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.

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 guaninylization 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).

SYNTHETIC APPROACHES TO TETRODOTOXIN

Controlling Diastereoselectivity with a \textit{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 \textit{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 (mCPBA) 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.

Epoxide 9, which was prepared from 8 in a 13 step sequence in 46% overall yield, underwent mCPBA-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.

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 iodoaminocyclization11 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 (Boc₂O) 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 mCPBA-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.

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)
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, conducive 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.

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.

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 sp² 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.

REFERENCES