Total Synthesis and Preliminary Antibacterial Evaluation of the RNA Polymerase Inhibitors (±)-Myxopyronin A and B

Tao Hu,† Jennifer V. Schaus,† Kelvin Lam,‡ Michael G. Palfreyman,‡ Mark Wuonola,‡ Gary Gustafson,‡ and James S. Panek*,†

Department of Chemistry, Metcalf Center for Science and Engineering, 590 Commonwealth Avenue, Boston University, Boston, Massachusetts 02215 and Scriptgen Pharmaceuticals Inc., 200 Boston Avenue, Medford, Massachusetts 02155

Received December 9, 1997

Introduction

In the early 1980s Höfle and co-workers reported the isolation of four novel polyketide derived antibiotics, myxopyronin A and B (**1a** and **1b**) as well as corallopyronin A and B from the gliding bacteria *Myxococcus fulvus* Mx f50 and *Corallococcus coralloides*, respectively.[1,2] This class of natural products which differ only by their length of the unsaturated side chain at the C3 position of the α-pyrone nucleus is virtually unexplored. The absolute stereochemistry of myxopyronin A and B has been determined by careful degradative and spectroscopic methods and was assigned as (*R*)-configuration at C7 (Figure 1).[1] Those molecules inhibited the growth of many Gram-positive and several Gram-negative bacteria.[3] No inhibitory activity was observed for yeasts and fungi (molds). They were also shown to be selective inhibitors of bacterial DNA-dependent RNA polymerase.[3] The broad spectrum of activity and selectivity for bacterial RNA polymerase over the human polymerase established the myxopyronins as promising candidates for development as antibacterial agents. The exhibition of activity against rifampicin- or streptolydigin-resistant bacteria by the myxopyronins suggests that these agents target a region of RNA polymerase distinct from the one by rifampicin. In this paper, we report the first total syntheses of myxopyronin A and B. To the best of our knowledge, no synthetic investigations have been reported on this class of natural products. Our convergent synthesis makes use of a 3-propionyl-4-hydroxy-α-pyrone **4** as the central building block from which both side chains are introduced.[4] In that regard, an alkylation strategy was used for the installation of the lower side chain followed by a titanium(IV)-promoted aldol condensation introducing the (*E*,*E*)-dienone of the upper side chain. Our retrosynthetic analysis of the myxopyronins is illustrated in Figure 1 with the first disconnection removing the terminal unsaturated carbamate. This detachment produced the advanced intermediate **2** bearing an unsaturated carboxylate functionality. The second disconnection produced the aldol synthons in the form of the pyrone **3** and the corresponding unsaturated aldehydes. Further disconnection of **3** produced the starting 3-propionyl-4-hydroxy-α-pyrone **4**.

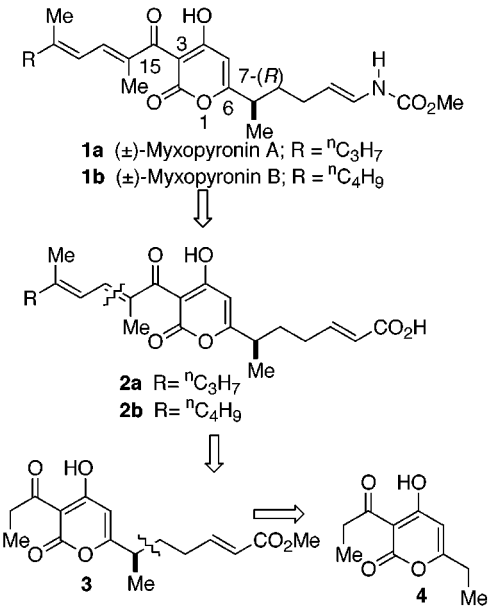


Figure 1.

Results and Discussion

Preparation of the O1−C14 Fragment. The synthesis of this material relied on a selective alkylation of the C6 ethyl group of pyrone **4** (Scheme 1). In the presence of LDA (3.2 equiv), the regioselective alkylation with primary iodide **5**[5] proceeds through a trianion intermediate to give the alkylated pyrone **6** in good yield.[6] Intermediate **6** was deprotected under mild acidic conditions to afford the corresponding primary alcohol in 91% yield. Oxidation of the primary hydroxyl with Dess−Martin reagent (2.0 equiv, 0 °C, 2 h)[7] gave aldehyde **7**. Subsequent Horner−Emmons−Wadsworth homologation using trimethyl phosphonoacetate (2.2 equiv, NaH 2.2 equiv, THF, rt) afforded the α,*β*-unsaturated ester **8** with an *E*:*Z* isomer ratio greater than 20:1. Silica gel chromatography provided geometrically pure **8** in 82% yield.

† Department of Chemistry, Metcalf Center for Science and Engineering, Boston University, Boston, Massachusetts.

‡ Scriptgen Pharmaceutical Inc., Department of Biology, Medford, Massachusetts.

(1) (a) Kohl, W.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1983**, 1656−1667. (b) Kohl, W.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1984**, 1088−1093. (c) For clarification, the natural products have been illustrated with the natural occurring (*R*)-configuration; however, all materials in this paper were synthesized in racemic form.

(2) Jansen, R.; Irschik, H.; Reichenbach, H.; Höfle, G. *Liebigs Ann. Chem.* **1985**, 822−836.

(3) Irschik, H.; Gerth, K.; Höfle, G.; Kohl, W.; Reichenbach, H. *J. Antibiot.* **1983**, *36*, 1651−1658.

(4) Pyrone **4** was prepared from commercially available ethyl 3-oxopropanoate see: Cook, L.; Ternai, B.; Ghosh, P. *J. Med. Chem.* **1987**, *30*, 1017−1022. See Experimental Section for further details.

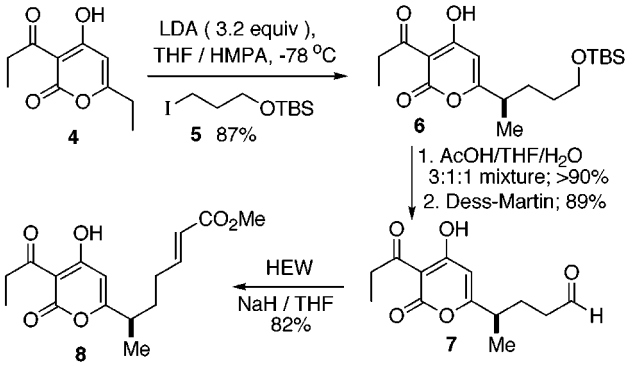
(5) Alkyl iodide **5** was prepared from 1,3-propanediol by a two-step reaction sequence: (i) selective protection with TBSCl (1.0 equiv), NaH (1.0 equiv), 98% yield; (ii) I_2, PPh_3, imidazole, 96% yield. See Supporting Information for details.

(6) Satisfactory spectroscopic data (^{1}H and ^{13}C NMR, IR, CIMS, and CIHRMS) were obtained for all new compounds.

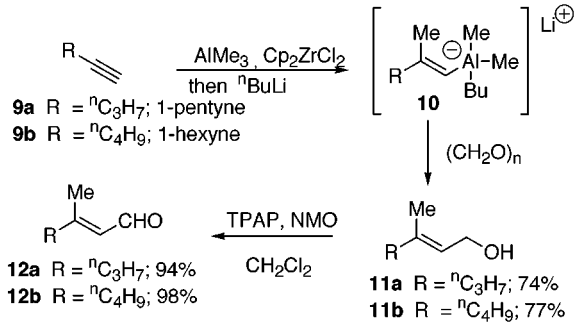
(7) (a) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155−4156. (b) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7287. For two improved procedures for the preparation of DMP, see: (c) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899. (d) Meyer, S. D.; Schreiber, S. L. *J. Org. Chem.* **1994**, *59*, 7549−7552.

S0022-3263(97)02161-0 CCC: $15.00
Published on Web 03/12/1998

Scheme 1



Scheme 2



This sequence completed the preparation of the O1−C14 fragment now set for the subsequent aldol condensation for the introduction of the C15−C24 and C25 side chains of myxopyronin A and B.

Synthesis of the α,β-Unsaturated Aldehydes 12a and 12b. The synthesis of these subunits relied on Negishi's carboalumination[8] of the terminal alkynes **9a** and **9b**. Treatment of the alkynes with zirconocene dichloride (1.0 equiv) in the presence of $AlMe_3$ (2.0 equiv) afforded the (*E*)-trisubstituted vinyl aluminates, which were then directly converted to the more reactive aluminate complex **10** by addition of ^nBuLi (1.0 equiv, THF) (Scheme 2). These intermediates were trapped with excess paraformaldehyde affording the (*E*)-trisubstituted allylic alcohol **11** in good overall yield. Oxidation of these materials with TPAP[9] (0.06 equiv) and NMO (2.0 equiv) afforded the corresponding aldehydes **12a** and **12b**, respectively. These experiments completed the preparation of the two volatile and labile aldehydes which were used immediately and without purification in the aldol condensation for installation of the C15−C24/25 upper side chain of the myxopyronins.

Titanium(IV)-Promoted Aldol Condensation and Completion of the Syntheses. The introduction of the upper side chains of myxopyronin A and B was carried out utilizing a Ti(IV) tetrachloride-promoted aldol condensation between the ethyl ketone **8** and aldehydes **12a** and **12b** (Scheme 3). The titanium enolate was generated at −78 °C by treatment of ethyl ketone **8** with $TiCl_4$ (4.0 equiv) and DIPEA (4.8 equiv); this excess of Lewis acid and base was found to be necessary to drive the reaction to completion. The derived enolate was condensed with freshly prepared aldehyde **12** at −78 °C for 48 → 56 h to directly afford after in situ dehydration the respective (*E*,*E*)-dienones **13a** and **13b**. With both side chains installed onto the α-pyrone core, completion of the individual synthesis required the conversion of the α,β-unsaturated methyl ester to the methyl carbamate. This was initiated by a LiOH (10 equiv, THF/H_2O 4:1, 35 h)-promoted hydrolysis of the methyl esters which afforded the free carboxylic acids **13a** and **13b** in quantitative yield. The vinyl carbamate was introduced by a modified Curtius rearrangement,[10] employing ethyl chloroformate and NaN_3. This sequence completed the assembly of the lower side chain and achieved the individual synthesis of (±)-myxopyronin A and B.

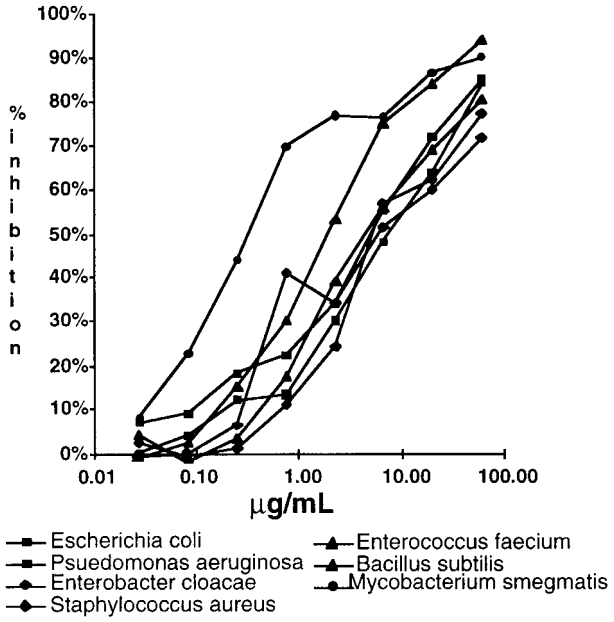


Figure 2. MICs at concentrations comparable to in vitro bacterial RNAP IC_{50}s.

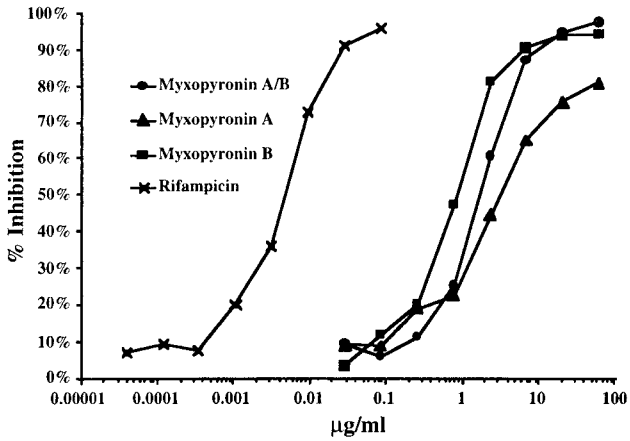


Figure 3. In vitro transcription assay with *Escherichia coli* RNA Polymerase.

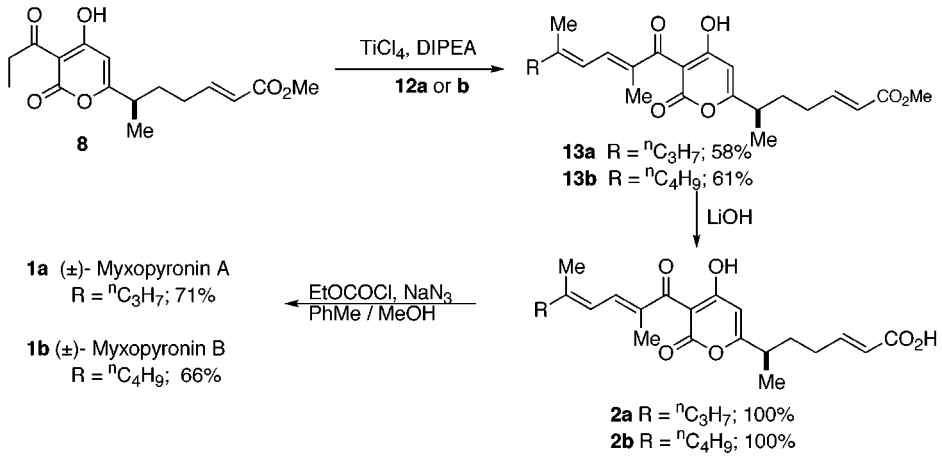
Antibacterial Evaluation of (±)-Myxopyronin A and B. The antibacterial activities of myxopyronins were evaluated with an in vitro transcription assay using *Escherichia coli* RNA polymerase (Figures 2 and 3). The myxopyronins A/B were isolated as a mixture of natural

(8) (a) Negishi, E.; Kondakov, D.; Choueiry, D.; Kasai, K.; Takahashi, T. *J. Am. Chem. Soc.* **1996**, *118*, 9577−9588. (b) Okukado, N.; Negishi, E. *Tetrahedron Lett.* **1978**, *27*, 2357−2360.

(9) (a) Ley, S.; Norman, J.; Griffith, W.; Marsden, S. *Synthesis* **1994**, 639−666. (b) Griffith, W.; Ley, S. *Aldrichimica Acta* **1990**, *23*, 13−19.

(10) Overman, L.; Taylor, G.; Petty, C.; Jessup, P. *J. Org. Chem.* **1978**, *43*, 2164−2167.

Scheme 3



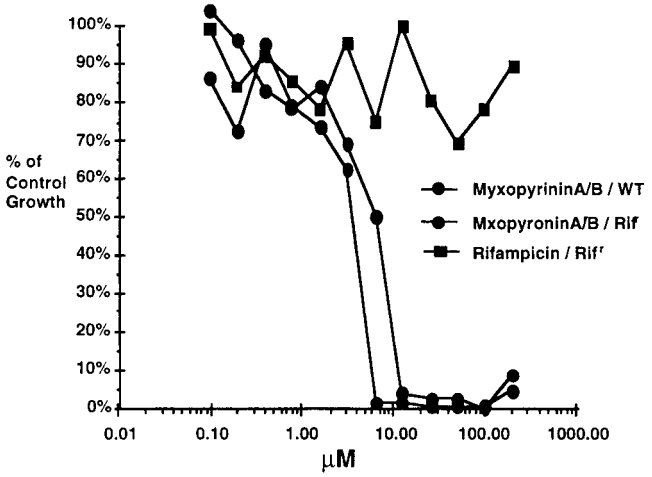


Figure 4. Myxopyronin A/B against rifampicin- and streptolydigin-resistant *Staphylococcus aureus*.

products containing a 9:1 ratio of A and B. The pyronins exhibited comparable micromolar inhibitory activity in enzymatic assays against a panel of seven different bacteria, in accordance with their previously established activity. These seven pathogens represent both Gram-positive and Gram-negative bacterial and illustrate the broad spectrum activity of the myxopyronins.

As a point of comparison, the synthetic myxopyronin A and B were tested against *E. coli* RNA polymerase separately and myxopyronin B is shown to be an approximately 4 times more potent molecule than A (Figure 3). As a validation of the in vitro transcription assay, a known transcription inhibitor, rifampicin, was assayed with the natural product mixture (myxopyronin A/B) against rifampicin and streptolydigin-resistant *S. aureus* (Figure 4).

Table 1 summarizes in vitro IC_{50} and MIC values obtained from the cell-based evaluation of the myxopyronins using both Gram-positive and Gram-negative bacteria. The data show that myxopyronins have in vivo cell-based activities against rifampicin-resistant bacteria.[11] In complement to the in vitro transcription activities, (±)-myxopyronin B is also shown to be at least 30-fold more potent in cell-based activities for the *S. aureus* Gram-positive strain than (±)-myxopyronin A (Table 1). Table 1 summarizes the in vitro IC_{50} and MIC values for myxopyronin A and B. The pyronins exhibited similar inhibition toward streptolygidin-resistant bacteria *S. aureus* than toward rifampicin-resistant strain (Figure 4).

Conclusion

We have described an efficient and highly convergent synthesis of myxopyronin A and B. Biological evaluation of these agents against RNA polymerase for a variety of bacteria including mammalian culture cells holds considerable promise as potential antibacterial agents. The asymmetric synthesis and SAR programs are currently underway in our laboratories and will be reported at the appropriate time.

Experimental Section

^1H and ^{13}C NMR spectra were taken in $CDCl_3$ at 400 and 75 MHz, respectively, unless specified otherwise. Chemical shifts are reported in parts per million using the solvent resonance internal standard (chloroform, 7.24 and 77.0 ppm for ^1H and ^{13}C NMR, respectively, unless specified otherwise). NMR data are reported as follows: chemical shift, multiplicity (app = apparent, par obsc = partially obscured, ovrlp = overlapping, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, abq = ab quartet), coupling constant, and integration. Infrared resonance (IR) spectra were recorded on a FTIR spectrophotometer. High-resolution mass spectra were obstained on a Finnegan spectrometer in the Boston University Mass Spectrometry Laboratory. Reversed phase preparative HPLC was conducted with a diode array detector, using a 22 × 250 mm C18 column (218TP1022). Methylene chloride (CH_2Cl_2), methanol (MeOH), benzene (C_6H_6), toluene, and hexane were distilled from calcium hydride, and tetrahydrofuran (THF) and hexamethylphosphoramide (HMPA) were distilled from sodium and benzophenone prior to use. Titanium tetrachloride ($TiCl_4$) was freshly distilled from copper powder under reduced pressure before each use. Anhydrous 1,2-dichloroethane ($ClCH_2CH_2Cl$), trimethylaluminum ($AlMe_3$, 2.0 M solution in hexanes), and zirconocene dichloride (Cp_2ZrCl_2) was purchased from Aldrich Chemical Co. Inc. All other reagents were used as supplied. All reactions were carried out in oven-dried glassware under argon atmosphere unless otherwise noted. Analytical thin-layer chromatography was performed on 0.25 mm silica gel

(11) *In vitro* transcription reactions were performed as described in the Supporting Information. [α-^{32}P] UTP-incorporated RNA was synthesized in 50 μL reaction volumes containing transcription buffer (50 mM Tris-HCl, pH 8.0, 200 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, and 1.5 μM BSA), 1 μg of DNA template, 4 μM UTP containing 5 μCi of [α-^{32}P] UTP, 400 μM each of ATP, GTP, and CTP. After incubation for 60 min at 25 °C, the reaction is terminated with 100 μL of 10% TCA, which also precipitates the newly transcribed RNA. See Supporting Information for details.

Table 1

compound	in vitro transcription (*E. coli* RNAP) IC_{50} (μg/mL)	MIC (μg/mL) *E. coli*	MIC (μg/mL) *E. coli*[a]	MIC (μg/mL) *S. aureus*[b]	MIC (μg/mL) *S. aureus*[b]
(*R*)-Myxo A/B natural mixture	8	200	5	4	5
(±)-Myxo B	5	>30	2	0.5	0.5
(±)-Myxo A	20	>30	4−8	15	8

[a] Mutant strain that has a permeabilized cell wall. [b] Mutant strain is rifampicin-resistant bacteria. See Supporting Information for details.

60-A plates. Flash chromatography was performed on silica gel 230−400 mesh. Due to the similarities of the two natural products, detailed experimental procedures are provided only for myxopyronin B; see Supporting Information.

3-(1-Propionyl)-4-hydroxy-6-ethyl-2-pyrone (4). Ethyl 3-oxopentanoate (5.0 g, 35 mmol) was dissolved in 30 mL of 1.5 M solution NaOH and stirred at rt for 30 h. The reaction was cooled to 0 °C, and 3 M HCl was slowly added until the reaction system reached a pH ∼2, and then solid KCl was added to saturate the solution. The reaction was extracted with EtOAc and $CHCl_3$, and the combined organic layers were dried with Na_2SO_4, filtered, and concentrated in vacuo to afford the crude 3-oxopentanoic acid (3.1 g, 76% yield) as a white solid. The material needed no further purification and was used directly in the next step: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.52 (s, 2H), 2.60 (q, J = 7.3 Hz, 2H), 1.10 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.3, 172.3, 47.9, 36.6, 7.5; IR (neat) ν_{max}: 3400, 1708, 1620 cm^{-1}.

To a stirring solution of the above 3-oxopentanoic acid (3.1 g, 26.7 mmol) in 50 mL of THF was added carbonyldiimidazole (5.6 g, 34.7 mmol). The reaction was left stirring for 18 h before being quenched with 2% HCl aq solution (pH measured 2∼3). It was extracted with EtOAc, dried (Na_2SO_4), filtered, and then concentrated in vacuo. The product needed no further purification as long as it was left on a vacuum pump for 2 h in order to remove any remaining starting acid. This protocol afforded compound **4** (2.22 g, 86%) as a pale yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.91 (s, 1H); 3.10 (q, J = 7.3 Hz, 2H); 2.53 (q, J = 7.2 Hz, 2H); 1.24 (t, J = 7.5 Hz, 3H); 1.15 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.4, 181.2, 173.5, 161.2, 99.9, 99.6; 35.4, 27.5, 10.5, 7.8; IR (neat) ν_{max}: 3420, 1731, 1640 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{10}H_{13}O_4$ ($M + H^+$) 197.0814, found: 197.0818.

1-Iodo-3-[(*tert*-butyldimethylsilyl)oxy]propane (5). To a suspension of NaH (4.0 g, 100 mmol, 60% dispersion in mineral oil) in THF (100 mL) at rt was added 1,3-propanediol (7.6 g, 100 mmol, dissolved in 50 mL of THF) via a cannula. The resulting mixture was stirred at rt for 45 min, before a solution of TBSCl (15.0 g, 100 mmol) in 50 mL of THF was added into the reaction mixture by a cannula. This resulting reaction was stirred for 1.0 h at rt. The reaction was quenched with saturated aqueous $NaHCO_3$ solution and extracted with ether. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (20% EtOAc in petroleum ether eluant) to afford the pure monoprotected alcohol (18.1 g, 98% yield) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73−3.80 (m, 4H); 2.71 (br s, 1H); 1.74 (m, 2H); 0.86 (s, 9H); 0.04 (s, 6H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 62.7, 62.2, 34.2, 25.9, 25.8, 25.8, 18.1, −5.6; IR (neat) ν_{max}: 3371, 2929, 1473, 1257, 1099 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_9H_{23}SiO_2$ ($M + H^+$) 191.1467, found: 191.1472.

To a solution of imidazole (10.2 g, 150 mmol) and triphenylphosphine (14.4 g, 55 mmol) in CH_2Cl_2 (200 mL) at 0 °C was added I_2 (14.0 g, 55 mmol). After 10 min, a solution of the above monoprotected alcohol (9.5 g, 50 mmol) in CH_2Cl_2 (100 mL) was added over 5 min. The mixture was warmed to rt, covered in aluminum foil, and stirred for an additional 15 h in the dark. The reaction was then diluted with 2.0 mL of saturated $Na_2S_2O_4$ aqueous solution before further dilution with water. The organic layer was separated, and the aqueous layer was back extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (2% EtOAc in hexanes as the eluant) to afford the primary iodide **5** (14.4 g, 96% yield) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.64 (t, J = 5.7 Hz, 2H), 3.25 (t, J = 6.6 Hz, 2H), 1.96 (m, 2H), 0.87 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 62.3, 36.1, 25.9, 8.3, 3.6, −5.3; IR (neat) ν_{max}: 2929, 1472, 1257, 1101 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_9H_{22}SiO_2I$ ($M + H^+$) 301.0485, found: 301.0470.

3-(1-Propionyl)-4-hydroxy-6-[1-methyl-4-[(*tert*-butyldimethylsilyl)oxy]butanyl]-2-pyrone (6). To a stirred solution of diisopropylamine (3.34 mL, 24 mmol) in 25 mL of THF at −78 °C under an argon atmosphere was added *n*-butyllithium (9.45 mL, 2.5 M solution in hexanes, 23.6 mmol). The mixture was allowed to warm to 0 °C for 30 min and then recooled to −78 °C. The resulting solution was treated with a solution of pyrone **4** (1.47 g, 7.5 mmol) in 10 mL of THF, and stirred for 1.0 h at −78 °C. The derived trianion was treated with iodide **5** (2.5 g, 8.25 mmol) in 10 mL of THF, followed by the addition of HMPA (4.0 mL, 23.2 mmol). The reaction mixture was allowed to stir for 30 min at −78 °C, before being diluted with saturated NH_4Cl aqueous solution. The organic layer was separated, and the aqueous layer was extracted with ether. The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting crude oil was purified by flash chromatography (20% EtOAc in hexanes as the eluant) to afford the pure alkylation product **6** (2.4 g, 87% yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90 (s, 1H), 3.57 (t, J = 6.0 Hz, 2H), 3.08 (q, J = 7.3 Hz, 2H), 2.56 (m, 1H), 1.43−1.73 (m, 4H), 1.22 (d, J = 7.3 Hz, 3H), 1.13 (t, 3H), 0.86 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 207.8, 180.6, 175.6, 160.6, 99.3, 62.2, 38.3, 34.8, 30.0, 29.7, 25.4, 17.8, 17.5, 7.3, −5.8; IR (neat) ν_{max}: 2936, 1743, 1636, 1100 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{19}H_{33}SiO_6$ ($M + H^+$) 369.2097, found: 369.2085.

3-(1-Propionyl)-4-hydroxy-6-(1-methyl-4-hydroxybutyl)-2-pyrone (7′). A solution of TBS ether **6** (1.66 g, 4.5 mmol) in 50 mL of AcOH/THF/H_2O (3:1:1) was stirred at rt for 22 h. The reaction was diluted with water and extracted with EtOAc. The combined organic layers were washed with a saturated solution of $NaHCO_3$. The organic layer was separated, and the aqueous layer was back extracted with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by flash chromatography (40% EtOAc in hexanes eluant) to afford diol **7′** (1.04 g, 91% yield) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90 (s, 1H), 3.63 (br t, 2H), 3.08 (q, J = 7.3 Hz, 2H), 2.58 (m, 1H), 1.75 (m, 1H), 1.50−1.62 (m, 3H), 1.33 (br s, 1H), 1.23 (d, J = 7.3 Hz, 3H), 1.13 (t, J = 7.3 Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 180.5, 175.2, 160.6, 99.3, 99.0, 61.6, 38.1, 34.7, 29.8, 29.5, 17.3, 7.1, 1.51; IR (neat) ν_{max}: 3425, 2940, 1734, 1636 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{13}H_{19}O_5$ ($M + H^+$) 255.1232, found: 255.1252.

3-(1-Propionyl)-4-hydroxy-6-(1-methyl-4-formylbutyl)-2-pyrone (7). To a solution of alcohol **7′** (483 mg, 1.9 mmol) in CH_2Cl_2 (20 mL) was added pyridine (845 μL, 10.45 mmol), followed with Dess−Martin periodinate reagent (2.8 mg, 6.65 mmol) in one portion. The resulting reaction mixture was stirred at rt for 2.0 h before being quenched with saturated $NaHCO_3$ aqueous solution. The reaction mixture was extracted with CH_2Cl_2, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was flash chromatographed (15% EtOAc in hexanes eluant) to afford aldehyde **11** (426 mg, 89% yield) as yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H), 5.90 (s, 1H), 3.07 (q, J = 7.3 Hz, 2H), 2.59 (m, 1H), 2.46 (t, J = 7.3 Hz, 2H), 1.83−2.02 (m, 2H), 1.23 (d, J = 7.3 Hz, 3H), 1.12 (t, J = 7.3 Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 200.7, 180.7, 174.4,

160.6, 99.9, 99.5, 40.8, 37.8, 35.0, 25.9, 17.5, 7.4, 1.8; IR (neat) ν_{max}: 2978, 1727, 1636, 1560 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{13}H_{17}O_6$ ($M + H^+$) 253.1076, found: 253.1076.

3-(1-Propionyl)-4-hydroxy-6-[5-(methoxycarbonyl)-1-methyl-4-pentenyl]-2-pyrone (8). To a solution of trimethyl phosphonoacetate (1.7 g, 9.3 mmol) in THF (20 mL) at room temperature was added NaH (360 mg, 8.95 mmol, 60% dispension in mineral oil). The resulting reaction was stirred at rt for 15 min, before aldehyde **7** (906 mg, 3.58 mmol) in 10 mL of THF was added, via cannula. The reaction was allowed to stir at rt for 3.0 h before being diluted with aqueous NH_4Cl solution. The mixture was extracted with EtOAc, dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was flash chromatographed (20% EtOAc in hexanes as the eluant) to afford compound **8** (904 mg, 82% yield) as a yellow oil: ^1H NMR (400 MHz, $CDCl_3$) δ 6.79 (dt, $J = 6.8$, 15.6 Hz, 1H), 5.83 (s, 1H), 5.70 (d, $J = 15.6$ Hz, 1H), 3.57 (s, 3H), 2.97 (q, $J = 7.1$ Hz, 2H), 2.49 (m, 1H), 2.11 (m, 2H); 1.78 (m, 1H), 1.58 (m, 1H), 1.14 (d, $J = 6.7$ Hz, 3H), 1.02 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 208.2, 180.8, 174.8, 166.6, 160.8, 147.4, 121.8, 100.0, 99.6, 51.4, 38.2, 35.2, 32.1, 29.5, 17.7, 7.6; IR (neat) ν_{max}: 2980, 1726, 1637, 1560 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{16}H_{20}O_6$ ($M + H^+$) 308.1260, found: 308.1234.

(*E*)-3-Methylhept-2-en-1-ol (11b). To a white slurry solution of zirconocene dichloride (7.3 g, 25 mmol) in 60 mL of $(CH_2)_2Cl_2$ was added $AlMe_3$ (25 mL, 2.0 M in hexanes, 50 mmol) at 0 °C, stirred for 30 min, and then warmed to rt for 1.0 h. To this lemon-yellow solution was added 1-hexyne (**9b**; 2.05 g, 25 mmol, dissolved in 20 mL of $(CH_2)_2Cl_2$) at rt. The reaction was allowed to stir at rt for 16 h. The volatile components were evaporated under reduced pressure (maximum 50 °C, 0.3 mmHg, 1−2 h). The remaining orange-yellow organic residue was extracted with dry hexanes (4 × 30 mL), and the yellow extract was transferred to a 500 mL round-bottom flask via a cannula. To this was added ^{n}BuLi (10 mL, 2.5 M in hexanes, 25 mmol) at 0 °C. This orange-yellow slurry solution was stirred from 0 °C to rt for 1.5 h, and then THF (50 mL) was added to dissolve the precipitate. The resulting solution (homogeneous, brown-yellow color) was cannulated to a suspension of paraformaldehyde (3.75 g, 125 mmol) in THF (50 mL) under a argon atmosphere. This orange-yellow suspension was allowed to stir at rt for 20 h before it was cooled to 0 °C (ice−water bath). Ice was added to dilute the reaction, and then saturated NH_4Cl was added. The ice bath was removed, and the reaction was further acidified with 3 M HCl until the reaction turned to a clear yellow (homogeneous) solution. At this time, the reaction pH was measured 2−3. The organic layer was separated, and the aqueous layer was extracted with ether. The organic extracts were combined, washed with a saturated solution $NaHCO_3$, and then dried with Na_2SO_4, filtered, and concentrated under reduced pressure to provide crude allylic alcohol **11b**. The crude product was purified by flash chromatography (20% EtOAc in hexanes as the eluant) to afford pure **11b** (2.46 g, 77% yield) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 5.37 (t, $J = 7.1$ Hz, 1H), 4.11 (d, $J = 7.1$ Hz, 2H), 1.98 (t, $J = 7.5$ Hz, 2H), 1.64 (s, 3H), 1.37 (m, 2H), 1.28 (m, 2H), 0.87 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 139.9, 123.1, 59.2, 39.2, 29.8, 22.3, 16.1, 13.9; IR (neat) ν_{max}: 3330, 2958, 1670, 1467 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_8H_{16}O_1$ (M^+) 128.1201, found: 128.1199.

(*E*)-3-Methylhept-2-en-1-al (12b). To a suspension solution of alcohol **11b** (739 mg, 5.7 mmol), 4 Å molecular sieves (2.9 g, activated), and NMO (1.33 g, 11.4 mmol) in CH_2Cl_2 (12 mL) at 0 °C was added TPAP (120 mg, 0.34 mmol) in one portion. The resulting dark reaction mixture was allowed to stir at 0 °C for 30 min, before being diluted with CH_2Cl_2, and then filtered through a short pad of silica gel. The filtrate was concentrated in vacuo to afford aldehyde **12b** (704 mg, 98% yield) as a colorless oil. The aldehyde was sufficiently pure and used immediately without further purification: ^1H NMR (400 MHz, $CDCl_3$) δ 9.94 (d, $J = 8.1$ Hz, 1H), 5.82 (d, $J = 8.1$ Hz, 1H), 2.16 (t, $J = 7.6$ Hz, 2H), 2.11 (s, 3H), 1.44 (m, 2H), 1.28 (m, 2H), 0.87 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.3, 164.5, 127.2, 40.3, 29.2, 22.3, 17.4, 13.8; IR (neat) ν_{max}: 2959, 1676 cm^{-1}.

3-[(*E*,*E*)-2,5-Dimethyl-2,4-nonadienoyl]-4-hydroxy-6-[5-(methoxycarbonyl)-1-methyl-4-pentenyl]-2-pyrone (13b). Compound **8** (178 mg, 0.578 mmol) was dissolved in CH_2Cl_2 (10 mL) and stirred at −78 °C under argon atmosphere. To this was added freshly distilled $TiCl_4$ (254 μL, 2.31 mmol), and the reaction turned to an yellow slurry mixture immediately. After stirring for 20 min at −78 °C, DIPEA (483 μL, 2.77 mmol) was added, and the reaction became a red-dark reaction mixture. This reaction mixture was allowed to stir at −78 °C for 3.0 h, and then aldehyde **12b** (254 mg, 2.0 mmol) dissolved in 2.0 mL CH_2Cl_2 was added via a cannula. This dark-red reaction mixture was stirred at −78 °C for 48 h and then warmed to 0 °C for 5 min, before it was quenched with distilled water. The reaction was extracted with CH_2Cl_2, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude product was flash chromatographed (25% EtOAc in hexanes as the eluant) to provide diene **13b** (147 mg, 61% yield) as a sticky yellow oil: ^1H NMR (400 MHz, $CDCl_3$): δ 6.97 (d, $J = 11.6$ Hz, 1H), 6.88 (dt, $J = 6.7$, 15.3 Hz, 1H), 6.13 (d, $J = 11.6$ Hz, 1H), 5.91 (s, 1H), 5.81 (d, $J = 15.3$ Hz, 1H), 3.70 (s, 3H), 2.58 (m, 1H), 2.20 (m, 2H), 2.14 (t, $J = 7.3$ Hz, 2H), 1.98 (s, 3H), 1.88 (m, 1H), 1.82 (s, 3H), 1.66 (m, 1H), 1.45 (m, 2H), 1.30 (m 2H), 1.24 (d, $J = 6.7$ Hz, 3 H), 0.89 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6, 180.7, 174.7, 166.7, 160.3, 149.4, 147.5, 133.6, 132.9, 121.9, 120.4, 100.0, 99.1, 51.5, 40.5, 38.2, 32.2, 30.0, 29.6, 22.4, 17.7, 17.3, 14.0, 13.5; IR (neat) ν_{max}: 2932, 1726, 1636, 1546 cm^{-1}; CIHRMS (NH_3 gas) calcd for $C_{24}H_{32}O_6$ (M^+) 416.2199, found: 416.2208.

3-[(*E*,*E*)-2,5-Dimethyl-2,4-nonadienoyl]-4-hydroxy-6-(5-carboxy-1-methyl-4-pentenyl)-2-pyrone (2b). To a stirred solution of **13b** (42 mg, 0.10 mmol) in THF (6.0 mL) was added LiOH aqueous solution (1.5 mL, 1.0 M aq, 1.5 mmol) at rt, and the resulting reaction mixture ($THF/H_2O = 4:1$) was allowed to stir at rt for 35 h before it was diluted with EtOAc and then quenched by saturated NH_4Cl aq solution. The reaction was further acidified to pH = 2 by slow addition of 5% HCl. It was extracted with EtOAc, dried (Na_2SO_4), filtered through a short pad of silica gel, and then concentrated in vacuo to afford the crude acid **2b** (40 mg, 100% yield) as a sticky yellow oil. This material was sufficiently pure and used without further purification: ^1H NMR (270 MHz, $CDCl_3$): δ 7.05−6.94 (m, 2H), 6.14 (d, $J = 11.5$ Hz, 1H), 5.92 (s, 1H), 5.81 (d, $J = 15.4$ Hz, 1H), 2.59 (m, 1H), 2.24 (m, 2H), 2.14 (t, $J = 7.3$ Hz, 2H), 1.97 (s, 3H), 1.89 (m, 1H), 1.82 (s, 3H), 1.68 (m, 1H), 1.43 (m, 2H), 1.28 (m 2H), 1.24 (d, $J = 6.8$ Hz, 3 H), 0.89 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.6, 180.7, 174.5, 171.4, 160.4, 150.2, 149.1, 133.6, 132.9, 128.3, 121.6, 120.6, 100.1, 99.1, 42.8, 38.2, 32.0, 29.7, 20.9, 17.7, 17.2, 13.8, 13.4; IR (neat) ν_{max}: 2960, 1724, 1636, 1561; CIHRMS (NH_3 gas) calcd for $C_{23}H_{31}O_6$ ($M + H^+$) 403.2121, found: 403.2136.

(±)-Myxopyronin B (1b). To a stirred solution of acid **2b** (22 mg, 0.0547 mmol) in dry acetone (1.0 mL) were sequentially added DIPEA (23 μL, 0.13 mmol) and ethyl chloroformate (11 μL, 0.12 mmol) at 0 °C. The reaction was stirred at 0 °C for 1.5 h, and then NaN_3 (17 mg, 0.263 mmol, dissolved in 300 μL of distilled H_2O) was added via a syringe. The resulting reaction mixture was stirred at 0 °C for 70 min before being quenched by ice−water, and extracted with distilled toluene, dried ($MgSO_4$), filtered, and concentrated in vacuo. The organic residue was taken up by dry toluene (6 mL) and refluxed for 2.0 h before fresh distilled MeOH (3.0 mL) was added to trap the isocyanate intermediate. The resulting reaction solution was further refluxed for 12 h and then concentrated in vacuo to provide crude (±)-myxopyronin B (**1b**) as a yellow oil. This crude material was purified by preparative reverse phase HPLC (70:30:4 MeOH:H_2O:AcOH) to provide pure (±)-myxopyronin B (**1b**, 15.6 mg, 66%) as a yellow sticky oil: ^1H NMR (400 MHz, CD_3OD, 3.27 ppm) δ 7.15 (d, $J = 11.6$ Hz, 1H), 6.36 (d, $J = 14.0$ Hz, 1H), 6.23 (d, $J = 11.6$ Hz, 1H), 6.01 (s, 1H), 5.02 (dt, $J = 7.3$, 14.0 Hz, 1H), 3.62 (s, 3H), 2.61 (m, 1H), 2.18 (t, $J = 7.3$ Hz, 2H), 1.97 (m, 2H), 1.90 (s, 3H), 1.78 (s, 3H), 1.72 (m, 1H), 1.55 (m, 1H), 1.49 (m, 2H), 1.31 (m, 2H), 1.21 (d, $J = 7.3$ Hz, 3H), 0.90 (t, $J = 7.0$ Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD, 49.15 ppm) δ 199.1, 175.2, 173.2, 165.0, 156.9, 151.6, 138.4, 135.2, 126.1, 122.3, 110.7, 102.6, 101.9, 52.8, 41.6, 39.2, 35.9, 31.3, 29.7, 23.6, 18.6, 17.4, 14.4, 11.9; IR (neat) ν_{max}: 3315, 2931, 1734, 1681 cm^{-1}; UV (methanol): λ_{max} (log ϵ) = 213, 298 nm; CIHRMS (NH_3 gas) calcd for $C_{24}H_{34}N_1O_6$ ($M + H^+$) 432.2386, found: 432.2377.

Acknowledgment. This work has been financially supported by Scriptgen Pharmaceuticals, Inc. We are grateful to Mr. Brian Mendelsohn for technical assistance.

Supporting Information Available: Experimental procedures and characterization for compounds **1a**, **2a**, **11a**, **12a**, and **13a** reported herein; copies of ^{1}H and ^{13}C NMR spectra for all reaction products. In addition, details for microdilution minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays (39 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9721610