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Synthesis and Biological Activity of Kalkitoxin and its Analogues

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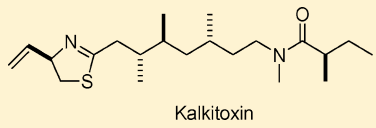
Supporting Information

ABSTRACT: Total syntheses of kalkitoxin, isolated from the Caribbean *Lyngbya majuscula*, and its analogues, 3-*epi*-, 7-*epi*-, 8-*epi*-, 10-*epi*-, 10-*nor*-, and 16-*nor*-kalkitoxin, were achieved via oxazolidinone-based diastereoselective 1,4-addition reaction of a methyl group and efficient $TiCl_4$-mediated thiazoline ring formation as the key steps. The biological activities of synthetic kalkitoxin and its analogues were evaluated with brine shrimp.



Kalkitoxin

INTRODUCTION

Kalkitoxin (**1**) was isolated from the cyanobacterium *Lyngbya majuscula* in the Caribbean Sea by Gerwick and co-workers in 2000.[1] It is reported that **1** possesses some interesting biological activities. For example, **1** shows strong ichthyotoxic activity toward the common goldfish (*Carassius auratus*, LC_{50} = 700 nM) and brine shrimp (*Artemia salina*, LC_{50} = 170 nM).[1] Also, **1** exhibits strong neurotoxicity (LC_{50} = 3.86 nM) in cerebellar granule neurons (CGN) as an inhibitor of *N*-methyl-D-aspartate (NMDA) receptor antagonists[3] and is a highly potent blocker of the voltage-dependent sodium channel in mouse neuro-2a cells (EC_{50} = 1 nM).[1,4] As a structural feature, **1** has five asymmetric centers: four methyl groups and a thiazoline ring containing a vinyl group. Due to its interesting biological activities as well as its intriguing structure, two total syntheses have been reported to date. The first total synthesis of **1** was reported by the Shioiri's group using Hruby's diastereoselective 1,4-addition reaction[5] of methyl cuprate controlled by Evans chiral oxazolidinone auxiliary[6] for construction of the stereogenic center at the C7 position and thiazoline ring formation via an oxazoline ring as the key steps.[1,2] In the course of the synthesis, the structure of **1** including absolute and relative configurations was unambiguously determined through the synthesis of its stereoisomers (kalkitoxin, 2′-*epi*-kalkitoxin, di-3,2′-*epi*-kalkitoxin, di-10,2′-*epi*-kalkitoxin, *ent*-kalkitoxin, 3-*epi*-*ent*-kalkitoxin, and di-3,8-*epi*-*ent*-kalkitoxin) based on the forecast with 1D and 2D NMR spectra of natural kalkitoxin.[1,2] They also reported LC_{50} values of the synthetic kalkitoxins toward brine shrimp (550 nM for 2′-*epi*-kalkitoxin, 1700 nM for di-3,2′-*epi*-kalkitoxin, 1100 nM for di-10,2′-*epi*-kalkitoxin, 9300 nM for *ent*-kalkitoxin, inactive for 3-*epi*-*ent*-kalkitoxin, and inactive for di-3,8-*epi*-*ent*-kalkitoxin). The second synthesis was achieved by White's group.[7] The key reaction in the synthesis was a diastereoselective 1,4-addition reaction of an alkyl cuprate to install the stereochemistry at C10 followed by alkylation of the resultant enolate with MeI (3.6:1 diastereomeric ratio) to construct the contiguous stereochemistry at C7 and C8 in a one-pot reaction.[8] This group also conducted a clonogenic assay using HCT-116 cells with **1** and its two intermediates, thiol amide and benzyl-protected thiol amide, and their IC_{50} values were found to be 1.0, 190, and 400 ng/mL, respectively. We have been attracted by the intriguing biological activities and structure–activity relationships of **1** and its congeners, such as diastereomers and demethylated kalkitoxins (*nor*-kalkitoxins). With the synthesis of various analogues in mind, a flexible synthetic route to easily change each stereogenic center was required. In the present paper, we describe the highly stereoselective synthesis of **1** and its analogues, 3-*epi*-kalkitoxin (**2**), 7-*epi*-kalkitoxin (**3**), 8-*epi*-kalkitoxin (**4**), 10-*epi*- kalkitoxin (**5**), 10-*nor*-kalkitoxin (**6**), and 16-*nor*-kalkitoxin (**7**), via the Hruby method with excellent stereochemical control of both the C7 and C10 stereogenic centers and a highly efficient thiazoline ring formation from trityl-protected thiol amide with $TiCl_4$ as the key steps (Figure 1). The biological activities, i.e., LC_{50} values, of the synthetic kalkitoxins were also evaluated with brine shrimp.

RESULTS AND DISCUSSION

The retrosynthetic analysis of **1** was designed to overcome the challenges for the synthesis of some isomers and is described in Scheme 1. We envisioned that **1** would be accessed from the amide **8** with a *S*-trityl group by highly effective $TiCl_4$-mediated thiazoline ring formation via removal of the trityl group followed by dehydration. The amide **8** would be synthesized by coupling between the carboxylic acid **9** and the amine **10** derived from L-cysteine with the stereochemical center set at C3. The stereogenic center at C7 of **9** would be installed by the application of the Hruby method to the α,β-unsaturated imide **11**, obtained through coupling between the known carboxylic acid **12** and the amine **13**. The stereochemistry at C10 of **13** was constructed in a similar manner, employing the α,β-unsaturated imide **14** prepared from the commercially available ester **15**. To access the epimers, we would employ either the Evans chiral oxazolidinone auxiliary with the opposite

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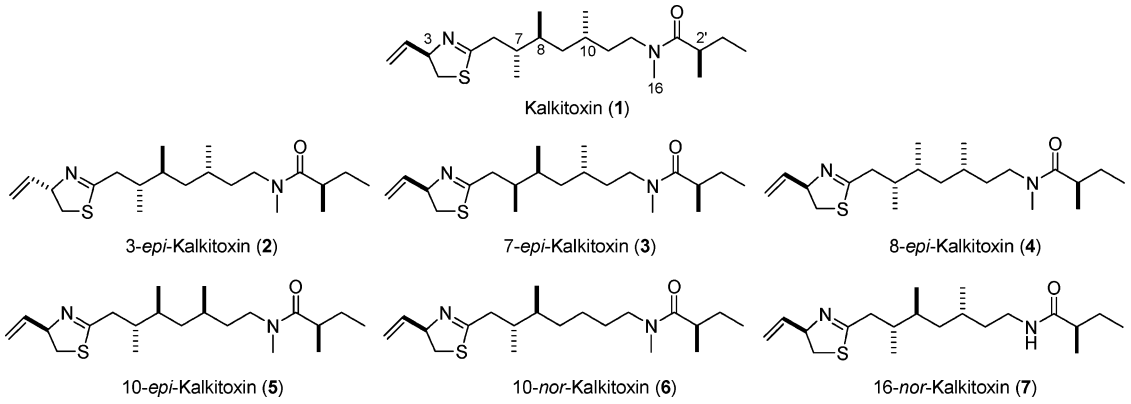
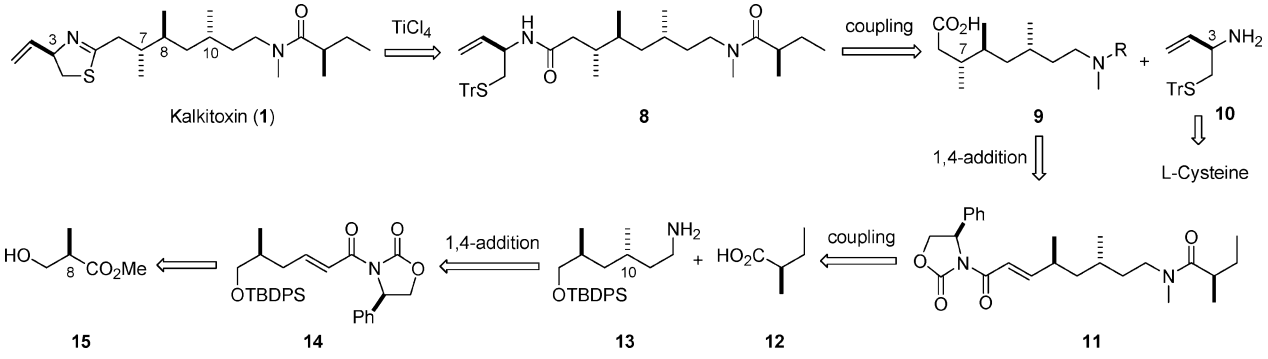


Figure 1. Kalkitoxin and its congeners.

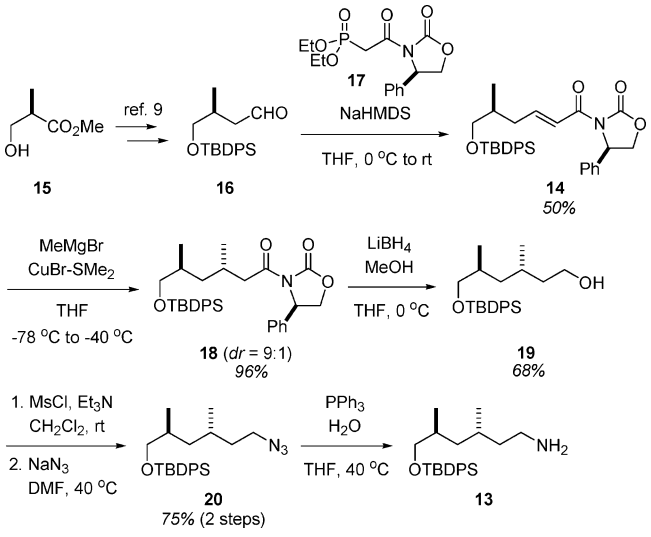
Scheme 1



stereochemistry or enantiomers of the building blocks (*ent*-**15** or D-cysteine).

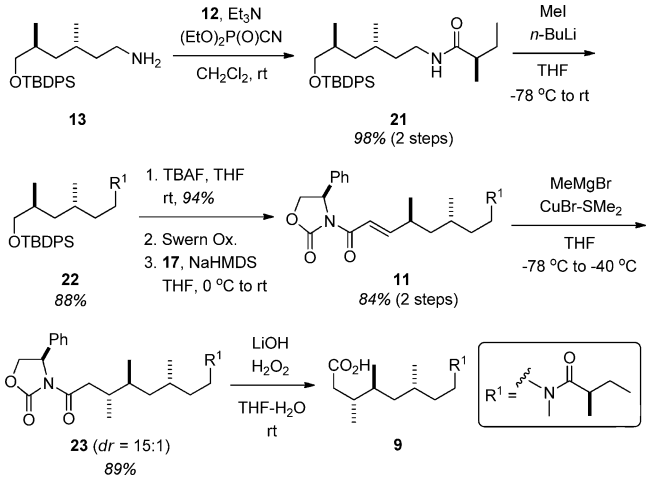
The synthesis of **1** started with the preparation of the α,β-unsaturated imide **14** according to a known procedure through the Horner–Wadsworth–Emmons (HWE) reaction of the aldehyde **16**,[9] derived from the commercially available ester **15**, with the phosphonate **17**[10] and NaHMDS (Scheme 2).

Scheme 2



1,4-Addition of **14** with methyl cuprate generated from CuBr·Me_2S and MeMgBr gave the imide **18** as a 9:1 mixture of diastereomers.[8,11,12] Reductive removal[13] of the chiral auxiliary from **18** with $LiBH_4$ gave the alcohol **19**, which was converted into the amine **13** by the following sequence of reactions: (1) mesylation of the primary alcohol with MsCl and Et_3N; (2) S_N2 reaction with NaN_3;[14] and (3) Staudinger reaction with PPh_3.[15] Coupling between **13** and the known carboxylic acid **12**[16] using $(EtO)_2P(O)CN$ and Et_3N under Shioiri's conditions[2,17] afforded the amide **21** in excellent yield (Scheme 3).

Scheme 3



After methylation of **21** with MeI and *n*-BuLi to give **22**,[18] the second 1,4-addition precursor **11** was synthesized in 3 steps: (1) deprotection of the TBDPS group with TBAF; (2) Swern oxidation; and (3) HWE reaction with **17** and NaHMDS. 1,4-Addition of **11** with methyl cuprate prepared from CuBr·Me_2S

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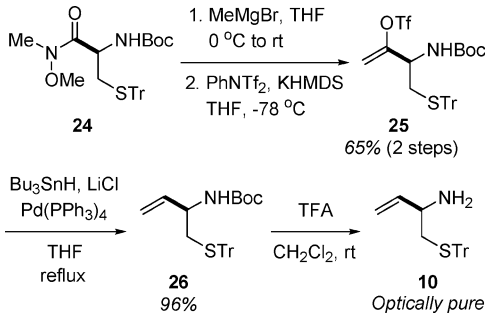
and MeMgBr occurred smoothly with 15:1 stereoselectivity,[12,19] and hydrolysis[20] of the resultant imide **23** with LiOH and H_2O_2 gave the carboxylic acid **9**.

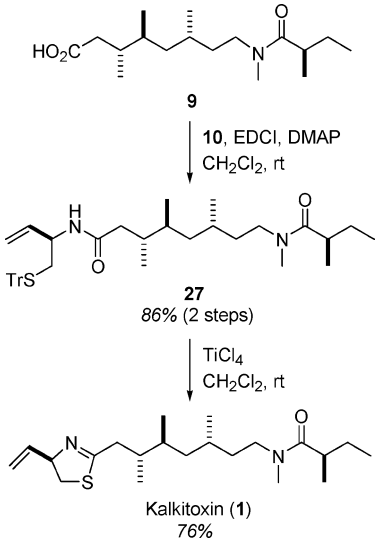
With the carboxylic acid **9** in hand, we then turned our attention to the synthesis of the amine **10** employing the Weinreb amide **24**, which was produced from L-cysteine following a known synthetic scheme.[21] First, we attempted Wittig olefination of the aldehyde obtained by reduction of **24** with $LiAlH_4$. However, partial racemization was observed under the strong basic conditions required for generation of $Ph_3P{=}CH_2$.[22] To avoid the racemization, a novel olefination procedure using **24** was developed. After addition of MeMgBr to **24**, conversion of the resultant methyl ketone into the enol triflate **25**[23] with $PhNTf_2$ and KHMDS, followed by successive reduction of **25** with Bu_3SnH and LiCl catalyzed by Pd(0),[24] successfully provided the olefin **26** in good yield (Scheme 4).

Scheme 4



Removal of the Boc group with TFA[25] yielded the amine **10** in enantiomerically pure form.[26] Condensation between **9** and **10** with EDCI and DMAP gave the amide **27** in excellent yield (Scheme 5). The deprotection of the trityl group of **27**

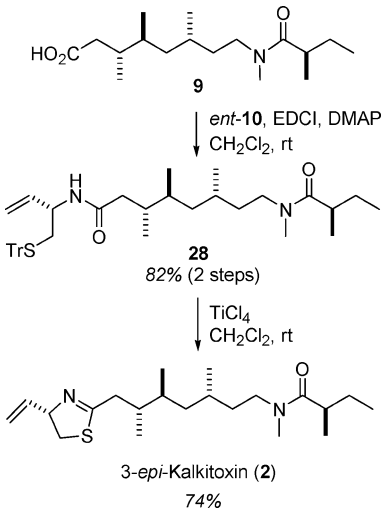
Scheme 5



proceeded efficiently accompanied by thiazoline ring formation in the presence of $TiCl_4$[27] to give **1** in high yield. All spectroscopic data of synthetic **1** were identical with those of natural **1**.[1,2]

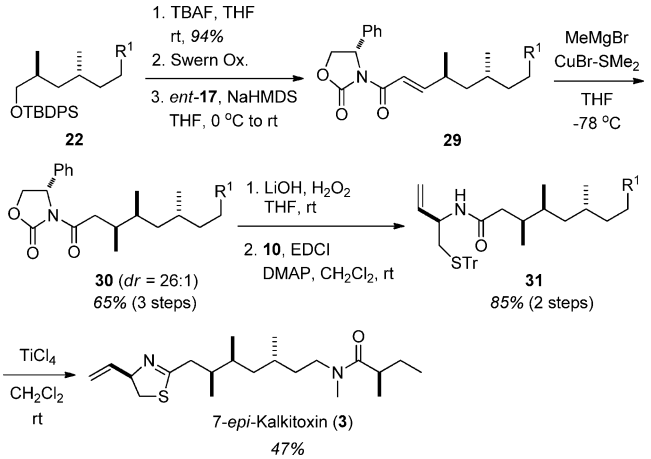
We then began the synthesis of the other kalkitoxins **2**–**7** based on the synthetic method developed for the total synthesis of **1** by changing the stereochemistry of the oxazolidinone auxiliaries or building blocks. First, 3-*epi*-kalkitoxin (**2**) was synthesized as shown in Scheme 6. To access **2**, the amine

Scheme 6



ent-**10** was prepared from D-cysteine following the same synthetic procedure as that for the preparation of **10** from L-cysteine (Scheme 4). The condensation reaction between **9** and *ent*-**10** with EDCI and DMAP gave the amide **28**, which was subjected to $TiCl_4$-promoted thiazoline ring formation to afford **2**.

Scheme 7



Scheme 7 illustrates the synthesis of 7-*epi*-kalkitoxin (**3**). HWE reaction of the aldehyde synthesized from **22** with the phosphonate *ent*-**17**[10] and NaHMDS gave the α,β-unsaturated imide **29**. 1,4-Addition of methyl cuprate to **29** took place with 26:1 stereoselectivity.[12] The imide **30** so obtained was transformed to **3** by the same reaction sequence as that employed in the total synthesis of **1**: (1) hydrolysis of imide with LiOH and H_2O_2; (2) condensation with the amine **10**, employing EDCI and DMAP; and (3) thiazoline ring formation induced by $TiCl_4$.

We next synthesized 8-*epi*-kalkitoxin (**4**). The synthesis commenced with the aldehyde *ent*-**16** prepared from the commercially

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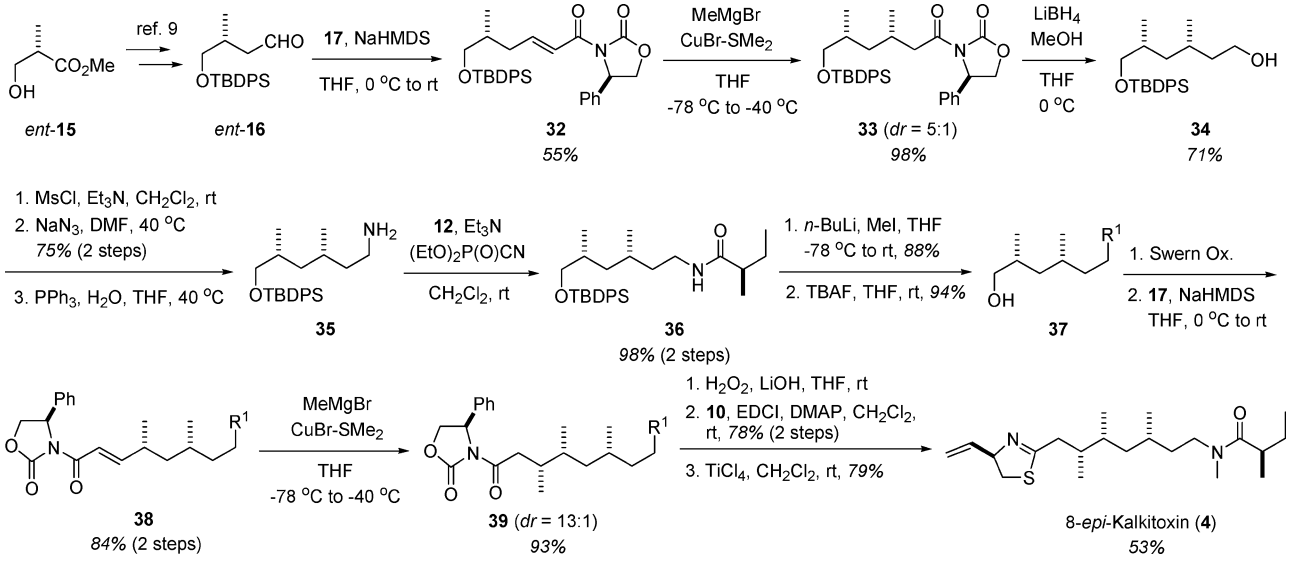
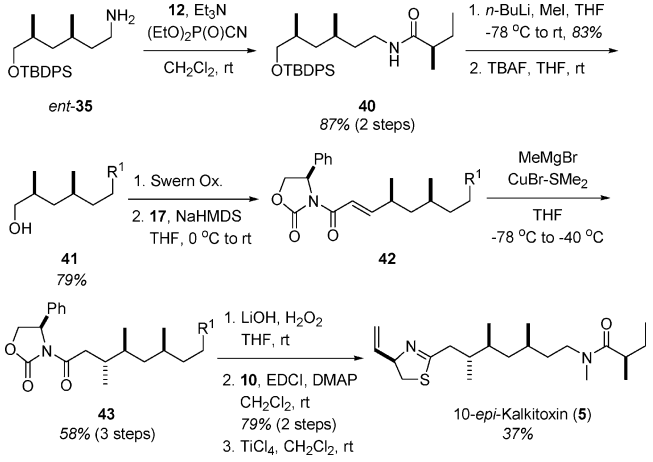
Scheme 8



available ester *ent*-**15** (Scheme 8). HWE reaction of *ent*-**16** with **17** afforded the α,β-unsaturated imide **32**. Installation of the methyl group via 1,4-addition was stereocontrolled to furnish the imide **33** with 5:1 selectivity,[12] which led to the amine **35** through the same sequence of reactions as for the preparation of **13** from **18** (Scheme 2). The amine **35** so obtained was converted to **4** via construction of C7 stereochemistry[12] using the same synthetic protocol as for the total synthesis of **1**.

The synthesis of 10-*epi*-kalkitoxin (**5**) is shown in Scheme 9. The synthesis started from the amine *ent*-**35**, derived from **16**

Scheme 9



and *ent*-**17** through the same reaction sequence as for the preparation of **35** from *ent*-**16** and **17** (Scheme 8). The amine *ent*-**35** was successfully converted to **5** following the synthetic procedure for the elaboration of **1**.[28]

Synthesis of novel congeners 10-*nor*-kalkitoxin (**6**) and 16-*nor*-kalkitoxin (**7**) was also performed. The synthesis of **6** commenced with **16** (Scheme 10). Wittig reaction of **16** with $Ph_3P{=}CHCO_2Me$ produced the α,β-unsaturated ester **44**, which was transformed into the alcohol **46** through hydrogenation of the olefin using Pd−C as catalyst and reduction of the ester moiety with DIBAL. The alcohol **46** was then converted to **6** according to the synthetic scheme developed for the total synthesis of **1**.[28] As shown in Scheme 11, **7** was elaborated from **21** using the same synthetic method as for the total synthesis of **1** without methylation of the amide moiety of **21**.[28]

With seven kalkitoxins in hand, we examined their biological activities. The biological activities were evaluated as LC_{50} (median lethal concentration) values against brine shrimp. The results are shown in Table 1. Our synthetic kalkitoxin (**1**) exhibited an LC_{50} value (0.18 μM) that corresponded closely to that of the natural kalkitoxin (0.17 μM) and the synthetic kalkitoxin (0.17 μM) previously synthesized by Shioiri. LC_{50} values for unnatural *epi*-kalkitoxins, 3-*epi*-kalkitoxin (**2**), 7-*epi*-kalkitoxin (**3**), 8-*epi*-kalkitoxin (**4**), and 10-*epi*-kalkitoxin (**5**) were 15, 3.6, 1.7, and 0.62 μM, respectively. These unnatural analogues were 3.3−80 times less potent than the natural kalkitoxin. The *nor*-kalkitoxins, 10-*nor*-kalkitoxin (**6**) and 16-*nor*-kalkitoxin (**7**), were also less active than kalkitoxin (LC_{50} 1.8 μM for **6**, and inactive for **7**). In addition, Shioiri reported an LC_{50} value (0.55 μM) for 2′-*epi*-kalkitoxin similar to those for the other *epi*-kalkitoxins. Clearly, all of the methyl groups within kalkitoxin and the stereochemistry of the five chiral centers is important for the biological activities of kalkitoxins. Furthermore, the *N*-methyl group was found to be essential.

■ CONCLUSION

In summary, the total synthesis of kalkitoxin and its analogues was achieved through the oxazolidinone-based diastereoselective 1,4-addition reaction of a methyl group and highly efficient thiazoline ring formation mediated by $TiCl_4$ from trityl-protected thiol amide. The flexible synthetic route allowed the synthesis of *epi*- and *nor*-kalkitoxins by simply changing the stereochemistry of the chiral auxiliaries or building blocks. The biological activities of the synthetic kalkitoxins revealed that all of the methyl groups and the stereochemistry of the five chiral centers, as well as the *N*-methyl group, were vital for the toxicity of kalkitoxin.

■ EXPERIMENTAL SECTION

General Methods. The IR spectra were recorded using a NaCl cell or KBr board. The ^1H and ^{13}C NMR spectra were recorded at

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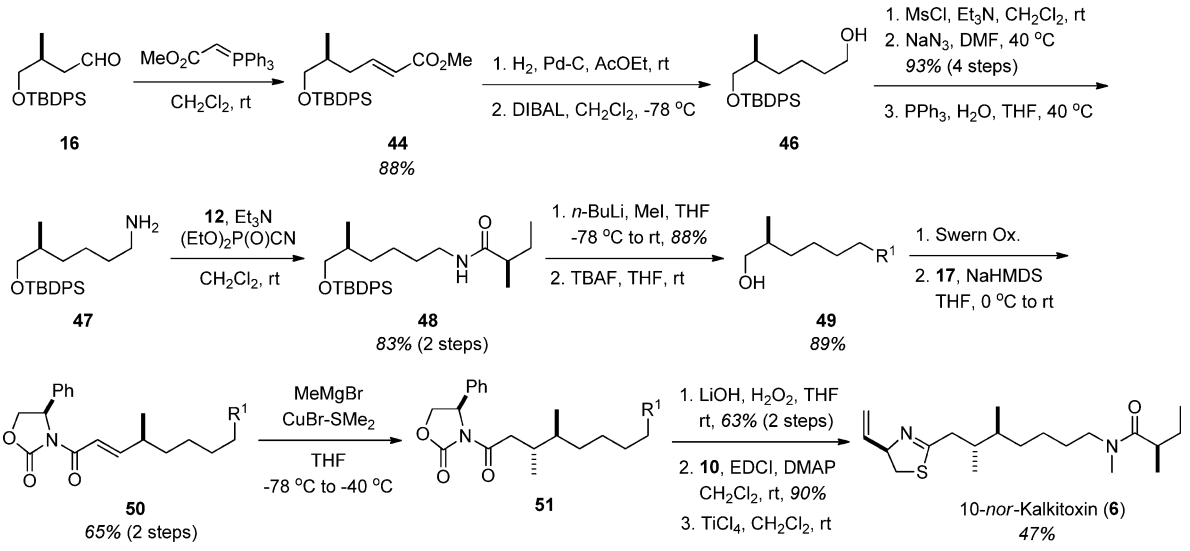
Scheme 10



Scheme 11

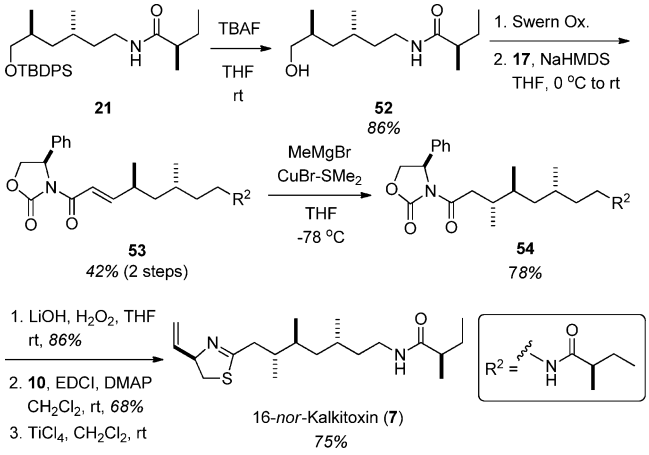


Table 1. Biological Activities of Synthetic Kalkitoxins

compound	LC_{50} (μM)
kalkitoxin (**1**)	0.18
3-*epi*-kalkitoxin (**2**)	15
7-*epi*-kalkitoxin (**3**)	3.6
8-*epi*-kalkitoxin (**4**)	1.7
10-*epi*-kalkitoxin (**5**)	0.62
10-*nor*-kalkitoxin (**6**)	1.8
16-*nor*-kalkitoxin (**7**)	inactive

400 and 100 MHz, respectively. Chemical shifts were reported in ppm downfield from the peak of Me_4Si used as the internal standard. Splitting patterns are designated as s, d, t, q, and m; these symbols indicate singlet, doublet, triplet, quartet, and multiplet, respectively. Tetrahydrofuran (THF) and ether were distilled from Na metal/benzophenone ketyl. Dichloromethane (CH_2Cl_2), triethylamine (Et_3N), iodomethane (MeI), and hexamethylphosphoramide (HMPA) were distilled from CaH_2. All commercially obtained reagents were used as received. Analytical and preparative TLC was carried out using precoated silica gel plates.

(*R*)-3-((*S*,*E*)-6-((*tert*-Butyldiphenylsilyl)oxy)-5-methylhex-2-enoyl)-4-phenyloxazolidin-2-one (14). To a solution of phosphonate **17** (68.9 mg, 0.202 mmol) in THF (0.5 mL) was added NaHMDS (0.99 M in THF, 0.204 mL) at 0 °C under Ar atmosphere. After 30 min, a solution of aldehyde **16** (57.1 mg, 0.168 mmol) in THF (0.5 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 2.5 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give α,β-unsaturated imide **14** (44.1 mg, 0.0836 mmol, 50%) as a colorless oil: $[\alpha]^{23}_D$ −21.9 (*c* 0.70, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, d, *J* = 6.8 Hz), 1.03 (9H, s), 1.84−1.87 (1H, m), 2.07−2.15 (1H, m), 2.49−2.54 (1H, m), 3.42−3.52 (2H, m), 4.28 (1H, dd, *J* = 3.9, 8.8 Hz), 4.70 (1H, t, *J* = 8.8 Hz), 5.48 (1H, dd, *J* = 3.9, 8.8 Hz), 7.03−7.09 (1H, m), 7.29−7.40 (12H, m), 7.61−7.63 (4H, m); ^{13}C NMR ($CDCl_3$) δ 164.3, 153.5, 150.8, 139.1, 135.5, 133.6, 129.5, 129.1, 128.6, 127.5, 125.9, 121.2, 69.9, 68.2, 57.8, 36.6, 35.5, 26.9, 19.3, 16.5; IR (neat) 3064, 2952, 2924, 2850, 1777, 1685, 1631, 1425, 1382, 1357, 1336, 1193, 1109, 1006, 897, 822, 704; FAB-MS *m*/*z* 550 (M^+ + Na); high-resolution FAB-MS *m*/*z* 550.2401 (M^+ + Na, calcd for $C_{32}H_{37}NO_4SiNa$ 550.2390).

(*R*)-3-((3*S*,5*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexanoyl)-4-phenyloxazolidin-2-one (18). To a solution of CuBr·Me_2S complex (890 mg, 4.33 mmol) in THF (14.4 mL) was added dropwise MeMgBr (0.84 M in THF, 8.65 mL, 7.26 mmol) at −78 °C under Ar atmosphere. After 10 min, a solution of α,β-unsaturated imide **14** (914 mg, 1.73 mmol) in THF (21.6 mL) was added to the mixture. The mixture was stirred for 30 min, warmed to −40 °C, stirred for 2 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give imide **18** (898 mg, 4.15 mmol, 96%) as a colorless oil: $[\alpha]^{23}_D$ −32.2 (*c* 0.80, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84 (6H, d, 6.6 Hz), 1.04 (9H, s), 1.24−1.28 (2H, m), 1.68−1.69 (1H, m), 2.07−2.15 (1H, m), 2.73−2.90 (2H, m), 3.34−3.43 (2H, m), 4.25 (1H, dd, *J* = 3.6, 8.8 Hz), 4.66 (1H, t, *J* = 8.8 Hz), 5.41 (1H, dd, *J* = 3.7, 8.6 Hz), 7.29−7.41 (11H, m), 7.63−7.64 (4H, m); ^{13}C NMR ($CDCl_3$) δ 171.9, 153.6, 139.1, 135.5, 134.0, 129.3, 129.1, 128.6, 127.5, 125.8, 69.8, 69.4, 57.6, 43.4, 40.0, 33.2, 27.1, 26.9, 19.4, 19.3, 16.3; IR (neat) 3064, 2952, 2924, 2850, 1780, 1705, 1458, 1425, 1383, 1321, 1193, 1109, 1004, 823, 703; FAB-MS *m*/*z* 544 (M^+ + H); high-resolution FAB-MS *m*/*z* 544.2858 (M^+ + H, calcd for $C_{33}H_{42}NO_4Si$ 544.2883).

(3*S*,5*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexan-1-ol (19). To a solution of imide **18** (898 mg, 1.65 mmol) in THF (8.0 mL) were added MeOH (133 μL, 3.30 mmol) and $LiBH_4$ (54.0 mg, 2.48 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 30 min, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column

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chromatography (AcOEt/hexane = 20:80) to give alcohol **19** (429 mg, 1.11 mmol, 68%) as a colorless oil: $[\alpha]^{23}_{D}$ −8.61 (*c* 2.31, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85 (3H, d, *J* = 6.6 Hz), 0.89 (3H, d, *J* = 6.8 Hz), 1.05 (9H, s), 1.20−1.28 (1H, m), 1.34−1.41 (1H, m), 1.48−1.60 (3H, m), 1.72−1.75 (1H, m), 3.40−3.50 (2H, m), 3.59−3.70 (2H, m), 7.35−7.43 (6H, m), 7.65−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 135.5, 134.0, 129.4, 127.5, 69.5, 61.1, 40.9, 40.8, 33.2, 27.0, 26.8, 19.5, 19.4, 16.7; IR (neat) 3336, 3064, 3044, 2922, 2852, 1468, 1425, 1386, 1359, 1188, 1109, 1007, 823, 740, 703; MS FAB-MS *m*/*z* 385 (M^+ + H); high-resolution FAB-MS *m*/*z* 385.2555 (M^+ + H, calcd for $C_{24}H_{37}O_2Si$ 385.2565).

(((2*S*,4*S*)-6-Azido-2,4-dimethylhexyl)oxy)(*tert*-butyl)-diphenylsilane (20). To a solution of alcohol **19** (363 mg, 0.945 mmol) in CH_2Cl_2 (9.5 mL) were added Et_3N (263 μL, 1.89 mmol) and MsCl (110 μL, 1.42 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 3 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mesylate was employed directly in the next reaction.

To a solution of crude mesylate in DMF (9.4 mL) was added NaN_3 (246 mg, 3.78 mmol) at room temperature. The mixture was heated to 40 °C for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give azide **20** (360 mg, 0.880 mmol, 93%) as a colorless oil: $[\alpha]^{23}_{D}$ −7.30 (*c* 1.23, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, *J* = 6.4 Hz), 0.93 (3H, d, *J* = 6.6 Hz), 1.05 (9H, s), 1.30−1.40 (3H, m), 1.54−1.61 (2H, m), 1.71−1.73 (1H, m), 3.20−3.29 (2H, m), 3.40−3.51 (2H, m), 7.35−7.44 (6H, m), 7.64−7.67 (4H, m); ^{13}C NMR ($CDCl_3$) δ 135.5, 134.0, 129.4, 127.5, 69.4, 49.5, 40.5, 36.6, 33.2, 27.7, 27.0, 19.4, 19.2, 16.6; IR (neat) 3064, 2924, 2852, 2090, 1461, 1425, 1387, 1359, 1188, 1110, 1008, 823, 738, 702; MS FAB-MS *m*/*z* 432 (M^+ + Na); high-resolution FAB-MS *m*/*z* 432.2440 (M^+ + Na, calcd for $C_{24}H_{35}N_3OSiNa$ 432.2447).

(*R*)-*N*-((3*S*,5*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexyl)-2-methylbutanamide (21). To a solution of azide **20** (550 mg, 1.34 mmol) in THF (13.4 mL) were added H_2O (120 μL) and PPh_3 (879 mg, 3.35 mmol) at room temperature. The mixture was heated to 40 °C for 12 h, cooled to room temperature, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude amine was employed directly in the next reaction.

To a solution of crude amine and caroboxlic acid **12** (164 mg, 1.61 mmol) in CH_2Cl_2 (13.4 mL) were added Et_3N (281 μL, 2.01 mmol) and $(EtO)_2POCN$ (244 μL, 1.61 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 2.5 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give amide **21** (595 mg, 1.27 mmol, 95%) as a colorless oil: $[\alpha]^{23}_{D}$ −11.6 (*c* 1.78, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.75−0.84 (9H, m), 0.98 (9H, s), 1.03 (3H, d, *J* = 6.8 Hz), 1.10−1.71 (8H, m), 1.90−2.00 (1H, m), 3.08−3.26 (2H, m), 3.31−3.42 (2H, m), 5.19 (1H, brs), 7.27−7.41 (6H, m), 7.55−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.1, 135.5, 134.0, 129.4, 127.5, 69.4, 43.3, 40.6, 37.7, 37.4, 33.2, 27.9, 27.4, 26.9, 19.4, 19.3, 17.6, 16.7, 12.0; IR (neat) 3290, 3064, 2954, 2924, 2852, 2090, 1640, 1549, 1459, 1425, 1385, 1263, 1234, 1109, 1008, 823, 739, 702; MS FAB-MS *m*/*z* 490 (M^+ + Na); high-resolution FAB-MS *m*/*z* 490.3104 (M^+ + Na, calcd for $C_{29}H_{45}NO_2SiNa$ 490.3117).

(*R*)-*N*-((3*S*,5*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexyl)-*N*,2-dimethylbutanamide (22). To a solution of amide **21** (321 mg, 0.685 mmol) in THF (6.8 mL) were added *n*-BuLi (2.73 M in hexanes, 451 μL) and MeI (171 μL, 2.74 mmol) at −78 °C under Ar atmosphere. The mixture was stirred for 10 min at −78 °C, warmed to room temperature, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give *N*-methyl amide **22** (310 mg, 0.644 mmol, 94%) as a colorless oil: $[\alpha]^{23}_{D}$ −11.5 (*c* 1.85, $CHCl_3$); ^{1}H NMR ($CDCl_3$) (rotamer) δ 0.82−0.92 (9H, m), 1.05, 1.06 (total 9H, each s), 1.05−1.11 (3H, m), 1.16−1.57 (4H, m), 1.58−1.80 (4H, m), 2.44−2.61 (1H, m), 2.90, 2.98 (total 3H, each s), 3.19−3.50 (4H, m), 7.32−7.44 (6H, m), 7.63−7.67 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.3, 176.0, 135.5, 134.0, 133.9, 129.5, 129.4, 127.5, 69.5, 69.4, 48.0, 46.2, 40.7, 40.6, 37.7, 37.4, 37.2, 37.0, 35.2, 33.7, 33.2, 28.1, 27.5, 27.1, 26.9, 19.4, 19.3, 17.9, 17.2, 16.6, 12.2, 12.1; IR (neat) 3064, 2954, 2924, 2852, 1642, 1462, 1425, 1408, 1259, 1191, 1110, 1008, 822, 740, 703; MS FAB-MS *m*/*z* 504 (M^+ + Na); high-resolution FAB-MS *m*/*z* 504.3262 (M^+ + Na, calcd for $C_{30}H_{47}NO_2SiNa$ 504.3274).

(*R*)-*N*-((3*S*,5*S*)-6-Hydroxy-3,5-dimethylhexyl)-*N*,2-dimethylbutanamide (S-1). To a solution of *N*-methyl amide **22** (1.30 g, 2.69 mmol) in THF (11.8 mL) was added TBAF (1 M in THF, 3.50 mL, 3.5 mmol) at room temperature. The mixture was stirred for 2 h, quenched with saturated NH_4Cl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 40:60) to give alcohol **S-1** (613 mg, 2.52 mmol, 94%) as a pale oil: $[\alpha]^{23}_{D}$ −33.5 (*c* 1.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) (rotamer) δ 0.85−0.94 (9H, m), 1.02 (3H, d, *J* = 6.3 Hz), 1.15−1.47 (6H, m), 1.51−1.73 (2H, m) 1.96 (1H, br), 2.49−2.52 (1H, m), 2.92, 3.01 (total 3H, each s), 3.24−3.52 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.4, 176.1, 68.8, 68.6, 48.1, 46.1, 40.6, 40.4, 37.4, 37.2, 37.0, 35.3, 35.1, 33.7, 33.1, 28.0, 27.9, 27.4, 27.1, 26.9, 19.5, 19.3, 17.8, 17.1, 16.4, 16.3, 12.2, 12.0; IR (neat) 3404, 2958, 2922, 2866, 1625, 1462, 1413, 1377, 1259, 1131, 1082; MS EI-MS *m*/*z* 243 (M^+); high-resolution EI-MS *m*/*z* 243.2200 (M^+, calcd for $C_{14}H_{29}NO_2$ 243.2198).

(*R*)-*N*-((3*S*,5*S*,*E*)-3,5-Dimethyl-8-oxo-8-((*R*)-2-oxo-4-phenyloxazolidin-3-yl)oct-6-en-1-yl)-*N*,2-dimethylbutanamide (11). To a solution of $(COCl)_2$ (28 μL, 0.318 mmol) in CH_2Cl_2 (0.2 mL) was added DMSO (30 μL, 0.424 mL) at −78 °C under Ar atmosphere. After 30 min, a solution of alcohol **S-1** (25.7 mg, 0.106 mmol) in CH_2Cl_2 (0.2 mL) was added to the mixture. After an additional 45 min, Et_3N (74 μL, 0.530 mmol) was added to the mixture. The mixture was warmed to room temperature, stirred for 1 h, quenched with saturated NH_4Cl, extracted with CH_2Cl_2 (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude aldehyde was employed directly in the next reaction.

To a solution of phosphonate **17** (54 mg, 0.159 mmol) in THF (0.5 mL) was added NaHMDS (0.99 M in THF, 160 μL, 0.159 mmol) at 0 °C under Ar atmosphere. After 30 min, a solution of aldehyde in THF (0.5 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give α,β-unsaturated imide **11** (40.2 mg, 0.0938 mmol, 89%). All spectral data were identical with those of the reported compound.[1]

(*R*)-*N*,2-Dimethyl-*N*-((3*S*,5*S*,6*R*)-3,5,6-trimethyl-8-oxo-8-((*R*)-2-oxo-4-phenyloxazolidin-3-yl)octyl)butanamide (23). To a solution of CuBr·Me_2S complex (298 mg, 1.45 mmol) in THF (2.9 mL) was added dropwise MeMgBr (0.96 M in THF, 2.03 mL, 1.95 mmol) at −78 °C under Ar atmosphere. After 20 min, a solution of α,β-unsaturated imide **11** (249 mg, 0.581 mmol) in THF (2.9 mL) was added to the mixture. The mixture was warmed to −40 °C, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give imide **23** (240 mg, 0.540 mmol, 93%). All spectral data were identical with those of the reported compound.[1]

(3*R*,4*S*,6*S*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4,6-trimethyloctanoic Acid (9). To a solution of imide **23** (216 mg, 0.486 mmol) in THF/H_2O (2.0 mL, 4:1) were added 30% H_2O_2 (278 μL) and 0.5 M LiOH (2.86 mL) at room temperature. The mixture was stirred for 12 h, 1 N NaOH was added, and the mixture was washed with AcOEt. The aqueous layer was acidified with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude carboxylic acid was employed directly in the next reaction.

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(*R*)-3-((*tert*-Butoxycarbonyl)amino)-4-(tritylthio)but-1-en-2-yl trifluoromethanesulfonate (25). To a solution of Weinreb amide **24** (681 mg, 1.34 mmol) in THF (6.0 mL) was added MeMgBr (1.08 M in THF, 6.23 mL, 6.72 mmol) at 0 °C under Ar atmosphere. The mixture was warmed to room temperature, stirred for 1 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude methyl ketone was employed directly in the next reaction.

To a solution of crude methyl ketone in THF (6.0 mL) was added $PhNTf_2$ (526 mg, 1.47 mmol) at room temperature under Ar atmosphere. The mixture was cooled to −78 °C, added KHMDS (0.5 M in toluene, 8.04 mL, 4.02 mmol), stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 3:97) to give enol triflate **25** (519 mg, 0.874 mmol, 65%) as a pale oil, which was immediately used for next reaction.

(*R*)-*tert*-Butyl (1-(Tritylthio)but-3-en-2-yl)carbamate (26). To a solution of LiCl (110 mg, 2.59 mmol) in degassed THF (0.5 mL) was added enol triflate **25** (515 mg, 0.868 mmol) in degassed THF, Bu_3SnH (257 μL, 0.954 mmol), and $Pd(PPh_3)_4$ (10.0 mg, 8.68 μmol) at room temperature under Ar atmosphere. The mixture was heated to reflux, stirred for 3 h, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 2:98) to give olefin **26** (371 mg, 0.833 mmol, 96%) as a colorless oil: $[\alpha]^{23}_D$ +10.2 (*c* 1.51, $CHCl_3$); ^1H NMR ($CDCl_3$) δ1.47 (9H, s), 2.32−2.49 (2H, m), 4.20 (1H, br), 4.66 (1H, d, *J* = 8.0 Hz), 5.10 (1H, d, *J* = 10.0 Hz), 5.12 (1H, d, *J* = 16.6 Hz), 5.67 (1H, ddd, *J* = 5.1, 10.0, 16.6 Hz), 7.24−7.50 (15H, m); ^{13}C NMR ($CDCl_3$) δ 144.4, 137.1, 129.4, 127.4, 127.1, 126.6, 123.5, 115.3, 77.2, 66.7, 37.0, 28.4; IR (neat) 3059, 1685, 1597, 1495, 1426, 1371, 1235, 1209, 1142, 1031, 948, 748, 699; MS EI-MS *m*/*z* 626 (M^+); high-resolution ESI-MS *m*/*z* 468.1968 (M^+ + Na, calcd for $C_{28}H_{31}NNaO_2S$ 468.1973).

(*R*)-1-(Tritylthio)but-3-en-2-amine (10). To a solution of olefin **26** (52.5 mg, 0.114 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (0.3 mL) at room temperature. The mixture was stirred for 30 min, concentrated *in vacuo* added 1 N NaOH, extracted with CH_2Cl_2 (×3), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude amine was employed directly in the next reaction.

(3*R*,4*S*,6*S*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4,6-trimethyl-*N*-((*R*)-1-(tritylthio)but-3-en-2-yl)octanamide (27). To a solution of crude amine and caroboxlic acid **9** (22.8 mg, 0.0761 mmol) in CH_2Cl_2 (0.7 mL) was added EDCI (22.0 mg, 0.114 mmol) and DMAP (1.0 mg, 8.1 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 5 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give amide **27** (40.8 mg, 0.0651 mmol, 86%) as a pale oil: $[\alpha]^{23}_D$ +12.2 (*c* 0.30, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.80−0.89 (12H, m), 1.08 (3H, t, *J* = 6.7 Hz), 1.23−1.50 (8H, m), 1.65−1.70 (1H, m), 1.90−1.95 (1H, m), 2.13−2.20 (1H, m), 2.37−2.39 (1H, m), 2.41−2.59 (2H, m), 2.90, 3.00 (total 3H, each s), 3.28−3.40 (2H, m), 4.54 (1H, br), 5.04−5.08 (2H, d, *J* = 14.9 Hz), 5.62−5.66 (1H, m), 7.20−7.41 (15H, m); ^{13}C NMR ($CDCl_3$) δ 176.3, 176.0, 171.9, 171.7, 144.4, 136.7, 129.4, 127.8, 126.7, 115.6, 66.7, 50.0, 48.1, 46.1, 40.8, 40.7, 37.4, 37.2, 36.6, 35.7, 35.3, 34.2, 33.7, 31.0, 29.7, 28.4, 27.4, 27.1, 19.2, 17.8, 17.2, 16.5, 16.3, 16.2, 16.1, 12.2, 12.0; IR (neat) 3290, 3052, 2956, 2920, 2868, 1624, 1535, 1487, 1443, 1413, 1377, 1296, 1081, 1033, 989, 922, 700; MS EI-MS *m*/*z* 626 (M^+); high-resolution EI-MS *m*/*z* 626.3906 (M^+, calcd for $C_{40}H_{54}N_2O_2S$ 626.3900).

(*R*)-*N*,2-Dimethyl-*N*-((3*S*,5*S*,6*R*)-3,5,6-trimethyl-7-((*R*)-4-vinyl-4,5-dihydrothiazol-2-yl)heptyl)butanamide (1). To a solution of amide **27** (23.4 mg, 0.0373 mmol) in CH_2Cl_2 (0.7 mL) was added $TiCl_4$ (12 μL, 0.112 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 12 h, quenched with saturated Rochelle salt, stirred for 1 h, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give kalkitoxin **1** (10.4 mg, 0.0283 mmol, 76%) as a colorless oil: $[\alpha]^{23}_D$ +11.6 (*c* 0.22, $CHCl_3$); ^1H NMR (benzene-d_6) δ 0.76 (3H, d, *J* = 6.8 Hz), 0.85 (3H, d, *J* = 6.1 Hz), 0.88 (3H, d, *J* = 7.5 Hz), 0.95(3H, d, *J* = 6.8 Hz), 1.02 (1H, m), 1.10 (1H,m), 1.10 (3H, d, *J* = 6.7 Hz), 1.24 (1H, m), 1.34 (1H, m), 1.38 (1H, m), 1.39 (1H, m), 1.54 (1H, m), 1.87 (1H, m), 2.05 (1H, m), 2.28 (1H, m), 2.31 (1H, m), 2.43 (3H, s), 2.55 (1H, m), 2.72 (1H, dd, *J* = 8.4, 10.7 Hz), 2.94 (1H, dd, *J* = 8.8, 10.5 Hz), 3.35 (2H, m), 4.75 (1H, dd, *J* = 7.5, 7.8 Hz), 5.01 (1H, d, *J* = 10.3 Hz), 5.24 (1H, ddd, *J* = 1.6, 1.6, 17.2 Hz), 5.85 (1H, ddd, *J* = 6.1, 10.3, 17.2 Hz); ^{13}C NMR (benzene-d_6) δ 176.1, 175.8, 170.7, 170.5, 139.2, 139.0, 116.2, 116.1, 80.2, 48.8, 47.0, 43.6, 41.4, 41.3, 39.9, 39.8, 39.6, 38.7, 38.6, 38.4, 37.0, 35.7, 35.6, 35.5, 34.6, 29.5, 29.1, 28.7, 20.6, 20.4, 19.5, 18.8, 17.6, 17.5, 13.7, 13.5; IR (neat) 2954, 2918, 2868, 1640, 1461, 1406, 1377, 1264, 1196, 1131, 1082, 1007, 924; MS FAB-MS *m*/*z* 367 (M^+ + H); high-resolution FAB-MS *m*/*z* 367.2767 (M^+ + H, calcd for $C_{21}H_{39}N_2OS$ 367.2783).

(3*R*,4*S*,6*S*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4,6-trimethyl-*N*-((*S*)-1-(tritylthio)but-3-en-2-yl)octanamide (28). To a solution of *ent*-**10** (36.1 mg, 0.105 mmol) and caroboxlic acid **9** (22.8 mg, 0.0761 mmol) in CH_2Cl_2 (0.7 mL) was added EDCI (20.0 mg, 0.105 mmol) and DMAP (1.0 mg, 8.1 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 5 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give amide **28** (36.2 mg, 0.0577 mmol, 82%) as a pale oil: $[\alpha]^{23}_D$ −23.5 (*c* 1.15, $CHCl_3$), ^1H NMR ($CDCl_3$) δ 0.67−0.98 (12H, m), 1.03 (3H, t, *J* = 6.9 Hz), 1.05−1.52 (8H, m), 1.53−1.60 (1H, m), 1.72−2.12 (2H, m), 2.28−2.31 (1H, m), 2.38−2.61 (2H, m), 2.84, 2.93 (total 3H, each s), 3.28−3.30 (2H, m), 4.47 (1H, s), 5.01 (2H, d, *J* = 12.2 Hz), 5.56−5.60 (1H, m), 7.11−7.34 (15H, m); ^{13}C NMR ($CDCl_3$) δ 176.4, 171.9, 144.4, 136.7, 129.4, 127.9, 126.7, 115.6, 66.7, 50.0, 48.1, 46.2, 42.2, 40.8, 40.6, 40.1, 37.4, 37.3, 36.6, 35.7, 35.4, 34.2, 33.7, 29.7, 28.3, 27.4, 27.1, 19.2, 17.8, 17.2, 16.6, 16.3, 14.5, 12.2, 12.1; IR (neat) 3288, 3052, 2956, 2918, 2868, 1625, 1535, 1488, 1457, 1443, 1413, 1378, 1294, 1081, 1033, 986, 920, 700; MS EI-MS *m*/*z* 626 (M^+); high-resolution EI-MS *m*/*z* 626.3906 (M^+, calcd for $C_{40}H_{54}N_2O_2S$ 626.3900).

(*R*)-*N*,2-Dimethyl-*N*-((3*S*,5*S*,6*R*)-3,5,6-trimethyl-7-((*S*)-4-vinyl-4,5-dihydrothiazol-2-yl)heptyl)butanamide (2). To a solution of amide **28** (10.2 mg, 0.0163 mmol) in CH_2Cl_2 (0.3 mL) was added $TiCl_4$ (5.4 μL, 0.0489 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 12 h, quenched with saturated Rochelle salt, stirred for 1 h, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give 3-*epi*-kalkitoxin **2** (4.4 mg, 0.0120 mmol, 74%) as a colorless oil: $[\alpha]^{23}_D$ −59.1 (*c* 0.425, $CHCl_3$), ^1H NMR (benzene-d_6) δ 0.45−1.53 (21H, m), 1.01−1.25 (1H, m), 1.53−1.60 (1H, m), 1.75 (1H, m), 1.97 (1H, m), 2.08 (1H, m), 2.12 (3H, s), 2.22 (1H, m), 2.40 (1H, dd, *J* = 10.7, 8.3 Hz), 2.63 (1H, dd, *J* = 10.7, 8.8 Hz), 3.04 (2H, m), 4.47 (1H, m), 4.70 (1H, d, *J* = 10.0 Hz), 4.94 (1H, d, *J* = 16.8 Hz), 5.53 (1H, ddd, *J* = 6.1, 10.0, 16.8); ^{13}C NMR (benzene-d_6) δ 176.1, 175.8, 170.7, 170.5, 139.2, 139.0, 116.2, 116.1, 80.2, 48.8, 47.1, 41.4, 41.3, 39.9, 39.6, 38.7, 38.3, 37.1, 35.8, 35.5, 34.8, 34.6, 34.3, 32.0, 31.4, 30.5, 29.5, 29.4, 28.7, 25.3, 24.5, 21.6, 20.5, 20.3, 19.5, 18.8, 17.6, 16.0, 13.7, 13.5; IR (neat) 2954, 2918, 2866, 1640, 1459, 1410, 1377, 1259, 1193, 1084, 1026, 921, 802, 701; MS EI-MS *m*/*z* 366 (M^+); high-resolution EI-MS *m*/*z* 366.2698 (M^+, calcd for $C_{21}H_{38}N_2OS$ 366.2705).

(*R*)-*N*,2-Dimethyl-*N*-((3*S*,5*S*,6*S*)-3,5,6-trimethyl-8-oxo-8-((*S*)-2-oxo-4-phenyloxazolidin-3-yl)octyl)butanamide (30). To a solution of $(COCl)_2$ (230 μL, 2.64 mmol) in CH_2Cl_2 (1.7 mL) was added DMSO (250 μL, 3.52 mL) at −78 °C under Ar atmosphere. After 15 min, a solution of alcohol **S-1** (214 mg, 0.881 mmol) in CH_2Cl_2 (1.7 mL) was added to the mixture. After an additional 45 min, Et_3N (616 μL, 4.41 mmol) was added to the mixture. The mixture was warmed to room temperature, stirred for 1 h, quenched with saturated NH_4Cl, extracted with CH_2Cl_2 (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude aldehyde was employed directly in the next reaction

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To a solution of phosphonate *ent*-**17** (451 mg, 1.32 mmol) in THF (4.4 mL) was added NaHMDS (0.99 M in THF, 1.33 mL, 1.32 mmol) at 0 °C under Ar atmosphere. After 30 min, a solution of crude aldehyde in THF (4.4 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude α,β-unsaturated imide **29** was employed directly in the next reaction.

To a solution of CuBr·Me_2S complex (452 mg, 2.20 mmol) in THF (4.4 mL) was added dropwise MeMgBr (0.96 M in THF, 3.07 mL, 2.95 mmol) at −78 °C under Ar atmosphere. After 20 min, a solution of crude α,β-unsaturated imide (249 mg, 0.581 mmol) in THF (4.4 mL) was added to the mixture. The mixture was warmed to −40 °C, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give imide **30** (255 mg, 0.573 mmol, 65%) as a colorless oil: $[\alpha]^{25}_D$ −55.5 (*c* 1.54, $CHCl_3$), ^{1}H NMR ($CDCl_3$), δ 0.63−0.97 (12H, m), 1.04 (3H, d, *J* = 6.9 Hz, 1 rotamer), 1.09 (3H, d, *J* = 6.9 Hz, 1 rotamer), 1.12−1.19 (2H, m), 1.24−1.75 (6H, m), 1.92−2.08 (1H, m), 2.50−2.62 (1H, m), 2.74−2.83 (2H, m), 2.85−2.95 (1H, m), 2.89, 2.97 (total 3H, each s), 3.14−3.47 (2H, m), 4.23−4.29 (1H, m), 4.66 (1H, t, *J* = 8.8 Hz), 5.38−5.47 (1H, m), 7.25−7.55 (5H, m) ; ^{13}C NMR ($CDCl_3$), 176.3, 176.0, 172.5, 172.4, 153.6, 139.1, 139.0, 129.1, 129.05, 129.02, 128.8, 128.6, 125.9, 125.85, 125.79, 125.77, 72.5, 69.8, 57.7, 56.3, 48.1, 48.0, 46.1, 45.8, 39.9, 39.8, 39.0, 38.3, 37.4, 37.24, 37.21, 37.18, 35.4, 35.2, 34.8, 34.7, 34.38, 34.35, 34.06, 34.04, 33.7, 33.3, 29.7, 28.2, 27.4, 27.1, 20.5, 18.9, 17.8, 17.2, 16.7, 16.4, 16.31, 16.27, 14.1, 12.2, 12.1; IR (neat) 2961, 2926, 2874, 1780, 1703, 1637, 1456, 1384, 1321, 1196, 1135, 1080, 1043, 963, 916, 762, 704; MS ESI-MS *m*/*z* 467 (M + Na^+); high-resolution ESI-MS *m*/*z* 467.2875 (M^+ + Na, calcd for $C_{26}H_{40}N_2O_4Na$ 467.2880)

(3*S*,4*S*,6*S*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4,6-trimethyl-*N*-((*R*)-1-(tritylthio)but-3-en-2-yl)octanamide (31). To a solution of imide **30** (255 mg, 0.574 mmol) in THF/H_2O (2.4 mL, 4: 1) was added 30% H_2O_2 (328 μL) and 0.5 M LiOH (3.4 mL) at room temperature. The mixture was stirred for 12 h, 1 N NaOH was added, and the mixture was washed with AcOEt. The aqueous layer was acidified with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude carboxylic acid was employed directly in the next reaction.

To a solution of amine **10** (47.5 mg, 0.138 mmol) and caroboxlic acid (27.4 mg, 0.0920 mmol) in CH_2Cl_2 (0.9 mL) were added EDCI (26.5 mg, 0.138 mmol) and DMAP (1.0 mg, 8.1 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 5 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give amide **31** (49.2 mg, 0.0577 mmol, 85%) as a pale oil: $[\alpha]^{26}_D$ +45.8 (*c* 0.70, $CHCl_3$), ^{1}H NMR ($CDCl_3$) δ 0.80−0.89 (12H, m), 1.08 (3H, t, *J* = 6.7 Hz), 1.23−1.50 (8H, m), 1.65−1.70 (1H, m), 1.90−1.95 (1H, m), 2.13−2.20 (1H, m), 2.37−2.39 (1H, m), 2.41−2.59 (2H, m), 2.90, 3.00 (total 3H, each s), 3.28−3.40 (2H, m), 4.54 (1H, s), 5.04−5.08 (2H, d, *J* = 14.9 Hz), 5.62−5.66 (1H, m), 7.20−7.41 (15H, m); ^{13}C NMR ($CDCl_3$) δ 176.3, 176.0, 171.8, 171.5, 144.5, 136.8, 136.7, 129.4, 127.9, 127.8, 126.7, 126.7, 126.0, 115.4, 66.7, 66.7, 56.4, 49.9, 49.9, 48.0, 46.2, 42.2, 42.2, 41.9, 37.4, 37.2, 36.9, 36.6, 35.3, 34.9, 34.9, 34.9, 33.9, 33.8, 33.7, 33.7, 29.7, 29,7, 28.2, 27.5, 27.2, 27.1, 19.5, 19.3, 17.8, 17.2, 14,6, 14.6, 14.4, 12.2, 12.1; IR (neat) 3288, 3052, 2956, 2919, 2868, 1625, 1535, 1489, 1457, 1443, 1413, 1378, 1294, 1081, 1033, 988, 922, 700; MS EI-MS *m*/*z* 626 (M^+); high-resolution EI-MS *m*/*z* 626.3906 (M^+, calcd for $C_{40}H_{54}N_2O_2S$ 626.3900).

(*R*)-*N*,2-Dimethyl-*N*-((3*S*,5*S*,6*S*)-3,5,6-trimethyl-7-((*R*)-4-vinyl-4,5-dihydrothiazol-2-yl)heptyl)butanamide (3). To a solution of amide **31** (49.2 mg, 0.0785 mmol) in CH_2Cl_2 (1.5 mL) was added $TiCl_4$ (25.3 μL, 0.230 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 12 h, quenched with saturated Rochelle salt, stirred for 1 h, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give 7-*epi*-kalkitoxin **3** (13.6 mg, 0.0371 mmol, 47%) as a colorless oil: $[\alpha]^{26}_D$ +17.7 (*c* 0.79, $CHCl_3$), ^{1}H NMR (benzene-d_6) δ 0.65−1.32 (21H, m), 1.46−1.53 (1H, m), 1.79−1.90 (1H, m), 1.98 (1H, m), 2.25 (1H, m), 2.31 (1H, m), 2.41 (3H, s), 2.48 (1H, m), 2.65 (1H, dd, *J* = 8.3, 15.6 Hz), 2.89 (1H, dd, *J* = 8.8, 15.6 Hz), 3.30 (2H, m), 4.73 (1H, m), 4.96 (1H, d, *J* = 10.5 Hz), 5.19 (1H, d, *J* = 17.3 Hz), 5.78 (1H, ddd, *J* = 6.3, 10.5, 17.3 Hz); ^{13}C NMR (benzene-d_6) δ 175.2, 174.9, 169.7, 169.4, 138.2, 138.1, 115.3, 115.2, 79.3, 71.7, 56.1, 47.8, 46.0, 42.5, 40.0, 38.9, 37.6, 37.0, 36.8, 36.4, 35.5, 34.7, 33.7, 33.6, 33.6, 28.4, 28.1, 27.7, 19.8, 19.6, 18.4, 17.7, 14.8, 14.7, 14.5, 14.5, 12.6, 12.4; IR (neat) 2954, 2918, 2868, 1640, 1460, 1410, 1377, 1260, 1193, 1084, 1026, 921, 802, 701; MS FAB-MS *m*/*z* FAB-MS *m*/*z* 367 (M^+ + H); high-resolution FAB-MS *m*/*z* 367.2802 (M^+ + H, calcd for $C_{21}H_{39}N_2OS$ 367.2783).

(*R*)-3-((*R*,*E*)-6-((*tert*-Butyldiphenylsilyl)oxy)-5-methylhex-2-enoyl)-4-phenyloxazolidin-2-one (32). To a solution of phosphonate **17** (104.0 mg, 0.304 mmol) in THF (1.3 mL) was added NaHMDS (1.06 M in THF, 0.287 mL) at 0 °C under Ar atmosphere. After 30 min, a solution of *ent*-**16** (93.9 mg, 0.276 mmol) in THF (1.3 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 2.5 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give α,β-unsaturated imide **32** (80.9 mg, 0.153 mmol, 55%) as a colorless oil: $[\alpha]^{23}_D$ −12.2 (*c* 1.78, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, d, 6.8 Hz), 1.05 (9H, s), 1.83−1.88 (1H, m), 2.07−2.16 (1H, m), 2.47−2.53 (1H, m), 3.43−3.53 (2H, m), 4.28 (1H, dd, *J* = 3.9, 8.8 Hz), 4.70 (1H, t, *J* = 8.8 Hz), 5.48 (1H, dd, *J* = 3.9, 8.8 Hz), 7.03−7.11 (1H, m), 7.30−7.43 (12H, m), 7.62−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 164.3, 153.5, 150.7, 139.1, 135.5, 133.6, 129.5, 129.1, 128.6, 127.6, 125.9, 121.2, 69.9, 68.3, 57.7, 36.6, 35.5, 26.9, 19.3, 16.5; IR (neat) 3064, 2950, 2924, 2852, 1776, 1685, 1630, 1425, 1382, 1358, 1336, 1193, 1109, 1005, 896, 823, 703; FAB-MS *m*/*z* 550 (M^+ + Na); high-resolution FAB-MS *m*/*z* 550.2374 (M^+ + Na, calcd for $C_{32}H_{37}NO_4SiNa$ 550.2390).

(*R*)-3-((3*S*,5*R*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexanoyl)-4-phenyloxazolidin-2-one (33). To a solution of CuBr·Me_2S complex (1.12 g, 5.45 mmol) in THF (30 mL) was added dropwise MeMgBr (0.96 M in THF, 9.54 mL, 9.16 mmol) at −78 °C under Ar atmosphere. After 10 min, a solution of α,β-unsaturated imide **32** (1.15 g, 2.18 mmol) in THF (30 mL) was added to the mixture. The mixture was stirred for 30 min, warmed to −40 °C, stirred for 2 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give imide **33** (1.16 mg, 2.13 mmol, 98%) as a colorless oil: $[\alpha]^{23}_D$ +19.4 (*c* 1.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, d, 6.6 Hz), 0.91 (3H, d, 6.6 Hz), 1.04 (9H, s), 1.31−1.35 (2H, m), 1.70−1.71 (1H, m), 2.00−2.08 (1H, m), 2.77−2.81 (2H, m), 3.34 (1H, dd, *J* = 6.8, 10.0 Hz), 3.49 (1H, dd, *J* = 5.4, 10.0 Hz), 4.24 (1H, dd, *J* = 3.9, 9.0 Hz), 4.65 (1H, t, *J* = 8.8 Hz), 5.39 (1H, dd, *J* = 3.9, 8.8 Hz), 7.29−7.42 (11H, m), 7.62−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 172.1, 153.5, 139.1, 135.5, 133.9, 129.4, 129.0, 128.5, 127.5, 125.8, 69.8, 68.8, 57.6, 42.4, 40.9, 33.2, 27.4, 26.9, 20.4, 19.4, 17.7; IR (neat) 3064, 2950, 2922, 2850, 1780, 1704, 1456, 1425, 1382, 1322, 1195, 1109, 1003, 823, 703; EI-MS *m*/*z* 544 (M^+ + H); high-resolution ESI-MS *m*/*z* 544.2863 (M^+ + H, calcd for $C_{33}H_{42}NO_4Si$ 544.2883).

(3*S*,5*R*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexan-1-ol (34). To a solution of imide **33** (964 mg, 1.78 mmol) in THF (18 mL) were added MeOH (162 μL, 3.56 mmol) and $LiBH_4$ (155 mg, 7.12 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 30 min, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give alcohol **34** (487 mg, 1.26 mmol, 71%) as a colorless oil: $[\alpha]^{23}_D$ +5.95 (*c* 1.60, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (3H, d, *J* = 6.6 Hz), 0.93 (3H, d,

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J = 6.6 Hz), 1.04 (9H, s), 1.30−1.42 (3H, m), 1.55−1.66 (2H, m), 1.72−1.76 (1H, m), 2.50 (1H, br), 3.42 (1H, dd, J = 6.4, 9.8 Hz), 3.50 (1H, dd, J = 5.1, 9.8 Hz), 3.58−3.70 (2H, m), 7.34−7.42 (6H, m), 7.62−7.67 (4H, m); ^{13}C NMR ($CDCl_3$) δ 135.5, 133.9, 129.4, 127.5, 68.8, 61.1, 41.2, 39.8, 33.2, 27.0, 26.9, 20.3, 19.4, 17.7; IR (neat) 3344, 3064, 3044, 2950, 2924, 2852, 1468, 1425, 1387, 1359, 1110, 1008, 823, 739, 702; MS FAB-MS m/z 385 (M^+ + H); high-resolution FAB-MS m/z 385.2573 (M^+ + H, calcd for $C_{24}H_{37}O_2Si$ 385.2563).

(((2*R*,4*S*)-6-Azido-2,4-dimethylhexyl)oxy)(*tert*-butyl)-diphenylsilane (S-2). To a solution of alcohol **34** (487 mg, 1.27 mmol) in CH_2Cl_2 (12 mL) were added Et_3N (353 μL, 2.54 mmol) and MsCl (148 μL, 1.91 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 3 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mesylate was employed directly in the next reaction.

To a solution of crude mesylate in DMF (12 mL) was added NaN_3 (330 mg, 5.08 mmol) at room temperature. The mixture was heated to 40 °C for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give azide **S-2** (392 mg, 0.957 mmol, 75%) as a colorless oil: $[\alpha]^{23}_D$ +11.4 (c 1.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (3H, d, J = 6.4 Hz), 0.93 (3H, d, J = 6.6 Hz), 1.05 (9H, s), 1.30−1.41 (3H, m), 1.54−1.62 (2H, m), 1.71−1.73 (1H, m), 3.20−3.29 (2H, m), 3.42−3.51 (2H, m), 7.35−7.44 (6H, m), 7.64−7.67 (4H, m); ^{13}C NMR ($CDCl_3$) δ 135.5, 133.9, 129.5, 127.5, 68.7, 49.4, 41.0, 35.5, 33.2, 27.9, 27.0, 20.1, 19.4, 17.7; IR (neat) 3064, 2950, 2924, 2852, 2090, 1460, 1425, 1387, 1359, 1189, 1110, 1007, 822, 739, 702; MS ESI-MS m/z 432 (M^+ + Na); high-resolution ESI-MS m/z 432.2436 (M^+ + Na, calcd for $C_{24}H_{35}N_3OSiNa$ 432.2547).

(*R*)-*N*-((3*S*,5*R*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexyl)-2-methylbutanamide (36). To a solution of azide **S-2** (392 mg, 0.957 mmol) in THF (9.5 mL) were added H_2O (86 μL) and PPh_3 (656 mg, 2.39 mmol) at room temperature. The mixture was heated to 40 °C for 12 h, cooled to room temperature, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude amine was employed directly in the next reaction.

To a solution of crude amine and caroboxlic acid **12** (117 mg, 1.15 mmol) in CH_2Cl_2 (9.5 mL) were added Et_3N (201 μL, 1.44 mmol) and $(EtO)_2POCN$ (174 μL, 1.15 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 2.5 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give amide **36** (438 mg, 0.937 mmol, 98%) as a colorless oil: $[\alpha]^{23}_D$ +0.211 (c 1.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.76−0.93 (9H, m), 1.05 (9H, s), 1.10 (3H, d, J = 6.8 Hz), 1.18−1.75 (8H, m), 1.95−2.02 (1H, m), 3.18−3.31 (2H, m), 3.38−3.52 (2H, m), 5.27 (1H, brs), 7.35−7.41 (6H, m), 7.64−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.1, 135.6, 135.5, 133.9, 129.4, 127.5, 68.9, 43.3, 41.2, 37.4, 36.7, 33.1, 28.2, 27.4, 26.9, 20.1, 19.4, 17.6, 12.0; IR (neat) 3290, 3064, 2954, 2924, 2852, 2090, 1640, 1549, 1459, 1425, 1385, 1263, 1234, 1109, 1008, 823, 739, 702; MS FAB-MS m/z 490 (M^+ + Na); high-resolution FAB-MS m/z 490.3104 (M^+ + Na, calcd for $C_{29}H_{45}NO_2SiNa$ 490.3117).

(*R*)-*N*-((3*S*,5*R*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexyl)-*N*,2-dimethylbutanamide (S-3). To a solution of amide **36** (438 mg, 0.957 mmol) in THF (9.5 mL) were added *n*-BuLi (2.69 M in hexanes, 625 μL, 1.68 mmol) and MeI (233 μL, 3.74 mmol) at −78 °C under Ar atmosphere. The mixture was stirred for 10 min at −78 °C, warmed to room temperature, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give *N*-methyl amide **S-3** (394 mg, 0.818 mmol, 88%) as a colorless oil: $[\alpha]^{23}_D$ −4.29 (c 1.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84−0.93 (9H, m), 1.05, 1.06 (total 9H, each s), 1.13−1.58 (9H, m), 1.65−1.74 (2H, m), 2.54 (1H, m), 2.90, 2.97 (total 3H, each s), 3.26−3.52 (4H, m), 7.35−7.41 (6H, m), 7.64−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.2, 175.9, 135.5, 133.9, 133.8, 129.5, 129.4, 127.5, 68.9, 68.6, 48.0, 46.1, 41.2, 41.0, 37.4, 37.2, 35.9, 35.2, 34.0, 33.7, 33.2, 28.3, 27.4, 27.1, 26.9, 20.3, 19.3, 17.9, 17.7, 17.2, 12.2, 12.1; IR (neat) 3064, 3044, 2952, 2924, 2852, 1643, 1462, 1425, 1408, 1259, 1191, 1110, 1087, 1008, 823, 739, 703; MS ESI-MS m/z 504 (M^+ + Na); high-resolution ESI-MS m/z 504.3254 (M^+ + Na, calcd for $C_{30}H_{47}NO_2SiNa$ 504.3274).

(*R*)-*N*-((3*S*,5*R*)-6-Hydroxy-3,5-dimethylhexyl)-*N*,2-dimethylbutanamide (37). To a solution of *N*-methyl amide **S-3** (278 mg, 0.577 mmol) in THF (2.9 mL) was added TBAF (1 M in THF, 0.75 mL, 0.75 mmol) at room temperature. The mixture was stirred for 2 h, quenched with saturated NH_4Cl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 40:60) to give alcohol **37** (132 mg, 0.542 mmol, 94%) as a colorless oil: $[\alpha]^{23}_D$ −4.29 (c 1.41, $CHCl_3$); ^{1} H NMR ($CDCl_3$) δ 0.87−0.95 (6H, m), 1.08 (3H, d, J = 6.6 Hz, 1 rotamer), 1.10 (3H, d, J = 6.6 Hz, 1 rotamer), 1.37−1.46 (4H, m), 1.67−1.73 (2H, m), 1.82 (3H, m), 2.58 (1H, m), 2.92, 3.01 (total 3H, each s), 3.24−3.52 (4H, m); ^{13} C NMR ($CDCl_3$) δ 176.7, 176.3, 67.8, 66.6, 48.0, 45.4, 40.9, 39.8, 37.5, 37.2, 35.7, 35.1, 34.5, 33.7, 33.0, 32.9, 28.2, 27.6, 27.4, 27.0, 20.7, 20.3, 18.0, 17.8, 17.3, 17.0, 12.2, 12.1; IR (neat) 3404, 2956, 2922, 2866, 1624, 1461, 1412, 1375, 1297, 1256, 1195, 1133, 1082, 1044, 985; MS EI-MS m/z 243 (M^+); high-resolution EI-MS m/z 243.2195 (M^+, calcd for $C_{14}H_{29}NO_2$ 243.2198).

(*R*)-*N*-((3*S*,5*R*,*E*)-3,5-Dimethyl-8-oxo-8-((*R*)-2-oxo-4-phenyloxazolidin-3-yl)oct-6-en-1-yl)-*N*,2-dimethylbutanamide (38). To a solution of $(COCl)_2$ (64 μL, 0.738 mmol) in CH_2Cl_2 (0.5 mL) was added DMSO (70 μL, 0.984 mL) at −78 °C under Ar atmosphere. After 15 min, a solution of alcohol **37** (59.8 mg, 0.246 mmol) in CH_2Cl_2 (0.5 mL) was added to the mixture. After an additional 45 min, Et_3N (172 μL, 1.23 mmol) was added to the mixture. The mixture was warmed to room temperature, stirred for 1 h, quenched with saturated NH_4Cl, extracted with CH_2Cl_2 (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude aldehyde was employed directly in the next reaction.

To a solution of phosphonate **17** (126 mg, 0.369 mmol) in THF (1.2 mL) was added NaHMDS (0.99 M in THF, 373 μL, 0.369 mmol) at 0 °C under Ar atmosphere. After 30 min, a solution of crude aldehyde in THF (1.2 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give α,β-unsaturated imide **38** (88.1 mg, 0.205 mmol, 84%) as an inseparable mixture. $[\alpha]^{25}_D$ −83.3 (c 0.54, $CHCl_3$), ^{1}H NMR ($CDCl_3$), δ 0.84−0.93 (6H, m), 0.99−1.09 (6H, m), 1.18−1.50 (4H, m), 1.59−1.72 (3H, m), 2.50−2.58 (2H, m), 2.90, 2.98 (total 3H, each s), 3.25 (1H, dt, J = 5.1, 10.3 Hz), 3.34 (1H, t, J = 10.3 Hz), 4.29 (1H, m), 4.70 (1H, dt, J = 2.2, 8.6 Hz), 5.48 (1H, dd, J = 3.4, 8.6 Hz), 6.89, 6.92 (total 1H, dd, J = 15.1 Hz), 7.20−7.26 (1H, m), 7.31−7.38 (5H, m); ^{13}C NMR ($CDCl_3$), 176.3, 176.0, 164.5, 156.8, 156.1, 153.5, 139.0, 138.9, 129.09, 129.07, 128.63, 128.55, 125.92, 125.91, 119.0, 118.6, 72.5, 69.97, 69.93, 57.79, 57.77, 47.9, 46.1, 43.6, 37.4, 37.2, 36.6, 35.3, 34.7, 34.6, 34.5, 33.7, 29.7, 28.65, 28.60, 27.4, 27.1, 22.7, 20.5, 20.3, 19.3, 19.2, 18.9, 17.8, 17.2, 14.2, 12.2, 12.0; IR (neat) 2962, 2925, 2872, 1779, 1687, 1636, 1456, 1381, 1362, 1326, 1197, 1102, 1044, 762, 706; MS ESI-MS m/z 451 (M + Na^+); high-resolution ESI-MS m/z 451.2566 (M^+, calcd for $C_{25}H_{36}N_2O_4Na$ 451.2567)

(*R*)-*N*,2-Dimethyl-*N*-((3*S*,5*R*,6*R*)-3,5,6-trimethyl-8-oxo-8-((*R*)-2-oxo-4-phenyloxazolidin-3-yl)octyl)butanamide (39). To a solution of CuBr·Me_2S complex (298 mg, 1.45 mmol) in THF (2.9 mL) was added dropwise MeMgBr (0.96 M in THF, 2.03 mL, 1.95 mmol) at −78 °C under Ar atmosphere. After 20 min, a solution of α,β-unsaturated imide **38** (249 mg, 0.581 mmol) in THF (2.9 mL) was added to the mixture. The mixture was warmed to −40 °C, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography

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(AcOEt/hexane = 20:80) to give imide **39** (240 mg, 0.540 mmol, 93%) as an inseparable mixture.

(3*R*,4*R*,6*S*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4,6-trimethyl-*N*-((*R*)-1-(tritylthio)but-3-en-2-yl)octanamide (S-4). To a solution of imide **39** (59.4 mg, 0.134 mmol) in THF/H_2O (0.5 mL, 4: 1) were added 30% H_2O_2 (77 μL) and 0.5 M LiOH (0.7 mL) at room temperature. The mixture was stirred for 12 h, 1 N NaOH was added, and the mixture was washed with AcOEt. The aqueous layer was acidified with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude carboxylic acid was employed directly in the next reaction.

To a solution of amine **10** (17.6 mg, 0.0510 mmol) and caroboxlic acid (10.1 mg, 0.0340 mmol) in CH_2Cl_2 (1.0 mL) were added EDCI (10.0 mg, 0.051 mmol) and DMAP (0.4 mg, 3.0 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 5 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give amide **S-4** (16.6 mg, 0.0265 mmol, 78%) as a pale oil: $[\alpha]^{23}_D$ +12.1 (*c* 1.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.77−0.95 (12H, m), 1.07 (3H, t, *J* = 7.1 Hz), 1.25−1.69 (9H, m), 1.92 (1H, m), 2.13−2.20 (1H, m), 2.37−2.39 (1H, m), 2.46−2.56 (2H, m), 2.91, 2.99 (total 3H, each s), 3.27−3.37 (2H, m), 4.54 (1H, s), 5.06 (2H, d, *J* = 14.9 Hz), 5.61−5.69 (1H, m), 7.20−7.56 (15H, m); ^{13}C NMR ($CDCl_3$) δ 176.3, 176.0, 171.8, 171.5, 144.5, 136.8, 129.5, 127.9, 126.7, 115.6, 66.8, 50.0, 48.1, 46.0, 42.5, 42.2, 37.4, 37.3, 36.6, 36.0, 35.3, 34.0, 33.9, 33.7, 33.2, 31.0, 29.7, 28.2, 27.5, 27.1, 20.4, 20.1, 17.9, 17.2, 15.3, 15.1, 14.2, 13.9, 12.3, 12.1; IR (neat) 3284, 3052, 2954, 2918, 2866, 1625, 1535, 1487, 1442, 1413, 1378, 1296, 1081, 1034, 988, 923, 700; MS EI-MS *m*/*z* 626 (M^+); high-resolution EI-MS *m*/*z* 626.3902 (M^+, calcd for $C_{40}H_{54}N_2O_2S$ 626.3900).

(*R*)-*N*,2-Dimethyl-*N*-((3*S*,5*R*,6*R*)-3,5,6-trimethyl-7-((*R*)-4-vinyl-4,5-dihydrothiazol-2-yl)heptyl)butanamide (4). To a solution of amide **S-4** (16.6 mg, 0.0265 mmol) in CH_2Cl_2 (0.5 mL) was added $TiCl_4$ (8.6 μL, 0.0780 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 12 h, quenched with saturated Rochelle salt, stirred for 1 h, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give kalkitoxin **4** (5.1 mg, 0.0139 mmol, 53%) as a colorless oil: $[\alpha]^{23}_D$ +25.3 (*c* 0.40, $CHCl_3$); ^{1}H NMR (benzene-d_6) δ 0.80−1.03 (15H, m), 1.17 (1H, d, *J* = 6.8 Hz), 1.26 (1H, d, *J* = 6.6 Hz), 1.39−1.74 (6H, m), 1.97 (1H, m), 2.18 (1H, m), 2.37 (1H, m), 2.52 (3H, s), 2.81 (1H, m), 2.90 (1H, m), 3.03 (1H, m), 3.45 (2H, m), 4.85 (1H, m), 5.09 (1H, d, *J* = 10.2 Hz), 5.32 (1H, d, *J* = 17.1 Hz), 5.94 (1H, m); ^{13}C NMR (benzene-d_6) δ 176.1, 175.8, 170.5, 170.3, 139.2, 139.0, 116.2, 116.1, 80.3, 48.8, 47.0, 43.7, 41.3, 41.1, 40.3, 39.9, 39.2, 37.7, 37.3, 36.3, 35.7, 35.1, 34.6, 34.3, 32.0, 31.4, 30.5, 29.7, 29.4, 29.1, 25.3, 24.5, 21.6, 21.4, 19.5, 18.8, 16.0, 15.8, 15.5, 13.7, 13.5; IR (neat) 2954, 2918, 2866, 1639, 1461, 1408, 1378, 1262, 1195, 1081, 923; MS FAB-MS *m*/*z* 367 (M^+ + H); high-resolution FAB-MS *m*/*z* 367.2773 (M^+ + H, calcd for $C_{21}H_{39}N_2OS$ 367.2783).

(*R*)-*N*-((3*R*,5*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexyl)-2-methylbutanamide (40). To a solution of *ent*-**35** (166 mg, 0.404 mmol) in THF (4.0 mL) were added H_2O (36 μL) and PPh_3 (265 mg, 1.01 mmol) at room temperature. The mixture was heated to 40 °C for 12 h, cooled to room temperature, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude amine was employed directly in the next reaction.

To a solution of crude amine and caroboxlic acid **12** (50.0 mg, 0.485 mmol) in CH_2Cl_2 (2.0 mL) were added Et_3N (85.0 μL, 0.606 mmol) and $(EtO)_2POCN$ (74.0 μL, 0.485 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 2.5 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give amide **40** (169 mg, 0.351 mmol, 87%) as a colorless oil: $[\alpha]^{22}_D$ −7.66 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.83−0.94 (9H, m), 1.05 (9H, s), 1.10 (3H, d, *J* = 6.8 Hz), 1.16−1.26 (1H, m), 1.32−1.53 (4H, m), 1.57−1.76 (2H, m), 1.98−2.03 (1H, m), 3.22−3.25 (2H, m), 3.40 (1H, dd, *J* = 9.7, 6.6 Hz), 3.50 (1H, dd, *J* = 9.7, 5.3 Hz), 5.40 (1H, brs), 7.34−7.43 (6H, m), 7.64−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.1, 135.5, 133.9, 129.5, 127.5, 68.8, 43.3, 41.1, 37.3, 36.7, 33.1, 28.1, 27.4, 26.9, 20.1, 19.3, 17.7, 16.8, 12.0. IR (neat) 3290, 3064, 2954, 2924, 2852, 2090, 1640, 1549, 1459, 1425, 1385, 1263, 1234, 1109, 1008, 823, 739, 702; MS FAB-MS *m*/*z* 468 (M^+ + H); high-resolution FAB-MS *m*/*z* 468.3289 (M^+ + H, calcd for $C_{29}H_{46}NO_2Si$ 468.3298).

(*R*)-*N*-((3*R*,5*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-3,5-dimethylhexyl)-*N*,2-dimethylbutanamide (S-5). To a solution of amide **40** (511 mg, 1.09 mmol) in THF (10.9 mL) were added *n*-BuLi (2.63 M in hexanes, 745 μL, 1.96 mmol) and MeI (271 μL, 4.36 mmol) at −78 °C under Ar atmosphere. The mixture was stirred for 10 min at −78 °C, warmed to room temperature, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give *N*-methyl amide **S-5** (437 mg, 0.908 mmol, 83%) as a colorless oil: $[\alpha]^{22}_D$ −13.4 (*c* 0.969, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84−0.93 (9H, m), 1.05, 1.06 (total 9H, each s), 1.07−1.09 (total 3H), 1.19−1.58 (5H, m), 1.62−1.76 (2H, m), 2.50−2.58 (1H, m), 2.90, 2.96 (total 3H, each s), 3.23−3.30 (2H, m), 3.39−3.52 (2H, m), 7.35−7.41 (6H, m), 7.64−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.3, 175.9, 135.49, 135.47, 133.9, 133.8, 129.4, 129.3, 127.5, 127.5, 68.9, 68.6, 48.0, 46.0, 41.2, 41.0, 37.4, 37.2, 36.0, 35.2, 33.9, 33.7, 33.2, 28.4, 27.5, 27.1, 26.9, 20.2, 19.4, 17.8, 17.7, 17.1, 12.2, 12.0; IR (neat) 3064, 3044, 2952, 2924, 2852, 1643, 1462, 1425, 1408, 1259, 1191, 1110, 1087, 1008, 823, 739, 703; MS FAB-MS *m*/*z* 482 (M^+ + H); high-resolution FAB-MS *m*/*z* 482.3451 (M^+ + H, calcd for $C_{30}H_{48}NO_2Si$ 482.3454).

(*R*)-*N*-((3*R*,5*S*)-6-Hydroxy-3,5-dimethylhexyl)-*N*,2-dimethylbutanamide (41). To a solution of *N*-methyl amide **S-5** (399 mg, 0.828 mmol) in THF (8.2 mL) was added TBAF (1 M in THF, 1.07 mL, 1.07 mmol) at room temperature. The mixture was stirred for 2 h, quenched with saturated NH_4Cl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 40:60) to give alcohol **41** (160 mg, 0.655 mmol, 79%) as a pale oil: $[\alpha]^{23}_D$ −29.3 (*c* 0.940, $CHCl_3$); ^{1} H NMR ($CDCl_3$) δ 0.83−0.96 (9H, m), 1.10 (3H, d, *J* = 6.8 Hz, 1 rotamer), 1.30−1.44 (4H, m), 1.54−1.74 (4H, m), 2.55−2.59 (1H, m), 2.92, 3.00 (total 3H, each s), 3.30−3.81 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.7, 176.4, 67.8, 66.6, 48.0, 45.2, 40.9, 39.7, 37.5, 37.2, 35.8, 34.9, 34.4, 33.7, 33.0, 32.9, 28.2, 27.6, 27.4, 27.0, 20.7, 20.3, 17.9, 17.8, 17.3, 17.0, 12.2, 12.0.; IR (neat) 3404, 2956, 2922, 2866, 1624, 1461, 1412, 1375, 1297, 1256, 1195, 1133, 1082, 1044, 985; MS FAB-MS *m*/*z* 244 (M^+ + H); high-resolution FAB-MS *m*/*z* 244.2267 (M^+ + H, calcd for $C_{14}H_{29}NO_2$ 244.2277).

(*R*)-*N*,2-Dimethyl-*N*-((3*R*,5*S*,6*R*)-3,5,6-trimethyl-8-oxo-8-((*R*)-2-oxo-4-phenyloxazolidin-3-yl)octyl)butanamide (43). To a solution of $(COCl)_2$ (66 μL, 0.756 mmol) in CH_2Cl_2 (0.5 mL) was added DMSO (72 μL, 1.01 mL) at −78 °C under Ar atmosphere. After 15 min, a solution of alcohol **41** (61.3 mg, 0.252 mmol) in CH_2Cl_2 (0.5 mL) was added to the mixture. After an additional 45 min, Et_3N (176 μL, 1.26 mmol) was added to the mixture. The mixture was warmed to room temperature, stirred for 1 h, quenched with saturated NH_4Cl, extracted with CH_2Cl_2 (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude aldehyde was employed directly in the next reaction.

To a solution of phosphonate **17** (129 mg, 0.378 mmol) in THF (1.7 mL) was added NaHMDS (0.99 M in THF, 382 μL, 0.378 mmol) at 0 °C under Ar atmosphere. After 30 min, a solution of crude aldehyde in THF (1.7 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude α,β-unsaturated imide **42** was employed directly in the next reaction.

To a solution of CuBr·Me_2S complex (130 mg, 0.630 mmol) in THF (1.2 mL) was added dropwise MeMgBr (0.96 M in THF, 1.10 mL, 1.06 mmol) at −78 °C under Ar atmosphere. After 20 min, a solution

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of α,β-unsaturated imide in THF (1.2 mL) was added to the mixture. The mixture was warmed to 0 °C, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give imide **43** (64.4 mg, 58%) as an inseparable mixture.

(3*R*,4*S*,6*R*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4,6-trimethyl-*N*-((*R*)-1-(tritylthio)but-3-en-2-yl)octanamide (S-6). To a solution of imide **43** (41.2 mg, 0.0927 mmol) in THF/H_2O (0.4 mL, 4: 1) were added 30% H_2O_2 (37 μL) and 0.5 M LiOH (0.3 mL) at room temperature. The mixture was stirred for 12 h, 1 N NaOH was added, and the mixture was washed with AcOEt. The aqueous layer was acidified with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude carboxylic acid was employed directly in the next reaction.

To a solution of amine **10** (10.3 mg, 0.0300 mmol) and crude caroboxlic acid in CH_2Cl_2 (1.0 mL) were added EDCI (6.0 mg, 0.0300 mmol) and DMAP (0.2 mg, 2.0 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 5 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give amide **S-6** (9.9 mg, 0.0265 mmol, 79%) as a pale oil: $[\alpha]^{24}_D$ +12.6 (*c* 1.43, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.75−0.89 (12H, m), 1.05−1.09 (3H, m), 1.25−1.83 (9H, m), 1.93−2.04 (1H, m), 2.13−2.22 (1H, m), 2.33−2.41 (1H, m), 2.45−2.60 (2H, m), 2.92, 2.99 (total 3H, each s), 3.24−3.45 (2H, m), 4.55 (1H, s), 5.05−5.09 (2H, m), 5.40, 5.89 (total 1H, each d, *J* = 7.5 Hz), 5.62−5.71 (1H, m), 7.21−7.44 (15H, m); ^{13}C NMR ($CDCl_3$) δ 176.3, 176.0, 172.1, 171.8, 144.5, 136.8, 129.5, 127.9, 126.7, 115.5, 66.7, 50.1, 48.0, 45.7, 41.3, 40.6, 40.1, 39.8, 37.4, 37.2, 36.6, 36.5, 35.5, 35.2, 34.9, 34.8, 34.6, 34.4, 33.7, 28.4, 27.5, 27.1, 20.8, 20.4, 17.8, 17.3, 17.2, 16.8, 16.2, 12.2, 12.1.; IR (neat) 3284, 3052, 2954, 2918, 2866, 1625, 1535, 1487, 1442, 1413, 1378, 1296, 1081, 1034, 988, 923, 700; MS EI-MS *m*/*z* 626 (M^+); high-resolution EI-MS *m*/*z* 626.3904 (M^+, calcd for $C_{40}H_{54}N_2O_2S$ 626.3900).

(*R*)-*N*,2-Dimethyl-*N*-((3*R*,5*S*,6*R*)-3,5,6-trimethyl-7-((*R*)-4-vinyl-4,5-dihydrothiazol-2-yl)heptyl)butanamide (5). To a solution of amide **S-6** (9.9 mg, 0.0159 mmol) in CH_2Cl_2 (0.3 mL) was added $TiCl_4$ (4.9 μL, 0.0450 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 12 h, quenched with saturated Rochelle salt, stirred for 1 h, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give 10-*epi*-kalkitoxin **5** (2.2 mg, 0.0060 mmol, 37%) as a colorless oil: $[\alpha]^{24}_D$ +12.6 (*c* 1.43, $CHCl_3$); ^1H NMR (benzene-d_6) δ 0.78−0.85 (3H, m), 0.92−1.02 (12H, m), 1.13−1.63 (7H, m), 1.97 (1H, m), 1.87−2.05 (1H, m), 2.13 (1H, br), 2.31−2.39 (2H, m), 2.45−2.57 (1H, m), 2.50, 2.90 (total 3H, each s), 2.73−2.80 (1H, m), 2.96−3.02 (2H, m), 3.20−3.27, 3.57−3.64 (total 1H, each m), 4.77−4.84 (1H, m), 5.09 (1H, d, *J* = 7.8 Hz), 5.31 (1H, d, *J* = 17.3 Hz), 5.82−5.94 (1H, m); ^{13}C NMR (benzene-d_6) δ 176.2, 175.9, 170.9, 170.5, 139.2, 139.0, 116.3, 116.1, 80.2, 48.8, 46.8, 42.0, 41.8, 39.8, 38.9, 38.8, 38.6, 38.5, 37.5, 37.4, 36.8, 35.8, 35.6, 34.6, 31.2, 29.6, 29.0, 28.6, 21.8, 21.6, 19.3, 18.6, 17.8, 17.7, 17.6, 13.5, 13.3; IR (neat) 2952, 2918, 2866, 1639, 1461, 1409, 1377, 1262, 1197, 1080, 923; MS FAB-MS *m*/*z* 367 (M^+ + H); high-resolution FAB-MS *m*/*z* 367.2763 (M^+ + H, calcd for $C_{21}H_{39}N_2OS$ 367.2783).

(*S*,*E*)-Methyl 6-((*tert*-Butyldiphenylsilyl)oxy)-5-methylhex-2-enoate (44). To a solution of aldehyde **16** (994 mg, 2.94 mmol) in CH_2Cl_2 (20 mL) was added (methoxycarbonylmethylene)-triphenylphosphorane (1.18 g, 3.52 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 17 h, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 5:95) to give α,β-unsaturated ester **44** (1.03 g, 2.59 mmol, 88%) as a colorless oil: $[\alpha]^{24}_D$ −4.7 (*c* 1.36, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.90 (3H, d, *J* = 6.8 Hz), 1.06 (9H, s), 1.80−1.88 (12, m), 2.01−2.09 (1H, m), 2.40−2.47 (1H, m), 3.43−3.54 (2H, m), 3.73 (3H, s), 5.82 (1H, dt, *J* = 15.6, 1.4 Hz), 6.94 (1H, dt, *J* = 15.6, 7.5 Hz), 7.35−7.43 (6H, m), 7.64 (4H, dd, *J* = 7.3, 0.7 Hz); ^{13}C NMR ($CDCl_3$) δ 166.8, 148.1, 135.3, 133.6, 129.5, 127.5, 122.0, 68.1, 51.3, 36.1, 35.4, 26.9, 19.3, 16.4; IR (neat) 3408, 3052, 2970, 2918, 2848, 1695, 1593, 1491, 1441, 1421, 1366, 1210, 1163, 1143, 743, 699; MS FAB-MS *m*/*z* 419 (M^+ + Na); high-resolution EI-MS *m*/*z* 419.2017 (M^+, calcd for $C_{24}H_{32}O_3SiNa$ 419.2018).

(*S*)-((6-Azido-2-methylhexyl)oxy)(*tert*-butyl)diphenylsilane (S-7). To a solution of α,β-unsaturated ester **44** (375 mg, 0.946 mmol) in EtOAc (5.0 mL) was added Pd−C (37.5 mg) at room temperature. The mixture was stirred for 17 h under H_2 atmosphere, fitered through a Celite pad, and concentrated *in vacuo*. The crude reductant was employed directly in the next reaction.

To a solution of crude reductant in CH_2Cl_2 (5.0 mL) was added DIBAL (0.99 M in toluene, 2.38 mL, 2.36 mmol) at 0 °C under Ar atmosphere. The mixture was stirred for 15 min, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. he crude alcohol was employed directly in the next reaction.

To a solution of crude alcohol in CH_2Cl_2 (5.0 mL) were added Et_3N (263 μL, 1.89 mmol) and MsCl (109 μL, 1.42 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude mesylate was employed directly in the next reaction.

To a solution of crude mesylate in DMF (5.0 mL) was added NaN_3 (184 mg, 2.83 mmol) at room temperature. The mixture was heated to 40 °C for 15 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give azide **S-7** (350 mg, 0.886 mmol, 93%) as a colorless oil: $[\alpha]^{23}_D$ −1.65 (*c* 1.19, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.91 (3H, d, *J* = 6.6 Hz), 1.05 (9H, s), 1.08−1.17 (2H, m), 1.28−1.41 (2H, m), 1.42−1. 54 (2H, m), 1.62−1.67 (1H, m), 3.23 (2H, d, *J* = 7.1 Hz), 3.43−3.51 (2H, m), 7.35−7.44 (6H, m), 7.64−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 135.5, 133.9, 129.4, 127.5, 68.7, 51.5, 35.6, 32.7, 29.2, 27.0, 24.2, 19.4, 16.8; IR (neat) 3064, 3012, 2926, 2852, 2092, 1468, 1425, 1387, 1359, 1188, 1109, 1008, 822, 738, 702; MS FAB-MS *m*/*z* 396 (M^+ + H); high-resolution FAB-MS *m*/*z* 396.2478 (M^+ + H, calcd for $C_{23}H_{34}N_3OSi$ 396.2471).

(*R*)-*N*-((*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-5-methylhexyl)-2-methylbutanamide (48). To a solution of azide **S-7** (1.15 g, 2.90 mmol) in THF (29 mL) were added H_2O (261 μL) and PPh_3 (1.90 g, 7.24 mmol) at room temperature. The mixture was heated to 40 °C for 12 h, cooled to room temperature, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude amine **47** was employed directly in the next reaction.

To a solution of crude amine **47** and caroboxlic acid **12** (355 mg, 3.48 mmol) in CH_2Cl_2 (14.5 mL) were added Et_3N (610 μL, 4.35 mmol) and $(EtO)_2POCN$ (530 μL, 3.48 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 2.5 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 10:90) to give amide **48** (1.09 g, 2.40 mmol, 83%) as a colorless oil: $[\alpha]^{23}_D$ −3.4 (*c* 1.18, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87−0.91 (6H, m), 1.05 (9H, s), 1.12 (3H, d, *J* = 7.1 Hz), 1.23−1.68 (9H, m), 2.01−2.06 (1H, m), 3.19−3.25 (2H, m), 3.41−3.51 (2H, m), 5.34 (1H, brs), 7.35−7.43 (6H, m), 7.64−7.65 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.1, 135.5, 134.0, 129.4, 127.5, 68.8, 43.4, 39.3, 35.7, 32.9, 30.1, 27.4, 26.9, 24.3, 19.4, 17.6, 16.9, 12.0; IR (neat) 3290, 3064, 2954, 2924, 2852, 2090, 1640, 1549, 1459, 1425, 1385, 1263, 1234, 1109, 1008, 823, 739, 702; MS ESI-MS *m*/*z* 476 (M^+ + Na); high-resolution ESI-MS *m*/*z* 476.2945 (M^+ + Na, calcd for $C_{28}H_{43}NO_2SiNa$ 476.2961).

(*R*)-*N*-((*S*)-6-((*tert*-Butyldiphenylsilyl)oxy)-5-methylhexyl)-*N*,2-dimethylbutanamide (S-8). To a solution of amide **48** (1.06 g, 2.34 mmol) in THF (23.4 mL) were added *n*-BuLi (2.63 M in hexanes, 1.60 mL, 4.21 mmol) and MeI (583 μL, 9.36 mmol) at −78 °C under Ar atmosphere. The mixture was stirred for 10 min at −78 °C, warmed to room temperature, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give

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N-methyl amide **S-8** (990 mg, 2.05 mmol, 88%) as a colorless oil: $[\alpha]^{23}_{D}$ −11.4 (*c* 1.06, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84−0.92 (6H, m), 1.05, 1.06 (total 9H, each s), 1.07−1.10 (3H, m), 1.21−1.70 (9H, m), 2.54−2.59 (1H, m), 2.91, 2.99 (total 3H, each s), 3.23−3.27 (2H, m), 3.40−3.51 (2H, m), 7.35−7.43 (6H, m), 7.64−7.66 (4H, m); ^{13}C NMR ($CDCl_3$) δ 176.4, 176.0, 135.5, 134.0, 133.9, 129.5, 129.4, 127.5, 68.9, 68.7, 49.8, 47.8, 37.4, 37.1, 35.8, 35.7, 35.3, 33.7, 33.0, 32.9, 29.4, 27.6, 27.5, 27.1, 26.9, 24.3, 24.2, 19.4, 17.9, 17.2, 16.8, 12.2, 12.1; IR (neat) 3064, 2954, 2924, 2852, 1642, 1462, 1425, 1408, 1259, 1191, 1110, 1008, 822, 740, 703; MS ESI-MS *m*/*z* 490 (M^+ + Na); high-resolution FAB-MS *m*/*z* 490.3113 (M^+ + Na, calcd for $C_{29}H_{45}NO_2SiNa$ 490.3117).

(*R*)-*N*-((*S*)-6-Hydroxy-5-methylhexyl)-*N*,2-dimethylbutanamide (49). To a solution of *N*-methyl amide **S-8** (960 mg, 2.05 mmol) in THF (10.3 mL) was added TBAF (1 M in THF, 2.67 mL, 2.67 mmol) at room temperature. The mixture was stirred for 2 h, quenched with saturated NH_4Cl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give alcohol **49** (418 mg, 1.82 mmol, 89%) as a pale oil: $[\alpha]^{23}_{D}$ −30.5 (*c* 1.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86−0.93 (6H, m), 1.08−1.11 (3H, m), 1.14−1.73 (10H, m), 2.55−2.64 (1H, m), 2.92, 3.01 (total 3H, each s), 3.28−3.31 (3H, t, *J* = 7.6 Hz), 3.38−3.52 (3H, m); ^{13}C NMR ($CDCl_3$) δ 176.5, 176.4, 67.7, 68.4, 49.8, 47.2, 37.4, 37.0, 35.6, 35.5, 35.1, 33.6, 32.9, 32.3, 29.2, 27.3, 27.2, 27.0, 24.2, 23.5, 17.7, 17.1, 16.6, 16.5, 12.0, 11.9; IR (neat) 3404, 2958, 2922, 2866, 1625, 1462, 1413, 1377, 1259, 1131, 1082; MS EI-MS *m*/*z* 229 (M^+); high-resolution EI-MS *m*/*z* 229.2040 (M^+, calcd for $C_{13}H_{27}NO_2$ 229.2042).

(*R*)-*N*,2-Dimethyl-*N*-((*S*,*E*)-5-methyl-8-oxo-8-((*R*)-2-oxo-4-phenyloxazolidin-3-yl)oct-6-en-1-yl)butanamide (50). To a solution of $(COCl)_2$ (148 μL, 1.70 mmol) in CH_2Cl_2 (1.2 mL) was added DMSO (161 μL, 2.27 mL) at −78 °C under Ar atmosphere. After 15 min, a solution of alcohol **49** (130 mg, 0.567 mmol) in CH_2Cl_2 (1.2 mL) was added to the mixture. After an additional 45 min, Et_3N (396 μL, 2.84 mmol) was added to the mixture. The mixture was warmed to room temperature, stirred for 1 h, quenched with saturated NH_4Cl, extracted with CH_2Cl_2 (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude aldehyde was employed directly in the next reaction.

To a solution of phosphonate **17** (290 mg, 0.851 mmol) in THF (2.8 mL) was added NaHMDS (0.99 M in THF, 860 μL, 0.851 mmol) at 0 °C under Ar atmosphere. After 30 min, a solution of crude aldehyde in THF (2.8 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give α,β-unsaturated imide **50** (154 mg, 0.371 mmol, 65%) as an inseparable mixture:

(3*R*,4*S*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4-dimethyloctanoic Acid (S-9). To a solution of CuBr·Me_2S complex (81.5 mg, 0.395 mmol) in THF (1.0 mL) was added dropwise MeMgBr (1.08 M in THF, 587 μL, 0.632 mmol) at −78 °C under Ar atmosphere. After 20 min, a solution of α,β-unsaturated imide **50** (65.7 mg as an inseparable mixture, theoretical 0.158 mmol) in THF (1.0 mL) was added to the mixture. The mixture was warmed to −40 °C, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude imide was employed directly in the next reaction.

To a solution of crude imide in THF/H_2O (1.5 mL, 4:1) were added 30% H_2O_2 (100 μL) and 0.5 M LiOH (1.0 mL) at room temperature. The mixture was stirred for 12 h, 1 N NaOH was added, and the mixture was washed with AcOEt. The aqueous layer was acidified with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give pure carboxylic acid **S-9** (28.4 mg, 0.0998 mmol, 63%) as a colorless oil: $[\alpha]^{25}_{D}$ −18.1 (*c* 1.03, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.77−0.94 (9H, m), 1.07−1.10 (3H, m), 1.15−1.54 (8H, m), 1.62−1.73 (1H, m), 1.98−2.20 (2H, m), 2.30−2.42 (1H, m), 2.54−2.62 (1H, m), 2.92, 3.01 (total 3H, each s), 3.26−3.31 (1.5H, m), 3.42−3.52 (0.5H, m); ^{13}C NMR ($CDCl_3$) δ 178.4, 178.0, 176.8, 176.6, 49.9, 47.9, 47.4, 37.7, 37.5, 37.2, 37.1, 36.4, 35.3, 34.2, 34.1, 33.8, 33.7, 32.9, 29.3, 27.4, 26.9, 24.7, 24.5, 17.7, 17.0, 16.9, 16.1, 16.0, 14.6, 14.5, 12.1, 12.0; IR (neat) 2963, 2932, 2874, 1726, 1610, 1460, 1404, 1378, 1269, 1186, 1082, 875; MS ESI-MS *m*/*z* 308 (M + Na^+); high-resolution ESI-MS *m*/*z* 308.2193 (M^+ + Na, calcd for $C_{16}H_{31}NO_3Na$ 308.2196).

(3*R*,4*S*)-8-((*R*)-*N*,2-Dimethylbutanamido)-3,4-dimethyl-*N*-((*R*)-1-(tritylthio)but-3-en-2-yl)octanamide (S-10). To a solution of amine **10** (34.9 mg, 0.0950 mmol) and crude caroboxlic acid **S-9** (18.9 mg, 0.0630 mmol) in CH_2Cl_2 (1.0 mL) were added EDCI (18.2 mg, 0.0950 mmol) and DMAP (0.7 mg, 5.8 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 5 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give amide **S-10** (34.9 mg, 0.0568 mmol, 90%) as a pale oil: $[\alpha]^{24}_{D}$ +15.7 (*c* 0.76, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86−0.94 (9H, m), 1.09−1.11 (3H, m), 1.41−2.00 (10H, m), 2.30 −2.54 (5H, m), 2.92, 3.03 (total 3H, each s), 3.27−3.47 (2H, m), 4.54 (1H, m), 5.06−5.10 (2H, m), 5.39−5.41 (1H, m), 5.61−5.68 (1H, m), 7.20−7.74 (15H, m); ^{13}C NMR ($CDCl_3$) δ 176.4, 176.1, 172.0, 171.8, 144.5, 144.4, 136.75, 136.72, 129.4, 127.8, 126.7, 126.6, 115.5, 66.7, 49.95, 49.90, 47.78, 47.69, 42.4, 42.3, 40.6, 40.4, 37.5, 37.4, 37.3, 37.1, 36.7, 36.6, 35.3, 35.1, 34.9, 34.5, 34.3, 34.2, 33.7, 33.2, 32.6, 29.7, 29.4, 27.4, 27.1, 24.8, 24.7, 17.8, 17.2, 16.9, 16.5, 16.3, 16.1, 14.8, 14.6, 14.5, 14.3, 12.1, 12.0; IR (neat) 3284, 3052, 2954, 2918, 2866, 1625, 1535, 1487, 1442, 1413, 1378, 1296, 1081, 1034, 988, 923, 700; MS EI-MS *m*/*z* 613 (M^+); high-resolution EI-MS *m*/*z* 613.3815 (M^+, calcd for $C_{39}H_{53}N_2O_2S$ 613.3828).

(*R*)-*N*-((5*S*,6*R*)-5,6-Dimethyl-7-((*R*)-4-vinyl-4,5-dihydrothiazol-2-yl)heptyl)-*N*,2-dimethylbutanamide (6). To a solution of amide **S-10** (15.2 mg, 0.0258 mmol) in CH_2Cl_2 (0.3 mL) was added $TiCl_4$ (8.5 μL, 0.0774 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 12 h, quenched with saturated Rochelle salt, stirred for 1 h, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give 10-*nor*-kalkitoxin **6** (5.4 mg, 0.0152 mmol, 59%) as a colorless oil: $[\alpha]^{24}_{D}$ +16.6 (*c* 0.54, $CHCl_3$); ^{1}H NMR (benzene-d_6) δ 0.76−0.80 (3H, m), 0.88−1.00 (6H, m), 1.14, 1.19 (total 3H, each d, *J* = 6.6 Hz), 1.23−1.48 (7H, m), 1.88−2.02 (1H, m), 2.07−2.15 (1H, m), 2.30 (3H, m), 2.52−2.58 (1H, m), 2.73−2.78 (1H, dd, *J* = 10.8, 8.0 Hz), 2.86−2.91 (1H, m), 2.98 (1H, dd, *J* = 10.8, 8.7 Hz), 2.46, 2.84 (total 3H, each s), 3.24−3.47 (1H, m), 4.76−4.84 (1H, m), 5.04 (1H, d, *J* = 10.2 Hz), 5.27 (1H, d, *J* = 17.3 Hz), 5.84−5.92 (1H, m); ^{13}C NMR (benzene-d_6) δ 175.2, 175.0, 169.9, 169.6, 138.2, 138.1, 115.2, 115.1, 79.17, 79.13, 49.5, 47.7, 40.1, 38.8, 38.4, 38.3, 37.6, 37.4, 37.0, 36.9, 36.6, 36.1, 35.9, 34.8, 34.7, 33.5, 32.99, 32.93, 29.6, 29.5, 28.0, 27.6, 25.2, 25.1, 18.4, 17.7, 16.8, 16.7, 16.5, 16.4, 14.6, 14.4, 12.5, 12.4; IR (neat) 2952, 2918, 2866, 1639, 1461, 1409, 1377, 1262, 1197, 1080, 923; MS FAB-MS *m*/*z* 353 (M^+ + H); high-resolution FAB-MS *m*/*z* 353.2639 (M^+ + H, calcd for $C_{20}H_{37}N_2OS$ 353.2627).

(*R*)-*N*-((3*S*,5*S*)-6-Hydroxy-3,5-dimethylhexyl)-2-methylbutanamide (52). To a solution of amide **21** (673 mg, 1.44 mmol) in THF (7.2 mL) was added TBAF (1 M in THF, 1.87 mL, 1.87 mmol) at room temperature. The mixture was stirred for 2 h, quenched with saturated NH_4Cl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give alcohol **52** (285 mg, 1.24 mmol, 86%) as a colorless oil: $[\alpha]^{23}_{D}$ −23.0 (*c* 0.920, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88−0.94 (9H, m), 1.13 (3H, d, *J* = 6.8 Hz), 1.18−1.46 (3H, m), 1.32−1.52 (2H, m), 1.59−1.75 (2H, m), 2.07−2.09 (2H, m), 3.17−3.37 (2H, m), 3.40−3.49 (2H, m), 5.39 (1H, brs); ^{13}C NMR ($CDCl_3$) δ 176.3, 68.8, 43.3, 40.5, 37.7, 37.4, 33.1, 27.8, 27.4, 19.4, 17.6, 16.4, 12.0; IR (neat) 3404, 2958, 2922, 2866, 1625, 1462, 1413, 1377, 1259, 1131, 1082; MS EI-MS *m*/*z* 229 (M^+); high-resolution EI-MS *m*/*z* 229.2037 (M^+, calcd for $C_{13}H_{27}NO_2$ 229.2042).

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(*R*)-*N*-((3*S*,5*S*,*E*)-3,5-Dimethyl-8-oxo-8-((*R*)-2-oxo-4-phenyloxazolidin-3-yl)oct-6-en-1-yl)-2-methylbutanamide (53). To a solution of $(COCl)_2$ (114 μL, 1.31 mmol) in CH_2Cl_2 (0.8 mL) was added DMSO (124 μL, 1.75 mL) at −78 °C under Ar atmosphere. After 15 min, a solution of alcohol **52** (100 mg, 0.438 mmol) in CH_2Cl_2 (0.8 mL) was added to the mixture. After an additional 45 min, Et_3N (306 μL, 2.19 mmol) was added to the mixture. The mixture was warmed to room temperature, stirred for 1 h, quenched with saturated NH_4Cl, extracted with CH_2Cl_2 (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude aldehyde was employed directly in the next reaction.

To a solution of phosphonate **17** (224 mg, 0.657 mmol) in THF (2.2 mL) was added NaHMDS (0.99 M in THF, 620 μL, 0.657 mmol) at 0 °C under Ar atmosphere. After 30 min, a solution of crude aldehyde in THF (2.2 mL) was added to the mixture. The mixture was warmed to room temperature, stirred for 12 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 20:80) to give α,β-unsaturated imide **53** (77.3 mg, 0.186 mmol, 42%) as an inseparable mixture:

(3*R*,4*S*,6*S*)-3,4,6-Trimethyl-8-((*R*)-2-methylbutanamido)-octanoic Acid (S-11). To a solution of CuBr·Me_2S complex (96.0 mg, 0.465 mmol) in THF (0.9 mL) was added dropwise MeMgBr (1.06 M in THF, 737 μL, 0.781 mmol) at −78 °C under Ar atmosphere. After 20 min, a solution of α,β-unsaturated imide **53** (77.3 mg as an inseparable mixture, theoretical 0.186 mmol) in THF (0.9 mL) was added to the mixture. The mixture was warmed to −40 °C, stirred for 1 h, quenched with saturated $NaHCO_3$, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude imide **54** was employed directly in the next reaction.

To a solution of crude imide **54** in THF/H_2O (0.3 mL, 4: 1) was added 30% H_2O_2 (29 μL) and 0.5 M LiOH (0.3 mL) at room temperature. The mixture was stirred for 12 h, 1 N NaOH was added, and the mixture was washed with AcOEt. The aqueous layer was acidified with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give pure carboxylic acid **S-11** (53.0 mg, 0.124 mmol, 67%) as a colorless oil: $[\alpha]^{25}_D$ −23.0 (*c* 1.08, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.78−0.91 (12H, m), 1.07−1.10 (6H, m), 1.23−1.52 (5H, m), 1.57−1.66 (1H, m), 1.90−1.98 (1H, m), 2.03−2.09 (1H, m), 2.33 (1H, dd, *J* = 14.7, 4.8 Hz), 3.20−3.35 (2H, m), 5.59 (1H, br); ^{13}C NMR ($CDCl_3$) δ 178.8, 176.6, 49.9, 43.2, 40.2, 38.0, 37.8, 37.4, 35.1 34.2, 19.0, 17.5, 16.6, 16.0, 14.5, 11.9; IR (neat) 3297, 2963, 2931, 2876, 1708, 1627, 1552, 1457, 1380, 1269, 1189, 1109, 891; MS ESI-MS *m*/*z* 308 (M + Na^+); high-resolution ESI-MS *m*/*z* 308.2195 (M^+ + Na, calcd for $C_{16}H_{31}NO_3Na$ 308.2196).

(3*R*,4*S*,6*S*)-3,4,6-Trimethyl-8-((*R*)-2-methylbutanamido)-*N*-((*R*)-1-(tritylthio)but-3-en-2-yl)octanamide (S-12). To a solution of amine **10** (34.2 mg, 0.0930 mmol) and crude caroboxlic acid **S-11** (17.8 mg, 0.0620 mmol) in CH_2Cl_2 (0.6 mL) were added EDCI (17.8 mg, 0.0950 mmol) and DMAP (0.7 mg, 5.8 μmol) at room temperature under Ar atmosphere. The mixture was stirred for 5 h, quenched with 1 N HCl, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give amide **S-12** (25.7 mg, 0.0419 mmol, 68%) as a pale oil: $[\alpha]^{23}_D$ +10.5 (*c* 0.270, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.79−0.93 (12H, m), 1.11 (3H, d, *J* = 6.8 Hz), 1.28−1.76 (10H, m), 1.87 (1H, m), 1.90−1.97 (1H, m), 2.01−2.08 (1H, m), 2.16 (1H, dd, *J* = 13.7, 4.1 Hz), 2.37 (1H, dd, *J* = 12.3, 5.1 Hz), 2.49 (1H, dd, *J* = 12.3, 6.4), 3.20−3.35 (2H, m), 4.54 (1H, br), 5.07 (1H, d, *J* = 12.4 Hz), 5.40 (1H, d, *J* = 8.0 Hz), 5.65 (1H, ddd, *J* = 12.4, 8.0, 5.1 Hz), 7.12−7.34 (15H, m); ^{13}C NMR ($CDCl_3$) δ 176.0, 171.7, 144.4, 136.6, 129.4, 127.8, 126.7, 115.5, 68.2, 50.0, 43.4, 40.8, 40.5, 37.9, 37.5, 36.6, 35.5, 34.4, 28.2, 27.5, 19.4, 17.7, 16.6, 16.4, 12.1; IR (neat) 3288, 3052, 2954, 2918, 2868, 1641, 1539, 1487, 1442, 1413, 1377, 1079, 1033, 989, 921, 700; MS ESI-MS *m*/*z* 635 (M^+ + Na); high-resolution ESI-MS *m*/*z* 635.3620 (M^+ + Na, calcd for $C_{39}H_{52}N_2O_2SNa$ 635.3647).

(*R*)-2-Methyl-*N*-((3*S*,5*S*,6*R*)-3,5,6-trimethyl-7-((*R*)-4-vinyl-4,5-dihydrothiazol-2-yl)heptyl)butanamide (7). To a solution of amide **S-12** (9.5 mg, 0.0150 mmol) in CH_2Cl_2 (0.3 mL) was added $TiCl_4$ (5.0 μL, 0.0450 mmol) at room temperature under Ar atmosphere. The mixture was stirred for 12 h, quenched with saturated Rochelle salt, stirred for 1 h, extracted with AcOEt (×3), washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 30:70) to give 16-*nor*-kalkitoxin **7** (4.0 mg, 0.0113 mmol, 75%) as a colorless oil: $[\alpha]^{23}_D$ +6.2 (*c* 0.120, $CHCl_3$)^{1}H NMR (benzene-d_6) δ 0.75 (3H, d, *J* = 6.8 Hz), 0.78 (3H, d, *J* = 6.6 Hz), 0.86 (3H, t, *J* = 7.3 Hz), 0.94 (3H, d, *J* = 6.8 Hz), 1.09 (3H, d, *J* = 6.5 Hz), 1.23- 1.78 (7H, m),, 2.03−2.13 (2H, m), 2.33 (1H, dd, *J* = 14.8, 8.8 Hz), 2.52 (1H, dd, *J* = 14.8, 5.4 Hz), 2.71 (1H, dd, *J* = 10.7, 8.2 Hz), 2.93 (1H, dd, *J* = 10.7, 8.8 Hz), 3.07−3.24 (2H, m), 4.29−4.33 (1H, m), 4.58 (1H, br), 4.73−4.76 (1H, m), 5.01 (1H, d, *J* = 10.2 Hz), 5.24 (1H, d, *J* = 17.1 Hz), 5.94 (1H, ddd, *J* = 17.1, 10.2, 6.3 Hz); ^{13}C NMR (benzene-d_6) δ 175.8, 171.0, 139.0, 116.3, 80.2, 51.7, 44.4, 41.3, 39.9, 39.7, 39.5, 35.4, 31.4, 29.4, 28.9, 20.5, 19.2, 17.8, 17.5, 13.5; IR (neat) 3290, 2954, 2918, 2848, 1641, 1452, 1380, 1258, 1090, 1023, 798; MS EI-MS *m*/*z* 352 (M^+); high-resolution EI-MS *m*/*z* 352.2548 (M^+, calcd for $C_{20}H_{36}N_2OS$ 352.2548).

Brine Shrimp Toxicity Assay. We tested the toxicity of the CSLs using a modified method.[29] Ten hatched brine shrimp, in ∼4.95 mL of seawater, were added to each well containing different concentrations of the compounds in 50 μL of EtOH or 50% EtOH to make a total volume of 5 mL. Samples and controls were tested in duplicate. The numbers of live and dead brine shrimp were counted after 24 h at 25 °C.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^{1}H NMR and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Wu, M.; Okino, T.; Nogle, L. M.; Marquez, B. L.; Williamson, R. T.; Sitachitta, N.; Berman, R. W.; Murray, T. F.; McGough, K.; Jacobs, R.; Colsen, K.; Asano, T.; Yokokawa, F.; Shioiri, T.; Gerwick, W. H. *J. Am. Chem. Soc.* **2000**, *122*, 12041−12042.

(2) Yokokawa, F.; Asano, T.; Okino, T.; Gerwick, W. H.; Shioiri, T. *Tetrahedron* **2004**, *60*, 6859−6880.

(3) Berman, R. W.; Gerwick, W. H.; Murray, T. F. *Toxicon* **1999**, *37*, 1645−1648.

(4) Lepage, K. T.; Goeger, D.; Yokokawa, F.; Asano, T.; Shioiri, T.; Gerwick, W. H.; Murray, T. F. *Toxicol. Lett.* **2005**, *158*, 133−139.

(5) (a) Nicolás, E.; Russell, K. C.; Hruby, V. J. *J. Org. Chem.* **1993**, *58*, 766−770. (b) Li, G.; Patel, D.; Hruby, V. J. *Tetrahedron: Asymmetry* **1993**, *4*, 2315−2319. (c) Li, G.; Jaroginski, M. A.; Hruby, V. J. *Tetrahedron Lett.* **1993**, *34*, 2561−2564.

(6) For recent examples for diastereoselective 1,4-addition reaction controlled by oxazolidinone, see: (a) Yamada, T.; Sakaguchi, K.; Shinada, T.; Ohfune, Y.; Soloshonok, V. A. *Tetrahedron: Asymmetry* **2008**, *19*, 2789−2795. (b) Esumi, T.; Shimizu, H.; Kashiyama, A.; Sasaki, C.; Yoyota, M.; Fukuyama, Y. *Tetrahedron Lett.* **2008**, *49*, 6846−6849. (c) Nakamura, S.; Kikuchi, F.; Hashimoto, S. *Tetrahedron: Asymmetry* **2008**, *19*, 1059−1067. (d) Morita, M.; Ishiyama, S.; Koshino, H.; Nakata, T. *Org. Lett.* **2008**, *10*, 1675−1678. (e) Wu, H.;

Moeller, K. D. *Org. Lett.* **2007**, *9*, 4599−4602. (f) Davies, S. G.; Roberts, P. M.; Smith, A. D. *Org. Biomol. Chem.* **2007**, *5*, 1405−1415.
(7) (a) White, J. D.; Lee, C. -S.; Xu, Q. *Chem. Commun.* **2003**, *16*, 2012−2013. (b) White, J. D.; Xu, Q.; Lee, C.; Valeriote, F. A. *Org. Biomol. Chem.* **2004**, *2*, 2092−2102.
(8) (a) Williams, D. R.; Turske, R. A. *Org. Lett.* **2000**, *2*, 3217−3220. (b) Williams, D. R.; Lowder, P. D.; Gu, Y. *Tetrahedron Lett.* **2000**, *41*, 9397−9401.
(9) (a) Qian, K.; Nagasawa-Goto, K.; Yu, D.; Morrus-Natschke, S. L.; Nitz, T. J.; Kilgore, N.; Allaway, G. P.; Lee, K.-H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6553−6557. (b) Marshall, J. A.; Sedrani, R. *J. Org. Chem.* **1991**, *56*, 5496−5498.
(10) For the preparation of phosphonate **16**, see refs 10a and 10b, for *ent*-**16**, see ref 10c: (a) Rzasa, R. M.; Shea, H. A.; Romo, D. *J. Am. Chem. Soc.* **1998**, *120*, 591−592. (b) Romo, D.; Rzasa, R. M.; Shea, H. A.; Park, K.; Langenham, J. M.; Sun, L.; Akhiezer, A.; Liu, J. O. *J. Am. Chem. Soc.* **1998**, *120*, 12237−12254. (c) Ishizaki, M.; Hara, Y.; Kojima, S.; Hoshino, O. *Heterocycles* **1999**, *50*, 779−790.
(11) Willams, D. R.; Kissel, W. S.; Li, J. J.; Mullins, R. *Tetraheron Lett.* **2002**, *43*, 3723−3727.
(12) In 1,4-addition reactions to **14** and **32**, diastereomeric ratios were determined from ^{1}H NMR spectra and HPLC chromatograms of the addition products **18** and **33**. In contrast, ^{1}H NMR spectra of the adducts **23**, **30**, and **39** produced by 1,4-addition of **11**, **29**, and **38** were too complex due to the presence of rotamers with regard to the amide moieties to estimate isomeric ratios. In these cases, diastereomeric ratios were determined by HPLC analysis of the corresponding methyl esters derived from **23**, **30**, and **39**; see Supporting Information.
(13) (a) David A., Kummer, D. A.; Brenneman, J. B.; Martin, S. F. *Tetrahedron* **2006**, *62*, 11437−11449. (b) Smith, A. B. III; Cantin, L-D; Pasternak, A.; Guise-Zawacki, L.; Yao, W.; Charnley, A. K.; Barbosa, J.; Sprengeler, P. A.; Hirschmann, R.; Munshi, S.; Olsen, D. B.; Schleif, W. A.; Kuo, L. C. *J. Med. Chem.* **2003**, *46*, 1831−1844.
(14) (a) Druais, V.; Hall, M. J.; Corsi, C.; Wendeborn, S. V.; Meyer, C.; Cossy, J. *Org. Lett.* **2009**, *11*, 935−938. (b) Higuchi, K.; Sato, Y.; Tsuchimochi, M.; Sugiura, K.; Hatori, M.; Kawasaki, T. *Org. Lett.* **2009**, *11*, 197−199.
(15) (a) Šolaja, B. A.; Opsenica, D.; Smith, K. S.; Milhous, W. K.; Terzić, N.; Opsenica, I.; Burnett, J. C.; Nuss, J.; Gussio, R.; Bavari, S. *J. Med. Chem.* **2008**, *51*, 4388−4391. (b) Fusz, S.; Srivatsan, S. G.; Ackermann, D.; Famulok, M. *J. Org. Chem.* **2008**, *73*, 5069−5077.
(16) (a) Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737−1739. (b) Evans, D. A.; Chapman, K. T.; Hung, D. T.; Kawaguchi, T. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 1184−1187. (c) Belelie, J. L.; Chong, J. M. *J. Org. Chem.* **2001**, *66*, 5552−5555. (d) Guerlavais, V.; Carroll, P. J.; Joullie, M. M. *Tetrahedron: Asymmetry* **2002**, *13*, 675−680.
(17) Takuma, S; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147−3153, and refereces therein.
(18) Kim, Y.; Shin, E.-K.; Beak, P.; Park, Y.-S. *Synthesis* **2006**, 3805−3808.
(19) We found that 1,4-addition reaction of **11** took place with high stereoselectivity by using only THF as a solvent, although the combination of THF and Me_2S was employed as a solvent in the 1,4-addition of **11** during the total synthesis of **1** by Shioiri's group; see ref 1.
(20) Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141−6144.
(21) Dose, C.; Seitz, O. *Org. Lett.* **2005**, *7*, 4365−4368.
(22) Moriwake, T.; Hamano, S.; Saito, S.; Torii, S. *Chem. Lett.* **1987**, 2085−2088.
(23) (a) Katagiri, T.; Fujiwara, K.; Kawai, H.; Suzuki, T. *Tetraheron Lett.* **2008**, *49*, 3242−3247. (b) Innitzer, A.; Brecker, L.; Mulzer, J. *Org. Lett.* **2007**, *9*, 4431−4434.
(24) (a) Cesati, R. R. III; Armas, J.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2004**, *126*, 96−101. (b) Choi, Y.; White, J. D. *J. Org. Chem.* **2004**, *69*, 3758−3764. (c) Yamamoto, K.; Heathcock, C. H. *Org. Lett.* **2000**, *2*, 1709−1712. (d) Oikawa, M.; Ueno, T.; Oikawa, H.; Ichihara, A. *J. Org. Chem.* **1995**, *60*, 5048−5068.
(25) Ionita, P.; Volkov, A.; Jeschke, G.; Chechik, V. *Anal. Chem.* **2008**, *80*, 95−106.
(26) Although an amide that was synthesized from (−)-*O*-methyl mandelic acid and **10** obtained through Wittig olefination was a mixture of epimers (1.5−9:1), condensation between (−)-*O*-methyl mandelic acid and **10** prepared via Pd(0)-catalyzed reduction gave the corresponding amide as a single diastereomer; see Supporting Information.
(27) Raman, P.; Razavi, H.; Kelly, J. W. *Org. Lett.* **2000**, *2*, 3289−3292.
(28) In 1,4-addition reactions of **42**, **50**, and **53**, diastereomeric ratios at C7 could not be estimated employing the same HPLC analysis method as that for determination of isomeric ratios of addition reactions to **11**, **29**, and **38**; see ref 12. However, in ^{1}H NMR spectra of the carboxylic acids yielded through hydrolysis of the addition products **43**, **51**, and **54**, peaks due to trace amounts of the corresponding 7-*epi* isomers only were observed. Therefore, it seems feasible that these addition reactions occurred with high diastereoselectivity.
(29) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31−34.