Total Synthesis of Ciguatoxin CTX3C, a Causative Toxin of Ciguatera Seafood Poisoning

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Ciguatera is a global disease caused by the consumption of certain warm-water fish (ciguateric fish) that have accumulated orally effective levels of sodium channel activator toxins (ciguatoxins) through the marine food chain.

The most prominent symptom is a sensory disorder, which effects temperature control; specifically, touching cold water arouses a pain similar to that of an electric shock. Other typical symptoms include diarrhea, vomiting, muscle pain, and itching; however, in severe cases, paralysis, coma, and death may occur. Takes 10 min to 24 hrs to develop.
Ciguatoxins

Pacific, Caribbean and Indian are different

**CTX3C**: R1=H, R2=H, n=1
**51-hydroxy CTX3C**: R1=H, R2=OH, n=1
**ciguatoxin CTX**: R1=R2=OH, n=0
**CTX4B**: R1=R2=H, n=0

1. **CTX** isolated in 1980 (1.3mg from 1,100 kg of moray eels)
   Mw=1,111, over 3 nm in length
   Characterized by FAB, NMR (1989) (on 0.35mg)
   Absolute structure by CD (1997)
3. Many more found by FAB MS
4. 2-40 times as toxic as tetrodotoxins. The most potent Na-channel toxins (persistent activation of the channels)

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**Gambierdiscus toxicus** (environmental/genetic) → Benthic blooms → Herbivores (fish/invertebrates) → oxidations, biotransformations → Carnivores (fish) → Human illness (≥ 0.1 ppb CTX in flesh)

Lewis, R. J. *Toxicon*, 2001, 39, 97
Scheuer, P. J. *Tetrahedron*, 1994, 50, 3
www.aqua.org
Ciguatoxin CTX3C

C\textsubscript{57}H\textsubscript{82}O\textsubscript{16}
Mol. Wt.: 1023.2514

1. 12 Oxygens and 52 Carbons in a polycyclic macromolecule
2. 30 stereocenters, 2 tetrasubstituted
3. 12 trans-fused rings from 6 to 9-membered
4. the 13\textsuperscript{th} 5-membered ring forms a spiro-system

Strategy:
1. Convergent synthesis of fragments, followed by coupling
   (three different coupling protocols were developed)
2. All the medium –sized rings (except for G and K) form via RCM
The Retro Synthesis
Left Wing. Starting Materials

Kishi, Y. et al JACS, 1982, 104, 4976

The AB fragment is ready byproduct Na cleaved

1st gen (1%) 
DCM, rt 97% 

1. I₂, DCM  
2. Zn, AcOH Et₂O/MeOH  70% 

BnBr, NaH  
THF-DMF, 0C→rt  84% 

1. I₂, DCM  
2. Zn, AcOH Et₂O/MeOH  70% 

Na, NH₃  
THF/EtOH, -78C  

p-MeOC₆H₄CH(OMe)₂  
TsOH, DMF, rt  59% 

BnO 4 O Bn
O
OBn
H H
OBn
C₃H₅Br, KH  
THF, 0C  
83%  

DIBALH,DCM  
-78 to -20C  82% 

I₂, PPh₃, Im  
PhMe  82% 

Synthesis of the E fragment

1. NaIO₄, MeOH
2. Ph₃PMeBr, KOt-Bu, THF

LDA, THF, -78°C

1st gen, 7%

DCM, +40°C, 2 days

Still E. Converging the isomers

1. TBSCI, Im, DMF (93%)
2. DIBALH (-78 to -50°C)
3. Ph₃PMeBr, KOt-Bu, THF
53% (two steps)

1. TBAF, THF, 99%
2. BrCH₂CO₂t-Bu
NaH, THF/DMF, 75%

Still E. Converging the isomers

Still E. Converging the isomers

1. TBAF, THF, 99%
2. BrCH$_2$CO$_2$t-Bu
NaH, THF/DMF, 75%

1. TBSCI, Im, DMF (93%)
2. DIBALH (-78 to -50°C)
3. Ph$_3$PMeBr, KOt-Bu, THF
53% (two steps)

1. LAH (95%)
2. TBDPSCI, TEA, DMAP, DCM, 87%
3. Dess-Martin (88%)

1. LAH (95%)
2. TBDPSCI, TEA, DMAP, DCM, 85%
3. Dess-Martin (83%)

The E fragment is ready. Let’s couple it to the AB

Synthesis of ABCDE fragment

Chiral auxiliaries can be used!

Synthesis of ABCDE fragment

1. PPTS, MeOH, 83%
2. TIPDSCI₂, Py, 92%
3. DDQ, DCM, 76%

R/S=6/1
Unstable to silica esp. on scale-up

R/S=1.8/1

1. RMgBr, 78%
2. CH(OMe)₃, 86%
3. Et₃SiH
BF₃/Et₂O, 87%

too bulky.
RCM 0%
not touched

1st gen. 280%

From C=>D to D=>C

From C=>D to D=>C

1. DDQ, DCM, 94%
2. CSA, HC(OMe)₃
   DCM, 64%
3. Et₃SiH, BF₃/Et₂O
   DCM, -78 to -30°C
   98%

From 18a to 22 total yield is 11%
From 18a to 27 total yield is 25%

Which fragment is next? HI!
The friendly fragment.

The friendly fragment.

Next fragment: LM.

Next fragment: LM.

\[
\text{BnO} \overset{\text{O}}{\underset{\text{47}}{\text{\longrightarrow}}} \quad \text{LDA, TMSCl} \quad \text{THF/HMPA} \quad -80^\circ \text{C to rt then CH}_2\text{N}_2 \quad 88\% \\
\]

\[
\text{BnO} \overset{\text{O}}{\underset{\text{47}}{\text{\longrightarrow}}} \quad \text{BnO} \overset{\text{O}}{\underset{\text{48}}{\text{\longrightarrow}}} \quad \text{BnO} \overset{\text{O}}{\underset{\text{49}}{\text{\longrightarrow}}} \quad \text{MeO} \overset{\text{Me}}{\underset{\text{50}}{\text{\longrightarrow}}} \\
\]

\[
\text{I}_2, \text{MeCN} \quad \text{rt} \quad 62\% \text{ of 51 sep. by LC} \\
\]

\[
\text{NaOH, aq EtOH} \quad \text{then AcOH} \quad 60^\circ \text{C, } 1 \text{h} \\
\]

\[
\text{H}^+ \\
\]

\[
\text{L is formed. Let’s attach M to it} \\
\]

Attaching M to L.

1. MOMCl, DIPEA, DCE, 50°C, 95%
2. AllylMgBr, THF, -70°C

1. (Sia)$_2$BH, THF, 0°C
2. NaHCO$_3$, H$_2$O$_2$
3. CSA, DCE, rt, 75%

1. H$_2$, Pd(OH)$_2$/C, EtOAc, rt, 100%
2. Swern, Me$_2$Br, PhMe, -80 to -70°C, 85%

Then NAPBr, NaH, DMF, THF, rt, 85%

1. OsO$_4$, NMO, tBuOH, water, rt, then NaIO$_4$
2. NaClO$_2$, NaH$_2$PO$_4$, 2-Me-2-butene, tBuOH, water, rt, 99%

Click me to see how the 51-hydroxy CTX3C is made

The pieces are almost ready! **JK** is next.
Initial attempts and some insights.

Scheme 9  Attempted cyclization of the J-ring via RCM reaction

Scheme 10  Mechanistic considerations of the J-ring cyclization
1. BH$_3$/Me$_2$S, THF, 0°C to rt
2. NaOH, H$_2$O$_2$, 76%, $\alpha/\beta$=3/1 (undesired) separable
3. Dess-Martin, DCM, rt, 90 and 97%

JK continued

1. HF/Py, THF, rt, 76%
2. von Doering
3. AllylSnBu3, MgBr2/Et2O
   MS4A, PhMe, -78 to rt
   62% + 21% of the epimer
4. NAPBr, TBAI, NaH
   THF/DMF, rt, 91%

66 oxoniumm to 66

All what is left to do is to couple the ABCDE and HIJKLJM fragments

Coupling and FG making
Model studies

Model studies. The key step #1.

Six-membered acetal is too stable! How about the seven-membered one?

Model studies. The key step #1.

After a lot of optimizations

Tight ion pair $S_{n1}$-like process

The model study continued. Approaching the FG.

1. PMBCl, DIPEA, TBABr, DCE, 40°C
2. TBAF, THF, 35°C
3. HCCO$_2$Me
   N-Me-morpholine, DCM 97%

nBu$_3$SnH, AlBN
PhMe, 80°C

extended s-trans

1. DIBAL, DCM
   -100°C to -80°C
2. NaHMDS
   Ph$_3$PMeBr
   THF, 0°C 86%

1. TMSBr, DCM
   -60 to -20°C, 78%
2. SO$_3$/Py, DMSO
   TEA, DCM, 0°C
3. Wittig, 75%

Finishing the model study. Making the FG.

The H-NMR is severely broadened at rt, like in the natural product. At -20°C in Py - mostly one conformer.

Synthesis of TrisNAP CTX3C. Why NAP?

Scheme 1. Model Study for Acetal Cleavage Reaction

Alternatives:
MPM- too acid labile

For hydrogenolysis
NAP is flat, bound to Pt better than Bn etc.

CAN: NAP cleaved first, then Bn
DDQ: PMB, then NAP

Synthesis of TrisNAP CTX3C.
Synthesis of TrisNAP CTX3C.

1. EVE, PPTS, DCM, rt, 73%
2. TBAF, THF, 40C,
3. HCCCO2Me, NMM, DCM
   DCM, rt, 89%

1. AIBN, nBu3SnH, PhMe, 85C
2. DiBALH, DCM, -80C
3. Ph3PMeBr, NaHMDS, THF, 0C
4. CSA, MeOH/THF, rt
   41%

1. SO3/Py, DMSO, TEA, DCM, rt
2. Ph3PMeBr, NaHMDS, THF, 0C, 100%
3. 1st gen, 30%, DCM, 40C, 90%

Total synthesis of CTX3C.

DDQ (600mol%), DCE/water=20/1, rt, 63%

1mg in one batch
0.7 mg have been isolated from nature

TLC, HPLC, HNMR, CNMR
CD, MS supported

The absolute structure was supported

Summary

Longest linear sequence: 47 steps (D-2-deoxyribose to CTX3C)

11 steps 5.1%
Summary

alkylation

9 steps
20%
coupling (+CD)

11-23 steps
10%
converging isomers

9 steps
19%
coupling (+JK)

23 steps
2%
18 steps
20%
carbonyl olefination

esterification

9 steps
20%

D-glucose

D-deoxyribose

benzyl-(S)-glycidol
45

D-glucose

D-deoxyribose

benzyl-(S)-glycidol
45
Summary from a different point of view.

1. Two major improvements:
   
   1.1. The right wing made more convergent (compared to the 2001 synthesis)
   1.2. The NAP-protective group used

2. Supply sufficient for further biological studies (1mg in one batch vs. 0.7mg from nature)

3. New synthetic methodologies:
   
   3.1. RCM to build the medium sized rings
   3.2. Three coupling protocols to assemble the fragments
   3.3. New(?) protective group strategy

4. An immunoassay to detect CTX3C has been developed already as a result

5. A lot of protection/deprotection

6. Limit of the art?