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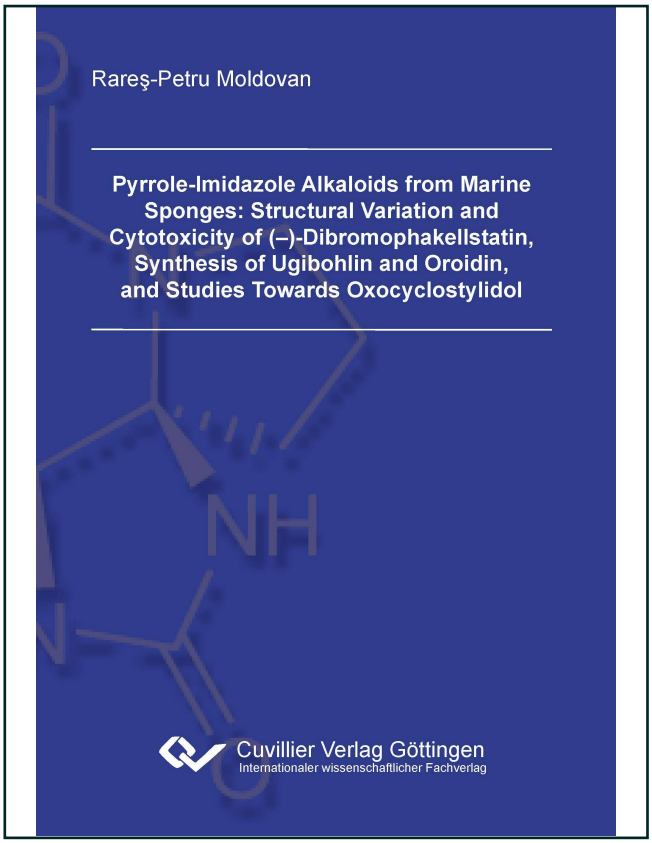
Pyrrole-Imidazole Alkaloids from Marine Sponges: Structural Variation and Cytotoxicity of (–)-Dibromophakellstatin, Synthesis of Ugibohlin and Oroidin, and Studies Towards Oxocyclostylidol



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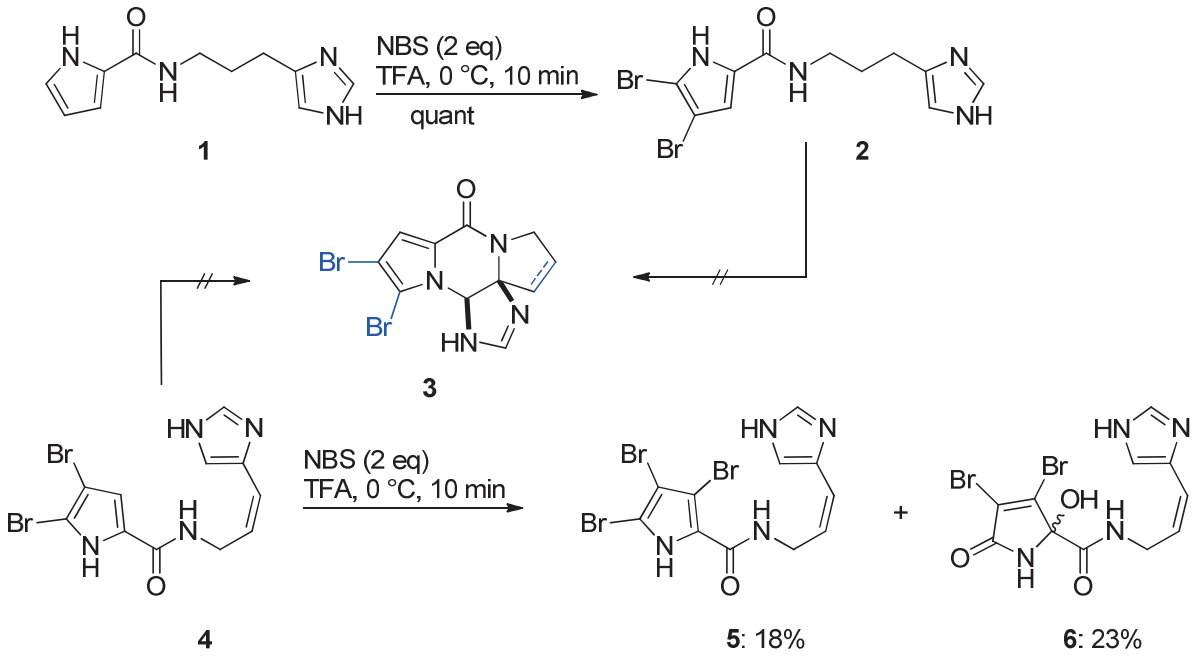
I. Introduction

1. Summary

a. Synthesis and oxidation of oroidin and analogues

Oroidin is considered to be the biogenetic key metabolite of the pyrrole-imidazole alkaloids. Several syntheses are known to date, all of which contain steps with only moderate yields. For a study on its reactivity large quantities are needed. Therefore, an improved synthesis would be beneficial. In the present work two different known routes to **8** were investigated. By following the method developed in our group (*via* Sonogashira coupling) several analogues arose as new products. By treating the two deaminated analogs **1** and **4** with NBS in TFA, no cyclization to the phakellin skeleton **3** took place. Instead, pyrrole oxidation occurred (Scheme 1).

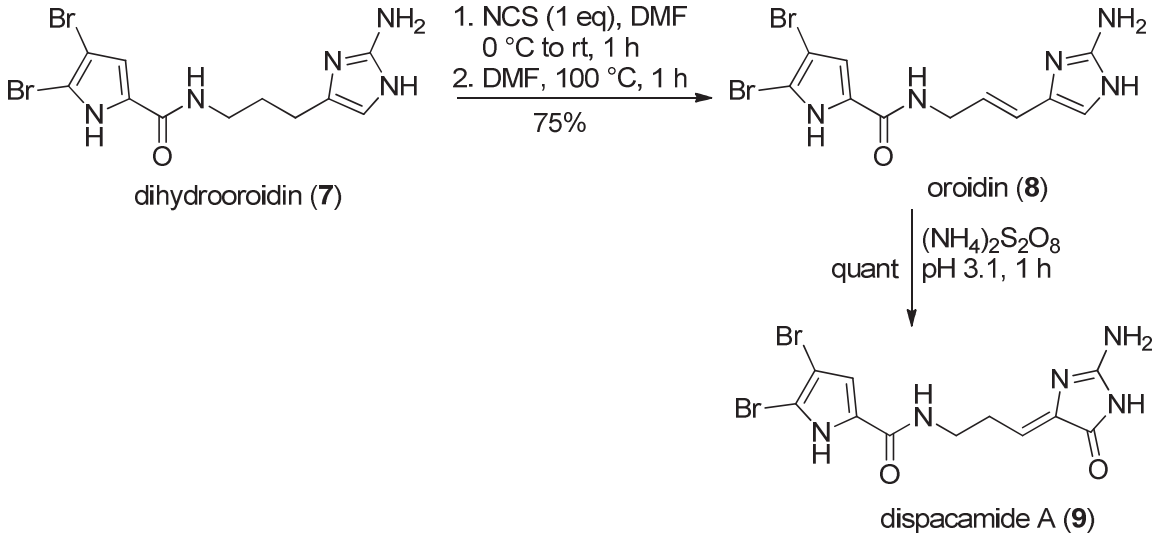


Scheme 1. Oxidation of oroidin analogues.

Alternatively, oroidin (**8**) was synthesized *via* dihydrooroidin (**7**) by modifying a procedure developed by Horne and co-workers. The yield of the oxidation step of **7** to **8** represented a great disadvantage of this approach. However, by treating **7** with an equimolar amount of *N*-chlorosuccinimide in DMF instead of MeOH, followed by warming up to 100 °C for one hour, oroidin (**8**) was formed in good yield (75%). The

reaction took place *via* chlorination of the 5-position on the 2-aminoimidazole followed by thermal elimination of hydrochloric acid (Scheme 2).

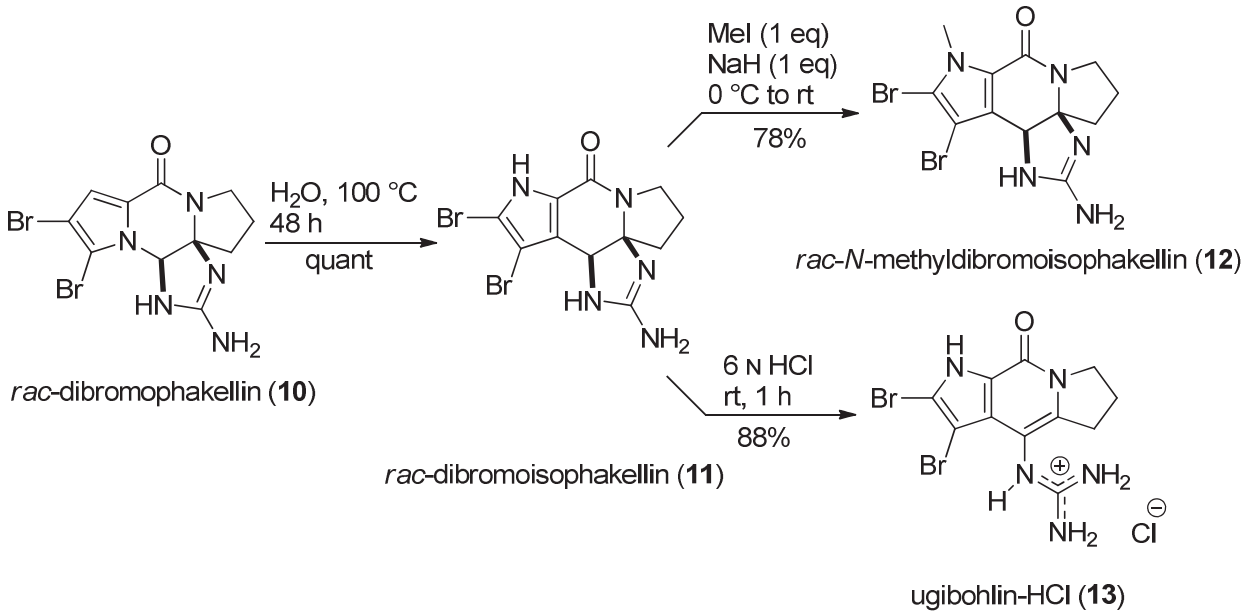
Several oxidation reactions were performed on oroidin (**8**) aiming at its conversion to more complex members of the family. Due to its high reactivity, complex product mixtures were formed or decomposition occurred. In a presumably biomimetic reaction with ammonium peroxodisulfate in an aqueous buffer the natural product dispacamide A (**9**) was formed in a quantitative manner.



Scheme 2. Improved conversion of **7** to oroidin (**8**) and oxidation to **9**.

b. Synthesis of ugibohlin and rac-*N-methylisophakellin*

Compound **7** was cyclized to *rac*-dibromophakellin (**10**) according to Horne's procedure and the reactivity of **10** was examined. Under oxidative reaction conditions, only pyrrole oxidation occurred. However, when **10** (the free base) was boiled in neutral or basic conditions (ammonia or $Ba(OH)_2$ aqueous solutions), isomerization to *rac*-dibromoisophakellin (**11**) took place (Scheme 3). By boiling the free base of **10** in water for 48 hours **11** was formed in a quantitative manner. *Rac-N*-methylisophakellin (**12**) was synthesized for the first time by treatment of **11** with NaH/MeI in good yield. When **11** was treated with 6 M hydrochloric acid for one hour at room temperature, the natural product ugibohlin (**13**) was obtained for the first time.



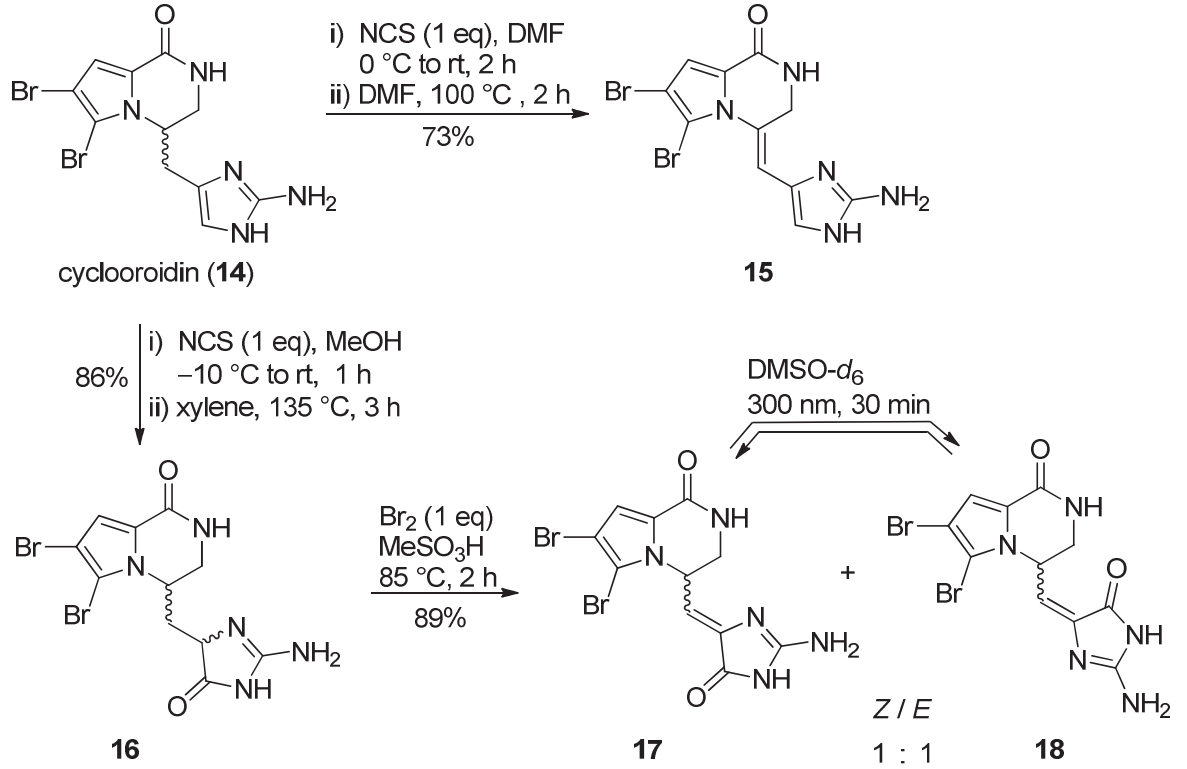
Scheme 3. Conversion of *rac*-dibromophakellin (**10**) to the natural products **11**, **12** and **13**.

c. Oxidation of rac-*cyclooroidin*

The biomimetic conversion of *rac*-cyclooroidin (**14**) to *rac*-oxocyclostylidol (**112**) was attempted. Initial efforts to introduce the olefinic double bond *via* dimethoxylation and elimination of methanol gave rise to the oxo compound **16**, which could be oxidized further to the dispacamide-like compounds **17** and **18**. Alternatively, **18** was formed directly from *rac*-cyclooroidin (**14**) in a much better yield (65%), together with small amount of **17** (10%, Scheme 25) by treatment with $Pb(OAc)_4$ in acetic acid. Upon irradiation at 300 nm for 30 minutes, the interconversion of **17** to **18** took place and an equilibrium ratio of 1:1 has been observed. Both **17** and **18** proved to be very stable and no further reaction was possible. The olefinic double bond was successfully introduced by the chlorination-dehydrochlorination procedure employing NCS and DMF, which had also worked for dihydrooroidin (**7**).

The oxidation of the pyrrole moiety proved to be difficult. The non-cyclized 2-aminoimidazole showed the much higher nucleophilicity. A favored oxidation product of cyclooroidin (**14**) employing different reagents was found to be **17** (Scheme 4). Several attempts were made in order to change the reactivity order of pyrrole versus 2-aminoimidazole. Boc-protection of the 2-aminoimidazole part of the

cyclooroidin (**14**) did not change the nucleophilicity of the 2-aminoimidazole at all. Also, removing the bromine atoms at the pyrrole unit did not increase its reactivity. Oxocyclostylidol (**112**) remains unsynthesized.



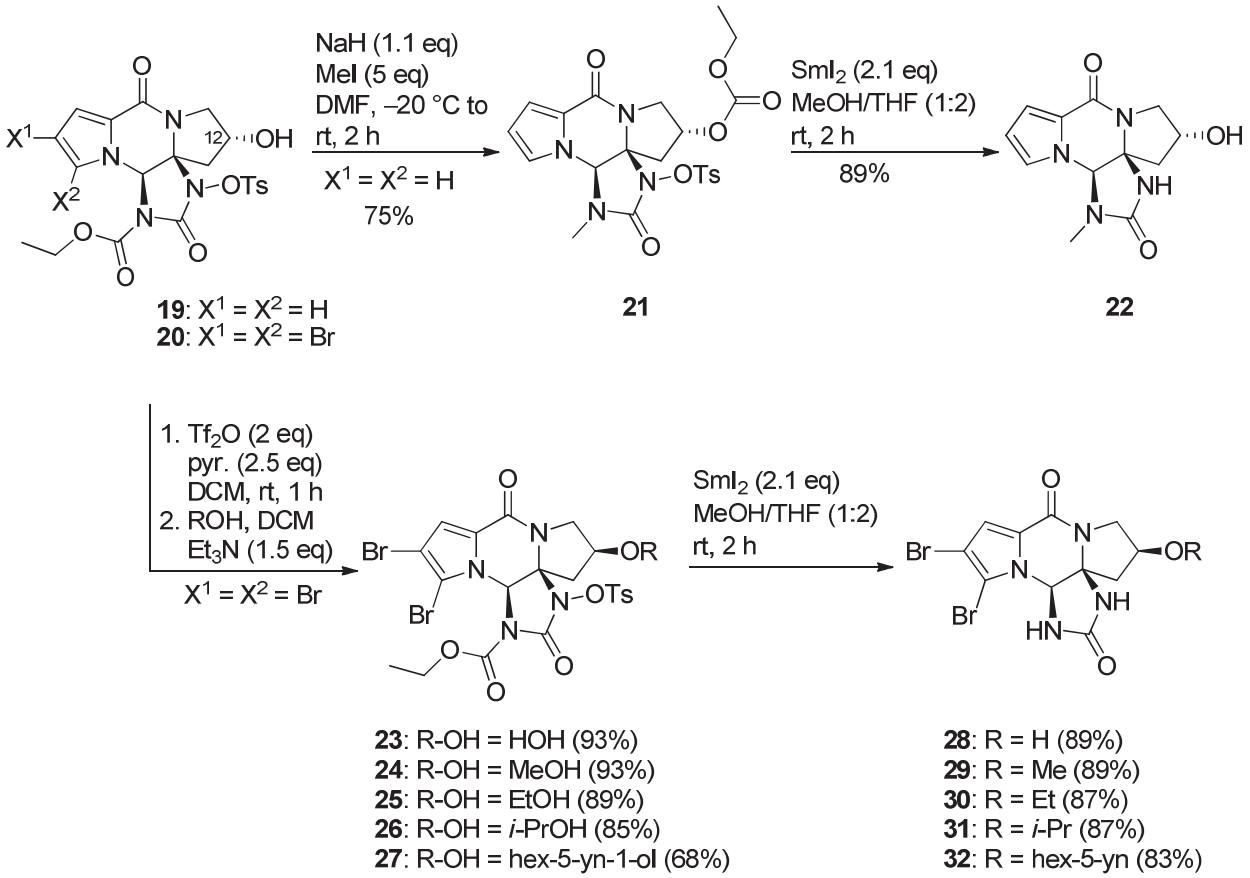
Scheme 4. Oxidation of cyclooroidin (**14**).

*d. Ring C functionalization of (–)-dibromophakellstatin (**33**)*

Initial efforts to functionalize the ring C hydroxyl function of (–)-dibromohydroxyphakellstatin (**34**) were based on the classical Williamson method. On treatment of **19** with NaH in DMF, an unexpected nucleophilic attack at the ring D carbamate took place forming a new carbonate moiety at ring C (**21**, Scheme 5). By quenching the reaction with electrophiles like methyl iodide, selective ring D alkylation occurred. Upon SmI_2–mediated deprotection of **21** (–)-*N*-methylhydroxyphakellstatin (**22**) was obtained (Scheme 5).

It was discovered that ring C functionalization of (–)-dibromophakellstatin (**33**) is possible in high yield starting from the hydroxy intermediate **20** *via* the corresponding triflate. Several ethers of different size were synthesized (Scheme 5). The mechanism through which **33** is acting as a cytostatic is not known. Aiming at a better understanding, several derivatives suitable for immobilization on protein beads

and binding protein isolation were synthesized. The simpler one is derivative **32** that bears a terminal alkyne (Scheme 61). Upon binding to the tagged protein, **32** should be subject of a Click reaction with an azide placed on a solid support, followed by the identification of a binding protein.



Scheme 5. Synthesis of ring C-functionalized dibromophakellstatin derivatives.

Another derivative (**35**) contains a fluorescent moiety (Figure 1). It is designed for visual identification of a binding protein. Compound **35** is the first fluorescent pyrrole-imidazole alkaloid derivative synthesized. The third and most complex synthesized derivative (**36**) contains a biotin moiety and a photoreactive group (Figure 1). It was designed for immobilization on avidin or streptavidin beads upon incubation with a protein mixture. Upon irradiation, the photoreactive group should ensure the covalent binding to the tagged protein, by generating the highly reactive nitrene.

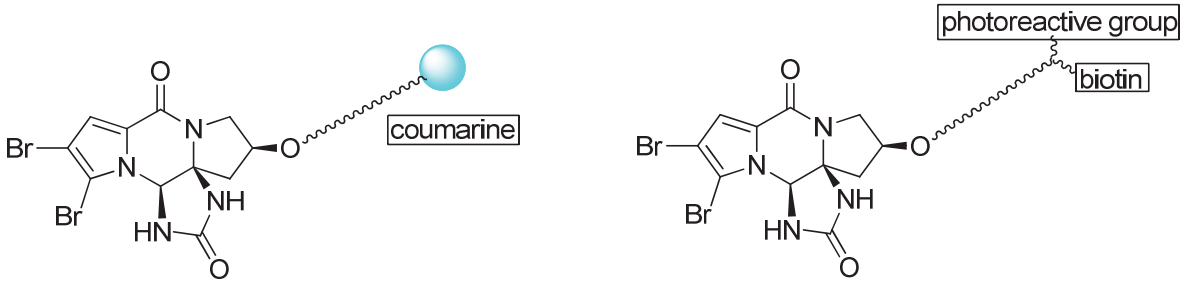


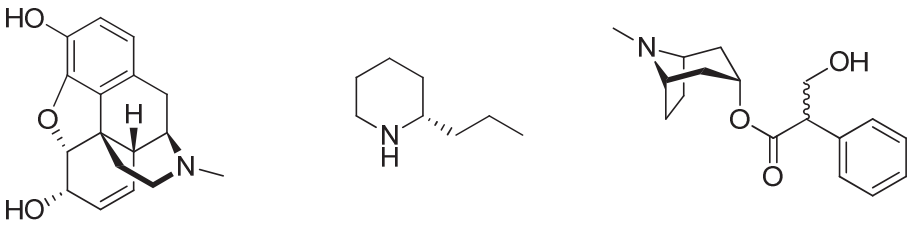
Figure 1. Schematic structures of the coumarine and biotine derivatives **35** and **36**.

The biological examination of the 12*S*-dibromohydroxyphakellstatin derivatives on twelve different cancer cell lines has shown no or low activity for the new derivatives. Only 12*R*-dibromohydroxyphakellstatin (**34**) exhibited cytotoxicity, with a medium value of IC_{50} = 1.34 μM compared to (–)-dibromophakellstatin (IC_{50} = 10.5 μM) (see Table 2). Low cytotoxicity was determined for the alkyne and coumarine (**32** and **35**, see Figure 17) derivatives and total activity loss was recorded for all other derivatives.

2. Alkaloids

Natural products represent a large variety of compounds found in living organisms. Whereas primary metabolites (sugars and amino acids) occur in all living organisms, secondary metabolites are limited to certain organisms and fulfill special biological and chemical roles. Secondary metabolites are responsible for the strong differentiation of species within the same class of organism, concerning chemical defence, chemical communication or other biochemical physiological functions. Considerable differentiation between non-marine and marine secondary metabolites can be made. Whereas the natural products extracted from terrestrial sources represent a large variety of secondary metabolites, compounds with unusual chemical diversity have been isolated from marine life.[1] Differences may be related to underwater pressure and the higher concentration of salt in the marine environment. Both marine and non-marine occurring natural products are investigated for biological activity in medicine. The difficult access to marine life is representing a great impediment, motivating scientists for research in the area of marine natural products.

Since ancient times Opium poppy and related plants (*Papaver somniferum*) have been used in medicine as analgesics.[2] However, the use of plants as medicinal drugs has a great drawback: the dose of the active compound cannot be precisely controlled. In 1806, morphine (**37** see Figure 2) was isolated from opium as a pure substance by Sertürner.[3] Although structural elucidation and synthesis of **37** were performed for the first time in the 20th century, it could be proven early that morphine (**37**) was responsible for the biological activity of the plant. Sertürner was the first scientist to isolate an active ingredient from a medicinal plant. A few years later a new class of compounds was established, called alkaloids, by the German chemist Carl F. W. Meissner.[4]



morphine (**37**) coniine (**38**) atropine (**39**)

Figure 2. Morphine (**37**), coniine (**38**), and atropine (**39**).

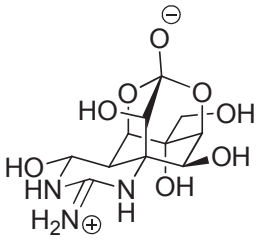
Another early example is coniine (**38**), which is a poison found in the yellow pitcher plant and was isolated by Giesecke in 1827.[5] Its structural elucidation was carried out by Blyth and Hoffmann.[6] It was the first alkaloid to be synthesized in the laboratory by Ladenburg in 1886.[7] The synthesis starts from 2-methylpyridine which reacted with formaldehyde in the presence of a base in a Knoevenagel condensation followed by reduction with sodium in ethanol. Atropine (**39**) is a secondary metabolite extracted from the *Solanaceae* plants isolated in pure form in 1833 by Geiger and Hess. It has also been known since ancient times to be a drug with a wide variety of medicinal effects.

Several other alkaloids were also isolated in the 19th century like quinine and strychnine.[8] However, a large number of alkaloids have been isolated in the 20th century due to the development of new chromatographic and analytical methods. Along with terpenes and polyketides, alkaloids are widely spread in nature. Despite the fact that a large number of alkaloids are toxic, numerous pharmaceuticals found important applicability in medicine during the 20th century.

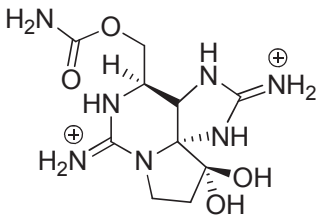
Only in the second half of the last century, the exploration of marine life attracted the attention of scientists. More than 22.000 marine natural products have been isolated to date.[9] Usually the isolation of natural products is bioassay-guided, and only the bio-active and the preponderant compounds are isolated.[10] The bioactive molecules might be carotenoids,[11] terpenoids,[12] guanidine derivatives,[13] indoles,[14] phenolic substances,[15] and others.

A large number of toxins have been isolated from marine organisms. Saxitoxin (**41**)[16] was isolated from the marine micro algae *Gonyaulax catenella* by Schantz and coworkers[17]. The structure of saxitoxin was assigned by X-ray crystallography.[18] It was shown to have an abnormal pK_a value of 8.1 for one of the guanidinium subunit.[19] The lethal doses (LD_{50}) to several animals are reported, including humans (death occurred upon ingestion of less than 1 mg of toxine).[20] Tetrodotoxin[21] occurs in both non-marine and marine bacteria[22] and it was first isolated in 1964 by several authors.[23] The structure of tetrodotoxin (**40**) was confirmed by single crystal X-ray diffraction studies by Woodward and coworkers.[24] Saxitoxin and tetrodotoxin are known to block the sodium channel in muscles and nerve.[25] Several toxins were isolated from the “red tide” dinoflagellate, *Karenia brevis* (formerly *Gymnodinium breve*), and named brevetoxines.[26] Brevetoxin A (Figure 3,

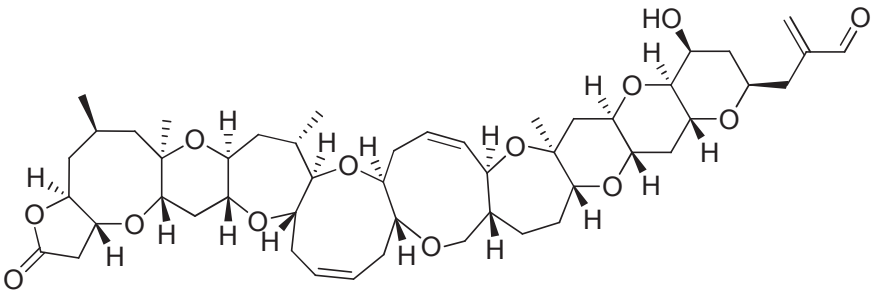
compound **42**)[27] appears to be the most toxic brevetoxine. The structural assignment was performed by NMR, IR, UV and MS and confirmed by X-ray analysis.[28]



tetrodotoxin (**40**)



saxitoxin (**41**)



brevetoxin A (**42**)

Figure 3. The structures of tetrodotoxin (**40**), saxitoxin (**41**), and brevetoxin A (**42**).

Maitotoxin[29] (not depicted in the Figure 3) was also isolated from the toxic dinoflagellate *Karenia brevis*.[28] Maitotoxin[29] is one of the most impressive natural products with a molecular mass of 3421.6 Da (as disodium salt), being the largest natural product to be isolated except for biopolymers.[30] Mouse lethality occurred when very small doses of maitotoxin were administrated (0.13 μg/kg, intraperitoneal injection) and proved to be the most active toxin besides the proteinous toxins.[30] The structural elucidation represented a challenge, largely due to the signal overlapping.[31] The toxins are produced by seafood as self-defense against predatory.[32] Although most of the fishes are killed by the poisonous plankton, some survive becoming poisonous for humans.[17] The mode of action has largely been investigated but still is not fully understood.

Many bioactive secondary metabolites have great medicinal potential. Wakayin (**43**, Figure 4), for example, was isolated from a *Clavelina* sponge (0.005% wet weight) by Ireland and co-workers.[33] Beside the unprecedented structure, wakayin (**43**) exhibited high *in vitro* cytotoxicity when tested against a human cancer cell line (HCT116 IC_{50} = 0.5 μg/mL). The mechanism of action has not been fully investigated, but it is supposed to exhibit biological activity by damaging

the DNA.[33] Marine life is also rich in bioactive terpenes.[34] An example is phyllofolactone A (**45**) which has been extracted from the marine sponge *Phyllospongia foliascens* by Wells and coworkers and[35] possesses activity against the murine cell line P 388 (IC_{50} = 5 μg/mL).[36] This sesterterpene also shows anti-fungal and anti-inflammatory activities.

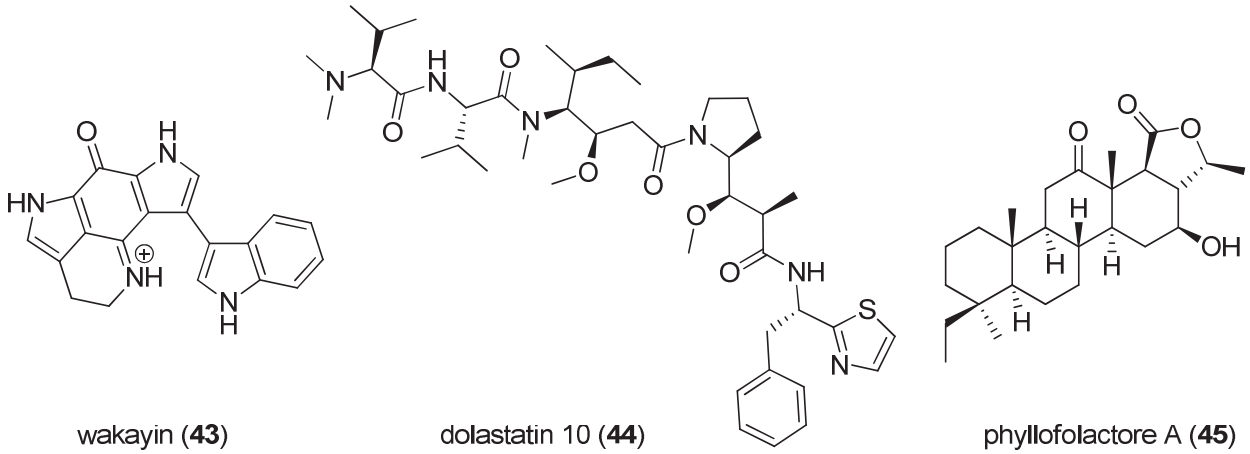


Figure 4. The structures of wakayin (**43**), dolastatin 10 (**44**), and phyllofolactone A (**45**).

Dolastatin 10[37] (**44**, Figure 4) is a cytotoxic peptide isolated by Pettit and coworkers from the marine sponge *Dolabella auricularia*, reported in 1987 along with other dolastatines (dolastatines 1-15, not pictured in the Figure 4).[38] Dolastatine 10 and 15 exhibited the most promising antiproliferative actions.[39] The isolation and structural characterization of those compounds proved to be difficult due to the small amounts produced by the sponge (each dolastatine <1x10^{-5}% of the wet weight).[38,40] Dolastatin 10 (**44**) was tested also for *in vitro* growth inhibition and efficacy against small lung cell cancer cell lines.[41] When tested against human prostate cancer cell lines (DU-145) *in vitro*, complete growth inhibition was observed at concentrations of 1 nM.[42] Several structural modifications were performed on dolastatines (especially dolastatine 10) leading to interesting cytostatic activities.[43,44,45]

In the last few years, the use of natural products in drug discovery took vast proportions, in particular with the improvement of biological screening methods. However, synthetic chemistry has to be employed for the generation of sufficient amounts of substances needed for studies in medicinal chemistry and also for ecological reasons.