Article

Total Synthesis and Biological Evaluation of Clavatadines C−E

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Cite This: *ACS Omega* 2022, 7, 22915−22929

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ABSTRACT: We described herein the application of a convergent and protecting-group avoidant approach that led to the first total synthesis of the marine natural products clavatadine D (**4**) and E (**5**), and the second total synthesis of clavatadine C (**3**). In each case, a key amide-coupling afforded an immediate precursor of each natural product in a rapid manner from structurally similar western and eastern portions that derived from an ester of L-tyrosine and butane-1,4-diamine, respectively. A deprotection step free of detectable byproducts cleanly provided the remaining known members of the clavatadine family of natural products. Each total synthesis required five steps (longest linear sequence) with overall yields of 30−37%, 26−39%, and 28−50% for clavatadine C (**3**), D (**4**), and E (**5**), respectively. A screen of their potential anticancer activity against the NCI-60 cell line panel revealed cytotoxicity levels up to 38% across a broad spectrum of tumor types. Although clavatadine C (**3**) was relatively benign, clavatadine D (**4**) exhibited 20−38% growth inhibition against a wide array of cancer cell types including leukemia, non-small-cell lung, colon, ovarian, and breast. Clavatadine E (**5**) was active against two types of human brain tumors.
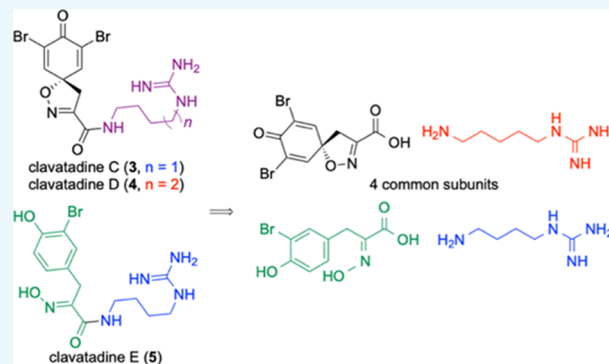
INTRODUCTION

Many marine sponges belonging to Order Verongida use the amino acid tyrosine as a building block to engineer an exotic array of biologically active secondary metabolites.[1−4] The vast majority of these natural compounds possess a mono- or dibrominated phenol and pendant oxime functionality or a six- or seven-membered-ring-containing spiroisoxazoline scaffold that also incorporates one or more bromine atoms. Together, these ring systems are linked by an amide bond to a wide variety of side chains, and they occasionally connect to form dimeric structures. Because many of these architecturally diverse natural products have an intriguing biological activity profile that warrants further exploration, they have attracted the attention of synthetic chemists.[1] Five members of one such family of natural products were discovered in the Great Barrier Reef sponge *Suberea clavata* and were named clavatadines A-E (**1−5**, respectively, Chart 1).[5−7] Clavatadine A (**1**), B (**2**), and C (**3**) were recently prepared by total synthesis using a convergent, early-stage guanidinylation approach.[8−11]

Due to substantial metabolite crossover that exists among members of this order, the quest to discover new compounds amid a hearty mix of biosynthetically related secondary metabolites often reacquaints researchers with previously discovered chemical entities. For example, the isolation effort that unearthed clavatadines C (**3**), D (**4**), and E (**5**) from *S. clavata* also led to the recovery of structurally related spirocyclic natural products purealidin L (**6**), aplysinamisine II (**7**), and aerophobin 1 (**8**) (Chart 2).[6] Notably, purealidin L (**6**) and aplysinamisine II (**7**) feature C-4 and C-5 linear

Chart 1. Clavatadines A−E
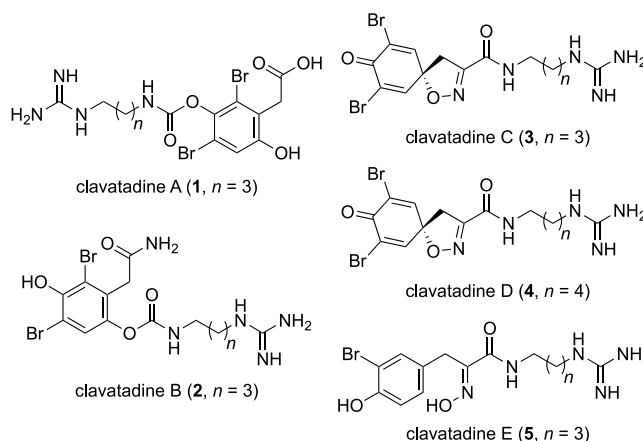
aminoguanidine side chains that are analogous to those found in clavatadines C (**4**) and D (**5**), respectively (Chart 2). Curiously, the chiral spiroisoxazoline ring system embedded within caissarine A (**9**)[12] and pseudoceratinamides A (**11**)[13]
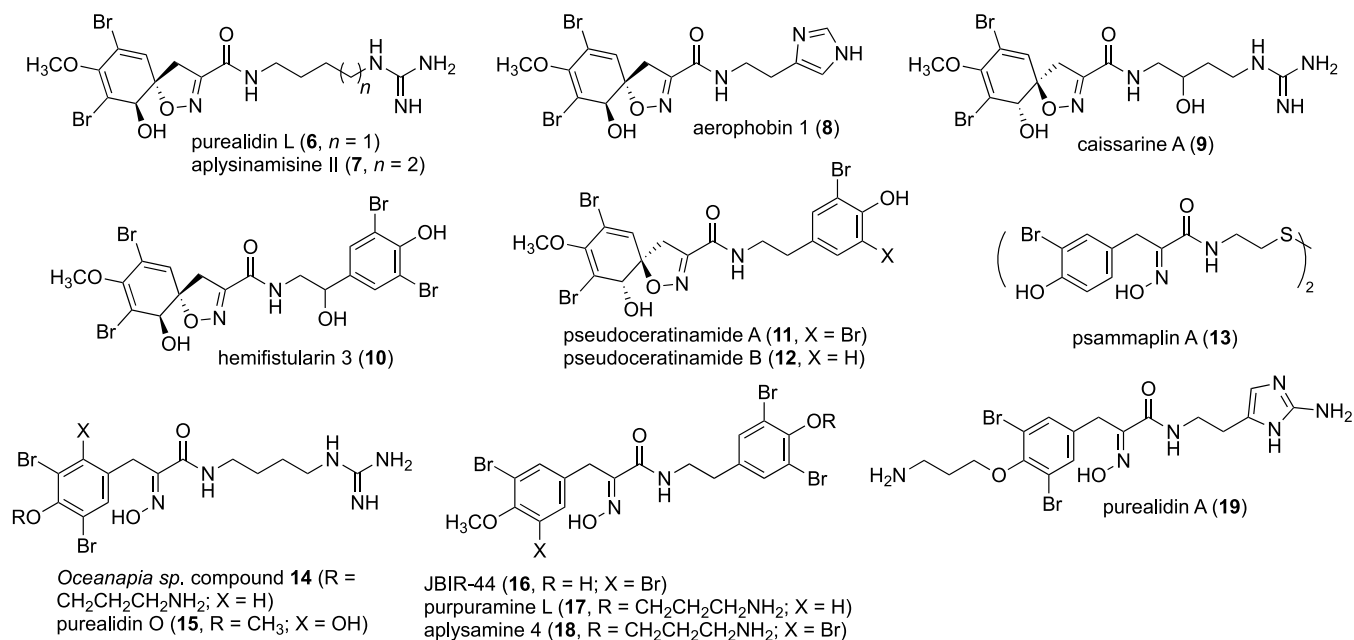
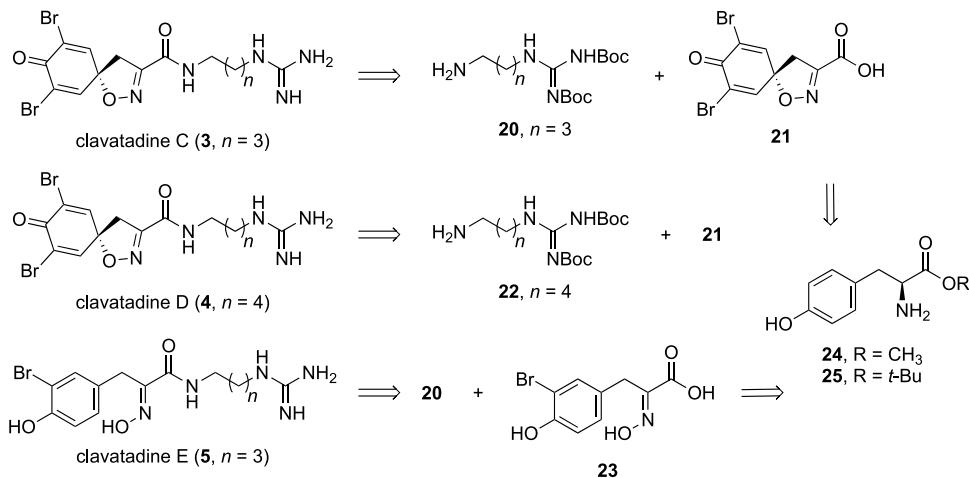**Chart 2. Examples of Naturally Occurring Spiroisoxazolines (6−12) That Resemble Clavatadine C (3) and D (4) and Marine Sponge-Derived Brominated Phenols (13−19) Reminiscent of Clavatadine E (5)**

purealidin L (6, n = 1)
aplysinamisine II (7, n = 2)

aerophobin 1 (8)

caissarine A (9)

hemifistularin 3 (10)

pseudoceratinamide A (11, X = Br)
pseudoceratinamide B (12, X = H)

psammaplin A (13)

Oceanapia sp. compound 14 (R = CH₂CH₂CH₂NH₂; X = H)
purealidin O (15, R = CH₃; X = OH)

JBIR-44 (16, R = H; X = Br)
purpuramine L (17, R = CH₂CH₂CH₂NH₂; X = H)
aplysamine 4 (18, R = CH₂CH₂CH₂NH₂; X = Br)

purealidin A (19)

Scheme 1. Retrosynthetic Analysis of Clavatadines C−E

clavatadine C (3, n = 3) ⟹ 20, n = 3 + 21

clavatadine D (4, n = 4) ⟹ 22, n = 4 + 21 ⟹ 24, R = CH₃ / 25, R = t-Bu

clavatadine E (5, n = 3) ⟹ 20 + 23

and B (12)[13] is the mirror image isomer of most members of this compound class (Chart 2).[2,3,5] A few steps further back on the proposed biogenetic pathway[13] of this natural product class lie mono- and dibrominated phenol-containing oxime derivatives such as psammaplin A (13),[14] compound 14,[15] which was found in *Oceanapia sp.* but unnamed, purealidin O (15),[16] JBIR-44 (16),[17] purpuramine L (17),[18] aplysamine 4 (18),[19] and purealidin A (19)[20] (Chart 2).

Fortuitously, many of the secondary metabolites that have been isolated from marine sponges were revealed to possess a wide range of useful biological activity, not just for the benefit of the organisms themselves, but to humans as well. For example, small-molecule constituents of Verongida sponges have been identified to possess potent antibiotic, antimalarial, antithrombotic, and antiviral activity, as well as a discriminate cytotoxicity that broadens their potential use further into the realm of cancer chemotherapeutic agents.[2,5] Unlike clavatadine A (1) and B (2), which were found to be potent (IC₅₀ = 1.3 and 27 μM, respectively) and selective inhibitors of human

blood coagulation factor XIa (FXIa), clavatadines C (3), D (4), and E (5) bind FXIa only weakly (17, 30, and 37% inhibition at 222 μM, respectively).[6] Although clavatadines C−E do not appear destined for medicinal use as antithrombotic agents to regulate homeostasis, recent efforts have shown that clavatadine C and dibrominated spiroisoxazoline-containing congeners of clavatadine C inhibit the growth of cancer cells *in vitro*.[8,21] Meanwhile, the potential bioactivity profile of clavatadine D (4) and E (5) remains wholly unexplored.

Synthetically speaking, at the heart of many Verongida-derived natural products that resemble clavatadines C−E (*vide infra*) is an oxime-containing bromophenol or dibrominated spiroisoxazoline ring system that links a wide array of structural appendages by an amide bond. To assemble these structural motifs in the laboratory, construction of the central amide bond is of particular strategic interest. In contrast to the method used to assemble the labile central carbamate that lies at the heart of clavatadine A (1) and B (2) and plays a critical role in their reported biological activity, a strategy that reversed

Scheme 2. Synthetic Precedent Revealing That the Oxime Hydroxy Group Is Likely More Nucleophilic Than the Phenol in Tyrosine-Derived Oximes
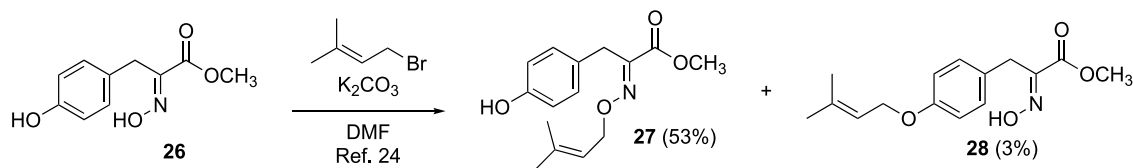
the polarity of the ring-containing and linear subunits was employed to construct the amide-containing natural products clavatadine C (**3**), D (**4**), and E (**5**).[6,7,22,23]

Encouraged by our prior efforts to prepare aminoguanidine-containing natural products using direct, early-stage guanidinylation, the retrosynthetic analysis of clavatadines C—E was designed to avoid unnecessary protecting groups and originate from common synthetic precursors (Scheme 1). Protected linear aminoguanidines **20** and **22**, which were successfully deployed in the total synthesis of clavatadine A (**1**) and B (**2**), would be linked with the requisite, known synthetic intermediate **23** or **21**, which in turn would be derived from the methyl or *tert*-butyl ester of L-tyrosine (compounds **24** or **25**, respectively). An additional focus was to develop a convergent approach that would be amenable to the preparation of analogues for biological evaluation. For example, because cytotoxicity relies most heavily upon the structure of the tail portion in clavatadine C analogues,[8] it was desired to construct the central amide bond as late as possible during each synthesis to maximize overall yield according to the principle of convergence. Thus, the principal goal in each synthesis was to incorporate the tail portion in the penultimate step. Deprotection of the guanidine moiety, the only protected functional group, would occur in the final step and reveal each natural product.

To enlist a protecting-group avoidant strategy and streamline each synthesis of clavatadine C—E required careful orchestration of reactivity among a diverse array of officious functional groups. As shown previously,[9,11] bis-Boc protection of the guanidine group ensured that the less nucleophilic amine would be oriented correctly when the tail portion was incorporated. In the approach to clavatadine C and D, sufficient precedent existed to suggest that amide coupling would dominate despite the presence of competing electrophiles in cyclic dienone **21**. In contrast, to prepare clavatadine E by amide coupling it was necessary that the nucleophilicity of the tail portion's amino group supersede the reactivity of the hydroxy groups present in the phenol and oxime housed within putative synthetic intermediate **23**. Interference by either group may cause homodimerization to occur between two molecules of compound **23**. One example of an etherification reaction revealed that the oxime is more nucleophilic than the phenol (Scheme 2).[24] Notably, neither hydroxy group appeared to react with the methyl ester in compound **26** to form dimers or polymeric byproducts.[24,25]

RESULTS AND DISCUSSION

Although many prior efforts to construct natural product scaffolds using a synthetic intermediate resembling compound **26** enlisted tetrahydropyranyl, benzyl, or methyl protecting groups to cloak either the phenolic hydroxy group, the oxime, or both, clavatadine E was prepared without using protecting groups in the bromophenol-containing half of the molecule. In

practice, L-tyrosine methyl ester (**24**) was oxidized to oxime **26** using a slight modification of the reported procedure (Scheme 3).[24] This chemoselective oxidation reaction did not require

Scheme 3. Synthesis of Clavatadine E (5)
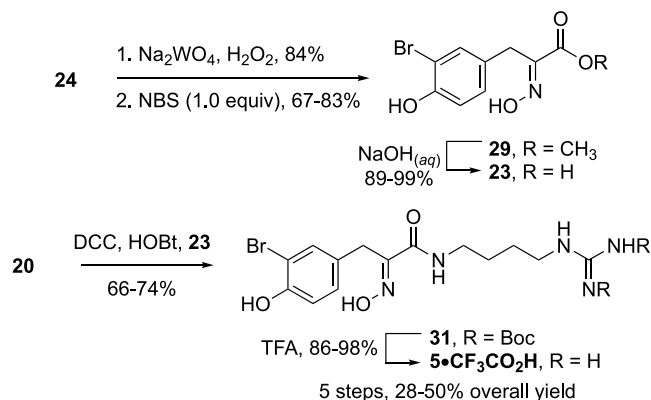
purification.[24] Next, attempts to monobrominate oxime **26** with *N*-bromosuccinimide (NBS) were inconsistent and compromised by crude reaction mixtures that contained unreacted starting material (**26**), the desired product **29**, and dibrominated compound **30**. Due to their similar polarity, these compounds were also difficult to separate fully using column chromatography. Table 1 illustrates the result of several experiments that were designed to consume unreacted starting material by increasing the amount of NBS that was used; however, this change seemed only to increase the

Table 1. Efforts to Optimize the Monobromination of Phenol 26

| entry	mol. equiv. NBS	26[a]	29[a]	30[a]
1	1.0[b]	ND	67	ND
2	1.0[b]	4	83	4
3	1.05[c]	4	67	7
4	1.05[b]	18	50	20
5	1.1[b]	12	60	13
6	1.15[b]	11	50	25
7	1.2[b]	ND	67	ND
8	1.2[b]	9	52	28

[a]Isolated yield. [b]2.75 mmol scale. [c]1 mmol scale, ND = not determined.

amount of dibrominated product that was formed without affecting the yield of monobrominated product substantially.

Although literature precedents describe successful direct aminolysis reactions between esters and primary amines, several attempts to apply this expedient strategy to prepare immediate precursors of clavatadine C−E were unsuccessful. For example, it was envisioned that aminolysis of monobrominated oxime methyl ester 29 by protected aminoguanidine 20 might afford the bis-Boc protected precursor of clavatadine E. Under reported conditions that employ one equivalent of ester in the presence of excess amine using either mild conditions, such as (CH₃OH/dioxane 1:1 v/v, 40 °C, 18 h),[26] (CH₃OH, 60 °C, 72 h),[27] or (50 mol % of ammonium nitrate[28] in CH₃OH, ethanol, or pyridine), or under forcing conditions (N,N-dimethylformamide (DMF), 130 °C, 30 min),[29] no aminolysis product 31 was observed. Unreacted starting materials were recovered when mild conditions were used. On the other hand, extensive decomposition was observed under high temperatures, presumably involving thermal Boc deprotection and competing side reactions. Similar results were observed when putative molecular scaffolds leading to clavatadine C or D were used.

Frustrated by unsuccessful direct ester aminolysis attempts, focus turned toward peptide coupling to construct clavatadine E (5).[30,31] To achieve this goal, ester 29 was hydrolyzed under basic conditions followed by acidification of the reaction mixture to provide acid 23 in near-quantitative yield (Scheme 3). Several classic and newer peptide coupling reagents were employed with varying degrees of success in this system (Table 2). Curiously, attempted amide formation in the presence of

Table 2. Efforts to Optimize Amide Formation to Prepare Compound 31
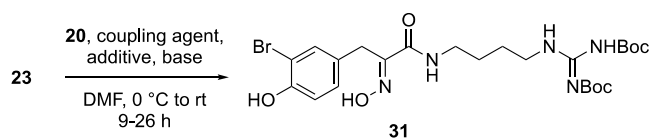
| entry	coupling agent	additive	base	yield (%)[a]
1	COMU	none	i-Pr₂NEt	17[c]−35[b]
2	COMU	none	TMP	11[b]
3	COMU	none	2,6-lutidlne	58[b]
4	HBTU	none	2,6-lutidlne	trace[b]
5	EDC	none	none	24[b]
6	EDC[d]	HOBt	none	29[b]−32[c]
7	DCC	none	none	trace[b]
8	DCC	N-hydroxyphthalimide	Et₃N	26[b]−28[c]
9	DCC	HOBt	none	66[b]−74[c]

[a]Isolated yield after chromatography. [b]Small scale (≤0.1 mmol). [c]Large scale (≥1 mmol). [d]CH₂Cl₂ was used.

newer reagents such as HBTU and COMU,[32,33] which often require a tertiary amine base such as Hünig's base (N-ethyl-N,N-diisopropylamine) or 2,2,6,6-tetramethylpiperidine (TMP), gave lower yields of product (Table 2, entries 1−4). One exception was when the less-basic 2,6-lutidine was used in place of a tertiary amine (Table 2, entry 3). It is likely that less-efficient examples such as these expose the limitation imposed by the unprotected oxime and phenol during amide formation using acid 23. Trials that incorporated a classic reagent such as EDC·HCl gave moderate yields regardless of whether an

additive such as 1-hydroxybenzotriazole (HOBt) was included (Table 2, entries 5 and 6). Moderate yields were also observed using N,N′-dicyclohexylcarbodiimide (DCC) in the presence of a reagent that could encourage the formation of an even more activated ester, such as HOBt,[34] but not N-hydroxyphthalimide/Et₃N[35−37] (Table 2, entries 8 and 9). The best result was observed when DCC was used in the presence of a stoichiometric amount of HOBt, which afforded N,N-diBoc clavatadine E (31) in a range of yields from 66−74% (Scheme 3 and Table 2, entry 9). Finally, deprotection of compound 31 with trifluoroacetic acid (TFA) completed the synthesis. After the reaction with TFA was judged to be complete by TLC, the reaction mixture was concentrated. The residue was oiled out by trituration with ether and the supernatant was removed to provide pure clavatadine E (5·CF₃CO₂H) as its hydrotrifluoroacetate salt.[9] 1D and 2D NMR spectra of unpurified clavatadine E (5) in DMSO-d_6 matched the data reported by Quinn and co-workers (see Table S3 for comparison).[6]

After several unsuccessful attempts to forge the central amide bond at the heart of clavatadine C and D expediently using direct aminolysis of an unhindered methyl ester (vide infra), the preparation of subunits suitable for peptide coupling was pursued. A well-precedented oxidative dearomatizing cyclization reaction of oximes derived from L-tyrosine was relied upon to introduce complexity rapidly and afford the spiroisoxazoline scaffold present in both clavatadine C and D.[38−40] Although treatment of oxime 26 with a slight excess of NBS cleanly afforded methyl ester 34, hydrolysis of the methyl ester under basic conditions followed by acidification led to a complex mixture of products that did not appear to include the desired carboxylic acid 21 (Scheme 4). Ethyl ester analogues of

**Scheme 4. Synthesis of Clavatadine C (3) and Clavatadine D (4)**
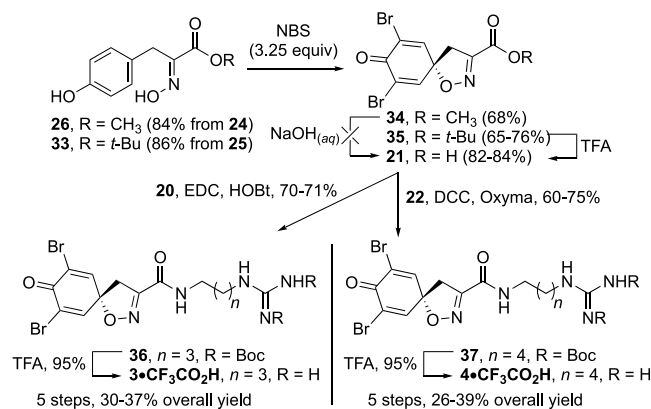
these precursors were also known; however, it was unlikely that basic hydrolysis of an ethyl ester would engender a different outcome because the same carboxylate intermediate would be formed during the reaction. On the other hand, the TFA-mediated cleavage of Boc groups and tert-butyl esters was well-known.[41] Fortunately, tert-butyl ester 35 was formed by dibromination followed by spirocyclization when phenol 33 was exposed to an excess amount of NBS. Removal of the acid-labile tert-butyl group in the presence of TFA cleanly afforded compound 21, which did not require purification (Scheme 4).[10]

With access to compound 21, peptide coupling of this carboxylic acid to C-4 aminoguanidine 20 or C-5 amino-

guanidine **22** was explored to construct clavatadine C or D, respectively (Scheme 4). The method that was developed and reported by Hawkins and co-workers' to prepare clavatadine C (**4**) using EDC·HCl was replicable and afforded *N,N*-diBoc clavatadine C (**36**) in 70−71% yield (Scheme 4). Cleavage of both Boc protecting groups in compound **36** proceeded efficiently in the presence of TFA and gave clavatadine C (**3·CF₃CO₂H**) as its hydrotrifluoroacetate salt, which was impurity-free. As before, the reaction mixture was concentrated, and then the unpurified residue was triturated with dry ether and dried under high vacuum. This process provided a product whose data matched that reported by Quinn[6] and co-workers and confirmed both the synthesis, data, and spectra published by Hawkins[10] and co-workers (see Table S1 for comparison).

The total synthesis of clavatadine D proceeded in a similar manner, but the amide-forming reaction to provide *N,N*-diBoc clavatadine D (**37**) required optimization to achieve a desirable yield. Despite its successful application in the clavatadine C synthesis, EDC·HCl gave low but consistent yields whether or not HOBt was used (Table 3, entries 1 and 2). Similar results

Table 3. Efforts to Optimize Amide Formation to Prepare Compound 37
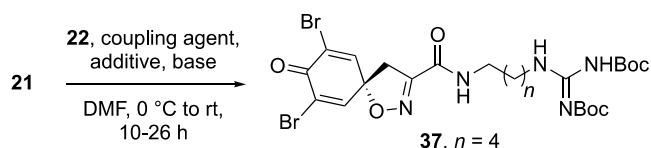
entry	coupling agent	additive	base	yield (%)[a]
1	EDC	none	none	19[b]
2	EDC[d]	HOBt	none	19[b]−31[c]
3	COMU	none	*i*-Pr₂NEt	76[b]−51[c]
4	HBTU	none	*i*-Pr₂NEt	29[b]
5	DCC	none	none	29[b]
6	DCC	oxyma	none	75[b]−60[c]
7	DCC	HOBt	none	57[c]

[a]Isolated yield after chromatography. [b]Small scale (≤0.1 mmol). [c]Large scale (≥1 mmol). [d]CH₂Cl₂ was used.

were observed with HBTU and DCC in the absence of additives (Table 3, entries 4 and 5); however, DCC couplings supplemented with HOBt or Oxyma gave higher yields (Table 3, entries 6 and 7). Acceptable results were also observed with COMU and provided compound **37** in yields ranging from 51−76% (Table 3, entry 4). In practice, it is likely that the classic peptide coupling reagent DCC with either Oxyma or HOBt would be favored over COMU because COMU is much more expensive. As before, deprotection with TFA cleanly afforded the hydrotrifluoroacetate salt of clavatadine D (**4·CF₃CO₂H**) in excellent yield (Scheme 4). ¹H NMR spectra

obtained on a dilute sample of unpurified clavatadine D matched the data reported by Quinn and co-workers (see Table S1 for comparison).[6] Only the chemical shift of the N−H near the guanidine moiety changed significantly (from δ 7.39 to δ 7.58) when a concentrated sample of clavatadine D (**4**) was prepared (see Figures S33 and S34). All other 1D and 2D NMR spectra matched the reported data.[6]

With facile access to pure, synthetic clavatadines C−E (**3−5**), a preliminary screen of their potential anticancer activity revealed growth inhibition across a broad spectrum of tumor types. A one-dose NCI-60 screen of each compound at 10 μM revealed low to moderate growth inhibition against several cell lines (Table 4). Earlier, Hawkins[10] and co-workers reported that clavatadine C (**3**) displayed significant cytotoxicity at 5 and 10 μM against several cancer cell lines; however, our sample of clavatadine C did not yield similar results in the NCI-60 screen. Instead, clavatadine C (**3**) was found to display at most 13% growth inhibition against any of the NCI-60 cell types, which is more in line with results obtained by Kiuru and co-workers in a more recent study involving clavatadine C and derivatives thereof.[8] Despite having a tail portion that is just one carbon longer than compound **3**, clavatadine D (**4**) exhibited activity against a wide array of cancer cell types including leukemia, non-small-cell lung, colon, ovarian, and breast, with approximately 20−40% growth inhibition. Clavatadine E (**5**), on the other hand, was inactive against most cancer cell types in the screen, but displayed low to moderate activity against the SF-268 and SNB-75 human brain tumor cells.

Finally, it is noteworthy that a minor discrepancy was observed in the ¹H NMR spectrum of synthetic *N,N*-diBoc clavatadine C (**36**) in samples independently prepared by Hawkins[10] and co-workers and in the present study. Notable differences in ¹H chemical shift occurred in the region between δ_H 3.3−3.6, which included three groups of methylene protons. The apparent difference in the chemical shifts of these methylene hydrogens appears to be of relatively minor concern for the following reasons. First, ¹³C-NMR data acquired independently and in the same solvent (CDCl₃) matches.[10] Next, both laboratories used the same procedure to convert compound **36** to the hydrotrifluoroacetate form of clavatadine C (**3·CF₃CO₂H**). Finally, the spectroscopic data of synthetic clavatadine C (**3·CF₃CO₂H**) prepared by both laboratories matched each other and agreed with data reported for the natural compound (see Table S1 for comparison).

It appeared that the minor difference in observed chemical shift could be attributable to two possible causes. One laboratory may have dissolved the purified sample of compound **36** in CDCl₃ that had trace amounts of HCl or DCl present because the CDCl₃ had not been "treated" prior to use. Pretreatment of commercially acquired CDCl₃ typically serves to remove water by adding activated 3 or 4 Å molecular

Table 4. NCI-60 Screening Results for Clavatadines C−E (3−5)[a]

cell type /compound	CCRF-CEM (leukemia)	K-262 (leukemia)	A-549 (lung)	NCI-H522 (lung)	HCT-15 (colon)	SF-268 (CNS)	SNB-75 (CNS)	OVCAR-3 (ovarian)	MCF-7 (breast)	MDA-MB-468 (breast)
Clavatadine C (**3**)	96	95	110[b]	87	105	95	107	103	98[c]	111
Clavatadine D (**4**)	62	62	110	69	73	92	98	80	80	76
Clavatadine E (**5**)	102	105	108	96	103	68	80	97	104	108

[a]Numbers reflect mean growth percent. 100 percent indicates no effect. [b]Ref 10 reported 39 ± 7% cell viability after 24 h upon exposure of this cell line to compound **3** [10 μM]. [c]Ref 10 reported 30 ± 5% cell viability after 24 h upon exposure of this cell line to compound **3** [10 μM].

sieves and/or neutralizing adventitious acid using a base such as potassium carbonate.[42] Organic synthesis laboratories who prepare acid-labile compounds frequently pretreat their CDCl$_3$ in this way. It is therefore possible that the use of untreated CDCl$_3$ led to decomposition and/or a change in conformation that altered the chemical shift of certain groups of hydrogens near the affected area. Alternatively, samples of compound **36** in the current and prior study were prepared at different concentrations. In this scenario, an observed change in the chemical shift of hydrogens bonded to carbon may represent an example of a rare phenomenon known as concentration-dependent chemical shift variation of nonexchangeable hydrogens.[43,44] These effects are often attributable to differences in how molecules fold or aggregate with changes in concentration.[44]

Slight concentration-dependent variation in ^{1}H chemical shifts is typically observed for exchangeable atoms, such as OH and NH hydrogens, but not hydrogens bound to carbon. For example, subtle chemical shift differences were observed in synthetic clavatadine E (**5**) for downfield OH resonances such as the phenol (δ_H 10.04 vs 10.11 ppm) and oxime (δ_H 11.76 vs 11.80 ppm) in dilute and concentrated samples, respectively. A similar downfield shift of approximately 0.2 ppm was observed for the N−H resonance near δ_H 7.5 ppm in concentrated samples of synthetic clavatadine C (**3**) and D (**4**). In all cases, the chemical shift of exchangeable resonances within synthetic samples most closely matched those of the natural samples when a dilute solution (1−2 mg/mL) of synthetic samples was prepared because the natural compounds were often isolated in small quantities at or below one milligram.[7]

To attempt to resolve the issue of the observed chemical shift differences between synthetic samples of *N,N*-diBoc clavatadine C (**36**), a series of NMR experiments was performed (see the Supporting Information for details). For purposes of comparison, standard protocol in the present study is to use "treated" CDCl$_3$ for NMR analysis. Furthermore, characterization by ^{1}H NMR is typically done using lower concentrations of sample (e.g., 1−10 mg/mL), and data acquisition is complete within minutes of sample preparation.[45] Higher solute concentrations (e.g., 20−50 mg/mL) are used for ^{13}C-NMR and 2D NMR analysis to increase signal-to-noise ratio and reduce acquisition time on a shared instrument.

To ascertain the impact that the condition of the CDCl$_3$ used to dissolve synthetic compound **36** may have on sample integrity, such as compound decomposition, ^{1}H NMR spectra were acquired on samples of varying concentration using treated or untreated CDCl$_3$ on the same day that the NMR samples were prepared and again one day after. The chemical shift of the singlet found at δ 3.48, which is assigned as the C-7 methylene (Figure 1), did not change significantly as a

function of concentration; however, an increase in concentration caused the signals arising from the remaining methylene hydrogens in this region, H-11 and H-14, to migrate upfield (Figures 2b,c and 3). Shimming quality and its effect on fine splitting in ^{1}H NMR spectra of these samples varied inversely as concentration increased. Although some differences in fine splitting quality were observed at various concentrations in samples dissolved in treated or untreated CDCl$_3$, treatment or nontreatment of the solvent did not affect the chemical shift of proton signals. This observation suggests that CDCl$_3$ did not need to be treated prior to sample preparation, yet it does not fully explain the observed differences in the reported spectra of compound **36** in the δ 3.3−3.6 region. Thus, it is more likely that the observed phenomenon is due to concentration-dependent chemical shift variation of nonexchangeable C−H bonds, though it also does not fully explain the differences in spectra acquired in this laboratory and in Hawkins'.[10] A separate series of experiments determined that ^{13}C-NMR chemical shifts do not change more than 0.1 or 0.2 ppm regardless of concentration or the condition of the CDCl$_3$ used to dissolve the samples.

■ CONCLUSIONS

In summary, the first total syntheses of clavatadines D (**4**) and E (**5**) have been completed, and we have successfully replicated the reported synthesis of clavatadine C (**3**) by Hawkins and co-workers.[10] All 1D and 2D NMR spectra of unpurified, synthetic clavatadine C (**3**), D (**4**), and E (**5**) were identical to those obtained by Quinn and co-workers[6] for the corresponding natural compounds (see Tables S1−S3 for comparison). Oximation and dibromospirocyclization of L-tyrosine *tert*-butyl ester ultimately led to both clavatadine C (**3**) and D (**4**) in a five-step, convergent synthesis from commercially available materials and in overall yields of 30−37 and 26−39%, respectively. In contrast to a prior report that found clavatadine C (**3**) to be moderately active against four cancer cell lines[10] but in agreement with recent work by Kiuru and co-workers,[8] our sample displayed little to no cytotoxicity. On the other hand, clavatadine D (**4**) moderately inhibited growth in a wide range of cancer cell types. The overall yield of our protecting-group avoidant, convergent synthesis of clavatadine E (**5**) is 28−50% over five steps from commercially available L-tyrosine methyl ester (**25**). Notably, the key amide-coupling step succeeded despite the presence of reactive oxime and phenol functionalities. Clavatadine E (**5**) did not possess broad-spectrum anticancer activity but exhibited moderate cytotoxicity against central nervous system (CNS) cancer cells. An overarching synthetic strategy that capitalized upon direct introduction of a bis-protected guanidine has thus far enabled total synthesis of several aminoguanidine-containing natural and non-natural products from this laboratory and should enable the rapid preparation of similarly functionalized natural products and natural product analogues in the future. It is planned to prepare derivatives of all known clavatadine natural products for further biological study, and these results will be reported in due course.

■ METHODS

General Experimental Procedures. ^{1}H and proton-decoupled ^{13}C{^{1}H} NMR spectra were recorded at 25 °C at 400 and 100 MHz, respectively, on a Bruker Avance III HD NMR spectrometer, and calibrated using tetramethylsilane
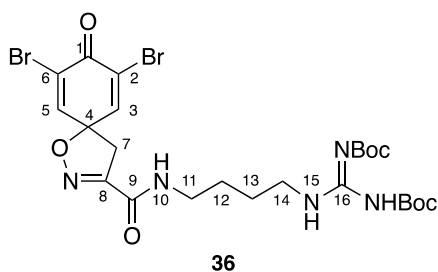

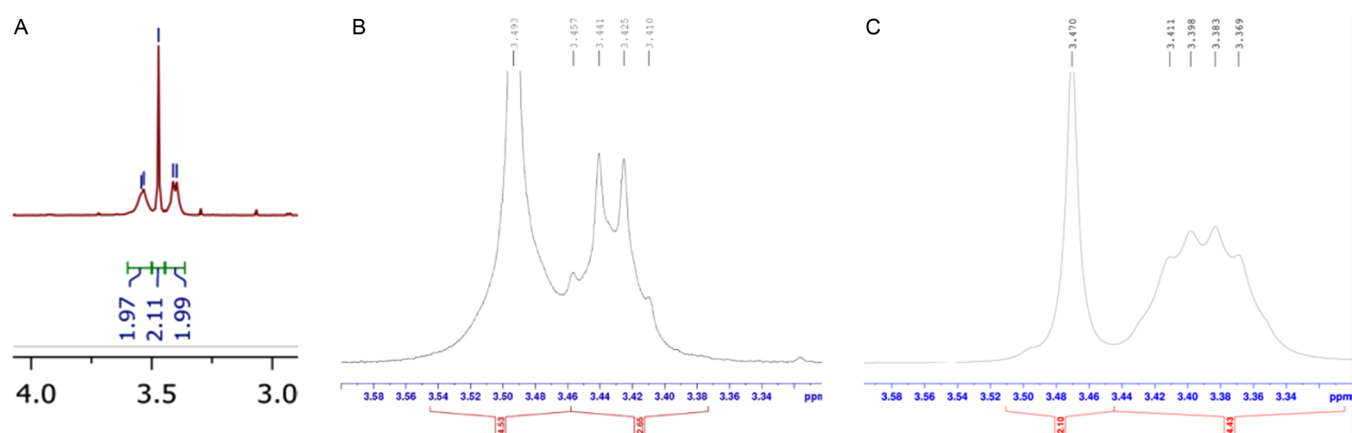**Figure 1.** Numbered backbone of *N,N'*-diBoc clavatadine C (**36**).

Figure 2. Concentration-dependent chemical shift variation may explain a difference in the recorded ^{1}H NMR spectra of compound **36** from the present study and from ref 10. (A) About 50 mg of compound **36**, identified in ref 10 as compound **12**, in CDCl$_3$, excerpted from ref 10, page S-16, Reprinted in part with permission (pending) from ref 10, Elsevier; (B) 0.8 mg of compound **36** in 0.6 mL of treated CDCl$_3$, day of preparation; and (C) 100 mg of compound **36** in 0.6 mL of treated CDCl$_3$, day of preparation.
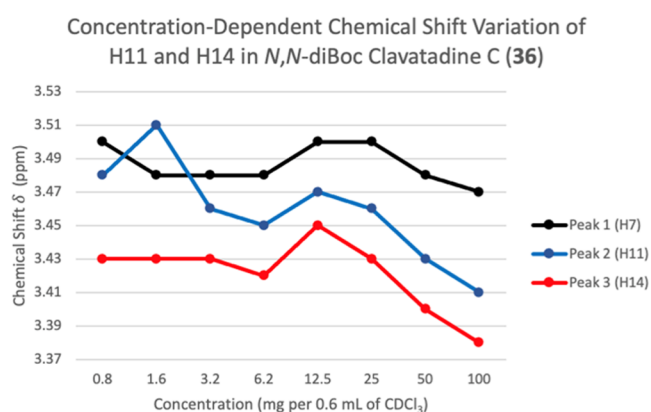

Figure 3. Concentration-dependent chemical shift variation of nonexchangeable methylene hydrogens observed in compound **36**.

(TMS) at δ 0.00 ppm, unless otherwise stated. For spectra calibrated using DMSO-d_6, ^{1}H- and ^{13}C-NMR data are referenced to residual internal CD$_3$SOCD$_2$*H* at δ 2.50 (^{1}H) and residual internal (CD$_3$)$_2$SO at δ 39.50 (^{13}C), respectively.[46] For spectra calibrated using acetone-d_6, ^{1}H- and ^{13}C-NMR data are referenced to residual internal CD$_3$COCD$_2$*H* at δ 2.05 (^{1}H) and residual internal (CD$_3$)$_2$CO at δ 29.84 (^{13}C), respectively.[46] For spectra calibrated using CD$_3$OD, ^{1}H- and ^{13}C-NMR data are referenced to residual internal CD$_2$*H*OD at δ 3.31 (^{1}H) and residual internal CD$_3$OD at δ 49.00 (^{13}C), respectively.[44] All chemical shifts are reported in ppm on the δ scale, multiplicity (v br = very broad, br = broad, s = singlet, d = doublet, t = triplet, m = multiplet, or combinations thereof), coupling constants in Hz, and integration. All 2D NMR spectra, including gradient correlation spectroscopy (COSY), gradient multiplicity-edited heteronuclear single-quantum coherence (HSQC), and gradient heteronuclear multiple bond correlation (HMBC) were recorded in CDCl$_3$ or DMSO-d_6 at ambient temperature. Infrared (IR) spectra were obtained on neat solids using a Bruker Tensor 27 attenuated total reflectance-infrared Fourier transform infrared (ATR-FTIR) spectrometer at ambient temperature. Accurate mass (high-resolution mass spectrometry (HRMS)) measurements were performed by the University of California, Irvine Mass Spectrometry Facility on a Waters LCT Premier time-of-

flight (TOF) instrument using electrospray ionization (ESI) in positive-ion mode (ES$^+$). Observed mass spectra were validated within ±5 ppm of the expected molecular formulae. Poly(ethylene glycols) were used for calibration mass standards. Liquid chromatography was performed using variable forced air flow (flash chromatography) of the indicated solvent system or solvent gradient through 60 Å silica gel (SiO$_2$) (40–63 μm, 230–400 mesh). Analytical thin-layer chromatography (TLC) was performed using 0.25 mm silica gel 60 (F254) plates. TLC spots were visualized by short-wave (254 nm) UV irradiation, exposure to iodine vapor in a closed container, and/or by dipping the plates in a cerium ammonium molybdate (CAM) solution followed by heating. All reaction mixtures not containing aqueous reagents were carried out under an atmosphere of dry argon using standard syringe/septa techniques. For reactions conducted under inert atmosphere, glassware was oven-dried overnight at 130 °C, sealed with a rubber septum, and then purged with dry argon using a vent needle. Alternatively, glassware was sealed with a rubber septum, placed under a positive pressure of dry argon with a vent needle, flame-dried using a propane torch, and allowed to cool under argon. Unless otherwise noted, all reagents were used as received from commercial suppliers. Reagents that were not commercially available were synthesized according to a known literature procedure. Anhydrous, anoxic CH$_2$Cl$_2$, tetrahydrofuran (THF), DMF, diethyl ether (Et$_2$O), and triethylamine (Et$_3$N) were obtained by passing the previously degassed solvents through an activated alumina column under argon. Hünig's base (*i*-Pr$_2$NEt) was dried over activated 4 Å molecular sieves and distilled under argon. CH$_3$CN was partially dried using a threefold treatment with activated 3 Å molecular sieves.[47] Although discrete chemical yields are reported here for specific experimental procedures, ranges of isolated yields are presented elsewhere in the manuscript when multiple trials of reactions were performed according to the same procedure regardless of scale. **Caution!** *O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HOBt, CAS number 123333-53-9) is explosive when dry and carries a risk of explosion if heated under confinement. For safety reasons, commercially available HOBt is sold wetted with at least 20% (w/w) of H$_2$O; thus, in reactions involving HOBt, 120% of the required amount was weighed. **Caution!** Some peptide coupling reagents, including dicyclohexylcarbo-

diimide (DCC, CAS number 538-75-0), N,N,N',N'-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, CAS number 94790-37-1), and possibly (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU, CAS number 1075198-30-9) are known or suspected immune sensitizers and may cause anaphylaxis.[48,49]

(E)-Methyl 2-Hydroxyimino-3-(4-hydroxyphenyl)-propionate (26). To a cooled (0 °C) suspension of L-tyrosine methyl ester (24) (4.083 g, 17.21 mmol, 1.00 equiv) in 50 mL of absolute EtOH in a round-bottom flask equipped with a magnetic stir bar were added, in sequence, Na_2WO_4 (5.675 g, 17.21 mmol, 1.00 equiv), 30% $H_2O_2(aq)$ (16.8 mL, 165 mmol, 9.60 equiv), and H_2O (34 mL), and the reaction mixture was stirred at 0 °C for 45 min. Seconds after H_2O_2 addition, the color of the reaction mixture changed from colorless to bright yellow. After 45 min, the cooling bath was removed, and the mixture was stirred with warming to ambient temperature for an additional 4.5 h. After 4.5 h, the pale-orange-colored solution was extracted with EtOAc (5 × 50 mL), and the combined organic extracts were washed with a 10% aqueous solution of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$, 5 × 20 mL) and saturated aqueous sodium chloride (1 × 75 mL), dried over anhyd $MgSO_4$, filtered, and concentrated in vacuo to provide the product as a cream-colored amorphous solid (3.720 g, 86%). The spectroscopic data for compound 26 matched previously reported data for compound 7 prepared by de Silva and Andersen.[24] R_f = 0.27, 3:1 (v/v) CH_2Cl_2/EtOAc; ^1H NMR (CD$_3$OD, 400 MHz): δ 7.06 (d, J = 8.4, 2H), 6.66 (d, J = 8.4, 2H), 3.81 (s, 2H), 3.76 (s, 3H); $^{13}C\{^1H\}$ NMR (CD$_3$OD, 100 MHz): δ 166.1 (C), 157.1 (C), 152.4 (C), 131.1 (CH), 128.6 (C), 116.2 (CH), 52.8 (CH$_3$), 30.3 (CH$_2$).

(E)-Methyl 3-(3-Bromo-4-hydroxyphenyl)-2-(hydroxyimino)propionate (29). In a round-bottom flask equipped with a magnetic stir bar and covered with aluminum foil was added phenol 26 (0.575 g, 2.75 mmol, 1.00 equiv) and 6 mL of partially dried CH$_3$CN, and then the mixture was cooled to 0 °C. To this mixture was added a solution of N-bromosuccinimide (0.489 g, 2.75 mmol, 1.00 equiv) in 4.5 mL of partially dried CH$_3$CN dropwise by syringe over 15 min. An additional 1 mL of partially dried CH$_3$CN was used to rinse the flask that contained NBS, and the rinse solution was added to the reaction flask in one portion. The reaction mixture was stirred at 0 °C with gradual warming to rt over 4 h. The reaction mixture was concentrated in vacuo and then partitioned between 20 mL of EtOAc and 20 mL of H$_2$O. The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 5 mL). The combined organic extracts were washed with H$_2$O (2 × 10 mL), a saturated aqueous solution of $Na_2S_2O_3 \cdot 5H_2O$ (1 × 10 mL), and brine (1 × 10 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo to provide the crude product (0.775 g) as a cream-colored solid. The crude product was dissolved in EtOAc and adsorbed onto 10 g of silica gel, carefully concentrated in vacuo, and then purified by flash column chromatography on silica gel using CH$_2$Cl$_2$/Et$_2$O (19:1 to 1:1) as eluent to provide compound 29 as a cream-colored amorphous solid (0.659 g, 83%). R_f = 0.38, 3:1 (v/v) CH$_2$Cl$_2$/EtOAc. The less polar dibrominated analogue, (E)-methyl 3-(3,5-dibromo-4-hydroxyphenyl)-2-(hydroxyimino)-propionate (30), was also obtained (0.038 g, 4% of theoretical) as well as recovered compound 26 (0.024 g, 4%), which was more polar than the desired product. The ^1H NMR

spectroscopic data for compound 29 matched previously reported data for compound 4 prepared by Altucci, de Lera, and co-workers.[36] $^{13}C\{^1H\}$ NMR (acetone-d_6, 100 MHz): δ 165.1 (C), 153.4 (C), 151.4 (C), 134.2 (CH), 130.30 (C), 130.26 (CH), 117.2 (CH), 110.1 (C), 52.5 (CH$_3$), 29.79 (CH$_2$).

(E)-3-(3-Bromo-4-hydroxyphenyl)-2-(hydroxyimino)-propanoic Acid (23). To a solution of methyl ester 29 (0.827 g, 2.87 mmol, 1.00 equiv) in 60 mL of THF in a round-bottom flask equipped with a magnetic stir bar at rt was added 3.3 M KOH(aq)[50] (12.5 mL, 41.5 mmol, 14.5 equiv) in one portion. The addition of the KOH solution produced a biphasic mixture with a pale-yellow-colored top layer and a dark-yellow-colored bottom layer. After 2 min, 5% HCl(aq) (83 mL, 49.8 mmol, 17.4 equiv) was added to the biphasic mixture, which led first to the formation of a pale-yellow-colored and then a colorless homogeneous solution when all of the HCl had been added. The reaction mixture was extracted with EtOAc (3 × 50 mL), and the combined organic extracts were washed with brine (1 × 50 mL), dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo to provide the product 23 as a cream-colored amorphous solid (0.775 g, 99%). The spectroscopic data for compound 23 in CD$_3$OD and DMSO-d_6 matched previously reported data for compound 11 prepared by Hong and co-workers.[35] R_f = 0.0, 4:1 (v/v) CH$_2$Cl$_2$/Et$_2$O; $^{13}C\{^1H\}$ NMR (DMSO-d_6, 100 MHz, referenced to solvent): δ 165.1 (C), 152.4 (C), 150.2 (C), 132.6 (CH), 129.0 (CH), 128.7 (C), 116.2 (CH), 108.9 (C), 28.5 (CH$_2$).

N,N'-DiBoc Clavatadine E (31). To a cooled (0 °C) solution of acid 23 (0.274 g, 1.00 mmol, 1.00 equiv) in 13 mL of anhyd DMF in a 50 mL round-bottom flask equipped with a magnetic stir bar were sequentially added COMU (0.429 g, 1.00 mmol, 1.00 equiv) and freshly distilled i-Pr$_2$NEt (0.350 mL, 2.00 mmol, 2.00 equiv), and the mixture was stirred for 5 min. Then, amine 20[11] (0.330 g, 1.00 mmol, 1.00 equiv) was added, the ice-water bath was maintained in place, and the reaction mixture was stirred with gradual warming to rt. After 26 h, the pale-orange-colored reaction mixture was partitioned between 50 mL of EtOAc and 50 mL of H$_2$O. The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (1 × 20 mL), dried over anhyd Na_2SO_4, filtered, and concentrated in vacuo to provide the crude product (1.068 g) as a pale-orange-colored residue. The crude product was purified by flash column chromatography on 100 g of silica gel using CH$_2$Cl$_2$/Et$_2$O (6:1 to 4:1) as eluent to provide amide 31 as a pale-orange-colored amorphous solid (102 mg, 17%). A smaller-scale reaction using 20 mg of compound 23 also afforded the product (15.1 mg, 35%).

To a cooled (0 °C) solution of amine 20[11] (24.1 mg, 0.0730 mmol, 1.00 equiv) in 3 mL of anhyd DMF in a 10 mL round-bottom flask equipped with a magnetic stir bar were sequentially added 2,2,6,6-tetramethylpiperidine (25 μL, 0.015 mmol, 2.0 equiv), acid 23 (20 mg, 0.073 mmol, 1.0 equiv), and COMU (32 mg, 0.073 mmol, 1.0 equiv). The cooling bath was removed, and the reaction mixture was stirred with gradual warming to rt. After 9.5 h, the pale-orange-colored reaction mixture was partitioned between 10 mL of tert-butyl methyl ether (TBME) and 10 mL of H$_2$O. The layers were separated, and the aqueous phase was extracted with TBME (3 × 5 mL). The combined organic extracts were washed with saturated aqueous NaHCO$_3$ (1 × 5 mL) and brine (1 × 5 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated

in vacuo to provide the crude product (39.7 mg) as a yellow-orange-colored residue. The crude product was purified by flash column chromatography on silica gel using 4:1 CH_2Cl_2/Et_2O as eluent to provide amide **31** as a maroon-colored amorphous solid (4.5 mg, 11%).

To a cooled (0 °C) solution of acid **23** (20 mg, 0.073 mmol, 1.0 equiv) in 1 mL of anhyd DMF in a one-dram vial equipped with a magnetic stir bar were added COMU (64 mg, 0.15 mmol, 2.0 equiv), 2,6-lutidine (17 μL, 0.15 mmol, 2.0 equiv), and the mixture was stirred for 15 min. Then, amine **20**[11] (24 mg, 0.073 mmol, 1.0 equiv) was added in one portion, the ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 24 h, the reaction mixture was partitioned between 3 mL of EtOAc and 3 mL of H_2O. The layers were separated, and the aqueous phase was extracted with EtOAc (3 × 1.5 mL). The combined organic extracts were washed with brine (1 × 1 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (73 mg) as a pale-yellow residue. The crude product was purified by flash column chromatography on 25 g of silica gel using 4:1 CH_2Cl_2/Et_2O as eluent to provide amide **31** as a pale-yellow-colored amorphous solid (25 mg, 58%).

To a cooled (0 °C) suspension of carboxylic acid **23** (20 mg, 0.073 mmol, 1.0 equiv) in 1 mL of anhyd DMF in a one-dram vial equipped with a magnetic stir bar were sequentially added HBTU (55 mg, 0.15 mmol, 2.0 equiv) in one portion and 2,6-lutidine (17 μL, 0.15 mmol, 2.0 equiv) dropwise by syringe, and the resulting mixture was stirred at 0 °C for 5 min. After 5 min, amine **20**[11] (24 mg, 0.073 mmol, 1.0 equiv) was added in one portion, the ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 24 h, the reaction mixture was partitioned between 3 mL of EtOAc and 3 mL of H_2O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 1.5 mL). The combined organic extracts were washed with H_2O (3 × 1 mL) and brine (1 × 1 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (67 mg) as an amber-colored residue. ¹H NMR analysis of the crude mixture revealed a complex mixture of products that did not appear to contain a significant amount of the desired amide **31**, so purification by chromatography was not pursued.

To a cooled (0 °C) solution of carboxylic acid **23** (27.4 mg, 0.100 mmol, 1.00 equiv) and amine **20**[11] (33 mg, 0.10 mmol, 1.0 equiv) in 1 mL of anhyd DMF in a one-dram vial equipped with a magnetic stir bar was added EDC·HCl (28.8 mg, 0.150 mmol, 1.50 equiv), and the resulting mixture was stirred at 0 °C for 1 h. After 1 h, the cooling bath was removed, and the solution was allowed to warm to ambient temperature with stirring. After 22 h, the pale-yellow-colored solution was partitioned between 2.5 mL of Et_2O and 2.5 mL of H_2O. The layers were separated, and the aqueous layer was extracted with Et_2O (3 × 1 mL). The combined organic extracts were washed with H_2O (2 × 1 mL) and brine (1 × 1 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product as a yellow-colored residue. The crude product was purified by flash column chromatography on 25 g of silica gel and 4:1 CH_2Cl_2/Et_2O as eluent to provide amide **31** as a pale-yellow-colored amorphous solid (14 mg, 24%).

To a cooled (0 °C) suspension of carboxylic acid **23** (0.301 g, 1.10 mmol, 1.10 equiv) in 17 mL of anhyd CH_2Cl_2 in a 50

mL round-bottom flask equipped with a magnetic stir bar were sequentially added HOBt (14 mg, 0.10 mmol, 0.10 equiv) and EDC·HCl (0.211 g, 1.10 mmol, 1.10 equiv), and the resulting mixture was stirred at 0 °C for 15 min. After 15 min, amine **20**[11] (0.330 g, 1.00 mmol, 1.00 equiv) was added in one portion, the cooling bath was removed, and the solution was allowed to warm to ambient temperature with stirring. After 19 h, the reaction mixture was diluted with 30 mL of CH_2Cl_2, washed with H_2O (1 × 20 mL) and brine (1 × 20 mL), then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (0.500 g) as a pale-orange-colored residue. The crude residue was purified by flash column chromatography on silica gel and 4:1 CH_2Cl_2/Et_2O as eluent to provide compound **31** as a cream-colored amorphous solid (92 mg, 32%). A smaller-scale reaction using 100 mg of compound **23** also afforded the product (56 mg, 29%).

To a cooled (0 °C) suspension of carboxylic acid **23** (27 mg, 0.10 mmol, 1.0 equiv) and amine **20**[11] (33 mg, 0.10 mmol, 1.0 equiv) in 3 mL of anhyd DMF in a 10 mL round-bottom flask equipped with a magnetic stir bar was added DCC (31 mg, 0.15 mmol, 1.5 equiv). The resulting mixture was stirred at 0 °C for 1 h and then allowed to warm gradually to ambient temperature with stirring. After 22 h, the pale-yellow-colored solution was partitioned between 2.5 mL of Et_2O and 2.5 mL of H_2O. The layers were separated, and aqueous layer was extracted with Et_2O (3 × 1 mL). The combined organic extracts were washed with H_2O (2 × 1 mL) and brine (1 × 1 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (43 mg) as a yellow-colored residue. ¹H NMR analysis of the crude mixture revealed a complex mixture of products that did not appear to contain a significant amount of the desired amide **31**, so purification by chromatography was not pursued.

To a cooled (0 °C) suspension of carboxylic acid **23** (0.274 g, 1.00 mmol, 1.00 equiv), amine **20**[11] (0.330 g, 1.00 mmol, 1.00 equiv), and *N*-hydroxyphthalimide (0.163 g, 1.00 mmol, 1.10 equiv) in 10 mL of anhyd DMF in a 25 mL round-bottom flask equipped with a magnetic stir bar were added DCC (0.206 g, 1.00 mmol, 1.00 equiv) in one portion and Et_3N (0.140 mL, 1.00 mmol, 1.00 equiv) dropwise by syringe. The ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 26 h, the transparent, red-orange-colored suspension was gravity filtered and the reaction flask was rinsed with a small volume of EtOAc. The EtOAc rinse was poured through the white filter cake. The filtrate was partitioned between 50 mL of EtOAc and 50 mL of H_2O. The layers were separated, and then the organic phase was washed with H_2O (2 × 20 mL). The combined aqueous washes were extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (0.752 g). The crude residue was purified by flash column chromatography on silica gel using 4:1 CH_2Cl_2/Et_2O as eluent to provide compound **31** as a cream-colored amorphous solid (0.166 g, 28%). A smaller-scale reaction using 55 mg of compound **23** also afforded the product (30 mg, 26%).

To a cooled (0 °C) suspension of carboxylic acid **23** (0.274 g, 1.00 mmol, 1.0 equiv) and HOBt (0.162 g, 1.00 mmol, 1.00 equiv) in 10 mL of anhyd DMF in a 25 mL round-bottom flask equipped with a magnetic stir bar was added amine **20**[11] (0.330 g, 1.00 mmol, 1.00 equiv) and DCC (0.310 g, 1.50

mmol, 1.50 equiv). The ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 26 h, the orange-brown-colored suspension was gravity filtered and the reaction flask was rinsed with a small volume of EtOAc. The EtOAc suspension was poured through the white filter cake. The filtrate was partitioned between 50 mL of EtOAc and 50 mL of H_2O. The layers were separated, and then the organic phase was washed with H_2O (3 × 20 mL). The combined aqueous washes were extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (1 × 10 mL), dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (0.996 g) as a light-brown-colored residue. The crude residue was purified by flash column chromatography using 100 g of silica gel using CH_2Cl_2/Et_2O (6:1 to 4:1) as eluent to provide compound **31** as a cream-colored amorphous solid (0.431 g, 74%). A smaller-scale reaction using 55 mg of compound **23** also afforded the product (77 mg, 66%). R_f = 0.35, 4:1 (v/v) CH_2Cl_2/Et_2O; 0.68, EtOAc; 0.83, 9:1 (v/v) EtOAc/MeOH; IR (neat) $\tilde{\nu}$ 3325, 3290, 2978, 2934, 1721, 1651, 1616, 1578, 1133, 801 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 11.74 (s, 1H), 11.49 (s, 1H), 10.01 (s, 1H), 8.27 (t, J = 5.2, 1H), 7.98 (t, J = 6.0, 1H), 7.28 (d, J = 1.6, 1H), 7.01 (dd, J = 8.4, 1.6, 1H), 6.83 (d, J = 8.4, 1H), 3.69 (s, 2H), 3.26 (dt, J = 6.0, 5.6, 2H), 3.13 (dt, J = 5.6, 5.6, 2H), 1.47 (s, 9H), 1.44 (m, 4H), 1.39 (s, 9H); $^{13}C\{^1H\}$ NMR (DMSO-d_6, 100 MHz, referenced to solvent): δ 163.12 (C), 163.07 (C), 155.2 (C), 152.3 (C), 152.08 (C), 152.05 (C), 132.7 (CH), 129.1 (CH), 128.8 (C), 116.1 (CH), 108.8 (C), 82.8 (C), 78.1 (C), 40.0 (CH_2), 38.3 (CH_2), 28.0 (CH_3), 27.7 (CH_2), 27.6 (CH_3), 26.4 (CH_2), 26.0 (CH_2); HRMS (TOF MS ES$^+$) *m/z*: [M + H]$^+$ calcd for $C_{24}H_{37}^{79}BrN_5O_7$ 586.1876; found 586.1876.

Clavatadine E Hydrotrifluoroacetate (3·CF₃CO₂H). To a round-bottom flask charged with DiBoc guanidine **31** (431 mg, 0.735 mmol, 1.00 equiv) was added 28 mL of CH_2Cl_2 and 3 mL of TFA. The flask was gently covered with a ground-glass stopper and was stirred at rt for 3 h, 30 min. Then, the reaction mixture was concentrated *in vacuo*. The resulting pale-yellow-colored residue was triturated with 10 mL of anhyd Et_2O, which caused the product to oil out. The supernatant solution was removed, and the resulting pale-yellow-colored oily residue was dried under high vacuum to afford pure clavatadine E hydrotrifluoroacetate (**3·CF₃CO₂H**) as a pale-yellow-colored fluffy solid (278 mg, 98%). A smaller-scale reaction using 73 mg of compound **31** also afforded the product (53.5 mg, 86%). R_f = 0.31, 9:1 (v/v) EtOAc/MeOH; IR (neat) $\tilde{\nu}$ 3350, 3188, 2981, 2939, 2872, 1653, 1625, 1423, 1184, 1135, 1006, 800, 721 cm^{-1}; ^1H NMR ([1 mg/0.75 mL] "dilute" in DMSO-d_6, 400 MHz, referenced to solvent): δ 11.76 (s, 1H), 10.04 (s, 1H), 8.02 (t, J = 6.0, 1H), 7.41 (t, J = 5.6, 1H), 7.28 (d, J = 2.0, 1H), 7.01 (dd, J = 8.4, 2.0, 1H), 6.83 (d, J = 8.4, 1H), 3.69 (s, 2H), 3.13 (dt, J = 7.2, 6.0, 2H), 3.08 (dt, J = 7.2, 5.6, 2H), 1.43 (m, 2H), 1.42 (m, 2H); ^1H NMR ([25 mg/0.75 mL] "concentrated" in DMSO-d_6, 400 MHz, referenced to solvent): δ 11.80 (s, 1H), 10.11 (s, 1H), 8.01 (t, J = 5.6, 1H), 7.63 (apparent br t, 1H), 7.5−6.8 (v br m, 4H), 7.28 (br s, 1H), 7.01 (br d, 1H), 6.83 (d, J = 8.4, 1H), 3.69 (s, 2H), 3.13 (dt, J = 7.2, 5.6, 2H), 3.08 (dt, J = 7.2, 5.2, 2H), 1.43 (m, 2H), 1.42 (m, 2H); $^{13}C\{^1H\}$ NMR (DMSO-d_6, 100 MHz, referenced to solvent, TFA resonances are omitted): δ 163.2 (C), 156.7 (C), 152.4 (C), 151.1 (C), 132.7 (CH), 129.1 (CH), 128.8 (C), 116.1 (CH), 108.8 (C), 40.4 (CH_2), 38.2 (CH_2), 27.7 (CH_2),

26.3 (CH_2), 26.0 (CH_2); HRMS (TOF MS ES$^+$) *m/z*: [M + H]$^+$ calcd for $C_{14}H_{21}^{79}Br_2N_5O_3$ 386.0828; found 386.0826.

***tert*-Butyl 2(*E*)-Hydroxyimino-3-(4-hydroxyphenyl)-propionate (33).** To a cooled (0 °C) suspension of L-tyrosine *tert*-butyl ester (**25**) (4.083 g, 17.21 mmol, 1.00 equiv) in 50 mL of absolute EtOH in a round-bottom flask equipped with a magnetic stir bar were added, in sequence, Na_2WO_4 (5.675 g, 17.21 mmol, 1.00 equiv), 30% $H_2O_2(aq)$ (16.8 mL, 0.165 mol, 9.60 equiv), and H_2O (34 mL), and the reaction mixture was stirred at 0 °C for 45 min. Seconds after H_2O_2 addition, the color of the reaction mixture changed from colorless to bright yellow. After 45 min, the cooling bath was removed, and the mixture was stirred with warming to ambient temperature for an additional 4.5 h. After 4.5 h, the pale-orange-colored solution was extracted with EtOAc (5 × 50 mL), and the combined organic extracts were washed with a 10% aqueous solution of sodium thiosulfate ($Na_2S_2O_3$·5H_2O, 5 × 20 mL) and brine (1 × 75 mL), dried over anhyd $MgSO_4$, filtered, and concentrated *in vacuo* to provide the product as a cream-colored amorphous solid (3.720 g, 86%). The product was judged to be sufficiently pure that additional purification was not required. The spectroscopic data for compound **33** matched previously reported data for compound **8** prepared by Hawkins and co-workers.[10] R_f = 0.34, 4:1 (v/v) CH_2Cl_2/Et_2O; $^{13}C\{^1H\}$ DEPTQ-135 NMR (CD$_3$OD, 100 MHz): δ 164.7 (C), 157.0 (C), 153.6 (C), 131.0 (CH), 128.7 (C), 116.1 (CH), 83.3 (C), 30.3 (CH_2), 28.2 (CH_3).

Methyl 7,9-Dibromo-8-oxo-1-oxa-2-azaspiro[4.5]-deca-2,6,9-triene-3-carboxylate (34). To a cooled (0 °C) solution of phenol **26** (40 mg, 0.19 mmol, 1.00 equiv) in 1 mL of anhyd DMF in a round-bottom flask equipped with a magnetic stir bar was added a solution of *N*-bromosuccinimide (120 mg, 0.67 mmol, 3.5 equiv) in 0.9 mL of anhyd DMF by syringe in one portion. The reaction mixture was stirred with gradual warming to rt over 1 h. Then, the reaction mixture was partitioned between 10 mL of Et_2O and 5 mL of H_2O. The layers were separated, and the aqueous phase was extracted with Et_2O (3 × 2 mL). The combined organic extracts were washed with H_2O (3 × 2 mL), a saturated aqueous solution of $Na_2S_2O_3$·5H_2O (2 × 2 mL), and saturated aqueous sodium chloride (1 × 2 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide dibrominated spirocycle **34** as a cream-colored solid (47.5 mg, 68%). The product was judged to be sufficiently pure that additional purification was not required. The spectroscopic data for compound **34** was consistent with previously reported data for compound **11** (R^1 = Br, R^2 = H, R^3 = CO_2Me) prepared by Forrester and co-workers.[39] R_f = 0.41, CH_2Cl_2.

***tert*-Butyl 7,9-Dibromo-8-oxo-1-oxa-2-azaspiro[4.5]-deca-2,6,9-triene-3-carboxylate (35).** To a cooled (0 °C) solution of phenol **33** (1.005 g, 4.00 mmol, 1.00 equiv) in 12.5 mL of anhyd DMF in a round-bottom flask equipped with a magnetic stir bar was added a solution of *N*-bromosuccinimide (2.313 g. 13.00 mmol, 3.25 equiv) in 12.5 mL of anhyd DMF dropwise by syringe over 15 min. The reaction mixture was stirred at 0 °C for 20 min and then warmed to ambient temperature over 10 min following removal of the reaction flask from the cooling bath. Then, the reaction mixture was partitioned between 50 mL of EtOAc and 50 mL of H_2O. The layers were separated, and the aqueous phase was extracted with EtOAc (9 × 20 mL). The combined organic extracts were washed with H_2O (5 × 30 mL), a saturated aqueous solution of sodium thiosulfate ($Na_2S_2O_3$·5H_2O, 4 × 25 mL), and

saturated aqueous sodium chloride (1 × 20 mL) and then dried over anhyd MgSO$_4$, filtered, and concentrated *in vacuo* to provide the crude product as a dark orange-brown-colored oil. Purification by flash column chromatography on silica gel using CH$_2$Cl$_2$ as eluent provided dibrominated spirocycle **35** as a cream-colored foamy amorphous solid (1.231 g, 76%). The spectroscopic data for compound **35** matched previously reported data for compound **10** prepared by Hawkins and co-workers.[10] R_f = 0.48, CH$_2$Cl$_2$; 0.94, 1:4 (v/v) Et$_2$O/CH$_2$Cl$_2$; ^{13}C{^{1}H} DEPTQ-135 NMR (CDCl$_3$, 100 MHz, referenced to solvent): δ 171.5 (C), 158.3 (C), 152.6 (C), 144.5 (CH), 123.8 (C), 86.1 (C), 84.9 (C), 43.5 (CH$_2$), 28.1 (CH$_2$).

7,9-Dibromo-8-oxo-1-oxa-2-azaspiro[4.5]-deca-2,6,9-triene-3-carboxylic Acid (21). To a solution of *tert*-butyl ester **35** (1.234 g, 3.031 mmol, 1.00 equiv) in 3 mL of anhyd CH$_2$Cl$_2$ was added 1.5 mL of TFA dropwise by syringe. After the solution was stirred at rt for 3.5 h, the yellow-colored, milky suspension was concentrated to dryness under a stream of argon to afford a pale-yellow-colored solid. Trituration of the residue with ice-cold, anhyd Et$_2$O afforded a bright-yellow-colored solution and a suspended fluffy white solid, which was recovered by vacuum filtration. The filter cake was rinsed with a small volume of ice-cold, anhyd Et$_2$O and dried to afford the product as a fluffy white amorphous solid (0.896 g, 84%). The product was judged to be sufficiently pure that additional purification was not required. The spectroscopic data for compound **21** matched previously reported data for compound **6** prepared by Hawkins and co-workers, but the chemical shift of the COOH proton was not included in their data or spectrum.[10,51] R_f = 0.18, 1:4 (v/v) Et$_2$O/CH$_2$Cl$_2$; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 13.86 (br s, 1H), 7.81 (s, 2H), 3.52 (s, 2H); ^{13}C{^{1}H} DEPTQ-135 NMR (DMSO-d_6, 100 MHz, referenced to solvent): δ 171.6 (C), 160.5 (C), 153.6 (C), 146.5 (CH), 121.7 (C), 86.0 (C), 42.8 (CH$_2$).

N,N′-DiBoc Clavatadine C (36). To a cooled (0 °C) suspension of carboxylic acid **21** (0.435 g, 1.239 mmol, 1.10 equiv) in 22 mL of anhyd CH$_2$Cl$_2$ in a 50 mL round-bottom flask equipped with a magnetic stir bar were sequentially added EDC (0.238 g, 1.239 mmol, 1.1 equiv), HOBt (18.3 mg, 0.113 mmol, 0.1 equiv), and the resulting mixture was stirred at 0 °C for 15 min. After 15 min, amine **20**[11] (0.372 g, 1.127 mmol, 1.0 equiv) was added, the cooling bath was removed, and the solution was allowed to warm to ambient temperature with stirring over 12 h. After 12 h, the transparent, dark-amber-colored reaction mixture was diluted with 45 mL of CH$_2$Cl$_2$, washed with 5% HCl(*aq*) (1 × 18 mL), saturated NaHCO$_3$(*aq*) (1 × 18 mL), and brine (1 × 18 mL) and then dried over anhyd Na$_2$SO$_4$, filtered, and concentrated *in vacuo* to provide the crude product (0.779 g) as a brown-colored solid. The crude residue was dissolved in CH$_2$Cl$_2$, adsorbed onto 15 g of silica gel, and then purified by flash column chromatography on silica gel using 3:2 EtOAc/hexanes as eluent to provide compound **36** as a cream-colored amorphous solid (0.523 g, 70%). A smaller-scale reaction using 50 mg of compound **21** also afforded the product (61.4 mg, 71%). The spectroscopic data for compound **36** matched previously reported data for compound **12** prepared by Hawkins and co-workers.[10] R_f = 0.36, 1:1 (v/v) hexanes/EtOAc; 0.83, 9:1 (v/v) EtOAc/MeOH; IR (neat) $\tilde{v}$ 3295, 2973, 2866, 1720, 1684, 1654, 1608, 1130, 1053, 663 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz): δ 11.49 (br s, 1H), 8.36 (t, *J* = 5.0, 1H), 7.32 (s, 2H), 6.80 (t, *J* = 5.8, 1H), 3.49 (s, 2H), 3.45 (m, 2H), 3.42 (m, 2H), 1.65 (m, 4H), 1.50 (s, 18H); ^{13}C{^{1}H}

DEPTQ-135 NMR (CDCl$_3$, 100 MHz, referenced to solvent): δ 171.5 (C), 163.7 (C), 158.3 (C), 156.4 (C), 154.0 (C), 153.5 (C), 144.5 (CH), 123.8 (C), 86.0 (C), 83.4 (C), 79.5 (C), 43.3 (CH$_2$), 40.3 (CH$_2$), 39.4 (CH$_2$), 28.4 (CH$_3$), 28.2 (CH$_3$), 26.70 (CH$_2$), 26.68 (CH$_2$); HRMS (TOF MS ES$^+$) *m/z*: [M + H]$^+$ calcd for C$_{24}$H$_{34}$^{79}Br$_2$N$_5$O$_7$ 662.0825; found 662.0802.

N,N′-DiBoc Clavatadine D (37). To a cooled (0 °C) suspension of carboxylic acid **21** (21.5 mg, 0.0613 mmol, 1.00 equiv) in 1 mL of anhyd DMF in a one-dram vial equipped with a magnetic stir bar was added EDC·HCl (17.6 mg, 0.092 mmol, 1.50 equiv), and the resulting mixture was stirred at 0 °C for 15 min. After 15 min, amine **22**[52] (21.1 mg, 0.0613 mmol, 1.00 equiv) was added in one portion, the cooling bath was removed, and the solution was allowed to warm to ambient temperature with stirring. After 23 h, the reddish-brown-colored solution was partitioned between 2.5 mL of Et$_2$O and 2.5 mL of H$_2$O. The layers were separated, and aqueous layer was extracted with Et$_2$O (3 × 1 mL). The combined organic extracts were washed with 5% HCl(*aq*) (1 × 1 mL), saturated NaHCO$_3$(*aq*) (1 × 1 mL), and brine (1 × 1 mL) and then dried over anhyd Na$_2$SO$_4$, filtered, and concentrated *in vacuo* to provide the crude product (13 mg) as a brown-colored residue. The crude residue was dissolved in EtOAc, adsorbed onto 1 g of silica gel, and then purified by flash column chromatography using 25 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound **37** as a cream-colored amorphous solid (8 mg, 19%).

To a cooled (0 °C) suspension of carboxylic acid **21** (0.435 g, 1.239 mmol, 1.10 equiv) in 22 mL of anhyd CH$_2$Cl$_2$ in a 50 mL round-bottom flask equipped with a magnetic stir bar were sequentially added EDC·HCl (0.238 g, 1.239 mmol, 1.1 equiv) and HOBt (18.3 mg, 0.113 mmol, 0.1 equiv), and the resulting mixture was stirred at 0 °C for 15 min. After 15 min, amine **22**[52] (0.388 g, 1.127 mmol, 1.0 equiv) was added in one portion, the cooling bath was removed, and the solution was allowed to warm to ambient temperature with stirring over 12 h. After 12 h, the transparent, dark-amber-colored reaction mixture was diluted with 45 mL of CH$_2$Cl$_2$, washed with 5% HCl(*aq*) (1 × 18 mL), saturated NaHCO$_3$(*aq*) (1 × 18 mL), and brine (1 × 18 mL) and then dried over anhyd Na$_2$SO$_4$, filtered, and concentrated *in vacuo* to provide the crude product (0.728 g) as a brown-colored residue. The crude residue was dissolved in EtOAc, adsorbed onto 10 g of silica gel, and then purified by flash column chromatography using 150 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound **37** as a cream-colored amorphous solid (0.233 g, 31%). A smaller-scale reaction using 78 mg of compound **21** also afforded the product (26 mg, 19%).

To a cooled (0 °C) suspension of carboxylic acid **21** (0.351 g, 1.00 mmol, 1.00 equiv) in 20 mL of anhyd DMF in a 50 mL round-bottom flask equipped with a magnetic stir bar were sequentially added COMU (0.470 g, 1.10 mmol, 1.10 equiv) in one portion and *i*-Pr$_2$NEt (0.350 mL, 2.00 mmol, 2.00 equiv) dropwise by syringe, and the resulting mixture was stirred at 0 °C for 15 min. After 15 min, amine **22**[52] (0.344 g, 1.00 mmol, 1.00 equiv) was added in one portion, the ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 26 h, the transparent, dark-amber-colored reaction mixture was partitioned between 50 mL of EtOAc and 50 mL of H$_2$O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic

extracts were washed with H_2O (3 × 20 mL), 5% HCl(aq) (1 × 20 mL), saturated $NaHCO_3$(aq) (1 × 20 mL), and brine (1 × 20 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (0.772 g) as a brown-colored residue. The crude residue was dissolved in EtOAc, adsorbed onto 10 g of silica gel, and then purified by flash column chromatography using 250 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound **37** as a cream-colored amorphous solid (0.345 g, 51%). A smaller-scale reaction using 35 mg of compound **21** and 2.0 molar equivalents of COMU also afforded the product (52 mg, 76%).

To a cooled (0 °C) suspension of carboxylic acid **21** (35 mg, 0.10 mmol, 1.0 equiv) in 2 mL of anhyd DMF in a one-dram vial equipped with a magnetic stir bar were sequentially added HBTU (76 mg, 0.20 mmol, 2.0 equiv) in one portion and *i*-Pr_2NEt (35 μL, 0.20 mmol, 2.0 equiv) dropwise by syringe, and the resulting mixture was stirred at 0 °C for 15 min. After 15 min, amine **22**[52] (34 mg, 0.10 mmol, 1.0 equiv) was added in one portion, the ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 25 h, the transparent, dark red-purple-colored reaction mixture was partitioned between 5 mL of EtOAc and 5 mL of H_2O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 2 mL). The combined organic extracts were washed with H_2O (3 × 2 mL), 5% HCl(aq) (1 × 2 mL), saturated $NaHCO_3$(aq) (1 × 2 mL), and brine (1 × 2 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (69 mg) as a brown-colored residue. The crude residue was dissolved in EtOAc, adsorbed onto 1.5 g of silica gel, and then purified by flash column chromatography using 40 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound **37** as a cream-colored amorphous solid (20 mg, 29%).

To a cooled (0 °C) suspension of carboxylic acid **21** (21.5 mg, 0.0613 mmol, 1.00 equiv) in 1 mL of DMF in a one-dram vial equipped with a magnetic stir bar was added DCC (19 mg, 0.092 mmol, 1.50 equiv), and the resulting mixture was stirred at 0 °C for 15 min. After 15 min, amine **22**[52] (21.1 mg, 0.0613 mmol, 1.00 equiv) was added in one portion, the cooling bath was removed, and the solution was allowed to warm to ambient temperature with stirring. After 23 h, the reddish-brown-colored solution was partitioned between 2.5 mL of Et_2O and 2.5 mL of H_2O. The layers were separated, and the aqueous layer was extracted with Et_2O (3 × 1 mL). The combined organic extracts were washed with 5% HCl(aq) (1 × 1 mL), saturated $NaHCO_3$(aq) (1 × 1 mL), and brine (1 × 1 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (37 mg) as an orange-red-colored residue. The crude residue was dissolved in EtOAc, adsorbed onto 1 g of silica gel, and then purified by flash column chromatography using 25 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound **37** as a cream-colored amorphous solid (12 mg, 29%).

To a cooled (0 °C) suspension of carboxylic acid **21** (0.116 g, 0.339 mmol, 1.10 equiv), amine **22**[52] (0.114 g, 0.330 mmol, 1.10 equiv), and ethyl (hydroxyimino)cyanoacetate [also known as Oxyma] (47 mg, 0.33 mmol, 1.1 equiv) in 3 mL of DMF in a 10 mL round-bottom flask equipped with a magnetic stir bar was added DCC (62 mg, 0.30 mmol, 1.0 equiv). The ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. Within 2 min, the reaction mixture turned from

yellow to orange and precipitate was visible. After 25 h, the transparent, orange-colored reaction mixture was partitioned between 5 mL of EtOAc and 5 mL of H_2O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 2 mL). The combined organic extracts were washed with H_2O (3 × 2 mL), 5% HCl(aq) (1 × 2 mL), saturated $NaHCO_3$(aq) (1 × 2 mL), and brine (1 × 2 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (0.239 g) as an orange-brown-colored residue. The crude residue was dissolved in EtOAc, adsorbed onto 4 g of silica gel, and then purified by flash column chromatography using 100 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound **37** as a cream-colored amorphous solid (0.122 g, 60%). A smaller-scale reaction using 35.1 mg of compound **21** also afforded the product (51 mg, 75%).

To a cooled (0 °C) suspension of carboxylic acid **21** (0.351 g, 1.00 mmol, 1.10 equiv), amine **22**[52] (0.344 g, 1.00 mmol, 1.10 equiv), and HOBt (0.162 g, 1.00 mmol, 1.10 equiv) in 9 mL of anhyd DMF in a 25 mL round-bottom flask equipped with a magnetic stir bar was added DCC (0.188 g, 0.910 mmol, 1.00 equiv). The ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 26 h, the orange-brown-colored suspension was gravity filtered and the reaction flask was rinsed with a small volume of EtOAc. The EtOAc suspension was poured through the white filter cake. The filtrate was partitioned between 50 mL of EtOAc and 50 mL of H_2O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with H_2O (3 × 20 mL), 5% HCl(aq) (1 × 20 mL), saturated $NaHCO_3$(aq) (1 × 20 mL), and brine (1 × 20 mL) and then dried over anhyd Na_2SO_4, filtered, and concentrated *in vacuo* to provide the crude product (0.602 g) as a light-brown-colored residue. The crude residue was dissolved in EtOAc, adsorbed onto 10 g of silica gel, and then purified by flash column chromatography using 250 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound **37** as a cream-colored amorphous solid (0.349 g, 57%). R_f = 0.53, 1:1 (v/v) EtOAc/hexanes; 0.28, 2:3 (v/v) EtOAc/hexanes; 0.84, 9:1 (v/v) EtOAc/MeOH; IR (neat) $\tilde{v}$ 3325, 2977, 2860, 1718, 1683, 1652, 1608, 1508, 1132, 1052, 696 cm^{-1}; ^1H NMR (CDCl$_3$, 400 MHz): δ 11.50 (br s, 1H), 8.32 (t, J = 4.4, 1H), 7.32 (s, 2H), 6.63 (t, J = 5.6, 1H), 3.49 (s, 2H), 3.43 (dt, J = 7.2, 5.6, 2H), 3.38 (dt, J = 6.8, 4.4, 2H), 1.67−1.57 (m, 4H), 1.51 (s, 9H), 1.50 (s, 9H), 1.48−1.39 (m, 2H); $^{13}C\{^1H\}$ NMR (CDCl$_3$, 100 MHz, referenced to solvent): δ 171.5 (C), 163.7 (C), 158.2 (C), 156.3 (C), 154.0 (C), 153.5 (C), 144.5 (CH), 123.8 (C), 86.0 (C), 83.3 (C), 79.5 (C), 43.3 (CH_2), 40.7 (CH_2), 39.6 (CH_2), 29.1 (CH_2), 28.8 (CH_2), 28.5 (CH_3), 28.2 (CH_3), 24.2 (CH_2); HRMS (TOF MS ES$^+$) m/z: [M + H]$^+$ calcd for $C_{25}H_{36}{}^{79}Br_2N_5O_7$ 676.0981; found 676.0993.

Clavatadine C Hydrotrifluoroacetate ($3\cdot CF_3CO_2H$). To a scintillation vial charged with DiBoc guanidine **36** (0.374 g, 0.564 mmol, 1.00 equiv) was added 8 mL of CH_2Cl_2 and 1.5 mL of TFA.[9] The vial was gently covered with its cap and was stirred at rt for 3 h, 40 min. Then, the reaction mixture was concentrated to dryness using a stream of dry argon, and the resulting green-black-colored residue was triturated with 6 mL of Et_2O. The supernatant solution was removed, and the resulting powder was dried under high vacuum to afford pure clavatadine C hydrotrifluoroacetate ($3\cdot CF_3CO_2H$) as a tan-

colored amorphous solid (0.267 g, 82%). Bumping occurred upon careful exposure to vacuum and caused some product loss into the vacuum line. A smaller-scale reaction using 45.6 mg of compound **36** also afforded the product (37.9 mg, 95%). R_f = 0.31, 9:1 (v/v) EtOAc/MeOH; IR (neat) $\tilde{\nu}$ 3447, 3306, 2924, 2853, 1782, 1673, 1651, 1196, 1141, 707 cm^{-1}; ^{1}H NMR ([2 mg/0.75 mL] "dilute" in DMSO-d_6, 400 MHz, referenced to solvent): δ 8.66 (t, J = 5.6, 1H), 7.80 (s, 2H), 7.46 (t, J = 5.6, 1H), 7.39−6.54 (v br d, 4H), 3.55 (s, 2H), 3.18 (dt, J = 6.0, 5.6, 2H), 3.10 (dt, J = 6.0, 5.6, 2H), 1.54−1.42 (m, 4H); ^{1}H NMR ([30 mg/0.75 mL] "concentrated" in DMSO-d_6, 400 MHz, referenced to solvent): δ 8.65 (t, J = 5.6, 1H), 7.80 (s, 2H), 7.64 (t, J = 5.6, 1H), 7.6−6.7 (v br d, 4H), 3.55 (s, 2H), 3.18 (dt, J = 6.0, 5.6, 2H), 3.10 (dt, J = 6.0, 5.6, 2H), 1.54−1.42 (m, 4H); ^{13}C{^{1}H} NMR (DMSO-d_6, 100 MHz, referenced to solvent, TFA resonances are omitted): δ 171.6 (C), 158.2 (C), 156.7 (C), 155.0 (C), 146.7 (CH), 121.6 (C), 85.2 (C), 43.2 (CH$_2$), 40.4 (CH$_2$), 38.3 (CH$_2$), 26.0 (CH$_2$), 25.9 (CH$_2$); HRMS (TOF MS ES$^+$) m/z: [M + H]$^+$ calcd for C$_{14}$H$_{18}$^{79}Br$_2$N$_5$O$_3$ 461.9776; found 461.9781.

Clavatadine D Hydrotrifluoroacetate (4·CF$_3$CO$_2$H). To a scintillation vial charged with DiBoc guanidine **37** (0.180 g, 0.266 mmol, 1.00 equiv) and a magnetic stir bar was added 4 mL of CH$_2$Cl$_2$ and 1.5 mL of TFA.[9] The vial was gently covered with its cap and was stirred at rt for 3 h, 40 min. Then, the reaction mixture was concentrated to dryness using a stream of dry argon, and the resulting orange-black-colored residue was triturated with 4 mL of Et$_2$O. Removal of the supernatant solution afforded pure clavatadine D hydro-trifluoroacetate (**4·CF$_3$CO$_2$H**) as a tan-colored amorphous solid (0.149 g, 95%). R_f = 0.25, 9:1 (v/v) EtOAc/MeOH; IR (neat) $\tilde{\nu}$ 3306, 3184, 2942, 2865, 1682, 1651, 1610, 1550, 1202, 1187, 1132, 801, 722 cm^{-1}; ^{1}H NMR ([2 mg/0.75 mL] "dilute" in DMSO-d_6, 400 MHz, referenced to solvent): δ 8.61 (t, J = 6.0, 1H), 7.80 (s, 2H), 7.39 (br t, 1H), 7.3−6.6 (v br d, 4H), 3.55 (s, 2H), 3.16 (dt, J = 7.2, 6.0, 2H), 3.08 (dt, J = 7.6, 6.4, 2H), 1.53−1.43 (m, 4H), 1.33−1.23 (m, 2H); ^{1}H NMR ([15 mg/0.75 mL] "concentrated" in DMSO-d_6, 400 MHz, referenced to solvent): δ 8.61 (t, J = 6.0, 1H), 7.80 (s, 2H), 7.58 (t, J = 5.6, 1H), 7.5−6.7 (v br d, 4H), 3.55 (s, 2H), 3.16 (dt, J = 6.8, 6.4, 2H), 3.08 (dt, J = 6.8, 6.0, 2H), 1.53−1.43 (m, 4H), 1.33-1.23 (m, 2H); ^{13}C{^{1}H} NMR (DMSO-d_6, 100 MHz, referenced to solvent, TFA resonances are omitted): δ 171.6 (C), 158.1 (C), 156.7 (C), 155.0 (C), 146.7 (CH), 121.6 (C), 85.1 (C), 43.2 (CH$_2$), 40.7 (CH$_2$), 38.6 (CH$_2$), 28.4 (CH$_2$), 28.1 (CH$_2$), 23.4 (CH$_2$); HRMS (TOF MS ES$^+$) m/z: [M + H]$^+$ calcd for C$_{15}$H$_{20}$^{79}Br$_2$N$_5$O$_3$ 475.9933; found 475.9931.

■ ASSOCIATED CONTENT

Ⓢ **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02913.

> Comparison of the NMR data of natural and synthetic clavatadine C (**3**), clavatadine D (**4**), and clavatadine E (**5**); NCI-60 one-dose screening data on compounds **3**−**5**; copies of ^{1}H and ^{13}C 1D-NMR spectra for new compounds and known compounds prepared using new or modified procedures, including 2D NMR data for synthetic clavatadine C (**3**), clavatadine D (**4**), clavatadine E (**5**), and their direct precursors; and copies of ^{1}H NMR spectra of N,N-diBoc clavatadine C

(**36**) at various concentrations using treated and untreated CDCl$_3$ (PDF)

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