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Synthesis and biological evaluation of triazole analogues of antillatoxin

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ABSTRACT

Antillatoxin **1**, a cyclic lipopeptide, is known as an activator of voltage-gated sodium channels and exhibits potent neurotoxicity toward Neuro 2a mouse neuroblastoma cells. To investigate the biological effects of the side-chain structures at C5 and C5′ in detail, we planned SAR studies of C5- and C5′-modified antillatoxin analogues. To diversify the structures at the last step of the synthesis, two key intermediates **4** and **6** possessing terminal alkynes at the C5- and C5′-positions were designed and synthesized using two distinct strategies. Sixteen side-chain derivatives were then prepared from **4** and **6** by coupling with a wide variety of azides via click chemistry, and subjected to the cytotoxicity assay. Although almost all of the C5-substituted analogues exhibited no cytotoxicity, the C5′-substituted analogues showed modest cytotoxicity. These results showed that C5′ is more tolerant than C5 to structural modifications. The present SAR study will provide valuable information for designing new antillatoxin-based molecular probes for neuroscience research.

1. Introduction

Antillatoxin (Fig. 1, **1**) is a cyclic lipopeptide isolated from the marine cyanobacterium *Lyngbya majuscule* as a potent ichthyotoxic compound.[1–3] Detailed biological studies of this peptide revealed that **1** was an activator of voltage-gated sodium channels (VGSC).[4,5] Consequently, **1** exhibited potent neurotoxicity toward Neuro 2a mouse neuroblastoma cells (EC_{50}=45 nM), which express VGSCs on their membranes. Furthermore, an enhancement of neurite outgrowth in cerebrocortical neurons by **1** was reported at a concentration of ~100 nM.[6] These findings emphasize the potential use of antillatoxin as a research tool in neuroscience.[7] Accordingly, structure–activity relationship (SAR) studies of antillatoxin analogues, which were exclusively provided by total syntheses, have been reported.[8,9]

The molecular structure of **1** is composed of glycine, *N*-methyl-L-valine and L-alanine, and a δ-hydroxycarboxylic acid (Fig. 1).[1] One of the most unusual features of **1** is a 9-*tert*-butyl-6,8-dimethyl-6,8-diene unit attached to C5 of the δ-hydroxycarboxylic acid. Recently, we revealed that this highly methylated diene adopts a twisted conformation at the C7–C8 bond due to the severe steric interaction between the C6- and C8-methyl groups and found that disruption of this twisted shape of the C5-side chain considerably decreases biological activity.[9] For instance, synthetic 8-demethyl-antillatoxin **2**, which has a planar side-chain conformation, exhibited 240-fold weaker cytotoxicity than antillatoxin (EC_{50}=11 μM, Neuro 2a). This result indicated the significance of the three-dimensional structure of the C5-side chain.

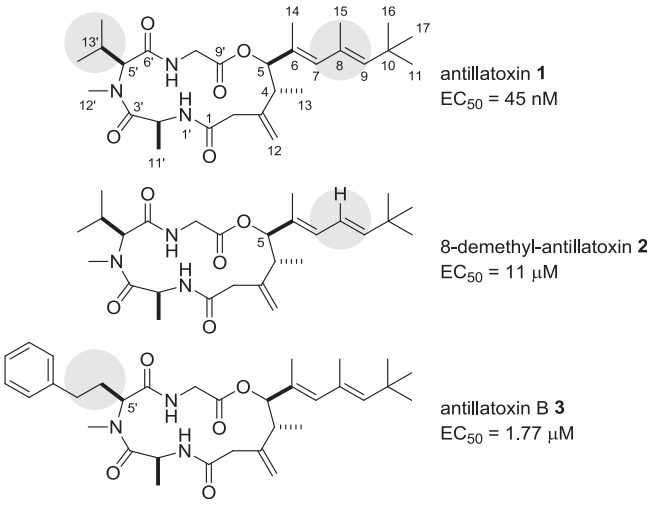

Fig. 1. Structures of antillatoxin (**1**) and its side chain analogues (**2** and **3**).

On the other hand, a naturally occurring homologue of **1** was isolated as antillatoxin B, **3** (Fig. 1), which contains *N*-methyl-L-homophenylalanine instead of *N*-methyl-L-valine.[10] Cytotoxicity and ichthyotoxicity of **3** were assessed to be one order of magnitude less than that of **1**. Because the only structural difference between **1** and **3** is the C5′-side chain, it is likely to be an important factor for the potent activities of **1**.

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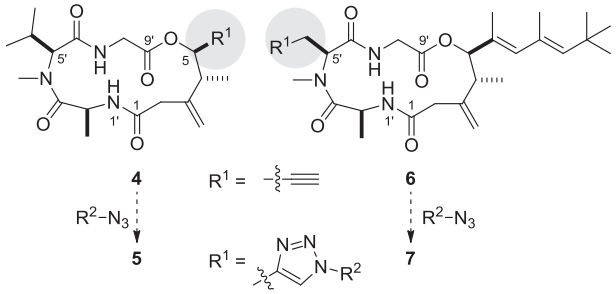
To investigate the biological effects of the structures at C5 and C5′ in detail, we decided to launch SAR studies of side-chain-modified antillatoxin analogues (Scheme 1). In doing so, the acetylene-bearing macrolactam cores **4** and **6** were designed as key common intermediates for syntheses of C5- and C5′-modified analogues **5** and **7**. Specifically, 1,3-dipolar coupling of azide reagents with the reactive terminal alkynes of **4** and **6** would enable diversification into many triazole derivatives **5** and **7**, respectively, with varied R^2 groups.[11] Substrates **4** and **6** for such click chemistry were envisioned to be constructed from the left tripeptide and right δ-hydroxycarboxylic acid fragments through C9′-esterification and C1-amidation. Here we report efficient syntheses and biological evaluations of C5- and C5′-side chain analogues of antillatoxin.



Scheme 1. Design of acetylene-bearing macrolactam cores **4** and **6** as precursors of C5-side chain analogues (**5**) and C5′-side chain analogues (**7**).

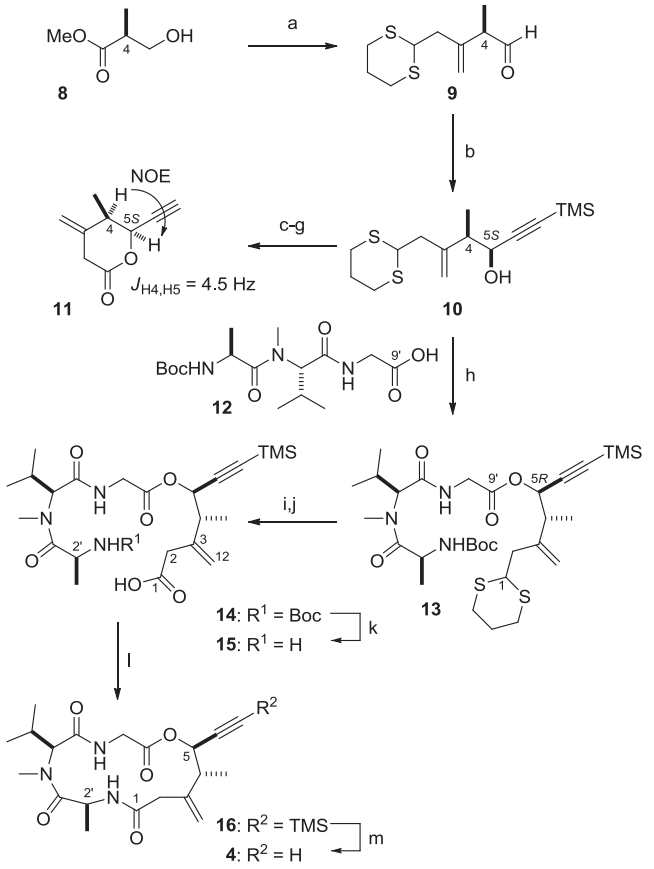
2. Results and discussion

2.1. Synthesis of alkyne 4

The δ-hydroxycarboxylic acid unit of **4** was synthesized from the dithiane-protected compound **9** (Scheme 2), which was derivatized from **8** through a four-step procedure developed by White et al.[2d] Diastereoselective addition of TMS-acetylene to aldehyde **9** set the C5-*S* stereochemistry of **10**. Namely, TMS-acetylene was treated with Et_2Zn to prepare the corresponding alkynyl zinc reagent, which was added to **9** in the presence of Ti(O*i*-Pr)$_4$ and (*R*)-BINOL, giving rise to propargyl alcohol **10** in a highly selective fashion (>20:1 dr).[12] The C5-stereochemistry was determined by the observed NOE between C4–H and C5–H of **11**, which was prepared from **10** by five standard synthetic manipulations. Next, condensation between alcohol **10** and *N*-Boc protected tripeptide **12**[9] under Mitsunobu conditions[13] provided C5-*R* ester **13** via inversion of the C5-configuration. After MeI-mediated deprotection of dithiane **13**,[14] the resultant aldehyde was oxidized to carboxylic acid **14** using $NaClO_2$. The Boc group of **14** was then cleaved to generate amino acid **15**, which was cyclized by the action of HATU and *i*-Pr_2NEt,[15] leading to macrolactam **16**. Finally, KF-promoted removal of the TMS group of **13** furnished C5-alkyne-bearing macrolactam **4**.[16]

2.2. Synthesis of alkyne 6

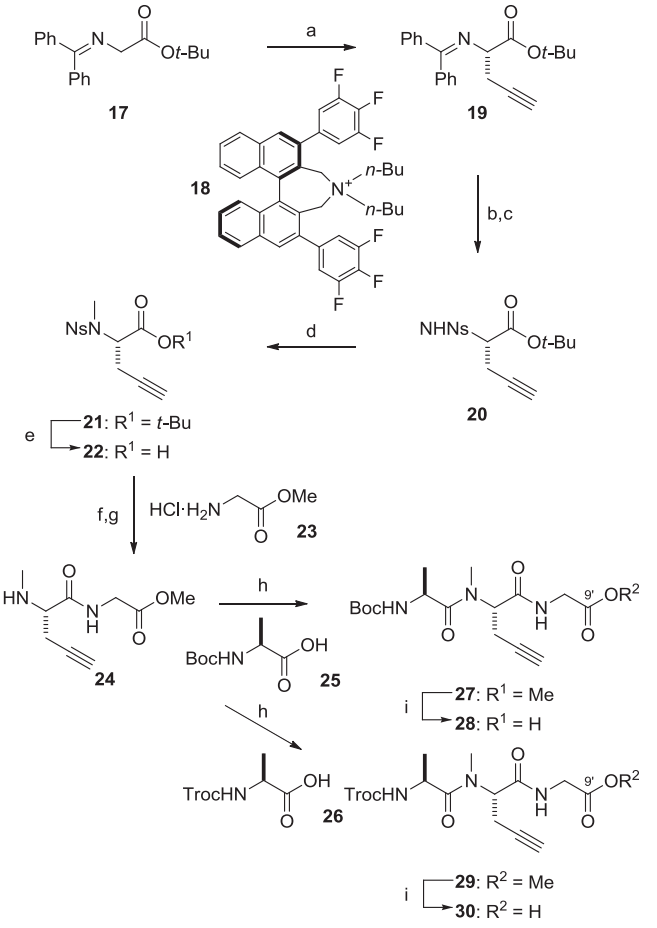
To synthesize the tripeptide fragment of C5′-propargyl analogue **6**, the protected L-propargylglycine **19** was first prepared (Scheme 3). The Maruoka phase-transfer catalyst **18**[17] was employed to induce the enantioselectivity in the alkylation of the glycine derivative **17** to yield **19** (>99% ee). After removal of the diphenylmethylene group of **19** using aqueous citric acid, the amine was protected with the Ns group[18] to generate **20**. Methylation of **20** with MeI in the presence of K_2CO_3, followed by removal of the *t*-Bu group of **21** with TFA, resulted in formation of carboxylic acid **22**. Compound **22** was then condensed with glycine methyl ester **23**



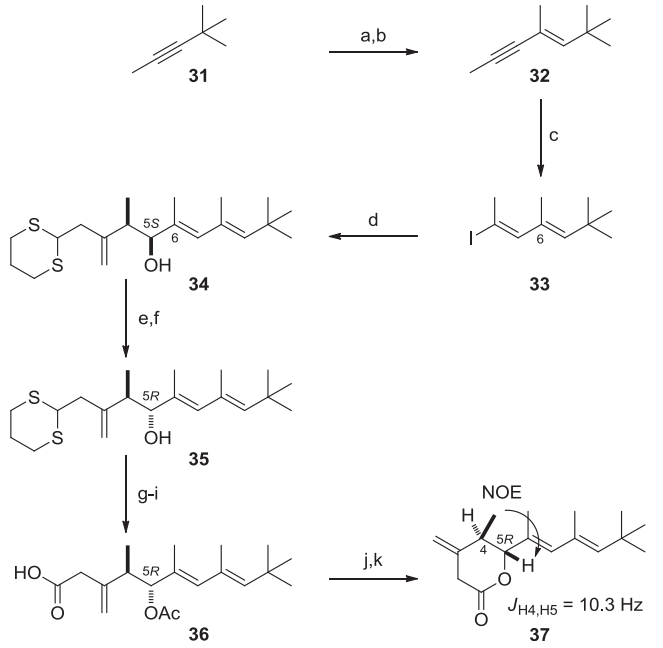
Scheme 2. Synthesis of alkyne **4**. (a) four steps (Ref. 2d); (b) TMS-≡-H, Et_2Zn, Ti(O*i*-Pr)$_4$, (*R*)-BINOL, toluene/Et_2O (1/4), 74%; (c) Ac_2O, pyridine, 93%; (d) MeI, $CaCO_3$, MeCN/H_2O (9/1); (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH/H_2O (5/1), 80% (two steps); (f) K_2CO_3, MeOH; (g) CSA, CH_2Cl_2, 51% (two steps); (h) **12**, DEAD, PPh_3, toluene, 93%; (i) MeI, $CaCO_3$, MeCN/H_2O (9/1); (j) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH/H_2O (5/1), 81% (two steps); (k) TFA/CH_2Cl_2 (1/10), 0 °C; (l) HATU, *i*-Pr_2NEt, DMF (5 mM), 0 °C, 66% (two steps); (m) KF, DMF/H_2O (10/1), 100%. BINOL=1,1′-bi-2-naphtol; Boc=*tert*-butoxycarbonyl; CSA=10-camphorsulfonic acid; DEAD=diethyl azodicarboxylate; HATU=*O*-(7-azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; TMS=trimethylsilyl.

using PyBrop,[19] and the Ns group of the adduct was removed using thiophenol to afford dipeptide **24**. The second amide bond formation between **24** and *N*-Boc alanine **25** provided tripeptide **27**. Saponification of **27** under basic conditions gave rise to the requisite Boc-protected carboxylic acid **28**. The same two-step protocol was applied to synthesize Troc-protected tripeptide **30** from dipeptide **24** and *N*-Troc alanine **26**.[20]

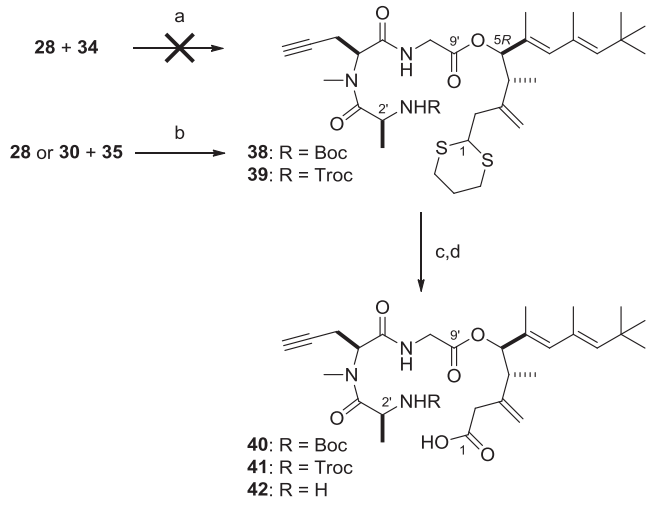
The δ-hydroxycarboxylic acid unit of **6** was synthesized by coupling aldehyde **9** (Scheme 2) and C5′-diene side chain **33** by applying the modified White procedure[2d] (Scheme 4). Hydrozirconation of alkyne **31** and in situ iodination,[21] followed by Pd-catalyzed cross-coupling with 1-propynylmagnesium bromide,[22] provided enyne **32**. Next stereoselective construction of vinyl iodide **33** was realized by hydroboration of **32** and subsequent iodine treatment.[23] The obtained **33** was then lithiated by the action of *t*-BuLi, and the generated vinyl lithium reacted with aldehyde **9** to give C5-*S* alcohol **34** in a stereoselective fashion (5*S*/5*R*=7/1). The epimeric C5-*R* alcohol **35** was also generated by IBX oxidation[24] of **34** and Luche reduction[25] (5*S*/5*R*=1/11).[2c] The C5-configuration of **35** was elucidated after derivatization of **35** to **37**. Acetyl protection of **35** and subsequent C1-deprotection/oxidation sequence generated Ac-protected carboxylic acid **36**, which was converted to **37** via Ac-removal and acid-induced lactonization. The C5-*R* stereochemistry was confirmed based on the NOE between C4-Me and C5-H of **37** and the value of $J_{H4,H5}$ (10.3 Hz).



Scheme 3. Synthesis of tripeptide fragments of **6**. (a) H–CH_2Br, **18** (0.1 mol %), toluene/50% aqueous KOH (1/1), >99% ee; (b) 15% aqueous citric acid, THF; (c) NsCl, Et_3N, CH_2Cl_2, 63% (three steps); (d) MeI, K_2CO_3, DMF, 96%; (e) TFA, CH_2Cl_2, 85%; (f) **23**, PyBrop, CH_2Cl_2; (g) PhSH, K_2CO_3, MeCN, 94% (two steps); (h) **25** or **26**, HATU, *i*-Pr_2NEt, DMF, 85% (**29**); (i) LiOH, THF/H_2O (1/1), 78% (**28**, two steps), 78% (**30**). Ns=*o*-nitrobenzenesulfonyl; PyBrop=bromo-tris-pyrrolidino-phosphonium hexafluorophosphate; Troc=2,2,2-trichloroethoxycarbonyl.



Scheme 4. Synthesis of δ-hydroxycarboxylic acid fragment of **6**. (a) Cp_2ZrHCl, THF; *N*-iodosuccinimide; (b) Me–MgBr, $Pd(PPh_3)_4$ (5 mol %), 83% (two steps); (c) catecholborane; I_2, pyridine, MeOH, 72%; (d) **9**, *t*-BuLi, Et_2O, 92% (from **9**), 5*S*/5*R*=7/1; (e) IBX, DMSO/THF (1/5); (f) $NaBH_4$, $CeCl_3\cdot 7H_2O$, THF/H_2O (1/1), 83% (two steps), 5*S*/5*R*=1/11; (g) Ac_2O, pyridine; (h) MeI, $CaCO_3$, MeCN/H_2O (9/1); (i) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH/H_2O (5/1), 74% (three steps); (j) K_2CO_3, MeOH; (k) CSA, CH_2Cl_2, 83% (two steps). IBX=1-hydroxy-1,2-benziodoxy-3(1H)-one-1-oxide.



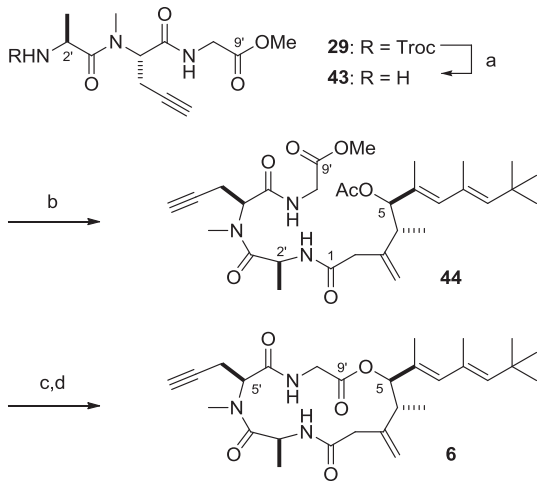
Scheme 5. Unsuccessful results for synthesis of **6**. (a) DEAD, PPh_3, toluene, 0%; (b) EDC, DMAP, CH_2Cl_2, 29% (**38**), 33% (**39**); (c) MeI, $CaCO_3$, MeCN/H_2O (9/1); (d) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-BuOH/H_2O (5/1), 62% (**40**, two steps), 73% (**41**, two steps). DMAP=4-(dimethylamino)pyridine; EDC=1-ethyl-3-(3-dimethylaminopropyl) carbodiimide.

Similar to the synthesis of **13** in Scheme 2, carboxylic acid **28** and C5-*S* alcohol **34** were subjected to Mitsunobu coupling conditions (Scheme 5). However, the adduct **38** was not obtained, and elimination of the C5-hydroxy group from **34** was observed presumably due to the more chemically labile nature of the diene-attached C5–OH of **34** in comparison to the propargylic C5–OH of **10**. Alternatively, C9′-esterification between acid (**28** or **30**) and the C5-*R* alcohol **35** was achieved using EDC and DMAP, leading to formation of the desired compound **38** or **39** in low yield. Both **38** and **39** were converted to carboxylic acids **40** and **41**, respectively, via thioacetal removal and oxidation. To our disappointment, removal of the Boc group of **40** or the Troc group of **41** to synthesize the macrolactam precursor **42** was unsuccessful in our hands, typically resulting in a mixture of undesired compounds. The failed deprotection of **40** and **41** appeared to originate from the instability of the diene-attached C5-acyloxy group toward the acidic or reductive reagents required for the deprotection.

These results forced us to adopt an alternative coupling strategy, in which the order of C9′-esterification and C1-amidation are reversed (Scheme 6). Before the intermolecular amidation, the required amine **43** was prepared from *N*-Troc protected **29** using Zn in phosphate buffer. Peptide coupling at C1 between **43** and C5-acetoxy carboxylic acid **36** was carried out with HATU in the presence of *i*-Pr_2NEt, giving rise to **44**. Finally, the methoxy group and the acetyl group of **44** were removed simultaneously by hydrolysis using LiOH, and the resultant seco acid was successfully macrolactonized by the action of MNBA[26] and DMAP to deliver C5′-alkyne-bearing macrolactam **6**.

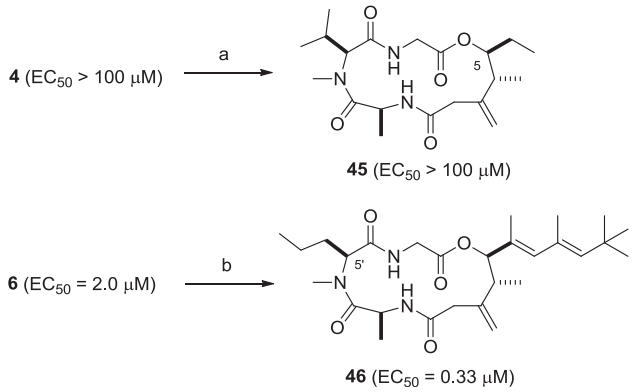
2.3. Cytotoxicity of 4 and 6 and their hydrogenated analogues

First, we evaluated the biological activities of acetylene-bearing **4** and **6** and their saturated analogues **45** and **46** by a neurotoxicity assay using Neuro 2a (Scheme 7). Chemoselective hydrogenation of



Scheme 6. Synthesis of alkyne **6**. (a) Zn, THF/1 M KH_2PO_4, 100%. (b) **36**, HATU, *i*-Pr_2NEt, DMF, 77%; (c) LiOH·H_2O, THF/MeOH/H_2O (3/1/1), 0 °C; (d) MNBA, DMAP, CH_2Cl_2 (1 mM), 54% (two steps). MNBA=2-methyl-6-nitrobenzoic anhydride.

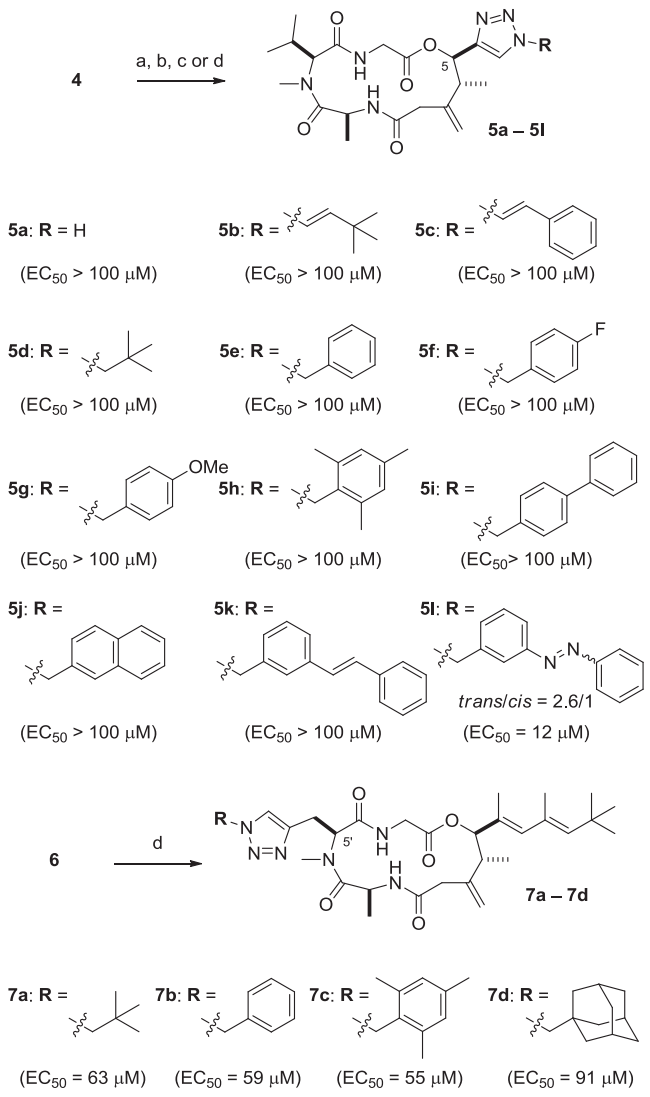
the terminal acetylenes of **4** and **6** was performed using Lindlar catalyst[27] under H_2 atmosphere, affording **45** and **46** in high yield without affecting the other olefins. Compounds **4** and **45**, the truncated C5-side chain analogues, showed almost no activity in concentrations up to 100 μM. These data are consistent with our previous results regarding the significant effect of the length of the C5-side chain on toxicity.[9] On the other hand, C5′-side chain analogues **6** and **46** exhibited strong cytotoxicity with EC_{50}s of ~1 μM, which are comparable to antillatoxin B **3**.[10] Interestingly, exchange of the *i*-Pr group at C5′ of antillatoxin **1** to a *n*-Pr group as in **46** decreased the toxicity 7-fold (45 nM vs 0.33 μM), suggesting the high importance of the branched alkyl structure of the C5′-side chain of **1**.[28]



Scheme 7. Chemoselective hydrogenation of acetylene moieties in **4** and **6**, and cytotoxicities of **4**, **6**, **45**, and **46**. (a) H_2, 5% Pd/$CaCO_3$ poisoned with Pb, EtOAc, 2 h, 86%; (b) H_2, 5% Pd/$CaCO_3$ poisoned with Pb, EtOAc, rt, 15 min, 71%.

2.4. Synthesis and cytotoxicity of triazole analogues 5 and 7

Next, the triazole analogues **5** and **7** were synthesized from alkynes **4** and **6** via 1,3-dipolar addition (Scheme 8). Whereas H-substituted triazole **5a** was synthesized from **4** using $TMSN_3$ and catalytic CuI in the presence of protic solvent,[29] the vinyl derivatives **5b** and **5c** were prepared by coupling of **4** with in situ generated vinyl azides.[30,31] The nine alkyl-substituted triazoles **5d**–**l** were produced using the corresponding alkylazides in the presence of CuI and *i*-Pr_2NEt.[32] Alternatively, macrolactam **6** was reacted with the four alkylazides under the standard conditions to furnish 5′-side chain analogues **7a**–**d**. Thus, diverse side-chain



Scheme 8. Synthesis and cytotoxicity of triazole analogues **5** and **7**. (a) $TMSN_3$, CuI, DMF/MeOH (9/1), 100 °C, 56% (**5a**); (b) NaN_3, *E*-2-(3,3-dimethylbutenyl)boronic acid pinacol ester, $CuSO_4$, sodium ascorbate, MeOH/H_2O (1/1), 75% (**5b**); (c) NaN_3, (1*E*-2-iodoethenyl)benzene, $CuSO_4$, sodium ascorbate, L-Pro, Na_2CO_3, DMSO/H_2O (9/1), 72% (**5c**); (d) RN_3, CuI (20 mol %), *i*-Pr_2NEt, THF, 93–27% (**5d**: 77%, **5e**: 65%, **5f**: 89%, **5g**: 85%, **5h**: 74%, **5i**: 76%, **5j**: 70%, **5k**: 65%, **5l**: 88%, **7a**: 46%, **7b**: 60%, **7c**: 74%, **7d**: 67%).

modifications either at C5 or at C5′ were efficiently realized in a single step from the two common alkyne intermediates.

The twelve C5-side chain analogues **5a**–**l** and four C5′-side chain analogues **7a**–**d** were subjected to the cytotoxicity assay against Neuro 2a, and the results are shown in Scheme 8. In comparison to the natural antillatoxin **1**, newly synthesized triazole derivatives **5a**–**l** and **7a**–**d** showed at least 270-fold less potent activities. Specifically, C5′-side chain analogues **7a**–**d** retained modest cytotoxicity with EC_{50} values of 55–91 μM, and C5-side chain analogues were almost inactive in this assay except for azobenzene analogue **5l** with an EC_{50} of 12 μM.[33]

It is interesting to note that C5′-substituted analogues **7a**–**d** all had comparable toxicities despite the large differences in the structures and steric bulkiness of their R groups. The activity indifference to the size of the C5′-side chains is important finding for future preparation of fluorescent and photoaffinity agents based on the C5′-modified antillatoxin structure. Additionally, new designs of active C5-modified antillatoxin analogues would be possible based on the *m*-azobenzene structure of C5-modified **5l**, although the structural rationale for the unusual toxicity of **5l** remains to be clarified.

Among the 11 non-active C5-substituted derivatives **5a**–**k**, we are particularly intrigued by **5d**, because the topology of the neopentyl-substituted triazole of **5d** is most similar to the 9-*tert*-butyl-6,8-dimethyl-6,8-diene moiety of natural **1**. To consider the relevance of **5d** as a structural mimic of **1**, ab initio calculations and NMR experiments were carried out (Fig. 2).[34,35] The ab initio simulation at the HF/6-31G** level showed that **5d** adopts a different side-chain conformation than **1**. The major difference was observed for the H5–C5–C6–C7 dihedral angles. The sp^3-C14 of **1** is bulkier than the sp^2-C7, such the H5–C5–C6–C7 angle was fixed in a *syn* conformation to avoid steric repulsion between C14–H and C5–H (Fig. 2a). On the other hand the sp^2-N14 of **5d** is less bulky than the sp^2-C7, and thus the preferable conformation of H5–C5–C6–C7 in **5d** is *anti* to avoid steric interaction between C5–H and C7–H (Fig. 2b). These stable three-dimensional structures of the side chains were further supported by NMR experiments. Whereas the *syn* conformation of H5–C5–C6–C7 in **1** was confirmed by a transannular NOE between H12′ and H14 (Fig. 2c), the *anti* conformation of H5–C5–C6–C7 in **5d** was revealed by the observation of a transannular NOE between H12′ and H7 (Fig. 2d).

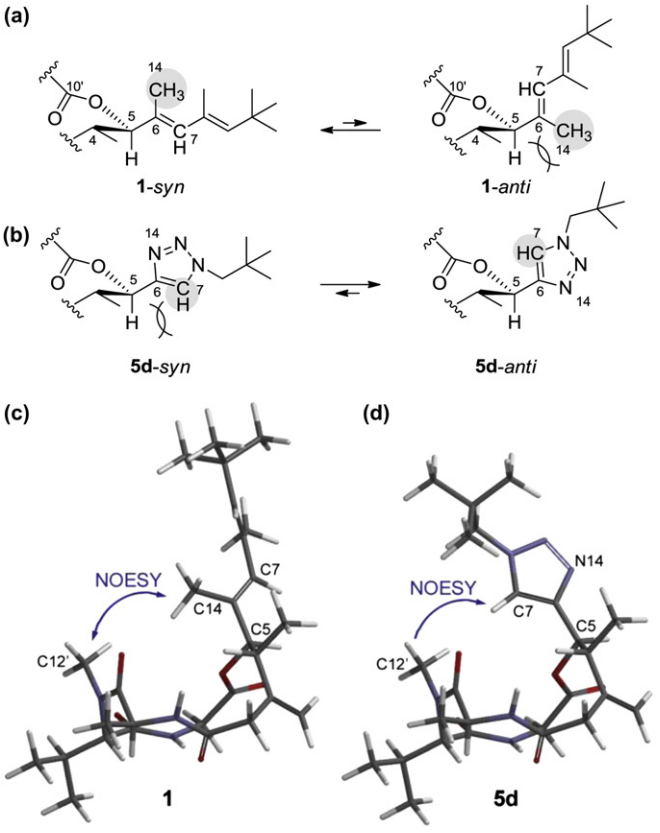


Fig. 2. Preferable conformations around the C5–C6 bond are illustrated for antillatoxin (a) and **5d** (b). NMR-assisted structures of antillatoxin (c) and **5d** (d) are shown with observed transannular NOEs (blue arrows).

Furthermore, we deduced contrasting electrostatic density surfaces of the most stable conformers of antillatoxin and **5d** at the DFT B3LYP/6-31G** level (Fig. 3). Although the C5-side chain of antillatoxin is neutral, the 1,4-substituted-1,2,3-triazole moiety of **5d** forms a negative potential region around nitrogen atoms N14 and N15 of the C5-side chain. Overall, the distinctly different stable conformation and electrostatic surface are both responsible for the negligible toxicity of **5d**.

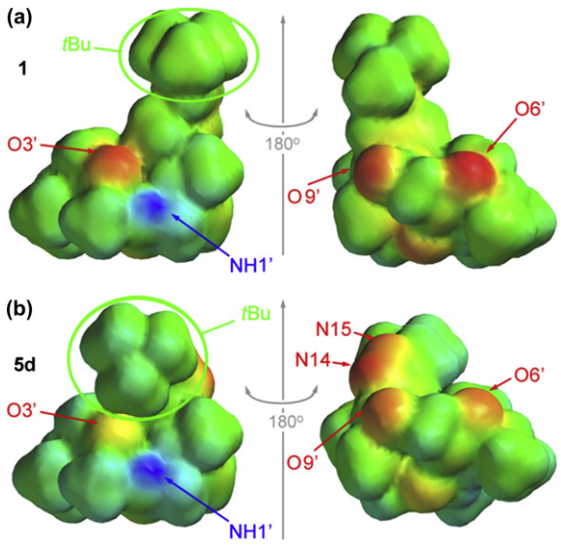


Fig. 3. Electron density surfaces of antillatoxin (a) and C5-side chain analogue **5d** (b). Color code represents the electrostatic potentials (red: negative, blue: positive, green: neutral).

3. Conclusion

In conclusion, we have achieved the synthesis and biological evaluation of antillatoxin analogues bearing triazole moieties in their side chains (**5a**–**l** and **7a**–**d**). In order to diversify the structures at the last step of the synthesis, two key intermediates **4** and **6** possessing terminal alkynes at the C5- and C5′-positions, respectively, were designed and synthesized using two distinct strategies. Sixteen side-chain derivatives were then prepared from **4** and **6** by coupling with a wide variety of azides via click chemistry, and subjected to the cytotoxicity assay. No cytotoxicity of **5a**–**k** and decent cytotoxicity of **7a**–**d** clearly showed that C5′ is more tolerant than C5 for introduction of the bulky side chains with the triazole moieties. The detailed NMR and ab initio studies of non-active **5d** indicated that large differences in the molecular shape and electrostatic nature of **5d** from **1** are responsible for its non-activity. The information obtained through these SAR studies will provide useful insight for designing new antillatoxin-based molecular probes for neuroscience.

4. Experimental section

4.1. General

All reactions sensitive to air or moisture were carried out under argon atmosphere under anhydrous conditions unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometer, a JEOL ECS 400 (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR) spectrometer, a JEOL ECX 500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometer or a JEOL ECA 500 (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) spectrometer. Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as internal standard ($CDCl_3$, ^{1}H δ 7.26, ^{13}C δ 77.0; CD_3OD, ^{1}H δ 3.31, ^{13}C δ 49.0; DMSO-d_6, ^{1}H δ 2.50, ^{13}C δ 40.0). IR spectra were recorded on a PERKIN ELMER Spectrum BX FT-IR System spectrometer, a JASCO FT/IR-410 spectrometer or a JASCO FT/IR-4100 spectrometer. Mass spectra were recorded on a PerSeptive Biosystems Mariner Biospectrometry Workstation instrument, a Bruker BioTOF-Q spectrometer or a Bruker microTOF II spectrometer. Optical rotations were recorded on a JASCO DIP-370 polarimeter, a JASCO P-1010 polarimeter or

a JASCO P-2100 polarimeter. All reactions were monitored by TLC on MERCK TLC Silica gel 60 F_{254}, MERCK HPTLC Silica gel 60 F_{254}, MERCK TLC Aluminum oxide 60 F_{254}, basic or MERCK TLC Silica gel 60 RP-18 F_{254s} under UV light (254 nm), and/or developed by 10% ethanolic phosphomolybdic acid, anisaldehyde solution (*p*-anisaldehyde (50 mL), AcOH (10 mL), EtOH (900 mL) and concentrated aqueous H_2SO_4 (50 mL)), cerium/molybdenum solution ($Ce(SO_4)_2 \cdot 2H_2O$ (1.0 g), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (21 g), H_2O (470 mL), and concentrated aqueous H_2SO_4 (16 mL)) or ninhydrin solution (ninhydrin (1.4 mg), AcOH (1.0 mL), *n*-BuOH (190 mL) and H_2O (9.0 mL)). Flash column chromatography was performed using MERCK Silica gel 60 particle size 0.040–0.063 mm (230–400 mesh ASTM), KANTO Silica gel 60 N (0.04–0.05 mm) or Wako Florisil particle size 0.075–0.150 mm (100–200 mesh).

4.2. Hydroxythioacetal 10

To a solution of TMS-acetylene (832 μl, 5.890 mmol) in toluene (5.9 mL) was added Et_2Zn (1.0 M solution in hexane, 5.9 mL, 5.90 mmol). The mixture was heated to reflux for 1 h, during which time a large amount of gray precipitate formed in the reaction flask. The mixture was cooled to room temperature, and the solution of (*R*)-BINOL (169 mg, 0.589 mmol) and $Ti(O\textit{i}\text{-}Pr)_4$ (405 μl, 1.473 mmol) in Et_2O (18.5 mL) was added via cannula. After 1 h, aldehyde **9**[2d] (254.8 mg, 1.178 mmol) in Et_2O (5 mL) was added via cannula, and the reaction mixture was stirred for 2 h. The reaction mixture was quenched with 1.0 M aqueous tartaric acid at 0 °C, and stirred for 30 min vigorously. The mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified with flash column chromatography to give **10** (275.4 mg) in 74% yield: $[\alpha]_D^{24}$ 15.4 (*c* 0.81, MeOH); FT-IR (film) ν 3430, 2960, 2899, 2172, 1643, 1422, 1249, 1024, 845, 761 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.17 (9H, s, TMS), 1.20 (3H, d, *J*=7.5 Hz, H13), 1.84 (1H, dtt, *J*=14.0, 11.5, 3.5 Hz, dithiane), 2.12 (1H, dtt, *J*=14.0, 4.5, 2.5 Hz, dithiane), 2.47 (1H, qd, *J*=7.5, 5.0 Hz, H4), 2.50 (1H, ddd, *J*=15.0, 8.0, 1.0 Hz, H2), 2.60 (1H, ddd, *J*=15.0, 7.0, 1.0 Hz, H2), 2.79–2.84 (2H, m, dithiane), 2.87 (1H, ddd, *J*=14.5, 11.5, 2.5 Hz, dithiane), 2.88 (1H, ddd, *J*=14.5, 11.5, 2.5 Hz, dithiane), 4.22 (1H, dd, *J*=8.0, 7.0 Hz, H1), 4.37 (1H, d, *J*=5.0 Hz, H5), 5.13 (1H, d, *J*=1.0 Hz, H12), 5.19 (1H, br s, H12); ^{13}C NMR (125 MHz, $CDCl_3$) δ −0.03, 15.15, 25.94, 30.58, 30.60, 42.30, 44.58, 45.67, 65.65, 90.78, 105.48, 115.97, 144.94; MS (MALDI-TOF), calcd for $C_{15}H_{20}Cl_3N_3NaO_6$ 314.1 (M^+), found 314.2.

4.3. Ester 13

To a solution of crude tripeptide Boc-Ala-*N*-MeVal-Gly-OH **12**[9] (384 mg, ca. 1.068 mmol), alcohol **10** (168.0 mg, 0.534 mmol), and PPh_3 (560.3 mg, 2.136 mmol) in toluene (2.7 mL) was added DEAD (40 wt% in toluene, 969 μl, 2.136 mmol) in one portion at 0 °C. The mixture was immediately warmed to room temperature and stirred for 30 min. The reaction mixture was concentrated. The residue was purified with flash column chromatography to give **13** (324.7 mg) in 93% yield: $[\alpha]_D^{20}$ −37.6 (*c* 1.20, $CHCl_3$); FT-IR (film) ν 3307, 2970, 1712, 1637, 1178, 847 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.16 (9H, s, TMS), 0.84 (3/4·3H, d, *J*=7.0 Hz, H14′ or H15′), 0.85 (1/4·3H, d, *J*=6.5 Hz, H14′ or H15′), 0.98 (3/4·3H, d, *J*=7.0 Hz, H14′ or H15′), 1.02 (1/4·3H, d, *J*=6.5 Hz, H14′ or H15′), 1.19 (3/4·3H, d, *J*=7.0 Hz, H13), 1.19 (1/4·3H, d, *J*=7.0 Hz, H13), 1.32 (3/4·3H, d, *J*=7.0 Hz, H11′), 1.35 (1/4·3H, d, *J*=6.0 Hz, H11′), 1.40 (1/4·9H, s, Boc), 1.43 (3/4·9H, s, Boc), 1.83 (1H, dtt, *J*=14.0, 11.5, 3.5 Hz, dithiane), 2.12 (1H, dtt, *J*=14.0, 5.0, 2.5 Hz, dithiane),2.31 (1H, dqq, *J*=11.0, 7.0, 7.0 Hz, H13′), 2.44–2.56 (3H, m, H2 and H4), 2.79–2.91 (4H, m, dithiane), 3.01 (3H, s, H12′), 3.84 (1H, dd, *J*=18.0, 5.0 Hz, H8′), 4.13 (1H, dd, *J*=18.0, 6.5 Hz, H8′), 4.19 (3/4H, dd, *J*=7.5, 7.5 Hz, H1), 4.22 (1/4H, dd, *J*=7.5, 7.5 Hz, H1), 4.60 (1H, d, *J*=11.0 Hz, H5′), 4.63 (3/4H, dq, *J*=8.0, 6.5 Hz, H2′), 4.80 (1/4H, dq, *J*=8.5, 6.0 Hz, H2′), 5.05 (1H, s, H12), 5.06 (1H, s, H12), 5.25 (1/4H, d, *J*=8.5 Hz, H1′), 5.34 (3/4H, d, *J*=8.0 Hz, H1′), 5.43 (3/4H, d, *J*=6.5 Hz, H5), 5.48 (1/4H, d, *J*=6.0 Hz, H5), 6.47 (3/4H, dd, *J*=6.5, 5.0 Hz, H7′), 8.13 (1/4H, dd, *J*=6.5, 5.0 Hz, H7′).

4.4. Carboxylic acid 14

To a solution of **13** (385.4 mg, 0.588 mmol) and $CaCO_3$ (558.5 mg, 5.880 mmol) in MeCN (18.6 mL) and H_2O (1.9 mL) was added MeI (3.66 mL, 58.80 mmol). The reaction mixture was stirred for 17 h. The mixture was filtered and washed with EtOAc. The filtrate was washed with brine, dried over Na_2SO_4, and concentrated. The crude aldehyde was used in the next reaction without further purification: ^1H NMR (500 MHz, $CDCl_3$) δ 0.16 (9H, s, TMS), 0.84 (7/10·3H, d, *J*=7.0 Hz, H14′ or H15′), 0.85 (3/10·3H, d, *J*=7.0 Hz, H14′ or H15′), 0.97 (7/10·3H, d, *J*=6.5 Hz, H14′ or H15′), 1.01 (3/10·3H, d, *J*=6.5 Hz, H14′ or H15′), 1.15 (3/10·3H, d, *J*=7.5 Hz, H13), 1.16 (7/10·3H, d, *J*=7.5 Hz, H13), 1.32 (7/10·3H, d, *J*=7.0 Hz, H11′), 1.35 (3/10·3H, d, *J*=6.5 Hz, H11′), 1.39 (3/10·9H, s, Boc), 1.43 (7/10·9H, s, Boc), 2.31 (7/10H, dqq, *J*=11.5, 7.0, 6.5 Hz, H13′), 2.44 (3/10H, dqq, *J*=11.5, 6.5, 6.5 Hz, H13′), 2.58 (1H, qd, *J*=7.5, 7.0 Hz, H4), 3.01 (3H, s, H12′), 3.10 (2H, s, H2), 3.80 (1H, dd, *J*=18.0, 5.0 Hz, H8′), 4.12 (1H, dd, *J*=18.0, 7.0 Hz, H8′), 4.59 (1H, d, *J*=11.5 Hz, H5′), 4.63 (7/10H, dq, *J*=8.0, 7.0 Hz, H2′), 4.79 (3/10H, dq, *J*=8.5, 6.5 Hz, H2′), 5.01 (3/10H, s, H12), 5.04 (7/10H, s, H12), 5.19 (3/10H, s, H12), 5.20 (7/10H, s, H12), 5.27 (3/10H, d, *J*=8.5 Hz, H1′), 5.33 (7/10H, d, *J*=8.0 Hz, H1′), 5.39 (7/10H, d, *J*=7.0 Hz, H5), 5.44 (3/10H, d, *J*=6.5 Hz, H5), 6.51 (7/10H, dd, *J*=7.0, 5.0 Hz, H7′), 8.17 (3/10H, dd, *J*=7.0, 5.0 Hz, H7′), 9.59 (7/10H, dd, *J*=2.5, 2.5 Hz, H1), 9.60 (3/10H, dd, *J*=3.0, 2.5 Hz, H1).

To a solution of above aldehyde, 2-methyl-2-butene (10 mL, 88.20 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (825.6 mg, 5.292 mmol) in *t*-BuOH (16.3 mL) and H_2O (3.3 mL) was added $NaClO_2$ (478.6 mg, 5.292 mmol). After being stirred for 20 min, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$. The mixture was extracted with EtOAc, dried over Na_2SO_4, and concentrated. The residue was purified with flash column chromatography to give **14** (276.1 mg) in 81% from **13**: $[\alpha]_D^{17}$ −44.7 (*c* 0.998, $CHCl_3$); FT-IR (film) ν 3307, 2975, 2252, 2179, 1713, 1636, 1517, 1370, 1250, 1176, 1062, 848, 759 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.15 (9H, s, TMS), 0.84 (3H, d, *J*=7.0 Hz, H14′ or H15′), 0.95 (5/7·3H, d, *J*=7.0 Hz, H14′ or H15′), 0.99 (2/7·3H, d, *J*=7.0 Hz, H14′ or H15′), 1.19 (3H, d, *J*=7.0 Hz, H13), 1.33 (3H, d, *J*=7.0 Hz, H11′), 1.41 (2/7·9H, s, Boc), 1.43 (5/7·9H, s, Boc), 2.30 (5/7H, dqq, *J*=11.0, 7.0, 7.0 Hz, H13′), 2.41 (2/7H, dqq, *J*=11.0, 7.0, 7.0 Hz, H13′), 2.61 (1H, dq, *J*=10.0, 7.0 Hz, H4), 2.99 (1H, d, *J*=13.5 Hz, H2), 3.08 (1H, d, *J*=13.5 Hz, H2), 3.10 (3H, s, H12′), 3.65 (1H, dd, *J*=18.0, 3.0 Hz, H8′), 4.40 (1H, dd, *J*=18.0, 8.5 Hz, H8′), 4.66 (5/7H, dq, *J*=8.5, 7.0 Hz, H2′), 4.71 (1H, d, *J*=11.0 Hz, H5′), 4.86 (2/7H, dq, *J*=8.5, 7.0 Hz, H2′), 5.00 (5/7H, s, H12), 5.04 (2/7H, s, H12), 5.07 (2/7H, s, H12), 5.10 (5/7H, s, H12), 5.26 (5/7H, d, *J*=8.5 Hz, H1′), 5.34 (2/7H, d, *J*=10.0 Hz, H5), 5.38 (5/7H, d, *J*=10.0 Hz, H5), 5.62 (2/7H, d, *J*=8.5 Hz, H1′), 7.13 (1H, dd, *J*=8.5, 3.0 Hz, H7′).

4.5. Macrolactam 16

A solution of **14** (276.1 mg, 0.475 mmol) in TFA (0.86 mL) and CH_2Cl_2 (8.6 mL) was stirred for 9 h at 0 °C. The reaction mixture was diluted with toluene and concentrated. The crude seco acid **15** (277.6 mg) was used in the next reaction without further purification: ^1H NMR (500 MHz, CD_3OD) δ 0.15 (9H, s, TMS), 0.89 (3H, d, *J*=6.5 Hz, H14′ or H15′), 1.00 (3H, d, *J*=6.5 Hz, H14′ or H15′), 1.21 (3H, d, *J*=7.0 Hz, H13), 1.50 (3H, d, *J*=7.0 Hz, H11′), 2.30 (1H, dqq, *J*=11.0, 6.5, 6.0 Hz, H13′), 2.66 (1H, dq, *J*=6.5, 6.5 Hz, H4), 3.04 (3H, s, H12′), 3.10 (2H, s, H2), 3.84 (1H, d, *J*=18.0 Hz, H8′), 4.03 (1H, d, *J*=18.0 Hz,

H8′), 4.42 (1H, q, *J*=7.0 Hz, H2′), 4.70 (1H, d, *J*=11.0 Hz, H5′), 5.10 (1H, s, H12), 5.12 (1H, s, H12), 5.43 (1H, d, *J*=6.5 Hz, H5).

The above seco acid **15** (277.6 mg) was dissolved in DMF (79 mL) and *i*-Pr_2NEt (414 μl, 2.375 mmol), and HATU (541.8 mg, 1.425 mmol) was added at 0 °C. The mixture was stirred for 30 min at 0 °C. The reaction mixture was quenched with 5% aqueous citric acid, and extracted with EtOAc. The extract was washed with H_2O, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was purified with flash column chromatography to give **16** (144.7 mg) in 66% yield from **14**: $[\alpha]_D^{23}$ −103.8 (*c* 1.03, MeOH); FT-IR (film) *ν* 3277, 3079, 2965, 2250, 2180, 1743, 1691, 1643, 1550, 1458, 1250, 1040, 846 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.15 (9H, s, TMS), 0.85 (3H, d, *J*=6.5 Hz, H14′ or H15′), 0.98 (3H, d, *J*=6.5 Hz, H14′ or H15′), 1.16 (3H, d, *J*=7.0 Hz, H13), 1.42 (3H, d, *J*=7.0 Hz, H11′), 2.20 (1H, dq, *J*=11.0, 7.0 Hz, H4), 2.44 (1H, dqq, *J*=10.5, 6.5, 6.5 Hz, H13′), 2.80 (1H, d, *J*=13.0 Hz, H2), 2.87 (3H, s, H12′), 2.95 (1H, d, *J*=13.0 Hz, H2), 3.51 (1H, dd, *J*=18.0, 2.0 Hz, H8′), 4.14 (1H, d, *J*=10.5 Hz, H5′), 4.73 (1H, dd, *J*=18.0, 10.0 Hz, H8′), 4.90 (1H, s, H12), 5.06 (1H, s, H12), 5.32 (1H, dq, *J*=9.5, 7.0 Hz, H2′), 5.43 (1H, d, *J*=11.0 Hz, H5), 6.59 (1H, d, *J*=9.5 Hz, H1′), 7.96 (1H, dd, *J*=10.0, 2.0 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* −0.24, 18.33, 18.82, 19.38, 19.52, 26.25, 29.02, 41.30, 42.40, 43.10, 46.16, 67.18, 67.25, 92.05, 100.90, 114.59, 143.75, 167.88, 168.13, 171.16, 173.06; HRMS (MALDI-TOF) calcd for $C_{23}H_{38}N_3O_5Si$ 464.2580 ($M+H^+$), found 464.2552.

4.6. Macrolactam 4

To a solution of **16** (57.1 mg, 0.123 mmol) in DMF (1.1 mL) and H_2O (0.11 mL) was added KF (10.7 mg, 0.185 mmol). The reaction mixture was stirred for 5 min, and quenched with saturated aqueous NH_4Cl. The mixture was extracted with EtOAc, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified with flash column chromatography to give **4** (50.0 mg) in 100% yield: $[\alpha]_D^{23}$ −140.5 (*c* 1.02, MeOH); FT-IR (film) *ν* 3276, 3079, 2970, 2249, 2121, 1741, 1683, 1642, 1553, 1458, 1244, 908, 733 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.86 (3H, d, *J*=6.5 Hz, H14′ or H15′), 0.98 (3H, d, *J*=6.5 Hz, H14′ or H15′), 1.16 (3H, d, *J*=7.0 Hz, H13), 1.41 (3H, d, *J*=7.0 Hz, H11′), 2.29 (1H, dq, *J*=9.5, 7.0 Hz, H4), 2.43 (1H, dqq, *J*=10.5, 6.5, 6.5 Hz, H13′), 2.47 (1H, d, *J*=2.5 Hz, H7), 2.85 (1H, d, *J*=13.5 Hz, H2), 2.87 (3H, s, H12′), 2.97 (1H, d, *J*=13.5 Hz, H2), 3.53 (1H, dd, *J*=18.0, 2.5 Hz, H8′), 4.14 (1H, d, *J*=10.5 Hz, H5′), 4.70 (1H, dd, *J*=18.0, 10.0 Hz, H8′), 4.98 (1H, s, H12), 5.10 (1H, s, H12), 5.34 (1H, dq, *J*=9.5, 7.0 Hz, H2′), 5.45 (1H, dd, *J*=9.5, 2.5 Hz, H5), 6.57 (1H, d, *J*=9.5 Hz, H1′), 7.91 (1H, dd, *J*=10.0, 2.5 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 18.47, 18.75, 18.83, 19.46, 26.38, 29.02, 41.28, 42.60, 43.02, 45.31, 66.83, 67.10, 75.33, 79.49, 115.60, 143.46, 167.86, 168.31, 171.02, 172.83; HRMS (MALDI-TOF) calcd for $C_{20}H_{29}N_3O_5$ 392.2185 ($M+H^+$), found 392.2111.

4.7. *N*-Nosyl-propargylglycine methyl ester 20

To a solution of **17** (2.00 g, 6.77 mmol) and catalyst **18** (5.1 mg, 0.0068 mmol) in toluene (22.4 mL) and 50% aqueous KOH (22.4 mL) at 0 °C was added propargyl bromide (0.612 mL, 8.12 mmol). After 1 h, additional propargyl bromide (0.612 mL, 8.12 mmol) was added. After being stirred for 17 h at 0 °C, the reaction mixture was quenched with H_2O and extracted with Et_2O. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration gave **19**, which was used in the next reaction without further purification. The ee value of **19** was determined to be >99% by HPLC analysis using a normal phase chiral column, Daicel Chiralcel-OD 4.6×250 mm, with an isocratic eluent of hexane/*i*-PrOH (499/1) at 0.5 mL/min of flow rate at 35 °C (t_R=28.7 min): ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.45 (9H, s), 1.95 (1H, dd, *J*=2.8, 2.8 Hz), 2.75 (1H, ddd, *J*=2.8, 8.2, 16.9 Hz), (1H, ddd, *J*=2.8, 5.5, 16.9 Hz), 4.17 (1H, dd, *J*=5.5, 8.2 Hz), 7.25–7.67 (10H, m); HRMS (MALDI-TOF) calcd for $C_{22}H_{24}NO_2$ 334.1802 ($M+H^+$), found 334.1828.

To a solution of the above crude **19** in THF (62 mL) at room temperature was added 15% citric acid (31 mL). After being stirred for 14 h at room temperature, the reaction mixture was quenched with 1 M aqueous HCl, and extracted with Et_2O. The aqueous layer was basified with K_2CO_3, and extracted with EtOAc. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration gave the crude amine, which was used in the next reaction without further purification.

To a solution of the crude amine and Et_3N (1.04 mL, 7.45 mmol) in CH_2Cl_2 (22.4 mL) at 0 °C was added NsCl (1.88 g, 8.46 mmol). After being stirred for 4 h at room temperature, the reaction mixture was cooled to 0 °C, quenched with H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4. Concentration and flash column chromatography (EtOAc) gave **20** (1.50 g) in 63% yield from **17**: $[\alpha]_D^{19}$ −176.1 (*c* 0.92, $CHCl_3$); IR (neat) *ν* 3294, 2981, 1735, 1542, 1426, 1361, 1157, 741 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.29 (9H, s, *t*-Bu), 2.06 (1H, dd, *J*=2.8, 2.8 Hz, H15′), 2.74 (1H, ddd, *J*=2.8, 5.5, 16.9 Hz, H13′), 2.79 (1H, ddd, *J*=2.7, 4.6, 16.9 Hz, H13′), 4.25 (1H, ddd, *J*=4.6, 5.5, 9.2 Hz, H5′), 6.39 (1H, d, *J*=9.2 Hz, N–H), 7.72–7.75 (2H, m, nosyl), 7.94 (1H, m, nosyl), 8.09 (1H, m, nosyl); ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 24.2, 27.7, 72.4, 83.3, 125.6, 130.3, 132.9, 133.6, 134.6, 147.7, 168.0; HRMS (MALDI-TOF) calcd for $C_{15}H_{18}N_2O_6SNa$ 377.0783 ($M+Na^+$), found 377.0790.

4.8. *N*-Nosyl-*N*-methyl-propargylglycine methyl ester 21

To a solution of **20** (1.50 g, 4.23 mmol) and K_2CO_3 in DMF (9.2 mL) at 0 °C was added MeI (1.04 mL, 16.9 mmol). After being stirred for 7 h at room temperature, the reaction mixture was cooled to 0 °C, quenched with saturated aqueous NH_4Cl, and extracted with EtOAc. The organic layer was washed with 0.5 M aqueous HCl, brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=2/1) gave **21** (1.56 g) in 96% yield: $[\alpha]_D^{20}$ +43.1 (*c* 1.96, $CHCl_3$); IR (neat) *ν* 3291, 2980, 1733, 1546, 1371, 1353, 1168, 585 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 1.35 (9H, s, *t*-Bu), 2.02 (1H, t, *J*=2.8 Hz, H15′), 2.74 (1H, ddd, *J*=2.8, 8.7, 17.4 Hz, H13′), 2.82 (1H, ddd, *J*=2.8, 5.5, 17.4 Hz, H13′), 3.06 (3H, s, H12′), 4.79 (1H, dd, *J*=5.5, 8.7 Hz, H5′), 7.61–7.64 (1H, m, nosyl), 7.68–7.70 (2H, m, nosyl), 8.08–8.10 (1H, m, nosyl); ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 20.4, 27.5, 30.8, 58.4, 71.6, 78.8, 82.7, 123.9, 130.7, 131.6, 132.5, 133.5, 147.8, 167.5; HRMS (MALDI-TOF) calcd for $C_{16}H_{20}N_2O_6SNa$ 391.0940 ($M+Na^+$), found 391.0954.

4.9. *N*-Nosyl-*N*-methyl-propargylglycine 22

To a solution of **21** (1.51 g, 4.09 mmol) in CH_2Cl_2 (30.8 mL) at room temperature was added TFA (6.2 mL). After being stirred for 6 h at room temperature, toluene (30 mL) was poured into the mixture. Concentration and flash column chromatography (hexane/EtOAc=1/1 to 1/4) gave **22** (1.08 g) in 85% yield: $[\alpha]_D^{20}$ +33.7 (*c* 2.83, $CHCl_3$); IR (neat) *ν* 3296, 1726, 1543, 1372, 1169, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 2.00 (1H, t, *J*=2.7 Hz, H15′), 2.78 (1H, ddd, *J*=2.7, 9.6, 17.8 Hz, H13′), 2.87 (1H, ddd, *J*=2.7, 5.0, 17.8 Hz, H13′), 3.07 (3H, s, H12′), 4.97 (1H, dd, *J*=5.0, 9.6 Hz, H5′), 7.67–7.74 (3H, m, nosyl), 8.06–8.08 (1H, m, nosyl); ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 19.8, 30.6, 57.8, 71.9, 78.1, 124.0, 130.5, 131.78, 131.81, 133.8, 147.5, 173.8; HRMS (MALDI-TOF) calcd for $C_{12}H_{12}N_2O_6SNa$ 335.0314 ($M+Na^+$), found 335.0317.

4.10. *N*-Methyl-propargylglycylglycine methyl ester 24

To a solution of **22** in CH_2Cl_2 (17.3 mL) and *i*-Pr_2NEt (2.41 mL, 13.8 mmol) at 0 °C were added Gly-OMe·HCl **23** (478 mg, 3.80 mmol) and PyBroP (1.86 g, 3. 98 mmol). After being stirred for

2 h at room temperature, the reaction mixture was cooled to 0 °C, quenched with 5% aqueous $KHSO_4$, and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=1/1 to 1/2) gave *N*–Ns-dipeptide (1.44 g), which was used in the next reaction without further purification: IR (neat) ν 3291, 1748, 1681, 1544, 1372, 1218 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.50 (1H, t, *J*=2.9 Hz, H15′), 2.64 (1H, ddd, *J*=2.9, 9.2, 17.7 Hz, H13′), 2.81 (1H, ddd, *J*=2.9, 6.3, 17.7 Hz, H13′), 3.05 (3H, s, H12′), 3.73 (3H, s, OMe), 3.96 (1H, dd, *J*=5.2, 18.4 Hz, H8′), 4.10 (1H, dd, *J*=5.7, 18.4 Hz, H8′), 4.70 (1H, dd, *J*=6.3, 9.2 Hz, H5′), 7.08 (1H, br s, N–H), 7.66–7.73 (3H, m, nosyl), 8.09–8.11 (1H, m, nosyl); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.4, 30.5, 41.3, 52.3, 58.4, 70.7, 78.8, 124.4, 131.5, 131.9, 132.7, 133.8, 147.9, 168.3, 169.6.

To a solution of the above *N*–Ns-dipeptide and thiophenol (0.85 mL, 8.3 mmol) in MeCN (69.2 mL) at 0 °C was added K_2CO_3 (1.43 g, 10.4 mmol). After being stirred for 14 h at room temperature, the mixture was passed through a pad of silica gel ($CHCl_3$/MeOH=10/1). Concentration gave **24** (643 mg) in 94% yield from **22**: $[\alpha]_D^{27}$ −3.77 (*c* 0.695, MeOH); IR (neat) ν 2957, 2927, 2859, 1730, 1273 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 2.04 (1H, t, *J*=2.8 Hz, H15′), 2.45 (3H, s, H12′), 2.55 (1H, ddd, *J*=2.8, 8.3, 17.0 Hz, H13′), 2.71 (1H, ddd, *J*=2.8, 4.6, 17.0 Hz, H13′), 3.18 (1H, dd, *J*=4.6, 8.3 Hz, H5′), 3.74 (3H, s, OMe), 4.00 (1H, dd, *J*=6.0, 18.8 Hz, H8′), 4.09 (1H, dd, *J*=6.0, 18.8 Hz, H8′), 7.77 (1H, br s, N–H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 21.6, 34.4, 40.4, 51.9, 62.1, 70.9, 79.8, 169.9, 172.5; HRMS (MALDI-TOF) calcd for $C_9H_{15}N_2O_3$ 199.1077 ($M+H^+$), found 199.1083.

4.11. *N*-Troc-alanyl-*N*-methyl-propargylglycylglycine methyl ester 29

To a solution of **24** (190 mg, 0.959 mmol), Troc-L-Ala **26** (397 mg, 1.44 mmol), and *i*-Pr_2NEt (0.501 mL, 2.88 mmol) in DMF (4.8 mL) at 0 °C was added HATU (547 mg, 1.44 mmol). After being stirred for 2 h at 0 °C, the reaction mixture was quenched with 5% aqueous citric acid, and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=1/2 to 1/4) gave **29** (376 mg) in 85% yield: $[\alpha]_D^{21}$ −90.3 (*c* 6.085, $CHCl_3$); IR (neat) ν 3305, 2953, 1737, 1647, 1533, 1214, 1092, 758 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.39 (3/5·3H, d, *J*=6.9 Hz, H11′), 1.48 (2/5·3H, d, *J*=6.9 Hz, H11′), 1.97 (3/5H, dd, *J*=2.3, 2.9 Hz, H15′), 2.01 (2/5H, dd, *J*=2.3, 2.9 Hz, H15′), 2.73–2.81 (2H, m, H13′), 2.82 (2/5·3H, s, H12′), 3.04 (3/5·3H, s, H12′), 3.679 (2/5·3H, s, OMe), 3.683 (3/5·3H, s, OMe), 3.82 (3/5H, dd, *J*=4.6, 18.3 Hz, H8′), 3.89 (2/5H, dd, *J*=4.9, 17.7 Hz, H8′), 4.02 (2/5H, dd, *J*=6.3, 17.7 Hz, H8′), 4.11 (3/5H, dd, *J*=6.3, 18.3 Hz, H8′), 4.59 (2/5H, d, *J*=12.0 Hz, Troc), 4.65 (3/5H, d, *J*=12.0 Hz, Troc), 4.68–4.74 (1H, m, H2′), 4.71 (3/5H, d, *J*=12.0 Hz, Troc), 4.76 (2/5H, d, *J*=12.0 Hz, Troc), 4.87 (2/5H, dd, *J*=5.8, 10.3 Hz, H5′), 5.19 (3/5H, dd, *J*=8.0, 8.1 Hz, H5′), 6.16 (3/5H, d, *J*=8.1 Hz, H1′), 6.40 (2/5H, d, *J*=6.9 Hz, H1′), 6.86 (3/5H, dd, *J*=4.6, 6.3 Hz, H7′), 7.83 (2/5H, dd, *J*=4.9, 6.3 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 17.7, 17.9, 18.2, 18.5, 28.6, 31.3, 40.9, 41.3, 46.3, 47.5, 52.1, 52.2, 55.9, 58.5, 71.0, 71.7, 74.4, 74.7, 79.1, 79.7, 95.0, 95.3, 153.7, 155.1, 168.4, 168.9, 169.6, 169.9, 173.3, 173.5; HRMS (MALDI-TOF) calcd for $C_{15}H_{20}Cl_3N_3NaO_6$ 466.0315 ($M+Na^+$), found 466.0294.

4.12. Alanyl-*N*-methyl-propargylglycylglycine methyl ester (43)

To a solution of **29** (122 mg, 0.274 mmol) and activated Zn (447 mg, 6.84 mmol) in THF (3.8 mL) at room temperature was added 1 M aqueous KH_2PO_4 (0.76 mL). After being stirred for 1 h at room temperature, the mixture was filtrated through glass wool three times. Concentration and flash column chromatography ($CHCl_3$/MeOH=20/1 to 10/1) gave **43** (91.8 mg) in 100% yield: $[\alpha]_D^{26}$ −68.8 (*c* 0.50, MeOH); IR (neat) ν 3278, 1747, 1668, 1615, 1532, 1217 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 1.49 (3/4·3H, d, *J*=7.3 Hz, H11′), 1.57 (1/4·3H, d, *J*=6.8 Hz, H11′), 2.41 (3/4H, dd, *J*=2.6, 2.6 Hz, H15′), 2.52 (1/4H, dd, *J*=2.6, 2.9 Hz, H15′), 2.60–2.93 (2H, m, H13′), 3.02 (1/4·3H, s, H12′), 3.20 (3/4·3H, s, H12′), 3.72 (3H, s, OMe), 3.89 (3/4H, d, *J*=17.4 Hz, H8′), 3.93 (1/4H, d, *J*=17.4 Hz, H8′), 4.03 (3/4H, d, *J*=17.4 Hz, H8′), 4.04 (1/4H, d, 17.4 Hz, H8′), 4.31 (1/4H, q, *J*=6.8 Hz, H2′), 4.36 (3/4H, q, *J*=7.3 Hz, H2′), 4.90 (3/4H, dd, *J*=6.9, 8.8 Hz, H5′), 4.96 (1/4H, dd, *J*=6.2, 9.5 Hz, H5′); ^{13}C NMR (500 MHz, CD_3OD) δ 17.7, 18.5, 19.4, 19.8, 30.6, 34.9, 42.0, 42.1, 47.9, 48.1, 52.7, 59.0, 60.9, 72.4, 73.8, 80.1, 80.6, 170.2, 170.6, 171.6, 171.7, 177.0; HRMS (MALDI-TOF) calcd for $C_{12}H_{19}N_3O_4$ 270.1448 ($M+H^+$), found 270.1476.

4.13. Conjugated vinyl iodide 33[2d]

The mixture of **32** (1.35 g, 9.93 mmol) and catecholborane (1.22 mL, 11.4 mmol) was stirred at 80 °C for 2 h. The mixture was poured into MeOH at room temperature. To the mixture at 0 °C was sequentially added pyridine (2.41 mL, 29.8 mmol) and I_2 (3.78 g, 29.8 mmol). After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$ and extracted with pentane. The organic layer was washed with H_2O, dried over Na_2SO_4. Concentration and alumina column chromatography (pentane) gave **33** (1.89 g) in 72% yield: ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (9H, s, H11), 1.79 (3H, s, H15), 2.52 (3H, s, H14), 5.30 (1H, s, H9), 6.60 (1H, s, H7); ^{13}C NMR (500 MHz, $CDCl_3$) δ 17.3, 29.0, 30.7, 32.6, 95.8, 131.6, 141.6, 146.4; MS (ESI) *m*/*z* 265 ($M+H^+$).

4.14. Hydroxythioacetal 34[2d]

To a solution of **33** (366.3 mg, 1.39 mmol) in Et_2O (5.2 mL) at −78 °C was added 1.7 M *t*-BuLi solution (1.63 mL). After the mixture was gradually warmed to −55 °C over 1 h, **9** (100 mg, 0.462 mmol) in Et_2O was added at −78 °C. The reaction mixture was gradually warmed to −65 °C over 0.5 h, and quenched with saturated aqueous NH_4Cl at −78 °C and extracted with EtOAc. The organic layer was washed with pH 7 phosphate buffer and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=30/1 to 10/1) gave **34** (151 mg, 5*S*/5*R*=7/1) in 92% yield: $[\alpha]_D^{23}$ −16.9 (*c* 0.84, $CHCl_3$); IR (neat) ν 3458, 2953, 2901, 1642, 1422, 1361, 1010, 904 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.02 (3H, d, *J*=7.3 Hz, H13), 1.14 (9H, s, H11, H16, H17), 1.72 (3H, s, H14), 1.80 (3H, s, H15), 1.86 (1H, m, dithiane), 2.13 (1H, m, dithiane), 2.44–2.52 (3H, m, H2, H4), 2.84–2.90 (4H, m, dithiane), 4.01 (1H, d, *J*=4.6 Hz, H5), 4.20 (1H, t, *J*=7.8 Hz, H1), 5.06 (1H, s, H12), 5.10 (1H, s, H12), 5.27 (1H, s, H9), 5.91 (1H, s, H7); ^{13}C NMR (500 MHz, $CDCl_3$) δ 12.9, 14.7, 18.0, 25.7, 30.5, 30.6, 31.0, 32.5, 41.3, 42.2, 45.9, 76.9, 114.2, 130.9, 131.3, 133.8, 139.9, 147.0; MS (ESI) *m*/*z* 377 ($M+Na^+$).

4.15. Hydroxythioacetal 35

To a solution of **34** (55.0 mg, 0.155 mmol) in THF (1.55 mL) and DMSO (0.31 mL) at 0 °C was added IBX (86.9 mg, 0.310 mmol). After being stirred for 1.5 h at room temperature, the reaction mixture was cooled to 0 °C and quenched with saturated aqueous $Na_2S_2O_3$, and extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. Concentration gave the ketone, which was used in the next reaction without further purification: IR (neat) ν 2954, 2900, 1661, 1611, 1362, 1042, 905 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.17 (9H, s, H11), 1.25 (3H, d, *J*=7.5 Hz, H13), 1.82–1.89 (1H, m, dithiane), 1.92 (3H, s, H14 or H15), 1.93 (3H, s, H14 or H15), 2.09–2.13 (1H, m, dithiane), 2.33–2.35 (1H, m, dithiane), 2.43 (1H, dd, *J*=7.4, 14.9 Hz, H2), 2.52 (1H, dd, *J*=7.4, 14.9 Hz, H2), 2.80–2.90 (4H, m, dithiane), 3.96 (1H, q, *J*=7.5 Hz, H4), 4.18 (1H, t, *J*=7.4 Hz, H1), 4.98 (1H, s, H12), 5.05 (1H, s, H12), 5.59 (1H, s, H9), 7.02 (1H, s, H7); ^{13}C NMR (400 MHz, $CDCl_3$) δ 13.4, 17.0, 17.4, 25.7,

30.3, 30.5, 30.9, 32.5, 41.0, 45.4, 45.5, 114.8, 131.0, 133.8, 144.5, 145.2, 145.8, 203.3.

To a solution of the above crude ketone and $CeCl_3 \cdot 7H_2O$ (86.6 mg, 0.233 mmol) in THF (0.7 mL) and H_2O (0.07 mL) at 0 °C was added $NaBH_4$ (8.8 mg, 0.23 mmol). After 30 min, additional $NaBH_4$ (1.8 mg, 0.046 mmol) was added to the mixture. The reaction mixture was stirred for 1 h at 0 °C, and quenched with H_2O, and extracted with EtOAc. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=50/1 to 20/1) gave **35** (45.7 mg), 5*S*/5*R*=1/11 in 83% yield: $[\alpha]_D^{27}$ −49.0 (*c* 1.24, $CHCl_3$); IR (neat) ν 2956, 2901, 1643, 1276, 1009, 904 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (1H, d, *J*=7.5 Hz, H13), 1.14 (9H, s, H11), 1.74 (3H, s, H14 or H15), 1.81 (3H, s, H14 or H15), 1.81–1.83 (1H, m, dithiane), 2.12–2.15 (1H, m, dithiane), 2.36 (1H, dq, *J*=7.5, 9.8 Hz, H4), 2.51 (2H, d, *J*=7.5 Hz, H2), 2.83–2.96 (4H, m, dithiane), 3.84 (1H, d, *J*=9.8 Hz, H5), 4.24 (1H, t, *J*=7.5 Hz, H1), 5.12 (1H, s, H12), 5.14 (1H, s, H12), 5.31 (1H, s, H9), 5.84 (1H, s, H7); ^{13}C NMR (500 MHz, $CDCl_3$) δ 12.0, 17.6, 17.9, 25.7, 30.3, 30.5, 30.9, 32.5, 40.9, 43.7, 45.5, 81.6, 114.8, 130.7, 133.7, 134.5, 140.3, 146.7.

4.16. Acetylcarboxylic acid 36

To a solution of **35** (38.0 mg, 0.107 mmol) in pyridine (1.1 mL) at 0 °C was added Ac_2O (0.54 mL). After being stirred for 7 h at 0 °C, the reaction mixture was quenched with 1 M aqueous HCl and extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$, H_2O and brine, and dried over Na_2SO_4. Concentration gave the crude dithiane, which was used in the next reaction without further purification: IR (neat) ν 2955, 2902, 1739, 1646, 1367, 1238, 1017, 971, 905, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.94 (3H, d, *J*=7.5 Hz, H13), 1.11 (9H, s, H11), 1.70 (3H, s, H14), 1.78 (3H, s, H15), 1.83 (1H, m, dithiane), 1.96 (3H, s, Ac), 2.10 (1H, m, dithiane), 2.45–2.49 (3H, m, H2, H4), 2.81–2.88 (4H, m, dithiane), 4.17 (1H, t, *J*=7.5 Hz, H1), 4.99 (1H, s, H12), 5.006 (1H, s, H12), 5.009 (1H, d, *J*=9.8 Hz, H5), 5.28 (1H, s, H9), 5.89 (1H, s, H7); ^{13}C NMR (500 MHz, $CDCl_3$) δ 12.9, 17.2, 17.8, 21.2, 25.8, 30.38, 30.44, 30.9, 32.5, 41.1, 41.7, 45.3, 83.5, 113.3, 130.3, 130.5, 136.0, 140.8, 146.4, 169.9.

To a solution of the above dithiane and $CaCO_3$ (107 mg, 1.07 mmol) in MeCN (3.2 mL) and H_2O (0.36 mL) at room temperature was added MeI (0.661 mL, 10.7 mmol). After being stirred for 15 h at room temperature, the mixture was filtered and washed with EtOAc. The filtrate was washed with H_2O, brine, and dried over Na_2SO_4. Concentration gave the crude aldehyde, which was used in the next reaction without further purification.

To a solution of the above aldehyde, 2-methyl-2-butene (1.81 mL, 16.1 mmol) and $NaH_2PO_4 \cdot 2H_2O$ (150 mg, 0.963 mmol) in *t*-BuOH (3.0 mL) and H_2O (0.6 mL) was added $NaClO_2$ (87.1 mg, 0.963 mmol). Until the complete consumption of the aldehyde was detected on TLC, additional 2-methyl-2-butene, $NaH_2PO_4 \cdot 2H_2O$ and $NaClO_2$ was added. After being stirred for 51 h at room temperature, the reaction mixture was quenched with saturated aqueous $Na_2S_2O_3$, and extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=10/1 to 3/1) gave **36** (25.7 mg) in 81% yield from **35**: $[\alpha]_D^{28}$ −26.7 (*c* 1.29, $CHCl_3$); IR (neat) ν 2959, 2867, 1739, 1712, 1647, 1370, 1238, 1018, 905 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (3H, d, *J*=6.9 Hz, H13), 1.13 (9H, s, H11), 1.72 (3H, s, H14 or H15), 1.80 (3H, s, H14 or H15), 1.99 (3H, s, Ac), 2.61 (1H, dq, *J*=6.9, 10.1 Hz, H4), 3.10 (2H, s, H2), 5.03 (1H, d, *J*=10.1 Hz, H5), 5.04 (1H, s, H12), 5.08 (1H, s, H12), 5.30 (1H, s, H9), 5.90 (1H, s. H7); ^{13}C NMR (500 MHz, $CDCl_3$) δ 12.8, 16.5, 17.8, 21.1, 30.9, 32.6, 40.3, 42.0, 83.1, 115.4, 130.1, 130.5, 136.2, 141.0, 143.4, 170.3, 177.1; MS (ESI) *m*/*z* 345 ($M+Na^+$).

4.17. Seco acid 44

To a solution of **36** (19.5 mg, 0.0605 mmol) and tripeptide **43** (32.6 mg, 0.121 mmol) in DMF (0.29 mL) at 0 °C was sequentially added *i*-Pr_2NEt (0.042 mL, 0.24 mmol) and HATU (46.0 mg, 0.121 mmol). After being stirred for 2 h at room temperature, the reaction mixture was quenched with 5% aqueous citric acid, and extracted with EtOAc. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=50/50) gave **44** (26.6 mg) in 77% yield: $[\alpha]_D^{19}$ −101 (*c* 1.33, $CHCl_3$); IR (neat) ν 3286, 2955, 1738, 1643, 1538, 1241, 1018, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (3/5·3H, d, *J*=6.9 Hz, H13), 0.90 (2/5·3H, d, *J*=6.9 Hz, H13), 1.12 (9H, s, H11), 1.37 (2/5·3H, d, *J*=6.9 Hz, H11′), 1.46 (3/5·3H, d, *J*=6.9 Hz, H11′), 1.72 (3H, s, H14), 1.78 (3H, s, H15), 2.00 (3H, 1H, m, Ac, H15′), 2.55 (1H, m, H4), 2.78–2.84 (2H, m, H13′), 2.84 (3/5·3H, s, H12′), 2.91 (3/5H, d, *J*=16.0 Hz, H2), 2.96 (2/5H, d, *J*=15.5 Hz, H2), 3.03 (2/5H, d, *J*=15.5 Hz, H2), 3.06 (3/5H, d, *J*=16.0 Hz, H2), 3.07 (2/5·3H, s, H12′), 3.70 (3/5·3H, s, OMe), 3.71 (2/5·3H, s, OMe), 3.83 (2/5H, dd, *J*=5.2, 18.3 Hz, H8′), 3.92 (3/5H, dd, *J*=5.7, 17.8 Hz, H8′), 4.06 (3/5H, dd, *J*=6.3, 17.8 Hz, H8′), 4.11 (2/5H, dd, *J*=6.9, 18.3 Hz, H8′) 4.82 (3/5H, dq, *J*=5.7, 6.9 Hz, H2′), 4.91–4.94 (3/5H, m, H5′), 4.94–4.98 (2/5H, m, H2′), 4.95 (3/5H, s, H12), 4.98 (2/5H, s, H12), 5.03 (3/5H, s, H12), 5.05 (2/5H, s, H12), 5.05–5.11 (2/5H, m, H5′), 5.07 (3/5H, d, *J*=10.3 Hz, H5), 5.08 (2/5H, d, *J*=10.3 Hz, H5), 5.29 (1H, s, H9), 5.88 (1H, s, H7), 6.66 (2/5H, dd, *J*=5.2, 6.9 Hz, H7′), 6.74 (3/5H, d, *J*=5.7 Hz, H1′), 6.83 (2/5H, d, *J*=7.5 Hz, H1′), 8.50 (3/5H, dd, *J*=5.7, 6.3 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 12.6, 12.7, 15.9, 16.2, 17.6, 17.72, 17.76, 17.78, 17.9, 18.5, 21.25, 21.33, 28.4, 30.4, 30.8, 30.9, 31.9, 32.6, 41.0, 41.3, 42.1, 42.4, 42.8, 45.0, 45.7, 52.1, 52.3, 56.7, 58.6, 70.9, 71.5, 79.4, 80.0, 82.2, 82.3, 114.9, 115.3, 129.7, 129.9, 130.3, 130.4, 136.5, 136.7, 141.1, 141.2, 144.5, 145.0, 168.9, 169.0, 169.8, 169.9, 170.1, 170.4, 170.6, 171.7, 173.1, 173.9; HRMS (MALDI-TOF) calcd for $C_{31}H_{47}N_3O_7Na$ 596.3312 ($M+Na^+$), found 596.3327.

4.18. Macrolactone 6

To a solution of **44** (2.5 mg, 4.83 μmol) in THF/MeOH/H_2O (3:1:1, 0.01 M) was added $LiOH \cdot H_2O$ at 0 °C. After being stirred for 19 h at 0 °C, the solution was acidified to pH 2–3 with 10 mM aqueous $KHSO_4$ at 0 °C, and extracted with EtOAc. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=1/1 to 0/100) gave the hydroxyl carboxylic acid (31.6 mg), which was used in the next reaction without further purification.

To a stirred solution of MNBA and DMAP in CH_2Cl_2 at 50 °C was added the hydroxyl carboxylic acid in CH_2Cl_2 dropwise by syringe pump over 16 h. After being stirred for additional 3 h, the reaction mixture was cooled to 0 °C, quenched with saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with H_2O and brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=1/1 to 0/100) gave **6** (0.78 mg) in 54% yield from **44**: $[\alpha]_D^{23}$ −202 (*c* 0.08, $CHCl_3$); IR (neat) ν 3273, 2960, 1736, 1643, 1541, 1255, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (3H, d, *J*=6.9 Hz, H13), 1.12 (9H, s, H11), 1.50 (3H, d, *J*=6.9 Hz, H11′), 1.58 (3H, s, H14), 1.79 (3H, s, H15), 2.02 (1H, dd, *J*=2.9, 2.9 Hz, H15′), 2.15 (1H, dq, *J*=6.9, 11.5 Hz, H4), 2.54 (1H, ddd, *J*=2.9, 4.1, 16.6 Hz, H13′), 2.81 (1H, d, *J*=13.2 Hz, H2), 2.84 (3H, s, H12′), 2.98 (1H, ddd, *J*=2.9, 11.5, 16.6 Hz, H13′), 3.02 (1H, d, *J*=13.2 Hz, H2), 3.56 (1H, d, *J*=18.9 Hz, H8′), 4.61 (1H, dd, *J*=9.8, 18.9 Hz, H8′), 5.03 (1H, s, H12), 5.06 (1H, s, H12), 5.10 (1H, dd, *J*=4.1, 11.5 Hz, H5′), 5.16 (1H, d, *J*=11.5 Hz, H5), 5.30 (1H, s, H9), 5.32 (1H, dq, *J*=6.9, 9.2 Hz, H2′), 5.95 (1H, s, H7), 6.45 (1H, d, *J*=9.2 Hz, H1′), 8.13 (1H, d, *J*=9.8 Hz, H7′); ^{13}C NMR (400 MHz, $CDCl_3$) δ 12.4, 17.7, 18.6, 18.9, 28.2, 30.8, 32.6, 39.2, 41.5, 43.4, 46.2, 58.8, 71.3, 79.8, 83.4,

113.9, 128.9, 130.4, 137.5, 141.6, 144.6, 166.6, 167.3, 171.3, 173.3; HRMS (MALDI-TOF) calcd for $C_{28}H_{41}N_3O_5Na$ 522.2944 ($M+Na^+$), found 522.2922.

4.19. Hydrogenation of 4

To a solution of **4** (38.5 mg, 98.3 μmol) in EtOAc (1 mL) was added 5% $Pd/CaCO_3$ poisoned with Pb (20.9 mg, 9.83 μmol) at room temperature. The reaction mixture was stirred under H_2 for 2 h, and filtrated through a pad of Celite with EtOAc. The filtrate was concentrated. The residue was purified with flash column chromatography (hexane/EtOAc=1/1 to 1/3) and HPLC (column: Inetrsil ODS-3 φ10×250 mm, flow rate: 2 mL/min, detection: UV 210 nm, eluent: MeOH/H_2O=7/3, t_R=12.4 min) to give **45** (33.3 mg) in 86% yield: $[\alpha]_D^{25.8}$ −148 (*c* 1.38, MeOH); IR (film) *ν* 3277, 2969, 1735, 1638, 1542, 1458, 1266 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) *δ* 0.75 (3H, t, *J*=7.4 Hz, H7), 0.85 (3H, d, *J*=6.9 Hz, H15′), 0.96 (3H, d, *J*=6.3 Hz, H13), 0.99 (3H, d, *J*=7.5 Hz, H14′), 1.34 (1H, ddq, *J*=14.9, 7.4, 7.4 Hz, H6), 1.39 (3H, d, *J*=6.9 Hz, H11′), 1.67 (1H, dqd, *J*=14.9, 7.4, 3.4 Hz, H6), 2.10 (1H, dq, *J*=7.4, 6.3 Hz, H4), 2.41 (1H, dqq, *J*=10.8, 7.5, 6.9 Hz, H13′), 2.82 (1H, d, *J*=13.2 Hz, H2), 2.85 (3H, s, H12′), 2.93 (1H, d, *J*=13.2 Hz, H2), 3.46 (1H, dd, *J*=18.3, 2.3 Hz, H8′), 4.12 (1H, d, *J*=10.8 Hz, H5′), 4.62 (1H, dd, *J*=18.3, 9.7 Hz, H8′), 4.958 (1H, ddd, *J*=7.4, 7.4, 3.4 Hz, H5), 4.964 (1H, s, H12), 5.03 (1H, s, H12), 5.37 (1H, dq, *J*=9.1, 6.9 Hz, H2′), 6.69 (1H, d, *J*=9.1 Hz, H1′), 7.90 (1H, dd, *J*=9.7, 2.3 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 8.9, 18.52, 18.54, 18.6, 19.2, 25.3, 26.1, 28.6, 41.0, 41.5, 42.7, 45.5, 67.1, 77.7, 114.9, 144.8, 168.0, 168.7, 171.1, 173.0; HRMS (ESI-TOF) calcd for $C_{20}H_{33}N_3O_5Na$ 418.2312 ($M+Na^+$), found 418.2309.

4.20. Hydrogenation of 6

To a solution of **6** (1.03 mg, 2.06 μmol) in EtOAc (2 mL) was added 5% $Pd/CaCO_3$ poisoned with Pb (8.8 mg, 4.12 μmol) at room temperature. The reaction mixture was stirred under H_2 for 10 min and filtrated through a pad of Celite with EtOAc. The filtrate was concentrated. The residue was purified with flash column chromatography (hexane/acetone=8/1 to 4/1) and HPLC (column: Inetrsil ODS-3 φ10×250 mm, flow rate: 2 mL/min, detection: UV 210 and 254 nm, eluent: MeOH/H_2O=8/1, t_R=20.2 min) to give **46** (0.88 mg) in 85% yield: $[\alpha]_D^{26.0}$ −145 (*c* 0.044, MeOH); IR (film) *ν* 3282, 2960, 2873, 1737, 1644, 1549, 1462, 1257, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.88 (3H, d, *J*=6.9 Hz, H13), 0.99 (3H, t, *J*=7.4 Hz, H15′), 1.12 (9H, s, H11, H16, H17), 1.26 (2H, m, H14′), 1.42 (3H, d, *J*=6.9 Hz, H11′), 1.56 (3H, s, H14), 1.76 (3H, s, H15), 1.76−1.88 (1H, m, H13′), 2.19 (1H, dq, *J*=10.9, 6.9 Hz, H4), 2.82 (1H, d, *J*=13.7 Hz, H2), 2.84 (3H, s, H12′), 2.99 (1H, d, *J*=13.7 Hz, H2), 3.51 (1H, dd, *J*=18.9, 1.1 Hz, H8′), 4.63 (1H, dd, *J*=18.9, 10.3 Hz, H8′), 4.74 (1H, dd, *J*=9.7, 6.3 Hz, H5′), 5.02 (1H, s, H12), 5.06 (1H, s, H12), 5.17 (1H, d, *J*=10.9 Hz, H5), 5.29 (1H, s, H9), 5.33 (1H, dq, *J*=9.1, 6.9 Hz, H2′), 5.94 (1H, s, H7), 6.65 (1H, d, *J*=9.1 Hz, H1′), 7.94 (1H, dd, *J*=10.3, 1.1 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 12.4, 13.8, 17.7, 18.7, 18.9, 28.5, 30.2, 30.86, 30.91, 32.6, 39.1, 41.4, 43.1, 46.5, 60.1, 83.5, 113.8, 129.1, 130.4, 137.3, 141.5, 144.8, 167.6, 168.0, 171.2, 173.1; HRMS (ESI-TOF) calcd for $C_{28}H_{45}N_3O_5Na$ 526.3251 ($M+Na^+$), found 526.3245.

4.21. Triazole 5a

To a solution of **4** (5.0 mg, 0.013 mmol) and CuI (0.25 mg, 1.3 μmol) in DMF/MeOH (9:1, 0.029 mL) at room temperature was added $TMSN_3$ (8.5 μl, 0.064 mmol). After 16.5 h at 80 °C, $TMSN_3$ (8.5 μl, 0.064 mmol) and MeOH (3 μl) were added to the solution. After being stirred for 4 h, the mixture was filtrated through Florisil with EtOAc. Concentration and flash column chromatography (hexane/EtOAc=50/50 to 0/100) gave **5a** (3.2 mg) in 56% yield: $[\alpha]_D^{22}$ −96.4 (*c* 0.240, $CHCl_3$); IR (neat) *ν* 3271, 2967, 2932, 1639, 1550, 1258, 756 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) *δ* 0.78 (3H, d, *J*=6.9 Hz, H13), 0.82 (3H, d *J*=6.9 Hz, H14′ or H15′), 0.89 (3H, d, *J*=6.3 Hz, H14′ or H15′), 1.30 (3H, d, *J*=6.9 Hz, H11′), 2.23 (1H, dqq, *J*=6.3, 6.9, 10.9 Hz, H13′), 2.57 (1H, dq, *J*=6.9, 12.0 Hz, H4), 2.59 (3H, s, H12′), 2.77 (1H, d, *J*=12.6 Hz, H2), 3.15 (1H, d, *J*=12.6 Hz, H2), 3.48 (1H, d, *J*=18.3 Hz, H8′), 4.30 (1H, dd, *J*=9.7, 18.3 Hz, H8′), 4.39 (1H, d, *J*=10.9 Hz, H5′), 4.94 (1H, s, H12), 5.00 (1H, s, H12), 5.37 (1H, dq, *J*=6.9, 8.6 Hz, H2′), 5.98 (1H, d, *J*=12.0 Hz, H5), 7.81 (1H, br, H7), 8.38 (1H, d, *J*=9.7 Hz, H7′), 9.20 (1H, d, *J*=8.6 Hz, H1′); ^{13}C NMR (500 MHz, DMSO-d_6) *δ* 17.7, 18.2, 18.4, 18.5, 25.4, 27.9, 40.5, 40.6, 42.3, 44.6, 65.5, 70.7, 112.1, 131.5, 145.3, 146.0, 167.4, 167.8, 170.2, 173.5; HRMS (ESI) calcd for $C_{20}H_{30}N_6O_5Na$ 457.2175 ($M+Na^+$), found 457.2195.

4.22. Triazole 5b

To MeOH (0.15 mL) at room temperature were added *E*-2-(3,3-dimethylbutenyl)boronic acid pinacol ester (32.5 mg 0.15 mmol), NaN_3 (3.9 mg, 0.06 mmol), and $CuSO_4$ (0.8 mg, 5.0 μmol), and the resultant mixture was stirred overnight, then **4** (19.6 mg, 0.05 mmol), sodium ascorbate (5.0 mg, 0.025 mmol) and H_2O (0.15 mL) was added. After being stirred for 7 h at room temperature, the reaction mixture was quenched with 1 M aqueous NH_3, and extracted with EtOAc. The organic layer was washed with H_2O and dried over Na_2SO_4. Concentration and flash chromatography (hexane/EtOAc=75/25 to 0/100) gave **5b** (19.3 mg) in 75% yield: $[\alpha]_D^{22}$ −65.5 (*c* −0.870, $CHCl_3$); IR (neat) *ν* 3273, 2964, 1742, 1637, 1554, 1461, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.90 (3H, d, *J*=6.9 Hz, H14′ or H15′), 0.94 (3H, d, *J*=6.9 Hz, H13), 0.98 (3H, d, *J*=6.9 Hz, H14′ or H15′), 1.15 (9H, s, *t*-Bu), 1.47 (3H, d, *J*=6.9 Hz, H11′), 2.42 (1H, dqq, *J*=6.9, 6.9, 10.9 Hz, H13′), 2.51 (1H, dq, *J*=6.9, 10.9 Hz, H4), 2.77 (3H, s, H12′), 2.86 (1H, d, *J*=13.2 Hz, H2), 3.04 (1H, d, *J*=13.2 Hz, H2), 3.51 (1H, d, *J*=18.9 Hz, H8′), 4.22 (1H, d, *J*=10.9 Hz, H5′), 4.62 (1H, dd, *J*=10.3, 18.9 Hz, H8′), 5.10 (1H, s, H12), 5.15 (1H, s, H12), 5.38 (1H, dq, *J*=6.9, 9.2 Hz, H2′), 6.16 (1H, d, *J*=10.9 Hz, H5), 6.34 (1H, d, *J*=14.9 Hz, H10), 6.52 (1H, d, *J*=9.2 Hz, H1′), 7.07 (1H, d, *J*=14.9 Hz, H9), 8.01 (1H, d, *J*=10.3 Hz, H7′), 8.16 (1H, s, H7); ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 18.58, 18.60, 18.8, 19.2, 25.8, 28.9, 29.3, 32.4, 40.9, 42.2, 43.3, 46.3, 67.2, 71.2, 114.5, 119.6, 121.3, 134.0, 144.0, 146.3, 167.5, 167.6, 171.2, 174.1; HRMS (ESI) calcd for $C_{26}H_{40}N_6O_5Na$ 539.2958 ($M+Na^+$), found 539.2966.

4.23. Triazole 5c

To a solution of **4** (66.5 mg, 0.17 mmol) in DMSO/H_2O (9:1, 0.38 mL) at room temperature were added (1*E*-2-iodoethenyl) benzene (115.9 mg, 0.5 mmol), L-proline (3.9 mg, 0.034 mmol), Na_2CO_3 (3.6 mg, 0.034 mmol), NaN_3 (39.7 mg, 0.61 mmol), sodium ascorbate (3.4 mg, 0.017 mmol), and $CuSO_4$ (1.4 mg, 9 μmol). After being stirred for 6 h at 60 °C, the reaction mixture was quenched with 1 M aqueous NH_4OH, and extracted with EtOAc. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=50:50 to 0/100) gave **5c** (66.4 mg) in 72% yield: $[\alpha]_D^{22}$ −24.1 (*c* 1.01, $CHCl_3$); IR (neat) *ν* 3273, 2969, 1744, 1681, 1636, 1553, 1237, 753 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.87 (6H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 0.94 (3H, d, *J*=6.3 Hz, H13 or H14′ or H15′), 1.42 (3H, d, *J*=6.9 Hz, H11′), 2.37 (1H, m, H13′), 2.57 (1H, m, H4), 2.75 (3H, s, H12′), 3.00 (2H, s, H2), 3.50 (1H, d, *J*=18.3 Hz, H8′), 4.25 (1H, d, *J*=10.9 Hz, H5′), 4.63 (1H, dd, *J*=9.2, 18.3 Hz, H8′), 5.06 (1H, s, H12), 5.12 (1H, s, H12), 5.36 (1H, dq, *J*=6.9, 9.7 Hz, H2′), 6.19 (1H, d, *J*=11.5 Hz, H5), 7.16 (1H, d, *J*=9.7 Hz, H1′), 7.24 (1H, d, *J*=14.9 Hz, H10), 7.28−7.44 (5H, m, phenyl), 7.79 (1H, d, *J*=14.9 Hz, H9), 8.14 (1H, d, *J*=9.2 Hz, H7′), 8.40 (1H, s, H7); ^{13}C NMR (500 MHz, $CDCl_3$) *δ* 18.50, 18.52, 18.7, 19.1, 25.6, 28.9, 40.8, 42.2, 43.4, 46.1, 67.2, 71.1, 114.4, 120.0, 121.8, 123.0, 126.7,

128.7, 128.9, 133.4, 144.0, 146.7, 167.5, 167.6, 171.3, 174.2; HRMS (ESI) calcd for $C_{28}H_{36}N_6O_5Na$ 559.2645 ($M+Na^+$), found 559.2643.

4.24. Triazole 5d

To a solution of **4** (20.5 mg, 0.051 mmol), CuI (1.9 mg, 0.01 mmol), and *tert*-butylmethylazide in THF (0.5 mL) at room temperature was added *i*-Pr_2NEt (0.088 mL, 0.51 mmol). After being stirred for 24.5 h at room temperature, the reaction mixture was quenched with 1 M aqueous HCl, and extracted with EtOAc. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=3/4 to 0/100) gave **5d** (20.0 mg) in 77% yield: $[\alpha]_D^{23}$ −112 (*c* 0.660, $CHCl_3$); IR (neat) ν 3439, 2965, 1740, 1634, 1553, 1259, 755 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.87 (3H, d, *J*=7.5 Hz, H14′ or H15′), 0.88 (3H, d, *J*=6.9 Hz, H13), 0.90 (3H, d, *J*=6.9 Hz, H14′ or H15′), 0.96 (9H, s, *t*-Bu), 1.44 (3H, d, *J*=6.9 Hz, H11′), 2.40 (1H, dqq, *J*=6.9, 7.5, 10.9 Hz, H13′), 2.60 (1H, dq, *J*=6.9, 10.9 Hz, H4), 2.75 (3H, s, H12′), 2.91 (1H, d, 13.2 Hz, H2), 3.02 (1H, d, *J*=13.2 Hz, H2), 3.52 (1H, dd, *J*=1.7, 18.3 Hz, H8′), 4.09 (1H, d, *J*=13.7 Hz, H9), 4.13 (1H, d, *J*=13.7 Hz, H9), 4.23 (1H, d, *J*=10.9 Hz, H5′), 4.62 (1H, dd, *J*=9.2, 18.3 Hz, H8′), 5.09 (1H, s, H12), 5.13 (1H, s, H12), 5.34 (1H, dq, *J*=6.9, 9.2 Hz, H2′), 6.16 (1H, d, *J*=10.9 Hz, H5), 6.74 (1H, d, *J*=9.2 Hz, H1′), 7.73 (1H, s, H7), 8.01 (1H, dd, *J*=1.7, 9.2 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.5, 18.6, 18.9, 19.2, 25.8, 27.5, 28.9, 32.5, 41.0, 41.9, 43.3, 46.1, 61.8, 67.1, 71.3, 114.4, 123.9, 144.2, 145.1, 167.67, 167.72, 171.3, 173.8; HRMS (ESI) calcd for $C_{25}H_{40}N_6O_5Na$ 527.2958 ($M+Na^+$), found 527.2949.

4.25. Triazole 5e

Triazole **5e** was obtained in 65% yield by the same procedure for **5d** using benzylazide: $[\alpha]_D^{23}$ −86.6 (*c* 0.82, $CHCl_3$); IR (neat) ν 3450, 3070, 1742, 1637, 1560, 1457, 1259, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, d, *J*=6.9 Hz, H15′), 0.89 (3H, d, *J*=7.5 Hz, H13), 0.94 (3H, d, *J*=6.3 Hz, H14′), 1.41 (3H, d, *J*=6.9 Hz, H11′), 2.23–2.37 (1H, m, H13′), 2.39 (3H, s, H12′), 2.53 (1H, dq, *J*=7.5, 11.5 Hz, H4), 2.87 (1H, d, *J*=12.6 Hz, H2), 3.00 (1H, d, *J*=12.6 Hz, H2), 3.48 (1H, dd, *J*=1.8, 18.3 Hz, H8′), 4.16 (1H, d, *J*=10.9 Hz, H5′), 4.58 (1H, dd, *J*=9.2, 18.3 Hz, H8′), 5.07 (1H, s, H12), 5.11 (1H, s, H12), 5.30 (1H, dq, *J*=6.9, 9.2 Hz, H2′), 5.41 (1H, d, *J*=14.9 Hz, H9), 5.61 (1H, d, *J*=14.9 Hz, H9), 6.16 (1H, d, *J*=11.5 Hz, H5), 6.67 (1H, d, *J*=9.2 Hz, H1′), 7.26–7.37 (5H, m, phenyl), 7.76 (1H, s, H7), 7.96 (1H, dd, *J*=1.8, 9.2 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.4, 18.6, 18.8, 19.2, 25.7, 28.5, 40.9, 41.8, 43.2, 46.0, 54.3, 67.1, 71.2, 114.4, 122.6, 128.2, 128.8, 129.1, 134.3, 144.1, 146.3, 167.5, 167.7, 171.3, 173.8; HRMS (ESI) calcd for $C_{27}H_{36}N_6O_5Na$ 547.2645 ($M+Na^+$), found 547.2636.

4.26. Triazole 5f

Triazole **5f** was obtained in 89% yield by the same procedure for **5d** using 4-fluorobenzylazide: $[\alpha]_D^{28}$ −70.5 (*c* 0.570, $CHCl_3$); IR (neat) ν 3271, 2968, 1743, 1682, 1636, 1556, 1512, 1226, 755 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.86 (3H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 0.87 (3H, d *J*=7.4 Hz, H13 or H14′ or H15′), 0.95 (3H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 1.42 (3H, d, *J*=6.9 Hz, H11′), 2.36 (1H, m, H13′), 2.45 (3H, s, H12′), 2.55 (1H, m, H4), 2.87 (1H, d, *J*=12.6 Hz, H2), 3.00 (1H, d, *J*=12.6 Hz, H2), 3.49 (1H, d, *J*=18.3 Hz, H8′), 4.18 (1H, d, *J*=11.5 Hz, H5′), 4.59 (1H, dd, *J*=9.8, 18.3 Hz, H8′), 5.06 (1H, s, H12), 5.11 (1H, s, H12), 5.31 (1H, dq, *J*=6.9, 9.2 Hz, H2′), 5.40 (1H, d, *J*=15.5 Hz, H9), 5.56 (1H, d, *J*=15.5 Hz, H9), 6.15 (1H, d, *J*=11.5 Hz, H5), 6.66 (1H, d, *J*=9.2 Hz, H1′), 7.05 (2H, t, *J*=8.6 Hz, phenyl), 7.27 (2H, dd, *J*=5.2, 8.6 Hz, phenyl), 7.76 (1H, s, H7), 7.97 (1H, d, *J*=9.8 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.4, 18.6, 18.8, 19.1, 25.7, 28.5, 40.8, 41.8, 43.2, 46.0, 53.5, 67.1, 71.2, 114.5, 116.1, 122.5, 130.1,130.2, 144.1, 146.4, 162.8, 167.6, 167.7, 171.3, 173.8; HRMS (ESI) calcd for $C_{27}H_{35}FN_6O_5Na$ 565.2551 ($M+Na^+$), found 565.2554.

4.27. Triazole 5g

Triazole **5g** was obtained in 85% yield by the same procedure for **5d** using 4-methoxybenzylazide: $[\alpha]_D^{25}$ −77.6 (*c* 0.42, $CHCl_3$); IR (neat) ν 3269, 2965, 1736, 1682, 1636, 1556, 1515, 1251, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, d, *J*=6.9 Hz, H14′ or H15′), 0.87 (3H, d, *J*=6.9 Hz, H13), 0.95 (3H, d, *J*=6.9 Hz, H14′ or H15′), 1.42 (3H, d, *J*=6.9 Hz, H11′), 2.36 (1H, dqq, *J*=6.9, 6.9, 10.9 Hz, H13′), 2.45 (3H, s, H12′), 2.50 (1H, dq, *J*=6.9, 11.5 Hz, H4), 2.85 (1H, d, *J*=13.2 Hz, H2), 3.01 (1H, d, *J*=13.2 Hz, H2), 3.49 (1H, d, *J*=18.9 Hz, H8′), 3.79 (3H, s, OMe), 4.17 (1H, d, *J*=10.9 Hz, H5′), 4.59 (1H, dd, *J*=10.3, 18.9 Hz, H8′), 5.07 (1H, s, H12), 5.11 (1H, s, H12), 5.32 (1H, dq, *J*=6.9, 9.8 Hz, H2′), 5.35 (1H, d, *J*=14.9 Hz, H9), 5.52 (1H, d, *J*=14.9 Hz, H9), 6.15 (1H, d, *J*=11.5 Hz, H5), 6.59 (1H, d, *J*=9.8 Hz, H1′), 6.87 (2H, d, *J*=8.6 Hz, phenyl), 7.23 (2H, d, *J*=8.6 Hz, phenyl), 7.74 (1H, s, H7), 7.96 (1H, d, *J*=10.3 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.5, 18.6, 18.9, 19.2, 25.7, 28.5, 40.9, 42.0, 43.2, 46.1, 53.8, 55.3, 67.1, 71.3, 114.4, 114.5, 122.2, 126.4, 129.7, 144.1, 146.3, 159.9, 167.5, 167.6, 171.3, 173.8; HRMS (ESI) calcd for $C_{28}H_{38}N_6O_6Na$ 577.2751 ($M+Na^+$), found 577.2757.

4.28. Triazole 5h

Triazole **5h** was obtained in 74% yield by the same procedure for **5d** using 2,4,6-trimethylbenzylazide: $[\alpha]_D^{23}$ −97.2 (*c* 1.63, $CHCl_3$); IR (neat) ν 3436, 2967, 1742, 1637, 1559, 1236, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.84 (3H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 0.86 (3H, d, *J*=7.5 Hz, H13 or H14′ or H15′), 0.94 (3H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 1.42 (3H, d, *J*=6.3 Hz, H11′), 2.27 (3H, s, CH_3Ph), 2.30 (6H, s, CH_3Ph), 2.32–2.36 (1H, m, H13′), 2.39 (3H, s, H12′), 2.49 (1H, m, H4), 2.86 (1H, d, *J*=12.6 Hz, H2), 2.99 (1H, d, *J*=12.6 Hz, H2), 3.47 (1H, dd, *J*=1.5, 18.9 Hz, H8′), 4.15 (1H, d, *J*=11.5 Hz, H5′), 4.57 (1H, dd, *J*=9.2, 18.9 Hz, H8′), 5.06 (1H, s, H12), 5.10 (1H, s, H12), 5.29 (1H, dq, *J*=6.3, 9.2 Hz, H2′), 5.49 (1H, d, *J*=14.9 Hz, H9), 5.58 (1H, d, *J*=14.9 Hz, H9), 6.10 (1H, d, *J*=11.5 Hz, H5), 6.69 (1H, d, *J*=9.2 Hz, H1′), 6.90 (2H, s, phenyl), 7.46 (1H, s, H7), 7.95 (1H, dd, *J*=1.5, 9.2 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.4, 18.6, 19.0, 19.2, 19.7, 20.9, 25.8, 28.3, 40.9, 41.6, 43.2, 46.1, 48.4, 67.0, 71.3, 114.1, 121.6, 127.2, 129.6, 137.9, 138.8, 144.4, 145.6, 167.6, 167.7, 171.3, 173.6; HRMS (ESI) calcd for $C_{30}H_{42}N_6O_5Na$ 589.3114 ($M+Na^+$), found 589.3106.

4.29. Triazole 5i

Triazole **5i** was obtained in 76% yield by the same procedure for **5d** using 4-biphenylazide: $[\alpha]_D^{24}$ −70.9 (*c* 0.76, $CHCl_3$); IR (neat) ν 3436, 2966, 1734, 1636, 1552, 1258, 750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.81 (3H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 0.91 (3H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 0.93 (3H, d, *J*=6.3 Hz, H13 or H14′ or H15′), 1.41 (3H, d, *J*=6.9 Hz, H11′), 2.29–2.32 (1H, m, H13′), 2.42 (3H, s, H12′), 2.56 (1H, m H4), 2.89 (1H, d, *J*=12.6 Hz, H2), 3.00 (1H, d, *J*=12.6 Hz, H2), 3.49 (1H, d, *J*=18.3 Hz, H8′), 4.16 (1H, d, *J*=10.9 Hz, H5′), 4.60 (1H, dd, *J*=9.2, 18.3 Hz, H8′), 5.07 (1H, s, H12), 5.11 (1H, s, H12), 5.30 (1H, dq, *J*=6.9, 9.8 Hz, H2′), 5.46 (1H, d, *J*=15.5 Hz, H9), 5.64 (1H, d, *J*=15.5 Hz, H9), 6.17 (1H, d, *J*=10.9 Hz, H5), 6.74 (1H, d, *J*=9.8 Hz, H1′), 7.35–7.59 (9H, m, diphenyl), 7.81 (1H, s, H7), 7.97 (1H, d, *J*=9.2 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.5, 18.6, 18.9, 19.2, 25.7, 28.5, 40.9, 42.0, 43.2, 46.1, 54.0, 67.1, 71.3, 114.6, 122.6, 127.1, 127.6, 127.8, 128.7, 128.8, 133.3, 140.2, 141.7, 144.0, 146.4, 167.5, 167.6, 171.3, 173.8; HRMS (ESI) calcd for $C_{33}H_{40}N_6O_5Na$ 623.2958 ($M+Na^+$), found 623.2947.

4.30. Triazole 5j

Triazole **5j** was obtained in 70% yield by the same procedure for **5d** using 2-naphthylazide: $[\alpha]_D^{21}$ −74.6 (*c* 0.70, $CHCl_3$); IR (neat) ν 3457, 2969, 1731, 1635, 1557, 1259, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.77 (3H, d, *J*=6.9 Hz, H13 or H14′ or H15′), 0.90 (3H, d, *J*=7.4 Hz, H13 or H14′ or H15′), 0.91 (3H, d, *J*=6.3 Hz, H13 or H14′ or H15′), 1.38 (3H, d, *J*=7.1 Hz, H11′), 2.23–2.27 (1H, m, H13′), 2.25 (3H, s, H12′), 2.51 (1H, m, H4), 2.83 (1H, d, *J*=12.6 Hz, H2), 3.00 (1H, d, *J*=12.6 Hz, H2), 3.47 (1H, d, *J*=16.6 Hz, H8′), 4.11 (1H, d, *J*=10.9 Hz, H5′), 4.58 (1H, dd, *J*=10.0, 16.6 Hz, H8′), 5.07 (1H, s, H12), 5.11 (1H, s, H12), 5.28 (1H, dq, *J*=7.1, 9.2 Hz, H2′), 5.59 (1H, d, *J*=14.9 Hz, H9), 5.75 (1H, d, *J*=14.9 Hz, H9), 6.18 (1H, d, *J*=10.9 Hz, H5), 6.51 (1H, d, *J*=9.2 Hz, H1′), 7.35 (1H, d, *J*=8.6 Hz, naphthalene), 7.49–7.51 (2H, m, naphthalene), 7.78–7.84 (5H, m, H7, naphthalene), 7.91 (1H, d, *J*=10.0 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.46, 18.55, 18.9, 19.2, 25.6, 28.3, 40.9, 42.1, 43.2, 46.1, 54.5, 67.0, 71.3, 114.6, 122.6, 125.4, 126.6, 127.5, 127.7, 128.0, 129.2, 131.7, 133.16, 133.19, 144.0, 146.5, 167.5, 167.6, 171.3, 173.7; HRMS (ESI) calcd for $C_{31}H_{38}N_6O_5Na$ 597.2801 ($M+Na^+$), found 597.2792.

4.31. Synthesis of (*E*)-3-stilbenemethylazide

To a solution of (*E*)-3-methylstilbene (300 mg, 1.55 mmol) in CCl_4 (15.5 mL) at room temperature were added NBS (302 mg, 1.7 mmol) and AIBN (36.1 mg, 0.078 mmol). In the course of the reaction, additional NBS (851 mg, 4.8 mmol) and AIBN (36.1 mg, 0.22 mmol) were added. After being stirred for 10.5 h at 90 °C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, and extracted with CCl_4. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane) gave (*E*)-3-(bromomethyl)stilbene (246 mg).

To a solution of (*E*)-3-(bromomethyl)stilbene in DMSO (2.6 mL) at room temperature was added NaN_3 (68 mg, 1.04 mmol). After being stirred for 3 h at room temperature, the reaction mixture was quenched with H_2O, and extracted with Et_2O. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane) gave (*E*)-3-stilbenemethylazide (94.7 mg) in 26% yield: IR (neat) ν 2095, 1602, 1496, 1448, 959 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.38 (2H, s), 7.11 (1H, d, *J*=16.6 Hz), 7.15 (1H, d, *J*=16.6 Hz), 7.22 (1H, d, *J*=7.5 Hz), 7.29 (1H, d, *J*=7.5 Hz), 7.36–7.40 (3H, m), 7.47 (1H, s), 7.50 (1H, d, *J*=8.0 Hz), 7.53 (2H, d, *J*=8.0 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ 54.7, 126.1, 126.4, 126.5, 127.2, 127.8, 128.0, 128.7, 129.1, 129.3, 135.8, 137.0, 137.9.

4.32. Triazole 5k

Triazole **5k** was obtained in 65% yield by the same procedure for **5d** using (*E*)-3-stilbenemethylazide: $[\alpha]_D^{27}$ −62.3 (*c* 0.960, $CHCl_3$); IR (neat) ν 1731, 1682, 1634, 1557, 1456, 754, 695 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.80 (3H, d, *J*=6.9 Hz, H14′ or H15′), 0.88 (3H, d *J*=6.6 Hz, H13), 0.92 (3H, d, *J*=6.3 Hz, H14′ or H15′), 1.39 (3H, d, *J*=6.9 Hz, H11′), 2.31 (1H, dqq, *J*=6.3, 6.9, 11.5 Hz, H13′), 2.48 (3H, s, H12′), 2.58 (1H, dq, *J*=6.6, 10.9 Hz, H4), 2.91 (1H, d, *J*=12.6 Hz, H2), 3.00 (1H, d, *J*=12.6 Hz, H2), 3.48 (1H, d, *J*=18.9 Hz, H8′), 4.17 (1H, d, *J*=11.5 Hz, H5′), 4.59 (1H, dd, *J*=9.2, 18.9 Hz, H8′), 5.05 (1H, s, H12), 5.10 (1H, s, H12), 5.29 (1H, dq, *J*=6.9, 9.2 Hz, H2′), 5.44 (1H, d, *J*=15.5 Hz, H9), 5.59 (1H, d, *J*=15.5 Hz, H9), 6.14 (1H, d, *J*=10.9 Hz, H5), 6.92 (1H, d, *J*=9.2 Hz, H1′), 7.06 (1H, d, *J*=16.6 Hz, vinyl), 7.11 (1H, d, 16.6 Hz, vinyl), 7.14 (1H, d, *J*=7.5 Hz, stilbene), 7.25–7.36 (4 H, m, stilbene), 7.44 (1H, s, stilbene), 7.47 (1H, d, *J*=8.0 Hz, stilbene), 7.50 (2H, d, *J*=7.5 Hz, stilbene), 7.82 (1H, s, H7), 7.99 (1H, d, *J*=9.2 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.4, 18.6, 18.9, 19.2, 25.7, 28.6, 40.9, 41.7, 43.2, 46.0, 54.3, 67.1, 71.2, 114.4, 122.6, 126.3, 126.6, 126.8, 127.2, 127.7, 127.9, 128.7, 129.5, 129.8, 134.8, 136.9, 138.3, 144.2, 146.7, 167.6, 167.7, 171.3, 174.2; HRMS (ESI) calcd for $C_{35}H_{42}N_6O_5Na$ 649.3114 ($M+Na^+$), found 649.3129.

4.33. Synthesis of 3-azobenzenemethylazide

To a solution of nitrosobenzene (214 mg, 2.00 mmol) and 3-hydroxymethylaniline (246 mg, 2.00 mmol) in toluene (15 mL) at room temperature was added three drops of acetic acid. After warming to 80 °C, additional nitrosobenzene (214 mg, 2.00 mmol) and acetic acid (seven drops) were added. After being stirred overnight at 80 °C, the reaction mixture was quenched with 1 M aqueous HCl, and extracted with EtOAc. The organic layer was washed with 3 M aqueous NaOH and H_2O, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=20/1 to 1/1) gave 3-hydroxymethylazobenzene (217 mg) in 51% yield: IR (neat) ν 3335, 1021, 796, 770, 693 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.82 (2H, s), 7.48–7.54 (5H, m), 7.86 (1H, d, *J*=7.5 Hz), 7.91–7.93 (3H, m); ^{13}C NMR (500 MHz, $CDCl_3$) δ 64.3, 120.5, 122.2, 122.7, 128.9, 129.0, 129.2, 130.9, 142.0, 152.3, 152.5.

To a solution of 3-hydroxymethylazobenzene (200 mg, 0.94 mmol) in CH_2Cl_2 (7.8 mL) at 0 °C were added Et_3N (0.196 mL, 1.41 mmol) and MsCl (0.088 mL, 1.13 mmol). In the course of the reaction, additional Et_3N (0.196 mL, 1.41 mmol) and MsCl (0.088 mL, 1.13 mmol) were added. After being stirred for 2 h at 0 °C, the mixture was diluted with H_2O, and extracted with EtOAc. The organic layer was washed with brine, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=1/1 to 1/2) gave methanesulfonyl methyl azobenzene (211 mg) in 77% yield: ^1H NMR (500 MHz, $CDCl_3$) δ 2.99 (3H, s), 5.35 (2H, s), 7.50–7.58 (5H, m), 7.93–7.97 (4H, m); ^{13}C NMR (500 MHz, $CDCl_3$) δ 38.1, 70.7, 122.5, 122.8, 123.9, 129.0, 129.6, 130.8, 131.3, 134.5, 152.2, 152.6.

To a mixture of 3-hydroxymethylazobenzene (200 mg, 0.69 mmol) and NaN_3 (224 mg, 3.45 mmol) at room temperature was added DMF (3.1 mL). After being stirred for 1 h at 50 °C, the mixture was diluted with H_2O, and extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4. Concentration and flash column chromatography (hexane/EtOAc=100/0 to 40/1) gave 3-azobenzenemethylazide (145 mg) in 89% yield: IR (neat) ν 2098, 692 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 4.47 (2H, s), 7.44–7.57 (5H, m), 7.88–7.94 (4H, m); ^{13}C NMR (500 MHz, $CDCl_3$) δ 54.4, 122.0, 122.9, 123.1, 128.7, 129.1, 129.5, 130.4, 131.2, 136.5, 152.4, 152.8.

4.34. Triazole 5l

Triazole **5l** was obtained in 88% yield by the same procedure for preparation of **5d** using 3-azobenzenemethylazide (*E*/*Z*=2.6:1): $[\alpha]_D^{27}$ −65.1 (*c* 0.610, $CHCl_3$); IR (neat) ν 3270, 2967, 1743, 1683, 1636, 1557, 1259, 756, 693 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 0.80 (3H, d, *J*=6.4 Hz, H13 or H14′ or H15′), 0.91 (3H, d, *J*=6.3 Hz, H13 or H14′ or H15′), 0.93 (3H, d, *J*=6.3 Hz, H13 or H14′ or H15′), 1.39 (3H, d, *J*=6.9 Hz, H11′), 2.31 (1H, m, H13′), 2.49 (3H, s, H12′), 2.57 (1H, m, H4), 2.89 (1H, d, *J*=13.2 Hz, H2), 3.00 (1H, d, *J*=13.2 Hz, H2), 3.49 (1H, d, *J*=18.9 Hz. H8′), 4.16 (1H, d, *J*=11.5 Hz, H5′), 4.59 (1H, dd, *J*=9.7, 18.9 Hz, H8′), 5.06 (1H, s, H12), 5.11 (1H, s, H12), 5.30 (1H, dq, *J*=6.9, 9.7 Hz, H2′), 5.56 (1H, d, *J*=15.5 Hz, H9), 5.69 (1H, d, *J*=15.5 Hz, H9), 6.18 (1H, d, *J*=11.5 Hz, H5), 6.67 (1H, d, *J*=9.7 Hz, H1′), 7.37 (1H, d, *J*=7.5 Hz, azobenzene), 7.49–7.54 (4H, m, azobenzene), 7.86–7.92 (5H, m, H7, azobenzene), 7.96 (1H, d, *J*=9.7 Hz, H7′); ^{13}C NMR (500 MHz, $CDCl_3$) δ 18.4, 18.6, 18.9, 19.2, 25.7, 28.7, 40.9, 42.0, 43.3, 46.0, 54.0, 67.1, 71.3, 114.7, 122.0, 122.7, 122.9, 123.7, 128.2, 128.8, 129.0, 129.1, 130.0, 130.4, 131.3, 152.4, 152.9, 167.5, 167.7, 171.3, 174.0; HRMS (ESI) calcd for $C_{33}H_{40}N_8O_5Na$ 651.3019 ($M+Na^+$), found 651.3018.

4.35. Triazole 7a

To a suspension of CuI (1.6 mg, 8.4 μmol) in THF (2 mL) was added *i*-Pr_2NEt (280 mL, 1.6 mmol) at room temperature. The mixture was sonicated for 10 s to give a cocktail of reagents. The flesh cocktail of CuI/*i*-Pr_2NEt in THF (120 μl) was added to a mixture of **6** (1.02 mg, 2.04 μmol) and *tert*-butylmethylazide (3.3 mg, 20.4 μmol) at room temperature. The reaction mixture was stirred at room temperature for 48 h. The reaction was quenched with 0.01 M aqueous $KHSO_4$. The aqueous layer was extracted four times with EtOAc. The combined organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtrated, and concentrated. The residue was purified with flash column chromatography (hexane/acetone=3/1 to 1/2) to give **7a** (0.76 mg) in 61% yield: $[\alpha]_D^{25.3}$ −179 (*c* 0.045, MeOH); IR (film) *ν* 3276, 2960, 1734, 1647, 1542, 1457, 1260, 750 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.87 (3H, d, *J*=6.9 Hz, H13), 0.95 (9H, s, *t*-Bu), 1.12 (9H, s, H11, H16, H17), 1.16 (3H, d, *J*=6.9 Hz, H11′), 1.56 (3H, s, H14), 1.79 (3H, s, H15), 2.15 (1H, dq, *J*=10.8, 6.9 Hz, H4), 2.78 (1H, d, *J*=13.2 Hz, H2), 2.84 (3H, s, H12′), 2.98 (1H, d, *J*=13.2 Hz, H2), 3.16 (1H, dd, *J*=15.5, 5.1 Hz, H13′), 3.33 (1H, dd, *J*=15.5, 10.3 Hz, H13′), 3.54 (1H, dd, *J*=18.9, 1.7 Hz, H8′), 4.05 (1H, d, *J*=13.7 Hz, CH_2), 4.11 (1H, d, *J*=13.7 Hz, CH_2), 4.64 (1H, dd, *J*=18.9, 9.7 Hz, H8′), 5.01 (1H, s, H12), 5.05 (1H, s, H12), 5.17 (1H, d, *J*=10.8 Hz, H5), 5.29 (1H, s, H9), 5.34 (1H, dq, *J*=9.7, 6.9 Hz, H2′), 5.43 (1H, dd, *J*=10.3, 5.1 Hz, H5′), 5.94 (1H, s, H7), 6.46 (1H, d, *J*=9.7 Hz, H1′), 7.30 (1H, s, triazole CH), 8.25 (1H, dd, *J*=9.7, 1.7 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 12.4, 17.7, 18.4, 18.9, 24.8, 27.5, 28.5, 30.9, 32.58, 32.61, 39.1, 41.4, 43.4, 46.4, 59.4, 61.7, 83.5, 113.8, 123.7, 129.1, 130.4, 137.3, 141.5, 142.2, 144.8, 167.53, 167.55, 170.9, 173.5; HRMS (ESI-TOF) calcd for $C_{33}H_{52}N_6O_5Na$ 635.3891 ($M+Na^+$), found 635.3894.

4.36. Triazole 7b

Triazole **7b** was obtained in 60% yield by the same procedure for preparation of **7a** using benzylazide: $[\alpha]_D^{25.5}$ −180 (*c* 0.055, MeOH); IR (film) *ν* 3279, 2960, 1734, 1640, 1543, 1458, 1260, 755 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.86 (3H, d, *J*=6.9 Hz, H13), 1.05 (3H, d, *J*=6.3 Hz, H11′), 1.12 (9H, s, H11, H16, H17), 1.55 (3H, s, H14), 1.78 (3H, s, H15), 2.14 (1H, dq, *J*=11.5, 6.9 Hz, H4), 2.77 (1H, d, *J*=13.2 Hz, H2), 2.81 (3H, s, H12′), 2.98 (1H, d, *J*=13.2 Hz, H2), 3.12 (1H, dd, *J*=16.1, 5.1 Hz, H13′), 3.30 (1H, dd, *J*=16.1, 10.3 Hz, H13′), 3.53 (1H, dd, *J*=18.9, 1.7 Hz, H8′), 4.63 (1H, dd, *J*=18.9, 9.7 Hz, H8′), 5.01 (1H, s, H12), 5.05 (1H, s, H12), 5.16 (1H, d, *J*=11.5 Hz, H5), 5.26 (1H, dq, *J*=9.7, 6.3 Hz, H2′), 5.29 (1H, s, H9), 5.39 (1H, dd, *J*=10.3, 5.1 Hz, H5′), 5.44 (1H, d, *J*=15.5 Hz, CH_2), 5.51 (1H, d, *J*=15.5 Hz, CH_2), 5.94 (1H, s, H7), 6.42 (1H, d, *J*=9.7 Hz, H1′), 7.22–7.24 (2H, m, Ph), 7.27 (1H, s, triazole CH), 7.34–7.37 (3H, m, Ph), 8.23 (1H, dd, *J*=9.7, 1.7 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 12.4, 17.7, 18.2, 18.9, 24.8, 28.5, 30.9, 32.6, 39.1, 41.4, 43.3, 46.4, 54.2, 59.4, 83.5, 113.8, 122.3, 128.0, 128.8, 129.1, 129.2, 130.4, 134.5, 137.3, 141.5, 143.3, 144.7, 167.45, 167.51, 170.9, 173.4; HRMS (ESI-TOF) calcd for $C_{35}H_{48}N_6O_5Na$ 655.3578 ($M+Na^+$), found 655.3578.

4.37. Triazole 7c

Triazole **7c** was obtained in 76% yield by the same procedure for preparation of **7a** using 2,4,6-trimethylbenzylazide: $[\alpha]_D^{26.9}$ −145 (*c* 0.08, MeOH); IR (film) *ν* 3272, 2960, 1736, 1640, 1549, 1462, 1321, 1259, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.86 (3H, d, *J*=6.8 Hz, H13), 1.10 (3H, d, *J*=6.9 Hz, H11′), 1.11 (9H, s, H11, H16, H17), 1.54 (3H, s, H14), 1.78 (3H, s, H15), 2.14 (1H, dq, *J*=10.9, 6.8 Hz, H4), 2.26 (6H, s, CH_3Ph), 2.29 (3H, s, CH_3Ph), 2.77 (1H, d, *J*=13.2 Hz, H2), 2.78 (3H, s, H12′), 2.98 (1H, d, *J*=13.2 Hz, H2), 3.06 (1H, dd, *J*=15.5, 4.6 Hz, H13′), 3.25 (1H, dd, *J*=15.5, 10.3 Hz, H13′), 3.52 (1H, dd, *J*=18.9, 1.7 Hz, H8′), 4.61 (1H, dd, *J*=18.9, 10.3 Hz, H8′), 5.00 (1H, s, H12), 5.05 (1H, s, H12), 5.16 (1H, d, *J*=10.9 Hz, H5), 5.28 (1H, s, H9), 5.29 (1H, dq, *J*=9.1, 6.9 Hz, H2′), 5.38 (1H, dd, *J*=10.3, 4.6 Hz, H5′), 5.47 (1H, d, *J*=14.9 Hz, CH_2Ph), 5.51 (1H, d, *J*=14.9 Hz, CH_2Ph), 5.93 (1H, s, H7), 6.45 (1H, d, *J*=9.1 Hz, H1′), 6.91 (2H, s, Ph), 6.95 (1H, s, triazole CH), 8.21 (1H, d, *J*=9.1 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 12.4, 17.7, 18.3, 18.9, 19.6, 21.0, 24.7, 28.4, 30.85, 30.90, 32.6, 39.1, 41.4, 43.3, 46.4, 48.2, 59.4, 83.5, 113.8, 121.4, 127.2, 129.1, 129.7, 130.4, 137.3, 137.7, 139.1, 141.5, 142.6, 144.8, 167.48, 167.54, 170.9, 173.4; HRMS (ESI-TOF) calcd for $C_{38}H_{54}N_6O_5Na$ 697.4048 ($M+Na^+$), found 697.4047.

4.38. Synthesis of (1-adamantyl)methylazide

To a solution of (1-adamantyl)methanol (300 mg, 1.80 mmol) in CH_2Cl_2 (18 mL) were added Et_3N (630 μl, 4.51 mmol) and MsCl (168 μl, 2.17 mmol) at room temperature. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous $NaHCO_3$. The aqueous layer was extracted three times with EtOAc. The combined organic layer was washed with saturated aqueous NH_4Cl, H_2O and brine, dried over Na_2SO_4, filtrated, and concentrated to give the crude mesylate.

The residue was dissolved in DMF (9 mL) and NaN_3 (579 mg, 8.91 mmol) was added to the solution. The reaction mixture was stirred at 50–130 °C for 24 h. The reaction was quenched with H_2O at room temperature. The aqueous layer was extracted three times with a mixture of hexane/EtOAc=4/1. The combined organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtrated, and concentrated. The residue was purified with flash column chromatography (hexane) to give (1-adamantyl)methylazide (269 mg) in 78% yield: IR (film) *ν* 2903, 2847, 2098, 1449, 1269 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 1.52 (6H, d, *J*=2.9 Hz, H18′, H22′, H23′), 1.63 (3H, d, *J*=12.6 Hz, H20′, H25′, H26′), 1.72 (3H, d, *J*=12.6 Hz, H20′, H25′, H26′), 1.99 (3H, d, *J*=2.9 Hz, H19′, H21′, H24′), 2.94 (2H, s, H16′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 28.1, 34.7, 36.8, 40.0, 64.3.

4.39. Triazole 7d

Triazole **7d** was obtained in 67% yield by the same procedure for preparation of **6** using (1-adamantyl)methylazide: $[\alpha]_D^{26.6}$ −147 (*c* 0.072, MeOH); IR (film) *ν* 3278, 2908, 1736, 1641, 1548, 1451, 1259, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 0.86 (3H, d, *J*=6.9 Hz, H13), 1.12 (9H, s, H11, H16, H17), 1.16 (3H, d, *J*=6.9 Hz, H11′), 1.46 (6H, m, H18′, H22′, H23′), 1.55 (3H, s, H14), 1.58 (3H, d, *J*=12.6 Hz, H20′, H25′, H26′), 1.70 (3H, d, *J*=12.6 Hz, H20′, H25′, H26′), 1.78 (3H, s, H15), 1.99 (3H, m, H19′, H22′, H24′) 2.14 (1H, dq, *J*=11.4, 6.9 Hz, H4), 2.77 (1H, d, *J*=13.2 Hz, H2), 2.84 (3H, s, H12′), 2.98 (1H, d, *J*=13.2 Hz, H2), 3.15 (1H, dd, *J*=15.5, 5.1 Hz, H13′), 3.34 (1H, dd, *J*=15.5, 10.3 Hz, H13′), 3.54 (1H, dd, *J*=18.3, 1.7 Hz, H8′), 3.94 (1H, d, *J*=13.8 Hz, H16′), 3.98 (1H, d, *J*=13.8 Hz, H16′), 4.65 (1H, dd, *J*=18.3, 9.7 Hz, H8′), 5.01 (1H, s, H12), 5.05 (1H, s, H12), 5.16 (1H, d, *J*=11.4 Hz, H5), 5.29 (1H, s, H9), 5.33 (1H, dq, *J*=9.2, 6.9 Hz, H2′), 5.41 (1H, dd, *J*=10.3, 5.1 Hz, H5′), 5.94 (1H, s, H7), 6.45 (1H, d, *J*=9.2 Hz, H1′), 7.25 (1H, s, H15′), 8.25 (1H, dd, *J*=9.7, 1.7 Hz, H7′); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 12.4, 17.7, 18.4, 18.9, 24.7, 28.1, 28.5, 30.9, 32.6, 34.1, 36.5, 39.1, 40.2, 41.4, 43.3, 46.4, 59.4, 62.2, 83.5, 113.8, 123.9, 129.1, 130.4, 137.3, 141.5, 142.1, 144.8, 167.5, 167.6, 170.8, 173.4; HRMS (ESI-TOF) calcd for $C_{39}H_{58}N_6O_5Na$ 713.4361 ($M+Na^+$), found 713.4366.

4.40. Ab initio calculation

The most stable geometry of **1** was taken from previous report.[9] The initial structure was built by the molecular mechanics simulation using a 1000-steps of Monte Carlo conformation search and TNCG energy minimization with MM3* (MacroModel 8.1). Four dihedral angle restraints from $^3J_{HH}$ coupling constants and sixteen distance restraints from NOESY spectrum were included into the simulation. The result was used as the starting structure for ab

initio calculation to obtain the most stable geometry of **1** at the Hartree-Fock (HF) level using the SPARTAN program (Spartan '08; Wavefunction Inc.: Irvine, CA) with internally stored 6-31G** basis set. Conformational analysis of **1** was carried out for the dihedral angle of H5–C5–C6=C7 with a step of 10° at HF/6-31G**. Electron density surface of **1** was calculated for the most stable geometry at DFT B3LYP/6-31G** level. The same procedure of ab initio calculation as **1** was applied to the C5-side chain analogue **5d**.

4.41. Neurotoxicity assay against Neuro 2a mouse neuroblastoma

Neuro 2a cells (ATCC, CCL131), obtained from Institute of Development Aging and Cancer (Tohoku university), were grown and maintained in 75 cm^2 tissue culture flasks (Falcon) at 37 °C in a humidified 5% CO_2 atmosphere using a growth medium, which was composed of RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum (Gibco), 2 mM L-glutamine, and 1% of antibiotic antifungal solution (10,000 U/mL penicillin G, 10 mg/mL streptomycin).

Cells were harvested in trypsin-EDTA solution (0.5%–0.2%, 2 min at 37 °C), and diluted to a concentration of 4×10^5 cell/mL with the growth medium. 100 μl of the cell suspension was inoculated into each well of a 96-well microplate (Falcon) and mixed with 100 μl of a solution of antillatoxin analogue to give a range of final concentrations between 10^{-4} and 10^{-9} M. The solutions of antillatoxin analogues were prepared from 100 μM DMSO stock solutions by sequential dilution with the growth media containing veratridine (40 μM), a site 2-specific sodium channel activator, ouabain (20 μM), a blocker of the Na^+/K^+ ATPase, and DMSO (2 v/v %). Three replicate samples were prepared for each antillatoxin analogues. After incubation for 20 h at 37 °C, cells were treated with 50 μl of 100 μM/3 mM PMS/XTT-containing growth medium, followed by further incubation for 4 h. Absorbance at 490 nm was measured on the microplate reader Model 550 (Bio Rad). The EC_{50} values were calculated using Prism v. 4.0 (GraphPad).

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.05.012.

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33. Because the length of the C5-side chain of antillatoxin had significant effect on toxicity (e.g., **1** vs **45**), we designed and synthesized **5b**–**l**, in which the long R groups were attached to the mono-substituted triazole analogue **5a**.
34. SPARTAN '08; Wavefunction: Irvine, CA.
35. Conformation analyses were carried out with respect to the H5–C5–C6–C7 dihedral angles. Global minima were found at 0°and 170° for antillatoxin and **5d**, respectively. Local minima were found at 170° (2.12 kJ/mol) and 0° (10.54 kJ/mol) for antillatoxin and **5d**, respectively.