# Further New Staurosporine Derivatives from the Ascidian *Eudistoma toealensis* and Its Predatory Flatworm *Pseudoceros* sp.

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Three new indolocarbazole alkaloids, 3-hydroxy-4′-*N*-methylstaurosporine (**3**), 3-hydroxy-4′-*N*-demethylstaurosporine (**4**), and 3′-demethoxy-3′-hydroxy-4′-*N*-demethylstaurosporine (**5**), were isolated from the marine ascidian *Eudistoma toealensis* and its predatory flatworm *Pseudoceros* sp. in addition to two known staurosporines. The structures were determined by 1D and 2D homonuclear and $^1$H-detected heteronuclear NMR spectroscopy and from comparisons with published data. CD measurements for these five staurosporine derivatives, as well as the previously described seven staurosporines, are reported, confirming that all derivatives possess the 2′*S*,3′*R*,4′*R*,6′*R* configuration.

## Introduction

In this paper we describe the isolation and structure elucidation of three new, as well as two known, staurosporine derivatives. Besides our previous report on staurosporines from *Eudistoma toealensis*[1] there have been two reports of staurosporine derivatives from ascidians of the same genus,[2,3] as well as other reports from marine sources.[4,5] Cantrell et al. reported 11-hydroxy-4′-*N*-demethylstaurosporine, which had been previously described by us, as well as 3,11-dihydroxystaurosporine from the prosobranch mollusk *Coriocella nigra*.[4] Prosobranch mollusks are predators of ascidians and are known to sequester compounds from their diets. In another study Williams et al. reported the isolation of three staurosporines and two holyrines (possible intermediates in staurosporine biosynthesis) from a marine actinomycete, isolated from a sediment core sample in the North Atlantic Ocean.[5]

On the other hand, numerous publications describe the isolation of staurosporines from terrestrial microorganisms such as actinomycetes of the genus *Saccharothrix*.[6−10] The isolation of staurosporine and derivatives from a marine actinomycete supports the hypothesis that associated marine microorganisms are the real producers of staurosporines in the ascidian *E. toealensis*. Whether the isolated staurosporine derivatives are produced by the ascidian *E. toealensis* or associated microorganisms needs to be verified. The flatworm *Pseudoceros* sp. seems to derive the staurosporines from *E. toealensis*, since they did not show any staurosporines when collected from a different ascidian found nearby in the same habitat.[1]

Staurosporine and its derivatives are strong kinase inhibitors which have been widely used as molecular tools. Other activities include the inhibition of platelet aggregation, inhibition of smooth muscle contraction, blocking of certain phases of the cell-growth cycle, and the reversal of multidrug resistance.[11−13] The potential of staurosporines as anticancer agents is clearly supported by the example of 7-hydroxystaurosporine (UNC-01), which is in clinical phase 1 trials at the NCI.[14]

Here we present spectroscopic data that allow structure elucidation of three new staurosporine derivatives. Cytotoxicity studies with several of the new derivatives are reported in Schupp et al.[15]

## Results and Discussion

The white colonial ascidian *Eudistoma toealensis*, Monniot and Monniot 1996, (Polycitoridae) and the flatworm *Pseudoceros* sp. (Pseudocerotidae) were observed in high densities on mangrove roots in areas of Chuuk, Micronesia. Sufficient quantities of the ascidian and the flatworm were collected by snorkeling, and the extraction of metabolites was performed as previously described.[1]

The marine ascidian *E. toealensis* and the marine flatworm *Pseudoceros* sp. were collected by snorkeling at depths of 1 to 2 m. Samples were frozen immediately and freeze-dried prior to extraction. Compounds **1**−**5** (Figure 1) were isolated from the methanol-soluble material using column chromatography and semipreparative HPLC. Although compounds **4** and **5** were present in only small amounts, we were able to resolve the structures by comparing the $^1$H NMR data of these compounds with compounds **1**−**3**, as well as with literature data.[1,16−19] Structures **1** and **2** showed the same $^1$H shift data (Table 1) and long-range correlations in the HMBC (Table 2) as reports from the literature and were identified as 11-hydroxystaurosporine and 4′-*N*-methylstaurosporine, respectively.[2,20]

Compounds **1**−**5** exhibited the typical UV spectra of indolocarbazole compounds of the staurosporine type. The MS indicated a relative mass of 496, 468, and 438 for compounds **3**−**5** respectively. This and different retention times in the HPLC chromatogram compared to previously isolated staurosporines[1] indicated the presence of more staurosporines derivatives. The structures of **1**−**5** followed directly from the shift data (Tables 1, 2) and long-range correlations in the HMBC spectra.

The molecular weight of 496 indicated that compound **3** was a staurosporine derivative with an additional methyl and hydroxyl group. The daughter ion with *m*/*z* 354 is characteristic of the oxygenated indolocarbazole moiety, suggesting for **3** an additional oxygen in the indolocarbazole moiety and a methyl group in the sugar moiety. The $^1$H NMR showed a spectrum similar to that in compound **2**.

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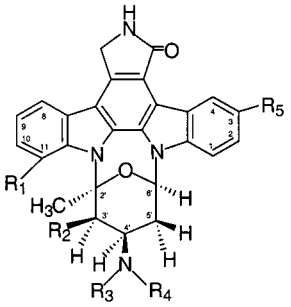

| comp. | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|
| **1** | OH | $OCH_3$ | H | $CH_3$ | H |
| **2** | H | $OCH_3$ | CH3 | $CH_3$ | H |
| **3** | H | $OCH_3$ | CH3 | $CH_3$ | OH |
| **4** | H | $OCH_3$ | H | H | OH |
| **5** | H | OH | H | H | H |

**Figure 1.** Structures of staurosporine derivatives.

**Table 1.** $^1$H Data for Compounds **1**−**5** in $CD_3OD$

| H no. | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 7.47 br d (8.1) | 7.48 br d (8.1) | 7.28 d (8.7) |
| 2 | 7.55 dt (1.0, 7.6) | 7.55 br t (8.1) | 7.07 dd (2.5, 8.7) |
| 3 | 7.36 br t (7.5) | 7.35 br t (7.2) | |
| 4 | 9.30 br d (7.9) | 9.32 br d (7.9) | 8.77 d (2.5) |
| 7 | 7A 5.09 d (17.6) | 5. 08 br s | 7A 5.02 (17.5) |
| | 7B 5.01 d (17.6) | | 7B 4.97 (17.5) |
| 8 | 7.61 d (7.7) | 8.10 br d (7.6) | 8.06 d (8.0) |
| 9 | 7.29 t (7.7) | 7.44 br t (7.4) | 7.44 br t (7.6) |
| 10 | 7.03 d (7.4) | 7.57 br t (8.4) | 7.57 ddd (1.0, 7.5, 8.0) |
| 11 | | 8.08 br d (8.8) | 8.05 d (8.2) |
| 3′ | 4.68 br s | 4.59 m | 4.60 br s |
| 4′ | 3.98 br m | 3.77 m | 3.92 m |
| 5′a | 3.35 br m | 3.32 m | 3.36 m |
| 5′b | 2.62 dt (4.5, 12.5) | 2.48 m | 2.41 ddd (≈2.5, ≈11.0, ≈13.0) |
| 6′ | 6.72 dd (4.5, 9.7) | 6.87 dd (2.8, 9.2) | 6.77 dd (2.5, 9.4) |
| 2′-$CH_3$ | 2.52 s | 2.59 s | 2.59 s |
| 3′-$OCH_3$ | 3.06 s | 2.45 br s | 2.35 s |
| 4′-$NCH_3$ | 2.94 s | 2.73 br s | 2.94 br s |

| H no. | **4** | **5** |
|---|---|---|
| 1 | 7.33 d (8.7) | 7.56 m*[a] |
| 2 | 7.07 dd (2.2, 8.7) | 7.56 m* |
| 3 | | 7.36 m* |
| 4 | 8.76 d (2.2) | 9.31 d (8.1) |
| 7 | 7A 5.01 (18.6) | 7A 5.13 (17.9) |
| | 7B 4.97 (18.6) | 7B 5.06 (17.9) |
| 8 | 8.05 br d (≈8.0) | 8.08 br d (8.1) |
| 9 | 7.44 br dd (7.5, 7.5) | 7.42 ddd (1.1, 7.5, 7.5) |
| 10 | 7.56 br dd (7.6, 7.6) | 7.54 m |
| 11 | 8.03 d (≈ 8.0) | 8.03 br d (8.6) |
| 3′ | 4.20 br s | 4.60 br s |
| 4′ | 4.12 m | 4.23 m |
| 5′a | 3.10 m | 2.86 m |
| 5′b | 2.37 m | 2.47 m |
| 6′ | 6.77 dd (2.5, 9.0) | 6.90 dd (5.2, 7.8) |
| 2′-$CH_3$ | 2.56 s | 2.41 s |
| 3′-$OCH_3$ | 2.55 s | |
| 4′-$NCH_3$ | | |

[a] * indicates second-order spin system in compound **5**.

There was a broad methyl signal at 2.94 ppm corresponding to two methyl groups (apparent from the integration). The methyl groups at 2.35 and 2.59 ppm showed typical correlations and were assigned to the 3′-$OCH_3$ and 2′-$CH_3$, respectively, from the HMQC and HMBC experiments (Tables 1, 2). All other proton signals of the sugar moiety were clearly visible in the $^1$H NMR spectra, and their chemical shifts were comparable to those of compound **2** and staurosporine.[1] The $^1$H NMR spectrum of **3** showed again the characteristic three-proton aromatic coupling pattern (2D COSY) in which there was an upfield shift of H-4 (8.77 ppm) and only one small coupling constant (2.5 Hz) comparable to shifts observed in 3-hydroxy derivatives of staurosporine.[1] The absence of H-3 and corresponding upfield shifts of H-4 and H-2 were only compatible with an additional hydroxyl group at C-3. Therefore compound **3** had a hydroxyl group at position C-3 and an additional methyl group at the 4′-nitrogen and is 3-hydroxy-4′-*N*-methylstaurosporine.

**Table 2.** $^{13}$C NMR Data of Staurosporine Derivatives **1**−**3** in $CD_3OD$

| C no. | **1** | **2** | **3** |
|---|---|---|---|
| 1 | 109.1 d | 109.1 d | 109.5 d |
| 2 | 126.5 d | 126.4 d | 116.1 d |
| 3 | 120.8 d | 120.7 d | |
| 4 | 126.9 d | 126.9 d | 111.7 d |
| 4a | 124.5 s | 124.4 s | |
| 4b | 116.9 s | | |
| 4c | 119.4 s | | |
| 5 | 175.0 s | 175.0 s | |
| 7 | 46.9 t | 46.9 t | 47.0 t |
| 7a | 134.4 s | 133.8 s | |
| 7b | 115.8 s | 115.7 s | |
| 7c | 128.3 s | 125.8 s | |
| 8 | 113.7 d | 122.3 d | 122.5 d |
| 9 | 123.0 s | 121.7 s | 121.9 s |
| 10 | 113.0 s | 126.2 s | 126.4 s |
| 11 | 143.7 s | 113.6 s | 113.4 s |
| 11a | 129.0 d | 139.6 d | |
| 12a | | | |
| 12b | | | |
| 13a | 138.7 s | 138.0 s | |
| 2′ | 95.5 s | 94.7 s | 95.0 s |
| 3′ | 81.3 d | 81.4 d | 81.0 d |
| 4′ | 55.7 d | | 63.5 d |
| 5′ | 27.9 t | | 29.2 t |
| 6′ | 82.6 d | 82.7 d | 82.7 d |
| 2′-$CH_3$ | 29.6 q | 28.3 q | 28.4 q |
| 3′-$OCH_3$ | 60.5 q | 59.7 q | 60.1 q |
| 4′-$NCH_3$ | 31.4 q | 35.1 q | 42.3 q |

The $^1$H NMR spectrum of **4** again showed a characteristic three-proton aromatic coupling pattern (2D COSY) in which there was an upfield shift of H-4 (8.76 ppm) and only one small coupling constant (2.2 Hz) comparable to shifts observed in compound **3**. The absence of H-3 and corresponding upfield shifts of H-4 and H-2 were only compatible with an additional hydroxyl group at C-3 (Table 1). In addition one of the methyl groups in the pyran ring was absent, as indicated by the molecular weight of 468. Since the retention time of compound **4** was clearly different from that of 3-hydroxy-3′-demethoxy-3′-hydroxystaurosporine by HPLC,[1] we assumed the loss of either a methyl group at the 2′-carbon or 4′-nitrogen. There was a slight upfield shift of H-4′ (4.12 ppm) compared to staurosporine, suggesting that the methyl group at the 4′-nitrogen was absent. On the other hand there was no downfield shift of H-3′ as in 3-hydroxy-3′-demethoxy-3′-hydroxystaurosporine. The methyl signal at 2.55 ppm corresponded to the 3′-$OCH_3$ signal observed in other isolated staurosporine derivatives. Furthermore, the other methyl group at 2.56 ppm showed a long-range coupling with C-2′, indicating it was 2′-$CH_3$. The remaining proton signals of the pyran ring were present and comparable to proton signals in 4′-*N*-demethylstaurosporine,[1] confirming that **4** had to be 3-hydroxy-4′-*N*-demethylstaurosporine.

Compound **5** again showed the typical UV spectrum of the other staurosporine derivatives (**1**−**4**). The molecular weight (MW 438) suggested two methyl groups were absent compared to staurosporine (MW 466). Fragmentation of the

**Table 3.** CD Measurements for Compounds **1**−**5** and Previously Described Staurosporine Derivatives (**6**, Staurosporine; **7**, 3-Hydroxystaurosporine; **8**, 3-Hydroxy-3′-demethoxy-3′-hydroxystaurosporine; **9**, 3′-Demethoxy-3′-hydroxystaurosporine; **10**, 3-Hydroxy-4′-*N*-demethylstaurosporine; **11**, 4′-*N*-Demethylstaurosporine; **12**, 11-Hydroxy-4′-*N*-demethylsaturosporine[a])

| **1** | | **2** | | **3** | | **4** | | **5** | | **6** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ |
| 211 | −7.45 | 208 | −6.50 | 210 | −4.92 | 210 | −1.88 | 209 | −0.56 | 206 | −4.90 |
| 233 | +4.11 | 231 | +2.38 | 226 | +1.86 | 232 | +0.53 | 227 | +0.18 | 231 | +2.14 |
| 254 | +0.34 | 241 | +0.38 | 249 | −0.82 | 249 | −0.37 | 246 | +0.12 | 243 | −0.18 |
| 265 | −2.81 | 249 | +1.17 | 258 | −0.14 | 258 | −0.17 | 267 | −0.23 | 250 | +1.29 |
| 280 | −2.75 | 266 | −1.08 | 271 | −0.86 | 266 | −0.40 | 283 | −0.02 | 263 | −0.92 |
| 302 | +6.83 | 279 | +0.25 | 278 | −0.82 | 298 | +0.41 | 297 | +0.27 | 275 | −0.71 |
| 324 | +1.22 | 295 | +2.66 | 289 | −0.34 | 303 | +0.29 | 319 | −0.08 | 295 | +2.07 |
| 358 | −0.94 | 319 | +0.16 | 298 | +0.42 | 308 | +0.39 | 335 | −0.03 | 359 | −0.42 |
| 363 | −0.78 | 326 | +0.47 | 309 | +0.88 | 320 | −0.04 | 376 | +0.02 | 364 | −0.31 |
| 372 | −1.79 | 358 | −0.38 | 342 | +0.31 | 326 | +0.10 | 382 | −0.06 | 373 | −0.56 |
| | | 365 | −0.09 | 365 | −0.29 | 332 | −0.03 | | | | |
| | | 371 | −0.62 | 382 | −0.50 | 385 | −0.23 | | | | |

| **7** | | **8** | | **9** | | **10** | | **11** | | **12** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ | $\lambda$ | $\Delta E$ |
| 209 | −13.31 | 211 | −1.66 | 208 | −4.12 | 210 | −1.88 | 205 | −6.54 | 208 | −2.71 |
| 231 | +4.40 | 231 | +0.47 | 230 | +0.98 | 232 | +0.53 | 229 | +2.15 | 227 | +1.10 |
| 249 | −1.79 | 248 | −0.21 | 237 | +0.22 | 249 | −0.37 | 241 | +0.39 | 246 | −0.84 |
| 255 | −0.61 | 261 | +0.03 | 249 | +0.90 | 258 | −0.17 | 249 | +1.42 | 261 | +0.11 |
| 269 | −2.03 | 274 | −0.22 | 263 | −0.45 | 266 | −0.40 | 268 | −1.13 | 277 | +0.26 |
| 282 | −0.88 | 302 | +0.41 | 281 | +0.04 | 298 | +0.41 | 295 | +3.73 | 292 | +0.28 |
| 285 | −1.19 | 312 | +0.41 | 297 | +2.31 | 303 | +0.29 | 320 | +0.18 | 305 | −0.14 |
| 308 | +3.10 | 323 | +0.17 | 319 | +0.29 | 308 | +0.39 | 325 | +0.52 | 321 | −0.50 |
| 331 | +0.68 | 341 | +0.22 | 334 | +0.59 | 320 | −0.04 | 358 | −0.30 | 329 | −0.45 |
| 345 | +0.95 | 361 | −0.08 | 358 | −0.06 | 326 | +0.10 | 362 | −0.07 | 375 | −0.03 |
| 383 | −1.05 | 371 | +0.02 | 366 | +0.06 | 332 | −0.03 | 370 | −0.44 | | |
| | | | | 372 | −0.18 | 385 | −0.23 | | | | |

[a] All spectra were recorded in MeOH.

molecular ion peak resulted in a daughter ion of *m*/*z* 338, which is characteristic of the indolocarbazole moiety. Hence structural changes had occurred in the sugar moiety. In the $^1$H NMR spectrum only one methyl signal at 2.41 ppm was visible. In HMBC experiments this methyl group coupled with C-2′ and was therefore assigned to 2′-$CH_3$ (Tables 1, 2). Other HMBC correlations were not detected due to the poor signal-to-noise. There were no methyl signals visible around 2.2 and 2.8 ppm, representing the 3′-$OCH_3$ and 4′-$NCH_3$ signals in staurosporine. The signals in the $^1$H−$^1$H COSY NMR again revealed two isolated ABCD aromatic spin systems. The aromatic system containing proton H-4 had the typical low-field shift at 9.31 ppm. The corresponding coupling constants could not be determined, due to a second-order spin system. Through comparison with NMR data for compounds **1**−**4**, literature data,[1,15−19] and considering the rigorous assignment of 2′-$CH_3$ and the missing 3′-$OCH_3$ and 4′-$NCH_3$ signals, compound **5** was identified as 3′-demethoxy-3′-hydroxy-4′-*N*-demethylstaurosporine.

Table 3 shows the CD spectra for compounds **1**−**5** as well as those of previously isolated staurosporines.[1] Comparison of our data with the literature[2,4,8,19−22] confirmed that the absolute configuration of all reported staurosporine derivatives was identical with the 2′*S*,3′*R*,4′*R*,6′*R* configuration described by Funato and colleagues.[23] In our original publication[1] absolute configurations were shown incorrectly (the mirror images of the correct configurations were shown in the figure) and should now be corrected.

## Experimental Section

**General Experimental Procedures.** $^1$H (1D and 2D COSY) and $^{13}$C (1D and 2D $^1$H-detected heteronuclear one-bond HMQC[24] and multiple-bond HMBC[25] correlations) NMR spectra (chemical shifts in ppm) were recorded on Bruker DPX 300, ARX 400, or DMX 600 NMR spectrometers using standard Bruker software. Mass spectra (ESIMS) were recorded on a Finnigan MAT TSQ-7000 triple-stage quadrupole mass spectrometer. The temperature of the heated capillary (20 V) was 200 °C, and the electrospray capillary voltage was set to 3.5 kV. Nitrogen served both as sheath (70 psi) and auxiliary gas; argon served as collision gas. For HPLC analysis, samples were injected into a HPLC system equipped with a photodiode-array detector (Gynkotek, Germany). Routine detection was at 254 and 292 nm. The separation column (125 × 4 mm, i.d.) was prefilled with Eurospher 100-$C_{18}$, 5 $\mu$m (Knauer GmbH, Germany). Separation was achieved by applying a linear gradient from 100% $H_2O$ (with 0.2% TFA) to 100% MeCN over 40 min. Semipreparative HPLC was conducted on a Merck Hitachi, La-Chrome L-7100 pump and Merck Hitachi, La-Chrome L-7400 UV detector. Chromatograms were recorded on a Merck Hitachi, D-2000 Chromato-Integrator. Separation columns (300 × 8 mm, i.d.) were prefilled with Eurospher 100-$C_{18}$, 7 $\mu$m (Knauer GmbH, Germany). Compounds were purified by isocratic separation with 33% MeCN, 67% $H_2O$ (with 0.2% TFA) for 40 min. TLC was performed on precoated TLC plates with Si gel 60 $F_{254}$ and Si gel RP-18 $F_{254}$ (Merck, Darmstadt, Germany). Compounds were detected from their UV absorbance at 254 and 366 nm. Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements.

**Animal Material.** *E. toealensis* is a colonial ascidian first described by Monniot and Monniot in 1996.[26] The zooids are whitish and slightly transparent. Several zooids converge, sitting on small, 2−4 cm short, brownish branches, which are attached to mangrove roots. A voucher specimen is kept under MNHN A3 Eud 134 at the Museum National de Histoire Naturelle, Paris, France. The flatworm *Pseudoceros* sp. is a new species.[27] It has a cream-colored body with uneven royal blue to dark purple spots along the margin. The spots continue over the pseudotentacles. Sizes ranged from 6 × 3 mm (immature) to 40 × 29 mm (mature), with an average length of 23 × 8 mm ($n$ = 44, STD = 1.4 mm and 0.3 mm, respectively). It was found in large numbers on colonies of *E.*

*toealensis* at depths of 1 to 2 m.[1] A voucher specimen (QM #G211100) is deposited at the Queensland Museum, Brisbane, Australia.

**Extraction and Isolation.** The freeze-dried samples of the ascidian *E. toealensis* (94 g) were extracted exhaustively with MeOH. The extract was evaporated under reduced pressure to give a residue of 19 g. The MeOH fraction was then partitioned sequentially between hexane, EtOAc, butanol, and $H_2O$. HPLC chromatograms showed that only the EtOAc fraction contained the compounds with the characteristic UV spectra. This fraction was chromatographed on RP-18 Si gel columns [elution with MeOH−$H_2O$−TFA (80:20:0.2)], yielding eight fractions. Final purification of the major UV-active fractions was achieved with semipreparative HPLC [MeCN−$H_2O$−TFA (33:67:0.2)]. Combined ascidian and flatworm fractions yielded compounds **1** (3.9 mg, 0.003%) and **3** (3.1 mg, 0.002%).

*Pseudoceros* sp. was also freeze-dried (60 g), then extracted with MeOH, and the MeOH extract was partitioned with $CH_2Cl_2$. Extracts were evaporated under reduced pressure to yield 6 g of the $CH_2Cl_2$ and 18 g of the MeOH fraction. HPLC chromatograms showed that only the $CH_2Cl_2$ fraction contained the compounds with the characteristic UV spectra. The $CH_2Cl_2$ fraction was chromatographed on RP-18 Si gel columns [elution with MeOH−$H_2O$−TFA (80:20:0.2)], yielding 13 fractions. Again the major UV-active fractions were purified with semipreparative HPLC [MeCN−$H_2O$−TFA (33:67:0.2)]. This yielded compounds **2** (3.3 mg, 0.005%), **4** (1.8 mg, 0.003%), and **5** (1.5 mg, 0.003%).

Compounds **1**−**5** were obtained by combining similar ascidian and flatworm fractions and subjecting them to repeated semipreparative HPLC. Therefore it was not possible to calculate their exact yields. Yields given are a conservative estimate of the real yields, since other minor fractions contained staurosporines as well; however, these fractions were not purified further. Compounds **2** and **4** were only detected in the flatworm crude extract, while compounds **1** and **3** could be detected in the ascidian crude extract as well. Although compound **5** was isolated from the flatworm crude extract, the concentration of it appeared to be too low in the crude extract of the flatworm to allow unambiguous identification. There were other trace peaks between the staurosporine peaks in the flatworm HPLC chromatogram, but the concentrations of these compounds were too small to obtain a UV spectrum and hence allow positive identification.

**3-Hydroxy-4′-*N*-methylstaurosporine (3).** This compound, which was detected in both the ascidian and the flatworm, was purified as a yellowish amorphous powder (percent purity: 100% as estimated by HPLC analysis): $UV_{max}$ (MeOH) 225 ($\epsilon$ 1570), 287 ($\epsilon$ 2070), 297 ($\epsilon$ 3150), 308 ($\epsilon$ 1950), 324 ($\epsilon$ 750), 342 ($\epsilon$ 660), 366 ($\epsilon$ 350), 385 ($\epsilon$ 370); ESIMS *m*/*z* 497 $[M + H]^+$; ESIMS/MS (collision energy 29 eV, 2 mTorr argon) *m*/*z* 354 (497−143); daughter ion *m*/*z* 354 is characteristic of the hydroxylated indolocarbazole moiety, based on detection of the non-hydroxylated indolocarbazole moiety (*m*/*z* 338) by Yang and Cordell.[28]

**3-Hydroxy-4′-*N*-demethylstaurosporine (4).** This compound, which was only detected in the flatworm, was purified as a yellowish amorphous powder (percent purity: 100% as estimated by HPLC analysis): $UV_{max}$ (MeOH) 204 ($\epsilon$ 2710), 287 ($\epsilon$ 3100), 297 ($\epsilon$ 4080), 342 ($\epsilon$ 1150), 366 ($\epsilon$ 700), 384 ($\epsilon$ 700); ESIMS *m*/*z* 469 $[M + H]^+$; ESIMS/MS (collision energy 29 eV, 2 mTorr argon) *m*/*z* 354 (469−115); daughter ion *m*/*z* 354 is characteristic of the hydroxylated indolocarbazole moiety, based on detection of the non-hydroxylated indolocarbazole moiety (*m*/*z* 338) by Yang and Cordell.[28]

**3′-Demethoxy-3′-hydroxy-4′-*N*-demethylstaurosporine (5).** This compound could not be detected in either the crude extract of the ascidian or the flatworm. The alkaloid was purified as a yellowish amorphous powder from the flatworm extract (percent purity: 99% as estimated by HPLC analysis): $UV_{max}$ (MeOH) 206 ($\epsilon$ 2190), 291 ($\epsilon$ 3440), 322 ($\epsilon$ 690), 334 ($\epsilon$ 760), 355 ($\epsilon$ 510), 372 ($\epsilon$ 550); ESIMS *m*/*z* 439 $[M + H]^+$; ESIMS/MS (collision energy 29 eV, 2 mTorr argon) *m*/*z* 338 (439−101); daughter ion *m*/*z* 338 is characteristic of the indolocarbazole moiety.[28]

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