Pergamon

SCIENCE DIRECT®

Tetrahedron 59 (2003) 8757–8762

TETRAHEDRON

# On the origin of quasi-racemic aplysinopsin cycloadducts, (bis)indole alkaloids isolated from scleractinian corals of the family Dendrophylliidae. Involvement of enantiodefective Diels–Alderases or asymmetric induction in artifact processes involving adventitious catalysts?

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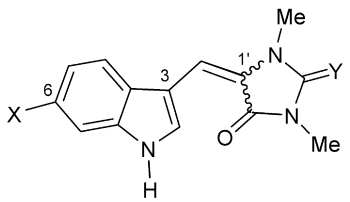
Received 27 May 2003; revised 22 August 2003; accepted 11 September 2003

**Abstract**—Reported here are two novel quasi-racemic (bis)indole alkaloids, cycloaplysinopsin A (**5**) and cycloaplysinopsin B (**6**), isolated from tropical Indo-Pacific (Comoros, Philippines) scleractinian corals of the family Dendrophylliidae. Although their structures suggest a Diels–Alder cycloaddition origin from aplysinopsin-type precursors, neither experiments, nor theory allowed us to clearly distinguish an enzymatic process with scarce enantioselection from the intrusion of an adventitious catalyst in the coral extracts, where the chiral environment caused a slight asymmetric induction.


## 1. Introduction

First isolated from the dictyoceratid sponge *Aplysinopsis reticulata* from the Great Barrier Reef,[1] aplysinopsin (**1**) and $N^{3'}$-methylaplysinopsin (**2**), belong together with brominated derivatives, like 6-bromoaplysinopsin (**3**), and oxo-forms, such as 3′-deimino-3-oxoaplysinopsin (**4**), to a class of indole alkaloids also found in other dictyoceratid and astrophorid sponges and in dendrophylliid scleractinian corals.[2] Aplysinopsin (**1**), and especially (*E*)-**2** as a reversible monoamine-oxidase inhibitor, were determined to protect mice against tetrabenazine-induced ptosis.[3] Because this constitutes a preliminary indication of antidepressant activity in humans, and **1** was also found to be cytotoxic against a panel of human tumor cells,[3] considerable interest arose toward this class of indole alkaloids both as potential drugs and leads.[4] The discovery of a thermally-reversible photoisomerization for this type of compounds also stimulated environmental interest.[2,3]




(*E*/*Z*)-**1** X = H,Y=NH aplysinopsin
(*E*/*Z*)-**2** X = H,Y=NMe *N*3'-methylaplysinopsin
(*E*/*Z*)-**3** X= Br,Y=NH 6-bromoaplysinopsin
(*E*/*Z*)-**4** X = H,Y= O 3'-deimino-3'-oxoaplysinopsin

At a recent meeting, we announced preliminarily modified Diels–Alder cycloadducts of these alkaloids, like cycloaplysinopsin A (**5**) and cycloaplysinopsin B (**6**), isolated from an unidentified dendrophylliid coral from the Comoro islands.[5†] These studies, extended to another dendrophylliid coral and the peculiar chiroptical properties of these alkaloids, pose challenging problems, specifically are they of natural origin or are they merely artifacts?

*Keywords*: (bis)indole alkaloids; Diels–Alder cycloaddition reactions; Diels–Alderases; chiral shift reagents; enantiomeric composition.
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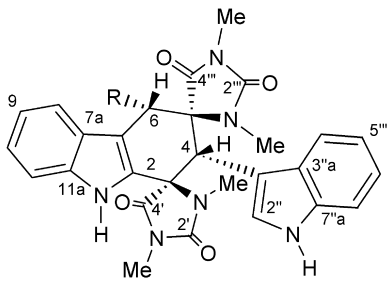
† Except for a few genera and species, the taxonomy of tropical scleractinian corals in the family Dendrophylliidae remains highly problematic.

0040–4020/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2003.09.038

**Table 1**. NMR spectral data for compound **6** in DMSO-$d_6$ $\delta$ in ppm, $J$ in Hz

| Atom | $\delta_H$ | $\delta_C$ | NOE[a] | Long-range heterocorrelation[b] |
|---|---|---|---|---|
| 2 | | 124.82 s | | |
| 3 | | 67.71 s | | H-4, Me–N1′ |
| 4 | 4.82 (s) | 37.30 d | H-6, Me–N1′,H-4″,Me–N1‴ | |
| 5 | | 71.31 s | | H-4, Me–N1‴ |
| 6 | 5.27 (d, $J_{5,6}$=5.5 Hz) | 64.35 d | HO-6, H-8, Me–N1‴ | |
| 7 | | 116.38 s | | H-6 |
| 7a | | 128.05 s | | H-6 |
| 8 | 7.67 (d, $J_{8,9}$=7.8 Hz) | 118.92 d | | |
| 9 | 7.11 (*t*, $J_{9,8}$=$J_{9,10}$=8.1 Hz) | 119.36 d | | |
| 10 | 7.21 (*td*, $J_{10,9}$=$J_{10,11}$=7.8, $J_{10,8}$=2.1 Hz) | 122.85 d | | |
| 11 | 7.38 (d, $J_{11,10}$=8.1 Hz) | 111.53 s | | |
| 11a | | 136.96 s | | |
| 2′ | | 154.77 s | | Me–N1′, Me–N3′ |
| 4′ | | 172.20 s | | H-4, Me–N3′ |
| 2″ | 6.89 (d, $J_{2'',NH}$=2.7 Hz) | 123.91 d | | H-4 |
| 3″ | | 105.15 s | | H-4 |
| 3″a | | 128.02 s | | |
| 4″ | 7.38 (d, $J_{4'',5''}$=8.1 Hz) | 117.32 d | | |
| 5″ | 7.00 (*t*, $J_{5'',4''}$=$J_{5'',6''}$=7.6 Hz) | 118.86 d | | |
| 6″ | 7.07 (*td*, $J_{6'',5''}$=$J_{6'',7''}$=7.6 Hz, $J_{6'',4}$=2.1 Hz) | 121.07 d | | |
| 7″ | 7.32 (d, $J_{7'',6''}$=7.6 Hz) | 111.26 d | | |
| 7″ | | 134.55 s | | |
| 2‴ | | 157.41 s | | Me–N1‴, Me–N3‴ |
| 4‴ | | 171.66 s | | H-4, Me–N3‴ |
| H–N1 | 11.53 (s) | | | |
| HO-6 | 5.72 (d, $J_{OH,6}$=5.5 Hz) | | | |
| Me–N1′ | 2.84 (s) | 25.14 q | H-6, H-2″ | |
| Me–N3′ | 2.50 (s) | 24.22 q | | |
| H–N1″ | 11.07 (d, $J_{NH,2''}$=2.7 Hz) | | | H-2″ |
| Me–N1‴ | 2.86 (s) | 28.32 q | H-4, H-6, H-2″ | |
| Me–N3‴ | 2.54 (s) | 24.44 q | | |

[a] NOE enhancement observed for the indicated H-atom(s) by irradiation of the proton(s) listed on the same row.
[b] Heterocorrelation of the indicated C-atom(s) with the proton(s) listed on the same row.




**5** R =H
**6** R =OH
**7** R=OMe

## 2. Results and discussion

Online LC–MS analysis of the AcOEt extracts from dendrophylliid corals, comprising an unidentified species (65M/19R) from the Comoro islands and previously investigated *Tubastraea* sp. from the Philippines,[2] revealed the presence of abundant cycloaplysinopsin A (**5**), less abundant cycloaplysinopsin B (**6**), and also, in trace amounts, their monobrominated derivatives, and furthermore the known 3′-deimino-3′-oxoaplysinopsin (**4**) and its 6-bromo derivative[2] (Section 4).

The composition $C_{28}H_{26}N_6O_5$ for cycloaplysinopsin B (**6**) was deduced from ESI-MS spectra, including tandem MS fragmentation experiments, high resolution EI-MS on the main fragment ions, and $^{13}C$ NMR spectra. The presence of two units related to 3′-deimino-3′-oxoaplysinopsin (**4**), which was detected in the extracts (Section 4), is supported by NMR signals for two indole units, four N-methyl groups, and two low-field methine protons ($\delta_H$=4.82 ppm, coupled to $\delta_C$=37.30 H-4)[‡] and $\delta_H$=5.27 ppm (coupled to $\delta_C$=64.35 H-6). A combination of NMR selected differential decoupling experiments, as well as $^1H$, $^1H$- and $^1H$, $^{13}C$-COSY experiments (Table 1), allowed us to define the structure; in particular, the carbon–carbon connectivity was based on long-range heterocoupling of C-7a with H-6, C-3 and C-5 with MeN-1′ and MeN-1‴, respectively, and, finally, C-4′, C-4‴, C-2″ and C-3″ with H-4. The relative configuration of the chiral centers was drawn from nOe data (Table 1) in the light of molecular mechanics (MM) minimized conformations. Thus, the *cis*-relationship between H-4 and H-6 was established from nOe enhancements of H-4 ($\delta_H$=4.82 ppm) with H-6, Me–N1′ and Me–N1‴, as well as of H-6 ($\delta_H$=5.27 ppm) with Me–N1‴, while the *cis*-position of the amidic N–Me groups in the rings at C-3 and C-5 was established from nOe enhancement with H-6 and H-2″.

The composition $C_{28}H_{26}N_6O_4$ for cycloaplysinopsin A (**5**) is supported by both HR-EI-MS data on the molecular ion and an intense retro-Diels–Alder fragmentation signal in the EI-MS spectrum at *m/z* 255, corresponding to the molecular ion of 3′-deimino-3′-oxoaplysinopsin (**4**). Due to the scarce amount available, NMR experimentation with **5** was less

[‡] Arbitrary numbering used here for convenience for compounds **5**–**7**.

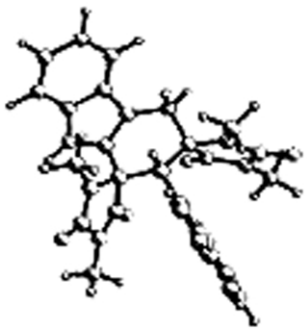


**Figure 1.** Strain-energy minimized conformation of compound **5**.

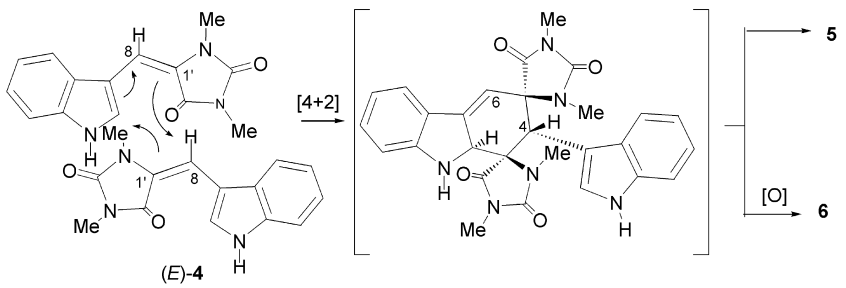
extensive than with **6**, but the analogy between the two compounds obviated the necessity of doing more. Thus, from experiments in $CD_3OD$, the $^1H$ NMR signals for H-6 and HO-6 of **6** are replaced in **5** by two resonances for the geminal methylene protons at $\delta_H$=3.65 and 3.38 ppm. *cis*-Arrangement of both Me–N1′ and Me–N1‴ with respect to H-4 ($\delta_H$=4.63 ppm) rests on nOe enhancements. This relationship is nicely simulated in the least strained conformer obtained from MM modeling (Fig. 1, where it is seen that the two imidazolidinone rings and the indolyl unit are nearly perpendicularly arranged with respect to the mean plane of the central six-membered ring).

Low optical rotation for **5** and the lack of optical rotation of **6** were observed (Section 4). Later we found that the methyl derivative **7**, obtained from **6** on prolonged storage in MeOH–$CDCl_3$, does rotate the plane of polarized light as much as **5**. However, the $^1H$ NMR spectra of both **5** and **7**, in $CDCl_3$ in the presence of the chiral shift reagent Eu(tfc)$_3$, showed splitting of signals in a ca. 65:35 ratio, revealing a low enantiomeric purity (Section 4).

Compounds **5** and **6** may be considered to derive from a Diels–Alder cycloaddition reaction between two molecules of (*E*)-**4**, followed by double bond shift to establish the fused indole unit and, for **6**, also by a subsequent, or preceding, oxidation reaction[§] (Scheme 1). Using this hypothesis, we have attempted to obtain these compounds from synthetic (*E*)-**4**[2] under non-enzymatic conditions. However, under the conditions of solvent, concentration of reagents, and temperature described above for the work-up of the scleractinian corals, or the higher temperature conditions used for the synthesis of aplysinopsins,[2,3] formation of cycloadducts was not observed, even at the high sensitivity of ESI-MS analysis. This rules out that compounds **5** and **6** may have formed during extract storage from non-catalyzed Diels–Alder reaction of aplysinopsin-type compounds. This conclusion is reinforced by a reexamination of HPLC analyses and EI MS spectra taken during the first studies of *Tubastraea* sp.,[2,3] which, on the light of present LC-ESI MS, clearly indicate that compound **6** was present in the scleractinian extracts at those early days, shortly after that the coral had been worked-up.

An alternative explanation is admitting catalysis of the above Diels–Alder process (Scheme 1). Let us first examine the possibility that a Diels–Alderase is involved. These have been often invoked,[7a] in particular a keramaphidin B synthase to explain features of the manzamine cascade,[7b] while proofs are restricted to three cases, in chronological order solanapyrone synthase,[7c] lovastatin nonaketide synthase,[7d] and macrophomate synthase.[7e] In all proven cases,[7c–e] it is a single enzyme that, however, does more than merely the electrocyclic reaction. This finds analogy in our case (Scheme 1), while the enantioselectivity of the process either does not pose,[7e] or is ambiguous, being not evidenced,[7d] or not discussed as to certain low ee values aside high ee values.[7c] To this respect, our case resembles that of keramaphidin B,[7b] with scarce enantioselectivity, but in neither case there is proof for a Diels–Alderase. Thus, at the present stage of knowledge, to explain the reaction course at the Scheme 1, we may conceive the adventitious intervention of a spurious Diels–Alder catalyst, where the chiral environment of other components, such as the steroids, created the conditions for a slight asymmetric induction.[¶]

The isolation of tubastrindole A (**8**), B (**9**), and C (**10**) from *Tubastraea* sp. from Japan has been recently reported.[9] Although a failure to mention the solvent used for the NMR

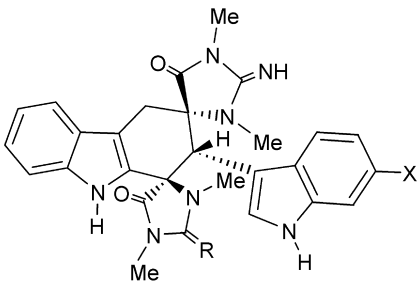



**Scheme 1.** Diels–Alder cycloaddition between two molecules of (*E*)-3′-deimino-3′-oxoaplysinopsin to give compounds **5** and **6**.

[§] Introduction of HO-6 might result from reaction at the trisubstituted double bond of the initial Diels–Alder cycloadduct, either via epoxidation reaction followed by a base-induced ring opening, or from $O_2^1$ addition, followed by reduction of the resulting tertiary hydroperoxide group.[6]

[¶] Our first report about the existence of aplysinopsin-type cycloadducts[5] was preceded by that of a 'dimer' of 6-bromo-2′-de-*N*-methylaplysinopsin, isolated from a dendrophylliid coral, *Tubastraea faulkneri* Wells, from Davies Reef on the Great Barrier Reef of Australia.[8] Although a signal at *m/z* 636, from otherwise undescribed mass spectra, may justify this contention, scarce other physical data, in particular lack of both NMR data,[8] and no commitment about the molecular structure, do not allow any comparison of this 'dimer'[8] with compounds **5** and **6**.

experiments[9] makes examination of this work difficult, the heterogeneous nature of **8**–**10** (formed from two different aplysinopsin-type molecules), the relative configuration at C-5 (as reported,[9] compounds **8**–**10** are in C-5 epimeric, or *ent*-epimeric, relationship to our compounds **5** and **6**), and the implied assumption of chiroptical purity of the reported alkaloids,[9] differ sharply from what we have observed and reported here for **5** and **6**.




**8** R =NH, X=Br
**9** R =NH, X=H
**10** R =O, X=H

Cycloaddition between the C1′═C8 double bond of one aplysinopsin-type molecule acting as dienophile and the *cis*-diene of another like molecule, finds precedence with synthetic 3-vinyl indoles as dienophiles,[10] while it is known that Diels–Alder cycloaddition reactions are stereospecific.[11] Formation of compounds **5**–**6** and **8**–**10**, with the indole unit at C-4 and the spiroimidazolidinone at C-3, rather than vice versa, also indicates regiospecificity.

Although the stereochemistry at C-2 is lost during the cycloaddition reaction, the stereochemical course toward **5** and **6** may be explained by the intervention of two (*E*)-**4** molecules, one acting as a diene and the other one as a dienophile, oriented as shown in the Scheme 1. Should instead the two (*E*)-**4** molecules approach with the dienophile turned by 180° along the longer axis (H-8 in relative *anti* orientation), the C-5 epimeric cycloadduct would be obtained, contrary to our observations. That this reaction course was kinetically driven, as is typical of Diels–Alder cycloaddition reactions, is suggested by MM modeling, which gave similar strain energies for the preferred conformations of the epimeric compounds. It is also worth mentioning that MM calculations predict significant attractive intermolecular forces (Van der Waals attractive and H-bonding) when the two molecules of (*E*)-**4** face a short distance, forcing them to assume a spatial arrangement wherein their long axis is parallel (Scheme 1). The total strain energy of this ensemble turned out from calculations to be lower than the total strain energy of the two isolated molecules. Differential H-bonding is indeed expected to play a role in the reactant non-covalent dimer leading to compounds **5** and **6**, involving the indolyl NH and amide C═O groups, whereas in the C-5 epimeric pathway leading to cycloadducts **8**–**10** a single H-bond between the indolyl NH and imidic C═O groups could take place.

Formation of cycloadducts **5**–**6** might also be conceived from the *anti* approach of a (*Z*)-**4** diene to a (*E*)-**4** dienophile, while cycloadducts **8**–**10** could derive from the *syn* approach of a (*Z*)-aplysinopsin-like diene to

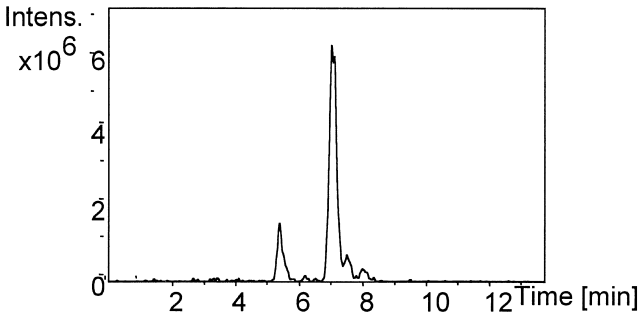



**Figure 2.** LC-ESI/MS trace at *m/z* 533 in positive mode from the AcOEt *Tubastraea* sp. extract, where the signal at $t_R$=7.2 min represents $[M+Na]^+$ for compound **5**.

(*E*)-aplysinopsin-like dienophile. Against this hypothesis is the observation that (*Z*)-aplysinopsins are generally less abundant that the (*E*)-isomers,[2,3] while the nearly planar disposition of (*E*)-aplysinopsin-like reactants allows closer contact between the reagents than non-planar (*Z*) isomers. The last possibility, deriving from reactions of aplysinopsin-like molecules having (*Z*)-stereochemistry at the diene with (*Z*)- or (*E*)-stereochemistry at the dienophile, would lead to other diasteroisomeric adducts, which have not yet been isolated.

Extensive LC–MS experimentation on our dendrophylliid extracts revealed a minor peak at shorter retention time than observed for **5** (Fig. 2). We believe that this corresponds to the C-5 epimer of **5**, originating from *anti* approach of two (*E*)-**4** molecules, in analogy with our hypothesis for the biogenesis of tubastrindoles A–C (**8**-**10**).

## 3. Conclusions

Lack of evidence for a Diels–Alderase leaves the explanation of artifact origin for cycloadducts **5**–**6** from aplysinopsin-type natural precursors by the aid of an adventitious Diels–Alder catalyst present in the coral extracts, where the chiral environment of steroids or other common metabolites, triggered a slight asymmetric induction. However, neither hypothesis easily explains the absence of aplysinopsin-type cycloadducts in the extracts of other dendrophylliid corals, in spite of the presence of free aplysinopsins in (*E*)-configuration, favorable for the Diels–Alder cycloaddition reaction to occur. The latter is work in progress, which will be reported once all samples of our vast collection of dendrophylliid corals are examined. Solution of these problems is not aided by either a mysterious ‘dimer’ previously reported from *Tubastraea faulkneri*,[8] or cycloadducts **8**–**10** reported from *Tubastraea* sp.,[9] until details of their structure and enantiomeric composition are given.

The name cycloaplysinopsins A–B for the (bis)indole alkaloids **5**–**6**[5] is more descriptive than tubastrindoles for **8**–**10**.[9] The former takes into account the possibility of artifact formation, or, should these alkaloids have a natural origin, their observation in extracts from dendrophylliid corals of other genera than *Tubastraea*. If nothing else, our proposal follows the rules of precedence.[5]

## 4. Experimental

### 4.1. General procedures

Preparative, reversed-phase HPLC was carried out on 25×1 cm columns packed with Merck LiChrosorbRP-18, under UV monitoring at $\lambda$=254 nm and solvent flux 5 mL $min^{-1}$, unless otherwise stated. ESI-MS experiments were performed in both positive and negative ion mode on a Bruker Esquire-LC™ ion trap spectrometer via an electrospray interface as either direct infusion or LC-ESI-MS. In the latter case, the spectrometer was coupled to a HP Mod. 1100 liquid chromatograph bearing a 250×4 mm column packed with 5 μm Merck LiChrospher RP-18; analysis was carried out under UV monitoring at $\lambda$=280 nm and solvent flux 1 mL $min^{-1}$, 7:3 split for UV and ESI detectors. EI-MS experiments were performed with a Kratos MS-80 mass spectrometer, equipped with a home-built computerized data system $^1H$ and $^{13}C$ NMR 1D and 2D spectra were recorded on a Varian XL-300 spectrometer operating at 299.94 and 75.43 MHz, respectively; $\delta$ values are given in ppm, with respect to either $Me_4Si$ as an internal standard or the residual solvent signals ($\delta_H$=2.49 ppm and $\delta_C$=39.50 ppm for DMSO-$d_6$, $\delta_H$=3.31 ppm and $\delta_C$=49.00 ppm for $CD_3OD$), while $J$ values are given in Hz. Multiplicities are from APT, $^1H$, $^1H$ correlations from COSY60 and selective decoupling irradiations, and $^1H$, $^{13}C$ assignments from one-bond and long-range COSY. nOe stands for differential 1D nuclear Overhauser effect, reported as 'irradiated proton/nOe observed on the proton(s)'. Polarimetric data were obtained with a Jasco-DIP-181 apparatus, reporting $[\alpha]_D$ in $dm^{-1}$ deg mL $g^{-1}$. Molecular mechanics (MM) calculations were carried out by the computer program PCMODEL 7.0, Serena Software, Bloomington, Indiana which is based on the MMX force field. Least-strain conformations for rotation around the C4–C3″ single bond of the compounds **5** and **6** were found by the 'dihedral driver' option with angle step size of 30°.

### 4.2. Biological material and work-up

*Tubastraea* sp. from the Philippines was taxonomically described in previous work,[2] and a voucher specimen is deposited at the Centre d'Océanologie de Marseille, Station Marine d'Endoume, F-13007 Marseille, France. Colonies of an unidentified dendrophylliid (65 M) were collected in the Comoro islands (Mayotte, station 19R, Reef environment, 3 m depth) during the French Benthedi expedition in 1977 and were immersed in 0.7 L of 95% EtOH. This dendrophylliid belongs to the large part of taxonomically problematic Indo-Pacific dendrophylliids, for which there will be no reliable generic attribution until a whole taxonomic revision is carried out. Unfortunately, the box containing this sample, in spite of a long search, was not found at the right place, accidentally misplaced among the other thousands boxes, and probably hidden into another box, which prevented a morphological description at this moment. The liquid was evaporated to dryness in July 1981, storing the residue at −20°C until it was examined in the year 2002 as follows.

Residues from evaporation of the AcOEt extracts of the scleractinian corals were subjected to RP-18 HPLC with $CH_3CN/H_2O$ 1:1, under photodiode array detection at $\lambda$=280 nm and LC-ESI-MS analysis. In positive mode, the chromatographic profile corresponding to $m/z$ 533 consisted of two peaks, a large one at $t_R$=7.2 min for compound **5**, and a smaller one at $t_R$=5.4 min (Fig. 2), while for $m/z$ 549 a single chromatographic peak was observed. LC-ESI-MS analysis in negative ion mode gave not only the corresponding signals at $m/z$ 525 $[M-H]^-$ and 509 $[M-H]^-$ for **6** and **5**, but also weak signals at $m/z$ 603/605 and 587/589 for monobrominated analogues of **5** and **6**, and, monitoring at $\lambda$=376 nm, signals for **4** and its 6-bromo analogue.[2] Preparative HPLC of 0.34 g residue from the AcOEt extract of *Tubastraea* sp.,[2] on Merck Lichrosorb RP18, $CH_3CN/H_2O$ 1:1, gave pure **6** ($t_R$=4.6 min, 1.8 mg) and pure **5** ($t_R$=8.0, 0.8 mg).

**4.2.1. Cycloaplysinopsin A (5).** Colorless powder. $[\alpha]_D$=−28, $[\alpha]_{546}$=−34 ($c$=0.05 mg/mL, MeOH). UV: (MeOH): 282 (2800), 220 (13200). ESI-MS (positive ion mode): $m/z$ 533 $[M+Na]^+$; (negative ion mode): $m/z$ 509 $[M-H]^-$; EI-MS: $m/z$ (%) 510 ($M^{+\cdot}$, 2.4), 255 (83),169 (7),155 (10). HR-EI-MS: $m/z$ 510.20103±0.005, calcd for $C_{28}H_{26}N_6O_4$ 510.20155. $^1H$ NMR (300 MHz, $CD_3$ OD and, within square bracket, data in $CDCl_3$) $\delta$: 2.48 [2.48] (3H, s, Me–$N3'$), 2.65 [2.72] (3H, s, Me–$N3'''$), 2.89 [2.91] (3H, s, Me–$N1'''$), 3.14 [3.17] (3H, s, Me–$N1'$), 3.38 [3.22] and 3.65 [3.72] (1H each, two d, $J_{gem}$=16.8 Hz, $H_2$-6), 4.63 [4.50] (1H, s, H-4), 7.07 (1H, s, H-2″), 7.04–7.14 (4H, series of m,), 7.23 (1H, dt, $J$=7.8, 2.1 Hz), 7.50 and 7.60 (1H each, two d, $J$=7.8 Hz). Because of the scarce amount, only the following $^{13}C$ NMR signals ($CDCl_3$) could be detected: 24.77, 24.84, 25.36, 29.24 (four Me–$N$), 27.57 (C-6), 41.91 (C-4), 110.95, 111.64, 117.70, 118.92, 120.52, 122.62, 123.90, 124.41, 125.24 (aromatic); nOe: 4.63 (H-4): Me–$N1'$, Me–$N1'''$; 7.07 (H-2″): Me–$N3'$.On addition of 0.1 equiv. of a 0.01 M solution of Eu(tfc)$_3$ in $CDCl_3$, the largest shifts—not taking into account aromatic signals—were observed for Me–$N3'''$ and Me–$N3'$. Once a total 0.2 mol equiv. of chiral reagent shift were added, each methyl-group signal was split in two, in a ca 65:35 ratio.

**4.2.2. Cycloaplysinopsin B (6).** Colorless powder. $[\alpha]_D$=0, $[\alpha]_{577}$=0 ($c$.=1.2 mg/mL, MeOH). UV (MeOH): 282 (2800), 272 (2700), 218 (12400). ESI-MS (positive ion detect.): $m/z$ 549 $[M+Na]^+$; MS/MS (549): 531 $[M-H_2O+Na]^+$, 294, 278; ESI-MS (negative ion detect.): $m/z$ 525 $[M-H^+]^-$; MS/MS (525): 507 $[M-H_2O-H^+]^-$, 270, 255. EI-MS: $m/z$ (%) 508 ($[M-H_2O]^{+\cdot}$, 0.1), 271 (2), 255 (16). HR-EI-MS: $m/z$ 271.0955±0.005, calcd for $C_{14}H_{13}N_3O_3$ 271.0956; $m/z$ 255.1006±0.005, calcd for $C_{14}H_{13}N_3O_2$ 255.1008. NMR: see Table 1.

**4.2.3. 6-Methoxy derivative of cycloaplysinopsin B (7).** When kept in MeOH–$CDCl_3$ at −20°C in the dark for some months, compound **6** was partially converted into **7**, which was isolated in 0.9 mg amount by HPLC with $CH_3CN/H_2O$ 1:1, collecting the eluate at $t_R$=13 min. $[\alpha]_D$=−15 ($c$=0.6 mg/mL, MeOH). $^1H$ NMR ($CDCl_3$) $\delta$: 2.51 and 2.72 (3H each, two s, Me–$N3'$, Me–$N3'''$); 2.94 and 3.05 (3H each, two s, Me–$N1'$, Me–$N1'''$); 3.46 (3H, s, OMe); 4.78 (1H, s, H-4); 5.09 (1H, s, H-6); 7.60–7.00 (9H, series of m, aromatic). On addition of 0.15 mol equiv. of a 0.01 M solution of Eu(tfc)$_3$ in $CDCl_3$ to **7** in $CDCl_3$, the largest shift

were observed for the signals corresponding to H-6, Me–$N1'$, and Me–$N1'''$; in particular, the amidic methyl group s at $\delta$ 3.04 ppm was split into two br.s in 35:65 ratio. Once 0.3 mol equiv. of the chiral shift reagent had been added, also the s corresponding to H-4 and the other methyl amidic group at $\delta$ 2.93 ppm were split into two br.s.

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