Asymmetric synthesis of batrachotoxin: Enantiomeric toxins show functional divergence against Na\textsubscript{v}

(-)-Batrachotoxin (BTX)
LD\textsubscript{50} = 2 µg/kg

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*Science, 2016, 354, 865-869*
Isolated in 1963 in the Chocó jungle region of Colombia from the skin extracts of the Colombian poison dart frog.

- *Phyllobates terribilis* (~1-2 mg BTX/frog), *Phylllobates aurotaenia* and *Phyllobates bicolor* (~10 fold less BTX)

Subsequently identified in birds (genus *Pitohui* and *Ifrita*) and beetles (genus *Choresine*); ~1.8 µg of (~)-BTX per beetle.

Levels of BTX tend to be reduced when frogs are maintained in captivity, possessing on average ~ 35% of the BTX contained in freshly captured frogs. In addition BTX was not detected in *phyllobates* frogs bred in captivity, suggesting that wild frogs possibly sequester the toxin from a dietary source.
Extremely potent cardio- and neurotoxin

Traditionally used by Native Chocó Indians who poison the tip of their arrows and blow-darts with the skin secretion of the frogs, which they call “kokoi.”

Selective and irreversible activation of voltage-gated sodium channels (Na⁺) in nerve and muscle cell membranes. Locking the ion channel in an ‘open’ state and causing membrane depolarization.

This ultimately results in the inability of the muscle and nerve cells to generate and respond to electrical signals ultimately resulting in death through heart failure and/or respiratory failure.

*Journal of Natural Products, 2010, 73, 299*
The structure and pharmacology of BTX was determined by John Daly’s group (NIH) between 1962-1973

- involved 7 more expeditions to the Choco jungle region and the collection/sacrifice of >10 000 frogs

*Scientists (from l) Bernhard Witkop, John Daly and Takashi Tokuyama study the structure of batrachotoxins*
Isolation/ structural determination

- The crude alkaloid extract contained 3 major constituents:

\[
\text{batrachotoxin (BTX)} \quad \text{LD}_{50} = 2 \, \mu g/kg \\
\text{homobatrachotoxin (hBTX)} \quad \text{LD}_{50} = 3 \, \mu g/kg \\
\text{batrachotoxinin A (BTX A)} \quad \text{LD}_{50} = 1000 \, \mu g/kg
\]

*most potent non-peptidic naturally occurring toxin currently known*

- Structural elucidation was challenging due to the minute quantities of alkaloid obtained e.g. in the early isolation/ purification process ~5000 frog skins yielded ~11 mg of BTX. Structure eventually solved by mass spectrometry & NMR analysis of BTX and X-ray analysis of the 20-\(p\)-bromobenzoate derivative of BTX A.

- Pregnane-type steroidal core functionalized with a homomorpholine ring, tertiary hemiketal & pyrrole ester

\[\text{Science, 1971, 172, 995-1002}\]
The structure was confirmed by semi-synthesis of BTX and analysis by TLC, MS, NMR and toxicity studies on the synthetic material.
Synthetic Efforts Towards Batrachotoxin

- **Wehrli group (1972)**
  - Total synthesis of 20S-batrachotoxinin A, 43 linear steps

- **Kishi group (1998)**
  - Total synth. of (±)-batrachotoxinin A
  - 48 linear steps
  - Formal synth. of (±)-batrachotoxinin

- Several approaches to the A/B/C framework by Kean, Magnus, Parsons, Deslongchamps, Schow and Lacrouts

- Approach to the C/D/E ring by Du Bois

**Synthetic studies are of continued importance:**

- Use of (−)-BTX as a Na\textsubscript{v} activator has led to depletion in the world supply from >1 g to 170 mg

- The *Phyllobates* species have been placed on the endangered species list, thus, collection of (−)-BTX from the natural source is restricted.

- The biosynthesis of (−)-BTX is unknown.
Retrosynthesis Analysis of (−)-Batrachotoxin

(-)-Batrachotoxin (BTX)
LD<sub>50</sub> = 2 µg/kg

C=O 1,2-addition

(-)-Batrachotoxinin (BTX) A (BTX-A)
LD<sub>50</sub> = >1000 µg/kg

regioselective esterification

tandem radical cyclization

reductive amination/ring closure

Steph McCabe@ Wipf Group
Asymmetric Total Synthesis of (−)-Batrachotoxin

1. t-BuLi, THF, -90 °C, then
2. 65%
3. K$_2$CO$_3$, MeOH, 94%

*(LiBr generated *in situ* during t-BuLi transmetallation was critical for obtaining yields >30% in the 1,2-addition)*

*(α-deprotonation was competitive with 1,2-addition (D$_2$O quench)*)

radical cyclization precursor 8.8 g scale
Key Radical Cyclization

Bu₃SnH, O₂, Et₃B, Ph₂O, 150 °C, 75%

1,4-HAT

6-endo-trig

5-exo-dig
Asymmetric Total Synthesis of \((-\)-Batrachotoxin

\[
\text{TBAF, THF} \quad 60 \degree C, \quad 94\%
\]

\[
\text{IBX, } t\text{-BuOH, } 65 \degree C \text{ then } OsO_4 (7 \text{ mol}), NaIO_4 \text{ pyridine/ } H_2O, \quad 57\%
\]

1. MeNH_2, CH_2Cl_2
2. NaB(O_2CCF_3)_3H
   CH_2Cl_2, -78 °C
3. ClCH_2COCl
   2,6-lutidine
   -78 to 0 °C, 52%
Asymmetric Total Synthesis of (-)-Batrachotoxin

1. NaOEt, EtOH
   1:1 THF/C₆H₆, 92%
2. KHMDS, PhNTf₂
   THF, -78 to 0 °C
   94%

1. NaClO₂, NaH₂PO₄
   DMSO/H₂O
2. SOCl₂, pyridine
3. NaN₃, acetone/H₂O
4. AcOH (aq), 1,4-dioxane
   90 °C, 15 h, 57% (4 steps)
5. p-TsOH, 4 A MS
   PMBCH₂OH, 89%

CuCl₂ (3.3 equiv.),
O₂, 1,4-dioxane
73 °C, 85%
Asymmetric Total Synthesis of (-)-Batrachotoxin

1. AlH₃, THF, -78 °C to 0 °C, 33%

2. pTsOH, 3:2 acetone/ H₂O, 83%

35 mg scale

2 mg synthesized
24 steps
0.25% overall yield

Steph McCabe@ Wipf Group
Asymmetric Total Synthesis of (−)-Batrachotoxin B & (+)-Batrachotoxin B

Electrophysiological characterization of synthetic alkaloids against Na$_v$ subtypes showed:

- (−)-BTX & BTX-B act as irreversible agonists of channel function. (−)-BTX-B showed similar potency to the natural product (−)-BTX

- The unnatural enantiomer (+)-BTX and derivative ent-BTX-B act as reversible antagonists of channel function by blocking the sodium channel.
- *ent*-BTX-B was tested against five rNa\textsubscript{v}1.4 single-point mutants that were previously shown to destabilize BTX binding.

- Mutation of N434, L1280, F1579, and N1584 to lysine resulted in a ~3- to 30-fold decrease in current block by 5 mM *ent*-BTX-B.

- However, against F1236K, *ent*-BTX-B retained significant activity (~34% current inhibition).

- Indicates an over-lapping, but nonidentical, binding region for *ent*-BTX-B and BTX-B within the inner pore cavity.
Conclusions

- First asymmetric synthesis of steroidal neurotoxin \((-\text{batrachotoxin}\) and it’s unnatural enantiomer \(+(\text{-batrachotoxin})\)
- Completed in 24 steps it is a significant improvement in terms of efficiency compared to prior racemic syntheses (>40 steps)
- Demonstrated that the unnatural enantiomer \(+(\text{-batrachotoxin})\) has a different mechanism of action acting as a reversible antagonist of \(\text{Na}_v\) ion channels.
- Synthesis and biological evaluation of derivative \(\text{BTX-B} \& \text{ent-BTX-B}\) which possess enhanced chemical stability and similar activity and potency to the natural/ unnatural enantiomers.

\((-\text{-Batrachotoxin (BTX)}\)

\(\text{LD}_{50} = 2 \mu g/kg\)