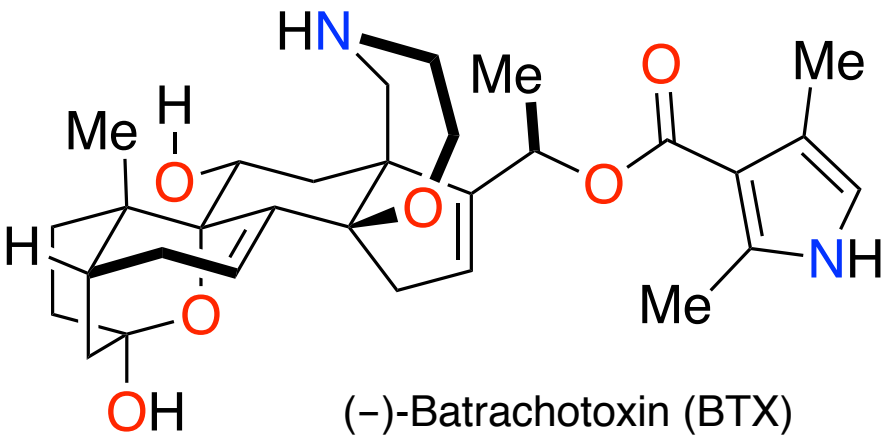
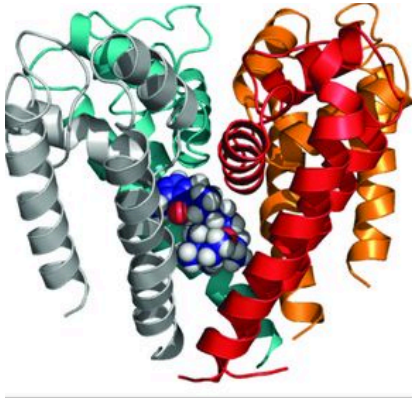
Asymmetric synthesis of batrachotoxin: Enantiomeric toxins show functional divergence against Na_V



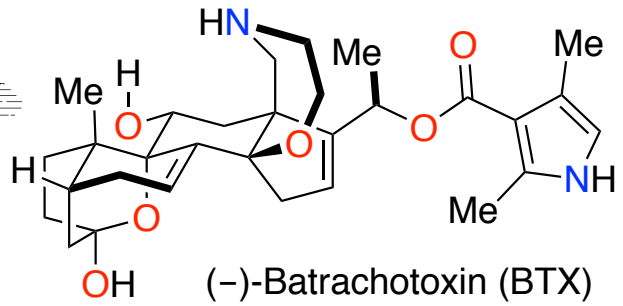
(−)-Batrachotoxin (BTX)
LD_{50} = 2 μg/kg



Matthew M. Logan, Tatsuya Toma, Rhiannon Thomas-Tran and J. Du Bois

Science, **2016**, *354*, 865-869

Natural Source



(−)-Batrachotoxin (BTX)

the golden poison dart frog,
Phyllobates terribilis

- 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), *Phyllobates aurotaenia* & *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.

Experientia, 1963, 19, 329
Science, 1980, 208, 1383

Biological Activity/ Uses

- 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 ($Na_v s$) 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

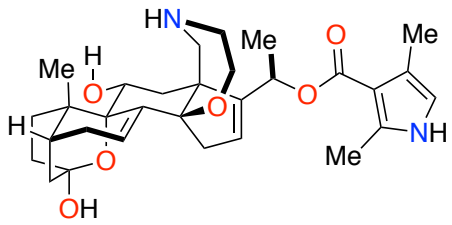
The structure and pharmacology of BTX was determined by John Daly's group (NIH) between 1962-1973

- 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

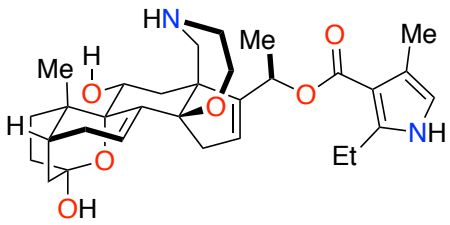
- The crude alkaloid extract contained 3 major constituents:



batrachotoxin
(BTX)

LD_{50} = 2 μg/kg

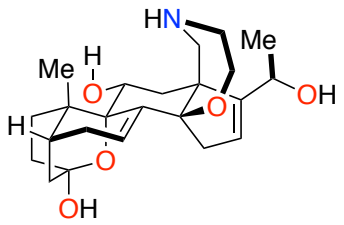
+



homobatrachotoxin
(hBTX)

LD_{50} = 3 μg/kg

+



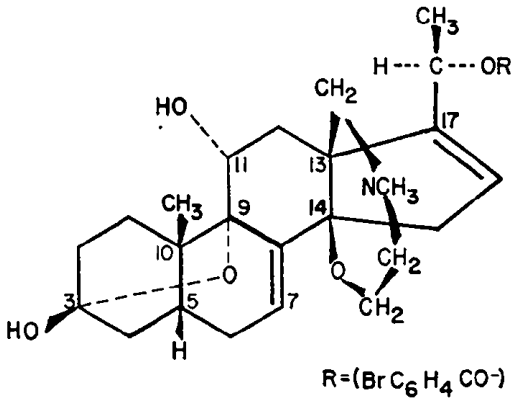
batrachotoxinin A
(BTX A)

LD_{50} = >1000 μg/kg

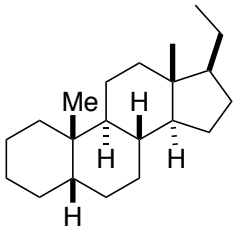
* most potent non-peptidic naturally occurring toxin currently known

- 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



R=(Br C_6H_4 CO-)

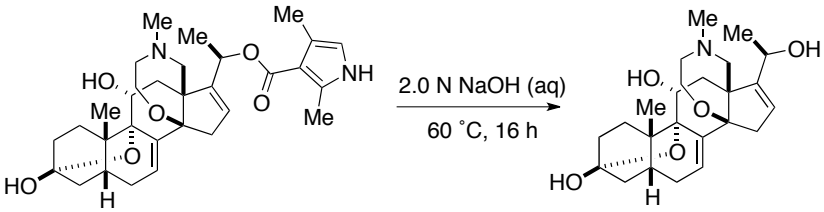


pregnane

Science, **1971**, *172*, 995-1002

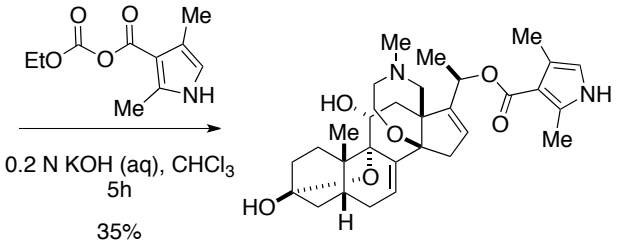
Structural Determination

- The structure was confirmed by semi-synthesis of BTX and analysis by TLC, MS, NMR and toxicity studies on the synthetic material.



batrachotoxin

batrachotoxinin A



batrachotoxin

Synthetic BTX
LD_{50} = 2 µg/kg

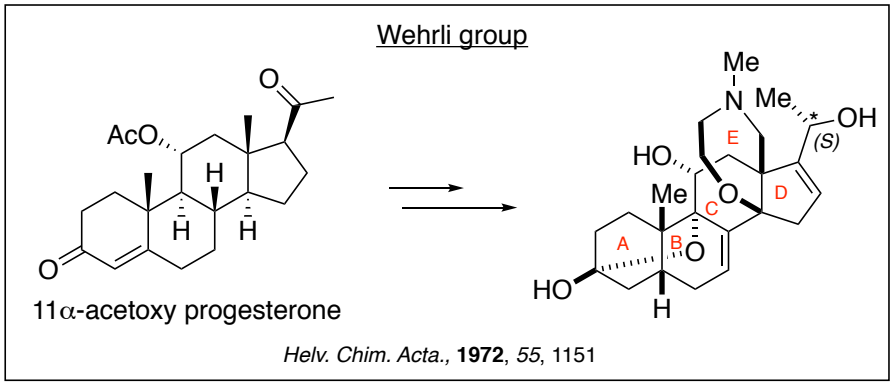
Figure 2. J.Daly, examining bioactivity of synthetic Batrachotoxin, at loft, Bldg.4, Aug.22 (1968).

Synthetic Efforts Towards Batrachotoxin

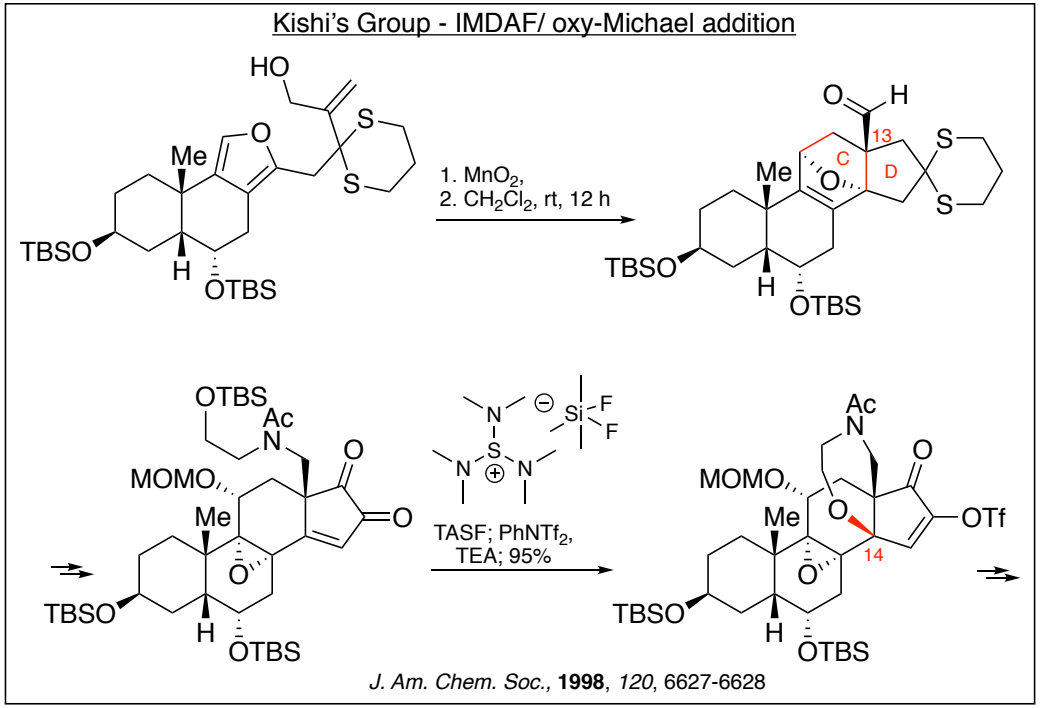
- Wehrli group (1972)
  Total synthesis of 20*S*-batrachotoxinin A,
  43 linear steps
- Kishi group (1998)
  Total synth. of (±)-batrachotoxinin A
  48 linear steps
  Formal synth. of (±)-batrachotoxinin
- Several approaches to the *A/B/C* framework by Keana, Magnus, Parsons, Deslongchamps, Schow and Lacrouts
- Approach to the *C/D/E* ring by Du Bois

Synthetic studies are of continued importance:

- Use of (−)-BTX as a Na_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.

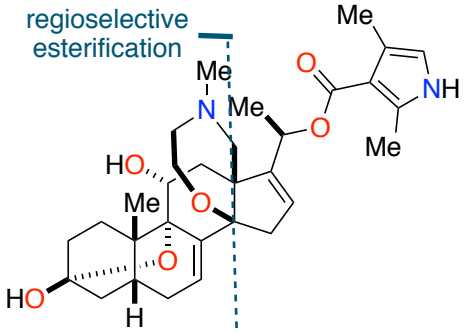


Helv. Chim. Acta., **1972**, *55*, 1151

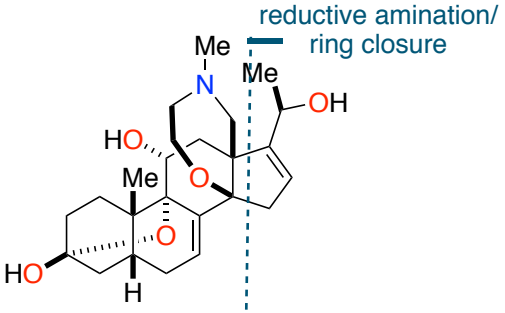


J. Am. Chem. Soc., **1998**, *120*, 6627-6628

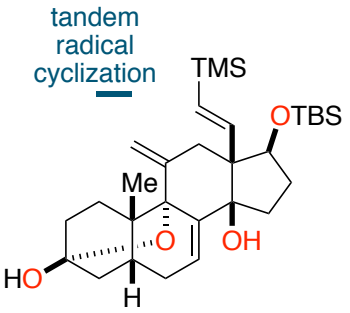
Retrosynthesis Analysis of (−)-Batrachotoxin

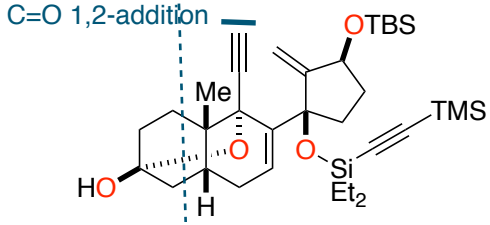


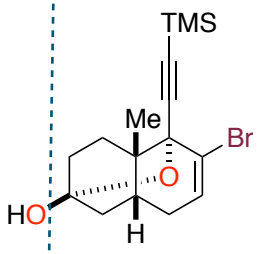
(−)-Batrachotoxin (BTX)
LD_{50} = 2 μg/kg



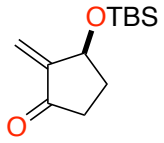
(−)-Batrachotoxinin (BTX) A (BTX-A)
LD_{50} = >1000 μg/kg



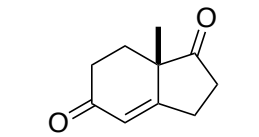




+

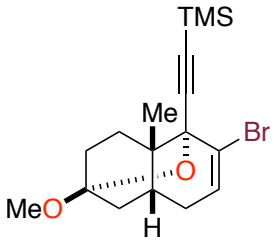


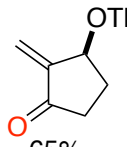
Asymmetric Total Synthesis of (−)-Batrachotoxin



(*S*)-(+)-Hajos-Parrish ketone

literature protocol



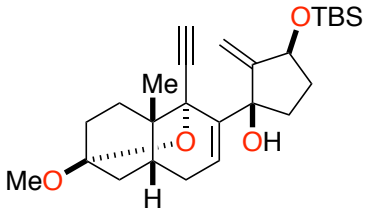
1. *t*-BuLi, THF, -90 °C, then
2. 

65%

3. K_2CO_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_2O quench)

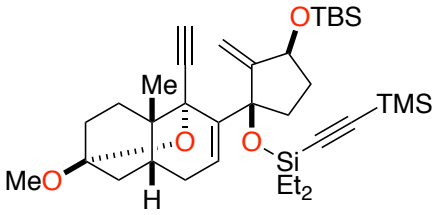


TMS

$SiEt_2Cl$

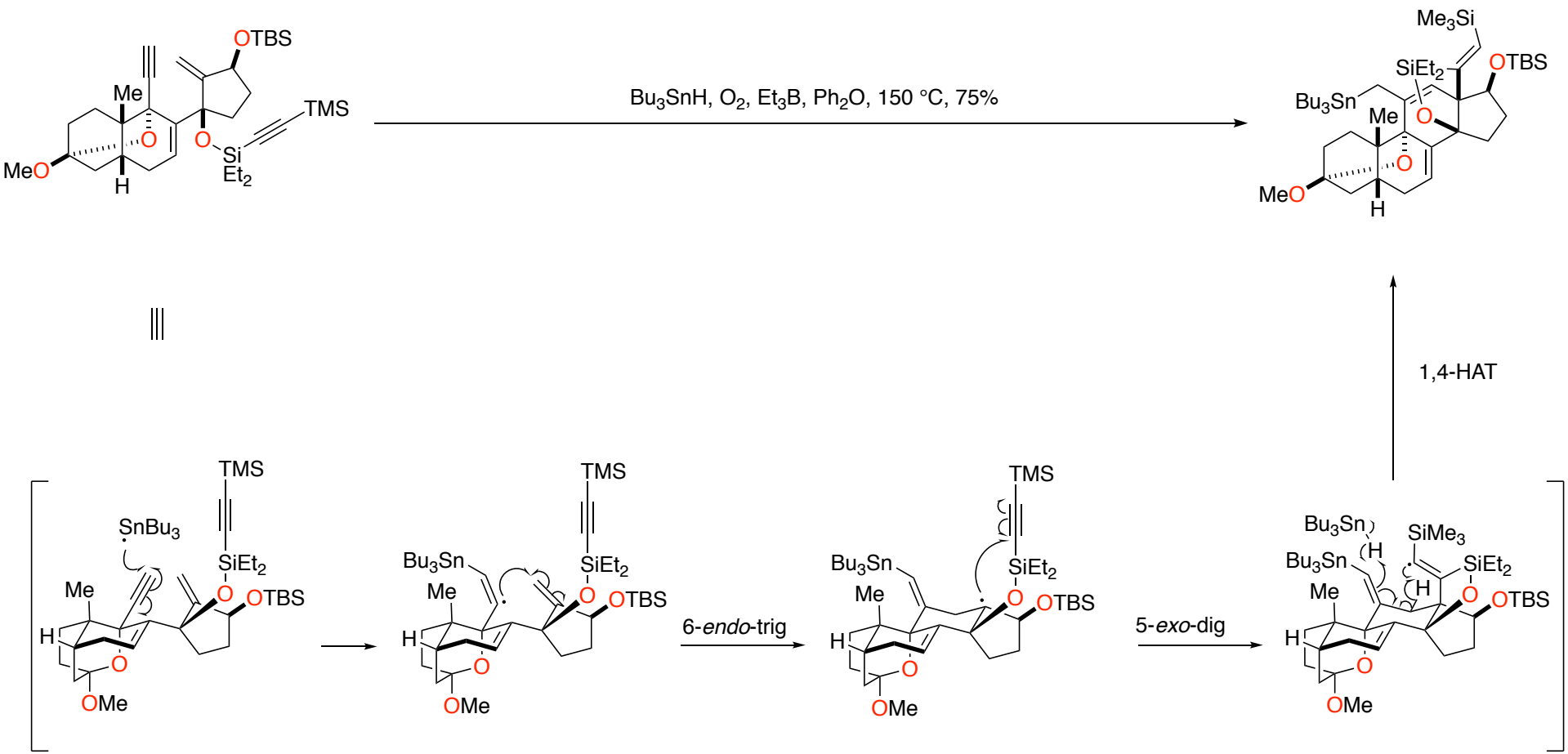
imidazole, CH_2Cl_2

93%

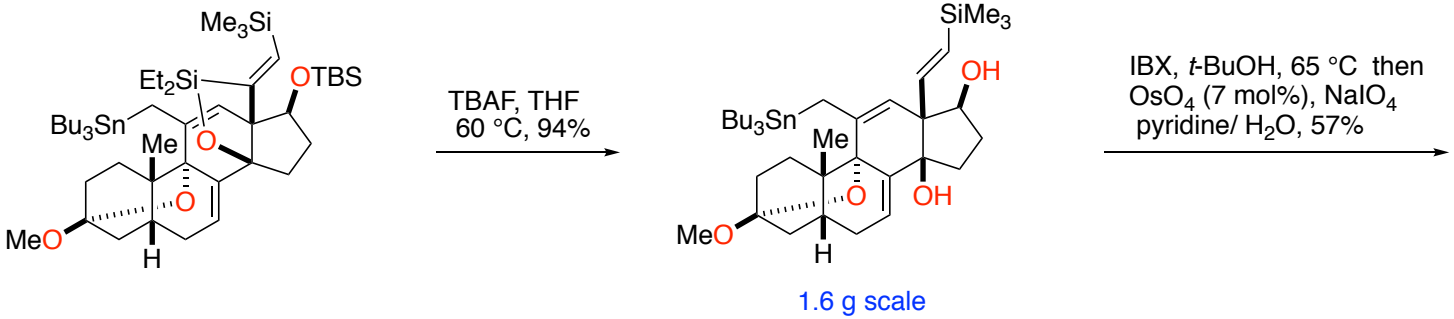


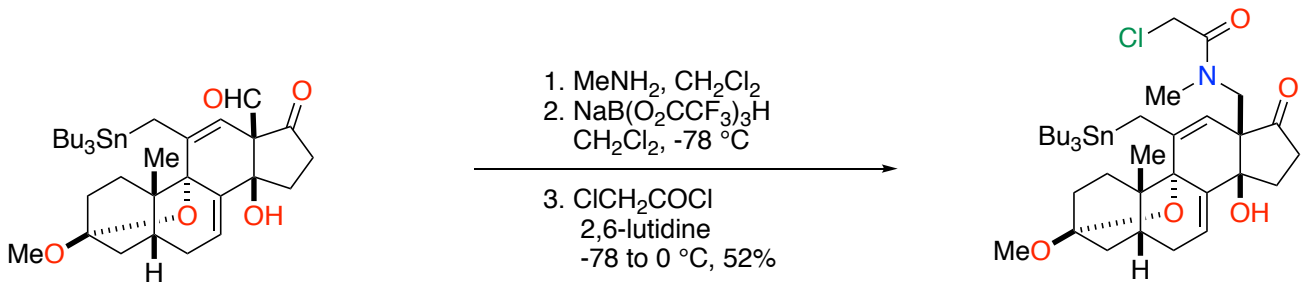
radical cyclization precursor
8.8 g scale

Key Radical Cyclization

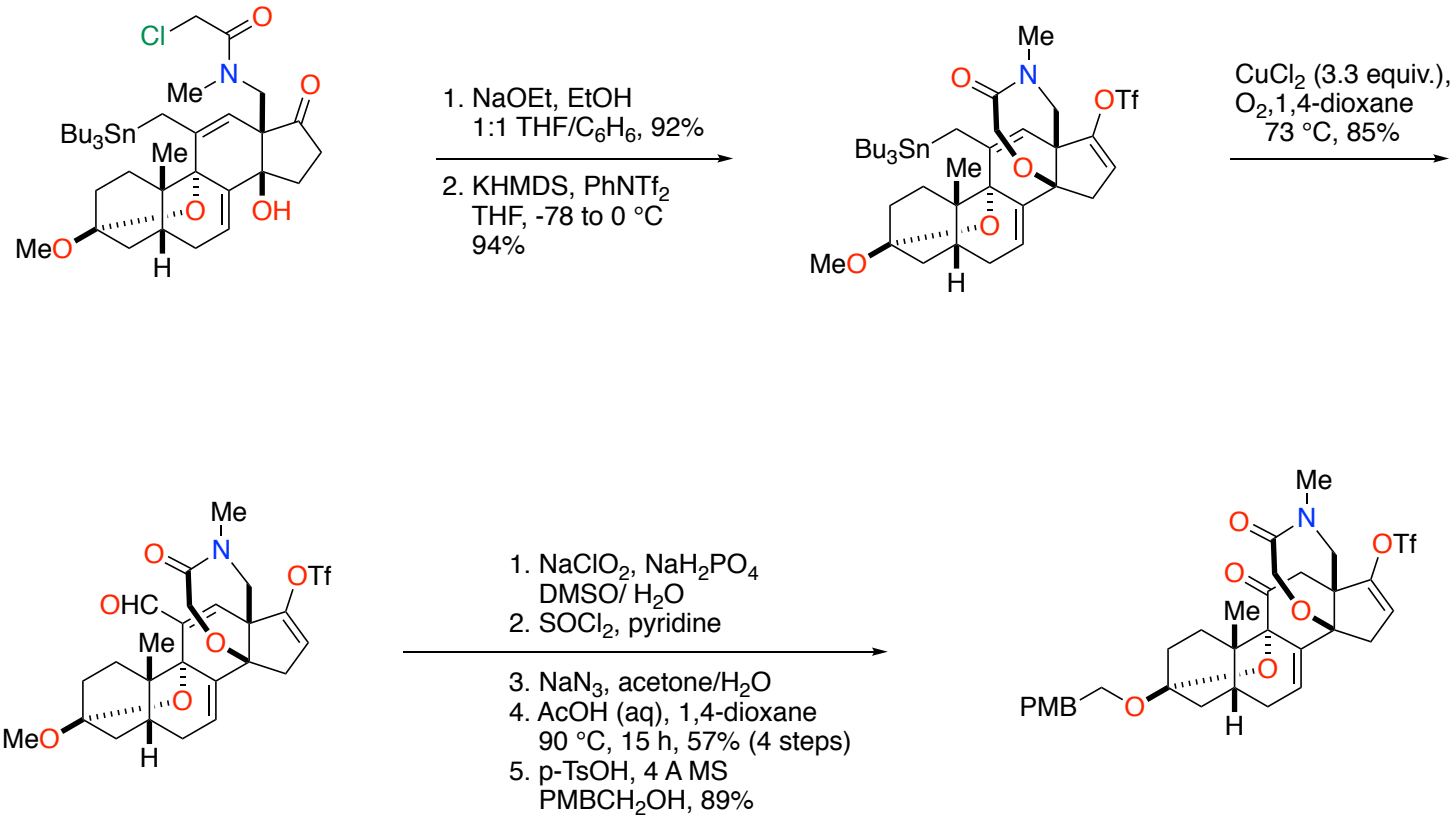


Asymmetric Total Synthesis of (−)-Batrachotoxin

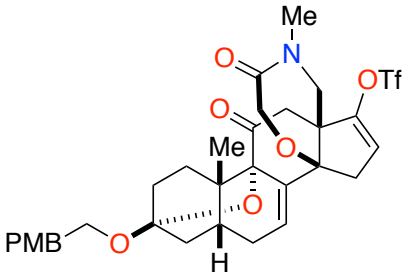




Asymmetric Total Synthesis of (−)-Batrachotoxin

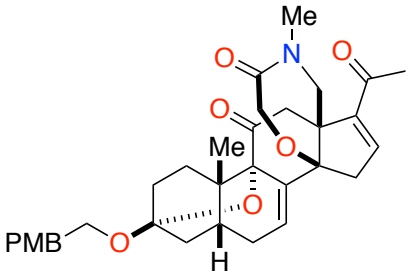


Asymmetric Total Synthesis of (−)-Batrachotoxin

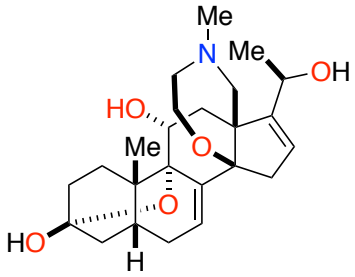


35 mg scale

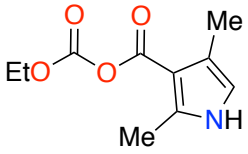
LiCl, CuCl, $Pd(PPh_3)_4$
tributyl(1-ethoxyvinyl)tin
THF, 60 °C, then
1M oxalic acid, 0 °C, 77%



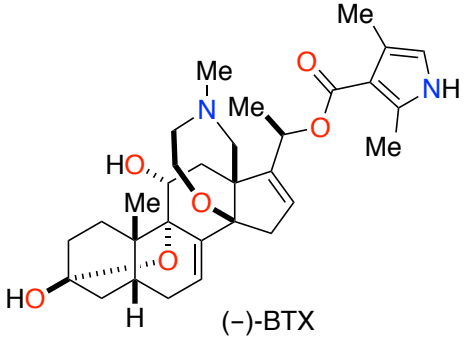
1. AlH_3, THF, -78 °C to 0 °C, 33%
2. pTsOH, 3:2 acetone/ H_2O, 83%



(−)-BTX A



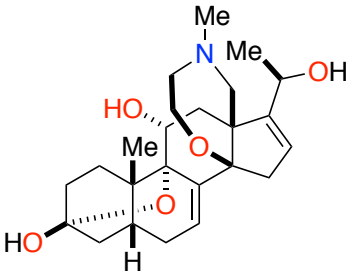
NEt_3, benzene, 45 °C, 79%



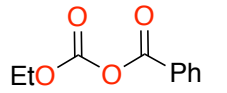
(−)-BTX

2 mg synthesized
24 steps
0.25% overall yield

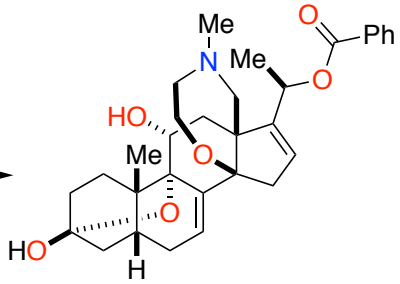
Asymmetric Total Synthesis of (−)-Batrachotoxin B & (+)-Batrachotoxin B



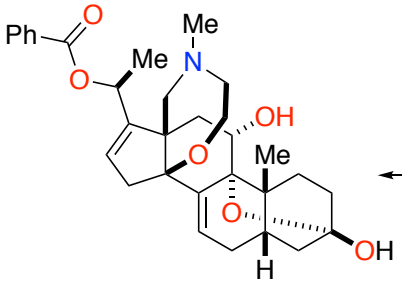
(−)-BTX A



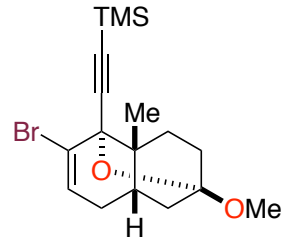
NEt_3, benzene
45 °C, 70%



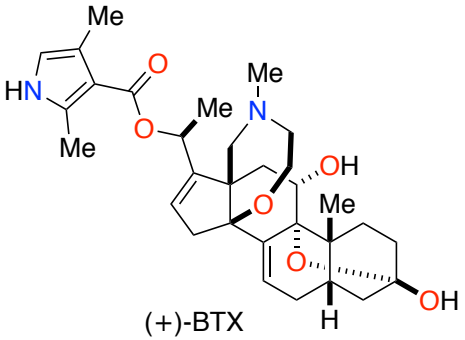
BTX B



ent-BTX B



ent-starting material



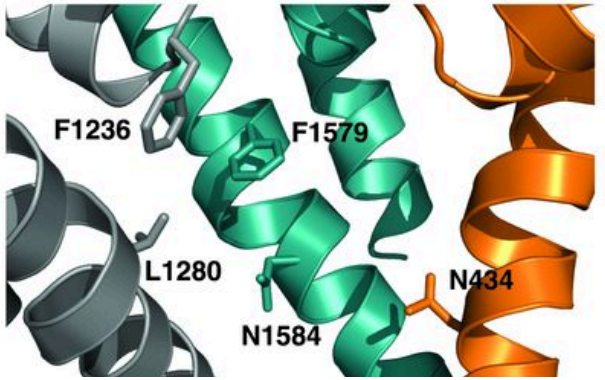
(+)-BTX

Electrophysiological characterization of synthetic alkaloids against Na_v subtypes showed:

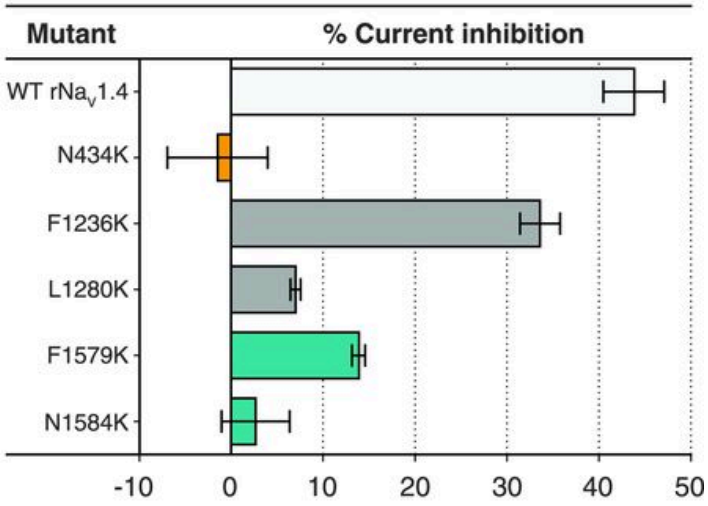
- (−)-BTX & BTX-B act as irreversible agonists of channel function. (−)-BTX-B showed similar potency to the natural product (−)-BTX
- The unnatural enantiomer (+)-BTX and derivative *ent*-BTX-B act as reversible antagonists of channel function by blocking the sodium channel.

Biological Activity of Synthetic Materials

- *ent*-BTX-B was tested against five rNa$_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

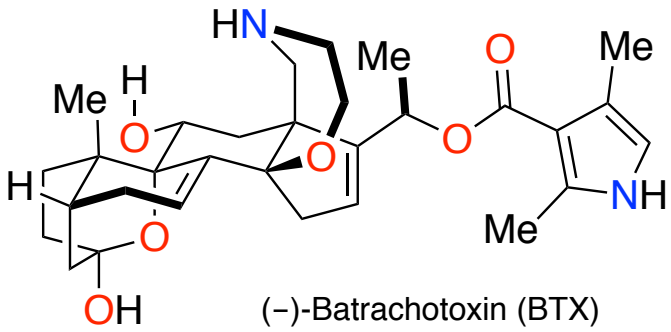


homology model highlighting residues that have previously been shown to abolish (–)-BTX activity



Percent current inhibition of rNa$_V$1.4 mutants by 5 μM *ent*-BTX-B

Conculsions



(−)-Batrachotoxin (BTX)
LD_{50} = 2 μg/kg

- First asymmetric synthesis of steroidal neurotoxin (-)-batrachotoxin and it's unnatural enantiomer (+)-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 (+)-batrachotoxin has a different mechanism of action acting as a reversible antagonist of Na_v ion channels.
- Synthesis and biological evaluation of derivative BTX-B & *ent*-BTX-B which possess enhanced chemical stability and similar activity and potency to the natural/ unnatural enantiomers.