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Total synthesis and biological evaluation of (+)-kalkitoxin, a cytotoxic metabolite of the cyanobacterium *Lyngbya majuscula*†

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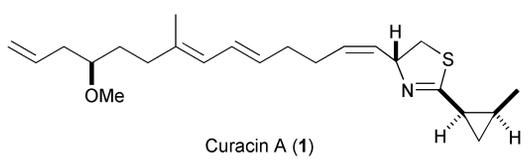
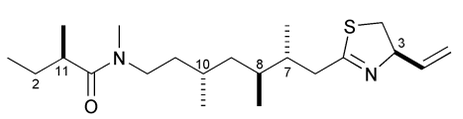
(+)-Kalkitoxin, a metabolite of the marine cyanobacterium *Lyngbya majuscula*, was synthesized from (*R*)-2-methylbutyric acid, (*R*)-cysteine, and (3*S*, 4*S*, 6*S*)-3,4,6-trimethyl-8-(methylamino)octanoic acid. A key step in the synthesis was installation of the *anti*,*anti* methyl stereotriad by means of a tandem asymmetric conjugate addition of an organocopper species to an α,β-unsaturated *N*-acyl oxazolidin-2-one followed *in situ* by α-methylation of the resultant enolate. The thiazoline portion of kalkitoxin was assembled by titanium tetrachloride catalyzed cyclization of a vinyl substituted amido thiol.

Introduction

The cyanobacterium *Lyngbya majuscula* is known to be a source of toxic metabolites such as lyngbyatoxin[1] and debromoaplysiatoxin,[2] but a wide variety of other biologically active metabolites including the antiproliferative agent curacin A (**1**)[3] have also been isolated from this prolific marine organism. Curacin A, in particular, has captured interest as a result of its potent cytotoxic activity.[4] In screening extracts of *L. majuscula* for additional substances with biological activity, Wu *et al.* discovered the lipopeptide kalkitoxin (**2**) and deduced both its gross structure and relative configuration by means of NMR experiments.[5] The absolute configuration of natural (+)-kalkitoxin was assigned as (3*R*, 7*R*, 8*S*, 10*S*, 2″*R*) by means of synthesis of all possible stereoisomers and comparison of their ^{13}C spectra with that of the natural product.



Curacin A (**1**)



Kalkitoxin (**2**)

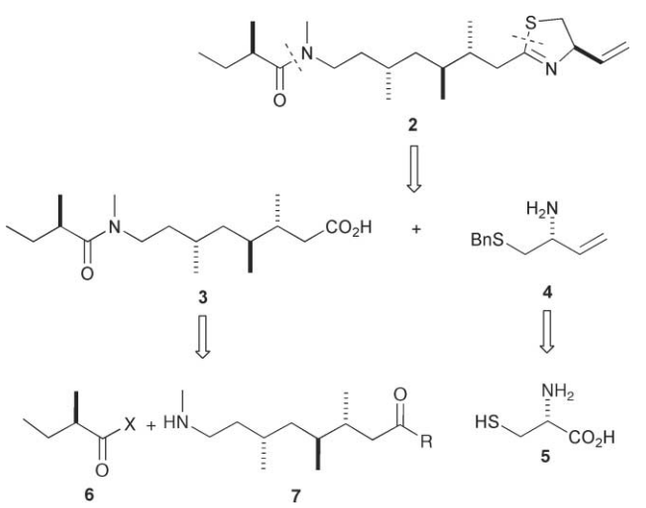
A striking similarity between kalkitoxin and curacin A emerges from these structural studies. For example, both structures contain a 2,4-disubstituted thiazoline with one of the substituents being a lipophilic chain and the other an unsaturated unit. However, a significant difference between **1** and **2** is the presence in the latter of three methyl substituents arranged in a 1,2,4-*anti*,*anti* sequence along the aliphatic chain. The synthetic interest presented by this stereochemical array, as well as intriguing reports of kalkitoxin's biological properties,[6–8] attracted our attention soon after the disclosure of its structure.[9] We now describe the full details of our work which has led to the total synthesis of natural (+)-kalkitoxin (**2**), and we also report studies of its pharmacological properties.

† Electronic supplementary information (ESI) available: ^1H NMR spectrum of synthetic (+)-kalkitoxin in C_6D_6. See http://www.rsc.org/suppdata/ob/b4/b404205k/

Results and discussion

Synthesis

A retrosynthetic analysis of kalkitoxin suggested that the thiazoline portion could be constructed from the constituent subunits **3** and **4** shown in Scheme 1, **4** being accessible in principle from (*R*)-(+)-cysteine (**5**). Amidocarboxylic acid **3** was foreseen as the coupling product of (*R*)-2-methylbutyric acid (**6**) with amine **7**, the latter bearing an *anti*,*anti* methyl triad whose absolute configuration would be set through the agency of a chiral reagent or auxiliary.



Scheme 1

Our plan from the outset envisioned installation of the *anti*-1,3-dimethyl array of **7** as the first operation, with insertion of the third methyl group as a subsequent and separate step. This strategy required an asymmetric method for positioning stereogenic methyl-bearing centers in a 1,3-relationship, for which there are a limited number of solutions. One of these is a tactic devised independently by Evans[10] and Sonnet[11] using prolinol as a chiral auxiliary, and which was employed with good results for the asymmetric assembly of an *anti*-1,3-dimethyl unit in our synthesis of bourgeanic acid.[12] Thus, the dianion of *N*-propionylprolinol (**8**) was reacted with the known iodide **9**[13] to give **10** as the sole detectable diastereomer (Scheme 2). Amides of prolinol, when exposed to hydrochloric acid, undergo transfer of the acyl group from the nitrogen atom to the primary alcohol

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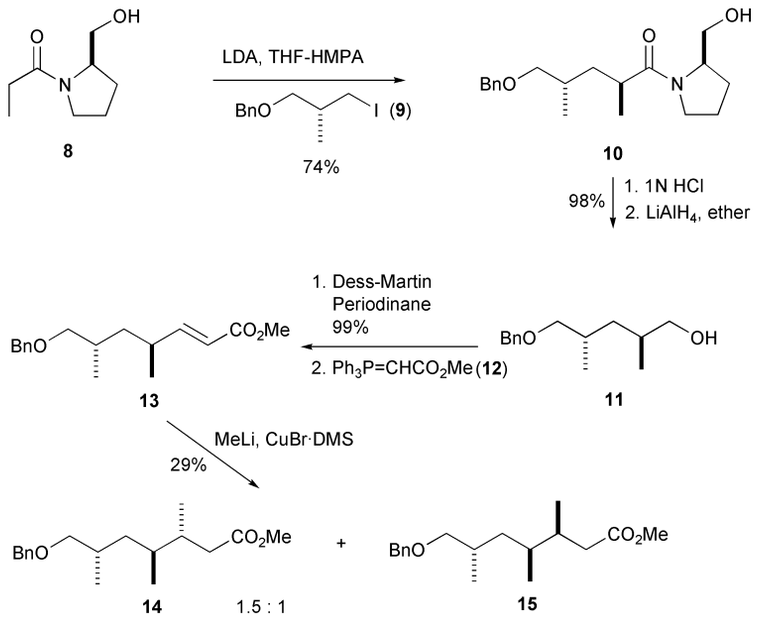
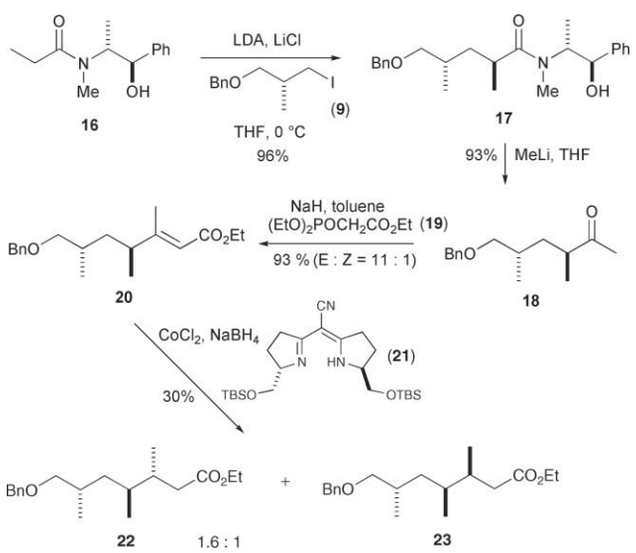


Scheme 2

in a process that is forced by generation of the pyrrolidinium salt. Reduction of the resultant ester therefore leads to a primary alcohol. When this protocol was applied to **10** using lithium aluminium hydride as the reducing agent, the alcohol **11** was produced in excellent yield along with recovered (*R*)-prolinol. Oxidation of **11** to the corresponding aldehyde with Dess–Martin periodinane,[14] followed by Wittig olefination with the phosphorane **12**, afforded *trans* α,β-unsaturated ester **13**. This substance now became the platform for a substrate-directed conjugate addition intended to introduce the third methyl substituent in the desired (*S*) configuration. Good precedent for this outcome appeared to exist in published work by Yamamoto,[15] who has shown that conjugate addition to γ-alkyl-α,β-unsaturated esters leads predominantly to an *anti* relationship between the new and preexisting alkyl substituents. However, it was found that the reaction of **13** with methyllithium and cuprous bromide under a variety of conditions resulted in a mixture of **14** and **15**, a typical ratio of products being 1.5 : 1 in favor of **14**. These esters were inseparable by chromatography, and the conjugate addition route was therefore abandoned as a means for installing the methyl stereotriad of kalkitoxin. Instead, a new pathway was sought which eschewed substrate stereocontrol for this purpose.

A tactical revision involving a reagent controlled reaction for setting configuration of the C7 methyl group of kalkitoxin appeared to be the logical alternative. Pfaltz has shown that reduction of β,β-disubstituted α,β-unsaturated esters can be accomplished in high yield and good enantiomeric excess[16] using the cobalt complex of a semicorrin and sodium borohydride, and this precedent became the basis for our next approach to **7**. Our route to the precursor required for this approach diverged from the alkylation sequence of Scheme 2 in that we now used Myers' pseudoephedrine auxiliary[17] for constructing our *anti*-1,3-dimethyl platform. The enolate of the *N*-propionyl derivative **16** of (1*R*, 2*R*)-pseudoephedrine was reacted with iodide **9** to give amide **17** as a single diastereomer in excellent yield (Scheme 3). An advantage of **17** over its prolinol analogue **10** is that the former can be converted directly to a methyl ketone, and when **17** was treated with methyllithium a clean reaction took place to yield **18**. Wadsworth–Emmons reaction of this ketone with the anion of phosphonate **19** furnished an (*E,Z*) mixture of α,β-unsaturated esters in which the (*E*) isomer **20** predominated by a ratio of 11 : 1. Catalytic hydrogenation of **20** over palladium-on-carbon not only produced a 1 : 1 mixture of inseparable stereoisomers from

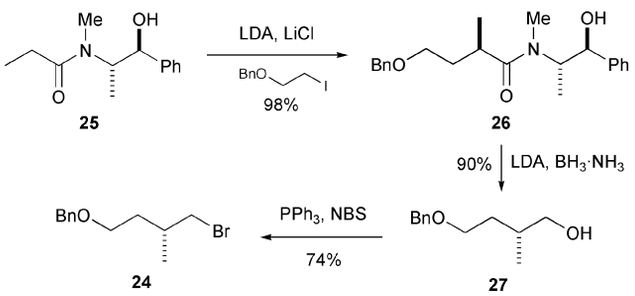


Scheme 3

saturation of the trisubstituted double bond but also cleaved the benzyl ether. We nevertheless hoped that the reducing system prepared from cobalt(II) chloride, sodium borohydride, and (*S,S*)-semicorrin **21** would effect stereoselective saturation of **20**. In practice, reduction of **20** with this set of reagents was very slow and resulted in only 30% conversion after three days. Furthermore, it gave a disappointing ratio of only 1.6 : 1 in favor of the desired product **22** over its isomer **23**. The reason for this unsatisfactory outcome probably lies in the steric hindrance presented by the substrate **20** to the bulky cobalt–semicorrin complex from **21** and suggests there is a practical limit to the application of this asymmetric reduction method in sterically unfavorable situations. In any event, we were forced to search again for methodology which would allow us to install the *anti*,*anti*-1,2,4-trimethyl array of **2** in an efficient asymmetric fashion.

At this point, we were drawn to a report by Hruby[18] in which it was shown that conjugate addition of an organocopper reagent to *trans*-3-crotonyl-4-phenyloxazolidin-2-one resulted in a high level of stereoinduction. Williams has generalized this reaction,[19] and in an elegant synthesis of sambutoxin[20] he made use of Hruby's finding for the asymmetric construction of an *anti*-1,3-dimethyl assemblage. For this strategy to be of greatest

practical value in our synthesis of kalkitoxin, the organocopper species used for conjugate addition should be one derived from halide **24**, *i.e.* a homologue of **9**. Since **24** was known only in racemic form,[21] its preparation as the (*R*) enantiomer was carried out using the sequence shown in Scheme 4. The enolate of *N*-propionylpseudoephedrine **25**[17] (enantiomeric with **16**) was alkylated with 2-iodoethyl benzyl ether,[22] and the resultant amide **26** was reduced with lithium amidotrihydroborate[23] to furnish (*R*)-**27**. This alcohol was shown to be enantiomerically pure within the limits of detection, and it was converted to bromide **24** with *N*-bromosuccinimide and triphenylphosphine in an overall yield of 64% from **25**.



Scheme 4

The alkylcopper reagent **28** was prepared by first converting bromide **24** to its Grignard reagent and then treating this with cuprous bromide dimethyl sulfide complex (Scheme 5). Addition of three equivalents of **28** to (*S*)-*trans*-3-crotonyl-4-phenyloxazolidin-2-one (**29**) followed by quenching of the intermediate enolate **30** with water, gave **31** as the sole detectable stereoisomer in high yield. The use of less than three equivalents of the organocopper species **28** resulted in a significant decrease in both stereoselectivity and yield of **31**. For example, the reaction of two equivalents of **28** with **29** afforded a (1.2 : 1) mixture of conjugate addition products only slightly favoring *anti* isomer **31**. Although a transition state of the reaction of **28** with **29** along lines proposed by Williams[20] suggests that this is a stereochemically matched case, in which both the methyl configuration in **28** and the phenyl configuration in **29** contribute to attack by the former at the *si* face of the latter (see Fig. 1), the need for an excess of **24** indicates that this rationale may be too simple. The assumed formation of (*Z*) enolate **30** in this process implies that *in situ* methylation of this intermediate will lead to incorporation of the third methyl

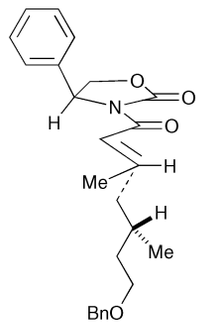
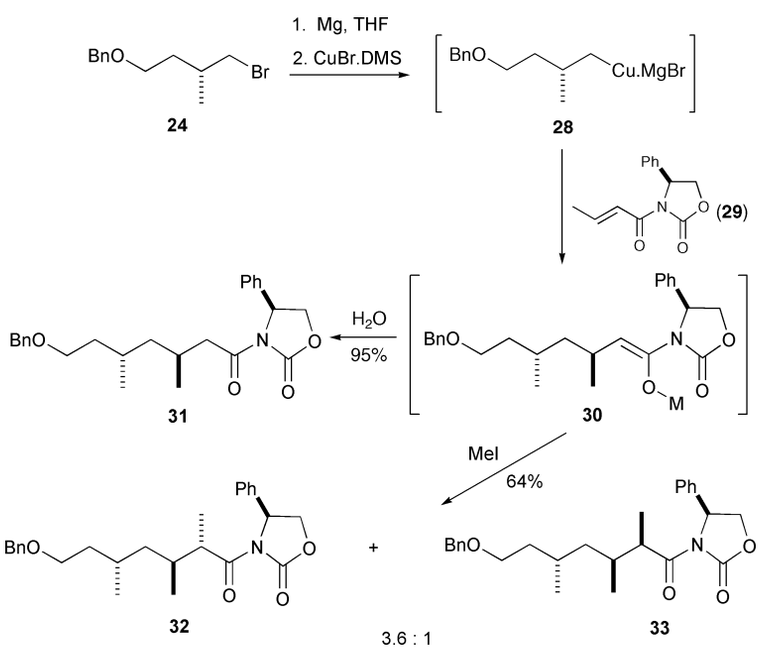


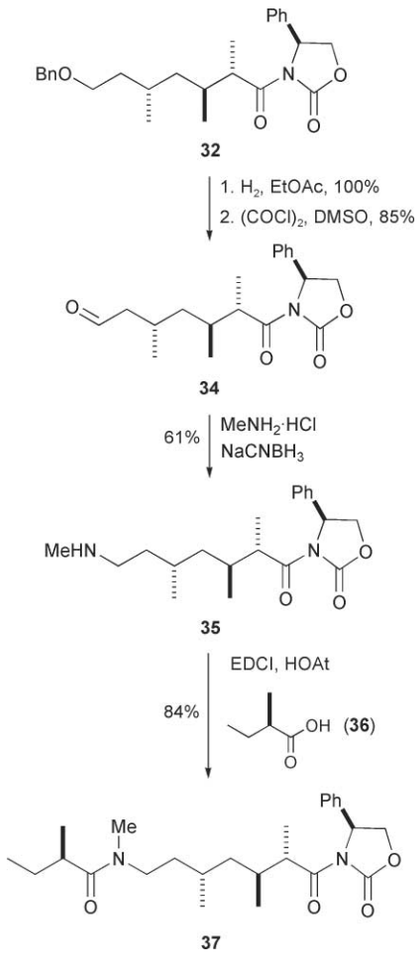
Fig. 1 Transition state for the reaction of **28** with **29** leading to **30**.

substituent in the desired (*R*) orientation, since both the oxazolidinone configuration and that adjacent to the enolate should favor attack from the rear face of **30**. In fact, addition of excess methyl iodide to the reaction mixture from **28** and **29** gave two methylated products in the ratio 3.6 : 1. These compounds were separated chromatographically, after which the major isomer crystallized. Its structure was established as **32** by X-ray crystallographic analysis.[9] For comparison, methylation of **30** using potassium hexamethyldisilylazide as the base gave a 2.8 : 1 mixture of **32** and **33**, respectively. As previously noted by Williams,[24] replacement of the phenyl substituent of the oxazolidinone **29** by a benzyl group resulted in decreased stereoselectivity in the conjugate addition step with **28**.

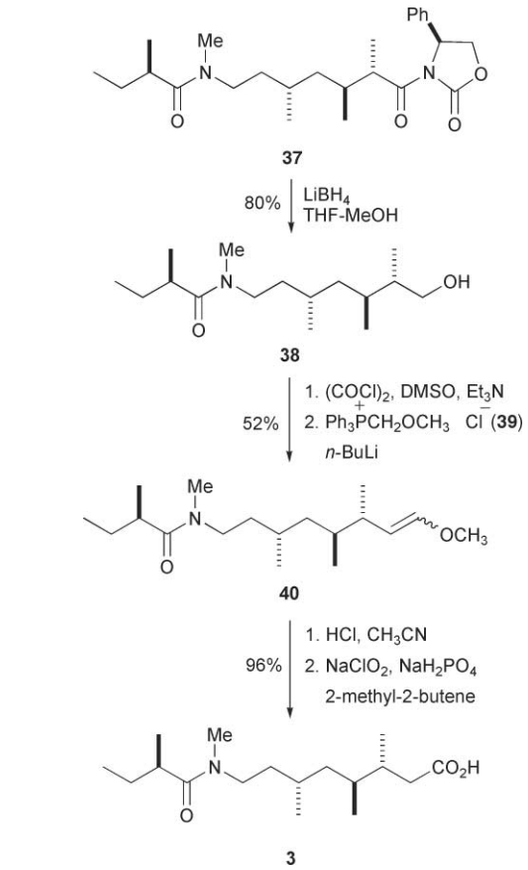
With the methyl stereotriad of kalkitoxin in place, attention next turned to elaboration of the benzyl ether terminus of **32**. To this end, the benzyl ether was cleaved by hydrogenolysis and the resulting alcohol was oxidized under Swern conditions to aldehyde **34** (Scheme 6). Reductive amination of this aldehyde with methylamine and sodium cyanoborohydride[25] furnished the secondary amine **35** which then became the partner for amide formation with (*R*)-2-methylbutyric acid (**36**). The reaction of activated α-branched carboxylic acids with secondary amines is typically difficult,[26] and this case was no exception. For example, the use of activating agents such as 1-hydroxybenzotriazole or diethyl cyanophosphate, as conventionally employed in peptide coupling,[27] resulted in sluggish reaction rates and low yields (29–46%) of amide **37**. It was discovered, however, that activation of **36** with Carpino's 1-hydroxy-7-azabenzotriazole[28] (HOAt) accomplished the desired coupling very efficiently with no detectable epimerization at the stereocenter α to the amide carbonyl. The product, as shown by



Scheme 5



Scheme 6

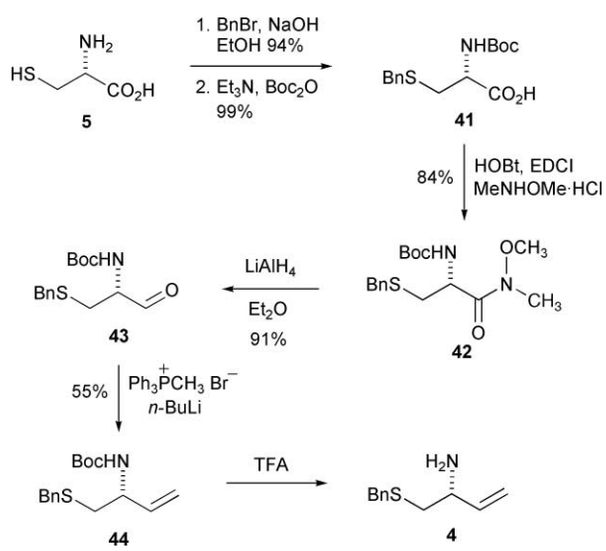


Scheme 7

NMR, was a 1 : 1 mixture of rotamers of **37** resulting from restricted rotation around the amide bond.

A trivial but necessary task for advancing **37** towards carboxylic acid **3** was a one-carbon homologation at the acyl oxazolidinone terminus, and for this purpose the auxiliary was cleaved from **37** by reduction with lithium borohydride. This gave primary alcohol **38** which was oxidized to the corresponding aldehyde under Swern conditions (Scheme 7). A Wittig reaction of this aldehyde with methoxymethylenephosphorane prepared from phosphonium chloride **39**[29] afforded the enol ether **40** as a 1 : 1 mixture of (*E*) and (*Z*) isomers, and acidic hydrolysis of the mixture furnished an aldehyde which was oxidized immediately with sodium chlorite to **3**. The five-step homologation sequence from **37** proceeded in an overall 51% yield.

The amine **4** required for coupling with **3** as a prelude to assembling the thiazoline moiety of kalkitoxin[30] was prepared from (*R*)-cysteine (**5**, Scheme 8). Thus, *S*-benzylation of **5** followed by protection of the amine as its *tert*-butoxycarbonyl (Boc) derivative gave carboxylic acid **41**[31] which was transformed to Weinreb amide **42**.[32] Reduction of **42** with lithium aluminium hydride yielded aldehyde **43**, and Wittig olefination of this substance with methylenetriphenylphosphorane gave **44**. Final cleavage of the Boc group from **44** led to benzyl (*R*)-2-amino-3-butenyl sulfide (**4**). Coupling of **4** with carboxylic acid **3** was accomplished by activating the latter with Carpino's *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU)[33] in the presence of Hunig's base and afforded bis amide **45** in excellent yield (Scheme 9). Reductive cleavage of the benzyl group from **45** with sodium–ammonia and treatment of the resulting thiol **46** with titanium tetrachloride in dichloromethane[34] led directly to (+)-kalkitoxin (**2**) with spectroscopic properties identical to those of



Scheme 8

the natural material (ESI †reports the ^{1}H NMR spectrum of synthetic (+)-kalkitoxin in C_6D_6; for that of natural (+)-kalkitoxin see the supporting information of ref. 5).

Biological studies

The cytotoxicity of kalkitoxin (**2**), thiol **46** and benzylthio ether **45** was measured against the human colon cell line HCT-116. The IC_{50} values for these three compounds are presented in Table 1, and a comparison of the data indicates that kalkitoxin is 200- to 400-fold more potent than either **45** or **46**. The latter are comparable in their toxicity. The data demonstrate that the intact thiazoline moiety of kalkitoxin is required for full cytotoxicity.

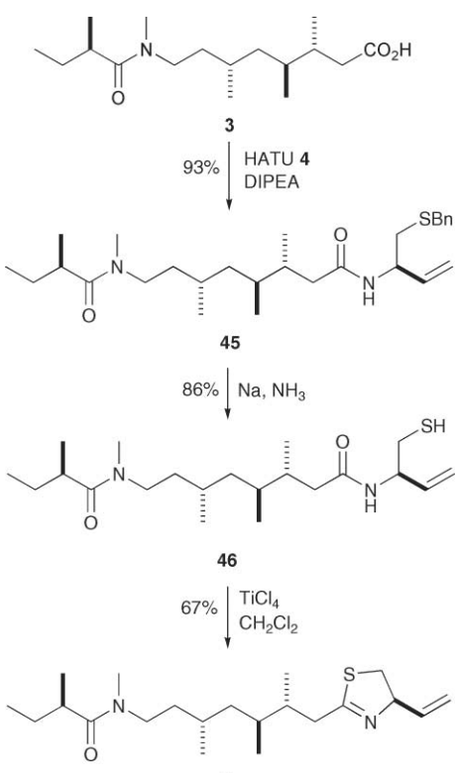
Table 1 IC_{50} values for kalkitoxin and synthetic precursors

Compound	IC_{50}/μg mL^{-1}
2	1.0×10^{-3}
46	1.9×10^{-1}
45	4.0×10^{-1}

Table 2 Zone differential results for kalkitoxin and synthetic precursors

Compound	μg/disk	$_{C38}\Delta s_{L1210}$ [a]	$_{C38}\Delta s_{CFU}$ [a]	$_{H116}\Delta s_{CEM}$ [a]
2	6	800	1000	350
46	45	—	—	300
	3	950	450	—
45	12	400	450	—

[a] See text for definition of Δs.



Scheme 9

An *in vitro* cell-based assay was employed to identify solid tumour selectivity for kalkitoxin and its synthetic precursors **45** and **46**. The differential cytotoxicity was determined by observing a zone differential between a solid tumour cell (Colon 38, Colon HCT-116, Lung H-125) and either leukemia cells (L1210 or CEM) or normal cells (CFU-GM).[35] A sample is designated as "solid tumour selective" if the zone units for the solid tumour (Colon 38, for example) minus the zone units for a leukemia cell (L1210, for example), expressed as $_{C38}\Delta s_{L1210}$, is greater than 250 units. The results of this assay are presented in Table 2 and show that kalkitoxin, **45** and **46** all show differential cytotoxicity for Colon 38 *versus* both L1210 leukemia and normal CFU-GM cells. Furthermore, kalkitoxin and thiol **46** show differential cytotoxicity for HCT-116 *versus* human leukemia CEM. While kalkitoxin is the most potent, **45** and **46** are only two- to eight-fold less potent by this assay.

Finally, a clonogenic assay was carried out in which concentration and time-survival measurements were made with kalkitoxin, **45** and **46** against HCT-116 cells. The results are shown in Fig. 2 and indicate that for either kalkitoxin or for **45** and **46** there was no cytotoxic effect for exposures up to 24 h at 10 μg mL^{-1}. However, when exposure was extended for 168 h, significant cytotoxicity appeared for kalkitoxin with 10% survival at 2 ng mL^{-1}. For **46** at this time interval there was less cytotoxicity, with 10% survival at 1 μg mL^{-1}, and for **45** there was 10% survival at 3 μg mL^{-1}. These results indicate that tumour cells need to be exposed to these compounds for greater than 24 h in order to produce cytotoxic effects. In the case of kalkitoxin, the cytotoxic effect could probably be maintained by daily administration of the drug *in vivo* since activity is at the ng mL^{-1} level. For **45** and **46**, which are *ca.* 1000-fold less potent than kalkitoxin, it appears unlikely that long term levels in the μg mL^{-1} range could be maintained.

Experimental

Starting materials and reagents were obtained from commercial sources and were used without further purification. Solvents were dried by distillation from the appropriate drying agents immediately prior to use. Tetrahydrofuran (THF), diethyl ether (Et_2O), and toluene were distilled from sodium benzophenone under an argon atmosphere. Diisopropylamine, triethylamine, benzene, acetonitrile and dichloromethane (CH_2Cl_2) were distilled from calcium hydride under argon. All solvents used for routine isolation of products and chromatography were reagent grade. Moisture and air sensitive reactions were carried out under an atmosphere of argon. Reaction flasks were flame dried under a stream of argon gas, and glass syringes were oven dried at 120 °C and cooled in a dessicator over anhydrous calcium sulfate prior to use. Unless otherwise stated, concentration under reduced pressure refers to a rotary evaporator at water aspirator pressure.

Analytical thin layer chromatography (TLC) was performed using precoated aluminium E. Merck TLC plates (0.2 mm layer thickness of silica gel 60 F-254). Compounds were visualized by ultraviolet light, and/or by heating the plate after dipping in a solution of 14% ammonium molybdate tetrahydrate and 1.4% cerium(IV) sulfate in 1.6 M sulfuric acid in water or 1% solution of vanillin in 0.1 M sulfuric acid in ethanol or 1% solution of potassium permanganate in 2% 1 N sodium hydroxide in water. Flash chromatography was carried out using E. Merck silica gel 60 (230–400 mesh ASTM). Radial chromatography was carried out on individual rotors with layer thickness of 1, 2, or 4 mm using a Chromatotron manufactured by Harrison Research, Palo Alto, California.

Melting points were measured using a Büchi melting point apparatus, and are uncorrected. Infrared (IR) spectra were recorded with a Nicolet 5DXB FT-IR spectrometer using a thin film supported between NaCl plates or KBr discs. Specific optical rotations were measured at ambient temperature (23 °C) from $CHCl_3$ solutions on a Perkin-Elmer 243 polarimeter using a 1 mL cell with 1 dm path length. Proton and carbon nuclear magnetic resonance (NMR) spectra were obtained using either a Bruker AC-300 or a Bruker AM-400 spectrometer. All chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane using the δ scale. ^{1}H NMR spectra data are reported in the order: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and br = broad), coupling constant (*J*) in Hertz (Hz) and number of protons.

Chemical ionization (CI) high and low resolution mass spectroscopy (HRMS and MS) were obtained using a Kratos MS-50 spectrometer with a source temperature of 120 °C and methane gas as the ionizing source. Perfluorokerosene was used as a reference. Electron impact (EI) mass spectra (HRMS and MS) were obtained using a Varian MAT311 or a Finnegan 4000 spectrometer. X-Ray crystallographic data were collected on a Siemens P4 instrument and these data were interpreted using the direct methods program contained in the SHELXTL (Silicon Graphics/Unix) software package.

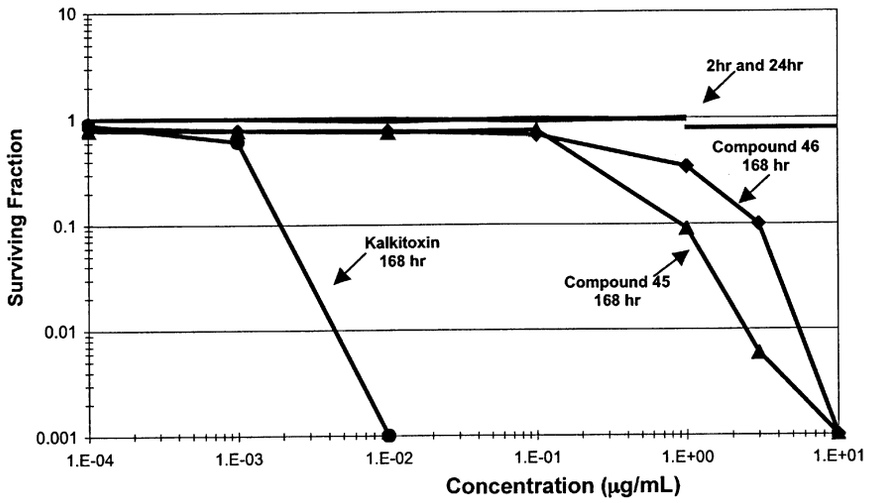


Fig. 2 Clonogenic dose response with HCT-116 cells for kalkitoxin, **45** and **46**.

(2*R*,2′*S*,4′*S*)-1-(5′-Benzyloxy-2′,4′-dimethylpentanoyl)-2-hydroxymethylpyrrolidine (10)

To a solution of diisopropylamine (1.12 mL, 8.0 mmol) in THF (2 mL) was added *n*-BuLi (2.5 M, 3.2 mL, 8 mmol) at 0 °C, and the resulting solution was stirred at 0 °C for 30 min. A solution of **8** (0.495 g, 3.15 mmol) in THF (3 mL) was added at 0 °C, the mixture was stirred for 1 h at room temperature, HMPA (1.4 mL, 8.0 mmol) was added, and the mixture was cooled to −78 °C. Iodide **9** (1.2 g, 4.1 mmol) was added, and the mixture was stirred for 1 h at −78 °C and allowed to warm to room temperature during 3 h. After stirring for 1 h at room temperature the reaction was quenched with saturated NH_4Cl solution and the mixture was extracted with EtOAc (100 mL). The organic layer was washed with brine, dried over $MgSO_4$, and concentrated. Purification of the residual oil by flash column chromatography (hexanes/ethyl acetate, 3 : 2) gave **10** as a colorless oil (0.738 g, 74%): $[\alpha]_D^{20}$ +50.4 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29 (m, 5H), 5.23 (br, 1H), 4.47 (q, J = 8.0 Hz, 2H), 4.18 (m, 1H), 3.55–3.25 (m, 6H), 2.68 (m, 1H), 2.05–1.43 (m, 7H), 1.09 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.3, 138.5, 128.3, 127.6, 127.5, 76.2, 73.1, 67.6, 60.9, 47.5, 38.0, 35.7, 31.4, 28.1, 24.3, 17.8, 17.5; IR (film, cm^{-1}) 2293, 2960, 2929, 2872, 1616, 1454, 1435, 1361, 1093; HRMS (FAB, M + H^+) calcd for $C_{19}H_{30}NO_3$ 320.2226, found 320.2223.

(2*S*,4*S*)-5-Benzyloxy-2,4-dimethylpentanol (11)

A solution of **10** (0.342 g, 1.07 mmol) in 1 N HCl (10 mL) was heated at reflux for 6 h, then was cooled to 0 °C, and treated with 15% NaOH solution for 10 min. The mixture was acidified to pH 3 and extracted with ether. The extract was dried over $MgSO_4$, and concentrated to give the crude carboxylic acid (0.253 g, 100%). To a solution of this acid in dry ether (10 mL) at 0 °C was added $LiAlH_4$ (0.085 g, 2.24 mmol) slowly during 10 min. The mixture was stirred for 30 min at 0 °C and then for 3.5 h at room temperature. The reaction was quenched by careful addition of water (2 mL) at 0 °C, and the mixture was treated with 15% NaOH solution (2 mL) for 20 min and diluted with ether (40 mL). The ethereal solution was washed with water and brine, dried over $MgSO_4$, and concentrated. Purification of the residual oil by flash column chromatography (hexanes/ethyl acetate, 4 : 1) yielded **11** as a colorless oil (0.232 g, 98%): $[\alpha]_D^{20}$ +50.4 (*c* 2.0, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.51(s, 2H), 3.45 (m, 2H), 3.30 (m, 2H), 1.90 (m, 1H), 1.75 (m, 1H), 1.56 (br, 1H), 1.20 (m, 2H), 0.91 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.6, 128.3, 127.6, 127.5, 76.6, 73.3, 68.9, 37.2, 33.0, 30.6, 17.0, 16.3; IR (film, cm^{-1}) 3381, 2956, 2924, 2854, 1454, 1363, 1099, 1029, 735, 697; HRMS (FAB, M + H^+) calcd for $C_{14}H_{23}O_2$ 223.1698, found 223.1696.

Methyl (4*S*,6*S*)-7-benzyloxy-4,6-dimethylhept-2-enoate (13)

To a stirred solution of Dess–Martin periodinane (0.541 g, 1.27 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added a solution of **11** (0.218 g, 0.98 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 9 h, and the reaction was quenched by the addition of 20% $Na_2S_2O_3$ solution (8 mL) and saturated $NaHCO_3$ solution (8 mL). The organic layer was separated and diluted with ether (80 mL), and the ethereal solution was washed with saturated $NaHCO_3$ solution and brine. After drying over $MgSO_4$, the solution was concentrated to give an aldehyde (0.217 g, 99%). To a solution of this aldehyde (16.0 mg, 0.073 mmol) in toluene (5 mL) was added **12** (69 mg, 0.20 mmol) and the mixture was heated to 80 °C for 3 h. After cooling to room temperature, the mixture was diluted with ether (20 mL) and washed with water. The separated organic phase was dried over $MgSO_4$ and concentrated, and the residual oil was purified by flash column chromatography (hexanes/ethyl acetate, 9 : 1) to give **13** (18.6 mg, 93%) as a pale yellow oil: $[\alpha]_D^{20}$ +31.7 (*c* 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 6.87 (dd, J = 15.7, 7.9 Hz, 1H), 5.77 (dd, J = 15.7, 1.1 Hz, 1H), 4.49 (s, 2H), 3.74 (s, 3H), 3.26 1.90 (m, 1H), 1.75 (m, 1H), 1.56 (br, 1H), 1.20 (m, 2H), 0.91 (d, J = 6.1 Hz, 3H), 0.89 (d, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.6, 128.3, 127.6, 127.5, 76.6, 73.3, 68.9, 37.2, 33.0, 30.6, 17.0, 16.3; IR (film, cm^{-1}) 3381, 2956, 2924, 2854, 1454, 1363, 1099, 1029, 735, 697; HRMS (FAB, M+H^+) calcd for $C_{14}H_{23}O_2$ 223.1698, found 223.1696.

Methyl (3*S*,4*S*,6*S*)-7-benzyloxy-3,4,6-trimethylheptanoate (14) and methyl (3*R*,4*S*,6*S*)-7-benzyloxy-3,4,6-trimethylheptanoate (15)

To a suspension of CuBr·DMS (0.042 g, 0.20 mmol) in THF (1 mL) was added MeLi (1.0 M solution, 0.4 mL, 0.4 mmol) at −40 °C, and the mixture was stirred for 20 min. To this mixture at −40 °C were added chlorotrimethylsilane (0.032 mL, 0.25 mmol), HMPA (0.044 mL, 0.25 mmol) and **13** (0.014 g, 0.051 mmol), and the mixture was stirred for 1.5 h at 0 °C. The reaction was quenched by the addition of saturated NH_4Cl solution, and the mixture was extracted with ether. The extract was dried over $MgSO_4$ and concentrated. Flash column chromatography (hexanes/ethyl acetate, 9 : 1) of the residual oil gave a mixture (0.004 g, 29%, 6 : 4 ratio) of **14** and **15**: ^{1}H NMR (asterisk denotes signals due to minor diastereomer, 300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.49 (s, 2H), 3.66 (s, 3H), 3.65* (s, 3H), 3.33* (m, 2H), 3.27 (m, 2H), 2.31* (m, 1H), 2.29 (m, 1H), 2.09 (m, 1H), 2.07* (m, 1H), 1.99 (m, 1H), 1.99* (m, 1H), 1.81* (m,

1H), 1.81* (m, 1H), 1.65 (m, 1H), 1.65* (m, 1H), 0.90* (d, J = 4.9 Hz, 3H), 0.88 (d, J = 4.4 Hz, 3H), 0.87 (d, J = 4.4 Hz, 3H), 0.85* (d, J = 4.7 Hz, 3H), 0.83 (d, J = 4.2 Hz, 3H), 0.79* (d, J = 4.7 Hz, 3H).

(1′*R*,2′*R*,2*S*,4*S*)-5-Benzyloxy-2,4-dimethylpentanoic acid (2′-hydroxy-1′-methyl-2′-phenylethyl)methylamide (17)

To a solution of lithium chloride (0.58 g, 13.7 mmol) and diisopropylamine (0.404 g, 4.0 mmol) in THF (2 mL) at −78 °C was added *n*-BuLi (2.1 M solution in hexane, 1.80 mL, 3.80 mmol) and the mixture was stirred for 10 min at −78 °C and then for 15 min at 0 °C. To this mixture at −78 °C was added a solution of **16** (0.450 g, 2.04 mmol) in THF (2 mL), and the mixture was stirred for 1 h at −78 °C, for 15 min at 0 °C and for 5 min at room temperature. The solution was cooled to 0 °C and a solution of **9** (0.298 g, 1.03 mmol) in THF (1 mL) was added. The mixture was stirred for 10 min at 0 °C and for 15 h at room temperature, and the reaction was quenched by the addition of saturated NH_4Cl solution. The mixture was extracted with EtOAc (60 mL × 3) and the extract was washed with water, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by flash column chromatography (hexanes/ethyl acetate, 3 : 2) gave **17** (0.451 g, 98%) as a pale yellow oil: $[a]_D^{20}$ −49.9 (*c* 1.1, $CHCl_3$); ^{1}H NMR (4 : 1 rotamer ratio, asterisk denotes signals due to minor rotamer, 300 MHz, $CDCl_3$) δ 7.31 (m, 10H), 4.58 (m, 1H), 4.56* (m, 1H), 4.50 (m, 3H), 4.40* (m, 2H), 4.09* (m, 1H), 3.32* (m, 2H), 3.21 (m, 2H), 3.09* (m, 1H), 2.88* (s, 3H), 2.73 (s, 3H), 2.70 (m, 1H), 1.86* (m, 1H), 1.70 (m, 1H), 1.45* (m, 2H), 1.40 (m, 2H), 1.09 (d, J = 6.9 Hz, 3H), 1.05* (overlapped, 3H), 1.03 (d, J = 6.8 Hz, 3H), 0.96* (m, 6H), 0.90 (d, J = 6.7 Hz, 3H); ^{13}C NMR (4 : 1 rotamer ratio, asterisk denotes signals due to minor rotamer, 75 MHz, $CDCl_3$) δ 179.0, 177.5*, 142.5, 141.4*, 138.5, 128.6*, 128.2, 128.1, 127.5, 127.4, 126.8*, 126.2, 76.3, 76.2*, 75.8, 75.1, 73.0, 72.9*, 58.6, 57.7*, 38.2, 34.2, 33.4*, 32.8*, 31.3, 29.6, 26.8*, 18.1*, 17.7*, 17.5, 17.3, 15.5*, 14.3; IR (film, cm^{-1}) 3384, 2956, 2929, 2871, 1617, 1455, 1097; HRMS (CI, M+H$^+$) calcd for $C_{24}H_{34}NO_3$ 384.2539, found 384.2541.

(3*R*,5*R*)-6-Benzyloxy-3,5-dimethylhexan-2-one (18)

To a solution of **17** (0.115 mg. 0.30 mmol) in THF (5 mL) at −78 °C was slowly added MeLi (0.55 mL, 1.3 M in hexane), and the mixture was stirred for 5 min at −78 °C and for 10 min at 0 °C. To the mixture at 0 °C was added a solution of diisopropylamine (0.06 mL, 0.043 mmol) in THF (1 mL), and the mixture was stirred for 15 min, diluted with EtOAc (20 mL) and washed with NH_4Cl solution. The separated organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. Flash column chromatography (hexanes/ethyl acetate, 4 : 1) of the residue gave **18** (0.065 g, 93%) as a colorless oil: $[a]_D^{20}$ +1.1 (*c* 1.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 5H), 4.49 (s, 2H), 3.28 (dd, J = 6.1, 1.3 Hz, 2H), 2.61 (m, 1H), 2.12 (s, 3H), 1.79 (m, 1H), 1.47 (m, 2H), 1.06 (d, J = 7.0 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.9, 138.5, 128.3, 127.5, 75.8, 73.0, 44.7, 36.7, 31.3, 27.9, 17.0, 16.2; IR (film, cm^{-1}) 2928, 2854, 1714, 1456, 1362, 1097; HRMS (CI, M$^+$) calcd for $C_{15}H_{22}O_2$, 234.1620, found 234.1617.

Ethyl (2*E*,4*R*,6*R*)-7-benzyloxy-3,4,6-trimethylhept-2-enoate (20)

To a solution of NaH (60% dispersion in mineral oil, 0.06 g, 1.50 mmol) in toluene (2 mL) at room temperature was added a solution of **19** (0.38 g, 1.50 mmol) in toluene (2 mL). The mixture was stirred for 1 h, and a solution of **18** (0.065 g, 0.28 mmol) in toluene (2 mL) was added. The mixture was heated for 8 h at 70 °C, cooled to room temperature, and diluted with ether (30 mL). The mixture was washed with NH_4Cl solution, and the separated organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. Column chromatography (hexanes/ethyl acetate, 93 : 7) of the residue gave **20** (0.078 g, 93%) as an oil (*E* : *Z* = 11 : 1): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 5.67 (s, 1H), 4.48 (s, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.27 (m, 2H), 2.29 (m, 1H), 2.09 (s, 3H), 1.79 (m, 1H), 1.44 (m, 1H), 1.26 (t, J = 7.2 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 164.6, 138.7, 128.3, 127.5, 127.4, 115.0, 75.5, 73.0, 59.4, 41.3, 38.8, 31.2, 19.0, 17.5, 15.5, 14.3. IR (film, cm^{-1}) 2962, 2928, 2854, 1715, 1643, 1455, 1222, 1158, 1096.

Ethyl (3*S*,4*S*,6*S*)-7-benzyloxy-3,4,6-trimethylheptanoate (22) and ethyl (3*R*,4*S*,6*S*)-7-benzyloxy-3,4,6-trimethylheptanoate (23)

To a solution of **20** (0.036 g, 0.118 mmol) in EtOH (0.05 mL) and diglyme (0.05 mL) were added **21** (1.3 mg, 2.8 μmol) and $CoCl_2 \cdot 6H_2O$ (0.85 mg, 3.5 μmol). The blue solution was degassed by three freeze–thaw cycles, $NaBH_4$ (9.8 mg, 0.26 mmol) was added, and the mixture was degassed again by three freeze–thaw cycles. The mixture was stirred for 3 days at room temperature, diluted with CH_2Cl_2 (10 mL) and washed with water. The separated organic phase was washed with saturated NaCl solution, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to give a 1.6 : 1 mixture of **22** and **23** (0.011 g, 30%). The ^{1}H NMR spectrum of the mixture of diastereomers was essentially identical to that of the homologous methyl esters **14** and **15**.

(1′*S*,2′*S*,2*R*)-4-Benzyloxy-*N*-(2′-hydroxy-1′-methyl-2′-phenylethyl)-2,*N*-dimethylbutyramide (26)

In a 100 mL round-bottomed flask was placed LiCl (2.16 g, 54.8 mmol) and the flask was flame-dried under argon. To the flask at −78 °C were added dry THF (8 mL) and diisopropylamine (2.24 mL, 16 mmol), and the resulting suspension was stirred as *n*-BuLi (7.6 mL, 15.2 mmol, 2.0 M hexane solution) was added. The mixture was stirred for 10 min at −78 °C and for 15 min at 0 °C, and was then cooled again to −78 °C as a solution of **25** in THF (8 mL) was added slowly by cannula. The mixture was stirred for 1 h at −78 °C, for 15 min at 0 °C and for 5 min at ambient temperature. To this mixture at 0 °C was added a solution of 2-benzyloxy-1-iodoethane (1.15 g, 4.4 mmol) in THF (4 mL), the mixture was stirred for 15 h at ambient temperature, and the reaction was quenched by the addition of aqueous NH_4Cl. The solution was extracted with EtOAc (200 mL × 2) and the extract was washed with NH_4Cl solution and brine. The extract was dried over $MgSO_4$, the solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (hexanes/ethyl acetate, 3 : 2) to give **26** (1.392 g, 98%) as a colorless oil: $[a]_D^{20}$ = +52.8 (*c* 0.8, $CHCl_3$); ^{1}H NMR (7 : 3 rotamer mixture, asterisk denotes signals due to minor rotamer, 300 MHz, $CDCl_3$) δ 7.29 (m, 10H), 4.78–4.40 (m, 4H), 4.12* (m, 1H), 3.68* (m, 1H), 3.55 (m, 1H), 3.45 (m, 1H), 3.29 (m, 1H), 3.21* (m, 1H), 2.88 (m, 1H), 2.83 (s, 3H), 2.81* (s, 3H), 2.25* (m, 1H), 1.93 (m, 1H), 1.68* (m, 1H), 1.65 (m, 1H), 1.1 (m, 6H).; ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.1, 176.9*, 142.4, 141.4*, 138.1, 128.3*, 128.1, 128.0*, 127.8, 127.5, 127.4*, 127.3, 127.2*, 126.7, 126.1*, 75.8, 75.0*, 72.6, 68.2*, 67.7, 57.9, 33.8, 32.8, 32.3*, 26.6*, 18.1*, 17.1, 15.5*, 14.1.; IR (film, cm^{-1}) 3386, 2968, 2932, 2869, 1618, 1454, 1089; HRMS (CI, M$^+$) calcd for $C_{22}H_{30}O_3N$ 356.2226, found 356.2224.

(2*R*)-4-Benzyloxy-2-methylbutanol (27)

To a solution of diisopropylamine (8.45 mL, 60.3 mmol) in THF (80 mL) at 0 °C was added *n*-BuLi (2.1 M, 28.7 mL, 60.3 mmol) at 0 °C and the resulting solution was stirred for 20 min. Borane–ammonia complex (90%, 1.90 g, 61.5 mmol) was added in one portion, and the suspension was stirred at 0 °C for 20 min and then warmed to room temperature. After 15 min, the

suspension was cooled to 0 °C and a solution of **26** (4.28 g, 12.06 mmol) in THF (12 mL) was added *via* cannula. The mixture was warmed to room temperature after 10 min, held at that temperature for 2.5 h, and then cooled to 0 °C while 3 N HCl was added slowly to quench excess hydride. The biphasic solution was stirred for 30 min at 0 °C and for 15 min at room temperature, and was then separated. The aqueous layer was extracted with ether and the ethereal extract was washed with brine, dried, and concentrated. Purification of the residual oil by flash column chromatography (hexanes/ethyl acetate, 2 : 1) afforded **27** as a colorless oil (2.11g, 90%): $[\alpha]_D^{20}$ +10.4 (*c* 7.5, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.38 (m, 5H), 4.51 (s, 2H), 3.38–3.62 (m, 4H), 2.94 (bs, 1H), 1.66–1.83 (m, 2H), 1.49–1.59 (m, 1H), 0.92 (d, 3H, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.9, 128.3, 127.6, 127.5, 72.9, 68.5, 67.8, 33.8, 17.0; IR (film, cm^{-1}) 3404, 2926, 2869, 1454, 1097; HRMS (CI, M^+) calcd for $C_{12}H_{18}O_2$ 194.1307, found 194.1303.

(2*R*)-4-Benzyloxy-1-bromo-2-methylbutane (24)

To a solution of **27** (0.25 g, 1.31 mmol) in CH_2Cl_2 (2 mL) was added PPh_3 (0.24g, 1.37 mmol) and *N*-bromosuccinimide (0.36 g, 1.37 mmol) at room temperature. The solution was stirred for 10 min and concentrated under vacuum. The crude solid was triturated with hexane, the mixture was filtered, and the filtrate was concentrated. The residue was purified by flash column chromatography (hexanes/ethyl acetate, 20 : 1) to provide **24** as a colorless oil (0.25 g, 74%): $[\alpha]_D^{20}$ −4.7 (*c* 0.32, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29–7.42 (m, 5H), 4.54 (s, 2H), 3.57 (dt, J = 3.3, 0.1 Hz, 2H), 3.47 (dd, J = 9.9, 4.8 Hz, 1H), 3.40 (dd, J = 9.9, 5.7 Hz, 1H), 2.03–2.11 (m, 1H), 1.83 (ddd, J = 12.9, 12.9, 6.3 Hz, 1H), 1.59 (ddd, J = 13.8, 13.8, 6.3 Hz, 1H), 1.07 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.9, 128.8, 128.0, 73.4, 68.3, 42.0, 35.1, 32.6, 19.2; IR (film, cm^{-1}) 2961, 2858, 1454; HRMS (FAB, M + H^+) calcd for $C_{12}H_{18}OBr$ 259.0521, found 259.0532.

(3′*S*,4*S*,5′*S*)-3-(7′-Benzyloxy-3′,5′-dimethylheptanoyl)-4-phenyloxazolidin-2-one (31)

To a suspension of magnesium (0.52 g, 21.4 mmol) in THF (10 mL) was added 1,2-dibromoethane (0.0384 mL, 0.44 mmol) and the mixture was heated at reflux for 10 min and then cooled to room temperature. A solution of **24** (2.29 g, 8.91 mmol) in THF (2 mL) was added *via* syringe, the mixture was heated at reflux for 20 min, then was cooled and added to a solution of CuBr·DMS (1.83 g, 8.91 mmol) in THF (8 mL) at −78 °C. The suspension was warmed to −20 °C, stirred for 25 min and cooled again to −78 °C. To this suspension was added dropwise a solution of **29** (0.68 g, 2.94 mmol) in THF (6 mL), the dark brown suspension was stirred for 2.5 h at −78 °C, and then was slowly warmed to −30 °C during 45 min. The reaction was quenched with saturated NH_4Cl, the mixture was extracted with ether, and the extract was washed with brine, dried over $MgSO_4$, and concentrated. Purification of the residual oil by flash column chromatography (hexanes/ethyl acetate, 3 : 1) gave **29** (1.16 g, 96%) as a colorless oil: $[\alpha]_D^{20}$ +20.0 (*c* 2.30, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29–7.45 (m, 10H), 5.47 (dd, J = 8.7, 3.6 Hz, 1H), 4.71 (t, J = 8.7 Hz, 1H), 4.52 (s, 2H), 4.30 (dd, J = 9.0, 3.9 Hz, 1H), 3.50 (dt, J = 7.5, 0.9 Hz, 2H), 2.96 (dd, J = 15.9, 5.4 Hz, 1H), 2.76 (dd, J = 15.9, 8.1 Hz, 1H), 2.09–2.22 (m, 1H), 1.66–1.75 (m, 1H), 1.58 (ddd, J = 12.6, 12.6, 6.6 Hz, 1H), 1.44 (ddd, J = 13.2, 13.2, 6.9 Hz, 1H), 1.22 (ddd, J = 13.5, 9.6, 4.5 Hz, 1H), 1.09 (ddd, J = 13.8, 9.3, 4.8 Hz, 1H), 0.88 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 153.6, 139.2, 138.6, 129.1, 128.6, 128.3, 127.6, 127.4, 125.9, 72.8, 69.8, 68.4, 57.5, 44.2, 43.2, 37.4, 27.1, 19.1; IR (film, cm^{-1}) 2925, 1782, 1705; HRMS (FAB, M + H^+) calcd for $C_{25}H_{32}NO_4$ 410.2331, found 410.2339.

(4*S*,2′*S*,3′*S*,5′*S*)-3-(7′-Benzyloxy-2′,3′,5′-trimethylheptanoyl)-4-phenyloxazolidin-2-one (32)[9] and (4*S*,2′*R*,3′*S*,5′*S*)-3-(7′-benzyloxy-2′,3′,5′-trimethylheptanoyl)-4-phenyloxazolidin-2-one (33)

To a suspension of magnesium (0.10 g, 4.14 mmol) in THF (2 mL) was added 1,2-dibromoethane (0.0074 mL, 0.086 mmol), and the mixture was heated at reflux for 10 min and then cooled to room temperature. A solution of **24** (0.443 g, 1.72 mmol) in THF (0.5 mL) was added *via* syringe, and the mixture was heated to reflux for 20 min and then cooled to −78 °C. This mixture was added to a solution of CuBr·DMS (0.34 g, 1.72 mmol) in THF (2 mL), and the resulting suspension was warmed to −20 °C, stirred for 25 min and cooled again to −78 °C. To the suspension was added dropwise a solution of **29** (0.14 g, 0.57 mmol) in THF (1.5 mL), and the suspension was stirred for 2.5 h at −78 °C and was then slowly warmed to −30 °C during 45 min. The mixture was cooled again to −78 °C, MeI (0.54 mL, 8.6 mmol) was added, and the mixture was allowed to warm to room temperature and was stirred overnight. Saturated NH_4Cl was added to quench the reaction, and the mixture was extracted with ether. The ether extract was washed with brine, dried over $MgSO_4$, and concentrated. Flash column chromatography (hexanes/ethyl acetate, 5 : 1) of the residue gave **32** (0.117 g, 50%) and **33** (0.038 g, 14%).

Recrystallization of **32** from warm hexanes gave colorless crystals: mp 75–76 °C; $[\alpha]_D^{20}$ +49.7 (*c* 1.65, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27–7.41 (m, 10H), 5.40 (dd, J = 8.4, 3.3 Hz, 1H), 4.62 (t, J = 8.7 Hz, 1H), 4.50 (s, 2H), 4.22 (dd, J = 9.0, 3.3 Hz, 1H), 3.70 (quint, J = 7.2 Hz, 1H), 3.51 (t, J = 6.6 Hz, 2H), 1.84–1.92 (m, 1H), 1.46–1.71 (m, 3H), 1.15 (ddd, J = 10.5, 10.5, 3.6 Hz, 2H), 1.04 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H), 0.84 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 153.4, 139.3, 138.6, 129.1, 128.5, 128.3, 127.6, 127.4, 125.6, 72.9, 69.6, 68.4, 57.7, 43.1, 39.6, 37.9, 32.7, 27.0, 18.9, 17.8, 13.6; IR (film, cm^{-1}) 2925, 1780, 1704; HRMS (FAB, M + H^+) calcd for $C_{26}H_{34}NO_4$ 424.2488, found 424.2509.

Data for **33**: $[\alpha]_D^{20}$ − 20.6 (*c* 1.88, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.41 (m, 10H), 5.46 (dd, J = 9.0, 4.5 Hz, 1H), 4.68 (t, J = 9.0 Hz, 1H), 4.51 (s, 2H), 4.28 (dd, J = 9.0, 4.5 Hz, 1H), 3.68 (quint, J = 6.6 Hz, 1H), 3.48 (t, J = 6.6 Hz, 2H), 1.89–1.97 (m, 1H), 1.46–1.62 (m, 2H), 1.36–1.42 (m, 1H), 1.16 (ddd, J = 13.5, 10.5, 3.6 Hz, 1H), 1.05 (d, J = 6.9 Hz, 3H), 0.83–0.96 (m, 1H), 0.74 (d, J = 6.3 Hz, 3H), 0.62 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.0, 153.8, 139.6, 139.1, 129.4, 129.1, 128.8, 128.0, 127.9, 126.8, 73.3, 69.9, 69.0, 58.1, 43.2, 42.9, 38.1, 33.4, 27.6, 19.3, 14.7, 12.3; IR (film, cm^{-1}) 2926, 1780, 1704; HRMS (FAB, M + H^+) calcd for $C_{26}H_{34}NO_4$ 424.2488, found 424.2490.

(4*S*,2′*S*,3′*S*,5′*S*)-3-(7′-Oxo-2′,3′,5′-trimethylheptanoyl)-4-phenyloxazolidin-2-one (34)

To a solution of **32** (0.753 g, 1.78 mmol) in EtOAc (12 mL) at room temperature was added $Pd(OH)_2$ on carbon (Pearlman's catalyst, 0.116 g), and the mixture was stirred for 2 h under an atmosphere of hydrogen. The catalyst was filtered off and the filtrate was concentrated. Flash column chromatography (hexanes/ethyl acetate, 1 : 1) of the residue gave (4*S*,2′*S*,3′*S*,5′*S*)-3-(7′-hydroxy-2′,3′,5′-trimethylheptanoyl)-4-phenyloxazolidin-2-one as a pale yellow oil (0.60 g, 100%): $[\alpha]_D^{20}$ +68.6 (*c* 2.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.41 (m, 5H), 5.42 (dd, J = 8.4, 3.3 Hz, 1H), 4.66 (t, J = 8.7 Hz, 1H), 4.24 (dd, J = 9.0, 3.3 Hz, 1H), 3.61–3.73 (m, 3H), 1.83–1.92 (m, 1H), 1.62–1.64 (m, 1H), 1.34–1.55 (m, 3H), 1.09–1.20 (m, 2H), 1.03 (d, J = 6.9 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.3, 153.4, 139.2, 129.1, 128.5, 125.6, 69.6, 60.8, 57.7, 43.0, 41.0, 40.0, 32.6, 26.6, 18.8, 17.7, 13.7; IR (film, cm^{-1}) 3385, 2926, 1779, 1702; HRMS (FAB, M + H^+) calcd for $C_{19}H_{28}O_4N$ 334.2018, found 334.2018.

To a solution of oxalyl chloride (0.261 mL, 3.0 mmol) in CH_2Cl_2 (3 mL) at −78 °C was added DMSO (0.284 mL, 4.0 mmol) and CH_2Cl_2 (3 mL). After stirring the mixture for 15 min, a solution of the alcohol prepared above (0.60 g, 1.78 mmol) in CH_2Cl_2 (8 mL) was added dropwise. The solution was stirred at −78 °C for 15 min, a solution of NEt_3 (1.09 mL, 8.0 mmol) in CH_2Cl_2 (4 mL) was added and the mixture was slowly warmed to 0° C during 30 min. The reaction was quenched by addition of saturated NH_4Cl, the layers were separated and the aqueous layer was extracted with ether. The extract was washed with brine, dried over $MgSO_4$, and concentrated. Flash column chromatography (hexanes/ethyl acetate, 3 : 1) of the residue yielded **34** as a pale yellow oil (0.51 g, 85%): $[a]_D^{20}$ +69.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (t, *J* = 2.1 Hz, 1H), 7.26–7.41 (m, 5H), 5.42 (dd, *J* = 8.7, 3.3 Hz, 1H), 4.67 (t, *J* = 9.0 Hz, 1H), 4.25 (dd, *J* = 9.0, 3.6 Hz, 1H), 3.69 (quin, *J* = 6.9 Hz, 1H), 2.29–2.33 (m, 2H), 2.08–2.19 (m, 1H), 1.84–1.93 (m, 1H), 1.28 (ddd, *J* = 13.5, 10.8, 3.0, 1H), 1.10 (ddd, *J* = 14.1, 10.8, 3.6, 1H), 1.03 (d, *J* = 6.9 Hz, 3H), 0.96 (d, *J* = 6.9 Hz, 3H), 0.90 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 203.0, 176.6, 153.8, 140.0, 129.6, 129.1, 126.1, 70.1, 58.2, 52.6, 43.5, 40.0, 33.0, 25.8, 19.4, 18.1, 14.2; IR (film, cm^{-1}) 2964, 2930, 1777, 1721, 1702, 1383; HRMS (FAB, M + H^+) calcd for $C_{19}H_{26}NO_4$ 332.1862, found 332.1872.

(4*S*,2′*S*,3′*S*,5′*S*)-3-(7′-Methylamino-2′,3′,5′-trimethylheptanoyl)-4-phenyloxazolidin-2-one (35)

To a solution of **34** (0.50g, 1.51 mmol) in MeOH (10 mL) at room temperature was added $MeNH_2$·HCl (0.204 g, 3.02 mmol), $MeNH_2$ (2 M solution in THF, 2.76 mL, 4.53 mmol) and Na_2SO_4 (0.4 g). The mixture was stirred for 20 min and then cooled to 0 °C as a solution of $NaCNBH_3$ (0.142 g, 2.26 mmol) in MeOH (1.5 mL) was added. The mixture was warmed to room temperature and stirred for 1 h, MeOH was removed under vacuum and the residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 20 : 1) to give **35** (0.314 g, 61%) as a viscous oil: $[a]_D^{20}$ +52.8 (*c* 1.70, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.44 (m, 5H), 5.51 (dd, *J* = 8.4, 3.6 Hz, 1H), 4.76 (t, *J* = 8.8 Hz, 1H), 4.26 (dd, *J* = 8.8, 3.6 Hz, 1H), 3.74 (quint, *J* = 6.8 Hz, 1H), 2.98 (dd, *J* = 8.4, 6.0 Hz, 2H), 2.72 (s, 3H), 1.85–1.95 (m, 1H), 1.60–1.80 (m, 3H), 1.22–1.34 (m, 2H), 1.14–1.16 (m, 1H), 1.08 (d, *J* = 7.2 Hz, 3H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.91 (d, *J* = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 154.2, 139.7, 129.6, 129.0, 126.0, 70.4, 58.3, 48.6, 43.4, 39.8, 34.2, 33.9, 33.0, 28.3, 18.8, 17.9, 14.3; IR (film, cm^{-1}) 2965, 2930, 2333, 2173, 1777, 1703, 1384; HRMS (CI, M^+) calcd for $C_{20}H_{30}N_2O_3$ 346.2256, found 346.2249.

(2*R*,3′*S*,5′*S*,6′*S*,4″*S*)-2,*N*-Dimethyl-*N*-[3′,5′,6′-trimethyl-7′-oxo-7′-(2″-oxo-4″-phenyloxazolidin-3-yl)heptyl]butyramide (37)

To a solution of **35** (0.314 g, 0.90 mmol) in DMF (4 mL) at 0 °C was added a solution of (*R*)-2-methylbutyric acid (**36**, 0.183 g, 1.80 mmol) in DMF (5 mL), followed by 1-hydroxy-7-azabenzotriazole (HOAt, 0.245 g, 1.80 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 0.345 g, 1.80 mmol) and diisopropylethylamine (DIPEA, 0.313 mL, 1.80 mmol). The mixture was kept at room temperature for 12 h and then diluted with EtOAc. The separated organic phase was washed with 1 N HCl and brine, and was dried over $MgSO_4$ and concentrated. Flash column chromatography (hexanes/ethyl acetate, 3 : 2) of the residual oil gave **37** (0.326 g, 84%) as a pale yellow oil: $[a]_D^{20}$ +40.0 (*c* 0.50, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26–7.40 (m, 5H), 5.43 (dd, *J* = 8.4, 3.0 Hz, 1H), 4.67 (t, *J* = 8.7 Hz, 1H), 4.25 (dd, *J* = 8.4, 4.2 Hz, 1H, rotamer), 4.24 (dd, *J* = 8.4, 3.3 Hz, 1H, rotamer), 3.66–3.72 (m, 1H), 3.37 (ddd, *J* = 10.2, 6.0, 3.6 Hz, 1H), 3.28 (ddd, *J* = 10.4, 6.0, 4.0 Hz, 1H), 3.00 (s, 3H, rotamer), 2.91 (s, 3H, rotamer), 2.50–2.63 (m, 1H), 1.81–1.90 (m, 1H), 1.64–1.75 (m, 1H), 1.31–1.48 (m, 3H), 1.01–1.11 (m, 9H), 0.84–0.93 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9, 176.6, 176.5, 153.9, 139.8, 139.6, 129.6, 129.1, 129.0, 126.1, 70.1, 58.2, 48.4, 46.5, 43.5, 40.2, 37.8, 37.7, 37.6, 35.9, 35.6, 34.1, 33.2, 33.0, 28.4, 27.8, 27.5, 19.2, 18.2, 18.0, 17.5, 14.4, 14.1, 12.6, 12.4; IR (film, cm^{-1}) 2926, 1779, 1704, 1639, 1456; HRMS (FAB, M + H^+) calcd for $C_{25}H_{39}N_2O_4$ 431.2910, found 431.2912.

(2*R*,3′*S*,5′*S*,6′*S*)-2,*N*-Dimethyl-*N*-[7′-hydroxy-3′,5′,6′-trimethylheptyl]butyramide (38)

To a solution of **37** (0.084 g, 0.20 mmol) in THF (1.7 mL) at 0 °C was added $LiBH_4$ (2 M solution in THF, 0.30 mL, 0.6 mmol), followed by MeOH (0.042 mL, 1.04 mmol). The solution was stirred for 40 min at 0 °C, for 10 min at room temperature, and the reaction was quenched with water. The mixture was extracted with EtOAc, and the extract was washed with brine, dried over $MgSO_4$, and concentrated. Flash column chromatography (hexanes/ethyl acetate, 3 : 2) of the residue afforded a mixture of rotamers of **38** as a pale yellow oil (0.042 g, 80%): $[a]_D^{20}$ −36.2 (*c* 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.58–3.62 (m, 1H), 3.25–3.48 (m, 3H), 3.02 (s, 3H, rotamer), 2.93 (s, 3H, rotamer), 2.58 (m, 1H), 1.92 (s, 1H), 1.33–1.73 (m, 6H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.09 (d, *J* = 6.8 Hz, 3H), 0.84–0.91 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 176.7, 66.3, 66.2, 48.5, 46.7, 41.5, 41.3, 40.5, 40.2, 37.8, 37.6, 35.7, 35.6, 34.1, 31.8, 31.7, 28.8, 28.6, 27.8, 27.4, 19.6, 19.4, 18.1, 17.5, 17.4, 17.3, 13.8, 13.6, 12.6, 12.4; IR (film, cm^{-1}) 3419, 2961, 1624, 1458; HRMS (FAB, M + H^+) calcd for $C_{16}H_{34}NO_2$ 272.2589, found 272.2605.

(2*R*,3′*S*,5′*S*,6′*S*)-2,*N*-Dimethyl-*N*-[8′-Methoxy-3′,5′,6′-trimethyloct-7-enyl]-butyramide (40)

To a solution of oxalyl chloride (0.073 mL, 0.84 mmol) in CH_2Cl_2 (0.8 mL) at −78 °C was added a solution of DMSO (0.079 mL, 1.12 mmol) in CH_2Cl_2 (0.8 mL). The mixture was stirred for 15 min at −78 °C and a solution of **38** (0.151 g, 0.56 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The mixture was stirred for an additional 15 min at −78 °C, and a solution of NEt_3 (0.314 mL, 2.24 mmol) in CH_2Cl_2 (0.8 mL) was added. The mixture was slowly warmed to −10 °C during 30 min and the reaction was quenched with saturated NH_4Cl. The mixture was extracted with ether, and the extract was washed with brine, dried over $MgSO_4$, and concentrated to give the crude aldehyde which was subjected immediately to the next reaction.

To a suspension of **39** (dried under vacuum at 50 °C, 0.955 g, 2.80 mmol) in THF (4.5 mL) at 0 °C was added *n*-BuLi (1.6 M in hexane, 1.57 mL, 2.52 mmol), and the resulting deep red solution was stirred for 30 min at room temperature. The mixture was cooled to −20 °C and a solution of the crude aldehyde prepared above in THF (2 mL) was added. The mixture was kept at −20 °C for 30 min and then warmed to room temperature. The reaction was quenched with MeOH and the mixture was diluted with ether (100 mL). The separated ethereal layer was washed with saturated NH_4Cl and brine, dried over $MgSO_4$, and concentrated. Flash column chromatography (hexanes/ethyl acetate, 5 : 1) of the residue gave **40** (1 : 1 mixture of *E* and *Z* isomers, 0.086 g, 52% over two steps): $[a]_D^{20}$ −20.4 (*c* 1.60, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.21 (dd, *J* = 12.4, 5.2 Hz, 1H), 5.85 (dd, *J* = 9.6, 6.4 Hz, 1H), 4.60 (dd, *J* = 12.8, 9.2 Hz, 1H), 4.18 (dd, *J* = 10.0, 6.4 Hz, 1H), 3.49–3.55 (s, 6H), 3.39 (dd, *J* = 7.6, 7.6 Hz, 2H), 3.26–3.30 (m, 2H), 3.02 (s, 6H, rotamer), 2.92 (s, 6H, rotamer), 2.50–2.63 (m, 3H), 1.90–2.01 (m, 1H), 1.66–1.74 (m, 2H), 1.25–1.56 (m, 10H), 1.03–1.18 (m, 10H), 0.86–0.99 (m, 18H), 0.75–0.80 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 176.5, 147.1, 146.9, 146.0, 145.8, 111.2, 110.7, 106.8, 106.5, 59.8, 56.4, 56.3, 48.5, 48.4, 46.6, 42.5, 42.4, 38.2, 38.1, 37.8, 37.6, 37.5, 37.4, 36.0, 35.9, 35.7, 35.6, 34.05, 34.00, 33.8, 28.6, 28.4, 27.8, 27.4, 19.8, 19.6, 19.5, 19.2, 19.1,

18.2, 17.5, 15.8, 15.7, 15.6, 12.5, 12.4; IR (film, cm^{-1}) 2929, 1647, 1463; HRMS (CI, M^+) calcd for $C_{18}H_{35}NO_2$ 297.2668, found 297.2663.

(2′*R*,3*R*,4*S*,6*S*)-3,4,6-Trimethyl-8-[methyl-2′-(methylbutyryl)amino]octanoic acid (3)

To a solution of **40** (0.086 g, 0.29 mmol) in CH_3CN (2.1 mL) at room temperature was added 1 N HCl (0.7 mL), and the mixture was stirred for 1 h and then diluted with ether. The ethereal layer was separated and was washed with brine, dried over $MgSO_4$, and concentrated to give the crude aldehyde. To a solution of the aldehyde in MeOH (10 mL) was added 2-methyl-2-butene (1.02 g, 14.5 mmol) followed by a freshly prepared solution of $NaClO_2$ (1.25 M in 20% NaH_2PO_4, 1.16 mL, 1.45 mmol). The mixture was stirred for 2 h at room temperature and then diluted with ether. The separated ethereal layer was washed with brine, dried over $MgSO_4$, and concentrated. Flash column chromatography (hexanes/ethyl acetate/MeOH, 47.5 : 47.5 : 5) of the residue gave **3** as a colorless oil (0.083 g, 96%): $[\alpha]_D^{20}$ −35.0 (*c* 0.40, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24–3.41 (m, 2H), 3.02 (s, 3H, rotamer), 2.93 (s, 3H, rotamer), 2.53–2.62 (m, 1H), 2.35 (dd, *J* = 10.8, 4.8 Hz, 1H), 2.10 (dd, *J* = 9.6, 7.2 Hz, 1H, rotamer), 2.06 (dd, *J* = 9.2, 6.8 Hz, 1H, rotamer), 1.91–2.01 (m, 1H), 1.65–1.74 (m, 1H), 1.30–1.58 (m, 5H), 1.04–1.16 (m, 2H), 1.10 (d, *J* = 6.8 Hz, 3H, rotamer), 1.08 (d, *J* = 6.8 Hz, 3H, rotamer), 0.80–0.94 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.0, 178.8, 177.3, 177.0, 48.6, 46.7, 40.8, 40.6, 38.6, 38.5, 37.8, 37.6, 37.5, 35.8, 35.7, 35.6, 35.4, 34.7, 34.6, 34.3, 28.6, 27.7, 27.4, 19.5, 18.0, 17.4, 17.0, 16.9, 16.44, 16.39, 13.5, 12.4; IR (film, cm^{-1}) 2963, 1728, 1611; HRMS (CI, M^+) calcd for $C_{17}H_{33}NO_3$ 299.2460, found 299.2466.

***S*-Benzyl-N_α-(*tert*-butyloxycarbonyl)cysteine *N*-methoxy-*N*-methylamide (42)**

To a solution of **41** (0.59 g, 1.90 mmol) in THF (38 mL) at 0 °C was added 1-hydroxybenzotriazole (HOBt, 0.282 g, 2.08 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 0.40 g, 2.08 mmol). The mixture was stirred at room temperature for 15 min, and MeNHOMe·HCl (0.208 g, 2.09 mmol) followed by diisopropylethylamine (0.362 mL, 2.08 mmol) were added. The solution was kept at room temperature for 12 h and then concentrated. Flash column chromatography (hexanes/ethyl acetate, 2 : 1) of the residue gave **42** (0.56 g, 84%) as an oil: $[\alpha]_D^{20}$ − 30.2 (*c* 1.04, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25–7.36 (m, 5H), 5.38 (d, *J* = 8.4 Hz, 1H), 4.92 (bs, 1H), 3.76 (s, 2H), 3.74 (s, 3H), 3.22 (s, 3H), 2.82 (dd, *J* = 14.0, 5.6 Hz, 1H), 2.65 (dd, *J* = 14.0, 7.2 Hz, 1H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 155.7, 138.3, 129.4, 128.9, 127.5, 80.2, 62.0, 50.0, 36.7, 33.9, 32.6, 28.8; IR (film, cm^{-1}) 3321, 2976, 1711, 1662, 1169; HRMS (CI, M^+) calcd for $C_{17}H_{26}NO_4N_2S$ 354.1613, found 354.1616.

(3*R*)-4-Benzylthio-3-(*N*-*tert*-butyloxycarbonyl)aminopropionaldehyde (43)

To a solution of **42** (0.632 g, 1.78 mmol) in ether (8 mL) at 0 °C was added $LiAlH_4$ (0.085 g, 2.23 mmol), and the mixture was stirred for 30 min at 0 °C. The mixture was extracted with ether, and the ethereal extract was washed with 1 N HCl and brine, dried over $MgSO_4$, and concentrated. Flash column chromatography (hexanes/ethyl acetate, 2 : 1) of the residue gave **43** (0.478 g, 91%) as a colorless oil: $[\alpha]_D^{20}$ +3.47 (*c* 1.96, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.21–7.34 (m, 5H), 5.39 (m, 1H), 4.24–4.32 (m, 1H), 3.72 (s, 2H), 2.86 (dd, *J* = 13.8, 5.7 Hz, 1H), 2.80 (dd, *J* = 14.1, 6.0 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.2, 155.8, 138.0, 129.4, 129.1, 127.8, 80.8, 59.5, 37.3, 31.0, 28.7; IR (film, cm^{-1}) 3354, 2977, 1709, 1495.

(3*R*)-4-Benzylthio-3-(*N*-*tert*-butyloxycarbonyl)amino-1-butene (44)

To a stirred suspension of methyltriphenylphosphonium bromide (1.27 g, 3.5 mmol) in THF (15 mL) was added KHMDS (0.5 M solution in toluene, 6.48 mL, 3.24 mmol), and the resulting yellow suspension was stirred at room temperature for 1 h and then cooled to −78 °C. A solution of **43** (0.478 g, 1.62 mmol) in THF (10 mL) was added, the cooling bath was removed, and the mixture was allowed to warm to room temperature. The mixture was diluted with ether, and the separated ethereal layer was washed with a half-saturated solution of Rochelle's salt, dried over $MgSO_4$, and concentrated. Purification of the residue by flash column chromatography (hexanes/ethyl acetate, 8 : 1) gave **44** (0.263 g, 55%) as a colorless oil: $[\alpha]_D^{20}$ +12.7 (*c* 2.63, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20–7.32 (m, 5H), 5.78 (ddd, *J* = 17.1, 10.5, 5.4 Hz, 1H), 5.19 (d, *J* = 17.4 Hz, 1H), 5.13 (d, *J* = 10.2 Hz, 1H), 4.80 (bs, 1H), 4.33 (bs, 1H), 3.73 (s, 2H), 2.58 (d, *J* = 6.0 Hz, 2H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.6, 138.4, 137.8, 129.4, 129.0, 127.5, 116.0, 80.0, 52.2, 37.0, 36.8, 28.8; IR (film, cm^{-1}) 3344, 2977, 1701, 1495; HRMS (CI, M^+) calcd. for $C_{16}H_{23}NO_2S$ 293.1449, found 293.1448.

(1″*R*,2′*R*,3*R*,4*S*,6*S*)-3,4,6-Trimethyl-8-[methyl-(2′-methylbutyryl)amino]octanoic acid (1″-benzylsulfanylmethylallyl)amide (45)

To a solution of **44** (0.244 g, 0.833 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added trifluoroacetic acid (TFA, 3 mL), and the mixture was stirred at 0 °C for 30 min. The solvent was removed, and the residue was taken up into CH_2Cl_2. The solution was washed with 5% NaOH aqueous solution and brine, dried over $MgSO_4$, and concentrated to give (3*R*)-4-benzylthio-3-amino-1-butene (**4**) which was used without further purification. To **3** (0.083 g, 0.278 mmol) at room temperature was added a solution of **4** in CH_2Cl_2 (2.5 mL), followed by *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium-hexafluorophosphate (HATU, 0.158 g, 0.417 mmol) and diisopropylethylamine (DIPEA, 0.073 mL, 0.417 mmol). The mixture was kept for 12 h at room temperature and the solvent was removed under vacuum. Flash column chromatography (hexanes/ethyl acetate, 1 : 1) of the residue afforded **45** (0.122 g, 93%): $[\alpha]_D^{20}$ −15.0 (*c* 1.16, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22–7.31 (m, 5H), 6.03 (d, *J* = 8.4 Hz, 1H, rotamer), 5.97 (d, *J* = 8.4 Hz, 1H, rotamer), 5.79 (ddd, *J* = 17.2, 10.4, 5.2 Hz, 1H), 5.18 (d, *J* = 17.2 Hz, 1H), 5.14 (d, *J* = 10.4 Hz, 1H), 4.71 (m, 1H), 3.73 (s, 2H), 3.26–3.41 (m, 2H), 3.00 (s, 3H, rotamer), 2.91 (s, 3H, rotamer), 2.53–2.66 (m, 3H), 2.22 (dt, *J* = 13.6, 3.6 Hz, 1H), 1.92–2.04 (m, 1H), 1.82–1.88 (m, 1H), 1.65–1.74 (m, 1H), 1.31–1.53 (m, 5H), 1.06–1.16 (m, 2H), 1.10 (d, *J* = 6.4 Hz, 3H, rotamer, 1.07 (d, *J* = 6.8 Hz, 3H, rotamer), 0.80–0.89 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.8, 176.5, 172.8, 172.7, 138.5, 138.4, 137.4, 129.4, 129.0, 127.5, 116.2, 50.3, 48.5, 46.5, 41.1, 41.0, 40.9, 40.6, 37.7, 37.6, 36.9, 36.4, 36.1, 35.8, 35.7, 35.6, 34.8, 34.7, 34.1, 28.6, 28.5, 27.8, 27.5, 19.64, 19.59, 18.2, 17.6, 16.9, 16.7, 16.6, 16.5, 12.6, 12.4; IR (film, cm^{-1}) 3302, 2917, 1625; HRMS (CI, M^+) calcd for $C_{28}H_{46}N_2O_2S$ 474.3280, found 474.3283.

(1″*R*,2′*R*,3*R*,4*S*,6*S*)-3,4,6-Trimethyl-8-[methyl-(2′-methylbutyryl)amino]octanoic acid (1″-mercaptomethylallyl)amide (46)

To a solution of sodium (0.023 g, 1 mmol) in liquid NH_3 (2 mL) at −78 °C was added a solution of **45** (0.018 g, 0.042 mmol) in THF (0.5 mL) and the deep blue solution was stirred for 30 min at −78 °C. Solid NH_4Cl was added to quench the reaction, and ammonia was evaporated in a stream of argon. The residue was triturated with CH_2Cl_2, and the separated organic phase was washed with saturated $NaHCO_3$, dried over Na_2SO_4, and concentrated. Flash column chromatography (hexanes/ethyl

acetate/MeOH, 47.5 : 47.5 : 5) of the residue afforded **46** as a colorless oil (0.0125 g, 86%): $[a]_D^{20}$ −4.3 (*c* 0.65, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.93 (d, J = 8.1 Hz, 1H, rotamer), 5.82 (d, J = 8.1 Hz, 1H, rotamer), 5.76 (ddd, J = 16.2, 11.1, 5.1 Hz, 1H), 5.20–5.26 (m, 2H), 4.72–4.83 (m, 1H), 3.21–3.45 (m, 2H), 3.01 (s, 3H, rotamer), 2.92 (s, 3H, rotamer), 2.51–2.85 (m, 3H), 2.25–2.30 (m, 1H), 1.88–2.04 (m, 2H), 1.61–1.75 (m, 2H), 1.28–1.57 (m, 6H), 1.12–1.16 (m, 1H), 1.10 (d, J = 7.8 Hz, 3H, rotamer, 1.07 (d, J = 6.9 Hz, 3H, rotamer), 0.80–0.94 (m, 12H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.9,176.6, 172.8, 172.5, 136.32, 136.26, 117.2, 51.9, 51.7, 48.5, 46.5, 41.2, 41.1, 41.0, 40.5, 37.8, 37.6, 36.1, 35.9, 35.7, 34.7, 34.6, 34.1, 30.0, 29.7, 28.6, 27.8, 27.5, 19.7, 19.6, 18.2, 17.5, 16.9, 16.8, 16.6, 12.6, 12.4; IR (film, cm^{-1}) 3297, 2961, 1640, 1624; HRMS (CI) calcd for $C_{21}H_{40}N_2O_2S$ 384.2810, found 384.2811.

(+)-Kalkitoxin (2)

To a solution of **46** (0.012 g, 0.031 mmol) in CH_2Cl_2 (1 mL) at room temperature was added freshly distilled $TiCl_4$ (0.034 mL, 0.31 mmol), and the orange coloured solution was stirred for 12 h at room temperature. The reaction was quenched by addition of saturated $NaHCO_3$, the mixture was extracted with CH_2Cl_2, and the extract was dried over Na_2SO_4 and concentrated. Flash column chromatography (hexanes/ethyl acetate, 1 : 1) of the residue furnished (+)-kalkitoxin (**2**) as a colorless oil (0.0076 g, 67%): $[a]_D^{20}$ +11.5 (*c* 0.34, $CHCl_3$) [lit.[5] $[a]_D^{25}$ +16 (*c* 0.07, $CHCl_3$)]; ^1H NMR (400 MHz, C_6D_6) δ 5.85 (ddd, J = 16.8, 10.4, 6.0 Hz, 1H), 5.24 (d, J = 17.2 Hz, 1H), 5.01 (d, J = 10.4 Hz, 1H), 4.75 (m, 1H), 3.35 (m, 1H), 2.94 (m, 1H), 2.93 (dd, J = 10.8, 8.4 Hz, 1H), 2.80 (s, 3H, rotamer), 2.71 (dd, J = 10.8, 8.4 Hz, 1H), 2.46–2.55 (m, 1H), 2.42 (s, 3H, rotamer), 2.20–2.37 (m, 2H), 2.06 (m, 1H), 1.84–1.99 (m, 1H), 1.21–1.59 (m, 5H), 1.18 (s, 3H, rotamer), 1.10 (m, 1H), 1.09 (s, 3H, rotamer), 0.69–0.96 (m, 13 H); ^{13}C NMR (100 MHz, C_6D_6) δ 175.5, 175.2, 170.1, 170.0, 138.5, 138.3, 115.5, 115.4, 79.4, 47.9, 46.1, 40.5, 40.4, 39.0, 38.8, 38.6, 38.2, 37.7, 37.6, 37.4, 36.1, 34.7, 34.6, 34.5, 33.6, 28.5, 28.1, 27.7, 19.6, 19.4, 18.4, 17.8, 16.6, 16.5, 12.6, 12.4; IR (film, cm^{-1}) 2961, 2874, 1643, 1463; HRMS (FAB M + H^+) calcd for $C_{21}H_{38}N_2OS$ 367.2783, found 367.2793.

IC_{50} Determination

HCT-116 cells were plated at 5×10^4 cells in T25 tissue culture flasks (Falcon Plastics, New Jersey) with 5 mL media RPMI 1640 (Cellgro, Virginia) supplemented with 15% BCS (Hyclone, Utah), 5% Pen. Strep. and 5% Glutamine (Cellgro). After three days (cells in logarithmic growth phase; 5×10^5 cells/flask), test compound was added to the flasks to achieve concentrations ranging from 10 to 10^{-4} μg mL^{-1}. At day 3, the flasks were washed, trypsinized, spun down and the cells counted for both viable and dead cells using 0.08% trypan blue (Gibco, Maryland). Viable cell number as a function of concentration was plotted and the IC_{50} values determined by interpolation.

Clonogenic assay

HCT-116 cells were seeded at 200 to 20 000 cells in 60 mm dishes. Drug was added to the medium (RPMI + 10% FBS) at concentrations of 1 mg mL^{-1} and 10-fold dilutions thereof. At either 2 h or 24 h, the drug-containing media was removed and fresh media without drug was added. The dishes were incubated for 7 days, media was removed and the colonies were stained with methylene blue. Colonies containing 50 cells or more were counted and the results were normalized to an untreated control. Plating efficiency for the untreated cells was *ca.* 90%. Repeat experiments were carried out to define the cell survival range between 10^0 and 10^{-3} survival. Values were determined for three exposure times (2 h, 24 h, 168 h).

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