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Note

Total Synthesis of Laucysteinamide A, a Monomeric Congener of Somocystinamide A

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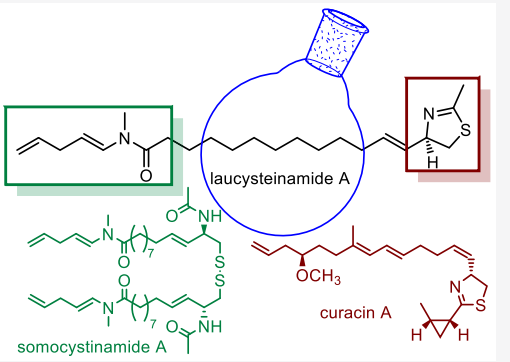
ABSTRACT: Laucysteinamide A (**4**) is a marine natural product isolated from the cyanobacterium *Caldora penicillata* and contains structural motifs found in promising cancer drug leads. The first total synthesis of **4** and its analogues was achieved, which also enabled a concise formal synthesis of somocystinamide A (**3**), a dimeric congener of **4** that previously showed extremely potent antiproliferative activities. This work provides further insights on structure−activity relationships in this class of natural products.



Marine cyanobacteria have been an incredibly prolific source of bioactive natural products and have yielded medicinally important compounds including those that feature enamide[1,2] and thiazoline moieties.[3] These structural features represent a synthetic challenge due to their labile nature. Curacin A (**1**), a potent tubulin inhibitor containing a thiazoline moiety and a highly lipophilic chain with four olefins, was isolated from the marine cyanobacterium *Moorena producens* (a subset of its former characterization, *Lyngbya majuscula*).[4−6] Tubulin dimerization and subsequent polymerization requiring GTP binding is a critical step in cell division.[7] Curacin A (**1**) inhibits this assembly process, thus inhibiting cell division and growth. This is of the utmost importance in combating cancers, as rapid growth is a key characteristic of tumor cells. The thiazoline and exocyclic olefin present in **1** were shown to be essential for its cytotoxicity.[8] This heterocycle moiety proved to be a source of significant instability and required great care during handling and storage of the molecule.[7,8] Kalkitoxin A (**2**), another thiazoline-containing metabolite with similar structural motifs and an isosteric profile to **1**, was shown to be a potent neurotoxic[9] and antiangiogenic agent.[10]

Somocystinamide A (**3**) is a dimeric natural product with two tertiary enamides. It was isolated from a mixed assemblage of *Lyngbya majuscula* and *Schitzothrix*.[11] Remarkably, metabolite **3** showed antiproliferative activity against primary human endothelial cells (HUVECs) at 500 fM.[12] Like **1**, this activity also inhibits cancer growth because tumor tissues need an increased supply of nutrients, resulting in rapid blood vessel proliferation. It was shown that compound **3** promotes apoptosis through activating caspase 8.[12] Recruitment of two procaspase-8 units and subsequent self-activation is necessary for this apoptosis pathway. This observation may imply that the dimeric character of **3** is important for its activity. Indeed, the sulfhydryl derivative of **3**, obtained by chemically reducing the disulfide, showed no cytotoxicity.[12]

More recently, laucysteinamide A (**4**) was also isolated from a related marine cyanobacterium, *Caldora penicillata*.[13,14] Laucysteinamide A (**4**) had an IC_{50} value of 11 μM against the H460 lung cancer cell line, but its full biological activity could not be evaluated due to sample decomposition.[13] Due to the similarities in structure to compounds **1**−**3**, which have shown promising cancer therapy potential, a more thorough evaluation of the biological properties of **4** was deemed desirable. Moreover, biological activity data obtained for **4** will be insightful toward understanding the structure−activity relationships of **3** because **4** is essentially a monomeric analogue of **3**. While showing a promising bioactivity profile, **3** has suffered from its poor solubility in water.[12,15] With the rise of therapeutics consisting of biologic macromolecules conjugated to small synthetic molecules, such as antibody−drug conjugates (ADCs), natural products with high lipophilicity such as **1**−**4** and their derivatives can redeem their in vivo efficacy through being employed as an ADC payload.[16] Herein reported are the first total syntheses of laucysteinamide A (**4**)

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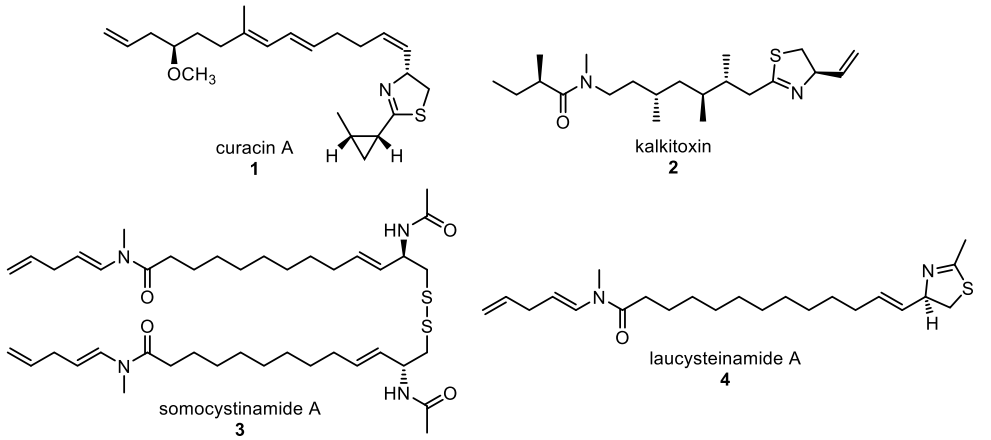
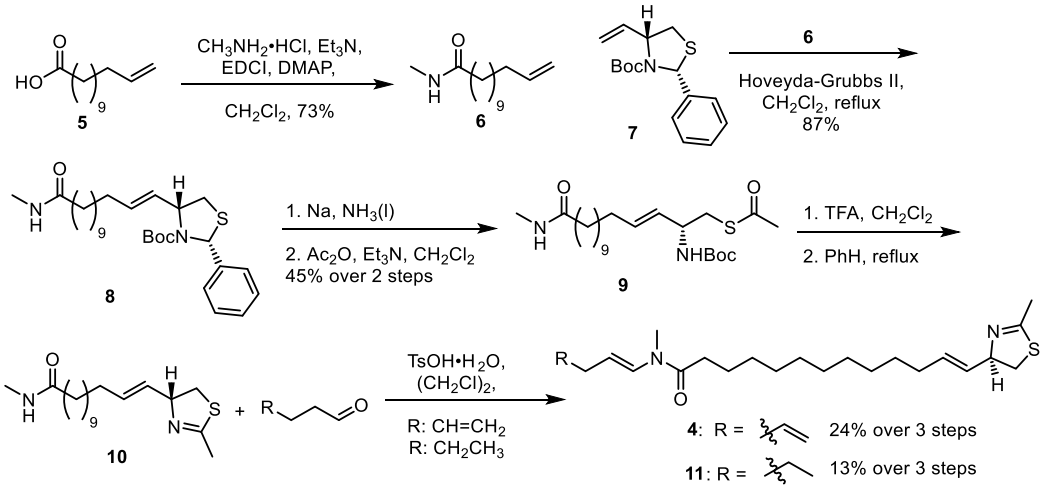
Chart 1



Scheme 1. Total Synthesis of Laucysteinamide A (4) and Its Partially Saturated Analogue 11



and its analogue as well as a more efficient and shorter formal synthesis of somocystinamide A (**3**).[15]

Initially, a synthesis was envisioned in which the thiazoline moiety would be constructed early to avoid the use of protecting groups. However, this approach was not successful, presumably due to the labile nature of the thiazoline moiety. This agrees with previous reports of thiazoline-containing natural products that have been found to decompose even under cold storage conditions.[4,7,8] Thus, a synthetic route was developed in which the thiazoline ring was installed at a late stage. Due to the labile nature of the enamide, we planned its installation last using the condensation method employed in the total synthesis of somocystinamide A (**3**).[15] The putative biosynthetic origin and assigned absolute configuration indicate laucysteinamide A (**4**) is likely derived from L-cysteine via an NRPS mechanism[13] and provided an obvious synthon. Stereochemical requirements prevented us from employing traditional olefination techniques such as the Wittig reaction. As with somocystinamide A (**3**), olefin cross metathesis was the chosen coupling method.[15]

Accordingly, olefin cross metathesis substrates **6** and **7** were prepared. The olefin **7** was synthesized from L-cysteine in five steps as reported with orthogonal protection by the phenyl thiazolidine and N-Boc.[15,17,18] Commercially available 11-bromo-1-undecene was homologated[19] by a malonate ester and subsequently decarboxylated to **5** (see Supporting Information) and coupled with methylamine to afford the amide **6**.[20,21] Coupling of **6** and **7** by Hoveyda–Grubbs II catalyst was achieved with an excellent yield. The resulting long-chain amide **8** was deprotected via Birch reduction to afford a thiol, which was then acetylated, forming thioester **9**.

Deprotection of **9** in TFA and subsequent reflux in benzene promoted dehydrative cyclization to afford the labile thiazoline **10** in a manner similar to the curacin A synthesis by White et al.[8] Without purification, the enamide moiety was immediately installed by treatment with 4-pentenal and toluenesulfonic acid in dichloroethane under reflux to afford the natural product **4**. In order to achieve removal of H_2O from this reversible reaction in a halogenated solvent, a Hickman still equipped with molecular sieves was employed in place of a Dean–Stark apparatus. While the yield for the last steps was not ideal, it is within the expected range based on the 30% yield for cyclization to form the thiazoline ring in curacin A.[8] This material was extensively studied by 1D and 2D NMR (see Supporting Information for 1D spectra) and was shown to have a structure identical to that of the natural product. A critique of the application of the exciton chirality method employed for **4** has been raised in the literature;[22] however, the original configurational assignment has been confirmed by this synthesis as evidenced by the specific rotation value of the synthetic sample (+41.3 vs natural +17.1).[13] The overall yield of the synthesis was 9.4% from the known olefin **7**.

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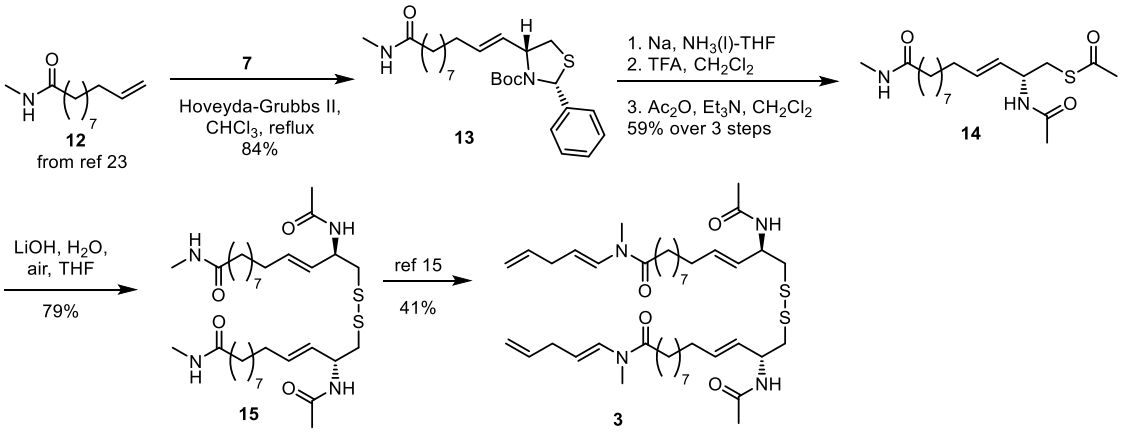
Scheme 2. Concise Formal Synthesis of Somocystinamide A



While handling **4**, we found that removing solvents in vacuo and storing it as a neat oil encouraged decomposition. According to the literature, **1** was stored frozen in benzene to avoid decomposition.[4,7,8] Likewise, we stored **4** in benzene that contained ∼0.1% triethylamine at −20 °C. The partially saturated enamide analogue **11** was synthesized from the secondary amide intermediate **10** by employing valeraldehyde rather than 4-pentenal. To investigate the role of the enamide functional group, the saturated amide (*N*,*N*-methyl-(4-pentenyl)amide) analogue of **8** was prepared and subjected to the Birch reduction conditions; however, this led to decomposition, presumably due to reduction of the amide. Furthermore, preparation of the carboxylic acid analogue of **9** in anticipation of amidation with *N*,*N*-methyl-(4-pentenyl)-amine was not fruitful.

The synthetic route employed for **4** was applied to somocystinamide A (**3**) as seen in Scheme 2.[23] This formal synthesis shortened the reported total synthesis of **3** by three steps and improved the overall yield from 11.6% to 16.1% (39% increase).[15] The use of a potentially dangerous and highly toxic reagent, diazomethane, was eliminated in this improved synthesis, and it makes a larger scale synthesis more practical for further biological testing.

We had postulated that the activity in the cytotoxicity assay observed for the natural sample of **4** (IC_{50} = 11 μM)[13] compared to that of **3** (IC_{50} = 46 nM against A549 lung carcinoma)[12] might have been due to partial decomposition of the sample and/or solubility issues. Indeed, a DMSO solution of synthetic **4** showed visual signs of insolubility at high concentrations in aqueous media. However, even with the aid of an emulsifying agent, PEG400, the synthetic sample of **4** and its analogue **11** only had IC_{50} values of 20 and 33 μM, respectively, against the H460 cell line (Table 1). In order to further investigate the importance of the terminal olefin-enamide moiety in **3** and **4**, small enamide analogues (**16** and **17**) were prepared in the same manner as **4** (Supporting Information). Both analogues showed no significant lethality in a brine shrimp assay. Interestingly, the highly antiproliferative agent **3** was also not lethal to brine shrimp even at 130 μM.[15] Because H460 cells are known to express procaspase-8,[24] compounds **4**, **16**, and **17** should show cytotoxicity against them while showing no toxicity to brine shrimp if they induce apoptosis in the same manner as **3**. However, **16** and **17** showed no activity in the H460 assay. Hence, the terminal PKS-NRPS enamide motif alone is likely not responsible for the observed activities of **3** or **4**. These results along with the apparent inactivity of the thiol monomeric derivative **18**[12] imply that the dimeric nature of **3** is important to impart high potency.

Table 1. IC_{50} Values of Enamide Natural Products and Analogues

compound	H460	brine shrimp	other
3	na	>130 μM15	A549: 46 nM[12]
			HUVEC: 500 fM[12]
4	11 μM (natural)[13]	>100 μM	na
	20 ± 3 μM (synthetic)		
11	33 ± 12 μM	na	na
16	>248 μM	>100 μM	na
17	>300 μM	>100 μM	na

This first total synthesis of the marine cyanobacterial metabolite laucysteinamide A (**4**) and its analogue has led to confirmation of its bioactivity profile as well as its absolute configuration. This work also led to a shorter and more robust formal synthesis of a potent congener of **4**, somocystinamide A (**3**). Finally, we gained further insight into structure−activity relationships with regard to **3**, an important compound with development potential as a novel cancer therapeutic.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were acquired on a JASCO J-810 spectrophotometer. IR spectra were acquired on a PerkinElmer Spectrum Two FT-IR spectrometer. NMR spectra were obtained using a Varian 600 MHz INOVA^{Unity} NMR instrument, and ^{1}H NMR reference values were 7.26 ppm for $CDCl_3$ and 7.16 ppm for C_6D_6. ^{13}C NMR reference values were 77.16 ppm for $CDCl_3$ and 128.06 ppm for C_6D_6. HRMS data were acquired on an Agilent 6230 TOF-MS under positive ion ESI-TOF-MS conditions and on a Thermo Scientific QExactive MS under positive ion HESI conditions. Optical density in the cytotoxicity assay was recorded on a SpectraMax M2 microplate reader with SoftMax Pro microplate data acquisition (Molecular Devices, LLC). NCI-H460 hypotriploid human lung carcinoma cells were acquired from the American Type Culture Collection. The second-generation Grubbs−Hoveyda ruthenium catalyst was purchased from Sigma-Aldrich. All other reagents and solvents were purchased from Fisher Scientific. CH_2Cl_2, dichloroethane, triethylamine, and benzene were distilled from CaH_2. NaH was purchased as a ∼60% w/w dispersion in oil and was used as-is. TFA and acetic anhydride were distilled immediately before use. Anhydrous THF was purchased and used as-is. All other reagents and solvents were used as purchased. All reactions were carried out under a N_2 atmosphere unless otherwise specified.

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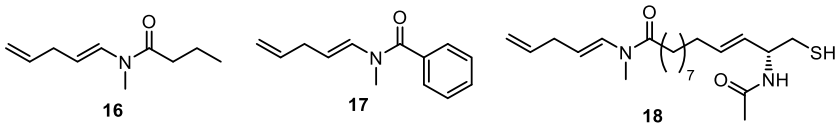
Chart 2



Synthesis. *N-Methyl 12-Tridecenamide (**6**).* To a suspension of **5** (1.24 g, 5.85 mmol) in CH_2Cl_2 (40 mL) were added CH_3NH_2·HCl (594 mg, 8.80 mmol, 1.50 equiv), Et_3N (1.63 mL, 11.7 mmol, 2.00 equiv), EDC·HCl (1.23 g, 6.44 mmol, 1.10 equiv), and DMAP (72 mg, 0.59 mmol, 0.10 equiv) successively at 0 °C. The solution was stirred overnight as it warmed to rt. Consumption of **5** was monitored and confirmed by TLC analysis. The solvents were removed in vacuo, and the resulting concentration was triturated with EtOAc to separate the urea byproduct. The resulting solution was washed with 0.5 M HCl (×3) followed by H_2O, $NaHCO_3$, H_2O, and brine successively. The organic layer was dried over Na_2SO_4 and concentrated to a solid, which was eluted through a plug of silica as an EtOAc solution to afford *N*-methyl 12-tridecenamide (**6**) as a white solid (956 mg, 4.24 mmol, 73% yield) upon removal of the solvents under vacuum. TLC R_f 0.22 EtOAc/hexanes (1:1) $KMnO_4$ stain; IR ν_{max} 3296, 3085, 2979, 2916, 2849, 1636, 1560, 912 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.43 (1H, s), 5.72 (1H, ddt, J = 17.0, 10.2, 6.7 Hz), 4.90 (1H, d, J = 17.0 Hz), 4.84 (1H, d, J = 10.2 Hz), 2.70 (3H, d, J = 4.8 Hz), 2.10 (2H, t, J = 7.7 Hz), 1.95 (2H, q, J = 7.2 Hz), 1.54 (2H, t, J = 7.3 Hz), 1.29 (2H, p, J = 6.9 Hz), 1.24−1.13 (12H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.2, 139.1, 114.0, 36.5, 33.7, 29.5, 29.5, 29.4, 29.4, 29.3, 29.1, 28.9, 26.1, 25.8; HR-ESI-TOFMS m/z $[M + H]^+$ 226.2166 (calcd for $C_{14}H_{28}NO$, 226.2165).

*(2R,4R)-3-tert-Butoxycarbonyl-4-((E)-13-amine-13-oxotridec-1-enyl)-2-phenylthiazolidine (**8**).* *N*-Methyl 12-tridecenamide (**6**) (730 mg, 3.24 mmol) was added to a solution of (2*R*,4*R*)-2-phenyl-4-vinylthiazolidine-3-*tert*-butyl carboxylate (**7**) (535 mg, 1.84 mmol) in CH_2Cl_2 (25 mL). The solution was degassed under vacuum with sonication three times. Hoveyda−Grubbs generation II catalyst (57 mg, 0.091 mmol) was added, turning the solution bright green. The solution was heated to reflux then refluxed overnight. The reaction progress was monitored by TLC analysis. The solution was concentrated under vacuum and was subjected to flash column chromatography (hexanes to 1:1 hexanes/EtOAc) to yield a pale yellow oil (759 mg, 1.55 mmol, 87%). TLC R_f 0.24 EtOAc/hexanes (2:1) $KMnO_4$ stain; $[\alpha]_D^{25}$ +198 (c 0.17, CH_2Cl_2); IR ν_{max} 3300 (br), 3031, 3064, 2976, 2924, 2852, 1733, 1696, 1648, 1365, 1157 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.36 (2H, d, J = 7.5 Hz), 7.30 (2H, t, J = 7.5 Hz), 7.23 (1H, t, J = 7.3 Hz), 6.07 (1H, s), 5.77 (1H, dt, J = 14.1, 6.2 Hz), 5.63 (1H, dd, J = 14.1, 7.5 Hz), 5.53 (1H, m,), 4.81 (1H, bs), 3.24 (1H, dd, J = 11.7, 6.4 Hz). 2.80 (1H, dd, J = 11.7, 4.6 Hz), 2.79/2.78 (3H, s, rotamers), 2.14 (2H, m), 2.06 (2H, q, J = 7.0 Hz), 1.60 (2H, m), 1.38 (2H, quin, J = 7.5 Hz), 1.34−1.26 (21H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.9, 153.8, 142.0, 134.2, 129.5, 128.3, 127.6, 126.4, 80.6, 66.2, 64.0, 36.9, 36.8, 32.3, 29.7, 29.6, 29.6, 29.5, 29.4, 29.2, 28.4, 28.4, 26.4, 25.9; HR-ESI-TOFMS m/z $[M + Na]^+$ 511.2959 (calcd for $C_{28}H_{44}N_2O_3SNa$, 511.2965).

*(R,E)-N-Methyl 14-((tert-Butoxycarbonyl)amino)-15-(acetylthio)-pentadec-12-enamide (**9**).* Liquid ammonia was prepared from combining solid NH_4Cl and powdered NaOH at room temperature. The resulting ammonia gas was passed through Drierite and was condensed at −78 °C. This liquid ammonia was distilled from sodium into the reaction flask at −78 °C. A solution of (2*R*,4*R*)-3-*tert*-butoxycarbonyl-4-((*E*)-13-amine-13-oxotridec-1-enyl)-2-phenylthiazolidine (**8**) (819 mg, 1.68 mmol) in THF (3 mL) was added to the liquid ammonia (30 mL) at −78 °C. Sodium metal (∼150 mg) was added to keep the solution a midnight blue color. The solution was allowed to warm to rt and refluxed for approximately 2 h, during which the blue color persisted. Solid NH_4Cl was added to quench the reaction. The solution was dried under a stream of nitrogen gas. Then, the residue was extracted with CH_2Cl_2 and filtered through a fritted funnel. The filtrate was then concentrated in vacuo to yield a pale brown oil. CH_2Cl_2 (40 mL) was added to the concentrated oil, and the solution was cooled. Subsequently, Ac_2O (750 μL, 7.93 mmol) and Et_3N (2.2 mL 16 mmol) were added successively. The solution was left to warm to rt under stirring overnight. The solvents were removed in vacuo, yielding a white solid, which was dissolved in Et_2O. To the organic phase were added successively aqueous Na_2CO_3, aqueous HCl, and brine to wash. The organic layer was then dried over $MgSO_4$. The resulting solution was concentrated in vacuo to yield a pale yellow solid, which was then subjected to flash column chromatography (hexanes to 1:9 MeOH/EtOAc), yielding a white amorphous solid (332 mg, 0.751 mmol, 45%). TLC R_f 0.49 EtOAc; $[\alpha]_D^{25}$ −2 (c 0.03, CH_2Cl_2); IR ν_{max} 3299 (br), 3094, 2924, 2853, 1695, 1650, 1551, 1241, 1171 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.62 (1H, td, J = 15.3, 6.9 Hz), 5.43 (1H, bs), 5.33 (1H, dd, J = 15.3, 6.1 Hz), 4.63 (1H, m), 4.25 (1H, bs), 3.09 (1H, dd, J = 14.0, 4.1 Hz), 3.04 (1H, dd, J = 14.0, 7.2 Hz), 2.81/2.80 (3H, s, rotamers), 2.34 (3H, s), 2.16 (2H, t, J = 7.6 Hz), 2.00 (2H, q, J = 7.1 Hz), 1.62 (4H, m), 1.44 (9H, s), 1.35−1.25 (12H, m); ^{13}C NMR (150 MHz, $CDCl_3$) δ 195.7, 173.9, 153.3, 133.1, 128.5, 56.1, 36.9, 34.2, 32.3, 30.7, 29.6, 29.6, 29.5, 29.5, 29.2, 28.5, 26.4, 25.9, 14.3; HR-ESI-TOFMS m/z $[M + Na]^+$ 465.2754 (calcd for $C_{23}H_{42}N_2O_4SNa$, 465.2757).

*(R)-2-Methyl-4-((E)-11-(methylamino)-11-oxotridec-1-enyl)-2-thiazoline (**10**).* To a solution of **9** (153.3 mg, 0.3466 mmol) in CH_2Cl_2 (16 mL) at 0 °C was added TFA (5.4 mL). The solution was allowed to warm to rt while stirring for 1 h, forming a pale pink film upon concentration in vacuo, which was immediately solubilized in benzene (5 mL). The benzene solution was then refluxed under N_2 for 2 h with a Hickman still filled with 3 Å molecular sieves on top of the reaction vessel. The reaction progress was monitored by TLC and LCMS analysis. Once the reaction appeared to reach completion, the solvent was removed in vacuo and the crude oil that resulted was immediately used in the next step without purification.

*General Procedures for Laucysteinamide A (**4**) and the Partially Saturated Analogue (**11**).* To a solution of crude thiazoline (**10**) in CH_2Cl (35 mL) were added 4-pentenal (150 μL, 1.43 mmol) and TsOH·H_2O (16.9 mg, 0.0981 mmol) at rt. The solution was degassed under vacuum with sonication three times. The reaction vessel was equipped with a Hickman still filled with 3 Å molecular sieves and a condenser. The reaction mixture was vigorously refluxed overnight, during which two additional amounts of 4-pentenal were added (a total of 225 μL, 2.13 mmol). A color change from pale yellow to dark brown was noted. The reaction was monitored using TLC and LCMS analysis, which showed the desired mass and diminished reactant concentration. Then the solution was cooled to 0 °C, and Et_3N (75 μL, 0.54 mmol) was added. The solution was concentrated in vacuo and was directly subjected to flash column chromatography (hexanes to EtOAc) in a triethylamine buffer, yielding a pale yellow oil **4** (32.8 mg, 0.0841 mmol, 24% across three steps). TLC R_f 0.60 EtOAc/hexanes (1:1); $[\alpha]_D^{25}$ +41 (c 0.02, $CHCl_3$); IR ν_{max} 3031, 3085, 3006, 2923, 2851, 1708, 1679, 1646, 1390, 1289, 1090 cm^{-1}; ^1H NMR (600 MHz, C_6D_6 including 0.1% Et_3N) δ 7.79 (0.3H, rotamer, d, J = 14.4 Hz), 6.43 (0.7H, rotamer, d, J = 13.8 Hz), 5.77 (1H, ddt, J = 16.6, 10.1, 6.2 Hz), 5.69 (1H, dt, J = 14.9, 6.8 Hz), 5.55 (1H, dd, J = 14.9, 6.7 Hz), 5.08−4.96 (2H, m), 4.80 (1H, q, J = 8.2 Hz), 4.67 (0.3H, rotamer, dt, J = 14.4, 7.0 Hz), 4.61 (0.7H, rotamer, dt, J = 13.8, 6.9 Hz), 3.02 (1H, dd, J = 10.8, 8.2 Hz), 2.90 (2.1H, rotamer, s), 2.79 (1H, dd, J = 10.8, 8.2 Hz), 2.68 (0.6H, rotamer, t, J = 6.6 Hz), 2.61 (1.4H, rotamer, dq, J = 6.6, 1.5 Hz), 2.36 (0.9H, rotamer, s), 2.09 (1.4H, rotamer, dd, J = 8.6, 6.1 Hz), 1.98 (2H, d, J = 1.6 Hz), 1.93 (0.6H, rotamer, t, J = 7.4 Hz), 1.73−1.65 (2H, m), 1.37−1.20 (12H, m); ^{13}C NMR (150 MHz, C_6D_6 including 0.1% Et_3N) δ 170.5, 170.3, 164.7, 138.1, 137.6, 132.1, 130.4, 130.1, 129.1, 115.2, 114.9, 107.2,

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107.0, 79.2, 40.4, 34.9, 34.8, 34.2, 33.7, 32.8, 31.5, 30.0, 29.9, 29.8, 29.6, 29.4, 25.3, 25.3, 20.2; HR-ESI-TOFMS m/z $[M + H]^+$ calcd for $C_{23}H_{39}N_2OS$ 391.2778, found 391.2774.

*Partially Saturated Laucysteinamide A Analogue (**11**).* Likewise, valeraldehyde was used in place of 4-pentenal with thiazoline **10** (44.0 mg, 0.136 mmol) to afford a colorless film (6.9 mg, 0.018 mmol, 13% from **9**). TLC R_f 0.69 EtOAc/hexanes (1:1); $[\alpha]_D^{25}$ +33 (c 0.03, $CHCl_3$); IR ν_{max} 3090, 3035, 2923, 2855, 1738, 1670, 1646, 1373, 1218, 1090 cm^{-1}; ^1H NMR (600 MHz, C_6D_6 including 0.1% Et_3N) δ 7.82 (0.3H, rotamer, d, J = 14.5 Hz), 6.47 (0.7H, rotamer, d, J = 13.8 Hz), 5.69 (1H, dt, J = 14.8, 6.7 Hz), 5.55 (1H, dd, J = 14.8, 6.7 Hz), 4.80 (1H, q, J = 8.1 Hz), 4.70 (0.3H, rotamer, dt, J = 14.5, 7.1 Hz), 4.64 (0.7H, rotamer, dt, J = 13.8, 7.2 Hz), 3.02 (1H, dd, J = 10.8, 8.1 Hz), 2.94 (2.1H, rotamer, s), 2.79 (1H, dt, J = 10.8, 8.1 Hz), 2.41 (0.9H, rotamer, s), 2.15 (2H, t, J = 7.4 Hz), 1.88 (2H, q, J = 7.2 Hz), 1.77−1.67 (2H, m), 1.41−1.16 (21H, m), 0.88 (3H, t, J = 7.3 Hz); ^{13}C NMR (150 MHz, C_6D_6 including 0.1% Et_3N) δ 170.5, 170.2, 164.6, 133.5, 132.1, 130.4, 140.1, 129.1, 110.0, 109.3, 79.2, 40.4, 34.3, 33.8, 32.9, 32.8, 30.0, 29.9, 29.8, 29.8, 29.6, 29.6, 29.5, 25.4, 25.3, 24.1, 23.9, 20.2, 13.8; HR-ESI-TOFMS m/z $[M + H]^+$ 393.2929 (calcd for $C_{23}H_{41}N_2OS$, 393.2934).

Cytotoxicity Assay Protocols. NCI-H460 cells were maintained in the lab. On the first day cells were seeded into 96-well plates at 6.66 × 10^3 cells/mL of RPMI 1640 medium with standard fetal bovine serum 180 μL/well and incubated for 24 h (37 °C, 5% CO_2). Then each one of the four tested compounds (**4**, **11**, **16**, or **17**) was dissolved in RPMI 1640 followed by nine half-log serial dilutions in the presence of 10% v/v DMSO and PEG400 in triplicates, and 20 μL of each of these formulations was added to the attached cells in duplicates. This way each concentration of each compound was tested six times in the presence of 1% v/v of DMSO and PEG400. The highest tested concentrations for compounds **4**, **11**, **16**, and **17** were 128, 127, 248, and 300 μM, respectively. Plates were incubated for an additional 48 h and then stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 25 min, and after being dissolved in DMSO the optical densities were recorded at 630 and 570 nm for each well. Dose−response curves and IC_{50} values were generated with GraphPad Software.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01317.

Experimental details for the synthesis of **5** and **13**−**17** and brine shrimp assay; dose−response curves for the H460 cytotoxicity assay; ^1H and ^{13}C NMR spectra for **4**−**6**, **8**, **9**, **11**, **13**, **14**, **16**, and **17** (PDF)

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

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