JOC The Journal of Organic Chemistry

Article

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# Synthesis of Psychrophilin E

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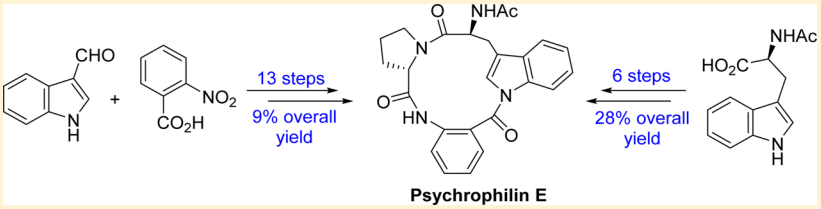
Supporting Information




**ABSTRACT:** The first total synthesis of psychrophilin E, a potent antiproliferative cyclic tripeptide isolated from *Aspergillus versicolor* ZLN-60, is reported herein. Key features of the synthesis include the installation of an amide bond between the indole-nitrogen of tryptophan and an anthranilic acid residue, and a high yielding macrolactamization of the linear tripeptide to the desired macrocycle.

## INTRODUCTION

In 2004, Dalsgaard et al. discovered a novel family of structurally related nitrated cyclic peptides, psychrophilins A–D (**1**–**4**), which were isolated from the psychrotolerant fungi *Penicillium ribeum* and *Penicillium algidum* (Figure 1).[1−3] In

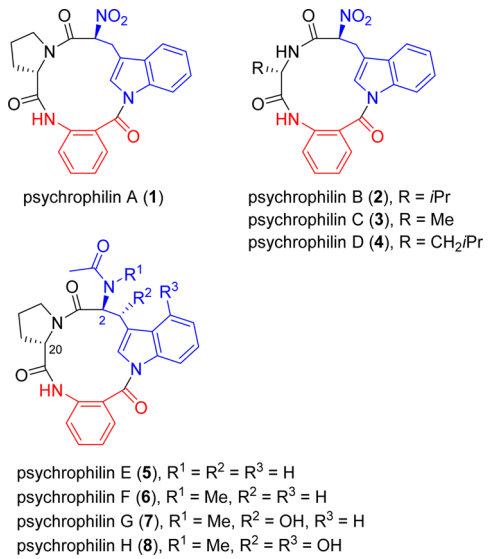



**Figure 1.** Structures of psychrophilins A–H (**1**–**8**).

2014, four structurally similar cyclic des-nitropeptides, psychrophilins E–H (**5**–**8**), were further discovered by Peng et al.[4] from the marine-derived fungus *Aspergillus versicolor* ZLN-60. Psychrophilin D (**4**) exhibits moderate cytotoxic activity against P388 murine leukemia cells ($ID_{50}$ = 10.1 μg/mL),[3] while psychrophilin G (**7**) exhibits potent lipid-lowering effects in HepG2 hepatocarcinomal cells ($IC_{50}$ = 10 μg/mL).[4] Interestingly, the isolation of psychrophilin E (**5**) was simultaneously reported by Ebada et al.[5] from the same fungal strain of the genus *Aspergillus* and was shown to demonstrate potent antiproliferative activity toward the HCT116 (colon) cell line ($IC_{50}$ = 28.5 μg/mL) with high selectivity. Importantly, psychrophilin E (**5**) demonstrated more potent cytotoxic activity than cisplatin, a well-known chemotherapeutic agent used clinically ($IC_{50}$ = 33.4 μg/mL).[5] In contrast, psychrophilins A–C, F, and H (**1**–**3**, **6**, **8**) did not display any biological activity.

Psychrophilins A–H (**1**–**8**) are 13-membered macrocycles that possess an unusual amide linkage between the indole-nitrogen of tryptophan and the anthranilic acid residues. Interestingly, psychrophilins A–D (**1**–**4**) contain an unprecedented nitro-tryptophan moiety and are the only naturally occurring cyclic peptides known to possess a nitro group. Furthermore, to the extent of our knowledge, the synthesis of an amide bond between the indole-nitrogen of tryptophan and an anthranilic acid has not been previously reported, but there is literature precedent on the synthesis of such a bond between an indole-nitrogen and anthranilic acid.[6,7] As part of our ongoing synthetic program to examine the pharmacological properties of macrocyclic peptides,[8−12] our attention focused on the synthesis of this structurally unique family of cyclic peptides.

Our attention initially focused on the synthesis of the nitrated cyclic peptide psychrophilin C (**3**). As far as we know, the cyclization of tripeptides employing a nitro alkylation-based strategy has no literature precedent. Thus, initial synthetic studies focused on the intramolecular Mitsunobu-type cyclization of nitroalcohol **9** and intramolecular base-assisted

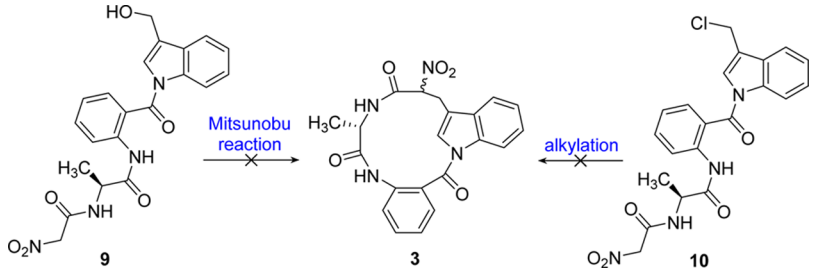
**Received:** June 7, 2016
**Published:** July 21, 2016



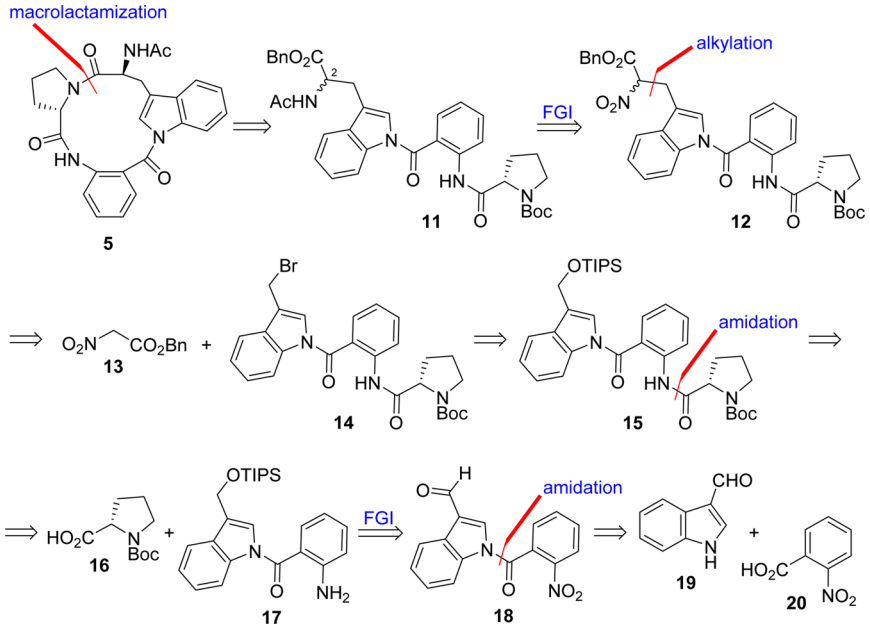


DOI: 10.1021/acs.joc.6b01369
J. Org. Chem. 2016, 81, 7635−7643

**Scheme 1. Attempted Cyclization of Precursors 9 and 10 To Afford Psychrophilin C (3)**




**Scheme 2. Retrosynthetic Analysis of Psychrophilin E (5)**




nitroalkylation of nitrochloride **10** (Scheme 1). Unfortunately, attempts to deliver psychrophilin C (**3**) using both of these strategies were unsuccessful. Nitroalcohol **9** was unreactive toward various azo-dicarboxylate reagents, and the use of diisopropyl azodicarboxylate (DIAD) led to the formation of an azo-dicarboxylate adduct $\alpha$ to the nitro moiety. Treatment of nitrochloride **10** with various bases at room temperature afforded no reaction, and the use of higher temperatures led only to decomposition.

In light of these difficulties, and the potent cytotoxic activity of the recently discovered psychrophilin E (**5**), we shifted our attention to the synthesis of this alternative target. We herein present the first synthesis of psychrophilin E (**5**). The synthesis of **5** provides further insight into synthetic strategies for the remaining members of the psychrophilin family.

One of the main challenges associated with the synthesis of macrocyclic peptides is tethering the two ends of the linear precursor. This is due to the need to overcome the ring strain of the cyclic structure to be formed, which is especially great in the case of tripeptides.[13] A study by Poteau and Trinquier showcased how energetically unfavorable it is to cyclize tri- and tetrapeptides that contain all *cis*-amide bonds.[14] These considerations highlight the problem that synthetic chemists face when trying to prepare cyclic tripeptides. Hence, the choice of an appropriate site to effect the key macrocyclization step is crucial.

Previous work in our laboratory has encountered difficulties involving amide coupling between the amino group of anthranilic acid residues and an activated carboxyl group. Therefore, building on our own demonstrated procedure to effect the macrolactamization of a linear hexapeptide via an *N*-terminal proline residue,[11] psychrophilin E (**5**) was thus retrosynthetically disconnected between the proline and tryptophan residues to linear precursor **11** (Scheme 2). The stereochemistry of the *N*-acetyl substituent at C-2 in tripeptide **11** was not taken into account at this stage. *N*-Acetyl benzyl ester **11** was envisioned to be accessible by reduction of the nitro group in tripeptide **12**. In turn, tripeptide **12** would be constructed by the addition of benzyl nitroacetate (**13**) to bromide **14**. Tripeptide **15** would then be assembled via amide coupling of *N*-Boc-proline (**16**) and aniline **17**, which is accessible through manipulation of the oxidation states of amide **18**. Amide **18** was further disconnected via the amide bond to afford commercially available compounds **19** and **20**. We also envisioned that deprotection of the benzyl ester and Boc amide groups in tripeptide **12** followed by macrolactamization would provide access to psychrophilin A (**1**).

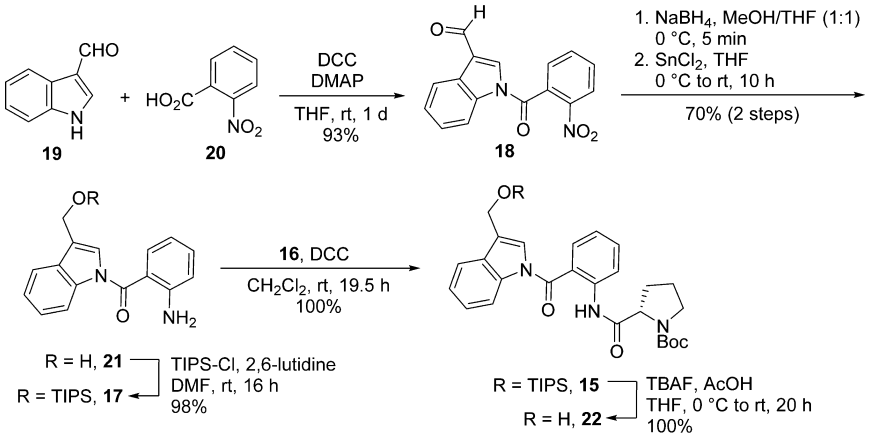
## RESULTS AND DISCUSSION

We embarked on the proposed synthetic route by coupling indole-3-carboxaldehyde (**19**) with 2-nitrobenzoic acid (**20**) using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and obtained amide **18** in 93% yield (Scheme 3). The presence of an

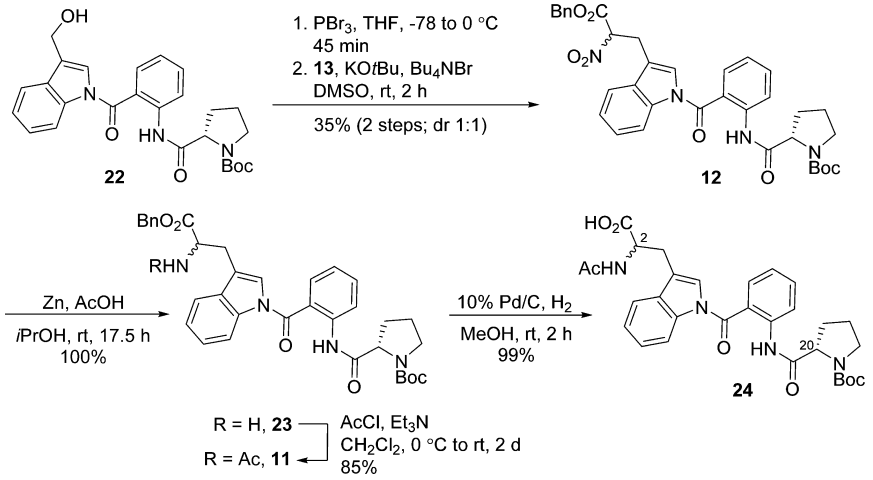


DOI: 10.1021/acs.joc.6b01369
J. Org. Chem. 2016, 81, 7635−7643

**Scheme 3. Synthesis of Alcohol 22**




**Scheme 4. Synthesis of Macrolactamization Precursor 24 as a Diastereomeric Mixture**

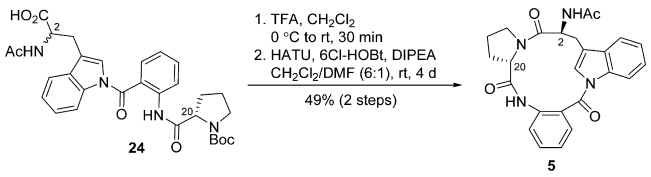



aldehyde in amine **19** and an *o*-nitro group in carboxylic acid **20**, respectively, were required to achieve a successful and high-yielding amidation. Using reduced and protected variants of **19** and **20** gave lower yields. Reduction of aldehyde **18** using sodium borohydride followed by reduction of the nitro group using tin(II) chloride afforded alcohol **21** in 70% yield over two steps. Silyl protection of alcohol **21** then furnished aniline **17** in excellent yield. Subsequent amide coupling of **17** with *N*-Boc-proline (**16**) using DCC followed by silyl deprotection provided alcohol **22** in near-quantitative yield over two steps.

Attention next turned to nitro alkylation of alcohol **22** to complete the backbone of the tripeptide (Scheme 4). Alcohol **22** was first converted to the corresponding bromide using phosphorus tribromide. The crude bromination mixture was then added to a prestirred mixture of benzyl nitroacetate (**13**), tetra-*n*-butylammonium bromide, and potassium *tert*-butoxide in DMSO. Nitro peptide **12** was isolated albeit in 31% yield as a 1:1 epimeric mixture over two steps. Fortunately, reduction of the nitro group in benzyl ester **12** with zinc powder and glacial acetic acid in isopropanol afforded amine **23** in quantitative yield. Acetylation of **23** gave *N*-acetyl tripeptide **11**, which underwent near-quantitative hydrogenolysis of the benzyl ester under standard conditions, providing carboxylic acid **24**.

With carboxylic acid **24** in hand, attention turned to removal of the *N*-terminal Boc group by treatment with TFA and the crude unprotected tripeptide was used directly in the key cyclization reaction, using our previously optimized macrolactamization conditions (Scheme 5).[12] Cyclization was performed under high dilution conditions (0.25 M) by slow

**Scheme 5. Synthesis of Psychrophilin E (5) from Precursor 24**




addition of a mixture of the deprotected linear precursor, HATU, and 6-chloro-1-hydroxybenzotriazole (6Cl-HOBt) in dichloromethane/DMF (1:1) to a stirred solution of *N*,*N*-diisopropylethylamine (DIPEA) in dichloromethane/DMF (7:1). Pleasingly, psychrophilin E (**5**) was isolated in 49% yield, over two steps after stirring at room temperature for 4.5 days. It was important to leave the reaction for an extended period of time, as the isolated yield was only 30% when the reaction time was 15.5 h. Although a 1:1 epimeric mixture of linear precursor **24** was used, surprisingly, only the desired 2*S*-epimer of macrocycle **5** was obtained. This observation suggested that only the 2*S*-epimer of the deprotected linear precursor underwent successful macrolactamization. The 2*R*-epimer of macrocycle **5** was never isolated, suggesting that the



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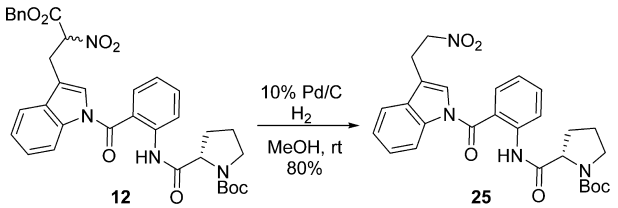
ring strain required to effect the formation of the macrocyclic system is too high in this case. Theoretical calculations lent support to this hypothesis; the 2*S*-epimer of macrocycle **5** was calculated to be more stable than the 2*R*-epimer by approximately 30 kJmol$^{-1}$.[15] The Boc-deprotected 2*R*-epimer of **24** was never isolated and is speculated to have not been extracted by ethyl acetate during the aqueous reaction workup step.

Gratifyingly, the $^{1}$H and $^{13}$C NMR spectroscopic data and the HRMS analysis of synthetic psychrophilin E (**5**) were in full agreement with those reported for the natural product.[4,5] In addition, the X-ray crystal structure of synthetic **5** matched perfectly with that of naturally occurring **5** reported by Peng et al.[4] Although Ebada et al.[5] misassigned the stereochemistry at C-2 in psychrophilin E as the *R* configuration, the NMR spectroscopic data recorded in $CDCl_3$ for our synthetic **5** completely matched the data they reported for the natural product. The optical rotation value measured for our synthetic material **5** in chloroform ($[\alpha]_D^{24}$ +92.0 (*c* 0.20 in $CHCl_3$)) was 3-fold higher than that reported for the natural product ($[\alpha]_D^{25}$ +32.0 (*c* 0.20 in $CHCl_3$)) by Ebada et al.[5] It was noted that a pure sample of synthetic **5** is insoluble in methanol; therefore, we were not able to obtain an accurate optical rotation value to be compared to that reported by Peng et al. for the natural product ($[\alpha]_D^{25}$ +96.0 (*c* 0.20 in MeOH)).[4] Therefore, based on the spectral data and the single-crystal X-ray structure of our synthetic material, the absolute configuration of psychrophilin E (**5**) is proposed to be (2*S*,20*S*).

In an attempt to synthesize psychrophilin A (**1**), nitrobenzyl ester **12** was subjected to standard hydrogenolysis conditions to effect benzyl ester deprotection. However, this resulted in decarboxylation and the corresponding nitroalkane product **25** was isolated in 80% yield (Scheme 6). This type of

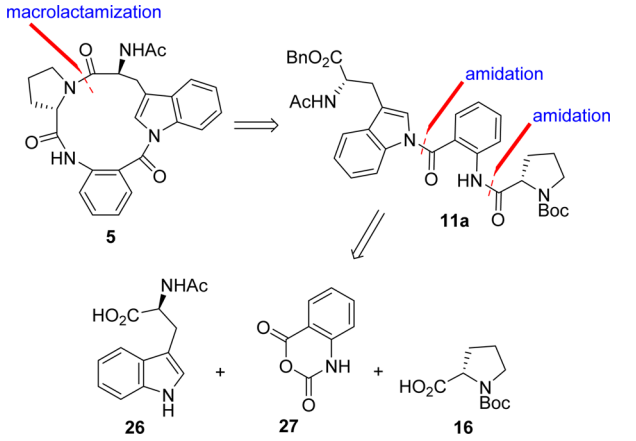
**Scheme 6. Attempted Benzyl Ester Deprotection on Nitrobenzyl Ester 12**




decarboxylation was also observed in our earlier synthetic studies focused on attempted cyclization of linear psychrophilin precursors via aminolysis of thioesters.

Although amide coupling between the indole-nitrogen of tryptophan and the carboxylic acid group of anthranilic acid has not been previously reported, there are examples of direct amide coupling between the indole-nitrogen of tryptophan and the carboxylic acid group of the amino acids alanine and leucine, using KF, 18-crown-6, and *N*,*N*-diisopropylethylamine (DIPEA) in MeCN at room temperature.[16,17] This observation therefore inspired us to investigate a more efficient and stereoselective synthetic strategy to access psychrophilin E (**5**) based on this approach. In our second generation synthesis, psychrophilin E (**5**) was retrosynthetically disconnected between the proline and tryptophan residues to give linear precursor **11a** (Scheme 7). Tripeptide **11a** in turn was envisioned to be accessible from commercially available compounds **16**, **26**, and **27**.

**Scheme 7. Retrosynthesis for Second Generation Synthesis of Psychrophilin E (5)**




Our revised synthesis commenced by protecting the *N*-acetyl tryptophan (**26**) as benzyl ester **28** (Scheme 8). The key amide coupling between the indole-amino group of tryptophan **28** and isatoic anhydride (**27**) proceeded only at elevated temperature. Pleasingly, the union of fragments **27** and **28** afforded dipeptide **29** in 53% yield, a moderate yield comparable to other similar examples reported in the literature.[16] Subsequent amide coupling of **29** with *N*-Boc-proline (**16**) using DCC afforded tripeptide **11a**, which underwent near-quantitative hydrogenolysis of the benzyl ester under standard conditions, providing carboxylic acid **24a** in 93% yield. Subjection of **24a** to the same method used earlier to prepare psychrophilin E (**5**) afforded **5** in an isolated yield of 79% over two steps after stirring at room temperature for 3 days. The overall yield of this revised six-step synthesis is 28%, compared to the initial 13-step synthesis which proceeded with an overall yield of 9%.

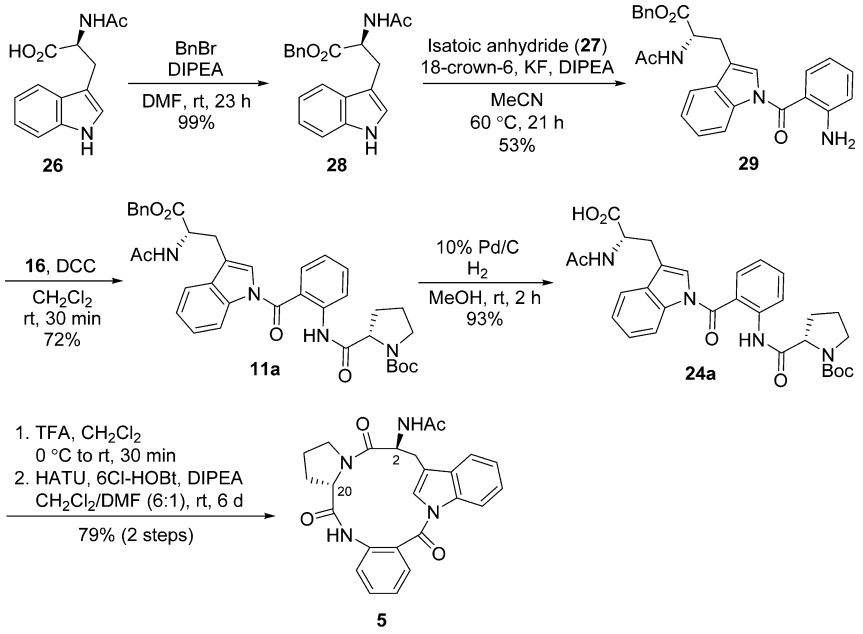
In summary, the first total synthesis of psychrophilin E (**5**) has been successfully executed in six steps with an overall yield of 28%. Highlights of the synthesis include installation of the rare amide bond between the indole-nitrogen of tryptophan and the anthranilic acid residue, and macrolactamization of the linear tripeptide to the desired macrocycle **5**. The overall synthetic route is scalable and is anticipated to be amenable to the production of other members of the psychrophilin family and analogues thereof.

## EXPERIMENTAL SECTION

**General Information.** All reactions were performed under an atmosphere of dry nitrogen in oven-dried (100 °C) glassware unless otherwise stated. Commercially available starting materials and reagents were used as received unless otherwise noted. All the solvents used were dried by passage through a column of activated alumina under nitrogen using an LC Technology solvent purification system. Thin layer chromatography (TLC) was performed using F254 0.2 mm silica plates, followed by visualization with UV irradiation at 254 nm, and staining with ethanolic vanillin solution. Flash column chromatography was performed using 63−100 $\mu$m silica gel. Optical rotations were measured with an automatic polarimeter at 589 nm, and the concentration was measured in g/100 mL. Infrared (IR) spectra were recorded with an FT-IR spectrometer using a diamond ATR sampling accessory. NMR spectra were recorded at ambient temperature as $CDCl_3$ solutions on either a spectrometer operating at 400 MHz for $^{1}$H nuclei and 100 MHz for $^{13}$C nuclei or a spectrometer operating at 500 MHz for $^{1}$H nuclei and 125 MHz for $^{13}$C nuclei. All chemical shifts are reported in ppm on the $\delta$ scale and were measured relative to the protium solvent in which the sample was


DOI: 10.1021/acs.joc.6b01369
J. Org. Chem. 2016, 81, 7635−7643

**Scheme 8. Synthesis of Psychrophilin E (5) from Precursor 26**




analyzed ($CDCl_3$: $\delta$ 7.26 for $^1H$ NMR and $\delta$ 77.0 for $^{13}C$ NMR). $^1H$ NMR data are reported as chemical shift in ppm, followed by multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad or combination thereof), coupling constants ($J$, in Hz) where applicable, and relative integral. NMR peak assignments were confirmed by 2D HSQC and 2D COSY NMR spectra. High-resolution mass spectra were recorded on a microTOF QII (electrospray ionization, ESI) mass spectrometer.

**(*R*)-*N*-(2-(3-(Hydroxymethyl)-1*H*-indole-1-carbonyl)phenyl)-2-(2-nitroacetamido)propanamide (9).** A solution of *tetra-n*-butylammonium fluoride (2.0 mL of 1.0 M in THF, 2.22 mmol) and glacial acetic acid (0.1 mL, 0.52 mmol) in THF (6 mL) was added to a stirred solution of TIPS-ether precursor (200 mg, 0.34 mmol) in THF (6 mL) at 0 °C. The reaction mixture was stirred for 25 h and allowed to warm to rt. The reaction was quenched with sat. aq. $NH_4Cl$ (20 mL), and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc) afforded the *title compound* **9** (139 mg, 0.33 mmol, 95%) as a white solid; $R_f$ 0.36 (EtOAc); mp = 182−189 °C; $[\alpha]_D^{23}$ −42.7 (*c* 1.00 in DMSO); $\nu_{max}$(neat)/cm$^{-1}$ 3278, 2973, 1680, 1648, 1564, 1535, 1386, 1363, 1332, 1143, 1007, 750, 666; $\delta_H$ (400 MHz; d-DMSO) 10.35 (s, 1 H, NH), 8.77 (d, $J$ = 7.5, 1 H, NH), 8.20 (d, $J$ = 7.9, 1 H, Ar−H), 7.67 (dd, $J$ = 7.1, 1.2, 1 H, Ar−H), 7.65 (td, $J$ = 7.8, 1.4, 1 H, Ar−H), 7.53 (dd, $J$ = 7.8, 1.4, 1 H, Ar−H), 7.46 (d, $J$ = 7.7, 1 H, Ar−H), 7.40−7.28 (m, 3 H, 3 × Ar−H), 7.17 (s, 1 H, Ar−H), 5.300, 5.258 (ABq, $J_{AB}$ = 13.6, 2 H, C$\underline{H}_2$NO$_2$), 5.09 (br t, $J$ = 5.1, 1 H, OH), 4.63 (d, $J$ = 4.0, 2 H, C$\underline{H}_2$OH), 4.44 (quintet, $J$ = 7.2, 1 H, C$\underline{H}$CH$_3$), 1.06 (d, $J$ = 7.1, 3 H, CHC$\underline{H}_3$); $\delta_C$ (100 MHz; *d*-DMSO) 170.4 (CO), 166.3 (CO), 160.8 (CO), 135.9 (C), 135.1 (C), 131.6 (Ar−CH), 129.7 (C), 129.2 (Ar−CH), 128.4 (C), 124.9 (Ar−CH), 124.7 (Ar−CH), 124.5 (Ar−CH), 124.3 (Ar−CH), 123.2 (Ar−CH), 122.2 (C), 119.6 (Ar−CH), 115.9 (Ar−CH), 78.2 (C$\underline{H}_2$NO$_2$), 55.2 (C$\underline{H}_2$OH), 48.6 (C$\underline{H}$CH$_3$), 17.8 (CH C$\underline{H}_3$); $m/z$ (ESI+) [M + Na]$^+$ 447.1286 calcd for $C_{21}H_{20}N_4NaO_6$ 447.1275.

**1-(2-Nitrobenzoyl)-1*H*-indole-3-carbaldehyde (18).** Indole-3-carboxaldehyde **19** (3.47 g, 23.9 mmol) was added to a stirred solution of 2-nitrobenzoic acid **20** (6.00 g, 35.9 mmol), *N*,*N*′-dicyclohexylcarbodiimide (8.15 g, 39.5 mmol), and 4-dimethylaminopyridine (0.58 g, 4.79 mmol) in THF (120 mL), and the reaction was stirred at rt for 24 h. Formation of a white precipitate (dicyclohexylurea) was observed, the solid was removed by filtration, and the filtrate was concentrated *in vacuo*. The crude residue was taken up in methanol, and the yellow precipitate formed was collected by filtration. Recrystallization of the yellow solid in methanol afforded the *title compound* **18** (6.52 g, 22.2 mmol, 93%) as pale yellow crystals; $R_f$ 0.53 (EtOAc/hexanes 4:1); mp = 205−207 °C; $\nu_{max}$(neat)/cm$^{-1}$ 3131, 3072, 2825, 1710, 1672, 1532, 1347; $\delta_H$ (400 MHz; $CDCl_3$) 10.01 (s, 1 H, CHO), 8.40 (dd, $J$ = 8.3, 1.1, 1 H, Ar−H), 8.30 (dd, $J$ = 7.7, 2.0, 2 H, 2 × Ar−H), 7.916 (td, $J$ = 7.5, 1.1, 1 H, Ar−H), 7.84 (td, $J$ = 8.4, 1.5, 1 H, Ar−H), 7.67 (dd, $J$ = 7.4, 1.5, 1 H, Ar−H), 7.55 (s, 1 H, Ar−H), 7.52−7.45 (m, 2 H, 2 × Ar−H); $\delta_C$ (100 MHz; $CDCl_3$) 185.4 (CO), 165.1 (CHO), 145.7 (C), 136.5 (C), 135.1 (Ar−CH), 134.9 (Ar−CH), 132.0 (Ar−CH), 130.2 (C), 128.9 (Ar−CH), 127.2 (Ar−CH), 126.5 (C), 126.1 (Ar−CH), 125.3 (Ar−CH), 123.4 (Ar−CH), 122.2 (Ar−CH), 116.1 (C); $m/z$ (ESI+) [M + Na]$^+$ 317.0534 calcd for $C_{16}H_{10}N_2NaO_4$ 317.0533.

**(2-Aminophenyl)(3-(hydroxymethyl)-1*H*-indol-1-yl)-methanone (21).** Sodium borohydride (431 mg, 11.4 mmol) was added to a stirred solution of nitro-aldehyde **18** (3.05 g, 10.4 mmol) in MeOH/THF 1:1 (40 mL) at 0 °C, and the mixture stirred for 5 min at 0 °C. The reaction was quenched with sat. aq. $NH_4Cl$ (15 mL), and the mixture was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc/hexanes 4:1) afforded the intermediate nitro-alcohol as an off-white foam that was used directly in the next step.

Anhydrous tin(II) chloride (19.6 g, 103 mmol) was added to a stirred solution of nitro-alcohol in THF (30 mL) at 0 °C. The reaction mixture was stirred for 10 h and allowed to warm to rt. The reaction was quenched with sat. aq. $NaHCO_3$ (30 mL), and the mixture was extracted with EtOAc (2 × 25 mL). The combined organic layers were washed with brine (30 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc/hexanes 3:2) afforded the *title compound* **21** (1.93 g, 7.26 mmol, 70% over two steps) as a yellow solid; $R_f$ 0.34 (EtOAc/hexanes 4:1); mp = 32.0−38.0 °C; $\nu_{max}$(neat)/cm$^{-1}$ 3471, 3370, 1663, 1613, 1588, 1449, 1350, 1327; $\delta_H$ (400 MHz; $CDCl_3$) 8.27 (dd, $J$ = 8.0, 1.0, 1 H, Ar−H), 7.66 (dd, $J$ = 7.1, 1.2, 1 H, Ar−H), 7.40−7.28 (m, 5 H, 5 × Ar−H), 6.77 (dd, $J$ = 8.6, 0.8, 1 H, Ar−H), 6.73 (td, $J$ = 8.0, 0.9, 1 H, Ar−H), 5.01 (br s, 2 H, $NH_2$), 4.83 (s, 2 H, $CH_2$), OH not observed; $\delta_C$ (100 MHz; $CDCl_3$) 169.1 (CO), 148.9 (C), 136.7 (C), 133.3 (Ar−CH), 131.2 (Ar−CH), 129.6 (C), 125.7 (Ar−CH), 125.1 (Ar−CH), 123.7 (Ar−CH), 121.2 (C), 119.3 (Ar−CH), 117.1 (Ar−CH), 116.8 (Ar−CH), 116.4 (Ar−CH), 116.0 (C),



DOI: 10.1021/acs.joc.6b01369
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57.3 ($CH_2$); *m/z* (ESI+) $[M + Na]^+$ 289.0951 calcd for $C_{16}H_{14}N_2NaO_2$ 289.0947.

**(2-Aminophenyl)(3-(((triisopropylsilyl)oxy)methyl)-1*H*-indol-1-yl)methanone (17).** Triisopropylsilyl chloride (2.11 mL, 9.87 mmol) was added to a stirred solution of alcohol **21** (2.19 g, 8.22 mmol) and 2,6-lutidine (1.90 mL, 16.4 mmol) in DMF (15 mL), and the reaction was stirred at rt for 16 h. The reaction was quenched with aq. lithium bromide (5% w/v, 20 mL), and the mixture was extracted with EtOAc (2 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc/hexanes 3:2) afforded the *title compound* **17** (155 mg, 0.37 mmol, 98%) as a yellow oil; $R_f$ 0.73 (EtOAc/hexanes 3:2); $\nu_{max}$(neat)/$cm^{-1}$ 3481, 3381, 2941, 2891, 2864, 1669, 1616, 1590, 1450, 1351, 1329, 1248, 1216, 1126, 1064, 884; $\delta_H$ (400 MHz; $CDCl_3$) 8.30 (d, *J* = 7.9, 1 H, Ar−H), 7.61 (d, *J* = 7.9, 1 H, Ar−H), 7.36 (td, *J* = 7.6, 1.4, 2 H, 2 × Ar−H), 7.32 (s, 1 H, Ar−H), 7.31−7.28 (m, 2 H, 2 × Ar−H), 6.78 (dd, *J* = 8.5, 0.8, 1 H, Ar−H), 6.72 (td, *J* = 8.0, 0.9, 1 H, Ar−H), 5.01 (br s, 2 H, $NH_2$), 4.96 (d, *J* = 1.3, 2 H, $CH_2$), 1.19−1.12 (m, 3 H, 3 × CH, TIPS), 1.08 (d, *J* = 2.9, 18 H, 6 × $CH_3$, TIPS); $\delta_C$ (100 MHz; $CDCl_3$) 169.1 (CO), 148.9 (C), 136.7 (C), 133.1 (Ar−CH), 131.4 (Ar−CH), 129.5 (C), 124.8 (2 × Ar−CH), 123.5 (Ar−CH), 122.3 (C), 119.5 (Ar−CH), 117.0 (Ar−CH), 116.6 (Ar−CH), 116.3 (Ar−CH), 58.5 ($CH_2$), 18.1 (6 × $CH_3$, TIPS), 12.3 (3 × CH, TIPS), one quaternary carbon not observed but confirmed by mass spectrometry; *m/z* (ESI+) $[M + Na]^+$ 445.2283 calcd for $C_{25}H_{34}N_2NaO_2Si$ 445.2282.

**(*S*)-*tert*-Butyl 2-((2-(3-(((triisopropylsilyl)oxy)methyl)-1*H*-indole-1-carbonyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (15).** A solution of *N,N′*-dicyclohexylcarbodiimide (685 mg, 3.32 mmol) in $CH_2Cl_2$ (60 mL) was added to a stirred solution of aniline **17** (936 mg, 2.21 mmol) and Boc-(L)-Proline **16** (715 mg, 3.32 mmol) in $CH_2Cl_2$ (40 mL). The reaction mixture was stirred at rt for 19.5 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography ($CH_2Cl_2$/EtOAc 9:1) afforded the *title compound* **15** (1.38 g, 3.21 mmol, 100%) as an off-white foam and as a 1:1.2 mixture of rotameric isomers; $R_f$ 0.45 ($CH_2Cl_2$/EtOAc 9:1); $[\alpha]_D^{22}$ −16.1 (*c* 1.00 in $CHCl_3$); $\nu_{max}$(neat)/$cm^{-1}$ 2940, 2865, 1696, 1666, 1602, 1583, 1520, 1449, 1365, 1352, 1329, 1297, 1255, 1216, 1159, 1124, 1085, 1064, 1031, 1014, 919, 880, 829, 747; $\delta_H$ (400 MHz; $CDCl_3$) 10.09 (br s, 1 H, NH)*, 9.93 (br s, 1 H, NH), 8.50 (br s, 1 H, Ar−H), 8.37 (d, *J* = 8.2, 1 H, Ar−H), 7.60−7.51 (m, 3 H, 3 × Ar−H), 7.39 (t, *J* = 7.6, 1 H, Ar−H), 7.32 (t, *J* = 7.4, 1 H, Ar−H), 7.22 (s, 1 H, Ar−H), 7.17 (br s, 1 H, Ar−H), 4.94 (s, 2 H, C$\underline{H}_2$OTIPS), 4.40 (s, 1 H, CH)*, 4.26 (s, 1 H, CH), 3.59−3.37 (m, 2 H, $CH_2$, C-3), 2.22 (s, 1 H, H-1a), 2.10 (s, 1 H, H-1b), 1.85 (m, 2 H, $CH_2$, C-2), 1.33 (s, 9 H, 3 × $CH_3$, Boc)*, 1.26 (s, 9 H, 3 × $CH_3$, Boc), 1.21−1.12 (m, 3 H, 3 × CH, TIPS), 1.07 (d, *J* = 6.6, 18 H, 6 × $CH_3$, TIPS); $\delta_C$ (100 MHz; $CHCl_3$) 168.0 (CO), 155.0 (CO), 154.2 (CO), 141.8 (C), 138.4 (C), 136.7 (C), 132.9 (Ar−CH), 130.6 (Ar−CH), 129.6 (C), 125.3 (Ar−CH), 124.1 (2 × Ar−CH), 123.0 (Ar−CH), 122.4 (C), 112.1 (Ar−CH), 119.5 (Ar−CH), 116.8 (Ar−CH), 80.4 (C, Boc), 62.4 (CH)*, 61.6 (CH), 58.4 (C$\underline{H}_2$OTIPS), 47.1 ($CH_2$, C-3), 29.7 ($CH_2$, C-1), 24.4 ($CH_2$, C-2), 23.8 ($CH_2$, C-2)*, 28.2 (3 × $CH_3$, Boc), 18.1 (6 × $CH_3$, TIPS), 12.0 (3 × CH, TIPS); *m/z* (ESI+) $[M + Na]^+$ 642.3350 calcd for $C_{35}H_{49}N_3NaO_5Si$ 642.3334. * = denotes the other rotameric isomer.

**(*S*)-*tert*-Butyl 2-((2-(3-(hydroxymethyl)-1*H*-indole-1-carbonyl)phenyl)carbamoyl)-pyrrolidine-1-carboxylate (22).** A solution of tetra-*n*-butylammonium fluoride (3.5 mL of 1.0 M in THF, 3.48 mmol) and glacial acetic acid (0.07 mL, 1.16 mmol) in THF (5 mL) was added to a stirred solution of TIPS-ether **15** (720 mg, 1.16 mmol) in THF (30 mL) at 0 °C. The reaction mixture was stirred for 20 h and allowed to warm to rt. The reaction mixture was concentrated *in vacuo*. Purification of the crude product by flash chromatography ($CH_2Cl_2$/EtOAc 7:3) afforded the *title compound* **22** (540 mg, 1.16 mmol, 100%) as an off-white foam and as a 1:1 mixture of rotameric isomers; $R_f$ 0.33 ($CH_2Cl_2$/EtOAc 7:3); $[\alpha]_D^{23}$ −135.1 (*c* 1.00 in $CHCl_3$); $\nu_{max}$(neat)/$cm^{-1}$ 330, 3011, 2923, 2874, 1669, 1602, 1583, 1520, 1450, 1353, 1330, 1253, 1216, 1158, 1121, 1051, 1016, 577, 749; $\delta_H$ (400 MHz; $CDCl_3$) 9.83 (br s, 1 H, NH), 9.42 (br s, 1 H, NH)*, 8.36−8.27 (m, 2 H, 2 × Ar−H), 7.68 (s, 1 H, Ar−H), 7.60−7.48 (m, 2 H, 2 × Ar−H), 7.41−7.32 (m, 2 H, 2 × Ar−H), 7.23 (br s, 2 H, 2 × Ar−H), 4.80 (s, 2 H, C$\underline{H}_2$OH), 4.34 (br s, 1 H, CH), 4.22 (br s, 1 H, CH)*, 3.35 (br s, 2 H, 2 × H-3), 2.17 (br s, 1 H, H-1a), 2.04 (br s, 1 H, H-1b), 1.79−1.67 (m, 2 H, $CH_2$, H-2), 1.32 (s, 9 H, 3 × $CH_3$, Boc); $\delta_C$ (100 MHz; $CDCl_3$) 171.8 (CO), 171.4 (CO), 167.8 (CO), 155.4 (C), 154.3 (C)*, 137.7 (C), 137.4 (C)*, 136.5 (C), 132.9 (Ar−CH), 132.8 (Ar−CH)*, 130.1 (Ar−CH), 129.7 (C), 125.6 (Ar−CH), 125.2 (Ar−CH),124.3 (Ar−CH), 123.8 (Ar−CH), 123.5 (C), 122.6 (Ar−CH), 119.6 (Ar−CH), 116.7 (Ar−CH), 80.6 (C, Boc), 62.2 (CH), 61.4 (CH)*, 57.0 (C$\underline{H}_2$OH), 47.1 ($CH_2$, C-3), 46.8 ($CH_2$, C-3)*, 31.2 ($CH_2$, C-1), 29.4 ($CH_2$, C-1)*, 28.2 (3 × $CH_3$, Boc), 24.4 ($CH_2$, C-2), 23.7 ($CH_2$, C-2)*; *m/z* (ESI+) $[M + Na]^+$ 486.1986 calcd for $C_{26}H_{29}N_3NaO_5$ 486.1999. * = denotes the other rotameric isomer.

**Benzyl 2-Nitroacetate (13).** A solution of *N,N′*-dicyclohexylcarbodiimide (147 mg, 0.71 mmol) in THF (1 mL) was added to a stirred solution of nitroacetic acid (406 mg, 0.48 mmol) and benzyl alcohol (0.05 mL, 0.48 mmol) in THF (3 mL) at 0 °C, and then stirred at 0 °C for 50 min. The reaction mixture was filtered through a pad of Celite and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography (PE/EtOAc 8:2) afforded the *title compound* **13** (74 mg, 0.48 mmol, 80%) as a yellow wax; $R_f$ 0.40 (PE/EtOAc 8:2); mp =33−36 °C; $\nu_{max}$(neat)/$cm^{-1}$ 3044, 2986, 1748, 1622, 1558, 1496, 1456, 1407, 1379, 1337, 1219, 1179, 1082, 1002, 970, 927, 904, 841, 792, 756, 701, 689; $\delta_H$ (400 MHz; $CDCl_3$) 7.41−7.34 (m, 5 H, 5 × Ar−H), 5.28 (s, 2 H, C$\underline{H}_2$Ph), 5.18 (s, 2 H, C$\underline{H}_2NO_2$); $\delta_C$ (100 MHz; $CDCl_3$) 161.7 (CO), 134.0 (C), 129.1 (Ar−CH), 128.8 (2 × Ar−CH), 128.7 (2 × Ar−CH), 76.3 (C$\underline{H}_2NO_2$), 68.8 (C$\underline{H}_2$Ph); *m/z* (ESI+) $[M + Na]^+$ 218.0428 calcd for $C_9H_9NNaO_4$ 218.0424.

***tert*-Butyl (2*S*)-2-((2-(3-(3-(Benzyloxy)-2-nitro-3-oxopropyl)-1*H*-indole-1-carbonyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (12).** Phosphorus tribromide (0.3 mL, 2.83 mmol) was added to a stirred solution of alcohol **22** (328 mg, 0.71 mmol) in THF (4 mL) at −78 °C and the mixture stirred for 45 min at 0 °C. The reaction was quenched with sat. aq. $NaHCO_3$ (10 mL) and the mixture extracted with $Et_2O$ (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude bromide product (354 mg) was used directly in the next step without any purification.

Potassium *tert*-butoxide (95 mg, 0.85 mmol) was added to a stirred solution of benzyl nitroacetate (166 mg, 0.85 mmol) and tetra-*n*-butylammonium bromide (274 mg, 0.85 mmol) in DMSO (5 mL) and the reaction was stirred at rt for 15 min. Crude bromide in DMSO (1 mL) was added and the reaction was stirred at rt for 2 h. The reaction was quenched with brine (20 mL) and the mixture extracted with $Et_2O$ (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification of the crude product by flash chromatography (PE/EtOAc 7:3) afforded the *title compound* **12** (159 mg, 0.25 mmol, 35% over two steps) as a white foam and as a 1:1 mixture of diastereoisomers; $R_f$ 0.46 (PE/EtOAc 3:2); $\nu_{max}$(neat)/$cm^{-1}$ 2972, 2934, 2885, 1752, 1692, 1604, 1583, 1561, 1518, 1488, 1451, 1364, 1328, 1300, 1258, 1216, 1156, 1120, 1088, 1039, 876, 751, 697; $\delta_H$ (400 MHz; $CDCl_3$) 10.03 (d, *J* = 15.7, 1 H, NH), 9.86 (s, 1 H, NH)*, 8.45 (d, *J* = 8.3, 1 H, Ar−H), 8.44 (d, *J* = 8.5, 1 H, Ar−H)*, 8.33 (d, *J* = 7.8, 1 H, Ar−H), 7.58 (s, 1 H, Ar−H), 7.54 (d, *J* = 8.7, 1 H, Ar−H), 7.41 (t, *J* = 8.6, 1 H, Ar−H), 7.37−7.33 (m, 5 H, Ar−H), 7.27−7.25 (m, 2 H, Ar−H), 7.19 (s, 1 H, Ar−H), 7.15 (s, 1 H, Ar−H), 5.46 (dd, $J_{AX}$ = 9.5, $J_{BX}$ = 5.5, 1 H, C$\underline{H}NO_2$), 5.24 (s, 2 H, $CH_2$, Bn), 4.38 (br s, 1 H, C$\underline{H}$C-1), 4.24 (br s, 1 H, C$\underline{H}$C-1)*, 3.62 (ABX, $\Delta\delta_{AB}$ = 0.05, $J_{AB}$ = 15.5, $J_{AX}$ = 9.5, $J_{BX}$ = 5.5, 2 H, C$\underline{H}_2$CHNO$_2$), 3.52 (br s, 1 H, H-3a), 3.36 (br s, 1 H, H-3b), 2.22 (br s, 1 H, H-1a), 2.08 (br s, 1 H, H-1b), 1.85 (br s, 2 H, $CH_2$, H-2), 1.32 (s, 9 H, 3 × $CH_3$, Boc), 1.26 (s, 9 H, 3 × $CH_3$, Boc)*; $\delta_C$ (100 MHz; $CDCl_3$) 172.1 (CO), 171.3 (CO)*, 167.8 (CO), 163.7 (CO), 154.3 (CO), 138.5 (C), 136.3 (C), 134.0 (C), 133.4 (Ar−CH), 133.2 (Ar−CH)*, 130.6 (Ar−CH), 130.3 (Ar−CH)*, 129.5 (C), 129.0 (Ar−CH), 128.8 (Ar−CH), 128.4 (2 × Ar−CH), 126.5 (C), 126.4 (Ar−CH), 125.9 (Ar−CH), 125.5


DOI: 10.1021/acs.joc.6b01369
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(Ar−CH), 124.4 (Ar−CH), 123.3 (Ar−CH), 122.4 (Ar−CH), 122.3 (Ar−CH)*, 118.3 (Ar−CH), 116.9 (Ar−CH), 114.6 (C), 87.2 (CHNO$_2$), 80.5 (C, Boc), 68.8 (CH$_2$, Bn), 62.3 (CHC-1), 61.5 (CHC-1)*, 47.1 (CH$_2$, C-3), 16.9 (CH$_2$, C-3)*, 31.3 (CH$_2$, C-1), 29.4 (CH$_2$, C-1)*, 28.2 (3 × CH$_3$, Boc), 26.1 (CH$_2$CHNO$_2$), 24.4 (CH$_2$, C-2), 23.8 (CH$_2$, C-2); $m/z$ (ESI+) [M + Na]$^+$ 663.2415 calcd for $C_{35}H_{36}N_4NaO_8$ 663.2425. * = denotes the other diastereoisomer.

***tert*-Butyl (2*S*)-2-((2-(3-(2-Amino-3-(benzyloxy)-3-oxopropyl)-1*H*-indole-1-carbonyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (23).** Glaciel acetic acid (1 mL, 17.3 mmol) was added to a stirred mixture of nitroester **12** (30 mg, 0.05 mmol) and zinc dust (18 mg, 0.28 mmol) in isopropanol (2 mL), and the reaction was stirred at rt for 17.5 h. The reaction was quenched with sat. aq. NaHCO$_3$ (10 mL), and the mixture extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (20 mL), dried over MgSO$_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc/MeOH 95:5) afforded the *title compound* **23** (28 mg, 0.05 mmol, 100%) as a white foam and as a 1:1 mixture of diastereoisomers; $R_f$ 0.39 (EtOAc/MeOH 95:5); $\nu_{max}$(neat)/cm$^{-1}$ 3357, 2976, 2937, 2885, 1735, 1690, 1604, 1582, 1514, 1492, 1450, 1364, 1330, 1300, 1255, 1214, 1156, 1119, 1088, 1040, 1020, 980, 924, 882, 853, 793, 751, 697; $\delta_H$ (400 MHz; CDCl$_3$) 10.03 (s, 1 H, NH), 9.85 (s, 1 H, NH)*, 8.45 (dd, $J$ = 8.4, 1.6, 1 H, Ar−H), 8.35 (br s, 1 H, Ar−H), 7.58 (d, $J$ = 7.6, 1 H, Ar−H), 7.56 (m, 1 H, Ar−H), 7.39 (t, $J$ = 7.4, 2 H, Ar−H), 7.35−7.27 (m, 4 H, 4 × Ar−H), 7.24−7.23 (m, 2 H, 2 × Ar−H), 7.15 (br s, 2 H, Ar−H), 5.16−5.06 (m, 2 H, CH$_2$, Bn), 4.38 (br s, 1 H, CHC-1), 4.24 (br s, 1 H, CHC-1)*, 3.86 (dd, $J_{AX}$ = $J_{BX}$ = 6.3, 1 H, CHNH$_2$), 3.57−3.46 (m, 1 H, H-3a), 3.35 (br s, 1 H, H-3b), 3.07 (ABX, $\Delta\delta_{AB}$ = 0.06, $J_{AB}$ = 14.5, $J_{AX}$ = 5.2, $J_{BX}$ = 7.5, 2 H, CH$_2$CHNH$_2$), 2.21 (br s, 1 H, H-1a), 2.06 (br s, 1 H, H-1b), 1.83 (br s, 2 H, CH$_2$, H-2), 1.32 (s, 9 H, 3 × CH$_3$, Boc), 1.27 (s, 9 H, 3 × CH$_3$, Boc)*; $\delta_C$ (100 MHz; CDCl$_3$) 174.7 (CO), 172.0 (CO), 171.3 (CO), 167.7 (CO), 138.3 (C), 136.3 (C), 135.4 (C), 133.1 (Ar−CH), 132.9 (Ar−CH)*, 130.9 (C), 130.5 (Ar−CH), 130.2 (Ar−CH)*, 128.6 (Ar−CH), 128.5 (Ar−CH), 128.2 (Ar−CH), 125.9 (Ar−CH), 125.7 (Ar−CH)*, 125.5 (Ar−CH), 125.2 (Ar−CH)*, 124.1 (Ar−CH), 123.2 (Ar−CH), 122.3 (Ar−CH), 122.3 (Ar−CH), 119.0 (Ar−CH), 118.0 (C), 117.7 (C), 116.8 (Ar−CH), 80.5 (C, Boc), 66.9 (CH$_2$, Bn), 62.3 (CHC-1), 61.5 (CHC-1)*, 54.5 (CHNO$_2$), 47.1 (CH$_2$, C-3), 46.9 (CH$_2$, C-3)*, 31.4 (CH$_2$, C-1), 30.4 (CH$_2$CHNO$_2$), 29.5 (CH$_2$, C-1)*, 28.2 (3 × CH$_3$, Boc), 24.4 (CH$_2$, C-2), 23.8 (CH$_2$, C-2)*; $m/z$ (ESI+) [M + H]$^+$ 611.2848 calcd for $C_{35}H_{39}N_4O_6$ 611.2864. * = denotes the other diastereoisomer.

***tert*-Butyl (2*S*)-2-((2-(3-(2-Acetamido-3-(benzyloxy)-3-oxopropyl)-1*H*-indole-1-carbonyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (11).** Acetyl chloride (4 μL, 0.06 mmol) was added to a stirred solution of amino ester **23** (31 mg, 0.05 mmol) and trimethylamine (11 μL, 0.08 mmol) in CH$_2$Cl$_2$ (2 mL) at 0 °C, and the mixture was stirred for 2 d at rt. The reaction was quenched with sat. aq. NaHCO$_3$ (10 mL), and the mixture was extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (20 mL), dried over MgSO$_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc/MeOH 95:5) afforded the *title compound* **11** (28 mg, 0.04 mmol, 85%) as an off-white foam and as a 1:1.2 mixture of diastereoisomers; $R_f$ 0.57 (EtOAc/MeOH 95:5); $\nu_{max}$(neat)/cm$^{-1}$ 2970, 1744, 1691, 1664, 1603, 1583, 1562, 1515, 1451, 1364, 1329, 1297, 1258, 1215, 1157, 1119, 1088, 1019, 919, 875, 794, 750, 698; $\delta_H$ (500 MHz; CDCl$_3$) 9.93 (s, 1 H, NH)$^\dagger$, 9.86 (s, 1 H, NH), 9.76 (s, 1 H, NH)*, 9.68 (s, 1 H, NH)*$^\dagger$, 8.38−8.30 (m, 2 H, 2 × Ar−H), 7.57 (br s, 1 H, Ar−H), 7.51 (dd, $J$ = 7.7, 4.2, 1 H, Ar−H), 7.40−7.27 (m, 6 H, 6 × Ar−H), 7.18−7.15 (m, 3 H, 3 × Ar−H), 6.98−6.93 (m, 1 H, Ar−H), 6.28 (br s, 1 H, NH), 6.22 (br s, 1 H, NH)*, 5.12−4.95 (m, 3 H, CH$_2$, Bn and CHNHAc), 4.36 (br s, 1 H, CHC-1), 4.24 (br s, 1 H, CHC-1)*, 3.50−3.33 (m, 2 H, CH$_2$, H-3), 3.23 (ABX, $\Delta\delta_{AB}$ = 0.03, $J_{AB}$ = 14.5, $J_{AX}$ = 5.0, $J_{BX}$ = 5.1, 2 H, CH$_2$CHNHAc), 2.18 (br s, 1 H, H-1a), 2.01 (br s, 1 H, H-1b), 1.952 (s, 3 H, CH$_3$, Ac), 1.947 (s, 3 H, CH$_3$, Ac)*, 1.81−1.77 (m, 2 H, CH$_2$, H-2), 1.32 (br s, 9 H, 3 × CH$_3$, Boc), 1.30 (br s, 9 H, 3 × CH$_3$, Boc)*; $\delta_C$ (125 MHz; CDCl$_3$) 172.1 (CO), 171.4 (CO), 169.9 (CO), 169.8 (CO)*, 167.6 (CO), 167.6 (CO)*, 155.4 (CO), 138.0 (C), 137.8 (C), 136.11 (C), 136.07 (C), 133.0 (Ar−CH), 132.8 (Ar−CH)*, 131.0 (2 × C), 130.3 (Ar−CH), 130.0 (Ar−CH), 128.6 (2 × Ar−CH), 128.2 (2 × Ar−CH), 125.9 (Ar−CH), 125.5 (Ar−CH), 125.2 (Ar−CH)*, 124.2 (Ar−CH), 123.6 (Ar−CH), 123.4 (Ar−CH)*, 123.0 (Ar−CH), 122.8 (Ar−CH)*, 118.9 (Ar−CH), 116.6 (Ar−CH), 80.5 (C, Boc), 67.4 (CH$_2$, Bn), 62.2 (CHC-1), 61.4 (CHC-1)*, 52.5 (CHNHAc), 47.1 (CH$_2$, C-3), 31.3 (CH$_2$, C-1), 28.4 (CH$_2$, C-1)*, 28.2 (3 × CH$_3$, Boc), 27.3 (CH$_2$CHNHAc), 27.2 (CH$_2$CHNHAc)*, 24.4 (CH$_2$, C-2), 23.8 (CH$_2$, C-2)*, 23.2 (CH$_3$, Ac), 23.1 (CH$_3$, Ac)*; $m/z$ (ESI+) [M + Na]$^+$ 675.2780 calcd for $C_{37}H_{40}N_4NaO_7$ 675.2789. * = denotes the other diastereoisomer. $^\dagger$ = denotes the other rotameric isomer.

***N*$^\alpha$-Acetyl-1-(2-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)benzoyl)tryptophan (24).** 10% Pd/C (10 wt %, 10 mg, 0.01 mmol) was added to a stirred solution of benzyl ester **11** (70 mg, 0.11 mmol) in MeOH (8 mL) at rt under a N$_2$ atmosphere, and the mixture stirred under a H$_2$ atmosphere for 2.3 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography (10% MeOH/CH$_2$Cl$_2$) afforded the *title compound* **24** (60 mg, 0.11 mmol, 99%) as a white foam and as a 1:1.4 mixture of diastereoisomers; $R_f$ 0.06 (CH$_2$Cl$_2$/MeOH 9:1); $\nu_{max}$(neat)/cm$^{-1}$ 3303, 2979, 2934, 2885, 1659, 1604, 1584, 1522, 1482, 1452, 1366, 1331, 1254, 1216, 1159, 1124, 1040, 920, 876, 752; $\delta_H$ (400 MHz; CDCl$_3$) 9.63 (s, 1 H, NH), 9.53 (s, 1 H, NH)*, 8.37−8.03 (m, 2 H, 2 × Ar−H), 7.57 (d, $J$ = 7.4, 2 H, 2 × Ar−H), 7.50 (br s, 1 H, Ar−H), 7.35 (t, $J$ = 7.4, 1 H, Ar−H), 7.30 (t, $J$ = 7.4, 1 H, Ar−H), 7.22 (br s, 1 H, Ar−H), 7.05−7.00 (m, 1 H, Ar−H), 6.60 (br s, 1 H, NH), 4.81 (dd, $J$ = 12.5, 5.5, 1 H, CHNHAc), 4.25 (br s, 1 H, CHC-1), 3.47 (br s, 2 H, CH$_2$, H-3)*, 3.39 (br s, 2 H, CH$_2$, H-3), 3.19 (ABX, $\Delta\delta_{AB}$ = 0.04, $J_{AB}$ = 14.8, $J_{AX}$ = 5.6, $J_{BX}$ = 5.3, 2 H, CH$_2$CHNHAc), 2.08 (br s, 2 H, CH$_2$, H-1), 1.93 (s, 3 H, CH$_3$, Ac)*, 1.92 (s, 3 H, CH$_3$, Ac), 1.80 (br s, 2 H, CH$_2$, H-2), 1.31 (br s, 9 H, 3 × CH$_3$, Boc)*, 1.29 (br s, 9 H, 3 × CH$_3$, Boc); $\delta_C$ (100 MHz; CDCl$_3$) 173.3 (CO), 173.2 (CO), 170.8 (CO), 167.63 (CO), 167.61 (CO), 137.0 (C), 135.9 (2 × C), 132.6 (Ar−CH), 131.2 (C), 130.0 (Ar−CH), 129.9 (Ar−CH), 125.9 (Ar−CH), 125.2 (Ar−CH), 124.3 (Ar−CH), 123.1 (Ar−CH), 119.1 (Ar−CH), 117.2 (C), 116.6 (Ar−CH), 81.1 (C, Boc), 62.0 (CHC-1)*, 61.3 (CHC-1), 52.7 (CHNHAc), 47.2 (CH$_2$, C-3), 46.9 (CH$_2$, C-3)*, 31.2 (CH$_2$, C-1)*, 29.7 (CH$_2$, C-1), 28.22 (3 × CH$_3$, Boc), 28.19 (3 × CH$_3$, Boc)*, 27.0 (CH$_2$CHNHAc), 24.3 (CH$_2$, C-2), 23.5 (CH$_2$, C-2)*, 23.0 (CH$_3$, Ac)*, 22.9 (CH$_3$, Ac); $m/z$ (ESI+) [M + Na]$^+$ 585.2303 calcd for $C_{30}H_{34}N_4NaO_7$ 585.2320. * = denotes the other diastereoisomer.

***tert*-Butyl (*S*)-2-((2-(3-(2-Nitroethyl)-1*H*-indole-1-carbonyl)-phenyl)carbamoyl)-pyrrolidine-1-carboxylate (25).** 10% Pd/C (10 wt %, 10 mg, 0.01 mmol) was added to a stirred solution of tripeptide benzyl ester precursor (19 mg, 0.03 mmol) in MeOH (3 mL) at rt under a N$_2$ atmosphere, and the mixture was stirred under a H$_2$ atmosphere for 2 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography (PE/EtOAc 3:2) afforded the *title compound* **25** (12 mg, 0.02 mmol, 80%) as an off-white foam and as a 1:1.3 mixture of rotameric isomers; $R_f$ 0.44 (PE/EtOAc 3:2); $[\alpha]_D^{20}$ −27.8 ($c$ 1.00 in CHCl$_3$); $\nu_{max}$(neat)/cm$^{-1}$ 3353, 2962, 2920, 2853, 1689, 1670, 1597, 1583, 1549, 1528, 1451, 1366, 1328, 1259, 1219, 1151, 1127, 1088, 928, 857, 751, 681; $\delta_H$ (500 MHz; $d$-DMSO) 10.08 (br s, 1 H, NH), 8.17 (d, $J$ = 7.8, 1 H, Ar−H)*, 8.12 (d, $J$ = 7.2, 1 H, Ar−H), 7.71 (d, $J$ = 6.7, 1 H, Ar−H), 7.65 (t, $J$ = 7.7, 1 H, Ar−H), 7.53 (d, $J$ = 7.5, 1 H, Ar−H)*, 7.50 (d, $J$ = 7.7, 1 H, Ar−H), 7.44 (d, $J$ = 7.5, 1 H, Ar−H), 7.37−7.30 (m, 3 H, 3 × Ar−H), 7.18 (s, 1 H, Ar−H), 4.86 (t, $J$ = 7.1, 2 H, CH$_2$NO$_2$), 4.16−4.12 (m, 1 H, CHC-1)*, 4.08 (dd, $J$ = 8.6, 4.1, 1 H, CHC-1), 3.32 (t, $J$ = 7.1, 2 H, CH$_2$CH$_2$NO$_2$), 3.25−3.19 (m, 1 H, H-3a), 3.18−3.13 (m, 1 H, H-3b), 1.91−1.83 (m, 1 H, H-1a), 1.56−1.48 (m, 1 H, H-1b), 1.45−1.38 (m, 2 H, 2 × H-2), 1.32 (s, 9 H, 3 × CH$_3$, Boc)*, 1.28 (s, 9 H, 3 × CH$_3$, Boc); $\delta_C$ (125 MHz; $d$-DMSO) 172.0 (CO), 166.8 (CO), 166.7 (CO), 136.0 (C), 135.9 (C), 132.3 (Ar−CH), 130.5 (C), 129.9 (Ar−CH), 128.6 (C), 126.1 (Ar−CH), 125.3 (Ar−CH), 125.2 (Ar−CH), 125.1 (C), 124.7 (Ar−CH), 124.3 (Ar−CH)*, 123.9 (Ar−CH), 119.5


DOI: 10.1021/acs.joc.6b01369
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(Ar−CH), 116.4 (Ar−CH), 79.0 (C, Boc), 75.0 ($CH_2$, $\underline{C}H_2NO_2$), 60.3 ($\underline{C}$HC-1)*, 60.1 ($\underline{C}$HC-1), 47.0 ($CH_2$, C-3)*, 46.8 ($CH_2$, C-3), 30.6 ($CH_2$, C1), 28.5 (3 × $CH_3$, Boc)*, 28.4 (3 × $CH_3$, Boc), 23.2 ($CH_2$, C-2), 22.6 ($\underline{C}H_2CH_2NO_2$); *m/z* (ESI+) $[M + Na]^+$ 529.2069 calcd for $C_{27}H_{30}N_4NaO_6$ 529.2058. * = denotes the minor rotameric isomer

***N*$^{\alpha}$-Acetyl-L-tryptophan Benzyl Ester (28).** Benzyl bromide (0.4 mL, 3.65 mmol) was added to a stirred solution of *N*-Ac-L-tryptophan (**26**) (600 mg, 2.44 mmol) and DIPEA (1.1 mL, 6.09 mmol) in DMF (30 mL) under a $N_2$ atmosphere, and the reaction mixture was stirred for 23 h. The reaction was diluted by the addition of water (60 mL), and the mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc) afforded the *title compound* **28** (814 mg, 2.44 mmol, 99%) as a white foam; $R_f$ 0.46 (EtOAc); $[\alpha]_D^{25}$ −13.5 (*c* 1.00 in $CHCl_3$); $\nu_{max}$(neat)/$cm^{-1}$ 3399, 3275, 3059, 2930, 1732, 1652, 1518, 1456, 1340, 1175, 1130, 1009, 739, 696; $\delta_H$ (500 MHz; $CDCl_3$) 8.44 (s, 1 H, NH, indole), 7.50 (d, *J* = 7.9, 1 H, Ar−H), 7.33−7.29 (m, 4 H, 4 × Ar−H), 7.23−7.21 (m, 2 H, 2 × Ar−H), 7.16 (td, *J* = 6.8, 1.0, 1 H, Ar−H), 7.08 (td, *J* = 7.6, 1.1, 1 H, Ar−H), 6.70 (d, *J* = 2.4, 1 H, Ar−H), 6.14 (d, *J* = 7.9, 1 H, NH), 5.09, 5.05 (ABq, *J* = 12.2, 2 H, $CH_2$, Bn), 5.00−4.96 (m, 1 H, C$\underline{H}$NHAc), 3.29 (ABX, $\Delta\delta_{AB}$ = 0.03, $J_{AB}$ = 14.7, $J_{AX}$ = 5.5, $J_{BX}$ = 5.3, 2 H, C$\underline{H}_2$CHNHAc), 1.89 (s, 3 H, $CH_3$, Ac); $\delta_C$ (125 MHz; $CDCl_3$) 172.0 (CO), 170.1 (CO), 136.2 (C), 135.3 (C), 128.6 (2 × Ar−CH), 128.5 (3 × Ar−CH), 127.8 (C), 123.0 (Ar−CH), 122.2 (Ar−CH), 119.6 (Ar−CH), 118.5 (Ar−CH), 111.4 (Ar−CH), 109.6 (C), 67.2 ($CH_2$, Bn), 53.3 (CH), 27.6 ($\underline{C}H_2$CHNHAc), 23.2 ($CH_3$, Ac); *m/z* (ESI+) $[M + Na]^+$ 359.1370 calcd for $C_{20}H_{20}N_2NaO_3$ 359.1366.

***N*$^{\alpha}$-Acetyl-1-(2-aminobenzoyl)-L-tryptophan Benzyl Ester (29).** Isatoic anhydride (**27**) (508 mg, 3.12 mmol), 18-crown-6 (2.06 g, 7.79 mmol), potassium fluoride (453 mg, 7.79 mmol), and DIPEA (0.54 mL, 3.12 mmol) were added to a solution of indole **28** (524 mg, 1.56 mmol) in MeCN (50 mL) at rt, and the reaction mixture was stirred for 21 h at 60 °C. The reaction was quenched with water (60 mL), and the mixture extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with brine (60 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc/PE 7:3) afforded the *title compound* **29** (369 mg, 0.83 mmol, 53%) as a white foam; $R_f$ 0.61 (EtOAc/PE 8:2); $[\alpha]_D^{24}$ −3.0 (*c* 1.00 in $CHCl_3$); $\nu_{max}$(neat)/$cm^{-1}$ 3473, 3355, 3061, 2925, 1738, 1656, 1617, 1589, 1535, 1490, 1451, 1364, 1327, 1248, 1215, 1181, 1162, 1127, 1029, 915, 885, 818, 787, 746, 697, 667; $\delta_H$ (500 MHz; $CDCl_3$) 8.25 (d, *J* = 8.0, 1 H, Ar−H), 7.35 (td, *J* = 7.0, 1.1, 1 H, Ar−H), 7.32−7.22 (m, 6 H, 6 × Ar−H), 7.16−7.14 (m, 2 H, 2 × Ar−H), 7.06 (s, 1 H, Ar−H), 6.77 (d, *J* = 8.5, 1 H, Ar−H), 6.70 (t, *J* = 7.8, 1 H, Ar−H), 6.14 (d, *J* = 7.6, 1 H, N$\underline{H}$Ac), 5.10−4.96 (m, 5 H, $CH_2$, Bn; Ar−N$\underline{H}_2$ and C$\underline{H}$NHAc), 3.24 (ABX, $\Delta\delta_{AB}$ = 0.03, $J_{AB}$ = 14.7, $J_{AX}$ = 5.6, $J_{BX}$ = 4.8, 2 H, C$\underline{H}_2$CHNHAc), 1.95 (s, 3 H, $CH_3$, Ac); $\delta_C$ (125 MHz; $CDCl_3$) 171.5 (CO), 169.7 (CO), 168.8 (CO), 149.0 (C), 136.1 (C), 134.8 (C), 133.4 (Ar−CH), 131.3 (Ar−CH), 131.0 (C), 128.65 (2 × Ar−CH), 128.61 (Ar−CH), 128.3 (Ar−CH), 126.1 (Ar−CH), 125.0 (Ar−CH), 123.7 (Ar−CH), 118.8 (Ar−CH), 117.1 (Ar−CH), 117.1 (Ar−CH), 116.7 (Ar−CH), 116.2 (Ar−CH), 116.0 (C), 115.6 (C), 67.5 ($CH_2$, Bn), 52.8 ($\underline{C}$HNHAc), 27.4 ($\underline{C}H_2$CHNHAc), 23.2 ($CH_3$, Ac); *m/z* (ESI+) $[M + Na]^+$ 478.1751 calcd for $C_{27}H_{25}N_3NaO_4$ 478.1737.

***tert*-Butyl (*S*)-2-((2-(3-((*S*)-2-Acetamido-3-(benzyloxy)-3-oxopropyl)-1*H*-indole-1-carbonyl)phenyl)carbamoyl)pyrrolidine-1-carboxylate (11a).** A solution of *N*,*N*′-dicyclohexylcarbodiimide (251 mg, 1.21 mmol) in $CH_2Cl_2$ (14 mL) was added to a stirred solution of aniline **29** (369 mg, 0.81 mmol) and Boc-L-proline **16** (261 mg, 1.21 mmol) in $CH_2Cl_2$ (14 mL). After stirring for 30 min at rt, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography (EtOAc/PE 7:3) afforded the *title compound* **11a** (381 mg, 0.58 mmol, 72%) as an off-white foam and as a 1:1.2 mixture of rotameric isomers; $R_f$ 0.28 (EtOAc/PE 7:3); $\nu_{max}$(neat)/$cm^{-1}$ 3302, 3062, 3011, 2931, 2855, 1738, 1657, 1518, 1457, 1439, 1371, 1341, 1213, 1190, 1130, 1010, 741, 696, 666; $[\alpha]_D^{23}$ −6.7 (*c* 1.00 in $CHCl_3$); $\delta_H$ (500 MHz; $CDCl_3$) 9.93 (br s, 1 H, NH), 9.76 (br s, 1 H, NH)*, 8.38 (d, *J* = 7.7, 1 H, Ar−H), 8.29 (br s, 1 H, Ar−H), 7.57 (br s, 1 H, Ar−H), 7.52 (d, *J* = 7.7, 1 H, Ar−H), 7.38 (t, *J* = 7.6, 1 H, Ar−H), 7.35−7.26 (m, 5 H, 5 × Ar−H), 7.17−7.15 (m, 3 H, 3 × Ar−H), 6.98−6.93 (m, 1 H, Ar−H), 6.17 (br s, 1 H, NH), 5.11, 5.018 (ABq, *J* = 12.3, 2 H, $CH_2$, Bn), 4.99−4.95 (m, 1 H, C$\underline{H}$NHAc), 4.36 (br s, 1 H, C$\underline{H}$C-1), 4.24 (br s, 1 H, C$\underline{H}$C-1)*, 3.50 (br s, 1 H, H-3a), 3.35 (br s, 1 H, H-3b), 3.28−3.17 (m, 2 H, C$\underline{H}_2$CHNHAc), 2.19 (br s, 1 H, H-1a), 2.04 (br s, 1 H, H-1b), 1.95 (s, 3 H, $CH_3$, Ac), 1.82 (br s, 2 H, 2 × H-2), 1.33 (s, 9 H, 3 × $CH_3$, Boc), 1.28 (s, 9 H, 3 × $CH_3$, Boc)*; $\delta_C$ (125 MHz; $CDCl_3$) 171.4 (CO), 169.8 (CO), 167.6 (CO), 136.1 (CO), 134.8 (CO), 133.4 (Ar−CH), 132.8 (Ar−CH), 131.0 (C), 130.5 (Ar−CH), 130.0 (Ar−CH), 128.6 (Ar−CH), 128.2 (Ar−CH), 125.8 (Ar−CH), 125.5 (Ar−CH), 125.2 (Ar−CH), 124.2 (Ar−CH), 123.4 (Ar−CH), 122.8 (Ar−CH), 118.9 (Ar−CH), 116.6 (Ar−CH), 80.5 (C, Boc), 67.5 ($CH_2$, Bn), 52.5 ($\underline{C}$HNHAc), 47.1 ($CH_2$, C-3), 29.4 ($CH_2$, C-1), 28.2 (3 × $CH_3$, Boc), 27.3 ($\underline{C}H_2$CHNHAc), 24.4 ($CH_2$, C-2), 23.8 ($CH_2$, C-2)*, 23.2 ($CH_3$, Ac), four quaternary carbons not observed but confirmed by mass spectrometry; *m/z* (ESI +) $[M + Na]^+$ 675.2803 calcd for $C_{37}H_{40}N_4NaO_7$ 675.2789. * = denotes the minor rotameric isomer.

***N*$^{\alpha}$-Acetyl-1-(2-((*S*)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxamido)benzoyl)-L-tryptophan (24a).** 10% Pd/C (10 wt %, 20 mg, 0.02 mmol) was added to a stirred solution of benzyl ester **11a** (83 mg, 0.13 mmol) in MeOH (15 mL) at rt under a $N_2$ atmosphere, and the mixture stirred under a $H_2$ atmosphere for 2 h. The reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. Purification of the crude product by flash chromatography (10% MeOH/$CH_2Cl_2$) afforded the *title compound* **24a** (67 mg, 0.12 mmol, 93%) as a white foam and as a 1:1.5 mixture of rotameric isomers; $R_f$ 0.06 ($CH_2Cl_2$/MeOH 9:1); $[\alpha]_D^{23}$ −28.2 (*c* 1.00 in $CHCl_3$); $\nu_{max}$(neat)/$cm^{-1}$ 3303, 2979, 2934, 2885, 1659, 1604, 1584, 1522, 1482, 1452, 1366, 1331, 1254, 1216, 1159, 1124, 1040, 920, 876, 752; $\delta_H$ (400 MHz; *d*-DMSO) 12.78 (s, 1 H, $CO_2H$), 10.10 (s, 1 H, NH), 8.21−8.14 (m, 2 H, Ac-NH, Ar−H), 7.67−7.63 (m, 2 H, 2 × Ar−H), 7.56−7.45 (m, 2 H, 2 × Ar−H), 7.36−7.30 (m, 3 H, 3 × Ar−H), 7.14 (s, 1 H, Ar−H), 4.48−4.43 (m, 1 H, C$\underline{H}$NHAc), 4.16 (d, *J* = 8.3, 1 H, CHC-1)*, 4.11 (d, *J* = 8.5, 1 H, CHC-1), 3.33−3.24 (m, 2 H, $CH_2$, H-3), 3.17−2.90 (m, 2 H, C$\underline{H}_2$CHNHAc), 1.92−1.86 (m, 1 H, H-1a), 1.77 (s, 3 H, $CH_3$, Ac), 1.56−1.41 (m, 3 H, $CH_2$, H-2, H-1b), 1.33 (s, 9 H, 3 × $CH_3$, Boc)* 1.30 (s, 9 H, 3 × $CH_3$, Boc); $\delta_C$ (100 MHz; *d*-DMSO) 173.1 (CO), 171.4 (CO), 170.9 (CO)*, 169.2 (CO), 166.3(CO)*, 166.2 (CO), 153.5 (CO)*, 153.0 (CO), 135.7 (C)*, 135.6 (C), 135.4 (C), 131.6 (Ar−CH), 130.5 (C), 129.3 (Ar−CH), 128.3 (C), 126.0 (Ar−CH), 124.7 (Ar−CH), 124.5 (Ar−CH), 124.2 (Ar−CH), 123.4 (Ar−CH), 118.8 (Ar−CH), 116.6 (C), 115.8 (Ar−CH), 18.7 (C, Boc)*, 78.5 (C, Boc), 59.8 ($\underline{C}$HC-1), 59.6 ($\underline{C}$HC-1)*, 51.8 ($\underline{C}$HNHAc), 46.5 ($CH_2$, C-3)*, 46.3 ($CH_2$, C-3), 30.1 ($CH_2$, C-1), 29.1 ($CH_2$, C-1)*, 28.0 (3 × $CH_3$, Boc)*, 27.9 (3 × $CH_3$, Boc), 26.4 ($\underline{C}H_2$CHNHAc), 23.4 ($CH_2$, C-2)*, 22.7 ($CH_2$, C-2), 22.3 ($CH_3$, Ac); *m/z* (ESI+) $[M + Na]^+$ 585.2303 calcd for $C_{30}H_{34}N_4NaO_7$ 585.2320. * = denotes the minor rotameric isomer.

**Psychrophilin E (5).** *Method A.* TFA (0.8 mL, 10 mmol) was added to a stirred solution of Boc-amide **24** (26 mg, 46 μmol) in $CH_2Cl_2$ (5.2 mL) at 0 °C, and the mixture was stirred at rt for 30 min. The reaction mixture was concentrated *in vacuo* and then put under high vacuum for 30 min to remove the excess TFA. The crude TFA salt (30 mg) was used directly in the next step without further purification.

Crude amine-TFA salt in $CH_2Cl_2$ (10.5 mL) was added to a mixture of HATU (53 mg, 138 μmol) and 6Cl-HOBt (19 mg, 115 μmol) in $CH_2Cl_2$ (10.5 mL) and DMF (21 mL). This activated linear peptide mixture was then transferred into two separate syringes, one of each was added dropwise (0.4 mL/h) using a syringe pump into two separate solutions of DIPEA (1.3 mL, 7.5 mmol) in $CH_2Cl_2$ (26 mL) and DMF (4 mL). The reaction mixtures were combined after stirring at rt for 4 d and then quenched with $H_2O$ (100 mL), and the mixture was extracted with EtOAc (4 × 80 mL). The combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, filtered, and


DOI: 10.1021/acs.joc.6b01369
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concentrated *in vacuo*. Purification of the crude product by flash chromatography ($CH_2Cl_2$/MeOH 19:1) afforded psychrophilin E (**5**) (10 mg, 49%, 22.5 μmol) as a white powder; $R_f$ 0.44 ($CH_2Cl_2$/MeOH 95:5); $[\alpha]_D^{24}$ +92.0 (*c* 0.20 in $CHCl_3$); $\nu_{max}$(neat)/cm$^{-1}$ 3351, 2924, 2853, 1698, 1665, 1623, 1604, 1523, 1486, 1450, 1366, 1332, 1285, 1251, 1224, 1172, 1153, 1120, 1104, 1058, 1038, 943, 895, 878, 843, 771; $\delta_H$ (500 MHz; $CDCl_3$) 8.63 (d, *J* = 8.4, 1 H, Ar−H), 8.52 (s, 1 H, NH-Abz), 7.83 (dd, *J* = 8.4, 1.2, 1 H, Ar−H), 7.68 (d, *J* = 7.7, 1 H, Ar−H), 7.58−7.56 (m, 2 H, 2 × Ar−H), 7.44 (td, *J* = 7.7, 1.1, 1 H, Ar−H), 7.39 (td, *J* = 6.7, 2.2, 2 H, 2 × Ar−H), 6.85 (s, 1 H, Ar−H), 6.21 (d, *J* = 8.0, 1 H, NH-Ac), 5.10 (ddd, *J* = 11.5, 8.1, 5.5, 1 H, H-2), 4.56 (dd, *J* = 8.3, 2.9, 1 H, H-20), 3.37 (dd, *J* = 12.8, 5.4, 1 H, H-3a), 3.25 (ddd, $J_A$ = 7.2, $J_B$ = $J_C$ = 9.4, 1 H, H-23a), 2.87 (t, *J* = 12.1, 1 H, H-3b), 2.17 (ddd, $J_A$ = 9.8, $J_B$ = 8.1, $J_C$ = 5.3, 1 H, H-23b), 2.09−2.05 (m, 1 H, H-21a), 2.06 (s, 3 H, $CH_3$, H-25), 1.70−1.67 (m, 1 H, H-21b), 1.54−1.50 (m, 1 H, H-22a), 1.24−1.20 (m, 1 H, H-22b); $\delta_C$ (125 MHz; $CDCl_3$) 172.3 (CO), 169.9 (CO), 167.4 (CO), 166.2 (CO), 135.5 (C), 133.4 (C), 132.6 (Ar−CH), 131.8 (Ar−CH), 129.7 (C), 128.2 (C), 126.4 (Ar−CH), 126.1 (Ar−CH), 125.6 (Ar−CH), 124.6 (Ar−CH), 123.8 (Ar−CH), 118.3 (Ar−CH), 117.2 (Ar−CH), 116.4 (C), 59.8 (CH, C-20), 51.0 (CH, C-2), 47.7 ($CH_2$, C-23), 29.8 ($CH_2$, C-3), 26.3 ($CH_2$, C-21), 24.7 ($CH_2$, C-22), 23.1 ($CH_3$, C-25); *m*/*z* (ESI+) $[M + H]^+$ 445.1879 calcd for $C_{25}H_{25}N_4O_4$ 445.1870.

*Method B.* TFA (0.7 mL, 9.4 mmol) was added to a stirred solution of Boc-amide **24a** (24 mg, 43 μmol) in $CH_2Cl_2$ (4.8 mL) at 0 °C, and the mixture was stirred at rt for 1 h. The reaction mixture was concentrated *in vacuo* and then put under high vacuum for 30 min to remove the excess TFA. The crude TFA salt (24 mg) was used directly in the next step without further purification.

Crude amine-TFA salt in $CH_2Cl_2$ (9.5 mL) was added to a mixture of HATU (49 mg, 128 μmol) and 6Cl-HOBt (18 mg, 107 μmol) in $CH_2Cl_2$ (9.5 mL) and DMF (19 mL). This activated linear peptide mixture was then added dropwise (0.4 mL/h) using a syringe pump to a solution of DIPEA (2.4 mL, 13.8 mmol) in $CH_2Cl_2$ (48 mL) and DMF (7 mL). After stirring at rt for 6 d, the reaction mixture was quenched with $H_2O$ (150 mL), and the mixture was extracted with EtOAc (4 × 80 mL). The combined organic layers were washed with brine (150 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Purification of the crude product by flash chromatography ($CH_2Cl_2$/MeOH 19:1) afforded psychrophilin E (**5**) (15 mg, 79%, 34 μmol) as a white powder. The spectroscopic data were in agreement with those reported above.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01369.

Crystallographic data for synthetic psychrophilin E (CIF)
$^1$H and $^{13}$C spectra for all new compounds and synthetic psychrophilin E, X-ray crystallographic analysis of synthetic psychrophilin E, and theoretical methods used for computational modeling of 2*S*-**5** and 2*R*-**5** (PDF)

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### Notes

The authors declare no competing financial interest.

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DOI: 10.1021/acs.joc.6b01369
*J. Org. Chem.* 2016, 81, 7635−7643