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Syntheses of novel myxopyronin B analogs as potential inhibitors of bacterial RNA polymerase

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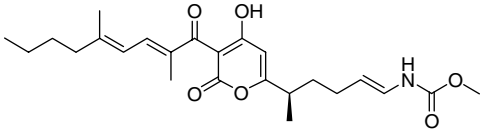
Abstract—Based upon observations from our initial findings, additional myxopyronin B analogs have been prepared and tested for in vitro inhibitory activity against DNA-dependent RNA polymerase and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Although antibiotic resistance has been around for more than 60 years since the discovery of penicillin, the problem has grown exponentially in recent years. Factors contributing to this rapid growth of antibiotic resistance include the overuse of powerful, broad-spectrum antibiotics to treat common infections (e.g., ear infections), as well as the use of antibiotics in inappropriate situations (e.g., viral infections like the common cold). Despite improved efforts for the rational use of existing agents, there remains a strong need for novel antibiotics. Myxopyronin B (Fig. 1, **1**), a bacterial metabolite isolated from *Myxococcus fulvus* Mx 150, represents such a candidate.[1]

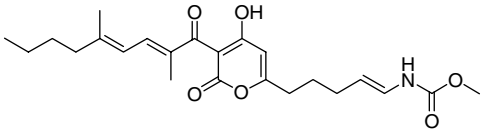
Myxopyronin B exhibits antibacterial activity against Gram-positive and Gram-negative bacteria through selectively inhibiting the bacterial DNA-dependent RNA polymerase (RNAP).[2] As an attractive lead for the development of RNAP inhibitors, myxopyronin B displays the following: selectivity versus human RNAP; good cell penetration that is reflected in a strong correlation between in vitro activity and cell potency; and potency against rifampicin-resistant *Staphylococcus aureus*.[3]



1 Myxopyronin B

Figure 1. The naturally occurring enantiomer (R) of myxopyronin B (**1**). All reported analogs were prepared as racemic mixtures.

Herein we report the synthesis and biological evaluation of eight new myxopyronin analogs compared to those presented in our initial report.[4] Among them, five compounds are derivatives of desmethyl myxopyronin B (Fig. 2, **2**) since this compound has enhanced potency and simplified structure. While our initial efforts focused on the changes to the side chains, our recent studies also included modifications to the pyrone core (see Schemes 1 and 2).



2 Desmethyl myxopyronin B

Figure 2. The synthetic desmethyl myxopyronin B (**2**).

Keywords: Myxopyronin B; Antibiotics; Antibacterial; Curtius rearrangement; Ortholithiation.
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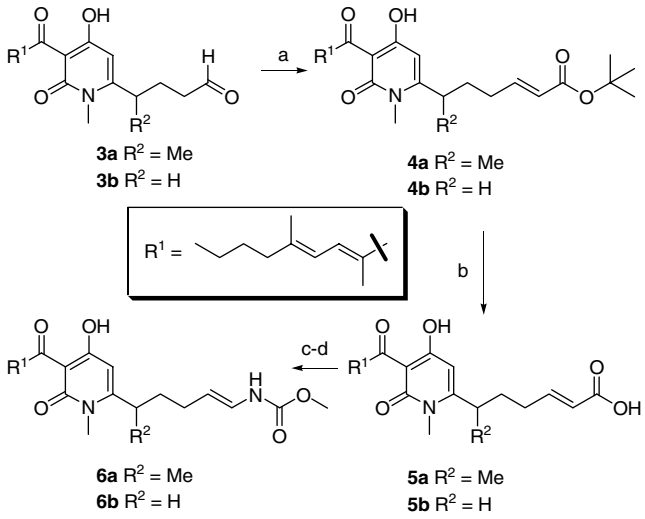
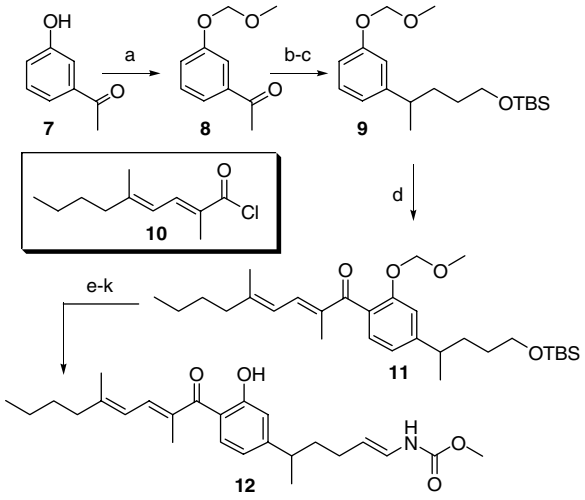


Scheme 1. Reagents and conditions: (a) *tert*-$BuOCOCH_2PO(OCH_3)_2$ (2.7 equiv), NaH (2.4 equiv), THF, 23 °C, 45 min; (b) TFA (10 equiv), CH_2Cl_2, 23 °C, 24 h, 79% two steps; (c) ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (4.8 equiv), H_2O, 0 °C, 90 min; (d) toluene, reflux, 2 h; then MeOH, 70 °C, 18 h, 60% two steps.

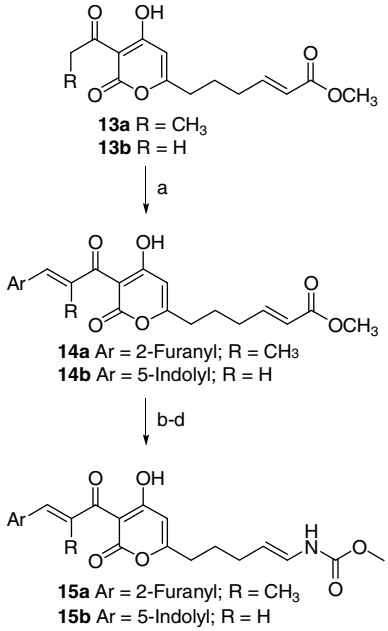


Scheme 2. Reagents and conditions: (a) MOMCl (1.2 equiv), DIPEA (1.4 equiv), CH_2Cl_2, 23 °C, 18 h, 93%; (b) $TBSO(CH_2)_3PPh_3^+I^-$ (2.0 equiv), *n*-BuLi (2.0 equiv), THF, −78 to 23 °C, 18 h, 82%; (c) H_2, 10% Pd/C (cat.), EtOAc, 23 °C, 18 h, 92%; (d) *tert*-BuLi (1.1 equiv), Et_2O, 0–23 °C, 1 h; then **10** (1.0 equiv), Et_2O, −78 to 23 °C, 18 h, 87%; (e) $TiCl_4$ (1.1 equiv), CH_2Cl_2, −78 °C, 5 min; (f) AcOH/THF/H_2O (3:1:1), 23 °C, 2 h, 95% two steps; (g) NMO (2.0 equiv), TPAP (cat.), 4 MS, CH_2Cl_2, 0 °C, 45 min; (h) $CH_3OCOCH_2PO(OCH_3)_2$ (2.6 equiv), NaH (2.5 equiv), THF, 23 °C, 2 h, 50% two steps; (i) 1 M LiOH/THF (1:3), 23 °C, 12 h, 95%; (j) ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (4.8 equiv), H_2O, 0 °C, 90 min; (k) toluene, reflux, 2 h; then MeOH, 70 °C, 18 h, 53% two steps.

Although pyrones have been used as a synthetic scaffold in HIV protease[5] and human sputum elastase inhibitors,[6] the 2-pyrone moiety of myxopyronin B is a potential electrophile.[7] To avoid this problem, we evaluated the importance of the pyrone core to the activity of myxopyronin B by preparing analogs **6a**,[8] **6b**[9] (Scheme 1) and **12**[10] (Scheme 2). The synthesis of **6a** is described as follows. Compound **3a** was prepared as previously reported[11] and subjected to Horner–Emmons–Wadsworth homologation, followed by acid hydrolysis to afford the carboxylic acid **5a** in 79% yield. It should be noted that although the ester hydrolysis could also be achieved in basic media (LiOH, THF/H_2O), the pyridone moiety was simultaneously converted to its corresponding pyrone.[11] Compound **5a** was converted to the enecarbamate **6a** in two steps by a Curtius rearrangement as reported by Panek and co-workers.[12]

To study the biologic necessity of the enolic pyrone core, we explored the possibility of replacing it with a simple phenol. Analog **12** was synthesized from commercial available 3′-hydroxyacetophenone (**7**) as shown in Scheme 2. The hydroxyl group of **7** was first protected as its methoxymethyl ether. A Wittig olefination, followed by catalytic hydrogenation, was utilized to introduce the right side chain. For the installation of the left side chain,[13] a directed ortholithiation–acylation strategy was adopted to give rise to compound **11**. Compound **11** was then treated with $TiCl_4$ in CH_2Cl_2, followed by AcOH/THF/H_2O to sequentially remove the MOM and TBS protecting groups. The resulting carboxylic acid was converted to the enecarbamate **12** under the same conditions as described for **6a** and **6b**.

Several analogs were made based on modifications to the left side chain. The syntheses of analogs **15a**[14] and **15b**[15] are depicted in Scheme 3. Aromatic side chains were readily prepared via aldol condensations catalyzed by piperidine. In the case of **15b**, diphenylphosphorazi-



Scheme 3. Reagents and conditions: (a) ArCHO (1.5 equiv), piperidine (cat.), $CHCl_3$, 65 °C, 18 h, 75%; (b) 1 M LiOH/THF (1:3), 23 °C, 12 h, 95%; (c) Ar = 2-furanyl: ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (4.8 equiv), H_2O, 0 °C, 90 min; Ar = 2-indolyl: $(PhO)_2PON_3$ (1.1 equiv), Et_3N (3.0 equiv), toluene, 65 °C, 1 h; (d) toluene, reflux, 2 h; then MeOH, 70 °C, 18 h, 53% for **15a** and 60% for **15b** two steps.

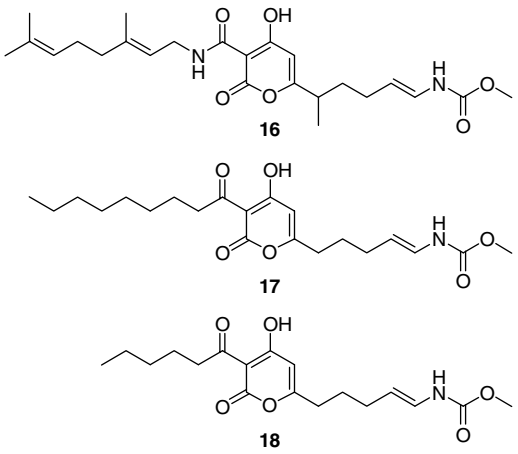


Figure 3. The structures of myxopyronin analogs **16**, **17**, and **18**.

date was utilized for the formation of the acyl azide intermediate of the Curtius rearrangement.

To further investigate the importance of the dienone system in the left side chain, and remove a potential electrophilic species from the molecule, analogs **16**,[16] **17**,[17] and **18**[18] (Fig. 3) were synthesized using techniques reported in our previous publication.[4]

In vitro inhibitory activity (IC_{50}) against RNAP (*Escherichia coli*) of the myxopyronin B analogs was measured utilizing an adapted nucleotide coupled NADPH/pyrophosphate release assay (Table 1) described in the literature.[19]

The antibacterial potency (MIC) of the analogs was determined in growth inhibition tests against *E. coli*, *E. coli* (Tol C), and *S. aureus*. All compounds were tested for cytotoxicity in a T-cell proliferation assay[20] and displayed no toxicity up to 40 μM (~18 μg/mL). The results are compiled in Table 1.

When the pyrone core of myxopyronin B was replaced with a more stable *N*-methyl pyridone, the analogs (Table 1, entries **6a** and **6b**) displayed substantial decrease in activity in the RNAP enzymatic assay and complete loss of potency against all three bacterial strains. When the pyrone core of compound **12** was replaced with phenol, it led to weak enzymatic inhibition and loss of antibacterial activity.

Attempts to modify the left chain of myxopyronin B and desmethyl myxopyronin B (Table 1, entries **16**, **15a**, **15b**, **17**, and **18**) were mostly fruitless as well. Compound **16** displayed mild antibacterial activity against *E. coli* (Tol C), but was inactive in the enzymatic assay and against *E. coli* and *S. aureus*.

In conclusion, the data generated from this continued study further confirmed that the antibacterial activities of myxopyronin B were very sensitive to both subtle and dramatic changes in its structure.

Table 1. In vitro activity against RNAP (IC_{50}) and antibacterial potency (MIC) of the myxopyronin analogs based on myxopyronin B (**1**) and desmethyl myxopyronin B (**2**)

Compound	IC_{50}[a] (μM)	MIC[b] (μg/mL)
1	0.92	>64/2/1
6a	20.5	>64/>64/>64
12	38%	>64/>64/>64
16	<20%	>64/16/>64
2	0.34	>64/1/4
6b	15	>64/>64/>64
15a	<20%	>64/>64/>64
15b	<20%	>64/>64/>64
17	<20%	>64/>64/>64
18	<20%	>64/>64/>64

[a] The IC_{50} value was not determined for weak inhibitors, instead the percentage of inhibition at 10 μM is listed.

[b] MIC: minimum inhibitory concentration (μg/mL), determined as average of triplicate measurements in serial dilution against *E. coli* (first value), *E. coli* Tol C (second value), and *S. aureus* (third value).

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8. Satisfactory spectroscopic data were obtained for all new compounds. All final analogs were purified by flash column chromatography and characterized by ^{1}H NMR and LC–MS. **6a**: ^{1}H NMR (400 MHz, CD_3OD) δ: 0.86 (t, J = 7.2 Hz, 3H), 1.20 (d, J = 6.8 Hz, 3H), 1.20–1.29 (m, 2H), 1.34–1.41 (m, 2H), 1.49–1.56 (m, 2H), 1.68–1.76 (m, 2H), 1.79 (s, 3H), 1.97–2.06 (m, 2H), 2.03 (s, 3H), 2.47–2.57 (m, 1H), 3.08 (s, 3H), 3.66 (s, 3H), 5.01–5.08 (m, 1H), 5.49–5.52 (m, 1H), 5.74 (s, 1H), 6.36–6.41 (m, 2H).
9. **6b**: ^{1}H NMR (400 MHz, CD_3OD) δ: 0.95 (t, J = 7.2 Hz, 3H), 1.32–1.38 (m, 2H), 1.44–1.50 (m, 2H), 1.66–1.72 (m, 2H), 1.77 (s, 3H), 1.98 (s, 3H), 2.02–2.07 (m, 2H), 2.17 (t, J = 7.2 Hz, 2H), 2.40 (t, J = 7.6 Hz, 2H), 3.13 (s, 3H), 3.66 (s, 3H), 5.04–5.11 (m, 1H), 5.73 (s, 1H), 6.12–6.20 (m, 2H), 6.35–6.44 (m, 1H).

10. **12**: ^{1}H NMR (400 MHz, CD_3OD) δ: 0.95 (t, J = 7.2 Hz, 3H), 1.24 (d, J = 6.8 Hz, 3H), 1.30–1.40 (m, 2H), 1.43–1.55 (m, 2H), 1.60–1.69 (m, 2H), 1.77 (s, 3H), 1.83–1.95 (m, 2H), 2.02 (s, 3H), 2.23 (t, J = 7.6 Hz, 2H), 2.73 (q, J = 7.2 Hz, 1H), 3.66 (s, 3H), 5.02–5.09 (m, 1H), 6.28–6.34 (m, 2H), 6.76–6.79 (m, 2H), 6.95 (dd, J = 1.6, 11.6 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H).
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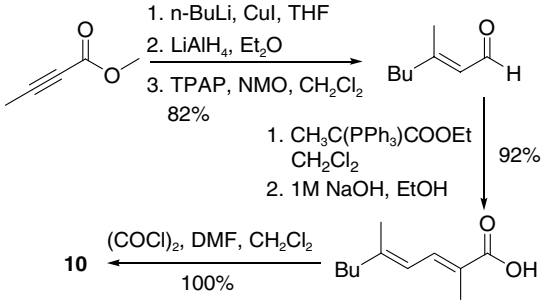
13. Compound **10** was synthesized based on the following route:



.
14. **15a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.57–1.64 (m, 2H), 1.98–2.03 (m, 2H), 2.09 (s, 3H), 2.44–2.50 (m, 2H), 3.58 (s, 3H), 5.00–5.07 (m, 1H), 6.05 (s, 1H), 6.33–6.39 (m, 1H), 6.67 (s, 1H), 6.94 (d, J = 3.2 Hz, 1H), 7.14 (s, 1H), 7.91 (s, 1H), 9.23 (d, J = 9.6 Hz, 1H).
15. **15b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.59–1.67 (m, 2H), 1.98–2.05 (m, 2H), 2.48–2.55 (m, 2H), 3.57 (s, 3H), 4.98–5.05 (m, 1H), 6.22 (s, 1H), 6.32–6.38 (m, 1H), 6.55 (s, 1H), 7.42–7.44 (m, 1H), 7.48–7.53 (m, 1H), 7.96 (s, 1H), 8.12 (dd, J = 30.4, 16.0 Hz, 2H), 9.22 (d, J = 8.8 Hz, 1H), 11.45 (s, 1H).
16. **16**: ^{1}H NMR (400 MHz, CD_3OD) δ: 1.60 (s, 3 H), 1.66 (s, 3H), 1.73 (s, 3H), 1.69–1.77 (m, 2H), 2.00–2.15 (m, 8H), 2.54 (t, J = 8.4 Hz, 2H), 3.66 (s, 3H), 3.96 (d, J = 8.0 Hz, 2H), 5.07–5.10 (m, 1H), 5.25–5.28 (m, 1H), 6.14 (s, 1H), 6.42 (d, J = 14.4 Hz, 1H).
17. **17**: ^{1}H NMR (400 MHz, CD_3OD) δ: 0.89 (t, J = 7.2 Hz, 3H), 1.28–1.36 (m, 10H), 1.74–1.82 (m, 4H), 2.07–2.13 (m, 2H), 2.65 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 3.66 (s, 3H), 5.04–5.11 (m, 1H), 6.42 (d, J = 12.8 Hz, 1H), 6.76 (s, 1H).
18. **18**: ^{1}H NMR (400 MHz, CD_3OD) δ: 0.90–0.93 (m, 3H), 1.35–1.40 (m, 4H), 1.74–1.82 (m, 4H), 2.07–2.12 (m, 2H), 2.65 (t, J = 7.6 Hz, 2H), 2.89 (t, J = 7.6 Hz, 2H), 3.66 (s, 3H), 5.03–5.11 (m, 1H), 6.42 (d, J = 12.8 Hz, 1H), 6.76 (s, 1H).
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20. Cell toxicity assays for CC_{50} determination were conducted on CEM-SS T lymphocyte cells. Cellular toxicity was measured using XTT (Sigma) and detected with a fluorometer at 450 nm/650 nm using Genios/Tecan (Xfluor). CEM-SS cells were incubated in 96-well microtiter plates for 3 days. At the end of that time, the cells were treated with an XTT solution, measurements were recorded and CC_{50} determinations were made.