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Potent Algicides Based on the Cyanobacterial Alkaloid Nostocarboline

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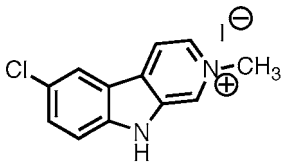
ABSTRACT



MIC 1 μM against *Microcystis aeruginosa*, *Synechococcus* and *Kirchneriella contorta*

Nostocarboline and seven derivatives were prepared and displayed minimal inhibitory concentration (MIC) values ≥100 nM against the growth of *Microcystis aeruginosa* PCC 7806, *Synechococcus* PCC 6911, and *Kirchneriella contorta* SAG 11.81, probably via the inhibition of photosynthesis. The natural product hybrid nostocarboline/ciprofloxacin displayed additional antibacterial activity against several Gram-negative bacteria (MICs ≥0.7 μM). Nostocarboline can thus be considered a potent, selective, readily available, natural algicide.

The unspecific adsorption of bacteria, cyanobacteria, algae, and higher organisms to submerged surfaces (i.e., biofouling) constitutes a significant challenge in marine and freshwater environments.[1] The impact of biofouling on ships, pipelines, oil platforms, nuclear power plants, and the like causes significant financial consequences.[1,2] These problems are accentuated as the use of organotin antifouling paints is increasingly banned all over the world.[2] In addition, resistance of aquatic species to Cu contributes to the need of novel antifouling compounds.[1,3] Many benthic aquatic organisms secrete such compounds for deterrence purposes, and the use of such natural products offers the possibility of identifying novel lead structures.[3] We recently reported the isolation of the cholinesterase inhibitor nostocarboline (**4**),[4] a carbolinium alkaloid from the cyanobacterium *Nostoc* 78-12A.[5] In this communication, we demonstrate that nostocarboline (**4**) and derivatives are quickly inhibiting the growth of phytoplanktal organisms (i.e., cyanobacteria and green algae) and thus possess potential antifouling activities.

The synthesis of derivatives of nostocarboline is straightforward (Scheme 1). Following modified literature procedures,[6] chlorination using different conditions of norharmane gives both the 6-chloronorharmane (**1**) and the 6,8-dichloronorharmane (**2**), which are both separable by flash chromato-

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(2) Overview: Champ, M. A. *Sci. Total Environ.* **2000**, *258*, 21−71.
(3) Abarzua, S.; Jakubowski, S.; Eckert, S.; Fuchs, P. *Bot. Mar.* **1999**, *42*, 459−465. Reviews: Bhadury, P.; Wright, P. C. *Planta* **2004**, *219*, 561−578. Gross, E. M. *Crit. Rev. Plant Sci.* **2003**, *22*, 313−319. Armstrong, E.; Boyd, K. G.; Burgess, J. G. *Biotechnol. Annu. Rev.* **2000**, *6*, 221−241. Smith, G. D.; Doan, N. T. *J. Appl. Phycol.* **1999**, *11*, 337−344.

(4) Becher, P. G.; Beuchat, J.; Gademann, K.; Jüttner, F. *J. Nat. Prod.* **2005**, *68*, 1793−1795.
(5) This *Nostoc* strain, isolated from a wastewater lagoon, was reported as anticyanobiotic, with the responsible compound not being determined; see: Flores, E.; Wolk, C. P. *Arch. Microbiol.* **1986**, *145*, 215−219.
(6) Nakano, K.; Suyama, K.; Fukazawa, H.; Uchida, M.; Wakabayashi, K.; Shiozawa, T.; Terao, Y. *Mutat. Res.* **2000**, *470*, 141−146. Ponce, M. A.; Tarzi, O. I.; Erra-Balsells, R. *J. Heterocycl. Chem.* **2003**, *40*, 419−426.

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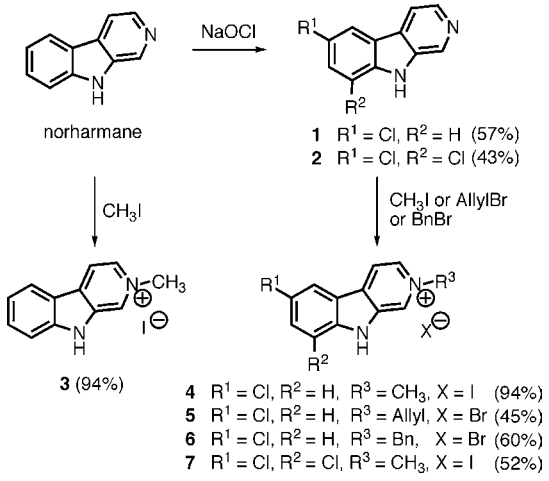
Scheme 1. Preparation of Nostocarboline (**4**) and Derivatives



graphy. Alkylation of the mono-Cl species **1** with different electrophiles resulted in nostocarboline (**4**), the allyl derivative **5**, and the Bn derivative **6**. Similarly, 8-Cl-nostocarboline (**7**) as well as the deschloronostocarboline **3** were also obtained in good yields.

Compounds **1**−**7** were then evaluated against the growth of the toxic cyanobacterium *Microcystis aeruginosa* PCC 7806 and the nontoxic cyanobacterium *Synechococcus* PCC 6911. In addition, *Kirchneriella contorta* SAG 11.81 was selected as a member of the eukaryotic green algae. Growth curves were measured over 280 h, and the minimal inhibitory concentration (MIC) and the minimal phytotoxic concentration (MPC) were determined at 170 h after addition of the different compounds (Table 1).[7]

Table 1. Biological Activity of Compounds **1**−**7**, **9**, and Ciprofloxacin to the Growth of *M. aeruginosa* (Ma), *Synechococcus* (SC), and *K. contorta* (KC)

	MIC (μM)[a]			MPC (μM)[b]		
compound	MA	SC	KC	MA	SC	KC
1	>100	>100	nd[c]	>100	>100	nd[c]
2	>100	>100	nd[c]	>100	>100	nd[c]
3	10	1	nd[c]	10	50	nd[c]
4	1	1	1	10	10	>100
5	10	1	nd[c]	10	10	nd[c]
6	10	0.1	nd[c]	10	10	nd[c]
7	10	1	nd[c]	10	10	nd[c]
9	1	1	10	1	50	10
ciprofloxacin	1	10	>100	>100	>100	>100

[a] MIC refers to the minimal inhibitory concentration at which reduction of growth (OD_{675nm}) vs the control is observed. [b] MPC refers to the minimal phytotoxic concentration at which reduction of the OD_{675nm} compared to the time of addition was observed. [c] nd = not determined.

Nostocarboline (**4**) was shown to be a potent inhibitor of cyanobacterial and algal growth (MIC = 1 μM against the three organisms tested) and induced rapid killing of cyanobacterial cells at 10 μM within 24 h. A change in the phenotype of *Synechococcus* could already be observed at a 100 nM concentration of nostocarboline (**4**). Structure activity studies revealed that the quaternary group appears to be essential for biological activity, as the demethylated compounds **1** and **2** were found to be inactive.[8] The degree of chlorination (e.g., **3** vs **4** vs **7**) had a small impact on biological activity, as a 10-fold decrease in activity for **3** and **7** against *M. aeruginosa* was observed. The replacement of the substituent on the 2-N atom led to an increase in activity of the Bn derivative **6** against *Synechococcus* (MIC = 100 nM) compared to the parent natural product. Replacing the methyl in **4** with the allyl in **5** results in a 10-fold loss of activity against *M. aeruginosa* but retained activity against *Synechococcus*. It is interesting to note that all compounds induced rapid killing of cells above the MPC concentration.

The activity of nostocarboline and derivatives can be compared to ciprofloxacin, a known and potent inhibitor of cyanobacterial growth.[9] Nostocarboline (**4**) displayed increased activity against *Synechococcus* and is also active against the eukaryote *K. contorta* compared to ciprofloxacin which is selective for prokaryotic organisms.

Moreover, IC_{50} values were obtained to better characterize the effect of nostocarboline (**4**) on growth (Figure 1). The

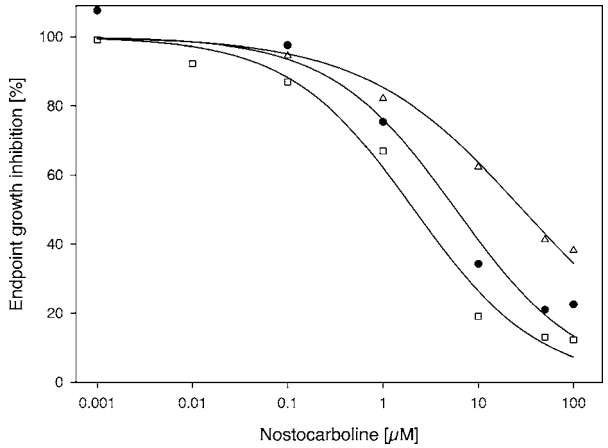


Figure 1. Effect of nostocarboline (**4**) on *Microcystis aeruginosa* (white squares), *Synechococcus* (black circles), and *Kirchneriella contorta* (white triangles). The graph shows the inhibition of the growth of the phytoplankton species in comparison to the control on day 7 after addition of nostocarboline.

IC_{50} values for the inhibition of the growth of the three different phytoplankton species were determined after 7 days

(7) See Supporting Information for full growth curves.

(8) In the literature, harmane was shown to possess cyanobacteriocidal activity (30 μg/disk); see: Kodani, S.; Imoto, A.; Mitsutani, A.; Murakami, M. *J. Appl. Phycol.* **2002**, *14*, 109−114.

(9) Robinson, A. A.; Belden, J. B.; Lydy, M. *J. Environ. Toxicol. Chem.* **2005**, *24*, 423−430. Halling-Sorensen, B.; Lutzhoft, H. C. H.; Andersen, H. R.; Ingerslev, F. *J. Antimicrob. Chemother*. **2000**, *46*, 53−58.

to be $IC_{50} = 2.1$ μM for *M. aeruginosa*, $IC_{50} = 5.8$ μM for *Synechococcus*, and $IC_{50} = 29.1$ μM for *K. contorta*. These results show the sensitivity of the toxic cyanobacterium *M. aeruginosa* to nostocarboline (**4**).

Nostocarboline (**4**) was also tested against the growth of its producer, *Nostoc* 78-12A, and showed a pronounced reduced activity as a MIC value of 50 μM and a high MPC value of 100 μM were determined. This large difference in MIC values for autoinhibition (50 μM) to the MPC values for competing organisms (10 μM) results in sustained growth of the producing organism with concomitant killing of competing phytoplankton. Moreover, significant amounts of nostocarboline (**4**) were detected in standing cultures of *Nostoc* 78-12A by HPLC-MS. Thus, these results point to the presumed ecological role of nostocarboline (**4**) by inducing competitive advantage via secretion.

To elucidate the mode of action of nostocarboline (**4**), growth experiments were performed with an initial growth phase in the dark. Virtually no inhibitory action was observed in that period for nostocarboline (**4**) for concentrations of up to 50 μM, indicating that nostocarboline activity is correlated to the photosynthesis of *M. aeruginosa*.[3,10] This notion is supported by the fact that nostocarboline (**4**) was not active against the growth of pathogenic nonphotosynthetic bacteria (different strains of *Staphylococcus*, *Enterococcus*, *Pseudomonas*, *Streptococcus*, *Haemophilus*, *Moraxella*, *Escherichia*) and yeast up to concentrations of 93 μM (see also Table 2). This selective inhibitory activity to photoautotrophs can be rationalized by the structural similarity of nostocarboline (**4**) to known and potent algicides such as diuron/monuron or herbicides such as paraquat. Detailed mechanistic studies on the photosynthesis inhibition of nostocarboline (**4**) are thus warranted.

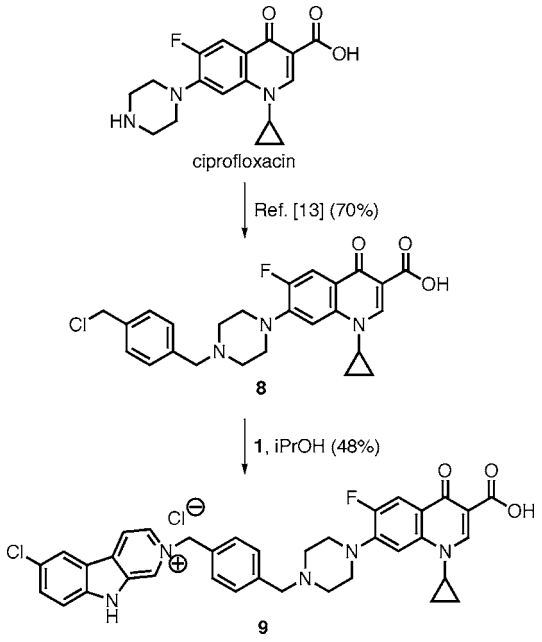
Table 2. Antibacterial Evaluation of Nostocarboline (**4**), the Nostocarboline Ciprofloxacin Hybrid **9**, and Ciprofloxacin[a]

strains[b]	**4**	hybrid **9**	cipro
S. aureus	>93	42.4	1.5
H. influenzae	>93	10.6	≤0.09
M. catarrhalis	>93	21.2	≤0.09
E. coli ATCC 25922	>93	10.6	≤0.09
E. coli A-337	>93	0.7	≤0.09

[a] Values are given in μM. [b] The strains surveyed were *Staphylococcus aureus* ATCC 29213, *Haemophilus influenzae* A-921, *Moraxella catarrhalis* A-894, *Escherichia coli* ATCC 25922, and *Escherichia coli* A-337 (TolC efflux deficient).

The natural product hybrid[11] **9** was targeted next, as this quinolone chimera[12] could possess a dual mode of action. The conjugate **9** was thus obtained via the known product **8** of the reaction of ciprofloxacin with 1,4-di(chloromethyl)-benzene[13] and subsequent alkylation of 6-Cl-norharmane (**1**), shown in Scheme 2. Compound **9** retained the broad activity of nostocarboline (**4**) against photoautotrophs and gained activity against eukaryotic organisms when compared to ciprofloxacin (Table 1). Moreover, the hybrid **9** displays antibacterial activity against several Gram-negative strains (Table 2), whereas the parent nostocarboline (**4**) was inactive. However, a loss of 1 to 2 orders of magnitude compared to ciprofloxacin was observed. Some of this reduced activity can be explained by efflux phenomena, as the hybrid **9** displayed increased activity against an efflux deficient *Escherichia coli* strain (0.7 μM).

Scheme 2. Preparation of the Quinolone Carboline Hybrid **9**



In conclusion, nostocarboline and its derivatives **3**−**7** display potent cyanobacteriocidal and algicidal activity against photosynthetic aquatic organisms. The benefits of nostocarboline (**4**) include (a) potent and fast reduction of phytoplankton growth, (b) cheap and simple preparation, (c) the biogenic nature offering benefits in its potential registration ("natural algicide"), (d) selectivity to photosynthetic organisms, and (e) a structure which is amenable to easy modification resulting in more potent derivatives such as **6** or the natural product hybrid **9**. For all these reasons, nostocarboline (**4**) can thus be considered a promising lead structure for the development of algicides addressing the worldwide need for effective, biogenic, and simple antifouling agents. Current efforts are directed toward the elucidation of the mode of action of nostocarboline (**4**), a general evaluation of its antifouling properties, and the related in

(10) Several cyanobacterial metabolites inhibit photosynthesis in competing organisms. For examples, see: Hagmann, L.; Jüttner, F. *Tetrahedron Lett.* **1996**, *36*, 6539−6542. Todorova, A. K.; Jüttner, F. *J. Org. Chem.* **1995**, *60*, 7891−7895.

(11) Review on natural product hybrids: Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3996−4028.

(12) For other quinolone chimeras, see, for example: Hubschwerlen, C.; Specklin, J.-L.; Sigwalt, C.; Schroeder, S.; Locher, H. H. *Bioorg. Med. Chem.* **2003**, *11*, 2313−2319.

(13) The electrophile was prepared according to: Kerns, R. J.; Rybak, M. J.; Kaatz, G. W.; Vaka, F.; Cha, R.; Grucz, R. G.; Diwadkar, V. U. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2109−2112.

vivo properties such as stability and biodegradation properties.

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Supporting Information Available: Synthetic procedures, full characterization of compounds, copies of spectra, biological experiments, and growth curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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