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Total Synthesis and Structural Revision of Lucentamycin A

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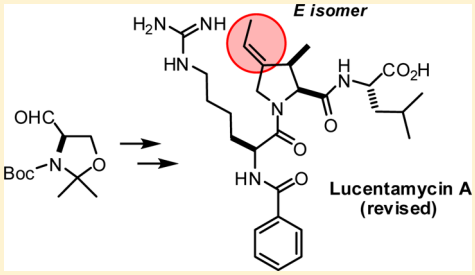
Supporting Information

ABSTRACT: Lucentamycin A is a marine-derived peptide natural product harboring a unique 4-ethylidene-3-methylproline (Emp) subunit. The proposed structure of lucentamycin A and the core Emp residue have recently been called into question through synthesis. Here, we report the first total synthesis of lucentamycin A, which confirms that the ethylidene substituent in Emp bears an *E* geometry, in contrast to the originally assigned *Z* configuration. Synthesis of the desired (*E*)-Emp subunit required the implementation of a novel strategy starting from Garner's aldehyde.



The lucentamycins are a family of tripeptides produced by *Nocardiopsis lucentensis* that harbor 4-ethylidene-3-methylproline (Emp) as a central residue.[1] The reported activity of lucentamycin A against HCT-116 human colon cancer cells coupled with the structural novelty of its proline core has prompted several synthetic efforts.[2] Our group recently completed the synthesis of the putative structure of lucentmycin A (**1**, Figure 1), establishing the need for structural

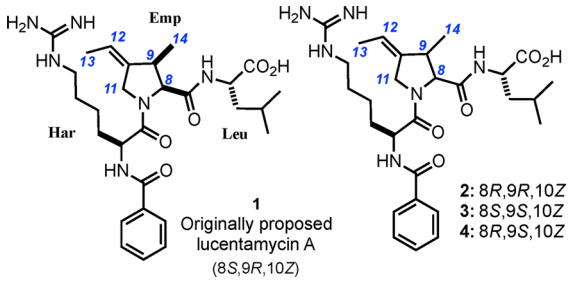


Figure 1. Originally proposed structure of lucentamycin A (**1**) and diastereomers.

revision on the basis of various discrepancies in the NMR spectral regions corresponding to the Emp residue.[2a] Lindsley and co-workers concurrently reported the synthesis of diastereomer **2**, which also showed key spectroscopic differences with the natural product.[2c] On the basis of NMR spectral data from synthetic isomers and the natural isolate, we hypothesized that the absolute stereochemistry of the proline core may be in need of revision. However, synthesis of all four diastereomers of Emp and their corresponding tripeptides revealed that these stereocenters were not the source of misassignment.[2b]

Two-dimensional NMR experiments originally carried out with natural lucentamycin A indicated a ROESY correlation between H-13 and H-11, indicative of a *Z* configuration.[1] However, a weak signal from this interaction combined with the conspicuous absence of correlation between H-12 and H-9 (or H-12 and H-14) left open the possibility of an (*E*)-alkene geometry. During review of this paper, Fenical and co-workers isolated an additional family member, lucentamycin E, and observed ROESY correlations indicative of an (*E*)-Emp configuration.[3] Reexamination of the spectra for **1** supported the same (*E*)-Emp structure for the other lucentamycin peptides. Here, we report the first total synthesis of lucentamycin A and confirmation of the (*E*)-Emp configuration of the natural product. Our synthetic strategy is based on a stereoselective enoate conjugate addition reaction followed by ester α-ethylenation to provide a linear precursor to the desired isomer. A detailed structural analysis of synthetic lucentamycin A resolves recent conflicting reports on the true structure of the natural product. We also prepared the simplified proline analogue for comparative biological studies and found, unexpectedly, that neither compound significantly inhibited the growth of HCT-116 colon cancer cells by XTT assay.

Synthesis of the originally proposed structure of lucentamycin A (**1**) has been reported using conceptually distinct strategies to establish the desired 8*S*,9*R*,10*Z* configuration. Our group had initially explored the utility of a stereoselective ester enolate-Claisen rearrangement for the preparation of highly substituted allylglycine derivatives, as shown in Scheme 1.[2b] These linear precursors were subsequently cyclized to afford various 3-alkyl-4-alkylideneprolines with moderate to high stereoselectivities. One drawback to this strategy is the inability to access the (*E*)-alkene due to the preferred equatorial disposition of the R^1 group in the chairlike transition state.[4] Thus, our first attempts to synthesize the desired compound relied on alkene cross-metathesis in the presence of 2-methyl-2-butene[5] with previously described tripeptides **5** and **6**.[2b] Despite exploration of various reaction conditions, no *E* isomers were detected by NMR. When compound **7** was

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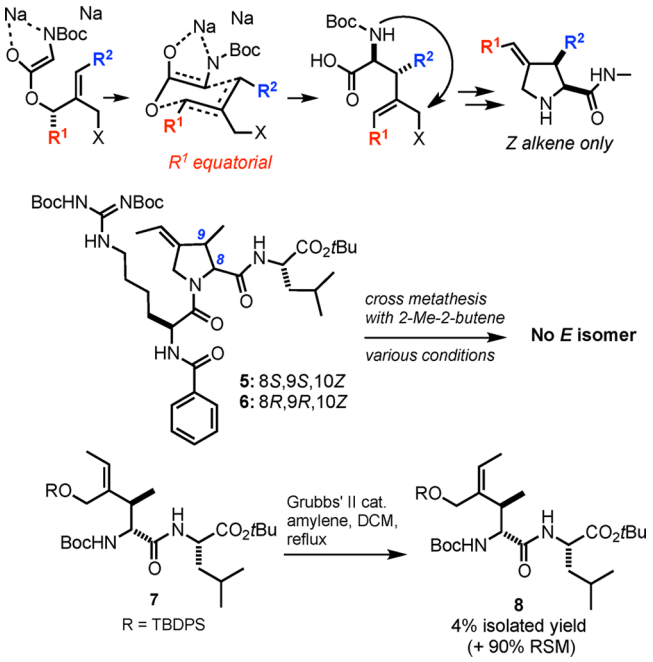
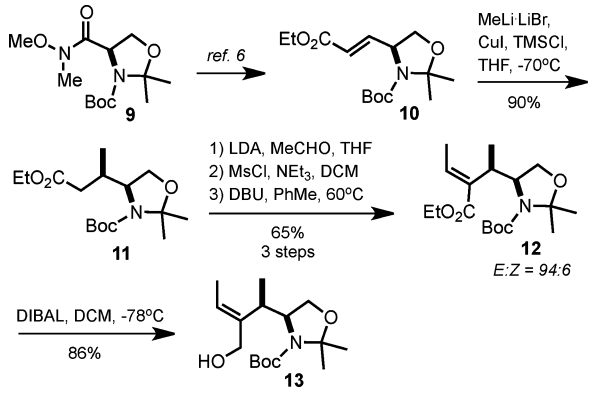
Scheme 1. Attempted Synthesis of Emp Isomers via Ester Enolate-Claisen Rearrangement and Alkene Cross-Metathesis



used as the substrate, we did isolate the alkene inversion product, albeit in very low yield.

The difficulties encountered with producing the *E* isomer by cross-metathesis prompted us to explore an alternative synthetic strategy employing D-serine as a chiral progenitor. As shown in Scheme 2, enoate **10**[6] was subjected to a

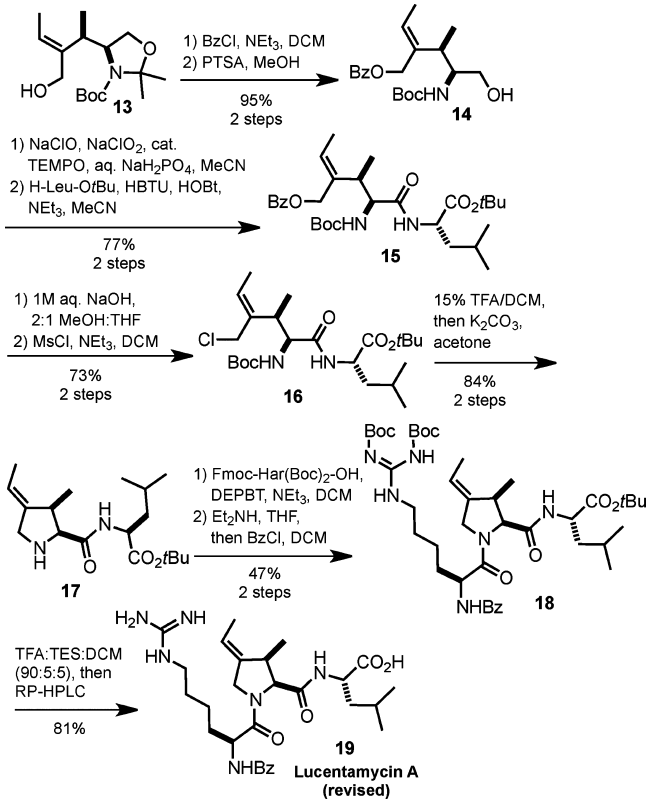
Scheme 2. Synthesis of Allylic Alcohol 13



diastereoselective conjugate addition reaction to afford **11** as a single syn isomer in 90% yield.[7] α-Ethylenation of **11** was carried out using a three-step sequence involving enolate alkylation with acetaldehyde, mesylation of the resulting secondary alcohol, and elimination with DBU. Enoate **12** was obtained in as an inseparable 94:6 *E*:*Z* mixture in 65% overall yield from **11**. Reduction of the ester group with DIBAL then provided primary allylic alcohol **13** in 86% yield.

Various attempts at activating the alcohol in **13** ($TsCl/NEt_3$, $MsCl/NEt_3$, CCl_4/PPh_3) ahead of pyrrolidine ring closure led to complex product mixtures. As an alternative, we prepared the leucyl dipeptide derivative prior to pyrrolidine ring formation. Thus, **13** was benzoylated and the acetonide removed in the presence of PTSA to give alcohol **14** in high yield. Oxidation to the carboxylic acid and condensation with leucine *tert*-butyl ester provided dipeptide **15** in 77% yield. After hydrolysis of the benzoyl group in **15**, the minor amount of *Z* isomer carried through from the α-ethylenation reaction could be separated by column chromatography. Chlorination of the now isomerically pure alcohol with MsCl and NEt_3 afforded allylic chloride **16**, which readily cyclized to the prolyl dipeptide upon acidolysis of the Boc group and neutralization with base. DEPBT-mediated condensation of **17** with Fmoc-Har(Boc)$_2$-OH[2a] and conversion to the N-benzoylated tripeptide provided **18**. Finally, global deprotection and purification by RP-HPLC on a semipreparative C_{18} column afforded **19** as a white solid upon lyophilization (Scheme 3).

Scheme 3. Synthesis of Lucentamycin A (19)



To prove that the structure of our synthetic material matched that of natural lucentamycin A, we closely analyzed the 2D NMR spectra for **19**. As shown in Figure 2A, we observed diagnostic ROESY correlations between H-11 and H-12 as well as between H-9 and H-13 for **19**. These correlations were also noted in the recent reexamination of the ROESY spectrum for natural lucentamycin A.[3] The optical rotation we obtained for **19** was slightly more pronounced than that of the natural isolate ($[\alpha]^{24}_D = -12.5$ vs $-6.3°$; *c* 0.175, MeOH). However, the ^1H, ^{13}C, COSY, HMQC, and HMBC NMR spectra obtained for **19** were all in agreement with those reported for the original sample (Figure 2B).[1]

Support for the configuration of the Emp residue in synthetic lucentamycin A (**19**) initially relied on our use of a highly syn-selective conjugate addition reaction onto a D-serine-derived synthon.[8] Sim and co-workers recently reported the synthesis of the same tripeptide employing an asymmetric Rh-catalyzed reductive cyclization strategy.[9] Notably, the spectral data

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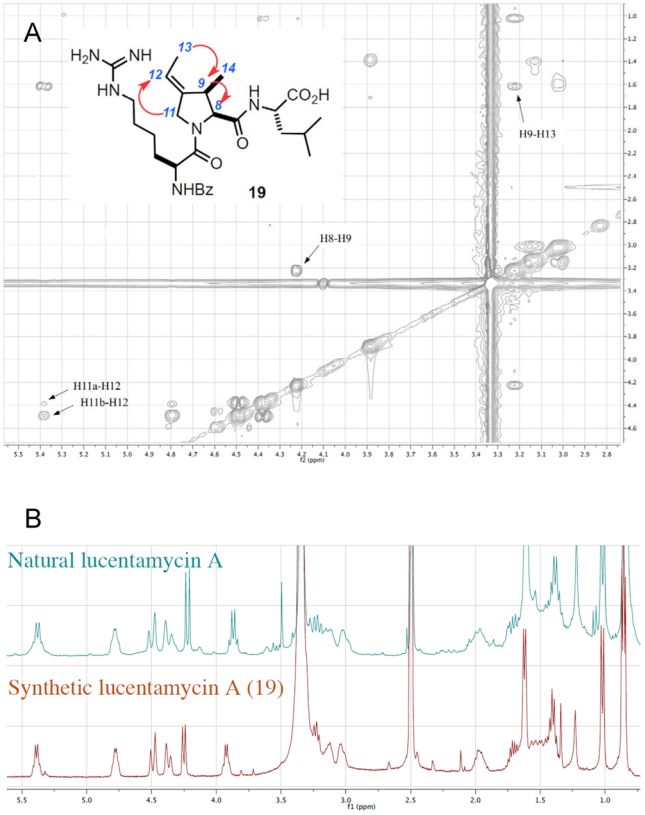

Figure 2. (A) Selected ROESY correlations observed for **19** in DMSO-d_6. (B) Comparison of ^{1}H NMR spectra (0.5−5.5 ppm) of natural lucentamycin A (blue) and **19** (red) in DMSO-d_6.

obtained for their material were not in agreement with that of natural lucentamycin A (or **19**), leading to the conclusion that the alkene geometry is not the source of missasignment. In light of this report, we sought an unequivocal configurational assignment of **19** by X-ray diffraction. Suitable crystals obtained from MeOH/H_2O led to a confirmation of the expected structure (Figure 3). Our data suggest that the compound

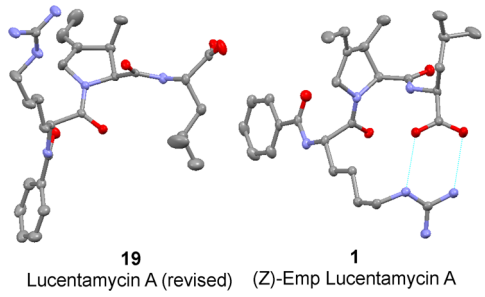

Figure 3. X-ray crystal structures of **1** and **19**.

recently described by Sim and co-workers as the (*E*)-Emp isomer of lucentamycin A[9] may instead be a structural (or conformational) isomer of **19**.

Comparison between the crystal structure of **19** and that of originally proposed lucentamycin A (**1**)[2b] revealed a significant conformational change resulting from distinct alkene geometries. Specifically, the pyrrolidine ring in **19** adopts an C$^\gamma$-exo puckered conformation, whereas the proline derivative in **1** exists as a C$^\gamma$-endo conformer in the solid state. Moreover, major changes in the ψ torsions for both the Har and Leu residues lead to a disruption of the head-to-tail salt bridge observed in the solid-state structure of **1**. The absence of this H-bond constraint may explain the notable differences in the NMR spectra of the two compounds. The signals for H-12 in **19** and natural lucentamycin A also exhibit a characteristic downfield shift of ∼0.1−0.2 ppm in the ^{1}H NMR spectrum relative to each of the (*Z*)-Emp analogues **1**−**4**.[2b]

Previously synthesized isomers of lucentamycin A have failed to show significant cytotoxicity against HCT-116 colon carcinoma cells. The change in global conformation shown in Figure 3 suggested that the presence of an (*E*)-alkene may be critical for bioactivity. However, in our hands, neither **19** or its simplified proline analogue (Bz-Har-Pro-Leu-OH)[2c] exhibited significant cytotoxicity toward HCT-116 cells at 20 μM by XTT assay (Figure 4A). Dose−response experiments at various

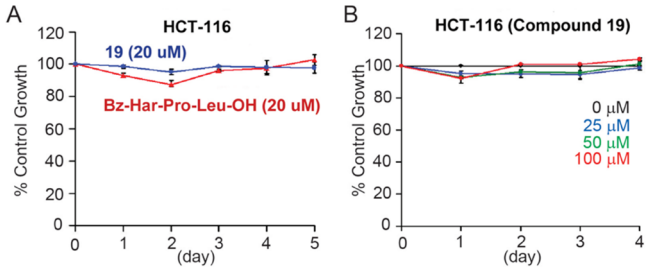

Figure 4. XTT assay with HCT-116 human colon carcinoma cells: (A) cells treated with **19** (20 μM) or Bz-Har-Pro-Leu-OH (20 μM) for a course of 5 days; (B) cells treated with 0, 25, 50, or 100 μM **19** for a course of 4 days. Percentages of cell growth were determined by comparing treated with untreated groups. Each data point derived from four independent groups receiving exactly the same treatment was plotted as mean ± SD.

time points showed compound **19** to have negligible growth inhibitory activity up to 100 μM, in stark contrast to the 0.2 μM GI_{50} previously reported by MTS assay (Figure 4B).[1] Compound **19** and Bz-Har-Pro-Leu-OH also failed to significantly inhibit the growth of two chronic lymphocytic leukemia cell lines, MEC2 and WaC3 (see the Supporting Information). This raises the possibility that the potent cytotoxicity observed with the natural isolate may be due to unidentified compounds in the original sample. Lucentamycin B is also reported to exhibit moderate growth inhibition of HCT-116 cells (GI_{50} = 10 μM), and efforts to synthesize and evaluate this compound by XTT are currently underway.

In conclusion, we have described the first total synthesis of lucentamycin A using D-serine as a chiral progenitor. Stereoselective introduction of the 3-methyl group in Emp was achieved by conjugate addition onto an enoate derived from Garner's aldehyde. The desired (*E*)-alkene geometry was established through base-promoted α-ethylenation of a linear Emp precursor. Importantly, a combination of compelling NMR and X-ray diffraction data obtained for **19** serves to resolve recent conflicting reports on the true structure of the natural product. We thus confirm, through synthesis, the alkene geometry as the sole source of lucentamycin A misassignment. In addition to providing access to lucentamycins B−E, our strategy should find application in the synthesis of other natural products featuring substituted (*E*)-4-alkylideneproline derivatives.[10]

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EXPERIMENTAL SECTION

General Techniques. Unless stated otherwise, reactions were performed in flame-dried glassware under a positive pressure of argon or nitrogen gas using dry solvents. Commercial grade reagents and solvents were used without further purification, except where noted. Diethyl ether, toluene, dimethylformamide, dichloromethane, and tetrahydrofuran were purified by a Glass Contour column-based solvent purification system. Other anhydrous solvents were purchased directly from chemical suppliers. Thin-layer chromatography (TLC) was performed using silica gel 60 F254 precoated plates (0.25 mm). Flash chromatography was performed using silica gel (60 μm particle size). The purity of all compounds was judged by TLC analysis (single-spot/two-solvent systems) using a UV lamp, CAM (ceric ammonium molybdate), ninhydrin, or basic $KMnO_4$ stain(s) for detection purposes. NMR spectra were recorded on a 400 MHz spectrometer. ^{1}H and ^{13}C NMR chemical shifts are reported as δ using residual solvent as an internal standard. Analytical (4 × 150 mm column, 1 mL/min flow rate) RP-HPLC was performed on a C_{18} column with acetonitrile/water (0.1% formic acid) as eluent.

(*S*)-*tert*-Butyl-4-((*R*)-4-ethoxy-4-oxobutan-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (11). Compound **11** was synthesized using a slight modification to the published procedure.[7] A slurry of CuI (6.11 g, 32.1 mmol) in 75 mL of THF under Ar was cooled to −15 °C and then treated with an 1.5 M solution of MeLi·LiBr in Et_2O (42.76 mL, 64.14 mmol). After it was stirred at −15 °C for 20 min, the solution was cooled to −70 °C and treated dropwise with TMSCl (12.2 mL, 96.2 mmol) followed by a solution of **10** (1.60 g, 5.34 mmol) in 8 mL of THF. The reaction mixture was stirred at −70 °C until TLC indicated completion (~30 min), and then quenched dropwise with 5 mL of 9/1 saturated aqueous NH_4Cl/2 M aqueous NaOH until gas evolution subsided. The mixture was then carefully diluted with 100 mL of the same aqueous solution, warmed to room temperature, and extracted with EtOAc. The organic layers were washed with saturated aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. Purification by flash chromatography over silica gel (8% EtOAc/hexanes) afforded **11** as an amber liquid (1.51 g, 90%). Spectral data for **11** matched those previously reported.[7]

(*S*)-*tert*-Butyl-4-((*R*,*E*)-3-(ethoxycarbonyl)pent-3-en-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (12). A solution of diisopropylamine (348 μL, 2.46 mmol) in 4 mL of THF was cooled to 0 °C and treated dropwise with a 1.6 M solution of *n*-BuLi in hexanes (1.49 mL, 2.38 mmol). After the mixture was stirred for 10 min at the same temperature, a solution of **11** (250 mg, 0.793 mmol) in 2 mL of THF was added dropwise. The reaction mixture was stirred at 0 °C for 15 min and then cooled to −78 °C and treated with acetaldehyde (445 μL, 7.93 mmol). After it was stirred for 20 min at −78 °C, the reaction mixture was quenched by dropwise addition of saturated aqueous NH_4Cl, warmed to room temperature, and extracted with EtOAc. The organic layers were washed with 1 M aqueous HCl, dried over Na_2SO_4, and concentrated. The crude alcohol was then taken up in 10 mL of DCM, cooled to 0 °C, and treated with NEt_3 (442 μL, 3.17 mmol) and methanesulfonyl chloride (184 μL, 2.38 mmol). After it was stirred for 1 h at room temperature, the reaction mixture was diluted with DCM, washed with 1 M aqueous HCl, dried over Na_2SO_4, and concentrated. The crude mesylate was then dissolved in 2.5 mL of toluene and treated with DBU (237 μL, 1.59 mmol). The reaction mixture was stirred at 60 °C for 4 h and then at room temperature for 16 h. The mixture was diluted with EtOAc, washed with 1 M aqueous HCl, dried over Na_2SO_4, and concentrated. Purification by flash chromatography over silica gel (5% EtOAc/hexanes) afforded **12** (94:6 *E*:*Z* mixture) as an amber oil (176 mg, 65%, three steps): ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 6.87 (m, 1H), 4.37 (m, 1H), 4.16 (m, 2H), 3.80 (m, 0.5H), 3.71 (m, 1H), 3.61 (m, 0.5H), 3.12 (m, 0.4H), 2.97 (m, 0.6H), 1.81 (d, J = 7.2 Hz, 3H), 1.70−1.37 (m, 15H), 1.26 (t, J = 7.1 Hz, 3H), 1.19 (d, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 167.6, 167.2, 153.1, 152.8, 139.4, 135.4, 135.2, 94.0, 93.5, 79.8, 66.5, 65.6, 60.7, 60.3, 59.9, 36.5, 35.7, 28.5, 27.7, 24.6, 23.2, 16.1, 15.8, 14.4, 14.4; HRMS (ESI-TOF) (m/z) $[MH]^+$ calcd for $C_{18}H_{31}NO_5$ 342.228 05, found 342.229 30; $[M + Na]^+$ calcd for $C_{18}H_{31}NO_5Na$ 364.209 44, found 364.210 63.

(*S*)-*tert*-Butyl-4-((*R*,*E*)-3-(hydroxymethyl)pent-3-en-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (13). A solution of **12** (354 mg, 1.04 mmol) in 10 mL of DCM at −78 °C under an Ar atmosphere was treated with a 1 M solution of DIBAL in THF (2.60 mL, 2.60 mmol) and stirred for 30 min. The reaction mixture was quenched with 50% Rochelle salt, diluted with Et_2O, and stirred vigorously for 2 h at room temperature. The organic layer was separated, and the aqueous layer was diluted with brine and extracted with DCM. The combined organic layers were dried over Na_2SO_4 and concentrated to give **13** (94:6 *E*:*Z* mixture) as a colorless oil (267 mg, 86% yield): ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 5.61 (q, J = 6.9 Hz, 1H), 4.08 (m, 2H), 3.96 (m, 1H), 3.84−3.58 (m, 2H), 3.07 − 2.81 (m, 1H), 2.75 (bs, 0.4H), 2.58−2.07 (m, 0.6H), 1.64 (m, 3H), 1.55 (m, 3H), 1.45 (m, 12H), 1.03 (d, J = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 153.4, 152.6, 141.0, 140.9, 125.1, 124.8, 94.1, 93.7, 80.1, 79.8, 66.7, 66.0, 64.5, 60.7, 60.2, 37.1, 36.7, 28.4, 27.6, 27.3, 24.6, 23.1, 16.1, 15.7, 13.4.; HRMS (ESI-TOF) (m/z) $[M + Na]^+$ calcd for $C_{16}H_{29}NO_4Na$ 322.198 88, found 322.197 60.

(3*R*,4*S*,*E*)-4-((*tert*-Butoxycarbonyl)amino)-2-ethylidene-5-hydroxy-3-methylpentyl Benzoate (14). A solution of **13** (249 mg, 832 μmol) in 8 mL of DCM at room temperature under Ar was treated with triethylamine (232 μL, 1.67 mmol) and benzoyl chloride (193 μL, 1.67 mmol). After being stirred at room temperature for 17 h, the reaction mixture was washed with saturated aqueous $NaHCO_3$ and extracted with DCM. The combined organic layers were dried over Na_2SO_4 and concentrated. Purification by flash chromatography over silica gel (5−20% EtOAc/hexanes) afforded the benzoylated intermediate as a colorless oil (329 mg, 98% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (m, 2H), 7.52 (m, 1H), 7.41 (m, 2H), 5.78 (q, J = 6.8 Hz, 1H), 4.75 (m, 2H), 4.24−4.00 (m, 1H), 3.94−3.62 (m, 2H), 2.98 (m, 1H), 1.71 (m, 3H), 1.59 (m, 3H), 1.53−1.32 (m, 12H), 1.13 (d, J = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 153.3, 152.6, 136.5, 136.3, 133.1, 132.9, 130.6, 130.4, 130.2, 130.0, 129.6, 128.9, 128.6, 128.5, 94.2, 93.7, 79.9, 79.8, 66.8, 66.7, 66.0, 60.3, 60.0, 37.4, 28.4, 28.1, 27.6, 24.6, 23.2, 16.1, 15.9, 14.4, 14.3, 13.6; HRMS (ESI-TOF) (m/z) $[MH]^+$ calcd for $C_{23}H_{34}NO_5$ 404.243 15, found 404.243 78; $[M + Na]^+$ calcd for $C_{23}H_{33}NO_5Na$ 426.225 09, found 426.225 06.

The above ester (194 mg, 481 μmol) in 2.5 mL of MeOH at 0 °C was treated with *p*-TsOH·H_2O (68.0 mg, 361 μmol) and stirred from 0 °C to room temperature over 4 h. The reaction mixture was concentrated under reduced pressure and loaded onto silica gel. Purification by flash chromatography over silica gel (20−35% EtOAc/hexanes) afforded **14** (94:6 *E*:*Z* mixture) as a white foam (167 mg, 96% yield): ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 8.00 (m, 2H), 7.52 (m, 1H), 7.41 (m, 2H), 5.75 (m, 1H), 5.07−4.62 (m, 3H), 3.81 (m, 1H), 3.72−3.45 (m, 2H), 2.99 (bs, 1H), 2.84 (m, 1H), 1.70 (d, J = 6.9 Hz, 3H), 1.46−1.29 (m, 9H), 1.16 (d, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 166.5, 156.6, 135.9, 133.1, 130.2, 129.7, 128.5, 127.8, 79.5, 67.1, 63.9, 55.4, 35.0, 28.5, 16.0, 13.6; HRMS (ESI-TOF) (m/z) $[M + Na]^+$ calcd for $C_{20}H_{29}NO_5Na$ 386.193 79, found 386.192 70.

(3*R*,4*S*,*E*)-5-(((*S*)-1-(*tert*-Butoxy)-4-methyl-1-oxopentan-2-yl)amino)-4-((*tert*-butoxycarbonyl)amino)-2-ethylidene-3-methyl-5-oxopentyl Benzoate (15). A solution of **14** (119 mg, 327 μmol) in 3 mL of MeCN at room temperature was treated with 1.5 mL of 0.67 M NaH_2PO_4 buffer followed by 20 mol % of TEMPO (15.0 mg). The mixture was placed at 40 °C and simultaneously treated with 700 μL of a solution of 6% bleach diluted with 3 mL of water and 900 μL of a 2 M solution of 80% $NaClO_2$ in water. After being stirred at 40 °C for 3 h, the reaction mixture was quenched with saturated aqueous Na_2SO_3 and partitioned with EtOAc. The aqueous layer was acidified with 1 M aqueous HCl (pH 4) and extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated. The crude carboxylic acid in 3.5 mL of MeCN at room temperature was treated with triethylamine (137 μL, 425 μmol); HBTU (161 mg, 425 μmol) and HOBt (8.8 mg, 65 μmol) were added, and the mixture was stirred for 3 min. H-Leu-Ot-Bu-HCl (95.0 mg,

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425 μmol) was then added into the mixture and stirred for 20 h. The solution was concentrated, and the resulting crude product was directly purified by flash chromatography over silica gel (10−20% EtOAc/hexanes) afforded **15** (94:6 *E*:*Z* mixture) as a white foam (138 mg, 77% yield over two steps): ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 8.06 (m, 2H), 7.53 (m, 1H), 7.42 (m, 2H), 6.43 (m, 1H), 5.75 (q, *J* = 7.0 Hz, 1H), 5.23 (m, 1H), 5.12−4.80 (m, 1H), 4.79−4.49 (m, 1H), 4.49−4.19 (m, 2H), 3.07 (m, 1H), 1.76−1.53 (m, 3H), 1.51−1.30 (m, 21H), 1.18 (d, *J* = 7.2 Hz, 3H), 0.97−0.84 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 171.4, 171.0, 166.6, 155.8, 134.3, 133.0, 130.5, 129.7, 129.1, 128.5, 127.1, 81.7, 79.9, 67.3, 57.5, 51.5, 42.2, 36.5, 28.4, 27.9, 24.8, 22.8, 22.3, 15.0, 13.7; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{30}H_{47}N_2O_7$ 547.338 50, found 547.339 00.

(*S*)-*tert*-Butyl 2-((2*S*,3*R*,*E*)-2-((*tert*-Butoxycarbonyl)amino)-4-(chloromethyl)-3-methylhex-4-enamido)-4-methylpentanoate (16). A solution of **15** (137 mg, 252 μmol) in 2 mL of 2/1 MeOH/THF at room temperature was treated with 2.0 mL of 1 M aqueous NaOH and stirred for 45 min. The reaction mixture was concentrated, neutralized with 1 M aqueous HCl, and extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated. Purification by flash chromatography over silica gel (10−15% then 40% EtOAc/hexanes) afforded the allylic alcohol as a white foam (93.0 mg, 84% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (d, *J* = 8.5 Hz, 1H), 5.54 (q, *J* = 6.8 Hz, 1H), 5.23 (d, *J* = 9.3 Hz, 1H), 4.46 (td, *J* = 9.0, 5.8 Hz, 1H), 4.39−4.10 (m, 2H), 3.96 (dd, *J* = 12.0, 2.7 Hz, 1H), 3.26 (m, 1H), 2.94 (m, 1H), 1.64 (m, 1H), 1.55 (d, *J* = 6.9 Hz, 3H), 1.51−1.37 (m, 21H), 1.12 (d, *J* = 7.0 Hz, 3H), 0.90 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.1, 171.9, 155.9, 138.9, 127.6, 82.2, 79.7, 65.3, 58.2, 51.0, 42.1, 37.1, 28.4, 28.1, 28.0, 24.8, 22.8, 22.0, 15.5, 13.3; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{23}H_{43}N_2O_6$ 443.311 56, found 443.312 09; $[M + Na]^+$ calcd for $C_{23}H_{42}N_2O_6Na$ 465.293 51, found 465.293 84.

A solution of the above allylic alcohol (102 mg, 230 μmol) in 2.5 mL of DCM at room temperature was treated with triethylamine (224 μL, 1.61 mmol) and MsCl (107 μL, 1.38 mmol). After being stirred at room temperature for 18 h, the reaction mixture was washed with 1 M aqueous HCl. The organic layer was dried over Na_2SO_4 and concentrated. Purification by flash chromatography over silica gel (10−20% EtOAc/hexanes) afforded **16** as a white foam (93.0 mg, 87% yield): ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 6.32 (d, *J* = 8.5 Hz, 1H), 5.76 (q, *J* = 7.0 Hz, 1H), 5.27−5.15 (m, 1H), 4.51−4.32 (m, 2H), 4.22 (m, 1H), 4.02 (m, 1H), 2.99 (m, 1H), 1.64 (d, *J* = 7.0 Hz, 3H), 1.62−1.35 (m, 21H), 1.21 (d, *J* = 7.1 Hz, 3H), 0.91 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$, mixture of rotamers) δ 171.5, 170.9, 155.8, 135.6, 131.7, 81.9, 79.9, 57.6, 51.7, 48.8, 42.4, 37.0, 28.4, 28.1, 24.9, 22.8, 22.3, 15.1, 14.1; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{23}H_{42}ClN_2O_5$ 461.277 68, found 461.277 67; $[M + Na]^+$ calcd for $C_{23}H_{41}ClN_2O_5Na$ 483.259 62, found 483.259 14.

(*S*)-*tert*-Butyl 2-((2*S*,3*R*,*E*)-4-Ethylidene-3-methylpyrrolidine-2-carboxamido)-4-methylpentanoate (17). A solution of **16** (92.0 mg, 199 μmol) in 2.0 mL of 15% TFA/DCM at 0 °C was stirred for 2.5 h and warmed to room temperature. The reaction was diluted with EtOAc and concentrated under reduced pressure. This dilution and evaporation sequence was repeated two more times, and the crude residue was dried under high vacuum for 1 h. The resulting trifluoroacetate salt was dissolved in 3 mL of acetone and treated with K_2CO_3 (275 mg, 1.99 mmol). After being stirred at room temperature for 24 h, the reaction mixture was filtered through a Celite pad with acetone rinsing. The organic filtrate was concentrated and purified by flash chromatography over silica gel (90% EtOAc/hexanes then 10−15% MeOH/EtOAc) to afford **17** as a yellow oil (54.0 mg, 84% yield over two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, *J* = 8.6 Hz, 1H), 5.27 (m, 1H), 4.49 (m, 1H), 4.01 (m, 1H), 3.76 (m, 1H), 3.62 (m, 1H), 3.17 (m, 1H), 1.73−1.48 (m, 6H), 1.44 (s, 9H), 1.00−0.86 (m, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.3, 170.9, 142.6, 115.8, 81.8, 65.3, 51.0, 49.4, 41.6, 36.7, 28.1, 25.0, 22.9, 21.9, 14.5, 14.3; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{18}H_{33}N_2O_3$ 325.248 57, found 325.248 48; $[M + Na]^+$ calcd for $C_{18}H_{32}N_2O_3Na$ 347.230 51, found 347.230 52.

(*S*)-*tert*-Butyl 2-((2*S*,3*R*,4*E*)-1-((*S*)-2-Benzamido-6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)hexanoyl)-4-ethylidene-3-methylpyrrolidine-2-carboxamido)-4-methylpentanoate (18). A solution of **17** (43.0 mg, 132 μmol) in 2 mL of THF at 0 °C was treated with triethylamine (37.0 μL, 264 μmol), DEPBT (60.0 mg, 199 μmol), and Fmoc-Har(Boc)$_2$-OH (97.0 mg, 158 μmol) and stirred for 5 h at 0 °C. The reaction mixture was quenched with saturated aqueous NH_4Cl and evaporated. The residue was dissolved in EtOAc and washed with 1 M aqueous HCl followed by saturated aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated. Purification by flash chromatography over silica gel (30−60% EtOAc/hexanes then 100% EtOAc) afforded **18** as a white foam (76.0 mg, 62% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.49 (s, 1H), 8.32 (s, 1H), 7.74 (d, *J* = 7.5 Hz, 2H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.47−7.34 (m, 2H), 7.28 (m, 2H), 6.02 (d, *J* = 8.4 Hz, 1H), 5.65 (d, *J* = 8.6 Hz, 1H), 5.40 (m, 1H), 4.51 (m, 3.5H), 4.43−3.99 (m, 4.5H), 3.43 (m, 2H), 3.24 (m, 1H), 2.02−1.78 (m, 1H), 1.77−1.53 (m, 7H), 1.46 (m, 31H), 1.09 (d, *J* = 7.2 Hz, 3H), 0.92 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 171.2, 168.3, 163.7, 156.2, 156.2, 153.3, 144.0, 143.9, 141.3, 139.0, 135.4, 132.8, 129.1, 127.8, 127.2, 125.9, 125.3, 125.2, 120.0, 117.8, 83.1, 82.0, 79.3, 67.1, 66.5, 64.6, 52.4, 51.6, 51.2, 47.2, 42.2, 40.8, 35.5, 32.6, 28.9, 28.4, 28.2, 28.1, 24.9, 22.8, 22.5, 22.4, 16.2, 16.1, 15.1, 13.7; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{50}H_{73}N_6O_{10}$ 917.538 27, found 917.540 34; $[M + Na]^+$ calcd for $C_{50}H_{72}N_6O_{10}Na$ 939.520 21, found 939.521 97.

The tripeptide above (50.0 mg, 55.0 μmol) was dissolved in 2 mL of THF at room temperature and was treated with diethylamine (226 μL, 2.18 mmol) and stirred for 5 h. The reaction mixture was concentrated under reduced pressure, and the resulting crude amine was treated with benzoyl chloride (13.0 μL, 109 μmol) at room temperature and stirred for 2 h. After the solvent was evaporated, the crude product was adsorbed onto silica gel and purified by flash chromatography over silica (50% EtOAc/hexanes and 100% EtOAc then 10−20% MeOH/EtOAc) to afford **18** as a white foam (33.0 mg, 76% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.47 (bs, 1H), 8.33 (m, 1H), 7.78 (m, 2H), 7.54−7.32 (m, 3H), 7.04 (d, *J* = 8.1 Hz, 1H), 6.00 (d, *J* = 8.3 Hz, 1H), 5.46 (m, 1H), 4.98 (m, 1H), 4.62−4.41 (m, 2H), 4.36 (m, 2H), 3.41 (m, 2H), 3.27 (m, 1H), 1.93 (m, 1H), 1.82−1.35 (m, 37H), 1.31 (m, 1H), 1.10 (d, *J* = 7.2 Hz, 3H), 0.95 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 171.3, 168.3, 167.2, 156.2, 153.3, 138.9, 134.1, 131.7, 128.6, 127.3, 117.9, 83.1, 82.1, 79.4, 64.7, 51.7, 51.3, 50.9, 42.3, 40.8, 35.6, 32.6, 28.9, 28.4, 28.2, 28.1, 25.0, 22.8, 22.6, 22.4, 15.2, 13.7; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{42}H_{67}N_6O_9$ 799.496 40, found 799.496 76; $[M + Na]^+$ calcd for $C_{42}H_{66}N_6O_9Na$ 821.478 35, found 821.478 80.

Synthetic Lucentamycin A (19). Tripeptide **18** (32 mg, 40 μmol) was treated with a 2 mL solution of TFA/TES/DCM (90/5/5) at room temperature and stirred for 7 h. The mixture was diluted with EtOAc and evaporated under reduced pressure. The dilution and evaporation sequence was repeated two more times. A portion of the crude residue (85% by weight) was purified by semipreparative RP-HPLC (15−70% MeCN/H_2O linear gradient over 20 min, retention time 7.4 min) to afford lucentamycin A (**19**) as a white fluffy solid (15 mg, 81% yield, based on the amount injected): ^{1}H NMR (400 MHz, d_6-DMSO) δ 10.36 (bs, 1H), 8.45 (d, *J* = 7.3 Hz, 1H), 8.27 (m, 1H), 7.86 (m, 2H), 7.52 (t, J = 7.3 Hz, 1H), 7.43 (dd, J = 11.4, 4.3 Hz, 2H), 7.18−6.88 (m, 3H), 5.39 (q, *J* = 6.4 Hz, 1H), 4.79 (dd, *J* = 11.3, 7.1 Hz, 1H), 4.49 (d, *J* = 13.8 Hz, 1H), 4.38 (d, *J* = 12.3 Hz, 1H), 4.24 (d, *J* = 8.5 Hz, 1H), 3.90 (dd, *J* = 13.3, 6.7 Hz, 2H), 3.22 (m, 2H), 3.12 (m, 2H), 3.02 (m, 2H), 1.98 (d, *J* = 7.0 Hz, 1H), 1.71 (m, 3H), 1.62 (d, *J* = 6.7 Hz, 3H), 1.59−1.45 (m, 3H), 1.44−1.21 (m, 3H), 1.02 (d, *J* = 7.2 Hz, 3H), 0.86 (d, *J* = 3.4 Hz, 3H), 0.85 (d, *J* = 3.5 Hz, 3H); ^{13}C NMR (101 MHz, d_6-DMSO) δ 175.9, 171.3, 167.3, 166.0, 157.2, 139.5, 133.8, 131.4, 128.3, 127.6, 116.3, 65.6, 52.4, 51.4, 51.0, 43.1, 41.2, 35.1, 31.4, 28.5, 24.5, 23.1, 22.7, 14.9, 13.2; HRMS (ESI-TOF) (*m*/*z*) $[MH]^+$ calcd for $C_{28}H_{43}N_6O_5$ 543.328 94, found 543.328 36; $[M + Na]^+$ calcd for $C_{28}H_{42}N_6O_5Na$ 565.310 89, found 565.309 33; $[\alpha]^{24}_D$ = −12.5° (*c* 0.175, MeOH).

Cell Proliferation Assays. Human colorectal carcinoma cell line HCT-116 was seeded and cultured in 96-well cell culture plates

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overnight in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, 1 mM sodium pyruvate, and 0.1 mM nonessential amino acids. On the next day, adherent HCT-116 cells were treated with fresh phenol red free DMEM complete media containing various concentrations of **19** and Bz-Har-Pro-Leu-OH, as indicated in the legends. Human MEC2 and WaC3 chronic lymphocytic leukemia cells were cultured in the RPMI 1640 media (Invitrogen) supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 0.1 mM β-mercaptoethanol (β-ME). Every 24 h, proliferative capabilities were assessed by XTT assays (Roche) according to the manufacturer's instructions. Briefly, 50 μL of XTT labeling reagent (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate), 1 μL of electron-coupling reagent (*N*-methyldibenzopyrazine methyl sulfate), and 100 μL of phenol red free culture media were combined and applied to each well of the 96-well plates. The assay was based on cleavage of the yellow tetrazolium salt XTT by mitochondrial dehydrogenases of the metabolic active cells to form the orange formazan compound, which can be quantified spectrophotometrically at 492 nm using a BioTek microplate reader.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra for all new compounds as well as HMQC, HMBC, and ROESY spectra for synthetic and natural lucentamycin A, dose−response curves for cell viability assays with HCT-116, MEC2, and WaC3 cell lines, and X-ray diffraction data for **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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