JOURNAL OF NATURAL PRODUCTS

Article

Cite This: J. Nat. Prod. XXXX, XXX, XXX−XXX

pubs.acs.org/jnp

Total Synthesis of Reniochalistatin E

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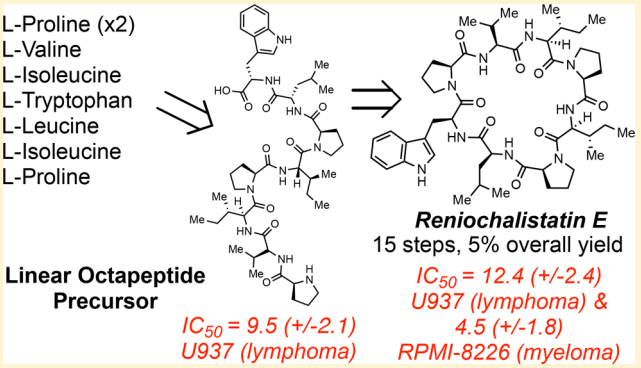
Supporting Information

ABSTRACT: Reniochalistatin E (**1**) is one of the five related cyclic peptides isolated from the marine sponge *Reniochalina stalagmitis*. The discovery of these compounds resulted from a screening program directed toward the identification of proline-rich bioactive compounds. Reniochalistatin E is the only member of the family to possess a tryptophan amino acid residue. Given the cytotoxicity observed for **1**, efforts were directed toward developing a synthetic route to **1**. The first total synthesis of **1** has been accomplished in a 15-step route in an overall 5.0% yield. The synthetic sample of reniochalistatin E was shown to have similar activity toward HeLa and RPMI-8226 cell lines compared to the natural sample, with IC_{50} values of 16.9 vs 17.3 μM and 4.5 vs 4.9 μM, respectively. Interestingly, both of the fully deprotected octapeptides constructed toward the synthesis of reniochalistatin E were shown to have cytotoxicity. The route provides a means to probe the structure−activity relationship of **1** and further biological investigations.



The reniochalistatin family of cyclic peptides was isolated from the marine sponge *Reniochalina stalagmitis* near Yongxing Island in the South China Sea in 2014 by Lin and co-workers.[1] The family was discovered as part of a screening program designed to find new bioactive cyclic peptides from marine sponges in the South China Sea.[2−5] Cyclic peptides that are proline-rich have been shown to have a variety and wide scope of biological activity, such as antiviral, antitumor, antimicrobial, and general cytotoxic properties.[6−8] The members of the reniochalstatin family were found to be proline-rich and composed of apolar and aromatic amino acid residues. Leucine and isoleucine are present in all five members, along with multiple proline units (two within reniochalistatins A, B, and C and three within D and E). The aromatic amino acid residue varies among the members of the family; B, C, and D contain phenylalanine, whereas E is the only one with a tryptophan residue.[8] Reniochalistatins A through D are cyclic heptaptides, whereas E is the only cyclic octapeptide that was isolated. Only reniochalistatin E showed biological activity, with cytotoxicity toward myeloma (RPMI-8226, IC_{50} of 4.9 μM) and gastric (MGC-803, IC_{50} of 9.7 μM) cancer cell lines. It was the activity toward myeloma that drew the attention of our laboratory and resulted in a total synthesis campaign to be undertaken.

We envisioned that **1** could be synthesized via successive amino acid couplings in tandem with selective deprotection steps. The cyclic compound could be obtained from the coupling and macrocyclization of the two desired tetrapeptides (**2** and **3**). Each of these tetrapeptides could be constructed from their corresponding commercially available L-amino acids, with selective protection about either the free carboxylic acid or amine (Scheme 1).

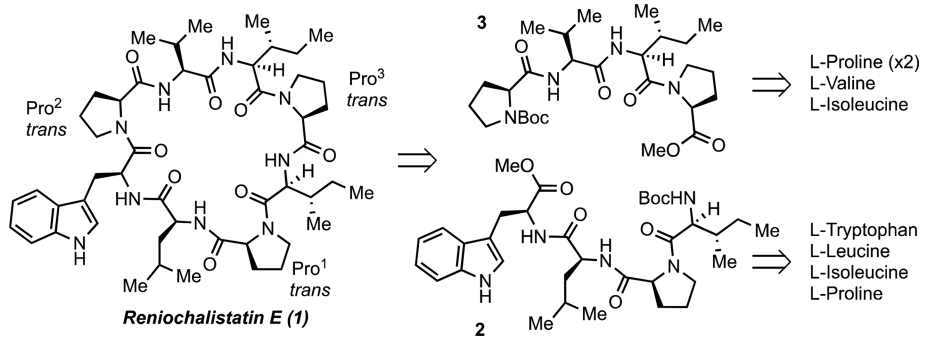
According to the retrosynthetic plan outlined in Scheme 1, efforts were directed toward the construction of tetrapeptide **2** (Scheme 2). Starting from the Boc-protected L-isoleucine (**4**) an amidation was performed though a benzotriazole-L-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP)-mediated coupling with the methyl ester of L-proline (**5**), affording the dipeptide **6** in 92% yield. Analogously, Boc-L-leucine (**7**) was coupled to the methyl ester of L-tryptophan (**8**) under the same conditions to access dipeptide **9** in 97% yield. Saponification of dipeptide **6** furnished the free acid **10**, and Boc deprotection of **9** accessed the amine hydrochloride salt **11**. Both reactions provided quantitative yields. The coupling of these two dipeptides was accomplished with PyBOP under standard conditions to give tetrapeptide **2** in 55% yield. Employing 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) with HOBt as the coupling conditions, yields were optimized at 86%.

With tetrapeptide **2** in hand, efforts were directed toward the construction of tetrapeptide **3** (Scheme 3), which will allow for access to **1**. The coupling of Boc-L-proline (**12**) to the methyl ester of L-leucine (**13**) was performed with PyBOP to furnish the dipeptide **14** in 97% yield. Saponification of **14** gave access to the free acid dipeptide **15** in quantitative yields. The second half of the tetrapeptide was accessed from the Boc deprotection of dipeptide **6** in quantitative yield. The coupling of **15** to **16** under the previously employed PyBOP conditions afforded the desired tetrapeptide **3**, but unfortunately as an impure mixture based upon the ^{1}H NMR spectrum obtained. All attempts at

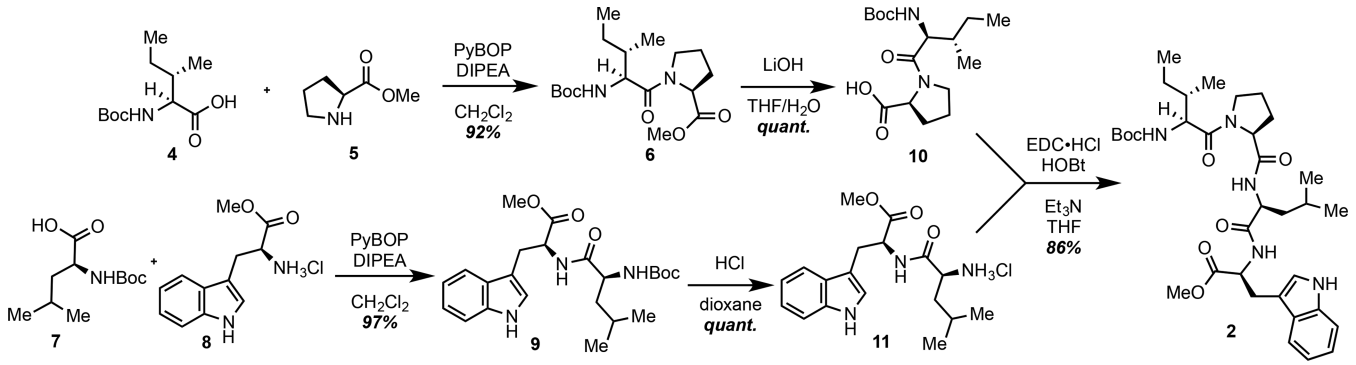
Received: July 29, 2017

DOI: 10.1021/acs.jnatprod.7b00656
J. Nat. Prod. XXXX, XXX, XXX−XXX

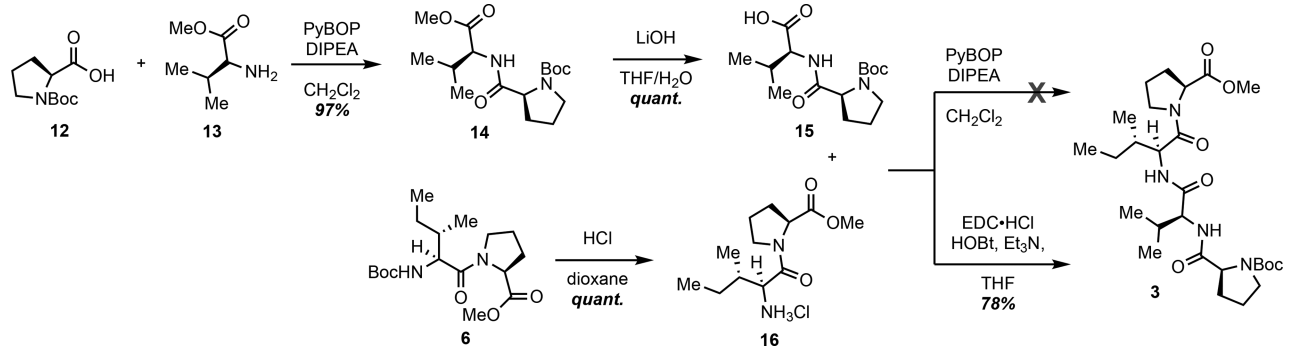
Scheme 1. Retrosynthetic Approach to Reniochalistatin E



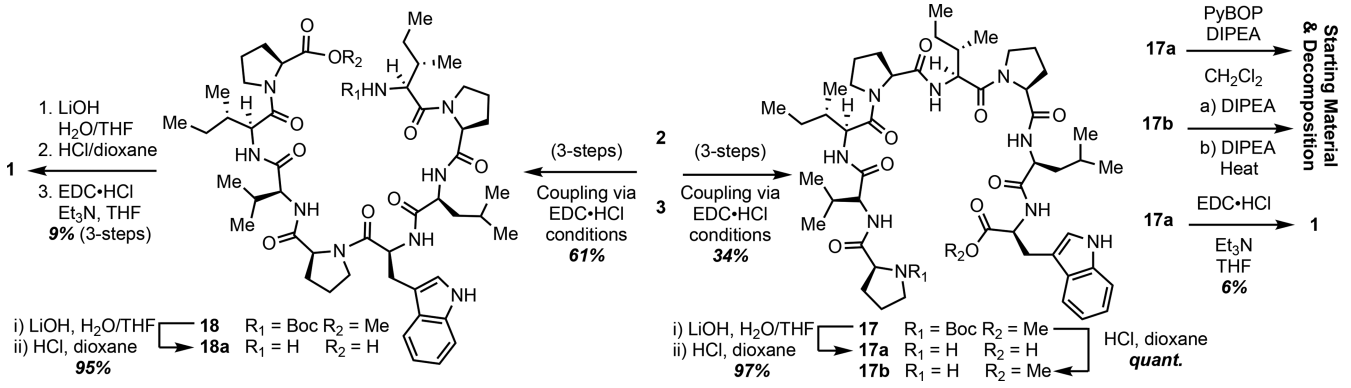
Scheme 2. Assembly of Tetrapeptide 2 with Successive PyBOP Coupling and Deprotection Steps



Scheme 3. Construction of Tetrapeptide 3 through Successive Amide Forming Coupling Reactions along with Deprotection Steps



Scheme 4. Tetrapeptide Couplings and Cyclization Strategies Accessing Reniochalistatin E (1)



purification (workup procedures, chromatographic eluents, and silica) of this material continued to provide the same contaminant peaks within the acquired ^{1}H NMR spectrum. When this material was carried forward, either through saponification or Boc deprotection, none of the desired material was collected. Pure tetrapeptide **3** was successfully accessed through the use of EDC·HCl and HOBt in 78% yield.

DOI: 10.1021/acs.jnatprod.7b00656
J. Nat. Prod. XXXX, XXX, XXX−XXX

Having accessed both of the desired tetrapeptides **2** and **3**, the coupling and macrocyclization were performed. The initial approach to the linear octapeptide of **1** was through the saponification of **2** and the Boc deprotection of **3**. Coupling of the free acid of **2** to the free base of **3** with EDC·HCl furnished octapeptide **17** in 34% yield over three steps (Scheme 4). Compound **17** was subjected to saponification followed by Boc deprotection to afford its fully deprotected form **17a** in 97% yield. The attempted macrocyclization of this material under PyBOP conditions failed to provide any of the desired product, but rather resulted in the return of starting material (55%) and decomposition. It is hypothesized that the bulk of both the secondary amine within the proline residue and the BOP-activated ester of the tryptophan residue could provide sufficient steric congestion, thereby preventing cyclization from occurring. To reduce the steric hindrance about these centers, aminolysis was then attempted for ring closure. For this, the Boc group on **17** was removed to afford **17b**, with the methyl ester intact. Unfortunately, all attempts at cyclization in this fashion failed to provide **1** and resulted in the return of starting material (67%) and decomposition. Recognizing the difficulty in macrocyclization of these two sterically encumbered proline and tryptophan residues of **17a**, it was envisioned that coupling of these two centers to access the linear octapeptide would be more attainable followed then by macrocyclization. As such, **2** was saponified to its free acid and Boc deprotection of **3** afforded the free amine, both accomplished in quantitative yields. The EDC·HCl coupling of these two units furnished the linear octapeptide **18** in 61% yield over three steps. Compound **18** was transformed into its free acid and free amine counterpart in 95% yield and then subjected to EDC·HCl coupling conditions to afford the desired natural product **1** in 9% yield. The ^{1}H and ^{13}C NMR spectra as well as the MS data of the synthesized **1** match with the literature data (see the Supporting Information). Confirmation of the *trans* prolines within **1** was confirmed based on the comparison of the reported $\Delta\delta_{C\beta\text{-}C\gamma}$ values of proline residues (4.9, 4.2, and 4.1 ppm for Pro,[1] Pro,[2] and Pro,[3] respectively). The successful macrocyclization of the free acid/amine of **18** with EDC·HCl on the first attempt led us to attempt the same conditions for cyclization upon the unsuccessful closure of the free acid/amine of **17**. To our surprise, treating **17a** with EDC·HCl afforded reniochalistatin E in 6% yield. All spectroscopic (NMR and IR) and MS data matched both the literature and the previously synthesized material.

Screening various coupling reagents and conditions was then performed to increase the yield of **1** (Table 1). Entries 1 and 9 highlight the coupling affording **1** outlined in Scheme 4 from **17a** and **18a**, respectively. The attempted PyBOP-mediated coupling of **18a** (entry 8) provided only recovered starting material (78%) and decomposition. Employing HOBt, a commonly used additive in peptide couplings, with EDC·HCl to cyclize **17a** and **18a** (entries 2 and 10) resulted in low yields of **1** of 2% and 6%, respectively. Entry 10 reflects the highest yield of **1** in our synthetic approach via **18a**. Next, macrocyclization was attempted employing 3-(diethoxyphosphoroyloxyl)-1,2,3-benzotriazine-4(3*H*)-one (DEPBT), a reagent that has been successful in the cyclization of similar amino acid based macrocycles.[9] Unfortunately, all attempts at cyclization with this reagent of **17a** and **18a** (entries 3 and 11) as well as using cesium chloride as an additive to promote carbonyl coordination (entries 4 and 12) failed to provide **1** in any increased yield. Uronium-based coupling reagents are well reported for their success in macrocyclizations, and as such, *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU)[10−13] and *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium tetrafluoroborate (TBTU)[13−16] were both attempted with **17a** and **18a** in the absence and presence of 4-(dimethylamino)pyridine (DMAP).[17,18] In all attempted cyclizations, these uronium-based reagents provided either none or low yields of **1**. Employing HBTU with DMAP to cyclize **18a** provided a 13% yield of **1** (entry 14), but in our hands, EDC·HCl with HOBt gave the best, albeit low, 15% yield of the natural product.

Table 1. Screening of Coupling Conditions of Fully Deprotected 17a and 18a

entry	cmpd	coupling agent	additive	result
1	**17a**	EDC·HCl	N/A	6%
2		EDC·HCl	HOBt	8%
3		DEBPT	N/A	
4		DEBPT	CsCl	
5		HBTU	N/A	
6		HBTU	DMAP	4%
7		TBTU	DMAP	trace
8	**18a**	PyBOP	N/A	trace
9		EDC·HCl	N/A	9%
10		EDC·HCl	HOBt	15%
11		DEBPT	N/A	
12		DEBPT	CsCl	5%
13		HBTU	N/A	
14		HBTU	DMAP	13%

With reniochalistatin E and numerous di-, tetra-, and octapeptides in both fully protected and deprotected states in hand, all compounds were screened for general cytotoxicity against six human cancer cell lines (A549, lung; HeLa, cervical; MiaPaca, pancreatic; U937, lymphoma, RPMI-8226, myeloma; and MM.1R, multiple myeloma). None of the di- or tetrapeptides in protected and partial deprotected states were found to have IC_{50} values lower than 30 μM against these cell lines. Our synthetic sample of reniochalistatin E possessed approximately the same IC_{50} value toward the HeLa and RPMI-8226 cell lines: IC_{50} of 16.9 μM ($\pm$1.9) relative to the reported 17.3 μM in HeLa and an IC_{50} of 4.5 ($\pm$2.2) relative to the reported 4.9 μM in RPMI-8226 (Table 1). Reniochalistatin E lacked cytotoxicity against all of the tested cell lines (cytotoxicity = IC_{50} < 10 μM). Both **17** and **18**, diprotected octapeptides, were shown to have no cytotoxicity. Interestingly, the free acid/amine **17a** was shown to have borderline cytotoxicity toward the U937 cell line with an IC_{50} of 9.5 $\pm$ 2.1 μM. In comparison to the free acid/amine **18a**, no cytotoxicity was observed. No further investigational studies at this time have been performed that could draw conclusions regarding structure−activity relationships based upon the results obtained.

In conclusion, we have accomplished the first total synthesis of reniochalistatin E in 15 steps with an overall 5.0% yield from commercially available materials. Cytotoxicity studies with reniochalistatin E and all intermediates accessed within the route revealed a general lack of cytotoxicity. However, the synthetic and isolated reniochalistatin E are shown to possess nearly the same activity toward the HeLa cervical cancer cell line, further supporting the total synthesis outlined herein. In addition, an interesting and unexpected cytotoxicity was observed for the free acid/amine of **17a** (linear octapeptide precursors to the natural product) toward the U937 cell line. With a route established to this cyclic octapeptide, efforts are

DOI: 10.1021/acs.jnatprod.7b00656
J. Nat. Prod. XXXX, XXX, XXX−XXX

Table 2. Evaluation of Reniochalistatin E and Linear Octapeptides for Cytotoxicity

	observed IC_{50} (μM)[a]					
cmpd	A549	HeLa	MiaPaca	U937	RPMI-8226	MM.1R
1	>20	16.9 ± 1.9	>20 μM	12.4 ± 2.4	4.5 ± 1.8	>11.2 ± 1.0
17	>20	>20	19.1 ± 1.3	>20	>20	>20
17a	>20	>20	>20	9.5 ± 2.1	>20	>20
18	>20	>20	>20	>20	>20	>20
18a	>20	18.4 ± 1.7	>20	>20	16.4 ± 3.5	>20

[a]Cytotoxicity evaluated in 384-well plates, 1500 (2000 U937) cells/well, 72 h incubation period, and evaluated via Alamar Blue.

currently being pursued toward probing the effects of single amino acid variations as well as the transposition of phenylalanine for tryptophan. The resulting analogues of **1** will be evaluated for cytotoxicity.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on an Atago Polax-2L polarimeter with a sodium lamp. IR spectra were recorded on a Cary 630 FT-IR spectrometer as thin films. Only the strongest and/or structurally important absorptions of IR spectra were reported in wavenumbers (cm^{-1}). ^1H (400, 500 MHz) and ^{13}C (100, 151 MHz) spectra were obtained on Varian and Bruker-Ascend spectrometers. The chemical shifts are given in parts per million (ppm) relative to residual $CHCl_3$ at δ 7.26 ppm for proton spectra and relative to $CDCl_3$ at δ 77.23 ppm for carbon spectra, unless otherwise noted. High-resolution mass spectra were obtained using an LCT Premier time-of-flight mass spectrometer. Flash column chromatography was performed with silica gel grade 60 (230−400 mesh). Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene (PhMe), *N*,*N*-dimethylformamide (DMF), CH_3CN, triethylamine (Et_3N), and MeOH were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves. All commercially available reagents were used as received.

***N*-Boc-L-Ile-L-Pro-OMe (6).** To a stirring solution of *N*-Boc-L-isoleucine (**4**; 1.0 g, 4.16 mmol, 1.0 equiv) in CH_2Cl_2 (42 mL) were added PyBOP (2.27 g, 4.37 mmol, 1.05 equiv) and iPr$_2$EtN (1.09 mL, 6.24 mmol, 1.5 equiv). To a separate round-bottom flask (RBF) were added L-proline methyl ester (**5**; 1.03 g, 6.24 mmol, 1.5 equiv), iPr$_2$EtN (2.17 mL, 12.48 mmol, 3.0 equiv), and CH_2Cl_2 (6.3 mL). Both reaction mixtures were left to stir for 1 h, at which time they were combined and left to stir overnight at room temperature (rt). The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2 (×2), washed with $NaHCO_{3(satd)}$, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:2 EtOAc/hexane) to afford **6** (1.30 g, 92% yield): $[\alpha]_D^{25}$ +40.3 (*c* 1.23, $CHCl_3$); IR (film) ν_{max} 3436, 2973, 1746, 1642, 1436, 1217 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.11 (d, *J* = 9.5 Hz, 1H), 4.52−4.44 (m, 1H), 4.24 (dd, *J* = 9.5, 7.2 Hz, 1H), 3.80−3.73 (m, 1H), 3.66 (s, 3H), 3.65−3.58 (m, 1H), 2.22−2.12 (m, 1H), 2.01−1.90 (m, 3H), 1.71 (ddq, *J* = 10.3, 6.5, 3.5 Hz, 1H), 1.53 (td, *J* = 7.2, 6.6, 3.2 Hz, 1H), 1.37 (s, 9H), 1.07 (ddd, *J* = 13.4, 6.7, 2.3 Hz, 1H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.64, 171.65, 156.01, 79.70, 59.04, 56.46, 52.35, 47.42, 38.13, 29.29, 28.56, 25.17, 24.38, 15.47, 11.46; HRESIMS *m*/*z* 343.2193 $[M + H]^+$ (calcd for $C_{17}H_{30}N_2O_5$, 343.2188).

***N*-Boc-L-Leu-L-Trp-OMe (9).** To a stirring solution of compound *N*-Boc-L-leucine (**7**; 1.0 g, 4.32 mmol, 1.0 equiv) in CH_2Cl_2 (43 mL) were added PyBOP (2.36 g, 4.54 mmol, 1.05 equiv) and iPr$_2$EtN (1.13 mL, 6.48 mmol, 1.5 equiv). To a separate RBF were added L-tryptophan methyl ester (**8**; 1.65 g, 6.48 mmol, 1.5 equiv), iPr$_2$EtN (2.26 mL, 12.96 mmol, 3.0 equiv), and CH_2Cl_2 (6.5 mL). Both reaction mixtures were left to stir for 1 h, at which time they were combined and left to stir overnight at rt. The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2 (×2), washed with $NaHCO_{3(satd)}$, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:1 EtOAc/hexane) to afford **9** (1.81 g, 97% yield): $[\alpha]_D^{25}$ +27 (*c* 0.15, $CHCl_3$); IR (film) ν_{max} 3418, 3018, 2872, 1739, 1513, 1215 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.06 (d, *J* = 7.4 Hz, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.07−7.02 (m, 1H), 6.96 (td, *J* = 7.5, 6.9, 1.1 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 1H), 4.51 (q, *J* = 7.1 Hz, 1H), 3.98 (td, *J* = 8.7, 6.0 Hz, 1H), 3.55 (s, 3H), 3.13−3.05 (m, 2H), 1.53 (dd, *J* = 13.6, 6.9 Hz, 2H), 1.35 (s, 9H), 1.26 (s, 1H), 0.84 (d, *J* = 6.6 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.99, 172.44, 155.95, 136.45, 127.76, 123.62, 122.15, 119.58, 118.63, 111.71, 109.49, 80.22, 53.46, 53.29, 52.54, 41.55, 28.52, 27.81, 24.92, 23.18, 22.04; HRESIMS *m*/*z* 454.2308 $[M + Na]^+$ (calcd for $C_{23}H_{33}N_3O_5Na^+$, 454.2312).

***N*-Boc-L-Ile-L-Pro-OH (10).** To a stirring solution of **6** (556 mg, 1.62 mmol, 1.0 equiv) in 1:1 THF/H_2O (16 mL) was added LiOH (194 mg, 8.12 mmol, 5.0 equiv). The mixture was left to stir at rt for 5 h. The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2, washed with $NaHCO_{3(satd)}$, dried over sodium sulfate, and concentrated under reduced pressure. The product **10** was obtained in quantitative yield as an oily residue, which was used without further purification. IR (film) ν_{max} 3456, 3304, 2977, 1704, 1314 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 5.35 (d, *J* = 9.5 Hz, 1H), 4.57 (t, *J* = 6.4 Hz, 1H), 4.32−4.25 (m, 1H), 3.82 (dt, *J* = 9.6, 7.2 Hz, 1H), 3.64 (ddd, *J* = 9.7, 7.5, 5.4 Hz, 1H), 2.18 (dd, *J* = 10.0, 3.9 Hz, 2H), 2.07−1.99 (m, 2H), 1.76 (td, *J* = 7.0, 3.4 Hz, 1H), 1.64−1.48 (m, 2H), 1.41 (d, *J* = 7.7 Hz, 9H), 0.95 (d, *J* = 6.7 Hz, 3H), 0.87 (t, *J* = 7.2 Hz, 3H).

***N*-Boc-L-Leu-L-Trp-OH (11).** To a RBF was added 1 M HCl in dioxane (4.6 mL), which cooled to 0 °C, at which point **9** (500 mg, 1.16 mmol) was added. The reaction was allowed to stir for 1.5 h. The solvent was removed under reduced pressure to afford a crude white solid (**11**), which was used without further purification. IR (film) ν_{max} 3416, 2879, 1651, 1176 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 9.04 (d, *J* = 7.2 Hz, 1H), 8.25 (s, 3H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.35 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.24 (d, *J* = 2.3 Hz, 1H), 7.07 (ddd, *J* = 8.1, 7.0, 1.3 Hz, 1H), 7.00 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 4.63−4.54 (m, 1H), 3.81 (t, *J* = 7.2 Hz, 1H), 3.58 (s, 3H), 3.21−3.13 (m, 2H), 1.68 (dt, *J* = 13.3, 6.6 Hz, 2H), 1.57 (s, 2H), 0.90 (dd, *J* = 7.9, 6.4 Hz, 6H).

***N*-Boc-L-Ile-L-Pro-L-Leu-L-Trp-OMe (2).** Compound **10** (1.32 g, 4.02 mmol, 1 equiv), compound **11** (1.08 g, 2.94 mmol, 1 equiv), and HOBt (473 mg, 3.09 mmol, 1.05 equiv) were dissolved in dry THF (40 mL) at rt under an argon atmosphere. The solution was cooled to 0 °C and stirred for 20 min, after which Et_3N (1.43 mL, 10.29 mmol, 3.5 equiv) was then added and stirred. Lastly, 20 min later EDC·HCl (592 mg, 3.09 mmol, 1.05 equiv) was added, and the reaction mixture was left to stir overnight. The reaction was quenched with H_2O (1 vol equiv), and the product was extracted with CH_2Cl_2, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (3:1 EtOAc/hexane) to give afford **2** (2.22 g, 86% yield) as a clear liquid: $[\alpha]_D^{25}$ +48.1 (*c* 1.75, $CHCl_3$); IR (film) ν_{max} 3305, 3017, 2876, 1741, 1631, 1506, 1392, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.29 (s, 1H), 7.51 (d, *J* = 8.6 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.16 (td, *J* = 8.2, 7.6, 1.3 Hz, 1H), 7.13−7.05 (m, 1H), 7.03 (d, *J* = 2.4 Hz, 1H), 6.97 (d, *J* = 7.8 Hz, 1H), 6.70 (d, *J* = 7.9 Hz, 1H), 5.20 (d, *J* = 9.4 Hz, 1H), 4.90 (dt, *J* = 7.9, 5.5 Hz, 1H), 4.37 (d, *J* = 6.0 Hz, 1H), 4.27 (dd, *J* = 9.3, 7.0 Hz, 1H), 3.71 (d, *J* = 8.9 Hz, 1H), 3.66 (s, 3H), 3.60−3.51 (m, 1H), 3.30 (d, *J* = 5.5 Hz, 2H), 2.12−1.87 (m, 3H), 1.73−1.56 (m, 5H), 1.43 (s, 9H), 1.13−1.05 (m, 1H), 0.92 (d, *J* = 6.8 Hz, 3H), 0.90−0.80 (m, 11H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.86, 172.49, 172.08, 171.74, 156.08, 136.32, 127.75, 123.57, 122.11,

DOI: 10.1021/acs.jnatprod.7b00656
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119.61, 118.60, 111.55, 109.58, 79.89, 60.26, 56.61, 52.95, 52.56, 52.15, 47.99, 41.15, 37.95, 28.60, 25.58, 27.76, 25.31, 24.79, 24.42, 23.10, 23.05, 22.06, 15.65, 11.31; HRESIMS m/z 642.3817 $[M + H]^+$ (calcd for $C_{34}H_{52}N_5O_7$, 642.3822).

***N*-Boc-L-Pro-L-Val-OMe (14).** Compounds **12** (3.0 g, 13.94 mmol, 1 equiv) and **13** (2.34 g, 13.94 mmol, 1 equiv) were added to a stirring solution of HOBt (2.24 g, 14.63 mmol, 1.05 equiv) in dry THF (139 mL) at rt under an argon atmosphere. The solution was then cooled to 0 °C and stirred for 20 min, after which Et_3N (6.8 mL, 48.79 mmol, 3.5 equiv) was added. After an additional 20 min of stirring EDC·HCl (2.8 g, 14.63 mmol, 1.05 equiv) was added, and the reaction mixture was left to stir overnight. The reaction was quenched with H_2O (1 vol equiv), and the product was extracted with CH_2Cl_2, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:1 EtOAc/hexane) to give **14** (4.45 g, 97% yield) as a clear liquid: $[\alpha]_D^{25}$ = +80.8 (*c* 2.05, $CHCl_3$); IR (film) ν_{max} 3680, 3323, 3415, 2973, 2879, 1740, 1681, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.50 (dd, *J* = 8.6, 5.1 Hz, 1H), 4.32 (s, 1H), 3.72 (s, 3H), 3.42 (s, 2H), 2.29 (s, 1H), 2.15 (pd, *J* = 6.9, 5.1 Hz, 2H), 1.95−1.84 (m, 3H), 1.48 (s, 9H), 0.92 (dd, *J* = 8.3, 6.8 Hz, 6H); ^{13}C NMR (DMSO-d_6, 101 MHz) δ 172.71, 78.98, 59.71, 58.16, 52.30, 47.18, 31.67, 30.41, 28.80, 28.68, 23.61, 19.77, 18.99, 14.76; HRESIMS m/z 329.2025 $[M + H]^+$ (calcd for $C_{16}H_{29}N_2O_5$, 329.2032).

***N*-Boc-L-Pro-L-Val-OH (15).** To a stirring solution of **14** (360 mg, 1.1 mmol, 1 equiv) in 1:1 THF/H_2O (4.4 mL) was added LiOH (131 mg, 5.5 mmol, 5 equiv), and the mixture was left to stir at rt for 3 h. The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2, washed with $NaHCO_{3(satd)}$, dried over sodium sulfate, and concentrated under reduced pressure. Crude **15** was obtained in quantitative yield as an oily residue and was used without further purification: IR (film) ν_{max} 3410 (b), 1676 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 10.37 (s, 1H), 7.51 (s, 1H), 4.50 (s, 1H), 4.40−4.19 (m, 1H), 3.50−3.28 (m, 2H), 2.31−2.09 (m, 3H), 1.94−1.79 (m, 2H), 1.43 (s, 9H), 0.91 (dd, *J* = 9.9, 6.9 Hz, 6H).

***H*$_2$*N*-L-Leu-L-Trp-OMe (16).** To a RBF containing **6** (857 mg, 2.5 mmol) was added 1.0 M HCl in dioxane (10 mL), and the mixture was cooled to 0 °C. The reaction mixture was allowed to stir at the same temperature for 3 h. The solvent was removed under reduced pressure to afford crude **16** in quantitative yield as a white solid, which was used without further purification. IR (film) ν_{max} 3416, 2879, 1651, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.34−8.27 (m, 3H), 4.61 (dd, *J* = 8.5, 5.3 Hz, 1H), 4.25 (s, 1H), 3.97 (s, 1H), 3.68 (s, 3H), 3.52 (d, *J* = 9.0 Hz, 1H), 2.28 (s, 1H), 2.05−1.91 (m, 4H), 1.68 (ddd, *J* = 13.5, 7.5, 3.3 Hz, 1H), 1.35 (ddd, *J* = 13.3, 10.0, 7.1 Hz, 1H), 1.12 (d, *J* = 6.8 Hz, 3H), 0.94 (t, *J* = 7.3 Hz, 3H); HSESIMS m/z 243.1658 $[M + H]^+$ (calcd for $C_{12}H_{23}N_2O_3$, 243.1664).

***N*-Boc-L-Pro-L-Val-L-Ile-L-Pro-OMe (3).** Compounds **16** (1.71 g, 7.06 mmol, 1 equiv) and **15** (2.24 g, 7.13 mmol, 1 equiv) were added to a stirring solution of HOBt (1.13 g, 7.41 mmol, 1.05 equiv) and dry THF (71 mL) at rt under an argon atmosphere. The solution was cooled to 0 °C and stirred for 20 min, after which Et_3N (3.46 mL, 24.71 mmol, 3.5 equiv) was added. After an additional 20 min of stirring, EDC·HCl (1.42 g, 7.41 mmol, 1.05 equiv) was added, and the mixture was stirred overnight. The reaction was quenched with H_2O (1 vol equiv), and the product was extracted with CH_2Cl_2, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (7:1 EtOAc/hexane) to give **3** (1.52 g, 78% yield) as a clear liquid: $[\alpha]_D^{25}$ +72.3 (*c* 3.21, $CHCl_3$); IR (film) ν_{max} 3673, 3412, 3306, 2879, 1743, 1632, 1368 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.48 (s, 1H), 6.65 (s, 1H), 6.42 (s, 1H), 4.58 (t, *J* = 8.2 Hz, 1H), 4.47 (dd, *J* = 8.6, 4.9 Hz, 1H), 4.31 (s, 1H), 4.26 (dd, *J* = 8.6, 5.6 Hz, 1H), 3.81 (dt, *J* = 9.8, 6.3 Hz, 1H), 3.68 (s, 3H), 3.64−3.59 (m, 1H), 3.46−3.28 (m, 2H), 2.33 (s, 1H), 2.19 (ddq, *J* = 12.9, 6.8, 3.7 Hz, 2H), 2.02−1.79 (m, 8H), 1.43 (s, 9H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.89−0.80 (m, 9H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.63, 172.40, 171.29, 171.25, 170.63, 80.70, 61.37, 59.96, 59.00, 58.70, 55.04, 52.35, 47.51, 47.25, 37.65, 31.41, 30.58, 29.29, 28.55, 25.17, 24.52, 19.50, 17.45, 15.41, 11.30; HSESIMS m/z 561.3198 $[M + H]^+$ (calcd for $C_{27}H_{46}N_4O_7Na$, 561.3264).

***H*$_2$*N*-L-Ile-L-Pro-L-Leu-L-Trp-OMe (Free Amine of Tetrapeptide 2).** To a RBF containing **2** (1.03 g, 1.6 mmol) was added 1.0 M HCl in dioxane (6.4 mL), and the solution was cooled to 0 °C. The reaction mixture was allowed to stir at the same temperature for 3 h. The solvent was removed under reduced pressure to afford a crude white solid (free base of **2**), which was used without further purification. IR (film) ν_{max} 3323, 3018, 2879, 1633, 1449, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.17 (d, *J* = 44.9 Hz, 2H), 7.51 (d, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 1H), 7.10−7.05 (m, 2H), 6.83 (d, *J* = 8.3 Hz, 1H), 4.96 (d, *J* = 7.8 Hz, 1H), 4.83−4.63 (m, 4H), 4.42 (q, *J* = 7.8 Hz, 2H), 3.91 (d, *J* = 8.3 Hz, 1H), 3.79−3.70 (m, 2H), 3.65 (s, 5H), 3.30 (d, *J* = 6.0 Hz, 2H), 2.96 (ddt, *J* = 22.3, 10.3, 6.4 Hz, 2H), 1.95−1.81 (m, 7H), 1.29−1.22 (m, 3H), 0.89−0.76 (m, 22H).

***N*-Boc-L-Pro-L-Val-L-Ile-L-Pro-OH (Free Acid of Tetrapeptide 3).** To a stirring solution of **3** (200 mg, 0.37 mmol, 1 equiv) in 1:1 THF/H_2O (1.5 mL) was added LiOH (45 mg, 1.86 mmol, 5 equiv), and the solution was left to stir at rt for 3 h. The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2, washed with $NaHCO_{3(satd)}$, dried over sodium sulfate, and concentrated under reduced pressure. The crude material (free acid of **3**), obtained in quantitative yield as an oily residue, was used without further purification. IR (film) ν_{max} 3311, 3016, 2965, 1632, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (s, 0H), 4.63 (t, *J* = 8.9 Hz, 0H), 4.41 (d, *J* = 65.5 Hz, 4H), 3.88 (d, *J* = 10.7 Hz, 1H), 3.67 (s, 2H), 3.43 (s, 2H), 2.16 (s, 7H), 1.96−1.74 (m, 5H), 1.46 (s, 9H), 1.21−0.74 (m, 12H).

Octapeptide *N*-Boc-L-Pro-L-Val-L-Ile-L-Pro-L-Ile-L-Pro-L-Leu-L-Trp-OMe (17). Free base of **2** (915 mg, 1.69 mmol, 1 equiv), free acid of **3** (784.5 mg, 1.5 mmol, 1 equiv), and HOBt (240 mg, 1.56 mmol, 1.05 equiv) were dissolved in dry THF (17 mL) at rt, under an argon atmosphere. The solution was then cooled to 0 °C and stirred for 20 min, after which Et_3N (0.731 mL, 5.25 mmol, 3.5 equiv) was added. After an additional 20 min of stirring, EDC·HCl (299 mg, 1.56 mmol, 1.05 equiv) was added, and the mixture was left to stir overnight. The reaction was quenched with H_2O (1 vol equiv), and the product was extracted with CH_2Cl_2, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (1:9 EtOAc/MeOH) to afford **17** (605 mg, 34% yield) as an amber liquid: $[\alpha]_D^{25}$ −26 (*c* 0.21, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (d, *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.13 (t, *J* = 7.2 Hz, 1H), 7.10−7.03 (m, 2H), 6.82 (s, 1H), 4.97 (s, 1H), 4.65 (s, 2H), 4.42 (s, 2H), 4.25 (s, 1H), 3.88 (d, *J* = 9.4 Hz, 1H), 3.69 (s, 2H), 3.66 (s, 3H), 3.30 (d, *J* = 4.9 Hz, 2H), 2.07 (d, *J* = 145.9 Hz, 24H), 1.44 (s, 4H), 1.31−1.21 (m, 1H), 0.90−0.75 (m, 25H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 17.38, 172.39, 151.82, 142.80, 140.68, 136.40, 127.78, 122.15, 119.63, 118.69, 111.44, 52.59, 48.15, 28.55, 24.87, 24.87, 23.06, 23.06, 19.62, 19.62, 15.33, 11.24; HRESIMS m/z 1070.6253 $[M + H]^+$ (calcd for $C_{55}H_{85}N_9O_{11}Na$, 1070.6267).

***H*$_2$*N*-L-Pro-L-Val-L-Ile-L-Pro-OMe (Free Amine of Tetrapeptide 3).** To an RBF containing **3** (151 mg, 0.28 mmol) was added 1.0 M HCl in dioxane (1.1 mL), and the solution was cooled to 0 °C. The reaction was allowed to stir at the same temperature for 3 h. The solvent was removed under reduced pressure, forming a crude white solid (free base **3**), which was used without further purification. IR (film) ν_{max} 3323, 2878, 1742, 1633, 1216 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 10.92 (s, 1H), 8.76 (s, 1H), 8.16 (s, 1H), 7.83 (d, *J* = 9.0 Hz, 1H), 4.92 (s, 1H), 4.76−4.68 (m, 1H), 4.52−4.35 (m, 2H), 3.87 (s, 2H), 3.69 (d, *J* = 2.2 Hz, 3H), 3.64 (dd, *J* = 8.5, 5.1 Hz, 1H), 3.44 (s, 3H), 2.63 (s, 1H), 2.46−2.17 (m, 5H), 2.00 (dd, *J* = 18.0, 6.0 Hz, 10H), 1.52−1.39 (m, 2H), 1.01−0.79 (m, 13H).

***N*-Boc-L-Ile-L-Pro-L-Leu-L-Trp-OH (Free Acid of Tetrapeptide 2).** To a stirring solution of **14** (100 mg, 0.16 mmol, 1 equiv) in 1:1 THF/H_2O (640 mL) was added LiOH (19 mg, 0.78 mmol, 5 equiv), and the solution was left to stir at rt for 3 h. The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2, washed with $NaHCO_{3(satd)}$, dried over sodium sulfate, and concentrated under reduced pressure. Crude **15** was obtained in quantitative yield as an oily residue, which was used without further purification. IR (film) ν_{max} 3311 (bs), 2965, 1632, 1215 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.42 (s, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.31 (s, 1H), 7.12 (dq, *J* = 24.7, 9.6, 7.3 Hz, 3H), 5.22 (s, 1H), 4.83 (d, *J* = 7.5 Hz, 1H), 4.31 (d, *J* = 41.9 Hz, 2H), 3.73 (s, 2H), 3.54 (s, 1H), 3.30 (s, 2H), 2.08−1.72 (m, 0H), 1.43 (s, 9H), 1.10−0.57 (m, 12H).

DOI: 10.1021/acs.jnatprod.7b00656
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Octapeptide *N*-Boc-L-Ile-L-Pro-L-Leu-L-Trp-L-Pro-L-Val-L-Ile-L-Pro-OMe (18). Free acid of **2** (137 mg, 0.31 mmol, 2.1 equiv) and free base of **3** (92 mg, 0.15 mmol, 1 equiv) were added to a stirring solution of HOBt (25 mg, 0.16 mmol, 1.05 equiv) in dry THF (3.1 mL) at rt under an argon atmosphere. The solution was cooled to 0 °C and stirred for 20 min, after which Et_3N (0.07 mL, 0.53 mmol, 3.5 equiv) was added and stirred. After an additional 20 min of stirring, EDC·HCl (31 mg, 0.16 mmol, 1.05 equiv) was added and the reaction mixture was left to stir overnight. The reaction was quenched with H_2O (1 vol equiv), and the product was extracted with CH_2Cl_2, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (9:1 EtOAc/MeOH) to afford **18** (198 mg, 61% yield) as an amber liquid: $[\alpha]_D^{25}$ −29 (*c* 0.78, MeOH); IR (film) ν_{max} 3423, 2925, 1741, 1687, 1612, 1452 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.67 (s, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.62−7.27 (m, 4H), 7.20−6.85 (m, 6H), 5.21 (d, *J* = 9.3 Hz, 1H), 5.01 (q, *J* = 7.2 Hz, 1H), 4.75−4.00 (m, 10H), 3.80 (dt, *J* = 35.8, 5.2 Hz, 3H), 3.72−3.66 (m, 3H), 3.58 (s, 3H), 3.18 (d, *J* = 7.3 Hz, 1H), 2.36−1.85 (m, 16H), 1.42 (d, *J* = 4.6 Hz, 9H), 1.04−0.83 (m, 24H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 172.76, 171.84, 171.74, 171.58, 170.90, 156.03, 136.30, 124.14, 123.58, 121.96, 119.58, 118.68, 111.55, 109.77, 79.70, 77.52, 77.04, 60.50, 60.27, 59.34, 59.11, 56.57, 55.13, 55.03, 52.31, 52.26, 52.10, 51.68, 48.01, 47.63, 47.55, 41.19, 37.93, 37.59, 37.53, 30.91, 30.26, 29.26, 28.58, 28.56, 28.11, 27.78, 25.36, 25.18, 24.80, 24.59, 24.53, 23.32, 23.01, 21.92, 19.59, 19.04, 18.36, 15.70, 15.60, 15.39, 11.33, 11.27; HSESIMS *m*/*z* 1070.6253 $[M + H]^+$ (calcd for $C_{55}H_{85}N_9O_{11}Na$, 1070.6267).

Reniochalistatin E (1) via 18. To a stirring solution of **18** (470 mg, 0.45 mmol, 1 equiv) in a 1:1 THF/H_2O (1.8 mL) was added LiOH (54 mg, 2.24 mmol, 5 equiv), and the solution was left to stir at rt for 3 h. The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2, dried with brine, dried over sodium sulfate, and concentrated under reduced pressure to afford the free acid of **18**, which was used without further purification. The free acid of **18** (365 mg, 0.72 mmol) was added to an RBF, and the flask was cooled to 0 °C, to which was added 1.0 M HCl in dioxane (2.9 mL). The reaction mixture was stirred for 3 h at rt. The solvent was removed under reduced pressure to afford the crude material **18a**, a white/orange solid that was used without further purification. Compound **18a** (115 mg, 0.12 mmol, 1 equiv) was added to a stirring solution of HOBt (18 mg, 0.12 mmol, 1 equiv) in dry THF (12 mL) at rt under an argon atmosphere. The solution was cooled to 0 °C and stirred for 20 min, after which Et_3N (0.06 mL, 0.42 mmol, 3.5 equiv) was added. After an additional 20 min of stirring, EDC·HCl (25 mg, 0.13 mmol, 1.05 equiv) was added and the reaction mixture was left to stir overnight. The reaction was quenched with H_2O (2 vol equiv), and the product was extracted with CH_2Cl_2, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography (85:15 EtOAc/MeOH) to afford **1** (39 mg, 9% yield): $[\alpha]_D^{25}$ −100 (*c* 0.16, MeOH); IR (film) ν_{max} 3290, 2960, 2927, 1670, 1615, 1501, 1445 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 10.96−10.84 (m, 1H), 8.11−7.78 (m, 1H), 7.52 (dd, *J* = 24.5, 7.9 Hz, 2H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.32 (t, *J* = 8.7 Hz, 1H), 7.02−6.92 (m, 2H), 4.38−4.33 (m, 1H), 3.36 (s, 23H), 3.33−3.22 (m, 1H), 2.48 (s, 1H), 1.99−1.70 (m, 2H), 1.24 (s, 2H), 0.96−0.75 (m, 8H), 0.83 (s, 27H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.37, 171.88, 171.81, 171.48, 171.31, 170.58, 170.22, 169.58, 136.59, 127.36, 124.18, 121.21, 118.60, 118.38, 111.71, 111.57, 61.20, 60.67, 59.59, 56.74, 55.85, 54.61, 54.39, 54.22, 47.81, 47.55, 47.41, 38.21, 37.49, 35.09, 33.21, 30.01, 23.92, 29.31, 29.28, 25.22, 25.12, 24.95, 24.83, 24.10, 23.77, 22.71, 20.87, 19.21, 18.88, 15.69, 15.10, 11.50, 9.81; HRESIMS *m*/*z* 938.5475 $[M + H]^+$ (calcd for $C_{49}H_{73}N_9O_8Na$, 938.5473).

Reniochalistatin E via 17. To a stirring solution of **17** (249 mg, 0.24 mmol, 1 equiv) in 1:1 THF/H_2O (1 mL) was added LiOH (29 mg, 1.2 mmol, 5 equiv), and the mixture was left to stir at rt for 3 h. The reaction was quenched with 1 M HCl (1 vol equiv), and the product was extracted with CH_2Cl_2, washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude material (free acid of **17**) was obtained in 68% yield as a white powder, which was used without further purification. The free acid of **17** (167 mg, 0.16 mmol) was added to an RBF, and the flask was cooled to 0 °C, to which 1.0 M HCl in dioxane (0.64 mL) and 1 mL of dioxane were added. The reaction mixture was stirred for 3 h at the same temperature, then allowed to warm to rt overnight. The solvent was removed under reduced pressure to afford the crude material **17a**, a white/orange solid that was used without further purification. Compound **17a** (152 mg, 0.16 mmol, 1 equiv) was added to the stirring solution of HOBt (25 mg, 0.16 mmol, 1 equiv) in dry THF (16 mL) at rt under an argon atmosphere. The solution was cooled to 0 °C and stirred for 20 min, after which Et_3N (0.08 mL, 0.56 mmol, 3.5 equiv) was added. After an additional 20 min of stirring, EDC·HCl (32 mg, 0.17 mmol, 1.05 equiv) was added, and the reaction mixture was left to stir overnight. The reaction was quenched with H_2O (1.5 vol equiv), and the product was extracted with CH_2Cl_2, washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. The crude material was purified via flash silica gel chromatography with a gradient solvent system (9:1 EtOAc/MeOH; 7:3 EtOAc/MeOH) to afford **1** (8.6 mg, 6% yield). All NMR and MS data matched those of **1** accessed via **18**.

Biological Assays. *Cell Culture Information.* Cells were grown in media supplemented with fetal bovine serum (FBS) and antibiotics (100 μg/mL penicillin and 100 U/mL streptomycin). Specifically, experiments were performed using the following cell lines and media compositions: HeLa, A549, RPMI-8226, MM.1R, and U-937 (RPMI-1640 + 10% FBS) and Mia PaCa-2 (DMEM + 10% FBS). Cells were incubated at 37 °C in a 5% CO_2, 95% humidity atmosphere for all experiments.

IC_{50} Value Determination for Adherent Cells Using Alamar Blue. Adherent cells were added to a 384-well plate (1500 cells/well) in 10 μL of media and were allowed to adhere for 2−3 h. Compounds were solubilized in DMSO (10 μM stock solutions) and added to a 96-well plate over a range of concentrations (31.6 nM to 200 μM) with media, and 40 μL was added to the 384-well plate in triplicate for each concentration of compound. After 69 h of continuous exposure, 5 μL of Alamar Blue was added to each well, and the cells were allowed to incubate for an additional 3 h. The plates were then read for fluorescence intensity with an excitation of 560 nm and emission of 590 nm on a BioTek Synergy H1 plate reader. Doxorubin and etoposide were both used as positive death controls, and wells with no compounds added as negative death controls. IC_{50} values were determined from three or more independent experiments using GraphPad Prism 7.0. (LaJolla, CA, USA)

IC_{50} Value Determination for Nonadherent Cells Using Alamar Blue. The same procedure for adherent cells was used, with the following modifications. Cells (2000 cell/well) in media (10 μL) were added after 40 μL of compound in media was added to the 384-well plate. No time was given to allow cells to adhere.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00656.

^1H NMR and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work could not have been undertaken without the gracious financial support from the Johnson Cancer Center of Kansas State University and Startup Capital from Kansas State University. This paper is warmly dedicated to Professor Richard

DOI: 10.1021/acs.jnatprod.7b00656
J. Nat. Prod. XXXX, XXX, XXX−XXX

M. Hyslop in recognition of his mentorship, guidance, and friendship.

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DOI: 10.1021/acs.jnatprod.7b00656
J. Nat. Prod. XXXX, XXX, XXX−XXX