The first F-ring modified ciguatoxin analogue showing significant toxicity†

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Ciguatoxins, the principal causative toxins of ciguatera seafood poisoning, are potent neurotoxic polycyclic ethers. We report herein the total synthesis of a 10-membered F-ring analogue of 51-hydroxyCTX3C, which constitutes the first example of an F-ring modified ciguatoxin that exhibits potent cytotoxicity as well as mouse acute toxicity.

Ciguatoxins (*e.g.*, **1**, Fig. 1), the principal causative toxins of ciguatera seafood poisoning,[1–4] are large ladder-like polycyclic ethers 3 nm in length with 13 rings ranging from 5- to 9-membered.[5] These toxins are produced by an epiphytic dinoflagellate, *Gambierdiscus toxicus*,[6] and transferred to herbivorous and carnivorous fish through the aquatic food chain, and eventually to humans. Ingestion of affected fish leads to gastrointestinal, cardiovascular and neurological disorders which may last for weeks or even years. The lethal potency of ciguatoxins (LD_{50} = 0.25–4 μg kg^{-1})[2–5] by intraperitoneal injection into mice was reported to be greater than that of the structurally related red-tide toxins, brevetoxins (LD_{50} > 200 μg kg^{-1}).[7–10]

These marine natural products exhibit their toxicities by binding to the voltage-sensitive sodium channels (VSSC) of excitable membranes, causing a continuous depolarization of membrane potential.[11,12] Unfortunately, the very limited supply of ciguatoxins from natural sources has prevented the biological and structure–activity relationship (SAR) studies that would aid in understanding the structural requirements necessary for ciguatoxin–VSSC interactions.[13–15]

Recently, we reported the unified total synthesis of ciguatoxins, CTX1B, CTX3C, and 51-hydroxyCTX3C (**1**), in quantities sufficient for testing.[16] Pharmacological studies revealed that the synthetic ciguatoxins exerted multimodal effects on VSSC with simultaneous stimulatory and inhibitory aspects.[17] Moreover, this unified synthetic strategy allowed us to prepare fully synthetic analogues of the ciguatoxins for SAR studies. The 8-membered ring (**2**) and the open chain O-linked analogue (**3**) suggested the critical importance of the 9-membered F-ring of **1** for potent biological activity.[18] Here, we report the total synthesis and biological activity of a new analogue, the 10-membered F-ring 51-hydroxyCTX3C (**4**), which shows more potent activity than **2** and **3**.

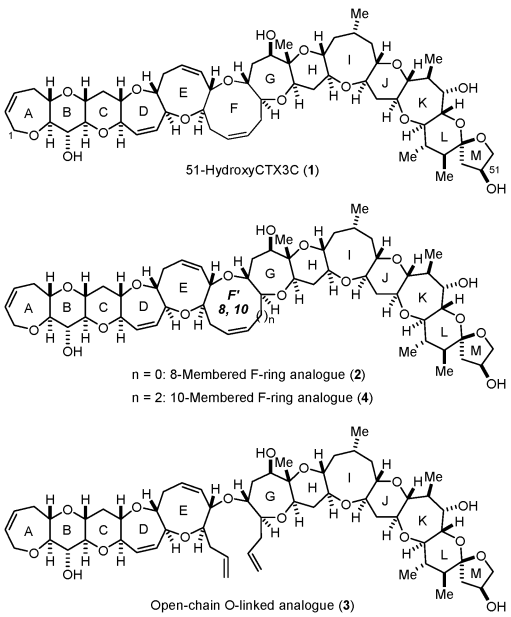

Fig. 1 Structures of ciguatoxin 51-hydroxyCTX3C (**1**) and its analogues.

The 10-membered F-ring analogue (**4**) was synthesized from the previously reported key intermediate **5**[16d,18] in seven steps (Scheme 1). DIBAL reduction of ester **5** followed by one-carbon homologation of alcohol **6** afforded nitrile **7**. DIBAL reduction of **7** and subsequent Wittig olefination of aldehyde **8** gave pentaene **9** in 90% overall yield. Ring-closing olefin metathesis using Grubbs' first generation catalyst[19] provided the desired tridecacyclic system **10** quantitatively. Finally, the removal of four 2-naphthylmethyl (NAP) groups[20] of **10** was realized by DDQ oxidation to generate the target molecule **4**.‡

Biological *in vitro* and *in vivo* activities of **4** were assayed (Table 1). To our surprise, the EC_{50} value, which was evaluated using mouse neuroblastoma Neuro-2A cells,[21] indicated that **4** exhibited 400–700 times greater toxicity than the other F-ring modified analogues **2** and **3**, although it was still 1/50 less cytotoxic than **1**. Moreover, **4** was the first synthetic analogue to exhibit some mouse acute toxicity.

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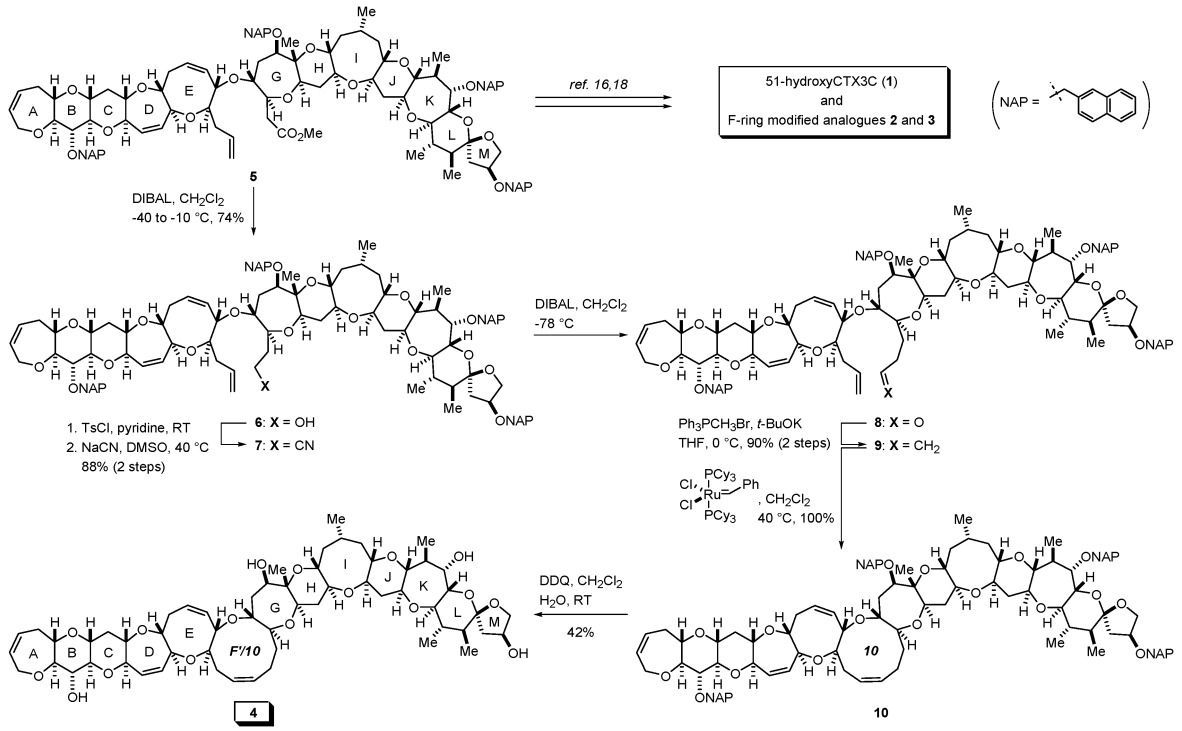

Scheme 1 Total synthesis of 10-membered F-ring analogue (**4**) of 51-hydroxyCTX3C. Cy = cyclohexyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DIBAL = diisobutylaluminium hydride, DMSO = dimethylsulfoxide, Ts = *p*-toluenesulfonyl.

Table 1 Biological assays of 51-hydroxyCTX3C (**1**), F-ring modified analogues **2**, **3** and **4**

Compounds	Cytotoxicity EC_{50}/nM[a]	Acute toxicity LD_{50}/μg kg^{-1}[b]
1	0.0049	0.31
2[c]	103	>667
3[c]	170	>667
4	0.24	600

[a] Cytotoxicity was determined as EC_{50} on mouse neuroblastoma cells Neuro-2A. [b] Acute toxicity was determined as LD_{50} on i.p. injected mice. [c] Ref. 18.

Modeling studies of **4** (MM2* in MacroModel Ver. 8.1)[22] suggested that the assay data could be related to the overall shapes of the molecules.[18] The most stable conformer of **4** was chosen from the pool of energy-minimized structures that satisfied the NOE experiment of the EF′GH-ring model **11** (Fig. 2 and 3A). Although **4** shows a bent form in the most stable state, a relatively straight rod-like structure similar to **1** was found at a higher level (2.74 kcal mol^{-1} more) than the most stable conformer (Fig. 3B). The root mean square

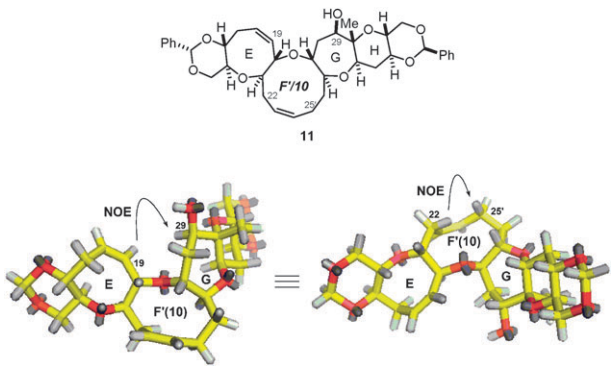

Fig. 2 Energy-minimized structure (MM2*, Macro Model Ver. 8.1) and NOE data for the model compound **11**. The phenyl groups were not included in the energy calculation.

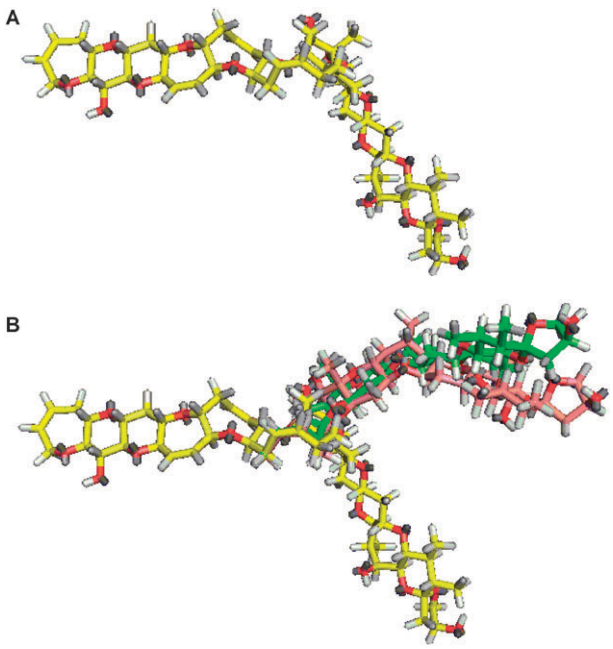

Fig. 3 (A) Energy-minimized structures of 10-membered F-ring analogue (**4**) of 51-hydroxyCTX3C. (B) Energy-minimized structures of 51-hydroxyCTX3C (**1**) (green) and **4** (yellow) and the straight conformer of **4** (pink) with ABCDE-rings superimposed (MM2*, Macro Model Ver. 8.1).

deviation (RMSD) of 17 oxygen atoms between the most stable structure of **1** and the straight conformer of **4** was calculated to be 0.65 Å. In contrast, the minimum RMSD between the most stable conformer of **1** and the 8-membered F-ring analogue **2** was found to be much larger (1.69 Å). The corresponding straight structure of **2** was not found within the 12.0 kcal mol^{-1} range from the most stable state.

In conclusion, we have synthesized the 10-membered F-ring analogue (**4**) of 51-hydroxyCTX3C and found an apparent relationship between the biological activities and the overall shapes of the polycyclic molecules (**2–4**). While a conformational change of **4** to the straight form might be induced by the interaction with VSSC, it is more likely that the minor straight rod-like conformer of **4** fits into and best interacts with VSSC using a hydrogen-bond network similar to natural product **1**. Further SAR studies of ciguatoxins are under active investigation in our laboratory.

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Notes and references

‡ Selected analytical data: **4**, colorless amorphous; ^{1}H NMR (500 MHz, C_6D_5N, 25 °C) δ 7.28 (1H, d, J = 3.5 Hz, OH7), 6.72 (1H, d, J = 3.5 Hz, OH44), 6.52 (1H, d, J = 4.0 Hz, OH51), 6.07 (1H, m, H19), 5.89 (1H, m, H13), 5.85 (1H, m, H23), 5.83 (1H, m, H18), 5.81 (1H, m, H2), 5.72 (1H, m, H3), 5.69 (1H, m, H14), 5.57 (1H, m, H24), 4.98 (1H, m, OH29), 4.84 (1H, m, H51), 4.46 (1H, ddd, J = 10.5, 10.5, 4.5 Hz, H41), 4.30 (1H, dd, J = 10.5, 5.5 Hz, H1), 4.22 (1H, m, H29), 4.21 (1H, m, H44), 4.19 (1H, m, H12), 4.16 (2H, m, H52 × 2), 4.16 (1H, m, H15), 4.05 (1H, m, H20), 4.05 (1H, m, H45), 4.02 (1H, m, H1), 3.99 (1H, m, H7), 3.95 (1H, dd, J = 10.5, 10.5 Hz, H46), 3.89 (1H, m, H27), 3.71 (1H, m, H16), 3.68 (1H, m, H26), 3.62 (1H, m, H6), 3.55 (1H, m, H5), 3.49 (1H, dd, J = 8.5, 8.5 Hz, H11), 3.44 (1H, ddd, J = 9.5, 9.5, 3.5 Hz, H21), 3.40 (1H, m, H34), 3.38 (1H, m, H9), 3.37 (1H, m, H8), 3.32 (1H, m, H39), 3.26 (1H, ddd, J = 12.5, 10.5, 4.5 Hz, H33), 3.20 (1H, m, H31), 3.19 (1H, m, H38), 3.17 (1H, m, H22), 3.15 (1H, m, H42), 2.87 (1H, m, H17), 2.66 (1H, m, H4), 2.62 (1H, m, H28), 2.60 (1H, m. H47), 2.58 (1H, m, H40), 2.57 (1H, m, H22), 2.50 (1H, ddd, J = 11.0, 4.5, 4.5 Hz, H10), 2.43 (1H, m, H4), 2.32 (1H, dd, J = 13.5, 3.5 Hz, H50), 2.29 (1H, m, H25′), 2.28 (1H, m, H28), 2.27 (1H, m, H17), 2.25 (1H, m, H32), 2.04 (1H, m, H25), 2.02 (1H, m, H25′), 2.01 (1H, m, H37), 1.99 (1H, m, H43), 1.91 (1H, m, H32), 1.90 (1H, m, H36), 1.83 (1H, m, H35), 1.78 (1H, m, H40), 1.74 (1H, m, H10), 1.71 (1H, m, H37), 1.66 (1H, m, H48), 1.61 (1H, m, H25), 1.60 (1H, m, H35), 1.46 (3H, s, Me53), 1.29 (3H, d, J = 7.5 Hz, Me56), 1.27 (3H, d, J = 6.5 Hz, Me55), 1.21 (3H, d, J = 7.0 Hz, Me57), 0.92 (3H, d, J = 7.5 Hz, Me54); HRMS (ESI), calcd. for $C_{58}H_{84}NaO_{17}$ 1075.5601 ($M+Na^+$), found 1075.5601.

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