The Total Synthesis of Tetrodotoxin

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Tetrodotoxin: Background

Certain varieties of puffer fish, especially the *tora fugu*, or tiger puffer (*S. rubripes*) and the closely related *ma fugu*, or common puffer (*S. porphyreus*), are highly prized as comestibles in Japan. The indulgence of the taste is fraught with some peril, since the livers and ovaries of the fish contain a powerful poison. The presence of this poison has been known through its effects since antiquity, but its labile nature and its extremely low concentration in its natural milieu made the isolation of the toxic principle extraordinarily difficult. Yokoo first succeeded in isolating the crystalline poisonous principle-now known as tetrodotoxin-on fourteen years ago (1950), and shortly thereafter Tsuda and Kawamura independently achieved its isolation in its pure state.

R. B. Woodward

Tetrodotoxin: Physical Characteristics

4,9-anhydo-Tetrodotoxin

- White crystalline solid
- Darkens above 220 °C, without melting
- Insoluble in all solvents except acids
- Weak base (pKa = 8.7)
- Exhibits no UV spectrum
- One of the most toxic compounds among poisons with low MW.
- Toxicity due to the blockage of sodium ion influx through sodium channel proteins.
Isolation Procedure

Ovaries of *Spheroides rubripes*  
(100 kg)

- Water
  - Residue
  - Extract
    - Boiled and filtered
      - Precipitate
      - Filtrate
        - (200 l.; ca. 10 mg/l.)
          - Amberlite IRC-50 (ammonium type)
            - 10% acetic acid
              - Active fraction
                - Non-active fraction
                  - (10 l.; 100–200 mg/l.)
                    - pH 8–9 with ammonia aq.
                      - Active charcoal (Norit A)
                        - Supernatant
                          - Charcoal
                            - dil. acetic acid
                              - Residue
                                - Eluates
                                  - (1 l.; 1–2 g/l.)
                                    - ammonia aq.
                                      - Crystalline ppt.
                                        - (1–2 g)

Fig. 1. Diagram of the isolation procedure.
General Retrosynthetic Analysis

Key Synthetic Challenges:

- Selection of acid labile protecting groups due to base senstivity of TTX
- Construction of the tetrasubstituted stereocenters C6 and C8a
- Introduction of the C8a amine
- Preventing epimerization at C9 and β-elimination of the hydroxyl at C5
- Determination of stereochemistry using coupling constants of vicinal hydrogens
Synthesis of the Cyclohexane Skeleton: Diels-Alder and Beckmann Rearrangement

Synthesis of the Cyclohexane Skeleton: Installation of the C8 Stereocenter and Introduction of the C11 Alcohol

1. Al[OCH(CH₃)₂]₃
2. Ac₂O
(95% in 2 steps)

1) SeO₂, 180 °C
2) NaBH₄, MeOH
(100% in 2 steps)

m-CPBA
90 °C
(95%)

1. CH₃C(OEt)₃, EtOH
2. Ac₂O, pyridine

1. Ac₂O, pyridine
2. CF₃CO₂H, H₂O, 70 °C
3. Ac₂O, pyridine
(80% in 3 steps)

C(8)H and C(7)H dihedral angle 90
J = 0 Hz

C(8)H and C(7)H dihedral angle 30
J = 6 Hz

Synthesis of the Cyclohexane Skeleton: Introduction of the C9 Hydroxyl

Stereoselective Synthesis of the Lactone Ring

Synthesis of the Guanidine Moiety

1) Et₃O BF₄ → Na₂CO₃ then AcOH/H₂O work-up

290-300 °C → high vacuum

(92%)

120 °C

12 h

150 °C

60 min

(20% 2 steps)

NH₃, MeOH/CH₂Cl₂

(100%)


Determination of C9 configuration

\[ W\text{-coupling} \quad J_{9,4a} = 1 \text{ Hz} \quad J_{9,8} = 0 \text{ Hz} \]
Completion of the Synthesis of DL-Tetrodotoxin

Summary of Kishi’s Total Synthesis

C8a and C4a: diastereoselective Diels-Alder rxn.
C8 and C5: stereospecific, substrate controlled hydride reductions.
C6: stereospecific, substrate controlled epoxidation
C7: carboxylate attack onto epoxide
C9: stereospecific, substrate controlled epoxidation of enol ether

32 Total steps

- Use of an unusual Diels-Alder dienophile containing an oxime.
- High degree of substrate control in creating stereocenters
- Development of a novel procedure to synthesize a guanidine moiety
Isobe’s Retrosynthetic Analysis - Asymmetric

Isobe, M., Ohyabu, T, Nishikawa, T. J. Am Chem. Soc. 2003, 125, 8798
Synthesis of the Cyclohexane Skeleton: Sonogashira Coupling and Claisen Rearrangement

Isobe, M., Ohyabu, T., Nishikawa, T. J. Am Chem. Soc. 2003, 125, 8798
Synthesis of the Cyclohexane Skeleton: Introduction of the C5 and C11 Hydroxyl Groups

Conformation of A and possible Interactions with m-CPBA

Isobe, M., Ohyabu, T, Nishikawa, T. J. Am. Chem. Soc. 2003, 125, 8798
Synthesis of the Cyclohexane Skeleton: Acetylene Hydration and Intramolecular Aldol Condensation

Proof of the Configuration at the C-5 position

Isobe, M., Ohyabu, T, Nishikawa, T. J. Am Chem. Soc. 2003, 125, 8798
Synthesis of the Cyclohexane Skeleton: Exoolefin Synthesis

Proof of the Configuration at the C-8 position

Isobe, M., Ohyabu, T, Nishikawa, T. J. Am Chem. Soc. 2003, 125, 8798
Introduction of the Nitrogen Functionality: Overman Rearrangement

Isobe, M., Ohyabu, T, Nishikawa, T.  J. Am Chem. Soc.  2003, 125, 8798
Introduction of the Nitrogen Functionality: Intramolecular Conjugate Addition

Isobe, M., Ohyabu, T, Nishikawa, T. J. Am Chem. Soc. 2003, 125, 8798
Stereoselective Synthesis of the Lactone Ring

All attempts at hydroxylation of C-9 failed

Stereoselective Synthesis of the Lactone Ring

Conformation of Enol precursor leading to Z-enolate

Introduction of the Guanidine Moiety

reaction temp. must be below 15 oC to prevent epimerization at C-9

Isobe, M., Ohyabu, T, Nishikawa, T. J. Am Chem. Soc. 2003, 125, 8798
Completion of the Total Synthesis

1) 4 M HCl
   THF

2) 4 M HCl dioxane MeOH

15% 4,9-anhydrotetrodotoxin

3) Ac₂O, Et₃N,
   Py.

65% tetrodotoxin

2% TFA-d/D₂O

4,9-anhydro-4-epitetrodotoxin  4-methoxytetrodotoxin

4 : 1 mixture

[α]²⁸°D = +1 (c 0.08, 0.05 N AcOH-H₂O) (+/-2)

Isobe, M., Ohyabu, T, Nishikawa, T. J. Am Chem Soc. 2003, 125, 8798
Summary of Isobe’s Total Synthesis

C4a: Claisen rearrangement
C5: epoxidation of an enol ether and inversion
C6: stereospecific, substrate controlled epoxidation
C7: intramolecular enolate attack on epoxide
C8: Stereospecific, substrate controlled hydride reduction
C8a: intramolecular conjugate addition of a carbamate
C9: stereospecific, substrate controlled hydride reduction

- 25 steps involved in protection group manipulations.
- Required many steps to make Overman rearrangement precursor which was unsuccessful.
Du Bois’ Retrosynthetic Analysis - Asymmetric

Synthesis of the Cyclohexane Skeleton: Aldol Addition and Rh-Catalyzed C-H Insertion

Synthesis of the Cyclohexane Skeleton: Installation of the C8a and C9 Stereocenters and Introduction of the C5 Alcohol

1) H₂ (1200 psi), Rh-C (5 mol%), CF₃CO₂H/MeOH
2) p-TsOH, THF
   MeO Me OMe
   Me
(77% in 2 steps)
3) Me₂NH, THF
   (83%)
(n-Pr₄N)RuO₄ (cat.)
NMO, 4 Å MS
(94%)

Completion of the Cyclohexane Skeleton
And Synthesis of the Lactone Ring

Rh-Catalyzed C-H Insertion Reaction for the Oxidative Conversion of Carbamates to Oxazolidinones

\[ \text{Reaction proceeds with retention of configuration, consistent with nitrene or nitrenoid intermediate.} \]

Rh catalyst is mediating the C-N insertion, a free carbamoylnitrene is not the active oxidant.
Completion of the Total Synthesis:  
Introduction of the Nitrogen and Guanidine Moiety

Hinman, A. and DuBois, J. Am. Chem. Soc. 2003, 125, 11510
Summary of Du Bois’ Total Synthesis

C6 and C7: chiral starting material
  C8: Diastereoselective aldol addition rxn.
C8a and C9: Stereoselective, substrate controlled hydrogenation
  C4a: Stereoselective, substrate controlled protonation after conjugate addn.
  C5: Stereoselective, substrate controlled hydride reduction

• Utilized C-H functionalization for key steps, allowing for a short and concise synthesis
• Very few protecting group manipulation steps (5)