Total Synthesis of Ciguatoxin CTX3C

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More than 20,000 people suffer annually from ciguatera seafood poisoning in subtropical and tropical regions. The extremely low content of the causative neurotoxins, designated as ciguatoxins, in fish has hampered the isolation, detailed biological studies, and preparation of anti-ciguatoxin antibodies for detecting these toxins. The large (3 nanometers long) and complicated molecular structure of ciguatoxins has impeded chemists from completing their total synthesis. Our highly convergent strategic approach featuring the chemoselective ring-closing metathesis reaction as a key tactic has enabled the total synthesis of ciguatoxin CTX3C, which will provide a practical supply for further studies.

More than 20,000 people in subtropical and tropical regions suffer annually from ciguatera, which is the most widespread human poisoning caused by the consumption of seafood (1, 2). The disease is characterized by gastrointestinal, neurological, and cardiovascular disturbances, which often persist for months or years, and in severe cases, paralysis, coma, and death may occur. More than 400 species of fish can be vectors of the ciguatera toxins, which are produced by a marine dinoflagellate, *Gambierdiscus toxicus*, living on macro-algae (3). These neurotoxins, designated as ciguatoxins (4–8), are far more dangerous [acute toxicity on mice median lethal dose $(LD_{50}) = 0.25 \sim 4$ μg/kg] than the structurally related red-tide toxins, brevetoxins $(LD_{50} > 100$ μg/kg) (9–12). Because reef fish are increasingly exported to other areas and ciguateric fish look, taste, and smell normal, ciguatera may become a worldwide health problem. Currently, there are no rapid and reliable methods of detecting ciguatoxins at fisheries. Furthermore, the content of ciguatoxins in fish is extremely low, which has hampered the isolation, detailed biological studies, and most importantly, preparation of the anti-ciguatoxin antibodies for detecting these toxins (13–15). Here we report the first total synthesis of ciguatoxin CTX3C, which will provide a practical supply of ciguatoxins for further studies.

Ciguatoxin (CTX, 1) (4–6) and CTX3C

(2) (7), along with brevetoxins, are structurally classified as ladderlike polyethers (Fig. 1). From pharmacological studies, ciguatoxins and brevetoxins exert their toxicities by

binding to a common site on the voltage-sensitive sodium channels (VSSCs) (16, 17), resulting in the persistent activation and/or prolonged open time of VSSCs (18). The significantly higher VSSC affinity of ciguatoxins than brevetoxins can be useful in the investigation of the VSSC function itself (19–22). Until now, the complicated and large architecture of ciguatoxins (over 3 nm in length) has impeded chemists from completing their total synthesis (23–25).

The synthetic challenge posed by 2 reflects the highly complex molecular structure; as shown in Fig. 1, 13 oxygen atoms and 52 carbon atoms are coiled in a polycyclic macromolecule that includes 13 rings and 30 stereogenic centers. During the course of our studies directed toward 2, we achieved the convergent syntheses of the multicyclic structural fragments 3 and 4, which are briefly described as follow: The A-E ring system 3 was afforded by an alkylative coupling between the AB ring 5 and the E ring 6, followed by the construction of the CD ring portion with ring-closing metathesis (RCM)

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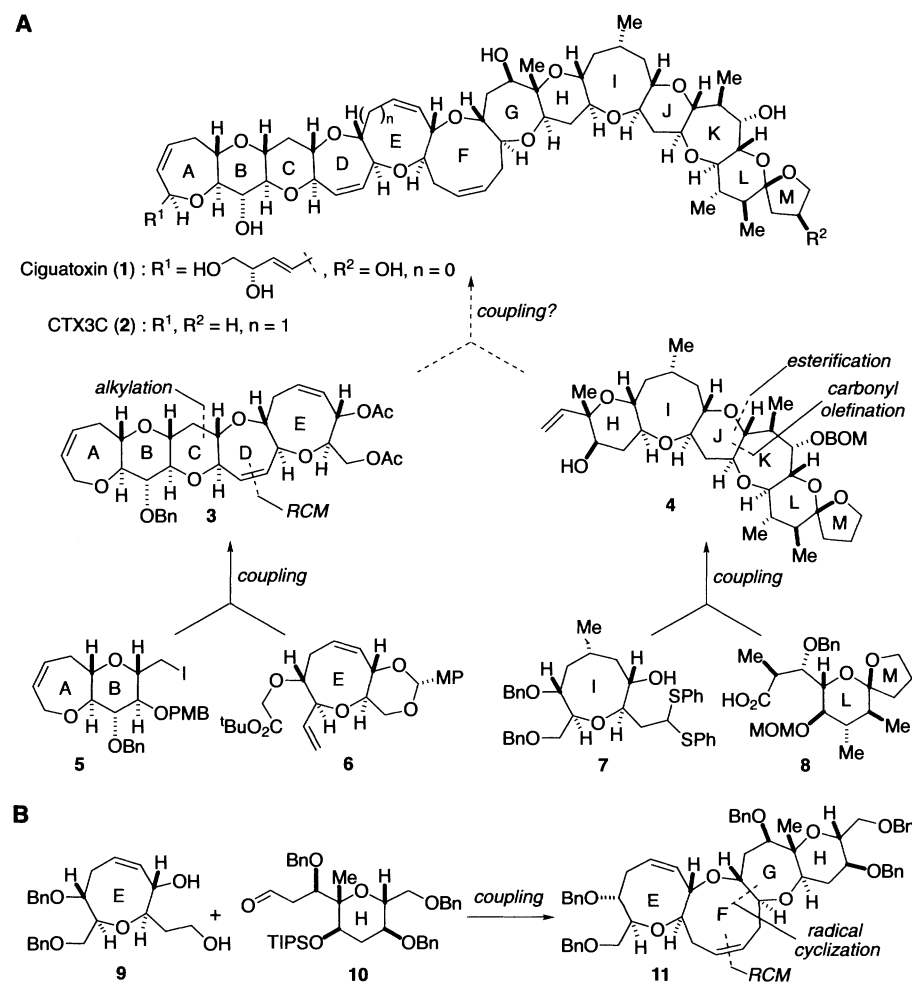
Fig. 1. (A) Molecular structures of CTX (1) and CTX3C (2) and synthetic strategy for the total synthesis of CTX3C. (B) Convergent strategy for construction of EFGH fragment (11): Bn, benzyl; BOM, benzyloxymethyl; tBu, t-butyl; Me, methyl; MOM, methoxy methyl; MP, p-methoxyphenyl; Ph, phenyl; PMB, p-methoxybenzyl; RCM, ring-closing metathesis.

(26) as a key transformation (27, 28). The H-M ring system **4** was synthesized via (i) esterification between the I ring **7** and the LM ring **8**, (ii) intramolecular carbonyl olefination with a low-valent titanium complex to form the J ring (29), and (iii) sequential construction of the K and H rings (30). The final and most challenging tasks in our synthetic venture were the coupling of these large and complex fragments, **3** and **4**, and simultaneously, the construction of the central FG ring system. As a model system for this particular coupling, the comparable E-H ring fragment **11** was successfully constructed from two monocyclic compounds, **9** and **10**, by using (i) acetal formation, (ii) radical reaction for constructing the G ring, and (iii) RCM reaction for cyclizing the F ring (31). With these proven synthetic materials and methods, we undertook our final goal of constructing the actual nanometer-scale ring system of **2**.

As shown in Fig. 2, the initial stage in the final assembly of **2** was the functionalization of both **3** and **4** to their derivatives suitable for coupling, **14** and **19**, respectively. The left-wing A-E ring diol **14** was obtained by means of protective group manipulation and one-carbon elongation. The hydroxyl groups of **3** were deacetylated with K_2CO_3 in methanol (MeOH) to afford **12** in 99% yield.

Deprotected **12** was selectively tosylated on the primary alcohol, then subsequently treated with NaCN in dimethylsulfoxide (DMSO) to give nitrile **13** (45% yield, two steps). Reduction of the nitrile moiety of **13** was carried out in two steps with diisobutylaluminum hydride (DIBAL), then $NaBH_4$ in MeOH to afford the A-E ring diol **14** (86% yield, two steps).

The right-wing H-M ring aldehyde **19** was obtained by means of functional group transformations of **4**, which included the establishment of the C29-stereocenter (Fig. 2). Protection of the C31–secondary alcohol of **4** as a triisopropylsilyl (TIPS) ether, followed by reductive removal of the benzyloxymethyl (BOM) group with lithium di-*t*-butylbiphenylide (LiDBB), generated C44-alcohol **15** (91% yield) (32), which in turn was protected as the benzyl ether to give **16** in 80% yield. Protected **16** was subjected to stepwise olefin cleavage [catalytic OsO_4, 4-methylmorpholine-N-oxide (NMO), then $NaIO_4$], resulting in the aldehyde, then directly subjected to chelation-controlled allylation with allyltributylstannane to yield a secondary alcohol, favoring the desired C29-isomer (4:1). Upon this treatment, partial isomerization of the C49-spiroketal was observed (17:C49-epi-**17** = 3:1), presumably due to the Lewis-acid nature of $MgBr_2$. The isomeric mixture was

treated under protic conditions [camphorsulfonic acid (CSA), (CH_2Cl_2)], which resulted in the desired and thermodynamically more stable ketal **17** (70% yield, three steps). The secondary alcohol of **17** was protected as its benzyl ether in 90% yield. The terminal olefin **18** was converted to the H-M ring aldehyde **19** through osmylation and oxidative diol-cleavage.

We have thus reached a critical stage in the total synthesis: unification of the two large fragments (**14**, **19**) and subsequent manipulations of the resulting compounds, which span over 3 nm (Fig. 3). First, the scandium trifluoromethanesulfonate–promoted condensation between the β-alkoxy aldehyde **19** and the 1,4-diol **14** afforded dioxepane acetal **20** as a C27 epimeric mixture (~4:1) in 57% yield (33–35). This seven-membered O,O-acetal **20** was converted to the O,S-acetal **21** as a single diastereomer by using a combination of phenylthiotrimethylsilane and trimethylsilyl trifluoromethanesulfonate in 61% yield (31, 36). Regioselective cleavage of the C27-acetal and the nonreaction of the C49-spiroketal under these conditions indicate the high degree of efficiency of this reaction in the complex matrix. Protection of the primary alcohol of **21** as the *p*-methoxybenzyloxymethyl (PMBM) ether, removal of the TIPS group, and then treatment with methyl propiolate and N-methylmorpholine provided β-(E)-alkoxyacrylate **22** in 59% overall yield (three steps) (37). Construction of the G ring, buried in the middle portion of the molecule, was realized by treating **22** in toluene with 2,2′-azobisisobutyronitrile (AIBN) and *n*-Bu₃SnH at 85°C to yield the desired oxepane **23**, in which the generated C27-radical added to the α,β-unsaturated ester in a stereo- and chemoselective manner in the presence of the other double bonds in the molecule (31, 38, 39).

With the 12 ether rings in place, the final steps were construction of the F ring by RCM reaction and deprotection (Fig. 3). DIBAL reduction of ester **23** to the aldehyde, followed by Wittig methylenation, produced olefin **24** in 61% overall yield (three steps). Removal of the PMBM group afforded alcohol **25**, which was converted to the RCM substrate **26** via a two-step sequence: an oxidation with SO_3·pyridine-DMSO, followed by a Wittig reaction. The final critical chemoselective RCM reaction of the pentaene **26** for closing the F ring, without affecting the preexisting disubstituted double bonds through ring-opening metathesis reaction, was successfully attained with the use of a Grubbs catalyst **27** (40) at 40°C in CH_2Cl_2 for 8 hours, affording the fully protected CTX3C (**28**) in 60% overall yield (three steps). This remarkable transformation indicates the efficiency and reliability of metathesis reactions, even in these highly elaborate
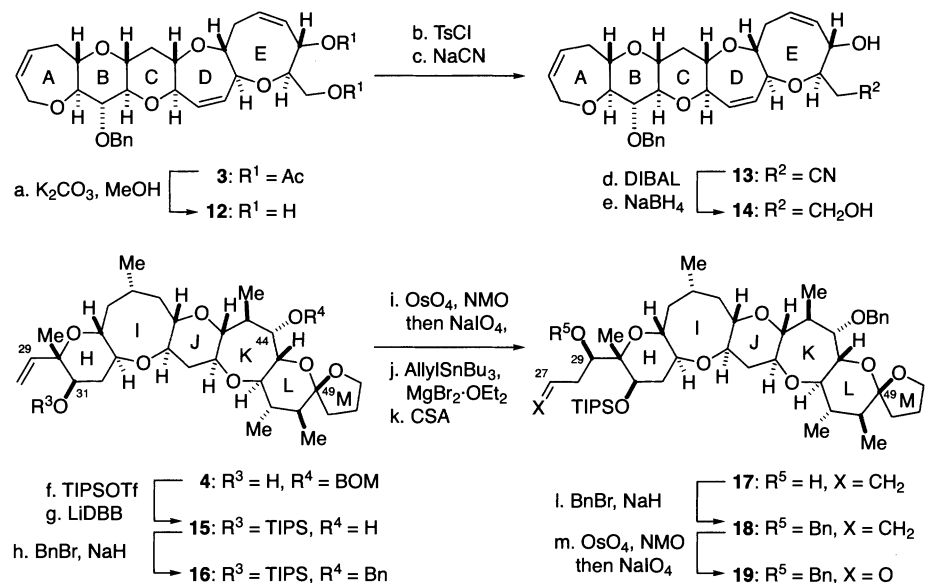

Fig. 2. Syntheses of A-E ring diol **14** and H-M ring aldehyde **19**. Reagents and conditions are as follows. (a) K_2CO_3, methanol (MeOH), room temperature (RT), 17 hours, 99%; (b) *p*-toluenesulfonyl chloride (TsCl), pyridine, 35°C, 10 hours; (c) NaCN, dimethyl sulfoxide (DMSO), 45°C, 18 hours, 45% (two steps); (d) diisobutylaluminum hydride (DIBAL), CH_2Cl_2, −80° to −60°C, 1 hour, 92%; (e) $NaBH_4$, MeOH, −80° to −10°C, 1 hour, 94%; (f) triisopropylsilyl trifluoromethanesulfonate (TIPSOTf), 2,6-lutidine, CH_2Cl_2, 0°C to RT, 12 hours; (g) excess lithium di-*t*-butylbiphenylide (LiDBB), THF (tetrahydrofuran), −60°C, 20 min, 91% (two steps); (h) benzyl bromide (BnBr), NaH, Bu₄NI, THF/N,N-dimethylformamide (DMF) (8:1), 0°C to RT, 13 hours, 80%; (i) OsO_4, 4-methylmorpholine-N-oxide (NMO), *t*-BuOMe, *t*-BuOH, H_2O, 40°C, 5 days, then $NaIO_4$, pH 7 phosphate buffer, RT, 2 hours; (j) AllylSnBu₃, $MgBr_2$·OEt₂, CH_2Cl_2, −80° to 10°C, 14 hours; (k) 10-camphorsulfonic acid (CSA), $(CH_2Cl)_2$, RT, 4 hours, 70% (three steps); (l) BnBr, NaH, THF/DMF (3:1), 0°C to RT, 6 hours, 90%; (m) OsO_4, NMO, *t*-BuOMe, *t*-BuOH, H_2O, RT, 19 hours, then $NaIO_4$, pH 7 phosphate buffer, RT, 1.5 hours.

situations. As observed for ciguatoxin (1) (4, 5), CTX3C (2) (7), and other model compounds (41), the ¹H nuclear magnetic resonance (NMR) signals of the F ring in **28** were severely broadened at room temperature due to the relatively slow conformational changes. Conversely, construction of the F ring in the last stage of the synthesis was practically favorable in avoiding complications in assigning the NMR spectra of the synthetic intermediates.

The final global deprotection of **28** was not a trivial step in our total synthesis (Fig. 3). In sharp contrast to the facile deprotec-

tions of the structural fragments, such as **4→15** and **30→31** (42), conversion of **28** to **2** was complicated by undesired side-reactions. The LiDBB-mediated reduction of the benzyl ethers of **28** was accompanied by reductive cleavage of the A ring allylic ether (32), leading only to compound **29**. On the other hand, oxidative treatment of **28** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) resulted in a complex mixture, presumably attributable to the involvement of the allylic ethers in the substrate. After considerable experimentation, conversion of **28** to the natural product **2**

was finally realized by a carefully controlled Birch reduction; treatment of **28** with sodium in NH₃-EtOH-THF at −90°C for 10 min accomplished the global deprotection to yield the target molecule **2** in ~30 to 40% crude yields. Further purification of **2** by high-performance liquid chromatography (HPLC, Asahipak ODP-50, 75% MeCN-H₂O) was carried out to isolate pure synthetic CTX3C (**2**) (7% yield from **28**), which was determined to be identical in all respects to the naturally occurring form by thin-layer chromatography, HPLC, ¹H-NMR, circular dichromism (CD), and
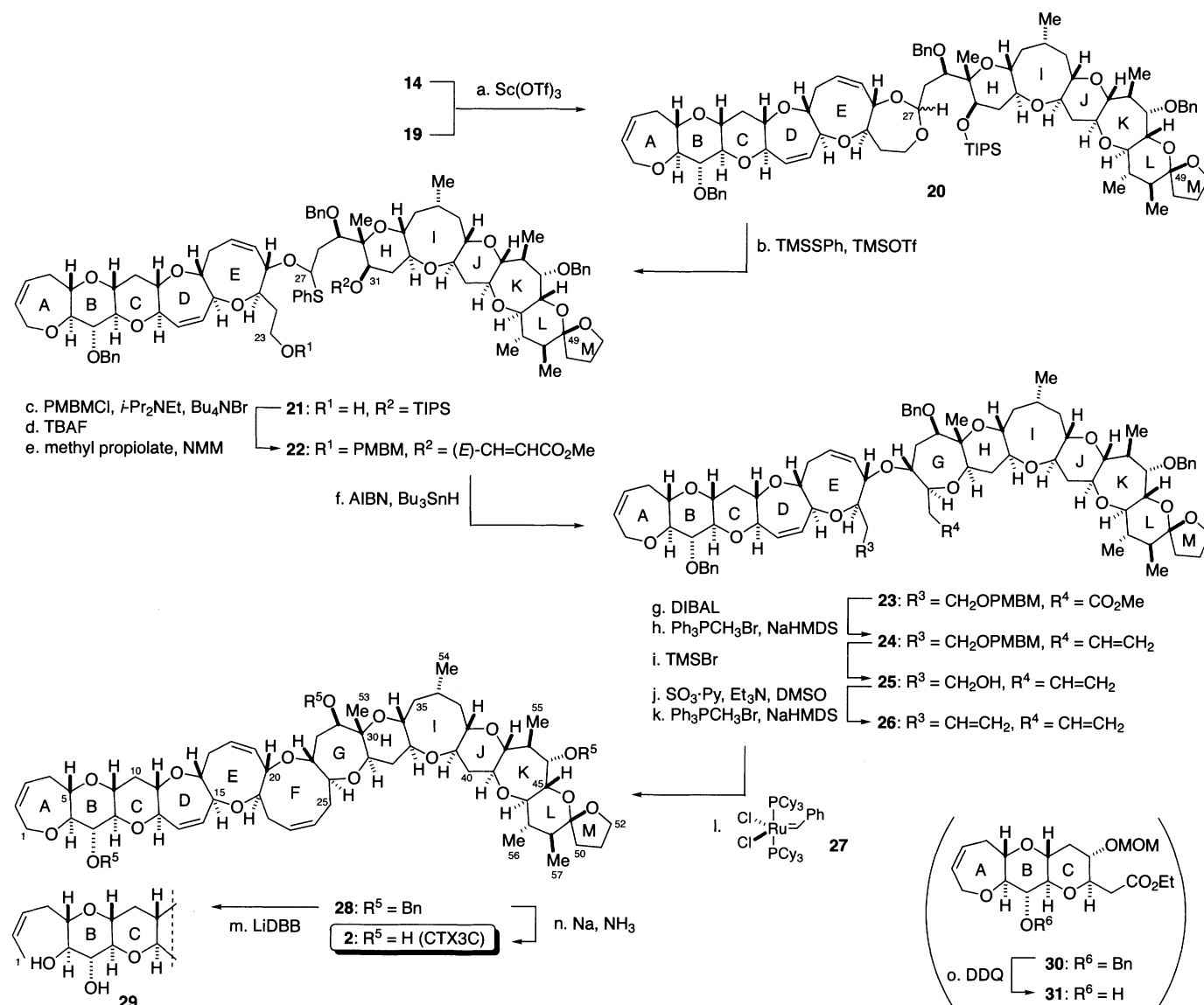

Fig. 3. Total synthesis of CTX3C (**2**). Reagents and conditions are as follows. (a) **19** (1.1 eq.), scandium trifluoromethanesulfonate [Sc(OTf)₃] (30 mol%), benzene, RT, 3 hours, 57%; (b) phenylthiotrimethylsilane (TMSSPh), trimethylsilyl trifluoromethanesulfonate (TMSOTf), CH₂Cl₂, 0°C to RT, 3 hours, then K₂CO₃, MeOH, RT, 1 day, 61%; (c) *p*-methoxybenzyloxymethyl chloride (PMBMCl), *i*-Pr₂NEt, Bu₄NBr, (CH₂Cl)₂, 40°C, 1 day, 70%; (d) tetra-*n*-butylammonium fluoride (TBAF), THF, 40°C, 14 hours, 92%; (e) methyl propiolate, 4-methylmorpholine (NMM), CH₂Cl₂, RT, 15 hours, 92%; (f) *n*-Bu₃SnH, 2,2'-azobisisobutylonitrile (AIBN),

toluene, 85°C, 2.5 hours; (g) DIBAL, CH₂Cl₂, −80°C, 1 hour; (h) Ph₃PCH₃Br, sodium hexamethyldisilazide (NaHMDS), THF, 0°C, 1 hour, 61% (three steps); (i) TMSBr, CH₂Cl₂, −60° to −20°C, 1.5 hours, 93%; (j) SO₃·Pyridine, Et₃N, DMSO, CH₂Cl₂, RT, 3 hours; (k) Ph₃PCH₃Br, NaHMDS, THF, 0°C, 1 hour; (l) **27** (20 mol%), CH₂Cl₂, 40°C, 8 hours, 60% (three steps); (m) LiDBB, THF, −80°C to −50°C, 1 hour; (n) Na, NH₃, THF, EtOH, −90°C, 10 min; HPLC purification (Asahipak ODP-50, 75% MeCN-H₂O), 7%; (o) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), CH₂Cl₂-H₂O (10:1), RT, 80%: Cy, cyclohexyl.