Synthetic studies of marine indole alkaloids and related systems

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Abstract

This thesis is mainly focussed on the synthesis of marine indole alkaloids and related systems, many of which display interesting biological activities.

In the first part of this thesis we could establish and confirm the stucture of barettin and 8,9-dihydrobarettin, isolated from the marine sponge *Geodia barretti*. The synthesis of barettin and 8,9-dihydrobarettin was performed in this work, involving a highly functionalised arginine derivative. This arginine derivative was used in a peptide coupling with 6-bromotryptophan methyl ester, as in the case of 8,9-dihydrobarettin, or as a phosphonate derivative in a Horner-Wadsworth-Emmons reaction with 6-bromoindole-3-carboxaldehyde, as in the key step in the synthesis of barettin. Both barettin and 8,9-dihydrobarettin are highly efficient in inhibiting the settlement of cyprid larvae of the barnacle *Balanus improvisus*, which makes them especially interesting as potential antifouling agents. The synthesis of an additional indole alkaloid, dipodazine (structurally related to barettin) is also described in this section. Dipodazine has been isolated and characterised as a major metabolite from *P. dipodomyis* and was here synthesized via a stereoselective aldol condensation from *N*-protected indole-3-carboxaldehyde and 1,4-diacetyl-2,5-piperazinedione in the presence of caesium carbonate. Several analogues of dipodazine, together with a few barettin analogues, have been prepared and tested for activity in antifouling assays, results and relevance of which are discussed in this thesis.

The second part of this thesis deals with the synthesis of the marine bis(indole) alkaloids rhopaladins A–D, isolated from the Okinawan marine tunicate *Rhopalaea* sp. The synthesis of the rhopaladins involved an imidate based cyclisation with tryptophan esters as the key step. A short and efficient new synthesis of indole-3-carbonyl nitriles from indole-3-carboxaldehydes and trimethylsilyl cyanide, followed by oxidation with DDQ is also described.

The last part of this thesis describes the synthetic approaches towards an indole alkaloid isolated from a Caribbean sponge, *Halichondria melanodocia*. Furthermore, an efficient method for preparation of certain carbazole derivatives, using indole and itaconic anhydride is described. Using this method, the 1-oxygenated carbazole alkaloid clausine E has been prepared in two steps.

Keywords: marine indole alkaloids, antifouling, synthesis, diketopiperazines, HWE-reaction, bis(indole) alkaloids, imidates, carbazole alkaloids

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List of publications

This thesis is based upon the following articles, which will be referred to in the text by their Roman numerals:

I. "Synthesis of barettin"
Johnson, A.-L.; Bergman, J.; Sjögren, M.; Bohlin, L.
Tetrahedron **2004**, *60*, 961

II. "Synthesis of the diketopiperazine dipodazine"
Johnson, A.-L.; Janosik, T.; Bergman, J.
ARKIVOC **2002**, *8*, 57

III. "Synthesis of the marine alkaloids rhopaladins A, B, C and D"
Janosik, T.; Johnson, A.-L.; Bergman, J.
Tetrahedron **2002**, *58*, 2813

IV. "Synthetic approaches towards lactams isolated from the marine sponge *Halichondria melanodocia*"
Johnson, A.-L.; Bergman, J.
Manuscript

Contents

1. Background

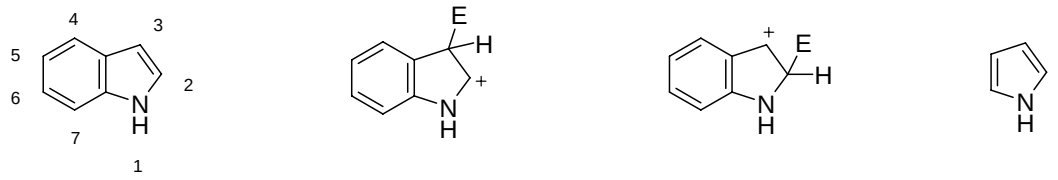
1.1 Indoles

Many commercially available indoles[1] are colourless crystalline solids with a pronounced smell; they are reasonably stable in air. Indole itself is produced by high-temperature vapour-phase cyclising dehydrogenation of 2-ethylaniline. Indole (**1**),[2] first described by Baeyer in 1869, has been thoroughly studied over the years and continues to attract attention from researchers all over the world. This focus on the indole ring system is partly due to its undisputable importance in Nature, where this particular heterocycle is embedded in countless natural products and medicinally relevant compounds.

1.2 Reactivity of indoles[3]

1.2.1 Electrophilic substitution

The chemistry of indole is dominated by electrophilic substitution. The heterocyclic ring of indole is very electron rich in comparison with its benzene counterpart; hence there is a strong preference for electrophilic substitution in the five-membered ring. Attack on the nitrogen would destroy the aromaticity of the pyrrole ring, hence the two other positions (C-2 and C-3) are the only remaining alternatives. When considering the stability of the two generalized cations (**2**) and (**3**), it is realised that the intermediate (**3**) cannot derive further resonance stabilisation without disrupting the aromatic ring, whereas (**2**) can derive contribution from the lone pair on nitrogen (Figure 1). The higher resonance stabilisation of this Wheland intermediate explains the preference for 3-substitution, which is in contrast to the substitution pattern preferred by its close relative pyrrole (**4**). Furthermore, electrophilic substitution at C-2 in 3-substituted indoles can occur in two ways, either *via* attack at the 3-position and subsequent 1,2-migration to the 2-position or *via* direct attack at the 2-position.



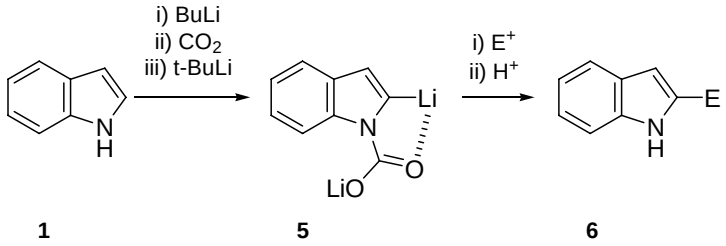
1 **2** **3** **4**

Figure 1

Although the 3-position is preferred in electrophilic substitutions, 3-unsubstituted indoles can also be substituted in 2-position, using suitable N-blocked indoles. Indolyl anions are readily formed by reacting indole with strong bases, which in turn will furnish the possibility of N-alkylation or acylation. Deprotonation can be effected at the 2-position of suitably N-blocked

[1] Sundberg, R. J. *The Chemistry of Indoles*, Academic Press, New York, 1970
[2] Baeyer, A.; Emmerling, A. *Chem. Ber.* **1869**, *2*, 679
[3] (a) For chemistry and reactivity of indoles, see: Joule, J. A.; Mills, K. *Heterocyclic Chemistry*, 4th ed. Blackwell Science, Oxford, 2000. (b) Joule, J. A. *Indole and its Derivatives*, In *Science of Synthesis*. Thomas, E. J. Ed.; Georg Thieme Verlag, Stuttgart, 2001, vol. 10, p. 361.

indoles, aided by the chelation with the N-substituent. One example is the well-known Katritzky protocol,[4] illustrated in scheme 1.



Scheme 1

1.2.2 Acylation

Acylation of indoles can be performed in many different ways, each with its merits and shortcomings. Although attack of electrophiles at the C-3 position of indole is perhaps the most characteristic reaction of this heterocycle, low yields can be encountered during acylations due to competitive formation of N-acylated, as well as 1,3-diacylated products. Moreover, self-oligomerisation of indole in an acidic environment can also be observed as a side reaction.[5] The use of N-protected indoles[6] is one effective strategy to overcome competing N-acylation and to limit di- and trimerization, although with the obvious drawback of extra protection-deprotection steps. The use of indole Grignard reagents,[7,8] Vilsmeier-Haack[9] or *N*-(α-haloacyl)pyridinium salts[10] are other useful synthetic methods for the preparation of 3-acylated indoles. Regioselective acylation at the 3-position under Friedel-Crafts conditions has been reported in the presence of various Lewis acids.[11,12] The high regioselectivity and absence of oligomerisation products is explained by the initial formation of a Lewis acid-indole complex (**7**)[11] (Figure 2), which would interfere in the oligomerization process.

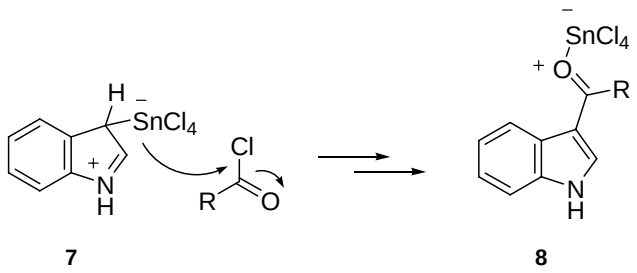


Figure 2

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[4] Katritzky, A. R.; Akutagawa, K. *Tetrahedron Lett*. **1985**, *26*, 5935
[5] Smith, G. F. *Adv. Heterocycl. Chem.* **1963**, *2*, 300
[6] Ketcha, D. M.; Gribble, G. W. *J. Org. Chem.* **1985**, *50*, 5451
[7] Heacock, R. A.; Kasparek, S. *Adv. Heterocycl. Chem.* **1969,** *10*, 43
[8] Bergman, J.; Venemalm, L. *Tetrahedron* **1990**, *46*, 6061
[9] Anthony, W. C. *J. Org. Chem.* **1960**, *25*, 2049
[10] Bergman, J.; Bäckwall, J.-E.; Lindström, J.-O. *Tetrahedron* **1973**, *29*, 971
[11] Ottoni, O.; de V. F. Neder, A; Dias, A. K. B.; Cruz, R. P. A.; Aquino, L. B. *Org. Lett.* **2001**, *3*, 1005
[12] Okauchi, T.; Itonaga, T.; Minami, T.; Owa, T.; Kitoh, K.; Yoshino, H. *Org. Lett.* **2000**, *2*, 1485

1.3 Tryptophan

Tryptophan (**9**) is an essential amino acid for vertebrates, i.e. it is not biosynthesised in the body, and as a consequence tryptophan has to be ingested *via* the food intake. Since tryptophan is an important building block of many proteins and also a biosynthetic precursor for several indole containing secondary metabolites, indole is one of the most commonly found heterocyclic system in Nature. Tryptophan is synthesised in bacteria from erythrose-4-phosphate and phosphoenolpyruvate.[13] The indole nitrogen is introduced *via* glutamine in the shikimate and chorismate pathway to form anthranilate (**10**) (Figure 3), which will subsequently condense with PRPP (phosphoribosyl pyrophosphate) to form indole-3-glycerol phosphate **11**. The final reaction in this sequence is catalysed by tryptophan synthase where the indole ring is formed by an aldol cleavage. The next step involves dehydration of serine with PLP, a pyridoxal phosphate cofactor. Indole then condenses with this PLP-aminoacrylate intermediate and the product is hydrolysed to release tryptophan (**9**).

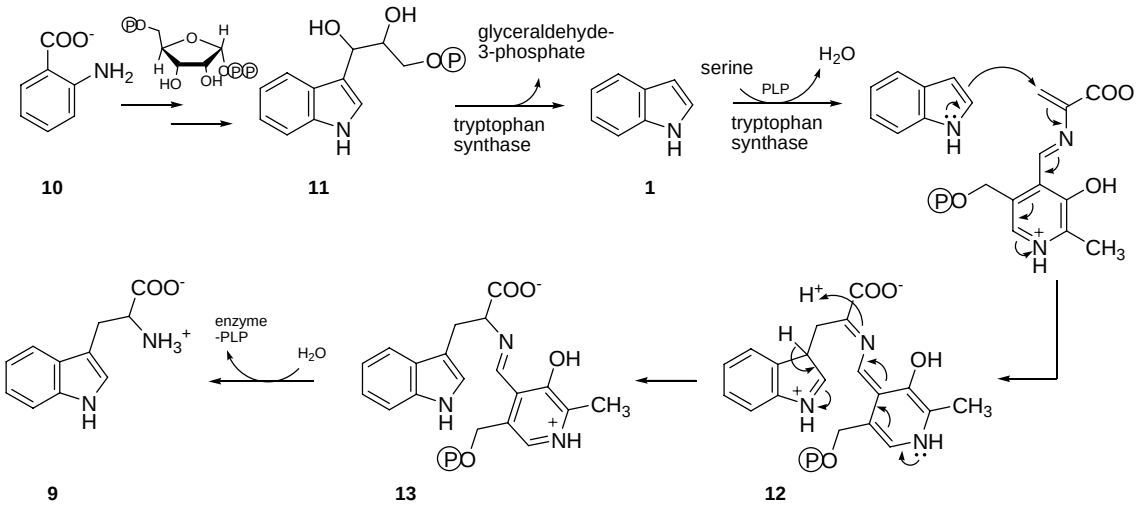
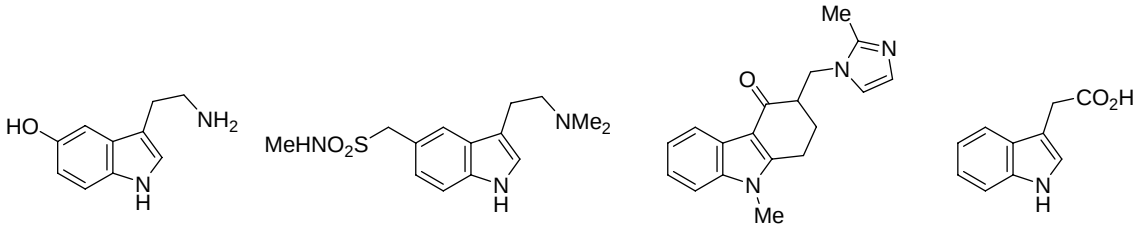


Figure 3

1.3.1 Tryptophan-derived metabolites

An important example of a tryptophan-derived metabolite is serotonin (**14**), an essential neurotransmitter in the central nervous system. Studies of serotonin and related ligands have resulted in several drugs, such as sumatriptan (**15**) for treatment of migraine, and odansetron (**16**) for suppression of nausea caused by chemotherapy (Figure 4). The tryptophan derivative indole-3-acetic acid (**17**) is the primary growth regulating hormone for the green herbs.

[13] Lehninger, A.; Nelson, D.; Cox, M. *Principles of Biochemistry* 2nd ed. Worth Publishers, New York, 1993



14 **15** **16** **17**

Figure 4

2. Alkaloids

2.1 "Alkali-like"

A natural product is a compound synthesised by a plant or an animal. Substances containing a basic nitrogen atom isolated from natural sources are called alkaloids. Since alkaloids are often amines, they react more or less as bases and give soluble salts when treated with acids. Hence the term alkaloid, derived from "alkali-like", was coined by the pharmacist W. Meissner in 1819.[14] This term was soon developed further and "true alkaloids" were long defined as compounds meeting four requirements:

The nitrogen atom is part of a heterocyclic system.
The compound has a complex molecular structure.
The compound manifests significant pharmacological activity.
The compound is restricted to the plant kingdom.

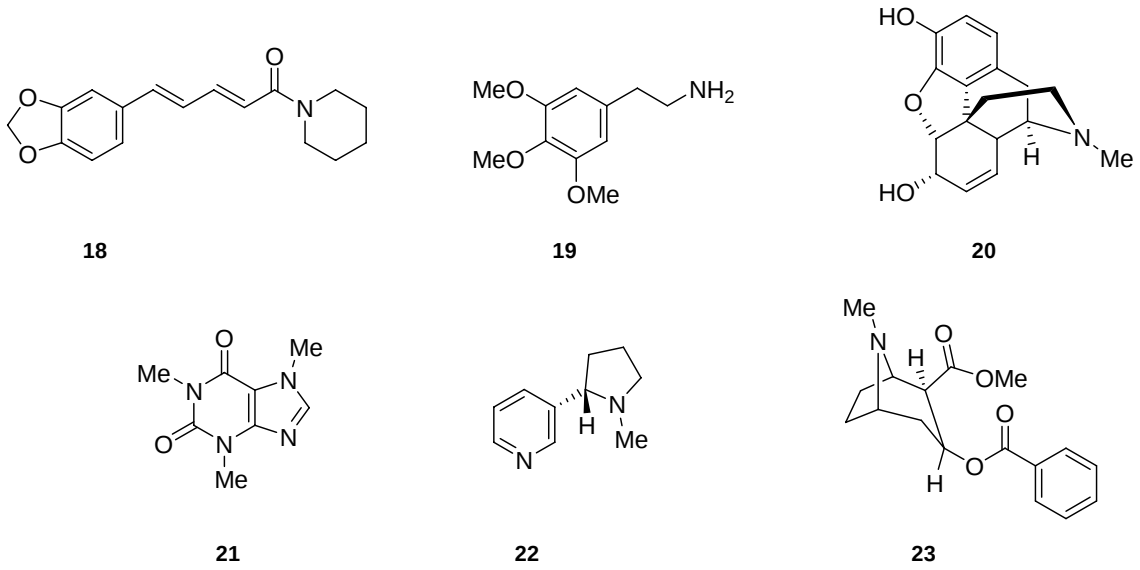
This definition is however not particularly valid today for several reasons.[15,16] Basicity, for example, is no longer regarded as a necessary property of an alkaloid, despite the fact that the word stems from *alkali-like*. Piperine (**18**) (isolated from black pepper *Piper nigrum*) is a good example of a compound that is considered to be an alkaloid despite being a neutral amide. Although many early classified alkaloids contained nitrogen in a heterocyclic system, there are today too many exceptions for this requirement to be mandatory, as exemplified by mescaline (**19**) (isolated from the cactus peyote, *Lophophora williamsii*)[17] (Figure 5). Also, vague concepts like *complexity* of molecular structure and *significant* pharmacological activity have no place in a modern definition. Furthermore, restriction to plant kingdom also seems inappropriate since too many compounds possessing classical alkaloid structures have been isolated from animal, fungal and bacterial sources.

[14] Hosztafi, S. *Pharmazie*, **1997**, *52*, 546
[15] Pelletier, S. W. *The nature and definition of an alkaloid*, In *Alkaloids: Chemical and biological perspectives*. Wiley, New York, 1983. vol. 1, p. 1
[16] Snieckus, V. *Heterocyclic Compounds in Alkaloid Synthesis*, In *Survey of Progress in Chemistry*. Scott, A. F. Ed.; Academic Press, New York, 1980, vol. 9, p.122
[17] Anderson, E. F. Peyote, the Divine Cactus, 2nd ed. The University of Arizona Press, Arizona, 1996

Thus, today the concept alkaloid has been expanded and it is generally considered to be a naturally occurring nitrogenous compound. However, a more specific definition has been proposed by Pelletier:[15]

An alkaloid is a cyclic organic compound containing nitrogen in a negative oxidation state which is of limited distribution among living organisms.

The first alkaloid to be isolated in pure form was morphine (**20**) (from opium poppy seeds) in 1805 by W. Sertürner.[14] Alkaloids typically have potent physiological properties and are often toxic when the dose is large enough. In spite of this, many alkaloids have found use in medicine. The sometimes striking physiological effects of alkaloids can be illustrated by the notoriously known alkaloids caffeine (**21**), nicotine (**22**) and cocaine (**23**), just to mention a few.



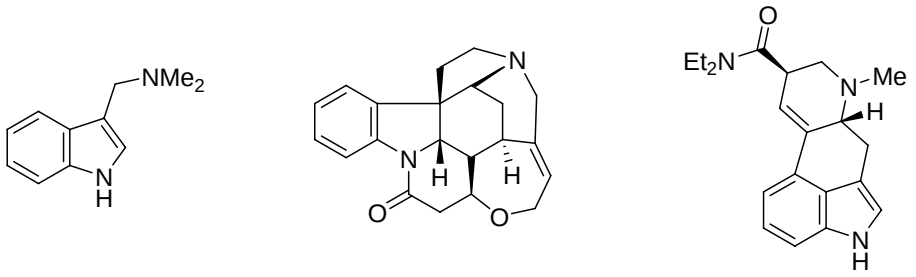
18 **19** **20**

21 **22** **23**

Figure 5

2.2 Indole alkaloids

Indole alkaloids can be relatively simple as exemplified by gramine (**24**), isolated from e.g. barley, or very complex such as strychnine (**25**), a highly poisonous compound isolated from the seeds of *Strychnos nux-vomica*. Lysergic acid diethylamide (LSD) (**26**) is another example of a synthetic derivative of an indole alkaloid well-known for its physiological activities (Figure 6).



24 **25** **26**

Figure 6

2.3 Marine indole alkaloids and bis(indole) alkaloids

Marine organisms provide a valuable source of natural products. The largest numbers of bioactive alkaloids with novel structures have been isolated from sponges and tunicates. Many of these compounds have generated interest as challenging problems for structure elucidation and synthesis, as well as for their biological activities.[18,19] For example, the indole alkaloids tryprostatins A (**27a**) and B (**27b**) isolated from a marine fungal strain completely inhibit cell cycle progression in the G2/M phase.[20] The cytotoxic molecule hyrtiosin B (**28**), isolated from the Okinawan marine sponge *Hyrtios erecta*,[21] is an exemple of a simple bis(indole) alkaloid. Several bis(indole) derivatives have a heterocyclic system between the indole units, e.g. an imidazole ring in the anti-inflammatory alkaloid topsentin (**29**) from the marine sponge *Topsentia genitrix*[22] and a piperazine ring in dragmacidin (**30**), a cytotoxic alkaloid isolated from the marine sponge *Dragmacidon* spp.[23] (Figure 7). A large number of related alkaloids from these species have been isolated and synthesised.[22,23]

[18] For reviews on marine natural products, see (a) Blunt, J. W., Copp, B. R.; Munro, M. H. G.; Northcote, P. N.; Prinsep, M. R. *Nat. Prod. Rep.* **2004**, *21*, 1 and previous issues in this series. For accounts on marine indole alkaloids, see also (b) Somei, M.; Yamada, F. *Nat. Prod. Rep*. **2005**, 22, 73 and previous issues in this series.
[19] Aygün, A.; Pindur, U. *Curr. Med. Chem.* **2003**, *10*, 1113
[20] (a) Isolation: Cui, C. B.; Kakeya, H.; Okada, G.; Onose, R.; Ubukata, M.; Takahashi, I.; Isono, K.; Osada, H. *J. Antibiot.* **1995**, *48*, 1382. Cui, C. B.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. *J. Antibiot.* **1996**, *49*, 527. Cui, C. B.; Kakeya, H.; Osada, H. *J. Antibiot.* **1996**, *49*, 534 (b) Synthesis: Caballero, E.; Avendano, C.; Menendez, J. C. *J. Org. Chem.* **2003**, *68*, 6944
[21] (a) Isolation: Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamutsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. *Tetrahedron*, **1990**, *46*, 7699 (b) Synthesis: Bergman, J.; Janosik, T.; Johnsson, A.-L. *Synthesis*, **1999**, 580
[22] (a) Isolation: Bartik, K.; Braekman, J.-C.; Daloze, D; Stoller, C.; Huysecom, J.; Vandevyver, G.; Ottinger, R. *Can. J. Chem.* **1987**, *65*, 2118 (b) Synthesis: Yang, C.-G.; Huang, H.; Jiang, B. *Curr. Org. Chem.* **2004**, *8*, 1691 and references cited therein.
[23] (a) Isolation: Kohmoto, S.; Kashman, Y.; McConnell, O. J.; Rinehart, K. L.; Wright, A.; Koehn, F. *J. Org. Chem.* **1988**, *53*, 3116 (b) Synthesis: Garg, N. K.; Caspi, D. D.; Stoltz, B. M. *J. Am. Chem. Soc.* **2005**, *127*, 5970 and references cited therein.

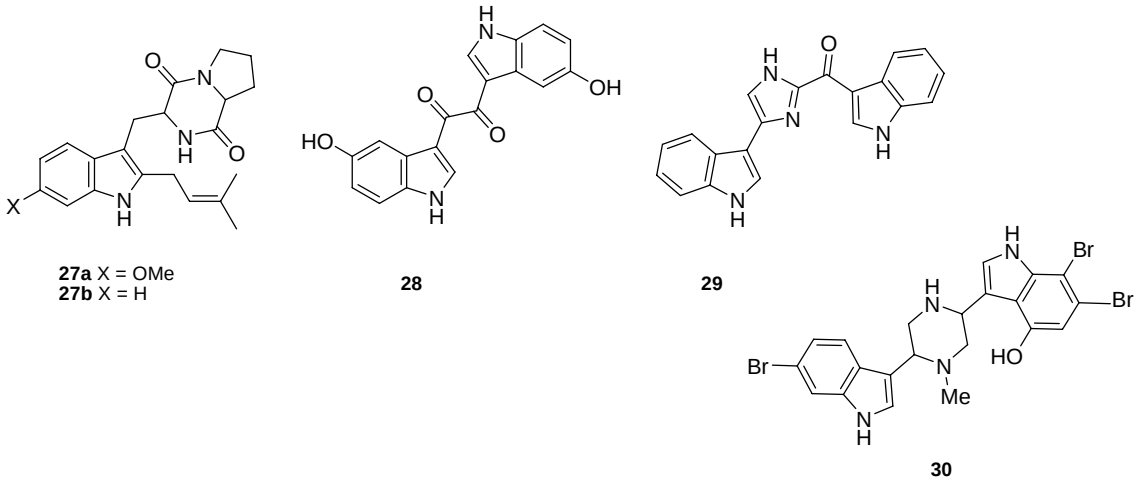


Figure 7

3. Barettin, dipodazine and antifouling (Papers I and II)

3.1 Biofouling

All man-made surfaces submerged in the sea will rapidly become covered by a biofilm typically consisting of algae, molluscs, ascidians and barnacles. This process, called biofouling, has substantial economic consequences and is an important problem of the marine technology today.[24] Fouling on the hulls of ships increases frictional drag, with a corresponding decrease in speed and manoeuvrability, and subsequent increase of fuel consumption.[25]

3.1.1 Organotin antifoulants

Historically, sailing vessels have been protected against fouling by primitive coatings containing arsenic or mercury. Since the early1960s, organotin compounds have been used extensively in marine paint formulations.[25] The heavy metal-based coatings largely in use today, e.g. TBT (tributyltin), TBTO (tributyltin oxide) and copper, undeniably effective against biofouling, have adverse environmental effects. These compounds will slowly leach from the paint formulas into the surrounding sea environment, killing barnacles and other marine organisms that are attached to the ship. However, these compounds persist in the environment with the possible risk of bioaccumulation. Specifically, organotin compounds have been shown to cause shell deformations in oysters; sex changes (imposex) in whelks and immune response, neurotoxic and genetic effects in other marine species.[26] Due to these unwanted side effects on non-target organisms, heavy metal-based coatings such as TBT are facing global bans.[27] The International Maritime Organisation resolution called for a global prohibition on the application of organotin compounds, which act as biocides in anti-fouling

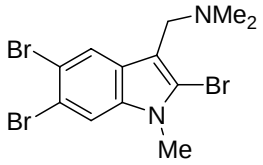
[24] Armstrong, E.; Boyd, K. G.; Burgess, J. G. *Biotechnol. Ann. Rev.* **2000**, *6*, 221
[25] Hellio, C.; Tsoukatou, M.; Maréchal, J.-P.; Aldred, N.; Beaupoil, C.; Clare, A. S.; Vagias, C.; Roussis, V. *Mar. Biotechnol.* **2005**, *7*, 297
[26] Evans, S. M.; Leksono, T.; McKinnell, P. D. *Mar. Poll. Bull.* **1995**, *1*, 14
[27] IMO 1999, International Maritime Organization; Resolution A. 895(21) "Anti-Fouling Systems Used on Ships"

systems on ships by 2003, and a complete prohibition by 2008.[27] As a part of the imminent prohibition of organotin containing antifouling coatings, it is of great importance to develop new, environmentally compatible alternatives, equally efficient against fouling organisms. The search for new antifoulants is intense in many different fields, the defence can involve physical, mechanical as well as chemical processes.[28]

3.1.2 Marine natural products as antifoulants

In the marine environment, where all surfaces are targets for colonization of fouling organisms, many sessile organisms still remain clean. For filter-feeding marine organisms a fouling free body surface is necessary for their food uptake, hence they are believed to produce secondary metabolites as part of a chemical defence against predation or growth of fouling organisms.[25,29,30] This putative chemical defence system of sessile marine organisms attracts attention from researchers in their efforts to find environmentally friendly antifouling substances. Secondary metabolites involved in such defence, as natural products, would be target oriented and biodegradable.[25,29]

A number of non-toxic secondary metabolite with antifouling properties isolated from marine organisms are known today; including the heterocycles 2,5,6-tribromo-1-methylgramine (**31**) isolated from the Californian bryozoan *Zoobotryon pellucidum*[31] and eudistomines G (**32a**) and H (**32b**) from the Caribbean tunicate *Eudistoma olivaceum*[32] (Figure 8).



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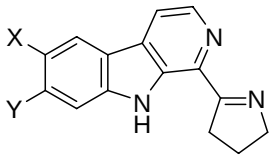

32a X = H, Y = Br
32b X = Br, Y = H

Figure 8

3.2 The marine sponge *Geodia barretti*

The marine sponge *Geodia barretti* Bowerbank (family Geodiidae, class Demospongiae, order Astrophorida) lives on the Atlantic continental shelf at depths between 10 and 500 m. It has a completely fouling-free body surface, which strongly suggests that some compound(s) produced by the sponge may be efficient against fouling growth. The sponge is already known to produce a variety of secondary metabolites such as histamine, inosine and taurine.[33] Also, a pharmacologically active indole alkaloid, named barettin, was isolated from *Geodia barretti* in the mid 1980s.[34]

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[28] Dahlström, M.; Mårtensson, L. G. E.; Jonsson, P. R.; Arnebrant, T.; Elwing, H. *Biofouling* **2000**, *16*, 191
[29] Fusetani, N. *Nat. Prod. Rep.* **2004**, *21*, 94
[30] Chanas, B.; Pawlik, J. R.; Lindel, T.; Fenical,W. *J. Exp. Mar. Biol. Ecol.* **1996**, *208*, 185
[31] Kon-ya, K.; Shimidzu, N.; Adachi, K.; Miki, W. *Fisheries Sci.* **1994**, *60*, 773
[32] Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Rinehart, K. L. *J. Am. Chem. Soc.* **1984**, *106*, 1526
[33] Hougaard, L.; Christophersen, C.; Nielsen, P. H.; Klintgaard, A.; Tendal, O. *Biochem. Sys. Ecol.* **1991**, *19*, 223
[34] Lidgren, G.; Bohlin, L.; Bergman, J. *Tetrahedron Lett.* **1986**, *27*, 3283

3.2.1 What is the structure if barettin?

The structure of the indole alkaloid isolated from *Geodia barretti* was long a subject of uncertainty. The originally reported structure **33**[34] was soon disproved by an independent total synthesis of Lieberknecht *et al*.[35] The German group suggested an alternative structure for barettin, namely **34** and with that the issue was dormant for over a decade. Recently, Sölter *et al*.[36] reported the isolation and structure characterisation of a compound from *G. barretti*, which showed NMR data similar to those originally reported for barettin. Consequently, structure **35** was suggested as the actual structure of barettin. In our work, which was made independently and in parallel with the report by Sölter *et al*.,[36] the bioassay-guided isolation of an ethanol extract of *G. barretti* led to the isolation of two indole alkaloids. Their structures were finally corroborated by total synthesis to be **35** and **36**. Compound **35** is thus identical with the structure suggested by Sölter *et al*.[36] and as shown by comparison with original data for barettin and the original indole alkaloid (isolated in the 1980s),[34] it also represents the actual structure of barettin. In addition to barettin, *G. barretti* produces another structurally related indole alkaloid, namely 8,9-dihydrobarettin (**36**) (Figure 9).[37]

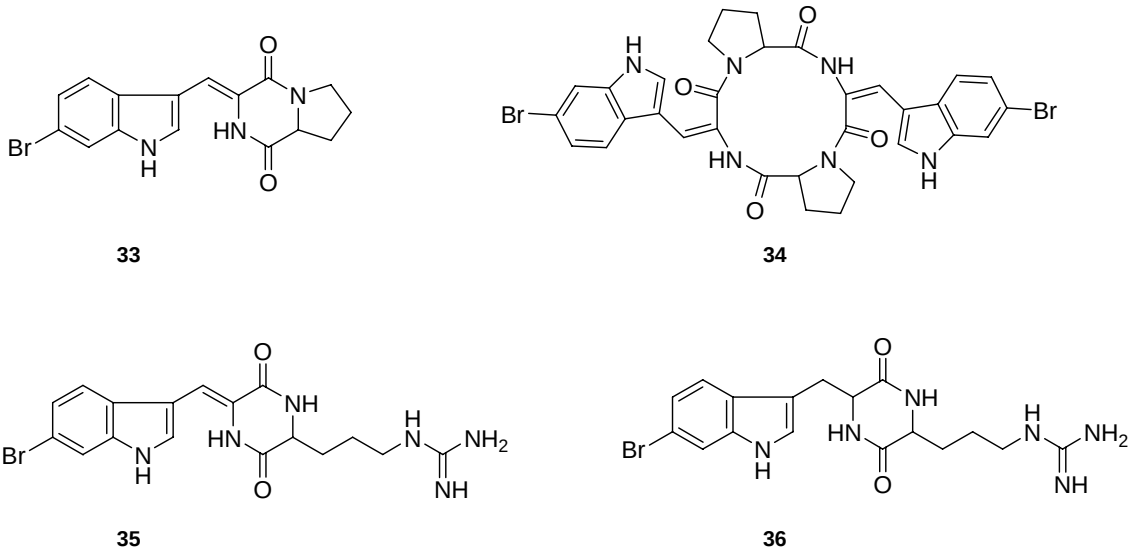

Figure 9

3.2.2 Antifouling activity of barettin and 8,9-dihydrobarettin

Guided by the effect of the aqueous ethanol extracts from *G. barretti* on the attachment and metamorphosis of the barnacle *Balanus improvisus*, the indole alkaloids barettin (**35**) and 8,9-dihydrobarettin (**36**) were isolated. Barettin and 8,9-dihydrobarettin displayed complete inhibition of the settlement of the cyprid larvae at 1.9 μM and 19 μM respectively. Furthermore, it was also found that the settlement inhibition evoked by barettin and 8,9-

[35] Lieberknecht, A.; Griesser, H. *Tetrahedron Lett*. **1987**, *28*, 4275
[36] Sölter, S.; Dieckmann, R.; Blumenberg, M; Francke, W. *Tetrahedron Lett.* **2002**, *43*, 3385
[37] Sjögren, M.; Göransson, U.; Johnson, A.-L.; Dahlström, M.; Andersson, R.; Bergman, J.; Jonsson, P. R.; Bohlin, L. *J. Nat. Prod.* **2004**, *67*, 368

dihydrobarettin was reversible. For both compounds the effect was reversed when the larvae were washed and transferred to fresh seawater, indicating that the compounds do not exert their action through a toxic, irreversible mechanism. The isolated compounds are hypothesized to be a part of the sponge's chemical defence to deter fouling organisms, which is supported by the fact that barettin (**35**) is found in water exposed to living specimens of *Geodia barretti* in concentrations that completely inhibit barnacles from settling. The ability of the sponge to release the active compound(s) into the surrounding water is most likely a function to deter attempts of attachment in an early phase. As many settling larvae have a sticky body surface or secrete a permanent glue preceding an often rapid metamorphosis, this would be highly advantageous in the sponge's defence.[37] It is interesting to note that the activities of barettin and 8,9-dihydrobarettin are in the same range as the antifouling agents used today. The widely used TBTO (tributyltin oxide) and Sea-Nine have LC_{50} values of 0.09 and 0.3 μg mL^{-1} respectively, whereas the corresponding EC_{50} value for **35** is 0.4 μg mL^{-1}.[37] The potential use of barettin (**35**) and 8,9-dihydrobarettin (**36**) as active components in non-toxic marine antifouling paint has been explored further in a recent study.[38] Under field conditions, the effect of the two compounds in combination with different paints were evaluated in terms of their ability to reduce the recruitment of the two most dominating fouling organisms in Swedish waters, the barnacle *B. improvisus* and the blue mussel *Mytilus edulis*. Mixed in non-toxic paint (concentrations of **35** and **36** ranging from 0.1- 0.01%), the recruitment of fouling organisms was significantly lower compared to the control panels after 8 weeks the field.[38]

3.3 Synthesis of barettin

3.3.1 Arginine in peptide synthesis

Barettin and 8,9-dihydrobarettin can be considered as cyclic dipeptides of the amino acids tryptophan and arginine. These amino acids contain two very characteristic units; indole in tryptophan and guanidine in arginine. These functional groups are responsible for the unique biochemistry and chemistry of these amino acid as well as the specific problems created by them during peptide synthesis.[39] The highly basic guanidino group of arginine (pKa ~13.5) is fully protonated under physiological conditions. Thus, the positive charge imposed on the molecule forms the basis for specific interactions between ligand and receptor or enzyme and substrate, mediated by hydrogen bonds. Not surprisingly, a vide array of structurally diverse guanidine-containing bioactive molecules have been isolated from natural sources.[40]

Different protective group strategies for indoles are readily available,[41] for arginine however, the alternatives are fewer. The basic guanidino group of arginine has to be thoroughly protected in peptide chemistry, as is displays strong nucleophilic character. The guanidine group of arginine is usually protected with a nitro group[42] or by protonation.[43]

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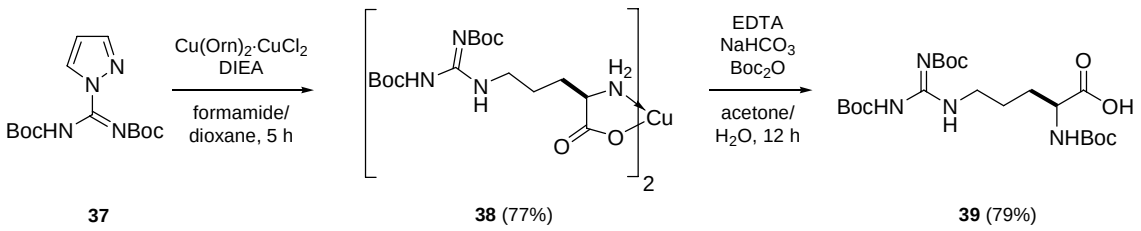
[38] Sjögren M.; Dahlström, M.; Göransson, U.; Jonsson, P. R.; Bohlin, L. *Biofouling* **2004**, *20*, 291
[39] Rzeszotarska, B.; Masiukiewicz, E. *Org. Prep. Proc. Int.* **1988**, *20*, 427
[40] Berlinck, G. S.; Kossuga, M. H. *Nat. Prod. Rep*. **2005**, *22*, 516
[41] Greene, T. W.; Wuts, P. G. M. *Protective groups in organic synthesis* 3rd ed, John Wiley Sons, Inc., New York, 1999
[42] Bergmann, M.; Zervas, L.; Rinke, H. *Z. Physiol. Chem.* **1934**, 40

However, problems with deprotection and poor solubility is frequently associated with those methods.[39] Preparation of arginine peptides by guanylation of the δ-amino groups of the appropriate ornithine-containing precursors is a strategy that has evolved as particularly attractive, as it eliminates the many problems associated with conventional arginine protecting methods.[44] In our synthetic approach, we utilised the ability of the copper (II) ion to mask the α-amino groups of amino acids.[45] Modifying the method described by Bernatowicz *et al.*[44], N^{α}-(*tert*-butoxycarbonyl)-N^{ω},$N^{\omega'}$-bis(*tert*-butoxycarbonyl)-L-arginine (**39**) was synthesised. Copper (II) protected L-ornithine, free to react at the N^{δ}, is treated with a pre-protected guanidinogroup in the form of the guanylderivative **37** (prepared from pyrazole and cyanamide),[44] which was subsequently released from copper and protected at the α-nitrogen to give the arginine derivative **39** (Scheme 2).



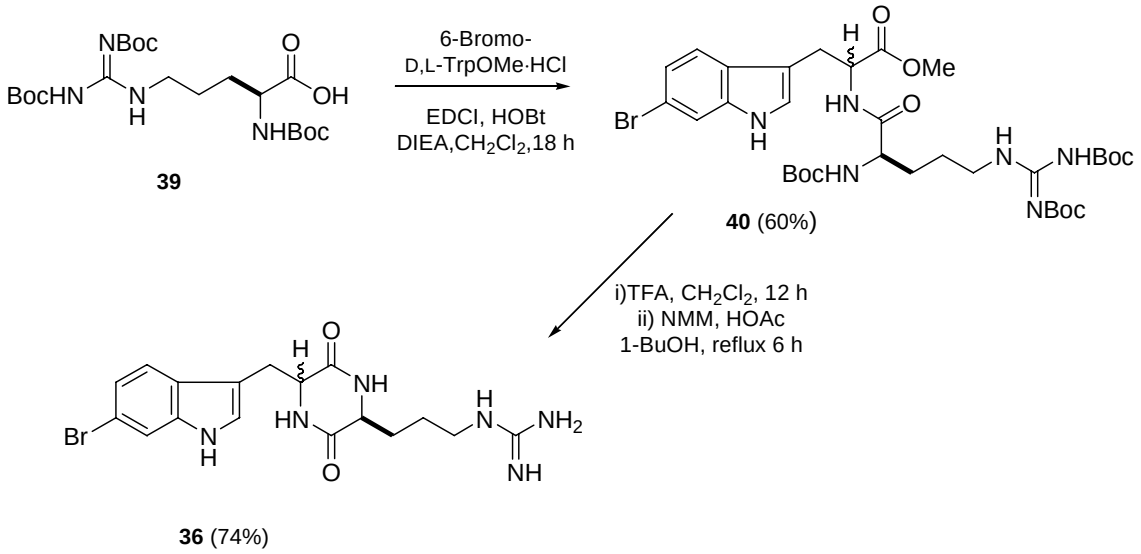
37 **38** (77%) **39** (79%)

Scheme 2

3.3.2 Synthesis of 8,9-dihydrobarettin

Using the protected derivative **39**, the unsaturated analogue of barettin, 8,9-dihydrobarettin, could be prepared *via* standard peptide coupling procedures with 6-bromotryptophan methyl ester. Removal of the protecting groups with trifluoroacetic acid (TFA) and subsequent cyclisation to the diketopiperazine in refluxing, slightly acidic butanol, afforded 8,9-dihydrobarettin (**36**), which we have however not been able to dehydrogenate into barettin (**35**) using reagents such as 2,3-dichloro-5,6-dicyano-2,4-benzoquinone (DDQ) or trichloroisocyanuric acid (TCCA) (Scheme 3).

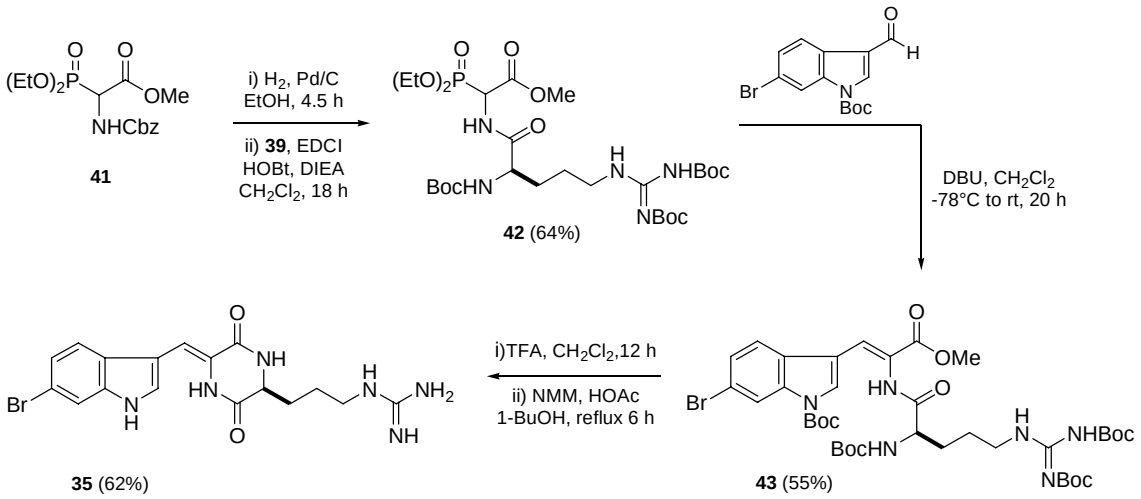
[43] Gish, D. T.; Carpenter, F. H. *J. Am. Chem. Soc.* **1953**, *75*, 5872
[44] (a) Bernatowicz, M.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *52*, 2497 (b) Wu, Y.; Matsueda, G. R.; Bernatowicz, M. *Synth. Commun.* **1993**, *23*, 3055 (c) See also: Bastiaans, H. M. M.; van der Baan, J. L.; Ottenheijm, H. C. J. *J. Org. Chem.* **1997**, *62*, 3880
[45] (a) Kurtz, A. C. *J. Biol. Chem.* **1938**, *122*, 47 (b) Kurtz, A. C. *J. Biol. Chem.* **1949**, *180*, 1253



Scheme 3

Consequently another strategy had to be employed in order to introduce the required double bond in barettin (**35**). Since there are literature reports describing the successful use of Horner-Wadsworth-Emmons (HWE) type reactions when preparing functionalised dehydroamino acids,[46] we opted for preparation of phosphonoglycinate **42** (prepared by hydrogenolysis of the known methyl 2-benzyloxycarbonylamino-2-(diethoxyphosphinyl)-acetate (**41**),[46] and subsequent coupling with the arginine derivative **39**). The HWE reaction of **42** with 6-bromo-1-(*tert*-butoxycarbonyl)-indole-3-carboxaldehyde afforded compound **43** as the desired *Z*-isomer. The yield in this particular reaction has so far been only moderate, around 55%. Although the low yield might indicate the co-formation of the *E*-isomer, we were however only able to detect the *Z*-isomer. The four Boc-protecting groups of **43** were removed by treatment with TFA. Cyclisation was thereafter performed in refluxing 1-butanol containing 0.1 M acetic acid affording barettin (**35**) in 62% yield (two steps) (Scheme 4).

[46] Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1984**, 53



Scheme 4

The analytical data agreed completely with the data reported by Sölter and with those recorded for the natural product isolated by us. Thus we can conclude that the structure of barettin is the diketopiperazine of 6-brominated dehydrotryptophan and arginine. Synthetic barettin and 8,9-dihydrobarettin displayed equal activity against the settlement inhibition of the barnacle larvae of *B. improvisus* as the compounds isolated from the natural source.[37]

3.4 Synthesis of dipodazine

The diketopiperazine dipodazine (**44**) has been isolated and characterised as a major metabolite from the mold *Penicillium dipodomyis*, and is also present in the meat-associated *Penicillium nalgiovese*.[47] Dipodazine (**44**) is composed of dehydrotryptophan and glycine and attracted our attention due to its close structural relationship with barettins and analogues. Structures related to **44** are known in the literature, e.g. neoechinulin (**45**)[48] and echinulines (**46**)[49] (Figure 10).

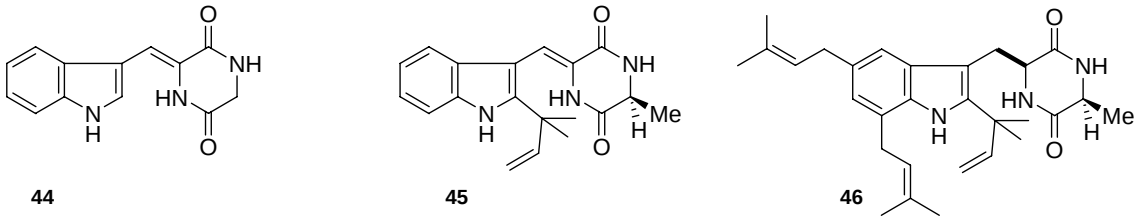


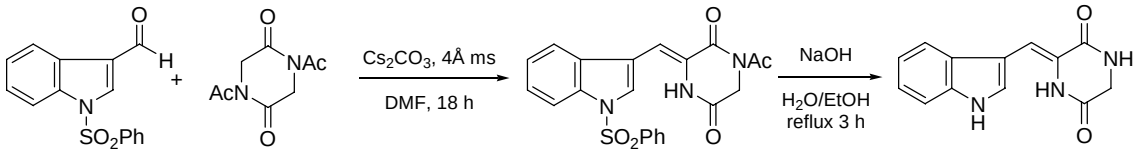
Figure 10

[47] Sørensen, D.; Larsen Ostenfeld, T.; Christophersen, C.; Nielsen Halfdan, P.; Anthoni, U. *Phytochemistry*, **1999**, *51*, 1181–1183

[48] (a) Isolation: Barbetta, M.; Casnati, G.; Pochini, A.; Selva, A. *Tetrahedron Lett*. **1969**, 4457 (b) Synthesis: Nakatsuka, S.; Miyazaki, H.; Goto, T. *Tetrahedron Lett.* **1980**, *21*, 2817

[49] Takamatsu, N.; Inoue, S.; Kishi, Y. *Tetrahedron Lett.* **1971**, 4665

The preparative routes to 3-ylidene-2,5-diketopiperazines, i.e. a 2,5-diketopiperazine with a double bond in 3-position, can be summarised by the description of the two commonly used basic synthetic methods; construction from an open-chain dehydroamino acid precursor, or introduction of the unsaturated side chain to a preformed diketopiperazine unit.[50]Our previous experience with dehydrotryptophan, i.e. difficulties during peptide coupling with amino acid derivatives (see **Paper I**), directed us towards the latter method. Although Wittig type reactions are excellent for introduction of ylidene functions in diketopiperazines,[35] we were aiming at a more straightforward synthesis with less exclusive starting materials. Hence, an aldol condensation with glycineanhydride seemed more appropriate.[51] The readily available 1,4-diacetyl-2,5-piperazinedione (**48**)[52] was condensed with the N-protected indole-3-carboxaldehyde **47** using caesium carbonate as the base[53,54] to afford the protected form of dipodazine **49** in good yield. The *Z*-isomer was formed exclusively in the condensation reaction, which was supported by a simple ROESY-experiment which displayed a cross-peak indicating a NOE between the 2-hydrogen of the indole and the 4-hydrogen of the diketopiperazine. Deprotection of **49** with base gave dipodazine (**44**), in all aspects identical with the data reported for the natural product (Scheme 5).



47 **48** **49** (77%) **44** (53%)

Scheme 5

3.5 Analogues of barettin and dipodazine

Barettin is very active in antifouling assays and the availability of synthetic material is a prerequisite for possible commercialisation. However, the synthetic route present today towards barettin has the drawback of being labour intensive and too expensive for large scale production (approximately 60 000 €/kg). The structural resemblance of dipodazine (**44**) to the above mentioned barettins led us to examine the antifouling activities of some dipodazine analoges. Since dipodazine can be regarded as a barettin derivative without the arginine side chain, the costs for preparation can be reduced significantly. The synthesis of dipodazine is fast and uncomplicated, hence structural analogues are readily available.

The main objectives of this analogue project have been both to design compounds with increased antifouling effect along with preserved non-toxic effect and to gain increased knowledge about the chemical basis of bioactivity. First, to assess possible structure-activity relationship, a small set of simple amino acids was tested in the antifouling

[50] Liebscher, J.; Jin, S. *Chem. Soc. Rev.* **1999**, *28*, 251 and references sited therein
[51] Gallina, C.; Liberatori, A. *Tetrahedron* **1974**, *30*, 667
[52] Dawson, I. M.; Pappin, A. J.; Peck, C. J.; Sammes, P. G. *J. Chem. Soc. Perkin Trans. 1* **1989**, 453
[53] Hayashi, Y.; Orikasa, S.; Tanaka, K.; Kanoh, K.; Kiso, K. *J. Org. Chem.* **2000**, *65*, 8402-8405
[54] Later studies (i.e. with dipodazine analogues) have shown that potassium-*tert*-butoxide works equally well as a base in the aldol condensation, both in respect to yields and stereoselectivity.

assay. None of the amino acids L-tryptophan, L-arginine or 6-bromo-D,L-tryptophan displayed any effect in relevant concentrations.[37] Next, analogues of barettin (**35**), 5-bromobarettin (**50**) and debromobarettin (**51**) were tested together with several dipodazine analogues; dipodazine (**44**), 5-bromodipodazine (**52**), 5-methoxydipodazine (**53**), 5-nitrodipodazine (**54**), 6-chlorodipodazine (**55**), 5-methyldipodazine (**56**), 6-bromo-1*H*-indole-3-carboxaldehyde (**57**), 3-[1-(6-bromo-1*H*-indol-3-yl)-meth-(*E*/*Z*)-ylidene]-hexahydro-pyrrolo[1,2-*a*]pyrazine-1,4-dione (**58a**/**58b**), benzo[*e*]dipodazine (**59**) and benzo[*g*]dipodazine (**60**) (Figure 11).[55]

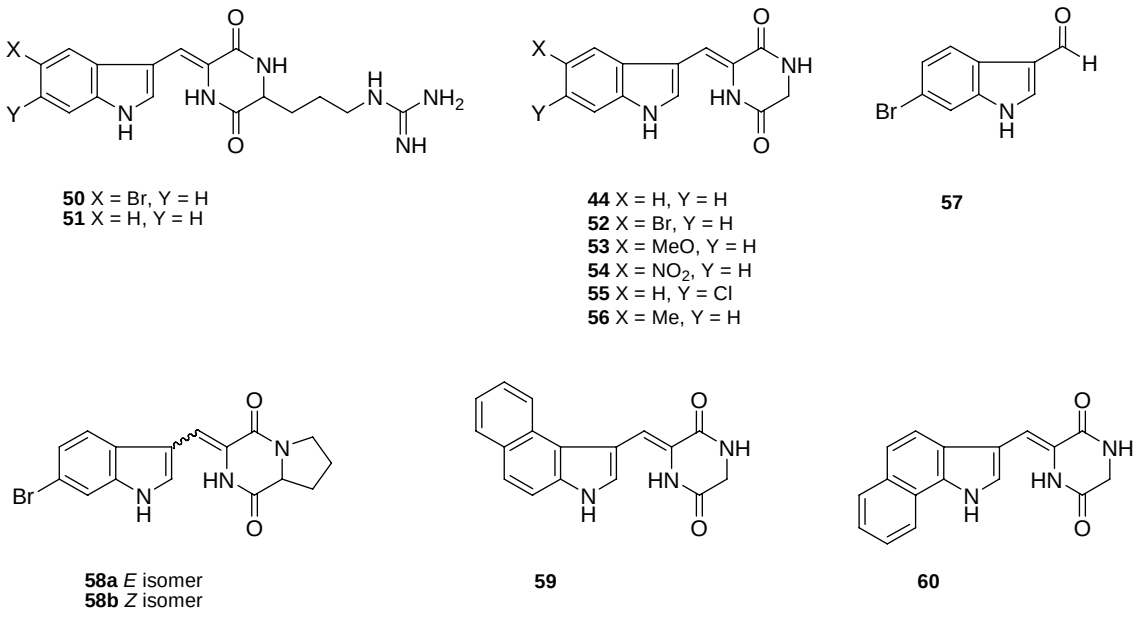


Figure 11

Only five of these compounds displayed inhibiting activity in relevant concentrations (Table 1). The test results suggest that the bromine atom plays a significant role in the antifouling activity, since removal of the bromine atom in debromobarettin (**51**) resulted in complete loss of activity. Shifting the position of the bromine atom as in 5-bromobarettin (**50**) also led to surprising results, as a stimulatory activity was obtained. This is rather surprising, but it has been speculated that the effect of the barettins is exerted through specific ligand-binding mechanisms to serotonin receptors in *B. improvisus*. If that is the case, the effect of 5-bromobarettin may be explained since serotonin is also substituted in the 5-position. In the dipodazine series, the significance of the bromine atom was further confirmed, since 5-bromodipodazine (**52**) showed inhibiting effects, whereas other 5-substituted dipodazines (OMe, Me, NO_2) failed to inhibit the settlement of the cyprid larvae of *B. improvisus*. The other analogues with a bromine atom (in 6-position) **57** and **58** also resulted in reduced settlement in the antifouling assay. The ability of 5-bromodipodazine (**52**) to significantly reduce settlement is however in contrast to the stimulatory effect of 5-bromobarettin (**50**), and further research is needed to gain increased knowledge of structure-activity relationship.

[55] Sjögren, M.; Johnson, A.-L.; Hedner, E.; Dahlström, M.; Göransson, U.; Shirani, H.; Bergman, J.; Jonsson, P. R.; Bohlin, L. *Manuscript in preparation*

Interestingly, the lack of activity of 6-chlorodipodazine (**55**) (and 5-methyldipodazine (**56**) for comparison) in the settlement assay indicated that the size of the substituent is of relevance, a hypothesis which was substantiated by the fact that both benzo[*e*]dipodazine (**59**) and benzo[*g*]dipodazine (**60**) were active in the antifouling assay. Benzo[*g*]dipodazine (**60**) was the most effective analogue and inhibited settlement of the barnacle larvae at even lower concentrations than barettin. This assay also indicated that the stereochemistry of the compounds might play an important role, since compound **58a** (*E*-isomer) is active, in contrast to the *Z*-isomer, which did not inhibit settlement in relevant concentrations. All dipodazine analoges are *Z*-isomers, whereas barettin was isolated as an *E/Z* mixture.

	EC_{50} (μM)	Inhibitors (I) / Stimulators (S)
Barettin (**35**)	0.9	I
8,9-Dihydrobarettin (**36**)	7.9	I
5-Bromobarettin (**50**)	4.1	S
Debromobarettin (**51**)	---	---
Dipodazine (**44**)	---	---
5-Bromodipodazine (**52**)	5.8	I
5-Methoxydipodazine (**53**)	---	---
5-Nitrodipodazine (**54**)	---	---
6-Chlorodipodazine (**55**)	---	---
5-Methyldipodazine (**56**)	---	---
6-Bromoindole-3-carboxaldehyde (**57**)	6.7	I
Compound **58a** *E*-isomer	2.4	I
Compound **58b** *Z*-isomer	---	---
Benzo[*e*]dipodazine (**59**)	1.5	I
Benzo[*g*]dipodazine (**60**)	0.034	I

Table 1. Results of antifouling assay with barettin and dipodazine analogues

4. Rhopaladins (Paper III)

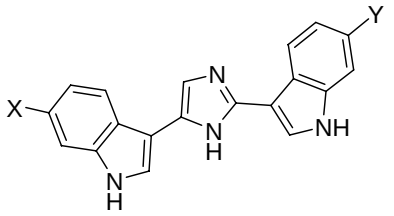
4.1 Marine indole alkaloids with an additional heterocyclic ring

As already mentioned, biologically active alkaloids have been frequently isolated from marine invertebrates and among these, 3-substituted indoles represent a major class, interesting from both a biological and a structural point of view. The substituent at the 3-position of indole is commonly an additional heterocyclic ring: e.g. imidazole (topsentin (**29**)[22], nortopsentin A-D (**61a-d**)[56]); dihydroimidazole (discodermindole (**62**)[57]); maleimide (didemnimides A-D (**63a-**

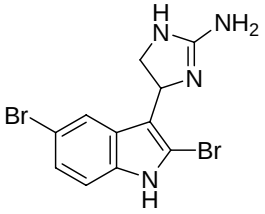
[56] (a) Isolation: Sakemi, S.; Sun, H. H. *J. Org. Chem.* **1991**, *56*, 4304 (b) Syntheses: Miyake, F. Y.; Yakushijin, K.; Horne, D. A. *Org. Lett.* **2000**, *2*, 2121; Kawaski, I.; Yamashita, M.; Ohta, S. *Chem. Pharm. Bull.* **1996**, *44*, 1831

[57] Sun, H. H.; Sakemi, S. *J. Org. Chem.* **1991**, *56*, 4307

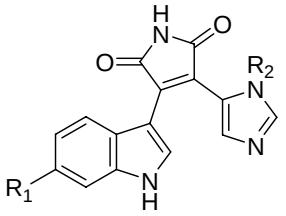
d)[58]); piperazine (dragmacidin (**30**)[23]); pyrimidine (meridians A-E (**64a-e**)[59]) and oxazole (martefragin (**65**)[60]). The first report of an indole alkaloid with an imidazolinone ring in 3-position was the isolation of 3-indolyl-imidazol-4-one (**66**)[61] from the tunicate *Dendrodoa grossularia*. The imidazolinone heterocyclic system is rarely seen among the plethora of indole and bis(indole) alkaloids (Figure 12).



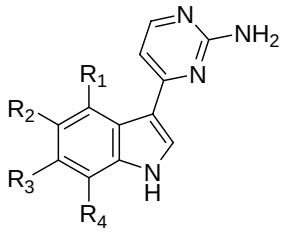
61a X = Br, Y = Br
61b X = Br, Y = H
61c X = H, Y = Br
61d X = H, Y = H



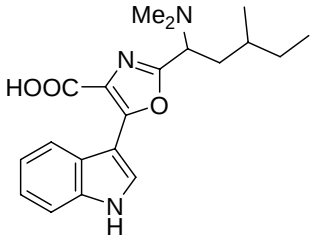
62



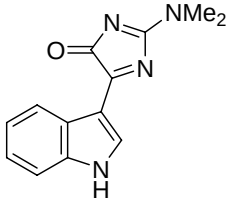
63a R_1 = H, R_2 = H
63b R_1 = Br, R_2 = H
63c R_1 = H, R_2 = CH_3
63d R_1 = Br, R_2 = CH_3



64a R_1 = OH, R_2 = H, R_3 = H, R_4 = H
64b R_1 = OH, R_2 = H, R_3 = Br, R_4 = H
64c R_1 = H, R_2 = Br, R_3 = H, R_4 = H
64d R_1 = H, R_2 = H, R_3 = Br, R_4 = H
64e R_1 = OH, R_2 = H, R_3 = H , R_4 = Br



65



66

Figure 12

4.2 Rhopaladins A-D, bis(indole) alkaloids from the marine tunicate *Rhopalaea* sp.

Since their isolation from the extracts of the Okinawan tunicate *Rhopalaea* sp. in 1998, the bis(indole) alkaloids rhopaladins A-D (**67a-d**) have been an interesting synthetic target (Figure 13). The structures were elucidated based upon spectroscopic data and they are the first reported bis(indole) alkaloids possessing an imidazolinone spacer between the two indole units. Furthermore, the rhopaladins showed antibacterial activity against *Sarcina lutea* and

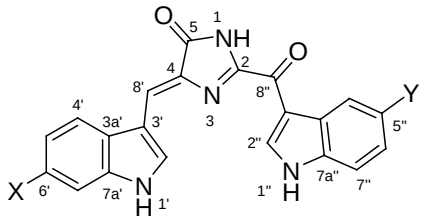
[58] (a) Isolation: Vervoort, H. C.; Richards-Gross, S. E.; Fenical, W. *J. Org. Chem.* **1997**, *62*, 1486 (b) Syntheses: Terpin, A.; Winklhofer, C.; Schumann, S.; Steglich, W. *Tetrahedron* **1998**, *54*, 1745; Hughes, T. V.; Cava, M. P. *Tetrahedron Lett.* **1998**, 39, 9629

[59] (a) Isolation: Franco, L. H.; Joffé, E. B. K.; Puricelli, L.; Tatian, M.; Seldes, A. M.; Palermo, J. A. *J. Nat. Prod.* **1998**, *61*, 1130 (b) Syntehesis: Fresneda, P. M.; Molina, P.; Bleda, J. A. *Tetrahedron* **2001**, *57*, 2355

[60] (a) Isolation: Takahashi, S.; Matsunaga, T.; Hasegawa, C.; Saito, H.; Fujita, D.; Kiuchi, F.; Tsuda, Y. *Chem. Pharm. Bull.* **1998**, *46*, 1527 (b) Synthesis: Nishida, A.; Fuwa, M.; Fujikaw, Y.; Nakahata, E.; Furuno, A.; Nakagawa, M. *Tetrahedron Lett.* **1998**, *39*, 5983

[61] Guyot, M.; Meyer, M. *Tetrahedron Lett.* **1986**, *27*, 2621

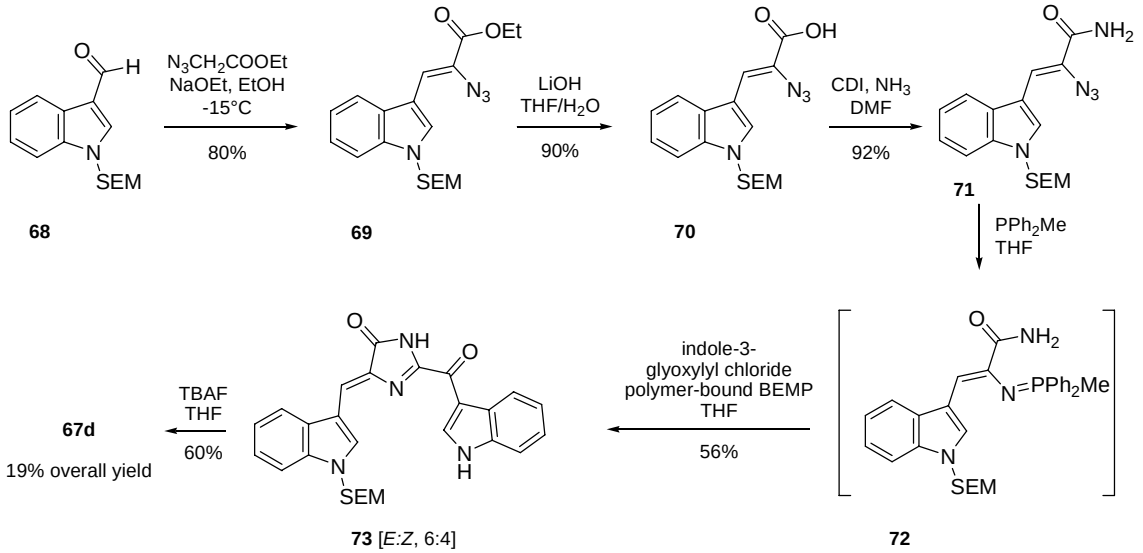
Corynebacterium xerosis and inhibitory activity against cyclin dependent kinase 4 and *c-erb*β-2 kinase.[62]



67a X = OH, Y = Br
67b X = OH, Y = H
67c X = H, Y = Br
67d X = H, Y = H

Figure 13

The first synthesis of rhopaladin D was reported by Fresneda *et al.*[63] As a key step, the construction of the central imidazolinone ring was based upon an aza-Wittig reaction of the iminophosphorane **72** with indole-3-glyoxylyl chloride in the presence of polymer-supported BEMP (2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine). An intramolecular cyclisation of the resulting imidoyl chloride followed immediately to give the protected rhopaladin **73** in a 6:4 mixture of *E/Z* isomers. After deprotection, rhopaladin D (**67d**) was obtained in an overall yield of 19% (Scheme 6).



Scheme 6

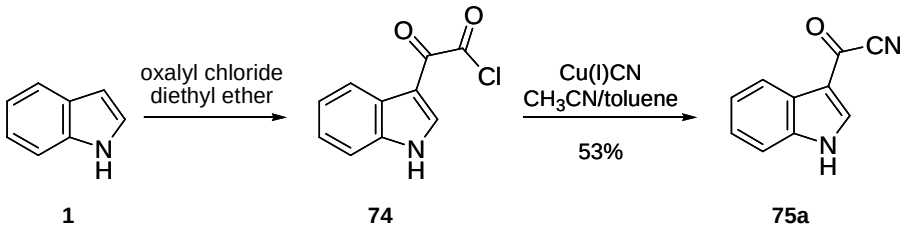
[62] Sato, H.; Tsuda, M.; Watanabe, K.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 8687
[63] Fresneda, P. M.; Molina, P.; Sanz, M. A. *Synlett* **2000**, 1190

4.3 Synthesis of rhopaladins A-D

As it has been suggested that the rhopaladins may be biosynthetically derived from two molecules of tryptophans, this amino acid looks like an appropriate starting point. Since imidates are well known to cyclise with amino acid esters to afford imidazolinone rings[64,65] it was envisaged that such a ring formation might prove to be useful in the total synthesis of rhopaladins A-D (**67a-d**). Tryptophan ester would provide one indole unit and ultimately the imidate would introduce the indolyl carbonyl group. As imidates are commonly prepared from nitriles, it was believed that the known indole-3-carbonyl nitrile could provide the indole unit, the carbonyl functionality as well as the nitrile for the imidate formation.

4.3.1 Efficient synthesis of indole-3-carbonyl nitriles

Indole-3-carbonyl nitrile (**75a**) is most commonly prepared *via* the synthetic route devised by Hogan and Sainsbury[66] where indole is treated with oxalyl chloride to give indole-3-glyoxylyl chloride and subsequent treatment with cuprous cyanide to afford (1*H*-indol-3-yl)carbonyl nitrile (**75a**) in 53% yield (Scheme 7).



Scheme 7

However, when using 5-bromoindole in the above reaction, a severe decrease in the yield was noted. The desired product was usually afforded in around 20–34% yield. Admittedly, we were also having problem repeating Sainsbury's method with regard to the yields stated in the literature. Thus a method for preparation of indole-3-carbonyl nitriles was required that would provide the compounds in reasonable yields and which would tolerate several different substituents. Synthesis of acyl cyanides through O-silylated cyanohydrins and subsequent oxidation is a well described topic.[67] Thus, indole-3-carboxaldehyde **76a** was treated with trimethylsilyl cyanide (TMSCN) to give the masked cyanohydrin silyl ether **77a** in good yield. The reaction can be performed in both refluxing acetonitrile and 1,2-dimethoxyethane (DME) with only minor differences in yield. During the development of this method, it was evident that the silyl ether had limited stability at room temperature and should thus preferably be used immediately or stored for a short period of time in a refrigerator. Oxidation

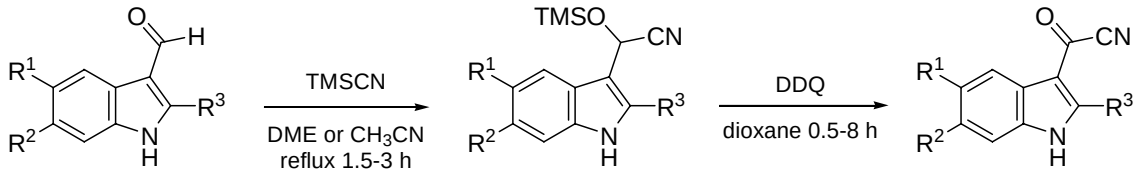
[64] For reviews on imidates see: (a) Roger, R.; Neilson, D. G. *Chem. Rev*. **1961**, *61*, 179 (b) Sandler, S. R.; Karo, W. *Organic Functional Group Preparation*, Academic Press, 1989, vol. 12, p. 314

[65] (a) Finger, H. *J. Prakt. Chem.* **1907**, *76*, 93 (b) Finger, H.; Zeh, W. *J. Prakt. Chem.* **1910**, *82*, 50 (c) Kjaer, A. *Acta. Chem. Scand.* **1953**, *7*, 1017

[66] Hogan, I. T.; Sainsbury, M. *Tetrahedron* **1984**, *40*, 681

[67] (a) Evans, D. A.; Truesdale, L. K.; Carroll, G. L. *J. Chem. Soc. Chem. Commun.* **1973**, 55 (b) Härle, H.; Jochims, J. C. *Chem. Ber.* **1986**, *119*, 1400 (c) Lee, J. G.; Lee, J. A.; Sohn, S. Y. *Synth. Commun.* **1996**, *26*, 543 (d) Piva, O.; Amougay, A.; Pete, J.-P. *Tetrahedron Lett*. **1991**, *32*, 3993

of the O-silylated cyanohydrin **77a** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane proceeded satisfactory, and the corresponding carbonyl nitrile **75a** could be isolated in good yield. Moreover, this method was shown to be applicable to indoles with several different substituents, e.g. 5-Br, 6-OMe, 2-Ph (**75b-d**), as illustrated in scheme 8.



76a R^1 = H, R^2 = H, R^3 = H
76b R^1 = Br, R^2 = H, R^3 = H
76c R^1 = H, R^2 = OCH_3, R^3 = H
76d R^1 = H, R^2 = H, R^3 = Ph

77a (67%)
77b (88%)
77c (66%)
77d (91%)

75a (91%)
75b (97%)
75c (58%)
75d (90%)

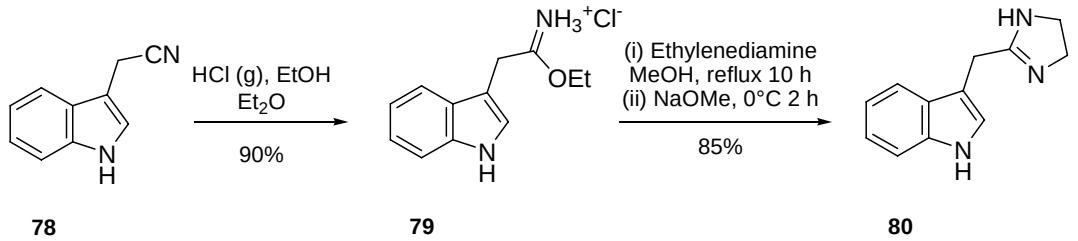
Scheme 8

Although this method was demonstrated to tolerate a number of differently substituted indoles, the presence of strongly electron withdrawing substituents (e.g. 4-NO_2) or N-protecting groups (e.g. SO_2Ph) on the cyanohydrin silyl ether prevented the DDQ-oxidation. An efficient synthesis of indolyl carbonyl nitriles has nevertheless been developed, and it is a good complement to the already existing one. Generally, this method has advantages such as good overall yields, shorter reaction times and compatibility with many substituents.

4.3.2 Reactions of indole-3-carbonyl imidates

The next step in our approach required transformation of the indolyl carbonyl nitriles into the corresponding imidates. Imidates are often prepared in a Pinner reaction wherein a nitrile and an alcohol are reacted under anhydrous conditions in the presence of hydrogen chloride.[64] So far, there are only a few cyclic acyl imidates reported in literature,[68] and no heterocyclic carbonyl imidates. However, the indole imidate **79**[69] is known and has been prepared using the Pinner reaction with indole-3-acetonitrile (**78**) (Scheme 9). In addition, 2-(3-indolylmethyl)imidazoline (**80**) has been prepared from compound **79** with ethylenediamine. This illustrates the potential use of imidates in e.g. synthesis of indole alkaloids containing additional heterocyclic systems (Scheme 9).

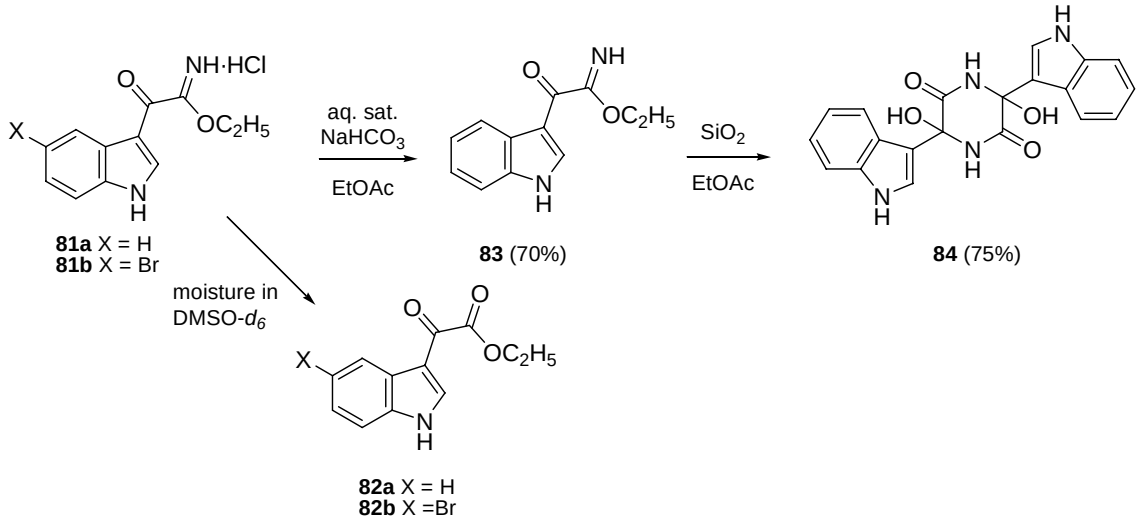
[68] (a) Vinkler, P.; Thimm, K.; Voss, J. *Justus Liebigs Ann. Chem*. **1976**, 2083 (b) Dirlam, J. P.; James, R. B.; Shoop, E. V. *J. Org. Chem.* **1982**, *47*, 2196 (c) Muller, A. J.; Nishiyama, K.; Griffin, G. W.; Ishikawa, K.; Gibson, D. *J. Org. Chem.* **1982**, *47*, 2342
[69] Kelarev, V. I.; Shvekhgeimer, G. A. *Chem. Heterocycl. Compd. Engl. Transl.* **1980**, *16*, 501



78 **79** **80**

Scheme 9

The Pinner protocol worked equally well with indolyl carbonyl nitriles **75a-b**. Thus, when a stream of dry gaseous hydrogen chloride was bubbled through a solution of **75a** or **75b** in dry ether in the presence of absolute ethanol the corresponding imidates **81a-b** were formed in respectable yields (Scheme 11). The imidates were found, as expected, to be rather sensitive towards moisture, but can be stored under dry conditions in a refrigerator. Rapid hydrolysis of the imine functionality of compounds **81a-b** into a carbonyl takes place in the presence of even small amounts of moisture, for example in DMSO-d_6 containing traces of water, affording the corresponding dicarbonyl compounds **82a-b**.[70] (Scheme 10). During work with the imidate **81a** it was noticed that a dimerisation reaction took place upon attempted purification of the free base on silica gel, giving the diketopiperazine derivative **84**, which is structurally related to several natural products (e.g. dragmacidins) (Scheme 10).



81a X = H
81b X = Br

83 (70%)

84 (75%)

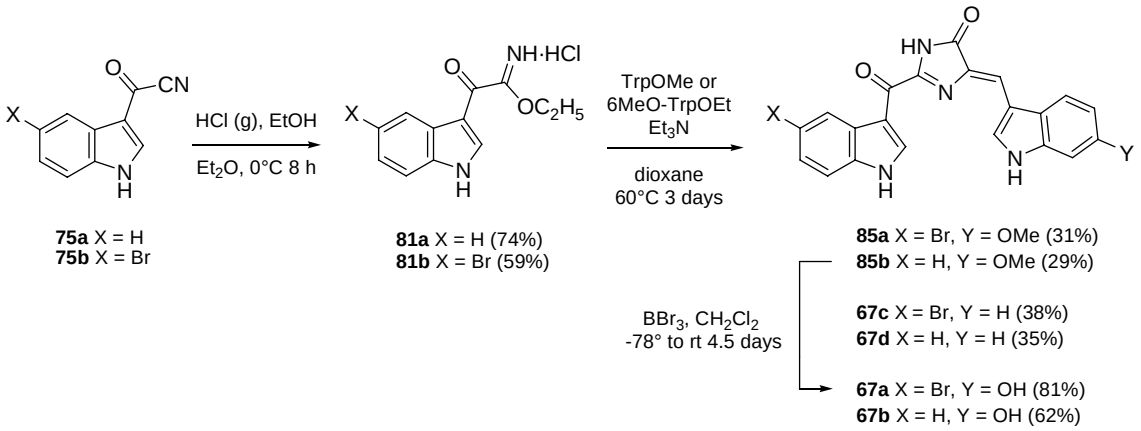
82a X = H
82b X =Br

Scheme 10

Treatment of the imidates **81a-b** with tryptophan methyl ester in presence of base gave the expected imidazolinone ring formation, and thus rhopaladins C (**67c**) and D (**67d**). Likewise, the imidates **81a-b** were coupled with 6-methoxytryptophan ethyl ester to compounds **85a-b**. A final demethylation of the 6-methoxy groups with BBr_3 in CH_2Cl_2 at -78°C gave

[70] (a) Nogrady, T.; Doyle, T. W. *Can. J. Chem.* **1964**, *42*, 485 (b) Da Settimo, A.; Saettone, M.; Nannipieri, E.; Barili, P. *Gazz. Chim. Ital.* **1967**, *97*, 1304

rhopaladins A (**67a**) and B (**67b**). The imidate based cyclisation reaction gave compounds **85a-b** and **67c-d** only in moderate yields (29-38%); prolonged reaction times (1.5 to 4 days) or higher reaction temperatures (from ~60°C to reflux) did however not improve the yields (Scheme 11).



Scheme 11

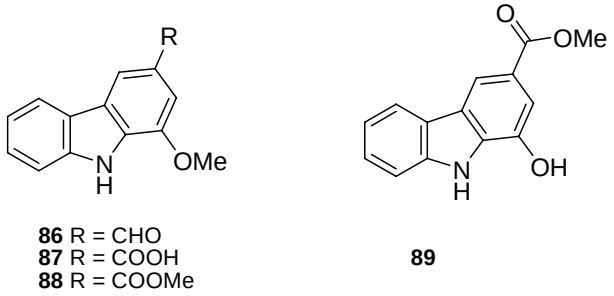
Interestingly, the reaction of the imidates with the tryptophan esters is the result of a coupling followed by a cyclisation and subsequent spontaneous dehydrogenation to give compounds **85a-b** and **67c-d** selectively in *Z*-conformation, which appears to be the most stable arrangement. Any presence of the corresponding *E*-isomer could not be detected. This spontaneous dehydrogenation to the most stable isomer is an advantage compared with the synthesis devised by Fresneda and Molina, described in scheme 6. These workers also reported a spontaneous cyclisation reaction, but since a preformed double bond already is present in the molecule, a mixture of *E/Z* isomers was obtained.[63] The *Z*-orientation of the *exo*-cyclic double bond could be deduced using difference-NOE experiments, as irradiation of the 2-hydrogen resonance of the carbonyl bridged indole unit produced enhancement of the signals corresponding to the 2- and 4-protons of the second indole. The presence of rotamers around the C-3´-C-8´ bond, as indicated previously,[62] was thus also confirmed.

Synthesis of all four known rhopaladins A-D (**67a-d**) in two synthetic steps has thus been attained, in overall yields of 13-26%. Also, the central imidates **81a** and **81b** may be considered as possible tools for creating other indole alkaloids containing additional heterocyclic rings.

5. Synthetic approaches towards lactams isolated from the marine sponge *Halichondria melanodocia* (Paper IV)

5.1 Carbazole alkaloids

The first carbazole alkaloid to be isolated was murrayanine (**86**), extracted from the steam-bark of the small tree *Murraya koenigii* in India.[71] Since then, the field has expanded enormously, largely due to the promising biological activities of many of the carbazole alkaloids. The carbazole alkaloids have primarily been isolated from plants of the genus *Murraya*, *Glycosmis* and *Clausena* from the family Rutaceae, particularly 1-oxygenated carbazole alkaloids like **86**, mukoeic acid (**87**),[72] and mukonine (**88**).[73,74] The shrub *Clausena excavata* is traditionally used in China for the treatment of snakebites, abdominal pain and as a detoxification agent. Extensive studies of the *Clausena* genus have resulted in several compounds with interesting biological activities. Clausine E (**89**), for instance, isolated from *C. excavata*[75,76,77] and *Clausena anisata*,[78] has displayed antiplatelet aggregating[75] and antitumor properties.[78](Figure 14)



86 R = CHO
87 R = COOH
88 R = COOMe

89

Figure 14

Widely used methods for synthesis of 1-oxygenated carbazoles include the classical Fischer indolisation with appropriate phenylhydrazones,[79] intramolecular cyclications of indoles,[80,81] and oxidadative cyclisation of diarylamines.[82] Increasingly important are transition metal-mediated and -catalysed processes for preparation of carbazoles.[74]

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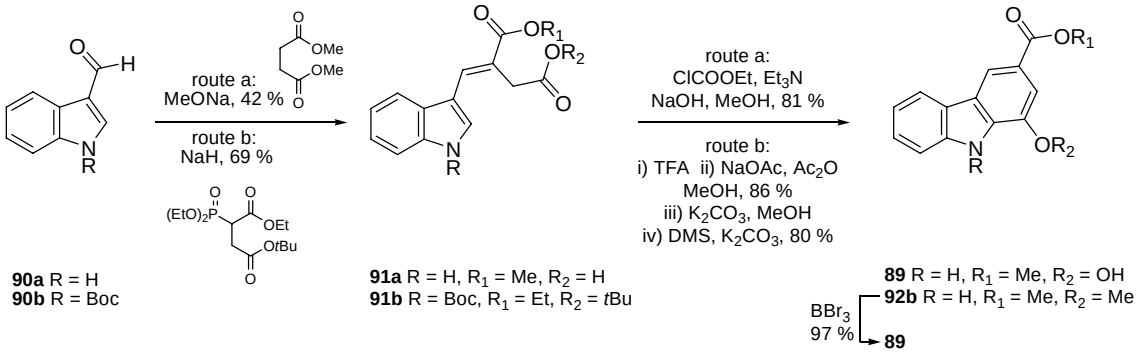
[71] Chakraborty, D. P.; Barman, B. K.; Bose, P. K. *Tetrahedron* **1965**, *21*, 681
[72] Choudhury, B. K.; Chakraborty, D. P. *Phytochemistry* **1971**, *10*, 1967
[73] Chakraborty, D. P.; Bhattacharyya, P.; Roy, S.; Bhattacharyya, S. P.; Biswas, A. K. *Phytochemistry* **1978**, *17*, 834
[74] For an in-depth review of biologically active carbazole alkaloids, which covers occurrence, biological acitivities and total synthesis of carbazole alkaloids, see Knölker, H. J.; Reddy, K. R. *Chem. Rev.* **2002**, *102*, 4303
[75] Wu, T.-S.; Huang, S.-C.; Wu, P.-L.; Teng, C.-M. *Phytochemistry* **1996**, *43*, 133
[76] Ito, C.; Katsuno, S.; Ohta, H.; Omura, M.; Kajura, I.; Furukawa, H. *Chem. Pharm. Bull.* **1997**, *45*, 48
[77] Wu, T.-S.; Huang, S.-C.; Wu, P.-L.; Kuoh, C.-S. *Phytochemistry* **1999**, *52*, 523
[78] Ito, C.; Katsuno, S.; Itoigawa, M.; Ruangrungsi, N.; Mukainaka, T.; Okuda, M.; Kitagawa, Y.; Tokuda, H.; Nishino, H.; Furukawa, H. *J. Nat. Prod.* **2000**, *63*, 125
[79] Chakraborty, D. P.; Choudhury, B. K. *J. Org. Chem.* **1968**, *33*, 1265
[80] Moody, C. J. *Synlett* **1994**, 681
[81] Brenna, E.; Fuganti, C.; Serra, S. *Tetrahedron* **1998**, *54*, 1585
[82] Furukawa, H.; Ito, C.; Yogo, M.; Wu, T.-S. *Chem. Pharm. Bull.* **1986**, *34*, 2672

5.1.1 A short synthesis of the carbazole alkaloid clausine E

Herein we would like to disclose a fast and uncomplicated synthesis of the carbazole alkaloid clausine E (**89**) in two synthetic steps. Synthesis of clausine E (**89**) has previously been reported by Brenna[81] (34%, 3 steps; route a) and by Bringmann[83] (46%, 6 steps; route b) (Scheme 12). Both methods involve the activation and intramolecular cyclisation of monoester acids **91a** and **91b**, obtained *via* a Stobbe condensation or a HWE reaction, respectively.



Scheme 12. (a) Synthesis of clausine E (**89**) Route a: Brenna *et al*.[81] Route b: Bringmann *et al*.[83]

Initially, we were interested in the outcome of the attempted Lewis acid-assisted acylation reaction of indole with itaconyl chloride (**93**). It was expected that the reaction should occur at either one of the carbonyls (affording **94** or **95**) or, perhaps more likely, a Michael type addition would predominate (Figure 15).

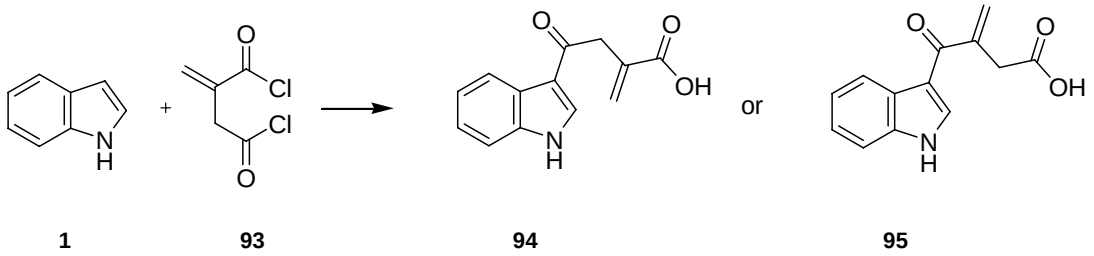
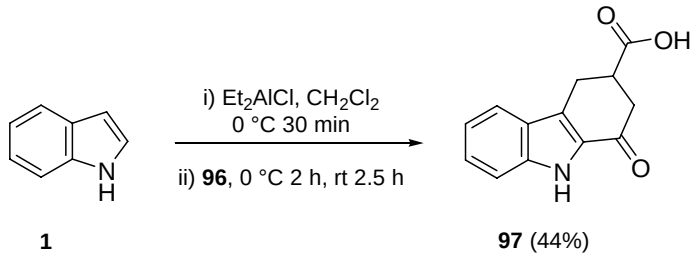


Figure 15

Following the protocol by Okauchi[12] and co-workers, the reaction of indole with itaconyl chloride in the presence of diethylaluminium chloride (Et_2AlCl) resulted in a Michael addition and the known carbazole **97**[84] could be isolated from the reaction mixture. The reaction worked better with itaconic anhydride (**96**), but still gave the carbazole **97** only in a modest yield of 44% (Scheme 13).

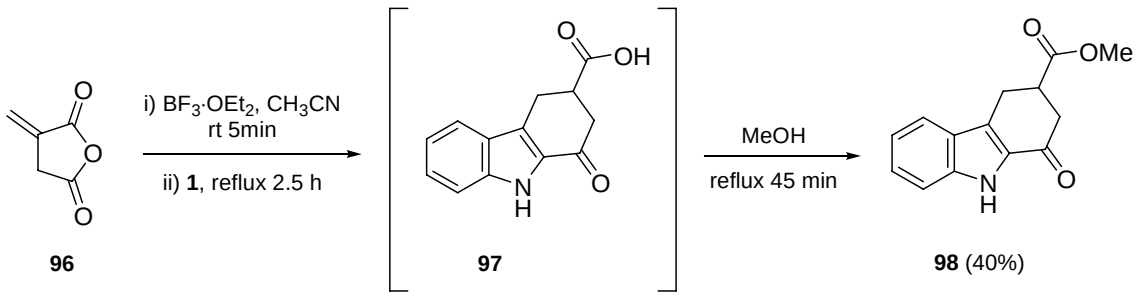
[83] Bringmann, G.; Tasler, S.; Endress, H.; Peters, K.; Peters, E.-M. *Synthesis* **1998**, 1501
[84] Erguen, Y.; Patir, S.; Okay, G. *Synth. Commun.* **2004**, *34*, 435



1 **97** (44%)

Scheme 13

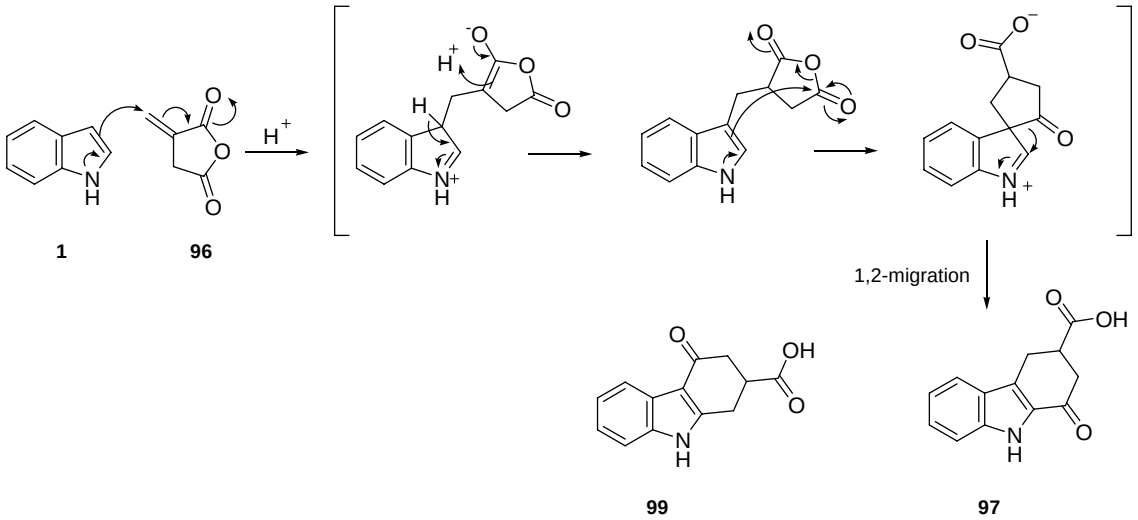
Changing the Lewis acid to boron trifluoride diethyletherate ($BF_3{\cdot}OEt_2$) instead of Et_2AlCl did not change the result significantly, but resulted in the advantage of utilising the residual acidity of the reaction mixture to directly transform the carboxylic acid **97** into the methyl ester **98**. (Scheme 14)



96 **97** **98** (40%)

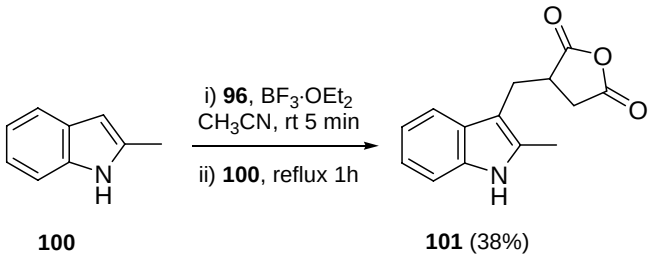
Scheme 14

It was considered that the reaction proceeds *via* initial addition to the double bond of itaconic anhydride in acidic environment, followed by subsequent 1,2-migration affording the six-membered ring **97** (Scheme 15), thus also implying the possibility of formation of the isomer **99**.



Scheme 15

In an attempt to support this reaction mechanism, indole was replaced by 2-methylindole (**100**) in the reaction with itaconyl anhydride in presence of $BF_3{\cdot}OEt_2$. Since the 2-methyl substituent of 2-methylindole would conceivably block the assumed 1,2-migration, it was speculated in the possibility of isolating the presumed spiro-indole derivative. However, only the anhydride **101** was isolated (Scheme 16), which is in line with the previously reported experiments in this area by Noland[85] and Kuryla.[86] These authors have described a simple procedure for heating 2-methylindole with itaconic anhydride, leading to the succinic anhydride **101** in good yields. Interestingly, **102** could reportedly not be prepared using this method.[86] These findings indeed support a reaction mechanism wherein an initial Michael addition of indole to the double bond of itaconic anhydride will take place. It is also tempting to speculate that the substituent of 2-methylindole will hinder the presumed reaction with the second carbonyl, thus halting at **101**.

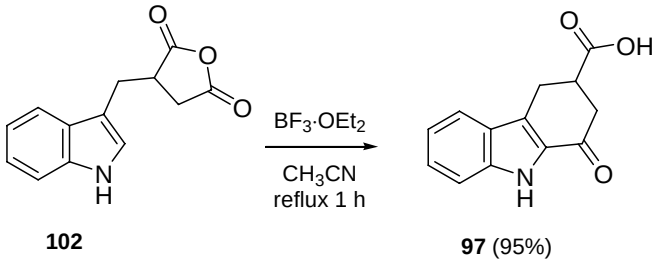


Scheme 16

Compound **102** has been synthesised previously by Lewis *et al.* from gramine and triethyl 1,1,2-ethanetricarboxylate, followed by saponification, decarboxylation and a final

[85] Noland, W.; Hammer, C. F. *J. Org. Chem.* **1960**, *25*, 1536
[86] Kuryla, W. Ph. D. Thesis, University of Minnesota, 1960

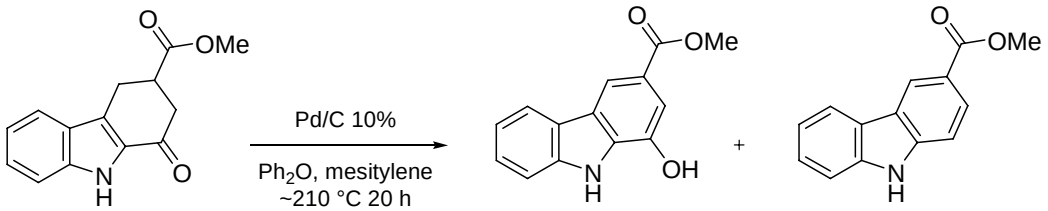
cyclisation.[87] It was expected that treatment of **102** with $BF_3{\cdot}OEt_2$ in refluxing acetonitrile, would result in the formation of **97**, which indeed was the case (Scheme 17). This further substantiates the reaction mechanism suggested in scheme 15.



102 **97** (95%)

Scheme 17

Dehydrogenation of the carbazole **98** was performed in diphenyl ether using Pd/C as the catalyst. Clausine E (**89**) was isolated in a good yield from the reaction mixture, together with small amounts of the carbazole **103**, which has previously been isolated from another *Clausena* species, *C. lansium*.[88] Addition of a diluent, here mesitylene, seems to be necessary for minimizing dehydrative side reactions (Scheme 18).[89,90,91]



98 **89** (76%) **103** (6%)

Scheme 18

Whether the reaction mechanism in scheme 15 is true or not, it could indeed be verified that **97** and not **99** was the isolated product. The presence of a 1-oxygenated carbazole was elucidated using difference-NOE experiments, as irradiation of the OH resonance of **89** produced enhancement of the signals corresponding to the NH of the indole and the proton at position 2 of the carbazole, as well as the methyl group, thus eliminating **99** as an alternative.

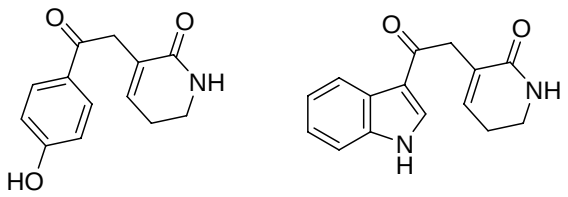
In conclusion, an efficient and uncomplicated synthesis of the carbazole alkaloid clausine E (30% yield, 2 steps) has been developed, utilising a Michel addition type reaction between itaconic anhydride and indole in the presence of a Lewis-acid catalyst. Although

[87] Lewis, R. T.; Macleod, A. M.; Merchant, K. J.; Kelleher, F.; Sanderson, I.; Herbert, R. H.; Cascieri, M. A.; Sadowski, S.; Ball, R. G.; Hoogsteen, K. *J. Med. Chem*. **1995**, *38*, 923
[88] (a) Isolation: Li, W.-S. McChesney, J. D.; Farouk, S. E.-F. *Phytochemistry* **1991**, *30*, 343 (b) Synthesis: Back, T. G.; Pandyra, A.; Wulff, J. E. *J. Org. Chem.* **2003**, *68*, 3299
[89] Linstead, R. P., Michaelis, K. O. A. *J. Chem. Soc.* **1940**, 1134
[90] Bringmann, G.; Ledermann, A.; François, F. *Heterocycles* **1995**, *40*, 293
[91] Scott, T. L.; Söderberg, B. C. G. *Tetrahedron* **2003**, *59*, 6323

explicit here, the method should have additional applications for preparation of other 1-oxygenated carbazole alkaloids.

5.2 Alkaloids isolated from the marine sponge *Halichondria melanodocia*

Back in the 1970's marine indole alkaloids were not as common in the literature as today, thus the indole derivative isolated from *Halichondria melanodocia* in 1979 was one of few marine indole alkaloids reported to date.[92] Despite of, or perhaps because of, first being mentioned for more that 25 years ago, this particular indole derivative has not been the subject of further investigations. To the best of our knowledge, the biological activity has not yet been evaluated, neither has the structure been confirmed *via* synthesis. The indole alkaloid in question was one of two alkaloids isolated from the isopropanol extracts of an algae-infested Caribbean sponge, *Halichondria melanodocia*. The structures of the alkaloids were assigned as the phenol and the indole derivatives **104** and **105,** respectively (Figure 16). It is uncertain whether the alkaloids are produced by the sponge itself or by the associated algae and bacteria.[92]



104 **105**

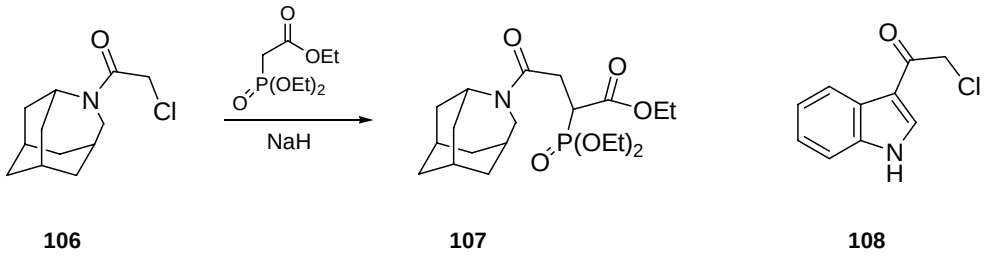
Figure 16

Our continuous interest in marine indole alkaloids[93] attracted our attention towards compound **105**. Since chloroacetylazahomoadamantane (**106**) (Figure 17) has been reported to react with triethyl phosphonoacetate at the α-position[94] to give compound **107**, we believed that 2-chloro-1-(1*H*-indol-3-yl)-ethanone (**108**)[10] could similarly provide the appropriate building block in our attempts to synthesise **105**.

[92] Gopichand, Y.; Schmitz, F. J. *J. Org. Chem.* **1979**, *44*, 4995

[93] (a) Wahlström, N.; Stensland, B.; Bergman, J. *Tetrahedron*, **2004**, *60*, 2147. (b) Janosik, T.; Johnson, A.-L.; Bergman, J. *Tetrahedron* **2002**, *58*, 2813. (c) Johnson, A.-L.; Bergman, J.; Sjögren, M.; Bohlin, L. *Tetrahedron* **2004**, *60*, 961

[94] Miryan, N. I.; Isaev, S. D.; Kovaleva, S. D.; Petukh, N. V.; Dvornikova, E. V.; Kardakova, E. V.; Yurchenko, A. G. *Russ. J. Org. Chem.* **1999**, *35*, 857

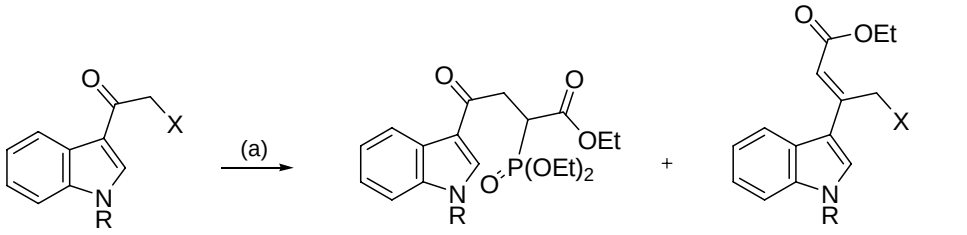


106 **107** **108**

Figure 17

5.2.1 Synthetic approaches towards the indole alkaloid from *H. melanodocia*

It was concieved that the indole variant of **107** could provide a possibility to introduce the double bond in the correct position of the lactam **105**. Hence, compound **109a** was treated with triethyl phosphonoacetate to give compound **110a**. However, the yields of **110a** were quite modest (seldom over 30 %), largely dependent on the formation of the side-product **111**[94,95] together with unreacted starting material (Scheme 19). Other bases, apart from NaH, were tried as well (BuLi, *t*-BuOK, LDA, DBU) with the result of either even lower yields or more side-product formation. Solvent and/or temperature changes did not improve the yields, but since it was observed that the yields improved in the presence of a catalyst (NaI or Bu_4NI) (less than 10% product without catalyst), most likely other 3-(2-haloacyl)indoles should give better results. As expected, changing the chlorine atom to bromine or iodine (compounds **109b-d**) improved the yields significantly. Furthermore, the formation of compound **111** was minimized. Changing to a polar aprotic solvent improved the yields even further.



109a X = Cl, R = Boc
109b X = Br, R = Boc
109c X = I, R = Boc
109d X = Br, R = SO_2Ph

110a R = Boc
110b R = SO_2Ph

111 X = Cl, R = Boc

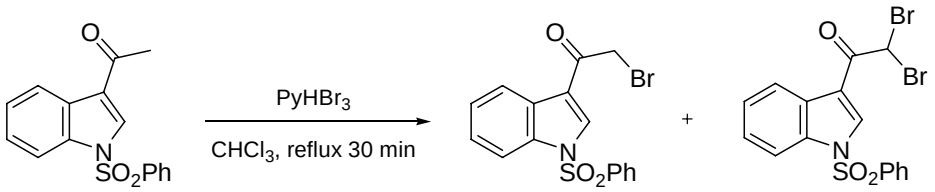
Scheme 19. (a) NaH, other reagents and conditions see Table 2

[95] Brooks, S.; Sainsbury, M.; Weerasinge, D. K. *Tetrahedron* **1982**, *38*, 3019

Indole derivative	Solvent	Other conditions	Yield **110a**/**110b**	Yield **111**
109a R = Boc, X = Cl	THF	Bu_4NI, rt 18 h	27%	14%
109a R = Boc, X = Cl	THF	Bu_4NI, rx 4 h	42%	n.i
109a R = Boc, X = Cl	THF	Bu_4NI, 0°C to rt 18 h	24%	n.i
109a R = Boc, X = Cl	toluene	Bu_4NI, reflux 3 h	22%	n.i
109a R = Boc, X = Cl	DMF	NaI, 60°C 36 h	--	--
109a R = Boc, X = Cl	THF	NaI, rt 18 h	26%	26%
109d R = SO_2Ph, X = Br	THF	NaI, rt 1.5 h	72%	--
109a R = Boc, X = Cl	THF	rt 18 h	8%	n.i
109c R = Boc, X = I	THF	rt 18 h	56%	n.i
109b R = Boc, X = Br	THF	rt 18 h	49%	n.i
109c R = Boc, X = I	DMF	rt 18 h	70%	--
109d R = SO_2Ph, X = Br	DMF	NaI, rt 18 h	56%	--
109b R = Boc, X = Br	DMF	rt 18 h	71%	--
109d R = SO_2Ph, X = Br	DMF	rt 18 h	64%	--

Table 2. rt = room temperature, n. i. = not isolated

Compound **109d**[96] was synthesised *via* bromination of 3-(1-benzenesulfonyl-1*H*-indol-yl)-ethanone (**112**)[6] using pyridinium hydrobromide perbromide.[97] The side-product, the dibromo derivative **113**, could easily be separated from the main product by column chromatography (Scheme 20).



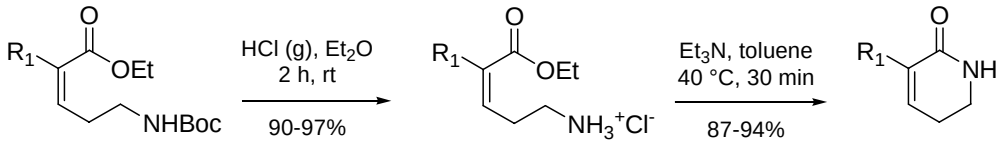
112 **109d** (55%) **113** (10%)

Scheme 20

The Horner-Wadsworth-Emmons olefination of **110a**, **b** with *N*-Boc-3-amino-propionaldehyde[98] did however only proceed in a modest yield. Using BuLi as the base afforded compounds **117a**, **b** in low yields, usually around 20%. Other bases tried (DBU, LDA, *t*-BuOK, $[(CH3)_3Si]_2NK$, NaH) failed to give the desired product (Scheme 22).

[96] Suzuki, H.; Furukawa, T.; Yamada, C.; Shibuya, I.; Kurumi, M.; Yokoyama, T.; Murakami, Y. *Heterocycles* **2002**, *56*, 519
[97] Ando, R.; Sakaki, T.; Jikihira, T. *J. Org. Chem*. **2001**, *66*, 3617
[98] Delfourne, E.; Kiss, R.; Le Corre, L.; Dujols, F.; Bastide, J.; Collignon, F.; Lesur, B.; Frydman, A.; Darro, F. *J. Med. Chem*, **2003**, *46*, 3536

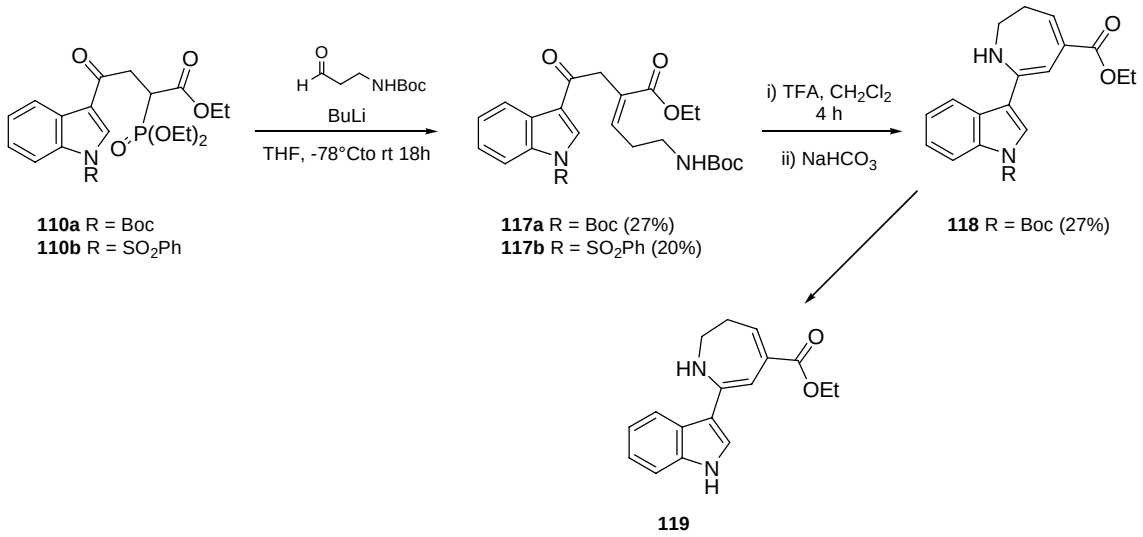
The plan was to remove the Boc-protecting groups and cyclise the amine to the desired lactam, which appeared to be a routine task according to the literature (Scheme 21).[99]



114 R_1= CH_3, Cl, F **115** R_1= CH_3, Cl, F **116** R_1= CH_3, Cl, F

Scheme 21

However, treatment of **117a** with 20 eq. TFA and subsequent treatment with $NaHCO_3$ gave the seven-membered heterocycle **118** instead of the expected free amine or six-membered ring **105** (Scheme 22) Also, quite surprisingly, the indole nitrogen remained Boc-protected despite the TFA treatment.



110a R = Boc
110b R = SO_2Ph

117a R = Boc (27%)
117b R = SO_2Ph (20%)

118 R = Boc (27%)

119

Scheme 22

Indolylazepine structures like **118** are rather uncommon. Only a few examples of non-fused indolylazepines are described in the literature, of which compound **121**[100] and **123**[101] can be mentioned as they possess similar structural features as **118** (Figure 18). Treatment of the two different 3-(1-methyl-2-pyrrolidinyl)indoles, **120** and **122**, with suitable 1,3-dicarbonyl derivatives (diethyl malonate or pentane-2,4-dione respectively) gave the indolylazepines **121** and **123**. In the latter case the authors have suggested an initial addition of the diketone enolate to the imine functionality of **122**.[101]

[99] Grison, C.; Genève, S.; Coutrot, R. *Tetrahedron Lett.* **2001**, *42*, 3831
[100] Hester, J. B. *J. Org. Chem.* **1967**, *32*, 4098
[101] Bishop, D. Al-Khawaja, I. K.; Joule, J. A. *J. Chem. Res., Synop.* **1981**, 361

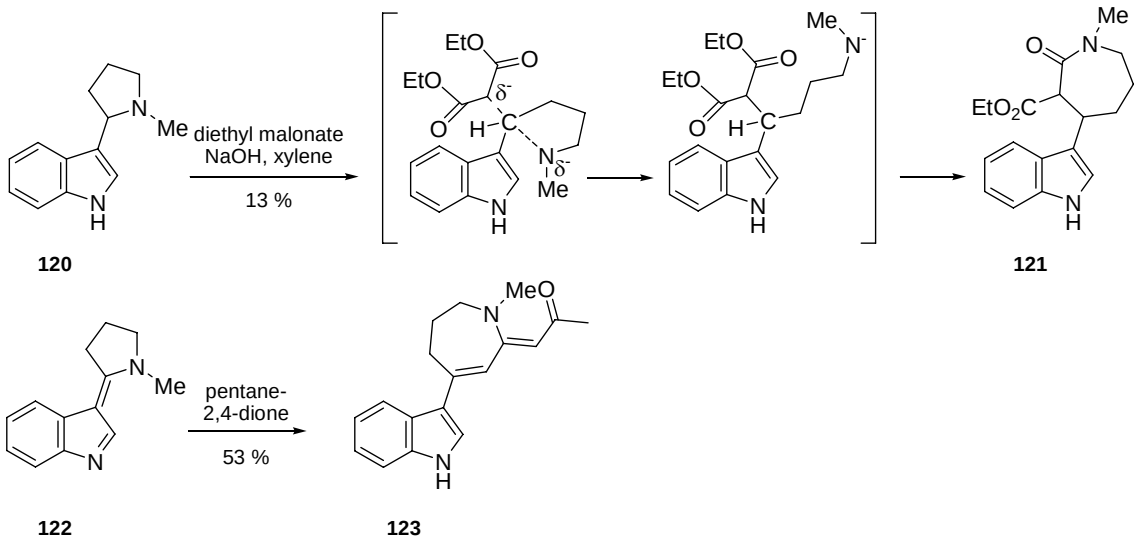
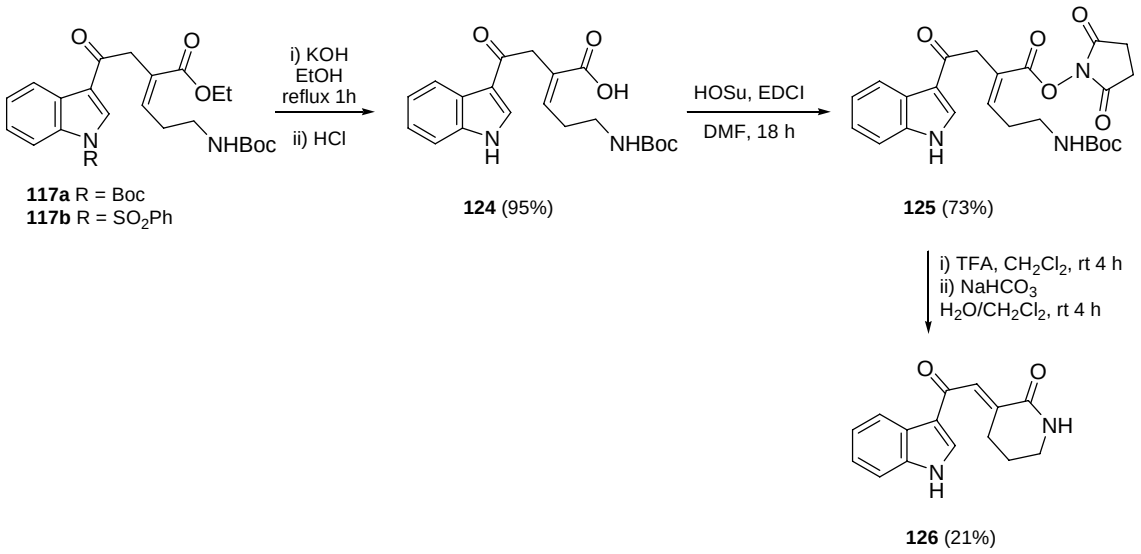


Figure 18

Compound **118** is rather unstable, which is probably due to its enamine character. Optimal conditions and proper purification methods for this compound are still an issue, as is the preparation of **119**. Protection of the free nitrogen of **118** would perhaps render the compound less prone to decomposition during isolation and purification. The azepine formation is likely to be induced by the electron withdrawing Boc-protecting group on the indole nitrogen, which would render the carbonyl at the 3-position of the indole more susceptible for attack than the ester functionality. Consequently, removal of the benzenesulfonyl group of **117b** under standard conditions afforded **124**, which was further reacted with *N*-hydroxysuccinimide (HOSu) (Scheme 23). Surprisingly, during hydrolysis of the ester of **117a** the acidic workup also removed the indole Boc-protecting group, affording compound **124**. This situation is in contrast with the treatment of **117a** with trifluoroacetic acid or formic acid where the amine group is more easily deprotected. The *N*-succinimide ester **125** was treated with TFA, followed by a biphasic mixture of aqueous $NaHCO_3$ solution and EtOAc. The exocyclic lactam **126** could thereafter be isolated from the organic phase (Scheme 23). Whether the exocyclic lactam **126** is the kinetic product is uncertain, but there are indications of similar cases in the literature.[102] Hence, the indole alkaloid **105** still remains to be synthesised. Changing the conditions in the final cyclisation reaction may have an impact on the outcome.

[102] Campi, E. M.; Chong, J. M.; Jackson, W. R.; van der Schoot, M. *Tetrahedron* **1994**, *50*, 2533



117a R = Boc
117b R = SO_2Ph

124 (95%)

125 (73%)

126 (21%)

Scheme 23

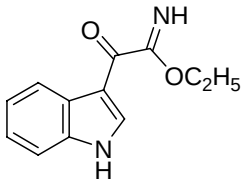
6. Concluding remarks

The study of the marine sponge *Geodia baretti* has led to the isolation of two indole alkaloids with the ability of efficiently inhibiting the settlement of the barnacle larvae of *Balanus improvisus*. The synthesis of barettin and 8,9-dihydrobarettin presented in this thesis renders further research in the antifouling area regarding these substances possible, as accessibility of synthetic material for further antifouling assays, field studies, analogue preparation and possible commercialisation is a prerequisite.

The synthesis of the marine bis(indole) alkaloids rhopaladins also includes a new and efficient method for preparation of indole-3-carbonyl nitriles. Moreover, the use of indole-3-carbonyl imidates in the central imidazolinone formation demonstrated that imidates might be applicable in the preparation of other heterocyclic systems.

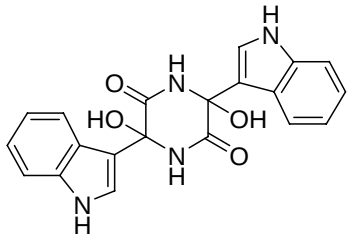
Although the alkaloids isolated from the marine sponge *Halichondria melanodocia* still remain to be synthesised, interesting observations have been made during the studies performed to date. In particular, an unexpected indolyl azepine formation deserves to be further studied. The reaction between itaconic anhydride and indole, affording a carbazole derivative, proved to be useful in the preparation of the carbazole alkaloid clausine E.

7. Supplementary material



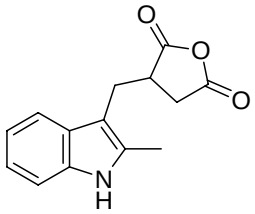
83

(1*H*-Indol-3-yl-carbonyl) ethyl imidate hydrochloride (**81a**) (50 mg, 0.20 mmol) was dissolved in aq. sat. $NaHCO_3$ (20 mL) and immediately extracted with EtOAc (20 mL). The organic phase was washed with brine (20 mL), dried over $MgSO_4$ and evaporated to give the free base of **83** as a slightly pinkish solid (30 mg, 70%).
Mp 182-184°C; IR (KBr): 3250, 2977, 1612, 1498, 1448, 1245, 1171, 1121, 936, 749 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 12.23 (s, 1H), 8.91 (s, 1H) 8.24 (d, *J* = 2.1 Hz, 1H); 8.24-8.21 (m, 1H), 7.55-7.53 (m, 1H), 7.30-7.23 (m. 2H), 4.37 (q, *J* = 7.1, 14.2, Hz, 2H), 1.40 (t, *J* = 7.1Hz, 3H); ^{13}C-NMR (DMSO-d_6): δ 178.6 (s), 165.2 (s), 137.8 (d), 136.4 (s), 126.2 (s), 123.6 (d), 122.6 (d), 121.4 (d), 112.5 (d), 112.3 (s), 61.9 (t), 14.0 (q); MS (ESI) *m/z* 215 $(M-H)^-$.



84

(1*H*-Indol-3-yl-carbonyl) ethyl imidate (23 mg, 0.11 mmol) was subjected to silica gel column chromatography using EtOAc as eluent to give diketopiperazine **84** as a beige solid (15 mg, 75 %).
Mp 185-186 °C; IR (KBr): 3394, 3199, 1667, 1579, 1408, 1235, 1133, 746 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 12.18 (s, 2H), 8.60 (s, 2H), 8.26-8.15 (m, 2H), 8.05 (br s, 2H), 7.69 (br s, 2H), 7.56-7.49 (m, 2H), 7.29-7.22 (m, 4H); ^{13}C-NMR (DMSO-d_6): δ 182.9 (s), 166.0 (s), 138.2 (d), 136.2 (s), 126.1 (s), 123.3 (d), 122.4 (d), 121.2 (d), 112.5(d), 112.1(s); MS (ESI) *m/z* 377 $(M+H)^+$.

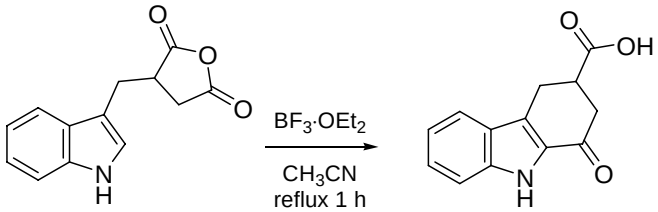


101

Itaconic anhydride (560 mg, 5 mmol) was dissolved in CH_3CN (15 mL) and $BF_3{\cdot}OEt_2$ (0.63 mL, 5 mmol) was added. After 5 min at room temperature was 2-methylindole (**100**) (656 mg, 5 mmol) added as a solid in one portion and the resulting mixture was heated at reflux for 1 h. The dark red solution was allowed to cool and was thereafter evaporated to dryness. The residue was dissolved in EtOAc (20 mL) and was washed with aq. sat. $NaHCO_3$ (2 ×10 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$ and evaporated. The residual red oil was purified on silica gel column chromatography using hexane/EtOAc (60:40) as the eluent to afford compound **101** as a pale yellow solid, which darkened upon standing. Yield: 461 mg (38%).

IR (KBr): 3399, 1844, 1773, 1462, 1219, 1068, 1039, 912, 744 cm^{-1}

^{1}H-NMR (DMSO-d_6): δ 10.82 (s, 1H), 7.44 (d, J = 7.4, 1H), 7.25 (d, J = 7.4, 1H), 7.03-6.92 (m, 2H), 3.57-3.51 (m, 1H), 3.14-3.11 (m, 2H), 2.89-2.80 (m, 1H), 2.73-2.65 (m, 1H), 2.34 (s, 3H); ^{13}C-NMR (DMSO-d_6): δ 175.1 (s), 171.1 (s), 135.2 (s), 133.1 (s), 127.9 (s), 120.2 (d), 118.4 (d), 117.2 (d), 110.5 (d), 105.9 (s), 41.7 (d), 33.8 (t), 24.1 (t), 11.3 (q).



102 **97**

Compound **102** (229 mg, 1 mmol) was dissolved in CH_3CN (15 mL) and $BF_3{\cdot}OEt_2$ (0.13 mL, 1 mmol) was added. The reaction mixture was heated at reflux for 1 h and was thereafter allowed to cool. The solvent was evaporated and the residue was dissolved in EtOAc (25 mL), washed with H_2O (20 mL) and brine (20 mL). The organic phase was dried over $MgSO_4$ and thereafter evaporated, affording **97** as a brownish solid (217 mg, 95%). The spectral data was identical to the compound prepared from indole and itaconic anhydride, see paper **IV**.

8. Acknowledgements

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