Synthetic studies on natural and non-natural compounds

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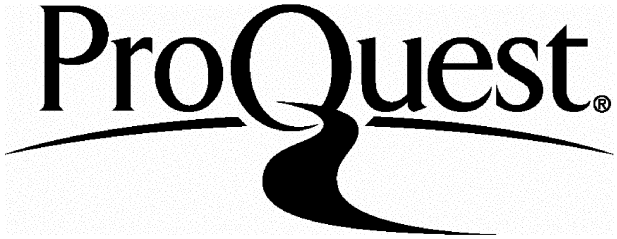

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ABSTRACT

PART I: THE STUDY OF CYCLOBUTYL SMALL RING COMPOUNDS

This part of the thesis describes a variety of routes that were attempted in the quest for a polymer containing cyclobutane rings which might have unusual properties as a conductor. These methods include the utilisation of the McMurry reaction, the Wittig reaction and the Barton-Kellog reaction. An attempt at zinc carbenoid carbonyl coupling was also performed. 3-*t*-Butoxycyclobutenone was successfully prepared as the potential "monomer" but all methods employed to obtain the polymeric material were unsuccessful.

PART II: MAPPING THE MELATONIN RECEPTOR

Melatonin, *N*-acetyl-5-methoxytryptamine, is the principal neurohormone secreted by the pineal gland. The indoleamine plays an important role in circadian and seasonal rhythm control in many animals. To investigate the mode of action of melatonin at cellular level, a number of indole derivatives have been synthesised to map the melatonin receptor. We continued this study by the preparation of compounds substituted at C-6 and C-7 of the indole nucleus. The compounds were tested for binding affinity on mt_1 and MT_2 cell lines and for potency on *Xenopus* melanophores. These compounds provide a further understanding of the nature and shape of the receptor pocket around these regions.

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PART I: THE STUDY OF CYCLOBUTYL SMALL RING COMPOUNDS

INTRODUCTION

I. Molecular wires

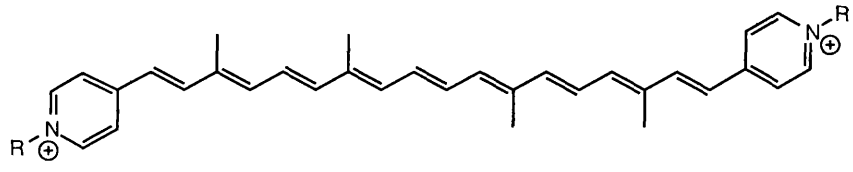
Besides having the obvious function of establishing electrical connections between components, wires in circuit boards also prevent short circuits which would otherwise damage the electronic device. Molecular electronics likewise requires not only molecular-scale switches, transistors and logic gates, but also nanoscale interconnections that provide essential electronic communication between components while blocking undesirable interactions. To create a functional molecular wire, one has to elaborate a molecule that would allow electron flow to take place at the molecular level, and to integrate this molecule into an organised support. The concept of a molecular wire became a reality nearly 25 years ago with the discovery of metallic conductivity in an organic polymer.[1] Since then, many approaches to the development of molecular wires have been undertaken.

II. Conjugated systems

The most popular strategy in the quest for molecular wires is to use polyunsaturated systems as a bridging unit along which charge transfer can take place by modification of bond alternation.

II.A. Polyalkenes

In 1986, Lehn and his colleagues[2] initiated a program of research into long, conjugated polyolefinic chains bearing pyridinium end groups, named caroviologens, which combined the structural features of carotenoids with the redox properties of methylviologen.



1a R = Me
1b R = $(CH_2)_3SO_3^-$

Such compounds (eg. **1a**) were synthesised and shown to be incorporated in a transmembrane fashion into dihexadecyl phosphate (DHDP) vesicles, although electron-transfer experiments of this system failed to yield positive results.

Zwitterionic caroviologen **1b** was then developed and was shown to conduct electrons in solution when it was embedded in a vesicle membrane. Electron-transfer from the external electron-rich solution (containing the reductant sodium dithionite) to the internal electron-poor solution (containing the oxidising agent potassium ferricyanide) is normally slow because of the insulating lipid-based membrane. Incorporating the wire into the membrane, so that the charged, redox-active termini protruded into the aqueous solution on either side resulted in a substantial increase in the rate of electron transfer across the membrane (Figure 1).[3]

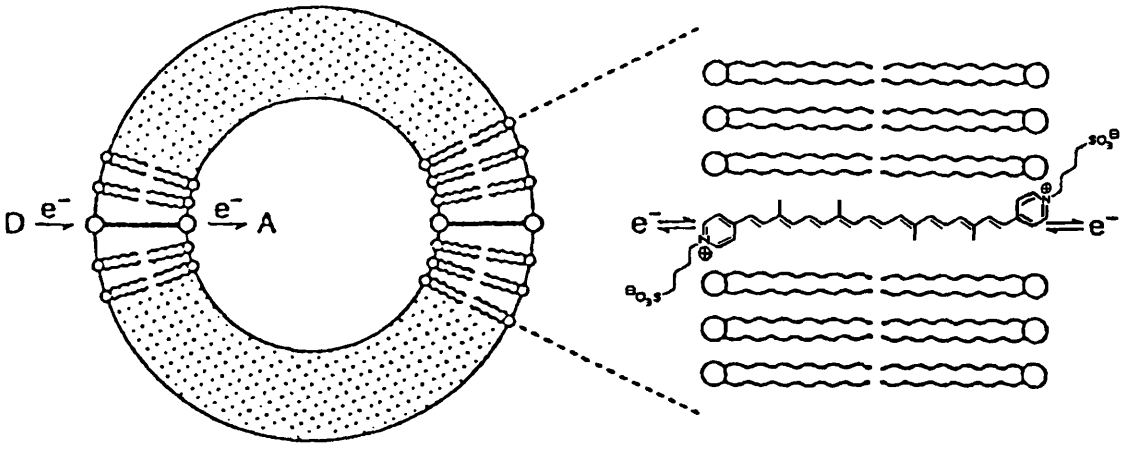
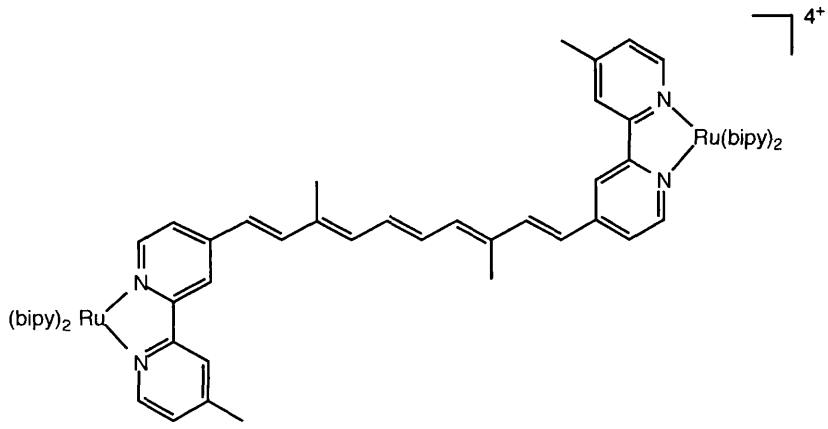


Figure 1. Schematic representation of electron conduction through the zwitterionic, caroviologen molecular wire **2** incorporated in a phospholipid vesicle membrane, from an outside reducing agent D (sodium dithionite) to an internal electron acceptor A (potassium ferricyanide). Taken from Kugimiya, S.-I.; Lazrak, T.; Blanchard, Desce, M.; Lehn, J.-M. J. Chem. Soc., Chem. Commun. 1991, **17**, 1179.[3]

Carotenoid polyenes have long been known to be important in photosynthesis. Photosynthetic reaction centres are molecular-scale photovoltaics that carry out charge separation across lipid bilayer membranes. Polyacetylene, an extended carotenoid polyene, has been investigated as an organic conductor, and carotenoids have been used as electron transfer agents both in solution and in synthetic membranes.[4]

The research of Lehn et al.[2,5] has also focused on modification of the terminal end groups of polyacetylene and optimisation of the polyene linker. These metallocarotenate-type molecular wires, such as **2**, combine the rich electrochemical and photochemical activities of metal complexes with the long-range conjugation properties of carotenoid chains, providing long range electron delocalisation. They thus represent switchable molecular wires responding to external stimuli. The polyene linker in **2** also acts as a spacer to prevent the ruthenium complexes from

coming into van der Waals contact via folding motions. The induced electronic interaction would be the molecular equivalent of a short circuit.



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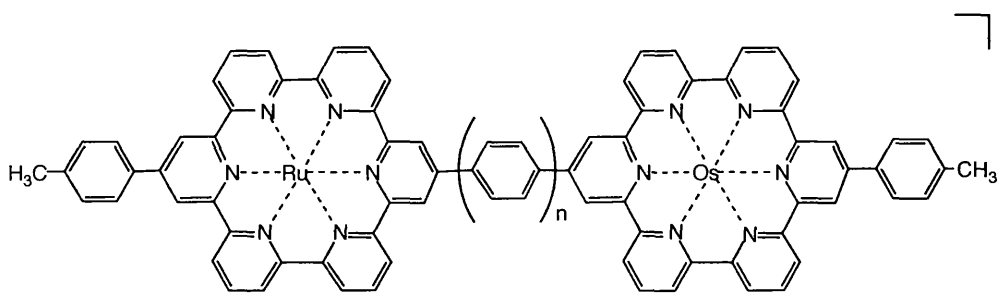
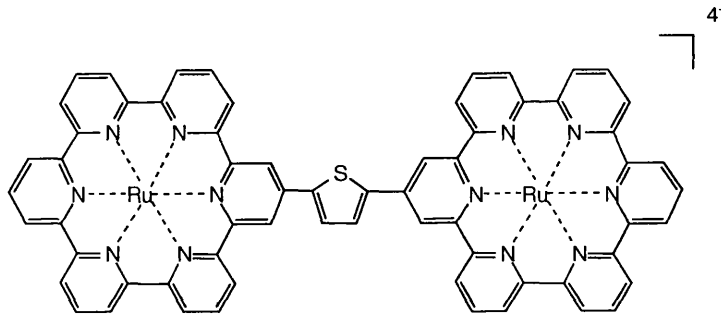
The mechanical role of the polyene is aided by its conjugated backbone. Due to overlap with the adjacent π-electron system, the formally single bonds in the chain exhibit some double-bond character. Rotation about these bonds involves orthogonal orientations in which the stabilising overlap is lost, and rotation is therefore restricted. In addition, planar s-cis arrangements about the single bonds are sterically unfavourable.

A similar approach to that of Lehn has been adopted by Barigelletti et al.[6] Using mono- and biphenylene as a spacer, they detected the electronic interaction between Ru(II)-Os(II) complexes in **3**, and monitored the electron transfer by luminescence spectroscopy. Upon excitation, the potentially luminescent ruthenium moiety of **3** showed no emission; instead emission was observed from the Os complex, indicating that the excitation energy absorbed by the Ru-based unit was transferred to the connected Os-based unit. Barigelletti and co-workers[7] later synthesised compounds **4** and **5** and found that using 2,5-thiophenediyl as a spacer group to connect the {Ru(terpy)$_2$} chromophores instead of the phenylenes significantly improved the luminescence properties.



3; n = 1, 2



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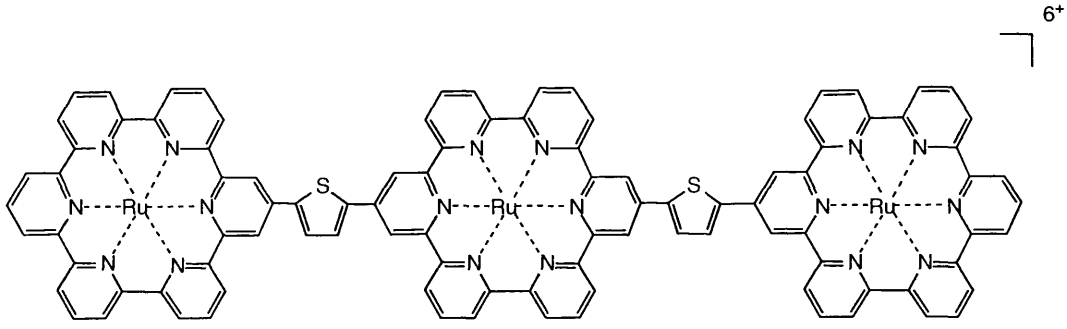

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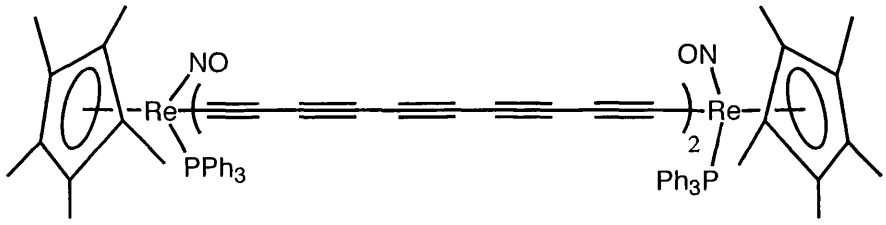
II.B. Polyalkynes

Being the only easily preparable all-carbon building block, the relative rigidity and linearity of the alkyne group is a further advantage in the synthesis of rigid and uniformly shaped structures. With the encouraging discovery of fullerenes, the research of carbon allotropes and carbon rich compounds with unusual structural, electronic and optical properties has undergone a resurgence over the past few years. Diederich and co-workers[8,9] have focused on the synthesis of infinite linear polyalkyne chains of pure carbon and three-dimensional carbon scaffolds. As a consequence of the presence of non-reactive terminal substituents, it has been shown that these chains can be stabilised against further reaction. Several research teams have used transition metal complexes,[10-12] while others have employed triethylsilyl,[6] trifluoromethyl[13] and nitrile[13] functionalities as end-capping groups.

By the use of Eglinton coupling,[14] Gladysz et al.[12] prepared the decayne **6**, with a "naked" C_{20} chain, the longest sp carbon chain tethered between two metals prepared to date.



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To make contact with these "wires", any desired substituents can be attached to the ends of the rods by changing the end-capping reagent.

III. Mixed valence species (intervalence charge transfer)

Macrocyclic transition metal complexes linked by linear organic bridging ligands exhibit semiconductor properties (Figure 2).

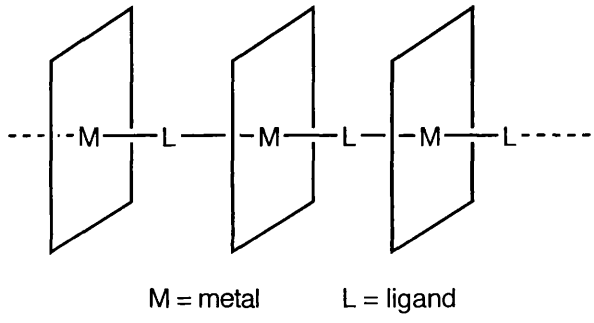


Figure 2. Bridged macrocyclic metal complexes

In very strongly interacting complexes, an odd electron is delocalised between both metals and this electron may be promoted from one orbital delocalised over the whole metal-bridge-metal system to a higher-energy orbital which is also delocalised, i.e. a π-π^* transition. However, in complexes where the interaction between the metals is weaker, the oxidation states of the metal alternate resulting in a mixed-valence state, and there is then the possibility of an electron transfer from the metal in the lower oxidation state to the one in the higher oxidation state, causing intervalence charge transfer. The electron transfer is therefore directional, from the lower to the higher oxidation state of the complex via the bridging ligand. An example of mixed valence linear chain compounds is Wolffram's red salt which has a structure consisting of alternate octahedral $[Pt(EtNH_2)_4Cl_2]^{2+}$ and square-planar $[Pt(EtNH_2)_4]^{2+}$ ions linked by chloride (Cl) bridges (Figure 3). It shows high electrical conductivity along the direction of the -Cl-Pt^{II}-Cl-Pt^{IV} chain.[15]

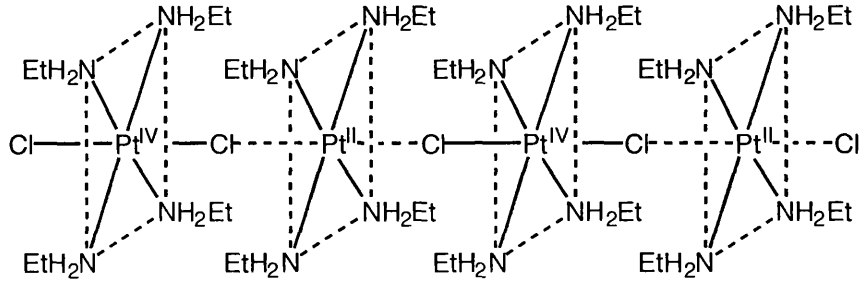


Figure 3. The coordination of platinum in Wolffram's red salt, $Pt(EtNH_2)_4Cl_3 \cdot 2H_2O$, showing alternating Pt^{II} and Pt^{IV} linked by Cl bridges. Four remaining Cl^- ions being within the lattice.

Hanack et al.[16] examined the conductivity of mixed valence polymers with M = Fe, Co in oxidation states +II, +III, and with L = cyanide, tetrazine, pyrazine [refer to Figure 2]. The ligand valence frequencies and electrical conductivities of the mixed valence compounds were compared to the values obtained from the corresponding monomers. It was found that the formation of the ligand bridge led to an increase of the ligand valence frequency. This can be explained by the decrease of electron density of the antibonding p-orbital (due to coordination to a second metal atom) which increases the bond order, and hence the valence frequency.

IV. Photosynthetic molecules (photoinduced electron transfer)

Photochemically-induced electron transfer supports excited-energy transfer rather than electron-transfer. Firstly, there is an absorption of a photon by a chromophore, which then enters a relatively long-lived electronically excited state, providing a natural means of inputting a signal to a molecular wire. This is followed b y long distance electron-transfer either to or from another group, thereby quenching the initial excited state. Since the chromophore and quencher are often linked by a bridging ligand, the electron transfer between them depends on the properties of the bridge.[17] Figure 4 shows a recent example of "photonic molecular wires" containing multiple porphyrin units which mimic the natural complexes. At one end of the wire, the boron-dipyrromethene dye absorbs blue-green light and the resulting excited-state energy is transmitted through a string of zinc porphyrins to a fluorescent metal-free porphyrin, which emits a photon of red light.

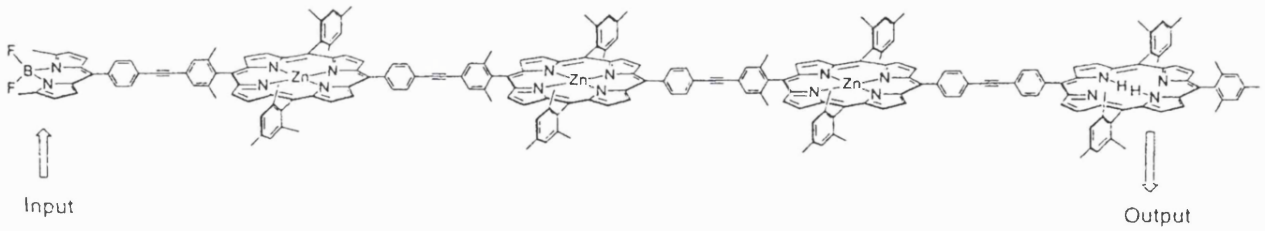


Figure 4.[17] An example of a molecular photonic wire, taken from Wagner, R.W.; Lindsey, J.S. J. Am. Chem. Soc. 1994, **116**, 9759.

In nature, photosynthetic light-harvesting complexes containing chlorophyll consist of hundreds of pigments in a solid array, absorb sunlight and quickly convey it to the reaction centres, where it is converted into chemical energy.

V. Biomolecular devices

The redox centres of most enzymes and proteins are located far enough from the outermost surface to be electrically inaccessible and as a result most centres do not exchange electrons with electrodes on which they are adsorbed. Electrical communication of the redox-site of enzymes and their environment is controlled by the participation of protein-associated cofactors, eg. flavin adenine dinucleotide (FAD) or pyrroloquinoline quinone (PQQ). Considering the difficulties in direct electron transfer, several strategies have been proposed to overcome the problem. These include the design of electron relay systems,[18] or redox mediators,[19] entrapment of enzymes in conductive polymers,[20,21] immobilisation of proteins in polymer arrays tethered by redox groups,[22-24] and reconstitution of proteins on chemically modified electrodes.[25-27]

Heller[18] proposed the incorporation of ferrocene/ferricinium carboxylate electron relays for the electrical communication between glucose oxidase (GOD), an enzyme which catalyses the transfer of electrons from glucose to oxygen, and platinum electrode (Figure 5). This reduced the electron-transfer distances between the FAD/$FADH_2$ redox centres of the enzyme involved in the process.

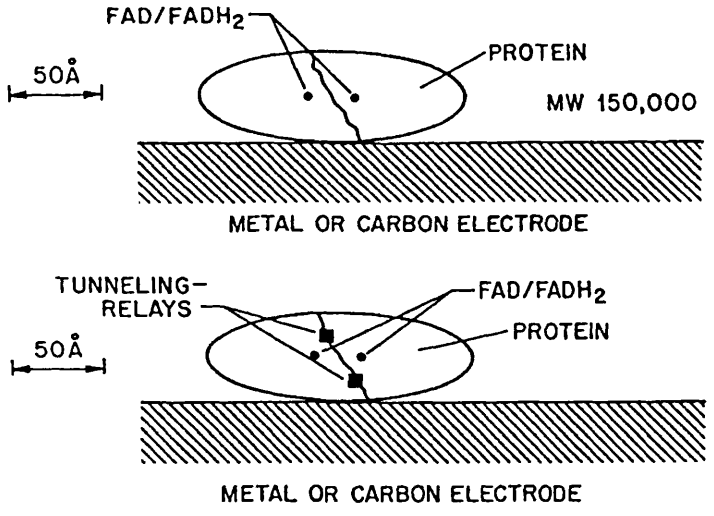


Figure 5.[18] Top: When a native redox enzyme (eg. GOD) is adsorbed on an electrode, the electron-transfer distances are excessive for electrical communication between the redox centres and the electrode. Bottom: Flow of electrons through a relay from a redox centre of an enzyme (GOD) to an electrode. A current is observed when the substrate (glucose) transfers a pair of electrons to an FAD centre of the enzyme that, in turn, transfers these either to a relay or to a molecular wire, which then transfers the electrons to the electrode. Taken from Heller, A. Acc. Chem. Res. 1990, **23**, 5, 134.

By covalently binding of ferrocene derivatives to sugar residues on the outer surface of GOD, Schuhmann et al.[19] modified the enzyme via spacer chains of different lengths and showed that the electrooxidation of the enzyme is rapid only when the spacer chain is long enough for the ferrocene to penetrate the enzyme sufficiently to approach the redox centre. Figure 6 shows the structure of their modified GOD.

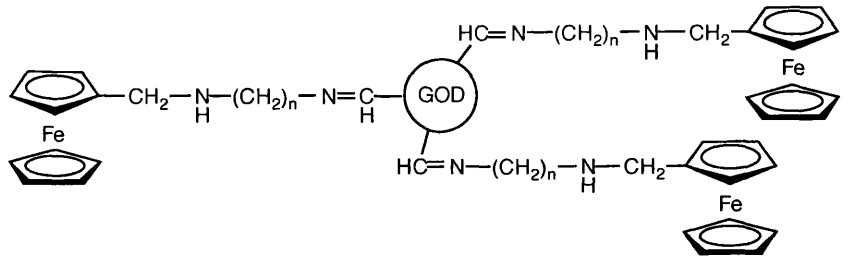
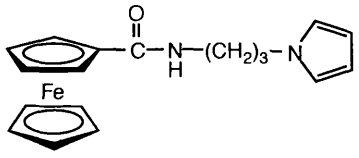


Figure 6. Structure of the glucose oxidase modified by peripherally bound ferrocenes

The entrapment of enzyme in polypyrrole films provides a simple method of enzyme immobilisation, which promotes proximity between the enzyme active site and the conducting surface of the electrode. It also provides a way of localising biologically active molecules to defined areas on electrodes. Foulds and Lowe[22] reported the entrapment of GOD in ferrocene-containing polypyrrole films made from

copolymerisation between pyrrole and **7** or **8**. This modified GOD electrode was found to rapidly oxidised glucose, eliminating hydrogen peroxide in the process.



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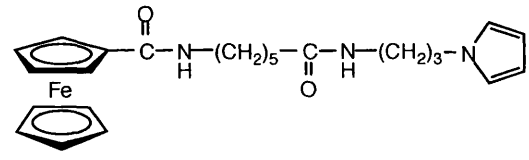

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A similar approach of enzyme entrapment was performed by Aizawa and co-workers[20] who synthesised an electro-conductive enzyme membrane at a platinum electrode surface by electrochemical polymerisation of pyrrole in the presence of GOD. The entrapped GOD showed enzyme activity and reversible electron transfer between the enzyme molecule and the electrode. Figure 7 shows the enzyme activity of the GOD/pyrrole membrane at various concentrations in electrolysis.

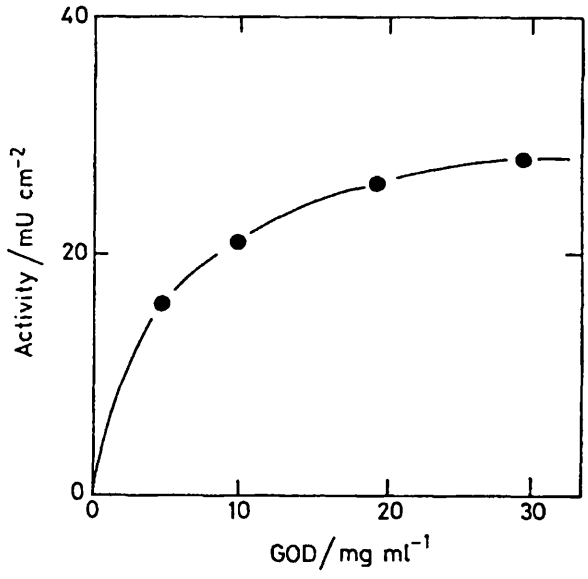


Figure 7.[20] Enzyme activities of GOD/polypyrrole membranes synthesised at different GOD concentrations in electrolysis, taken from Yabuki, S.-I.; Shinohara, H.; Aizawa, M. J. Chem. Soc. Chem. Comm. 1989, 945.

Recently, novel methods to transform redox enzymes into "electroenzyme" biocatalysts were reported by Willner and co-workers.[26,28] The conversion to "electroenzymes" was achieved by the reconstitution of apo-glucose and apo-D-amino acid oxidase with a relay-modified FAD co-factor[28] as well as by the reconstitution of apo-flavo-enzyme onto relay-modified-FAD monolayers associated with electrode surfaces (Figure 8).[26]

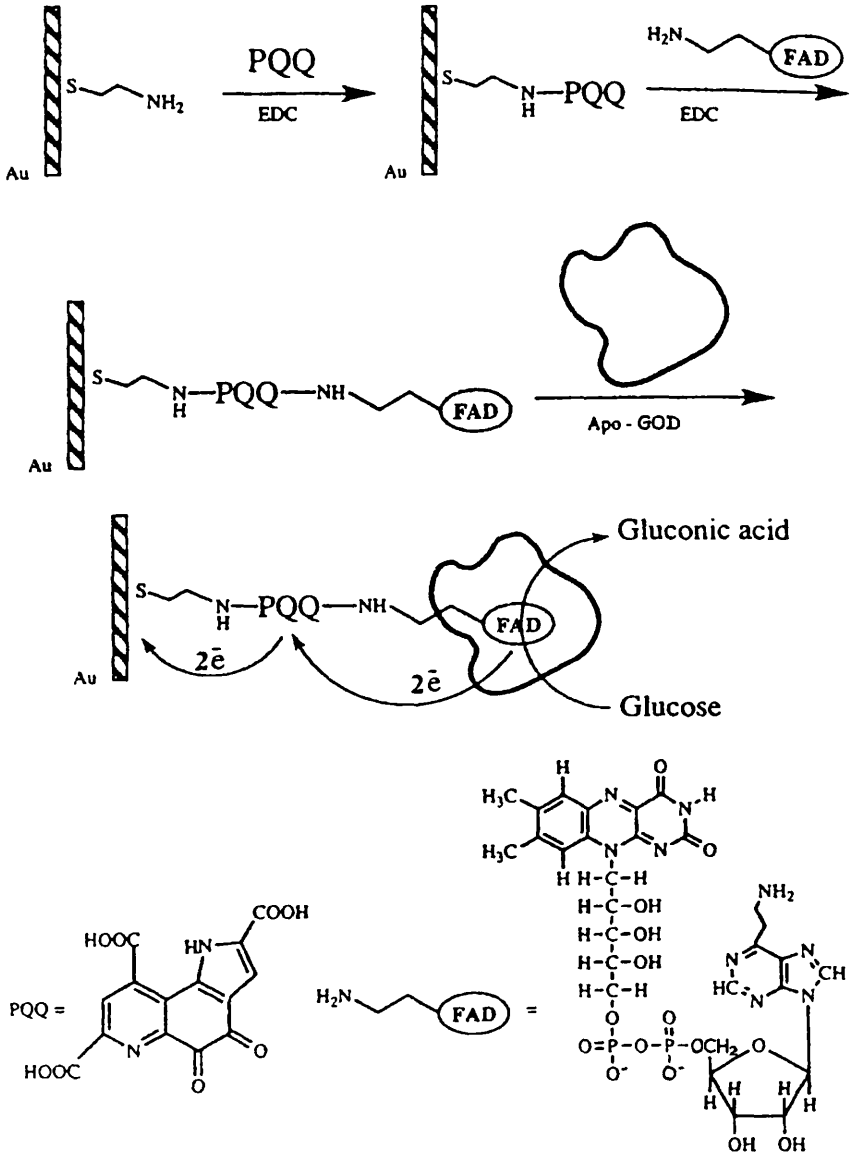


Figure 8.[26] Reconstitution of GOD onto a PQQ/FAD monolayer Au-electrode and direct electrocatalysed oxidation of glucose by the modified electrode, taken from Willner, I.; Heleg-Shabtai, V.; Blonder, R.; Katz, E.; Tao, G. J. Am. Chem. Soc. 1996, **118**, 10321.

An impressive achievement in this field by Moore and co-workers[29] involved the construction of a biomimetic "proton pump", which is driven by vectorial photoinduced electron transfer in a carotene-porphyrin-naphthoquinone molecular triad (C-P-Q),[30] and the incorporation of the proton pump and ATP synthase into liposomes (Figure 9).

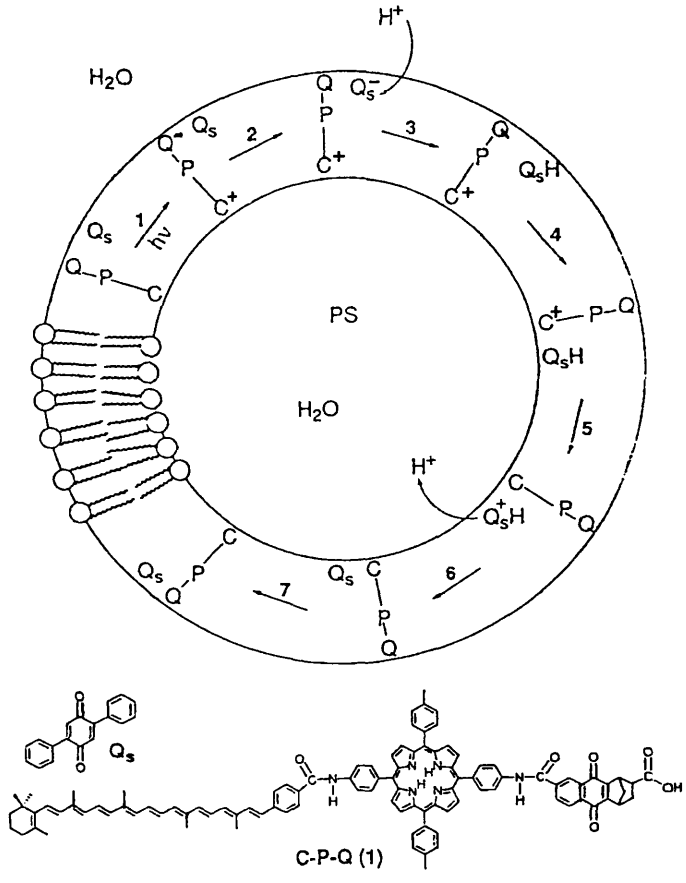
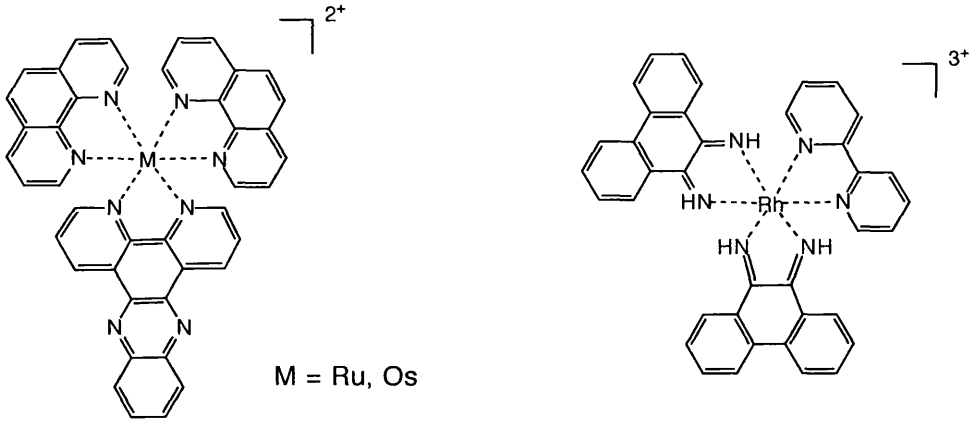


Figure 9.[31] Photoelectrochemical cycle of the "proton pump" generating a pH gradient across the liposomal bilayer with the structures of the essential components (bottom), taken from Piotrowiak, P. Chem. Soc. Rev. 1999, **28**, 143.

Excitation by visible light leads to the formation of the species C^+-P-Q^- (Step 1). The intramolecular redox potential represented by the carotenoid cation and the naphthoquinone anion is controlled by the proton translocation via a lipophilic quinone (Q_S) which alternates between reduced and oxidised states. Reduction of Q_S occurs by electron transfer from the naphthoquinone anion of the C^+-P-Q^- near the external bilayer-water interface to form Q_S^- (Step 2). The species Q_S^- then accepts a proton from the external aqueous solution to form the semiquinone HQ_S which diffuses through the membrane and delivers the proton and electron to the site

of potential (C^+-P-Q) located near the inner membrane surface (Steps 3 and 4). Oxidation of HQ_S to Q_SH^+ near the internal aqueous interface causes proton ejection to the intraliposomal volume and the imbalance of electrochemical potential of protons across the membrane (Steps 5 and 6). On sufficient imbalance of electrochemical potential, the production of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (P_i) begins. Random diffusion of the regenerated Q_S completes the cycle (Step 7). This is the first completed biomimetic system, which effectively couples electrical potential derived from photoinduced electron transfer to the chemical potential associated with the ADP-ATP conversion.

It has been suggested that the π-π interaction between the stacked base-pairs of double-stranded DNA could provide a pathway for rapid, one dimensional charge migration.[32-38] Barton and co-workers[39,40] designed a family of metal complexes of types $[M(L)_2(dppz)_2]^{2+}$ and $[Rh(phi)_2(L)]^{3+}$ which binds to DNA via intercalative stacking within the helix, to probe the DNA-mediating electron transfer (Figure 10). The stacked aromatic heterocycles in the interior of DNA were shown to efficiently promote electron transfer reactions over 40 Å. The charge transfer between donors and acceptors was demonstrated to be independent upon the molecular distances but is sensitive to the degree of overlapping of the DNA-binding agent with the π-stack.[41,42] If the double helix really functions as a "molecular wire", this will open up the possibility of using DNA as a biological sensor.



$[Ru(phen)_2(dppz)_2]^{2+}$ $[Rh(phi)_2(bpy)]^3$

Figure 10.[43] Structures of two donor and acceptor complexes, $[Ru(phen)_2(dppz)_2]^{2+}$ and $[Rh(phi)_2(bpy)]^{3+}$. The Ru^{II} complex binds DNA by preferential stacking of the dppz ligand between adjacent base pairs. In the metal-ligand charge transfer excited state, the complex directs an electron into the DNA π-stack. The phi complex of Rh^{III} binds to the DNA by intercalation. The phi ligand serves to anchor the complex in the major groove of DNA.

VI. Cyclic systems

In the quest for highly conducting charge transfer complexes, Hünig[44] proposed a general structural principle for reversible, two-step electron transfer organic systems (Figure 11).

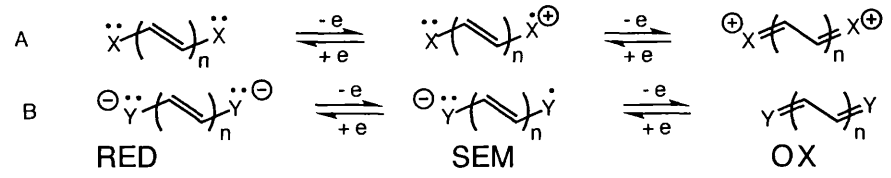


Figure 11.[44] General structures for reversible two-step redox systems, adapted from Hünig, S. Pure & Appl. Chem. 1990, 62, 3, 395.

Two general classes of compounds could be deduced from Figure 11: (i) Wurster-type systems, which are aromatic in the reduced form; and (ii) Weitz-type systems, which are aromatic in the oxidised form.

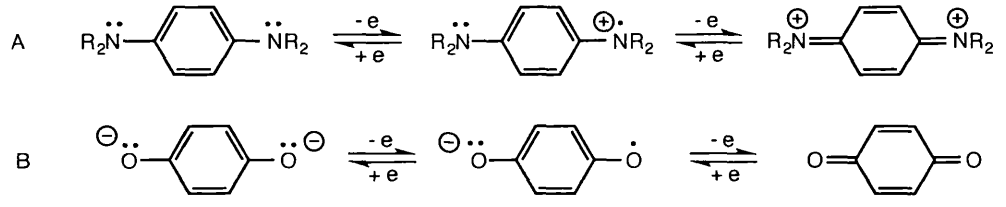


Figure 12.[44] Examples of Wurster-type redox systems.

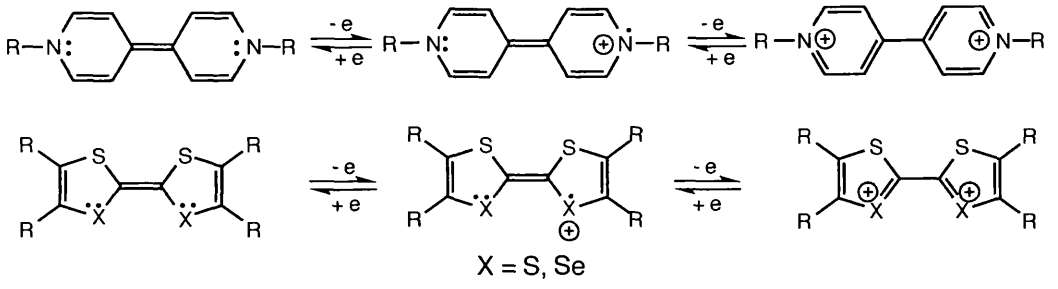
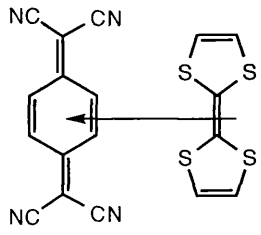


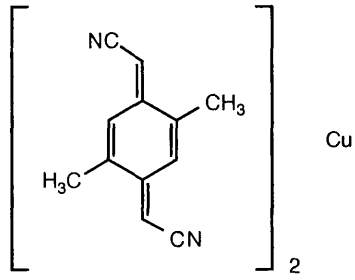
Figure 13.[44] Examples of Weitz-type redox system.

Since the discovery of a highly conducting complex **9**[45] formed by tetracyano-p-quinodimethane (TCNQ), as an electron acceptor, and tetrathiafulvalene (TTF), as an electron donor, which showed high metallic conductivity, a number of different charge transfer complexes have been prepared in the search for better conducting properties. These include the N,N'-dicyanoquinonediimine (DCNQI)-TTF complex whose copper salt derivative **10** displays conductivity as high as 1000 S cm^{-1} at room temperature and up to 500 000 S cm^{-1} at approximately 10 K.[46] While a good number of electron-accepting p-quinodimethanes and analogues have been synthesised, relatively few electron-donating p-quinodimethanes have been known.[47] Recent research[47] showed that

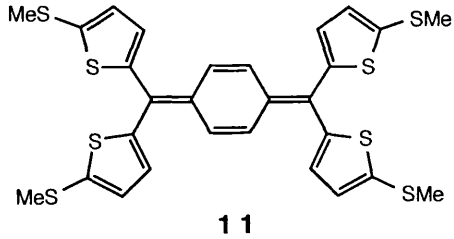
the electron rich tetra(2-thienyl)-p-quinodimethanes (TTQDM) display good amphoteric redox properties, in particular the tetrakis(methylthio) derivative **11** which is a good electron donor to form charge transfer complexes of considerable electrical conductivity. When TTQDM was complexed with molecular iodine and TCNQ, the observed conductivities were 3.5×10^{-3} and 4.8×10^{-4} S cm^{-1}, respectively.



TCNQ-TTF **(9)**
$\sigma = 1000$ S cm^{-1}



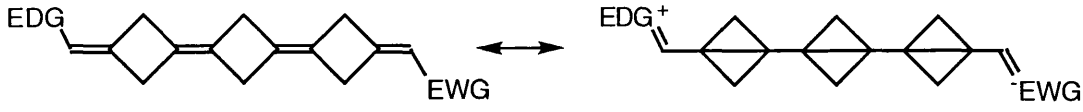
$[2,5\text{-}Me_2DCNQI]_2Cu$ **(10)**
$\sigma = 1000$ S cm^{-1} at rt.



11

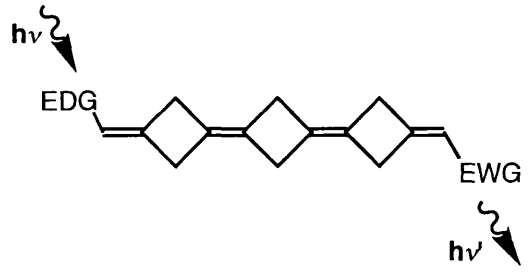
VII. Polymeric small ring compounds

One of the basic yet fundamental components for signal transmission at molecular level is a connector or junction which allows electron flow to occur between different parts of the system. We have been interested in polymers, such as **12**, in which signal transfer would result via a second molecular state involving π to σ bond conversion in a "skipped" polyene, as illustrated below. These type of systems may have the potential to serve as molecular wires or switches.



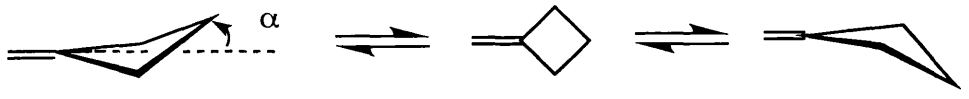
12

By introducing different electron withdrawing groups (EWG) and electron donating groups (EDG) to the two ends of the molecule, colours produced could be "tuned" with the aid of a light source.

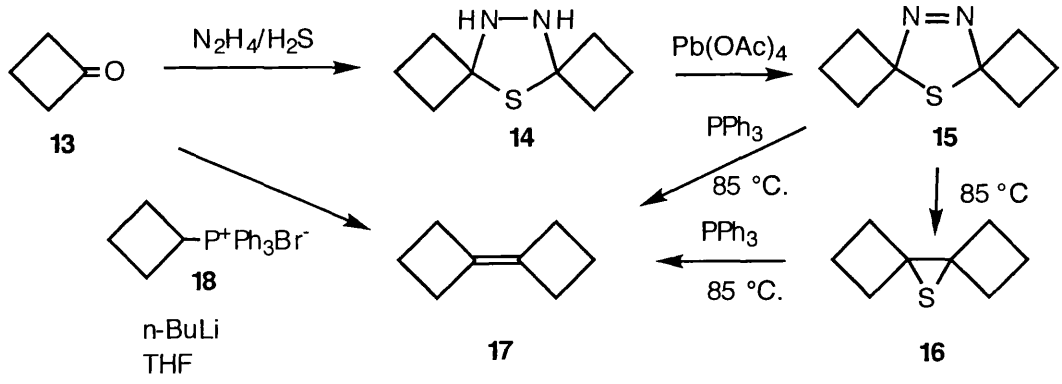


1 2

In cyclobutane, the 1,3-carbons are at a reasonable distance for "bond formation" (ca. 218 pm). Having a puckered geometry, it has a dihedral angle (α) of 30°.[48,49] For methylenecyclobutane, the energy barrier to the transition from the puckered to the planar conformation is -0.8 kJ mol^{-1} lower than for unsubstituted cyclobutane by a factor of 4 to 5. The distinction is due to the smaller changes in both the angular strain and the torsional interactions which accompany the transition of methylenecyclobutane from the non-planar to the planar configuration. An even lower energy barrier would be expected for 1,3-diethylene cyclobutane, and thus the proposed system **12**.

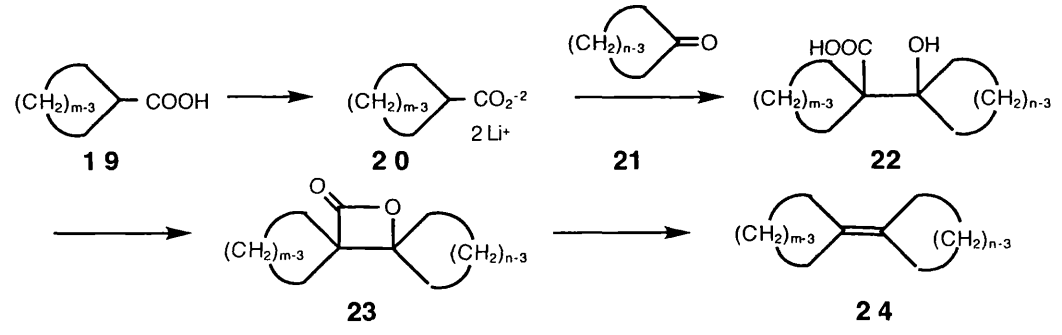


The electron transfer properties of polymeric small ring compounds are of particular interest due to the possible spatial interactions between the double bonds when they are in close proximity within the molecule.[50] To this end, Garratt and co-workers[51-53] devised two synthetic routes to bicyclobutylidene **(17)** (Scheme 1). Reaction of cyclobutanone **(13)** with hydrazine hydrate and hydrogen sulphide at -20 °C gave thiadiazolidine **14** which was oxidised with lead tetraacetate to furnish thiadiazoline **15**. Heating **15** with triphenylphosphine afforded bicyclobutylidene **(17)** in 69% overall yield. In the absence of triphenylphosphine, episulphide **16** is formed which can be converted to **17** by heating with triphenylphosphine. The second route involved a Wittig reaction between the butyllithium-generated phosphorane of **18** and cyclobutanone **13**. This one-step synthesis gives a lower yield of 31% but is a convenient way of preparing large amount of **17**.



Scheme 1. Garratt's syntheses of bicyclobutylidene.[51-53]

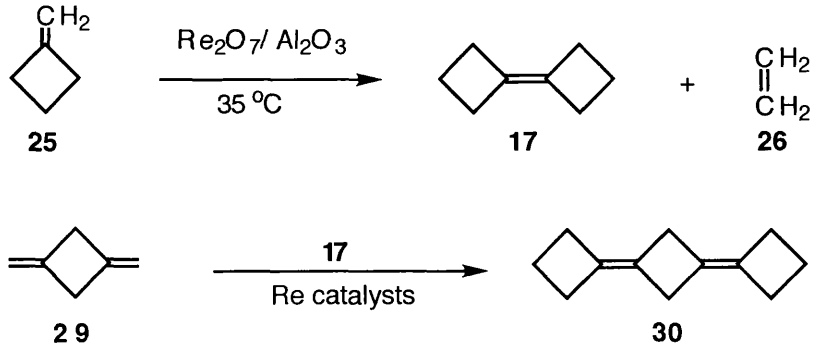
Krapcho[54] published a general synthetic route to cycloalkylidenes (Scheme 2). Treatment of the cycloalkanecarboxylic acids **19** with lithium diisopropylamide in THF leads to the formation of α-lithiated cycloalkanecarboxylic acid salts **20,** which reacts with **21** to give the β-hydroxyl acids **22**. The β-hydroxyl acids **22** can then cyclise to the β-lactones **23**. Both symmetrical (m = n = 4, 5, or 6) and unsymmetrical (m ≠ n = 4, 5, or 6) compounds of type **22** were obtained in overall yields of 50 - 70%.



Scheme 2. Krapcho's synthetic route to cycloalkylidenes.

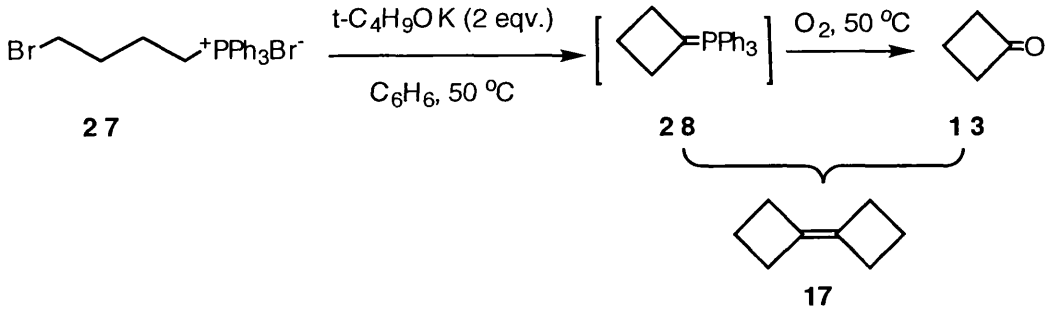
Finkel'shtein and co-workers[55] have also studied the behaviour of cyclobutane derivatives containing exo- and endo- double bonds. Under conditions of metathesis and with the continual withdrawing of the ethylene **26**, bicyclobutylidene **17** was obtained in 70% yield via the route illustrated in Scheme 3.

Using the method, Finkel'shtein and co-workers[57] then examined the structure of **17** based on gas-phase electron diffraction data. Their results showed that the exocyclic double bond in bicyclobutylidene alters the puckered geometry of the cyclobutyl ring by reducing the dihedral angle from 30° to 15° whereas the endocyclic double bond leads to complete flattening of the four-membered ring system. Recently, they[58] claimed to have obtained 1,3-bicyclobutylidene-cyclobutane **30** in presence of rhenium catalysts, although no spectroscopic information was reported.



Scheme 3. Finkel'shtein's synthetic route to cycloalkylidenes. [55,58]

The most recent synthesis of bicyclobutylidene by Fitjer et al.[56] in 1987 involved treating two equivalents of potassium t-butoxide with **27** in benzene followed by auto-oxidation of the resulting cyclobutylidenetriphenyl phosphorane **28** with molecular oxygen. This resulted in an efficient formation of cyclobutanone **13**, which was trapped with unreacted **28** to produce **17** in 75% yield (Scheme 4).



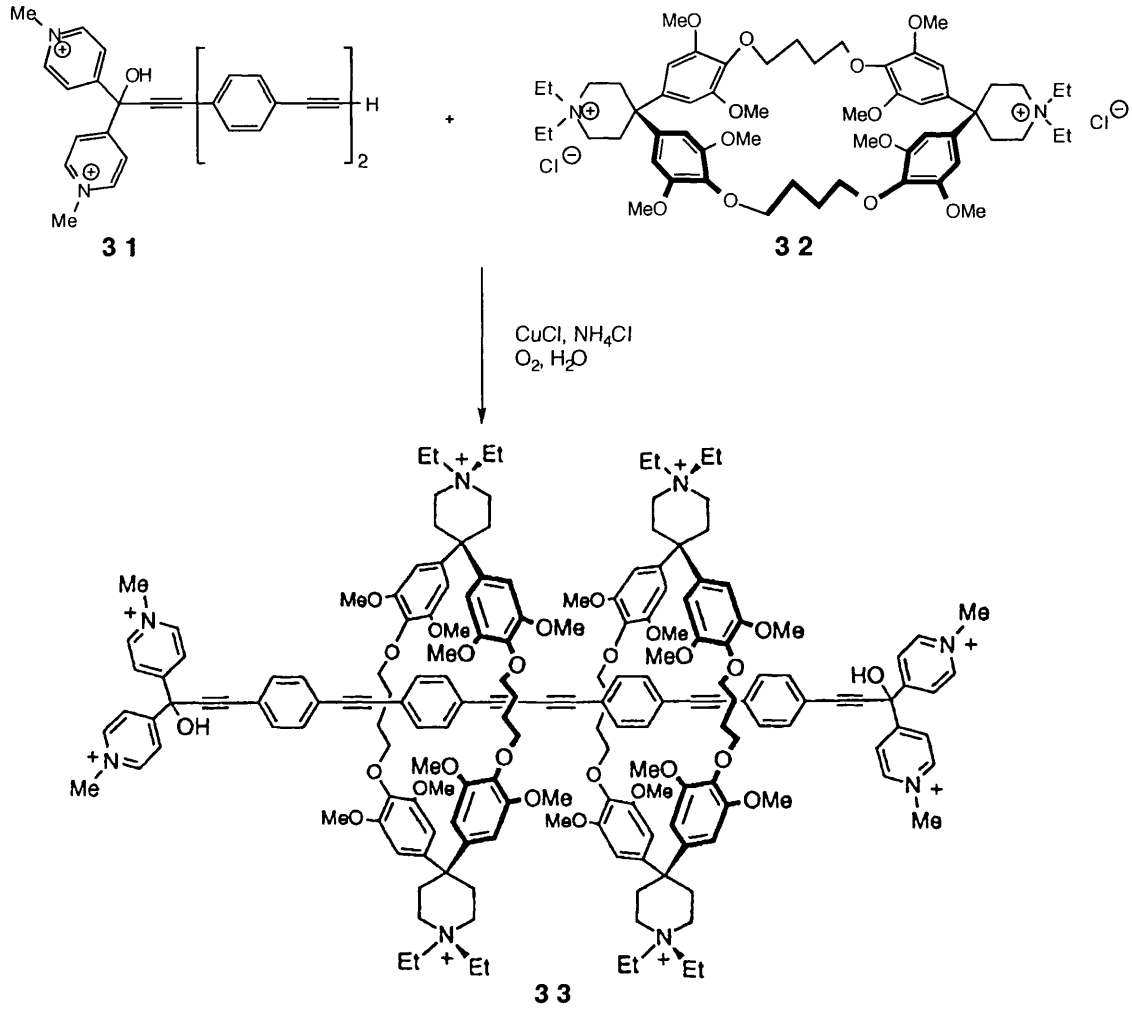
Scheme 4. Fitjer's synthesis of bicycloalkylidene.[56]

VIII. Insulated molecular wires

The organic "molecular wires" mentioned so far may have many potential applications in non-linear optics, in organoluminescent display devices, and as organic semiconductors,[59-61] but their unique electronic properties could also lead to chemical reactivity and instability, which can limit their usefulness. Several research groups have designed potential molecular wires which are insulated from their neighbours and from external environment.

The approach by Anderson et al.[62] is to thread a series of conjugated molecular "beads" onto a conjugated backbone to form a molecular "necklace" and add large bulky end-groups to prevent the insulators from unthreading. The researchers synthesised **31** as a conjugated wire with an encapping group and cyclophan **32** as the insulating "bead" and linked the two together by Glaser

coupling[63] at a 30% yield. The polyrotaxane showed higer fluorescence efficiency than the non-insulated counterpart.



31 32 33

Scheme 5. Synthesis of polyroxatane **33**.

Another approach to insulate molecular wires was to protect them with ligand shells as shown in Figure 14. Finniss et al.[64] reported the synthesis of the material $\{[Rh(MeCN)_4](BF_{4)1.5}\}_\infty$ **(33)** from galvanostatic reduction at a platinum electrode. This material was demonstated to display irreversible redox process that correspond to a one-electron reduction.[65,66] The authors also suggested that the MeCN ligands serve as an organic "sheath" around the central core of the ruthenium chain to stabilise the material. Figure 14 shows a portion of the ruthenium chain contained in each unit cell.

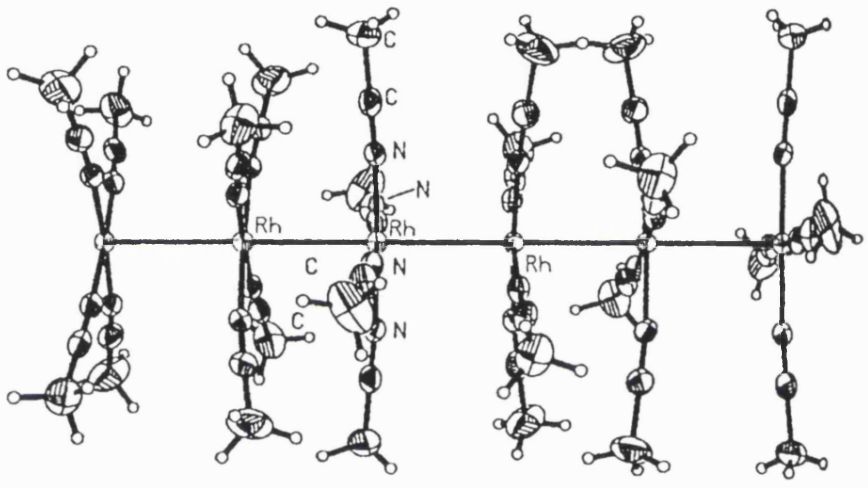
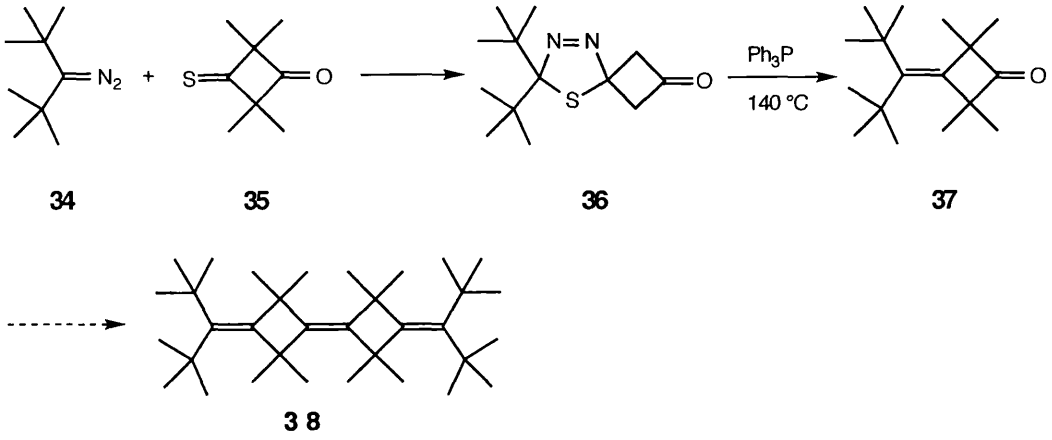


Figure 14.[64] Oak Ridge Thermal Ellipsoid Plot Program (ORTEP) representation of a $[Rh(MeCN)_4]^{9+}$ segment of **33**, taken from Finniss, G.M.; Canadell, E.; Campana, C; Dunbar, K.R. Angew. Chem. Int. Ed. Engl. 1996, **35**, 23/24, 2772.

The approach of Garratt et al.[67] to access insulating molecular wires was to prepare cyclic polymers with substituents which protects the conducting "inner core". To this end, they have synthesised 3-methylenecyclobutanone **37** as a precursor by Barton-Kellogg reaction with an overall yield of 19% (Scheme 6). However, attempts to couple the ketone **37** to form the target molecule **38** were unsucessful.



34 **35** **36** **37**

38

Scheme 6. Synthetic approach to the cyclic polyalkene **38**.

IX. Objectives

The aim of the present work was to investigate synthetic routes to cyclobutyl chain polymers and undertake studies into the chemistry of their derivatives. Although an increasing number of studies have been made concerning small ring compounds, very little is known about the properties of such linear polymers.

This work focused on the preparation of target small ring compounds which were envisioned to act as suitable monomeric units for the construction of linear polymers. In addition, the reaction chemistry of these small ring, cyclobutyl compounds and their derivatives were investigated.

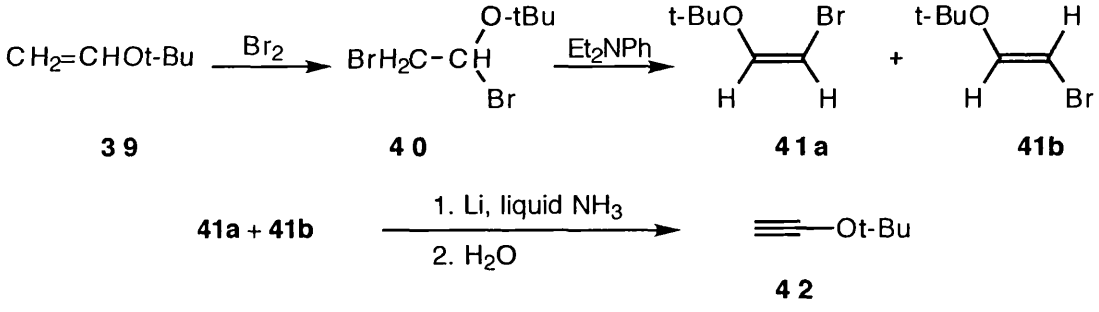
It is envisaged that insulated cyclobutyl molecular rods of defined length could serve as molecular wires in molecular electronic applications.

RESULTS AND DISCUSSIONS

X. Synthesis of 3-t-butoxycyclobutenone (46)

At the outset of this work, synthetically attractive routes to versatile, small ring cyclobutyl monomeric compounds were investigated, in order to generate intermediates that could act as "building blocks" for cyclobutyl chain polymers. A review of the available chemical literature highlighted 3-t-butoxycyclobutenone **(46)** as an enticing target molecule.

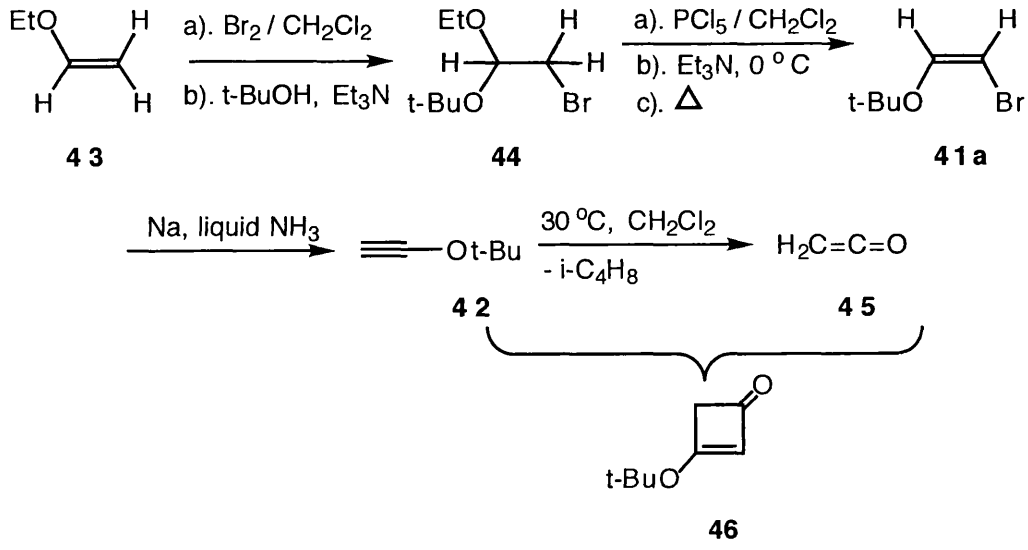
Two approaches for the preparation of **(46)** have been described; both of which utilise t-butoxyethyne **(42)** as a precursor. The synthesis of **(42)** by Arens and coworkers,[68] was undertaken initially (Scheme 7).



Scheme 7. Synthesis of 3-t-butoxyethyne **(42)**.

In this procedure, t-butyl vinyl ether **(39)** was treated with bromine to furnish 1,2-dibromo-1-t-butoxyethane **(40)**. Subsequent base-induced dehydrobromination of **(40)** yielded both the cis **41a** and trans **41b** isomers of 1-bromo-2-t-butoxyethene **(41)** in 52% yield after purification. An attempt to further dehydrobrominate the mixture of isomers **41a** and **41b** by lithium amide to give **(42)** was unsuccessful, with no desired product isolated. This was probably due to the decomposition of t-butoxyethyne when distillation was performed under normal atmospheric pressure.

A modified procedure as described by Pericàs et al.[69,70] was then investigated (Scheme 8). The attractiveness of the method lay in the insensitivity to the steric hindrance of the tertiary alcohol employed, and the low cost of the starting materials compared to those used in the former method.



Scheme 8. Synthesis of 3-t-butoxycyclobutenone **(46)**.

In this synthetic scheme, ethyl vinyl ether **(43)** was brominated at low temperature in dichloromethane, and the resulting solution of 1,2-dibromo-1-ethoxyethane then treated with a mixture of t-butyl alcohol and triethylamine. The resultant 2-bromo-1-t-butoxy-1-ethoxyethane **(44)** was obtained in 70% yield along with some (Z)-1-bromo-2-ethoxyethene, arising from a base-induced dehydrobromination of the 1,2-dibromo-1-ethoxyethane. Reaction of **44** with phosphorus pentachloride in dichloromethane occurred with high chemoselectivity, the primary ethoxy group being displaced. Subsequent treatment with triethylamine at 0 °C, followed by heating under reflux, resulted in (Z)-1-bromo-2-t-butoxyethene **(41a)** in 84% yield. Treatment of **41a** with sodium amide in liquid ammonia led to a final dehydrobromination reaction affording t-butoxyethyne **(42)** in a very low yield (1%).

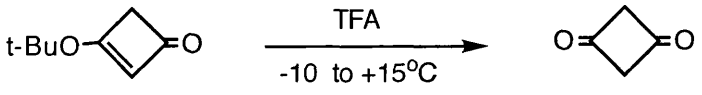
The poor yield of **(42)** was unexpected since sodium amide is the most commonly used and efficient means of triple-bond formation. This base causes 1-alkynes to predominate (where possible), because it forms the salt of the alkyne, shifting any equilibrium between 1- and 2-alkynes. However, by modification of the reaction conditions, a 64% yield of **(42)** was eventually obtained. These modifications were: (i) performing the sodium amide reaction under a continuous flow of nitrogen to ensure an oxygen-free atmosphere and (ii) using decahydronaphthalene as an extraction solvent instead of the lower boiling xylene. The overall yield from the vinyl ether **43** to the desired t-butoxyethyne **(42)** was 38%.

A dichloromethane solution of the acetylenic ether **42** was reacted in a closed atmosphere of nitrogen at 30 °C for 86 hours to furnish 3-t-butoxycyclobutenone **(46)**. An analysis of the infrared spectrum of the reaction mixture at this stage revealed the absence of the acetylenic absorption band. The reaction conditions were crucial in order to obtain a high yield. Under these conditions, the generation of ketene from t-butoxyethyne **(42)** was slow enough to ensure, in the presence of excess t-butoxyethyne **(42)**, a high selectivity in the cycloaddition reaction whilst the low concentration of ketene in the reaction mixture prevented its oligomerisation. The volatile isobutene was removed by rotary evaporation at the completion of the reaction to give a brown coloured solution. Initially, however, no crystalline substance was obtained. Subsequent distillation of the brown solution gave the alkoxycyclobutenone **46** in 20% yield. It was later discovered that crystallisation could be induced by cooling the crude material to -20°C for 24 hours. By this procedure, high vacuum distillation was avoided and the yield of **46** increased to 48%.

XI. Synthesis of 1,3-cyclobutanedione (47)

Following the successful synthesis of 3-t-butoxycyclobutenone **(46)**, the next synthetic target was the generation of 1,3-cyclobutanedione **(47)**. The general approach involves acid hydrolysis of alkoxycyclobutenones. Thus, Wasserman et al.[71] employed chilled, ethereal hydrochloric acid to achieve this transformation. Under these conditions, hydrolysis takes place almost immediately. However, the formation of **(47)** may also occur more slowly when the precursor alkoxycyclobutenone is permitted to stand in a moist atmosphere at -10 °C.

After some preliminary investigations, it was found that treatment of **46** with trifluoroacetic acid (TFA)[69] at -10° to +15 °C yielded 1,3-cyclobutanedione **(47)** in 80% yield (Scheme 9).



46 **47**

Scheme 9. Synthesis of cyclobutanedione **(47)**.

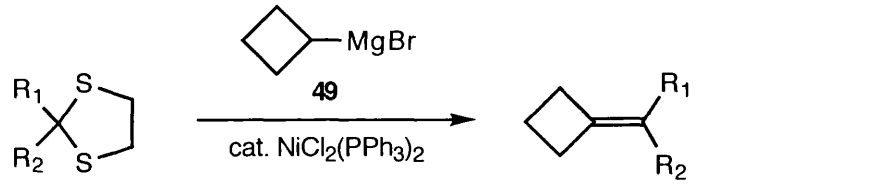
An infrared spectroscopic analysis of **(47)** in dichloromethane exhibits only very weak bands attributable to the enolic form of cyclobutanedione. In chloroform, the ^{1}H NMR spectrum reveals a singlet resonance at δ 3.85, indicative of the methylene protons.

XII. Synthesis of cyclobutylidenes and cyclobutenylidenes

There are many approaches to the preparation of cyclobutylidenes although in contrast, cyclobutenylidenes have not yet been synthesised. For the preparation of cyclobutylidenes, one can use, for example, the reaction of cyclobutyl Grignard reagents with dithioacetals,[72] the Barton-Kellog reaction,[53] the spiropentane rearrangement,[73] and also the Wittig reaction.[53,74,75]

XII.A. Reaction of cyclobutyl Grignard reagent with dithioacetals

In an investigation of coupling reactions of dithioacetals with Grignard reagents, Ng and Luh[72] discovered that cyclobutylidenes were obtained in 70 - 72% yield as the sole products, when cyclobutyl Grignard reagents were reacted with dithioacetals in the presence of a nickel catalyst (Scheme 10).



48a $R_1 = R_2 = Ph$ **50a** $R_1 = R_2 = Ph$
48b R_1 = 2-Naph, R_2 = H **50b** R_1 = 2-Naph, R_2 = H

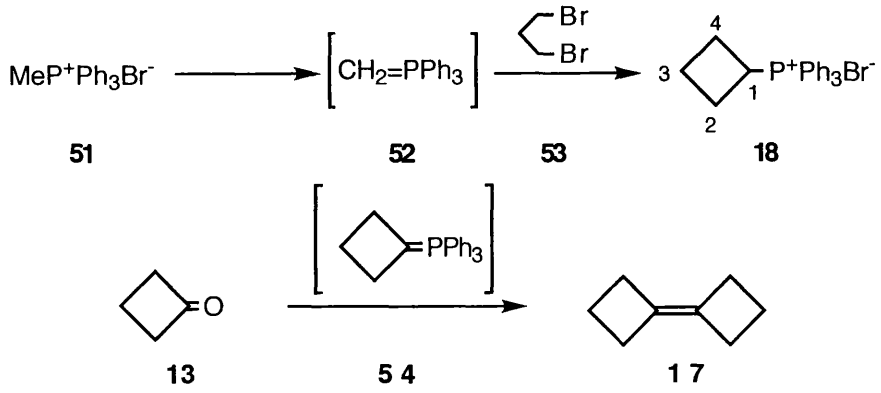
Scheme 10. Synthesis of cyclobutylidene via cyclobutyl Grignard reagent **(50)**.

XII.B. The Barton-Kellog reaction

Using the Barton-Kellog[76] reaction, Garratt and co-workers[53] synthesised bicyclobutylidene as described in Chapter 1 (Scheme 1). The overall yield from cyclobutanone **13** to bicyclobutylidene **17** was 68%.

XII.C. The Wittig reaction

Another synthetic route to bicyclobutylidene **17** was reported by Bee and Garratt[53] (Scheme 11). Reaction of n-butyllithium generated ylid **52** from methyltriphenylphosphonium bromide **51** with 1,3-dibromopropane **53** gave phosphonium salt **18**. Subsequent treatment of the n-butyllithium generated ylid **54** with cyclobutanone **13** gave bicyclobutylidene **17** in 30% yield. Despite the low yield obtained, the Wittig reaction was the method of choice, because of the convenience of the synthesis and the low cost of the starting material.



Scheme 11. Synthesis of bicyclobutylidene **(17)**.

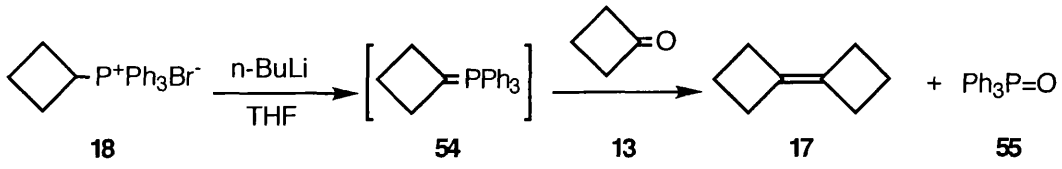
XIII.A. Synthesis of cyclobutylidenes and cyclobutenylidenes via the Wittig reaction

XIII.A.A. Synthesis of cyclobutyltriphenylphosphonium bromide (18)

Following the synthetic procedure of Garratt,[53,75] cyclobutyltriphenyl-phosphonium bromide **(18)** was prepared from commercially available methyltriphenyl-phosphonium bromide **(51)** and 1,3-dibromopropane **(53)**, via the Wittig reaction at 50 - 60 °C, as a starting material for further experiments (Scheme 10). The ^{1}H NMR spectrum of **18** shows a multiplet resonance at δ 7.70, assignable to the benzylic protons; a multiplet at δ 6.86, assignable to the C-1 proton; and a multiplet in the region of δ 1.60 - 2.90, assignable to the methylene protons.

XIII.A.B. Synthesis of bicyclobutylidene (17)

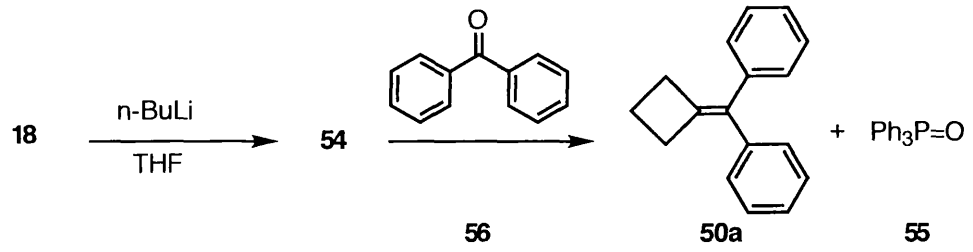
Reaction of cyclobutanone **(13)** with ylid **54** gave bicyclobutylidene **(17)** in 11% yield (Scheme 12). The low yield in comparison to that reported in the literature[53] is probably due to the small scale performed and loss of material in the distillation step. Two sets of environmentally different protons are predicted in the ^{1}H NMR spectrum of **(17)** due to the symmetry of the molecule. The recorded ^{1}H NMR spectrum showed no resonance in the region of δ 7.70 - δ 6.86 Instead, a triplet resonance occurred at δ 2.5, assignable to the allylic protons and the quartet resonance occurred at δ 1.93, assignable to the methylene protons. The ^{13}C NMR spectrum showed three signals as expected.



Scheme 12. Synthesis of bicyclobutylidene **(17)**.

XIII.A.C. Synthesis of diphenylmethylenecyclobutane (50a)

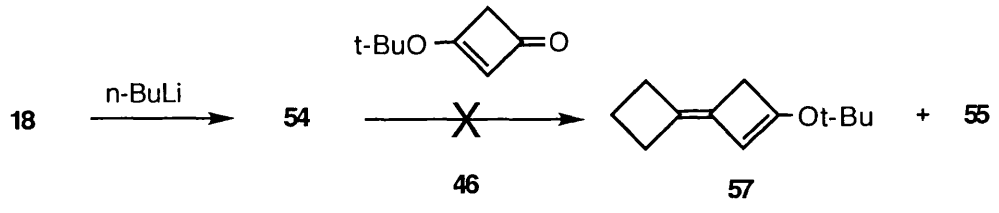
The reaction of benzophenone **(56)** with the n-butyllithium-generated phosphorane **54** gave a mixture of the desired diphenylmethylenecyclobutane **50a** and triphenylphosphine oxide **(55)** (Scheme 13), which were separated by column chromatography, furnishing diphenylmethylenecyclobutane **50a** in a 95% yield. The ^{1}H NMR spectrum of **50a** showed two multiplet resonances at δ 7.36 and δ 7.20, suggesting the presence of the two phenylic groups; a triplet resonance at δ 2.90, assignable to the allylic protons; and a quartet at δ 2.00, assignable to the methylene protons. The electron ionisation mass spectrum of **50a** showed the molecular ion at 220 and fragmentation ions at m/z 143, indicative of the loss of a phenyl group.



Scheme 13. Synthesis of diphenylmethylenecyclobutane **(50a)**.

XIII.A.D. Attempted synthesis of 3-t-butoxybicyclobutyliden-2-ene (57)

3-t-Butoxycyclobutenone **(46)** was then reacted with the ylid **54** under similar conditions (Scheme 14). Many undesired products were obtained which could not be identified. Extraction with water in the work up procedure led to the loss of the t-butoxy functional group which was earlier revealed in the ^{1}H NMR of the crude product.

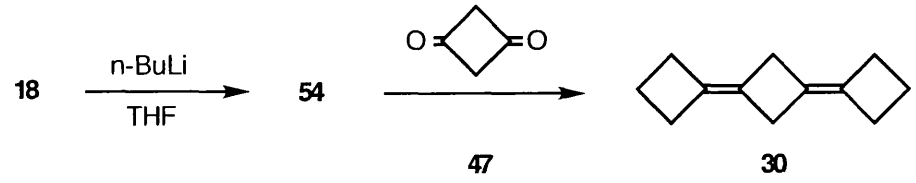


Scheme 14. Attempted synthesis of 3-t-butoxybicyclobutyliden-2-ene **(57)**.

XIII.A.E. Synthesis of 1,3-bicyclobutylidene-cyclobutane (30)

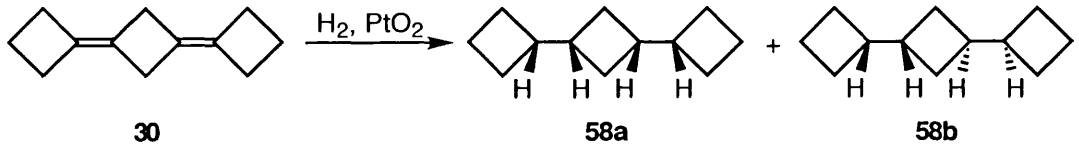
In order to obtain 1,3-bicyclobutylidene-cyclobutane **(30)**, the Wittig reaction between cyclobutanedione **(47)** and the cyclobutylidenetriphenylphosphorane **(54)** was investigated (Scheme 15). Heating the reaction mixture under reflux for 7 hours resulted in many products, which were separated by column chromatograophy. The first fraction collected was postulated to contain **30**, based on a preliminary analysis.

The predicted ^1H NMR spectrum of **30** should show three sets of environmentally different protons. However, the recorded spectrum of **30** showed a singlet resonance at δ 1.95 - δ 2.12, a multiplet resonance at δ 1.57 and δ 1.55, a singlet resonance at δ 1.26 and a multiplet resonance at δ 0.89.



Scheme 15. Attempted synthesis of 1,3-bicyclobutylidene-cyclobutane **(30)**.

In order to show the presence of **30**, hydrogenation was performed using platinum dioxide in dichloromethane. Shifts to higher field resonances was expected in the ^1H NMR spectrum of the resulting hydrogenation products due to the removal of the double bonds. Unfortunately, owing to the small amount of **30** available, the hydrogenation products **58a** and **58b** could not be obtained.

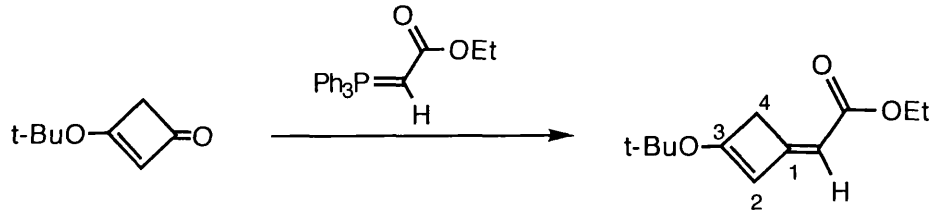


Scheme 16. Attempted hydrogenation of purportive 1,3-bicyclobutylidenene-cyclobutane **(58)**.

A second attempt to synthesise bicyclobutylidene-cyclobutane **(30)** on a much larger scale gave a mixture of volatile compounds. Only triphenylphosphine and triphenylphosphine oxide could be isolated from the mixture after purification using column chromatography.

After the unsuccesful attempts to synthesise 3-t-butoxybicyclobutyliden-2-ene **(53)**, a test to investigate whether the enone **46** undergoes a Wittig reaction with a simple ylid was carried out. A mixture of 3-t-butoxycyclobutenone **(46)** and commercially available carbethoxymethylenetriphenyl phosphorane **(59)** was heated at 65 - 70 °C under nitrogen for 12 hours (Scheme 17). (3-t-Butoxy-cyclobut-2-enylidene)-acetic acid ethyl ester **(60)** was isolated from the reaction mixture by column chromatography in 1% yield. Despite the low yield, enone **46** was shown to undergo a Wittig reaction, though probably to a limited extent. The ^1H NMR spectrum of **60** showed a doublet resonance at δ 6.12, assignable to the C-2 proton; a singlet at δ 4.64, assignable to the two C-4 methylene protons; and a singlet at δ 1.35, characteristic of the t-butoxy resonance. Additionally, a doublet at δ 5.88, assignable to the proton α to the carbonyl group; a quartet at δ 4.22, and a triplet at δ 1.20,

assignable to the five ethoxy protons were observed. The EI mass spectrum of **60** showed molecular ions at m/z 210 and fragment ions at m/z 137, 92, 64 and 39, indicative of the loss of the t-butoxy group followed by the ethoxy group, the ester funtionality and the entire C-1 side chain.



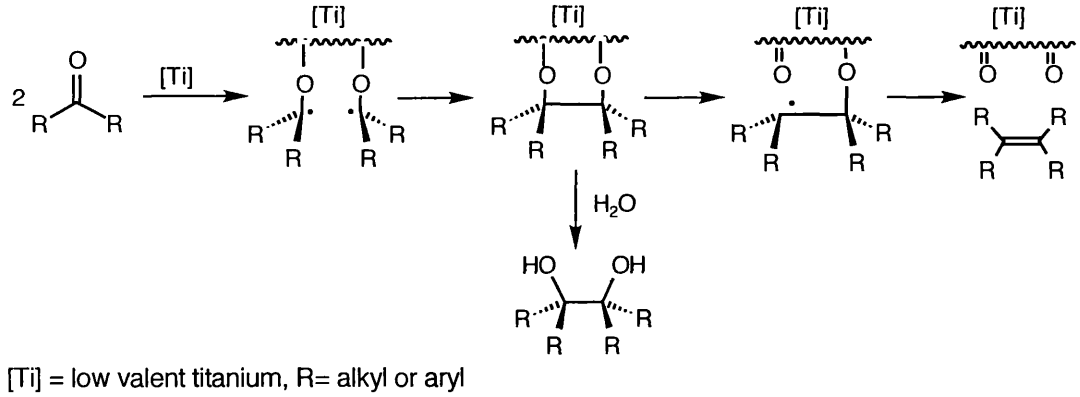
46 **59** **60**

Scheme 17. Synthesis of (3-t-butoxy-cyclobut-2-enylidene)-acetic acid ethyl ester **(60)**.

XIII.B Attempted synthesis of cyclobutylidenes and cyclobutenylidenes via carbonyl coupling

XIII.B.A. Carbonyl coupling reactions using low-valent titanium

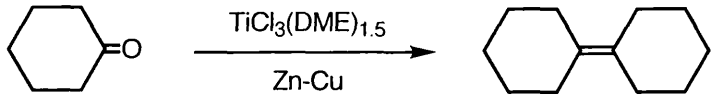
One of the most powerful methods for carbonyl coupling is the reductive dimerisation of ketones to yield olefins on treatment with low-valent titanium reagents, discovered almost simultaneously by McMurry et al.,[77] and two other groups[78,79] in the 1970s. The reaction can be used in both an inter- and intramolecular coupling. The reaction proceeds via the dimerisation of the starting ketone to form a C-C bond followed by stepwise cleavage of the C-O bond to yield the alkene and an oxide coated titanium surface (Scheme 18). The intermediate pinacols can be isolated in high yield if the reaction is performed at 0 °C rather than reflux. Subsequent treatment with low-valent titanium at 60 °C leads to deoxygenation, giving the olefin.[80]



[Ti] = low valent titanium, R= alkyl or aryl

Scheme 18.[80] The proposed mechanism of the dimerisation of ketones with low-valent titanium reagents, adapted from McMurry, J.E. Chem. Rev. 1989, **89**, 1513.

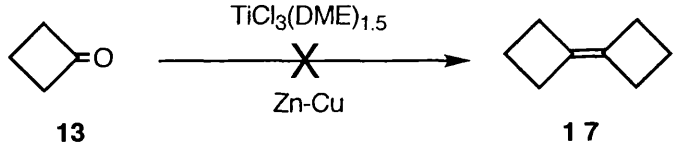
The utilisation of the McMurry reaction[80] was considered for the coupling of cyclobutanones. In order to examine the reaction conditions, cyclohexylidene **62** was prepared under the conditions described by McMurry et al.[81] (Scheme 19). A mixture of freshly made titanium trichloride-dimethoxyethane complex with zinc-copper couple was heated to reflux in dimethoxyethane for 2 hours. Cyclohexanone was then added and the mixture was heated to reflux for a further 12 hours. Cyclohexylidene **(62)** was obtained in 97% after purification. The ^{1}H NMR spectrum of the alkene **62** showed a triplet resonance at δ 2.18, assignable to the eight allylic protons; and a quartet resonance at δ 1.51, assignable to the twelve methylene protons. The ^{13}C NMR spectrum of **62** showed four signals ascribed to the four environmentally different carbon atoms.



61 **62**

Scheme 19. Synthesis of cyclohexylidene **(62)**.

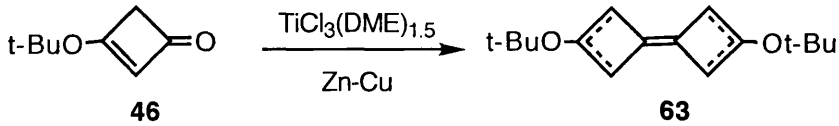
However, attempts to synthesise bicyclobutylidene **(17)** from cyclobutanone **(13)** under the same conditions yielded a complex mixture which could not be purified by column chromatography.



13 **17**

Scheme 20. Attempted synthesis of bicyclobutylidene via the McMurry reaction.

Under the same conditions, many attempts to dimerise the enone **46** also resulted a complex mixture.

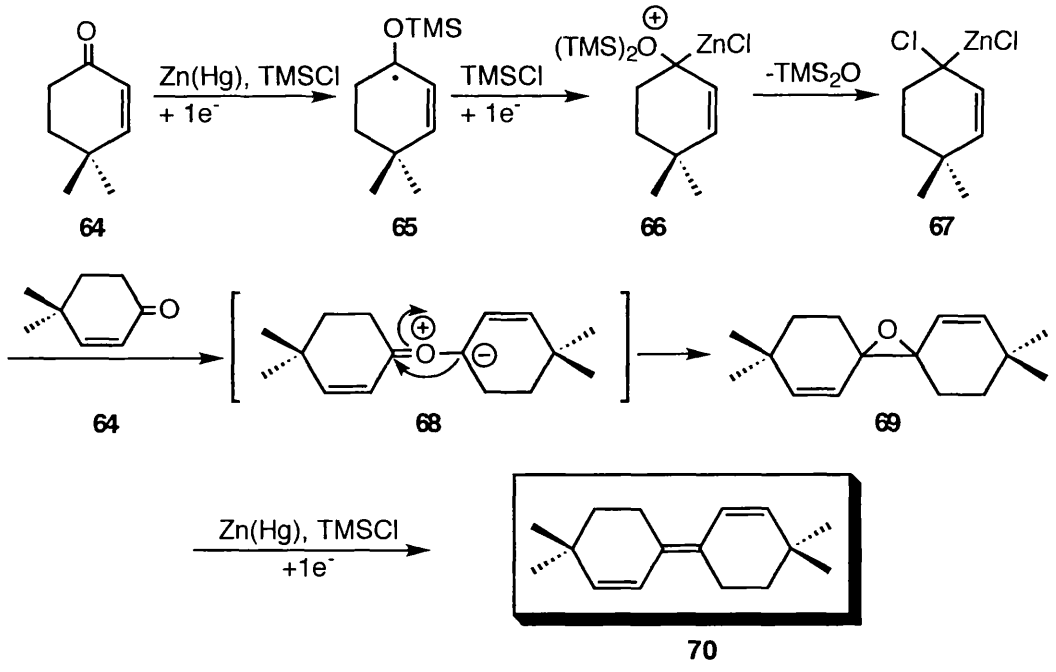


46 **63**

Scheme 21. Attempted synthesis of bicyclobutenylidene **63** via the McMurry reaction.

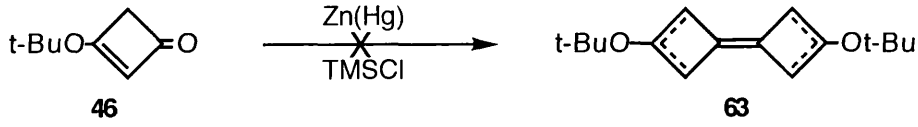
XIII.B.B. Carbonyl coupling reactions using zinc carbenoid[82]

The fruitless attempts to couple enone **46** prompted the investigation into another synthetic route, which involves the carbonyl coupling reaction using organozinc carbenoid. Motherwell and co-workers[83] examined the dicarbonyl coupling reactions using modified Clemmensen conditions involving zinc carbenoid. Unlike the McMurry reaction, the formation of olefins in the zinc / chlorotrimethylsilane system involved trapping the intermediate organozinc carbenoid by a second molecule of carbonyl compound, followed by subsequent deoxygenation of the resulting epoxide, via a reaction not involving pinacols. The proposed mechanism of this reaction is illustrated in Scheme 22. Initial transfer of an electron from the zinc surface to the carbonyl group of **64** leads to the formation of a ketyl radical anion **65** which reacts with trimethylsilylchloride (TMSCl) to furnish species **66**. Futher reaction with TMSCl then allows the disiloxane to become a leaving group. Loss of zinc chloride from **67** followed by coupling with another molecule of **64** gives the epoxide **69**. A similar reaction occur again with the epoxide, accepting an electron from the zinc surface. Reaction of TMSCl followed by loss of zinc chloride yields the alkene **70**.



Scheme 22. An example of the zinc carbenoid dicarbonyl coupling reaction.

An attempt was made to synthesise the disubstituted cyclobutenylidene via direct deoxygenation of the cyclic ketone **46** by the action of zinc and TMSCl as mentioned above. Thus t-butoxycyclobutenone **46** was added to a mixture of refluxing TMSCl and zinc / mercury amalgam in diethyl ether and the reflux was continued for 15 hours. No identified products were obtained.



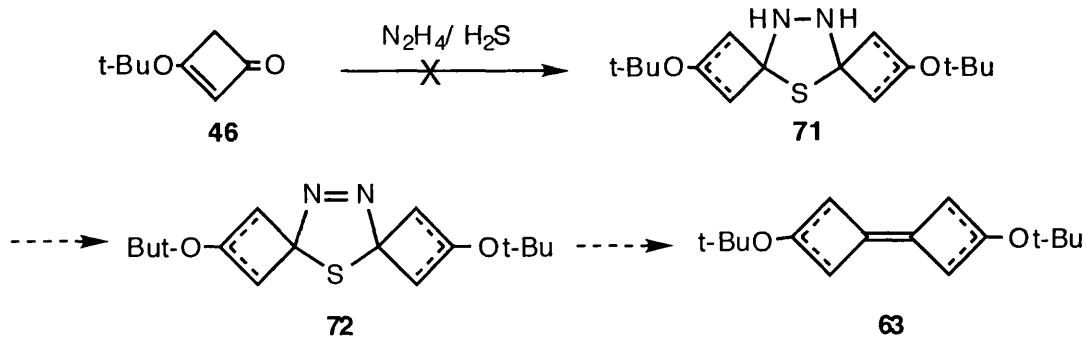
46 **63**

Scheme 23. Attempted synthesis of bicyclobutenylidene **63** via the zinc carbenoid dicarbonyl coupling reaction

XIII.C Attempted synthesis of cyclobutylidenes and cyclobutenylidenes via the Barton-Kellog reaction

An alternative route of coupling 3-t-butoxycyclobutenone **(46)** was then sought. The Barton-Kellog reaction[76] was considered (see P. 23, Scheme 1), although the synthesis involves more steps than the Wittig reaction, the McMurry reaction, and the zinc carbenoid dicarbonyl coupling reaction previously described. To examine the reaction conditions, cyclobutanone **(13)** was reacted with hydrazine hydrate and H_2S at -15 °C to furnish the thiazolidine **14** in 40% yield. The yield is relatively low compare to the literature,[53] probably due to loss of the cyclobutanone when passing the H_2S through the solution. The ^1H NMR spectrum of thiazoline **14** showed a distinctive broad singlet at δ 3.80, assignable to the amino protons; a multiplet signal at δ 2.50; assignable to the C-1 protons; a multiplet signal at δ 2.23; assignable to the C-3 protons; and a multiplet signal at δ 1.86, assignable to the C-2 protons. Subsequent oxidation of the thiazolidine with lead(IV) acetate resulted in thiadiazoline **15** in 90% yield, after removing the metal residue by filtration through celite.

In order to obtain the bicyclobutenylidene **63**, the same reaction conditions were applied to t-butoxycyclobutenone **46.** However, no thiazolidine was observed. Varying the solvent (pentane, ether, methanol) and reaction temperature did not alter the result. Only starting material **46** was recovered.



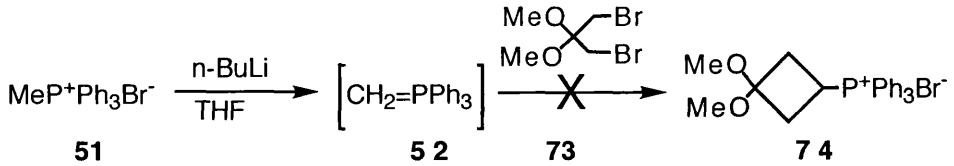
Scheme 24. Attempted synthesis of bicyclobutenylidene **63** via the Barton-Kellog reaction.

XIV. Attempted syntheses of 3-substituted cyclobutyl phosphonium salts

During the search for a coupling reaction that could be applied to 3-t-butoxycyclobutenone **(46)**, the syntheses for a number of 3-substituted cyclobutyl phosphonium bromide salts were attempted. it was hoped that a 3-substituted phosphonium ylid could be reached where the functional groups could allow further reactions to proceed, thereby leading to the congregation of more cyclobutyl moieties.

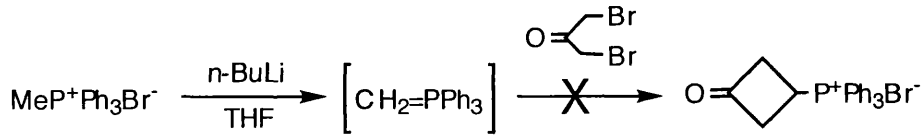
With the synthesis of cyclobutyltriphenyl phosphonium bromide in mind, it was hoped that a 3-substituted phosphonium ylid could be reached where the functional groups could allow further reactions to proceed, thereby leading to the congregation of more cyclobutyl moieties.

Many attempts to synthesise 3,3-dimethoxycyclobutyltriphenyl phosphonium bromide **(74)** via this route were unsuccessful. Refluxing for 83 hours resulted in no reaction. Only the starting materials were recovered. The lack of reactivity of the dibromide **73** could be due to the electron donating nature of the methoxy groups. The proposed scheme is given below:



Scheme 25. Attempted synthesis of 3-substituted cyclobutyltriphenyl phosphonium salt **74**.

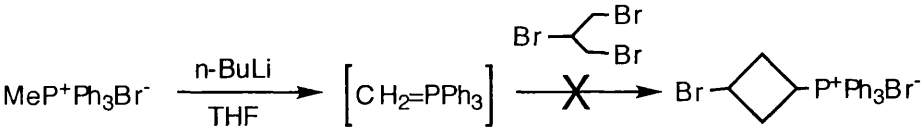
Attempts to synthesise cyclobutyl-oxo-3-triphenylphosphonium bromide **(76)** via the same method using 1,3-dibromo-2-acetone **(75)** as a reactant resulted a brown solid, which oxidised readily in air. Recrystallisation of the crude solid using water, ethanol / water and pure ethanol were unsuccessful.



51 **52** **75** **76**

Scheme 26. Attempted synthesis of 3-substituted cyclobutyltriphenyl phosphonium salt **76**.

Under the same conditions, using 1,2,3-tribromopropane **(77)** as a reactant, attempts to synthesise 3-bromocyclobutyl-triphenylphosphonium bromide **(78)** also proved to be unsuccessful. A light brown material was obtained which was shown to be a mixture of the starting material and an unidentified compound.



51 **52** **77** **78**

Scheme 27. Attempted synthesis of 3-substituted cyclobutyltriphenyl phosphonium salt **78**.

XV. Conclusion

Although the quest for cyclobutyl polymers has not been successful, several novel methods were examined. The unsuccessful attempts of carbonyl coupling involving the McMurry reaction or zinc carbenoid could be due to the sensitivity of the starting material and the labile nature of the products. In most cases, purification and low yields also made identification difficult. This led to the exploration of more conventional approaches: the Wittig reaction and the Barton-Kellog reaction. The latter synthetic approach gave moderate yield of the bicyclobutylidene **(17)** in spite of the inconvenience. However, no t-butoxycyclobutenone **(46)** coupling was observed with the Barton-Kellog reaction.

Promising results have been achieved using the Wittig reaction. Diphenthymethylenecyclobutane **(50a)** and bicyclobutylidene **(17)** were synthesised by this route. Furthermore, enone **46** was also demonstrated to undergo the Wittig reaction to give the ethyl (3-t-butoxy-cyclobut-2-enylidene)-acetic acid ethyl ester **(60)**, although in very low yield. However, attempts to prepare 3-substituted cyclobutyl phosphonium salts as building blocks were not successful. Further investigation into the utilisation of the Wittig reaction in coupling together small ring compounds are recommended.

EXPERIMENTAL

XVI. Instrumentation and experimental techniques

^1H NMR spectra were recorded at 300 MHz on a Bruker AC300 or at 400 MHz on a Varian VXR-400; ^{13}C NMR spectra were recorded either at 100 MHz on a Varian VXR-400 or at 75 MHz on a Bruker AC300. Spectra were recorded in the solvent specified. All chemical shifts are reported in parts per million (δ) relative to the internal standard. The residual protic solvent signal i.e. $C\underline{H}Cl_3$ (δ_H = 7.27 ppm), and the resonances of $\underline{C}DCl_3$ (δ_C = 77.0 ppm, t) were used as internal standards. Coupling constants (J) are given in hertz (Hz). The following abbreviations are used in signal assignments; s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sex (sextet), m (multiplet), and b (broad).

Infrared spectra were recorded on a Perkin-Elmer PE-983G or Perkin-Elmer 1605 FTIR spectrophotometer using potassium bromide (KBr) pellets unless otherwise stated, and are reported in cm^{-1}. Mass spectra were recorded using one of three techniques; E.I. (electron ionisation) on a VG7070H mass spectrometer with Finnigan Incos II data system; A.P.C.I. (atmospheric pressure chemical ionisation) on a Micromass Quatro L/C mass spectrometer, or F.A.B. (fast atom bombardment) on a VG ZAB-SE mass spectrometer, at UCL. Only those peaks due to the molecular ions (M^+), base peaks and other major peak fragmentations are reported and are measured as a percentage (%) of the base peak. High resolution mass spectra were performed using a VG ZAB-SE mass spectrometer at the London School of Pharmacy.

Elemental analyses were carried out by the Microanalytical Section of the Chemistry Department, UCL on a Perkin-Elmer 2400 CHN elemental analyser. Melting points were determined on a Reichert melting point apparatus and are uncorrected.

Analytical thin layer chromatography (t.l.c.) was performed on pre-coated aluminium backed Merck Kieselgel 60 F_{254} plates and the plates were visualised with ultra-violet light (254 nm), iodine, or by heat development using one of PMA [phosphomolybolic acid (12 g), EtOH (250 mL)], vanillin [vanillin (15 g), EtOH (250 mL), conc. H_2SO_4 (2.5 mL)] or potassium permanganate [$KMnO_4$ (3 g), K_2CO_3 (20 g), 5% aqueous NaOH (5 mL), water (300 mL)] stains. Column chromatography was performed either using Merck flash silica (200-400 mesh) or neutral alumina (Al_2O_3) as the stationary phase.

All experiments involving the usage of moisture-sensitive reagents were carried out under an atmosphere of dry nitrogen. Pentane and petroleum ether (b.p. 40-60 °C) used for column chromatography were distilled prior to use. Solvents used in a dry atmosphere were distilled under nitrogen over drying agents immediately prior to use, as follows: pentane, triethylamine and dichloromethane over calcium hydride and diethyl ether, benzene, tetrahydrofuran and dimethoxyethane over sodium metal and benzophenone.

Zinc-copper couple

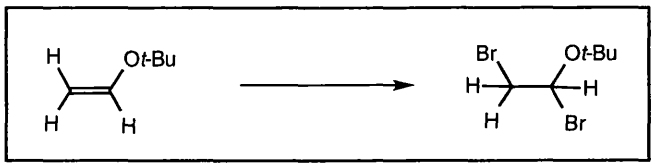
Zinc powder (4.1 g, 62.5 mmol) was washed sucessively with hydrochloric acid (3%, 4 x 3 mL), distilled water (4 x 4 mL), copper sulphate solution (2%, 2 x 7 mL), distilled water (4 x 4 mL), pure acetone (4 x 4 mL) and diethyl ether (4 x 3 mL). The zinc-copper couple was dried under vacuum and stored under nitrogen.

Zinc-amalgam

Zinc powder (10 g, 0.153 mol) was added to a vigorously stirred solution of mercuric chloride (2.0 g, 7.2 mmol) and hydrochloric acid (0.5 mL, 10 M) in water (30 mL). The mixture was stirred for 10 minutes, and any zinc aggregates were crushed with a glass rod. The aqueous layer was decanted. The amalgam was transferred to a sintered funnel and was sucessively washed with distilled water (4 x 20 mL), pure acetone (4 x 20 mL) and diethyl ether (4 x 20 mL). The zinc-amalgam was dried under vacuum and stored under nitrogen.

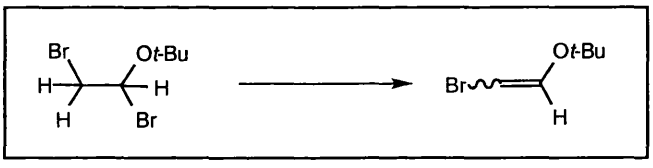
XVII. Compounds synthesised

1,2-Dibromo-1-*t*-butoxyethane (40)[68]



t-Butyl vinyl ether **(39)** (10.03 g, 0.10 mol) was treated with bromine (8 g, 0.1 mol) at -40 to -45 °C with stirring under nitrogen. The resultant dibromoether was used immediately without further purification.

1-Bromo-2-*t*-butoxyethene (41)[68]

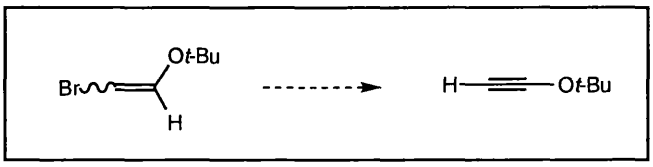


The cold, crude dibromoether **40** above was added to a warm solution of diethylaniline (14.90 g, 0.20 mol) in benzene (8 mL). The mixture was then heated to 80 - 100 °C for 2 hours and then allowed to cool to room temperature. The resultant crystalline diethylaniline-hydrobromide was filtered and washed with benzene. The combined filtrates were distilled under diminished pressure affording **41** as a colourless liquid (b.p. 24 - 36 °C, 250 mmHg).

Yield: 9.32 g, 0.05 mol (52%).

IR (cm^{-1}, liquid film): 2974, 1730, 1368, 1371, 1201, 1150, 912, 739.

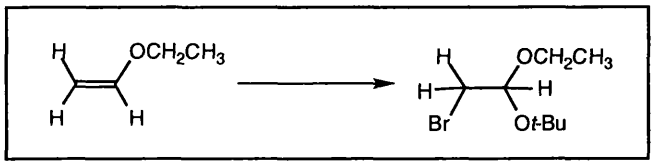
Attempted synthesis of *t*-butoxyethyne (42) *via* lithium amide dehydrobromination



Lithium metal (0.72 g, 0.10 mol) was added portionwise to liquid ammonia (80 mL) containing ferric nitrate (0.01 g, 0.03 mmol) in a round-bottomed flask equipped with a water condenser. Crude 1-bromo-2-*t*-butoxyethene **(41)** (9.32 g, 0.052 mol) was added dropwise to the stirred suspension of the resultant lithium amide over 15

minutes. The mixture was then stirred for an additional 15 minutes before pouring onto crushed ice (150 g). The cold solution was extracted with pentane (3 x 10 mL) and the combined extracts washed with water and dried ($MgSO_4$). The pentane was removed by distillation under atmospheric pressure. No material was obtained above the boiling point of pentane.

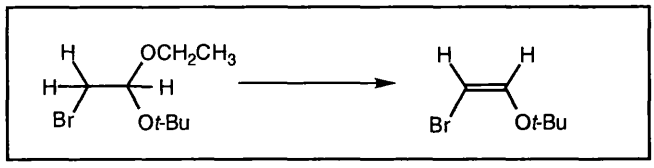
1-Bromo-2-*t*-butoxy-2-ethoxyethane (44)[70]



With stirring, liquid bromine (27.9 g, 0.34 mol) was added to ethyl vinyl ether **(43)** (24.0 g, 0.33 mol) in dichloromethane (90 mL) at -78 °C under nitrogen until the bromine colour persisted. The mixture was then decolourised by the addition of a few drops of ethyl vinyl ether. To this mixture was added a solution of *t*-butyl alcohol (74.0 g, 1.00 mol) in triethylamine (52 mL, 0.37 mol) dropwise over 1 hour at -78 °C. The cooling bath was removed and the mixture was allowed to warm up to 0 °C. Dichloromethane (86 mL) was added prior to the quenching of the mixture with ice-cold water (250 mL). The organic layer was separated and the aqueous fraction extracted with dichloromethane (2 x 18 mL). The combined organic extracts were washed with 0.5M HCl (2 x 7 mL) and saturated $NaHCO_3$ before drying ($MgSO_4$). After solvent evaporation under reduced pressure, the crude material was distilled under reduced pressure, yielding **44** as a colourless oil (b.p. 83 - 86 °C, 18 mmHg). The material which distilled below this temperature was a mixture of **44** and (*Z*)-1-bromo-2-ethoxyethene.

Yield:	52.11 g, 0.23 mol (70%).
IR (cm^{-1}, liquid film):	2977, 2940, 2890, 1415, 1392, 1057, 1016.
^1H NMR (200 MHz, $CDCl_3$): δ	4.80 (t, *J* = 6 Hz, 1H, CH(OEt)O*t*-Bu), 3.60 - 3.18 (m, 4H, $C\underline{H}_2Br$, $OC\underline{H}_2CH_3$), 1.22 (s, 9H, $OC(C\underline{H}_3)_3$), 1.17 (t, *J* = 8 Hz, 3H, $OCH_2C\underline{H}_3$).

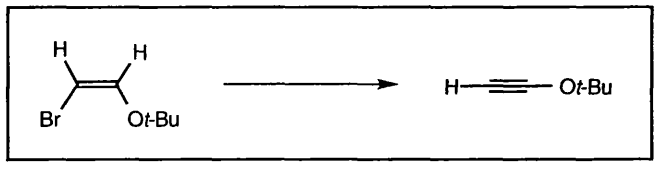
(*Z*)-1-Bromo-2-*t*-butoxyethene (41a)[70]



A solution of **44** (26.63 g, 0.12 mol) in dichloromethane (34 mL) was added dropwise to a suspension of phosphorus pentachloride (27.45 g, 0.13 mol) in dichloromethane (116 mL) at 0 °C under nitrogen over 15 minutes and stirring continued for 45 minutes until all the starting material had disappeared. Triethylamine (82.4 mL, 0.59 mol) was then added dropwise at the same temperature. The cooling bath was removed and the reaction mixture heated under reflux for 105 minutes before pouring onto crushed ice (120 g). The organic phase was separated and the aqueous layer extracted with dichloromethane (2 x 35 mL). The combined organic extracts were washed with 0.5 M HCl (2 x 15 mL) and saturated $NaHCO_3$ solution before drying over $MgSO_4$. After solvent evaporation at reduced pressure, the crude material was distilled, furnishing pure (*Z*)-1-bromo-2-*t*-butoxyethene **(41a)** (b.p. 61 - 64 °C, 22 mmHg). Compound **41a** was dried over molecular sieves and stored under nitrogen. The material distilled below this temperature was a mixture of **41a** and 1-bromo-2-ethoxyethene.

Yield:	17.79 g, 0.1 mol (84%).
IR (cm^{-1}, liquid film):	3107, 2980, 2936, 1639, 1465, 1394, 1371, 1262, 1231, 1177, 1093.
^1H NMR (200 MHz, $CDCl_3$): δ	6.75 (d, *J* = 6 Hz, 1H, C<u>H</u>Br), 5.08 (d, *J* = 6 Hz, 1H, C<u>H</u>O*t*-Bu), 1.30 (s, 9H, OC(C<u>H</u>$_3$)$_3$).

***t*-Butoxyethyne (42)**[70]



Anhydrous liquid ammonia (150 mL) was placed in a 250 mL flask equipped with a mechanical stirrer and a pressure-equalising dropping funnel. A small piece of sodium metal was added, followed by $Fe(III)(NO_3)_3 \cdot 9H_2O$ (0.1g). More sodium was

introduced portionwise until a total of 2.6 g (0.11 moles) was added. The observed change from a blue coloured solution to a grey coloured suspension indicated the formation of sodium amide. Pure **41a** (9.36 g, 0.05 mol) was then added in dropwise after 30 minutes. Stirring was continued for an additional 25 minutes, during which time further additions of ammonia were made as required. The reaction mixture was then cautiously poured into crushed ice (100 g) and the mixture extracted with xylene (4 x 10 mL). The combined organic layers were washed with water (1 x 50 mL), a phosphate buffer (pH 7.4, 100 mL), and then dried ($MgSO_4$). Pure *t*-butoxyethyne **(42)** was collecting at -78 °C as a colourless liquid upon distillation at 1.0 mmHg .

Yield: 0.06 g, 0.61 mmol (1%).

IR (cm^{-1}, liquid film): 2981, 1760, 1560, 1372, 1327.

^1H NMR (400 MHz, $CDCl_3$): δ 1.55 (s, 1H, C≡C<u>H</u>), 1.42 (s, 9H, OC(C<u>H</u>$_3$)$_3$).

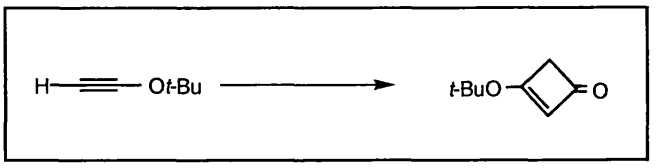
^{13}C NMR (100 MHz, $CDCl_3$): δ 87.7, 86.1, 29.2, 26.9.

Modified synthesis of *t*-butoxyethyne (42)

Into a 2-L three-necked flask, equipped with a mechanical stirrer and a pressure-equalising dropping funnel, was introduced anhydrous liquid ammonia (~1000 mL) at -33 °C under a continuous purge of nitrogen. A small piece of sodium metal was added, followed by $Fe(III)(NO_3)_3 \cdot 9H_2O$ (0.6 g, 0.0015 mol). Further additions of sodium were made until a total amount of 22.5 g had been introduced. The observed change from a blue coloured solution to a grey coloured suspension indicated the formation of sodium amide. The suspension was stirred for an additional 30 minutes. Pure **41a** (77.88 g, 0.44 mol) was then added dropwise. Stirring was continued for an additional 25 minutes, during which time more ammonia was added as required. The reaction mixture was then cautiously poured onto ice (1000 g) and extracted with dekalin (3 x 45 mL). The combined organic layers were washed with water (1 x 50 mL) followed by a phosphate buffer (pH = 7, 50 mL). Upon filtration, the organic fraction was dried ($MgSO_4$). Pure *t*-butoxyethyne **(42)** was obtained by distillation at 1.0 torr, collecting at -78 °C. The ^1H NMR and IR spectra were identical to those obtained for the previous sample.

Yield: 27.48 g, 0.28 mol (64%)

3-*t*-Butoxycyclobutenone (46)[69]



A solution of *t*-butoxyethyne **(42)** (4.90 g, 50 mmol) containing dry dichloromethane (0.8 mL) was kept in a sealed pyrex tube filled with nitrogen at 30 ± 0.5 °C for 86 hours, after which time no C≡C absorption band could be detected in the IR spectrum. The volatile material was removed under reduced pressure. Upon cooling to -40 °C for 24 hours, crude **46** was obtained as a brown crystalline substance. Pure **46** was obtained by vacuum sublimation (30 °C, 0.05 mmHg) and dried in a desiccator.

Yield: 1.68 g, 0.012 mmol (48%).

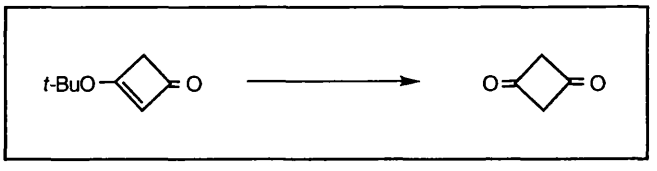
mpt: 44 - 46 °C (lit.[69] 45 - 46 °C)

IR (cm^{-1}, CH_2Cl_2): 2982, 2925, 2853, 1760, 1565, 1373, 1328, 1261, 1161, 1037, 1006.

^1H NMR (400 MHz, $CDCl_3$): δ 4.82 (s, 1H, C<u>H</u>C=O), 3.14 (s, 2H, C<u>H</u>$_2$C=O), 1.49 (s, 9H, OC(C<u>H</u>$_3$)$_3$).

EIMS (*m/z*): 140 (M^+, <1), 125 (M^+ - CH_3, <1), 112 (4), 85 (2), 67 (M^+ - O*t*-Bu, 11), 57 (*t*-Bu, 86), 39 (M^+ - O*t*-Bu - C=O,100), 27 (89).

1,3-Cyclobutanedione (47)[69]

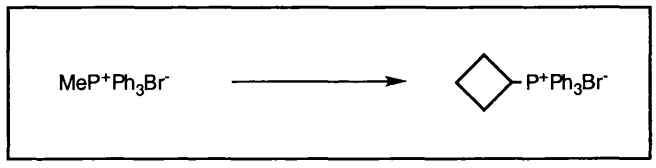


Trifluoroacetic acid (1.5 mL) was added dropwise with stirring to **46** (0.2450g, 1.7 mmol) at -10 °C. The mixture was allowed to warm to 15 °C with continual stirring for 3 hours. The volatile material was removed under reduced pressure followed by high vacuum (0.5 torr) evaporation, furnishing the diketone **47** as a off-white solid. A second crystalline crop was obtained by concentration of the mother liquor at -20 °C.

Yield: 0.1068 g, 1.27 mmol (73%).

mpt:	119 - 120 °C (lit.[69] 121 - 122 °C)
IR (cm^{-1}, $CHCl_3$):	3021, 1761, 1216, 1159, 1046.
^1H NMR (400 MHz, $CDCl_3$): δ	3.85 (s).
^{13}C NMR (100 MHz, $CDCl_3$): δ	198.0, 64.0.

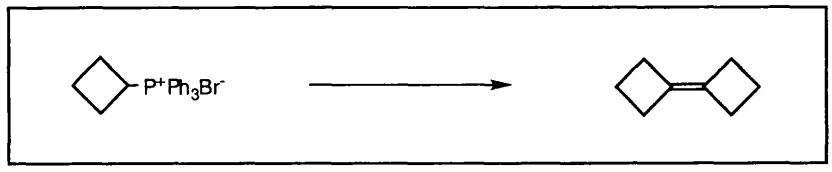
Cyclobutyltriphenyl phosphonium bromide (18)[75]



n-BuLi (42 mL, 1.6 M in *n*-hexane, 0.06 mol) was added to a stirred suspension of methyltriphenyl phosphonium bromide **(51)** (21.4 g, 0.06 mol) in dry THF (300 mL) under nitrogen, and the mixture was stirred for 1 hour at room temperature. 1,3-Dibromopropane **(53)** (6.07 g, 0.03 mol) in dry THF (100 mL) was added dropwise to the clear orange solution at 50 - 60 °C over 1 hour and stirring maintained for a further 18 hours. The resultant white precipitate formed was collected by filtration and recrystallised from water to furnish **18** as a crystalline solid.

Yield:	8.64 g, 0.022 mmol (36%).
mpt:	264 - 265 °C (lit.[75] 264 - 265 °C)
IR (cm^{-1}, $CHCl_3$):	3021, 1761, 1216, 1159, 1046.
^1H NMR (200 MHz, $CDCl_3$): δ	7.70 (m, 15H, $(C_6\underline{H}_5)_3$), 6.86 (m, 1H, $C\underline{H}PPh_3Br$), 2.90 (m, 2H, $C\underline{H}_2$), 2.50 (m, 1H, $C\underline{H}$), 2.10 (m, 2H, $C\underline{H}_2$), 1.60 (m, 1H, $C\underline{H}$).

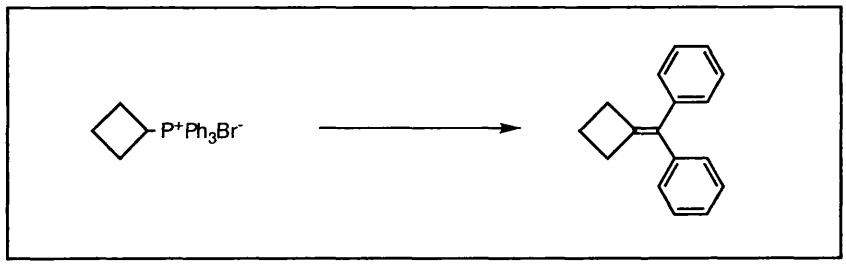
Synthesis of bicyclobutylidene (17) *via* the Wittig reaction[53]



n-BuLi (2.20 mL, 2.0 M in cyclohexane, 4.40 mmol) was added to a stirred suspension of cyclobutyltriphenyl phosphonium bromide **(18)** (1.76 g, 4.43 mmol) in dry THF (20 mL) and stirring continued until all the phosphonium bromide had dissolved (15 minutes). Cyclobutanone **(13)** (0.30 mL, 4.02 mmol) in dry THF (7 mL) was then added dropwise to the deep-red ylid solution over 5 minutes. The mixture was stirred for 30 minutes at room temperature before heating to 60 - 65 °C under nitrogen for 12 hours. Upon cooling to room temperature, dichloromethane (25 mL) was added and the resulting mixture was washed with water (3 x 10 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residual liquid was distilled under vacuum to afford **17** as a clear liquid (85 °C, 10 mmHg).

Yield:	0.051 g, 0.47 mmol (11%).
^{1}H NMR (400 MHz, $CDCl_3$): δ	1.93 (q, 4H, J = 7.2 Hz, 2 x C$\underline{H}_2$CH$_2$C=C), 2.5 (t, 8H, J = 7.7 Hz, 4 x C$\underline{H}_2$C=C).
^{13}C NMR (100 MHz, $CDCl_3$): δ	17.1, 28.9, 129.1.

Diphenylmethylenecyclobutane (50a)[73]

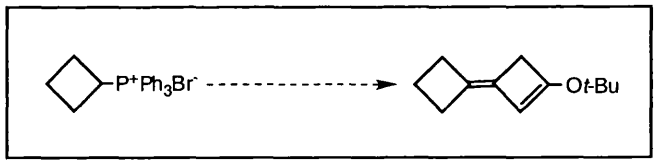


A suspension of cyclobutyltriphenyl phosphonium bromide **(18)** (0.1026 g, 0.259 mmol) in dry THF (1 mL) was treated with *n*-BuLi (0.12 mL, 2.0 M in cyclohexane, 0.24 mmol) with stirring, and the resultant mixture was stirred for a further 30 minutes at room temperature. Benzophenone **(56)** (0.04750 g, 0.261 mmol) in dry THF (0.5 mL) was then added dropwise, and the mixture was stirred for 15 minutes before heating at 60 - 65 °C under nitrogen for 12 hours. The solution was

cooled to room temperature, and dichloromethane (2 mL) was added followed by extraction with water. The organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure. Column chromatography of the residue using pentane as an eluent furnished pure diphenylmethylenecyclobutane **(50a)** as a white solid.

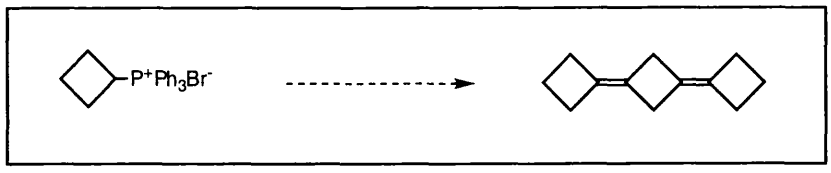
Yield:	0.05420 g, 0.246 mmol (95%).
mpt:	57 - 58 °C (lit.[42] 58 - 58.5 °C)
IR (cm^{-1}):	2956, 1653, 1492, 1441, 1072, 772, 702.
^1H NMR (400 MHz, $CDCl_3$): δ	7.36 (m, J = 8.6 Hz, 5H, $C_6\underline{H}_5$), 7.20 (m, J = 8.6 Hz, 5H, $C_6\underline{H}_5$), 2.90 (t, J = 8.6 Hz, 4H, 2 x C=C$C\underline{H}_2$), 2.00 (q, J = 8.6 Hz, 2H, $CH_2C\underline{H}_2CH_2$).
EIMS (*m/z*):	220 (M^+, 100), 165 (34), 154 (47), 143 (M^+ - C_6H_5, 42), 77 (C_6H_5, 38).

Attempted synthesis of 3-*t*-butoxybicyclobutyliden-2-ene (57)



n-BuLi (0.17 mL, 2.0 M in cyclohexane, 0.34 mmol) was added to a stirried suspension of cyclobutyltriphenyl phosphonium bromide **(18)** (0.1437 g, 0.36 mmol) in dry THF (1.8 mL) and stirring continued at room temperature until all the phosphonium bromide had dissolved (40 min). 3-*t*-Butoxycyclobutenone **(46)** (0.050 g, 0.36 mmol) in dry THF (0.8 mL) was added dropwise over 2 minutes to the deep-red ylid solution before heating at 60 - 65 °C under nitrogen for 12 hours. The solution was allowed to cool to room temperature, after which time dichloromethane (5 mL) was added. The mixture was washed with water (2 x 5 mL) and the organic layer dried ($MgSO_4$). Solvent evaporation under reduced pressure followed by column chromatography using pentane : ether (97:3) yielded several fractions. The major fraction was collected and analysed. Spectroscopic data showed unidentified compound with no *t*-butoxy functional group. An alternative extraction with a $NaHCO_3$ solution also resulted undesired products which were not identified.

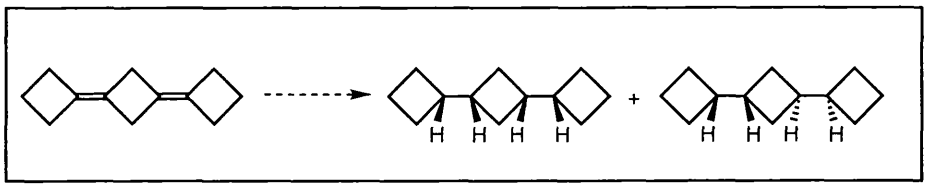
1,3-Bicyclobutylidene-cyclobutane (30)



n-BuLi (1.12 mL, 2.0 M in cyclohexane, 2.24 mmol) was added dropwise to a stirred suspension of cyclobutyltriphenyl phosphonium bromide **(18)** (0.94 g, 2.37 mmol) in dry THF (12 mL) over 5 minutes and stirring continued for a further 30 minutes at room temperature. Cyclobutyl-1,3-dione **(47)** (0.10 g, 1.19 mmol) in dry THF (2 mL) was then added dropwise to the reaction mixture and the mixture was stirred for 5 minutes before heating at 60 - 65 °C under nitrogen for 19 hours. The solution was cooled to room temperature, followed by addition of dichloromethane (10 mL) and extraction with water (5 x 10 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure. Column chromatography was performed on silica with gradient elution (pentane:EtOAc, 100:0 to 50:50). The first fraction was obtained as a clear oil and was analysed.

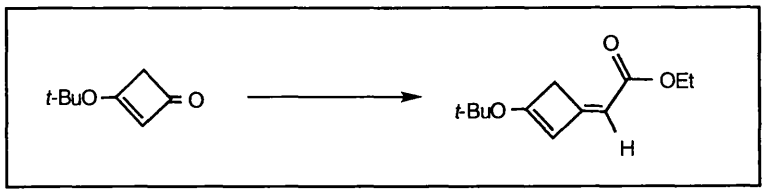
Yield:	0.002 g, 0.013 mmol (1%).
IR (cm^{-1}, CCl_4):	1550, 1251, 1005, 790.
^1H NMR (200 MHz, $CDCl_3$): δ	1.95 - 2.12 (bs), 1.57 (br, m), 1.55 (br, m), 1.26 (br, s), 0.89 (m).

Attempted hydrogenation of 1,3-bicyclobutylidene-cyclobutane (30)



A 2 mL flask fitted with a magnetic stirrer bar was charged with the cyclobutylidene derivative **30** (2 mg, 0.0125 mmol), platinum dioxide (~1 mg) and dichloromethane (1.5 mL). The flask was fitted with a 3-way tap connected to a vacuum line, and balloon filled with hydrogen. The reaction flask was evacuated and flushed with hydrogen (x3), and then left under a slight positive pressure of hydrogen for 24 hours. No notable change was observed in the ^1H NMR spectrum of the final reaction mixture.

Synthesis of (3-*t*-butoxy-cyclobut-2-enylidene)-acetic acid ethyl ester (60)



t-Butoxycyclobutenone **(46)** (0.20 g, 1.43 mmol) and carbeoxymethylene-triphenyl phosphorane were dissolved in dry THF (5 mL) with stirring under nitrogen. The solution was then heated to 70 °C for 12 hours. The resulting mixture was washed with water (2 x 5 mL) and dried ($MgSO_4$). The solvent was evaporated under reduced pressure. Purification by column chromatography on silica with petroleum ether : ether (8:1) yielded **60** as a clear oil.

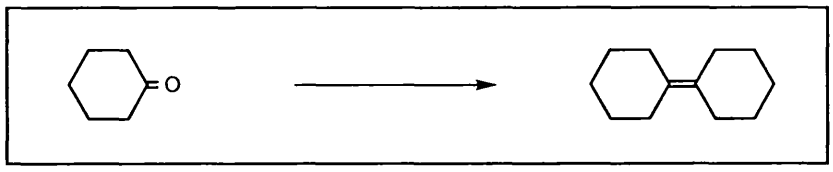
Yield:	0.0040 g, 0.019 mmol (1%).
IR (cm^{-1}, $CHCl_3$):	2982, 2400, 1732, 1605, 1476, 1369, 1257, 1161.
^1H NMR(400 MHz, $CDCl_3$): δ	6.12 (d, 1H, *J* = 7 Hz, *t*-BuO-C=C<u>H</u>), 5.88 (d, 1H, *J* = 7 Hz, C<u>H</u>=CO_2Et), 4.64 (s, 2H, C<u>H</u>$_{2cyclobutane}$), 4.22 (q, 2H, *J* = 7 Hz, C<u>H</u>$_2$$CH_3$), 1.35 (s, 9H, *t*-OBu), 1.20 (t, 3H, CH_2C<u>H</u>$_3$).
EIMS (*m/z*):	210 (M^+, 50), 137 (M^+ - O*t*-Bu, 10), 92 (M^+ - O*t*-Bu - OEt, 22), 64 (M^+ - O*t*-Bu - CO_2Et, 62), 39 (M^+ - O*t*-Bu - C=CHCO_2Et, 100).

Preparation Of $TiCl_3(DME)_{1.5}$

Titanium trichloride (0.990 g, 6.41 mmol) weighed in a glovebox was added to dry DME (14 mL) under nitrogen. The mixture was then refluxed for 48 hours under nitrogen. Upon cooling to room temperature, the mixture was filtered under nitrogen via a cannular, washed with dry pentane and dried under vacuum furnishing $TiCl_3(DME)_{1.5}$ as a fluffy light blue solid, which was used directly in the following coupling reaction.

Yield: 1.5676 g, 5.42 mmol (85%).

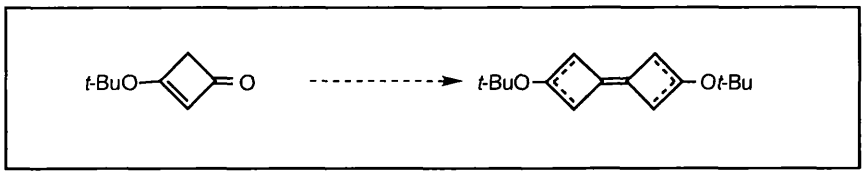
Synthesis of bicyclohexylidene[81] (62)



Zinc-copper couple (1.48 g, 20 mmol) was transferred to the $TiCl_3(DME)_{1.5}$ obtained above under nitrogen, and dry DME (30 mL) was added with vigorous stirring. The resulting mixture was refluxed for 2 hours during which time a black suspension was formed. Cyclohexanone (0.13 g, 1.33 mmol, 0.14 mL) in dry DME (3 mL) was added dropwise and the resulting mixture was refluxed for 12 hours. Upon cooling to room temperature, the reaction mixture was diluted with pentane (20 mL), filtered through Celite and concentrated under reduced pressure to afford bicyclohexylidene **(62)** as white crystals.

Yield:	0.2148 g, 1.31 mmol (98%).
mpt:	54 - 55 °C (lit.[81] 52 - 53 °C)
^{1}H NMR (400 MHz, $CDCl_3$): δ	2.18 (t, 8H, *J* = 6.1 Hz, 4 x $C\underline{H}_2C$=C), 1.51 (q, 12H, *J* = 5.6 Hz, 2 x $(C\underline{H}_2)_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	27.7, 28.6, 30.1, 129.4.

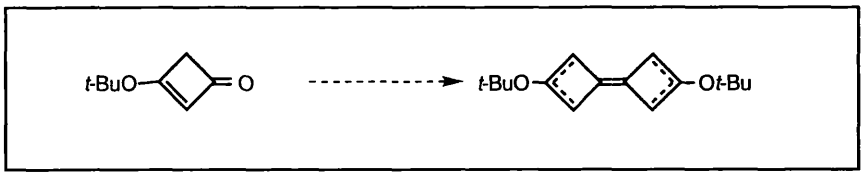
Attempted synthesis of 3,3'-di-*t*-butoxy-bicyclobutylidene-2,2'-diene (63) *via* the McMurry reaction



Zn-Cu couple (2.55 g, 34 mmol) was transferred to freshly prepared $TiCl_3(DME)_{1.5}$ (2.7 g, 9.34 mmol) under nitrogen and dry DME (50 mL) was added with vigorous stirring. The resulting mixture was refluxed for 2 hours to form a black suspension. *t*-Butoxyclobutenone **(46)** (0.25 g, 1.79 mmol) in dry DME (4 mL) was added in one portion and the resulting mixture was refluxed for a further 2 hours. After cooling to room temperature, the reaction mixture was quenched with 0.05% aqueous ammonia (50 mL). Diethyl ether (20 mL) was added, the organic layer was separated, and the aqueous layer was further extracted with diethyl ether (3 x 10 mL). The combined extracts were dried ($MgSO_4$) and concentrated by reduced

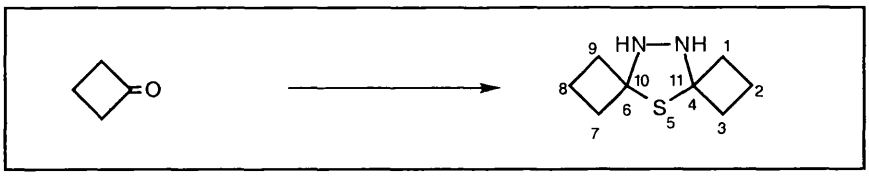
pressure. Column chromatography was performed on silica using pentane : ether, 9:1 to give an unidentified oil.

Attempted synthesis of 3,3'-di-*t*-butoxy-bicyclobutylidene-2,2'-diene (63) *via* organozinc carbenoids



Zinc-mercury amalgam (1.3 g, 20 mmol) and zinc chloride (280 mg, 2 mmol) were flame-dried in a 25 cm^3 conical flask. Ether (10 mL) was added and the mixture was heated to reflux. TMSCl (10 mL) was added followed by the dropwise addition of a solution of *t*-butoxycyclobutenone **(46)** (280 mg) in ether (2 mL). After refluxing for 15 hours, the reaction was quenched by the addition of saturated $NaHCO_3$ (5 mL). The mixture was stirred for an additional further 15 minutes, and the solids removed by filtration through celite. The ethereal layer was separated, washed with brine and dried ($MgSO_4$). Column chromatography was performed on silica, eluting with pentane : ether (9:1) to give two fractions. The ^1H NMR spectra of the material in the two fractions could not be interpreted.

Synthesis of 5-thia-10,11-dispiro[3.1.3.2]undecane (14) *via* the Barton-Kellog reaction[53]



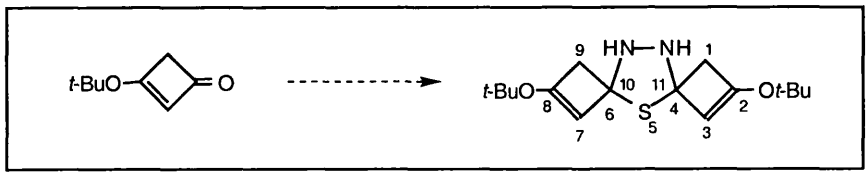
Hydrogen sulphide was bubbled through stirred cyclobutanone **(13)** (0.52 g, 0.55 mL, 7.37 mmol) at -10 °C for 20 minutes. With the continual passage of H_2S, aqueous hydrazine (0.48 mL of 7.5 M, 3.76 mmol) was added in a dropwise fashion over 20 minutes. More H_2S was introduced for a further 20 minutes, after which time a solid appeared. Dichloromethane (5 mL) was added to this crude mixture. The organic layer was separated and the aqueous fraction extracted with dichloromethane (3 x 5 mL). The combined layers were dried ($MgSO_4$) and concentrated under reduced pressure to give **14** as white crystals.

Yield: 0.23 g, 1.35 mmol (37%).

mpt: 96 - 97 °C (lit.[53] 95 - 97 °C)

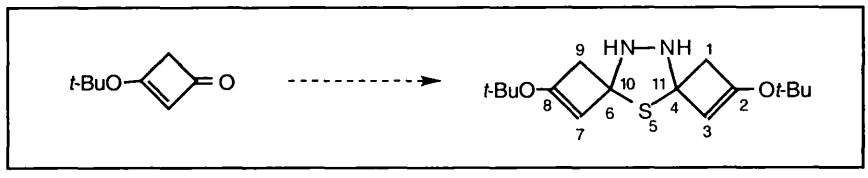
^{1}H NMR (400 MHz, $CDCl_3$): δ 3.80 (bs, 2H, NH), 2.50 (m, 4H, H-1, H-9), 2.23 (m, 4H, H-3, H-7), 1.86 (m, 4H, H-2, H-8).

Attempted synthesis of 71 *via* the Barton-Kellog reaction (I)



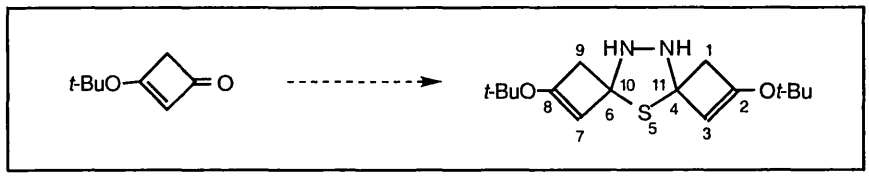
Hydrogen sulphide was bubbled through a stirred solution of *t*-butoxycyclobutenone **(46)** (0.40 g, 2.86 mmol) in dichloromethane at -10 °C for 15 minutes. With the continual addition of H_2S, aqueous hydrazine (0.21 mL of 7.5 M, 1.58 mmol) was added in a dropwise fashion over 20 minutes. H_2S was bubbled into the mixture for a further 20 minutes. Dichloromethane (5 mL) was added to the crude mixture and the organic layer separated. The aqueous layer was extracted with dichloromethane (3 x 5 mL) and the combined layers were dried ($MgSO_4$) and concentrated under reduced pressure. The starting material was recovered unreacted.

Attempted synthesis of 71 *via* the Barton-Kellog reaction (II)



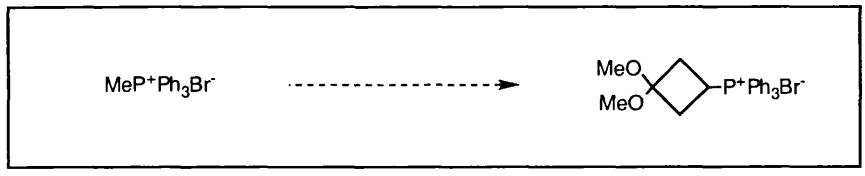
Hydrogen sulphide was bubbled through a stirred solution of *t*-butoxycyclobutenone **(46)** (0.40 g, 2.86 mmol) in methanol (6 mL) at -10 °C for 20 minutes. With continual bubbling of H_2S into the reaction mixture, aqueous hydrazine (0.21 mL of 7.5 M, 1.58 mmol) was added dropwise over 20 minutes. H_2S was added for a further 20 minutes. The solvent was removed under reduced pressure. The starting material was recovered unreacted.

Attempted synthesis of 71 *via* the Barton-Kellog reaction (III)



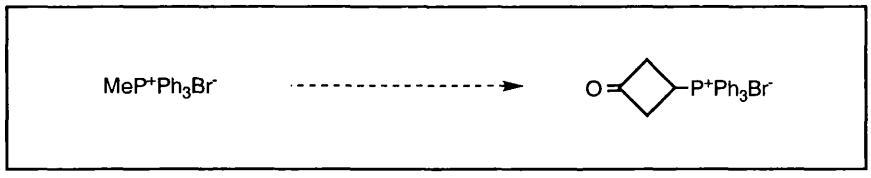
Hydrogen sulphide was bubbled through a stirred solution of *t*-butoxycyclobutenone **(46)** (0.40 g, 2.86 mmol) in ether (3 mL) at -5 °C for 15 minutes. With continual bubbling of H_2S, aqueous hydrazine (0.21 mL of 7.5 M, 1.58 mmol) was added dropwise over 20 minutes. H_2S was introduced for a further 20 minutes and the reaction mixture allowed to warm to room temperature. Ether (3 mL) was added to the crude mixture and the organic layer was separated. The aqueous portion was extracted with ether (2 x 3 mL) and the combined layers were dried ($MgSO_4$) and concentrated by reduced pressure. The ^1H NMR spectrum of the residue revealed a complex mixture with no sign of hydrazine formation.

Attempted synthesis of 3-substituted cyclobutylphosphonium salt (74)



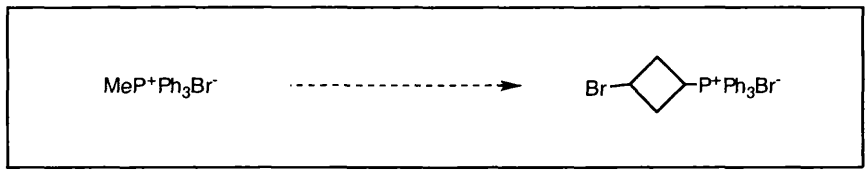
n-BuLi (1.5 mL, 2.0 M in cyclohexane, 0.003 mol) was added to a stirred suspension of methyltriphenylphosphonium bromide **(51)** (1.07 g, 0.003 mol) in dry THF (15 mL) at room temperature and stirring maintained for 1 hour. 1,3-Dibromo-2,2-dimethoxypropane **(73)** (0.39 g, 0.015 mol) in dry THF (6 mL) was then added to the yellow solution of the ylid at 50 - 60 °C over 20 minutes. The reaction mixture was heated to reflux for 83 hours and then stirred at room temperature for 24 hours. The resultant white precipitate was filtered under nitrogen and vacuum dried. Only the starting materials were recovered.

Attempted synthesis of 3-substituted cyclobutylphosphonium salt (76)



n-BuLi (6.9 mL, 2.0 M in cyclohexane, 0.012 mol) was added to a stirred suspension of methyltriphenylphosphonium bromide **(51)** (4.30 g, 0.012 mol) in dry THF (60 mL) and the stirring was continued for 1 hour. 1,3-Dibromo-2-acetone **(75)** (1.30 g, 0.006 mol) in dry THF (24 mL) was added dropwise to the yellow solution of the ylid at 50 - 60 °C over 20 minutes. The reaction mixture was stirred at this temperature for a further 3 hours and then at room temperature for an additional 12 hours. The resultant brown precipitate was filtered and dried under vacuum. Attempted recrystallisations using water or ethanol or an ethanol-water mixture were all unsuccessful.

Attempted synthesis of 3-substituted cyclobutylphosphonium salt (78)



n-BuLi (6.9 mL, 2.0 M in cyclohexane, 0.012 mol) was added to a stirred suspension of methyltriphenylphosphonium bromide **(51)** (4.28 g, 0.012 mol) in dry THF (60 mL) and stirring continued for 1 hour. 1,2,3-Tribromopropane **(77)** (1.68 g, 0.006 mol) in dry THF (24 mL) was added in a dropwise fashion to the yellow solution of the ylid at 50 - 60 °C over 20 minutes. Stirring was maintained at this temperature for a further 3 hours and then at room temperature for 12 hours. The resultant brown precipitate was filtered and dried under vacuum. Attempted recrystallisations using water or ethanol or an ethanol-water were all unsuccessful.

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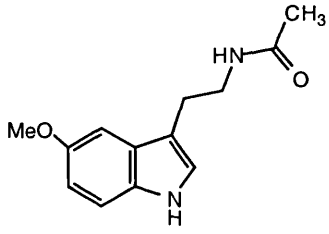
PART II: MAPPING THE MELATONIN RECEPTOR

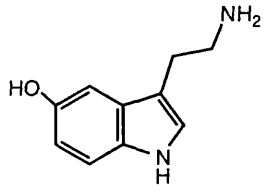
INTRODUCTION

I.A. History of melatonin

Melatonin was first discovered in 1917 by McCord *et al.*,[1] who observed that an extract of pineal gland caused blanching of tadpole skin, but it did not attract much interest until 1958 when Lerner *et al.*[2] isolated melatonin from bovine pineal tissue and identified the structure of the hormone to be *N*-acetyl-5-methoxytryptamine **(1)**. Lerner also named the molecule *melatonin*; *mela* because it lightens *mela*nophores and *tonin* because it is a derivative of sero*tonin* **(2)**.



1



2

Melatonin was tried as a therapeutic agent for treating irregular skin pigmentation until its anti-gonadotrophic activity was noticed by Wurtman *et al.*,[3] who reported that persistent estrus induced by constant light could be reduced by administering pineal extracts. Quay *et al.*[4] then demonstrated the light-dark cycle dependence of serotonin, the precursor of melatonin, in the pineal gland of mice with the highest level occurring during daytime and lowest during the night. In an investigation of the influence of the pineal gland on reproductive functions under the effects of light, Hoffman and Reiter[5] encountered the photoperiod-dependence of melatonin level. These observations provided clues to the pineal function.

I.B. Nature and properties of melatonin

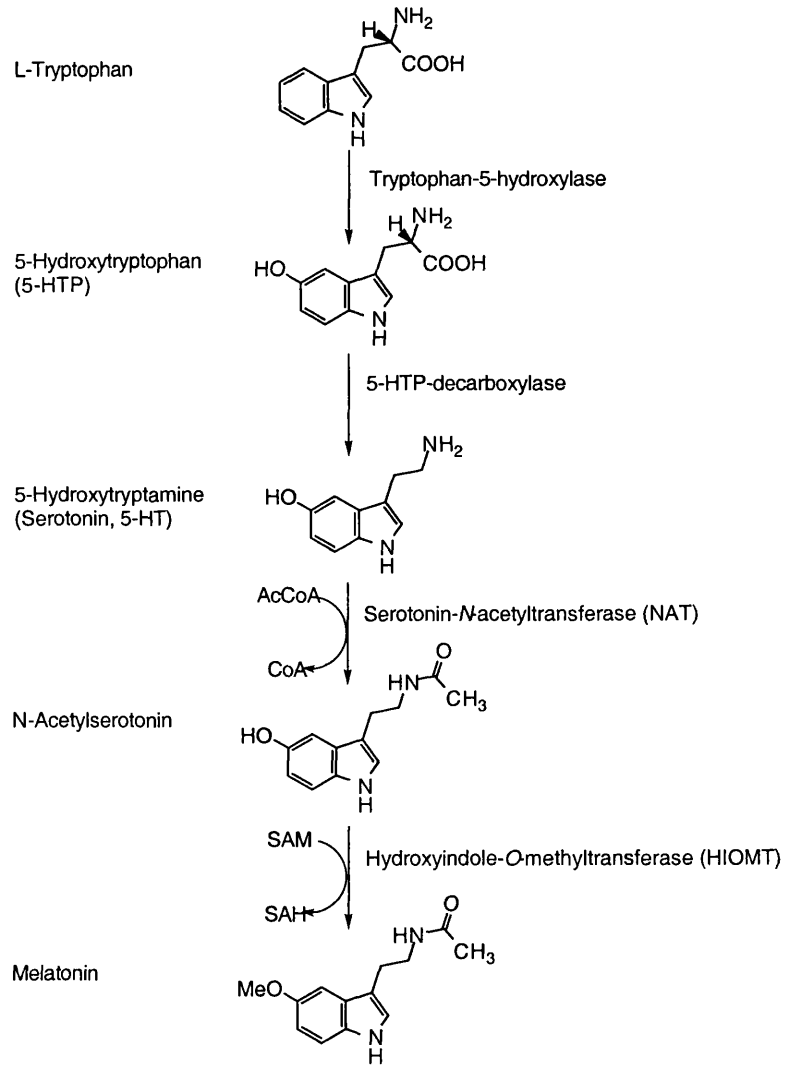
Melatonin is known to have a diverse range of actions which can be thought of as adaptive responses to the physiological system of all species.[6] The most important physiological function of this hormone is to convey the photoperiodic and seasonal information to physiological systems that regulates various processes such as reproduction,[7] sleep/rest activity,[8] body temperature[9] and circadian rhythm in animals.[10] In mammals, the effect of melatonin during prenatal development involves melatonin secreted primarily by the mother. Through melatonin, the foetus gets information about the periodicity of the environment.[11] Recent research on the sites and mechanisms of its circadian clock function suggested that melatonin alleviates jet-

lag[12] and promotes sleep.[13] How melatonin is involved in sleep regulation is unclear, although there has been a suggestion that it promotes sleep by shutting off wakefulness, rather than by actively inducing sleep.[13] As a rhythm regulator, melatonin controls the phase and amplitude of circadian rhythm by acting both on the suprachiasmatic nucleus (SCN) and on different cells and tissues of the body. Because of its action, melatonin has been named as a "chronobiotic" or "internal zeitgeber".[14] This property has been extended to its use as retraining agent for the disrupted circadian rhythms in the blind,[15] shift workers,[16] and subjects with delayed sleep phase syndrome.[17] Studies of melatonin rhythms in depressed patients indicate that melatonin also plays a part in the control of human mood and behaviour.[18,19] Melatonin is also found to be a powerful free radical scavenger and preventive antioxidant which detoxifies a variety of neurotoxic free radicals and reactive intermediates, including the hydroxyl radical ($^{\cdot}OH$), peroxynitrite anion ($ONOO^-$), singlet oxygen ($^1O_2$) and nitric oxide ($NO^{\cdot}$).[20] It has been shown to markedly protect both membrane lipids and nuclear DNA from oxidative damage. Poeggeler *et al.*[21] suggested that the acetyl group on the side chain and the methoxy group at the C-5 position of the melatonin molecule are essential for its radical scavenging action. In the reaction with free radicals, it has been proposed that melatonin contributes an electron to suppress the toxicity of the free radical. In doing so, it is converted into a weak indolyl cation radical which then scavenges superoxide radicals. The indolyl cation radical is then converted into a melatonin metabolite, *N*1-acetyl-*N*2-formyl-5-methoxykynurenamine. Acting as an antioxidant, melatonin stimulates the activities of antioxidative enzymes and these in tum decompose free radicals.[22] The protective effects of melatonin against free radicals has led to suggestions of its use in the treatment of cancer[23] and to slow down the process of ageing.[24] All of these claims have, however, been disputed.

Recent investigations of Pappolla *et al.*[25] on the mechanism of action of melatonin revealed the hormone's cytoprotective properties which may also lead to its potential use in the treatment of Alzheimer's disease. Deposits of amyloid β protein (Aβ), a neurotoxic amino acid peptide, are found to be widely distributed as amyloid fibrils in the brains affected with Alzheimer's disease. Aβ peptides with high contents of β- sheets are partially resistant to proteolytic degradation. The report has shown that at pharmacological concentration, melatonin protects neurons against Aβ toxicity and inhibits the progressive formation of β-sheets and amyloid fibrils which occurs in patients with Alzheimer's disease.[26]

I.C. Biosynthesis

The potential roles of melatonin may be understood by the way it is produced and secreted in the pineal gland. In all species examined, melatonin is only secreted during the hours of darkness and hence is termed "darkness hormone". It is generated mainly in the pineal gland although synthesis also occurs in the retina,[27] the gastrointestinal tract,[28] in blood platelets,[29] and in erythrocytes.[30] In mammals, these sources contribute far less to the melatonin levels in the blood than the pineal gland, but may be of local importance.



Scheme 1. Biosynthetic pathway of melatonin **(1)** in the pineal gland.

The biosynthetic pathway of melatonin was first elucidated by Axelrod[31] in 1974 (Scheme 1). The first step in the formation of melatonin is the uptake of the dietary amino acid *L*-tryptophan from the circulation into the melatonin-synthesising tissues. Catalytic oxidation of *L*-tryptophan is then carried out by the enzyme tryptophan hydroxylase to give *L*-5-hydroxytryptophan. This is followed by decarboxylation to form serotonin, which is then acetylated with acetyl coenzyme A (AcCoA) by the enzyme *N*-acetyl transferase (NAT), whose activity is highest during the dark hours of the day-night cycle. The final step in the pathway to form melatonin is the *O*-methylation of the *N*-acetylserotonin by the enzyme hydroxyindole-*O*-methyltransferase which transfers a methyl group from *S*-adenosyl methionine (SAM). The resultant lipophilic melatonin is not stored in the pineal gland but, once formed, diffuses freely into the bloodstream and general circulation.

I.D. Control of melatonin biosynthesis

The most distinctive features of the melatonin-generating system are its diurnal or circadian variation and its sensitivity to light, which suppresses its activity. The pineal glands of birds, reptiles, amphibians and fish have photoreceptive cells that respond directly to environmental light to produce nerve impulses.[32] However, photoreceptive elements are not found in mammalian pineal cells. In mammals, the circadian "clock", which establishes its rhythmicity of approximately 24 hours, is located in the hypothalamic suprachiasmatic nuclei (SCN). Photic information is received by the SCN directly from the retina *via* the retinohypothalamic tract. Neurochemical and chronobiological information is then sent from the SCN to the paraventricular nuclei (PVN) through fibres in the medial forebrain bundle and reticular formation to the intermediolateral nucleus of the spinal cord. From there, signals pass to preganglionic adrenergic fibres of the sympathetic nervous system, which conduct them to the superior cervical ganglia (SCG), and then through postganglionic fibres to the pineal body (Figure 1).

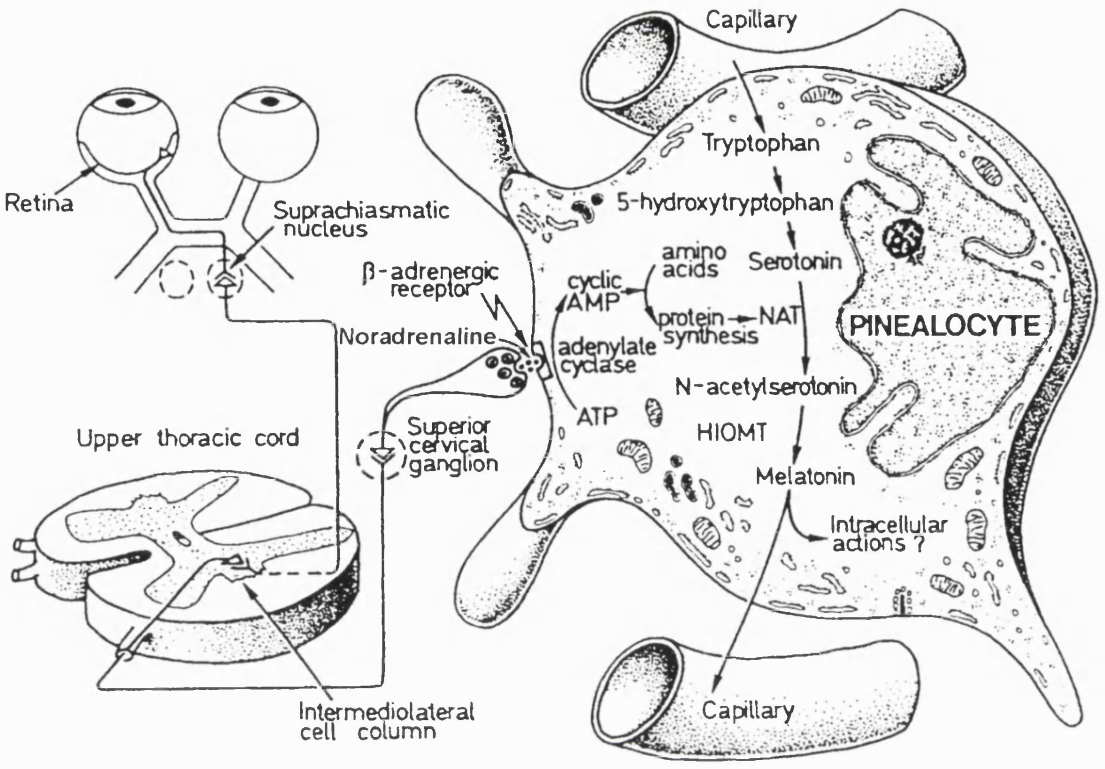


Figure 1.[33] Regulation of the pineal gland, taken from Reiter, R.J. *Ann. Med.* 1998, **30**, 103.

Noradrenaline (NA) is discharged from the postganglionic fibres as a neurotransmitter which binds to the β-adrenergic receptors, activating the enzyme adenylate cyclase *via* a stimulatory guanine nucleotide binding protein, Gs, resulting in the synthesis of cyclic adenosine monophosphate (cAMP).[34] The magnitude of melatonin secretion induced by NA has been shown to be modulated by neuropeptide Y acting as a co-transmitter also in the postganglionic fibres. The recent observation[35] that some species display a strong annual variation in pineal neuropeptide Y innervation and in the length and amplitude of the nocturnal melatonin peak indicates that neuropeptide Y could be a transmitter involved in regulating the seasonal rhythm of melatonin secretion.

In the adenylate cyclase-cyclic AMP system, adenosine triphosphate (ATP) is converted into cyclic AMP which in turns activates the enzyme *N*-acetyltransferase (NAT). NAT catalyses the acetylation of 5-hydroxytryptamine (5-HT), a precursor to melatonin biosynthesis.[36]

NA also concomitantly acts through postsynaptic α_1-adrenergic receptors to stimulate the breakdown of membrane phosphatidylinositol, transiently forming diacylglycerol which interacts with protein kinase C (PKC) in the presence of membrane phospholipids and Ca^{2+}.[37] This PKC activation amplifies the β-receptor-induced cyclic AMP production activity[32] but also has a negative feedback effect, inhibiting further α_1-adrenergic stimulation. The α_1-adrenergic receptors are less significant compared to the β-receptors.[38] However, this does not preclude the possibility that α-mediated events may be very important for some undefined pinealocyte functions. The α-receptor binding sites have been little investigated and of the three case studies in rats, only one reported a variation in α-adrenergic receptor numbers over a 24-hour period.[39-41] Pharmacological studies suggest that the α_1-adrenergic receptors in the pineal gland are predominantly of the α_{1B} subtype.[42]

Besides α_1- and β-adrenergic receptors, other neurotransmitter or neuromodulator receptors which may have influence on the secretion of melatonin have been described in the mammalian pineal gland. These include the muscarinic,[34] vasoactive intestinal polypeptide (VIP),[43] γ-amino butyric acid (GABA),[44] dopaminergic,[45] benzodiazepinergic,[46,47] serotoninergic,[48] and glutaminergic[49] receptors.

Muscarinic receptors resemble the α_1-adrenergic receptors in that they induce the hydrolysis of membrane phosphoinositides.[50] The muscarinic receptors respond to both acetylcholine and noradrenaline with an increase in intracellular Ca^{2+} and this increases the noradrenaline-dependent accumulation of cyclic AMP and cyclic guanosine monophosphate (cGMP). However, it is not clear whether cyclic GMP plays an important role in transduction of any of the effects of melatonin.[34]

It has been shown in early studies of the rat pineal gland that VIP receptors, present in the nerve endings, resemble β-adrenergic receptors that interact with the α_1-adrenergic receptors, inducing a rapid increase in the pinealocyte cyclic AMP which again stimulates the NAT activity.[43] Pévet and co-workers[51] further demonstrated that VIP receptors induce melatonin secretion from perfused pineals or cultured pinealocytes. The nature of the stimuli which cause the release of VIP from the nerve endings in the pineal and whether VIP receptors participate in the physiological regulation of melatonin remains unknown.

GABA receptors appear to be of major importance in synaptic processing within the brain and are present at both post- and presynaptic sites. It has been demonstrated in rat and bovine pineals that GABA, released by exposure to NA,

behaves as a modulating inhibitory pineal signal which impairs NA postsynaptic effects. It also impairs Ca^{2+} inflow and the release of 5-HT.[44] However, its role in the process of melatonin synthesis is unclear.

Dopamine is shown to be partially responsible for the light-induced suppression of retinal melatonin production. In 1986, Iuvone and Besharse demonstrated that dopamine, the predominant catecholamine in the vertebrate retina, inhibits the nocturnal increase of the retinal NAT activation.[45] Recent studies on the retina of mice[52] and chicken[53] also suggest that stimulation of dopaminergic receptors decreases the intracellular cyclic AMP level, although more research is needed to determine the molecular mechanism(s) of underlying dopaminergic receptor-induced suppression of NAT activation.

The existence of benzodiazepine receptors in the pineal glands of human,[46] cattle[54] and rats[47] have been reported. In rats these receptors, located in the pinealocytes, are shown to be suppressed by light stimulation[47] and are known to enhance NA stimulation of NAT *in vitro*.[47] The role and physiological relevance of pinealocyte benzodiazepine receptors in this response and its mechanism await full investigation.

L-Glutamate is a negative regulator of melatonin synthesis through glutaminergic receptor-mediated inhibition of cyclic AMP. It has been reported that the nicotinic acetylcholine receptor can trigger the peripheral glutaminergic systems in cultured rat pinealocytes. It has also been shown that cholinergic stimulation by acetylcholine or nicotine inhibits the NA-dependent NAT activation, which results in decreased melatonin synthesis.[49]

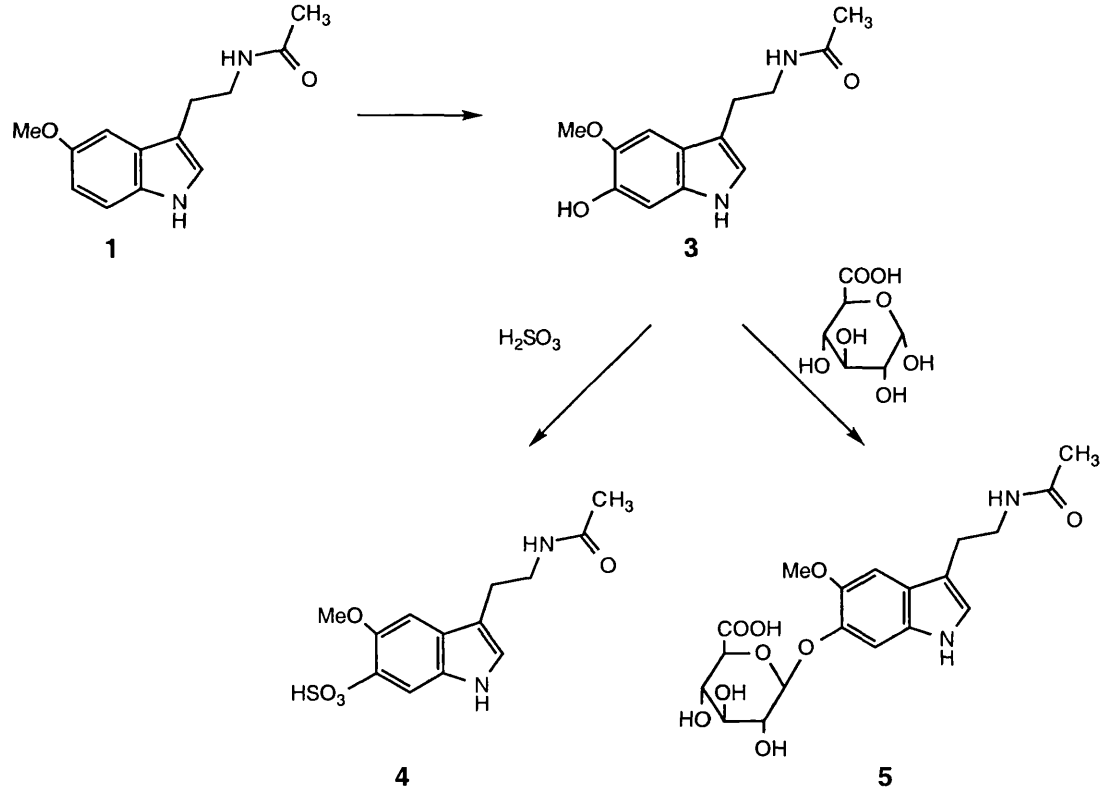
In hamsters, SCN photic-dependent phenomena are regulated by the serotoninergic input. This originates from the SCN neurons whereas in rats, the serotoninergic regulation is mediated through a serotoninergic control of the pathway conveying photic input to the SCN. The mechanism and the role of the serotoninergic receptors in melatonin synthesis is, however, still unknown.[48]

Recently, the retinal photoreceptors that transmit light information to SCN have been identified.[55] Research showed light-induced melatonin suppression in people with colour blindness, indicating that the photoreceptors contain either rod-like photoreceptors or short wavelength blue cone receptors.[55] Studies conducted on blind people further demonstrated an entire light-suppressed melatonin secretion in some patients. The above findings revealed that photoreceptors which mediate photo-transduction to SCN are distinct from the receptors that mediate visual information.[56]

I.E. Melatonin metabolism

I.E.A. Liver metabolism

Melatonin of pineal origin is metabolised primarily within the liver and secondarily within the kidney by a two-step reaction.[57] Initially, it undergoes hydroxylation at the C-6 position to form 6-hydroxymelatonin **(3)**. Subsequent conjugation of **3** with sulphonic acid or glucuronic acid give the more hydrophilic sulphate **4** or glucuronide **5**. The resulting conjugated metabolites are then excreted by the kidney (Scheme 2). The ratio of the two metabolites formed probably depends on the species. In human and rodents the main metabolite is 6-sulphatoxymelatonin, whereas in sheep 6-hydroxymelatonin glucuronide predominates.[57]

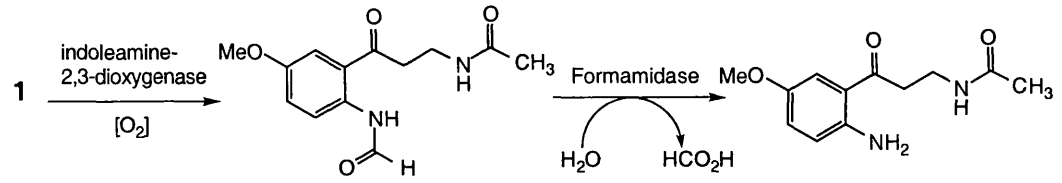


Scheme 2. Metabolism of melatonin **(1)** in the liver.

I.E.B. Brain metabolism

A minor route for melatonin metabolism occurs in the brain. In this process, melatonin undergoes enzymatic cleavage of the C2-C3 bond by indoleamine-2,3-dioxygenase to form *N*^{1}-acetyl-*N*^{2}-formyl-5-methoxykynurenamine **(6)** which is then degraded to *N*^{1}-acetyl-5-methoxykynurenamine **(7)** by the action of formamidase[58,59] (Scheme 3). It has been reported that these metabolites may have

similar activities to melatonin in the regulation of circadian rhythms and are also inhibitors of prostaglandin synthesis.[59]



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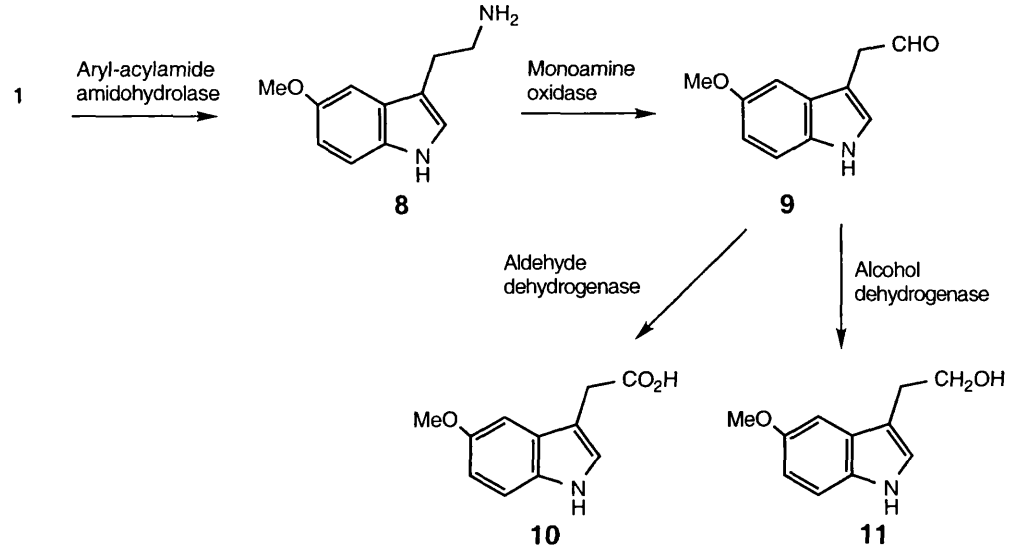
Scheme 3. Metabolism of melatonin **(1)** in the brain.

I.E.C. Retinal metabolism

Studies on cultured eye cups of frogs, goldfish, lizards and chickens[60,61] indicated that the retinae of these animals can rapidly metabolise melatonin. This metabolic route involves three reaction steps. Initially, aryl-acylamide amidohydrolase catalyses deacetylation of melatonin to 5-methoxytryptamine **(8)**. The resulting 5-methoxytryptamine **(8)** is then oxidatively deaminated by monoamine oxidase to form 5-methoxyindole acetylaldehyde **(9)** which is then either further oxidised to 5-methoxyindoleacetic acid **(10)** or reduced to 5-methoxytryptophol **(11)** (Scheme 4).[62]



8 9 10 11

Scheme 4. Metabolism of melatonin **(1)** in the retina.

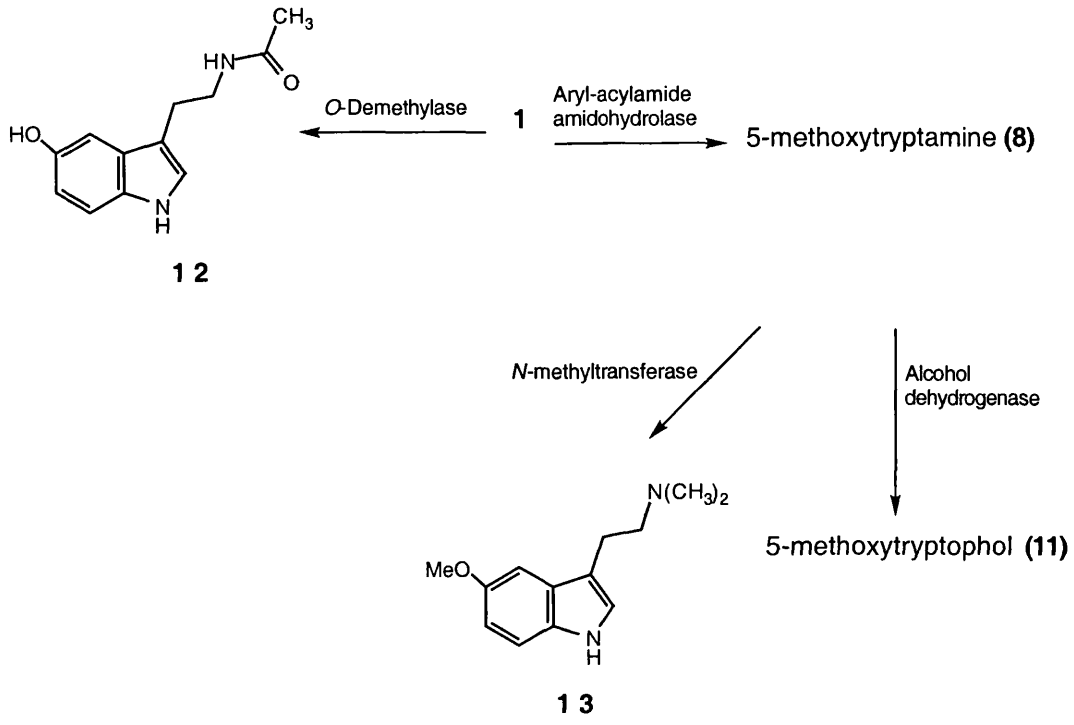
I.E.D. Bioactive melatonin metabolites

The spectrum of actions exerted by melatonin is certainly not limited to the molecule itself, but also seems to be due to its active metabolites. Among these active metabolites, several are indolic compounds (Scheme 5).[63] Melatonin can undergo rapid reconversion to its precursor *N*-acetylserotonin **(12)** in mammals and birds, especially in their retinae.[64,65] Although it has been shown in blood lymphocytes that *N*-acetylserotonin has a better extra- and intracellular antioxidant activity than melatonin,[66] the exact physiological role remains to be discovered.

Much better understood are the effects of 5-methoxylated indoles, especially of 5-methoxytryptamine **(8)**, which is formed either by direct *O*-methylation of serotonin **(2)**,[67-69] or by deacetylation of melatonin **(1)**.[60,61,70] The circadian rhythm of 5-methoxytryptamine biosynthesis is out of phase with the melatonin rhythm, that is the level of the amine peaks during daytime. However, like melatonin, 5-methoxytryptamine shows antigonadotropic activity[71-73] and promotion of sleep in vertebrates.[74] Other effects demonstrated by 5-methoxytryptamine include autoreceptor-mediated inhibition of serotonin release,[75] and the reduction of vasopressin immunoreactivity.[76] It also causes stimulation of light emission and encystment of cells in the bioluminescent unicellular alga *Gonyaulax polyhedra*,[77,78] and a suppression of thyroid hormones in a teleost fish, *Clarias batrachus*.[79]

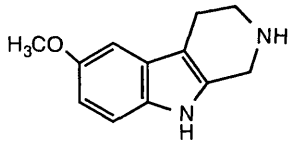
Another indole compound with biological activity is 5-methoxytryptophol **(11)**, which is produced either by monoamine oxidase and alcohol dehydrogenase from **8** or by *O*-methylation of 5-hydroxytryptophol. In vertebrates, 5-methoxytryptophol is found in the pineal gland,[80,81] the retina[82] and the Harderian glands.[83] 5-Methoxytryptophol exhibits circadian rhythmicity,[80-85] seasonal rhythmicity[80] and both pro- and antigonadotropic activity in fish, birds and mammals.[71,86-89] It is also found to be a potent activator of rod disc shedding in *Xenopus laevis* eyecup preparations[90] and an inhibitor of phagocytic activity of cultured chick retina pigment epithelium cells.[91] The effects of 5-methoxytryptophol are again very similar to those of melatonin.

N,N-Dimethyl-5-methoxytryptamine **(13)**, derived from *N*-methylation of **8**, is an extremely potent serotoninergic ligand. This, together with other *N,N*-dimethylated indoleamines constitute a class of endogenous hallucinogens which are found in elevated concentrations in the urine of schizophrenics. The link between over-production of these compounds and mental disorders remain to be elucidated.

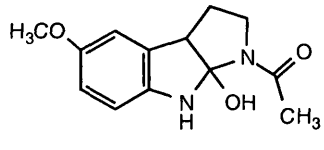


Scheme 5. Catabolism of melatonin **(1)** to form bioactive metabolites.

Another pineal indole metabolite, 6-methoxy-1,2,3,4-tetrahydro-β-carboline (pinoline, **14**) is formed from the cyclisation of serotonin followed by methylation. It has been found in similar concentrations to those of melatonin in all mammalian and avian organs which produce large amounts of melatonin.[92,93] β-Carbolines, in general, are well known for their psychomimetic actions[92,94] owing to their binding to the benzodiazapine receptor[95] and their modulation of the affinity of the impramine binding site related to the serotonin transporter.[96,97] Recent *in vitro* studies of the antioxidant effect of pinoline **14** showed that it efficiently reduces H_2O_2-induced lipid peroxidation and may therefore be a potential neuroprotective agent in the prevention of oxidative brain damage.[98]



pinoline **(14)**



cyclic 2-hydroxymelatonin **(15)**

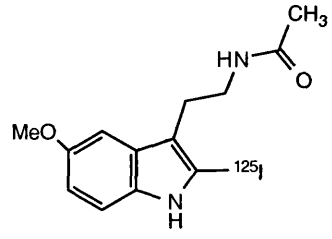
The metabolism of melatonin is not restricted to the formation of indolic compounds. Cyclisation can occur in conjunction with hydroxylation and saturation of the 2,3-double bond of melatonin to form a second 5-membered ring resulting in **15**, a cyclic isomer of 2-hydroxymelatonin, which is found in the urine of human and rat.[99] The biological activity of the cyclic metabolite has not been fully established but structure-activity studies[100] suggested that it may have skin-lightening effect like melatonin.

II. Melatonin binding sites

II.A. Discovery

While melatonin has been known and studied rather extensively, the search over many years for its physiological receptor sites had been unproductive. The lack of success reflected technical problems and the lack of necessary pharmacological tools, such as bioassays, high affinity ligands with high specific activity, and selective pharmacological agents. There was no information on the localisation of melatonin-responsive tissues and cells. Although several groups reported the existence of melatonin receptors in a number of tissues such as brain[101,102] and cytoplasm[103] using [^{3}H]-melatonin as radioligands to measure receptor binding, the results obtained were often contradictory because of technical difficulties and the low specificity of the ligand.

A major breakthrough in the discovery of melatonin receptors occurred in 1984 when Vakkuri and co-workers[104] investigated the preparation of radioiodinated melatonin for use as a tracer in radioimmunoassays and found that the iodine was incorporated at the C-2 position of the indole moiety to produce 2-[^{125}I]iodomelatonin **16**. The 2-[^{125}I]iodomelatonin was shown to have a higher binding affinity (K_i = 2.5 nM) to the melatonin receptors in the chick brain membrane binding assay than melatonin (K_i = 6.3 nM). Three years later, the biological activity of 2-[^{125}I]iodomelatonin was shown to mimic melatonin as a potent inhibitor of evoked dopamine release in the chicken retina.[105] The development of 2-[^{125}I]iodomelatonin as a selective, high-affinity ligand of high specific activity has provided the long-awaited tool to allow the identification and localisation of melatonin-binding sites.[106] ^{125}Iodine produces both β and γ radiation of high specific activity. 2-[^{125}I]Iodomelatonin is of fundamental importance in detecting low density receptors in both tissue homogenate-binding assays and in receptor autoradiography.[107,108]



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2-[^{125}I]Iodomelatonin-binding sites are very species-specific but have been localised in a wide range of brain sites and in peripheral tissues. In lower vertebrates, binding sites are found in several neural and peripheral structures, whereas in

mammals their distribution seems to be much more restricted.[107,108] Consistent with melatonin's role in transducing photoperiodic information, receptors have been detected in the retina and retinorecipient structures of the hypothalamus and thalamus of chicken[109] and goldfish.[110] On the other hand, the regions which show saturable and reversible 2-[^{125}I]iodomelatonin labelling in most of the mammals studied are the SCN[111] and the pars tuberalis (PT) of the pituitary gland.[112] This finding is consistent with the roles of the SCN and the PT in circadian rhythmicity and photoperiodic responses, respectively. Melatonin receptors have also been identified in the pre-optic area,[57] cerebellum,[113] pineal gland,[106,114] and pars distalis (PD) of the pituitary.[115] Peripheral melatonin binding sites have been localised in a number of tissues such as the spleen,[116,117] Harderian gland,[118] thymus,[119] kidney,[120,121] jejunum,[122], granulosa cells,[123] prostate,[124] ovary and testis.[125] The precise anatomical distribution of specific labelling within each tissue still remains to be determined. Specific 2-[^{125}I]iodomelatonin binding sites identified by *in vitro* autoradiography include the cerebral and caudal arteries, and these may be involved in cardiovascular and thermoregulatory functions.[126]

II.B. High and low affinity melatonin receptors

Based on pharmacological and kinetic analysis of 2-[^{125}I]iodomelatonin binding data, Dubocovich classified the binding sites to ML_1 and MT_3 subtypes (Table 1).[127] For recent nomenclature and classification of melatonin receptor approved by the Nomenclature Committee of the International Union of Pharmacology, see Appendix.] The ML_1 binding site corresponds to the high-affinity receptor binding (K_d 20 - 400 pM) and is reversible and saturable to the binding of 2-[^{125}I]iodomelatonin. The kinetics of association and dissociation are slow and are temperature-dependent, with an increasing affinity with rising temperature.[105,128-131] On the other hand, the MT_3 receptor has a low affinity for 2-[^{125}I]iodomelatonin (K_d > 1 nM) and its fast kinetics of association and dissociation is demonstrated in brain membranes of hamsters and in the melanoma cell line.[132-135]

Table 1. Comparison of the high-affinity and low-affinity binding sites.*

	ML_1	MT_3
Common names	High affinity (pM)	Low affinity (nM)
Kinetics		
Association	Slow ($t_{1/2}$ = 9 - 60 min)	Fast ($t_{1/2}$ = 1 - 2 min)
Dissociation	Slow ($t_{1/2}$ = < 40 min)	Fast ($t_{1/2}$ = 1 - 2 min)
Regulation		
GTP	yes	no
Na^+	yes	no
Ca^{2+}	yes	no
Mg^{2+}	no	no
Temperature	yes (affinity increase)	yes (affinity decrease)
Ranked order of affinity	I > M ≥ 6 > N >> Prazosin > 5HT	I > 6 > MIA > AO > 5-M-MCA-NAT > Prazosin ≥ N ≥ M >> 5HT

I = 2-iodomelatonin; M = melatonin; 6 = 6-chloromelatonin; N = *N*-acetyl-5-hydroxytryptamine; AO = acridine orange; 5HT = 5-hydroxytryptamine; MIA = methyl-isobutyl-amiloride; 5-M-MCA-NAT = 5-methylcarbonylamino-*N*-acetyltryptamine.

*Data taken from Dubocovich, M. L. *Trends Pharm. Sci.* 1995, **16**, *2*, 50.[127] and Paul *et al. J. Pharmcol. Exp. Ther.* 1999, **290**, *1*, 334.[136]

II.B.A. High affinity (ML_1) binding sites

Signal transduction studies revealed that the high-affinity melatonin receptors negatively coupled to a pertussis toxin-sensitive G protein to inhibit cyclic AMP formation.[127] Such receptors may also inhibit the adenylyl cyclase activity[139] and electrical activity of cells in the SCN.[137]

Cloning of a melatonin receptor was first achieved by Reppert and co-workers.[138] They successfully expressed a receptor from the *Xenopus* dermal melanophores, which is now designated Mel_{1C}. Subsequently, receptors were cloned from birds[147] and mammals[144] and two other subtypes were recognised, now designated mt_1 and MT_2. These three subtypes form a distinct family family in the large superfamily of G protein-coupled receptors. When members of the three subtypes are compared, the amino acid identity is 53 - 74%.[141,142]

G protein-coupled melatonin receptors are made up of a single polypeptide chain which has seven transmembrane domains connected by intra- and extracellular loops, an intracellular C-terminus, an extracellular N-terminus and several amino acid residues.[143]

Full length mt_1 receptors have now been cloned from human,[144] mouse,[145] sheep,[144] hamster[144,146] and chicken.[147] Receptor fragments of mt_1 have also been cloned from rat,[144] pig,[148] bovine,[148] *Xenopus laevis*[147] and zebrafish.[147] The cloned mammalian receptors are more than 80% identical with each other in the amino acid sequence, and can therefore be classified as the same receptor species. This mt_1 receptor is expressed in the rodent SCN and pars tuberalis which are the presumed sites of the circadian and reproductive actions of melatonin; hence the receptor is likely to mediate some circadian and reproductive responses in mammals.[144] The mt_1 receptor may account for all the 2-[^{125}I]iodomelatonin binding observed by *in vitro* autoradiography in mammals.

A full-length MT_2 receptor was only cloned from human tissues[149] although receptor fragments have been cloned from rats,[149] mouse,[146] hamsters,[146] chicken,[150] *Xenopus laevis*[147] and zebrafish.[147] The human MT_2 receptor is 60% identical in amino acid sequence to the human mt_1 receptor and has very similar ligand binding characteristics. The MT_2 receptor mRNA has been detected in human retina, brain and hippocampus but not in the brain or pituitary of the rat. This receptor may mediate the known actions of melatonin in the retina of some mammals.

The Mel_{1c} receptor has only been detected in non-mammalian vertebrates and has not yet been cloned in mammals. The full-length Mel_{1c} receptors have been cloned from chicken[147] and *Xenopus laevis*[138,151] and a receptor fragment has been cloned from zebrafish.[147] These receptors are found to be 80% identical in their amino acid sequence with the frog receptor at the amino acid level but only 60% identical to mt_1 and MT_2 receptors. However, they have similar pharmacological and functional properties to the mammalian mt_1 and MT_2 receptors (Table 2).

Table 2. Characteristics of the cloned G protein-coupled melatonin receptors.*

Characteristics	Melatonin receptor subtypes		
	mt_1	MT_2	Mel_{1c}
Clones			
human	yes	yes	no
rat	yes[a]	yes[a]	no
mouse	yes	yes[a]	no
hamster	yes	yes[a]	no
sheep	yes	no	no
pig	yes[a]	no	no
bovine	yes[a]	no	no
chicken	yes	yes[a]	yes
Xenopus	yes[a]	yes[a]	yes
zebrafish	yes[a]	yes[a]	yes[a]
K_d[b] (pM)	20 - 40	160	20 - 60
inhibit cyclic AMP	yes	yes	yes
Pharmacology	I > M ≥ 6 > N >> 5HT	I > M = 6 > N >> 5HT	I > M ≥ 6 > N >> 5HT

[a]Only fragments of complementary DNA were cloned.

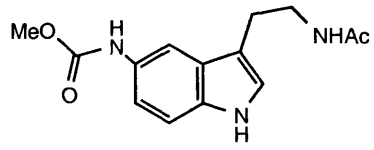
[b]K_d values were determined using 2-[^{125}I]iodomelatonin as the ligand. I = 2-iodomelatonin; M = melatonin; 6 = 6-chloromelatonin; N = *N*-acetyl-5-hydroxytryptamine; 5HT = 5-hydroxytryptamine.

*Data taken from Kokkola, T.; Laitinen, J.T. *Ann. Med.* 1998, **30**, 88[141] and Reppert, S.M.; Weaver, D.R.; Godson, C. *Trends Pharm. Sci.* 1996, **17**, 100.[140]

II.B.B. Low affinity (MT_3) binding sites

In contrast to the cloned high-affinity G protein-coupled melatonin receptors, a distinct low-affinity melatonin binding site, MT_3, has been identified in the hamster hypothalamus,[132] RPMI-1846 Syrian hamster melanoma cells,[134] and peripheral organs such as the intestine and kidneys.[136] This MT_3 binding site is characterised by its rapid association and dissociation rates for the radio ligand, with peak melatonin specific binding reached at 4 °C.[132] Pharmacological studies have shown that the rank of order for the potency of compounds at the MT_3 site is different from any of the cloned melatonin receptor family[127,134] (Table 1). While the high-affinity sites of melatonin receptors are negatively coupled to the activity of adenylate cyclase *via* a G protein as aforementioned, the low-affinity binding sites in chick

brains are demonstrated to mediate the stimulation of phosphoinositide breakdown *via* a G protein.[152] To further discriminate MT_3 from mt_1 and MT_2, a ligand specific for MT_3, 5-methoxycarbonyl amino-*N*-acetyltryptamine **(17)** and its 2-iodinated analogue **(18)** have been developed.[153] Prazosin, an adrenergic antagonist, has been shown to be one of the most potent inhibitors of 2-[^{125}I]iodomelatonin binding to MT_3. Although studies suggesting the coupling of phosphoinositide hydrolysis to MT_3 binding site,[152,154,155] no physiological functions has been found to be related to MT_3 at present. The sequence of binding affinities also suggests that MT_3 may not in fact be a real melatonin binding site.



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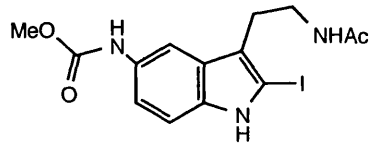

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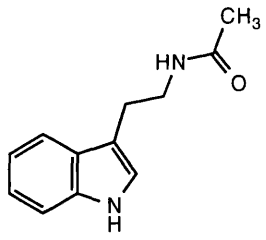
III. Melatonin receptor ligands

Binding data alone is insufficient to enable a full understanding of receptor pharmacology since it does not indicate function. In the study of structure-activity relationship (SAR) of melatonin with its receptor, a number of functional assays have been used. These include the aggregation of pigment granules in amphibian melanophores,[34,156,157] the inhibition of calcium-dependent release of [^{3}H]-dopamine in the rabbit retina,[129] the inhibition of forskolin-stimulated cyclic AMP production in the ovine PT,[108] and the inhibition of cyclic AMP and 3',5'-cyclic GMP accumulation in neonatal *pars distalis* of rats stimulated with luteinising hormone.[158] The different nature of the functional assays presents a problem in comparing results from different groups and the lack of binding and functional data from the same receptor subtype adds to the difficulties. In addition, some model systems may contain a heterogenous population of receptors which further complicates the issue.

III.A. Structure-activity reiationship

Early structure-activity analysis of melatonin receptors was carried out using semi-quantitative frog skin bioassays.[4,159] The determination of the minimum concentration that was effective *in vivo* gave the following order of potency: melatonin (0.1 pg/ml) << 5-methoxytryptamine << serotonin (1 μg/ml).[4] It was realised early that the 5-methoxy and *N*-acetyl groups are important for optimal biological activity.[160,161] With the *in vitro* studies of SAR of melatonin and related indoleamines on the frog (*Rana pipiens*), Heward and Hadley[160] demonstrated that *N*-

acetyltryptamine **(19)**, an analogue of melatonin lacking a 5-OMe group, had no intrinsic activity but was able to antagonise the skin-lightening effect of melatonin.



19

They then concluded that this activity of indolic compounds on the melatonin receptor is determined primarily by the moiety substituted at the C-5 position, whereas the affinity for the receptor binding site is determined primarily by the moiety substituted at the C-3 position of the indole nucleus. Their proposed structural functions for the melatonin molecule is summarised in Figure 2.[160]

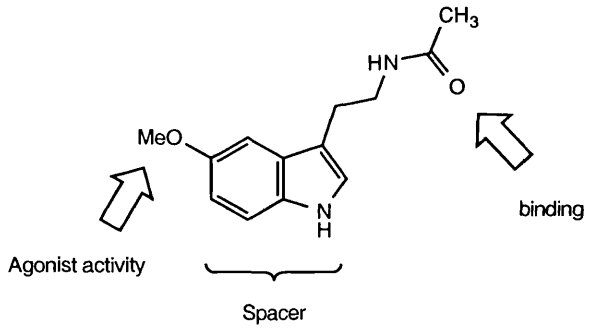


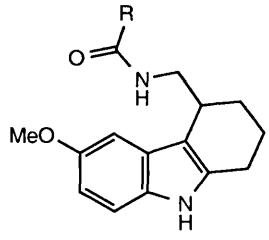
Figure 2. Heward and Hadley's[160] proposed melatoninergic pharmacophore.

This concept of the biological activity of melatonin was generally accepted and a number of indolic and nonindolic compounds with improved potency and antagonistic properties were synthesised based on this hypothesis.[129,162,163,178] However, by comparing the binding affinity and agonist activity of a series of *N*-acyltryptamines and the equivalent melatonin homologues, Garratt *et al.*[164] have shown that the 5-methoxy group is not an essential requirement for agonist activity but is a major binding site in the attachment of melatonin to its receptor. This has been confirmed by the high affinity observed for 5-halogenated derivatives. Spadoni and co-workers[197] then demonstrated that moving the methoxy group to C-4, C-6 or C-7 of the indole ring results in a dramatic drop in affinity, but by maintaining an appropriate relative distance of the methoxy group to the amido side chain, the two functionalities can be moved to different indole positions without loss of binding affinity.[175] Garratt *et al.*[164,173] have also found that the 5-methoxy group and the 3-amidoethane side chain have a mutual action, docking the melatonin molecule into the receptor pocket to trigger the biological response. It has been noted that the indole nucleus is not necessary for the binding but appears to hold the functional groups in

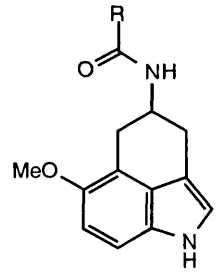
the correct orientation for binding to the receptor[166] and can be replaced by either a naphthalene group[169] or other bicyclic systems, such as tetralin.[169] It was also discovered that the region of the receptor in which the 3-aminoethane chain is accommodated is larger than necessary for the acetyl group and increasing the length of the aliphatic side chain up to three carbons improves binding, presumably through enhanced van der Waals attraction.[173]

Certain substitutions at the C-2 position of the indole ring also resulted in increased binding affinity, including halogen,[156,251] methyl,[251] or an aromatic ring.[173,251] Garratt *et al.*[173] proposed that the large substituents at the C-2 position of these compounds increases the population of the appropriate conformation required for the interaction with the receptor. Recent work of Spadoni *et al.*[251] suggested that there may also be a putative binding site at the C-2 position which these substituents can occupy.

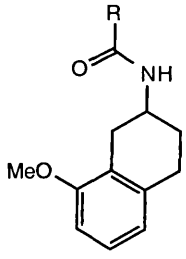
Over the last few years, several groups of melatoninergic ligands have been developed where the amido side chain is incorporated in a ring. These were either derivatives of tricyclic indoles, such as tetrahydrocarbazole[252,194] and tetrahydrobenzindole,[253] or the non-indolic 2-amido-8-methoxytetralin[178] and phenalene.[179]



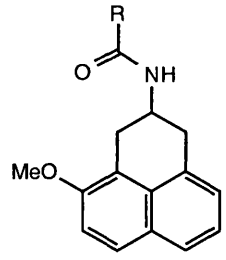
tetrahydrocarbazole



tetrahydrobenzindole



2-amido-8-methoxytetralin



phenalene

By examining the potency of these constrained melatoninergic agents, the preferred conformation of the 3-aminoethane was shown to be **a**, where the amido group is close to the 5-methoxy moiety, rather than **b**, the ground state conformation, where the amido group is remote from the 5-methoxy moiety (Figure 3).

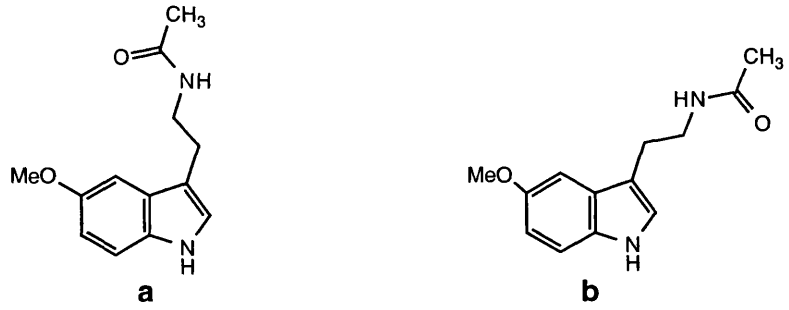


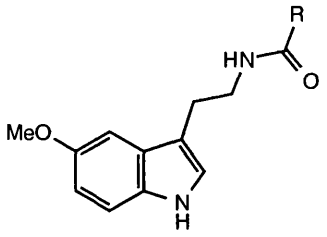
Figure 3. The preferred conformation **a** and the lower energy state **b** of melatonin.

Recently, Sugden *et al.*[167] reported the first systematic comparison of the structure-activity relationship of the three cloned, high-affinity ML_1 receptor subtypes. Radioligand binding assays performed on cloned human mt_1, human MT_2 and *Xenopus* Mel_{1c} using 2-[^{125}I]iodomelatonin indicated that MT_2 receptor subtype has less stringent requirements than the mt_1 receptor; removal or replacement of the 5-methoxy group by another functionality, such as hydroxy, methyl and benzyloxy group, reduces the binding affinity less at the MT_2 than either the mt_1 or Mel_{1c} subtype. It was shown that the 'pocket' into which the *N*-acetyl group fits is very similar for each subtype. However, there is a clear difference in the binding pocket of the subtypes in the region surrounding the 5-position. At present, there are no selective agents available for the characterisation of the pharmacology of melatonin receptors. Further investigation of the receptor subtype binding sites for the 5-substituent may lead to more subtype-specific agonists and antagonists which will be valuable tools for the characterisation and classification of functional melatonin receptors.[167]

III.B. Agonists

Structure-activity relationship studies on derivatives of melatonin have elucidated several key interactions between the ligand and the receptors for the high-affinity binding sites. A large number of melatonin analogues have been tested using 2-[^{125}I]iodomelatonin as a radioligand, including compounds of type **21** which utilise the naphthalene nucleus as the parent structure instead of indole. The ranked order of potency in membrane binding and functional bioassay of two types of compounds **20** and **21** are shown (Table 3). Native receptors in chicken retina and brain, and in sheep *pars tuberalis*, show this same rank order.

Table 3. Structure of melatonin and naphthalenic analogues and their affinities determined by competition binding to ovine PT using 2-[^{125}I]iodomelatonin as a radioligand.



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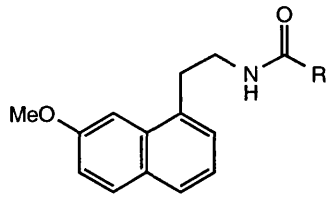


21

Entry	-R	K_d/K_i (pM)	K_d (pM)[b]
a	$-CH_3$	517[a]	100
b	$-CH_2CH_3$	486[a]	22
c	$-(CH_2)_2CH_3$	164[a]	6.2
d	$-(CH_2)_3CH_3$	23400[a]	3.4
e	$-(CH_2)_4CH_3$	555000[c]	283000
f	$-(CH_2)_5CH_3$	-	2330000
g	cyclopropyl	0.50[b]	0.42
h	cyclobutyl	26300[c]	24000

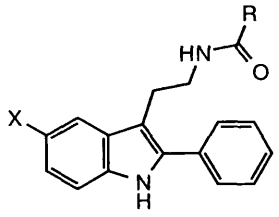
[a]Data taken from Sugden, D.; Chong, N.W.S. *Brain Res.* 1991, **539**, 151.[168]

[b]Data taken from Yous, S.; Andrieux, J.; Howell, H.E.; Morgan, P.J.; Renard, P.' Pfeiffer, B.; Lesieur, D.; Guardiola-Lemaitre, B. *J. Med. Chem.* 1992, **35**, 1484.[169]

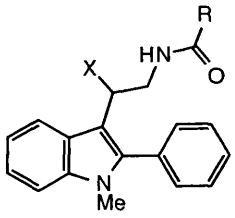
[c]Sugden, D Personal communication.

From the results, it can be seen that analogues of melatonin with a greater binding affinity can be produced by increasing the *N*-acetyl chain length, with butanoyl (R = $(CH_2)_2CH_3$) and pentanoyl (R = $(CH_2)_3CH_3$) having the highest affinity in the melatonin and naphthalene series, respectively. This difference between the two series could be caused by the difference of the distance between the 5-methoxy group and the 3-amido side chain. Interestingly, replacing the methyl group on the *N*-acylamino side chain by a cyclopropyl group also increased the binding affinity for both of the analogues.[108,171,172] Compound **21a** was also shown to express *in vivo* melatonin agonist activity by promoting free-running locomotor activity in rats.[170]

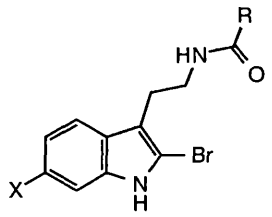
Indole analogues with substituents at positions 1 and 2, as well as other non-indolic compounds substituted in a similar way, have been tested as melatonin agonists.



22 X = H
23 X = OMe



24 X = H
25 X = Me



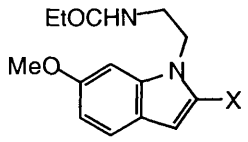
26 X = H
27 X = Br

Compounds of type **22** demonstrate a higher affinity for the melatonin receptor in the chicken brain binding assay than *N*-alkanoyl tryptamines. When R = Me, the binding affinity, K_i, was 100 nM compared to K_i of 701 nM for *N*-acetyl tryptamine.[164,173] Similarly, compounds of type **23** possess considerably higher binding affinities than melatonin. When R = Me, the binding affinity, K_i, was 0.06 nM compared to K_i of 0.59 nM for melatonin.[173]

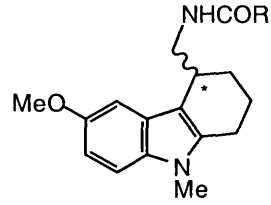
Methylation of melatonin and most substituted melatonin analogues to their corresponding *N*-methylmelatonin analogues led to slight loss of binding affinity.[174] Unexpectedly, compounds of type **24** have slightly greater binding affinities than their non-methylated type **22** analogues in the chicken brain binding assay. In compound **24a**, where R = Me, the binding affinity, K_i, is 63 nM compared to the K_i of 100 nM for **22a** (R = Me). The introduction of a methyl group at the β-carbon of the amide side chain also increases the binding affinities. Thus compounds of type **25** have higher binding affinities (when R = Me, K_i = 5.9 nM) than their type **24** analogues.[173]

For compounds of type **26**, with bromine at C-2, the binding affinities are much greater than their non-brominated analogues in the chicken brain binding assay, in agreement with the observation that 2-iodomelatonin has a greater binding affinity than melatonin. In compound **26a**, where R = Me, the binding affinity, K_i, is 138 nM compared to K_i of 701 nM for *N*-acetyltryptamine.[164] Further bromination at the C-6 position of the indoleamine resulted in a slight increase in the binding affinities. Hence compounds of type **27** have slightly higher binding affinities than their type **26** counterparts. In compound **27a**, where R = Me, the binding affinity, K_i, is 122 nM compared to **26a**, 138 nM shown above.[164]

Compounds **29** (X = COOMe; K_i = 0.23 nM), **30** (X = Br; K_i = 0.04 nM) and **31** (X = Ph; K_i = 0.01) have greater binding affinities than the unsubstituted analogue **28** (K_i = 1.91 nM) in a quail brain membrane binding assay.[175] All of the compounds **28** - **31** have higher binding affinities for the melatonin receptor in the quail brain than melatonin (K_i = 0.61 nM).[175]

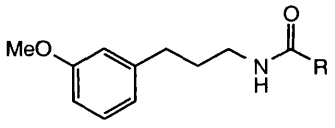


28 X = H
29 X = COOMe
30 X = Br
31 X = Ph



32

The S-(-)-enantiomers of the *N*-methylated carbazoles of type **32** were reported to possess comparable affinities (when R = Me, K_i = 0.37 nM; when R = Pr, K_i = 0.38 nM) to melatonin.[176] However, the R-(+)-enantiomers in this type of compounds were found to have a much lower affinity compare to the S-(-)-enantiomers.



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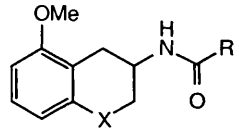
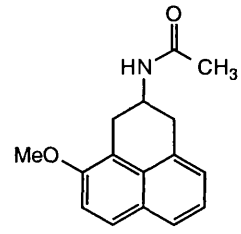


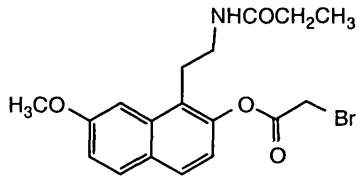
34 X = O
35 X = CH_2



36

Compounds of type **33** are very simple analogues of melatonin lacking the 1,2-indole atoms. When R = Pr, the compound has a binding affinity of 63 nM, only 100 fold less than that of melatonin, which is a remarkably high affinity for such a simple system.[177]

Non-indolic compounds of type **34** (K_i = 46 nM) and **35** (K_i = 94 nM) show moderate affinity for the melatonin receptor in chicken retina[178] and chicken brain,[166] respectively. A series of non-indolic phenylenes were shown to be good melatonin ligands in chicken brain binding assay. An example of such a compound is the phenalene **36** which has a high binding affinity (K_i = 0.7 nM).[179]



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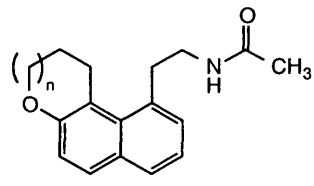

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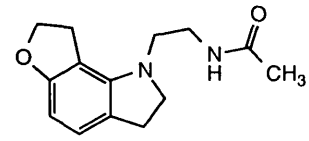


39

N-[2-[2-(bromoacetoxy)-7-methoxynapthyl]ethyl]-propionamide **37** was found to be a high-affinity ligand that binds irreversibly to the MT_2 receptor with a binding affinity that is close to melatonin (**37**, K_i = 1.3 nM; melatonin, K_i = 0.08 nM) in

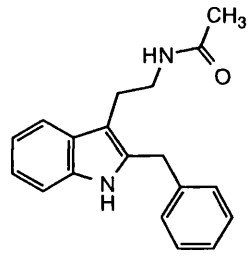
the Chinese hamster ovary cells binding assay. Witt-Enderby *et al.*,[180] who synthesised this compound, found that **37** selectively alkylated human MT_2 melatonin receptors expressed in Chinese hamster ovary cells. This agent could be useful in molecular characterisation and the determination of tissue distribution of the MT_2 melatonin receptor.

Tricyclic compounds of type **38** and **39**, which have the methoxy functionality incorporated into a ring, were also reported to have excellent binding affinities for the high affinity mt_1, MT_2 and Mel_{1c} receptors.[181-183] When n = 1 for compounds of type **38** (K_i = 0.1 nM), the compound is equipotent to the corresponding methoxynaphthalene derivative **20a** (see Table 3) for the receptor in ovine PT.[181] Another tricyclic compound, the indoline **39**, was found to possess better binding affinity (K_i = 0.42 nM) than melatonin (K_i = 6.3 nM) for the receptor in chick retina.[183] Compounds of type **38** and **39** provide an indication of the directionality of the hydrogen bond between the receptor and the methoxy group of melatonin.

III.C. Antagonists

In contrast to the situation described earlier in which we find many available melatonin agonists, at present there are no potent and selective antagonists of melatonin receptors. Although several compounds have been reported to block the action of melatonin *in vivo* and *in vitro*, their usefulness is restricted by the fact that their potency of action varied markedly with different tests or animal species[108] and their binding affinities for high-affinity melatonin receptors are only modest.[127,184] It is therefore difficult to judge the minimal structural requirements for a competitive melatonin receptor antagonist.

Luzindole **(40)**, 2-benzyl-*N*-acetyltryptamine, was the first competitive and selective antagonist of melatonin to have been investigated in detail. It inhibits the Ca^{2+}-dopamine release from rabbit retina with a dissociation constant, K_b, of 20 nM, more effective as an antagonist compared to 6-chloromelatonin (40 nM) but not as effective as 2-methyl-6,7-dichloromelatonin (16 nM).[127] This antagonizing effect has also been reported to occur in the retina *in vivo*[185] and on melanophores *in vitro*.[186] However, its antagonist effect has not proven to be general as it fails to block the action of melatonin on cyclic AMP production even at concentrations as high as 1 mM.[187]



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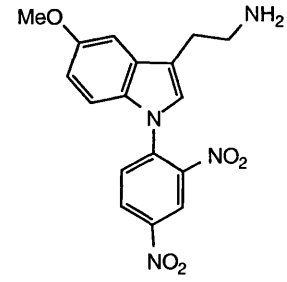
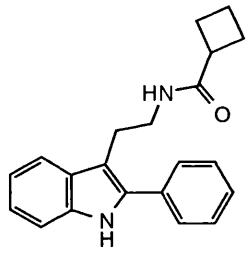


41

Another antagonist of melatonin is *N*-(2,4-dinitrophenyl)-5-methoxytryptamine **41**. Like luzindole **40**, mixed effects were observed. It was reported to block the effect of melatonin-mediated processes of sexual maturation of rats *in vivo*.[188] However, it fails to counteract reproductive effects of melatonin in rams[189] or hamsters[190] and has low displacement activity with respect to the high affinity melatonin receptor.[57]

Garratt and co-workers[173,242] have discovered that several melatonin analgoues with cyclobutane carbonyl side chain exhibit antagonist activity in the *Xenopus melanophore* model and **42** has been subsequently used by others for comparative studies.



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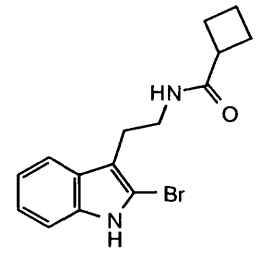

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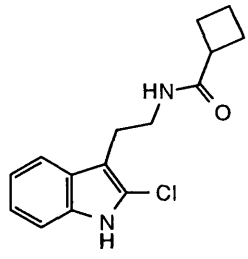

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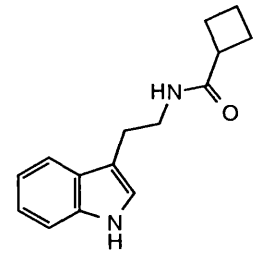
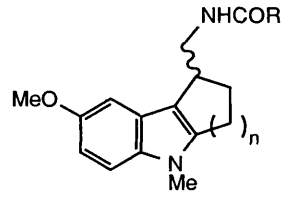


45

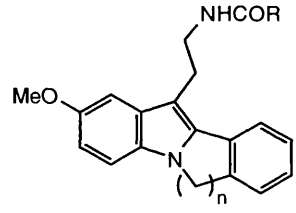
A recent report by Garratt and co-workers[176] has indicated the tendency of compound of type **46c** to be antagonists. While most of the available antagonist ligands bind melatonin receptors with low to moderate affinity, *N*-propanoyl-10-(aminomethyl)-2-methoxy-5-methyl-hexahydrocyclohept[b]indole, **46c** with R = $CH_2CH_3CH_3$, has a much higher binding affinity (K_i = 84 nM) compare to luzindole **40** (K_i = 1606 nM) in the chicken brain membrane binding assay but has lower antagonist activity. It was found that while compounds of type **46a** and **46b** are

either partial or full agonists, the presence of the cycloheptane ring fused to the [b] face of the indole ring as in **46c**, converts the system into an antagonist.



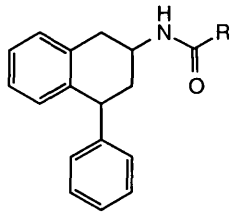
46a n = 1
46b n = 2
46c n = 3

Garratt and co-workers[254] also found that compounds of type **47c** possess similar or better antagonist activity than luzindole **40** in the *Xenopus* melanophore binding assay. When R = $CH_2CH_2CH_3$, **47c** has an exceptional binding affinity (**47c**, K_i = 0.50 nM; **40**, K_i = 44.7 nM) to the human MT_2 receptor and a 100-fold selectivity for the human MT_2 over the mt_1 receptor. Like **46**, the results obtained showed that an increase in the size of ring attached to the [a] face of the indole from cyclopentane to cycloheptane also convert the system from agonist to antagonist.



47a n = 1
47b n = 2
47c n = 3

4-Phenylamidotetralines **48** with R = Me, Et, CH_2Cl were synthesised by Copinga *et al.*[178] who demonstrated the remarkable correlation between the MT_2 binding affinity of these compounds (K_i = 120 nM - 500 nM) in the chick retina binding assay and their activity in antagonising melatonin-induced inhibition of dopamine release from rabbit retina, in a similar way to luzindole.



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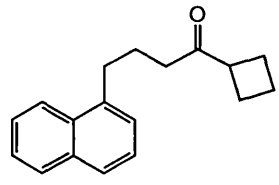

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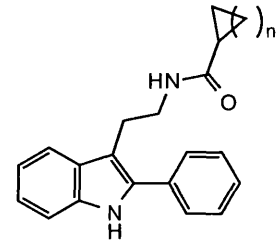


50

The naphthalene derivative **49** blocks the inhibitory effect of melatonin on forskolin-induced cyclic AMP production in sheep PT cells and reverses the melatonin-triggered pigment aggregation in *Xenopus* melanophores.[191]

N-cycloalkancarbonyl-2-phenyltryptamine **50** with n = 1, 2, 3 and 4 were demonstrated in *Xenopus* melanophore assay to be full antagonists, with the cyclopropyl derivative having the highest affinity and the binding decreasing with increasing ring size.[168]

IV. Melatonin receptor model

Apart from SAR studies,[173,176,177] analogies with other G-protein coupled receptors for small molecular weight ligands (such as the catecholamines and serotonin) and the use of the comparative molecular field analysis (CoMFA)[142,196,197] were employed to formulate models for the binding of melatonin to its receptor. Five different models based on the seven transmembrane (TM) domains of either bacteriorhodopsin[166,198-200] or rhodopsin[142] have been proposed hitherto.

By comparing the amino acid sequence of the cloned melatonin and serotonin receptors, Sugden *et al.*[173] proposed the possible sites of interaction between the melatonin molecule and the amino acid residues. The model predicted hydrogen bonding between the 5-methoxy oxygen of melatonin and Ser_{115} in helix III and between the amido hydrogen of melatonin and Asn_{167} in helix IV. π-π Stacking of the indole ring of melatonin and Trp_{256} in helix VI and stablising interactions with Ile_{89}, Val_{70} and Lle_{194} were also predicted (Figure 4).

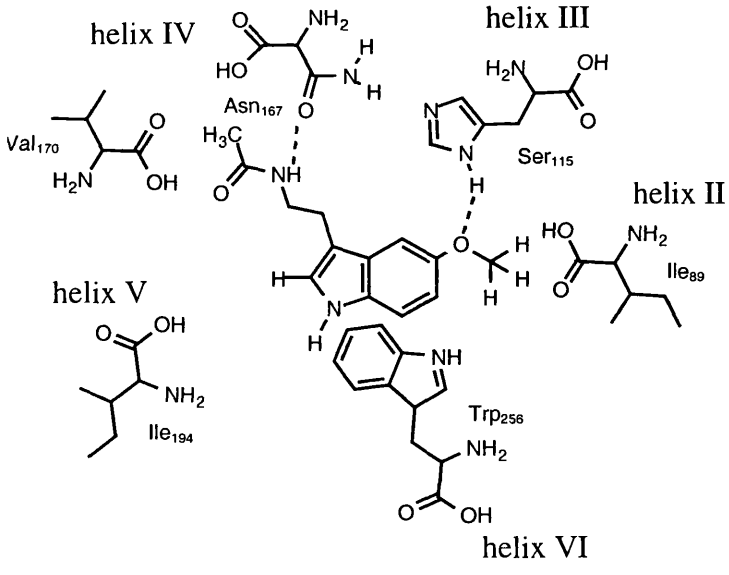


Figure 4. Sudgen and Lewis' [165] postulated melatonin receptor binding site model.

A second model based on the amino acid sequence of cloned melatonin receptor was proposed by Grol *et al.*.[198] The model predicted hydrogen bondings between Ser_{115} and Ser_{119} in helix III and the amide function, and between His_{200} in helix V and the methoxy oxygen in melatonin. It also predicted stabilising interactions with the aromatic rings of Phe_{168}, Phe_{287} and Trp_{280}.

Using CoMFA, Sicsic *et al.*[199] created a model similar to that of Grol *et al.*.[198] The only difference is that in Grol's model, the carbonyl oxygen of the carboxamide group is turned away from the indole ring; whereas in Sisic's model, the carboxamide group is turned towards the indole ring.[199]

Also using CoMFA, Navajas *et al.*[142] generated a model which postulated hydrogen bondings between His_{10} in helix V and the methoxy oxygen in melatonin, and between Ser_6 in helix VII and the carbonyl oxygen of the carboxamide group of melatonin. The model also suggested interactions between Val_7 and His_{10} and between Ala_{10} and Ser_6, which provide subsidiary anchors for methoxymethyl group at the 5-position and the amidomethyl group respectively, and a possible interaction between the indole ring and the Phe_9 in TMVI.

The final model was proposed by Vasilescu and Broch[200] who deduced a preferred minimal energy conformation of melatonin from quantum molecular modelling studies. Their model is in good agreement with that proposed by Grol[198] and Sicsic.[198,199]

Recent site-directed mutagenesis studies on the ovine mt_1 subtype indicated the likeliness of Val_{208} to be in the melatonin receptor binding pocket and be involved in the ligand binding site but is not essential for melatonin-mediated receptor function, while His_{211} maybe necessary for such function.[201]

From these models, it can be seen that there are clear differences in both the active conformation of melatonin deduced and its orientation within the binding pocket among the helices, which also led to the difference in the choice of amino acids. Nonetheless, these models provide interesting insights and an understanding of the interaction between melatonin and its receptor.

V. Objectives

Previous studies have been directed towards the binding sites around the C-3 amido side chain and the 5-methoxy functionality and latterly, around N-1. By comparison, there is little information on the binding 'pocket' around the 6- and 7-positions of the indole moiety. Previous research[172,178,251,197] has shown that the introduction of a bromine or chlorine atom at the C-6 position is well-tolerated and has only little effect on the binding affinity, whereas the presence of a hydroxy group at this position showed a reduced potency and affinity.[172] The only information available for the C-7 position is that the substitution of a methoxy group for hydrogen lowered both the potency and the binding affinity by 200 fold.[197]

The major aim of this project was to examine the spatial requirements of melatonin analogues around these two positions in order to provide a further understanding of the nature and shape of the receptor pocket. Six sets of compounds substituted at C-6 and C-7 of the indole nucleus were prepared and their binding affinities compared (Figure 5).

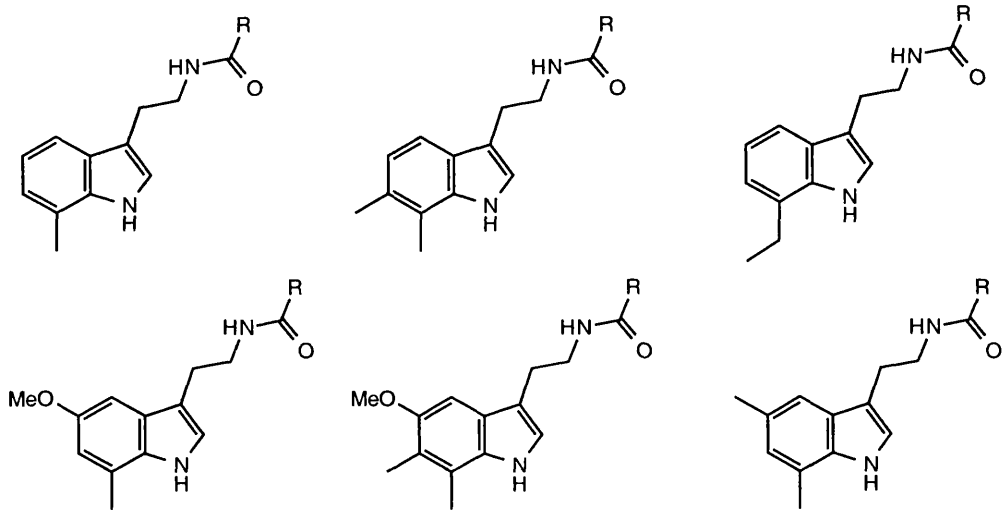


Figure 5. Sets of 6- and 7-substituted indoleamines synthesised.

The biological activity of a group of ring-cleaved analogues of melatonin and indoleamines, the kynurenamines, which are potential melatonin receptor binding ligands were also of interest. Representatives were prepared and their binding affinities investigated (Figure 6).

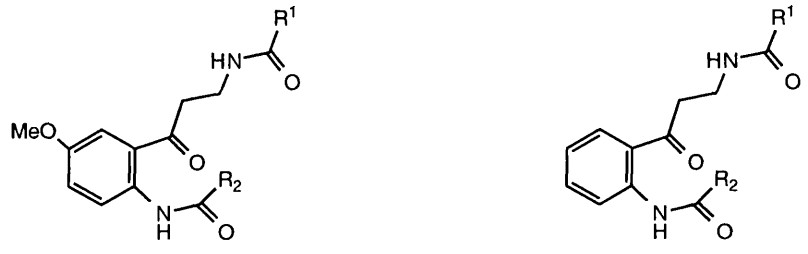


Figure 6. Kynurenamines synthesised.

RESULTS AND DISCUSSION

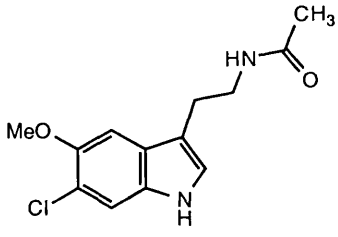
VI. 5-, 6- and 7-Substituted indole analogues

As part of a study to investigate the melatonin receptor binding site, a series of 5-, 6- and 7-alkylated indoles have been synthesised.

VI.A. Literature review

VI.A.A. 6- and 7-Substituted melatonin anaiogues

A variety of melatonin derivatives substituted at the 6-position have previously been prepared. Thus Flaugh *et al.*,[202] Huegel *et al.* [203] and Kirk *et al.* [204] have prepared 6-halogen substituted derivatives (**51** and **52**) and a number of groups[205-209] have prepared the 6-OH or 6-OR substituted derivatives (**3**, **53** and **54**). 6-Methylmelatonin **(55)** has been prepared by Flaugh *et al.*[202] Little work has been reported on 7-substituted melatonin derivatives, although 7-chloromelatonin **(56)** has been prepared.[210]



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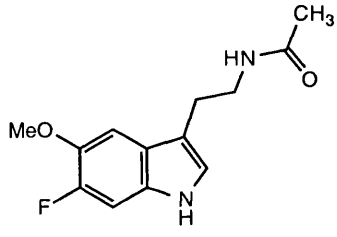

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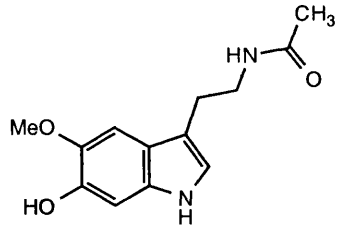

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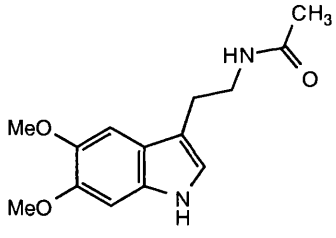

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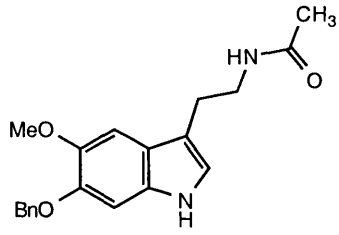

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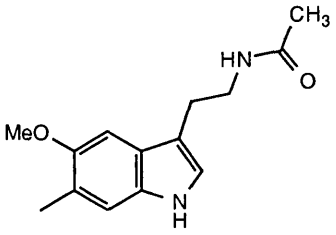

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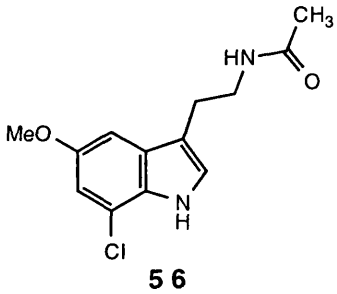
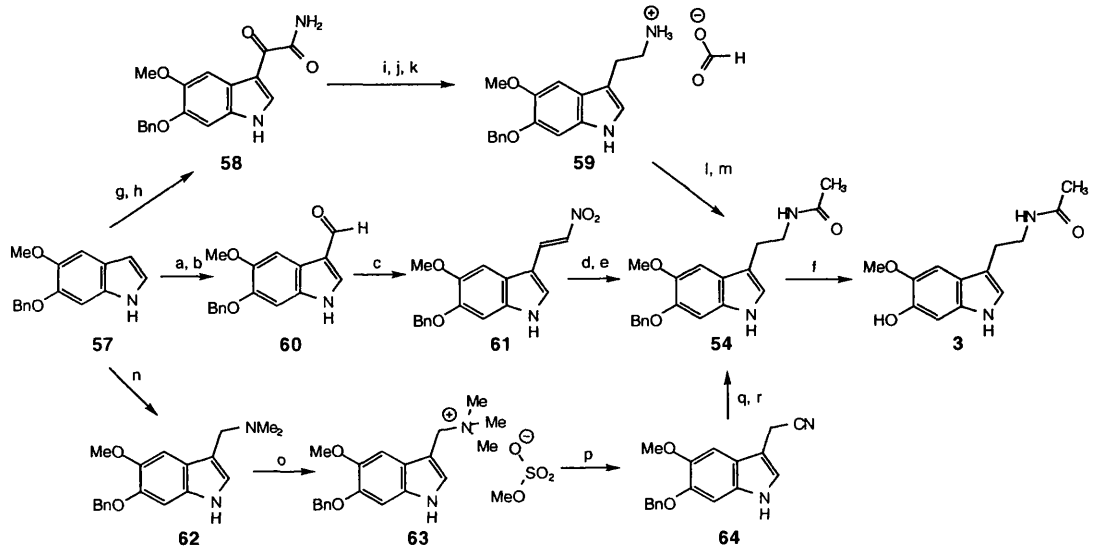


56

6-Hydroxymelatonin **(3)** has been prepared from 6-benzyl-5-methoxyindole **(57)** by introduction of the 3-ethylamine side chain via three methods. Benigni *et al.*[205] treated **57** with oxalyl chloride followed by ammonia to give **58** which was then reduced with $LiAlH_4$ and the amine isolated as the formate **59**. Addition of base followed by acetic anhydride gave 6-benzyloxymelatonin **54** which was deprotected with hydrogen on palladium to give **3** (Scheme 6).



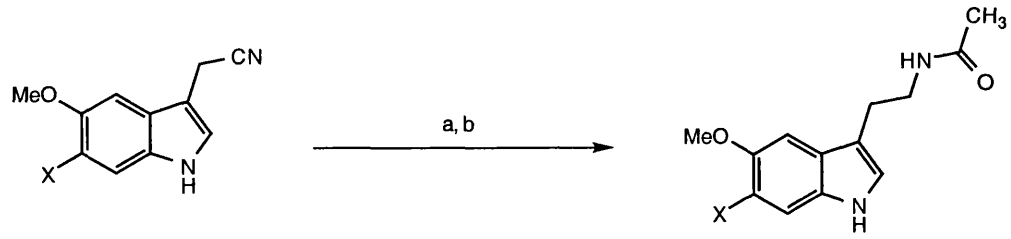
Reagents and conditions: a) $POCl_3$, DMF, reflux; b) NaOH, H_2O; c) NH_4OAc, CH_3NO_2; d)$LiAlH_4$, THF, reflux, 4h; e) Ac_2O, py; f) H_2, Pd/C; g) $(CO)_2Cl_2$; h) NH_3; i) $LiAlH_4$, THF, reflux, 15 h; j) H_2O, reflux, 30 min; k) HCO_2H, $CHCl_3$; l) NaOH, H_2O; m) Ac_2O; n) CH_2O, $(CH_3)_2NH$, AcOH; o) Me_2SO_4, AcOH, THF, 5 °C; p) NaCN, H_2O, 67 °C, 1 h; q) $LiAlH_4$, Et_2O, reflux, 18 h; r) Ac_2O; f) H_2, Pd/C.

Scheme 6. Synthesis of 6-benzyloxymelatonin and 6-hydroxymelatonin.

Hall *et al.*[207] introduced the side chain by a Vilsmeier-Haack formylation[212] to give the aldehyde **60** which was then subject to a Henry nitro-olefination[213] and $LiAlH_4$ reduction to give **54** which was acylated and deprotected to give **3**.

Taborski *et al.*[209] treated **57** with formaldehyde and dimethylamine to give **62**. Introduction of the cyanide functionality to **62** followed by reduction with $LiAlH_4$ and acetylation gave **54** which was converted into **3** as above.

For the synthesis of 6-chloromelatonin **(51)** and 6-fluoromelatonin **(52)**, Flaugh and co-workers[202] prepared acetonitriles **65a** and **65b** from the corresponding substituted indoles by same method described in Taborski's synthesis of acetonitrile **64**. Compounds **65a** and **65b** were then converted to **51** and **52** by reduction and acetylation (Scheme 7).



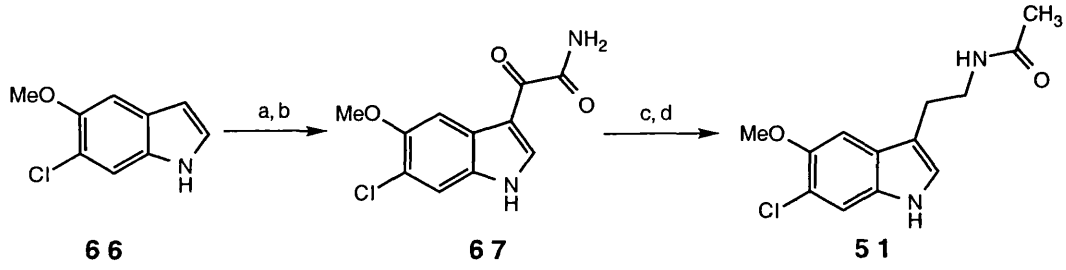
65a X = Cl
65b X = F

51 X = Cl
52 X = F

Reagents and conditions: a) $LiAlH_4$; b) Ac_2O, py.

Scheme 7. Synthesis of 6-fluoro- and 6-chloromelatonin.

Huegel[203] modified Flaugh's synthesis of 6-chloromelatonin **51** by reaction of **66** with oxalyl chloride and liquid ammonia to give **67** which was reduced with dimethylsulphide-borane and acetylation to give **51** (Scheme 8).

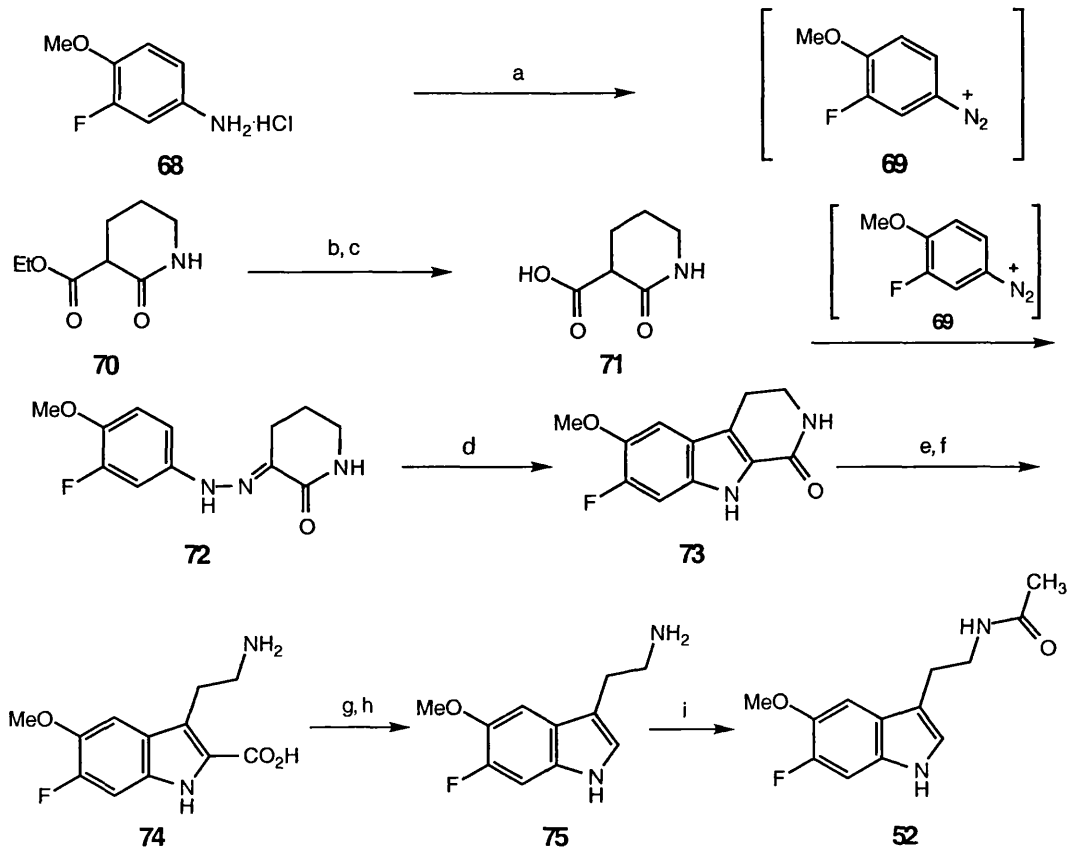


66 **67** **51**

Reagents and conditions: a) $(CO)_2Cl_2$, 0 °C; b) NH_3; c) $(H_3C)_2S{\cdot}BH_3$/THF, reflux; d) Ac_2O/py.

Scheme 8. Huegel's synthesis of 6-chloromelatonin.

Kirk[204] prepared 6-fluoromelatonin **(52)** using the Fischer indole synthesis. 3-Fluoro-4-methoxyaniline hydrochloride **(68)** was diazotised and the resulting diazonium intermediate coupled to 2-oxopiperidine-3-carboxylic acid to give the phenylhydrazone **72**. Formic acid-catalysed cyclisation of **72** gave a single ketocarboline **73**. Base-catalysed ring opening of **73** and acid-catalysed decarboxylation of the resulting amino acid **74** produced 5-methoxy-6-fluorotryptamine **(75)**, which gave 6-fluoromelatonin **(52)** on reaction with acetic anhydride (Scheme 9).

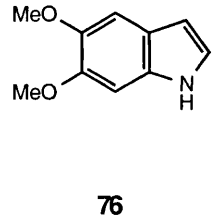


Reagents and conditions: a) conc. HCl, H_2O, $NaNO_2$, 0 °C; b) NaOH, rt, 12 h; c) AcOH, 4 °C, 15 h; d) HCO_2H, Δ, 30 min; e) KOH, EtOH, reflux, 15 h; f) H_2O, AcOH; g) HCl, reflux, 12 h; h) 30% NaOH; i) Ac_2O, EtOAc.

Scheme 9. Kirk's synthesis of 6-fluoromelatonin.

The synthesis of 7-chloromelatonin **(56)** was achieved by Magidson *et al.*[210] using the same reaction sequence conditions as that applied by Kirk *et al.*[204] for the synthesis of 6-fluoromelatonin **(52)** in Scheme 9.

6-Methoxymelatonin **(53)** was prepared by Huebner *et al.*[208] from 5,6-dimethoxyindole **(76)** by the route used by Taborski *et al.* for the synthesis of 6-benzyloxymelatonin **(54)**.



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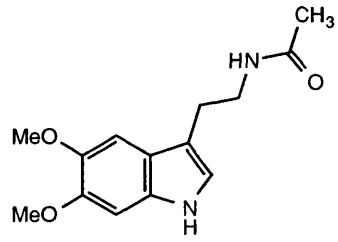
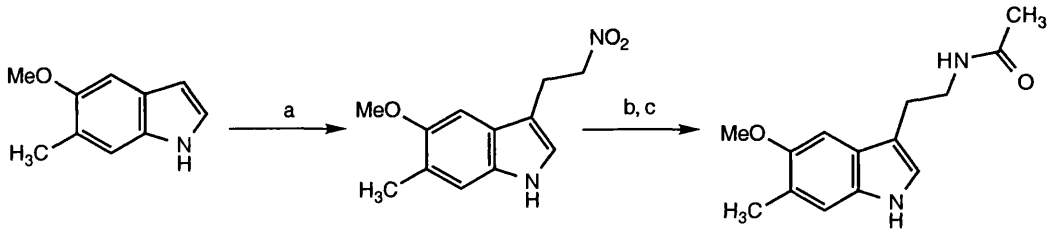


53

When 6-hydroxymelatonin **(3)** became commercially available, Campaigne *et al.*[206] methylate it with dimethyl sulfate to **53**.

Besides 6-halomelatonin, Flaugh *et al.*[202] has also synthesised 6-methylmelatonin **(55)** from 5-methoxy-6-methylindole **77** by introducing the ethyl acetamide side chain at C-3 in the way described in Scheme 6. Hydrogenation of the nitro group of **78** over platinum oxide, followed by acetylation with acetic anhydride gave **55** (Scheme 10).



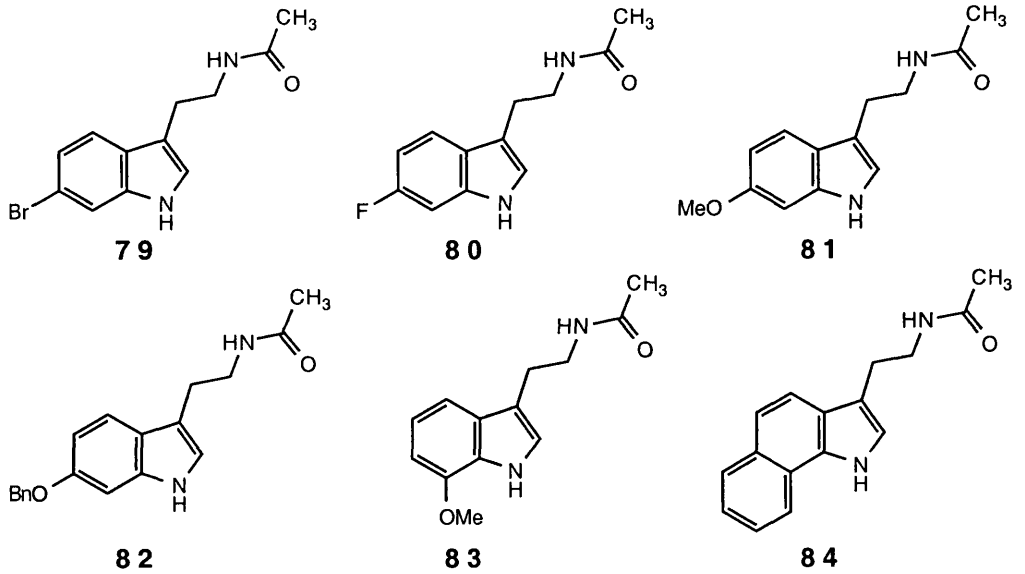
77 **78** **55**

Reagents and conditions: a) $AcOC_2H_4NO_2$, xylene, reflux; b) H_2, PtO_2; c) Ac_2O, py.

Scheme 10. Synthesis of 6-methylmelatonin.

VI.A.B. 6- and 7-Substituted *N*-acetyltryptamines

Several groups have synthesised 6- and 7-substituted *N*-acetyltryptamines as potential melatonin analogues.

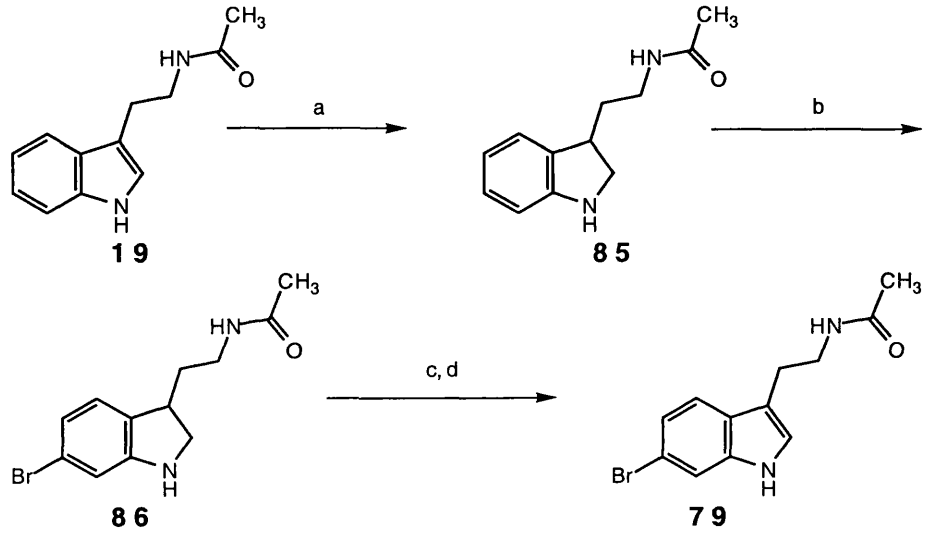


79 **80** **81**

82 **83** **84**

In the synthesis of *N*-acetyl-6-bromotryptamine **(79)**, Kikugawa and co-workers[215] converted *N*-acetyltryptamine **(19)** to the corresponding indoline **85** by

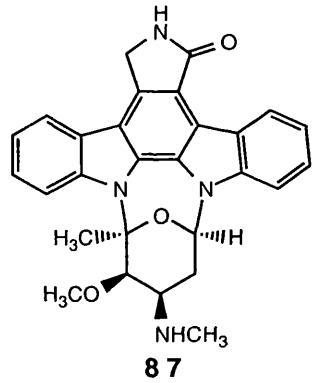
reduction with pyridine-borane under acidic conditions. Bromination of **85** in either (i) 97% sulphuric acid in the presence of silver sulfate, or (ii) superacid (HF-SbF_5), gave the 6-bromoindoline **(86)** which was then dehydrogenated with dimethylsulfide and *tert*-butyl hypochlorite to give **79** (Scheme 11).



Reagents and conditions: a) Py·BH_3, H^+; b) Br_2, HF-SbF_5 or Br_2, Ag_2SO_4; c) $(CH_3)_2S$, ^tBuOCl; d) $NaOC_2H_5$.

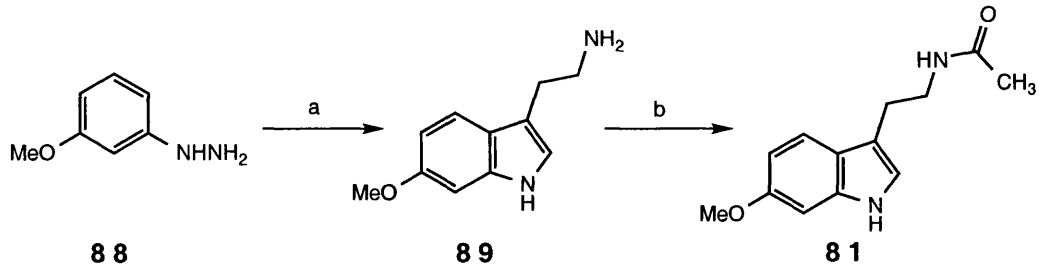
Scheme 11. Synthesis of *N*-acetyl-6-bromotryptamine.

In a tryptophan metabolic study by Yang *et al.*,[216] *N*-acetyl-6-fluorotryptamine **(80)** was obtained as a major product from the fermentation of commercially available 6-fluorotryptamine with *Streptomyces staurosporeus*, a producer of the potent protein kinase inhibitor staurosporine **87**.



In 1930, Späth and Lederer[217] prepared *N*-acetyl-6-methoxytryptamine **(81)** as an intermediate in the synthesis of carbolines. This was achieved using a modified Fischer indole methodology as shown in Scheme 12. Lewis acid-catalysed condensation of the hydrazine **88** with 4,4-diethoxybutylamine followed by an

internal electrophilic substitution with the elimination of ammonia yielded 6-methoxytryptamine **(89)**. Subsequent acetylation of **89** with acetic anhydride gave **81** (Scheme 12).



88 **89** **81**

Reagents and conditions: a) $(EtO)_2CH(CH_2)_3NH_2$, $ZnCl_2$, 180 °C; b) Ac_2O.

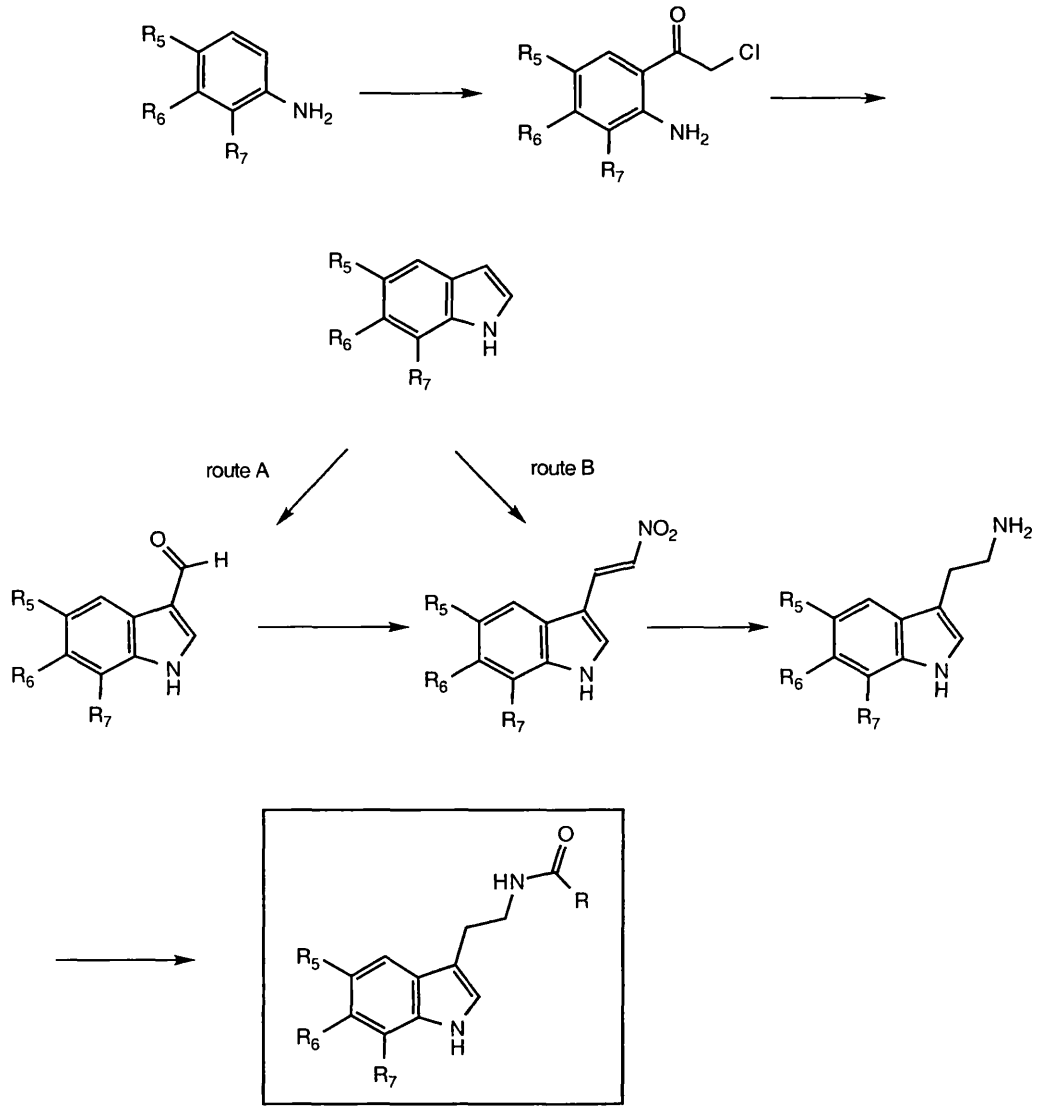
Scheme 12. Synthesis of *N*-acetyl-6-methoxytryptamine.

In a report on the synthesis and pharmacological activity of alkylated tryptamines, Kalir *et al.*[218] prepared *N*-acetyl-6-benzyloxytryptamine **(82)** by acetylation of 6-benzyloxytryptamine sulphate with acetic anhydride.

Yamada *et al.* [219] prepared 7-methoxytryptamine **(83)** from 7-methoxyindole by the Vilsmeier-Haack, Henry nitro-olefination route described earlier. Hiremath *et al.*[220] also prepared *N*-acetyl-β-benz[g]-tryptamine **(84)** from benz[g]indole by this route.

VI.B. Synthesis

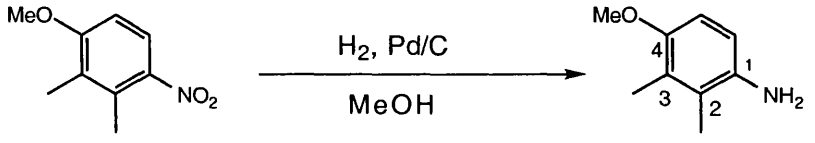
To access the target 5-, 6- and 7-alkylated indoles, a five-step synthetic pathway was devised (Scheme 13) which involved chloroacetylation of the appropriate substituted amine followed by reduction of the resulting acetophenone to the indole, nitro-olefination to the nitrovinylindole, hydrogenation to the tryptamine and finally acylation. The nitro-olefination was performed by two methods which will be discussed later in this chapter.



Scheme 13. Projected synthetic route to 5, 6 and 7-alkylated indole analogues.

VI.B.A. Amine precursors

All the amine precursors were purchased from Aldrich Chemical Company except 4-methoxy-2,3-dimethylaniline **(91)** which was readily made by the method of Knölker.[221] Hydrogenation of the commercially available 2,3-dimethyl-4-nitroanisole **(90)** over palladium-on-carbon afforded the pure aniline **91** in 99% yield after recrystallisation from petroleum ether (Scheme 14). The spectroscopic data obtained is consistent with that reported for **91**.

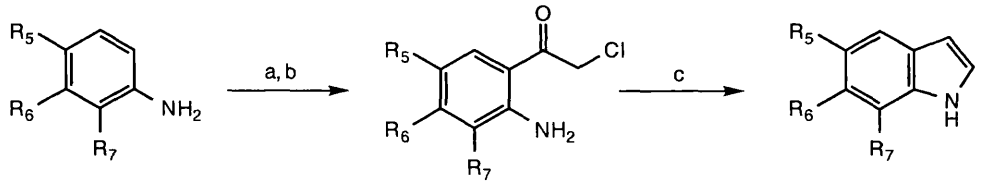


90 **91**

Scheme 14. Synthesis of 4-methoxy-2,3-dimethylaniline.

VI.B.B. Synthesis of 5-, 6- and 7-alkylated indoles

The substituted indoles were prepared as described by Glennon *et al.*[222] in two steps *via* a Friedel-Crafts reaction. Reaction of the anilines **91** - **96** with chloroacetonitrile in the presence of titanium tetrachloride gave the corresponding 2-amino-α-chloroacetophenones **97** - **102**. Reduction of the 2-amino-α-chloro-acetophenones with sodium borohydride gave the corresponding substituted indoles **103** - **108** (Scheme 15).



92 R_5 = OMe, R_6 = H, R_7 = CH_3
91 R_5 = OMe, R_6 = R_7 = CH_3
93 R_5 = R_7 = CH_3, R_6 = H
94 R_5 = R_6 = H, R_7 = CH_3
95 R_5 = H, R_6 = R_7 = CH_3
96 R_5 = R_6 = H, R_7 = CH_2CH_3

97 R_5 = OMe, R_6 = H, R_7 = CH_3
98 R_5 = OMe, R_6 = R_7 = CH_3
99 R_5 = R_7 = CH_3, R_6 = H
100 R_5 = R_6 = H, R_7 = CH_3
101 R_5 = H, R_6 = R_7 = CH_3
102 R_5 = R_6 = H, R_7 = CH_2CH_3

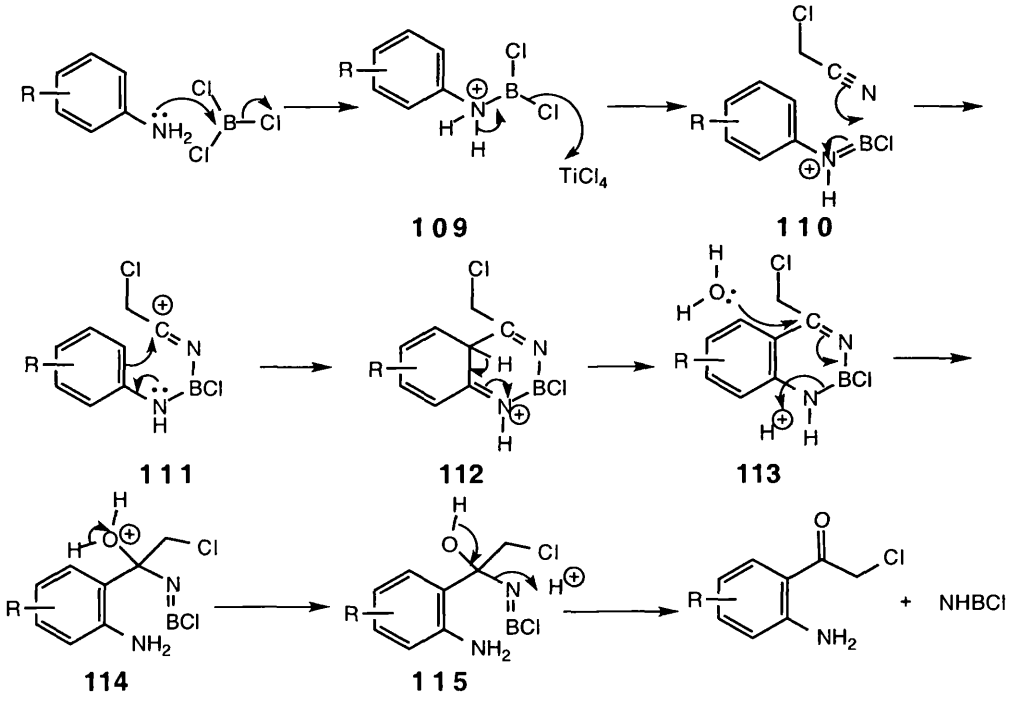
103 R_5 = OMe, R_6 = H, R_7 = CH_3
104 R_5 = OMe, R_6 = R_7 = CH_3
105 R_5 = R_7 = CH_3, R_6 = H
106 R_5 = R_6 = H, R_7 = CH_3
107 R_5 = H, R_6 = R_7 = CH_3
108 R_5 = R_6 = H, R_7 = CH_2CH_3

Reagents and conditions: a) BCl_3, CH_2Cl_2, $ClCH_2CN$, $TiCl_4$, reflux, 5 h; b) H^+; c) $NaBH_4$, dioxane, H_2O, 85 °C, 2 h.

Scheme 15. Synthesis of 5-, 6- and 7- alkylated indoles.

A possible mechanism for the chloroacetylation step is illustrated in Scheme 16. An S_N2 reaction of the amine with boron trichloride gives the anilinodichloroborane **109** which loses HCl to furnish the iminium salt **110**. The salt **110** reacts with chloroacetonitrile with the formation of a second N-B bond to give intermediate **111** which then undergoes electrophilic substitution at the *ortho* position with the resultant carbocation to give the cyclic derivatives **112**. Re-aromatisation followed by hydrolysis under acidic conditions gives the desired 2-amino-α-chloroacetophenone.

The α-chloroacetophenones gradually decomposed in air and attempted purification led to a vast decrease in yields and hence they were not purified prior to use.

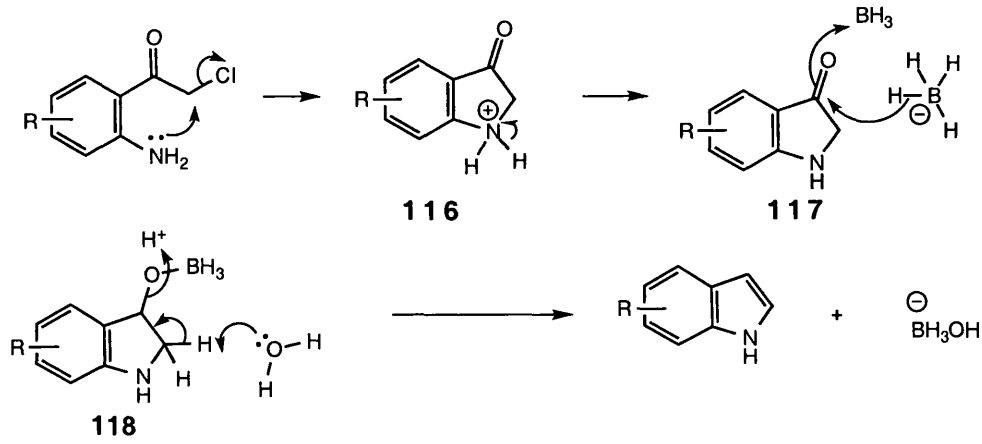


Scheme 16. Proposed mechanism for chloroacetylation of anilines.

The deshielded protons of the $C\underline{H}_2Cl$ group of the α-chloroacetophenones appear as a distinct singlet at approximately δ 4.7 in the ^1H NMR spectra. Two main absorptions band at around 3500 and 1650 cm^{-1} occur in the IR spectra and are attributed to the N-H and C=O stretching frequencies respectively. The mass spectra of all the α-chloroacetophenones showed a molecular ion (M^+) and base ions at $M^+ - CH_2Cl$.

In the reduction step, the borohydride ion can abstract the proton from the amino group of the acetophenone, giving intermediate **116** which can then cyclise to

form **117** with the elimination of the chloride ion. Hydrolysis of the inoxyl-borane complex **118** gave the substituted indole (Scheme 17).



Scheme 17. Proposed mechanism for the borohydride reduction of α-chloroacetophenones.

The ^1H NMR spectra of the substituted indoles were assigned by comparison of the data with the literature values for indole. The indolylic NH protons of all the substituted indoles prepared appear as broad singlets at approximately δ 8.0. The IR spectra also showed an absorption peak at about 3350 cm^{-1} assigned to the NH stretching frequency. The mass spectra of all the indoles show molecular ions (M^+) and no other major ions were observed.

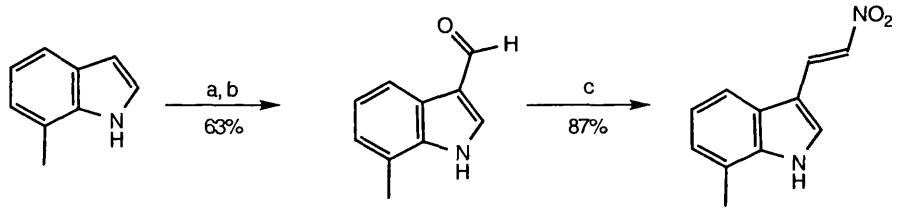
VI.B.C. Synthesis of nitrovinylindoles

Two approaches were undertaken for the synthesis of nitrovinylindoles. The first synthetic method employed involved the Vilsmeier-Haack formylation followed by a Henry nitro-olefination. The second procedure comprised a one step electrophilic substitution of the indole with dimethylaminonitroethylene.

VI.B.C.A. The Vilsmeier-Haack Reaction and the Henry Reaction

7-Methyl-3-(2-nitrovinyl)indole **(120)** was prepared as shown in Scheme 18. Treating phosphorus oxychloride with *N,N*-dimethylformamide gave a complex of *N,N*-dimethylacetamide which was then allowed to react with 7-methylindole **(106)**. Base hydrolysis of the resulting iminium salt, using aqueous sodium hydroxide, gave the desired aldehyde **119**. The ^1H NMR spectrum of the carbaldehyde shows a singlet at δ 10.09, attributed to the aldehydic proton. Similarly, the aldehydic carbon was observed in the ^{13}C NMR spectrum downfield at δ 185.2. A strong absorption

at 1647 cm^{-1} in the IR spectrum was attributed to the carbonyl stretching frequency. The mass spectrum showing molecular ion at 159 (M^+) and a base ion at 158 (M^+ - H), gave further support for the identity of carbaldehyde **119**. Condensation of nitromethane with **119** in the presence of ammonium acetate gave the nitrovinylindole **120** as an orange crystalline solid with a melting point of 210 - 211 °C. The ^1H NMR spectrum of **120** revealed the loss of the aldehyde singlet, with the appearance of two sets of doublets at δ 8.30 and δ 7.81 with a large coupling constant of J = 13.5 Hz, assigned to the two vinylic protons with an (*E*)-configuration. Introduction of the nitro function also resulted in two characteristic absorption bands at 1271 and 1302 cm^{-1} in the IR spectra. The mechanism of the individual reactions are detailed in Schemes 19 and 20.

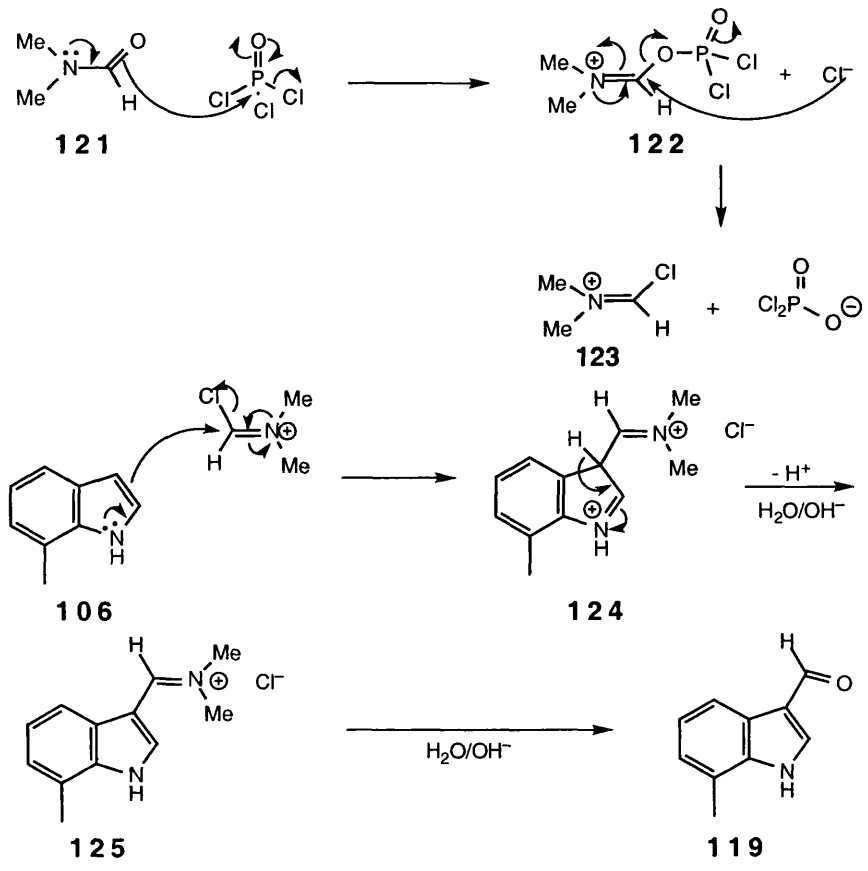


106 **119** **120**

Reagents and conditions: a) $POCl_3$, DMF, 0 °C; b) NaOH, H_2O; c) CH_3NO_2, NH_4OAc.

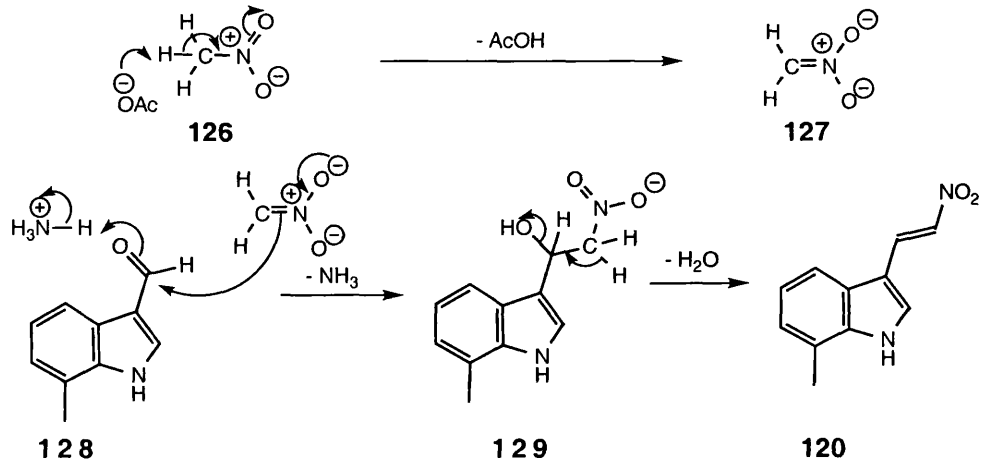
Scheme 18. Synthesis of nitrovinylindoles.

The proposed mechanism of the Vilsmeier-Haack formylation[212] is depicted in Scheme 19. The initial addition-elimination of the *N,N*-dimethylformamide **121** on the phosphorous oxychloride gave intermediate **122** which then undergoes nucleophilic attack by the chloride ion and loses $PCl_2O_2^-$ to generate the reactive species **123**. Electrophilic attack of **123** on the indole **106** gave the unstable iminium salt **124** which hydrolyses under basic conditions to yield the desired aldehyde **119**.



Scheme 19. Mechanism for the Vilsmeier-Haack formylation.

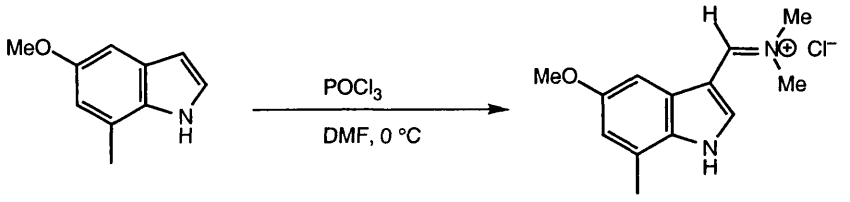
The Henry nitro-olefination[213] is believed to be initiated by the species **127**, generated by the removal of a proton from nitromethane **126** using ammonium acetate. The carbanion **127** adds to the carbonyl group of **128** which acquires a proton to form the alcohol **129**. Subsequent elimination of water gave the nitrovinylindole **120**.



Scheme 20. Mechanism for the Henry nitro-olefination.

An attempt to synthesise 5-methoxy-7-methylindole-3-carboxaldehyde by this route resulted in the isolation of the intermediate iminium salt **130**. The ^{1}H NMR

spectrum of this product showed a singlet at δ 3.87 which indicates the presence of a methoxy group. The spectrum also showed, however, two signals at δ 3.79 and δ 3.67, indicating the presence of the two non-equivalent N-$C\underline{H}_3$ groups. The mass spectrum showed ions at *m/z* 218 and 217, attributed to the $M^+ + 1$ and M^+ species. Attempts to hydrolyse the iminium salt **130** to the aldehyde were unsuccessful.

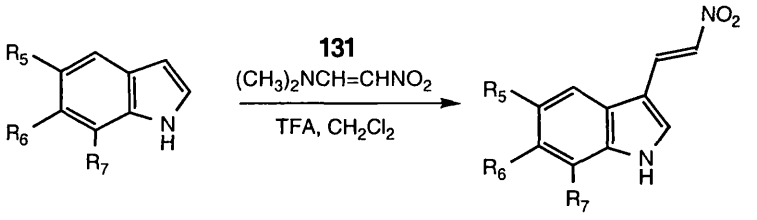


103 **130**

Scheme 21. Vilsmeier-Haack reaction of 5-methoxy-7-methylindole.

VI.B.C.B. One step nitro-olefination of indoles

The problem with hydrolysis of the iminium intermediate **130** prompted the exploration of the one-step synthesis of nitrovinylindoles described by Büchi *et al.*.[223] Condensation of the substituted indoles with dimethylaminonitroethylene **131** in the presence of trifluoroacetic acid afforded the corresponding nitrovinylindoles in good yields (Scheme 22). The reaction works successfully with all the indole derivatives examined (**103**, **104**, **105**, **107**, **108**). The ^1H NMR spectra of the nitrovinylindoles show two characteristic doublets in the region δ 8.24 - 8.33 and δ 7.77 - 7.88, both with a coupling constant of J ~ 13.4 Hz, assigned to the vinylic protons. Two new absorption bands at ~1250 and ~1300 cm^{-1} , attributed to the two N=O stretching frequencies, were also observed in the IR spectra. The EI mass spectra of the nitrovinylindoles showed molecular ions, while the FAB spectra show $M^+ + 1$ ions.



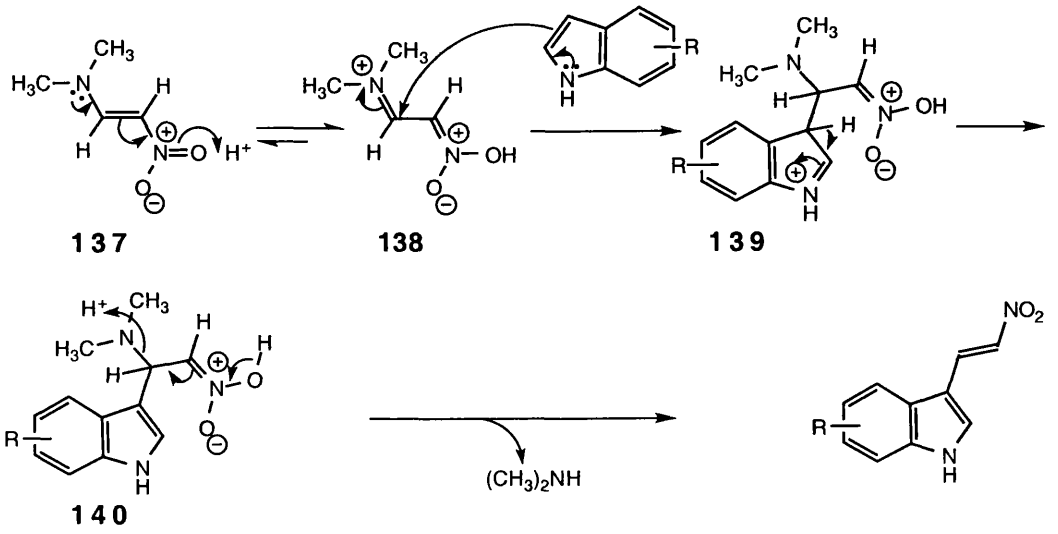
103 R_5 = OMe, R_6 = H, R_7 = CH_3
104 R_5 = OMe, R_6 = R_7 = CH_3
105 R_5 = R_7 = CH_3, R_6 = H
107 R_5 = H, R_6 = R_7 = CH_3
108 R_5 = R_6 = H, R_7 = CH_2CH_3

132 R_5 = OMe, R_6 = H, R_7 = CH_3
133 R_5 = OMe, R_6 = R_7 = CH_3
134 R_5 = R_7 = CH_3, R_6 = H
135 R_5 = H, R_6 = R_7 = CH_3
136 R_5 = R_6 = H, R_7 = CH_2CH_3

Scheme 22. Nitro-olefination of indoles to nitrovinylindoles.

The proposed mechanism of the condensation is shown in Scheme 23. The trifluoroacetic acid initiates the protonation of the oxygen on the nitro group giving

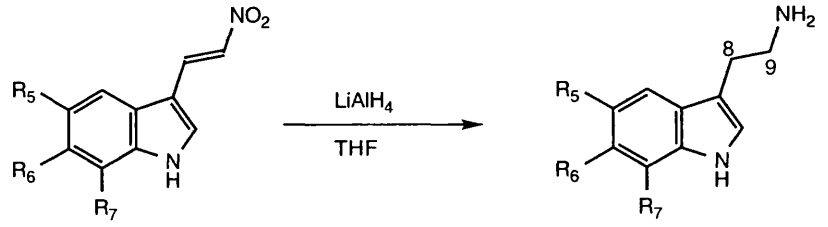
intermediate **138** which acts as an electrophile to react with the indole yielding the C-3 substituted species **139**. Re-aromatisation of the indole ring followed by protonation of the amino group and subsequent elimination of dimethylamine gives the nitrovinylindole.



Scheme 23. Proposed mechanism for the one step nitro-olefination of substituted indoles.

VI.B.D. Synthesis of the tryptamines

Reduction of the nitrovinylindoles to the corresponding tryptamines was readily achieved with lithium aluminium hydride (Scheme 24). Due to the reactivity of the resulting amines in contact with the atmosphere, presumably through oxidation, they were used in the subsequent acylations without further purification.



132 R_5 = OMe, R_6 = H, R_7 = CH_3
133 R_5 = OMe, R_6 = R_7 = CH_3
134 R_5 = R_7 = CH_3, R_6 = H
120 R_5 = R_6 = H, R_7 = CH_3
135 R_5 = H, R_6 = R_7 = CH_3
136 R_5 = R_6 = H, R_7 = CH_2CH_3

141 R_5 = OMe, R_6 = H, R_7 = CH_3
142 R_5 = OMe, R_6 = R_7 = CH_3
143 R_5 = R_7 = CH_3, R_6 = H
144 R_5 = R_6 = H, R_7 = CH_3
145 R_5 = H, R_6 = R_7 = CH_3
146 R_5 = R_6 = H, R_7 = CH_2CH_3

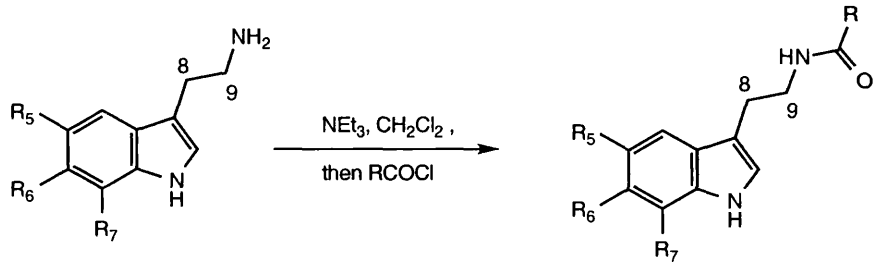
Scheme 24. Synthesis of tryptamines.

The ^1H NMR spectra of the tryptamines showed a characteristic NH resonance in the region of δ 7.84 - δ 8.50 as a broad singlet. Two new triplets with coupling constants of J ~ 6.5 Hz at about δ 2.90 and δ 3.00 were observed which

were assigned to CH_2-8 and CH_2-9 respectively. The spectroscopic data of the synthesised 5-methoxy-7-methyltryptamine[225] **(141)**, 5,7-dimethyltryptamine[225] **(143)** and 7-methyltryptamine[224] **(144)** agree with that of the literature. The mass spectra of all the tryptamines showed a characteristic base peak for $M^+ - CH_2NH_2$.

VI.B.E. Synthesis of *N*-acyltryptamines

Acylation of the tryptamines **141** - **146** with the appropriate acyl chlorides in the presence of triethylamine gave the melatonin analogues **147** - **169** (Scheme 25).

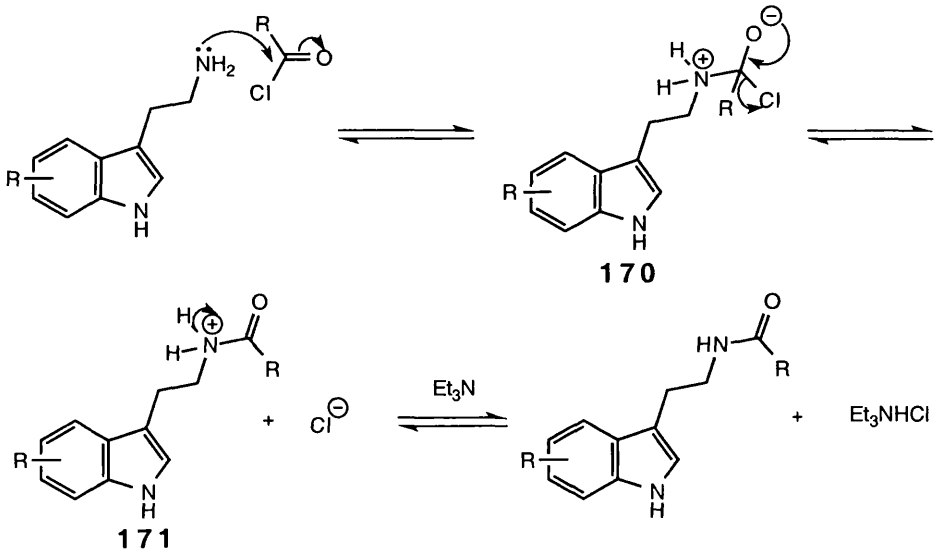


141 R_5 = OMe, R_6 = H, R_7 = CH_3
142 R_5 = OMe, R_6 = R_7 = CH_3
143 R_5 = R_7 = CH_3, R_6 = H
144 R_5 = R_6 = H, R_7 = CH_3
145 R_5 = H, R_6 = R_7 = CH_3
146 R_5 = R_6 = H, R_7 = CH_2CH_3

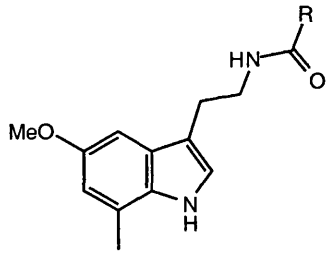
147 - **148** R_5 = OMe, R_6 = H, R_7 = CH_3, R = CH_3
149 - **152** R_5 = OMe, R_6 = R_7 = CH_3, R = CH_3
153 - **155** R_5 = R_7 = CH_3, R_6 = H, R = CH_3
156 - **160** R_5 = R_6 = H, R_7 = CH_3
161 - **164** R_5 = H, R_6 = R_7 = CH_3, R = CH_3
165 - **169** R_5 = R_6 = H, R_7 = CH_2CH_3, R = CH_3

Scheme 25. Synthesis of *N*-acyltryptamines.

The reaction is believed to proceed by an addition-elimination mechanism (Scheme 26). Initial addition of the tryptamine on the carbonyl group of the acyl chloride gave a tetrahedral intermediate **170** which expels Cl^- to afford the protonated tryptamine **171**. The reaction is driven to *N*-acyltryptamine by triethylamine.

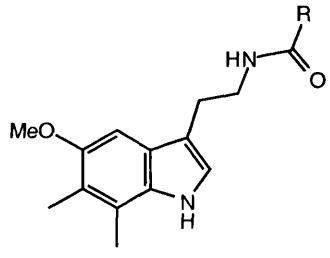


Scheme 26. Proposed mechanism for the acylation of tryptamines.



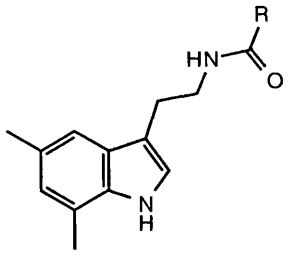
147 R = CH_3
148 R = CH_2CH_3

The ^1H NMR spectra of the *N*-acyl-5-methoxy-7-methyltryptamines were assigned on the basis of the literature spectrum of 5-methoxy-7-methyltryptamine.[225] The spectra of *N*-acyl-5-methoxy-7-methyltryptamines **147** and **148** showed an indolyl NH resonance at ~ δ 7.9, two doublets at ~ δ 7.4 (*J* ~ 1.5 Hz) and δ 7.0 (*J* ~ 1.7 Hz) attributed to H-4 and H-6 respectively, a singlet at ~ δ 6.7 assigned to H-2, an amido NH resonance at around δ 5.6, a quartet at ~ δ 3.6 (*J* ~ 6.4 Hz) assigned to CH_2-9, and a triplet at ~ δ 3.0 (*J* ~ 6.7 Hz), assigned to CH_2-8. The IR spectra of compounds **147** and **148** showed strong absorptions at approximately 3310, 3270 and 1610 cm^{-1} assigned to the two NH and C=O stretching frequencies, respectively. The mass spectra (FAB) all showed molecular ions, fragment ions at *m/z* 188 and *m/z* 187, representing the loss of RCONH, and a fragment at *m/z* 174, representing the loss of $RCONHCH_2$.



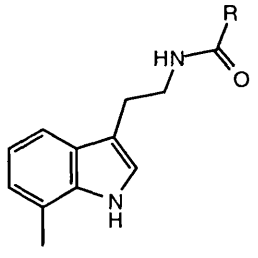
149 R = CH_3
150 R = CH_2CH_3
151 R = cyclopropyl
152 R = cyclobutyl

The ^1H NMR spectra of the *N*-acyl-5-methoxy-6,7-dimethyltryptamines **149** - **152** showed an indolyl NH resonance at around δ 8.3, a doublet at ~ δ 7.4 (*J* ~ 2.2 Hz) attributed to H-2, a singlet at ~ δ 6.9, assigned to H-4, an amido NH resonance centred at δ 5.7, a quartet at ~ δ 3.6 (*J* ~ 6.4 Hz) assigned to CH_2-9 and a triplet at ~ δ 3.0 (*J* ~ 6.7 Hz) assigned to CH_2-8. The IR spectra of **149** - **152** showed strong absorptions at around 3400, 3285 and 1650 cm^{-1} assigned to the stretching modes of the two NH and the C=O bands, respectively. The mass spectra (FAB and EI) all showed molecular ions, fragment ions at *m/z* 202 and *m/z* 201, indicating the loss of RCONH, and ions at *m/z* 188 and *m/z* 174, indicating the loss of $RCONHCH_2$.



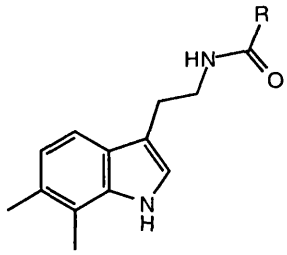
153 R = CH_2CH_3
154 R = cyclopropyl
155 R = cyclobutyl

The ^1H NMR spectra of the *N*-acyl-5,7-dimethyltryptamines **153** - **155** were in accord with the spectroscopic data for 5,7-dimethyltryptamine.[225] All the spectra obtained in the series showed an indolyl NH resonance at around δ 8.1, a singlet at ~ δ 7.3 assigned to H-4, a doublet at ~ δ 7.0 (*J* ~ 2.1 Hz) assigned to H-2, a singlet at ~ δ 6.9 assigned to the H-6 resonance, an amido NH resonance at approximately δ 5.6, together with a quartet at ~ δ 3.6 (*J* ~ 6.5 Hz) and a triplet at ~ δ 3.0 (*J* ~ 6.5 Hz) assigned to CH_2-9 and CH_2-8, respectively. The IR spectra of **153** - **155** showed strong absorptions at approximately 3320, 3280 and 1620 cm^{-1} assigned to the two NH and the C=O bands, respectively. The mass spectra (FAB and EI) all showed molecular ions, fragment ions at *m/z* 172 and *m/z* 171, indicating the loss of RCONH and *m/z* 158, indicating the loss of $RCONHCH_2$.



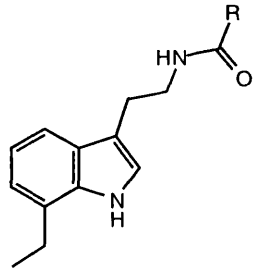
156 R = CH_3
157 R = CH_2CH_3
158 R = $CH_2CH_2CH_3$
159 R = cyclopropyl
160 R = cyclobutyl

The ^1H NMR spectra of N-acyl-7-methyltryptamines **156** - **160** were assigned on the basis of the literature spectrum of 7-methyltryptamine.[224] The indolyl NH resonance of the compounds occurred as a broad singlet centred at δ 8.0; H-2, H-5 and H-6 appeared as a multiplet at approximately δ 7.1 and the amido NH resonance occurred as a broad singlet at approximately δ 5.6. The quartet at around δ 3.6 (*J* ~ 6.4 Hz) was assigned to CH_2-9 and the triplet at around δ 3.0 (*J* ~ 6.7 Hz) was assigned to CH_2-8. The IR spectra of compound **156** - **160** showed strong absorptions at approximately 3400, 3250 and 1650 cm^{-1} assigned to the two NH and the C=O bands, respectively. The EI mass spectra all showed molecular ions and fragment ions at *m/z* 157 for loss of the amido function, and at *m/z* 144 for loss of $RCONHCH_2$.



161 R = CH_3
162 R = CH_2CH_3
163 R = cyclopropyl
164 R = cyclobutyl

The ^1H NMR spectra of the *N*-acyl-6,7-dimethyltryptamines **161** - **164** showed a characteristic indolyl NH resonance at approximately δ 8.1, a doublet at about δ 7.4 (*J* ~ 8.0 Hz) assigned to H-4, and a multiplet at about δ 7.0 which was assigned to H-2 and H-5. The amido NH resonance occurred as a broad singlet at approximately δ 5.6. The quartet at around δ 3.6 (*J* ~ 6.4 Hz) and the triplet at approximately δ 3.0 (*J* ~ 6.7 Hz) were assigned to CH_2-9 and CH_2-8. The IR spectra of compound **161** - **164** showed strong absorptions at around 3400, 3300 and 1650 cm^{-1} assigned to the two NH and C=O bands, respectively. The mass spectra (FAB) all showed molecular ions, fragment ions at *m/z* 172 and *m/z* 171 due to the loss of RCONH, and ions at *m/z* 158 and *m/z* 144 due to fragmentation of the amido side chain.



165 R = CH_3
166 R = CH_2CH_3
167 R = $CH_2CH_2CH_3$
168 R = cyclopropyl
169 R = cyclobutyl

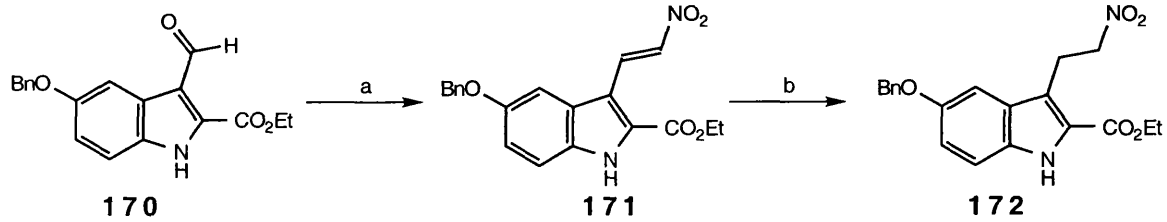
The ^1H NMR spectra of the *N*-acyl-7-ethyltryptamines **165** - **169** showed an indolyl NH resonance at about δ 8.4, a doublet at about δ 7.5 (*J* ~ 7.6 Hz) assigned to the H-4, a multiplet at about δ 7.1, assigned to H-5 and H-6, a doublet at approximately δ 7.0 (*J* ~ 2.2 Hz) assigned to H-2, and an amido NH resonance at approximately δ 5.7. The quartet at about δ 3.6 (*J* ~ 6.4 Hz) and the triplet at about δ 3.0 (*J* ~ 6.7 Hz) were assigned to CH_2-9 and CH_2-8 respectively. The IR spectra of compound **165** - **169** showed strong absorptions at 3400, 3000 and 1650 cm^{-1} assigned to the two NH and C=O bands, respectively. The mass spectra (FAB and EI) all showed molecular ions, ions at *m/z* 172 and *m/z* 171 due to the loss of RCONH, and ions at *m/z* 158 and *m/z* 144 due to fragmentation of the amido side chain.

VII. Synthesis of 2,3-dialkylsubstituted indoles

Non-isotopic immunoassay methods such as enzyme and chemiluminescent immunoassay provide good alternatives to radioimmunoassay, owing to their competitive specificity and sensitivity and their ease of handling. A recent example was demonstrated by Cuisset *et al.*[255] who coupled 11-ketotestosterone to acetylcholinesterase (AChE) and applied the complex as an enzyme tracer for the determination of the plasma concentration in Siberian sturgeon. It has been shown that melatonin can be directly coupled to an enzyme in a similar fashion *via* the Mannich reaction.[226] We were interested in melatonin analogues substituted at the 2-position with a small carbon chain terminating in a functional group that could be selectively conjugated to acetyl cholinesterase. Such a system could then be used in an immunosorbant assay. Nitroethylindoles with alkyl substituents at the 3-position were selected to be intermediates to compounds of this type.

VII.A Literature review

There has been extensive research on indole analogues bearing substituents at the 2- and 3-position. In 1975, Nantka-Namirski *et al.*[227] described a two-step synthesis of 5-benzyl-3-(2-nitroethyl)-1H-indole-2-carboxylic acid ethyl ester **(172)**. A Henry reaction[213] of carboxaldehyde **170** followed by reduction with sodium borohydride gave **172** (Scheme 27).



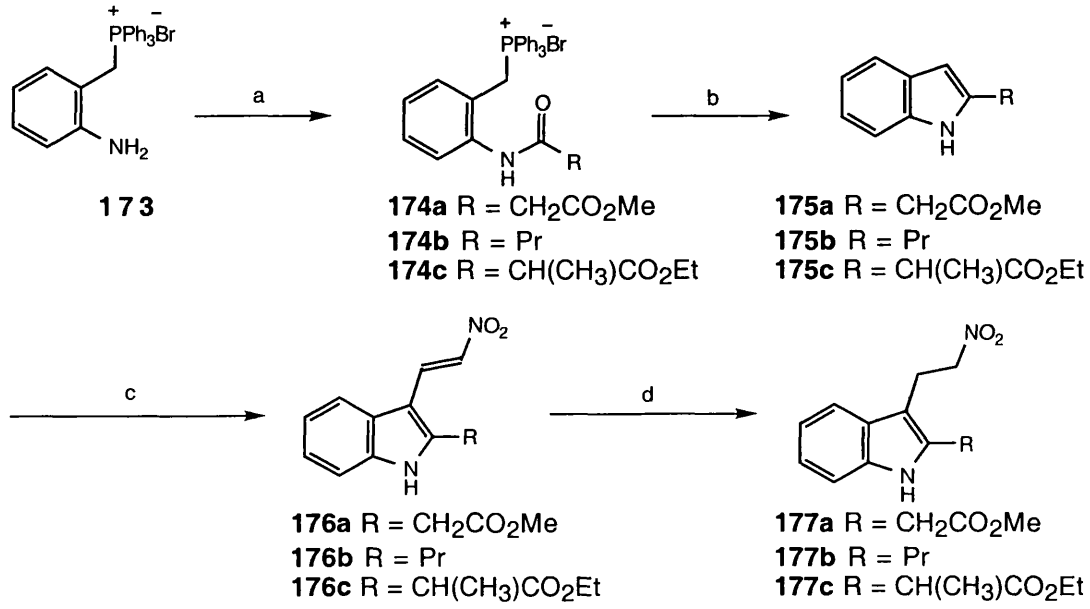
170 **171** **172**

Reagents and conditions: a) CH_3NO_2, $EtNO_2$, AcOH, AcONa; b) $NaBH_4$, EtOH.

Scheme 27. Synthesis of 5-benzyl-3-(2-nitroethyl)-1H-indole-2-carboxylic acid ethyl ester.

In a report on tricyclic indole derivatives and alkaloids, Mahboobi *et al.*[228] described the synthesis of methyl 3-(2-nitroethyl)-1H-indole-2-acetate **(177a)** (Scheme 28). Condensation of the (2-aminobenzyl)triphenyl phosphonium bromide **(173)** with methyl 3-chloro-3-oxo-propanoate gave the amide **174a** which in an intramolecular Wittig-type reaction induced by sodium tert-pentylate in toluene yielded **175a**. Reaction of **175a** with *N,N*-dimethyl-2-nitroethenamine in the presence of trifluoroacetic acid gave the nitrovinylindole **176a**. Selective hydrogenation of the nitroethylene double bond using Wilkinson's catalyst gave the nitroethylindole **177a**.

This method was also used by Freund *et al.*[229] in the synthesis of 3-(2-nitroethyl)-2-propyl-1H-indole **(177b)** (Scheme 28) and by Danieli *et al.*[230] in the synthesis of ethyl 2-[3-(2-nitroethyl)-indol-1H-2-yl]propanoate **(177c)** as a precusor to the alkaloid (±)-3-oxovincadifformine.



173

174a R = CH_2CO_2Me
174b R = Pr
174c R = $CH(CH_3)CO_2Et$

175a R = CH_2CO_2Me
175b R = Pr
175c R = $CH(CH_3)CO_2Et$

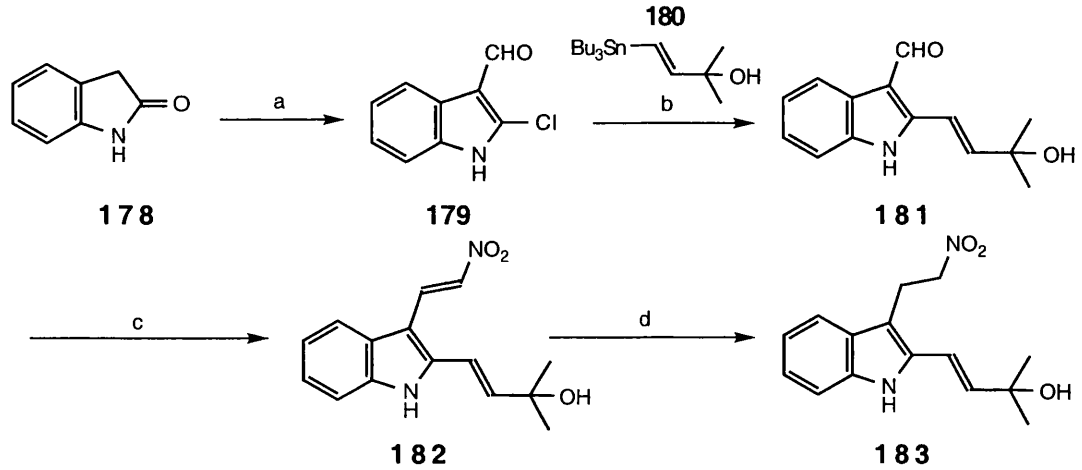
176a R = CH_2CO_2Me
176b R = Pr
176c R = $CH(CH_3)CO_2Et$

177a R = CH_2CO_2Me
177b R = Pr
177c R = $CH(CH_3)CO_2Et$

Reagents and conditions: a) MeO_2CCH_2COCl, CH_2Cl_2, or PrCOCl, or $EtO_2C(CH_3)CHCOCl$; b) $C_2H_5C(CH_3)_2ONa$, toluene, reflux, 3 h; c) $(CH_3)_2NCH{=}CHNO_2$, TFA, CH_2Cl_2; d) $(PPh_3)_3RhCl$, H_2 or $NaBH_4$, THF-MeOH 9:1.

Scheme 28. Synthesis of methyl 3-(2-nitroethyl)-1H-indole-2-acetate.

In 1988, Somei and co-workers[231] took a different approach to the synthesis of the 2-substituted nitroethylindole **(211)**, a precusor to the alkaloid borrerine (Scheme 29). A Vilsmeier-Haack formylation[212] of the oxindole **178** followed by Stille coupling[232] with (3-hydroxy-3-methyl-1-buten-1-yl)tributyltin **(180)** in the presence of tetra-*n*-butylammonium chloride and a catalytic amount of palladium acetate gave the 2-substituted carboxaldehyde **181**. Subsequent Henry nitro-olefination[213] followed by reduction with sodium borohydride gave **183**.

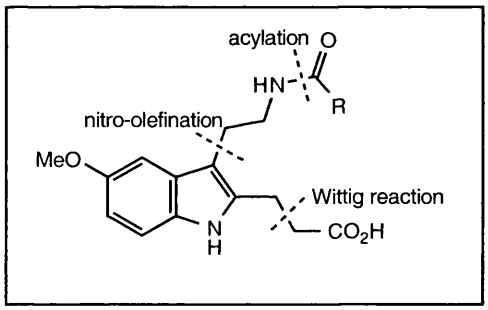


Reagents and conditions: a) $POCl_3$, *N,N*-dimethylformamide; b) Bu_4NH_4Cl, cat. $Pd(OAc)_2$; c) CH_3NO_2, NH_4OAc; d) $NaBH_4$, MeOH.

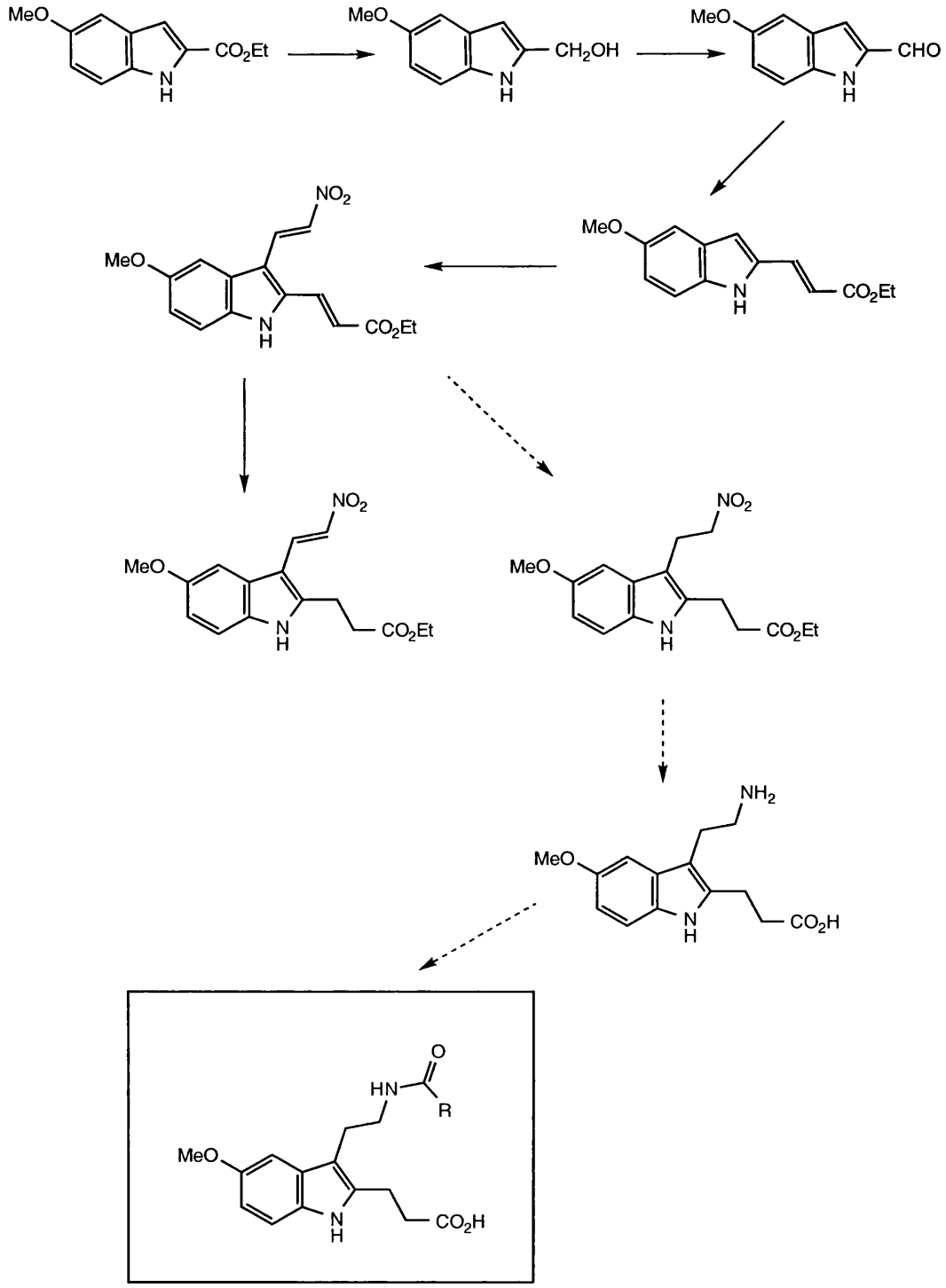
Scheme 29. Synthesis of 2-methyl-4-[3-(2-nitroethyl)-1H-indol-2-yl]-but-3-en-2-ol.

VII.B Synthesis

Retroanalysis of the 2,3-dialkylsubstituted shows three key steps: a nitro-olefination and a Wittig reaction for introducing the side chains at the C-2 and C-3 positions, and an acylation step for the introduction of R groups.



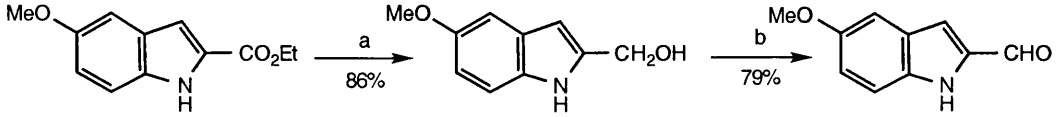
The synthetic plan was to start with the 2-substituted indole ester and convert it into the corresponding alcohol and then to the aldehyde which can be transformed into the vinyl ester. Introduction of the nitrovinyl group at the C-3 position followed by reduction of the double bonds of the C-2 and C-3 side chain should lead to the tryptamine which can then be acylated to give the target compounds (Scheme 30).



Scheme 30. Synthetic plan for the synthesis of 2,3-dialkylsubstituted indoles.

VII.B.A. Preparation of 5-methoxy-indole-2-carboxaldehyde

Following the method described by Meyer *et al.*,[233] the starting 5-methoxy-indole-2-carboxylic acid ethyl ester **(184)** was reduced with lithium aluminium hydride. Oxidation of the resulting 2-(hydroxymethyl)-5-methoxyindole **(185)** with manganese dioxide afforded the carboxaldehyde **186** in 68% overall yield (Scheme 31).



184 **185** **186**

Reagents and conditions: a) $LiAlH_4$, THF; b) MnO_2, CH_2Cl_2.

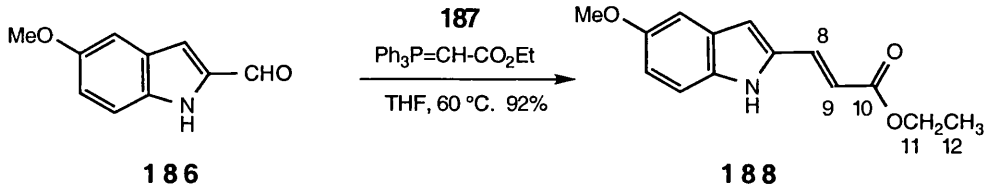
Scheme 31. Synthesis of 5-methoxy-indole-2-carboxaldehyde.

The ^1H NMR spectrum of the alcohol **185** showed the characteristic broad indolyl signal at δ 8.22, two doublets at δ 7.24 (J = 8.7 Hz) and at δ 7.05 (J = 2.4 Hz) which were assigned to H-7 and H-4 respectively, a doublet of doublet at δ 6.85 (J = 8.8, 2.5 Hz) which was assigned to H-6, a singlet at δ 6.35, which was assigned to H-3, a doublet at δ 4.83 which was assigned to the CH_2 protons, a distinctive methoxy singlet at δ 3.85 and a triplet at δ 1.76 which was assigned to the OH group. The IR spectrum showed absorptions at 3353 and 3198 cm^{-1}, assigned to the OH and NH bands, respectively.

Compound **186** shows the expected changes in spectroscopic data from those of **185**. In the ^1H NMR spectrum the OH triplet signal at δ 1.76 was absent and the aldehydic proton appeared as a singlet at δ 9.82. The aldehydic carbon appeared at δ 181.8 in the ^{13}C NMR spectrum. The IR spectrum shows an additional strong absorption for the carbonyl bond at 1674 cm^{-1}.

VII.B.B. Preparation of the carboxylate

A Wittig reaction of 5-methoxyindole-2-carboxaldehyde **(186)** with carbethoxymethylenetriphenyl phosphorane **(187)** gave selectively the *trans* ester **188** in 92% yield (Scheme 32).



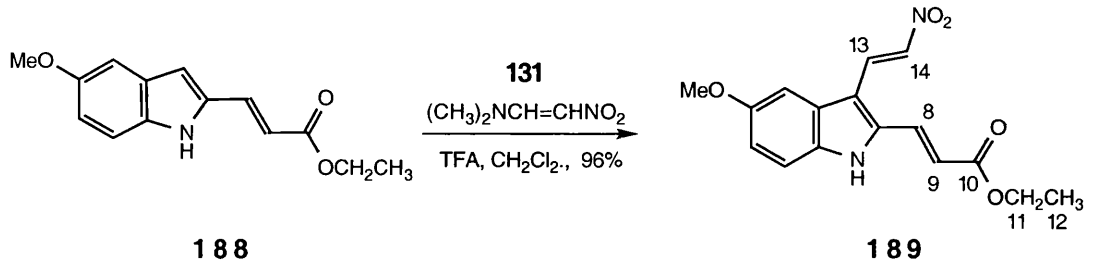
186 **188**

Scheme 32. Synthesis of ethyl (*E*)-(5-methoxy-indol-2-yl)-2-propenoate.

No singlet at δ 9.82 was observed in the ^{1}H NMR spectrum of **188** and two new doublets at δ 7.67 and δ 6.25 (both with J =16.0 Hz) had appeared, assigned to the H-9 and H-8, respectively. A strong absorption was observed at 1685 cm^{-1} in the IR spectrum assigned to the C=O group of the conjugated ester. The mass spectra (FAB) showed molecular ions and fragment ions at *m/z* 200, indicating the loss of the OEt group.

VII.B.C. Synthesis of the nitrovinylindolyl ester

The synthesis of the nitrovinyl ester **189** proceeded smoothly by the reaction of **188** and 1-dimethylamino-2-nitroethylene **(131)** in the presence of an excess amount of trifluoroacetic acid (Scheme 33). The mechanism of the nitro-olefination was discussed previously (See Scheme 26).



188 **189**

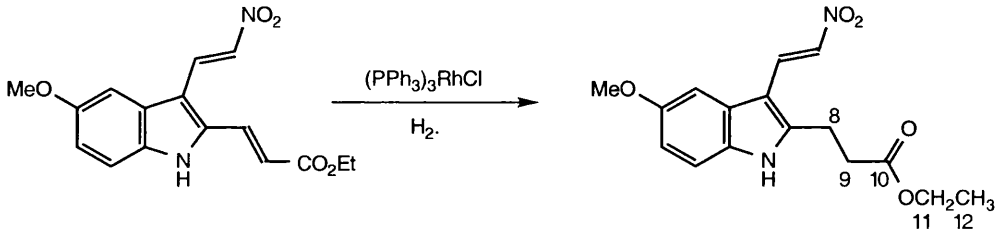
Scheme 33. Synthesis of the nitrovinylindolyl ester.

The introduction of the nitrovinyl group at C-3 gave rise to two new doublets at δ 8.45 and δ 7.80, both with J = 13.4 Hz in the ^{1}H NMR spectrum which were assigned to H-14 and H-13, respectively. Similarly, in the ^{13}C NMR spectrum two new signals in the aromatic region were observed. The IR spectrum of **189** showed

two new absorptions at 1305 and 1254 cm^{-1}, ascribed to the stretching mode of the N=O bonds. The mass spectra (FAB) showed molecular ions and fragment ions at *m/z* 271, indicating the loss of the nitro group, and at *m/z* 258, indicating the loss of $CHNO_2$.

VII.B.D. Hydrogenation of the nitrovinylindolyl ester

The first strategy was to reduce the double bonds in nitrovinylindolyl ester **189** with lithium aluminium hydride in tetrahydrofuran and acylate the resulting amine with acetyl chloride. However, none of the desired product was observed. Another synthetic route was sought so that more control of the hydrogenation process could be achieved. The second strategy was to follow the catalytic hydrogenation procedure of Mahboobi *et al.*[228] for the reduction of the double bonds with Wilkinson's catalyst, tris(triphenylphosphine)rhodium(I) chloride (Scheme 34).



189 **190**

Scheme 34. Hydrogenation of the nitrovinylindolyl ester.

The ^1H NMR spectrum of the product reveals the disappearance of the doublets at δ 7.92 and δ 6.38, the appearance of two triplets at δ 3.24 and δ 2.78 (J = 5.9 Hz) assigned to H-9 and H-8, and the retention of the two doublets with a coupling constant of J ~ 13.2 Hz, arising from the nitrovinyl protons at δ 8.33 and δ 7.76. From this, it can be concluded that the vinyl ester side chain was reduced but not the nitrovinyl functionality.

Lack of time precluded further investigation of compound **190**.

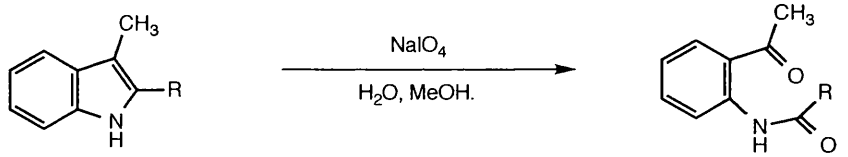
VIII. Kynurenamines

The hypothesis that melatonin acts as a prohormone and that two of the metabolites, N^{γ}-acetyl-N^{2}-formyl-5-methoxykynurenamine **(6)** and N-acetyl-5-methoxykynurenamine **(7)**, may modulate many of its effects was suggested by Kelly et al. [59] who demonstrated that **7** inhibits prostaglandin (PG) synthesis but melatonin does not. Kennaway et al. [234] then found that both **6** and **7** significantly retard reproductive maturation in prepubertal rats when injected subcutaneously. Kynurenamine **7** was also shown to have in vitro activity in inhibiting benzodiazepine receptor binding in crude membrane homogenates. No binding data had previously been published for this group of melatonin metabolite. We decided to synthesise kynurenamines in order to examine their potencies as melatonin receptor ligands.

VIII.A. Literature review

The direct synthetic route to kynurenamine is by oxidative cleavage of the double bond between C-2 and C-3 of melatonin. Numerous methods have been reported for the cleavage of this bond in indole derivatives.

In 1966, Dolby et al.[235] successfully cleaved the indolic double bond of 3-methylindole **(191a)** and 2,3-dimethylindole **(191b)** using an aqueous solution of sodium periodate in methanol to give o-formaminoacetophenone **(192a)** and o-acetaminoacetophenone **(192b)** (Scheme 35).

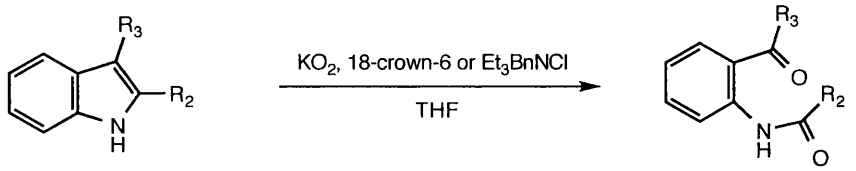


191a R = H
191b R = CH_3

192a R = H
192b R = CH_3

Scheme 35. Oxidation of indoles with sodium periodate.

Ring cleavage of indoles was also achieved by Balogh-Hergovich et al.[236] using potassium superoxide. The indoles **193a** - **193d** were treated with superoxide ions in the presence of either 18-crown-6 or triethyl(benzyl)ammonium chloride in THF. When R_2 = H or Ph, the corresponding o-acylaminoketones **194a** - **194d** were formed (Scheme 36).

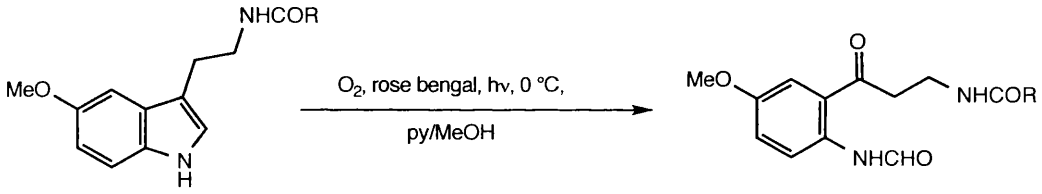


193a R_2 = H, R_3 = CH_3
193b R_2 = Ph, R_3 = H
193c R_2 = Ph, R_3 = CH_3
193d R_2 = Ph, R_3 = Ph

194a R_2 = H, R_3 = CH_3
194b R_2 = Ph, R_3 = H
194c R_2 = Ph, R_3 = CH_3
194d R_2 = Ph, R_3 = Ph

Scheme 36. Oxidation of indoles with superoxides.

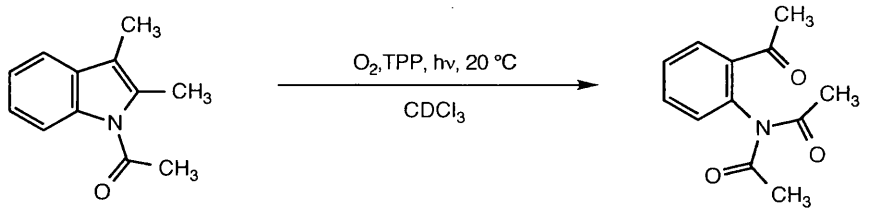
Photo-oxygenation of indoles via singlet oxygen was reported by two groups[237,238] using the sensitisers tetrachlorotetraiodo-fluorescein (Rose Bengal) or 5,10,15,20-tetraphenyl-21H,23H-porphine (TPP). Nakagawa et al.[238] converted melatonin **1** and its analogue **195** to kynurenamines **6** and **196** respectively with Rose Bengal in pyridine-MeOH at 0 °C under oxygen (Scheme 37), whereas Adam et al.[237] converted N-acylated indole **197** to the corresponding ketoimide **198** with TPP at 20 °C (Schemes 38).



1 R = Me
195 R = OMe

6 R = Me
196 R = OMe

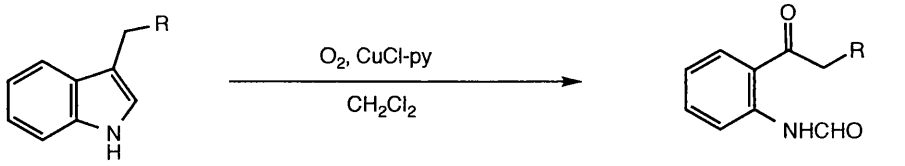
Scheme 37. Photo-oxygenation of indole derivatives with Rose Bengal.



197 **198**

Scheme 38. Photo-oxygenation of an indole derivative with TPP.

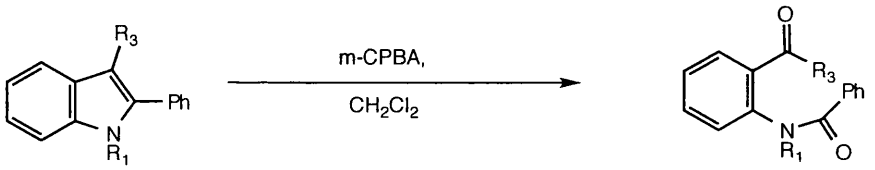
The oxidative cleavage of 3-substituted indoles was reported by Tsuji et al.[239] N-acetyltryptamine **(199a)**, methyl 3-indolylacetate **(199b)** and methyl-2-acetamido-3-(3-indolyl)propionate **(199c)** were cleaved by catalytic oxidation under oxygen using copper (I) chloride - pyridine complex to give the corresponding aminoacetophenones **200a - 200c** (Scheme 39).



199a R = CH_2NHAc **200a** R = CH_2NHAc
199b R = CO_2Me **200b** R = CO_2Me
199c R = $CH(NHAc)CO_2Me$ **200c** R = $CH(NHAc)CO_2Me$

Scheme 39. Oxidative cleavage of indole derivatives with CuCl/py complex.

Hino et al.[240] successfully oxidised N-methyl-2,3-diphenylindole **(201b)** and the N-unsubstituted derivative **201a** with m-chloroperbenzoic acid (m-CPBA) to give the corresponding aminoacetophenones **202b** and **202a**.



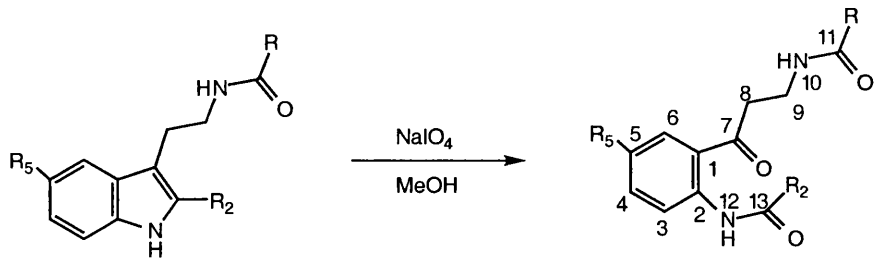
201a R_1 = H, R_3 = Ph **202a** R_1 = H, R_3 = Ph
201b R_1 = Me, R_3 = Ph **202b** R_1 = Me, R_3 = Ph

Scheme 40. Oxidative cleavage of indole derivatives with m-CPBA.

VIII.B. Synthesis

An investigation into different reaction conditions with melatonin itself was undertaken which showed that the sodium periodate oxidation afforded the most convenient method. With two equivalents of periodate, melatonin was cleaved in 21 hours to give kynurenamine **6** in 30% yield. N-cyclobutanecarbonyl-2-(5-methoxy-phenyl-indol-3-yl)ethanamine **(203)** and cyclopentanecarbonyl-2-(phenyl-indol-3-yl)ethanamine **(204)** were also cleaved under the same conditions, giving kynurenamines **205** and **206** in respectable yields (Scheme 41).

However, when the periodate oxidation was applied to N-propanoyl-2-(7-methylindol-3-yl)ethanamine **(158)**, N-cyclopropanecarbonyl-2-(7-methylindol-3-yl)ethanamine **(159)** and N-cyclobutanecarbonyl-2-(7-methylindol-3-yl)ethanamine **(160)**, a mixture of compounds was obtained but none of the desired products could be isolated.



1 R = CH_3, R_2 = H, R_5 = OMe
203 R = cyclobutyl, R_2 = Ph, R_5 = OMe
204 R = cyclopentyl, R_2 = Ph, R_5 = H

6 R^1 = CH_3, R_2 = H, R_5 = OMe
205 R = cyclobutyl, R_2 = Ph, R_5 = OMe
206 R = cyclopentyl, R_2 = Ph, R_5 = H

Scheme 41. Synthesis of kynurenamines.

The spectroscopic data of kynurenamine **6** was consistent with the published data.[238] The ^{1}H NMR spectra of the kynurenamine derivatives **6**, **205** and **206** all showed a broad singlet for the benzamide proton in the region of δ 11.0 -13.0, and a broad singlet for the ethanamide proton at ~ δ 6.0. The ^{13}C NMR spectra displayed three downfield signals between δ 160.0 - δ 204.0, assignable to the three carbonyl carbon atoms. Additionally, the IR spectrum also showed three strong C=O stretching frequencies at around 1650 cm^{-1}. Mass spectrum (EI) of the synthesised kynurenamines showed the molecular ion and fragment ions indicating the loss of CH_2CH_2NHCOR and $COCH_2CH_2NHCOR$.

IX. Biological Assays

IX.A. Introduction

The biological activity of the melatonin derivatives and the kynurenamines were determined in a specific *in vitro* model of melatonin action, the pigment aggregation response, in a clonal *Xenopus laevis* dermal melanophore cell line[156,157] developed and carried out by Dr David Sugden and Mr Muy-Teck Teh of King's College, London.

IX.A.A. Cell cultures

Xenopus laevis fibroblast and melanophore cell lines were grown at 24 °C - 27 °C in the dark without any gaseous exchange. The medium, which consisted of 0.7 x Leibovitz medium (L-15) containing 100 i.u. mL^{-1} penicillin, 0.1 mg mL^{-1} streptomycin, 4 mM *L*-glutamine and 10% heat inactivated (56 °C for 30 minutes) fetal calf serum ('Myoclon'; GIBCO/BRL), was conditioned by fibroblasts and used to feed the melanophores every 3 - 5 days. All cell types were maintained as monolayers in flasks with a 175 cm^2 growth area.

IX.A.B. Measurement of pigment aggregation

IX.A.B.A. Agonism

In the melanophores, thousands of black pigment granules are normally distributed throughout the cell cytosol and addition of melatonin triggers their rapid movement towards the centre of the cell. This aggregation response was quantified by measuring the change in light absorbance of the cells at 630 nm before and after drug treatment. The fractional change in absorbance, $1-(A_f/A_i)$, where A_i is the initial absorbance before drug treatment and A_f is the final absorbance 60 minutes after drug treatment, was calculated for each concentration of drug tested. The negative logarithm of the concentration of analogue producing 50% of the maximum agonist response (pEC_{50}) was determined using a curve-fitting program.[241] In the present study, a clonal melanophore cell line was used. Cells were grown in 96-well tissue culture plates containing the growth medium described in section IX.A.A., the original medium was replaced with 0.7 x L-15 medium (containing 1 mg ml^{-1} bovine albumin, 100 i.u. ml^{-1} penicillin, and 0.1 mg ml^{-1} streptomycin) 18 hours before the analogues were tested. All experiments used triplicate wells at six concentrations of analogue.

IX.A.B.A. Antagonism

For evaluation of antagonist potency, cells were treated with vehicle (1% dimethyl sulphoxide or methanol) or varying concentrations (10^{-4} - 10^{-9} M) of the analogues before melatonin (10^{-9} M) was added. Vehicle did not alter pigment granule distribution itself or inhibit responses to melatonin. The concentration of analogue reducing melatonin-induced pigment aggregation by 50% was found and an IC_{50} value determined (Table 4 - 12).

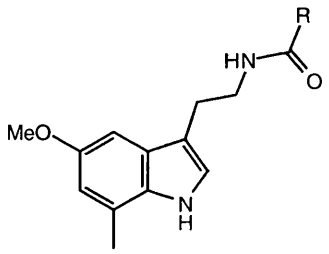
Each data point on the graphs represents a mean of $n = 3$ measurements. All standard errors (s.e.) were nearly always less than 0.02 ($p < 0.02$) because of the large number of cells involved and hence are omitted. All the pEC_{50} values are reported in molar (M) concentrations unless otherwise stated (Figures 7 - 14).

The compounds prepared as described in the synthetic section VII.B and VIII.B were evaluated as agonists or antagonists in these assays. The pEC_{50} and pIC_{50} values are recorded in the following tables.

N-Acyltryptamines

Table 4 shows the biological effect of two *N*-acyl-5-methoxy-7-methyltryptamines. The results are summarised in Figure 7. Both of the *N*-cycloalkane carbonyl-5-methoxy-7-methyltryptamines **147** and **148** were weak antagonists. Comparing the values found for **147** and **148** to those for *N*-cyclopropyl-5-methoxytryptamine **(209)** and *N*-cyclobutyl-5-methoxytryptamine **(210)** (Table 5), which were both found to be agonists in the *Xenopus* melanophore assay,[156] it can be seen that the introduction of a methyl group in the 7-position dramatically lowers the agonist potency for these compounds. Compound **148** resembles one of the numerous cyclobutyl derivatives that show antagonist rather than agonist potency. In the case of the cyclopropyl derivatives, these more frequently show agonist behaviour but the activity depends on the general structure. For example, compound **211** (P.124) is an agonist, with both the 5-methoxy and 2-phenyl group promoting agonist potency, whereas **212**, in which chlorine replaces methoxy at the 5-position, is potent both as an agonist and antagonist. Compound **147** shows partial agonist activity (31% aggregation).

Table 4. Potency of *N*-acyl-5-methoxy-7-methyltryptamines on *Xenopus* melanophores.



Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
147	cyclopropyl	PA	4.9
148	cyclobutyl	NA	4.8

NA = Not agonist or not antagonist; PA = Partial agonist.

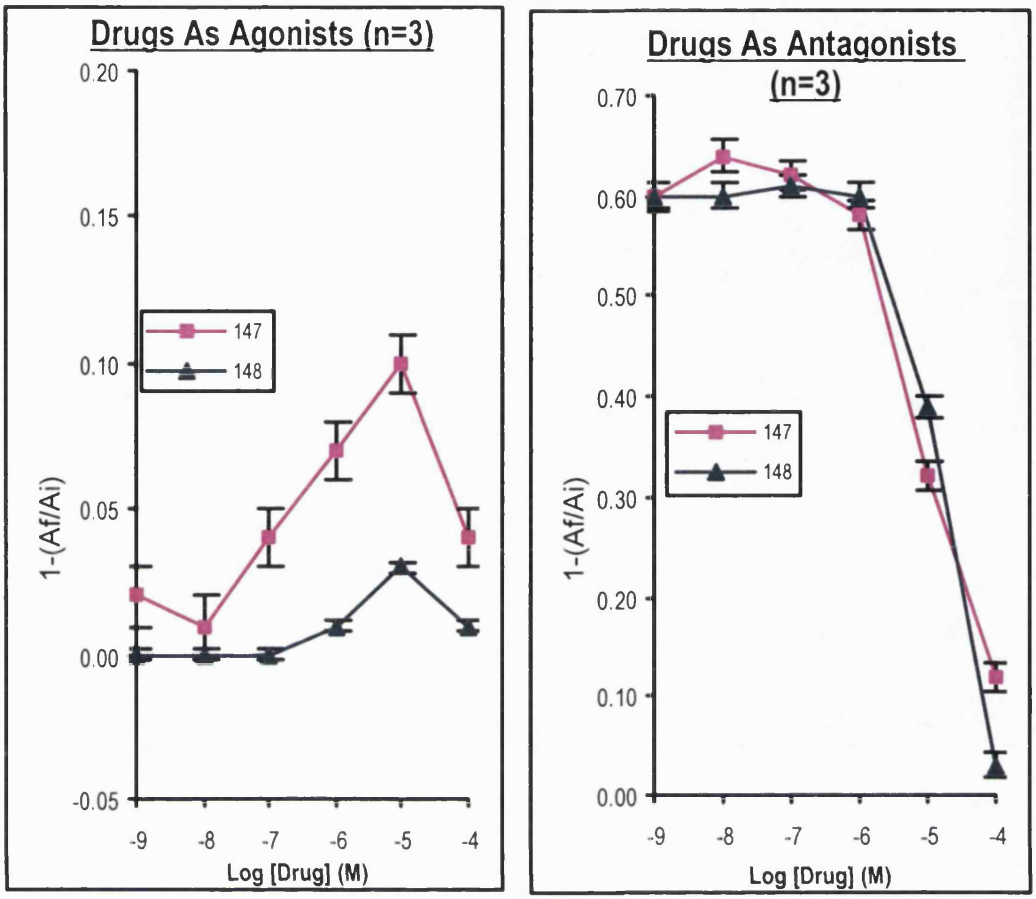
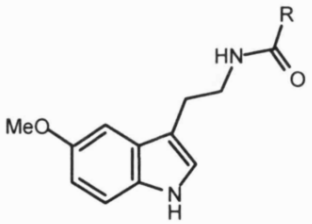


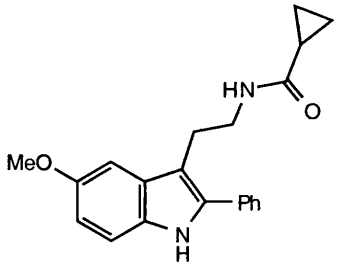
Figure 7. Concentration-response curves for pigment aggregation by *N*-acyl-5-methoxy-7-methyltryptamines in *Xenopus leavis* melanophores.

Table 5. Potency of *N*-acyl-5-methoxytryptamines on *Xenopus* melanophores.



Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})	Ref
1	CH_3	9.1	NA	172
207	CH_2CH_3	9.8	NA	172
208	$CH_2CH_2CH_3$	10.3	NA	172
209	cyclopropyl	9.0	NA	172
210	cyclobutyl	8.1	3.9	*

* Private communication from Sugden, D.; Jones, R.J.



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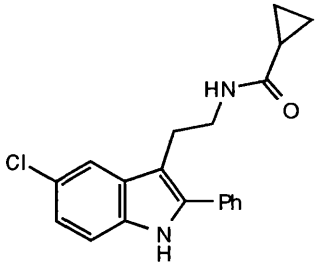
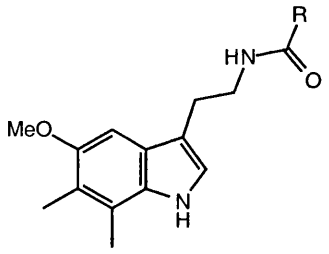


212

The biological response of a series of *N*-acyl-5-methoxy-6,7-dimethyltryptamines is given in Table 6 and the concentration-response curves in the pigment aggregation model are shown in Figure 8. Derivatives **149** and **150** are agonists with similar potencies, but showed no antagonist activity. Compound **149** (pEC_{50} = 8.2) exhibits a nine-fold decrease in agonist activity when compared to melatonin **1**[172] (pEC_{50} = 9.2). Similarly, compound **150** (pEC_{50} = 8.2) is 42-fold less active than the corresponding 5-methoxy-*N*-propionyltryptamine **(207)** (pEC_{50} = 9.8). However, changing from a ethyl **150** to a cyclopropyl **151** *N*-acyl side chain caused a complete loss in agonist activity. The effect in this series thus differs from that in the series of simple melatonin analogues (Table 5) when changing R from propyl to cyclopropyl reduces (pEC_{50} = 9.2 to 9.0) rather than eliminates the agonist activity. Compounds **151** and **152** are devoid of agonist activity but act as melatonin antagonists, the cyclopropyl derivative **151** being more potent. A similar difference was observed with the corresponding *N*-cycloalkyl-5-methoxy-7-methyltryptamine homologues **147** and **148**. It can also be seen that introduction of the methyl group at C-6 further increases the antagonist potency.

Table 6. Potency of *N*-acyl-5-methoxy-6,7-dimethyltryptamines on *Xenopus* melanophores.



Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
149	CH_3	8.2	NA
150	CH_2CH_3	8.2	NA
151	cyclopropyl	NA	5.6
152	cyclobutyl	NA	5.4

NA = Not agonist or not antagonist.

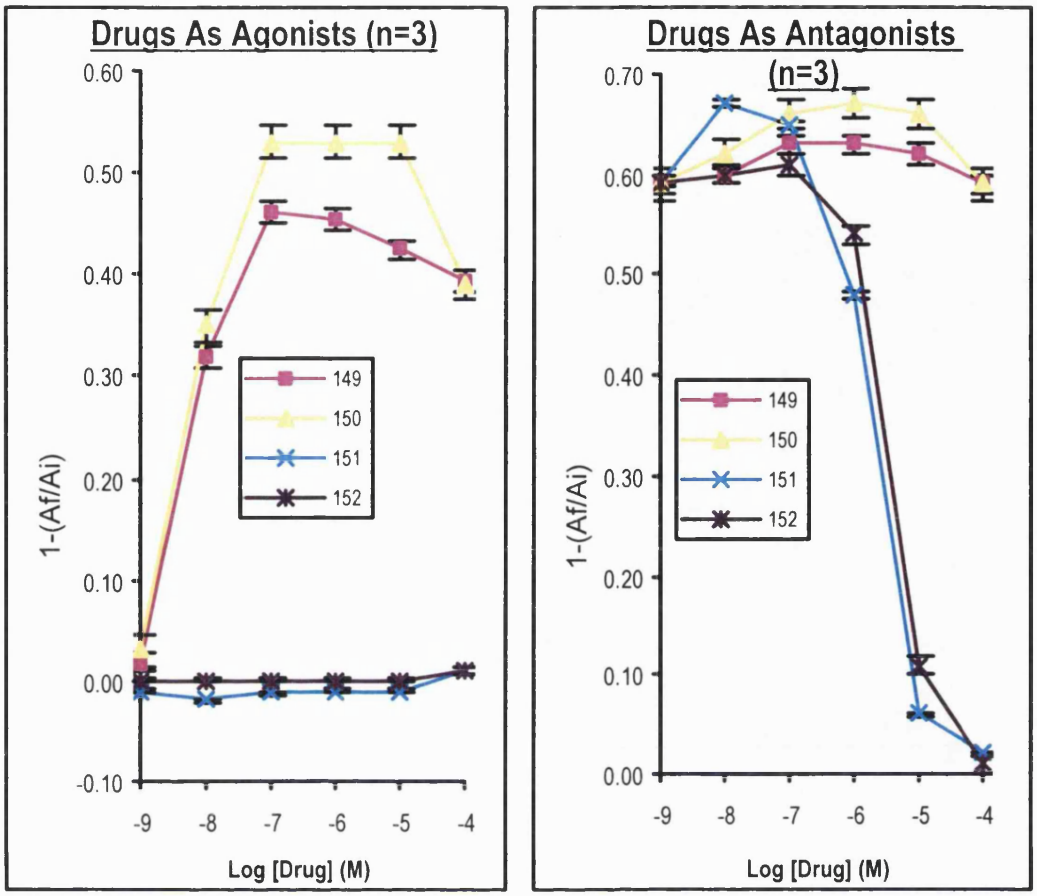
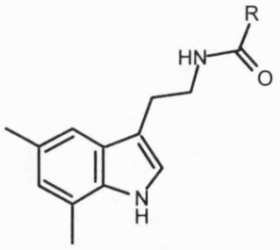


Figure 8. Concentration-response curves for pigment aggregation by *N*-acyl-5-methoxy-6,7-dimethyltryptamines in *Xenopus leavis* melanophores.

Figure 9 summarised the biological responses of the three *N*-acyl-5,7-dimethyltryptamines shown in Table 7. Compound **153**, in which a methyl group replaces the methoxy group at C-5, is an agonist, but with a considerably lowered potency (pEC_{50} = 6.2) compared to **207** (Table 5) (pEC_{50} = 9.8) or melatonin **1** (pEC_{50} = 9.2). Compound **154**, unlike **147**, is not a partial agonist and, further, shows a slight increase in antagonist potency.

Table 7. Potency of *N*-acyl-5,7-dimethyltryptamines on *Xenopus* melanophores.



Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
153	CH_2CH_3	6.2	NA
154	cyclopropyl	NA	5.1
155	cyclobutyl	NA	5.3

NA = Not agonist or not antagonist.

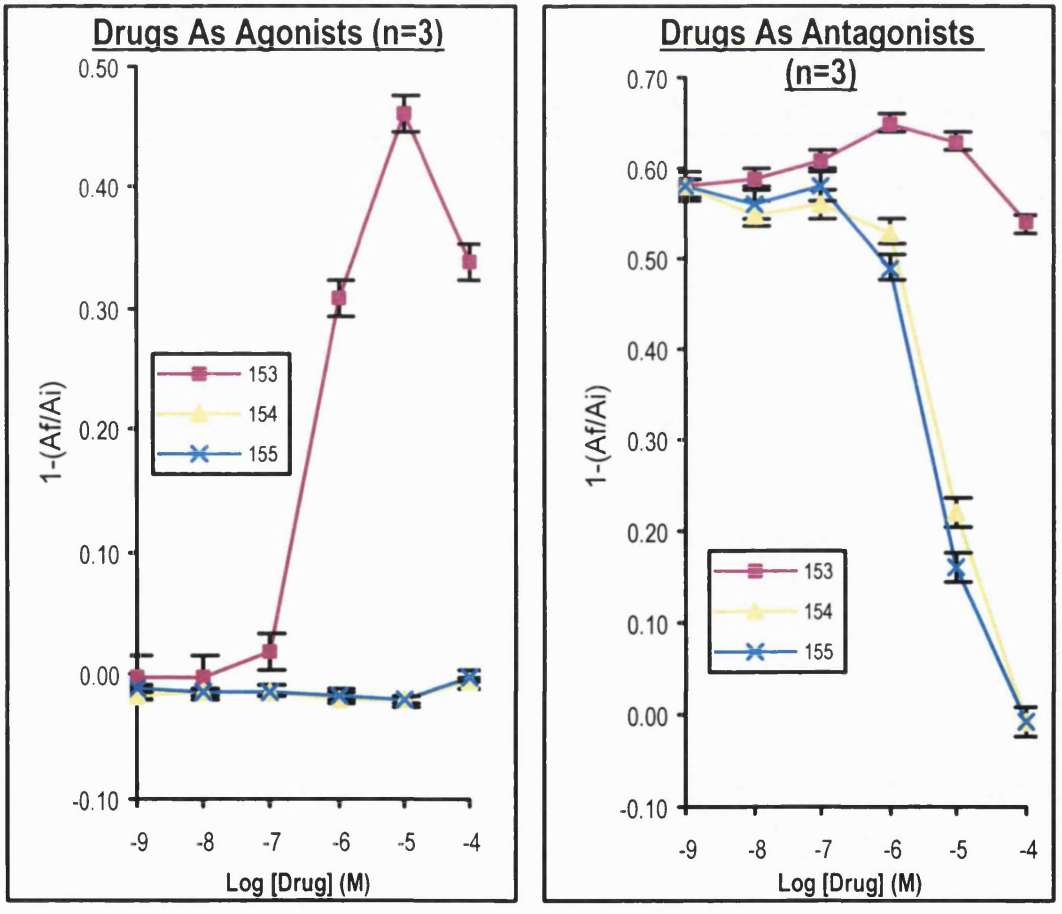
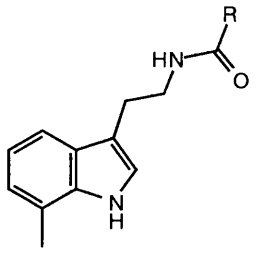


Figure 9. Concentration-response curves for pigment aggregation by *N*-acyl-5,7-dimethyltryptamines in *Xenopus leavis* melanophores.

Figure 10 represents the biological response of the series of *N*-acyl-7-methyltryptamines shown in Table 8. When compared to the *N*-acyl tryptamines (Table 9), it can be seen that the introduction of the 7-methyl group causes the loss of the weak agonist behaviour of *N*-propanoyltryptamine **214**. It can also be seen that an increase in the length of the C-3 amido side chain causes a progressive decrease in the antagonist potency, the reverse of that found in the tryptamine series **213** - **215**. Compound **159** (pIC_{50} = 5.0) is also a more potent antagonist than the corresponding 5-methoxy derivative **147** (pIC_{50} = 4.9), whereas in the case of the cyclobutyl derivatives, the methoxy analogue **148** is a slightly more potent antagonist than compound **160**.

Table 8. Potency of *N*-acyl-7-methyltryptamines on *Xenopus* melanophores.



Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
156	CH_3	NA	5.3
157	CH_2CH_3	NA	4.9
158	$CH_2CH_2CH_3$	NA	4.7
159	cyclopropyl	NA	5.0
160	cyclobutyl	NA	4.8

NA = Not agonist or not antagonist.

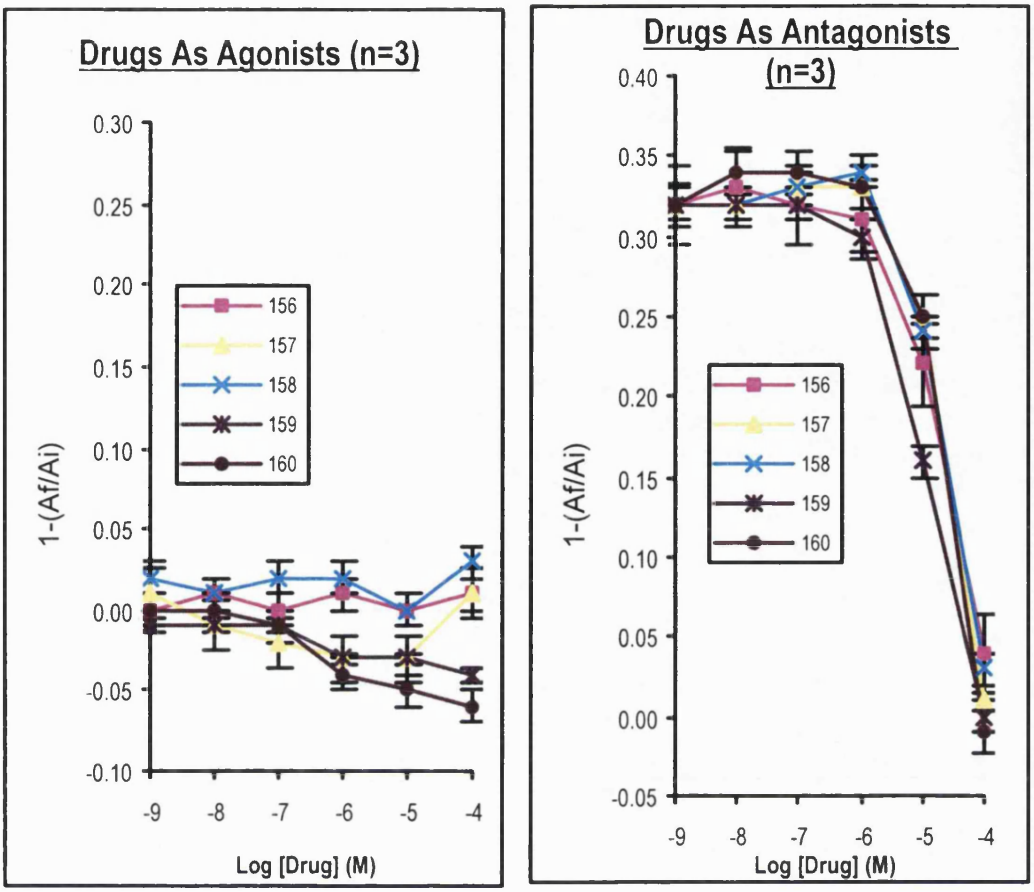
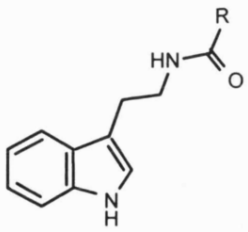


Figure 10. Concentration-response curves for pigment aggregation by *N*-acyl-7-methyltryptamines in *Xenopus laevis* melanophores.

Table 9.[242] Potency of *N*-acyl-tryptamines on *Xenopus* melanophores.

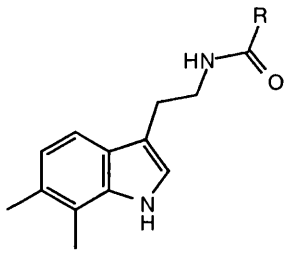


Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
213	CH_3	PA	4.4
214	CH_2CH_3	6.2	4.5
215	$CH_2CH_2CH_3$	NA	6.1
216	cyclopropyl	NA	5.5
217	cyclobutyl	NA	4.4

NA = Not agonist or not antagonist.

The compounds shown in Table 9 form the only set for which there is a complete comparison with the melatonin and the 5-methoxy-6,7-dimethyl derivatives shown in Table 6. Introduction of the 6- and 7-methyl groups into the melatonin skeleton (Table 6, **149**), caused a ten-fold decrease in the agonist potency. Removing the 5-methoxy group to give **161** removes the agonist potency and turns it into an antagonist. The cyclopropyl derivative **151** shows no agonist activity while the melatonin analogue **209** (Table 5) is a potent agonist. Compound **151** is an antagonist with approximately ten-fold greater potency than the 5-H derivative **163**, while the cyclobutyl derivatives **152** and **164** have similar antagonist potencies.

Table 10. Potency of *N*-acyl-6,7-dimethyltryptamines on *Xenopus* melanophores.



Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
161	CH_3	NA	4.0
162	CH_2CH_3	NA	3.8
163	cyclopropyl	NA	4.7
164	cyclobutyl	NA	5.2

NA = Not agonist or not antagonist.

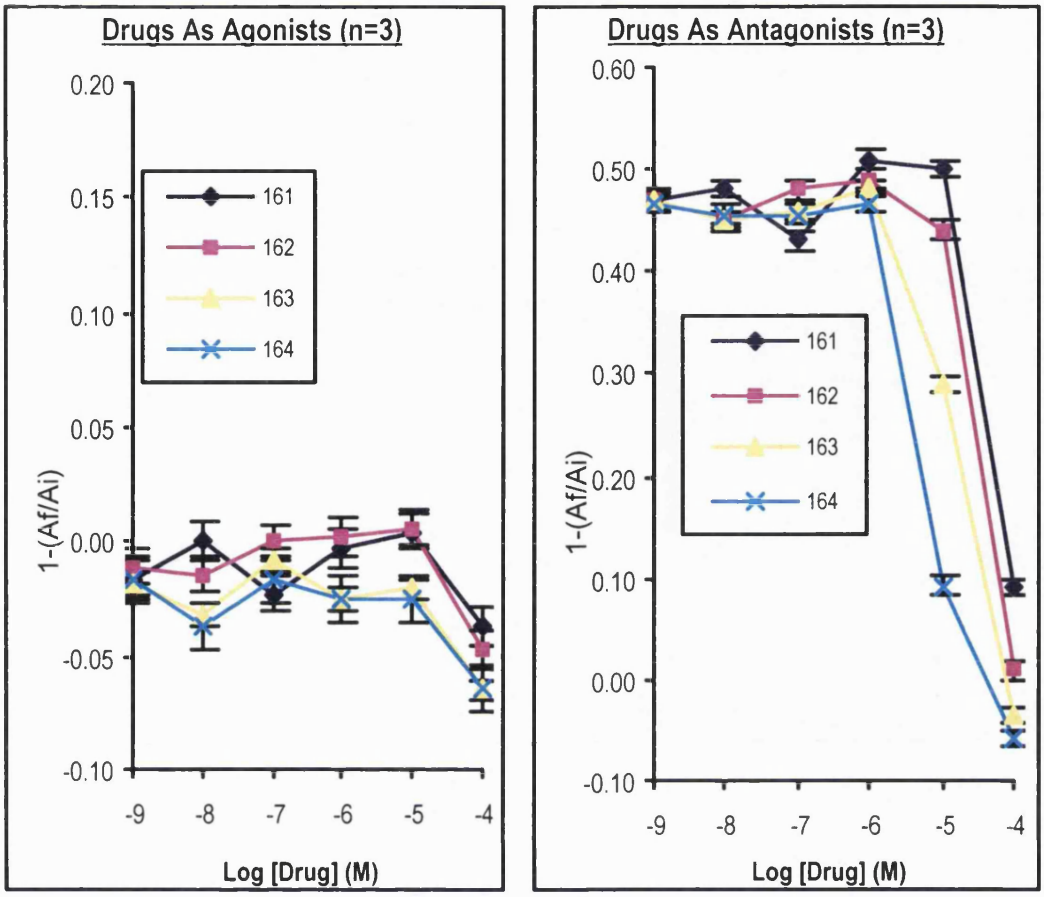
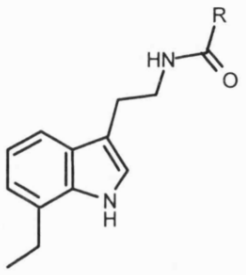


Figure 11. Concentration-response curves for pigment aggregation by *N*-acyl-6,7-dimethyltryptamines in *Xenopus leavis* melanophores.

Figure 12 shows the biological response of the series of *N*-acyl-7-ethyltryptamines shown in Table 11. The antagonist potencies of this set of compounds are much less than either those of the *N*-acyltryptamines or the *N*-acyl-7-methyltryptamines. Interestingly, *N*-propanoyl-2-(7-ethylindol-3-yl)ethanamine **(166)** and *N*-cyclopropanecarbonyl-2-(7-ethylindol-3-yl)ethanamine **(167)** both showed partial agonist activity (**166**, 40% aggregation; **167**, 17% aggregation). By changing a methyl group to an ethyl group at the 7- position, it can be seen that the agonist potency has been partially restored.

Table 11. Potency of *N*-acyl-7-ethyltryptamines on *Xenopus* melanophores.



Compound	R	Agonist (pEC_{50})	Antagonist (pIC_{50})
165	CH_3	NA	4.6
166	CH_2CH_3	PA	4.2
167	$CH_2CH_2CH_3$	PA	4.5
168	cyclopropyl	NA	4.9
169	cyclobutyl	NA	5.4

NA = Not agonist or not antagonist; PA = Partial agonist.

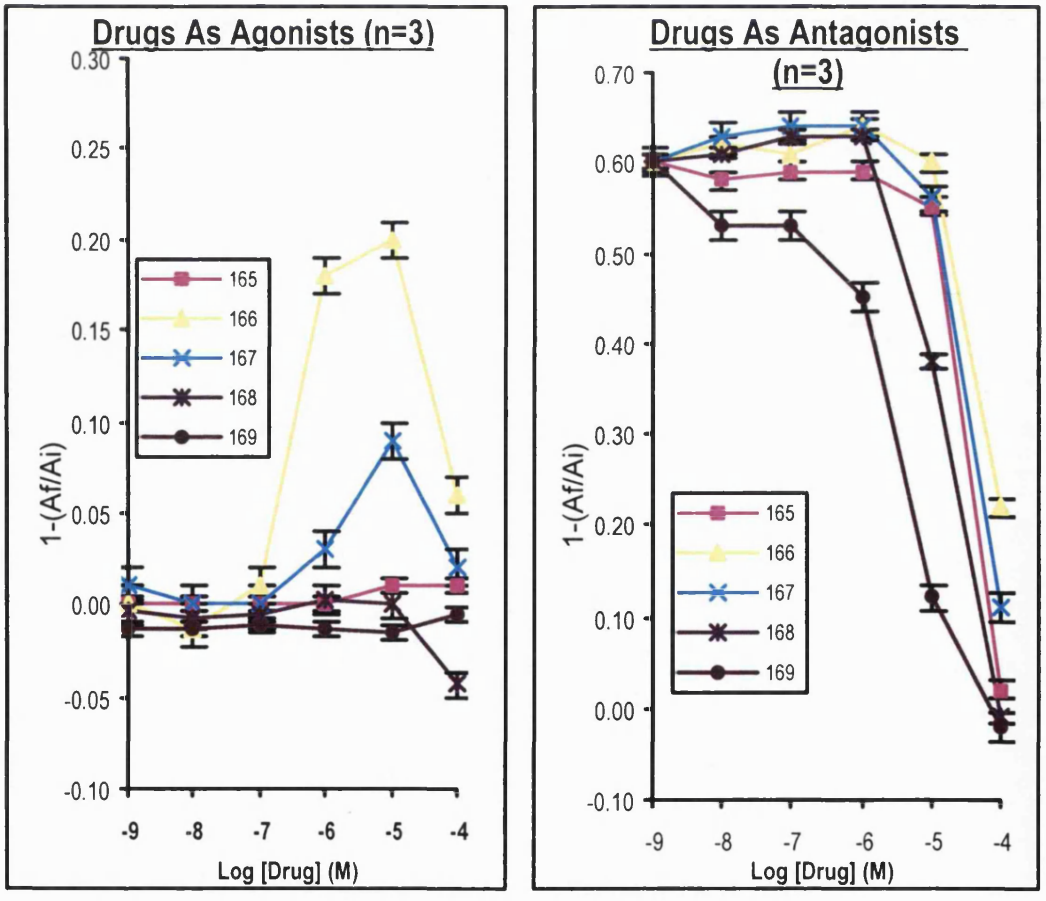
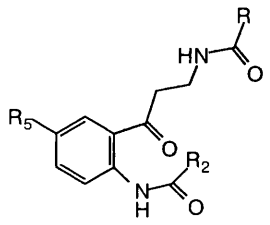


Figure 12. Concentration-response curves for pigment aggregation by *N*-acyl-7-ethyltryptamines in *Xenopus leavis* melanophores.

Kynurenamines

Table 12 shows the biological response of kynurenamines **6**, **218**[258] and **206**. The result for kynurenamine **6**, which is synthesised *via* the oxidation of melatonin, is also summarised in Figure 13. The kynurenamine **6** is 700-fold less active than melatonin in the biological assay, which shows that, at least in this biological model, that **6** is not the active form of melatonin. Comparison of the agonist potencies of **6** and **218** shows that a phenyl substituent increases the agonist activity ca. 500-fold.

Table 12. Potency of kynurenamines on *Xenopus* melanophores



Compound	R	R_2	R_5	Agonist (pEC_{50})	Antagonist (pIC_{50})
6	CH_3	H	OMe	6.3	NA
218	CH_3	Ph	OMe	9.1	NA
206	cyclopentyl	Ph	H	NA	5.0

NA = Not agonist or not antagonist.

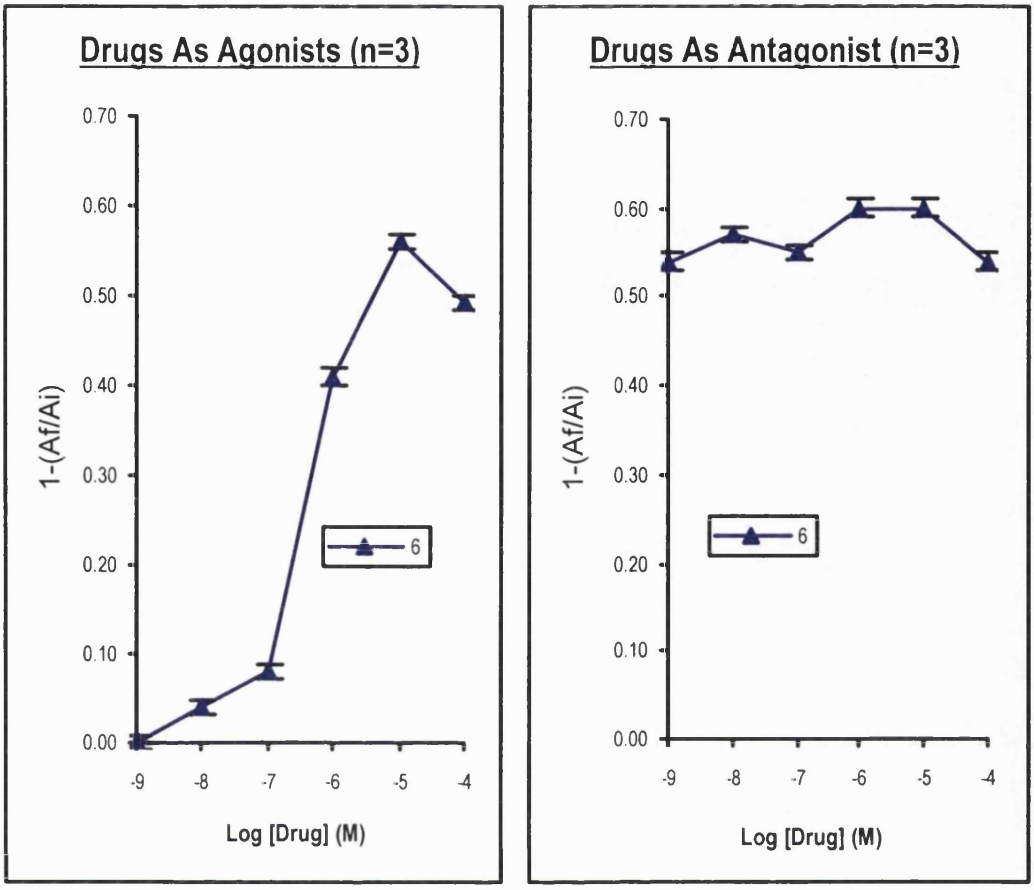


Figure 13. Concentration-response curves for pigment aggregation by kynureamine **6** in *Xenopus leavis* melanophores

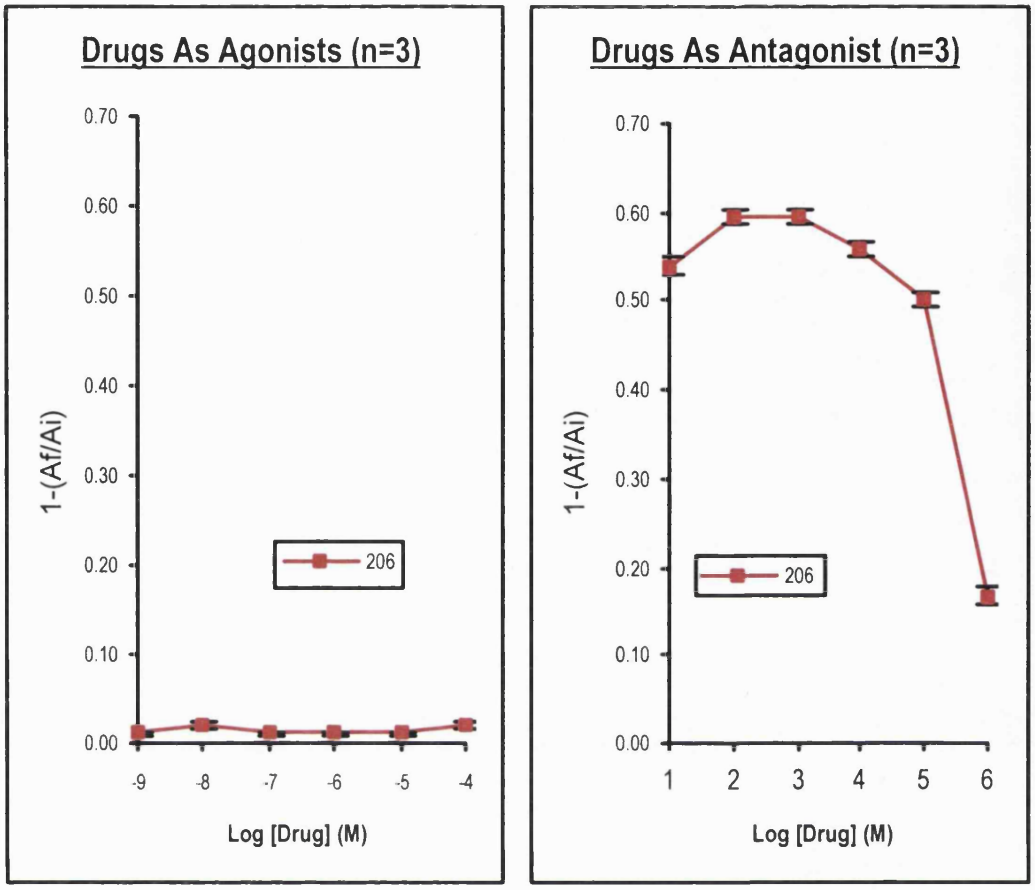


Figure 14. Concentration-response curves for pigment aggregation by kynureamine **206** in *Xenopus leavis* melanophores

X. Conclusion

It was previously known that the 5-methoxy group is important for the binding in melatonin although it is not essential for the biological activity. It is thus of interest that the acetyl derivative **213**, with hydrogen at position 5, is a partial agonist as well as an antagonist, whereas, the propanoyl derivative **214**, again with hydrogen at position 5, is both a full agonist and antagonist. As was previously shown,[172, 257] increasing the length of the alkanoyl group increases the binding affinity of melatonin analogues.

Cyclobutanoyl derivatives of melatonin analogues are often antagonists as, to a lesser extent, are cyclopropanoyl compounds. Thus while the melatonin analogues **209** and **210**, with cyclopropanoyl and cyclobutanoyl group replacing acetyl, are both agonists, with **210** also showing weak antagonist properties, introduction of a 7-methyl group into the corresponding derivatives **147** and **148** changes these compounds into antagonists.

Replacing the 5-methoxy group by methyl in the 5-methoxy-7-methyl derivatives **147** and **148** to give the 5,7-dimethyl derivatives **154** and **155** slightly increases the antagonist potency and **154**, unlike **147**, is not a partial agonist.

Introducing a second methyl group at position 6 to give 5-methoxy-6,7-dimethyl derivatives increases the antagonist potency of the cyclopropanoyl **151** and cyclobutanoyl **152** compounds compared to the corresponding 5-methoxy-7-methyl derivative **147** and **148**. The acetyl **149** and propanoyl **150** derivatives are both agonists but are less potent than melatonin and the propanoyl analogue **207**, respectively. Interestingly, **149** and **150** are equipotent and this series does not show the increase in potency found when the acetyl side chain of melatonin is replaced by longer alkanoyl groups.

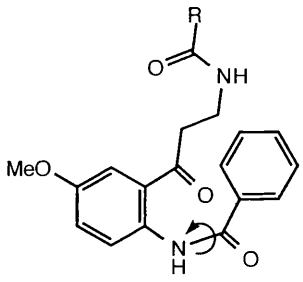
The 6,7-dimethyl derivatives without the 5-methoxy group, **161** - **164**, are all weak antagonists, with the cyclobutyl derivative **164** being the most potent. The 7-ethyl derivative **165** - **169** are also weak antagonists wiith, surprisingly, some partial agonist activity for the propanoyl **166** and butanoyl **167** derivatives. The corresponding 7-ethyl-5-methoxy derivatives therefore deserve investigation.

Current interest has moved to studying compounds which can discriminate between MT_1 and MT_2 receptors. At present, there are a number of compounds which show a preference for the MT_2 subreceptor either as agonists or antagonists. It appears that, in all of the regions of the melatonin molecule so far investigated, the

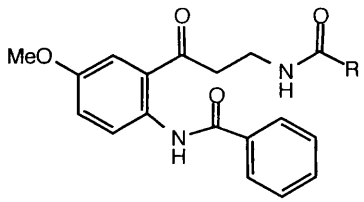
MT_2 subreceptor is more amiable to molecular change than the MT_1 subreceptor. This suggests that the active site in the MT_1 receptor is more constrained to the introduction of additional groups than the MT_2 receptor, although both subreceptors have very high binding affinities for melatonin. It may be that the receptor site in MT_2 can be more easily distorted by the additional groups or that "solvent" molecules can displaced.

We have obtained only potency data but no binding data yet for any of the series of compounds that have been synthesised in this thesis, since these series are not complete. Further studies are in progress to complete the synthesis of the series of 5-methoxy-7-methyl derivatives and, after potency evaluation, all the derivatives will be screened for MT_1 and MT_2 binding affinities.

For the kynurenamine type compounds, it can be seen that compound **218**, derived from 2-phenylmelatonin, has a much higher potency than the melatonin metabolite **6**. This may indicate that the phenyl group can fit into a receptor site, as suggested by Spadoni et al.[251] for the melatonin model, or it might imply that the phenyl group produces a greater preference for the side chain in the active conformation **a**, rather than conformation **b**. Compound **206**, derived from the 5-hydrogen analogue **204**, is an antagonist. Both the lack of a 5-methoxy group and the presence of the cyclopentyl group favour antagonism.



a



b

The Mel_{1c} receptor of Xenopus with which the potency of these compounds has been evaluated appeared similar in its potency profile to the MT_1 rather than the MT_2 receptor.[256] It may be possible, therefore, to find compounds that are antagonists at the Mel_{1c} receptor, and thus at the MT_1 receptor, which are agonists at the MT_2 receptor. Compounds that are highly potent at the Mel_{1c} receptor will be screened for their potency in human MT_1 and MT_2 cell lines.

XI. General procedures

This section describes several general procedures which were used to obtain substituted indoles. Specific conditions are described individually in the experimental section where applicable.

XI.A. Preparation of indoles

XI.A.A. Synthesis of substituted 2-amino-α-chloroacetophenones

The substituted aniline (40 mmol) in dry benzene (40 mL) was added to a solution of boron trichloride in CH_2Cl_2 (1 M, 40 mL, 40 mmol) at 0 °C followed by chloroacetonitrile (2.8 mL, 44 mmol) and titanium tetrachloride (4.43 mL, 40 mmol). The mixture was heated to reflux for 5 hours under nitrogen when the brown precipitate dissolved and was then allowed to cool to room temperature. Cold HCl (2 M) was added until a yellow precipitate formed. The mixture was heated to 80 °C until the precipitate dissolved (~ 1 hour). The cooled mixture was neutralised to pH7 by aqueous NaOH (2 M). The mixture was filtered and the filtrate separated. The organic layer collected was washed with sat. $NaHCO_3$ solution (3 x 30 mL) and dried ($MgSO_4$). Evaporation of the solvent gave the crude substituted 2-amino-α-chloroacetophenone.

XI.A.B. Cyclisation of α-chloroacetophenones to substituted indoles

The crude acetophenone (17 mmol), sodium borohydride (0.70 g, 18 mmol), dioxane (85 mL) and water (8.5 mL) were heated to reflux for 1 hour. The solvent was removed under reduced pressure. Water (90 mL) was added to the residue and the resulting mixture was extracted with CH_2Cl_2 (4 x 30 mL) and the organic layer was dried ($MgSO_4$). Evaporation of solvent under reduced pressure yielded a brown residue which was then dissolved in benzene (5 mL) and passed through a silica gel column to remove a polar fraction, eluting with benzene. The eluant was evaporated to give the indole.

XI.B. Preparation of nitrovinylindoles

Trifluoroacetic acid (3.3 mL) in distilled CH_2Cl_2 (10 mL) was added dropwise to a suspension of 1-dimethylamino-2-nitroethylene (0.64 g, 5.5 mmol) in distilled CH_2Cl_2 (10 mL) at 0 °C. A solution of indole (5.5 mmol) in CH_2Cl_2 (5 mL) was then added and the mixture was stirred at 0 °C for 30 min and then at rt for a further 10

hours. Ice (2 g) was added into the mixture and the organic layer was separated. The aqueous layer was then extracted with EtOAc (3 x 50 mL) and the combined organic layers were washed with sat. $NaHCO_3$ solution (2 x 30 mL) and dried ($MgSO_4$).

XI.C. Preparation of substituted tryptamines

A solution of the nitrovinylindole (1.5 mmol) in dry THF (8 mL) was added dropwise to a solution of $LiAlH_4$ (0.27 g, 7.2 mmol) in THF (12.5 mL) at 0 °C under nitrogen and the mixture was allowed to warm to rt and stirred for 24 hours. H_2O (5 mL), aqueous NaOH (2 M, 4 mL) and Et_2O (10 mL) were added sequentially to the solution. The inorganic precipitate was removed by filtration and was washed with THF (5 mL) and Et_2O (20 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with brine (20 mL) and dried ($MgSO_4$). Solvent evaporation under reduced pressure gave the crude tryptamine. Most of the tryptamine intermediates were converted into their amide analogues without further purification.

XI.D. Preparation of N-acyl amides

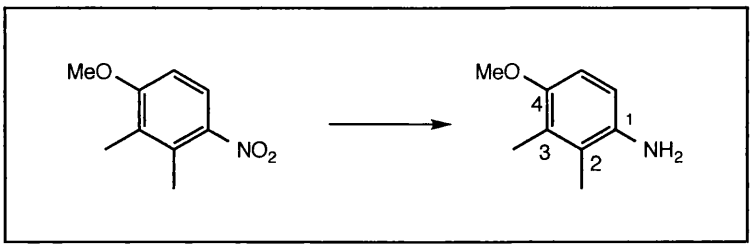
Triethylamine (0.12 mL, 0.86 mmol) was added dropwise to a stirred solution of crude tryptamine (0.88 mmol) in dry CH_2Cl_2 (4 mL) at 0 °C and the mixture was stirred for another 10 min. The acid chloride or acid anhydride (0.88 mmol) was then added at the same temperature. The resultant mixture was warmed to rt and stirred for 4 hours. The solution was then washed sequentially with aqueous HCl (10%, 5 mL), sat. $NaHCO_3$ solution (5 mL), sat. NaCl solution (5 mL) and dried ($MgSO_4$).

XI.E. Preparation of kynurenamines

A solution of sodium metaperiodate (0.51 g, 2.40 mmol) in H_2O (4.5 mL) was added to a solution of the melatonin analogue (1.08 mmol) in methanol (4.5 mL). The resulting solution was stirred at rt for 21 hours. The mixture was extracted with CH_2Cl_2 (4 x 10 mL) and the combined organic extracts were washed with brine (20 mL) and dried ($MgSO_4$). Evaporated in vacuo gave a residual purple solid which was then purified by spinning plate chromatography eluting with EtOAc.

XII. Compounds synthesised

4-Methoxy-2,3-dimethylaniline[221] (91)

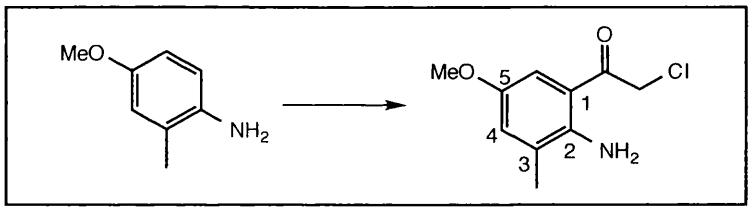


Compound **91** was prepared by the method described of Knolker et al.[221] from 2,3-dimethyl-4-nitroanisole (5.00 g, 27.6 mmol) in methanol (130 mL) as colourless crystals.

Yield: 4.13 g, 27.4 mmol (99%)

mp: 65 - 66 °C (lit.[221]: 67 - 68 °C)

2-Amino-5-methoxy-3-methyl-α-chloroacetophenone[222] (97)



Compound **97** was prepared from 4-methoxy-2-methylaniline (5.15 mL, 40 mmol) by method XI.A.A. The crude material obtained was triturated with hexane (2 x 250 mL) and the hexane portion was decanted. Evaporation of the solvent gave crude **97** as green crystals of sufficient purity to be used in subsequent reactions.

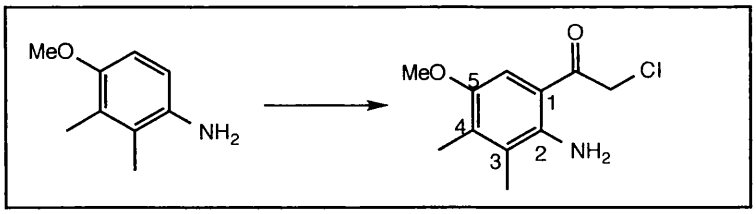
Yield: 2.31 g, 10.8 mmol (27%)

mp: 70 - 73 °C (lit.[222]: 71 - 73 °C)

IR ($CHCl_3$,cm^{-1}): 3507, 3362, 2836, 1652, 1606, 1560, 1506.

^1H NMR (400 MHz, $CDCl_3$): δ 6.98 (s, 2H, Ar$\underline{H}$), 6.19 (bs, 2H, N$\underline{H_2}$), 4.69 (s, 2H, C$\underline{H_2}$Cl), 3.78 (s, 3H, OC$\underline{H_3}$), 2.19 (s, 3H, C$\underline{H_3}$).

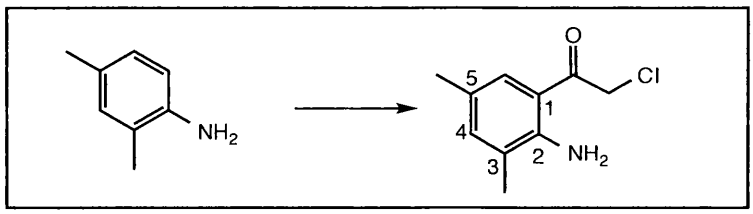
2-Amino-5-methoxy-3,4-dimethyl-α-chloroacetophenone (98)



Compound **98** was prepared from 4-methoxy-2,3-dimethylaniline (3.90 g, 25.8 mmol) in dry benzene (25.8 mL), boron trichloride in CH_2Cl_2 (1 M, 25.8 mL, 25.8 mmol), chloroacetonitrile (1.8 mL, 28.2 mmol) and titanium tetrachloride (2.8 mL, 25.8 mmol) by method XI.A.A. Evaporation of the solvent gave crude **98** as a green solid of sufficient purity to be used in subsequent reactions.

Yield:	5.66 g , 24.9 mmol (97%)
mp:	134 - 135 °C
^{1}H NMR (300 MHz, $CDCl_3$): δ	6.90 (s, 1H, H-6), 6.30 (bs, 2H, $N\underline{H}_2$), 4.66 (s, 2H, $C\underline{H}_2Cl$), 3.78 (s, $OC\underline{H}_3$), 2.23 (s, 3H, $C\underline{H}_3$), 2.09 (s, 3H, $C\underline{H}_3$).
IR (cm^{-1}):	3430, 2945, 1649, 1602, 1545, 1464, 1415, 1371, 1247, 1184, 1132, 1104.
EIMS (m/z):	229 ($M^+[^{37}Cl]$, 31), 227 ($M^+[^{35}Cl]$, 93), 191 (M^+ - HCl, 9), 178 (M^+ - CH_2Cl, 100), 150 (M^+ - $COCH_2Cl$, 29), 135 (M^+ - CH_3COCH_2Cl, 30).

2-Amino-3,5-dimethyl-α-chloroacetophenone[244] (99)

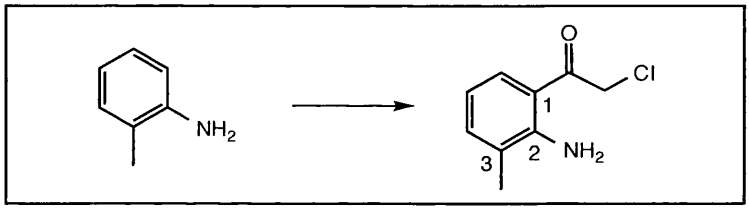


Compound **99** was prepared from 2,4-dimethylaniline (5.4 mL, 44 mmol), boron trichloride in CH_2Cl_2 (1 M, 44 mL, 44 mmol), chloroacetonitrile (3.05 mL, 48 mmol) and titanium tetrachloride (4.85 mL, 44 mmol) by method XI.A.A. The crude material obtained was triturated with hexane (2 x 250 mL) and the hexane portion

was decanted. Evaporation of the solvent gave crude **99** as a white solid of sufficient purity to be used in subsequent reactions.

Yield:	8.51 g, 43.1 mmol (98%)
mp:	61 - 63 °C
^{1}H NMR (300 MHz, $CDCl_3$): δ	7.43 (s, 1H, H-6), 7.13 (s, 1H, H-4), 6.66 (bs, 2H, $N\underline{H}_2$), 4.68 (s, 2H, $C\underline{H}_2Cl$), 2.35 (s, 3H, $C\underline{H}_3$), 2.19 (s, 3H, $C\underline{H}_3$).
EIMS (m/z):	197 ($M^+[^{35}Cl]$, 15), 148 (M^+ - CH_2Cl, 100), 120 (M^+ - $COCH_2Cl$, 17).

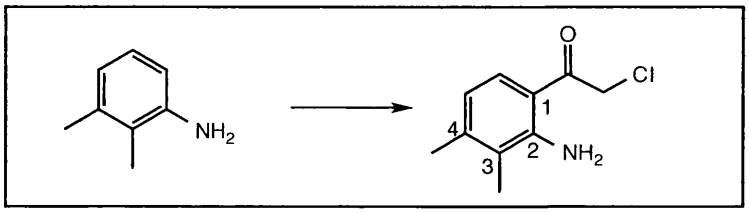
2-Amino-3-methyl-α-chloroacetophenone[243] (100)



Compound **100** was prepared from o-toluidine (4.3 mL, 40 mmol) by method XI.A.A. but using dry aluminium trichloride (5.34 g, 40 mmol) instead of titanium tetrachloride. The crude material obtained was triturated with hexane (2 x 250 mL) and the hexane portion was decanted. Evaporation of the solvent gave crude **100** as yellow crystals of sufficient purity to be used in subsequent reactions.

Yield:	3.64 g, 19.8 mmol (50%)
mp:	56 - 58 °C (lit.[243]: 57 - 58 °C)

2-Amino-3,4-dimethyl-α-chloroacetophenone (101)

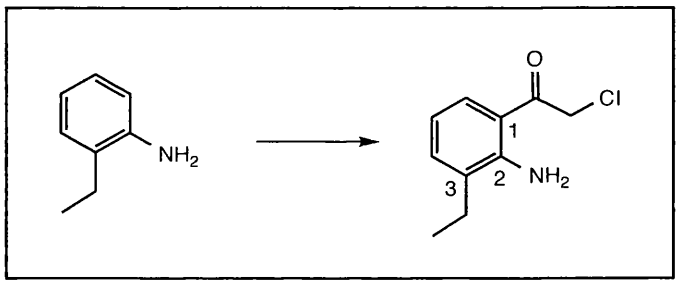


Compound **101** was prepared from 2,3-dimethylaniline (4.9 mL, 40 mmol), boron trichloride in CH_2Cl_2 (1 M, 40 mL, 40 mmol), chloroacetonitrile (2.8 mL, 44 mmol) and titanium tetrachloride (4.43 mL, 40 mmol) by method XI.A.A. Evaporation of

the solvent gave crude **101** as yellow solid of sufficient purity to be used in subsequent reactions.

Yield:	6.88 g, 34.8 mmol (87%)
mp:	65 - 67 °C
IR ($CHCl_3$, cm^{-1}):	3512, 3348, 2965, 1651, 1604, 1543, 1418, 1318, 1255, 1097, 1020.
^1H NMR (300 MHz, $CDCl_3$): δ	7.42 (d, 1H, J = 8.4 Hz, H-6), 6.53 (d, 3H, J = 8.3 Hz, H-5, $N\underline{H}_2$), 4.68 (s, 2H, $C\underline{H}_2Cl$), 2.32 (s, 3H, $C\underline{H}_3$), 2.07 (s, 3H, $C\underline{H}_3$).
EIMS (m/z):	199 ($M^+[^{37}Cl]$, 7), 197 ($M^+[^{35}Cl]$, 22), 161 (M^+ - HCl, 4), 148 (M^+ - CH_2Cl, 100), 120 (M^+ - $COCH_2Cl$, 22).

2-Amino-3-ethyl-α-chloroacetophenone[222] (102)

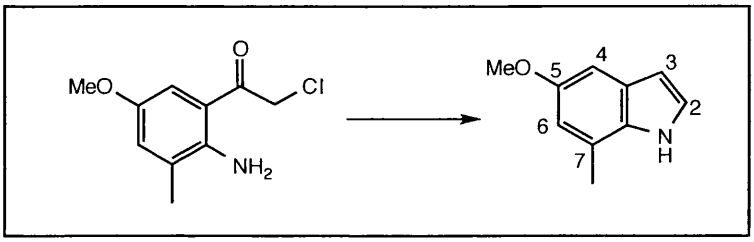


Compound **102** was prepared from ethylaniline (4.5 mL, 36 mmol) in dry benzene (30 mL), boron trichloride in CH_2Cl_2 (1 M, 36 mL, 36 mmol), chloroacetonitrile (2.5 mL, 40 mmol) and titanium tetrachloride (3.9 mL, 36 mmol) by method XI.A.A., except that the mixture was heated to reflux for 3 hours instead of 5 hours. The crude material obtained was triturated with hexane (2 x 150 mL) and the hexane portion was decanted. Evaporation of the solvent gave crude **102** as an off-white solid of sufficient purity to be used in subsequent reactions.

Yield:	2.87 g, 14.5 mmol (40%)
mp:	86 - 88 °C

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, 1H, J = 8.1 Hz, H-4),
7.26 (d, 1H, J = 8.1 Hz, H-6),
6.66 (t, 1H, J = 7.3 Hz, H-5),
6.55 (bs, 2H, $N\underline{H}_2$), 4.73 (s, 2H, $C\underline{H}_2Cl$),
2.53 (q, 2H, J = 7.5 Hz, $C\underline{H}_2$),
1.29 (t, 3H, J = 7.5 Hz, $C\underline{H}_3$).

5-Methoxy-7-methylindole[222] (103)



Compound **103** was prepared as a colourless crystalline solid from 2-amino-5-methoxy-3-methyl-α-chloroacetophenone **(97)** (0.42 g, 1.95 mmol), $NaBH_4$ (0.081 g, 2.14 mmol), dioxane (9.8 mL) and water (0.98 mL) by method XI.A.B., except that the mixture was heated to reflux for 2 hours instead of 1 hour.

Yield: 0.27 g, 1.67 mmol (87%)

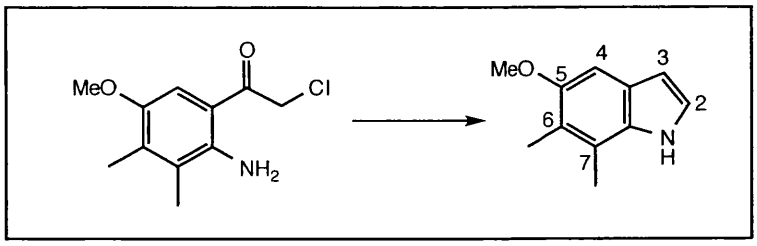
mp: 65 - 66.5 °C (lit.[222]: 65-66 °C)

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (bs, 1H, N$\underline{H}$),
7.20 (t, 1H, J = 2.8 Hz, H-2),
7.00 (d, 1H, J = 2.0 Hz, H-4),
6.70 (d, 1H, J = 1.4 Hz, H-6),
6.50 (t, 1H, J = 2.7 Hz, H-3),
3.85 (s, 3H, $OC\underline{H}_3$), 2.48 (s, 3H, $C\underline{H}_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 154.3, 130.7, 127.6, 124.3, 121.2, 113.0, 102.9
99.8, 55.8, 16.8.

EIMS (m/z): 161 (M^+, 100), 146 ($M^+ - CH_3$, 75).

5-Methoxy-6,7-dimethylindole (104)

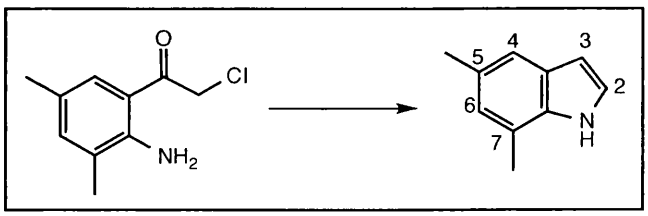


Compound **104** was prepared from 2-amino-5-methoxy-3,4-dimethyl-α-chloroacetophenone **(98)** (4.71 g, 20.7 mmol), $NaBH_4$ (0.87 g, 23 mmol), dioxane (103 mL) and water (10.3 mL) by method XI.A.B. as a white crystalline solid.

Yield:	2.35 g, 13.4 mmol (65%)
mp:	67 - 68 °C
IR (cm^{-1}):	3392, 2931, 1583, 1456, 1431, 1303, 1132.
^1H NMR (400 MHz, $CDCl_3$): δ	7.87 (bs, 1H, NH), 7.42 (s, 1H, H-4), 7.19 (t, 1H, J = 2.5 Hz, H-2), 6.89 (s, 1H, H-3), 4.24 (s, OCH_3), 2.78 (s, 3H, CH_3), 2.58 (s, 3H, CH_3).
^{13}C NMR (MHz, $CDCl_3$): δ	152.7, 130.6, 124.6, 123.6, 119.9, 119.1, 102.0, 98.5, 55.7, 12.9, 11.8.
MS (FAB) (m/z):	176 (M^+ + H, 49), 175 (M^+, 100).

Calcd. for $C_{11}H_{13}NO$, (M^+): 175.1002. Found: 175.0997.

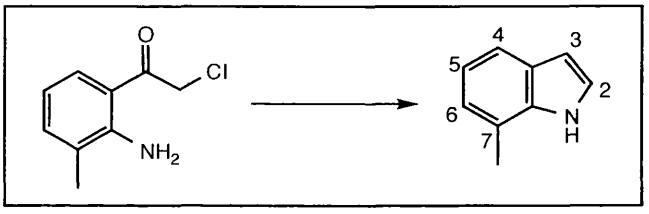
5,7-Dimethylindole[246] (105)



Compound **105** was prepared from 2-amino-3,5-dimethyl-α-chloroacetophenone **(99)** (7.90 g, 40 mmol), $NaBH_4$ (1.68 g, 44 mmol), dioxane (200 mL) and water (20 mL) by method XI.A.B. as a yellow oil.

Yield:	4.59 g, 31.6 mmol (79%)
^{1}H NMR (400 MHz, $CDCl_3$): δ	7.72 (bs, 1H, NH), 7.64 (s, 1H, H-4), 7.16 (m, 2H, H-2, H-6), 6.77 (dd, 1H, J = 3.1, 1.0 Hz, H-3), 2.77 (s, 3H, CH_3), 2.61 (s, 3H, CH_3).
^{13}C NMR (100 MHz, $CDCl_3$): δ	133.5, 129.0, 128.2, 127.5, 124.1, 119.8, 117.8, 102.1, 21.3, 16.3.
EIMS (m/z):	145 (M^+, 100), 130 (M^+ - CH_3, 53).

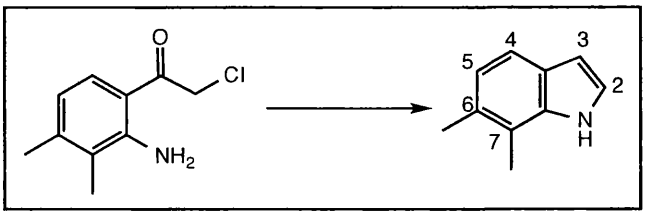
7-Methylindole[245] (106)



Compound **106** was prepared from 2-amino-3-methyl-α-chloroacetophenone **(100)** (3.13 g, 17 mmol) by method XI.A.B. as a colourless crystalline solid.

Yield:	0.96 g, 7.32 mmol (43%)
mp:	81.5 - 83 °C (lit.[245]: 82 °C)
^{1}H NMR (300 MHz, $CDCl_3$): δ	8.09 (bs, 1H, NH), 7.56 (d, 1H, J = 7.5 Hz, H-4), 7.25 (t, 1H, J = 2.9 Hz, H-2), 7.10 (td, 1H, J = 7.6, 0.9 Hz, H-5), 7.07 (d, J = 7.4 Hz, H-6), 6.55 (td, 1H, J = 2.2, 0.8 Hz, H-3), 2.45 (s, 3H, CH_3).
^{13}C NMR (100 MHz, $CDCl_3$): δ	135.4, 127.3, 123.8, 122.4, 120.1, 120.0, 118.4, 103.1, 16.7.
IR (cm^{-1}):	3397, 1491, 1458, 1428, 1341, 1109.
EIMS (m/z):	131 (M^+, 98), 130 (M^+ - H, 100).

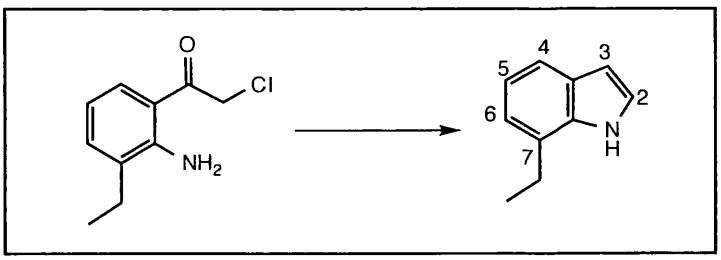
6,7-Dimethylindole[246] (107)



Compound **107** was prepared as colourless crystals from 2-amino-3,4-dimethyl-α-chloroacetophenone **(101)** (6.10 g, 30.9 mmol), $NaBH_4$ (1.28 g, 34 mmol), dioxane (155 mL) and water (15.6 mL) by method XI.A.B., except that the mixture was heated to reflux for 2 hours instead of 1 hour.

Yield:	4.08 g, 28.1 mmol (91%)
mp:	64 - 65 °C (lit.[246]: 64 °C)
IR (cm^{-1}):	3413, 2911, 2856, 1618, 1502, 1435, 1331, 1165, 1111, 1077.
^1H NMR (400 MHz, $CDCl_3$): δ	7.97 (bs, 1H, NH), 7.44 (d, 1H, J = 8.1 Hz, H-5), 7.16 (t, 1H, J = 2.8 Hz, H-2), 7.00 (d, 1H, J = 8.1 Hz, H-4), 6.55 (dd, 1H, J = 3.2, 1.1 Hz, H-3), 2.45 (s, 3H, CH_3), 2.42 (s, 3H, CH_3).
^{13}C NMR (75 MHz, $CDCl_3$): δ	136.0, 129.2, 125.6, 123.4, 122.6, 117.9, 117.7, 102.9, 19.3, 13.1.
MS (FAB) (m/z):	146 (M^+ + H, 27), 145 (M^+, 100).

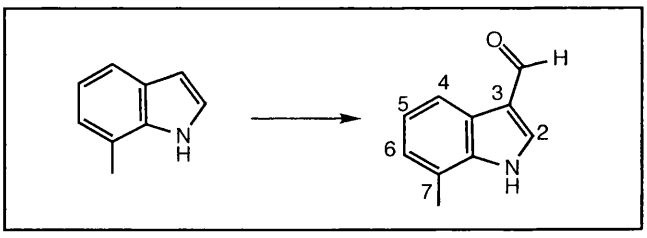
7-Ethylindole[222] (108)



Compound **108** was prepared from 2-amino-3-ethyl-α-chloroacetophenone **(102)** (1.69 g, 8.55 mmol), $NaBH_4$ (0.56 g, 14.8 mmol), dioxane (68 mL) and water (10.3 mL) by method XI.B. as a colourless oil.

Yield:	1.08 g, 7.44 mmol (87%)
^{1}H NMR (400 MHz, $CDCl_3$): δ	8.00 (bs, 1H, NH), 7.71 (d, 1H, J = 7.5 Hz, H-4), 7.29 (t, 1H, J = 7.6 Hz, H-5), 7.20 (m, 2H, H-2, H-6), 6.74 (m, 1H, J = 2.0, 1.0 Hz, H-3), 2.96 (q, 2H, J = 7.6 Hz, CH_2CH_3), 1.51 (t, 3H, J = 7.6 Hz, CH_2CH_3).
^{13}C NMR (100 MHz, $CDCl_3$): δ	134.5, 127.5, 126.4, 123.8, 120.3, 120.0, 118.4, 102.8, 23.9, 13.7.

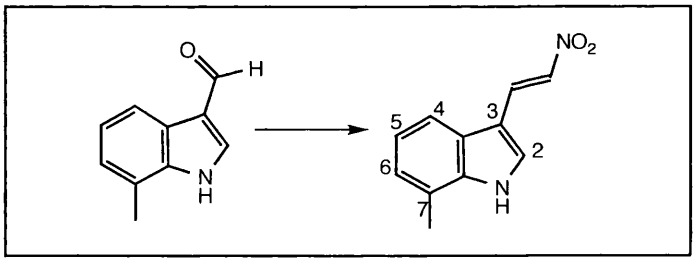
7-Methylindol-3-carbaldehyde[247] (119)



Phosphorus oxychloride (0.14 mL, 1.5 mmol) was added slowly to DMF (0.46 mL, 5.8 mmol) with stirring [illegible]0 °C under nitrogen and the solution was stirred at this temperature for a further 18 min. A solution of 7-methylindole **(106)** (0.20 g, 1.5 mmol) in DMF (0.3 mL) was then added in dropwise over 5 min and the resultant mixture was stirred at rt for 13 hours before adding to aqueous NaOH (2 M, 3.2 mL). The mixture was cooled and the resulting precipitate was filtered, washed with water and dried by evaporation under reduced pressure with benzene (3 x 5 mL) giving the aldehyde **119** as a white crystalline solid.

Yield:	0.15 g, 0.94 mmol (63%)
mp:	210 - 211 °C (lit.[246] 212 - 214 °C)
IR (cm^{-1}):	3226, 1647, 1526, 1445, 1385, 1234, 1132, 1015.
^1H NMR (400 MHz, $CDCl_3$): δ	10.09 (s,1H, CHO), 8.73 (bs, 1H, NH), 8.17 (d, 1H, J = 7.9 Hz, H-4), 7.87 (d, 1H, J = 3.1 Hz, H-2), 7.28 (m, 1H, H-5), 7.14 (d, 1H, J = 7.1 Hz, H-6), 2.55 (s, 3H, CH_3).
^{13}C NMR (100 MHz, $CDCl_3$): δ	185.2, 134.8, 125.0, 124.5, 121.4, 120.3, 119.6, 116.0, 110.0, 16.9.
EIMS (m/z):	159 (M^+, 92), 158 (M^+ - H, 100), 130 (M^+ - CHO, 55).

7-Methyl-3-(2-nitrovinyl)indole (120)



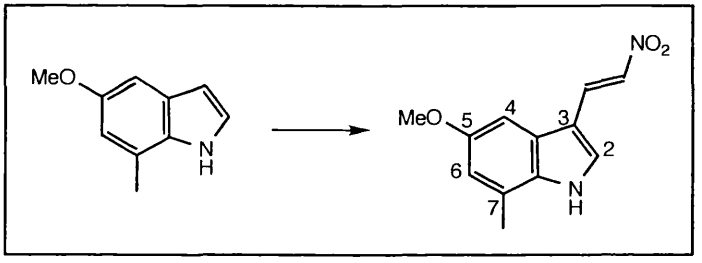
7-Methylindol-3-carboxaldehyde **(119)** (0.70 g, 4.37 mmol) was dissolved in nitromethane (10.9 mL). To this solution was added ammonium acetate (0.54 g, 7.0 mmol) and the mixture was heated to reflux for 2 hours. The solvent was evaporated under reduced pressure. CH_2Cl_2 (60 mL) was added and the solution was washed with H_2O (25 mL) and dried ($MgSO_4$). Solvent evaporation gave **120** as an orange cystalline solid.

Yield:	0.77 g, 3.81 mmol (87%)
mp:	210 - 211 °C
IR (cm^{-1}):	3314, 2925, 1612, 1475, 1302, 1271, 1217, 1106.

^{1}H NMR (400 MHz, $CDCl_3$): δ	8.70 (bs, 1H, NH), 8.30 (d, 1H, J = 13.5 Hz, CH=CHNO$_2$), 7.81 (d, 1H, J = 13.5 Hz, CH=CHNO$_2$), 7.69 (d, 1H, J = 3.0 Hz, H-2), 7.65 (d, 1H, J = 7.9 Hz, H-4), 7.25 (t, 1H, J = 7.9 Hz, H-5), 7.17 (d, 1H, J = 7.9 Hz, H-6), 2.55 (s, 3H, CH$_3$)
^{13}C NMR (100 MHz, $CDCl_3$): δ	138.0, 136.0, 133.5, 133.0, 131.9, 124.9, 122.8, 121.5, 118.2, 110.2, 16.9.
EIMS (m/z):	202 (M^+, 100), 155 (M^+ - H - NO_2, 65), 129 (M^+ - H - $CHCHNO_2$, 30).

Calcd. for $C_{11}H_{11}N_2O_2$, (M^+ + H): 203.0815. Found: 203.0820.

5-Methoxy-7-methyl-3-(2-nitrovinyl)indole (132)



Compound **132** was prepared via method XI.B. by adding sequentially a solution of TFA (1.2 mL) in CH_2Cl_2 (3.6 mL) and a solution of 5-methoxy-7-methylindole **(103)** (0.33 g, 2.0 mmol) in CH_2Cl_2 (1.8 mL) to a mixture of dimethylamino-2-nitroethylene (0.64 g, 5.5 mmol) in CH_2Cl_2 (3 mL). Solvent evaporation gave **132** as an orange crystalline solid.

Yield:	0.41g, 1.77 mmol (88%)
mp:	225 - 226 °C
IR (cm^{-1}):	3256, 2923, 1612, 1475, 1295, 1271, 1226, 1194, 1118.

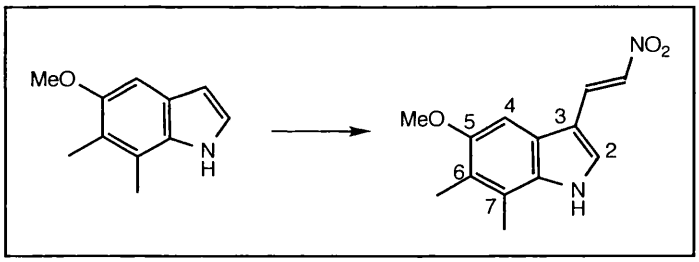
^{1}H NMR (400 MHz, d_6-acetone): δ 11.20 (bs, 1H, NH),
8.35 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
8.08 (d, 1H, J = 2.9 Hz, H-2),
7.86 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.25 (d, 1H, J = 2.2 Hz, H-4),
6.77 (d, 1H, J = 2.0 Hz, H-6),
3.89 (s, 3H, OCH$_3$), 2.83 (s, 3H, CH$_3$).

^{13}C NMR (100 MHz, d_6-acetone): δ 157.2, 135.0, 134.9, 133.1, 132.9, 126.2, 124.0, 115.0, 110.0, 100.7, 56.0, 16.8.

MS (FAB) (m/z): 233 (M^+ + H, 70), 232 (M^+, 53), 207 (100), 180 (70).

Calcd. for $C_{12}H_{13}N_2O_3$, (M^+ + H): 233.0921. Found: 233.0926.

6,7-Dimethyl-5-methoxy-3-(2-nitrovinyl)indole (133)



Compound **133** was prepared via method XI.B. by adding sequentially a solution of TFA (7.9 mL) in CH_2Cl_2 (23 mL) and a solution of 5-methoxy-6,7-dimethylindole **(104)** (2.30 g, 13 mmol) in CH_2Cl_2 (12 mL) to a mixture of dimethylamino-2-nitroethylene (1.52 g, 13 mmol) in CH_2Cl_2 (19 mL). Solvent evaporation gave **133** as an orange crystalline solid.

Yield: 2.01 g, 8.16 mmol (62%)

mp: 228 - 229 °C

IR (cm^{-1}): 3449, 1612, 1476, 1306, 1269, 1231, 1110.

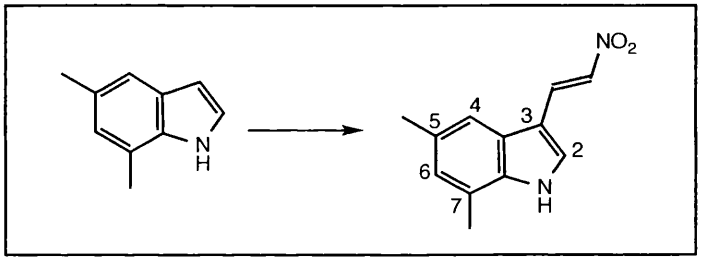
^{1}H NMR (400 MHz, d_6-acetone): δ 11.07 (bs, 1H, NH),
8.33 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.99 (s, 1H, H-4),
7.84 (d, 1H, J = 13.3 Hz, CH=CHNO$_2$),
7.23 (s, 1H, H-2), 3.94 (s, 1H, OCH$_3$),
2.44 (s, 3H, CH$_3$), 2.23 (s, 3H, CH$_3$).

^{13}C NMR (100 MHz, d_6-acetone): δ 155.5, 135.1, 134.5, 133.3, 131.9, 123.5, 122.6, 121.9, 110.0, 99.3, 56.3, 13.7, 12.0.

EIMS (m/z): 246 (M^+, 100), 199 (M^+ - H - NO_2, 30), 173 (M^+ - H - $CHCHNO_2$, 30).

Calcd. for $C_{13}H_{15}N_2O_3$, (M^+ + H): 247.1081. Found: 247.1083.

5,7-Dimethyl-3-(2-nitrovinyl)indole (134)



Compound **134** was prepared via method XI.B. by adding sequentially a solution of TFA (18 mL) in CH_2Cl_2 (54 mL) and a solution of 5,7-dimethylindole **(105)** (4.34 g, 30 mmol) in CH_2Cl_2 (27 mL) to a mixture of dimethylamino-2-nitroethylene (3.47 g, 30 mmol) in CH_2Cl_2 (43 mL). Solvent evaporation gave **134** as an orange crystalline solid.

Yield: 3.10 g, 14.3 mmol (48%)

mp: 211 - 213 °C

IR (cm^{-1}): 3245 2910, 1613, 1466, 1303, 1272, 1213, 1110.

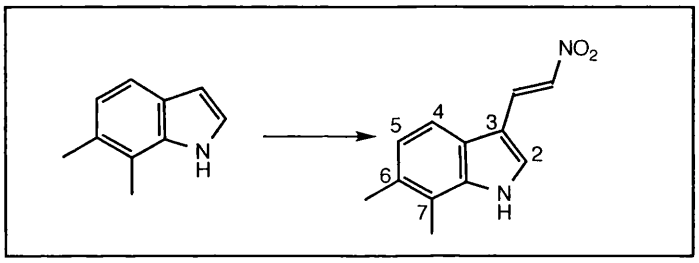
^{1}H NMR (400 MHz, d_6-acetone): δ 11.21 (bs, 1H, NH),
8.32 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
8.05 (s, 1H, H-4),
7.88 (d, 1H, J = 13.5 Hz, CH=CHNO$_2$),
7.58 (s, 1H, H-6), 6.94 (s, 1H, H-2),
2.49 (s, 3H, CH$_3$), 2.44 (s, 3H, CH$_3$).

^{13}C NMR (75 MHz, d_6-acetone): δ 136.6, 135.6, 135.0, 132.6, 132.3, 126.7, 125.9, 122.6, 118.7, 109.6, 21.5, 16.7.

EIMS (m/z): 216 (M^+, 100), 170 (M^+, - NO_2, 48), 156 (M^+ - H - $CHNO_2$, 27).

Calcd. for $C_{12}H_{13}N_2O_2$, (M^+ + H): 217.0975. Found: 217.0977.

6,7-Dimethyl-3-(2-nitrovinyl)indole (135)



Compound **135** was prepared via method XI.B. by adding sequentially a solution of TFA (8.3 mL) in CH_2Cl_2 (25 mL) and a solution of 6,7-dimethylindole **(107)** (2.00 g, 14 mmol) in CH_2Cl_2 (12.5 mL) to a mixture of dimethylamino-2-nitroethylene (1.60 g, 14 mmol) in CH_2Cl_2 (20 mL). Solvent evaporation gave **135** as an orange crystalline solid.

Yield: 1.51 g, 6.98 mmol (50%)

mp: 189 - 190 °C

IR (cm^{-1}): 3415, 2921, 1616, 1553, 1448, 1376, 1309, 1226, 1115.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.63 (bs, 1H, NH),
8.24 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.77 (d, 1H, J = 13.5 Hz, CH=CHNO$_2$),
7.58 (s, 1H, H-2), 7.51 (d, 1H, J = 7.9 Hz, H-4),
7.15 (d, 1H, J = 8.1 Hz, H-5),
2.42 (s, 3H, CH$_3$), 2.40 (s, 3H, CH$_3$).

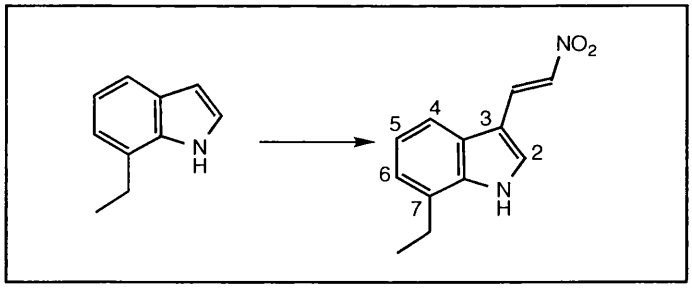
^{1}H NMR (400 MHz, d_6-acetone): δ 11.19 (bs, 1H, NH),
8.32 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
8.05 (s, 1H, H-2),
7.85 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.65 (d, 1H, J = 8.1 Hz, H-4),
7.11 (d, 1H, J = 8.1 Hz, H-5),
2.44 (s, 3H, CH$_3$), 2.38 (s, 3H, CH$_3$).

^{13}C NMR (75 MHz, d_6-acetone): δ 138.9, 135.4, 134.9, 132.4, 132.2, 125.6, 123.9, 120.8, 118.3, 109.2, 19.3, 13.2.

MS (FAB) (m/z): 217 (M^+ + H, 33), 154 (86), 136 (100).

Calcd. for $C_{12}H_{13}N_2O_2$, (M^+ + H): 217.0974. Found: 217.0977.

7-Ethyl-3-(2-nitrovinyl)indole (136)



Compound **136** was prepared from 7-ethylindole (0.80 g, 5.5 mmol) by method XI.B. Solvent evaporation gave **136** as a dark yellow solid.

Yield: 0.97 g, 4.49 mmol (82%)

mp: 204 - 205 °C

IR (cm^{-1}): 3325, 2943, 1612, 1474, 1304, 1222, 1103.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (bs, 1H, NH),
8.31 (d, 1H, J = 13.5 Hz, CH=CHNO$_2$),
7.81 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.69 (d, 1H, J = 2.8 Hz, H-2),
7.67 (d, 1H, J = 8.1 Hz, H-4),
7.30 (dd, 1H, J = 8.1, 7.0 Hz, H-5),
7.21 (d, 1H, J = 7.0 Hz, H-6),
2.90 (q, 2H, J = 7.6 Hz, CH$_2$CH$_3$),
1.40 (t, 3H, J = 7.6 Hz, CH$_2$CH$_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 138.0, 133.6, 132.9, 132.00, 127.7, 124.5, 122.9, 118.2, 110.2, 23.9, 13.9.

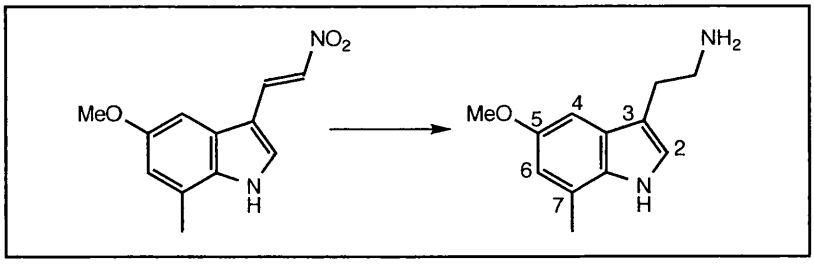
^{1}H NMR (400 MHz, d_6-acetone): δ 11.30 (bs, 1H, NH),
8.36 (d, 1H, J = 13.5 Hz, CH=CHNO$_2$),
8.13 (s, 1H, H-2),
7.89 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.78 (d, 1H, J = 8.1 Hz, H-4),
7.24 (dd, 1H, J = 8.1, 7.3 Hz, H-5),
7.16 (d, 1H, J = 7.3 Hz, H-6),
2.95 (q, 2H, J = 7.6 Hz, CH$_2$CH$_3$),
1.31 (t, 3H, J = 7.6 Hz, CH$_2$CH$_3$).

^{13}C NMR (100 MHz, d_6-acetone): δ 135.3, 135.1, 134.7, 132.7, 129.3, 125.8, 123.3, 123.2, 118.9, 110.0, 24.6, 14.7.

EIMS (m/z): 216 (M^+, 100), 169 (M^+ - H - NO_2, 65),
154 (M^+ - H - NO_2 - CH_3 , 66),
141 (M^+ - H - $CHNO_2$ - CH_3 , 24).

Calcd. for $C_{12}H_{13}N_2O_2$, (M^+ + H): 217.0975. Found: 217.0977.

5-Methoxy-7-methyltryptamine[225] (141)

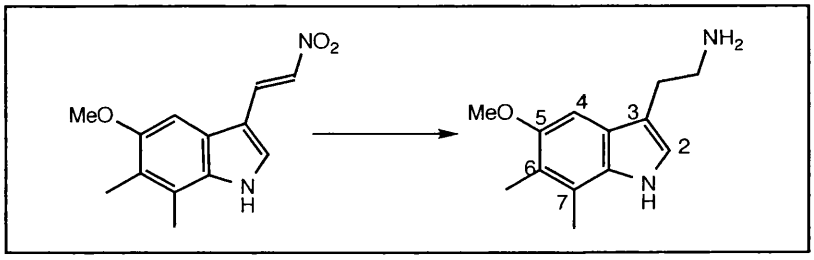


Compound **141** was prepared via method XI.C. by adding a solution of 5-methoxy-7-methyl-3-(2-nitrovinyl)indole **(132)** (0.22 g, 0.93 mmol) in THF (3.7 mL) to a solution of $LiAlH_4$ (0.20 g, 5.6 mmol) in THF (8.5 mL). Solvent evaporation gave **141** as a light brown solid. The tryptamine was used in subsequent reactions without further purification.

Yield: 0.16 g, 0.77 mmol (83%)

mp: 163 - 164 °C (lit.[225] 164.4 °C)

6,7-Dimethyl-5-methoxytryptamine (142)



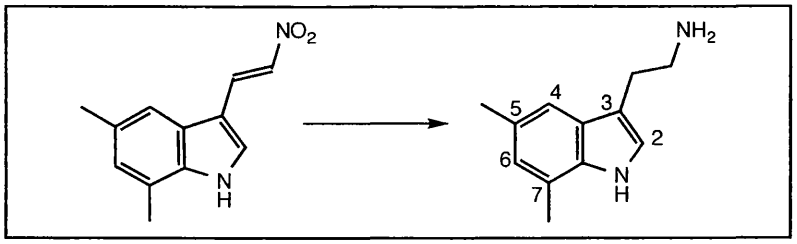
Compound **142** was prepared via method XI.C. by adding a solution of 6,7-dimethyl-5-methoxy-3-(2-nitrovinyl)indole **(133)** (1.93 g, 7.82 mmol) in THF (35 mL) to a solution of $LiAlH_4$ (1.75 g, 45.9 mmol) in THF (70 mL) and then stirring at rt for 10 hours. Solvent evaporation gave **142** as a light brown solid. The tryptamine was used in subsequent reactions without further purification.

Yield: 1.67 g, 7.65 mmol (98%)

^1H NMR (300 MHz, $CDCl_3$): δ 7.86 (bs, 1H, $N\underline{H}_{indole}$), 6.93 (s, 1H, H-4), 6.87 (s, 1H, H-2) 3.89 (s, 1H, $OC\underline{H}_3$), 3.02 (t, 2H, J = 6.6 Hz, $CH_2C\underline{H}_2NH_2$), 2.88 (t, 2H, J = 6.4 Hz, $C\underline{H}_2CH_2NH_2$), 2.38 (s, 3H, $C\underline{H}_3$), 2.32 (s, 3H, $C\underline{H}_3$).

EIMS (m/z): 218 (M^+, 44), 188 ($M^+ - CH_2NH_2$, 100), 174 ($M^+ - CH_2CH_2NH_2$, 36).

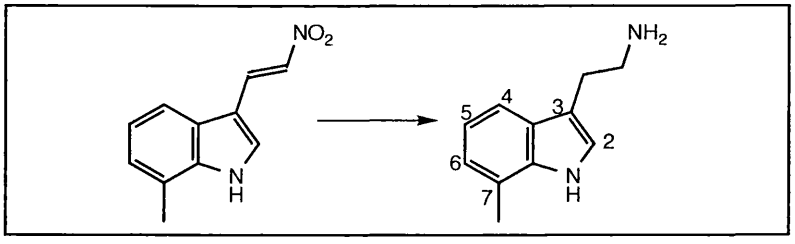
5,7-Dimethyltryptamine[225] (143)



Compound **143** was prepared via method XI.C. by adding a solution of 5,7-dimethyl-3-(2-nitrovinyl)indole **(134)** (2.50 g, 11.6 mmol) in THF (50 mL) to a solution of $LiAlH_4$ (2.58 g, 68.0 mmol) in THF (100 mL) and then stirring at rt for 10 hours. Solvent evaporation gave **143** as a light brown solid. The tryptamine was used in subsequent reactions without further purification.

Yield:	2.13 g, 11.3 mmol (98%)
mp:	236 - 238 °C (lit.[225] 237 - 238 °C)
^{1}H NMR (300 MHz, $CDCl_3$): δ	8.55 (bs, 1H, N<u>H</u>$_{indole}$), 7.41 (s, 1H, H-4), 6.90 (s, 1H, H-2), 6.87 (s, 1H, H-6), 3.02 (t, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH_2$), 2.90 (t, 2H, J = 6.4 Hz, $C\underline{H}_2CH_2NH_2$), 2.48 (s, 3H, $C\underline{H}_3$), 2.42 (s, 3H, CH_3).
EIMS (m/z):	188 (M^+, 18), 158 (M^+ - CH_2NH_2, 100).

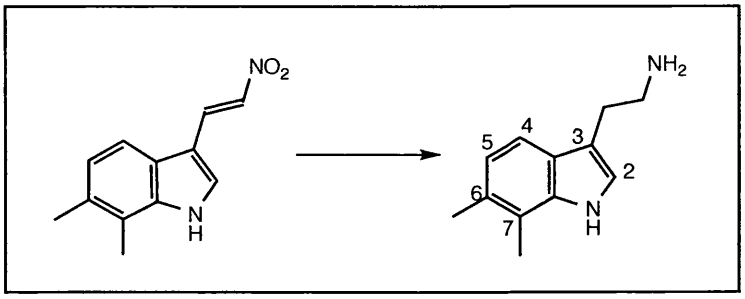
7-Methyltryptamine (144)[224]



Compound **144** was prepared from 7-methyl-3-(2-nitrovinyl)indole **(120)** (0.30 g, 1.48 mmol) by method XI.C. Solvent evaporation gave **144** as a light brown solid. The tryptamine was used in subsequent reactions without further purification.

Yield:	0.24 g, 1.38 mmol (93%)
mp:	169 - 170 °C
EIMS (m/z):	174 (M^+, 45), 144 (M^+ - CH_2NH_2, 100), 130 (M^+ - $CH_2CH_2NH_2$, 35).

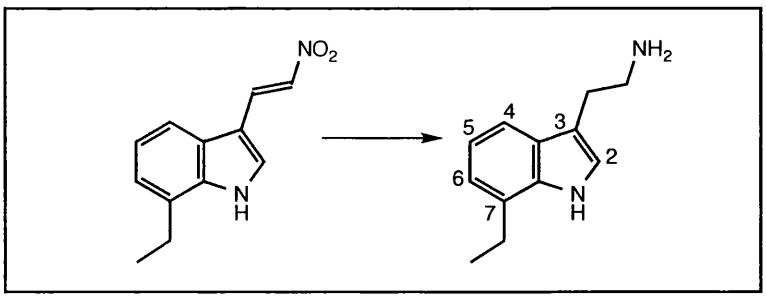
6,7-Dimethyltryptamine (145)[248]



Compound **145** was prepared via method XI.C. by adding a solution of 6,7-dimethyl-3-(2-nitrovinyl)indole **(135)** (1.56 g, 1.48 mmol) in THF (39 mL) to a solution of $LiAlH_4$ (1.60 g, 42.2 mmol) in THF (60 mL) and then stirring at rt for 10 hours. Solvent evaporation gave **145** as a light brown solid. The tryptamine was used in subsequent reactions without further purification.

Yield:	1.31 g, 6.96 mmol (97%)
^{1}H NMR (300 MHz, $CDCl_3$): δ	7.84 (bs, 1H, N<u>H</u>indole), 7.36 (d, 1H, J = 7.4 Hz, H-4), 6.97 (m, 2H, H-2, H-5), 3.00 (t, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH_2$), 2.89 (t, 2H, J = 6.4 Hz, $C\underline{H}_2CH_2NH_2$), 2.41 (s, 3H, $C\underline{H}_3$), 2.38 (s, 3H, $C\underline{H}_3$).
EIMS (m/z):	188 (M^+, 20), 158 (M^+ - CH_2NH_2, 100).

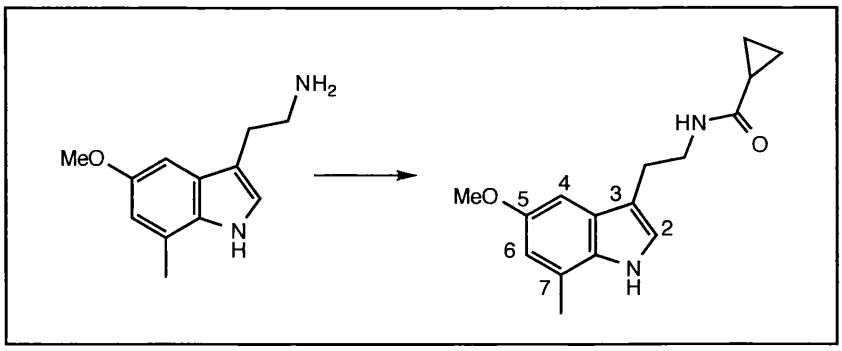
7-Ethyltryptamine (146)



Compound **146** was prepared from 7-ethyl-3-(2-nitrovinyl)indole **(136)** (0.84 g, 3.86 mmol) by method XI.C. Solvent evaporation gave **146** as a light brown solid. The tryptamine was used in subsequent reactions without further purification.

Yield:	0.71 g, 3.77 mmol (98%)
^{1}H NMR (300 MHz, $CDCl_3$): δ	8.44 (bs, 1H, N<u>H</u>$_{indole}$), 7.54 (d, 1H, J = 7.4 Hz, H-4), 7.15 (m, 2H, H-5, H-6), 7.00 (d, 1H, J = 2.3 Hz, H-2), 3.10 (t, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH_2$), 2.97 (t, 2H, J = 6.5 Hz, $C\underline{H}_2CH_2NH_2$), 2.89 (q, 2H, J = 7.5 Hz, $C\underline{H}_2$), 1.39 (t, 3H, J = 7.6 Hz, $C\underline{H}_3$).
EIMS (m/z):	188 (M^+, 20), 158 (M^+ - CH_2NH_2, 100), 144 (M^+ - $CH_2CH_2NH_2$, 14).

N-Cyclopropanecarbonyl-2-(5-methoxy-7-methylindol-3-yl)ethanamine (147)

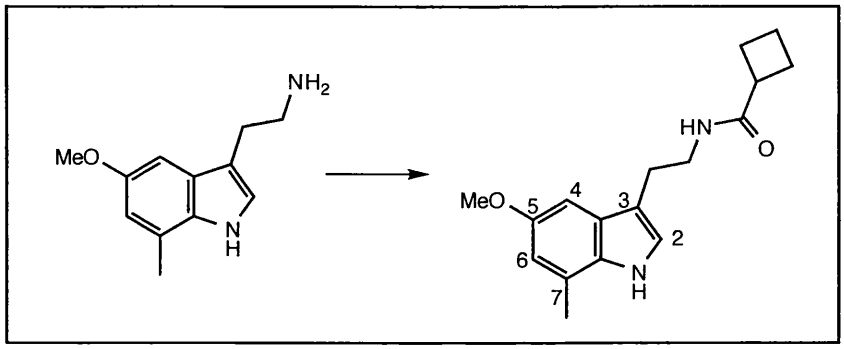


Compound **147** was prepared from cyclopropanecarbonyl chloride (0.09 g, 0.08 mL, 0.88 mmol) and 5-methoxy-7-methyltryptamine **(141)** (0.13 g, 0.64 mmol) via method XI.D. Purification by column chromatography on silica with gradient elution (CH_2Cl_2 to Et_2O) gave **147** as a light brown foam. Trituration of the foam with diethyl ether yielded **147** as a beige crystalline solid.

Yield:	0.036 g, 0.13 mmol (20%)
mp:	147 - 148 °C
IR (cm^{-1}):	3319, 3272, 3119, 2916, 1612, 1488, 1444, 1314, 1270, 1205, 1172, 1101.
^1H NMR (400 MHz, $CDCl_3$): δ	7.90 (bs, 1H, $N\underline{H}_{indole}$), 7.05 (d, 1H, J = 1.5 Hz, H-4), 6.91 (d, 1H, J = 1.4 Hz, H-6), 6.71 (s, 1H, H-2), 5.70 (bs, 1H, $N\underline{H}_{amide}$), 3.86 (s, 3H, $OC\underline{H}_3$), 3.63 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.96 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.47 (s, 3H, $C\underline{H}_3$), 1.22 (s, 1H, $C\underline{H}C{=}O$), 0.97 (m, 2H), 0.71 (m, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	173.4, 154.3, 131.2, 127.2, 122.3, 121.5, 113.5, 113.2, 97.9, 55.9, 39.9, 25.5, 16.7, 14.8, 7.0.
MS (APCI) (m/z):	272 (M^+, 100), 187 (M^+ - H - $NHCOC_3H_5$, 64), 174 (M^+ - $CH_2NHCOC_3H_5$, 98).
MS (FAB) (m/z):	273 (M^++ H, 76), 272 (M^+, 64), 188 (M^+ - $NHCOC_3H_5$, 59), 187 (M^+ - H - $NHCOC_3H_5$, 100), 174 (M^+ - $CH_2NHCOC_3H_5$, 60).

Calcd. for $C_{16}H_{21}N_2O_2$, (M^+ + H): 273.1595. Found: 273.1603.

N-Cyclobutanecarbonyl-2-(5-methoxy-7-methylindol-3-yl)ethanamine (148)



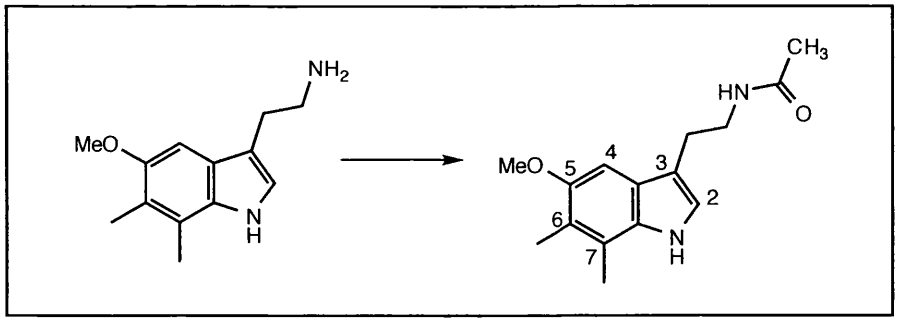
Compound **148** was prepared via method XI.D. by the sequential addition of Et_3N (0.12 mL, 0.86 mmol), cyclobutanecarbonyl chloride (0.09 g, 0.09 mL, 0.79 mmol) and 5-methoxy-7-methyltryptamine **(141)** (0.16 g, 0.77 mmol) in CH_2Cl_2 (4 mL). Purification by column chromatography on silica with gradient elution (CH_2Cl_2 to Et_2O) gave **148** as a light brown foam. Trituration of the foam with diethyl ether yielded **148** as a beige crystalline solid.

Yield:	0.033 g, 1.15 mmol (15%)
mp:	146 - 147 °C
IR (cm^{-1}):	3310, 3269, 3099, 2943, 1613, 1570, 1490, 1440, 1390, 1311, 1269, 1205, 1169, 1104, 1053.
^1H NMR (400 MHz, $CDCl_3$): δ	7.90 (bs, 1H, N<u>H</u>indole), 7.03 (d, 1H, J = 1.5 Hz, H-4), 6.89 (d, 1H, J = 1.9 Hz, H-6), 6.71 (s, 1H, H-2) 5.44 (bs, 1H, N<u>H</u>amide), 3.86 (s, 3H, OC<u>H</u>3), 3.61 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.94 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.91 (m, 1H, C<u>H</u>C=O), 2.45 (s, 3H, C<u>H</u>3), 2.24 (m, 2H), 2.08 (m, 2H), 1.91 (m, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	174.8, 154.3, 131.2, 127.1, 122.3, 121.5, 113.4, 113.2, 97.9, 55.9, 40.0, 39.5, 25.5, 25.3, 18.1, 16.7.
MS (FAB) (m/z):	287 (M^+ + H, 68), 286 (M^+, 28), 188 (M^+, - $NHCOC_4H_7$, 30), 185 (100), 174 (M^+, - $CH_2NHCOC_4H_7$, 24).

Calcd. for $C_{17}H_{23}N_2O_2$, (M^+ + H): 287.1768. Found: 287.1760.

$C_{17}H_{22}N_2O_2$ requires: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.16; H, 7.52; N, 9.27.

N-Acetyl-2-(5-methoxyindol-6,7-dimethyl-3-yl)ethanamine (149)



Compound **149** was prepared via method XI.D. by the sequential addition of Et_3N (0.27 mL, 1.94 mmol) and acetyl chloride (0.14 g, 0.13 mL, 1.78 mmol) into a solution of 5-methoxy-6,7-dimethyltryptamine **(142)** (0.34 g, 1.81 mmol) in CH_2Cl_2 (16 mL). Purification by column chromatography on silica with gradient elution (Et_2O to EtOAc) gave **149** as a white foam.

Yield: 0.36 g, 1.38 mmol (76%)

IR ($CHCl_3$, cm^{-1}): 3479, 3286, 3002, 2933, 1662, 1521, 1438, 1370, 1274, 1230, 1208, 1091.

^1H NMR (300 MHz, $CDCl_3$): δ 8.52 (bs, 1H, $N\underline{H}_{indole}$), 6.99 (d, 1H, J = 2.3 Hz, H-2), 6.89 (s, 1H, H-4), 5.87 (bs, 1H, $N\underline{H}_{amide}$), 3.90 (s, 3H, $OC\underline{H}_3$), 3.62 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.97 (t, 2H, J = 6.6 Hz, $C\underline{H}_2CH_2NH$), 2.48 (s, 3H, $C\underline{H}_3$), 2.47 (s, 3H, $C\underline{H}_3$), 1.95 (s, 3H, $COC\underline{H}_3$).

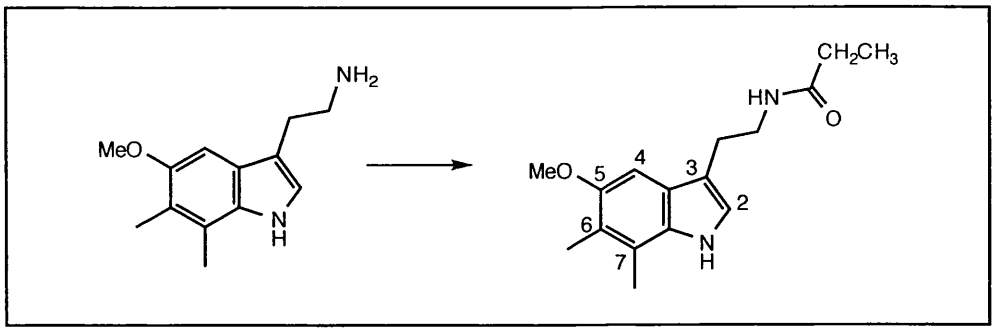
^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 134.3, 128.9, 127.1, 124.4, 122.0, 120.2, 115.9, 112.8, 60.4, 39.8, 25.3, 23.3, 21.0, 16.5.

MS (FAB) (m/z): 261 (M^+ + H, 18), 260 (M^+, 25), 202 (M^+ - $NHCOCH_3$, 23), 201 (M^+ - H - $NHCOCH_3$, 25), 188 (M^+ - $CH_2NHCOCH_3$, 68), 174 (M^+ - $CH_2CH_2NHCOCH_3$, 14), 172 (100), 158 (71).

Calcd. for $C_{15}H_{20}N_2O_2$, (M^+): 260.1523. Found: 260.1525.

$C_{15}H_{20}N_2O_2$ requires: C, 69.20; H, 7.74; N, 10.76. Found: C, 70.03; H, 7.76; N, 11.17.

N-Propanoyl-2-(5-methoxyindol-6,7-dimethyl-3-yl)ethanamine (150)



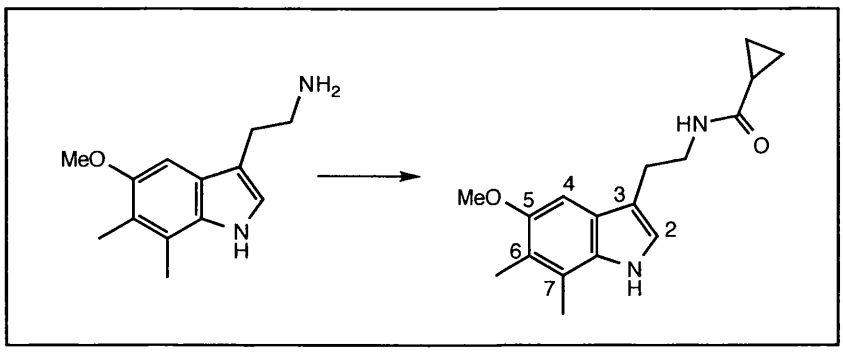
Compound **150** was prepared via method XI.D. by the sequential addition of Et_3N (0.27 mL, 1.94 mmol) and propionyl chloride (0.18 g, 0.17 mL, 1.95 mmol) into a solution of 5-methoxy-6,7-dimethyltryptamine **(142)** (0.42 g, 1.92 mmol) in CH_2Cl_2 (16 mL). Purification by column chromatography on silica with gradient elution (Et_2O to EtOAc) gave **150** as a white crystalline solid after crystallisation from CH_2Cl_2/ hexane.

Yield:	0.37 g, 1.35 mmol (71%)
mp:	116 - 117 °C
IR (cm^{-1}):	3440, 3286, 2976, 1942, 1658, 1556, 1528, 1453, 1379, 1313, 1219, 1169, 1098.
^1H NMR (400 MHz, $CDCl_3$): δ	8.17 (bs, 1H, N<u>H</u>$_{indole}$), 6.95 (d, 1H, J = 2.1 Hz, H-2), 6.90 (s, 1H, H-4), 5.65 (bs, 1H, N<u>H</u>$_{amide}$), 3.88 (s, 1H, OC<u>H</u>$_3$), 3.62 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.95 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.47 (s, 3H, C<u>H</u>$_3$), 2.45 (s, 3H, C<u>H</u>$_3$), 2.15 (q, 2H, J = 7.6 Hz, $C\underline{H}_2CH_3$), 1.33 (t, 3H, J = 7.6 Hz, $CH_2C\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	173.8, 152.8, 131.4, 124.2, 121.3, 120.6, 119.5, 112.8, 96.6, 56.0, 39.6, 29.7, 25.4, 13.52, 11.8, 9.9.
EIMS (m/z):	274 (M^+, 24), 201 (M^+ - H - $NHCOCH_2CH_3$, 100), 188 (M^+ - $CH_2NHCOC_2H_5$, 89).

Calcd. for $C_{16}H_{22}N_2O_2$, (M^+): 274.1673. Found: 274.1681.

$C_{16}H_{22}N_2O_2$ requires: C, 70.04; H, 8.08; N, 10.21. Found: C, 69.56; H, 8.19; N, 10.09.

N-Cyclopropanecarbonyl-2-(5-methoxyindol-6,7-dimethyl-3-yl)ethanamine (151)



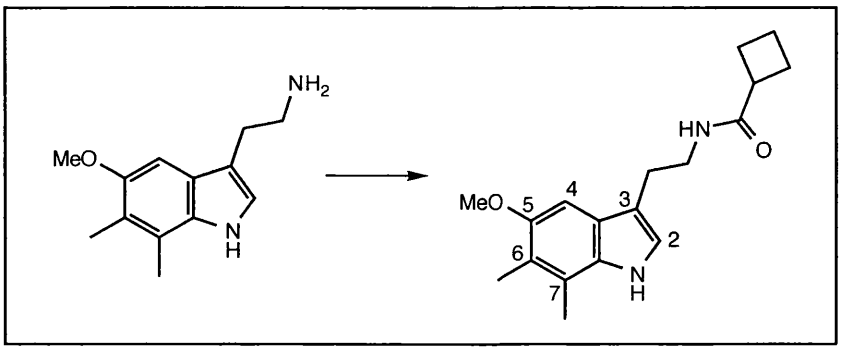
Compound **151** was prepared via method XI.D. by the sequential addition of Et_3N (0.27 mL, 1.94 mmol) and cyclopropanecarbonyl chloride (0.20 g, 0.18 mL, 1.98 mmol) into a solution of 5-methoxy-6,7-dimethyltryptamine **(142)** (0.42 g, 1.92 mmol) in CH_2Cl_2 (16 mL). Purification by column chromatography on silica with gradient elution (Et_2O to EtOAc) gave **151** as a white crystalline solid after crystallisation from CH_2Cl_2/ hexane.

Yield:	0.23 g, 0.80 mmol (42%)
mp:	155 - 156 °C
IR (cm^{-1}):	3380, 3285, 2944, 1650, 1534, 1455, 1313, 1235, 1172, 1101.
^1H NMR (400 MHz, $CDCl_3$): δ	8.01 (bs, 1H, N$\underline{H}_{indole}$), 6.98 (d, 1H, J = 2.1 Hz, H-2), 6.91 (s, 1H, H-4), 5.77 (bs, 1H, N$\underline{H}_{amide}$), 3.89 (s, 1H, OC$\underline{H}_3$), 3.62 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.96 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.41 (s, 3H, C$\underline{H}_3$), 2.30 (s, 3H, C$\underline{H}_3$), 1.24 (m, 1H, C$\underline{H}$C=O), 0.98 (m, 2H), 0.70 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$): δ	173.4, 152.9, 131.4, 124.3, 121.4, 120.7, 119.5, 113.1, 96.7, 56.1, 40.0, 25.5, 14.8, 13.6, 11.9, 7.0.
EIMS (m/z):	286 (M^+, 27), 201 (M^+ - H - $NHCOC_3H_5$, 100), 188 (M^+ - $CH_2NHCOC_3H_5$, 69).

Calcd. for $C_{17}H_{22}N_2O_2$, (M^+): 286.1853. Found: 286.1881.

$C_{17}H_{22}N_2O_2$ requires: C, 71.30; H, 7.74; N, 9.78. Found: C, 70.92; H, 7.90; N, 9.70.

N-Cyclobutanecarbonyl-2-(5-methoxyindol-6,7-dimethyl-3-yl)ethanamine (152)



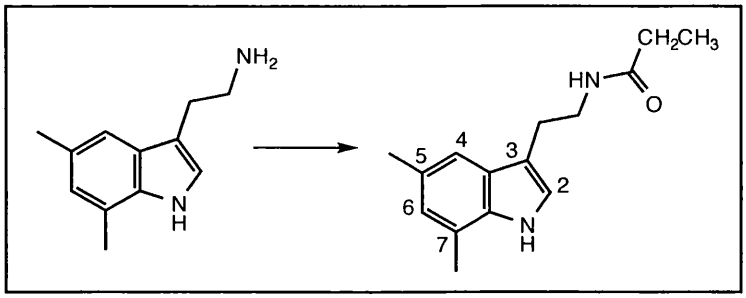
Compound **152** was prepared via method XI.D. by the sequential addition of Et_3N (0.27 mL, 1.94 mmol) and cyclobutanecarbonyl chloride (0.23 g, 0.22 mL, 1.93 mmol) into a solution of 5-methoxy-6,7-dimethyltryptamine **(142)** (0.42 g, 1.92 mmol) in CH_2Cl_2 (16 mL). Purification by column chromatography on silica with gradient elution (Et_2O to EtOAc) gave **152** as a white crystalline solid after crystallisation from CH_2Cl_2/ hexane.

Yield:	0.18 g, 0.60 mmol (31%)
mp:	166 - 168 °C
IR (cm^{-1}):	3372, 3280, 2338, 1650, 1584, 1530, 1493, 1456, 1369, 1311, 1220, 1169, 1100.
^1H NMR (300 MHz, $CDCl_3$): δ	8.04 (bs, 1H, N<u>H</u>$_{indole}$), 6.96 (d, 1H, J = 2.2 Hz, H-2), 6.89 (s, 1H, H-4), 5.49 (bs, 1H, N<u>H</u>$_{amide}$), 3.88 (s, 1H, OC<u>H</u>$_3$), 3.60 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.94 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.91 (m, 1H, C<u>H</u>C=O), 2.40 (s, 3H, C<u>H</u>$_3$), 2.29 (s, 3H, C<u>H</u>$_3$), 2.26 (m, 2H), 2.10 (m, 2H), 1.90 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$): δ	174.9, 152.6, 131.4, 124.2, 121.3, 120.7, 119.5, 112.9, 96.6, 56.1, 40.0, 39.5, 25.4, 25.3, 18.1, 13.6, 11.9.
EIMS (m/z):	300 (M^+, 21), 201 (M^+ - H - $NHCOC_4H_7$, 100), 189 (M^+ - $CHNHCOC_4H_7$, 56).

Calcd. for $C_{18}H_{25}N_2O_2$, (M^+ + H): 301.1903. Found: 301.1916.

$C_{18}H_{24}N_2O_2$ requires: C, 71.97; H, 8.05; N, 9.33. Found: C, 71.45; H, 8.10; N, 9.21.

N-Propanoyl-2-(5,7-dimethylindol-3-yl)ethanamine (153)



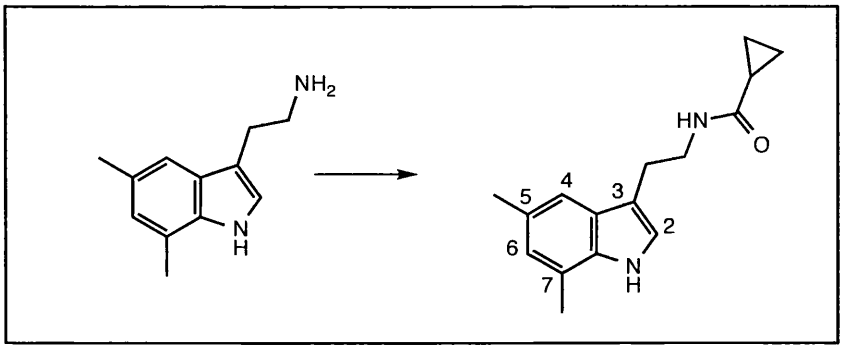
Compound **153** was prepared via method XI.D. by the sequential addition of Et_3N (0.40 mL, 2.87 mmol) and propionyl chloride (0.26 g, 0.25 mL, 2.86 mmol) into a solution of 5,7-dimethyltryptamine **(143)** (0.54 g, 2.87 mmol) in CH_2Cl_2 (25 mL). Purification by column chromatography on silica with gradient elution (CH_2Cl_2 to Et_2O) gave **153** as a white crystalline solid after crystallisation from CH_2Cl_2/ hexane.

Yield:	0.42 g, 1.72 mmol (60%)
mp:	108 - 109 °C
IR (cm^{-1}):	3314, 3280, 2921, 1640, 1540, 1447, 1371, 1235, 1104.
^1H NMR (400 MHz, $CDCl_3$): δ	8.20 (bs, 1H, N<u>H</u>indole), 7.26 (s, 1H, H-4), 7.00 (d, 1H, J = 2.0 Hz, H-2), 6.88 (s, 1H, H-6), 5.60 (bs, 1H, N<u>H</u>amide), 3.60 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.95 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.47 (s, 3H, $C\underline{H}_3$), 2.45 (s, 3H, $C\underline{H}_3$), 2.15 (q, 2H, J = 7.6 Hz, $C\underline{H}_2CH_3$), 1.33 (t, 3H, J = 7.6 Hz, $CH_2C\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	173.8, 134.3, 128.9, 127.1, 124.4, 122.0, 120.2, 116.0, 112.9, 39.8, 29.8, 25.4, 21.4, 16.5, 9.9.
MS (FAB) (m/z):	245 (M^+ + H, 71), 244 (M^+, 56), 171 (M^+ - H - $NHCOCH_2CH_3$, 100), 158 (M^+ - $CH_2NHCOCH_2CH_3$, 55).

Calcd. for $C_{15}H_{21}N_2O$, (M^+ + H): 245.1646. Found: 245.1654.

$C_{15}H_{20}N_2O$ requires: C, 73.74; H, 8.25; N, 11.47. Found: C, 73.22; H, 8.01; N, 11.25.

N-Cyclopropanecarbonyl-2-(5,7-dimethylindol-3-yl)ethanamine (154)



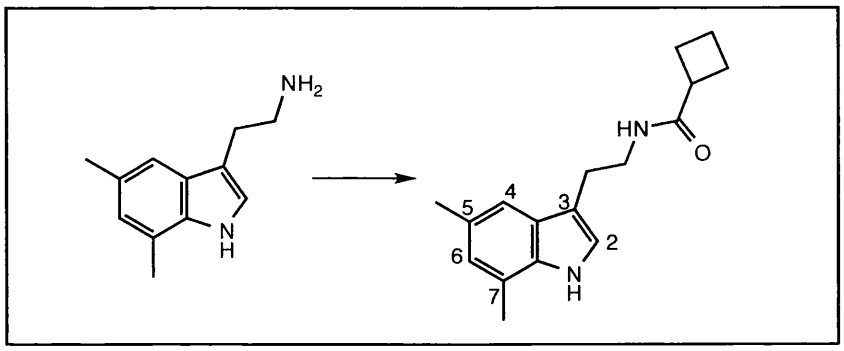
Compound **154** was prepared via method XI.D. by the sequential addition of Et_3N (0.40 mL, 2.87 mmol) and cyclopropanecarbonyl chloride (0.30 g, 0.26 mL, 2.86 mmol) into a solution of 5,7-dimethyltryptamine **(143)** (0.55 g, 2.92 mmol) in CH_2Cl_2 (25 mL). Purification by column chromatography on silica with gradient elution (CH_2Cl_2 to Et_2O) gave **154** as a white crystalline solid after recrystallisation from EtOAc.

Yield:	0.30 g, 1.17 mmol (40%)
mp:	132 - 133 °C
IR (cm^{-1}):	3314, 3279, 2912, 1620, 1444, 1273, 1243, 1207, 1100, 1060, 1017.
^1H NMR (300 MHz, $CDCl_3$): δ	8.16 (bs, 1H, $N\underline{H}_{indole}$), 7.26 (s, 1H, H-4), 7.00 (d, 1H, J = 2.1 Hz, H-2), 6.87 (s, 1H, H-6), 5.78 (bs, 1H, $N\underline{H}_{amide}$), 3.61 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.95 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.46 (s, 3H, $C\underline{H}_3$), 2.43 (s, 3H, $C\underline{H}_3$), 1.23 (m, 1H, $C\underline{H}C{=}O$), 0.98 (m, 2H), 0.70 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$): δ	173.5, 134.3, 128.9, 127.2, 124.4, 122.0, 120.2, 116.0, 113.0, 40.1, 25.5, 21.4, 16.5, 14.8, 7.0.
EIMS (m/z):	256 (M^+, 28), 172 (M^+- $NHCOC_3H_5$, 100).

Calcd. for $C_{16}H_{21}N_2O$, (M^+ + H): 257.1648. Found: 257.1654.

$C_{16}H_{20}N_2O$ requires: C, 74.97; H, 7.86; N, 10.93. Found: C, 74.62; H, 7.68; N, 10.75.

N-Cyclobutanecarbonyl-2-(5,7-dimethylindol-3-yl)ethanamine (155)



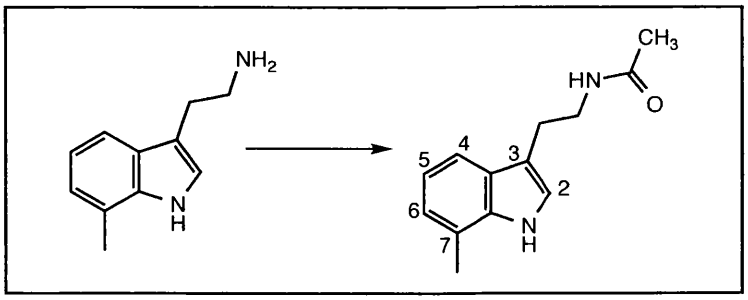
Compound **155** was prepared via method XI.D. by the sequential addition of Et_3N (0.40 mL, 2.87 mmol) and cyclobutanecarbonyl chloride (0.34 g, 0.33 mL, 3.07 mmol) into a solution of 5,7-dimethyltryptamine **(143)** (0.55 g, 2.92 mmol) in CH_2Cl_2 (25 mL). Purification by column chromatography on silica with gradient elution (CH_2Cl_2 to Et_2O) gave **155** as a white crystalline solid after recrystallisation from EtOAc.

Yield:	0.13 g, 0.48 mmol (16%)
mp:	142 - 143 °C
IR (cm^{-1}):	3326, 3280, 2946, 2360, 1618, 1442, 1387, 1267, 1235, 1108.
^1H NMR (300 MHz, $CDCl_3$): δ	8.01 (bs, 1H, N<u>H</u>$_{indole}$), 7.25 (s, 1H, H-4), 7.01 (d, 1H, J = 2.2 Hz, H-2), 6.87 (s, 1H, H-6), 5.44 (bs, 1H, N<u>H</u>$_{amide}$), 3.59 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.92 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.91 (m, 1H, C<u>H</u>C=O), 2.47 (s, 3H, C<u>H</u>$_3$), 2.45 (s, 3H, C<u>H</u>$_3$), 2.28 (m, 2H), 2.09 (m, 2H), 1.90 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$): δ	174.9, 134.3, 128.9, 127.1, 124.4, 121.9, 120.1, 116.0, 113.1, 40.0, 39.7, 25.44, 25.3, 21.4, 18.1, 16.5.
EIMS (m/z):	270 (M^+, 35), 172 (M^+ - $NHCOC_4H_7$, 100), 160 (91).

Calcd. for $C_{17}H_{23}N_2O$, (M^+ + H): 271.1816. Found: 271.1810.

$C_{17}H_{22}N_2O$ requires: C, 75.52; H, 8.20; N, 10.36. Found: C, 73.97; H, 8.06; N, 10.09.

N-Acetyl-2-(7-methylindol-3-yl)ethanamine (156)



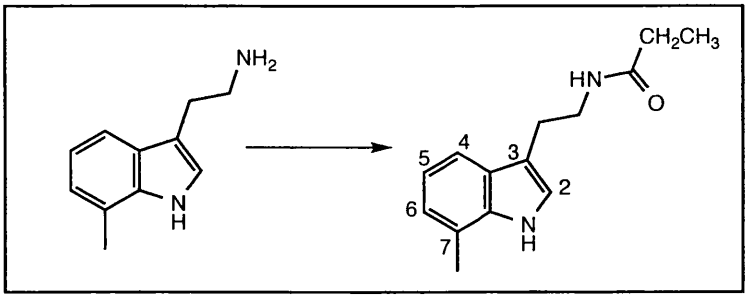
Compound **156** was prepared via method XI.D. by sequential addition of Et_3N (0.13 mL, 0.93 mmol) and acetic anhydride (0.09 g, 0.09 mL, 0.88 mmol) into a solution of 7-methyltryptamine **(144)** (0.15 g, 0.86 mmol) in CH_2Cl_2 (1.2 mL) as an yellow oil. Trituration of the oil with diethyl ether gave **156** as a white crystalline solid.

Yield:	0.15 g, 0.69 mmol (81%)
mp:	87 - 88 °C
IR (cm^{-1}):	3397, 3251, 2937, 2860, 1665, 1530, 1452, 1367, 1274, 1233, 1105, 1068.
^1H NMR (300 MHz, $CDCl_3$): δ	8.41 (bs, 1H, N<u>H</u>$_{indole}$), 7.47 (d, 1H, J = 7.6 Hz, H-4), 7.05 (m, 3H, H-2, H-5, H-6), 5.69 (bs, 1H, N<u>H</u>$_{amide}$), 3.60 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.98 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.51 (s, 3H, $C\underline{H}_3$), 1.92 (s, 3H, $COC\underline{H}_3$).
^{13}C NMR (75 MHz, $CDCl_3$): δ	170.1, 136.0, 126.8, 122.6, 121.8, 120.6, 119.6, 116.3, 113.2, 39.8, 25.3, 23.3, 16.6.
EIMS (m/z):	216 (M^+, 20), 157 (M^+ - H - NHCOCH 97), (M^+ - $CH_2NHCOCH_3$, 100).

Calcd. for $C_{13}H_{17}N_2O$, (M^+ + H): 217.1349. Found: 217.1341.

$C_{13}H_{16}N_2O$ requires: C, 72.19; H, 7.46; N, 12.95. Found: C, 71.50; H, 7.42; N, 12.76.

N-Propanoyl-2-(7-methylindol-3-yl)ethanamine (157)



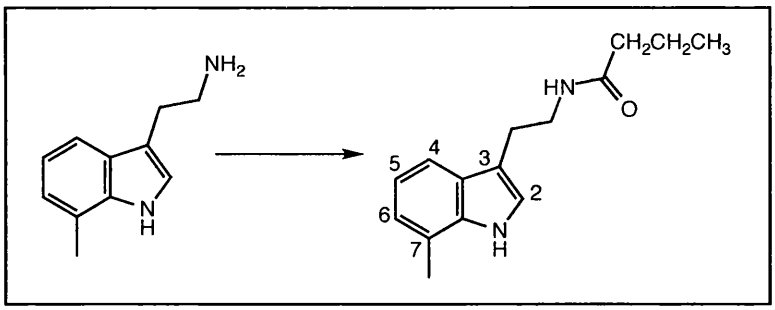
Compound **157** was prepared via method XI.D. by sequential addition of Et_3N (0.13 mL, 0.93 mmol) and propionyl chloride (0.09 g, 0.08 mL, 0.90 mmol) into a solution of 7-methyltryptamine **(144)** (0.15 g, 0.86 mmol) in CH_2Cl_2 (1.2 mL) as a dark grey oil. Trituration of the oil with diethyl ether gave **157** as a white crystalline solid.

Yield:	0.16 g, 0.70 mmol (81%)
mp:	92 - 93 °C
IR ($CHCl_3$, cm^{-1}):	3384, 3258, 2935, 1664, 1529, 1452, 1355, 1236, 1131, 1109.
^1H NMR (400 MHz, $CDCl_3$): δ	8.00 (bs, 1H, N<u>H</u>indole), 7.49 (d, 1H, J = 7.6 Hz, H-4), 7.04 (m, 3H, H-2, H-5, H-6), 5.49 (bs, 1H, N<u>H</u>amide), 3.61 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.99 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.51 (s, 3H, $C\underline{H}_3$), 2.23 (q, 2H, J = 7.5 Hz, $C\underline{H}_2CH_3$), 1.12 (t, 3H, J = 7.5 Hz, $CH_2C\underline{H}_3$).
^{13}C NMR (75 MHz, $CDCl_3$): δ	δ 173.7, 136.0, 126.9, 122.8, 121.7, 120.4, 119.8, 116.5, 113.7, 39.7, 29.8, 25.4, 16.6, 9.8.
EIMS (m/z):	230 (M^+, 10), 157 (M^+ - H - $NHCOCH_2CH_3$, 100), 144 (M^+ - $CH_2NHCOCH_2CH_3$, 75).

Calcd. for $C_{14}H_{19}N_2O$, (M^+ + H): 231.1486. Found: 231.1497.

$C_{14}H_{18}N_2O$ requires: C, 73.01; H, 7.88; N, 12.16. Found: C, 72.97; H, 7.84; N, 11.85.

N-butyryl-2-(7-methylindol-3-yl)ethanamine (158)



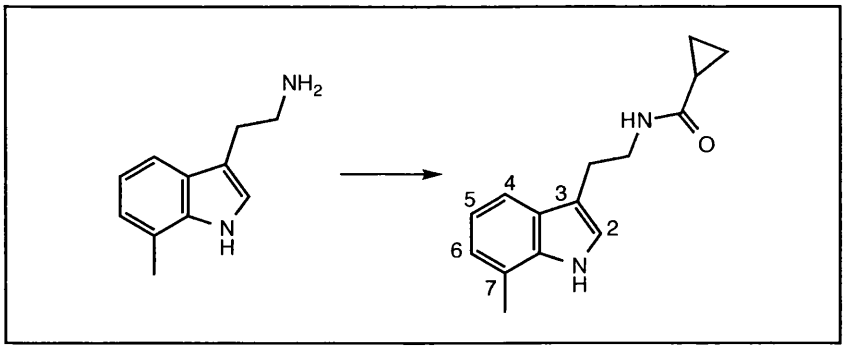
Compound **158** was prepared via method XI.D. by sequential addition of Et_3N (0.25 mL, 1.82 mmol) and butyryl chloride (0.14 g, 0.14 mL, 1.29 mmol) into a solution of 7-methyltryptamine **(144)** (0.23 g, 1.29 mmol) in CH_2Cl_2 (2 mL) as a white solid.

Yield:	0.28 g, 1.10 mmol (85%)
mp:	95 - 96 °C
IR (cm^{-1}):	3414, 3275, 2964, 1625, 1573, 1453, 1359, 1294, 1212, 1108, 1070.
^1H NMR (400 MHz, $CDCl_3$): δ	8.01 (bs, 1H, $N\underline{H}_{indole}$), 7.47 (d, 1H, J = 7.6 Hz, H-4), 7.05 (m, 3H, H-2, H-5, H-6), 5.50 (bs, 1H, $N\underline{H}_{amide}$), 3.62 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.98 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.51 (s, 3H, $C\underline{H}_3$), 2.01 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2$), 1.62 (m, 2H, $C\underline{H}_2CH_3$), 0.92 (t, 3H, J = 7.4 Hz, $CH_2C\underline{H}_3$).
^{13}C NMR (75 MHz, $CDCl_3$): δ	172.9, 136.0, 126.9, 122.7, 121.7, 120.4, 119.7, 116.5, 113.6, 39.6, 38.8, 25.5, 19.1, 16.6, 13.8.
EIMS (m/z):	244 (M^+, 5), 157 (M^+ - H - $NHCOC_3H_7$, 54), 144 (M^+ - $CH_2NHCOCH_3$, 33), 84 (89), 49 (100).

Calcd. for $C_{15}H_{21}N_2O$, (M^+ + H): 245.1646. Found: 245.1654.

$C_{15}H_{20}N_2O$ requires: C, 73.74; H, 8.25; N, 11.47. Found: C, 73.77; H, 8.28; N,11.06.

N-Cyclopropanecarbonyl-2-(7-methylindol-3-yl)ethanamine (159)



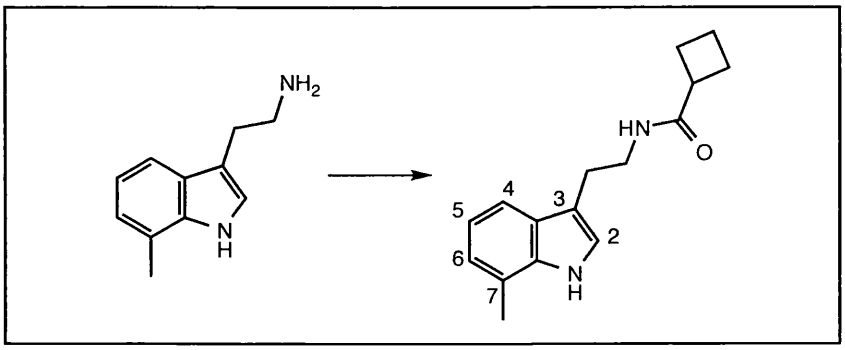
Compound **159** was prepared via method XI.D. by sequential addition of Et_3N (0.20 mL, 1.43 mmol) and cyclopropanecarbonyl chloride (0.14 g, 0.12 mL, 1.32 mmol) into a solution of 7-methyltryptamine **(144)** (0.23 g, 1.29 mmol) in CH_2Cl_2 (2.1 mL) as a light brown solid. Recrystallisation of the solid from $CHCl_3$/ petroleum ether gave **159** as a white crystalline solid.

Yield:	0.25 g, 1.03 mmol (78%)
mp:	133 - 134 °C
IR (cm^{-1}):	3373, 3250, 3008, 2935, 1659, 1536, 1452, 1451, 1381, 1349, 1239, 1133, 1103, 1058.
^1H NMR (400 MHz, $CDCl_3$): δ	8.00 (bs, 1H, N<u>H</u>$_{indole}$), 7.50 (d, 1H, J = 7.6 Hz, H-4), 7.06 (m, 3H, H-2, H-5, H-6), 5.70 (bs, 1H, N<u>H</u>$_{amide}$), 3.63 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.99 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.51 (s, 3H, $C\underline{H}_3$), 1.23 (m, 1H, C<u>H</u>C=O), 0.98 (m, 2H) 0.70 (m, 2H).
^{13}C NMR (75 MHz, $CDCl_3$): δ	173.5, 136.0, 126.9, 122.6, 121.8, 120.5, 119.6, 116.4, 113.4, 40.0, 25.5, 16.6, 14.8, 7.0.
MS (APCI) (m/z):	243 (M^+ + H, 100), 158 (M^+ - $NHCOC_3H_5$).

Calcd. for $C_{15}H_{19}N_2O$, (M^+ + H): 243.1487. Found: 243.1497.

$C_{15}H_{18}N_2O$ requires: C, 74.35; H, 7.49; N, 11.56. Found: C, 73.58; H, 7.37; N, 11.18.

N-Cyclobutanecarbonyl-2-(7-methylindol-3-yl)ethanamine (160)



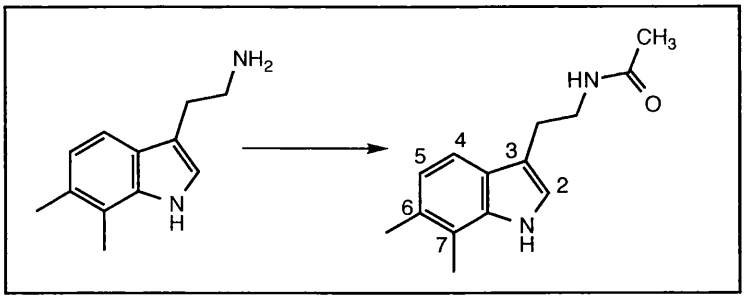
Compound **160** was prepared via method XI.D. by sequential addition of Et_3N (0.20 mL, 1.43 mmol) and cyclobutanecarbonyl chloride (0.16 g, 0.15 mL, 1.31 mmol) into a solution of 7-methyltryptamine **(144)** (0.23 g, 1.29 mmol) in CH_2Cl_2 (2.1 mL) as a beige crystalline solid.

Yield:	0.28 g, 1.09 mmol (85%)
mp:	117 - 118 °C
IR (cm^{-1}):	3412, 3295, 2937, 1623, 1548, 1442, 1348, 1262, 1218, 1070.
^1H NMR (400 MHz, $CDCl_3$): δ	8.06 (bs, 1H, N<u>H</u>$_{indole}$), 7.47 (d, 1H, J = 7.6 Hz, H-4), 7.05 (m, 3H, H-2, H-5, H-6), 5.70 (bs, 1H, N<u>H</u>$_{amide}$), 3.63 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.99 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.91 (m, 1H, C<u>H</u>C=O), 2.51 (s, 3H, C<u>H</u>$_3$), 2.24 (m, 2H), 2.10 (m, 2H), 1.91 (m, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	174.8, 136.0, 128.3, 126.9, 122.7, 121.7, 120.4, 119.7, 116.5, 113.7, 40.0, 39.6, 25.4, 18.1, 16.6.
MS (APCI) (m/z):	257 (M^+ + H, 100), 158 (M^+- $NHCOC_4H_7$, 44).

Calcd. for $C_{16}H_{21}N_2O$, (M^+ + H): 257.1647. Found: 257.1654.

$C_{16}H_{20}N_2O$ requires: C, 74.97; H, 7.86; N, 10.93. Found: C, 74.52; H, 7.83; N, 10.85.

N-Acetyl-2-(6,7-dimethylindol-3-yl)ethanamine (161)



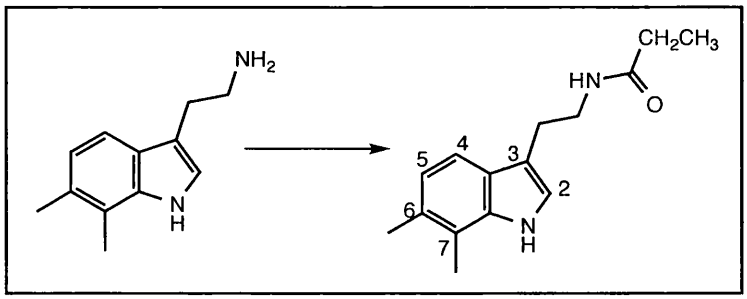
Compound **161** was prepared via method XI.D. by sequential addition of Et_3N (0.26 mL, 1.79 mmol) and acetyl chloride (0.14 g, 0.13 mL, 1.78 mmol) into a solution of 6,7-dimethyltryptamine **(145)** (0.34 g, 1.81 mmol) in CH_2Cl_2 (16 mL). Purification by column chromatography on silica with gradient elution (EtOAc : MeOH, 100:0 to 98:2) gave **161** as a white foam.

Yield:	0.27 g, 1.17 mmol (66%)
IR (cm^{-1}):	3478, 3337, 2992, 2931, 2866, 1661, 1522, 1440, 1368, 1275, 1231, 1089.
^1H NMR (400 MHz, $CDCl_3$): δ	8.27 (bs, 1H, N<u>H</u>$_{indole}$), 7.35 (d, 1H, J = 8.0 Hz, H-4), 6.98 (m, 2H, H-2, H-5), 5.60 (bs, 1H, N<u>H</u>$_{amide}$), 3.59 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.95 (t, 2H, J = 6.6 Hz, $C\underline{H}_2CH_2NH$), 2.41 (s, 3H, C<u>H</u>$_3$), 2.40 (s, 3H, C<u>H</u>$_3$), 1.92 (s, 3H, $COC\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	170.2, 136.6, 129.5, 125.2, 122.2, 121.4, 118.3, 115.6, 113.1, 39.8, 25.3, 23.3, 19.4, 13.0.
MS (FAB) (m/z):	231 (M^+ + H, 96), 230 (M^+, 87), 172 (M^+ - $NHCOCH_3$, 76), 171 (M^+ - H - $NHCOCH_3$, 100), 158 (M^+ - $CH_2NHCOCH_3$, 68), 144 (M^+ - $CH_2CH_2NHCOCH_3$, 12).

Calcd. for $C_{14}H_{19}N_2O$, (M^+ + H): 231.1486. Found: 231.1497.

$C_{14}H_{18}N_2O$ requires: C, 73.01; H, 7.88; N, 12.16. Found: C, 72.96; H, 7.92; N, 12.20.

N-Propanoyl-2-(6,7-dimethylindol-3-yl)ethanamine (162)



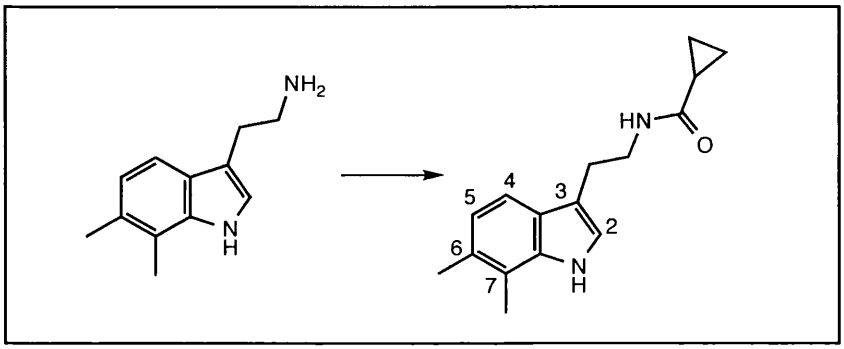
Compound **162** was prepared via method XI.D. by the sequential addition of Et_3N (0.25 mL, 1.82 mmol) and propionyl chloride (0.17 g, 0.16 mL, 1.83 mmol) into a solution of 6,7-dimethyltryptamine **(145)** (0.34 g, 1.81 mmol) in CH_2Cl_2 (10 mL). Purification by column chromatography on silica with gradient elution (EtOAc : MeOH, 100:0 to 98:2) gave **162** as a yellow oil.

Yield:	0.26 g, 1.06 mmol (59%)
mp:	101 - 102 °C
IR (cm^{-1}):	3412, 3303, 2931, 1634, 1562, 1443, 1344, 1235, 1218, 1169, 1107, 1060.
^1H NMR (300 MHz, $CDCl_3$): δ	8.01 (bs, 1H, N<u>H</u>$_{indole}$), 7.36 (d, 1H, J = 7.8 Hz, H-4), 6.98 (m, 2H, H-2, H-5), 5.53 (bs, 1H, N<u>H</u>$_{amide}$), 3.60 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.96 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.42 (s, 3H, $C\underline{H}_3$), 2.40 (s, 3H, $C\underline{H}_3$), 2.14 (q, 2H, J = 7.6 Hz, $C\underline{H}_2CH_3$), 1.15 (t, 3H, J = 7.6 Hz, $CH_2C\underline{H}_3$).
^{13}C NMR (75 MHz, $CDCl_3$): δ	173.7, 136.6, 129.6, 125.2, 122.3, 121.4, 118.3, 115.7, 113.3, 39.6, 29.8, 25.4, 19.2, 13.0, 9.8.
MS (FAB) (m/z):	245 (M^+ + H, 67), 244 (M^+, 56), 172 (M^+ - $NHCOCH_2CH_3$, 73), 171 (M^+ - H - $NHCOCH_2CH_3$, 100), 158 (M^+ - $CH_2NHCOCH_2CH_3$, 55), 144 (M^+ - $CH_2CH_2NHCOCH_2CH_3$, 12).

Calcd. for $C_{15}H_{21}N_2O$, (M^+ + H): 245.1646. Found: 245.1654.

$C_{15}H_{20}N_2O$ requires: C, 73.74; H, 8.25; N, 11.47. Found: C, 72.86; H, 7.98; N, 11.28.

N-Cyclopropanecarbonyl-2-(6,7-dimethylindol-3-yl)ethanamine (163)



Compound **163** was prepared via method XI.D. by sequential addition of Et_3N (0.25 mL, 1.79 mmol) and cyclopropanecarbonyl chloride (0.19 g, 0.17 mL, 1.87 mmol) into a solution of 6,7-dimethyltryptamine **(145)** (0.34 g, 1.81 mmol) in CH_2Cl_2 (10 mL). Purification by column chromatography on silica with gradient elution (Et_2O to EtOAc) gave **163** as a yellow oil which crystallised as a white solid from CH_2Cl_2/ hexane.

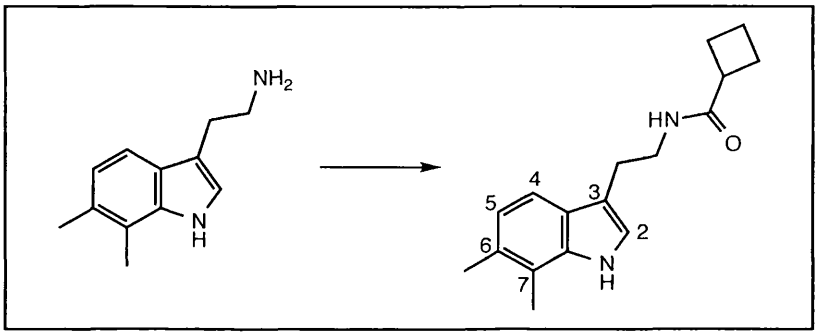
Yield:	0.24 g, 0.94 mmol (52%)
mp:	157 - 158 °C
IR (cm^{-1}):	3394, 3290, 3030, 2932, 2889, 2845, 1653, 1524, 1447, 1376, 1338, 1267, 1220, 1175, 1100, 1060.
^1H NMR (400 MHz, $CDCl_3$): δ	8.05 (bs, 1H, N<u>H</u>$_{indole}$), 7.35 (d, 1H, J = 8.0 Hz, H-4), 6.98 (m, 2H, H-2, H-5), 5.74 (bs, 1H, N<u>H</u>$_{amide}$), 3.60 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.95 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.41 (s, 3H, C<u>H</u>$_3$), 2.39 (s, 3H, C<u>H</u>$_3$), 1.27 (m, 1H, C<u>H</u>C=O), 0.98 (m, 2H), 0.70 (m, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	173.5, 136.6, 129.6, 125.2, 122.3, 121.4, 118.3, 115.8, 113.4, 39.94, 25.5, 19.2, 14.8, 13.0, 7.0.

MS (FAB) (m/z): 257 (M^++ H, 61), 256 (M^+, 55),
187 (M^+ - COC_3H_5, 11),
172 (M^+ - $NHCOC_3H_5$, 70),
171 (M^+ - H - $NHCOC_3H_5$, 100),
158 (M^+ - $CH_2NHCOC_3H_5$, 66),
144 (M^+ - $CH_2CH_2NHCOC_3H_5$, 12).

Calcd. for $C_{16}H_{21}N_2O$, (M^+ + H): 257.1648. Found: 257.1654.

$C_{16}H_{20}N_2O$ requires: C, 74.97; H, 7.86; N, 10.93. Found: C, 72.91; H, 7.73; N, 10.50.

N-Cyclobutanecarbonyl-2-(6,7-dimethylindol-3-yl)ethanamine (164)



Compound **164** was prepared via method XI.D. by the sequential addition of Et_3N (0.25 mL, 1.79 mmol) and cyclobutanecarbonyl chloride (0.19 g, 0.17 mL, 1.87 mmol) into a solution of 6,7-dimethyltryptamine **(145)** (0.34 g, 1.81 mmol) in CH_2Cl_2 (10 mL).

Yield: 0.22 g, 0.81 mmol (45%)

mp: 146 - 148 °C

IR (cm^{-1}): 3409, 3273, 3090, 2936, 2862, 1636, 1562, 1440, 1375, 1345, 1261, 1216, 1080.

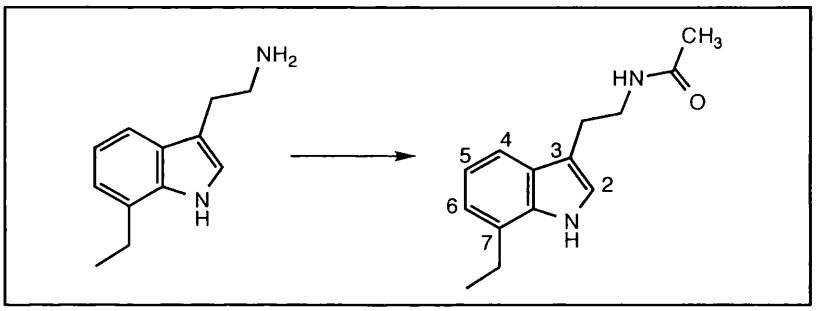
^1H NMR (300 MHz, $CDCl_3$): δ 8.12 (bs, 1H, N$\underline{H}_{indole}$),
7.36 (d, 1H, J = 8.0 Hz, H-4),
6.98 (m, 2H, H-2, H-5), 5.48 (bs, 1H, N$\underline{H}_{amide}$),
3.60 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$),
2.95 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$),
2.91 (m, 1H, C$\underline{H}$C=O), 2.41 (s, 3H, C$\underline{H}_3$),
2.39 (s, 3H, C$\underline{H}_3$), 2.24 (s, 3H, C$\underline{H}_3$),
2.20 (m, 2H), 2.09 (m, 2H), 1.91 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ	174.9, 156.2, 136.7, 129.6, 125.2, 122.3, 121.4, 118.3, 115.8, 113.4, 40.0, 39.6, 25.4, 19.3, 18.1, 13.0.
MS (FAB) (m/z):	271 (M^+ + H, 63), 270 (M^+, 41), 187 (M^+ - COC_4H_7, 13), 172 (M^+ - $NHCOC_4H_7$, 75), 171 (M^+ -H - $NHCOC_4H_7$, 100), 158 (M^+ - $CH_2NHCOC_4H_7$, 67), 144 (M^+ - $CH_2CH_2NHCOC_4H_7$, 13).

Calcd. for $C_{17}H_{23}N_2O$, (M^+ + H): 271.1816. Found: 271.1810.

$C_{17}H_{22}N_2O$ requires: C, 75.52; H, 8.20; N, 11.56. Found: C, 74.67; H, 8.40; N, 11.54.

N-Acetyl-2-(7-ethylindol-3-yl)ethanamine (165)



Compound **165** was prepared via method XI.D. by the sequential addition of Et_3N (0.13 mL, 0.93 mmol) and acetyl chloride (0.08 g, 0.07 mL, 0.98 mmol) into a solution of 7-ethyltryptamine **(146)** (0.18 g, 0.94 mmol) in CH_2Cl_2 (4 mL). Purification by column chromatography on silica with hexane : EtOAc, 1:3 gave **165** as a yellow oil.

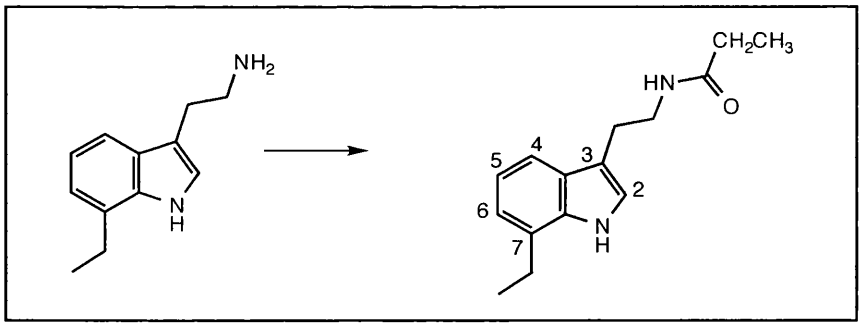
Yield:	0.18 g, 0.78 mmol (83%)
IR (cm^{-1}, $CHCl_3$):	3480, 3020, 1662, 1521, 1435, 1367, 1230, 1207, 1085.

^{1}H NMR (400 MHz, $CDCl_3$): δ	8.64 (bs, 1H, N<u>H</u>$_{indole}$), 7.47 (d, 1H, J = 7.5 Hz, H-4), 7.10 (m, 2H, H-5, H-6), 7.01 (d, 1H, J = 2.3 Hz, H-2), 5.85 (bs, 1H, N<u>H</u>$_{amide}$), 3.61 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 2.98 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.88 (q, 2H, J = 7.6 Hz, $C\underline{H}_2CH_3$), 1.93 (s, 3H, $COC\underline{H}_3$), 1.39 (t, J = 7.6 Hz, 3H, $C\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	170.2, 135.2, 127.0, 126.8, 121.8, 120.5, 119.6, 116.2, 112.9, 39.8, 25.3, 23.9, 23.2, 13.8.
EIMS (m/z):	230 (M^+, 14), 171 (M^+ - H - $NHCOCH_3$, 96), 158 (M^+ - $CH_2NHCOCH_3$, 100).

Calcd. for $C_{14}H_{19}N_2O$, (M^+ + H): 231.1486. Found: 231.1497.

$C_{14}H_{18}N_2O$ requires: C, 73.01; H, 7.88; N, 12.16. Found: C, 71.73; H, 7.91; N, 11.15.

N-Propanoyl-2-(7-ethylindol-3-yl)ethanamine (166)



Compound **166** was prepared via method XI.D. by the sequential addition of Et_3N (0.13 mL, 1.94 mmol) and propionyl chloride (0.08 g, 0.08 mL, 0.92 mmol) into a solution of 7-ethyltryptamine **(146)** (0.17 g, 0.90 mmol) in CH_2Cl_2 (4 mL). Purification by column chromatography on silica with Et_2O gave **166** as a white crystalline solid.

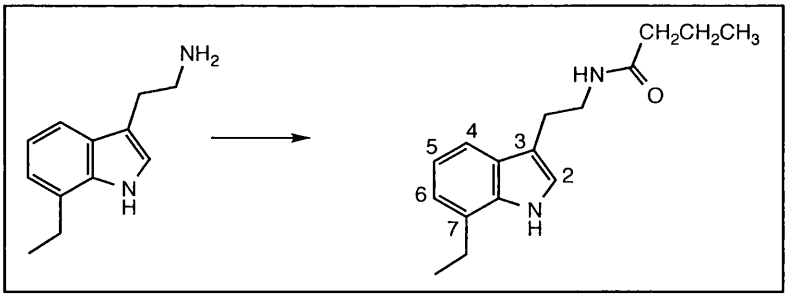
Yield:	0.12 g, 0.49 mmol (55%)
mp:	88 - 89 °C
IR (cm^{-1}):	3381, 3261, 2965, 2932, 1665, 1527, 1455, 1361, 1244, 1230, 1131, 1105.

^{1}H NMR (400 MHz, $CDCl_3$): δ	8.34 (bs, 1H, N<u>H</u>$_{indole}$), 7.48 (d, 1H, J = 7.6 Hz, H-4), 7.09 (m, 2H, H-5, H-6), 7.04 (d, 1H, J = 2.1 Hz, H-2), 5.61 (bs, 1H, N<u>H</u>$_{amide}$), 3.64 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.99 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.89 (q, 2H, J = 7.6 Hz, $COC\underline{H}_2CH_3$), 2.16 (q, 2H, J = 7.6 Hz, $C\underline{H}_2CH_3$), 1.38 (t, 3H, J = 7.6 Hz, $COCH_2C\underline{H}_3$), 1.13 (t, 3H, J = 7.6 Hz, $CH_2C\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	173.7, 135.3, 127.1, 126.7, 121.7, 120.7, 119.8, 116.4, 113.5, 39.7, 29.8, 25.4, 24.0 13.8, 9.8.
EIMS (m/z):	244 (M^+, 14), 171 (M^+ - H - $NHCOCH_3$, 100), 158 (M^+ - $CH_2NHCOC_2H_5$, 56).

Calcd. for $C_{15}H_{21}N_2O$, (M^+ + H): 245.1646. Found: 245.1654.

$C_{15}H_{20}N_2O$ requires: C, 73.74; H, 8.25; N, 11.47. Found: C, 73.57; H, 8.05; N, 11.33.

N-butyryl-2-(7-ethylindol-3-yl)ethanamine (167)



Compound **167** was prepared via method XI.D. by the sequential addition of Et_3N (0.19 mL, 0.14 mmol) and butylryl chloride (0.08 g, 0.08 mL, 0.92 mmol) into a solution of 7-ethyltryptamine **(146)** (0.17 g, 0.90 mmol) in CH_2Cl_2 (4 mL). Purification by column chromatography on silica with CH_2Cl_2 : Et_2O, 95:5 gave **167** as a white crystalline solid.

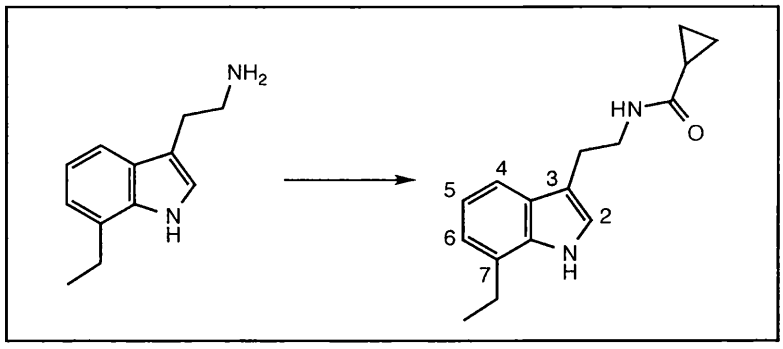
Yield:	0.18 g, 0.71 mmol (51%)
mp:	125 - 126 °C
IR (cm^{-1}):	3275, 2961, 2932, 1628, 1572, 1455, 1364, 1289, 1215, 1107.

^{1}H NMR (400 MHz, $CDCl_3$): δ	8.19 (bs, 1H, N$\underline{H}_{indole}$), 7.49 (d, 1H, J = 7.6 Hz, H-4), 7.08 (m, 2H, H-5, H-6), 7.04 (d, 1H, J = 2.1 Hz, H-2), 5.54 (bs, 1H, N$\underline{H}_{amide}$), 3.62 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.99 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.89 (q, 2H, J = 7.5 Hz, $C\underline{H}_2CH_3$), 2.10 (t, 2H, J = 7.6 Hz, $C\underline{H}_2CH_2CH_3$), 1.64 (m, 2H, $CH_2C\underline{H}_2CH_3$), 1.38 (t, 3H, J = 7.6 Hz, $CH_2C\underline{H}_3$), 0.92 (t, 3H, J = 7.5 Hz, $CH_2CH_2C\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	172.9, 135.3, 127.1, 126.7, 121.6, 120.8, 119.8, 116.5, 113.6, 39.6, 38.8, 30.9, 25.4, 24.0, 19.1, 13.8.
MS (FAB) (m/z):	259 (M^+ + H, 68), 258 (M^+, 41), 187 (M^+ - COC_3H_7, 12), 172 (M^+- $NHCOC_3H_7$, 57), 171 (M^+ - H - $NHCOC_3H_7$, 100).

Calcd. for $C_{16}H_{23}N_2O$, (M^+ + H): 259.1805. Found: 259.1810.

$C_{16}H_{22}N_2O$ requires: C, 74.38; H, 8.58; N, 10.84. Found: C, 73.52; H, 8.34; N,10.72.

N-Cyclopropanecarbonyl-2-(7-ethylindol-3-yl)ethanamine (168)



Compound **168** was prepared via method XI.D. by the sequential addition of Et_3N (0.13 mL, 1.94 mmol) and cyclopropanecarbonyl chloride (0.08 g, 0.08 mL, 0.92 mmol) into a solution of 7-ethyltryptamine **(146)** (0.17 g, 0.90 mmol) in CH_2Cl_2 (4 mL). Purification by column chromatography on silica with Et_2O gave **168** as a white crystalline solid.

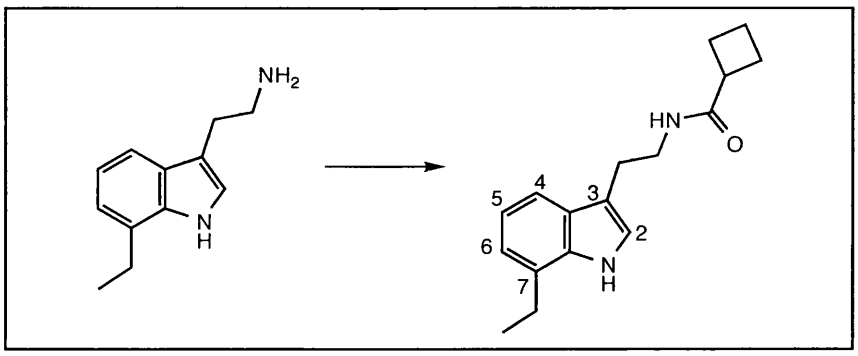
Yield: 0.11 g, 0.43 mmol (48%)

mp:	128 - 129 °C
IR (cm^{-1}):	3379, 3262, 2957, 1657, 1533, 1448, 1376, 1237, 1104.
^1H NMR (400 MHz, $CDCl_3$): δ	8.19 (bs, 1H, $N\underline{H}_{indole}$), 7.50 (d, 1H, J = 7.6 Hz, H-4), 7.10 (m, 2H, H-5, H-6), 7.04 (d, 1H, J = 2.3 Hz, H-2), 5.74 (bs, 1H, $N\underline{H}_{amide}$), 3.64 (q, 2H, J = 6.5 Hz, $CH_2C\underline{H}_2NH$), 3.00 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.88 (q, 2H, J = 7.6 Hz, $C\underline{H}_2CH_3$), 1.40 (t, 3H, J = 7.6 Hz, $CH_2C\underline{H}_3$), 1.25 (m, 1H, $C\underline{H}C{=}O$), 0.98 (m, 2H), 0.70 (m, 2H).
^{13}C NMR (100 MHz, $CDCl_3$): δ	173.4, 135.2, 127.1, 126.7, 121.7, 120.7, 119.8, 116.5, 113.6, 39.9, 25.5, 24.0, 14.8, 13.8, 7.0.
EIMS (m/z):	256 (M^+, 2), 171 (M^+ - H - $NHCOC_3H_5$, 45), 158 (M^+ - $CH_2NHCOC_3H_5$, 26), 144 (M^+ - $CH_2CH_2NHCOC_3H_5$, 100).

Calcd. for $C_{16}H_{21}N_2O$, (M^+ + H): 257.1648. Found: 257.1654.

$C_{16}H_{20}N_2O$ requires: C, 74.97; H, 7.86; N, 10.93. Found: C, 73.94; H, 7.94; N, 10.96.

N-Cyclobutanecarbonyl-2-(7-ethylindol-3-yl)ethanamine (169)



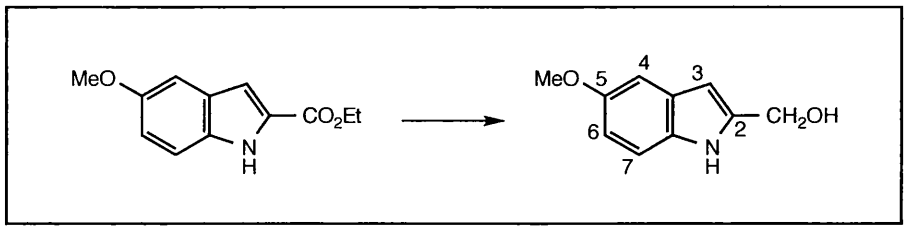
Compound **169** was prepared via method XI.D. by the sequential addition of Et_3N (0.19 mL, 1.36 mmol) and cyclobutanecarbonyl chloride (0.18 g, 0.17 mL, 1.51 mmol) into a solution of 7-ethyltryptamine **(146)** (0.26 g, 0.96 mmol) in CH_2Cl_2 (4 mL). Purification by column chromatography on silica with gradient elution (CH_2Cl_2 to Et_2O) gave **169** as a white crystalline solid.

Yield:	0.13 g, 0.48 mmol (34%)
mp:	93 - 94 °C
IR (cm^{-1}):	3380, 3266, 2956, 1657, 1527, 1452, 1432, 1367, 1254, 1226, 1132, 1105, 1082.
^1H NMR (400 MHz, $CDCl_3$): δ	8.18 (bs, 1H, N<u>H</u>indole), 7.48 (d, 1H, J = 7.6 Hz, H-4), 7.10 (m, 2H, H-5, H-6), 7.04 (d, 1H, J = 2.0 Hz, H-2), 5.46 (bs, 1H, N<u>H</u>amide), 3.61 (q, 2H, J = 6.4 Hz, $CH_2C\underline{H}_2NH$), 2.96 (t, 2H, J = 6.7 Hz, $C\underline{H}_2CH_2NH$), 2.89 (m, 3H, $C\underline{H}_2CH_3$, C<u>H</u>C=O), 2.26 (m, 2H), 2.10 (m, 2H), 1.90 (m, 2H), 1.40 (t, 3H, J = 7.6 Hz, $CH_2C\underline{H}_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	174.9, 135.2, 127.1, 126.7, 121.6, 120.7, 119.7, 116.5, 113.5, 40.0, 39.6, 25.5, 25.3, 24.0, 18.1, 13.8.
EIMS (m/z):	270 (M^+, 11), 172 (M^+ - $NHCOC_4H_7$, 100), 160 (51), 145 (M^+ - $CHCH_2NHCOC_3H_5$, 10).

Calcd. for $C_{17}H_{23}N_2O$, (M^+ + H): 271.1807. Found: 271.1810.

$C_{17}H_{23}N_2O$ requires: C, 75.52; H, 8.20; N, 10.36. Found: C, 74.81; H, 8.21; N, 10.22.

2-(Hydroxymethyl)-5-methoxyindole[249] (185)

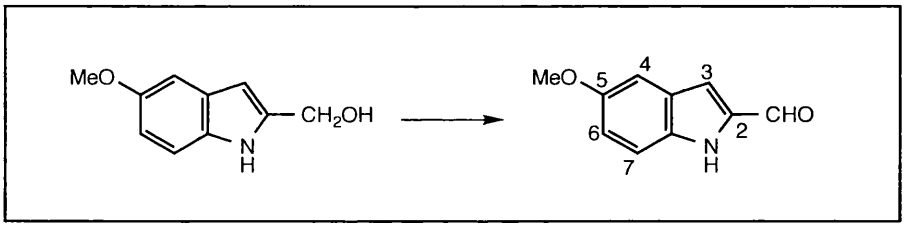


A solution of the commercially available 5-methoxy-indole-2-carboxylic acid ethyl ester (2.0 g, 9.2 mmol) in THF (30 mL) was added dropwise over 15 min to a solution of $LiAlH_4$ (0.4 g, 10.5 mmol) in THF (20 mL) at 0 °C. The mixture was stirred for a further 45 min at room temperature and quenched by adding sat. NH_4Cl (5 mL) followed by H_2O (10 mL). A precipitate formed which was removed by filtration and washed with THF (2 x 10 mL). The combined filtrate was extracted with Et_2O (4 x 10

mL) and dried ($MgSO_4$). Solvent evaporation yielded **185** as a white crystalline solid.

Yield:	1.41 g, 7.96 mmol (86%)
mp:	82 - 83 °C (lit.[249] 83 °C)
IR (cm^{-1}):	3353, 3198, 2933, 1622, 1586, 1489, 1449, 1326, 1292, 1226, 1199, 1166, 1128.
^1H NMR (400 MHz, $CDCl_3$): δ	8.22 (bs, 1H, NH), 7.24 (d, 1H, J = 8.7 Hz, H-7), 7.05 (d, 1H, J = 2.4 Hz, H-4), 6.85 (dd, 1H, J = 8.8, 2.5 Hz, H-6), 6.35 (s, 1H, H-3), 4.83 (d, J = 5.9 Hz, 2H, CH_2), 3.85 (s, 3H, OCH_3), 1.76 (t, 1H, OH).
^{13}C NMR (100 MHz, $CDCl_3$): δ	154.2, 138.3, 131.5, 128.6, 112.3, 111.6, 102.3, 100.3, 58.8, 55.8.

5-Methoxyindole-2-carbaldehyde[250] (186)



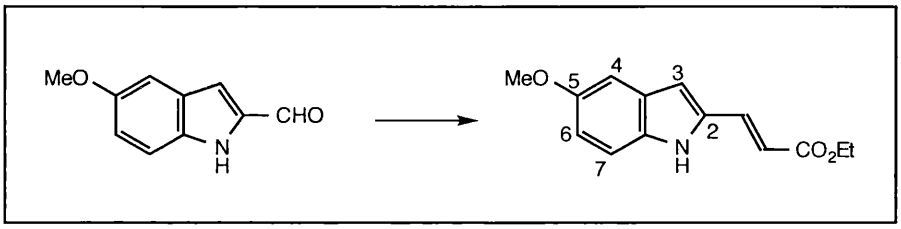
A solution of 2-(hydroxymethyl)-5-methoxyindole **(185)** (1.40 g, 7.9 mmol) in CH_2Cl_2 (60 mL) was added dropwise to a stirred solution of MnO_2/C (50% w/w, 6.32 g, 36.3 mmol) in CH_2Cl_2 (30 mL). The resultant mixture was stirred rapidly at 35 °C for a further 10 h and filtered. The filter cake was washed with hot acetone (4 x 50 mL) and the combined filtrate concentrated under reduced pressure yielding **186** as a yellow crystalline solid.

Yield:	1.10 g, 6.28 mmol (79%)
mp:	141 - 143 °C (lit.[250] 140 - 141 °C)
IR (cm^{-1}):	3202, 2948, 2853, 1675, 1525, 1455, 1291, 1205, 1168, 1119, 1025.

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.82 (s, 1H, C<u>H</u>O), 9.06 (bs, 1H, N<u>H</u>), 7.36 (d, 1H, J = 8.9 Hz, H-7), 7.20 (s, 1H, H-2), 7.12 (d, 1H, J = 2.5 Hz, H-4), 7.09 (dd, 1H, J = 8.9, 2.5 Hz, H-6), 3.87 (s, 3H, OC<u>H</u>$_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ 181.8, 154.9, 138.3, 133.4, 127.7, 119.4, 114.1, 113.4, 102.8, 55.7.

Ethyl (E)-(5-methoxy-indol-2-yl)-2-propenoate (188)



A solution of (carbethoxymethylene)triphenyl phosphorane (1.20 g, 3.44 mmol) in THF (36 mL) was added in one portion to a stirred solution of 5-methoxy-indole-2-carbaldehyde **(186)** (0.60 g, 3.42 mmol) in THF (36 mL). The mixture was stirred at room temperature for a further 10 min before heating to 60 °C for 24 h. It was then allowed to cool to room temperature and washed with H_2O (3 x 50 mL) and dried ($MgSO_4$). Purification by column chromatography on silica eluting with CH_2Cl_2 : Et_2O, 4:1 gave **188** as a white solid.

Yield: 0.77 g, 3.14 mmol (92%)

mp: 134 - 135 °C

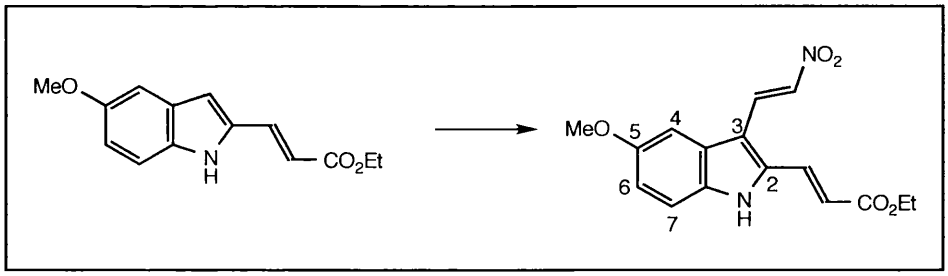
IR (cm^{-1}): 3337, 2952, 1685, 1618, 1521, 1454, 1374, 1281, 1235, 1202, 1171, 1138, 1034.

^{1}H NMR (400 MHz, $CDCl_3$): δ	8.50 (bs, 1H, NH), 7.67 (d, 1H, J = 16.1 Hz, CHCO$_2$Et), 7.25 (d, 1H, J = 8.8 Hz, H-7), 7.05 (d, 1H, J = 2.5 Hz, H-4), 6.94 (dd, 1H, J = 8.8, 2.4 Hz, H-6), 6.75 (s, 1H, H-2), 6.25 (d, 1H, J = 16.0 Hz, CH=CHCO$_2$Et), 4.30 (q, J = 7.2 Hz, 2H, CH$_2$), 3.86 (s, 3H, OCH$_3$), 1.36 (t, J = 7.1 Hz, 3H, CH$_3$).
^{13}C NMR (100 MHz, $CDCl_3$): δ	167.2, 154.5, 134.5, 133.9, 128.8, 115.6,115.1, 113.1, 112.0, 108.3, 102.1, 60.6, 55.7, 14.3.
MS (FAB) (m/z):	246 (M^+ + H, 48), 245 (M^+, 100), 200 (M^+ - OEt, 42).

Calcd. for $C_{14}H_{15}NO_3$, (M^+): 245.1053. Found: 245.1052.

$C_{14}H_{15}NO_3$ requires: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.10; H, 6.05; N, 5.64.

Ethyl 3-[2-(5-Methoxy-3-(2-nitrovinyl)-indol-yl)-(E)-propenoate (189)



Compound **189** was prepared via method XI.D. by adding sequentially a solution of TFA (1.5 mL) in CH_2Cl_2 (4.4 mL) followed by a solution of ethyl (E)-(5-methoxy-indol-2-yl)-2-propenoate **(188)** (0.60 g, 2.45 mmol) in CH_2Cl_2 (3.3 mL) into a mixture of dimethylamino-2-nitroethylene (0.29 g, 2.47 mmol) in CH_2Cl_2 (5.0 mL). Solvent evaporation gave **189** as a red crystalline solid.

Yield:	0.74 g, 2.34 mmol (96%)
mp:	219 - 220 °C
IR (cm^{-1}):	3314, 1704, 1605, 1573, 1496, 1465, 1305, 1254, 1227, 1180, 1104, 1037.

^{1}H NMR (400 MHz, $CDCl_3$): δ
8.78 (bs, 1H, NH),
8.45 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.92 (d, 1H, J = 16.0 Hz, CHCO$_2$Et),
7.80 (d, 1H, J = 13.4 Hz, CH=CHNO$_2$),
7.36 (d, 1H, J = 9.0 Hz, H-7),
7.15 (s, 1H, H-4),
7.07 (dd, 1H, J = 8.8, 2.4 Hz, H-6),
6.38 (d, 1H, J = 16.0 Hz, CH=CHCO$_2$Et),
4.35 (q, J = 7.1 Hz, 2H, CH$_2$),
3.93 (s, 3H, OCH$_3$),
1.39 (t, J = 7.1 Hz, 3H, CH$_3$).

^{1}H NMR (400 MHz, d_6-acetone): δ
8.50 (d, 1H, J = 13.5 Hz, CH=CHNO$_2$),
7.99 (d, 1H, J = 16.0 Hz, CHCO$_2$Et),
7.98 (d, 1H, J = 13.3 Hz, CH=CHNO$_2$),
7.44 (d, 1H, J = 9.0 Hz, H-7),
7.41 (d, 1H, J = 2.0, H-4),
7.02 (d, 1H, J = 8.8, 2.5 Hz, H-6),
6.71 (d, 1H, J = 16.0 Hz, CH=CHCO$_2$Et),
4.26 (q, J = 7.1 Hz, 2H, CH$_2$),
3.92 (s, 3H, OCH$_3$),
1.31 (t, J = 7.1 Hz, 3H, CH$_3$).

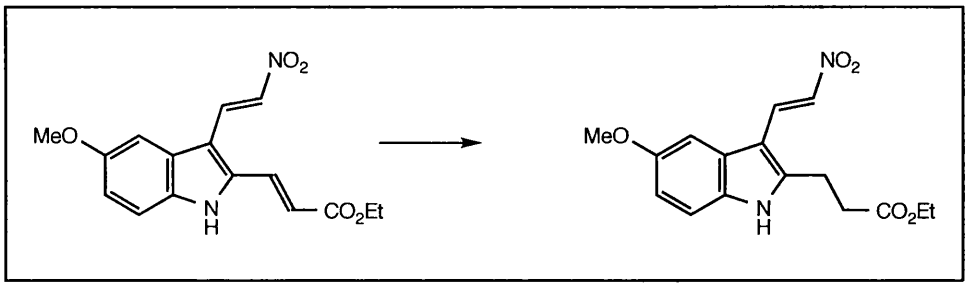
^{13}C NMR (100 MHz, d_6-acetone): δ
166.4, 157.4, 134.7, 133.8, 132.7, 131.3, 130.0, 126.9, 121.1, 117.2, 114.1, 113.9, 103.5, 61.3, 56.1, 14.5.

MS (FAB) (m/z):
317 (M^+ + H, 100), 316 (M^+, 99),
271 (M^+ - H - NO_2, 47),
258 (M^+ - H - $CHNO_2$, 40).

Calcd. for $C_{16}H_{17}N_2O_5$, (M^+ + H): 317.1124. Found: 317.1137.

$C_{16}H_{16}N_2O_5$ requires: C, 60.75; H, 5.10; N, 8.86. Found: C, 60.52; H, 5.10; N, 8.82.

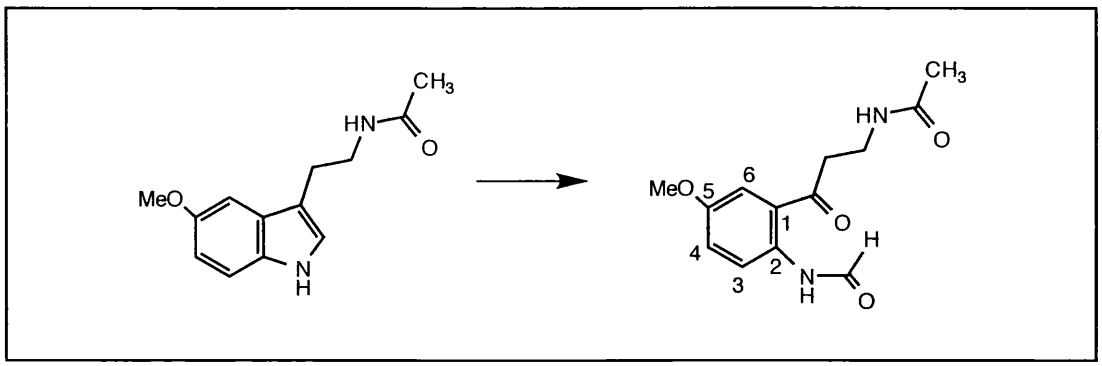
Ethyl 3-[2-(5-Methoxy-3-(2-nitrovinyl)-indol-yl]-propanoate (190)



Tris(triphenylphosphine) rhodium(I) chloride (0.005 g) was added to a solution of ethyl 3-[2-(5-methoxy-3-(2-nitrovinyl)-indol-yl)-(E)-propenoate **(189)** (0.050 g, 0.15 mmol) in EtOH (5 mL) under nitrogen. The mixture was then stirred under H_2 at 50 °C for 24 h. The catalyst was removed by column filtration on silica, eluting with Et_2O, yielding compound **190** as a dark orange solid after evaporation of the solvent.

Yield:	0.013 g, , 0.04 mmol (27%)
mp:	decomp. 260 - 270 °C
IR (cm^{-1}):	3307, 2930, 1727, 1605, 1466, 1375, 1309 1228, 1183, 1134, 1033.
^1H NMR (400 MHz, $CDCl_3$): δ	9.63 (bs, 1H, NH), 8.33 (d, 1H, J = 13.2 Hz, CH=C<u>H</u>NO_2), 7.76 (d, 1H, J = 13.2Hz, C<u>H</u>=CHNO_2), 7.30 (d, 1H, J = 8.7 Hz, H-7), 7.13 (d, 1H, J = 2.0, H-4), 6.94 (dd, 1H, J = 8.9, 2.3 Hz, H-6), 4.22 (q, J = 7.2 Hz, 2H, C<u>H</u>$_2$), 3.24 (t, J = 5.9 Hz, 2H, C<u>H</u>$_2$$CO_2$Et), 2.78 (t, J = 5.9 Hz, 2H, C<u>H</u>$_2$$CH_2CO_2$Et), 1.29 (t, J = 7.1 Hz, 3H, C<u>H</u>$_3$).

N-Acetyl-N'-formyl-5-methoxy-kynurenamine (6)



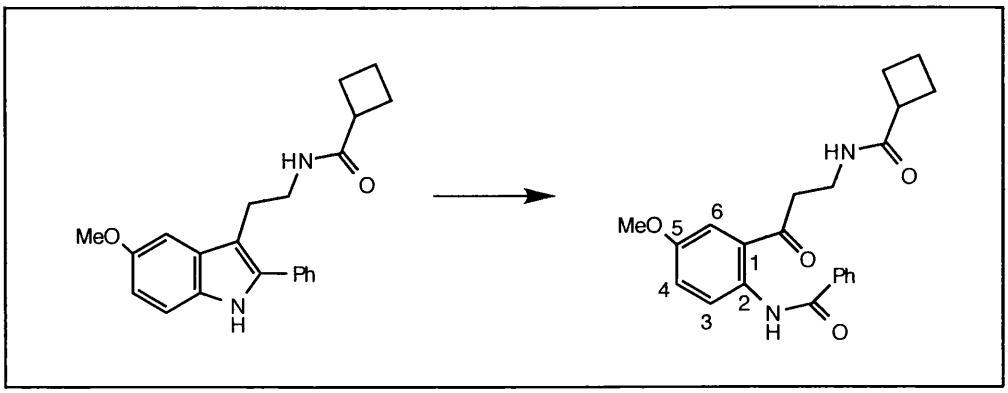
Compound **6** was prepared as a white solid from melatonin (0.25 g, 1.08 mmol) by method XI.E.

Yield:	0.085 g, 0.32 mmol (30%)
mp:	145 - 146 °C
IR (cm^{-1}):	3449, 3330, 1696, 1671, 1655, 1560, 1534, 1430, 1370, 1296, 1262, 1202, 1186, 1048.
^1H NMR (300 MHz, $CDCl_3$): δ	11.23 (bs, 1H, C<u>H</u>O), 8.69 (d, 1H, J = 9.2 Hz, H-6), 8.46 (d, 1H, J = 1.9 Hz, N<u>H</u>CHO), 7.39 (d, 1H, J = 2.9 Hz, H-3), 7.15 (dd, 1H, J = 9.2, 2.9 Hz, H-4), 6.05 (bs, 1H, N<u>H</u>ethanamide), 3.85 (s, 3H, OC<u>H</u>3), 3.66 (q, 2H, J = 5.8 Hz, $CH_2C\underline{H}_2NH$), 3.29 (t, 2H, J = 5.6 Hz, $C\underline{H}_2CH_2NH$), 1.98 (s, 3H, C<u>H</u>3).
^{13}C NMR (75 MHz, $CDCl_3$): δ	203.7, 170.2, 159.4, 154.9, 133.3, 123.2, 122.6, 120.9, 115.5, 55.7, 39.6, 34.4, 23.3.
EIMS (m/z):	264 (M^+, 48), 192 (M^+ - $CH_2NHCOCH_3$, 48), 177 (M^+ - $CH_2CH_2NHCOCH_3$, 72), 176 (100), 150 (M^+ - $COCH_2CH_2NHCOCH_3$, 44).

Calcd. for $C_{13}H_{17}N_2O_4$, (M^+): 265.1186. Found: 265.1188.

$C_{13}H_{17}N_2O_4$ requires: C, 59.08; H, 6.10; N, 10.60. Found: C, 60.52; H, 5.10; N, 8.82.

N-Cyclobutanecarbonyl-5-methoxy-N'-phenyl-kynurenamine (205)



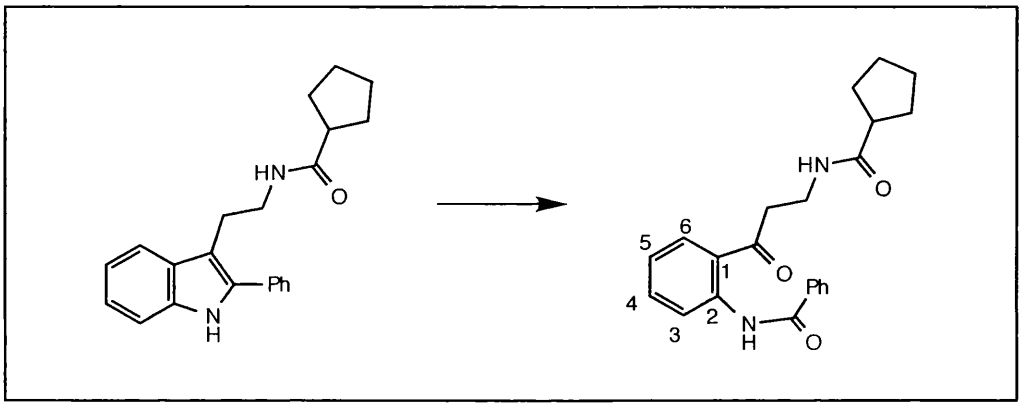
Compound **205** was prepared via method XI.E. as a white solid from N-cyclobutanecarbonyl-2-(5-methoxy-phenyl-indol-3-yl)ethanamine[242] **(203)** (0.0091 g, 0.026 mmol) in methanol (0.12 mL) and sodium metaperiodate (0.024 g, 0.11 mmol) in H_2O (0.22 mL). The mixture was stirred for 92 h and then extracted with EtOAc (4 x 3 mL). The combined organic extracts were washed with brine (10 mL) and dried ($MgSO_4$). Solvent evaporation in vacuo gave the impure kynurenamine **205** which was then purified by spinning plate chromatography eluting with EtOAc .

Yield:	0.0038 g, 0.010 mmol (38%)
mp:	150 - 151 °C
IR (cm^{-1}):	3287, 2960, 1676, 1642, 1612, 1586, 1534, 1451, 1302, 1204.
^1H NMR (300 MHz, $CDCl_3$): δ	12.28 (bs, 1H, N$\underline{H}_{benzamide}$), 8.91 (d, 1H, J = 9.4 Hz, H-6), 8.06 (dd, 2H, J = 7.7, 1.4 Hz, Ar$\underline{H}_{phenyl}$), 7.56 (m, 3H, Ar$\underline{H}_{phenyl}$), 7.44 (d, 1H, J = 2.9 Hz, H-3), 7.22 (dd, 1H, J = 9.2, 2.9 Hz, H-4), 6.05 (bs, 1H, N$\underline{H}_{ethanamide}$), 3.87 (s, 3H, OC$\underline{H}_3$), 3.69 (q, 2H, J = 5.8 Hz, $CH_2C\underline{H}_2NH$), 3.33 (t, 2H, J = 5.6 Hz, $C\underline{H}_2CH_2NH$), 2.97 (m, 1H, C$\underline{H}$C=O), 2.25 (m, 2H), 2.13 (m, 2H), 1.90 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$): δ	203.9, 182.8, 175.1, 154.6, 134.9, 131.9, 131.2, 129.0, 128.8, 127.4, 122.6, 121.2, 115.6, 112.5, 55.8, 39.9, 34.3, 25.3, 18.1.
EIMS (m/z):	380 (M^+, 27), 254 (M^+ - $CH_2CH_2NHCOC_4H_7$, 14), 226 (M^+ - $COCH_2CH_2NHCOC_4H_7$, 7), 105 (COPh, 71), 77 (Ph, 28), 28 (100).

Calcd. for $C_{22}H_{25}N_2O_4$, (M^+ + H): 381.1808. Found: 381.1814.

N-Cyclopentanecarbonyl-N'-phenyl-kynurenamine (206)



Compound **206** was prepared via method XI.E. as a white solid from N-cyclopentanecarbonyl-2-(phenyl-indol-3-yl)ethanamine[242] **(204)** (0.080 g, 0.24 mmol) in methanol (3 mL) and sodium metaperiodate (0.18 g, 0.84 mmol) in H_2O (1.6 mL). The mixture was stirred for 98 h and then extracted with EtOAc (4 x 5 mL). The combined organic extracts were washed with brine (10 mL) and dried ($MgSO_4$). Solvent evaporation in vacuo gave the impure kynurenamine **206**. Purification of the impure solid by column chromatography on silica with eluting with petroleum ether : EtOAc, 50:50 gave **206** as a white solid.

Yield:	0.083 g, 0.23 mmol (96%)
mp:	164 - 165 °C
IR (cm^{-1}):	3422, 3281, 2943, 1654, 1697, 1560, 1542, 1458, 1291, 1262, 1198, 1174.

^{1}H NMR (300 MHz, $CDCl_3$): δ

12.60 (bs, 1H, N$\underline{H}_{benzamide}$),
8.97 (d, 1H, J = 8.5 Hz, H-6),
8.07 (dd, 2H, J = 7.5, 1.4 Hz, Ar$\underline{H}_{phenyl}$),
7.97 (d, 1H, J = 8.0 Hz, H-3),
7.60 (m, 4H, Ar$\underline{H}_{phenyl}$,H-4),
7.15 (t, 1H, J = 7.2 Hz, H-5),
6.19 (bs, 1H, N$\underline{H}_{ethanamide}$),
3.69 (q, 2H, J = 5.8 Hz, $CH_2C\underline{H}_2NH$),
3.33 (t, 2H, J = 8.1 Hz, $C\underline{H}_2CH_2NH$),
2.49 (quin, 1H, J = 7.9 Hz, C$\underline{H}$C=O),
1.73 (m, 8H, $C\underline{H}_{2cyclopentane}$).

^{13}C NMR (75 MHz, $CDCl_3$): δ

204.2, 176.4, 166.0, 141.3, 135.5, 134.7, 132.0, 131.1, 128.8, 127.4, 122.6, 121.5, 120.8, 45.7, 39.7, 34.4, 30.4, 25.8.

EIMS (m/z):

364 (M^+, 5), 267 (M^+ - COC_5H_9, 11),
251 (M^+ - H - COC_5H_9, 6),
238 (M^+ - $CH_2COC_5H_9$, 3),
224 (M^+ - $CH_2CH_2COC_5H_9$, 34),
196 (M^+ - $COCH_2CH_2COC_5H_9$, 13),
146 (27), 105 (100).

Calcd. for $C_{22}H_{25}N_2O_3$, (M^+ + H): 365.1843. Found: 365.1865.

$C_{22}H_{24}N_2O_3$ requires: C, 72.50; H, 6.64; N, 7.69. Found: C, 71.76; H, 6.28; N, 7.10.

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ABBREVIATIONS

AMMTC	N-Acetyl-4-aminomethyl-6-methoxy-9-methyl-1,2,3,4-tetrahydrocarbazole
2-Br-MCA-NAT	2-Bromo-methoxy-carbonylamino-N-acetyltryptamine
GR128107	3-(1-Acetyl-3-methyl-piperidine)-5-methoxyindole
GR135531	5-Methoxy-carbonylamino-N-acetyltryptamine
GR196429	N-[2-[2,3,7,8,-Tetrahydro1H-furo(2,3-g)indol-1-yl]ethyl]acetamide
8-M-ADOT	8-Methyl-2-acetamidotetralin
8-M-CADOT	8-Methyl-2-carboxamidotetralin
5-MCA-NAT	5-Methoxy-carbonylamino-N-acetyltryptamine
8-M-PDOT	4-Phenyl-2-propionamidotetralin
4-P-ADOT	4-Phenyl-2-acetamidotetralin
4-P-CADOT	4-Phenyl-2-carboxamidotetralin
4-P-PDOT	4-Phenyl-2-propionamidotetraline
S20098	N-[2-(7-methoxy-1-napthyl)-ethyl]acetamide

Table of the chemical name and IUPAC three-letters abbreviation of the common amino acids.

A = Ala = Alanine	G = Gly = Glycine	P = Proline
R = Arg = Arginine	H = His = Histidine	S = Ser = Serine
N =Asn = Asparagine	L = Leu = Leucine	T = Thr = Theonine
D = Asp = Aspartic acid	K = Lys = Lysine	W = Trp = Tryptophan
C = Cys = Cysteine	M = Met = Methionine	Y = Tyr = Tyrosine
E = Glu = Glutamic acid	F = Phe = Phenylalanine	V = Val = Valine
Q = Gln = Glutamine		

APPENDIX

Melatonin receptor classification:

Receptor subtype	mt_1
Previous name(s)	Mel_{1a}, MEL_{1A}, ML_{1A}
Structural information	7 transmembranes[1-3]
Functional assays	potentiation of vasoconstriction of rat caudal artery[4,5]
	inhibition of forskolin-stimulated cAMP from sheep pars tuberalis cells[6]
	inhibition of neuronal firing in mouse suprachiasmatic nucleus slice[7]
Selective agonists	none
Agonists potency ratios	iodomelatonin (0.14) > (-)AMMTC (0.43) ≥ melatoinin (1.0) >> 6-hydroxymelatonin (26) > (+)AMMTC (229) > N-acetylserotonin (1450)[4,5]
Selective antagonists	none
Antagonist potencies	luzindole (pA_2 6.4 - 6.9 human recombinant mt_1 receptor[4,8] and rat caudal artery constriction[9]
Radioligands	2-[^{125}I]-iodomelatonin; [^{3}H]-melatonin (both are non-selective)[10,11]
Radioligand assays	recombinant sheep, human, mouse, hamster receptors; sheep pars tuberalis[1-3,12]

Ligand affinities	2-[^{125}I]-iodomelatonin (K_d = 26 - 55.6 pM) [^{3}H]-melatonin (K_d = 129 pM) 2-iodomelatonin > S20098 = melatonin ≥ 6-chloro-2-methylmelatonin ≥ GR196429 ≥ 6,7-dichloro-2-methylmelatonin ≥ 8-M-CADOT> 6-chloromelatonin ≥ 5-methoxyluzindole ≥ 6-hydroxymelatonin >> 8-M-ADOT > 8-M-PDOT > GR128107 > luzindole > N-acetyltryptamine ≥ 6-methoxymelatonin ≥ N-acetylserotonin ≥ 4-P-CADOT ≥ 4-P-PDOT > 5-MCA-NAT > 5-methoxytryptophol >> 5-methoxytryptamine[10,11,13]
Transduction mechanism(s)	inhibition of cAMP through recombinant receptor activation[2,3,14-16]
Receptor distribution	suprachiasmatic nucleus, pars tuberalis, paraventricular thalamic nucleus (human, rat, mouse, hamster, sheep), cerebellum (human) (in situ hybridisation);[1-3,17,18] hypothalamus (human), kidney (human, rat, guinea-pig), intestinal epithelium (guinea-pig) (immunocytochemistry);[19] occipital cortex, parietal cortex, temporal cortex, thalamus, frontal cortex, hippocampus (human) (quantitative polymerase chain reaction).[20]
Tissue function(s)	not established
Comments	There are no selective radioligands, or a radioligand binding or functional assay in native tissue in which mt_1 receptor protein has been characterised. Relative binding affinities differ depending on the ligand and assay used.[10,11,21]

Receptor subtype	MT_2
Previous name(s)	Mel_{1b}, MEL_{1B}, ML_{1B}
Structural information	7 transmembranes[22]
Functional assays	inhibition of dopamine release from rabbit retina[10,23,24]
Selective agonists	none
Agonists potency ratios	2-iodomelatonin (0.73) ≥ S20098 (0.38) ≥ melatoinin (0.95) ≥ 8-M-PDOT (2.2) ≥ 6-chloromelatonin (2.4) > 6,7-dichloro-2-methylmelatonin (2.8) > 6-chloro-2-methylmelatonin (8.9) ≥ 8-M-CADOT > 6-hydroxymelatonin >> N-acetylserotonin (738)[10,23,24]
Selective antagonists	4P-CADOT, 4-P-ADOT, 4-P-PDOT, GR128107, 5-methoxyluzindole (partial agonist)[10]
Antagonist potencies	4-P-ADOT (pA_2 8.8), 4-P-PDOT (9.5), GR128107 (10.2), 5-methoxyluzindole (10.2), N-acetyl-tryptamine[10]
Radioligands	2-[^{125}I]-iodomelatonin; [^{3}H]-melatonin (both are non-selective)[10]
Radioligand assays	human recombinant MT_2 receptor[10,22]
Ligand affinities	2-[^{125}I]-iodomelatonin (K_d = 160 pM)[22] [^{3}H]-melatonin (K_d = 275 pM)[11] S20098 ≥ melatonin ≥ 6-chloromelatonin ≥ 2-iodomelatonin ≥ 6,7-dichloro-2-methylmelatonin ≥ 5-methoxyluzindole ≥ GR196429 ≥ 8-M-CADOT ≥ GR128107 ≥ 4-P-CADOT ≥ 4P-ADOT ≥ 4P-PDOT ≥ 6-chloro-2-methylmelatonin ≥ 8-M-PDOT ≥ 6-methoxymelatonin ≥ 8-M-ADOT ≥ 6-hydroxymelatonin ≥ luzindole > N-acetyltryptamine > N-acetylserotonin > 5-methoxytryptamine > 5-MCA-NAT >> 5-methoxytryptophol[10,11,13]

Transduction mechanism(s)	inhibition of cAMP[22]
Receptor distribution	retina, hippocampus, brain (reverse transcription polymerase chain reaction),[1,12] suprachiasmatic nucleus[7,18]
Tissue function(s)	inhibition of dopamine release from retina,[10,25,26] phase shift of circadian rhythms[18]
Comments	There is no selective radioligand agonist or radioligand assay in a native tissue in which the MT_2 receptor protein has been characterised. Relative binding affinities differ depending on the ligand and assay used.[10,11,13]

Receptor subtype	MT_3
Previous name(s)	ML_2
Structural information	none
Functional assays	increase in PI turnover from hamster melanoma cells[27]
Selective agonists	GR135531[24,28]
Agonists potency ratios	N-acetylserotonin (0.64) > 6-chloromelatonin (0.84) ≥ 2-iodomelatonin (0.87) ≥ melatonin (1.0)[27]
Selective antagonists	prazosin[27-29]
Antagonist potencies	see comments
Radioligands	2-[^{125}I]-iodomelatonin (non-selective)[10] 2-[^{125}I]-MCA-NAT (selective)[28]
Radioligand assays	hamster brain, testes, kidney, melanoma cells[28-31]
Ligand affinities	2-[^{125}I]-iodomelatonin (K_d = 1.48 nM) 2-[^{125}I]-MCA-NAT (K_d = 116 pM)[28] 2-Br-MCA-NAT ≥ 6-chloro-2-methylmelatonin ≥ 2-iodomelatonin ≥ 6-chloromelatonin ≥ 6-methoxymelatonin > MCA-NAT = N-acetyl-serotonin > melatonin = 6,7-dichloro-2-methylmelatonin ≥ 6-hydroxymelatonin ≥ 5-methoxyluzindole ≥ 5-methoxytryptamine ≥ N-acetyltryptamine> luzindole[28,30,31]
Transduction mechanism(s)	increase in PI turnover in hamster melanoma cells[27]
Receptor distribution	hamster brain, kidney, testes; mouse brain (radioligand binding)[28,30,31]
Tissue function(s)	not established
Comments	This receptor has not been cloned. Antagonist potencies have not yet been established.

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