Natural Products

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The Twisted Side Chain of Antillatoxin is Important for Potent Toxicity: Total Synthesis and Biological Evaluation of Antillatoxin and Analogues**

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Voltage-gated sodium channels (VGSC) are transmembrane proteins which initiate action potentials in nerve, muscle, and other electrically excitable cells, thereby playing a central role in neurotransmission.[1] VGSC modulators are therefore useful research tools for neuroscience, as well as therapeutic agents for neurological disorders.[2] Its six distinct specific binding sites are classified with respect to the binding characteristics of the molecules.[3]

Antillatoxin (**1**, Scheme 1), a cyclic peptide and potent toxin, was isolated from the marine cyanobacterium *Lyngbya majuscula*.[4,5] It has been shown to act as a VGSC activator and to bind to a hitherto unknown site of the protein distinct from other known binding sites.[6] The specific functional properties of **1** confer it with the potential as a new tool for investigating the VGSC functions.

One of the characteristic structural features of antillatoxin is a 9-*tert*-butyl-6,8-dimethyl-6,8-diene unit attached to C5 of the cyclic peptide backbone. We speculated that the unique three-dimensional shape of the lipophilic side chain may play an important role in allosteric activation of the channels through hydrophobic interactions. Herein we report total syntheses of **1** and three side-chain analogues, which were used to uncover the structural basis for the importance of the side chain in the neurotoxicity of **1**.

We planned to devise a unified strategy for synthesizing **1** and its analogues with distinct side chains (Scheme 1). Therefore, the diene was envisioned to be appended to the common intermediate **21** through C7–C8 bond formation by a Suzuki–Miyaura coupling reaction.[7] This synthetic scheme would allow us to access a variety of side chain modified analogues by varying the final reaction. As an initial phase in the study of the structure-activity relationship at an atomic level, we designed the C8-demethyl antillatoxin **2** to investigate the impact of one methyl substitution. Deletion of the CH_3 group (C15) would specifically modify the conformation of the 6,8-diene without changing the overall shape and the chemical properties of **1**.

The synthesis of **1** and **2** started from an *anti*-selective asymmetric aldol reaction[8] between aldehyde **4**[9] and silyl enol ether **5** to set the two stereocenters at C4 and C5. The resulting adduct **6** (96% *ee*) was recrystallized to give an enantiopure material. After protection of the secondary alcohol of **6** as a TES ether, ester **7** was reduced to the primary alcohol **8**. One-carbon homologation from **8** to **10**, through tosylate **9**, and subsequent DIBAL-H reduction of the nitrile afforded aldehyde **11**. Next, a Mannich reaction of **11** at C3 installed the requisite *exo*-methylene moiety to provide **12**,[10] which was reduced to alcohol **13** using DIBAL-H. The primary alcohol of **13** was in turn converted into the highly unstable allylic triflate, which was displaced in situ by the carbanion generated from methylthiomethylphenyl sulfone,[11] leading to **14**. Finally, TBAF treatment of TES ether **14** produced fragment **15**, the right half of antillatoxin and its analogues.

Coupling of the fragment **16**,[5] the left half of the molecule, with **15** using EDC·HCl and DMAP resulted in the formation of ester **17**, and the next two steps efficiently transformed the phenylsulfonyl methylthiomethyl group at C1 of **17** into the thioester of **19**. Chemoselective *m*CPBA oxidation of sulfide **17** generated sulfoxide **18**, which underwent a Pummerer reaction by the action of trifluoroacetic anhydride and 2,6-di-*tert*-butyl-pyridine in toluene.[12] The reaction led to thioester **19** after PhSH attack on the resultant trifluoroacetate with concomitant ejection of phenyl sulfinic acid.[13] In the sequence going from **14** to **18**, the use of the C1-acetal structure as a thioester surrogate was particularly important for the success of the synthesis, because premature formation of an sp^2 carbon center at C1 allowed the C3/C12 *exo* olefin to isomerize into a C2/C3 conjugated olefin under the conditions used.

After removal of the Boc group of **19** under acidic conditions, thioester **20** was effectively activated by silver nitrate in the presence of HOAt and $i\mathrm{Pr_2NEt}$ to give the common intermediate **21** through a high yielding macrolactam formation.

The two boronic esters **22**[14] and **23** were then coupled with vinyl iodide **21** using a reagent combination of $[PdCl_2(dppf)]$, Ph_3As, and Cs_2CO_3,[15] giving rise to antillatoxin **1** and its C8-demethylated analogue **2**, respectively. Hence, a unified synthetic route to the antillatoxin structures was successfully developed.

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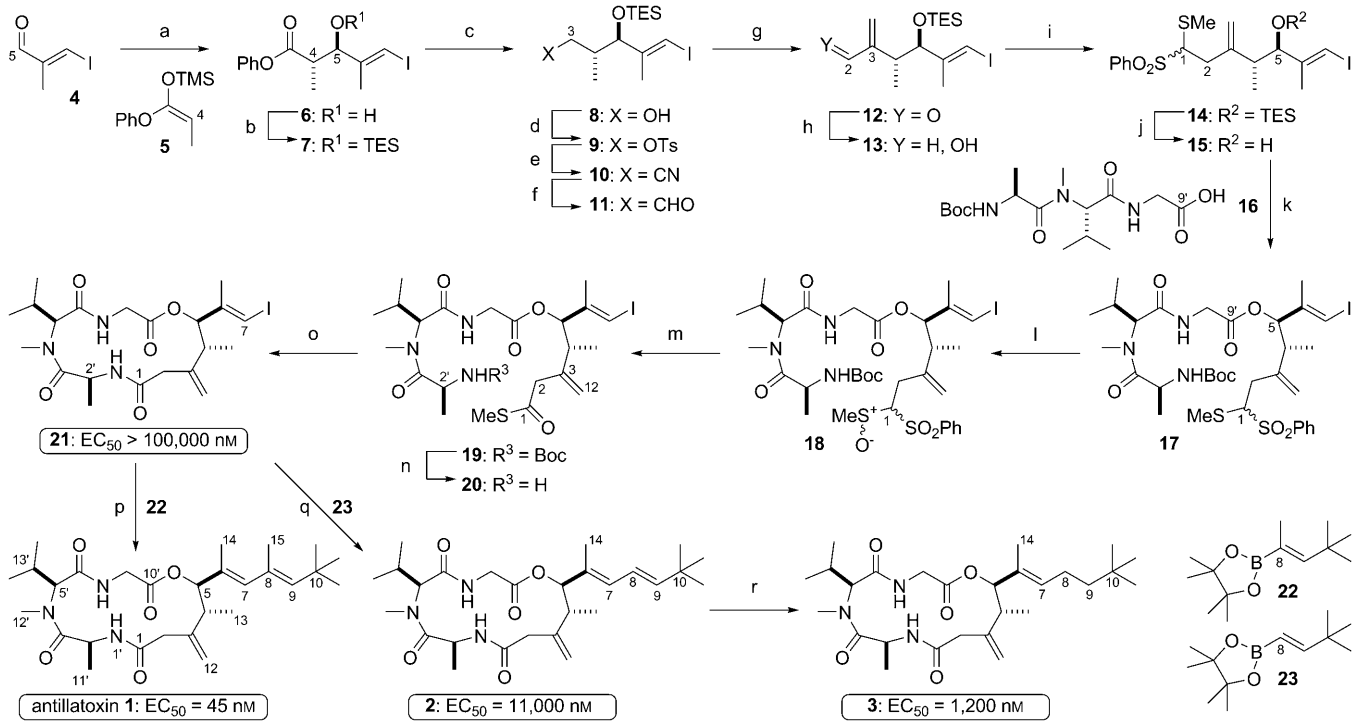

Scheme 1. Total synthesis and cytotoxicity of antillatoxin **1** and the side chain modified analogues. Reagents and conditions: a) **5** (1.2 equiv), $Zr(OtBu)_4$ (0.1 equiv), (*R*)-3,3′-I_2-binol (0.12 equiv), *n*PrOH (0.8 equiv), H_2O (0.2 equiv), toluene, 0 °C, 80% (83% *de*, 96% *ee*); recrystallization from AcOEt, 89% (>99% *ee*); b) TESCl, imidazole, DMF, RT, 100%; c) DIBAL-H, CH_2Cl_2, −78 °C, 100%; d) TsCl, pyridine, RT, 94%; e) NaCN, DMSO, 45 °C, 94%; f) DIBAL-H, CH_2Cl_2, −78 °C; g) pyrrolidine, propionic acid, aq HCHO, *i*PrOH, 45 °C, 82% (2 steps); h) DIBAL-H, CH_2Cl_2, −78 °C, 87%; i) Tf_2O, 2,6-lutidine, 4 Å M.S., CH_2Cl_2, −78 °C then $PhSO_2CH_2SMe$, NaH, DMF, −78 °C to −50 °C, 69%; j) TBAF, THF, 0 °C, 96%; k) **16** (2.0 equiv), EDC·HCl, DMAP, CH_2Cl_2, RT, 83%; l) *m*CPBA, CH_2Cl_2, −78 °C to −20 °C; m) $(CF_3CO)_2O$, 2,6-di-*tert*-butylpyridine, toluene, −78 °C to −40 °C, then PhSH, −40 °C, 67% (2 steps); n) CF_3COOH/CH_2Cl_2 = 1:5, RT; o) $AgNO_3$, HOAt, *i*Pr_2NEt, DMF, 0 °C, 71% (2 steps); p) **22** (4.0 equiv), $[PdCl_2(dppf)\cdot CH_2Cl_2]$ (0.25 equiv), Ph_3As (0.5 equiv), Cs_2CO_3, THF, RT, 78%; q) **23** (4.0 equiv), $[PdCl_2(dppf)\cdot CH_2Cl_2]$ (0.25 equiv), Ph_3As (0.5 equiv), Cs_2CO_3, THF, RT, 92%; r) H_2, 20% $Pd(OH)_2$/C, benzene, RT, 72%. binol = 2,2-dihydroxy-1,1-binaphthyl, *m*CPBA = *meta*-chloroperbenzoic acid, DIBAL-H = diisobutylaluminum hydride, DMF = *N*,*N*-dimethylformamide, DMAP = 4-dimethylaminopyridine, DMSO = dimethylsulfoxide, dppf = 1,1′-bis(diphenylphosphino)ferrocene, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOAt = 1-hydroxy-7-azabenzotriazole, M.S. = molecular sieves, TBAF = tetra-*n*-butylammonium fluoride, TES = triethylsilyl, THF = tetrahydrofuran, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl, Ts = *para*-toluenesulfonyl.

The biological activities of **1**, **2**, and **21** as VGSC activators were evaluated by a neurotoxicity assay using Neuro 2a mouse neuroblastoma cells, which express VGSCs on their membrane (Scheme 1).[6c] In sharp contrast to potent toxicity of the parent natural product **1** (EC_{50} = 45 nM), vinyl iodide **21** exhibited more than 2000-fold weaker activity (EC_{50} > 100000 nM), indicating the significance of the bulky *tert*-butyl-substituted group at C7. Most interestingly, the C8-demethylated analogue **2** was found to be approximately 250 times less toxic than **1** (EC_{50} = 11000 nM), even though the dienes of **1** and **2** had the similar steric bulk. This result clearly shows that the methyl substituent at C8 has a decisive role in the potent neurotoxicity of antillatoxin.

To gain insight into the relationship between the assay data and the conformational behavior of the molecules, detailed NMR analyses of **1** and **2** were carried out (Figure 1). The ^1H NMR chemical shifts for the core of **2** were in excellent agreement with those of **1**.[16] Thus, the structure of the macrolactam core of **2** was virtually identical to that of **1**, and deletion of the CH_3 group (C15) had no influence over the specific conformation of the macrocycle. In contrast, the conformations of the side-chains of **1** and **2** were different

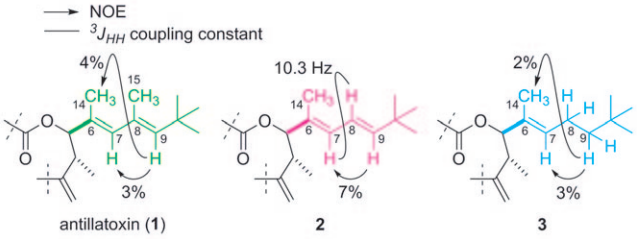

Figure 1. NOE and $^3J_{HH}$ coupling data of the diene moieties of antillatoxin (**1**), 8-demethyl-antillatoxin (**2**), and 8-demethyl-8,9-dihydro-antillatoxin (**3**). The side chains are shown in green (**1**), magenta (**2**), and cyan (**3**).

based on the ^1H NMR chemical shifts as well as $^3J_{HH}$ and NOE data (Figure 1). The C8-demethylated antillatoxin **2** shows a NOE only between the hydrogen atoms on C9 and C7 (designated C9–H and C7–H), and the value of $^3J_{HH}$ between C7–H and C8–H was measured as 10.3 Hz. Both of these findings indicated that the conjugated diene moiety of **2** adopts a planar *s-trans* conformation around the C7–C8 bond

as a single stable isomer, and thus that the C6=C7–C8=C9 torsion angle (ϕ) should be approximately 180°. In contrast, the diene of antillatoxin **1** apparently deviates from the planar conjugated conformation, since C9–H of **1** is in spatial proximity not only to C7–H in a 1,3-relationship, but also to the C14–H in a 1,5-relationship, based on the NOE data.

To clarify the conformational preference of the dienes of **1** and **2**, ab initio calculations were carried out using model structures at the RHF/6-31G** level (Figure 2a).[16] The C6=C7–C8=C9 torsion angle (ϕ) was varied from 0° to 360° and the energy was minimized using MMFF[17] prior to the MO calculations, while the coordinates of the macrolactam core (shown in gray in Figure 2a) were fixed as the energy minimized conformation of **1** which satisfied the NMR data. The diene moiety of C8-demethylated **2** was found to exhibit a single most-stable *s-trans* conformation ($\phi = 181°$), which agreed with the results of the NOE experiment, while the diene of **1** was found to have a distinctive energy profile. The high energy of the *s-trans* conformation ($\phi = 180°$) of **1** would be explained by a greater destabilization energy from the steric interactions between the CH_3 groups (C14 and C15) in a 1,3-relationship compared to the stabilizing conjugation energy gained from the planar conformation.[18] Instead, the diene of **1** has two stable twisted conformations ($\phi = 57°$ and 296°), in which C9–H is proximal to both C7–H and C14–H, as shown in the NOE data. Importantly, the three-dimensional orientation of the bulky *tert*-butyl group in these two conformations of **1** is significantly different from that of **2**.[19]

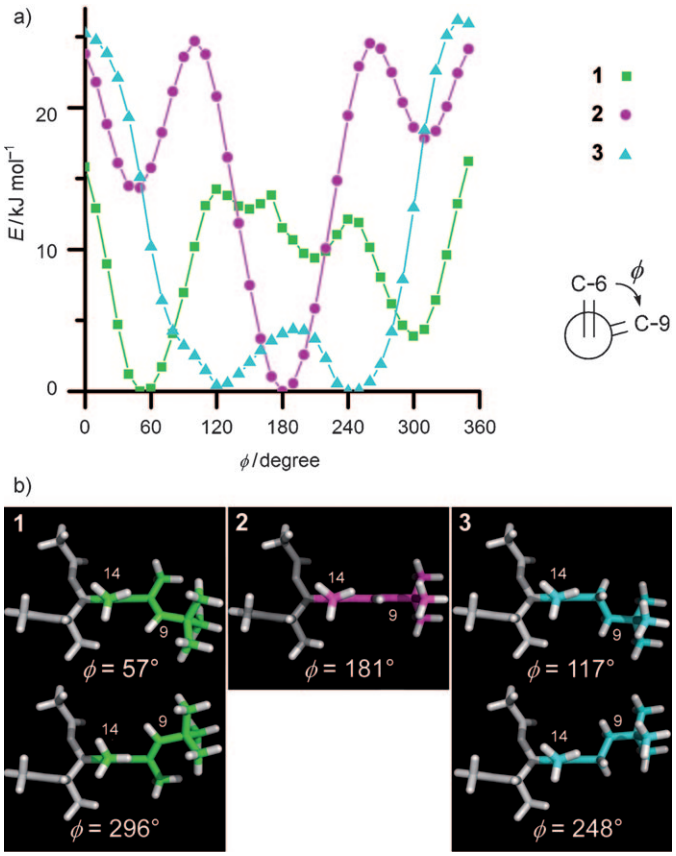

Figure 2. a) Ab initio conformation energy profiles of the ϕ [C6=C7–C8=C9] dihedral angles of **1**, **2**, and **3**. b) The optimized geometries for the most stable conformations are presented in stick model (**1**: 57°, **2**: 181°, **3**: 248°). The second most stable conformations are also shown for **1** (296°, 3.2 kJ mol^{-1}) and **3** (117°, 0.54 kJ mol^{-1}).

Based on the NMR and simulation data for **1** and **2**, we hypothesized that the twisted shape of the *tert*-butyl-substituted diene of **1** was critical in the allosteric activation of the sodium channel, resulting in neurotoxicity.

This hypothesis was additionally corroborated by the synthesis and biological evaluation of analogue **3**. It had tetrahedral sp^3 carbon atoms at C8 and C9 in place of the planar sp^2 carbon atoms of **2**, and is therefore expected to provide more rotational freedom around the C8–C9 bond (Scheme 1). Chemoselective hydrogenation of the C8–C9 double bond of triene **2** was accomplished using $Pd(OH)_2/C$ under a hydrogen atmosphere in benzene, giving rise to 8-demethyl-8,9-dihydro-antillatoxin **3**. Whereas the ^1H NMR spectrum of the cyclic peptide core of **3** indicated that the molecular topology of the cycle of **1** and **2** is preserved,[16] **3** showed NOEs between C9–H and both C7–H and C14–H, suggesting a twisted conformation of the side chain (Figure 1). Nonplanar gauche conformations with $\phi = 117°$ and 248° were found to be the two most stable conformations based on ab initio calculations (Figure 2b). Notably, the shapes of these two conformations of **3** are similar to those of **1** at 57° and 298°. Compound **3**, possessing the saturated twisted side chain, indeed exhibited approximately 10 times stronger neurotoxicity than the planar demethyl compound **2** (EC_{50} = 1200 nM, Scheme 1). Therefore, we were able to regain the biological activity for **3** only by introducing two hydrogen atoms at C8 and C9 of **2**.

In summary, we achieved the unified total synthesis of antillatoxin **1** and several analogues. Use of a phenylsulfonyl methylthiomethyl group as a thioester surrogate and the introduction of carbon chains by Suzuki–Miyaura coupling reaction at the last stage are two key features of this synthesis. Structural and biological studies of **1**, **2**, **3**, and **21** revealed that the twisted shape of the *tert*-butyl-substituted diene groups plays a critical role in the potent neurotoxicity of **1**. Our strategy for total synthesis is expected to generate other new antillatoxin analogues having a variety of structures at the lipophilic side chain without changing the overall molecular topology of the macrolactam core. Additional manipulation of the side-chain structure of **1** is currently underway in our group with the aim of developing useful molecular probes for modulating the structures and functions of VGSCs.

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