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Tandem electrocyclic ring opening/radical cyclization: application to the total synthesis of cribrostatin 6

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This manuscript is dedicated to Gilbert Stork on the occasion of his 90th birthday and in honor of his many significant contributions to the methods and the art of organic synthesis

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ABSTRACT

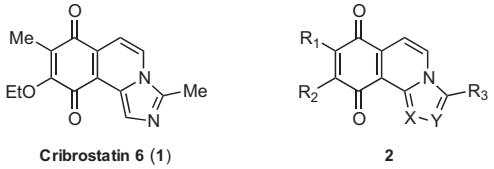
A concise total synthesis of cribrostatin 6 (**1**), an antimicrobial and antineoplastic agent, was accomplished using a tandem electrocyclic ring opening/radical cyclization sequence. Specifically, intermediate **4** underwent a 4π-electrocyclic ring opening, radical cyclization, and homolytic aromatic substitution sequence followed by an oxidation to afford the natural product **1** in one pot. Owing to the rapid buildup of complexity in the key step, **1** could be synthesized from commercially available starting materials in only four linear steps. Application of this chemistry to the concise syntheses of analogs of cribrostatin 6 (**1**) is also reported.

1. Introduction

The spread of multi-drug-resistant bacteria has fueled the need for new structural classes of antibiotics. Resistant strains of *enterococci* and *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* are emerging with increased frequency. *S. pneumoniae*, which is the most common bacterial cause of acute respiratory infection and otitis media, causes millions of deaths each year.[1] Indeed, pneumonia is the eighth leading cause of death in the United States.[2] A growing resistance of *S. pneumoniae* against trimethoprim-sulfamethoxazole, penicillin, macrolide, and tetracycline antibiotics, has spurred the search for novel antibiotic leads.

In 2003 Pettit and co-workers reported the isolation and characterization of the novel heterocycle cribrostatin 6 (**1**) from the marine sponge *Cribrochalina* sp. (Fig. 1).[3] The dark blue solid was found to inhibit the growth of a number of antibiotic-resistant, Gram-positive bacteria and pathogenic fungi. Most notably, cribrostatin 6 (**1**) was active against *S. pneumoniae* with minimum bactericidal concentration (MBC)/MIC ratios of ≤2 for 75% of *S. pneumoniae* clinical isolates.[1] Cribrostatin 6 also displayed significant antineoplastic activity against several human cancer cell lines (GI_{50}=0.2–0.4 μg/mL). Hergenrother and co-workers have recently found that **1** has broad anticancer activity, and they have also made the important discovery that it induces apoptosis through a reactive oxygen species (ROS)-mediated mechanism.[4] Given the success of known ROS producers as anticancer agents,[5] their findings strongly suggest that cribrostatin 6 (**1**) has significant potential as a novel chemotherapeutic agent.

The important biological activity of cribrostatin 6 (**1**) coupled with its tricyclic imidazo[5,1-*a*]isoquinolinedione architecture,



Cribrostatin 6 (1) 2

Fig. 1. Natural product cribrostatin 6 (**1**) and potential analogs (**2**).

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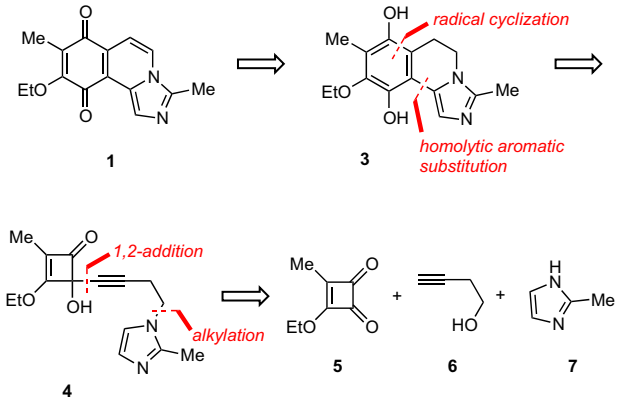
which is unprecedented among known natural products, has attracted the attention of the synthetic community. Nakahara and co-workers were the first to complete a total synthesis of **1**, using a modified Pomeranz–Fritsch isoquinoline synthesis as the key step to give **1** in 18 steps and 0.79% overall yield.[6] Kelly and co-workers subsequently reported an alternative synthesis that employed two Stille couplings and a one-pot process for oxidative olefin cleavage, *N*-Boc deprotection, and hemiaminal formation to furnish **1** via a longest linear sequence of 13 steps (15 steps total) and an improved 3.1% yield.[7]

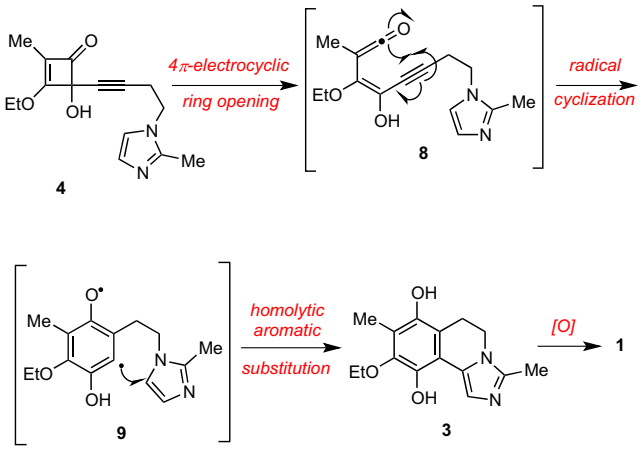
In the context of our general interest in quinone-derived natural products,[8] we were attracted to the challenge of developing a novel route to cribrostatin 6 (**1**) that would be both more concise and more efficient than previous syntheses. Because of the clear biological potential of **1**, we were also interested in designing an approach that would be readily amenable to the construction of analogs of **1** having the general structure **2** for further biological evaluation. We now report a detailed account of our concise total synthesis of cribrostatin 6 (**1**) and the facile modification of the approach to the construction of several cribrostatin 6 analogs.[9]

Our retrosynthetic analysis of cribrostatin 6 (**1**) is outlined in Scheme 1. In particular we envisioned that **1** would be accessed from **3** via oxidation of the hydroquinone and dehydrogenation of the hydroisoquinoline ring. The key step of the synthesis drew upon the prior work of Moore and co-workers, who studied extensively the conversions of squarate derivatives to quinones,[10] and involved the transformation of the squarate **4** into the hydroquinone **3** via a tandem electrocyclic ring opening/radical cyclization. Intermediate **4** would then be prepared from the known squarate derivative **5**[11] and commercially available alcohol **6** and 2-methylimidazole (**7**). By simply modifying the structure of the inputs **5** and **7**, we envisioned that it would be possible to quickly access numerous analogs of **1**.



Scheme 1.

As noted previously, our approach to **1** was influenced by the seminal work of Moore and co-workers. Namely, there was ample precedent for the thermal ring opening of squarate derivatives closely related to **4** via a 4π-electrocyclic ring opening reaction to furnish ketene intermediates, such as **8** (Scheme 2). Radical cyclization of **8** would then deliver the diradical **9**,[12] which we anticipated would undergo a homolytic aromatic substitution onto the pendant imidazole to afford hydroquinone **3**, which possesses the tricyclic core of cribrostatin 6 (**1**). Although cyclizations of phenyl radicals related to **9** onto pendant alkenyl and alkynyl groups had been documented,[12] there were only a few examples of cyclizations onto aryl rings, none of which involved azoles.[13] Hence, the successful transformation of **9** to deliver **3** would significantly expand the utility of such cyclizations. Oxidation and dehydrogenation of **3**, ideally in the same pot, would then deliver cribrostatin 6 (**1**).

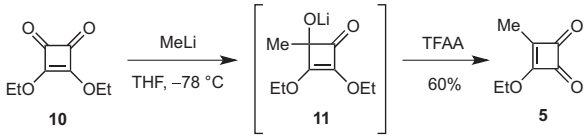


Scheme 2.

2. Results and discussion

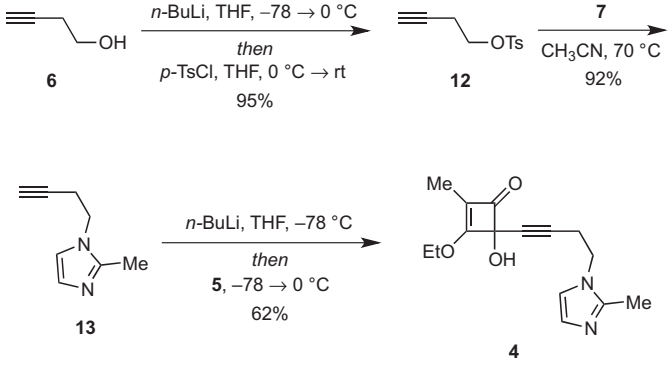
2.1. Synthesis of cribrostatin 6: Optimization of tandem ring opening/radical cyclization sequence

In accordance with our plan (Scheme 1), efforts toward cribrostatin 6 (**1**) commenced with the synthesis of the requisite squarate analog **5** following a literature procedure (Scheme 3).[11] In the event, commercially available diethyl squarate (**10**) was treated with MeLi to give the intermediate adduct **11**, which underwent elimination upon reaction with TFAA to deliver **5** in 60% yield.



Scheme 3.

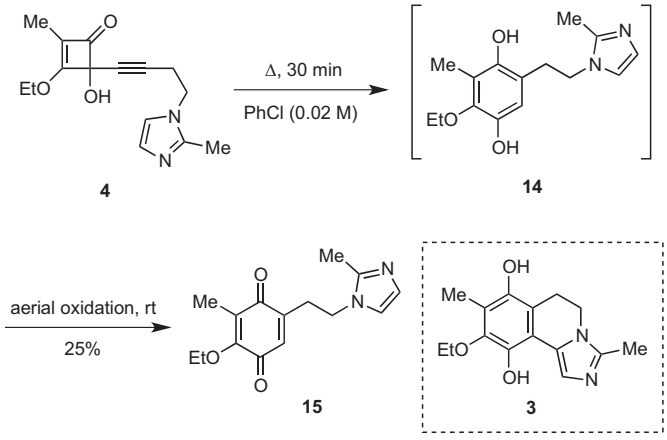
The next task required the preparation of **4**. Toward this end, commercially available alcohol **6** was converted to tosylate **12** in 95% yield using a slightly modified version of a known procedure (Scheme 4).[14] Initial experiments to prepare the alkyne **13** focused on the reaction of tosylate **12** with 2-methylimidazole (**7**) in the presence of bases, such as *t*-BuOK, Cs_2CO_3, and NaH. However, none of these reactions produced significant quantities of the desired **13**. After some experimentation, we discovered that heating the tosylate **12** with an excess of **7** in acetonitrile at 70 °C gave the requisite alkyne **13** in 92% yield. Deprotonation of the acetylenic proton of **13** with *n*-BuLi and regioselective



Scheme 4.

addition of the anion thus formed to the squarate derivative **5** in accordance with literature precedent[10,11] afforded the key intermediate **4** in 62% yield.

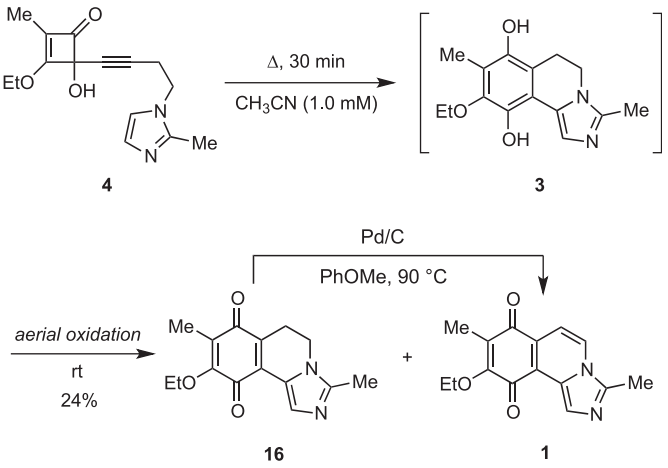
With the squarate derivative **4** in hand, the stage was set to explore its pivotal transformation into **3**. In the event, **4** was heated in refluxing PhCl (0.02 M) for 30 min to give hydroquinone **14** (Scheme 5). Based upon examination of the ^{1}H NMR spectrum of the crude reaction mixture, **14** was the only identifiable product of the reaction; none of the desired **3** was detected. However, it was not possible to isolate **14** in pure form because it underwent facile aerial oxidation to give quinone **15**. Although obtaining the quinone **15** was not the desired outcome, we were able to convert **4** cleanly into **15** by the simple expedient of heating **4** in refluxing PhCl for 30 min, followed by stirring the resultant solution at room temperature open to air overnight. Quinone **15** was thus isolated as the sole product of the reaction in 25% yield (unoptimized).



Scheme 5.

This result suggests that the diradical intermediate **9** (cf. Scheme 2) did indeed form, but rather than undergoing the desired cyclization via homolytic aromatic substitution onto the pendant imidazole, two intermolecular hydrogen atom transfers ensued to deliver hydroquinone **14**. Because Moore and Xiong had also isolated hydroquinone products,[13a] we were not completely surprised by this finding. Mechanistic considerations clearly suggested that we should conduct the reaction under more dilute conditions as the desired cyclization was unimolecular, whereas the hydrogen atom transfer was intermolecular.[12b] Accordingly, we conducted a number of exploratory experiments on a small scale to identify the conditions (solvent, temperature, time, and concentration) that would provide the cyclized material **3**. Indeed, we heated a more dilute solution of **4** in PhCl (1.0 mM) under reflux, and the ^{1}H NMR spectrum of the crude reaction mixture revealed that we had formed a mixture (ca. 1:3) that contained both **14** and the desired hydroquinone **3**. Further experimentation demonstrated that the solvent also played an important role in this tandem ring opening and radical cyclization sequence. For example, use of dichloroethane, dimethylformamide, and anisole as solvent (1.0 mM) gave similar mixtures in which **3** was formed in increased amounts relative to **14**. Eventually we discovered that CH_3CN gave the best results as heating a solution of **4** in refluxing CH_3CN (1.0 mM) gave a mixture containing **3** and **14** in an approximate ratio of 8:1.

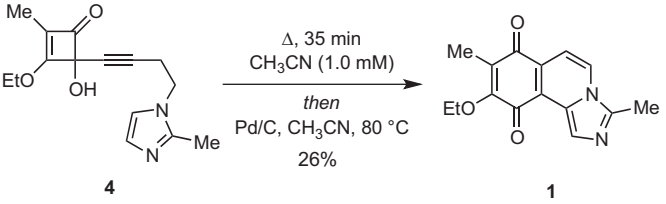
Having identified the optimal conditions for the ring opening/radical cyclization sequence, we then conducted a preparative experiment in which a solution of **4** in CH_3CN (1.0 mM) was heated to give hydroquinone **3** as the major product (Scheme 6). During our preliminary experiments, we discovered that **3** like **14** was also labile toward facile aerial oxidation. We thus stirred the solution of **3** open to air overnight at room temperature. We were somewhat surprised to discover that a mixture (2:1) of quinone **16** and cribrostatin 6 (**1**) was produced in 24% combined yield. This result not only confirmed that our plan for preparing cribrostatin 6 (**1**) was viable, but it also suggested that **1** might be obtained directly from **4** in one pot. However, extended stirring of the mixture of **16** and **1** in an open flask gave at best a mixture of **1** and **16** in a 2:1 ratio.



Scheme 6.

In as much as dehydrogenation of **16** to naturally-occurring **1** was only partially successful using air, we screened additional oxidants. Gratifyingly, after screening a number of potential oxidants (e.g., CAN, DDQ, MnO_2, Ag_2O), we found that heating the crude mixture of **16** and **1** with Pd/C in anisole gave exclusively cribrostatin 6 (**1**) in 17% overall yield from **4**.

At this juncture we wanted to further streamline the conversion of **4** to cribrostatin 6 (**1**). In the event, a solution of **4** in CH_3CN (1.0 mM) was first heated until starting material had been consumed (TLC) (Scheme 7). The majority of the solvent was evaporated under reduced pressure, Pd/C was added, and the reaction mixture was heated at 80 °C for 4 h to give cribrostatin 6 (**1**) in one pot and 26% overall yield. The synthetic **1** thus obtained gave ^{1}H and ^{13}C NMR spectra consistent with those reported in the literature.[3,6,7] This total synthesis of cribrostatin 6 is remarkably concise as it requires only four steps in the longest linear sequence and only five steps from commercially available starting materials, and it proceeds in 14.1% overall yield. Notably, no protecting groups are used.

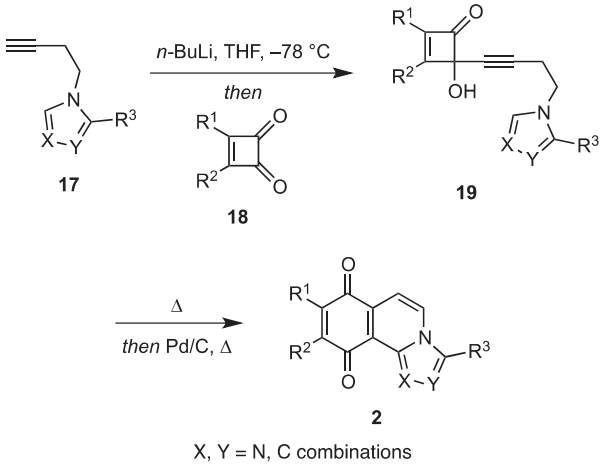


Scheme 7.

2.2. Synthesis of cribrostatin 6 analogs

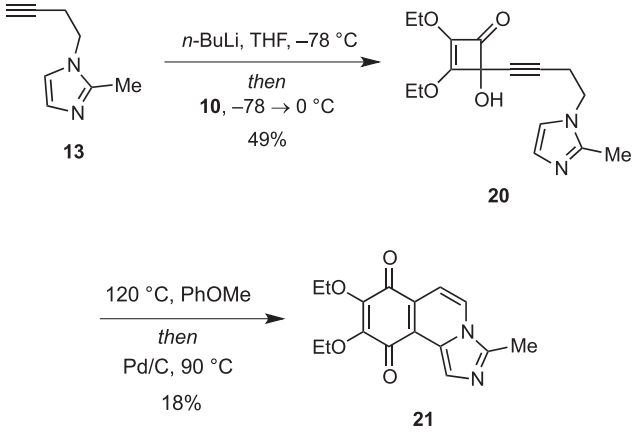
Owing to the extraordinarily concise nature of our approach to cribrostatin 6 (**1**), the synthesis of its analogs became a practical

matter. As noted previously, we had envisioned that changing the starting materials would give rapid access to a variety of cribrostatin 6 analogs (**2**) (Scheme 8). More specifically, we envisioned that the *N*-alkylated azole **17** could be readily modified by varying X, Y, and R_3. It would also be a simple matter to alter R_1 and R_2 on the squarate input **18**, because a large number of squarate derivatives are known and can be readily accessed.[11] Combining these inputs would then furnish a number of different intermediates **19** that should deliver cribrostatin 6 analogs **2** upon ring opening, radical cyclization, and oxidation.



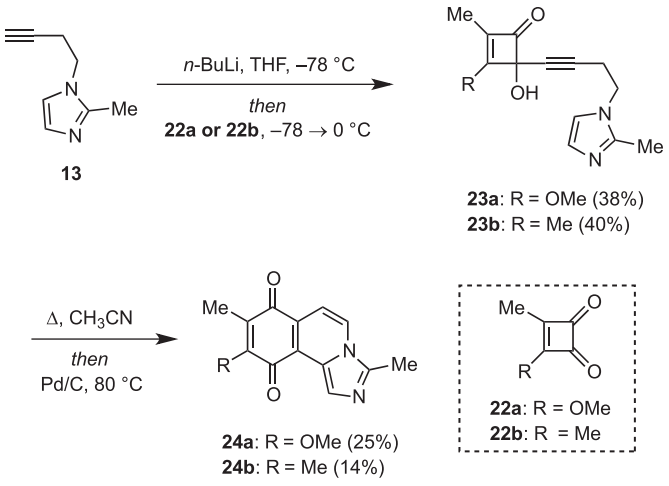
Scheme 8.

Toward the objective of synthesizing cribrostatin 6 analogs, we first explored a route to the diethoxy analog **21** (Scheme 9). Thus, commercially available diethyl squarate (**10**) was coupled with the anion of alkyne **13** to afford cyclobutenol **20**. When **20** was heated under reflux in CH_3CN for 24 h, only unreacted starting material was recovered. On the other hand, heating a solution of **20** in anisole (1.0 mM), followed by evaporating most of the solvent, and heating the resulting solution with Pd/C afforded cribrostatin 6 analog **21** in 18% overall yield from **20**.



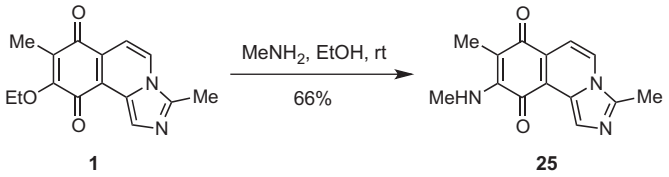
Scheme 9.

Several additional analogs of cribrostatin 6 were then prepared using the known squarate derivatives **22a** and **22b** as inputs (Scheme 10).[11] The standard coupling protocol with **13** was employed to afford key intermediates **23a**,**b**. Thermolysis of these cyclobutenols followed by oxidation in one pot provided the expected cribrostatin 6 analogs **24a**,**b** in acceptable overall yields (unoptimized).



Scheme 10.

Ready access to cribrostatin 6 (**1**) itself creates the possibility that other analogs might be prepared from the natural product itself. In order to explore the feasibility of conducting such transformations, **1** was treated with methylamine to afford **25** in 66% yield (Scheme 11). This important discovery now opens the door for synthesizing a wide range of cribrostatin 6 analogs via analogous addition/elimination sequences using alcohols, thiols, and other amines.



Scheme 11.

3. Summary

We have completed a remarkably concise synthesis of cribrostatin 6 (**1**) and several analogs by a novel approach that involves the rapid buildup of molecular complexity. The synthesis of **1** required a mere four steps in the longest linear sequence (five total steps from commercially available starting materials) and proceeded in 14.1% overall yield. The approach features the assembly of the natural product by a convenient, one-pot operation that proceeds via a tandem 4π-electrocyclic ring opening of a squarate derivative and a radical cyclization/homolytic aromatic substitution, followed by oxidation. The use of an azole as a reaction partner in the cyclization of phenyl radicals generated from squarates also represents a significant expansion of a related process discovered by Moore and co-workers. Further applications of this chemistry to the synthesis of additional cribrostatin 6 analogs as well as evaluation of the biological potency of these novel compounds is currently underway and will be reported in due course.

4. Experimental

4.1. General

Solvents and reagents were reagent grade and used without purification unless otherwise specified. THF was passed through two columns of neutral alumina. CH_3CN and DMF were passed through two columns of molecular sieves. Reactions involving air- or moisture-sensitive reagents or intermediates were performed

under an inert atmosphere of argon or nitrogen in glassware that had been oven dried. Reaction temperatures refer to bath temperatures. Melting points are uncorrected. Infrared (IR) spectra were recorded neat on sodium chloride plates and are reported in wave numbers (cm^{-1}). ^1H and ^{13}C NMR spectra were obtained as solutions in $CDCl_3$ unless otherwise noted, and chemical shifts are reported in parts per million (ppm) in reference to $CDCl_3$ (7.24 ppm and 77.0 ppm, respectively). Coupling constants are reported in hertz (Hz). Spectral splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; app, apparent; br, broad; m, multiplet; comp, overlapping multiplets of magnetically nonequivalent protons. All products that were used without further purification were >95% pure by ^1H NMR spectroscopy.

4.2. Experimental procedures

*4.2.1. But-3-ynyl-4-methylbenzenesulfonate (**12**).* A solution of *n*-BuLi (2.80 mL, 2.60 M, 7.27 mmol) in hexanes was added dropwise to a solution of 6 (0.50 mL, 6.61 mmol) in THF (7.5 mL) at −78 °C. After 5 min at −78 °C, stirring was continued for 30 min at 0 °C. *p*-TsCl (1.51 g, 7.93 mmol) in THF (8 mL) was added, and the reaction was stirred for 30 min at 0 °C. H_2O (2 mL) was added, and the mixture was extracted with Et_2O (3×10 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure to give 1.41 g (95%) of **12** as a pale yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J=8.0 Hz, 2H), 7.33 (d, J=8.0 Hz, 2H), 4.08 (t, J=7.1 Hz, 2H), 2.53 (dt, J=7.1, 2.6 Hz, 2H), 2.43 (s, 3H), 1.95 (t, J=2.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.0, 132.7, 129.9, 127.9, 78.3, 70.7, 67.4, 21.6, 19.4; IR (neat) 3290, 2962, 2919, 1598, 1359, 1190, 1176, 980, 904, 815 cm^{-1}; mass spectrum (ESI) m/z 247.0399 [$C_{11}H_{12}O_3S$ (M+23) requires 247.0400], 471 (base), 247, 225.

*4.2.2. 1-(But-3-ynyl)-2-methyl-1H-imidazole (**13**).* 2-Methylimidazole (**7**) (4.456 g, 54.27 mmol) was added to a solution of **12** (2.434 g, 10.85 mmol) in CH_3CN (15 mL). The reaction was heated for 18 h at 70 °C, whereupon it was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give an oil that was further purified by short path distillation under reduced pressure (2 Torr) at 150 °C to give 1.346 g (92%) of **13** as a clear oil; ^1H NMR (400 MHz, $CDCl_3$) δ 6.85 (d, J=1.2 Hz, 1H), 6.82 (d, J=1.2 Hz, 1H), 3.95 (t, J=7.0 Hz, 2H), 2.53 (dt, J=7.0 Hz, 2H), 2.35 (s, 3H), 1.99 (t, J=2.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.3, 127.1, 118.9, 79.6, 71.1, 44.3, 20.9, 12.8; IR (neat) 3287, 2923, 1501, 1425, 1281 cm^{-1}; mass spectrum (CI) m/z 135.0926 [$C_8H_{10}N_2$ (M+1) requires 135.0922], 135 (base).

*4.2.3. 3-Ethoxy-4-hydroxy-2-methyl-4-(4-(2-methyl-1H-imidazol-1-yl)but-1-ynyl)cyclobut-2-enone (**4**).* A solution of *n*-BuLi (0.53 mL, 2.44 M, 1.30 mmol) in hexanes was added dropwise to a solution of **13** (145 mg, 1.08 mmol) in THF (5.4 mL) at −78 °C. After 35 min at −78 °C, a solution of 3-ethoxy-4-methylcyclobutene-1,2-dione (**5**) (242 mg, 1.73 mmol) in THF (5 mL) at −78 °C was added dropwise via cannula. After 10 min at −78 °C, stirring was continued for 1.5 h at 0 °C. Saturated aqueous NH_4Cl (5 mL) and brine (5 mL) were added, and the mixture was extracted with EtOAc (3×20 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 185 mg (62%) of **4** as an amber oil; ^1H NMR (400 MHz, $CDCl_3$) δ 6.85 (d, J=1.4 Hz, 1H), 6.84 (d, J=1.4 Hz, 1H), 4.56–4.38 (comp, 2H), 3.99 (t, J=6.7 Hz, 2H), 2.67 (t, J=6.7 Hz, 2H), 2.39 (s, 3H), 1.65 (s, 3H), 1.43 (t, J=7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.9, 181.1, 144.4, 126.6, 123.9, 119.1, 85.0, 82.7, 78.6, 69.0, 44.3, 21.6, 15.1, 12.7, 6.4; IR (neat) 2986, 1763, 1620, 1327 cm^{-1}; mass spectrum (ESI, CI) m/z 275.1390 [$C_{15}H_{18}O_3N_2$ (M+1) requires 275.1393], 275 (base).

*4.2.4. 2-Ethoxy-3-methyl-5-(2-(2-methyl-1H-imidazol-1-yl)ethyl)-cyclohexa-2,5-diene-1,4-dione (**15**).* A solution of **4** (40 mg, 0.15 mmol) in chlorobenzene (6.2 mL) was heated for 30 min at 130 °C in a preheated oil bath. After cooling to room temperature, the reaction was stirred open to air for 16 h. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 10 mg (25%) of **15** as a yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (d, J=1.2 Hz, 1H), 6.75 (d, J=1.2 Hz, 1H), 6.27 (s, 1H), 4.29 (q, J=7.0 Hz, 2H), 4.00 (t, J=7.3 Hz, 2H), 2.78 (t, J=7.3 Hz, 2H), 2.34 (s, 3H), 1.94 (s, 3H), 1.33 (t, J=7.0 Hz, 3H); mass spectrum (ESI) m/z 275.1395 [$C_{15}H_{18}N_2O_3$ (M+1) requires 275.1390].

*4.2.5. Cribrostatin 6 (**1**).* A solution of **4** (96 mg, 0.35 mmol) in CH_3CN (350 mL) was heated under reflux for 35 min in a preheated oil bath (130 °C). After cooling to room temperature, the solution was concentrated to approximately 5 mL by evaporation under reduced pressure. Pd/C (15 mg, 10 wt % loading) was added, and the reaction was heated for 4 h at 80 °C. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 25 mg (26%) of **1** as a blue solid: mp 165–167 °C (lit.[3] 169–171 °C, lit.[7] 165–167 °C); ^1H NMR (600 MHz, $CDCl_3$) δ 8.23 (d, J=0.9 Hz, 1H), 7.83 (dd, J=7.3, 0.9 Hz, 1H), 7.17 (d, J=7.3 Hz, 1H), 4.38 (q, J=7.0 Hz, 2H), 2.67 (s, 3H), 2.04 (s, 3H), 1.40 (t, J=7.0 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 184.9, 180.7, 156.2, 137.7, 130.1, 125.9, 125.0, 124.7, 123.9, 123.5, 107.6, 69.6, 16.0, 12.6, 9.2; IR (neat) 2923, 1662, 1626, 1611, 1527, 1172 cm^{-1}; mass spectrum (ESI) m/z 271.1077 [$C_{15}H_{14}N_2O_3$ (M+1) requires 271.1078], 271 (base), 243, 215.

*4.2.6. 2,3-Diethoxy-4-hydroxy-4-(4-(2-methyl-1H-imidazol-1-yl) but-1-ynyl)cyclobut-2-enone (**20**).* A solution of *n*-BuLi (1.27 mL, 2.29 M, 2.91 mmol) in hexanes was added dropwise to a solution of **13** (340 mg, 2.53 mmol) in THF (12 mL) at −78 °C. After 35 min at −78 °C, a solution of 3,4-diethoxycyclobut-3-ene-1,2-dione (**10**) (862 mg, 5.07 mmol) in THF (6 mL) at 0 °C was added dropwise via cannula. After 10 min at −78 °C, stirring was continued for 1.5 h at 0 °C. Saturated aqueous NH_4Cl (5 mL) and brine (5 mL) were added, and the mixture was extracted with EtOAc (3×30 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with CH_2Cl_2/MeOH (10:1→5:1) to give 379 mg (49%) of **20** as a yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ 6.83 (d, J=1.4 Hz, 1H), 6.79 (d, J=1.4 Hz, 1H), 4.53–4.38 (comp, 2H), 4.25 (q, J=7.1 Hz, 2H), 3.96 (t, J=6.6 Hz, 2H), 2.63 (t, J=6.6 Hz, 2H), 2.35 (s, 3H), 1.40 (t, J=7.0 Hz, 3H), 1.27 (t, J=7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.3, 165.1, 144.4, 134.2, 126.5, 119.1, 84.2, 78.6, 78.1, 69.4, 67.0, 44.4, 21.5, 15.5, 15.2, 12.6; IR (neat) 3119, 2982, 1776, 1634, 1324, 1045 cm^{-1}; mass spectrum (ESI) m/z 305.1496 [$C_{16}H_{20}O_4N_2$ (M+1) requires 305.1499], 305 (base).

*4.2.7. 8,9-Diethoxy-3-methylimidazo[5,1-a]isoquinoline-7,10-dione (**21**).* A solution of **20** (89 mg, 0.29 mmol) in degassed anisole (290 mL) was heated at 120 °C for 2.5 h in a preheated oil bath. After cooling to room temperature, the solution was concentrated to approximately 5 mL by evaporation under reduced pressure. Pd/C (12 mg, 10 wt % loading) was added, and the reaction was heated for 15 h at 90 °C. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 16 mg (18%) of **21** as an aquamarine solid: mp 125–127 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.85 (d, J=7.4 Hz, 1H), 7.18 (d, J=7.4 Hz, 1H), 4.35 (q, J=7.1 Hz, 2H), 4.34 (q, J=7.1 Hz, 2H), 2.69 (s, 3H), 1.42 (t, J=7.1 Hz, 3H), 1.41 (t, J=7.1 Hz, 3H); ^{13}C NMR (151 MHz,

$CDCl_3$) δ 181.7, 181.3, 145.9, 145.6, 137.9, 126.6, 124.5, 124.0, 123.5, 123.3, 107.5, 69.91, 69.88, 15.6 (2C), 12.7; IR (neat) 2925, 1666, 1621, 1601, 1531, 1271, 1176 cm^{-1}; mass spectrum (CI) *m/z* 301.1182 [$C_{16}H_{16}N_2O_4$ (M+1) requires 301.1183].

*4.2.8. 4-Hydroxy-3-methoxy-2-methyl-4-(4-(2-methyl-1H-imidazol-1-yl)but-1-ynyl)cyclobut-2-enone (**23a**).* A solution of *n*-BuLi (1.30 mL, 2.61 M, 3.39 mmol) in hexanes was added dropwise to a solution of **13** (396 mg, 2.95 mmol) in THF (10 mL) at −78 °C. After 35 min at −78 °C, a solution of 3-methoxy-4-methylcyclobut-3-ene-1,2-dione (**22a**) (595 mg, 4.72 mmol) in THF (5 mL) at 0 °C was added dropwise via cannula. After 10 min at −78 °C, stirring was continued for 1.5 h at 0 °C. Saturated aqueous NH_4Cl (5 mL) and brine (5 mL) were added, and the mixture was extracted with EtOAc (3×20 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1→5:1) to give 241 mg (38%) of **23a** as an orange oil; ^1H NMR (400 MHz, $CDCl_3$) δ 6.79 (d, *J*=1.4 Hz, 1H), 6.74 (d, *J*=1.4 Hz, 1H), 4.11 (s, 3H), 3.93 (t, *J*=6.7 Hz, 2H), 2.61 (t, *J*=6.7 Hz, 2H), 2.30 (s, 3H), 1.59 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.0, 181.7, 144.4, 126.4, 124.1, 119.0, 84.9, 82.5, 78.6, 59.5, 44.3, 21.5, 12.5, 6.3; IR (neat) 3115, 2956, 1765, 1625, 1339 cm^{-1}; mass spectrum (ESI) *m/z* 261.1237 [$C_{14}H_{16}N_2O_2$ (M+1) requires 261.1234], 261 (base).

*4.2.9. 4-Hydroxy-2,3-dimethyl-4-(4-(2-methyl-1H-imidazol-1-yl) but-1-ynyl)cyclobut-2-enone (**23b**).* A solution of *n*-BuLi (1.54 mL, 2.59 M, 4.00 mmol) in hexanes was added dropwise to a solution of **13** (447 mg, 3.33 mmol) in THF (17 mL) at −78 °C. After 35 min at −78 °C, a solution of 3,4-dimethylcyclobut-3-ene-1,2-dione (**22b**) (550 mg, 5.00 mmol) in THF (5 mL) at −78 °C was added dropwise via cannula. After 10 min at −78 °C, stirring was continued for 1.5 h at 0 °C. Saturated aqueous NH_4Cl (5 mL) and brine (5 mL) were added, and the mixture was extracted with EtOAc (3×30 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (5:1) to give 322 mg (40%) of **23b** as a yellow oil; ^1H NMR (400 MHz, $CDCl_3$) δ 6.74 (d, *J*=1.4 Hz, 1H), 6.66 (d, *J*=1.4 Hz, 1H), 3.86 (t, *J*=6.7 Hz, 2H), 2.53 (t, *J*=6.7 Hz, 2H), 2.23 (s, 3H), 2.03 (s, 3H), 1.60 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.1, 178.1, 149.0, 144.1, 126.0, 118.9, 85.4, 84.0, 79.3, 44.2, 21.3, 12.3, 10.3, 7.5; IR (neat) 3520, 2922, 1760, 1644, 1426 cm^{-1}; mass spectrum (ESI) *m/z* 245.1290 [$C_{14}H_{16}N_2O_2$ (M+1) requires 245.1285].

*4.2.10. 9-Methoxy-3,8-dimethylimidazo[5,1-a]isoquinoline-7,10-dione (**24a**).* A solution of **23a** (240 mg, 0.92 mmol) in CH_3CN (400 mL) was heated under reflux for 35 min in a preheated oil bath (130 °C). After cooling to room temperature, the solution was concentrated to approximately 5 mL by evaporation under reduced pressure. Pd/C (31 mg, 10 wt % loading) was added, and the reaction was heated for 20 h at 80 °C. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (20:1) to give 60 mg (25%) of **24a** as a green-blue solid: mp 149–151 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.82 (d, *J*=7.2 Hz, 1H), 7.15 (d, *J*=7.2 Hz, 1H), 4.09 (s, 3H), 2.67 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.8, 180.5, 156.6, 137.7, 129.4, 125.9, 124.8, 124.7, 123.8, 123.5, 107.6, 61.1, 12.6, 9.0; IR (neat) 2925, 1666, 1628, 1291, 1156 cm^{-1}; mass spectrum (ESI) *m/z* 257.0924 [$C_{14}H_{12}N_2O_3$ (M+1) requires 257.0921].

*4.2.11. 3,8,9-Trimethylimidazo[5,1-a]isoquinoline-7,10-dione (**24b**).* A solution of **23b** (108 mg, 0.44 mmol) in CH_3CN (400 mL) was heated under reflux for 35 min in a preheated oil bath (130 °C). After cooling to room temperature, the solution was concentrated to approximately 5 mL by evaporation under reduced pressure. Pd/C (10 mg, 10 wt % loading) was added, and the reaction was heated for 15 h at 80 °C. After cooling to room temperature, the solvent was removed under reduced pressure, and the residue was purified by flash column chromatography eluting with ($CHCl_3$/MeOH 10:1) to give 15 mg (14%) of **24b** as a purple solid: mp >300 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (s, 1H), 7.82 (d, *J*=7.4 Hz, 1H), 7.16 (d, *J*=7.4 Hz, 1H), 2.67 (d, 3H), 2.12 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, one ArC not observed) δ 184.5, 183.9, 142.3, 141.3, 137.8, 126.4, 124.8, 124.5, 124.1, 107.6, 12.64, 12.60, 12.5; IR (neat) 2922, 1649, 1609, 1298, 1284 cm^{-1}; mass spectrum (ESI) *m/z* 241.0975 [$C_{14}H_{12}N_2O_2$ (M+1) requires 241.0972], 241 (base).

*4.2.12. 9-(Dimethylamino)-3,8-dimethylimidazo[5,1-a]isoquinoline-7,10-dione (**25**).* A solution of $MeNH_2$ (28 μL, 8.03 M, 0.23 mmol) in EtOH was added to a solution of **1** (12 mg) in EtOH (2.5 mL). After 18 h at room temperature, the solvent was removed under reduced pressure and the residue was purified by flash column chromatography eluting with $CHCl_3$/MeOH (10:1) to give 8 mg (66%) of **25** as a green solid: mp 208–210 °C; ^1H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 8.10 (s, 1H), 7.87 (d, *J*=7.5 Hz, 1H), 7.27 (d, *J*=7.5 Hz, 1H), 5.79 (d, *J*=4.0 Hz, 1H), 3.24 (d, *J*=4.0 Hz, 1.5H), 3.23 (d, *J*=4.0 Hz, 1.5H), 2.68 (s, 3H), 2.22 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.2, 181.5, 145.9, 137.2, 127.6, 125.5, 124.2, 123.7, 121.6, 108.8, 108.4, 32.8, 12.6, 10.5; IR (neat) 3583, 3325, 2922, 1620, 1515 cm^{-1}; mass spectrum (ESI) *m/z* 256.1087 [$C_{14}H_{13}N_3O_2$ (M+1) requires 256.1081], 256 (base).

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