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Convergent Synthesis and Biological Activity of the WXYZA′B′C′ Ring System of Maitotoxin

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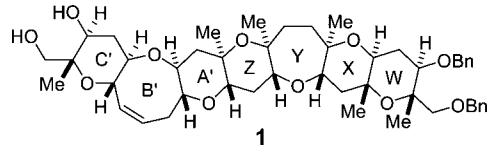
ABSTRACT



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The WXYZA′B′C′ ring system (1) of maitotoxin (MTX) was synthesized in a convergent manner via successive coupling of the W, Z, and C′ ring fragments through construction of the XY and A′B′ ring systems. The synthetic segment 1 blocked the hemolytic activity elicited by MTX.

Maitotoxin (MTX) was first discovered as one of the toxins responsible for ciguatera seafood poisoning and later found to be a product of the epiphytic dinoflagellate *Gambierdiscus toxicus*.[1] MTX is the largest non-biopolymer known to date (MW 3422),[2] and its remarkable biological activities have been reported, namely, that (i) it elicited hemolysis of mouse blood cells at 15 nM,[3] (ii) it induced Ca^{2+} influx in rat glioma C6 cells at 0.3 nM,[4] and particularly, (iii) its lethality against mammals was extremely potent (50 ng/kg, mice, i.p.).[2c,5] The structure of MTX was elucidated by the Yasumoto group through extensive NMR analysis,[2] and its complete stereochemistry was determined independently by the Tachibana[6] and Kishi[7] groups. Its unique, gigantic molecular structure has attracted considerable attention from the synthetic community, and recent syntheses of partial structures of MTX by the Nicolaou[8] and Nakata[9] groups have given support to the originally proposed structure.[10] Despite a large number of pharmacological and biophysical investigations, the precise mode of action of MTX has not been elucidated at the molecular level. Preparation of photoaffinity probes and

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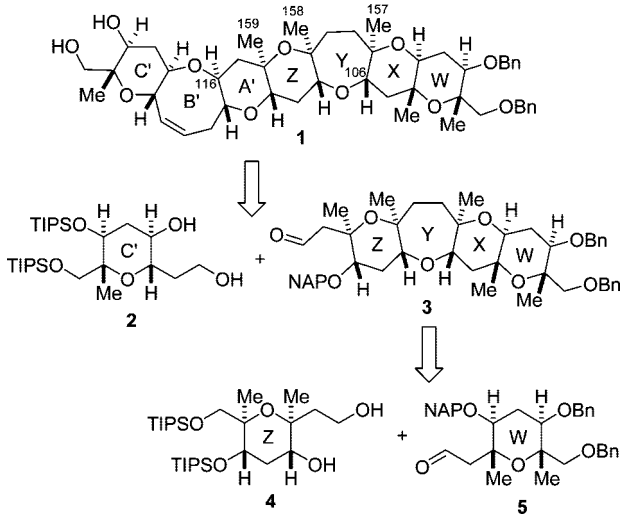
radioisotope-labeled derivatives for identification of its target molecules has been hampered chiefly due to the short supply of this material from natural sources, although non-selective cation channels are proposed to be its target protein.[11] It is reported that Ca^{2+} influx in rat glioma C6 cells induced by MTX was inhibited by brevetoxin-B (BTXB) and the EC_{50} value was estimated to be 13 μM.[4] The hydrophobic part of MTX resembles other ladder-shaped polyethers such as BTXB, which is known to bind to voltage-sensitive sodium channels.[12] Therefore, this part was expected to be the binding domain for target (transmembrane) proteins and to be utilized as a molecular probe to identify these proteins.[13] Herein, we describe the convergent synthesis of the WXYZA′B′C′ ring system (**1**, Scheme 1) corresponding to

Scheme 1. Synthesis Plan for the WXYZA′B′C′ Ring System (**1**) of MTX



a portion of the hydrophobic part of MTX and the evaluation of its biological activity.

From the synthetic point of view, it is a daunting challenge to construct the WXYZ ring system in a convergent manner because of the presence of contiguous angular methyl groups, i.e., C157, C158, and C159, on the Y and Z rings, although a linear synthesis of the WXYZA′ ring system has recently been reported.[9b] Our synthesis plan of the WXYZA′B′C′ ring system (**1**) of MTX is shown in Scheme 1. We envisaged extensive utilization of the convergent method via α-cyano ethers[14] to construct the heptacyclic ether **1**, which was to be derived from the C′ (**2**) and WXYZ (**3**) ring units through construction of the A′B′ ring system. In turn, the tetracyclic ether **3** was to be synthesized in an analogous sequence from the W and Z ring units via formation of the XY ring system. However, it remained uncertain whether our method was applicable to the WXYZ ring system with its contiguous angular methyl groups.

As shown in Scheme 2, synthesis of the WXYZ ring system **3** started with coupling of the Z ring diol **4**[15] and the W ring aldehyde **5**[15] through (i) acetal formation, (ii) regioselective opening of the seven-membered ring acetal using TMSCN and $Sc(OTf)_3$,[16] and (iii) elimination of the resulting primary alcohol, giving a terminal olefin by Nishizawa−Grieco protocol,[17] to yield α-cyano ether (**6**) as an inseparable mixture of C106 diastereomers (70%, three steps).[18] Reduction of the nitrile **6** with DIBALH gave aldehyde **7** (70%), which was treated with vinyllithium to afford allylic alcohol **8** (86%). Ring-closing metathesis (RCM) of the diene **8** by the action of second-generation Grubbs catalyst **9**[19] proceeded smoothly in toluene under reflux for 20 min. Oxidation of the alcohol with Dess−Martin periodinane (DMP)[20] giving enone, followed by hydrogenation of the double bond using PtO_2 catalyst, furnished saturated ketones as an inseparable mixture of the desired **10** and its C106 epimer in a 1:2 ratio. Subsequent DBU-mediated isomerization in toluene at 110 °C improved the ratio to 8:1. Removal of the NAP[21] group of **10** with DDQ afforded **11** (73%), which was separated from C106 epimer (12%) by silica gel chromatography. When hydroxyketone **11** was treated with ethanethiol in the presence of $Zn(OTf)_2$, the reaction was sluggish, and mixed thioacetal **12** was obtained in 42% yield with recovery of the starting material, in contrast to the behavior of a similar system lacking angular methyl groups.[22] Recovered **11** was recycled (twice) to provide **12** in 64% total yield as an inseparable mixture of isomers (5:1) with respect to the stereogenic center on the acetal carbon, with concomitantly formed dithioacetal **13** (12%) and recovery of **11** (10%). The crucial step of the present synthesis, introduction of the angular methyl group in axial orientation,[23] was achieved under carefully controlled conditions. Oxidation of the mixed thioacetal to the sulfone was carried out with MCPBA in dichloromethane at −78 to −40 °C. The reaction mixture was treated with Me_3Al at

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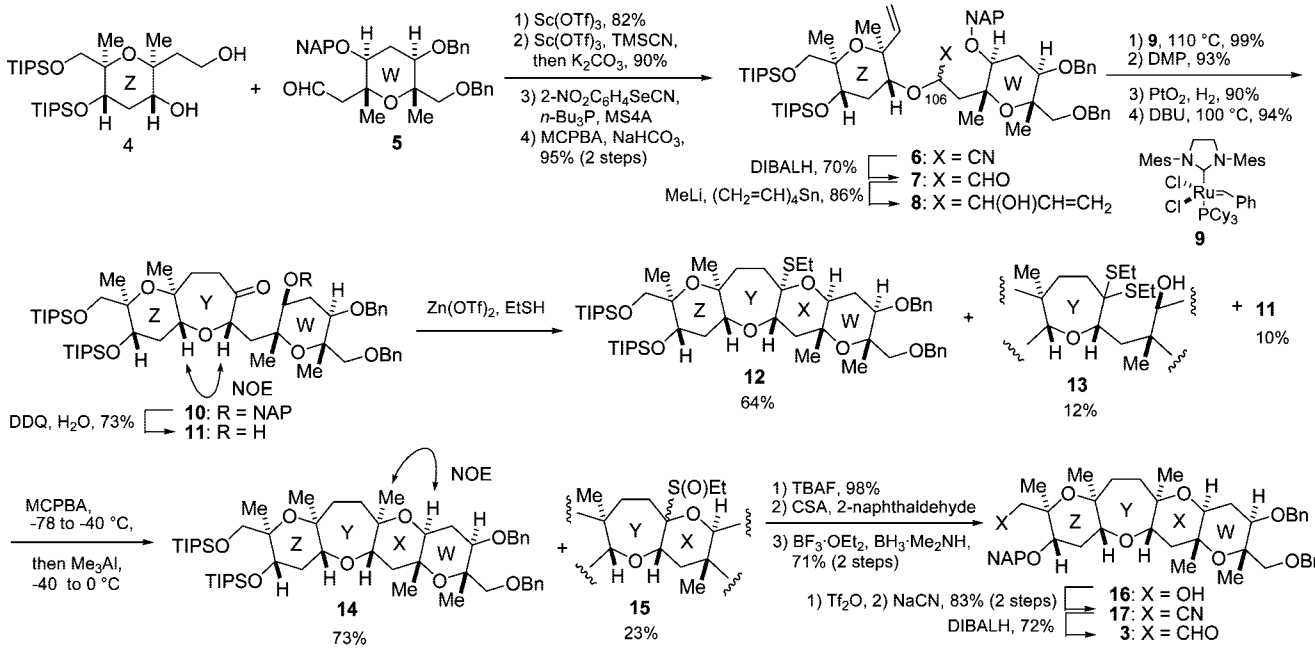
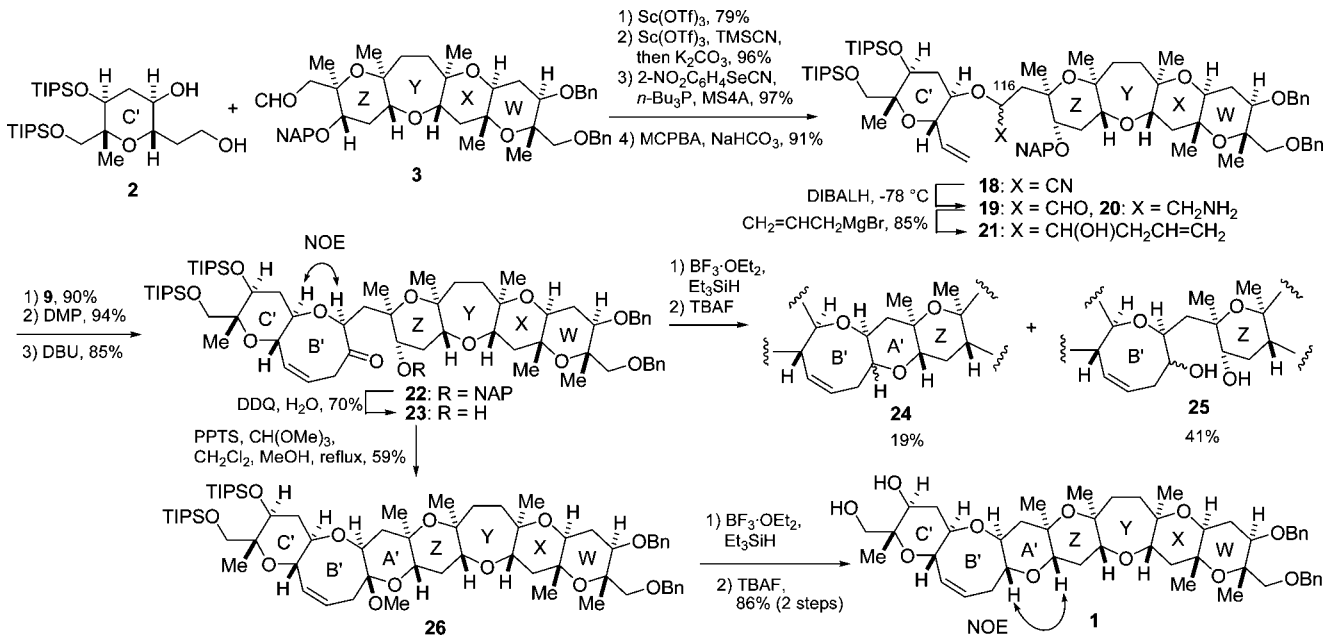
Scheme 2



−40 °C and warmed to 0 °C to give **14** as a single isomer in 73% yield along with the sulfoxide intermediate **15** (23%). The structure of **14** was unambiguously determined by NOE experiments. Thus, convergent synthesis of the WXYZ ring system possessing the requisite five angular methyl groups was achieved. Side chain elongation of the Z ring was carried out next. Removal of the TIPS groups of **14** was followed by 2-naphthylidene acetal formation and regioselective reductive cleavage[24] of the acetal to provide primary alcohol **16** (**16**:regioisomer ≥10:1). Conversion of alcohol **16** to aldehyde **3** was performed by standard procedures: (i) triflation, (ii) substitution with NaCN giving nitrile **17** (83%, two steps), and (iii) DIBALH reduction of **17** (72%).

Having synthesized the WXYZ ring fragment, we turned our attention to the WXYZA′B′C′ ring system (Scheme 3). The C′ ring diol **2**[15] and the WXYZ ring aldehyde **3** were coupled through (i) acetal formation, (ii) regioselective cleavage, and (iii) elimination of the primary alcohol to afford

Scheme 3



α-cyano ether **18** (67%, three steps). Reduction of nitrile **18** with DIBALH was not an easy task, in contrast to the previous results, furnishing aldehyde **19** in 51% yield with concomitant formation of primary amine **20** (42%) due to overreduction, which was recovered by Dess−Martin oxidation to give nitrile **18** in 77% yield.[25] Treatment of the aldehyde **19** with allylmagnesium bromide resulted in the formation of homoallylic alcohol **21**. RCM of the diene **21** with Grubbs catalyst **9** was followed by Dess−Martin oxidation to yield eight-membered ring enones as a mixture of **22** and its C116 epimer in a 1:2 ratio. Isomerization by treatment with DBU in toluene at 110 °C afforded **22** as the major product in 85% yield, and removal of the NAP group with DDQ gave hydroxyketone **23** (70%), which was separated from C116 epimer (12%).

The final step, construction of the A′ ring by reductive etherification of the hydroxyketone **23**, turned out to be problematic.[26] Treatment of **23** with Et_3SiH in the presence of $BF_3{\cdot}OEt_2$ was followed by removal of the TIPS groups with TBAF to furnish **24** in low yield (19%) as a mixture of C116 epimers (2:1) with concomitant formation of diol **25** (41%, a mixture of diastereomers). Although conversion of **23** to mixed thioacetal under the identical conditions for transforming **11** to **12** (Scheme 2) using $EtSH/Zn(OTf)_2$ failed, with recovery of starting material, treatment of **23** with $CH(OMe)_3$ in the presence of PPTS in CH_2Cl_2/MeOH under reflux for 65 h afforded methylacetal **26** in 59% yield. Reduction of the methylacetal[27] with $Et_3SiH/BF_3{\cdot}OEt_2$ and subsequent removal of the TIPS groups with TBAF afforded the WXYZA′B′C′ ring system (**1**) as a single isomer (86%, two steps), whose structure was determined by NOESY experiments.

Differences in proton (500 MHz) and carbon (125 MHz) NMR chemical shifts in 1:1 C_5D_5N/CD_3OD between MTX and the synthetic WXYZA′B′C′ fragment **1** are shown in Figure 1. The ^1H and ^{13}C NMR chemical shifts of the XYZA′B′ ring moiety were in good accordance with those of MTX, supporting the proposed structure, although the values corresponding to both terminal rings (W and C′) were different due to the absence of the D′E′F′ and UV ring systems.[7]

The biological activity of **1** was evaluated next (see Supporting Information).[3,4] MTX induced hemolysis of human red blood cells at 10 nM, and this value was taken as 100%. The WXYZA′B′C′ moiety **1** blocked this hemolytic activity in a dose-dependent manner, and ca. 80% inhibition was observed at 10 μM. However, **1** itself induced no hemolysis at the same concentration. Considering that BTXB did not inhibit MTX-induced hemolytic activity even at 10 μM, the hydrophobic portions (e.g., **1**) of MTX are expected to be promising molecular probes for identifying the target proteins of MTX.

In conclusion, a highly convergent synthesis of the WXYZA′B′C′ ring system of MTX was achieved by extensive utilization of the α-cyano ether method through union of the W, Z, and C′ ring fragments via construction of the XY and A′B′ ring systems. Further studies on the biological activities of **1** and synthesis of the W−F′ ring system of MTX to improve the antagonistic activity are currently in progress in our laboratory.

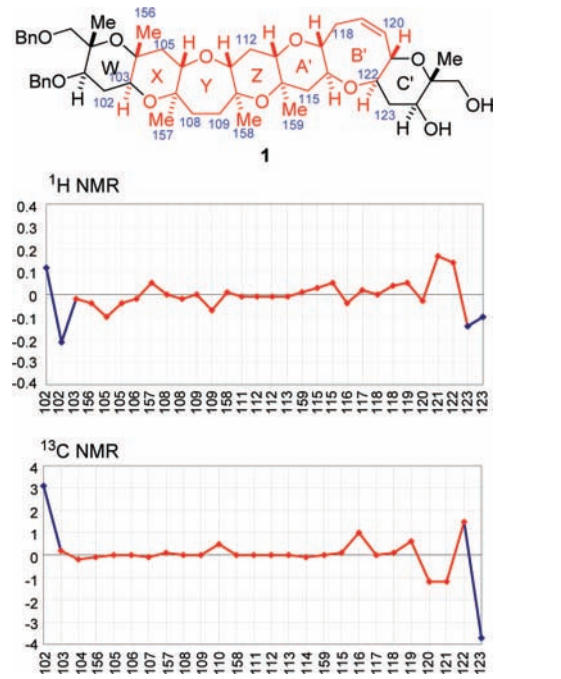


Figure 1. Differences in proton (500 MHz, 1:1 C_5D_5N/CD_3OD) and carbon (125 MHz, 1:1 C_5D_5N/CD_3OD) NMR chemical shifts between MTX and the WXYZA′B′C′ ring system (**1**). The *x*- and *y*-axes represent carbon number and $\Delta\delta$ ($\Delta\delta = \delta$MTX $- \delta$synthetic **1** in ppm), respectively.

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Supporting Information Available: Experimental details and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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