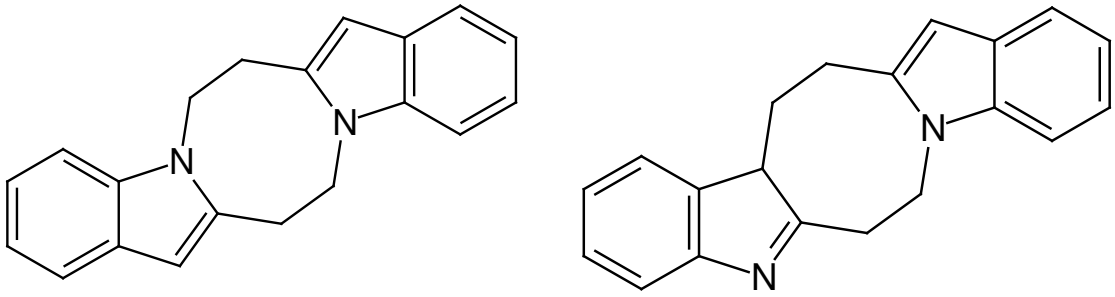
NOVEL HETEROCYCLIC RING SYSTEMS DERIVED FROM CARACURINE V AS LIGANDS FOR THE ALLOSTERIC SITE OF MUSCARINIC M_2 RECEPTORS



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For my parents

Table of Contents

1. Introduction

The cholinergic neuronal system is a part of the central nervous system (CNS) and peripheral nervous system (PNS) which consists of nerves outside the cerebrospinal axis, the somatic nerves and the autonomic nervous system. In this neuronal system, acetylcholine (ACh) serves as a neurotransmitter in all ganglia, the neuromuscular junction, and the postganglionic synapses. The actions of ACh are the result of activation of the cholinergic receptors which have been characterized as nicotinic (ionotropic family) and muscarinic (metabotropic family) on the basis of binding ability of the plant alkaloids nicotine and muscarine, respectively (Fig.1). These receptors are located in different areas, nicotinic receptors are found in all autonomic ganglia, adrenal medulla, causing release of adrenaline, and at neuromuscular endplate of striated muscle. The main location of muscarinic receptors are in postsynaptic cell membrane of smooth muscle, cardiac muscle and glandular tissue at the ends of parasympathetic nerves. Agonists and antagonists of cholinergic receptors can modify the output of neurotransmitters, including ACh. In the PNS, muscarinic receptor mediate smooth muscle contraction, glandular secretion, and modulation of cardiac rate and force. In the CNS, there is evidence that muscarinic receptors are involved in motor control, temperature regulation, cardiovascular regulation, and memory. Receptor subtypes that differ in location and specificity to agonists and antagonists have been identified for both nicotinic and muscarinic receptors.[1,2]

1.1 Muscarinic acetylcholine receptor

Muscarinic receptors belong to the large superfamily of plasma membrane-bound G protein-coupled receptors (GPCR), comprised seven α–helically arranged transmembrane domains (TM I-VII), connected by three extracellular (o) and three intracellular (i) loops. The protein sequence has an extracellular amino terminal end and an intracellular carboxy terminal end (Fig. 1). The α–helixes are arranged around a central pocket that serves as the point of entry for the agonist or antagonist and specific amino acid residues provide the groups for the drug receptor interactions. The coupling of muscarinic receptors to the pharmacological response is through the G protein primarily at the third intracellular loop (i_3) (Fig. 1).

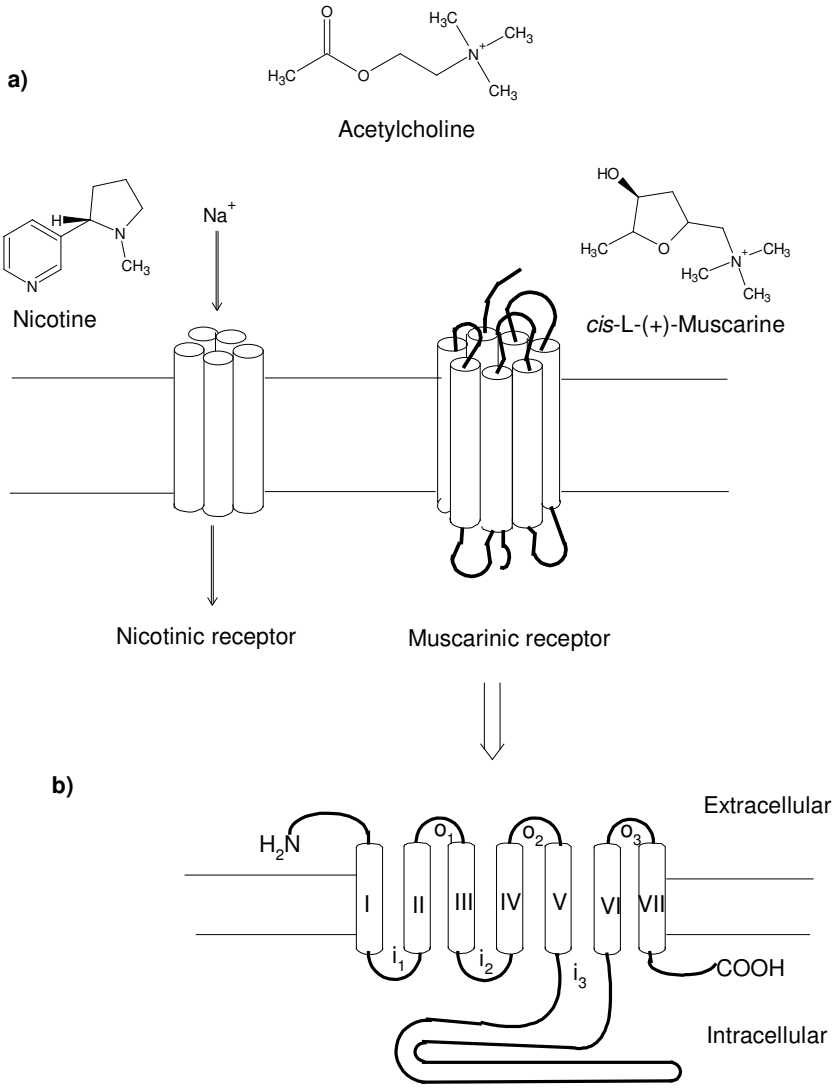


Figure 1. a) The model of the hypothetical arrangement of the transmembrane (TM) segments within the plane of membrane of nicotinic and muscarinic receptors, respectively; b) The model of seven TM of muscarinic receptor.

To date, five subtypes of muscarinic receptor have been cloned and sequenced, designated as m_1-m_5, which encode the corresponding muscarinic receptor. Structural and pharmacological criteria have suggested the presence of five subtypes, designated as M_1, M_2, M_3, M_4, and M_5.[3] The five muscarinic receptors can be classified into two biochemical classes based upon structural similarity and second messenger coupling. The M_1, M_3, and M_5 are members of the subclass that couple to the G_q subfamily which transmits the consequence signal by the β-type of phospholipase C. The M_2 and M_4 couple to inhibitory G-protein (G_i) subfamily which displays inhibitory effect on adenylate cyclase (Fig. 2). The agonists can activate receptor through G-protein stimulation which leads to the release of the second messenger.

Phospholipase C_β (PLC_β) activation releases the second messengers inosital triphosphate (IP_3) and diacylglycerol (DAG) of M_1, M_3, and M_5 receptors. IP_3 can release Ca^{2+} from the intracellular sarcoplasmic reticulum to initiate smooth muscle contraction and glandular secretion (by M_3 receptors). DAG stimulates protein kinase C which initiates phosphorylation of key proteins involved in muscle contraction and Ca^{2+} influx (Fig. 2). The M_2 and M_4 receptors inhibit adenylate cyclase activity reducing concentration of cAMP, which is a second messenger for a number of receptor types including β–adrenoceptors and histamine H_2 receptors. There is evidence that the M_2 receptor-mediated inhibition of voltage-gate calcium channels in the heart is the result of adenylate cyclase inhibition.[1,4]

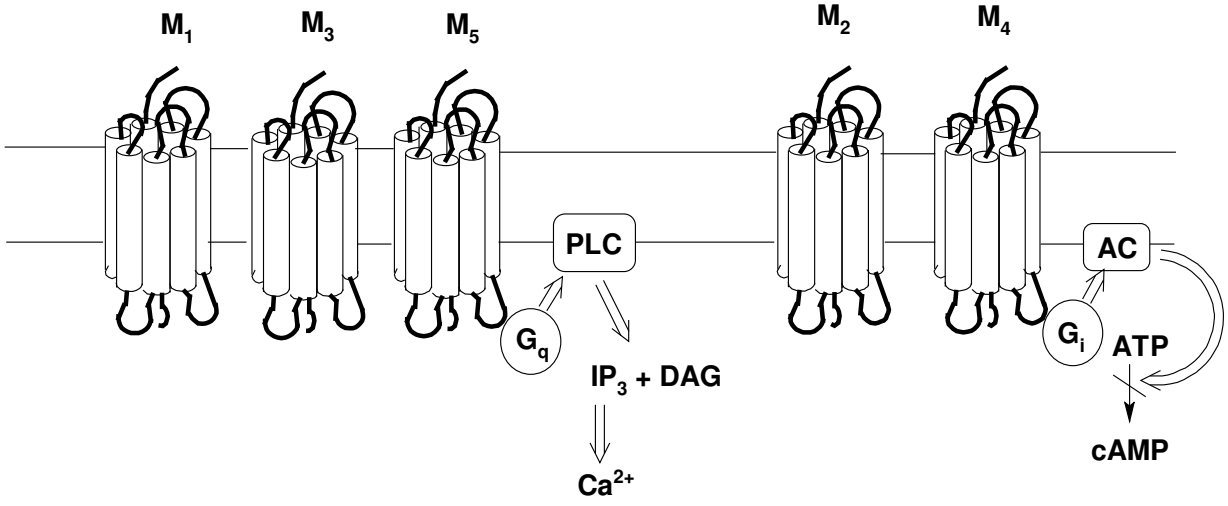


Figure 2. Signal transduction of muscarinic receptor subtypes.

The five subtypes of muscarinic receptors have a distinct regional distribution. In addition to the CNS, M_1 receptors are located in exocrine glands and seem to affect arousal attentions, rapid eye movement (REM) sleep, emotional responses, affective disorders including depression, and modulation of stress. They also participate in higher brain functions, such as memory and learning. M_2 receptors are called cardiac muscarinic receptors because they are located in the atria and conducting tissue of heart. M_3 receptors, referred to as glandular muscarinic receptors, located in exocrine glands and smooth muscle. Their effect on these organ systems is mostly stimulatory, e.g. glandular secretions from lacrimal, salivary, bronchial, pancreatic, and mucosal cells in the GI tract. There is an evidence that M_4 and M_5 receptors have been found in CNS.[2]

1.2 Allosteric Modulators

Due to the low selectivity of ligands (agonists or antagonists) that are active at five subtypes of muscarinic acetylcholine receptors, it is possible that selective compounds may be developed by targeting their allosteric site. The development of subtype-selective allosteric modulators may be a more promising prospect because the amino acid sequence is less conserved in the extracellular domains of the muscarinic subtypes where the allosteric modulators are presumed to bind to the receptor.[5] Another potential advantage of allosteric modulators is the potential for either increase or decrease of a particular subtype muscarinic effect by acetylcholine or the muscarinic agonists or antagonists.

1.2.1 Definition and functions

An allosteric modulator is defined as a compound that interacts with a second binding site (allosteric site) on a protein molecule to influence the affinity of classical orthosteric ligand for a topographically distinct site. The simplest model of describing this interaction is the allosteric ternary complex model[6,7] (Fig. 3), in which two ligands, Z and A, are capable of binding simultaneously to separate binding sites on the receptor, where K_A and K_Z are the affinity constants for the binding of the allosteric ligand A and the orthosteric ligand Z, respectively. The binding of one ligand to the receptor changes the affinity of the other ligand by a factor α, the cooperativity factor. If $\alpha < 1$, there is positive cooperativity, i.e., A and Z increase the binding of each other. In turn, if $\alpha > 1$, there is negative cooperativity, i.e., A and Z inhibit the binding of each other.

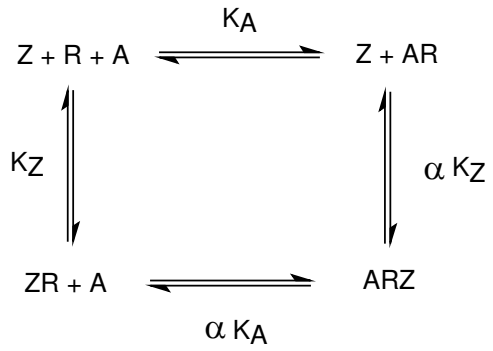


Figure 3. Ternary complex model of allosteric action of a classical orthosteric ligand Z with an allosteric modulator A at a receptor R.

The allosteric modulators can influence both the ligand association and dissociation resulting either in a reduction or in an elevation of ligand equilibrium binding. For example, in combination with antagonist such as atropine, the therapy of organophosphorus poisoning can take advantage of the retarding of the dissociation. The allosteric elevation of endogenous ACh binding might be beneficial in the treatment of pain and dementia.[8] Results of biochemical[9], mutagenesis[10-14], and chemical modification[15] studies suggest that allosteric modulators interact with a common allosteric site on the extracellular face[16,17,18], while the orthosteric binding site is located in a narrow cavity created by the seven transmembrane domains (TM) of muscarinic receptors.[19] Fig. 4 displays the binding model of the allosteric modulators and orthosteric ligand, *N*-methylscopolamine, at the muscarinic M_2 receptor.

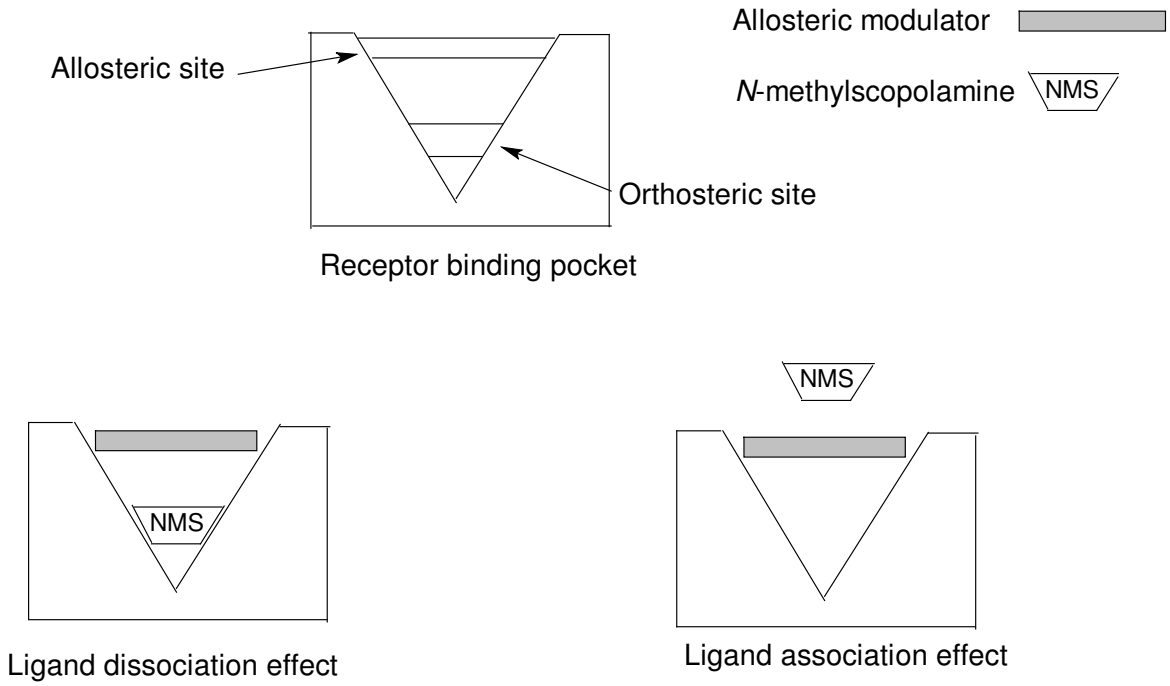
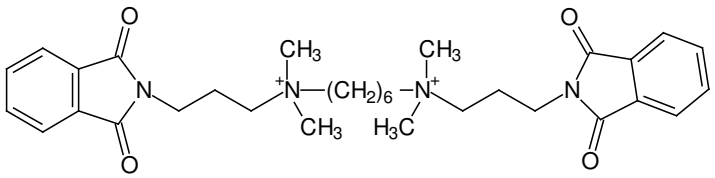


Figure 4. Sketch model of the receptor binding pocket of muscarinic M_2-receptors.

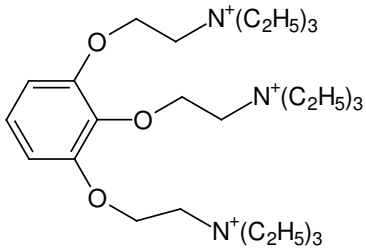
1.2.2 Classical allosteric modulators

The earliest evidence suggesting an allosteric binding site on muscarinic receptors was derived from functional studies on the M_2 receptor in guinea-pig isolated atria. *Lüllmann et al.*[20] observed in mice that combinations of atropine with alkane-bisammonium compounds such as W84, and C7/3′-phth induced an unexpected protection against organophosphate poisoning. W84 can antagonize the action of muscarinic agonist carbachol, in beating atria isolated from guinea pig hearts. By contrast to conventional antagonists, the shift of the

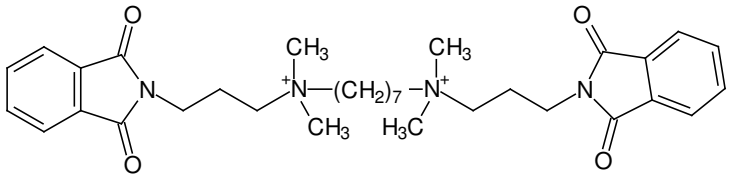
agonist curve did not steadily increase with increasing concentration. Furthermore, combinations of atropine and W84 had a over additive antimuscarinic action. *Clark* and *Mitchelson*[21] reported that the progressive increase in the degree of inhibition produced by increasing the concentration of gallamine, a neuromuscular blocking agent, was less than that expected from experiments with gallamine or atropine alone. These observations led to the conclusion that the action of gallamine is allosteric.



W84



Gallamine

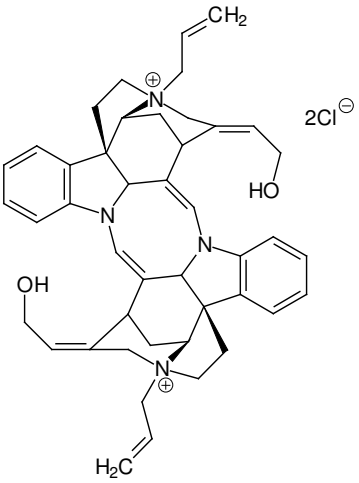


C7/3'-phth

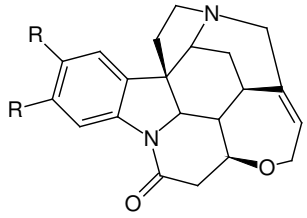
The effect of an allosteric action is indicated by the alteration of the dissociation characteristics of a ligand-receptor complex, which requires binding to a site apart from the ligand binding site. *Jepsen et al.*[22] reported the allosteric activity of W84, showing an inhibiting effect on [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) dissociation in guinea pig cardiac homogenates. *Stockton et al.*[23] demonstrated the allosteric interaction between gallamine and [^{3}H]NMS in equilibrium binding and dissociation experiment.

A number of other neuromuscular blocking agents have been reported as allosteric ligands at muscarinic receptors.[24] For example, alcuronium increases the binding of [^{3}H]NMS to muscarinic M_2 and M_4 receptor but inhibits binding to M_1, M_3, and M_5 receptor, indicating that this allosteric effect is subtype and not tissue specific.[25] Strychnine, which is the prerequisite starting material for the synthesis of alcuronium, showed allosteric properties similar to alcuronium at muscarinic receptors.[26] However, the cooperativities of the two compounds are different. Strychnine shows neutral cooperativity at M_1 receptor and positive cooperativity at M_4 receptor with NMS as antagonist, whereas alcuronium inhibits NMS

binding to M_1 receptor and is neutral cooperativity at M_4 receptor. In addition, alcuronium diminishes the affinity of ACh at all muscarinic subtypes, whereas strychnine manifests neutral cooperativity with ACh at M_1 and M_4 receptor, respectively.[26,27] Brucine increases the affinity of ACh for muscarinic M_1 and M_3 receptors but produces different patterns of affinity augmentation at receptor subtypes with other muscarinic agonists.[28]



Alcuronium



Brucine; R = CH_3O—

Strychnine; R = H

1.2.3 Development of allosteric modulators

Since the molecular modelling studies revealed two positively charged nitrogens and two aromatic systems arranged in a sandwich-like geometry,[29] various allosteric modulators with increased affinity for the allosteric site in NMS occupied at muscarinic M_2 receptor were developed.[30] Within the series of alkane-bisammonio compounds, the following structural modifications based on pharmacophoric hypothesis were performed: (i) variation of the number of methylene groups between positively charged nitrogen atoms;[31] (ii) substitution of the aromatic imides in lateral positions;[32] (iii) alkylation of the lateral propyl chains;[33,34] (iv) replacement of the lateral aromatic rings of phthalimide residues by differently substituted imide moieties in a series of symmetrical and nonsymmetrical compounds (Fig. 5).[34,35]

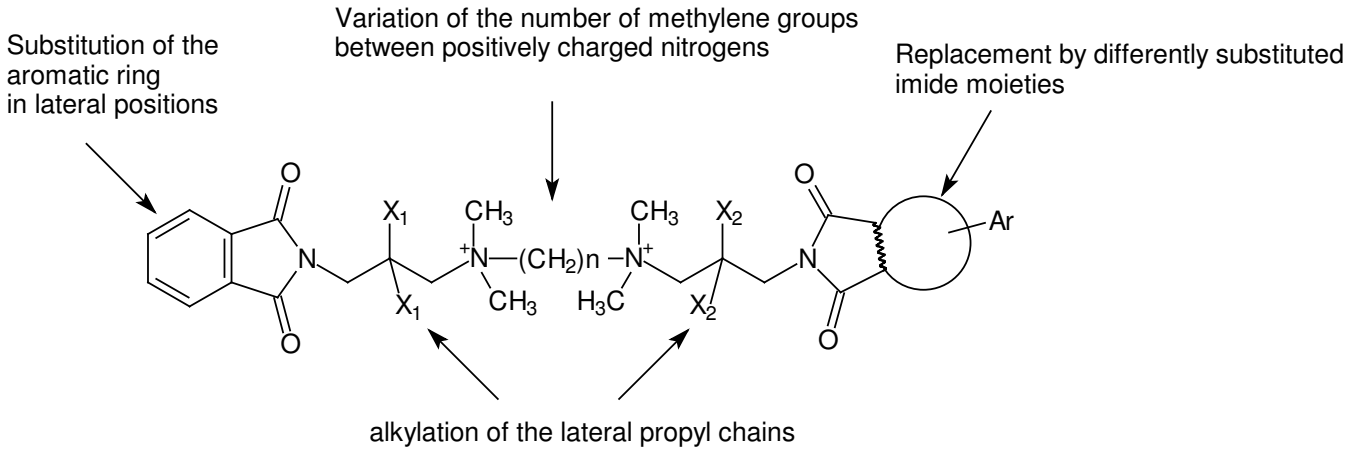
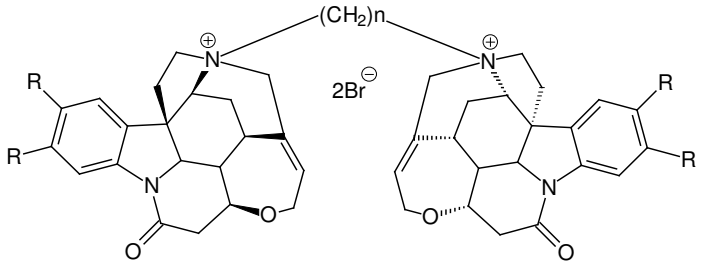


Figure 5. Structural modifications of alkane-bisammonio compounds.

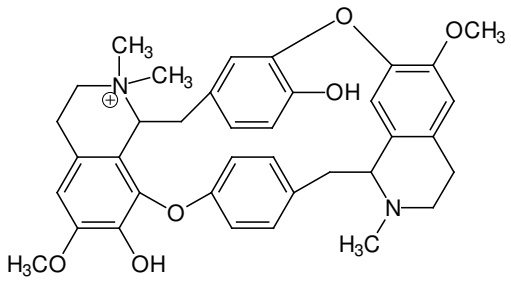
Recently, bisquaternary dimers of strychnine and brucine were synthesized and examined for their allosteric activity. All compounds exhibited higher affinity to the allosteric site of [^{3}H]NMS-occupied M_2 receptors than the monomeric strychnine and brucine, while their positive cooperativity with NMS was fully maintained. [36]



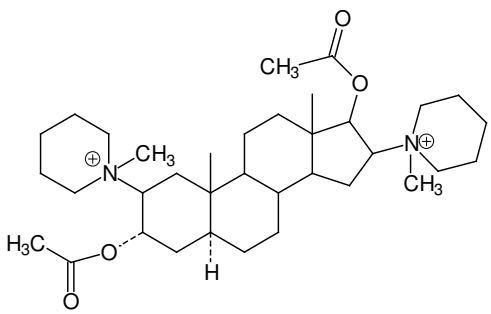
Bisquarternary dimers of strychnine and brucine

Brucine; R = CH_3O—
Strychnine; R = H

As aforementioned, alcuronium and gallamine are neuromuscular blocking agents which block the neuronal stimulation of skeleton muscle fibers by action of ACh, at the motor end plate on cholinergic-nicotinic receptors. These two compounds and the other neuromuscular blockers such as *d*-tubocurarine and pancuronium compete with ACh for the recognition site on the nicotinic receptor by preventing depolarization of the end plate by the neurotransmitter.



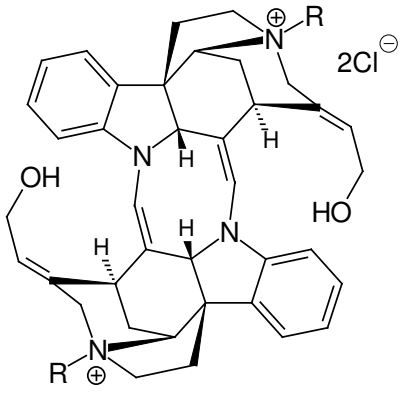
(+)-Tubocurarine



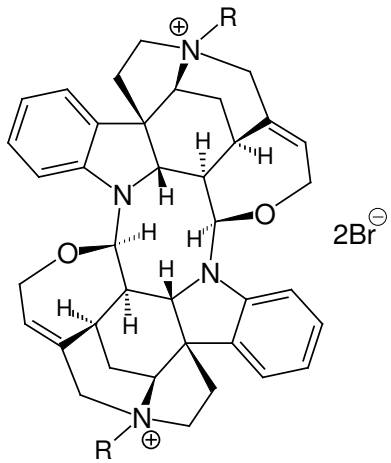
Pancuronium

Thus, by decreasing the effective ACh-receptor interactions, the end-plate potential becomes too small to initiate the propagated action potential. These results in a paralysis of neuromuscular transmission. There are several clinical applications for neuromuscular blockade. The most important by far is the induction of muscle relaxation during anesthesia for effective surgery. However, these neuromuscular blockers also find limited utility in virtue convulsant action and paralyzant action.[2] Although, several compounds in this group reveal allosteric effects on M_2 muscarinic receptors,[8] the therapeutic use as an allosteric modulator is impossible, due to their above-mentioned toxicity.

There is an interesting phenomena of some neuromuscular blocking agents. For instance, on the one hand, caracurine V methochloride which is a cyclization product of the calabash curare alkaloid C-toxiferine I, was reported to have a 50-fold lower neuromuscular blocking activity than C-toxiferine I.[37] On the other hand both alcuronium and its cyclization product, diallylcaracurinium V dibromide are very potent, with NMS positive cooperative, allosteric ligands.[38] The different effects of the caracurine V analogues at allosteric M_2-muscarinic receptors and at neuromuscular end-plate on cholinergic-nicotinic receptors open a new perspective to develop selective compounds for therapeutic purposes. Therefore, the allosteric effect of several different substituted bisquaternary analogues of caracurine V was examined. SAR studies revealed small unpolar N-substituents such as methyl, allyl, and propagyl groups to be important for good allosteric potency. Furthermore, based on the rigidity of caracurine V ring skeleton, its 3D-structure[39] was used as a tool to verify a model of the human M_2 muscarinic receptor by docking into the entrance of the ligand binding cavity.[40]



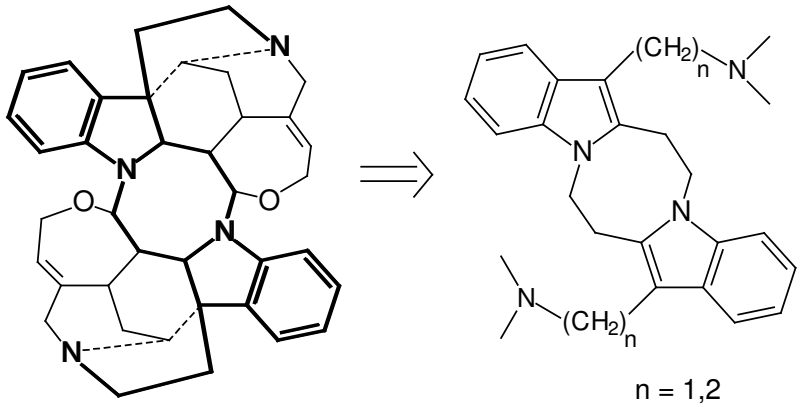
C-toxiferine I, R = methyl
Alcuronium, R = allyl



Caracurine V methochloride, R = methyl
Diallylcaracurinium V, R = allyl

1.3 Goals and objectives of the present study

The caracurine V skeleton, which comprises the pharmacophore model for potent allosteric modulators of muscarinic M_2 receptor is an excellent pharmacological tool for exploring the allosteric mechanism of the ligand-receptor interaction. The aim of this study was to synthesize a novel pentacyclic ring system derived from the rigid ring skeleton of caracurine V and to test it for the allosteric potency on muscarinic M_2 receptors. Considering the allosteric pharmacophore model,[29,30] the design strategy was to simplify the complexed caracurine V ring structure to a novel pentacyclic ring system (Fig. 6). Furthermore, the influence of the length of the side-chains (n = 1 or 2 in Fig. 6) of the novel ring system and of the N-substituents on the allosteric activity should be examined. This novel ring skeleton could open a new perspective for highly potent allosteric modulators at muscarinic acetylcholine M_2 receptors.



Caracurine V

Figure 6. Structure relationship between caracurine V and the desired novel pentacyclic ring system.

Retrosynthetic analysis (Fig. 7) suggested that the desired pentacyclic ring system should be available by double intermolecular *N*-alkylation of bromoethyl indole, which should be easily prepared from the corresponding indolmethylacetate after reduction to an alcohol. An alternative pathway for the critical dimerization step involves the intermolecular lactame formation of the corresponding indole acetic acid.

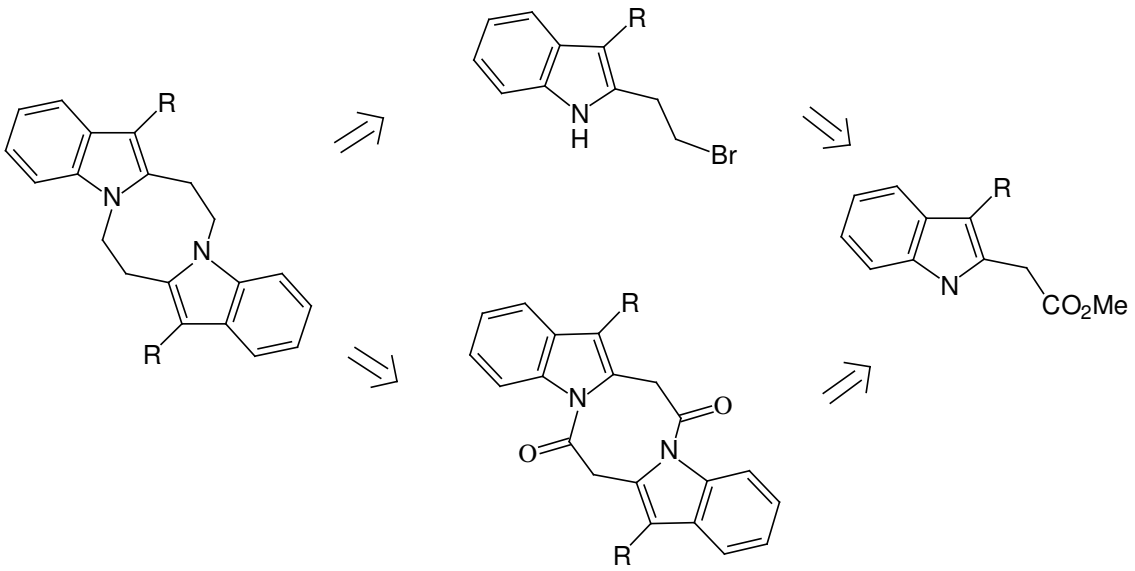


Figure 7. Retrosynthesis of desired novel pentacyclic ring system.

Similar to the synthesis of toxiferine I, which was prepared by condensation of two molecules of Wieland-Gumlich aldehyde methochloride, another possible route for building the diazocinodiindole ring skeleton involves a double enamine formation from indolyl acetaldehyde. The resulting ring system has two additional double bonds in the central eight-memberded ring, which are also present in the ring skeleton of alcuronium and toxiferine. (Fig 8).

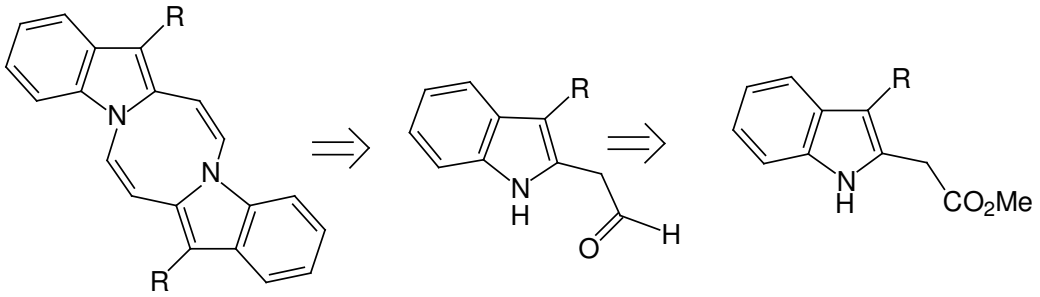


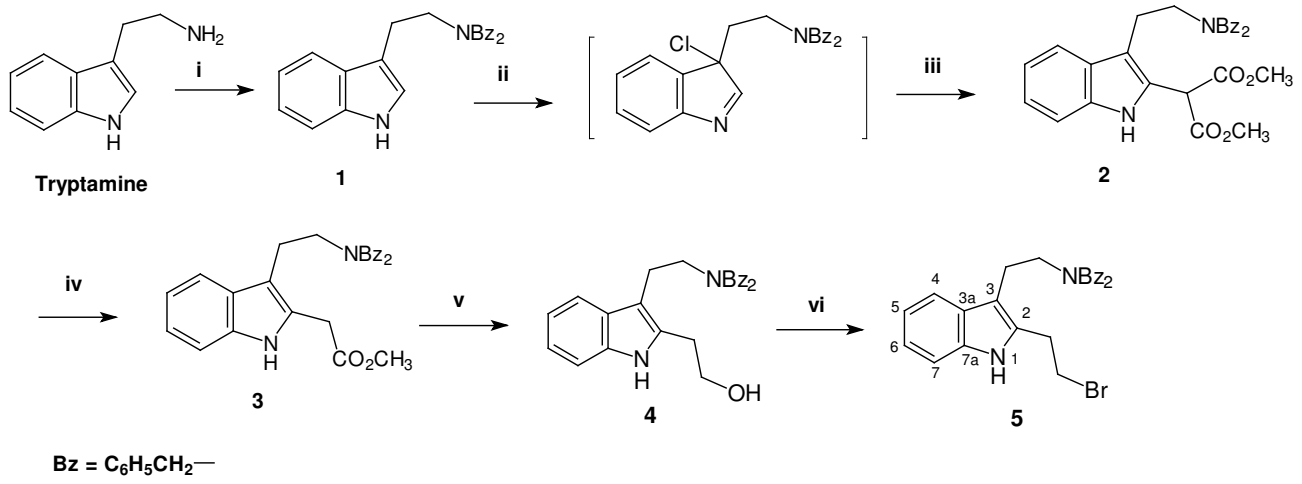
Figure 8. Retrosynthesis of a novel alcuronium derived ring system involving double enamine formation.

2. Results and Discussion

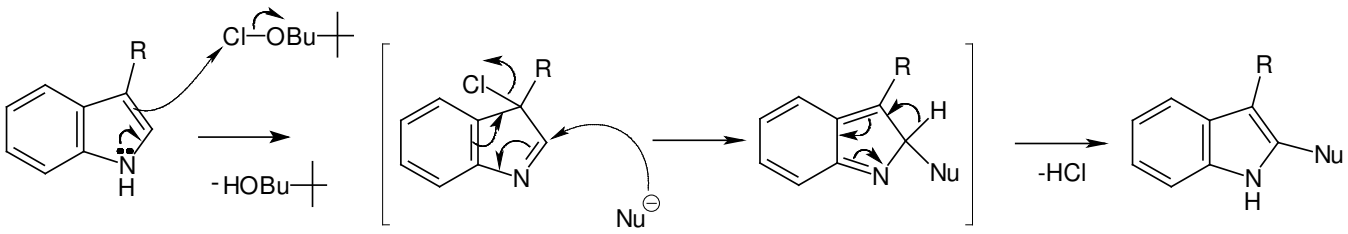
2.1 Synthesis

2.1.1 Synthesis of 6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]diindole ring system

Retrosynthetic analyses of the desired pentacyclic ring system revealed methyl [3-(2-dibenzylaminoethyl)-1*H*-indol-2-yl]-acetate **3** as a key intermediate of our synthetic approach (Fig. 7, p 11). This compound was already prepared as an intermediate in the synthesis of various *Strychnos*-type alkaloids by *Kuehne* and co-workers.[41] The synthetic pathway is illustrated in Scheme 1. Dibenzylation of commercially available tryptamine using benzyl bromide and K_2CO_3 in refluxing methanol provided *N,N*-dibenzyltryptamine **(1)** in a good yield. Introduction of the malonester moiety at C-2 of the indole ring proceeded by chlorination of **1** with *tert*-butyl hypochlorite (*tert*-BuOCl)/triethylamine in dry THF at –78 °C and reaction of the resulting chloroimine with thalium dimethylmalonate (TlDMM)[41], giving **2** in high yield. The reaction mechanism of the latter alkylation is displayed in Scheme 2.[42] Finally, demethoxycarbonylation of indol-2-yl malonate **2** by refluxing with lithium iodide hydrate in dimethylacetamide afforded the desired methyl indol-2-yl acetate **3** in a fair yield.



Scheme 1. (i) benzyl bromide, K_2CO_3, dry MeOH, reflux, 72 h; (ii) *tert*-BuOCl, NEt_3, dry THF, -78 °C, 3 h; (iii) TlDMM, dry THF, -78 °C 1 h, room temperature 12 h; (iv) LiI xH_2O, DMA, 130 °C, 3 h; (v) $LiAlH_4$, dry THF, room temp., 3 h; (vi) CBr_4, $P(NMe_2)_3$, dry CH_2Cl_2, room temp., 16 h.



Scheme 2. General mechanism of the alkylation of indole at C-2.

Reduction of **3** was carried out with $LiAlH_4$ in THF giving alcohol **4** (Scheme 1). The structure of **4** was confirmed by ^{1}H NMR as shown in Appendix 1. Conversion of **4** to the corresponding bromide **5** could be achieved by using carbon tetrabromide (CBr_4) and triphenylphosphine (PPh_3) in CH_2Cl_2. Replacement of PPh_3 by *tris*-(dimethylamino)phosphine ($P(NMe_2)$) gave **5** in a much better yield (87 %) (Scheme 1). ^{1}H-NMR of **5** is shown in Fig. 9. **5** undergoes readily a HBr elimination as indicated by the presence of olefinic protons in ^{1}H NMR spectrum at δ = 6.62, 5.40, and 5.16 ppm, respectively.

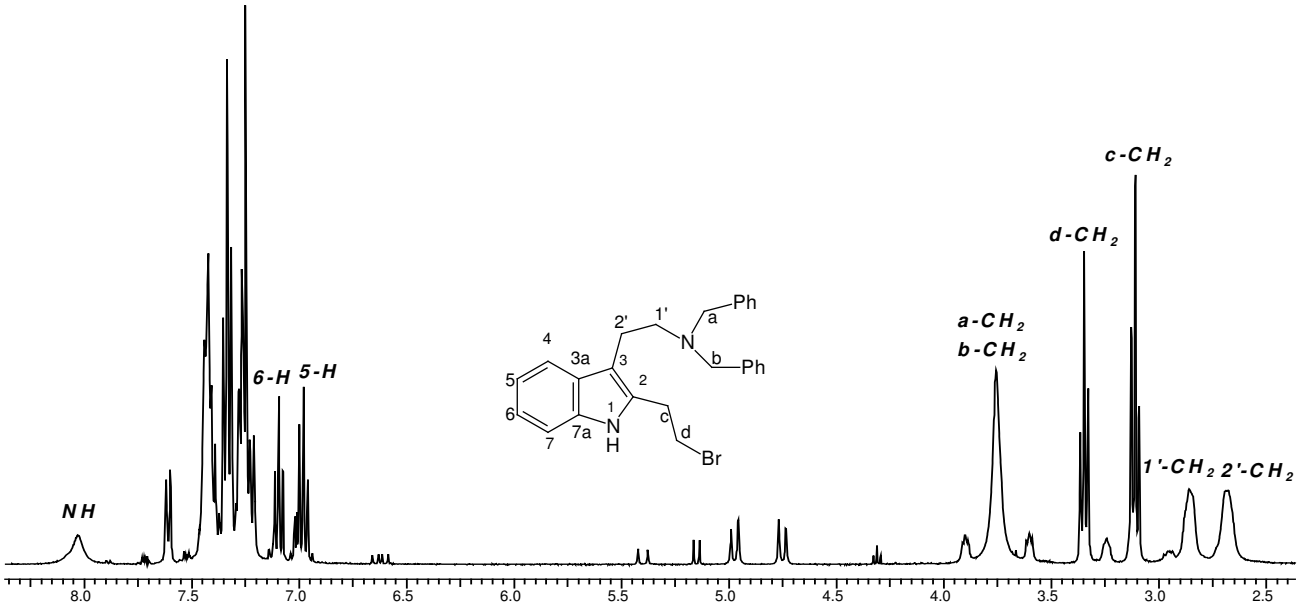
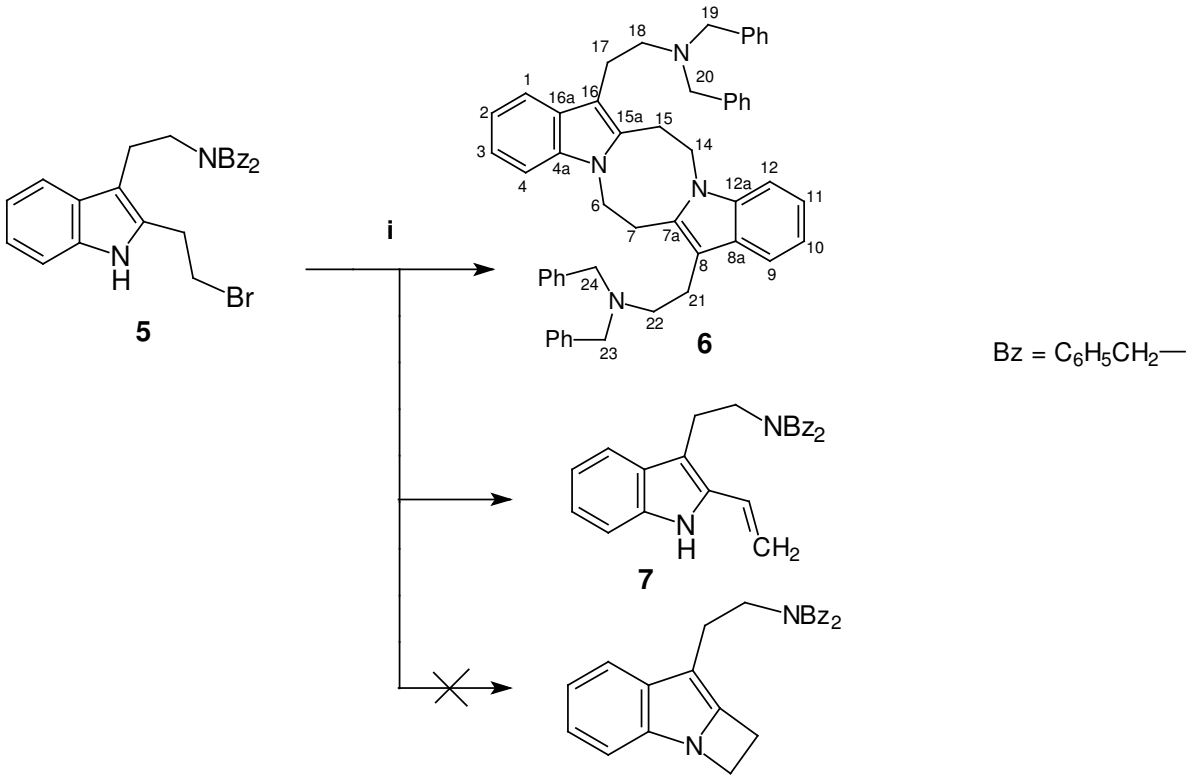


Figure 9. 400 MHz ^{1}H-NMR spectrum of **5** ($CDCl_3$).

The intermolecular double *N*-alkylation of **5** is a key step for the synthesis of the desired pentacyclic ring system. The self-condensation could be achieved under strong base conditions. Treatment of **5** with NaH in DMF provided 6,7,14,15-

tetrahydro[1,5]diazocino[1,2-a:6,5-a′]diindole **6** as a first representative of a novel heterocyclic ring system. The moderate yield is due to a side-reaction involving the HBr elimination from the side chain of **5**. The resulting 2-vinylindole **7** could be separated from (**6**) by column chromatography on silica gel. Another possible side-product with a four membered ring, resulting from the intramolecular *N*-alkylation of **5** was not observed (Scheme 3).



Scheme 3. (i) NaH, dry DMF, 0 °C, 15 min, room temp. 20 min.

Mass spectrometry was used as a first tool to confirm the structure of compound **6**. Due to the absence of the molecular peak in the EI mass spectrum, the CI mass spectrum of **6** was recorded using NH_3 as ionisation gas. The molecular peak at *m/z* 733 as well as the $[M+1]^+$ and $[M+2]^+$ peaks indicated the expected molecular formula of $C_{52}H_{52}N_4$. The EI mass spectrum (Fig. 10) showed prominent peaks at m/z 91 (base peak), 210, 522, and 641, respectively. These molecular fragments could be assigned according to the fragmentation mechanism proposed in Fig. 11.

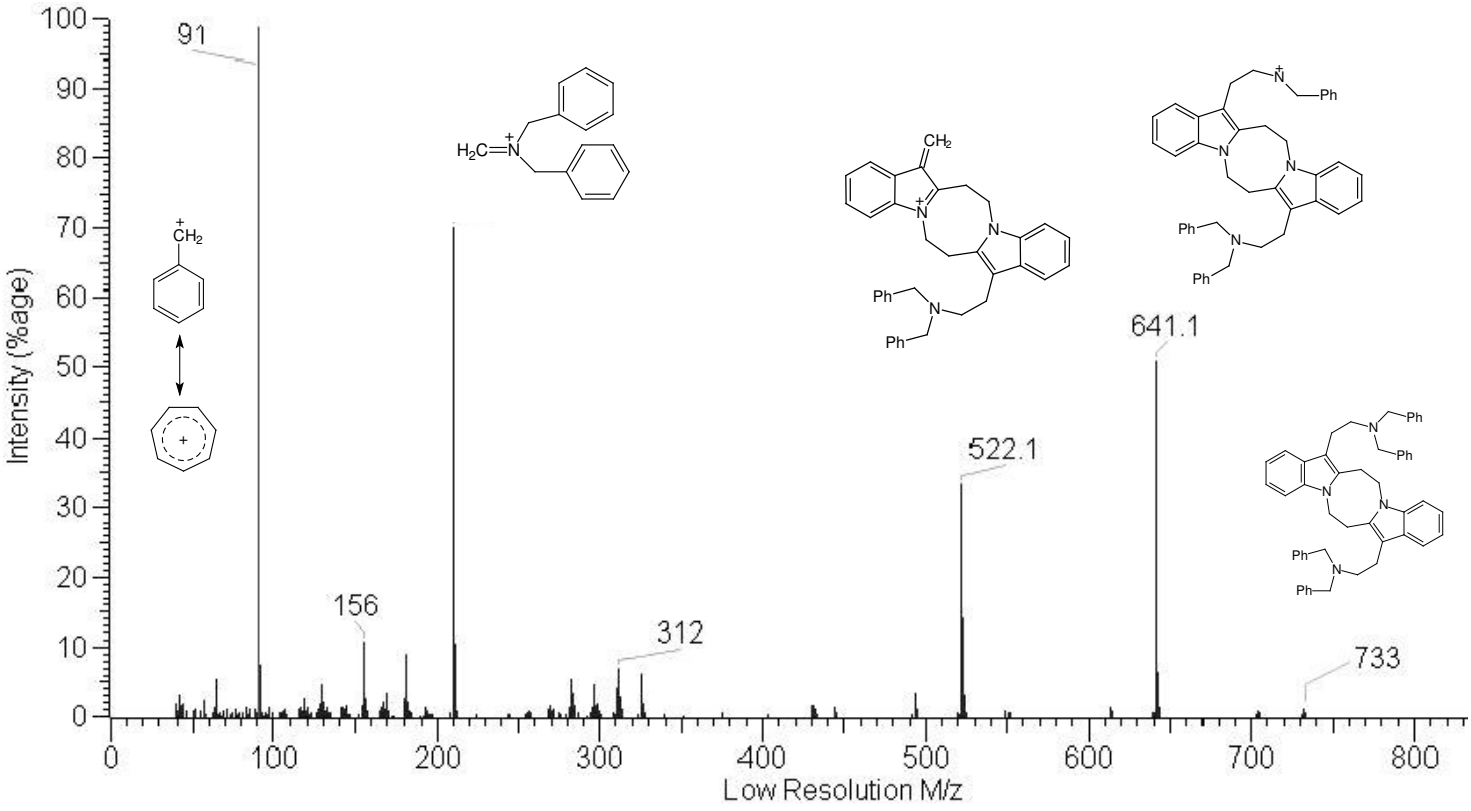


Figure 10. EI (70 eV) mass spectrum of **6**.

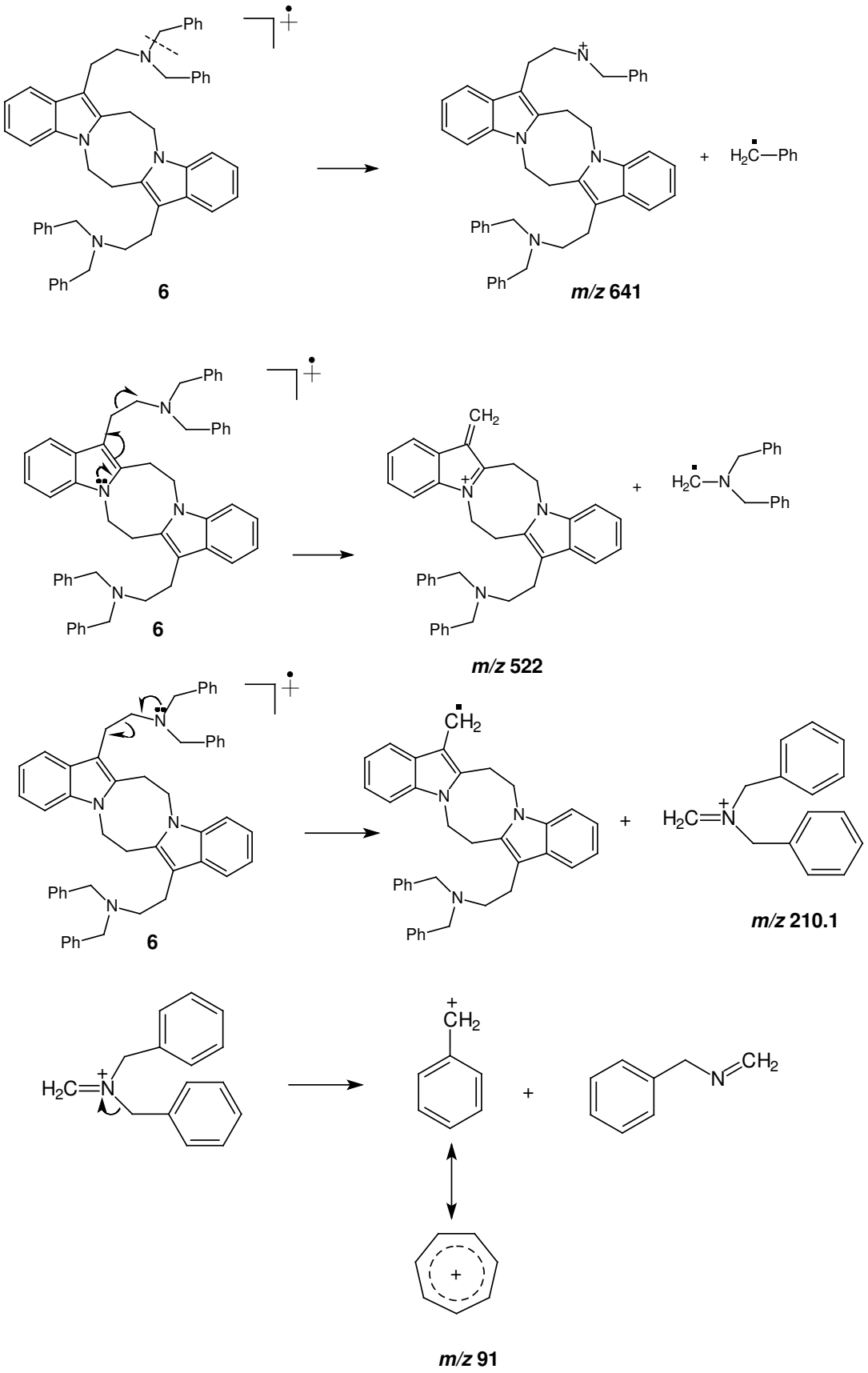


Figure 11. MS-Fragmentation patterns of **6** (EI, 70 eV).

Unlike caracurine V, which is a highly symmetrical ring system with a C_2 symmetry axis, the novel ring skeleton shows no symmetry as indicated by NMR spectroscopy. Both ^1H (Fig. 12) and ^{13}C (Appendix 2) NMR spectra of **6** revealed two sets of signals, each for half the molecule.

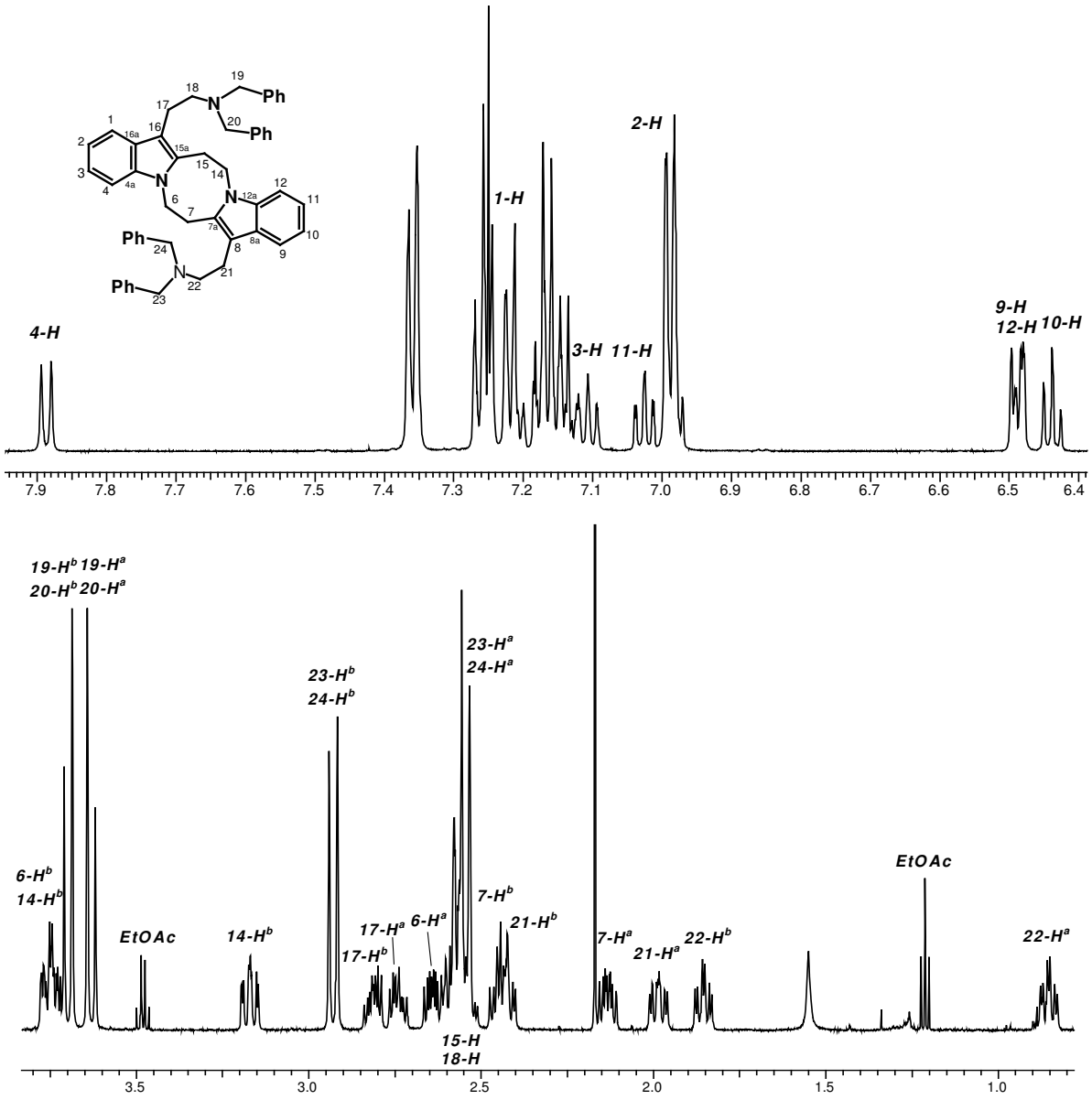


Figure 12. 600 MHz ^1H NMR spectrum of **6** ($CDCl_3$).

The ^{13}C NMR spectrum showed 34 signals, which are fewer than the number of carbon atoms in the molecule, due to coinciding resonances of some carbons belonging to the different benzyl groups. The signals of quaternary carbons could be verified by their absence in the DEPT-135 spectrum. All resonance signals in the aliphatic region were assigned as methylene

carbons by DEPT-135 spectrum. The most intensive negative peaks represent the benzylic carbons C-19, C-22, and C-23, C-24, respectively (Appendix 2a).

Due to the unsymmetrical structure of **6**, the complete assignment required several 2D-NMR experiments, such as H,H-COSY, ROESY, HMQC, and HMBC. Interpretation of the ^{1}H NMR spectrum was only possible by using a high resolution 600 MHz NMR spectrometer. A good starting point for the NMR assignment is the carbon resonance at 112.3 ppm, which is in a typical range for the aromatic indole atom C-4 (according to the numbering of the new ring system). HMQC cross peak from C-4 revealed H-4 as an isolated doublet at δ = 7.89 ppm (Fig. 13).

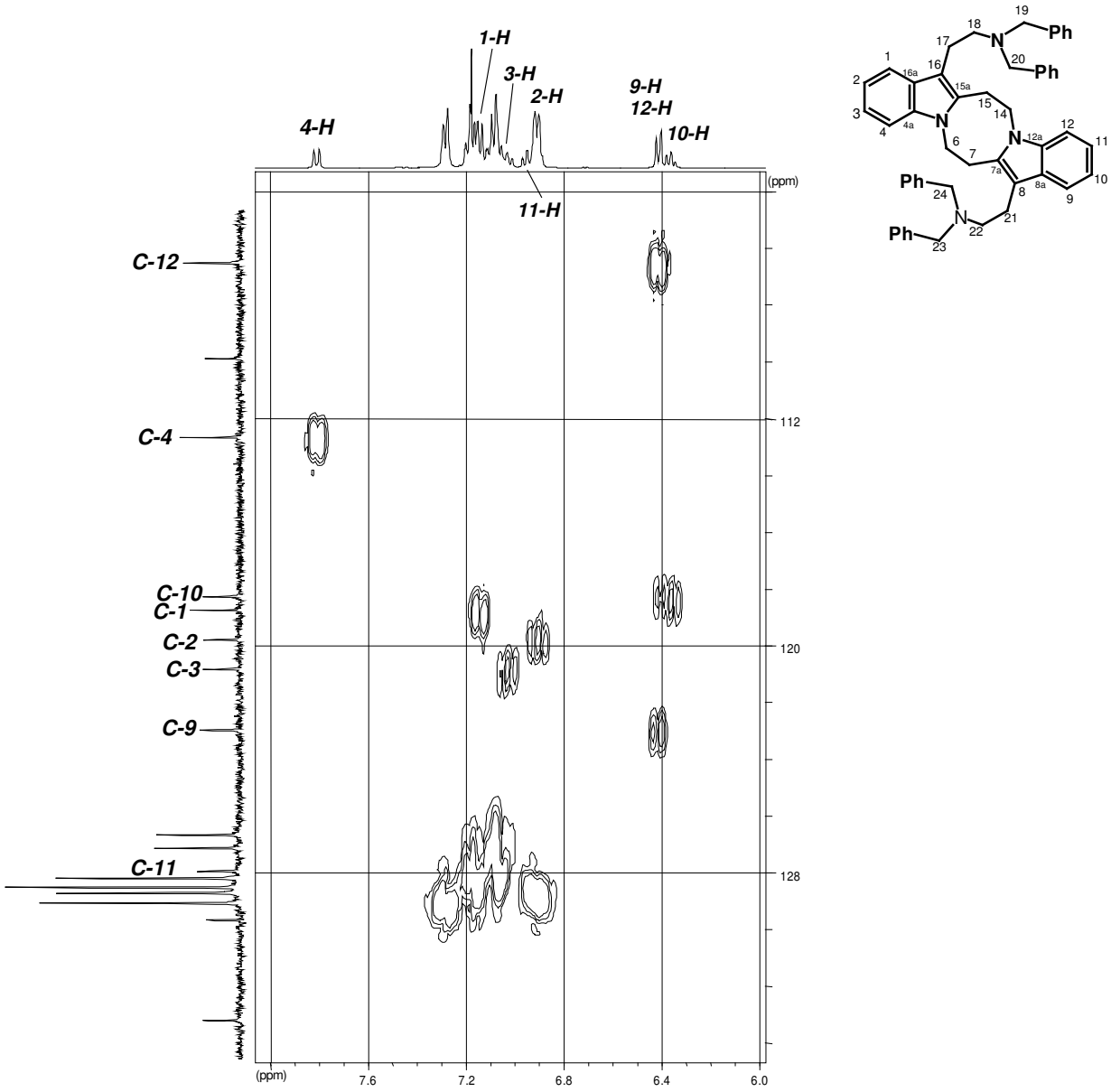
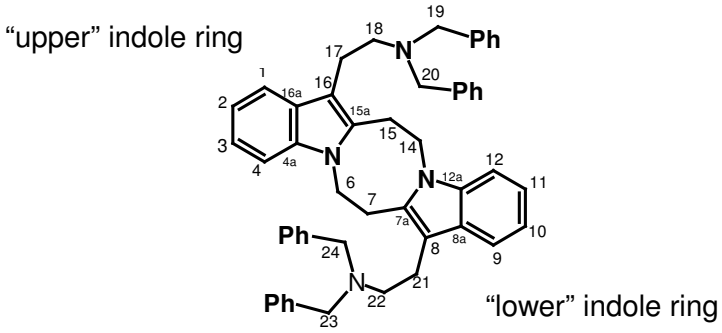


Figure 13. Expanded section of the aromatic region of 400 MHz HMQC contours plot of **6** ($CDCl_3$).

The remaining aromatic indole protons H-1 (δ = 7.22 ppm), H-2 (δ = 6.99 ppm), H-3 (δ = 7.11 ppm) could be identified based on HH-COSY cross peaks within the "upper" indole ring (Fig.14). The "lower" indole ring was assigned similarly, starting with the HMQC correlated atoms C-12 (δ = 106.2 ppm) and H-12 (δ = 6.49 ppm) (Fig. 13).



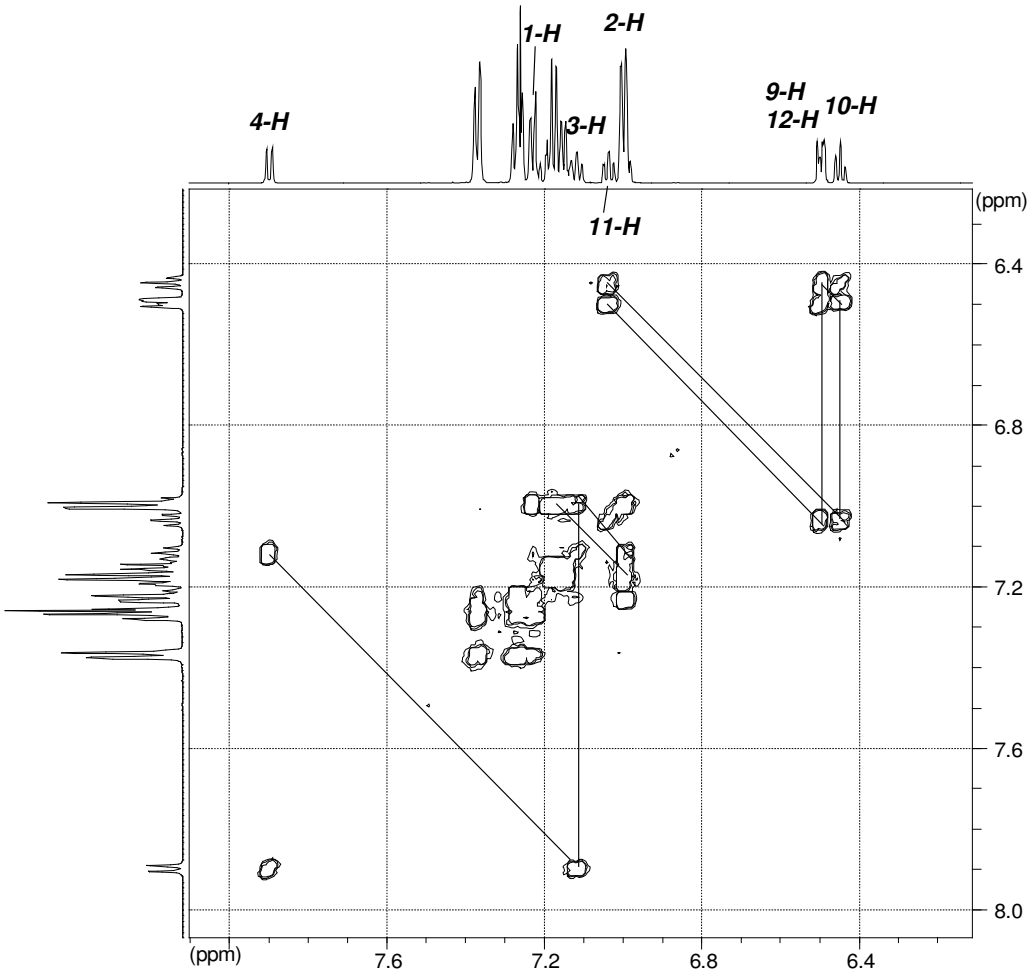


Figure 14. Expanded section of the aromatic region of 600 MHz HH-COSY contours plot of **6** ($CDCl_3$).

The two side chains were identified by ROESY correlations between H-1(H-9) and the respective methylene hydrogens at C-17 and/or C-18 (C-21 and/or C-22) in combination with aliphatic sections of HH-COSY, ROESY and HMQC experiments (Fig. 15 and 16).

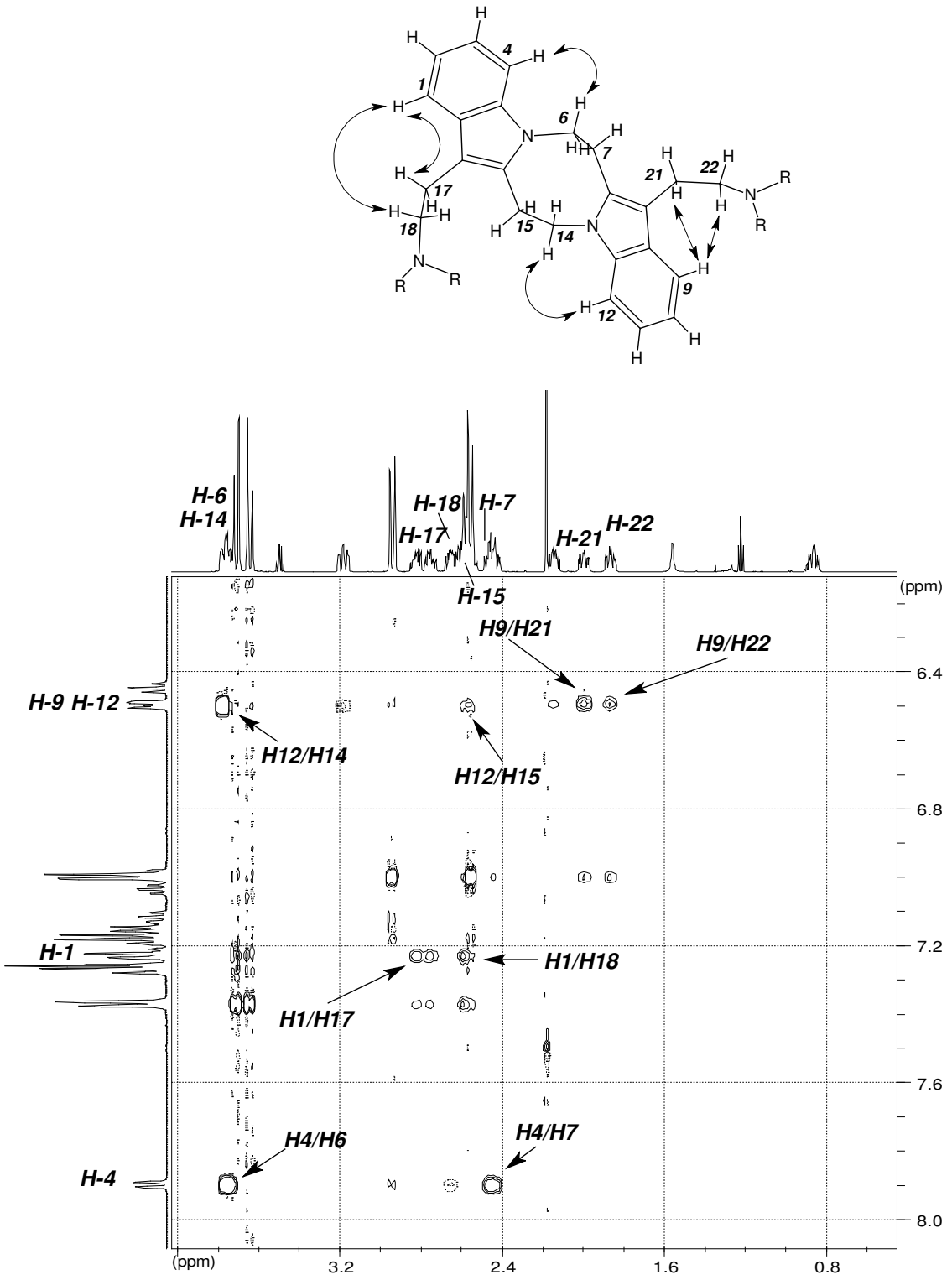


Figure 15. Expanded section of 600 MHz ROESY contours plot of **6** ($CDCl_3$).

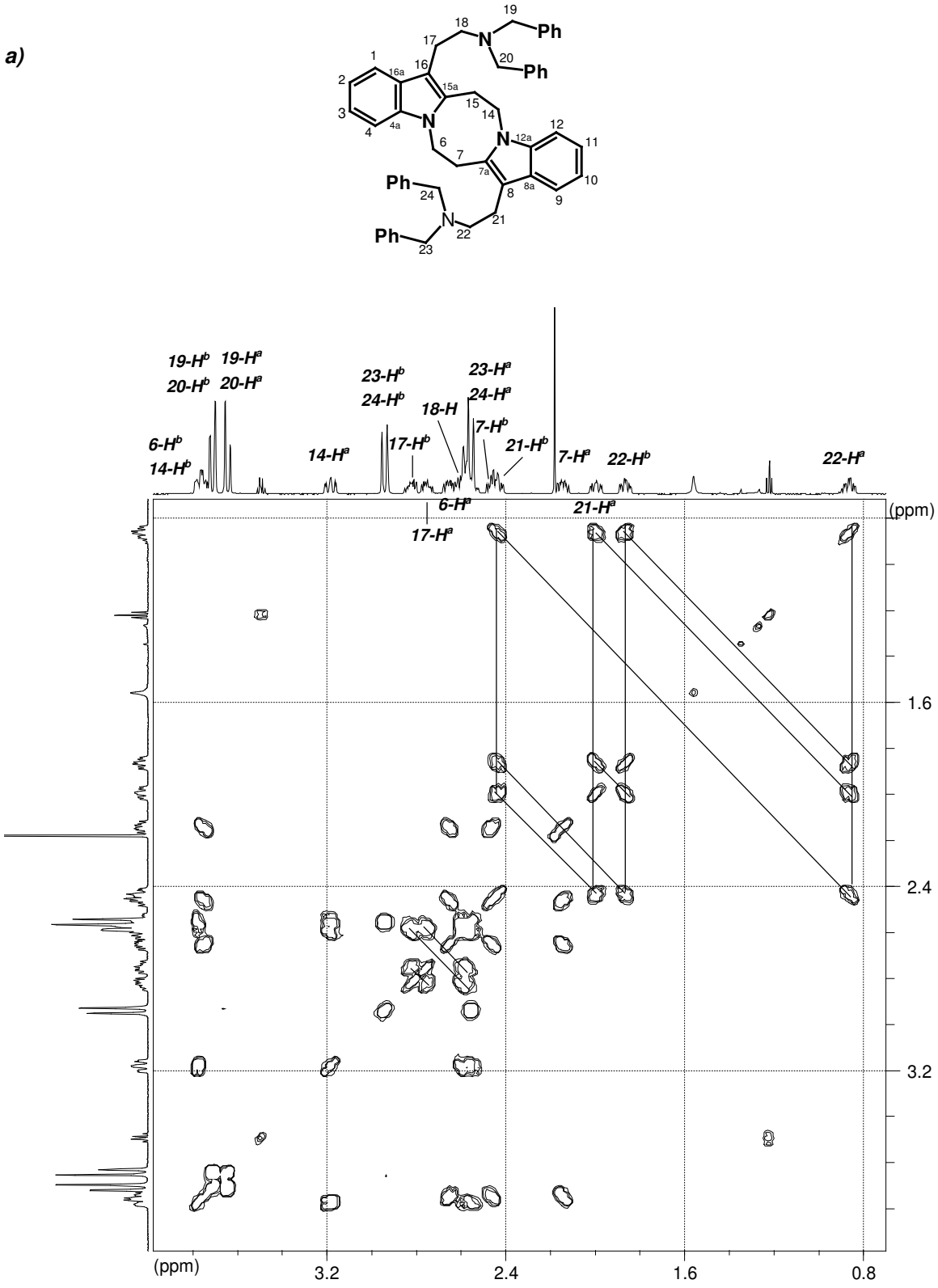


Figure 16. (a) Expanded section of aliphatic regions of 600 MHz HH-COSY contours plot of **6** ($CDCl_3$).

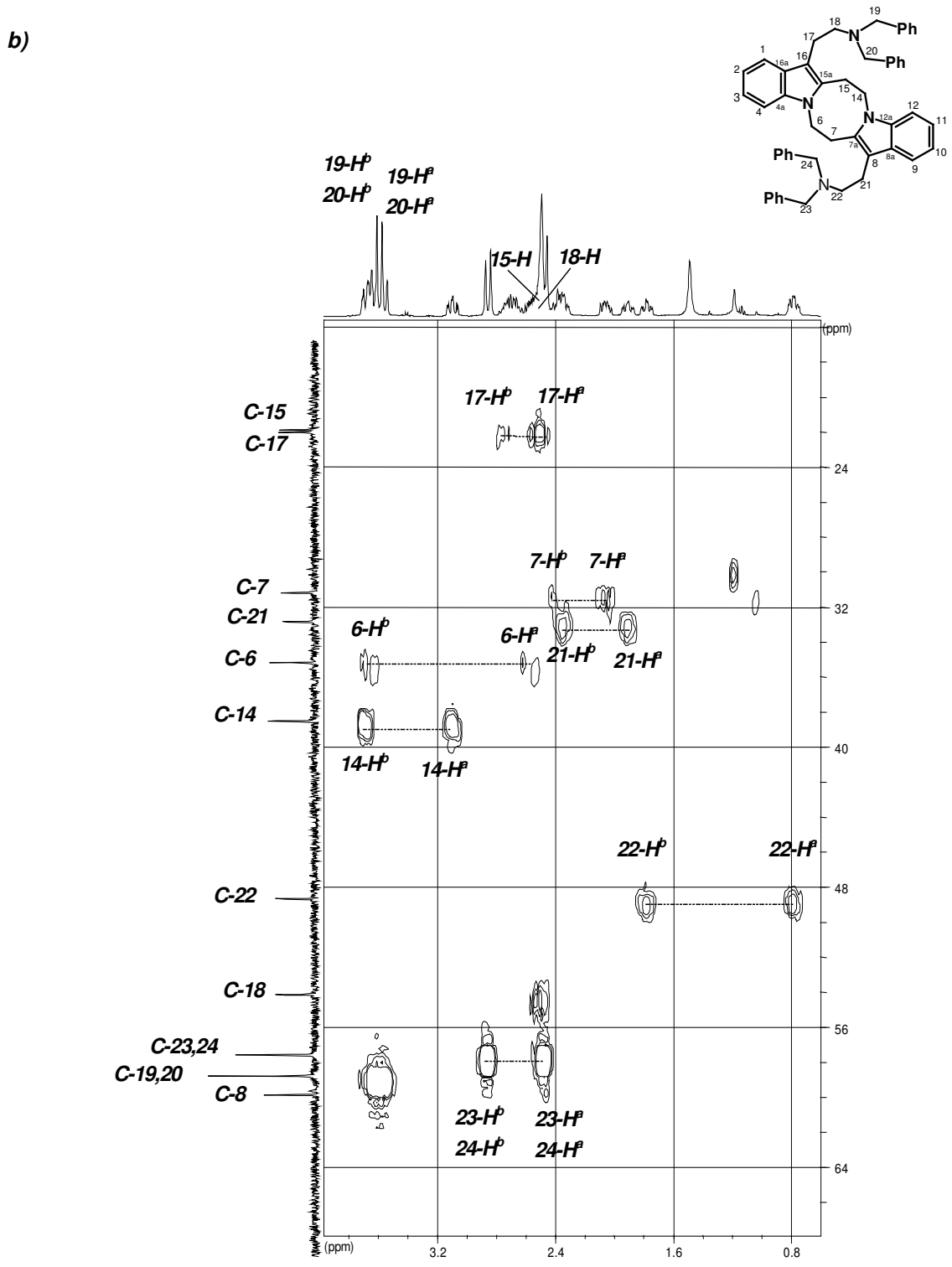


Figure 16. (b) Expanded section of aliphatic regions of 400 MHz HMQC contours plot of **6** ($CDCl_3$).

The assignment of ethylene groups within the central diazocine ring was carried out by ROESY correlations of H-4 and H-12, respectively. ROEs between H-4 and the HH-COSY correlated signals at δ = 2.64 ppm and δ = 3.72-3.79 ppm led to their assignment as H-6^a and H-6^b, respectively. The resonance signals of H-7^a and H-7^b could be readily determined by COSY correlations to H-6^a and H-6^b. The 14-CH_2 and 15-CH_2 ethylene groups were assigned in a similar manner, starting from ROEs between H-12 and the HH-COSY correlated signals of H-14^a at δ = 3.17 ppm and H-14^b at δ = 3.72-3.79 ppm (Fig. 15). Surprisingly, all geminal hydrogens belonging to side chains and central diazocine ring appear widely separated (Fig 16b). This indicates a high rigidity of this ring system, as well as limited flexibility of the side chains.

The HMBC experiment allowed to assign the signals of the quaternary carbons and to confirm the previous assignment. For instance, HMBC correlations of the ^{13}C resonance signal at δ = 85.5 to H-6^a, H-6^b, H-7^a, and H-7^b let to its assignment as C-7a. The HMBC spectrum of the aliphatic region of **6** is shown in Appendix 3.

The complexity of the ^1H NMR spectrum of **6** can be explained by two reasons. First, the pentacyclic ring system adopts a fixed conformation, which doubles the NMR signals. Second, the hydrogens within the central diazocine ring, as well as the ethylene side-chain protons could be affected by ring currents of the four benzylic groups, which seem to have a defined spatial arrangement, as indicated by non-equivalence of the methylene protons at C-19, C-20, C-21, and C-22.

The elimination side-product **7** showed a typical NMR spectrum of a compound substituted with a vinyl group (Appendix 4). The proton Hc, attached to a carbon bearing the indole ring, is assigned the largest chemical shift at δ = 6.70 ppm, since it is affected by the deshielding ring current generated by the π electrons of the indole double bond. The remaining protons H_A and H_B are distinguishable by different vicinal coupling constants with Hc. The doublet at δ = 5.41 ppm could be assigned as H_A due to a typical trans coupling J_{AC} = 17.5 Hz, whereas the more narrow doublet (J_{BC} = 11.5 Hz) at δ = 5.22 ppm revealed a cis-coupled H_C. The geminal coupling between H_A and H_B could be rarely observed ($J_{AB} \cong 0$), suggesting a H-C-H angle larger than 120°.

2.1.2 Conformational analysis of the 6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]diindole ring system

The conformation of the central eight-membered ring of 6 which is crucial for the geometry of the whole molecule was elucidated by means of NMR spectroscopy and semiempirical calculations. AM1 calculations carried out by means of PC SPARTAN[43] revealed two possible symmetrical conformations for the diazocinodiindole ring: a chair, possessing a center of inversion (i), and a twisted boat with a 2-fold symmetry axis (C_2) (Fig. 17).

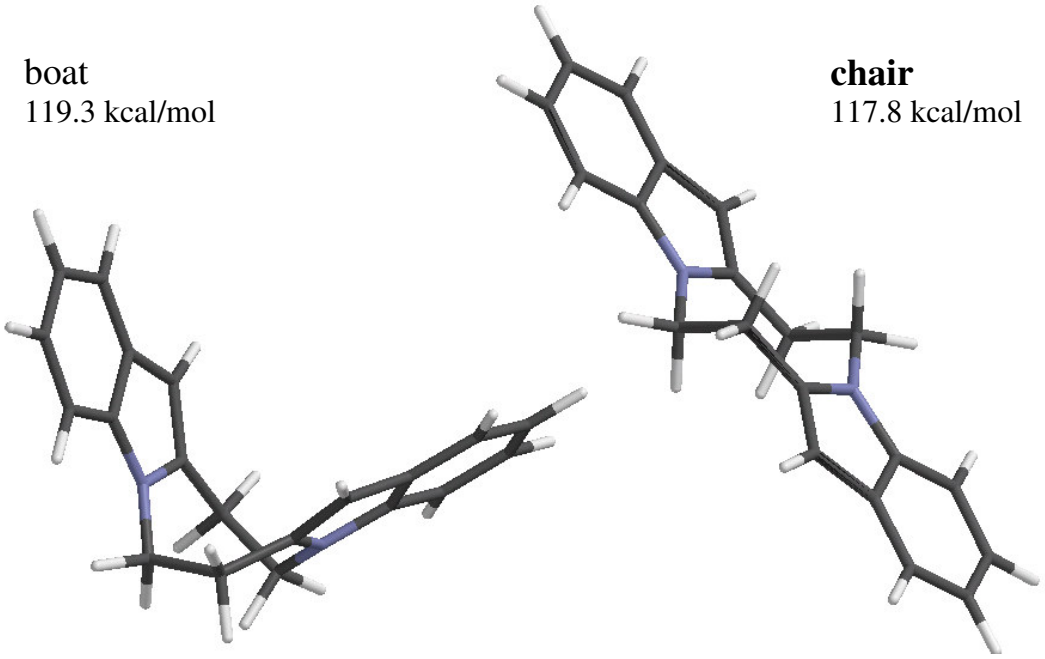
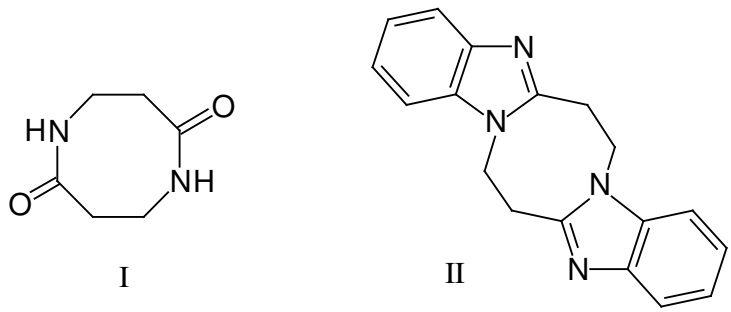


Figure 17. Possible conformations of the 6,7,14,15-Tetrahydro[1,5]diazocino-[1,2-a:6,5-a′]diindole ring system obtained by semiempirical calculations (AM1).

Both conformational minima are consistent with 3D structures known for other 1,5-diconstrained eight-membered ring systems.[44,45] For instance, the eight-membered ring diamide I was shown by X-ray crystallography to exist in the solid state as a twisted boat with C_2 symmetry.[45] In comparison, the central eight-membered ring of bisbenzimidazodiazocine II adopts in a crystal structure a chair conformation (Fig. 18).[45]



I II

Figure 18. Related eight-membered rings with known solid state conformations (I: twisted boat, II: chair).

NMR spectra of II recorded in different solvents and at different temperatures revealed coinciding resonance signals for both halves of the molecule, indicating the existence of a symmetrical conformation in solution. However, splitting patterns of the resonances belonging to the ethylene groups of the diacozine ring (C-CH_2: triplet, J = 3.7 Hz, N-CH_2: triplet, J = 4.6 Hz) implied a rapid interconversion between the symmetrical conformations.[45]

Both ^{1}H and ^{13}C NMR spectra of compound 6 in $CDCl_3$ revealed two sets of signals, each for half the molecule. (Fig. 12, p. 17). Moreover, all methylene hydrogens belonging to the central diazocine ring and to the side-chains appeared as clear separated resonances, except for 15-CH_2 and 18-CH_2, that is indicative of a non-symmetrical and rigid conformation. The conformation of the central diazocine ring was elucidated by a 600 MHz ROESY experiment. ROEs between H-4 and H-7^b as well as between H-12 and H-15 (Fig. 15, p. 20) are only consistent with the twisted boat conformation in which the respective hydrogens are at a distance of 3.0 Å (in the chair conformation, the corresponding protons are 3.8 Å away from each other) (Fig. 19). The boat conformation was confirmed by the vicinal coupling constants within the C6-C7 ethylene group. The experimental values are in agreement with those calculated for the boat conformation by means of the Karplus equation implemented in PC MODEL[43] (Table 1).

If one considers that the diazocine ring exists in a symmetrical conformation possessing a C_2 axis, the doubled NMR resonances of 6 are surprising. However, the side chains which have not been included in the conformational analysis so far, might assume a non-symmetrical

arrangement, resulting in loss of symmetry of the whole molecule. The possible spatial arrangement of the side chains will be discussed later.

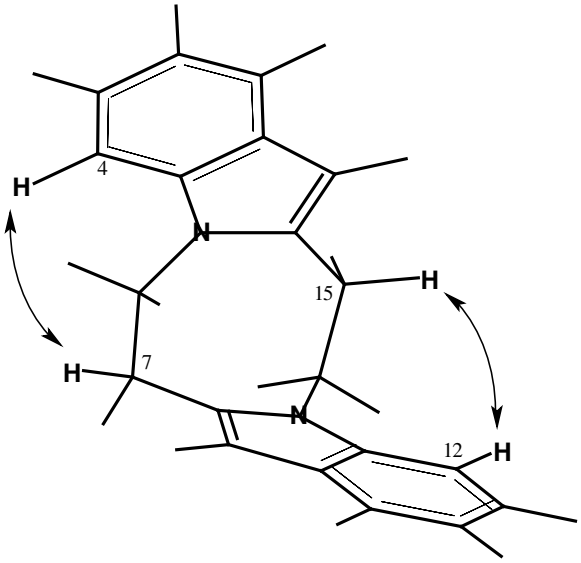


Figure 19. ROEs indicating the twisted boat conformation of the 6,7,14,15-tetrahydro[1,5]-diazocino[1,2-a:6,5-a′]diindole ring system.

Table 1. Experimental and calculated vicinal coupling constants (Hz) (PC MODEL) within the C6-C7 ethylene group of 6.

	$6\text{-}H^a\text{-}7\text{-}H^a$	$6\text{-}H^a\text{-}7\text{-}H^b$	$6\text{-}H^b\text{-}7\text{-}H^a$	$6\text{-}H^b\text{-}7\text{-}H^b$
calc.	10.5	6.9	7.6	0.5
found	10.2	6.5	6.6	-*

*not determinable because resonances within a complex group of signals

The twisted-boat conformation of the diazocine ring of 6 is a helical structure, giving rise to chirality of the whole molecule. Depending on the twist direction of the pentacyclic framework, two enantiomeric pentahelicenes, displayed in Fig. 20, are possible.

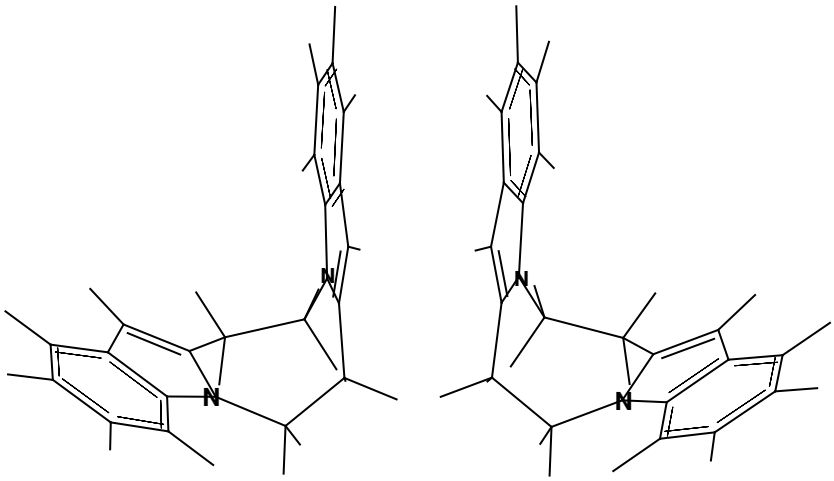


Figure 20. The enantiomeric helical structures of the 6,7,14,15-tetrahydro[1,5]-diazocino[1,2-a:6,5-a′]diindole ring system.

The positions of the ethylamine side chains of 6 were estimated by ROEs from H-1 and H-9 to the ethylene protons H-17, H-18 and H-21 and H-22, respectively, as shown in Fig. 21 and subsequent semiempirical calculation (AM1). The limited flexibility of the side chains was confirmed by the non-equivalence of all hydrogen atoms belonging to both ethylene groups.

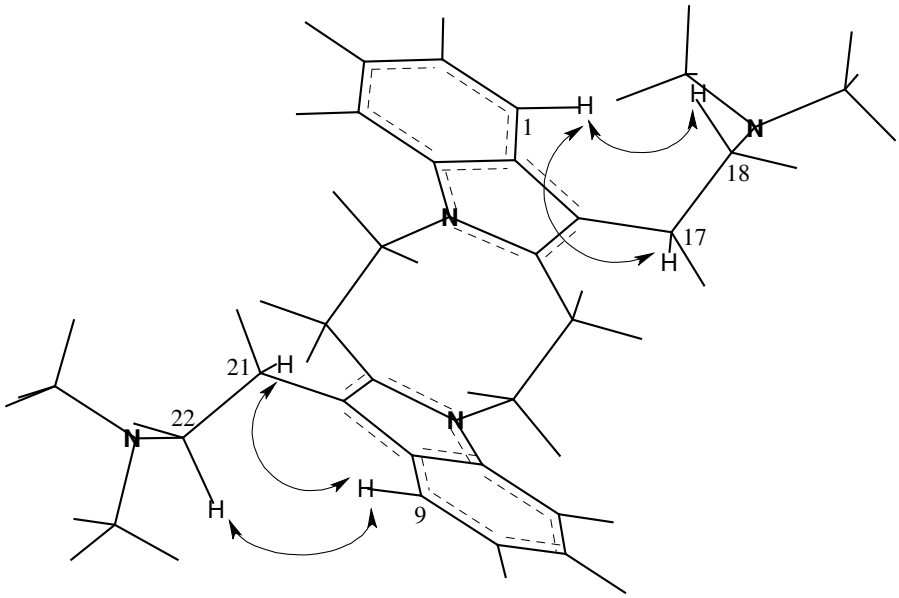


Figure 21. ROEs indicating the positions of the ethylamine side chains of 6.

Finally, the four benzyl groups were attached to the nitrogen atoms in such positions that their ring current effects helped to explain the wide separation of the resonance signals for the methylene protons at C-22 and C-21. Subsequent AM1 calculation carried out by Hyperchem[46] led to the conformation of 6 shown in Fig. 22.

It should be mentioned that several other conformations with different positions of the benzyl groups, having similar heats of formation, are also possible. X-ray crystallographic analyses should help gaining more insight into the structure of the new ring systems in a solid state, and to confirm the proposed solution structure. However, it was not yet possible to obtain suitable crystals for the X-ray analysis.

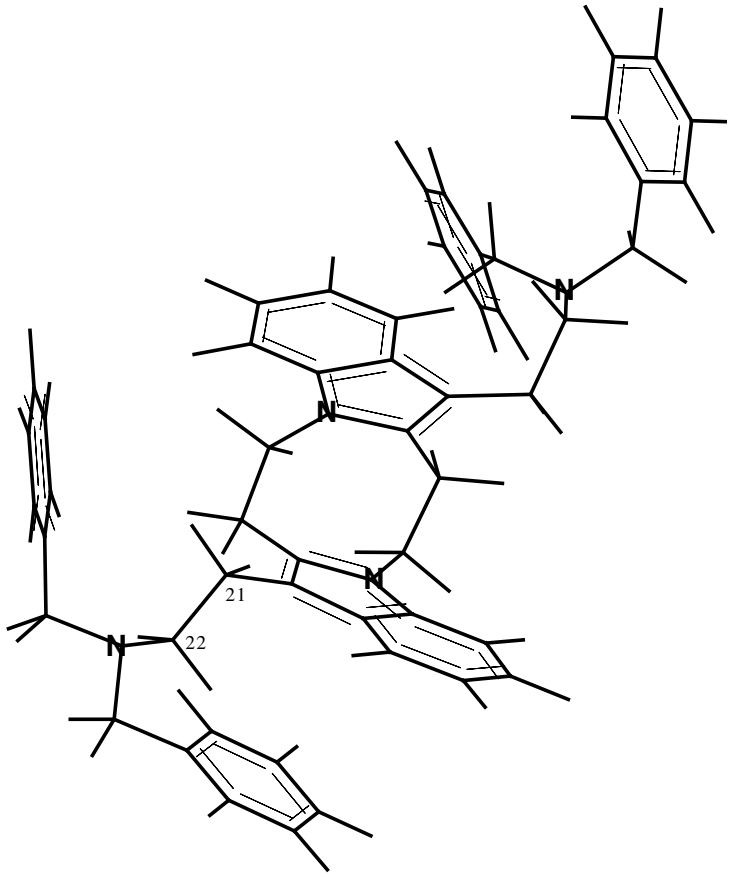
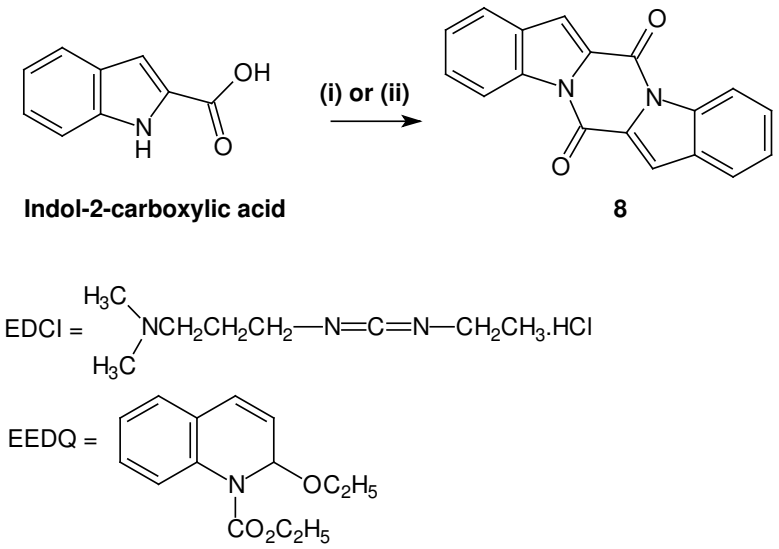


Figure 22. The possible conformation of 6 in chloroform solution.

The distance between the nitrogen atoms in the side chains of 6 (10.4 Å) is in agreement with the pharmacophore model. It is slightly higher than the corresponding distance in caracurine V (9.6 Å).

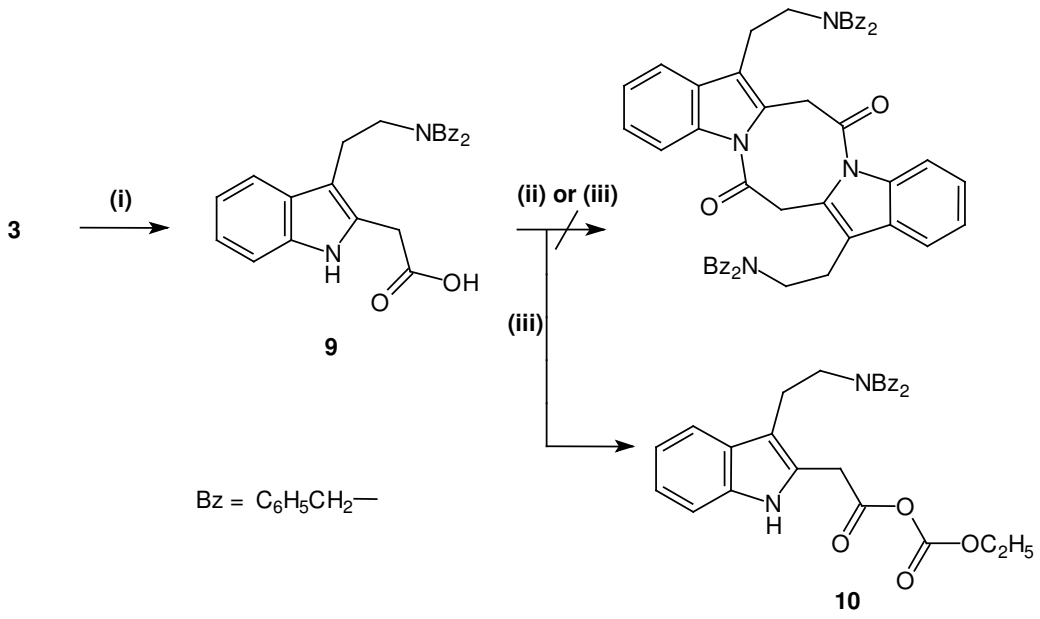
2.1.3 Investigation of the double N-acylation approach

Another route for building the desired ring system *via* an intermolecular double *N*-acylation of the monoester **3** and its corresponding acid was investigated (Fig. 7 p. 11). In order to find out the optimal reaction conditions, the retrohomologous indole-2-carboxylic acid was dimerized to give the corresponding pyrazino[1,2-a;4,5-a´]diindole-6,13-dione **8**. *Whitlock* prepared **8** from the dimerization of indole-2-carbonyl chloride giving an extremely insoluble orange solid in only 5 % yield.[47] **8** was synthesized directly from indole-2-carboxylic acid using different peptide coupling reagents (scheme 4). Treatment of indole-2-carboxylic acid with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ),[48] in refluxing THF for 7 h provided **8** in a low yield (21 %). A much better yield could be obtained by using 1[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI)/(dimethylamino)pyridine (DMAP)[49] as reagents (95 %).



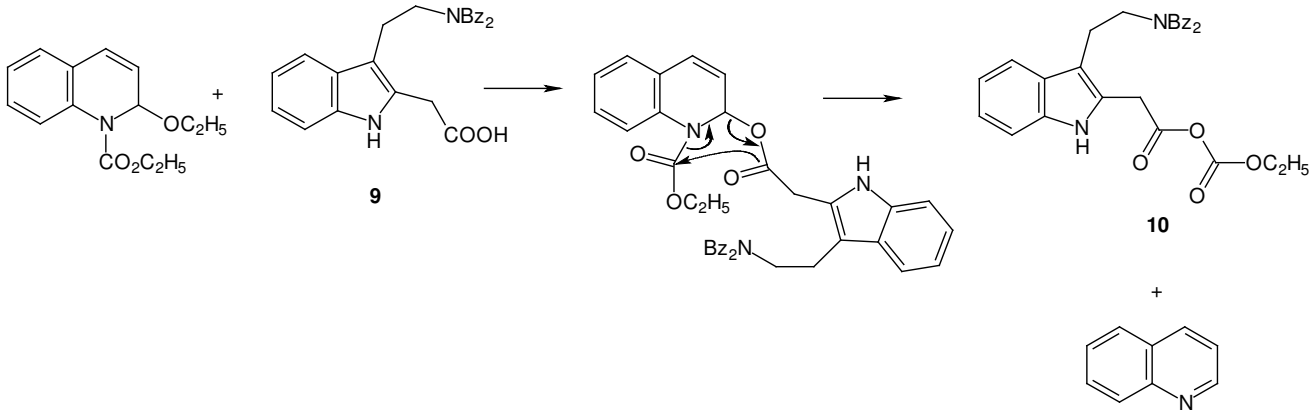
Scheme 4. (i) EDCI, DMAP, dry DMF, room temp., 16 h; (ii) EEDQ, dry THF, reflux, 7 h.

The starting material for the dimerization step was the acid **9** which could be prepared by a saponification of the ester **3** using methanolic KOH. Unfortunately, after treatment of **9** with the aforementioned coupling reagents no dimerization product was observed. Using EEDQ, an anhydride intermediate **10** could be isolated (Scheme 5). Furthermore, treatment of **3** with other coupling reagents, i.e., DCC and Mukaiyama's reagent [50, 51] was also unsuccessful.



Scheme 5. (i) 3% KOH (aq)/MeOH, reflux, 1 h; (ii) EDCI, DMAP, dry DMF, room temp., 16 h; (iii) EEDQ, dry THF, reflux, 6 h.

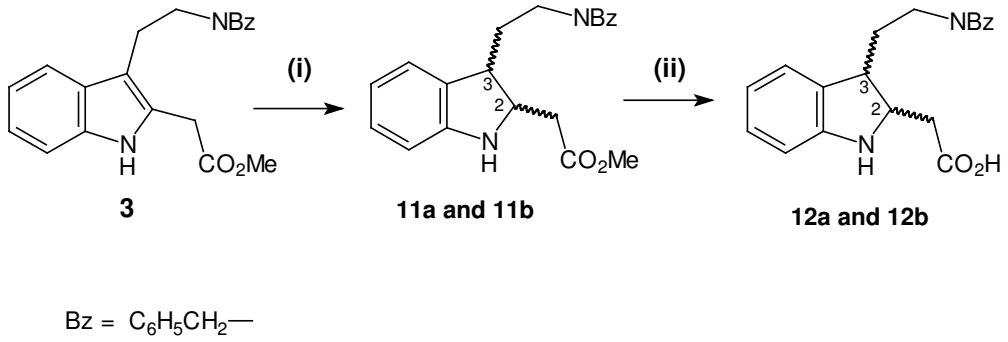
Due to the very small amounts of the anhydride intermediate **10**, an attempt to cyclize two molecules of **10** in the next step was not investigated. **10** was formed according to the following mechanism (Scheme 6).



Scheme 6. Mechanism for the formation of **10**.

The reason, why the self-condensation of **9** did not occur, might be the weak nucleophilic character of the indole nitrogen. Therefore, the indole double bond was reduced to the corresponding indoline, expecting that the increased nucleophilic character of the indole nitrogen would facilitate the lactame formation. The reduction of indole to the corresponding indoline was carried out by treatment of ester **3** with $NaBH_4$ in CF_3COOH giving a mixture of

trans and *cis* indoline **11**[52], which could be readily hydrolized to the corresponding acids **12**. However, after treatment of **12** with coupling reagents EEDQ and EDCI, no intermolecular dimerization product could be observed (Scheme 7).



Scheme 7. (i) $NaBH_4/CF_3COOH$, 0-10 °C, 7 h; (ii) 3% aq. KOH /MeOH, reflux, 1h.

^{1}H NMR spectra of both isomers of **11** (Fig. 23a) showed two sets of signals for the hydrogen atoms at C-2 and C-3, which could be assigned by means of HH-COSY diagram (Fig. 23b). Due to the very similar vicinal constants $J_{2H,3H}$ for both isomers (isomer 1 = 7.3 Hz, isomer 2 = 6.3 Hz), a clear *cis* and *trans* assignment as described by *Anet* and *Muchowski*[53] for other indoline derivatives was impossible.

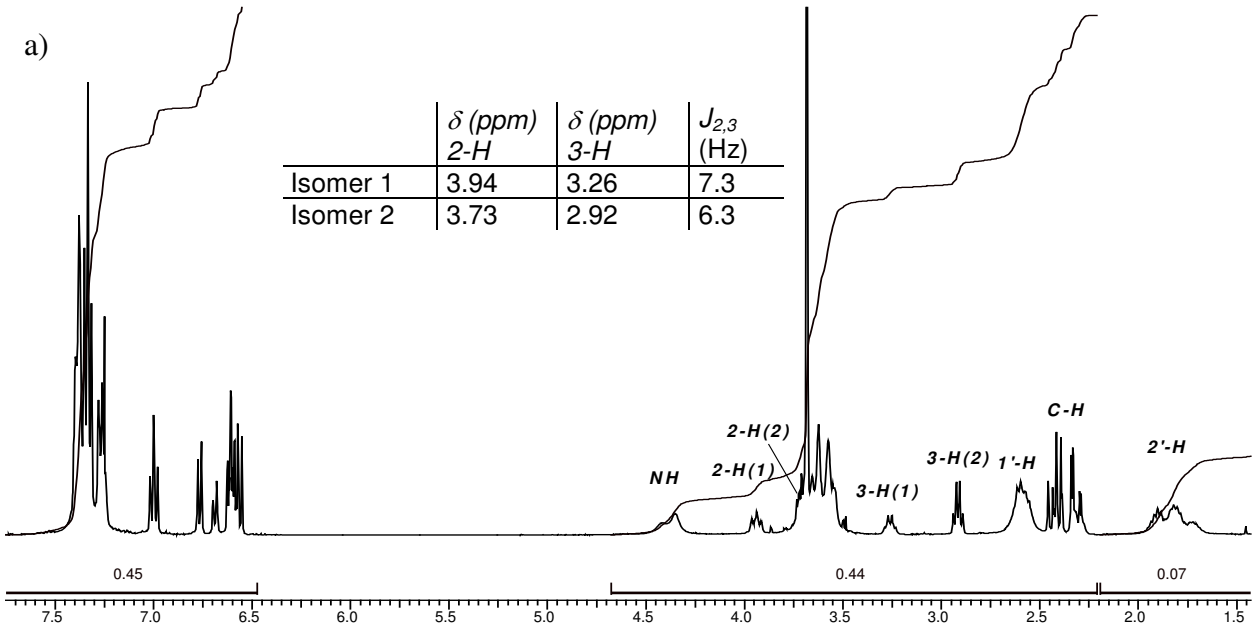


Figure 23. (a) 400 MHz ^{1}H-NMR spectrum of 11 ($CDCl_3$).

b)

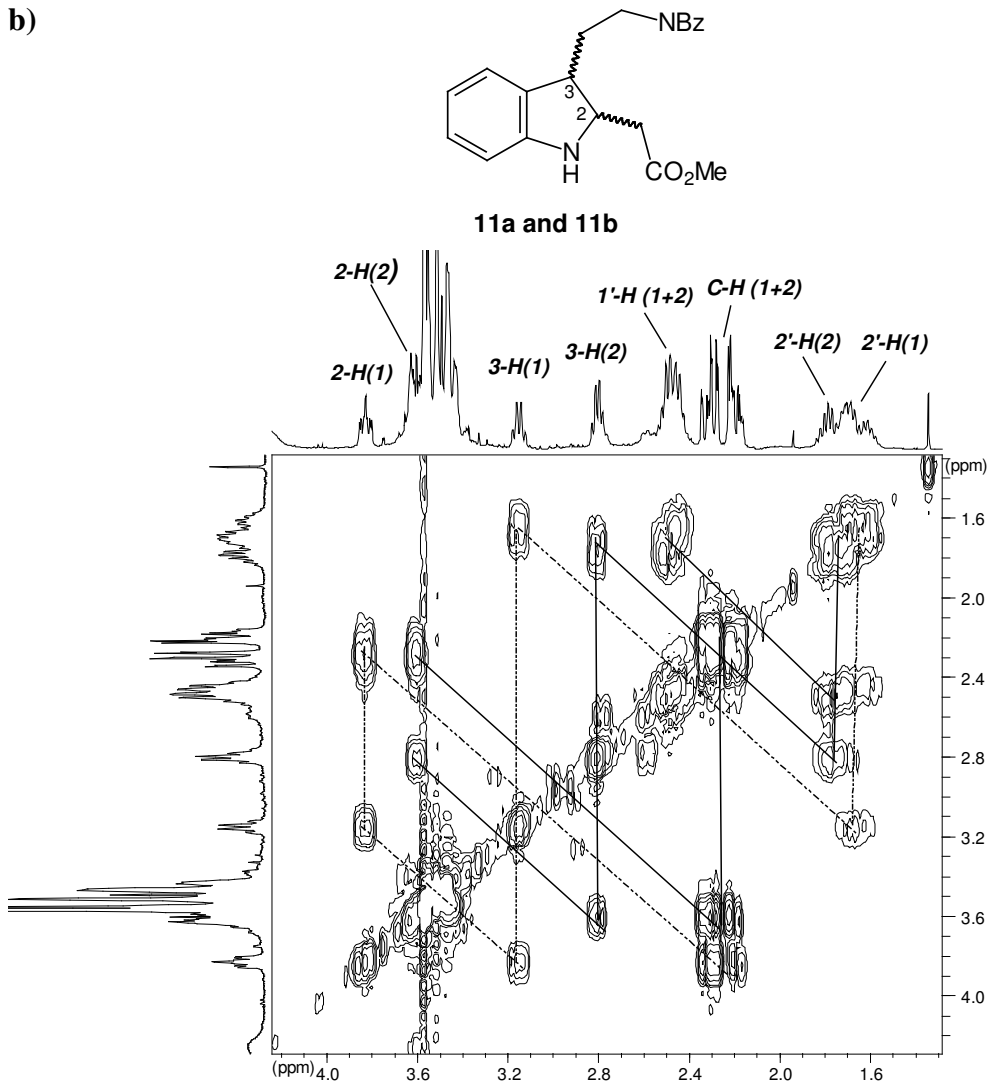
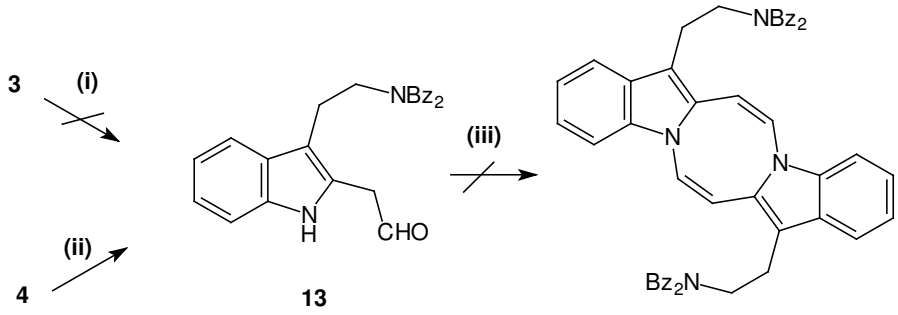


Figure 23. (b) Expanded region of a 400 MHz H,H-COSY contours plot of **11** ($CDCl_3$); (1) = isomer 1, (2) = isomer 2.

2.1.4 Investigation of the double enamine-formation approach

Another possible route for building the diazocinodiindole ring skeleton involves an intermolecular double enamine-formation from two molecules of indol-2yl-acetaldehyde (Fig 8, p. 11). The resulting ring system has two additional double bonds in the central eight-membered ring. We first investigated different routes to prepare the starting material, indol-2-yl acetaldehyde **13** from ester **3** and alcohol **4**, respectively (Scheme 8). Reduction of ester **3** with diisobutylaluminum hydride (DIBAL) at a low temperature should stop on an aldehyde

level. As indicated by ^{1}H-NMR spectrum of the reaction mixture, after treatment of **4** with DIBAL-solution in toluene at –60 °C, no aldehyde was formed. More successful were the oxidation attempts of alcohol **5**. While treatment of **4** with pyridine-SO_3 complex in DMSO and in methanesulfonic acid anhydride provided no aldehyde, the Swern oxidation using oxalyl chloride and DMSO led to the desired product **13**, as indicated by the resonance signals of the aldehyde proton at δ = 9.23 ppm (Fig. 24). The two doublets at δ = 6.39 and 6.01 ppm in ^{1}H NMR spectrum might result from the desired enamine. The crude product was used for the following dimerization step, because of a difficult purification of **13**.



Scheme 8. (i) DIBAL, dry toluene, -60 °C, 4 h; (ii) oxalyl chloride, DMSO, -60 °C, 15 min, NEt_3, 5 min, -60 °C, room temp.; (iii) pivalic acid, 120 °C, 17 h.

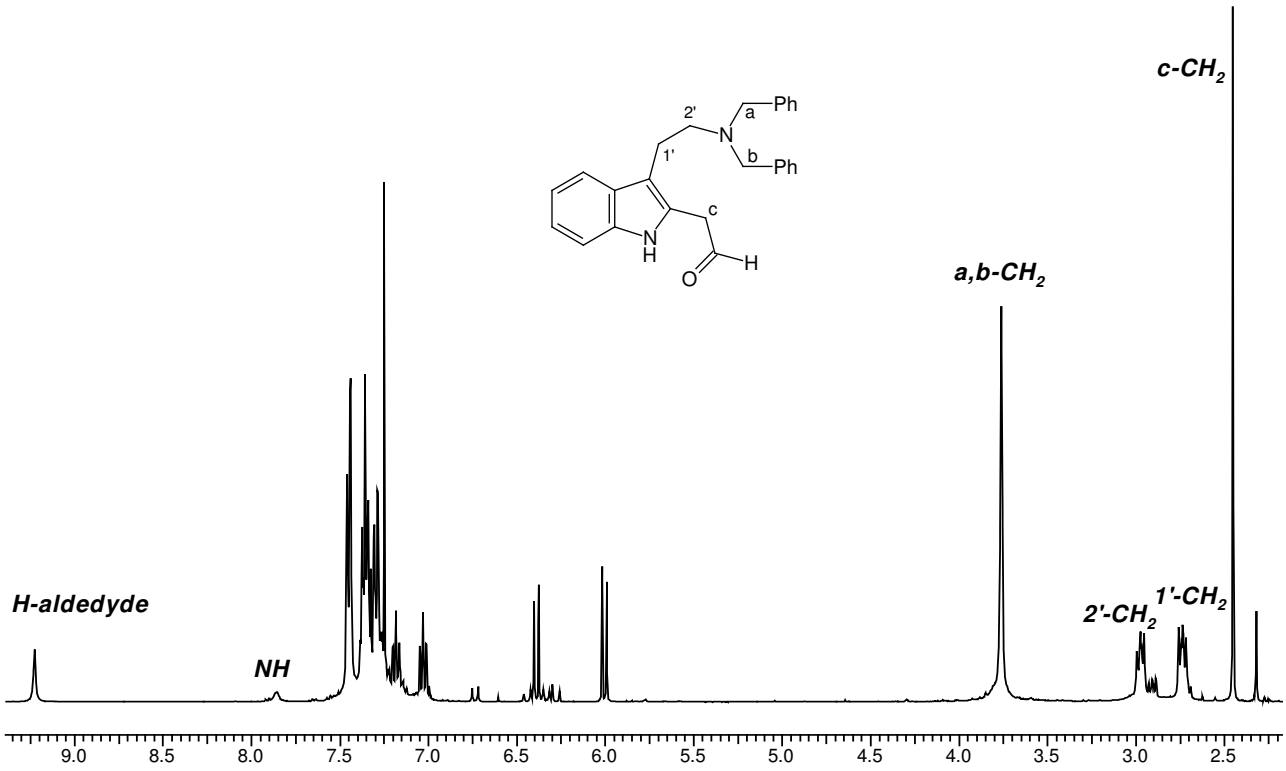
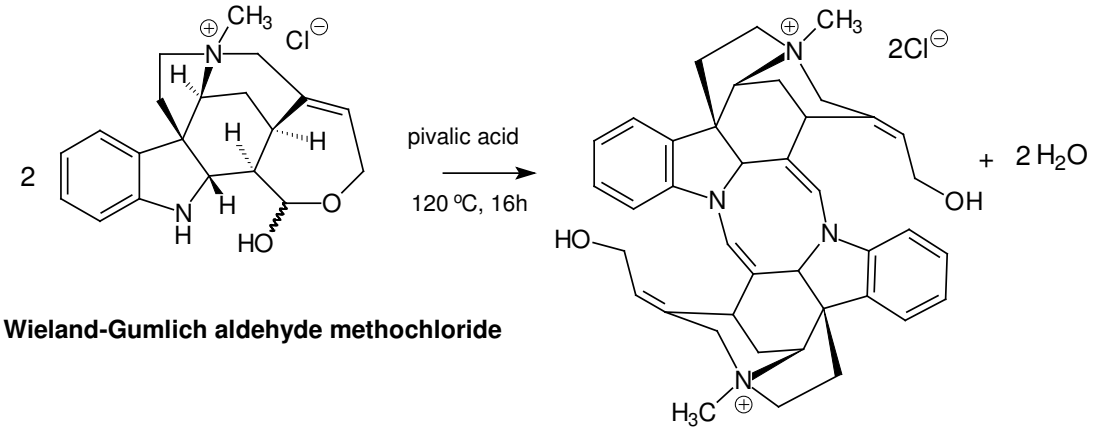


Figure 24. 400 MHz ^{1}H NMR spectrum of **13** ($CDCl_3$)

Battersby and *Hodson* synthesized the calabash-curare alkaloid toxiferine I by heating of Wieland-Gumlich aldehyde methochloride with pivalic acid in an evacuated sealed tube[54] (Fig 25). The same procedure was applied to aldehyde **13**. However, no condensation product was observed and the small amount of enamine from the latter step was probably decomposed.



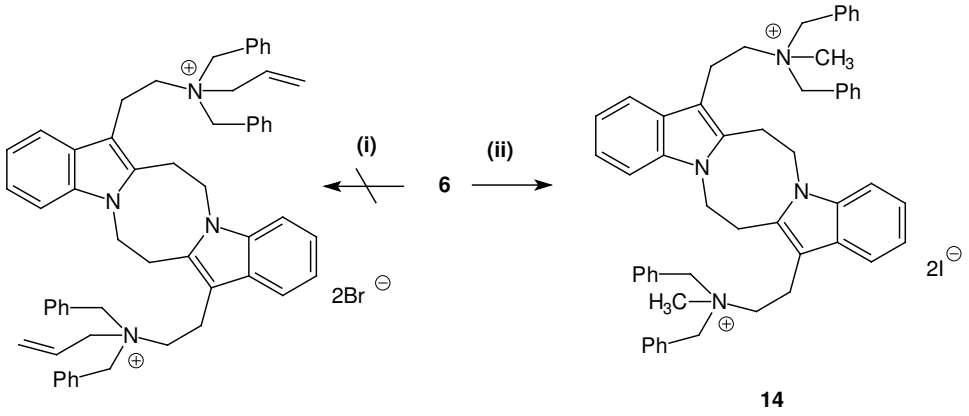
Wieland-Gumlich aldehyde methochloride

Toxiferine I

Figure 25. Synthesis of toxiferine I by condensation of Wieland-Gumlich aldehyde methochloride.

2.1.5 Quaternization of 6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]diindole

In a series of bisquaternary caracurine V salts, double quaternization of caracurine V base with small alkyl substituents, such as methyl or allyl groups, caused an approximately 50-fold increase in allosteric potency.[38] Expecting the same effect on the new heterocyclic ring system, it was aimed to quaternize **6** with methyl and allyl groups, respectively. Since treatment of **6** with methyl iodide under the reaction conditions used for the quaternization of caracurine V (2.5-fold excess, chloroform solution, room temperature) gave no quaternization product, pure methyl iodide was used as alkylation agent. After stirring of **6** in methyl iodide for three days at room temperature, the desired methoiodide could be isolated from the reaction mixture by adding diethyl ether (Scheme 9).



Scheme 9. (i) allyl bromide, room temp., 3 days; (ii) methyl iodide, room temp., 3 days.

The FABMS spectrum confirmed that the alkylation took place at both nitrogen atoms. Like the starting compound **6**, **14** is a non-symmetrical compound, as indicated by NMR-spectra (Fig. 26).

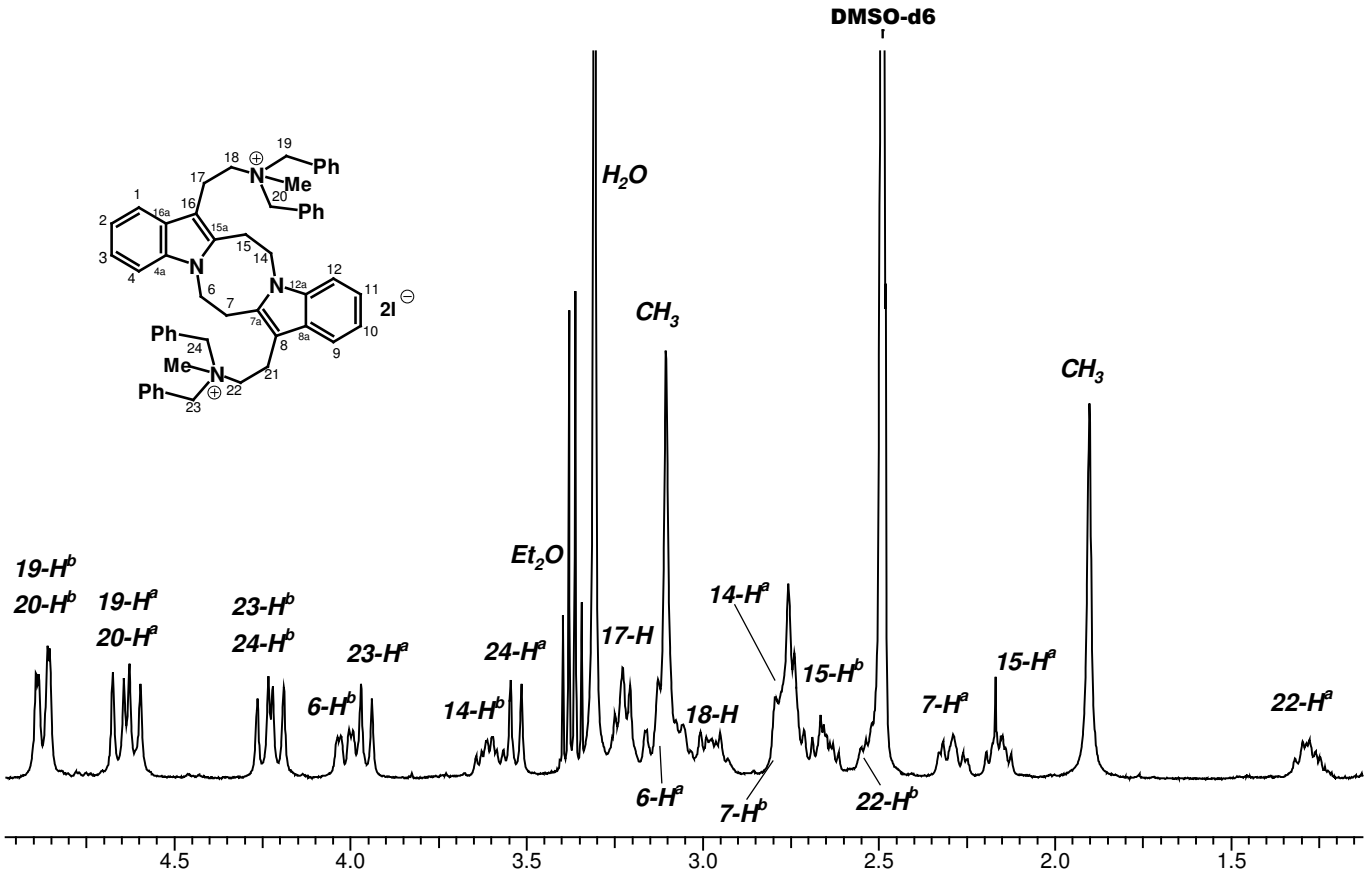


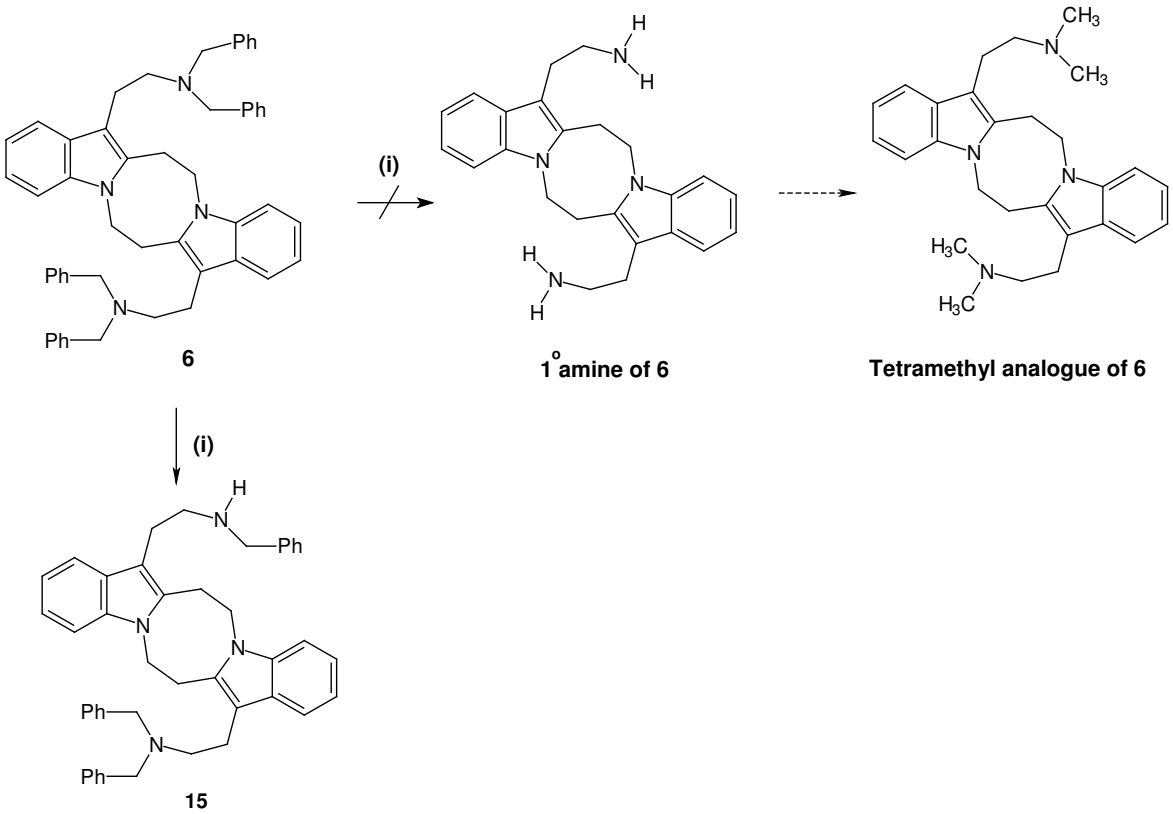
Figure 26. Expanded aliphatic section of 400 MHz ^{1}H NMR spectrum of **14** (DMSO-d_6).

2.1.6 Attempts to synthesize the tetramethyl analogue of 6

Since in the series of caracurine V salts, replacement of *N*-benzyl groups by smaller substituents caused an increase in allosteric potency, it was also tried to exchange the benzyl substituents of **6** by smaller groups.

Debenzylation of 6 by catalytic hydrogenation

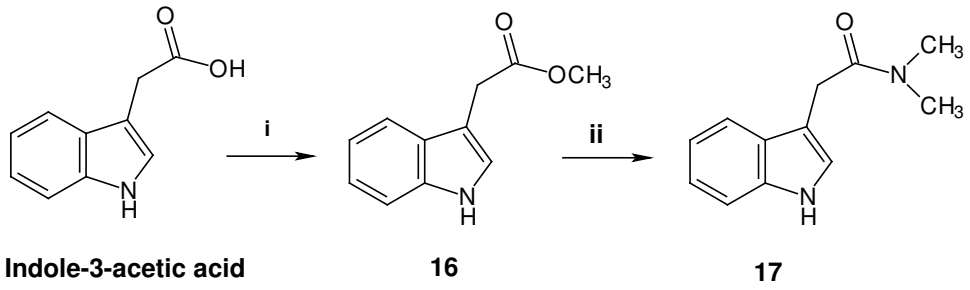
The first synthetic approach involved the debenzylation of **6** by catalytic hydrogenation and a subsequent reductive methylation using sodium cyanoborohydride/formaldehyde in acetic acid. The catalytic hydrogenation was performed by heating (60 °C) a solution of **6** in glacial acetic acid with 10% palladium on charcoal under 50 bar hydrogen pressure. However, the expected primary amine of **6** was not obtained. The only compound isolated was the monodebenzylation product **15** (Scheme 10).



Scheme 10. (i) 10 % Pd/C, CH_3COOH, H_2 (50 bar), 60 °C, 3 days.

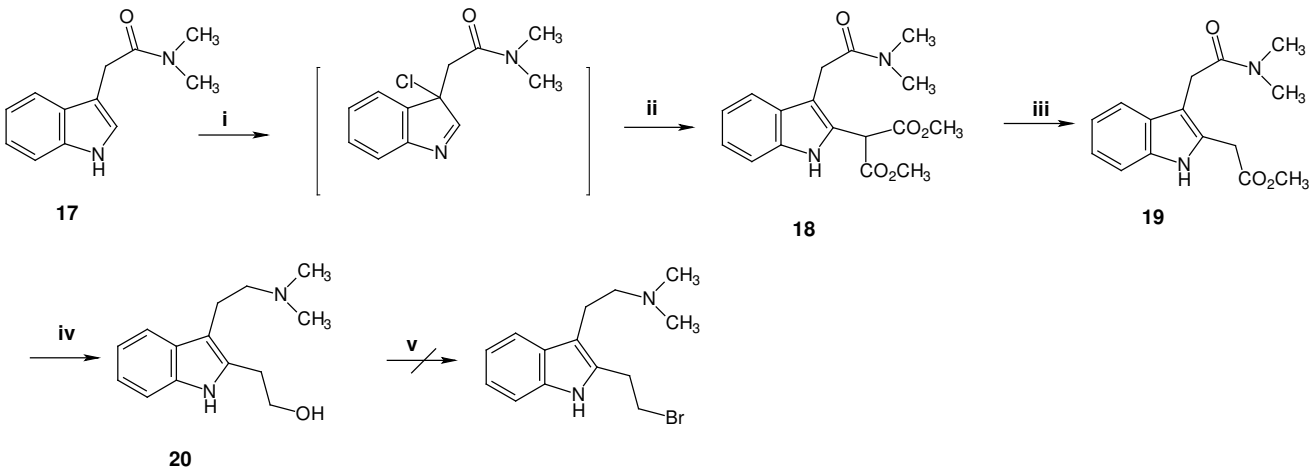
2-(1*H*-Indol-3-yl)-*N,N*'-dimethyl acetamide as a starting material

Another approach for the synthesis of the tetramethyl analogue of **6** started from the known 2-(1*H*-indol-3yl)-*N,N*-dimethyl acetamide **17**, which was prepared according to the procedure previously described by *Sintas* and *Vitale*[55]. A commercially available, indole-3-acetic acid was esterified by refluxing with conc. H_2SO_4 in MeOH and subsequently treated with 40 % aqueous dimethylamine to give the corresponding amide **17** in an overall yield of 33% (Scheme 11).



Scheme 11. (i) conc. H_2SO_4, dry MeOH, reflux, 5 h; (ii) 40% aq. NMe_2, room temp., overnight.

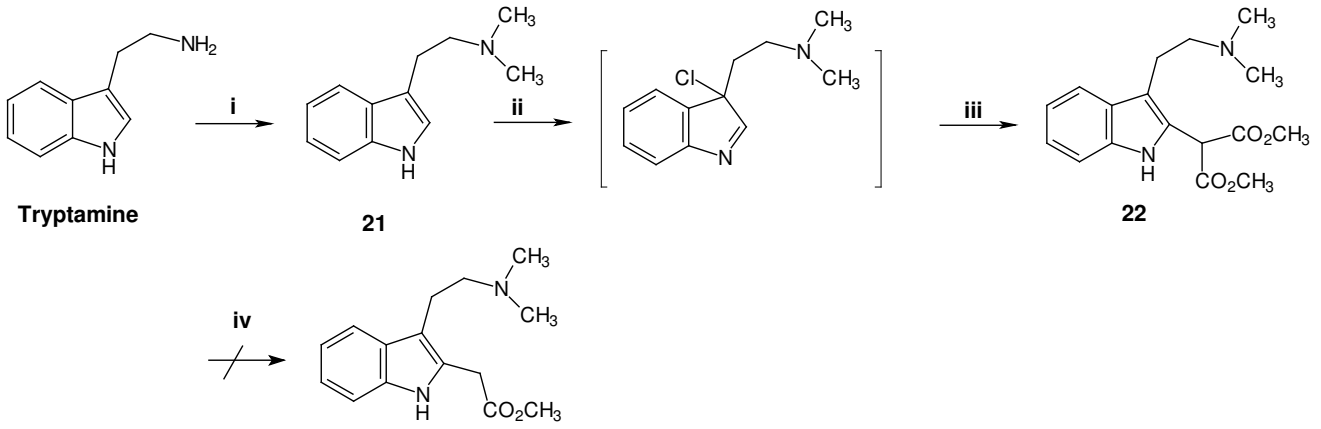
For the following steps (Scheme 12), the same procedure as described for the synthesis of **6** was applied. Chlorination of **17** with *tert*-BuOCl in the presence of triethylamine gave the chloroimine intermediate, which was treated by TlDMM to give diester **18** in 61 %. Demethoxycarbonylation was carried out by heating diester **19** with lithium iodide hydrate in dimethylacetamide to give only a small amount (17 %) of the corresponding monoester **19**. Simultaneous reduction of both carbonyl functions in **19** was achieved by means of $LiAlH_4$ in THF to obtain the corresponding compound **20** in 49 % yield. Surprisingly, the alcohol **20** failed to react with $P(NMe_2)_3$ and CBr_4 or with PBr_3 to give the corresponding bromide product. Hence, the final cyclization step could not be carried out.



Scheme 12. (i) *tert*-BuOCl, NEt_3, dry THF, -78 °C, 3 h; (ii) TlDMM, dry THF, -78 °C 1 h, room temp. 12 h; (iii) LiI xH_2O, DMA, 130 °C, 3 h. (iv) $LiAlH_4$, dry THF, room temp., 3 h; (v) CBr_4, $P(NMe_2)_3$, dry CH_2Cl_2, room temp., 16 h.

Tryptamine as a starting material

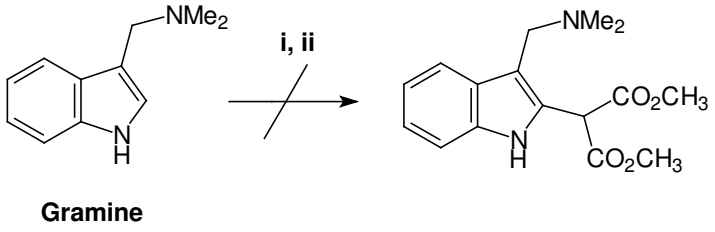
Yet another attempt to synthesize the tetramethyl analogue of **6** involved tryptamine as a starting compound. Reductive methylation of tryptamine could be accomplished using formaldehyde and sodium cyanoborohydride in acetic acid to produce *N,N*-dimethyl tryptamine **21**. Introduction of the malonate moiety was successful by utilizing the same procedure as for the synthesis of **2** to give diester **22** in 46%. However, this synthetic route terminated, since the demethoxycarbonylation of **22** failed (Scheme 13), probably due to difficulties by the isolation of the product from the DMF solution.



Scheme 13. (i) $NaCNBH_3$, HCHO, CH_3COOH, room temp., 3 h; (ii) *tert*-BuOCl, NEt_3, dry THF, -78 °C, 3 h; (iii) TlDMM, dry THF, -78 °C 1 h, room temp., 12 h; (iv) LiI xH_2O, DMA, 130 °C, 3 h.

2.1.7 Synthesis of 6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole ring system (35)

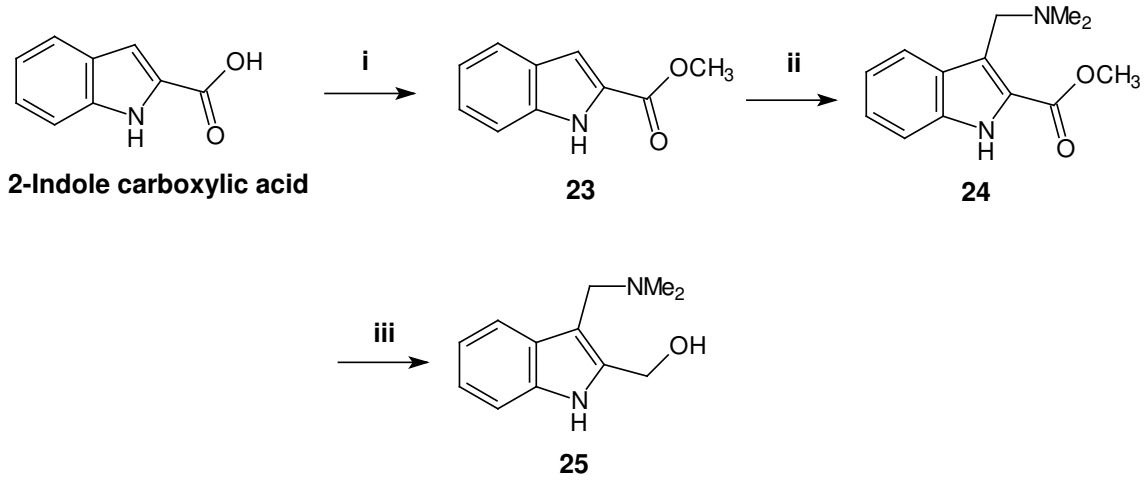
In order to examine the influence of the length of the side-chains on muscarinic activity, the ethylamine moieties of **6** should be replaced by methyl amino groups (Fig. 6, p. 10). Similar to the synthesis of **2**, it was first tried to introduce the malonester moiety to C-2 of gramine using *tert*-BuOCl/TlDMM. However, the desired diester could not be obtained (Scheme 14).



Scheme 14. (i) *tert*-BuOCl, NEt_3, dry THF, -78 °C, 3 h; (ii) TlDMM, dry THF, -78 °C 1 h, room temp., 12 h.

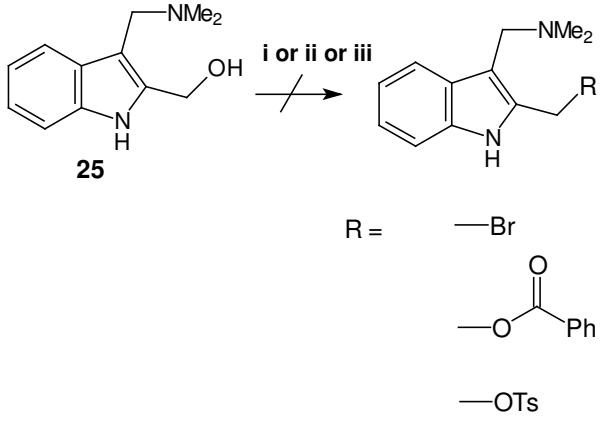
An alternative strategy employing 2-indole carboxylic acid as a starting compound was used. After esterification of the acid by refluxing with conc. H_2SO_4 in MeOH, the dimethylamino moiety was introduced at C-3 of the indole ring by means of a Mannich reaction. Treatment of

2-indole carboxylic acid methylester (**23**) with a mixture of formaldehyde and dimethylamine under acid conditions at high temperature gave methyl gramine carboxylate (**24**) in an excellent yield (86 %). Reduction of **24** by means of $LiAlH_4$ in THF gave alcohol **25** (Scheme 15).



Scheme 15. (i) conc. H_2SO_4 , dry MeOH, reflux, 3 h; (ii) 40 % aq. NMe_2 (1.2 equiv.), 40 % aq. HCHO (1.2 equiv.), CH_3COOH, warm until clear, room temp., 2h; (iii) $LiAlH_4$, dry THF, room temp., 3 h.

For the dimerization step the side-chain at C-2 had to be increased by one carbon atom. The homologation of **25** might be achieved by the reaction sequence developed by *Kutney et al*[56] involving the nucleophilic substitution of the corresponding appropriate leaving groups, e.g., bromide, benzoylate, and tosylate, with KCN. Nevertheless, attempts to convert the hydroxy group of **25** to several leaving groups mentioned above, failed (Scheme 16).



Scheme 16. (i) CBr_4, $P(NMe_2)_3$, dry CH_2Cl_2, room temp. 16 h; (ii) PhCOCl, NEt_3, dry THF, room temp., 4h; (iii) TsCl, NEt_3, dry CH_2Cl_2, room temp., overnight.

Alternatively, the introduction of dimethylamino moiety by means of Mannich reaction might be carried out after the dimerization step (Fig. 27).

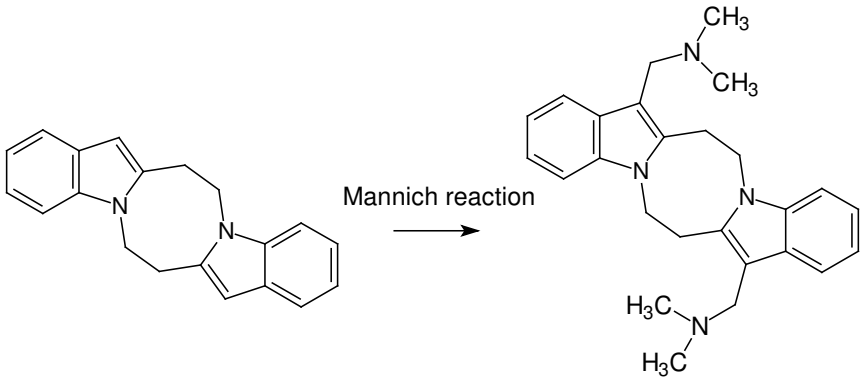
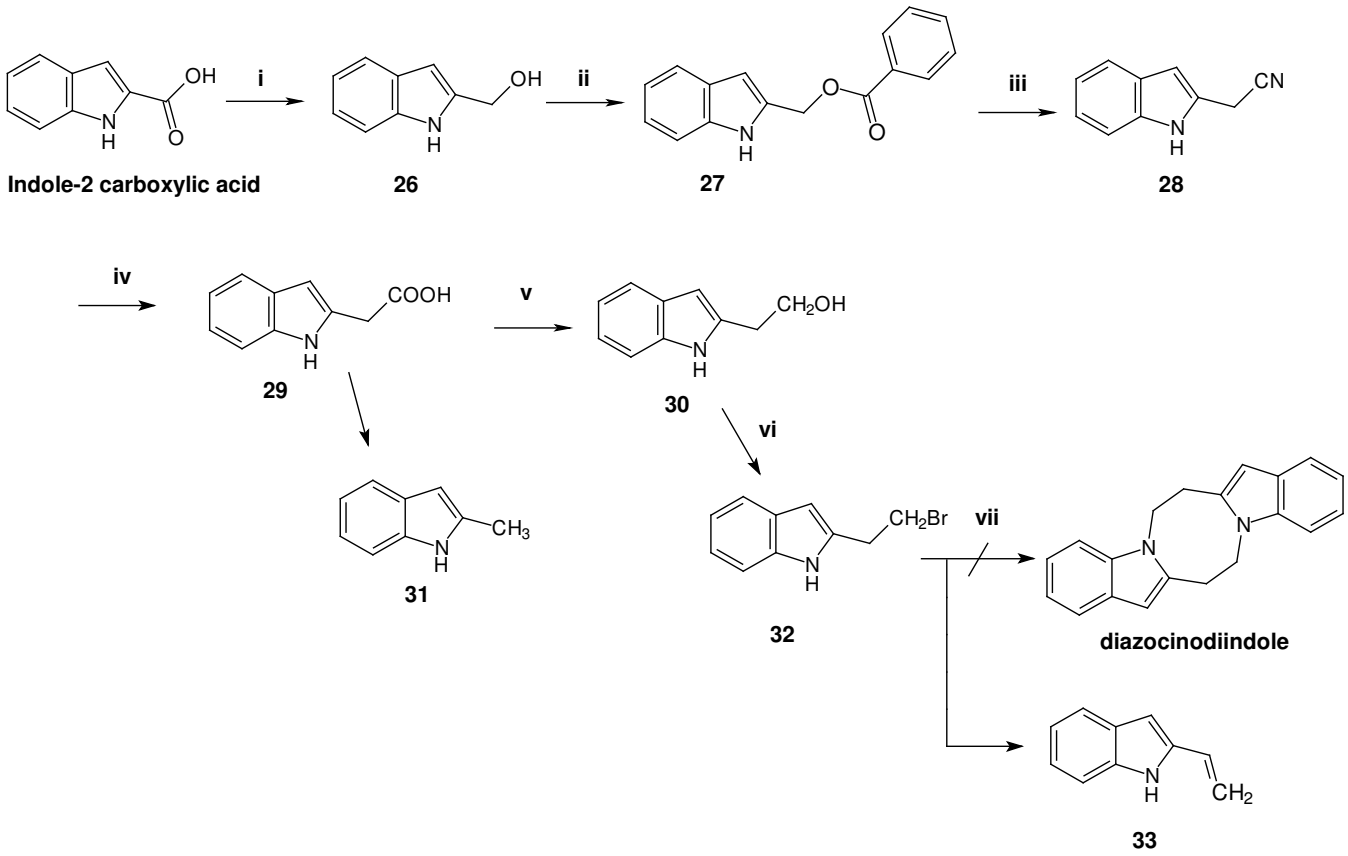


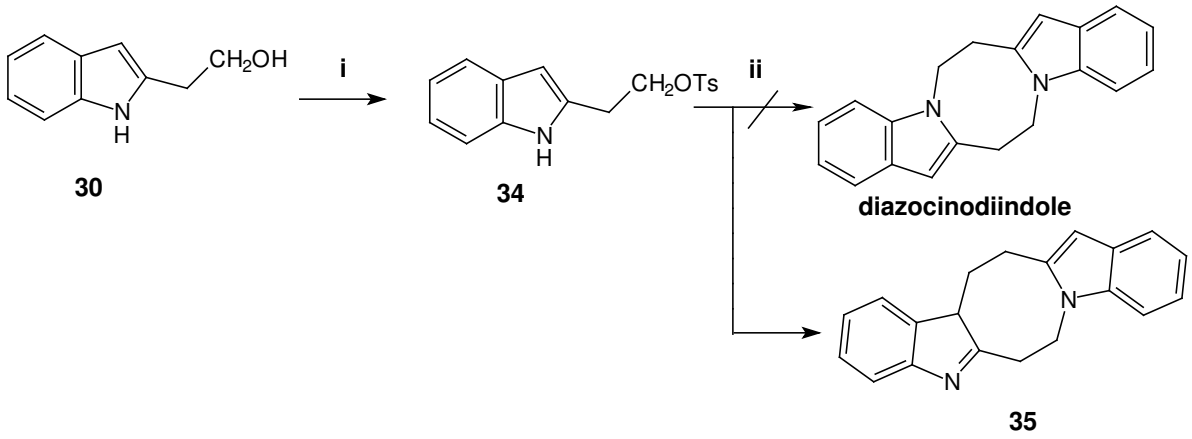
Figure 27. An alternate synthesis plan of diazocinodiindole ring with methylamine side-chains.

Conversion of indole-2-carboxylic acid to the homologous indol-2-yl acetic acid could be accomplished using the reaction sequence by *Kutney et al.*[56] Reduction of indole-2-carboxylic acid with $LiAlH_4$ by refluxing in THF for 5 h gave indol-2-yl methanol (**26**). Benzoylation of the hydroxy group of **26** using benzoyl chloride in THF gave the corresponding benzoylate **27** quantitatively. Reaction of **27** with KCN in DMSO led to indol-2-ylacetonitrile (**28**) in rather good yields. Finally, hydrolysis of **28** by refluxing in methanolic NaOH gave indole-3-acetic acid (**29**) in 79 %. It should be noted that the acid **29** was unstable to storage, due to an easy decarboxylation to 2-methyl indole (**31**). Thus, after hydrolyzation of **28**, reduction of **29** using $LiAlH_4$ in THF to give indol-2ylethanol (**30**) was carried out immediately. The hydroxy group of the alcohol **30** was replaced with bromine under mild condition using $P(NMe_2)_3$ and CBr_4 in CH_2Cl_2 to give the corresponding bromide **32** in 24 %. Attempts to dimerize **32** under the reaction conditions applied for the synthesis of **6** failed. After treatment of **32** with sodium hydride in DMF only 2-vinyl-indole **33** was isolated as a result of the HBr elimination from **32** (Scheme 17).



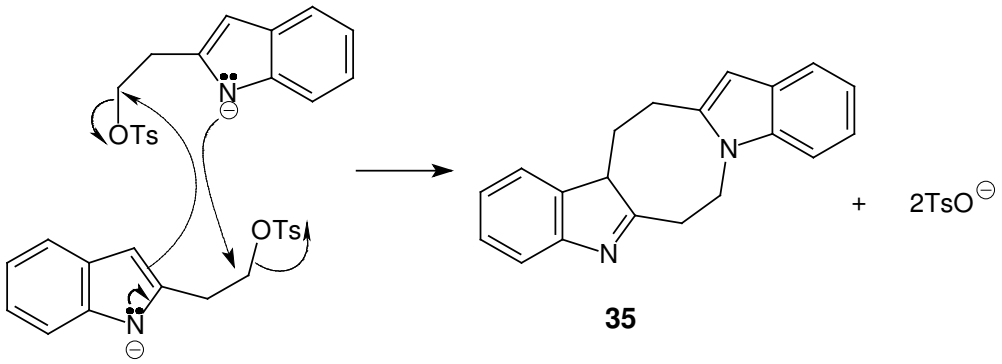
Scheme 17 (i) $LiAlH_4$, dry THF, reflux, 6h; (ii) PhCOCl, NEt_3, dry THF, room temp., 4h; (iii) KCN, dry DMSO, 60 ^oC, 7 h; (iv) 30% aq. NaOH, MeOH, reflux, 6h; (v) $LiAlH_4$, dry THF, reflux, 6h; (vi) CBr_4, $P(NMe_2)_3$, dry CH_2Cl_2, room temp. 16 h; (vii) NaH, dry DMF, 0 ^oC 15 min, room temp. 20 min.

In the cyclization step, there is a competition reaction between elimination and substitution reaction under strong base condition. The rate of E2 elimination depends on the acidic property at the aliphatic β-position. The bromide group, which is a good electron withdrawing group, can increase the rate of E2 eliminations, due to the increasing of the acidity at the β-position, whereas other leaving groups, for example, tosylate group exhibit only small rates of E2 elimination.[57,58] Thus, in order to suppress the side-product **33** the tosylate of alcohol **30** (Compound **34**) was used as a starting material for the crucial double alkylation step. Reaction of **30** with tosyl chloride in the presence of triethylamine in CH_2Cl_2 gave **34** in 70 % yield. The dimerization step was carried out by treatment of **34** with NaH in DMF. Interestingly, instead of the expected diazocinodiindole, an isomeric pentacyclic ring system: 6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole (**35**), was exclusively formed (Scheme 18).



Scheme 18. (i) TsCl, NEt_3, dry CH_2Cl_2, room temp., overnight; (ii) NaH, dry DMF, 0 °C 15 min, room temp. 20 min.

The formation of this novel ring system **35** could be explained by a reaction mechanism shown in Scheme 19. Because of the ambident nucleophilic character of the unsubstituted-indolyl anion, it can be alkylated either at nitrogen or at β–position.[59] In contrast, when the 3-position of the indole ring was blocked, as given in compound **6**, the dimerization led to the symmetrical double *N*-alkylation product.



Scheme 19. Reaction mechanism of the synthesis of **35**.

The HREIMS showed the expected molecular ion $[M-1]^+$ at *m/z* 285.1393 and confirmed the proposed structural formula $C_{20}H_{18}O_2$. Inspection of the 400 MHz ^1H NMR spectrum and COSY data established the presence of four independent ^1H spin systems, two aromatic (2 x indole) and two aliphatic, in addition to an isolated proton at δ = 6.16 ppm, which could be assigned to H-13. The connecting pathway in the COSY diagram starting from H-15a at δ = 4.73 ppm to H-15^a and H-15^b, and further to H-14^a and H-14^b revealed the first aliphatic spin

system as a 15aH-15CH_2-16CH_2 chain. The remaining aliphatic signals correspond to the ethylene protons at C-6 and C-7 (Fig. 28).

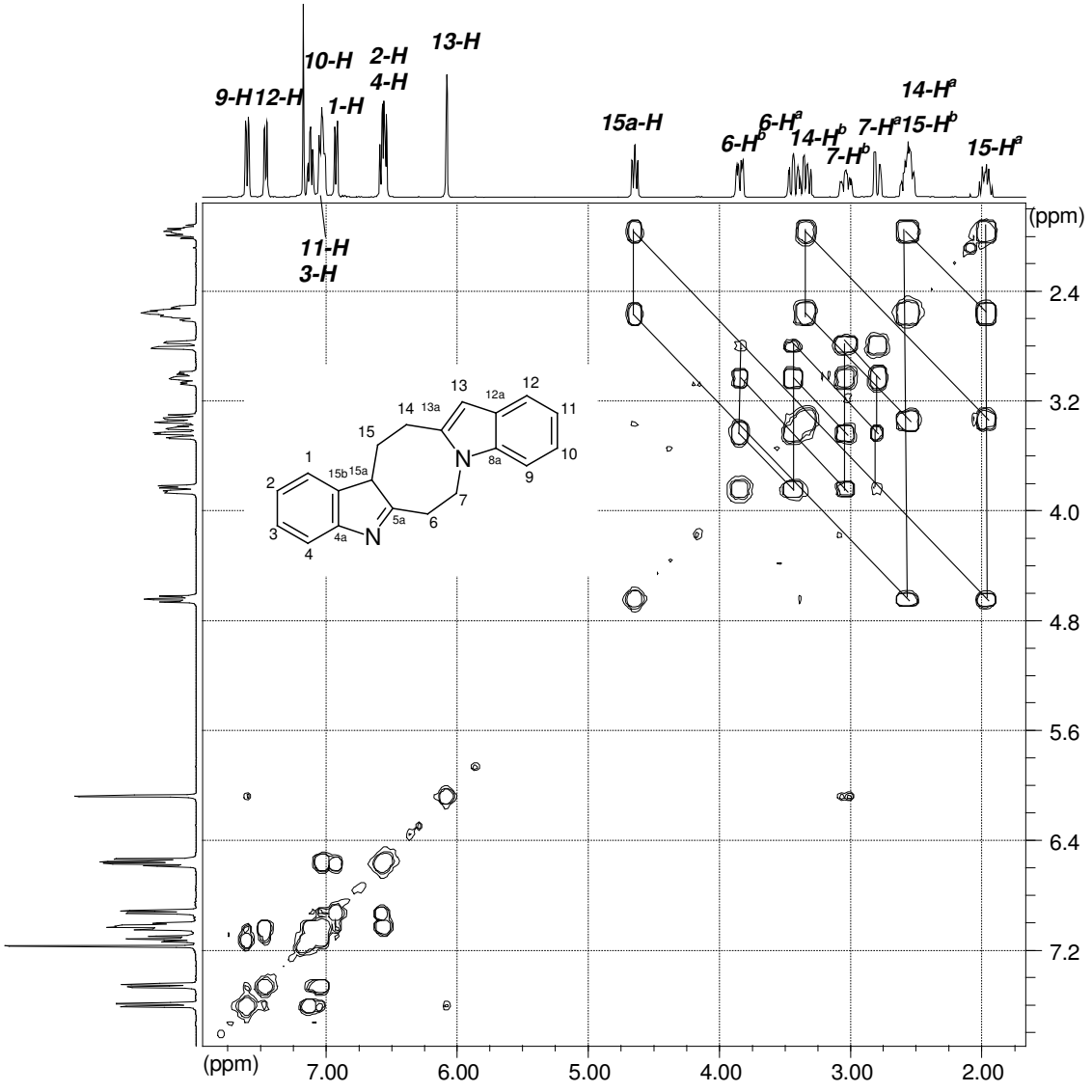


Figure 28. 400 MHz H,H-COSY contours plot of **35** ($CDCl_3$).

Furthermore, the HMBC-cross peaks from H-15^a and H-15^b to C-15a, C-15b, and C-5a indicated the substitution of the lower indole ring at C-15a (Fig 29). The substitution of the upper indole ring at nitrogen could be confirmed by the HMBC-cross peak from H-7 to C-13a (Fig 29).

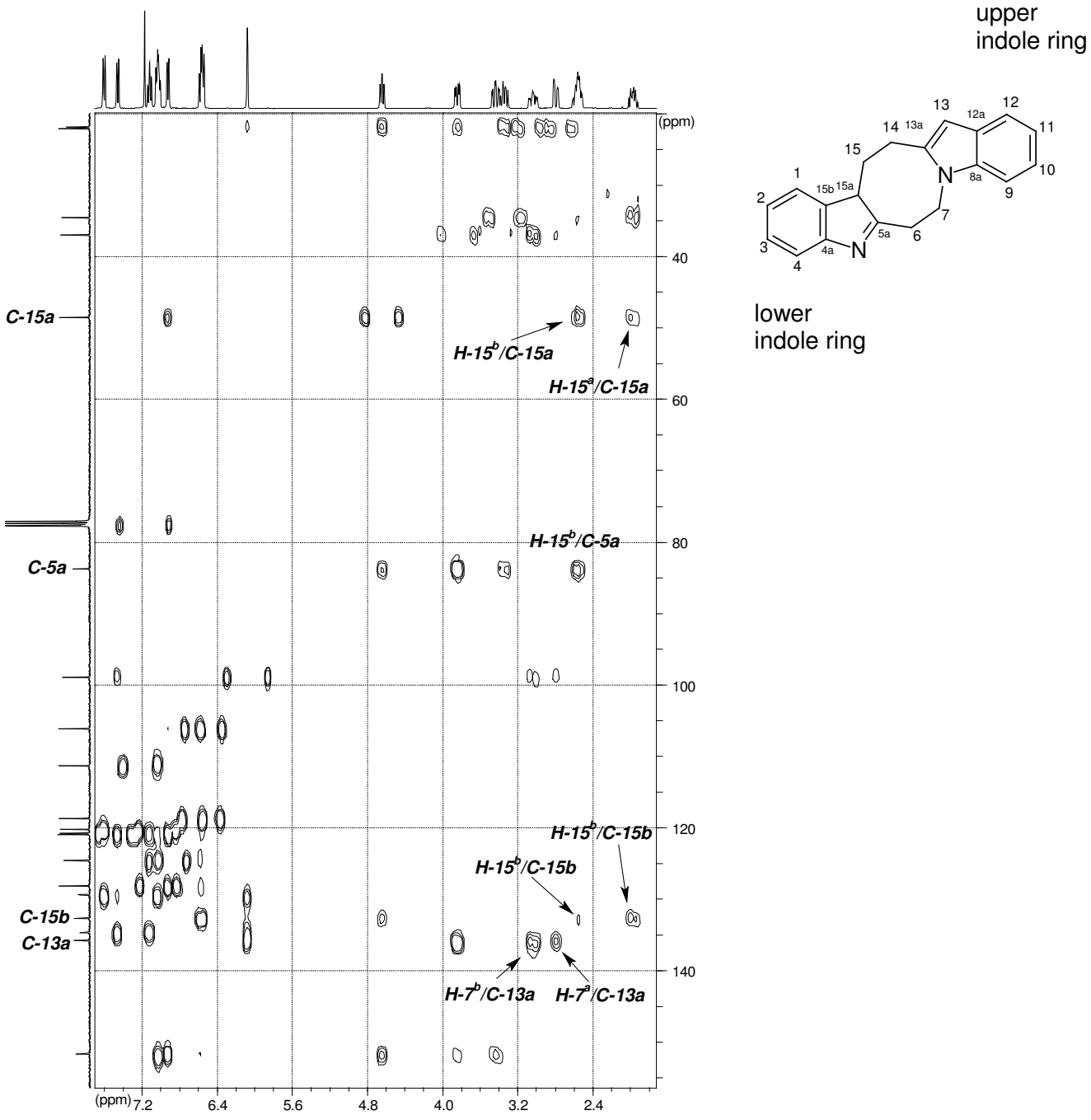
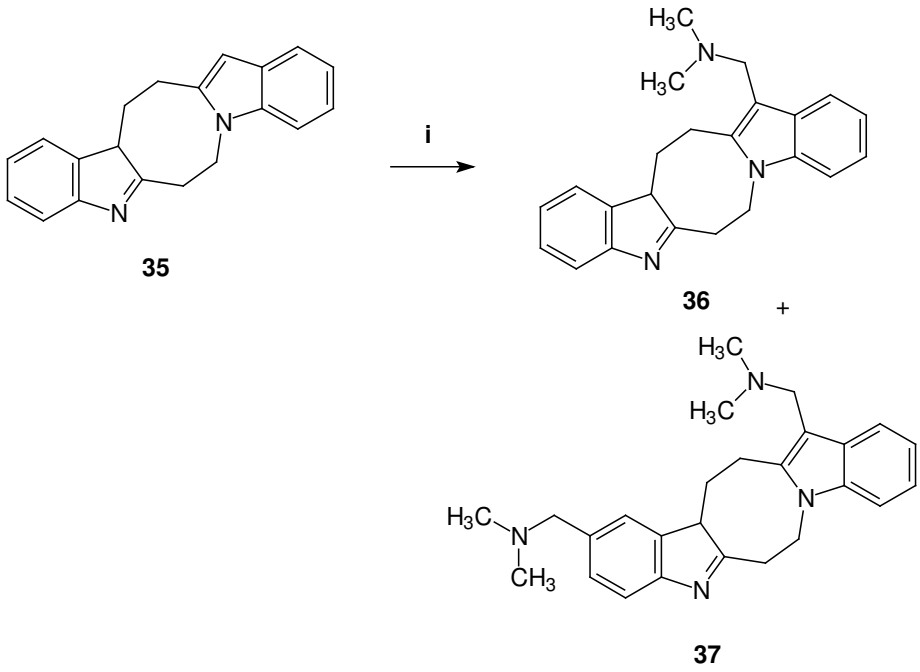


Figure 29. 400 MHz HMBC contours plot of **35** ($CDCl_3$).

2.1.8 Mannich reaction of 6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole

In order to obtain potential muscarinic active compounds, the new ring system was subjected to a Mannich reaction (Scheme 20). Treatment of **35** with dimethylamine and formaldehyde in acetic acid provided two products, **36** and **37,** which could be separated by column chromatography on silica gel with $CHCl_3$: MeOH: 25% NH_3 / 100: 10: 1 as eluent. The more

polar compound **37** was the desired double-aminoalkylation product with the 2,13-disubstitution pattern. The presence of a small amount of the 13-monosubstituted product **36** indicated that the first aminomethylation occurred at C-13.



Scheme 20. (i) 40 % aq. $N(CH_3)_2$ (3 equiv.), 40 % aq. HCHO (3 equiv.), CH_3COOH, 4h.

The position of the second aminomethyl group at the aromatic ring (C1-C15b) of **37** could be elucidated by NMR. HMBC correlations from C-4a and C-15a revealed H-1 as a narrow doublet at δ = 6.95 with a typical *meta* coupling constant $J_{1H\text{-}3H}$ = 1.5 Hz. This coupling pattern is only possible when proton H-2 is absent. The C-2 substitution could be confirmed by HMBC-cross peaks from H-1 and H-3 to the methylene protons of C-16 (Fig. 30).

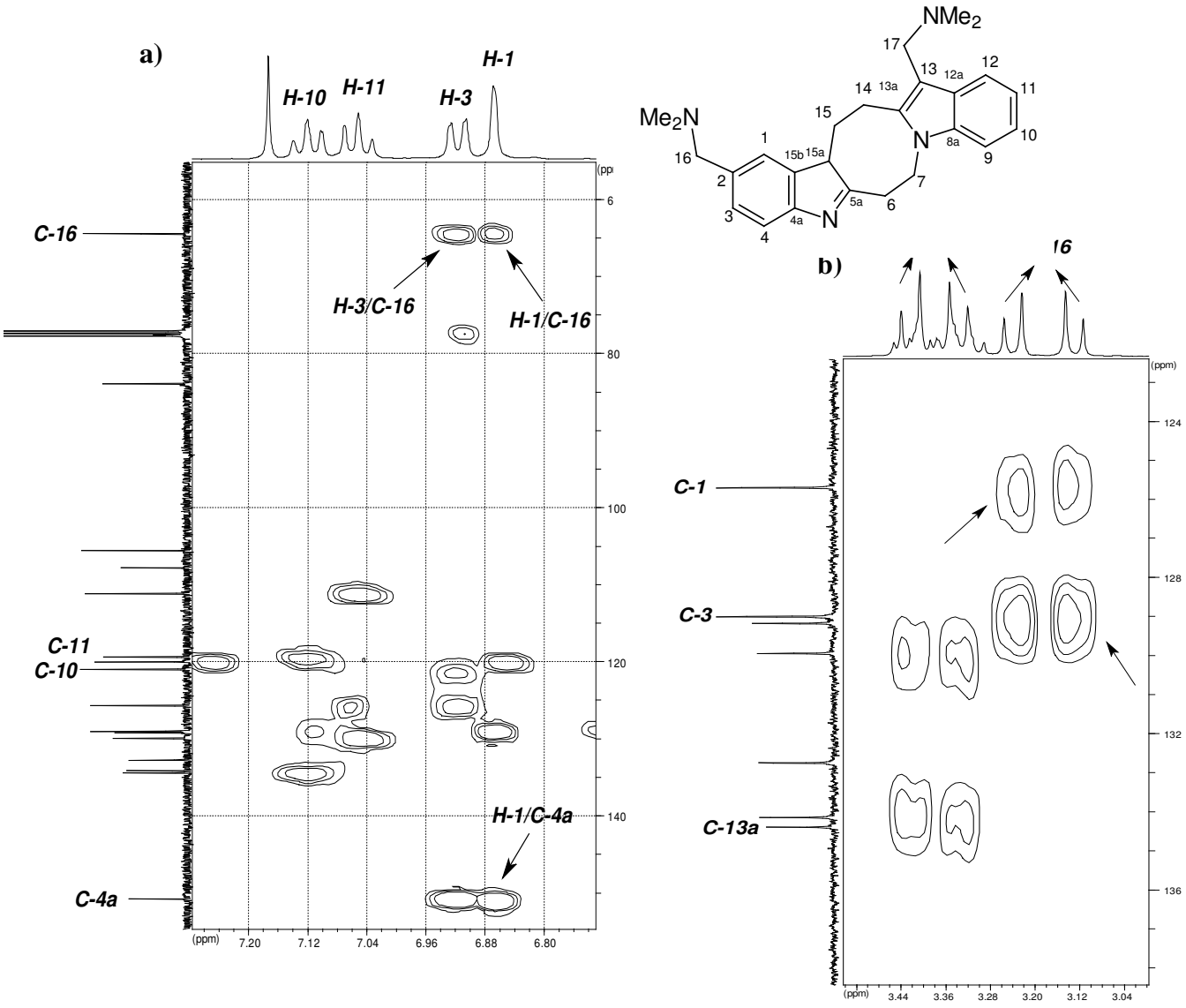
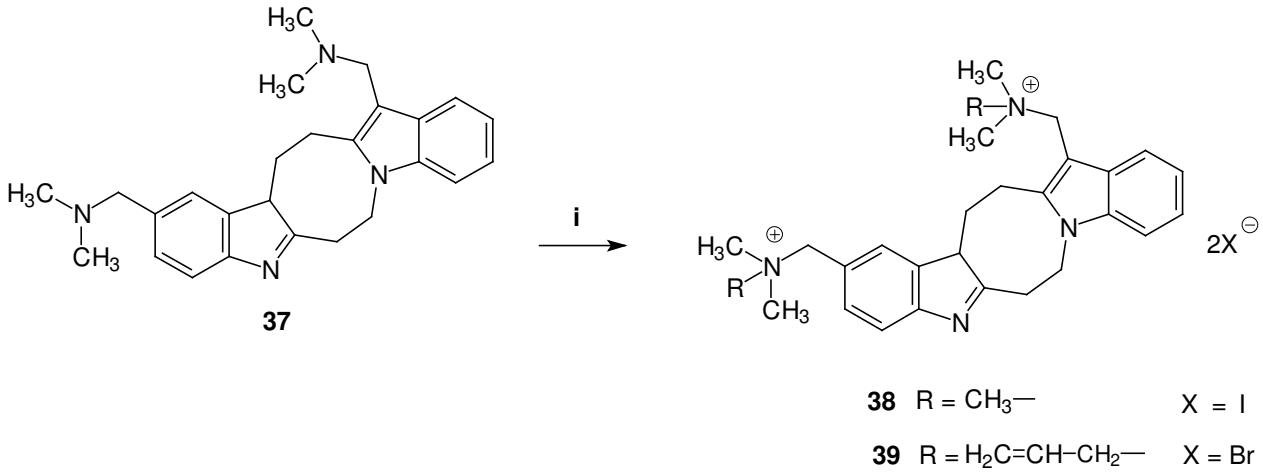


Figure 30. (a) Expanded region of aromatic region of 400 MHz HMBC contours plot of **37** ($CDCl_3$);

(b) Long range ^{1}H-^{13}C couplings of the methylene protons at C-13 and C-2 of **37** in 400 MHz HMBC diagram ($CDCl_3$).

2.1.9 Quaternization of 2,13-bis-(dimethylaminomethyl)-6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole

Double quaternization of **37** was accomplished by stirring of **37** for 1h at room temperature in the pure methyliodide and allylbromide, respectively, to give the methyl **38** (59 %) and the allyl **39** (72 %) ammonium salts of **37**, respectively (Scheme 21). The structure of the desired bisquarternary salts could be confirmed by FABMS and NMR spectra (see Experimental section).



Scheme 21. (i) methyliodide, room temp., 1h and allylbromide, room temp., 1h.

2.2 Pharmacological Studies

All pharmacological experiments were carried out by the group of Prof. Dr. K. Mohr, Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Bonn.

In order to determine the allosteric potency of the compounds **14**, **38**, and **39**, their ability to allosterically retard the dissociation of [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) from porcine cardiac M_2 receptors was measured. Dissociation assays were conducted in a buffer composed of 4 mM Na_2HPO_4 and 1 mM KH_2PO_4 (pH 7.4) at 23 ^oC. Cardiac membranes were preincubated with [^{3}H]NMS (0.2 nM) for 30 min; radioligand dissociation was then revealed by the addition of 1 μM atropine, in the presence or absence of the allosteric modulator. The time course of dissociation was observed by collecting aliquots at various times over a period of 120 min. Membranes were separated by vacuum filtration and membrane bound radioactivity was determined by liquid scintillation counting. Experimental results were analyzed by nonlinear regression analysis (Prism 2.01, Graph Pad®). Dissociation data were fitted using a monoexponential decay function that yielded the apparent rate constant of dissociation k_{-1}. To obtain concentration-effect curves (Fig. 31) for the retardation of radioligand dissociation, curve fitting was based on a four parameter logistic function. The concentration which retard [^{3}H]NMS dissociation by a factor of 2 ($EC_{50,diss}$) served as a measure of allosteric potency.

The effect of the allosteric test compound on [^{3}H]NMS equilibrium binding was investigated at equieffective concentration ($EC_{25,diss}$). $EC_{25,diss}$ is the concentration of test compound at which the rate of [^{3}H]NMS dissociation is reduced to 25 % of the control value. Equilibrium binding data in the presence of allosteric modulator were expressed as a percentage of the value under control conditions, which was set as 100 %. If the allosteric agent enhances [^{3}H]NMS equilibrium binding ($EC_{25,diss}$ >100 %), there is a positive cooperativity between the allosteric and the orthosteric compound. In the case of negative cooperativity, [^{3}H]NMS binding is lowered by allosteric agent and $EC_{25,diss}$ <100 %. Neutral cooperativity means that [^{3}H]NMS binding is unchanged despite allosteric agent binding to the receptors and $EC_{25,diss}$ =100 %.

All investigated compounds were able to retard the dissociation of [^{3}H]NMS at similar $EC_{50,diss}$ concentrations. Screening of the [^{3}H]NMS equilibrium binding at equieffective concentrations showed that compounds **38** and **39** are negative cooperativity with the [^{3}H]NMS. The pharmacological results are complied in Table 2.

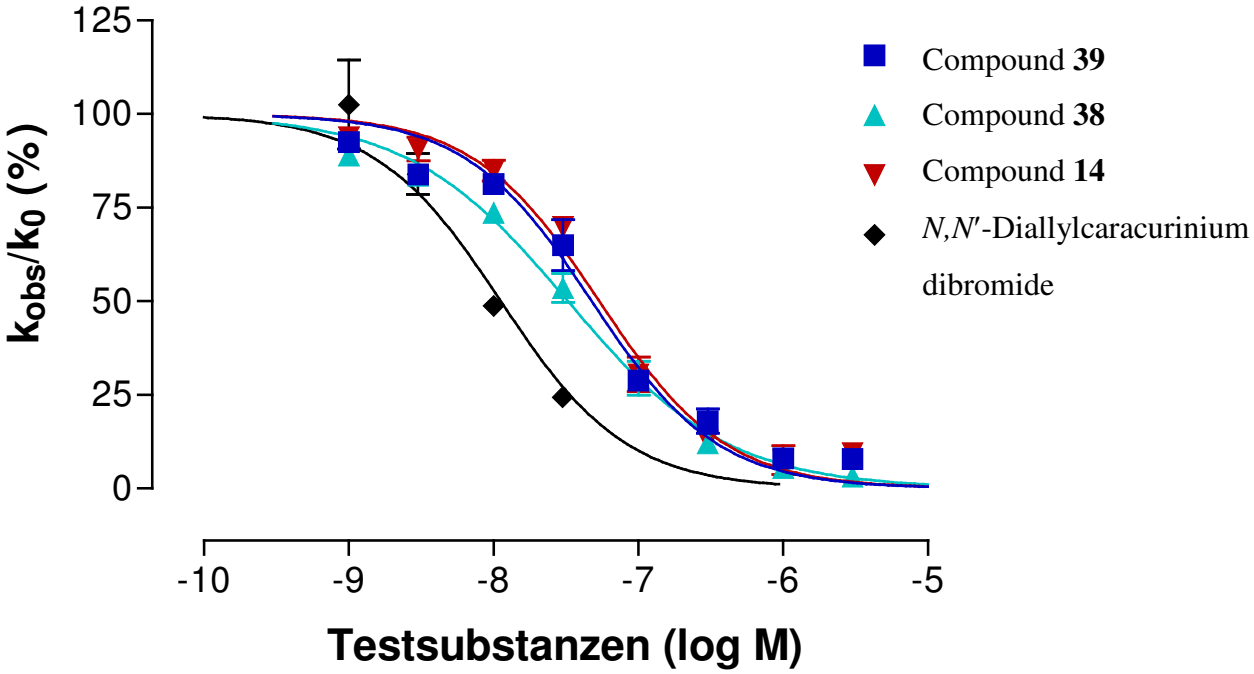


Figure 31. Concentration-effect curves for the allosteric retardation of the apparent rate constant of ligand dissociation k_{-1}.

Table 2. Parameters characterizing the allosteric interaction of the indicated test compounds with [^{3}H]NMS at porcine heart M_2 receptors.

Compounds	$EC_{50,diss}$	$pEC_{50,diss}$ [a] n = 3 ± SEM	[^{3}H]NMS equilibrium binding (%)$EC_{25,diss}$ n = 3 ± SEM
N,N'-Diallylcaracurinium dibromide	10 nM	7.95±0.08	108.8±4.5
14	54 nM	7.27±0.04	-[b]
38	35 nM	7.46±0.04	61.31±0.57
39	48 nM	7.32±0.05	22.18±1.4

[a] minus log value of the concentration reducing [^{3}H]NMS dissociation half maximally
[b] not determined

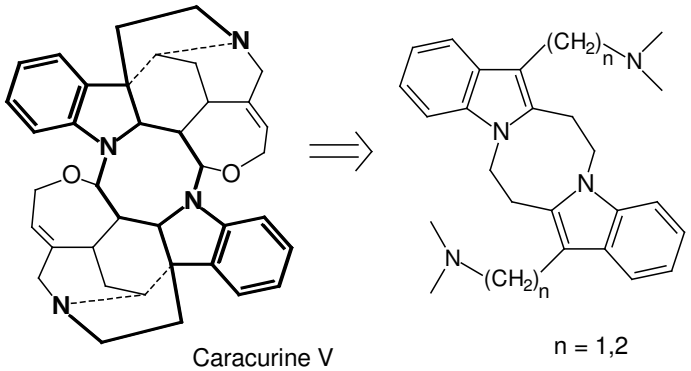
Compound **14** is an analogue of a novel caracurine V derived ring system which comprises all pharmacophoric elements, i.e., two positively charged nitrogens in a distance of approximately 10 Å surrounded by two aromatic ring systems. In order to compare the allosteric potency of the new ring system with that of caracurine V, the binding affinities of equally substituted derivatives should be considered. Since each nitrogen in the side chains of **14** is substituted with two benzyl groups, its binding affinity can be best compared with that of *N,N'*-dibenzylcaracurinium V dibromide salt (CARBEN) ($EC_{50,diss}$ = 69 nM). Similar binding affinities of 14 and CARBEN indicated that the allosteric potency of both ring systems is comparable. However, since dimethyl- and diallylcaracurinium salts showed 5-fold increase of binding affinity relative to the dibenzyl analogue, replacement of the bulky benzyl groups of **14** by smaller substitutents should result in a pronounced increase of allosteric potency.

Compound **38** and **39** are analogues of a novel unsymmetrical pentacyclic ring system which is not included in the caracurine V ring skeleton. Nevertheless, the new acozinodiindole ring skeleton also comprised the essentially pharmacophoric elements, although their relative spatial arrangement is different from that in caracurine V. **38** and **39** exhibited a 4-fold lower M_2 binding affinity ($EC_{50,diss}$ = 35 and 48 nM, respectively) than the corresponding caracurine V analogues (dimethylcaracurinium diiodide: 8 nM, diallylcaracurinium dibromide: 10 nM), which is probably due to the different spatial arrangements of the aromatic rings, as well as to different internitrogen distances in both ring systems.

3. Summary

The study deals with the area of the allosteric modulation of the muscarinic M_2 receptors. The allosteric modulators have an influence on binding of orthosteric ligands (agonists and antagonists) to the classical orthosteric binding site of the muscarinic M_2-receptors. The modulators are able to enhance (positive cooperativity) or decrease (negative cooperativity) the affinity of ligands to the orthosteric binding site. The allosteric binding site is located at the entrance of the receptor binding pocket. It is less conserved than the orthosteric binding site which is located in a narrow cavity created by the seven transmembrane domains. Consequently, development of subtype selective allosteric ligands is easier than subtype-selective muscarinic agonists or antagonists. Furthermore, subtype selectivity can be achieved by differently cooperative interactions between the allosteric and orthosteric ligand at different receptor subtypes. For example, the allosteric modulators that are positively cooperative with ACh at M_1 receptors and neutrally cooperative at the other receptor subtypes could be beneficial for treatment of the Alzheimer's disease.

Bisquaternary analogues of the *Strychnos* alkaloid caracurine V are among the most potent allosteric modulators of muscarinic M_2-receptors. The very rigid ring skeleton comprises the pharmacophoric elements of two positively charged nitrogens at an approximate distance of 10Å surrounded by two aromatic ring systems in a distinct spatial arrangement. Owing to the close structural relationship of caracurine V salts to the strong muscle relaxants toxiferine and alcuronium, they are likely to exhibit neuromuscular blocking activity, which would limit their usefulness as research tools and make the therapeutical use impossible. Reduction of the caracurine V ring skeletons to structural features responsible for good allosteric potency could possibly lead to compounds with negligible neuromuscular blocking activity and very high affinity to the allosteric binding site at M_2 receptor. Thus, the aim of this study was to synthesize and pharmacologically evaluate analogues of a novel heterocyclic ring system, which comprises the pharmacophoric elements mentioned previously.

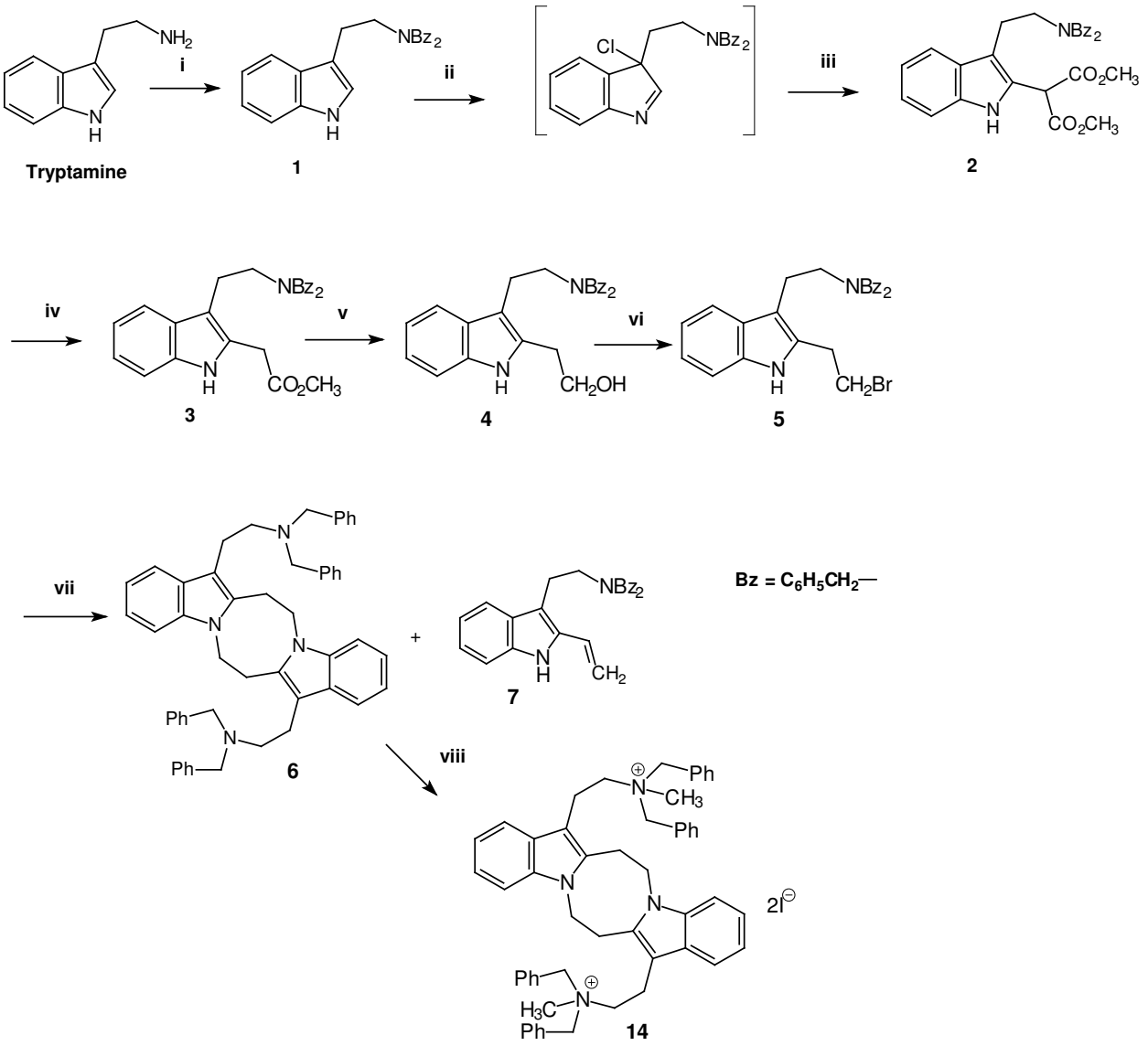


Caracurine V n = 1,2

The key step of the synthesis of the desired 6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]-diindole ring system (**6**) involved the intermolecular double *N*-alkylation of the bromoethyl indole (**5**), which was prepared from the known indolyl methylacetate (**3**) by reduction of the ester group to alcohol and subsequent substitution by bromine. **3** could be prepared in three steps involving *N*,*N*-dibenzylation of tryptamine followed by introduction of the dimethyl malonate moiety at C-2 of indole ring and a subsequent demethoxycarbonylation. The total synthesis of 6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]diindole ring system (**6**) is shown in Scheme 24.

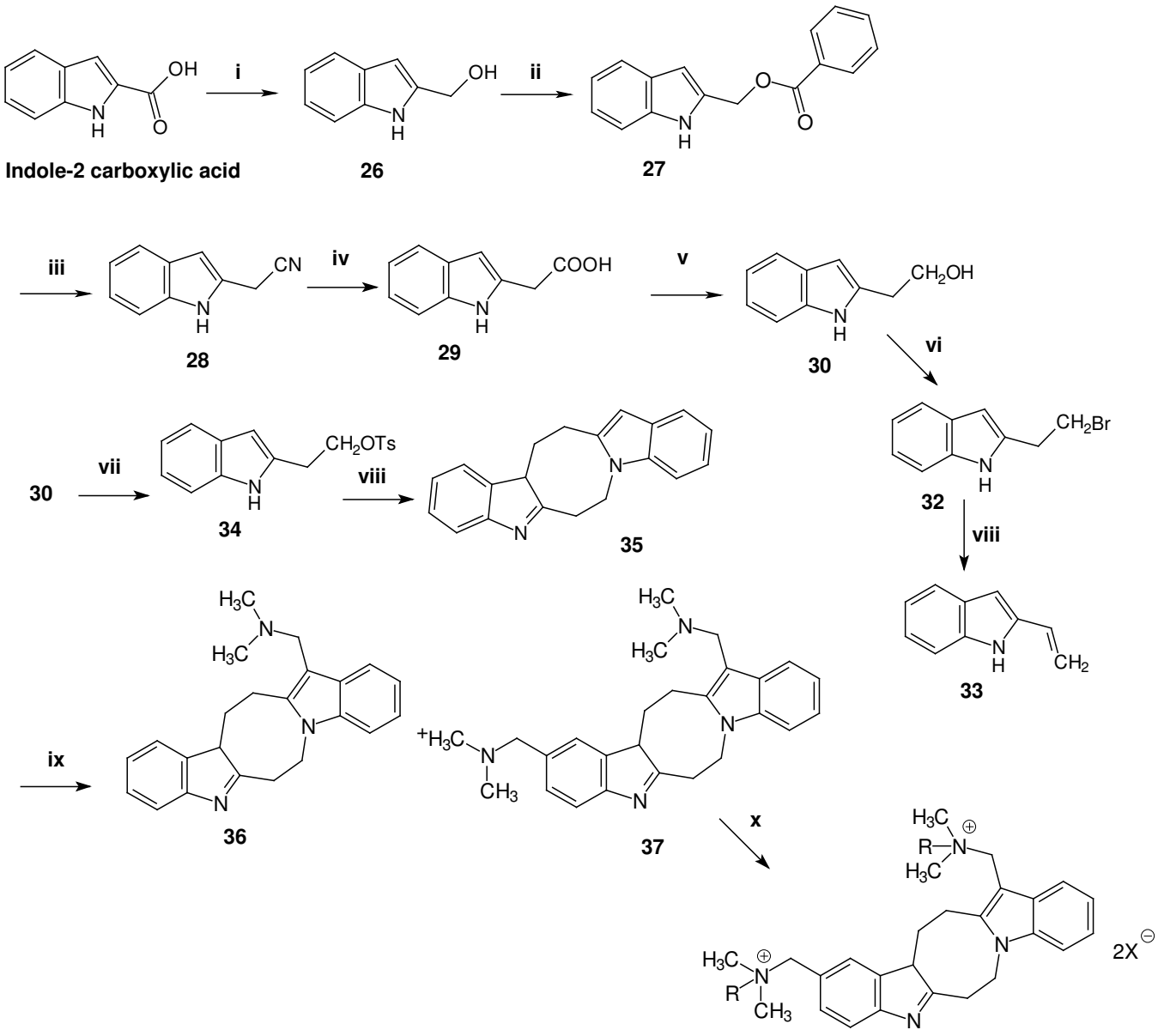
In order to examine the influence of the length of the side-chain on muscarinic activity, exchange of the ethylamine moieties of **14** by the methylamino groups was planned. This should be accomplished by dimerization of the unsubstituted 2-bromoethylindole (**32**), and subsequent Mannich aminomethylation of the resulting unsubstituted pentacyclic ring. The total synthesis of the 6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole ring system (**35**) is shown in Scheme 25. **32** was prepared from indole-2-carboxylic acid in six steps involving reduction of the acid to the corresponding alcohol **26**, benzoylation of **26** followed by nucleophilic substitution with KCN, hydrolysis of the cyanide **28** to indolyl acetic acid **29**, reduction of **29** to the corresponding alcohol **30**, and finally bromination of **30** to give the bromide **32**. Since dimerization attempts of **32** provided only 2-vinylindole (**33**), the tosylate **34** was used as starting material for the intermolecular alkylation to give exclusively an isomeric pentacyclic ring system, 7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole (**35**). The formation of the novel, asymmetric ring skeleton can be explained by the ambident nucleophilic character of the indolyl anion that can be alkylated either at nitrogen or at C-3 of indole ring. **35** was subjected to a Mannich reaction to give 2,13-dimethylaminoalkylated product **37** as well as small amounts of the 13-monosubstituted compound (**36**).

The geometry of novel ring systems **6** was elucidated by means of NMR spectroscopy and semiempirical calculations. The diazocinodiindole ring skeleton of **6** exists in chloroform solution at room temperature in a twisted-boat conformation, as indicated by 600 MHz ROESY experiment, vicinal coupling constants within the eight-membered ring, and AM1 calculations.



Step	Reagents/Conditions	% yield
i	Benzyl bromide, K_2CO_3, dry MeOH, reflux, 72 h.	81
ii	*tert*-BuOCl, triethylamine, dry THF, -78 ^oC, 3 h	-
iii	TlDMM, dry THF, -78 ^oC for 1 h, room temp., 12 h	84
iv	LiI xH_2O, dimethylacetamide, 130 ^oC, 3 h	62
v	$LiAlH_4$, dry THF, room temp., 3 h	81
vi	CBr_4, $P(NMe_2)_3$, dry CH_2Cl_2, room temp., 16 h	87
vii	NaH, dry DMF, 0 ^oC, 15 min, room temp. 20 min	47
viii	Methyl iodide, room temp., 3 days	52

Scheme 24. Total synthesis of the 6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]diindole ring system (**6**).



Step	Reagents/Conditions	% yield
i	$LiAlH_4$, dry THF, reflux, 6 h	90
ii	PhCOCl, triethylamine, dry THF, room temp., 4 h	96
iii	KCN, dry DMSO, 60 °C, 7 h	57
iv	30 % aq. NaOH, MeOH, reflux, 6 h	79
v	$LiAlH_4$, dry THF, reflux, 6 h	56
vi	CBr_4, $P(NMe_2)_3$, dry CH_2Cl_2, room temp., 16 h	24
vii	TsCl, triethylamine, dry CH_2Cl_2, room temp., 16 h	70
viii	NaH, dry DMF, 0 °C, 15 min, room temp., 20 min	47
ix	40 % aq. NMe_2 (3equiv.), 40 % HCHO (3 equiv.), acetic acid, room temp., 4 h	43
x	Methyliodide, room temp., 1 h Allylbromide, room temp., 1 h	59 72

Scheme 25. Total synthesis of the 6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole ring system (**35**).

In order to obtain potent allosteric ligands, the new heterocycles **6** and **37** were quarternized with methyliodide to the corresponding ammonium salts **14** and **38**, respectively. Additionally, the *N,N'*-diallylsalts of **37** (compound **39**) was prepared.

The allosteric effect of **14**, **38**, and **39** on the dissociation of the orthosteric radioligand [^{3}H]*N*-methylscopolamine ([^{3}H]NMS) and their effects on [^{3}H]NMS equilibrium binding were studied in homogenates of porcine heart ventricles. The concentration of an allosteric agent for a half-maximum effect on orthosteric ligand dissociation ($EC_{50,diss}$) corresponds to a 50 % occupancy of the liganded receptors by the respective allosteric test compounds. Due to the presence of two benzyl groups on each nitrogen in the side chains of **14**, its binding affinity can be best compared with that of *N,N'*-dibenzylcaracurinium V dibromide ($EC_{50,diss}$ = 69 nM). Compound **14** exhibited the comparable affinity to *N,N'*-dibenzylcaracurinium V dibromide with $EC_{50,diss}$ = 54 nM. This result suggested that replacement of the bulky benzyl groups of **14** by smaller substitutents will probably increase the allosteric potency, since dimethyl- and diallylcaracurinium salts showed a 5-fold increase of binding affinity relative to the dibenzyl analogue.

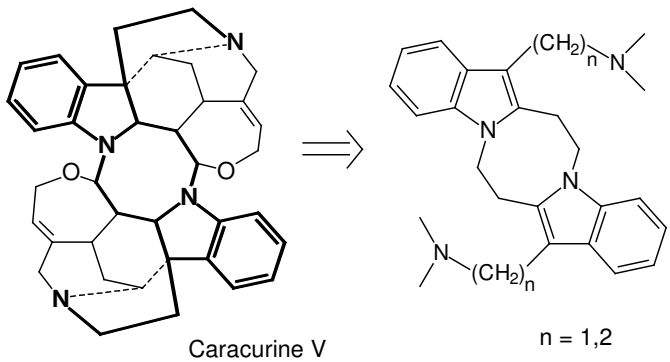
Even though the new azocinodiindole ring system of **38** and **39**, is not included in the caracurine V ring skeleton, it comprises the essentially pharmacophoric elements of allosteric potency. Due to the different spatial arrangements of the aromatic rings, as well as to different internitrogen distances in both ring systems, compound **38** and **39** exhibited 4-fold lower M_2 binding affinity ($EC_{50,diss}$ = 35 and 48 nM, respectively) than the corresponding caracurine V analogues.

This study deals with the synthesis of the first representative (Compound **6**) of a novel pentacyclic ring system derived from caracurine V. The high allosteric potency of its dimethyl analogue reveals the [1,5]diazocino[1,2-a:6,5-a′]-diindole ring system as a new promising lead structure for allosteric modulators of muscarinic M_2 receptors. Future research will be focused on structural modifications of the new ring system in order to increase the affinity to the muscarinic receptors. Furthermore, the binding affinities of the new synthesized compounds to the muscle type of nicotinic ACh-receptor should reveal structural features responsible for the muscarinic/nicotinic selectivity.

4. Zusammenfassung

Die vorliegende Arbeit befasst sich mit dem Gebiet allosterischer Modulation des muscarinischen M_2 Rezeptors. Allosterische Liganden beeinflussen das Bindungsverhalten eines orthosterischen Liganden (Agonisten oder Antagonisten) an die klassische Bindungsstelle des muscarinischen Rezeptors, indem sie seine Affinität entweder erhöhen (positive Kooperativität) oder erniedrigen (negative Kooperativität). Die allosterische Bindungsstelle befindet sich extrazellulär am Eingang der Rezeptor-Bindungstasche. Sie ist weniger konserviert als die orthosterische Bindungsdomäne, die tiefer im Rezeptorkanal zwischen den sieben transmembranalen Domänen lokalisiert ist. Demzufolge ist die Entwicklung subtyp-spezifischer allosterisch wirkenden Liganden leichter als subtyp-spezifischer Agonisten oder Antagonisten. Die Subtypselektivität kann darüber hinaus über unterschiedliche Kooperativitäten zwischen dem orthosterischen und allosterischen Liganden an verschiedenen muscarinischen Subtypen erreicht werden. Ein am M_1-Rezeptor mit Acetylcholin positiv kooperativer allosterer Modulator, der sich an anderen muscarinischen Subtypen neutral kooperativ verhält, könnte z.B. für die Therapie von Morbus Alzheimer eingesetzt werden.

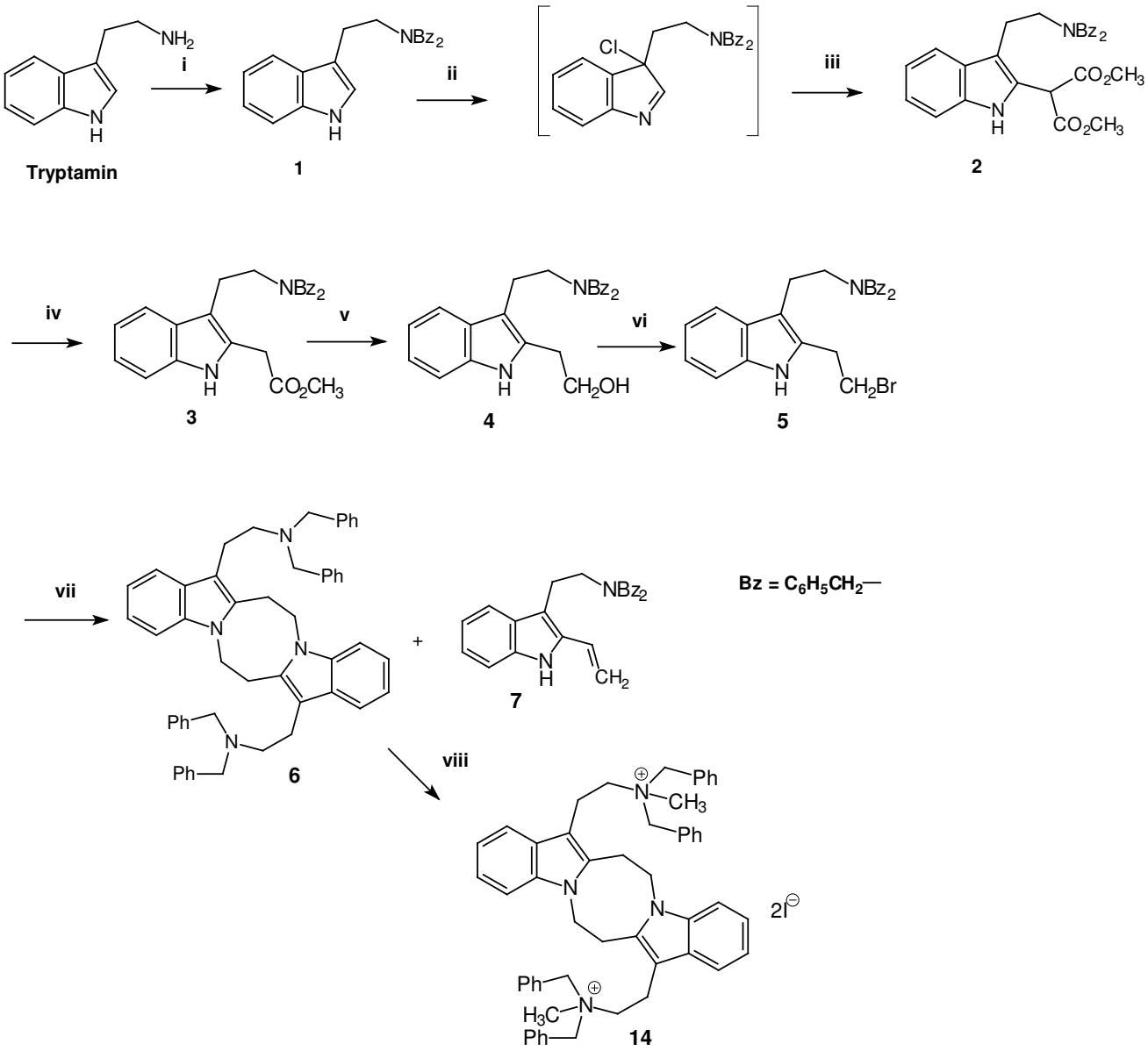
Bisquartäre Ammoniumsalze des *Strychnos*-Alkaloids Caracurin-V gehören zu den potentesten allosterischen M_2-Liganden. Die relative Stellung der aromatischen Indolringe und der Abstand zwischen den positiv geladenen Stickstoffatomen (ca. 10 Å) in dem sehr starren Caracurin-V-Ringsystem definieren den Pharmakophor für potente allosterische Modulatoren. Caracurin-V-Salze sind strukturell sehr verwandt mit den starken Muskelrelaxantien Toxiferin-I und Alcuronium und besitzen vermutlich selbst neuromuskulär-blockierende Eigenschaften, was ihre Anwendung in der pharmakologischen Forschung einschränken würde. Reduktion des Caracurin-V-Ringsystems auf die wesentlichen Pharmakophorelemente könnte zu allosterisch wirksamen Verbindungen mit vernachlässigbarer muskelrelaxierender Wirkung führen. Ziel dieser Arbeit war die Synthese und pharmakologische Testung von Derivaten eines neuen, von Caracurin V abgeleiteten, heterocyclischen Ringsystems.



Caracurine V n = 1,2

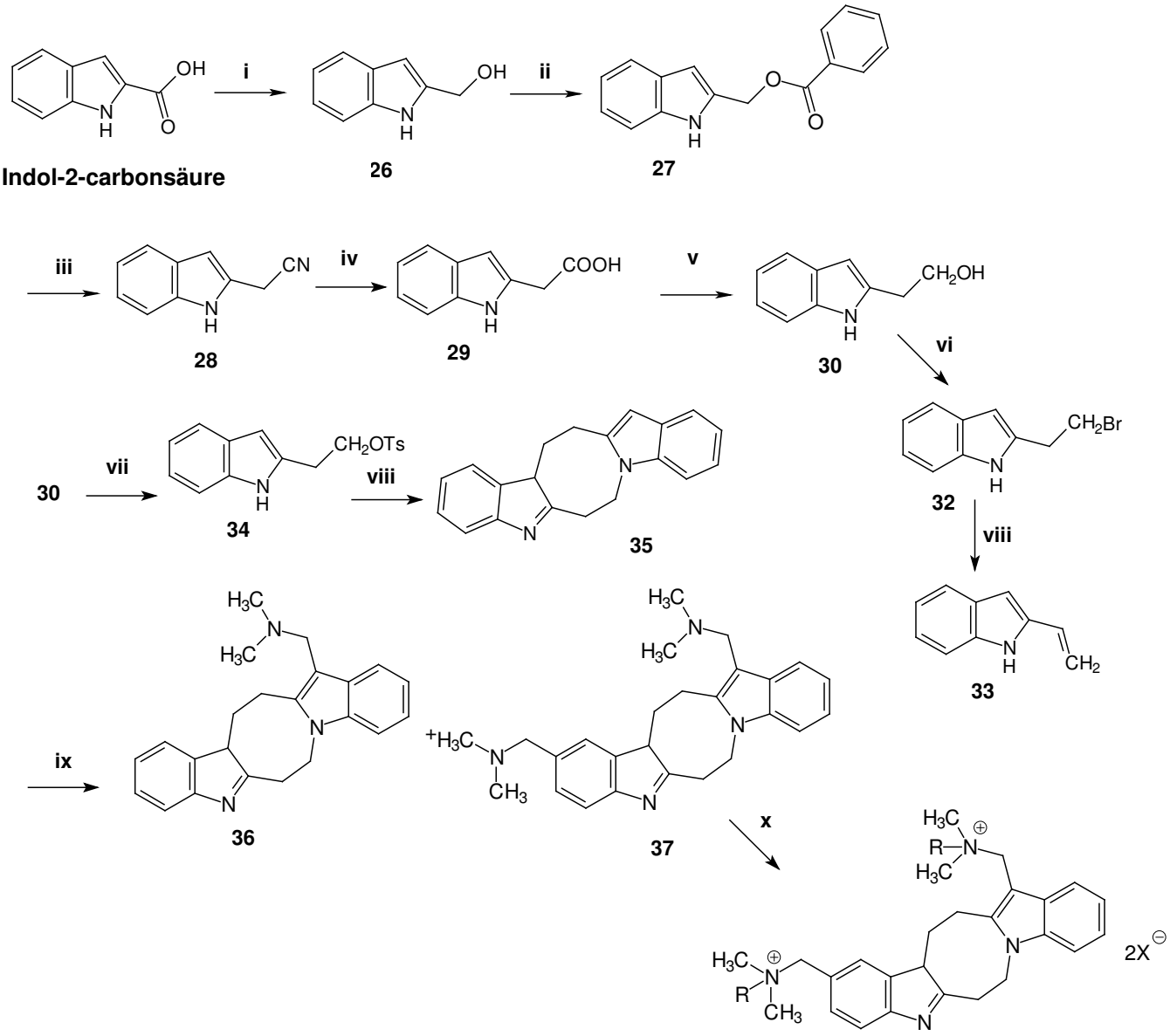
Das neue gewünscht 6,7,14,15-Tetrahydro[1,5]diazocino[1,2-a:6,5-a′]-diindole-Ringsystem (**6**) wurde in einer intermolekularen *N*-Alkylierung von zwei Molekülen Bromethylindol **5** aufgebaut. Die Ausgangsverbindung **5** konnte aus dem Indolylessigsäuremethylester **3** durch Reduktion der Estergruppe zum Alkohol und anschließende Substitution durch Brom dargestellt werden. Der bekannte Ester **3** wurde ausgehend von Tryptamin erhalten. Die dreistufige Synthese umfasste *N*-Dibenzylierung, Einführung der Malonestergruppe am C-2 von Indol und anschließende Demethoxycarbonylierung. Die Totalsynthese des neuen Pentacyclus ist im Schema 24 dargestellt. Die 3D-Struktur des neuen Ringgerüstes konnte mit Hilfe von NMR-Spektroskopie und semiempirischen Rechnungen (AM1) aufgeklärt werden. Verbindung **6** liegt in Lösung in einer verdrehten Wanne-Konformation mit unsymmetrisch angeordneten Seitenketten vor.

Um den Einfluss der Seitenkettenlänge des neuen Ringsystems auf die allosterische Wirksamkeit zu untersuchen, war es geplannt, die Ethylamin-Gruppen durch Methylamin-Einheiten zu ersetzen. Der entsprechende Syntheseplan bestand darin, das unsubstituierte Ringsystem in einer doppelten Mannich-Reaktion zu aminomethylieren. Der Ausgangsstoff für die Dimerisierung, Bromethylindol **32**, wurde aus Indol-2-carbonsäure hergestellt. Die Synthese umfasste folgende Reaktionsschritte: Reduktion der Carboxylgruppe und Benzoylierung des resultierenden Alkohols, nucleophile Substitution mit Kaliumcyanid, alkalische Hydrolyse des Cyanids zu Indolacetessigsäure, erneute Reduktion zum Alkohol und abschließende Substitution mit Brom. Da Dimerisierungsversuche von **32** nur zur Bildung des HBr-Eliminierungsproduktes **33** führten, wurde das entsprechende Tosylat als Ausgangsstoff eingesetzt. Überraschenderweise entstand nicht das erwartete Diazocinodiindol-Ringgerüst, sondern ausschließlich ein isomeres, noch nicht bekanntes 6,7,14,15-Tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindol-Ringsystem **35**. Die Bildung des neuen unsymmetrischen Ringsystems ist auf den ambidenten Charakter des Indolylanions zurückzuführen, das entweder am Sticksoff oder an C3 alkyliert werden kann. Umsetzung von **35** nach Mannich lieferte das bisaminoalkylierte Produkt **37**, neben einer kleinen Menge der monoalkylierten Verbindung **36**. Die Totalsynthese des zweiten Ringsystems ist im Schema 25 dargestellt.



Stufe	Reagenzien	% Ausbeute
i	Benzylbromid, K_2CO_3, abs. MeOH, erhitzt, 72 h.	81
ii	*tert*-BuOCl, triethylamin, abs. THF, -78 °C, 3 h.	-
iii	TLDMM, abs. THF, -78 °C, 1 h, RT, 12 h.	84
iv	LiI xH_2O, dimethylacetamid, 130 °C, 3 h.	62
v	$LiAlH_4$, abs. THF, RT, 3 h.	81
vi	CBr_4, $P(NMe_2)_3$, abs. CH_2Cl_2, RT, 16 h.	87
vii	NaH, abs. DMF, 0 °C, 15 min., RT, 20 min.	47
viii	Methyliodid, RT, 3 Tage	52

Schema 24. Die Totalsynthese des 6,7,14,15-Tetrahydro[1,5]diazocino[1,2- a:6,5-a′]diindol-Ringsystems (**6**).



Stufe	Reagenzien	% Ausbeute
i	$LiAlH_4$, abs. THF, erhitzt, 6 h.	90
ii	PhCOCl, triethylamin, abs. THF, RT, 4 h.	96
iii	KCN, abs. DMSO, 60 °C, 7 h.	57
iv	30%iger Natronlauge, MeOH, erhitzt, 6 h.	79
v	$LiAlH_4$, abs. THF, erhitzt, 6 h.	56
vi	CBr_4, $P(NMe_2)_3$, abs. CH_2Cl_2, RT, 16 h.	24
vii	TsCl, triethylamin, abs. CH_2Cl_2, RT, 16 h	70
viii	NaH, abs. DMF, 0 °C, 15 min., RT, 20 min	47
ix	40%ige wäβrige Lösung NMe_2 (3 Äquiv.), 40%ige wäβrige Lösung HCHO (3 Äquiv.), Essigsäure, RT, 4 h.	43
x	Methyliodid, RT, 1 h. Allylbromid, RT, 1 h.	59 72

Schema 25. Die Totalsynthese des 6,7,14,15-Tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindol-Ringsystems (**35**).

Um potentere Verbindungen zu erhalten, wurden beide Endstufen **6** bzw. **37** mit Methyliodid zu **14** bzw. **38** quaternisiert. **37** wurde zusätzlich mit Allylgruppen zu **39** substituiert.

Die pharmakologische Testung von **14, 37,** und **38** erfolgte über Radioligandbindungsstudien an Membransuspensionen der Herzventrikel des Hausschweins. Der allostere Effekt der Testverbindungen wurde über die Hemmung der Dissoziation von [^{3}H]-*N*-Methylscopolamin ([^{3}H]-NMS) von den damit gesättigten Rezeptoren gemessen. Die erhaltenen $EC_{50,diss}$-Werte geben die Konzentration des allosteren Modulators an, bei der die [^{3}H]-NMS-Dissoziation auf die Hälfte des Kontrollwertes reduziert ist. Sie sind ein Maβ für die Affinität der Testsubstanzen zur allosterischen Bindungsstelle des M_2 Rezeptors.

Für die einzige Verbindung mit dem Diazocinodiindole-Ringsystem **14** wurde ein $EC_{50,diss}$-Wert von 54 nM gemessen. Da **14** über vier Benzylsubstituenten verfügt, kann seine Bindungsaffinität am besten mit der von Dibenzylcaracurinium-Dibromid verglichen werden, die ganz ähnlich ist (69 nM). Aufgrund der Tatsache, dass die Verkleinerung des *N*-Substituenten am Caracurin-V-Gerüst zur erheblichen Steigerung der allosterischen Potenz führte, ist zu erwarten, dass der Austausch der voluminösen Benzylgruppen von **14** durch z.B. Methyl- oder Allylsubstituenten, eine deutliche Affinitätssteigerung bewirken würde. Damit scheint die allosterische Potenz des neuen Ringsystems mindestens genauso gut zu sein, wie die von Caracurin V.

Die beiden Vertreter des Azocinodiindol-Ringsystems, **38** und **39**, sind bereits mit den Gruppen substituiert, die die beste allosterische Potenz bei dem Caracurin-V-Ringsystem zeigten (Methyl- und Allyl). Ihre $EC_{50,diss}$-Werte (35 nM für **38**, 48 nM für **39**) sprechen jedoch für eine ca. 4-fach schwächere Bindungsaffinität als die der entsprechenden Caracurine, was vermutlich auf einen anderen Abstand zwischen den quartären Stickstoffatomen und eine andere relative Stellung der Indolaromaten in den beiden Ringsystemen zurückzuführen ist. Anders als die entsprechenden Caracurin-V-Salze, sind **38** und **39** negativ kooperativ mit dem Antagonisten [^{3}H]NMS.

Zusammenfassend lässt sich feststellen, dass von den beiden neu synthetisierten heterocyclischen Ringsystemen das direkt von Caracurin V abgeleitete Tetrahydro-[1,5]diazocino[1,2-a:6,5-a′]diindol eine bessere und vielversprechende Leitstruktur für die Entwicklung neuer potenter allosterischen Liganden des M_2-Rezeptors darstellt. Weitere

synthetische Arbeiten an dem Ringsystem wie z.B. Variation des Sticksstoffsubstituenten und der Seitenkettenlänge sollten zu einer Steigerung der Bindungsaffinität in den sub-nanomolaren Bereich führen. Darüber hinaus sind die Ergebnisse der pharmakologischen Testung an dem muskulären Typ des nicotinischen Acetylcholinrezeptors abzuwarten.

5. Experimental Section

5.1 Instrumentation and Chemicals

^{1}H-NMR-Spectra: Bruker AV-400 spectrometer (400.132 MHz) and
Bruker AV-600 spectrometer (600.133 MHz) for compound **6**

^{13}C-NMR-Spectra: Bruker AV-400 spectrometer (100.621 MHz)

Hydrogen chemical shifts are referenced to C*H*Cl_3 (δ = 7.24 ppm) and DMSO-d_6 (δ = 2.55 ppm); carbon chemical shifts are referred to $^{13}CDCl_3$ (δ = 77.0 ppm) and DMSO-d_6 (δ = 39.50 ppm), respectively. The NMR assignments were based on H,H-COSY, HMQC, and HMBC experiments of the representative compounds. Abbreviation for data quoted are s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The NMR solvents used were deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-d_6).

IR-Spectra: Bio Rad, PharmalyZir Instrument

KBr disc was used for neat liquid substances. ATR technique was used for solid substances.

Mass spectra: Finnigan MAT 8200 spectrometer

Using EI, CI, and FAB ionization techniques.

Elemental analyses: Leco CHNS-932

Melting point: Capillary melting apparatus (Gallenkamp, Sanyo). All values are uncorrected.

Column chromatography: Silica gel 60 (0.063-0.200 mm) (Nr. 7734) and
Aluminium oxide 90 active neutral (0.063-0.200 mm) (Nr. 101076), obtained from Merck

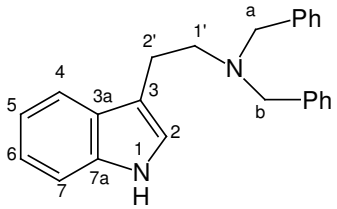
TLC: Silica gel coated aluminium sheet (Silica gel 60 F_{254}, Nr. 105554), obtained from Merck

All solvents were freshly distilled prior to use: tetrahydrofurane (THF) and diethyl ether were distilled under argon from sodium metal, dichloromethane (CH_2Cl_2) was distilled from calcium hydride powder, *N,N*-dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride powder. All starting compounds and reagents were purchased from Aldrich, Merck, Fluka, and Acros.

5.2 3-(2-Dibenzylaminoethyl)-indole (1)[41]

A mixture of tryptamine (10.0 g, 0.062 mol), benzyl bromide (26.5 g, 0.155 mol), and K_2CO_3 (24.0 g) in dry methanol (340 mL), was stirred and refluxed for 70 h. After cooling to room temperature, the mixture was filtered and the solvent was evaporated. The residue was dissolved in dichloromethane and filtered. The brown oily residue was purified by column chromatography (Silica gel, EtOAc:Hex / 1:2) to give a golden oil **1** (19.6 g, 93%); TLC R_f = 0.38 (Silica gel, EtOAc:Hex / 1:2); FT-IR (KBr) ν (cm^{-1}) 3424, 3057, 3027, 2923, 2799, 1601, 1490, 1452, 1122, 1011, 740.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



1

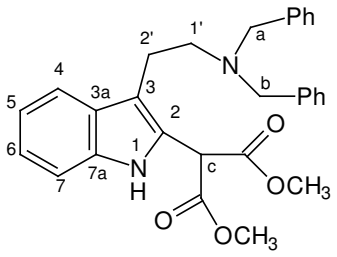
	^{1}H(400 MHz)	^{13}C(100 MHz)
1	7.83 s, br	-
2	7.44-6.88 m	121.8
3	-	110.9
3a	-	126.7
4	7.44-6.88 m	119.0
5		121.4
6		118.8
7		114.5
7a	-	139.8
1‘	3.05-2.96 m	53.9
2‘	2.88-2.79 m	23.0
a	3.72 s	58.3
b	3.72 s	58.3
Ph	7.44-6.88 m	128.8, 128.1

5.3 Dimethyl [3-(2-dibenzylaminoethyl)-1*H*-indol-2-yl]-propanedioate (2)[41]

tert-Butyl hypochlorite[60] (9.4 g, 0.087 mol) was added dropwise to a solution of **1** (19.6 g 0.058 mole) and triethylamine (6.0 ml) in dry THF (200 mL) at -78 °C (dry ice/acetone). The reaction mixture was stirred at –78 °C for 2 h, and then concentrated in *vacuo* without heating. The residual brown solution was transferred into a stirred suspension of thallium dimethyl malonate[61] (21.4 g, 0.064 mol) in dry THF (350 mL) at –78 °C. The mixture was stirred at –78 °C for 1 h, warmed to room temperature, and stirring was continued for 12 h. The thallium salt was filtered off through a pad of Celite and the solvent was removed *in vacuo*. The residue was purified by column chromatography (Silica gel, EtOAc:Hex / 1:2) to give compound **2** as a pale yellow solid (21.2 g, 78%); mp 105 – 106 °C (Lit.[41] 104°C) ; TLC R_f =

0.26 (silica gel, EtOAc:Hex / 1:2), FT-IR (ATR) ν (cm^{-1}) 3367, 3026, 2953, 1753, 1722, 1493, 1454, 1435, 1238, 1141, 734, 699.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}CNMR (100 MHz, $CDCl_3$).



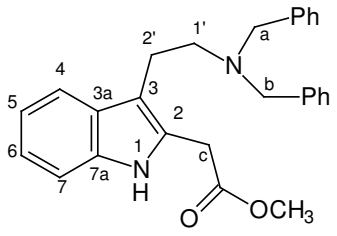
2

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.76 s, br	-
2	-	126.7
3	-	110.6
3a	-	127.4
4	7.43-7.00 m	119.2
5		122.3
6		118.8
7		111.0
7a	-	139.7
1‘	2.93-2.85 m	53.8
2‘	2.70-2.62 m	22.1
a	3.72 s	58.5
b	3.72 s	58.5
c	4.80 s	48.6
Ph	7.43-7.00 m	128.6, 128.1
2 x C=O	-	167.5
2 x $–CH_3$	3.67 s	53.0

5.4 Methyl [3-(2-dibenzylaminoethyl)-1*H*-indol-2-yl]-acetate (3)[41]

To a solution of **2** (6.54 g, 0.014 mol) in *N,N*-dimethylacetamide (60 mL), lithium iodide hydrate (3.13 g, 0.017 mol) and triethylamine hydrochloride (0.5 g) were added. The reaction mixture was heated to 130 °C for 3 h. Cooling to room temperature, brine (60 mL) and 25% aq. NH_4OH (60 mL) were added. The dark two phase solution was extracted with diethyl ether (10 x 100 ml). The combined organic phase was washed with water (5 x 30 mL), dried over $MgSO_4$ and evaporated. The residual dark oil was purified by column chromatography (Silica gel, EtOAc:Hex / 1:3), to give compound **3** as a brown solid (3.67 g, 64%); mp 81 – 82 °C (Lit.[41] 122-123°C); TLC R_f = 0.22 (Silica gel, EtOAc:Hex / 1:3); FT-IR (ATR) ν (cm^{-1}) 3380, 3030, 2954, 2841, 1733, 1460, 1436,1206, 1171, 739, 698; MS (EI, 70 eV) *m/z* (rel int) 412 [M^+] (6), 210 (97), 91 (100).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



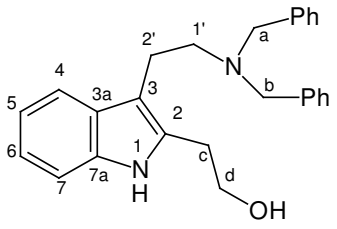
3

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.45 s, br	-
2	-	126.8
3	-	110.6
3a	-	135.6
4	7.44-7.00 m	119.1
5		121.6
6		118.5
7		111.7
7a	-	139.8
1‘	2.94-2.86 m	53.8
2‘	2.72-2.65 m	22.1
a	3.74 s	58.5
b	3.74 s	58.5
c	3.61 s	31.4
Ph	7.44-7.00 m	128.7, 128.2
C=O	-	171.0
$-CH_3$	3.68 s	52.2

5.5 2-[3-(2-Dibenzylaminoethyl)-1*H*-indol-2-yl]-ethanol (4)

The solution of **3** (1.72 g, 4.2 mmol) in dry THF (20 mL) was added dropwise to the suspension of $LiAlH_4$ (0.23 g, 6.3 mmol) in dry THF (30 mL) at 0 ^{o}C under argon. The mixture was stirred at room temperature for 3 h. After cooling to 0 ^{o}C, 2 ml of water was added slowly, followed by the careful addition of 2 ml of 15% aq. NaOH and water (4 mL). The reaction mixture was stirred at room temperature for 1 h and filtered off. The precipitant was washed with THF and the combined THF solutions were dried over Na_2SO_4 and evaporated. Purification by column chromatography (Silica gel, $CHCl_3$:MeOH / 10:1) gave 1.30 g (81%) of compound **4** as a pale yellow solid. Crystallization from diethyl ether/hexane afforded an analytical sample: mp 77-78 ^{o}C; TLC R_f = 0.56 (Silica gel, $CHCl_3$:MeOH / 10:1); FT-IR (ATR) ν (cm^{-1}) 3348, 3052, 2889, 1456, 1356, 1063, 1015, 735, 699; MS (EI, 70 eV) *m/z* (rel int) 384 [M^+], (1), 210 (60), 91 (100); Anal. Calcd. for $C_{26}H_{28}N_2O$: C, 81.21; H, 7.34; N, 7.29. Found: C, 81.06; H, 7.02; N, 7.22.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



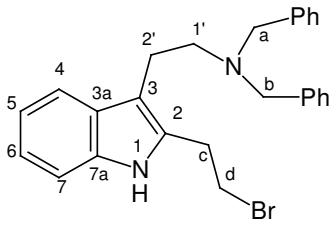
4

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.25 s, br	-
2	-	133.0
3	-	126.8
3a	-	135.3
4	7.40-6.97 m	118.9
5		121.1
6		118.1
7		110.3
7a	-	139.5
1‘	2.87 t, 7.1 Hz	54.0
2‘	2.67 t, 7.1 Hz	22.1
a	3.72 s	58.5
b	3.72 s	58.5
c	3.71 t, 6.1 Hz	62.3
d	2.75 t, 6.1 Hz	28.8
Ph	7.40-6.97 m	128.9, 128.7, 128.1
-OH	1.79 s	-

5.6 2-(2-Bromoethyl)-3-(2-dibenzylaminoethyl)-indole (5)

The solution of *tris*-(dimethylamino)-phosphine (2.61 g, 16 mmol) in dry dichloromethane (30 mL) was added dropwise to the mixture of **6** (1.52 g, 4.0 mmol) and carbon tetrabromide (2.62 g, 8.0 mmol) in dry CH_2Cl_2 (60 mL) at 0 ^{o}C. After stirring for 16 h at room temperature, the reaction mixture was washed with water (3x50 mL) and brine (30 mL), dried over Na_2SO_4 and evaporated *in vacuo*. Purification by column chromatography (Silica gel, EtOAc:hexane / 1:5) gave the compound **6** (1.55 g, 87 %) as a pale yellow solid. Crystallization from diethyl ether/hexane afforded an analytical sample: mp 85-86 ^{o}C; TLC R_f = 0.32 (Silica gel, EtOAc:hexane / 1:5); FT-IR (ATR) ν (cm^{-1}) 3221, 2900, 1456, 750, 698, 654; MS (CI, NH_3 gas, 150 eV) *m/z* (rel int) 449 $[M+2]^+$ (8),447 $[M^+]$ (11), MS (EI, 70 eV) *m/z* (rel int) 367, (6), 210 (100), 91 (92). Anal. Calcd. for $C_{26}H_{27}BrN_2$: C, 69.8; H, 6.1; N, 6.3. Found: C, 69.22; H, 6.42; N, 5.82.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



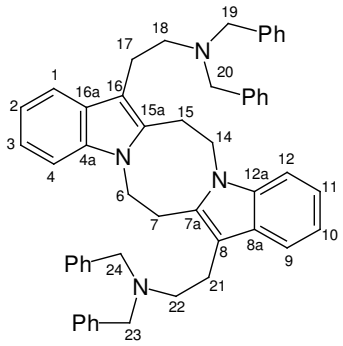
5

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.02 s, br	-
2	-	127.2
3	-	108.0
3a	-	133.2
4	7.61-6.95 m	119.2
5		121.6
6		118.4
7		110.6
7a	-	135.4
1‘	2.86-2.84 m	54.4
2‘	2.69-2.68 m	22.5
a	3.75 s	59.1
b	3.75 s	59.1
c	3.34 t, 7.07 Hz	32.0
d	3.10 t, 7.07 Hz	30.1
Ph	7.61-6.95 m	129.0, 128.3

5.7 8,16-Bis-(2-dibenzylaminoethyl)-6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]-diindole (6) and 2-vinyl-3-(2-dibenzylaminoethyl)-indole (7)

The suspension of NaH (0.07 g, 2.9 mmol) in dry DMF (10 mL) was added dropwise to the solution of **5** (0.5 g, 1.1 mmol) in dry DMF (20 mL) at 0 °C. The reaction mixture was stirred for 20 min at 0 °C and then for 1 h at room temperature. Diethyl ether (30 mL) and cold water (30 mL) were added and the aqueous phase was extracted with diethyl ether (2 x 30 mL). The combined ether extracts were washed with water (2 x 15 mL) and brine (15 mL), dried over Na_2SO_4 and evaporated. Purification by column chromatography (Silica gel, EtOAc:hexane / 1:3) gave the desired dimer compound **6** (0.2 g, 47 %) as a white solid and the side-product **7** (0.09 g, 22 %) as a yellow solid. Compound **6** was crystallized from diethyl ether/hexane to afford an analytical sample: mp 130 °C; TLC R_f = 0.56 (Silica gel, EtOAc:hexane / 1:3); FT-IR (ATR) ν (cm^{-1}) 3221, 2900, 1456, 750, 698, 654; MS (CI, NH_3 gas, 150 eV) *m/z* (rel int) 733 [M^+] (39), MS (EI, 70 eV) *m/z* (rel int), 641 (51), 522 (33), 210 (70); 91 (100); Anal. Calcd. for $C_{52}H_{52}N_4$: C, 85.21; H, 7.15; N, 7.64. Found: C, 85.00; H, 7.32; N, 7.61.

^{1}H NMR (600 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



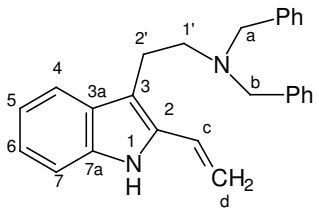
6

	^{1}H(600 MHz)	^{13}C(100 MHz)
1	7.23-7.09 m[a]	118.4
2	7.00-6.97 m[a]	119.5
3	-	120.5
4	7.89 d, 8.5 Hz	112.3
4a	-	135.0
5		
6	H^a 2.64 ddd, 13.7, 10.2, 6.5 Hz H^b 3.75 m	35.0
7	H^a 2.13 ddd, 12.5, 10.2, 6.6 Hz H^b 2.44 m	29.7
7a	-	85.5
8		59.7
8a	-	134.6
9	6.48 dd, 7.2, 1.0 Hz	122.7
10	6.43 t, 7.2 Hz	117.9
11	7.03 ddd, 8.3, 7.1, 1.0 Hz	127.6
12	6.49 d, 8.3 Hz	106.2
12a	-	149.4
13		
14	H^a 3.17 ddd, 13.8, 12.1, 3.3 Hz H^b 3.75 m	38.4
15	2.56 m	21.7
15a	-	132.8
16		109.6
16a	-	129.3
17	H^a 2.74 ddd, 13.0, 9.7, 6.1 Hz H^b 2.76 m	21.9
18	2.61-2.51 m	54.0
19/20	H^a 3.63 d, 13.7 Hz H^b 3.70 d, 13.7 Hz	58.6
21	H^a 1.99 ddd, 14.1, 11.9, 4.5 Hz H^b 2.44 m	30.7
22	H^a 0.86 m H^b 1.86 ddd, 12.5, 12.5, 4.5 Hz	48.5
23/24	H^a 2.54 d, 13.9 Hz H^b 2.90 d, 13.9 Hz	57.4
Ph	7.23-7.09 m and 7.00-6.97 m	140.0, 139.9, 128.7, 128.4, 128.2, 127.9, 126.8, 126.3

[a] Overlapping with signals of benzene rings

Compound **8** was crystallized from $CHCl_3$/hexane to afford an analytical sample: mp 111-113 ^oC; TLC R_f = 0.45 (Silica gel, EtOAc:hexane / 1:3); FT-IR (ATR) ν (cm^{-1}) 3411, 3010, 2818, 1450, 1026, 881, 737, 696; MS (EI, 70 eV) *m/z* (rel int) 366 [M^+], (2), 210 (100), 149 (33), 91 (97); Anal. Calcd. for $C_{26}H_{26}N_2$: C, 84.74; H, 7.66; N, 7.60. Found: C, 84.37; H, 7.37; N, 7.22.

^1H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



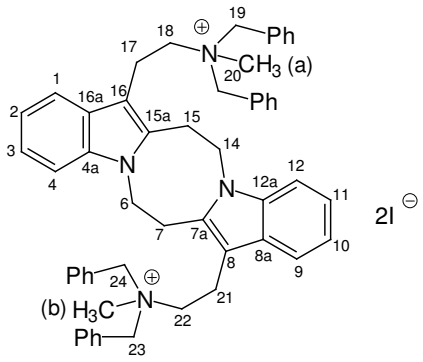
7

	^1H(400 MHz)	^{13}C(100MHz)
1	7.92 bs	-
2	-	132.2
3	-	126.8
3a	-	136.1
4	7.44-6.98 m	119.3
5		122.9
6		119.0
7		110.4
7a	-	139.8
1‘	2.98 m	54.2
2‘	2.74 m	22.1
a	3.74 s	58.6
b	3.74 s	58.6
c	6.70 dd, 17.4, 11.4 Hz	125.4
d	H^a 5.41 d, 17.7 Hz H^b 5.22 d, 11.6 Hz	110.7
Ph	7.44-6.98 m	128.8, 128.1

5.8 5,13-Dimethyl-8,16-bis-(2-dibenzylaminoethyl)-6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]-diindole diiodide (14)

Compound **6** (0.11 g, 0.15 mmol) was stirred in an excess of methyl iodide (4 mL) at room temperature for 70 h. Diethyl ether (20 mL) was added and the precipitated product was collected by filtration, washed with diethyl ether (5 x 20 mL), and dried *in vacuo*. The pure compound was obtained as yellow crystals (0.08 g, 52 %): mp >220 ^oC; FT-IR (ATR) ν (cm^{-1}) 3031, 2947, 2881, 1601, 1484, 1455, 1315, 1252, 1213, 1155, 1026, 909, 746, 721, 702; MS (FAB, *m*-nitrobenzyl alcohol (MNOBA) matrix) *m/z* (rel int) 890 [M-I] $^+$.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



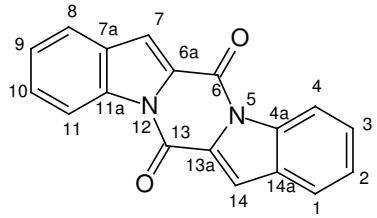
14

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	7.31 d, 7.6 Hz	118.2
2	7.03 t, 7.6 Hz	119.9
3	7.19 m	121.5
4	8.01 d, 8.3 Hz	112.6
4a	-	135.0
5	-	-
6	H^a 2.59 m H^b 3.55 m	34.7
7	2.75 m	26.7
7a	-	104.6
8	-	84.4
8a	-	134.6
9	7.11 m	122.8
10	6.68 t, 7.6 Hz	117.9
11	7.11 m	128.6
12	6.82 d, 7.8 Hz	106.5
12a	-	148.7
13	-	-
14	H^a 2.76 m H^b 4.02 dd, 6.8, 4.0 Hz	37.1
15	H^a 2.16 m H^b 2.98 m	17.2
15a	-	132.8
16	-	109.6
16a	-	129.3
17	H^a 2.29 ddd, 15.4, 11.4, 4.8 Hz H^b 2.75 m	21.2
18	3.23 t, 8.8 Hz	60.0
19/20	H^a 4.64 d, 12.9 Hz H^b 4.88 dd, 12.9, 2.8 Hz	58.6
21	H^a 2.16 m H^b 2.65 m	30.4
22	H^a 1.28 m H^b 2.53 m	59.8
23/24	H^a 2.54 d, 13.9 Hz H^b 2.90 d, 13.9 Hz	57.4
$-CH_{3\,(a)}$	3.10 s	46.0
$-CH_{3\,(b)}$	1.90 s	44.2
Ph	7.67-7.40 m	130.3, 130.2, 129.0,128.9, 128.8,128.7, 128.0, 127.2 126.7

5.9 Pyrazino[1,2-a;4,5-a']diindole-6,13-dione (8)

The solution of 2-indole carboxylic acid (0.2 g, 1.24 mmol) in dry DMF (8 mL) was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (0.48 g, 2.48 mmol) and dimethylaminopyridine (DMAP) (0.38 g, 3.1 mmol). The reaction mixture was stirred for 16 h at room temperature. The pale-yellow precipitate was filtered off, washed with 10% aqueous hydrochloric acid (6x50 mL) and saturated aqueous sodium hydrogen carbonate (6 x 50 mL) and dried *in vacuo*, to give compound **8** (0.17 g, 95%); mp >250 ^{o}C (Lit.[43] >340 ^{o}C); FT-IR (ATR) ν (cm^{-1}) 3123, 3102, 3059, 1696, 1560, 1450, 1197, 1078, 745; MS (EI, 70 eV) *m/z* (rel int) 286 [M^{+}], (100), 258 (11), 143 (45), 115 (46).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



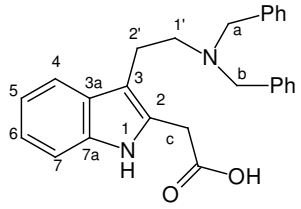
8

	^{1}H(400 MHz)	^{13}C(100 MHz)
1/8	8.47 d, 7.83 Hz	125.5
2/9	7.45 t, 7.83 Hz	129.0
3/10	7.63 t, 7.83 Hz	124.0
4/11	7.88 d, 7.83 Hz	117.0
4a/11a	-	136.2
6/13	-	153.8
6a/13a	-	129.3
7/14	7.84 s	116.4
7a/14a	-	129.7

5.10 [3-(2-Dibenzylaminoethyl)-1*H*-indol-2-yl]-acetic acid (9)

A solution of **3** (0.73 g, 1.77 mmol) in MeOH (30 mL) was refluxed with 3% aqueous potassium hydroxide solution (5.2 ml) for 1 h. After cooling to room temperature, water (30 mL) was added and the reaction mixture was acidified with 2M aq. HCl to pH 2-3. The insoluble white solid was filtered off, washed several times with water and dried *in vacuo*. The crude product **9** (0.57 g, 80%) was used for the next reaction without further purification; mp 161 ^{o}C; TLC R_f = 0.21 (Silica gel, $CHCl_3$:MeOH:CH_3COOH / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3251, 3232, 3052, 2947, 1710, 1610, 1458, 738, 700, 684; MS (EI, 70 eV) *m/z* (rel int) 354 (1), 210 (62), 91 (100), 44 (13).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



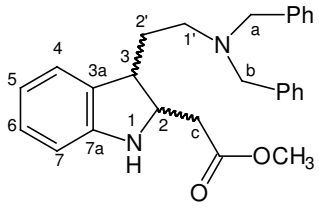
9

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	10.74 s, br	-
2	-	127.2
3	-	110.1
3a	-	135.8
4	7.43-6.79 m	118.4
5		120.8
6		118.0
7		111.1
7a	-	139.8
1‘	2.84 m	53.6
2‘	2.57 m	21.6
a	3.71 s	57.9
b	3.71 s	57.9
c	3.59 s	32.2
-COOH	12.3 s	171.8
Ph	7.43-6.79 m	129.1, 128.9, 128.5, 128.0

5.11 *trans*- and *cis*-Methyl {3-[2-(dibenzylamino)ethyl]-2,3-dihydro-1*H*-indol-2-yl}acetate (11a) and (11b)

$NaBH_4$ (0.46 g, 0.012 mole) was added to a solution of compound **3** (1.0 g, 2.4 mmol) in trifluoroacetic acid (10 ml) at 0 ^oC in a way that the reaction temperature remained below 10 ^oC. After stirring for 7 h, the reaction mixture was diluted with water (30 mL), basified to pH 9 by adding NaOH pellets and then extracted with diethyl ether (4 x 50 mL). After washing with water (2 x 30 mL), and brine (30 mL). The reaction mixture was dried over Na_2SO_4, and concentrated *in vacuo*. The brown residue was purified by column chromatography (Silica gel, EtOAc:petroleum ether / 1:3) to give diastereomeric mixture of **11a** and **11b** as a neat brown liquid (0.94 g, 94 %). TLC Rf = 0.35 (Silica gel, EtOAc: petroleum ether / 1:3).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



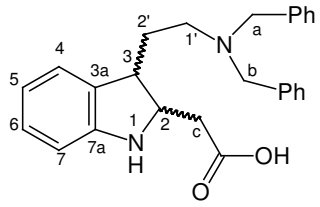
11a and 11b

	Isomer 1		Isomer 2	
	^{1}H(400 MHz)[a]	^{13}C(100 MHz)	^{1}H(400 MHz)	^{13}C(100 MHz)
1	4.42 s, br	-	4.35 s, br	-
2	3.94 dt, 4.99, 2.8 Hz	61.0	3.73-3.70 Hz	58.9
3	3.26 q, 7.3 Hz	45.3	2.92 q, 6.3 Hz	41.7
3a	-	139.6	-	139.6
4	7.40-6.55 m	124.1	7.40-6.55 m	124.1
5		126.9		126.9
6		118.5		118.4
7		109.5		109.1
7a	-	149.8	-	149.5
1‘	1.94-1.71 m	53.8	1.94-1.71 m	53.8
2‘	2.61-2.55 m	51.1	2.61-2.55 m	50.8
a	3.68 s	58.6	3.68 s	58.5
b	3.68 s	58.6	3.68 s	58.5
c	2.46-2.29 m	40.2	2.46-2.29 m	34.5
Ph	7.40-6.55 m	128.7, 128.2, 127.6, 127.4	7.40-6.55 m	128.7, 128.2, 127.6, 127.4
$-CH_3$	3.68 s	51.6	3.68 s	51.6
C=O	-	173.1	-	172.8

5.12 *trans*- and *cis*-[3-(2-Dibenzylaminoethyl)-2,3-dihydro-1*H*-indol-2-yl]acetic acid (12a and 12b)

A solution of the diastereomeric mixture **11a** and **11b** (0.2 g, 0.48 mmol) in MeOH (10 mL) was refluxed with 3% aq. KOH (1.5 mL) for 1 h. After cooling to room temperature, water (30 mL) was added and the reaction mixture was acidified with 2M HCl to pH 2-3. The insoluble solid was filtered off, washed several times with water, dried *in vacuo*. The crude product (0.13 g, 68%) was used for the next reaction without further purification; TLC R_f = 0.06 (Silica gel, $CHCl_3$:MeOH:CH_3COOH / 30:1:0.1). FT-IR (ATR) ν (cm^{-1}) 3351, 3031, 2962, 2926, 1709, 1457, 1259, 1087, 1018, 798, 698; MS (EI, 70 eV) *m/z* (rel int) 400 $[M]^+$ (0.3), 341 (16), 309 (100), 210 (58), 144 (13), 91 (100).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$)



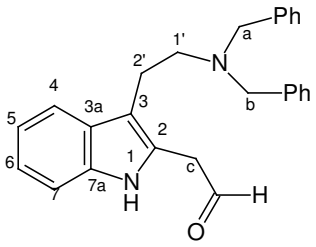
12a and 12 b

	Isomer 1		Isomer 2	
	^{1}H(400 MHz)	^{13}C(100 MHz)	^{1}H(400 MHz)	^{13}C(100 MHz)
1	4.10 s, br	-	4.10 s, br	-
2	3.91 m	61.2	-	59.4
3	3.10 q, 7.3 Hz	45.3	2.81 q, 6.1 Hz	41.8
3a	-	137.1	-	136.9
4	7.29-6.47 m	124.3	7.29-6.47 m	124.2
5		119.8		118.8
6		127.6		127.6
7		110.1		109.8
7a	-	149.7	-	149.3
1‘	2.67-2.51 m	50.6	2.67-2.51 m	50.4
2‘	1.78-1.69 m	30.8	1.85-1.80 m	25.1
a,b -H^a	3.57 d, 13.4 Hz	57.8	3.61 d, 14.7 Hz	57.7
a,b- H^b	3.72 d, 13.4 Hz	57.8	3.64 d, 16.4 Hz	57.7
c	2.45-2.31 m	35.4	2.45-2.31 m	35.4
-COOH	7.71 s	176.6	7.71 s	176.5
Ph	7.43-6.79 m	132.2, 130.9, 129.5, 128.5	7.43-6.79 m	131.5, 129.6, 129.5, 128.5

5.13 2-[3-(2-Dibenzylaminoethyl)-1*H*-indol-2-yl]-ethanal (13)

A solution of dry DMSO (6.4 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a solution of oxalyl chloride (400 mg, 3.2 mmol) in dry CH_2Cl_2 (10 mL) at –60 ^{o}C while the temperature was kept under –60 ^{o}C. After stirring at –60 ^{o}C for 10 min, a solution of alcohol **5** (1.0 g, 2.6 mmol) in dry CH_2Cl_2 (10 mL) was added. Stirring was continued for 15 min and afterwards dry triethylamine (2.0 mL) was added within 5 min. The cooling bath was removed and the reaction mixture was allowed to reach 25 ^{o}C. Water (20 mL) was added and the aqueous phase was separated and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were washed with water (2 x 20 mL), dried over $MgSO_4$, and evaporated to give **15** as a dark solid (0.6 g, 60%). The crude product was used for the next reaction without further purification; TLC R_f = 0.52 (Silica gel, EtOAc:petroleum ether / 1:2).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



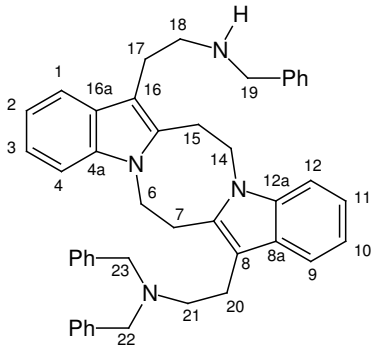
13

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	7.89 bs	-
2	-	127.5
3	-	110.8
3a	-	135.9
4	7.45-7.00 m	119.3
5		122.6
6		118.7
7		116.7
7a	-	139.7
1‘	2.97 m	54.2
2‘	2.73 m	22.2
a	3.75 s	58.6
b	3.75 s	58.6
c	2.44 s	32.2
-CHO	9.22 s	192.2
Ph	7.45-7.00 m	128.8, 128.2, 126.8

5.14 8-(2-Dibenzylaminoethyl),16-(*N*-benzylaminoethyl)-6,7,14,15-tetrahydro[1,5]diazocino[1,2-a:6,5-a′]-diindole (15)

The solution of **6** (0.29 g, 0.4 mmol) in acetic acid (20 mL) was added to a hydrogenation vessel containing an argon-flushed, followed by 0.1 g of 10 % palladium on charcoal. The vessel was saturated with hydrogen at 50 bar, and the mixture was stirred at 60^{o}C for 72 h. After cooling to room temperature, the reaction mixture was filtered through Celite and the filter pad was washed with acetic acid (2 x 10 mL). The filtrate was poured onto crushed ice and adjusted pH to 9-10 with 25% aq. NH_3. After extraction with CH_2Cl_2 (3 x 30 mL), the combined organic phases were washed with water (2 x 20 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude product was purified by column chromatography (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1) to obtain **15** (0.09 g, 36 %) as a pale yellow neat liquid; TLC R_f = 0.31 (Silica gel, $CHCl_3$: MeOH / 10:1); MS (EI, 70 eV) *m/z* (rel int), 641 $[M-1]^+$ (51), 522 (33), 210 (70); 91 (100).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



15

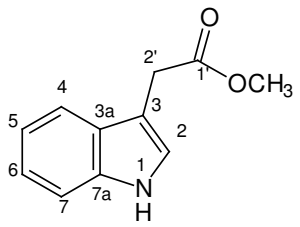
	^{1}H(400 MHz)	^{13}C(100 MHz)
1	7.27-7.11 m [a]	118.4
2	6.94 d, 7.1 Hz	119.7
3	7.27-7.11 m [a]	120.8
4	7.93 d, 8.3 Hz	112.2
4a	-	134.8
5	-	-
6	H^a 2.80 dd, 12.0, 4.3 Hz H^b 3.87 dd, 13.8, 3.3 Hz	31.0
7	H^a 2.53 m H^b 2.72 m	23.3
7a	-	85.3
8	-	59.4
8a	-	134.3
9	6.59 d, 10, 7.8 Hz	122.5
10	6.67 t, 7.3 Hz	118.1
11	7.27-7.11 m [a]	127.2
12	6.87 d, 6.1 Hz	106.0
12a	-	149.1
13	-	-
14	H^a 3.28 ddd, 13.8, 12.1, 3.3 Hz H^b 3.75 ddd, 13.8, 6.0, 2.5 Hz	34.8
15	2.03-1.96 m	22.0
15a	-	132.6
16	-	106.8
16a	-	129.5
17	2.25-2.14 m	22.5
18	2.96-2.85 m	54.1
19	3.04 d, 3.3 Hz	53.4
20	H^a 2.41 ddd, 14.4, 12.0, 4.3 Hz H^b 2.74-2.70 m	29.5
21	H^a 0.96 m H^b 2.20 m	47.5
22/23	H^a 2.88 d, 14.5 Hz H^b 2.93 d, 11.6 Hz	52.9
NH	3.02 s, br	-
Ph	7.27-7.11 m	141.0, 139.9, 128.9, 128.4, 128.3, 127.9, 126.8, 126.3

[a] Overlapping with signals of benzene rings

5.15 Methyl (1*H*-indol-3yl)-acetate (16)[55]

A solution of concentrated sulfuric acid (0.64 mL) in dry methanol (10 mL) was added dropwise to a solution of indole-3-acetic acid (10.0 g, 0.06 mol) in dry MeOH (100 mL). The reaction mixture was refluxed for 5 h. After quantitative removal of methanol, the residue was poured into crushed ice, and extracted with diethyl ether (4 x 75 mL). The combined diethyl ether layers were washed with saturated sodium hydrogen carbonate (3 x 25 mL), water (2 x 25 mL), and brine (25 mL), dried over $CaCl_2$ and evaporated *in vacuo*. The brown oily residue was purified by column chromatography (Silica gel, $CHCl_3$:MeOH / 15:1) to obtain compound **16** as a colorless neat liquid (9.8 g, 86%); TLC R_f = 0.38 (Silica gel, $CHCl_3$:MeOH / 15:1); FT-IR (KBr) ν (cm^{-1}); 3410, 3055, 2951, 2844, 1730, 1454, 1433, 1338, 1165, 1095, 1008, 728.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



16

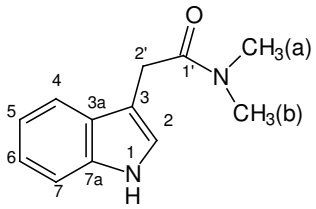
	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.16 s	-
2	6.96 s	123.2
3	-	107.8
3a	-	127.0
4	7.64 d, 7.6 Hz	119.5
5	7.26-7.15 m	121.9
6		118.6
7		111.2
7a	-	136.0
1‘	-	172.8
2‘	3.85 d, 1.01 Hz	31.0
-OCH_3	3.72 s	23.0

5.16 2-(1*H*-Indol-3yl)-*N*,*N*-dimethyl acetamide (17)[55]

Compound **16** (9.1 g, 0.048 mol) was stirred with 40% aq. dimethylamine (60 mL) at room temperature for 16 h. After adding water (50 mL) the reaction mixture was extracted with CH_2Cl_2 (3 x 50 mL), washed with water (2 x 50 mL) and brine (50 mL), dried over $MgSO_4$, and the solvent was evaporated *in vacuo*. After purification by column chromatography (Silica gel, $CHCl_3$:MeOH:NH_3 / 10:1:0.1) compound **17** was obtained as a pale brown solid (3.7 g, 38%); mp 120-123 ^{o}C (Lit.[55] 119-120 ^{o}C); TLC R_f = 0.55 (Silica gel, $CHCl_3$:MeOH:25 %

NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}); 3187, 2930, 2876, 1616, 1445, 1391, 1124, 1055, 1007, 735, 675.

^1H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



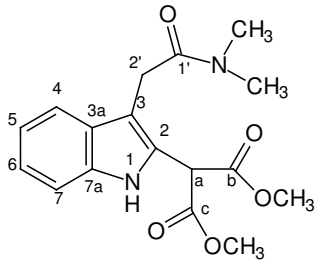
17

	^1H(400 MHz)	^{13}C(100 MHz)
1	8.56 s	-
2	6.97 s	122.7
3	-	109.0
3a	-	127.2
4	7.62 d, 7.1 Hz	119.4
5	7.09 dd, 7.1, 1.3 Hz	122.0
6	7.15 dd, 7.1, 1.3 Hz	118.6
7	7.30 d, 7.1 Hz	111.3
7a	-	136.2
1‘	-	171.7
2‘	3.81 s	31.2
-$N(CH_3)_2$	(a) 3.00 s (b) 2.97 s	(a) 37.8 (b) 35.6

5.17 Dimethyl [2-(3-dimethylcarbamoylmethyl)-1*H*-indol-2-yl]-propanedioate (18)

18 was prepared similar to the synthesis of **2** using a solution of **17** (4.32 g, 0.021 mol) and triethylamine (2 mL) in dry THF (100 mL), *tert*-butyl hypochlorite (3.5 g, 0.032 mol), and thallium dimethyl malonate (7.75 g, 0.023 mole) in dry THF (100 ml). The crude product was purified by column chromatography (Silica gel, EtOAc:MeOH / 30:1) to give **18** as a pale brown solid (4.25 g, 61%); mp 173-175 °C TLC R_f = 0.43 (Silica gel, EtOAc:MeOH / 30:1); FT-IR (ATR) ν (cm^{-1}) 3216, 3025, 2957, 1760, 1724, 1631, 1453, 1432, 1311,1128, 1015, 752; MS (EI, 70 eV) *m/z* (rel int) 332 (1), 300 (44), 260 (48), 228 (100), 200 (20), 169 (23), 72 (45).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



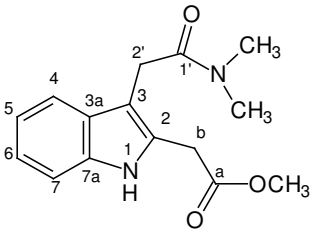
18

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	9.03 s	-
2	-	126.1
3	-	108.1
3a	-	127.2
4	7.55 d, 8.2 Hz	119.8
5	7.09 ddd, 8.2, 7.6, 1.3 Hz	122.5
6	7.17 ddd, 8.2, 7.6, 1.3 Hz	118.4
7	7.34 d, 8.2 Hz	111.3
7a	-	135.6
1‘	-	171.1
2‘	3.81 s	30.0
a	5.38 s	49.0
b	-	167.8
c	-	167.8
2 x –OCH_3	3.75 s	53.1
-$N(CH_3)_2$	(a) 2.98 s (b) 2.91 s	(a) 37.7 (b) 35.7

5.18 Methyl 3-(dimethylcarbamoylmethyl)-1*H*-indol-2yl-acetate (19)

Compound **19** was prepared similar to the synthesis of **3**. Starting compound, reagents and solvent were as follows: **18** (4.23 g, 0.013 mol), lithium iodide hydrate (4.9 g, 0.026 mol), triethylamine hydrochloride (0.45 g), and *N,N*-dimethylacetamide (60 mL). The reaction was worked up by adding brine (60 mL) and 25% aq. NH_3, and then extracted with diethyl ether (10 x 80 mL). The combined diethylether extracted were washed with water (3 x 50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The brown residue was purified by column chromatography (Silica gel, EtOAc:MeOH / 30:1) to obtain compound **19** as a pale brown solid (0.6 g, 17%); mp 144-146 ^{o}C; TLC R_f = 0.56 (Silica gel, $CHCl_3$:MeOH:25 % NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3235, 3059, 2930, 1736, 1625, 1491, 1462,1398,1195, 1144, 738; MS (EI, 70 eV) *m/z* (rel int) 274 (5), 216 (19), 202 (15), 144 (100), 72 (12).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



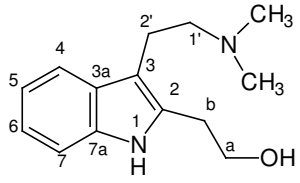
19

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.86 s	-
2	-	127.7
3	-	106.4
3a	-	128.1
4	7.51 d, 8.1 Hz	119.5
5	7.06 ddd, 8.1,7.6, 1.0 Hz	121.8
6	7.12 ddd, 8.1, 7.6, 1.0 Hz	118.1
7	7.26 d, 8.1 Hz	110.9
7a	-	135.4
1‘	-	171.3
2‘	3.76 s	30.2
a	-	171.0
b	3.83 s	31.7
$-OCH_3$	3.69 s	52.2
$-N(CH_3)_2$	2.99 s, 2.93 s	37.7, 35.7

5.19 2-[3-(2-Dimethylaminoethyl)-1*H*-indol-2yl]-ethanol (20)

20 was prepared similar to the synthesis of **4** using **19** (0.6 g, 2.3 mmol), $LiAlH_4$ (0.17 g, 4.5 mmol), and dry THF (100 mL). The reaction was terminated by carefully adding of water (2 mL) and 20% aq. NaOH. The mixture was stirred at room temperature for 1 h and filtered. The precipitate was washed with THF and the combined THF layers were dried over Na_2SO_4 and evaporated *in vacuo*. After purification by column chromatography (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:2:0.1) compound **20** was obtained as yellow brown solid (0.26 g, 49%); mp 130-133 ^{o}C; TLC R_f = 0.27 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:2:0.1); FT-IR (ATR) ν (cm^{-1}) 3219, 3194, 3113, 2942, 2821, 1464, 1362, 1328, 1242, 1174, 1055, 1003, 731; MS (EI, 70 eV) *m/z* (rel int) 232 (2), 214 (2), 202 (2), 174 (2), 58 (100).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



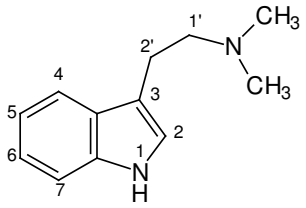
20

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.40 s	-
2	-	125.5
3	-	110.6
3a	-	128.1
4	7.47 d, 7.1 Hz	119.0
5	7.12-7.04 m	121.2
6	7.12-7.04 m	117.8
7	7.24 d, 7.8 Hz	110.9
7a	-	135.7
1‘	2.84 t, 6.8 Hz	60.2
2‘	2.55 t, 6.8 Hz	22.4
a	3.83 t, 7.0 Hz	61.5
b	2.91 t, 7.0 Hz	29.4
-$N(CH_3)_2$	2.22 s	45.5
-OH	4.15 bs	-

5.20 3-(2-Dimethylaminoethyl)-indole (21)

A solution of $NaCNBH_3$ (3.1 g, 0.05 mol) in MeOH (12 mL) was added to a solution of tryptamine (3.0 g, 0.02 mol) in glacial acetic acid (5 mL). A solution of 40 % aq. formaldehyde (7.5 mL, 0.1 mol) in MeOH (50 mL) was added to the reaction mixture. After stirring at room temperature for 3 h, MeOH was removed *in vacuo*. The residue was basified by 1N NaOH and extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1) to give **21** as a brown solid (2.7 g, 72%); mp 43-44 ^{o}C (Lit[62]. 44-46 ^{o}C); TLC R_f = 0.36 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3409, 3047, 2962, 1615, 1560, 1483, 1454, 1421, 1089, 1005, 914, 750, 736.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



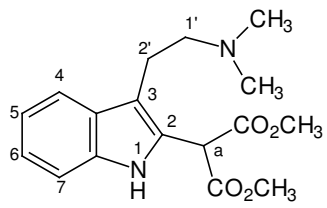
21

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.61 s, br	-
2	6.95 d, 1.3 Hz	121.7
3	-	113.9
3a	-	127.4
4	7.64 d, 8.1 Hz	119.0
5	7.20 ddd, 8.1, 7.0, 1.0 Hz	121.6
6	7.14 ddd, 8.1, 7.0, 1.0 Hz	118.6
7	7.31 d, 8.1 Hz	111.1
7a	-	136.3
1‘	3.00 t, 8.3 Hz	58.5
2‘	2.70 t, 8.3 Hz	24.1
2 x CH_3	2.39 s	45.8

5.21 Dimethyl [3-(2-dimethylaminoethyl)-1*H*-indol-2-yl]-propanedioate (22)

22 was prepared similar to the synthesis of **2** using **21** (2.7 g, 0.014 mol), *tert*-butylhypochlorite (3.11, 0.03 mol), triethylamine (1.5 mL) in dry THF (50 mL), and thallium dimethylmalonate (5.8g, 0.02 mol) in dry THF (50 mL). The crude residue was purified by column chromatography (Silica gel, $CHCl_3$: MeOH:25% NH_3 / 10:1:0.1) to give **22** as a pale brown solid (0.63 g, 14%); TLC R_f = 0.61 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



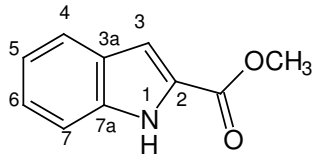
22

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.93 s, br	-
2	-	124.6
3	-	113.3
3a	-	127.4
4	7.57 d, 8.1 Hz	119.2
5	7.18 ddd, 8.1, 7.0, 1.0 Hz	122.5
6	7.09 ddd, 8.1, 7.0, 1.0 Hz	118.7
7	7.35 d, 8.1 Hz	111.2
7a	-	135.9
1‘	2.92 m	60.2
2‘	2.51 m	22.7
a	5.06 s	48.9
C=O	-	167.7
2 x CO_2CH_3	3.77 s	53.1
2 x CH_3	2.33	45.4

5.22 Methyl 1*H*-indole-2-carboxylate (23)

A solution of conc. H_2SO_4 (3.5 mL) in MeOH (10 mL) was added to the solution of indole-2-carboxylic acid (5.0 g, 0.03 mol) in MeOH (65 mL), and then refluxed for 3 h. After removing MeOH, water (50 mL) was added. The reaction mixture was extracted with chloroform (3 x 50 mL), washed with saturated sodium hydrogen carbonate (2 x 50 mL) and brine 50 (mL), dried over $MgSO_4$, and then evaporated *in vacuo* to give **23** as a the pale-yellow product was obtained (5.06 g, 93 %); mp 153-155 °C (Lit.[63] 150-152 °C); TLC R_f = 0.27 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:2:0.1); FT-IR (ATR) ν (cm^{-1}) 3312, 2954, 1686, 1526, 1437,1312, 1251, 1208, 772, 745.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



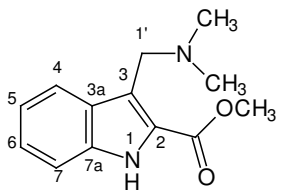
23

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	9.21 bs	-
2	-	125.5
3	6.47 d, 1.1 Hz	111.7
3a	-	128.5
4	7.85 d, 8.1 Hz	121.6
5	7.30 ddd, 8.1, 7.4, 1.0 Hz	124.4
6	7.14 ddd, 8.1, 7.4, 1.0 Hz	120.6
7	7.35 d, 8.1 Hz	120.4
7a	-	135.6
1‘	3.97 s	52.8
-OCH_3	3.92 s	51.7
2 x (-NCH_3)	2.33 s	45.6

5.23 Methyl 3-[(dimethylamino)methyl]-1*H*-indole-2-carboxylate (24)

23 (5.56 g, 0.032 mol) was treated with a mixture of 40 % aq. dimethylamine (12 mL), glacial acetic acid (12 mL), and 40 % aq. formaldehyde (12 mL) in an ice-bath. The reaction mixture was warmed until clear, then stirred at room temperature for 2 h. 20 % aq. NaOH was added to pH 10 and the reaction mixture was extracted with CH_2Cl_2 (2 x 100 mL). The combined organic phases were dried over MgSO4, and evaporated *in vacuo*. The crude product was purified by column chromatography (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1) obtained **24** as a pale-yellow solid (6.33 g, 86 %); mp 103-105 °C (Lit.[64] 103-104 °C); TLC R_f = 0.36 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3349, 3061, 2813, 2760, 1672, 1539,1252,1019, 747.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



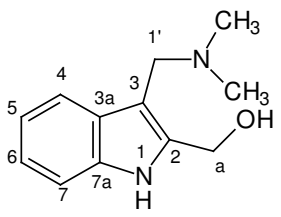
24

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	9.21 bs	-
2	-	125.5
3	-	111.7
3a	-	128.5
4	7.85 d, 8.1 Hz	121.6
5	7.30 ddd, 8.1,7.5,1.0 Hz	124.4
6	7.14 ddd, 8.1,7.5,1.0 Hz	120.6
7	7.35 d, 8.1 Hz	120.4
7a	-	135.6
1‘	3.97 s	52.8
-OCH_3	3.92 s	51.7
2 x (-NCH_3)	2.33 s	45.6

5.24 (3-[(Dimethylamino)methyl]-1*H*-indol-2yl)-methanol (25)

The solution of **24** (2.0 g, 8.6 mmol) in dry THF (25 mL) was added dropwise to the suspension of $LiAlH_4$ (0.4 g, 10.0 mmol) in dry THF (25 mL) at 0 °C under argon. The mixture was stirred at room temperature for 3 h. After cooling to 0 °C, water (1 mL) was slowly added, followed by the careful addition of 15% NaOH (1 mL) and water (2 mL). The reaction mixture was stirred at room temperature for 1 h and filtered. The precipitate was washed with THF and the combined THF solutions were dried over Na_2SO_4 and evaporated *in vacuo*. The product **25** was obtained as a white solid (1.50 g, 87 %); mp 77-78 °C; TLC R_f = 0.11 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3189, 3060, 2946, 2779, 1456, 1345, 1312, 1038, 1015, 1000, 834, 737; MS (EI, 70 eV) *m/z* (rel int) 204 $[M+1]^+$ (9), 160 (77), 142 (100), 130 (56), 115 (27), 46 (95).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



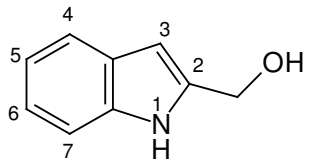
25

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	10.93 s	-
2	-	128.4
3	-	107.9
3a	-	135.1
4	7.52 d, 8.1 Hz	118.4
5	7.02 ddd, 8.1, 7.0, 1.0 Hz	120.6
6	6.94 ddd, 8.1, 7.0, 1.0 Hz	118.3
7	7.31 d, 8.1 Hz	111.0
7a	-	137.6
1‘	3.51 s	52.8
a	4.64 s	55.1
2 x (-NCH_3)	2.13 s	44.8
-OH	5.52 bs	-

5.25 1*H*-Indol-2-yl-methanol (26)

A solution of indole-2-carboxylic acid (4.84 g, 0.03 mol) in dry THF (40 mL) was added dropwise into a suspension of $LiAlH_4$ (1.7 g, 0.04 mol) in dry THF (100 mL) under argon at 0°C. The reaction mixture was stirred and refluxed for 4 h. After cooling at 0 °C, water (5 mL) was carefully added, followed by 15% aq. NaOH (4 mL). Vigorously stirring was continued at room temperature for 1 h. The reaction mixture was filtered and the precipitate was washed with THF. The combined THF solutions were dried over $MgSO_4$ and evaporated *in vacuo*. The product **26** was obtained as a pale-yellow solid (3.97 g, 90 %); mp 76-78 °C (Lit.[65] 76-77 °C); TLC R_f = 0.11 (Silica gel, $CHCl_3$:MeOH:NH_3/10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3373, 3232, 3049, 2870, 1452, 1414, 1338, 1283,1134, 1053, 1005, 742, 735.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



26

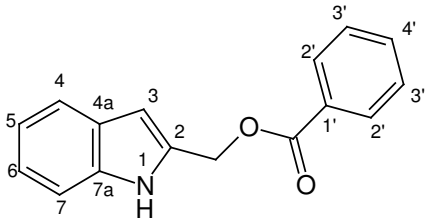
	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.26 s, br	-
2	-	128.4
3	6.24 d, 1.3 Hz	101.0
3a	-	136.8
4	7.52 dd, 8.0, 1.0Hz	118.4
5	7.01 ddd, 8.0, 7.3, 1.3 Hz	122.6
6	6.08 ddd, 8.0, 7.3, 1.3 Hz	120.4
7	7.14 dd, 8.0, 1.0 Hz	111.5
7a	-	137.9
-CH_2	4.58 s	58.9
-OH	2.41 s, br	-

5.26 1*H*-Indol-2-yl-methyl-benzoate (27)

Benzoyl chloride (7.6 g, 0.054 mole) was added dropwise to a solution of **26** (5.28 g, 0.036 mol) and triethylamine (7.3 g, 0.072 mol) in dry THF (150 mL) at 0 °C. The reaction mixture was stirred for 4 h at room temperature. Afterwards the solvent was evaporated *in vacuo*. The residue was treated with 10% aq. NaOH solution (80 mL) and the product was extracted with CH_2Cl_2 (5 x 80 mL). After washing with water (3 x 50 mL), the combined organic phases were dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by column chromatography (Silica gel, EtOAc:Hex / 1:3) to give **27** as a pale-brown solid (8.7 g, 96 %); mp 129-130 °C; TLC R_f = 0.63 (Silica gel, EtOAc:Hex / 1:3); FT-IR (ATR) ν (cm^{-1}) 3347,

3057,1702,1452, 1276, 1100, 1070, 920, 732, 705; MS (EI, 70 eV) *m/z* (rel int) 251 $[M]^+$ (47), 130 (49), 129 (100), 105 (40), 77 (24).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



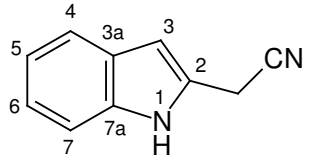
27

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.74 s, br	-
2	-	127.5
3	6.64 d, 1.3 Hz	104.1
3a	-	133.3
4	7.63 d, 8.0 Hz	120.9
5	7.22 ddd, 8.0, 7.6, 1.0 Hz	122.8
6	7.15 ddd, 8.0, 7.6, 1.0 Hz	120.0
7	7.33 dd, 8.0, 0.8 Hz	111.1
7a	-	136.6
-CH_2-	5.49 s	60.2
1‘	-	116.5
2‘	8.08 dd, 7.83, 1.5 Hz	129.6
3‘	7.44 t, 7.83 Hz	128.4
4‘	7.57 tt, 7.83, 1.5 Hz	133.0

5.27 1*H*-Indol-2-yl acetonitrile (28)

KCN (9.0 g, 0.138 mol) was added to a solution of **27** (8.7 g, 0.035 mol) in dry DMSO (200 mL). The reaction mixture was stirred at 60 ^{o}C for 8 h. After cooling to room temperature, the reaction mixture was poured into 5% aq. $NaHCO_3$ solution (800 mL) and extracted with CH_2Cl_2 (8x100 mL). The combined organic layers were washed with brine (3x50 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The dark residue was purified by column chromatography (Silica gel, EtOAc:Hex / 1:2) to give **28** as a red-brown solid (3.1 g, 57 %); mp 97-98 ^{o}C (Lit.[66] 96-98 ^{o}C); TLC R_f = 0.31 (Silica gel, EtOAc:Hex / 1:2); FT-IR (ATR) ν (cm^{-1}) 3340, 2922, 2853, 2255, 1450, 1433,1344, 1294, 793, 744, 720.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



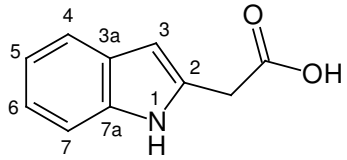
28

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.24 s, br	-
2	-	125.8
3	6.47 dd, 1.1, 1.0 Hz	102.6
3a	-	128.0
4	7.59 d, 8.0 Hz	120.5
5	7.23 ddd, 8.0, 7.2, 1.0 Hz	122.6
6	7.15 ddd, 8.0, 7.2, 1.0 Hz	120.4
7	7.33 dd, 8.0, 0.8 Hz	111.0
7a	-	136.5
-CH_2-	3.83 d, 0.8 Hz	17.4
-CN	-	116.5

5.28 1*H*-Indol-2-yl acetic acid (29)

30 % aq. NaOH solution (110 mL) was added to a solution of **28** (3.1 g, 0.02 mol) in MeOH (50 mL). The reaction mixture was stirred at reflux for 6 h. After cooling to 0 ^{o}C, the reaction mixture was acidified to pH 1-2 with 10% aq. HCl and extracted with CH_2Cl_2 (3 x 100 mL). Washing with brine (2 x 50 mL), drying over $MgSO_4$ and evaporation *in vacuo*, gave the odour acid **29** as a brown crystal solid (2.76 g, 79%); mp 91-92 ^{o}C (Lit.[67] 93 ^{o}C); TLC R_f = 0.4 (Silica gel, $CHCl_3$:MeOH:CH_3COOH / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3384, 3227, 3054, 2923, 2853, 1667, 1612, 1489, 1454,1329, 1281, 748, 731. Acid **29** was used directly for the next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



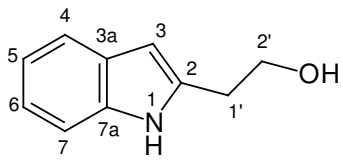
29

	^{1}H(400 MHz)	^{13}C (100 MHz)
1	10.98 s	-
2	-	128.7
3	6.26 dd, 1.0, 0.8 Hz	102.6
3a	-	135.5
4	7.44 d, 8.0 Hz	119.4
5	7.03 ddd, 8.0, 7.1, 1.0 Hz	120.5
6	6.98 ddd, 8.0, 7.1, 1.0 Hz	119.7
7	7.38 d, 8.0 Hz	118.9
7a	-	136.2
-CH_2-	3.74 s	34.0
-COOH	12.50 s, br	171.5

5.29 2-(1*H*-Indol-2-yl)ethanol (30)

The solution of **29** (1.6 g, 9.13 mmol) in dry THF (40 mL) was added dropwise to the suspension of $LiAlH_4$ (0.42 g, 11.1 mmol) in dry THF (80 mL) under argon at 0 ^{o}C. The mixture was stirred at room temperature for 6 h. After cooling to 0 ^{o}C, 3 ml of water was slowly added, followed by the careful addition of 20% aq. NaOH (3 mL). The reaction mixture was stirred at room temperature for 1 h and filtered. The precipitate was washed with THF and the combined THF solutions were dried over Na_2SO_4 and evaporated *in vacuo*. The product **30** was obtained as a brown solid (0.82 g, 56 %) after purification by column chromatography (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1): mp 54-56 ^{o}C (Lit.[68]

55-56 °C); TLC R_f = 0.36 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3379, 3050, 2937, 2882,1549, 1454, 1412, 1341, 1288, 1037, 1016, 776, 749.
^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



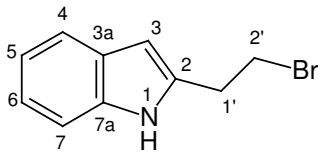
30

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.36 s, br	-
2	-	128.4
3	6.29 d, 1.0 Hz	100.1
3a	-	136.0
4	7.57 d, 8.0 Hz	119.6
5	7.17 ddd, 8.0, 7.1, 1.1 Hz	121.2
6	7.11 ddd, 8.0, 7.1, 1.1 Hz	119.8
7	7.30 d, 8.0 Hz	110.6
7a	-	136.9
1‘	2.92 t, 5.8 Hz	31.1
2‘	3.87 t, 5.8 Hz	62.1
-OH	2.05 s, br	-

5.30 2-(2-Bromoethyl)-1*H*-indole (32)

The solution of *tris*-(dimethylamino)-phosphine (1.21 g, 7.4 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to the mixture of **30** (0.3 g, 1.9 mmol) and carbon tetrabromide (1.23 g, 3.7 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C. After stirring for 16 h at room temperature, the reaction mixture was washed with water (3x20 mL) and brine (20 mL), dried over Na_2SO_4 and evaporated *in vacuo*. Purification by column chromatography (Silica gel, EtOAc:hexane / 1:5) gave the compound **32** (0.1 g, 24 %) as a brown solid: mp 65-66 °C; TLC R_f = 0.26 (Silica gel, EtOAc:hexane / 1:5); FT-IR (ATR) ν 3375, 3010, 2937, 1545, 1454, 1412, 1340, 1251, 1190, 776.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}CNMR (100 MHz, $CDCl_3$).



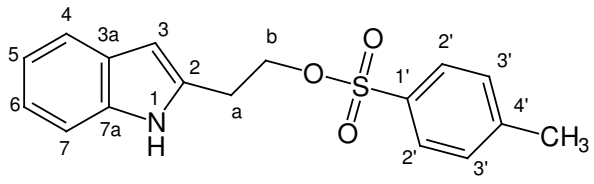
32

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.07 s, br	-
2	-	121.8
3	6.33 d, 1.3 Hz	101.0
3a	-	128.4
4	7.55 d, 8.0 Hz	119.6
5	7.15 ddd, 8.0, 6.9, 1.3 Hz	120.2
6	7.09 m	120.0
7	7.28 dd, 8.0, 0.8 Hz	110.6
7a	-	136.0
1‘	3.35 t, 7.1 Hz	31.2
2‘	3.65 t, 7.1 Hz	32.0

5.31 2-(1*H*-Indol-2-yl)ethyl-4-methylbenzenesulfonate (34)

p-Toluenesulfonyl chloride (1.2 g, 6.3 mmol) was added dropwise to a solution of **30** (0.82 g, 5.1 mmol) and triethylamine (1.6 mL, 11.3 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into ice-H_2O (300 mL) under stirring and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (2 x 70 mL) and the combined organic phases were washed with 2M aq. HCl (15 mL), 5% aq. $NaHCO_3$ (15 mL), brine (2 x 30 mL), dried over $MgSO_4$, and evaporated *in vacuo*. The crude product was purified by column chromatography (Silica gel, $CHCl_3$) to give **34** as a brown solid (1.13 g, 70 %); mp 115-116 °C (Lit.[68] 114-116 °C); TLC R_f = 0.36 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 3377, 3053, 2909, 1460, 1412, 1378, 1343,1171, 1096, 982, 914, 812, 796.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



34

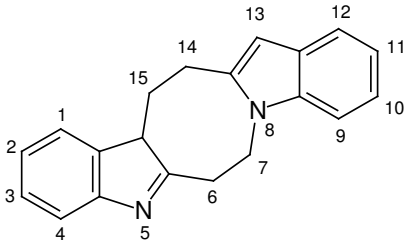
	^{1}H(400 MHz)	^{13}C(100 MHz)
1	8.36 s, br	-
2	-	128.4
3	6.29 d, 1.0 Hz	100.1
3a	-	132.6
4	7.57 d, 8.0 Hz	119.6
5	7.17 ddd, 8.0, 7.3, 1.0 Hz	121.2
6	7.11 ddd, 8.0, 7.3, 1.0 Hz	119.8
7	7.30 d, 8.0 Hz	110.6
7a	-	136.2
a	2.92 t, 5.8 Hz	31.1
b	3.87 t, 5.8 Hz	62.1
-OH	2.05 bs	-
1‘	-	145.0
2‘	7.27 d, 8.1 Hz	129.8
3‘	7.20 d, 8.1 Hz	127.8
4‘	-	131.3
-CH_3	2.38 s	21.6

5.32 6,7,14,15-Tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole (35)

The suspension of NaH (0.1 g, 4.2 mmol) in dry DMF (20 mL) was added dropwise to the solution of **35** (1.0 g, 3.2 mmol) in dry DMF (20 mL) at 0 °C. The reaction mixture was allowed to stir for 15 min. at 0 °C and for 20 min at room temperature. Cool water (60 mL) was added dropwise and the reaction mixture was extracted with diethyl ether (4 x 50 mL). The combined ether extracts were washed with water (2 x 50 mL) and brine (50 mL), dried

over Na_2SO_4 and evaporated *in vacuo*. Purification by column chromatography (Silica gel, $CHCl_3$:hexane / 1:2) gave the desired ring **35** (0.17 g, 44 %) as a pale brown solid: mp 148-150 °C; TLC R_f = 0.43 (Silica gel, $CHCl_3$:hexane / 1:2); FT-IR (ATR) ν (cm^{-1}) 3042, 2992, 2967, 1607, 1480, 1454, 1387, 1351, 1320, 1199,1018, 739; MS (EI, 70 eV) *m/z* (rel int), 286 $[M]^+$ (3), 258 (100), 257 (26), 129 (15), 128 (16); HREIMS *m/z* 285.1393 $[M-1]^+$ (calc. for $C_{20}H_{17}N_2$, 285.1392).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



35

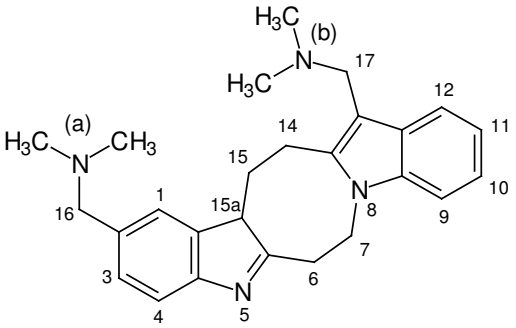
	^{1}H(400 MHz)	^{13}C(100 MHz)
1	7.00 d, 7.1 Hz	124.6
2	6.65 m	113.7
3	7.10 m	128.1
4	6.65 m	106.1
4a	-	151.6
5	-	-
5a	-	83.8
6	H^a 3.52 ddd, 14.3, 13.5, 3.0 Hz H^b 3.93 dd, 14.3, 5.1 Hz	37.8
7	H^a 2.70 dd, 15.9, 3.0 Hz H^b 3.12 m	22.2
8	-	-
8a	-	134.6
9	7.68 d, 8.1 Hz	111.3
10	7.20 t, 8.1 Hz	120.9
11	7.10 m	120.2
12	7.54 d, 7.8 Hz	120.8
13	6.16 s	98.9
13a	-	135.7
14	H^a 2.65 m H^b 3.43 m	34.6
15	H^a 2.05 m H^b 2.65 m	22.0
15a	4.73 t, 8.3 Hz	48.5
15b	-	132.6

5.33 13-(Dimethylaminomethyl)-6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole (36) and 2,13-Bis-(dimethylaminomethyl)-6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole (37)

A mixture of 40 % aq. dimethylamine (0.3 mL, 2.0 mmol), glacial acetic acid (12 mL), and 40% aq. formaldehyde (0.2 mL, 2.0 mmol) was added to compound **35** (0.2 g, 0.7 mmol) at

0 °C. The reaction mixture was warmed until clear, and stirred at room temperature for 4 h. The reaction mixture was made alkaline to pH 10 by 15% aq. NaOH. Extraction with CH_2Cl_2 (3 x 25 mL), drying over $MgSO_4$, and evaporation *in vacuo* gave the crude product which was purified by column chromatography (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1) obtained **37** as a pale-yellow solid (60 mg, 43 %); mp 75-76 °C; TLC R_f = 0.14 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1); FT-IR (ATR) ν (cm^{-1}) 2940, 2853, 2809, 2761, 1612, 1485, 1455, 1320, 1173,1013,737; MS (EI, 70 eV) *m/z* (rel int) 400 $[M]^+$ (2), 372 (60), 328 (100), 284 (34), 142 (34); HREIMS *m/z* 372.2312 $[M-14]^+$ (calc. for $C_{20}H_{17}N_2$, 372.2314).

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).

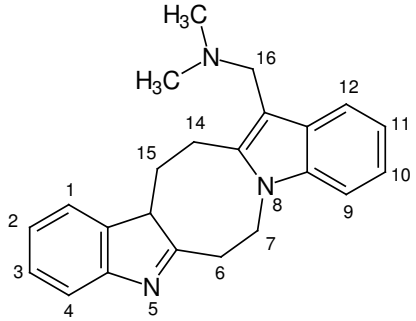


37

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	6.95 d, 1.5 Hz	125.3
2	-	128.8
3	6.98 dd, 8.1, 1.5 Hz	128.6
4	6.55 d, 8.0 Hz	105.1
4a	-	150.3
5	-	-
5a	-	83.5
6	H^a 3.48 m H^b3.94 dd, 14.4, 3.8 Hz	36.6
7	H^a 2.95 m H^b 3.02 m	20.1
8	-	-
8a	-	134.0
9	7.57 m	110.7
10	7.20 t, 7.3 Hz	120.6
11	7.13 t, 7.3 Hz	119.6
12	7.57 m	119.0
12a		129.5
13	-	107.4
13a	-	133.7
14	H^a 2.63 m H^b 3.41 m	34.3
15	H^a 2.03 m H^b 2.60 m	21.6
15a	4.69 t, 8.3 Hz	48.1
15b	-	132.3
16	H^a 3.21 d, 12.6 Hz H^b 3.33 d, 12.6 Hz	64.1
17	H^a 3.42 d, 13.1 Hz H^b 3.50 d, 13.1 Hz	52.9
N-CH_3 (a)	2.21 s	45.5
N-CH_3 (b)	2.18 s	45.1

The monoalkylated product **36** was obtained as a pale-yellow solid (10 mg, 8.3%); TLC R_f = 0.36 (Silica gel, $CHCl_3$:MeOH:25% NH_3 / 10:1:0.1).

^1H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



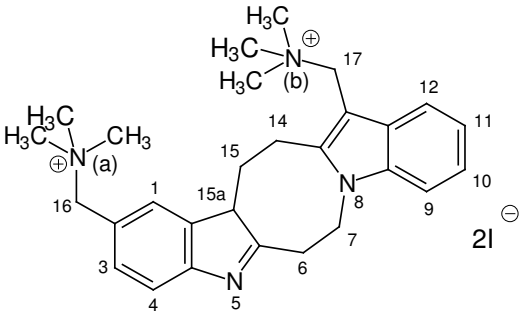
36

	^1H(400 MHz)	^{13}C(100 MHz)
1	6.99 d, 7.1 Hz	124.2
2	6.63 m	105.7
3	7.08 dd, 7.8, 1.0 Hz	127.7
4	6.63 m	118.2
4a	-	151.1
5	-	-
5a	-	83.3
6	H^a 3.51 m H^b 3.95 m	36.5
7	H^a & H^b 3.01-2.97 m	20.2
8	-	-
8a	-	134.0
9	7.65 m	110.8
10	7.20 ddd, 8.1, 7.5, 0.8 Hz	120.6
11	7.13 ddd, 8.1, 7.5, 0.8 Hz	119.7
12	7.65 m	119.0
12a	-	128.9
13	-	107.1
13a	-	133.9
14	H^a 2.64 m H^b 3.41 m	34.3
15	H^a 2.04 m H^b 2.64 m	21.5
15a	4.72 t, 8.3 Hz	48.2
15b	-	132.2
16	H^a 3.44 d, 12.9 Hz H^b 3.53 d, 12.9 Hz	52.8
2 x N-CH_3	2.22 s	45.4

5.34 *N,N′*-Dimethyl-2,13-bis-(dimethylaminomethyl)-6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole diiodide (38)

Compound **37** (20 mg, 0.05 mmol) was stirred in an excess of methyl iodide (1 mL) at room temperature for 1 h. Diethyl ether (5 mL) was added and the precipitate was collected by filtration, washed with diethyl ether (5 x 5 mL), and dried *in vacuo*. The pure compound **38** was obtained as a pale yellow (0.02g, 59%): mp >230 ^oC FT-IR (ATR) ν (cm^{-1}) 2945, 1614, 1483,1402, 1219, 1068, 1032, 976, 863, 751; MS (FAB, MNOBA matrix) *m/z* (rel int) 557 $[M-I]^+$.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



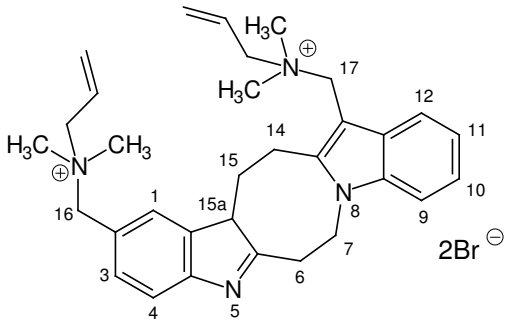
38

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	7.14 d, 1.5 Hz	127.9
2	-	128.8
3	7.23 m	133.8
4	6.97 d, 8.1 Hz	105.9
4a	-	152.2
5	-	-
5a	-	83.0
6	H^a 3.59 m H^b 4.18 dd, 14.1, 5.3 Hz	35.2
7	3.17 m	20.5
8	-	-
8a	-	139.3
9	7.90 d, 8.3 Hz	111.8
10	7.20 t, 7.3 Hz	121.8
11	7.23 m	121.0
12	7.82 d, 7.8 Hz	118.8
12a	-	129.0
13	-	99.0
13a	-	117.3
14	H^a 2.63 m H^b 3.59 m	33.9
15	2.80 m	21.3
15a	4.81 t, 7.6 Hz	47.1
15b	-	132.1
16	4.33 s	68.2
17	H^a 4.57 d, 13.6 Hz H^b 4.63 d, 13.9 Hz	59.1
N-CH_3 (a)	2.99 s	51.5
N-CH_3 (b)	2.93 s	51.3

5.35 *N,N′*-Diallyl-2,13-bis-(dimethylaminomethyl)-6,7,14,15-tetrahydro-15a*H*-azocino[1,2-a:6,5-b′]diindole dibromide (39)

Compound **37** (20 mg, 0.05 mmol) was stirred in an excess of allyl bromide (1 mL) at room temperature for 1 h. Diethyl ether (5 mL) was added and the precipitate was collected by filtration, washed with diethyl ether (5 x 5 mL), and dried *in vacuo*. The pure compound **39** was obtained as pale yellow solid (0.023 g, 72 %): mp >230 ^{o}C FT-IR (ATR) ν FT-IR (ATR) ν (cm^{-1}) 3014, 2948, 1612, 1477, 1458, 1402, 1323, 1241, 1199, 1070, 960; MS (FAB, MNOBA matrix) *m/z* (rel int) 563, 561[M-Br] $^{+}$.

^{1}H NMR (400 MHz, $CDCl_3$), ^{13}C NMR (100 MHz, $CDCl_3$).



39

	^{1}H(400 MHz)	^{13}C(100 MHz)
1	7.17 d, 1.5 Hz	126.1
2	-	128.2
3	7.25 m	127.5
4	6.96 d, 8.1 Hz	105.9
4a	-	152.2
5	-	-
5a	-	83.0
6	H^a 3.55 ddd, 14.4, 13.5, 1.7 Hz H^b 4.19 dd, 14.4, 4.8 Hz	36.6
7	2.98 m	20.1
8	-	-
8a	-	139.5
9	7.91 d, 8.3 Hz	116.8
10	7.31 t, 8.3 Hz	121.0
11	7.25 m	120.6
12	7.82 d, 7.8 Hz	118.8
12a	-	129.1
13	-	109.4
13a	-	133.8
14	H^a 2.64 m H^b 3.32[a]	34.3
15	H^a 1.90 m H^b 2.54[b]	21.6
15a	4.81 t, 8.3 Hz	48.1
15b	-	133.3
16	H^a 4.32 d, 13.6 Hz H^b 4.35 d, 13.6 Hz	65.5
17	H^a 3.32[a] H^b 3.20[a]	54.9
2 x $\underline{CH}$=CH_2	6.14-6.05 m	121.9
2 x CH=$\underline{CH_2}$	5.64-5.58 m	132.2
2 x $\underline{CH_2}$-CH=	3.18 d, 7.1 Hz	66.9
2 x N-CH_3	2.85 s	48.3

[a] Overlapping with signals of H_2O
[b] Overlapping with signals of DMSO

List of Abbreviations

abs.	Absolut
ACh	Acetylcholine
ATR	Attenuated total reflectance
CARBEN	*N,N'*-dibenzylcaracurinium V dibromide
CIMS	Chemical ionization mass spectrum
CNS	Central nervous system
DAG	Diacylglycerol
DCC	Dicyclohexylcarbodiimide
DEPT	Distortionless enhancement by polarisation transfer
DIBAL	Diisobutylaluminum hydride
DMA	*N,N*-dimethylacetamide
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-dimethylformamide
DMSO	Dimethylsulfoxide
$EC_{25,diss}$	Effective concentration of test compound at which the rate of [^{3}H]NMS dissociation is reduced to 25% of the control value.
$EC_{50,diss}$	Effective concentration of test compound at which the rate of [^{3}H]NMS dissociation is reduced to 50% of the control value.
EDCI	1[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
EEDQ	2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
EIMS	Electron ionization mass spectrometry
EtOAc	Ethyl acetate
FABMS	Fast atom bombardment mass spectrometry
FT-IR	Fourier-transform infrared spectroscopy
GPCR	G-protein-coupled receptors
G-Protein	Guanine-nucleotide-binding protein
H,H COSY	Homonuclear (proton) correlation spectroscopy
Hex	Hexane
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum correlation
HREIMS	High resolution electron ionization mass spectrometry
IP_3	Inosital triphosphate

Lit	Literature
m/z	Mass/charge
MeOH	Methanol
MNOBA	*m*-nitrobenzyl alcohol
mp	Melting point
NEt_3	Triethylamine
nM	Nanomolar
NMe_2	Dimethylamine
NMR	Nuclear magnetic resonance
NMS	*N*-methylscopolamine
NOESY	Nuclear Overhauser effect spectroscopy
$pEC_{50,diss}$	minus log value of the concentration reducing [^{3}H]NMS dissociation half maximally.
PLC_β	Phospholipase C_β
PNS	Peripheral nervous system
rel int	Relative intensity
REM	Rapid eye movement
ROESY	Rotating-frame overhauser effect spectroscopy
RT	Raumtemperatur
SAR	Structure activity relationships
tert-BuOCl	*tert*-butylhypochlorite
THF	Tetrahydrofurane
TLC	Thin layer chromatography
TlDMM	Thalium dimethylmalonate
TM	Transmembrane
TsCl	Tosyl chloride (*p*-toluene sulfonylchloride)
µM	Micromolar

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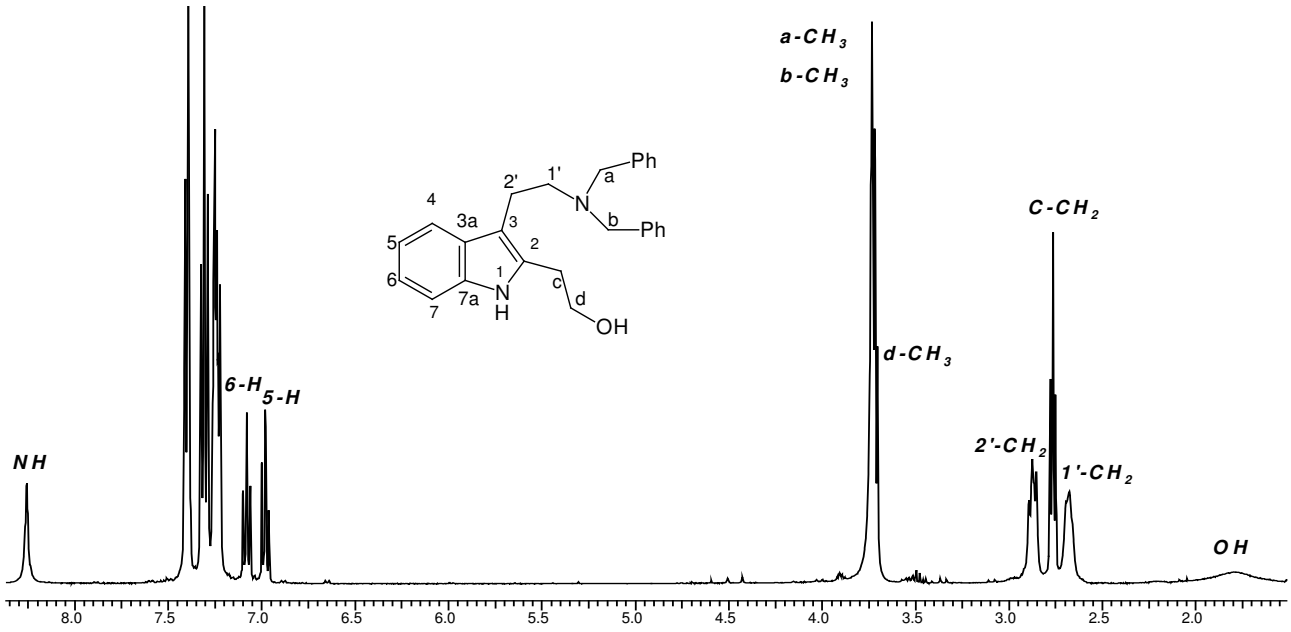
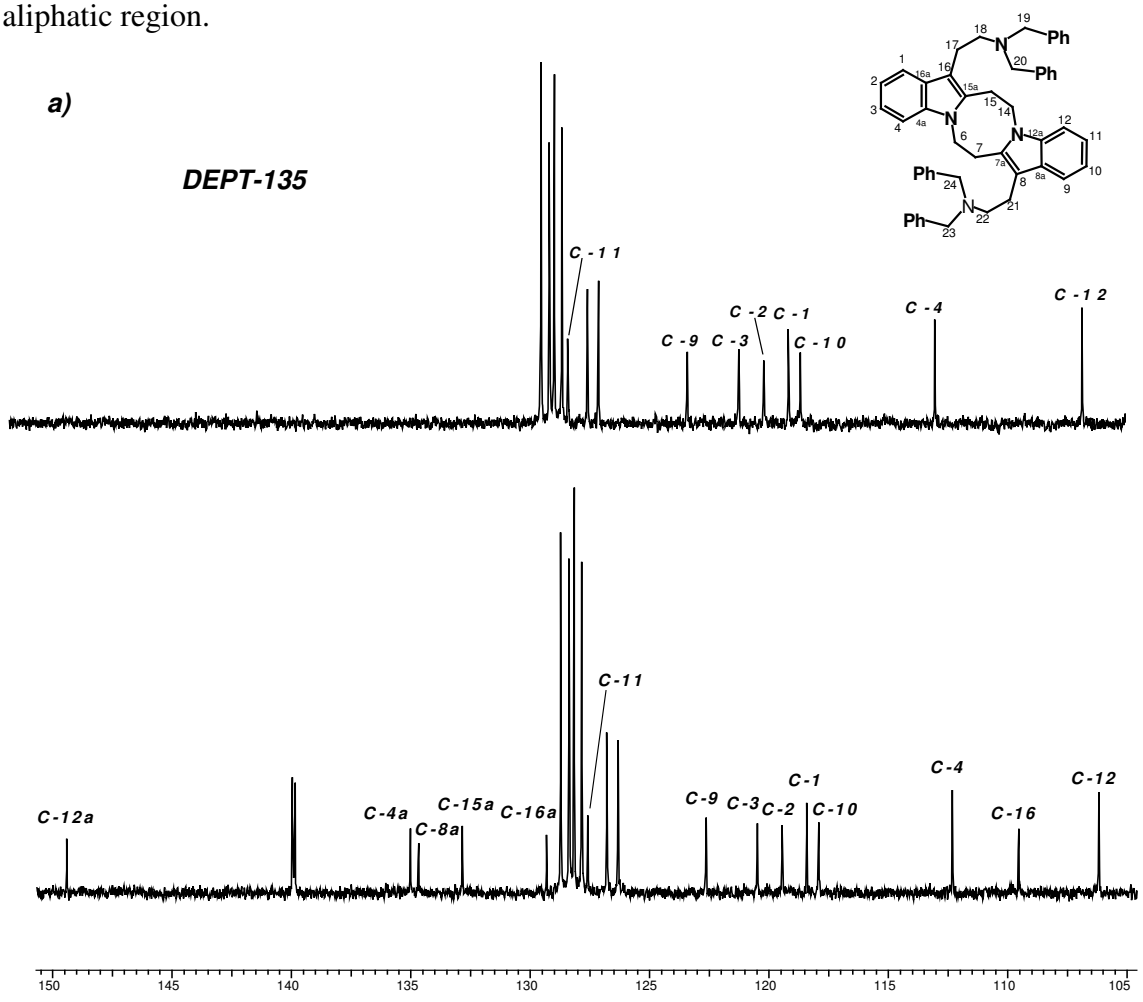
Appendix

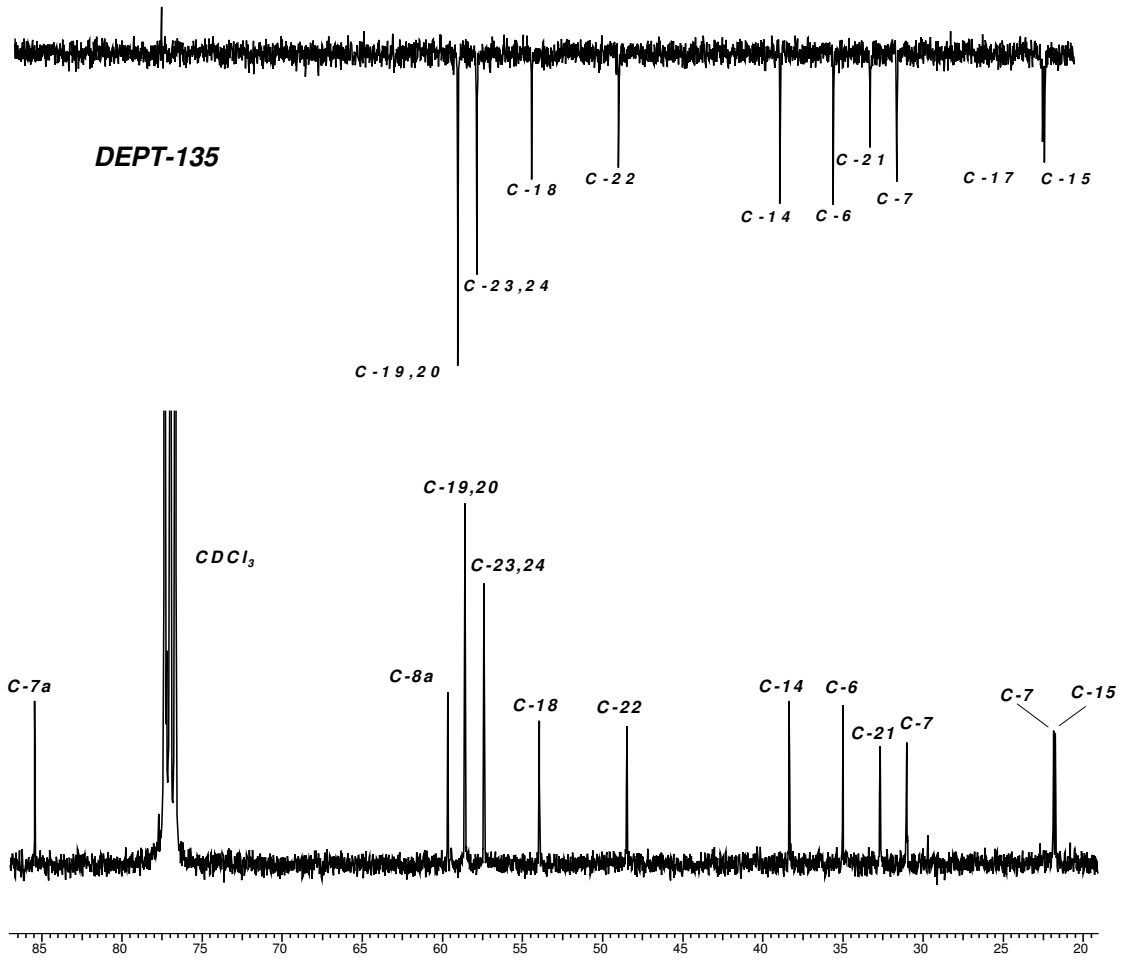
1. 400 MHz ^{1}H-NMR spectrum of **4** ($CDCl_3$)



2. Conventional 100 MHz ^{13}C and 135-DEPT spectra of **6** ($CDCl_3$). (a) aromatic region; (b) aliphatic region.



b)


DEPT-135
C-19,20
C-23,24
C-18
C-22
C-14
C-6
C-21
C-7
C-17
C-15
C-7a
CDCl3
C-19,20
C-23,24
C-8a
C-18
C-22
C-14
C-6
C-21
C-7
C-7
C-15
85
80
75
70
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60
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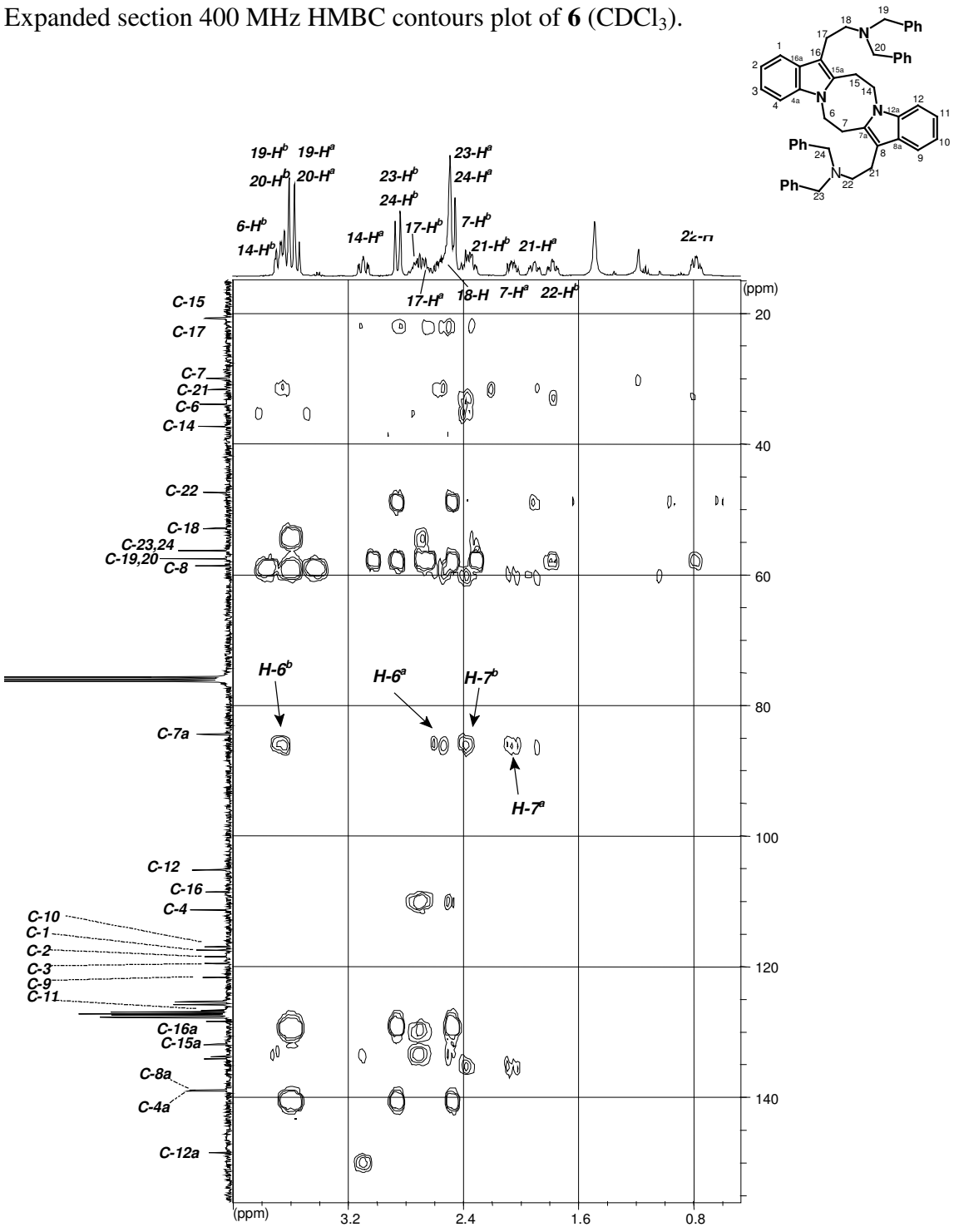
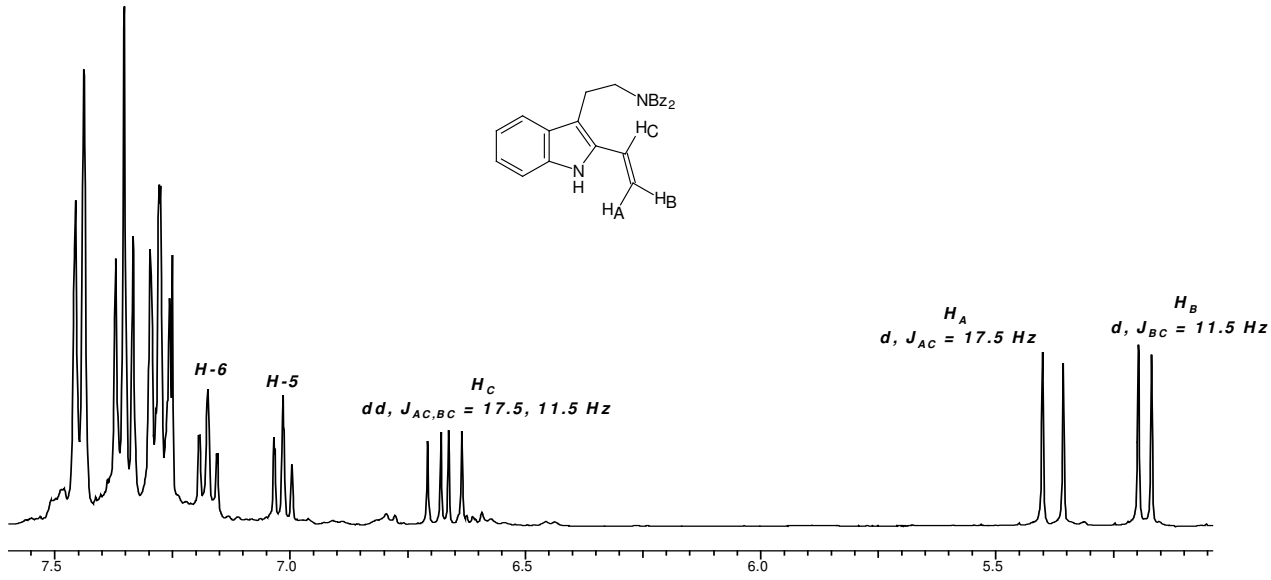
25
20

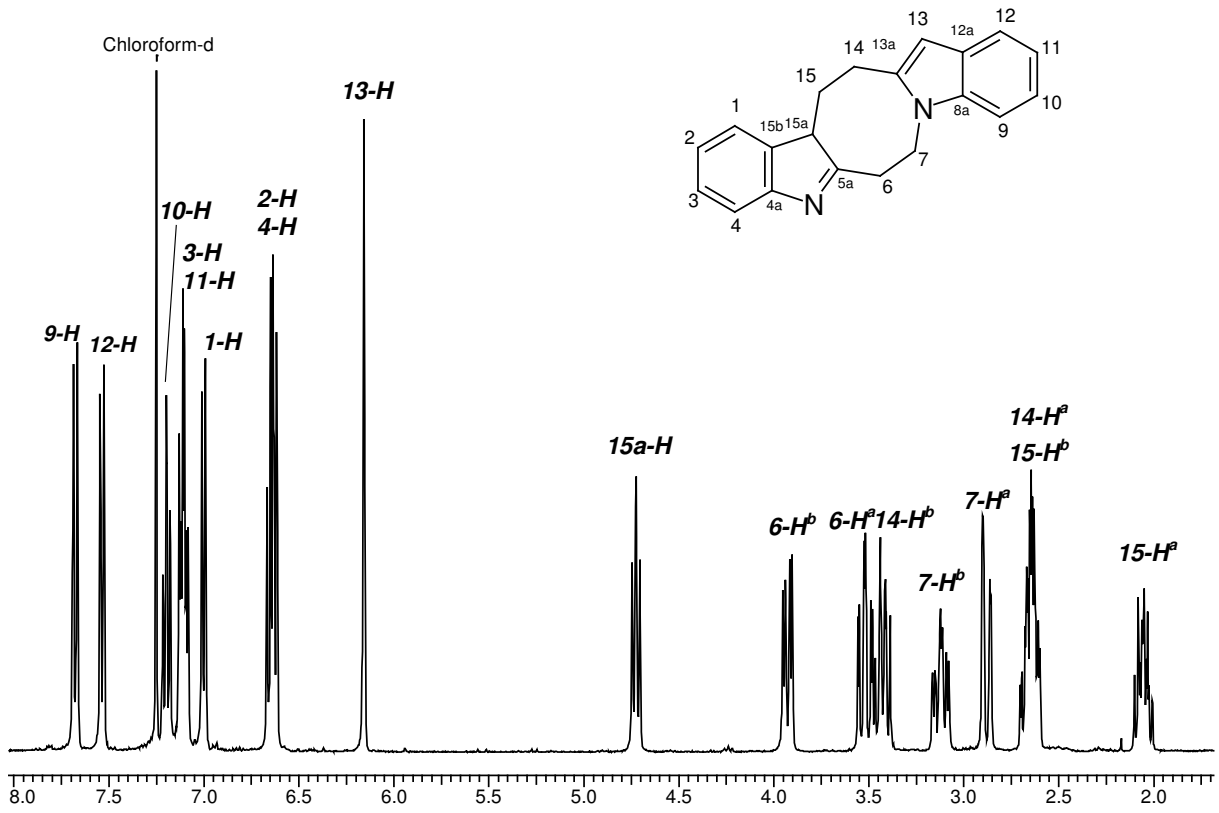
3. Expanded section 400 MHz HMBC contours plot of **6** ($CDCl_3$).



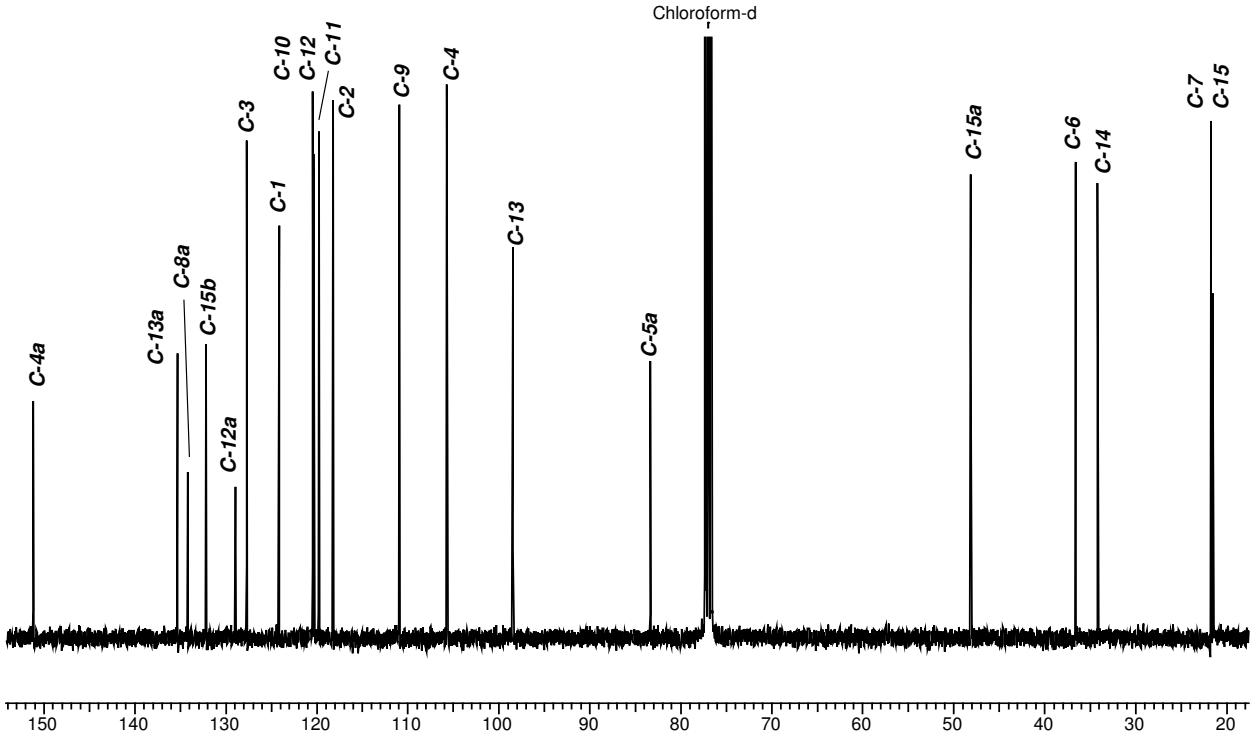
4. Aromatic/olefinic region of 400 MHz ^{1}H NMR spectrum of **7** ($CDCl_3$).



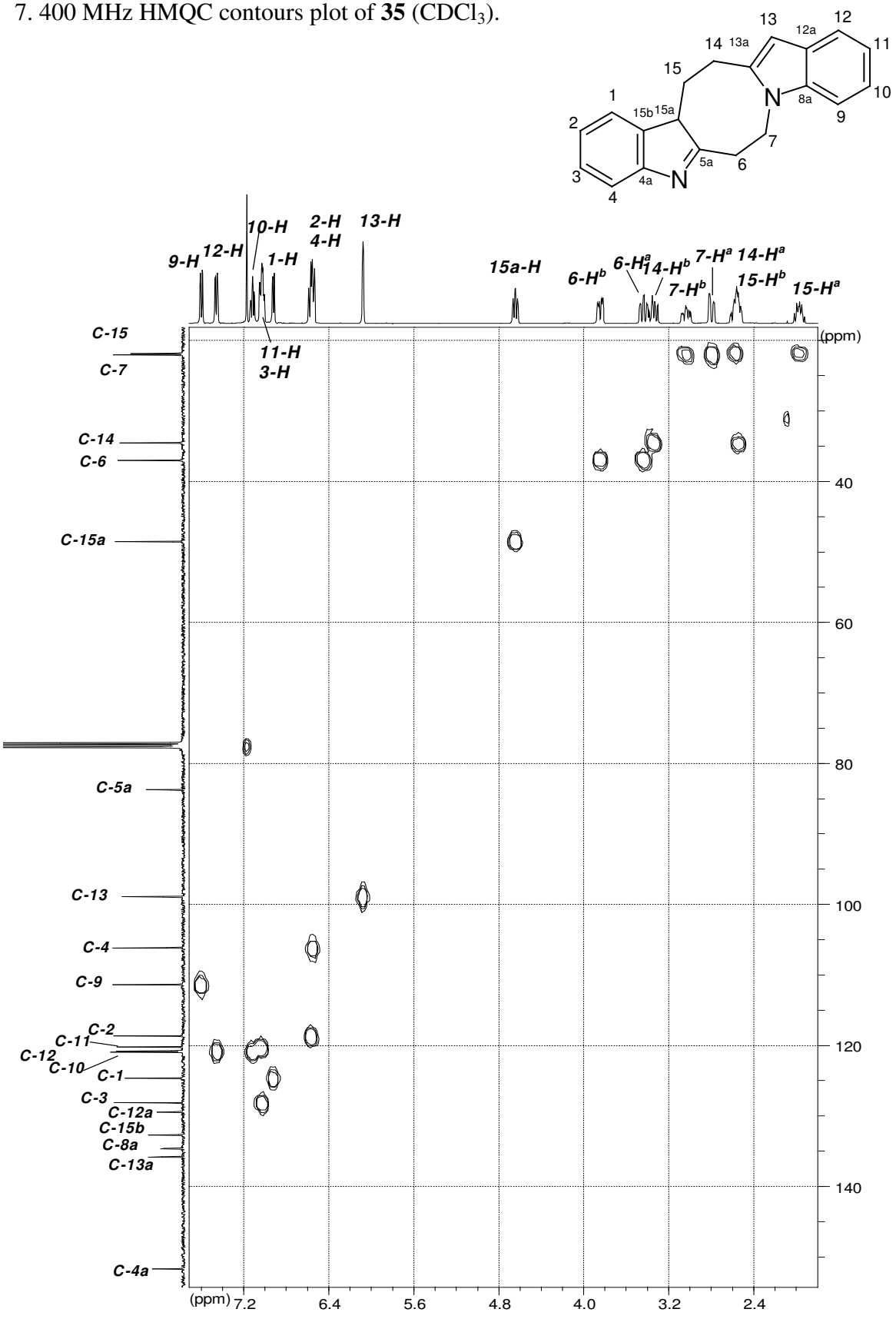
5. 400 MHz ^{1}H NMR spectrum of **35** ($CDCl_3$).



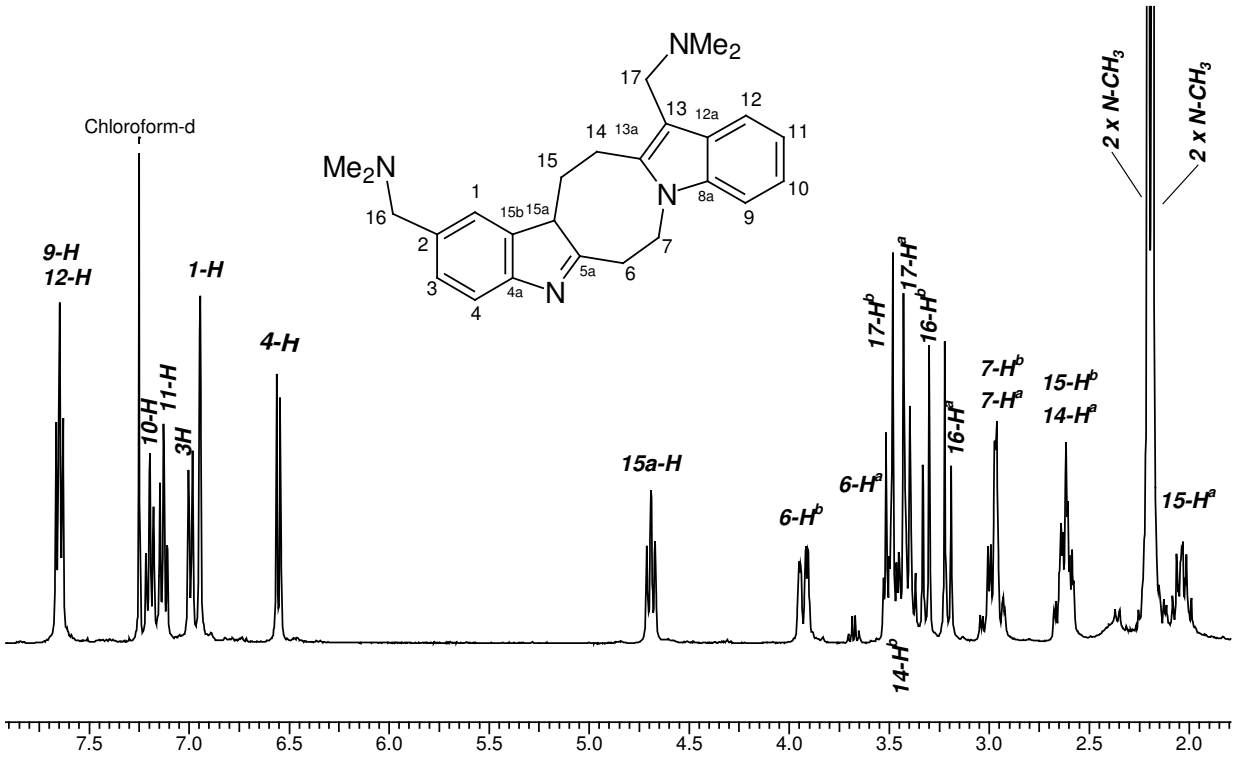
6. 400 MHz ^{13}C NMR spectrum of **35** ($CDCl_3$).



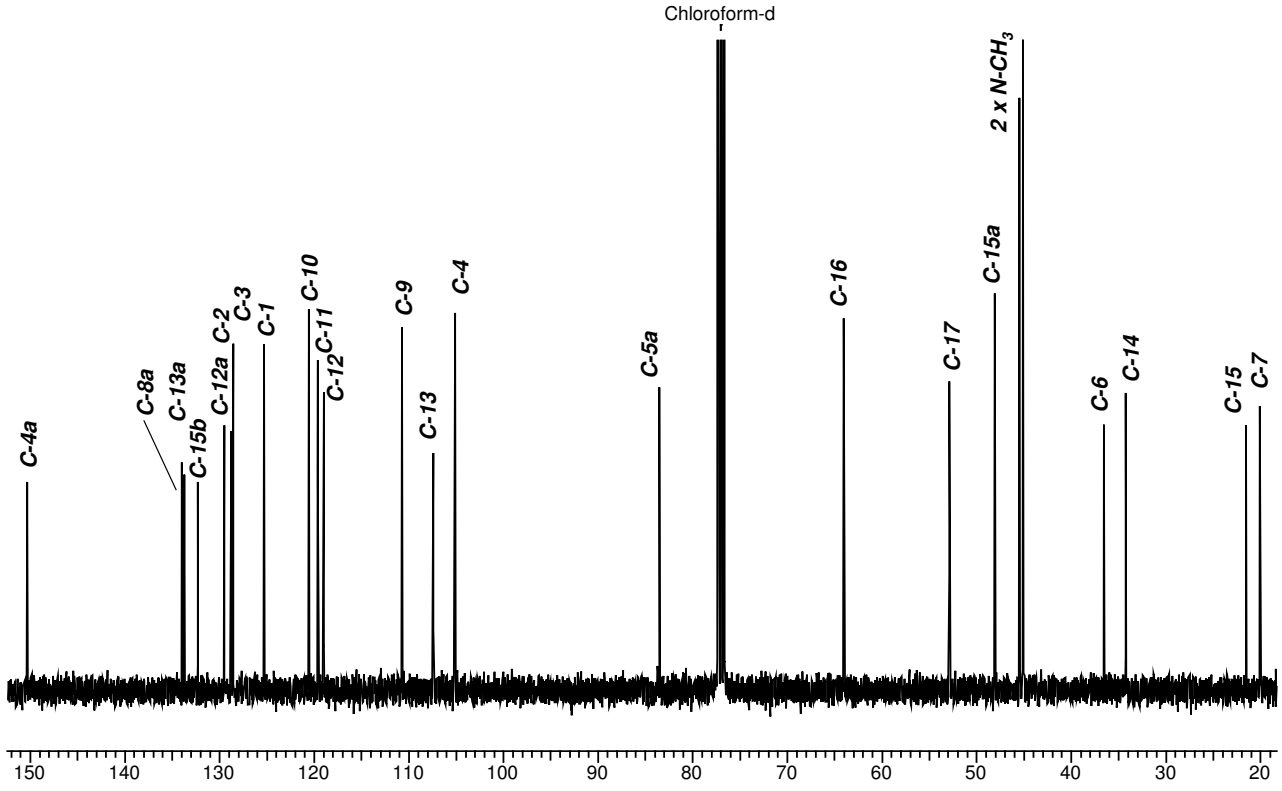
7. 400 MHz HMQC contours plot of **35** ($CDCl_3$).



8. 400 MHz ^{1}H NMR spectrum of **37** ($CDCl_3$).



9. 400 MHz ^{13}C NMR spectrum of **37** ($CDCl_3$).



Curriculum Vitae

Personal information

Surname:	Sripha
Name:	Kittisak
Date of Birth:	30.10.1970
Place of Birth:	Bangkok, Thailand
Nationality:	Thai
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Education

1976 - 1982	Primary school in Bangkok, Thailand
1982 - 1985	Secondary school "Suankularb Witthayalai Nonthaburi", Nonthaburi, Thailand
1985 - 1988	High school "Suankularb Witthayalai Nonthaburi", Nonthaburi, Thailand
1988 - 1993	B.Sc.(Pharmacy), Faculty of Pharmacy, Mahidol University, Bangkok, Thailand Approbation as Pharmacist: 25.03.1993
1993 - 1996	M.Sc.(Pharmacy), Pharmaceutical Chemistry, Faculty of Pharmacy,Mahidol University, Bangkok, Thailand Thesis title: Synthesis of Phthalimidoalkyl Derivatives as Potential HIV-1 Reverse Transcriptase Inhibitors

Professional occupation experience

1996 - 1999	Lecturer in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

Promotion

October 1999 -	Pharmazeutische Chemie Work group of Prof. Dr. Ulrike Holzgrabe, Institut für Pharmazie und Lebensmittelchemie der Bayerische Julius-Maximilians-Universität Würzburg

Scholarship

April 1999 - September 2003	Deutscher Akademischer Austauschdienst (DAAD)

Study of German language

April - September 1999	Intensive course, Goethe Institut, Bremen