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Total Synthesis of Ustiloxin D Utilizing an Ammonia–Ugi Reaction

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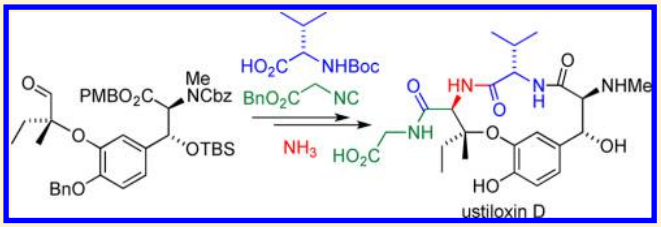
Supporting Information

ABSTRACT: Total synthesis of the highly functionalized cyclic peptide natural product, ustiloxin D, has been achieved in a convergent manner. Our strategy incorporates an asymmetric allylic alkylation to construct the *tert*-alkyl aryl ether linkage between the dopa and isoleucine residues. The elaborated β-hydroxydopa derivative is rapidly converted to a linear tripeptide through an ammonia–Ugi reaction. Subsequent cyclization and global deprotection affords ustiloxin D in six steps from a known β-hydroxydopa derivative.



INTRODUCTION

The ustiloxins are a family of cyclic peptides isolated from the fungus *Ustaliginoidea virens*, which causes the growth of false smut balls on rice plants.[1] The ustiloxins are potent antimitotic agents that bind to the vinca/rhizoxin site on tubulin, inhibiting the assembly of α,β-tubulin dimers into microtubules at low micromolar concentrations.[2] Ustiloxins A–F possess a common 13-membered cyclic core, generated by cross-linking of a β-hydroxydopa residue with an isoleucine residue through an uncommon *tert*-alkyl aryl ether linkage (Figure 1).

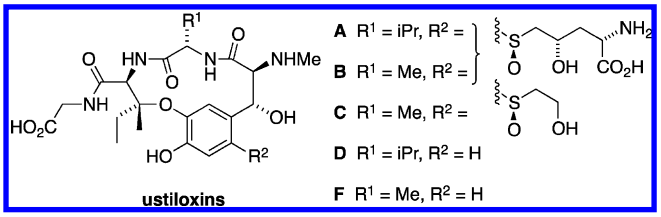
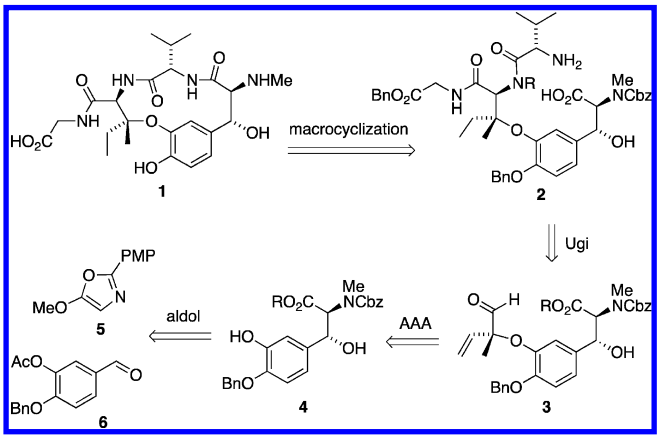


Figure 1. Ustiloxins A–F.

The intriguing structures of the ustiloxins together with their potent biological activity has led to numerous investigations of their partial[3] or total synthesis[4] and preparation of analogues.[5] Joullié reported the first total synthesis of ustiloxin D,[4a] which employed a chiral pool approach to the β-functionalized isoleucine residue, aminohydroxylation to generate the β-hydroxydopa residue, and an S_NAr strategy to link these moieties through the *tert*-alkyl aryl ether. Wandless next reported a different approach to ustiloxin D,[4b] employing a Suga–Evans aldol-type construction of the β-hydroxydopa residues and an asymmetric allylic alkylation (AAA) route to generate the *tert*-alkyl aryl ether. Joullié's convergent,[4d] second-generation approach to ustiloxin D also used the efficient Suga–Evans aldol approach to the dopa residue, which was coupled to a chiral pool-derived aziridine carboxylate to construct the isoleucine–dopa ether linkage. These routes highlight challenges in the asymmetric assembly of *tert*-alkyl aryl ethers and also in the step-economical construction of highly functionalized peptides. In our approach to ustiloxin D, we sought conditions for a highly selective AAA coupling to generate the *tert*-alkyl aryl ether, employing an easily accessible allyl donor combined with a convergent strategy to prepare the functionalized Val-Ile*-Gly tripeptide moiety.

Prior studies have shown that the most efficient macrolactamization occurs between the valine and dopa residues, such that the ether-linked tripeptide–dopa intermediate **2** is a key precursor to the macrocycle (Scheme 1).[4d,e] We proposed accessing the tripeptide moiety of **2** in a single step, employing an Ugi reaction of aldehyde **3**. The aldehyde **3** would be accessed from the corresponding primary alcohol, which would be generated through a Tsuji–Trost asymmetric allylic alkylation (AAA) reaction of β-hydroxydopa derivative **4**. β-Hydroxydopa derivative **4** has been efficiently generated in the

Scheme 1. Retrosynthesis



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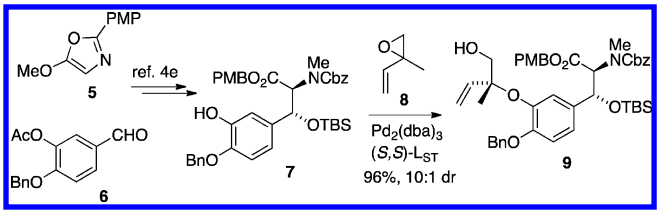
prior syntheses of ustiloxin D through aldol-type reaction of oxazole **5** and benzaldehyde **6**.

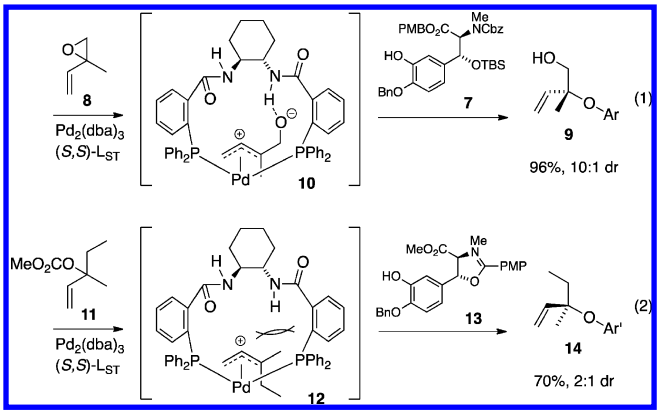
RESULTS AND DISCUSSION

The β-OH dopa derivative **7** was our first target and was synthesized according to the method of Joullié.[4e] Gratifyingly, allylation of **7** with isoprene epoxide **8** in the presence of $Pd_2(dba)_3$ and the (*S*,*S*)-Trost ligand[6] proceeded in high yield and good diastereoselectivity to generate the *tert*-alkyl aryl ether **9** (Scheme 2). The high degree of stereoselectivity in this case

Scheme 2. Construction of the Alkyl Aryl Ether



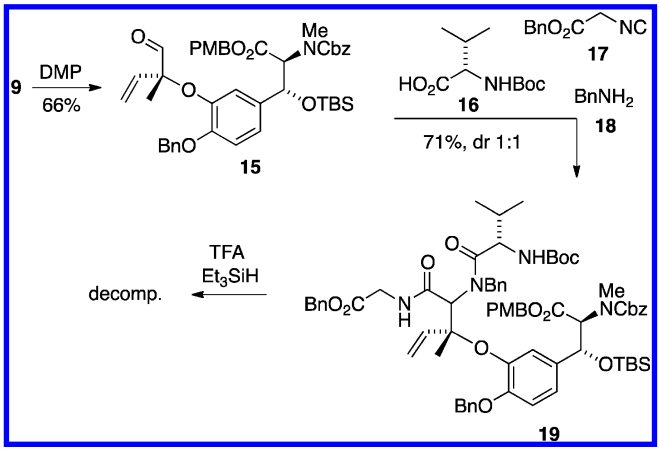
Scheme 3. Facial Selectivity in Aryl Ether Formation



(Scheme 3, eq 1) is in contrast to the 1:2 ratio obtained when allylic carbonate **11** was employed in Wandless' synthesis[4b,c] (Scheme 3, eq 2). Despite remarkably similar π-allyl intermediates, differentiation when employing isoprene epoxide is between pendant methyl and hydroxymethyl groups on the π-allyl intermediate, rather than between methyl and ethyl groups. We propose that hydrogen bonding between the π-allyl intermediate derived from **8** and chiral ligand–Pd complex (see **10**) imparts significant facial selectivity upon attack of the phenoxide nucleophile, whereas facial selectivity upon attack of the complex **12** (derived from **11**) is governed only by steric discrimination between methyl and ethyl groups, in accord with Lloyd-Jones' analysis of the role of the chiral ligand in such asymmetric allylations.[7] Further, the effect of the H-bonding in the π-allyl-Pd intermediate reverses the facial selectivity of the allylation, though as the olefin is converted to the isoleucine α- and carbonyl carbons in Wandless' synthesis, and the isoleucine side chain "ethyl group" in ours, the (*S*,*S*)-Trost ligand is employed in both cases.

With the ether **9** in hand we proceeded to construct the functionalized isoleucine-containing peptide fragment. Oxidation of the primary alcohol with Dess–Martin periodinane gave aldehyde **15** (Scheme 4). The Ugi four-component condensation was then investigated. The requisite coupling

Scheme 4. Ugi Reaction

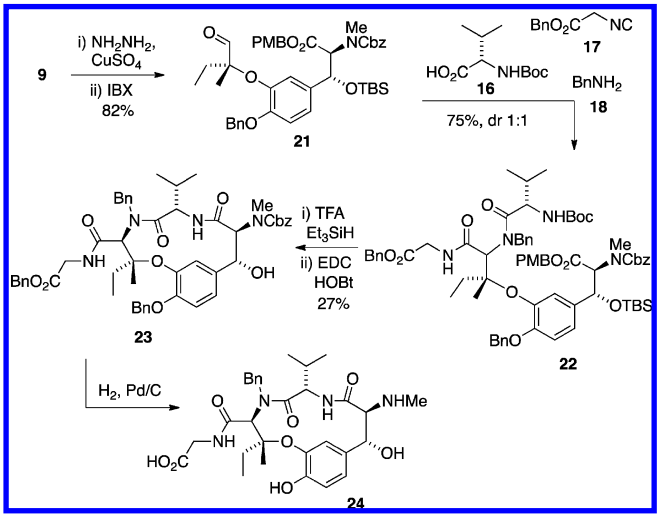


partners in addition to the aldehyde **15** are a protected valine, a glycine derived isonitrile, and an amine. A suitable protecting group strategy led to the choice of N-Boc-valine **16**, benzyl isocyanoacetate **17**, and benzylamine **18**, all of which are commercially available (though the benzyl isocyanoacetate **17** is more economically prepared from glycine benzyl ester[8]). Ugi reaction of these components yielded the desired dopa–tripeptide **19** as a 1:1 mixture of diastereoisomers. The lack of stereoselectivity in Ugi reactions is a common feature.[9]

With the tripeptide–dopa adduct **19** assembled, treatment with TFA in order to remove the *N*-Boc and PMB ester groups was undertaken. However, subjecting **19** to acidic conditions resulted in cleavage of the tertiary allylic ether. The decomposition of ether **19** highlights the effect of subtle differences on carbocation stabilization (vida infra); the conditions employed were identical to those used by Joullié in which the equivalent tertiary propargylic ether is stable. Nevertheless, in order to decrease the acid lability of the newly constructed tertiary ether it was decided to reduce the terminal olefin of **9** with diimide. Subsequent oxidation of the resultant primary alcohol **20** to the corresponding aldehyde **21** then proceeded in good yield with either Dess–Martin periodinane or IBX (Scheme 5).

The Ugi reaction of saturated aldehyde **21** with the remaining components **16**–**18** generated the desired dopa–

Scheme 5. Ugi Reaction and Macrolactamization



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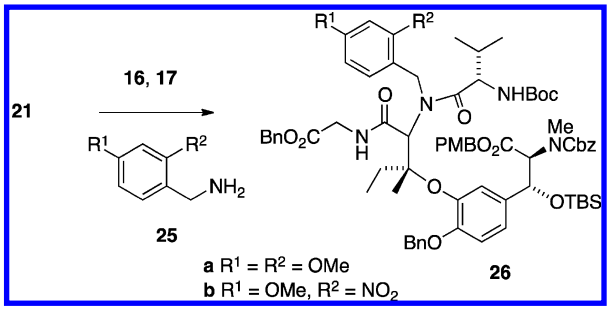
tripeptide **22** in 75% yield, again as a 1:1 mixture of diastereoisomers. Treatment of Ugi adduct **22** with TFA resulted in facile removal of the PMB and Boc protecting groups, with no cleavage of the teriary ether evident, validating the requirement for reduction of the olefin. Macrolactamization with EDC then generated protected ustiloxin D (**23**). Intriguingly, upon treatment of the mixture of epimers from deprotection of **22** with EDC it was found that only the isoleucine (αS)-isomer underwent macrolactamization, whereas the (αR)-isomer underwent oligomerization. That is, only the epimer that possesses the requisite (*S*)-confuguration at the isoleucine α-carbon to generate the stereochemistry present in the natural product underwent macrocyclization. This divergent reactivity was fortuitous in that it enabled facile separation of the epimeric products at this stage. The macrolactamization of the correct stereoisomer proceeded to give **23** in 54% yield, in accordance with the related macrolactamizations of Wandless and Joullié.[4] Subsequent hydrogenolysis of **23** under standard conditions then generated *N*-benzylustiloxin D (**24**). Though cleavage of the benzyl ether, ester, and carbamate protecting groups proceeded efficiently, the *N*-benzylamide group was resistant to cleavage under a range of different conditions (e.g., palladium-catalyzed hydrogenolysis under high pressure, strongly acidic conditions, or radical cleavage using NBS[10]). Accordingly, a variety of substituted benzylamines were investigated as Ugi reaction components to generate a range of amide-protected ustiloxin derivatives.

Synthesis of the 2,4-dimethoxybenzyl- and 4-methoxy-2-nitrobenzylamides **26a** and **26b** thorough analogous Ugi condensations with amines **25a** and **25b** was investigated (Scheme 6). Dimethoxybenzyl (DMB) groups have commonly

Scheme 6. Alternative Amines in Ugi Reaction

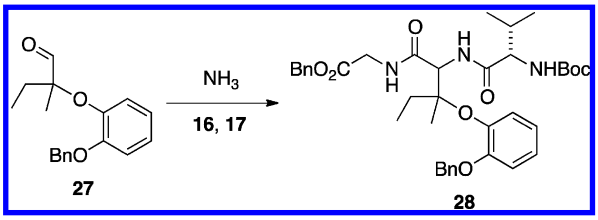


been employed as acid-labile amide protecting groups,[11] and the 4-methoxy-2-nitrobenzyl group has been employed as a photocleavable amide protecting group.[12] However, neither of these was found to be suitable for our purposes. While the Ugi reaction with **25a** proceeded well, deprotection of the resultant DMB-amide **26a** with TFA led to concomitant cleavage of the alkyl aryl ether, further highlighting subtle substituent effects on the relative stabilities of closely related tertiary alkyl aryl ethers. The 4-methoxy-2-nitrobenzylamine **25b** was found to be unstable under the Ugi reaction conditions, with decomposition generating a complex mixture of products.

The problematic amide deprotection led us to investigate the use of ammonia as the amine component in the Ugi reaction. We were initially hesitant to explore this chemistry due to the precedent for low to moderate yields when using ammonia as the amine component in Ugi reactions.[9a,13] It is thought that high reactivity of the intermediate imine leads to undesired reaction pathways, such as reaction with the solvent (e.g., methanol) generating hemiaminals, which react further to give a mixture of products.[13b] However, Whittaker and Kazmaier have demonstrated that ammonia Ugi condensations can proceed in reasonable yield for simple systems where sterically bulky aldehydes are employed.[13]

Optimization of the ammonia–Ugi reaction was undertaken with the simplified aldehyde component **27**, together with Boc-valine **16** and the isonitrile **17** (Table 1). Use of methanolic

Table 1. Optimization of the Ammonia–Ugi Reaction[a]



entry	solvent	NH_3 (equiv)	**16** (equiv)	conc (M)	yield (%)
1	MeOH	1	1	1	58[b]
2	TFE	1	1	1	50
3	TFE	1.2	1	1	60
4	TFE	1.4	1	1	55
5	TFE	1.2	1	0.2	60
6	TFE	1.2	1	0.02	75
7	TFE	1.2	1	0.01	73
8	TFE	1.2	1.2	0.02	**85**
9	TFE	1.2	1.4	0.02	71

[a]Standard conditions: NH_3 in TFE added to **27** in TFE at 0 °C, then **16**, **17** added, stirred at rt, 18 h. [b]NH_3 in MeOH added to **27** in MeOH; product is the methyl ester analogue of **28**.

ammonia according to the Whittaker protocol[13a] was found to promote an undesired transesterification of the benzyl ester of the tripeptide product **28** to the corresponding methyl ester (entry 1). Accordingly, use of ammonia in trifluoroethanol[13b,d] was investigated. A slight excess of ammonia (entries 2–4) and carboxylic acid substrates (entries 7–9) was found to be optimal. A low substrate concentration (0.02 M) gave improved yields (entries 5–7), with optimal conditions generating the Ugi adduct in high yield (entry 8).

With a high-yielding model ammonia–Ugi reaction in hand, we turned our attention to the natural product. Treatment of the dopa–aldehyde **21** under the optimized ammonia–Ugi conditions gave the tripeptide–dopa adduct **29** as a 1:1 mixture of inseparable diastereomers in good yield (Scheme 7). The structural complexity generated in this multicomponent reaction compensates for the lack of stereoselectivity commonly associated with the Ugi reaction. Deprotection of the TFA-labile Boc, PMB, and TBS groups was followed by macrolactamization to give protected ustiloxin D **30**. As was found during macrocyclization of **22**, only the desired (αS)-isomer of **29** underwent macrolactamization, with the (αR)-isomer undergoing oligomerization. Subsequent hydrogenolysis of the benzylic protecting groups of macrocycle **30** afforded ustiloxin D (**1**) to complete the total synthesis.

CONCLUSIONS

In summary, a novel, efficient total synthesis of ustiloxin D has been completed, incorporating a highly stereoselective Tsuji–Trost AAA reaction and an ammonia–Ugi reaction as key steps. From the common phenol intermediate **7**, our sequence and that reported by Joullié each require six steps to attain

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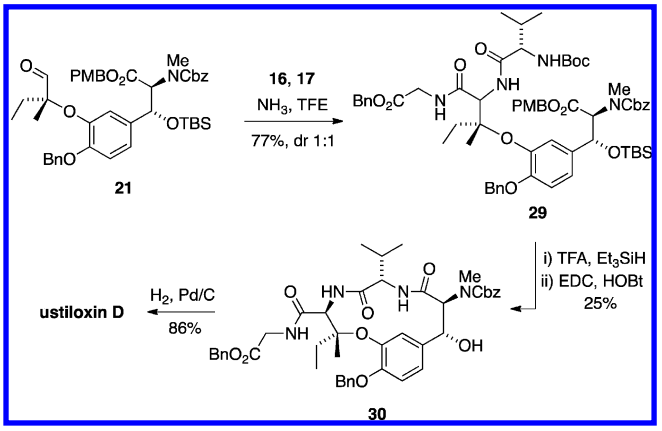
Scheme 7. Ammonia Ugi Route



ustiloxin D, in 14 and 12% yields, respectively. In addition to a slight increase in overall yield, our route does away with the need for the lengthy 10-step sequence required to generate a substrate for construction of the alkyl aryl ether. Additionally, incorporation of a hydrogen-bonding allyl donor in the AAA reaction results in significant improvements in diastereoselectivity. Further improvements in our route are facilitated through incorporation of the step-economical, high-yielding ammonia–Ugi multicomponent reaction. Work is underway in our laboratory both toward a systematic study of the ammonia–Ugi reaction and toward the application of this strategy to the members of the ustiloxin family that have not yet succumbed to total synthesis.

EXPERIMENTAL SECTION

General Information. ^{1}H NMR spectra were recorded at 400 or 500 MHz. Residual solvent peaks were used as internal references: chloroform (δ 7.26 ppm), methanol-d_3 (δ 3.31 ppm), DMSO-d_5 (δ 2.50 ppm). ^{13}C NMR spectra were recorded at 100 or 125 MHz, with solvent used as an internal reference: chloroform-d (δ 77.00), methanol-d_4 (δ 49.00 ppm), and DMSO-d_6 (δ 39.52). Chemical shifts (δ) are reported in parts per million (ppm) relative to the internal standard, and splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; br d, broad doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; q, quartet; m, multiplet. IR spectra were obtained as thin films. All mass spectra were recorded on an ESI-TOF mass spectrometer. All data were acquired and reference mass corrected via a dual spray electrospray ionization (ESI) source. Anhydrous THF, Et_2O, and CH_2Cl_2 were obtained from a solvent drying and dispensing system where the solvent was dried by passage through two packed columns of neutral alumina. All other anhydrous solvents were dried by storage over activated sieves.

*(2S,3R)-2-[[(Benzyloxycarbonyl)methyl]amino]-3-[4-(benzyloxy)-3-[(1R)-1-(hydroxymethyl)-1-(methylallyl)oxy]phenyl]-3-[(tert-butyldimethylsilanyl)oxy]propionic Acid 4-Methoxybenzyl Ester (**9**).* Under an atmosphere of argon, $Pd_2dba_3{\cdot}CHCl_3$ (32 mg, 0.031 mmol 5%) and Trost ligand (*S,S*)-L_{ST} (56 mg, 0.090 mmol) were added to an oven-dried round-bottom flask. Dry, deoxygenated DCM (8 mL) was added to the reaction vessel by cannula, followed by methylvinyloxirane (235 μL, 2.43 mmol). A solution of phenol **7** (417 mg, 0.61 mmol) in deoxygenated DCM (4 mL) was added to the flask by means of a syringe pump at a rate of 600 μL/h. The reaction was stirred for 18 h, and the solvent was evaporated under reduced pressure. The crude material was purified by flash chromatography (25:75 EtOAc/petroleum spirits) to give the olefin **9** (447 mg, 0.58 mmol, 96%) as a clear oil: ^{1}H NMR (500 MHz, 100 °C, DMSO) δ 7.46 (d, J = 7.4 Hz, 2H) 7.38–7.28 (m, 8H), 7.16 (d, J = 8.4 Hz, 2H), 7.07 (s, 1H), 6.98–6.92 (m, 2H), 6.95 (d, J = 10.8 Hz, 2H) 6.01 (dd, J = 17.4, 11.2 Hz, 1H), 5.21–5.16 (m, 2H), 5.08–5.06 (m, 3H), 5.03–4.98 (m, 2H), 4.93–4.90 (m, 2H), 4.24 (br s, 1H), 3.75 (s, 3H), 3.54 (d, J = 11.0 Hz, 1H), 3.50 (d, J = 11.0 Hz, 1H), 3.04 (s, 3H), 1.29 (s, 3H), 0.80 (s, 9H), −0.01 (s, 3H), −0.26 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (rotamers observed, minor rotamers are reported in brackets) δ 169.5 (169.2), 159.8 (159.8), 157.0 (156.2), 151.8 (151.8), 144.5 (144.3), 140.4 (140.0), 136.8 (136.6), 136.3 (136.3), 133.8 (133.9), 130.4 (130.3), 128.5 (128.5), 128.5, 128.0 (127.9), 127.8 (127.6), 127.9 (127.9), 127.7 (127.6), 127.5 (127.4), 122.8 (123.0), 122.5 (122.7), 116.6 (116.4), 114.0 (114.0), 113.1 (113.3), 84.3 (84.2), 74.5 (74.0), 71.3, 67.4 (67.3), 67.2 (67.1), 66.9, 64.8 (65.0), 55.4, 33.4, 25.8 (25.7), 19.5 (19.9), 18.0, −4.2 (−4.3), −5.4 (−5.3); $[\alpha]_D^{23}$ −38.1 (c 1.2, $CHCl_3$); IR (neat) 3690, 2933, 1703, 1613, 1515, 1455, 1401, 1304, 1250, 1144, 1101, 1004, 836, 778, 698 cm^{-1}; HRMS (ESI positive ion) $C_{44}H_{55}NNaO_9Si^+$ requires m/z 792.3539, found 792.3540.

*(2S,3R)-2-[[(Benzyloxycarbonyl)methyl]amino]-3-[4-(benzyloxy)-3-[(1R)-1-formyl-1-(methylallyl)oxy]phenyl]-3-[(tert-butyldimethylsilanyl)oxy]propionic Acid 4-Methoxybenzyl Ester (**15**).* To an oven-dried flask was added Dess–Martin periodinane (DMP) (134 mg, 0.32 mmol), and the flask was evacuated and backfilled with argon. DCM (500 μL) was added to the reaction mixture. A flask containing primary alcohol **9** (163 mg, 0.212 mmol) was evacuated and backfilled with argon. To this flask was added DCM (2 × 200 μL), and the solution was slowly added to the DMP suspension. The reaction was stirred for 1 h before the reaction mixture was diluted with ether (10 mL) and added to 1 M $NaHCO_3/Na_2S_2O_3$ (10 mL). The biphasic mixture was stirred vigorously for 10 min. The organic layer was separated and washed with 1 M $NaHCO_3/Na_2S_2O_3$ (10 mL) and distilled water (10 mL). The organic phase was dried over $MgSO_4$ and filtered and the solvent removed under reduced pressure. The crude material was purified by flash chromatography (20:80 EtOAc/petroleum spirits) to furnish aldehyde **15** (121 mg, 0.158 mmol, 75%): ^{1}H NMR (500 MHz, $CDCl_3$) (rotamers observed, minor rotamers are reported in brackets) δ 9.60 (9.60) (s, 1H), 7.43–7.12 (m, 12H), 6.98–6.81 (m, 5H), 5.87 (dd, J = 17.4, 10.8 Hz, 1H), 5.53–5.46 (m, 1H), 5.34–5.31 (m, 2H), 5.19–4.81 (m, 7H), 3.80 (3.79) (s, 3H), 3.12 (3.10) (s, 3H), 1.33 (1.36) (s 3H), 0.84 (0.82) (s, 9H), 0.00 (−0.01) (s, 3H), −0.26, (−0.29) (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (rotamers observed, minor rotamers are reported in brackets) δ 199.8 (199.6), 169.5 (169.2), 159.8 (159.8), 157.0 (156.1), 151.0 (151.0), 144.1 (144.3), 136.8 (136.6), 136.7 (136.7), 136.2 (135.9), 133.9 (134.0), 130.3, 128.7 (128.6), 128.5 (128.4), 128.1 (128.2), 127.9, 127.7 (127.8), 127.6, 127.5 (127.4), 122.7 (122.7), 121.5 (121.4), 118.3 (118.4), 114.0 (114.0), 113.9 (113.7), 86.5 (86.4), 74.4 (74.0), 70.9 (70.9), 67.4 (67.3), 66.9, 64.8 (65.0), 55.4, 33.3, 25.8 (25.7), 19.7 (19.5), 18.0 (18.0), −4.3 (−4.4), −5.4 (−5.3); $[\alpha]_D^{25}$ −11.9 (c 0.9, $CHCl_3$); HRMS (ESI positive ion) $C_{44}H_{53}NNaO_9Si^+$ requires m/z 790.3387, found 790.3360.

*(2S,3R)-3-[4-(Benzyloxy)-3-[(1R)-1-[[(benzyloxycarbonyl)-methylcarbamoyl]-((S)-2-[(tert- butoxycarbonyl)amino]-3-methyl-butyryl-(N-benzylamino)methyl]-1-methylprop-2-enyloxy]phenyl]-2-[[(benzyloxycarbonyl)methyl]amino]-3-[(tert-butyldimethylsilanyl)oxy]propionic Acid 4-Methoxybenzyl Ester (**19**).* To an oven-dried round-bottom flask was added powdered 3 Å sieves (90 mg). The sieves were activated with a heat gun under vacuum, and the flask was backfilled with N_2. A solution of aldehyde **15** (121 mg, 0.158 mmol) in DCM (900 μL) was added to the flask, followed by benzylamine (17.5 μL, 0.164 mmol). The reaction mixture was allowed to stir for 18 h. The reaction mixture was filtered using a glass frit and the solvent removed. To the residual imine (125 mg) was added dry MeOH (600 μL). A solution of *N*-Boc-valine **16** (32 mg, 0.147 mmol) in dry MeOH (500 μL) was added to the flask, and the mixture was allowed to stir for 10 min. A solution of isonitrile **17** (26 mg, 0.147 μL) in MeOH (400 μL) was added, and the reaction mixture was allowed to stir for 96 h. The solvent was removed under reduced pressure. The crude material was purified using flash chromatography (35:65 EtOAc/petroleum spirits), yielding two epimers of tripeptide **19**. First eluting epimer **19a**: pale yellow oil (R_f = 0.35, 64 mg, 35%); ^{1}H NMR (500 MHz, $CDCl_3$) (rotamers observed, only major rotamer reported) δ 7.53–6.68 (m, 27H), 5.89–

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5.47 (m, 2H), 5.22−4.66 (m, 12H), 4.55−4.00 (m, 3H), 3.79−3.76 (m, 3H), 3.39−2.98 (m, 5H), 1.93−1.91 (m, 1H), 1.79−1.71 (m, 3H), 1.44−1.37 (m, 9H) 0.94−0.75 (m, 15H), −0.02 to −0.06 (m, 3H), −0.26 to −0.30 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (rotamers observed, minor rotamers are reported in brackets) δ 174.0 (173.6), 169.7 (169.6), 169.5 (169.5), 169.3, 169.3 (169.2), 159.9, 157.2, 156.2 (155.8), 145.0, 143.7 (143.3), 137.6, 137.0, 135.5 (135.3), 133.5, 132.3 (132.1), 131.9, 131.6 (131.5), 130.5 (130.3), 128.8 (128.7), 128.6, 128.5 (128.4), 128.2 (128.2), 128.0, 127.9 (127.8), 127.6 (127.5), 127.4 (127.2), 126.9 (126.8), 126.5 (126.3), 123.0 (122.6), 118.5, 114.1, 109.6, 79.5, 71.9, 71.2 (71.1), 68.1, 67.7 (67.5), 67.3 (67.2), 66.9 (67.1), 63.7, 56.2, 55.4, 51.2, 41.6, 34.1, 31.7 (31.5), 29.9, 28.5, 25.8 (25.7), 19.9 (19.8), 18.0, 17.7 (17.5), −4.3 (−4.4), −5.2 (−5.3); $[\alpha]_D^{25}$ −21.7 (*c* 0.7, $CHCl_3$); IR (neat), 3364, 2954, 1742, 1698, 1516, 1497, 1456, 1390, 1366, 1304, 1249, 1174, 1094, 1003, 837, 778, 754, 698 cm^{-1}; HRMS (ESI positive ion) $C_{71}H_{89}N_4O_{14}Si^+$ requires *m*/*z* 1249.6140, found 1249.6138. Second eluting epimer **19b**: pale yellow oil (R_f = 0.27, 65 mg, 36%); ^{1}H NMR (500 MHz, $CDCl_3$) (rotamers observed, only major rotamer reported) δ 7.52−6.71 (m, 27H), 5.83−5.55 (m, 2H), 5.33−4.74 (m, 12H), 4.59−4.42 (m, 2H), 3.90−3.68 (m, 4H), 3.42−3.01 (m, 5H), 2.10−2.00 (m, 1H), 1.44−1.37 (m, 12H), 0.94−0.75 (m, 15H), −0.02 to −0.06 (m, 3H), −0.26 to −0.30 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (rotamers observed, minor rotamers are reported in brackets) δ 174.5, 169.7, 169.6, 169.4, 169.1, 160.0, 157.1, 156.2 (155.4), 145.2, 143.7, 137.8, 136.5, 135.7, 132.4 (132.8), 132.1 (132.2), 131.8 (131.9), 130.7 (130.8), 130.5 (130.3), 128.8 (128.7), 128.6 (128.6), 128.5, 128.4 (128.3), 128.2 (128.0), 127.6 (127.5), 127.4 (127.3), 127.3 (127.1), 126.7 (126.4), 123.0 (122.6), 118.4 (118.8) 114.2, 109.7, 79.3, 72.0, 71.2, 67.5, 67.2, 66.9, 66.6, 63.5, 56.3, 55.4, 49.4, 41.7, 34.5, 32.4, 29.9, 28.4, 25.9, 19.9, 18.1, 17.3, −4.3 (−4.4), −5.4 (−5.5); $[\alpha]_D^{25}$ −16.5 (*c* 0.5, $CHCl_3$); IR (neat), 3363, 2960, 1742, 1698, 1513, 1499, 1457, 1250, 1174, 838, 738, 699 cm^{-1}; HRMS (ESI positive ion) $C_{71}H_{89}N_4O_{14}Si^+$ requires *m*/*z* 1249.6140, found 1249.6146.

*(2S,3R)-2-[[(Benzyloxycarbonyl)methyl]amino]-3-[4-(benzyloxy)-3-[[(1R)-1-(hydroxymethyl)-1-methylpropyl]oxy]phenyl]-3-[(tert-butyldimethylsilanyl)oxy]propionic Acid 4-Methoxybenzyl Ester (**20**).* To a flask containing olefin **9** (379 mg, 0.49 mmol) was added EtOH (10 mL). The solution was cooled to 0 °C before addition of hydrazine monohydrate (355 μL) and saturated aqueous $CuSO_4$ (100 μL). The reaction mixture was allowed to stir vigorously for 48 h. The crude reaction mixture was diluted with DCM (50 mL) and washed with brine (50 mL) and distilled water (50 mL). The organic layer was dried over $MgSO_4$ and filtered and the solvent evaporated at the pump. The crude material was purified by flash chromatography (30:70 EtOAc/petroleum spirits) to yield primary alcohol **20** (346 mg, 0.45 mmol, 91%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ (rotamers observed, minor rotamers are reported in brackets) 7.45−6.84 (m, 17H), 5.34−4.81 (m, 9H), 3.80 (3.79) (s, 1H), 3.43−3.41 (m, 1H), 3.36−3.32 (m, 1H), 3.14 (3.12) (s, 3H), 1.71 (1.67) (q, *J* = 7.5 Hz, 2H), 1.13 (1.12) (s, 3H), 0.97−0.96 (m, 3H), 0.85 (0.82) (s, 9H), 0.01 (0.00) (s, 3H), −0.25 (−0.27) (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (rotamers observed, minor rotamers are reported in brackets) 169.5 (169.2), 159.9 (159.8), 157.0 (156.2), 152.5 (152.4), 143.9 (144.0), 136.8 (136.7), 136.1 (136.1), 134.1 (134.2), 130.3, 128.8 (128.8), 128.6, 128.6 (128.5), 128.2 (128.0), 127.9 (127.9), 127.7 (127.6), 127.5 (127.4), 124.1 (124.3), 122.7 (123.0), 114.1 (114.0), 113.6 (113.5), 85.7 (85.7), 74.5 (74.0), 71.6, 67.4 (67.3), 67.2 (67.1), 66.9, 64.8 (65.0), 55.4, 33.4, 29.8 (29.4), 25.8 (25.7), 20.2 (19.9), 18.0 (18.0), 8.7 (8.7), −4.3 (−4.3), −5.4 (−5.3); $[\alpha]_D^{25}$ −34.1 (*c* 1.0, $CHCl_3$); IR (neat) 3697, 2950, 2343, 1702, 1508, 1463, 1304, 1259, 1033, 778, 700; HRMS (ESI positive ion) $C_{44}H_{57}NO_9SiNH_4^+$ requires 789.4146, found 789.4141.

*(2S,3R)-2-[[(Benzyloxycarbonyl)methyl]amino]-3-[4-(benzyloxy)-3-((1R)-1-formyl-1-methylpropyl]oxy]phenyl]-3-[(tert-butyldimethylsilanyl)oxy]propionic Acid 4-Methoxybenzyl Ester (**21**).* To a flask containing olefin **20** (340 mg, 0.44 mmol) was added EtOH (10 mL). The solution was cooled to 0 °C before addition of hydrazine monohydrate (355 μL) and saturated aqueous $CuSO_4$ (100 μL). The reaction mixture was allowed to stir vigorously for 48 h. The crude reaction mixture was diluted with DCM (50 mL) and washed with brine (50 mL) and distilled water (50 mL). The organic layer was dried over $MgSO_4$ and filtered and the solvent evaporated at the pump. The crude material (346 mg) was added to an oven-dried flask containing IBX (186 mg, 0.66 mmol) in dry MeCN (2.4 mL). The mixture was stirred at 80 °C for 90 min. The suspension was allowed to cool to room temperature, silica was added, and the solvent was removed at the pump. Purification by flash chromatography (20:80 EtOAc/petroleum spirits) afforded aldehyde **21** (303 mg 0.39 mmol, 89%) as a light yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) (rotamers observed, minor rotamers are reported in brackets) δ 9.76 (9.74) (s, 1H), 7.39−6.78 (m, 17H), 5.33−4.81 (m, 8H), 3.80 (3.79) (s, 3H), 3.12 (3.10) (s, 3H), 1.87−1.79 (m 1H), 1.71−1.64 (m 1H), 1.11 (1.13) (s, 3H), 0.99−0.95 (m, 3H), 0.85 (0.82) (s, 9H), 0.00 (−0.01) (s, 3H), −0.25 (−0.28) (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (rotamers observed, minor rotamers are reported in brackets) δ 203.7 (203.5), 169.5 (169.2), 159.8 (159.8), 157.0 (156.1), 151. Four (151.4), 144.1 (144.3), 136.8 (136.3), 136.5 (136.6), 133.9 (134.1), 130.4 (130.3), 128.5 (128.5), 128.6, 128.6 (128.5), 128.2 (128.2), 127.9 (127.9), 127.8 (127.8), 127.7 (127.6), 123.0 (122.8), 122.2 (122.2), 114.0 (114.0), 113.6 (113.4), 86.6 (86.5), 74.4 (74.0), 70.8 (70.8), 67.3 (67.4), 66.9, 64.8 (65.0), 55.4, 33.3 (33.4), 29.9 (29.7), 25.8 (25.7), 18.0 (18.0), 17.3 (17.2), 7.5 (7.5), −4.3 (−4.4), −5.4 (−5.3); $[\alpha]_D^{25}$ −15.9 (*c* 1.0, $CHCl_3$); IR (neat) 2931, 1733, 1703, 1613, 1506, 1455, 1381, 1304, 1249, 1143, 1004, 827, 778, 737, 697 cm^{-1}; HRMS (ESI positive ion) $C_{44}H_{56}NO_9Si^+$ requires *m*/*z* 770.3719, found 770.3721.

*(2S,3R)-3-[4-(Benzyloxy)-3-[(R)-1-[[(benzyloxycarbonyl)methyl]-carbamoyl]-(S)-2-[(tert- butoxycarbonyl)amino]-3-(methylbutyryl)-[(N-benzylamino)methyl]-1-methylpropoxy]phenyl]-2-[[(benzyloxycarbonyl)methyl]amino]-3-[(tert-butyldimethylsilanyl)-oxy]propionic Acid 4-Methoxybenzyl Ester (**22**).* To an oven-dried round-bottom flask was added powdered 3 Å sieves (60 mg). The sieves were activated with a heat gun under vacuum and the flask backfilled with nitrogen. A solution of aldehyde **21** (61 mg, 0.079 mmol) in DCM (600 μL) was added to the flask, followed by benzylamine (9 μL, 0.080 mmol). The reaction mixture was allowed to stir for 18 h. The reaction mixture was filtered using a glass frit and the solvent removed. To a flask containing *N*-Boc-valine **16** (16 mg, 0.079 mmol) was added a solution of crude imine (67 mg) in dry MeOH (600 μL), and the mixture was allowed to stir for 10 min. Subsequently, a solution of isonitrile **17** (13 mg, 0.079 μL) in MeOH (400 μL) was added, and the reaction mixture was allowed to stir for 96 h. The solvent was removed under reduced pressure. The crude material was purified by flash chromatography (35:65 EtOAc/petroleum spirits), yielding a 1:1 mixture of epimers of tripeptide **22** (68 mg, 0.054 mmol, 68%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67−6.26 (m, 27 H), 5.75−4.38 (m, 14H), 3.84−3.69 (m, 3H), 3.43−3.19 (m, 1H), 3.15−3.08 (m 3H), 3.02−2.67 (m, 1H) 2.18−1.29 (m, 15H) 1.00−0.73 (m, 18H), 0.07−0.01 (m, 3H), −0.17 to −0.30 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (diastereomers and rotamers present, only distinct resolvable peaks reported) δ 159.9, (133.8, 133.7, 133.5, 133.3), 114.1, (70.9, 70.7), (55.8, 55.4), (50.4, 49.8, 49.5, 48.3), (40.8, 40.6, 40.5, 40.5), (28.5, 28.4, 28.4); IR (neat) 3363, 2960, 1708, 1514, 1457, 1366, 1250, 1174, 1004, 833, 7450, 697 cm^{-1}; HRMS (ESI positive ion) $C_{71}H_{91}N_4O_{14}Si^+$ requires *m*/*z* 1251.6296, found 1251.6298.

*[[(3R,4S,7S,10S,11R)-15-(Benzyloxy)-10-[[(benzyloxycarbonyl)-methyl]amino]-3-ethyl-11-hydroxy-7-isopropyl-3-methyl-6,9-dioxo-2-oxa-5-(benzylamino)-8-aminobicyclo[10.3.1]hexadeca-1-(16),12,14-triene-4-carbonyl]amino]acetic Acid Benzyl Ester (**23**).* To a solution of tripeptide **22** (36 mg, 0.03 mmol) and DCM (500 μL) at 0 °C were added TFA (153 μL) and Et_3SiH (129 μL). The reaction mixture was stirred for 7 h and then added to distilled water (5 mL). The organic layer was evaporated, the resulting suspension was dispersed by addition of acetonitrile (3 × 1 mL), and the solution was lyophilized. The resulting white solid was dissolved in DMF (16 mL) and the mixture cooled to 0 °C. To this solution were added EDCI (20 mg, 0.11 mmol), HOBt (14 mg, 0.11 mmol), and $NaHCO_3$ (30 mg, 0.35 mmol). The reaction mixture was stirred and allowed to

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return to room temperature over 18 h. The solvent was evaporated and the resulting yellow oil dissolved in EtOAc (100 mL) and washed with HCl (0.5 M, 100 mL), $NaHCO_3$ (sat. 100 mL), and brine (100 mL). The organic layer was dried with $MgSO_4$ and filtered and the solvent evaporated under reduced pressure. Purification by flash chromatography (acetone/petroleum spirits, 35:65) afforded macrocycle **23** (7 mg, 27%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40−7.12 (m, 22H), 6.99 (s, 1H), 6.39 (d, J = 9.6 Hz, 1H), 5.74 (s, 1H), 5.17−4.80 (m, 10H), 4.45 (t, J = 9.7 Hz, 1H), 3.83−3.66 (m, 2H), 3.09 (s, 3H), 1.84−1.71 (m, 3H), 1.58 (s 3H, obscured by water peak), 1.07−1.05 (m, 3H), 0.66−0.59 (m, 3H), 0.27−0.20 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.9, 169.2, 168.9, 168.5, 157.8, 152.3, 145.6, 139.2, 136.8, 136.5, 135.3, 133.2, 128.8, 128.7, 128.7, 128.6, 128.2, 127.9, 127.8, 127.2, 126.8, (4 Obs C), 122.2, 120.8, 114.5, 87.1, 71.2, 70.9, 67.9, 67.3, 62.5, 55.3, 54.9, 50.2, 41.2, 33.0, 31.2, 29.9, 23.0, 19.5, 18.0, 8.4; $[\alpha]_D^{25}$ −40.1 (c 0.1, $CHCl_3$). IR (neat) 3313, 2973, 1676, 1393, 1228, 1065, 698 cm^{-1}; HRMS (ESI positive ion) $C_{52}H_{59}N_4O_{10}^+$ requires m/z 899.4226, found 899.4224.

*[[(3R,4S,7S,10S,11R)-3-Ethyl-11,15-dihydroxy-7-isopropyl-3-methyl-10-(methylamino)-6,9-dioxo-2-oxa-5-(benzylamino)-8-aminobicyclo[10.3.1]hexadeca-1(16),12,14-triene-4-carbonyl]-amino]acetic Acid (**24**).* A suspension of palladium black (4 mg) in THF/H_2O (1:1, 700 μL) was added to a flask containing macrocycle **23** (9.0 mg, 10.0 μmol). The flask was filled with hydrogen and the reaction mixture stirred overnight. The slurry was filtered through Celite, using H_2O as the eluent. The solvent was evaporated, and the crude yellow oil was dissolved in distilled H_2O (2 mL) and lyophilized. The crude material was purified by HPLC (Phenomenex, C18, 100 Å, AXIA, 150 × 21.2 mm, 5−15% MeCN in H_2O with 0.1% TFA over 5 min and then 15−25% MeCN over 15 min) to afford benzyl-protected macrocycle **24** (3.2 mg, 5.5 μmol, 55%): ^{1}H NMR (500 MHz, DMSO) δ 9.34 (app t, J = 5.7, 1H), 8.79 (s, 1H), 7.32−7.20 (m, 5H), 7.01 (dd, J = 8.2, 1.7 Hz, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.44 (br s, 1H), 5.75 (s, 1H), 5.43 (d, J = 15.9 Hz, 1H), 5.10 (d, J = 15.9 Hz, 1H), 4.76 (br s, 1H), 4.39 (app t, J = 9.9 Hz, 1H), 3.78 (dd, J = 17.3, 6.3 Hz, 1H), 3.62 (dd, J = 17.3, 5.3, 1H), 2.35 (s, 3H), 1.89−1.84 (m, 2H), 1.61 (dq, J = 13.8, 7.0 Hz, 1H), 1.40 (s, 3H), 1.04 (t, J-7.29 Hz, 3H), 0.56 (d, J = 6.7 Hz, 3H), 0.16 (d, J-6.7 Hz, 3H); ^{13}C NMR (125 MHz, DMSO) δ 172.5, 172.3, 170.2, 167.9, 148.9, 142.7, 140.3, 131.8, 131.8, 128.1, 126.6, 126.5, 119.7, 115.2, (1 obscured C), 85.6, 71.6, 69.3, 61.8, 55.0, 48.5 (1 obscured C), 34.7, 28.7, 20.2, 19.2, 18.5, 7.7; $[\alpha]_D^{25}$ + 46.5 (c 0.15, DMSO-d_6); HRMS (ESI positive ion) $C_{30}H_{41}N_4O_8^+$ requires 585.2919, found 585.2915.

*1-(Benzyloxy)-2-[[(1-formyl-1-methyl)propyl]oxy]benzene (**27**).* Under an atmosphere of argon, $Pd_2dba_3{\cdot}CHCl_3$ (42 mg, 0.040 mmol) and Trost ligand (*R,R*)-L_{ST} (56 mg, 0.090 mmol) were added to an oven-dried round-bottom flask. Dry, deoxygenated DCM (60 mL) was added to the reaction vessel by cannula, followed by methylvinyloxirane (1.16 mL, 16 mmol). A solution of 2-(benzyloxy)-phenol (700 μL, 4 mmol) in deoxygenated DCM (20 mL) was added to the flask. The solution was stirred for 18 h, and the solvent was evaporated under reduced pressure. The crude material was purified by flash chromatography (20:80 EtOAc/petroleum spirits) to give the corresponding olefin (1.016 mg, 3.6 mmol, 90%) as a clear oil. To a flask containing the olefin (997 mg, 3.5 mmol) was added EtOH (73 mL). The solution was cooled to 0 °C before addition of hydrazine monohydrate (2.55 mL) and saturated aqueous $CuSO_4$ (680 μL). The reaction mixture was allowed to stir vigorously for 48 h. DCM (100 mL) was added. The organic layer was separated, dried over $MgSO_4$, and filtered and the solvent evaporated under reduced pressure. The crude alcohol (908 mg) was used in future reactions without purification.

An oven-dried flask containing IBX (144 mg, 0.51 mmol) was added a solution of the crude alcohol (98 mg, 0.34 mmol) in dry MeCN (2.0 mL). The mixture was stirred at 80 °C for 90 min. The solvent was removed under reduced pressure. Purification by flash chromatography (5:95 EtOAc/petroleum spirits) afforded aldehyde **27** (70 mg 0.25 mmol, 57% (over three steps)) as a light yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.42−7.33 (m, 5H), 7.01 (ddd J = 8.1, 7.3, 1.7, 1H), 6.96 (dd, J = 7.9, 1.7, 1H), 6.94 (dd, J = 8.1, 1.6, 1H), 6.88 (ddd, J = 7.9, 7.3, 1.6, 1H), 5.07 (s, 2H), 1.88 (dq, J = 14.3, 7.3, 1H), 1.75 (dq, J = 14.3, 7.3, 1H), 1.23 (s, 3H), 1.02 (t, 7.3, 3H); ^{13}C NMR (175 MHz, $CDCl_3$) δ 203.7, 151.9, 144.6, 136.8, 128.6, 128.1, 127.6, 124.5, 123.6, 121.4, 114.3, 86.5, 70.8, 29.7, 17.3, 7.5; IR (neat); 2976, 1732, 1593, 1495, 1452, 1381, 1254, 1204, 1112, 1006, 743; HRMS (ESI positive ion) $C_{18}H_{20}NaO_3$ requires 307.1310, found 307.1312.

*N-Boc-valyl-[3-[2-(benzyloxy)phenoxy]isoleucyl]glycine Benzyl Ester (**28**).* Trifluoroethanol (TFE) (85 mL) was dried over 3 Å sieves for 96 h. The solvent was transferred by cannula into a dry round-bottom flask. Approximately 10 mL of condensed ammonia was transferred by cannula into the TFE. The concentration of ammonia was determined to be 2.7 M by titration with HCl (0.5 M) using bromothymol blue as an indicator. To a solution of aldehyde **27** (33 mg, 0.12 mmol) and TFE (6 mL) at 0 °C was added this solution of NH_3 in TFE (2.7 M, 51 μL, 0.14 mmol). The solution was allowed to stand for 10 min before addition of *N*-Boc-valine **16** (30.3 mg 0.14 mmol). The mixture was allowed to stand for a further 10 min before addition of isonitrile **17** (20.6 mg, 0.12 mmol). The reaction mixture was allowed to warm to room temperature over the course of 18 h. The solvent was evaporated, and flash chromatography (35:65 EtOAc/petroleum spirits) afforded a mixture of diastereoisomers of tripeptide **28** (66 mg, 0.10 mmol, 85%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58−6.89 (m, 14H), 5.17−4.82 (m, 5H), 4.11−3.48 (m, 3H), 2.10−1.61 (m, 3H), 1.42−0.83 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) (diastereomers present, only distinct resolvable peaks reported) δ (172.0, 171.9, 171.8, 171.7), (155.9 155.7), (152.7, 152.7, 151.5, 151.4), (144.0, 143.7 143.6 143.5), (136.5, 136.1, 136.0, 135.5), (114.1, 113.7, 113.4, 113.0), 113.4 (113.7), (86.3, 86.1, 85.5, 85.3), (80.0, 79.9), (71.5, 71.2, 71.1, 71.0), (66.8, 66.8), (41.4, 41.1), 8.7, 8.6, 8.5, 8.4); IR (neat) 3328, 2968, 1660, 1497, 1389, 1249, 1176, 748 cm^{-1}; HRMS (ESI positive ion) $C_{38}H_{50}N_3O_8^+$ requires m/z 676.3593, found 676.3595.

*(2S,3R)-3-[4-(Benzyloxy)-3-[[(R)-1-[[(benzyloxycarbonyl)methyl]-carbamoyl]-(S)-2-[[[[(tert- butoxycarbonyl)amino]-3-methyl-butyryl]amino]methyl]-1-methylpropyl]oxy]phenyl]-2-[[(benzyloxy-carbonyl)methyl]amino]-3-[(tert-butyldimethylsilanyl)oxy]-propionic Acid 4-Methoxybenzyl Ester (**29**).* Trifluoroethanol (TFE) (85 mL) was dried over 3 Å sieves for 96 h. The solvent was transferred by cannula into a dry round-bottom flask. Approximately 10 mL of liquid ammonia was transferred by cannula into the TFE. The concentration of ammonia was determined to be 2.7 M by titration with HCl (0.5M) using bromothymol blue as an indicator. To a solution of aldehyde **21** (105 mg, 0.094 mmol) and TFE (6.8 mL) at 0 °C was added this solution of NH_3 in TFE (2.7M, 60 μL, 0.16 mmol). The solution was allowed to stand for 10 min before addition of *N*-Boc-valine **16** (36 mg 0.16 mmol) The mixture was allowed to stand for a further 10 min before addition of isonitrile **17** (24 mg, 0.14 mmol). The reaction mixture was allowed to warm to room temperature over the course of 18 h. The solvent was evaporated under reduced pressure. Flash chromatography (35:65 EtOAc/petroleum spirits) afforded a mixture of epimers of tripeptide **29** (122 mg, 0.10 mmol, 77%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48−6.82 (m, 22H), 5.34−4.76 (m, 11H), 3.88−3.75 (m, 4H), 3.61−3.51 (m, 1H), 3.48−3.32 (m, 1H), 3.15−3.08 (m, 3H), 2.17−1.11 (m, 15H), 1.01−1.79 (m, 18H), 0.02−0.01 (m, 3H), −0.24 to −0.28 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) (diastereomers and rotamers present, only distinct resolvable peaks reported) δ (171.9, 171.7, 171.6), 170.0, 159.8, 157.0, (143.9, 143.7, 143.5, 143.3), 114.0, (74.4, 73.7), (71.7, 71.4, 71.1, 71.0), 55.4, (41.4, 41.2), (33.6, 33.5), 28.4, 25.8, (25.7), (−4.2, −4.3), (−5.3, −5.4, −5.4, −5.4); IR (neat) 2957, 1740, 1699, 1497, 1454, 1389, 1366, 1248, 1173, 1004, 827, 778, 736, 697 cm^{-1}; HRMS (ESI positive ion) $C_{64}H_{85}N_4O_{14}Si^+$ requires m/z 1161.5827, found 1161.5836.

*[[(3R,4S,7S,10S,11R)-15-(Benzyloxy)-10-[[(benzyloxycarbonyl)-methyl]amino]-3-ethyl-11-hydroxy-7-isopropyl-3-methyl-6,9-dioxo-2-oxa-5,8-diazabicyclo[10.3.1]hexadeca-1(16),12,14-triene-4-carbonyl]amino]acetic acid benzyl ester (**30**).* To a solution of tripeptide **29** (124 mg, 0.11 mmol) and DCM (5 mL) at 0 °C were added TFA (1.6 mL) and Et_3SiH (1.3 mL). The reaction mixture was stirred for 7 h and then added to distilled water (5 mL). The organic

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layer was evaporated, the resulting suspension was dispersed by addition of acetonitrile (3 × 1 mL), and the solution was lyophilized overnight. The resulting white solid was dissolved in DMF (50 mL) and the mixture cooled to 0 °C. To this solution were added EDCI (75 mg, 0.39 mmol), HOBt (53 mg, 0.39 mmol), and $NaHCO_3$ (9 mg, 0.11 mmol). The reaction mixture was stirred and allowed to return to room temperature over 18 h. The solvent was evaporated and the resulting yellow oil dissolved in EtOAc (100 mL) and washed with HCl (0.5 M, 100 mL), $NaHCO_3$ (satd 100 mL), and brine (100 mL). The organic layer was dried with $MgSO_4$ and filtered and the solvent evaporated under reduced pressure. Purification by flash chromatography (acetone/petroleum spirits, 40:60) afforded the macrocycle **30** (22 mg, 0.27 mmol, 25%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40−7.30 (m, 17H), 6.94 (d, J = 8.4 Hz, 1H), 6.59 (d, J = 9.5, 1H), 6.48 (d, J = 4.0, 1H), 5.17−4.87 (m, 7H), 4.72 (d, J = 10.2, 1H), 4.66 (d J = 9.5, 1H), 4.35 (br d, J = 17.9, 1H) 3.92 (dd J = 17.9, 4.8, 1H), 3.85 (t J = 5.49, 1H), 3.24 (s, 3H), 2.23−2.16 (m, 1H), 1.98−1.92 (m, 1H), 1.80 (s, 3H), 1.59−1.52 (m, 1H), 0.79−0.74 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1, 169.6, 169.3, 169.2, 157.4, 153.3, 144.6, 136.7, 136.0, 135.4, 133.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.1, 128.0, 127.5, 127.4, 124.9, 121.9, 116.4, 85.7, 71.2, 70.3, 67.8, 67.0, 65.3, 60.4, 58.5, 41.3, 31.7, 30.2, 28.4, 24.2, 19.1, 17.2, 8.8; $[\alpha]_D^{25}$ −93.2 (c 1.0, $CHCl_3$); IR (neat) 3320, 2964, 1747, 1659, 1506, 1455, 1384, 1263, 1193, 1154, 1032, 736, 697 cm^{-1}; HRMS (ESI positive ion) $C_{45}H_{53}N_4O_{10}^+$ requires m/z 809.3762, found 809.3765.

[[(3R,4S,7S,10S,11R)-3-Ethyl-11,15-dihydroxy-7-isopropyl-3-methyl-10-(methylamino)-6,9-dioxo-2-oxa-5,8-diazabicyclo-[10.3.1]hexadeca-1(16),12,14-triene-4-carbonyl]amino]acetic Acid (Ustiloxin D, 1). A suspension of THF/H_2O (1:1, 1 mL) and palladium black (5 mg) was added to a flask containing macrocycle **30** (9.0 mg, 11.1 μmol). The flask was filled with hydrogen and the reaction mixture stirred overnight. The slurry was filtered by Celite, using H_2O as the eluent. The solvent was evaporated, and the crude yellow oil was dissolved in distilled H_2O (2 mL) and lyophilized. The crude material was purified by HPLC (Phenomenex, C18, 100 Å, AXIA, 150 × 21.2 mm, 5−10% MeCN in H_2O with 0.1% TFA over 5 min and then 15−25% MeCN over 15 min) to afford ustiloxin D (**1**) (4.7 mg, 9.5 μmol, 86%): ^{1}H NMR (500 MHz, D_2O) δ 7.19 (dd J = 8.5, 2.0 Hz, 1H), 7.07 (d, J = 8.5 Hz, 1H), 7.02 (br s, 1H), 4.85−4.75 (m, 2H, obscured by solvent peak), 4.08 (d, J = 10.0 Hz, 1H), 3.92 (d, J = 9.5 Hz, 1H), 3.90 (s, 2H), 2.75 (s, 3H), 2.14 (dq, J = 14.3, 7.3 Hz, 1H), 1.72−1.67 (m, 1H), 1.61−1.52 (m, 4H), 1.07 (t, J = 7.3 Hz, 3H), 0.84 (d, J = 6.7 Hz, 3H), 0.75 (d, J = 6.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.6, 171.2, 171.1, 166.2, 150.9, 142.6, 130.6, 124.0, 122.7, 119.0, 85.9, 73.0, 68.9, 60.1, 59.4, 42.8, 32.4, 32.3, 29.0, 21.3, 18.4, 18.0, 8.0; $[\alpha]_D^{25}$ −46.1 (c 0.14, H_2O). IR 3319, 1651, 1433, 1282, 1183, 1132, 958, 839, 800, 722 cm^{-1}; HRMS (ESI positive ion) $C_{23}H_{35}N_4O_8^+$ requires m/z 495.2450, found 495.2447.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b01519.

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

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Notes

The authors declare no competing financial interest.

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