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Total Synthesis and Biological Evaluation of Hybrubin A

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Abstract

Here we report the first total synthesis of hybrubin A, a bipyrrole tetramic acid alkaloid, representing a new carbon framework derived from convergent (truncated *red* cluster and exogenous *hbn* cluster) biosynthetic pathways. A highly convergent synthesis was developed employing 4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one (**13**) as a single starting material to provide hybrubin A in three steps from **13** and 20.8% overall yield. As no biological activity was prescribed to hybrubin A, except a lack of cytotoxicity, we further profiled this unique alkaloid across panels of discrete molecular targets. Interestingly, hybrubin A was found to be a ligand for a variety of GPCRs, with a propensity for potent binding across therapeutically relevant adenosine receptors (A₁, A_{2a} and A₃) as well as a potent activity at a kinase, FLT3. This pattern of biological activity is distinct from other related prodigiosin natural and unnatural products, and even more intriguing in the absence of cytotoxicity.

INTRODUCTION

Prodigiosin (1) and related tripyrrole natural products (**Figure 1**) have been the subject of renewed biological interest by virtue of their wide breadth of activities including cytotoxicity and antibacterial properties. In fact, the synthetic prodigiosin analogue obatoclax (2) was granted orphan drug status by the U.S. Food and Drug Administration for the treatment of chronic lymphocytic leukemia.¹ Marineosin A (3) is a novel macrocyclic spirolactam spiroiminal alkaloid structurally related to the prodigiosin family, with potent inhibition against human colon carcinoma cell growth, with an IC₅₀ of 0.5 μ M in HCT-116 cells.² A structurally related family, the tambjamines 4-6, are also highly cytotoxic and of interest in oncology.³ The surprising properties of these structural diverse classes of 2,2²-bipyrrolic alkaloid natural products have garnered significant attention to probe deeper into the potential applications of prodigiosin-like natural products.



Figure 1. Structures of prodigiosin (1) and related 2,2'bipyrrole cytotoxic alkaloids obatoclax (2), marineosin A (3), tambjamine A (4), the generic tambjamine core (5) and tambjamine K (6).



Figure 2. Structures of hybrubin A (7), hybrubin B (8) and hybrubin C (9).

To this end, structural analogs of prodigiosin, termed the hybrubins (**Figure 2**), and designated hybrubin A (7), hybrubin B (8) and hybrubin C (9), were recently discovered as the result of bacterial artificial chromosome library screening.⁴ These "unnatural" natural products are of interest due to their novel chimera structure which joins a 2,2'-bipyrrole motif common to prodigiosin biosynthesis (truncated *red* cluster) with a tetramic acid ring (exogenous *hbn* cluster). All three hybrubins **7-9** were isolated as rapidly interchanging C6' olefin isomers, with ratios of 1:1 to 3.5:1 by ¹H NMR, and further designated as

hybrubin A_1 and A_2 , hybrubin B_1 and B_2 and hybrubin C_1 and C_2 . While both pharmacophores of these chimera alkaloids are known to possess biological activity, initial studies demonstrated that, surprisingly, the hybrubins were devoid of the cytotoxic or antibacterial properties typical of the broader class.⁵ Therefore the need for independent synthesis to further probe for biological activity was apparent. Here we report the first total synthesis of hybrubin A via a highly convergent synthetic approach that enabled detailed biological evaluation and the discovery of a unique and unexpected pharmacological profile.

RESULTS AND DISCUSSION

Our first generation retrosynthetic analysis of **7** (**Figure 3**) begins with an elimination step to provide *tert*butyl ether **10**. A strategic disconnection across the C3'-C6' olefin then affords known bipyrrole **11** and the corresponding tetramic acid **12**, which can be joined through aldol chemistry. The synthesis began with efforts to generate bipyrrole **11**, which conveniently had been synthesized *en route* to marineosins A and B previously reported by our laboratory (**Scheme 1**).² The core 2,2'-bipyrrole of **11** has been the subject of numerous other syntheses and methodologies spanning over five decades both in the context presented here as well as in the context of porphyrinoids, among others.⁶⁻¹¹ Conversion of the commercially available **13**



Figure 3. First generation retrosynthetic analysis of hybrubin A (1).

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began with modified Vilsmeier conditions to formulate the pyrrolidine with concurrent installation of a bromine atom to afford 14. Treatment of 14 with standard Suzuki coupling conditions with the commercially available N-Boc-2-pyrrole boronic acid vielded desired bipyrrole 11 in 44% vield, after the desired coupling and hydrolysis of enamine were achieved with complete conversion of 14. Upon purification, a small amount of N-Boc deprotected 11 could be collected; further explanation of the modest yield is possible homocoupling or protodeboronation occasionally seen in cross-couplings of heteroarvl halides. То address the latter. facile transformations utilizing potassium heteroaryltrifluoroborates¹² or MIDA boronates¹³ as opposed to standard boronic acids have been reported which help avoid these side reactions. As seen with our previously reported synthesis using this route (see ref. 2) yields were generally low; therefore this sequence was performed on gram scale to supply material for the completion of the total synthesis. Of note is the brevity of the sequence in which four distinct chemical transformations are made in only two sets of reaction conditions.⁶

Scheme 1. Synthesis of the key bipyrrole 11.



With bipyrrole **11** in hand, our attention now turned to syntheses of the tetramic acid motif and ultimately hybrubin A (Scheme 2). Initially it was envisioned that the tetramic acid moiety **12** could be prepared in a single step from the amino acid *L*-threonine and Meldrum's acid using a previously reported cyclization reaction which has found use in several related syntheses.^{14,15} After a subsequent aldol condensation and removal of protecting groups a mixture of hybrubins A (7) and B (**8**) would be obtained. **15** was smoothly transformed to **12**, however it was quickly realized **12** was prone to decomposition from

silica gel chromatography, and as such, was taken on crude into the next step. Since **15** was used directly from a commercial source as the depicted diastereomer and the desired final product is devoid of the two stereogenic centers d.r. ratios of proceeding products were not obtained. The following Aldol condensation with **11** was the first reaction in the sequence which required significant optimization (**Table 1**). The use of stoichiometric amounts of heterogeneous and homogenous bases in various solvents and temperatures was unable to afford the desired condensation to deliver **10** in acceptable yields. Here it is worthwhile to mention that sodium hydride, when employed in two one equivalent portions, resulted in chiefly recovered starting materials presumably on basis of the reversibility of the aldol reaction. When employing catalytic reagents, yields increased noticeably; however, when exposed to reaction temperatures above 80 °C, the labile Boc protecting group was also removed. This observation was later used to our advantage when the transformation was carried out in refluxing ethanol with piperidine to elicit both the desired aldol condensation and removal of the labile pyrrole Boc group in good yields (68% for two steps). With the carbon skeleton of the hybrubins in place, completion of the synthesis required only deprotection of the *tert*-butyl ether moiety and the Boc group on the tetramic acid, followed by dehydration to give **7** and **8**.

Table 1. Reaction optimization for Aldol condensation to produce 10.



Entry	Base	Temp (°C)	Solvent	Yield
1	NEt ₃ (3 eq)	65	THF	3.2%
2	NEt ₃ (3 eq)	80	1,4 dioxane	10%
3	N,N-Diisopropylethylamine (3 eq)	80	1,4 dioxane	3.1%
4	Cs_2CO_3 (2 eq)	23	THF	3%
5	NaH (2 eq)	60	THF	7.8%
6	NaOtBu (2.5 eq)	62	MeOH	0
7	<i>L</i> -proline (0.05 eq)	30	DMSO	5.5%
8	<i>L</i> -proline (0.20 eq)	90	DMSO	23.4%
9	(S)-5-(pyrrolidine-2-yl)-1H-tetrazole)	90	DMSO	32%
	(0.20eq)			





After screening numerous conditions for the removal of both protecting groups in **10** simultaneously it became evident a two-step procedure would be more advantageous.¹⁶ Owing to the high aqueous solubility of **16** it was further desired to perform these transformations in absence of aqueous workups. With these goals in mind, a deprotection sequence which entailed thermal removal of the Boc group followed directly by treatment of crude material with TMSI was developed which furnished **16** in 85% yield across both steps.^{17,18} Subsequent attempts at dehydration of the free alcohol were once again subject to optimization. Traditional acidic or basic conditions (i.e. HCl in dioxanes, TFA in CHCl₃, and NaOMe in MeOH) failed to affect the desired dehydration. Dehydration reagents such as SOCl₂, POCl₃, and Martin Sulfurane were also unable to efficiently dehydrate **16**, and provided only unreacted starting material.¹⁹ It was not until **16** was treated with acetic anhydride and heated to 110 °C that the desired conversion of **16** to a mixture of **7** and **8** was achieved, albeit in low yields (19%). The route presented in

Scheme 3 is capable of successful total synthesis of 7 and 8, but is dominated by protecting group manipulations and culminates in harsh, low yielding dehydration conditions. Additionally, compounds 7 and 8 were unable to be separated via HPLC despite following analogous conditions to those reported by Tao and coworkers.⁴

Thus, a second generation approach would be required to access 7 in high yield and purity for biological study. While developing the first approach, a secondary strategy towards hybrubin A (7) was simultaneously explored (**Figure 4**). This approach utilized the same starting material **13** as the complementary arm of the convergent synthesis for key fragments **11** and **17**, and prioritized the



Figure 4. Second generation retrosynthetic analysis of hybrubin A (7).

installation of the exocyclic olefin. Therefore, treatment of **13** (Scheme 3) with acetaldehyde and sodium *tert*-butoxide to furnish **18** provided an appealing alternative to the multi-step deprotection and dehydration sequence previously developed. The anticipated transformation proceeded smoothly and purification of the crude reaction mixture on silica gel yielded fractions of one pure olefin isomer and fractions of complex mixtures. NOESY experiments showed the pure isomer thus obtained was the desired *Z*-olefin; proton NMR experiments were never able to confirm noticeable presence of the *E* olefin.²⁰ This observation is supported by $A_{1,3}$ strain considerations which would favor the *Z*-olefin.²¹

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While the collection of only a single olefin geometry at this stage denies access to hybrubin B, it also provides a complementary approach to the isolation of hybrubin A from B via HPLC performed by Tao and coworkers. After facile transformation of **18** to **17** by brief treatment with concentrated HCl in THF and subsequent aldol condensation with the previously optimized conditions, **7** was obtained in 70% yield. In summary, hybrubin A (**7**) was selectively obtained in a convergent fashion from a shortest linear three step reaction sequence from **13**. The second generation sequence provides **7** in 20.8% overall yield in only 20.5 total hours of reaction time, without need for chromatographic separation of the exocylic olefins or discrete protecting group manipulations from a single starting material **13**. The synthetic **7** was identical to the natural product in all aspects.^{4,22}

Scheme 3. Second generation approach to the tetramic acid moiety and the synthesis of hybrubin A (7).



With sufficient quantities of 7 in hand, we initiated biological studies. First, we evaluated cytotoxicity, as related prodigiosin alkaloids **1-6** displayed robust cytotoxicity against HCT-116 colon cancer cell lines. To our surprise, 7 (at concentrations up to 10 μ M) was devoid of cytotoxicity after

incubation in HCT-116 cells for 24 and 48 hours as measured by cell viability (WST-1 metabolism).²³ Thus not only does 7 differentiate structurally from 1-6, but also differs in terms of general bioactivity. Based on this outcome, we then evaluated 7 in radioligand binding assays against 68 discrete targets (GPCRs, ion channels and transporters) at an initial concentration of 10 μ M.²⁴ We were excited to see that 7 afforded significant activity (**Table 2**) at only 4 of the 68 targets (>70% inhibition@10 μ M), with the greatest activity against the adenosine receptors (A₁, A_{2a} and A₃) as well as the serotonin 5-HT_{2B} receptor. Follow-up K_i determinations showed that 7 was a potent ligand to these GPCRs, affording micromolar

Table 2. Summary of significant results of 7 against a panel of GPCRs, ion channels and transporters.

Target	Species	% inhibiton@10 µM	$K_i (nM)^a$
Adenosine A ₁	human	80	2,610
Adenosine A _{2A}	human	92	550
Adenosine A ₃	human	101	54
Serotonin 5-HT _{2B}	human	97	1,050

potency at A₁ (2.6 μ M) and 5-HT_{2B} (1.05 μ M) and nanomolar binding to A_{2A} (550 nM) and A₃ (54 nM). Thus, 7 represents a new chemotype for a selective A₃ ligand, with 10-fold selectivity over A_{2A} and 48-fold selectivity over A₁. We also screened 7 against a panel of 369 wildtype and mutant kinases at a concentration of 10 μ M, and noted very limited kinase activity (significant reduction of enzyme activity at only 7 of the 369 kinases) and upon IC₅₀ determinations, 7 proved to only inhibit FLT3 with submicromolar potency (IC₅₀ = 505 nM), as the other six kinase hits had IC₅₀ values in the 1.6 to 7.9 μ M range (and the remaining 362 kinases had IC₅₀S > 10 μ M).²⁵ Coupled with the lack of cytotoxicity of 7 and the high kinome selectivity for FLT3, a target for acute myeloid leukemia (AML), 7 represents an attractive lead compound derived from nature.²⁶ These data also suggest that additional FLT3 ligands might be mined from screening known A₃ ligands, and *vice versa*. Overall, the biological profile of 7 is unique and very exciting, and argues for the synthesis of unnatural analogs to develop SAR and new *in vivo* tool compounds.

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In conclusion, we performed the first total synthesis of hybrubin A (7) via a rapid and highly convergent approach (3 steps, 20.8% overall yield from **13**) employing a single starting material to access two chemically distinct, advanced intermediates. Extensive biological studies showed that 7 lacked general cytotoxicity, and was a potent ligand at a number of therapeutically relevant GPCRs, with selective binding to the A₃ receptor. In addition, a broad kinase panel found 7 to be a selective inhibitor of FLT3, a therapeutic target for AML. Based on these data, we are currently evaluating the *in vivo* efficacy of 7 in animal models as well as developing routes to access hybrubins B and C selectively, as well as unnatural analogs. Studies are underway and additional results and refinements will be reported in due course.

EXPERIMENTAL SECTION

General. All reagents and solvents were commercial grade and purified prior to use when necessary. Thin layer chromatography (TLC) was performed on glassbacked silica gel. Visualization was accomplished with UV light, and/or the use ninhydrin or potassium permanganate solution followed by brief heating with a heat gun. Flash chromatography on silica gel was performed using Silica Gel 60 (230-400 mesh). Preparative reversed-phase HPLC was run with a system containing a C₁₈ column (30 x 100mm). IR spectra were acquired from samples which were applied to NaCl plates as DCM solutions followed by generous air drying. Melting points were obtained with an automated melting point system. ¹H and ¹³C NMR spectra were recorded on either 400 MHz or 600MHz instruments. Chemical shifts are reported in ppm relative to residual solvent peaks as an internal standard at the following chemical shifts (¹H and ¹³C respectively): 7.26 and 77.1 ppm for CDCl₃; 2.50 and 39.5 ppm for DMSO-*d*₆, 3.31 and 49.2 ppm for CD₃OD. Data are reported as follows: chemical shift, multiplicity (s = singlet, d =

doublet, t = triplet, q = quartet, p = pentet, qd = quartet of doublets, br = broad, m = multiplet), coupling constant (Hz), integration. Low resolution mass spectra (LCMS) were obtained using a quadrupole with electrospray ionization generating $[M+H]^+$ or $[M+Na]^+$ ions. High resolution mass spectra (HRMS) were obtained using a Q-TOF with electrospray ionization generating $[M+H]^+$ or $[M+Na]^+$ ions.

(5Z)-5-ethylidene-3-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)pyrrolidine-2,4*dione* (7). To a stirred solution of (5Z)-5-ethylidenepyrrolidine-2,4-dione 17 (47.1 mg, 0.380 mmol) in ethanol (1.07 mL) and piperidine (86.8 μ L, 0.540 mmol) was added 11 (78.0 mg, 0.270 mmol) and the resulting mixture warmed to 82 °C and allowed to stir for 8 hours. Upon reaction completion as determined by LC/MS the mixture was cooled to ambient temperature and all volatiles were removed in vacuo. The resulting deep red gel was dissolved in a 1 mL of methanol and purified over a strong cation exchange column. Desired material eluted after addition of 2M ammonia solution in MeOH; the organics collected from this method were brought to dryness under a steady stream of air before being resuspended in a 15% MeOH in DMSO solution for purification using Gilson reverse-phase chromatography system (30 x 100 mm column, 32-51% MeCN / 0.1% trifluoroacetic acid (aq), 9 min gradient, 300 nm wavelength) to afford hybrubin A, (5Z)-5-ethylidene-3-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5yl)methylene)pyrrolidine-2,4-dione, as a red solid (56.2 mg, 0.189 mmol, 70% yield). m.p. 246-248 °C: ¹H NMR (400.1 MHz, DMSO-d₆ major regioisomer) δ (ppm): 13.58 (br.s, 1H), 12.01 (br.s, 1H), 9.89 (s, 1H), 7.1924 (s, 1H), 7.1919 (s, 1H), 6.79 (br.s, 1H), 6.58 (d, J= 2.4 Hz, 1H), 6.35 (m, 1H), 5.55 (q, J=7.4 Hz, 1H), 3.98 (s, 3H), 1.78 (d, J=7.4 Hz, 3H). ¹³C {¹H} NMR .(150 MHz, DMSO-d₆, major regioisomer) δ (ppm): 182.0, 169.0, 161.6, 137.9, 136.5, 124.1, 123.5, 122.5, 121.1, 111.0, 110.4, 105.5, 103.1, 93.3, 58.8, 12.1. FTIR: (neat) v 2916, 2848,

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2356, 2335, 1670, 1613, 1549, 1504, 1454, 1365, 1212, 1133, 952, 808 cm⁻¹. LCMS: R_T: 0.883 min., $m/z = 298.1 [M + H]^+$, >99% abs @ 215, 254, and 300n, HRMS-ESI: calcd for C₁₆H₁₅N₃O₃Na [M + Na]⁺ m/z 320.1013; found m/z 320.1003.

tert-butyl (S)-2-((R)-1-(tert-butoxy)ethyl)-4-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)-3,5-dioxopyrrolidine-1-carboxylate (10). Piperidine (389.2 µL, 2.41 mmol) was added to a stirring solution of **12** (613.55 mg, 2.05 mmol) in ethanol (4.82 mL). The resulting solution was allowed to briefly stir before 11 (350 mg, 1.21 mmol) was introduced and the reaction warmed 82 °C. After 6 hours of stirring LC/MS confirmed reaction completion and all volatiles were removed *in vacuo* to produce crude product as a pitch colored oil. Upon purification using Teledyne ISCO Combi-Flash system (silica gel loading, 12G column, 15-40% EtOAc, 30 min run) tert-butyl (S)-2-((R)-1-(tert-butoxy)ethyl)-4-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5vl)methylene)-3,5-dioxopyrrolidine-1-carboxylate was obtained as a red solid and mixture of interchanging C6' olefin isomers (388 mg, 0.820 mmol, 68% yield). m.p. 166-168 °C ¹H NMR (400.1 MHz, CDCl₃, integrations based off more abundant C6' isomer) δ (ppm): 13.36 (s, 1H), 9.64 (s, 1H), 7.39 (s, 1H), 7.09-7.07 (m, 1H), 6.75-6.73 (m, 1H), 6.38-6.36 (m, 1H), 6.07 (d, J=2.4Hz, 1H), 4.43 (q.d, 1H, J = 6.6, 1.5 Hz), 4.10 (d, 1H, J = 1.5Hz), 3.92 (s, 3H), 1.60 (s, 9H), 1.16 (s, 9H), 1.10 (s, 3H). ¹³C {¹H} NMR .(100.6 MHz, CDCl₃) δ (ppm): 192.4, 170.0, 163.2, 150.3, 140.4, 124.4, 124.1, 123.8, 122.7, 112.8, 111.2, 106.1, 92.9, 82.4, 74.6, 68.2, 66.7, 58.5, 28.5, 28.3, 20.1. LCMS: R_T: 1.098 min., $m/z = 472.3 [M + H]^+$, >99% abs @ 215 and 254nm. **HRMS-ESI:** calcd for C₂₅H₃₄N₃O₆ $[M + H]^+$ *m/z* 472.2449; found *m/z* 472.2443.

tert-butyl 5'-formyl-4'-methoxy-1H,1'H-[2,2'-bipyrrole]-1-carboxylate (11).

1-*Boc*-pyrrole-2-boronic acid (3.13 g, 14.8 mmol) and **14** (2.56 g, 9.88 mmol) were dissolved in degassed 1,4-dioxane (74.1 mL) and water (8.2 mL). Sodium carbonate (3.14 g, 29.6 mmol) was

then added and the mixture purged with argon for one minute before

Tetrakis(triphenylphosphine)palladium(0) (1.14 g, 0.988 mmol) was added and the reaction vessel sealed and heated to 85 °C for 3.5 hours. The mixture was then cooled to ambient temperature and poured into water (130 mL) followed by the slow addition of 1 N aq. HCl to achieve pH = 7. The solution was next transferred to a separatory funnel and extracted with DCM (3 x 70 mL). The resulting organic layers were dried over magnesium sulfate, filtered, and condensed directly onto silica gel for purification using Teledyne ISCO Combi Flash system (solid loading, 40G column, 0-30% EtOAc, 35 min run) to afford *tert*-butyl 5'-formyl-4'- methoxy-1*H*,1'*H*-[2,2'-bipyrrole]-1-carboxylate as a light orange solid (1.27 g, 4.37 mmol, 44% yield).^{2,6} ¹**H** NMR (400.1 MHz, CDCl₃) δ (ppm): 10.74 (br.s, 1H), 9.51 (s, 1H), 7.32 (m, 1H), 6.65 (m, 1H), 6.23 (t, *J*= 3.6Hz, 1H). 6.06 (d, *J*= 2.6 Hz, 1H), 3.87 (s, 3H), 1.59 (s, 9H). ¹³C {¹H} NMR (100.6 MHz, CDCl₃) δ (ppm): 174.3, 157.7, 149.7, 130.3, 126.0, 124.5, 118.3, 116.9, 111.5, 94.8, 85.7, 57.9, 27.9 **LCMS:** R_T: 0.988 min., *m/z* = 291.1 [M + H]⁺. **HRMS-ESI:** calcd for C₁₅H₁₉N₂O₄ [M + H]⁺ *m/z* 291.1347; found *m/z* 291.1340.

tert-butyl (S)-2-((R)-1-(tert-butoxy)ethyl)-3,5-dioxopyrrolidine-1-carboxylate **(12).** To a solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid, 1.73 g, 12.0 mmol) and 4dimethylaminopyridine (1.86 g, 15.3 mmol) in DCM (72.6 mL) at 0 °C was added **15** (3.00 g, 10.9 mmol) and then *N*-(3-dimethylaminopropyl-*N*-ethylcarbodiimide hydrochloride (EDC, 2.53 g, 13.1 mmol). The resulting mixture was warmed to ambient temperature and allowed to stir for 21 hours. At this time all solvent was removed *in vacuo* and the resulting yellow oil resuspended in EtOAc (175 mL) and washed with brine (2 x 80 mL), 1M citric acid (3 x 100 mL), and again brine (100 mL). The resulting organic layer was passed through a phase separator and heated to 77 °C for 90 minutes. Upon cooling all volatiles were removed *in vacuo*

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to afford crude *tert*-butyl (*S*)-2-((*R*)-1-(*tert*-butoxy)ethyl)-3,5-dioxopyrrolidine-1-carboxylate as a yellow foamy oil (2.93 g, 9.78 mmol, 89% yield). ¹**H NMR** (400.1 MHz, CDCl₃) δ (ppm): 4.59 (p, *J* = 6.2Hz, 1H), 4.34 (d, *J* = 5.2 Hz, 1H), 3.60 (s, 2H), 1.50 (s, 9H), 1.27 (s, 9H). 0.99 (d, *J*= 6.4 Hz, 3H) **LCMS:** R_T: 0.919 min., *m/z* = 621.2 [2M + Na]⁺, >99% abs @ 215 and 254nm. **HRMS-ESI:** calcd for C₁₅H₂₅NO₅Na [M + Na]⁺ *m/z* 322.1633; found *m/z* 322.1627.

N-((5-bromo-3-methoxy-2H-pyrrol-2-vlidene)methyl)-N-ethylethanamine (14). A 250 mL round bottom flask containing a stir bar was charged with N,N-diethylformamide (4.72 mL, 42.4 mmol) and DCM (35 mL). The mixture was then cooled in an ice bath and a solution of phosphorus (V) oxybromide (15.2 g, 53.1 mmol) in DCM (10.6 mL) was added dropwise over the course of several minutes. After addition was complete the reaction was allowed to stir for twenty additional minutes before a solution of 4-methoxy-3-pyrrolin-2-one (3.00 g, 26.5 mmol) in DCM (26 mL) was introduced dropwise over the course of several minutes. Once the addition was complete the reaction mixture was transferred directly from the ice bath to metal round bottom warmer preheated to 42 °C and allowed to stir for 3.5 hours. Upon cooling of the reaction mixture to ambient temperature the material was transferred to a 1 L round bottom flask, cooled in an ice bath, and quenched with the dropwise addition of water (30 mL). Then, a solution of aqueous sodium hydroxide (15% w/w, 350 mL) was carefully added and the resulting mixture allowed to stir at ambient temperature for 20 minutes. The material was then transferred to a separatory funnel and extracted with DCM (3 x 120 mL). The combined organic layers were dried over magnesium sulfate, filtered, and condensed directly onto silica gel for purification using Teledyne ISCO Combi Flash system (solid loading, 40G column, 0-20% EtOAc, 25 min run) to afford N-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-N-ethylethanamine as pale vellow crystals (3.33 g, 12.8 mmol, 49% yield).^{2,6 1}**H NMR** (400.1 MHz, CDCl₃) δ (ppm): 6.99

(s, 1H), 5.59 (s, 1H), 4.11 (q, J= 7.1Hz, 2H), 3.75 (s, 3H), 3.39 (q, J= 7.2Hz, 2H), 1.28 (m, 6H) ¹³C {¹H} NMR (100.6 MHz, CDCl₃) δ (ppm): 165.2, 138.6, 133.4, 120.7, 96.3, 57.9, 51.1, 44.5, 14.5, 12.4. LCMS: R_T: 0.552 min., m/z = 259.2 [M + H]⁺ >99% abs @ 215 and 254nm. HRMS-ESI: calcd for C₁₀H₁₆BrN₂O [M + H]⁺ m/z 259.0448; found m/z 259.0439.

(S)-5-((R)-1-hydroxyethyl)-3-((4-methoxy-1H, 1'H-[2, 2'-bipyrrol]-5-yl)methylene)pyrrolidine-

2.4-dione (16). Solid 10 (59.5 mg, 0.160 mmol) was heated neat in a two dram vial fully immersed in a pie-block at 180°C for 90 minutes. The resulting crude solid was cooled and immediately dissolved chloroform (1.62 mL) and treated with trimethylsilvl iodide (47.6 µL, 0.320 mmol) and allowed to stir at 45 °C for one hour. The reaction mixture was then cooled to ambient temperature and diluted with MeOH (1 mL) before all volatiles were removed in vacuo. The resulting deep red solid was dissolved in MeOH (1 mL) and purified over a strong cation exchange cartridge with product eluting following addition of 2M ammonia in MeOH solution after several rinses of pure MeOH. In this manner, (S)-5-((R)-1-hydroxyethyl)-3-((4-methoxy-1H,1'H-[2,2'-bipyrrol]-5-yl)methylene)pyrrolidine-2,4-dione was obtained as a fine red solid (43.3 mg, 0.137 mmol, 85.7%) after complete removal of all volatiles. m.p 242-244 °C ¹H NMR (400.1 MHz, CD₃OD, inseparable mixture of olefins) δ (ppm): 7.30 (s, 2H), 7.10 (s, 2H), 6.88 (s, 2H), 6.35-6.33 (m, 3H), 6.33 (s, 1H), 4.16 (br.s, 2H), 4.01 (s, 3H), 3.99 (s, 3H), 3.77 (d, J=3.3 Hz, 1H), 1.34 (d, J=6.5 Hz, 3H), 1.12 (d, J=6.4 Hz, 3H), ¹³C {¹H} NMR (150 MHz, DMSO-d₆, inseparable mixture of olefins) δ (ppm):195.8, 195.3, 173.3, 171.3, 161.5, 161.0, 137.4, 137.1, 123.9, 123.3, 123.1, 122.1, 120.8, 120.4, 117.7, 117.4, 110.8, 110.5, 109.8, 108.8, 107.7, 107.2, 93.2, 92.4, 67.2, 66.9, 66.7, 65.0, 58.6, 58.5, 20.6, 17.1. LCMS: R_T: 0.833 min., m/z = 316.1 [M $+ H_{1}^{+}$, >99% abs @ 215, 254, and 300nm. **HRMS-ESI:** calcd for C₁₆H₁₈N₃O₄ [M + H]⁺ m/z 316.1299; found *m/z* 316.1292.

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(Z)-5-ethvlidenepvrrolidine-2,4-dione (17). To a stirred solution of (Z)-5-ethvlidene-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-one **18** (451 mg, 3.24 mmol) in THF (64.5 mL) at ambient temperature was carefully added concentrated HCl (38 mL). The resulting faint yellow solution was stirred at ambient temperature for 90 minutes at which time TLC confirmed reaction completion. Saturated aqueous sodium bicarbonate (40 mL) was then slowly added to quench the reaction. The reaction mixture was transferred to a separatory funnel and extracted with EtOAc (3 x 50 mL) and the resulting pooled organics were dried over magnesium sulfate. Crude product was purified using Teledyne ISCO Combi-Flash system (solid loading on silica gel, 4G column, 20-70% EtOAc, 25 min run) to afford (Z)-5-ethylidenepyrrolidine-2,4-dione as a light yellow solid (269 mg, 2.15 mmol, 66% vield). Spectroscopic data of this compound reported in compliance with previously reported data.²⁷ m.p 184-186 °C ¹H NMR (400.1 MHz, CDCl₃) δ (ppm): 9.32 (s, 1H), 5.76 (q, J=7.5 Hz, 1H), 3.10 (s, 2H x 1/2), 1.82 (d, J=7.5Hz, 3H). ¹³C {¹H} NMR .(100.6 MHz, CDCl₃) δ (ppm): 192.4, 170.7, 136.5, 106.7, 40.6, 12.0. LCMS: R_T: 0.116 min., $m/z = 126.2 [M + H]^+$, >99% abs @ 215 and 254 nm. **HRMS-ESI:** calcd for C₆H₈NO₂ [M + H]⁺ *m/z* 126.0557; found *m/z* 126.0548.

(*Z*)-5-ethylidene-4-methoxy-1,5-dihydro-2H-pyrrol-2-one (**18**). To a 100 mL round bottom flask equipped with a magnetic stir bar was added ethanol (44 mL), 4-methoxy-3-pyrrolin-2-one (1.00 g, 8.84 mmol), sodium *tert*-butoxide (1.70 g, 17.7 mmol), and lastly acetaldehyde (2.00 mL, 35.4 mmol). The resulting mixture was warmed to 40 °C and allowed to stir for four hours. Following this, all volatiles were removed via rotovaporation. The resulting orange oil was condensed directly onto silica gel for purification using Teledyne ISCO Combi-Flash system (12G column, 30-90% EtOAc, 45 min run). Careful TLC analysis of the resulting column fractions was used to selectively collect (*Z*)-5-ethylidene-4-methoxy-1,5-dihydro-2*H*-pyrrol-2-

one which upon concentration was afforded as a white solid (551 mg, 3.96 mmol, 45%). m.p 137-139 °C; ¹H NMR (400.1 MHz, CDCl₃) δ (ppm): 9.01 (s, 1H), 5.46 (q, *J*= 7.5 Hz, 1H), 5.09 (d, 1H, *J*= 1.5 Hz), 3.81 (s, 3H), 1.86 (d, *J*= 7.5Hz, 3H). ¹³C {¹H} NMR .(100.6 MHz, CDCl₃) δ (ppm): 172.8, 166.0, 134.1, 105.9, 92.4, 57.8, 12.4. LCMS: R_T: 0.112 min., *m/z* = 140.1 [M + H]⁺, >99% abs @ 215 nm. HRMS-ESI: calcd for C₇H₁₀NO₂ [M + H]⁺*m/z* 140.0713; found *m/z* 140.0703.

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Supporting Information. Supplemental Tables, data and copies of all ¹H and ¹³C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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