Natural Products

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A Synthetic Route to the Saxitoxin Skeleton: Synthesis of Decarbamoyl α-Saxitoxinol, an Analogue of Saxitoxin Produced by the Cyanobacterium *Lyngbya wollei***

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Dedicated to the researchers affected by the devastating 2011 earthquake of Japan

Saxitoxin (**1**), which was first isolated as a paralytic shellfish poison,[1] is a potent and specific blocker of voltage-gated sodium channels,[2] similar to tetrodotoxin, the puffer fish toxin. This unique biological activity prompted us to use saxitoxin and tetrodotoxin as biochemical tools for the study of voltage-gated sodium channels and other ion channels in the field of neurophysiology.[3] Recently, molecular genetic analyses have revealed that ten subtypes of voltage-gated sodium channels are expressed in mammals, and that these subtypes appear to have specific functions in different organs.[4] However, the specific functions of these channels have yet to be clarified. Our group is interested in developing a subtype-selective blocker of voltage-gated sodium channels based on natural compounds, such as tetrodotoxin and saxitoxin, and have previously established synthetic methods for tetrodotoxin and its analogues.[5] To expand the range of available candidate subtype-selective blockers against voltage-gated sodium channels, we are attempting to develop a novel synthetic route for saxitoxin and its analogues, including neo-saxitoxin, gonyautoxin 3, and zetekitoxin AB, which was isolated from a Panamanian golden frog (Figure 1).[6]

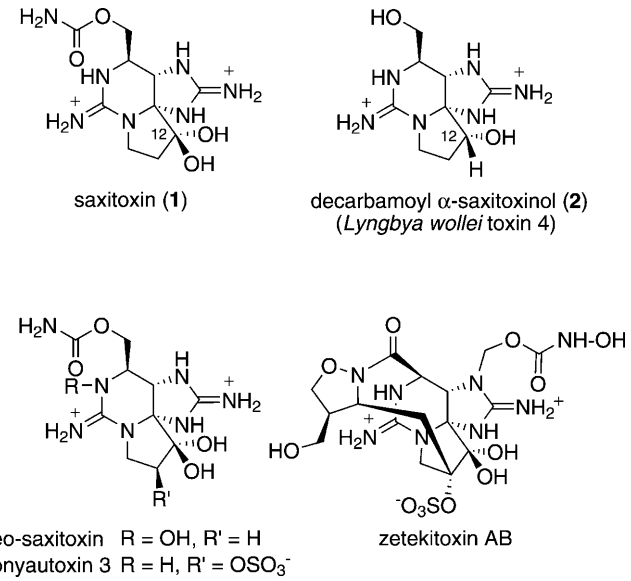

Figure 1. Structures of saxitoxin (**1**) and its naturally occurring analogues.

Since its structural elucidation in 1975, saxitoxin has been an attractive target molecule for total synthesis owing to its potent biological activity, as well as its unique densely functionalized structure, which features a bicyclic guanidinium condensed with a pyrrolidine and a hydrated form of the C12 carbonyl group. The total synthesis of saxitoxin was first reported by Kishi and co-workers in 1977[7] and subsequently by Jacobi et al. in 1984.[8] During the last five years, Du Bois and co-workers,[9] and Nagasawa and co-workers[10] have independently studied the synthesis; these studies culminated in the total syntheses of saxitoxin and its analogues, including gonyautoxin 3. Herein, we describe a novel synthetic route for the saxitoxin skeleton; this route enabled us to synthesize decarbamoyl α-saxitoxinol (**2**),[11] which is a nontoxic, naturally occurring analogue of saxitoxin produced by the cyanobacterium *Lyngyba wollei*.

To synthesize saxitoxin (**1**) and its analogues, we developed a novel bromocyclization strategy for the syntheses of cyclic guanidines from simple acyclic substrates; five- and six-membered cyclic guanidines were formed from propargyl and homopropargyl guanidine, respectively.[12] Scheme 1 shows an example of the bromocyclization; this reaction yielded the six-membered cyclic guanidine **4** possessing a spiro-aminal structure, which is fully functionalized for the saxitoxin skeleton. However, subsequent reduction of the azide group of **4** in preparation for the installation of the second guanidine functional group proved to be difficult, probably because of the severe steric hindrance arising from the geminal dibromo substituents. In the course of these synthetic experiments, we incidentally found a novel reaction involving the transformation of the *gem*-dibromomethylene group of **4** into a carbonyl functionality, as shown in Scheme 1. In this reaction, dibromo spiro-aminal compound **4** was treated with acetic anhydride and triethylamine in dichloromethane at room temperature to afford enol acetate **5** as an unstable product,[13] and alcohol **6**[14] was isolated after a reduction with $NaBH_4$ and a

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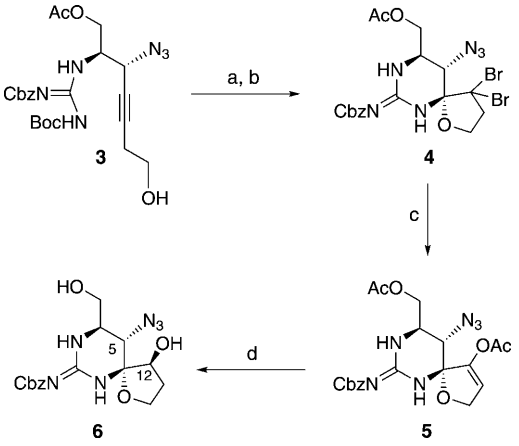
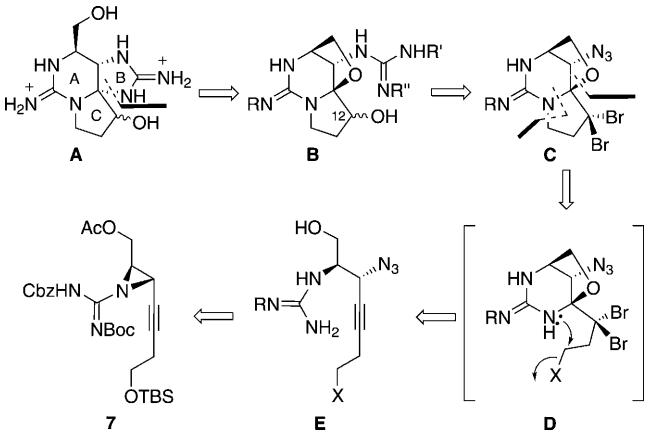


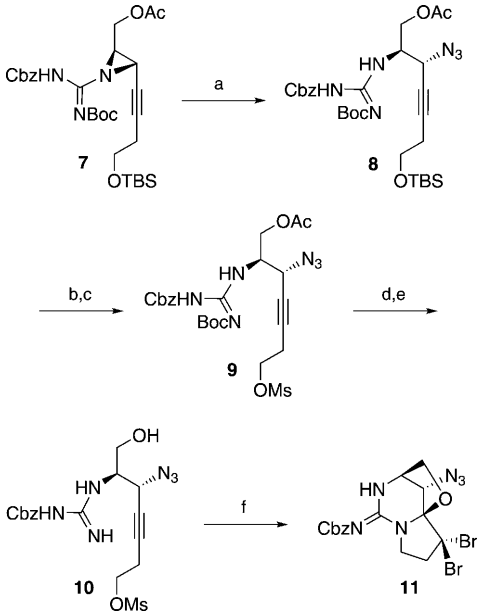
Scheme 1. Bromocyclization for the synthesis of the cyclic guanidine **4** and the novel transformation of *gem*-dibromomethylene into a carbonyl functionality. Reagents and reaction conditions: a) TFA, CH_2Cl_2, RT, 2 h; b) $PyHBr_3$ (5 equiv), K_2CO_3 (10 equiv), CH_2Cl_2, H_2O, RT 1 h, 71% (for 2 steps); c) Ac_2O, Et_3N, CH_2Cl_2, RT, 30 min; d) $NaBH_4$ (2 equiv), MeOH, RT, 10 min; then K_2CO_3 (1.3 equiv), RT, 1 h, 64% (for 2 steps). Boc = *tert*-butyloxycarbonyl, Cbz = benzyloxycarbonyl, Py = pyridine, TFA = trifluoroacetic acid.

subsequent hydrolysis of the acetate with K_2CO_3 in methanol. However, further efforts to synthesize the saxitoxin skeleton from **6** were unsuccessful.

Based on the above results, we devised an alternative synthetic route for the saxitoxin skeleton, as shown in Scheme 2. We envisaged that the tricyclic skeleton **A** of saxitoxin would be formed by a guanidine cyclization of **B** via an iminium ion intermediate under acidic conditions.[15] Precursor **B** could then be prepared from the tricyclic intermediate **C** by the transformation of the *gem*-dibromomethylene group under the reaction conditions mentioned above followed by the installation of the guanidine functionality. We planned to synthesize **C** by a cascade cyclization of **E**, which was initiated by a bromonium ion, as the expected oxaazabicyclo[3.2.1] product **D** would undergo an intramolecular N alkylation to give pyrrolidine, which corresponds to the C ring of saxitoxin. Precursor **E** could then be prepared from guanidino aziridine **7**, an intermediate for the synthesis of **3**, as described in our previous report.[12]



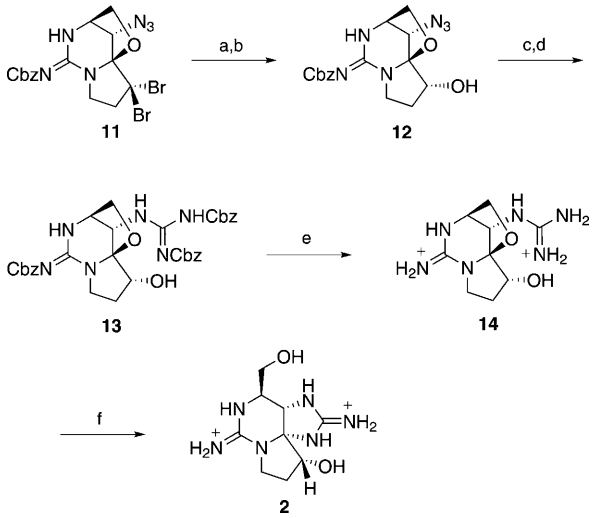
Scheme 2. Synthetic plan for the saxitoxin skeleton. TBS = *tert*-butyldimethylsilyl.



Scheme 3. Synthesis of **10** and its bromocyclization to give tricyclic compound **11**. Reagents and reaction conditions: a) NaN_3 (1.5 equiv), DMF, RT, 4.5 h; b) TBAF (1.5 equiv), THF, RT, 30 min; c) MsCl (1.05 equiv), Et_3N (3 equiv), CH_2Cl_2, 0°C to RT, 40 min; d) KCN (1.05 equiv), EtOH, RT, 12 h; e) TFA, CH_2Cl_2, RT, 2 h; f) $PyHBr_3$ (3 equiv), K_2CO_3 (10 equiv), CH_2Cl_2, H_2O, RT, 1 h, 24% for 6 steps. DMF = *N*,*N*-dimethylformamide, TBAF = tetrabutylammonium fluoride, THF = tetrahydrofuran, Ms = methanesulfonyl.

The precursor for the bromocyclization reaction (**10**), which has a similar structure to **E**, was synthesized from **7** in five steps, as shown in Scheme 3. Compound **7** underwent ring opening with NaN_3 in *N*,*N*-dimethylformamide at room temperature, and the siloxy group of product **8** was transformed to the corresponding mesylate **9** in two steps by desilylation and mesylation under conventional reaction conditions. Sequential deprotection of the acetate with KCN in EtOH[16] and the Boc group with trifluoroacetic acid yielded **10**, which served as the precursor for the bromocyclization. As anticipated, upon treatment of **10** with pyridinium tribromide ($PyHBr_3$) in a biphasic medium of CH_2Cl_2 and aqueous K_2CO_3 at room temperature, the key bromocyclization reaction and subsequent intramolecular N alkylation took place to afford **11** as a single product. Since intermediates **9** and **10** were unstable, the optimal result was obtained when these intermediates were not purified, and this approach allowed for the preparation of **11** in 24% overall yield in 6 steps from **7** (average yield of 79% in each step).

After obtaining the key intermediate **11**, we next examined the transformation of the *gem*-dibromomethylene group of **11** into a carbonyl functionality under the reaction conditions used in Scheme 1. Using this approach, compound **11** was successfully transformed into alcohol **12**[17] in two steps: 1) Ac_2O, Et_3N in CH_2Cl_2 and 2) reduction of the resulting enol acetate with $NaBH_4$ in MeOH (Scheme 4). The azide group of **12** was then reduced under Staudinger conditions (PMe_3 in CH_2Cl_2 and subsequent hydrolysis with aqueous HCl and MeOH) followed by a conventional



Scheme 4. Synthesis of decarbamoyl α-saxitoxinol (**2**). Reagents and conditions: a) Ac_2O, Et_3N, CH_2Cl_2, RT, 15 min; b) $NaBH_4$ (30 equiv), MeOH, RT, 30 min, 32% (for 2 steps); c) Me_3P (3 equiv), CH_2Cl_2, RT, 30 min; then MeOH/12 M HCl (5:1), RT, 30 min; d) CbzN=C(SMe)-NHCbz (2 equiv), $HgCl_2$ (2 equiv), Et_3N (10 equiv), DMF, 60 °C, 51% (for 2 steps); e) H_2 (1 atm), 10% Pd/C, MeOH, EtOAc, RT, 27 h; f) $B(OCOCF_3)_3$, TFA, RT, 24 h, 73% (for 2 steps).

guanidinylation to give **13**. Deprotection of the benzyloxycarbonyl groups under hydrogenolytic conditions yielded **14**, a precursor for the next cyclization reaction. Upon treatment with $B(OCOCF_3)_3$ in TFA at room temperature,[18] **14** underwent ring opening of the hemiaminal and subsequent cyclization of the second guanidine to furnish decarbamoyl α-saxitoxinol **2** (*L. wollei* toxin 4), a metabolite of the cyanobacterium *L. wollei*, in 73% yield. The spectroscopic data of compound **2** synthesized in this study were in good agreement with those of the natural product.[11]

In summary, we have developed a concise synthetic route for the saxitoxin skeleton, featuring two key reactions: cascade cyclization to give tricyclic intermediate **11**, and transformation of the *gem*-dibromomethylene group into enol acetate. Because the hemiaminal of **11** is stable owing to the electron-withdrawing nature of the *gem*-dibromo substituents, the intermediate should be suitable for the incorporation of additional functionality, which will provide diverse analogues of saxitoxin for the development of subtype-selective blockers of voltage-gated sodium channels. Further studies toward the synthesis of other saxitoxin analogues, such as gonyautoxin 3 and zetekitoxin AB, employing this method are currently under investigation in our laboratory.

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[13] The generality and mechanistic studies of this reaction are currently under investigation in this laboratory.

[14] The configuration of the C12-position was determined by NOESY correlation between protons of the C5- and C12-positions. See the Supporting Information.

[15] Du Bois reported the synthesis of a similar compound (ketone at C12-position) to **B**, however, cyclization of the five-membered guanidine was unsuccessful. We speculated that the hydrate functionality (ketone equivalent) at the C12-position exhibited resistance to the formation of an iminium ion intermediate owing to the electron-withdrawing nature of the hydrate (see Ref. [8b]). We anticipated that **B** could be a substrate for this cyclization reaction because of the weak electron-withdrawing nature of the hydroxy group at the C12-position.

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[17] The configuration of the C12-position was determined by analysis of NOESY spectra of **13**. See the Supporting information.

[18] The conditions were identical to those employed in the total syntheses of saxitoxin by Du Bois and co-workers, and Nagasawa and co-workers (see Ref. [9a–c and 10b].

