rev.* **1992**, *92*, 1021.

(6) Snider, B. B.; Rodini, D. J.; Conn, R. S. E.; Sealfon, S. *J. Am. Chem. Soc.* **1979**, *101*, 5283.

(7) Watson, L. J.; Harrington, R. W.; Clegg, W.; Hall, M. J. *Org. Biomol. Chem.* **2012**, *10*, 6649.

(8) Lu, X. *Org. Lett.* **2004**, *6*, 2813.

(9) Thaler, W. A.; Franzus, B. *J. Org. Chem* **1964**, *29*, 2226.

(10) Adam, W.; Krebs, O. *Chem. Rev.* **2003**, *103*, 4131.

(11) Strecker, A. *Liebigs Ann.* **1850**, *75*, 27.

(12) Wang, J.; Liu, X.; Feng, X. *Chem. Rev.* **2011**, *111*, 6947.

(13) E.;, D. S.; Thorarensen, A. *J. Am. Chem. Soc.* **1997**, *119*, 125.

(14) Denmark, S. E.; Middleton, D. S. *J. Org. Chem* **1998**, *63*, 1604.

(15) Denmark, S. E.; Hurd, A. R.; Sacha, H. J. *J. Org. Chem* **1997**, *62*, 1668.

(16) Denmark, S. E.; Thorarensen, A. *Chem. Rev.* **1996**, *96*, 137.

(17) Avalos, M.; Babiano, R.; Cintas, P.; Jiminez, J. L.; Palacios, J. C.; Silva, M. A. *Chem. Comm.* **1988**, 459.

(18) Roush, W. R.; Sciotti, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 7411.

(19) Ruggeri, R. B.; Hansen, M. M.; Heathcock, C. H. *J. Am. Chem. Soc.* **1988**, *110*, 8734.

(20) Kraus, G. A.; Kim, J. *Org. Lett.* **2004**, *6*, 3115.

(21) Suzuki, K.; Inomata, K.; Endo, Y. *Org. Lett.* **2004**, *6*, 409.

(22) Nichols, D. E.; Nichols, C. D. *Chem. Rev.* **2008**, *108*, 1614.

(23) Razzaque, Z.; Heald, M. A.; Makell, L.; Beer, M. S.; Hill, R. G.; Longmore, J. *Br. J. Clin. Pharmacol.* **1999**, *47*, 75.

(24) Duffy, N. H.; Lester, H. A.; Dougherty, D. A. *ACS Chem. Biol.* **2012**, *7*, 1738.

(25) Howars-Jones, A. R.; Walsh, C. T. *J. Am. Chem. Soc.* **2006**, *128*, 12289.

(26) Merino, A.; Madden, K. R.; Lane, W. S.; Champoux, J. J.; Reinberg, D. *Nature* **1993**, *365*, 227.

(27) Bailly, C.; Riou, J.-F.; Colson, P.; Houssier, C.; Rodrigues-Pereira, E.; Prudhomme, M. *Biochemistry* **1997**, *36*, 3917.

(28) Gani, O. A. B. S. M.; Engh, R. *Nat. Prod. Rep.* **2010**, *27*, 489.

(29) Tamaoki, T.; Nomoto, H.; Takahashi, I.; Kato, Y.; Morimoto, M.; Tomita, P. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 397.

(30) Tanramluk, D.; Schreyer, A.; Pitt, W.; Blundell, T. L. *Chem. Biol. Drug Des.* **2009**, *74*, 16.

(31) Deslandes, S.; Chassaing, S.; Delfourne, E. *Mar. Drugs* **2009**, *7*, 754.

(32) Zhang, Z.; Hunter, T. *Int. J. Cancer* **2014**, *134*, 1013.

(33) Conchon, E.; Anizon, F.; Aboab, B.; Golsteyn, R. M.; Leonce, S.; Pfeiffer, B.; Prudhomme, M. *Eur. J. Med. Chem* **2008**, *43*, 282.

(34) Caballero, E.; Adeva, M.; Calderon, S.; Sahagun, H.; Tome, F.; Medarde, M.; Fernandez, J. L.; Lopez-Lazaro, M.; Ayuso, M. J. *Bioorg. Med. Chem.* **2003**, *11*, 3413.

(35) Ty, N.; Dupeyre, G.; Chabot, G. G.; Seguin, J.; Quentin, L.; Chiaroni, A.; Tilequin, F.; Scherman, D.; Michel, S.; Cachet, X. *Eur. J. Med. Chem.* **2010**, *45*, 3726.

(36) Eitel, M.; Pindur, U. *J. Org. Chem* **1990**, *55*, 5368.

(37) Grieco, P. A.; Kaufman, M. D. *J. Org. Chem* **1999**, *64*, 7586.

(38) Kaufman, M. D.; Grieco, P. A. *J. Org. Chem* **1994**, *59*, 7197.

(39) Kinsman, A. C.; Kerr, M. A. *Org. Lett.* **2001**, *3*, 3189.

(40) Le Strat, F.; Maddaluno, J. *Org. Lett.* **2002**, *4*, 2791.

(41) Cotterill, L. J.; Harrington, R. W.; Clegg, W.; Hall, M. J. *J. Org. Chem* **2010**, *75*, 4604.

(42) Lovely, C. J.; Du, H.; Sivappa, R.; Bhandari, M. R.; He, Y.; Dias, H. V. R. *J. Org. Chem* **2007**, *72*, 3741.

(43) He, Y.; Chen, Y.; Wu, H.; Lovely, C. J. *Org. Lett.* **2003**, *5*, 3623.

(44) Lovely, C. J.; Du, H.; Dias, H. V. R. *Org. Lett.* **2001**, *3*, 1319.

(45) Lovely, C. J.; Du, H.; He, Y.; Dias, H. V. R. *Org. Lett.* **2004**, *6*, 735.

(46) Poverlein, C.; Breckle, G.; Lindel, T. *Org. Lett.* **2006**, *8*, 819.

(47) Diaz-Ortiz, A.; Carillo, J. R.; Diez-Barra, E.; De La Hoz, A.; Gomez-Escalonilla, M. J.; Moreno, A.; Langa, F. *Tetrahedron* **1996**, *52*, 9237.

(48) Simon, M. M.; Arques, J. S. *Tetrahedron* **1989**, *42*, 6683.

(49) Dilley, A. S.; Romo, D. *Org. Lett.* **2001**, *3*, 1535.

(50) Dransfiled, P. J.; Wang, S.; Dilley, A.; Romo, D. *Org. Lett.* **2005**, *7*, 1679.

(51) Noland, W. E.; Xia, G.-M.; Gee, K. R.; Konkel, M. J.; Wahlstrom, M. J.; Condoluci, J. J.; Reiger, D. L. *Tetrahedron* **1996**, *52*, 4555.

(52) Pindur, U.; Lutz, G.; Fischer, G.; Schollmeyer, D.; Massa, W.; Schroder, L. *Tetrahedron* **1993**, *49*, 2863.

(53) Zhang, Y. *Tetrahedron Lett.* **2005**, *46*, 6483.

(54) Lambert, J. D.; Porter, Q. N. *Aust. J. Chem* **1981**, *34*, 1483.

(55) Yang, L.; Deng, G.; Wang, D.-X.; Huang, Z.-T.; Zhu, J.-P.; Wang, M.-X. *Org. Lett.* **2007**, *9*, 1387.

(56) Merlic, C. A.; You, Y.; McInnes, D. M.; Zechman, A. L.; Miller, M. M.; Deng, Q. *Tetrahedron* **2001**, *57*, 5199.

(57) Pindur, U.; Kim, M. H.; Rogge, M.; Massa, W.; Molinier, M. *J. Org. Chem.* **1992**, *57*, 910.

(58) Nakayama, J.; Hirashima, A. *J. Am. Chem. Soc.* **1990**, *112*, 7648.

(59) Medio-Simon, M.; de Lavida, M. J. A.; Sequlveda-Arques, J. *J. Chem. Soc., Perkin Trans. 1* **1990**, *10*, 2749.

(60) Bain, J. P. *J. Am. Chem. Soc.* **1946**, *68*, 638.

(61) Arnold, R. T.; Dowdall, J. F. *J. Am. Chem. Soc.* **1948**, *70*, 2590.

(62) Jackson, A. C.; Goldman, B. E.; Snider, B. B. *J. Org. Chem* **1984**, *49*, 2446.

(63) Snider, B. B.; Rodini, D. J.; Kirk, T. C.; Cordova, R. *J. Am. Chem. Soc.* **1982**, *104*, 555.

(64) Orfanopoulos, M.; Smonou, I.; Foote, C. S. *J. Am. Chem. Soc.* **1990**, *112*, 3607.

(65) Lovely, C. J.; Du, H.; He, Y.; Dias, H. V. R. *Org. Lett.* **2006**, *6*, 735.

(66) Wang, J.; Liu, X.; Feng, X. *Chem. Rev.* **2011**, *111*, 6947.

(67) Evans, C. G.; Gestwiki, J. E. *Org. Lett.* **2009**, *11*, 2957.

(68) Verkade, J. M. M.; van Hermet, L. J. C.; Quadfleig, P. J. L. M.; Rutjes, F. P. J. T. *Chem. Soc. Rev.* **2008**, *37*, 29.

(69) Zhu, J. *Eur. J. Org. Chem* **2003**, 1133.

(70) Tietze, L. F. *Chem. Rev.* **1996**, *96*, 115.

(71) Denmark, S. E.; Thorarensen, A. *Chem. Rev.* **1996**, *96*, 137.

(72) Bienayme, H.; Hulme, C.; Oddon, G.; Schmitt, P. *Chem. Eur. J.* **2000**, *6*, 3321.

(73) Ramachary, D. B.; Jain, S. *Org. Biomol. Chem.* **2011**, *9*, 1277.

(74) Giraud, F.; Akue-Gedu, R.; Nauton, L.; Candelon, N.; Debiton, E.; Thery, V.; Anizon, F.; Moreau, P. *Eur. J. Med. Chem* **2012**, *56*, 225.

(75) Dulon, M.; Haamann, F.; Peters, C.; Schablon, A.; Nienhaus, A. *BMC Infectious Diseases* **2011**, *11*, 138.

(76) Kumarasamy, K. K.; Toleman, M. A.; Walsh, T. R.; Bagaria, J.; Butt, F.; Balakrishnam, R.; Chaudhary, U.; Doumith, M.; Giske, C. G.; Irfan, S.; Krishnan, P.; Kumar, A. V.; Maharjan, S.; Mushtaq, S.; Noorie, T.; Paterson, D. L.; Pearson, A.; Perry, C.; Pike, R.; Rao, B.; Ray, U.; Sarma, J. B.; Sharma, M.; Sheridan *The Lancet Infectious Diseases* **2010**, *10*, 597.

(77) Linder, R. 2013 Unpublished work.

(78) Zhu, D.; Liu, Q.; Luo, B.; Chen, M.; Pi, R.; Huang, P.; S., W. *Adv. Synth. Catal.* **2013**, *355*, 2172.

(79) Nyasse, B.; Grehn, L.; Ragnarsson, U. *Chem. Comm.* **1997**, *11*, 1017.

(80) MacKay, J. A.; Bishop, R. L.; Rawal, V. H. *Org. Lett.* **2005**, *7*, 3421.

(81) Reactions performed by Morton, S.

(82) MIC experiments performed by Kepplinger, B.

(83) Wissner, A.; Fraser, H. L.; Ingalls, C. L.; Dushin, R. G.; Floyd, M. B.; Cheung, K.; Nittoli, T.; Ravi, M. R.; Tan, X.; Loganzo, F. *Bioorg. Med. Chem.* **2007**, *15*, 3635.

(84) Schirmer, A.; Kennedy, J.; Murli, S.; Reid, R.; Santi, D. V. *PNAS* **2006**, *103*, 4234.

(85) Pan, Z.; Scheerens, H.; Shy-Jiann, L.; Schultz, B. E.; Sprengler, P. A.; Burrill, L. C.; Mendonca, R. V.; Sweeney, M. D.; Scott, K. C. K.; Grothaus, P. G.; Jeffery, D. A.; Spoerke, J. M.; Honigberg, L. A.; Young, P. R.; Dalrymple, S. A.; Palmer, J. T. *ChemMedChem* **2007**, *2*, 58.

(86) Kwak, E. L.; Sordella, R.; Bell, D. W.; Gordin-Heymann, N.; Okimoto, R. A.; Brannigan, B. W.; Harris, P. L.; Driscoll, D. R.; DFidias, P.; Lynch, T. J.; Rabindran, S. K.; McGinnis, J. P.; Wissner, A.; Sharma, S. V.; Isselbacher, K. J.; Settleman, J.; Haber, D. A. *PNAS* **2005**, *102*, 7665.

(87) Felip, E.; Snantarpia, M.; Rosell, R. *Expert Opin. Emerging Drugs* **2007**, *12*, 449.

(88) Fousteris, M. A.; Papakyriakou, A.; Koutsourea, A.; Manioudaki, M.; Lampropoulou, E.; Papadimitriou, E.; Stpyroulias, G. A.; Nikolaropoulos, S. S. *J. Med. Chem* **2008**, *51*, 1048.

(89) Zhang, J.; Yang, P. L.; Gray, N. S. *Nature Reviews Cancer* **2009**, *9*, 28.

(90) Davis, M. I.; Hunt, J. P.; Herrgard, S.; Ciceri, P.; Wodicka, L. M.; Pallares, G.; Hocker, M.; TReiber, D. K.; Zarrinkar, P. P. *Nat. Biotechnol.* **2011**, *29*, 1046.

(91) Siddiquee, K. A.; Arauzo-Bravo, M. J.; Shimizu, K. *FEMS Microbiol. Lett.* **2004**, *235*, 25.

(92) Shen, T. L.; Park, A. Y.; Peng, X.; Jang, I.; Koni, P.; Flavell, R. A.; Gu, H.; Guan, J. L. *J. Cell Biol.* **2005**, *169*, 941.

(93) Aleman, J.; Cabrera, S. *Chem. Soc. Rev.* **2013**, *42*, 774.

(94) Wilson, R. M.; Jen, W. S.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2005**, *127*, 11616.

(95) Thayumanavan, R.; Dhevalapally, B.; Sakthivel, K.; Tanaka, F.; Barbas III, C. F. *Tetrahedron Lett.* **2002**, *43*, 3817.

(96) Unni, A. K.; Yamamoto, H.; Takenaka, N.; Rawal, V. H. *J. Am. Chem. Soc.* **2005**, *127*, 1336.

(97) Jia, Z.-J.; Jiang, H.; Li, J.-L.; Gschwend, B.; Li, Q.-Z.; Yin, X.; Grouleff, J.; Chen, Y.-C.; Jorgensen, K. A. *J. Am. Chem. Soc.* **2011**, *133*, 5053.

(98) Ahrendt, K. A.; Borths, C. J.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2000**, *122*, 4243.

(99) Enders, D.; Joie, C.; Deckers, K. *Chem. Eur. J.* **2013**, *19*, 10818.

(100) Jui, N. T.; Lee, E. C. Y.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2010**, *132*, 10015.

(101) Jui, N. T.; Garber, J. A. O.; Finelli, F. G.; MacMillan, D. W. C. *J. Am. Chem. Soc.* **2012**, *134*, 11400.

(102) Tan, B.; Hernandez-Torres, G.; Barbas III, C. F. *J. Am. Chem. Soc.* **2011**, *133*, 12354.

(103) Gioia, C.; Hauville, A.; Bernardi, L.; Fini, F.; Ricci, A. *Angew. Chem. Int. Ed* **2008**, *47*, 9236.

(104) Gioia, C.; Bernardi, L.; Ricci, A. *Synthesis* **2010**, *1*, 161.

(105) Vakulya, B.; Varga, S.; Csampai, A.; Soos, T. *Org. Lett.* **2005**, *7*, 1967.

(106) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Advanced Drug Delivery Reviews* **2001**, *46*, 3.

(3a*S,10b*S**)-2methyl-10-tosyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione - 148**

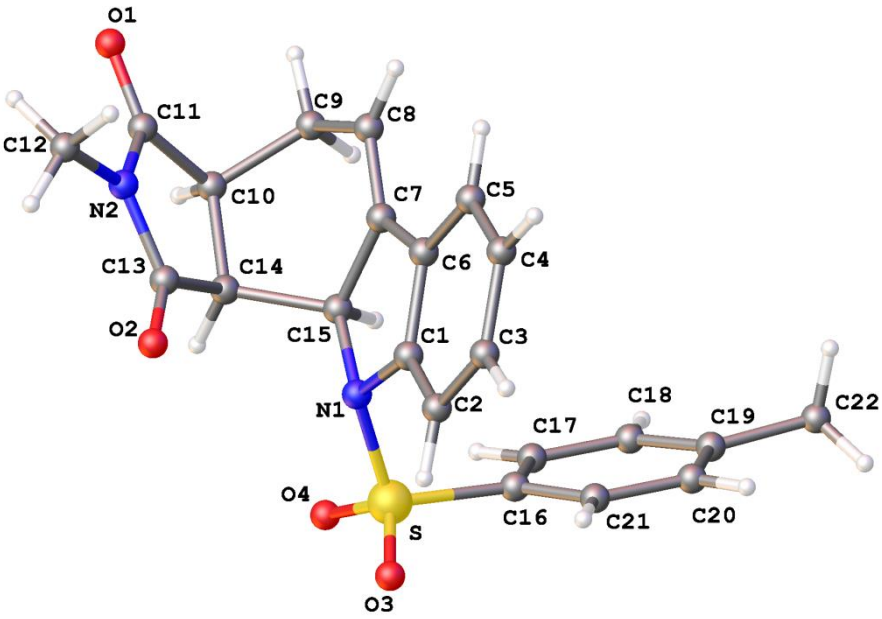


Table 1. Crystal data and structure refinement for mjh80.

Identification code	mjh80	
Chemical formula (moiety)	$C_{22}H_{20}N_2O_4S$	
Chemical formula (total)	$C_{22}H_{20}N_2O_4S$	
Formula weight	408.46	
Temperature	150(2) K	
Radiation, wavelength	MoK□, 0.71073 Å	
Crystal system, space group	monoclinic, $P12_1/n1$	
Unit cell parameters	a = 10.5141(8) Å	□ = 90°
	b = 17.9454(11) Å	□ = 113.865(9)°
	c = 11.0976(8) Å	□ = 90°
Cell volume	1914.9(2) $Å^3$	
Z	4	
Calculated density	1.417 g/cm^3	
Absorption coefficient □	0.202 mm^{-1}	
F(000)	856	
Crystal colour and size	colourless, 0.30 × 0.30 × 0.30 mm^3	
Reflections for cell refinement	3401 (□ range 3.0 to 28.4°)	
Data collection method	Xcalibur, Atlas, Gemini ultra thick-slice □ scans	
□ range for data collection	3.0 to 28.5°	
Index ranges	h –13 to 10, k –23 to 21, l –14 to 13	
Completeness to □ = 25.0°	99.8 %	
Reflections collected	10008	
Independent reflections	4083 (R_{int} = 0.0256)	
Reflections with F^2>2□	3395	

Absorption correction	semi-empirical from equivalents
Min. and max. transmission	0.9419 and 0.9419
Structure solution	direct methods
Refinement method	Full-matrix least-squares on F^2
Weighting parameters a, b	0.0407, 1.1952
Data / restraints / parameters	4083 / 0 / 265
Final R indices [F^2>2□]	R1 = 0.0408, wR2 = 0.0939
R indices (all data)	R1 = 0.0522, wR2 = 0.1012
Goodness-of-fit on F^2	1.040
Extinction coefficient	0.0023(7)
Largest and mean shift/su	0.000 and 0.000
Largest diff. peak and hole	0.35 and –0.43 e $Å^{-3}$

(3a*S,10a*S**,10b*S**)-2-phenyl-10-tosyl-4,10,10a,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 149**

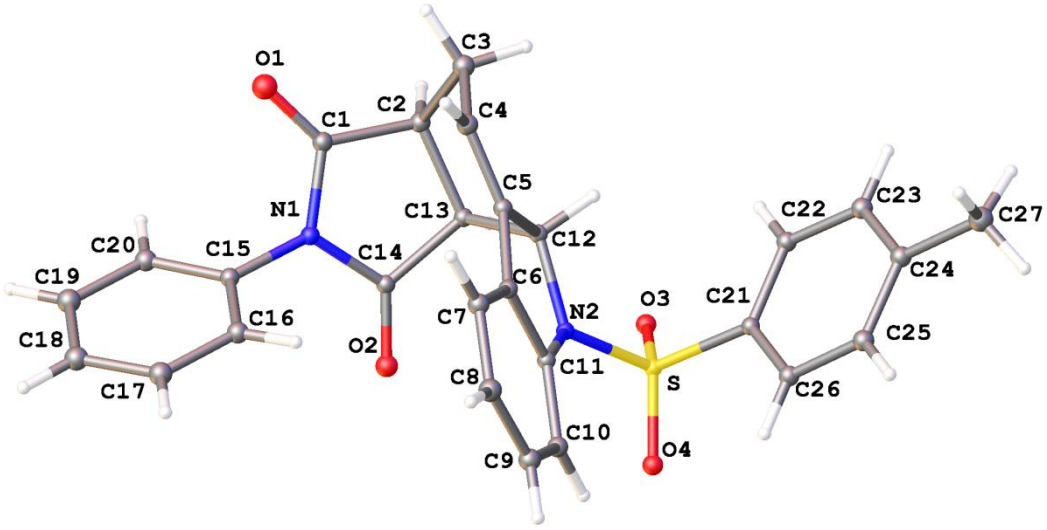


Table 1. Crystal data and structure refinement for mjh120022.

Identification code	mjh120022	
Chemical formula (moiety)	$C_{27}H_{22}N_2O_4S$	
Chemical formula (total)	$C_{27}H_{22}N_2O_4S$	
Formula weight	470.53	
Temperature	150(2) K	
Radiation, wavelength	MoK□, 0.71073 Å	
Crystal system, space group	monoclinic, $P12_1/c1$	
Unit cell parameters	a = 10.5762(6) Å	□ = 90°
	b = 20.2985(8) Å	□ = 116.781(7)°
	c = 11.4519(6) Å	□ = 90°
Cell volume	2194.79(19) Å^3	
Z	4	
Calculated density	1.424 g/cm^3	
Absorption coefficient □	0.187 mm^{-1}	
F(000)	984	
Crystal colour and size	colourless, 0.30 × 0.30 × 0.20 mm^3	
Reflections for cell refinement	4397 (□ range 2.9 to 28.4°)	
Data collection method	Xcalibur, Atlas, Gemini ultra thick-slice □ scans	
□ range for data collection	3.0 to 28.5°	
Index ranges	h –10 to 14, k –21 to 26, l –15 to 15	
Completeness to □ = 25.0°	99.8 %	
Reflections collected	13075	
Independent reflections	4661 (R_{int} = 0.0310)	
Reflections with F^2>2□	3757	
Absorption correction	semi-empirical from equivalents	
Min. and max. transmission	0.9461 and 0.9636	
Structure solution	direct methods	
Refinement method	Full-matrix least-squares on F^2	

Weighting parameters a, b	0.0413, 0.9706
Data / restraints / parameters	4661 / 0 / 309
Final R indices [F^2>2□]	R1 = 0.0410, wR2 = 0.0901
R indices (all data)	R1 = 0.0566, wR2 = 0.0993
Goodness-of-fit on F^2	1.040
Extinction coefficient	0.0022(6)
Largest and mean shift/su	0.000 and 0.000
Largest diff. peak and hole	0.32 and −0.48 e $Å^{-3}$

2-phenyl-11-tosyl-11,11a-dihydro-1H,5H-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-*b*]indole-1,3(2*H*)-dione - 151

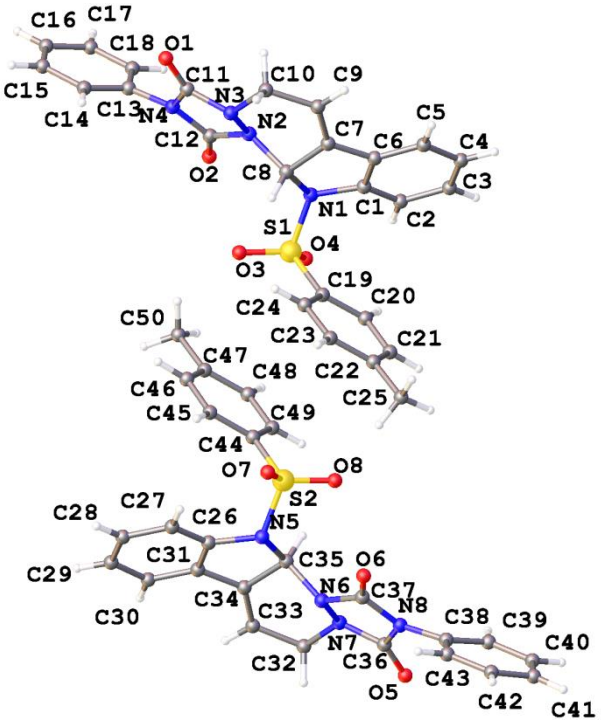


Table 1. Crystal data and structure refinement for mjh105.

Identification code	mjh105	
Chemical formula (moiety)	$C_{25}H_{20}N_4O_4S \cdot 0.5CH_2Cl_2$	
Chemical formula (total)	$C_{25.50}H_{21}ClN_4O_4S$	
Formula weight	514.97	
Temperature	150(2) K	
Radiation, wavelength	CuK□, 1.54178 Å	
Crystal system, space group	monoclinic, $P2_1/c$	
Unit cell parameters	a = 6.2586(2) Å	□ = 90°
	b = 43.8481(9) Å	□ = 93.143(2)°
	c = 17.0987(3) Å	□ = 90°
Cell volume	4685.3(2) $Å^3$	
Z	8	
Calculated density	1.460 g/cm^3	
Absorption coefficient □	2.634 mm^{-1}	
F(000)	2136	
Crystal colour and size	colourless, 0.30 × 0.30 × 0.10 mm^3	
Reflections for cell refinement	5338 (□ range 2.0 to 62.3°)	
Data collection method	Xcalibur, Atlas, Gemini ultra thick-slice □ scans	
□ range for data collection	2.0 to 62.3°	
Index ranges	h –3 to 6, k –41 to 49, l –19 to 19	
Completeness to □ = 62.3°	96.9 %	
Reflections collected	13837	
Independent reflections	7206 (R_{int} = 0.0228)	
Reflections with F^2>2□	5704	

Absorption correction	semi-empirical from equivalents
Min. and max. transmission	0.5055 and 0.7787
Structure solution	direct methods
Refinement method	Full-matrix least-squares on F^2
Weighting parameters a, b	0.0522, 9.6468
Data / restraints / parameters	7206 / 0 / 641
Final R indices [F^2>2□]	R1 = 0.0566, wR2 = 0.1451
R indices (all data)	R1 = 0.0705, wR2 = 0.1532
Goodness-of-fit on F^2	1.086
Extinction coefficient	0.00006(3)
Largest and mean shift/su	0.001 and 0.000
Largest diff. peak and hole	0.40 and –0.41 e $Å^{-3}$

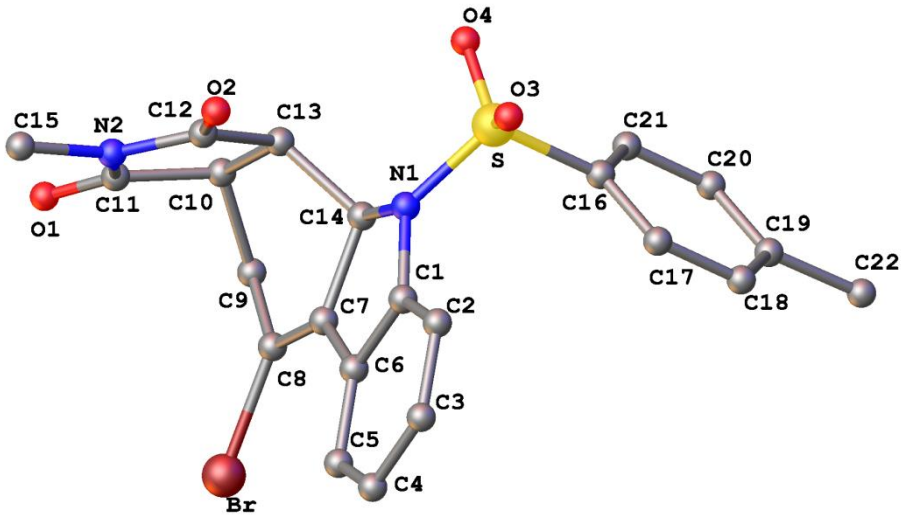


Table 1. Crystal data and structure refinement for mjh12.

Identification code	mjh120038	
Chemical formula (moiety)	$C_{22}H_{19}BrN_2O_4S{\cdot}0.5CH_2Cl_2$	
Chemical formula (total)	$C_{22.50}H_{20}BrClN_2O_4S$	
Formula weight	529.83	
Temperature	150(2) K	
Radiation, wavelength	CuK□, 1.54178 Å	
Crystal system, space group	monoclinic, $P12_1/c1$	
Unit cell parameters	a = 6.5785(2) Å	□ = 90°
	b = 25.1833(8) Å	□ = 93.607(2)°
	c = 13.7502(4) Å	□ = 90°
Cell volume	2273.46(12) $Å^3$	
Z	4	
Calculated density	1.548 g/cm^3	
Absorption coefficient □	4.681 mm^{-1}	
F(000)	1076	
Crystal colour and size	colourless, 0.30 × 0.30 × 0.04 mm^3	
Reflections for cell refinement	5759 (□ range 1.8 to 67.2°)	
Data collection method	Xcalibur, Atlas, Gemini ultra thick-slice □ scans	
□ range for data collection	3.5 to 66.7°	
Index ranges	h –7 to 7, k –26 to 29, l –16 to 10	
Completeness to □ = 33.5°	100.0 %	
Reflections collected	13891	
Independent reflections	3990 (R_{int} = 0.0325)	
Reflections with F^2>2□	3461	
Absorption correction	semi-empirical from equivalents	
Min. and max. transmission	0.3341 and 0.8349	
Structure solution	direct methods	
Refinement method	Full-matrix least-squares on F^2	
Weighting parameters a, b	0.0758, 3.2435	
Data / restraints / parameters	3990 / 2 / 298	

Final R indices [F^2>2□]	R1 = 0.0447, wR2 = 0.1252
R indices (all data)	R1 = 0.0518, wR2 = 0.1322
Goodness-of-fit on F^2	1.056
Largest and mean shift/su	0.001 and 0.000
Largest diff. peak and hole	1.10 and −0.50 e $Å^{-3}$

6-(3,5-dioxo-4-phenyl-1,2,4-triazolidin-1-yl)-2-phenyl-11-tosyl-6,11-dihydro-1H,5H-[1,2,4]triazolo[1',2':1,2]pyridazino[3,4-b]indole-1,3(2*H*)-dione – 191

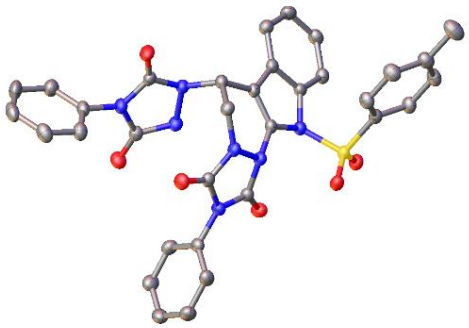


Table 1 Crystal data and structure refinement for mjh140002.

Identification code	mjh140002
Empirical formula	$C_{33}H_{25}N_7O_6S$
Formula weight	647.66
Temperature/K	150.00(10)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	13.1236(3)
b/Å	20.8432(5)
c/Å	12.0391(2)
α/°	90
β/°	100.991(2)
γ/°	90
Volume/Å^3	3232.74(13)
Z	4
ρ_{calc}mg/mm^3	1.331
m/mm^{-1}	1.360
F(000)	1344.0
Crystal size/mm^3	0.25 × 0.1 × 0.06
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection	6.862 to 132.494°
Index ranges	-14 ≤ h ≤ 15, -24 ≤ k ≤ 24, -14 ≤ l ≤ 10
Reflections collected	23327
Independent reflections	5646 [R_{int} = 0.0383, R_{sigma} = 0.0297]
Data/restraints/parameters	5646/0/428
Goodness-of-fit on F^2	1.036
Final R indexes [I>=2σ (I)]	R_1 = 0.0357, wR_2 = 0.0863
Final R indexes [all data]	R_1 = 0.0464, wR_2 = 0.0927
Largest diff. peak/hole / e Å^{-3}	0.35/-0.36

(3a*S,5*S**,10b*S**)-5-(hydroxy(*o*-tolyl)amino)-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2*H*,3a*H*)-dione – 200**

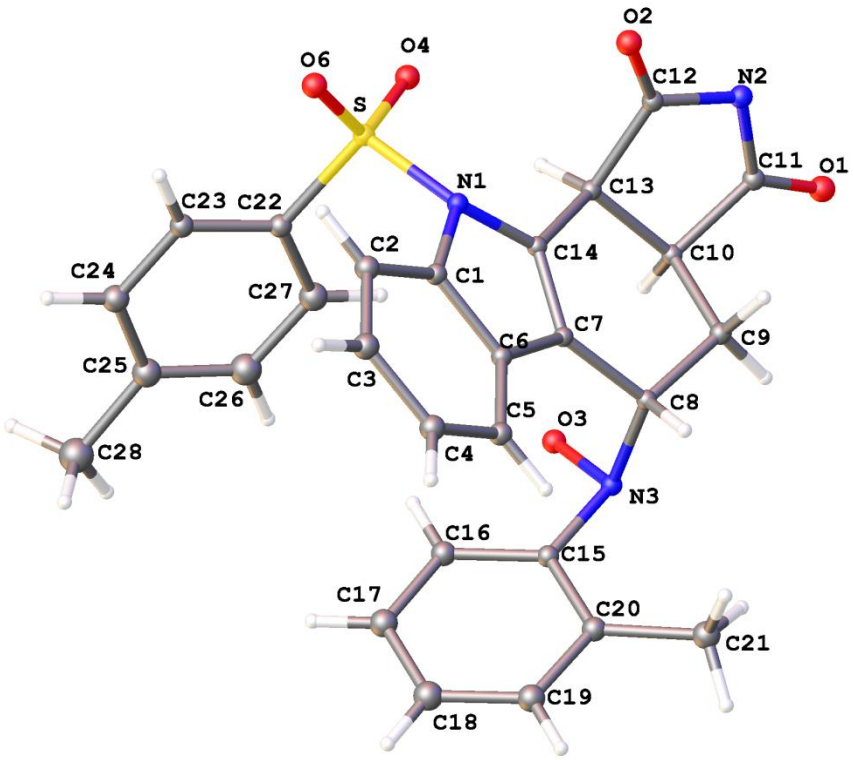


Table 1. Crystal data and structure refinement for mjh12.

Identification code	mjh120026	
Chemical formula (moiety)	$C_{28}H_{23}N_3O_5S \cdot CH_2Cl_2$	
Chemical formula (total)	$C_{29}H_{25}Cl_2N_3O_5S$	
Formula weight	598.48	
Temperature	150(2) K	
Radiation, wavelength	CuK□, 1.54178 Å	
Crystal system, space group	triclinic, $P\bar{1}$	
Unit cell parameters	a = 10.0614(5) Å	□ = 104.231(4)°
	b = 10.2208(5) Å	□ = 95.071(4)°
	c = 15.2649(7) Å	□ = 109.240(4)°
Cell volume	1411.79(12) $Å^3$	
Z	2	
Calculated density	1.408 g/cm^3	
Absorption coefficient □	3.133 mm^{-1}	
F(000)	620	
Reflections for cell refinement	7995 (□ range 3.0 to 66.9°)	
Data collection method	Xcalibur, Atlas, Gemini ultra thick-slice □ scans	
□ range for data collection	3.0 to 67.0°	
Index ranges	h –11 to 11, k –11 to 12, l –17 to 18	
Completeness to □ = 67.0°	98.6 %	
Reflections collected	13046	
Independent reflections	4954 (R_{int} = 0.0293)	
Reflections with F^2>2□	4280	

Absorption correction	semi-empirical from equivalents
Min. and max. transmission	0.86739 and 1.00000
Structure solution	direct methods
Refinement method	Full-matrix least-squares on F^2
Weighting parameters a, b	0.0620, 1.1284
Data / restraints / parameters	4954 / 0 / 336
Final R indices [F^2>2□]	R1 = 0.0485, wR2 = 0.1283
R indices (all data)	R1 = 0.0546, wR2 = 0.1315
Goodness-of-fit on F^2	1.093
Largest and mean shift/su	0.001 and 0.000
Largest diff. peak and hole	0.67 and −0.37 e $Å^{-3}$

(3a*S,5*S**,10b*S**)-5-((*R*)-hydroxy(perfluorophenyl)methyl)-2-methyl-10-tosyl-4,5,10,10b-tetrahydropyrrolo[3,4-*a*]carbazole-1,3(2*H*,3a*H*)-dione – 215**

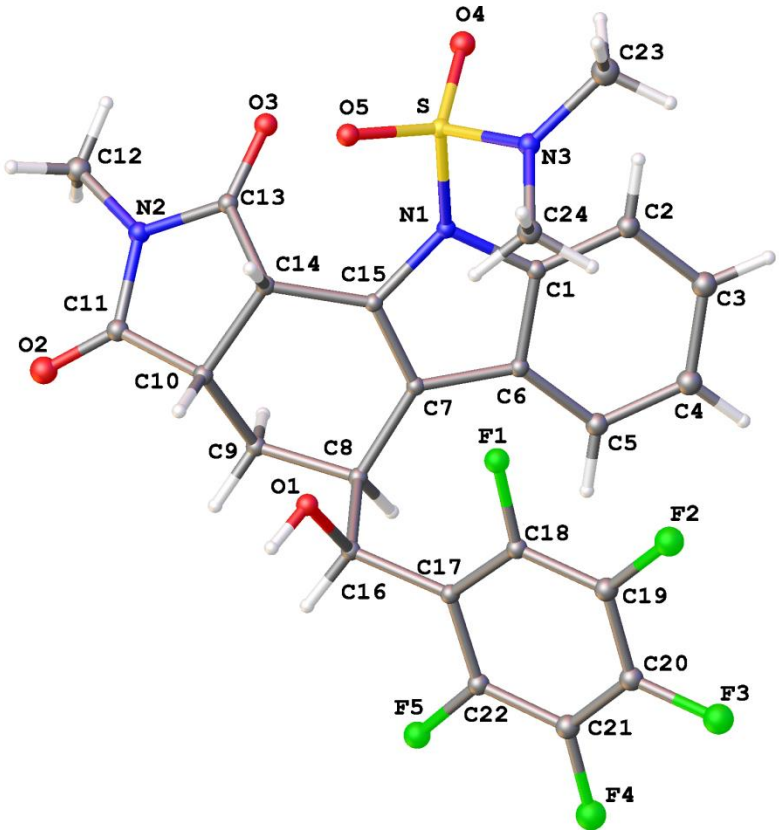


Table 1. Crystal data and structure refinement for mjh12.

Identification code	mjh120042	
Chemical formula (moiety)	$C_{24}H_{20}F_5N_3O_5S$	
Chemical formula (total)	$C_{24}H_{20}F_5N_3O_5S$	
Formula weight	557.49	
Temperature	150(2) K	
Radiation, wavelength	MoK□, 0.71073 Å	
Crystal system, space group	monoclinic, $P12_1/n1$	
Unit cell parameters	a = 7.9070(4) Å	□ = 90°
	b = 14.2862(7) Å	□ = 101.016(5)°
	c = 20.6839(10) Å	□ = 90°
Cell volume	2293.4(2) $Å^3$	
Z	4	
Calculated density	1.615 g/cm^3	
Absorption coefficient □	0.227 mm^{-1}	
F(000)	1144	
Reflections for cell refinement	5543 (□ range 2.8 to 28.5°)	
Data collection method	Xcalibur, Atlas, Gemini ultra thick-slice □ scans	
□ range for data collection	2.8 to 28.6°	
Index ranges	h –10 to 10, k –18 to 18, l –26 to 24	
Completeness to □ = 25.0°	99.2 %	
Reflections collected	23890	

Independent reflections	5125 (R_{int} = 0.0596)
Reflections with $F^2>2$□	3582
Absorption correction	semi-empirical from equivalents
Min. and max. transmission	0.98002 and 1.00000
Structure solution	direct methods
Refinement method	Full-matrix least-squares on F^2
Weighting parameters a, b	0.0401, 1.5195
Data / restraints / parameters	5125 / 0 / 351
Final R indices [$F^2>2$□]	R1 = 0.0492, wR2 = 0.0953
R indices (all data)	R1 = 0.0864, wR2 = 0.1108
Goodness-of-fit on F^2	1.027
Extinction coefficient	0.0001(5)
Largest and mean shift/su	0.000 and 0.000
Largest diff. peak and hole	0.34 and −0.37 e $Å^{-3}$

(3aS,10bS)-5-(hydroxy(o-tolyl)amino)-2-methyl-4,5,10,10b-tetrahydropyrrolo[3,4-a]carbazole-1,3(2H,3aH)-dione – 256

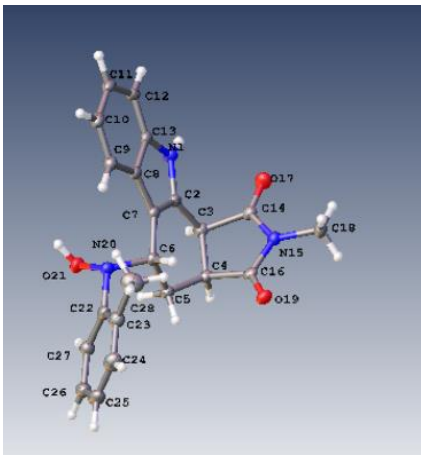


Table 1 Crystal data and structure refinement for MJH130014

Identification code	MJH130014
Empirical formula	$C_{22}H_{21}N_3O_3$
Formula weight	375.42
Temperature/K	150.01(10)
Crystal system	triclinic
Space group	P-1
a/Å	8.4756(2)
b/Å	9.6962(3)
c/Å	12.5017(3)
α/°	68.321(3)
β/°	71.529(3)
γ/°	81.860(2)
Volume/Å^3	905.20(5)
Z	2
ρ_{calc}mg/mm^3	1.377
m/mm^{-1}	0.756
F(000)	396.0
Crystal size/mm^3	0.5681 × 0.5364 × 0.2474
2Θ range for data collection	7.94 to 132.56°
Index ranges	-10 ≤ h ≤ 10, -11 ≤ k ≤ 11, -14 ≤ l ≤ 14
Reflections collected	30625
Independent reflections	3153[R(int) = 0.0292]
Data/restraints/parameters	3153/0/326
Goodness-of-fit on F^2	1.075
Final R indexes [I>=2σ (I)]	R_1 = 0.0365, wR_2 = 0.0921
Final R indexes [all data]	R_1 = 0.0373, wR_2 = 0.0928

Largest diff. peak/hole / e $Å^{-3}$ 0.19/-0.28

Appendix II

Other Publications

Oxley A, Berry P, Taylor GA, Cowell J, Hall MJ, Hesketh J, Lietz G, Boddy AV; An LC/MS/MS method for stable isotope dilution studies of β-carotene bioavailability, bioconversion and vitamin A status in humans. *Journal of Lipid Research*, 2014, **55**, 2, 319.

methods

An LC/MS/MS method for stable isotope dilution studies of β-carotene bioavailability, bioconversion, and vitamin A status in humans[S]

Anthony Oxley,* Philip Berry,† Gordon A. Taylor,† Joseph Cowell,§ Michael J. Hall,§ John Hesketh, Georg Lietz,[1,]* and Alan V. Boddy†**

Human Nutrition Research Centre,* Northern Institute for Cancer Research,† School of Chemistry,§ and Institute for Cell and Molecular Biosciences,** Newcastle University, Newcastle Upon Tyne, UK

Abstract **Isotope dilution is currently the most accurate technique in humans to determine vitamin A status and bioavailability/bioconversion of provitamin A carotenoids such as β-carotene. However, limits of MS detection, coupled with extensive isolation procedures, have hindered investigations of physiologically-relevant doses of stable isotopes in large intervention trials. Here, a sensitive liquid chromatography-tandem mass spectrometry (LC/MS/MS) analytical method was developed to study the plasma response from coadministered oral doses of 2 mg [$^{13}C_{10}$]β-carotene and 1 mg [$^{13}C_{10}$]retinyl acetate in human subjects over a 2 week period. A reverse phase C_{18} column and binary mobile phase solvent system separated β-carotene, retinol, retinyl acetate, retinyl linoleate, retinyl palmitate/retinyl oleate, and retinyl stearate within a 7 min run time. Selected reaction monitoring of analytes was performed under atmospheric pressure chemical ionization in positive mode at *m/z* 537→321 and *m/z* 269→93 for respective [^{12}C]β-carotene and [^{12}C] retinoids; *m/z* 547→330 and *m/z* 274→98 for [$^{13}C_{10}$]β-carotene and [$^{13}C_5$] cleavage products; and *m/z* 279→100 for metabolites of [$^{13}C_{10}$]retinyl acetate. A single one-phase solvent extraction, with no saponification or purification steps, left retinyl esters intact for determination of intestinally-derived retinol in chylomicrons versus retinol from the liver bound to retinol binding protein. Coadministration of [$^{13}C_{10}$] retinyl acetate with [$^{13}C_{10}$]β-carotene not only acts as a reference dose for inter-individual variations in absorption and chylomicron clearance rates, but also allows for simultaneous determination of an individual's vitamin A status.—** Oxley, A., P. Berry, G. A. Taylor, J. Cowell, M. J. Hall, J. Hesketh, G. Lietz, and A. V. Boddy. **An LC/MS/MS method for stable isotope dilution studies of β-carotene bioavailability, bioconversion, and vitamin A status in humans. *J. Lipid Res.* 2014.** 55: **319–328.**

Supplementary key words β-carotene 15,15′-monooxygenase • carotenoid metabolism • retinol metabolism • retinyl esters • tandem mass spectrometry

Vitamin A deficiency is a major public health issue in the developing world due to inadequate intake of both preformed vitamin A and provitamin A carotenoids in the diet (1). However, detection of subclinical deficiency is problematic because ~85% of vitamin A is stored in the liver while the level of vitamin A circulating in the blood is under strict homeostatic control and not indicative of hepatic reserves (2). Increasing the intake of provitamin A carotenoids, primarily through β-carotene, is seen as a safe way of restoring the vitamin A reserves of an individual because high doses of preformed vitamin A have adverse health effects (3). Although the current vitamin A equivalency ratio for β-carotene is estimated at 12:1 (by weight) (4), large inter-individual variations in both absorption and conversion have been observed (5–8).

In the intestinal mucosa, a proportion of absorbed β-carotene undergoes centric cleavage by the β-carotene 15,15′-monooxygenase 1 (BCMO1) enzyme to produce two molecules of retinal which are further reduced to retinol (vitamin A) (9). For export into the circulation, retinol is esterified to a long chain fatty acid, typically palmitate, and incorporated, along with intact β-carotene, into chylomicrons (10). Subsequently, retinyl esters are either stored in hepatic stellate cells or hydrolyzed back to retinol by the liver for repartition to other tissue compartments bound to retinol binding protein (RBP).

Currently, stable isotope dilution offers the most accurate determination of β-carotene bioefficacy and vitamin A status irrespective of high endogenous circulating levels of these micronutrients (1, 2). However, the minimum dose to be administered has been dictated by the detection limit of the analytical method (2). Furthermore, isolation

Abbreviations: APCI, atmospheric pressure chemical ionization; BCMO1, β-carotene 15,15′-monooxygenase 1; BHT, butylated hydroxytoluene; LOD, limit of detection; LOQ, limit of quantitation; RBP, retinol binding protein; RSD, relative standard deviation; SRM, selected reaction monitoring; TRL, triglyceride-rich lipoprotein.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of two figures.

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of carotenoids/retinoids from the plasma matrix for MS analysis often involves extensive and time-consuming extraction/purification procedures that have included: saponification, solid-phase extraction, preparative HPLC, and, in the case of GC-MS analysis, further conversion to *tert*-butyl-dimethylsilyl derivatives (11–18). The aim was to develop an analytical method that involved a simplified extraction procedure, sensitive MS/MS for detection of physiological doses of stable isotopes, and short LC run-times so as to be suitable for high-throughput of samples from human intervention studies.

MATERIALS AND METHODS

Chemicals

The following carotenoid and retinoid (>95% all-*trans*) standards were purchased from Sigma (St. Louis, MO): β-carotene, lycopene, retinol, retinyl acetate, and retinyl palmitate. The [12-, 12′-, 13-, 13′-, 14-, 14′-, 15-, 15′-, 20-, 20′-$^{13}C_{10}$]β-carotene and [8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 19-, 20-$^{13}C_{10}$]retinyl acetate (**Fig. 1**) to be administered to human subjects were custom synthesized by Buchem BV (Apeldoorn, The Netherlands) and certified fit for human consumption. Similarly, the [8-, 8′-, 9-, 9′-, 10-, 10′-, 11-, 11′-, 12-, 12′-, 13-, 13′-, 14-, 14′-, 15-, 15′-, 19-, 19′-, 20-, 20′-$^{13}C_{20}$]β-carotene, [12-, 13-, 14-, 15-, 20-$^{13}C_5$]retinol, and [10-, 19-, 19-, 19-d4]retinyl palmitate stable isotopes (Fig. 1) were also purchased from Buchem BV. Methanol, propan-2-ol, chloroform, ethanol, ethyl acetate, toluene, and acetic acid were all of HPLC grade and purchased from Fisher Scientific (Loughborough, UK). Butylated hydroxytoluene (BHT), Novozyme 435, and ammonium acetate were obtained from Sigma-Aldrich (St. Louis, MO). Aberlyst A-21, linoleic acid, oleic acid, and stearic acid for synthesis of retinyl esters were obtained from Alfa Aesar (Heysham, Lancashire, UK).

Synthesis of retinyl esters

Retinyl esters were synthesized via an enzyme-catalyzed transesterification (19) as follows. Into a dry Schlenk flask, retinyl acetate (33 mg, 0.10 mmol), Novozyme 435 (120 mg), and Aberlyst A-21 (50 mg) were suspended in dry toluene (5 ml). The reaction mixture was stirred under an atmosphere of N_2, and five equivalents (0.50 mmol) of the appropriate acid (palmitic, stearic, linoleic, or oleic) were added. After 20 h at room temperature, the reaction mixture was filtered and the solvent was removed under reduced pressure to give a mixture (approximately 1:4) of the desired retinyl ester and unreacted acid. The resulting mixtures were used without further purification as LC/MS/MS standards for the corresponding retinyl esters.

Subjects and blood collection

Healthy male and female volunteers with an age range of 18–45 years were recruited into the "BetaSNP" dietary intervention study where written informed consent was obtained. Exclusion criteria were: pregnancy, smoking, high blood pressure, diabetes, BMI >30, liver/kidney/gastrointestinal disease, lipid metabolic disorders, and consumption of multivitamins (containing vitamins A, C, E) or β-carotene supplements 3 months prior to the study start. The study was conducted according to the guidelines set forth in the Declaration of Helsinki, and all procedures involving human subjects were approved by the National Research Ethics Service (NRES), North East - Sunderland Committee (REC 09/H0904/20) before registration with the UK Clinical Research Network (UKCRN: 7413). The [$^{13}C_{10}$]β-carotene and [$^{13}C_{10}$]retinyl acetate were prepared for oral administration in sunflower oil, at respective concentrations of 2 mg ml^{-1} and 1 mg ml^{-1}, by sonication in amber bottles at room temperature for 30 min. Oil solutions were then stored in sterile 1 ml tip-cap amber oral syringes (Becton Dickinson, Oxford, UK) and used within 1 week of preparation. Fasted subjects were cannulated via the antecubital vein and blood was drawn into 10 ml EDTA Vacutainer tubes (Becton Dickinson). Subjects then received the dual isotopic oral dose of 2 mg [$^{13}C_{10}$]β-carotene and 1 mg [$^{13}C_{10}$]retinyl

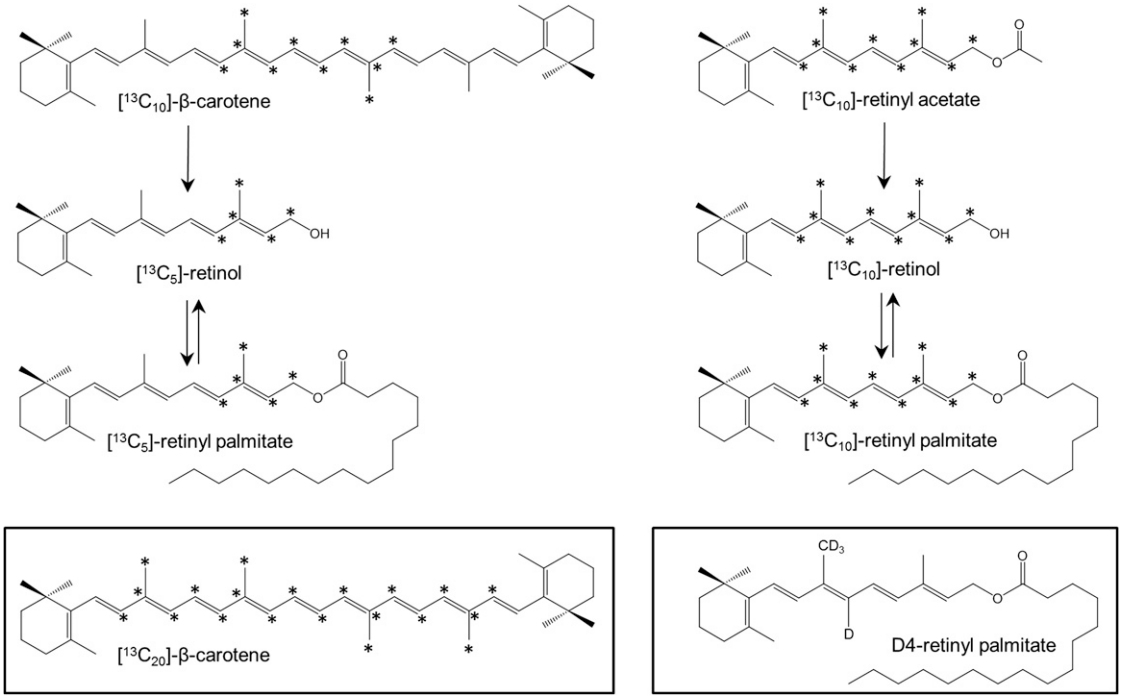

Fig. 1. β-carotene and retinyl acetate metabolism. Position of [^{13}C] labels are shown for [$^{13}C_{10}$]β-carotene and [$^{13}C_{10}$]retinyl acetate, and derived metabolites. Inserts show the [$^{13}C_{20}$]β-carotene and d4-retinyl palmitate used for method validation. Asterisks (*) denote position of [^{13}C] labels.

acetate along with a standardized breakfast meal consisting of a muffin and yogurt smoothie. The meal was designed to reflect the same nutrient content as described by Borel et al. (5) containing 46.3 g of fat (55.5% of total energy intake). Blood was subsequently collected at 2, 4, 6, 8, 10, and 12 h postdose via cannulation, and at 24, 48, 168, and 336 h by simple venipuncture. Each blood sample was immediately centrifuged at 4°C upon collection and the plasma stored at −80°C until analysis.

Plasma extraction and analyte recovery

An ethanol/ethyl acetate (1:1) solvent extraction was applied to plasma samples to ensure adequate recovery of all analytes without coextraction of lipids known to interfere with LC/MS analyses. All extraction procedures were performed under yellow lighting. To 1 ml of plasma, 10 μl (50 pmol) each of the [$^{13}C_{10}$]retinyl acetate and [$^{13}C_{20}$]β-carotene internal standards were added before denaturing with 5 ml of ethanol and 5 ml of ethyl acetate. The sample was then shaken on an orbital shaker for 10 min and centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was transferred to a clean glass tube and the solvent evaporated to dryness under a stream of nitrogen. The residue was resuspended in 100 μl of ethyl acetate, by vortexing briefly, and transferred to amber glass vials ready for LC/MS/MS injection.

Due to endogenous levels of [^{12}C]β-carotene, retinol, and retinyl palmitate always being present in "control" plasma, recovery of target analytes from the plasma matrix was assessed using the following stable isotopes: [$^{13}C_{10}$]β-carotene, [$^{13}C_5$]retinol, and d4-retinyl palmitate. Blank plasma was generously provided by the Blood Transfusion Service, Newcastle upon Tyne Hospitals (UK). For extraction efficiency experiments, 10 μl of [$^{13}C_{10}$]β-carotene, [$^{13}C_5$]retinol, and d4-retinyl palmitate in ethanol were spiked into 1 ml of control plasma at a final concentration of 5 μM. Plasma was then extracted as described above.

LC/MS/MS analysis

Chromatographic separation of β-carotene and retinoids was achieved using a Perkin Elmer Series 200 LC (Beckonsfield, UK) equipped with a Gemini C_{18} column (3 μm; 50 mm × 2 mm i.d.) and SecurityGuard C_{18} column (4 × 3 mm) both from Phenomenex (Cheshire, UK) maintained at 30°C. Reverse phase elution of analytes was performed with mobile phases of 0.1M aqueous ammonium acetate pH 5 (A) and 50:50 (w/w) methanol/isopropanol (B). The mobile phase system consisted of a 1 min linear gradient from 80% to 99% B, held at 99% B for 3 min, then immediately returned to 80% B for 3 min to re-equilibrate. Flow rate was 1.0 ml min^{-1} with an injection volume of 10 μl.

An API4000 triple quadrupole LC/MS/MS (Applied Biosystems, Carlsbad, CA) was used for analysis with atmospheric pressure chemical ionization (APCI) performed in positive ion mode using nitrogen gas with the following optimum settings: collision gas, 7; curtain gas, 10; ion source gas 1, 60; ion source gas 2, 15. Temperature of the heated nebulizer was 400°C with an ionspray voltage of 5,500. Optimization of MS/MS parameters for all analytes was performed by selecting precursor ions of $[M+H]^+$ for β-carotene, $[M+H-18]^+$ for retinol, $[M+H-256]^+$ for retinyl palmitate, and $[M+H-60]^+$ for retinyl acetate to obtain product ion spectra. Quantitation of analytes was performed in selected reaction monitoring (SRM) mode; mass transitions and optimized MS/MS parameters are given in **Table 1**. Analyst® software v1.4.1 (AB SCIEX, Framingham, MA) was used for SRM, peak integration, and analyte quantitation. Peak areas were adjusted according to internal standard recovery ([$^{13}C_{10}$]retinyl acetate for retinoids and [$^{13}C_{20}$]β-carotene for carotenes) and quantified against external calibration curves of [^{12}C]β-carotene, [^{12}C]retinol, and [^{12}C]retinyl palmitate (**Table 2**).

LC/MS/MS validation

The [^{12}C] species of β-carotene, retinol, and retinyl palmitate were used to assess linear dynamic ranges, limits of detection, limits of quantitation, intra-/inter-day assay precision, and to construct external calibration curves. Stock solutions of β-carotene and retinyl palmitate were prepared in chloroform containing 0.1% BHT at respective concentrations of 0.2 mg ml^{-1} and 1.0 mg ml^{-1}. Retinol was dissolved in ethanol containing 0.1% BHT at 1.0 mg ml^{-1}. Stock solutions were diluted in ethanol for spectrophotometric determination of absolute concentration at λ_{max} 450 nm for β-carotene and λ_{max} 325 nm for retinol and retinyl palmitate. Concentrations were calculated from published extinction coefficients ($E^{1\%}_{1cm}$) for these compounds in ethanol (20, 21). A standard mix of analytes was prepared in ethanol to study linear dynamic range via serial dilution (11 μM–5 nM), and for determination of intra- and inter-day assay precision (1 μM) through multiple injections.

RESULTS

APCI in positive mode offered greater linear dynamic range for both β-carotene and retinoids compared with electrospray ionization (ESI). APCI of retinoids resulted in the elimination of terminal functional groups to produce

TABLE 1. LC retention times, SRM mass ion transitions (Q1/Q3), and MS parameters of analytes

Analyte	Retention Time (min)	SRM Transitions (*m/z*)	Declustering Potential (V)	Entrance Potential (V)	Collision Energy (eV)	Collision Exit Potential (V)
[^{12}C]retinol	0.63	269→93	51	10	27	6
[$^{13}C_5$]retinol	0.62	274→98	51	10	27	6
[$^{13}C_{10}$]retinol	0.62	279→100	41	10	27	6
[$^{13}C_{10}$]retinyl acetate	0.91	279→100	41	10	27	6
[^{12}C]retinyl linoleate	2.20	269→93	51	10	27	6
[$^{13}C_5$]retinyl linoleate	2.20	274→98	51	10	27	6
[$^{13}C_{10}$]retinyl linoleate	2.20	279→100	41	10	27	6
[^{12}C]retinyl palmitate/oleate	2.36	269→93	51	10	27	6
[$^{13}C_5$]retinyl palmitate/oleate	2.36	274→98	51	10	27	6
[$^{13}C_{10}$]retinyl palmitate/oleate	2.35	279→100	41	10	27	6
d4-Retinyl palmitate	2.34	273→94	41	10	31	2
[^{12}C]retinyl stearate	2.63	269→93	51	10	27	6
[$^{13}C_5$]retinyl stearate	2.63	274→98	51	10	27	6
[$^{13}C_{10}$]retinyl stearate	2.63	279→100	41	10	27	6
[^{12}C]β-carotene	2.96	537→321	46	10	33	32
[$^{13}C_{10}$]β-carotene	3.00	547→330	86	10	33	18
[$^{13}C_{20}$]β-carotene	2.99	557→335	66	10	29	24

TABLE 2. Limits of detection, limits of quantitation, linear dynamic ranges, calibration curves, correlation coefficients, and intra-/inter-day variations of [^{12}C] standards used for quantitation of analytes

Analyte	LOD[a] (pmol)	LOQ[b] (pmol)	Linear Range (pmol)	Slope[c] ($a \times 10^5$)	Intercept[c] ($b \times 10^4$)	Correlation Coefficient (r^2)	Intra-day[d] (%RSD)	Inter-day[e] (%RSD)
[^{12}C]retinol	0.01	0.03	0.03–110	7.937	4.219	0.999	3.8	6.5
[^{12}C]retinyl palmitate	0.03	0.10	0.10–100	4.388	1.689	0.999	3.7	7.1
[^{12}C]β-carotene	0.05	0.17	0.17–90	1.701	0.455	1.000	3.7	7.8

[a]Limit of detection (S/N = 3; n = 5)
[b]Limit of quantitation (S/N = 10; n = 5)
[c]Calibration curves (y = ax + b).
[d]Intra-day, n = 50.
[e]Inter-day, n = 8.

identical Q1 precursor ions of $[M+H-H_2O]^+$ for retinol, $[M+H-CH_3CO_2H]^+$ for retinyl acetate, and $[M+H-CH_3(CH_2)_{14}CO_2H]^+$ for retinyl palmitate. Consequently, it was necessary to adequately separate retinoids by LC before selected reaction monitoring (SRM) at *m/z* 269→93, *m/z* 274→98, and *m/z* 279→100 for respective [^{12}C], [$^{13}C_5$], and [$^{13}C_{10}$] isotopologues (Table 1). The abundant Q3 product ion for retinoids was due to cleavage at the C_9-C_{10} double bond where the selected polyene chain fragment contained all [^{13}C] labels from *m/z* 274 and seven of the [^{13}C] labels from *m/z* 279 (**Fig. 2**).

APCI of β-carotene resulted in protonation of the molecule $[M+H]^+$ with an abundant Q3 product ion at *m/z* 177 irrespective of isotopic composition (*m/z* 537→177 [^{12}C] and *m/z* 547→177 [^{13}C]; **Fig. 3**). The geometric isomer of β-carotene, lycopene, also produced a fragment Q3 ion at *m/z* 537→177 and possessed an identical LC retention time to β-carotene. Furthermore, an unidentified compound was observed in "blank" plasma at *m/z* 547→177 which could not be separated from β-carotene by LC. Therefore, an alternative less abundant fragment of higher *m/z* was selected for [^{13}C]β-carotene at *m/z* 330 (Fig. 3). This product ion was the result of cleavage at C_{12}-C_{13} and contained the majority of the [^{13}C] labeling from *m/z* 547 and also from *m/z* 557 as internal standard. The corresponding fragment for [^{12}C]β-carotene at *m/z* 321 was not present for lycopene. Both *trans*- and *cis*-β-carotene isomers produced the same Q3 product ions (supplementary Fig. I). Optimized MS/MS parameters and SRM transitions for all analytes are given in Table 1.

Retinol and retinyl acetate were separated to baseline on a C_{18} reversed-phase column with a 1 min linear gradient of 80–99% methanol/isopropanol (50:50, w/w); their respective retention times were 0.63 and 0.91 min (**Fig. 4**). Retinyl palmitate and β-carotene eluted at 2.36 min and 2.96 min respectively under isocratic conditions of 99% methanol/isopropanol. From extracted control plasma, two additional peaks were observed at *m/z* 269→93 that flanked the retinyl palmitate peak. As these peaks were suspected to be alternative fatty acid esters of retinol, it was necessary to synthesize noncommercially available retinyl esters. The presence of the postulated retinyl esters was confirmed through the use of natural abundance ^{13}C NMR measured in $CDCl_3$ using a Jeol ECS-400 MHz. ^{13}C NMR analysis of the reaction between palmitic acid and retinyl acetate revealed a signal at 174.0 ppm which correlates to the carbonyl carbon of retinyl palmitate (in comparison to commercial standards) and was clearly distinct from retinyl acetate (171.2 ppm) and palmitic acid (180.4 ppm). Similar ^{13}C NMR signals were observed for retinyl stearate (174.0 ppm), retinyl oleate (174.0 ppm), and retinyl linoleate (173.9 ppm), confirming the production of each of the retinyl esters. Synthetic retinyl palmitate was compared against commercially-available retinyl palmitate by LC/MS/MS providing the same retention time and mass spectra, further confirming the formation of the desired retinyl esters. Consequently, LC/MS/MS peaks at 2.20 and 2.63 min were confirmed as retinyl linoleate and retinyl stearate while retinyl oleate coeluted with retinyl palmitate at 2.36 min. Total LC run-time was 7 min, which included a column re-equilibration period of 3 min.

From extraction efficiency experiments (n = 6), the recoveries of [$^{13}C_5$]retinol, d4-retinyl palmitate, and [$^{13}C_{20}$]β-carotene were 39% (±1.9% SD), 36% (±2.3% SD), and 30% (±1.6% SD) respectively. Although recovery of analytes was relatively low, the mild extraction procedure employed negated the detrimental effects associated with co-extracted lipids during MS analysis. Furthermore, total analyte concentrations were calculated using the internal standards [$^{13}C_{10}$] retinyl acetate and [$^{13}C_{20}$]β-carotene, thus correcting for the low recovery. On-column validation of linear dynamic range, limit of detection, and intra- and inter-day precision for [^{12}C] analytes are given in Table 2. Limits of detection ranged from 10 fmol for retinol to 50 fmol for β-carotene. Linear dynamic ranges were over two to three orders of magnitude with r^2 values of >0.999 (supplementary Fig. II). Intra- and inter-day precision ranged from 3.7 to 3.8% relative standard deviation (RSD) and from 6.5 to 7.8% RSD, respectively.

Administered 2 mg [$^{13}C_{10}$]β-carotene could be detected in plasma from 2 h to 2 weeks postdose (**Fig. 5**). The [$^{13}C_{10}$]β-carotene plasma response exhibited an initial increase to 10 nmol/l at 6 h, followed by a brief plateau to 8 h, then a steady rise to a maximum of 25 nmol/l at 24 h. The [$^{13}C_{10}$]β-carotene cleavage product, [$^{13}C_5$] retinyl palmitate, rapidly attained a maximum concentration of 50 nmol/l at 4 h postdose, while [$^{13}C_5$]retinol started to appear at 3–4 h in plasma and peaked at 10 h. Metabolites of the 1 mg [$^{13}C_{10}$]retinyl acetate dose reached plasma concentrations 4- to 6-fold higher than [$^{13}C_{10}$]β-carotene and derived cleavage products. Plasma kinetics of [$^{13}C_{10}$]retinol and [$^{13}C_{10}$]retinyl palmitate mirrored those observed for [$^{13}C_5$]retinol and [$^{13}C_5$]retinyl palmitate. Retinol secreted from the intestine was predominantly esterified to palmitate and oleate. However, retinyl

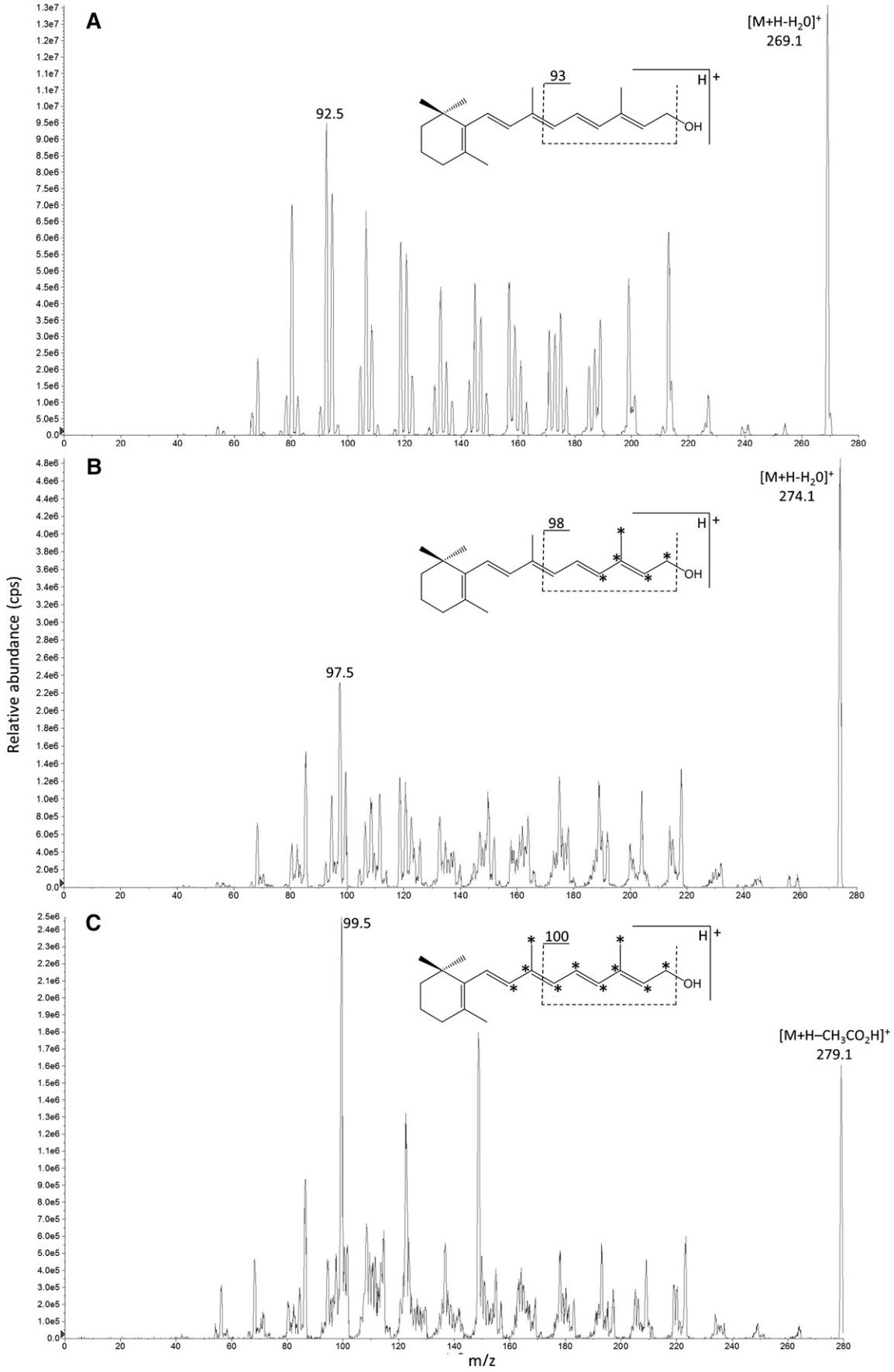

Fig. 2. Flow-injection APCI-MS/MS product ion mass spectra of *m/z* 269 [^{12}C]retinol (A), *m/z* 274 [$^{13}C_5$]retinol (B), and *m/z* 279 [$^{13}C_{10}$] retinol (C) in positive mode. Asterisks (*) denote position of [^{13}C] labels.

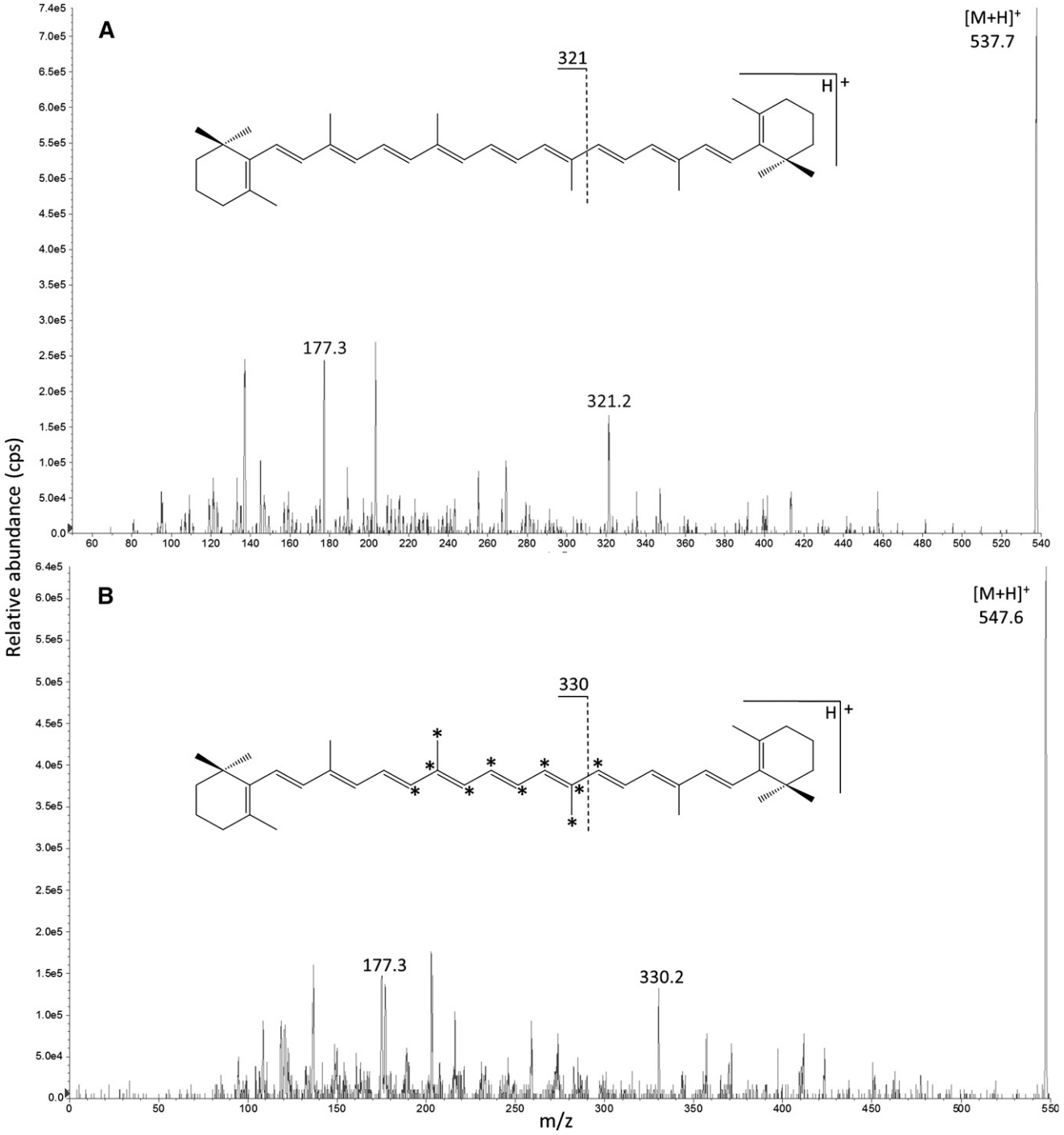

Fig. 3. Flow-injection APCI-MS/MS product ion mass spectra of *m/z* 537 [^{12}C]β-carotene (A) and *m/z* 547 [$^{13}C_{10}$]β-carotene (B) in positive mode. Asterisks (*) denote position of [^{13}C] labels.

linoleate levels were higher than retinyl stearate for [$^{13}C_5$] cleavage products while retinyl stearate was higher than retinyl linoleate for [$^{13}C_{10}$]retinol.

DISCUSSION

In human intervention studies, the size of stable isotope dose given is largely determined by the limit of detection of the analytical method (1, 2). Although carotene absorption and metabolism may be tracked by the very sensitive method of accelerator MS (22, 23), this method involves the administration of radiolabeled material, albeit at micro-doses, and requires laborious sample fractionation to distinguish metabolites, followed by very expensive analysis using highly specialized equipment that is not widely available. Even if other MS methods such as gas chromatography/combustion/isotope-ratio MS and electron capture negative chemical ionization MS allow effective use of physiological doses of retinol (24, 25) and β-carotene (26) tracers, these methods have the disadvantage of requiring extensive sample preparation,

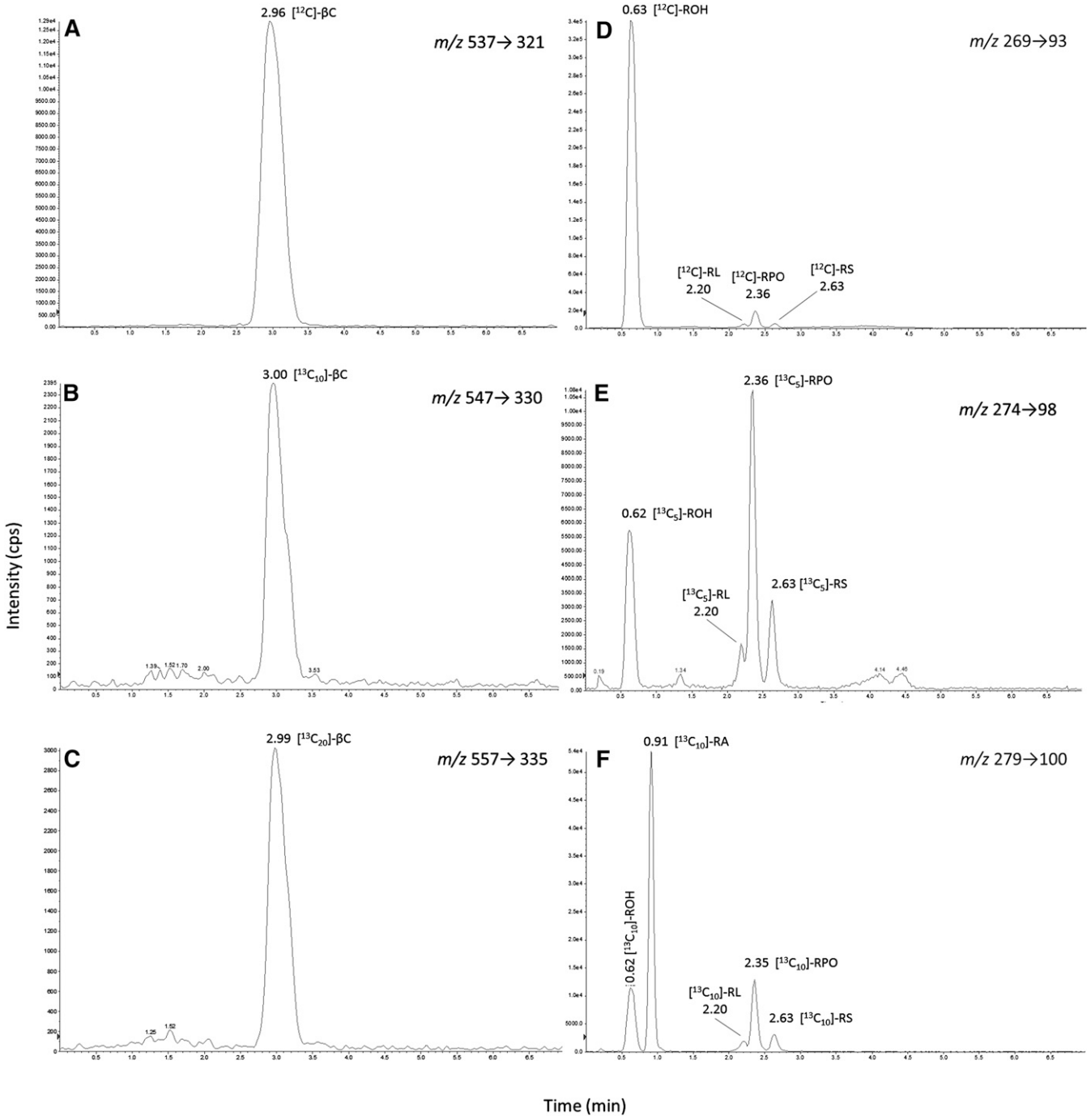

Fig. 4. APCI (positive mode) LC/MS/MS chromatograms from a human subject plasma sample 6 h postdose showing [^{12}C], [$^{13}C_{10}$], and [$^{13}C_5$] isotopologues of β-carotene (βC), retinol (ROH), retinyl linoleate (RL), retinyl palmitate/oleate (RPO), and retinyl stearate (RS). [$^{13}C_{10}$]retinyl acetate (RA) and [$^{13}C_{20}$]β-carotene were used as internal standards. SRM transitions are given for each chromatogram.

including HPLC purification and derivatization, before injection into the MS. In contrast, the application of liquid chromatography mass spectrometry (LC/MS) to the analysis of retinoid and carotenoid tracers offers the advantages of high sensitivity and selectivity without the need for hydrolysis and derivatization (17, 27–30). However, isolation of carotenoids and retinoids from the plasma matrix is frequently carried out individually leading to separate injections, use of different LC systems, MS ionization methods (APCI/ESI) and modes (positive/negative) (11–18). The current method allows for the first time the analysis of both [^{13}C] retinoid and β-carotene tracers simultaneously using chemical ionization (APCI) in positive mode. Furthermore, the new method is more sensitive than comparable LC/MS methods, with detection limits of 10 fmol for retinol and 50 fmol for β-carotene compared with 233 (27) and 672 fmol (29) for retinol and 250 (17), 559 (28), and 57 fmol (27) for β-carotene in previous methods.

The single solvent extraction procedure developed here for both carotenoids and retinoids negated the effect of

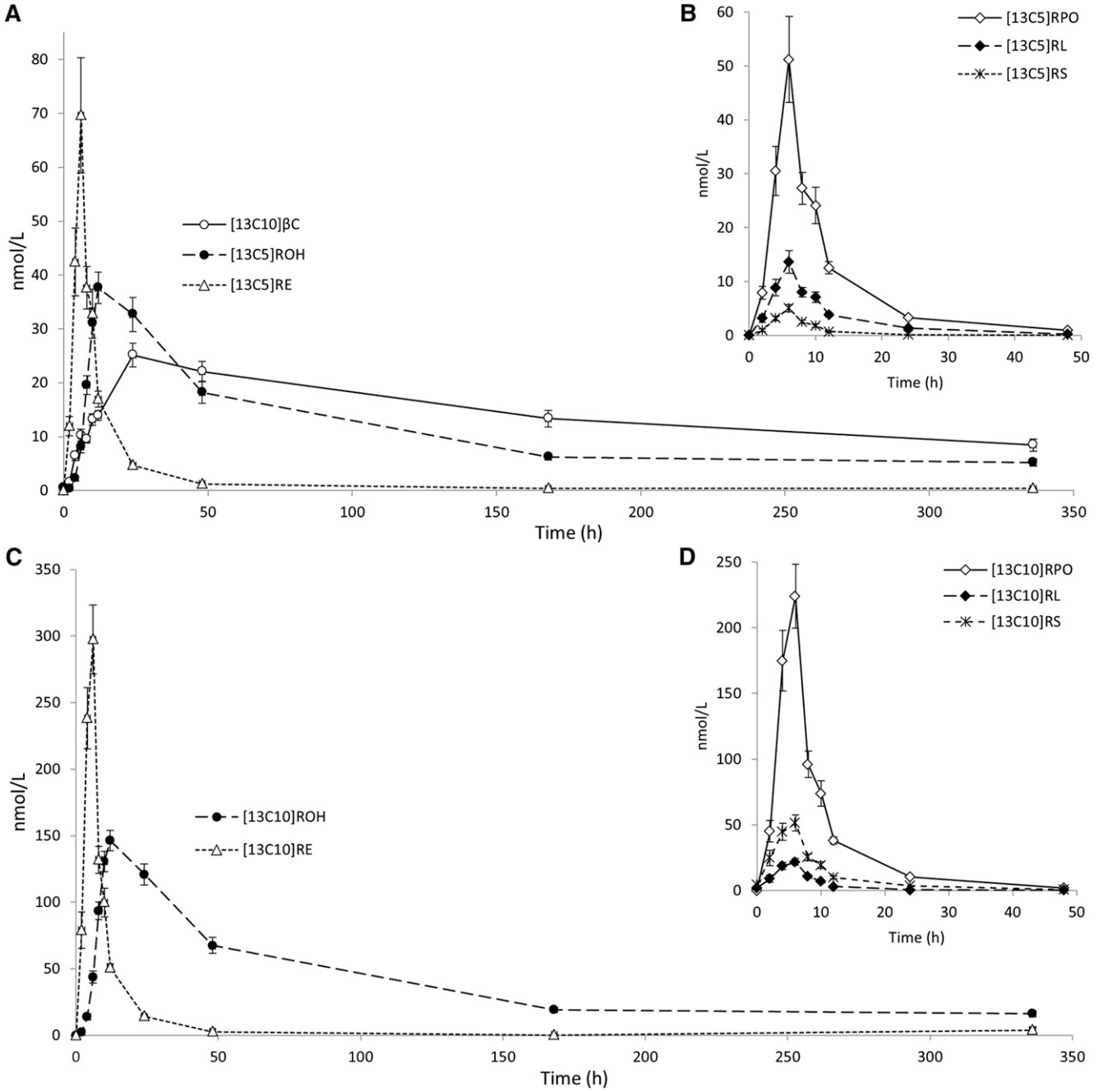

Fig. 5. Quantitative LC/MS/MS analysis of mean plasma responses from 45 human subjects (± SEM) over the whole 14 day study period (A, C) and during the first 48 h (B, D). Administered [$^{13}C_{10}$]β-carotene (βC) and resulting [$^{13}C_5$] cleavage products (ROH, retinol; RE, total retinyl esters; RL, retinyl linoleate; RPO, retinyl palmitate/retinyl oleate; RS, retinyl stearate) are shown in (A) and (B). [$^{13}C_{10}$] metabolites of administered [$^{13}C_{10}$]retinyl acetate are shown in (C) and (D).

interfering plasma lipids (31), without saponification, leaving retinyl esters intact. Consequently, it was not necessary to prepare triglyceride-rich lipoprotein (TRL) fractions to discriminate newly-absorbed intestinally-derived retinyl esters from retinol secreted by the liver bound to RBP. However, it is recognized that small amounts (~3%) of unesterified retinol, derived from administered retinyl acetate and β-carotene, may be present in lymph chylomicrons (32, 33). Although TRL fractions, obtained by ultracentrifugation at a solution density of <1.006 g ml^{-1}, contain >83% of retinyl esters in the first 6 h postprandial period, a large percentage of plasma retinyl esters is progressively and irreversibly transferred to the denser LDL fraction resulting in 32% of the plasma retinyl esters localized to the LDL fraction 12 h after fat load (34). This transfer of retinyl esters is even more substantial in subjects with familial hypercholesterolemia (35). Furthermore, inter-individual variation in chylomicron clearance kinetics, such as delayed chylomicron remnant clearance in subjects with endogenous hypertriglyceridemia (36) or variation in chylomicron recovery during TRL preparation and analysis, reduces the accuracy of this approach to directly measure the mass of retinyl

esters or β-carotene absorbed (37). Thus, the current method can detect intestinally-derived retinyl esters with more accuracy compared with methods employing TRL separations (27, 37, 38).

The current method also allows β-carotene bioefficacy and vitamin A dilution to be studied concurrently due to differential extrinsic [^{13}C] labeling of administered compounds. [^{13}C] isotopes were selected because deuterated compounds are subject to hydrogen-deuterium exchange and possess different physicochemical characteristics resulting in altered LC retention times and solvent extraction efficiencies (2, 11, 28). Position of [$^{13}C_{10}$] labels around the centric 15,15′ double bond on the β-carotene molecule allowed BCMO1 [$^{13}C_5$] cleavage products to be distinguished from [$^{13}C_{10}$] metabolites of [$^{13}C_{10}$]retinyl acetate. Although both [$^{13}C_{10}$] and [$^{13}C_5$] metabolites displayed similar plasma kinetic profiles, concentrations of [$^{13}C_5$] retinol and retinyl esters were 3- to 4-fold lower even though twice the dose of [$^{13}C_{10}$]β-carotene was administered. It is known that intestinal absorption of synthetic β-carotene is limited although bioavailability is distinctly enhanced when dissolved in oil (39). Regarding retinyl esters, both [$^{13}C_{10}$] and [$^{13}C_5$]retinol were preferentially esterified to palmitate and oleate. However, subsequent specificities of [$^{13}C_5$]retinol for linoleate and [$^{13}C_{10}$]retinol for stearate were observed, which suggests differences in subcellular compartmentalization between preformed retinol and retinol from provitamin A sources in the enterocyte before incorporation in chylomicrons.

Retinyl acetate was coadministered with β-carotene as a reference dose to correct for inter- and intra-individual variations in intestinal absorption and chylomicron clearance rates (37). The [$^{13}C_{10}$]retinyl acetate dose can also be used to determine total body vitamin A reserves after a sufficient period (circa 3 days) of isotope dilution with endogenous pools (1). In some previous studies, the reference dose was not administered concomitantly with β-carotene to avoid competition during intestinal absorption (12, 14). Single doses of β-carotene have ranged from 5 to 126 mg due to analytical detection limits dictating the minimum dose that can be administered to human subjects. However, β-carotene bioefficacy is dose-dependent when >4 mg is ingested (40), while doses >6 mg perturb the steady-state equilibrium in the blood (41). The 2 mg utilized in the current study represents a true physiological dose according to the estimated daily intake of β-carotene in UK and US populations (39). Although lower doses have been administered daily over a prolonged period to reach a plateau of isotopic enrichment in the blood (15, 16), multiple dosing cannot establish uptake kinetics.

In summary, this new sensitive analytical method allows for the simultaneous study of β-carotene bioefficacy and vitamin A status in human subjects at physiological doses for at least 2 weeks. The simple extraction procedure and single 7 min LC/MS run-time for all analytes makes the method applicable to the high throughput of samples generated in large human intervention studies.

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REFERENCES

1. Furr, H. C., M. H. Green, M. Haskell, N. Mokhtar, P. Nestel, S. Newton, J. D. Ribaya-Mercado, G. W. Tang, S. Tanumihardjo, and E. Wasantwisut. 2005. Stable isotope dilution techniques for assessing vitamin A status and bioefficacy of provitamin A carotenoids in humans. *Public Health Nutr.* **8:** 596–607.
2. van Lieshout, M., C. E. West, and R. B. van Breemen. 2003. Isotopic tracer techniques for studying the bioavailability and bioefficacy of dietary carotenoids, particularly beta-carotene, in humans: a review. *Am. J. Clin. Nutr.* **77:** 12–28.
3. Haskell, M. J. 2012. The challenge to reach nutritional adequacy for vitamin A: β-carotene bioavailability and conversion–evidence in humans. *Am. J. Clin. Nutr.* **96:** 1193S–1203S.
4. Tang, G. 2010. Bioconversion of dietary provitamin A carotenoids to vitamin A in humans. *Am. J. Clin. Nutr.* **91:** 1468S–1473S.
5. Borel, P., P. Grolier, N. Mekki, Y. Boirie, Y. Rochette, B. Le Roy, M. C. Alexandre-Gouabau, D. Lairon, and V. Azais-Braesco. 1998. Low and high responders to pharmacological doses of beta-carotene: proportion in the population, mechanisms involved and consequences on beta-carotene metabolism. *J. Lipid Res.* **39:** 2250–2260.
6. Hickenbottom, S. J., J. R. Follett, Y. M. Lin, S. R. Dueker, B. J. Burri, T. R. Neidlinger, and A. J. Clifford. 2002. Variability in conversion of beta-carotene to vitamin A in men as measured by using a double-tracer study design. *Am. J. Clin. Nutr.* **75:** 900–907.
7. Leung, W. C., S. Hessel, C. Meplan, J. Flint, V. Oberhauser, F. Tourniaire, J. E. Hesketh, J. von Lintig, and G. Lietz. 2009. Two common single nucleotide polymorphisms in the gene encoding beta-carotene 15,15′-monoxygenase alter beta-carotene metabolism in female volunteers. *FASEB J.* **23:** 1041–1053.
8. Lietz, G., A. Oxley, W. Leung, and J. Hesketh. 2012. Single nucleotide polymorphisms upstream from the β-carotene 15,15′-monoxygenase gene influence provitamin A conversion efficiency in female volunteers. *J. Nutr.* **142:** 161S–165S.
9. Lietz, G., A. Oxley, C. Boesch-Saadatmandi, and D. Kobayashi. 2012. Importance of β,β-carotene 15,15′-monooxygenase 1 (BCMO1) and β,β-carotene 9′,10′-dioxygenase 2 (BCDO2) in nutrition and health. *Mol. Nutr. Food Res.* **56:** 241–250.
10. Harrison, E. H. 2012. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. *Biochim. Biophys. Acta.* **1821:** 70–77.
11. Dueker, S. R., A. D. Jones, G. M. Smith, and A. J. Clifford. 1994. Stable isotope methods for the study of beta-carotene-d8 metabolism in humans utilizing tandem mass spectrometry and high-performance liquid chromatography. *Anal. Chem.* **66:** 4177–4185.
12. Lin, Y., S. R. Dueker, B. J. Burri, T. R. Neidlinger, and A. J. Clifford. 2000. Variability of the conversion of beta-carotene to vitamin A in women measured by using a double-tracer study design. *Am. J. Clin. Nutr.* **71:** 1545–1554.
13. Novotny, J. A., S. R. Dueker, L. A. Zech, and A. J. Clifford. 1995. Compartmental analysis of the dynamics of beta-carotene metabolism in an adult volunteer. *J. Lipid Res.* **36:** 1825–1838.
14. Tang, G., J. Qin, G. G. Dolnikowski, and R. M. Russell. 2000. Vitamin A equivalence of beta-carotene in a woman as determined by a stable isotope reference method. *Eur. J. Nutr.* **39:** 7–11.
15. van Lieshout, M., C. E. West, S. Muhilal, D. Permaesih, Y. Wang, X. Xu, R. B. van Breemen, A. F. Creemers, M. A. Verhoeven, and J. Lugtenburg. 2001. Bioefficacy of beta-carotene dissolved in oil studied in children in Indonesia. *Am. J. Clin. Nutr.* **73:** 949–958.
16. Van Loo-Bouwman, C. A., T. H. J. Naber, R. B. van Breemen, D. Zhu, H. Dicke, E. Siebelink, P. J. M. Hulshof, F. G. M. Russel, G. Schaafsma, and C. E. West. 2010. Vitamin A equivalency and apparent absorption of beta-carotene in ileostomy subjects using a dual-isotope dilution technique. *Br. J. Nutr.* **103:** 1836–1843.
17. Wang, Y., X. Y. Xu, M. van Lieshout, C. E. West, J. Lugtenburg, M. A. Verhoeven, A. F. L. Creemers, and R. B. van Breemen. 2000. A liquid chromatography-mass spectrometry method for the quantification of bioavailability and bioconversion of beta-carotene to retinol in humans. *Anal. Chem.* **72:** 4999–5003.

18. Zhu, D., Y. Wang, Y. Pang, A. Liu, J. Guo, C. A. Bouwman, C. E. West, and R. B. van Breemen. 2006. Quantitative analyses of beta-carotene and retinol in serum and feces in support of clinical bioavailability studies. *Rapid Commun. Mass Spectrom.* **20:** 2427–2432.
19. Boaz, N. W., and S. K. Clendennen. 2006. Preparation of retinyl esters. United States patent application 20080085534. 2006 June 10.
20. Barua, A. B., and H. C. Furr. 1998. Properties of retinoids. Structure, handling, and preparation. *Mol. Biotechnol.* **10:** 167–182.
21. Craft, N. E., and J. H. Soares. 1992. Relative solubility, stability, and absorptivity of lutein and β-carotene in organic-solvents. *J. Agric. Food Chem.* **40:** 431–434.
22. Dueker, S. R., Y. M. Lin, B. A. Buchholz, P. D. Schneider, M. W. Lame, H. J. Segall, J. S. Vogel, and A. J. Clifford. 2000. Long-term kinetic study of beta-carotene, using accelerator mass spectrometry in an adult volunteer. *J. Lipid Res.* **41:** 1790–1800.
23. Ho, C. C., F. F. de Moura, S-H. Kim, B. J. Burri, and A. J. Clifford. 2009. A minute dose of C-14-beta-carotene is absorbed and converted to retinoids in humans. *J. Nutr.* **139:** 1480–1486.
24. Tang, G., J. Qin, and G. Dolnikowski. 1998. Deuterium enrichment of retinol in humans determined by gas chromatography electron capture negative chemical ionization mass spectrometry. *J. Nutr. Biochem.* **9:** 408–414.
25. Tanumihardjo, S. A. 2000. Vitamin A status assessment in rats with C-13(4)-retinyl acetate and gas chromatography/combustion/isotope ratio mass spectrometry. *J. Nutr.* **130:** 2844–2849.
26. Parker, R. S., J. E. Swanson, B. Marmor, K. J. Goodman, A. B. Spielman, J. T. Brenna, S. M. Viereck, and W. K. Canfield. 1993. Study of beta-carotene metabolism in humans using C-13-beta-carotene and high-precision isotope ratio mass-spectrometry. *Ann. N. Y. Acad. Sci.* **691:** 86–95.
27. Fleshman, M. K., K. M. Riedl, J. A. Novotny, S. J. Schwartz, and E. H. Harrison. 2012. An LC/MS method for d8-β-carotene and d4-retinyl esters: β-carotene absorption and its conversion to vitamin A in humans. *J. Lipid Res.* **53:** 820–827.
28. Pawlosky, R. J., V. P. Flanagan, and J. A. Novotny. 2000. A sensitive procedure for the study of beta-carotene-d8 metabolism in humans using high performance liquid chromatography-mass spectrometry. *J. Lipid Res.* **41:** 1027–1031.
29. van Breemen, R. B., D. Nikolic, X. Y. Xu, Y. S. Xiong, M. van Lieshout, C. E. West, and A. B. Schilling. 1998. Development of a method for quantitation of retinol and retinyl palmitate in human serum using high-performance liquid chromatography atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr. A.* **794:** 245–251.
30. van Breemen, R. B., L. Dong, and N. D. Pajkovic. 2012. Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids. *Int. J. Mass Spectrom.* **312:** 163–172.
31. Hagiwara, T., T. Yasuno, K. Funayama, and S. Suzuki. 1998. Determination of lycopene, alpha-carotene and beta-carotene in serum by liquid chromatography atmospheric pressure chemical ionization mass spectrometry with selected-ion monitoring. *J. Chromatogr. B Biomed. Sci. Appl.* **708:** 67–73.
32. Huang, H. S., and D. S. Goodman. 1965. Vitamin A and carotenoids. I. Intestinal absorption and metabolism of 14C-labelled vitamin A alcohol and beta-carotene in the rat. *J. Biol. Chem.* **240:** 2839–2844.
33. Goodman, D. S., R. Blomstrand, B. Werner, H. S. Huang, and T. Shiratori. 1966. The intestinal absorption and metabolism of vitamin A and beta-carotene in man. *J. Clin. Invest.* **45:** 1615–1623.
34. Krasinski, S. D., J. S. Cohn, R. M. Russell, and E. J. Schaefer. 1990. Postprandial plasma vitamin A metabolism in humans: a reassessment of the use of plasma retinyl esters as markers for intestinally derived chylomicrons and their remnants. *Metabolism.* **39:** 357–365.
35. Rubinsztein, D. C., J. C. Cohen, G. M. Berger, D. R. Vanderwesthuyzen, G. A. Coetzee, and W. Gevers. 1990. Chylomicron remnant clearance from the plasma is normal in familial hypercholesterolemic homozygotes with defined receptor defects. *J. Clin. Invest.* **86:** 1306–1312.
36. Cortner, J. A., P. M. Coates, N. A. Le, D. R. Cryer, M. C. Ragni, A. Faulkner, and T. Langer. 1987. Kinetics of chylomicron remnant clearance in normal and in hyperlipoproteinemic subjects. *J. Lipid Res.* **28:** 195–206.
37. Edwards, A. J., C. S. You, J. E. Swanson, and R. S. Parker. 2001. A novel extrinsic reference method for assessing the vitamin A value of plant foods. *Am. J. Clin. Nutr.* **74:** 348–355.
38. van Vliet, T., W. H. P. Schreurs, and H. Vandenberg. 1995. Intestinal beta-carotene absorption and cleavage in men: response of beta-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of beta-carotene. *Am. J. Clin. Nutr.* **62:** 110–116.
39. Grune, T., G. Lietz, A. Palou, A. C. Ross, W. Stahl, G. Tang, D. Thurnham, S-a. Yin, and H. K. Biesalski. 2010. Beta-carotene is an important vitamin A source for human. *J. Nutr.* **140:** 2268S–2285S.
40. Brubacher, G. B., and H. Weiser. 1985. The vitamin A activity of beta-carotene. *Int. J. Vitam. Nutr. Res.* **55:** 5–15.
41. Tang, G. 2012. Techniques for measuring vitamin A activity from β-carotene. *Am. J. Clin. Nutr.* **96:** 1185S–1188S.