THE ALKALOIDS

Chemistry and Biology

VOLUME **70**

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Edited by

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PREFACE

Over a period of 60 years, from 1950 to 2010, 69 volumes of *The Alkaloids* have appeared. The present volume marks the 70th of this series and is the first under my editorship. Thus, it is a good occasion to take a look at the history of this series and to describe its development to the leading book series in the important field of alkaloid chemistry.

The Alkaloids was founded by the late R.H.F. Manske and started with volume 1 in 1950 under the subtitle *Chemistry and Physiology*. The founding editor R.H.F. Manske was in charge of this series for the first 16 volumes (volume 16 appeared in 1977). The following four volumes, from volume 17 in 1979 to volume 20 in 1981, were edited by R.G.A. Rodrigo. With volume 21 (published in 1983), when Arnold Brossi took over the editorship, the subtitle of the series was changed to *Chemistry and Pharmacology*. The late Arnold Brossi was responsible as the editor of *The Alkaloids* till volume 41 published in the year 1992. In the same year, with the publication of volume 42, Geoffrey A. Cordell took over as Editor-in-Chief. Starting with volume 50 in 1998, the subtitle of the series was changed to *Chemistry and Biology*. Geoffrey A. Cordell served as editor for this series until 2010 (publication of volume 69), when he decided to step back.

As the new editor of this series, I would like to thank profoundly my predecessor, Geoffrey A. Cordell, for the marvelous and outstanding job he has done for this series over a period of 19 years (from 1992 to 2010). During this time, Geoffrey was not only running this series to maintain it, he was still increasing its quality and broadening its scope within the area of alkaloids. In consequence, *The Alkaloids* today is covering all aspects of the field: isolation, structural elucidation, biosynthesis, chemical total synthesis, biological activity, and pharmacological potential including medical application of alkaloids. Thus, the series has attracted great interest by scientists from different research areas. I would also like to thank Geoffrey personally for his advice and supportive hints to ensure a smooth transition when taking over the editorship.

The most important goal for the future of this series is to keep its reputation as the leading publication forum for the chemistry and biology of alkaloids and to continue in publishing high-quality reviews written by leading experts.

The present volume consists of two chapters. The first article by Daniel M. Evans and Patrick J. Murphy from Bangor University in Wales (UK) covers the cylindrospermopsin alkaloids. This family of alkaloids is not only of interest due to their challenging structure that has prompted several research groups to develop synthetic approaches, they are also remarkable by their biosynthesis and their environmental impact. Cylindrospermopsin is a potent hepatotoxin produced by cyanobacteria and thus, its increased production can lead to poisoning of drinking water. Therefore, this class of natural products is being looked at from different perspectives.

The second article is from our own group in Dresden (Germany) and describes the isolation, proposed biosyntheses, and the development of different strategies for the total synthesis of pyrrolo[2,1-*a*] isoquinoline alkaloids. These alkaloids have attracted strong interest due to the antitumor and antibacterial activities exhibited by some of their derivatives. Crispine A, a plant alkaloid isolated by a Chinese group in 2002, represents the parent alkaloid for this class. Interestingly, this compound had already been prepared by synthesis seven times prior to its isolation as a natural product (the first synthesis was reported in 1931!). The identification of Crispine A as natural product induced a tremendous development in this area, which has led to 25 total syntheses from 2005 until September 2011.

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CHAPTER 1

The Cylindrospermopsin Alkaloids

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Contents

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I. INTRODUCTION

The cylindrospermopsin alkaloids are a family of three structurally unique water soluble toxins comprising of cylindrospermopsin (**1**), 7-*epi*-cylindrospermopsin (**2**), and 7-*deoxy*-cylindrospermopsin (**3**), which are known to be produced by an ever evolving collection of cyanobacterial species (Figure 1). The toxicological properties of these secondary metabolites are of great concern and have been investigated extensively, as have methods enabling their detection as well as their removal from drinking water sources. The unique structural features of these compounds, comprising of a sulfonated tricyclic guanidine attached to a uracil ring combined with their potent biological activity, have made them a synthetic target of considerable interest. This reviewed chapter discusses the initial isolation of these alkaloids and their environmental impact with a strong emphasis on the methodologies that have been employed in their syntheses [1,2].

II. ISOLATION AND CHARACTERIZATION

Cylindrospermopsin (**1**) was initially isolated from cyanobacterium *Cylindrospermopsis raciborskii* and characterized by Moore and co-workers

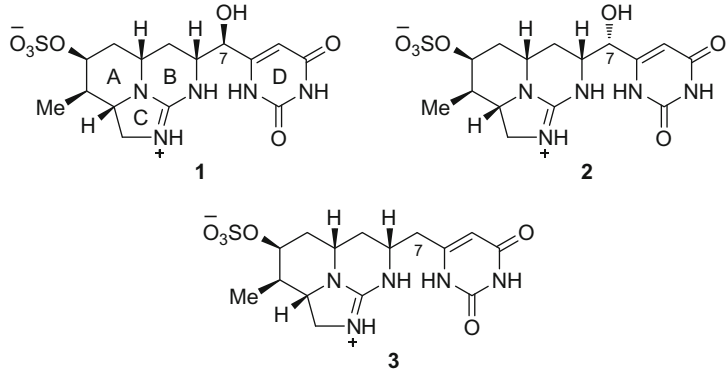


Figure 1 The cylindrospermopsin alkaloids cylindrospermopsin (**1**), 7-*epi*-cylindrospermopsin (**2**) and 7-*deoxy*-cylindrospermopsin (**3**).

in 1992 using mass spectrometry and a combination of 1D and 2D NMR techniques [3]. It was this toxin that was retrospectively identified as the causative agent of an outbreak of hepatoenteritis on Palm Island, Australia, 13 years earlier; the outbreak affected 148 inhabitants of the island with the large majority of these being children requiring hospital care [4]. The outbreak occurred a few days after the treatment of a dense algal bloom with copper sulfate on the town's water supply Solomon Dam. It is believed that the copper sulfate treatment commonly used to treat nuisance algal blooms caused the lysis of the cyanobacterial cells and the release of the toxin into the drinking water supply, although conjecture was raised that the illness the towns' folk were suffering from was in fact acute copper sulfate poisoning [5].

An intense interest surrounding this newly characterized toxin led to the discovery and isolation of 7-*deoxy*-cylindrospermopsin (**3**) which had been routinely observed by high-performance liquid chromatography (HPLC) during the isolation of cylindrospermopsin (**1**) from *C. raciborskii*, but had not been isolated and characterized until 1999 [6]. The group that isolated this compound believed that it exhibited the tautomeric forms of **4** and **5** as a vinylic proton at the uracil ring was not observed in the ^{1}H NMR spectrum of the isolated material (Figure 2). However, the absorbance maximum (λ_{max}) of **4** was consistent with the presence of a uracil group and through total synthesis it is most likely represented by structure **3** (Figure 1). Although it was clear that the natural material was a mixture of components, it was not possible to ascertain whether **3** was a minor component of that mixture [7].

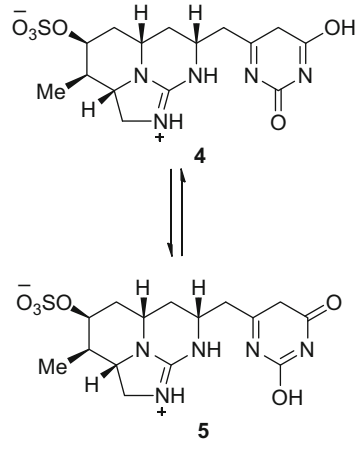


Figure 2 Proposed tautomeric forms of 7-*deoxy*-cylindrospermopsin (**3**).

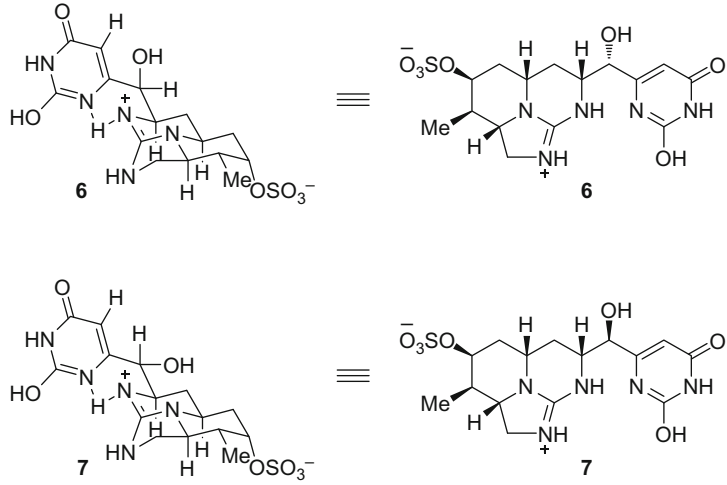


Figure 3 Proposed structures of cylindrospermopsin (**1**) and 7-*epi*-cylindrospermopsin (**2**).

The C-7 epimer of cylindrospermopsin (**1**), 7-*epi*-cylindrospermopsin (**2**) was initially found to be produced by a different species of cyanobacterium, *Aphanizomenon ovalisporum* in Israel in the year 2000 [8]. The initial assignment of the relative stereochemistry of cylindrospermopsin (**1**) with particular regard to the C-7 hydroxyl group was made on the basis that the uracil D ring existed as an unusual enol tautomer which was intramolecularly hydrogen bonded to a nitrogen terminus of the guanidine moiety as shown in structure **6**. In this configuration, the molecule satisfied the NMR evidence obtained by the group leading them to propose structure **7** as the most likely representation of cylindrospermopsin (**1**) (Figure 3). This in turn led to the belief that the C-7 epimer, 7-*epi*-cylindrospermopsin (**2**) displayed a conformation similar to **7** and thus was assigned the structure **6** (Figure 3). However, upon stereoselective total synthesis it became clear that the stereochemical nature of the C-7 hydroxy group was in fact reversed [9], nullifying the theory that these alkaloids existed as uracil tautomers and establishing with certainty that cylindrospermopsin (**1**) was accurately represented by **1** and it's epimer by structure **2** (Figure 1).

III. OCCURRENCE AND PRODUCTION OF CYLINDROSPERMOPSIN

Since the initial isolation of cylindrospermopsin (**1**) from *C. raciborskii* in 1992, several other species of cyanobacteria have been found to produce

the three known cylindrospermopsin alkaloids in varying quantities (Table 1). The table is as exhaustive as possible and includes all geographical regions where the presence of cylindrospermopsin alkaloids has been reported. The toxin has also been detected in Poland [10], although the species of cyanobacteria responsible for its production was not verified. There have also been successive isolations of these compounds in countries where their presence has already been verified but where the organism was not identified [11,12] or where more than one possible species was present [13,14].

IV. DETECTION TECHNIQUES

Cylindrospermopsin (**1**) and cyanobacteria capable of producing the toxin are prevalent throughout the world, making its detection and the detection of species capable of producing the toxin of paramount importance.

A. Chromatographic

1. HPLC-PDA

The use of high-performance liquid chromatography coupled with a photodiode array detector (HPLC-PDA) has been investigated as a detection method for the toxin [44]. The technique has been successful in identifying cylindrospermopsin (**1**) from lyophilized cyanobacterial cells in several different labs [45] and establishing the presence of the toxin in purified samples [29]. However, results from the analysis of environmental samples have been shown to suffer from a considerable matrix background effect. Peaks eluting close to cylindrospermopsin (**1**) and in some cases completely masking the signal of the toxin [44], as well as the presence of unknown compounds displaying similar UV spectra have been observed [38]. The extraction method employed in sample preparation has been shown to play a role in the presence of contaminant peaks [45], something that has been improved upon with the use of solid phase extraction (SPE) cartridges [46,47], and recently the use of a double cartridge system consisting of a styrene polymer cartridge and an anion exchange cartridge [48].

2. HPLC-MS and HPLC-MS^2

Kaya and co-workers have demonstrated the use of 2-(4-(2-hydroxyethyl)piperazin-1-yl)ethanesulfonic acid (HEPES) as an internal standard in HPLC-MS, allowing for the successful determination of the cylindrospermopsin (**1**) content of lyophilized *C. raciborskii* cells with a quantitative limit of 0.16 ng [49]. Single stage mass spectrometric determination has also been coupled with hydrophilic interaction liquid

Table 1 Species and reported locations of the cylindrospermopsin alkaloids

Cyanobacterial species	Location	Year	Metabolite			Concentration	Reference
			1	**2**	**3**		
Cylindrospermopsis raciborskii	Australia	1992	Yes			Not reported	[3]
		1997	Yes			5.5 mg g^{-1}[a]	[15]
		1999	Yes		Yes	Not reported	[6]
		2000[b]	Yes			3.4 μg L^{-1}	[16]
		2009[c]	Yes		Yes	**1** 38.2 μg L^{-1} **3** 42.2 μg L^{-1}	[17]
	New Zealand	2001	Yes			Not reported	[18]
		2001	Yes			1–2 μg L^{-1}	[19]
		2003	Yes		Yes	Not reported	[20]
	Thailand	2001	Yes		Yes	**1** 1.02 mg g^{-1}[a]	[21]
		2004	Yes			Not reported	[22]
	Germany	2003	Yes			49–741 μg g^{-1}[a]	[23]
	Japan	2004	Yes			Not reported	[22]
	Italy	2005	Yes			0.46–15 ng mL^{-1}	[24]
		2010[c]	Yes			2.6–126 μg L^{-1}	[25]
	Egypt	2007[d]				Not reported	[26]
Cylindrospermopsis	Brazil	2001	Yes			Not reported	[27]
	Mexico	2010[e]	Yes			21.34±2.00 ng L^{-1}[f]	[28]
Umezakia natans	Japan	1994	Yes			Not reported	[29]
Aphanizomenon ovalisporum	Israel	1997	Yes			Not reported	[30]
		2000		Yes		Not reported	[8]
	Australia	1999	Yes			4–120 μg L^{-1}	[31]

	Spain	2007	Yes			1.5–9.4 μg L^{-1}	[32]
	USA	2008	Yes			7.39–9.33 μg mg^{-1}[a]	[33]
	Australia	2009[c]	Yes		Yes	**1** 38.2 μg L^{-1} **3** 42.2 μg L^{-1}	[17]
	Italy	2010[c]	Yes			2.6–126 μg L^{-1}	[25]
Aphanizomenon	Germany	2007	Yes			0.08–11.75 μg L^{-1}	[34]
	Czech Republic	2009	Yes			0.01–0.14 μg L^{-1}	[35]
Raphidiopsis curvata	China	2001	Yes		Yes	**1** 0.56 μg g^{-1}[a] **3** 1.3 mg g^{-1}[a]	[36]
Anabaena bergii	Australia	2001	Yes			Not reported	[37]
Anabaena lapponica	Finland	2006	Yes			242 μg g^{-1}[a]	[38]
Aphanizomenon flos-aquae	Germany	2006	Yes			2.3–6.6 mg g^{-1}[a]	[39]
Lyngbya wollei	Australia	2007	Yes		Yes	**1** 20 μg g^{-1}[a] **3** 550 μg g^{-1}[a]	[40]
Anabaena planctonica	France	2009[g]	Yes			1.88–1.95 μg L^{-1}	[41]
Oscillatoria sp.	Various	2010	Yes	Yes	Yes	**1** 0.13–0.72 μg mL^{-1} **2** 0.01–0.69 μg mL^{-1}	[42]
Raphidiopsis mediterranea	Australia	2011	Yes		Yes	**1** 917 μg g^{-1}[a] **3** 1065 μg g^{-1}[a]	[43]

[a]Based on dry weight of cyanobacterial cells.
[b]Median toxin concentration of 14 sampled lakes.
[c]More than one cylindrospermopsin producers co-existed in the same water body.
[d]Shown to be hepatotoxic by mouse bioassay.
[e]Two dominant species, *Cylindrospermopsis catemaco* and *Cylindrospermopsis philippinensis* were present in the water body.
[f]Determined by ELISA.
[g]In the presence of other potential cylindrospermopsin producers.

chromatography (HILIC-MS) and been shown to be an effective and efficient method for the detection of cylindrospermopsin (**1**) even in the presence of other cyanobacterial toxins, with samples requiring no clean up or preconcentration step [50].

HPLC tandem mass spectrometry (HPLC-MS^2) was initially investigated by Norris and co-workers, who accurately and reproducibly detected the presence of cylindrospermopsin (**1**) at concentrations as low as 1 $\mu g\ L^{-1}$, allowing for the direct detection of the toxin at low concentrations in surface water samples [51]. The sensitivity of this technique was further improved upon by Sonzogni and co-workers who were able to detect cylindrospermopsin (**1**) at concentrations as low as 0.5 $\mu g\ L^{-1}$ in directly injected aqueous samples through the use of mass filtering capacity to reduce background noise [52]. The further addition of ion trap analysis has resulted in the lowest published limits of quantification of 0.10 ng mL^{-1} and 1.0 ng g^{-1} for detecting the toxin in surface water and fish muscle, respectively, which is hoped, will prove useful in monitoring early stages of contamination and bioaccumulation [12]. Ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS^2) has also been applied for the detection of both cylindrospermopsin (**1**) and deoxycylindrospermopsin (**3**) [53]. The technique has been expanded to detect 10 different cyanotoxins from three distinct groups, namely, microcystins, anatoxin-a, and cylindrospermopsin (**1**) at 5 ppm without sample clean up by Westrick and co-workers [54]. Intriguing work by Pinto and co-workers investigated the different fragmentation pathways for protonated and cationic cylindrospermopsin (**1**), observing that the differential ion mobility of group 1A metals leads to different fragmentation pathways, giving rise to mass spectra with different profiles. This is of great importance when analyzing the toxin in matrices with a high metal content and will prove useful in the future identification of cylindrospermopsin derivatives or metabolites [55].

B. Biological Assays

The traditional assay used for cyanobacterial toxin detection is the mouse bioassay. While offering a better indication of toxicity this assay suffers from difficulties such as time constrains in the interpretation of the results and ethical considerations [56,57]. These difficulties have led to the investigation of alternative bioassays; recently the use of the speckled cockroach (*Nauphoeta cinerea*) has been investigated. Unfortunately this species was found to be highly tolerant to cylindrospermopsin (**1**) but has shown promise for the detection of the neurotoxin saxitoxin (**13**) [58]. The inhibition of tobacco pollen germination by cylindrospermopsin (**1**) has demonstrated potential as a bioassay for the toxin, although a preconcentration step would be required for the monitoring of environmental samples [59]. Another plant-based test has demonstrated a concentration-dependent

inhibition in the growth of etiolated mustard plant seedlings (*Sinapis alba*), with regard to cylindrospermopsin (**1**). The IC_{50} of seedling growth was observed at 18.2 μg mL^{-1}. This assay was used in conjunction with gel electrophoresis to separate and investigate fractions of interest from the cyanobacterial culture [60]. The combination of other electrophoresis techniques, namely, capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) have simultaneously resolved environmental samples containing mixtures of three cyanotoxins including cylindrospermopsin (**1**) using UV detection [61]. The use of commercially available test kits based on the rabbit reticulocyte translation system, the brine shrimp (*Artemia salena*), and the larvae of the beavertail fairy shrimp (*Thamnocephalus platyurus*) have all been shown to quickly and cost-effectively detect cylindrospermopsin (**1**) with comparable sensitivity to HPLC analysis [62–64]. Similar sensitivity has also been reported using cell substrate impedance sensing (ECIS) and the *Chinese hamster ovary* (CHO) cell line where an $ECIS_{50}$ of ~2 μg mL^{-1} was observed after 20 h [65].

Enzyme-linked immunosorbent assay (ELISA) test kits are commercially available for several cyanotoxins, providing highly sensitive rapid real-time testing. A recent publication by Bláha and co-workers compared this immunochemical method of detection with conventional HPLC-MS methods. The group observed good qualitative agreement between the two tests but quantitatively the ELISA determination was 10 times higher than the concentrations determined chromatographically. The group postulated that this may be the result of cross reactivity between the antibodies of the test kit and unidentified cylindrospermopsin congeners or isomers present in the sample [35]. Discrepancies in toxin concentration between these two techniques have also been observed by two other research groups [33,66].

Saint *et al* have shown a long standing interest in developing a polymerase chain reaction (PCR) assay capable of detecting cylindrospermopsin (**1**) producing cyanobacterial species [67–69]. The group's initial investigations concluded with the production of a PCR test capable of detecting *C. raciborskii*. While being more accurate than the morphological identification of cyanobacteria, it did not differentiate between strains based on their toxin producing capabilities [67]. An ensuing publication detailed the further development of a multiplex PCR assay that took less than one day to perform and was capable of distinguishing *C. raciborskii* from other cyanobacterial strains capable of producing cylindrospermopsin (**1**) [68]. Finally, the most recent publication by the group adapted their multiplex PCR assay [68] into a real-time assay that was capable of rapidly detecting cylindrospermopsin (**1**) producing cyanobacteria both in the lab and in the field [69]. Real-time PCR analysis has also been used by Orr et al. to investigate toxic *C. raciborskii* blooms in three Australian lakes [70].

V. WATER QUALITY

Toxins produced by cyanobacteria [71] are a prevalent problem [72,73], one which is likely to be intensified by the effects of climate change [74]. There are several routes of exposure to such toxins [75,76] with the main being consumption of tainted drinking water [77]. Traditional methods of water treatment such as flocculation, sedimentation, and filtration have been shown to be successful in the removal of cyanobacterial cells but ineffective in removing dissolved cyanobacterial toxins [78–80]. Unfortunately these techniques may even facilitate the release of intracellular material leading to higher observed toxin concentrations [80,81].

The concentration of cylindrospermopsin (**1**) in water bodies varies throughout the world with reported toxin concentrations ranging from 0.01 to 126 $\mu g\ L^{-1}$, the highest of these being observed in Italy in 2010 [25,35]. The effects of light and temperature gradients [82] as well as nutrient composition on toxic bloom growth and cylindrospermopsin (**1**) production have been investigated [83,84] along with the seasonal dynamics of this process [85]. Recently, Everson et al. have reported the varying concentration of cylindrospermopsin with depth in a toxic bloom involving both *A. ovalisporum* and *C. raciborskii* and the issues this discovery poses for drinking water quality [86]. A link between cell division and toxin production during the fast growth phase has also been observed during batch culture [87]. Saker and Neilan found that *C. raciborskii* growth rates were lowest in the absence of a fixed-nitrogen source, but it was under these conditions that the highest concentrations of cylindrospermopsin (**1**) were observed [83]. Deprivation of sulfate and phosphate has also been shown to reduce the amount of cylindrospermopsin (**1**) produced with the effects being most prevalent in a sulfate-starved medium [84]. Recently, an intriguing and novel allelopathic effect of cylindrospermopsin (**1**) has been observed, and the release of the toxin has been shown to cause the release of phosphatase enzymes in neighboring phytoplankton species in order to generate inorganic phosphate that can in part be used by the toxin producer [88,89].

The need for further toxicological studies in order to determine a safe guideline value for the presence of the toxin in drinking water and the difficulties that need to be overcome in this regard have been highlighted by Falconer and Humpage [90]. However, recognizing the threat this metabolite poses to human safety some countries have already employed guideline values for its presence in drinking water; these include Brazil (15 $\mu g\ L^{-1}$), New Zealand (2.0 $\mu g\ L^{-1}$), and European Union (0.1 $\mu g\ L^{-1}$) [91]. Research to determine a definitive safe water quality concentration is still ongoing with a practical guideline value of 1.0 $\mu g\ L^{-1}$ recently being proposed [92]. Exposure to cylindrospermopsin (**1**) and cylindrospermopsin-producing *C. raciborskii* has

also been shown to cause irritant and hypersensitivity reactions, which is a concern for recreational water use [93].

A. Chemical Methods

1. Chlorination (ClO_3^-, ClO_2, NH_2Cl)

Chlorination is a common process used to disinfect drinking water and as such its effect of cyanotoxins has been greatly investigated [94–96]. Initial work by Senogles et al. showed that the addition of free chlorine in the form of sodium hypochlorite was successful in removing pure cylindrospermopsin (**1**) at concentrations of up to 185 $\mu g\ L^{-1}$. A greater concentration of chlorine (4 $mg\ L^{-1}$) was needed to remove cylindrospermopsin (**1**) at concentrations up to 130 $\mu g\ L^{-1}$ when cell-free algal extract was used [97]. These findings are mirrored in recent work that observed 100% inactivation of *C. raciborskii* at chlorine concentrations as low as 4 $mg\ L^{-1}$. Interestingly, this oxidation did not lead to detectable levels of cylindrospermopsin (**1**) in solution [98]. The efficiency of degradation using free chlorine has been shown to decrease with pH, with optimal results being obtained at approximately pH 7, where the half-life of cylindrospermopsin (**1**) has been shown to be 1.7 min [94,97,99]. Currently, the chemical structures of two chlorination by-products have been elucidated, 5-*chloro*-cylindrospermopsin (**8**) and cylindrospermic acid (**9**) (Figure 4) [100]. In addition a new as yet uncharacterized by-product of cylindrospermopsin (**1**) chlorination has been observed, having the molecular formula $C_{13}H_{18}N_4O_7S$ [101].

Of great concern is the toxicity of the generated oxidation products. Banker et al. determined that both 5-*chloro*-cylindrospermopsin (**8**) and cylindrospermic acid (**9**) were virtually nontoxic by mouse bioassay, even at high doses of up to 10 $mg\ kg^{-1}$ intraperitoneally (IP). All study participants survived with no physiological or pathological toxic symptoms being observed within 10 days of treatment [100]. However, further work by Senogles-Derham et al. observed fatty vacuolation in 40% of male mice treated with water containing cylindrospermopsin

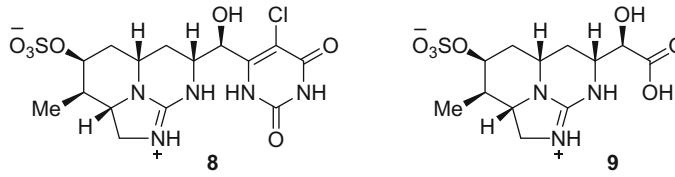


Figure 4 Characterized cylindrospermopsin chlorination by-products 5-*chloro*-cylindrospermopsin (**8**) and cylindrospermic acid (**9**).

chlorination products over a 170-day period, highlighting the need for further studies to investigate this phenomenon [102].

The effectiveness of the less powerful chlorinated oxidants, chlorine dioxide, and monochloramine in the removal of cylindrospermopsin (**1**) has also been investigated [94,98–100]. Treatment with ClO_2 at varying concentrations resulted in no removal of the toxin [98,99], although an almost 75% inactivation of *C. raciborskii* with no release of cylindrospermopsin (**1**) into the environment was observed [98]. A high level of resistance to oxidation was also observed with monochloramine, even at elevated concentrations. Similarly to treatment with chlorine dioxide, partial elimination of *C. raciborskii* was observed [98]. Cylindrospermopsin (**1**) has been shown to have a half-life of 14.4 h in the presence of ClO_2; however, this value increases when the oxidant is monochloramine [94].

2. Ozonolysis

Ozone has been shown to deactivate cylindrospermopsin (**1**) through the oxidation of the double bond and amino moieties of its structure [103]. The half lives of the toxin have been shown to be 0.1 s and 10.5 min when exposed to ozone and hydroxyl radicals, respectively, at concentrations of 1 mg L^{-1} [94]. Doses of 0.4 mg L^{-1} were needed for 95% oxidation of the toxin in water having a dissolved organic carbon (DOC) content of 1.6 mg L^{-1}; when the concentration of DOC increased to 13.1 mg L^{-1} dosing in excess of 2 mg L^{-1} was required for the same level of toxin removal [103]. Exposure of 1 mg L^{-1} min^{-1} has also been seen to cause complete inactivation of *C. raciborskii*, combined with no observable buildup of the toxin in solution, either as it is not released from the cyanobacterial cells or that it is almost instantly oxidized. Therefore, treatment with ozone at the tested levels poses a highly effective method for the removal of cylindrospermopsin (**1**) and the disinfection of the producing cyanobacterial species [98].

3. Permanganate (MnO_4^-)

Permanganate is used as an alternative oxidant in the drinking water treatment process, and thus has not been as rigorously investigated for its ability to successfully remove cylindrospermopsin (**1**) [94,98,99]. It was observed that the reactivity of permanganate toward cylindrospermopsin (**1**) was very similar over the pH range investigated (pH 6–8), with the positive effect of increased temperature on the second-order rate constant [99]. The half-life of cylindrospermopsin (**1**) has been seen to be 4.2 days in the presence of 1 mg L^{-1} permanganate, with a dose of 1.5 mg L^{-1} only being effective at removing 10% of the toxin; however, this level of dosing was shown to fully remove other cyanobacterial toxins such as microcystin-LR (**10**) and anatoxin-a [94]. Recent work by Cheng et al. displayed similar findings, allowing the group to conclude

that the use of permanganate at typical drinking water treatment exposure doses is ineffective for the removal of cylindrospermopsin (**1**). Partial inactivation of *C. raciborskii* was observed under these conditions without the buildup of toxin in solution [98].

B. Chemical-free Alternatives

1. Photodegradation

The photodegradation of cylindrospermopsin (**1**) by sunlight was initially investigated by Chiswell et al. The group observed that algal extracts containing low concentrations of toxin degraded rapidly with a half-life of only 1.5 h, whereas pure cylindrospermopsin (**1**) in dam water containing natural plant pigments had a much greater half-life of 11−15 days [104]. The most recent work regarding the photodegradation of cylindrospermopsin (**1**) has shown that the process is highly dependent on UV-A radiation (320−400 nm), although the degradation was very low under natural conditions with only 27.3±4.35% of the toxin being degraded over the 22-day study period [105].

UV light is commonly used in the water treatment process, and as such its ability to degrade cylindrospermopsin (**1**) has also been investigated [98,103]. The most recent work details the degradation of both the toxin and the producing species of cyanobacteria (*C. raciborskii*); however, this was only achieved at levels of UV radiation much greater than those used in water treatment disinfection, making UV irradiation an ineffective method for the removal of cylindrospermopsin (**1**) [98]. Titanium dioxide (TiO_2) has been shown to be an effective photocatalyst for the degradation of cylindrospermopsin (**1**) with UV light. In the presence of 0.1 g L^{-1} TiO_2, the half-life of the toxin was shown to be only 0.7 min [106].

2. Bioremediation

Significant interest has been expressed toward the exploitation of microbes for the removal of cyanobacterial toxins from water supplies, in the hope of providing a green alternative to the currently employed chemical techniques [107,108]. A recent 40-day study by Quesada and co-workers investigated the effect of naturally occurring bacteria on cylindrospermopsin (**1**); disappointingly, no toxin degradation was observed on the different toxin sources used [109]. Interestingly, the biodegradation of cylindrospermopsin (**1**) has been observed in water bodies that have had a history of toxic *C. raciborskii* blooms; however, the degradation was shown to be concentration dependant suggesting the existence of a threshold toxin concentration that is needed to induce degradation [110].

The use of the large ciliate *Paramecium caudatum* as a biological control agent for the removal of cylindrospermopsin (**1**) producing *C. raciborskii* has been investigated by Fabbro et al. The group observed that the eukaryote was capable of consuming over 100 cyanobacterial cells per animal per hour and that the toxin concentration was reduced as a result of the grazing process, suggesting that this grazer may be an effective control measure against toxic cylindrospermopsin (**1**) producing cyanobacterial strains [111]. The ability of several different strains of probiotic bacteria to remove cylindrospermopsin (**1**) has also recently been investigated, the most efficient species was found to be *Bifidobacterium longum* 46, being capable of removing 31.6% of cylindrospermopsin (**1**) over a 24-h period, demonstrating efficient cyanotoxin removal [108].

3. Interval Immobilization

The interval immobilization technique represents a novel molecular recognition method capable of recognizing a target molecule based on the "interval" or distance between its ionic functional groups. Hosoya and co-workers have prepared a cross-linked polymer molecularly imprinted with a template molecule having a similar ionic interval to cylindrospermopsin (**1**), and demonstrated by HPLC and Scatchard analysis that it accurately recognizes cylindrospermopsin (**1**), and thus confirmed the utility of this technique as a possible water purification tool [112,113]. Currently, the group is applying this methodology to other cyanobacterial toxins [114].

4. Nanofiltration

The use of nanofiltration membranes for the removal of a variety of cyanotoxins including cylindrospermopsin (**1**) has proven to be of great interest [115–118]. Dixon et al. investigated the effectiveness of six different molecular weight cut-off (MWCO) membranes for the removal of the toxin. They determined that nanofiltration membranes with a MWCO of 100 Da were capable of removing between 90% and 100% toxin at concentrations of 16 $\mu g\, L^{-1}$ [117]. A follow-up study by the group further investigated four nanofiltration membranes with two different water sources, one had undergone ultrafiltration and the other had been subjected to conventional water treatments of flocculation, dissolved air flotation, and sand filtration. They found that the membranes having a MWCO of 300 Da or less were effective in removing between 90% and 100% toxin from both water sources, demonstrating the feasibility of this approach for water treatment [118].

5. Adsorption

The sorption and degradation of cylindrospermopsin (**1**) by sediments has recently been investigated [119,120]. Work by Klitzke et al.

determined that cylindrospermopsin (**1**) adsorption to silty sediments was negligible; however, the sediments did prove effective in degrading this toxin. The use of preconditioned sediments proved most effective for toxin degradation that could be achieved in 4.7 days, with degradation time increasing to 8 days with the inclusion of reservoir water enriched with dissolved organic carbon (DOC) [119]. Further work by this group indicated that the organic carbon content of sediments was the main parameter in determining cylindrospermopsin (**1**) adsorption, e.g. organic mud (44.5% organic carbon) showed the greatest adsorption of the toxin (0−360.5 $\mu g\,kg^{-1}$), although desorption is likely if the organic matter is being decomposed [120].

Another adsorption medium that has received attention regarding its ability to remove cyanobacterial toxins from drinking water is powdered activated carbon (PAC) [121−124]. The ability of two different PAC's to successfully remove cylindrospermopsin (**1**) (20 $\mu g\,L^{-1}$) from raw ultra filtered water has recently been investigated. PAC doses required to lower toxin levels to below 1 $\mu g\,L^{-1}$ varied from 25 to 50 $mg\,L^{-1}$ depending on the water source used, demonstrating the effectiveness of this adsorbent, although relatively high doses would be required [121]. These findings are corroborated by the group's earlier work, which concluded that the removal of cylindrospermopsin (**1**) with PAC is largely dependent on the DOC content of the feed water used [124].

VI. BIOACCUMULATION

The bioaccumulation of cylindrospermopsin (**1**) is a very expansive topic which is beyond the scope of this review, an excellent article regarding this area of cylindrospermopsin toxicity has been written by Kinnear [125].

VII. TOXICITY

The toxins produced by cyanobacteria represent a structurally diverse collection of natural products including hepatotoxic cyclic peptides, such as microcystin-LR (**10**) as well as an array of alkaloids ranging from neurotoxic saxitoxin (**11**) and anatoxin-a (s) (**12**) to the dermatotoxic tumor promoter lyngbyatoxin (**13**) (Figure 5).

Cylindrospermopsin (**1**) is a potent hepatotoxin produced by freshwater cyanobacterial species and has been implicated in the death of livestock [126,127] as well as an outbreak of hepatoenteritis on Palm Island, Australia, which led to its discovery [3,4]. The toxic modes of

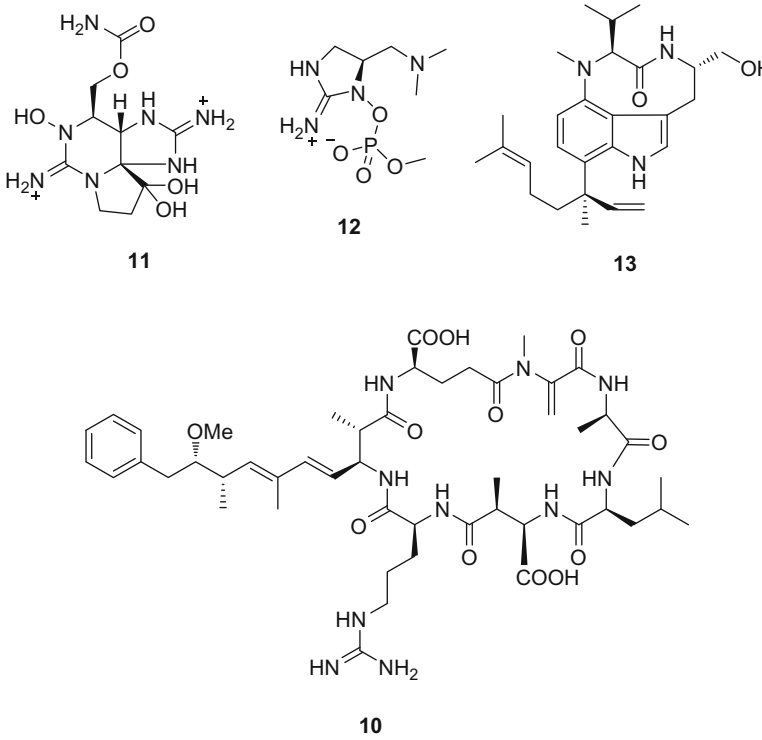


Figure 5 Related prominent cyanobacterial toxins microcystin-LR (**10**), saxitoxin (**11**), anatoxin-a (s) (**12**), and lyngbyatoxin (**13**).

action of cylindrospermopsin (**1**) are not fully understood but the metabolite has been shown to affect several organ systems, primarily the liver but also the kidneys, thymus, heart, and spleen [56,128,129]. The toxin has also been shown to elicit irritant and hypersensitivity reactions [93] and as a result is under continuing research due to public health implications. The potency of the toxin has been quantified, with several LD_{50} values being reported from varying toxin isolates and exposure routes in mouse models (Table 2).

A. Cytotoxicity

1. In Vitro

As a result of the hepatic symptoms initially displayed by cylindrospermopsin (**1**) exposure, some of the first cell lines used to investigate the cytotoxicity of this alkaloid were primary hepatocytes derived from mice and rat livers [23,130–134]. Initial findings by Runnegar et al. demonstrated that treatment of cultured rat hepatocytes with

Table 2 Reported half lethal dose values for cylindrospermopsin and its derivatives

Metabolite	LD_{50}	Time	Toxin Type	Dosing Method	Reference
1	64 mg kg^{-1}	24 h	Cell-free algal extract	IP	[57]
1	116 mg kg^{-1}	24 h	Crude extract	IP	[30]
1	50–110 mg kg^{-1}	24 h	Cell-free algal extract	IP	[129]
	20–65 mg kg^{-1}	7 days			
1	52 mg kg^{-1}	24 h	Cell-free algal extract	IP	[15]
	32 mg kg^{-1}	7 days			
1	4.4–6.9 mg kg^{-1}	2–6 days	Alkaloid equivalent	Oral	[56]
2	200 μg kg^{-1}	5 days	Pure	IP	[100]
3	>800 μg kg^{-1}	5 days[a]	Pure	IP	[6]
8	>10,000 μg kg^{-1}	5 days[a]	Pure	IP	[100]
9	>10,000 μg kg^{-1}	5 days[a]	Pure	IP	[100]

[a]No toxicity observed.

2.2–5.0 μM of cylindrospermopsin (**1**) caused significant cell death (40–67%) with toxin exposure above 10 μM essentially causing complete cell death within 18 h. It was also observed that toxicity was preceded by a decrease in cell-reduced glutathione (GSH), and that lowering cell glutathione predisposed cells to cylindrospermopsin toxicity [130]. A follow-up study led the group to conclude that this was the result of the inhibition of glutathione synthesis and not a result of increased consumption [131]. Similar depletions in GSH levels were observed in primary mouse hepatocytes by Humpage and co-workers; however, even treatment with cylindrospermopsin (**1**) at concentrations up to 5 μM did not elevate levels of lipid peroxidation leading them to conclude that reactive oxygen species (ROS) are not involved in cylindrospermopsin (**1**) toxicity [133]. The involvement of cytochrome P450 (CYP450) in the metabolism of cylindrospermopsin (**1**) was first investigated by Runnegar et al. who found that treatment with the CYP450 inhibitor α-naphthoflavone partially protected from cylindrospermopsin (**1**) mediated toxicity and from the fall of cell GSH [131].

Significant concentration-dependant cytotoxicity has also been observed toward primary mouse hepatocytes at cylindrospermopsin (**1**) concentrations ranging from 1 to 5 μM causing between 52% and 82% cell death. Again treatment with the potent CYP450 inhibitors proadifen and ketoconazole at 50 μM diminished the cytotoxicity of cylindrospermopsin (**1**), but did not affect the toxins ability to inhibit protein synthesis conferring the involvement of CYP450 metabolites in its cytotoxicity, while highlighting the importance of protein synthesis inhibition in cells deficient in CYP450 enzymes [132]. Interestingly, the group also observed that the inhibition of protein synthesis caused by toxin exposure was irreversible, even after toxin removal [132]. A similar investigation by Humpage and co-workers determined the EC_{50} of cylindrospermopsin (**1**) toward primary mouse hepatocytes to be 0.47 μM after 18 h. They also observed a diminished cytotoxic effect from cylindrospermopsin (**1**) in day-old cells, as primary hepatocytes are known to lose their CYP450 metabolizing capabilities in culture; this supports earlier findings that CYP450 enzymes are involved in the metabolic activation of the toxin [133]. Further exploring the involvement in CYP450 enzymes they also investigated the effectiveness of several CYP450 inhibitors to protect against cylindrospermopsin (**1**) induced cytotoxicity. Omeprazole (100 μM) was found to be the most effective being approximately twice as effective as proadifen and ketoconazole [133].

Cylindrospermopsin (**1**) has also demonstrated toxicity toward a number of other mammalian cell lines. Methanolic extracts of German strains of *C. raciborskii* have been shown to be toxic to human hepatoblastoma (HepG2) and human adenocarcinoma (Caco-2) cell lines with LC_{50} values ranging from 0.3 to 1.3 mg DW mL^{-1} [23]. Froscio et al.

investigated the cytotoxicity of cylindrospermopsin (**1**) toward seven different mammalian cell lines, six of which were human derived using the MMT cytotoxicity assay. They found that the most sensitive cell lines were human hepatocellular carcinoma C3A and HepG2 cells, which after exposure to 0.25 μM of cylindrospermopsin (**1**) for 7 days showed a reduction of 70% in cell viability with exposure to toxin concentrations of 2.5 μM and above displaying a progressive loss of viability before almost complete cell death was induced [135]. Cylindrospermopsin (**1**) has also been found to be toxic toward human granulose cells, treatment with 1 μg mL^{-1} toxin led to a 59% reduction in cell viability after 24 h, the decreased toxicity observed compared to primary hepatocytes was rationalized by the difference in the CYP450 profile of the granulose cell line [136]. It was also noted that after 6 h this dose caused no cytotoxicity but did display an inhibitory effect on estrogen production, suggesting that cylindrospermopsin (**1**) has the potential to be an endocrine distributor by changing the progesterone: estrogen ratio in women [136]. Toxicity studies have shown that cylindrospermopsin (**1**) can induce effects in a wide range of different cell types; primary hepatocytes have been shown to be the most sensitive; however, the way in which this toxin enters the intracellular environment still remains unclear [137]. The bile acid transport system has been implicated in the uptake of the toxin in primary hepatocytes, although another mechanism, possibly passive diffusion as a result of the toxins low molecular weight may be involved [134]. Kiss et al. have also shown that material purified by HPLC matching the reported data for cylindrospermopsin (**1**) displays a neurotoxic effect toward the neurons of two snail species studied [138].

Only recently has work detailing the toxic effects of cylindrospermopsin (**1**) in fish been reported. Pichardo and co-workers utilized the fish liver-derived PLCH-1 cell line, to which cylindrospermopsin (**1**) was shown to be highly cytotoxic, toward MTS assay having EC_{50} values of 8.0 and 2.6 ng mL^{-1} for 24 and 48 h exposure, respectively. Cells exposed to cylindrospermopsin (**1**) also displayed a concentration- and time-dependant increase in the inhibition of protein synthesis [139]. Levels of GSH also decreased with increasing cylindrospermopsin (**1**) exposure, this was combined with an increase in the enzyme activity of γ-glutamylcysteine synthase and the levels of ROS, suggesting an involvement of ROS in cylindrospermopsin (**1**) cytotoxicity [139] contrary to earlier observations by Humpage and co-workers [133]. Filipak Neto and co-workers have also used *Prochilodus lineatus* derived hepatocytes to investigate cylindrospermopsin (**1**) toxicity *in vitro*. They observed a significant decrease in cell viability at toxin concentrations of 0.1 and 1.0 μg L^{-1} with toxin exposure at the highest dose of 10 μg L^{-1} causing an increase in reactive oxygen and nitrogen species

combined with an increase in lipid peroxidation, further suggesting that at least in this hepatocyte model ROS species play a role in cylindrospermopsin (**1**) toxicity [140].

Further evidence of the ability of cylindrospermopsin (**1**) to inhibit protein synthesis *in vitro* has been reported by Froscio et al. who observed that mice hepatocyte cultures exposed to the toxin at concentrations between 0.5 and 5.0 μM demonstrated inhibition of protein synthesis between 74% and 88%, with inhibition being maximal at 4 h [133]. Further work by Froscio et al. established that cylindrospermopsin (**1**) displayed a similar IC_{50} value for protein synthesis inhibition in both plant and animal extracts being 334 nM in wheat germ extract and 110 nM in rabbit reticulocyte lysate assay. Using ^{14}C-labeled cylindrospermopsin (**1**) they found that the toxin does not target the ribosome but possibly targets a high molecular weight protein associated with the eukaryotic protein translation system. They found that ^{14}C labeled toxin could be displaced by treatment with an excess of its unlabeled equivalent, meaning that although a strong association to the binding site is formed the link is not covalent [141]. Cylindrospermopsin (**1**) has also been shown to inhibit protein synthesis in pollen from the tobacco plant at concentrations between 5 and 100 μg mL^{-1} [59]. Treatment with 41.5 μg mL^{-1} of cylindrospermopsin (**1**) has also been shown to inhibit protein synthesis in *E. coli* 70S extracts by 25% compared to the control [142].

Another aspect pertinent to the toxicity of this compound is the effects it solicits on plant life; however, to our knowledge only one study has investigated the effect of cylindrospermopsin (**1**) on plant life *in vitro* [143]. Máthé and co-workers utilized plantlets cultured from the common reed (*Phragmites australis*) which demonstrated a decrease in root elongation and an increase in root number upon exposure to cylindrospermopsin (**1**). Treatment with toxin concentrations above 5 μg mL^{-1} led to the formation of a callus-like tissue and necrosis in the root cortex; the group attributed these alterations in root growth to the alteration of microtubule organization [143].

Attempts to equate the toxic action of cylindrospermopsin (**1**) to parts of the compounds molecular structure have been undertaken, initial findings by Sukenik indicate that the uracil moiety of the toxin is partially responsible for its potent biological activity, reasoning competitive or inhibitory binding to a catalytic site [100]. Runnegar et al. further probed the importance of the various functionalities of cylindrospermopsin (**1**), investigating the biological properties of cylindrospermopsin diol **14** and 7-*epi*-cylindrospermopsin diol **15** as well as a host of synthetic model compounds, the AB model **16**, uracil model **17**, and the functionalized AC model **18** (Figure 6) [144].

On comparing the toxicity of racemic synthetic cylindrospermopsin (±**1**) to that of native 7-*epi*-cylindrospermopsin (**2**) they concluded that

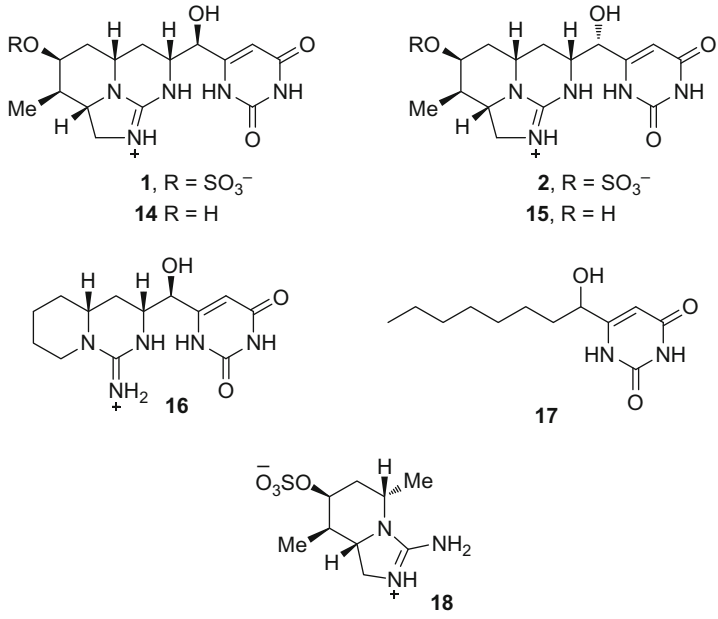


Figure 6 Analogs used to investigate the biological properties of cylindrospermopsin.

the orientation of the hydroxyl group at C-7 had no impact on the biological activity or transport of toxin. They also compared cylindrospermopsin (±**1**) and 7-*epi*-cylindrospermopsin (**2**) to their corresponding diols, compounds ±**14** and **15**, they found that **1** and the corresponding diol ±**14** both inhibited protein synthesis with comparable IC_{50} values of 0.20 and 0.21 μM. In addition, 7-*epi*-cylindrospermopsin (**2**) and the corresponding diol **15** both depleted cell GSH by similar amounts, leading them to conclude that the sulfate group does not play a significant role in the biological activity or uptake of the toxin. The AB model compound ±**16** also displayed an inhibitory effect on protein synthesis but at concentrations 500–1000-fold higher than natural cylindrospermopsin (**1**), while the uracil ±**17** and AC model ±**18** compounds lacked biological activity with no inhibitory effect on protein synthesis being observed at concentrations up to 800 and 2000 μM, respectively. These finding suggest that an intact tricyclic ABC ring system is crucial for the biological activity of these compounds [144]. Shaw and co-workers investigated the toxicity of 7-*deoxy*-cylindrospermopsin (**3**) toward four mammalian cell lines. For all the cell lines tested, clear dose–response curves were obtained with 7-*deoxy*-cylindrospermopsin (**3**) being at least as potent as cylindrospermopsin (**1**) as determined by the MTS cell proliferation assay. Both compounds were also shown to inhibit protein

synthesis to a similar degree with cylindrospermopsin (**1**) and 7-*deoxy*-cylindrospermopsin (**3**) having IC_{50} values of 340 and 220 nM, respectively [145]. Williams and co-workers also found that 7-*deoxy*-cylindrospermopsin (**3**) exhibited levels of protein synthesis inhibition within one order of magnitude to that of cylindrospermopsin (**1**), while displaying a similar inhibitory effect on cell GSH [7].

2. In Vivo

The mouse bioassay has revealed that cylindrospermopsin (**1**) exerts toxicity on a range of organs, primarily the liver but also the kidneys, thymus, heart, and spleen [56,128,129]. Initial work by Harada showed that there were four distinct pathological stages observed in the liver of exposed mice, the first was the inhibition of protein synthesis, the second being the inhibition of membrane proliferation, and the third phase being fat droplet accumulation finally leading to cell death [128]. To further understand the metabolism of cylindrospermopsin (**1**), Norris et al. investigated the distribution of ^{14}C-labeled toxin in the mouse model, treating mice with 0.2 mg kg^{-1} intraperitoneally. The majority of cylindrospermopsin (**1**) (65.2%) was found to be excreted in the first 12 h in the urine, with significant excretion *via* the fecal route (8.1%) being observed in some individuals. After 6 h, accumulation of the toxin was primarily observed in the liver (20.6%) and to a lesser extent the kidneys (4.3%). The loss of some radio-labeled material was observed in the protein precipitate upon methanol extraction suggesting the presence of a protein-bound metabolite, which could be a result of metabolic activation. The excretion patterns observed by the group showed a substantial degree of interindividual variability; however, the data obtained did not show a simple relationship between this and toxin response [146].

Mice exposed to drinking water containing cylindrospermopsin (**1**), where the daily consumption was in the range of the previously proposed no observed adverse effect level (NOAEL) of 30 μg kg^{-1} day^{-1} [92] for three weeks, displayed abnormal red blood cell formation known as acanthocytosis that is associated with a significant increase in the hematocrit. A significant increase in the cholesterol levels in red blood cell membranes, plasma, and the liver was also observed, it was postulated that cylindrospermopsin (**1**) elicits an effect on the hepatic uptake of lipoproteins *via* an alteration of cholesterol-mediated metabolism caused by reduced levels of GSH in the liver as a result of toxin exposure [147]. A further study by the group investigated the effects of long-term exposure to the toxin; experimental groups of mice were exposed to an increasing level of cylindrospermopsin in their drinking water (10–55 μg kg^{-1} day^{-1}) over a period of 42 weeks. No significant differences in weight were observed between the control and

experimental groups, although both genders of the experimental group had enlarged livers and kidneys. Significantly elevated hematocrit levels were also observed in both genders after 16 weeks of exposure to low doses of toxin, which was accompanied by a deformation in red blood cells. At the end of the 42-week period almost all red blood cells had turned acanthocyte, these findings in accordance with their earlier study should be considered early and prime responses to cylindrospermopsin (**1**) exposure [148].

Norris et al. have investigated the role of GSH and CYP450 *in vitro* in the mouse. They found that pre-depletion of hepatic GSH by treatment with buthionine sulfoximine and diethyl maleate before administration of a 200 $\mu g\ kg^{-1}$ IP dose of cylindrospermopsin (**1**) had no significant effect in the 7-day survival rates, suggesting that depletion of GSH by cylindrospermopsin is not a primary mechanism of its toxicity. The group observed a protective effect when test subjects were treated with piperonyl butoxide, a CYP450 inhibitor before administration of 200 $\mu g\ kg^{-1}$ dose of the toxin increasing the 7-day survival rate to 100% compared to 40% in the control, and the protective effect was also observed in doses up to 800 $\mu g\ kg^{-1}$ confirming the importance of CYP450 activation in living systems as well as *in vitro* [149]. Cylindrospermopsin (**1**) toxicity has also been investigated in tilapia (*Oreochromis niloticus*). Animals were exposed orally (gavage) to doses of 200 and 400 $\mu g\ kg^{-1}$ of toxin and sacrificed 24 h later. A dose-dependent increase in lipid peroxidation products was observed in both the liver and kidney, combined with altered activity in the antioxidant enzymes glutathione peroxidase (GPx) and soluble glutathione-*S*-transferases (sGST) and the sGST protein abundance that varied between both organs [150].

Chernoff and co-workers have investigated the effect of cylindrospermopsin (**1**) exposure on the litter during pregnancy. Maternal exposure to 50 $\mu g\ kg^{-1}$ (IP) on gestational days (GD) 8–12 reduced the litter size; however, exposure later in pregnancy GD 13–17 produced a variety of other adverse effects, including blood in the gastrointestinal tract and hematomas in the tips of the tails. All pups that were exposed to cylindrospermopsin (**1**) *in eutero* were cross fostered to control dams; however, after weaning a persistent reduced body weight was observed up to one-and-a-half years after birth, suggesting that some toxin-induced injury may have prevented normal growth [151]. A follow-up study by the group investigated the postexposure toxicity and subsequent recovery of the postpartum females. They observed a much greater incidence of clinical toxicity in the animals dosed on GD 8–12, with both groups displaying a significant elevation in serum makers indicative of hepatic injury and alteration in the profiles of genes involved in ribosomal biogenesis, xenobiotic and lipid metabolism, inflammatory response, and oxidative stress after the final toxin dose.

One week after dosing serum parameters had returned to normal with gene expression changes returning to normal after 4 weeks [152].

The effect of cylindrospermopsin (**1**) on plant species is not as widely studied as in mammalian test systems. However, cylindrospermopsin (**1**) exposure has also been shown to have an inhibitory effect on the growth of the Common Duckweed (*Lemna minor*) and the Rootless Duckweed (*Wolffia arrhiza*) with exposure to extracts of *A. ovalisporum* containing 20 $\mu g\ mL^{-1}$ inhibiting growth by 60% and 54% with exposure to pure toxin having a less pronounced inhibition of 30% and 34% in the two species, respectively. No significant differences in soluble protein were observed in either species; however, a number of protease isozymes were found to be more active with new ones appearing in cylindrospermopsin (**1**) treated plants, demonstrating the toxins ability to disrupt proteolysis in the investigated plant species [153]. Recently, Borbély and co-workers have observed a significant concentration-dependant reduction in fresh mass and length of cotyledon, hypocotyls, and main roots of white mustard (*S. alba*) seedlings, with the IC_{50} for various organs ranging from 5 to 10 $\mu g\ mL^{-1}$ of pure toxin. Exposure to concentrations higher than 20 $\mu g\ mL^{-1}$ caused a decrease in peroxide enzyme activity [154], which is in agreement with their earlier work that reported an IC_{50} value of 18.2 $\mu g\ mL^{-1}$ for seedling growth [60]. Water Thyme (*Hydrilla verticillata*) exposed to whole cell extracts of *C. raciborskii* with a maximum cylindrospermopsin (**1**) concentration of 400 $\mu g\ L^{-1}$ displayed a significant increase in root growth, combined with a decrease in plant chlorophyll and changes in the relative abundances of chlorophyll *a* and chlorophyll *b*. Kinnear et al. postulated that the redistribution of plant resources observed as a result of toxin exposure may be the result of the production of root compounds as an adaptive strategy to minimize toxicity to cylindrospermopsin (**1**) or *C. raciborskii* containing whole cell extracts [155].

The toxicity of cylindrospermopsin (**1**) producing *C. raciborskii* has also been demonstrated toward the small planktonic crustacean *Daphnia magna*, which displayed a 10% survival rate of 48 h after exposure to cyanobacterium [156], a related study showed that after 5-day exposure no juvenile *D. magna* survived [157]. The addition of cyanobacterial lipopolysaccharide (LPS) has been shown to have a preventative effect showing a reduction in toxic response in *D. magna* to purified cylindrospermopsin (**1**) [158]. Toxic effects have also been expressed toward the cane toad (*Bufo marinus*), 66% mortality was observed upon exposure to live cultures of *C. raciborskii* (232 $\mu g\ L^{-1}$ total cylindrospermopsin (**1**)) [159]. Although exposed tadpoles survived, a related study showed that they exhibited multiorgan toxicity further demonstrating cylindrospermopsin (**1**) toxicity toward amphibians [160]. Similar cultures have also proved toxic to two bivalve species, the Mediterranean Muscle (*Mytilus galloprovincialis*) and the Asian Clam (*Corbicula fluminea*). However, this

study did not exclude the presence of other toxic species [161]. Cylindrospermopsin (**1**) also displays toxicity toward the zebra fish (*Danio rerio*) embryo, but only if exposure is through direct injection into the embryo, which demonstrated dose-dependant mortality with a dose of 4.2×10^3 nM resulting in 100% mortality or severe deformity [162].

7-*epi*-cylindrospermopsin (**2**) has been shown to be equally toxic as cylindrospermopsin by mouse bioassay [100], and has been used interchangeably with cylindrospermopsin (**1**) in toxicity experiments prior to structural reassignment. However, the intermediary metabolite 7-*deoxy*-cylindrospermopsin (**3**) was shown to elicit no toxic effects at doses up to 800 $\mu g\ kg^{-1}$ [6]. However, due to the recent *in vitro* toxicity data regarding this metabolite further higher dose animal trials are required to clarify the potential toxicity risk to humans.

B. Genotoxicity

1. In Vitro

Humpage et al. investigated the genotoxicity of cylindrospermopsin in the human WIL2-NS lymphoblastoid cell line using the cytokinesis block micronucleus assay (CBMN) and fluorescent *in situ* hybridization (FISH). The group observed a significant increase in micronucleus induction (MNi) in cells exposed to the highest cylindrospermopsin (**1**) concentrations. A significant increase in centromere positive MNi was observed at all toxin concentrations investigated (1, 3, 6, and 10 $\mu g\ mL^{-1}$); however, at higher cylindrospermopsin (**1**) concentrations the magnitude of centromere positive MNi did not account for the total increase in MNi indicating the presence of both centromere positive and centromere negative MNi. This infers cylindrospermopsin (**1**) displays clastogenic and aneugenic activities, events known to be implicated in carcinogenesis [163]. Degradation of the major cytoskeletal components by cylindrospermopsin (**1**) have been observed in the CHO-K1 cell line, which could lead to aneuploidy [164]. Subsequent work by Humpage and co-workers found that the genotoxic effects of a non-cytotoxic dose of cylindrospermopsin (**1**) (0.2 μM) could be prevented in primary mouse hepatocytes by treatment with the CYP450 inhibitors proadifen (50 μM) and omeprazole (100 μM) for 18 h suggesting that cylindrospermopsin (**1**) is pro-genotoxic requiring metabolic activation [133]. It was also suggested that genotoxicity is a specific and primary effect of cylindrospermopsin (**1**) as this takes place at sub-cytotoxic levels of exposure [133].

Further evidence supporting the pro-genotoxicity of cylindrospermopsin (**1**) has been observed with investigations using the CHO-K1 cell line [165,166]. Fessard and Bernard detected no DNA damage in this cell line using alkaline comet assay (ACA) after 24-h treatment with

cylindrospermopsin (**1**) at concentrations of 0.5 and 1.0 $\mu g\,mL^{-1}$. Their rational for this was that CYP450 metabolizing enzymes are low in this cell line, confirming the need for metabolic activation of the parent compound in order to elicit genotoxic effects [165]. Lankoff et al. also failed to observe any cylindrospermopsin (**1**) induced genotoxic effects in this cell line even in the presence of rat liver-derived S9 fraction [166]. It has been recently highlighted that this may not exclude the involvement of cylindrospermopsin (**1**) metabolites in genotoxicity, but it could be due to the lack of the appropriate metabolic systems in the S9 activation fraction used [167]. Cylindrospermopsin (**1**) genotoxicity has also been investigated in both differentiated and undifferentiated Caco-2 and HepaRG cells with and without the presence of CYP450 inhibitor ketoconazole. Due to the lower level of metabolic enzymes in undifferentiated HepaRG cells no genotoxicity was observed; however, the differentiated cells from this cell line showed a significant increased MNi after 24-h exposure to toxin concentrations ranging from 0.04 to 0.3 $\mu g\,mL^{-1}$. Cylindrospermopsin (**1**) also generated a significant increase in MNi in both differentiated and undifferentiated Caco-2 cells, although higher doses of toxin (1.12−1.50 $\mu g\,mL^{-1}$) were required. The addition of ketoconazole was observed to reduce MNi in this cell line by 50% [168], agreeing with earlier work by Humpage that CYP450 metabolism of cylindrospermopsin (**1**) is involved in its genotoxic effect [133]. The most recent publication involving the genotoxicity of cylindrospermopsin (**1**) has shown that exposure to the toxin leads to the upregulation of cytochrome genes CYP1A1 and CYP1A2 providing further evidence that these enzymes are involved in the metabolism of cylindrospermopsin (**1**) into genotoxic intermediates [169].

Recent work has also demonstrated that cylindrospermopsin induced a concentration-dependant increase in mRNA levels for DNA damage response P53 target genes (CDKN1A, GADD45α, BAX, and MDM2); however, despite induction of P53 regulated genes no accumulation of P53 protein was detected [170]. Similar results have been obtained by Žegura and co-workers who did not observe changes in the expression of the P53 tumor suppressor gene but in the downstream regulated DNA damage response genes GADD45α and MDM2 [169]. This study supports earlier findings by Bain and co-workers [170] and confirms the induction of DNA damage by exposure to cylindrospermopsin (**1**).

2. In Vivo

Shaw et al. investigated the formation of DNA adducts in mice treated with a single 1 $\mu g\,kg^{-1}$ IP dose of cylindrospermopsin (**1**) using ^{32}P-postlabelling assay. A single adduct spot was observed in the liver DNA of mice periodically sacrificed between 24 and 96 h post dosing, suggesting that cylindrospermopsin (**1**) or one of its metabolites is

capable of forming covalent adducts with DNA [171]. DNA strand breaks have also been observed in the liver DNA of mice treated with 200 μg kg^{-1} of cylindrospermopsin (**1**), animals were sacrificed at 6, 12, 24, 48, and 72 h intervals and strand breakage monitored *via* alkaline gel electrophoresis (pH 12). A significant decrease in the median molecular length (MML) value compared to control animals was observed and found to be maximal after 24 h of exposure, suggesting the DNA strand breakage is a key mechanism of cylindrospermopsin (**1**) genotoxicity [172]. The most recent work regarding *in vitro* genotoxicity of cylindrospermopsin (**1**) has been reported by Fessard and co-workers [173]. Male Swiss albino mice were treated with 50, 100, and 200 μg kg^{-1} doses of cylindrospermopsin (IP) and 1, 2, and 4 mg kg^{-1} orally (gavage), after 24 h samples of liver, blood, bone marrow, kidney, intestine, and colon were examined. The group observed that the highest doses of toxin, 100 and 200 μg kg^{-1} (IP) and 4 mg kg^{-1} (oral) caused significant DNA damage in the colon and oral doses of 1 and 2 mg kg^{-1} caused DNA damage to the bone marrow as determined by ACA, however no statistically significant increase in MNi was observed in any of the organ systems investigated [173].

C. Carcinogenicity

1. In Vitro

Initial investigations determined that cylindrospermopsin (**1**) did not inhibit protein phosphatase 2A, which suggested that it was unlikely to be a potential tumor promoter, as an imbalance in protein phosphorylation/dephosphorylation has been linked to tumor promotion [134]. However, more recent work by Maire and co-workers utilized the Syrian hamster embryo (SHE) assay to evaluate the carcinogenic potential of cylindrospermopsin (**1**) *in vitro*. The assay demonstrated the cell transforming potential of the toxin following a 7-day continuous treatment with non-cytotoxic or genotoxic doses of cylindrospermopsin (1×10^{-7} to 1×10^{-2} ng mL^{-1}), indicating the toxin is a carcinogenic hazard at very low doses [174]. Recently, cyanobacterial extracts of *Aphanizomenon flos-aquae* have been shown to effect *in vitro* biomarkers for tumor promotion that could not be attributed to cylindrospermopsin (**1**) or other known cyanotoxins further highlighting the need for research in this area [175].

2. In Vivo

Limited evidence is available regarding *in vivo* carcinogenic potential of cylindrospermopsin (**1**) exposure. However, initial investigations by Falconer and Humpage discovered tumors in 5 mice out of 53 who were orally dosed with cell-free extracts of cylindrospermopsin (**1**)

producing *C. raciborskii*, 3 of the mice were given doses 1500 mg kg^{-1} and 2 were given 500 mg kg^{-1}. Although the number of animals used in the study was insufficient to provide statistical significance, it highlights the need for further investigation due to public health considerations raised [176].

A recent review by Žegura and co-workers discusses the genotoxicity and potential carcinogenicity of cylindrospermopsin (**1**) in the context of other harmful freshwater cyanobacterial toxins [167].

VIII. BIOSYNTHESIS

Initial investigations into the biosynthesis of cylindrospermopsin (**1**) were undertaken by Moore and co-workers who indicated that the toxin was of polyketide origin, with guanidino acetic acid serving as the starter unit for the formation of the polyketide chain [177]. Acetate feeding experiments showed that C-4 through C-13 and the oxygen atoms attached to C-4 and C-12 arise from five contiguous acetate units attached from head to tail. Glycine feeding experiments indicate that C-14, C-15, and N-16 are the result of the incorporation of one intact glycine unit, with the methyl group at C-13 also of glycine origin (Figure 7). However, the group was unable to determine the biological origin of the guanidino unit in guanidino acetic acid or to show how the uracil ring is constructed.

Information obtained from the various acetate feeding experiments carried out allowed for several plausible pathways to be proposed for the biosynthesis of cylindrospermopsin (**1**). The backbone of the polyketide chain is most probably assembled by either a modular (type I) or nonmodular (type II) polyketide synthase from guanidino acetic acid and five acetate units. This comprises of two processing steps (C-methylation and keto reduction) and three cyclizations involving the guanidine group, either as the polyketide chain is being elongated or after it has been completed. Molecular modeling studies carried out by the group suggest that thioester **19** is a particularly attractive intermediate,

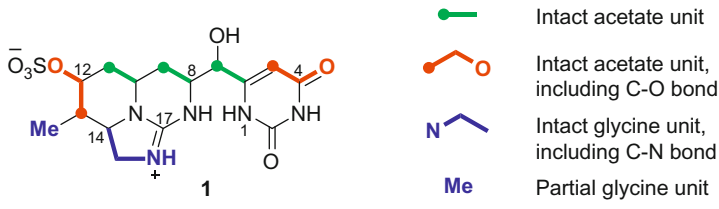
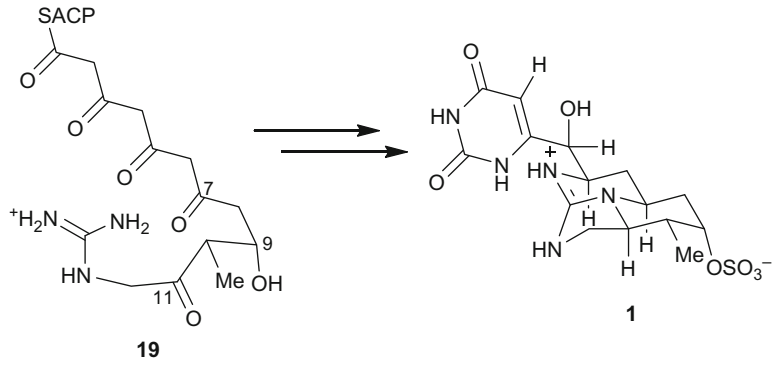


Figure 7 Incorporation of labeled precursors into cylindrospermopsin (**1**).



Scheme 1 Possible spontaneous cyclization proposed by Moore.

due to strong hydrogen bonding between the guanidine group, the hydroxyl group at C-9 and the ketone carbonyl oxygens at C-7 and C-11 (Scheme 1). The modeling studies suggested that polyketide **19** readily assumes a favorable conformation for the formation of rings A, B, and C in **1**, and thus the cyclizations may be spontaneous events that are not under enzymatic control [177].

Recent work by Neilan and co-workers has been directed toward characterizing the gene cluster (*cyr*) responsible for the biosynthesis of cylindrospermopsin (**1**) in *C. raciborskii* AWT205 [178]. The biosynthesis of cylindrospermopsin (**1**) involves an amidinotransferase, an NRPS (nonribosomal peptide synthetase), and a PKS (polyketide synthase) [37,179–181]. The first step in the formation of the carbon skeleton of cylindrospermopsin (**1**) involves the transamination of glycine (**20**) forming guanidinoacetic acid (**21**), which proceeds *via* a novel L-arginine:glycine amidinotransferase enzyme CyrA [180,181]. The next step in the biosynthesis is carried out by CyrB, which is responsible for the activation of guanidinoacetate and extension of **22** by one acetate unit, followed by the reduction of the ketone group to an alcohol and elimination of water resulting in a double bond to give **23**. Methylation of the double bond in **23** followed by Michael addition of the guanidine unit installs the C ring of cylindrospermopsin (**1**). CyrC is the next enzyme involved in the biosynthesis and is responsible for inclusion of a further acetate unit followed by keto reduction giving intermediate **24**. Addition of a further acetate unit is achieved by CyrD and is accompanied by further ketone reduction followed by dehydration giving alkene **25**. The newly formed double bond then undergoes Michael addition forming the piperidine A ring of cylindrospermopsin (**1**). This intermediate is the substrate for the next enzyme, CyrE, which catalyzes the addition of a further acetate unit and formation of a double bond

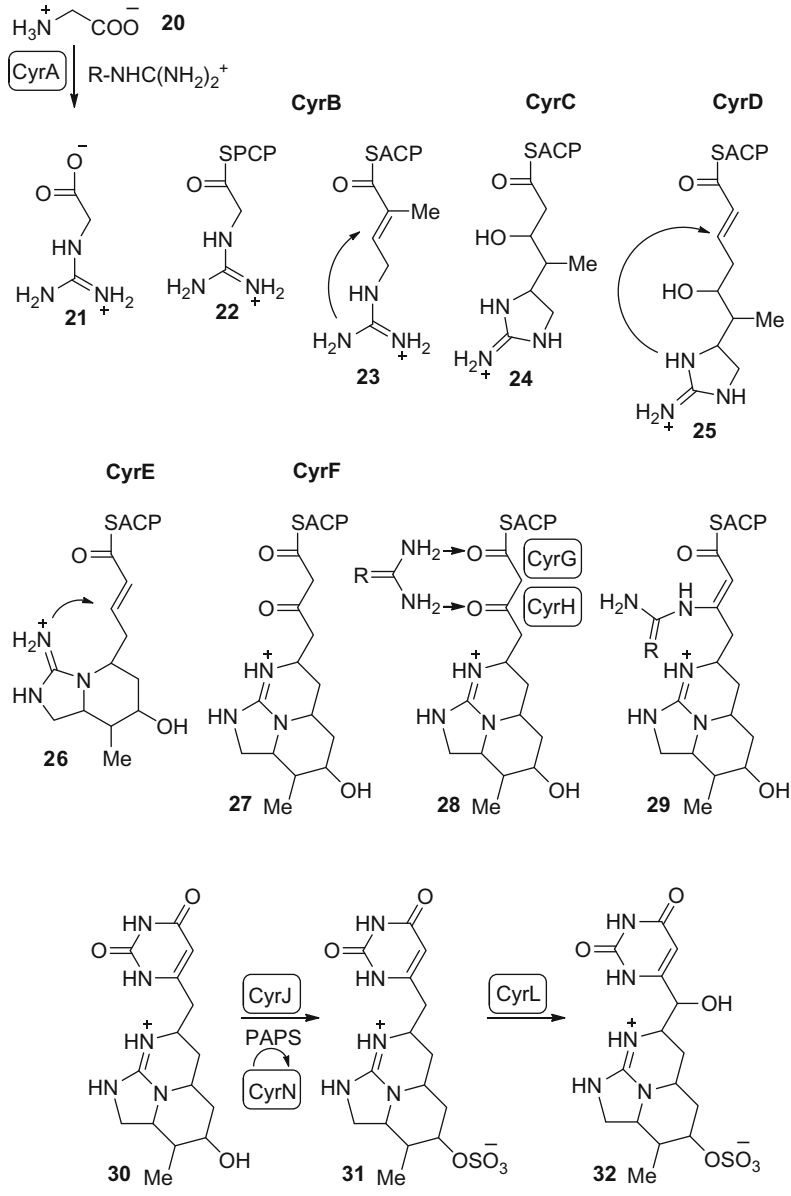
26, with a further ring closure resulting in the formation of the tricyclic guanidine portion of the molecule. The polyketide chain is then extended by a further acetate unit by the final PKS module CyrF producing intermediate **27**. The next step in the biosynthetic pathway involves the formation of the uracil D ring, a reaction that has proven to be elusive to characterize. The enzymes CyrG and CyrH are believed to transfer a second guanidino donor such as arginine or urea to **28**, which undergoes elimination of water to give **29**. The second reaction involves the formation of a second bond completing the six-membered ring system of the uracil group, while concomitantly releasing the molecule **30** from the enzyme complex **29**. A third reaction may be required for the cleavage of the guanidino group from a donor molecule other than urea. The lack of a thioesterase or cyclization domain led the group to believe that the formation of the ring system is achieved in a stepwise manner and is not a spontaneous event as had been previously postulated. The finalization of the biosynthesis requires two tailoring reactions. The first is sulfonation carried out by CyrJ that is believed to be a 3′-phosphoadenosine-5′-phosphosulfate (PAPS)-dependant sulfotransferase, which is accompanied by CyrN, an adenylylsulfate kinase that creates the pool of PAPS needed for sulfonation leading to **31**. The final step of cylindrospermopsin (**1**) biosynthesis involves hydroxylation of the bridging carbon atom between the two ring systems giving rise to **32**. This hydroxylation is carried out by CyrL that has been shown to be a 2-oxoglutarate-dependant iron oxygenase; the stereoselectivity of this enzyme varies from strain to strain giving rise to either cylindrospermopsin (**1**) or 7-*epi*-cylindrospermopsin (**2**). [182] (Scheme 2).

IX. TOTAL SYNTHESES

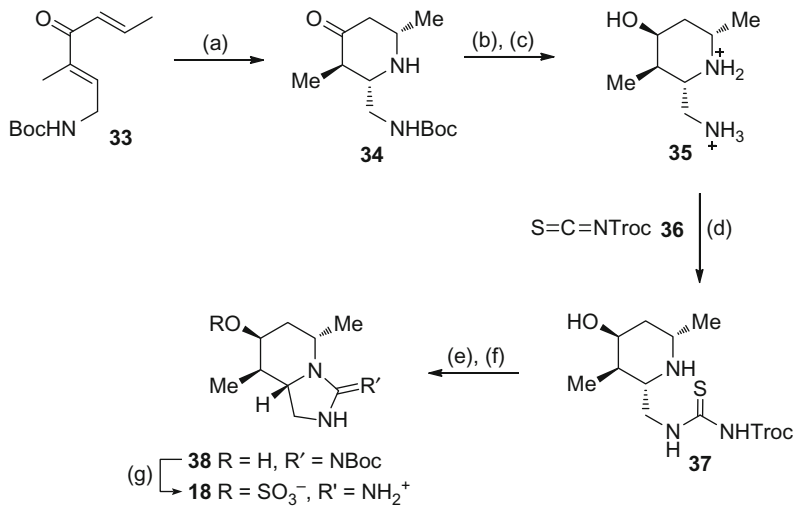
Currently, four total syntheses pertaining to this family of natural products have been reported [9,183–188]. Their unique structure containing a tricyclic guanidine moiety combined with continually evolving toxicological parameters makes them an intriguing and worthwhile synthetic target. An intense interest in developing an efficient high-yielding synthesis is still being undertaken [189–192] (Scheme 3).

A. The Snider Research Group

Snider and co-workers reported the first total synthesis of (±)-cylindrospermopsin (**1**) [183]. Their initial synthetic endeavors began with the preparation of a bicyclic model **18** of the AC ring system of this family of natural products [193] (Scheme 4). They reported the double Michael addition of ammonia to dienone **33** (prepared in five steps



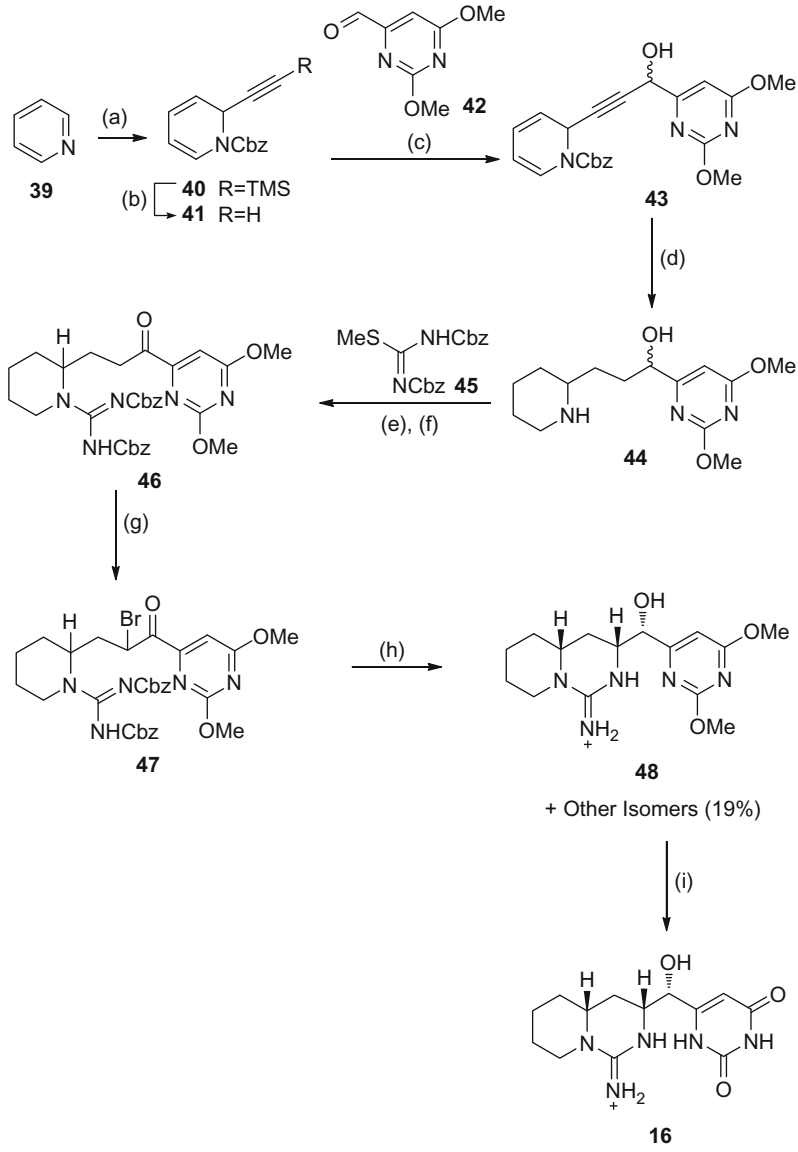
Scheme 2 Proposed biosynthesis of cylindrospermopsin with sequential polyketide extensions and subsequent cyclizations.



Scheme 3 Reagents and conditions: (a) NH_4OH, NH_4Cl, MeOH, 67 °C, 16 h, 55%; (b) L-Selectride, 88%; (c) TFA, 100%; (d) **36**; (e) $HgCl_2$, NEt_3, DMF, 40% (two steps from **35**); (f) Zn, AcOH, H_2S, HCl (aq), 81%; (g) $SO_3 \cdot$ DMF, DMF, 100%.

form (*E*)-1,3-dibromobut-2-ene) that afforded a diastereomeric mixture of piperidones with the desired product **34** as the major isomer, having all three substituents equatorial as is necessary for cylindrospermopsin (**1**). To prevent a retro-Michael reaction occurring during guanidine formation, the ketone **34** was reduced with L-Selectride, followed by removal of the Boc-protecting group to give diamino alcohol **35**. Diamino alcohol **35** was then reacted with isothiocyanate **36** that was generated *in situ* to yield the protected thiourea **37**, subsequent treatment of **37** with $HgCl_2$ and NEt_3 in DMF closed the second ring to give the key bicyclic guanidine **38** in 40% yield from **35**. Deprotection of **38** with zinc dust in aqueous acetic acid gave the corresponding guanidinium chloride, which upon sulfonation with $SO_3 \cdot$ DMF in DMF gave the desired bicyclic model compound **18** in a quantitative yield.

Snider next addressed the construction of the B-ring and the uracil-containing side chain of cylindrospermopsin (**1**), reporting the preparation of the ABD tricyclic model compound **16** [194] (Scheme 4). The group began by treating pyridine (**39**) with benzyl chloroformate in the presence of trimethylsilylethynylmagnesium bromide to give silylacetylene **40**, which was desilylated with methanolic potassium carbonate giving terminal alkyne **41** in high yield. Treatment of **41** with ethylmagnesium bromide followed by exposure to aldehyde **42** gave compound **43** as a mixture of diastereomers. Hydrogenation of **43** over 5% Pd/C



Scheme 4 Reagents and conditions: (a) CbzCl, THF, TMSC≡CMgBr, 0 °C, 2 h, 95%; (b) K_2CO_3, MeOH, 25 °C, 20 min, 97%; (c) EtMgBr, **42**, 85%; (d) 5% Pd/C, H_2, MeOH, 94%; (e) **45**, $HgCl_2$, NEt_3, DMF; (f) Dess–Martin periodinane, 74% (two steps from **44**); (g) $CuBr_2$, EtOAc, 40 °C, 15 min; (h) 5% Pd/C, H_2, MeOH, 2 h, 81% (two steps from **46**); (i) conc. HCl, △, 6 h, 95%.

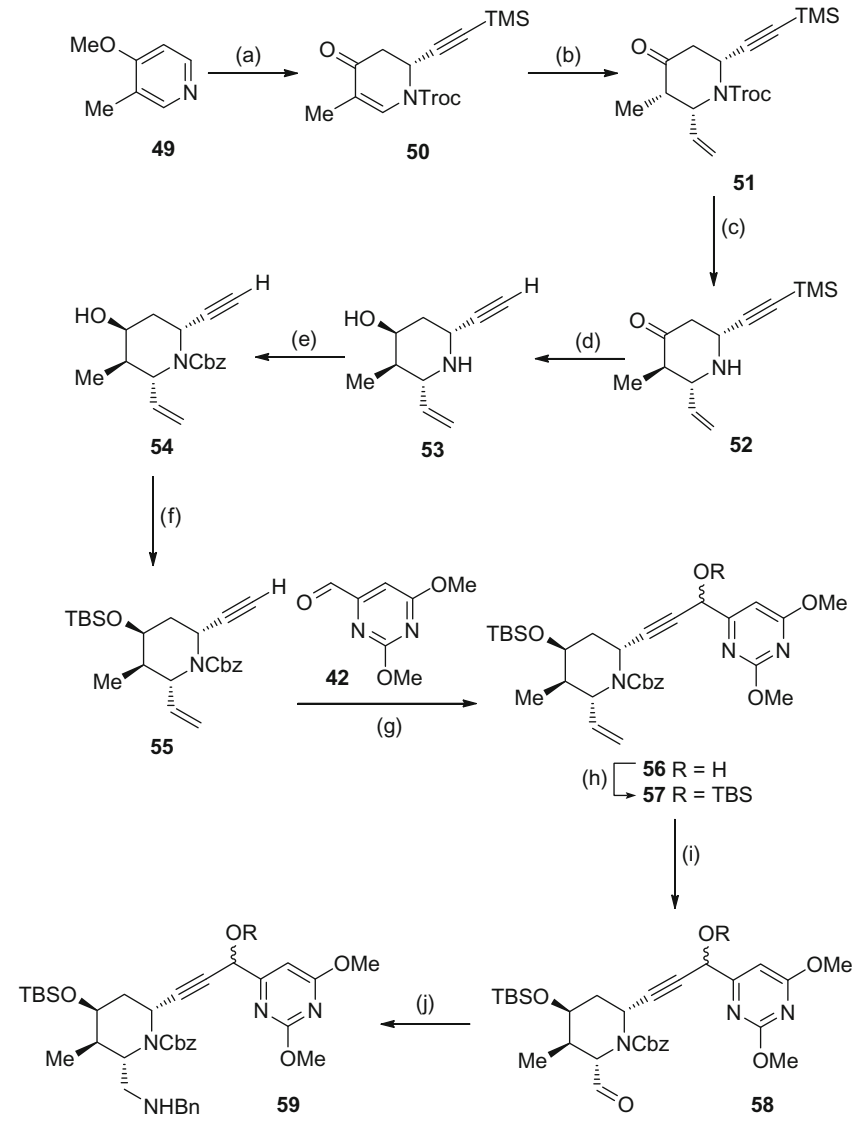
reduced the dihydropyridine and acetylene groups and cleaved the Cbz protecting group generating piperidine **44** in 94% yield as a mixture of diastereomers. The guanidine functionality was introduced *via* a mercuric chloride mediated coupling between **44** and *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea (**45**) followed by oxidation of the hydroxymethyluracil side chain with Dess−Martin periodinane to afford ketone **46**. Bromination of **46** with $CuBr_2$ furnished the unstable bromo ketone **47**, subsequent hydrogenation cleaved the Cbz groups liberating the free guanidine that spontaneously cyclized generating the B ring of the model compound. Under these conditions the ketone group was also reduced generating alcohol **48** in 81% yield, together with a 19% yield of three other isomers. Hydrolysis of the dimethoxypyrimidine was achieved by heating at reflux in concentrated hydrochloric acid for 6 h with no decomposition or isomerization being observed under these forcing conditions. It was noted that protonation of the guanidine and uracil rings shielded the alcohol from solvolysis. This process gave the ABD tricycle **16** that displayed ^{1}H and ^{13}C NMR spectral data very similar to those reported for cylindrospermopsin (**1**). Snider originally [194] reported a structure for **16** which was epimeric at the C-7 position; however, in the later paper [144] the structure was revised in line with the reassignment of the structures of **1** and **2** [9].

Following on from the group's second reported model synthesis, Snider and co-workers reported the first total synthesis of (±)-cylindrospermopsin (**1**) from 4-methoxy-3-methylpyridine (**49**) [183]. The group prepared their starting material 4-methoxy-3-methylpyridine (**49**) in three steps from 3-picoline *via* a modified literature procedure. The disubstituted pyridine **49** was then treated with TrocCl followed by trimethylsilylethynylmagnesium bromide to give dihydropyridine **50** in 49% yield (87% based on recovered **49**). A cuprate-catalyzed conjugate addition of vinylmagnesium bromide to the enone of **50** successfully generated piperidone **51** in 92% yield. Cleavage of the Troc protecting group from **51** was achieved by treatment with zinc dust in acetic acid affording the free piperidone, in which the methyl group adjacent to the ketone had equilibrated under the acidic conditions to give the thermodynamically more stable isomer **52** with all three equatorial substituents. Reduction of **52** with L-Selectride in THF at −78 °C followed by basic hydrolysis generated the desired axial alcohol and cleaved the alkynylsilane furnishing the desired piperidine **53** with all four stereogenic centers of the A ring of cylindrospermopsin (**1**) in place. The amino group of **53** was then protected by treatment with CbzCl generating carbamate **54** followed by conversion of the remaining hydroxyl function to the corresponding silyl ether giving alkyne **55**. Alkyne **55** was then coupled with aldehyde **42** *via* the corresponding alkynylmagnesium bromide giving alcohol **56** as a 1:1 mixture of diastereomers in 83% yield. The newly formed alcohol

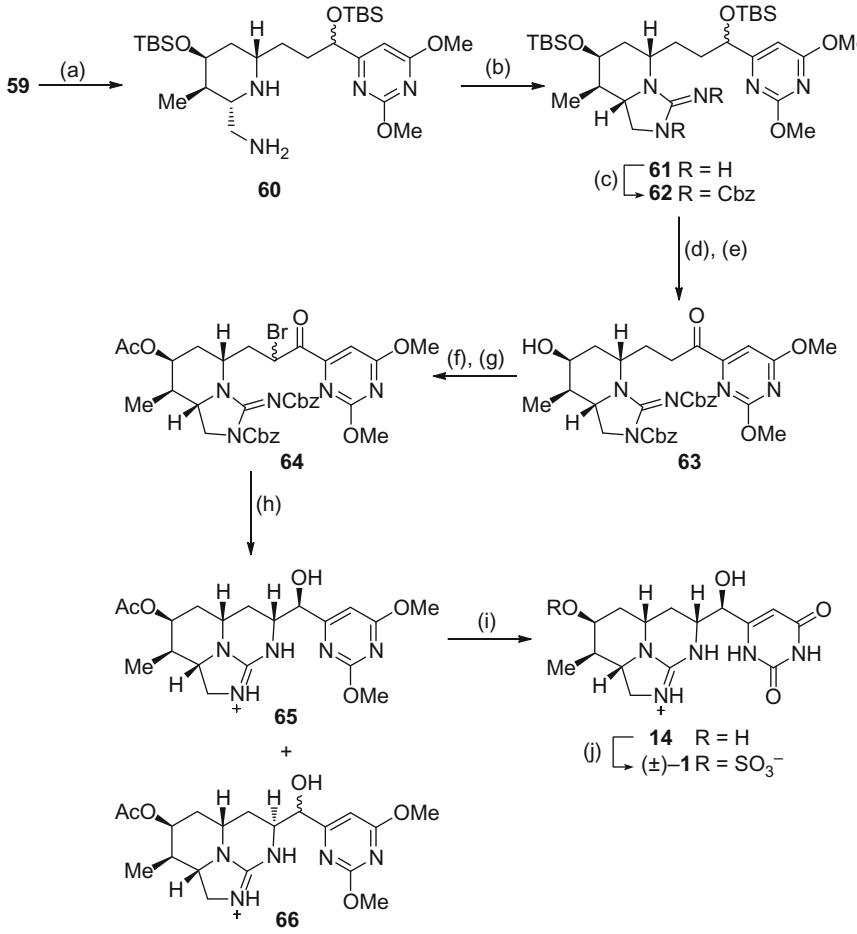
function of **56** was then protected by treatment with TBSCl, imidazole, and a catalytic amount of DMAP giving **57** in 88% yield. Ozonolysis of the alkene substituent of **57** in DCM followed by reductive workup with Me_2S gave aldehyde **58** that subsequently underwent reductive amination providing benzylamine **59** in 68% yield (Scheme 5).

Hydrogenation (1 atm) of **59** over 5% Pd/C in methanol reduced the alkyne bond and cleaved the benzyl and Cbz groups generating unpurified diamine **60** in 65–75% yield. Formation of the desired guanidine proved difficult but was successfully achieved by the slow addition of one molecular equivalent of cyanogen bromide to a dilute benzene solution of **60** to give a primary cyanamide, which spontaneously cyclized to give guanidine **61** in which both the A and C rings of cylindrospermopsin (**1**) have been established. The newly formed guanidine function was then protected as the corresponding carbamate by treatment with CbzCl to afford protected guanidine **62** in 45% yield from benzylamine **59**. Treatment with TBAF in THF at room temperature removed the silyl protecting groups allowing for the selective oxidation of the benzylic hydroxyl group with MnO_2 in DCM to afford ketone **63** in high yield. Bromination of ketone **63** could not be accomplished; therefore, the remaining alcohol group was acetylated under standard conditions followed by successful bromination with $CuBr_2$ in EtOAc to give an unstable mixture of α-bromo ketones **64**. Immediate hydrogenation of this mixture over 5% Pd/C in methanol liberated the free guanidine which, in turn, underwent an S_N2 cyclization, with concomitant ketone reduction to give an easily separable 3:2 mixture of tricyclic guanidines **65** and **66** in 70% yield over two steps. Hydrolysis of **65** was achieved in concentrated hydrochloric acid at 100 °C for 6 h, affording uracil diol **14** in 95% yield. The final step of the synthesis involved monosulfonation of the ring alcohol of **14**, this was achieved by exposure to $SO_3 \cdot DMF$ in pyridine and DMF generating the compound now known to be cylindrospermopsin (**1**) racemically in 1.5–2.0% overall yield from 4-methoxy-3-methylpyridine (**53**) in 21 steps (Scheme 6).

Snider and co-workers reported the first total synthesis of (±)-cylindrospermopsin (**1**) in 21 steps and 1.5–2.0% overall yield. They noted that the chemical shift of the H-7 proton of intermediate **65** δ 4.68 (d, $J = 3.70$ Hz) corresponded closely to that initially reported for that of the natural product δ 4.70 (d, $J = 3.90$ Hz) suggesting that their synthetic material displayed the same C-7 stereochemistry. However, they were unable to establish conclusively the C-7 stereochemistry of any of their intermediates. The key step in their synthesis was α-bromination and an intramolecular nucleophilic substitution reaction installing the cylindrospermopsin B ring and completing the tricyclic portion of the molecule (**63** → **65**).



Scheme 5 Reagents and conditions: (a) TrocCl, THF, −30 °C, TMSC≡CMgBr, 87%; (b) $CuBr \cdot SMe_2$, −78 °C, vinylmagnesium bromide, TMSCl, THF, 92%; (c) Zn, AcOH; (d) L-Selectride 90% (two steps from **51**); (e) CbzCl, Na_2CO_3, THF, 96%; (f) TBSCl, imidazole, DMAP, DCM, 89%; (g) EtMgBr, THF, 0 °C, **42**, 83%; (h) TBSCl, imidazole, DMAP, DCM, 88%; (i) O_3, DMS, DCM, −78 °C, 72%; (j) NH_2Bn, AcOH, benzene; (k) $NaCNBH_3$, MeOH, 68% (two steps from **58**).



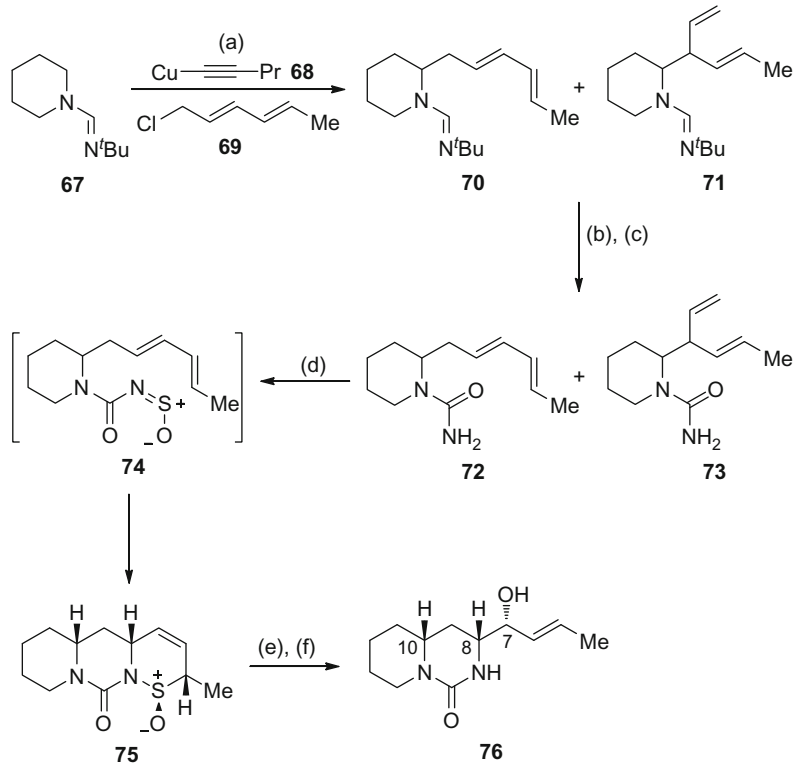
Scheme 6 Reagents and conditions: (a) 5% Pd/C, H_2, MeOH; (b) CNBr, benzene; (c) NaH, CbzCl, THF, 45% (three steps from **63**); (d) TBAF, THF, 83%; (e) MnO_2, DCM, 87%; (f) Ac_2O, pyridine, rt, 87%; (g) $CuBr_2$, EtOAc, rt 30 min; (h) $Pd(OH)_2$, H_2, MeOH, 42% (two steps); (i) conc. HCl, Δ, 95%; (j) $SO_3 \cdot DMF$, pyridine, DMF, 60–80% (**1**).

B. The Weinreb Research Group

Following the work of Snider, Weinreb and co-workers reported the total synthesis of both cylindrospermopsin (**1**) and 7-*epi*-cylindrospermopsin (**2**) [9,184]. The group's work also led to the stereochemical reassignment of the orientation of the bridging hydroxymethylene group, showing that cylindrospermopsin was in fact structure **1** and not structure **2** as was originally assigned by Moore and co-workers [3].

Weinreb's initial synthetic work toward **1** was to investigate the preparation of an AB model compound using the *N*-sulfinyl dienophile hetero Diels–Alder methodology previously developed in their laboratories [195]. They began with amidine **67** that was lithiated with *t*-BuLi and exposed to copper acetylide **68** to give the corresponding cuprate. The cuprate was then alkylated with 1-chlorohexa-2,4-diene (**69**) generating diene **70** together with the isomeric unconjugated diene **71** in 67% yield as an inseparable 4:1 mixture. Hydrolysis of the mixture with aqueous KOH generated the corresponding primary amine that upon reaction with sodium cyanate afforded ureas **72** and **73** in 48% yield again as an inseparable mixture. Subsequent reaction of urea **72** with thionyl chloride and imidazole generated *N*-sulfinyl urea **74** *in situ*, which cyclized to give a single Diels–Alder adduct **75** (67% based on **72** present in the mixture) whose structure was established by X-ray crystallography. Treatment of **75** with phenylmagnesium bromide gave the corresponding allylic sulfoxide that underwent a [2,3]-sigmatropic rearrangement to give the AB model compound **76**, displaying the correct C-7, C-8, and C-10 relative configurations for the synthesis of the originally proposed cylindrospermopsin structure **2** (Scheme 7).

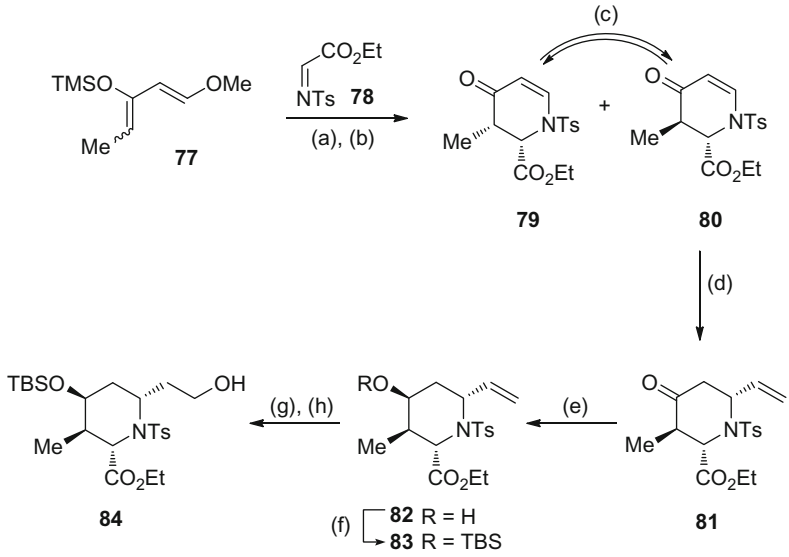
Following on from their initial publication detailing the preparation of a bicyclic AB model compound, Weinreb and co-workers further described the stereoselective preparation of the piperidine A ring moiety of cylindrospermopsin (**1**) using an imino Diels–Alder based methodology [196]. Their approach began from oxygenated diene **77**, which is a 4:1 mixture of *Z*/*E* isomers. Treatment of **77** with ethyl (*N*-tosylimino)-acetate (**78**) followed by acidic workup gave a mixture of the epimeric keto esters **79** and **80**. The yields and ratios of these cycloadducts were found to be dependent upon the specific reaction conditions employed. For example, with $AlCl_3$ as catalyst in toluene at −78 °C a 53% yield of a 7:1 mixture of **79** and **80** was obtained; however, using $ZnCl_2$ under similar conditions gave **79** and **80** in a ratio of 22:1 in 60% overall yield. The uncatalyzed condensation between diene **77** and imine **78** in toluene at room temperature led to a 4.7:1 mixture of **79** and **80**. The major *cis* product **79** arises from the *Z*-diene *via* a transition state having the ester group of the imine **78** endo, and the minor *trans* adduct **80** is derived from the *E*-diene isomer. It was postulated that the variable ratios of **79** and **80** might result from *E*/*Z* isomerization of the diene under the reaction conditions and/or epimerization of the adducts upon workup and purification. In support of the latter hypothesis, it was found that when the major keto ester **79** was treated with *p*-TsOH in refluxing benzene a 4:1 mixture of **79** and **80** was produced; the same 4:1 equilibrium mixture was observed when the minor keto ester **80** was exposed to the same reaction conditions. A cuprate-catalyzed conjugate addition of vinylmagnesium bromide to **80** successfully generated vinyl ketone **81**, which



Scheme 7 Reagents and conditions: (a) *t*-BuLi, **68** then **69**, 67%; (b) KOH, MeOH/H_2O, 94%; (c) NaOCN, HCl, 48%; (d) $SOCl_2$, imidazole, DCM, −78 °C, 67%; (e) PhMgBr, THF, −78 °C; (f) $(MeO)_3P$, MeOH, △, 65% (two steps from **75**).

upon reduction with L-Selectride gave alcohol **82** that displays the correct relative stereochemistry of cylindrospermopsin (**1**) A ring. Further elaboration of **82** by conversion of the ring hydroxyl group to the corresponding silyl ether **83** followed by hydroboration gave the primary alcohol **84** in good yield (Scheme 8).

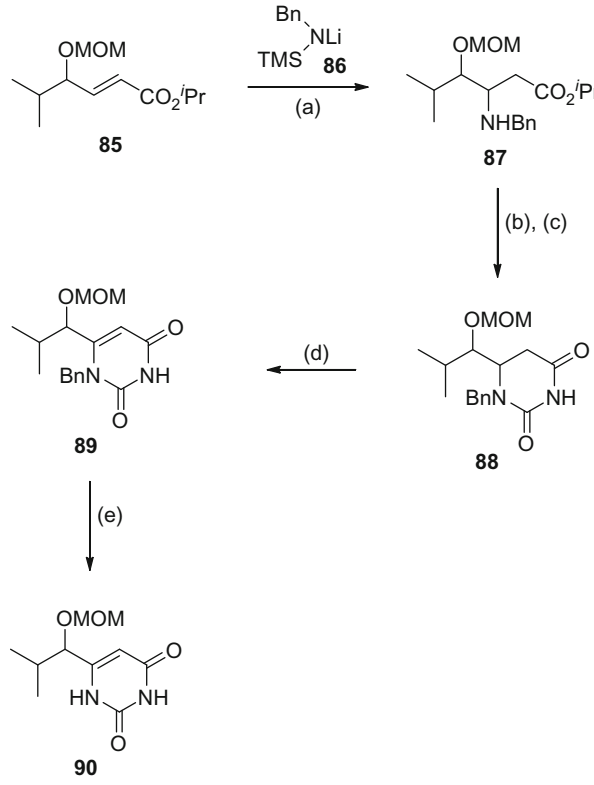
Having demonstrated the preparation of a model AB ring system and the stereoselective preparation of a suitable A ring precursor, Weinreb and co-workers set about investigating novel routes to uracils related to the D ring of cylindrospermopsin (**1**). The key step in their methodology involved the addition of N-nucleophiles to α,β-unsaturated esters [197]. Their initial route commenced using enoate **85** as a model for cylindrospermopsin (**1**). Thus, reaction between **85** and silylated lithium amide reagent **86** afforded adduct **87** which, upon treatment with trichloroacetyl isocyanate, followed by basic hydrolysis furnished the desired dihydrouracil **88** in 88% yield. It was possible to oxidize **88** by treatment



Scheme 8 Reagents and conditions: (a) **78**, toluene, rt, 3 h; (b) H_3O^+, 51%, **79**:**80** (4.7:1 ratio), (two steps from **77**); (c) *p*-TsOH, benzene, $\triangle$, 57% **79**:**80** (4:1 ratio); (d) vinylmagnesium bromide, CuI, THF, 88%; (e) L-Selectride, THF, 49%; (f) TBSOTf, DIPEA, DCM, 83%, (g) $BH_3 \cdot THF$, THF; (h) NaOH, H_2O_2, 50% (two steps from **83**).

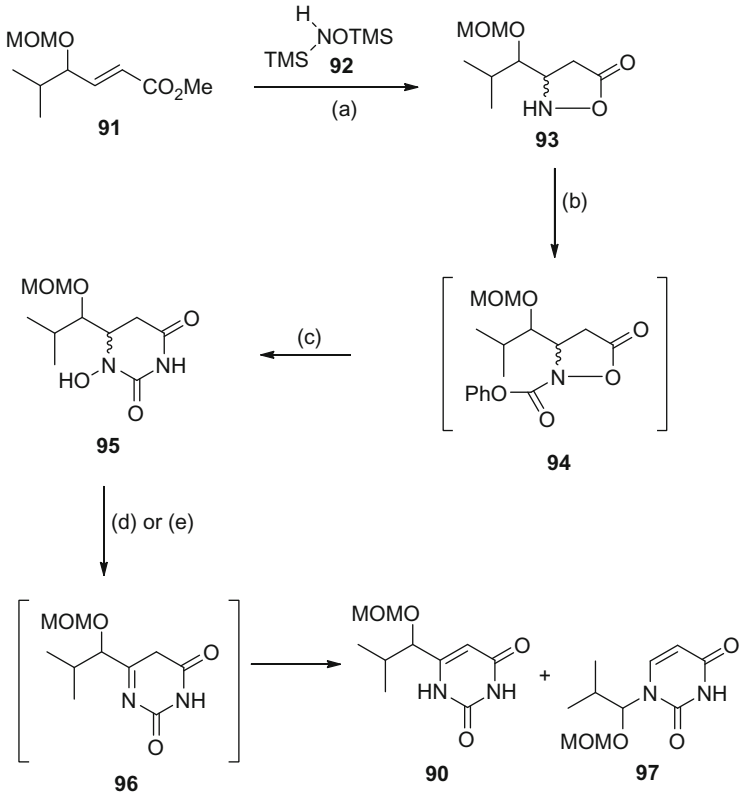
with benzeneseleninic anhydride to give protected uracil **89**, which could be deprotected under transfer hydrogenation conditions with ammonium formate over 10% Pd/C in methanol giving the desired uracil system **90** (Scheme 9).

Although this route proved successful, a more efficient alternative where both the oxidation step and nitrogen protection could be avoided was devised [197]. Thus, treating methyl ester **91** with commercially available *N*,*O*-bis-(trimethylsilyl)hydroxylamine (**92**) in refluxing ethanol afforded adduct **93** as a 1:1 mixture of stereoisomers in 68% yield. Treatment of **93** with phenyl chloroformate led to the intermediate *N*-acylation product **94**, which upon ammonolysis afforded *N*-hydroxy dihydrouracil **95**. It was envisaged that the conversion of dihydrouracil **95** to the desired product **90** could be achieved *via* dehydration and subsequent tautomerization of the intermediate imine **96**. This process was initially investigated by treatment with triflic anhydride and pyridine in DCM at room temperature. These conditions gave **90** in appreciable yield along with the unusual rearrangement by-product **97**. The formation of **97** could be prevented by adopting different reaction conditions, namely the use of tosyl chloride and DMAP in refluxing 1,2-dichloroethane, which gave **90** as the only product in 81% yield (Scheme 10).



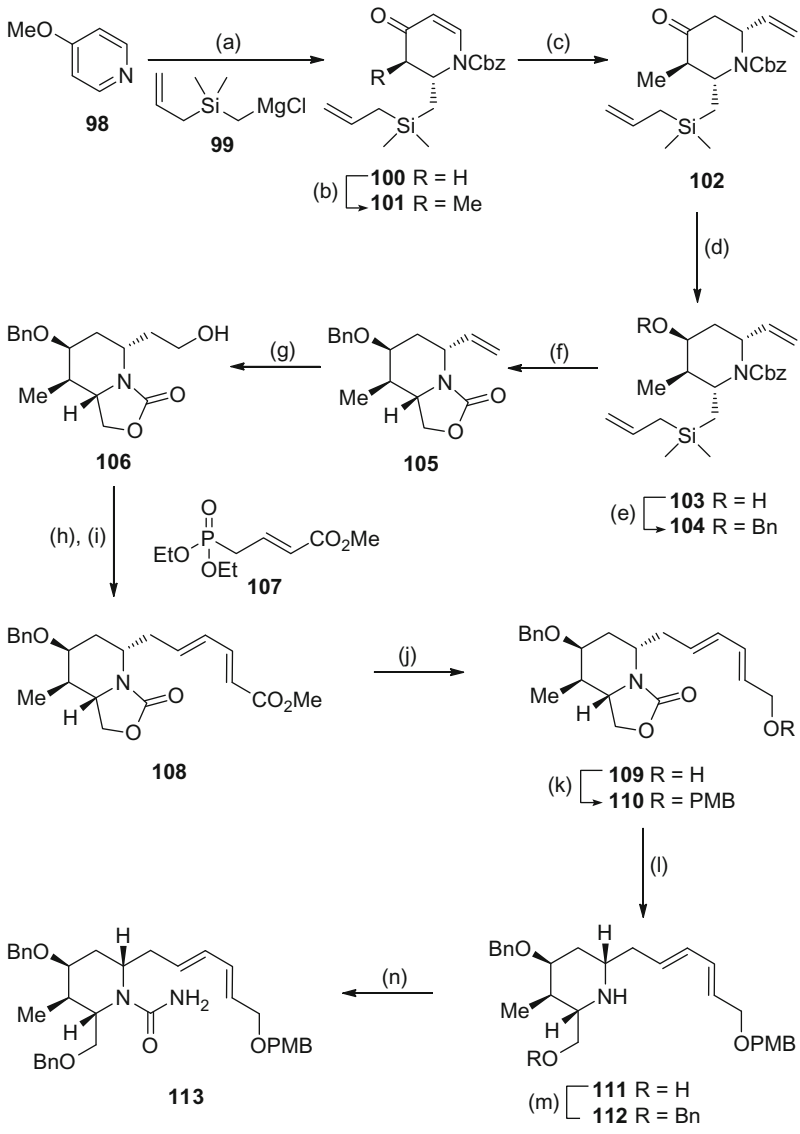
Scheme 9 Reagents and conditions: (a) THF, −78 °C to rt, 63%; (b) trichloroacetyl isocyanate, DCM; (c) KOH, H_2O, △, 88% (two steps from **87**); (d) $(PhSeO)_2O$, toluene, △, 59%; (e) 10% Pd/C, NH_4HCO_3, MeOH, △, 54%.

An initial communication published by the Weinreb's group detailed the stereoselective total synthesis of 7-*epi*-cylindrospermopsin (**2**) proving conclusively that the initial stereochemical assignment at the C-7 position was incorrect and that cylindrospermopsin is represented by structure **1** [9]. Thus, 4-methoxypyridine (**98**) was treated with CbzCl, followed by the addition of a hydroxymethyl anion equivalent in the form of Grignard reagent **99** to give dihydropyridinone **100** in 94% yield. Generation of the enolate anion of **100** with NaHMDS followed by treatment with MeI afforded the desired *trans* product **101**. A cuprate-catalyzed conjugate addition of vinylmagnesium bromide to **101** gave the requisite adduct **102** in very high yield. Reduction of ketone **102** with L-Selectride in THF gave the corresponding alcohol **103** having the four correct A ring stereocenters of this family of natural products. Alcohol **103** was then protected giving benzyl ether **104**, with subsequent Tamao oxidation [198] of the silane



Scheme 10 Reagents and conditions: (a) EtOH, △, 68%; (b) PhOCOCl, THF, NEt_3; (c) NH_4OH, *i*-PrOH, 65% (two steps from **93**); (d) Tf_2O, pyridine, DCM, rt, 54% (**90**) 34% (**97**); (e) TsCl, DMAP, DCE, △, 21 h, 81% (**90**).

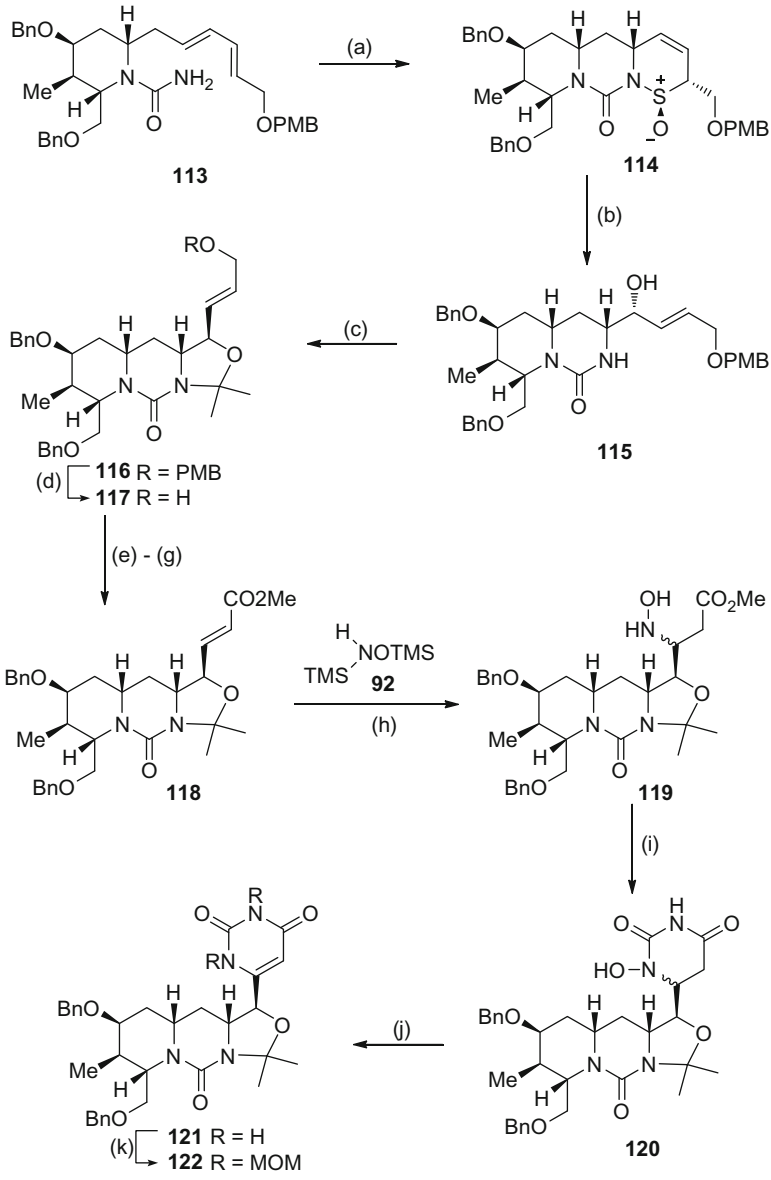
group leading directly to cyclic carbamate **105**. Elaboration to the required side chain began with the hydroboration of alkene **105** with disiamylborane successfully generating primary alcohol **106** in near quantitative yield. Oxidation of **106** to the corresponding aldehyde was achieved under Swern conditions. A subsequent HWE reaction between phosphonate **107** and the aldehyde cleanly generated the desired (*E*,*E*)-diene ester **108**. The ester group of **108** was then reduced with DIBAL providing allylic alcohol **109** that was protected as *p*-methoxybenzyl ether **110**. Hydrolysis of the cyclic carbamate functionality of **110** was achieved by treatment with ethanolic sodium hydroxide generating the primary alcohol **111** quantitatively, with subsequent protection of the hydroxy group with BnBr furnishing dibenzyl compound **112** in 65% yield. Finally, conversion of **112** to the corresponding urea successfully generated the required Diels–Alder precursor **113** in 85% yield (Scheme 11).



Scheme 11 Reagents and conditions: (a) (i) CbzCl, THF, −20 °C, (ii) **99**, Et_2O, −20 °C, 94% (two steps from **98**); (b) NaHMDS, MeI, THF, −78 °C, 88%; (c) vinylmagnesium bromide, CuI, THF, −78 °C to −20 °C, 98%; (d) L-Selectride, THF, 80%; (e) NaH, BnBr, TBAI, THF, △, 95%; (f) (i) KHF_2, $CHCl_3$, TFA, (ii) MeOH, $NaHCO_3$, THF, 30% H_2O_2, 88% (two steps); (g) (i) Sia_2BH, THF, (ii) H_2O_2, NaOH, −20 °C to rt, 97% (two steps from **104**); (h) $(COCl)_2$, DMSO, NEt_3, DCM, −55 °C to rt, 84%; (i) **107**, LiOH-H_2O, 4 Å MS, THF, △, 80%; (j) DIBAL, $BF_3 \cdot Et_2O$, DCM, −78 °C, 83%; (k) NaH, PMBCl, TBAI, THF, △, 96%; (l) NaOH, H_2O, EtOH, △, 100%; (m) NaH, BnBr, TBAI, THF, 0 °C to rt, 65%; (n) KOCN, HOAc, pyridine, NEt_3, 85%.

Treatment of (*E*,*E*)-diene urea **113** with thionyl chloride and imidazole in DCM at −78 °C followed by slowly warming to room temperature led to a single cycloadduct **114** in excellent yield and stereoselectivity. At this juncture it was possible to remove the PMB protecting group from **114** by treatment with DDQ generating the corresponding alcohol whose structure and stereochemistry could be firmly established by X-ray crystallography, allowing the group to continue with their synthesis. Dihydrothiazine oxide **114** then underwent a stereospecific ring opening/[2,3]-sigmatropic rearrangement to afford bicyclic urea **115** having all six stereocenters of the natural product in place. The stereochemistry of **115** was verified by X-ray crystallography of its MOM-protected derivative. To elaborate the uracil moiety, protection of the newly introduced alcohol function was required which was achieved by converting bicyclic urea **115** into cyclic acetonide **116**. Subsequent removal of the PMB group gave allylic alcohol **117** that was converted *via* a short sequence to the required α,β-unsaturated methyl ester **118** in 81% yield. Treatment of ester **118** with *N*,*O*-bis-(trimethylsilyl) hydroxylamine (**92**) provided the desired conjugated addition product **119**, subsequent exposure of which to phenyl chloroformate, followed by ammonium hydroxide provided *N*-hydroxydihydrouracil **120**. Finally, dehydration of **120** with triflic anhydride completed the installation of the uracil D ring **121**, with subsequent exposure to MOMCl generating bis-*N*-MOM-protected derivative **122** (Scheme 12).

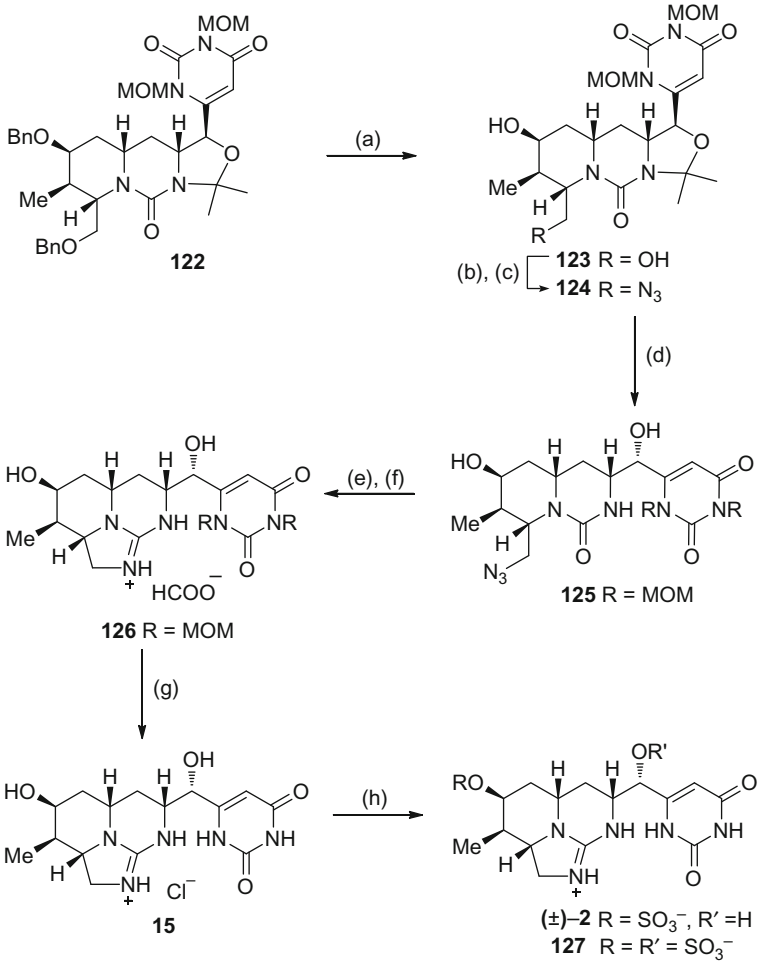
The final challenge for the group to overcome was the construction of the 5-membered C ring. This sequence began with the hydrogenation of **122** over Pearlman's catalyst furnishing the crystalline diol **123**, the analysis of which further verified the structures of the group's late stage synthetic intermediates. The primary alcohol group of **123** was then converted to the corresponding azide by treatment with triphosgene followed by exposure to NaN_3 giving the requisite azide **124** in 86% yield. Selective acid hydrolysis of the acetonide protecting group of **124** was achieved by treatment with dilute HCl producing urea diol **125**. Subsequent activation of the urea moiety of **125** with methyl triflate followed by catalytic hydrogenation of the azide over 10% Pd/C proceeded smoothly, leading directly to the tetracyclic guanidinium compound **126**. Deprotection of the uracil D ring of **126** was achieved with vigorous acid hydrolysis to afford guanidinium diol **15** in 43% yield from urea **125**. The spectral properties of **15** were found to be incongruous to the corresponding intermediate in the Snider group's total synthesis of cylindrospermopsin (±**1**), in particular compound **15** had δ_{C7} 4.50 ppm with ($J_{7,8}$=6.6 Hz) in line with that reported for 7-*epi*-cylindrospermopsin, versus δ_{C7} 4.70 ppm ($J_{7,8}$=4.0 Hz) for both the Snider diol and natural cylindrospermopsin. Moreover, the monosulfate ±**2** that was prepared in high yield and isolated along with 25% of the corresponding *bis* sulfate **127**, provided NMR spectral data identical to those



Scheme 12 Reagents and conditions: (a) $SOCl_2$, imidazole, DCM, −78 °C to rt, 81%; (b) (i) PhMgBr, THF/DCM, −55 °C; (ii) $(MeO)_3P$, MeOH, 50 °C, 84% (c) $Me_2C(OMe)_2$, acetone, CSA, △, 93%; (d) DDQ, H_2O, DCM, 78%; (e) Dess–Martin periodinane, DCM; (f) $NaClO_2$, *t*-BuOH, H_2O; (g) DIPEA, MeI, DMF, 81% (three steps from **117**); (h) THF, EtOH, 82%; (i) (i) PhOCOCl, NEt_3, THF, (ii) NH_4OH, *i*-PrOH, 65% (two steps from **119**); (j) Tf_2O, pyridine, DCM, 73%; (k) TMSCl, MOMCl, DIPEA, DCM, 80%.

of natural 7-*epi*-cylindrospermopsin (**2**) and significantly different from cylindrospermopsin (**1**) (Scheme 13).

A subsequent publication by Weinreb detailed the conversion of a late synthetic intermediate, compound **122** into the newly assigned cylindrospermopsin structure **1** [184]. First, the acetonide functionality of **122** was removed under acid hydrolysis to afford alcohol **128** in 85% yield, and then the stereochemistry of the C-7 alcohol was cleanly inverted by a Mitsunobu process to afford the desired epimer **129**.



Scheme 13 Reagents and conditions: (a) $Pd(OH)_2$, H_2, EtOH, 71%; (b) triphosgene, THF, rt; (c) NaN_3, DMF, 65 °C, 86%; (d) dil. HCl, THF, H_2O, 85 °C, 72%; (e) MeOTf, 2,6-di-*tert*-butylpyridine, DCM, −78 °C to rt; (f) 10% Pd/C, H_2, EtOH; (g) 12 M HCl, 95 °C, 43% (three steps from **125**); (h) $SO_3 \cdot$ DMF, pyridine, Na_2SO_4, DMF, rt, 70% (±**2**).

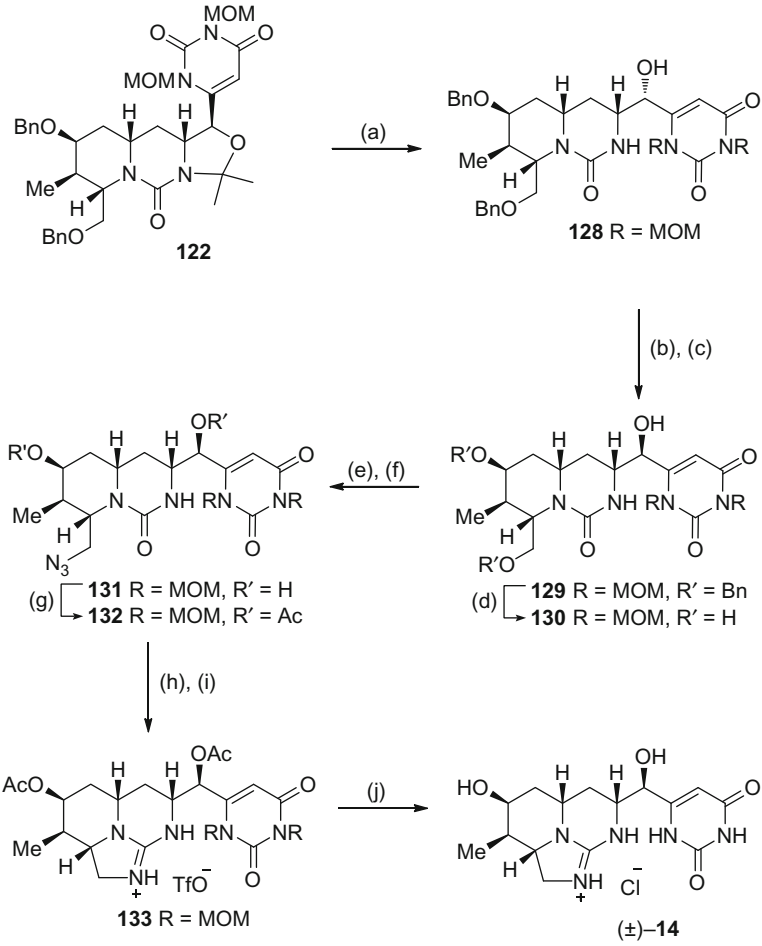
Removal of the two benzyl groups of **129** by hydrogenation over Pearlman's catalyst provided triol **130** that upon treatment with triphosgene followed by sodium azide in DMF cleanly gave azide diol **131**, whose structure and stereochemistry were verified by X-ray crystallography. Conversion of diol **131** to the corresponding diacetate **132** allowed for activation of the urea functionality with methyl triflate followed by hydrogenation to directly furnish guanidinium salt **133**. Finally, acidic hydrolysis of the acetyl and MOM groups afforded tetracyclic diol **14** in 61% overall yield from diacetate **131**, which had spectra identical to those reported by Snider and co-workers [183] in their total synthesis of cylindrospermopsin, thus confirming that the toxin should be represented by structure **1** (Scheme 14). In light of this it also seems unlikely that the toxins exist as uracil tautomers as previously believed, which contributed to the incorrect stereochemical assignment of the C-7 hydroxy group.

Weinreb and co-workers published the first synthesis where the C-7 stereochemistry was definitively assigned *via* X-ray crystallography of their late stage synthetic intermediates. This led to the reassignment of the orientation of the C-7 hydroxy group meaning that cylindrospermopsin is represented by **1** and its epimer by structure **2**. The key reaction in the groups synthesis was their *N*-sulfinyl Diels–Alder cyclization installing the B ring followed by a Grignard ring opening/allylic sulfoxide [2,3]-sigmatropic rearrangement that stereoselectively installed the C-7 hydroxy group (**113**→**115**). This led them to report the first racemic synthesis of 7-*epi*-cylindrospermopsin (±**2**) in 33 steps and 0.26% overall yield, as well as a formal synthesis of racemic cylindrospermopsin (±**1**) from a common late synthetic intermediate in 36 steps and 0.2–0.25 % overall yield.

C. The White Research Group

White and Hansen detailed the first asymmetric total synthesis of 7-*epi*-cylindrospermopsin (**2**), which became their natural target after the toxins stereochemistry was reassigned by Weinreb [9]. They employed a convergent approach splitting the target molecule into two fragments, namely hydroxylamine **140** termed as the "western" fragment and pyrimidine aldehyde **149** termed as the "eastern" fragment. The pivotal step in the synthesis was an intramolecular nitrone cycloaddition between these fragments, which allowed them to establish both the C-10 and C-12 stereochemistry of 7-*epi*-cylindrospermopsin (**2**) [185,186].

The preparation of the group's first substrate began with the mono *p*-bromobenzyl protection of ethylene glycol (**134**) followed by the oxidation of the residual alcohol group with Dess–Martin periodinane to afford aldehyde **135** in 90% yield. Aldehyde **135** successfully

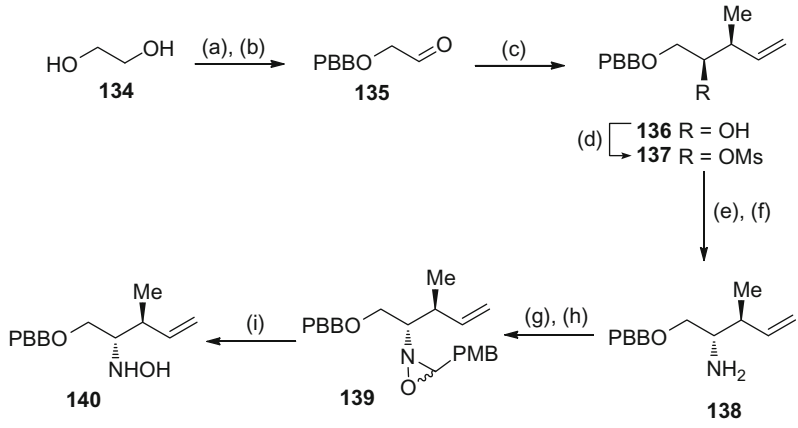


Scheme 14 Reagents and conditions: (a) dil. HCl, THF, H_2O, 85%; (b) PPh_3, *p*-NBA, DEAD, benzene; (c) MeOH, K_2CO_3, 61% (two steps form **128**); (d) $Pd(OH)_2$, EtOH, cyclohexene, 95%; (e) triphosgene, THF; (f) NaN_3, DMF, Δ, 70% (two steps from **130**); (g) Ac_2O, pyridine, DMAP, 78%; (h) MeOTf, 2,6-di-*tert*-butylpyridine, DCM; (i) 10% Pd/C, H_2, EtOH; (j) 12 M HCl, Δ, 61% (three steps from **132**).

underwent asymmetric crotylation [199] to give the *syn* homoallylic alcohol **136** in 94% enantiomeric excess. Treatment of alcohol **136** with methanesulfonic anhydride and pyridine in DCM generated the corresponding mesylate **137** quantitatively. Displacement of the mesylate functionality of **137** with sodium azide, followed by a Staudinger reduction with triphenylphosphine afforded the inverted primary amine **138** in 56% yield. Condensation of amine **138** with *p*-anisaldehyde, followed

by *in situ* oxidation of the resulting imine with *m*-chloroperbenzoic acid gave oxaziridine **139**. Finally, treatment of oxaziridine **139** with hydroxylamine hydrochloride furnished the group's western fragment **140** in 60% yield from amine **138** (Scheme 15).

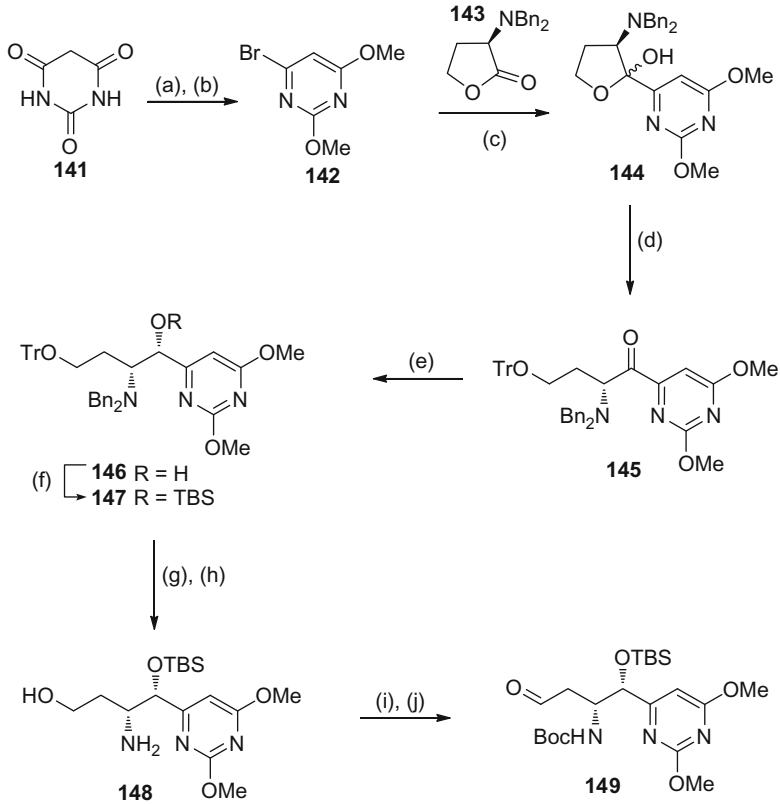
The synthesis of the group's eastern fragment began with the treatment of barbituric acid (**141**) with phosphorus oxybromide and triethylamine followed by exposure of the resulting tribromopyrimidine to two equivalents of sodium methoxide furnishing the desired 4-bromo-2,6-dimethoxypyrimidine (**142**) in high yield. Preparation of γ-lactone **143** was achieved by treatment of (*R*)-(−)-methionine with excess benzyl bromide, which upon recrystalization allowed the desired product to be isolated in >98% enantiomeric excess. Subsequent halogen−metal exchange of bromopyrimidine **142** by treatment with *n*-BuLi followed by exposure of the resulting lithio-pyrimidine to **143** in the presence of cerium trichloride gave lactol **144** in quantitative yield as a mixture of stereoisomers. Treatment of **144** with trityl chloride gave the primary trityl ether **145**; subsequent reduction of the ketone functionality of **145** with L-Selectride at −78 °C gave the major *syn* amino alcohol **146** and its *anti* epimer in the ratio 12:1, respectively. The newly formed hydroxyl group was protected as the corresponding silyl ether **147** in 87% yield. Subsequent treatment with formic acid followed by hydrogenation over Pearlman's catalyst removed both the trityl and benzyl groups to afford alcohol **148**. Boc protection of the newly unmasked secondary amine followed by oxidation generated the desired pyrimidine



Scheme 15 Reagents and conditions: (a) NaH, *p*-$BrC_6H_4CH_2Br$, THF, 65%; (b) Dess−Martin periodinane, DCM, 90%; (c) *cis*-2-butene, *t*-BuOK, *n*-BuLi, (+)-$MeOB(Ipc)_2$, Et_2O/THF, 45%; (d) Ms_2O, pyridine, DCM; (e) NaN_3, DMF, 85 °C; (f) Ph_3P, THF/H_2O, 56% (three steps from **136**); (g) *p*-anisaldehyde, Na_2CO_3, MeOH, 60 °C; (h) *m*-CPBA, DCM, 0 °C to rt; (i) $HONH_2 \cdot HCl$, MeOH, 0 °C to rt, 60% (three steps from **138**).

aldehyde **149** in high yield, completing the synthesis of the eastern fragment (Scheme 16).

The coupling of the eastern and western fragments **149** and **140** proceeded efficiently in refluxing methanol in the presence of 3 Å molecular sieves to remove the water of condensation; under these condition the (*Z*)-nitrone **150** was obtained as a single isomer. Substantial effort was devoted to optimizing the reaction conditions of the key 1,3-dipolar cycloaddition, it was found that toluene was the optimum reaction solvent and that the reaction proceeded in a narrow temperature window. Above 110 °C nitrone **150** decomposed rapidly, whereas below 95 °C there was little reaction, within this temperature range isomerization of the (*Z*)-nitrone to the (*E*)-isomer was observed, this process was



Scheme 16 Reagents and conditions: (a) $POBr_3$, NEt_3, toluene, Δ, 99%; (b) NaOMe, MeOH, 84%; (c) *n*-BuLi, **143**, $CeCl_3$, Et_2O/THF, −78 °C to rt, 97%; (d) TrCl, NEt_3, DMAP, DCM, 93%; (e) L-Selectride, THF, −78 °C, 84%; (f) TBSOTf, NEt_3, THF, 87%; (g) HCO_2H, THF, 100%; (h) $Pd(OH)_2$, H_2, EtOH, 81%; (i) Boc_2O, NEt_3, DCM, 68%; (j) TPAP, NMO, MS, DCM, 91%.

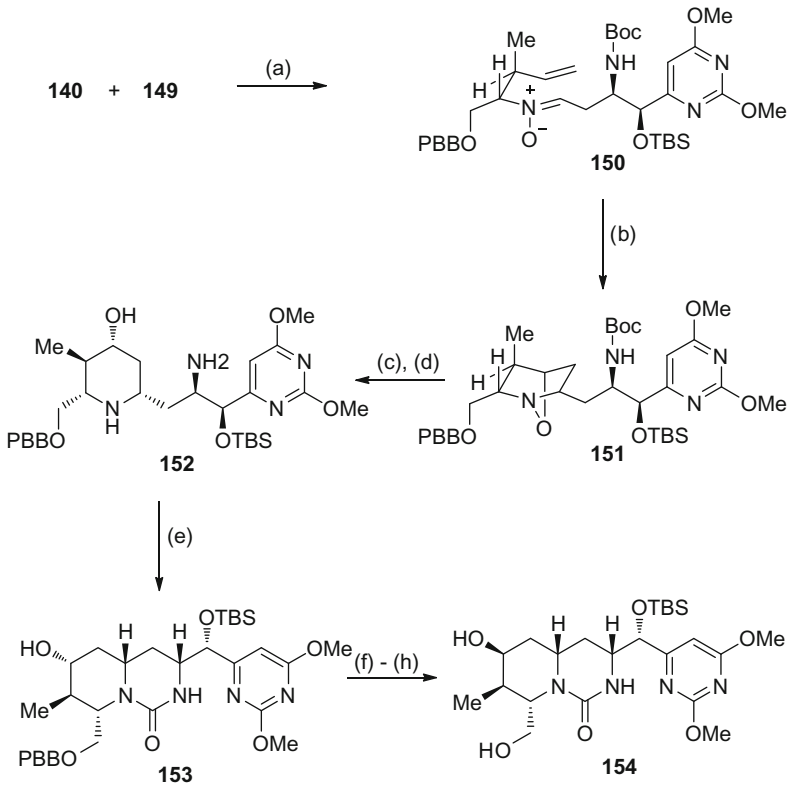
accelerated by Lewis acid catalysts such as 5 M lithium perchlorate, with no benefit to the cycloaddition process. The optimized conditions for intramolecular cycloaddition of **150** gave oxazabicyclo[2.2.1]heptane **151** as a 2:1 mixture of isomers that arose from *exo* and *endo* cycloaddition, respectively. The *in situ* reduction of **151** with zinc and ammonium chloride followed by acidic removal of the Boc group furnished piperidine **152** in 68% from nitrone **150**. Treatment of piperidine **152** with carbonyldiimidazole successfully bridged the piperidine nitrogen and the C-8 amine group generating bicyclic urea **153** in high yield. A short sequence involving oxidation of the piperidyl hydroxyl group of **153** with Dess–Martin periodinane followed by reduction with L-Selectride successfully inverted the hydroxy group to correct the configuration for the natural product. Subsequent cleavage of the *p*-bromobenzyl ether by hydrogenation over palladium hydroxide gave diol **154** containing all six stereocenters of 7-*epi*-cylindrospermopsin (**2**), as was confirmed by X-ray crystallography (Scheme 17).

Treatment of diol **154** with triphosgene followed by exposure to sodium azide in hot DMF gave azide **155** in appreciable yield. Subsequent protection of the remaining alcohol function was achieved by treatment with triethylsilyl triflate giving silyl ether **156** quantitatively. The urea functionality of **156** was then converted to *O*-methylisourea derivative **157** that was hydrogenated immediately over palladium on carbon, reducing the azide functionality to the corresponding primary amine which spontaneously cyclized to afford tricyclic guanidine **158**. Global deprotection of guanidine **158** was achieved with vigorous acid hydrolysis in concentrated HCl producing tetracyclic diol **15** in 21% overall yield from azide **156**. The final step in the synthesis involved the installation of the sulfonic acid moiety, which was achieved *via* known chemistry to afford 7-*epi*-cylindrospermopsin (**2**) as a 2.5:1 ratio with the corresponding *bis* sulfate (Scheme 18).

In conclusion, White and Hansen reported the asymmetric total synthesis of 7-*epi*-cylindrospermopsin (**2**) in 25 steps (longest linear sequence) and 0.39% overall yield using a convergent methodology, with the key step of their synthesis being an intramolecular nitrone 1,3-dipolar cycloaddition (**150**→**151**). The synthesis allowed them to assign the absolute stereochemistry of **2** as 7*S*, 8*R*, 10*S*, 12*S*, 13*R*, and 14*S*.

D. The Williams Research Group

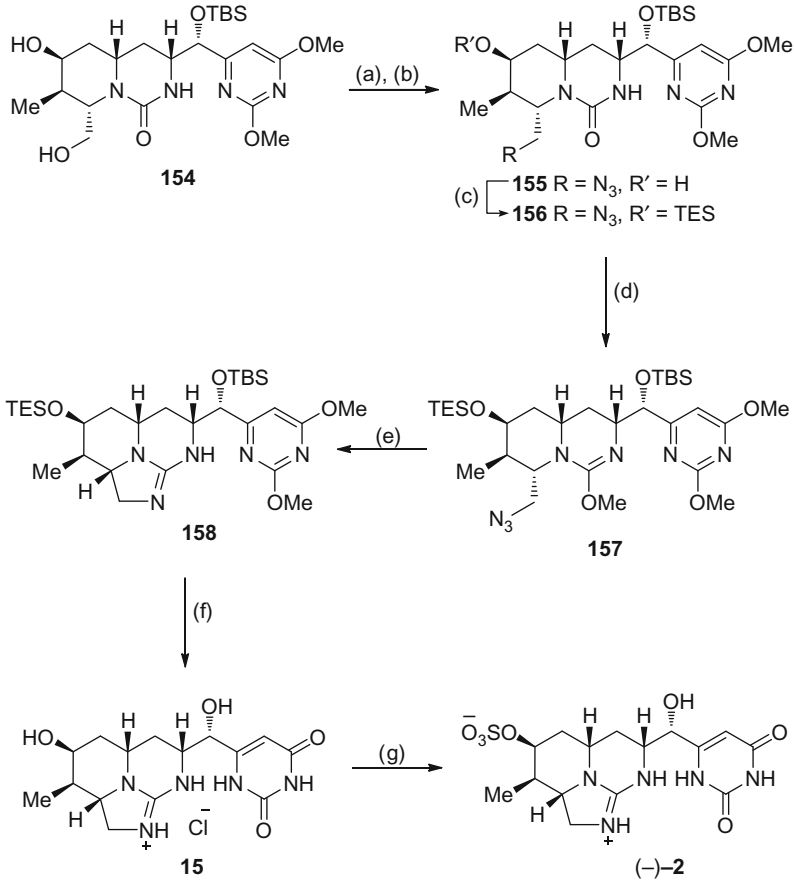
Williams and co-workers have published the most recent work detailing the total synthesis of all three of the currently known cylindrospermopsin alkaloids [7,187,188]. Their syntheses allow for the preparation of the target molecules with the fewest synthetic manipulations and



Scheme 17 Reagent and conditions: (a) MeOH, 3 Å MS, △, 60%; (b) toluene, 3 Å MS, △; (c) Zn, NH_4Cl, THF/H_2O, △; (d) HCl, MeOH, 68% (three steps from **150**); (e) (i) CDI, DCM, (ii) K_2CO_3, MeOH, 85%; (f) Dess–Martin periodinane, DCM, 98%; (g) L-Selectride, THF, 85%; (h) $Pd(OH)_2$, H_2, EtOH, 76%.

utilizing a reductive guanidinylation as one of the key steps in forming the tricyclic guanidine core of each metabolite.

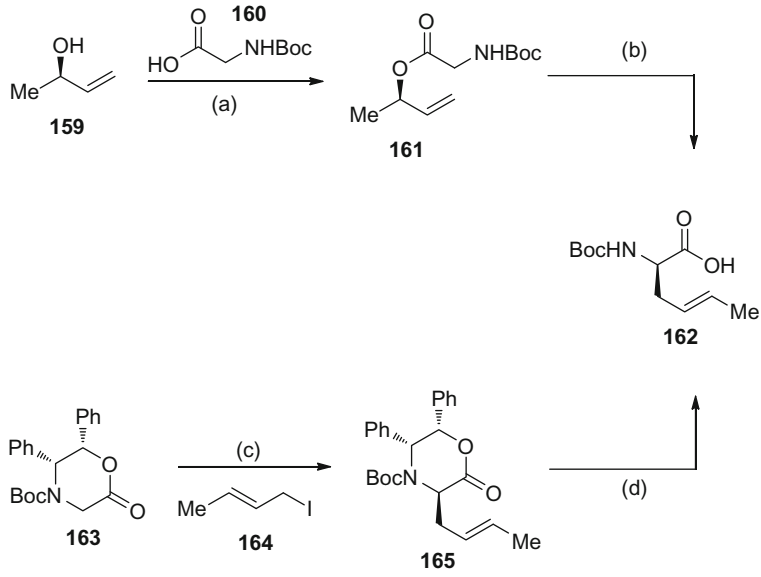
The synthetic approaches reported [7,187,188,200] rely upon the preparation of Boc crotyl glycine derivative **162**, which could be achieved efficiently *via* two different pathways. The first approach involved coupling of (*R*)-3-buten-2-ol (**159**) with Boc glycine (**160**) by treatment with DIC giving ester **161** in high yield. Subsequent enolate-Claisen rearrangement of **161** gave the desired crotyl glycine derivative **162** quantitatively with rearrangement of the optically pure ester proceeding through the chelated *Z*-enolate to give (*R*)-**162** in 92:8 *ee*. Initially the group used this methodology with racemic **159** to generate large quantities of racemic **162** for their initial synthetic investigations. Alternatively, the oxazinone **163** can be alkylated with crotyl iodide **164** to give **165** as a single diastereomer in 92% yield. Removal of the auxiliary with lithium in ammonia delivered



Scheme 18 Reagents and conditions: (a) triphosgene, THF; (b) NaN_3, DMF, 49% (over two steps from **154**); (c) TESOTf, NEt_3, THF, 99%; (d) KHMDS, $Me_3O^+ BF_4^-$, DCM, 0 °C to rt; (e) Pd/C, H_2, EtOH; (f) conc. HCl, △, 21% (three steps from **156**); (g) $SO_3 \cdot$ pyridine, DMF, 63%.

(*R*)-**162** with very high enantioselectivity, making this the group's preferred method for the preparation of intermediate **162** (Scheme 19).

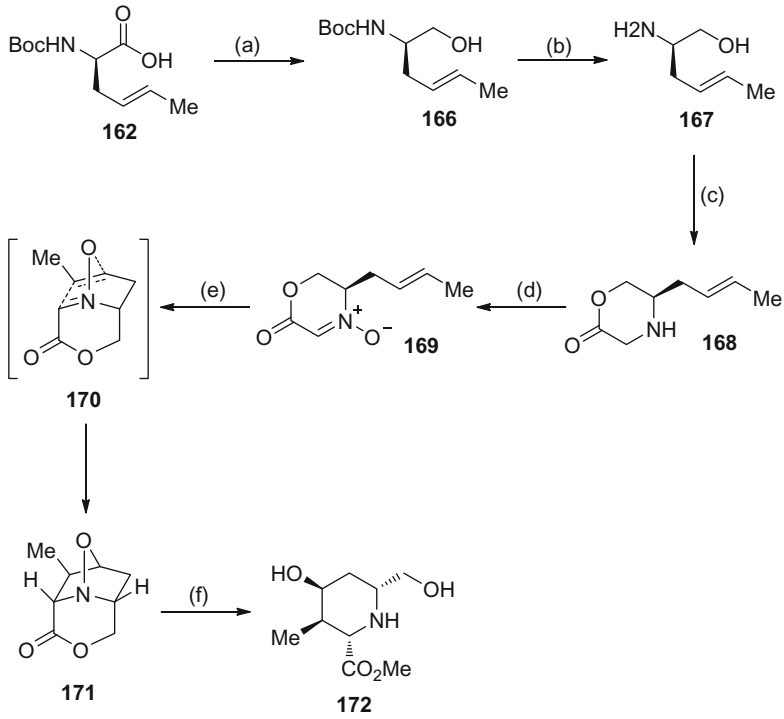
Initially, Looper and Williams investigated the utility of a 1,3-dipolar cycloaddition to stereoselectively construct a suitable A ring synthon for use in the synthesis of the cylindrospermopsin alkaloids [200]. The Boc-protected crotyl glycine derivative **162** was reduced with $LiAlH_4$ at 0 °C to give the corresponding alcohol **166** that upon removal of the Boc-protecting group with boron trifluoride etherate afforded amino alcohol **167**. Treatment of the resulting amino alcohol **167** with phenyl bromoacetate furnished oxazin-2-one **168** in moderate yields. As **168** was prone to



Scheme 19 Reagents and conditions: (a) **160**, DIC, DMAP, DCM, 0 °C, 96%; (b) NaHMDS, THF, 0 °C to rt, 100%; (c) KHMDS, **164**, THF, −78 °C to rt, 92%; (d) Li, NH_3(l), THF, EtOH, 68−87%.

dimerization, oxidation was carried out immediately after isolation using Davis oxaziridine or *m*-CPBA leading to the conjugated oxazinone-*N*-oxide **169** in 75% or 84% yield, respectively. In contrast to **168**, the *N*-oxide **169** was surprisingly stable and no dimerization or spontaneous cyclization was observed. Subsequent exposure of oxazinone-*N*-oxide **169** to elevated temperatures cleanly affected the 1,3-dipolar cycloaddition. It was postulated that the nitrone added suprafacially to the alkene predominantly through the chair-like *exo*-transition state **170** to give the tricyclic isoxazolidine **171** in 78% yield as a 10:1 mixture of regioisomers. Isoxazolidine **171** could also be prepared in the improved ratio of 12:1 by treatment of **168** with scandium triflate although the reaction took 3 days to reach completion at ambient temperatures [188]. The stereochemistry of **171** was confirmed by X-ray crystallography [200]. Finally, hydrogenation of **171** over Pd/C in methanol led directly to ester **172**, which has the correct absolute stereochemistry for the cylindrospermopsin A ring (Scheme 20).

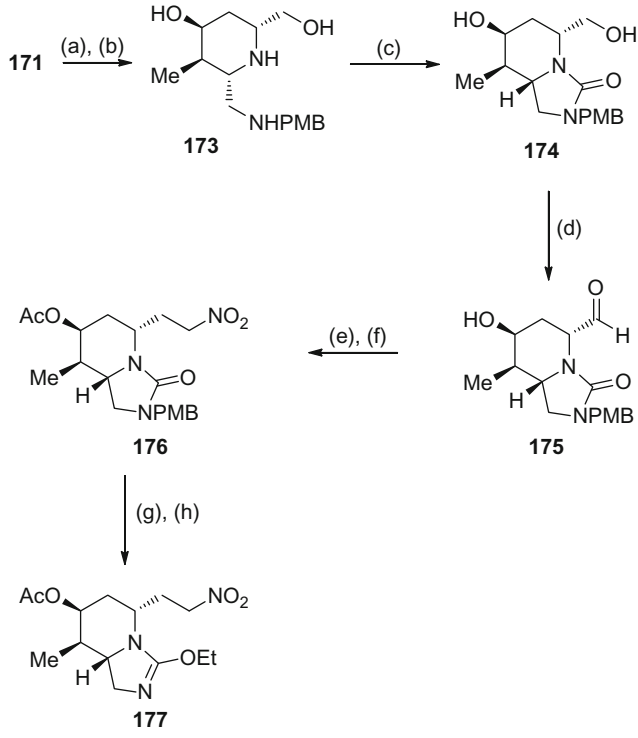
Ester **172** was unsuitable for further modification to the natural products. An alternative strategy involving the reduction of isoxazolidine **171** with DIBAL gave an intermediate lactol which underwent reductive amination with *p*-methoxybenzylamine furnishing diol **173**. Conversion of diol **173** to bicyclic urea **174** was achieved in 67% yield by treatment with bis-*p*-nitrophenyl carbonate in acetonitrile. Oxidation



Scheme 20 Reagents and conditions: (a) $LiAlH_4$, 0 °C, THF, 62–83%; (b) $BF_3 \cdot Et_2O$, DCM, 5 eq. 1,3-dimethoxybenzene; (c) phenyl bromoacetate, DIPEA, MeCN, 63% (two steps from **166**); (d) Davis oxaziridine, THF, 0 °C, 75%, or *m*-CPBA, Na_2HPO_4, DCM, −78 °C, 84%; (e) toluene, 200 °C, sealed tube, 78%; (f) Pd/C, H_2, MeOH, 98%.

of the primary alcohol group in **174** to the corresponding aldehyde **175** proved problematic but could be achieved in high yield by treatment with TEMPO and 1.5 equivalents of DAIB in the presence of 1 mol% methanesulfonic acid. Homologation of the resultant aldehyde **175** by addition of lithiated nitromethane produced a scalemic mixture of nitro alcohols that could be dehydrated by treatment with acetic anhydride, concomitant to acetylating of the piperidyl alcohol group. The newly formed nitroalkenes were then reduced with $NaBH_4$ without purification furnishing nitroalkane **176** in 56% yield from aldehyde **175**. Finally, removal of the *p*-methoxybenzyl protecting group by treatment with refluxing TFA, followed by exposure to triethyloxonium tetrafluoroborate afforded the key reductive guanylation substrate, activated *O*-ethylisourea **177** in 62% yield (Scheme 21).

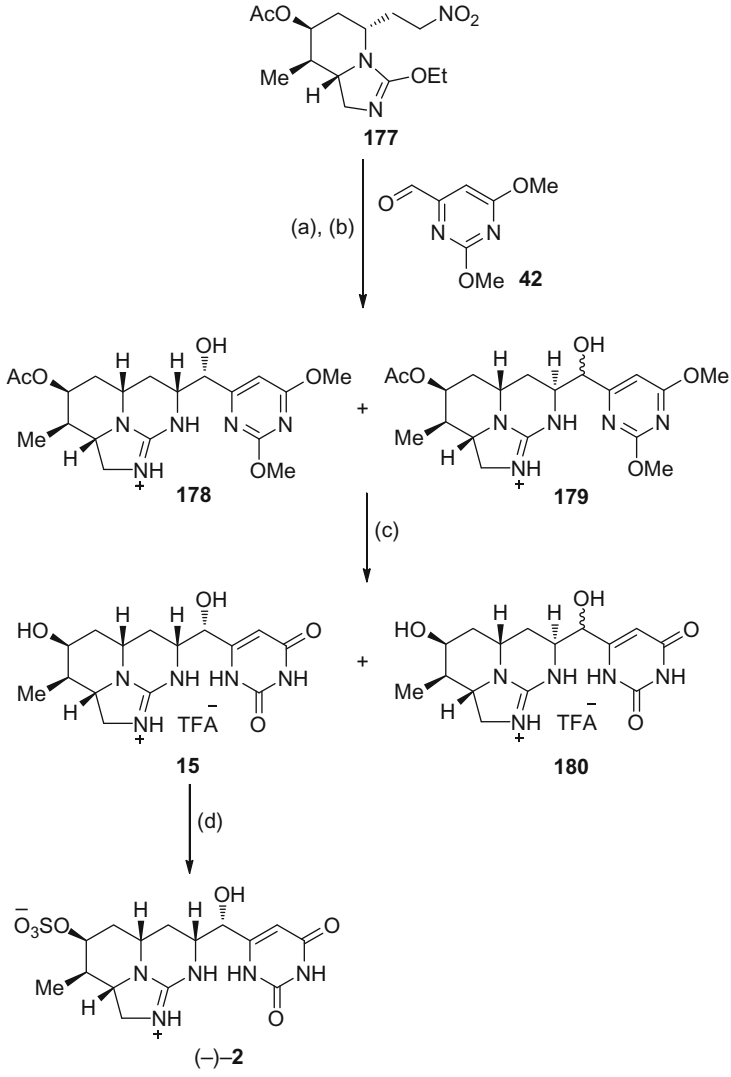
With the *O*-ethylisourea **177** in hand the construction of the C7–C8 bond of the target was undertaken *via* a nitro-aldol (Henry) reaction. Thus, **177** was reacted with 2,6-dimethoxypyrimidine-4-carbaldehyde



Scheme 21 Reagents and conditions: (a) DIBAL, −78 °C; (b) 10% Pd/C, H_2 (1 atm), $PMBNH_2$, EtOAc; (c) (p-$O_2NC_6H_4O)_2CO$, MeCN, 67% (three steps from **171**); (d) 40 mol-% TEMPO, 1.5 eq. $PhI(OAc)_2$, 1 mol% $MeSO_3H$, $CDCl_3$, 75%; (e) $MeNO_2$, n-BuLi; (f) Ac_2O, DMAP then $NaBH_4$, EtOH, 56% (two steps from **175**); (g) TFA, △; (h) Et_3OBF_4, Cs_2CO_3, 62% (two steps from **176**).

(**42**), in the presence of 2 eq. of TBAF for 15 minutes, followed by an acidic quench and reductive guanylation using $Pd(OH)_2/H_2$ in methanol. It was found that short reaction times gave the best selectivity in the nitro-aldol reaction, furnishing the tetracyclic guanidines **178** and **179** as an inseparable 1:0.8 mixture, which favored **178**, the diastereoisomer required for the synthesis of 7-*epi*-cylindrospermopsin (**2**). The acidic quench was also found to be imperative as omitting this step gave an approximate 1:1:1:1 mixture of the four possible diastereomers, indicating that the reaction was highly reversible. Acidic hydrolysis of pyrimidines **178** and **179** furnished a separable mixture of **15** and **180** isolated in 32% and 29% yield, respectively, from **177**. The synthesis was completed by treatment of **15** with sulfur trioxide pyridine complex in the presence of 3 Å molecular sieves reproducibly giving (−)-7-*epi*-cylindrospermopsin (**2**) in 59% yield (Scheme 22).

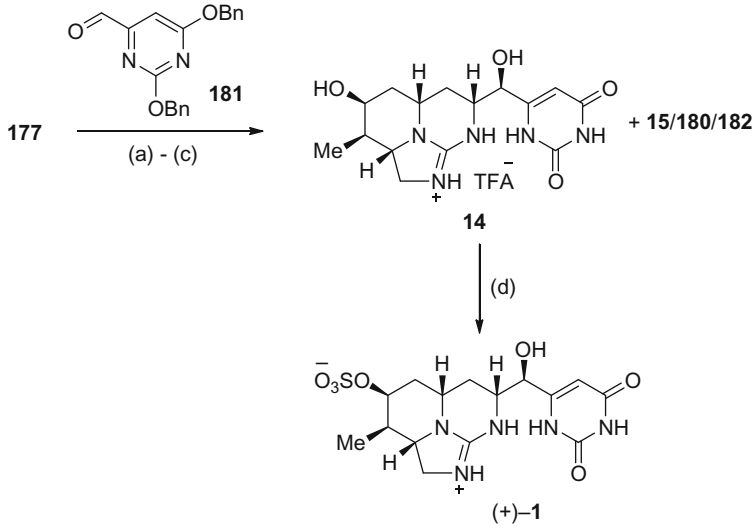
Following this work, the methodology was adapted to the synthesis of cylindrospermopsin (**1**) [188] by modifying the uracil precursor in such a way that when the reductive guanylation is performed, a simultaneous deprotection of the uracil group occurs. Thus, treatment of *O*-ethylisourea **177** with 2,6-dibenzyloxypyrimidine-4-carbaldehyde (**181**) in the presence



Scheme 22 Reagents and conditions: (a) **42**, 2.0 eq. TBAF, THF, −15 °C, 15 min; (b) $Pd(OH)_2$, H_2, MeOH, 5% HOAc; (c) conc. HCl, △, 12 h, 32% (**15**), 29% (**180**) (three steps from **177**); (d) $SO_3 \cdot$ pyridine, 3 Å MS, DMF, 59%.

of 1 eq. of TBAF for 30 minutes, followed by reductive guanylation led to a mixture of diastereomers. Partial cleavage of the acetate protecting group was observed under the reaction conditions, but it was not possible to drive this to completion. Therefore, the mixture was exposed to concentrated HCl briefly to fully remove the acetate. Although this three-step sequence produced an approximate 1:1:1:0.5 mixture of the four possible diasterioisomers **14:15:182:180**, the overall chemical yield was excellent and the cylindrospermopsin diol **14** was isolated in 20% overall yield. Finally, sulfonation under previously reported conditions afforded (+)-cylindrospermopsin (**1**) in 60% yield, representing the first enantioselective synthesis of this metabolite (Scheme 23).

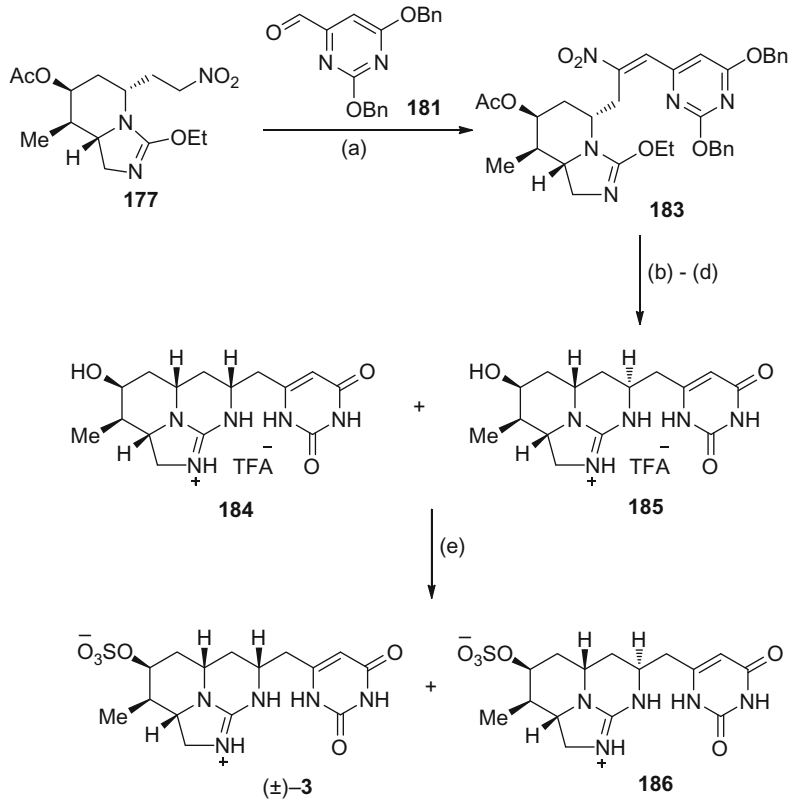
Having completed the synthesis of the two oxygenated cylindrospermopsin alkaloids, effort was directed toward the synthesis of the previously unprepared metabolite 7-*deoxy*-cylindrospermopsin (**3**) [7,188]. Treatment of racemic **177** with 2,6-dibenzyloxypyrimidine-4-carbaldehyde (**181**) in the presence of acetic anhydride and excess cesium fluoride coupled the two units together and allowed dehydration to occur in a single operation, thus affording nitroalkene **183** in 67% yield. Attempts to reduce **183** directly to **184** *via* their previously utilized reductive guanylation conditions returned a complex mixture, containing products arising from hydrolysis of a supposed enamine intermediate. This hydrolysis step was circumvented by subjecting nitroalkene



Scheme 23 Reagents and conditions: (a) **181**, 1.0 eq. TBAF, −15 °C, 0.5 h; (b) $Pd(OH)_2$, H_2, MeOH, 5% AcOH; (c) conc. HCl, Δ, 0.5 h, 20% (**14**) (three steps from **177**); (d) $SO_3 \cdot$ pyridine, 3 Å MS, DMF, 60%.

183 to a two-stage conjugate reduction/reductive guanylation using sodium borohydride followed by hydrogenation over Pearlman's catalyst and a brief HCl deprotection leading to a 1:1 mixture of diastereomers **184** and **185**. The final sulfonation step was simplified by the lack of a C-7 hydroxyl group affording racemic 7-*deoxy*-cylindrospermopsin (±**3**) and **186** in a combined yield of 66% (Scheme 24).

The methodology developed by Williams represents the most efficient to date, in terms of numbers of steps and overall yields and uses the least number of protecting group manipulations. It has led to the first enantioselective synthesis of cylindrospermopsin (+**1**) in 19 steps and 0.34−0.57% overall yield, 7-*epi*-cylindrospermopsin (−**2**) in 19 steps and 0.47−0.82% overall yield, as well as the first racemic synthesis of the biosynthetic intermediate 7-*deoxy*-cylindrospermopsin (±**3**) in 20 steps and 0.62−1.05% overall yield. The key reactions of these total



Scheme 24 Reagents and conditions: (a) CsF, Ac_2O, MeCN, 67%; (b) $NaBH_4$, EtOH; (c) $Pd(OH)_2$, H_2, MeOH, 5% AcOH; (d) conc. HCl, △, 0.5 h; (e) $SO_3 \cdot$ pyridine, 3 Å molecular sieves, DMF, 33% (±**3**) (four steps from **183**).

syntheses are the 1,3-dipolar cycloaddition used to construct the A ring of these metabolites (**169**→**170**) and a Henry reaction of an elaborated nitroalkane with a pyrimidine aldehyde (**177**→**178**/**14**/**185**) followed by intramolecular reductive guanylation to form the B ring.

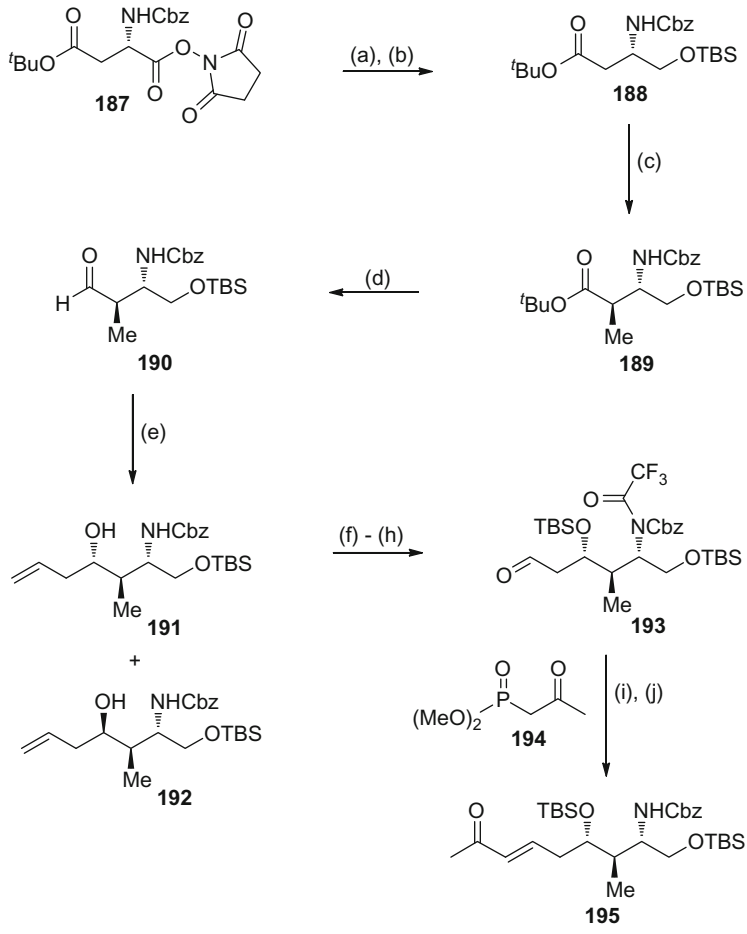
X. MODEL SYSTEMS

In addition to the total syntheses reported, several groups have detailed diverse methodologies for the preparation of model compounds directed toward the synthesis of cylindrospermopsin alkaloids [189–192].

A. The Armstrong Research Group

Armstrong and McAlpine reported a stereoselective synthesis of a tricyclic guanidinium moiety similar to that found in cylindrospermopsin alkaloids. They employed an intramolecular conjugate addition reaction as a key step in the creation of the piperidine A ring, combined with a Mitsunobu strategy to construct both the B and C rings of the tricycle [189]. The $NaBH_4$ reduction of the commercially available activated aspartic acid derivative **187** followed by silyl ether protection afforded the ester **188** in 93% yield. Subsequent addition of methyl iodide to the dianion of **188** at −78 °C gave **189** as the major product. Reduction of the *tert*-butyl ester of **189** with DIBAL gave aldehyde **190** that was allylated using allyl (−)-isopinocampheylborane giving alcohols **191** and **192** in a 2:1 ratio and 70% yield. This transformation could also be achieved by treatment with allyltributyltin and boron trifluoride etherate in superior yield but with a diminished diastereoselectivity, producing **191** and **192** in a 1.5:1 ratio. Subsequent protection of the alcohol and carbamate groups allowed ozonolysis to proceed smoothly, generating aldehyde **193** in 91% yield. HWE olefination of aldehyde **193** with phosphonate **194** successfully furnished the key cyclization substrate, enone **195** in 64% yield after workup with methanolic sodium carbonate (Scheme 25).

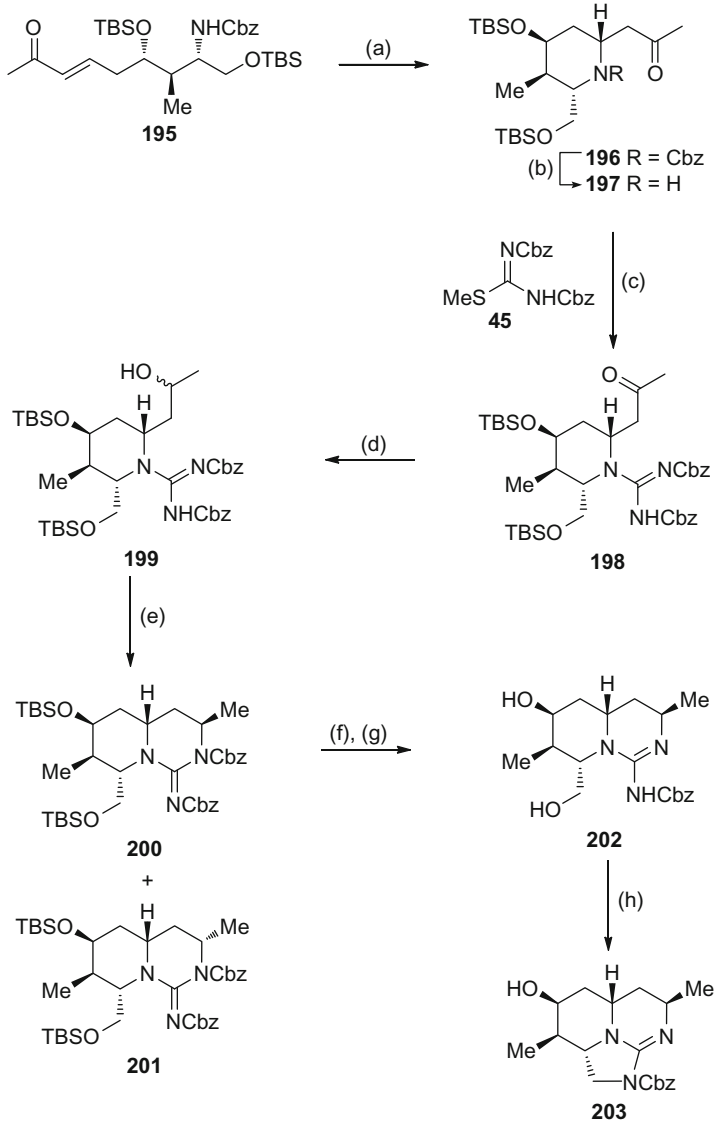
Having prepared enone **195**, the key cyclization reaction was effected by treatment with a catalytic amount of *p*-TsOH in refluxing benzene, giving Cbz protected pyrimidine **196** as a single diastereomer having all four stereocenters of the cylindrospermopsin A ring in place. Hydrogenation of **196** quantitatively generated the corresponding free piperidine **197**, subsequent guanylation of which with methylthioisourea **45** gave the corresponding *bis*-Cbz guanidine **198** in 85% yield. Reduction of the methyl ketone function of guanidine **198** was conducted under non-asymmetric conditions giving an inseparable mixture of alcohols **199**, which on cyclization under Mitsunobu conditions gave bicyclic



Scheme 25 Reagents and conditions: (a) $NaBH_4$, THF, 0 °C; (b) TBSOTf, NEt_3, 2,6-lutidine, 93% (two steps from **187**); (c) (i) LDA, LiCl, THF, −78 °C, (ii) MeI, 72%; (d) DIBAL, toluene, −78 °C, 89%; (e) $BF_3 \cdot OEt_2$, allylBu_3Sn, DCM, −78 °C, 51% (**191**), 33% (**192**); (f) TBSOTf, 2,6-lutidine, 100%; (g) TFAA, NEt_3, 90%; (h) O_3, PPh_3, DCM, −78 °C, 91%; (i) LDA, **194**, THF; (j) Na_2CO_3, MeOH, 64%.

guanidines **200** and **201** in a 5:1 ratio and 75% combined yield. Unfortunately, it was the minor bicyclic product **201** that was shown to display the desired stereochemistry in the newly formed B ring. Despite this, the feasibility of the approach was demonstrated using the major product **200** to construct the C-ring found in the parent alkaloids. Selective removal of one Cbz group with sodium hydride in a 1:1 mixture of THF and methanol, followed by treatment with TBAF furnished bicyclic diol

202. A further Mitsunobu cyclization using triphenylphosphine and DIAD installed the final C ring giving tricyclic guanidine **203** as the only isolated product in 27% yield (Scheme 26).



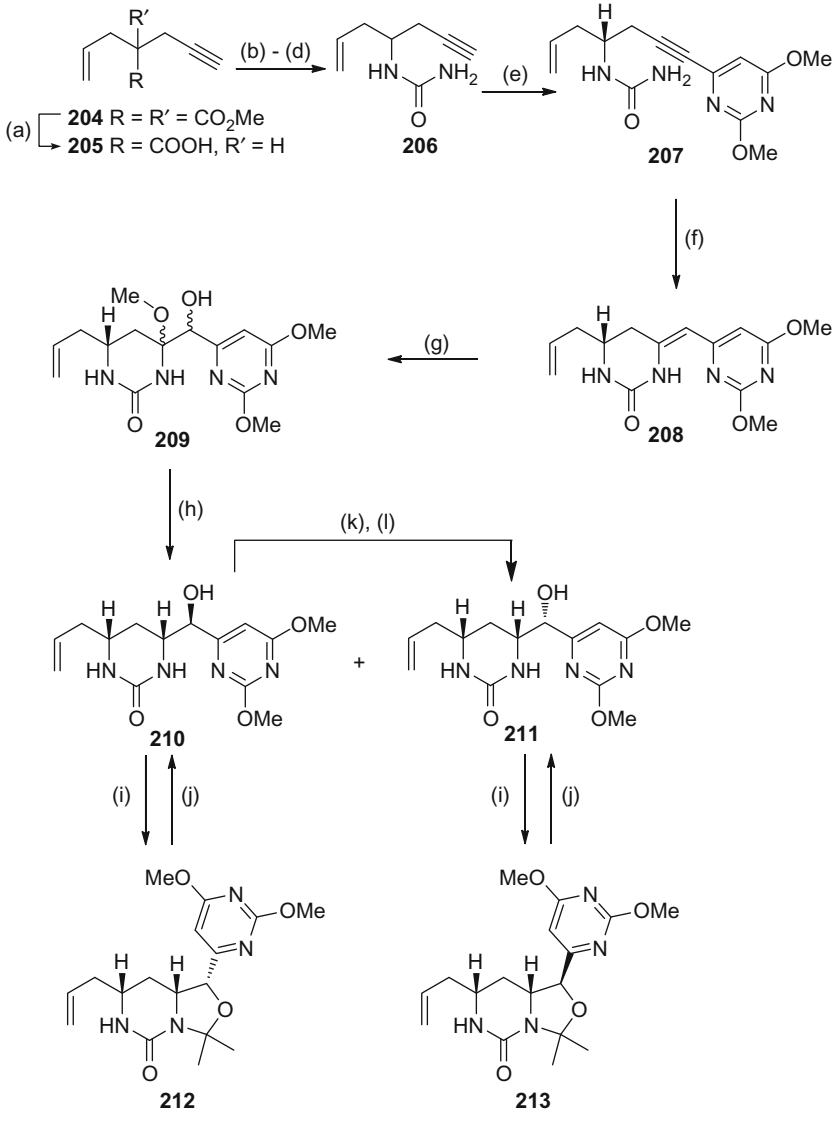
Scheme 26 Reagents and conditions: (a) *p*-TSA, benzene, 74%; (b) Pd/C, H_2, 100%; (c) **45**, $HgCl_2$, NEt_3, DMF, 85%; (d) $NaBH_4$, MeOH, 100%; (e) PPh_3, DIAD, 63% (**200**), 12% (**201**); (f) NaH, THF, MeOH, 67%; (g) TBAF, THF, 84%; (h) PPh_3, DIAD, 27%.

B. The Hart Research Group

A further synthetic approach toward cylindrospermopsin (**1**) was reported by Hart and co-workers, who developed a methodology for the synthesis of the BD ring system employing an intramolecular conjugate addition of urea to an alkynyl pyrimidine as the key reaction [190]. Saponification of the known diester **204**, followed by acidification and decarboxylation furnished carboxylic acid **205** in 90% yield which was converted into alkynyl urea **206** in high overall yield via a Curtius rearrangement, trapping the intermediate isocyanate with ammonia. Coupling of **206** with 6-bromo-2,4-dimethoxypyrimidine (**142**) under Sonogashira conditions gave alkynyl pyrimidine **207** in 90% yield. The key step of the sequence was affected by the treatment of **207** with sodium hydride in THF resulting in the clean formation of cyclic urea 2**08** in very high yield. Treatment of **208** with DMDO in the presence of methanol gave an unstable mixture of *N*,*O*-acetals **209**, which could be reduced with $NaCNBH_3$ to give a 78:22 mixture of isomeric alcohols **210** and **211** which were inseparable by column chromatography. Separation was achieved by conversion of the mixture of alcohols to the corresponding cyclic *N*,*O*-acetals **212** and **213**, which were isolated in 58% and 16%, respectively, after column chromatography. Regeneration of the alcohols was then possible by acidic hydrolysis to give **210** and **211** in 70% and 60% yields, respectively. The major isomer **210** was then converted to epimeric alcohol **211** *via* a Mitsunobu inversion procedure (Scheme 27).

C. The Troin Research Group

A more recent approach toward this family of secondary metabolites has been detailed by Henon and Troin who successfully constructed an ABD ring system comparable to that found in this family of natural products. Key steps in their sequence involve an intramolecular Mannich reaction to form the A ring, followed by addition of guanidine to an activated double bond to form the B ring [191]. Starting from the commercially available methyl-3-oxopentanoate (**214**), the keto function of which was protected with ethylene glycol followed by $LiAlH_4$ reduction of the methyl ester to afford alcohol **215** in 85% yield. Oxidation of alcohol **215** with manganese dioxide gave the corresponding aldehyde, which underwent Wittig olefination to give unsaturated ester **216** in 56% yield. Diastereoselective conjugate addition of lithiated (*R*)-*N*-benzyl-1-phenylethylamine (**217**) to this ester was achieved in 72% yield, which was followed by hydrogenation over Pearlman's catalyst to give amine **218** in 75% yield. The key intramolecular Mannich reaction of amine **218** with benzaldehyde under conditions previously developed by the group cleanly afforded a 3:2 mixture of piperidines

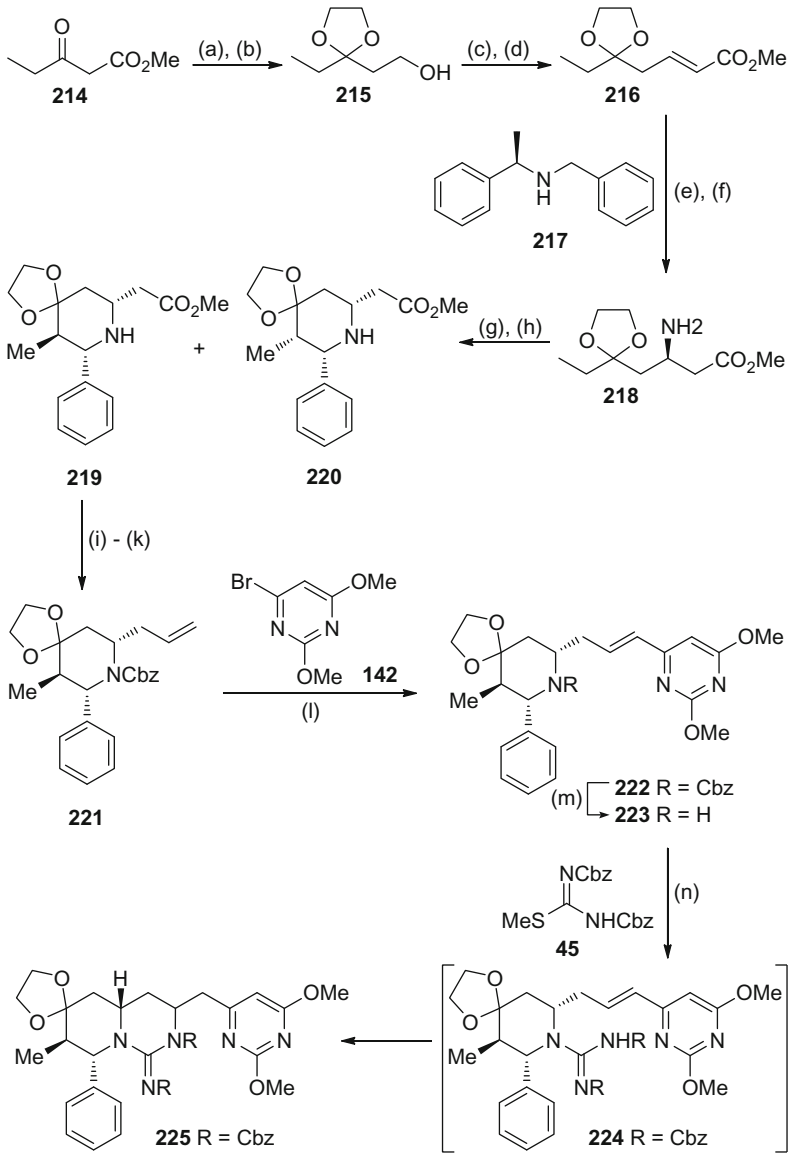


Scheme 27 Reagents and conditions: (a) (i) NaOH, (ii) H_3O^+, (iii) $\triangle$; (b) $(COCl)_2$; (c) NaN_3, $\triangle$; (d) NH_3, 86% (three steps from **204**); (e) $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, 6-bromo-2,4-dimethoxypyrimidine (**142**), 90%; (f) NaH, THF, 97%; (g) DMDO, MeOH/DCM, acetone; (h) $NaCNBH_3$, MeOH, H_2O, pH 4, 97% (**210** and **211**: 78:22) (two steps from **208**); (i) $Me_2C(OMe)_2$, CSA, 58% (**212**) 16% (**213**) (three steps from **208**); (j) $HCl_{(aq)}$, 70% (**210**) 60% (**211**); (k) *p*-NBA, DEAD, PPh_3, THF, $\triangle$; (l) K_2CO_3, MeOH, 91% (two steps).

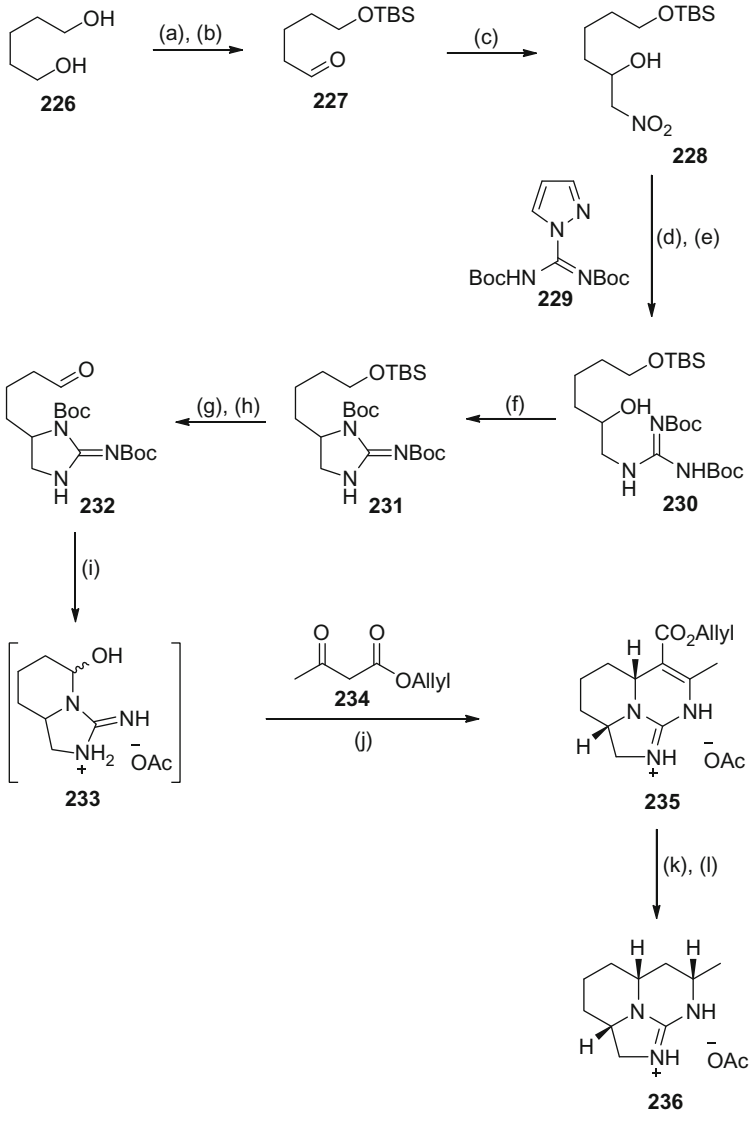
219 and **220**, which were isolated in 62% yield and separated chromatographically. The major isomer **219** that displayed the correct orientation around the piperidine A ring was used by the group in the following reactions. Subsequent Cbz protection of the secondary amine functionality of **219**, followed by DIBAL reduction, furnished the corresponding aldehyde, which was used without purification in a Wittig olefination with methyl triphenylphosphonium bromide giving piperidine **221** in 55% yield over three steps. Heck cross-coupling of **221** with 4-bromo-2,6-dimethoxypyrimidine (**142**) gave the desired piperidine **222** in poor yield of 31%, which could not be improved by modification of the reaction conditions. Removal of the carbamate protecting group was achieved by exposure to TMSI in MeCN giving the corresponding free piperidine **223** in 67% yield. Treatment of piperidine **223** with methylthioisourea **45** in the presence of $HgCl_2$ successfully guanylated the hindered piperidine nitrogen and the resulting guanidine **224** spontaneously underwent cyclization to give bicyclic guanidine **225** in 45% yield on attempted purification on silica gel (Scheme 28).

D. The Murphy Research Group

The most recent approach toward the synthesis of cylindrospermopsin (**1**) employs a biomimetic approach in the preparation of a tricyclic guanidinium unit similar to that found in this class of natural products, with the key stage of the synthesis being a tethered intramolecular Biginelli condensation [192]. Commercially available 1,5-pentane diol (**226**) was monosilylated and the remaining alcohol oxidized under Swern conditions giving aldehyde **227** in high yield. A subsequent nitro-aldol reaction between aldehyde **227** and nitromethane gave the corresponding β-hydroxy nitro compound **228**. Reduction of the nitro function of **228** to the corresponding primary amine followed by treatment with the guanylating agent **229** generated the protected guanidine **230** in 78% yield. Cyclodehydration by treatment with I_2, PPh_3, and imidazole in DCM at $-20\,^{\circ}C$ successfully generated the C ring heterocycle **231** in near quantitative yield. Removal of the silyl protecting group with TBAF followed by oxidation with Dess–Martin periodinane furnished the aldehyde **232**. Removal of the Boc-protecting groups proceeded smoothly upon exposure to acetic acid yielding the hemiaminal **233**, this intermediate was then condensed with allyl acetoacetate **234** under tethered Biginelli conditions successfully generating the tricyclic guanidine **235** in 43% overall yield from **232**. Finally, removal of the ester and double bond functionality was achieved by treatment with a catalytic amount of $Pd(PPh_3)_4$ in THF/MeOH, followed by reduction of the enamine intermediate with $NaCNBH_3$ under acidic conditions giving the saturated tricyclic guanidine **236** in 57% yield (Scheme 29).



Scheme 28 Reagents and conditions: (a) $(CH_2OH)_2$, *p*-TSA, toluene; (b) $LiAlH_4$, THF, 85% (two steps from **214**); (c) MnO_2; (d) $Ph_3P{=}CHCO_2Me$, 56% (two steps from **215**); (e) **217**, BuLi, 0 °C, 72%; (f) $Pd(OH)_2$, H_2 (5 atm), MeOH/H_2O/AcOH, 75%; (g) $MgSO_4$, PhCHO, DCM, △; (h) *p*-TSA, toluene, 65 °C, 62% 3:2 (**219**:**220**) (two steps from **218**); (i) CbzCl, Na_2CO_3; (j) DIBAL; (k) $MePPh_3^+Br^-$, *n*-BuLi, THF, 0 °C, 55% (three steps from **219**); (l) **142**, $Pd(OAc)_2$, PPh_3, Na_2CO_3, DMF, 31%; (m) TMSI, MeCN, 67%; (n) **45**, $HgCl_2$, NEt_3, DMF, 0 °C, 45%.



Scheme 29 Reagents and conditions: (a) TBSCl, NaH, THF, 2 h, 61%; (b) oxalyl chloride, DMSO, NEt_3, DCM, −78 °C, 3 h, 89%; (c) $MeNO_2$, DIPEA, DCM, 36 h, 81%; (d) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, NEt_3, 0 °C, 3 h; (e) **229**, 48 h, 78% (two steps from **228**); (f) PPh_3, I_2, imidazole, DCM, −20 °C, 1 h, 96%; (g) TBAF, THF, 0 °C to rt, 24 h, 99%; (h) Dess–Martin periodinane, pyridine, DCM, 24 h, 96%; (i) AcOH, 24 h; (j) morpholinium acetate, **234**, Na_2SO_4, CF_3CH_2OH, 70 °C, 12 d, 43%; (k) $Pd(PPh_3)_4$, pyrrolidine, THF/MeOH, 1.5 h; (l) $NaBH_3CN$, AcOH/MeOH, 16 h, 57% (two steps from **235**).

Of the model systems reported, the work of Murphy and Evans represents the most concise method (12 steps) for the construction of the tricyclic core of the alkaloids, introducing two ring systems (A and B) and the requisite overall ring stereochemistry in three steps and in a convergent manner. However, the introduction of the A ring substituents and the hydroxyl-uracil fragments of the molecule need to be addressed in order for this methodology to be of use.

XI. CONCLUSION AND IDEALITY IN SYNTHESIS

The cylindrospermopsin family of alkaloids display potent toxicity toward several organs *in vivo* and are suspected carcinogens. These properties alone make its effective removal from drinking water of paramount importance. Additionally, the widening occurrence of these metabolites combined with the increase in reports of the number of cyanobacterial species known to produce them has led the Environment Protection Agency (EPA) to place cylindrospermopsin on their list of "unregulated contaminants." Furthermore, the World Health Organization (WHO) is working toward establishing a drinking water guideline value for the presence of cylindrospermopsin.

These structurally unique alkaloids have also generated a great deal of interest from synthetic chemists, which has led to four different groups reporting elegant multi-step total syntheses of all three metabolites. Their unique molecular architecture, particularly the high density of functional groups and stereocenters present in a relatively small molecule makes them particularly challenging targets. As such, it is difficult to judge the efficiency and ease of total synthesis as the goals of the practitioner are not to optimize each step to an industrial level and the ease of a transformation is often dependent on scale. Certain factors such as number of synthetic steps, yields, and number of protecting group steps employed are used as guidelines but these are sometimes not reliable methods of judgment.

The issue of efficiency in chemical synthesis was first addressed by Hendrickson when he defined "ideal synthesis" [201]:

> "The ideal synthesis creates a complex molecule ... in a sequence of only construction reactions involving no intermediary refunctionalisations, and leading directly to the target, not only its skeleton but also its correctly placed functionality."

Numerous attempts have been made to quantify and evaluate various parameters of efficiency [202–205] and economy in chemical synthesis [206–208]. Recent work by Baran and Gaich of the Scripps Research Institute has reported an attempt to quantify numerically the

Table 3 Percentage ideality of published cylindrospermopsin syntheses

Target	Research group	Reference	Steps	Chemical yield (%)	Nonstrategic redox	PG-manipulation	FGI	Strategic redox	Construction reactions	% Ideality
Cylindrospermopsin	Snider[a](2000)	[183]	20	1.50−2.00	2	8	1	2	7	45%
	Weinreb[a](2002)	[184]	36	0.20−0.25	3	12	8	2	11	36%
	Williams (2006)	[188]	19	0.34−0.57	3	5	2	0	9	47%
7-epi-cylindrospermopsin	Weinreb[a] (2001)	[9,188]	33	0.26	3	10	8	2	10	36%
	Williams (2004)	[187,188]	19	0.47−0.82	3	5	2	0	9	47%
	White (2002)	[185,186]	25	0.39[b]	3	9	5	2	6	32%
7-deoxy-cylindrospermopsin	Williams[a] (2005)	[7,188]	20	0.62−1.05	3	5	2	1	9	50%

[a]Racemic.
[b]Based on longest linear sequence.

ideality of a chemical synthesis [209]. The purpose of their work is to aid practitioners of synthesis to easily make comparisons and pinpoint areas of improvement. Thus, they determined the percentage "ideality" of synthesis with the following equation:

$$\%\text{ideality} = \frac{[(\text{No. of construction reactions}) + (\text{No. of strategic redox reactions})]}{(\text{total No. of steps})} \times 100$$

Construction reactions, as defined by Hendrickson, are those which form skeletal bonds (C−C and C−heteroatom). *Strategic redox reactions* (another form of construction reactions) have previously been defined as those that directly establish the correct functionality found in the final product. All other reactions fall into the category of a *concession step*: (1) *nonstrategic redox manipulations* (2) *functional group interconversions*, and (3) *protecting group manipulations*.

Although the concept of percentage ideality does not provide an ultimate measure of synthesis, it does provide a method for comparing the syntheses of the same target molecule. We applied this method to the reported synthesis of cylindrospermopsin, 7-*epi*-cylindrospermopsin, and 7-*deoxy*-cylindrospermopsin and the results are shown in Table 3.

It is pleasure to note that all the reported synthetic routes score above 30% efficiency, with the most efficient being those of Williams et al., while the highest overall yield of cylindrospermopsin was achieved by Snider, although this synthesis was racemic. As one might predict, the synthetic routes with the fewest steps and the least protecting group manipulations have the highest efficiency and overall yields. It is hoped that this review and the excellent work of the chemists we have featured will point the way to a more detailed understanding of this fascinating group of metabolites and ultimately lead to more valuable synthetic methodology.

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CHAPTER 2

The Pyrrolo[2,1-*a*]isoquinoline Alkaloids

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I. INTRODUCTION

The present chapter describes the isolation and syntheses of natural products with a pyrrolo[2,1-*a*]isoquinoline framework. In addition, total syntheses of some structural analogs of these alkaloids are discussed as well. Natural products containing a pyrrolo[2,1-*a*]isoquinoline core with additionally annulated rings are not considered.

Pyrrolo[2,1-*a*]isoquinolines have been synthesized long before they were isolated as natural products. The interest in novel syntheses of compounds with this structural framework increased tremendously when the first report of their antitumor activity appeared [1]. In 2002, Zhao and co-workers described the isolation of five new alkaloids from the extracts of *Carduus crispus* L. which are known to have antitumor activity. Two of these alkaloids have a pyrrolo[2,1-*a*]isoquinoline skeleton and were named crispine A [(+)-**1**] and crispine B (**2**) (Figure 1) [1].

In 2004, Cai and co-workers isolated trolline [(−)-**3**] a new pyrrolo[2,1-*a*]-isoquinoline alkaloid from the Chinese plant *Trollius chinensis* Bunge [2]. Independently, Ding and co-worker isolated the same natural product from another source and named the compound salsoline A [(−)-**3**] [3]. The isolation of the opposite enantiomer (+)-**3** was published by Du and co-workers in 2005. They named this alkaloid oleracein E [(+)-**3**] [4]. A regioisomer with the two hydroxyl groups at C-9 and C-10 instead of C-8 and C-9, named salsoline B (**4**), has been isolated by Xiang et al. in 2007 (Figure 2) [5].

Natural products containing a pyrrolo[2,1-*a*]isoquinoline framework have been known for a long time. However, until 2002, except one report [6], they have always been incorporated in larger ring systems. Prominent examples are the lamellarins that were isolated first by Faulkner and co-workers in 1985 (Figure 3) [7]. These polycyclic natural products from marine invertebrates show interesting biological activities [8].

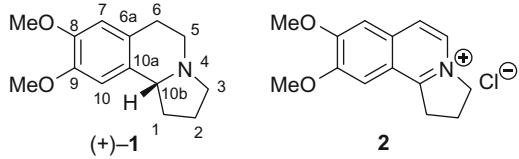


Figure 1 Crispine A [(+)-**1**] with pyrrolo[2,1-*a*]isoquinoline numbering and crispine B (**2**).

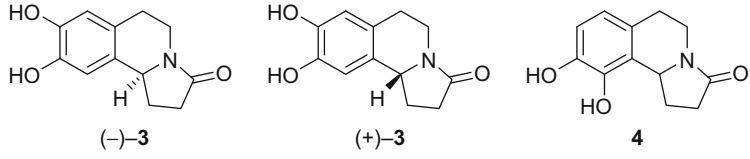
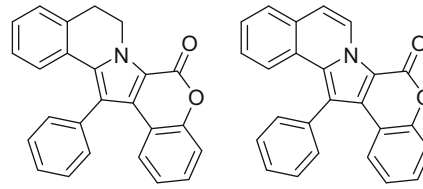
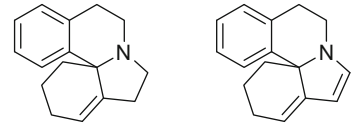


Figure 2 Trolline (salsoline A) [(−)-**3**], oleracein E [(+)-**3**], and salsoline B (**4**).



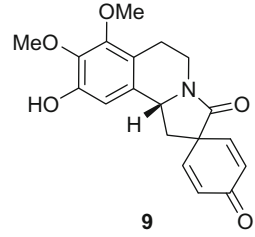
5 **6**

Figure 3 Structural motif of lamellarins type I **5** and type II **6** [8].



7 **8**

Figure 4 Structural motifs **7** and **8** of the erythrina alkaloids [11].



9

Figure 5 Annosqualine (**9**).

Further natural products that may also contain a pyrrolo[2,1-*a*]-isoquinoline core are erythrina alkaloids, first isolated by Folkers and co-workers in 1937 (Figure 4) [9]. These alkaloids are interesting due to their polycyclic structures and biological activities [10]. More information about lamellarins and erythrina alkaloids can be found in recently published reviews [8,10–13]. Spiro-annulated compounds are known as well, e.g., annosqualine (**9**), isolated by Wu and co-workers from the stem of *Annona squamosa* L. in 2004 (Figure 5) [14].

II. ISOLATION, BIOGENESIS, AND BIOLOGICAL ACTIVITY

The first report on non-annulated pyrrolo[2,1-*a*]isoquinoline alkaloids dates back to 1968 [6]. Kapadia and Fales identified peyoglutam (**10**)

and mescalotam (**11**) in the nonbasic fractions of a peyote cactus (Figure 6). The molecular formulae were determined by HR-MS. The structures were assigned by comparison of the mass spectra and gas–liquid chromatography retention times of both natural products **10** and **11** with synthetic samples (cf. Scheme 6). No further reports on these two alkaloids have been published since then.

A. Isolation of the Crispines

It was not until three decades later that further alkaloids with a pyrrolo[2,1-*a*]isoquinoline framework were isolated from a natural source. In 2002, Zhao and co-workers described the extraction of the natural products (+)-8,9-dimethoxy-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline [(+)-**1**] named crispine A [(+)-**1**] and 8,9-dimethoxy-2,3-dihydro-1*H*-pyrrolo[2,1-*a*]isoquinolinium chloride (**2**) named crispine B (**2**) from the ethanol fraction of *C. crispus* L. (Figure 1). This thistle, collected in Hohhot, China, in August, has been used in Chinese folk medicine for the treatment of cold, stomach ache, and rheumatism [1]. The two pyrrolo[2,1-*a*]isoquinoline alkaloids were isolated together with three new isoquinoline alkaloids called crispine C (**12**), crispine D (**13**), and crispine E (**14**) (Figure 7) [1].

The structures of the five novel natural products **1**, **2** and **12**–**14** were assigned based on advanced NMR measurements (including ^{1}H, ^{13}C with DEPT, COSY, HMBC, and HMQC spectra) and HRSI-MS experiments. A value of $[\alpha]_D^{25}$=+91.0 (HOMe) is reported for the optical

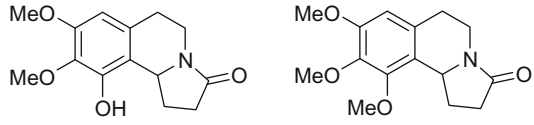


Figure 6 Peyoglutam (**10**) and mescalotam (**11**).

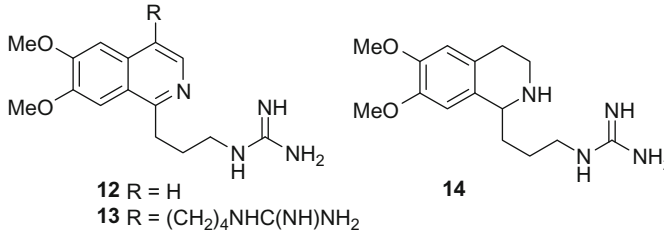


Figure 7 Crispine C–E (**12**–**14**).

rotation of crispine A [(+)-**1**]. Later on, asymmetric syntheses of the two enantiomeric forms of crispine A [(+)-**1** and (−)-**1**] led to the determination of the absolute configuration for the natural product as (+)-(*R*)-crispine A [(+)-(*R*)-**1**] [15,16]. No value of optical rotation is reported for the chiral crispine E (**14**).

In 2004, Jia and Xie isolated crispine B (**2**) and crispine C (**12**) from the same plant source and named the two compounds carcrisine A (**2**) and carcrisine B (**12**) without giving reference to the first isolation [17]. Nevertheless, in later publications the same authors called these alkaloids crispines [18].

The three isoquinoline alkaloids **12**−**14** are structurally related to crispine A [(+)-**1**] and crispine B (**2**). Thus, formation of these natural products via a common biogenetic pathway can be assumed (cf. Section II.E).

B. Isolation of Trolline and Salsoline A

Trolline or (*S*)-8,9-dihydroxy-1,2,5,6-tetrahydropyrrolo[2,1-*a*]isoquinolin-3(10b*H*)-one [(−)-**3**] was isolated independently by Zhao and Ding [3] and by Cai and co-workers [2] in 2004. Cai and co-workers isolated trolline [(−)-**3**] from the ethanol extract of the flowers of *T. chinensis* Bunge. This yellow blooming plant is widely distributed in China and belongs to the family ranunculaceae. The plant is known in traditional Chinese medicine for its effect against respiratory infections, pharyngitis, tonsillitis, and bronchitis [2]. The molecular formula was assigned by HR-ESI-MS. The structural assignment was performed based on advanced NMR measurements (including ^{1}H, ^{13}C with DEPT, COSY, HMBC, and HMQC spectra) and measurement of the optical rotation. The value of optical rotation of natural trolline [(−)-**3**] ($[\alpha]_D^{20} = -197$, $c = 0.8$, HOMe) was compared with that of a synthetic compound of similar structure. Based on this, an *S* configuration was proposed for the stereogenic center (C-10b). A single-crystal X-ray analysis confirmed this structural assignment [2].

Zhao and Ding isolated the same alkaloid from *Salsola collina* Pall., and named it salsoline A [(−)-**3**]. The authors also used advanced NMR experiments in combination with mass spectrometry and IR spectroscopy for structural identification. The value of optical rotation of $[\alpha]_D^{25} = -82.6$ ($c = 0.1$, HOMe) shows, as well as for the isolated trolline [(−)-**3**], a negative sign, confirming that both compounds are the same enantiomer.

C. Isolation of Oleracein E

One year after the isolation of trolline [(−)-**3**], its enantiomer called oleracein E [(+)-**3**] was isolated from *Portulaca oleracea* L., a widely distributed annual succulent [4]. Purslane (*P. oleracea*) is an edible plant used also in ancient medicine as diuretic, febrifuge, antiseptic, antispasmodic, and

antihelminthic [4]. The molecular formula of oleracein E [(+)-**3**] was determined by HR-EI-MS. NMR measurement of (+)-**3** including ^{1}H, ^{13}C with DEPT, COSY, and HMBC led to the same structural assignment as for trolline [(−)-**3**]. The value of optical rotation of oleracein E [(+)-**3**] ($[\alpha]_D^{26}$ = +61.12, *c* = 0.32, HOMe) has an opposite sign as compared to trolline [(−)-**3**]. Therefore, it was concluded that oleracein E [(+)-**3**] is the enantiomer. The melting points published for the two alkaloids are significantly different. For oleracein E [(+)-**3**] a melting point of 238−240 °C is reported, which is much higher than the melting point of trolline [(−)-**3**] (165−167 °C).

In 2009, Zhang and co-workers also isolated oleracein E [(+)-**3**] from another plant source called *Meconopsis integrifolia* (Maxim.) Franch [19].

D. Isolation of Salsoline B

Salsola collina belongs to the family chenopodiaceae and is known as remedy for treatment of hypertension in Chinese folk medicine. In 2007, Xiang et al. isolated salsoline A (trolline) [(−)-**3**] along with a new alkaloid from this plant. This new alkaloid is named salsoline B (**4**) (Figure 2) [5]. The structure for salsoline B (**4**) was assigned using 1D, 2D NMR experiments and mass spectroscopy. A value of the optical rotation for salsoline B (**4**) has not been published. Syntheses and biological activities of salsoline B (**4**) have not been described.

E. Biogenesis

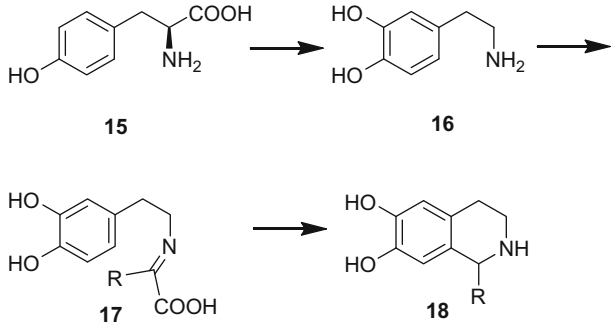
So far, no biogenetic study has been published for the pyrrolo[2,1-*a*]isoquinoline alkaloids crispine A [(+)-**1**], crispine B (**2**), trolline [(−)-**3**], oleracein E [(+)-**3**], salsoline B (**4**), peyoglutam (**10**) or mescalotam (**11**). Herein, we propose a biogenetic route to these alkaloids. Our biogenetic proposals toward the pyrrolo[2,1-*a*]isoquinolines **1**−**4** and **10**−**11** and toward the isoquinoline alkaloids **12** and **14** are not supported experimentally.

The isoquinoline alkaloids crispine E (**14**) and crispine C (**12**) are considered as potential biogenetic precursors for crispine A [(+)-**1**] and B (**2**). The biogenetic pathway for the formation of isoquinolines is well-known (Scheme 1) [20]. L-Tyrosine (**15**) can be transformed to dopamine (**16**) via oxygenation and decarboxylation. Condensation of **16** with an α-keto acid to imine **17** followed by Pictet−Spengler cyclization generates the second ring. Subsequent oxidative decarboxylation affords a dihydroisoquinoline that can be reduced to the corresponding tetrahydroisoquinoline **18**. The final enzymatic step forms a stereogenic center at C-1 [20].

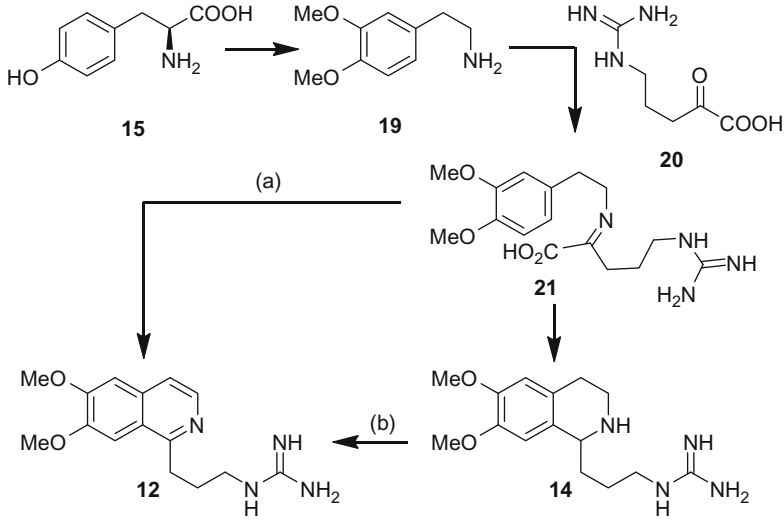
The α-keto acid required for the biosynthesis of crispine E (**14**) is 5-guanidino-2-oxo-valeric acid (**20**) that is derived from the amino acid arginine. Transformation of L-tyrosine (**15**) into amine **19** followed by condensation, Pictet−Spengler cyclization, decarboxylation, and

subsequent reduction furnishes crispine E (**14**). Oxidation to the aromatic system instead of the reduction as last step leads to the fully aromatic crispine C (**12**) (Scheme 2, route a). Alternatively, crispine C (**12**) could be formed by aromatization of crispine E (**14**) (Scheme 2, route b).

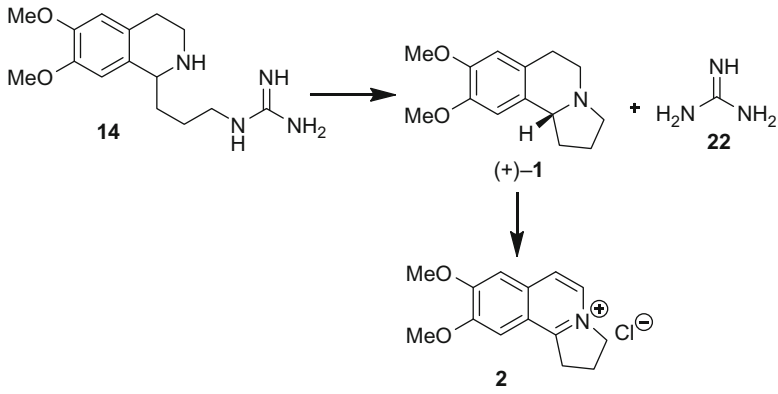
The pyrrole ring of crispine A [(+)-**1**] and B (**2**) could be formed by an intramolecular nucleophilic attack of the piperidine nitrogen atom at C-3 of the side chain with concomitant elimination of guanidine (**22**). Subsequently, crispine A [(+)-**1**] is oxidized to crispine B (**2**) (Scheme 3). A potential alternative route involving cyclization of crispine C (**12**) by an intramolecular nucleophilic attack of the pyridine nitrogen atom would lead directly to crispine B (**2**).



Scheme 1 Biogenetic pathway to tetrahydroisoquinolines **18**.



Scheme 2 Proposed biogenetic pathway to crispine E (**14**) and crispine C (**12**).



Scheme 3 Proposed biogenetic pathway to crispine A [(+)-**1**] and crispine B (**2**) via crispine E (**14**).

An identical configuration of the stereogenic centers of crispine E (**14**) and crispine A [(+)-**1**] would support this hypothesis. Unfortunately, only for the pyrrolo[2,1-*a*]isoquinoline alkaloid (+)-**1** an optical activity is reported [1].

An alternative biosynthetic pathway for crispine A [(+)-**1**] and B (**2**) involves condensation of amine **19** with α-ketoglutaric acid (**23**) (from glutamic acid) followed by Pictet–Spengler cyclization forming the isoquinoline core. Lactamization of the resulting isoquinoline **25** leads to pyrrolidinone **26** (Scheme 4).

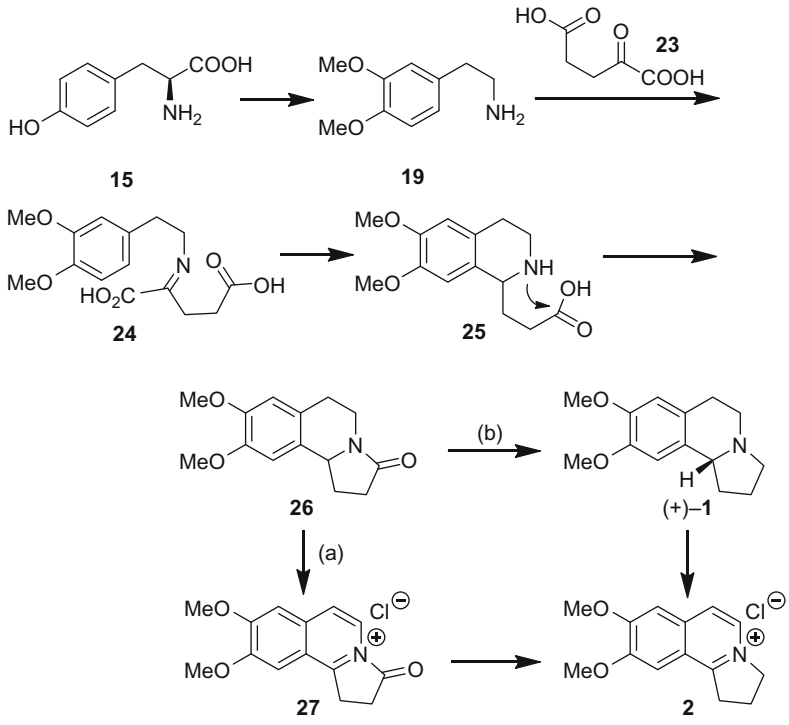
Compound **26** is subsequently oxidized to the isoquinolinium salt **27** that by reduction affords crispine B (**2**) (Scheme 4, route a). Alternatively, **26** could also be reduced first to crispine A [(+)-**1**] and then oxidized to crispine B (**2**) (Scheme 4, route b).

Isoquinoline formation is also known for free hydroxyphenethylamines [20]. Thus, the biosynthesis for trolline [(−)-**3**], oleracein E [(+)-**3**], and salsoline B (**4**) could follow similar lines. Pictet–Spengler cyclization as described before using dopamine (**16**) as substrate followed by oxidative decarboxylation and reduction of the double bond leads to tetrahydroisoquinoline **29**. Subsequent lactamization generates trolline [(−)-**3**] and oleracein E [(+)-**3**]. Cyclization of the regioisomeric isoquinoline **30** leads to salsoline B (**4**) (Scheme 5).

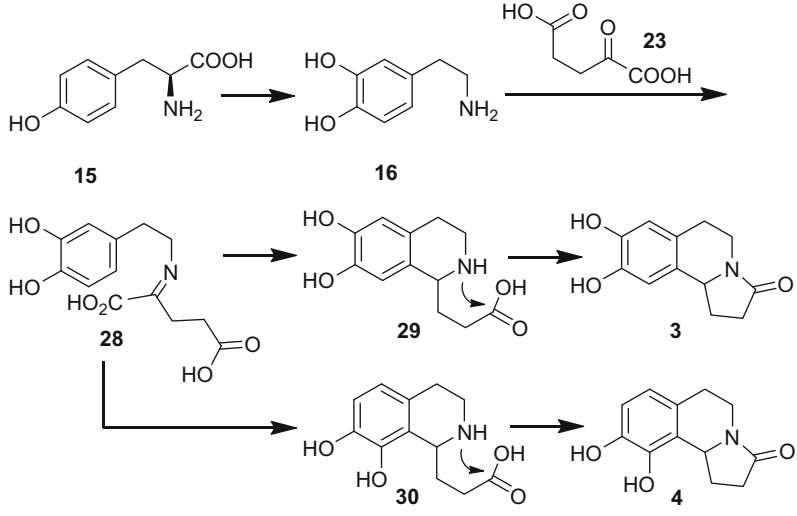
The biogenesis of the structurally closely related alkaloids peyoglutam (**10**) and mescalotam (**11**) is proposed to follow similar lines.

F. Biological Activities

Crispine A [(+)-**1**], crispine B (**2**), trolline [(−)-**3**], oleracein E [(+)-**3**], as well as some derivatives with the same parent framework have been



Scheme 4 Proposed biogenetic pathways to crispine A [(+)-**1**] and crispine B (**2**) based on α-ketoglutaric acid (**23**).



Scheme 5 Proposed biogenetic pathways for the dihydroxypyrroloisoquinolinons **3** and **4**.

tested for their bioactivities. In 2002, the cytotoxic activity of the natural products **1** and **2** on SKOV3, KB, and HeLa human cancer cell lines has been tested in a sulforhodamine B (SRB) assay. Crispine B (**2**) showed significant cytotoxicity at micromolar levels [1]. In 2005, Jia and co-workers investigated the antitumor activity of crispine B (**2**) and crispine C (**12**) using a sulforhodamine B assay with the human ovarian cancer cell line HO-8910 and the human hepatoma cancer cell line Bel-7402. Only crispine B (**2**) showed significant activity [18]. Crispine A [(+)-**1**] has been tested as potential ligand for the human dopamine receptors D_1, D_{2L}, D_4, and D_5, but no significant activity could be determined [21].

In 2004, the antibacterial and antiviral activities of trolline [(−)-**3**] were tested. *In vitro* antibacterial experiments with the Gram-negative bacterial strains *Klebsiella pneumoniae* 02-63, *Pseudomonas aeruginosa* 02-123, and *Haemophilus influenzae* 02-102 and the Gram-positive bacterial strains *Staphylococcus aureus* 01-159, *Streptococcus pneumoniae* 02-19, and *Streptococcus pyogenes* M1371 showed significant inhibitory activity of trolline [(−)-**3**] for Gram-negative and Gram-positive bacteria. Moreover, trolline [(−)-**3**] exhibited moderate antiviral activity against influenza virus A (JF 190-15) [2].

Xiang and co-workers found for oleracein E [(+)-**3**] a potent DPPH free radical scavenging activity and a strong inhibitory activity on hydrogen peroxide-induced lipid peroxidation in rat brain. Thus, oleracein E [(+)-**3**] can be regarded as antioxidant [22].

For salsoline B (**4**), peyoglutam (**10**), and mescalotam (**11**) no biological activity is reported. For other non-annulated but non-natural pyrrolo[2,1-*a*]-isoquinolines various biological activities have been found, e.g., cardiotonic activity [23], antitumor activity [24], antidepressant activity [25], antileukemic activity [26], and activity as α_2-adrenoreceptor antagonist [27].

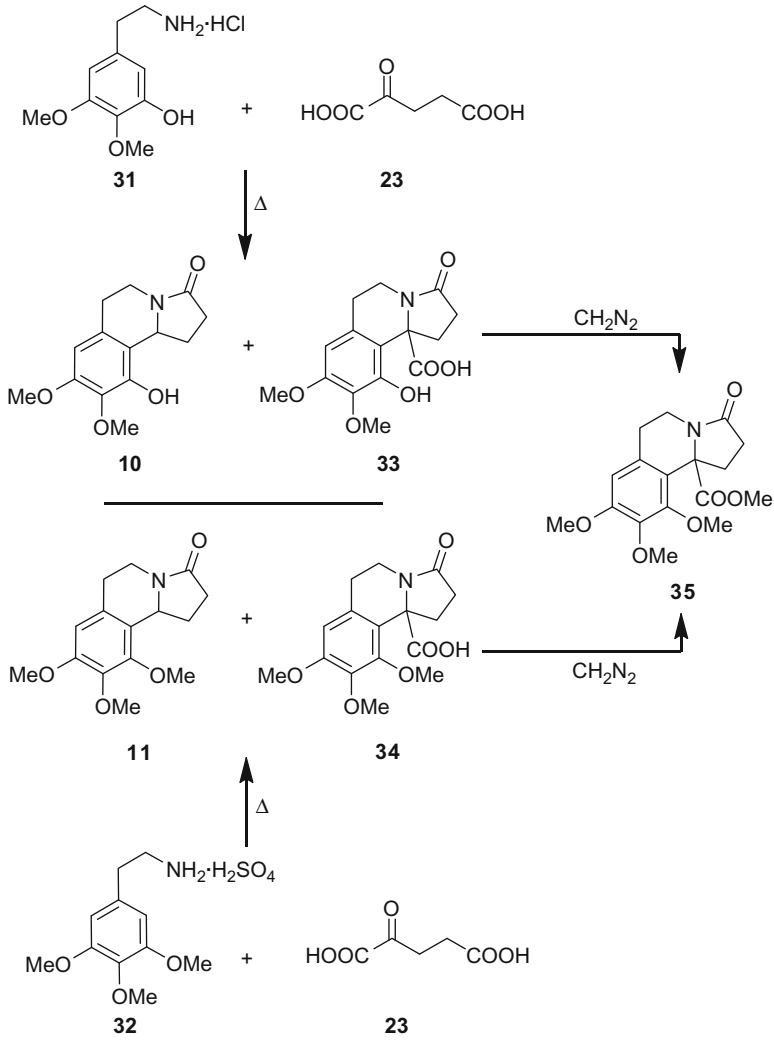
III. SYNTHESES OF THE NATURAL PRODUCTS

To discuss all synthetic efforts toward pyrrolo[2,1-*a*]isoquinoline derivatives would go far beyond the scope of the present chapter. A review summarizing selected pyrrolo[2,1-*a*]isoquinoline syntheses has been published by Mikhailovskii and Shklyaev in 1997 [28]. Transformations of pyrrolo[2,1-*a*]isoquinoline derivatives, like ring openings [29], will not be discussed here.

In the following sections, we describe the syntheses of the natural products peyoglutam (**10**), mescalotam (**11**), crispine A [(+)-**1**], crispine B (**2**), trolline (salsoline A) [(−)-**3**], oleracein E [(+)-**3**], salsoline B (**4**), and of selected analogs.

A. Syntheses of Peyoglutam and Mescalotam

The only synthesis of peyoglutam (**10**) and mescalotam (**11**) was reported along with the isolation of these alkaloids by Kapadia and Fales in 1968 [6]. The authors obtained both natural products **10** and **11** in small quantities by treatment of the acid **23** with the arylethylamines **31** and **32**, respectively. The main products of this transformation were the corresponding acids **33** and **34** that were methylated with diazomethane to provide methyl ester **35** in both cases. These transformations supported the structural assignments (Scheme 6).



Scheme 6 Syntheses of peyoglutam (**10**) and mescalotam (**11**) [6].

B. Racemic and Enantioselective Syntheses of Crispine A

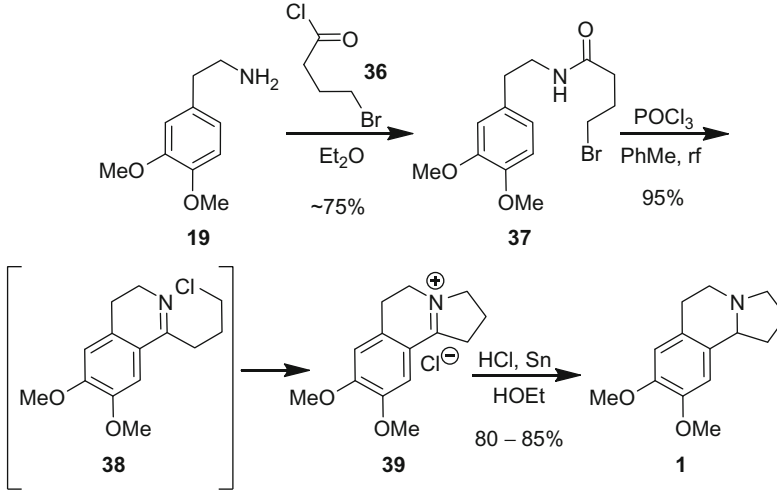
The first racemic synthesis of crispine A (**1**) after its isolation as natural product was published by Knölker and Agarwal in 2005 [30], followed by an enantioselective synthesis published by Czarnocki and co-workers in the same year [31]. Prior to its isolation from nature [1], compound **1** had already been synthesized by Child and Pyman in 1931 [32] and afterwards by others [33–38]. In the following section all synthetic efforts toward crispine A (**1**) are discussed.

1. Child's Synthesis of (±)-Crispine A (1931)

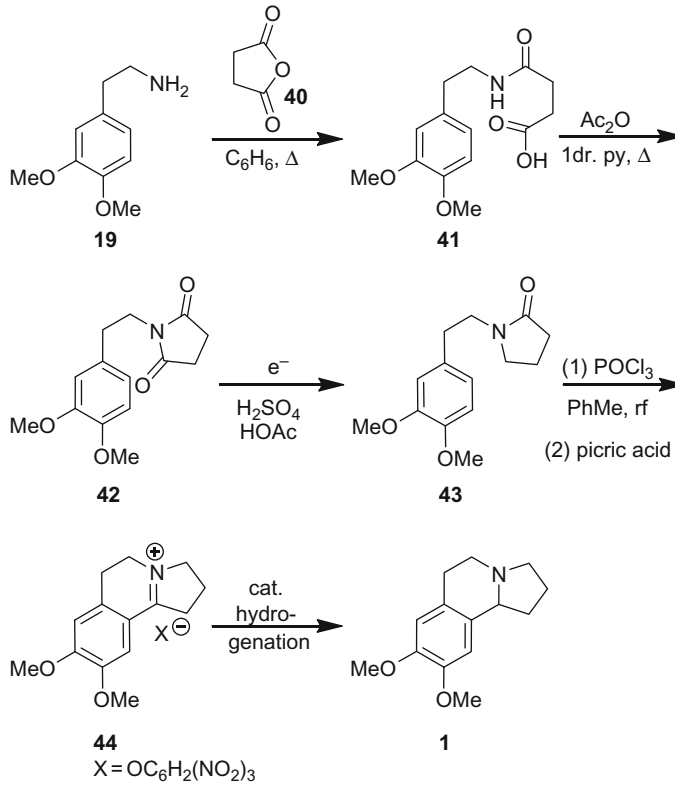
Already in 1931, the first synthesis of the racemic compound **1** was published by Child and Pyman [32]. The key step of their reaction sequence is a double cyclization consisting of a Bischler–Napieralski reaction with subsequent formation of the second ring by nucleophilic attack of the imine nitrogen atom. Condensation of veratrylethylamine (**19**) with γ-bromobutyryl chloride (**36**) led to amide **37** (Scheme 7). Compound **37** was then treated with phosphorus oxychloride to provide the pyrrolo[2,1-*a*]isoquinolinium chloride **39** in 95% yield. Reduction of the salt **39** gave crispine A (**1**) in an overall yield of ~59%, more than 70 years before its isolation as natural product.

2. Sugasawa's Synthesis of (±)-Crispine A (1939)

In 1939, Sugasawa et al. developed a new synthetic route toward compound **1** using the same starting material as Child and Pyman



Scheme 7 Child's synthesis of the pyrrolo[2,1-*a*]isoquinoline **1** [32].



Scheme 8 Sugasawa's synthesis of the pyrrolo[2,1-*a*]isoquinoline **1** [33].

[33]. The key step of their synthesis is an electrolytic reduction of the preformed succinimide **42**. Veratrylethylamine (**19**) was treated with succinic anhydride (**40**) to provide amide **41** (Scheme 8). Heating of **41** with acetic anhydride and traces of pyridine gave succinimide **42**. Subsequent electrolytic reduction in a mixture of acetic and sulfuric acid afforded lactam **43** in good yield. Treatment of **43** with phosphorus oxychloride provided iminium salt **44** that was isolated as picrate (X = $OC_6H_2(NO_2)_3$). Catalytic reduction of salt **44** afforded compound **1**.

3. Zymalkowski's Synthesis of (±)-Crispine A (1967)

Zymalkowski and Schmidt modified the synthesis of Child [32] by selecting veratrylethylamine (**19**) and γ-butyrolactone (**45**) as starting materials [34]. Reaction of **19** with lactone **45** led to amide **46** that on

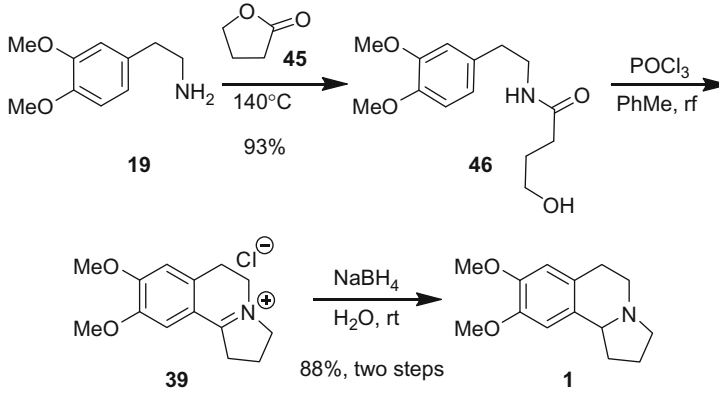
treatment with phosphorous oxychloride underwent a double cyclization to the same pyrrolo[2,1-*a*]isoquinolinium salt **39** as described previously. Reduction of the crude product **39** using sodium borohydride afforded **1** in three steps and 82% overall yield based on compound **19** (Scheme 9).

4. Shono's Synthesis of (±)-Crispine A (1983)

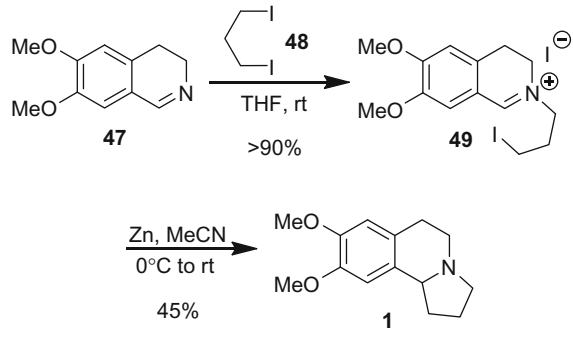
Shono et al. developed a zinc-promoted reductive coupling of alkyl halides and iminium salts [35]. Exploration of the applicability of their method led to the syntheses of various alkaloids, including compound **1**. Intramolecular reductive couplings like that leading to **1** showed significantly lower yields compared to intermolecular couplings. The iminium salt **49** was prepared from 3,4-dihydro-6,7-dimethoxyisoquinoline (**47**) and 1,3-diiodopropane (**48**). Reaction of **49** with zinc in acetonitrile afforded **1** in moderate yield (Scheme 10).

5. Schell's Synthesis of (±)-Crispine A (1983)

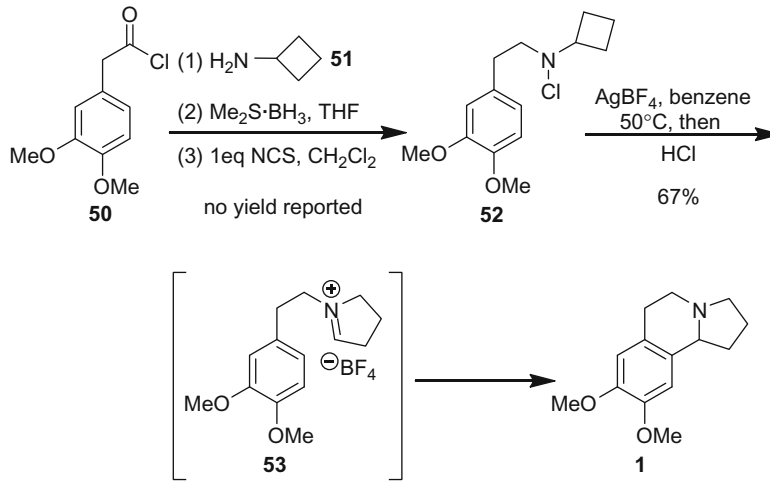
The key step of the synthesis of compound **1** developed by Schell and Smith in 1983 is a silver-induced ring enlargement of the cyclobutylchloroamine **52** [36]. Reaction of veratrylacetyl chloride (**50**) and cyclobutylamine (**51**) led to the corresponding amide that on reduction and subsequent chlorination with *N*-chlorosuccinimide afforded *N*-chloroamine **52**. Treatment of compound **52** with silver tetrafluoroborate initiated a rearrangement to the iminium salt **53**. Addition of concentrated hydrochloric acid to the mixture completed the reaction



Scheme 9 Zymalkowski's synthesis of the pyrrolo[2,1-*a*]isoquinoline **1** [34].



Scheme 10 Shono's synthesis of the pyrrolo[2,1-*a*]isoquinoline **1** [35].

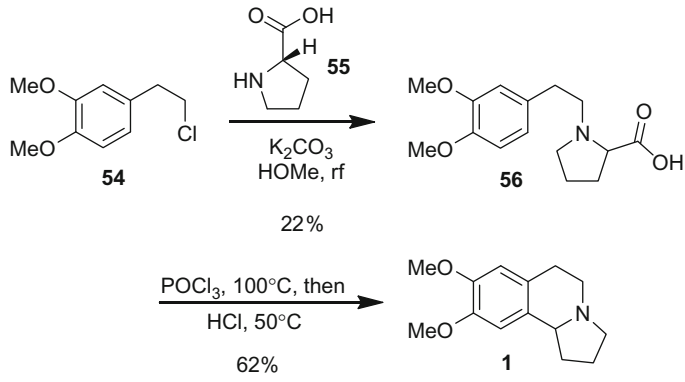


Scheme 11 Schell's synthesis of the pyrrolo[2,1-*a*]isoquinoline **1** [36].

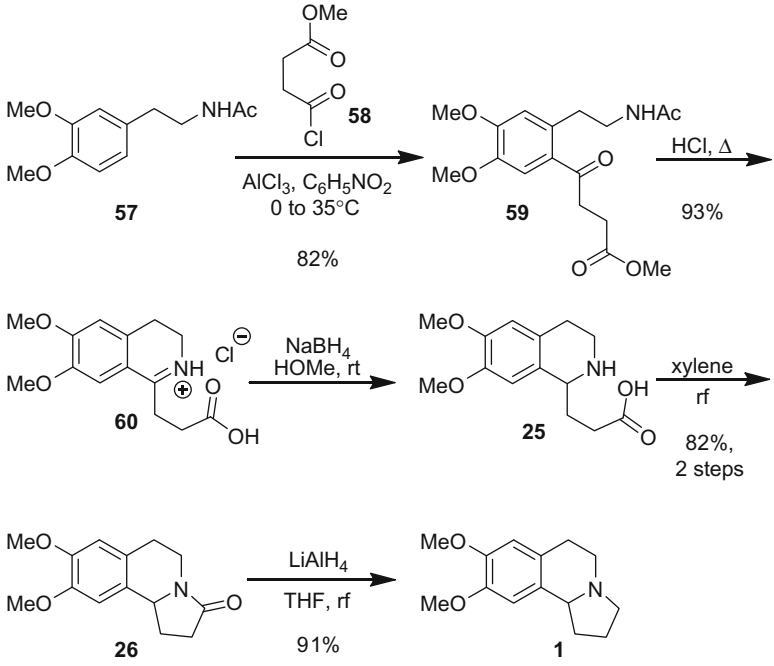
sequence. Ring closure by Pictet–Spengler cyclization afforded **1** in 67% yield after recrystallization (Scheme 11) [36].

6. Proctor's Synthesis of (±)-Crispine A (1984)

In 1984, Proctor and co-workers accomplished the synthesis of compound **1** using precursor **56** which they had described 9 years earlier [39]. Reaction of L-proline (**55**) with veratrylethyl chloride (**54**) afforded **56**. Cyclization of the proline derivative **56** with phosphorus oxychloride provided **1** in 62% yield (Scheme 12) [37]. Although, the authors started with the enantiopure amino acid **55** they did not comment on the preservation or the loss of the stereochemical information.



Scheme 12 Proctor's synthesis of the pyrrolo[2,1-*a*]isoquinoline **1** [37].



Scheme 13 Orito's synthesis of the pyrrolo[2,1-*a*]isoquinoline **1** [38].

7. Orito's Synthesis of (±)-Crispine A (1988)

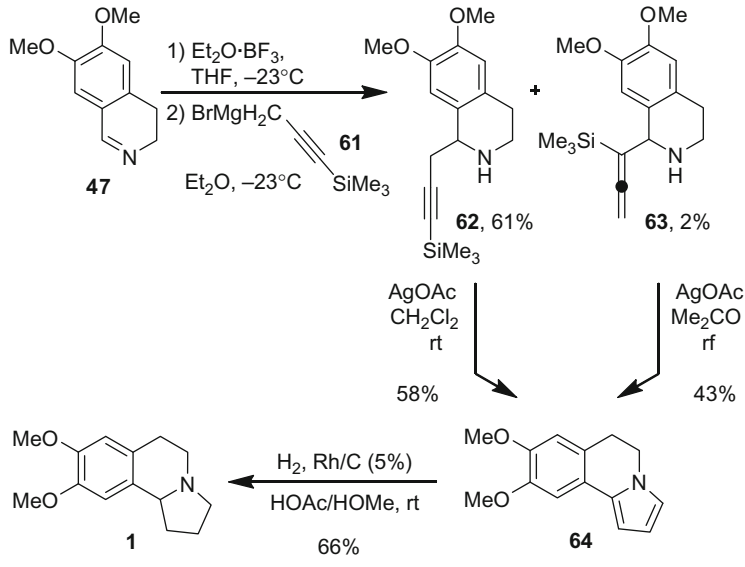
Orito et al. studied the Friedel–Crafts acylation of *N*-acetylveratrylethylamine (**57**). During the study, they found a new approach to compound **1** [38]. Treatment of **57** with acyl chloride **58** and aluminum trichloride provided **59** (Scheme 13). Cyclization of **59** by heating in hydrochloric

acid afforded isoquinolinium salt **60**. Reduction with sodium borohydride led to tetrahydroisoquinoline **25**. The crude product **25** was heated at reflux in xylene using a Dean–Stark apparatus to effect the formation of lactam **26**. Finally, reduction of **26** provided **1** in five steps and 57% overall yield based on amide **57** [38].

8. Knölker's Synthesis of (±)-Crispine A (2005)

In 2004, Knölker and Agarwal developed a new procedure for the synthesis of pyrroles from homopropargylamines using stoichiometric amounts of silver acetate [40]. Five years later, they described a modified pyrrole synthesis that is induced by catalytic amounts of silver [41–43]. The silver-mediated oxidative cyclization to pyrroles was applied to the first synthesis of racemic crispine A (**1**) after its isolation as natural product [30].

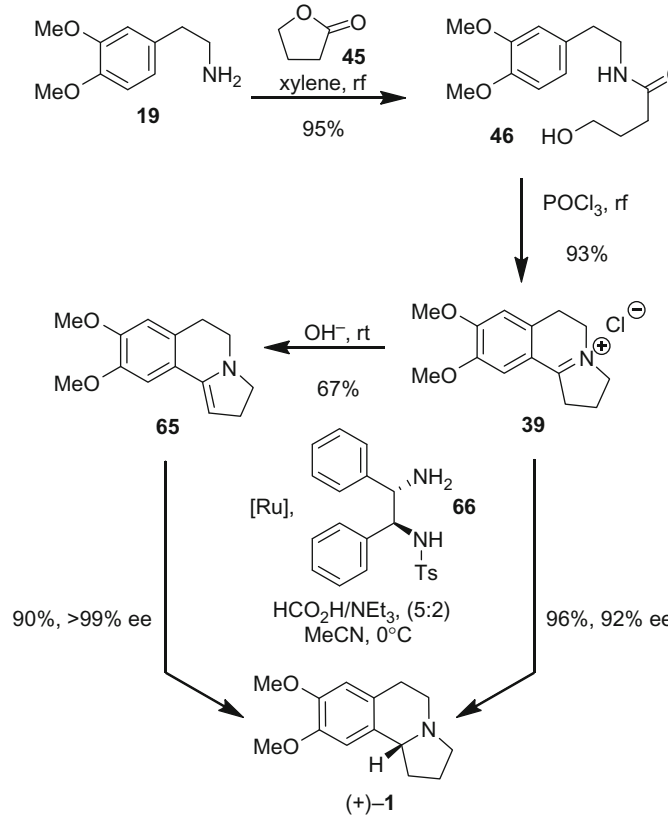
The starting material, 3,4-dihydro-6,7-dimethoxyisoquinoline (**47**), is not only commercially available but also readily prepared on a multigram scale by Bischler–Napieralski cyclization [44]. Boron trifluoride-promoted addition of the propargyl Grignard reagent **61** led to compound **62** in 61% yield as major product along with 2% of the allene **63**. Using silver acetate, both compounds were transformed to pyrrolo[2,1-*a*]isoquinoline **64**. For the major product **62**, the silver-mediated oxidative cyclization was carried out at room temperature, whereas the cyclization of the allene **63** required elevated temperatures. Hydrogenation of the pyrrole ring using rhodium on activated carbon as catalyst provided crispine A (**1**) in 66% yield (Scheme 14).



Scheme 14 Knölker's synthesis of crispine A (**1**) [30].

9. Czarnocki's Synthesis of (+)-Crispine A (2005)

In 2005, Czarnocki and co-workers published the first enantioselective synthesis of crispine A [(+)-**1**]. The key step is a catalytic asymmetric reduction generating the stereogenic center at the final stage of their synthesis [31]. Reaction of veratrylethylamine (**19**) and γ-butyrolactone (**45**) afforded amide **46**. Cyclization of **46** led to the tricyclic compound **39**. This reaction sequence follows Zymalkowski's route (cf. Scheme 9) [34]. Both, the iminium salt **39** and the enamine **65** have been subjected to asymmetric reduction. A ruthenium-catalyzed asymmetric transfer hydrogenation using ligand **66** turned out to be the best choice. (+)-Crispine A [(+)-**1**] could be obtained in 96% yield and 92% ee starting from iminium salt **39**, and in 90% yield and 99% ee starting from the enamine **65** (Scheme 15). In 2007, the authors could confirm their structural assignment for crispine A [(+)-**1**] as the *R* enantiomer by an X-ray analysis of a single crystal of the corresponding picrate [45].



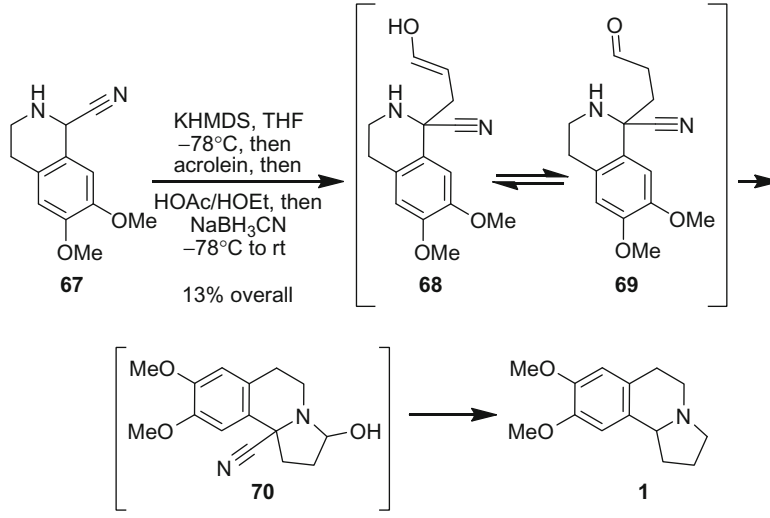
Scheme 15 Czarnoki's synthesis of (+)-crispine A [(+)-**1**] [31].

10. Opatz' Synthesis of (±)-Crispine A (2006)

In 2006, Meyer and Opatz published a one-pot synthesis of crispine A (**1**) and several C-ring substituted analogs [46]. The nitrile **67** used as starting material for the one-pot reaction was formed by addition of hydrogen cyanide to dihydroisoquinoline **47**. Deprotonation of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carbonitrile (**67**) with potassium bis(trimethylsilyl)amide followed by 1,4-addition to acrolein and nucleophilic attack of the secondary amine at the carbonyl group generates pyrrolo[2,1-*a*]isoquinoline **70**. Treatment with sodium cyanoborohydride led to the elimination of hydrogen cyanide and water to form racemic crispine A (**1**) (Scheme 16) [46].

11. Chong's Synthesis of (+)-Crispine A (2006)

In 2006, Wu and Chong reported a new method for asymmetric allylboronation of cyclic imines. They demonstrated the synthetic applicability of their method by the syntheses of some alkaloids including crispine A [(+)-**1**]. Treatment of 6,7-dimethoxy-3,4-dihydroisoquinoline (**47**) with allylboronate **71** (Figure 8) afforded allyltetrahydroisoquinoline **72** in 78% yield and 98% ee. Hydroboration and oxidative work-up under basic conditions followed by intramolecular Mitsunobu reaction with the nitrogen atom of the piperidine ring as nucleophile led to the natural product (+)-**1** in three steps and 44% overall yield (Scheme 17) [47].



Scheme 16 Opatz' synthesis of crispine A (**1**) [46].

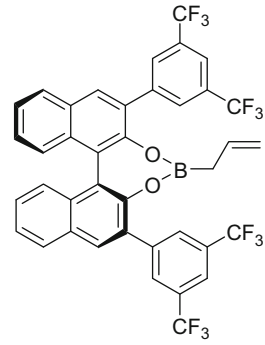
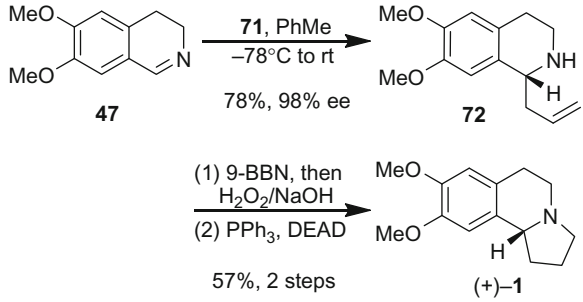


Figure 8 Allylboronate **71**.



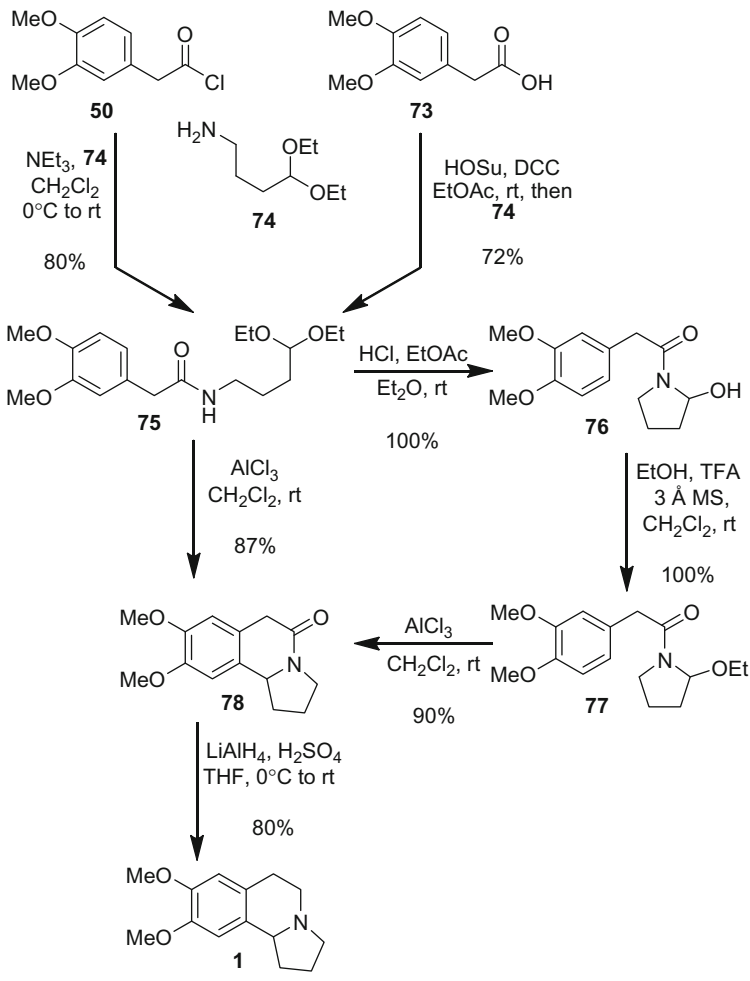
Scheme 17 Chong's synthesis of (+)-crispine A [(+)-**1**] [47].

12. King's Synthesis of (±)-Crispine A (2007)

King used a Lewis acid-promoted Friedel–Crafts-type cyclization as key step for his approach toward crispine A (**1**) [48]. The acid chloride **50** or the acid **73** that was activated by *N*,*N*′-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (HOSu), and amine **74** have been used as starting materials. Reaction of either compound, **50** or **73**, with **74** led to amide **75** in 80% or 72% yield, respectively. Formation of the pyrrolidine ring followed by Lewis acid-promoted electrophilic substitution of the arene provided the pyrrolo[2,1-*a*]isoquinoline **78**. Lewis acid-promoted reaction of **75** under the same conditions led directly to amide **78** in good yield. Reduction of the amide **78** with *in situ* prepared alane provided crispine A (**1**) in three steps and 56% overall yield (five steps and 58% overall yield via the longer route) (Scheme 18) [48].

13. Turner's Synthesis of (+)-Crispine A (2007)

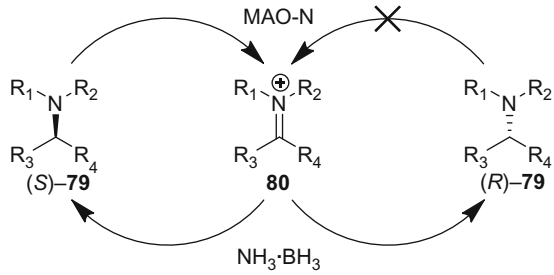
Turner and co-workers developed a general method for the chemoenzymatic deracemization of tertiary [49] and secondary [50] amines. The



Scheme 18 King's synthesis of crispine A (**1**) [48].

enzyme monoamine oxidase from *Aspergillus niger* (MAO-N) selectively oxidizes the *S* enantiomer of a racemic mixture of amine **79** to the corresponding iminium salt **80**. Subsequently, this iminium salt **80** is nonselectively reduced by the ammonia–borane complex. The overall process leads to an accumulation of enantiomer (*R*)-**79** in the reaction mixture (Scheme 19) [51].

To demonstrate the applicability of this method to the synthesis of natural products, racemic crispine A [(±)-**1**] was prepared following literature procedures [29,32,34,52]. Enzymatic resolution of (±)-**1** provided within 40 h (+)-crispine A [(+)-**1**] in an enantiomeric excess of 97% [51].



Scheme 19 Chemoenzymatic deracemization of tertiary amines [51].

14. Allin's Synthesis of (+)-Crispine A (2007)

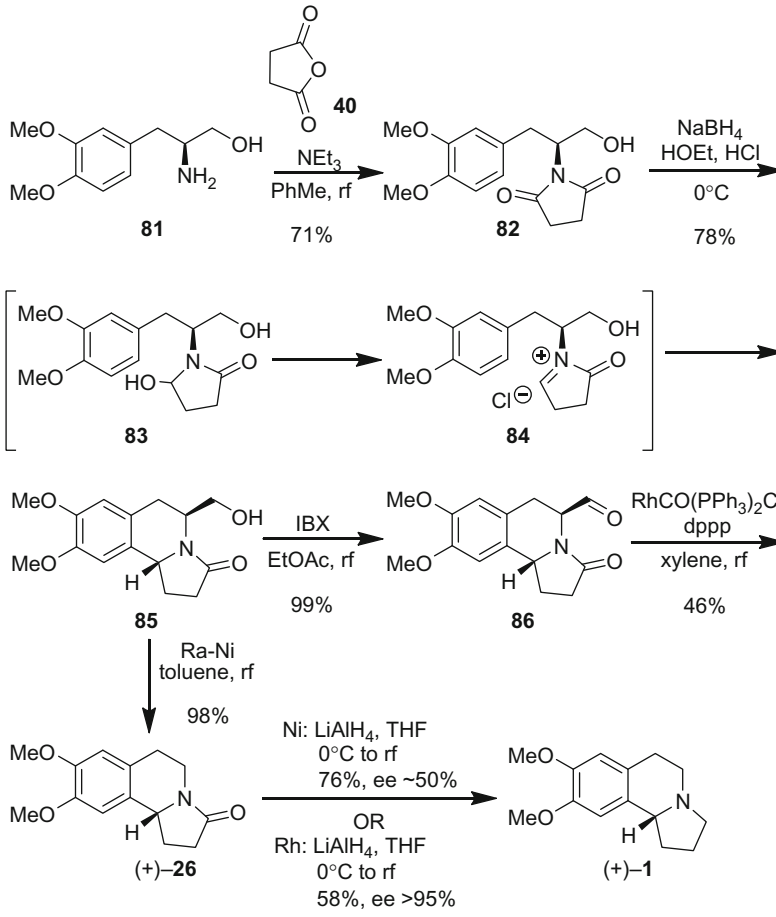
Allin et al. used the stereogenic information of the amino acid derivative **81** and obtained (+)-crispine A [(+)-**1**)] in excellent enantiopurity and an overall yield of 21% starting from the readily available imide **82**. The imide **82** was prepared by condensation of (*S*)-3,4-dimethoxyphenylalaninol (**81**) and succinic anhydride (**40**) [53,54]. Reduction with sodium borohydride proceeded via the corresponding hemiaminal **83** to afford iminium salt **84**. Electrophilic substitution at the aromatic ring provided the tricyclic compound **85**. Removal of the redundant C-1 moiety is achieved via direct decarbonylation using Raney-Nickel or via oxidation to the corresponding aldehyde **86** and subsequent rhodium-catalyzed decarbonylation. In both cases, the resulting amide (+)-**26** was reduced with lithium aluminum hydride to afford (+)-crispine A [(+)-**1**]. The Raney-Nickel route provided (+)-**1** in good yield but only in ~50% ee. The Rh-catalyzed route led to (+)-**1** in only moderate yield (58%) and with an enantiomeric excess of more than 95% (Scheme 20) [15].

15. Itoh's Synthesis of (−)-Crispine A (2007)

In 2007, Itoh and co-workers developed a synthesis of (−)-crispine A [(−)-**1**] together with the syntheses of trolline [(−)-**3**] and (−)-crispine E [(−)-**14**] [55]. The synthesis of trolline [(−)-**3**] is discussed in Section III.D.2.

Nonnatural (−)-crispine A [(−)-**1**] was synthesized in nine steps and an overall yield of 45% starting from 3,4-dihydro-6,7-dimethoxyisoquinoline (**47**) (Scheme 21). Addition of hydrogen cyanide to **47** in the presence of Jacobsen's catalyst **87** (Figure 9) followed by *in situ* acylation with trifluoroacetic anhydride provided the tetrahydroisoquinoline-1-carbonitrile **88** in 95% ee.

Hydrolysis of the nitrile **88** followed by acid-catalyzed esterification with concomitant cleavage of the trifluoroacetamide and *N*-Boc-protection of the tetrahydroisoquinoline provided the methyl ester **89**. Reduction of **89** with diisobutylaluminum hydride (DIBAL) afforded the aldehyde **90**.

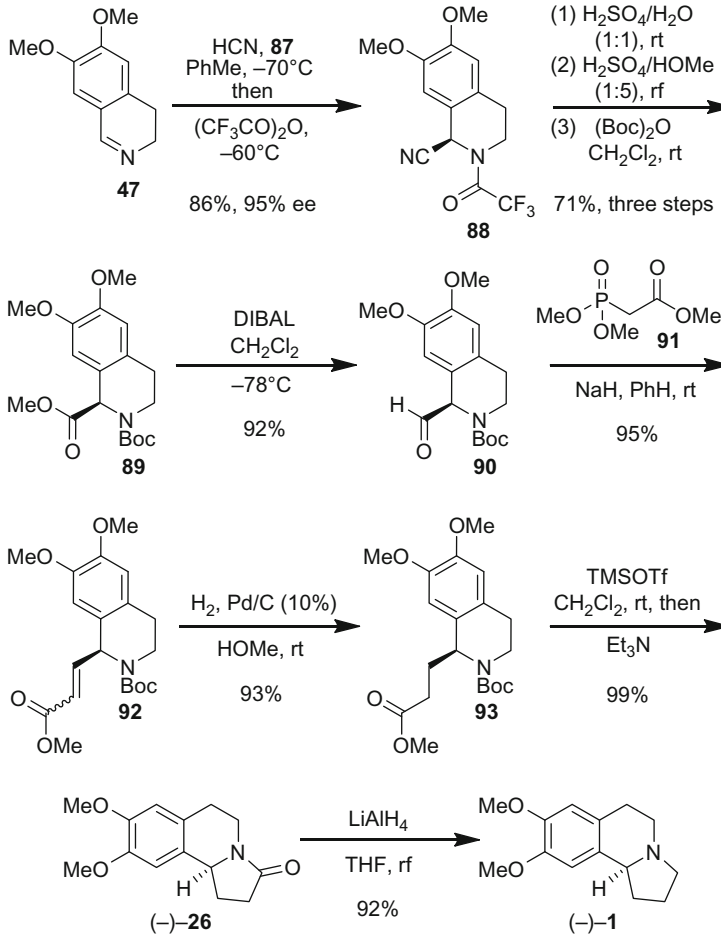


Scheme 20 Allin's synthesis of (+)-crispine A [(+)-**1**] [15].

Horner–Wadsworth–Emmons reaction with trimethyl phosphonoacetate (**91**) and subsequent hydrogenation of the double bond afforded tetrahydroisoquinoline **93**. Treatment with trimethylsilyl triflate led to cleavage of the Boc group with concomitant cyclization to pyrrolo[2,1-*a*]isoquinoline (−)-**26**. Subsequent reduction of amide (−)-**26** completed the first synthesis of nonnatural (−)-crispine A [(−)-**1**] (Scheme 21) [55].

16. Xu's Synthesis of (±)-Crispine A (2008)

Xu et al. developed a new method for the cyclodehydration of amino alcohols using thionyl chloride [56]. The addition of amino alcohol **94** to a solution of thionyl chloride provided the chlorosulfinates **95**. Nucleophilic attack of chloride at **95** led to the chlorides **96** which are in



Scheme 21 Itoh's synthesis of (−)-crispine A [(−)-**1**] [55].

equilibrium with each other. Cyclization of the intermediate chlorides **96** by treatment with base afforded racemic crispine A (**1**) in a one-pot procedure in 95% overall yield (Scheme 22) [56].

17. Banwell's Synthesis of (±)-Crispine A (2008)

In 2008, Banwell and co-workers published a novel synthesis of racemic crispine A (**1**) starting from veratrylethylamine (**19**) [57]. A modified Paal–Knorr synthesis by reaction of **19** with 2,5-dimethoxytetrahydrofuran (**97**) afforded the pyrrole **98**. Oxidative cyclization of compound **98** provided pyrrolo[2,1-*a*]isoquinoline **64** in low yield (Scheme 23).

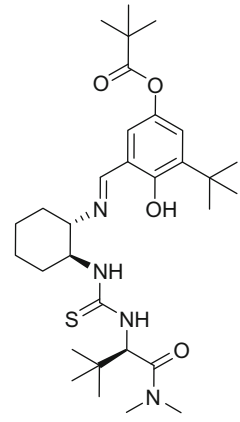
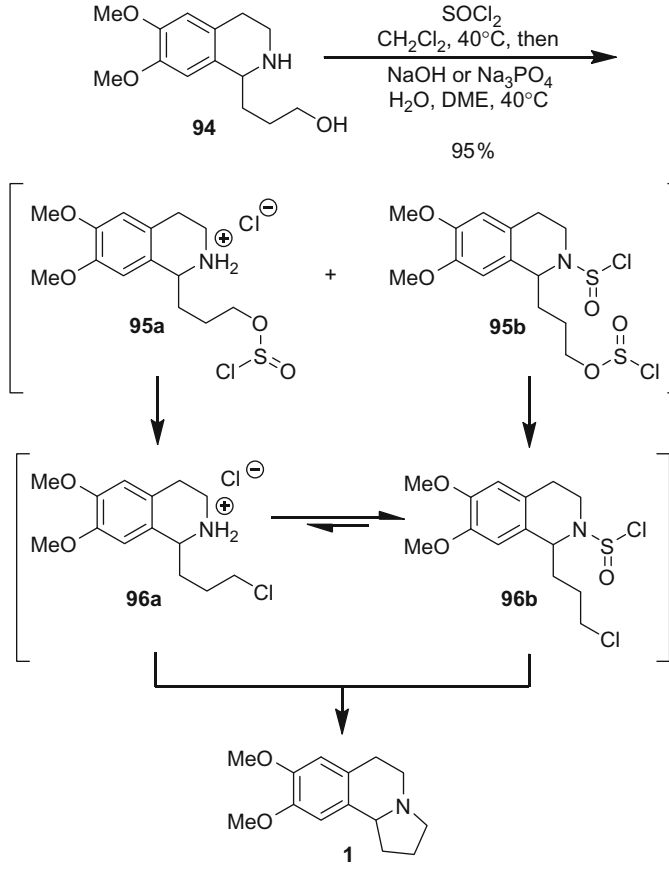
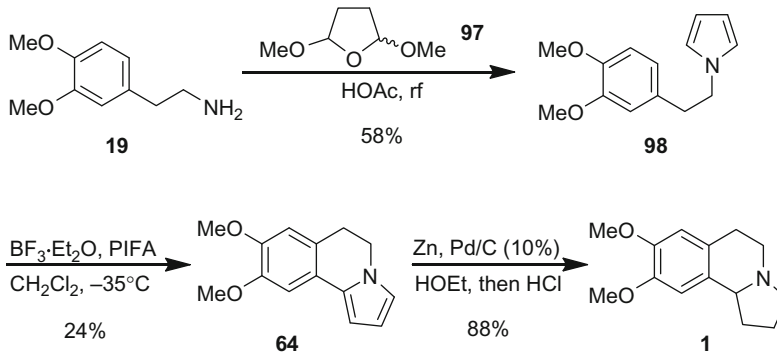


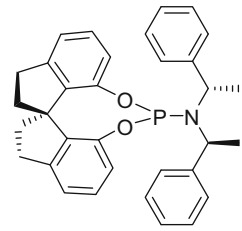
Figure 9 Jacobsen's catalyst **87**.



Scheme 22 Xu's synthesis of crispine A (**1**) [56].



Scheme 23 Banwell's synthesis of crispine A (**1**) [57].



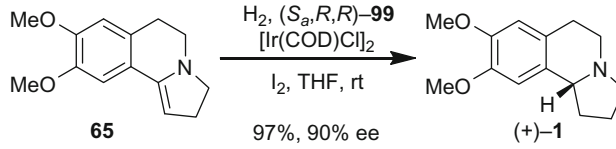
(*R*$_a$,*S*,*S*)–**99**

Figure 10 Spirophosphoramidite ligand (*R*$_a$,*S*,*S*)-**99**.

Compound **64** served already as intermediate for the synthesis of crispine A (**1**) as reported by Knölker and Agarwal in 2005 (cf. Scheme 14) [30]. Banwell et al. employed activated zinc powder and aqueous hydrogen chloride together with palladium on activated carbon in ethanol for the one-pot reduction of the pyrrole ring to provide racemic crispine A (**1**) in 88% yield **64** [57]. Knölker's route afforded intermediate **64** in 36% overall yield, whereas Banwell obtained **64** in only 14% overall yield. However, the reduction of **64** to racemic crispine A (**1**) was significantly improved by Banwell's approach (88% yield versus 66% yield in Knölker's route) [30,57].

18. Zhou's Synthesis of (+)-Crispine A (2009)

Zhou and co-workers developed an iridium-catalyzed asymmetric hydrogenation of cyclic enamines [58]. Various mono- and bidentate ligands have been tested of which the commercially available spirophosphoramidite (*R*$_a$,*S*,*S*)-**99** showed the best results (Figure 10).



Scheme 24 Zhou's asymmetric hydrogenation [58].

For a demonstration of the applicability of their method in the synthesis of natural products they prepared enantiopure crispine A [(+)-**1**]. Enamine **65** was obtained following Czarnocki's approach (cf. Scheme 15) [45]. The asymmetric iridium-catalyzed hydrogenation of **65** afforded crispine A [(+)-**1**] in 97% yield and 90% ee (Scheme 24).

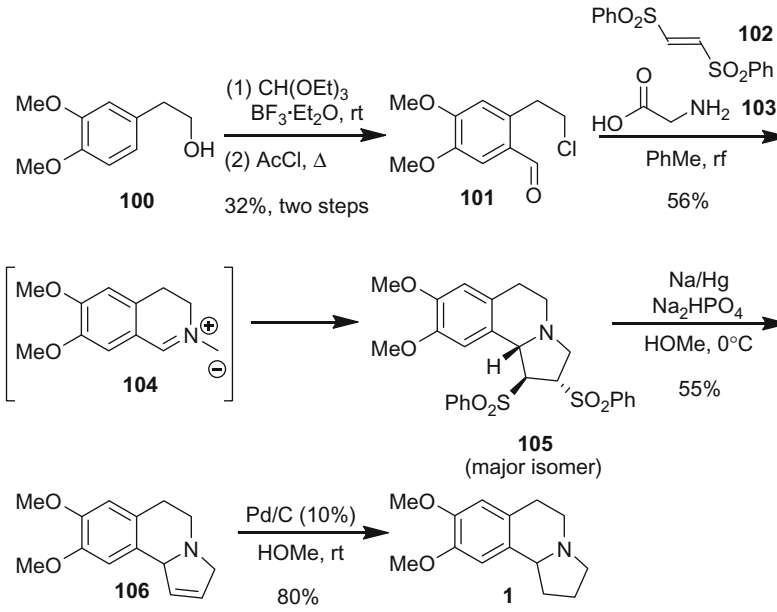
19. Coldham's Synthesis of (±)-Crispine A (2009)

In 2009, Coldham et al. published a general method for the synthesis of nitrogen-containing heterocycles like indolizines and pyrrolizines. The key step of their approach is an intermolecular dipolar cycloaddition of nitrones or azomethine ylides and activated dipolarophiles. This method was applied to the total synthesis of crispine A (**1**) [59].

The benzaldehyde **101**, prepared using Yamato's procedure [60], was used as starting material. The mechanism of the intermolecular cycloaddition is thought to proceed via azomethine ylide **104** that can be formed by condensation of aldehyde **101** and glycine (**103**) followed by intramolecular *N*-alkylation and decarboxylation. Subsequent [3+2] cycloaddition of ylide **104** and the electron-deficient olefin **102** gives rise to intermediate **105**. Reductive removal of the benzenesulfonyl groups afforded the alkene **106**. Hydrogenation of the double bond provided crispine A (**1**) in five steps and 8% overall yield based on compound **100** (Scheme 25).

20. Chiou's Synthesis of (±)-Crispine A (2009)

In 2009, Chiou et al. described the rhodium-catalyzed cyclohydrocarbonylation of aromatic carbon nucleophiles [61]. Using this method crispine A (**1**) was synthesized. The starting material, 3,4-dimethoxyphenylacetic acid (**73**), was treated with thionyl chloride to give the corresponding acid chloride that was subsequently condensed with allylamine (**108**) to furnish the amide **109** in 74% yield. Rhodium-catalyzed carbonylation using BIPHEPHOS (**107**) (Figure 11) as ligand and *p*-toluenesulfonic acid as additive in acetic acid under a carbon monoxide/dihydrogen atmosphere led to the amide **78**. The mechanism of this carbonylation reaction is believed to proceed via the *in situ* formed aldehyde **110** that cyclizes to the iminium cation **111** via the hemiaminal **76**. Pictet–Spengler-type cyclization of the intermediate iminium cation **111** provides the known lactam



Scheme 25 Coldham's synthesis of crispine A (**1**) [59].

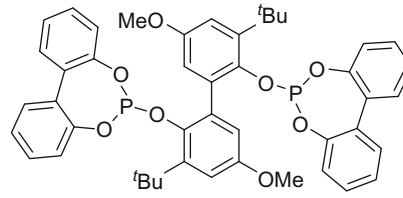
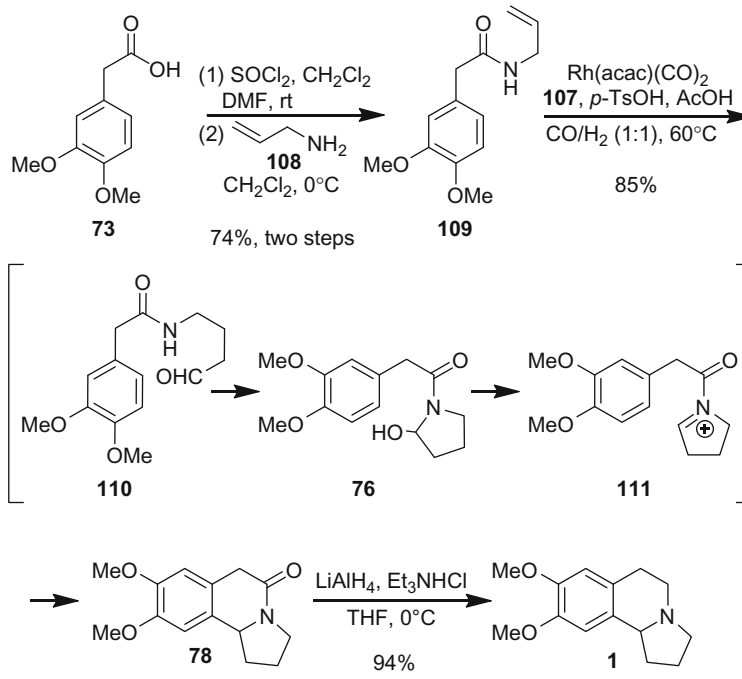


Figure 11 BIPHEPHOS (**107**).

78. Reduction of the amide **78** with lithium aluminum hydride afforded crispine A (**1**) in four steps and 59% overall yield (Scheme 26) [61].

21. Pihko's Synthesis of (−)-Crispine A (2009)

In 2009, Pihko and co-workers studied the asymmetric synthesis of tetrahydroisoquinolines via transfer hydrogenation [62]. Starting from the iminium salt **39** they synthesized (−)-crispine A [(−)-**1**] using the same synthetic pathway as Czarnocki and co-workers in 2005 in their synthesis of (+)-**1** (cf. Scheme 15) [31]. Both groups used a ruthenium catalyst. Czarnocki and co-workers performed the asymmetric hydrogenation using formic acid and triethylamine in acetonitrile. In contrast, Pihko et al. used sodium formate in aqueous medium as hydride source and

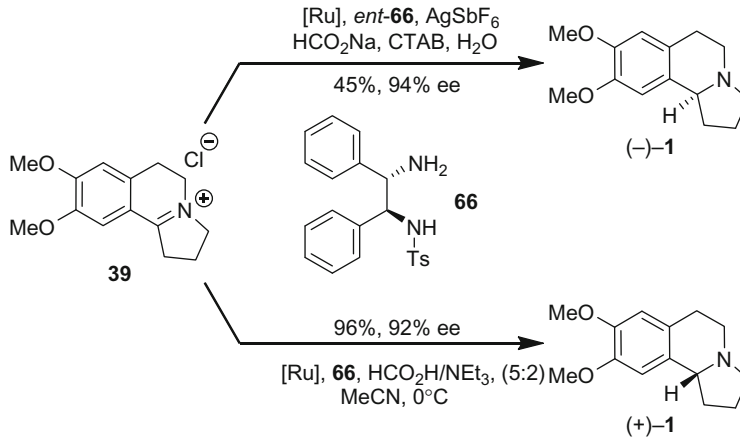


Scheme 26 Chiou's synthesis of crispine A (**1**) [61].

cetyltrimethylammonium bromide (CTAB) as cationic surfactant. Commercially available diamine ligands gave excellent ee values and good yields for simple tetrahydroisoquinolines. However, the results for (−)-crispine A [(−)-**1**] were only moderate. While the enantioselectivity was excellent (94% ee) and slightly better than Czarnocki's result, the yield (45%) was significantly lower than reported by Czarnocki (96%) (Scheme 27) [31,62].

22. Amat's Synthesis of (−)-Crispine A (2010)

In 2010, Amat et al. developed a general method for the enantioselective synthesis of 1-substituted tetrahydroisoquinoline alkaloids [63]. The key step of their approach is the stereoselective addition of a Grignard reagent to enantiopure oxazolopiperidone lactam **114**. Using this method several dimethoxytetrahydroisoquinoline alkaloids were synthesized including (−)-crispine A [(−)-**1**], in four steps and 15% overall yield. The enantiomeric excess was not determined but comparison of the optical rotation indicated an excellent enantioselectivity [63]. Reaction of methyl 2-formyl-4,5-dimethoxybenzoate (**112**) and (*R*)-phenylglycinol (**113**) afforded the enantiomerically pure key intermediate **114** in 52% yield. The retention of configuration during the nucleophilic

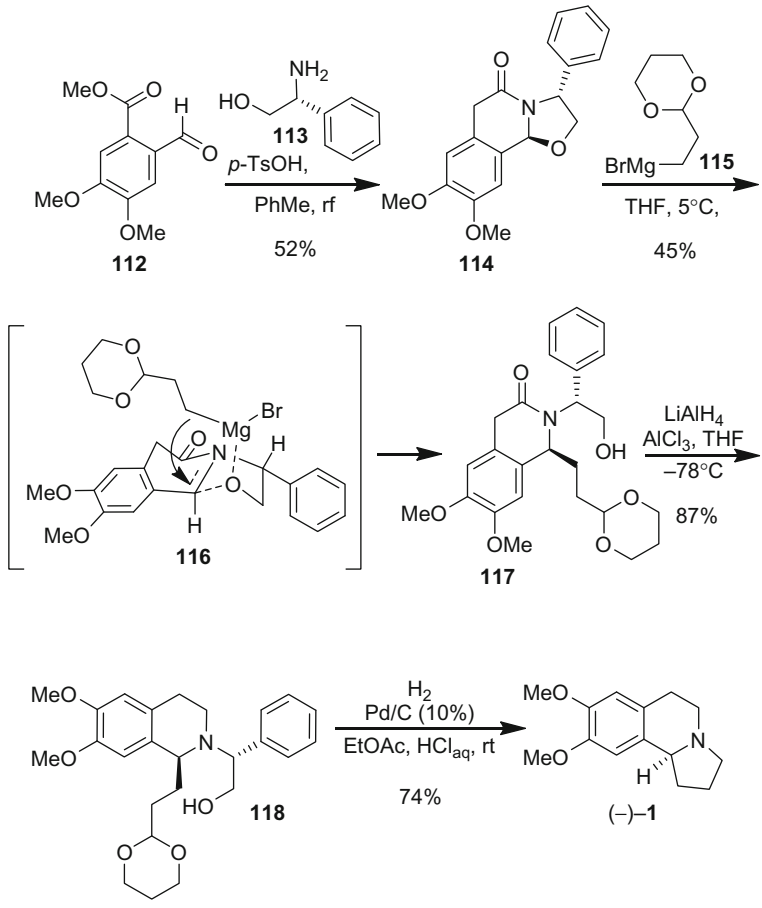


Scheme 27 Comparison of Pihko's (top) and Czarnocki's (bottom) asymmetric syntheses of (−)-crispine A [(−)-**1**] and (+)-crispine A [(+)-**1**] [31,62].

attack of the Grignard reagent **115** was explained by **116** as possible transition state. Reduction of amide **117** to the corresponding tertiary amine **118** followed by deprotection of the formyl group and concomitant removal of 1-hydroxymethylbenzyl group afforded directly (−)-crispine A [(−)-**1**] in 74% yield (Scheme 28) [63].

23. Jones' Synthesis of (±)-Crispine A (2010)

Jones and co-workers developed an unprecedented approach to crispine A (**1**). The product was formed by Diels–Alder reaction with subsequent retro-Diels–Alder reaction and reduction [64]. To circumvent endo/exo selectivity issues, the authors used the substituted anthracene **121** as auxiliary group for their synthesis. Enantioselective reduction of **119** with borane and the chiral ligand **120** followed by *O*-methylation afforded compound **121**. Subsequent [2+4] cycloaddition with *N*-(veratrylethyl) maleimide (**122**) [65] as dienophile afforded the Diels–Alder adduct **123**. The selectivity of this reaction is controlled by the electronic repulsion of the lone pairs of the methoxy group and the carbonyl oxygen atom. Reduction of **123** afforded the hemiaminal **124** that on treatment with trifluoracetic acid (TFA) underwent an intramolecular electrophilic aromatic substitution to the tetrahydroisoquinoline **125**. Subsequent removal of the anthracene moiety **121** by a flash vacuum pyrolysis occurred with an undesired isomerization of the double bond and thus resulted in a loss of the stereochemical information. The 1:1 mixture of the enamide **126** and the auxiliary **121** was subjected directly to hydrogenation using palladium on activated carbon as catalyst. Chromatographic separation provided

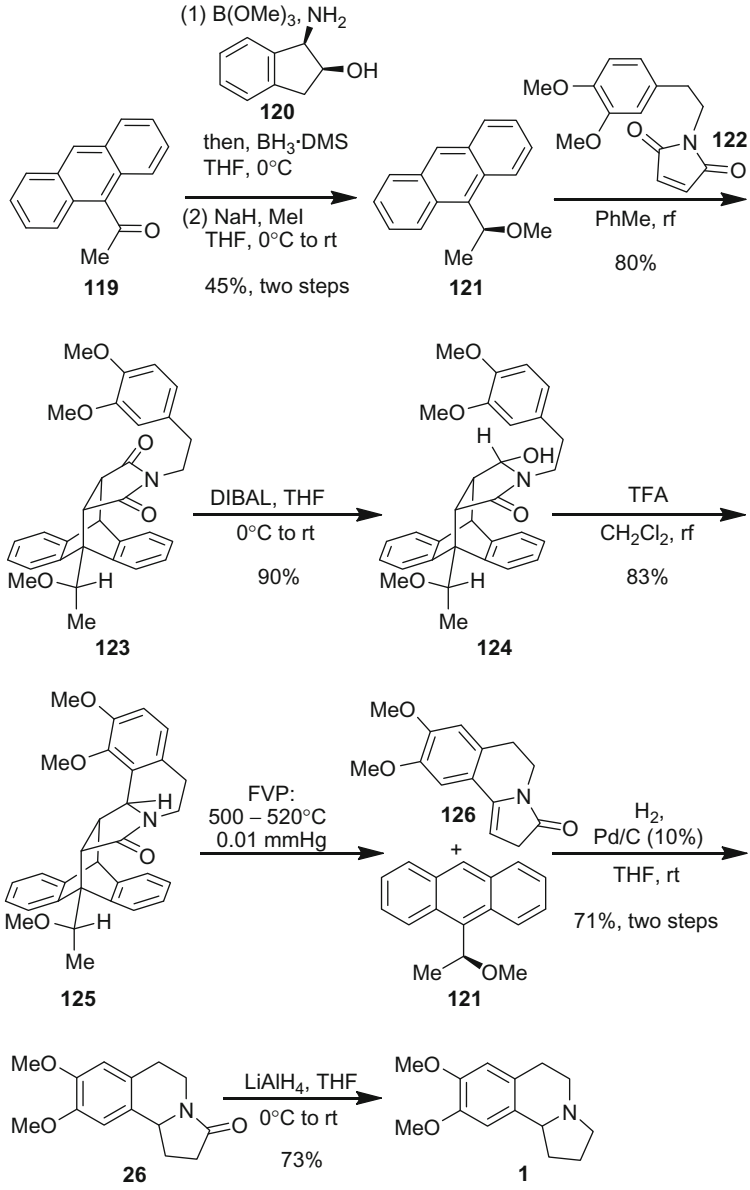


Scheme 28 Amat's synthesis of (−)-crispine A [(−)-**1**] [63].

the pure amide **26**. Reduction with lithium aluminum hydride afforded racemic crispine A (**1**) in six steps and 31% overall yield (Scheme 29).

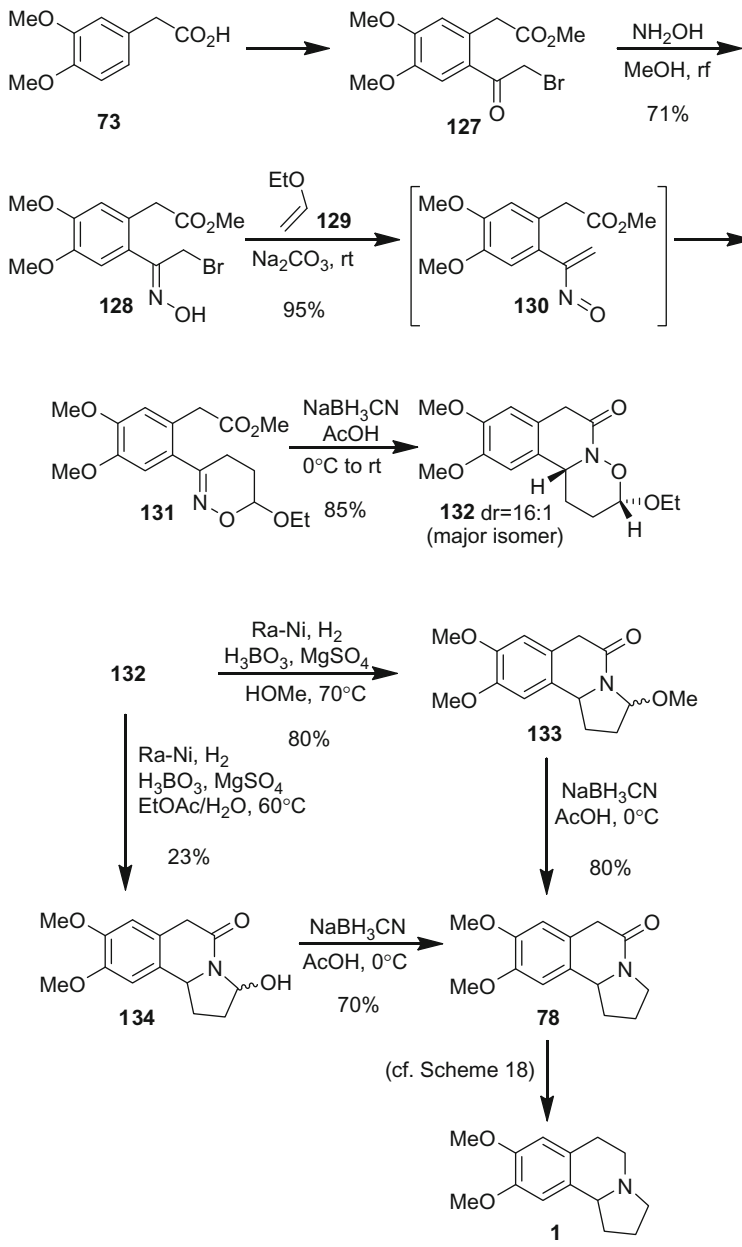
24. Gallos' Synthesis of (±)-Crispine A (2011)

Gallos and co-workers described a formal synthesis of crispine A (**1**) via a hetero-Diels–Alder reaction as key step (Scheme 30) [66]. Using 3,4-dimethoxyphenylacetic acid (**73**) as starting material, the authors followed literature procedures to synthesize the α-bromoketone **127** [67,68]. Ketone **127** was converted into the oxime **128**. Treatment of the α-bromooxime **128** with sodium carbonate in the presence of ethyl vinyl ether (**129**) afforded by elimination of hydrogen bromide the intermediate unstable nitrosoalkene **130** that underwent instantaneously a



Scheme 29 Jones' synthesis of crispine A (**1**) [64].

hetero-Diels–Alder reaction to the dihydrooxazine **131**. Reduction with sodium cyanoborohydride led to the oxazino[3,2-*a*]isoquinoline **132**. The isoquinolinone **132** was transformed to the amide **78** either via alcohol **134** or, in much better yields, via the corresponding methyl ether **133**



Scheme 30 Gallos' synthesis of crispine A (**1**) [66].

[66]. The final reduction of the amide **78** to racemic crispine A (**1**) was described previously by King in 2007 (cf. Scheme 18) [48].

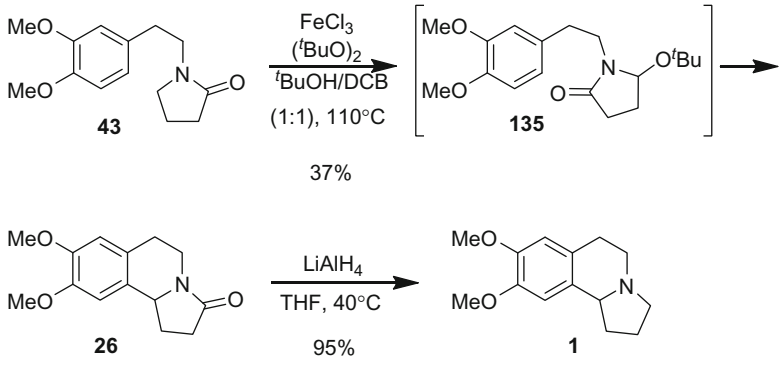
25. Hayashi's Synthesis of (±)-Crispine A (2011)

In 2011, Hayashi et al. developed an iron catalyzed one-pot procedure for the alkoxylation of amides followed by Friedel–Crafts coupling [69]. An intramolecular version of the Friedel–Crafts reaction was applied to the synthesis of crispine A (**1**). The starting material **43**, prepared following literature procedures [21,29,70], was treated with catalytic amounts of iron(III) chloride and di-*tert*-butyl peroxide as oxidant in a mixture of *tert*-butanol and 1,2-dichlorobenzene (DCB). The mechanism is described as a radical reaction. Alkoxylation in α-position to the lactam nitrogen atom afforded the intermediate **135**. Subsequently, Lewis acid-promoted Friedel–Crafts alkylation provided amide **26** in 37% yield as best result. The low yield for this transformation results from overoxidation of the substrate. Reduction with lithium aluminum hydride afforded crispine A (**1**) in 95% yield (Scheme 31).

26. Itoh's Synthesis of (+)-Crispine A (2011)

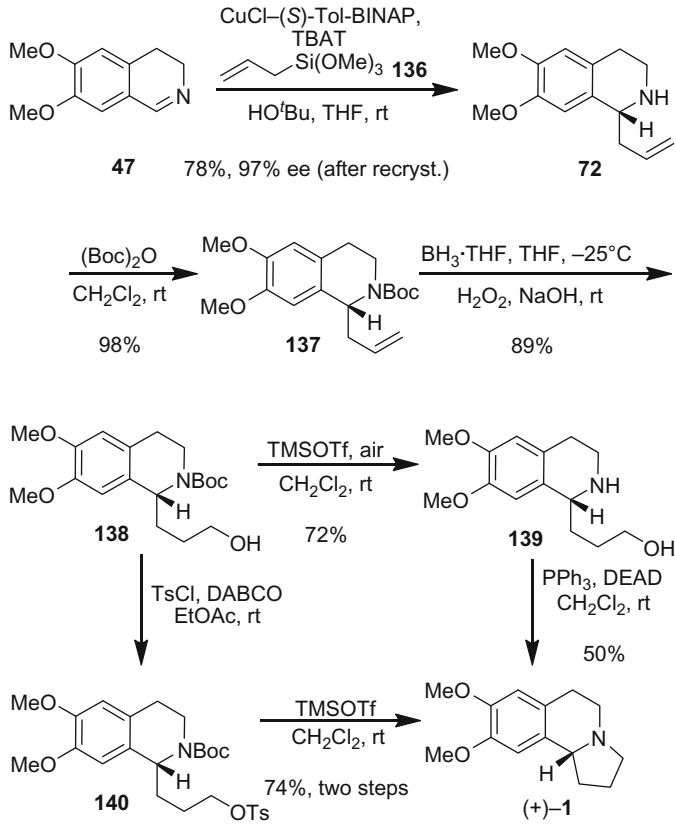
Following their earlier publication of the synthesis of (−)-crispine A [(−)-**1**] together with related alkaloids in 2007 (cf. Scheme 21) [55], Itoh and co-workers developed a copper-catalyzed asymmetric allylation of 3,4-dihydroisoquinolines. Using this method, they synthesized several enantiomerically pure 1-substituted tetrahydroisoquinolines. Application to natural product synthesis led to a novel approach to the alkaloid (+)-crispine A [(+)-**1**)] in six steps and 45% overall yield based on amine **19** [71].

Like Chong and co-workers in their asymmetric allylation (cf. Scheme 17), Itoh et al. used dihydroisoquinoline **47** as starting material for their key step [72]. Coupling of isoquinoline **47** and allyltrimethoxysilane (**136**) using copper(I) chloride, tetrabutylammonium difluorotriphenylsilicate



Scheme 31 Hayashi's synthesis of crispine A (**1**) [69].

(TBAT), and (*S*)-Tol-BINAP as chiral ligand provided the desired product **72** in 91% yield and 71% ee [71,73]. The enantiopurity could be enhanced by recrystallization with (−)-dibenzoyl-L-tartaric acid. After this purification, the allyl derivative **72** was obtained in 97% ee (78% yield). For the total synthesis of (+)-crispine A [(+)-**1**] the amino functionality of tetrahydroisoquinoline **72** was protected as *tert*-butyl carbamate. Hydroboration of compound **137** followed by oxidative work-up led to the chiral tetrahydroisoquinolin-1-ylpropanol **138**. Two different routes were explored for the transformation of alcohol **138** to the desired natural product (+)-crispine A [(+)-**1**]. Lewis acid-promoted cleavage of the Boc group and subsequent Mitsunobu reaction afforded (+)-**1** in 36% yield over two steps. Alternatively, alcohol **138** was converted into the tosylate **140**. Treatment of **140** with Lewis acid resulted in removal of the Boc group and cyclization. The latter route proved to be superior and provided (+)-crispine A [(+)-**1**] in 74% yield over two steps (Scheme 32).



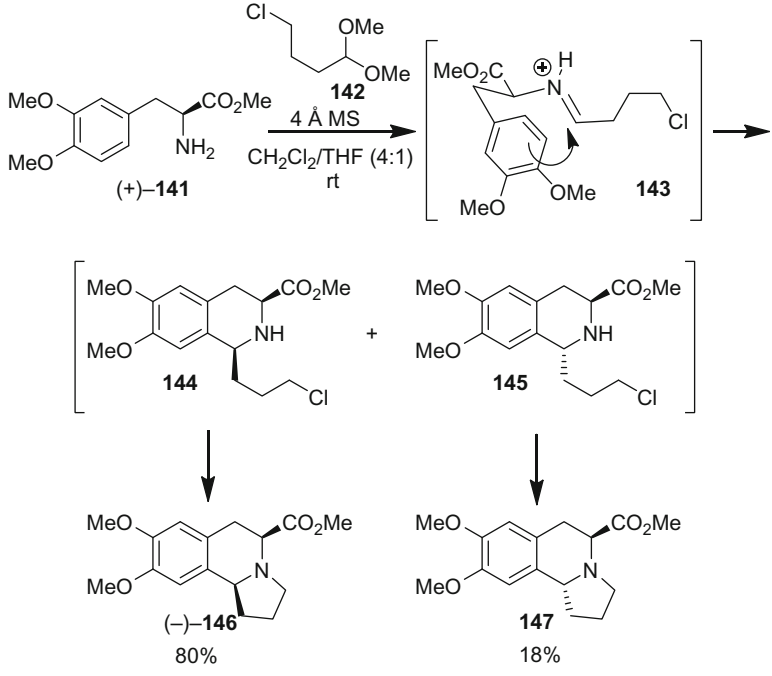
Scheme 32 Itoh's synthesis of (+)-crispine A [(+)-**1**] [71].

27. Herr's Synthesis of (+)- and (−)-Crispine A (2011)

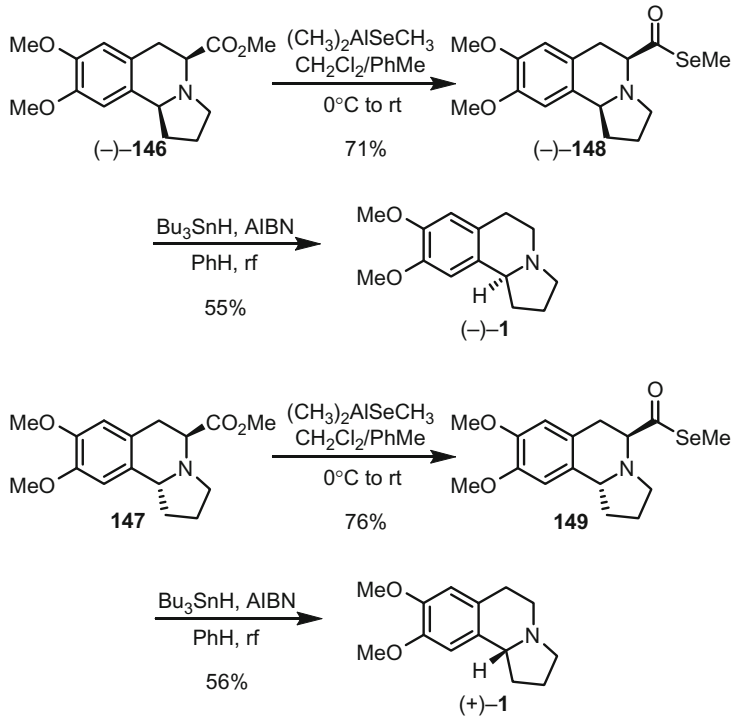
Herr and co-workers synthesized (+)-crispine A [(+)-**1**] and its enantiomer (−)-crispine A [(−)-**1**] starting from the commercially available amino acid esters (*S*)-(+)-methyl 2-amino-3-(3,4-dimethoxyphenyl)propanoate [(+)-**141**] (Schemes 33 and 34) and its enantiomer (−)-**141**, respectively [74]. Condensation of amine (+)-**141** with the protected aldehyde **142** led to the intermediate iminium salt **143**. Pictet–Spengler cyclization of **143** provided the *syn*-isomer **144** as major product. Subsequent intramolecular nucleophilic substitution afforded the pyrrolo[2,1-*a*]isoquinoline (−)-**146**. The minor isomer **145** led to the ester **147**. The diastereoselectivity of the Pictet–Spengler cyclization most likely derives from the preferred chair conformation of intermediate **143**.

The two diastereoisomeric esters (−)-**146** and **147** are formed in a ratio of 4.5:1. Transformation of (−)-**146** and **147** to the methyl selenoates (−)-**148** and **149** followed by radical defunctionalization with tributylstannane provided the natural product (+)-**1** in 8% overall yield from (+)-**141** and the non-natural enantiomer (−)-**1** in 31% overall yield based on (+)-**141** (Scheme 34).

Following the route described above, the natural enantiomer (+)-**1** was obtained only as minor product. Therefore, the same reaction



Scheme 33 Herr's synthesis of (+)- and (−)-crispine A [(+)-**1**, (−)-**1**] [74], Part I.

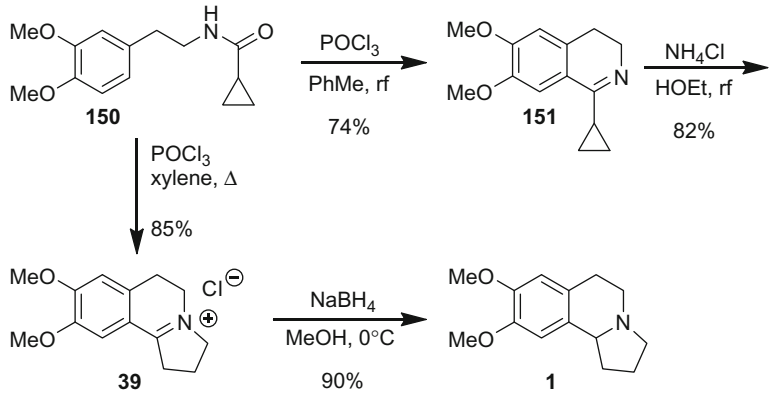


Scheme 34 Herr's synthesis of (+)- and (−)-crispine A [(+)-**1**, (−)-**1**] [74], Part II.

sequence was carried out starting from the more expensive (*R*)-(−)-isomer of **141**. (*R*)-(−)-**141** was transformed to the *syn*-isomer (+)-**146** in 82% yield. Transformation of (+)-**146** into (+)-crispine A [(+)-**1**] was achieved via the same sequence of steps as described for the (−)-enantiomer. Thus, (+)-crispine A [(+)-**1**] was obtained in three steps and with 32% overall yield.

28. Patro's Synthesis of (±)-Crispine A (2011)

Patro and co-workers described a synthesis of crispine A (**1**) using cyclopropanecarboxamide **150** as starting material [75]. The amide **150** is easily prepared from veratrylethylamine (**19**) and cyclopropanecarbonyl chloride [76]. Bischler–Napieralski cyclization of amide **150** using phosphorus oxychloride in toluene afforded the cyclopropyldihydroisoquinoline **151** in 74% yield. Heating of **151** in ethanol at reflux in the presence of ammonium chloride induced the desired rearrangement to the tetrahydropyrrolo[2,1-*a*]isoquinolinium salt **39**. This two-step procedure was converted to a one-pot reaction by change of solvent and temperature. Bischler–Napieralski cyclization with concomitant rearrangement was

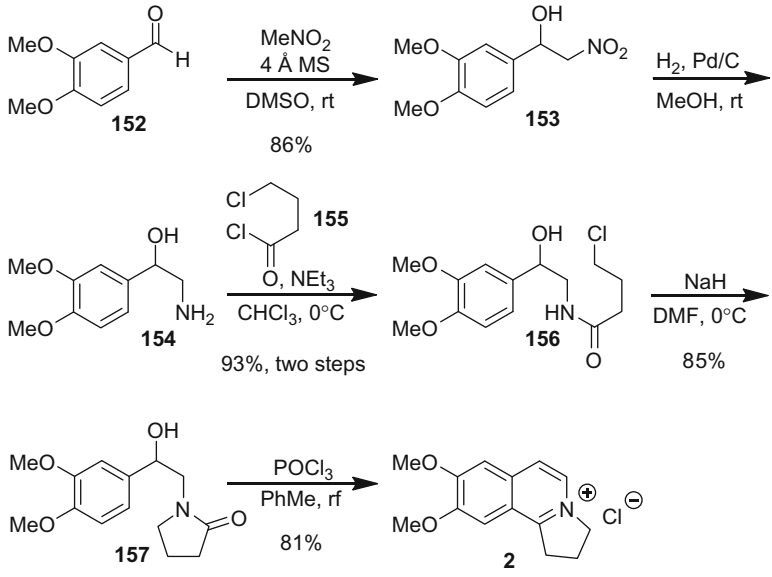


Scheme 35 Patro's synthesis of crispine A (**1**) [75].

achieved by heating amide **150** with phosphorus oxychloride in xylene as solvent and led to the iminium salt **40** in 85% yield. Reduction with sodium borohydride provided racemic crispine A (**1**) (Scheme 35) [75].

C. Syntheses of Crispine B

To the best of our knowledge, only one synthesis of the antitumor active alkaloid crispine B (**2**) has been reported so far.



Scheme 36 Muraoka's synthesis of crispine B (**2**) [77].

1. Muraoka's Synthesis of Crispine B (2009)

Muraoka and co-workers developed a facile five-step synthesis of crispine B (**2**). Starting from 3,4-dimethoxybenzaldehyde (**152**), crispine B (**2**) was obtained in 55% overall yield. The Henry reaction of 3,4-dimethoxybenzaldehyde (**152**) and nitromethane using a literature procedure provided compound **153**. Hydrogenation of the nitro group followed by acylation of amine **154** with γ-chlorobutyryl chloride (**155**) led to the chlorobutyramide **156**. The cyclization of the pyrrole ring by intramolecular nucleophilic substitution was induced by deprotonation of amide **156** with sodium hydride and afforded the lactam **157**. Pictet–Gams cyclization with phosphorus oxychloride provided crispine B (**2**) in 81% yield (Scheme 36) [77].

D. Syntheses of Trolline and Oleracein E

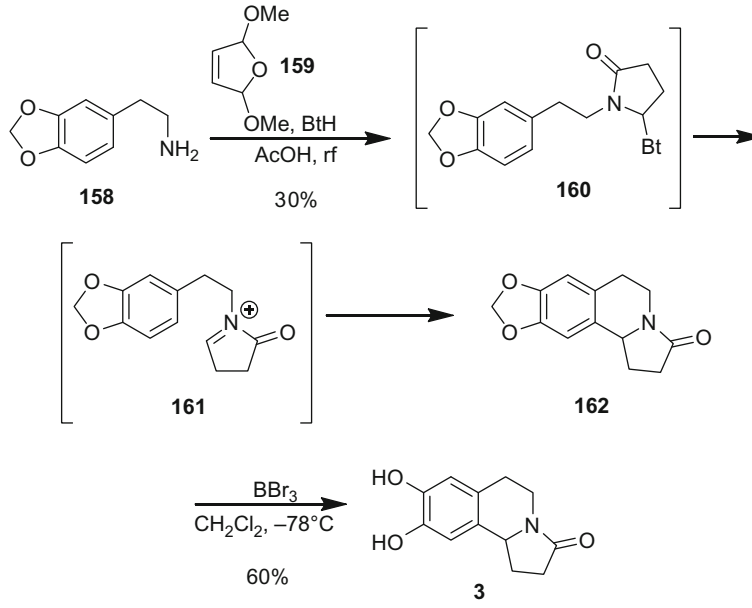
Even though the structure of trolline (−)-**3** is much related to that of crispine A [(+)-**1**], only three syntheses have been reported for this natural product. The first total synthesis by Liu et al. and the synthesis by Hayashi et al. provided racemic products. Thus, although it is not mentioned in the original publications, they can also be regarded as total syntheses of racemic oleracein E (**3**). The synthesis of enantiopure (+)-oleracein E [(+)-**3**] has not been reported so far. For reasons of clarity, only the name trolline (**3**) is used in the following section.

1. Liu's Synthesis of (±)-Trolline (2007)

In 2007, Liu and co-workers developed the first total synthesis of (±)-trolline [(±)-**3**] [78]. For the construction of the pyrrolo[2,1-*a*]isoquinoline framework a method developed by Katritzky et al. in 2001 [79] was adapted. Treatment of 2-(3,4-methylendioxyphenyl)ethylamine (**158**) with the dihydrofurane **159** and benzotriazole (BtH) in acetic acid at reflux afforded the amide **162**. Most likely, this transformation proceeds via the intermediates **160** and **161**. Cleavage of the methylene acetal with boron tribromide afforded (±)-trolline [(±)-**3**] in two steps and 18% overall yield (Scheme 37) [78].

2. Itoh's Synthesis of (−)-Trolline (2007)

In 2007, Itoh and co-workers developed a synthesis of (−)-trolline [(−)-**3**] together with syntheses of (−)-crispine A [(−)-**1**] and (−)-crispine E [(−)-**14**]. The synthesis of (−)-crispine A [(−)-**1**] has already been discussed above (cf. Chapter III.B.15). The key step of the enantioselective synthesis is an asymmetric addition of hydrocyanic acid to 6,7-dimethoxy-3,4-dihydroisoquinoline (**47**) followed by trifluoroacetylation. The construction of

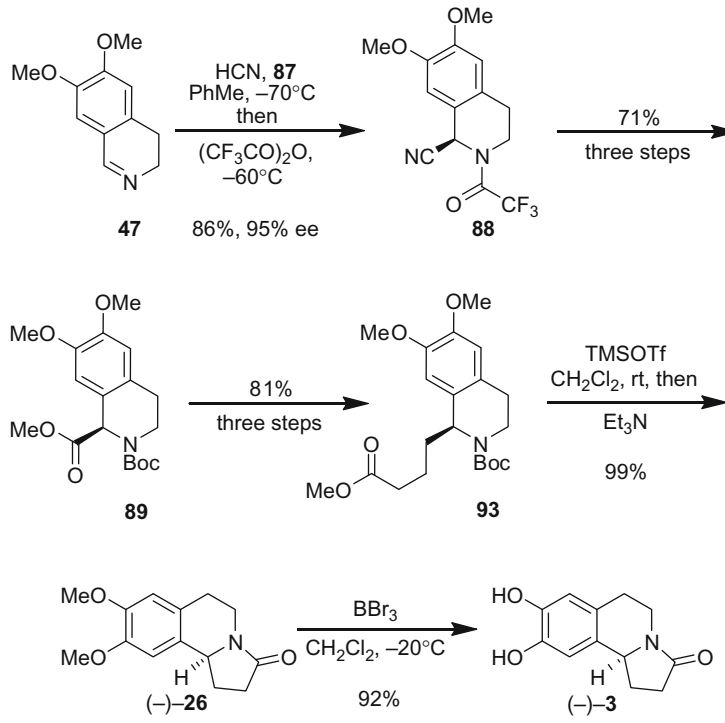


Scheme 37 Liu's synthesis of (±)-trolline [(±)-**3**] [78].

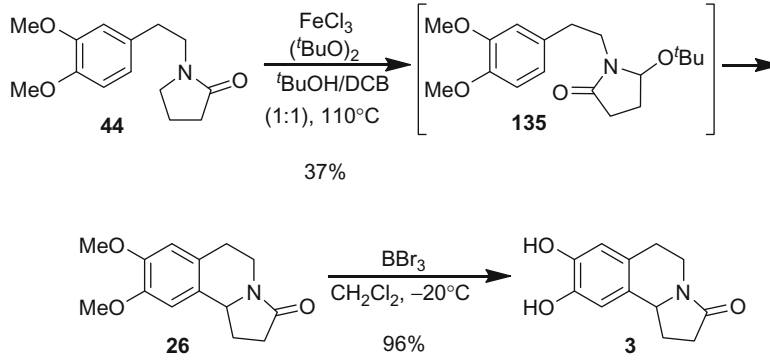
the stereogenic center was achieved using Jacobsen's catalyst **87** (Figure 9) to afford compound **88** in 86% yield and 95% ee. The following steps for the transformation of nitrile **88** to amide (−)-**26** are identical to those described for (−)-crispine A [(−)-**1**]. An ether cleavage using boron tribromide converted the amide (−)-**26** into (−)-trolline [(−)-**3**] in 92% yield (Schemes 21 and 38) [55].

3. Hayashi's Synthesis of (±)-Trolline (2011)

In 2011, Hayashi and co-workers developed a one-pot procedure for the alkoxylation of amides with concomitant Friedel–Crafts alkylation. Using this method, the racemic alkaloids crispine A (**1**) and trolline [(±)-**3**] were synthesized [69]. The synthesis of crispine A (**1**) has already been discussed (cf. Chapter III.B.25). The synthesis of trolline (**3**) follows the same route. The one-pot oxidation/cyclization of amide **44** afforded pyrrolo[2,1-*a*]isoquinoline **26** in 37% yield. Cleavage of the methyl ethers using boron tribromide provided (±)-trolline [(±)-**3**] in 96% yield (Scheme 39).



Scheme 38 Itoh's synthesis of (−)-trolline [(−)-**3**] [55].



Scheme 39 Hayashi's synthesis of (±)-trolline [(±)-**3**] [69].

IV. SYNTHESES OF ANALOGS

A series of pyrrolo[2,1-*a*]isoquinoline derivatives has been synthesized as structural analogs of the natural products. Some of them also show interesting biological activities.

A. Syntheses of Non-Substituted Pyrrolo[2,1-*a*]-isoquinolines

To the best of our knowledge, only the four non-substituted pyrroloisoquinolines **163**–**166** have been synthesized so far (Figure 12).

1. Syntheses of Pyrrolo[2,1-*a*]isoquinoline

For the parent pyrrolo[2,1-*a*]isoquinoline (**163**) three syntheses have been published. The first synthesis of **163** was reported by Boekelheide and Godfrey in 1953 [80]. Their approach is related to the syntheses of crispine A [(+)-1] developed by Child [32] and Sugasawa [33] which already have been described in Section III.B. Starting from β-phenethylamine (**167**) and γ-butyrolactone (**45**) the pyrrolidinone **168** was prepared via the method developed by Späth and Lintner [81]. Treatment of **168** with phosphorus pentoxide in tetraline at reflux provided tetrahydropyrrolo[2,1-*a*]isoquinoline **165** in 80% yield. Catalytic dehydrogenation using palladium on activated carbon in naphthalene at reflux under a carbon dioxide atmosphere afforded the aromatized pyrrolo[2,1-*a*]isoquinoline (**163**) in three steps and 24% overall yield (Scheme 40).

The second synthesis of compound **163** was published by Fröhlich and Kröhnke in 1971. They studied the 1,3-dipolar addition of acrylonitriles and *N*-ylides [82]. The isoquinolinium salt **169** was treated with base to give a 1,3-dipole. Addition of the nitriles **170** and **171** afforded the pyrrolo[2,1-*a*]isoquinolines **172** and **174**, respectively. Both compounds can be converted to the disubstituted pyrrolo[2,1-*a*]isoquinoline **175** by dehydrogenation using palladium on activated carbon as catalyst. In the case of **172**, a two-step procedure consisting of catalytic dehydrogenation and subsequent treatment with base also leads to **175**. By heating the disubstituted pyrroloisoquinoline **175** in sulfuric acid (40%) at reflux, compound **163** was obtained in 83% yield (Scheme 37). A comparison of the three routes reveals that **163** was obtained in a

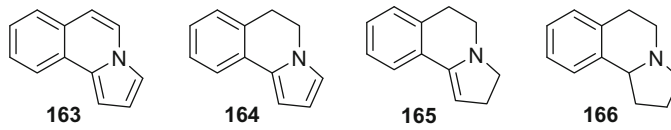
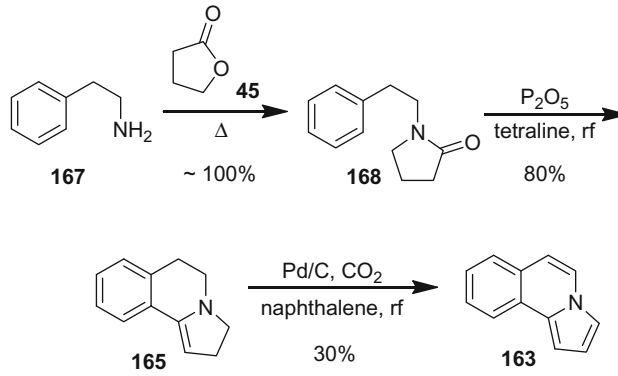
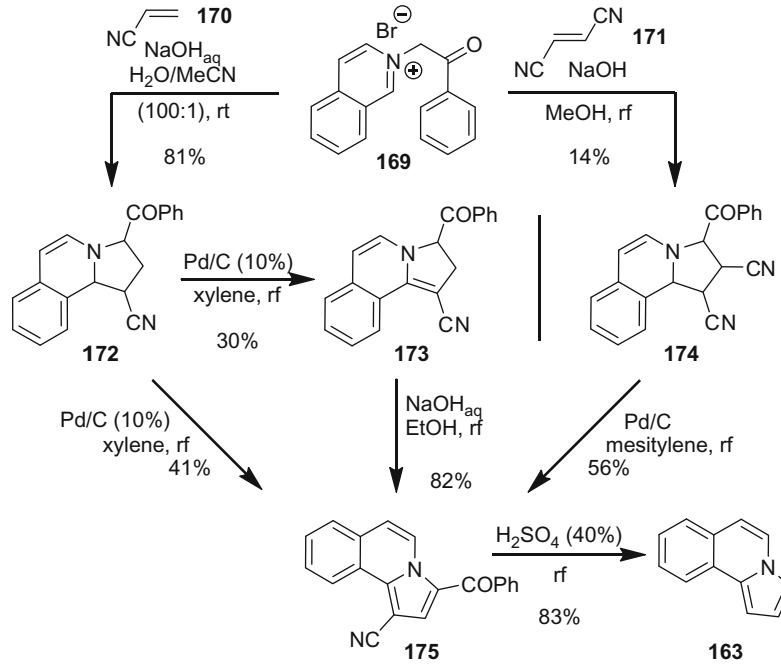


Figure 12 Non-substituted pyrrolo[2,1-*a*]isoquinolines **163**–**166**.



Scheme 40 Boekelheide's synthesis of pyrrolo[2,1-*a*]isoquinoline (**163**) [80].

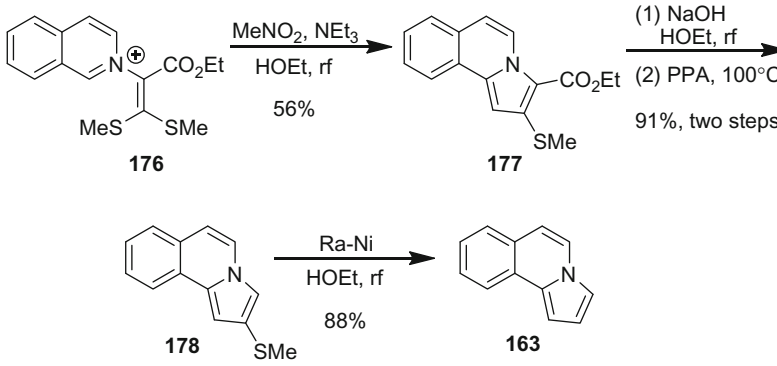


Scheme 41 Kröhnke's synthesis of pyrrolo[2,1-*a*]isoquinoline (**163**) [82].

maximum yield of 28% over three steps by cycloaddition of **169** and acrylonitrile (**170**) via intermediate **172** (Scheme 41).

In 1989, Tominaga et al. published the third synthesis of **163** [83]. Cyclization of the ketene dithioacetal **176** with nitromethane provided the pyrrolo[2,1-*a*]isoquinoline **177**. Ester cleavage of **177** and subsequent

decarboxylation by treatment with polyphosphoric acid at high temperature led to compound **178**. Removal of the thioether with Raney-Nickel provided **163** in 88% yield (Scheme 42).



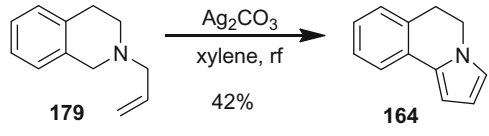
Scheme 42 Tominaga's synthesis of pyrrolo[2,1-*a*]isoquinoline (**163**) [83].

2. Syntheses of 5,6-Dihydropyrrolo[2,1-*a*]isoquinoline

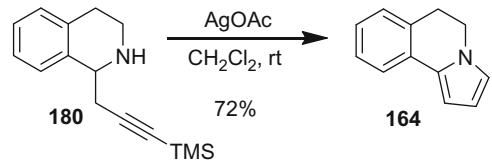
Three different syntheses for non-substituted 5,6-dihydropyrroloisoquinoline (**164**) are known. The first synthesis was described by Grigg et al. in 1992. Allylamine **179**, prepared from tetrahydroisoquinoline and allyl bromide, was treated with silver carbonate to afford compound **164** in 48% yield (Scheme 43) [84].

The second synthesis was developed by Knölker and Agarwal in 2004. As discussed above (cf. their synthesis of crispine A (**1**), Section III.B.8), addition of the propargyl Grignard reagent **61** to 3,4-dihydroisoquinoline led to the homopropargylamine **180**. The silver-mediated oxidative cyclization of **180** afforded compound **164** in 72% yield (Scheme 44) [40].

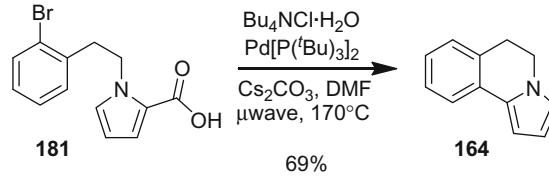
A third possibility for the synthesis of compound **164** was developed by Bilodeau et al. in 2010. Cyclization of the pyrrole carboxylic acid **181** by an intramolecular palladium-catalyzed decarboxylative cross coupling reaction provided the dihydropyrrolo[2,1-*a*]isoquinoline **164** in 69% yield (Scheme 45) [85].



Scheme 43 Grigg's synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**164**) [84].



Scheme 44 Knölker's synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**164**) [40].



Scheme 45 Bilodeau's synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinoline (**164**) [85].

3. Syntheses of 2,3,5,6-Tetrahydropyrrolo[2,1-*a*]isoquinoline

Since compound **165** is not very stable, only a few syntheses have been reported for this compound. The first was described by Boekelheide et al. as discussed in Section IV.A.1. In their approach, tetrahydropyrrolo[2,1-*a*]isoquinoline **165** was obtained as an intermediate toward the fully aromatic pyrrolo[2,1-*a*]isoquinoline (**163**) [80].

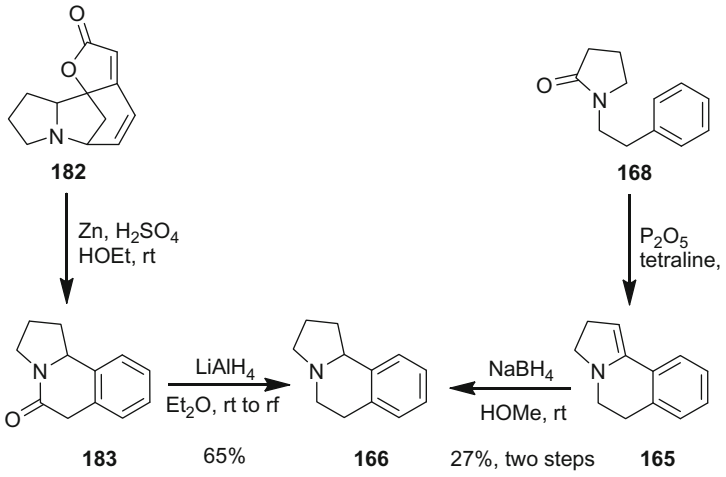
In 1965, Tamura and co-workers obtained compound **165** as an intermediate in their synthesis of hexahydropyrrolo[2,1-*a*]isoquinoline **166** [86]. Compound **165** was also an intermediate in the asymmetric synthesis of (−)-**166** developed by Zhou and co-workers [58]. Both syntheses are discussed in the following section.

4. Syntheses of 1,2,3,5,6,10b-Hexahydropyrrolo[2,1-*a*]isoquinoline

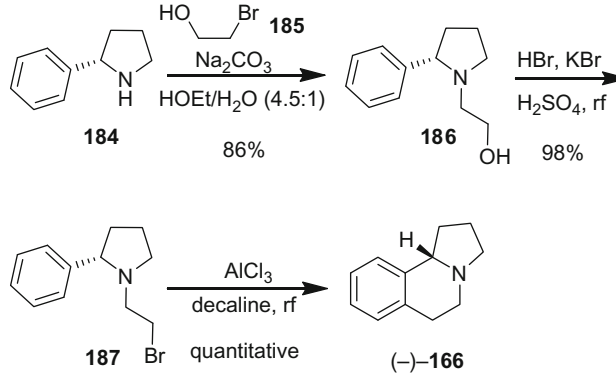
Out of the four non-substituted pyrrolo[2,1-*a*]isoquinolines **163**, **164**, **165**, and **166**, compound **166** has been synthesized most often.

In 1965, while proving the structure of alkaloid norsecurinine (**182**), compound **166** was synthesized for the first time (Scheme 46) [86]. The isolated natural product **182** was degraded with zinc dust and sulfuric acid leading to lactam **183**. Reduction of **183** using lithium aluminum hydride provided the compound **166**. Cyclization of **168** with phosphorous pentoxide, as described by Boekelheide and Godfrey [80], led to the unstable tetrahydro derivative **165**. Reduction with sodium borohydride afforded the identical product **166**, thus supporting the structure proposed for norsecurinine (**182**).

In 1976, Morlacchi and Losacco described the second synthesis of **166** using the enantioenriched (*S*)-2-phenylpyrrolidine (**184**) as starting



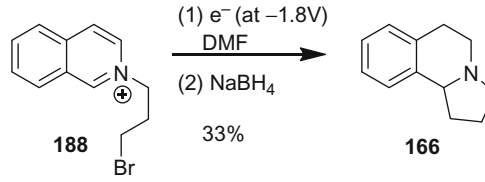
Scheme 46 Tamura's synthesis of 1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (**166**) [86].



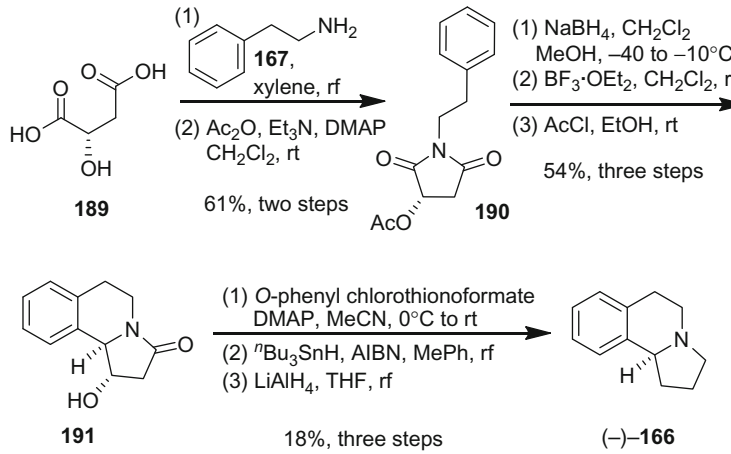
Scheme 47 Morlacchi's synthesis of (−)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]-isoquinoline [(−)-**166**] [87].

material (Scheme 47) [87]. Reaction of **184** with 2-bromoethanol **185** led to the *N*-alkylated product **186**. Replacement of the hydroxy group by bromine and subsequent Friedel–Crafts reaction furnished (*S*)-(−)-**166** without loss of stereochemical information.

In 1978, Shono et al. reported a new method for the formation of alkaloid frameworks via electrolytic cyclizations. Starting from the *N*-substituted isoquinoline **188**, the hexahydropyrrolo[2,1-*a*]isoquinoline **166** was obtained in 33% yield by cyclization and subsequent reduction with sodium borohydride (Scheme 48) [88].



Scheme 48 Shono's synthesis of 1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (**166**) [88].



Scheme 49 Lee's synthesis of (−)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline [(−)-**166**] [89,90].

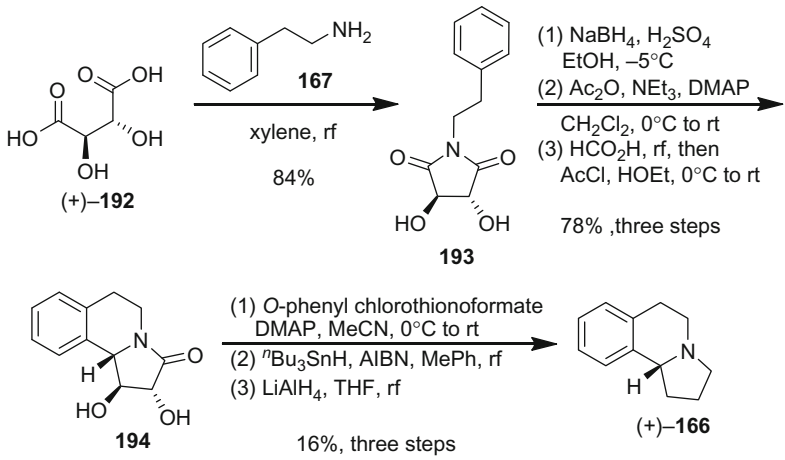
In 1987, Maryanoff et al. synthesized compound **166** using Boekelheide and Godfrey's approach (cf. Scheme 40) [80]. Starting from amine **167** and lactam **45**, they obtained intermediate **165**. Reduction of **165** using sodium borohydride provided **166** [25].

In 1995, Lee et al. developed a new asymmetric strategy for the synthesis of both enantiomers of **166** starting from L-malic acid (**189**) and L-tartaric acid [(+)-**192**], respectively. L-Malic acid (**189**) was transformed into imide **190** by condensation with phenethylamine **167** and subsequent acetylation. Chemoselective reduction of the sterically more hindered carbonyl group followed by Lewis acid-promoted stereoselective cyclization and cleavage of the ester led to lactam **191**. Removal of the redundant hydroxy group was achieved by Barton–McCombie deoxygenation. Subsequent reduction of the intermediate amide with lithium aluminum hydride provided enantiomerically pure compound (−)-**166** in eight steps and 6% overall yield (Scheme 49) [89].

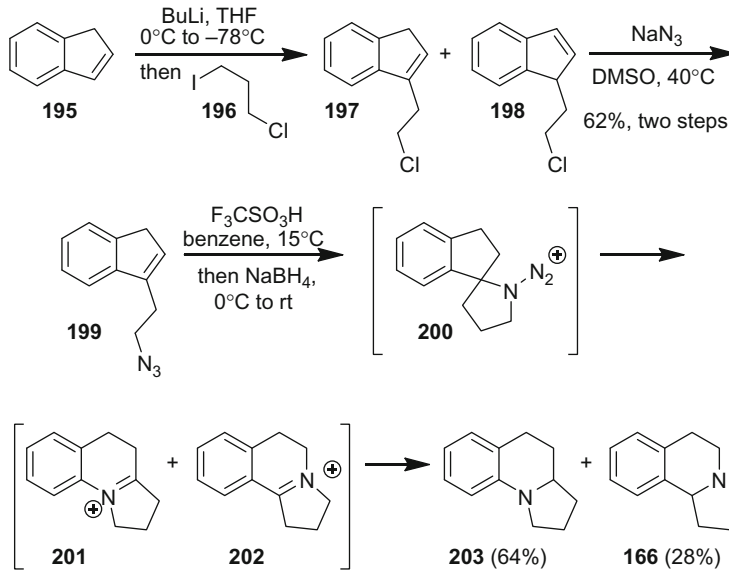
The synthesis of the enantiomer (+)-**166** follows similar lines. Formation of imide **193** and subsequent reduction, acetylation, and cyclization with *in situ* ester cleavage led to the enantiomerically pure compound **194** [90]. Twofold Barton–McCombie deoxygenation followed by reduction using lithium aluminum hydride provided the enantiomerically pure product (+)-**166** in seven steps and 11% overall yield (Scheme 50) [89].

In 2000, Pearson and Fang synthesized various nitrogen-containing benzoannulated heterocycles using a Schmidt rearrangement as key step [91]. Metallation of indene (**195**) with butyllithium and subsequent alkylation with **196** led to a mixture of **197** and **198**. The crude product mixture was transformed into the corresponding azides with concomitant thermal double bond isomerization [92] to the more stable azide **199**. Treatment of **199** with trifluoromethanesulfonic acid induced the Schmidt rearrangement to the regioisomeric iminium ions **201** and **202** by loss of dinitrogen. Reduction with sodium borohydride afforded the two products **203** and **166** in a combined yield of 92%. Following the this route, **166** was obtained in three steps as minor product with an overall yield of 17% (Scheme 51) [91].

Sato and co-workers investigated the addition of chiral allyltitanium compounds to various cyclic and acyclic aldehydes, ketones, aldimines, and ketimines [93–97]. In the course of their studies they synthesized the enantiomerically pure compound (+)-**166** [97]. The acrolein-derived allylacetal **205** was used as starting material for the synthesis of the chiral titanium species **207**. Reaction of **205** with (η^2-propene)titanium diisopropoxide, prepared *in situ* from titanium(IV) isopropoxide and isopropylmagnesium bromide, afforded **207** that was then treated with



Scheme 50 Lee's synthesis of (+)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline [(+)-**166**] [89,90].

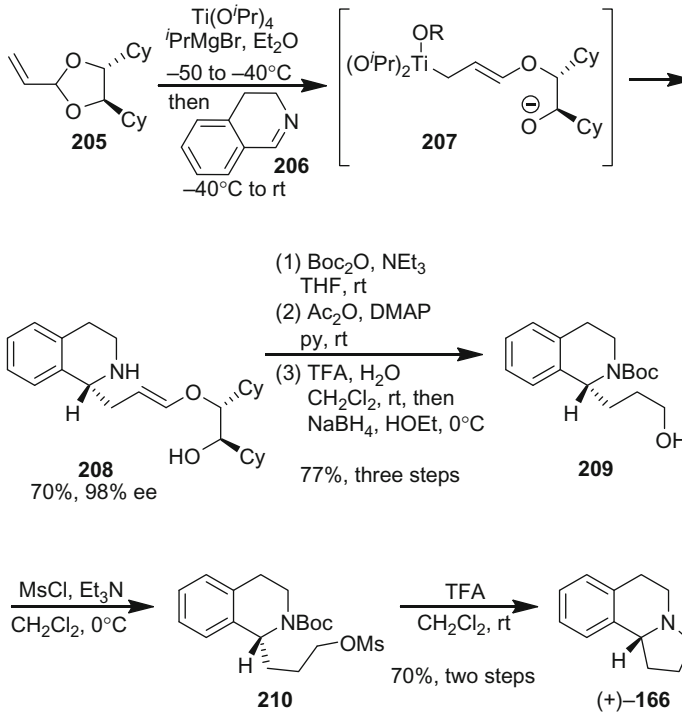


Scheme 51 Pearsons's synthesis of 1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (**166**) [91].

3,4-dihydroisoquinoline (**206**). The isoquinoline **208** is obtained in 70% yield and 98% ee (Scheme 52). The stereoselectivity of the reaction is explained by the proposed transition state **204** (Figure 13) [97].

A sequence of *N*-Boc-protection, *O*-acetylation, and treatment with aqueous trifluoroacetic acid followed by reduction using sodium borohydride generated the free alcohol **209**. Transformation of the alcohol **209** into the mesylate **210** followed by removal of the Boc group with concomitant cyclization led to (+)-**166** (Scheme 52) [97].

In 2002, Ohsawa and co-workers published a further asymmetric synthesis of (+)-**166**. The key step is the asymmetric addition of allyltributyltin to a substituted isoquinoline. Following a literature procedure, electrophilic bromination of isoquinoline (**211**) with bromine in the presence of aluminum trichloride afforded the dibromo derivative **212** in 55% yield [98]. Compound **212** was chosen for the reaction due to the improved stereoselectivity of the subsequent allylation [99,100]. Addition of freshly prepared chiral acid chloride **213** and allyltributyltin (**214**) to the isoquinoline **212** furnished adduct **215** in excellent yield and diastereoisomeric excess. Hydroboration of the homoallylamine **215** followed by oxidative work-up provided alcohol **216**. Hydrogenation of **216** using palladium on activated carbon as catalyst provided via the initially formed debrominated isoquinoline **217**, the tetrahydroisoquinoline **218**. Reduction with lithium aluminum hydride followed by intramolecular



Scheme 52 Sato's synthesis of (+)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline [(+)-**166**] [97].

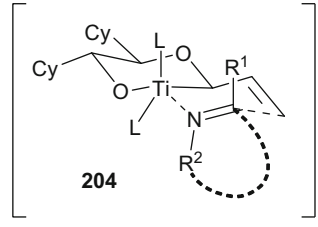
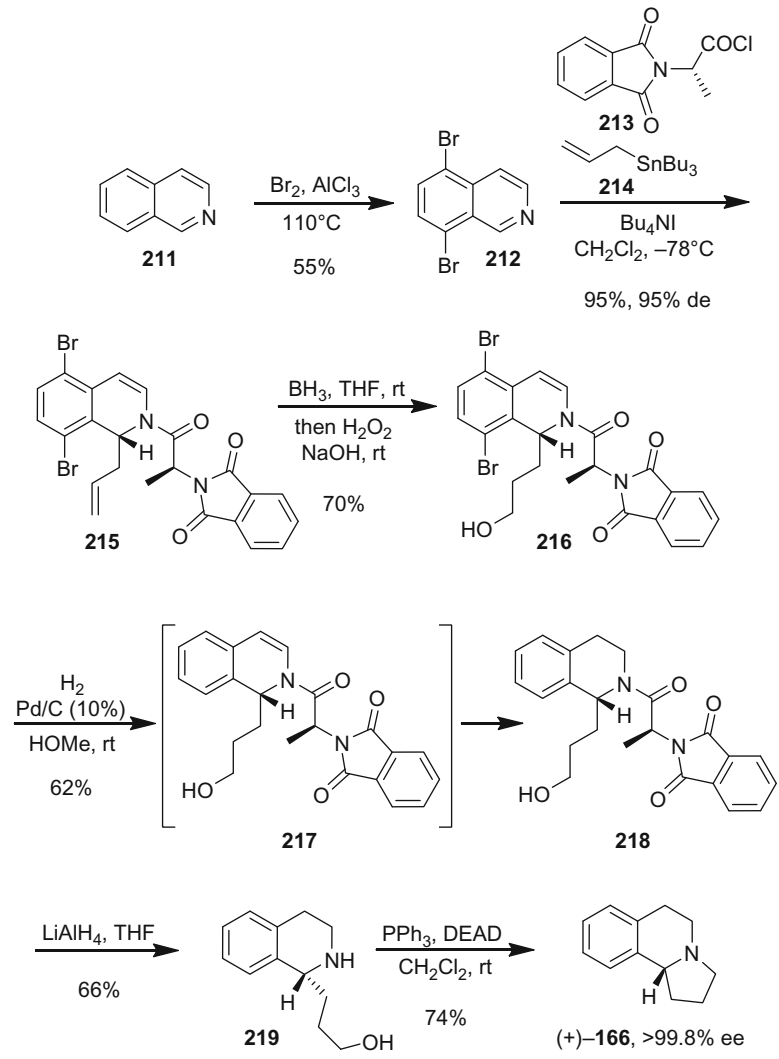


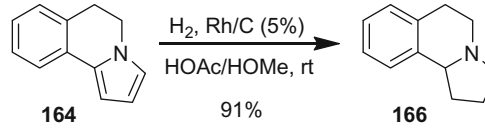
Figure 13 Sato's proposed transition state **204** [97].

Mitsunobu reaction provided (+)-**166** in six steps and 11% overall yield (Scheme 53) [101,102].

In 2005, Knölker and Agarwal described a synthesis of **166**. The starting material **164** was prepared using their silver-mediated oxidative cyclization (cf. Section IV.A.2) [40]. Rhodium-catalyzed hydrogenation of **164** under the same conditions as described for their synthesis of crispine A (**1**) (cf. Section III.B.8) provided compound **166** in 91% yield (Scheme 54) [30].



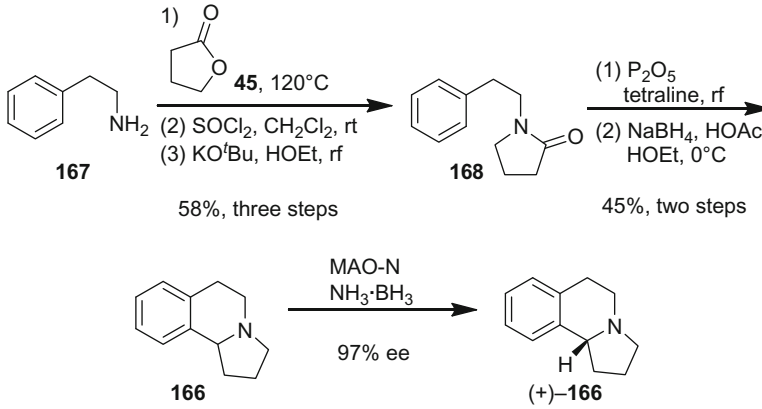
Scheme 53 Ohsawa's synthesis of (+)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline [(+)-**166**] [101,102].



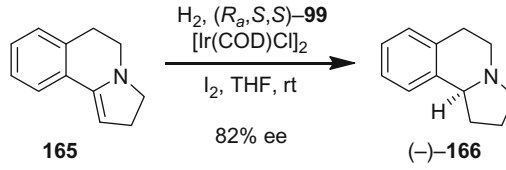
Scheme 54 Knölker's synthesis of 1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (**166**) [30].

In 2007, Turner and co-workers developed a chemoenzymatic deracemization of tertiary amines [51]. This approach using the enzyme monoamine oxidase from *A. niger* (MAO-N) was applied not only to the synthesis of (+)-crispine A [(+)-**1**] (cf. Section III.B.13) but also to the synthesis of enantiomerically pure (+)-**166**. Racemic **166** was prepared using the same starting materials as Boekelheide and Godfrey [80]. Cyclization of compound **168** with phosphorous oxychloride and subsequent reduction using sodium borohydride afforded **166** in 45% yield over two steps. From the racemic mixture of **166**, the pure (*R*)-(+)-enantiomer (+)-**166** was obtained by selective enzymatic oxidation of the *S*-(−)-enantiomer (−)-**166** to the corresponding non-chiral iminium salt **202**. Nonselective reduction of the iminium salt **202** to both enantiomeric forms leads to an enrichment of (+)-**166** in the reaction mixture. In only 6 h, an enantiomeric excess of 97% could be achieved (Scheme 55) [51].

In 2009, Zhou and co-workers described an enantioselective synthesis of (−)-**166** along with their synthesis of crispine A [(+)-**1**] described in Section III.B.18 [58]. Asymmetric hydrogenation of enamine **165** using an iridium complex and chiral ligand (R_a,*S*,*S*)-**99** (cf. Figure 10) afforded (−)-**166** (Scheme 56).



Scheme 55 Turner's synthesis of (+)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]-isoquinoline [(+)-**166**] [51].



Scheme 56 Zhou's synthesis of (−)-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline [(−)-**166**] [58].

B. Syntheses of Substituted Analogs

This section covers selected analogs of the natural products **1**–**4** and **10**–**11** that are methylated and/or oxygenated and that have saturated B- and C-rings (cf. Figure 14).

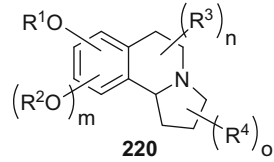


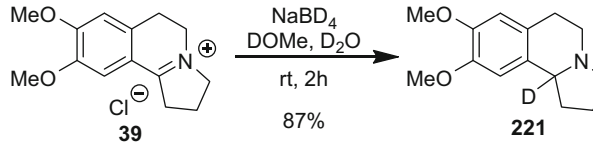
Figure 14 Substitution pattern of the discussed pyrrolo[2,1-*a*]isoquinolines.

1. Deuterated Crispine A

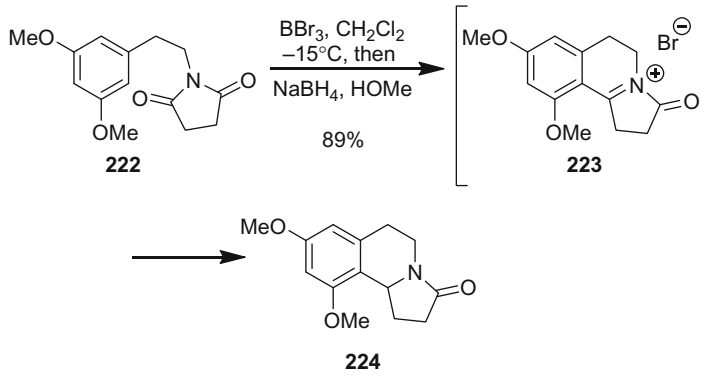
Bremner and Winzenberg studied the photolysis of bridgehead quaternary ammonium salts. In the course of their studies, crispine A (**1**) was synthesized using Child and Pyman's approach (cf. Scheme 7) [32]. Additionally, reduction of the iminium salt **39** using sodium borodeuteride afforded the deuterated crispine A derivative **221** (Scheme 57) [29].

2. Oxygenated Derivatives

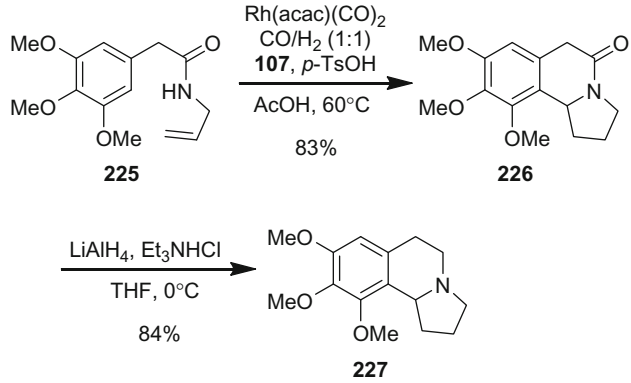
The four pyrrolo[2,1-*a*]isoquinoline derivatives **224**, **226**, **227**, and **230** are the closest analogs of peyoglutam (**10**) and mescalotam (**11**) reported in the literature. Compound **224** was synthesized first by Ramanathan and co-workers in 2010. They developed a one-pot procedure for the cyclization of methoxy-substituted *N*-phenethylimides to nitrogen-containing heterocycles. Using boron tribromide as Lewis acid and sodium borohydride as reducing agent they obtained compound **224** via the iminium salt **223** in good yield (Scheme 58) [103].



Scheme 57 Bremner's synthesis of 1,2,3,5,6,10b-hexahydro-8,9-dimethoxy-[10b–*d*]-pyrrolo[2,1-*a*]isoquinoline (**221**) [29].



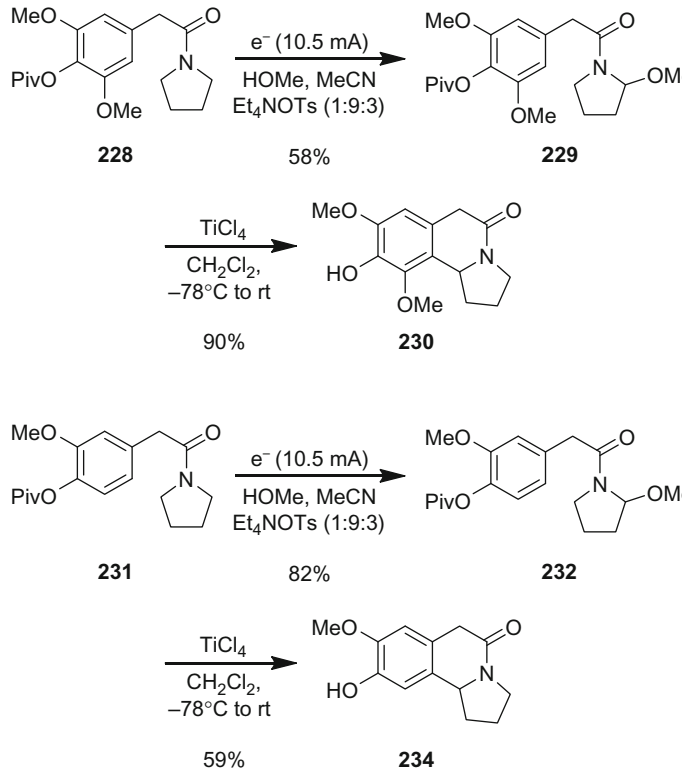
Scheme 58 Ramanathan's synthesis of **224** [103].



Scheme 59 Chiou's synthesis of **226** and **227** [61].

The 8,9,10-trimethoxypyrrolo[2,1-*a*]isoquinoline **227** was synthesized first by Chiou et al. in 2009 (Scheme 59) [61]. They used the same rhodium-catalyzed cyclohydrocarbonylation-cyclization of *N*-allylamides as already discussed for their synthesis of crispine A (**1**) (cf. Section III. B.20). The double ring closure furnished **226** in 83% yield. Reduction with *in situ* generated alane–triethylamine complex afforded **227** in 84% yield.

A compound with similar structure was synthesized previously by Moeller et al. in 1991. Anodic oxidation of the amides **228** and **231** in the presence of methanol and tetraethylammonium tosylate in acetonitrile resulted in methoxylation. Both intermediates were cyclized with titanium tetrachloride to the lactams **230** and **234** (Scheme 60) [104].

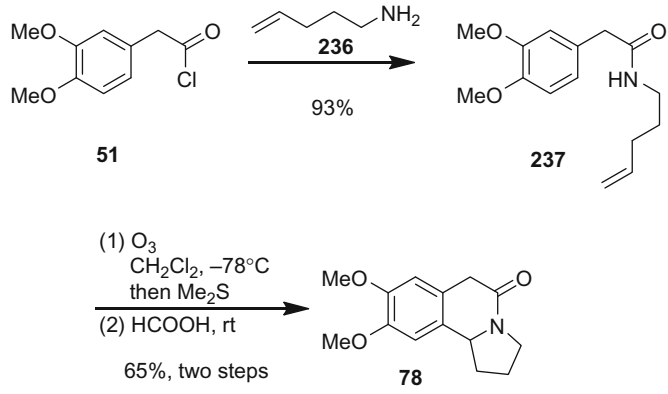


Scheme 60 Moeller's synthesis of **230** and **234** [104].

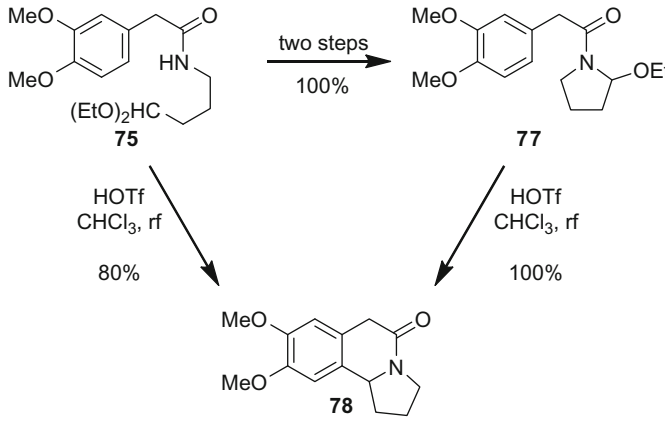
Lactam **78**, which is closely related to compound **234**, has been an intermediate in some of the syntheses of crispine A (**1**) discussed in Section III.B.12, 20, and 24 [48,61,66]. In 1985, lactam **78** has already been synthesized by Kano et al. using an ozonolysis as key step. Condensation of the acid chloride **51** with amine **236** provided amide **237**. Subsequent ozonolysis with reductive work-up was followed by treatment with formic acid to provide lactam **78** in 60% yield over two steps (Scheme 61) [105].

King et al. described a modification of their earlier synthesis of lactam **78** (cf. Scheme 18, Section III.B.12). An improvement was achieved by treatment of compound **77** with trifluoromethanesulfonic acid in chloroform at reflux which provided lactam **78** quantitatively. The direct conversion of acetal **75** into **78** could not be improved (Scheme 62) [106].

Compound **134** is a close structural analog of lactam **78**, discussed above. The synthesis of **134** has been discussed in Section III.B.24 (Scheme 30) [66]. Further hydroxylated pyrrolo[2,1-*a*]isoquinoline



Scheme 61 Kano's synthesis of **78** [105].



Scheme 62 King's synthesis of **78** [106].

derivatives reported in the literature are the compounds **238** and **239** (Figure 15).

In 1997, Lete and co-workers described the synthesis of the hemiaminal **238**. Key step of their approach is a lithium–halogen exchange at the iodinated succinimide **240** with subsequent cyclization by intramolecular nucleophilic attack at the carbonyl group of the imide (Scheme 63). As **238** is prone to spontaneous dehydration, a full characterization was not possible [107].

In 1976, Dörnyei and Szántay described the synthesis of the dihydroxylated compound **239** using D-tartaric acid [(−)-**192**] as starting material. However, in all schemes of their publication the L-derivative (+)-**192** has been drawn. Following a literature procedure, D-tartaric acid [(−)-**192**] was

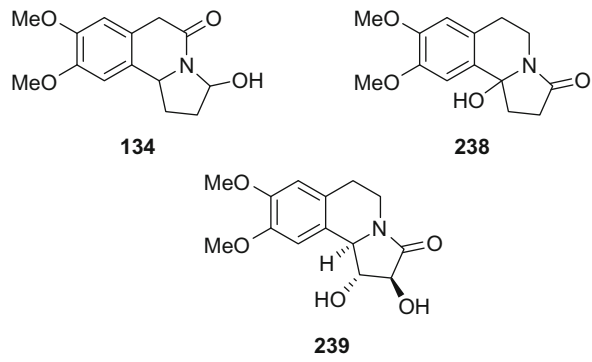
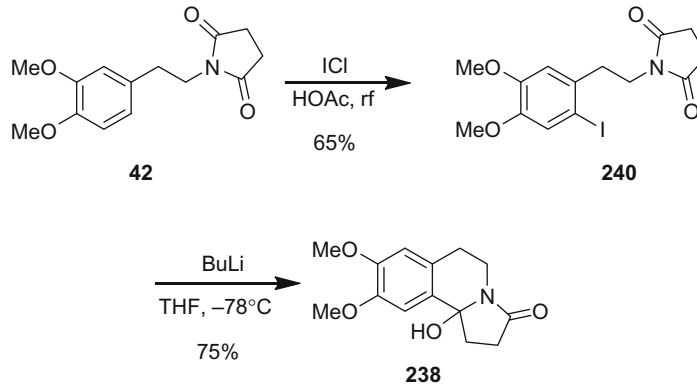


Figure 15 Hydroxylated derivatives **134**, **238**, and **239**.

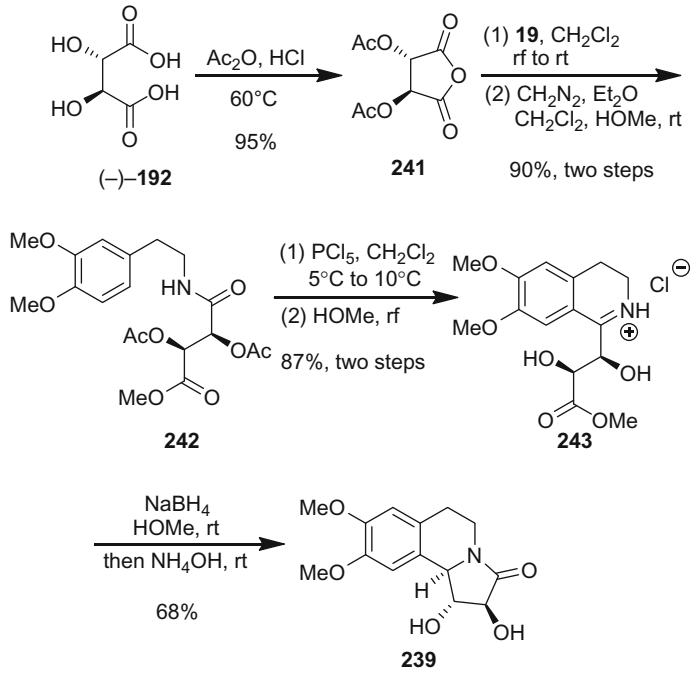


Scheme 63 Lete's synthesis of **238** [107].

transformed into the diacetylated anhydride **241** [108]. Amidation with veratrylethylamine (**19**) provided the free acid that on treatment with diazomethane furnished the methyl ester **242**. Cyclization with phosphorous pentachloride followed by methanolysis of the acetyl groups led to isoquinolinium salt **243**. Reduction with sodium borohydride provided a tetrahydroisoquinoline which on standing overnight at pH 10 cyclized to the pyrrolo[2,1-*a*]isoquinoline **239** (Scheme 64) [109].

3. Methylated Derivatives

In this section, the methylated pyrrolo[2,1-*a*]isoquinolines **244**–**249** are discussed as structural analogs of the natural products **1**–**4** and **10**–**11** (Figure 16).



Scheme 64 Dörney's synthesis of **239** [109].

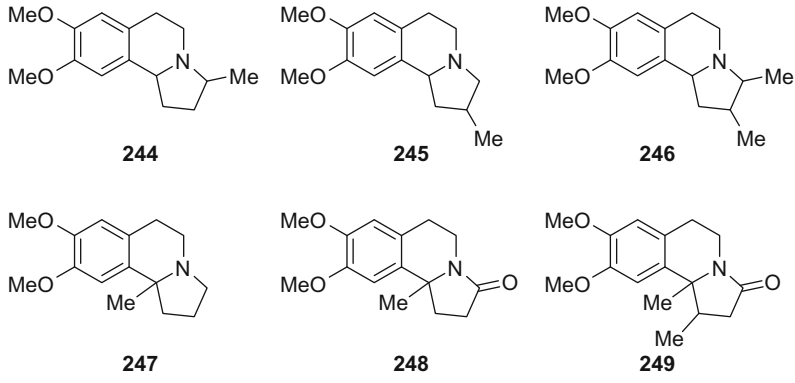


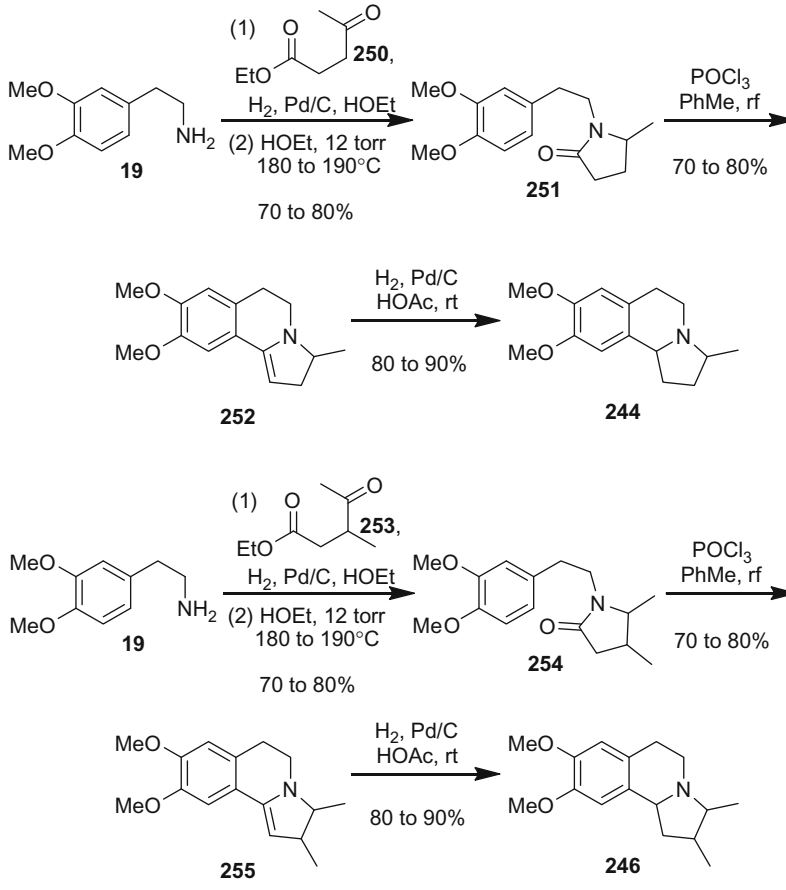
Figure 16 Methylated derivatives **244**–**249**.

The monomethylated compound **244** was synthesized in 1952 along with the dimethylated compound **246**. Pailer and Brandstetter used veratrylethylamine (**19**) for condensation with the γ-ketoesters **250** and **253**, respectively. The resulting lactams **251** and **254** were subjected to a

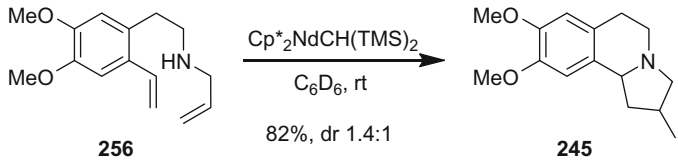
Bischler–Napieralski cyclization. Catalytic hydrogenation of the resulting enamines **252** and **255** led to the methylated derivatives **244** and **246** in good yields (Scheme 65) [110].

Compound **245** was synthesized first by Molander and Pack in 2003. The allylamine **256** was prepared in four steps and 12% yield starting from (3-bromo-4,5-dimethoxyphenyl)methanol. A neodymium-catalyzed cyclization of **256** provided **245** in a diastereoisomeric ratio of *cis*:*trans*=1.4:1 (Scheme 66) [111].

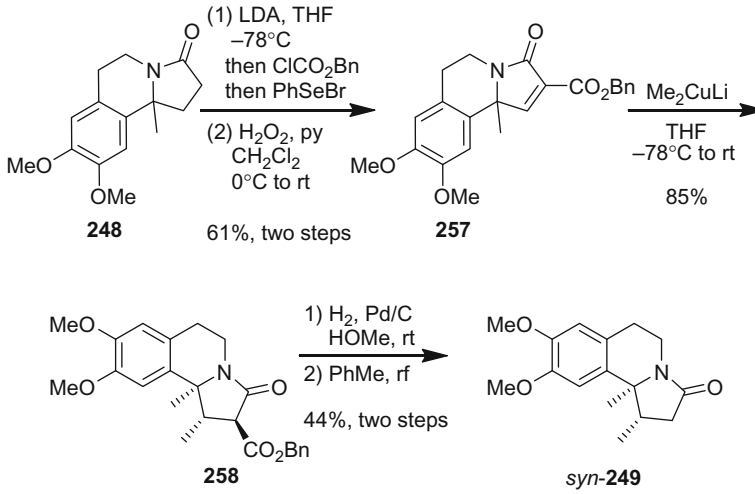
In 2006, Meyer and Opatz described an alternative synthesis of compound **245**. Using the same approach as reported for their synthesis of crispine A (**1**) (cf. Section III.B.10), **245** was prepared in 41% yield and with a diastereoisomeric ratio of *syn*:*anti* = 4.5:1 [46].



Scheme 65 Pailer's synthesis of **244** and **246** [110].



Scheme 66 Molander's synthesis of **245** [111].



Scheme 67 Lete's synthesis of *syn*-**249** [121].

The following syntheses have been reported for the compounds **247** and **248**. Most of the approaches have been described above. 8,9-Dimethoxy-10b-methyl-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinoline (**247**): Bremner and Winzenberg in 1984 [29], Meyers et al. in 1991 [112], Sato and co-workers in 2001 [97], Baskaran and co-workers in 2007 [113a, 113b]. 8,9-Dimethoxy-10b-methyl-1,2,3,5,6,10b-hexahydropyrrolo[2,1-*a*]isoquinolin-3-one (**248**): synthesized first by Winn and Zaugg in 1968 [114] and later on by several others [107,115–120].

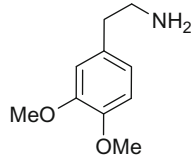
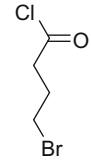
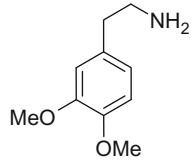
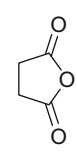
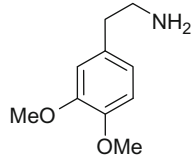
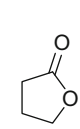
In 2009, Lete and co-workers described the first synthesis of the double methylated compound *syn*-**249** [121]. Starting from **248**, the Michael-acceptor **257** is accessible in 61% yield. Diastereoselective 1,4-addition of Gilman's reagent is followed by debenzylation and decarboxylation to afford racemic *syn*-**249** in five steps and 23% overall yield (Scheme 67) [121]. By a similar 1,4-addition using an enantiopure precursor the enantiomerically pure (1*R*, 10b*R*)-**249** was obtained (>99% ee) [121].

V. SUMMARY

The present chapter describes isolation, biogenetic proposals, and syntheses of the natural products **1**–**4** and **10**–**11** with a pyrrolo[2,1-*a*]-isoquinoline framework. Moreover, the syntheses of some structural analogs are discussed. The pyrrolo[2,1-*a*]isoquinolines are of interest due to their promising biological activities. For crispine A (**1**), many total syntheses have been reported and for trolline (**3**), only three. Only one total synthesis has been reported for each of the following natural products: peyoglutam (**10**), mescalotam (**11**), and the antitumor active crispine B (**2**). Some of the pyrrolo[2,1-*a*]isoquinoline alkaloids have not been synthesized yet.

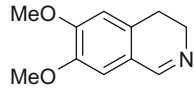
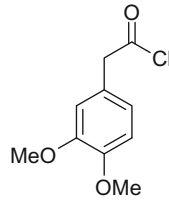
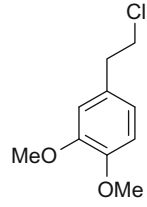
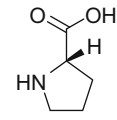
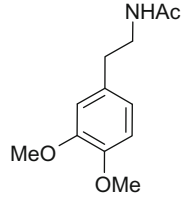
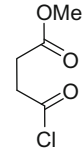
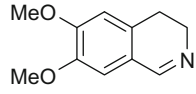
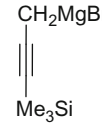
The following three tables summarize the synthetic efforts toward crispine A (**1**) (Table 1: racemic syntheses; Table 2: enantioselective syntheses) and trolline (**3**) (Table 3).

Table 1 Racemic syntheses of crispine A [**1**]

Authors (Year)	Starting materials	Number of steps	Overall yield
Child, Pymann (1931)	 **19**  **36**	3	~59%
Sugasawa (1939)	 **19**  **40**	5	Not reported
Zymalkowski (1967)	 **19**  **45**	3	82%

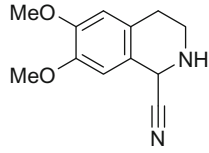
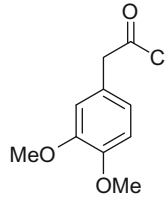
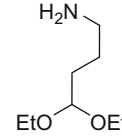
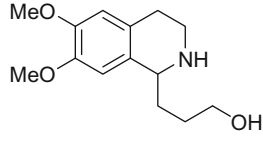
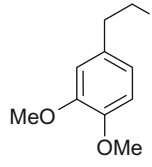
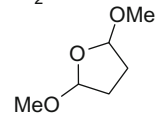
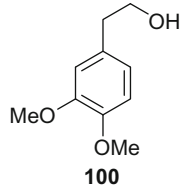
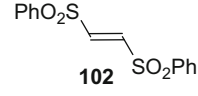
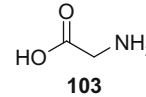
(*Continued*)

Table 1 (Continued)

Authors (Year)	Starting materials		Number of steps	Overall yield
Shono (1983)	 **47**	 **48**	2	~ 41%
Schell (1983)	 **50**	 **51**	4	Not reported
Proctor (1984)	 **54**	 **55**	2	14%
Orito (1988)	 **57**	 **58**	5	57%
Knölker (2005)	 **47**	 **61**	3	24%

(*Continued*)

Table 1 (Continued)

Authors (Year)	Starting materials	Number of steps	Overall yield
Opatz (2006)	 **67**	1	13%
King (2007)	 **50**  **74**	3 or 5	56% or 58%
Xu (2008)	 **94**	1	95%
Banwell (2008)	 **19**  **97**	3	12%
Coldham (2009)	 **100**  **102**  **103**	5	8%

(*Continued*)

Table 1 (Continued)

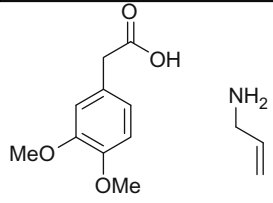
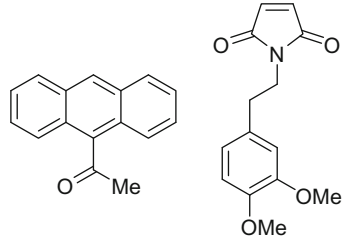
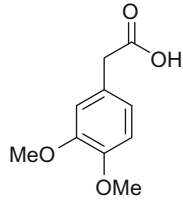
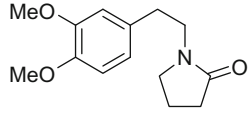
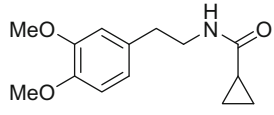
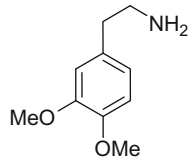
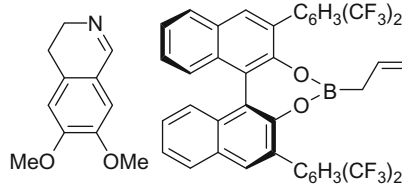
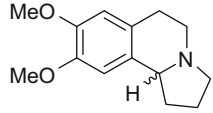
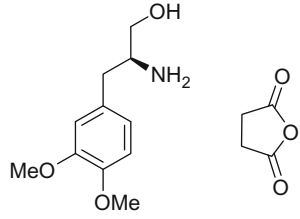
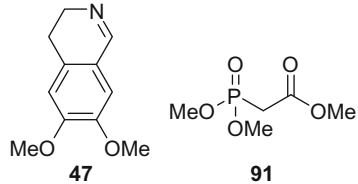
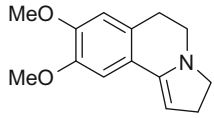
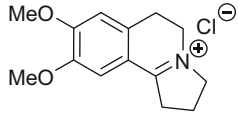
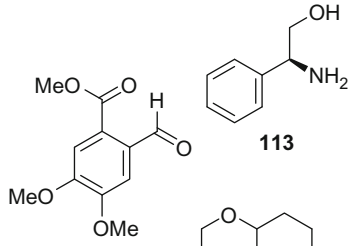
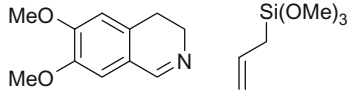
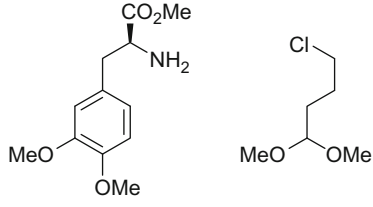
Authors (Year)	Starting materials	Number of steps	Overall yield
Chiou (2009)	 **73** **108**	4	59%
Jones (2010)	 **119** **122**	6	31%
Gallos (2011)	 **73**	9	24%
Hayashi (2011)	 **43**	2	35%
Patro (2011)	 **150**	2	77%

Table 2 Enantioselective syntheses of (+)-crispine A [(+)-**1**] and (−)-crispine A [(−)-**1**]

Authors (Year)	Starting materials	Number of steps	Overall yield (% ee)
Czarnocki (2005)	 **19** **45**	3 or 4	85% (92) or 53% (>99)
Chong (2006)	 **47** **71**	3	44% (98)
Turner (2007)	 (±)**1**	1	Not reported (97)
Allin (2007)	 **81** **40**	4 or 5	41% (50) or 15% (>95)
Itoh (2007)	 **47** **91**	9	45% (95)

(*Continued*)

Table 2 (Continued)

Authors (Year)	Starting materials	Number of steps	Overall yield (% ee)
Zhou (2009)	 **66**	1	97% (90)
Pihko (2009)	 **39**	1 or 1	45% (94) or 85% (58)
Amat (2010)	 **112** **113** **115**	4	15% (not reported)
Itoh (2011)	 **47** **136**	5	50% (97)
Herr (2011)	 (+)–**141** **142**	3	8% (>93)

(*Continued*)

Table 2 (Continued)

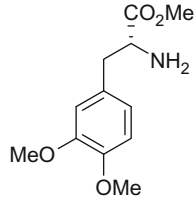
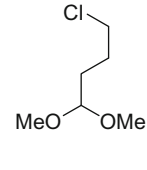
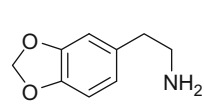
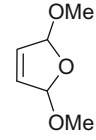
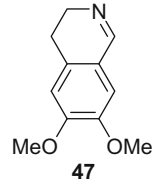
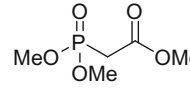
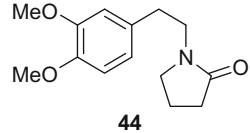
Authors (Year)	Starting materials	Number of steps	Overall yield (% ee)
Herr (2011)	 (–)–**141**  **142**	3	32% (>93)

Table 3 Racemic and enantioselective syntheses trolline (**3**)

Authors (Year)	Starting materials	Number of steps	Overall yield (% ee)
Liu (2007)	 **158**  **159**	2	18% (rac.)
Itoh (2007)	 **47**  **91**	9	45% (95)
Hayashi (2011)	 **44**	2	36% (rac.)

VI. ADDENDUM

After the present manuscript was accepted, five publications dealing with the synthesis of pyrrolo[2,1-*a*]isoquinoline alkaloids have appeared. Using a ruthenium-catalyzed ring-closing metathesis/isomerization/*N*-acyliminium cyclization sequence, Nielsen et al. synthesized mescalotam (**11**) [122]. O'Brien et al. reported a synthesis of (*R*)-crispine A [(+)-**1**] via an enantioselective palladium-catalyzed arylation of *N*-Boc pyrrolidine [123]. Fülöp et al. described a total synthesis of both crispine A enantiomers [(+)-**1**] and [(−)-**1**] using a lipase-catalyzed kinetic resolution as key step [124]. A novel synthesis of compound **26** has been described using a cyclization via an *N*-acyliminium ion as key step [125]. Thus, this work represents a formal synthesis of (±)-crispine A [(±)-**1**] (compare Scheme 13) and of (±)-trolline [(±)-**3**] (compare Scheme 39). Most recently, Kawai et al. described the enantioselective syntheses of (−)-trolline [(−)-**3**] and (+)-crispine A [(+)-**1**] together with the first synthesis of enantiomerically pure (+)-oleracein E [(+)-**3**] [126].

ABBREVIATIONS

9-BBN 9-borabicyclo[3.3.1]nonane
acac acetylacetonate
AIBN azobisisobutyronitrile
aq aqueous
Boc *tert*butyloxycarbonyl
BtH benzotriazole
cat. catalytic
cf. [lat.] confer, compare
COD 1,5-cyclooctadiene
COSY homonuclear correlation spectroscopy
CTAB cetyltrimethylammonium bromide
Cy cyclohexyl
DABCO 1,4-diazabicyclo[2.2.2]octane
DCB 1,2-dichloroisobutane
DCC *N*,*N*′-dicyclohexylcarbodiimide
de diastereomeric excess
DEAD diethyl azodicarboxylate
DEPT distortionless enhancement by polarization transfer
DIBAL diisobutylaluminum hydride
DMAP 4-(*N*,*N*-dimethylamino)pyridine
DME 1,2-dimethoxyethane
DMF *N*,*N*-dimethylformamide

DMS dimethyl sulfide
dppp 1,3-bis(diphenylphosphino)propane
dr. drop
ee enantiomeric excess
et al. [lat.] et alii, and others
FVP flash vacuum pyrolysis
HMBC heteronuclear multiple-bond correlation spectroscopy
HMQC heteronuclear multiple-quantum correlation spectroscopy
HOSu *N*-hydroxysuccinimide
HR-ESI-MS high resolution-electron spray ionization-mass spectrometry
HR-MS high resolution mass spectrometry
HRSI-MS high resolution single ion-mass spectrometry
IBX 2-iodoxybenzoic acid
KHMDS potassium bis(trimethylsilyl)amide
LDA lithium diisopropylamide
MS molar sieves
Ms methanesulfonyl
NCS *N*-chlorosuccinimide
NMR nuclear magnetic resonance
Pd/C palladium on activated carbon
PPA polyphosphoric acid
py pyridine
rac. racemic
Ra-Ni Raney-Nickel
recryst. recrystallized
rf reflux
Rh/C rhodium on activated carbon
rt room temperature
SRB assay sulforhodamine B assay
TBAT tetrabutylammonium difluorotriphenylsilicate
Tf trifluoromethanesulfonyl
TFA trifluoroacetic acid
THF tetrahydrofuran
TMS trimethylsilyl
Tol-BINAP 2,2′-bis(di-*p*-tolylphosphino)-1,1′-binaphthyl
Ts toluenesulfonyl
μ wave microwave

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