Synthesis of Cyclic Guanidine Intermediates of Anatoxin-a(s) in Both Racemic and Enantiomerically Pure Forms

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Abstract: The alkyl chain of anatoxin-a(s) (cyclic guanidines), which can be used as an intermediate in the total synthesis of anatoxin-a(s), was synthesized in both racemic and enantiomerically pure forms. These enantiomerically pure cyclic compounds can be used as chiral inductors in some reactions. The two racemic routes disclosed herein have the advantages of high overall yield and mild reaction conditions. Both routes proceed through an intermediate 2,3-diaminoacid – an important synthetic scaffold – with good yields. Furthermore, the *N*,*N*-dimethyl-2(tosylimino)imidazolidine-4-carboxamide might be obtained from 2-(tosylimino)imidazolidine-4-carboxylic acid followed by selective reduction of the carbonyl functionality. All synthesized compounds were analyzed by mass spectrometry and ^{1}H NMR and ^{13}C NMR spectroscopy.

Key words: cyclic guanidines, anatoxin-a(s), neurotoxin, racemic synthesis, chiral synthesis

Anatoxin-a(s) is a phosphonate ester of cyclic *N*-hydroxyguanidine, which is considered to be the most potent natural acetylcholinesterase inhibitor known.[1] Anatoxin-a(s) irreversibly binds the acetylcholinesterase enzyme, which would normally hydrolyze acetylcholine. As a result, sodium channels remain permanently open, producing a flow of sodium ions into neurons, which generate action potentials until energetic exhaustion of the nerve cell occurs.[2] Anatoxin-a(s) is a very potent inhibitor, with very low LD_{50} (20 μg/kg in mouse i.p.). Intoxication of vertebrates by anatoxin-a(s) after ingestion of cyanobacteria-contaminated waters can lead to death.[3] This alkaloid was first reported by Mahmood and Carmichael in 1986 and is produced mainly by *Anabaena flosaquae* and *Anabaena lemmermanniin*.[4] Later, the same authors reported its chemical structure.[5] The chemical structure of anatoxin-a(s) – with emphasis on the alkyl chain – is shown in Figure 1.

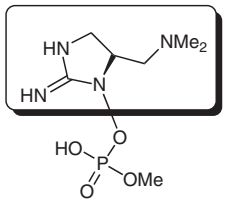

Figure 1 Chemical structure of anatoxin-a(s)

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The described methods for qualitative analysis of this hazardous toxin are based on unspecific acetylcholinesterase inhibition measurements using spectrophotometric or electrochemical techniques.[6]

Recently, cyclic guanidines have attracted considerable attention due to their presence in several natural products. Representative compounds such as dibromophakellin[7] and the potent neurotoxin saxitoxin[8] are shown in Figure 2. These two natural products are good examples of the molecular complexity found in many cyclic guanidine-containing compounds.

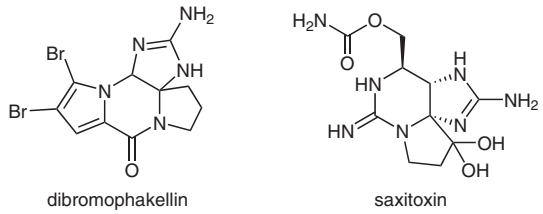


Figure 2 Two natural products with cyclic guanidine in its chemical structure

Because of their hydrogen-bonding ability, these molecules are also used as pharmacophores, for instance as coagulation factor Xa inhibitors.[9]

Enantiomerically pure guanidines have found applications as chiral base catalysts for asymmetric processes[10] and as chiral superbases for asymmetric synthesis as well.[11]

The value of asymmetric synthesis is clear, especially because of the gain in organic synthesis when dominated this characteristic. These types of methods are of great importance in the preparation of compounds with pharmacological functions because many of these molecules have specific sites in which stereochemical definition is necessary for activity. However, some methodologies for the synthesis of enantiomerically pure compounds are very tedious and exhaustive. On the other hand, routes in which racemic materials are formed are normally less expensive, faster, and lead to a better overall yield.

Our research group has been interested in synthetic routes to cyanotoxins, as well as their use as internal standards for analytical protocols by GC-MS and LC-MS.[12] Therefore, the aim of this work was to determine a more attractive route, either chiral or racemic, for the synthesis of the

alkyl chain present in anatoxin-a(s). In this endeavor, we also wanted to develop a synthesis of a 2,3-diamino acid as a potential intermediate in the synthesis of anatoxin-a(s).

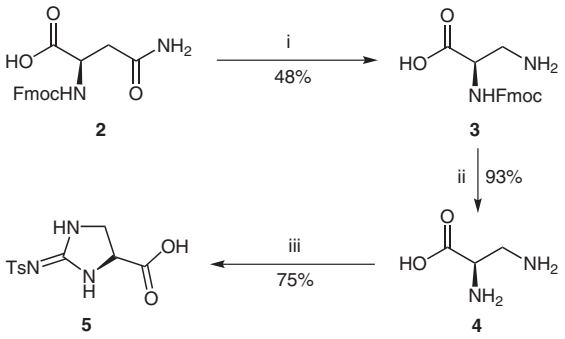
The chiral synthesis of 2,3-diamino acids has gained the attention of researchers in the last few years because of their versatile applications. Using classical Mitsunobu conditions for the synthesis of the lactone intermediate, Strazzolini et al.[13] prepared 2,3-diamine with 22% yield over four steps. They used 1-arylaziridine-2-carboxamides that were obtained from *Rhodococcus rhodochrous* IFO 15564-catalyzed hydrolysis. These carboxamides were used as starting intermediates in the synthesis of the chloride of (*R*)-2,3-diamino acid with good enantiomeric excess but poor yield.[14] In the same way, *N*-arylsulfonyl aziridines were reacted with chiral isocyanates. Subsequent hydrolysis of the 2-imidazolidinones intermediates gave the desired diamino acids as racemic mixtures in good yields but with variable diastereoselectivities.[15]

Several approaches to the synthesis of cyclic guanidines have appeared in the literature. One of the more efficient methods for the synthesis of these cyclic molecules involves reacting L-α,β-diaminopropionic acid with cyanogen bromide in alkaline media.[16] The major drawback of this method is the toxicity of cyanogen halides. Isobe et al.[17] described the synthesis of N-acylated guanidines through the cyclization of thioureas or ethoxycarbonylated thioureas using 2-chloro-1,3-dimethylimidazolinium chloride (DMC). Finally, the same authors reported the conversion of an amino ester to fused cyclic guanidines over several steps.

In the same way, some solid-phase processes have been published. One of these procedures employed combinatorial chemistry techniques that led to a one-step synthesis of alkylated analogues with high purity but poor yields.[18] Acharia et al.[19] described the reaction of aza-Wittig reagents derived from azidobenzoic acids with bifunctional amines to produce guanidines. However, this method is limited by the instability of the starting materials.

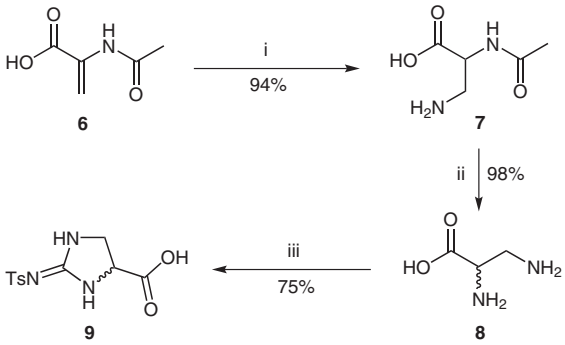
The most concise route to stereodefined 4-carboxyamideguanidines begins with amino acids. Carmichael employed asparagine to prepare these toxins for the purposes of identification and elucidation of absolute configuration.[5]

Following the same strategy, the first step in our modified route to the synthesis of the enantiomerically pure alkyl chain of anatoxin-a(s) was Fmoc protection of L-asparagine in 83% yield (Scheme 1).[20] Fmoc-*N*-L-asparagine (**2**) was then treated with [bis(trifluoroacetoxy)iodo]benzene,[21] which mediated a Hoffmann rearrangement to provide **3** in 48% yield. Subsequently, this compound was deprotected[22] to afford 2,3-diamino acid **4** as a single enantiomer. Finally, **4** was cyclized with *S*,*S*′-dimethyl-*N*-tosyliminodithiocaronimidate[23] generating the cyclic *N*-Ts-guanidine **4** with an overall yield of 27.8% over four steps.



Scheme 1 *Reagents and conditions*: (i) $[CF_3COO]_2IPh$, pyridine, H_2O,AcOH, ACN, 4 h; (ii) Et_2NH, EtOH, 6 h; (iii) $TsNC(SMe)_2$, NaOH, H_2O, 5 h.

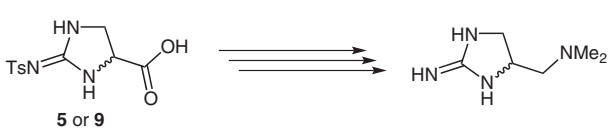
The production of molecules in racemic form has been used in several areas of interest. This strategy can lead to gains in overall efficiency. As such, we also wanted to show that cyclic *N*-Ts-guanidine acid **5**, which was prepared in enantiomerically pure form from asparagine, can be obtained from acetoamidoacrylic acid in higher overall yield. Thus, our racemic synthesis began from compound **6**, which was elaborated by way of a simple Michael addition to obtain **7** in excellent yield (94%).[24] The acetamide was then hydrolyzed to generate **8**,[25] which was transformed into racemic cyclic *N*-Ts-guanidine **9**, as reported earlier, in 69% overall yield.



Scheme 2 *Reagents and conditions*: (i) NH_4OH, 24 h, 60 °C; (ii) HCl, 4 h, 80 °C; (iii) $TsNC(SMe)_2$, NaOH, H_2O, 5 h.

In order to finish the alkyl chain of anatoxin-a(s), **5** or **9** could be envisioned to undergo amidation, followed by selective reduction of the amide carbonyl with Red-Al. Subsequent removal of the tosyl group could then give the key cyclic guanidine intermediate necessary for the total synthesis of anatoxin a(s) (Scheme 3).

In conclusion, we have demonstrated two different methods for obtaining key alkyl intermediates of anatoxin-a(s), which are practical procedures for the synthesis of other



Scheme 3

cyclic guanidines. The choice between racemic or stereospecific synthesis will depend on the application of the obtained products. Our stereospecific route, which was a modification of a route from L-asparagine previously carried out, provided the cyclic guanidine product in almost 28% overall yield over four steps. The racemic form of the same compound could be prepared from acetoamidoacrylic acid in 69% yield over three steps.

The 2,3-diamino acid **8** was obtained in higher yield through these new procedures than had been reported previously. Thus, these methods should find widespread application in the production of this important scaffold.

The preparation of the racemic forms, like was prepared here from acetoamidoacrylic acid (Scheme 2), is expected to find application in cases where the production of enantiomerically pure analytical standards is not cost-effective. These products can also be useful as internal standards in analytical techniques associated with mass spectrometry, where chiral resolution is not necessary. The procedures presented in this work can be used for the production of modified alkaloid anatoxin-a(s), thereby aiding identification. Since the current method for anatoxin-a(s) analyses is by the acetylcholinesterase inhibition assay, the products obtained can be used as internal standards in new and more reliable analytical methods. In addition, these products can be helpful in the elucidation of the mechanism of action of anatoxin-a(s).

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(22) Zvilichovsky, G.; Gurvich, V. *Tetrahedron* **1997**, *53*, 4457.
(23) **Synthesis of Compound 5 or 8: 2-(Tosylimino)-imidazolidine-4-carboxylic Acid**
The 2,3-diamine acid (312 mg, 3 mmol) was dissolved in H_2O (5 mL) and 1 N NaOH (10 mL) was added. *S*,*S*-Dimethyl-*N*-tosyliminoditiohcarbonimidate (0.813 mg, 3 mmol) in EtOH was added. The mixture was refluxed for 15 h. With reduction of original volume in one-third, the product precipitated. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.33 (3 H, s), 3.68 (2 H, dd, J_1 = 7.2 Hz), 4.04 (1 H, dd, J_1 = 7.8 Hz), 7.21 (2 H, d, J = 8.0 Hz), 7.83 (2 H, d, J = 7.3 Hz) ppm. ^{13}C NMR (300 MHz, $CDCl_3$): δ = 20.4, 57.3, 61.8, 125.1 (2 C), 127.0 (2 C), 134.2, 143.7, 143.9, 165.9 ppm. ESI-MS: *m/z* = 284 $[M + H]^+$, 238 $[M - COOH]^+$, 155 $[SO_2PhCH_3]^+$, 91.2 $[PhCH_3]^+$.
(24) **Synthesis of Compound 7: 2-Acetamido-3-amino-propanoic Acid**
α-Acetamidoacrylic acid (1.5 g) was dissolved in a solution of NH_4OH (37%, 50 mL). This mixture was allowed to stand at 40 °C for 72 h. The amine excess was removed for evaporation in vacuum. This compound was used without purification in the next step.
(25) **Synthesis of Compound 8: (*R*,*S*)-2,3-Diaminopropanoic Acid**
The compound **6** was hydrolyzed by boiling for 2 h with a solution of 2 N HCl (30 mL). ^{1}H NMR (300 MHz, D_2O): δ = 3.04 (1 H, dd, J_1 = 7.2 Hz), 3.15 (1 H, dd, J_1 = 7.2 Hz), 3.74 (1 H, dd, J_1 = 7.2 Hz). Mp 225–227 °C.