

Synthesis and Biological Evaluation of QRSTUVWXYZA' Domains of Maitotoxin

K. C. Nicolaou,^{*,†} Philipp Heretsch,[†] Tsuyoshi Nakamura,[‡] Anna Rudo,[‡] Michio Murata,[§] and Keiichi Konoki^{||}

[†]Department of Chemistry, BioScience Research Collaborative, Rice University, 6100 Main Street, Houston, Texas 77005, United States

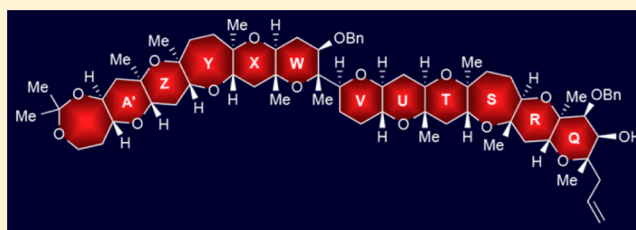
[‡]Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

[§]Department of Chemistry, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043, Japan

^{||}Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan

S Supporting Information

ABSTRACT: The synthesis of QRSTUVWXYZA' domains 7, 8, and 9 of the highly potent marine neurotoxin maitotoxin (1), the largest secondary metabolite isolated to date, is described. The devised synthetic strategy entailed a cascade Takai–Utimoto ester olefination/ring closing metathesis to construct ring Y, a hydroxydithioketal cyclization/methylation sequence to cast ring X, a Horner–Wadsworth–Emmons coupling of WXYZA' ketophosphonate 11 with QRSTU aldehyde 12 to form enone 10, and a reductive hydroxyketone ring closure to forge ring V. 2D NMR spectroscopic analysis and comparison of ¹³C chemical shifts with those of the corresponding carbons of maitotoxin revealed close similarities supporting the originally assigned structure of this region of the natural product. Biological evaluations of various synthesized domains of maitotoxin in this and previous studies from these laboratories led to fragment structure–activity relationships regarding their ability to inhibit maitotoxin-elicited Ca²⁺ influx in rat C6 glioma cells.



1. INTRODUCTION

The isolation¹ and biological evaluation² of maitotoxin (1, Figure 1a) inspired numerous studies directed toward its structural elucidation^{3–6} and synthesis.^{7,8} As the largest secondary metabolite isolated to date and one of the most potent neurotoxins known, this impressive marine natural product represents the ultimate synthetic target and provides opportunities for discovery and invention in organic synthesis and chemical biology. As part of a program to explore such opportunities, we have developed several methods for the construction of maitotoxin's structural motifs and synthesized a number of its polycyclic domains, including fragments 2–6 (Figure 1b).^{7a–f} In this article we report the synthesis of the QRSTUVWXYZA' domains 7–9 (Figure 1c) of maitotoxin and the biological evaluation of these and other previously synthesized fragments as inhibitors of maitotoxin-elicited Ca²⁺ ion influx in rat C6 glioma cells. We also report cytotoxic properties of some of these fragments as revealed by assays with the NCI-60 DTP Human Tumor Cell Line panel.

2. RESULTS AND DISCUSSION

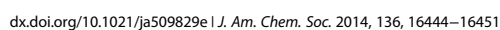
2.1. Retrosynthetic Analysis. The synthetic strategy for the synthesis of the QRSTUVWXYZA' domains 7–9 (Figure 1c) of maitotoxin, desired either for further elaboration into larger maitotoxin domains or to serve as tools for biological

investigations, was devised based on the retrosynthetic analysis shown in Figure 2. Thus, dismantling of ring V within 8 through a hydroxyketone reductive cyclization⁹ led to enone 10 as a potential precursor. The latter was to be derived from the corresponding partners (ketophosphonate 11 and aldehyde 12) for a Horner–Wadsworth–Emmons (HWE) olefination. Ketophosphonate 11 was traced back to the previously synthesized building blocks 14^{7f} and 15^{7f} through projected (a) esterification; (b) Takai–Utimoto ester olefination/ring closing metathesis;^{10,11} and (c) hydroxydithioketal cyclization followed by stereoselective replacement of the remnant thio group with a methyl group as indicated in Figure 2.¹² Fragment 12 was to be prepared from the previously synthesized advanced intermediate 13^{7d} through standard manipulations.

2.2. Synthesis of WXYZA' Ketophosphonate 11. Scheme 1 summarizes the construction of the required ketophosphonate 11 from the previously reported ZA' and W fragments 14^{7f} and 15.^{7f} Thus, TBDPS ether 14 was efficiently converted to nitrile 16 through a sequence involving bis-desilylation (TBAF, quant. yield), monotosylation (TsCl, 90% yield), resilylation (TBSOTf, 91% yield) and substitution of the tosylate group with a cyano group (KCN, 99% yield). Stepwise reduction of nitrile 16, first

Received: September 23, 2014

Published: November 6, 2014



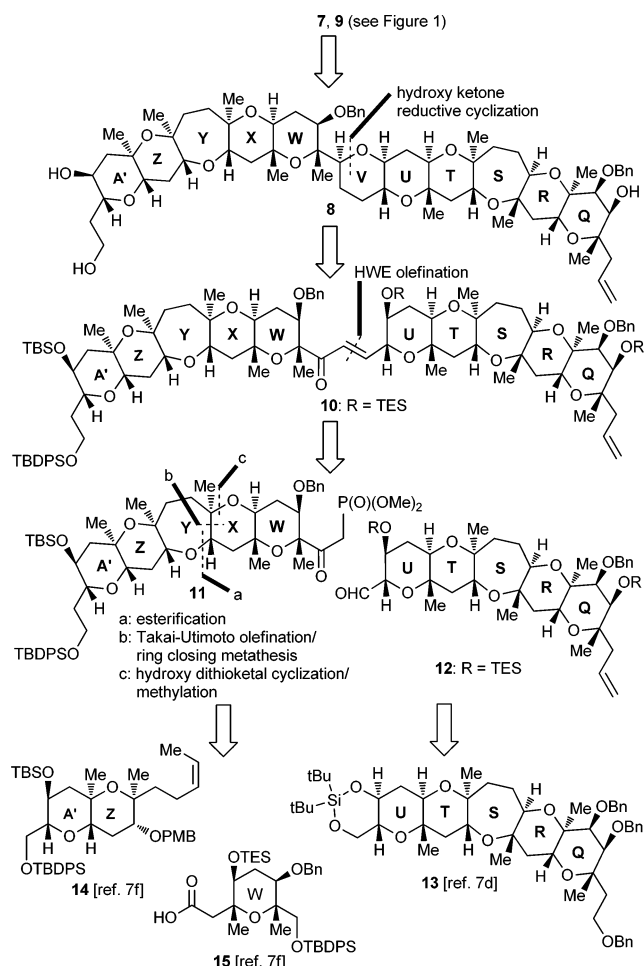


Figure 2. Retrosynthetic analysis of the QRSTUVWXYZA' domains 7, 8, and 9 of maitotoxin. Abbreviations: PMB = *para*-methoxybenzyl; TBDPS = *tert*-butyldiphenylsilyl; TBS = *tert*-butyldimethylsilyl; TES = triethylsilyl.

The rather labile hydroxyenol ether **20** was then protected as a TMS ether (TMSOTf, quant. yield) and converted stereoselectively to ketone **22** by hydroboration/oxidation (CyBH₂; H₂O₂/NaOH, 74% yield) followed by oxidation of the resulting alcohol (DMP, 90% yield). The configuration of the newly established chiral center at C₁₀₆ was confirmed by NMR spectroscopic analysis (i.e., NOE studies as indicated on structure **22**, Scheme 1). Parenthetically, it is interesting to note that substrate **21** proved resistant to hydroboration with Cy₂BH, presumably due to the bulkiness of this reagent as compared to CyBH₂.¹⁴ Removal of the TMS group from **22** with *p*-TsOH gave the corresponding hydroxyketone (96% yield), which was subjected to mixed thioacetalization [EtSH, Zn(OTf)₂] to furnish the corresponding S,O-ketal as a mixture of C₁₀₇ diastereoisomers (74% yield, **23a**: α -SEt, **23b**: β -SEt, ca. 1:2 dr, inconsequential). Exposure of this mixture to *m*-CPBA in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (−78 → −10 °C) followed by addition of Me₃Al at −78 °C furnished pentacycle **24** in 78% overall yield and as a single diastereoisomer.^{7g,12b}

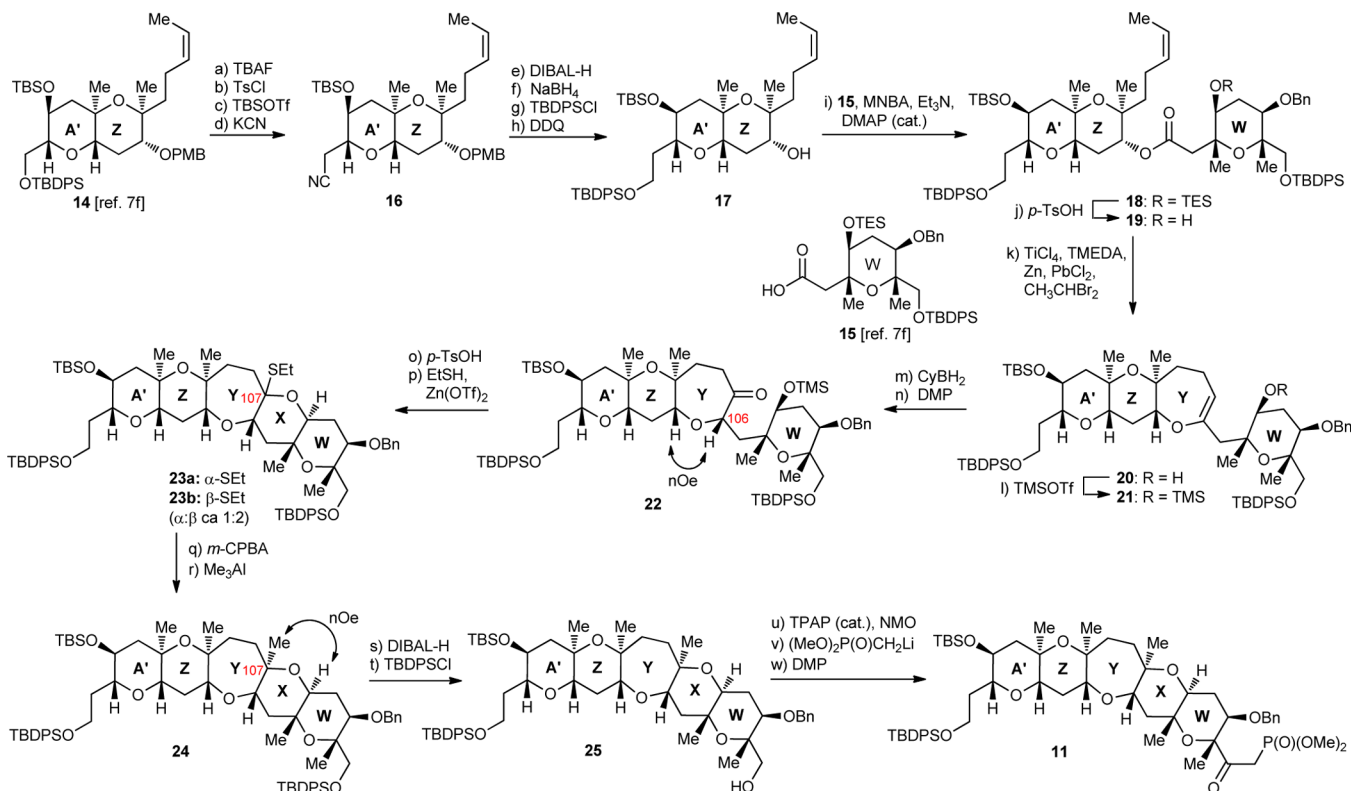
The configuration of the newly introduced C₁₀₇ methyl group was established by NMR spectroscopic analysis (i.e., NOE studies as indicated on structure **24**, Scheme 1). Differentiation of the two primary hydroxyl groups of the growing intermediate was achieved in the presence of the secondary TBS ether on ring A' by selective cleavage of both primary TBDPS ethers of

Table 1. C₇₈ to C₁₁₈ and C₁₅₀ to C₁₅₉ Chemical Shifts (δ) for Maitotoxin (MTX, **1**) and QRSTUVWXYZA' Ring System **9** and Their Differences ($\Delta\delta$, ppm)^a

| carbon | δ for MTX (1) (ppm) | δ for 9 (ppm) | difference ($\Delta\delta$, ppm) |
|--------|-------------------------------------|-----------------------------|------------------------------------|
| 78 | 49.8 | 23.6 | 26.2 |
| 79 | 75.4 | 79.1 | −3.7 |
| 150 | 19.8 | 19.9 | −0.1 |
| 80 | 81.4 | 71.9 | 9.5 |
| 81 | 74.8 | 76.5 | −1.7 |
| 82 | 76.4 | 76.0 | 0.4 |
| 151 | 15.2 | 14.5 | 0.7 |
| 83 | 64.7 | 64.2 | 0.5 |
| 84 | 41.0 | 41.3 | −0.3 |
| 85 | 78.4 | 78.2 | 0.2 |
| 152 | 16.5 | 16.5 | 0.0 |
| 86 | 74.1 | 74.1 | 0.0 |
| 87 | 25.9 | 26.2 | −0.3 |
| 88 | 38.5 | 38.9 | −0.4 |
| 89 | 79.5 | 79.7 | −0.2 |
| 153 | 19.5 | 19.7 | −0.2 |
| 90 | 72.1 | 71.9 | 0.2 |
| 91 | 43.2 | 43.3 | −0.1 |
| 92 | 75.0 | 75.0 | 0.0 |
| 154 | 16.0 | 16.0 | 0.0 |
| 93 | 71.9 | 71.6 | 0.3 |
| 94 | 32.0 | 32.1 | −0.1 |
| 95 | 80.2 | 80.2 | 0.0 |
| 96 | 71.4 | 71.4 | 0.0 |
| 97 | 30.3 | 30.3 | 0.0 |
| 98 | 25.1 | 25.1 | 0.0 |
| 99 | 87.8 | 87.8 | 0.0 |
| 100 | 78.8 | 78.8 | 0.0 |
| 155 | 19.5 | 19.7 | −0.2 |
| 101 | 74.7 | 74.5 | 0.2 |
| 102 | 31.0 | 31.0 | 0.0 |
| 103 | 72.6 | 72.6 | 0.0 |
| 104 | 74.6 | 74.6 | 0.0 |
| 156 | 20.2 | 20.3 | −0.1 |
| 105 | 42.8 | 42.8 | 0.0 |
| 106 | 84.5 | 84.3 | 0.2 |
| 107 | 79.6 | 79.7 | −0.1 |
| 157 | 18.3 | 18.5 | −0.2 |
| 108 | 39.1 | 39.1 | 0.0 |
| 109 | 40.4 | 40.3 | 0.1 |
| 110 | 79.6 | 79.7 | −0.1 |
| 158 | 23.5 | 23.1 | 0.4 |
| 111 | 87.7 | 87.7 | 0.0 |
| 112 | 30.3 | 31.0 | −0.7 |
| 113 | 83.8 | 82.6 | 1.2 |
| 114 | 74.1 | 73.9 | 0.2 |
| 159 | 217. | 22.0 | −0.3 |
| 115 | 46.7 | 45.3 | 1.4 |
| 116 | 76.8 | 70.3 | 6.5 |
| 117 | 84.7 | 82.6 | 2.1 |
| 118 | 31.5 | 37.0 | −5.5 |

^a150 MHz, 1:1 methanol-*d*₄:pyridine-*d*₅.

24 (excess DIBAL-H, −10 °C, 88%)¹⁵ followed by selective monosilylation (TBDPSCI) of the resulting diol (steric control), leading to primary alcohol **25** (quant. yield). Finally, keto-phosphonate **11** was generated from alcohol **25** through a three-step sequence involving oxidation (TPAP cat., NMO),

Scheme 1. Synthesis and Advancement of Fragment 14 to Ketophosphonate 11^a

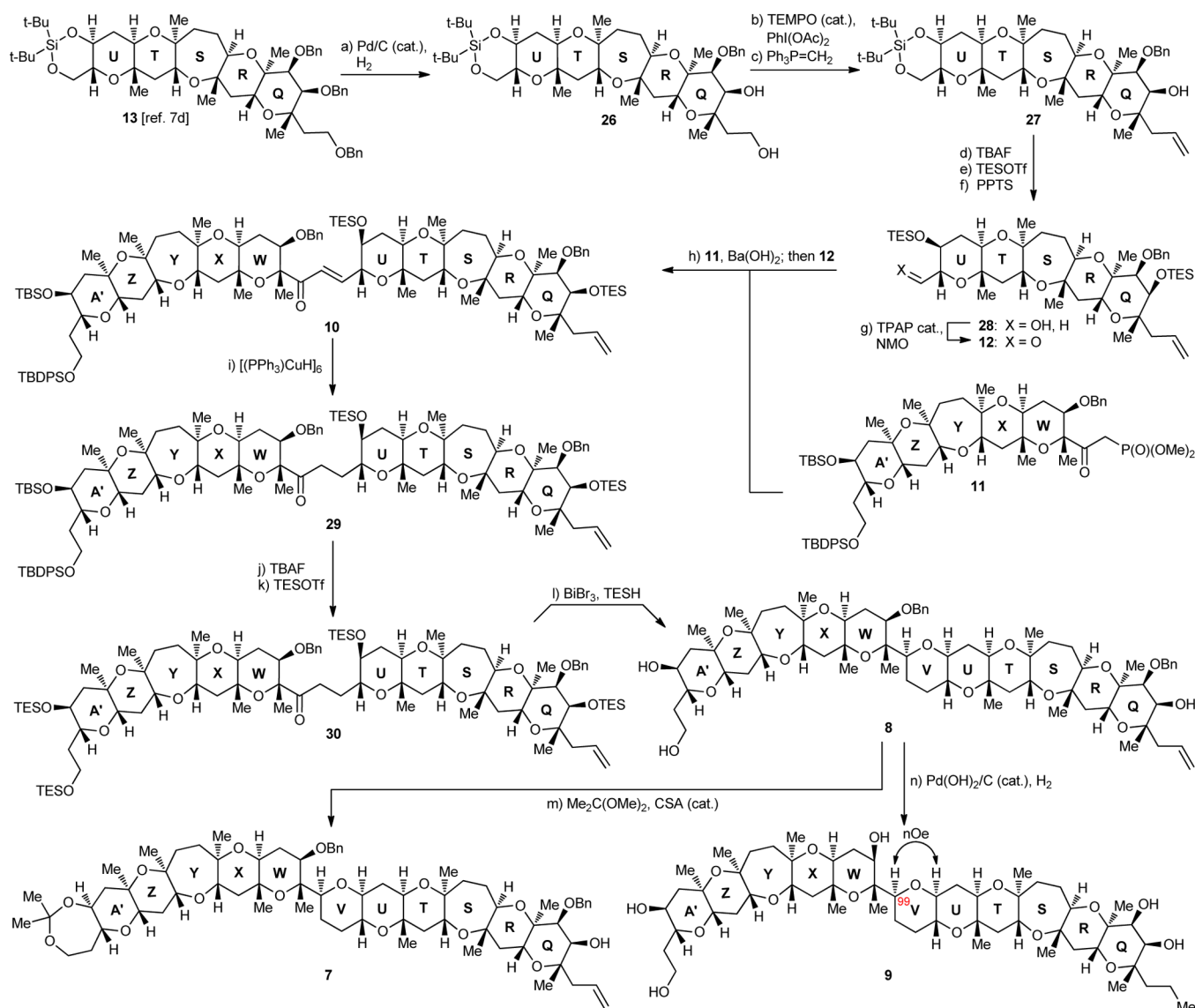
^aReagents and conditions: (a) TBAF (1.0 M in THF, 5.0 equiv), THF, 25 °C, 1 h, quant.; (b) TsCl (4.0 equiv), pyridine, 25 °C, 3.5 h, 90%; (c) TBSOTf (1.3 equiv), 2,6-lutidine (2.5 equiv), CH₂Cl₂, 0 °C, 30 min, 91%; (d) KCN (20 equiv), DMF, 100 °C, 5 h, 99%; (e) DIBAL-H (1.0 M in CH₂Cl₂, 1.1 equiv), CH₂Cl₂, -50 → -30 °C, 1 h; then aq. workup; then citric acid (2.0 equiv), THF:H₂O (2:1), 25 °C, 30 min; (f) NaBH₄ (2.0 equiv), MeOH:THF (4:1), 0 °C, 45 min, 86% over two steps; (g) TBDPSCl (2.0 equiv), imidazole (3.0 equiv), CH₂Cl₂, 25 °C, 15 min, 95%; (h) DDQ (2.0 equiv), CH₂Cl₂:phosphate buffer pH 7 (3:1), 0 °C, 2 h, 90%; (i) 15 (1.0 equiv), MNBA (1.05 equiv), Et₃N (2.7 equiv), DMAP (0.1 equiv), 4 Å MS, PhMe, 25 °C, 20 min; then 17 (1.0 equiv), 14 h, 85%; (j) *p*-TsOH:H₂O (2.0 equiv), MeOH:CH₂Cl₂ (3:1), 0 °C, 40 min, 94%; (k) TiCl₄ (1.0 M in CH₂Cl₂, 50 equiv), TMEDA (285 equiv), Zn (110 equiv), PbCl₂ (5.0 equiv), CH₃CHBr₂ (50 equiv), THF, 0 → 65 °C, 1.5 h, 78%; (l) TMSOTf (3.0 equiv), 2,6-lutidine (4.0 equiv), CH₂Cl₂, 30 min, 0 °C, quant.; (m) CyBH₂ (5.0 equiv), THF, 0 → 25 °C, 1 h; then NaOH (1 M aq.), H₂O₂ (30% aq., excess), 0 → 25 °C, 1 h, 74%; (n) DMP (3.0 equiv), NaHCO₃ (5.0 equiv), CH₂Cl₂, 0 → 25 °C, 30 min, 90%; (o) *p*-TsOH:H₂O (0.1 equiv), MeOH:CH₂Cl₂ (1:1), 0 °C, 30 min, 96%; (p) Zn(OTf)₂ (5.0 equiv), EtSH:CH₂Cl₂ (4:1), 25 °C, 1.5 h, 74% (23a:23b ca. 1:2 dr); (q) *m*-CPBA (4.0 equiv), 2,6-di-*tert*-butyl-4-methylpyridine (5.0 equiv), CH₂Cl₂, -78 → -10 °C, 20 min; (r) Me₃Al (30 equiv), -78 → 0 °C, 1 h, 78% over two steps; (s) DIBAL-H (1.0 M in CH₂Cl₂, 50 equiv), CH₂Cl₂, -40 → -10 °C, 2 h, 88%; (t) TBDPSCl (1.2 equiv), imidazole (3.0 equiv), CH₂Cl₂, 0 °C, quant.; (u) NMO:H₂O (3.0 equiv), TPAP (0.05 equiv), 4 Å MS, CH₂Cl₂, 0 → 25 °C, 1 h; (v) (MeO)₂P(O)Me (10 equiv), *n*-BuLi (9.0 equiv), THF, -78 °C, 10 min; then crude aldehyde, -78 °C, 30 min; (w) DMP (3.0 equiv), CH₂Cl₂, 0 → 25 °C, 2 h, 67% over three steps. Abbreviations: DDQ = 2,3-dichloro-5,6-dicyano-*para*-benzoquinone; DIBAL-H = diisobutylaluminum hydride; DMAP = *N,N*-dimethyl-4-aminopyridine; DMF = *N,N*-dimethylformamide; DMP = Dess–Martin periodinane; MS = molecular sieves; *m*-CPBA = *meta*-chloroperoxybenzoic acid; MNBA = 2,6-methylnitrobenzoyl anhydride; NMO = *N*-methylmorpholine-*N*-oxide; NOE = nuclear Overhauser effect; TBAF = tetra-*n*-butylammonium fluoride; Tf = trifluoromethanesulfonyl; THF = tetrahydrofuran; TMEDA = tetramethylethylenediamine; TMS = trimethylsilyl; TPAP = tetra-*n*-propylammonium perruthenate; Ts = 4-toluenesulfonyl.

reaction of the resulting aldehyde with the lithioderivative of methyl dimethylphosphonate [(MeO)₂P(O)CH₂Li] and oxidation (DMP) of the so-formed hydroxyphosphonate in 67% overall yield.

2.3. Synthesis of QRSTU Aldehyde 12, Fragment Coupling, and Completion of the Syntheses of Maitotoxin QRSTUVWXYZA' Domains. With WXYZA' ketophosphonate fragment 11 in hand, we then turned our attention to its coupling with a suitable QRSTU fragment (i.e., aldehyde 12, Scheme 2) and elaboration of the product to the targeted QRSTUVWXYZA' domains (7–9) as shown in Scheme 2. The synthesis of the required fragment 12 began with the previously synthesized QRSTU fragment 13,^{7d} whose two out of three benzyl ethers were selectively cleaved by hydrogenolysis (Pd/C, H₂, 80% yield) to afford diol 26. The remaining benzyl

ether within 26 residing on ring Q adjacent to a quaternary center proved resistant to cleavage under these conditions, most likely due to steric hindrance. Selective oxidation of the primary alcohol of 26 [TEMPO cat., Ph(OAc)₂] followed by Wittig reaction of the resulting aldehyde with Ph₃P=CH₂ gave hydroxyolefin 27 in 77% overall yield. Desilylation of the latter followed by persilylation of the generated triol (TESOTf) led to the corresponding tri-TES ether (quant. yield for the two steps), from which the primary TES group was selectively removed (PPTS, MeOH, -10 °C) to afford primary alcohol 28 (76% yield). Oxidation of this alcohol (TPAP cat., NMO) then furnished the coveted aldehyde 12, which was directly condensed with ketophosphonate 11 in the presence of Ba(OH)₂ to give α,β-unsaturated ketone 10 (78% overall yield).¹⁶ The latter compound was treated with Stryker's reagent {[(PPh₃)CuH]₆ }¹⁷

Scheme 2. Synthesis of Aldehyde Fragment 12, Coupling with Ketophosphonate 11 to Afford Enone 10, and Completion of the Synthesis of Maitotoxin Domains 7, 8, and 9^a



^aReagents and conditions: (a) 10% Pd/C (0.2 equiv), H₂, EtOH, 25 °C, 4 h, 80%; (b) TEMPO (0.3 equiv), PhI(OAc)₂ (2.0 equiv), CH₂Cl₂, 40 °C, 2.5 h; then additional TEMPO (0.5 equiv), 40 °C, 1 h; (c) CH₃PPh₃Br (10 equiv), NaHMDS (0.6 M in PhMe, 9.0 equiv), THF, 0 °C, 10 min; then crude aldehyde, 0 °C, 1 h, 77% over two steps; (d) TBAF (1.0 M in THF, 4.0 equiv), THF, 25 → 45 °C, 4 h; (e) TESOTf (6.0 equiv), 2,6-lutidine (8.0 equiv), 25 °C, 1 h, quant. over two steps; (f) PPTS (0.07 equiv), MeOH, -10 °C, 1 h, 76%; (g) NMO·H₂O (3.0 equiv), TPAP (0.05 equiv), 4 Å MS, CH₂Cl₂, 0 → 25 °C, 1 h; (h) 11 (1.0 equiv), Ba(OH)₂·8H₂O (1.5 equiv), THF:H₂O (6:1); then 12, 25 °C, 4.5 h, 78% over two steps; (i) [(PPh₃)CuH]₆ (1.5 equiv), PhMe, 25 °C, 3 h, 97%; (j) TBAF (1.0 M in THF, 6.0 equiv), THF, 25 °C, 5 h; (k) TESOTf (8.0 equiv), 2,6-lutidine (10 equiv), CH₂Cl₂, 0 °C, 1 h, 88% over two steps; (l) BiBr₃ (0.5 M in MeCN, 3.0 equiv), TESH (50 equiv), MeCN:CH₂Cl₂ (4:1), -10 °C, 2 h, 81%; (m) 2,2-dimethoxypropane (50 equiv), CSA (0.2 equiv), CH₂Cl₂, 25 °C, 1 h, 83%; (n) 20% Pd(OH)₂/C (0.6 equiv), H₂, EtOH, 25 °C, 28 h, quant. Abbreviations: CSA = (±)-camphor-10-sulfonic acid; NaHMDS = sodium bis(trimethylsilyl)amide; PPTS = pyridinium *para*-toluene sulfonate; TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxy.

to furnish selectively saturated ketone 29 (97% yield). In preparation for and in order to improve for a cleaner reaction in the pending cyclization to forge ring V, it was desired to exchange the TBS and TBDPS ethers (ring A') with TES ethers. To this end, intermediate 29 was globally desilylated with TBAF and then persilylated with excess TESOTf to afford tetra-TES substrate 30 (88% overall yield). Exposure of precursor 30 to excess Et₃SiH (TESH) in the presence of BiBr₃ effected the desired formation of ring V through stereoselective reductive cyclization with concomitant global desilylation, furnishing

bis-benzyl ether QRSTUVWXYZA' domain 8 of maitotoxin in 81% yield. Hydrogenation of the olefinic moiety with concomitant cleavage of the two benzyl ethers [20% Pd(OH)₂/C, H₂; note the more active catalyst required for this hydrogenolysis as compared to 10% Pd/C used in the conversion of 13 to 26 (Scheme 2) mentioned above] within 8 then led to domain 9 (quant. yield). Protection of the two hydroxyl groups extended from ring A' of 8 as a cyclic ketal [Me₂C(OMe)₂, CSA cat.] furnished acetonide bis-benzyl ether QRSTUVWXYZA' domain 7 (83% yield). The stereochemical configurations assigned to

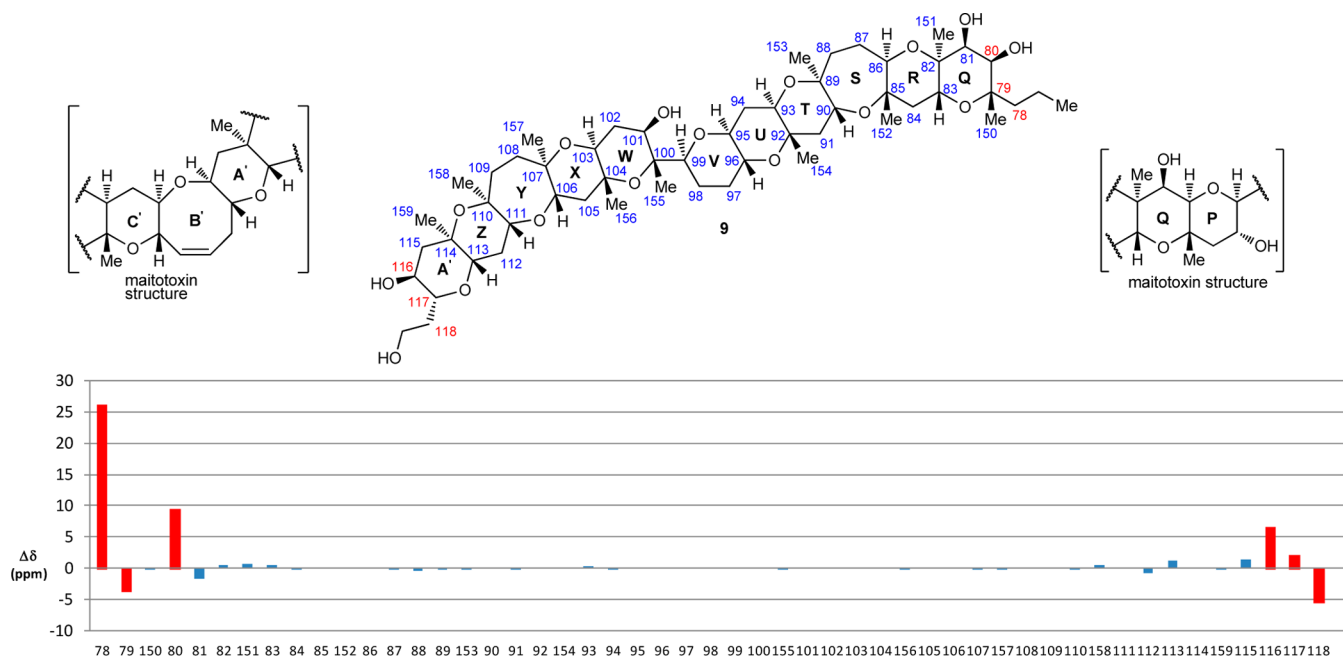


Figure 3. Graphically depicted ^{13}C chemical shift differences ($\Delta\delta$, ppm) for each carbon between C_{78} and C_{118} and C_{150} and C_{159} for maitotoxin (1) and QRSTUVWXYZA' ring system 9. Data were collected at 150 MHz in 1:1 methanol- d_4 :pyridine- d_5 solvent mixture.

compounds 7, 8, and 9 were established by NMR spectroscopic analysis of domain 9 (i.e., NOE studies as indicated on structure 9, Scheme 2).

The C'D'E'F' (36–43) and QRSTUVWXYZA' (34) derivatives, required for the biological investigations (see Figure 4) were prepared from 6^{7e} (derivatives 36–42), 35^{7e} (derivative 43) and 7 (derivative 34) by standard methods as described in the Supporting Information.

2.4. Comparison of the ^{13}C NMR Chemical Shifts of the QRSTUVWXYZA' Domain 9 with Those Corresponding to the Same Region of Maitotoxin. In order to provide further support for the original structural assignment of maitotoxin (1),^{3d} we determined the chemical shifts of carbons C_{78} to C_{118} and C_{150} to C_{159} through 2D NMR spectroscopic analysis (see Table 1) of the QRSTUVWXYZA' domain 9. Comparison of these values with those corresponding to the same region of maitotoxin led to the differences for each carbon of these two compounds ($\Delta\delta$) as shown in Table 1. These differences are also shown graphically in Figure 3 reflecting only small deviations between the two sets. Thus, the average chemical shift difference ($\Delta\delta$) between the two sets of values for C_{81} to C_{115} and C_{150} to C_{159} (blue bars in Figure 3) is 0.24 ppm and the maximum difference ($\Delta\delta$) for any of these carbons is 1.7 ppm (C_{81}). The aberrant values for C_{78} , C_{79} , C_{80} , C_{116} , C_{117} and C_{118} (red bars in Figure 3) are attributed to the significant differences of the edges of the two molecules under comparison (see Figure 3).

2.5. Biological Evaluation of Synthesized Maitotoxin Fragments. Maitotoxin is presumed to elicit its neurotoxicity through binding to and activating membrane ion channels within neurons, thereby causing rapid Ca^{2+} influx.¹⁸ In order to gather information regarding the ability of various maitotoxin fragments synthesized in our laboratories [7–9 (this work), 34 (this work), 36–43 (this work), 2,^{7c} 4,^{7d} 6,^{7e} 31,^{7c} 32,^{7c} 33,^{7f} 35,^{7e} Figure 4] to inhibit maitotoxin-induced influx of calcium ions, we subjected them to appropriate assays with rat C6 glioma cells. As shown in Figure 4, fragments 31 (ABCDE), 2 (ABCDEFG) and 32 (ABCDEFG) were found to be inactive¹⁹ (2 and 32) or only

slightly active¹⁹ (i.e., $\text{IC}_{50} > 30 \mu\text{M}$, 31). Fragment 4 (QRSTU) and fragment 33 (WXYZA') were found to be slightly active ($\text{IC}_{50} > 30 \mu\text{M}$). Fragment 8 (QRSTUVWXYZA') was active at IC_{50} ca. $30 \mu\text{M}$, whereas its debenzylated saturated counterpart 9 exhibited potency at $\text{IC}_{50} = 3.2 \mu\text{M}$.¹⁹ Fragment 7 was slightly active while fragment 34 was active at $\text{IC}_{50} = 5.4 \mu\text{M}$. Fragment 6 (C'D'E'F') showed only slight activity ($\text{IC}_{50} > 30 \mu\text{M}$) in contrast to its *tert*-butyldiphenyl silyl ether derivative 35, which proved to be the most potent compound we tested in this study ($\text{IC}_{50} = 2.3 \mu\text{M}$). These results are, for the most part, consistent with Murata's hypothesis²⁰ that maitotoxin anchors itself in the neuron membrane using its lipophilic domain (i.e., QRSTUVWXYZA'B'C'D'E'F') which presumably binds to the membrane-bound ion channel causing it to open, whereas its hydrophilic domain (i.e., ABCDEFGHIJKLMNOP) remains outside the cell membrane. The latter most likely serves to sequester and facilitate the influx of Ca^{2+} ions into the cell through the channel once opened. Thus, fragment 31 (ABCDE) shows only weak activity despite its lipophilic protective groups, presumably due to its inability to bind to the Ca^{2+} ion channel. Similarly, fragment 2 shows no activity due to its hydrophilic nature that does not allow it to easily enter the membrane, or, if it does, has no significant affinity for the ion channel. Its polybenzyl counterpart 32 shows no activity, presumably due to its weak binding affinity to the ion channel. Fragment 4 is probably too polar to enter the cell membrane due to its five hydroxyl groups, whereas fragment 33 might have weak affinity for the ion channel. Fragments 9 and 34 exhibit significant inhibition of maitotoxin in this assay, as expected, with the former being the more potent of the two. Their benzylated counterparts, 7 and 8, show only slight activity. Most interesting, however, is the relatively high potency of fragment 6, whose structural features (i.e., *tert*-butyldiphenylsilyl ether moiety), overall shape and lipophilicity may allow it to anchor into the membrane and bind to the ion channel, presumably at the same site as the corresponding region of maitotoxin. The remaining compounds (35–43, Figure 4) proved to be either inactive (41, 42) or weakly

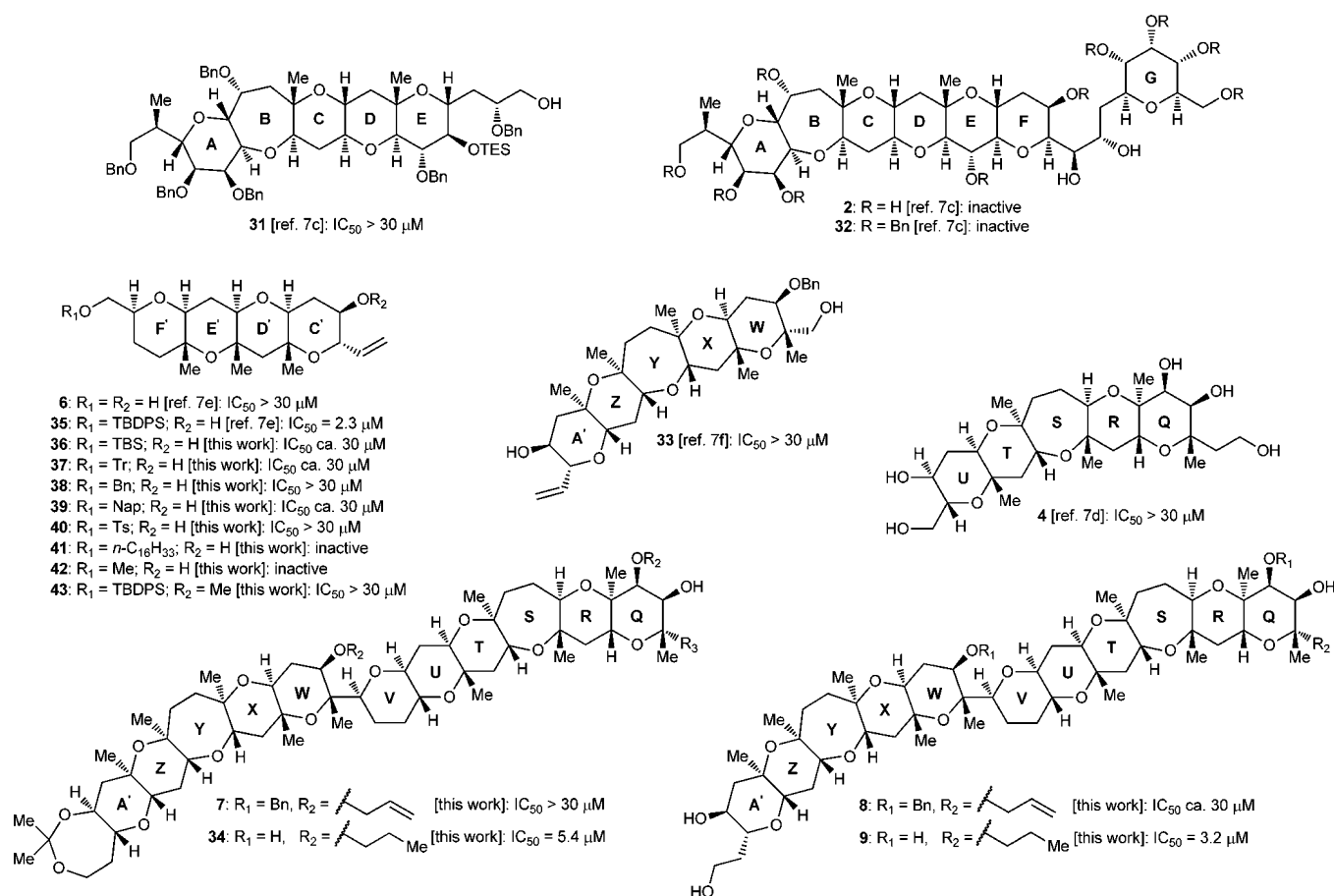


Figure 4. Structures of maitotoxin (1) and synthesized fragments with their ability to inhibit maitotoxin-elicited $^{45}\text{Ca}^{2+}$ influx in rat C6 glioma cells.¹⁹ More details can be found in the Supporting Information. Abbreviations: Nap = 2-naphthylmethyl; Tr = triphenylmethyl.

active (IC_{50} ca. $30 \mu\text{M}$, 36, 37, 39 or $\text{IC}_{50} > 30 \mu\text{M}$, 38, 40, 43), a phenomenon presumably depending on their ability to reach and bind to the intended receptor site as dictated by their specific structural features.

A number of these maitotoxin fragments (i.e., 2, 4, 6–9, 32, 33 and 35, Figure 4) were also submitted to NCI for screening. Most exhibited only relatively low growth inhibition (GI_{50}) and cytotoxicity against the NCI-60 DTP Human Tumor Cell Line panel, with QRSTUVWXYZA' domain 9 being the most potent. Thus, compound 9 exhibited significant growth inhibition against leukemia ($\text{GI}_{50} = 2.15 \mu\text{M}$, cell line RPMI-8226), nonsmall cell lung cancer ($\text{GI}_{50} = 2.44 \mu\text{M}$, cell line HOP-92), colon cancer ($\text{GI}_{50} = 2.34 \mu\text{M}$, cell line HCT-116), CNS cancer ($\text{GI}_{50} = 4.50 \mu\text{M}$, cell line SF-539), melanoma ($\text{GI}_{50} = 2.13 \mu\text{M}$, cell line SK-MEL-5), ovarian cancer ($\text{GI}_{50} = 3.84 \mu\text{M}$, cell line OVCAR-3), renal cancer ($\text{GI}_{50} = 1.86 \mu\text{M}$, cell line SN12C), prostate cancer ($\text{GI}_{50} = 1.83 \mu\text{M}$, cell line PC-3) and breast cancer ($\text{GI}_{50} = 1.26 \mu\text{M}$, cell line MDA-MB-468). More details of these assays can be found in the Supporting Information.

3. CONCLUSION

The described chemistry renders the QRSTUVWXYZA' domain of maitotoxin readily available as three derivatives 7, 8, and 9 for further chemical and biological studies. The devised convergent strategy demonstrates the power of the hydroxydithioketal cyclization/methylation, reductive hydroxyketone cyclization, and Takai–Utimoto ester olefination/ring closing metathesis methods as means to construct complex, ladder-like polyether

structures.²¹ Biological investigations with the synthesized maitotoxin fragments from these and previous studies in these laboratories revealed significant inhibitory activities against maitotoxin-elicited Ca^{2+} influx in rat glioma cells. The structure–activity relationships obtained from these investigations can be rationalized, for the most part, by Murata's hypothesis (amphoteric hydrophilic–lipophilic nature of maitotoxin)²⁰ whereby the molecule is embedded within the neuron cell membrane through its “lower” rather lipophilic domain, whereas the “top” rather hydrophilic domain resides outside the membrane, presumably serving to sequester Ca^{2+} ions and facilitate their influx into the cell. On the basis of these results, further advances toward the total synthesis of maitotoxin and elucidation of its precise mode of action are both possible and warranted.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization data for all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

■ AUTHOR INFORMATION

Corresponding Author

kcn@rice.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Drs. Lawrence B. Alemany and Quinn Kleerekoper for NMR-spectroscopic assistance and Dr. Christopher L. Pennington for mass-spectrometric assistance. Financial support was provided by The Cancer Prevention Research Institute of Texas (CPRIT), The Welch Foundation, Rice University and the National Institutes of Health (U.S.A.). A postdoctoral fellowship to P.H. from Deutsche Akademie der Naturforscher Leopoldina is gratefully acknowledged. K.K. received funding from the "ERATO Murata Lipid Active Structure Project" (Japan Science and Technology Agency). Part of this work was carried out at The Scripps Research Institute (TSRI). T.N. and A.R. participated at TSRI only; K.C.N. and P.H. participated at both TSRI and Rice University.

■ REFERENCES

- (1) (a) Murata, M.; Yasumoto, T. *Nat. Prod. Rep.* **2000**, *17*, 293. (b) Yasumoto, T.; Bagnins, R.; Randal, J. E.; Banner, A. H. *Nippon Suisan Gakkaishi* **1971**, *37*, 724. (c) Yasumoto, T.; Bagnins, R.; Vernoux, J. P. *Nippon Suisan Gakkaishi* **1976**, *42*, 359. (d) Yasumoto, T.; Nakajima, I.; Bagnis, R.; Adachi, R. *Nippon Suisan Gakkaishi* **1977**, *43*, 1021. (e) Yokoyama, A.; Murata, M.; Oshima, Y.; Iwashita, T.; Yasumoto, T. *J. Biochem.* **1988**, *104*, 184.
- (2) (a) Takahashi, M.; Ohizumi, Y.; Yasumoto, T. *J. Biol. Chem.* **1982**, *257*, 7287. (b) Gusovsky, F.; Daly, J. W. *Biochem. Pharmacol.* **1990**, *39*, 1633. (c) Ueda, H.; Tamura, S.; Fukushima, N.; Takagi, H. *Eur. J. Pharmacol.* **1986**, *122*, 379. (d) Konoki, K.; Hashimoto, M.; Nanomura, T.; Sasaki, M.; Murata, M.; Tachibana, K. *J. Neurochem.* **1998**, *70*, 409. (e) Murata, M.; Gusovsky, F.; Yasumoto, T.; Daly, J. W. *Eur. J. Pharmacol.* **1992**, *227*, 43.
- (3) (a) Murata, M.; Iwashita, T.; Yokoyama, A.; Sasaki, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1992**, *114*, 6594. (b) Murata, M.; Naoki, H.; Iwashita, T.; Matsunaga, S.; Sasaki, M.; Yokoyama, A.; Yasumoto, T. *J. Am. Chem. Soc.* **1993**, *115*, 2060. (c) Murata, M.; Naoki, H.; Matsunaga, S.; Satake, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1994**, *116*, 7098. (d) Satake, M.; Ishida, S.; Yasumoto, T. *J. Am. Chem. Soc.* **1995**, *117*, 7019.
- (4) (a) Zheng, W.; DeMattei, J. A.; Wu, J.-P.; Duan, J. J.-W.; Cook, L. R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946. (b) Cook, L. R.; Oinuma, H.; Semones, M. A.; Kishi, Y. *J. Am. Chem. Soc.* **1997**, *119*, 7928. (c) Kishi, Y. *Pure Appl. Chem.* **1998**, *70*, 339.
- (5) (a) Sasaki, M.; Nonomura, T.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1995**, *36*, 9007. (b) Sasaki, M.; Nonomura, T.; Murata, M.; Tachibana, K.; Yasumoto, T. *Tetrahedron Lett.* **1995**, *36*, 9011. (c) Sasaki, M.; Nonomura, T.; Murata, M.; Tachibana, K. *Tetrahedron Lett.* **1994**, *35*, 5023. (d) Sasaki, M.; Matsumori, N.; Muruyama, T.; Nonomura, T.; Murata, M.; Tachibana, K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1672. (e) Nonomura, T.; Sasaki, M.; Matsumori, N.; Murata, M.; Tachibana, K.; Yasumoto, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1675.
- (6) (a) Gallimore, A. R.; Spencer, J. B. *Angew. Chem., Int. Ed.* **2006**, *45*, 4406. (b) Nicolaou, K. C.; Frederick, M. O. *Angew. Chem., Int. Ed.* **2007**, *46*, 5278.
- (7) (a) Nicolaou, K. C.; Cole, K. P.; Frederick, M. O.; Aversa, R. J.; Denton, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 8875. (b) Nicolaou, K. C.; Frederick, M. O.; Burtoloso, A. C. B.; Denton, R. M.; Rivas, F.; Cole, K. P.; Aversa, R. J.; Gibe, R.; Umezawa, T.; Suzuki, T. *J. Am. Chem. Soc.* **2008**, *130*, 7466. (c) Nicolaou, K. C.; Aversa, R. J.; Jin, J.; Rivas, F. *J. Am. Chem. Soc.* **2010**, *132*, 6855. (d) Nicolaou, K. C.; Gelin, C. F.; Seo, J. H.; Huang, Z.; Umezawa, T. *J. Am. Chem. Soc.* **2010**, *132*, 9900. (e) Nicolaou, K. C.; Seo, J. H.; Nakamura, T.; Aversa, R. J. *J. Am. Chem. Soc.* **2011**, *133*, 214. (f) Nicolaou, K. C.; Baker, T. M.; Nakamura, T. *J. Am. Chem. Soc.* **2011**, *133*, 220.
- (8) (a) Nakata, T.; Nomura, S.; Matsukura, H. *Chem. Pharm. Bull.* **1996**, *44*, 627. (b) Nagasawa, K.; Hori, N.; Shiba, R.; Nakata, T. *Heterocycles* **1997**, *44*, 105. (c) Sakamoto, Y.; Matsuo, G.; Matsukura, H.; Nakata, T. *Org. Lett.* **2001**, *3*, 2749. (d) Morita, M.; Ishiyama, S.; Koshino, H.; Nakata, T. *Org. Lett.* **2008**, *10*, 1675. (e) Morita, M.; Haketa, T.; Koshino, H.; Nakata, T. *Org. Lett.* **2008**, *10*, 1679. (f) Satoh, M.; Koshino, H.; Nakata, T. *Org. Lett.* **2008**, *10*, 1683. (g) Oishi, T.; Hasegawa, F.; Torikai, K.; Konoki, K.; Matsumori, N.; Murata, M. *Org. Lett.* **2008**, *10*, 3599. (h) Kunitake, M.; Oshima, T.; Konoki, K.; Ebine, M.; Torikai, K.; Murata, M.; Oishi, T. *J. Org. Chem.* **2014**, *79*, 4948.
- (9) (a) Evans, P. A.; Cui, J.; Gharpure, S. J.; Hinkle, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 11456. (b) Lian, Y.; Hinkle, R. J. *J. Org. Chem.* **2006**, *71*, 7071.
- (10) Takai-Utimoto reagent: (a) Takai, K.; Kakiuchi, T.; Kataoka, Y.; Utimoto, K. *J. Org. Chem.* **1994**, *59*, 2668. For a review summarizing the applications of olefin metathesis in the synthesis of fused polyethers, see: (b) Clark, J. S. *Chem. Commun.* **2006**, 3571. For earlier methods using ring-closing metathesis to form enol ethers, see: (c) Fu, G. C.; Grubbs, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 5426. (d) Fu, G. C.; Nguyn, S. T.; Grubbs, R. H. *J. Am. Chem. Soc.* **1993**, *115*, 9856. (e) Fujimura, O.; Fu, G. C.; Grubbs, R. H. *J. Org. Chem.* **1994**, *59*, 4029. (f) Nicolaou, K. C.; Postema, M. H. D.; Claiborne, C. F. *J. Am. Chem. Soc.* **1996**, *118*, 1565. (g) Nicolaou, K. C.; Postema, M. H. D.; Yue, E. W.; Nadin, A. J. *Am. Chem. Soc.* **1996**, *118*, 10335. (h) Clark, J. S.; Kettle, J. G. *Tetrahedron Lett.* **1997**, *38*, 123. (i) Clark, J. S.; Kettle, J. G. *Tetrahedron* **1999**, *55*, 8231.
- (11) (a) Majumder, U.; Rainier, J. D. *Tetrahedron Lett.* **2005**, *46*, 7209. (b) Iyer, K.; Rainier, J. D. *J. Am. Chem. Soc.* **2007**, *129*, 12604.
- (12) (a) Nicolaou, K. C.; Duggan, M. E.; Hwang, C.-K. *J. Am. Chem. Soc.* **1986**, *108*, 2468. (b) Nicolaou, K. C.; Prasad, C. V. C.; Hwang, C.-K.; Duggan, M. E.; Veale, C. A. *J. Am. Chem. Soc.* **1989**, *111*, 5321. (c) Nicolaou, K. C.; Veale, C. A.; Hwang, C.-K.; Hutchinson, J.; Prasad, C. V. C.; Ogilvie, W. W. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 299. (d) Nicolaou, K. C.; Yang, Z.; Shi, G.-Q.; Gunzner, J. L.; Agrios, K. A.; Gärtner, P. *Nature* **1998**, *392*, 264.
- (13) (a) Shiina, I.; Ibuka, R.; Kubota, M. *Chem. Lett.* **2002**, 286. (b) Shiina, I.; Kubota, M.; Ibuka, R. *Tetrahedron Lett.* **2002**, *43*, 7535. (c) Shiina, I.; Oshiumi, H.; Hashizume, M.; Yamai, Y. S.; Ibuka, R. *Tetrahedron Lett.* **2004**, *45*, 543. (d) Shiina, I.; Kubota, M.; Oshiumi, H.; Hashizume, M. *J. Org. Chem.* **2004**, *60*, 1822.
- (14) We note that the same is true for the hydroboration of a similar compound described in ref 7f. In that paper, Cy₂BH was erroneously mentioned inadvertently instead of CyBH₂.
- (15) Kuranaga, T.; Ishihara, S.; Ohtani, N.; Satake, M.; Tachibana, K. *Tetrahedron Lett.* **2010**, *51*, 6345.
- (16) Paterson, I.; Yeung, K.-S.; Smaill, J. B. *Synlett* **1993**, 774.
- (17) Mahoney, W. S.; Brestensky, D. M.; Stryker, J. M. *J. Am. Chem. Soc.* **1988**, *110*, 291.
- (18) Sinkins, W. G.; Estacion, M.; Prasad, V.; Goel, M.; Shull, G. E.; Kunze, D. L.; Schilling, W. P. *Am. J. Physiol.: Cell Physiol.* **2009**, *297*, C1533.
- (19) If the relative ⁴⁵Ca²⁺ influx caused by maitotoxin in the presence of 30 μM of the tested compound was >90% compared to the control, its IC₅₀ is addressed as "inactive". If it was 60–90%, it is addressed as ">30 μM". If it was 40–60%, it is addressed as "ca. 30 μM". If an inhibition curve could be drawn, it was fitted to the Hill equation to determine the IC₅₀ value. For experimental details, see the Supporting Information.
- (20) Murata, M.; Matsumori, N.; Konoki, K.; Oishi, T. *Bull. Chem. Soc. Jpn.* **2008**, *81*, 307.
- (21) For selected reviews on the synthesis of fused polyether natural products, see: (a) Nicolaou, K. C.; Aversa, R. J. *Isr. J. Chem.* **2011**, *51*, 359. (b) Nicolaou, K. C.; Frederick, M. O.; Aversa, R. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 7182. (c) Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 588. (d) Nakata, T. *Chem. Rev.* **2005**, *105*, 4314. (e) Inoue, M. *Chem. Rev.* **2005**, *105*, 4379. (f) Sasaki, M. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 856.