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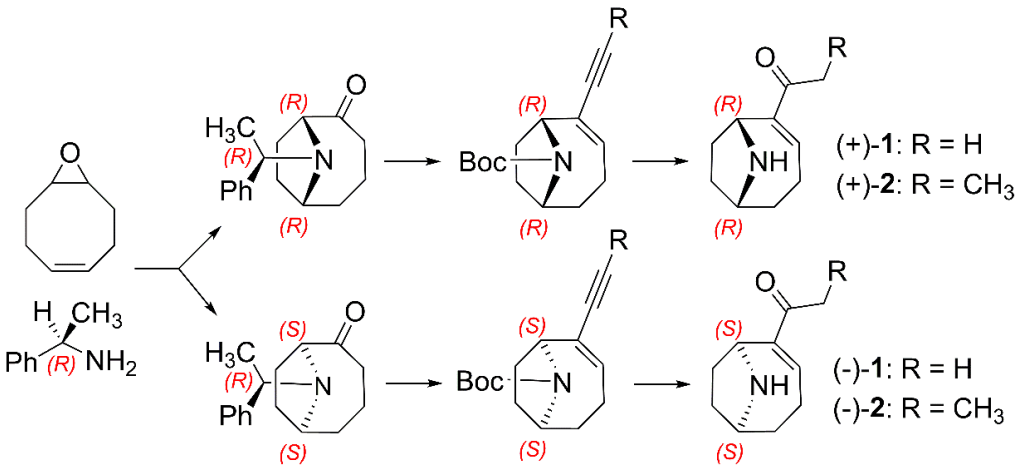
Graphical Abstract



Title:

A unified approach to the synthesis of both enantiomers of anatoxin-a and homoanatoxin-a cyanotoxins

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Abstract:

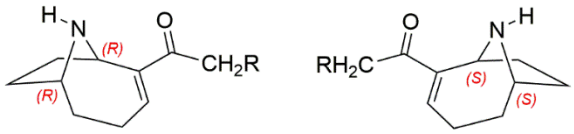
Anatoxin-a and homoanatoxin-a are highly neurotoxic compounds produced by cyanobacteria, principally during surface water-blooms (SWBs). Owing to their powerful biological activity and unique structural characteristics, these natural alkaloids have been the subject of extensive research work in both pharmacological and synthetic studies. In this contribution we report a simple and efficient synthetic approach for the preparation of both the natural and unnatural enantiomers of these cyanotoxins, [(+)-**1** and (+)-**2**] and [(−)-**1** and (−)-**2**] respectively. Key features of this approach include: i) construction of the azabicyclic homotropane framework in both enantiomeric forms from *cis*-5,6-epoxycyclooctene, based on a microwave mediated epoxide ring opening reaction by a chiral benzyl amine followed by a transannular amine-alkene cyclization; ii) elaboration of the characteristic methyl or ethyl enone by means of a Sonogashira cross-coupling reaction of an enol-triflate with a C2 or C3 terminal alkyne, followed by a chemo- and regio-selective hydration of the resulting conjugated enyne group.

Keywords:

Homotropane; Epoxide ring opening–transannular cyclization; Sonogashira coupling; Regioselective enyne hydration

1. Introduction

(+)-Anatoxin-a [(+)-**1**], initially called "Very Fast Death Factor (VFDF)" due to its ability to induce death within a few minutes after intraperitoneal injection in mice,[1] and structurally related (+)-homoanatoxin-a [(+)-**2**] are cyanobacterial neurotoxins produced by several species of both benthic and planktonic cyanobacteria (formerly known as blue-green algae).[2,3] They are potent neuromuscular blocking agents which act as a nicotinic (cholinergic) agonist by binding to neuronal nicotinic acetylcholine receptors (nAChR) and blocking the electrical signals between nerve cells, which may result in respiratory paralysis, asphyxiation and ultimately death. (+)-Anatoxin-a is approximately 50 times more potent than (−)-nicotine and 20 times more potent than acetylcholine.[4,5] This makes (+)-anatoxin-a one of the most potent agonists of nicotinic acetylcholine receptors and the smallest toxic alkaloid so far characterized. (+)-Homoanatoxin shares almost identical toxicological properties.[6] Both neurotoxins are mainly produced in cyanobacterial surface water blooms and are responsible of several incidents of fatal poisoning of cattle, domestic animals, and wildlife, as well as adverse human health effects.[7,8] Currently, different countries have begun to take action by developing regulations and implementing controls for these toxins in drinking and recreational waters.[9]



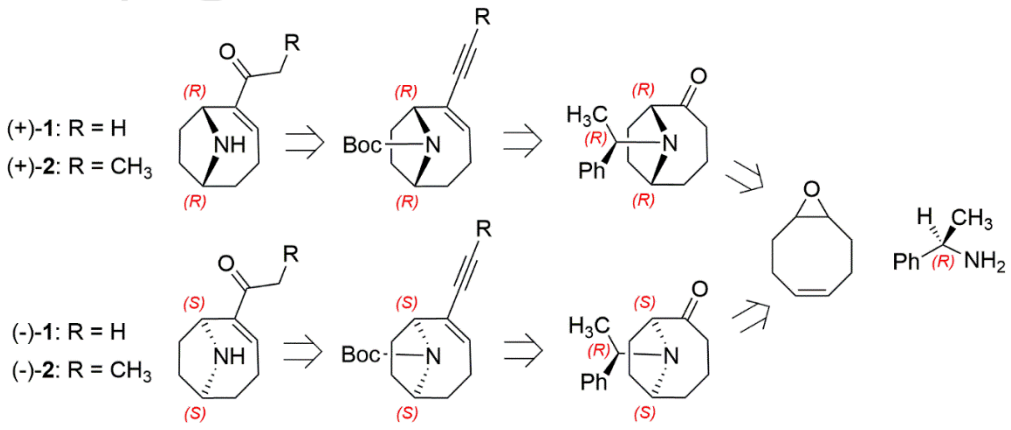
(+)-**1**: R = H anatoxin-a (-)-**1**: R = H
(+)-**2**: R = CH_3 homoanatoxin-a (-)-**2**: R = CH_3

Figure 1. Anatoxin-a and homoanatoxin-a enantiomer structures

Owing to their powerful biological activity and unique structural characteristics – the bicyclic homotropane moiety is exclusive of these natural alkaloids[10] – anatoxin-a, and to a lesser extent, homoanatoxin-a, have been the subject of extensive research work in both pharmacological[11,12,13] and synthetic studies.[14,15,16] Concerning anatoxin-a, numerous synthetic strategies have been described so far involving different approaches for the construction of the 9-azabicyclo[4.2.1]nonane ring system, including: ring expansion of tropanes, transannular cyclization of amino-cyclooctenes derivatives, intramolecular cyclization of iminium cations, intramolecular alkylation reaction, cycloaddition reactions,

enyne metathesis, and carbenoid insertion into amide C-N bonds.[14,17,18] Most of these syntheses are racemic, but some of them are enantiospecific leading to (+)-anatoxin-a[19] and/or, in some cases, to the unnatural enantiomeric form, (−)-anatoxin-a,[19b,19e,19f,9i,20] in high enantiomeric purity. In contrast, only two racemic syntheses have been described so far for homoanatoxin-a. In the first one, published before its isolation from natural sources, homoanatoxin was prepared as a synthetic analogue of (±)-anatoxin-a via alkylation of the methyl carbonyl moiety of its *N*-Boc derivative.[21] In the second one, the synthesis of racemic homoanatoxin-a was completed using a cyclization reaction between an in-situ generated enol silyl ether and a sulfonyliminium cation as the key step for the construction of the azabicyclic ring system.[22]

In the context of a project aimed at developing an immunoassay for (+)-anatoxin-a,[23] we required anatoxin-a and homoanatoxin-a in both enantiomeric forms. Here, we describe the development of a unified synthetic approach for the preparation of these compounds, which is one of the simplest strategies published so far. This approach relies on the previous work of Carroll *et al*.[24] for the construction of the chiral 9-azabicyclo[4.2.1]nonane ring system and on a new strategy for the elaboration of the methyl and ethyl enone moieties. According to the retrosynthetic analysis shown in Scheme 1, the enone group of both anatoxin-a and homoanatoxin-a can be generated through a regioselective hydration of the appropriate conjugate enyne moiety, in turn available from a carbonyl group through coupling of the corresponding enol triflate derivative with a C2 or C3 alkyne. As previously described, the azabicyclic ketone framework can be prepared in each of the two required enantiomeric forms from commercially available prochiral *cis*-5,6-epoxycyclooctene and (*R*)-(+)-α-methylbenzylamine through an epoxide ring opening–transannular cyclization sequence.



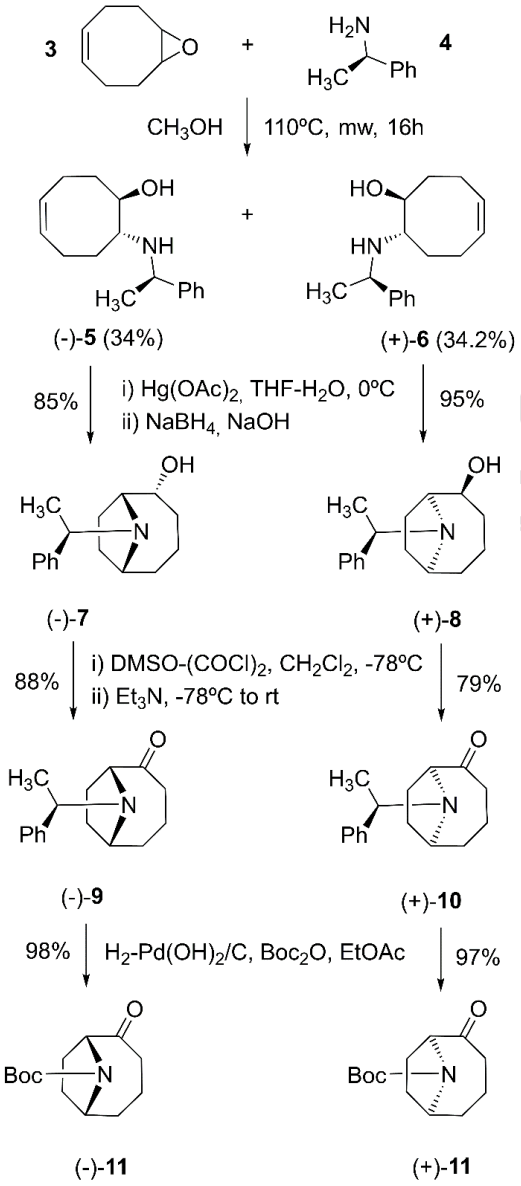
Scheme 1. Retrosynthetic approach to both enantiomers of anatoxin-a (**1**) and homoanatoxin-a (**2**)

2. Results and discussion

As mentioned above, the preparation of the (1*R*,6*R*) and (1*S*,6*S*) enantiomers of the 9-aza-bicyclo[4.2.1]nonane ring system (homotropane) was based on the work developed by Carroll *et al*. for the preparation of this moiety during the synthesis of nicotinic acceptors of acetylcholine based on the pyrido[3,4-b]homotropane skeleton.[24] According to this approach, the preparation of the homotropane skeleton of enantiomeric azabicyclic ketones (−)-**11** and (+)-**11**, which are common intermediates for the preparation of anatoxin-a and homoanatoxin-a in their natural and unnatural forms, respectively, started from the prochiral epoxide 9-oxabicyclo[6.1.0]non-4-ene (**3**), whose desymmetrization was carried out by bimolecular nucleophilic opening of the oxirane ring with chiral (*R*)-(+)-α-methylbenzylamine (**4**) using a modification of the original procedure involving microwave irradiation, with simultaneous external air-cooling, of a methanolic solution of equimolecular amounts of **3** and **4** (Scheme 2). These conditions substantially shorten the reaction times previously reported for this transformation, providing in only 16 h a nearly 1:1 mixture of diastereoisomeric amino-alcohols **5** and **6**, readily separable in a high degree of diastereomeric purity via a combination of crystallization and column chromatography.

Preparation of the homotropane skeleton corresponding to each enantiomeric series was undertaken in parallel from the corresponding diastereoisomeric amino-alcohol. First, the construction of the nitrogen bridge was carried out by means of an intramolecular cyclization reaction between the amino nitrogen and the double bond promoted by Hg^{2+}. The initially formed mercurial intermediate was reduced in situ with sodium borohydride to afford amino-alcohols **7** or **8** from **5** or **6**, respectively, in high yield. It is important to note that, in contrast to the originally described procedure, the aminomercuration reaction requires only 30 min at 0 °C to complete; an increase in the reaction time and/or temperature prior to the demercuration step with sodium borohydride results in a significant decrease in the yield of the azabicyclic alcohol. Subsequent oxidation of the secondary hydroxyl group of amino-alcohols **7** and **8** to the corresponding ketone under Swern conditions followed by amine protecting-group exchange through hydrogenolysis of the benzylamino moiety in the presence of di-*tert*-butyl dicarbonate provided *N*-Boc ketones (−)-**11** and (+)-**11**. All spectroscopic data of both enantiomeric *N*-Boc ketones correspond with those previously described for the racemic compound.[18] It should be noted that, as earlier observed for this and other *N*-Boc derivatives of the azabicyclic system,[19b] peak

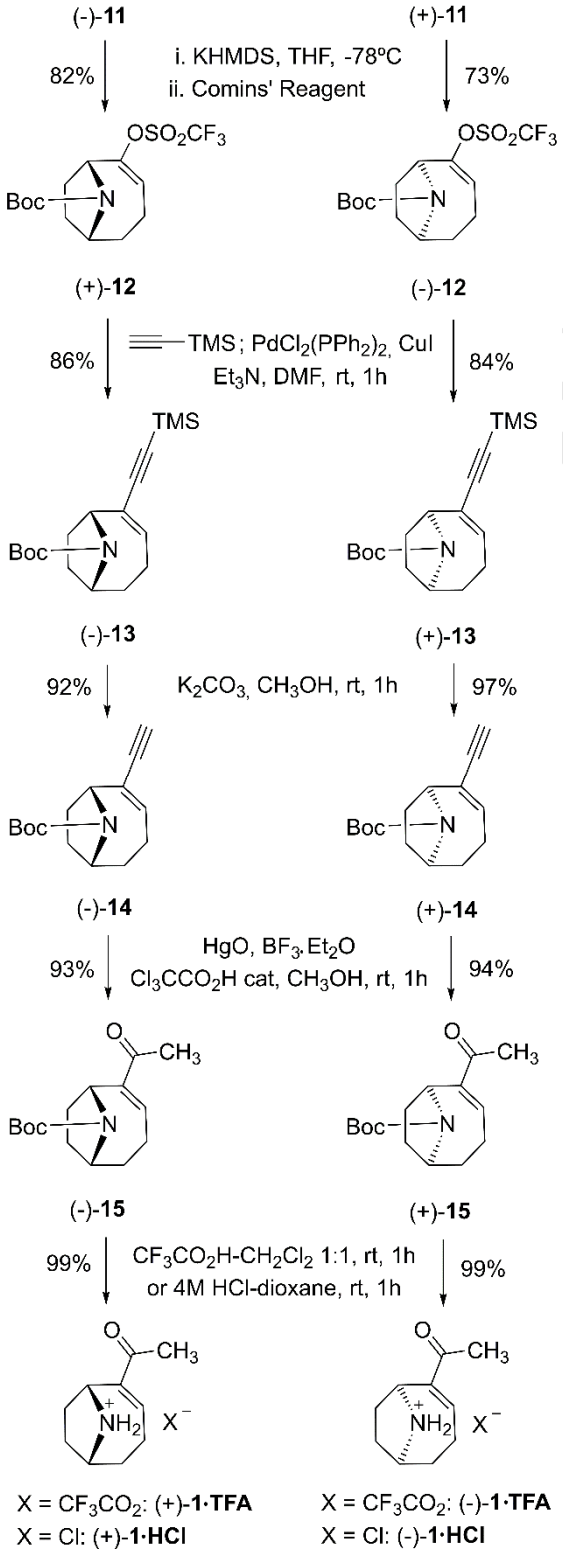
doubling or broadening is observed in the NMR spectra owing to the presence of rotational isomers of the carbamate moiety (about a 2−3:1 mixture of rotamers).



Scheme 2. Synthesis of (1*R*,6*R*) and (1*S*,6*S*) enantiomeric homotropane frameworks

Once the homotropane skeleton was available in both enantiomeric forms, the elaboration of the characteristic methyl enone group of (+)- and (−)-anatoxin-a was addressed. First, the carbonyl group of *N*-Boc ketones (−)-**11** and (+)-**11** was transformed into an enyne moiety. This was done via the Pd(0)-catalyzed Sonogashira cross-coupling reaction of the corresponding enol triflate, readily obtained by trapping of the kinetic potassium enolate with Comins' reagent under standard conditions, with trimethylsilylacetylene, a convenient liquid acetylene surrogate (Scheme 3).[25] The terminal

alkyne group of thus obtained trimethylsilyl enynes (−)-**13** and (+)-**13** was unmasked upon treatment with base in MeOH at room temperature, to afford enynes (−)-**14** and (+)-**14** in 65% and 60% overall yield, respectively, for the three step sequence.



Scheme 3. Synthesis of (+)- and (-)-anatoxin-a trifluoroacetate and hydrochloride salts

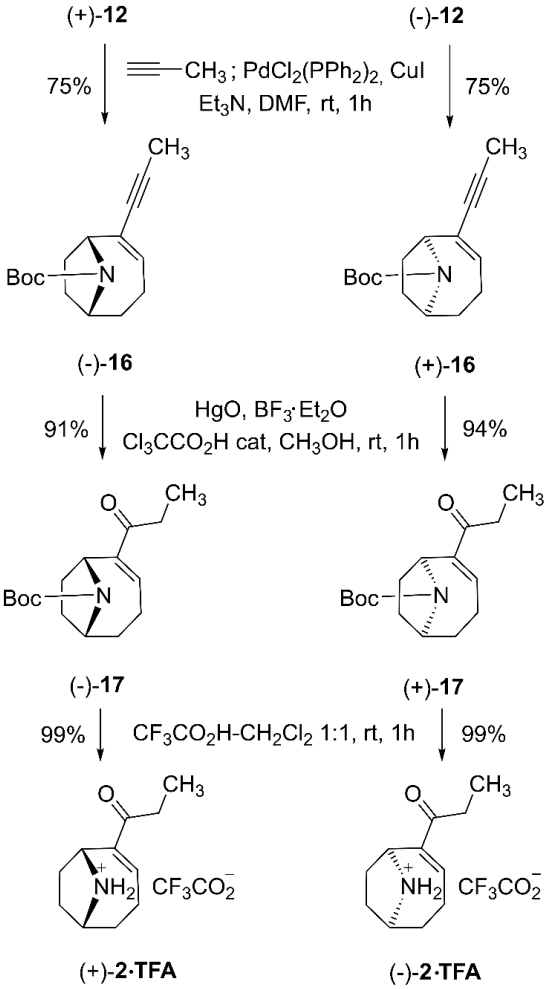
After completing the formation of the enyne moiety, the triple bond was hydrated to generate the corresponding ketone carbonyl group. This transformation was effected chemo- and regioselectively by treatment of the enyne compound with a methanolic solution of mercury(II) oxide and boron trifluoride etherate in the presence of catalytic amounts of trichloroacetic acid (0.2–0.3 equivalents relative to HgO). Under these conditions, the triple bond hydration reaction proceeds very efficiently, providing, after aqueous work up and purification by column chromatography, the corresponding methyl enone, (−)-**15** or (+)-**15**, with an excellent yield. The effectiveness of this catalytic system (Hennion-Nieuwland catalysts)[26,27] for the hydration of the triple bond was much higher than that of other catalyst systems tested, *e.g.* HCO_2H-H_2O, $PdCl_2$/MeCN-H_2O, $Pt_2Cl_4(C_2H_4)_2$/THF-H_2O or $NaAuCl_4$/MeOH-H_2O.[28] The hydration reaction proceeds through the initial formation of a dimethyl ketal and/or methyl enol ether, from the addition of a single molecule of MeOH to the triple bond, that are hydrolyzed to the ketone carbonyl group during the aqueous work up. In fact, small percentages of dimethyl ketal and/or enol-ether intermediates could be identified in the ^{1}H NMR spectra of the hydration crude product of some reaction batches. The physical and spectral data of enantiomeric enones (−)-**15** and (+)-**15**, which are the *N*-Boc derivatives of natural and unnatural anatoxin-a, respectively, were identical to those previously reported.[19b] The enantiomeric purity of both *N*-Boc derivatives of anatoxin-a was found to be >99%, based on chiral HPLC analysis (see experimental part).

Finally, the synthesis of both enantiomers of anatoxin-a was completed by removal of the *N*-Boc protecting group in acidic medium. Thus, treatment of (−)-**15** and (+)-**15** with a 1:1 mixture of trifluoroacetic acid and CH_2Cl_2 at room temperature directly afforded the corresponding trifluoroacetate salts, (+)-**1·TFA** and (−)-**1·TFA**, respectively. Alternatively, treatment of (−)-**15** and (+)-**15** with 4M HCl in dioxane at room temperature gave the equivalent hydrochlorides, (+)-**1·HCl** and (−)-**1·HCl**, whose physical and spectroscopic data were in agreement with those previously reported in the literature.[19b,19i,19m]

The synthesis of homoanatoxin-a in each of the enantiomeric forms was carried out according to a synthetic route similar to that used for the preparation of anatoxin-a, which was based on the Sonogashira reaction of the same intermediate enoltriflate, (+)-**12** and (−)-**12** – in this case with propyne – followed by hydration reaction of the triple bond (Scheme 4). Apparently, as shown by TLC analysis of the reaction mixture, the reaction between the

enoltriflate (+)-**12** and propyne occurred quite efficiently using both propyne gas or a commercial 4% solution of propyne in DMF under relatively similar conditions to those described for the synthesis of anatoxin-a. However, in contrast to the analogous reaction of the same enoltriflate with trimethylsilylacetylene, the initially isolated cross-coupling product experimented a rapid degradation after work up and exposition of the crude extract to the air. In fact, after a few hours, TLC of the crude extract showed the nearly complete transformation of the initially formed enyne into a mixture of compounds from which a major, more polar compound, was isolated. It was tentatively characterized as epoxide (**i**) on the basis of its molecular formula and NMR data (Figure 2); in particular, the CH-epoxide signal in the ^{1}H NMR spectrum (a broad doublet at δ 3.17 ppm) and the signals of the epoxide carbon atoms in the ^{13}C NMR spectrum (C-2 and C-4 at δ 57.4 and 62.1 ppm, respectively). We speculate with the possibility that the formation of epoxide (**i**) could be related to the use of a large excess of alkyne in this Sonogashira reaction, which could favor the formation of complexes of copper with propyne or hexa-2,4-diyne, formed via a Glaser-Hay homocoupling of propyne,[29] that could be extracted within the organic phase and catalyze the reaction of the enyne moiety with oxygen.[30] In fact, this transformation was substantially reduced by including a washing step with a strong copper complexing agent such as EDTA during the work up of the reaction mixture. With this modification, the cross-coupling reaction of enoltriflates (+)-**12** or (−)-**12** with propyne afforded the corresponding enynes, (−)-**16** or (+)-**16**, respectively, in about 75% yield after column chromatography.

With enantiomeric enynes (−)-**16** and (+)-**16** in hand, the synthesis of both enantiomers of homoanatoxin-a was readily completed following the same procedure that was used for the above described synthesis of structurally related anatoxin-a enantiomers. Thus, chemo- and regioselectively hydration of the triple bond of enynes (−)-**16** and (+)-**16** using the Hennion-Nieuwland catalytic system afforded *N*-Boc ethyl enones (−)-**17** and (+)-**17**, respectively, whose treatment with a 1:1 mixture of trifluoroacetic acid and CH_2Cl_2 at room temperature gave the corresponding trifluoroacetate salts of natural and unnatural homoanatoxina-a, (+)-**2·TFA** and (−)-**2·TFA** respectively, in 67−70% overall yield from the starting enol triflates. The spectroscopic NMR data of both homoanatoxin-a enantiomers were practically identical and consistent with those previously described for the natural product[31] and the synthetic racemic form.[21,22]



Scheme 4. Synthesis of (+)- and (-)-homoanatoxin-a trifluoroacetate salts

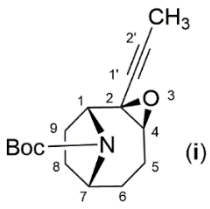


Figure 2

3. Conclusions

In summary, we have successfully established a simple approach for the preparation of both natural and unnatural enantiomers of the cyanotoxins anatoxin-a [(+)-**1** and (−)-**1**] and homoanatoxin-a [(+)-**2** and (−)-**2**]. This approach is based on two synthetic transformations:

first, the construction of the homotropane skeleton in both enantiomeric forms from *cis*-5,6-epoxycyclooctene, via a microwave mediated epoxide ring opening reaction by a chiral amine and a transannular amine-alkene cyclization; second, the elaboration of the required methyl or ethyl enone moiety through a Sonogashira cross-coupling reaction of an enol-triflate with a C2 or C3 terminal alkyne followed by chemo- and regio-selective hydration of the resulting conjugated enyne group.The synthetic sequences for the preparation of the enantiomers of anatoxin-a and homoanatoxin-a from initially prepared diastereomeric amino-alcohols (−)-**5** and (+)-**6** comprised only eight and seven steps, respectively, and were accomplished in all cases with an overall yield ranging from 37% to 42%. These strategies represent one of the most simple ways so far described for the preparation of (+)- and (−)-anatoxin-a and the first reported synthesis of (+)- and (−)-homoanatoxin-a.

4. Experimental Section

4.1. General Information

All organic solvents required in anhydrous conditions were dried and distilled prior to use using standard techniques.[32] CH_2Cl_2 was distilled over CaH_2 under nitrogen and THF and toluene were distilled over Na/benzophenone. MeOH was dried and stored over 3Å molecular sieve. Other solvents and reagents were obtained from commercial sources and used without purification. Operations with air-and/or moisture-sensitive reagents were performed under an inert atmosphere of dry N_2 or Ar, using syringes or cannulas, glassware dried at 130°C and freshly distilled solvents and dried according to the techniques described above. Microwave-assisted chemistry was performed in a laboratory monomode microwave reactor (CEM corporation, model Discover). Reactions were monitored by thin-layer chromatography (TLC) on precoated silica plates (0.25 mm layer thickness, Silica Gel 60 F_{254}) using UV light as the visualizing agent and ethanolic phosphomolybdic acid or aqueous ceric ammonium molybdate solutions and heat as developing agents. Except for cases where the crude product showed a sufficiently high purity (greater than 95% by ^{1}H-NMR), the synthesized compounds were purified by flash column chromatography using silica gel 60 (particle size 0.043–0.063 mm). Melting points were determined on a Büchi M-560 or Kofler hot-stage apparatus and are uncorrected. Optical rotations were recorded on a Perkin Elmer Mod. 343 polarimeter at a temperature of 20 °C, using a 1 dm cell and the solvent specified in each case; concentrations of the solutions are expressed in g/100 mL. Proton, carbon and

fluorine nuclear magnetic resonance (^{1}H, ^{13}C and ^{19}F NMR) spectra were recorded at room temperature (rt) on a Bruker DPX300 spectrometer operating at 300.1, 75.5 and 282 MHz, respectively, or on a Bruker DRX500 spectrometer operating at 500.1, 125.8 and 470.5 MHz, respectively. Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Carbon substitution degrees were established by DEPT pulse sequences. A combination of COSY and HSQC experiments was used in most cases for the assignment of ^{1}H and ^{13}C chemical shifts. Infrared (IR) spectra were measured without prior preparation using the total attenuated reflectance (ATR) technique on a Thermo Scientific Nicolet i510 FTIR Spectrometer from ThermoFisher Scientific (IR band intensities: w = weak, m = medium, s = strong). High resolution mass spectra (HRMS) were recorded by the electrospray (ES) ionization mode, which was obtained with a Q-TOF premier mass spectrometer with an electrospray source (Waters, Manchester, UK).

4.2. Preparation of the (*1R,6R) and (1S,6S) enantiomeric form of the homotropane framework*: *Synthesis of N-Boc homotropanones (−)-**11** and (+)-**11***

*4.2.1. (1R,8R,Z)-8-(((R)-1-phenylethyl)amino)cyclooct-4-en-1-ol [(−)-**5**] and (1S,8S,Z)-8-(((R)-1-phenylethyl)amino)cyclooct-4-en-1-ol [(+)-**6**)]*

A solution of (*Z*)-9-oxabicyclo[6.1.0]non-4-ene (**3**, 2.00 g, 16.1 mmol) and (*R*)-(+)-α-methylbenzylamine (**4**, 2.1 mL, 16.5 mmol, 1.05 equiv) in anhydrous MeOH (15 mL) placed in a sealed microwave quartz vessel was irradiated with microwave (300 W, 110 °C) for 16h with simultaneous cooling of the reaction vessel with compressed air. The reaction mixture was cooled to rt and concentrated to dryness at reduced pressure. The resulting oily residue (3.273 g) was dissolved in hexane (7−8 mL) and kept in the fridge overnight. Then, the formed crystals of (+)-**6** formed (0.500 g) were separated and washed with cold hexane, the filtrate and washing were concentrated under vacuum and the obtained residue was chromatographed on silica gel, using CH_2Cl_2-acetone mixtures from 95:5 to 70:30 as eluent, to afford, in order of elution, (−)-**5** (1.120 g, 34%) as a viscous oil, followed by (+)-**6** (0.613 g, 34.2% taking into account the crystals initially obtained) as a white solid.

Characterization data of (−)-**5**: $[\alpha]_D$ −20.1 (c 3.36, $CHCl_3$) {Lit.[24] $[\alpha]_D$ −20.1}; IR (ATR) ν_{max} (cm^{-1}) 3350 (br, m), 3017 (w), 2926 (m), 1450 (m), 1104 (m), 1048 (m), 761 (s), 735 (s), 702 (s); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.15 (5H, m, Ph), 5.70−5.58 (1H, m, H-4), 5.46−5.35 (1H, m, H-5), 3.72 (1H, q, *J* = 6.6 Hz, C*H*Me), 3.29 (1H, ddd, *J* = 8.9, 7.4, 3.0 Hz, H-1), 2.54 (1H,ddd,

J = 11.3, 7.4, 4.3 Hz, H-8), 2.35−2.10 (4H, m, H-6, H_2-3, H-2), 1.98−1.86 (1H, ddt, *J* = 14.0, 7.0, 4.8 Hz, H'-6), 1.78−1.65 (1H, m, H-7), 1.36 (1H, m. H'-2), 1.31 (3H, d, *J* = 6.6 Hz, Me), 1.24 (1H, m, H'-7); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145.5 (C-1 Ph), 130.8 (C-4), 128.7 (C-3/C-5 Ph), 128.2 (C-5), 127.5 (C-4 Ph), 126.6 (C2/C-6 Ph), 71.8 (C-1), 58.8 (C-8), 57.9 (*C*HMe), 35.0 (C-2), 33.5 (C-7), 23.5 (Me), 23.1 (C-3), 22.9 (C-6); HRMS (TOF MS ES+) calcd for $C_{16}H_{24}NO$ $[M+H]^{\cdot+}$ 246.1852, found 246.1859.

Characterization data of (+)-**6**: $[\alpha]_D$ +102.8 (c 1.38, $CHCl_3$), mp 88.6–89 °C (crystallized from hexane) {Lit.[24] $[\alpha]_D$ +104.5, mp 91–92 °C}; IR (ATR) ν_{max} (cm^{-1}) 3369 (br, m), 2929 (m), 1639 (w), 1454 (w), 1218 (w), 1028 (w), 754 (s), 704(s); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.20 (5H, m, Ph), 5.56–5.38 (2H, m, H-4, H-5), 3.90 (1H, q, *J*= 6.6 Hz, C*H*Me), 3.29 (1H, ddd, *J*= 8.8, 7.5, 3.1 Hz, H-1), 2.43−2.27 (2H, m, H-6, H-8), 2.1−1.90 (5H, m, H-7, H_2-3, H'-6), 1.34 (3H, d, *J* = 6.6 Hz, Me), 1.37−1.30 (1H, m, H'-7), 1.07 (1H, m, H'-2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.7 (C-1 Ph), 130.6 (C-4), 128.6 (C-3/C-5 Ph), 128.1 (C-4 Ph), 127.3 (C-5), 127.1 (C2/C-6 Ph), 71.7 (C-1), 57.8 (C-8), 56.4 (*C*HMe), 34.6 (C-2), 32.8 (C-7), 25.0 (Me), 23.1 (C-3), 23.0 (C-6); HRMS (TOF MS ES+) calcd for $C_{16}H_{24}NO$ $[M+H]^{\cdot+}$ 246.1852, found 246.1859.

*4.2.2. (1R,2R,6R)-9-((R)-1-phenylethyl)-9-azabicyclo[4.2.1]nonan-2-ol [(−)-**7**]*

A solution of amino alcohol (−)-**5** (0.654 g, 2.665 mmol) in THF (5.3 mL) was added dropwise to a stirred suspension of $Hg(OAc)_2$ (1.00 g, 3.145 mmol, 1.18 equiv) in a 1:1 mixture of THF-H_2O (16 mL) at 0 °C. The mixture was stirred at the same temperature for 30 min and the resulting transparent and pale yellow resulting mixture was treated with a 3M aqueous solution of NaOH (3.1 mL). Then, the obtained cloudy, dark-orange mixture was treated with a solution of $NaBH_4$ (120 mg, 3.158 mmol, 1.18 equiv) in the same NaOH solution (6.3 mL) at 0 °C. After stirring at this temperature for a few minutes the reaction mixture turned black and the stirring was continued at rt until completion of the reaction (about 30 min), as confirmed by TLC ($CHCl_3$-CH_3OH 95:5 as eluent). The reaction mixture was allowed to stand, the metallic mercury was separated by decantation and the liquid phase was extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated at reduced pressure to give a residue that was purified by chromatography, using $CHCl_3$ as eluent, to give azabicyclic alcohol (−)-**7** (0.556 g, 85%) as a low melting point solid. $[\alpha]_D$ −9.5 (c 1.13, $CHCl_3$) {Lit.[24] $[\alpha]_D$ −9.2}; IR (ATR) ν_{max} (cm^{-1}) 3295 (br, m), 2927 (s), 1452 (m), 1370 (m), 1081 (m), 1025 (s), 875 (m), 700 (s); ^{1}H NMR (300 MHz,

$CDCl_3$) δ 7.40−7.15 (5H, m, Ph), 3.86−3.80 (1H, m, H-2), 3.79 (1H, q, *J* = 6.5 Hz, C*H*Me), 3.48 (1H, br t, *J* = 8.3 Hz, H-1), 3.32 (1H, br t, *J* = 6.5 Hz, H-6), 2.08 (1H, tdd, *J* = 12.6, 9.0, 5.7 Hz, H-5), 1.92−1.35 (8H, m, H_2-3, H_2-4, H-5, H_2-7, H-8), 1.32 (3H, d, *J* = 6.5 Hz, Me), 1.25−1.15 (1H, m, H'-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.0 (C-1 Ph), 128.3 (C-3/C-5 Ph), 127.3 (C-2/C-6 Ph), 126.7 (C-4 Ph), 74.4 (C-2), 64.5 (C-1), 61.4 (*C*HMe), 59.2 (C-6), 36.0 (C-8), 33.5 (C-5), 33.2 (C-3), 23.3 (C-7), 22.9 (Me), 20.4 (C-4); HRMS (TOF MS ES+) calcd for $C_{16}H_{24}NO$ $[M+H]^{+}$ 246.1852, found 246.1845.

*4.2.3. (1S,2S,6S)-9-((R)-1-phenylethyl)-9-azabicyclo[4.2.1]nonan-2-ol [(+)-**8**]*

This diastereomer was prepared (658 mg, 95%) in an analogous way as described for (−)-**7** employing the (1*S*,8*S*,*Z*)-isomer (+)-**6** (687 mg); (+)-**8** was obtained as a colorless viscous oil; $[\alpha]_D$ +19.3 (c 1.05, $CHCl_3$), {Lit.[24] $[\alpha]_D$ +20.5}; IR (ATR) ν_{max} (cm^{-1}) 3285 (br, w), 2924 (s), 1450 (m), 1048 (m), 761 (s), 733 (s), 702 (s); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38–7.28 (4H, m, Ph), 7.24–7.17 (1H, m, Ph), 3.85–3.77 (1H, m, H-2), 3.78 (1H, q, *J* = 6.5 Hz, C*H*Me), 3.49 (1H, br t, *J* = 8.2 Hz, H-1), 3.31 (1H, br t, *J* = 6.4 Hz, H-6), 2.01 (1H, tdd, *J* = 12.5, 9.1, 5.5 Hz, H-5), 1.91−1.36 (8H, m, H_2-3, H_2-4, H-5, H_2-7, H-8), 1.32 (3H, d, *J* = 6.5 Hz, Me), 1.16 (1H, m, H'-8); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.9 (C-1 Ph), 128.3 (C-3/C-5 Ph), 127.3 (C-2/C-6 Ph), 126.8 (C4 Ph), 73.8 (C-2), 66.1 (C-1), 61.3 (*C*HMe), 57.4 (C-6), 36.4 (C-8), 33.5 (C-5), 33.4 (C-3), 23.0 (C-7), 22.7 (Me), 20.3 (C-4); HRMS (TOF MS ES+) calcd for $C_{16}H_{24}NO$ $[M+H]^{+}$ 246.1852, found 246.1851.

*4.2.4. (1R,6R)-9-((R)-1-Phenylethyl)-9-azabicyclo[4.2.1]nonan-2-one [(−)-**9**]*

DMSO (0.446 mL, 6.28 mmol) was added dropwise to a solution of oxalyl chloride (0.266 mL, 3.14 mmol) in anhydrous CH_2Cl_2 (6.8 mL) at −78 °C under nitrogen. After stirring at this temperature for 10−15 min, a solution of alcohol (−)-**7** (0.513 g, 2.09 mmol) in CH_2Cl_2 (3.5 mL) was added and the mixture was stirred for an additional 30 min before the addition of Et_3N (1.75 mL, 12.56 mmol). The reaction mixture was stirred at −78 °C for 1 h and then allowed to slowly warm to rt, quenched by the addition of a saturated aqueous solution of Na_2CO_3 (5 mL), diluted with water and extracted with Et_2O. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated under vacuum to give an oily residue that was chromatographed on silica gel, using CH_2Cl_2 as eluent, to give ketone (−)-**9** (0.452 g, 88%) as a colorless oil. $[\alpha]_D$ −21.1 (c 1.99, $CHCl_3$) {Lit.[24] $[\alpha]_D$ −20.7}; IR (ATR) ν_{max} (cm^{-1}) 2969 (m), 2929 (m), 1700 (s), 1456 (m), 1374 (m), 1121 (s), 1072 (s), 704 (s);

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.15 (5H, m, Ph), 3.80 (1H, q, *J* = 6.6 Hz, C*H*Me), 3.67 (1H, br dd, *J* = 10.2, 1.8 Hz, H-1), 3.62 (1H, br dd, *J* = 5.8, 2.5 Hz, H-6), 3.02 (1H, ddd, *J* = 14.0, 13.1, 3.0 Hz, H-3), 2.31 (1H, br dd, *J* = 14.0, 5.5 Hz, H'-3), 2.25−2.10 (1H, m, H-8), 1.90−1.67 (4H, m, H'-8, H-7, H-5, H-4), 1.67−1.45 (3H, m, H'-7, H'-5, H'-4), 1.34 (3H, d, *J* = 6.6 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 218.6 (C-2), 145.7 (C-1 Ph), 128.5 (C-3/C-5 Ph), 127.3 (C-2/C-6 Ph), 127.2 (C-4 Ph), 70.0 (C-1), 61.6 (C-6), 60.7 (*C*HMe), 42.6 (C-3), 35.5 (C-5), 30.9 (C-8), 27.2 (C-7), 22.7 (Me), 20.1 (C-4); HRMS (TOF MS ES+) calcd for $C_{16}H_{22}NO$ $[M+H]^{\cdot+}$ 244.1696, found 244.1687.

*4.2.5. (1S,6S)-9-((R)-1-Phenylethyl)-9-azabicyclo[4.2.1]nonan-2-one [(+)-**10**]*

This diastereomer was prepared (505 mg, 79%) in an analogous way as described for (−)-**9** employing the (1*S*,2*S*,6*S*)-isomeric amino-alcohol (+)-**8** (644 mg); (+)-**10** was obtained as a colorless viscous oil. $[\alpha]_D$ +106.9 (c 0.49, $CHCl_3$) {Lit.[24] $[\alpha]_D$ +107}; IR (ATR) ν_{max} (cm^{-1}) 2969 (w), 2928 (s), 1698 (s), 1454 (m), 767 (w), 704 (m); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.19 (5H, m, Ph), 3.94 (1H, q, *J* = 6.6 Hz, C*H*Me), 3.74 (1H, dd, *J* = 10.1, 2.4 Hz, H-1), 3.58–3.50 (1H, m, H-6), 2.83 (1H, ddd, *J* = 15.2, 12.8, 3.4 Hz, H-3), 2.40 (1H, br dd, *J* = 15.1, 5.7 Hz, H'-3), 2.26–2.15 (1H, m, H-8), 1.96−1.50 (7H, m, H_2-4, H_2-5, H_2-7, H'-8), 1.34 (3H, d, *J* = 6.5 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 218.5 (C-2), 145.5 (C-1 Ph), 128.6 (C-3/C-5 Ph), 127.2 (C-2/C6 Ph), 127.1 (C-4 Ph), 70.2 (C-1), 59.7 (C-6), 59.4 (*C*HMe), 43.2 (C-3), 34.2 (C-5), 30.2 (C-8), 27.9 (C-7), 22.8 (Me), 20.0 (C-4); HRMS (TOF MS ES+) calcd for $C_{16}H_{22}NO$ $[M+H]^{\cdot+}$ 244.1696, found 244.1687.

*4.2.6. tert-Butyl (1R,6R)-2-oxo-9-azabicyclo[4.2.1]nonane-9-carboxylate [(−)-**11**]*

A mixture of benzyl amine (−)-**9** (0.280 g, 1.15 mmol), di-*tert*-butyl dicarbonate (0.296 mL, 1.29 mmol) and $Pd(OH)_2$ on charcoal (50*%* moisture, 20% loading, 50 mg) in EtOAc (5 mL) was stirred under a pressure of about 60 psi of hydrogen at rt for 14 h. The mixture was filtered through a short pad of celite using EtOAc for washing. The filtrate and washing were combined and washed with 5% aqueous $NaHCO_3$ solution and brine and dried over anhydrous $MgSO_4$. Evaporation of the solvent in vacuo led to (−)-**11** (0.270 g, 98%) as a viscous colorless oil that solidifies upon standing in the refrigerator. The compound thus obtained was pure by NMR and was used in the next step without further purification. Mp 72.1–73.0 °C (crystallized from cold Hexane-EtOAc) {Lit.[18] for (±)-**11**, mp 49–51 °C}; $[\alpha]_D$ −54.2 (c 0.77, $CHCl_3$); IR (ATR) ν_{max} (cm^{-1}) 2935 (m), 2972 (m), 1693 (s), 1395 (s), 1171 (m), 1108 (m), 774 (w); ^{1}H NMR (300 MHz, $CDCl_3$) (a mixture of rotamers, only signals of the major

rotamer are given) δ 4.52 (1H, br d, *J* = 8.4 Hz, H-1), 4.25 (1H, br dd, *J* = 10.5, 2.3 Hz, H-6), 2.60–2.45 (1H, m, H-3), 2.45−2.25 (2H, m, H'-3, H-5), 2.25−2.00 (2H, m, H-7, H-8), 1.98−1.85 (1H, m, H'-5), 1.82−1.60 (4H, m, H_2-4, H'-7, H'-8), 1.43 (9H, s, C*Me*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 215.3 (C-2), 153.0 (CO Boc), 80.4 (*C*Me_3), 65.0 (C-1), 56.6 (C-6), 41.8 (C-3), 33.0 (C-8), 30.0 (C-5), 28.5 (C*Me*$_3$), 26.9 (C-7), 19.4 (C-4); HRMS (TOF MS ES+) calcd for $C_{13}H_{22}NO_3$ $[M+H]^{+}$ 240.1594, found 240.1587.

*4.2.6. tert-Butyl (1S,6S)-2-oxo-9-azabicyclo[4.2.1]nonane-9-carboxylate [(+)-**11**]*

This enantiomer (261.2 mg, 97%) was prepared in an analogous manner as described for (−)-**11** employing the (1*S*,6*S*)-isomer (+)-**10** (273.9 mg); (+)-**11** was obtained as a semisolid that solidifies upon standing in the refrigerator. Mp 71.5–72.5 °C (crystallized from cold Hexane-EtOAc) $[\alpha]_D$ +54.6 (c 0.88, $CHCl_3$). Its spectroscopic data correspond with those of enantiomer (−)-**11**.

*4.3. Synthesis of the natural and unnatural enantiomer of anatoxin-*a *[(+)-**1** and (–)-**1**]*

*4.3.1. tert-Butyl (1R,6R)-2-(((trifluoromethyl)sulfonyl)oxy)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(+)-**12**]*

A 0.5 M solution of potassium *bis*(trimethylsilyl)amide (KHMDS) in toluene (3.8 mL, 1.9 mmol, 1.5 equiv) was added dropwise to a solution of ketone (−)-**11** (276 mg, 1.252 mmol) in anhydrous THF (15 mL) at −78 °C under nitrogen. The mixture was stirred under this conditions for 1 h and then treated with a solution of *N*-(5-chloro-2-pyridyl)*bis*(trifluoromethanesulfonimide) (Comins' Reagent) (763 mg, 1.94 mmol, 1.55 equiv) in THF (1 mL). After stirring at −78 °C for 30 min, the reaction mixture was quenched by the addition of saturated aqueous $ClNH_4$ (4 mL), diluted with water (60 mL) and extracted with Et_2O. The combined organic phases were washed with brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to afford a brownish oil that was purified by chromatography on silica gel, using hexane-EtOAc 9:1 as eluent, to give enol triflate (+)-**12** (351.2 mg, 82%) as a white solid. Mp 63.1–64.2 °C (crystallized from hexane-EtOAc); $[\alpha]_D$ +19.5 (c 1.31, $CHCl_3$); IR (ATR) ν_{max} (cm^{-1}) 2972 (w), 1679 (s), 1400 (s), 1366 (s), 1243 (m), 1207 (s), 1168 (s), 1138 (s), 1138 (s), 10210 (m), 884 (m), 827 (m); ^{1}H NMR (300 MHz, $CDCl_3$) (a mixture of rotamers, only signals of the major rotamer are given) δ 5.76 (1H, br t, *J* = 6.2 Hz, H-3), 4.51 (1H, br d, *J* = 7.9 Hz, H-1), 4.38 (1H, m, H-6), 2.29−2.20 (2H, m, H_2-4), 2.25−2.10

(3H, m, H-5, H-7, H-8), 2.10−2.00 (1H, m, H'-5), 1.80−1.58 (2H, m, H'-7, H'-8), 1.46 (9H, s, C*Me*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.6 (C-2), 153.1 (CO), 120.8 (C-3), 118.6 (q, *J* = 320.2 Hz, CF_3), 80.5 (*C*Me$_3$), 58.9 (C-1), 54.9 (C-6), 31.1 (C-8), 30.5 (C-5), 28.5 (C-7), 28.3 (C*Me*$_3$), 19.8 (C-4); ^{19}F NMR (282 MHz, $CDCl_3$) δ −74.3 (s); HRMS (TOF MS ES+) calcd for $C_{14}H_{21}F_3NO_5S$ $[M+H]^{\cdot+}$ 372.1087, found 372.1088.

*4.3.2. tert-Butyl (1S,6S)-2-(((trifluoromethyl)sulfonyl)oxy)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(−)-**12**]*

The preparation of this enantiomer (312.7 mg, 73%) was carried out in an analogous manner to that described for (+)-**12** employing the (1*S*,6*S*)-enantiomer (+)-**11** (346.7 mg); (−)-**12** was obtained as a white solid. Mp 65.3–65.6 °C (crystallized from hexane-EtOAc); $[\alpha]_D$ −18.5 (c 1.04, $CHCl_3$). Its spectroscopic data correspond with those of enantiomer (+)-**12**.

*4.3.3. tert-Butyl (1R,6R)-2-((trimethylsilyl)ethynyl)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(−)-**13**]*

A mixture of enol triflate (+)-**12** (174 mg, 0.468 mmol), $PdCl_2(PPh_3)_2$, (34 mg, 48.6 µmol, 0.1 equiv), CuI (4.6 mg, 24.2 µmol, 0.05 equiv) and trimethylsilylacetylene (0.264 mL, 184 mg, 1.872 mmol, 4 equiv) in anhydrous DMF (3 mL) was exhaustively degassed by ultrasound under nitrogen atmosphere. Et_3N (0.196 mL 1.407 mmol, 3 equiv) was added to the resulting yellowish suspension to give a clear solution that was stirred for 1 h at rt (during this time, the color of the reaction mixture changed from light yellowish to orange, then brown, and finally black). After this time, the solution was diluted with water and then extracted with Et_2O. The combined organic layers were washed with a 5% aqueous solution of LiCl and brine, dried over anhydrous $MgSO_4$ and concentrated under vacuum. The crude product was purified by chromatography, eluting first with hexane and then with hexane-EtOAc 9:1, to give alkyne (−)-**13** (128 mg, 86%) as a slightly colored amorphous solid. $[\alpha]_D$ −64.0 (c 1.05, $CHCl_3$); IR (ATR) ν_{max} (cm^{-1}) 2989 (s), 2363 (s), 2343 (s), 1685 (m), 1408 (m), 1069 (s), 845 (m), 756 (s); ^{1}H NMR (300 MHz, $CDCl_3$) (a mixture of rotamers, only signals of the major rotamer are given) δ 6.13 (1H, t, *J* = 6.2 Hz, H-3), 4.48 (1H, br d, *J* = 8.5 Hz, H-1), 4.35 (1H, m, H-6), 2.31−2.20 (1H, m, H_2-4), 2.22−1.97 (3H, m, H-5, H-7, H-8), 1.95−1.83 (1H, m, H'-5), 1.75−1.50 (2H, m, H'-7, H'-8), 1.46 (9H, s, C*Me*$_3$), 0.16 (9H, s, SiMe$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4 (CO), 138.0 (C-3), 131.7 (C-2), 106.6 (C-1'), 92.6 (C-2'), 79.6 (*C*Me$_3$), 59.8 (C-1), 55.6 (C-6),

31.4 (C-8), 31.3 (C-5), 28.8 (C-7), 28.6 (C*Me*$_3$), 24.3 (C-4), 0.15 (SiMe$_3$); HRMS (TOF MS ES+) calcd for $C_{18}H_{30}NO_2Si$ $[M+H]^{+}$ 320.2040, found 320.2042.

*4.3.4. tert-Butyl (1S,6S)-2-((trimethylsilyl)ethynyl)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(+)-**13**]*

This enantiomer (126.3 mg, 84.4%) was prepared in an analogous manner as described for (−)-**13** employing the (1*S*,6*S*)-isomer (−)-**12** (379.1 mg); (+)-**13** was obtained as a pale colored low melting point solid, $[\alpha]_D$ +66.3 (c 0.99, $CHCl_3$). Its spectroscopic data correspond with those of enantiomer (−)-**13**.

*4.3.5. tert-Butyl (1R,6R)-2-ethynyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(−)-**14**]*

A mixture of trimethyl(alkynyl)silane (−)-**13** (239 mg, 0.748 mmol), K_2CO_3 (516 mg, 3.740 mmol, 5 equiv) in MeOH (6.5 mL) was stirred at rt for 1 h. The reaction mixture was poured into water and extracted with Et_2O, the organic phase was washed with brine, dried over anhydrous $MgSO_4$ and concentrated at reduced pressure. The residue was purified by silica gel column chromatography, eluting first with hexane and then with hexane-EtOAc 9:1, to obtain alkyne (−)-**14** (216 mg, 92%) as a pale yellow oil that solidified upon freezing. $[\alpha]_D$ −35.8 (c 1.01, $CHCl_3$); IR (ATR) ν_{max} (cm^{-1}) 3311 (w), 2976 (m), 2935 (w), 1693 (s), 1408 (s), 1367 (m), 1173 (m), 1119 (m; ^{1}H NMR (300 MHz, $CDCl_3$) δ (a mixture of rotamers, only signals of the major rotamer are given) δ 6.15 (1H, t, *J* = 6.1 Hz, H-3), 4.50 (1H, br d, *J* = 8.0 Hz, H-1), 4.35 (1H, m, H-6), 2.88 (1H, s, H-2'), 2.32−2.23 (2H, m, H_2-4), 2.23−2.05 (3H, m, H-5, H-7, H-8), 1.93−1.83 (1H, m, H'-5), 1.75−1.65 (1H, m, H'-7), 1.65−1.55 (1H, m, H'-8), 1.46 (9H, s, C*Me*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5 (CO), 138.3 (C-3), 130.1 (C-2), 85.1 (C-1'), 79.7 (*C*Me$_3$), 76.1 (C-2'), 60.0 (C-1), 55.3 (C-6), 31.8 (C-8), 31.2 (C-5), 29.2 (C-7), 28.6 (C*Me*$_3$), 24.2 (C-4); HRMS (TOF MS ES+) calcd for $C_{15}H_{22}NO_2$ $[M+H]^{+}$ 248.1645, found 248.1645.

*4.3.6. tert-Butyl (1S,6S)-2-ethynyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(+)-**14**]*

This enantiomer (198.9 mg, 97%) was prepared in an analogous way as described for (−)-**14** employing the (1*S*,6*S*)-isomer (+)-**13** (264.7 mg); (+)-**14** was obtained as an oil; $[\alpha]_D$ +35.5 (c 1.02, $CHCl_3$). Its spectroscopic data correspond with those of enantiomer (−)-**14**.

*4.3.7. tert-Butyl (1R,6R)-2-acetyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(−)-**15**]*

Freshly distilled $BF_3{\cdot}Et_2O$ (23 µL, 0.184 mmol, 0.3 equiv) was dropwise added to a solution of Cl_3CCO_2H (0.89 mg, 5.49 µmol, $9{\cdot}10^{-3}$ equiv) in anhydrous MeOH (2 mL) at rt under nitrogen. The resulting solution was added dropwise via syringe to a stirred suspension of HgO (64 mg, 0.294 mmol, 0.5 equiv) in MeOH (10 mL) under the same conditions and, after stirring for a few minutes, a solution of enyne (−)-**14** (147.5 mg, 0.596 mmol) in anhydrous MeOH (4 mL) was added and the mixture vigorously stirred at rt for about 1.5 h. After completion of the reaction (TLC, hexane-EtOAc 8:2), the orange suspension was poured into cold water (about 15 mL), stirred at rt for 10–15 min and extracted with Et_2O. The combined organic layers were washed with brine, dried and concentrated to leave a residue that was purified by silica gel chromatography, eluting with CH_2Cl_2 and then with $CHCl_3$, to give *N*-Boc methyl enone (−)-**15** (147 mg, 93%) as a colorless oil. The enantiomeric purity was determined by HPLC analysis (Daicel Chiralpak AD-H (250x4.6 mm), hexane/2-propanol 99/1, flow rate=1 mL/min, λ = 241 nm; retention time: 26.4 min [(1*S*,6*S*)-enantiomer, <1%] and 30.6 min [(1*R*,6*R*)-enantiomer, >99%]. $[\alpha]_D$ −60.4 (c 0.96, $CHCl_3$), −46.7 (c 1.02, CH_2Cl_2) {Lit[19g]. $[\alpha]_D$ −46.8 (CH_2Cl_2); Lit.[19b] $[\alpha]_D$ −47.2 (CH_2Cl_2)}; IR (ATR) ν_{max} (cm^{-1}) 2974 (w), 2933 (w), 1689 (s), 1665 (s), 1633 (m), 1391 (s), 1365 (s), 1169 (s), 933 (w), 772 (w); ^1H NMR (300 MHz, $CDCl_3$) (a mixture of rotamers, only signals of the major rotamer are given) δ 6.82 (1H, t, *J* = 6.0 Hz, H-3), 5.13 (1H, br d, *J* = 9.2 Hz, H-1), 4.41 (1H, m, H-6), 2.50−2.40 (2H, m, H_2-4), 2.37−2.25 (1H, m, H-8), 2.29 (3H, s, Me), 2.22−2.04 (2H, m, H-5, H-7), 1.75−1.58 (3H, m, H'-5, H'-7, H'-8), 1.37 (9H, s, C*Me*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.9 (*C*OMe), 153.3 (CO Boc), 150.5 (C-2), 142.3 (C-3), 79.5 (*C*Me_3), 55.8 (C-6), 53.2 (C-1), 31.6 (C-8), 30.5 (C-5), 28.9 (C-7), 28.6 (C*Me*$_3$), 25.5 (Me), 24.3 (C-4); HRMS (TOF MS ES+) calcd for $C_{15}H_{24}NO_3$ $[M+H]^+$ 266.1751, found 266.1747.

*4.3.8. tert-Butyl (1S,6S)-2-acetyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(+)-**15**]*

This enantiomer (190 mg, 94%) was prepared in an analogous manner as described above for (−)-**15** employing the (1*S*,6*S*)-isomer (+)-**14** (188.1 mg); (+)-**15** was obtained as a slightly yellowish oil. The enantiomeric purity was determined by HPLC analysis (same conditions as above for (−)-**15**; retention time: 26.4 min [(1*S*,6*S*)-enantiomer, >99%] and 30.6 min [(1*R*,6*R*)-enantiomer, <1%]. $[\alpha]_D$ +60.0 (c 0.96, $CHCl_3$), $[\alpha]_D$ +43.8 (c 0.99, CH_2Cl_2), {Lit.[19b] $[\alpha]_D$ +51.9 (c 0.79, CH_2Cl_2)}. The spectroscopic data of (+)-**15** correspond with those of enantiomeric (−)-**15**.

*4.3.9. (+)-Anatoxin-a trifluoroacetate [(+)-**1·TFA**] and hydrochloride [(+)-**1·HCl**] salts*

CF_3CO_2H (1.5 mL) was added dropwise to a solution of (−)-**15** (106 mg, 0.40 mmol) in anhydrous CH_2Cl_2 (1.5 mL) and the mixture was stirred at rt under nitrogen until consumption of the starting material, as showed by TLC using $CHCl_3$-MeOH-HCO_2H 90:10:1 as eluent. After about 1 h, the solvents were evaporated under reduced pressure and the residue kept under vacuum overnight to afford (+)-**1·TFA** (112 mg, nearly quantitative yield) as a viscous oil with a purity higher than 95% according to the ^{1}H NMR spectra. $[\alpha]_D$ +10.6 (c 0.45, $CHCl_3$); IR (ATR) ν_{max} (cm^{-1}) 3686 (w), 3410 (br, m), 2989 (s), 2903 (s), 1660 (m), 1396 (m), 1069 (s); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 and 8.83 (1H each, each br s, NH_2^+), 7.15 (1H, ddd, *J* = 8.2, 4.4, 1.3 Hz, H-3), 5.24 (1H, br t, *J* = 6.9 Hz, H-1), 4.30 (1H, m, H-6), 2.61−2.55 (2H, m, H_2-4), 2.50 (1H, ddd, *J* = 12.9, 9.2, 3.1 Hz, H-8), 2.43−2.32 (1H, m, H-7), 2.29 (1H, s, Me), 2.21−2.12 (1H, m, H-5), 1.96 (1H, m, H'-7), 1.89 (1H, ddd, *J* = 14.2, 6.3, 2.8 Hz, H'-8), 1.85 (1H, m, H'-5); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.9 (CO), 146.6 (C-3), 144.2 (C-2), 59.0 (C-6), 52.5 (C-1), 30.4 (C-8), 28.0 (C-5), 27.6 (C-7), 25.0 (Me), 23.6 (C-4); ^{19}F NMR (282 MHz, $CDCl_3$) δ −76.32 (s); HRMS (TOF MS ES+) calcd for $C_{10}H_{16}NO$ $[M+H-CF_3CO_2H]^+$ 166.1226, found 166.1222.

Alternatively, a 4M solution of HCl in dioxane (220 µL, 0.88 mmol) was added to a solution of (−)-**15** (25.6 mg, 0.097 mmol) in anhydrous dioxane (300 µL) and the mixture was stirred at rt for 1.30 h. The excess HCl and dioxane were removed under nitrogen steam, then benzene was added and the solvent removed under reduced pressure (2 mL x 3). The residue obtained was kept under high vacuum ($5.0x10^{-3}$ Torr) overnight to give (+)-**1·HCl** (19.2 mg, 99%) as a white powder which showed NMR spectral data identical to those previously reported (see Supplementary Data File).[19b,19c,19m] This powder was suspended in a 95:5 mixture of Et_2O and MeOH (2.2 mL) at 50-60 °C and MeOH was added to obtain a cleair solution (about 200 µL). The solution was kept at rt for 3 h and then at 4 °C overnight. The formed white prism crystals were separated and washed with a little Et_2O and dried under vacuum to give crystalline (+)-**1·HCl**. Mp 156.5–158.5 °C (with darkening), $[\alpha]_D$ +40.8 (c, 0.49 EtOH) {Lit.[19c] mp 152-153 °C, Lit.[19b] $[\alpha]_D$ +43.2 (c 0.67, EtOH); Lit.[19m] mp, 151-153 °C, $[\alpha]_D$ +37.3 (c, 2.08, EtOH)}.

*4.3.10. (−)-Anatoxin-a trifluoroacetate [(−)-**1·TFA**] and hydrochloride salts [(−)-**1·HCl**]*

The trifluoroacetate (17.8 mg, 99%) and hydrochloride (44.1 mg, 99%) salts of this enantiomer were prepared in an analogous manner as described above for the equivalent

salts of (+)-anatoxin-a employing the (1*S*,6*S*)-isomer (+)-**15** (18 mg and 59.0 mg, respectively). (−)-**1·TFA** was obtained as a viscous oil; $[\alpha]_D$ −9.6 (c 1.44, $CHCl_3$). (−)-**1·HCl** was obtained as a white foam; it forms large colorless prisms after recrystallization from cold Et_2O – 10% MeOH, mp 157.5−159.5 °C (with darkening), $[\alpha]_D$ –42.9 (c 0.64, EtOH) {Lit.[19b] $[\alpha]_D$ −46.3 (c 0.57 EtOH); Lit.[20b] $[\alpha]_D$ −39.0 (c 0.55 EtOH)}. The spectroscopic data of the unnatural enantiomer, (−)-**1·TFA** and (−)-**1·HCl**, correspond with those of the natural enantiomer (+)-**1·TFA** and (+)-**1·HCl**, respectively (see Supplementary Data File).

*4.4. Synthesis of the natural and unnatural enantiomer of homoanatoxin-a [(+)-**2** and (+)-**2**]*

*4.4.1. tert-Butyl (1R,6R)-2-(prop-1-yn-1-yl)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(−)-**16**]*

A mixture of enol triflate (+)-**12** (174 mg, 0.468 mmol), $PdCl_2(PPh_3)_2$, (34 mg, 48.6 µmol, 0.1 equiv), CuI (14.7 mg, 77.44 µmol, 0.16 equiv) and DMF (6 mL), contained in a Büchi 'Tiny Clave' reactor equipped with a magnetic stirring bar, was exhaustively degassed by vacuum-filling nitrogen cycles. Et_3N (0.653 mL, 4.690 mmol, 10 equiv) was added to the resulting yellowish suspension to give a clear solution that was first cooled at −78 °C and degassed again by vacuum (200 mmHg)-filling nitrogen cycles. Then, the mixture was stirred and homogenized (during this time the reaction mixture color changed from light yellowish to orange, then brown, and finally black), and then cooled at −78 °C and propyne gas was condensed (about 1/3 of the total volume, >10 equiv). Finally, the reaction mixture was allowed to reach rt and then stirred overnight. The reactor was opened to the atmosphere and the excess of propyne allowed to evaporate. Then, the resulting solution was diluted with water and extracted with Et_2O. The combined organic layers were washed with a 5% aqueous solution of LiCl, a 2M aqueous solution of EDTA and brine, dried over anhydrous $MgSO_4$ and a small spoon of Dowex© M4195 resin, filtered and concentrated under vacuum. The crude product was purified by chromatography, eluting first with hexane and then with hexane-EtOAc 9:1, to give alkyne (−)-**16** (83.7 mg, 75%) as a viscous light yellow oil. $[\alpha]_D$ −36.4 (c 0.60, $CHCl_3$). IR (ATR) ν_{max} (cm^{-1}) 2969 (w), 2931 (w), 2363 (s), 2343 (s), 1698 (m), 1408 (m), 1365 (w), 789 (m), 758 (s); ^{1}H NMR (300 MHz, $CDCl_3$) (a mixture of rotamers, only signals of the major rotamer are given) δ 5.95 (1H, t, *J* = 6.0 Hz, H-3), 4.46 (1H, br d, *J* = 7.8 Hz, H-1), 4.33 (1H, m, H-6), 2.30–2.18 (2H, m, H_2-4), 2.18–2.05 (3H, m, H-5, H-7, H-8), 1.93 (3H, s, Me), 1.92–1.80 (1H, m, H'-5), 1.77–1.55 (2H, m, H'-7, H'-8), 1.46 (9H, s, CMe_3); ^{13}C

NMR (75 MHz, $CDCl_3$) δ 153.6 (*C*O Boc), 134.8 (C-3), 131.2 (C-2), 84.4 (C-1'), 80.9 (C-2'), 79.4 (*C*Me_3), 60.5 (C-1), 55.2 (C-6), 32.2 (C-8), 31.2 (C-5), 29.5 (C-7), 28.6 (C*Me*$_3$), 23.9 (C-4), 4.33 (Me); HRMS (TOF MS ES+) calcd for $C_{16}H_{24}NO_2$ $[M+H]^{\cdot+}$ 262.1802, found 262.1798.

A more polar compound, characterized as epoxide **i**, was aisolated from some reaction batches (see the results and discussion part for details). Epoxide **i** (*tert-butyl (1R,2R,4S,7S)-2-(prop-1-yn-1-yl)-3-oxa-10-azatricyclo[5.2.1.02,4]decane-10-carboxylate*) showed the following spectroscopic properties: ^{1}H NMR (300 MHz, $CDCl_3$) (a mixture of rotamers, only signals of the major rotamer are given) δ 4.40 (1H, d, *J* = 8.7 Hz, H-1), 4.11 (1H,dddd, *J* = 5.6, 2.7, 1.7, 1.7 Hz, H-7), 3.17 (1H, br d, *J* = 4.9 Hz, H-4), 2.30−2.17 (2H, m, H-9, H-5), 2.08 (1H, m, H-6), 2.05−1.85 (3H, m, H´-5, H-8, H´-9), 1.84 (3H, s, Me), 1.54 (1H, m, H´-8), 1.50 (9H, s, CMe_3), 1.38 (1H, m, H´-6); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0 (*C*O Boc), 79.9 (C-1'), 79.5 (*C*Me_3), 78.5 (C-2'), 62.1 (C-4), 57.7 (C-1), 57.4 (C-2), 54.3 (C-7), 28.6 (C-9), 28.6 (C*Me*$_3$), 27.7 (C-6), 25.1 (C-8), 22.8 (C-5), 3.9 (Me); HRMS (TOF MS ES+) calcd for $C_{12}H_{16}NO_3$ $[M+H-C_4H_8]^{\cdot+}$ 222.1125, found 222.1126; calcd for $C_{16}H_{27}N_2O_3$ $[M+NH_4]^{\cdot+}$ 295.2016, found 295.2016.

*4.4.2. tert-Butyl (1S,6S)-2-(prop-1-yn-1-yl)-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(+)-**16**]*

This enantiomer (151.3 mg, 75%) was prepared in an analogous manner as described for (−)-**16** employing the (1*S*,6*S*)-isomer (−)-**12** (188.1 mg); (+)-**16** was obtained as a slightly yellowish oil; $[\alpha]_D$ +37.9 (c 0.60, $CHCl_3$). The spectroscopic data of enantiomer (+)-**16** correspond with those of (−)-**16**.

*4.4.3. tert-Butyl (1R,6R)-2-propionyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(−)-**17**]*

Freshly distilled $BF_3{\cdot}Et_2O$ (23 µL, 0.184 mmol, 0.3 equiv) was dropwise added to a solution of Cl_3CCO_2H (0.89 mg, 5.49 µmol, $9{\cdot}10^{-3}$ equiv) in anhydrous MeOH (2 mL) at rt under nitrogen. The resulting solution was added dropwise via syringe to a stirred suspension of HgO (64 mg, 0.294 mmol, 0.5 equiv) in MeOH (10 mL) under the same conditions and, after stirring for a few minutes, a solution of enyne (−)-**16** (147.5 mg, 0.596 mmol) in anhydrous MeOH (4 mL) was added and the mixture vigorously stirred at rt for about 1.5 h. After completion of the reaction (TLC, hexane-EtOAc 8:2), the orange suspension was poured into cold water and processed as described above for (−)-**15**. The crude product was purified by silica gel chromatography, eluting with CH_2Cl_2 and then with

$CHCl_3$, to afford *N*-Boc ethyl enone (−)-**17** (144 mg, 91%) as a slightly yellowish oil. The enantiomeric purity was determined by HPLC analysis (Daicel Chiralpak AD-H (250x4.6 mm), hexane/2-propanol 99/1, flow rate=1 mL/min, λ = 235 nm; retention time: 16.6 min [(1*S*,6*S*)-enantiomer, <1%] and 22.6 min [(1*R*,6*R*)-enantiomer, >99%). $[\alpha]_D$ −36.5 (c 0.80, $CHCl_3$); IR (ATR) ν_{max} (cm^{-1}) 2933 (w), 2976 (w), 1672 (s), 1393 (s), 1365 (m),1169 (s), 1110 (s), 752 (s); ^{1}H NMR (300 MHz, $CDCl_3$) (a mixture of rotamers, only signals of the major rotamer are given) δ 6.80 (1H, t, *J* = 6.0 Hz, H-3), 5.12 (1H, br d, *J* = 9.3 Hz, H-1), 4.43–4.37 (1H, m, H-6), 2.77–2.55 (2H, m, *CH*$_2$Me), 2.46–2.36 (2H, m, H_2-4), 2.37–2.26 (1H, m, H-8), 2.20–2.02 (2H, m, H-5, H-7), 1.73−1.58 (3H, m, H'-5, H'-7, H'-8), 1.36 (9H, s, *C*Me_3), 1.10 (3H, t, *J* = 7.2 Hz, CH_2*Me*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.7 (CO), 153.3 (COBoc),149.9 (C-2), 140.6 (C-3), 79.4 (*C*Me_3), 55.8 (C-6), 53.4 (C-1), 31.7 (C-8), 30.5 (*C*H_2Me), 30.4 (C-5), 28.9 (C-7), 28.6 (C*Me*$_3$), 24.2 (C-4), 9.00 (CH_2*Me*);HRMS (TOF MS ES+) calcd for $C_{16}H_{25}NO_3$ $[M+H]^{+}$ 280.1907, found 280.1901.

*4.4.4. tert-Butyl (1S,6S)-2-propionyl-9-azabicyclo[4.2.1]non-2-ene-9-carboxylate [(+)-**17**]*

This enatiomer (189.6 mg, 94%) was prepared in an analogous manner as described for (−)-**17** employing the (1*S*,6*S*)-isomer (+)-**16** (188.1 mg); (+)-**17** was obtained as a slightly yellowish oil. The enantiomeric purity was determined by HPLC analysis (the same conditions as above for (−)-**17**; retention time: 16.6 min [(1*S*,6*S*)-enantiomer, >99%] and 22.6 min [(1*R*,6*R*)-enantiomer, <1%]. $[\alpha]_D$ +35.6 (c 0.60, $CHCl_3$). Its spectroscopic data correspond with those of enantiomer (−)-**17**.

*4.4.5. (+)-Homoanatoxin-a trifluoroacetate salt [(+)-**2·TFA**]*

CF_3CO_2H (400 µL) was added dropwise to a solution of (−)-**17** (25 mg, 0.089 mmol) in anhydrous CH_2Cl_2 (400 µL) and the mixture was stirred at rt under nitrogen until consumption of the starting material, as showed by TLC using $CHCl_3$-MeOH-HCO_2H 90:10:1 as eluent. After about 1 h, the solvents were evaporated under reduced pressure and the residue kept under vacuum overnight to afford (+)-**2·TFA** (26 mg, 99%) as a viscous yellowish oil, that was shown to be almost pure by NMR. $[\alpha]_D$ +13.2 (c 0.91, $CHCl_3$); IR (ATR) ν_{max} (cm^{-1}) 3665 (w), 3406 (br, m), 2974 (s), 2903 (m), 1668 (s), 1409 (m), 1383 (m), 1195 (s), 1134 (s), 1080 (s), 1069 (s), 724 (w); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27 and 8.68 (1H each, each br s, NH_2^+), 7.17 (1H, t, *J* = 6.0 Hz, H-3), 5.29 (1H, m, H-1), 4.35 (1H, m, H-6), 2.66 (2H, m, *CH*$_2$Me), 2.63−2.46 (3H, m, H_2-4, H-8), 2.45−2.35 (1H, m, H-7), 2.18−2.07 (1H, m, H-5), 1.97 (1H, m, H'-

7), 1.97−1.82 (2H, m, H'-5, H'-8), 1.07 (3H, t, *J* = 7.2 Hz, CH_2*Me*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.5 (CO), 145.1 (C-3), 143.6 (C-2), 59.1 (C-6), 52.9 (C-1), 30.4 (C-8), 30.0 (*C*H_2Me), 28.0(C-5), 27.6 (C-7), 23.5 (C-4), 8.4 (CH_2*Me*); 19F NMR (282 MHz, $CDCl_3$) δ −76.21 (s); HRMS (TOF MS ES+) calcd for $C_{11}H_{18}NO$ $[M+H\text{-}CF_3CO_2H]^{+}$ 180.1383, found 180.1376.

*4.4.6. (−)-Homoanatoxin-a trifluoroacetate salt [(−)-**2·TFA**]*

This enantiomer (15 mg, 99%) was prepared in an analogous way as described for (+)-**2·TFA** employing the (1*S*,6*S*)-enatiomer (+)-**17** (15.6 mg); (−)-**2·TFA** was obtained as a viscous yellowish oil; $[\alpha]_D$ −15.3 (c 0.74, $CHCl_3$). Its spectroscopic data correspond with those of enantiomer (+)-**2·TFA**.

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Appendix A. Supplementary data

Supplementary data related to this article (copies of ^{1}H and ^{13}C NMR spectra) can be found at the Supplementary Data File.

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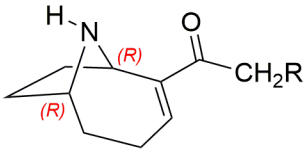
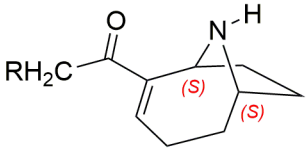
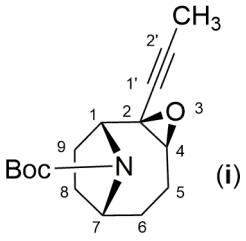


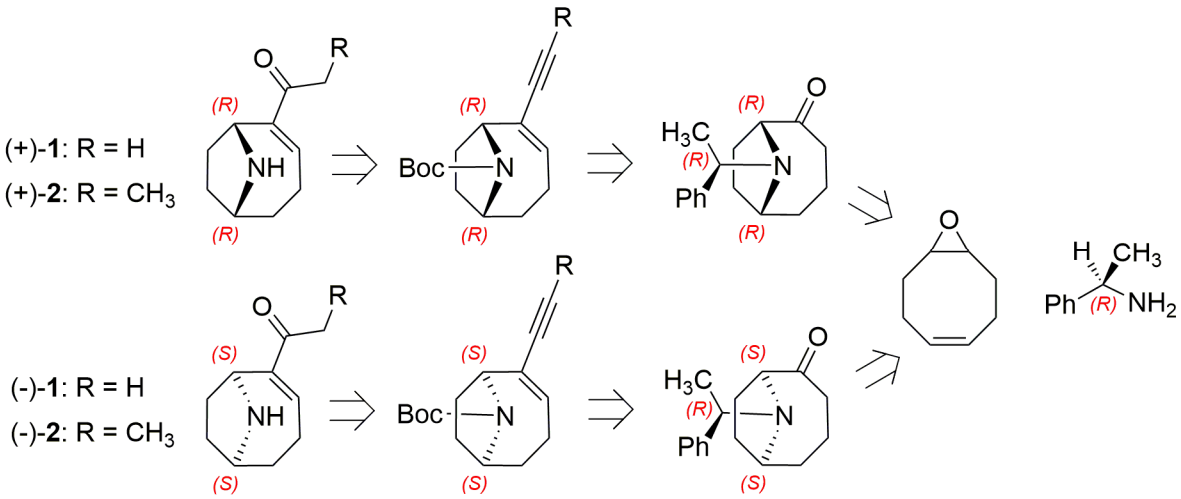


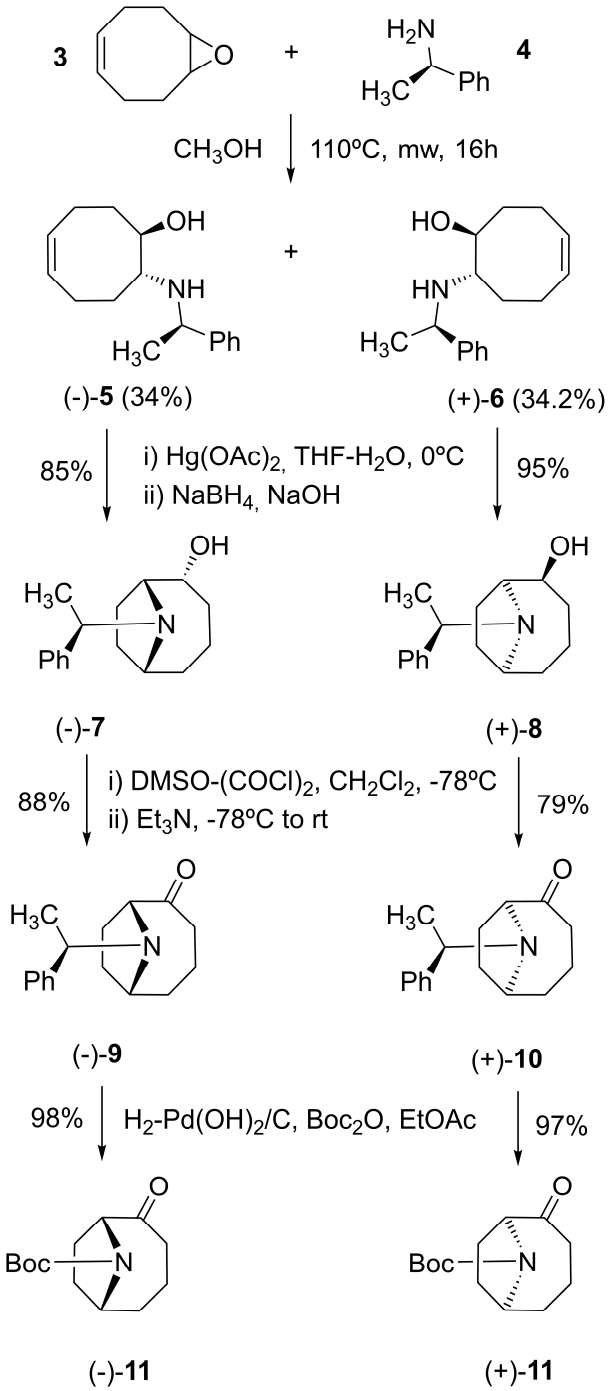
(+)-**1**: R = H anatoxin-a (-)-**1**: R = H

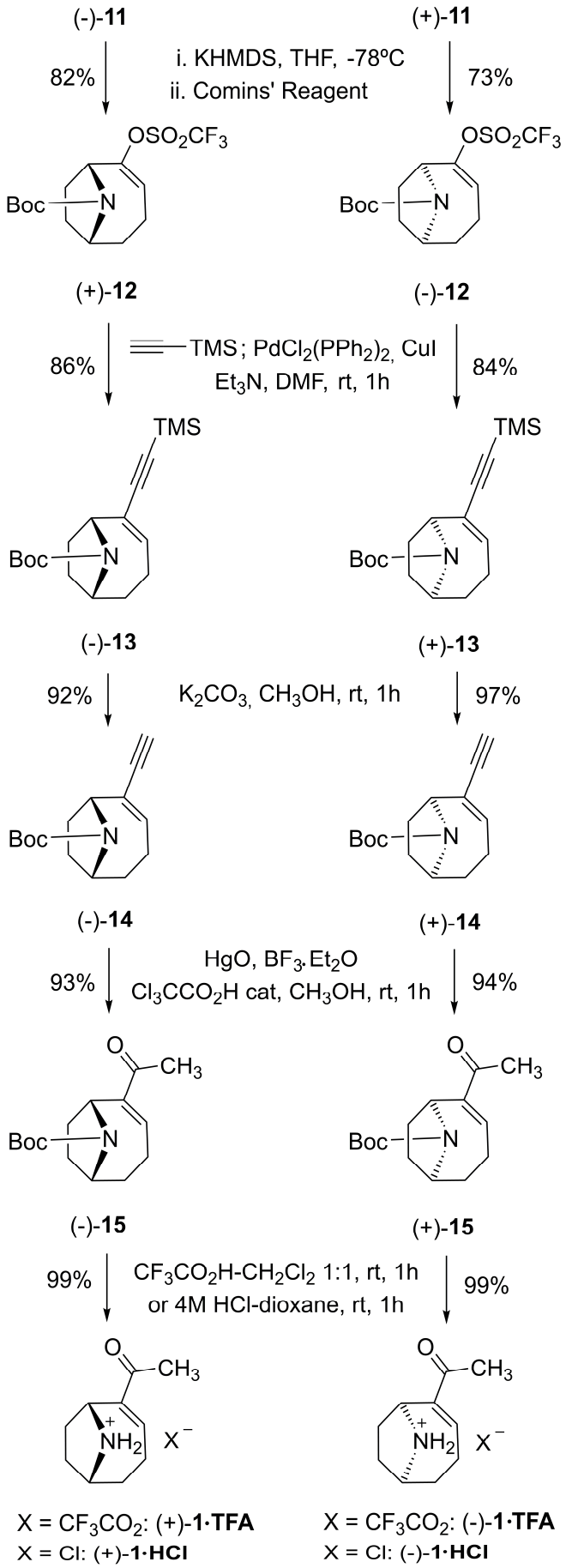
(+)-**2**: R = CH_3 homoanatoxin-a (-)-**2**: R = CH_3



(**i**)







X = CF_3CO_2: (+)-**1·TFA**
X = Cl: (+)-**1·HCl**

X = CF_3CO_2: (-)-**1·TFA**
X = Cl: (-)-**1·HCl**