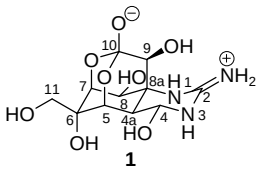
# RECENT APPROACHES TOWARD THE TOTAL SYNTHESIS OF TETRODOTOXIN

Reported by Ryan J. Carra December 8, 2003

## INTRODUCTION

Tetrodotoxin (Figure 1) is a naturally occurring compound isolated from the liver and ovaries of puffer fish.[1] Due to its potent toxicity as a selective blocker of voltage-gated $Na^+$ ion channels[2] and the high regard for puffer fish as a culinary delicacy, tetrodotoxin has become a molecule of general, as well as scientific, interest. Structural determination revealed a densely functionalized dioxa-adamantane core containing an ortho-acid and a pendant guanidine.[1] The challenging architecture, as well as a demand for structural analogues to study the mechanism of its action, make tetrodotoxin a synthetic target. The first total synthesis of racemic tetrodotoxin was accomplished by Kishi and co-workers in 1972.[3] Since then, though recent attempts from many labs have been disclosed,[4-8] the work of Du Bois[9] and Isobe[10] have provided the only two completed syntheses, each within the past year. This review will briefly summarize the Kishi synthesis and highlight recent approaches to the natural product.

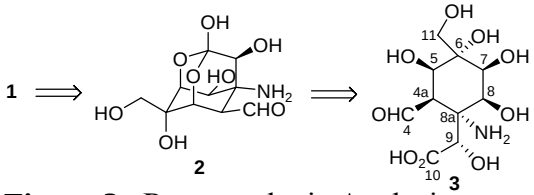


**1**

**Figure 1.** Tetrodotoxin

## RETROSYNTHETIC ANALYSIS

Due to the polarity of the guanidine and ortho-acid functionalities, these groups have typically been installed late in tetrodotoxin syntheses (Figure 2). The guanidine may result from guaninylation of a masked form of tetrodamine **2**, which itself may result from a masked form of cyclohexane derivative **3**. Given this established end-game, the main challenge of synthetic approaches to tetrodotoxin is access to the highly substituted cyclohexane derivative **3**. Key challenges include the tetrasubstituted stereogenic centers at C-(6) and C-(8a) as well as the introduction of the nitrogen substituent at C-(8a).



**2** **3**

**Figure 2.** Retrosynthetic Analysis

## SYNTHETIC APPROACHES TO TETRODOTOXIN

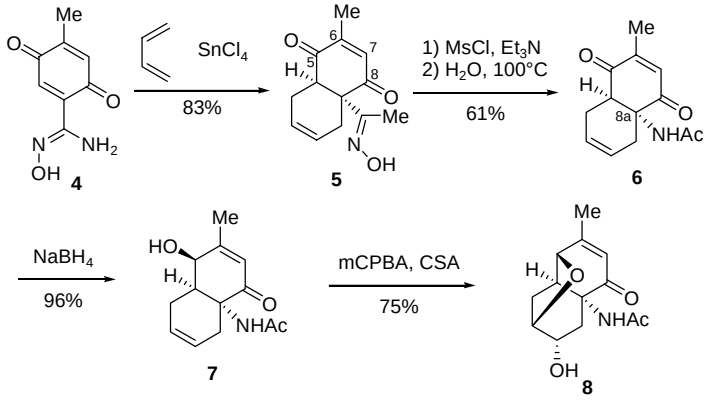
### Controlling Diastereoselectivity with a *cis*-Decalin Ring System: Highlights of Kishi's Synthesis

Kishi's racemic synthesis of tetrodotoxin began with a Lewis acid catalyzed Diels-Alder cycloaddition between quinone derivative **4** and 1,3-butadiene (Scheme 1). The resulting *cis*-decalin skeleton of **5** remained intact for over half of the synthetic route and helped set the C-(5), C-(6), C-(8) and C-(9) stereogenic centers. Installation of the nitrogen functionality at the quaternary stereogenic center C-(8a) was accomplished via Beckmann rearrangement of oxime **5**, affording acetamide **6** in 61% yield over two steps. Regio- and stereoselective reduction of quinone **6** with sodium borohydride

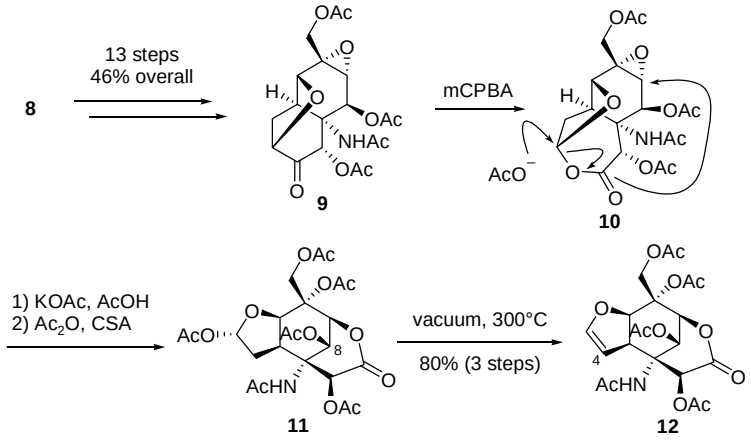
afforded alcohol **7** in 96% yield. The stereoselectivity of the reduction can be rationalized by preferential approach of the hydride from the convex face of the decalin system. Facial selectivity of the epoxidation of **7** was also controlled by the decalin system as the approach of *meta*-chloroperoxybenzoic acid (*m*CPBA) was preferentially from the *exo* face of the more electron-rich olefin. Spontaneous epoxide ring-opening by the pendant secondary hydroxyl afforded cyclic ether **8** in 75% yield.

**Scheme 1. Functionalization of *cis*-Decalin System**




Epoxide **9**, which was prepared from **8** in a 13 step sequence in 46% overall yield, underwent *m*CPBA-mediated Baeyer-Villiger oxidation to furnish lactone **10** in quantitative yield (Scheme 2). External acetate opening of the acetal followed by intramolecular carboxylate opening of the epoxide within **10**, and subsequent protection of the resulting tertiary alcohol provided tricyclic compound **11** in 90% yield over two steps. Pyrolysis of **11** under vacuum led to selective acetate elimination, affording dihydrofuran **12** in 80% yield.

**Scheme 2. Intramolecular Epoxide Opening**

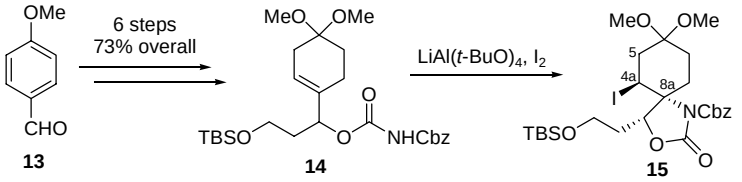



Intermediate **12** contains every stereogenic center within the advanced cyclohexane intermediate **3** and only C-(4) requires manipulation of its oxidation state. Indeed, **12** was converted to tetrodotoxin (**1**) in 11 steps in 3% overall yield. Most of the yield loss occurred in the final three steps of the synthesis, which included oxidation of C-(4) to the aldehyde oxidation state as well as global acetate deprotection to form the ortho-acid of tetrodotoxin. Kishi's elegant synthetic plan involved incorporation of a six-membered ring onto the cyclohexane core of tetrodotoxin (**1**) and exploitation of the concave/convex properties of the resulting *cis*-decalin to set four of the stereogenic centers. This work, which was published only eight years after the structural elucidation of the natural product, required 37 steps from known quinone **4** in 0.44% overall yield.

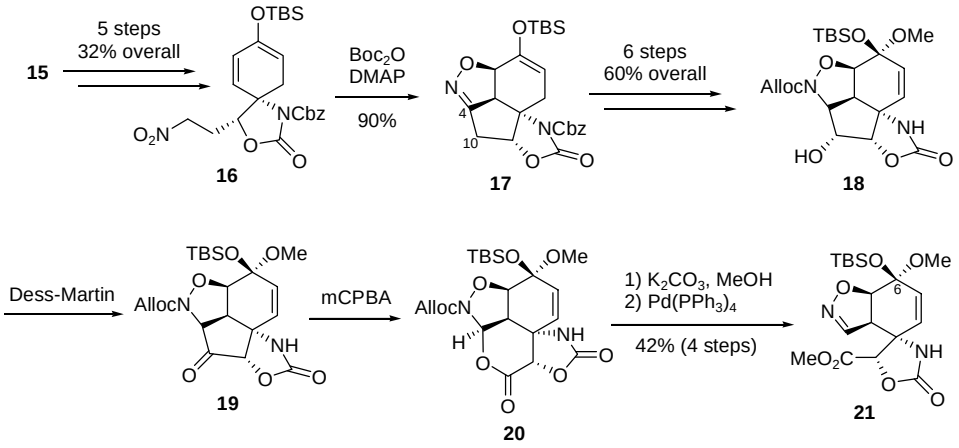
**1,3-Dipolar Cycloaddition to Install the C-(4a) and C-(5) Substituents**

In 2002, Fukuyama and co-workers reported a partial synthesis of tetrodotoxin utilizing a nitrile oxide [3+2] cycloaddition as a key step to build the β-hydroxy aldehyde functionality in **3**. Starting from *para*-anisaldehyde (**13**), the racemic carbamate **14** was generated in 6 steps in 73% overall yield (Scheme 3). Taguchi's protocol for iodoaminocyclization[11] installed the C-(8a) nitrogen substituent in modest diastereoselectivity (dr = 3:1). The protected primary alcohol **15** was converted to nitro compound **16** in 32% yield over 5 steps (Scheme 4). Treatment of **16** with di-*tert*-butyl dicarbonate ($Boc_2O$) and a catalytic quantity of *N*,*N*-dimethylaminopyridine (DMAP) provided a transient nitrile oxide, which upon intramolecular 1,3-dipolar cycloaddition gave isoxazoline **17** in 90% yield as a single diastereomer. Unfortunately, multiple steps were required to achieve the correct oxidation states at C-(10) and C-(4). Accordingly, the alcohol **18** was prepared in 60% yield over six steps from **17**. Dess-Martin oxidation of **18** furnished the corresponding ketone **19**, which upon *m*CPBA-mediated Baeyer-Villiger oxidation formed the lactone **20**. Methanolysis of **20**, followed by palladium-mediated allyl carbamate deprotection and subsequent loss of water, afforded isoxazoline **21** in 42% yield over 4 steps from **18**.

**Scheme 3. Stereospecific Iodoaminocyclization**




**Scheme 4. Nitrile Oxide Dipolar Cycloaddition**



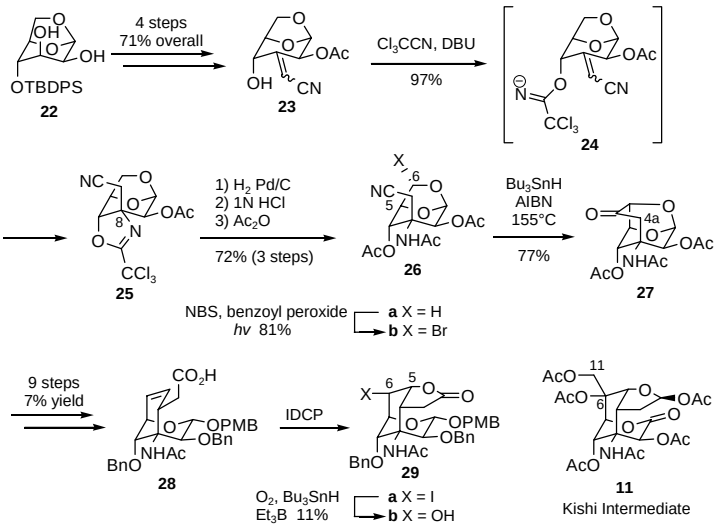

The advanced intermediate **21**, accessed in 23 steps from *para*-anisaldehyde in 5.3% overall yield, contains four of the stereogenic centers of tetrodotoxin (**1**), including the tetrasubstituted center at C-(8a) and contains substituents which could lead to formation of the natural product. One could imagine setting the C-(6) stereogenic center by deprotecting acetal **21** would require a C-(6) ketone followed by olefination and diastereoselective dihydroxylation. Unfortunately, the *cis*-ring skeleton of **21** necessitates either a *cis*-dihydroxylation of the C-(7)—C-(8) olefin from the concave face or a dihydroxylation from the convex face, followed by double inversion. The Fukuyama route produced advanced intermediate **21** *via* stereospecific iodoaminocyclization to form the tetrasubstituted C-(8a)

stereogenic center and set the stage for a key nitrile oxide [3+2] cycloaddition. It appears, however, that problems involving conversion of **21** to tetrodotoxin have truncated this approach.

**Radical Cyclization to Form the Cyclohexane Core of Tetrodotoxin**

In 1996, Fraser-Reid and co-workers reported the synthesis of an advanced intermediate towards the synthesis of tetrodotoxin (**1**) from a carbohydrate precursor. 1,6-Anhydro-β-D-mannopyranose derivative **22** was converted to nitrile **23** as a mixture of *E*/*Z* isomers in 71% overall yield over four steps (Scheme 5). Treatment of **23** with trichloroacetonitrile resulted in formation of transient imidate **24**, which underwent conjugate addition to the α,β-unsaturated nitrile, affording oxazoline **25** in 97% yield. This reaction succeeded in setting the tetrasubstituted C-(8a) stereogenic center of the natural product and placed the nitrile carbon in an axial position, condusive to C-(5)—C-(6) bond construction. Hydrogenolysis of the trichloromethyl group of **25**, was followed by hydrolysis of the oxazoline and acetylation of the resulting secondary alcohol, furnishing nitrile **26a** in 72% yield over three steps. Methodology developed by Ferrier and Furneaux,[12] was applied to **26a** to achieve regioselective bromination in 81% yield. Heating **26b** in the presence of the radical initiator 2,2'-azobisisobutyronitrile (AIBN) and tributyl tin hydride generated a C-(6) radical, which underwent intramolecular addition to the nitrile, providing, after workup, cyclohexanone **27** in 77% yield.

**Scheme 5. Radical Cyclization Toward Tetrodotoxin**



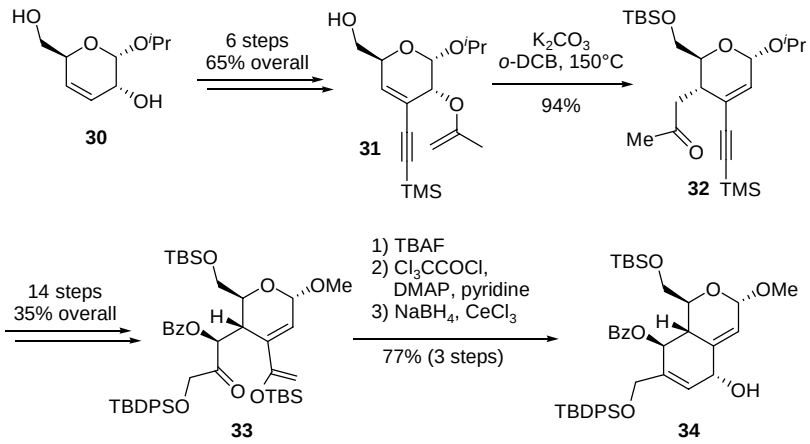

Ketone **27** was converted to the γ,δ-unsaturated acid **28** in 7% yield over nine steps including bromination at C-(4a) and radical allylation. Iodonium dicollidine perchlorate- (IDCP) mediated iodolactonization set the stereochemistry at C-(5) and provided functionality for C-(6) oxygenation, furnishing lactone **29a** in 86% yield. Conversion of the secondary iodide **29a** to the secondary alcohol **29b** resulted in only 11% yield with 77% recovered starting material.

Intermediate **29b** requires only C-(6)—C-(11) bond construction and oxidation state manipulations of two other carbons to complete a formal synthesis following the Kishi route (Scheme 2). However, the Fraser-Reid route to **29b** required 21 steps in an overall yield of 0.20% while Kishi accessed **11** in the same number of steps in approximately 13% yield. Thus, while the route contained several successful key steps, including conjugate addition to install the nitrogen functionality, radical cyclization to form the cyclohexane core and iodolactonization to set the C-(5) stereochemistry, the Kishi route is ultimately more efficient.

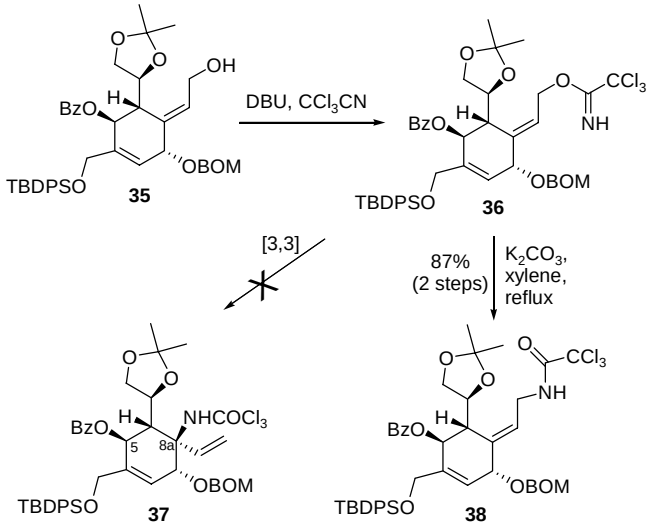
**Attempted Overman Rearrangement to Install the Angular Nitrogen: The Isobe Synthesis**

In 2003, Isobe and coworkers published the first total asymmetric synthesis of tetrodotoxin (**1**). Conversion of D-glucal derivative **30** to enol **31** was achieved in 65% overall yield over six steps (Scheme 6). A stereospecific Claisen rearrangement generated ketone **32** in 94% yield. After the silyl enol ether **33** was accessed in 14 further steps in 35% overall yield, the cyclohexane core of the natural product was constructed *via* intramolecular aldol condensation. The stereoselectivity of the subsequent sodium borohydride reduction, which provided secondary alcohol **34** in 77% yield from **33,** can be rationalized by the hydride approaching the convex face of the pseudo-boat conformation of the cyclohexene. Eight more steps were required for formation of the primary alcohol **35** (Scheme 7). Earlier model studies had suggested that treating **35** with trichloroacetonitrile and heating the resulting imidate **36** under basic conditions would effect an Overman rearrangement to install the C-(8a) nitrogen substituent, however, only undesired 1,3-shifts were observed providing amide **38** in 87% yield from **35**.

**Scheme 6. Construction of Cyclohexane Core**



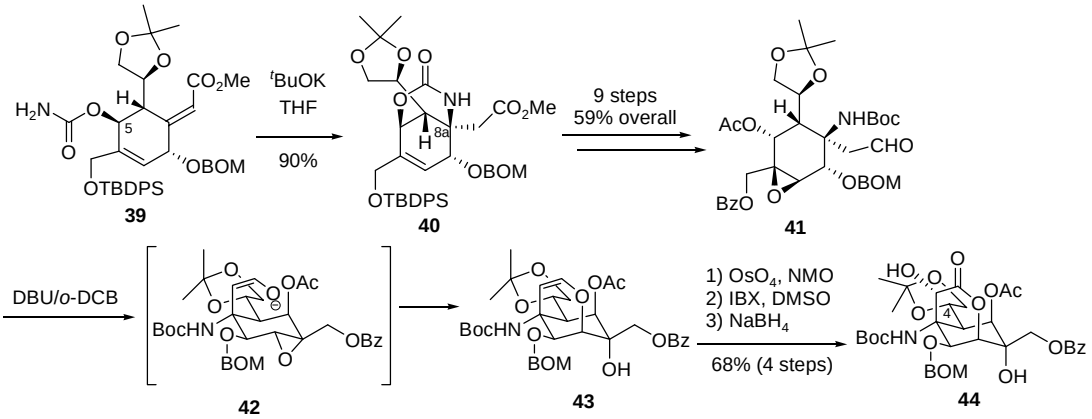

**Scheme 7. Attempted Overman Rearrangement**




An alternative route for nitrogen installation involved taking advantage of the stereochemistry of the C-(5) oxygen substituent. Accordingly, α,β-unsaturated ester **39** was prepared from **37** *via* a six-step route in 77% yield. The stereochemistry at the quaternary C-(8a) center was set by intramolecular 1,4-conjugate addition of **39** under basic conditions (Scheme 8). The carbamate **40** was converted to epoxide **41** in a nine-step sequence including hydrolysis of the carbamate, hydroxyl-directed epoxidation of the olefin and epimerization of the C-(5) oxygen substituent to achieve the configuration of tetrodotoxin (**1**). The final oxygen substituent on the cyclohexane core was installed *via* intramolecular epoxide opening by the oxygen of enolate **42**, affording enol **43**. Dihydroxylation of **43** followed by hypervalent iodine-mediated double oxidation afforded an α-ketolactone, which was subsequently reduced to α-hydroxylactone **44** by sodium borohydride in 68% yield from **41**. The remaining 14 steps

to complete the synthesis were all protecting group manipulations except for guanidine installation and oxidative cleavage to form a C-(4) aldehyde.

**Scheme 8. Installation of the Nitrogen Functionality and C-(7) Oxygen**




Compared to Kishi's route to tetrodotoxin (**1**), Isobe's is characterized by its length and extensive use of protective groups. Of the 68 steps in the total synthesis, 33 involved installation or cleavage of protective groups. Formation of the cyclohexane core **34** of the natural product was not accomplished until 22 steps into the synthesis. The key steps of the synthesis were intramolecular aldol condensation to initially form the cyclohexane core, conjugate addition to install the C-(8a) nitrogen functionality, hydroxyl-directed epoxidation to set the quaternary C-(6) stereogenic center, and intramolecular epoxide ring-opening to install the C-(5) oxygen substituent.

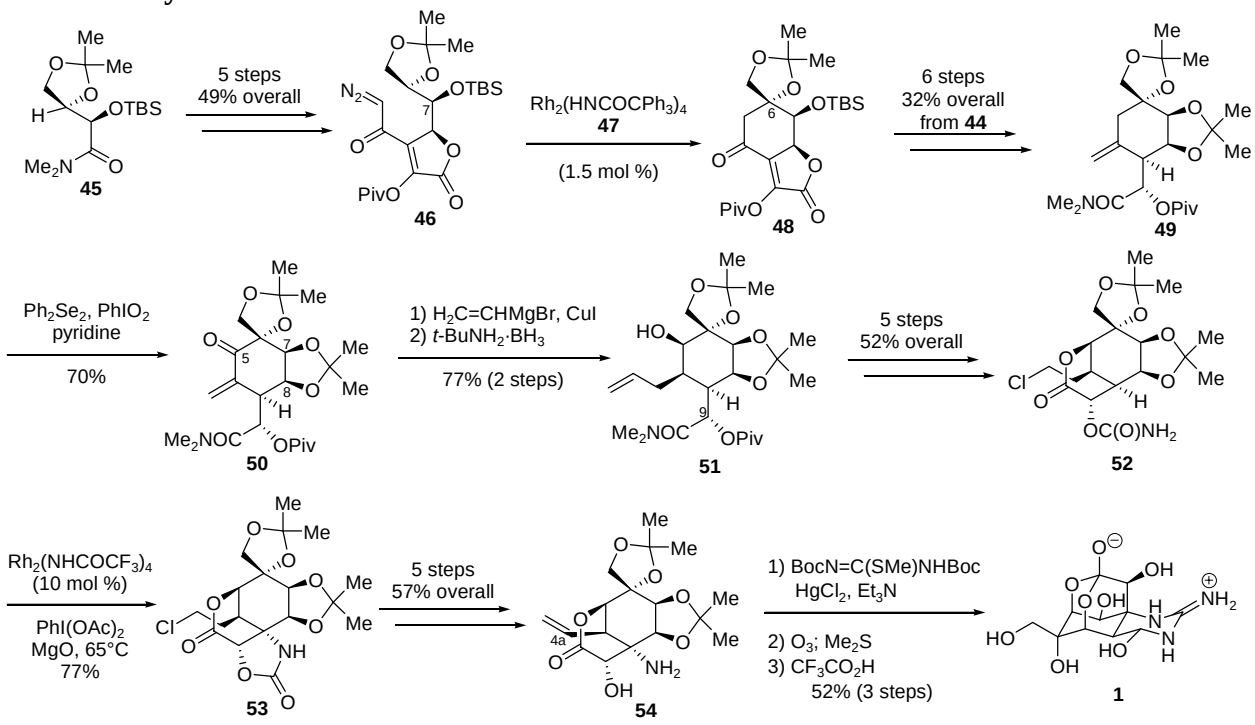
**Showcasing the Utility of C-H Activation: The Du Bois Synthesis**

Du Bois and coworkers reported the second asymmetric synthesis of tetrodotoxin (**1**) in 2003. By exploiting the advantages of C-H activation methodology, they have minimized the use of protecting groups. First a rhodium carbenoid derived from an α-diazoketone was utilized to form the cyclohexane core of the natural product. Second, the C-(8a)—C-(9) carbinolamine moiety in **3** was recognized as a potential adduct of nitrene C-H insertion chemistry previously developed in their laboratory.[13]

The synthetic route began with the elaboration of the known amide **45** to α-diazoketone **46** in 49% yield over five steps (Scheme 9). Decomposition of the diazo functionality by Rh-acetamide catalyst **47** gave exclusive formation of cyclohexane **48** in over 75% yield. In simpler systems, intramolecular rhodium-carbenoid C-H insertion is known to form five-membered rings preferentially, even if formation of a six-membered ring is electronically favored.[14] Du Bois and coworkers did not comment on the apparent reversal of selectivity in this system, but one might postulate that the $sp^2$ carbon alpha to the carbonyl increases the strain in the five-membered transition state sufficiently to favor six-membered ring formation. The route to **48** comprised an efficient 7-step synthesis of the

cyclohexane core of tetrodotoxin, with three key stereogenic centers, including the tetrasubstituted C-(6) center, set correctly.

**Scheme 9. Synthesis of Tetrodotoxin *via* C-H Activation**




Ketone **48** was elaborated to terminal olefin **49** in 32% yield over six steps from diazoketone **46**. Subsequent allylic oxidation provided the C-(5) ketone **50** in 70% yield. At this point, clever use was made of the concave/convex fused ring system created by the acetonide-protected C-(7)—C-(8) diol. Conjugate addition of vinyl cuprate was followed by stereoselective protonation of the resulting enolate from the more sterically accessible convex face. Borane reduction of the resulting ketone also proceeded from the convex face of the ring system, furnishing secondary alcohol **51** in 77% yield over two steps. It is worth noting that after 14 steps, all but one stereogenic center of advanced intermediate **3** had been set correctly.

Late-stage introduction of the nitrogen functionality was the largest remaining synthetic hurdle and the C-(9) oxygen substituent present in **51** was available to act as a tether for nitrene C-H insertion. Thus, carbamate **52** was generated in five steps from **51** in 52% overall yield. The rigid ring system of **52** may be invoked to explain the observed regioselectivity of the C-H amination as exposure to dirhodium (tetrakis)trifluoroacetamide afforded carbamate **53** exclusively in 77% yield. Functional group manipulations including generation of a C-(4a) olefin as a direct precursor to the desired aldehyde, provided primary amine **54** in 57% yield over five steps. Introduction of the guanidine moiety was accomplished by treating **54** with isothiourea in the presence of mercuric chloride. Ozonolysis of

the olefin was followed by global deprotection with trifluoroacetic acid, to furnish tetrodotoxin **1** in 52% yield over three steps.

While Kishi and Isobe gradually built up oxygen substituents, Du Bois' use of C-H activation chemistry allowed for the introduction of heteroatom functionality at an early stage, thus minimizing the protecting group manipulations which have characterized other routes. At 30 steps, this asymmetric total synthesis is the shortest and the highest yielding at 1.0% overall.

## CONCLUSIONS

For 31 years the Kishi route stood as the only synthesis of tetrodotoxin (**1**). That this route still compares favorably to most current efforts which have access to modern methodology, speaks of the elegance of its design. The efficiency of the Kishi synthesis, however, was recently surpassed by the Du Bois approach, which showcased the applicability of C-H bond activation to complex molecule construction.

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