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Total Synthesis of Clavatadine A

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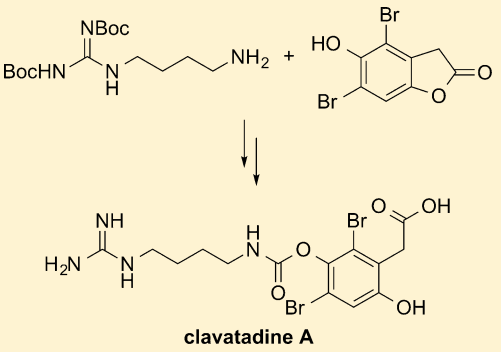
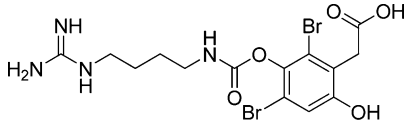
Supporting Information

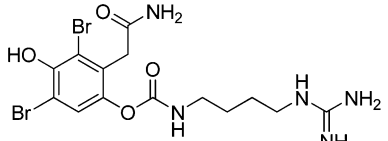
ABSTRACT: The first total synthesis of the potent and selective human blood coagulation factor XIa inhibitor clavatadine A (**1**) is described. Direct, early-stage guanidinylation enabled rapid, convergent access to an immediate clavatadine A precursor. Concomitant lactone hydrolysis and guanidine deprotection with aqueous acid cleanly provided clavatadine A (**1**) in only four steps (longest linear sequence, 41−43% overall yield).



In 2008, after an extensive screen of over 38 000 biota extracts for anticoagulant activity, Quinn and co-workers reported the isolation of two new dibromophenol alkaloids, clavatadines A (**1**) and B (**2**).[1] These new alkaloids were obtained from an extract of the marine sponge *Suberea clavata* collected at Swain Reefs, Great Barrier Reef, Queensland, Australia. Despite a relatively minor difference in the functionality of these molecules, only clavatadine A (**1**) was found to be a potent (IC_{50} = 1.3 μM) and selective inhibitor of human blood coagulation factor XIa (FXIa). X-ray crystallographic studies revealed that precise placement of the linear, arginine-like chain and the phenylacetic acid moiety at opposing termini of clavatadine A (**1**) maximizes two key noncovalent interactions within the FXIa active site.[1]



clavatadine A (**1**)



clavatadine B (**2**)

Our laboratory was drawn to clavatadine A (**1**) due to its relative structural simplicity and noted activity toward a promising new protein target in anticoagulant drug development. Recent studies suggest that targeted FXIa inhibition may reduce the likelihood of excessive clot formation (thrombosis), but still allow coagulation to occur.[2] Further, because bleeding associated with genetic FXIa deficiency (hemophilia C) is rarely spontaneous and results only from significant trauma, such as major surgery or a car accident, treatment with a specific FXIa inhibitor may not present an excessive bleeding risk like current drugs on the market.[2c] Accordingly, clavatadine A (**1**) may represent a lead compound in the development of the next-generation anticoagulant drug to treat thrombosis.

In conjunction with its intriguing biological activity, clavatadine A (**1**) was an ideal template to probe the generality of our early-stage guanidinylation approach for organic synthesis.[3] The retrosynthetic analysis of clavatadine A (**1**) was also guided by a desire to construct the central carbamate moiety in a convergent fashion in the key step. Before uniting both halves of the molecule, however, it was sought to prepare the terminal guanidine-containing, linear portion of clavatadine A (**1**) by installing the guanidine moiety in the first step. Direct guanidinylation would obviate the circuitous approach conventionally used in synthesis to incorporate a terminal guanidine. Namely, two unnecessary steps involving amine protecting groups were eliminated by allowing a protected guanidinylation reagent to react with a commercially available or readily prepared primary amine rather than with an amine that had first been introduced as a latent amine precursor (azide, phthalimide, etc.). A recent proof of concept study demonstrated that this strategy could be used successfully to prepare the fully functionalized terminal guanidine-containing portion of the indole alkaloids phidianidines A and B and ultimately led to the total synthesis of these bioactive natural products.[3]

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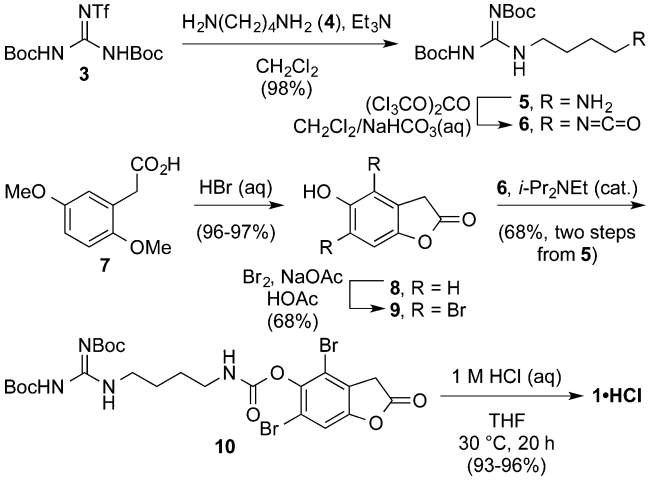
Adopting this enabling and efficient synthetic strategy to prepare clavatadine A (**1**) in a similar fashion became necessary after an advanced azide-containing intermediate could not be converted into the primary amine needed to host the guanidine found in clavatadine A (**1**).

RESULTS AND DISCUSSION

To explore whether a direct guanidinylation approach could be used to prepare clavatadine A (**1**), the known[4,5] *N,N′*-di-Boc-protected guanidine **5** was assembled by chemoselective guanidinylation of 1,4-butanediamine (**4**) using Goodman's reagent[6,7] (**3**) and Et_3N under high dilution conditions (Scheme 1). A modified isolation protocol combining an aqueous $NaHCO_3$ wash with subsequent column chromatography on silica gel was necessary to separate compound **5** from excess diamine **4** and from the principal byproduct of the reaction, triethylammonium trifluoromethanesulfonamide.

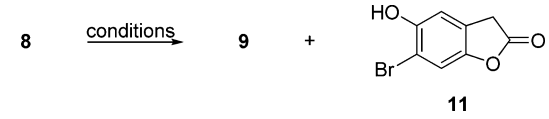
Scheme 1. Synthesis of Clavatadine A (1)



It was envisaged that the aromatic portion of clavatadine A (**1**) could be prepared from the known homogentisic acid lactone[8] (**8**), which was prepared from commercially available 2,5-(dimethoxyphenyl)acetic acid (**7**).[9,10] Ideally suited for the present purposes, compound **8** cloaks the highly polar and reactive *o*-hydroxyphenylacetic acid moiety as a lactone, thereby facilitating handling of derivatives and enabling purification by silica gel chromatography. It also provides an activating, *ortho,para*-directing hydroxy group that orchestrates regioselective installation of two bromine atoms: the first bromine adds *ortho* to the hydroxy at the most sterically accessible position (C-6), and the second is introduced at a more hindered location (C-4), but at a site that is also *ortho* to the strongest activator, the hydroxy group.

Although dibromination of compound **8** has been explored, prior efforts have not addressed the need for an efficient two-step, one-flask method to prepare valuable synthetic intermediate **9**. For example, Krohn observed that regiospecific monobromination of the electron-rich phenol **8** occurred within 20−30 min when the reaction was conducted in diethyl ether with Br_2 to provide compound **11** in 85% yield (Table 1, entry 1a).[11] Even in the presence of excess bromine, decomposition rather than the second bromination ensued if the reaction mixture was left to stir for 2 days.[11] To address this problem, Krohn isolated bromophenol **11** by washing the reaction mixture with aqueous sodium sulfite and then carried out the second bromination in a separate flask.[11] Subsequent bromination of the resulting attenuated arene **11** to form **9** was considerably slower (6−8 h) (Table 1, entry 1b).[11] Results from the present study confirm these observations (Table 1, entries 2 and 3). Even upon prolonged exposure (6 h) of a solution of phenol **8** to excess Br_2, the formation of compound **9** was never observed. In this instance, only monobrominated phenol **11** was recovered, albeit in good yield (Table 1, entry 3).[12] Without a base to neutralize the HBr generated during the reaction, perhaps the strong Brønsted acid deactivated the aromatic ring by protonating the hydroxy group to form an oxonium ion.[13] Strongly acidic solutions may also cause hydrolytic cleavage of the benzofuranone ring, leading to decomposition.[14] A recent attempt by Lebouvier and co-workers to perform both bromination reactions in one flask using NBS and *p*-toluenesulfonic acid (*p*-TsOH) led to a mixture of **9** and **11** in low yield (Table 1, entry 4); thus, a more efficient two-step, one-flask method was desired.[15] Accordingly, a slight excess of a weak base, sodium acetate, was added to a solution of compound **8** and bromine that neutralized both equivalents of HBr formed during the reaction and led to the desired lactone **9** (Table 1, entry 5).[16] This improvement to the reported sequential[11] and nonspecific[15] bromination of homogentisic acid lactone (**8**) provided 4,6-dibromohomogentisic acid lactone (**9**) in good yield.

Table 1. Bromination of Phenol 8



11

		yield (%)[a]	
entry	conditions	**9**	**11**
1[b]	(a) Br_2, Et_2O, rt, 2 h;	0	85
	(b) **11**, Br_2, Et_2O, rt, 6−8 h	70	0
2[c]	**8**, Br_2, Et_2O, rt, 25 min	0	90
3[c]	**8**, Br_2, Et_2O, rt, 6 h	0	96
4[d]	NBS (2 equiv), *p*-TsOH, CH_2Cl_2, rt, 12 h	21	12
5[c]	Br_2, NaOAc, HOAc, rt, 7.25 h	68	11

[a]Isolated yields of purified reaction products. [b]Ref 11. [c]This work. [d]Ref 15.

Armed with an efficient method to prepare both amine **5** and lactone **9**, the two halves were joined together to build the complete clavatadine A (**1**) scaffold. In practice, exposure of amine **5** to triphosgene afforded the semistable isocyanate **6**, which was isolated in quantitative yield (Scheme 1). Immediately, isocyanate **6** was added to a solution of lactone **9** and a catalytic amount of base to give the carbamate **10**.[17,18] Subsequent attempts to hydrolyze the lactone with aqueous base followed by acidification of the reaction mixture led only to quantitative recovery of carbamate **10**.[19] It was then discovered that addition of a solution of carbamate **10** to dilute aqueous hydrochloric acid promoted hydrolysis of the lactone and deprotected the di-Boc guanidine to provide clavatadine A hydrochloride (**1**·HCl) in 93−96% yield.[14]

Analysis of the ^{1}H NMR spectrum of unpurified, synthetic clavatadine A (**1**) in DMSO-d_6 revealed relatively few impurities, but the spectrum did not precisely match the reported spectrum of the natural product. There were two prominent differences. First, the ^{1}H NMR spectrum of synthetic **1** lacked an apparent 1:1:1 triplet at approximately δ_H 7.1 that was visible in the ^{1}H NMR spectrum[20] of natural **1**,

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which was isolated by HPLC as its hydrotrifluoroacetate salt.[1] This triplet is often observed in the ^{1}H NMR spectrum of natural compounds purified by HPLC when a dilute methanolic or aqueous solution of trifluoroacetic acid is used as the eluent. The sharp, spin 1 triplet ($J \approx 48$ Hz) observed at δ_H 7.1 in the natural sample likely arises from ^{1}H coupling to ^{14}N.[1,21] Sharp quadrupole coupling such as this is only observed in small, symmetric molecules, not complex molecules such as clavatadine A (**1**).[22] Thus, the peak at δ_H 7.1 in the ^{1}H NMR spectrum of natural **1** is likely attributable to the presence of an artifact: ammonium trifluoroacetate.[21,22] Second, the singlet at δ_H 6.51 in the ^{1}H NMR spectrum of natural **1** is a common impurity that appeared only in highly diluted samples in DMSO-d_6. Its presence was ascribed to the manufacturer of the DMSO-d_6 that was used to dissolve natural clavatadine A (**1**), because the peak at δ_H 6.51 was no longer observed in ^{1}H NMR spectra after the supplier was changed. Aside from impurities found only in the natural sample of **1**, slight differences were observed in the chemical shift values reported for some NH and OH resonances in the synthetic sample: carboxylic acid (δ_H 12.41 vs 12.38), phenol (δ_H 10.45 vs 10.42), and guanidinium NH (δ_H 7.52 vs 7.45). Sample concentration seemed to have the biggest impact on the observed ^{1}H NMR chemical shifts. Accordingly, the present data most closely matched the reported values for the natural compound when dilute solutions (1−2 mg/mL) of synthetic clavatadine A (**1**) were prepared in DMSO-d_6. Although the 400 MHz NMR spectrometer used could not completely resolve the peaks at approximately δ_H 3.1 (δ_H 3.11 and 3.08 for natural **1**) or those near δ_H 1.5 (δ_H 1.52 and 1.49 for natural **1**), data gathered on a 600 MHz NMR instrument exhibited matching δ_H and J values to the natural compound.[1] All other 1D (^{13}C and DEPT) and 2D NMR spectra of unpurified, synthetic clavatadine A (**1**) were identical to those reported for natural **1**. In addition, the ^{1}H NMR spectrum of synthetic clavatadine A (**1**) that had been purified by HPLC to provide the corresponding hydrotrifluoroacetate salt (**1**·CF_3CO_2H) revealed coincidental resonances with the natural material. The present preparation of the title compound was further confirmed by an FXIa enzyme-inhibition assay, which revealed nearly identical IC_{50} values for the natural (1.3 μM)[1] and synthetic (6.3 μM) samples of clavatadine A (**1**) (Figure 1).

CONCLUSIONS

In conclusion, the first total synthesis of clavatadine A (**1**) has been completed in four steps (longest linear sequence). The overall yield of the convergent approach used was 41−43% from commercially available compound **7**. Direct guanidinylation circumvented the cumbersome protected amine-to-amine-to-guanidine sequence traditionally employed in the synthesis of terminal guanidine-containing molecules and should enable the rapid preparation of analogues for biological evaluation. It is planned to use this basic approach to prepare clavatadine B (**2**) and to synthesize non-natural analogues of clavatadine A (**1**) as novel, reversible FXIa inhibitors.

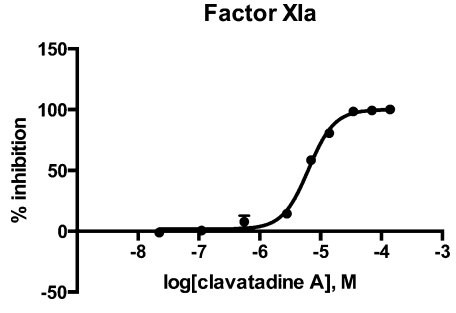

Figure 1. Results of FXIa inhibition assay (IC_{50} 6.3 μM) using synthetic clavatadine A (**1**).

EXPERIMENTAL SECTION

General Experimental Procedures. Infrared (IR) spectra were obtained on neat solids or liquids using an ATR FT-IR spectrometer. One- and two-dimensional ^{1}H and ^{13}C NMR spectra were recorded at ambient temperature at 400 and 100 MHz, respectively, and calibrated using tetramethylsilane at δ 0.00, unless otherwise stated. All chemical shifts are reported in ppm on the δ scale, integration, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, or combinations thereof), and coupling constants in Hz. All 2D NMR spectra were recorded in DMSO-d_6. Carbon−hydrogen coupling constant values in inverse-detection 2D NMR experiments, which included gradient HSQC ($^1J_{CH}$ = 140 Hz) and gradient HMBC ($^nJ_{CH}$ = 8.3 Hz), matched those used in the clavatadine A (**1**) isolation paper by Quinn and co-workers.[1] Accurate mass (MS) measurements were performed on an LCT premier time-of-flight (TOF) instrument using electrospray ionization in positive ion mode (ES+) by Dr. John Greaves and Ms. Shirin Sorooshian. 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. Spots were visualized by short-wave (254 nm) UV irradiation and/or by dipping the plates in phosphomolybdic acid (PMA), cerium ammonium molybdate (CAM), ferric chloride, or ninhydrin solutions followed by heating. All reaction mixtures not containing aqueous reagents were carried out under an atmosphere of dry nitrogen using standard syringe/septum techniques. Glassware was oven-dried overnight at 130 °C, flame-dried using a propane torch, and then allowed to cool to ambient temperature under a stream of dry nitrogen. Unless otherwise noted, all reagents were used as received from commercial suppliers. Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), and Hünig's base (i-Pr_2NEt) were dried over anhydrous CaH_2 and distilled under N_2. Triethylamine (Et_3N) was dried over KOH and distilled under N_2. When anhydrous reaction conditions were required, solvents were first distilled under nitrogen onto activated 3 Å molecular sieves and then were transferred under nitrogen by cannula into amber-colored glass bottles for storage. These bottles were capped with an Aldrich (St. Louis, MO, USA) Sure-Seal PTFE disk and a metal crown cap using an Aldrich crown-cap crimper. Factor XIa was purchased from Haematologic Technologies, Inc. (Essex Junction, VT, USA), and substrate s2366 from Chromogenix (manufactured by Instrumentation Laboratory, Bedford, MA, USA). Clear 96-well Nunc microplates were purchased from Thermo Scientific (Waltham, MA, USA). Absorbance was measured on a PerkinElmer (Waltham, MA, USA) Envision 2104 multilabel reader.

N,N′-Di-Boc Guanidine **5**. The known *N,N′*-di-Boc guanidine **5** was prepared using an adaptation of the procedures of Botta and co-workers and Muñoz and co-workers.[4,5] Spectroscopic data and copies of spectra are also provided for compound **5** because complete data have not yet been published. To a solution of 1,4-butanediamine (**4**) (2.32 mL, 23.1 mmol, 3.0 equiv) in 320 mL of CH_2Cl_2 was added Et_3N (1.07 mL, 7.69 mmol, 1.0 equiv). Then, a solution of *N,N′*-di-Boc-*N″*-triflylguanidine[7] (**3**) (3.01 g, 7.69 mmol, 1.0 equiv) in 25 mL of CH_2Cl_2 was added dropwise by addition funnel over approximately 1−2 h (the reaction mixture grew cloudy), and the reaction mixture was stirred at ambient temperature for 12 h. After 12 h, the reaction mixture was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), H_2O (2 × 50 mL), and brine (1 × 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography on silica gel (5:3:2 EtOAc−MeOH−Et_3N) afforded the product as a pale yellow oil (2.49 g, 98%): IR (neat) ν_{max}

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3333, 2977, 2932, 1719, 1636, 1613, 1572, 1414, 1365, 1328, 1131, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.50 (1H, br s), 8.35 (1H, br s), 3.43 (2H, td, J = 7.2, 4.8 Hz), 2.74 (2H, t, J = 7.0 Hz), 1.63 (2H, m), 1.53 (2H, m), 1.51 (9H, s), 1.49 (9H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.6 (C), 156.1 (C), 153.3 (C), 83.1 (C), 79.3 (C), 41.8 (CH_2), 40.7 (CH_2), 30.8 (CH_2), 28.3 (CH_3), 28.1 (CH_3), 26.4 (CH_2); (+)-HRTOFESIMS m/z 353.2168 (calcd for $C_{15}H_{30}N_4O_4Na$ $[M + Na]^+$, 353.2165); R_f = 0.39, 5:3:2 EtOAc−MeOH−Et_3N.

6-Bromohomogentisic Acid Lactone (11). To a solution of homogentisic acid lactone **8** (0.150 g, 1.00 mmol, 1.00 equiv)[9,10] in 10 mL of Et_2O was added bromine (0.154 mL, 3.00 mmol, 3.00 equiv) dropwise by syringe. The reaction mixture was stirred at ambient temperature for 25 min and then was poured into a separatory funnel containing 10 mL of saturated aqueous Na_2SO_3. The layers were separated, and the organic phase was washed with an additional 2 × 10 mL of saturated aqueous Na_2SO_3 and 1 × 10 mL of brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to provide the product as a brown-colored solid (0.207 g, 90%): R_f of **11** = 0.34, 1:1 EtOAc−hexanes. Krohn first reported this method to prepare compound **11**, but did not provide experimental details.[11] Spectroscopic data for **11** match previously reported data for compound **6b** prepared by Lebouvier.[15] If, instead, the above reaction mixture was stirred at ambient temperature for 6 h, compound **11** was formed in 96% yield.

4,6-Dibromohomogentisic Acid Lactone (9). To a suspension of homogentisic acid lactone **8** (0.601 g, 4.00 mmol, 1.00 equiv)[9,10] and sodium acetate (0.673 g, 8.20 mmol, 2.05 equiv) in 30 mL of acetic acid was added a solution of bromine (0.62 mL, 12.0 mmol, 3.00 equiv) in 1.25 mL of acetic acid dropwise by syringe. The reaction mixture was stirred at ambient temperature for 7 h, 15 min and then was concentrated in vacuo to provide a brown solid (1.146 g). This solid was dissolved in EtOAc, adsorbed onto 5 g of silica gel, and then purified by flash column chromatography on silica gel (1:4 EtOAc−hexanes, to recover the desired product, compound **9**, and then 1:1 EtOAc−hexanes to elute 6-bromohomogentisic acid lactone (**11**)). This purification provided the desired product, compound **9**, as a brownish-purple-colored powder (0.834 g, 68%, based on a theoretical yield of 1.232 g, 4.00 mmol), and compound **11**, as a brown-colored powder (0.102 g, 11%, based upon a theoretical yield of 0.916 g, 4.00 mmol): R_f of **9** = 0.66, 1:1 EtOAc−hexanes. The spectroscopic data for **9** matched previously reported data for compound **6a** prepared by Lebouvier.[15]

N,N′-Di-Boc Guanidine-Containing Carbamate 10. To a cooled (0 °C) solution of *N,N′*-di-Boc guanidine **5** (1.00 g, 3.03 mmol, 1.00 equiv) in 25 mL of CH_2Cl_2 was added triphosgene (0.297 g, 1.01 mmol, 0.33 equiv; **CAUTION:** *highly toxic; open, weigh, and handle only in a fume hood and while wearing two pairs of nitrile gloves*). Then, a solution of saturated aqueous $NaHCO_3$ (25 mL) was added, and the biphasic reaction mixture was stirred vigorously at 0 °C for 30 min. After 30 min, the reaction mixture was partitioned between 100 mL of CH_2Cl_2 and 100 mL of H_2O, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to provide semistable, crude isocyanate **6** as a light-brown-colored oil (1.11 g, 103% of theoretical). This oil was used immediately in the next step without purification. Diagnostic data: IR (neat) ν_{max} 3331, 2978, 2934, 2265, 1719, 1635, 1613, 1574, 1327, 1129, 731 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.50 (1H, br s), 8.36 (1H, br s), 3.46 (2H, m), 3.37 (2H, m), 1.67 (4H, m), 1.51 (9H, s), 1.50 (9H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.5 (C), 156.2 (C), 153.4 (C), 122.0 (NCO), 83.3 (C), 79.4 (C), 42.6 (CH_2), 40.1 (CH_2), 28.5 (CH_2), 28.3 (CH_3), 28.1 (CH_3), 26.2 (CH_2).

To a round-bottomed flask containing a solution of dibromophenol **9** (0.933 g, 3.03 mmol, 1.00 equiv) and *N,N*-diisopropylethylamine (0.103 mL, 0.606 mmol, 0.200 equiv) in 30 mL of CH_2Cl_2 was added a solution of freshly prepared isocyanate **6** (1.11 g, 3.03 mmol, 1.00 equiv) in 30 mL of CH_2Cl_2 dropwise by cannula over 1 h. The flask containing isocyanate **6** was rinsed with CH_2Cl_2 (2 × 5 mL), and these washings were added to the reaction flask containing dibromophenol **9**. The resulting solution was stirred at ambient temperature for 3 h and was concentrated in vacuo to provide a foamy, dark-brown-colored solid (2.20 g, 110% mass balance, presuming *i*-Pr_2NEt was removed in vacuo). This solid was purified by flash column chromatography on silica gel (1:0 to 1:1 CH_2Cl_2−Et_2O) to provide carbamate **10** as a maroon-colored, foamy solid (1.36 g, 68% from amine **5**): IR (neat) ν_{max} 3335, 2927, 1822, 1755, 1720, 1639, 1616, 1583, 1413, 1158, 733 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.48 (1H, br s), 8.40 (1H, apparent br t, J value indecipherable), 7.32 (1H, s), 5.78 (1H, br t, J = 6.0 Hz), 3.69 (2H, s), 3.45 (2H, dt, J = 6.6, 6.0 Hz), 3.38 (2H, dt, J = 6.6, 6.0 Hz), 1.75−1.64 (4H, m), 1.50 (18H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.3 (C), 163.5 (C), 156.3 (C), 153.3 (C), 152.3 (C), 151.7 (C), 142.7 (C), 125.5 (C), 118.3 (C), 115.0 (C), 114.4 (CH), 83.2 (C), 79.4 (C), 40.7 (CH_2), 40.0 (CH_2), 34.7 (CH_2), 28.3 (CH_3), 28.1 (CH_3), 26.5 (CH_2), 26.1 (CH_2); ^1H NMR (DMSO-d_6, 400 MHz) δ 11.51 (1H, br s), 8.32 (1H, apparent br t, J value indecipherable), 8.13 (1H, t, J = 5.6 Hz), 7.66 (1H, s), 3.91 (2H, s), 3.28 (2H, m), 3.10 (2H, m), 1.58−1.50 (2H, m), 1.48 (9H, s, and 2H, m), 1.40 (9H, s); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.2 (C), 163.1 (C), 155.1 (C), 152.1 (C), 152.0 (C), 151.2 (C), 141.8 (C), 127.0 (C), 116.6 (C), 114.3 (C), 113.5 (CH), 82.8 (C), 78.0 (C), 40.1 (CH_2), 39.4 (CH_2), 34.7 (CH_2), 27.9 (CH_3), 27.5 (CH_3), 26.5 (CH_2), 25.8 (CH_2); (+)-HRTOFESIMS m/z 685.0468 (calcd for $C_{24}H_{32}{}^{79}Br_2N_4O_8Na$ $[M + Na]^+$, 685.0485); R_f = 0.36, 2:3 EtOAc−hexanes, visualized with short-wave UV light, I_2, and CAM/heat.

Clavatadine A (1). To a round-bottomed flask charged with carbamate **10** (1.205 g, 1.810 mmol) was successively added 12 mL of THF and 48 mL of 1 M HCl(aq). The flask was gently covered with a glass stopper to prevent evaporation and to avoid plasticizer leaching into the mixture, the latter of which occurred when a standard rubber septum or plastic yellow cap was used to cover the aperture. The resulting solution was heated at 30 °C (water bath) and stirred vigorously for 20 h. After 20 h, the resulting suspension was vacuum filtered to remove 9 mg of a black-colored solid. The yellow-orange-colored filtrate was then concentrated in vacuo to afford clavatadine A hydrochloride (**1**·HCl) as a peach-colored, amorphous solid (0.866 g, 93%). The ^1H NMR chemical shifts for dilute solutions of synthetic clavatadine A (1−2 mg of **1**·HCl per milliliter of DMSO-d_6) differed very slightly from those found in the previously reported ^1H NMR spectrum of natural clavatadine A hydrotrifluoroacetate (**1**·CF_3CO_2H) (1.5 mg in DMSO-d_6).[1] All other NMR data, including ^{13}C, DEPT-135, and all correlations within gradient COSY, gradient HSQC, and gradient HMBC spectra of unpurified synthetic clavatadine A (**1**), matched previously reported spectra for natural clavatadine A (**1**):[1] IR (neat) ν_{max} 3166, 2942, 1714, 1648, 1398, 1200, 953, 754 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz, referenced to residual solvent peak at δ 2.50) δ 12.41 (1H, v br s), 10.45 (1H, s), 8.02 (1H, t, J = 5.6 Hz), 7.52 (1H, t, J = 5.6 Hz), 7.40−7.10 (2H, v br s), 7.10 (1H, s), 6.95−6.65 (2H, v br s), 3.66 (2H, s), 3.12 (2H, dt, J = 5.6, 5.6 Hz), 3.07 (2H, dt, J = 5.6, 5.6 Hz), 1.50 (4H, m); ^{13}C NMR (DMSO-d_6, 100 MHz, referenced to residual solvent peak at δ 39.50) δ 170.8 (C), 156.8 (C), 154.2 (C), 152.7 (C), 138.0 (C), 123.2 (C), 121.9 (C), 117.1 (DEPT-135, *C*H), 115.7 (C), 40.4 (DEPT-135, CH_2), 40.0 (DEPT-135, CH_2), 35.4 (DEPT-135, CH_2), 26.5 (DEPT-135, CH_2), 25.8 (DEPT-135, CH_2); (+)-HRTOFESIMS m/z 480.9716 (calcd for $C_{14}H_{19}{}^{79}Br_2N_4O_5$ $[M + H]^+$, 480.9722).

Purification. An aliquot (4.0 mg) of synthetic clavatadine A hydrochloride salt (**1**·HCl) was subjected to purification by mass-directed semipreparative HPLC to yield 3.4 mg of clavatadine A hydrotrifluoroacetate (**1**·CF_3CO_2H) as a colorless oil (Agilent Technologies (Santa Clara, CA, USA) 1200 system, G1311A pump, G1315D diode array detector, Agilent 6120 quadrupole MS). HPLC conditions: Zorbax C_{18} column (9.4 × 50 mm), flow rate 5 mL/min, solvent solvent A = 0.1% TFA in H_2O, B = 0.1% TFA in CH_3CN, linear gradient from 95% solvent A, 5% solvent B to 5% solvent A, 95% solvent B over 6 min, then to 95% solvent A, 5% solvent B in 2 min. The oil was dissolved in DMSO to give a 10 mM stock solution.

Factor XIa Assay. Factor XIa enzyme (received as a 3.9 mg/mL solution in glycerol−H_2O, 1:1) was serially diluted to a working concentration of 0.54 nM in assay buffer (pH 7.4 at rt) consisting of 50 mM Tris, 100 mM NaCl, 5 mM $CaCl_2$, and 0.1 mg/mL BSA. The

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assay was performed in triplicate in 96-well microplates and consisted of the following: 1 μL of compound in DMSO, 31 μL of factor XIa buffer, 20 μL of factor XIa, followed by incubation at rt for 15 min. Then, 20 μL of substrate S2366 (0.9 mM in FXIa assay buffer) was added, followed by incubation for 2 h at rt. Absorbance was then read at 405 nM. In-plate controls consisted of 0% inhibition (1 μL of DMSO, 31 μL of buffer, 20 μL of factor XIa, and 20 μL of substrate S2366) and 100% inhibition (1 μL of DMSO, 51 μL of buffer, 20 μL of substrate S2366).

Data Analysis. Percent inhibition was calculated as follows:

$$\%\text{inhibition} = 100 - \{[(\text{Abs}_{\text{compound}} - \text{Abs}_{100\%\text{inhibition}}) / (\text{Abs}_{0\%\text{inhibition}} - \text{Abs}_{100\%\text{inhibition}})] \times 100\}$$

■ ASSOCIATED CONTENT

Supporting Information

Comparison of the NMR data of natural and synthetic clavatadine A and copies of ^{1}H and ^{13}C NMR spectra for new compounds and compounds prepared using modified literature procedures, including 2D NMR data for synthetic clavatadine A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(19) The consumption of carbamate **10** under basic (0.1 M NaOH(aq)) and reducing (0.21 M Na_2SO_3(aq)) conditions was confirmed qualitatively by TLC. In one experiment, in lieu of acidification, the reaction mixture was concentrated to provide a beige-colored solid. Analysis of the ^{1}H NMR spectrum of the resulting unstable compound, which decomposed upon exposure to air and within hours in DMSO-d_6 solution, revealed a highly shielded singlet at δ_H 6.74 (1H, aromatic C−H) and a deshielded singlet at δ_H 3.99 (2H, benzylic CH_2). In comparison, the aromatic C−H and benzylic methylene resonances of synthetic 4,6-dibromohomogentisic acid, which is similar to the hydrolysis product of compound **10**, are δ_H 6.99 and 3.63, respectively, in DMSO-d_6. These results suggest that the lactone was hydrolyzed under basic reaction conditions to provide a carboxylate/phenolate dianion intermediate that cyclized upon acidification.

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