ISSN:2790-1688 Volume-15-(2025)

Progress in the Total Synthesis of Saxitoxin

Zehua Zhou

Southeast University, 211189, China

*Corresponding author e-mail: 213242137@seu.edu.com

Abstract. Saxitoxin (STX) is a typical guanidinium neurotoxin initially identified in shellfish poisoning incidents and primarily biosynthesized by organisms such as freshwater cyanobacteria and marine dinoflagellates. As one of the most representative members of the natural paralytic shellfish toxin (PST) family, over 50 structural analogues of STX and its derivatives have been discovered to date. This toxin exerts its neurotoxicity by selectively inhibiting voltage-gated sodium channels (NaV), making it a valuable molecular tool in neuropharmacology and ion channel research. The compact tricyclic fused skeleton and highly polar diguanidinium structure of the STX molecule present significant challenges for its chemical total synthesis. Concurrently, the potential application value of this class of toxins and their derivatives in drug development has attracted considerable attention from synthetic chemists for decades. This review systematically summarizes the progress in the total synthesis of STX, critically evaluates the design concepts and key steps of different synthetic strategies, and provides an outlook on future directions in this field.

Keywords: Saxitoxin; Total Synthesis; Marine Toxin; Guanidinium toxin; Sodium ion channel.

1. Introduction

1.1 Discovery and Sources of Saxitoxin

Research on Saxitoxin (STX) originated from investigations of early paralytic shellfish poisoning events [1]. In 1957, researchers including Sommer first isolated the toxic component from the Alaskan butter clam (*Saxidomus giganteus*) and named it "saxitoxin" [2]. In 1975, Schantz and colleagues determined the chemical structure of STX for the first time using X-ray crystallography and NMR techniques, revealing its unique tricyclic fused skeleton and diguanidinium groups [3–5]. Subsequently, Kishi's team accomplished the first racemic total synthesis of the molecule in 1977 [6], marking the beginning of a new era in artificial STX synthesis.

STX is primarily produced by various algal species, including marine dinoflagellates (e.g., *Alexandrium* spp., *Gymnodinium catenatum*) and freshwater cyanobacteria (e.g., *Anabaena circinalis*, *Aphanizomenon* spp., *Raphidiopsis raciborskii*) [7–10]. These toxin-producing algae accumulate toxins in bivalve shellfish (such as mussels, clams, and oysters) through the food chain, leading to human intoxication. According to statistics, global ingestion of PSP toxin-contaminated seafood causes approximately 2000 poisoning cases annually, with a mortality rate reaching 15% [7]. Therefore, monitoring and synthetic studies of STX and its analogues also hold significant public safety importance.

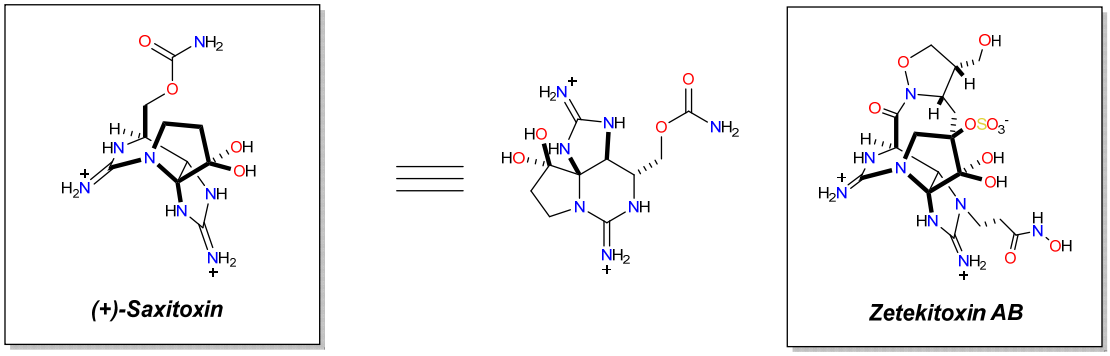

Figure 1 The stereochemical structures of STX and ZTX

1.2 Structure, Physicochemical Properties, and Biological Activity

STX commonly exists as a dihydrochloride salt, appearing as a white, hygroscopic amorphous solid. Its structural features include a tricyclic fused skeleton (comprising two fused six-membered pyridine rings and one six-membered guanidinium ring) and highly polar diguanidinium groups at the C7, C8, and C9 positions, as shown in Figure 1. The molecule is strongly basic, highly soluble in water, slightly soluble in methanol and ethanol, insoluble in non-polar organic solvents, and relatively stable under acidic conditions.

The neurotoxicity of STX stems from its high-affinity blockade of voltage-gated sodium channels (NaV). The diguanidinium moiety in the molecule can interact via hydrogen bonding and electrostatic forces with key amino acid residues in the pore region of NaV channels, thereby blocking sodium ion influx, inhibiting the generation and propagation of action potentials, and ultimately leading to muscle paralysis and respiratory failure. Severe poisoning can be fatal within hours [7].

Beyond its notable toxicity, STX and its structural analogues, due to multiple modifiable sites, are widely used in developing NaV subtype-selective probes to study channel structure and function relationships. By specifically binding to NaV, STX serves as an important neurobiological tool for blocking sodium currents. For instance, Zetekitoxin AB (ZTX), isolated from the Panamanian poison frog *Atelopus zeteki*, shares high structural similarity with STX and also exhibits potent NaV inhibitory activity. These complex nitrogen-containing heterocyclic natural products continue to stimulate research interest in organic synthesis and medicinal chemistry due to their significant bioactivity and synthetic challenges.

2. Total Synthesis Strategies for Saxitoxin

2.1 Pioneering Work: Kishi's First-Generation Synthesis (1977)

In 1977, Y. Kishi's team achieved the first racemic total synthesis of STX [6, 11]. Given the limitations of purification techniques at the time, Kishi adopted a strategy of first constructing the fused ring skeleton followed by functional group modifications, effectively circumventing purification difficulties associated with introducing highly polar guanidinium groups late in the synthesis [11].

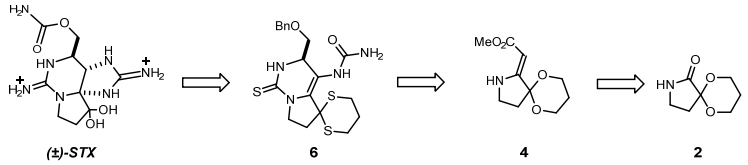

Figure 2 The retrosynthetic analysis of (±)-STX by Kishi's team

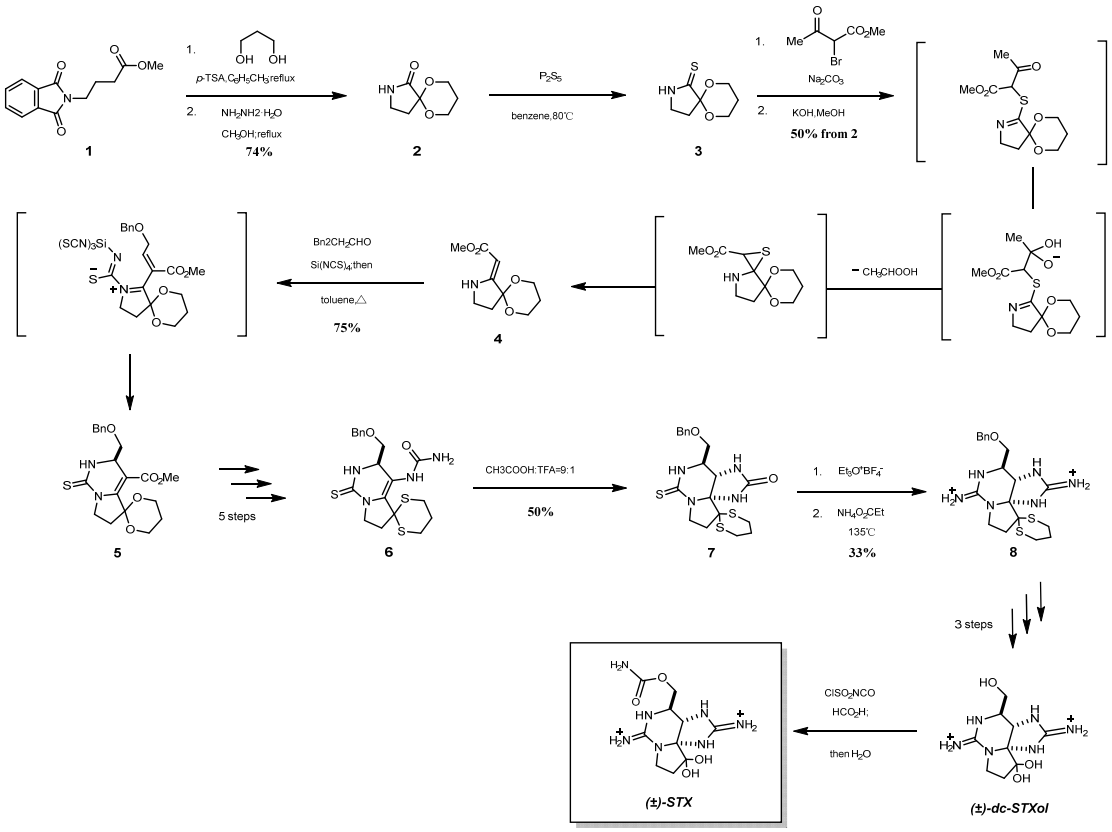

Figure 3 The total synthesis route of (±)-STX by Kishi's team

The synthesis commenced with methyl 2-oxo-4-phthalimidobutanoate 1. Intramolecular condensation yielded thiourea 3, successfully constructing the first five-membered ring. Subsequent Eschenmoser desulfurization [12, 13], utilizing a low-valent phosphorus reagent, generated vinylogous formate 4 in situ. This step, replacing the carbonyl in a classical Mannich reaction with a thiocarbonyl group, significantly improved the yield [11]. Kishi then cleverly employed a Si(NCS)4-promoted 6 π -electrocyclization to stereoselectively build the six-membered ring skeleton and establish the relative configuration of the first chiral center. Construction of the third ring started from thiourea 5. Following Curtius rearrangement to afford intermediate 6, cyclization under weak acid conditions proceeded with high stereoselectivity to give 7. The ratio of the target product to its stereoisomer was 5:1 [6], speculated to result from differing torsional strain experienced by the chiral center under varying acidic conditions. Finally, thiourea ammonolysis and further modifications yielded (±)-STX.

Although this route was lengthy, had low overall yield, and was difficult to adapt for synthesizing carbamate derivatives like dc-STX due to solvent limitations, Kishi's pioneering achievement in synthesizing STX and (±)-dc- α -STXol remains a milestone.

2.2 Subsequent Improvements and Innovations

With advances in chiral synthesis and separation techniques, total synthetic routes for STX gradually became more efficient and concise, with the work of J. Du Bois [14–16] and S. Yokoshima [17, 18] being particularly notable.

2.2.1 J. Du Bois (2006, 2007, 2008)

J. Du Bois reported the first asymmetric total synthesis of (+)-STX in 2006 [14]. The core strategy involved first constructing a 9-membered medium ring containing key substituents, followed by intramolecular condensation to form the pentacyclic-fused hexacyclic skeleton. Retrosynthetic

analysis identified the key medium-ring intermediate as accessible from two different starting materials, significantly broadening the substrate scope [14, 15].

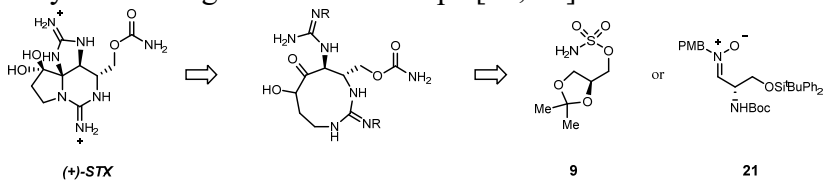

Figure 4 The retrosynthetic analysis of (+)-STX by D.Bois's team

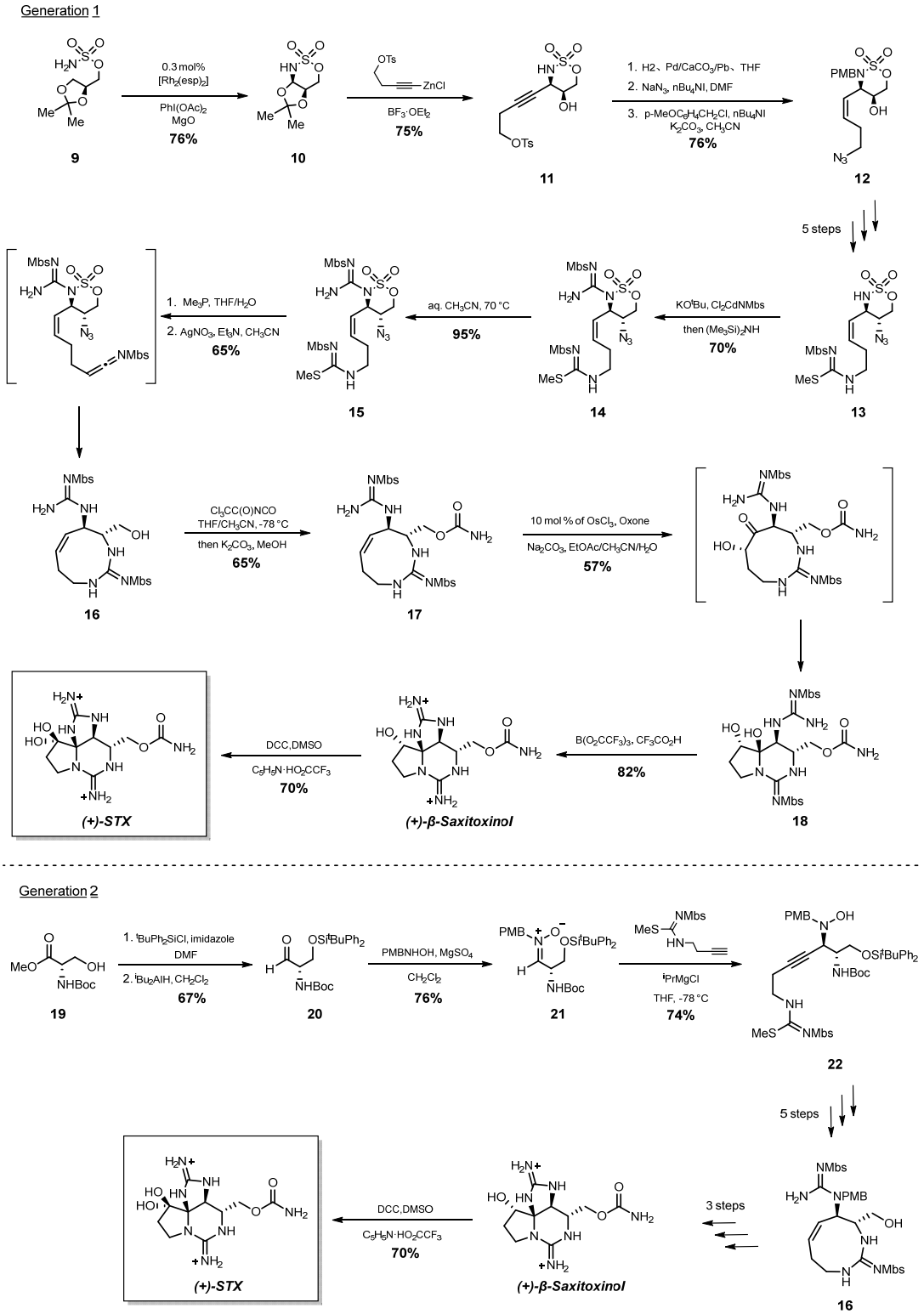

Figure 5 The total synthesis route of (+)-STX by D.Bois's team

In the synthesis, Du Bois introduced both guanidinium groups early, avoiding difficulties associated with late-stage skeleton modifications. Intermediate 17 underwent stereoselective intramolecular condensation under oxidative conditions, predominantly yielding 18. Other oxidant combinations like OsO4 and t-BuOOH produced the N1-C12 linked byproduct. Although the mechanism remained unclear, treatment with B(O2CCF3)2 converted the mixture uniformly to (±)-β-saxitoxinol [1], which was then efficiently transformed into the ketal via Swern oxidation, completing the total synthesis of (+)-STX.

2.2.2 Toshio Nishikawa (2011)

Nishikawa developed a strategy employing an alkyne as a C4, C12-diketone equivalent, constructing the STX skeleton stereoselectively via an oxidative cascade reaction.

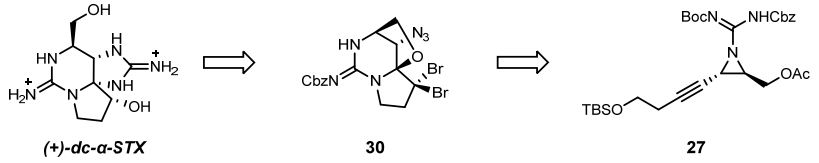

Figure 6 The retrosynthetic analysis of (+)-STX by Nishikawa's team

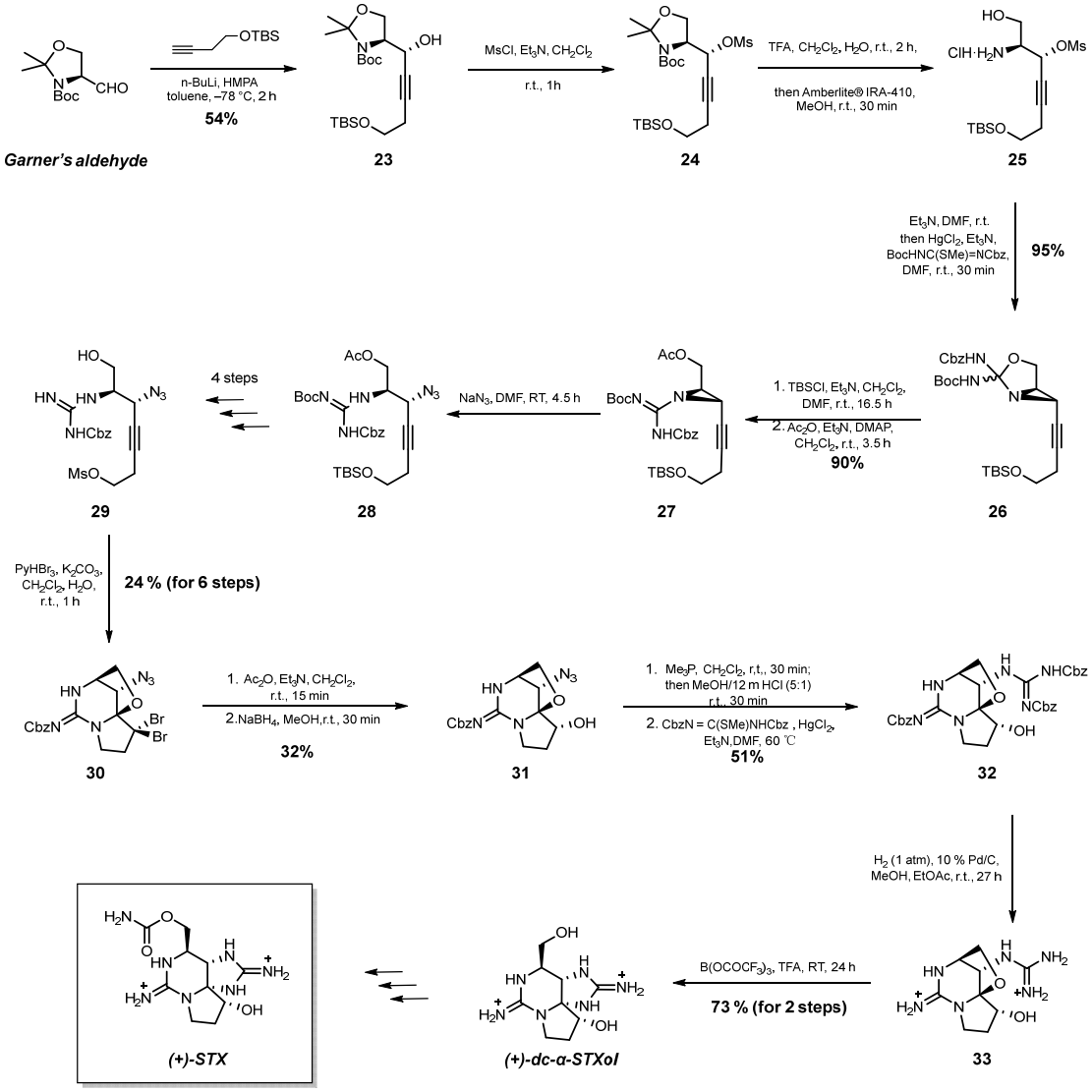

Figure 7 The total synthesis route of (+)-STX by Nishikawa's team

The synthesis started from Garner's aldehyde. High diastereoselective addition (>10:1) of lithium acetylide provided 23 [17], which was subsequently mesylated, deprotected, and treated with ion-

exchange resin to afford amine hydrochloride 25. Base-induced cyclization yielded aziridine 26, and protection gave the key intermediate 27. After azide opening, desilylation, and deacetylation, precursor 29 underwent bromocyclization and intramolecular N-alkylation in one pot using Py • HBr3, constructing the bicyclic system 30 in 79% yield [17]. Subsequent reduction and B(OCOCF3)3/TFA-catalyzed acetal hydrolysis/guanidinium cyclization ultimately yielded (+)-dc-α-STXol, which could be further derivatized to STX, ZTX, and other analogues.

Both Du Bois and Nishikawa's strategies started from chiral pool materials, established stereochemistry early, and enabled the diverse synthesis of STX family molecules, providing a foundation for physiological studies. However, the former relied on expensive metal reagents, involved harsh conditions for medium-ring condensation, and was lengthy. The latter, while achieving efficient one-pot cyclization, faced challenges in the difficult preparation and poor stability of the key intermediate 27, limiting synthetic efficiency.

2.3 Recent Efficient Syntheses

Recent developments in photocatalysis and radical chemistry have driven innovation in STX synthesis strategies. In 2025, Tuoping Luo and P. S. Baran's groups independently reported highly efficient and concise asymmetric syntheses of (+)-STX.

2.3.1 Tuoping Luo (2025)

Luo's team utilized 8-bromoxanthine as the starting material, simplifying the synthesis to the construction of essentially a single ring system. They innovatively employed a chiral boron reagent-induced photocatalytic [2+2] cycloaddition to replace the Michael addition, overcoming the thermodynamic unfavorability of forming the tetrasubstituted alkene in the fused ring and achieving high stereocontrol. The C8-carbonyl acted as a strong hydrogen bond acceptor, stabilizing the transition state and enhancing stereoselectivity. Synthesis involved borylcupration/protonation to give 36 (Z:E >20:1), ligand exchange, followed by [2+2] cycloaddition under 295 nm irradiation, and oxidative cleavage/reduction to prepare single diastereomer 37 on a gram scale. Subsequent steps included cyano substitution, methanolysis, reduction, protection, and guanidinylation. A one-pot ozonolysis/deprotection sequence, followed by conversion under known conditions, finally yielded (+)-STX, achieving the synthesis of an STX tetracyclic analogue in 16 steps [19, 21].

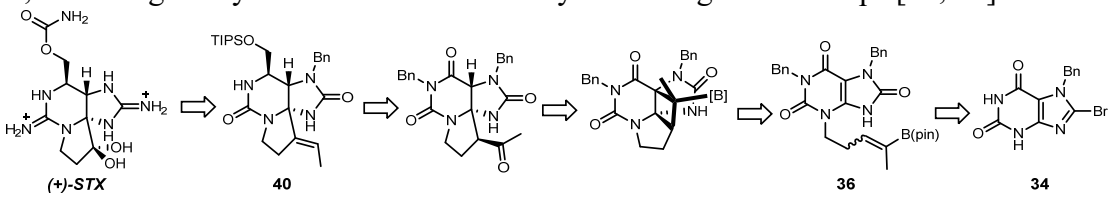

Figure 8 The retrosynthetic analysis of (+)-STX by Luo's team

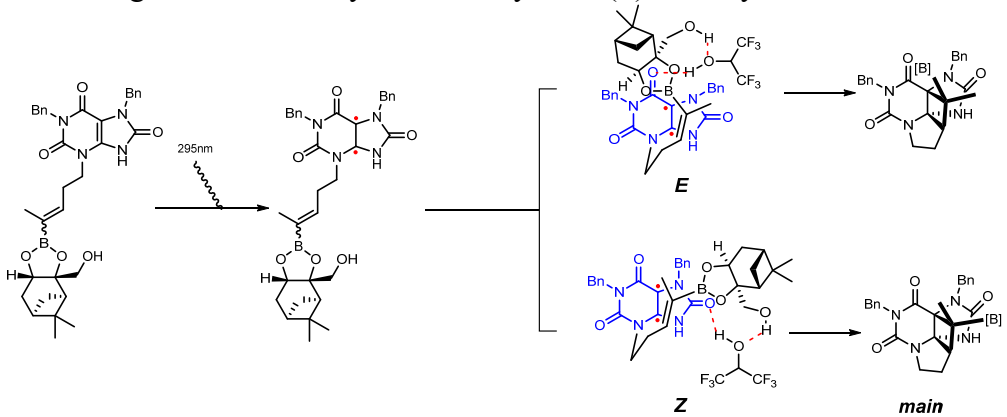

Figure 9 The mechanism of photocatalytic [2+2] reaction induced by chiral boronic groups

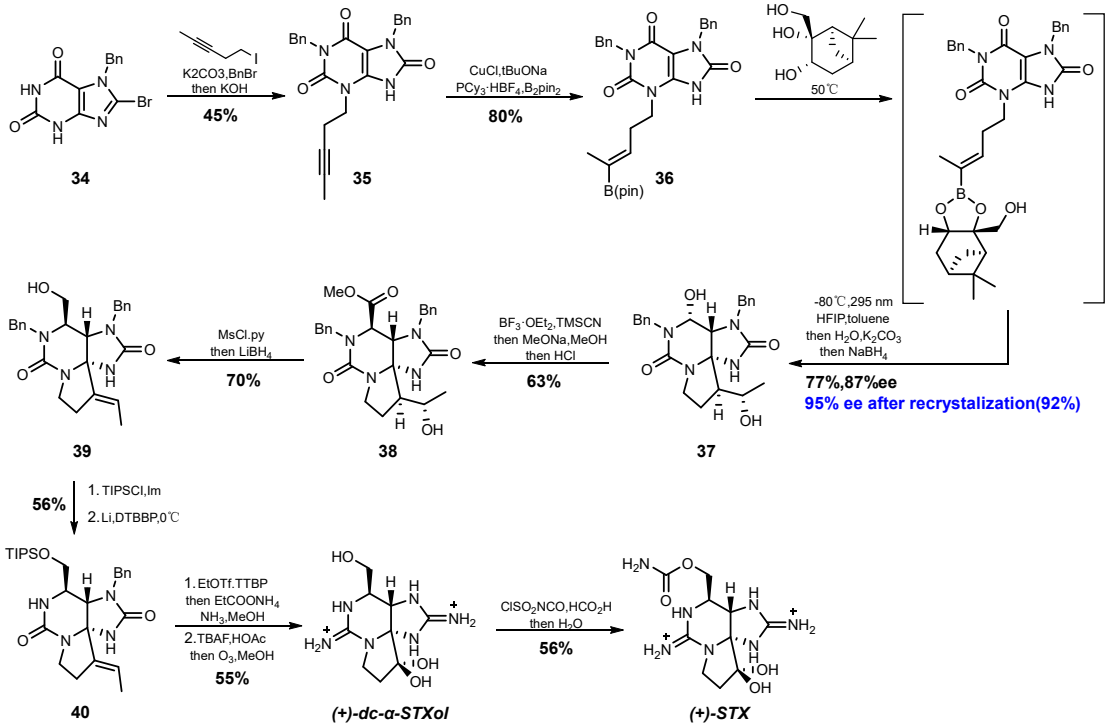

Figure 10 The total synthesis route of (+)-STX by Luo's team

2.3.2 Phil S. Baran (2025)

Baran's team integrated three major strategies-retrosynthetic radical disconnections, C-H functionalization, and biocatalysis-to achieve efficient preparation of STX family molecules. Key features included using radical cross-coupling to efficiently join serine and proline derivatives for rapid modular skeleton assembly, employing C-H oxidation to simplify C-N bond formation, and utilizing an engineered fungal hydroxylase for decagram-scale highly selective hydroxylation of L-proline (20:1 d.r.). Starting from 40 and 43, radical cross-coupling afforded 46. Deprotection/guanidine-mediated cyclization yielded 47, followed by Anelli oxidation and PIFA-promoted cyclization to give 48. Final Boc deprotection/guanidinylation completed the synthesis of (+)-STX. The entire route required only 7 steps with an average yield of 71%, representing the most efficient STX synthesis reported to date [20].

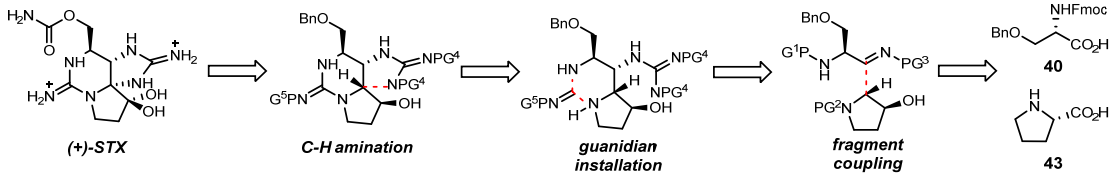

Figure 11 The retrosynthetic analysis of (+)-STX by Baran's team

The work of both Luo and Baran, relying on modern methodologies, achieved efficient and highly selective construction of the critical C4-C5 bond, highlighting the powerful potential and generality of new strategies in the synthesis of complex natural products.

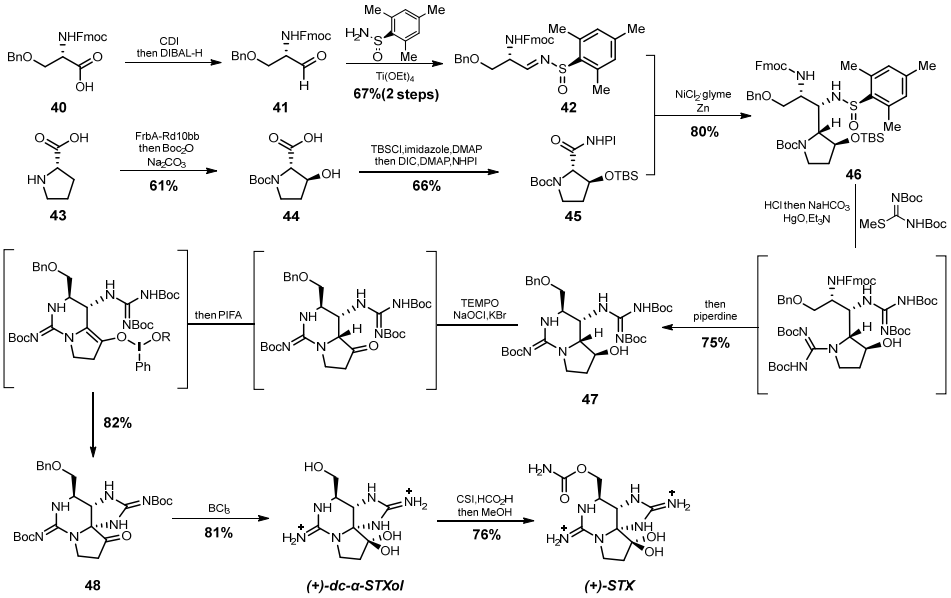

Figure 12 The total synthesis route of (+)-STX by Baran's team

3. Comparison and Discussion of Different Synthetic Routes

A comparison of representative total synthesis routes from Kishi (1977), Du Bois (2006), Nishikawa (2010), Jacobsen (2014), Luo (2020), and Baran (2023), as summarized in Table 1, clearly outlines the evolution of this field over nearly five decades. Table 1 shows that modern synthetic methods such as photocatalytic [2+2] reactions, retrosynthetic radical approaches, C – H functionalization, and biocatalysis offer significant advantages in substantially shortening the number of steps. For instance, Luo's group utilized a chiral boron reagent-induced asymmetric photocatalytic [2+2] cycloaddition to efficiently construct the core tricyclic skeleton of STX in just one step, contrasting sharply with the multi-step construction required in the early Kishi route. Baran's work represents a pinnacle of modern synthesis, skillfully integrating retrosynthetic radical disconnections, C-H functionalization, and biocatalytic enzymatic techniques, dramatically compressing processes that traditionally required multiple steps for functional group introduction and skeletal modification. Furthermore, their adoption of a convergent synthesis strategy, splitting the molecule into two modules prepared in parallel and finally joining them via a key coupling step, effectively shortened the longest linear sequence and improved overall efficiency.

Regarding overall yield, the methods of J. Du Bois and Baran are particularly advantageous, both exceeding 5%. Du Bois's group achieved a high cumulative yield by employing efficient and reliable transformations at each step (e.g., Rh-catalyzed C-H amination, addition-condensation reactions), maintaining high average yield per step. Baran's group, representing the cutting edge of synthetic concepts, innovatively merged synthetic biology with modern radical chemistry using a modular convergent strategy. This route, preparing one key module via engineered enzyme catalysis and converging it with a chemically synthesized module via highly selective radical cross-coupling, not only drastically shortened the longest linear sequence but also achieved a record-high overall yield of approximately 7%. Its key to its success lay in avoiding low-yielding steps common in traditional routes, such as challenging alkyne additions or cyclizations.

Throughout its development history, STX total synthesis exhibits several distinct overarching trends. Firstly, the synthetic objective has evolved from initial structural confirmation of the racemate to the high enantioselective construction of chiral target molecules, as evidenced by Jacobsen's first use of organocatalytic asymmetric Diels-Alder reactions, and the direct production of optically pure products in the Luo and Baran routes. Secondly, synthesis strategies increasingly emphasize one-pot/tandem reactions and modular design, aiming to minimize purification steps and enhance overall efficiency, exemplified by the clever tandem deprotection/addition/intramolecular condensation

sequence in Baran's route. Finally, reaction conditions show a significant trend towards milder protocols, transitioning from early reliance on strong acids/bases or stoichiometric metal reagents to the use of visible light photocatalysis, organocatalysis, and biocatalysis -- more green and controllable means.

In summary, the history of STX total synthesis is a microcosm of modern synthetic methodology continually challenging the limits of complex natural product molecules. Its evolution fully embodies the core guiding principles of step economy, atom economy, and green chemistry in contemporary organic synthesis.

Table 1 Comparison of Different Synthesis Routes

Group	Year	Total Steps	Overall Yield	Key Strategy/Reaction
Y.Kishi	1977	18 steps	0.24%	The 6-π electrophilic cyclization reaction promoted by Si(NCS)4
J. Du Bois	2006 and 2007	18 steps and 13 steps	1.89% and 6.46%	Intramolecular cyclization reaction
Toshio Nishikawa	2011	18 steps to (+)-dc-α-STXol	2.86% from 27	Oxidation cascade reaction
Luo tuoping	2025	10 steps	2.11%	Chiral boronic group-induced photocatalytic [2+2] reaction
Phil S.Baran	2025	7 steps	9.10%	Free radical retrosynthesis, C–H functionalization and enzymatic catalysis

4. Conclusion and Outlook

Reflecting on the decades-long development of STX total synthesis, from its first achievement in 1977 to the highly efficient synthetic routes from the Baran group in 2025, the synthetic strategy has undergone a remarkable transformation from arduous exploration to concise efficiency. This progress embodies the wisdom and courage of generations of synthetic chemists who, facing the challenges posed by highly toxic compounds, have driven the field forward through continuous technological innovation and methodological optimization. Although the current level of synthesis has significantly improved, STX total synthesis still faces numerous challenges. While the Baran group achieved gram-scale preparation, the large-scale acquisition and engineering of the fungal hydroxylase used in the key biocatalytic step remain technical bottlenecks requiring breakthrough. Simultaneously, the synthesis of structurally more complex STX family derivatives, further optimization of key steps like isocyanation, and the development of decagram-scale preparation processes are all directions requiring future in-depth exploration. Furthermore, effectively controlling the toxicity risks of STX and its intermediates during large-scale synthesis, for instance by developing new routes that avoid high-toxicity intermediates like (+)-dc-α-STXol, is a crucial issue pertaining to experimental safety and practical application.

Looking ahead, STX research shows a trend towards diversification. As its biosynthetic pathway becomes increasingly elucidated, utilizing synthetic biology techniques for the large-scale production of STX demonstrates significant potential. This approach could not only reduce safety risks associated with traditional synthesis but also improve production efficiency. In terms of application prospects, the differential binding characteristics of STX family compounds to different NaV subtypes provide unique opportunities for developing novel therapeutics for neurological diseases such as neuropathic pain and epilepsy. Overall, with the continuous innovation in synthesis technologies and deepening of biological research, STX and its derivatives are poised to show increasingly broad application prospects in physiological research and drug development.

References

[1] Thottumkara A P, Parsons W H, Du Bois J. Saxitoxin[J]. Angewandte Chemie (international Ed. in English), 2014, 53(23): 5760-5784.

[2] Schantz E J, Mold J D, Stanger D W, et al. Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues[J]. Journal of the American Chemical Society, 1957, 79(19): 5230-5235.

[3] Schantz E J, Ghazarossian V E, Schnoes H K, et al. Structure of saxitoxin[J]. Journal of the American Chemical Society, 1975, 97(5): 1238-1239.

[4] Bordner J, Thiessen W E, Bates H A, et al. Structure of a crystalline derivative of saxitoxin. Structure of saxitoxin[J]. Journal of the American Chemical Society, 1975, 97(21): 6008-6012.

[5] Distribution of paralytic toxins in california shellfish[J]. Toxicon, 1985, 23(2): 346-349.

[6] Tanino H, Nakata T, Kaneko T, et al. A stereospecific total synthesis of d,l-saxitoxin[J]. Journal of the American Chemical Society, 1977, 99(8): 2818-2819.

[7] Deng H, Shang X, Zhu H, et al. Saxitoxin: a comprehensive review of its history, structure, toxicology, biosynthesis, detection, and preventive implications[J]. Marine Drugs, 2025, 23(7): 277.

[8] Ciminiello P, Fattorusso E, Forino M, et al. Saxitoxin and neosaxitoxin as toxic principles of alexandrium andersoni (dinophyceae) from the gulf of naples, italy[J]. Toxicon, 2000, 38(12): 1871-1877.

[9] Landsberg, J.H.; Hall, S.; Johannessen, J.N.; White, K.D.; Conrad, S.M.; Abbott, J.P.; Flewelling, L.J.; Richardson, R.W.; Dickey, R.W.; Jester, E.L.; et al. Saxitoxin Puffer Fish Poisoning in the United States, with the First Report of Pyrodinium bahamense as the Putative Toxin Source. Environ. Health Perspect. 2006, 114, 1502–1507.

[10] The globally distributed genus alexandrium: multifaceted roles in marine ecosystems and impacts on human health[J]. Harmful Algae, 2012, 14: 10-35.

[11] Y. Kishi, Heterocycles 1980, 14, 1477 1495.

[12] Yamada Y, Miljkovic D, Wehrli P, et al. Ein neuer synthetischer zugang zum corrinsystem[J].

[13] Roth M, Dubs P, Götschi E, et al. Sulfidkontraktion via alkylative kupplung: eine methode zur darstellung von β-dicarbonylderivaten. Über synthetische methoden, 1. Mitteilung[J]. Helvetica Chimica Acta, 1971, 54(2): 710-734.

[14] Fleming J J, Du Bois J. A synthesis of (+)-saxitoxin[J]. Journal of the American Chemical Society, 2006, 128(12): 3926-3927.

[15] Fleming J J, McReynolds M D, Du Bois J. (+)-saxitoxin: a first and second generation stereoselective synthesis[J]. Journal of the American Chemical Society, 2007, 129(32): 9964-9975.

[16] Mulcahy J V, Du Bois J. A stereoselective synthesis of (+)-gonyautoxin 3[J]. Journal of the American Chemical Society, 2008, 130(38): 12630-12631.

[17] Sawayama Y, Nishikawa T. A synthetic route to the saxitoxin skeleton: synthesis of decarbamoyl α-saxitoxinol, an analogue of saxitoxin produced by the cyanobacterium lyngbya wollei[J]. Angewandte Chemie (international Ed. in English), 2011, 50(31): 7176-7178.

[18] Sawayama Y, Nishikawa T. Bromocyclization of alkynyl guanidine: a new approach to the synthesis of cyclic guanidines of saxitoxin[J]. Synlett, 2011, 2011(5): 651-654.

[19] Jiao Y, Liu J, Mao W, et al. Synthesis of (+)-saxitoxin facilitated by a chiral auxiliary for photocycloadditions involving alkenylboronate esters[J]. Journal of the American Chemical Society, 2025, 147(11): 9091-9097.

[20] Guo Y, Li Y, Chen S, et al. Scalable total synthesis of saxitoxin and related natural products[J]. Nature, 2025.

[21] Fang R, Jiao Y, Xia T, et al. Synthesis of anhydro-dc-tetracyclic saxitoxin via a photoinduced cycloaddition[J]. JACS Au, 2025: jacsau.5c01028.