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A Novel Method for the Synthesis of Indolo[2,1-a]isoquinolines and Modelling Studies of 3-Substituted Oxindoles against PfPK5

Thato Saoeni Sello

2008

A Novel Method for the Synthesis of Indolo[2,1-a]isoquinolines and Modelling Studies of 3-Substituted Oxindoles against PfPK5

Thato Saoeni Sello

Supervised by Prof. C. B. de Koning and Dr. W. A. L. van Otterlo

A dissertation submitted to the Faculty of Science

University of the Witwatersrand, Johannesburg

In fulfilment of the requirements for

Degree of Master of Science

Declaration

I declare that the work presented in this dissertation was carried out by myself with help from Dr. S. Pelly under the supervision of Prof. C. B. de Koning and Dr. W. A. L. van Otterlo. It is being submitted for the degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Thato Saoeni Sello

January 2008

Acknowledgements

I am truly grateful to my supervisors, Prof. C. B. de Koning and Dr W. A. L. van Otterlo, for all their help and encouragement throughout this dissertation and advice outside the field of chemistry. I am also grateful to Prof. J. P. Michael for his input in the dissertation.

I would like to thank Mr Richard Mampa for running the NMR spectra and for always making time when I needed help in interpreting spectral data, Dr. A. Dinsmore for running some of my mass spectra and Dr. S. Pelly for helping me with the modelling studies.

I would like to thank the entire organic group, especially my lab mates, Mrs Sameshnee Pelly (always wanted to call you that way), Susan Travis, Siyanda Mthembu and Garreth Morgans. Life would be hard in the lab without you guys.

Thanks to the University of the Witwatersrand, CSIR, as well as NRF for their financial support. Also many thanks to the chemistry department for all the great braais and Christmas parties we had together.

To Thabiso Phadi, Winston Nxumalo, Sizwe Buqwana, Mxolisi Maake-ka Ncube, Mojalefa Huma and the rest of my friends, thank you for making my life a living pleasure.

A special thanks goes out to my family for all their consistent love, support and understanding, especially my mom and sister (Stumzin) who have been with me through good and bad times. This one is for you.
Miss Tumeka (MaNdlovu) Poswa, thank you for being there when I needed you the most. Your presence in my life is greatly appreciated.

And thank you God for giving me the life that I have.

List of Abbreviations

AcOH	Acetic acid
$CDCl_3$	Deuterated chloroform
CDK	Cyclin dependent kinase
CH_2Cl_2	Dichloromethane
CaH_2	Calciumhydride
DMF	Dimethylformamide
DME	1,2-Dimethoxyethane
Et_3N	Triethylamine
EtOAc	Ethyl acetate
EtOH	Ethanol
HCl	Hydrochloric acid
HRMS	High resolution mass spectroscopy
IR	Infrared
$KOBu^t$	Potassium *t*-butoxide
LDA	Lithium diisopropylamide
NBS	*N*-bromosuccinimide
$NaHCO_3$	Sodium hydrogen carbonate
n-BuLi	n-Butyllithium
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
PfPK5	*Plasmodium falciparum* protein kinase five
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV	Ultra violet

Abstract

Many naturally occurring and synthetically made azapolycyclic aromatic ring systems display important biological activities. One class of naturally occurring azapolycyclic aromatic ring systems is the dibenzopyrrocoline alkaloids, made from an indole nucleus fused to an isoquinoline system sharing the same nitrogen, i.e. the indolo[2,1-*a*]isoquinoline nucleus. The indolo[2,1-*a*]isoquinoline and its analogues have been reported to possess antileukemic, tubulin polymerization inhibitory and antitumor activity.

A variety of indolo[2,1-*a*]isoquinolines have been synthesized in our labs. This includes, the 5,12-dimethyl-6-phenylindolo[2,1-*a*]isoquinoline, using the Suzuki-Miyaura cross-coupling reaction and reaction conditions for the formation of aromatic rings ($KOBu^t$ in DMF) developed in our laboratories. In this dissertation, we outline the syntheses of (±)-5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol and 2-(1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde. We also discuss the synthesis and the modelling studies, (docked *in silico*) of the 3-substituted oxindoles in the X-ray crystal structure of the PfPK5 cyclin dependent kinase (CDK).

The synthesis of indolo[2,1-*a*]isoquinolines started with *N*-protection of isatin and benzimidazole with a benzyl group to afford 1-benzylindoline-2,3-dione and 1-benzyl-1*H*-benzo[*d*]imidazole, respectively. The next step was the synthesis of the brominated compound, 1-benzyl-2-bromo-1*H*-indole, and the iodated compound, 1-benzyl-2-iodo-1*H*-benzo[*d*]imidazole. 1-Benzyl-2-bromo-1*H*-indole was synthesized by means of a functional group interconversion of the oxygen in the 3-position of isatin to two chlorine atoms initially, followed by removal of those chlorine atoms with activated zinc, followed by the conversion of the carbonyl of the oxindole to give a 2-bromoindole using $POBr_3$. 1-Benzyl-2-iodo-1H-benzo[*d*]imidazole was synthesized in two ways. Firstly, 1-benzyl-1*H*-benzo[*d*]imidazole was exposed to LDA followed by iodinating the 2-position by

exposure of the intermediate to diiodoethane. The second method uses a halogenating method developed in our labs. 1-Benzyl-1*H*-benzo[*d*]imidazole was exposed to isopropylmagnesium chloride lithium chloride followed by I_2. Having obtained the halogenated products, both sets of halogenated precursors were coupled with 2-formylphenylboronic acid using the Suzuki-Miyaura cross-coupling reaction to obtain the products, 2-(1-benzyl-1*H*-indol-2-yl)benzaldehyde and 2-(1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde in 98 and 67% yield, respectively. Aromatization of 2-(1-benzyl-1*H*-indol-2-yl)benzaldehyde occurred easily using ^{t}BuOK in DMF at room temperature to afford (±)-5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol in 75% yield (7:3 ratio of *anti*-: *syn*-) but exposing 2-(1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde to the same reaction conditions did not afford the desired product. Dehydrating (±)-5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol using methanesulfonyl chloride in CH_2Cl_2 was unsuccessful. Further attempts at dehydrating (±)-5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol were prevented due to time constraints.

In the last part of the project, a library of 3-substituted oxindoles (13 molecules) was synthesized successfully and the compounds were docked *in silico* in the active site of an X-ray crystal structure of PfPK5, a cyclin dependent kinase of the *Plasmodium falciparum*, the agent causing the most severe form of human malaria. Eleven of the thirteen compounds were synthesized by condensation of oxindole and a suitable aldehyde in the presence of piperidine. The other two, 3-(propan-2-ylidene)indolin-2-one and 5,6-dimethoxy-3-(methylthio)indolin-2-one, were synthesized differently. 3-(Propan-2-ylidene)indolin-2-one was synthesized by reacting the oxindole with acetone in the presence of HCl and 5,6-dimethoxy-3-(methylthio)indolin-2-one was synthesized following Gassman's methodology. Two molecules scored well in the molecular modelling studies using the X-ray crystal structure of PfPK5, namely, (*E/Z*)-3-(3,4-dimethoxybenzylidene)indolin-2-one and (*Z*)-3-(4-hydroxybenzylidene)indolin-2-one.

In conclusion, we managed to synthesize (±)-5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol using the Suzuki Miyaura cross-coupling reaction and reaction conditions that lead to aromatization (^{t}BuOK in DMF at room temperature) as key steps and 2-(1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde using the Suzuki-Miyaura cross-coupling reaction. A library of 3-substituted oxindoles was made and using molecular modelling were docked *in silico* into the crystal structure of the active site of PfPK5 with 2 compounds showing promise, for further studies.

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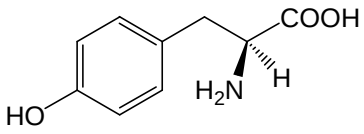
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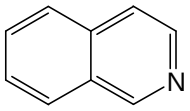
Chapter 1: Introduction

1.1 The Biological Importance of Indole and its Derivatives

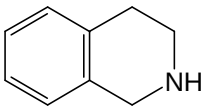
Many naturally occurring and synthetically made azapolycyclic aromatic ring systems display very important biological activities and have found application in the pharmaceutical and other industries. Many different types of alkaloids, which are compounds seen to be “like alkali” (having an amine group),[64] since the first extraction of these compounds was done using acid. The alkaloids have been isolated from various sources like fungi, mosses, frogs, and even mammals. The majority of these alkaloids have their biosynthetic origin from two amino acids, tyrosine and tryptophan. Isoquinoline **(2)** and tetrahydroisoquinoline **(3)** are examples of alkaloids which have their origin from the amino acid tyrosine **(1)** **(Figure 1)**.[1,2]



1



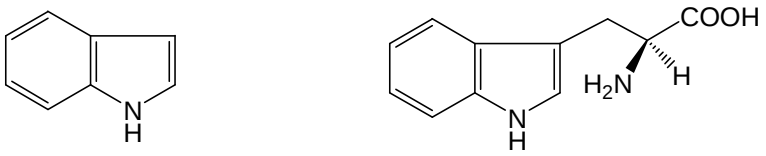
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3

Figure 1

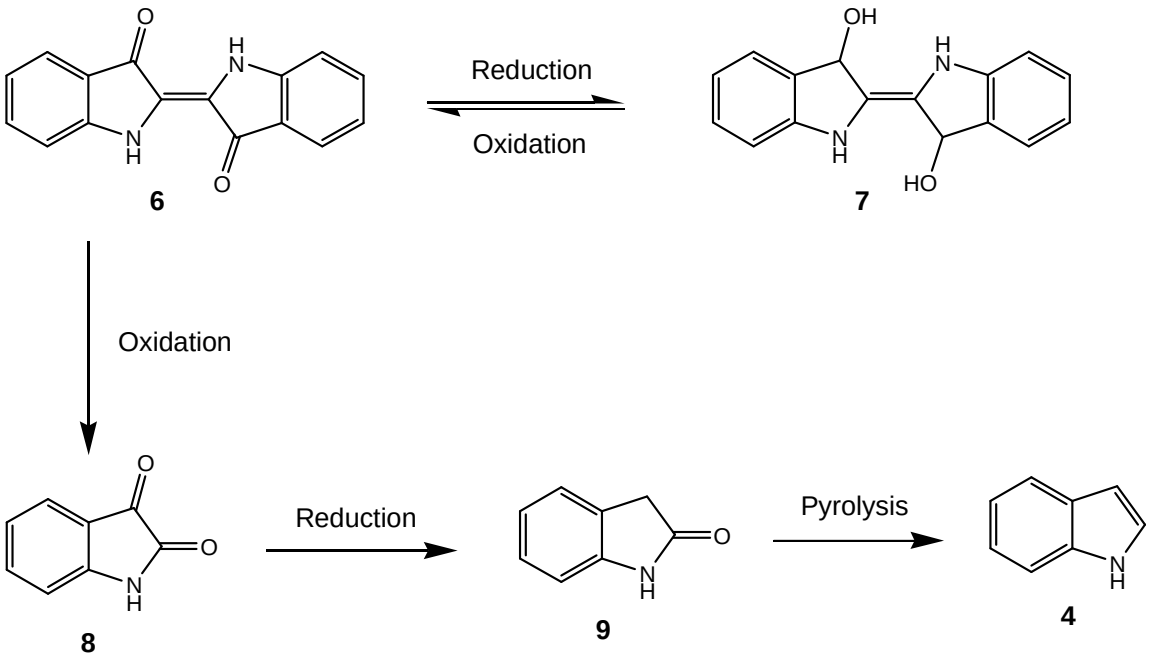
The indole nucleus **(4)**, which is another type of alkaloid structure that is present in many naturally occurring products, owes its biosynthetic origin to tryptophan **(5)**.[1,2]



4 **5**

Figure 2

The indole **(4)** nucleus is made up of a benzopyrrole in which a pyrrole ring is fused to a benzene ring through the α- and β-position of the pyrrole. The chemistry of indole began to develop when the science community was sensitised by indigo **(6)**, a dye used in the textile industry for its deep blue colour **(Scheme 1)**. It is found in a variety of indigo and *Polygonum* plants.[3,4]



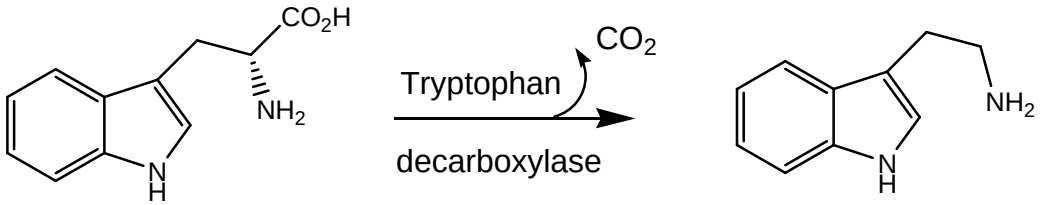
6 **7** **8** **9** **4**

Scheme 1

For example, in the process of dyeing textiles giving cotton a blue colour, indigo **(6)** is reduced to leucoindigo **(7)**. Then the fabric is soaked for a specified time

and allowed to air dry, which causes leucoindigo **(7)** to oxidize and thus precipitate in the fibers. In 1886 Adolf von Bayer obtained the indole nucleus by synthesis from oxindole, by the process of pyrrolysis using zinc dust. In this case, indigo was converted into isatin **(8),** via leucoindigo **(7)**. Isatin **(8)** was then reduced to give oxindole **(9),** which was converted to indole **(4) (Scheme 1).** [4]

As mentioned indole alkaloids occur in nature and have been isolated from a variety of sources, having different levels of complexity. Most of these structures are based on the structure of tryptophan or the decarboxylated form of tryptophan **(5)**, named tryptamine **(10)**. In nature, tryptamine **(10)** is formed by the catalysed decarboxylation of tryptophan mediated by the enzyme tryptophan decarboxylase **(Scheme 2)**.[5]



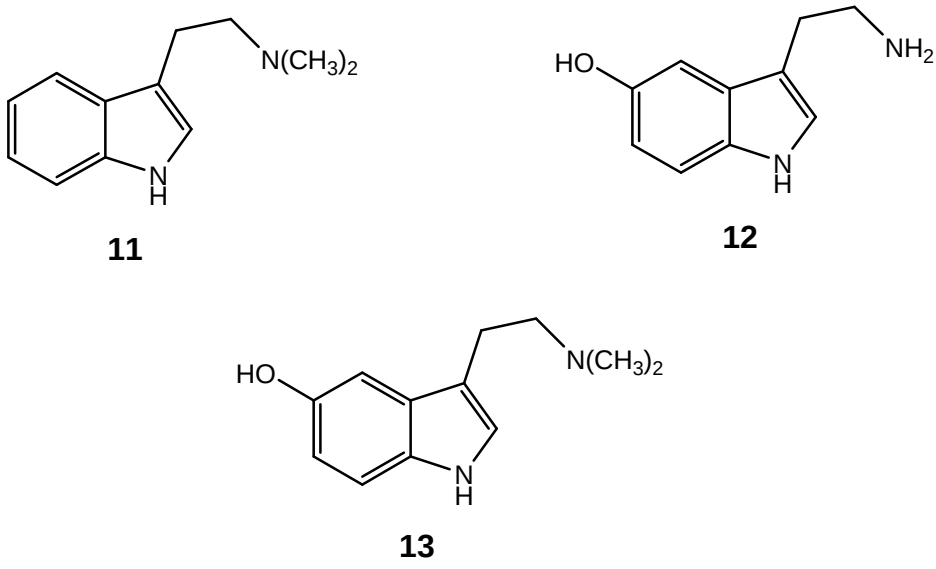
5 **10**

Scheme 2

Indole **(4)** has also been isolated from a variety of plant species. This includes *Jasminum* and *Citrus* plants**,** *Narcissus jonquilla* L., and *Chimonanthus fragrans* Lindl.[1] Industrially, indole is mainly produced from tar by distillation.[4]

Tryptamine **(10)** occurs in both plants and fungi and in many edible fruits, including plums and tomatoes. It also undergoes biological transformations which result in the formation of complex alkaloids.[1,5] Simple tryptamine derivatives include *N*,*N*- dimethyltryptamine **(11),** which is one of the hallucinogenic constituents found in the seed pods of *Piptadenia peregrine* (Benth), a plant used as a narcotic snuff by the American Indian tribes.[1] Other examples include 5-

hydroxytryptamine (serotonin) **(12)** found in bananas and stinging nettle. In mammals it serves as an important neurohormone. *N*,*N*-dimethylserotonin **(13)** is also an hallucinogen, isolated from certain fungi and secretions of the common toad obtained by N-methylation of **(12) (Figure 1)**.[1,6]

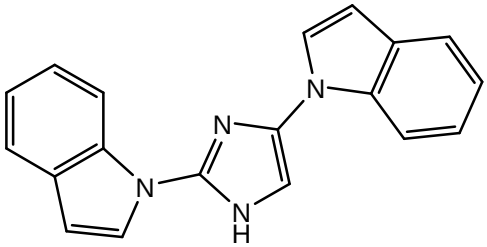


11 **12** **13**

Figure 3

Due to their varying complexity the indole alkaloids have been divided into smaller classes of alkaloid families, such that compounds which fall under the same family are structurally similar. A few recently discovered and naturally-occurring important indole alkaloids will be discussed in the following paragraphs.

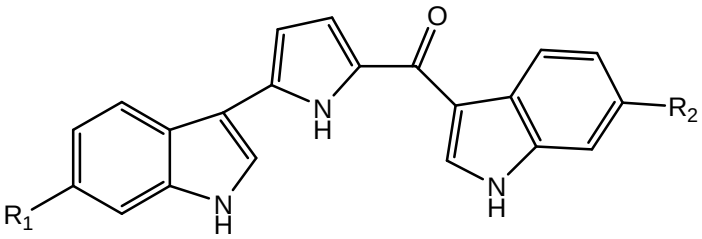
Marine sponges are a rich natural source of bis-indole alkaloids. Recently, new bis-indole alkaloids of the class topsentins,[7,8] nortopsentins,[8] dragmacidins[8] and hamacanthins[8] have been reported to be isolated from marine sponges. Topsentin **(14)**, is a *bis*-(indolyl)imidazole which was isolated from *Spongosorites genitrix* about a decade ago **(Figure 4)**. It exhibits with its related analogues, potent and diverse bioactivity including antiviral, antitumor, antibacterial, antifungal and antiinflammatory activity.[7,8]



14

Figure 4

Examples of these compounds which fall under the topsentin class include bromodeoxytopsentin **(15)**, and isobromodeoxytopsentin **(16)** which were recently isolated from a sponge *Spongosorites genitrix* Schmidt found at the coast of the Jaeju Islands in Korea. These compounds were found to have moderate activity against the human leukemia cell line K-562 **(Figure 5)**.[7]



15 R_1 = Br, R_2 = H
16 R_1 = H, R_2 = Br

Figure 5

(S)-6',6"-Debromohamacanthin A **(17)** and 1,2-bis-(1*H*-indol-3-yl)ethane-1,2-dione **(18)** are other interesting examples of bis-indole alkaloids isolated from the sponges *Spongosorites sp*[8] and *Smenospongia sp* (Thorectidae),[9] respectively. Hamacanthin **(17)** was shown to have marginal cytotoxicity against solid tumor cell lines **(Figure 6)**.[8]

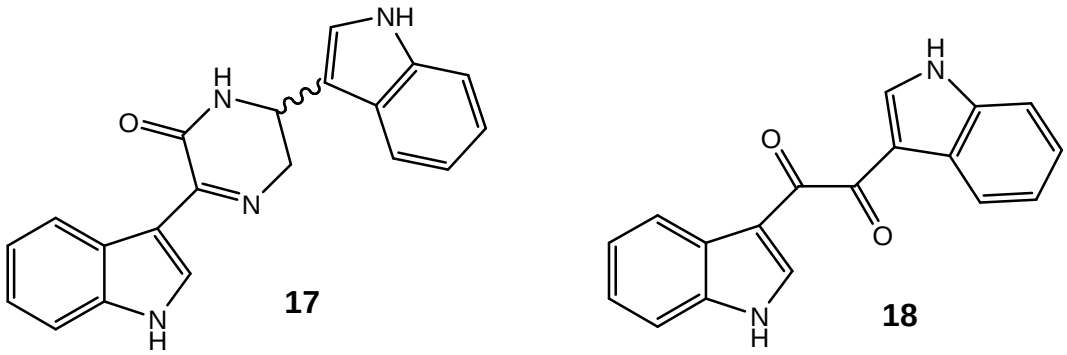


Figure 6

Another topical example is a bis-indole with a pyrimidine moiety named hyrtinadine A **(19)**, and was isolated from an Okinawan marine sponge *Hyrtios* sp. This natural product has been shown to exhibit cytotoxicity against murine leukemia L1210 cells **(Figure 7)**.[10]

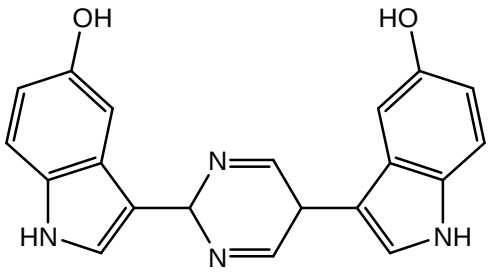


Figure 7

Other examples of recently discovered indole alkaloids, in no particular order are:

- The epipolythiodioxopiperazines - Chaetocochins C **(20)** and dethio-tetra(methylthio)chetomine **(21),** with one containing a sulfur bridge and being absent in the other. Both were isolated from the fermented rice culture of the fungus *Chaetomium cochliodes*. Compound **(21)** was found

to have very high toxicity against the cancer cell lines Bre-04 (MDA-MB-231), Lu-04 (NCI-H460) and N-04 (SF-268) **(Figure 8)**.[11]

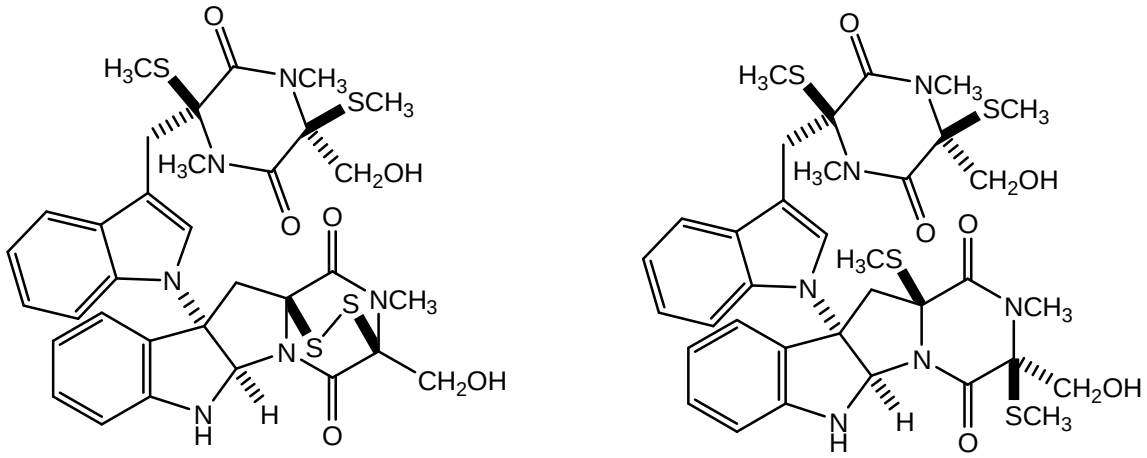


Figure 8

- Integerriomides A **(22)** and B **(23)** are cyclic heptapeptides which differ in the position of amino acids, forming a macrocyclic bonded to the 3-position of the indole nucleus. The compounds were isolated from the latex of *J. integerrima*. The natural-occurring compounds were found to have activity against neurite growth, cell proliferation and cell migration **(Figure 9)**.[12]

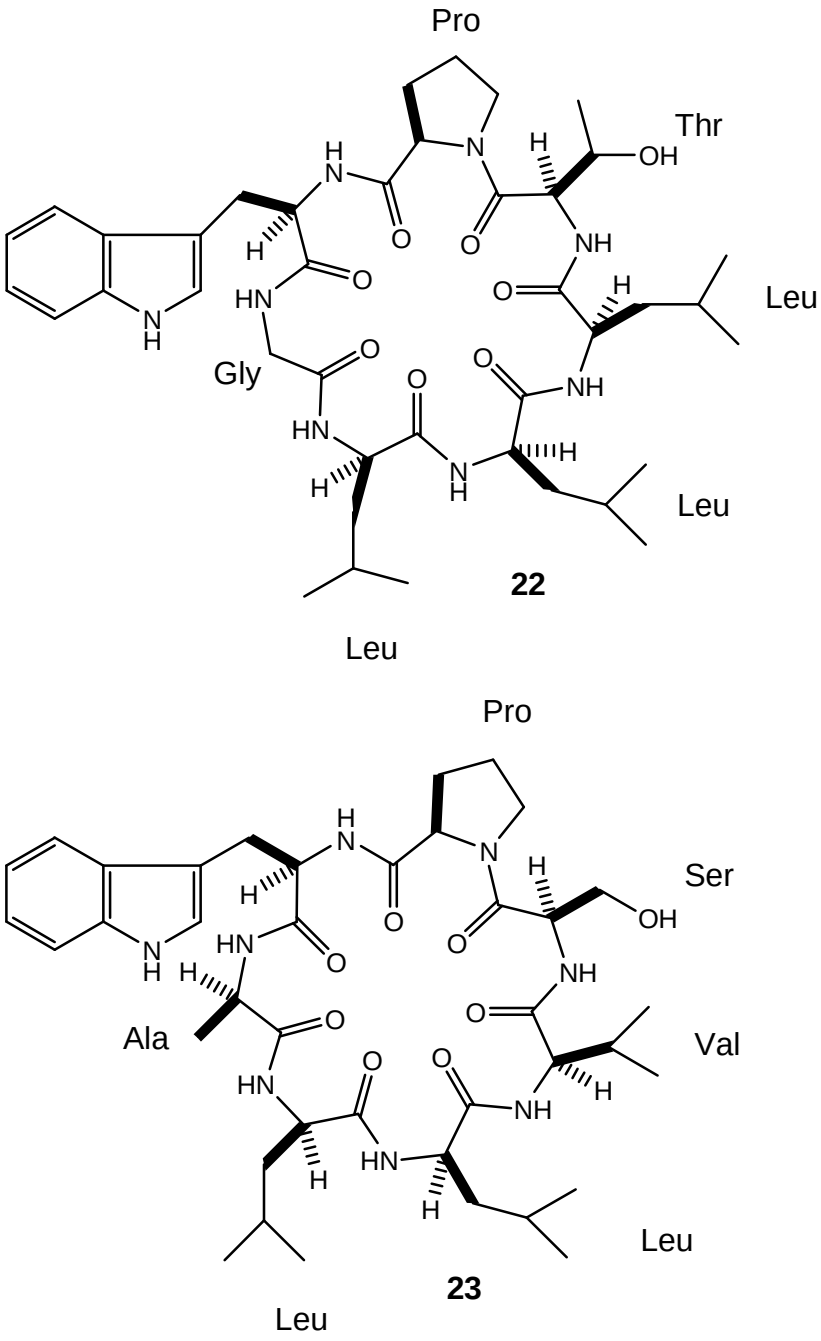
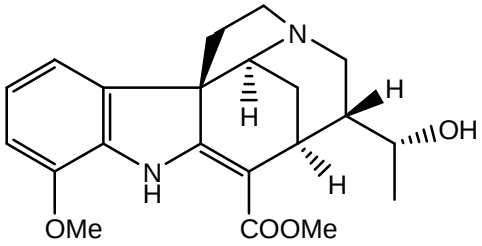


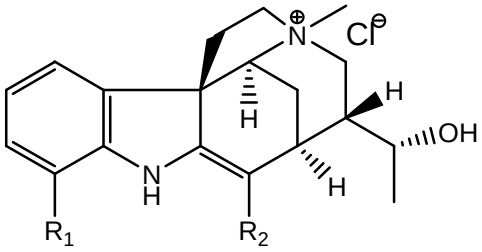
Figure 9

- *N*-(4)-Dimethyl-12-methoxyalstogustine **(24)**, 17-carboxy-12-methoxy-*N*-(4)-methylechitamidine chloride **(25)** and 12-methoxyechitamidine chloride **(26)**, all of which were isolated from stem bark of *Winchia calophylla* (a

plant found in China). Compound **(26)** showed weak cytotoxic activity against the cancer cell line A-549 **(Figure 10)**.[13]



24

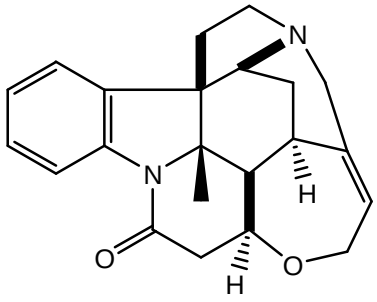


25 R_1 = OMe R_2 = COOH
26 R_1 = OMe R_2 = COOMe

Figure 10

Containing over 1800 members of rich structural diversity, monoterpenoid indole alkaloids are a great source of physiologically active molecules and are of major importance in the pharmaceutical industry. These alkaloids originate from the amino acid tryptophan **(5)** and a monoterpenoid, secologanin. In the second half of the century, the monoterpenoid indole alkaloids stimulated the development of a handful of drugs which became registered phamaceuticals.[1,5]

One such important natural occurring product which falls under the group of monoterpenoid alkaloids is strychnine **(27)**, which is one of the most complex alkaloids known, having seven fused rings and six stereogenic centers **(Figure 11)**.[14] It is used as a component in rat poisoning products and a homoeopathic drug.



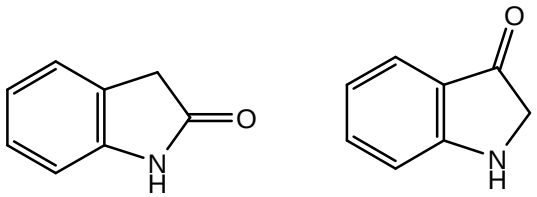
27

Figure 11

Even though strychnine **(27)** has been synthesized before,[14,15,16,17] many of these naturally occuring compounds have yet to be assemblied in the laboratory. Hence this creates a formidable challenge to synthetic chemists to develop viable synthetic routes to make these compounds, even to be used in large scale processes.

1.2 Biological Importance of Oxindole and its Derivatives

The oxindole system is an analogue of the indole nucleus which is a prominent structural motif, like indole, found in a large number of naturally occurring products. It is a benzopyrrolidinone, with the pyrrolidinone ring fused to the benzene ring, and the carbonyl group being either in the 2- **(9)** or the 3-position **(28)** of the nucleus **(Figure 12)**. [16]

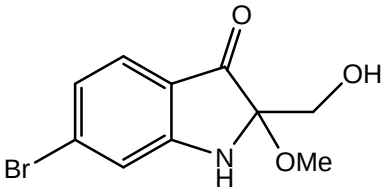


9 **28**

Figure 12

Naturally occurring oxindole alkaloids have been isolated from various sources and have been found to show biological activity against a large number of diseases.[16] Some examples of these will be illustrated below.

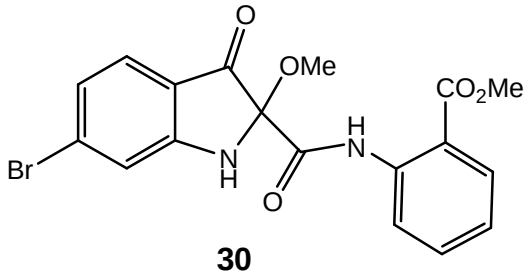
Recently discovered matemone **(29)**, which is bromine-containing 3-oxindole alkaloid, was isolated from an Indian Ocean sponge *Iotrochota purpurea*, found off the coast of Mozambican Matemo Islands **(Figure 13).**[17] Matemone **(29)** is marginally active against the bacterium *Staphylococcus aureus* and has significant binding affinity to DNA. It also showed activity against a panel of cancer cell lines.[17]



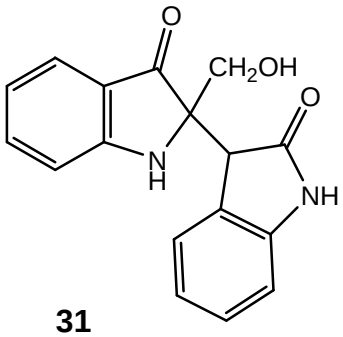
29

Figure 13

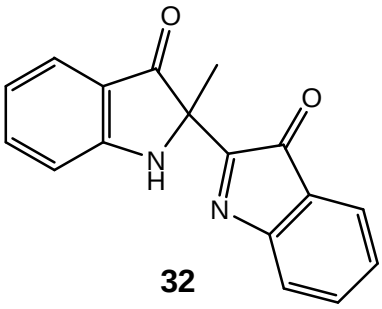
Another example of the 3-oxindole containing alkaloids are the cephalinones B **(30)**, C **(31)** and D **(32)** which occur as dimers. These compounds were isolated from *Cephalanceropis gracilis*, a native orchid found in Taiwan **(Figure 14)**. Only compound **(32)** showed cytotoxicity against cancer cell lines, namely MCF-7, NCI-H460 and SF-268.[18]



30



31



32

Figure 14

The last examples of naturally-occurring oxindole alkaloids described, are the paratumides A **(33)**, B **(34)**, C **(35)** and D **(36)**, having a 2-oxindole nucleus

instead of the 3-oxindole nucleus, linked to a secologanin unit **(Figure 15)**. These four compounds were isolated from the bark of *Cinnamodendron axillare* which is a plant found in Brazil also known as “paratude”. This plant is still used as a stomachic and for the treatment of tonsillitis.[19]

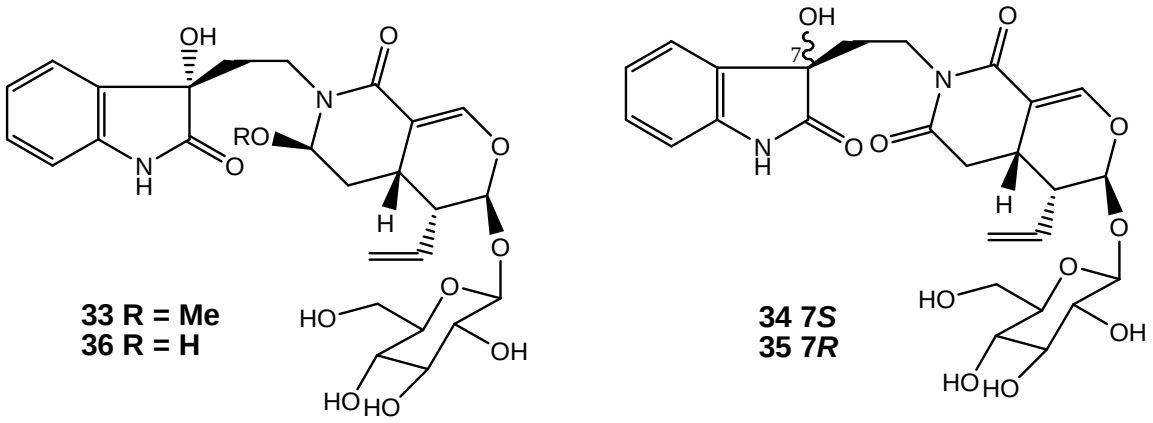


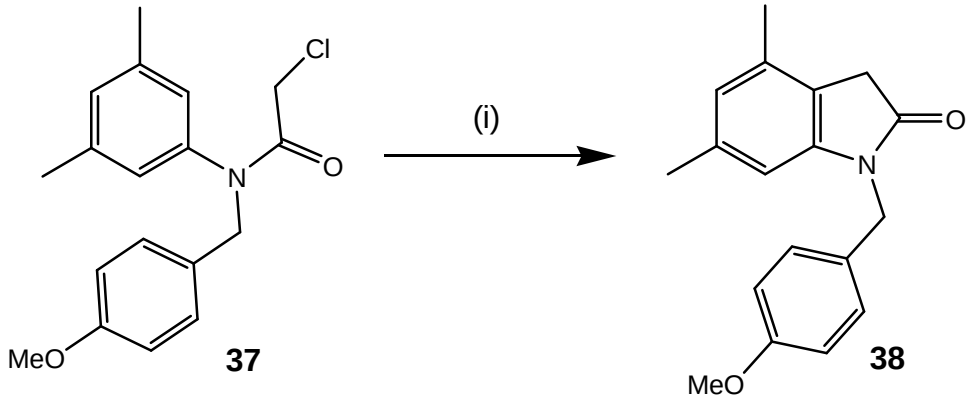
Figure 15

1.2.1 Previous synthesis of the oxindole nucleus

Due to the significant number of biologically active oxindole-containing alkaloids, it has been of importance that simple and efficient synthetic routes to create the oxindole nucleus (**9**) be available, especially from readily available materials.[16] In the following paragraphs, previous syntheses designed for the creation of the oxindole (or substituted oxindole) nucleus will be discussed.

In the past, oxindole syntheses have been limited, especially in creating substituted analogues. Among techniques commonly used is the Wolf-Kishner reduction of isatin,[20,21] or the oxidation of the corresponding indole nucleus.[20] Other classical techniques include the Friedel-Crafts cyclization of α-halo-[20,22] and hydroxyl-acetanilides.[20]

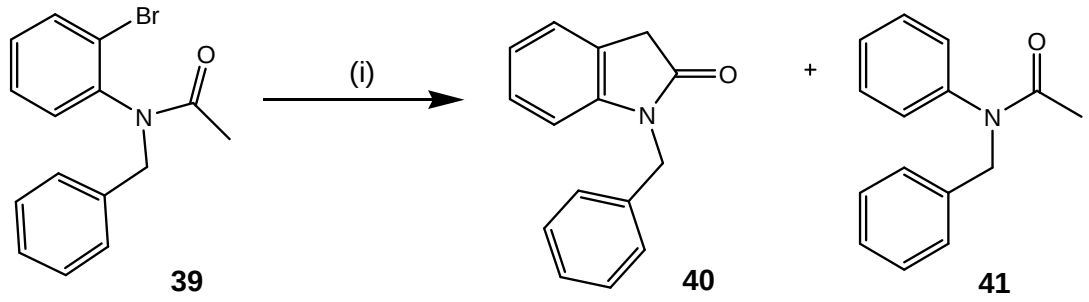
Recently Buchwald *et al.*[20] have used different "Friedel-Crafts cyclization methodology" to synthesize substituted oxindoles employing a palladium complex as a catalyst. This methodology was used on *N*-alkyl and *N*-aryl chloroacetanilides, which have advantages over the classical Friedel-Crafts approach as it uses lower temperatures and many functional groups (electron donating or electron withdrawing) can tolerate the reaction conditions, unlike the strong Lewis acids used in the classical Friedel-Crafts conditions. For example, when *N*-(*p*-methoxybenzyl)-3,5-dimethylchloroacetanilide **(37)** was subjected to the reaction conditions described above, it gave the desired oxindole **(38)** in 84% yield **(Scheme 3)**.[20]



Scheme 3: (i) 3 mol% $Pd(OAc)_2$, 3 mol% 2-(di-tert-butylphosphinobiphenyl)biphenyl, 1.5 equiv. Et_3N, toluene, 80^oC, 3hrs.

An intramolecular enolate arylation to produce oxindole, employing palladium metal as a catalyst was demonstrated by Hartwig *et al.*[22] 2-Bromoanilides were subjected to an organopalladium complex with a base to give 3-substituted oxindoles **(Scheme 4)**. The yields of the reactions depended on the combination of base and ligand used. Isoquinoline-type compounds were synthesized following the same procedure and the yields were also dependant on the base and ligand. For example *N*-benzyl-2-bromoacetanilide **(39)** was subjected to $Pd(dba)_2$ with BINAP, in the presence of sodium *tert*-butoxide, to give the desired

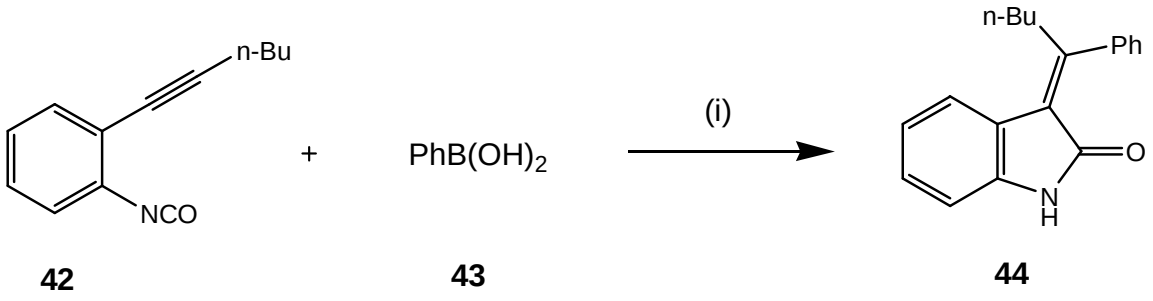
N-benzyl oxindole **(40)** in 66% yield, as well as compound **(41)** in 27% yield (debrominated starting material) **(Scheme 4)**.[22]



Scheme 4: (i) 5% $Pd(dba)_2$, 7.5% BINAP, 1.5 equiv., sodium *tert*-butoxide.

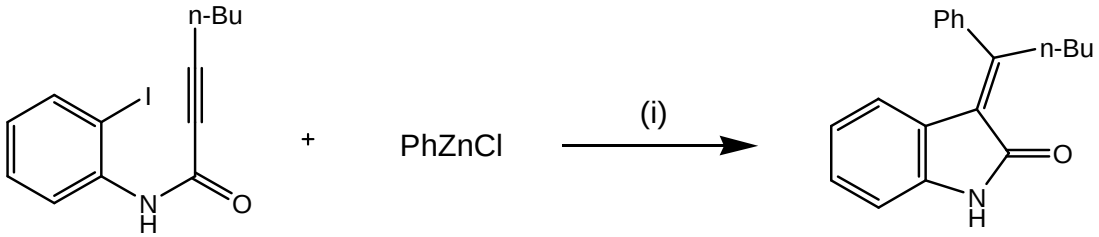
Another transition metal that is gaining popularity for the synthesis of oxindole and oxindole analogues is rhodium, as it is found to catalyse the addition of organoboron[23] and organometallic[24] reagents to unsaturated organic compounds. Two examples are described below.

With 2-alkynylaryl isocyanates and arylboronic acids as substrates, the reaction proceeds via a rhodium/boron transmetalation to give an organorhodium(I) species. This is followed by the carborhodation step after the co-ordination of rhodium by the alkyne and isocyanate groups of the two substrates to give a 3-substituted oxindole system. 2-(1-Hexynyl)phenyl isocyanate **(42)** was reacted with phenylboronic acid **(43)** in the presence of the rhodium complex to give 3-alkylideneoxindole **(44)** as a single stereoisomer **(Scheme 5)**.[23]



Scheme 5: (i) 2.5 mol% $[Rh(OH)(cod)]_2$, THF, rt, 2hrs, 78%.

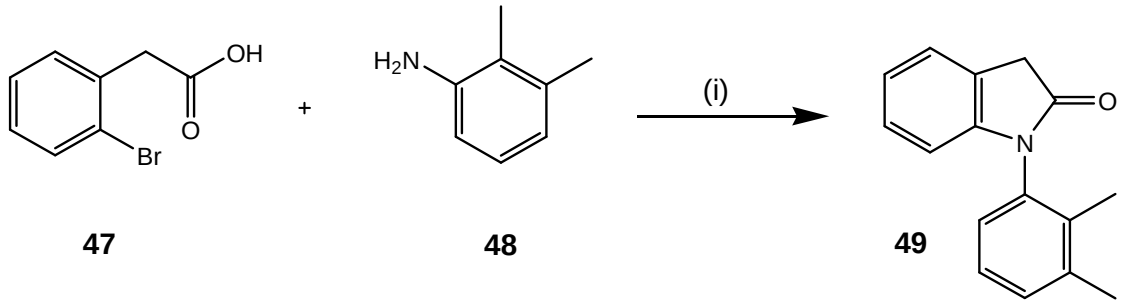
Using slightly different reagents, organoalkynyl halides with organometallic reagents in the presence of a different rhodium catalyst, one can get similar products (or other stereoisomers as in this case) with slightly higher yields. The reaction sequence starts at the same point with transmetallation between the organometallic reagent **(46)** and the rhodium catalyst, followed by the insertion of the organorhodium species into the alkynyl part of the organoalkynyl halide species **(45)**. Then oxidative addition follows and a subsequent reductive elimination gives the desired 3-substituted oxindole compound **(44) (Scheme 6).**[24]



45 **46** **44**

Scheme 6: (i) $[RhCl(C_2H_4)_2]_2$, DPPF, dioxane, 40^{o}C, 20hrs, 87%.

Other synthetic strategies have been developed by many groups to synthesize the oxindole nucleus or its analogues. One of the methods developed is the use of microwaves to drive reactions to completion in less time when compared to reactions that are heated by conventional methods for longer periods of time. This includes palladium-catalysed amidation reactions that take approximately 20 hrs to run to completion.[16,25] However, Turner *et al*. managed to synthesize oxindole analogues using a microwave reactor. First, a 2-haloarylacetic acid **(47)** was coupled to dimethylaniline **(48)** to form an amide using microwave-mediation. This was followed by a microwave-assisted palladium-catalysed amidation reaction to furnish the oxindole **(49)** in moderate to good yields **(Scheme 7)**.[16]



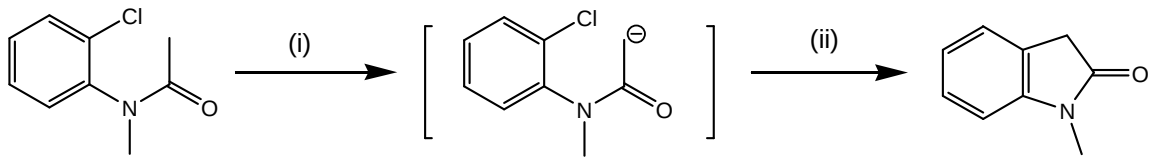
47 **48** **49**

Scheme 7: (i) (a) MW, 200W, 100^{o}C, 30 min.; (b) $Pd(OAc)_2$, phosphine ligand, Cs_2CO_3, toluene, MW, 200W, 100^{o}C, 30 min, 82%.

This confirms that the use of transition metals is of major importance in organic synthesis because transition metals provide alternative methods to get to target molecules such as the oxindole nucleus.

Some organic groups have dedicated their efforts in devising synthetic strategies that do not employ the use of transition metals for the synthesis of the oxindole nucleus.

The first example of this sort to be described here is the cyclization of the *N*-acyl-*o*-chloroaniline **(50)**, which was done by treating the precursor with excess lithium diisopropylamide, followed by irradiation of the reaction contents with light to give the oxindole **(51) (Scheme 8)**.



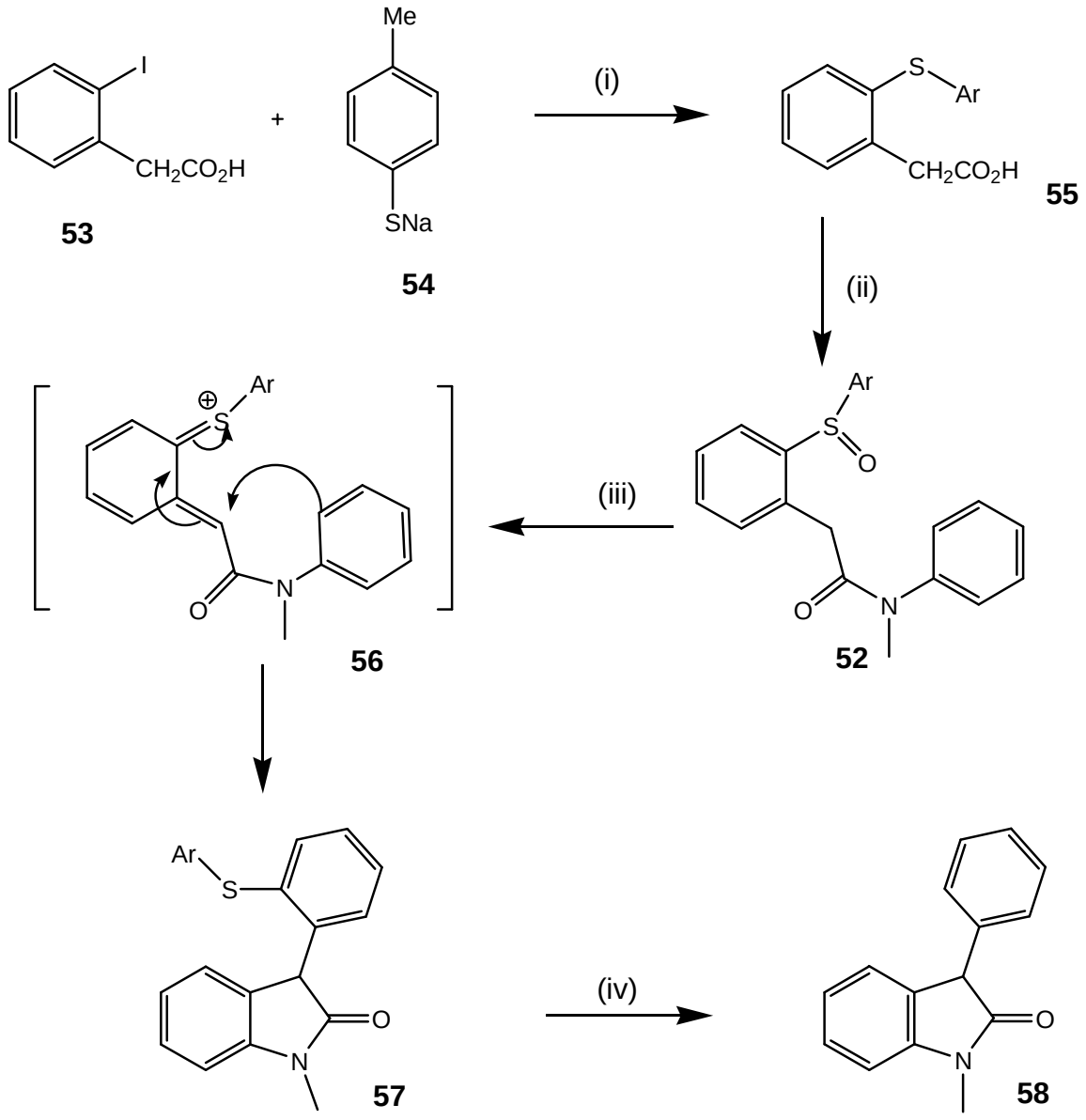
50 **51**

Scheme 8: (i) LDA, THF, -78^{o}C; (ii) hv, -78^{o}C, 83%.

The excess LDA produces a carbonanion intermediate which is generated after removal of the acidic proton on the α-carbon of the carbonyl system, which is

then followed by photo-stimulated ring closure process.[26] This methodology was also extended to making azaoxindole from 2-chloro-3-(*N*-methylacetamido)pyridines.[26]

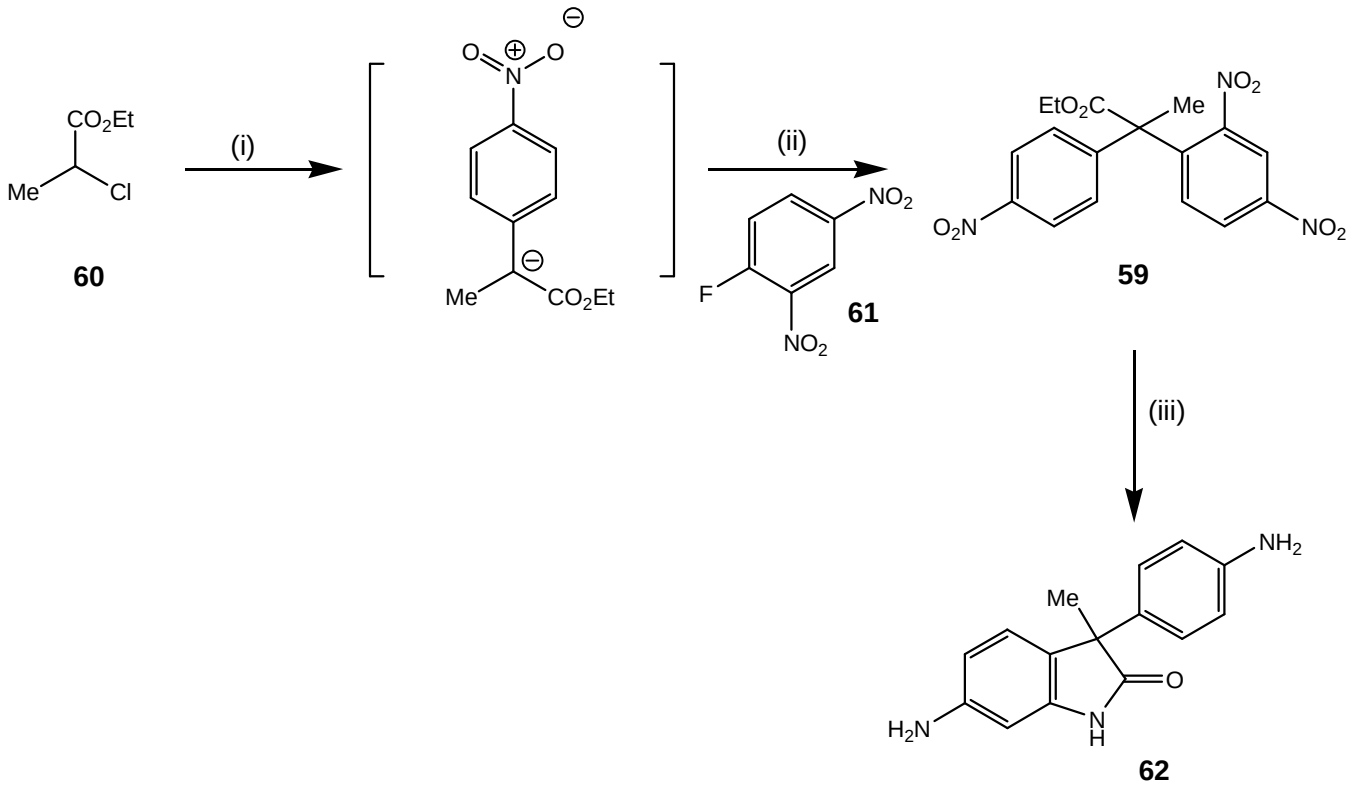
Oxindole derivatives have also been synthesized employing a Pummerer rearrangement by Padwa *et*. *al*., methodology which has been widely studied and given much attention of late as a useful synthetic process.[27]



Scheme 9: (i) $(bpy)_2NiBr_2$, 83%. (ii) a. ClCOCOCl/MeNHPh; b.Ti(III)-H_2O_2, 81%. (iii) TFAA, DCM, 25^oC, 78%. (vi) Ra/Ni, 80%.

Padwa started by synthesizing an aryl amido sulfoxide **(52)**, generated from the bis(bipyridyl)nickel(II) catalysed thioarylation of 2-iodophenylacetic acid **(53)** with *p*-thiocresolate **(54)** to give **(55)**. This was followed by the conversion of **(55)** to the amide and the titanium(III)-hydrogen peroxide oxidation to afford **(52)**. This compound was reacted with trifluoroacetic acid anhydride to give 3-substituted oxindole **(57)** via intermediate **(56)**. Removal of the sulfur substituent was done by treating **(57)** with Raney Nickel to afford the final product **(58) (Scheme 9)**. [27]

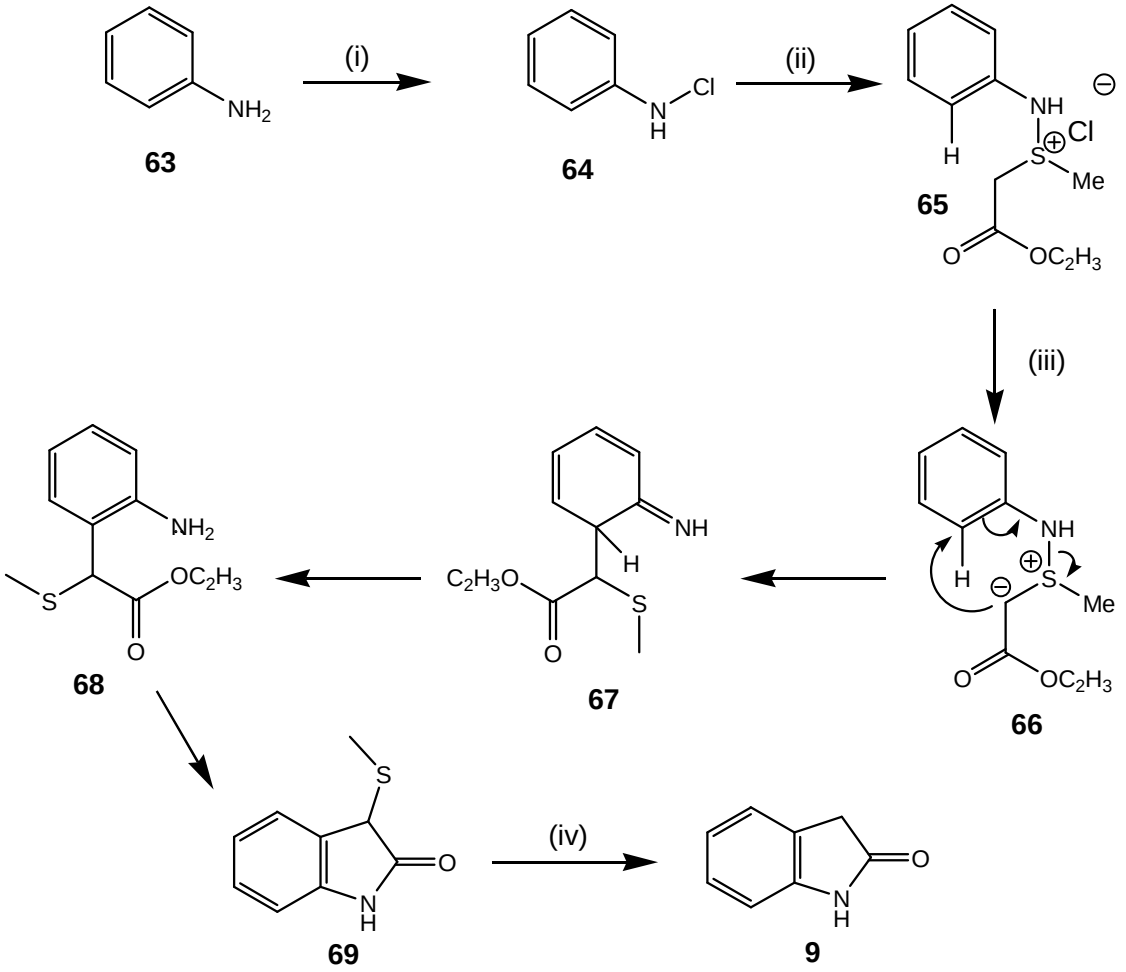
Diarylacetates generated from a vicarious nucleophilic substitution process, followed by a normal nucleophilic substitution reation (VNS_{Ar}-S_NAr), have been transformed into oxindole systems. VNS_{Ar} is a process that provides a direct and efficient route to the coupling of functionalized aromatics and generally a process in which simple precursors can be elaborated a three-component coupling reactions.



Scheme 10: (i) NaH, nitrobenzene; (ii) 2,4-dinitrofluorobenzene; (iii) H_2, Pd/C, EtOAc, AcOH, r.t., 16-20 h

The synthesis of the diarylacetate **(59)** involved the reaction of the ester **(60)** with nitrobenzene in the presence of a base followed by the addition of 2,4-dinitrofluorobenzene **(61)**. The product **(59)** was then exposed to reducing conditions to give the 3,3-disubstituted oxindole **(62) (Scheme 10)**.[28]

Gassman *et al*. developed a simple one pot reaction for the synthesis of substituted indoles, oxindoles and isatins. [29,30,31,32] The reaction mechanism for the synthesis of indole and the oxindole follow the same pathway, with 3 distinct steps. In the first step, the aniline **(63)** was reacted with *tert*-butyl hypochlorite or a suitable chlorinating agent to give the chloroamine **(64)**. The second step was the addition of the disubstituted sulfide to react with the chloroamine **(64)** to give the azasulfonium salt **(65)**, which has acidic protons on the carbon adjacent to the sulfur. When the sulfide contains a β-keto function 3-thioindoles are formed, but when the sulfur is β to the carboalkoxy group then acid is added to produce a 3-thiooxindole system.[29] The 3-thioindole and oxindole were exposed to Raney nickel for the removal of the sulfur group to give the desired indole and oxindole molecules, respectively.[29,30] The addition of a base, in this case triethylamine (third step), deprotonates the carbon adjacent to the sulfur atom to form an intermediate sulfonium ylide **(66)**. The sulfonium ylide **(66)** quickly undergoes an intramolecular Sommelet-Hauser-type rearrangement[29,30] with attack on the aromatic ring *ortho* to the amino functionality to give imine species **(67)**. Then the ring of **(67)** re-aromatizes to the amine **(68)**, and is followed by a ring closure to form the 3-thiomethyloxindole **(69)**. Treating the 3-thiomethyloxindole **(69)** with Raney nickel then affords the desired oxindole **(9) (Scheme 11)**. [30]



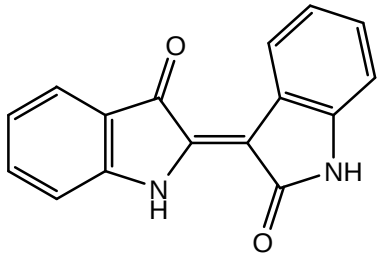
Scheme 11: (i) ^{t}BuOCl; (ii) $CH_3SCH_2CO_2C_2H_3$; (iii) Et_3N; (iv) Ra/Ni, EtOH, rt.

1.2.2 Oxindoles in Medicinal Chemistry

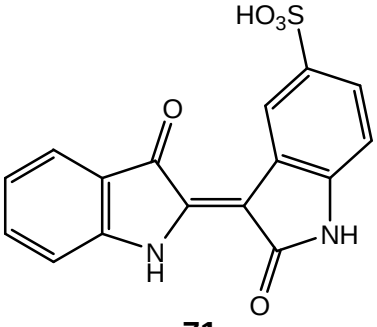
Recently organic chemistry has shifted from just the classical synthesis of target molecules to include the synthesis of low molecular weight molecules for biological testing against a large number of bacteria, viruses and other diseases such as cancer that cause harm to the normal functioning of the human body or can even cause death.

One such class of compounds is the 3-substituted oxindoles. Many simple and complex 3-oxindoles have thus been designed, synthesized and screened for activity against diseases targeting specific enzymes. For example, efforts related to this MSc have been made to design molecules that inhibit cyclin dependent kinases (CDKs) which have a vital role of regulating cell cycle events in malaria[33] and a variety of human cancer cells.[34] The activity of CDKs depend on a regulatory subunit called cyclin which binds to and activates the relevant CDKs. Direct inhibitors are preferably low molecular weight compounds that block the enzymatic activity of the CDKs.[36]

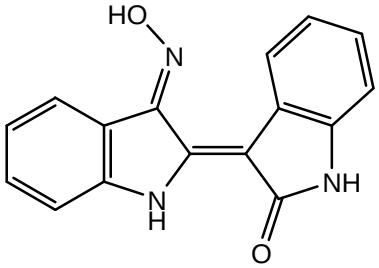
Indirubin **(70)**, which is an active ingredient from Danggui Longhui Wan, a mixture of plants that was first used for the treatment of chronic diseases, such as leukemia, centuries ago, has demonstrated high selectivity for CDKs.[34,36] Indirubin and a few synthesized indirubin analogues like indirubin sulfonic acid **(71)** and indirubin monoxime **(72)** were tested and found to inhibit CDK-1, -2 and -5 by binding to the ATP site of the enzyme and inhibiting the normal functioning of the enzyme ATP complex **(Figure 16)**. [34]



70



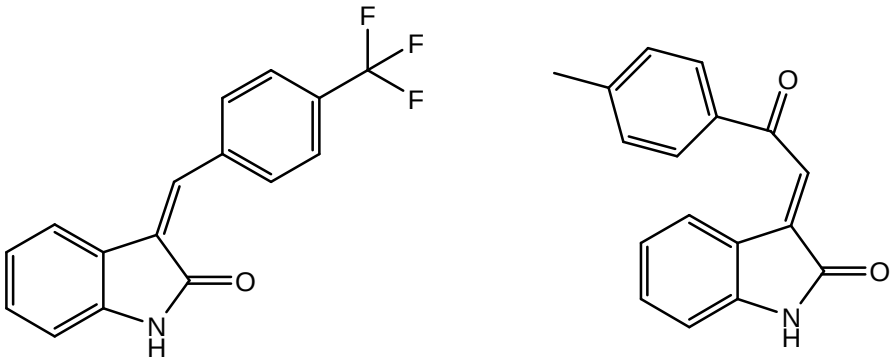
71



72

Figure 16

PfPK5 and Pfmrk are two CDKs found in *Plasmodium falciparum*, the agent causing the most severe form of human malaria. Pfmrk is most homologous to CDK-7 (mammalian kinase), which has the function of activating kinases in mammalian cells. 3-Subsituted oxindoles were synthesized and screened against Pfmrk, and compound **(73)** and **(74)** were the most active against Pfmrk but did not inhibit PfPK5 **(Figure 17)**. [33]

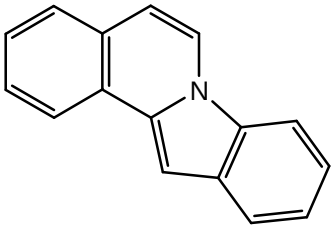


73 **74**

Figure 17

1.3 Dibenzopyrrocoline Alkaloids, Indolo[2,1-a]isoquinolines and Related Analogues

Thus far we have discussed the biological importance of the indole and oxindole nucleus in nature and in the pharmaceutical industry. We will now discuss the importance of the dibenzopyrrocoline alkaloids, the indolo[2,1-*a*]isoquinoline **(75)** derivatives **(Figure 18)** and its related analogues as they form part of the topic of this MSc dissertation.

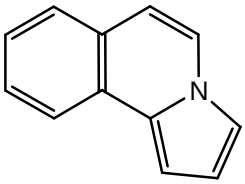


75

Figure 18

These compounds are made from an indole nucleus fused to an isoquinoline system sharing the same nitrogen. The dibenzopyrrocoline alkaloids form part of the same family as the isoquinolines and the tetrahydroisoquinolines, possessing a 5,6,12,12*a*-tetrahydro-indolo[2,1-*a*]isoquinoline skeleton.

The indolo[2,1-*a*]isoquinoline **(75)** and the related pyrrolo[2,1-*a*]isoquinoline **(76)** **(Figure 19)**, without an additional benzene ring fused to the five membered ring, are some examples which form part of this family and these types of compounds have been found to display important biological activities.

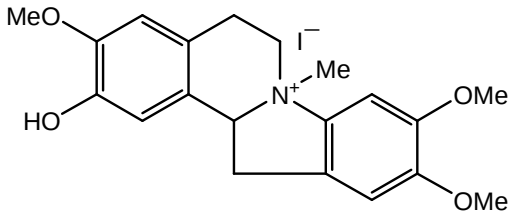


76

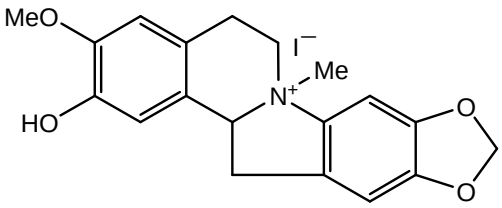
Figure 19

For example, natural-occuring products which possess the indole[2,1-*a*]isoquinoline backbone, cryptaustoline **(77)** and cryptowoline **(78)**, were isolated from a bark of *Cryptocaria bowie*, a tree indigenous to Australia, as their water soluble salts.[37] These two alkaloids have been found to possess antileukemic,[38] tubulin polymerization inhibitory[39] and antitumor activity.[40,41] In addition, the

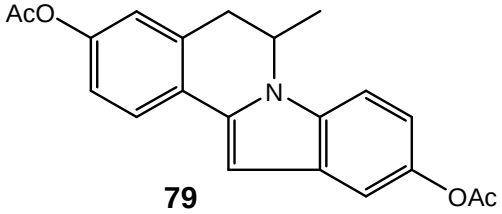
synthetic acetoxy-substituted 5,6-dihydro[2,1-*a*]isoquinoline **(79)** showed a strong binding affinity for the oestrogen receptor of MDA-MB 231 and MCF-7 mammary tumor cells **(Figure 20)**.[42] It is important to note that apart from the oxidation state of the nucleus, the examples shown below differ only in the oxygen containing substituents attached on the main backbone.



77



78



79

Figure 20

Other related examples include cryptolepine (5-methyl-5*H*-indolo[3,2-*b*]quinoline) **(80)**, neocryptolepine (cryptotackeine, 5-methyl-5*H*-indolo[3,2-*b*]quinoline) **(81)** and isocryptolepine (cryptosanguinolentine, 5-methyl-5*H*-indolo[3,2-*b*]quinoline) **(82)** isolated from the root of a tree in West Africa known as *Cryptolepis sanguinoleta*. These compounds are also made from an indole and isoquinoline nucleus but contain two nitrogen atoms rather than one shared atom **(Figure 21)**. The decoction of the root of this tree was used to treat fevers caused by malarial infection and it was later found that the three isomeric indoloisoquinolines are responsible for the activity against the fever. Recently these compounds have also been found to have antiplasmodial activity.[43]

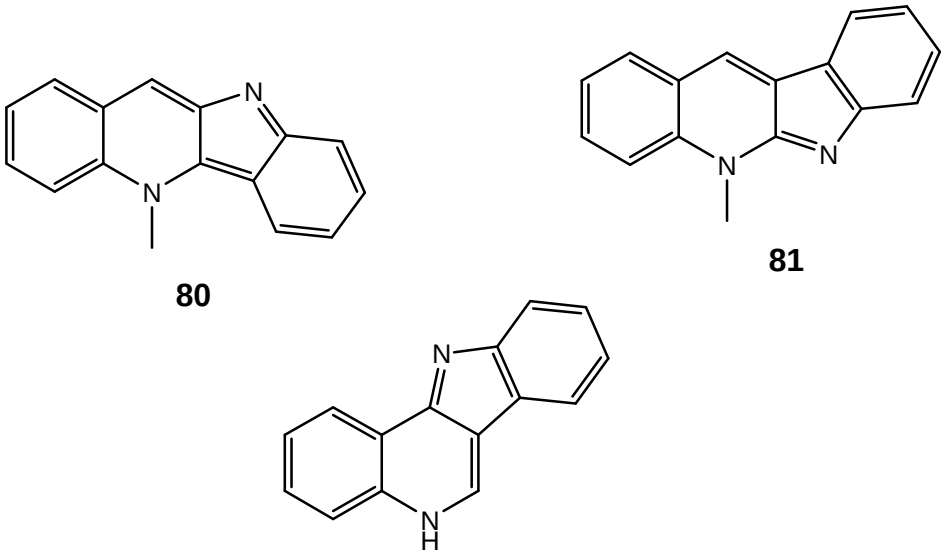


Figure 21

Other related compounds such as the hexahydropyrrol[2,1-*a*]isoquinoline alkaloids **(83)** and **(84)**, which do not have an additional aromatic ring fused to the five membered pyrrole ring, have been reported to inhibit neural uptake of serotonin (5-HT), norepinephrine and dopamine, making them potential candidates for the treatment of obesity and depression **(Figure 22)**.[44,45]

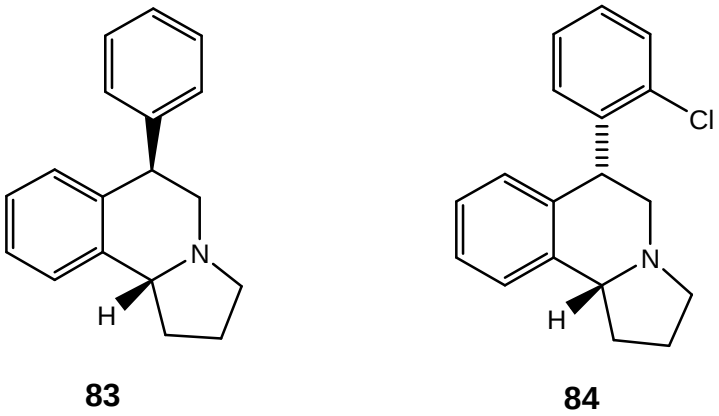
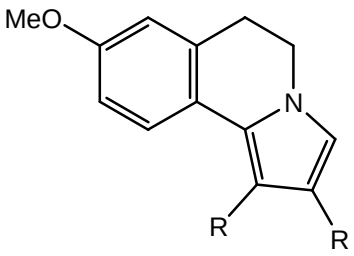


Figure 22

Another example of this class of alkaloids is the pyrrolo[2,1-*a*]isoquinoline bis(carbamate) **(85),** which was found to exhibit antineoplastic activity and possess antileukemic activity **(Figure 23)**.[46] These kinds of alkaloids are related to the indolo[2,1-*a*]isoquinolines and dibenzopyrrocoline alkaloids and fall under the pyrrolo[2,1-*a*]isoquinoline class.



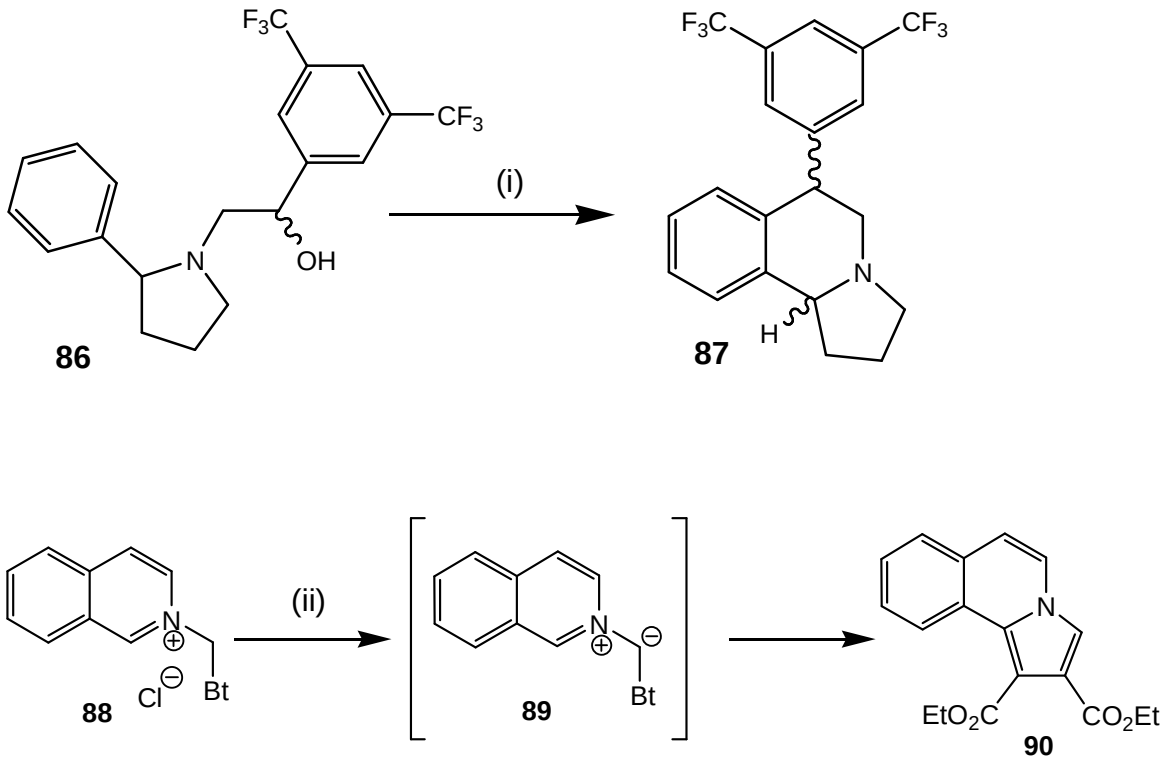
R = $CH_2OCONHCH(CH_3)_2$

85

Figure 23

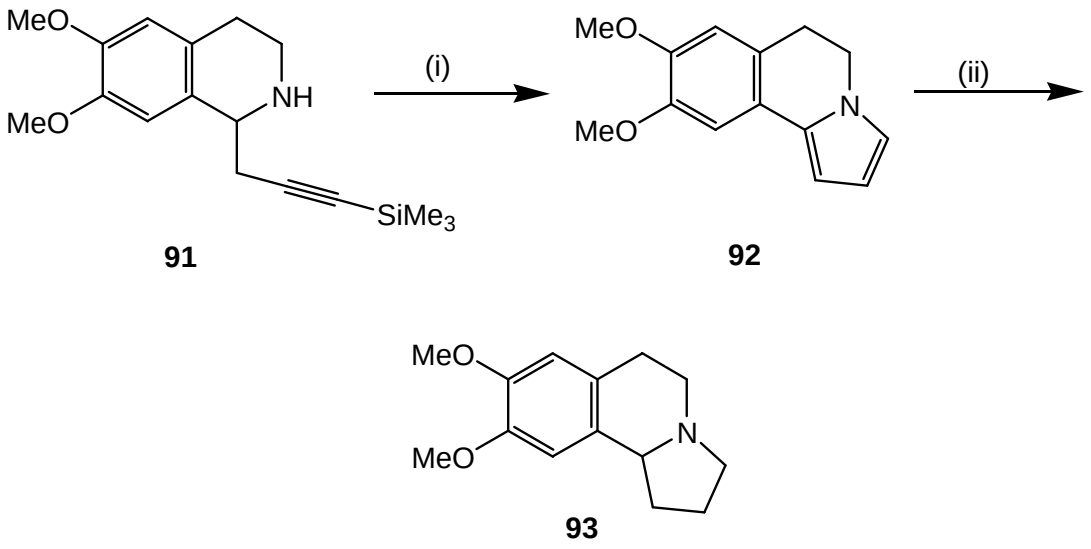
1.3.1 Previous Syntheses of the Indolo[2,1-a]isoquinolines and Related Analogues

A number of syntheses have been reported for obtaining the pyrrolo[2,1-*a*]isoquinoline and indolo[2,1-*a*]isoquinoline skeletons. One of the many examples involves using an acid-catalyzed reaction for the final ring closure to obtain pyrrolo[2,1-*a*]isoquinolines. The key step which involved the cyclization of compound **(86)**, gave the product **(87)** as a mixture of the *cis* and the *trans* isomers **(Scheme 12)**.[44] The 1,3-dipolar cycloaddition has also been one of the major methods used to afford these types of alkaloid systems. For example, the reaction of **(88)** with diethyl acetylenedicarboxylate, in the presence of triethylamine, gave the desired pyrrolo[2,1-*a*]isoquinoline **(90)**, by way of intermediate (**89)**.



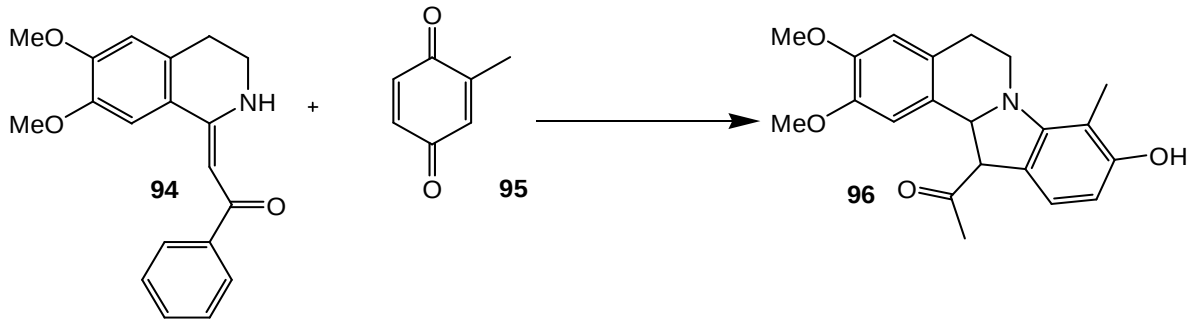
Scheme 12: (i) Polyphosphoric acid, heat, 47%; (ii) $EtCO_2C{\equiv}CCO_2Et$, Et_3N, MeCN, 67%.

Another route was used by Knölker *et al*. in the synthesis of a naturally-occurring pyrrolo[2,1-*a*]isoquinoline analogue, Crispine A. [47] This compound was isolated from a plant in China, *Carduus crispus*, and has been used in the treatment of colds, stomach aches and rheumatism.[48] The synthesis includes a key step which uses a silver(I)-promoted oxidative cyclization of the trimethylsilyl alkyne **(91)**, followed by the hydrogenation of **(92)**, which gave the final product **(93)**, as shown by **Scheme 13**.



Scheme 13: (i) AgOAc, CH_2Cl_2, rt, 14hrs; (ii) 5 mol % Rh/C, H_2, AcOH/MeOH, rt, 192hrs.

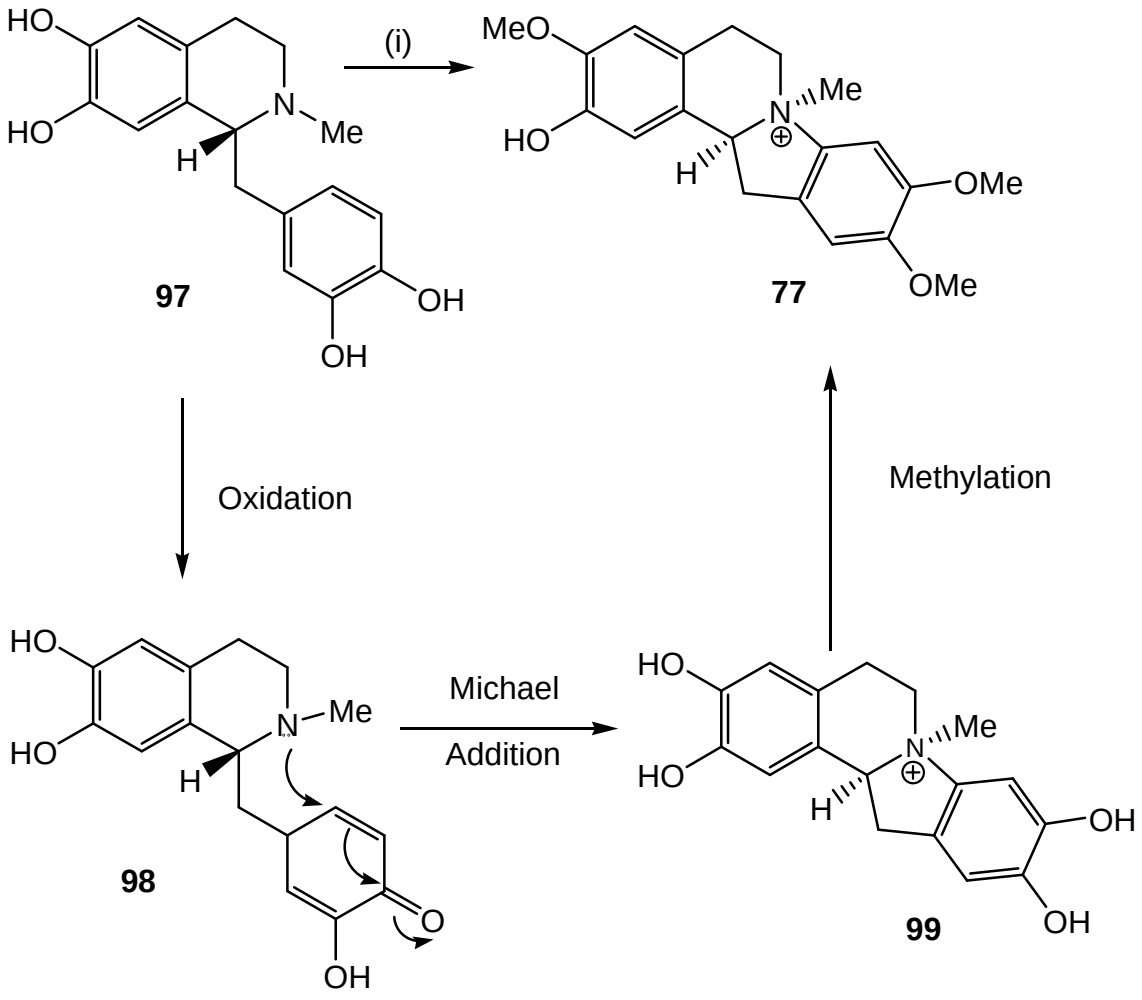
One route which is used by researchers at the Indian Institute of Technology to construct the indolo[2,1-*a*]isoquinoline skeleton, employed the Nenitzescu reaction between tetrahydroisoquinoline-derived enaminones like compound **(94)** and *p*-benzoquinone **(95)**.[49] For example, the synthesis of indolo[2,1-*a*]isoquinoline **(96)** was achieved by reacting the enaminone **(94)** with *p*-benzoquinone **(95)** at room temperature in nitromethane, as depicted in **Scheme 14**.



Scheme 14: CH_3NO_2, rt, 2 days.

Another route employed benzylisoquinoline as an advanced starting material. For example, the synthesis of (–)-cryptaustoline by Brossi *et al*.,[50] started with (+)-

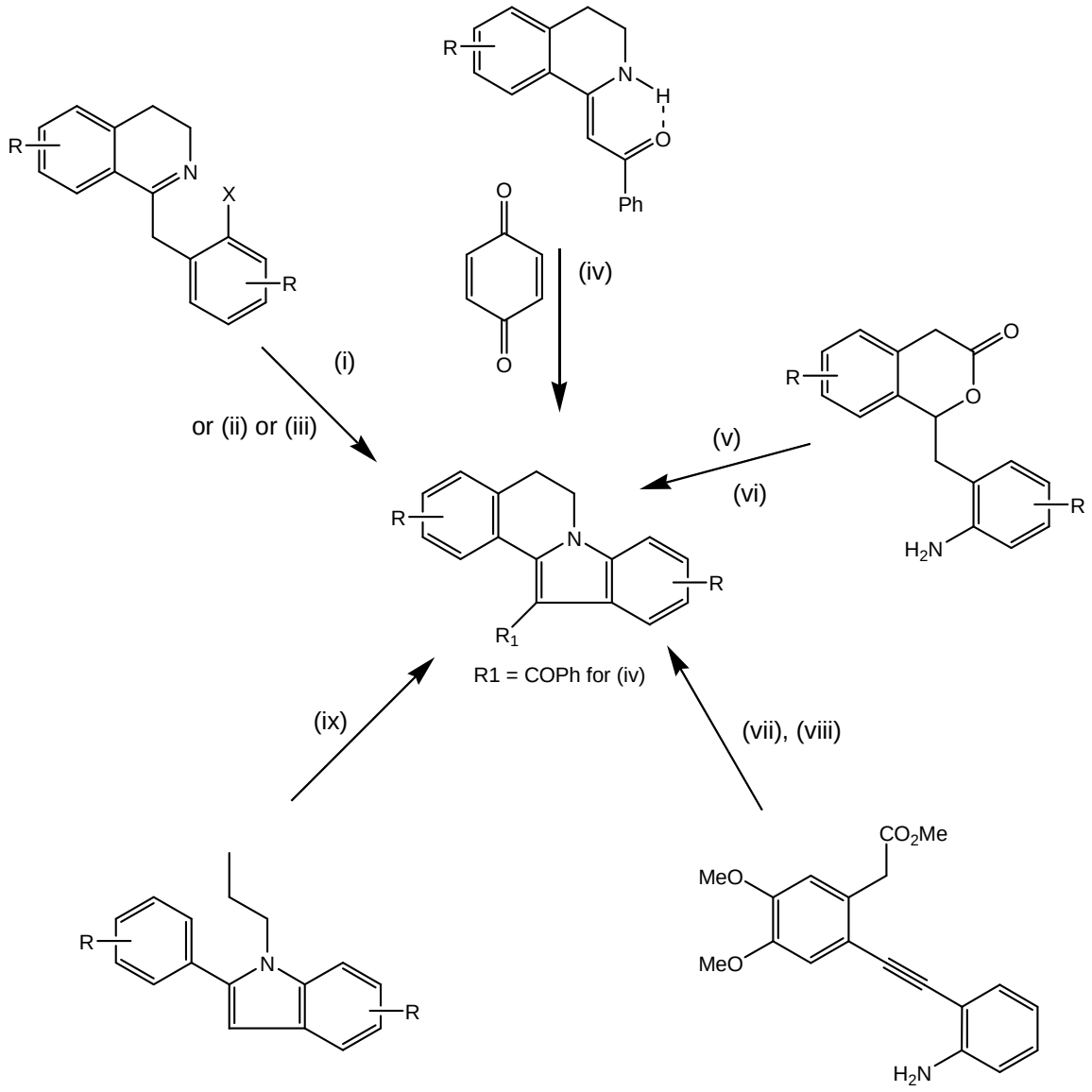
laudanolosine **(97)**. They then performed an oxidative cyclization using a peroxidase-hydrogen peroxide mixture, which resulted in laudanosoline being oxidized to the Michael acceptor intermediate **(98)**. The cyclised product **(99)** then formed by way of a Michael addition, and the subsequent selective methylations of the phenol functional groups resulted in (–)-cryptaustoline **(77)** being obtained.[51,52]



Scheme 15: (i) HRP-H_2O_2, MeI.

Various other synthetic routes have been used to obtain the target indolo[2,1-*a*]isoquinolines. Other general methods for the formation of the final ring which forms the indolo[2,1-*a*]isoquinolines are shown in **Scheme 16**. It is evident that

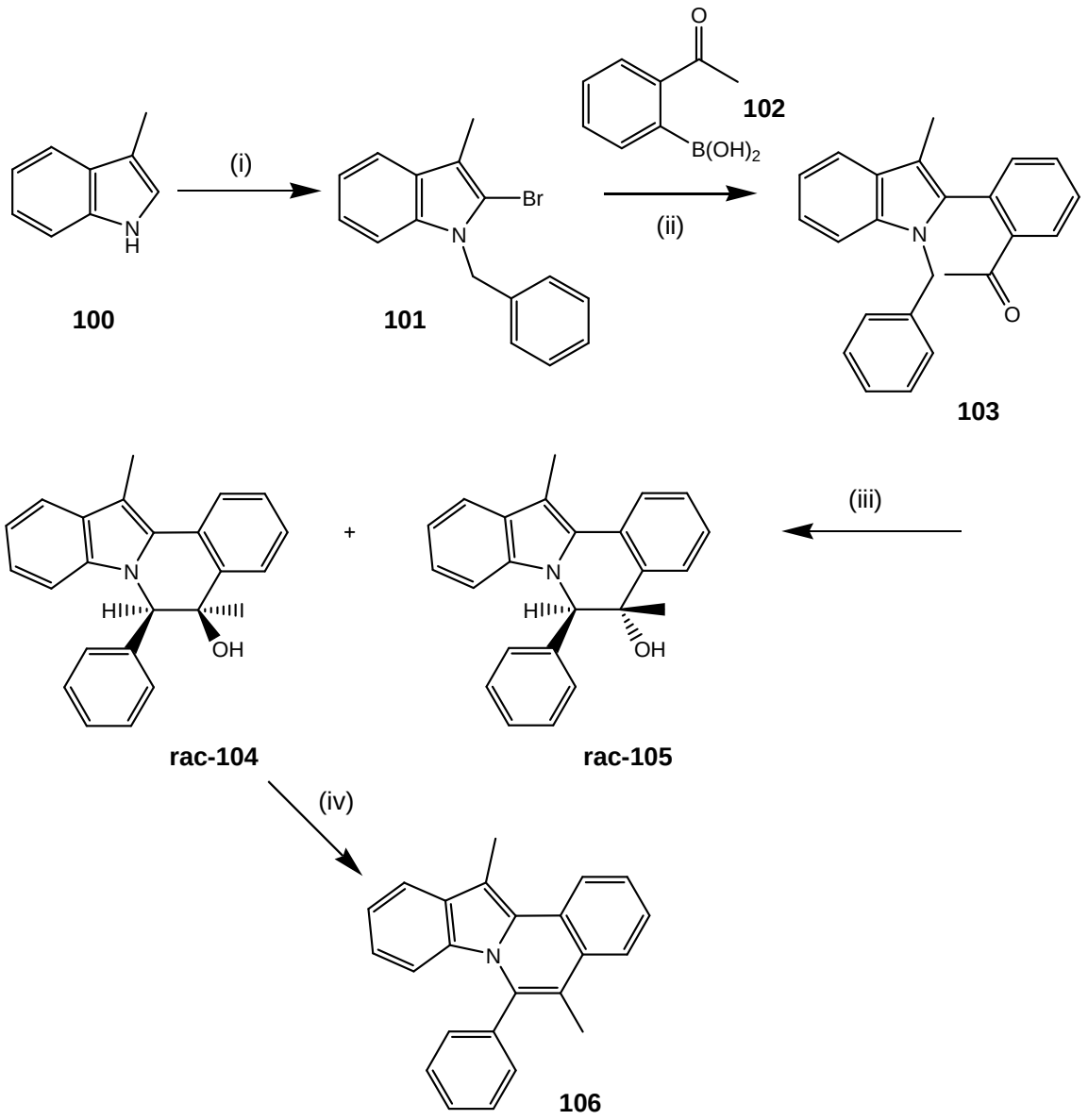
the common initial disconnection is between the isoquinoline nitrogen and the benzene ring which forms part of the indole system. The final bond can be formed in various ways and these include being formed by base-, radical-, or benzyne-mediated ring closure methods **(Scheme 16)**.



Scheme 16: (i) X=Br, K_2CO_3, DMF, reflux, 3 days, Orito;[53] or (ii) X=Br, AIBN, Bu_3SnH, Ambros[42, 54] or (iii) X=Cl, nBuLi, THF, -100^oC, 98%, Meyers;[55] (iv) $MeNO_2$, rt, 2 days, 72%, Junjappa;[56] (v) TFA-CH_2Cl_2, 18hrs, 53%; BH_3-THF, Elliot;[57] (vii) CuI, DMF, reflux, 2hrs; (viii) *p*-TsOH, CH_2Cl_2, Reboredo;[58] (ix) dicumyl peroxide, chlorobenzene, heat, 69-85%, Menes-Arzate.[59]

It is also evident from previous paragraphs that the indolo[2,1-*a*]isoquinoline and its analogues have interesting biological activities. Although many synthetic routes have been developed, it is of importance to search or develop new and simpler synthetic strategies to access the indolo[2,1-*a*]isoquinoline skeleton. This skeleton and a variety of analogues have been successfully synthesized in our group of which one will be discussed in the following paragraphs.

A recent method which is used extensively by researchers in the synthesis of such skeletons as the indolo[2,1-*a*]isoquinolines alkaloid uses as a key step the Suzuki-Miyaura cross-coupling reaction which employs palladium(0) as the metal responsible for catalysing the reaction. This key step has also been used extensively in our labs by de Koning and co-workers and two sets of general reaction conditions had since been developed extensively for forming carbon-carbon bonds between aromatic systems.[60] **Scheme 17** shows how the use of the Suzuki-Miyaura cross-coupling reaction was a key step in synthesizing the indolo[2,1-*a*]isoquinoline analogue **(106)**. The first step in this synthesis was to make the 1-benzyl-2-bromoskatole **(101)** using known chemistry,[57] and then to couple **(101)** with a boronic acid **(102)** using the Suzuki reaction to obtain **(103)** in 64% yield. Compound **(103)** was then exposed to $KOBu^t$ in DMF and a light source[58] to form the two diastereomers **(104)** and **(105)** in a 1:3 ratio. The major diastereomer **(105)** was then exposed to 15 mol% TsOH to afford the indolo[2,1-*a*]isoquinoline derivative **(106)** in 79% yield.

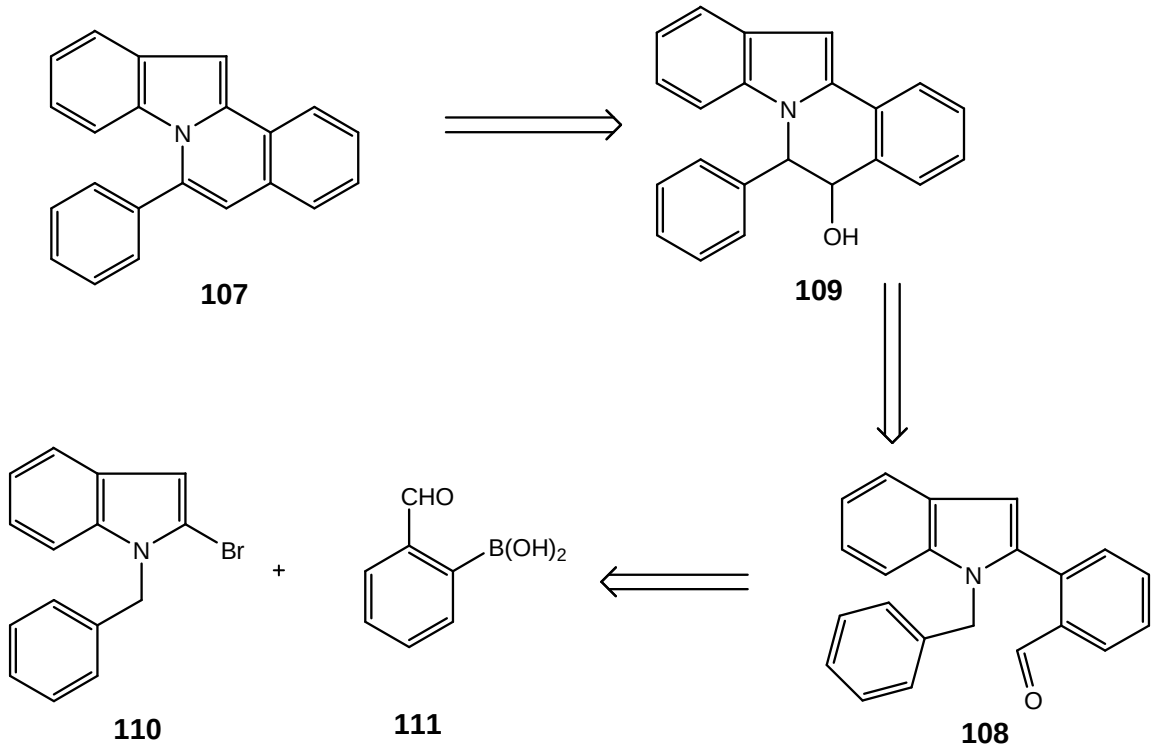


Scheme 17: (i) NBS, CCl_4, 3h then aq. NaOH, BnBr, 66%; (ii) **102**, 10 mol % $Pd(PPh_3)_4$, aq. Na_2CO_3, DME, 64%; (iii) $KOBu^t$, DMF, 80℃; (iv) 15 mol % TsOH, CH_2Cl_2, rt, 24h.

1.4 Aims of this MSc

The initial aim of this project was to extend the novel synthesis for the indolo[2,1-*a*]isoquinoline skeleton described in the last paragraph to related analogues and investigate the scope and limitations of this methodology. Therefore, the

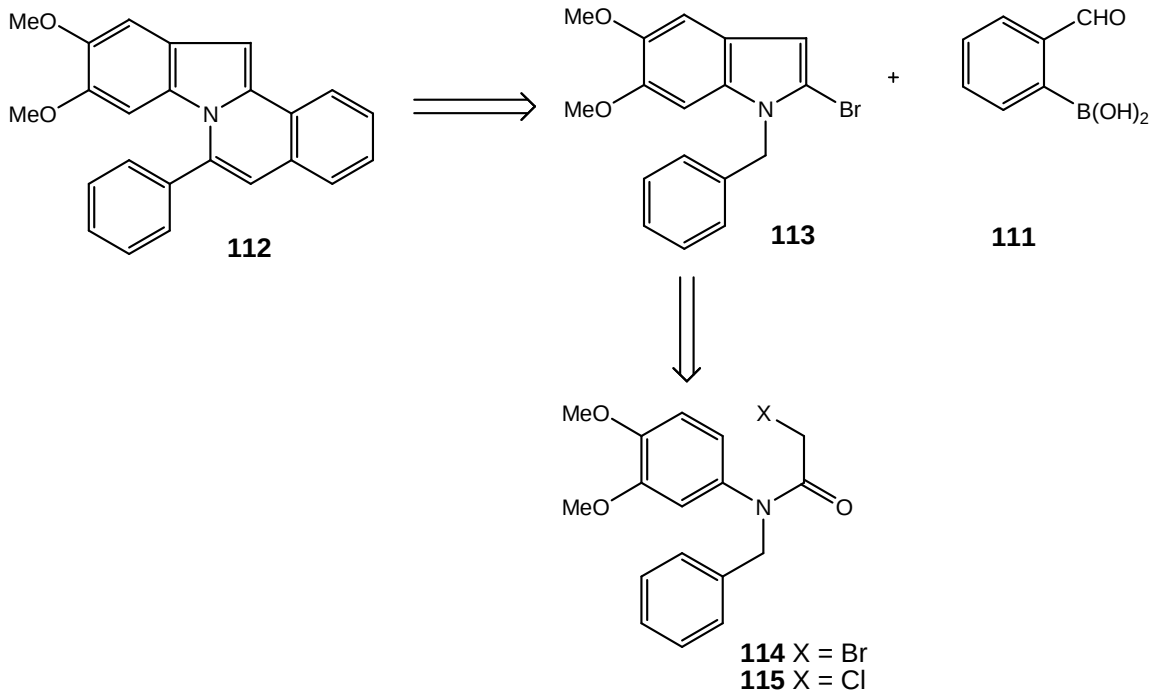
methodology we wish to follow is an extension of that described in **Scheme 17**. The first part of the project will involve the synthesis of phenyl substituted indolo[2,1-*a*]isoquinoline **(107)** lacking two methyl substituents found on **(106)**. 2-(1-Benzyl-1*H*-indol-2-yl)benzaldehyde **(108)** is one key precursor for the ring-closing reaction to form the 5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol **(109),** which in turn leads to **(107)** by removal of water. Target **(108)** can be disconnected to form two precursors namely, the 1-benzyl-2-bromo-1*H*-indole **(110)** and the formylphenylboronic acid **(111)**, which is commercially available. Hence the initial challenge will be to synthesize indole **(110)**.



Scheme 18: Retrosynthesis of 6-phenylindolo[2,1-*a*]isoquinoline.

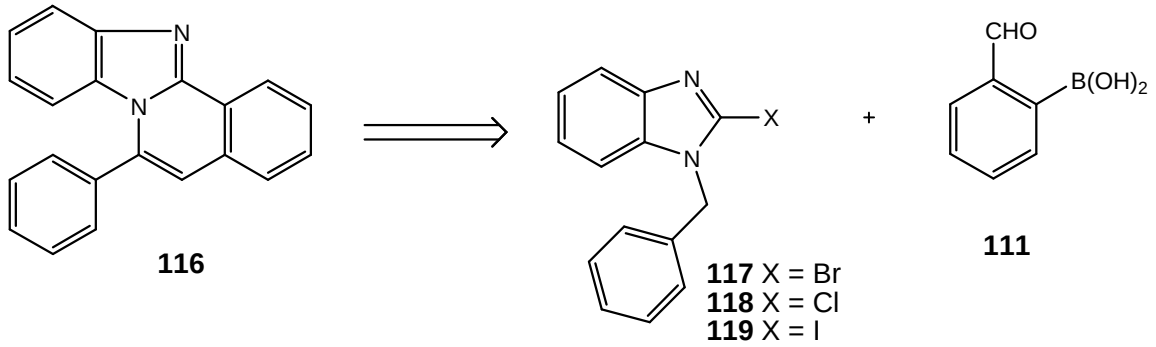
Once the indolo[2,1-*a*]isoquinoline **(107)** has successfully been synthesized, the synthesis of dimethoxy-phenyl substituted indolo[2,1-*a*]isoquinoline **(112)** will be attempted. As before, target **(112)** can be disconnected to 1-benzyl-2-bromo-5,6-dimethoxy-1*H*-indole **(113)** and the formylphenylboronic acid **(111)**. We believed

that compound **(113)** could be accessed from *N*-benzyl-2-halo-*N*-(3,4-dimethoxyphenyl)acetamide **(114)**, or **(115)** in two steps from 3,4-dimethoxyaniline, using Buchwald methodology.[20]



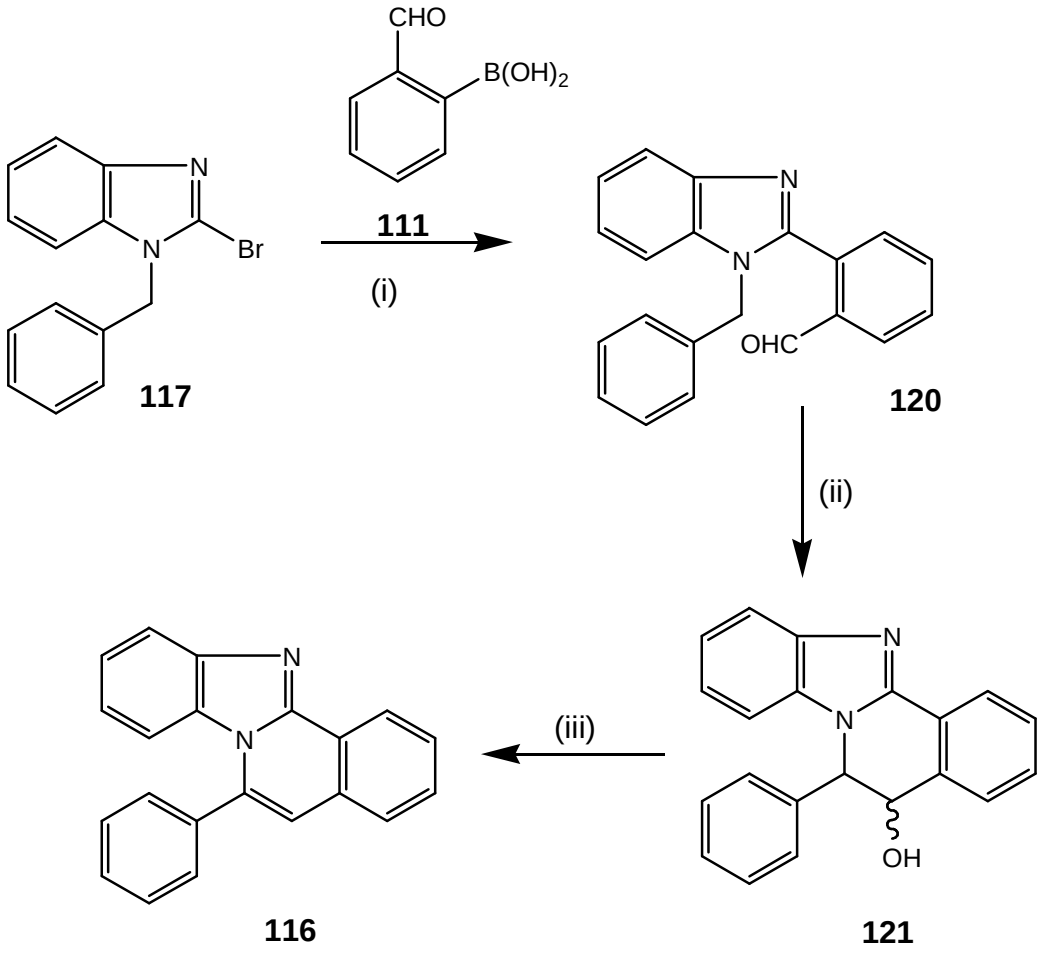
Scheme 19: Retrosynthesis of 9,10-dimethoxy-6-phenylindolo[2,1-*a*]isoquinoline.

The next aim of the project will be to synthesize the 6-phenyl benzimidazo[2,1-*a*]isoquinoline **(116)** containing two nitrogen atoms instead of one as in target **(112)**. The disconnection of **(116)** can lead to compound **(117)**, **(118)**, or **(119)** and formylphenylboronic acid **(111) (Scheme 20).**



Scheme 20: Retrosynthesis of 6-phenylbenzimidazo[2,1-*a*]isoquinoline.

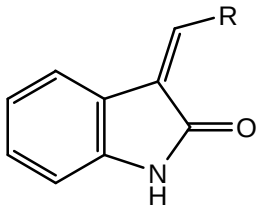
From the retrosynthesis, benzyl-brominated benzimidazole **(117)** could be reacted, using transition metal mediated methodology, to the formylphenylboronic acid **(111)** to give compound **(120)**. The ring closure reaction of **(120)** could then be done using $KOBu^t$ at room temperature to give **(121)** which would be treated with a strong acid to give the final product **(116)**.



Scheme 21: (i) $Pd(PPh_3)_4$ (10 mol %), aq. Na_2CO_3, DME, reflux; (ii) $KOBu^t$, DMF, rt; (iii) Δ, H^+.

The next aim of the MSc was to use the experience gained in our work on indoles to synthesize a library of 3-substituted oxindoles using oxindole as the core nucleus **(122) (Figure 24)**. In fact, we believed that oxindoles would be

precursors for the synthesis of indoles needed in the other parts of this MSc as will be discussed later.



122

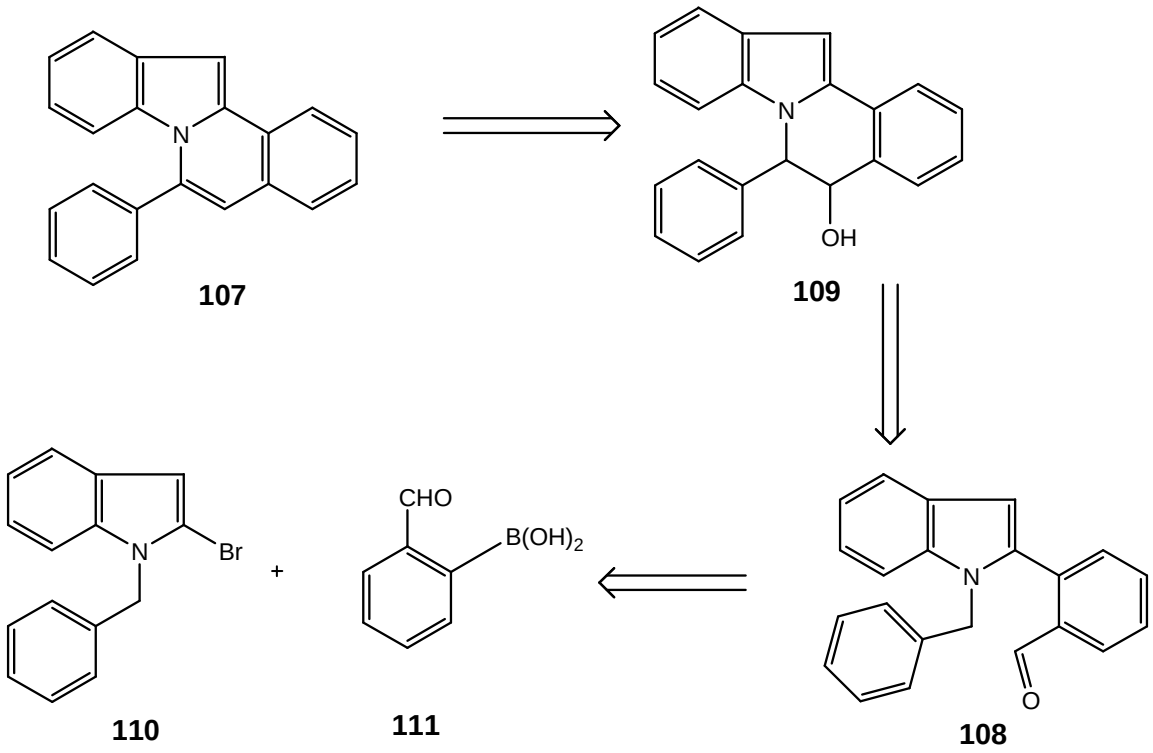
Figure 24

The reason for us wishing to accomplish the synthesis of these products will be outlined in detail in chapter 3. In summary, in collaboration with the CSIR in Modderfontein, we have been conducting some molecular modelling studies on a malarial parasite kinase known as PfPK5. This kinase is the only known malarial kinase whose structure has been solved by x-ray crystallography. Using the x-ray crystal structure of PfPK5 in molecular modeling studies, we found that the 2-oxindole type structures, substituted at the 3-position, docked well in the active site of PfPK5 and therefore we wished to synthesize these compounds

Chapter 2: Synthesis of Indolo[2,1-a] isoquinoline analogues

2.1 Attempted Synthesis of 6-Phenylindolo[2,1-a]isoquinoline (107)

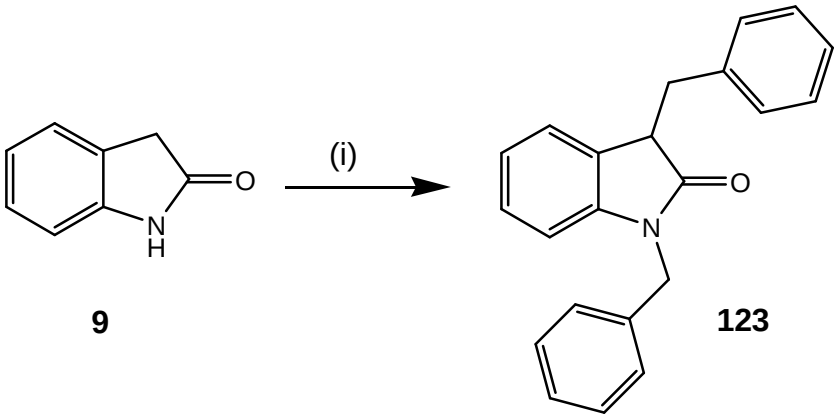
Firstly, in this section the synthesis of the 6-phenylindolo[2,1-*a*]isoquinoline **(107)** will be discussed, a compound for which the retro-synthesis was discussed in the previous chapter, but is shown again here in more detail.



Scheme 18: Retrosynthesis of 6-phenylindolo[2,1-*a*]isoquinoline.

As the first step, we believed that the synthesis of *N*-benzyloxindole could be easily achieved by treatment of oxindole with benzyl bromide under basic conditions. However, the synthesis of the benzylated oxindole did not succeed as expected as the 3-position of the oxindole nucleus was also benzylated due to its acidic nature, with a yield of 60%, the ratio of the dibenzylated product being greater than 90% **(Scheme 22)** in relation to the desired product. The base used

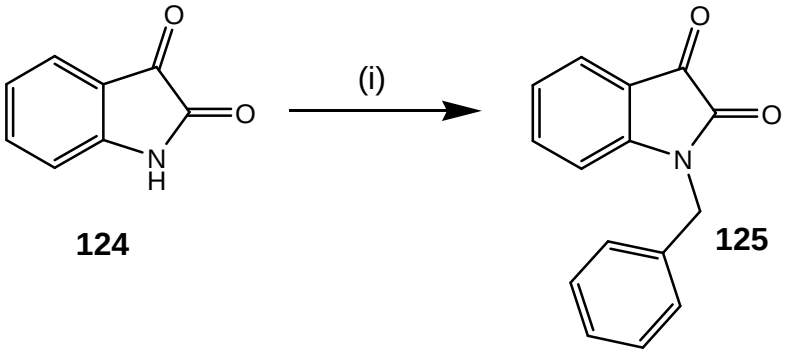
in the benzylation deprotonated not only the nitrogen atom but also the acidic hydrogens of the carbon in the 3-position. Using different bases did not eliminate this problem. A paper by Grunda revealed that the reactivity of the 3-position of oxindole could cause problems during the protection of the amide. [23]



9 **123**

Scheme 22: (a) NaH, THF, rt; (b) BnBr, reflux, 24hrs, 60%.

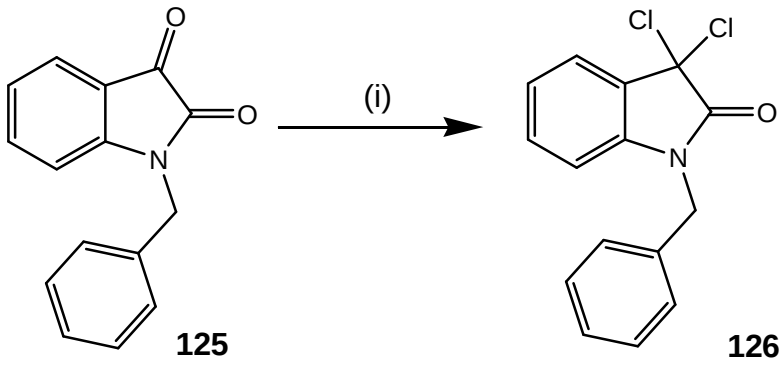
This led us to start the synthesis of the 6-phenylindolo[2,1-*a*]isoquinoline using methodology developed by Garden *et al*.[90] Isatin **(124)** in which the 3-position of oxindole is replaced by a carbonyl, and CaH_2 were dissolved in DMF and benzyl bromide was added. The resulting mixture was stirred for 4 hours before aqueous HCl was added to afford an orange solid, which was recrystallized, to yield 1-benzylindoline-2,3-dione **(125)** as an orange solid in 89% yield **(Scheme 23)**. ^{1}H NMR spectroscopy confirmed the addition of the benzyl group as a singlet appeared at 4.94 ppm (2H) and a multiplet was evident between 7.25 and 7.38 ppm which integrated for 5 protons.



124 **125**

Scheme 23: (i) (a) CaH_2 (1.0 equiv.), DMF, 100^{o}C, 1hr; (b) 40^{o}C, BnBr, then 100^{o}C, 4hrs.

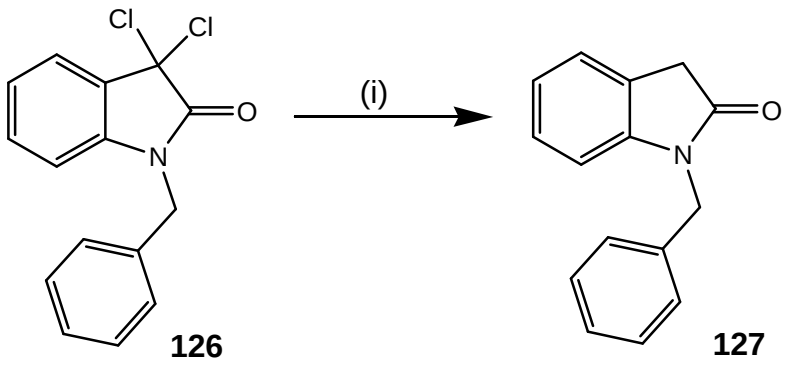
The next step was to do a functional group interconversion. This was done to replace the carbonyl with a methylene substituent, by initially replacing the carbonyl group in the 3-position with two chlorine atoms, to form 1-benzyl-3,3-dichloroindolin-2-one **(126)**, following Power's procedure.[61] Compound **(125)** was therefore treated with large excess of PCl_5, added portion-wise, resulting in a brown residue. The residue was purified by column chromatography to afford a light yellow oil which solidified and which was recrystallized to afford *N*-benzyl-3,3-dichloroindolin-2-one **(126)** as a white solid, in 81% yield. The ^{13}C NMR spectrum of **(124)** was slightly different from the ^{13}C NMR spectrum of **(125)** as the quaternary carbon belonging to the carbonyl in the 3-position was not evident and now coincided with a signal at 124.2 ppm (CCl_2).



Scheme 24: (i) PCl_5, benzene, 50^oC, 24 hrs.

Now that **(126)** was in hand, the chlorine atoms had to be removed from the carbon in the 3-position. This was done using the Zimmerman reaction which uses activated zinc to remove the halogens.[62] 1-Benzyl-3,3-dichloroindolin-2-one **(126)** was therefore dissolved in AcOH, followed by the addition of activated zinc. After purification by column chromatography **(127)** was obtained as a white solid, in 79% yield. Evidence of a new signal in the ^1H NMR spectrum of the product **(127)**, at 3.62 ppm (2H), suggested that the chlorine atoms were no longer present. The ^{13}C NMR spectrum also provided evidence of a new peak at 43.7

ppm which was absent in the spectrum of 1-benzyl-3,3-dichloroindolin-2-one **(126).**



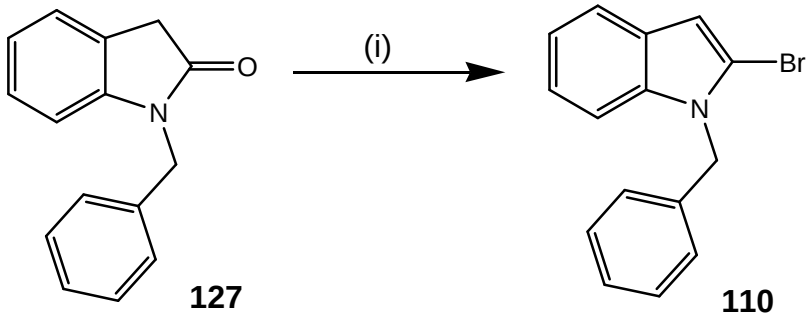
126 **127**

Scheme 25: (i) AcOH, Zn, 2 min.

The bromination of the 2-position of the oxindole **(127)** was performed twice to try and optimize the reagents and reaction conditions for the formation of 1-benzyl-2-bromo-1*H*-indole **(110)**. This reaction presumably proceeded via an enol intermediate. Firstly, 1-benzylindolin-2-one **(127)** was dissolved in CH_2Cl_2 with the addition of $POBr_3$ followed by imidazole. The reaction mixture was stirred for 48 hours. An orange residue resulted which was purified using column chromatography to afford 1-benzyl-2-bromo-1*H*-indole **(110)** in a yield of 88%. The solid formed was found to be unstable as it decomposed after a couple of days; but if stored under refrigerated conditions the compound was reasonably stable.

The second method of bromination was performed by dissolving 1-benzylindolin-2-one **(127)** in CH_2Cl_2 and the temperature kept at 0^oC. DMF and PBr_3 were added together at 0^oC and then this mixture was added to **(127)**. The desired product **(110)**, which was the white solid, was unfortunately obtained in very low yields using this methodology. The ^1H NMR spectrum of the desired product differed from that of precursor **(127)** in that the singlet at 3.62 ppm had disappeared and a new peak, now integrating for one proton, appeared at 6.65 ppm. The ^{13}C NMR spectrum showed that the peak at 35.7 ppm, corresponding

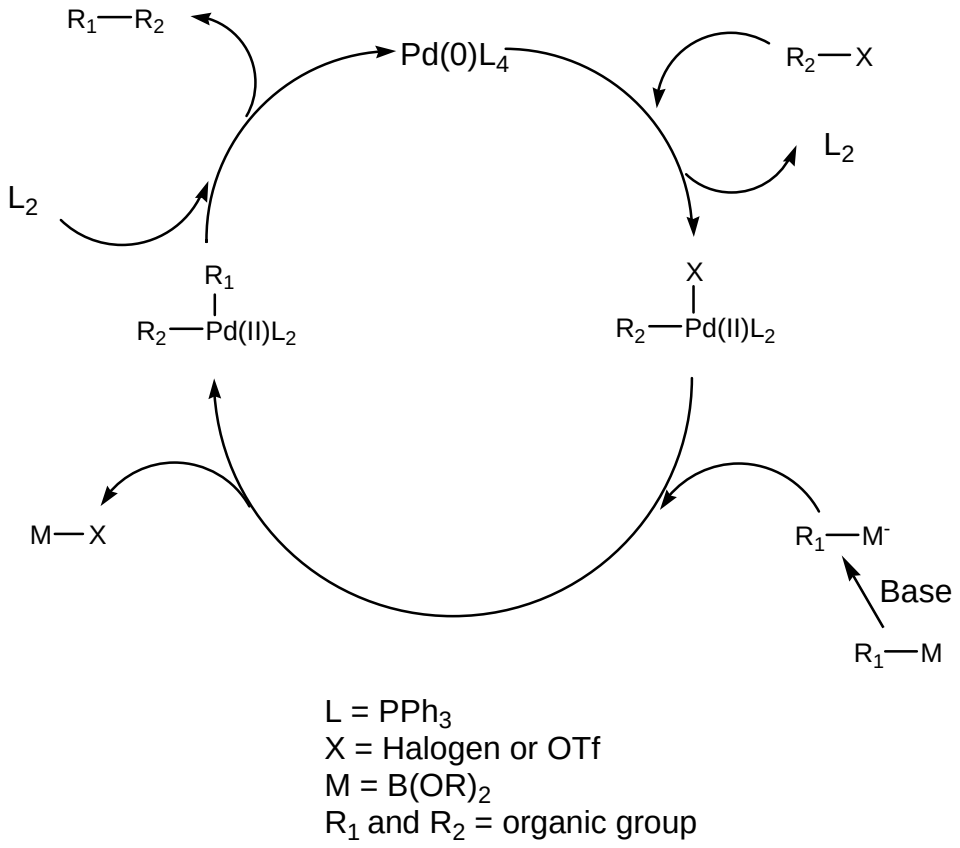
to the **C**H_2 of the starting material had disappeared and that a new peak corresponding to the carbon at the 3-position of the indole nucleus had appeared at 104.5 ppm. The carbonyl peak at 175.1 ppm had also disappeared and a new quaternary peak, for a carbon bonded to the bromine atom had appeared at 137.0 ppm. HRMS showed a molecular species at m/z 285.0091 ($C_{15}H_{12}N^{79}Br$ requires 285.0153) and provided evidence for **(110)**. The IR spectrum showed the absence of the C=O stretch (1650-1750 cm^{-1}) and with a C-Br stretch being present at 691 cm^{-1} and the ArC=C stretch at 1605 cm^{-1}. This confirmed that **(127)** had been successfully converted to the desired product **(110)**.



Scheme 26: (i) (a) CH_2Cl_2, $POBr_3$, reflux, 1hr, rt, then imidazole, 48 hrs or (b) CH_2Cl_2, DMF and PBr_3, 0^oC, 3 min, <5%.

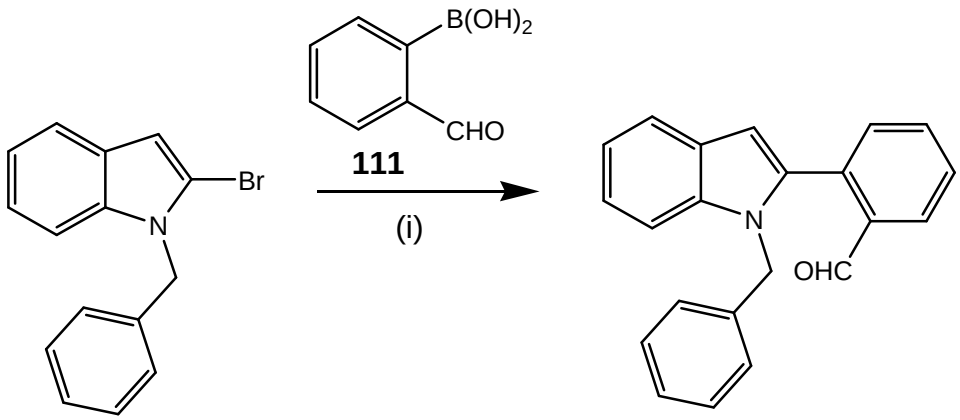
Now that the 1-benzyl-2-bromo-1H-indole **(110)** had been successfully synthesized, the next step was to couple **(110)** with 2-formylphenylboronic acid **(111)** using Suzuki-Miyaura coupling reaction conditions.[63] This coupling involves a palladium catalyst which aids in the cross-coupling of a halogen (or triflate) and a boron-containing compound, which in this case is a boronic acid. The reaction makes use of a base which is believed to form a borate of the boronic acid. Firstly the halogen (or triflate) reacts with the Pd(0) phosphine complex by way of an oxidative addition to form Pd(II) species **(Scheme 27)**. The nucleophile (R_1), which is a boron-containing species, is transferred from the boron to the Pd(II) complex in a step known as transmetallation where the boron is considered to be a “metal”. The generated Pd(II) complex with the two R groups then combines

the two R groups to form the coupled product with Pd(0) being regenerated for the next catalytic cycle through a reductive elimination process.[64]



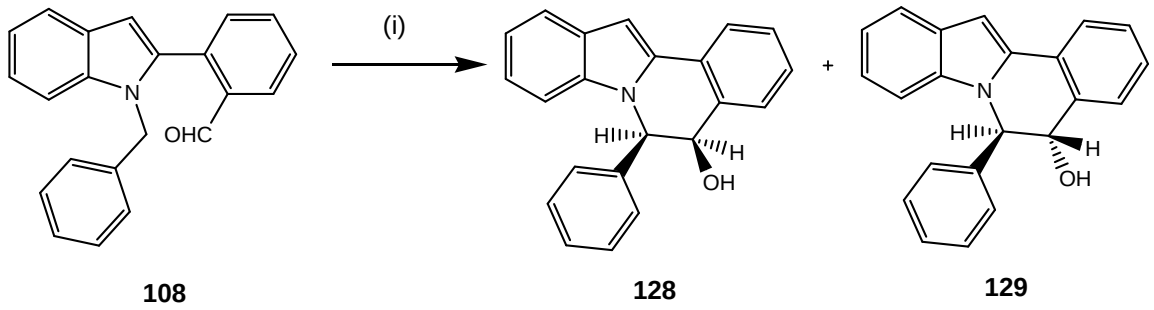
Scheme 27: Catalytic cycle of Pd in Suzuki-Miyaura coupling.

Using the Suzuki-Miyaura approach, 1-benzyl-2-bromo-1*H*-indole **(110)** and 2-formylphenylboronic acid **(111)** were dissolved in DME and transferred to a flask containing $Pd(PPh_3)_4$. An aqueous oxygen free solution of Na_2CO_3 was then introduced and the contents stirred under reflux. A brown oil resulted which was purified using column chromatography to give 2-(1-benzyl-1*H*-indol-2-yl)benzaldehyde **(108)** as yellow viscous oil, in an excellent yield of 98%. The ^{1}H NMR spectrum of the product **(108)** confirmed that the reaction was successful with an aldehyde peak appearing at 9.85 ppm and the ^{13}C NMR spectrum showed the same aldehyde peak at 191.6 ppm.



Scheme 28: (i) 10 mol % $Pd(PPh_3)_4$, DME, Na_2CO_3, reflux, 24 hrs.

The next step was to synthesize 5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol **(128)** and **(129)** which were the expected products of the ring closing reaction **(Scheme 29)**. 2-(1-Benzyl-1*H*-indol-2-yl)benzaldehyde **(108)** was therefore dissolved in DMF and ^{t}BuOK was added. The mixture was subsequently stirred for 2 minutes. The solvent was removed to give a residue of the two diastereomers which were separated using column chromatography to afford the *syn*- **(128)** and *anti*-5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol **(129)** as diastereomers in a 78% combined yield.



Scheme 29: (i) ^{t}BuOK, DMF, rt, 2 min.

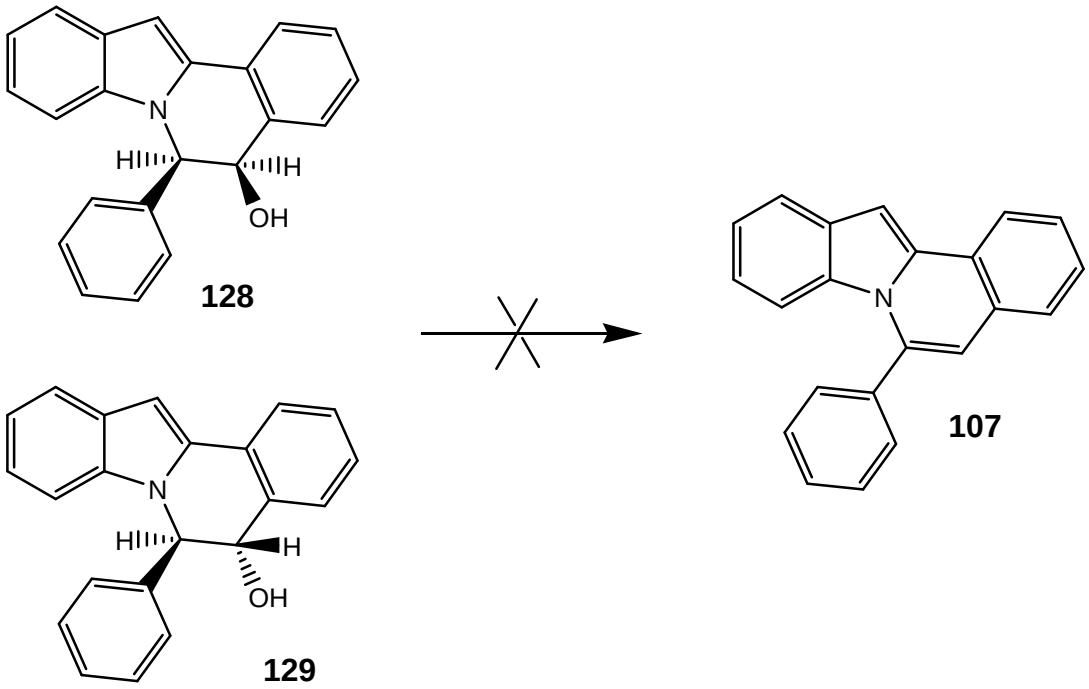
Both diastereomers were found to be yellow-green resins in a ratio of 7:3 i.e. 53% *anti* and 27% *syn*. The R_f values on the TLC plate were also found to be

different (0.13 difference when run with 30% mixture of EtOAc/hexane). The IR spectra of both compounds showed the presence of a broad OH signal at 3548 and 3364 cm^{-1} for the *syn*- and *anti*-diastereomers respectively. The ^{1}H NMR spectrum of the *syn*-diastereomer **(128)** showed a peak at 5.26 ppm which corresponded to the methylene protons of the benzyl group and that the aldehyde proton had disappeared. It showed the presence of the hydroxyl group, as a doublet at 1.66 ppm (J = 11.0 Hz), benzylic proton attached to the carbon, which is bonded to the hydroxyl group, as a double doublet at 5.38 ppm (J = 7.0 and 11.0 Hz) and the benzylic proton attached to the nitrogen as a doublet at 5.71 ppm (J = 6.9 Hz). From the coupling constants, it can be seen that the hydroxyl proton is coupled to the benzylic proton attached to the hydroxyl, which is also coupled to the benzylic proton attached to the nitrogen. The aromatic region was complicated but integrated for 14 protons as expected. The ^{13}C NMR spectrum clearly showed that the aldehyde peak and the methylene peak at 191.6 and 47.6 ppm respectively for **(108),** had been replaced by two new peaks; at 59.5 ppm, corresponding to the carbon bonded to the hydroxyl group, and the second at 69.5 ppm, corresponding to the carbon bonded to the phenyl. The spectrum also showed the correct number of 6 quaternary carbons and 12 aromatic carbons (CH). HRMS showed a molecular ion for the *syn*-compound at *m/z* 311.1302 ($C_{22}H_{17}NO$ requires 311.1310).

The ^{1}H NMR spectrum for the *anti*-diastereomer **(129)** was significantly different from the *syn*-diastereomer. It showed the presence of a broad singlet corresponding to the hydroxyl group at 2.18 ppm, a singlet at 4.91 ppm corresponding to the benzylic proton attached to the hydroxyl and a doublet at 5.81 ppm corresponding to the benzylic proton attached to the nitrogen (J = 1.5 Hz). Comparing the coupling constants of the benzylic protons attached to the nitrogen of **(128)** (J = 6.9 for) and **(129)** (J = 1.5), we could see that the coupling constant of the *anti*-diastereomer **(129)** is far smaller than the coupling constant of the *syn*-diastereomer **(128)** and based on this the *anti*- and the *syn*- were assigned. The ^{13}C NMR spectrum showed new peaks corresponding to the

benzylic carbon attached to the hydroxyl group (62.0 ppm) and the benzylic carbon attached to the phenyl group (73.9 ppm). The rest of the signals were correct in number but had slightly different shifts compared to the *syn*-diastereomer. The HRMS depicted the molecular ion for **(129)** to be at *m/z* 311.1339, where $C_{22}H_{17}NO$ required 311.1310.

The last step required to synthesize the 6-phenylindolo[2,1-*a*]isoquinoline **(107)** by the dehydration of both **(128)** and **(129)**. Diastereomers **(128)** and **(129)** were dissolved in CH_2Cl_2 followed by the addition of triethylamine. Methanesulfonyl chloride was added to convert the hydroxyl substituent into a better leaving group. After the reaction, the mixture was extracted and solvent removed to obtain a green residue which was purified by column chromatography to give an un-characterizable material. The reaction was repeated several times without any success and time did not allow us to attempt other methods of removing water from the diastereomers **(128)** and **(129)**.

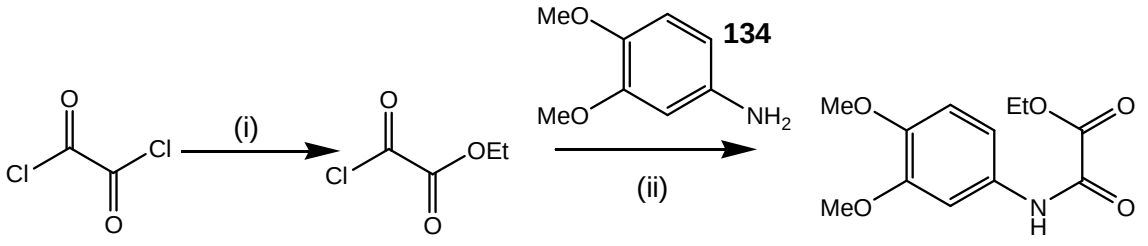


Scheme 30: Et_3N, methanesulfonyl chloride, DMF, 3 min, rt.

2.2 Attempted Synthesis of 9,10-Dimethoxy-6-phenylindolo[2,1-a]isoquinoline

Dimethoxy-substituted isatin **(130)** was seen as a reasonable staring point for the synthesis of 9,10-dimethoxy-6-phenylindolo[2,1-*a*]isoquinoline **(112)** as the chemistry was reasonably known. The first step was to synthesize the 5,6-dimethoxyisatin **(130)** using Taylor's methodology,[65] which will then be used as a precursor using the reaction conditions described for the synthesis of 6-phenylindolo[2,1-*a*]isoquinoline **(107)**.

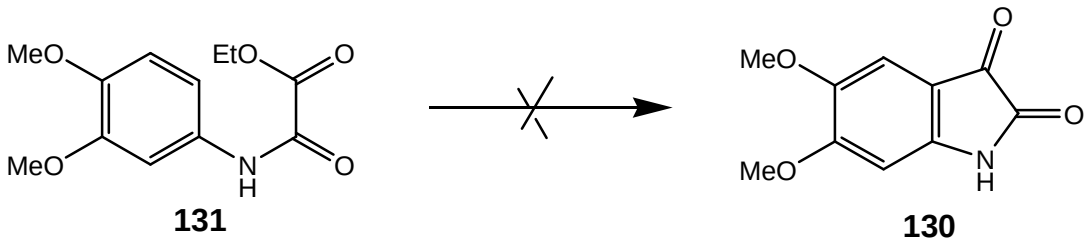
The synthesis of **(130)** started by initially making (3,4-dimethoxyphenylcarbamoyl) formate **(131) (Scheme 31)**. First, oxalyl chloride **(132)**, in excess, was reacted with ethanol. Unreacted oxalyl chloride **(132)** was removed to afford ethyl(chlorocarbonyl) formate **(133)** as a yellow-brown oil which was dissolved in THF. Then 3,4-dimethoxyaniline **(134)** was added slowly and was left to react for 30 minutes. The reaction mixture was extracted and the solvent was removed to give (3,4-dimethoxyphenylcarbamoyl) formate **(131)** as an orange solid, which was not purified further, in quantitative yield. The IR spectrum showed an expected carbonyl stretch at 1710 cm^{-1} and a C-O stretch at 1150 cm^{-1}. The ^{1}H NMR spectrum showed a doublet and a triplet at 4.42 ppm and 1.43 ppm, corresponding to the OCH_2 and CH_3 of the ester group. The ^{13}C NMR spectrum confirmed the presence of the two carbonyls at 161.5 ppm (amide carbonyl) and 154.1 ppm (ester carbonyl), respectively.



132 **133** **131**

Scheme 31: (i) EtOH, 20 min; (ii) THF, 0^{o}C, 30min.

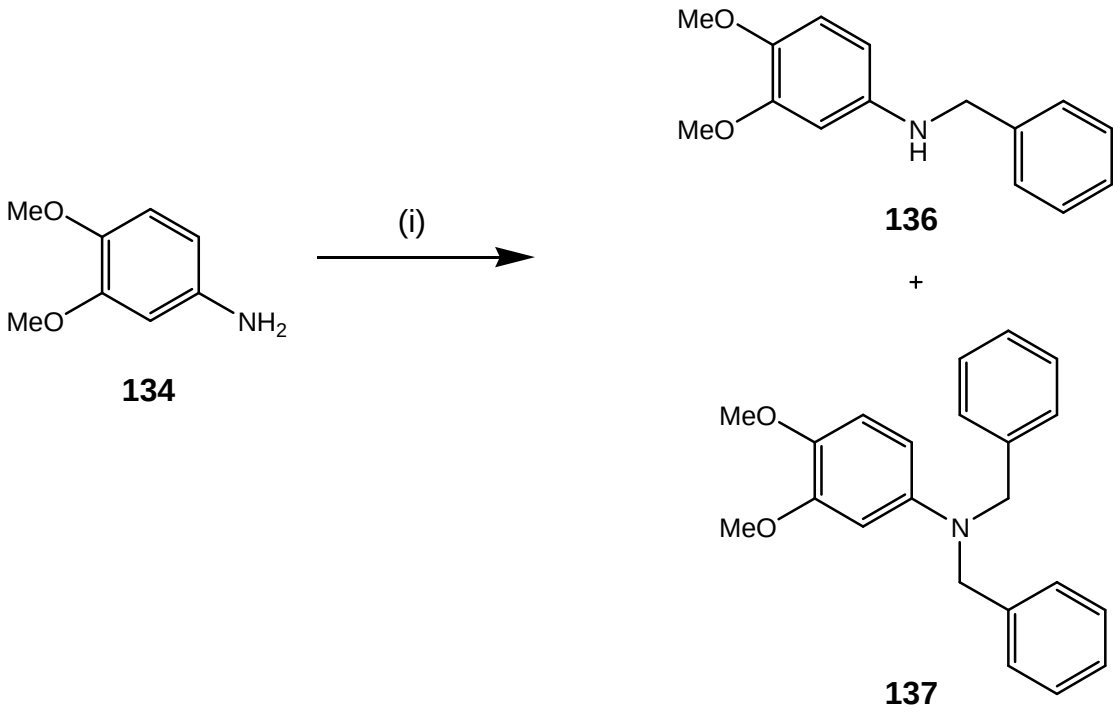
The next step was to perform a ring-closing reaction on compound **(131)** to give the desired dimethoxy-substituted isatin **(130)** using Taylor's methodology **(Scheme 32)**. This was attempted several times using different conditions to try to obtain the product. (3,4-Dimethoxyphenylcarbamoyl) formate **(131)** was treated with BF_3.OEt and the reaction was left to stir. The reaction mixture was extracted and purified, only to obtain starting material. It was decided to replace the BF_3.OEt with $AlCl_3$ using the same reaction conditions. However, the reaction was unsuccessful with 90% of the starting material recovered. The last attempt at the ring closing reaction was done by treating **(131)** with polyphosphoric acid which hopefully would mediate the cyclization. The result was unsuccessful and this route was thus abandoned, forcing us to develop another strategy.



Scheme 32: (a) BF_3.OEt, CH_2Cl_2, 1.5hrs, rt; (b) $AlCl_3$, CH_2Cl_2, 1.5hrs, rt.

The next attempt was to now access the dimethoxy-substituted oxindole **(135)** instead of the dimethoxy-substituted isatin **(130)**. This was going to be done using Buchwald's methodology,[20] which is a modified Friedel-Crafts cyclization of α-chloroacetanilides to oxindoles via a palladium-catalysed C-H functionalization. 3,4-Dimethoxyaniline **(134)** was therefore reacted with benzyl bromide, in water, in the presence of $NaHCO_3$. The reaction mixture was extracted and the solvent was removed resulting in a brown residue which was purified by column chromatography to give the desired monobenzylated dimethoxyaniline as a brown oil **(136)**, in 91% yield **(Scheme 33)**. A side product, the dibenzyl dimethoxyaniline **(137)** was obtained, as a orange-brown oil, in 7% yield. The ^{1}H NMR spectrum was sufficient to confirm that the reaction had been successful as

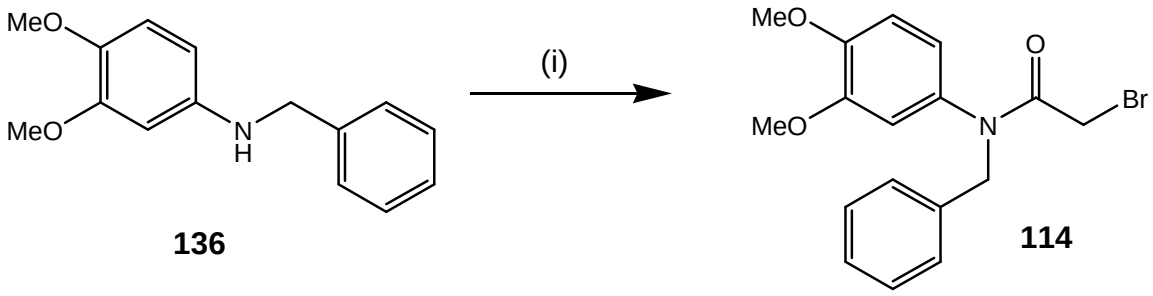
a peak at 4.29 ppm corresponded to the benzylic protons α to the nitrogen and an additional five protons at 7.31 ppm were evident for compound **(136)**.



134 **136** + **137**

Scheme 33: (i) (a) water, $NaHCO_3$, 95^{o}C; (b) BnBr, 3hrs.

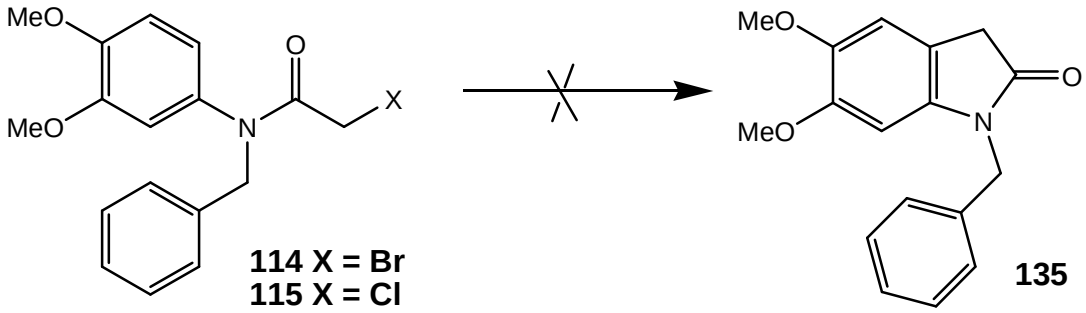
Since the *N*-benzyl-3,4-dimethoxyaniline **(136)** had been successfully synthesized, the next step was to synthesize *N*-benzyl-3,4-dimethoxybromoacetanilide **(114) (Scheme 34)**. Compound **(136)** was thus reacted with bromoacetyl bromide in the presence of triethylamine. A yellow oil resulted which was purified using column chromatography to give an oil which solidified to a white solid. This solid was recrystallized to afford *N*-benzyl-3,4-dimethoxybromoacetanilide **(114)** in 95% yield. The IR spectrum showed a new signal corresponding to the carbonyl at 1746 cm^{-1}. In addition, the ^{1}H NMR spectrum showed a new signal at 3.89 ppm corresponding to the α-carbon of the carbonyl bonded to the bromine. The ^{13}C NMR spectrum also showed a carbonyl peak at 167.3 ppm which meant the reaction had been successful.



136 **114**

Scheme 34: (i) CH_2Cl_2, 0^oC, bromoacetyl bromide, 20 min.

It was now time to attempt the ring-closing reaction on compound **(114)** using Buchwald's methodology to try and obtain oxindole **(135) (Scheme 35)**.[20] Compound **(114)** was introduced to a flask with $Pd(OAc)_2$, and a ligand, [(2-(di-*tert*-butylphosphino)biphenyl)] in the presence of anhydrous triethylamine. The contents were placed in a pre-heated oil bath. The reaction was repeated many times without any success (even though it has been reported in the literature), with starting material, compound **(114)** being recovered. *N*-benzyl-3,4-dimethoxychloroacetanilide **(115)** was also synthesized by reacting *N*-benzyl-3,4-dimethoxyaniline **(136)** with chloroacetyl chloride, thinking that the size of the halogen might have an effect in the mechanism of the ring closing reaction and also proved to be unsuccessful in this reaction.



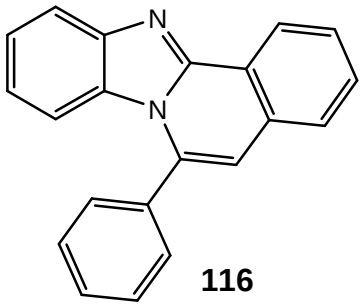
114 X = Br
115 X = Cl

135

Scheme 35: $Pd(OAc)_2$, 2-(di-*tert*-butylphosphino)biphenyl, Et_3N, toluene, 80^oC, 3hrs.

2.3 Attempted Synthesis of Benzimidazo[2,1-a]isoquinoline (116)

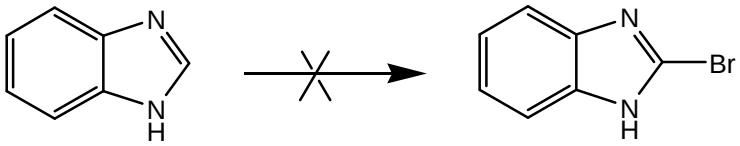
After the unsuccessful attempt of synthesizing the 9,10-dimethoxy-6-phenylindolo[2,1-*a*]isoquinoline **(112)**, another analogue, the benzimidazo[2,1-*a*]isoquinoline **(116)** was thought to be a possible synthetic target, employing some of the chemistry that was developed when synthesizing the 6-phenylindolo[2,1-*a*]isoquinoline **(107)**.



116

Figure 25

Benzimidazole **(138)** was to be a starting material towards the synthesis of the target compound **(116)**. Treating **(138)** with NBS in the presence of silica gel[91] yielded uncharacterizable material. The reaction was performed several times, changing the amounts of the reagents without any success in obtaining the desired product **(139) (Scheme 36)**.

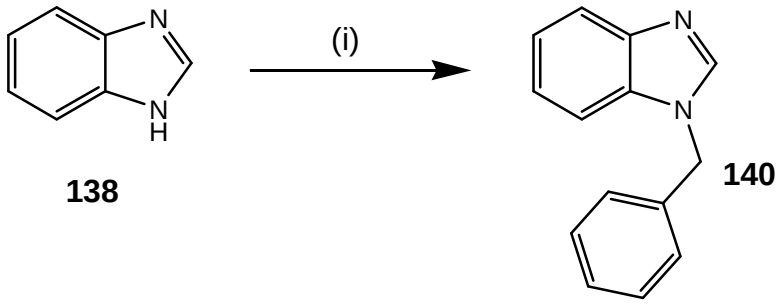


138 **139**

Scheme 36: NBS, silica gel, CH_2Cl_2, 2 hrs.

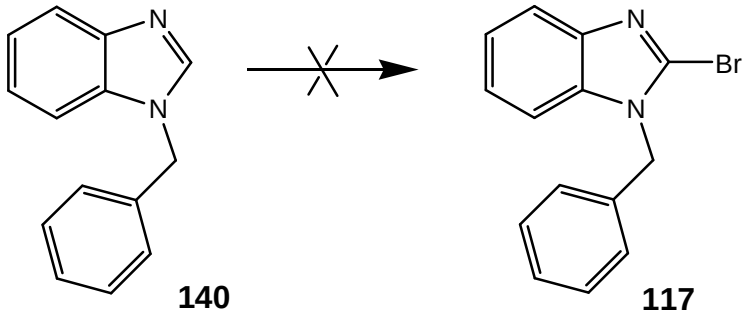
It was then decided that before halogenating the 2-position of the benzimidazole **(138)**, the amine nitrogen should be protected. This was done by reacting

benzimidazole **(138)** with benzyl bromide in the presence of sodium hydroxide **(Scheme 37)**.[67] The reaction mixture was extracted and on removal of the solvent a colorless oil was obtained which on standing became a white solid. The solid was recrystallised from acetone to give 1-benzyl-1*H*-benzo[*d*]imidazole **(140)** as a white solid in 98% yield. The ^{1}H NMR spectrum showed a peak at 5.33 ppm corresponding to benzylic protons bonded to nitrogen and an integration of 5 protons in the aromatic region between 7.83 and 7.33 ppm confirmed the structure of **(140)**.



Scheme 37: (i) (a) acetone, NaOH, 1hr; (b) BnBr, reflux, 24 hrs.

Since the synthesis of 1-benzyl-1*H*-benzo[*d*]imidazole **(140)** was successful, the next step was to halogenate the 2-position of the 1-benzyl-1*H*-benzo[*d*]imidazole **(140)** prior to the Suzuki-Miyaura coupling with the boronic acid **(111)**. Compound **(140)** was exposed again to the same reaction conditions described in **(Scheme 36)** with no success of brominating the 2-position of 1-benzyl-1*H*-benzo[*d*]imidazole **(140) (Scheme 38)**.

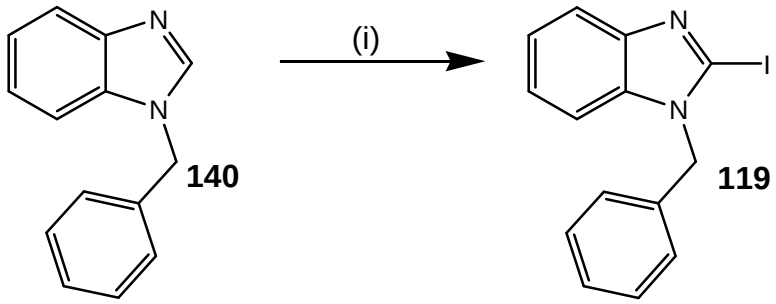


Scheme 38: NBS, silica gel, CH_2Cl_2, 2 hrs.

It was then decided that another halogenating procedure would be used instead of the previous one, of employing NBS as a brominating agent. Two different reaction conditions were used to halogenate the 2-position of precursor **(140)** using diiodoethane and iodine as halogenating agents.

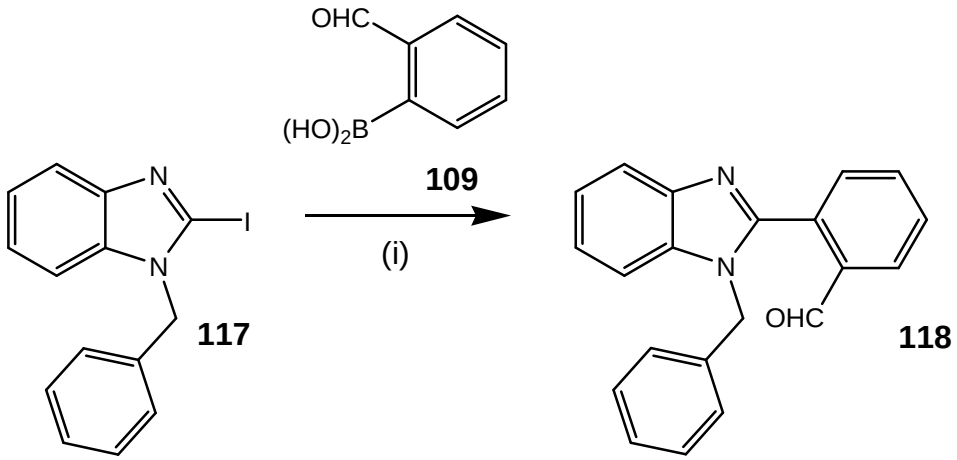
Firstly, 1-benzyl-1*H*-benzo[*d*]imidazole **(140)** was initially treated with LDA at -78^{o}C. 1,2-Diiodoethane was added to the reaction mixture with stirring, allowing the reaction to warm up to room temperature.[92] The solvent was removed and the resulting residue was purified using column chromatography to give 1-benzyl-2-iodo-1*H*-benzo[*d*]imidazole **(119)** as a white solid in 40% yield **(Scheme 39)**.

The second method, developed in our labs, commenced by treating 1-benzyl-1*H*-benzo[*d*]imidazole **(140)** with isopropylmagnesium chloride and lithium chloride followed by the addition of iodine. The reaction mixture was washed with sodium thiosulfate to remove the unreacted iodine. The solvent was removed and the residue was purified using column chromatography to afford 1-benzyl-2-iodo-1*H*-benzo[*d*]imidazole **(119)** as a white solid in 75% yield **(Scheme 39)**. The ^{1}H NMR spectrum showed that the peak at 7.29 ppm which corresponds to the proton in between the two nitrogen atoms was no longer present. The ^{13}C NMR spectrum showed that a peak at 144.4 ppm which corresponded to the carbon between the two nitrogen atoms had been replaced with a peak at 146.2 ppm. The HRMS found the molecular ion for **(119)** to be at m/z 335.1425 $(M + 1)^{+}$.



Scheme 39: (i) (a) LDA, THF, -78^{o}C, 20 min; ICH_2CH_2I, 20 min. -78^{o}C to rt or (b) *i*-PrMgCl.LiCl, 24 hrs, I_2, 1 hr.

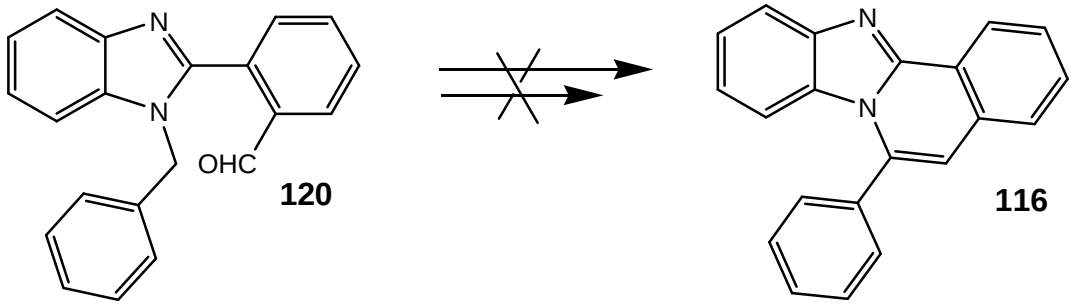
The next step was to facilitate a Suzuki-Miyaura coupling between 1-benzyl-2-iodo-1*H*-benzo[*d*]imidazole **(119)** and the boronic acid **(111)**. The reaction conditions followed that of the synthesis of 2-(1-benzyl-1*H*-indol-2-yl)benzaldehyde **(108)**. 1-Benzyl-2-iodo-1*H*-benzo[*d*]imidazole **(119)** was reacted with 2-formylphenyl boronic **(111)** in the presence of $Pd(PPh_3)_4$ and Na_2CO_3. The solvent was removed and the black residue was purified using column chromatography to give 2-(1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde **(120)** as a viscous orange oil in 67% yield **(Scheme 40)**. The IR spectrum of **(120)** showed a C=O stretch at 1694 cm^{-1}. The ^{1}H NMR spectrum also showed a peak at 10.29 ppm which belongs to the aldehyde proton. The aromatic region was complicated but integrated for 13 protons which was the required number of protons. The ^{13}C NMR spectrum also showed that the peak at 146.2 ppm for the carbon attached to the iodine was no longer evident but a peak at 190.7 ppm, corresponding to the aldehyde carbon was now present. Finally the HRMS displayed a molecular ion for **(118)** at m/z 313.2430 $(M + 1)^{+}$.



Scheme 40: (i) 10 mol % Pd $(PPh_3)_4$, DME, Na_2CO_3, reflux, 24 hrs.

The ring-closing reaction on **(120)** was also done with the same reaction conditions as in the synthesis of 5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol **(128/129)**. As with the synthesis of **(128/129)**, we had thought that the reaction

would yield an alcohol, which then would need to be dehydrated for the formation of the final product **(116)**. 2-(1-Benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde **(120)** was therefore treated with ^{t}BuOK and the reaction mixture was allowed to stir for 3 minutes. The reaction mixture was then extracted and the solvent was removed resulting in a green residue. This was purified using preparative column chromatography to give material which was uncharacterizable **(Scheme 41)**. The reaction was performed several times but proved to be unsuccessful. Due to time constraints, we could not explore other reaction conditions that might aid in the formation of the target molecule.

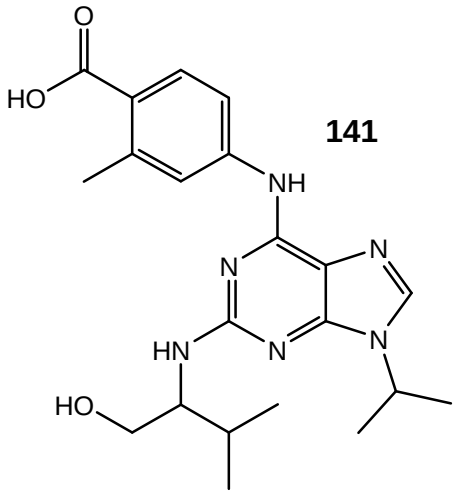


Scheme 41: ^{t}BuOK, DMF, rt, 3 min.

Chapter 3: Synthesis and Modelling of 3-substituted oxindole against PfPK5

3.1 Modelling and Preparation of the PfPK5 protein

PfPK5 (malarial *Plasmodium falciparum* protein kinase 5) is a cyclin dependent kinase of the *Plasmodium falciparum*, the agent causing the most severe form of human malaria. PfPK5 is responsible for regulating part of the malaria parasite's cell cycle. As 4-[2-(1-hydroxy-3-methylbutan-2-ylamino)-9-isopropyl-9*H*-purin-6-ylamino)-2-methylbenzoic acid **(141)** was crystallized together with the protein PfPK5 and the x-ray crystal structure solved, we decided to screen a number of related compounds in molecular modelling docking studies.



141

Figure 26

Therefore a library of 3-substituted oxindoles was docked using Accerlys software into the active site of the PfPK5 in order to determine if any of these molecules might be potential candidates for biological screening once they had been synthesized. The x-ray crystal structure of the protein PfPK5 from the protein databank (with PDB code 1V0P) was prepared and used for the docking

procedure. Preparation of the protein involved the changing of the amino acid valence errors, definition of the binding site using a space filling algorithm and optimization of the binding site by removing the original inhibitor, 4-[2-(1-hydroxy-3-methylbutan-2-ylamino)-9-isopropyl-9*H*-purin-6-ylamino)-2-methylbenzoic acid **(154)**, co-crystallized with the protein and minimizing the energy of the molecule using a Dreiding force field. The inhibitor, 4-[2-(1-hydroxy-3-methylbutan-2-ylamino)-9-isopropyl-9*H*-purin-6-ylamino)-2-methylbenzoic acid **(154)**, was then used to optimize the binding parameters and further optimize the binding site of the PfPK5 so that the highest scoring pose in the relevant scoring functions correctly matched the known crystal pose for this compound. Docking of other known inhibitors also helped to choose which scoring functions correctly identified known inhibitors from random compounds. **Figure 27** is a picture of the PfPK5 protein with the prepared binding site (the ball like shape). While PfPK5 is a unique kinase, there are similarities with human CDK2, a kinase that is essential for the reproduction of healthy mammalian cells. However, CDK2 has a flatter, wider active site. From **Figure 27**, it is noticeble that the PfPK5 active site shown in green differs slightly from the human CDK site in that the site is rounder in shape.

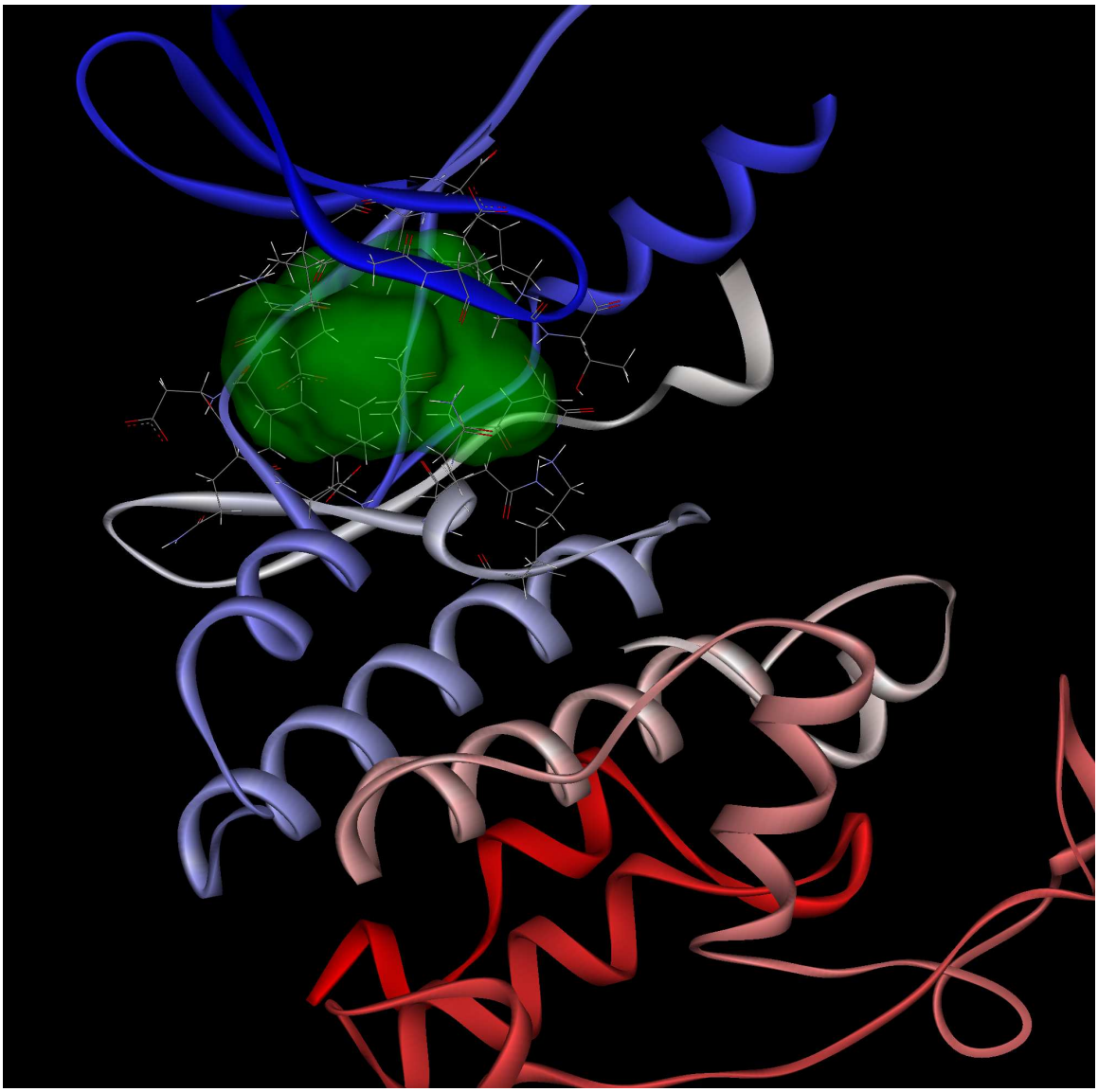


Figure 27 (produced by Dr. Steve Pelly, CSIR.)

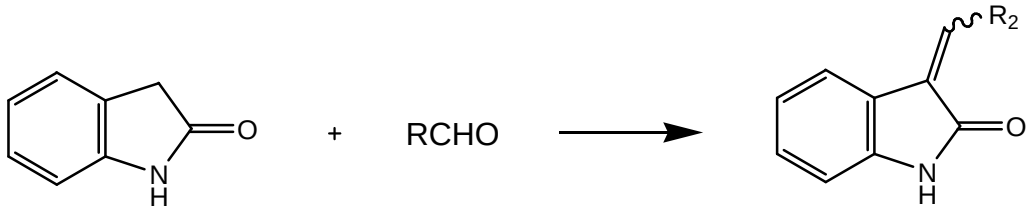
Using the Accerlys program suite, Ligscore2 (with the CFF force field), -PLP2 and Ludi3 were found to accurately represent the known inhibitor **(141)**, with the correct pose, against a random set of decoy compounds.

The molecules were then converted to SD format (a 3D file format), and minimised using CHARMm before docking them into the prepared PfPK5 protein. For each molecule, up to 10 of the highest scoring poses were retained based upon dock score and then this list was minimized within the shape of the binding site using the CHARMm force field. These docked and minimised molecules

were then scored using Ligscore2 (with the CFF force field), -PLP2 and Ludi3. Molecules that scored well should have scored within 35 % of the three scoring functions (consensus scoring). This aspect of the work was supervised by Dr. S. Pelly at the CSIR, in Modderfontein.

3.2 Synthesis of the 3-Substituted Oxindoles

The molecules tested in the *in silico* screening were synthesized using a well documented literature method.[68-72] Condensing 2-oxindoles **(9)** and suitable benzaldehydes in the presence of a base, piperidine, as depicted by **Scheme 42**. The results are listed in Table 1.



9 **142-154**

Scheme 42: EtOH, piperidine, reflux, 24 hrs

Compound Number	**R_2-group**	**(*E/Z*); Yield**
142	phenyl	(*E/Z*) 46%
143 and **144***	3-methylphenyl	51%
145	3,4-dimethoxyphenyl	(*E/Z*) 48%
146	2,5-dimethoxyphenyl	(*E/Z*) 51%
147	Benzo[d][1,3]dioxole	(*E/Z*) 51%
148	2-methoxyphenyl	(*Z*) 58%
149 and **150***	4-methoxyphenyl	43%
151	2-hydrxyphenyl	(*Z*) 51%
152	3-hydroxyphenyl	(*Z*) 45%
153	4-hydroxyphenyl	(*Z*) 46%
154	2-hydroxy-3-methoxyphenyl	(E/Z) 26%

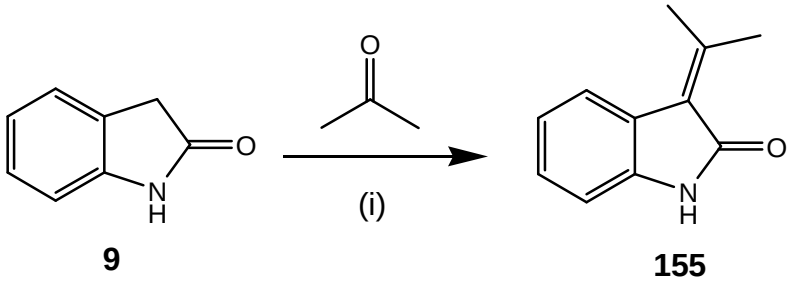
Table 1: *E* and *Z* = geometry of the R group, (*) **143** and **144** (similarly to **149** and **150**) separated isomers, (*E/Z*) = mixture of isomers not separated.

From Table 1, 3-benzylideneindolin-2-one **(142)** was synthesized as a mixture of (*E*)- and (*Z*)-isomers in 46% yield. This was confirmed when the ^{13}C NMR spectrum was obtained with double the number of expected peaks of the compound. (*Z*)-3-(3-Methylbenzylidene)indolin-2-one **(144),** and (*E*)-3-(3-methylbenzylidene)indolin-2-one **(143)** were synthesized and separated successfully by column chromatography. The R_f values of the two isomers were sufficiently different to allow for separation. The proton on the benzylidene carbon was found at 8.08 ppm and at 7.81 ppm of the (*E*)- and (*Z*)-isomers, respectively. Other peaks had different chemical shifts integrating for the correct number of protons. The ^{13}C NMR spectra were also different showing also that the (*E*)-isomer as the major isomer, and the geometric configuration, was confirmed using NOE experiments. 3-(3,4-Dimethoxybenzylidene)indolin-2-one **(145)** was synthesized as a mixture of (*E*)- and (*Z*)-isomers in a ratio of close to 1:1 in a yield of 48%, with the expected number of signals in the ^{13}C NMR spectrum for both the (*E*)- and (*Z*)-isomers. The two isomers were not separated as the R_f values of the two compounds were similar. 3-(2,5-Dimethoxybenzylidene)indoline-2-one **(146)** was obtained as a mixture of (*E*)- and (*Z*)-isomers with similar R_f values in a yield of 51%. This was evident from the ^{13}C NMR spectrum with one isomer being produced in a greater (90%) amount than the other isomer. 3-[(Benzo[d][1,3]dioxol-6-yl)methylene]indolin-2-one **(147)** was also obtained in a mixture of (*E*)- and (*Z*)-isomers, as seen in the ^{13}C NMR spectrum, in a yield of 51%. With the R_f values of the two isomers being the same, it was not possible to separate the isomers. (*Z*)-3-(2-Methoxybenzylidene)indolin-2-one **(148)** was synthesized successfully in 58% yield. The NOE experiments concluded that the geometry was *Z* even though the ^{13}C NMR spectrum showed traces of the (*E*)-isomer being present (less than 5%). Compound **(148)** was recrystallized with the aim of removing traces of the other isomer but this was unsuccesful. (*Z*)-3-(4-Methoxybenzylidene)indolin-2-one **(149)**, and (*E*)-3-(4-methoxybenzylidene)indolin-2-one **(150)** were synthesized and separated successfully in a combined yield of 43%. The ^{1}H NMR spectra of the two had the benzylidene proton at 7.53 and 7.79 for the (*E*)-

and the (*Z*)-isomer, respectively, with little difference between the carbon signals corresponding the benzylidene carbon. (*E*/*Z*)-3-(2-Hydroxybenzylidene)indolin-2-one **(151)** was obtained in a yield of 51%. The ^{1}H NMR spectrum showed the OH peak at 3.24 ppm as a broad singlet with the benzylidene proton at 7.86 ppm. The ^{13}C NMR spectrum showed traces of the (*E*)-isomer but in less than 3% yield. (*E*/*Z*)-3-(3-Hydroxybenzylidene)indolin-2-one **(152)** and (*E*/*Z*)-3-(4-hydroxybenzylidene)indolin-2-one **(153)** were synthesized with yields of 45 and 46% respectively. The ^{1}H NMR for compound **(152)** showed an OH peak 9.26 ppm and 7.62 ppm for the benzylidene proton while that of **(153)** showed the OH peak at 3.32 ppm and the benzylidene was not clear as it was overlapping with signals in the aromatic region.

Compound **(154)** was obtained in a low yield in a mixture of (*E*)- and (*Z*)-isomers. Even after several attempts, 26% was the highest yield obtained. 2-Formyl-6-methoxyphenyl acetate was reacted with 2-oxindole followed by the removal of the acetate to give 3-(2-hydroxy-3-methoxybenzylidene)indolin-2-one **(154)** as an orange solid. The ^{1}H NMR spectrum showed both the OMe and OH signals at 3.95 and 1.74 ppm, respectively. The ^{13}C NMR spectrum displayed the right number of quaternary signals (7 signals) and the total number of carbons (15 signals) for compound **(154)**.

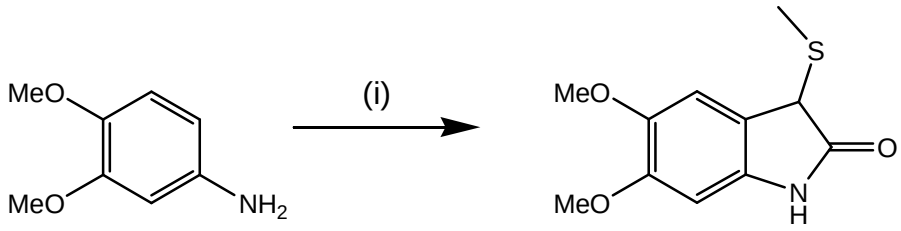
Two other related compounds were also synthesized. 3-(Propan-2-ylidene)indolin-2-one **(155)** was obtained using Trost's methodology.[75] Compound **(155)** was obtained in 76% yield after reacting 2-oxindole with acetone as depicted in **Scheme 43**. The ^{1}H NMR spectrum had two new signals at 2.64 and 2.39 ppm corresponding to the two methyl goups. The ^{13}C NMR spectrum also confirmed the presence of the two methyl groups on the oxindole **(9)** molecule. The two peaks were visible at 25.6 and 23.6 ppm, respectively.



9 **155**

Scheme 43: (i) HCl, rt, 48 hrs.

5,6-Dimethoxy-3-(methylthio)indolin-2-one **(156)** was obtained following a modified Gassman oxindole synthesis.[76] 3,4-Dimethoxyaniline **(134)** was chlorinated using sulfuryl chloride, then reacted with ethyl (methylthio)acetate, with the heterocyclic ring formed after the addition of triethylamine and stirring in acetic acid to form compound **(156)** in a low yield of 31% **(Scheme 44)**. The ^{1}H NMR spectrum showed a peak at 1.97 ppm corresponding to the methyl group bonded to the sulfur atom. Another peak, integrating for one proton at 4.26 was visible with two singlets at 6.96 and 6.58 ppm corresponding to the two aromatic protons. A peak at 3.82 was also seen which integrated for 6 protons, corresponding to the 2-methoxy groups. The ^{13}C NMR spectrum showed the CH and the CH_3 peak at 47.2 and 11.7 ppm, respectively.



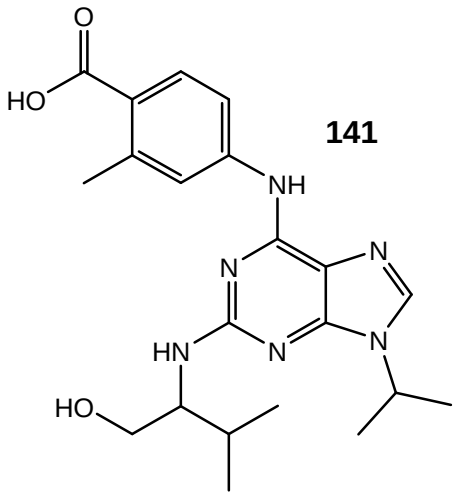
134 **156**

Scheme 44: (i) (a) SO_2Cl_2, $CH_3SCH_2CO_2Et$, 1,8-bis(dimethylamino)naphthalene; (b) Et_3N; (c) AcOH.

3.3 Modelling results

As mentioned before, the docking of the synthesized compounds **(142-154)**, was done prior to the preparation of the the 3-substituted oxindoles. In this section details of the molecular modelling will be described.

Figure 28 below shows the original inhibitor, 4-[2-(1-hydroxy-3-methylbutan-2-ylamino)-9-isopropyl-9*H*-purin-6-ylamino)-2-methylbenzoic acid **(141)** which was used in the preparation of the active site of PfPK5 for the molecular modelling studies.



141

Figure 28

Figure 29 shows the original ligand **(141)** docked in the active site of the PfPK5. Strong hydrogen bonding was seen on the left side of the active site (green dotted lines). The front of the active site favours the polar interactions, for example with polar constituents of the inhibitor. The back of the active site favors the lipophilic constituents of the inhibitor, in the case of the original inhibitor **(141)**, this is the isopropyl group.

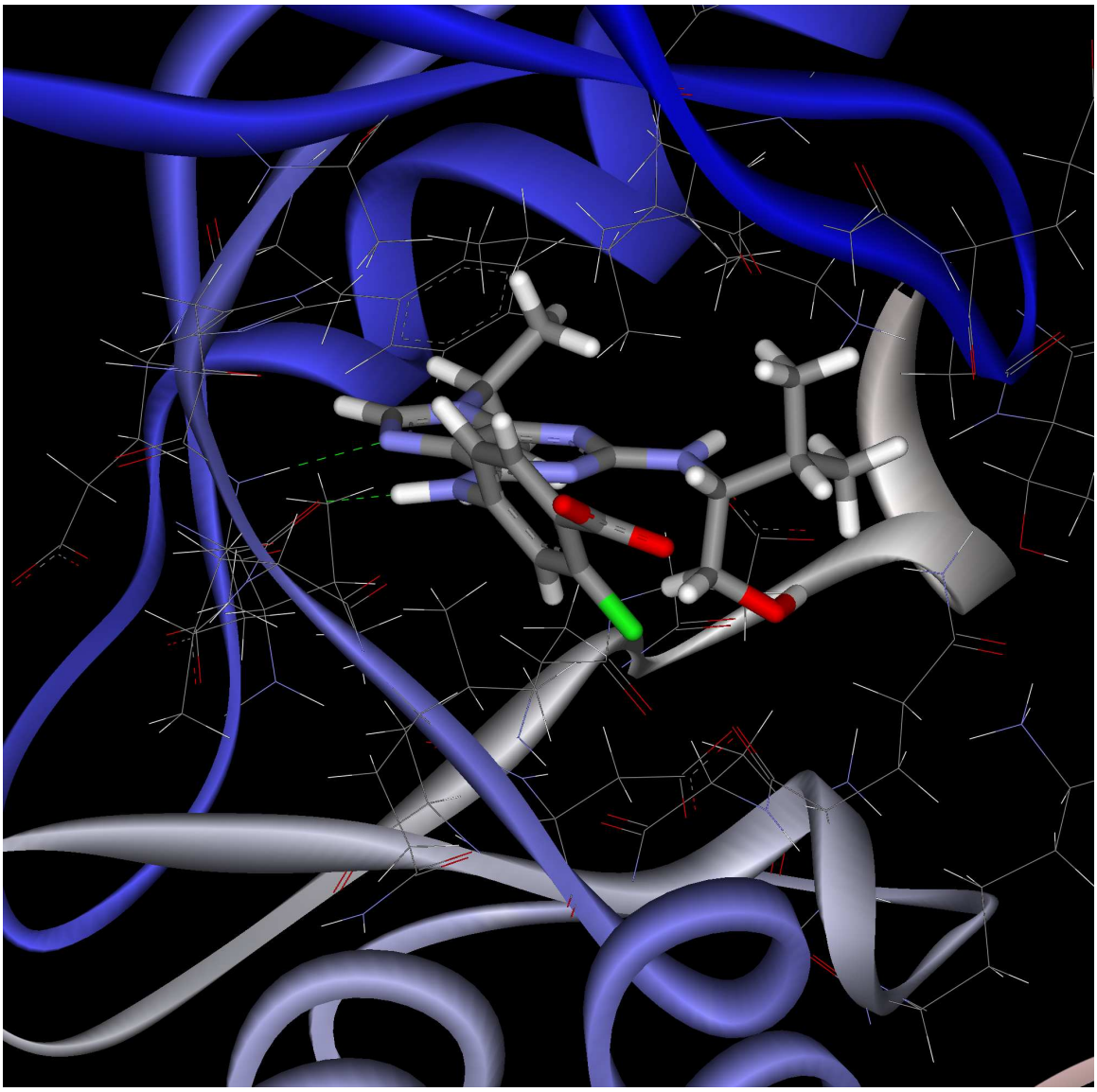


Figure 29: (produced by Dr. Steve Pelly, CSIR.)

The diagram below **(Figure 30)** shows the actual pose of the original inhibitor **(141a)** taken from the crystal structure of the PfPK5 with the inhibitor in the active versus the docked original inhibitor **(141)** .The highest scoring pose (in green) **(141a)** is a very good match for the actual pose of the inhibitor in the active site,

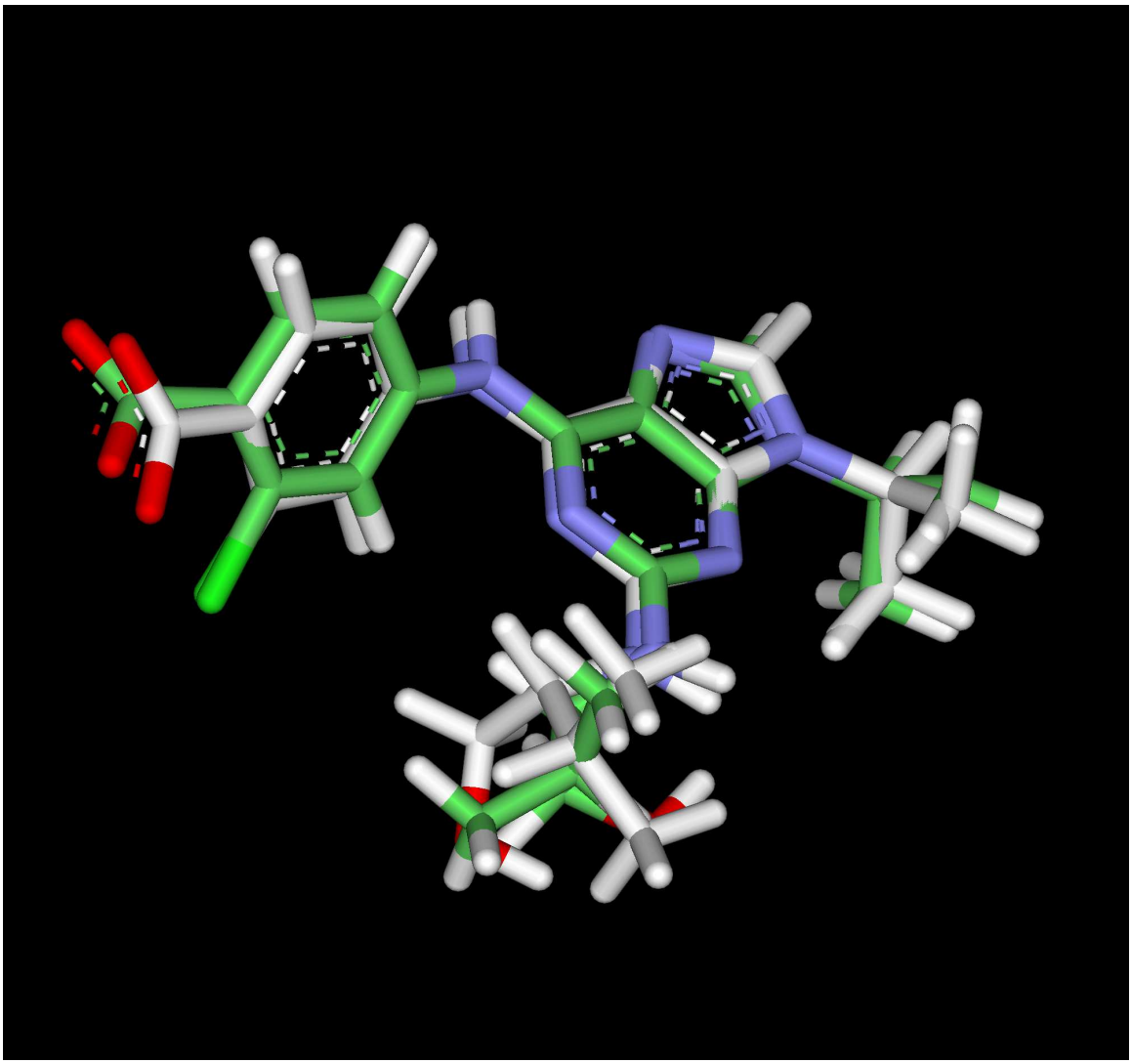


Figure 30: (Produced by Dr. Steve Pelly, CSIR.)

Two of the molecules were identified (Table 2) in the modelling process as docking well in the active site of PfPK5, as well at the actual inhibitor. The inhibitor was docked along with the oxindoles we had synthesized to check that there was no malfunctioning of the docking algorithm.

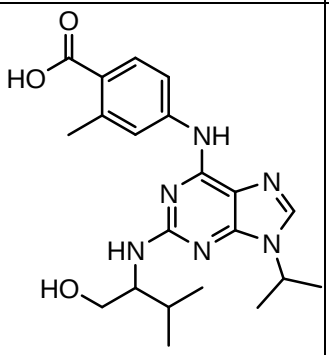
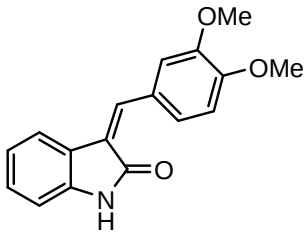
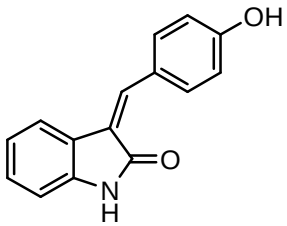
Molecule	Consensus	Number	Ligscore2	PLP2	Dock Score	Ludi3	MWt
 **141**	3	154	6.57	89.97	89.438	711	431.9
 **145**	3	142	5.32	62.81	66.959	452	281.3
 **153**	3	150	5.14	61.57	83.022	422	237.3

Table 2: Scores obtained after docking in the active site of PfPK5.

Of all the synthesized oxindoles, compound **(145)** and **(153)** were the two which scored significantly high using the parameters utilized by the Accerlys software. One of the important reasons for the other compounds not scoring high, and the downfall of for example compound **(153)**, was that the molecules do not utilize the majority of the space available in the site. 3-(3,4 Dimethoxybenzylidene)indolin-2-one **(145)** was one compound seen to make better use of the space available in the site **(Figure 31)**.

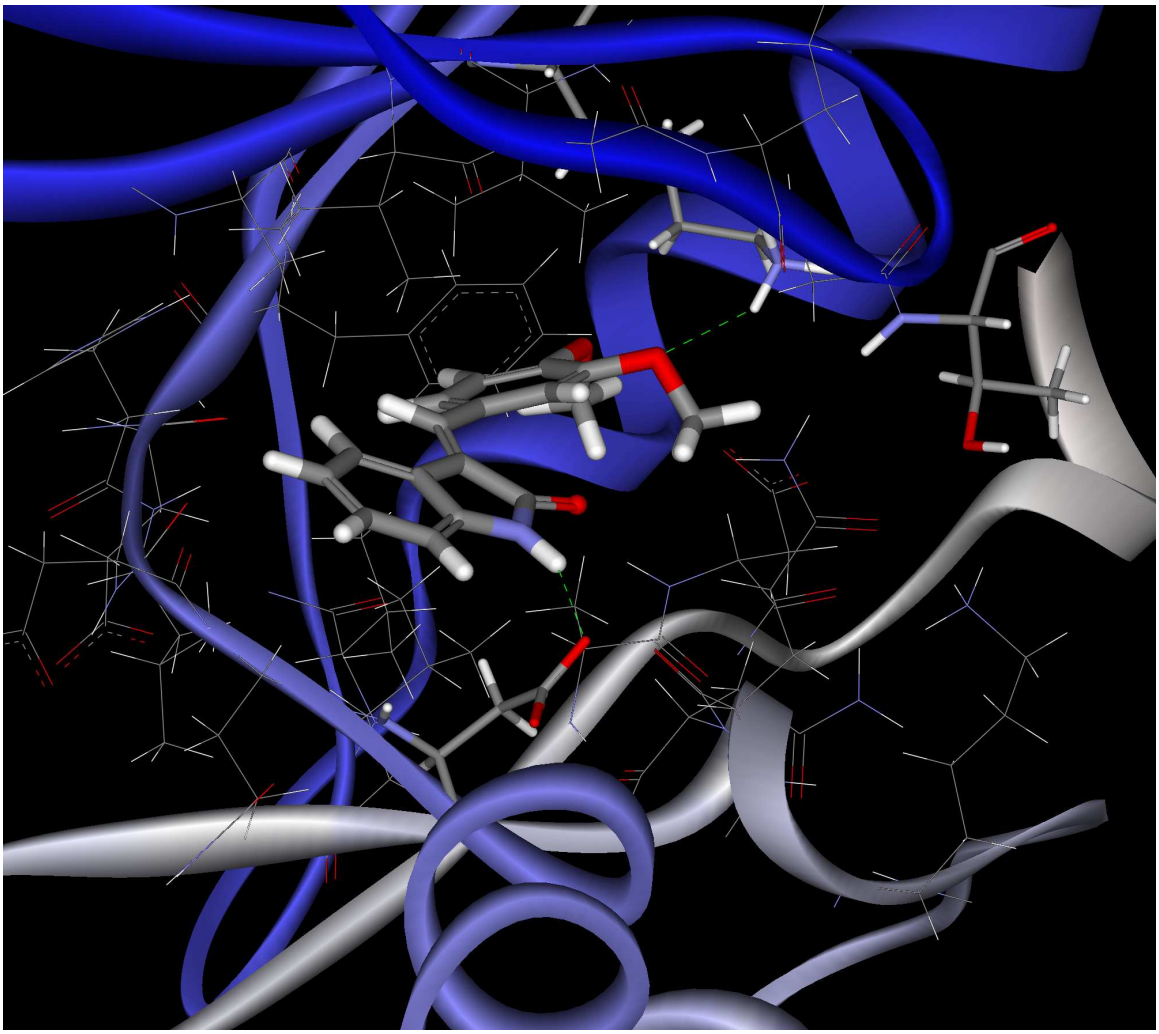


Figure 31: (produced by Dr. Steve Pelly.)

Another important reason for the low scoring was the poor ability of the compounds to fill the hydrophobic and hydrophilic regions of the active site correctly. The phamacore map of the binding site, which is shown bellow **(Figure 32)**, show regions where certain groups of the compound should be found. The turquoise ball at the back of the active site represents a region of space where a hydrophobic group should be placed, since the back region of the binding sites rather hydrophobic, and would prefer to interact with a non-polar part of the inhibitor. The purple balls represent hydrogen bond donor locations, for example, the –OH, or –NH type groups. The green balls represent regions where an H-

bond acceptor should be found, for example, -N=, or C=O. For the hydrogen bonding features, the smaller balls are those parts of the inhibitor which should be in space (inside the active site) and the larger balls are the matching interaction site on the protein itself.

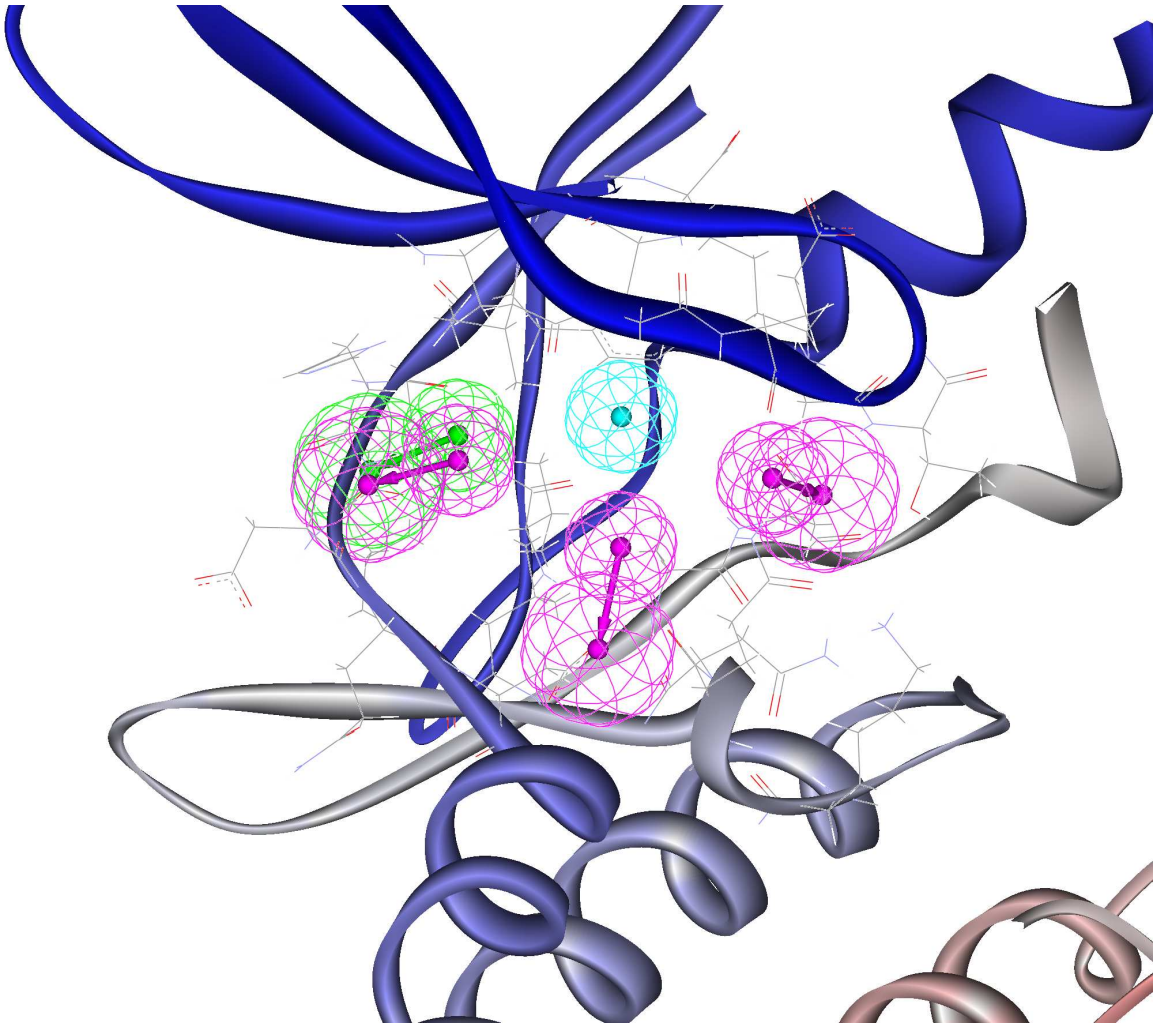


Figure 32: (Produced by Dr. Steve Pelly, CSIR.)

Figure 33 shows how well the actual inhibitor **(141)** fits into the phamacophore. The hydrophobic isopropyl group is located on the hydrophobic part of the active site at the back (turquoise ball). On the left of the picture, the hydrogen bond donor and acceptor regions are exactly where they need to be (purple and green balls respectively). At the bottom of the picture another hydrogen bond donor

group which in this case is the –OH group, was located exactly in the correct space. A feature that is not utilized was the hydrogen bond feature which is shown on the right side of **Figure 33**, but this is believed not to be problematic, as the hydrophobic isopropyl group is not on this polar pharmacophore, but close to it.

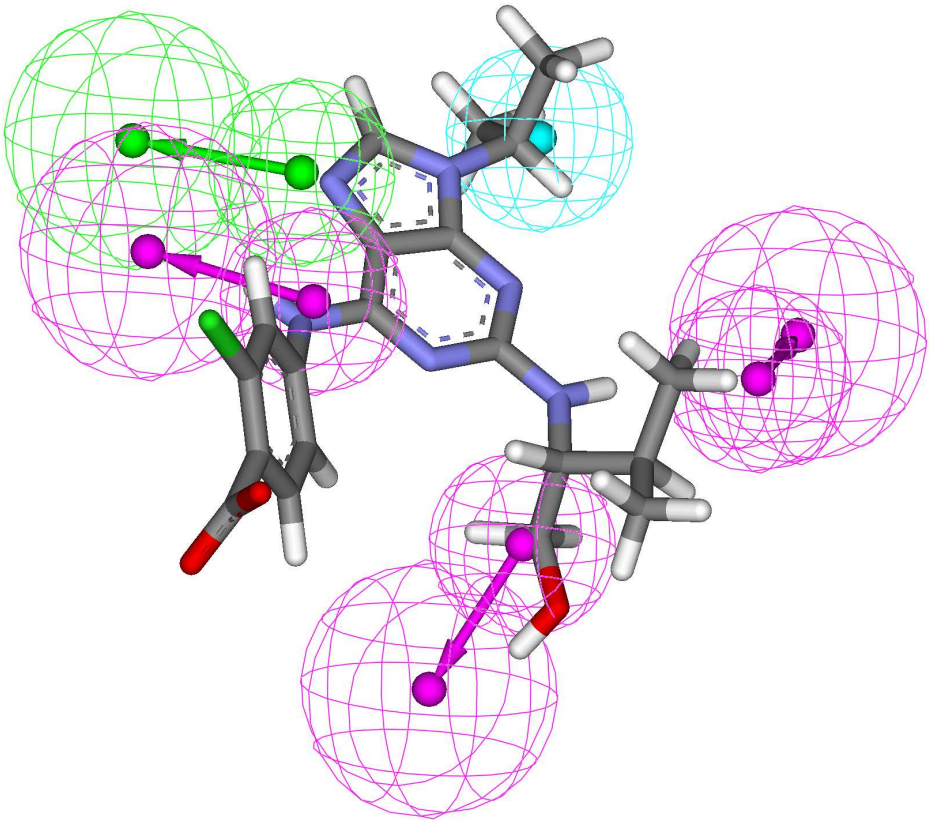


Figure 33: (Produced by Dr. Steve Pelly, CSIR.)

As mentioned earlier, molecules which were synthesized in this project do not possess the shape to map out the pharmacophore features. An example is compound **(145)** in the pharmacophore as shown in **Figure 34**. Compound **(145)** displayed only one good mapping at the bottom of the picture, which was the hydrogen bond donor being the oxindole NH group. Bad interactions were noticed for the hydrogen bonding features on the left and right hand side and a

poorly matched hydrophobic feature was seen at the back of the diagram. Improvements may be to change the 3-methoxy to a 3-hydroxy to generate a hydrogen bond donor substituent which would suit the required features on the right hand side of **Figure 34** and to remove the 4-methoxy substituent, as it is located where there should be hydrophobic interactions perhaps even replace it with a methyl substituent to occupy the available space.

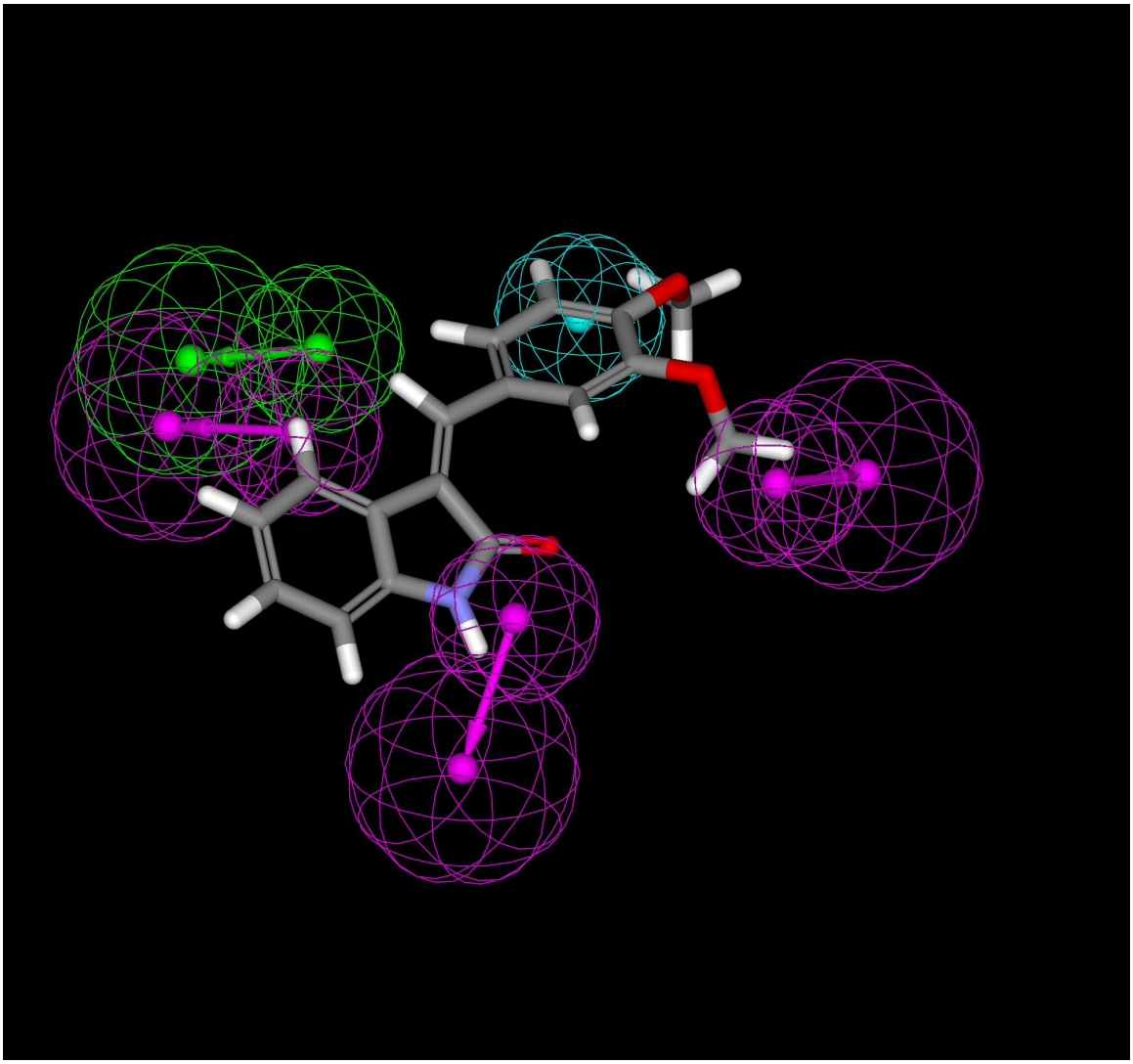
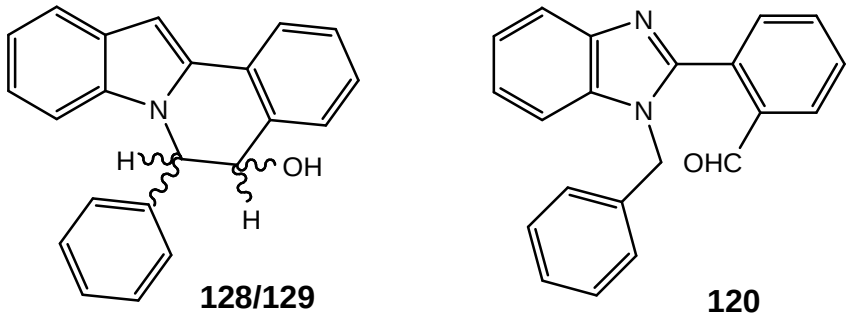


Figure 34: (Produced by Dr. Steve Pelly, CSIR.)

Due to time constraints, we could not further explore modelling other compounds based on the 2-oxindole core, which might have better results in the docking process.

Chapter 4: Conclusion and Future Work

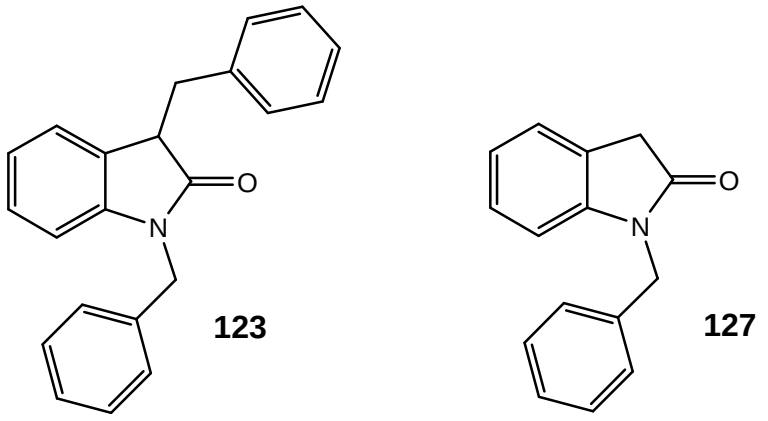
In conclusion we have managed to synthesize 2-(1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde **(120)** and 5,6-dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol **(128/129)** in moderate yields, using the Suzuki-Miyaura cross-coupling reaction and the cyclization reaction conditions (^{t}BuOK in DMF at room temperature).



128/129 **120**

Figure 35

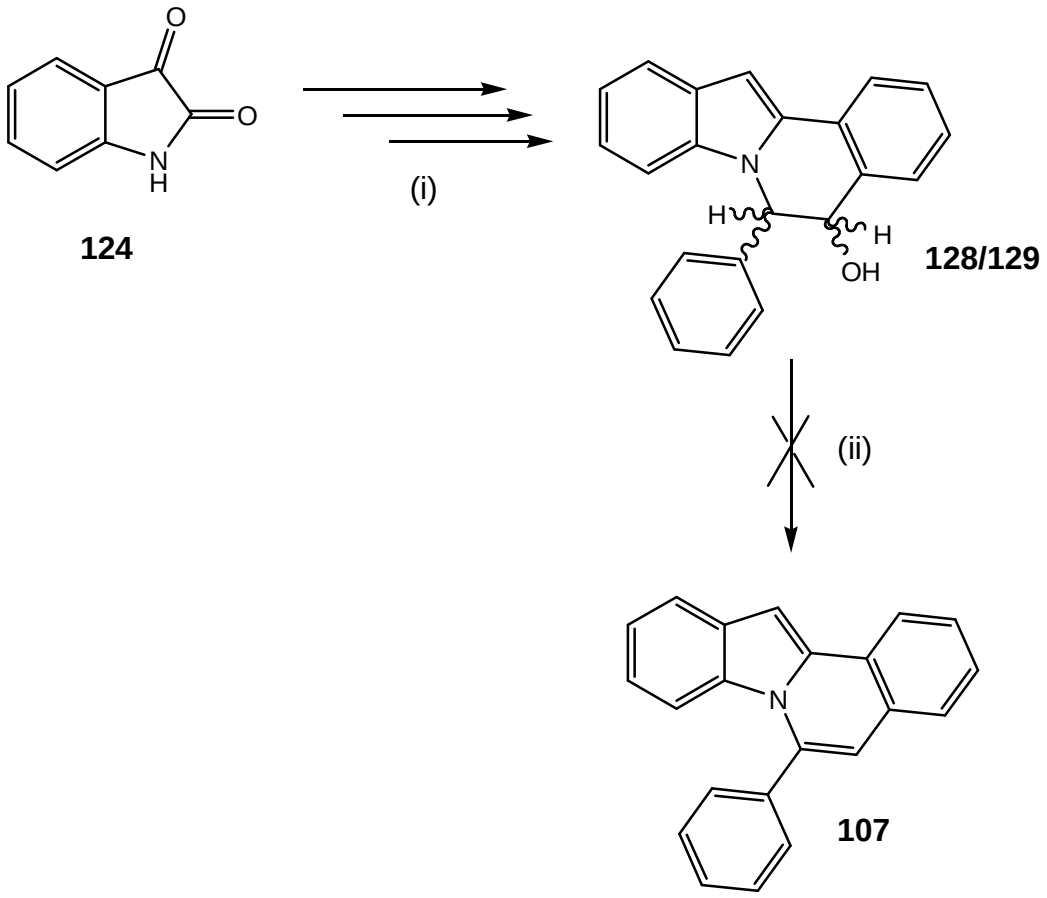
The attempted synthesis 6-phenylindolo[2,1-*a*]isoquinoline **(107)** from oxindole **(9)** was found to be unsuccessful as **(1123)** was obtained when starting with oxindole, when we only required *N*-benzyloxindole **(127) (Figure 36)**.



123 **127**

Figure 36

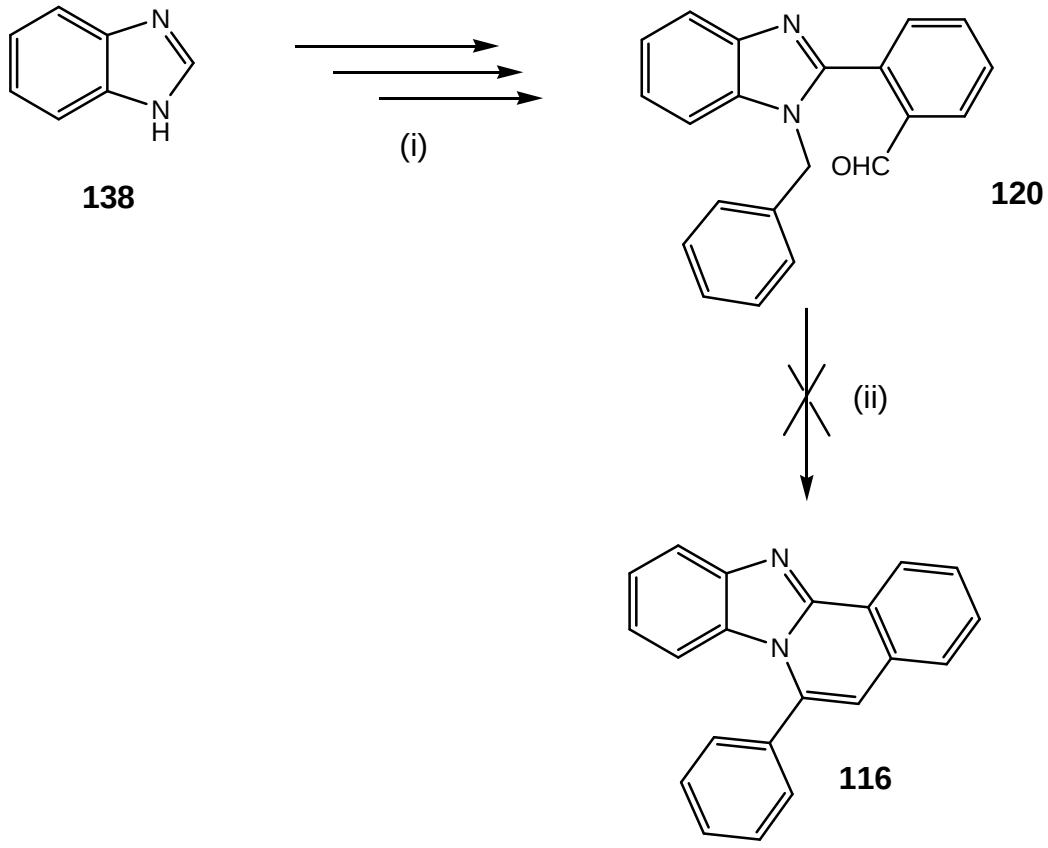
The alternative route starting from isatin **(124)** was found to be successful. The only hurdle was the tricky bromination reaction to afford 1-benzyl-2-bromo-1*H*-indole **(110)** from **(127)** in high yield of 88%. We managed to synthesize diastereomers **(128/129)** in a combined yield of 75% from **(108)**, but we could not remove water from either of the diastereomers to afford to the target compound **(107)** by stirring it in CH_2Cl_2 in the presence of triethylamine and methanesulfonyl chloride.



Scheme 42: (i) A route used to synthesize **(128/129)**. (ii) Final step in the synthesis of **107** failed using Et_3N, methanesulfonyl chloride, CH_2Cl_2, 0^oC.

Alternative reaction conditions that could be employed to convert **(128/129)** to **(107)** might be by using various acids to protonate the alcohol so as to convert it to a leaving group.

Compound **(120)** was synthesized starting from benzimidazole **(138)**. The iodination of the protected benzimidazole **(140)** was achieved using two different reactions, which gave product **(119)** in moderate to good yields of the desired products (40% and 75%). Ring closure of compound **(120)** failed to yield the desired alcohols using the cyclization reaction conditions (^{t}BuOK in DMF at room temperature) used in our previous work **(Scheme 43)**.

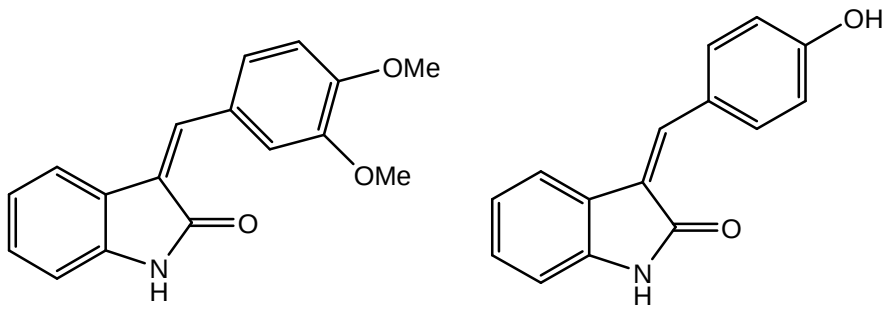


138 **120** **116**

Scheme 43: (i) A route used to synthesize **(120)**. (ii) Failed cyclization reaction to give alcohol and dehydration to afford compound **(116)**.

Future work could be to use bases to attempt the required ring closure reaction which will lead to the desired alcohol, which then can be dehydrated to form the final product **(116)**.

Thirteen small molecule indole derivatives were successfully synthesized and modelled against PfPK5. Molecule **(145)** and **(153)** scored significantly high against the parameters measured by the Accerlys software.



145 **153**

Figure 37

Future work includes the actual testing of the molecules in a PfPK5 assay and the subsequent comparison of the modelling results with the test results. More similar small molecules (3-substituted oxindoles) can also be synthesized having different substituents on the benzene ring of the oxindole system or the 3-position itself as has been done in this project **(Figure 38)**.

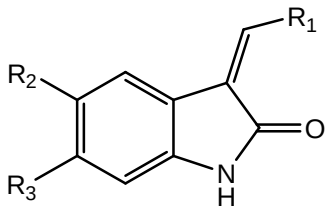


Figure 38

Chapter 5: Experimental Procedures

5.1. General Experimental Procedures

5.1.1. Purification of Solvents and Reagents

All the solvents which were used for reactions, unless otherwise stated, were distilled before use and solvents used for silica gel chromatography were distilled prior to use. Tetrahydrofuran was distilled from sodium benzophenone ketal radical and dichloromethane from calcium hydride. Potassium tertiary butoxide was sublimed before use. Other reagents were obtained from commercial sources and unless stated, were used without further purification.

5.1.2. Chromatographic Separation

The quoted R_f values are from thin layer chromatography (TLC) on aluminium-backed Macherey-Nagel Alugram Sil G/UV_{254} plates pre-coated with 0.25 mm silica gel 60, and detection was done by UV absorbtion. Column chromatography was done on wet columns using Macherey-Nagel Kieselgel 60 (particle size 0.063-0.200 mm) as the stationary phase. Preparative silica gel chromatography was carried out on dry packed columns using Macherey-Nagel Kieselgel 60 silica gel 60 (particle size 0.063-0.200 mm) as the adsorbent. Different percentages of ethyl acetate and hexane were used as the mobile phase.

5.1.3. Spectroscopic and Physical Data

All melting points were obtained on a Reichert hot stage microscope and are uncorrected.

^{1}H NMR spectral data were recorded on a Bruker AVANCE 300 (300.13Hz) spectrometer. Unless specified, spectra were recorded in deuterated chloroform and chemical shifts were expressed as parts per million from the reference signal from tetramethylsilane. Coupling constants are given in hertz and the splitting partens are assigned as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). NMR spectra data are reported as follows: chemical shifts (integration of signal, coupling constant where applicable, assignments).

^{13}C NMR spectra were recorded on a Bruker AVANCE 300 (75.47 Hz) spectrometer. Spectra were recorded in deuterated chloroform ($CDCl_3$) unless otherwise stated. Spectra data are expressed in parts per million in relation to the reference signal of deuterated chloroform, at δ 77.00.

IR (infrared) spectra were recorded using a Bruker IFS-25 Fourier Transform Spectrometer. Liquid and solid samples were recorded as is. The signals are reported on the wavenumber scale (v / cm^{-1}). The data is reported in the following manner: wavenumber (assignment).

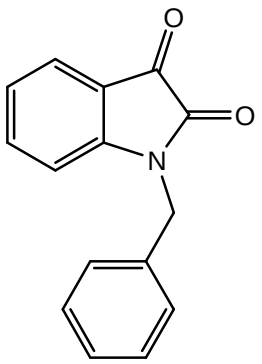
High resolution mass spectra (HRMS) were recorded on a VG70 MS (Mass Spectrum CC Pyramid data system) or on a VG70 SEQ (VG 11-250J or Marc II data system). Mass spectroscopy values are measured as *m/z*.

5.1.4. Other General Procedures

The term removal of solvent under reduced pressure refers to removing the solvent using an aspirator pressure (*ca*. 25 Torr) with a rotary evaporator at 40-60^{o}C. The term *in vacuo* refers to the solvent being removed by rotary evaporation followed by removal of the residual solvent using an oil pump at pressures of between *ca*. 0.1-1.0 Torr at ambient temperature, until constant mass was achieved.

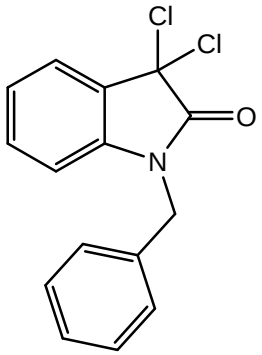
5.2. Attempted synthesis of 6-phenylindolo[2,1-a]isoquinoline.

5.2.1. Synthesis of 1-benzylindoline-2,3-dione (125)[73]



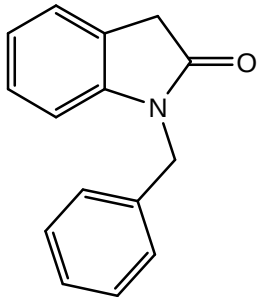
Isatin (8.00 g, 54.0 mmol) and CaH_2 (2.29 g, 54.0 mmol, 1.00 equiv.) were dissolved in DMF in a round bottom flask equipped with a stirrer bar and a condenser. The solution was then stirred at 100^{o}C for 1 hr, then the reaction contents were left to cool to 40^{o}C so that benzyl bromide (60.2 mmol, 7.10 cm^3, 1.10 equiv.) could be added portion-wise over a period of 30 mins. The resulting mixture was stirred for a further 4 hrs and then allowed to cool to rt. The solution was then poured into an aqueous 0.5 M HCl (200 cm^3) solution with vigorous stirring, which resulted in an orange solid precipitating. The precipitate was filtered and washed with H_2O. The crude solid was then purified further by recrystallization (CH_2Cl_2/Hexane) to afford 1-benzylindoline-2,3-dione **(125)** as orange crystals. (11.4 g, 89%). R_f 0.42 (EtOAc/Hexane, 30 : 70); m.p. 133-134 ^{o}C (Lit. m.p. 132-133 ^{o}C)[73]; δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.61 (1H, d, J=7.5, ArH), 7.48 (1H, dt, J=1.2, and 7.8, ArH, 7.25-7.38 (5H, m, 5×ArH), 7.09 (1H, t, J=7.6, ArH), 6.78 (1H, d, J=7.9, ArH), 4.94 (2H, s, ArC*H*$_2$N); δ_c (75 MHz, $CDCl_3$) 183.2 (C=O), 158.2 (BnNC=O), 150.7 (ArC), 138.3 (ArCH), 134.5 (ArC), 129.0 (2×ArCH), 128.1 (ArCH), 127.4 (2×ArCH), 125.3 (ArCH), 123.8 (ArCH), 117.6 (ArC), 110.0 (ArCH), 44.0 (CH_2).

5.2.2. Synthesis of 1-benzyl-3,3-dichloroindolin-2-one (126)



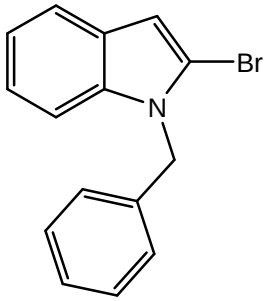
1-Benzylindoline-2,3-dione **(125)** was dissolved in benzene (50 cm^3) in a flame-dried round bottom flask. Phosphorus pentachloride (12.8 g, 62.1 mmol, 2.30 equiv.) was added portion-wise with fizzing and then the reaction mixture was left for a further 24 hrs at 50^oC. The solvent was then removed under reduced pressure to obtain a brown-yellowish residue which was then purified using column chromatography (EtOAc/Hexane, 30: 70) to give a yellow oil which solidified. This was purified further by recrystallization using EtOH to give 1-benzylindoline-3,3 dichloroindolin-2-one **(126)** as a white solid (6.37 g, 81%). R_f 0.58 (EtOAc/Hexane, 30: 70); m.p. 125-126 ^oC; v_{max} (film)/cm^{-1}: 2399 (m, C-H), 1744 (s, C=O), 1523 (w, C=C), 1099 (w, C-N), 772 (s, C-Cl); δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.64 (1H, d, J=7.5, ArH), 7.22-7.39 (6H, m, 6×ArH), 7.14 (1H, t, J=7.6, ArH), 6.72 (1H, d, J=7.9, ArH), 4.94 (2H, s, CH_2); δ_c (75 MHz, $CDCl_3$) 169.2 (BnNC=O), 139.8 (ArC), 134.4 (ArC), 131.8 (ArCH), 129.3 (ArC), 129.0 (2×ArCH), 128.1 (ArCH), 127.1 (2×ArCH), 124.9 (ArCH), 124.2 (CCl_2), 110.1 (ArCH), 44.5 (CH_2).

5.2.3. Synthesis of 1-benzylindolin-2-one (127)[80,81]



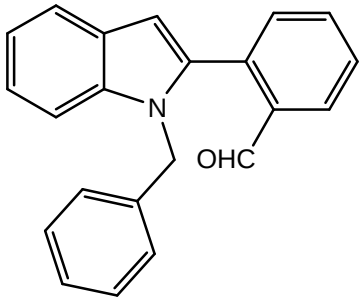
1-Benzylindoline-3,3-dichloroindolin-2-one **(126)** (3.00 g, 10.3 mmol) was dissolved in AcOH (100 cm^3). Activated Zn (10.0 g, 154 mmol, 15.0 equiv.) was added over a 10 min. period and then the mixture was stirred for another 10 mins. The Zn was filtered off and the filtrate was washed with AcOH (20 cm^3) and EtOAc (30 cm^3). The solution was then extracted using CH_2Cl_2 (3 × 50 cm^3). The organic layers were combined and dried using anhydrous $MgSO_4$. The $MgSO_4$ was filtered and the solvent was removed under reduced pressure; the resulting residue was purified by column chromatography (EtOAc/Hexane, 30: 70) to give 1-benzylindolin-2-one **(127)** as a white solid (1.75 g, 76%). R_f 0.40 (EtOAc/Hexane, 30: 70); m.p. 60-62 ^oC (Lit. m.p. 61-63 ^oC)[80]; δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.21-7.34 (6H, m, 6×ArH); 7.16 (1H, t, J=7.8, ArH), 7.00 (1H, t, J=7.5, ArH), 6.72 (1H, d, J=7.8, ArH), 4.91 (2H, s, CH_2), 3.62 (2H, s, COCH_2); δ_c (75 MHz, $CDCl_3$) 175.1 (C=O), 144.3 (ArC), 135.9 (ArC), 128.7 (2×ArCH), 127.8 (ArCH), 127.6 (ArCH), 127.3 (2×ArCH), 124.4 (ArC), 124.3 (ArCH), 122.3 (ArCH), 109.0 (ArCH), 43.7 (CH_2), 35.7 (COCH_2).

5.2.4. Synthesis of 1-benzyl-2-bromo-1H-indole (110)[61,77]



A solution of 1-benzylindolin-2-one **(127)** (0.19 g, 0.68 mmol) was dissolved in CH_2Cl_2 (20.0 cm^3) in a flame-dried round bottom flask. Phosphorus oxybromide (2.81 g, 10.0 mmol, 1.5 equiv.) was added and the resulting mixture was stirred for 1 hr and let to cool down to room temperature. Imidazole (0.040 g, 0.50 mmol, 1.20 equiv.) was added and reaction contents were stirred for 48 hrs under reflux. The reaction was quenched with 15% aqueous solution of $NaHCO_3$ (40 cm^3) until the effervescence had stopped. The solution was then extracted using CH_2Cl_2 (3 × 30 cm^3). The organic extracts were combined and dried using $MgSO_4$. It was then filtered and the solvent removed under reduced pressure to give an orange residue which was purified by column chromatography (EtOAc/Hexane, 2: 98) to afford the brominated compound **(110)** (0.07 g, 88%) as a white solid. m.p. 87-89 ºC; ν_{max} (film)/cm^{-1}: 3000 (ArC-H), 1605 (ArC=C), 780 (ArH), 691 (C-Br); δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.55 (1H, dd, J=2.2 and 6.4, ArH), 7.20-7.28 (4H, m, 4×ArH), 7.06-7.13 (4H, m, 4×ArH), 6.65 (1H, s, 3H), 5.41 (2H, s, CH_2); δ_c (75 MHz, $CDCl_3$) 137.0 (ArC), 136.7 (ArC), 128.7 (2×ArCH), 128.2 (ArC), 127.5 (ArCH), 126.4 (2×ArCH), 122.0 (ArCH), 120.3 (ArCH), 119.8 (ArCH), 113.5 (ArC), 109.0 (ArCH), 104.5 (CH), 48.1 (CH_2); m/z 285 (M^+, 42%), 204 (11), 91 (100), 65 (9); Found M^+, 285.0091. $C_{15}H_{12}N^{79}Br$ requires 285.0153.

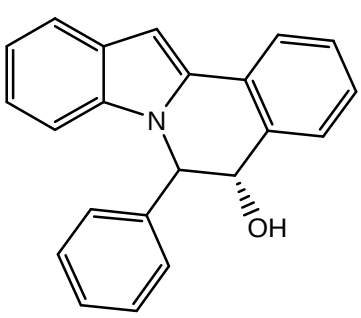
5.2.5. Synthesis of 2-(1-benzyl-1H-indol-2-yl)benzaldehyde (108)[63]

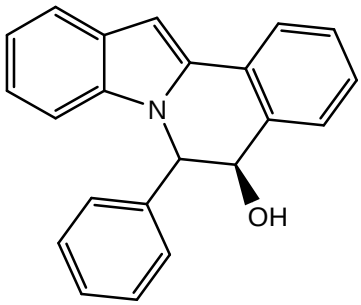


Palladium(0) tetrakis (triphenylphosphine) (0.10 g 0.087 mmol, 10 mol%) was introduced into a flame-dried two-necked round bottom flask equipped with a dropping funnel and a condenser. A solution of 1-benzyl-2-bromo-1*H*-indole **(110)** (0.25 g, 0.87 mmol), 2-formylphenylboronic acid (0.150 g, 1.30 mmol, 1.50 equiv.) and DME (2.40 cm^3, 1.1% of Na_2CO_3 solution) was prepared in the dropping funnel, degassed with N_2 for 3 mins and transferred to the flask. An aqueous solution of 2M Na_2CO_3 (0.461 g, 4.40 mmol, 5.00 equiv.) was added to the dropping funnel, degassed for 3 mins with N_2 and then added to the flask. The reaction contents were stirred for a further 24 hrs. The reaction was quenched with water (10 cm^3) and was extracted with CH_2Cl_2 (3 × 30 cm^3). The organic layers were collected and dried using $MgSO_4$. This was filtered and the solvent was removed under reduced pressure to give a brown residue which was purified using column chromatography (EtOAc/Hexane, 10: 90) to afford 2-(1-benzyl-1*H*-indol-2-yl)benzaldehyde **(108)** as a yellow viscous oil (0.49 g, 98%). R_f 0.74 (EtOAc/Hexane, 30: 70); v_{max} (film)/cm^{-1}: 3060 (ArCH), 1710 (C=O), 1550 (ArC=C), 750 (ArH); δ_H (300 MHz; $CDCl_3$; Me_4Si) 9.85 (1H, s, CHO), 8.01 (1H, dd, J=1.4 and 7.6, ArH), 7.69 (1H, dd, J=1.1 and 6.9, ArH), 7.50-7.61 (2H, m, 2×ArH), 7.40 (1H, dd, J=1.2 and 7.4, ArH), 7.32 (1H, d, J=8.0, ArH), 7.16-7.27 (5H, m, 5×ArH), 6.83 (2H, dd, J=3.9 and 5.3, 2×ArH), 6.63 (1H, s, NCCH), 5.26 (2H, s, CH_2); δ_c (75 MHz, $CDCl_3$) 191.6 (C=O), 137.8 (ArC), 137.4 (ArC), 135.8 (ArC), 135.5 (ArC), 135.4 (ArC), 133.2 (ArCH), 131.4 (ArCH), 129.0 (ArCH), 128.7 (2×ArCH), 127.9 (ArC), 127.5 (ArCH), 127.4 (ArCH), 126.1 (2×ArCH),

122.7 (ArCH), 120.8 (ArCH), 120.5 (ArCH), 110.4 (ArCH), 106.8 (BnNC*C*H), 47.6 (CH_2); m/z 311 (M^+, 33%), 283 (58), 282 (65), 221 (19), 220 (100), 206 (39), 182 (53), 181 (72), 180 (20), 153 (20), 152 (30), 91 (87), 86 (27), 84 (41), 76 (15), 57 (22), 43 (18); Found M^+, 311.1335, $C_{22}H_{17}NO$ requires 311.1310.

5.2.6. Synthesis of 5,6-Dihydro-6-phenylindolo[2,1-a]isoquinolin-5-ol (128/129)[78]



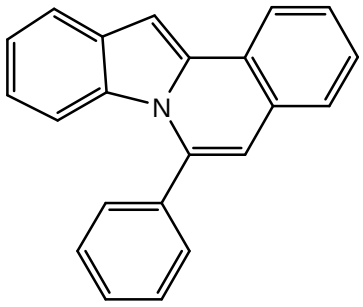


A solution of 2-(1-benzyl-1*H*-indol-2-yl)benzaldehyde **(108)** (0.19 g, 0.640 mmol) in DMF was prepared in a flame-dried round bottom flask. ^{t}BuOK (0.086 g, 0.77 mmol, 1.2 equiv.) was added and the reaction mixture was stirred for a further 2 mins at rt. The solution was quenched with water (40 cm^3) and extracted with CH_2Cl_2 (3 × 40 cm^3). After this, the organic layers were combined and dried using $MgSO_4$. This was filtered and the solvent removed under reduced pressure; the residue thus obtained was purified by column chromatography (EtOAc/Hexane, 5:95) to afford the *anti*- and the *syn*-diastereomers as yellow-green resins in a 7:3 ratio (53% *anti* and 22% *syn*). R_f (*syn*) 0.49 (EtOAc/Hexane, 30: 70); ν_{max} (film)/cm^{-1}: 3548 (OH), 3060 (ArC-H), 1605 (ArC=C), 747 (ArH); δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.75 (1H, d, J=7.6, ArH), 7.54 (1H, dd, J=2.2 and 6.4, ArH), 7.38 (1H, d, J=7.8, ArH), 7.29 (1H, t, J=7.4, ArH), 7.11-7.21 (2H, m, 2×ArH), 6.97-7.05 (5H, m, 5×ArH), 6.92 (1H, s, ArH), 6.84 (2H, m, 2×ArH), 5.71 (1H, d, J=6.9, NCH), 5.38 (1H, dd, J=7.0 and 11.0, C*H*-OH), 1.66 (1H, d, J=11.0, OH); δ_c (75 MHz, $CDCl_3$) 136.4 (ArC), 135.0 (ArC), 134.5 (ArC), 133.4 (ArC), 129.1 (ArC), 128.6 (2×ArCH), 128.4 (ArCH), 128.1 (ArCH), 128.0 (ArCH), 127.9 (2×ArCH),

127.3 (ArC), 125.0 (ArCH), 123.9 (ArCH), 122.3 (ArCH), 120.8 (ArCH), 120.5 (ArCH), 109.4 (ArCH), 97.4 (ArCH), 69.5 (N*C*H), 59.5 (*C*OH); m/z 311 (M^+, 100%), 310 (18), 283 (17), 282 (47), 280 (14), 234 (17), 220 (42), 206 (41), 204 (30), 178 (15), 165 (18), 155 (14), 91 (20); Found M^+, 311.1310, $C_{22}H_{17}NO$ requires 311.1310.

R_f (*anti*) 0.36 (EtOAc/Hexane, 30: 70); v_{max} (film)/cm^{-1}: 3364 (OH), 3058 (ArC-H), 1607 (ArC=C), 747 (ArH); δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.79 (1H, d, J=7.7, ArH), 7.58-7.60 (1H, m, ArH), 7.29-7.35 (1H, m, ArH), 7.12-7.18 (3H, m, 3×ArH), 7.00-7.09 5H, m, 5×ArH), 6.97 (1H, s, ArH), 6.70 (2H, dd, J=2.6 and 6.6, 2×ArH), 5.81 (1H, d, J=1.5, NC*H*), 4.91 (1H, s, C*H*OH), 2.18 (1H, bs, OH); δ_c (75 MHz, $CDCl_3$) 137.8 (ArC), 137.4 (ArC), 133.7 (ArC), 131.0 (ArC), 129.9 (ArCH), 129.7 (ArCH), 129.1 (ArC), 128.7 (2×ArCH), 128.1 (ArCH), 128.0 (ArC), 127.7 (ArCH), 125.9 (2×ArCH), 124.4 (ArCH), 122.4 (ArCH), 120.9 (ArCH), 120.5 (ArCH), 109.6 (ArCH), 97.6 (ArCH), 73.9 (NCH), 62.0 (COH); m/z 311 (M^+, 100%), 310 (20), 283 (18), 282 (47), 280 (14), 234 (18), 220 (42), 217 (14), 206 (42), 204 (31), 178 (16), 165 (19), 155 (15), 91 (21); Found M^+, 311.1339, $C_{22}H_{17}NO$ requires 311.1310.

5.2.7. Attempted synthesis of 6-phenylindolo[2,1-a]isoquinoline (107)

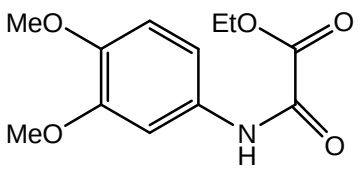


5,6-Dihydro-6-phenylindolo[2,1-*a*]isoquinolin-5-ol (0.12 g, 0.32 mmol) was dissolved in CH_2Cl_2 (15 cm^3) in a flame-dried round bottom flask. The temperature was lowered to 0^oC, followed by the addition of triethylamine (4 µL,

0.32 mmol, 1.0 equiv.) and stirred for a further 30 mins. Methanesulfonyl chloride (25 µL, 0.32 mmol, 1.0 equiv.) was added and the reaction stirred for a further 30 mins at the same temperature. The reaction was quenched with $NaHCO_3$ (40 cm^3) and extracted using CH_2Cl_2 (3 × 30 cm^3). The organic layers were combined, dried and the solvent removed under reduced pressure to obtain a green residue which was purified by column chromatography (EtOAc/Hexane, 10: 90) to give an un-characterizable product.

5.3. Attempted synthesis of 9,10-dimethoxy-6-phenylindolo[2,1-a]isoquinoline

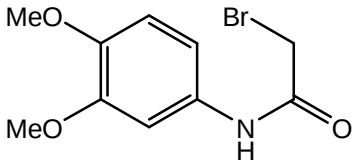
5.3.1. Synthesis of ethyl (3,4-dimethoxyphenylcarbamoyl)formate (131)[65]



Oxalyl chloride (20.0 cm^3, 40.0 mmol, 2.00 equiv.) was added to a flame-dried round bottom flask and the temperature was dropped to 0 ºC. EtOH (1.60 cm^3, 20.0 mmol) was added dropwise to the flask and the mixture was left for a further 20 mins at the same temperature. The oxalyl chloride was removed *in vacuo* to give ethyl (chlorocarbonyl)formate as yellow-brown oil which was dissolved in THF in a flame-dried round bottom flask equipped with a stirrer bar. The temperature of the reaction mixture was lowered to 0 ºC and 3,4-dimethoxyaniline (6.13 g, 40.0 mmol, 2.00 equiv.) was added slowly to the solution. This was left to react for a further 30 mins. After $NaHCO_3$ (30 cm^3) was added, the mixture was extracted with CH_2Cl_2 (3 × 30 cm^3) and dried with $MgSO_4$. The mixture was then filtered and the solvent removed in *vacuo* to give an orange brown solid in quantitative yield (2.58 g, 100%). R_f 0.54 (EtOAc/Hexane, 50 : 50); ν_{max} (film)/cm^{-1} 3425 (s, N-H), 1701 (m, C=O), 1514 (w,

aromatic), 1303 (w, C-N) and 1150 (w, C-O); δ_H (300 MHz; $CDCl_3$; Me_4Si) 8.82 (1H, s, N-H), 7.44 (1H, s, ArH), 7.04 (1H, d, J=2.3, ArH), 6.86 (1H, d, J=8.6, ArH), 4.42 (2H, q, J=7.1, C*H*$_2$CH$_3$), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3) and 1.43 (3H, t, J=7.1, CH_2C*H*$_3$). δ_c (75 MHz, $CDCl_3$) 161.5 (*C*OOEt), 154.1 (*C*ON), 149.5 (ArC), 147.1 (ArC), 130.3 (ArC), 112.3 (ArCH), 111.7 (ArCH), 104.7 (ArCH), 64.0 (*C*H_2CH$_3$), 56.4 (O*C*H_3), 56.3 (O*C*H_3) and 14.4 (CH_2*C*H_3); m/z 253 (M^+, 97%), 254 (95), 272 (5), 276 (5), 289 (17), 307.1 (19); Found M^+, 253.0950, $C_{12}H_{15}NO_5$ requires 253.0951.

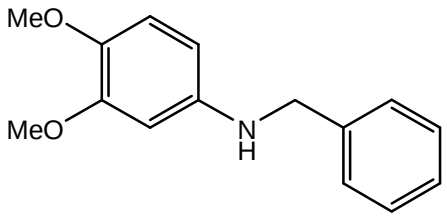
5.3.2. Synthesis of 2-bromo-N-(3,4-dimethoxyaniline)acetamide



3,4-Dimethoxyaniline (1.80 g, 6.58 mmol) was dissolved in CH_2Cl_2 in a flame-dried round bottom flask equipped with a stirrer bar. The temperature was lowered to 0 ºC and bromoacetyl bromide (0.60 cm^3, 4.3 mmol, 1.00 equiv.) was added drop-wise. The reaction was left to run for a further 30 min. $NaHCO_3$ (30 cm^3) was added, extracted with CH_2Cl_2 (3 × 30 cm^3) and dried with $MgSO_4$. It was then filtered to give purple crude product. This material was then recrystalized (methanol), the crystals filtered and washed with cold methanol to afford 2-bromo-*N*-(3,4-dimethoxyaniline)acetamide as white crystals (1.62 g, 91%). R_f 0.50 (EtOAc/Hexane, 50 : 50); m.p. 153^{o}C; ν_{max} (CH_2Cl_2)/cm^{-1} 3423 (s, N-H), 1655 (m, C=O) and 668 (w, C-Br); δ_H (300 MHz; $CDCl_3$; Me_4Si) 8.06 (1H, s, N-H), 7.26 (1H, s, ArH), 6.94 (1H, d, J=10.9, ArH), 6.85 (1H, d, J=8.6, ArH), 4.03 (2H, s, C*H*$_2$), 3.89 (3H, s, OCH_3), 3.87 (3H, s, OCH_3); δ_c (75 MHz, $CDCl_3$) 163.6 (C=O), 149.5 (ArC), 146.9 (ArC), 130.8 (ArC), 112.7 (ArCH), 111.7 (ArCH), 105.4 (ArCH), 56.5 (OCH_3), 56.4 (OCH_3), 29.9 (CH_2Br); m/z (APCI) 274 (^{79}Br-M^+

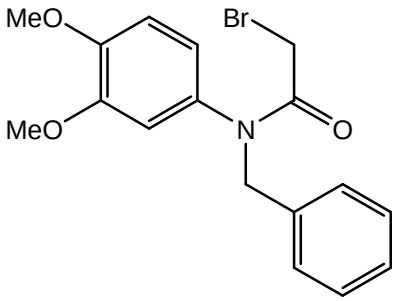
+ 1, 16%), 272.8 (16), 307 (23.5), 289 (20), 287 (2.5), 242 (2.1), 249 (2.5); Found $^{+}M^{79}Br$, 274 $C_{10}H_{13}O_3N^{79}Br$ (M + H) requires 274.0080.

5.3.3. Synthesis of N-benzyl-3,4-dimethoxyaniline (136)[66]



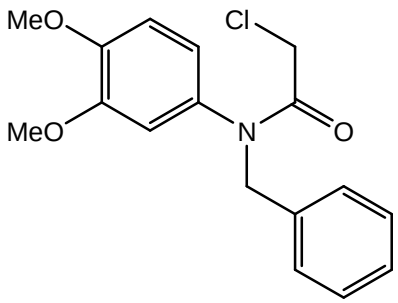
A two-necked round bottom flask was equipped with a magnetic stirrer bar and a condenser. 3,4-Dimethoxyaniline (1.46 g, 6.03 mmol, 3.53 equiv.) was dissolved in water (5 cm^3) and $NaHCO_3$ (0.197 g, 2.35 mmol, 1.27 equiv.) was added. The temperature was raised to 95 ºC and then benzyl bromide (0.20 cm^3, 1.85 mmol, 1.00 equiv.) was added over a period of 1 hr with vigorous stirring. The reaction mixture was stirred for a further 3 hrs. The reaction mixture was cooled to rt and water (30 cm^3) was added. The mixture was extracted with CH_2Cl_2 (3 × 40 cm^3) and dried with anhydrous $MgSO_4$. The $MgSO_4$ was filtered and then the solvent removed *in vacuo*. The crude product was then purified using column chromatography (10% EtOAc/Hexane) to afford *N*-benzyl-3,4-dimethoxyaniline **(136)** as a brown-orange oil (0.41 g, 91%). R_f 0.67 (EtOAc/Hexane, 50 : 50); ν_{max} (CH_2Cl_2)/cm^{-1} 3401 (w, N-H), 2933 (w, C-H), 1517 (s, aromatic), 1233 (s, C-N) and 1168 (w, C-O); δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.31 (5H, m, 5×ArH), 6.75 (1H, d, J=8.4, ArH), 6.28 (1H, s, ArH), 6.18 (1H, d, J=8.4, ArH), 4.29 (2H, s, NC*H*$_2$), 3.80 (6H, s, 2×OCH_3); δ_c (75 MHz, $CDCl_3$) 150.4 (ArC), 143.5 (ArC), 142.1 (ArC) 139.9 (ArC), 129.0 (2×ArCH), 127.9 (2×ArCH), 127.6 (ArCH), 119.7 (ArCH), 104.1 (ArCH), 99.5 (ArCH), 57.1 (OCH_3), 56.1 (OCH_3), 49.6 (NCH_2); m/z 243 (M^+, 96%), 228 (55), 218 (7) 152 (37), 125 (5), 91 (100), 65 (6) Found M^+, 243.1260. $C_{15}H_{17}NO_2$ requires 243.1259.

5.3.4. Synthesis of N-benzyl-3,4-dimethoxybromoacetanilide (114)



N-Benzyl-3,4-dimethoxyaniline (0.615 g, 1.69 mmol) was dissolved in CH_2Cl_2 (40 cm^3) in a flame-dried round bottom flask equipped with a stirrer bar. The temperature was lowered to 0 ºC and bromoacetyl bromide (0.24 cm^3, 1.7 mmol, 1.0 equiv.) was added followed by triethylamine. The reaction was then left for a further 20 min at 0 ºC. Then water (30 cm^3) was added, the mixture extracted with CH_2Cl_2 (3 × 30 cm^3) and then dried with $MgSO_4$. The mixture was filtered and the solvent removed *in vacuo*. The brown crude product was purified using column chromatography (30% EtOAc/Hexane) and further purified by recrystallization (ethanol) to afford *N*-benzyl-3,4-dimethoxybromoacetanilide **(114)** (0.61 g, 95%). R_f 0.50 (EtOAc/Hexane, 50 : 50); m.p. 65-68^oC; ν_{max} (CH_2Cl_2)/cm^{-1} 1746 (s, C=O), 1612 (m, aromatic), 1361 (m, C-N), 1175 (w, C-O), 675 (w, C-Br); δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.22 (5H, m, 5×ArH), 6.80 (1H, d, J=8.4, ArH), 6.63 (1H, d, J=8.4, ArH), 6.44 (1H, s, ArH), 4.85 (2H, s, NCH_2Ar), 3.89 (2H, s, $COCH_2Br$), 3.87 (3H, s, OCH_3) and 3.71 (3H, s, OCH_3); δ_c (75 MHz, $CDCl_3$) 167.3 (C=O), 149.7 (ArC), 149.5 (ArC), 137.2 (ArC), 134.2 (ArCH), 129.6 (ArCH), 128.9 (ArCH), 128.1 (ArCH), 120.1 (ArC), 111.9 (ArCH), 111.6 (ArCH), 56.4 (OCH_3), 56.3 (OCH_3), 54.1 (NCH_2), 27.6 (CH_2Br); m/z 364 (^{79}Br-M^+, 100%), 367 (17), 366 (95), 307 (25), 284 (30), 272 (5), 242 (16), 226 (17) Found ^{79}Br-M^+, 364. $C_{17}H_{18}NO_3{}^{79}Br$ requires 364.0470.

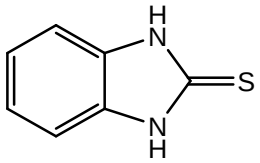
5.3.5. Synthesis of N-benzyl-3,4-dimethoxychloroacetanilide (115)[20]



N-Benzyl-3,4-dimethoxyaniline (3.11g, 12.7 mmol) was dissolved in CH_2Cl_2 (30 cm^3) in flame-dried round bottom flask equipped with a stirrer bar. The temperature was then lowered to 0 ^oC using an ice bath and chloroacetyl chloride (1.02 cm^3, 12.8 mmol, 1.00 equiv.) was added portion-wise followed by triethylamine (few drops). Water (30 cm^3) was then added, the mixture extracted with CH_2Cl_2 (3 × 30 cm^3) and dried using $MgSO_4$. The organic component was filtered and the solvent removed under reduced pressure. The residue was purified using column chromatography (30% EtOAc/Hexane) to give a brown oil which solidified; the solid was further purified by recrystalization using ethanol to give *N*-benzyl-3,4-dimethoxychloroacetanilide **(115)** as a white solid. (3.88 g, 95%). Rf 0.42 (EtOAc/Hexane, 40 : 60); m.p. 146-149^oC; δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.19-7.28 (5H, m, 5×ArH), 6.77-6.80 (1H, d, J=8.7, ArH), 6.56-6.60 (1H, d, J=9.0, ArH), 6.39 (1H, s, ArH), 4.86 (2H, s, $NCH_2C_6H_5$), 3.88 (6H, s, $2\times OCH_3$), 3.69 (2H, s, $COCH_2Cl$); δ_c (75 MHz, $CDCl_3$) 164.9 (C=O), 147.8 (ArC), 147.6 (ArC), 135.3 (ArC), 131.9 (ArC), 127.7 (2×ArCH), 126.9 (2×ArCH), 126.2 (ArCH), 118.9 (ArCH), 109.9 (ArCH), 109.7 (ArCH), 54.4 (OMe), 54.4 (OMe), 52.2 (NCH_2), 40.5 ($COCH_2Cl$); m/z (APCI) 320 (^{35}Cl-M^++1, 100%) 286 (12), 242 (10), 166 (1), 97 (1); Found ^{35}Cl-M^++1, 320.199 $C_{17}H_{19}NO_3Cl$ (M +H) 320.1054.

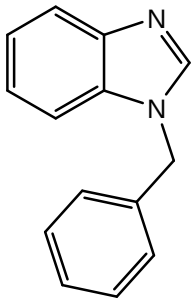
5.4. Attempted synthesis of 6-phenyl benzimidazo[2,1-a]isoquinoline

5.4.1. Synthesis of 1H benzo[d]imidazole-2(3H)-thione[74]



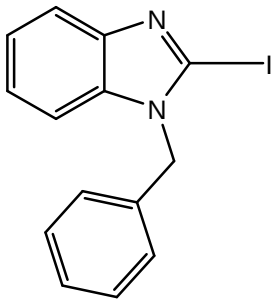
A solution of ethanol (20 cm^3), NaOH (0.88 g, 22.0 mmol, 1.16 equiv.) and carbon disulphide (1.32 cm^3, 22.0 cm^3, 1.16 equiv.) was formed in a round bottom flask equipped with a stirrer bar and a condenser. *o*-Phenylinediamine (2.05 g, 19.0 mmol) and water (3 cm^3) were then added and the reaction was left to run for a further 3 hrs. Then charcoal was added cautiously and the reaction left for a further 15 mins. The charcoal was filtered and the filtrate was warmed to 60 ^oC and quenched with warm water (9 cm^3), followed by 50% AcOH (25 cm^3) with vigorous stirring to give a white solid. The solution was put in the refrigerator to maximize crystallization. This was collected by filtration to afford 1*H*-benzo[*d*]imidazole-2(3*H*)-thione (2.85 g, 41%). R_f 0.30 (EtOAc/Hexane, 40: 60); δ_H (300 MHz; DMSO-d_6; Me_4Si) 12.50 (2H, s, 2×NH), 7.13 (4H, m, 4×ArH); δ_c (75 MHz, DMSO) 173.7 (C=S), 137.8 (ArC), 127.9 (ArCH), 115.1 (ArCH); m/z (APCI) 151 (M^++1, 100%), 150 (2), 149 (99), 148 (36); Found M^++1, 151.166 $C_7H_7N_2S$ (M + H) 151.0331.

5.4.2. Synthesis of 1-benzyl-1H-benzo[d]imidazole (140)[82,83]



In a flame-dried round bottom flask equipped with a stirrer bar and a condenser, benzimidazole (6.05 g, 51.2 mmol) was dissolved in acetone (60 cm^3), followed by the addition of NaOH (2.05 g, 51.2 mmol, 1.00 equiv.). This was left stirring for an hour at rt; then benzyl bromide (5.53 cm^3, 51.2 mmol 1.00 equiv.) was added portion-wise and the reaction mixture was left to stir for a further 24 hrs under reflux. The reaction mixture was let to cool to rt, followed by the addition of water (40 cm^3). This mixture was then extracted using CH_2Cl_2 (3 × 30 cm^3). The organic layers were combined and dried using $MgSO_4$, filtered and the solvent removed in *vacuo* to produce yellow oil which was left overnight. A solid formed which was recrystallised (EtOH) to give 1-benzyl-1*H*-benzo[*d*]imidazole **(140)** as white crystals (10.48 g, 98 %). Rf 0.13 (EtOAc/Hexane, 40 : 60); m.p. 89-91 ^oC; δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.29 (1H, s, NCHN), 7.83 (1H, d, J=8.1, ArH), 7.29-7.33 (3H, m, 3×ArH), 7.23-7.27 (3H, m, 3×ArH), 7.16-7.13 (2H, m, 2×ArH), 5.33 (2H, s, NCH_2Bn); δ_c (75 MHz, $CDCl_3$) 144.4 (NCHN), 143.6 (ArC), 135.9 (ArCH), 134.4 (ArC), 129.44 (2×ArCH), 128.7 (ArCH), 127.5 (2×ArCH), 123.5 (ArCH), 122.7 (ArCH), 120.9 (ArC), 110.4 (ArCH), 49.2 (NCH_2Bn); m/z (ESI+) 209 (M^++1, 100%), 149 (6),132 (1), 131 (8), 129 (2), 93(2), 92 (8) 91 (2), 65 (2); Found M^++1, 209.19. $C_{14}H_{13}N_2$ (M + H) requires 209.1079.

5.4.3. Synthesis of 1-benzyl-2-iodo-1H-benzo[d]imidazole (119)



Method 1

A solution of diisopropylamine (0.60 cm^3, 4.20 mmol, 2.0 equiv.) and THF (18 cm^3) were added to a flame-dried two-necked round bottom flask

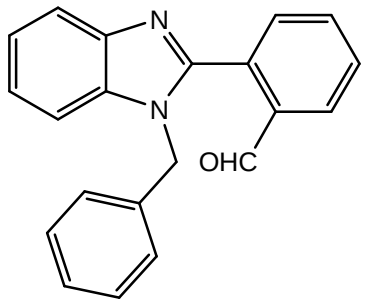
equipped with a stirrer bar. The temperature was lowered to -30^{o}C and the solution was left to stir for 10 mins. n-BuLi (0.91 M, 3.4 cm^{3}, excess) was added to the solution and was left to stir for a further 30 mins. The temperature was then lowered to -78^{o}C and was left for a further 10 mins. A solution of 1-benzyl-1*H*-benzo[*d*]imidazole **(140)** (0.500 g, 2.40 mmol) in THF (10 cm^{3}) was added to the flask and left to stir for a further 20 mins at the same temperature. 1,2-Diiodoethane (0.681 g, 2.40 mmol, 1.00 equiv.) was added to the reaction flask and was also left to stir for a further 20 mins and let to warm up to rt with stirring. The reaction was quenched with saturated NH_4Cl (30 cm^{3}), followed by extraction using CH_2Cl_2 (3 × 30 cm^{3}). The organic layers were combined and dried with $MgSO_4$. This was filtered and the solvent removed under reduced pressure. The residue was purified using column chromatography with (EtOAc/Hexane 40:60) to give 1-benzyl-2-iodo-1*H*-benzo[*d*]imidazole **(119)** as a white solid (0.35 g, 40%) with identical spectroscopic data to that reported under method 2.

Method 2

To a round bottom flask equipped with a stirrer bar with 1-benzyl-1*H*-benzo[*d*]imidazole (1.00 g, 4.80 mmol) was dried under high vacuum with occasional heating. The diisopropyl magnesium chloride lithium chloride (5.52 cm^{3}, 7.20 mmol, 1.50 equiv.) was added and the reaction temperature rose. This was left to stir overnight. I_2 (1.83 g, 7.20 mmol, 1.5 equiv.) was added and reaction was left to stir for a further hour. The reaction was then quenched with saturated NH_4Cl (30 cm^{3}) and washed with sodium thiosulphate (70 cm^{3}). It was then extracted with CH_2Cl_2 (3 × 30 cm^{3}). The organic fractions were collected and dried using $MgSO_4$. This was filtered and the solvent removed under reduced pressure. The residue was residue was purified using column chromatography (EtOAc/Hexane 40: 60) to give 1-benzyl-2-iodo-1*H*-benzo[*d*]imidazole **(119)** as a white solid (1.2 g, 75%), R_f 0.52 (EtOAc/Hexane 40:60) ; m.p. 118-119 ^{o}C; δ_H (300 MHz; $CDCl_3$; Me_4Si) 7.73-7.76 (1H, d, J=7.2,

ArH), 7.13-7.32 (8H, m, 8×ArH), 5.39 (2H, s, NC*H*$_2$); δ_c (75 MHz, $CDCl_3$) 146.2 (N*C*lN), 136.3 (ArC), 135.6 (ArC), 129.4 (2×ArCH), 128.5 (ArCH), 127.1 (2×ArCH), 123.7 (ArCH), 122.9 (ArCH), 119.8 (ArCH), 110.3 (ArCH), 104.6 (ArC), 51.0 (N*C*H$_2$Bn); m/z (ESI+) 335 (M^++1, 100%), 327 (9), 285 (1.5), 252 (4), 251 (19), 246 (1); Found ^{127}I-M^++1, 335.1 $C_{14}H_{12}N_2I$ (M + H) 335.0046.

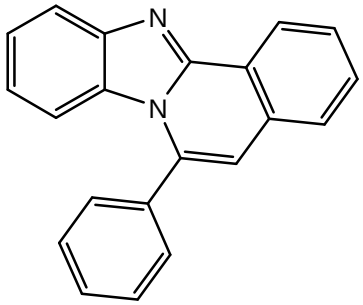
5.4.4. Synthesis of 2-(1-benzyl-1H-benzo[d]imidazol-2-yl)benzaldehyde (120)



In a flame-dried two-necked round bottom flask equipped with a stirrer bar, a condenser and a dropping funnel, Pd(PPh_3)$_4$ (0.027 g, 0.089 mmol, 10 mol%) was introduced. 1-Benzyl-2-iodo-1*H*-benzo[*d*]imidazole **(119)** (0.27 g, 0.81 mmol) and 2-formylphenyl boronic (0.20 g, 1.4 mmol, 1.50 equiv.) was dissolved in DME (8 cm^3) and the solution was transferred to the dropping funnel and degassed with N_2, before being transferred to the flask. Then a solution of 2M Na_2CO_3 (0.80 g, 7.56 mmol, 8.5 equiv.) was prepared and transferred to the dropping funnel, degassed with N_2 and transferred to the flask. The resulting mixture was left to stir under reflux for a further 24 hrs. Water (10 cm^3) was added and the mixture wasextracted using CH_2Cl_2 (3 × 30 cm^3). The organic layers were collected and dried over $MgSO_4$. This was filtered and the solvent removed under reduced pressure. The residue was purified using column chromatography (EtOAc/Hexane, 40: 60) to give 2-(1-benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde **(120)** as an orange oil which solidified on standing (0.18 g, 67%). R_f 0.32 (EtOAc/Hexane, 40: 60); v_{max} (film)/cm^{-1}: 2861 (w, C-H), 1694 (s, C=O), 1600 (m, C=C), 1161 (m, C-N); δ_H (300 MHz; $CDCl_3$; Me_4Si) 10.29 (1H, s, C*H*O), 8.42-8.43 (1H, m, ArH), 8.22 (1H, d, J=6.6, ArH), 7.97-7.99 (2H, m,

2×ArH), 7.86-7.85 (1H, m, ArH), 7.66-7.70 (3H, m, 3×ArH), 7.58-7.59 (3H, m, 3×ArH), 7.29 (2H, m, 2×ArH), 5.65 (2H, s, NC*H*$_2$C$_6$H$_5$); δ_c (75 MHz, $CDCl_3$) 190.7 (CHO), 150.4 (ArC), 143.1 (ArC), 135.8 (ArC), 135.7 (ArC), 135.5 (ArC), 133.4 (ArCH), 132.8 (ArC), 130.8 (ArCH), 130.4 (ArCH), 128.9 (2×ArCH), 128.8 (ArCH), 128.1 (ArCH), 126.4 (2×ArCH), 123.6 (ArCH), 122.9 (ArCH), 120.4 (ArCH), 110.6 (ArCH), 48.3 (NCH_2C$_6$H$_5$); m/z (APCI) 313 (M^++1, 100%), 312 (2), 311 (8), 297 (4); Found M^++1, 313.243 $C_{21}H_{17}N_2O$ (M + H) requires 313.1342.

5.4.5. Attempted synthesis of 6-phenylbenzimidazo[2,1-a]isoquinoline (116)



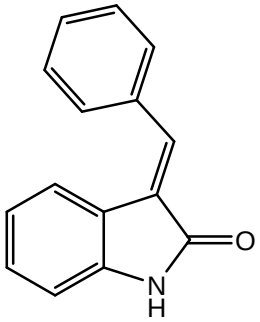
2-(1-Benzyl-1*H*-benzo[*d*]imidazol-2-yl)benzaldehyde **(120)** (0.053 g, 0.18 mmol) was dissolved in DMF (3 cm^3) in a flame-dried round bottom flask equipped with a stirrer bar. ^{t}BuOK (0.024 g, 0.22 mmol, 1.20 equiv.) was added to the solution and was let to stir for 3 mins. The solution was quenched with water (8 cm^3) followed by extraction using CH_2Cl_2 (3 × 30 cm^3). The organic layers were collected and dried with $MgSO_4$. This was filtered and the solvent was removed under reduced pressure and a green residue resulted. This was further purified using preparative column chromatography (EtOAc/Hexane, 10: 90) to give products which were uncharacterizable.

5.5. Synthesis of 3-(substituted)indolin-2-one

5.5.1. General Procedure

Oxindole (1.00 equiv.) and a suitable benzaldehyde (1.00 equiv.) were dissolved in EtOH (50 cm^3) in a flame-dried round bottom flask, equipped with a stirrer bar and a condenser. This was followed by the addition of piperidine (1.62 equiv.) and the reaction mixture was allowed to reflux overnight. Then the contents were allowed to cool to rt and the solid that was formed was filtered and washed with cold EtOH (40 cm^3) three to four times. If precipitation did not occur then the reaction was quenched with water (30 cm^3), followed by extraction with CH_2Cl_2 (3 × 30 cm^3). The organic extracts were combined and dried with $MgSO_4$. This was filtered and the solvent was removed under reduced pressure to give a residue which was then purified using column chromatography (EtOAc/Hexane) to give the desired product.

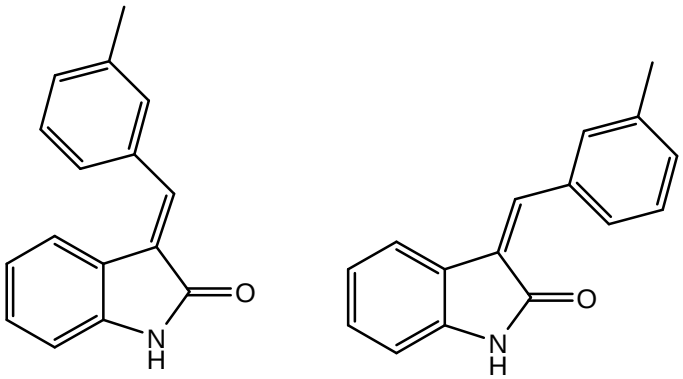
5.5.2. Synthesis of (E/Z)-3-benzylideneindolin-2-one (142)[79]



This was obtained following the general procedure using 2-oxindole (1.01 g, 7.51 mmol), benzaldehyde (0.80 g, 7.51 mmol, 1.00 equiv.) and piperidine (1.20 cm^3, 12.2 mmol). The reaction was left overnight and on cooling a solid precipitated and was filtered and washed three times with cold EtOH (40 cm^3) to give 3-benzylideneindolinindolin-2-one **(142)** (0.59 g, 46%) as a yellow solid which was not purified further. R_f 0.58 (EtOAc/Hexane 40: 60); m.p. 147-149 ^oC; ν_{max} (film)/cm^{-1}: 3151 (w, NH), 1703 (m, C=O), 1636 (m, aromatic), 1361 (m, C-N); δ_H

(300 MHz; $CDCl_3$; Me_4Si) 9.09 (1H, s, NH), 7.85 (1H, s, COCC*H*), 7.62-7.68 (3H, m, 3×ArH), 7.43-7.49 (3H, m, 3×ArH), 7.19-7.26 (1H, m, ArH), 6.92-6.95 (1H, d, J=9, ArH), 6.84-6.89 (1H, t, J=7.6, ArH); δ_c (75 MHz, $CDCl_3$) 170.9 (N*C*OC), 142.2 (ArC); 137.9 (COC*C*H), 135.3 (ArC), 130.3 (ArCH), 130.1 (ArCH), 129.7 (2× ArCH), 129.1 (2× ArCH), 128.1 (CO*C*CH), 123.4 (ArCH), 122.2 (ArCH); 122.1 (ArC); 110.8 (ArCH); m/z (ESI+) 222 (M^++1, 100%), 206 (2), 205 (16), 204 (99), 194 (49), 179 (1); Found M^++1, 222.21 $C_{15}H_{12}NO$ (M + H) requires 222.0920.

5.5.3. Synthesis of 3-(3-methylbenzylidene)indolin-2-one (E-143/Z-144)[88]

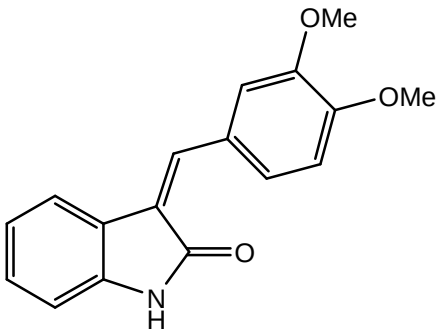


The compound was obtained following the general procedure. 2-Oxindole (1.01 g, 7.51 mmol), *m*-tolualdehyde (0.902 g, 7.51 mmol, 1.00 equiv.) and piperidine (1.20 cm^3, 12.2 mmol) were dissolved in EtOH (50 cm^3) and the reaction mixture was allowed to reflux overnight. Water (30 cm^3) was added, extracted with CH_2Cl_2 (3 × 30 cm^3) and dried with $MgSO_4$. This was filtered and the solvent removed under reduced pressure to give a red residue which was purified and separated using column chromatography (EtOAc/Hexane 10-50%) to give the *Z*- and the *E*- isomers as yellow solids in a combined yield of (0.91 g, 51%). (*E* -isomer) **(140)** R_f 0.67 (EtOAc/Hexane 40: 60); m.p. 149-152 ^oC; ν_{max} (film)/cm^{-1}: 3150 (w,NH), 1686 (m, C=O), 1615 (s, C=C); 1197 (m, C-N); δ_H (300 MHz; $CDCl_3$+DMSO-d_6, Me_4Si) 10.25 (1H, s, NH), 8.17 (1H, d, J=7.8, ArH), 8.08 (1H, s, COCC*H*), 7.50-7.52 (2H, m, 2×ArH), 7.31 (1H, t, J=7.4, ArH), 7.20 (1H, d,

J=7.5, ArH), 7.16 (1H, d, J=7.8, ArH), 6.97 (1H, t, J=7.3, ArH), 6.83 (1H, d, J=7.5, ArH), 2.39 (3H, s, CH_3); δ_c (75 MHz,$CDCl_3$+DMSO-d_6, Me_4Si) 167.6 (C=O), 140.9 (ArC), 137.4 (ArC), 136.6 (ArCH), 133.9 (ArC), 132.5 (ArCH), 131.0 (ArCH), 129.0 (ArCH), 128.7 (ArCH), 128.0 (ArCH), 126.9 (ArC), 125.1 (ArC), 121.1 (ArCH), 119.1 (ArCH), 109.6 (ArCH), 21.3 (CH_3).

(*Z* -isomer) **(141)** R_f 0.53 (EtOAc/Hexane 40: 60); m.p. 133-136^oC; ν_{max} (film)/cm^{-1}: 3012 (w, NH), 1697 (s, C=O), 1606 (m, C=O), 1141 (s, C-N); δ_H (300 MHz; $CDCl_3$, Me_4Si) 10.12 (1H, s, NH), 7.81 (1H, s, COCC*H*), 7.62 (1H, d, J=7.8, ArH), 7.44-7.47 (2H, m, 2×ArH), 7.32 (1H, t, J=7.5, ArH), 7.20 (1H, d, J=6.3, ArH), 7.16 (1H, d, J=7.8, ArH), 6.97 (1H, d, J=7.8, ArH), 6.83 (1H, t, J=7.6, ArH), 2.38 (3H, s, CH_3); δ_c (75 MHz,$CDCl_3$, Me_4Si) 169.6 (C=O), 142.9 (ArC), 138.2 (ArC), 136.2 (ArCH), 134.7 (ArC), 132.1 (ArCH), 130.2 (ArCH), 129.8 (ArCH), 127.9 (ArCH); 126.2 (ArC); 128.5 (ArCH), 126.1 (ArC), 122.6 (ArCH), 121.4, (ArCH), 110.2 (ArCH), 29.5 (CH_3).

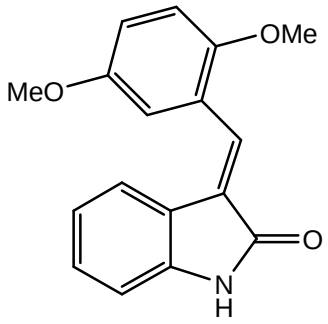
5.5.4. Synthesis of (E/Z)-3-(3,4-dimethoxybenzylidene)indolin-2-one (145)



3-(3,4-Dimethoxybenzylidene)indolin-2-one was obtained following the general procedure using 2-oxindole (0.902 g, 7.51 mmol, 1.00 equiv.), 3,4-dimethoxy benzaldehyde (1.24 g, 7.51 mmol) and piperidine (1.20 cm^3, 12.2 mmol, 1.62 equiv.). The reaction was stirred overnight and a precipitate formed which was filtered and washed three times (cold EtOH) to give the desired product **(145)** as a bright yellow solid which was not purified further (1.01 g, 48%). R_f 0.22

(EtOAc/Hexane 40: 60); m.p. 221-224^{o}C; v_{max} (film)/cm^{-1}: 3476 (NH), 1684 (w, aromatic), 1335 (m, C-N), 1142 (s, C-O); δ_H (300 MHz; $CDCl_3$+DMSO-d_6; Me_4Si) 10.26 (1H, s, NH), 8.77 (COCC*H*), 7.66 (1H, d, J=8.4, ArH), 7.51-7.54 (2H, m, 2×ArH), 7.14 (1H, t, J=7.8, ArH), 6.94-6.99 (2H, m, 2×ArH), 6.84 (1H, d, J=7.5, ArH), 3.95 (3H, s, OMe), 3.92 (3H, s, OMe); δ_c (75 MHz, $CDCl_3$ + DMSO-d_6; Me_3Si) 167.85 (C=O), 151.2 (ArC), 148.2 (ArC), 140.5 (ArC), 137.3 (COC*C*H), 128.2 (ArCH), 127.7 (ArCH), 125.7 (ArC), 124.4 (ArC), 123.3 (ArC), 121.0 (2×ArCH), 115.1 (ArCH), 110.9 (ArCH), 109.4 (ArCH), 55.8 (OMe), 55.7 (OMe); m/z (ESI+) 222 (M^++1, 100%), 269 (3), 268 (18), 267 (98), 254 (6), 250 (14), 249 (48), 238 (2), 222 (1), 165 (1), 146 (3), 137 (5), 130 (1); Found M^++1, 282.22 $C_{17}H_{16}NO_3$ (M + H) requires 282.1131.

5.5.5. Synthesis of (E/Z)-3-(2,5-dimethoxybenzylidene)indolin-2-one (146)[70]

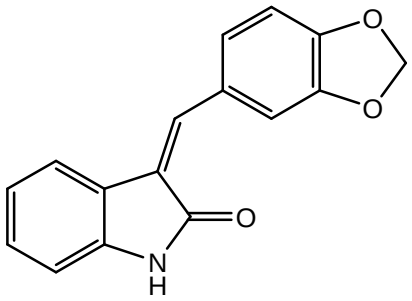


This was obtained following the general procedure with 2-oxindole (2.030 g, 15.02 mmol), 2,5-dimethoxybenzaldehyde (2.496 g, 15.02 mmol), piperidine (2.42 cm^3, 24.2 mmol) and MeOH and was left to reflux for a further 24 hrs. Then the mixture was allowed to cool to rt and then put in an ice bath. A solid precipitated, was filtered off and washed (cold EtOH) several times to afford the 3-(2,5-dimethoxybenzylidene)indoline-2-one as a orange solid **(143)** which was not purified further (2.09 g, 51 %). Rf 0.28 (EtOAc/Hexane 40: 60); v_{max} (film)/cm^{-1}: 3387 (m, NH), 1689 (m, C=O), 1608 (m, aromatic), 1326 (w, C-N), 1124 (C-O); δ_H (300 MHz; DMSO-d_6) 10.58 (1H, s, NH), 7.63 (1H, s, COCC*H*), 7.46 (1H, d, J=7.8, ArH), 7.19-7.25 (2H, m, 2×ArH), 7.04-7.12 (2H, m, 2×ArH), 6.84-6.89 (2H, m, 2×ArH), 3.81 (3H, s, OMe), 3.74 (3H, s, OMe); δ_c (75 MHz, DMSO-d_6) 174.2

(C=O), 158.2 (ArCH), 157.5 (ArC), 148.4 (ArC), 137.0 (COC*C*H), 135.6 (ArC), 133.1 (ArC), 128.9 (ArCH), 128.0 (ArC), 126.7 (ArCH), 126.6 (ArCH), 122.5 (ArC), 119.9 (ArCH), 118.3 (ArCH), 115.7 (ArC), 61.6 (OMe), 61.2 (OMe); m/z (ESI+) 282 (M^++1, 100%), 269 (2), 268 (18), 267 (98), 254 (3); Found M^++1, 282.21 $C_{17}H_{16}NO_3$ (M + H) requires 282.1131.

5.5.6. Synthesis of 3-[(benzo[d][1,3]dioxol-6-yl)methylene]indolin-2-one (147)[79]

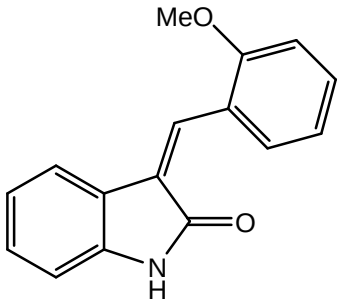
…



This was obtained following the general procedure with 2-oxindole (0.902 g, 7.51 mmol, 1.00 equiv.), piperonal (1.18 g, 7.51 mmol, 1.00 equiv.), piperidine (1.20 cm^3, 12.2 mmol, 1.62 equiv.) dissolved in EtOH and was left to reflux for a further 24 hrs. Then the mixture was allowed to cool to rt. A solid precipitated, filtered off and washed (cold EtOH) several times to afford the 3-[(benzo[*d*][1,3]dioxol-6-yl)methylene]indolin-2-one (1.04 g, 51%) as a orange solid **(147)** which was not purified further. R_f 0.33 (EtOAc/Hexane 40: 60); v_{max} (film)/cm^{-1}: 3663 (s, NH), 2898 (w, C-H), 1739 (s, C=O), 1113 (w, C-N), 1097 (m, C-O); δ_H (300 MHz; $CDCl_3$+DMSO-d_6; Me_4Si) 10.39 (1H, s, NH), 7.66 (1H, d, J= 7.8, ArH), 7.54 (1H, s, COCC*H*), 7.15-7.24 (3H, m, 3×ArH), 6.97 (1H, d, J= 7.8, ArH), 6.87 (1H, s, ArH), 6.83 (1H, d, J= 8.1, ArH); 6.09 (2H, s, CH_2); δ_c (75 MHz, $CDCl_3$ + DMSO-d_6; Me_3Si) 169.4 (C=O), 148.9 (ArC), 147.9 (ArC), 143.0 (ArC), 135.9 (COC*C*H), 129.7 (ArCH), 128.5 (ArC), 126.6 (ArC), 124.6 (ArCH), 122.5 (ArCH), 121.4 (ArC), 121.1 (ArCH), 110.2 (ArC), 109.3 (ArCH), 108.7 (ArCH), 101.8 (O*C*H_2O); m/z (ESI+) 266 (M^++1, 100%), 250 (3), 249 (14), 248 (83), 239 (4), 238 (24), 237 (17), 236 (99), 221 (4), 220 (15), 210 (1), 209 (6), 208 (21), 192 (1), 182 (1), 149

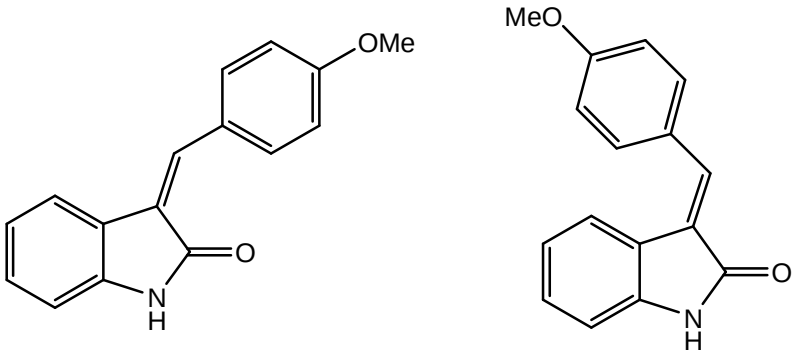
(3), 133 (2), 132 (38), 117 (1); Found M^++1, 266.19 $C_{16}H_{12}NO_3$ (M + H) requires 266.0818.

5.5.7. Synthesis of (Z)-3-(2-methoxybenzylidene)indolin-2-one (148)



The compound was obtained following the general procedure. 2-Oxindole (0.902 g, 7.51 mmol, 1.00 equiv.), 2-methoxybenzaldehyde (1.01 g, 7.51 mmol, 1.00 equiv.) and piperidine (1.25 cm^3, 12.2 mmol, 1.62 equiv.) were dissolved in EtOH (50 cm^3). This was refluxed and on standing a solid precipitated. This was filtered and washed several times with cold EtOH (40 cm^3) to afford **(***Z*)-3-(2-methoxybenzylidene)indolin-2-one **(148)** (1.09 g, 58%). R_f 0.61 (EtOAc/Hexane 40: 60); m.p. 206-209 ^oC; v_{max} (film)/cm^{-1}: 3597 (s, NH), 1697 (m, C=O), 1327 (w, C-N),1110 (w, C-O); δ_H (300 MHz; $CDCl_3$+DMSO-d_6; Me_4Si) 10.09 (1H, s, NH), 7.83 (1H, s, COCC*H*), 7.70 (1H, d, J=7.5, ArH), 7.50 (1H, d, J=7.8, ArH), 7.44 (1H, t, J=8.2, ArH), 7.16 (1H, t, J=7.8, ArH), 6.99-7.06 (2H, m, 2×ArH), 6.87 (1H, d, J=7.8, ArH), 6.80 (1H, t, J=7.6, ArH), 3.88 (3H, s, OMe); δ_c (75 MHz, $CDCl_3$+DMSO-d_6, Me_4Si), 169.4 (C=O), 157.9 (ArC), 142.8 (ArC), 132.2 (COC*C*H), 131.5 (ArCH), 129.6 (ArCH), 129.6 (ArCH), 127.6 (ArC), 123.4 (ArC), 122.5 (ArCH), 121.5 (ArCH), 121.0 (ArCH), 120.2 (ArCH), 111.1 (ArCH), 110.1 (ArCH), 55.5 (OMe); m/z (ESI+) 252 (M^++1, 100%), 239 (3), 238 (17), 237 (99), 235 (12), 234 (54), 225 (2), 224 (12), 220 (13), 219 (8), 206 (3), 146 (2), 107 (1); Found M^++1, 252.22 $C_{16}H_{14}NO_2$ (M + H) requires 252.1025.

5.5.8. Synthesis of 3-(4-methoxybenzylidene)indolin-2-one (Z-149/E-150)[88,89]

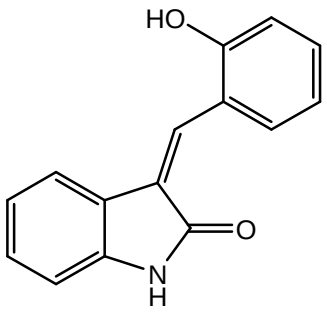


The compound was obtained following the general procedure. 2-Oxindole (0.9023 g, 7.51 mmol, 1.00 equiv.), 4-methoxybenzaldehyde (1.0124 g, 7.51 mmol, 1.00 equiv.) and piperidine (1.25 cm^3, 12.2 mmol, 1.62 equiv.) were dissolved in EtOH (50 cm^3). This was refluxed and then water (20 cm^3) was added after being left to cool to rt. It was extracted using CH_2Cl_2 and dried with $MgSO_4$, filtered and the solvent was removed under reduced pressure. The residue was purified and separated using column chromatography 10-50% (EtOAc/ Hexane) to give the (*E*)- and (*Z*)-isomers of 3-(4-methoxybenzylidene)indolin-2-one as a bright yellow solid in a combined yield of (0.75 g, 43%).

(E-isomer) R_f 0.58 (EtOAc/Hexane 40: 60); m.p. 130-132 ^oC; v_{max} (film)/cm^{-1}: 3411 (NH), 1682 (m, C=O), 1613 (w, aromatic), 1305 (C-N), 1050 (C-O); δ_H (300 MHz; DMSO-d_6) 10.38 (1H, s, NH), 8.42 (1H, d, J=8.1, ArH), 7.61-7.69 (2H, m, 2×ArH), 7.53 (1H, s, COCC*H*), 7.15 (1H, t, J=7.1, ArH), 6.93-7.02 (3H, m, 3×ArH), 6.81-6.88 (1H, m, ArH); 3.85 (3H, s, OMe) δ_c (75 MHz, $CDCl_3$+DMSO-d_6, Me_4Si): 170.8 (C=O), 161.1 (ArC), 142.5 (ArC), 137.3 (COC*C*H), 134.7 (ArCH), 131.7 (2×ArCH), 129.7 (ArCH), 126.4 (ArC), 122.8 (ArCH) 122.2 (ArC), 119.0 (ArC),114.4 (2×ArCH), 110.6 (ArCH) 55.8 (OMe); m/z (APCI) 252 (M^++1, 100%), 251 (29), 250 (99), 249 (10), 248 (1), 247 (1), 237 (1); Found M^++1, 252.231 $C_{16}H_{14}NO_2$ (M + H) requires 252.1025.

(Z-isomer) R_f 0.48 (EtOAc/Hexane 40: 60); m.p. 182-184^oC; v_{max} (film)/cm^{-1}: 3411 (w, NH), 1682 (s, C=O), 1166 (m, C-O), 1150 (m, C-N); δ_H (300 MHz; $CDCl_3$, Me_4Si) 9.93 (1H, s, NH), 7.79 (1H, s, COCC*H*), 7.74 (1H, d, J= 7.8, ArH),7.64-7.67 (2H, m, ArH), 7.18 (1H, t, J= 7.6, ArH), 6.96-6.99 (3H, m, 3×ArH), 6.87 (1H, t, J= 7.6, ArH) 3.86 (3H, s, OMe); (75 MHz, $CDCl_3$+DMSO-d_6, Me_4Si), 168.6 (ArC), 161.8 (ArC), 140.4 (ArC),137.4 (COCCH), 128.5 (ArCH), 127.6 (ArCH), 127.4 (ArC), 126.0 (ArC), 124.7 (ArC), 121.6 (ArCH), 114.1 (2 × ArCH), 110.6 (2 × ArCH), 109.9 (ArCH), 55.7 (OMe); m/z (APCI) 252 (M^++1, 100%), 250 (1), 237 (2), 161 (1), 120 (1); Found M^++1, 252.188 $C_{16}H_{14}NO_2$ (M + H) requires 252.1025.

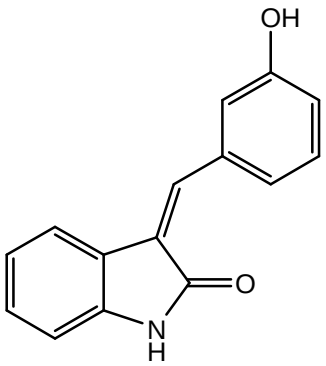
5.5.9. Synthesis of (E/Z)-3-(2-hydroxybenzylidene)indolin-2-one (151)



The compound was obtained following the general procedure. 2-Oxindole (0.902 g, 7.51 mmol, 1.00 equiv.), 2-hydroxybenzaldehyde (0.909 g, 7.45 mmol, 1.00 equiv.) and piperidine (1.25 cm^3, 12.2 mmol, 1.62 equiv.) were dissolved in EtOH (50 cm^3). This was refluxed for 18 hrs and allowed to cool to rt which resulted in a precipitate forming. The solid was filtered and washed (cold EtOH) several times to give (*E/Z*)-3-(2-hydroxybenzylidene)indolin-2-one **(151)** (0.90 g, 51%) as a light green solid. R_f 0.20 (EtOAc\Hexane 40:60); m.p. 192-197 ^oC; v_{max} (film)/cm^{-1}: 2529 (vs, NH/OH), 1694 (m, C=O), 1227 (m, C-O); δ_H (300 MHz; DMSO-d_6) 10.13 (1H, s, NH), 7.86 (1H, s, COCCH), 7.64 (1H, d, J= 6.6, ArH), 7.57 (1H, d, J=7.8, ArH), 7.39 (1H, t, J= 7.2, ArH), 7.14 (1H, t, J=7.4, ArH), 6.98 (1H, d, J= 7.8, ArH), 6.85-6.92 (2H, m, 2×ArH), 6.81 (1H, t, J= 7.6, ArH), 3.24 (1H, bs, OH); δ_c (75 MHz, DMSO-d_6) 169.7 (C=O), 156.6 (ArC), 142.5 (ArC), 133.2 (COCCH),

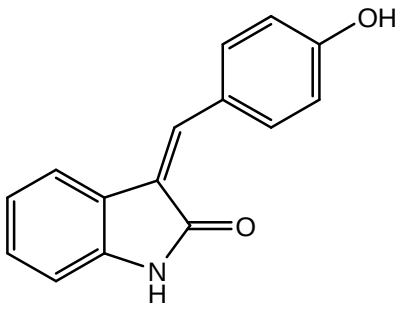
131.2 (ArCH), 129.6 (ArCH), 129.2 (ArCH), 126.9 (ArCH), 122.7 (ArCH), 121.9 (ArC), 121.8 (ArC), 120.9 (ArCH), 118.8 (ArCH), 116.1 (ArCH), 109.9 (ArCH); m/z (APCI) 238 (M^++1, 100%) 237 (6), 236 (4), 221 (8), 220 (44), 210 (6), 148 (1), 106 (1); Found M^++1, 238.154 $C_{15}H_{12}NO_2$ (M + 1) requires 238.0869.

5.5.10. Synthesis of (E/Z)-3-(3-hydroxybenzylidene)indolin-2-one (152)



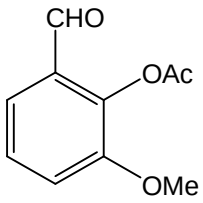
The compound was obtained following the general procedure. 2-oxindole (0.902 g, 7.51 mmol, 1.00 equiv.), 3-hydroxybenzaldehyde (0.909 g, 7.45 mmol, 1.00 equiv.) and piperidine (1.25 cm^3, 12.2 mmol, 1.62 equiv.) were dissolved in EtOH (50 cm^3). This was refluxed for 18 hrs and a solid had already formed. This was allowed to cool to rt and the solid was filtered. It was washed (EtOH) several times to afford the desired product **(152)** as a greenish solid (0.80 g, 45%). Rf 0.21 (EtOAc\Hexane 40:60); m.p. 250-254 ^oC ν_{max} (film)/cm^{-1}: 3191 (s, NH/OH), 1681 (m, C=O), 1094 (m, C-O), 1141 (m, C-N); δ_H (300 MHz; DMSO-d_6) 10.22 (1H, s, NH), 9.26 (1H, bs, OH), 7.63 (1H, d, J= 7.8, ArH), 7.62 (1H, s, COCCH), 7.27 (1H, t, J= 7.8, ArH), 7.17 (1H, t, J= 7.6, ArH), 7.08-7.11 (2H, m, 2×ArH), 6.85-6.90 (2H, m, 2×ArH), 6.80 (1H, d, J= 7.5, ArH); δ_c (75 MHz, DMSO-d_6) 169.7 (C=O), 157.6 (ArC), 142.9 (ArC), 136.3 (COCCH), 135.9 (ArC), 129.7 (ArCH), 129.6 (ArCH), 127.8 (ArC), 123.1 (ArCH), 121.4 (ArC), 121.1 (ArCH), 120.1 (ArCH),116.8 (ArCH), 115.8 (ArCH), 110.1 (ArCH); m/z (APCI) 238 (M^++1, 100%) 237 (1), 236 (2); Found M^++1, 238.177 $C_{15}H_{12}NO_2$ (M + 1) requires 238.0869.

5.5.11. Synthesis of (E/Z)-3-(4-hydroxybenzylidene)indolin-2-one (153)



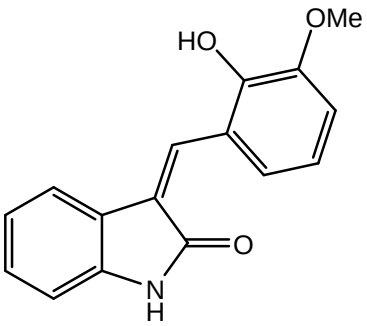
3-(4-Hydroxybenzylidene)indolin-2-one **(153)** was obtained using the general procedure. 2-Oxindole **(9)** (0.902 g, 7.45 mmol, 1.00 equiv.), 4-hydroxybenzaldehyde (0.909 g, 7.45 mmol, 1.00 equiv.) and piperidine (1.25 cm^3, 12.2 mmol, 1.62 equiv.) were dissolved in EtOH (50 cm^3). This was heated for 18 hrs during which time a solid had already formed. The reaction mixture was allowed to cool to rt and the solid was filtered. It was washed (EtOH) several times to afford the desired product **(153)** (0.81 g, 46%) as a greenish solid. Rf 0.03 (EtOAc\Hexane 40:60); v_{max} (film)/cm^{-1}: 2760 (s, OH), 1679 (s, C=O), 1281 (m, C-N), 1104 (w, C-O); δ_H (300 MHz; $CDCl_3$+DMSO-d_6, Me_4Si) 10.33 (1H, s, NH), 7.72 (1H, d, J=7.8, ArH), 7.51-7.58 (3H, m, 3×ArH), 7.16 (1H, t, J= 7.6, ArH), 6.82-6.92 (4H, m, 4×ArH), 3.32 (1H, bs, OH); δ_c (75 MHz, $CDCl_3$+DMSO-d_6) 169.7 (C=O), 159.5 (ArCOH), 142.7 (ArC), 136.7 (COC*C*H), 134.8 (ArC), 131.7 (2×ArCH), 129.2 (ArCH), 125.8 (ArCH), 125.4 (ArCH), 125.2 (ArC), 123.4 (ArCH), 121.8 (ArCH), 115.8 (2×ArCH), 110.1 (ArC); m/z (ESI+) 238 (M^++1, 100%), 222 (1), 221 (16), 220 (99), 211 (3), 210 (25), 209 (25), 192 (4); Found M^++1, 238.17 $C_{15}H_{12}NO_2$ (M + H) requires 238.0869.

5.5.12. Synthesis of 2-formyl-6-methoxyphenyl acetate [84]



2-Formyl-6-methoxyphenyl acetate (2.00 g, 13.1 mmol) was dissolved in CH_2Cl_2 (40 cm^3) in a round bottom flask equipped with a stirrer bar. Then Et_3N (1.37 cm^3, 13.1 mmol, 1.00 equiv.) was introduced and the solution was left to stir for 5 mins. The temperature was lowered to 0 ^oC using an ice bath followed by the slow addition of acetyl chloride (0.93 cm^3, 13.1 mmol, 1.00 equiv.). After the addition of acetyl chloride the ice bath was removed and the reaction was left to run for a further 2 hrs. This was then quenched with water (30 cm^3), followed by extraction using CH_2Cl_2 (3 × 30 cm^3). The organic layer was then dried using $MgSO_4$. This was then filtered and the solvent was removed in *vacuo* to afford 2-formyl-6-methoxyphenyl acetate as a brown-greenish solid which was not purified further (2.89 g, 97%). Rf 0.55 (EtOAc\Hexane 40:60); v_{max} (film)/cm^{-1}: 2901 (w, C-H), 1764 (m, C=O), 1163 (s, C-O); δ_H (300 MHz; $CDCl_3$; Me_4Si) 10.14 (1H, s, CHO), 7.45-7.48 (1H, dd, J= 1.5 and 1.6, ArH), 7.31-7.37 (1H, t, J= 7.8, ArH), 7.20-7.24 (1H, dd, J= 1.2 and 1.5, ArH), 3.88 (3H, s, OMe), 2.41 (3H, s, COC*H*$_3$); δ_c (75 MHz, $CDCl_3$) 189.1 (CHO), 169.1 (O*C*OMe), 152.1 (ArC), 141.9 (ArC), 129.6 (ArC), 127.2 (ArCH), 121.7 (ArCH), 118.2 (ArCH), 56.8 (OMe), 20.9 (CO*C*H$_3$).

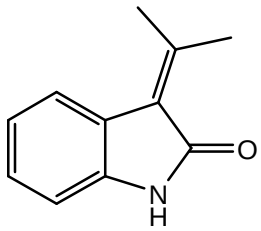
5.5.13. Synthesis of (E/Z)-3-(2-hydroxy-3-methoxybenzylidene)indolin-2-one (154)



(*E*/*Z*)-3-(2-Formyl-3-methoxybenzylidene)indolin-2-one **(154)** was obtained following the general procedure. 2-Oxindole **(9)** (0.90 g, 7.45 mmol, 1.00 equiv.), 2-formyl-6-methoxyphenyl acetate (1.34 g, 7.45 mmol, 1.00 equiv.) and piperidine (1.25 cm^3, 12.2 mmol, 1.62 equiv.) were dissolved in MeOH (50 cm^3) and the reaction was left to reflux for 24 hrs. Water (30 cm^3) was added and the mixture was then extracted using CH_2Cl_2 (3 × 30 cm^3). The solution was dried with $MgSO_4$, filtered and the solvent removed in *vacuo* to give 3-(2-acetoxy-3-methoxybenzylidene)indolin-2-one (0.100 g, 0.32 mmol) as a light brown solid which was dissolved in MeOH (3 cm^3) in a flame-dried round bottom flask equipped with a stirrer bar. Potassium carbonate (0.0661 g, 0.481 mmol, 1.50 equiv.) was then added to the solution and this was left to stir for a further 3 hrs at rt. The solution was quenched with water (5 cm^3) and the resulting solution was left to stand overnight to give a pure orange solid **(151)** which was not purified further (0.22 g, 26 %). R_f 0.21 (EtOAc\Hexane 40:60); m.p. 181-196^oC ν_{max} (film)/cm^{-1}: 3663 (m, NH\OH), 1703 (s, C=O), 1616 (w, C=C), 1172 (s, C-N), 1095 (m, C-O); δ_H (300 MHz; $CDCl_3$+DMSO-d_6, Me_4Si) 8.65 (1H, s, NH), 7.99 (1H, s, COCCH), 7.58 (1H, d, J=7.5, ArH), 7.26-7.31 (1H, m, ArH), 7.19 (1H, t, J=7.5, ArH), 6.83-6.93 (4H, m, 4×ArH), 3.95 (3H, s, OCH_3), 1.74 (1H, bs, OH); δ_c (75 MHz, $CDCl_3$+DMSO-d_6) 169.5 (C=O), 147.1 (ArC), 145.1 (ArC), 141.9 (ArC), 132.4 (COC*C*H), 128.9 (ArCH), 127.2 (ArC), 124.5 (ArC), 122.7 (ArCH), 121.6 (ArC), 121.2 (ArCH), 120.7 (ArCH), 118.5 (ArCH), 111.8 (ArCH),109.6 (ArCH);

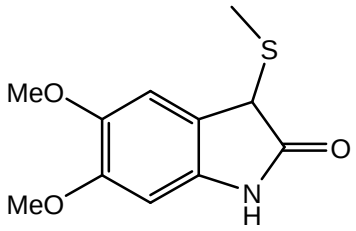
55.8 (OMe); m/z (APCI) 268 (M^++1, 100%), 267 (1), 266 (2), 250 (10); Found M^++1, 268.228 $C_{16}H_{14}NO_3$ (M + H) requires 268.0974.

5.5.14. Synthesis of 3-(propan-2-ylidene)indolin-2-one (155)[85]



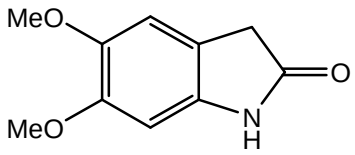
2-Oxindole **(9)** (1.18 g, 8.78 mmol) was dissolved in acetone (100 cm^3) in a flame-dried round bottom flask equipped with a stirrer bar. Concentrated hydrochloric acid (7 cm^3) was then added. The resulting mixture was stirred for a further 48 hrs at rt. The solvent was then evaporated under reduced pressure to afford 3-(propan-2-ylidene)indoline as a dark red solid **(152)** which was not purified further (1.17 g, 76%). R_f 0.50 (EtOAc/Hexane 40:60); m.p. 186-187^{o}C; ν_{max} (film)/cm^{-1}: 2894 (w, C-H), 1690 (m, C=O), 1335 (w, C-N); δ_H (300 MHz; $CDCl_3$; Me_4Si) 8.56 (1H, s, NH), 7.50-7.53 (1H, d, J=7.6, ArH), 7.18 (1H, t, J=7.2, ArH), 7.01 (1H, t, J=7.5, ArH), 6.87 (1H, d, J=7.5, ArH), 2.64 (3H, s, CH_3), 2.39 (3H, s, CH_3); δ_c (75 MHz, $CDCl_3$) 170.8 (C=O), 155.9 (ArC), 139.8 (ArC), 128.2 (ArC), 127.9 (ArCH), 124.9 (ArC), 124.1 (ArCH), 121.9 (ArCH), 109.8 (ArCH), 25.6 (CH_3), 23.6 (CH_3); m/z (ESI+) 174 (M^++1, 100%), 159 (2), 156 (17), 147 (5), 146 (35), 133 (11), 132 (100), 131 (1); Found M^++1, 174.16 $C_{11}H_{12}NO$ (M + H) requires 1740920.

5.5.15. Synthesis of 5,6-dimethoxy-3-(methylthio)indolin-2-one (156)[86]



CH_2Cl_2 (200 cm^3) was cooled to -78°C under N_2. Ethyl (methylthio)acetate (3.95 cm^3, 30.7 mmol, 1.00 equiv.) was added by a syringe followed by sulfuryl chloride (2.47 cm^3, 30.7 mmol, 1.00 equiv.). The resulting mixture was stirred for 15 mins. A solution of 3,4-dimethoxyaniline **(134)** (4.70 g, 30.7 mmol) and 1,8 bis(dimethylamino)naphthalene (2.00 g) was added over a period of an hour and the mixture was stirred over a period of 2 hrs. A solution of Et_3N (4.28 cm^3, 30.7 mmol, 1.00 equiv.) and CH_2Cl_2 (8 cm^3) was added drop-wise for a period of 30 mins and the solution allowed warming up to rt. The reaction was quenched with water (30 cm^3). The resulting solution was extracted with CH_2Cl_2 (3 × 30 cm^3). The organic layers were combined and dried using $MgSO_4$, filtered and the solvent removed under reduced pressure. The residue was purified using column chromatography (EtOAc/Hexane 40:60) as eluent to give 5,6-dimethoxy-3-(methylthio)indolin-2-one **(156)** as a brown solid which was recrystalized using toluene to give a white solid (2.00 g, 31%). R_f 0.28 (EtOAc/Hexane 40:60); ν_{max} (film)/cm^{-1}: 2910 (s, NH), 1721 (s, C=O), 1110 (s, C-O), 999 (m, C-S) δ_H (300 MHz; $CDCl_3$; Me_4Si) 9.30 (1H, s, NH), 6.96 (1H, s, ArH), 6.58 (1H, s, ArH), 4.26 (1H, s, SCH), 3.82 (6H, s, 2×OCH_3), 1.97 (3H, s, CH_3). δ_c (75 MHz, $CDCl_3$) 178.8 (CHO), 150.2 (ArC), 145.6 (ArC), 135.2 (ArC), 116.5 (ArC), 109.4 (ArCH), 95.7 (ArCH), 56.7 (OCH_3), 56.3 (OCH_3), 47.2 (SCH), 11.7 (SCH_3).

5.5.18. Synthesis of 5,6-dimethoxyindolin-2-one (157)[87]



5,6-Dimethoxy-3-(methylthio)indolin-2-one **(157)** (1.512 g, 6.74 mmol) was dissolved in EtOH (20 cm^3) in a flame-dried round bottom flask equipped with a stirrer bar and a condenser. Raney nickel (large excess) was added and the suspension was stirred for 4 hrs under reflux. The reaction mixture was allowed to cool to rt and was filtered. The solvent was removed in *vacuo* to give 5,6-dimethoxyindolin-2-one **(157)** as a white solid which was not purified further. R_f 0.16 (EtOAc/Hexane 40:60); δ_H (300 MHz; $CDCl_3$+DMSO-d_6, Me_4Si) 10.08 (1H, s, NH), 6.85 (1H, s, ArH), 6.49 (1H, s, ArH), 3.84 (3H, s, OMe), 3.81 (3H, s, OMe), 3.41 (2H, s, $COCH_2$); δ_c (75 MHz, $CDCl_3$ + DMSO-d_6) 177.6 (C=O), 148.9 (ArC), 144.2 (ArC), 137.2 (ArC), 115.9 (ArC), 109.8 (ArCH), 95.7 (ArCH), 56.7 (OMe), 56.1 (OMe), 36.4 ($CO\mathit{C}H_2$).

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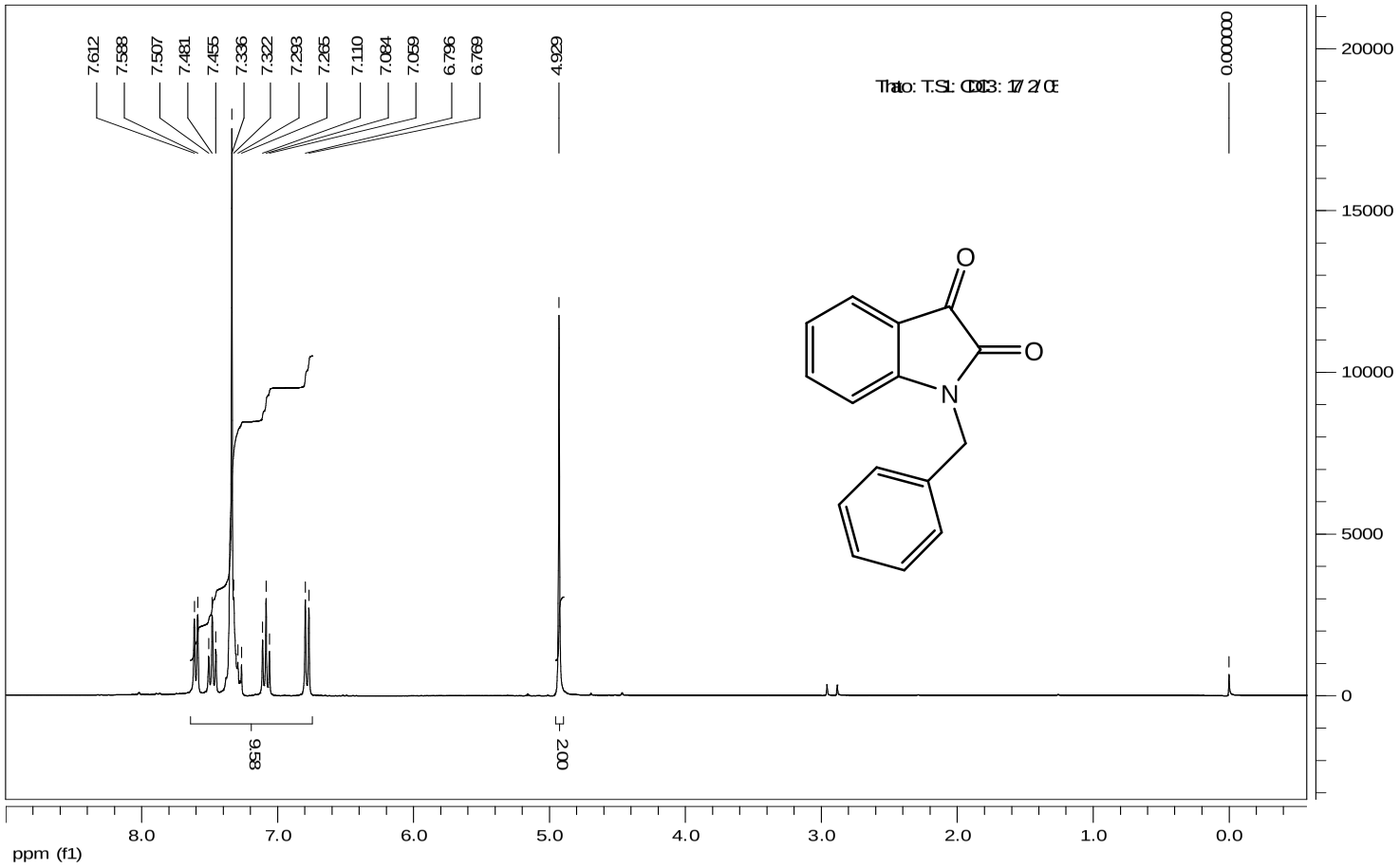
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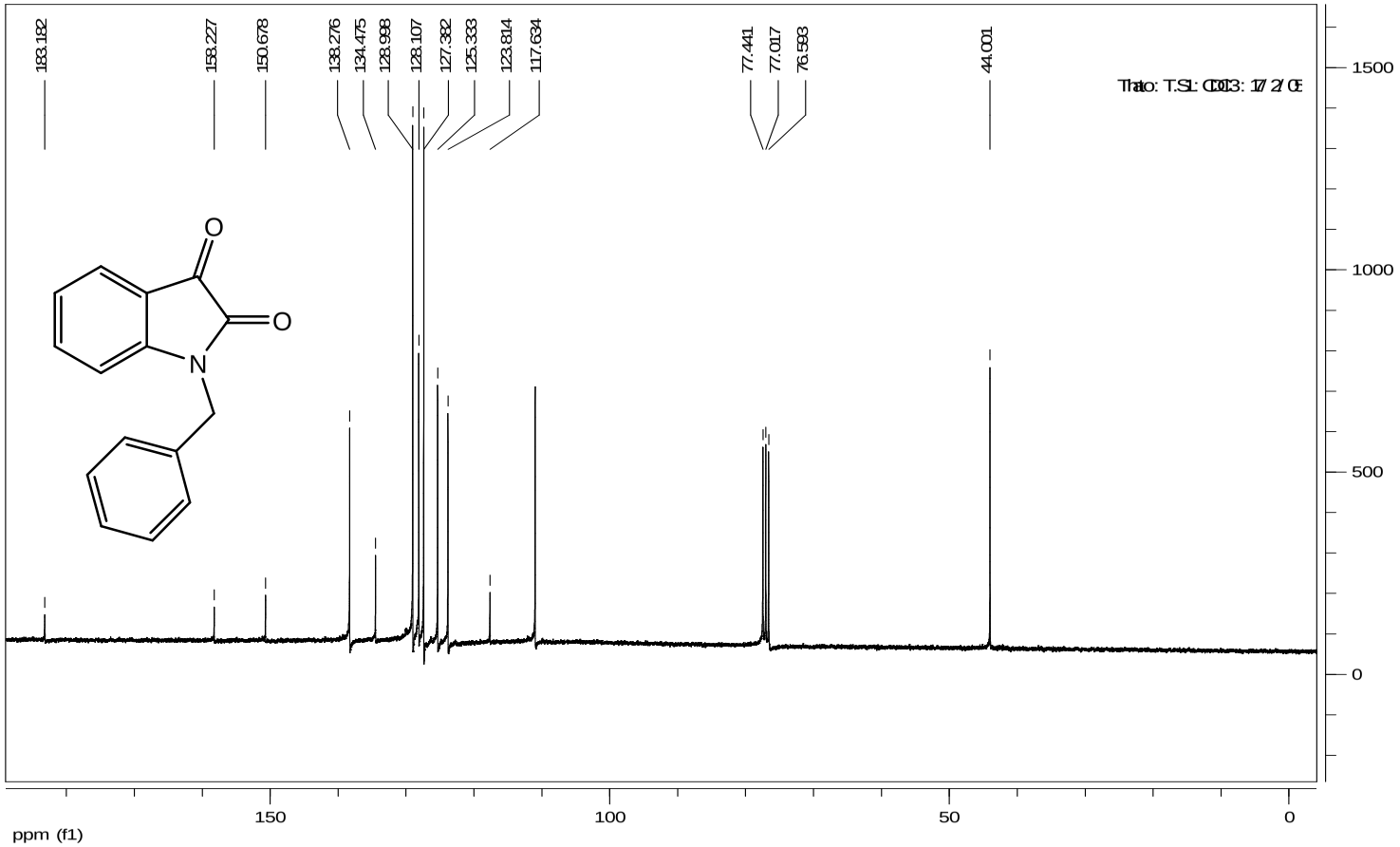
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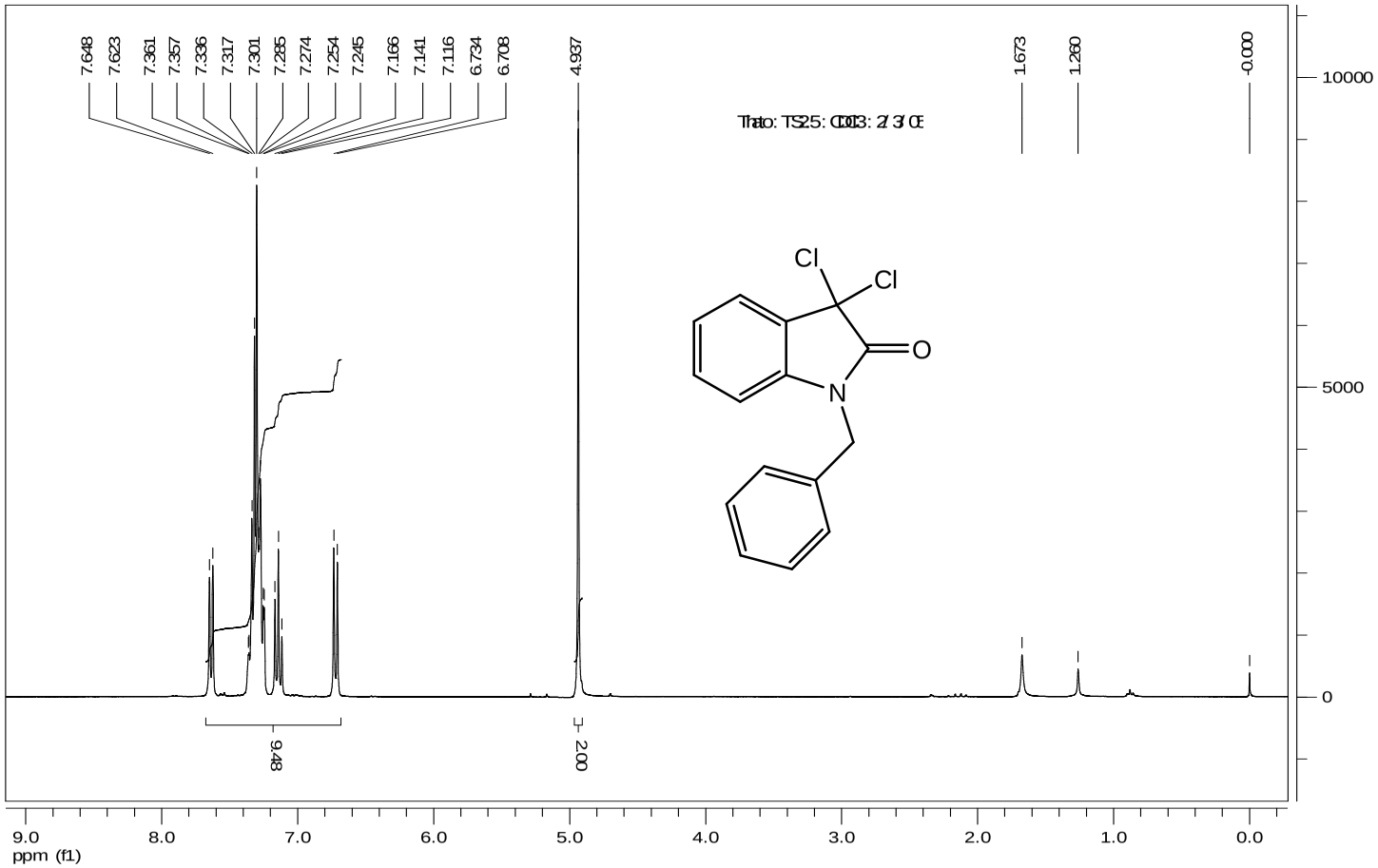
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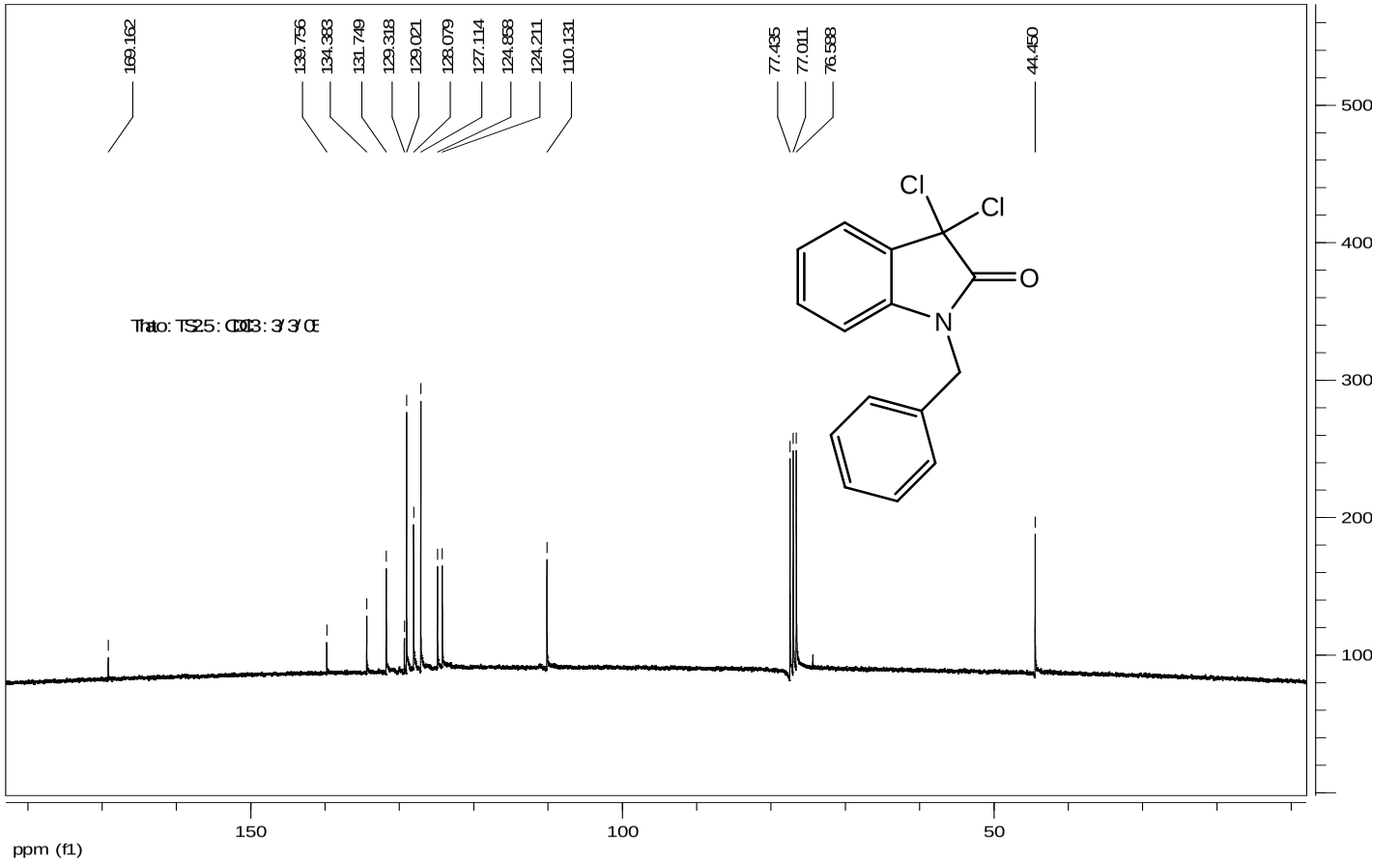
Appendix

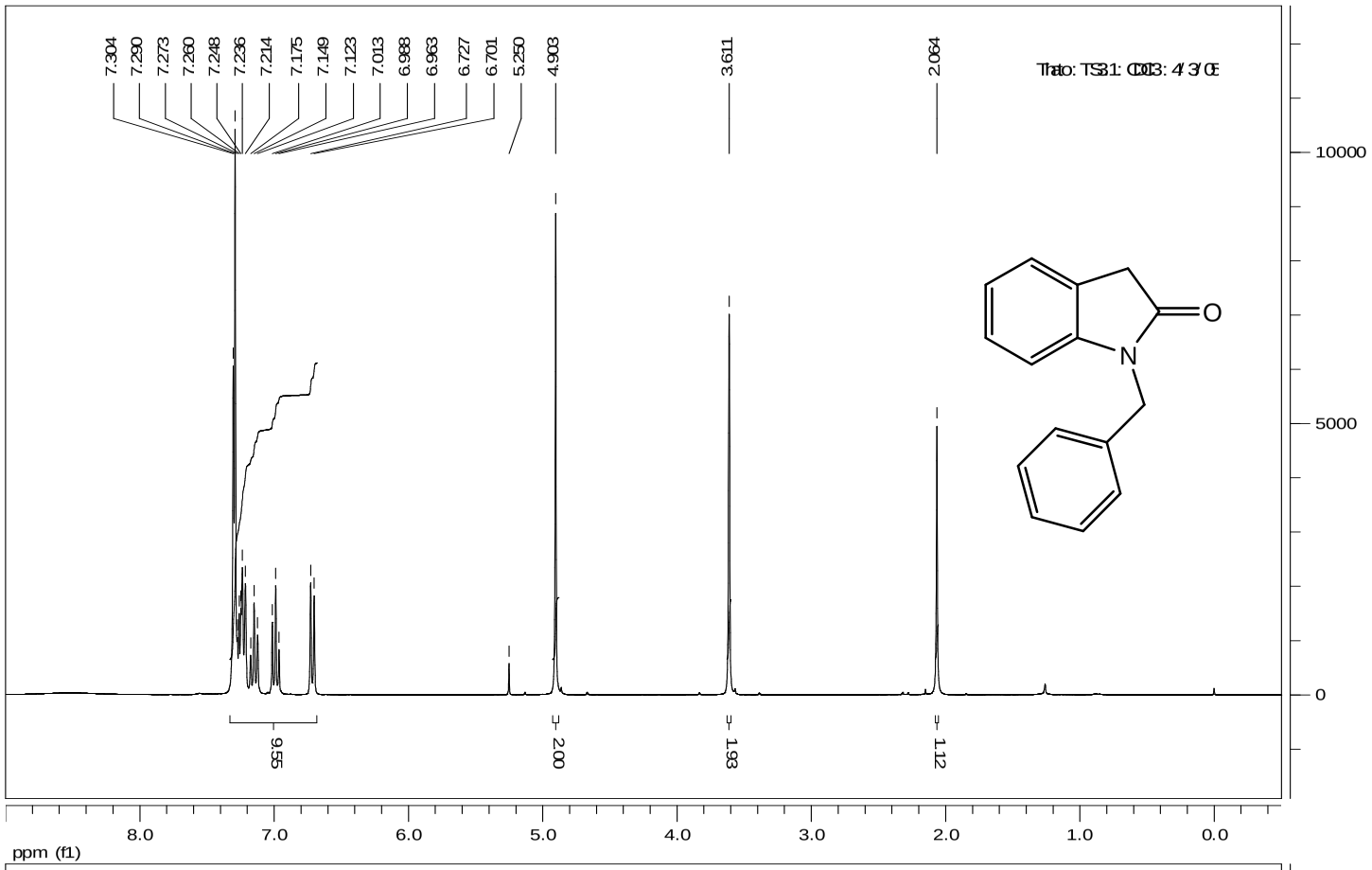
Appendix I – Selected NMR Spectra

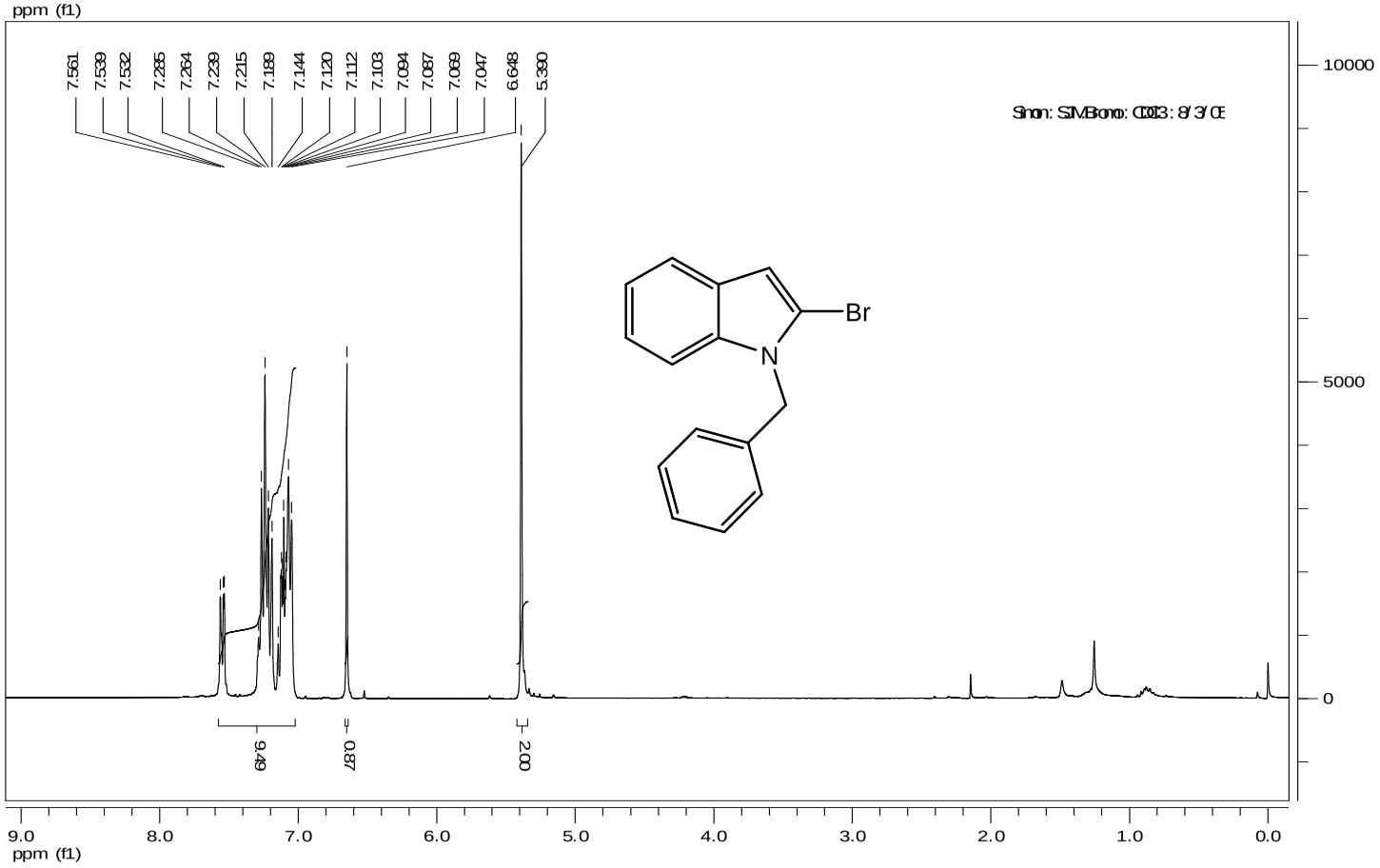

7.612
7.588
7.507
7.481
7.455
7.336
7.322
7.293
7.265
7.110
7.084
7.059
6.796
6.769
4.929
0.000000
9.58
2.00
20000
15000
10000
5000
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

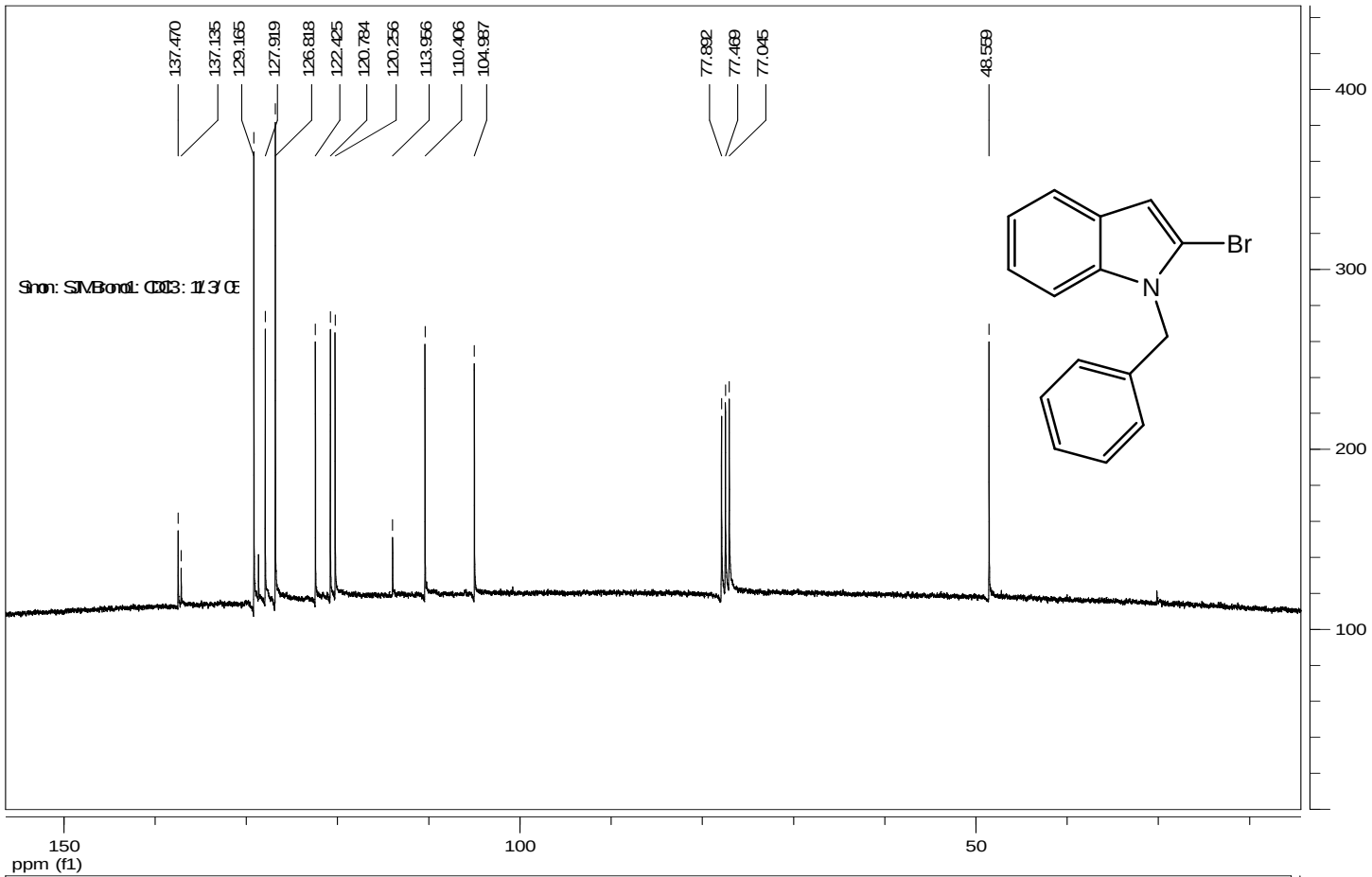

183.182
158.227
150.678
138.276
134.475
128.998
128.107
127.382
125.333
123.814
117.634
77.441
77.017
76.593
44.031
1500
1000
500
0
150
100
50
0
ppm (f1)

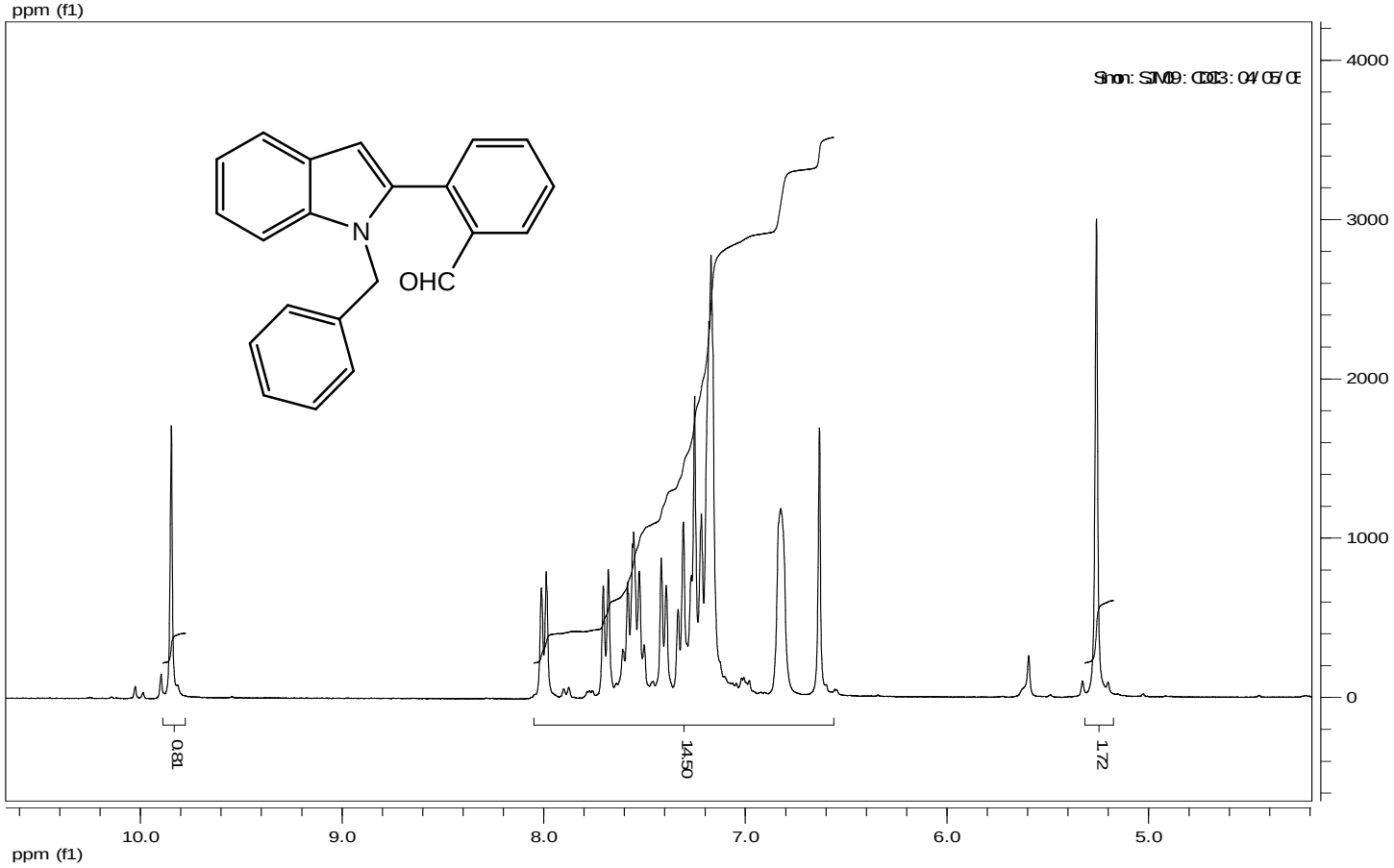

7.648
7.623
7.361
7.357
7.336
7.317
7.301
7.285
7.274
7.254
7.245
7.166
7.141
7.116
6.734
6.708
4.937
1.673
1.260
-0.000
Cl
Cl
O
N
9.48
2.00
10000
5000
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

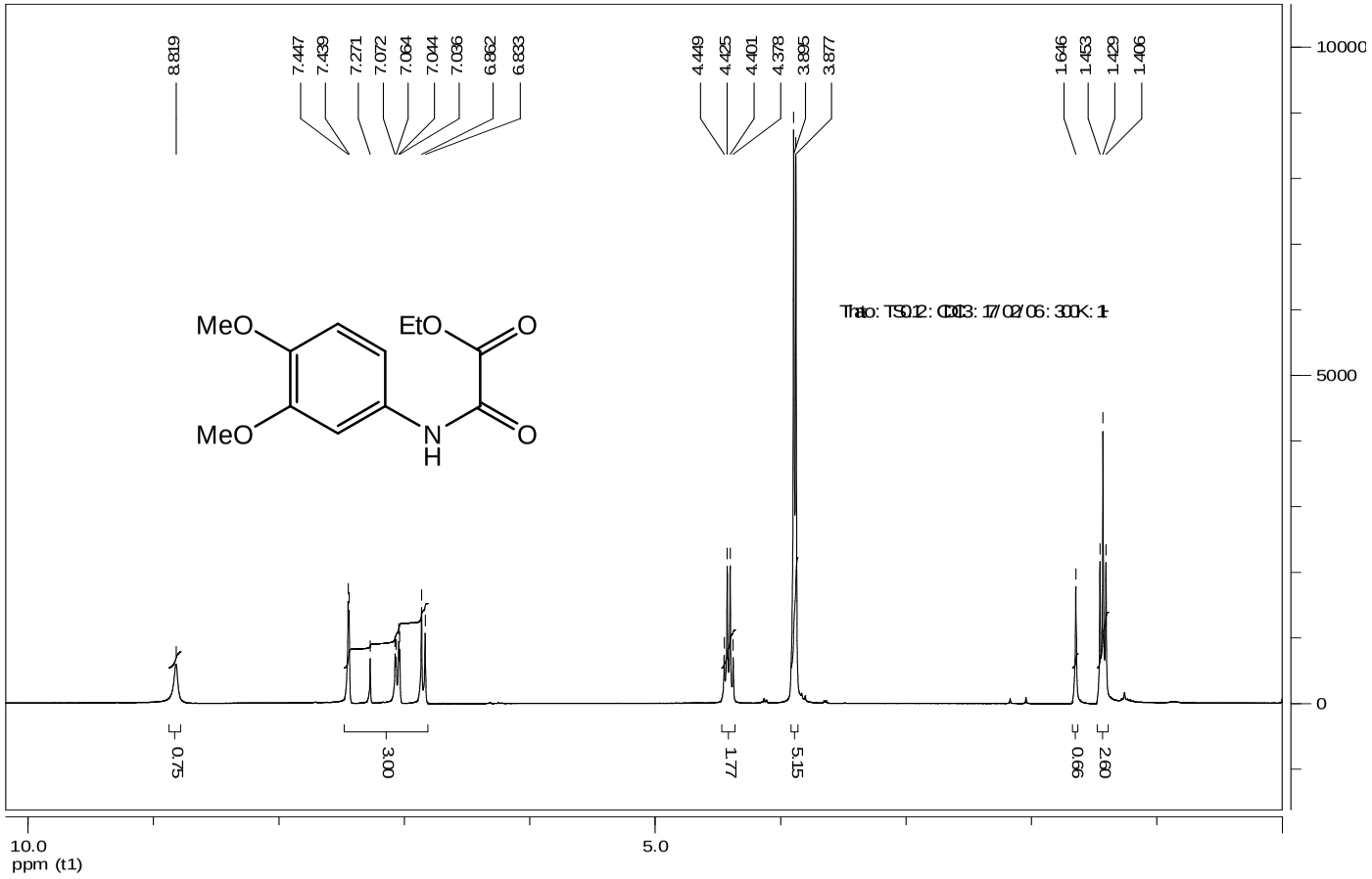

169.162
139.756
134.383
131.749
129.318
129.021
128.079
127.114
124.858
124.211
110.131
77.435
77.011
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100
150
100
50
ppm (f1)

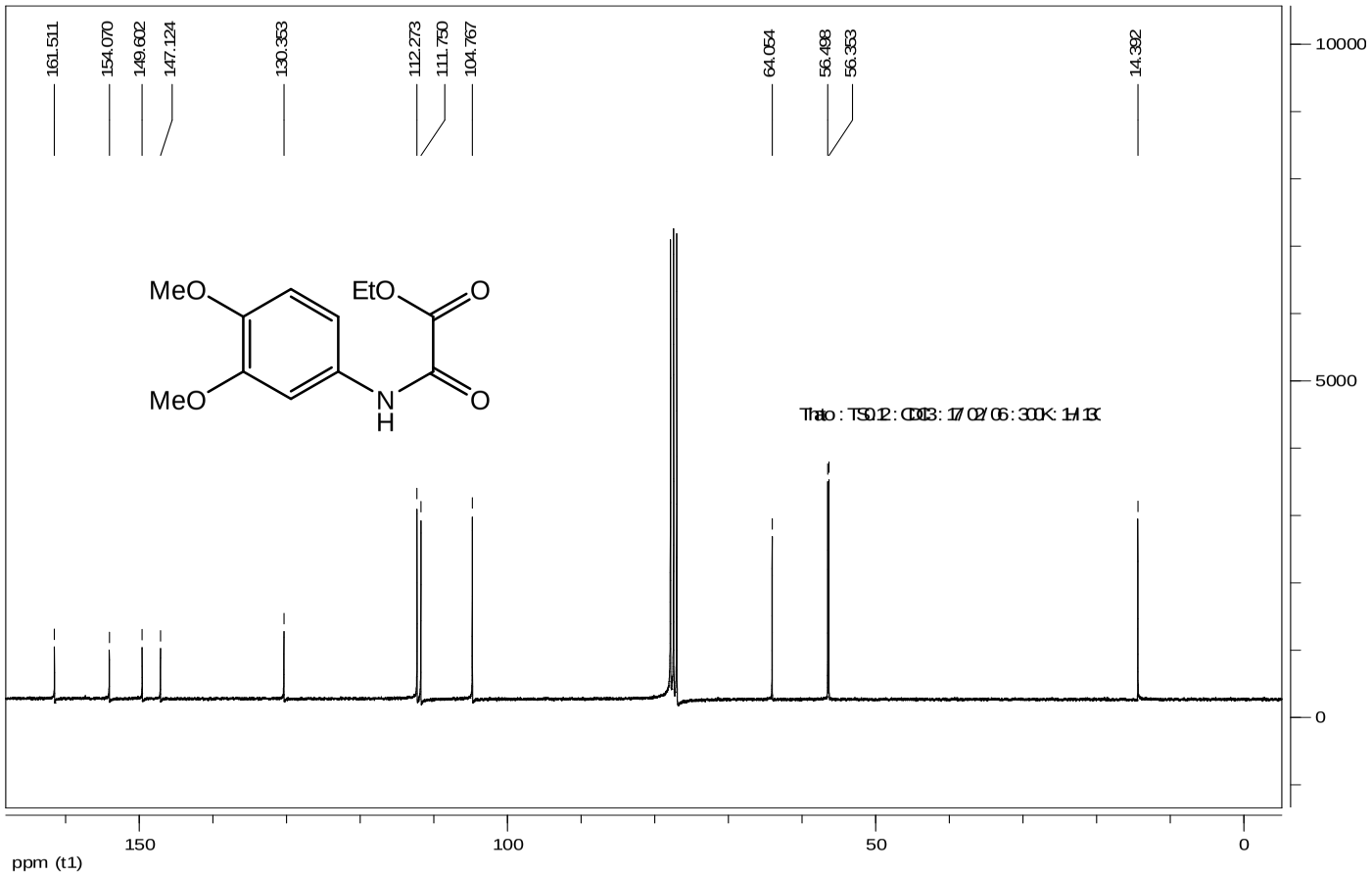

7.304
7.290
7.273
7.260
7.248
7.236
7.214
7.175
7.149
7.123
7.013
6.988
6.963
6.727
6.701
5.250
4.903
3.611
2.064
9.55
2.00
1.93
1.12
O
N
10000
5000
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

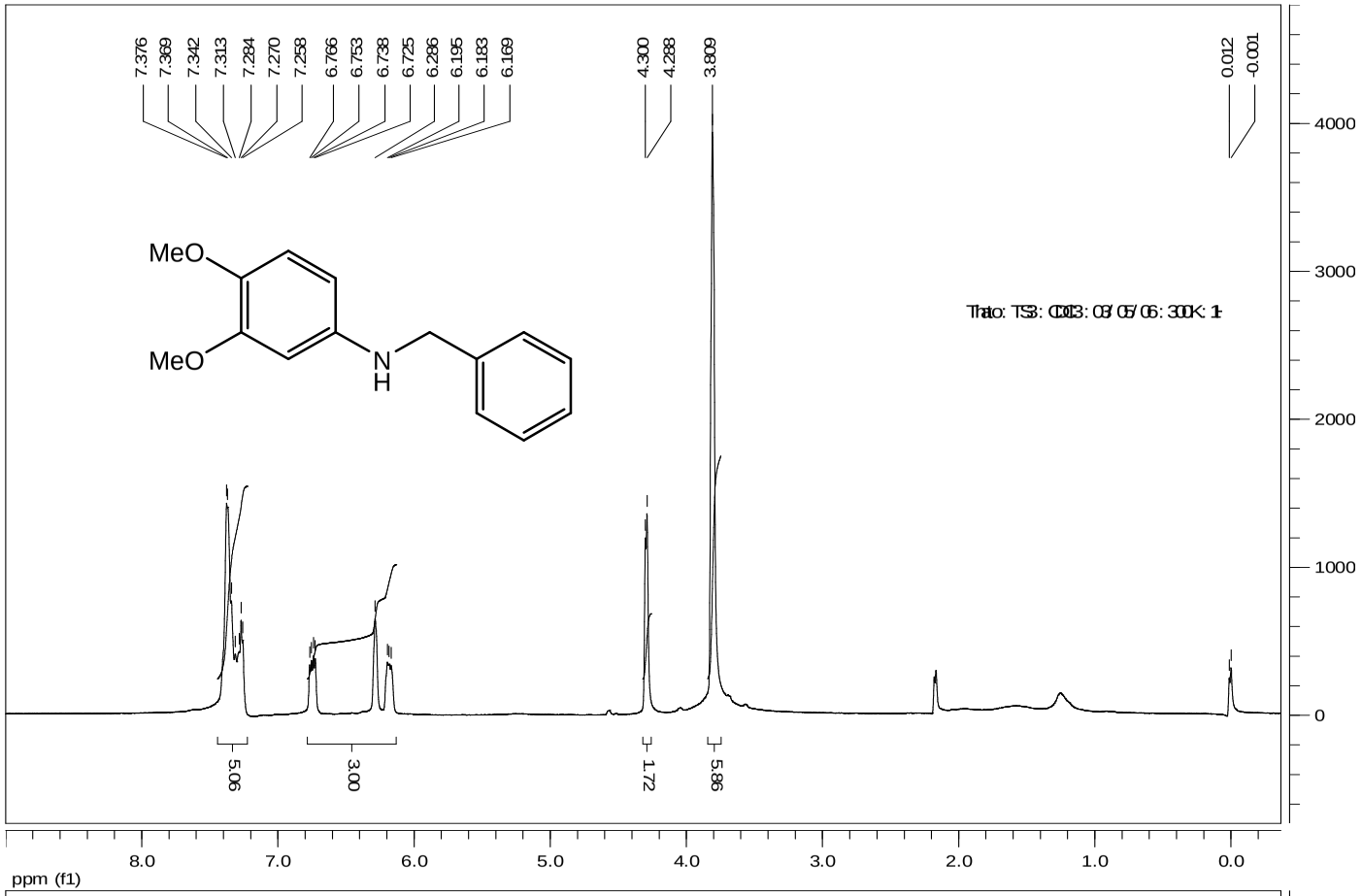

7.561
7.539
7.532
7.285
7.264
7.239
7.215
7.189
7.144
7.120
7.112
7.103
7.094
7.087
7.069
7.047
6.648
5.390
9.49
0.87
2.00
Br
N
10000
5000
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
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ppm (f1)

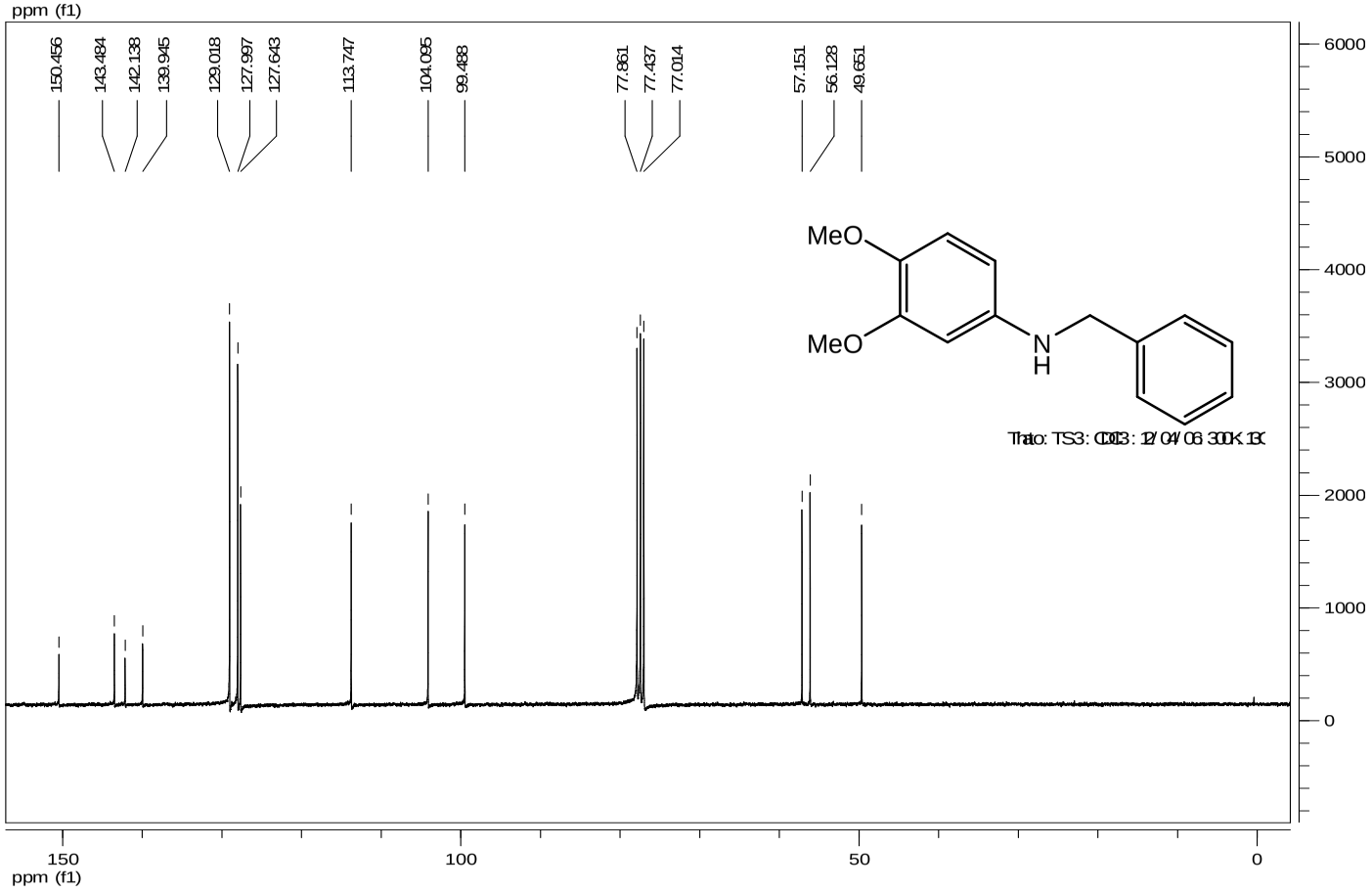

137.470
137.135
129.165
127.919
126.818
122.425
120.784
120.256
113.956
110.406
104.987
77.892
77.469
77.045
48.559
Br
N
400
300
200
100
150
100
50
ppm (f1)

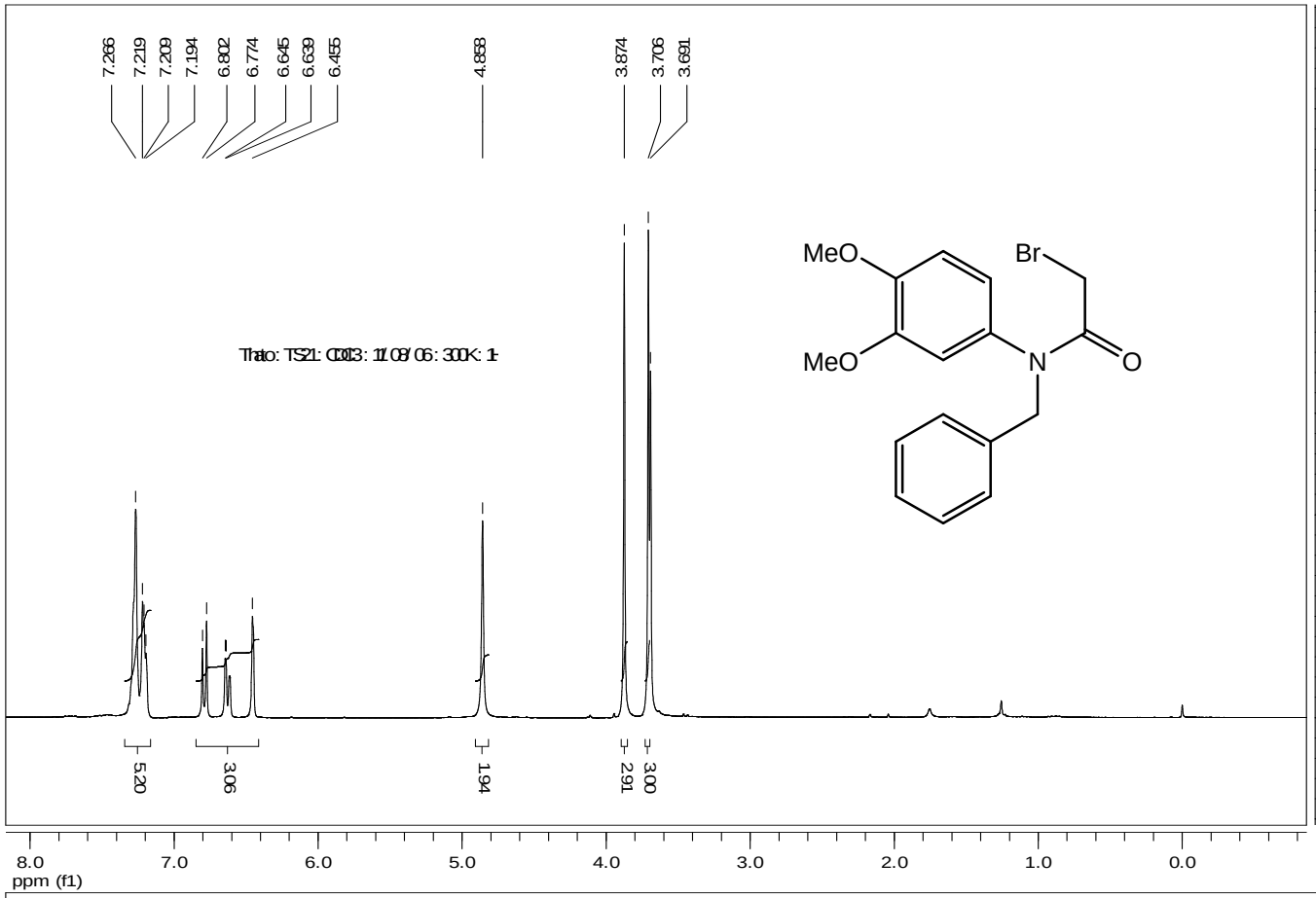

N
OHC
4000
3000
2000
1000
0
0.81
14.50
1.72
10.0
9.0
8.0
7.0
6.0
5.0
ppm (f1)

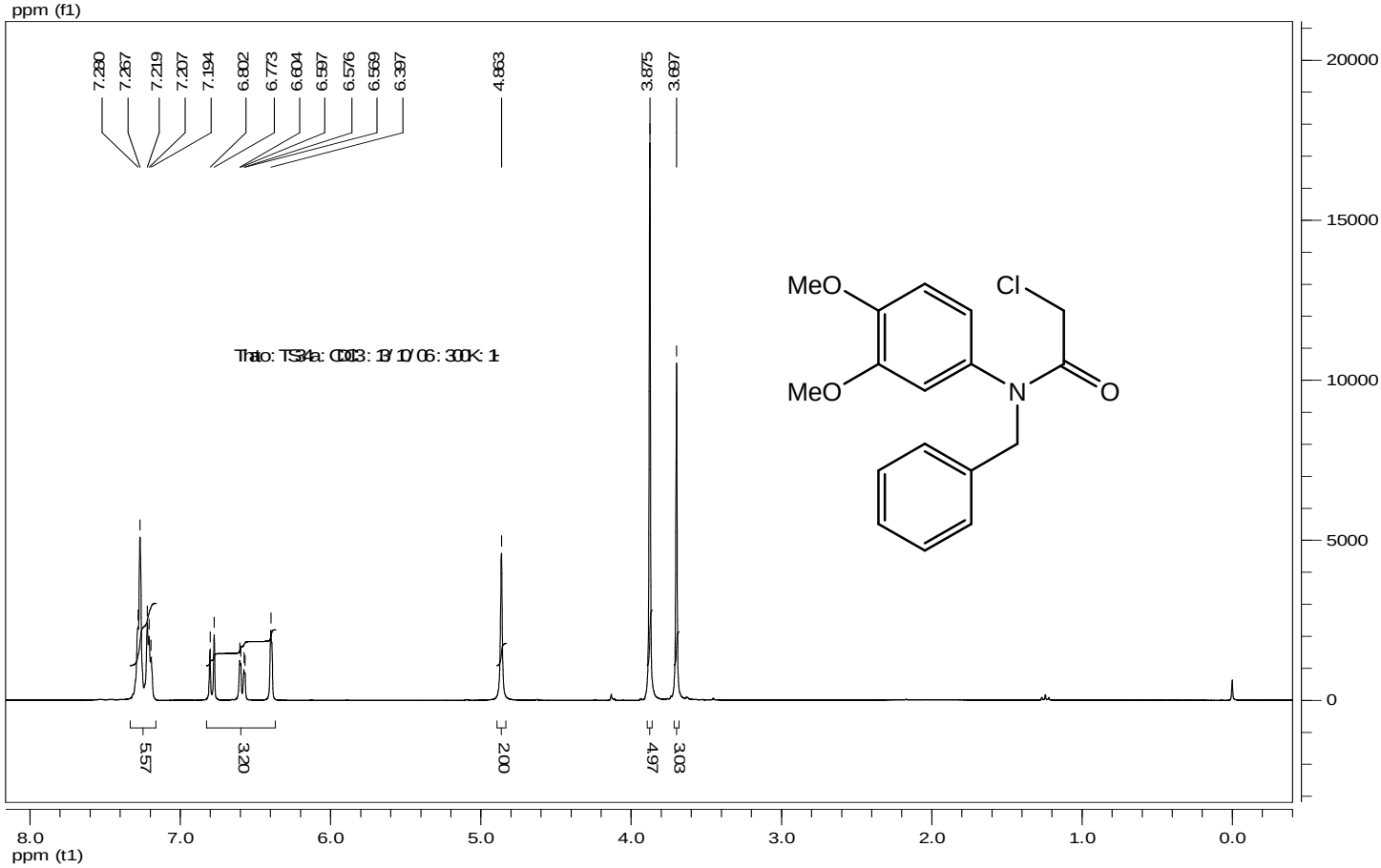

8.819
7.447
7.439
7.271
7.072
7.064
7.044
7.036
6.862
6.833
4.449
4.425
4.401
4.378
3.895
3.877
1.646
1.453
1.429
1.406
MeO
MeO
EtO
O
O
N
H
0.75
3.00
1.77
5.15
0.66
2.60
10000
5000
0
10.0
5.0
ppm (t1)

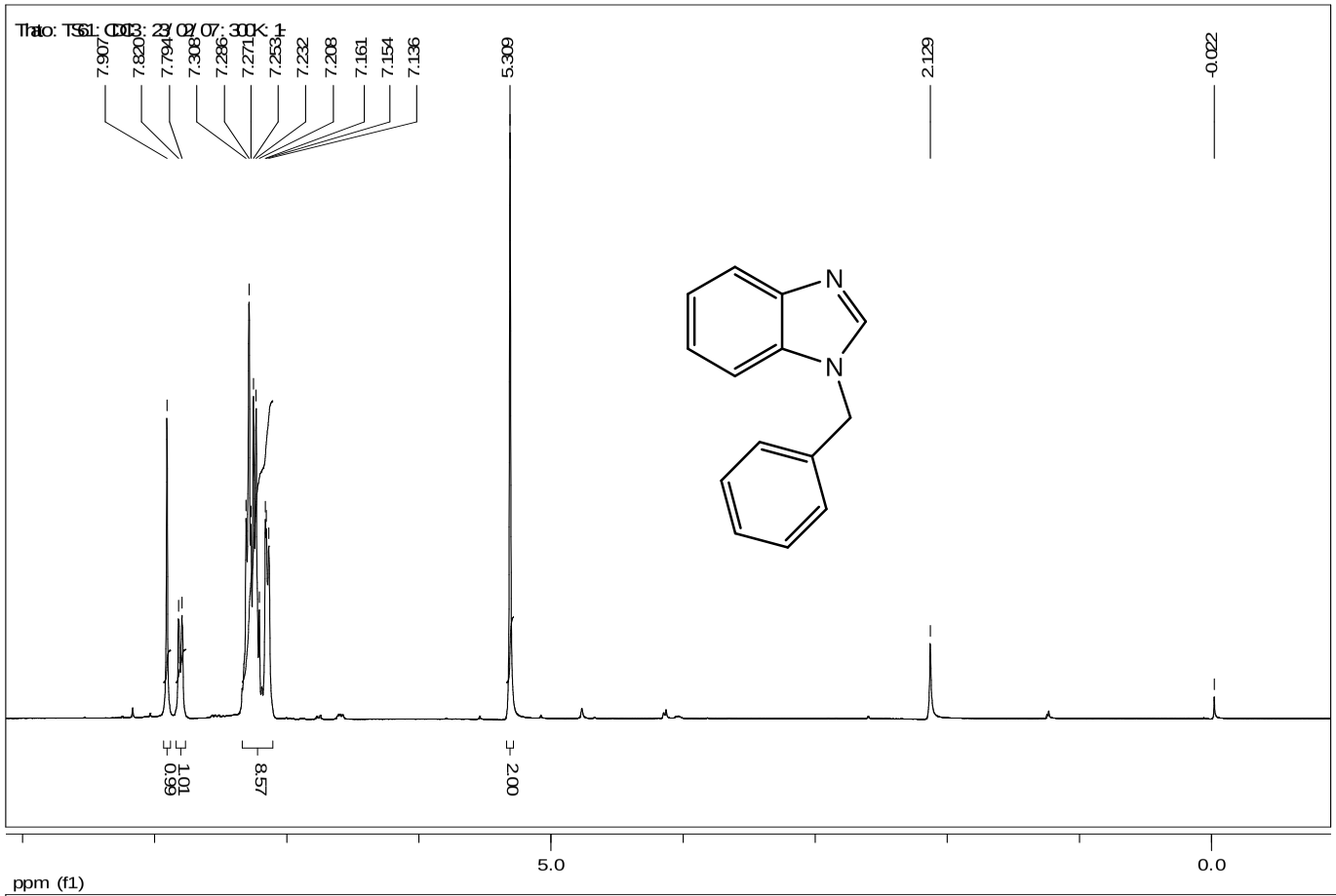

161.511
154.070
149.602
147.124
130.353
112.273
111.750
104.767
64.054
56.498
56.353
14.392
MeO
MeO
EtO
O
O
N
H
10000
5000
0
150
100
50
0
ppm (t1)

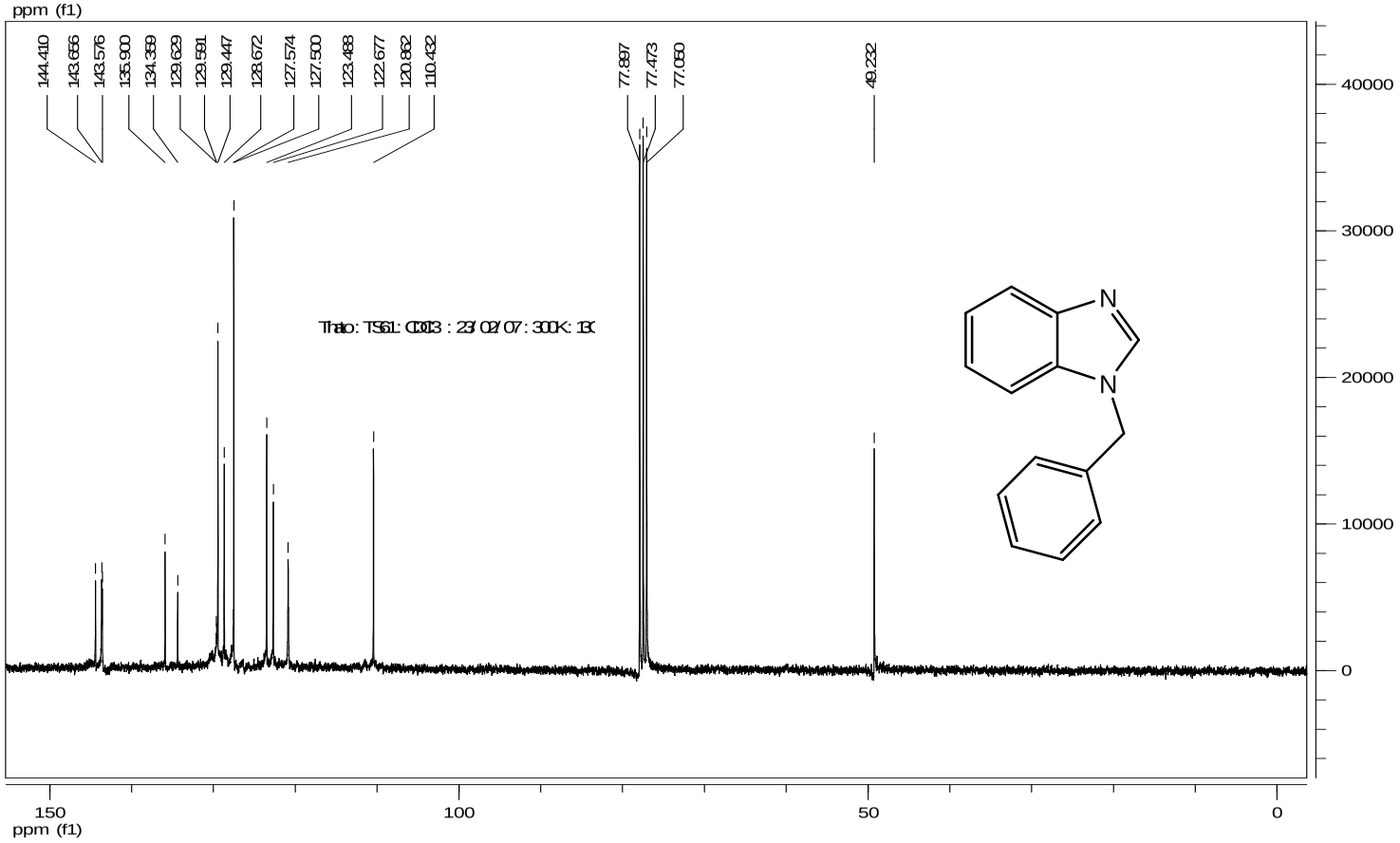

7.376
7.369
7.342
7.313
7.284
7.270
7.258
6.766
6.753
6.738
6.725
6.286
6.195
6.183
6.169
4.300
4.288
3.809
0.012
-0.001
MeO
MeO
N
H
5.06
3.00
1.72
5.86
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

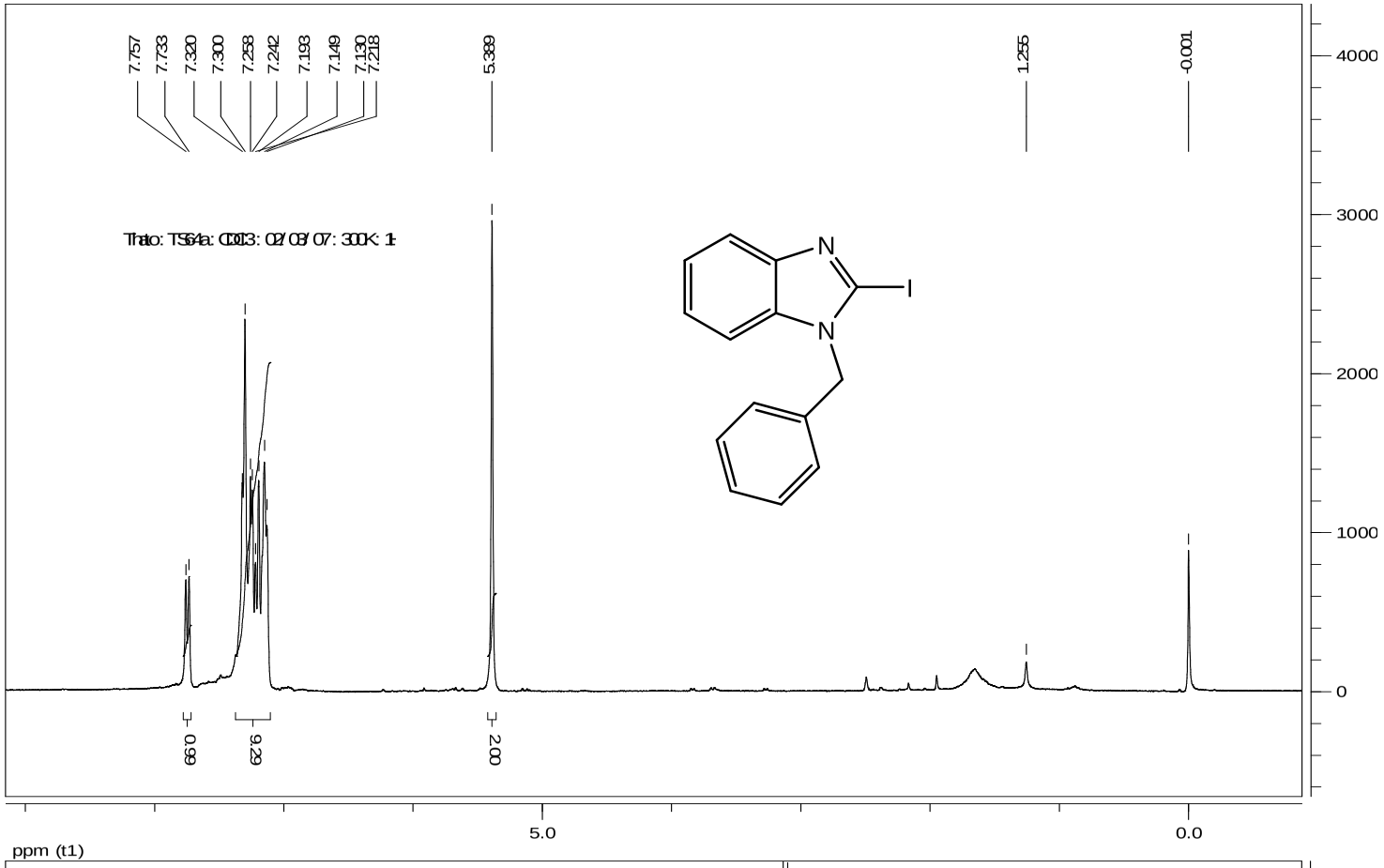

150.456
143.484
142.138
139.945
129.018
127.997
127.643
113.747
104.095
99.488
77.861
77.437
77.014
57.151
56.128
49.651
MeO
MeO
N
H
150
100
50
0
ppm (f1)

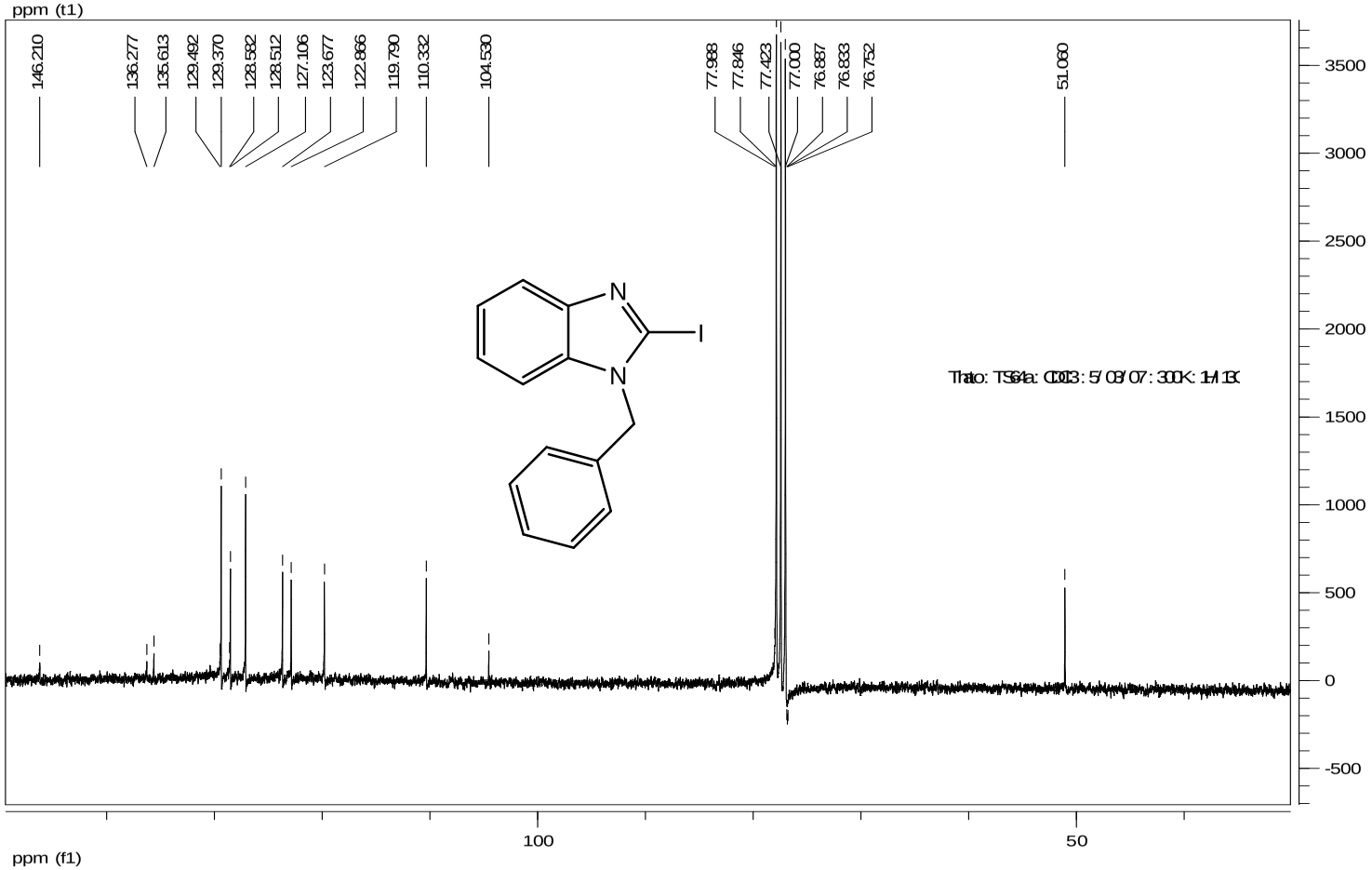

7.266
7.219
7.209
7.194
6.802
6.774
6.645
6.639
6.455
4.858
3.874
3.706
3.691
MeO
MeO
Br
N
O
5.20
3.06
1.94
2.91
3.00
6000
5000
4000
3000
2000
1000
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

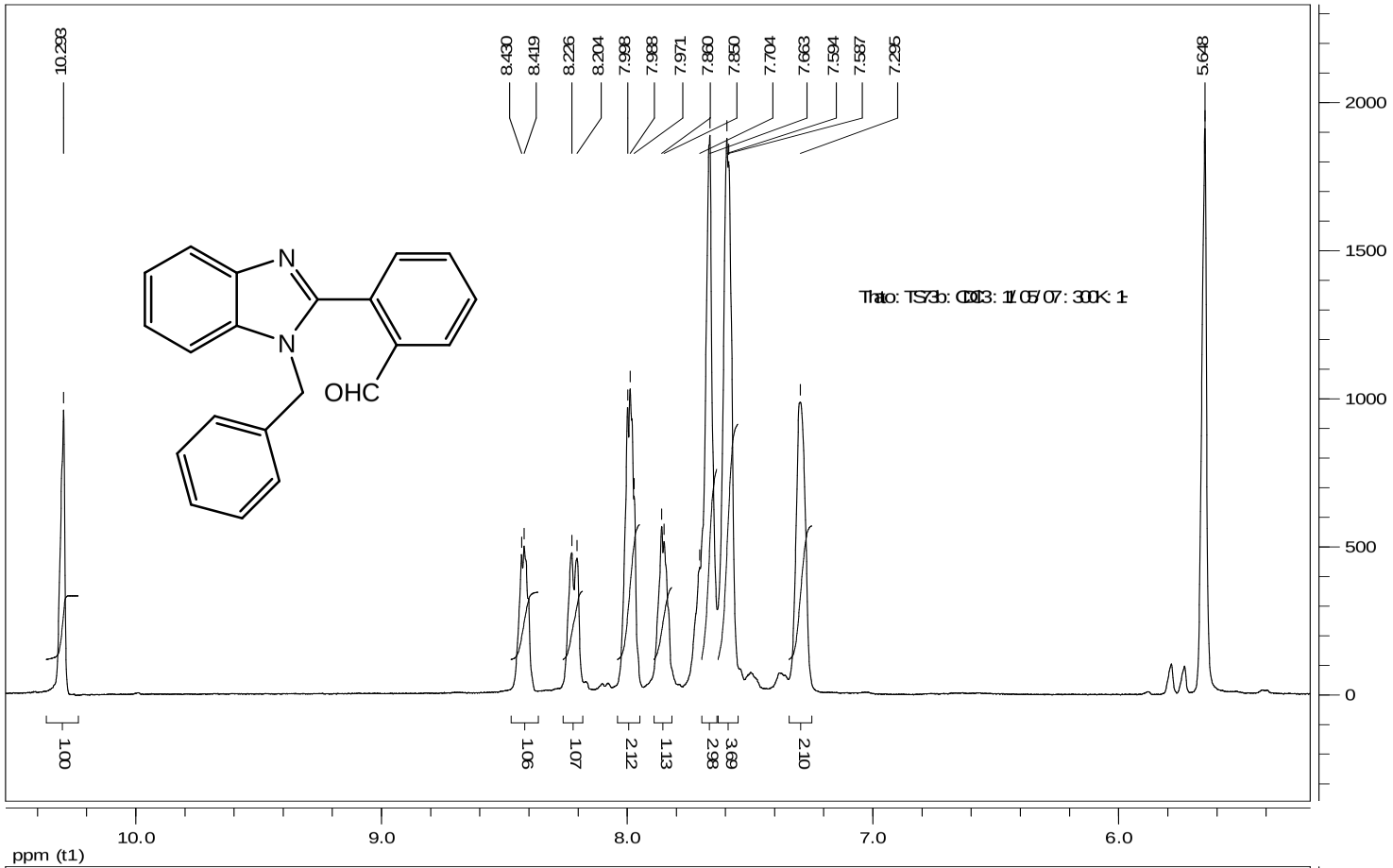

7.280
7.267
7.219
7.207
7.194
6.802
6.773
6.604
6.597
6.576
6.569
6.397
4.863
3.875
3.697
MeO
MeO
Cl
N
O
5.57
3.20
2.00
4.97
3.03
20000
15000
10000
5000
0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

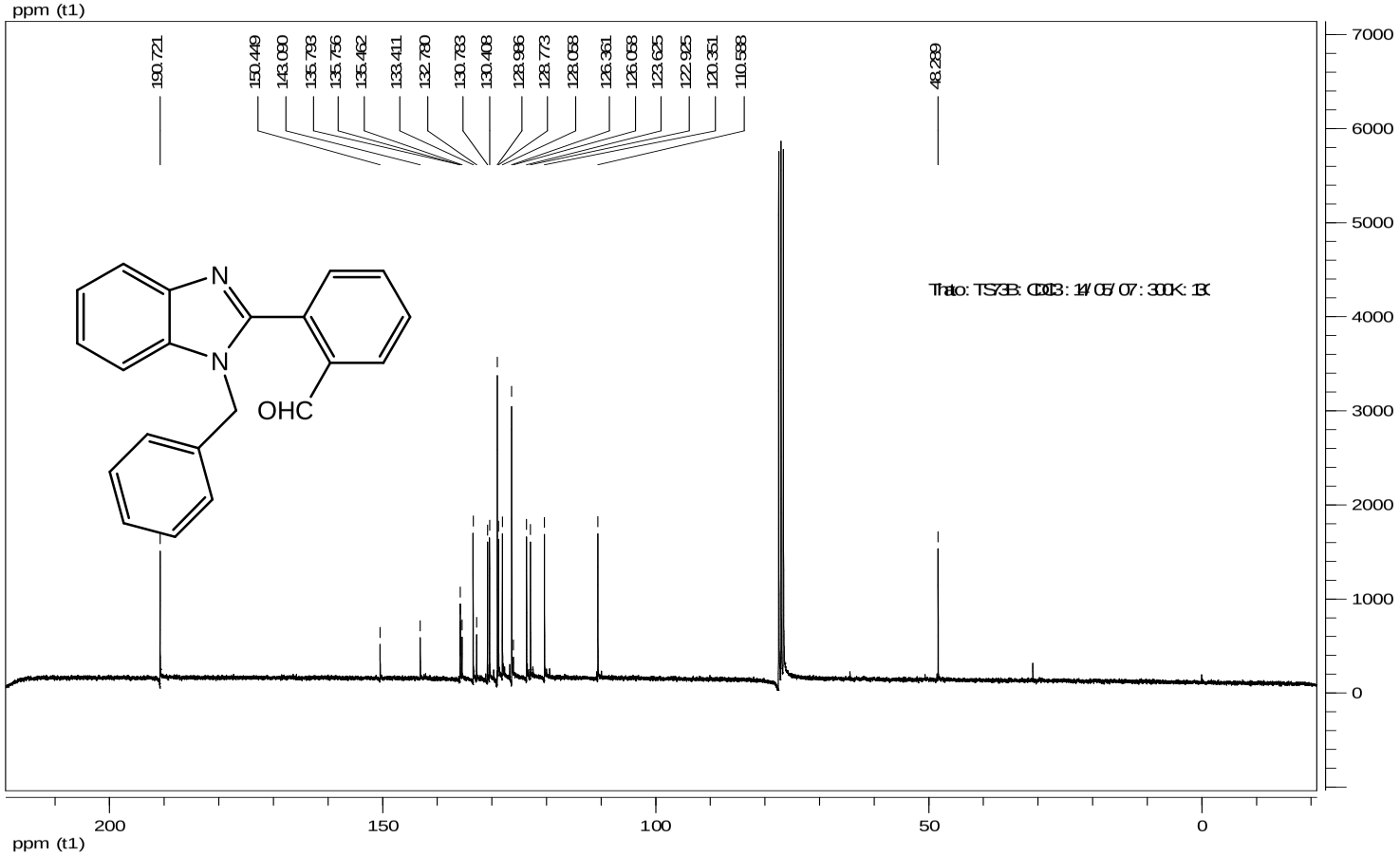

7.907
7.820
7.794
7.308
7.286
7.271
7.253
7.232
7.208
7.161
7.154
7.136
5.309
2.129
-0.022
N
N
5000
0
0.99
1.01
8.57
2.00
5.0
0.0
ppm (f1)

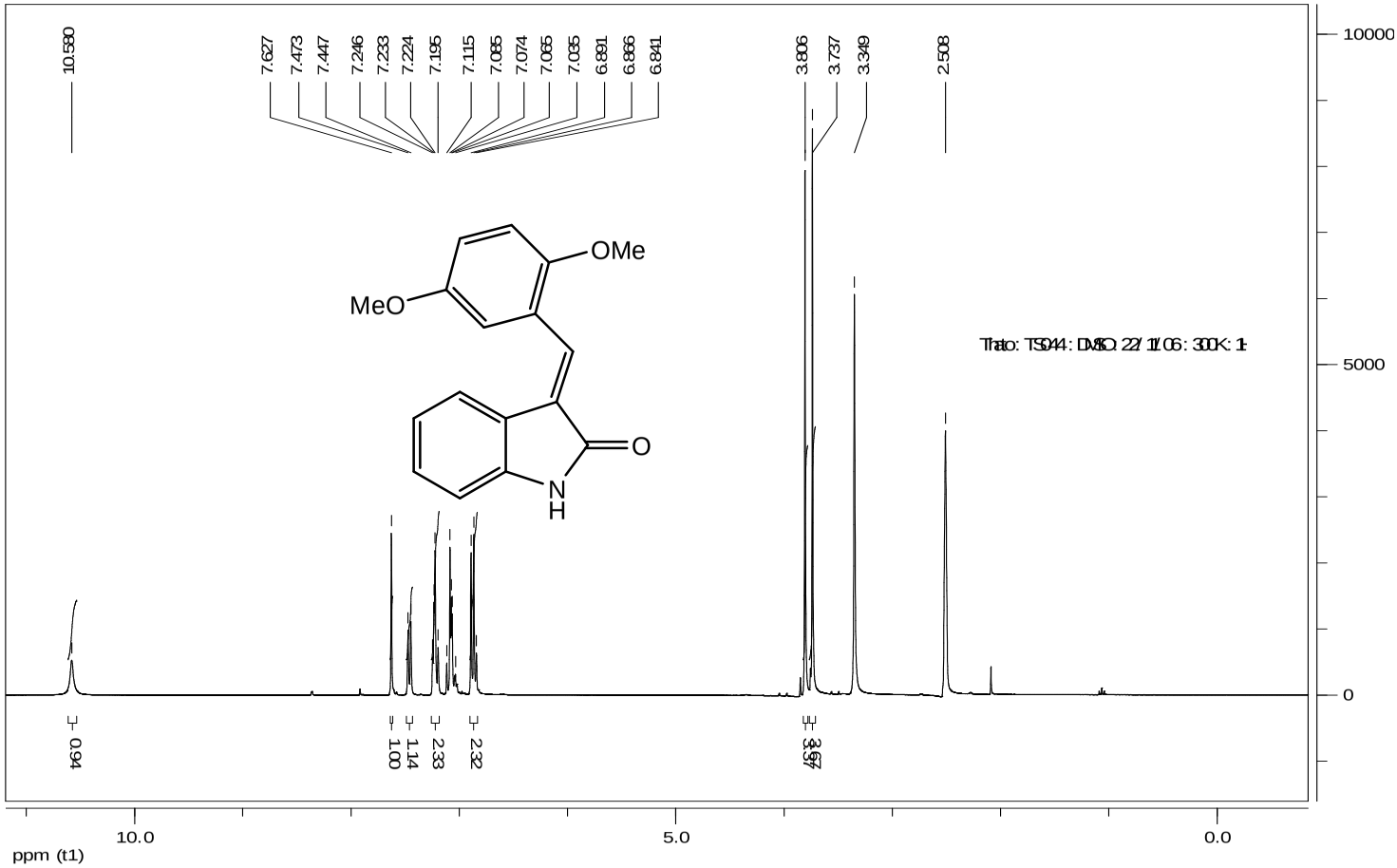

144.410
143.666
143.576
135.900
134.389
129.629
129.591
129.447
128.672
127.574
127.500
123.488
122.677
120.862
110.432
77.897
77.473
77.050
49.232
N
N
40000
30000
20000
10000
0
150
100
50
0
ppm (f1)

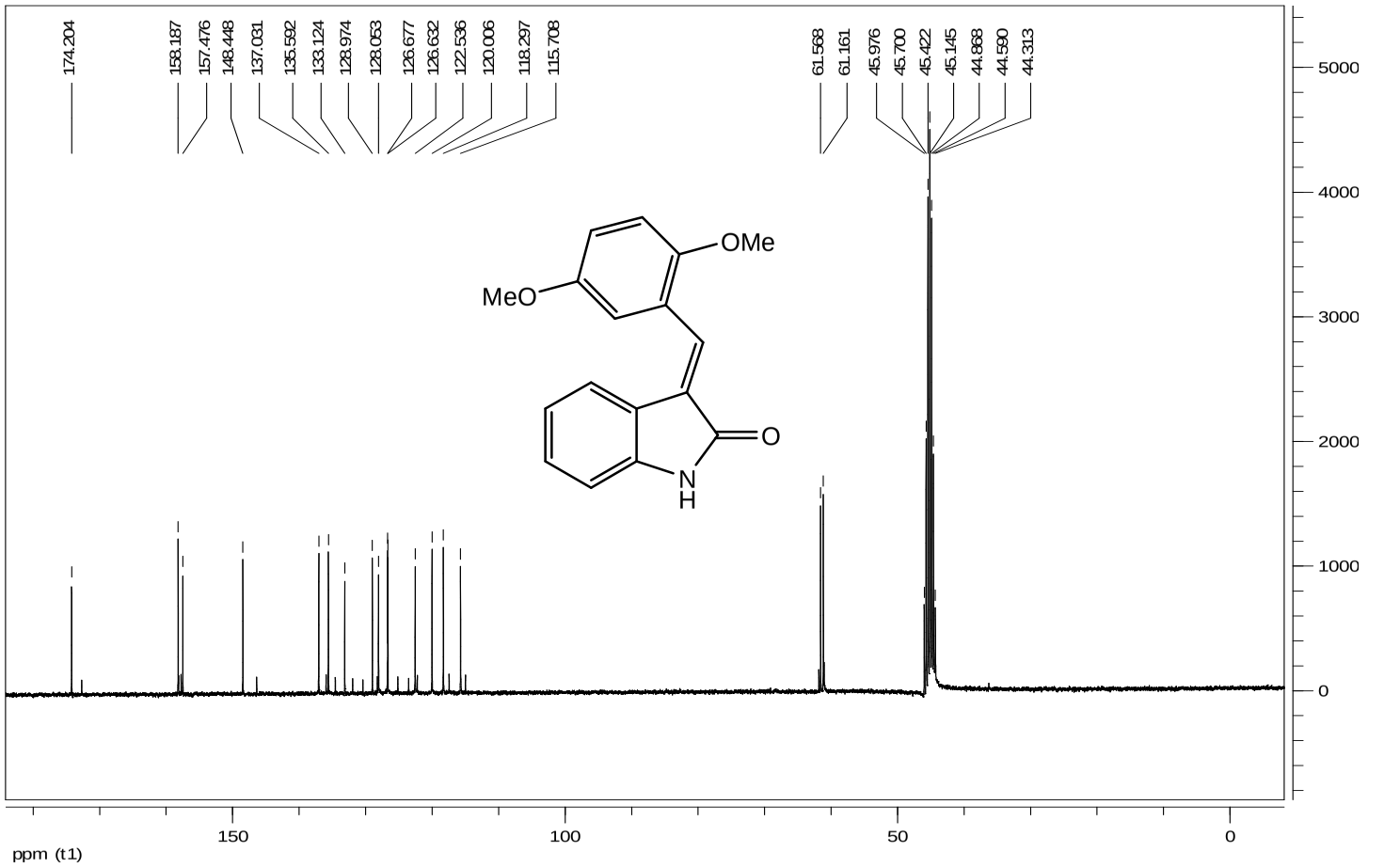

7.757
7.733
7.320
7.300
7.258
7.242
7.193
7.149
7.130
7.218
5.389
1.255
-0.001
N
I
N
0.99
9.29
2.00
4000
3000
2000
1000
0
5.0
0.0
ppm (t1)

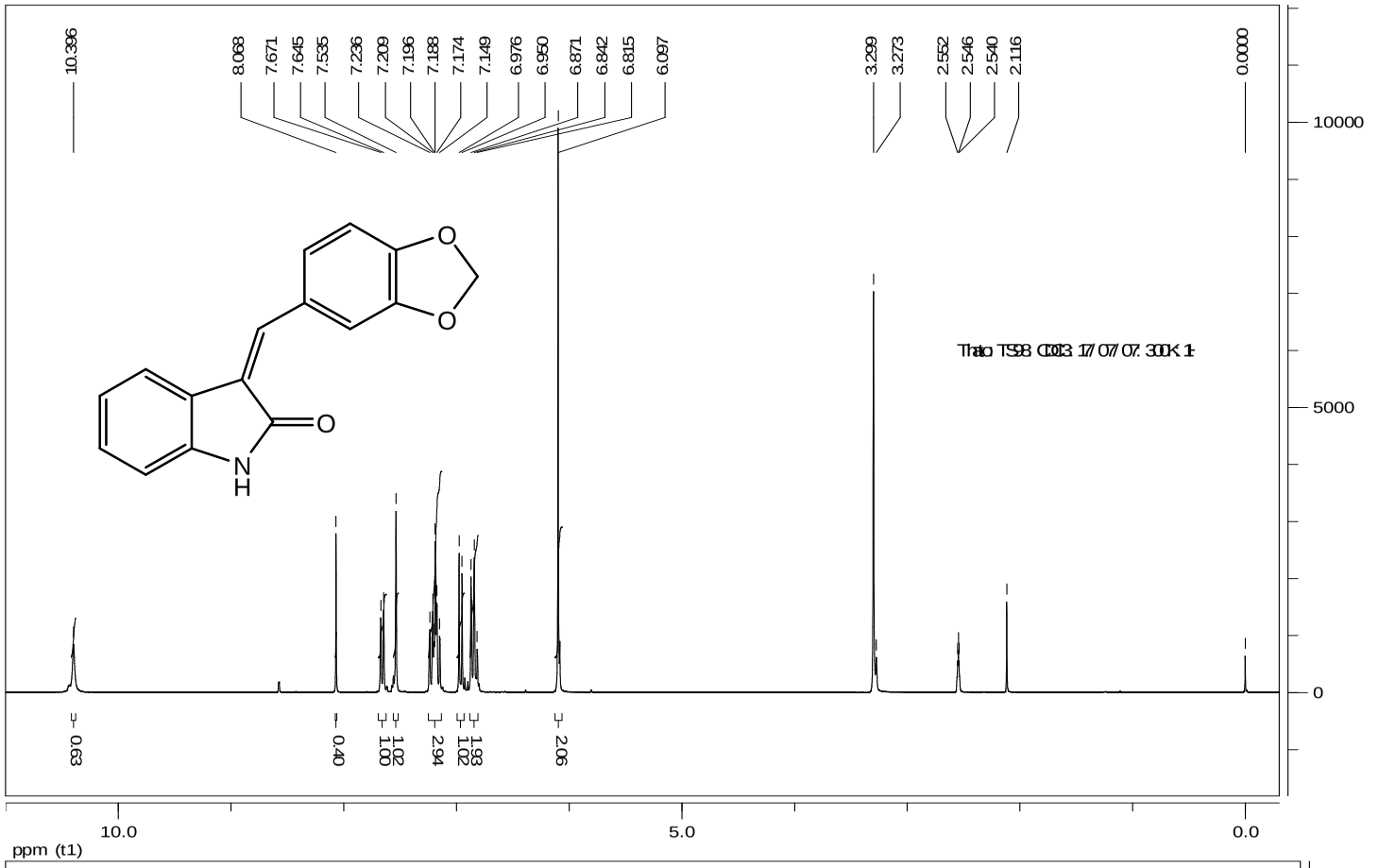

146.210
136.277
135.613
129.492
129.370
128.582
128.512
127.106
123.677
122.866
119.790
110.332
104.530
77.988
77.846
77.423
77.000
76.887
76.833
76.752
51.060
N
I
N
3500
3000
2500
2000
1500
1000
500
0
-500
100
50
ppm (f1)

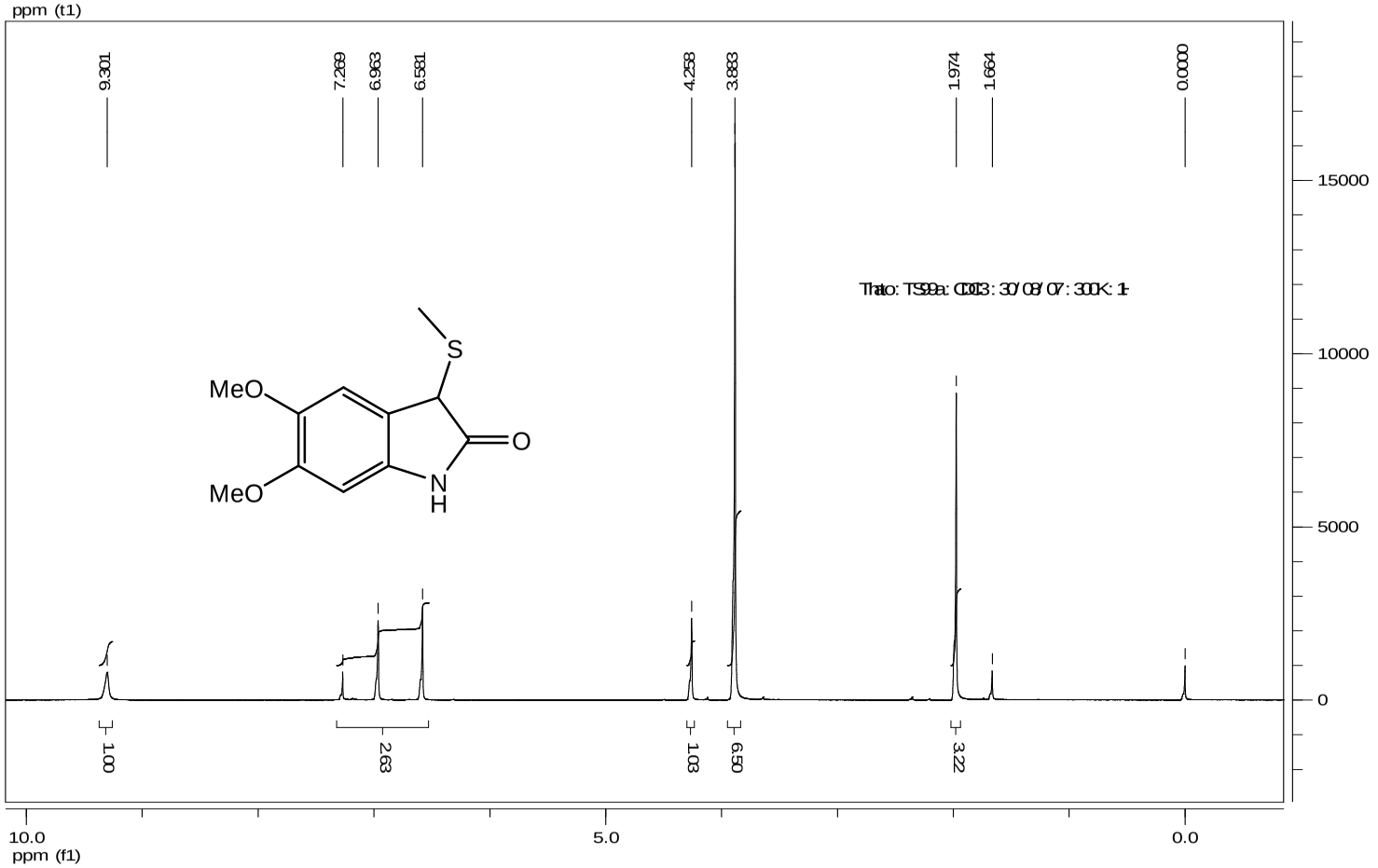

10.293
8.430
8.419
8.226
8.204
7.998
7.988
7.971
7.860
7.850
7.704
7.663
7.594
7.587
7.295
5.648
N
N
OHC
1.00
1.06
1.07
2.12
1.13
2.98
3.69
2.10
10.0
9.0
8.0
7.0
6.0
ppm (t1)
2000
1500
1000
500
0

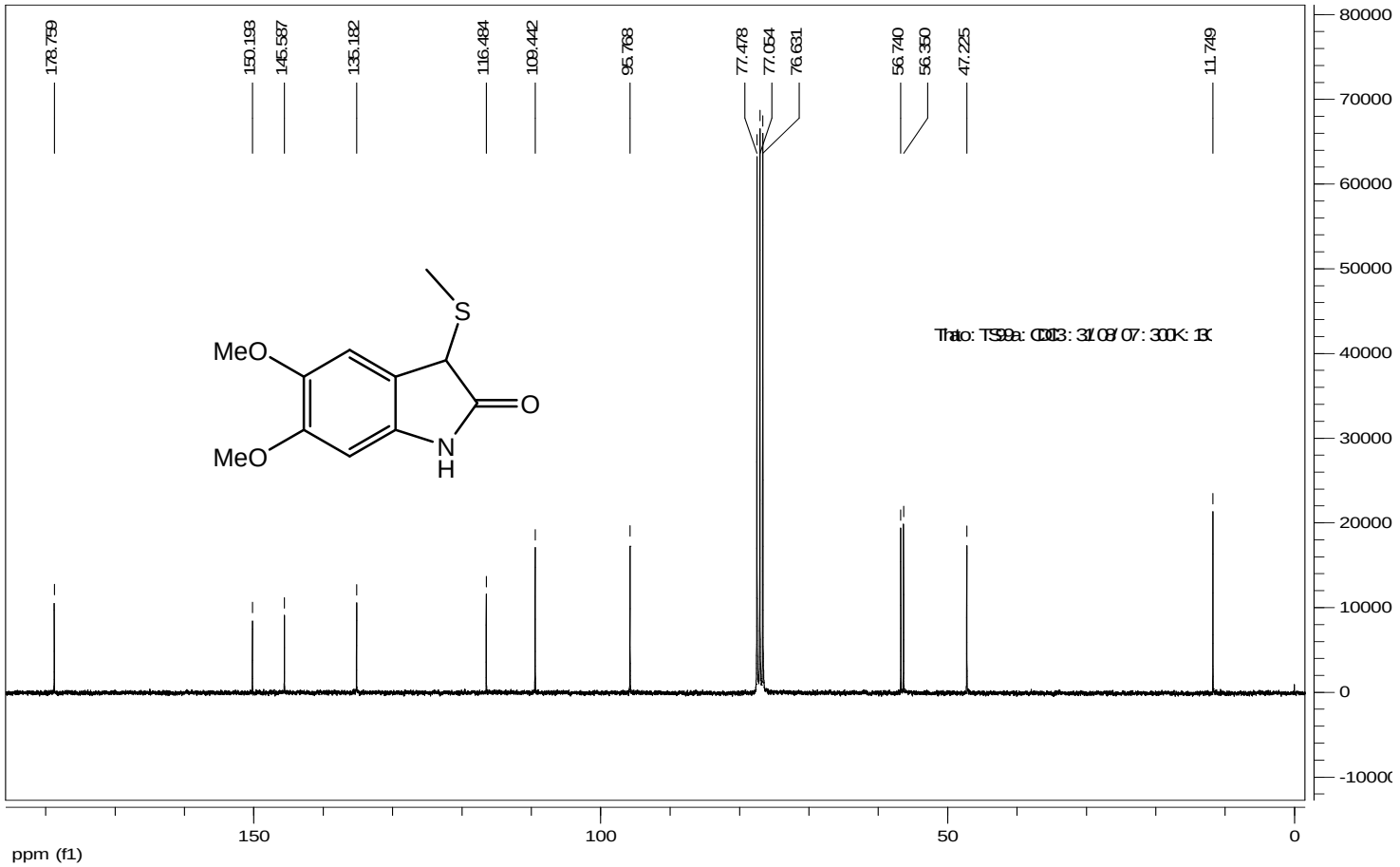

190.721
150.449
143.080
135.793
135.756
135.462
133.411
132.780
130.783
130.408
128.986
128.773
128.068
126.361
126.068
123.625
122.925
120.351
110.588
48.289
N
N
OHC
200
150
100
50
0
ppm (t1)
7000
6000
5000
4000
3000
2000
1000
0

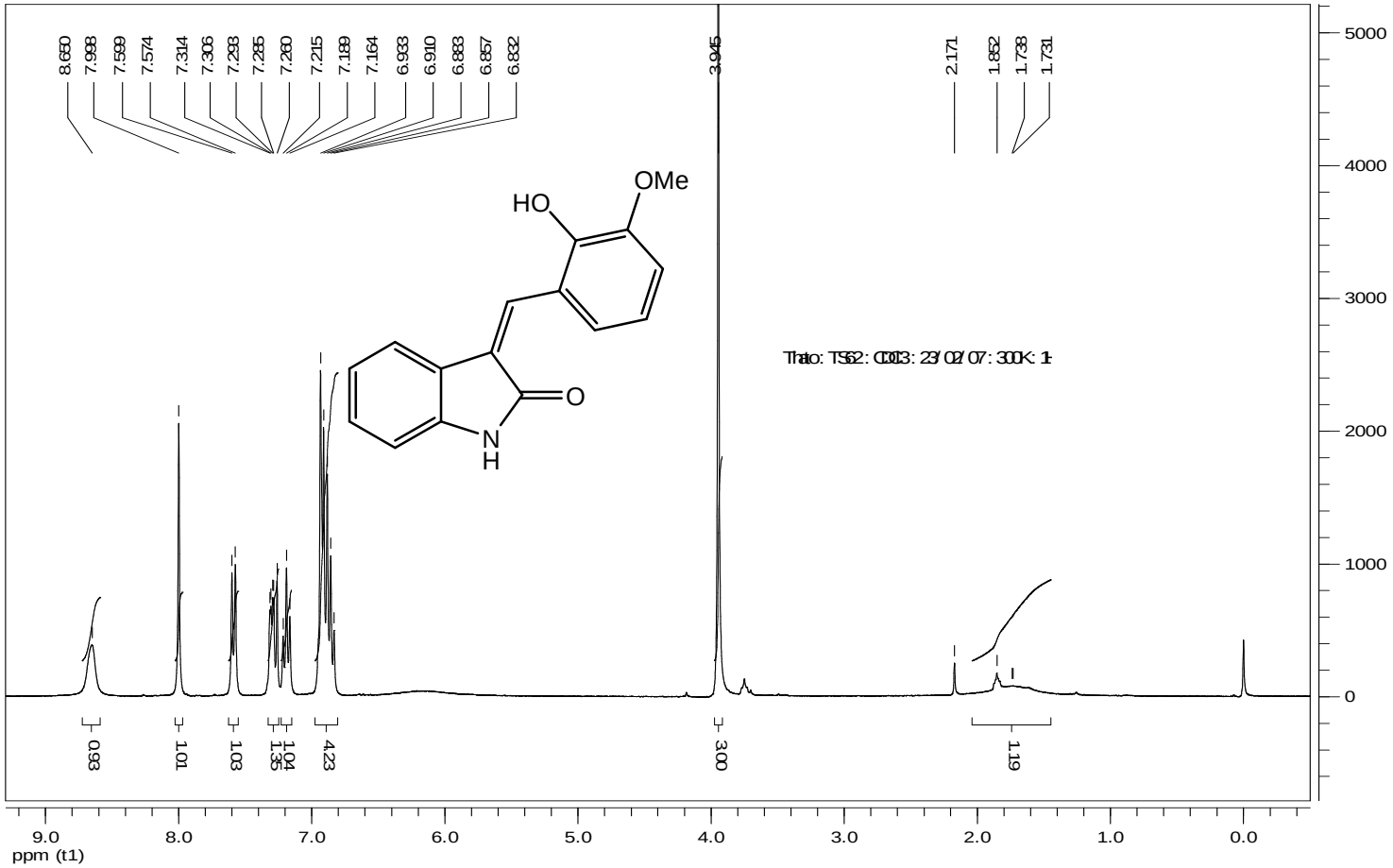

10.590
7.627
7.473
7.447
7.246
7.233
7.224
7.195
7.115
7.085
7.074
7.065
7.035
6.891
6.866
6.841
3.806
3.737
3.349
2.508
OMe
MeO
O
N
H
0.94
1.00
1.14
2.33
2.32
3.37
3.37
10000
5000
0
10.0
5.0
0.0
ppm (t1)

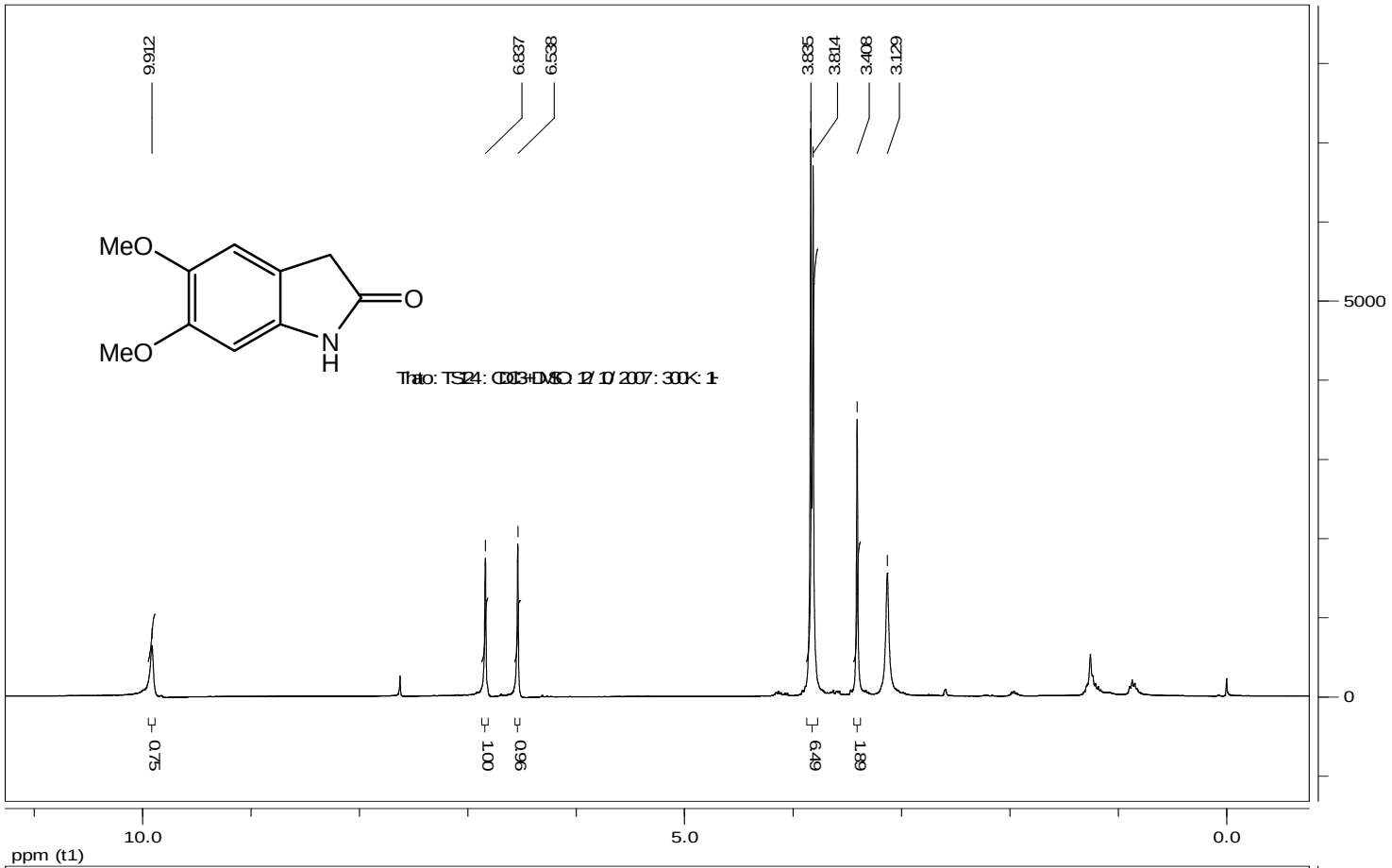

174.204
158.187
157.476
148.448
137.031
135.592
133.124
128.974
128.053
126.677
126.632
122.536
120.006
118.297
115.708
61.568
61.161
45.976
45.700
45.422
45.145
44.868
44.590
44.313
OMe
MeO
O
N
H
5000
4000
3000
2000
1000
0
150
100
50
0
ppm (t1)

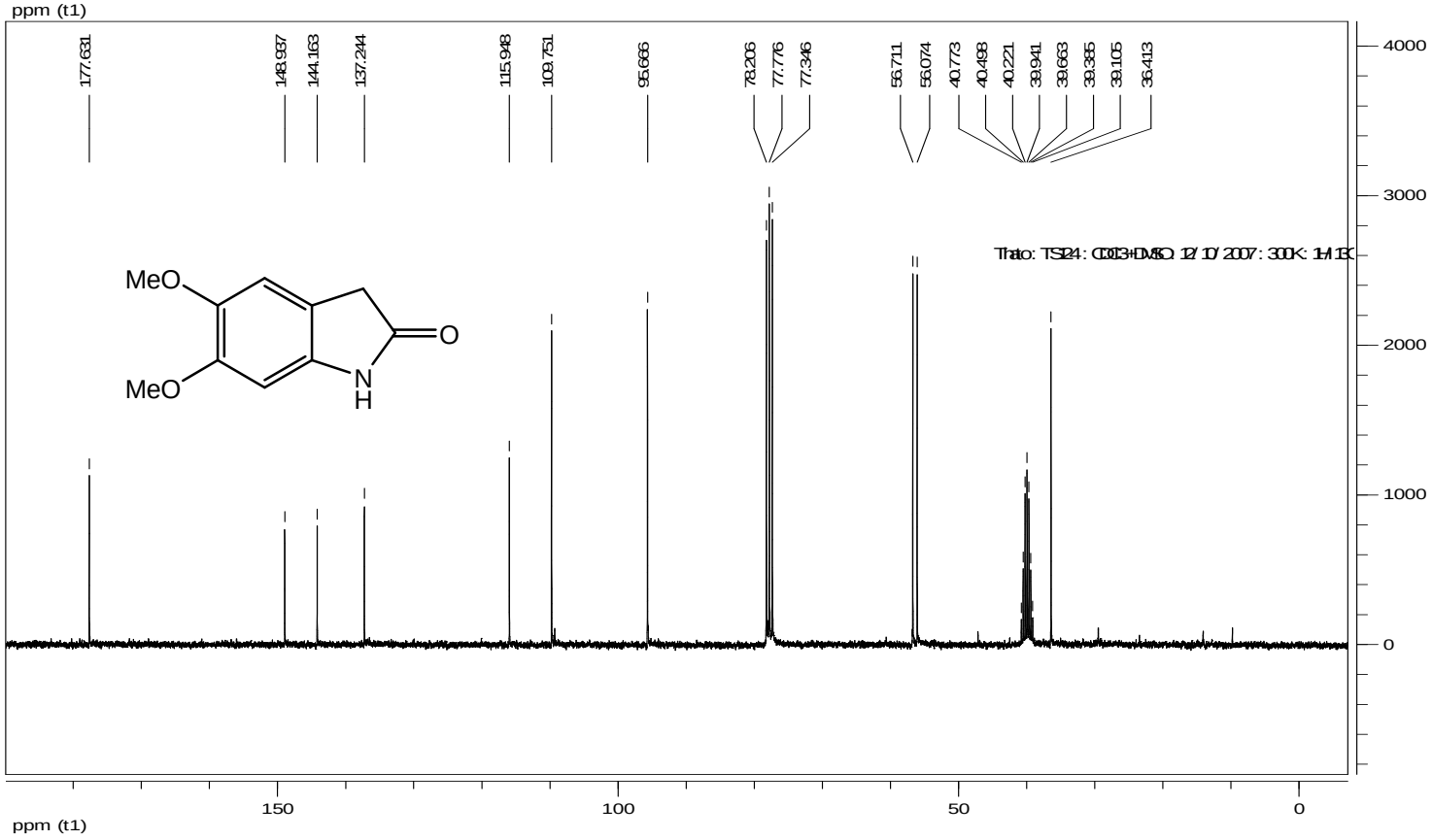

10.396
8.068
7.671
7.645
7.535
7.236
7.209
7.196
7.188
7.174
7.149
6.976
6.950
6.871
6.842
6.815
6.097
3.299
3.273
2.552
2.546
2.540
2.116
0.000
O
O
O
N
H
10000
5000
0
0.63
0.40
1.00
1.02
2.94
1.02
1.93
2.06
10.0
5.0
0.0
ppm (t1)

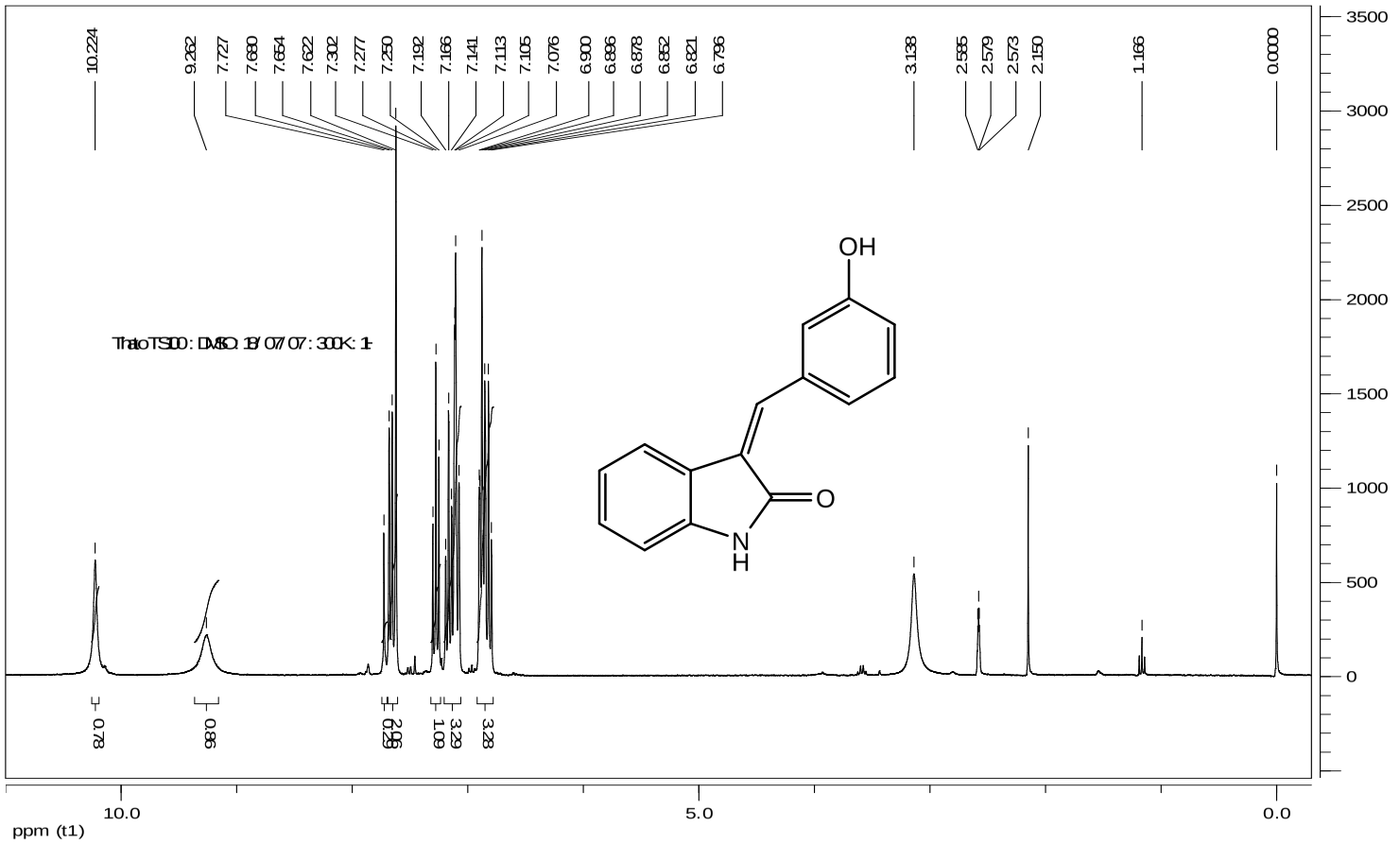

9.301
7.269
6.963
6.581
4.258
3.883
1.974
1.664
0.000
S
MeO
MeO
O
N
H
15000
10000
5000
0
1.00
2.63
1.03
6.50
3.22
10.0
5.0
0.0
ppm (f1)

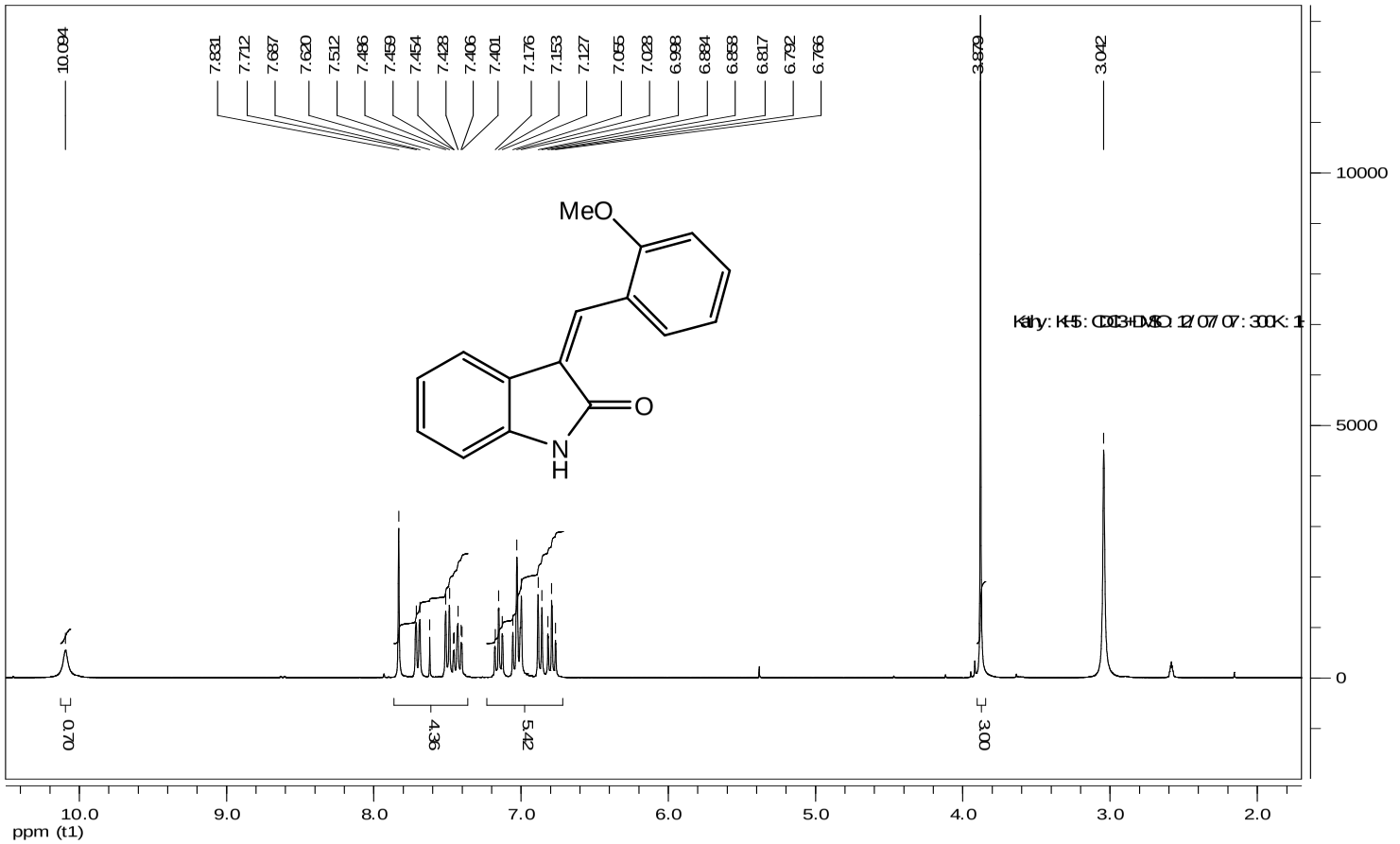

178.759
150.193
145.587
135.182
116.484
109.442
95.768
77.478
77.054
76.631
56.740
56.350
47.225
11.749
MeO
MeO
S
O
N
H
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
150
100
50
0
ppm (f1)

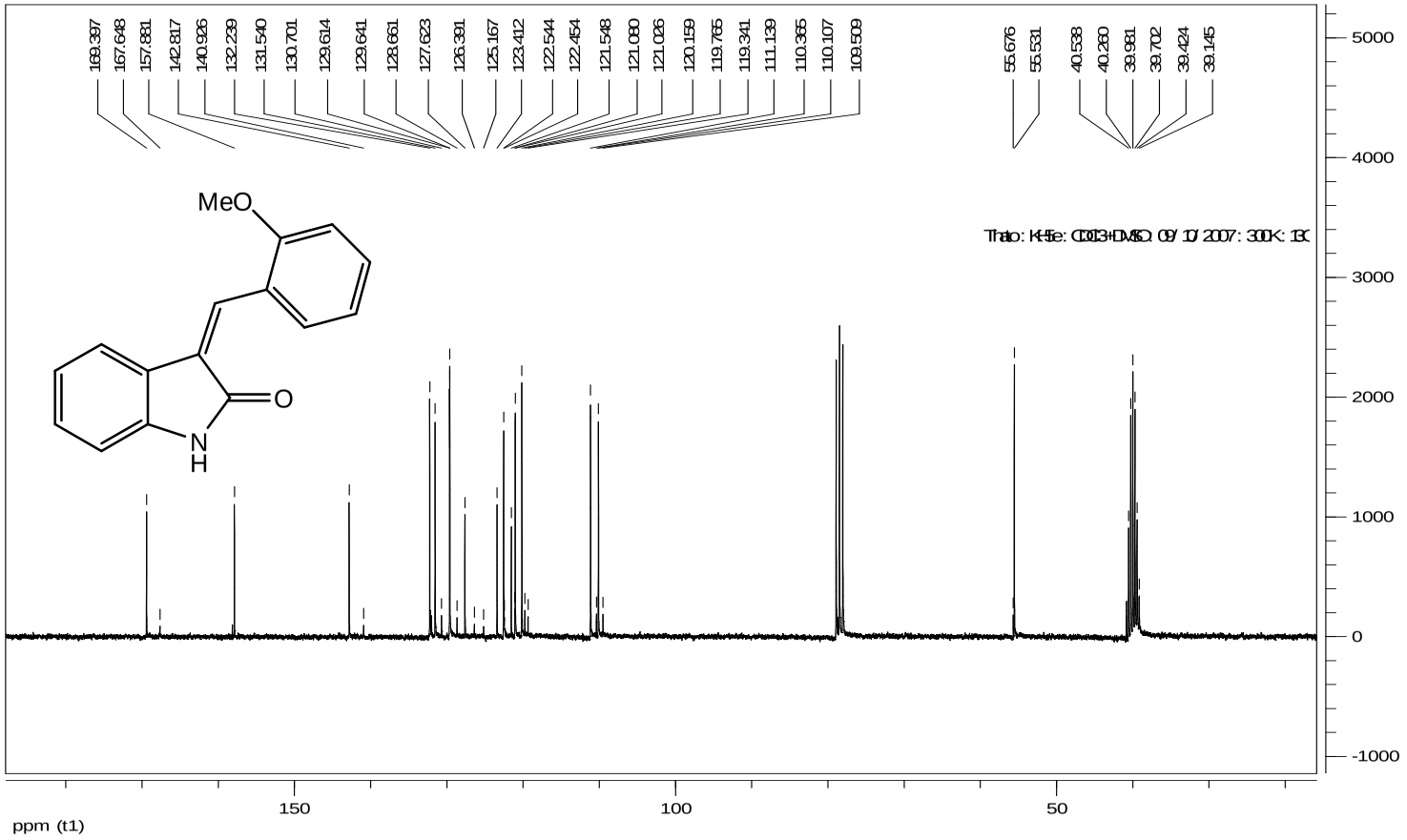

8.650
7.998
7.599
7.574
7.314
7.306
7.293
7.285
7.260
7.215
7.189
7.164
6.933
6.910
6.883
6.857
6.832
3.945
2.171
1.852
1.738
1.731
HO
OMe
O
N
H
0.93
1.01
1.03
1.35
1.04
4.23
3.00
1.19
5000
4000
3000
2000
1000
0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)


9.912
6.837
6.538
3.835
3.814
3.408
3.129
MeO
MeO
O
N
H
0.75
1.00
0.96
6.49
1.89
5000
0
10.0
5.0
0.0
ppm (t1)


177.631
148.937
144.163
137.244
115.948
109.751
95.666
78.206
77.776
77.346
56.711
56.074
40.773
40.488
40.221
39.941
39.663
39.385
39.105
36.413
MeO
MeO
O
N
H
4000
3000
2000
1000
0
150
100
50
0
ppm (t1)


10.224
9.262
7.727
7.680
7.654
7.622
7.302
7.277
7.250
7.192
7.166
7.141
7.113
7.105
7.076
6.900
6.896
6.878
6.852
6.821
6.796
3.138
2.585
2.579
2.573
2.150
1.166
0.000
OH
O
N
H
0.78
0.86
2.00
2.06
1.09
3.29
3.28
10.0
5.0
0.0
ppm (t1)


10.094
7.831
7.712
7.687
7.620
7.512
7.486
7.459
7.454
7.428
7.406
7.401
7.176
7.153
7.127
7.055
7.028
6.998
6.884
6.858
6.817
6.792
6.766
3.879
3.042
MeO
O
N
H
0.70
4.36
5.42
3.00
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (t1)


169.397
167.648
157.881
142.817
140.926
132.239
131.540
130.701
129.614
129.641
128.661
127.623
126.391
125.167
123.412
122.544
122.454
121.548
121.080
121.026
120.159
119.765
119.341
111.139
110.365
110.107
109.509
55.676
55.531
40.538
40.280
39.981
39.702
39.424
39.145
MeO
O
N
H
5000
4000
3000
2000
1000
0
-1000
150
100
50
ppm (t1)