Total Synthesis and Biological Evaluation of Clavatadines C–E
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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. 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. 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). Clavatadine A (1), B (2), and C (3) were recently prepared by total synthesis using a convergent, early-stage guanidinylation approach. 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), aplysaminamide II (7), and aerophobin 1 (8) (Chart 2). Notably, purealidin L (6) and aplysaminamide II (7) feature C-4 and C-5 linear

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) and pseudoceratinamides A (11) 
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)

Scheme 1. Retrosynthetic Analysis of Clavatadines C–E

and B (12)\(^\text{13}\) is the mirror image isomer of most members of this compound class (Chart 2).\(^\text{2,3,5}\) A few steps further back on the proposed biogenetic pathway\(^\text{13}\) of this natural product class lie mono- and dibrominated phenol-containing oxime derivatives such as psammaplin A (13),\(^\text{14}\) compound 14,\(^\text{15}\) which was found in Oceanapia sp. but unnamed, purealidin O (15, \(R = CH_3, X = OH\)),\(^\text{16}\) JBIR-44 (16, \(R = H; X = Br\)),\(^\text{17}\) purpuramine L (17, \(R = CH_2CH_2CH_2NH_2; X = H\)),\(^\text{18}\) alysamine 4 (18, \(R = CH_2CH_2CH_2NH_2; X = Br\)),\(^\text{19}\) and purealidin A (19).\(^\text{20}\) (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.\(^\text{2,5}\) Unlike clavatadine A (1) and B (2), which were found to be potent (IC\(_{50} = 1.3\) and 27 \(\mu M\), respectively) and selective inhibitors of human blood coagulation factor Xla (FXIa), clavatadines C (3), D (4), and E (5) bind FXIa only weakly (17, 30, and 37% inhibition at 222 \(\mu M\), respectively).\(^\text{6}\) 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 \textit{in vitro}.\(^\text{8,21}\) 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 (\textit{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
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 amino guanidine-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 group may cause homodimerization to occur between two molecules of compound 23. In contrast, to prepare 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 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...
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 amino-guanidine 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 20 equivalents of acid chlorides or under forcing conditions (N,N-dimethylformamide (DMF), 130 °C, 30 min), 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). 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 newer reagents such as HBTU and COMU, which often require a tertiary amine base such as Hünig's base (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, but not N-hydroxysuccinimide/Et3N (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) as its hydrofluoroacetate salt. 1D and 2D NMR spectra of unpurified clavatadine E (5) in DMSO-d6 matched the data reported by Quinn and co-workers (see Table S3 for comparison).

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 spirooxazoline scaffold present in both clavatadine C and D. 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 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. 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).

With access to compound 21, peptide coupling of this carboxylic acid to C-4 aminoguanidine 20 or C-5 aminogu...
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 and co-workers and confirmed both the synthesis, data, and spectra published by Hawkins 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 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).

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.

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 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. 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 and co-workers and in the present study. Notable differences in 1H chemical shift occurred in the region between δH 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. 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 sieves to prepare clavatadine C

Table 3. Efforts to Optimize Amide Formation to Prepare Compound 37

| entry | coupling agent | additive | base | yield (%) |
|-------|----------------|----------|------|-----------|
| 1     | EDC            | none     | none | 19        |
| 2     | EDC           | HOBt     | none | 19−31     |
| 3     | COMU           | i-Pr2NEt | none | 76−51     |
| 4     | HBTU           | i-Pr2NEt | none | 29        |
| 5     | DCC            | none     | none | 29        |
| 6     | DCC            | oxyma    | none | 75−60     |
| 7     | DCC            | HOBt     | none | 57        |

*Isolated yield after chromatography. Small scale (≤0.1 mmol). Large scale (≥1 mmol). 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).

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

| cell type / compound | CCRF-CEM (leukemia) | K-262 (leukemia) | A-549 (lung) | A-431 (lung) | HCT-15 (colon) | SF-268 (CNS) | SNB-75 (CNS) | OVCA3-3 (ovarian) | MCF-7 (breast) | MDA-MB-468 (breast) |
|----------------------|---------------------|-----------------|--------------|--------------|---------------|--------------|-------------|-------------------|---------------|-------------------|
| Clavatadine C (3)    | 96                  | 95              | 110          | 87           | 105           | 95           | 107         | 103               | 98            | 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               |

*Numbers reflect mean growth percent. 100 percent indicates no effect. Ref 10 reported 39 ± 7% cell viability after 24 h upon exposure of this cell line to compound 3 [10 μM]. 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. Organic synthesis laboratories who prepare acid-labile compounds frequently pretreat their CDCl₃ in this way. It is therefore possible that the use of untreated CDCl₃ 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. These effects are often attributable to differences in how molecules fold or aggregate with changes in concentration.

Slight concentration-dependent variation in ¹H 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 (δH 10.04 vs 10.11 ppm) and oxime (δH 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 δH 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.³⁶

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” CDCl₃ for NMR analysis. Furthermore, characterization by ¹H 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 ¹³C-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 CDCl₃ used to dissolve synthetic compound 36 may have on sample integrity, such as compound decomposition, ¹H NMR spectra were acquired on samples of varying concentration using treated or untreated CDCl₃ 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 line splitting in ¹H 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 CDCl₃, treatment or nontreatment of the solvent did not affect the chemical shift of proton signals. This observation suggests that CDCl₃ 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 ¹³C-NMR chemical shifts do not change more than 0.1 or 0.2 ppm regardless of concentration or the condition of the CDCl₃ 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-workers⁶ for the corresponding natural compounds (see Tables S1–S3 for comparison). Oximation and dibromopirocylazation 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 lines¹⁴ 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 amidocoupling 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.** ¹H and proton-decoupled ¹³C(¹H) 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.
NMR data are referenced to residual internal CD<sub>3</sub>COCD<sub>2</sub>D and residual internal (CD<sub>3</sub>)<sub>2</sub>SO at δ 39.50 (13C), respectively. For spectra calibrated using acetone-D<sub>6</sub>, 1H- and 13C-NMR data are referenced to residual internal CD<sub>3</sub>SOCD<sub>2</sub>D at δ 2.50 (1H) and residual internal (CD<sub>3</sub>)<sub>2</sub>CO at δ 29.84 (13C), respectively. For spectra calibrated using acetone-D<sub>6</sub>, 1H- and 13C-NMR data are referenced to residual internal CD<sub>3</sub>COCD<sub>2</sub>H at δ 2.05 (1H) and residual internal (CD<sub>3</sub>)<sub>2</sub>CO at δ 29.84 (13C), respectively. For spectra calibrated using acetone-D<sub>6</sub>, 1H- and 13C-NMR data are referenced to residual internal CD<sub>3</sub>COCD<sub>2</sub>H at δ 2.05 (1H) and residual internal (CD<sub>3</sub>)<sub>2</sub>CO at δ 29.84 (13C), respectively. 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<sub>3</sub> or DMSO-d<sub>6</sub> 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 (ESI<sup>+</sup>). 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<sub>2</sub>) (40–63 μm, 230–400 mesh). Analytical thin-layer chromatography (TLC) was performed using 0.25 mm silica gel 60 (F<sub>254</sub>) 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<sub>2</sub>Cl<sub>2</sub>, tetrahydrofuran (THF), DMF, diethyl ether (Et<sub>2</sub>O), and triethylamine (Et<sub>3</sub>N) were obtained by passing the previously degassed solvents through an activated alumina column under argon. Hüning’s base (i-Pr<sub>2</sub>NEt) was dried over activated 4 Å molecular sieves and distilled under argon. CH<sub>3</sub>CN was partially dried using a threefold treatment with activated 3 Å molecular sieves. 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<sub>2</sub>O; thus, in reactions involving HOBt, 120% of the required amount was weighed. Caution! Some peptide coupling reagents, including dicyclohexylcarbo-
diimide (DCD, CAS number 538-75-0), N,N,N’,N’-tetrameth- 
yl-O-(1H-benzo triazol-1-yl)uronium hexafluorophosphate 
(HBTU, CAS number 94790-37-1), and possibly (1-cyano-2-
ethoxy-2-oxoethylidenaminoxy)dimethylamino-morpholino-
carbenium hexafluorophosphate (COMU, CAS number 
1075198-30-9) are known or suspected immune sensitizers 
and may cause anaphylaxis.26,46,49

(E)-Methyl 2-Hydroxyimino-3-(4-hydroxyphenyl)- 
propionate (26). To a cooled (0 °C) suspension of 
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, Na2WO4 
(5.675 g, 17.21 mmol, 1.00 equiv), 30% H2O2 (aq) (16.8 mL, 
165 mmol, 96.00 equiv), and H2O (34 mL), and the reaction 
mixture was stirred at 0 °C for 45 min. Seconds after H2O2 
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 (Na2S2O3 
aq) (26) (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 Na2SO4, filtered, and 
concentrated in vacuo to provide the product as a cream-colored 
amorphous solid (0.775 g, 86%). The spectroscopic data for compound 26 
matched previously reported data for compound prepared by de Silva and Andersen.24

(E)-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) (22) (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 Na2SO4, filtered, and 
concentrated in vacuo to provide the product as a cream-colored 
amorphous solid (0.775 g, 99%). The spectroscopic data for compound 23 in CD2OD and DMSO-d6 matched previously 
reported data for compound prepared by Hong and co-workers.26 Rf = 0.0, 4:1 (v/v) CH3Cl/EtO2; 13C{1H} 
NMR (DMSO-d6, 100 MHz, referenced to solvent): δ 165.1 
(C), 152.4 (C), 150.2 (C), 132.6 (C), 129.0 (CH), 128.7 
(C), 116.2 (CH), 108.9 (C), 28.5 (CH3).

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-Pr2NET (0.350 
ml, 2.00 mmol, 2.00 equiv), and the mixture was stirred for 5 
min. Then, amine 20 (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 H2O. 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 Na2SO4, 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 CH2Cl2/EtO2 (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 (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 H2O. The layers were 
separated, and the aqueous phase was extracted with TBME (3 
× 5 mL). The combined organic extracts were washed with 
saturated aqueous NaHCO3 (1 × 5 mL) and brine (1 × 5 mL) and then dried over anhyd Na2SO4, 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 CH₂Cl₂/Et₂O 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 sequentially 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 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 H₂O. The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 1.5 mL). The combined organic extracts were washed with brine (1 × 1 mL) and then dried over anhyd Na₂SO₄, 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 CH₂Cl₂/Et₂O 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 2011 (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 H₂O. The layers were separated, and then 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 Na₂SO₄, filtered, and concentrated in vacuo to provide amide 31 as a pale-yellow-colored amorphous solid (25 mg, 58%).

To a cooled (0 °C) suspension of carboxylic acid 23 (27.4 mg, 0.100 mmol, 1.0 equiv) and amine 2011 (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 Et₂O and 2.5 mL of H₂O. The layers were separated, and the aqueous layer was extracted with Et₂O (3 × 1 mL). The combined organic extracts were washed with H₂O (2 × 1 mL) and then dried over anhyd Na₂SO₄, 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, 1.0 equiv) and amine 2011 (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 Et₂O and 2.5 mL of H₂O. The layers were separated, and then the aqueous layer was extracted with Et₂O (3 × 1 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried over anhyd Na₂SO₄, filtered, and concentrated in vacuo to provide the crude product (43 mg) as a yellow-colored residue. The crude residue was purified by flash column chromatography on silica gel using CH₂Cl₂/Et₂O 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 2011 (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 Et₂O and 2.5 mL of H₂O. The layers were separated, and then the aqueous layer was extracted with Et₂O (3 × 1 mL). The combined organic extracts were washed with H₂O (2 × 1 mL) and then dried over anhyd Na₂SO₄, filtered, and concentrated in vacuo to provide the crude product (43 mg) as a yellow-colored residue. The crude residue was purified by flash column chromatography on silica gel using 4:1 CH₂Cl₂/Et₂O as eluent to provide compound 31 as a cream-colored amorphous solid (56 mg, 29%).

To a cooled (0 °C) suspension of carboxylic acid 23 (27 mg, 0.10 mmol, 1.0 equiv) and amine 2011 (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 Et₂O and 2.5 mL of H₂O. The layers were separated, and then the organic layer was extracted with Et₂O (3 × 1 mL). The combined organic extracts were washed with H₂O (2 × 1 mL) and then dried over anhyd Na₂SO₄, filtered, and concentrated in vacuo to provide the crude product (43 mg) as a yellow-colored residue. The crude residue was purified by flash column chromatography on silica gel using 4:1 CH₂Cl₂/Et₂O 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 (27.4 mg, 0.100 mmol, 1.0 equiv) and amine 2011 (0.330 g, 1.00 mmol, 1.0 equiv) and HOBt (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 2011 (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 CH₂Cl₂, washed with H₂O (1 × 20 mL) and brine (1 × 20 mL), then dried over anhyd Na₂SO₄, 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 CH₂Cl₂/Et₂O as eluent to provide compound 31 as a cream-colored amorphous solid (92 mg, 32%). The smaller-scale reaction using 100 mg of compound 23 also afforded the product (56 mg, 29%).
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 orangebrown-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 H2O. The layers were separated, and then the organic phase was washed with H2O (3 × 20 mL). The combined aqueous washes were exchanged with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (1 × 10 mL), dried over anhyd Na2SO4, and concentrated in vacuo to provide a 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 CH2Cl2/EtO (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%). Rf = 0.35, 4:1 (v/v) CH2Cl2/EtO; 0.68, EtOAc; 0.83, 9:1 (v/v) EtOAc/MeOH; IR (neat) = 3325, 3290, 2978, 2934, 1721, 1651, 1616, 1578, 1133, 801 cm−1; 1H NMR (DMSO-d6, 400 MHz, referenced to solvent): 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), 1.27 (s, 9H); HRMS (TOF MS ES+) m/z: [M + H]+ calculated for C32H37BrN2O5, 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 CH2Cl2 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 EtOAc, which caused the product to oil out. The supernatant solution 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 (Na2S2O3·5H2O, 5 × 20 mL) and brine (1 × 75 mL), dried over anhyd MgSO4, and concentrated in vacuo to provide the crude product (77 mg, 66%).

tert-Butyl 2(E)-Hydroxyimino-3-(4-hydroxyphenyl)-propionate (33). To a cooled (0 °C) suspension of l-tyrosine tert-butyl ester (2S) (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, Na2WO4 (5.675 g, 17.21 mmol, 1.00 equiv), 30% H2O2 (16.8 mL, 0.165 mol, 9.60 equiv), and H2O (34 mL), and the reaction mixture was stirred at 0 °C for 45 min. Seconds after H2O2 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 (Na2S2O3·5H2O, 5 × 20 mL) and brine (1 × 75 mL), dried over anhyd MgSO4, and concentrated in vacuo to provide the 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 Rf = 0.34, 4:1 (v/v) CH2Cl2/EtO; 5% 1H DEPTQ-135 NMR (CD3OD, 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 (CH2), 28.2 (CH3).

Methyl 7,9-Dibromo-8-oxo-1-oxa-2-azaspiro[4.5]-deca-2,6,9-triene-3-carboxylic acid (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 for 1 h. Then, the reaction mixture was partitioned between 10 mL of EtO and 5 mL of H2O. The layers were separated, and the aqueous phase was washed with EtO (3 × 2 mL). The combined organic extracts were washed with H2O (3 × 2 mL), a saturated aqueous solution of Na2S2O3·5H2O (2 × 2 mL), and an aqueous sodium chloride (1 × 2 mL) and then dried over anhyd Na2SO4, 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 (R1 = Br, R2 = H, R3 = CO2Me) prepared by Forrester and co-workers.9 Rf = 0.41, CH2Cl2.

tert-Butyl 7,9-Dibromo-8-oxo-1-oxa-2-azaspiro[4.5]-deca-2,6,9-triene-3-carboxylic acid (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 H2O. The layers were separated, and the aqueous phase was washed with EtOAc (9 × 20 mL). The combined organic extracts were washed with H2O (5 × 30 mL), a saturated aqueous solution of sodium thiosulfate (Na2S2O3·5H2O, 4 × 25 mL), and
saturated aqueous sodium chloride (1 × 20 mL) and then dried over anhyd MgSO₄, 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 CH₂Cl₂ 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 = 0.48, CH₂Cl₂; 0.94, 1:4 (v/v) EtO/CH₂Cl₂; ¹³C{¹H} DEPTQ-135 NMR (CDCl₃, 100 MHz, referenced to solvent): δ 171.5 (C), 158.3 (C), 156.4 (C), 154.0 (C), 153.5 (C), 144.5 (C), 123.8 (C), 86.0 (C), 83.4 (C), 79.5 (C), 43.3 (CH₂), 40.3 (CH₂), 39.4 (CH₂), 28.4 (CH₂), 28.2 (CH₂), 26.70 (CH₃), 26.68 (CH₃); HRMS (TOF MS ES⁺) m/z: [M + H]⁺ calcd for C₂₂H₃₄Br₂N₅O₇ 662.0825; found 662.0820.

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 (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 Et₂O and 2.5 mL of H₂O. The layers were separated, and aqueous layer was extracted with Et₂O (3 × 1 mL). The combined organic extracts were washed with 5% HCl(aq) (1 × 1 mL), saturated NaHCO₃(aq) (1 × 1 mL), and brine (1 × 1 mL) and then dried over anhyd Na₂SO₄ 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 CH₂Cl₂ 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 20 (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 CH₂Cl₂, washed with 5% HCl(aq) (1 × 18 mL), saturated NaHCO₃(aq) (1 × 18 mL), and brine (1 × 18 mL) and then dried over anhyd Na₂SO₄ 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 25 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-Pr₂NEt (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 21 (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 H₂O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic
extracts were washed with H₂O (3 × 20 mL), 5% HCl(aq) (1 × 20 mL), saturated NaHCO₃(aq) (1 × 20 mL), and brine (1 × 20 mL) and then dried over anhyd Na₂SO₄ 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 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-Pr₂NET (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 (34 mg, 0.10 mmol, 1.0 equiv) was added in one portion, the cooling 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 H₂O. The layers were separated, and then the aqueous phase was extracted with 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 (62 mg, 0.30 mmol, 1.00 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, 1.10 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 filtrate was partitioned between 50 mL of EtOAc and 50 mL of H₂O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with H₂O (3 × 2 mL), 5% HCl(aq) (1 × 2 mL), saturated NaHCO₃(aq) (1 × 2 mL), and brine (1 × 2 mL) and then dried over anhyd Na₂SO₄ 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 25 g of silica gel and 1:2 EtOAc/hexanes as eluent to provide compound 37 as a cream-colored amorphous solid (0.602 g, 76%). A smaller-scale reaction using 25 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%).

To a cooled (0 °C) suspension of carboxylic acid 21 (0.114 g, 0.330 mmol, 1.00 equiv) in 2 mL of DMF in a one-dram vial equipped with a magnetic stir bar was added DCC (19 mg, 0.10 mmol, 1.00 equiv) in 1 mL of DMF in a one-dram vial equipped with a magnetic stir bar was added DCC (0.188 g, 0.910 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, 1.10 equiv). The ice-water bath was kept in place, and the resulting mixture was allowed to warm gradually to ambient temperature with stirring. After 23 h, the reddish-brown-colored solution was partitioned between 2.5 mL of Et₂O and 2.5 mL of H₂O. The layers were separated, and the aqueous layer was extracted with Et₂O (3 × 2 mL). The resulting mixture was stirred at rt for 3 h, 40 min. Then, the reaction mixture was allowed to warm gradually to ambient temperature with stirring. After 25 h, the transparent, dark red-orange-colored reaction mixture was partitioned between 5 mL of EtOAc and 5 mL of H₂O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with H₂O (3 × 20 mL), 5% HCl(aq) (1 × 20 mL), saturated NaHCO₃(aq) (1 × 20 mL), and brine (1 × 20 mL) and then dried over anhyd Na₂SO₄ filtered, and concentrated in vacuo to provide the crude product (52 mg, 76%). The transparent, dark red-orange-colored reaction mixture was partitioned between 5 mL of EtOAc and 5 mL of H₂O. The layers were separated, and then the aqueous phase was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with H₂O (3 × 20 mL), 5% HCl(aq) (1 × 20 mL), saturated NaHCO₃(aq) (1 × 20 mL), and brine (1 × 20 mL) and then dried over anhyd Na₂SO₄ 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%). Rₜ = 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) ν 3325, 2977, 2860, 1718, 1683, 1652, 1608, 1132, 1052, 966 cm⁻¹; ¹H NMR (CDCl₃, 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), 1.36 (s, 9H). ¹³C{¹H} NMR (CDCl₃, 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 (CH₂), 40.7 (CH₂), 39.6 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 28.5 (CH₂), 28.2 (CH₂), 24.2 (CH₂); HRMS (TOF MS ES⁺) m/z: [M + H]+ calcd for C₂₅H₃₆CF₃N₃O₄ 796.2991, found 796.2993.

**Clavatidine C Hydrotrifluoroacetate (3-CF₃CO₂H).** To a scintillation vial charged with DiBoc guanidine 36 (0.374 g, 0.564 mmol, 1.00 equiv) was added 8 mL of CH₂Cl₂ and 1.5 mL of TFA. 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 EtO₂. The supernatant solution was removed, and the resulting powder was dried under high vacuum to afford pure clavatidine C hydrotrifluoroacetate (3-CF₃CO₂H) 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%); Rf = 0.31, 9:1 (v/v) EtOAc/Methanol; IR (neat) λ 3447, 3306, 2924, 2853, 1782, 1673, 1651, 1196, 1141, 707 cm−1; 1H NMR ([2 mg/0.75 mL] “dilute” in DMSO-d6 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 (4H, 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); 13C{1H} NMR (30 mg/0.75 mL] “concentrated” in DMSO-d6 400 MHz, referenced to solvent): δ 171.6 (C), 158.2 (C), 156.7 (C), 155.0 (C), 146.7 (CH), 121.6 (C), 85.2 (C), 43.2 (CH2), 40.7 (CH2), 38.3 (CH2), 28.4 (CH2), 28.1 (CH2), 23.4 (CH3); HRMS (TOF MS ES+) m/z: [M + H]+ calcd for C14H18Br2N5O3 461.9776; found 461.9781.

**Clavatadine D Hydrotrifluoroacetate (4-CF3CO2H).** To a scintillation vial charged with DiBoc guanidine (0.266 mmol, 1.00 equiv) and a magnetic stir bar was added 400 μL of CH2Cl2 and 1.5 mL of TFA. 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 Et2O. Removal of the supernatant solution afforded pure clavatadine D hydrotrifluoroacetate (4-CF3CO2H) as a tan-colored amorphous solid (0.149 g, 95%). Rf = 0.25, 9:1 (v/v) EtOAc/Methanol; IR (neat) λ 3306, 3184, 2942, 2865, 1682, 1651, 1610, 1550, 1202, 1187, 1132, 801, 722 cm−1; 1H NMR ([2 mg/0.75 mL] “dilute” in DMSO-d6 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); 13C{1H} NMR ([15 mg/0.75 mL] “concentrated” in DMSO-d6 400 MHz, referenced to solvent): δ 171.6 (C), 158.1 (C), 156.7 (C), 155.0 (C), 146.7 (CH), 121.6 (C), 85.2 (C), 43.2 (CH2), 40.4 (CH2), 38.3 (CH2), 26.0 (CH2), 25.9 (CH3); HRMS (TOF MS ES+) m/z: [M + H]+ calcd for C14H18Br2N5O3 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 3–8; copies of 1H and 13C 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 1H NMR spectra of N,N-diBoc clavatadine C (36) at various concentrations using treated and untreated CDCl3 (PDF)

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