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Total Synthesis of Pyrophen and Campyrones A−C

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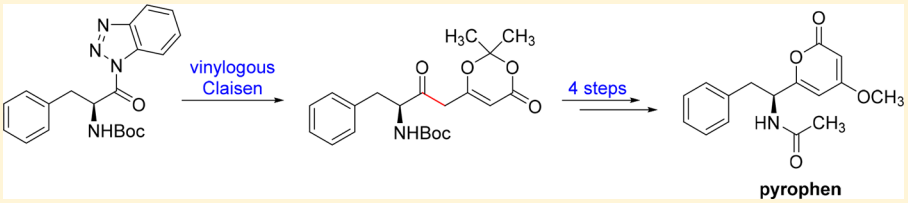
Supporting Information



ABSTRACT: The first total syntheses of the natural products pyrophen and campyrones A−C, isolated from the fungus *Aspergillus niger*, have been achieved in six steps starting from commercially available *N*-Boc amino acids. Key steps in this sequence include a vinylogous Claisen condensation to achieve fragment coupling and a dioxinone thermolysis/cyclization cascade to form the α-pyrone ring. The route described herein afforded the natural products in 15−25% overall yield, furnishing sufficient material for testing in biological assays.

Fungi of the genus *Aspergillus* produce a wide variety of bioactive polyketides, many of which exhibit antifungal and anticancer activity.[1] In 1990, Barnes isolated the natural product pyrophen (**1**) from cultures of *A. niger*, which causes black mold disease in several important commercial crops (Figure 1).[2] Since this initial report, pyrophen has been reisolated from several other *Aspergillus* species[3] as well as from the fungus *Alternaria alternata*.[4] In 2013, Laatsch and co-workers isolated the structurally related natural products campyrones A−C (**2**−**4**) from *A. niger*,[5] and compounds **2** and **4** have subsequently been isolated from *A. tubingensis*[6] and reisolated from *A. niger*.[7]

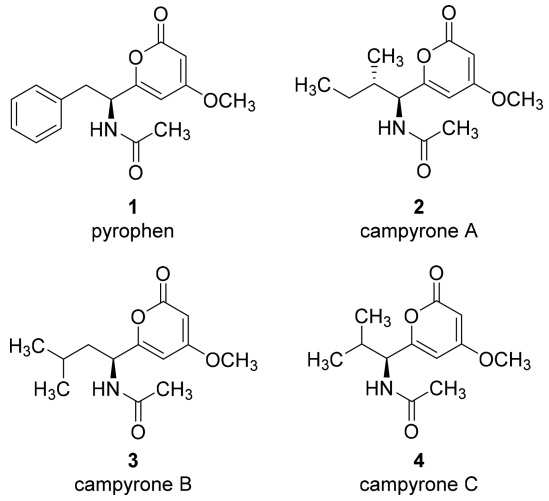


Figure 1. Structures of pyrophen and campyrones A−C.

Biosynthetically, **1** is likely derived from the amino acid L-phenylalanine and features a characteristic α-pyrone ring of polyketide origin.[8] Notably, compounds **2**−**4** contain the same α-pyrone functionality and could be derived from the amino acids L-isoleucine, L-leucine, and L-valine, respectively. Although the biosynthetic pathway leading to the formation of these compounds has not been fully elucidated, a plausible route is depicted in Figure 2. In this pathway, a polyketide synthase would mediate the sequential condensation of two equivalents of malonyl coenzyme A with an activated *N*-acetyl amino acid to form a 1,3,5-tricarbonyl intermediate. Intramolecular cyclization would release the substrate from the enzyme, forming a 4-hydroxy-α-pyrone after tautomerization. Natural

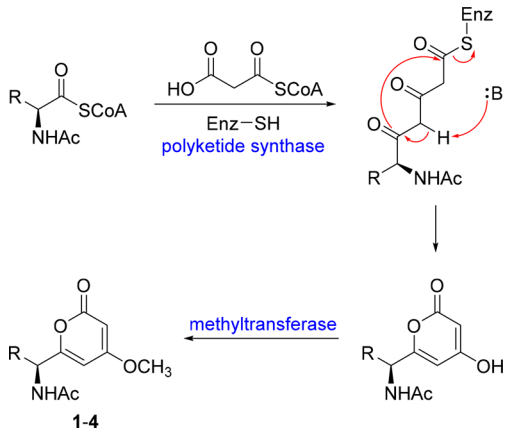


Figure 2. Putative biosynthesis of pyrophen and campyrones A−C.

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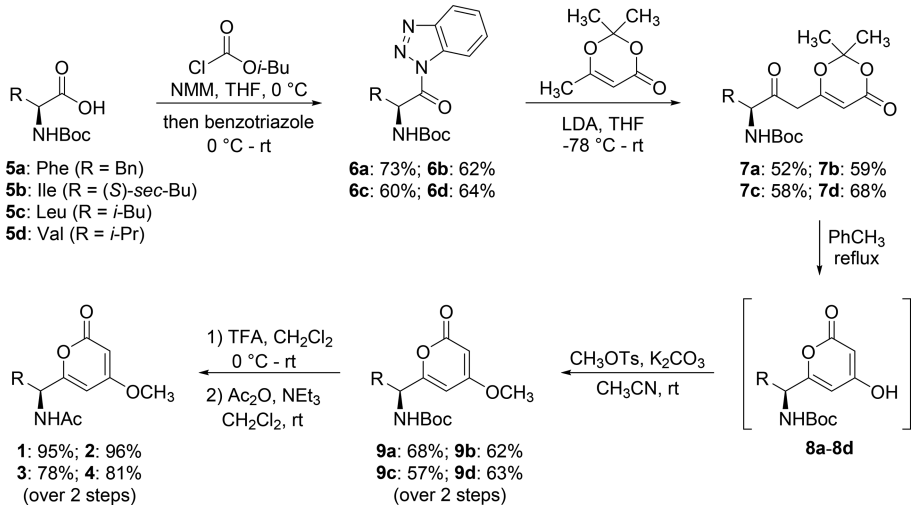
Scheme 1. Synthesis of Pyrophen and Campyrones A−C



products **1**−**4** would then be formed via a final methylation step.

In initial bioactivity assays, **1** was found to inhibit the growth of *Candida albicans*, the most common cause of hospital-acquired fungal infections.[4] More recently, Astuti reported that **1** exhibits promising cytotoxicity against T47D breast cancer cells, inducing S-phase arrest at a concentration of 1.39 μM.[3e] Compounds **2**−**4** were toxic when tested against brine shrimp larvae (40−42 μM) but exhibited no significant antimicrobial or antifungal activity.[5] To the best of our knowledge, no synthetic efforts toward pyrophen or the campyrones have been reported. In order to provide authentic samples of compounds **1**−**4** for further evaluation in anticancer assays, we set out to develop a scalable synthetic route to this family of natural products.

RESULTS AND DISCUSSION

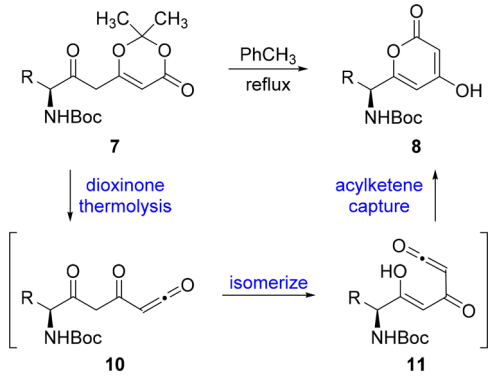
Inspired by the proposed biosynthesis of **1**−**4**, our strategy was to couple an activated amino acid derivative with a carbon-based nucleophile comprising two acetate units. Our route began with commercially available *N*-Boc amino acids **5a**−**d** (Scheme 1). After evaluating several different activated carboxylic acid derivatives[9] as potential electrophiles (including methyl esters, phenyl esters, pentafluorophenyl esters, acyl imidazoles, Weinreb amides, and Leuch anhydrides), we obtained the best results using acyl benzotriazoles.[10] Indeed, acyl benzotriazoles have been extensively used as electrophiles in Claisen condensations with enolates to form 1,3-dicarbonyl compounds.[11] Amino acid-derived acyl benzotriazoles have also been utilized in coupling reactions as neutral acylating agents that are resistant to epimerization at the α-stereocenter.[12]

We found that acyl benzotriazoles **6a**−**d** could be easily prepared via the intermediacy of a mixed anhydride and purified by either recrystallization (**6a** and **6d**) or column chromatography (**6b** and **6c**). It is interesting to note that attempts to form the acyl benzotriazoles starting from the corresponding *N*-acetyl amino acids (as found in the natural products) resulted in extensive epimerization at the α-stereocenter.[13] Presumably, the use of a carbamate protecting group disfavors the formation of an acidic azlactone intermediate, allowing the acyl benzotriazoles to be isolated without epimerization.

With acylbenzotriazoles **6a**−**d** in hand, we turned our attention to the key Claisen condensation reaction that would couple the amino acid and polyketide-derived fragments. Initially, we attempted to use the Weiler dianion derived from methyl acetoacetate as the nucleophilic component.[14] However, this reaction resulted in low yields due to difficulties in purifying the tricarbonyl product. As an alternative, we utilized a dioxinone-derived enolate as a protected β-keto ester dianion equivalent.[15] This vinylogous Claisen condensation proved to be a reliable and scalable method for fragment coupling, giving keto dioxinones **7a**−**d** in 52−68% yield after purification by column chromatography.

Upon thermolysis, keto dioxinones undergo a retro-Diels−Alder/cyclization cascade to form 4-hydroxy-α-pyrones (Scheme 2), as originally reported by Sato.[15a,16] This

Scheme 2. Dioxione Thermolysis/Acylketene Capture Cascade to Form 4-Hydroxy-α-pyrones



transformation is believed to proceed through a highly reactive acylketene intermediate **10**, which is captured intramolecularly (presumably via enol isomer **11**) to form the pyrone following tautomerization.[17] This strategy has found several applications in the synthesis of pyrone-containing natural products, including pyripyopene A,[15e] myxopyronin B,[18] and chatancin.[19] Thus, heating dilute solutions (10 mM) of dioxinones **7a**−**d** in refluxing toluene cleanly afforded the corresponding 4-hydroxy-α-pyrones **8a**−**d**, provided that the reaction was conducted under strictly anhydrous conditions.

Alternatively, pyrone formation could be achieved via the two-step one-pot sequence depicted in Scheme 3. In the first stage of the reaction, thermolysis of dioxonine **7** was carried out

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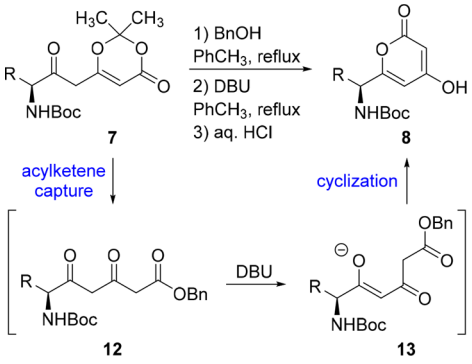
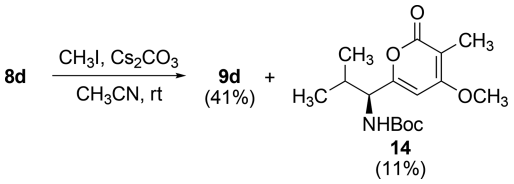
Scheme 3. Two-Step Synthesis of 4-Hydroxy-α-pyrones via a Tricarbonyl Intermediate



in the presence of benzyl alcohol, which reacted with the acylketene intermediate to form tricarbonyl compound **12**.[17] Subsequent addition of the amidine base DBU resulted in cyclization (likely via enolate **13**) to afford the same 4-hydroxy-α-pyrone after acidification.[20] Notably, this method of α-pyrone formation is somewhat biomimetic in that similar tricarbonyl cyclizations are mediated by polyketide synthases, as depicted in Figure 2.[8]

Since hydroxypyrones **8a–d** are highly polar and therefore difficult to purify, we found it convenient to use the crude compounds directly in the subsequent methylation step. After removal of the solvent, **8a–d** were dissolved in acetonitrile and treated with methyl *p*-toluenesulfonate and potassium carbonate at room temperature, affording the desired methoxypyrones **9a–d** in 57–68% yield over the two-step sequence. Interestingly, when this methylation step was carried out using iodomethane and cesium carbonate, the dimethylated pyrone byproduct **14** was formed in 11% yield along with the desired product **9d** (Scheme 4).[21] Presumably, the use of a soft

Scheme 4. Formation of a Dimethylated Byproduct under Soft Alkylation Conditions



electrophile and counterion resulted in some initial C-methylation of the extended enolate prior to O-methylation. Formation of this byproduct was completely suppressed in the presence of the harder alkylating agent methyl *p*-toluenesulfonate.[22] At this stage of the synthesis, all that remained was to install the *N*-acetyl group found in the natural products. Treatment of **9a–d** with trifluoroacetic acid resulted in clean removal of the Boc protecting group, and subsequent acetylation of the primary ammonium salt with acetic anhydride afforded pyrophen (**1**) and campyrones A–C (**2–4**) as crystalline solids in 78–96% yield over the two-step sequence.

After obtaining full characterization data for **1**, we noted several discrepancies in the reported NMR spectra for this natural product. Although the ^{1}H NMR chemical shifts for synthetic **1** match those reported by Barnes in the original isolation paper,[2] many of the observed ^{13}C NMR chemical shifts deviate by at least 0.5 ppm (and in some cases by more than 1.0 ppm). To date, pyrophen has been reisolated and characterized by five other groups, and NMR data have been reported in three different solvents (Table 1).[3a,b,e,f,4] Although there is good general agreement between the reported spectral data of **1** and those observed for our synthetic material, the most significant deviations are observed in acetone-d_6, particularly at C7 and C10. A more detailed discussion clarifying some additional inconsistencies in the reported NMR data and optical rotation of **1** is included in the Supporting Information. Gratifyingly, the ^{1}H and ^{13}C NMR spectral data of **2–4** were identical to those reported in the isolation paper; however, the observed optical rotations of synthetic **2–4** were of significantly higher magnitude [e.g., $[\alpha]^{25}_{D}$ −189 (*c* 0.11, CH_3OH) for synthetic **4** vs $[\alpha]^{20}_{D}$ −16 (*c* 0.11, CH_3OH) for natural **4**].[5]

In summary, we have achieved the first total syntheses of the fungal metabolites pyrophen (**1**) and campyrones A–C (**2–4**). The Claisen condensation of an extended enolate with amino acid-derived acyl benzotriazoles was used to form a key carbon–carbon bond, and subsequent pyrone formation could be achieved through either a one- or two-step dioxinone thermolysis/cyclization sequence. This robust and scalable six-step synthetic route allows for the preparation of larger quantities of these scarce natural products and their unnatural analogues. Efforts to further evaluate the biological activity of these compounds are currently underway in our laboratory.

EXPERIMENTAL SECTION

General Experimental Procedures. All reactions were carried out in flame-dried glassware with magnetic stirring under a positive pressure of argon. ACS reagent grade or anhydrous acetonitrile (CH_3CN), dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), hexanes, methanol (CH_3OH), tetrahydrofuran (THF), and toluene ($PhCH_3$) were used without further purification. Reactions were monitored by thin layer chromatography (TLC) using glass plates precoated with a 0.25 mm layer of silica gel containing a fluorescent indicator. TLC plates were visualized by exposure to ultraviolet light and subsequently stained with *p*-anisaldehyde or ninhydrin solution followed by heating on a laboratory hot plate. Silica gel for flash column chromatography had a 60 Å pore size and a 40–63 μm particle size and was 230–400 mesh.

Procedure A: Synthesis of Acyl Benzotriazoles. A 0.5 M solution of *N*-Boc amino acids **5a–d** (1.00 equiv) in anhydrous THF was cooled to 0 °C in an ice bath and treated with *N*-methylmorpholine (1.50 equiv) to give a colorless, homogeneous solution. Isobutyl chloroformate (1.10 equiv) was added dropwise over 10 min, resulting in the immediate formation of the morpholinium hydrochloride salt as a white precipitate. The reaction mixture was stirred at 0 °C for 45 min before the addition of solid benzotriazole (1.10 equiv) in a single portion. The cooling bath was allowed to slowly expire, and the reaction mixture was stirred at room temperature overnight, at which point TLC showed complete consumption of the *N*-Boc amino acid and formation of the acyl benzotriazole. The accumulated solids were removed by filtration through a pad of Celite, which was washed with additional THF. The solvent was removed under reduced pressure, and the crude product was purified via either recrystallization or column chromatography to afford the *N*-Boc acyl benzotriazoles **6a–d**.

*tert-Butyl (S)-(1-(1H-benzo[d][1,2,3]triazol-1-yl)-1-oxo-3-phenylpropan-2-yl)carbamate (**6a**).* The acyl benzotriazole was synthesized according to procedure A using *N*-Boc phenylalanine **5a** (2.00 g, 7.54 mmol, 1.00 equiv), *N*-methylmorpholine (1.24 mL, 11.3 mmol, 1.50 equiv), isobutyl chloroformate (1.08 mL, 8.29 mmol, 1.10 equiv), and benzotriazole (988 mg, 8.29 mmol, 1.10 equiv). The crude product was obtained as a viscous oil after evaporation of the solvent. Addition of hexanes gave a cloudy suspension, and the product precipitated as white crystals upon standing at room temperature for 10 min. The

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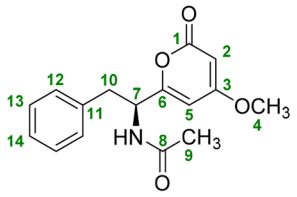
Table 1. Comparison of ^{13}C NMR Chemical Shifts of Natural and Synthetic Pyrophen (1) in Three Different Solvents[a]



	acetone-d_6		$CDCl_3$				DMSO-d_6		
	Barnes[b] (1990)	Current Work[c]	Crews[d] (2000)	Wang[e] (2010)	Shaaban[f] (2012)	Current Work[c]	Berlinck[g] (2016)	Astuti[h] (2016)	Current Work[c]
C1	163.2	163.8	164.4	164.1	164.7	164.5	164.2	163.8	163.8
C2	88.0	88.6	88.5	88.5	88.0	88.6	88.4	88.0	88.0
C3	170.8	171.1	170.5	171.0	171.0	171.0	170.0	170.8	170.8
C4	56.4	56.7	56.0	55.9	55.7	56.1	56.9	56.5	56.5
C5	99.4	100.4	101.2	101.0	100.6	101.2	99.8	99.4	99.4
C6	163.8	164.4	161.3	161.4	161.9	161.5	163.8	163.2	163.2
C7	52.1	53.4	52.4	52.5	52.3	52.6	52.5	53.0	52.1
C8	169.1	169.7	169.6	169.4	170.7	169.8	169.0	169.0	169.1
C9	22.4	22.7	23.2	23.1	22.3	23.2	22.8	22.4	22.4
C10	37.9	39.2	38.9	39.0	38.1	38.9	39.0	37.9	37.9
C11	137.2	138.2	135.8	135.9	136.0	136.0	137.0	137.3	137.3
C12	126.5	127.5	129.1	129.0	128.6	129.1	129.4	128.3[i]	129.1
C13	128.3	129.2	128.7	128.7	128.2	128.8	128.7	129.1[i]	128.3
C14	129.0	130.0	127.2	127.1	126.5	127.2	127.2	126.6	126.6

[a]Chemical shifts differing from synthetic **1** with 0.5 ppm ≤ |Δδ| < 1.0 ppm are highlighted in blue. Chemical shifts differing from synthetic **1** with |Δδ| ≥ 1.0 ppm are highlighted in red. [b]Collected at 75 MHz; ref 2. [c]Collected at 100 MHz. [d]Collected at 125 MHz; ref 3a. [e]Collected at 125 MHz; ref 3b. [f]Collected at 125 MHz; ref 4. [g]Collected at 100 MHz; ref 3f. [h]Collected at 100 MHz; ref 3e. [i]These assignments may have been inadvertently transposed in ref 3e.

solid was collected by vacuum filtration and recrystallized from 9:1 hexanes−EtOAc to give pure **6a** as a white, crystalline solid (2.02 g, 73%).

*tert-Butyl ((2S,3S)-1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-methyl-1-oxopentan-2-yl)carbamate (**6b**).* The acyl benzotriazole was synthesized according to procedure A using *N*-Boc isoleucine **5b** (2.00 g, 8.65 mmol, 1.00 equiv), *N*-methylmorpholine (1.43 mL, 13.0 mmol, 1.50 equiv), isobutyl chloroformate (1.23 mL, 9.51 mmol, 1.10 equiv), and benzotriazole (1.13 g, 9.51 mmol, 1.10 equiv). The crude product was obtained as an oil, which was purified by column chromatography (19:1 hexanes−EtOAc → 9:1) to give pure **6b** as a colorless oil that solidified upon standing (1.79 g, 62%).

*tert-Butyl (S)-(1-(1H-benzo[d][1,2,3]triazol-1-yl)-4-methyl-1-oxopentan-2-yl)carbamate (**6c**).* The acyl benzotriazole was synthesized according to procedure A using *N*-Boc leucine **5c** (2.00 g, 8.65 mmol, 1.00 equiv), *N*-methylmorpholine (1.43 mL, 13.0 mmol, 1.50 equiv), isobutyl chloroformate (1.23 mL, 9.51 mmol, 1.10 equiv), and benzotriazole (1.13 g, 9.51 mmol, 1.10 equiv). The crude product was obtained as an oil, which was purified by column chromatography (19:1 hexanes−EtOAc → 9:1) to give pure **6c** as a colorless oil (1.73 g, 60%).

*tert-Butyl (S)-(1-(1H-benzo[d][1,2,3]triazol-1-yl)-3-methyl-1-oxobutan-2-yl)carbamate (**6d**).* The acyl benzotriazole was synthesized according to procedure A using *N*-Boc valine **5d** (2.00 g, 9.21 mmol, 1.00 equiv), *N*-methylmorpholine (1.52 mL, 13.8 mmol, 1.50 equiv), isobutyl chloroformate (1.31 mL, 10.1 mmol, 1.10 equiv), and benzotriazole (1.21 g, 10.1 mmol, 1.10 equiv). The crude product was obtained as a viscous oil after evaporation of the solvent. Addition of hexanes resulted in the immediate precipitation of the product as white crystals. The solid was collected by vacuum filtration and recrystallized from 9:1 hexanes−EtOAc to give pure **6d** as a white, crystalline solid (1.88 g, 64%).

Procedure B: Claisen Condensation of Acyl Benzotriazoles. A 0.15 M solution of diisopropylamine (2.00 equiv) in anhydrous THF was cooled to 0 °C in an ice bath, and a 2.5 M solution of *n*-butyllithium in hexanes (2.00 equiv) was added dropwise over 5 min. After addition was complete, the resulting solution of lithium diisopropylamide (LDA) was stirred at 0 °C for 15 min before cooling to −78 °C in a dry ice−2-propanol bath. Neat 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (2.00 equiv) was added dropwise over 5 min, and the reaction mixture was stirred at −78 °C for 1.5 h. To the resulting enolate solution was added a 0.2 M solution of the acyl benzotraizoles **6a−d** (1.00 equiv) in anhydrous THF, and the reaction mixture was stirred at −78 °C for an additional 1.5 h. The cooling bath was allowed to slowly expire, and the reaction mixture was stirred at room temperature overnight, at which point TLC showed complete consumption of the acyl benzotriazole and formation of the dioxinone product. The reaction was quenched with 1 M aqueous HCl solution and diluted with water and EtOAc. The layers were separated, and the aqueous phase was extracted with one additional portion of EtOAc before the combined organics were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the resulting brown oil was purified by column chromatography (3:1:1 hexanes−EtOAc−CH_2Cl_2) to afford dioxinones **7a−d**.

*tert-Butyl (S)-(4-(2,2-dimethyl-4-oxo-4H-1,3-dioxin-6-yl)-3-oxo-1-phenylbutan-2-yl)carbamate (**7a**).* The dioxinone was synthesized according to procedure B using diisopropylamine (1.08 mL, 7.71 mmol, 2.00 equiv), 2.5 M *n*-butyllithium in hexanes (3.08 mL, 7.71 mmol, 2.00 equiv), 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (1.02 mL, 7.71 mmol, 2.00 equiv), and acyl benzotriazole **6a** (1.41 g, 3.85 mmol, 1.00 equiv). After purification by column chromatography, dioxinone **7a** was obtained as a viscous yellow oil that gradually solidified upon standing (781 mg, 52%).

*tert-Butyl ((3S,4S)-1-(2,2-dimethyl-4-oxo-4H-1,3-dioxin-6-yl)-4-methyl-2-oxohexan-3-yl)carbamate (**7b**).* The dioxinone was synthesized according to procedure B using diisopropylamine (1.46 mL, 10.5 mmol, 2.00 equiv), 2.5 M *n*-butyllithium in hexanes (4.18 mL, 10.5 mmol, 2.00 equiv), 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (1.39 mL, 10.5 mmol, 2.00 equiv), and acyl benzotriazole **6b** (1.74 g, 5.23 mmol, 1.00 equiv). After purification by column chromatography, dioxinone **7b** was obtained as a viscous yellow oil that gradually solidified upon standing (1.09 g, 59%).

*tert-Butyl (S)-(1-(2,2-dimethyl-4-oxo-4H-1,3-dioxin-6-yl)-5-methyl-2-oxohexan-3-yl)carbamate (**7c**).* The dioxinone was synthesized

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according to procedure B using diisopropylamine (931 μL, 6.65 mmol, 2.00 equiv), 2.5 M *n*-butyllithium in hexanes (2.66 mL, 6.65 mmol, 2.00 equiv), 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (883 μL, 6.65 mmol, 2.00 equiv), and acyl benzotriazole **6c** (1.10 g, 3.32 mmol, 1.00 equiv). After purification by column chromatography, dioxinone **7c** was obtained as a viscous yellow oil (685 mg, 58%).

*tert-Butyl (S)-(1-(2,2-dimethyl-4-oxo-4H-1,3-dioxin-6-yl)-4-methyl-2-oxopentan-3-yl)carbamate (**7d**).* The dioxinone was synthesized according to procedure B using diisopropylamine (2.02 mL, 14.4 mmol, 2.00 equiv), 2.5 M *n*-butyllithium in hexanes (5.78 mL, 14.4 mmol, 2.00 equiv), 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (1.92 mL, 14.4 mmol, 2.00 equiv), and acyl benzotriazole **6d** (2.30 g, 7.22 mmol, 1.00 equiv). After purification by column chromatography, dioxinone **7d** was obtained as a viscous yellow oil (1.69 g, 68%).

Procedure C: Dioxinone Thermolysis/Hydroxypyrone Methylation. A two-neck round-bottom flask was filled ∼3/4 of the way with $PhCH_3$ and equipped with a septum on one neck and a Dean−Stark trap/reflux condenser on the other. The $PhCH_3$ was heated to reflux and azeotropically dried by removing $PhCH_3$ from the side arm of the Dean−Stark trap until a final volume of ∼100 mL of $PhCH_3$ per 1 mmol of dioxinone remained in the two-neck flask. A 0.1 M solution of dioxinones **7a−d** (1.00 equiv) in $PhCH_3$ (reserved from the last draining of the Dean−Stark trap) was added dropwise via syringe through the septum to the refluxing $PhCH_3$ at a rate of 1 mL/min. After addition was complete, the reaction mixture was heated at reflux for 45 min, at which point TLC showed complete consumption of the dioxinone.

After cooling to room temperature, the solvent was removed under reduced pressure, and the crude hydroxypyrones **8a−d** were dissolved in anhydrous CH_3CN at a concentration of 0.05 M. Solid K_2CO_3 (3.00 equiv) was added followed by neat CH_3OTs (3.00 equiv), and the resulting orange solution was stirred at room temperature overnight, at which point TLC showed that the reaction was complete. The reaction mixture was diluted with water and EtOAc, and brine was added to ensure complete separation of the layers. The aqueous phase was extracted with one additional portion of EtOAc before the combined organics were dried over Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (2:1 hexanes−EtOAc) to afford methoxypyrones **9a−d**.

*tert-Butyl (S)-(1-(4-methoxy-2-oxo-2H-pyran-6-yl)-2-phenylethyl)carbamate (**9a**).* The methoxypyrone was synthesized according to procedure C using dioxinone **7a** (731 mg, 1.88 mmol, 1.00 equiv) in approximately 250 mL of azeotropically dried $PhCH_3$, K_2CO_3 (778 mg, 5.63 mmol, 3.00 equiv), and CH_3OTs (1.05 g, 5.63 mmol, 3.00 equiv). After purification by column chromatography, methoxypyrone **9a** was obtained as an off-white solid (442 mg, 68% over two steps).

*tert-Butyl ((1S,2S)-1-(4-methoxy-2-oxo-2H-pyran-6-yl)-2-methylbutyl)carbamate (**9b**).* The methoxypyrone was synthesized according to procedure C using dioxinone **7b** (1.32 g, 3.71 mmol, 1.00 equiv) in approximately 400 mL of azeotropically dried $PhCH_3$, K_2CO_3 (1.54 g, 11.1 mmol, 3.00 equiv), and CH_3OTs (2.07 g, 11.1 mmol, 3.00 equiv). After purification by column chromatography, methoxypyrone **9b** was obtained as a light yellow gum (719 mg, 62% over two steps).

*tert-Butyl (S)-(1-(4-methoxy-2-oxo-2H-pyran-6-yl)-3-methylbutyl)carbamate (**9c**).* The methoxypyrone was synthesized according to procedure C using dioxinone **7c** (643 mg, 1.81 mmol, 1.00 equiv) in approximately 250 mL of azeotropically dried $PhCH_3$, K_2CO_3 (750 mg, 5.43 mmol, 3.00 equiv), and CH_3OTs (1.01 g, 5.43 mmol, 3.00 equiv). After purification by column chromatography, methoxypyrone **9c** was obtained as a yellow gum (719 mg, 57% over two steps).

*tert-Butyl (S)-(1-(4-methoxy-2-oxo-2H-pyran-6-yl)-2-methylpropyl)carbamate (**9d**).* The methoxypyrone was synthesized according to procedure C using dioxinone **7d** (1.69 g, 4.95 mmol, 1.00 equiv) in approximately 600 mL of azeotropically dried $PhCH_3$, K_2CO_3 (2.05 g, 14.9 mmol, 3.00 equiv), and CH_3OTs (2.77 g, 14.9 mmol, 3.00 equiv). After purification by column chromatography, methoxypyrone **9d** was obtained as a light yellow solid (926 mg, 63% over two steps).

Alternative Hydroxypyrone Synthesis via Two-Step Dioxinone Thermolysis/Cyclization. To a 0.1 M solution of dioxinones **7a−d** (1.00 equiv) in anhydrous $PhCH_3$ was added neat benzyl alcohol (3.00 equiv). The reaction mixture was heated at reflux for 30 min, at which point TLC (2:1 hexanes−EtOAc, UV/ninhydrin) showed complete consumption of the dioxinone and formation of the tricarbonyl compounds **12a−d** as streaky spots that stained yellow/orange. After cooling to room temperature, neat DBU (4.00 equiv) was added, and the reaction mixture was heated at reflux for a further 1.5 h, at which point TLC showed that cyclization of the tricarbonyl compounds to the hydroxypyrones was complete. After cooling to room temperature, the reaction mixture was quenched with 1 M aqueous HCl and diluted with EtOAc. The layers were separated, and the aqueous phase was extracted with one additional portion of EtOAc. The combined organics were washed once with brine before drying over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to afford the crude hydroxypyrones **8a−d**, which were used directly in the subsequent methylation step (as described in procedure C) without purification. Crude yields of **8a−d** were generally 75−85% over the two steps, which are comparable to those obtained in the direct dioxinone thermolysis described in procedure C.

*tert-Butyl (S)-(1-(4-methoxy-3-methyl-2-oxo-2H-pyran-6-yl)-2-methylpropyl)carbamate (**14**).* Thermolysis of valine-derived dioxinone **7d** (104 mg, 0.30 mmol, 1.00 equiv) was carried out as described in procedure C to give the crude hydroxypyrone **8d**. To a solution of this compound in 8 mL of anhydrous CH_3CN was added solid Cs_2CO_3 (297 mg, 0.91 mmol, 3.00 equiv), and the solution immediately turned from pale yellow to dark yellow/orange. Neat iodomethane (57 μL, 0.91 mmol, 3.00 equiv) was added, and the reaction mixture was stirred at room temperature overnight. After this time, TLC (1:1 hexanes−EtOAc, UV/ninhydrin) showed complete consumption of the hydroxypyrone (R_f = 0.05) and formation of methoxypyrone **9d** (R_f = 0.39, stains orange in ninhydrin) and the dimethylated byproduct **14** (R_f = 0.52, stains orange in ninhydrin). The reaction was worked up and purified by column chromatography as described in procedure C to afford **9d** (37.5 mg, 41%) and the dimethylated byproduct **14** as a yellow oil (10.3 mg, 11%).

Procedure D: *N*-Boc Deprotection/Acetylation. A 0.08 M solution of *N*-Boc methoxypyrones **9a−d** (1.00 equiv) in anhydrous CH_2Cl_2 was cooled to 0 °C in an ice bath, and neat trifluoroacetic acid (10.00 equiv) was added dropwise. After 30 min, the cooling bath was removed, and the reaction mixture was allowed to warm to room temperature. The reaction was monitored by TLC (1:1 hexanes−EtOAc, UV/ninhydrin stain) at 30 min intervals for disappearance of the starting material and formation of the tifluororoacetate salt on the baseline. If starting material remained, an additional portion of trifluoroacetic acid (10.00 equiv) was added. After the reaction was complete, the solvent was evaporated under reduced pressure, and the resulting ammonium salt was further dried under high vacuum to remove residual trifluoroacetic acid.

This crude material was dissolved in anhydrous CH_2Cl_2 at a concentration of 0.1 M, and triethylamine (8.00 equiv) was added followed by acetic anhydride (3.00 equiv). The reaction mixture was stirred at room temperature overnight, at which point TLC showed that the acetylation was complete. The reaction was quenched with 1 M aqueous HCl solution, and the layers were separated. The aqueous phase was extracted with one additional portion of CH_2Cl_2 before the combined organics were dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (1:3 hexanes−EtOAc → EtOAc) to afford the natural products **1−4**.

*Pyrophen (**1**).* Pyrophen was synthesized according to procedure D using *N*-Boc methoxypyrone **9a** (147 mg, 0.42 mmol, 1.00 equiv), three aliquots of trifluoroacetic acid (3 × 325 μL, 12.8 mmol, 30.00 equiv), NEt_3 (473 μL, 3.40 mmol, 8.00 equiv), and Ac_2O (120 μL, 1.27 mmol, 3.00 equiv). After purification by column chromatography, pyrophen was obtained as an off-white solid (116 mg, 95% over two steps).

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Campyrone A (2). Campyrone A was synthesized according to procedure D using *N*-Boc methoxypyrone **9b** (205 mg, 0.66 mmol, 1.00 equiv), three aliquots of trifluoroacetic acid (3 × 504 μL, 19.8 mmol, 30.00 equiv), NEt_3 (734 μL, 5.27 mmol, 8.00 equiv), and Ac_2O (187 μL, 1.98 mmol, 3.00 equiv). After purification by column chromatography, campyrone A was obtained as a white solid (160 mg, 96% over two steps).

Campyrone B (3). Campyrone B was synthesized according to procedure D using *N*-Boc methoxypyrone **9c** (214 mg, 0.69 mmol, 1.00 equiv), two aliquots of trifluoroacetic acid (2 × 526 μL, 13.7 mmol, 20.00 equiv), NEt_3 (765 μL, 5.49 mmol, 8.00 equiv), and Ac_2O (195 μL, 2.06 mmol, 3.00 equiv). After purification by column chromatography, campyrone B was obtained as a white solid (136 mg, 78% over two steps).

Campyrone C (4). Campyrone C was synthesized according to procedure D using *N*-Boc methoxypyrone **9d** (128 mg, 0.43 mmol, 1.00 equiv), two aliquots of trifluoroacetic acid (2 × 330 μL, 8.60 mmol, 20.00 equiv), NEt_3 (480 μL, 3.44 mmol, 8.00 equiv), and Ac_2O (122 μL, 1.29 mmol, 3.00 equiv). After purification by column chromatography, campyrone C was obtained as a white solid (83 mg, 81% over two steps).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.7b00720.

Full characterization data for all new compounds, ^1H and ^{13}C NMR spectra, a discussion of inconsistencies in the reported NMR spectra and optical rotation of pyrophen, and spectral comparisons for pyrophen and campyrones A–C (PDF)

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