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Myxopyronin B analogs as inhibitors of RNA polymerase, synthesis and biological evaluation

Thomas Doundoulakis,[a] Alan X. Xiang,[a] Ricardo Lira,[a] Konstantinos A. Agrios,[a] Stephen E. Webber,[a] Wes Sisson,[b] Robert M. Aust,[b] Amit M. Shah,[b] Richard E. Showalter,[b] James R. Appleman[b] and Klaus B. Simonsen[a,*]

[a]*Department of Medicinal Chemistry, Anadys Pharmaceuticals Inc., 3115 Merryfield Row, San Diego, CA 92121, USA*
[b]*Department of Discovery Biology, Anadys Pharmaceuticals Inc., 3115 Merryfield Row, San Diego, CA 92121, USA*

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Abstract—A series of myxopyronin B analogs has been prepared via a convergent synthetic route and were tested for in vitro inhibitory activity against DNA-dependent RNA polymerase and antibacterial activity against *E. coli* and *S. aureus*. The parent lead compound proved to be very sensitive to even small changes. Only the achiral desmethyl myxopyronin B (**1a**) provided enhanced potency.

Since the discovery of penicillin, natural sources have become the preferred choice in the search for new antibiotics. Today more than half of all approved drugs from anticancer and antiinfective programs originate from natural products.[1]

Myxopyronin B (**1b**), a bacterial metabolite isolated from *Myxococcus fulvus* Mx 150,[2a,b] exhibits antibacterial activity against Gram-positive and Gram-negative bacteria.[3] Myxopyronin B belongs to an interesting class of 3-acyl-4-hydroxy-α-pyronens natural products, which include the corallopyronins A–C[2c] and myxopyronin A.[2] Myxopyronin B, inhibits the bacterial DNA-dependent RNA polymerase (RNAP)[3] and represents a new lead for drug discovery directed at that target. DNA-dependent RNAP is the principal enzyme of transcription in all living organisms, and a key target in many regulatory pathways that control gene expression. Currently, rifampicin is the only known drug that inhibits RNAP, but its use has been limited by the rapid development of resistance.[4] Therefore, new leads that inhibit RNAP are important. Myxopyronin B represents an attractive lead for the development of RNAP inhibitors. Myxopyronin B displays: selectivity versus human RNAP, good cell penetration, reflected in a good correlation between in vitro *activity* and cell potency, and potency against rifampicin-resistant *S. aureus*.[4] Although myxopyronin B was isolated more than 20 years ago, a total synthesis was reported only recently by Panek and co-workers.[5]

Here, we report the synthesis and biological evaluation of 15 new myxopyronin analogs (Fig. 1 and Table 1) in which the pyrone core remains constant, and the dienone and enecarbamate side chains have been modified. This convergent route relies on two synthetic differentiations steps, including an alkylation step to install the enecarbamate part of the molecule followed by an aldol condensation to introduce the dienone part (Fig. 1).[5]

Several features within the structure of myxopyronin B suggest possible modification in order to modify the chemical stability of metabolically liable functionalities. Specifically, the enecarbamate moiety may be prone to

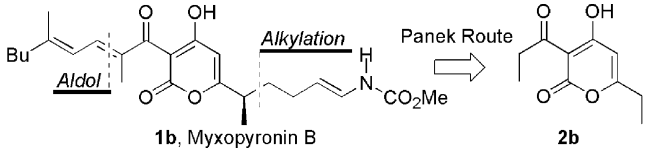

Figure 1. The naturally occurring enantiomer (*R*) of Myxopyronin B (**1b**). All reported analogs were prepared as racemic mixtures.

Keywords: RNA polymerase; Antibiotics; Medicinal chemistry.
* Corresponding author at present address: Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark. E-mail: kbs@dfuni.dk

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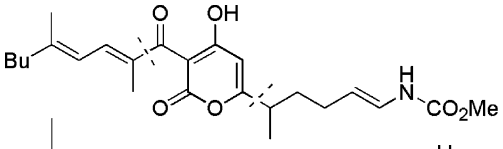
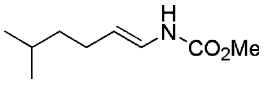
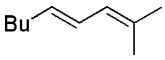
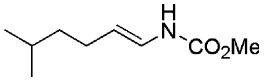
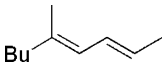
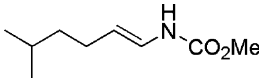
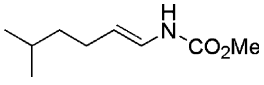
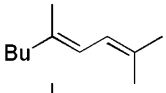
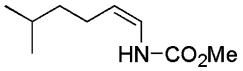
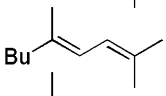
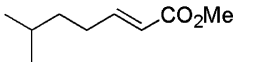
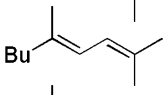
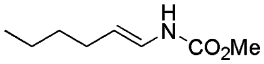
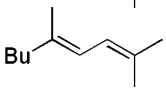
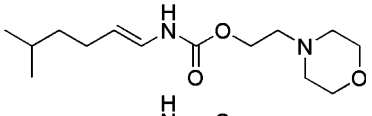
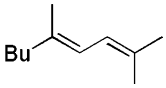
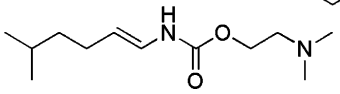
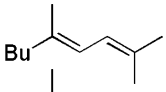
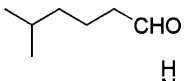
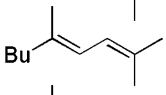
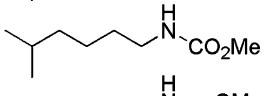
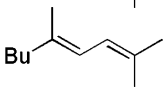
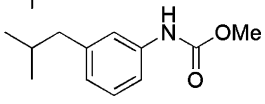
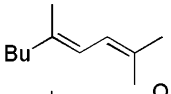
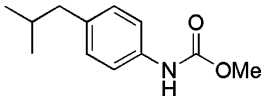
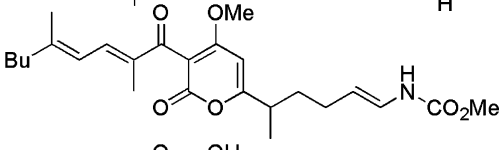
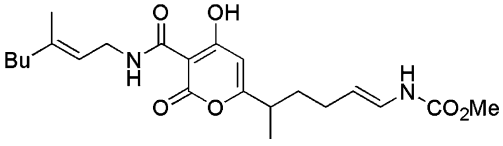
Table 1. In vitro activity against RNAP (IC_{50}) and antibacterial potency (MIC) of the myxopyronin analogs

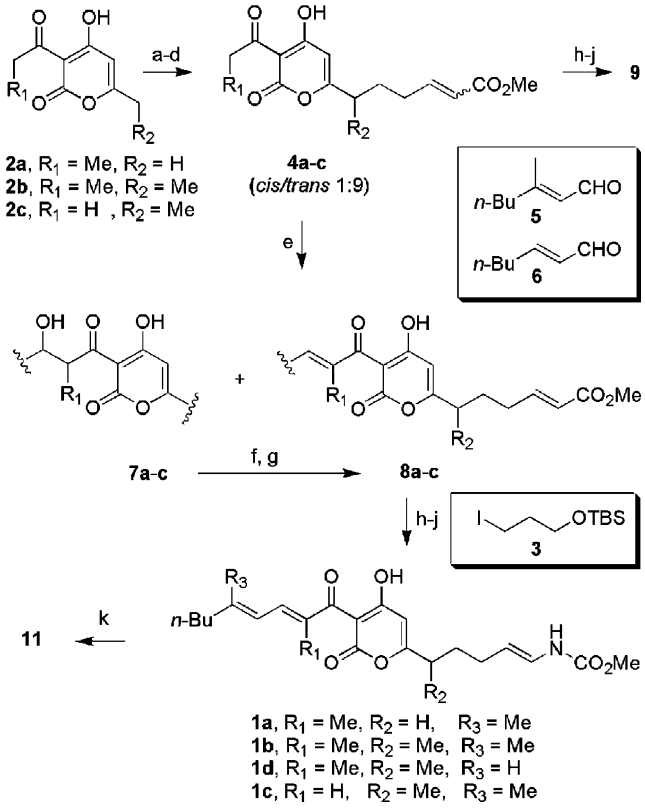
Compd	Structure		IC_{50} (μM)[a]	MIC (μg/mL)[b]
	Dienone	Enecarbamate		
1b			0.92	>64/2/1
1e			2.5	>64/16/16
1d			1.04	>64/2/16
1c			70%	>64/32/64
9			<20%	>64/>64/>64
10			1.26	>64/4/4
8b			<20%	>64/>64/>64
1a			0.34	>64/1/4
13			<20%	>64/>64/>64
12			<20%	>64/>64/>64
18			<20%	>64/>64/>64
19			4.0	>64/8/64
26			<20%	>64/>64/>64
27			<20%	>64/>64/>64
11			4.2	>64/>64/>64
33			<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).

hydrolysis. Although pyrones have been used as synthetic scaffold in HIV protease[6] and human sputum elastase inhibitors,[7] the 2-pyrone moiety and the dienone could act as possible Michael acceptors.

The syntheses of analogs **1a**–**d** are depicted in Scheme 1. We will discuss the synthesis of desmethyl myxopyronin B (**1a**) as a representative example. Lithiation of 4-hydroxy-6-methyl-3-propionyl-2-pyrone[8] (**2a**) followed by



Scheme 1. Reagents and conditions: (a) LDA (3.2 equiv), HMPA (3.2 equiv), THF, −78 °C, 1 h; then **3** (1.1 equiv), −78 °C, 1 h, 65–80%; (b) AcOH/THF/H_2O (3:1:1), 23 °C, 12 h, 85–90%; (c) Dess–Martin periodinane (2.0 equiv), $NaHCO_3$ (5.0 equiv), CH_2Cl_2, 23 °C, 2 h, 90–95%; (d) trimethyl phosphonoacetate (2.6 equiv), NaH (2.5 equiv), THF, 23 °C, 15 min; then aldehyde (1.0 equiv), THF, 23 °C, 3 h, 65–78% (*cis*/*trans* ~ 1:9); (e) **4a–c** (1 equiv), $TiCl_4$ (4.0 equiv), CH_2Cl_2, −78 °C, 30 min; then DIPEA (5.0 equiv), −78 °C, 3 h; then **5** or **6** (2–4 equiv), CH_2Cl_2, −78 °C, 48 h; −78 to 23 °C, 1 h, 15–35%; (f) MsCl (2.0 equiv), Et_3N (3.0 equiv), CH_2Cl_2, 23 °C, 4 h, 78%; (g) DBU (3.0 equiv), THF, reflux, 2 h, 75%; (h) 1 M LiOH/THF (1:3), 23 °C, 12 h, 90–95%; (i) ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (10 equiv), H_2O, 0 °C, 90 min; (j) toluene, 2 h, reflux; then MeOH, 18 h, 70 °C, 50–65% two steps; (k) MeI (3.0 equiv), K_2CO_3 (10 equiv), acetone, reflux, 12 h, 55%.

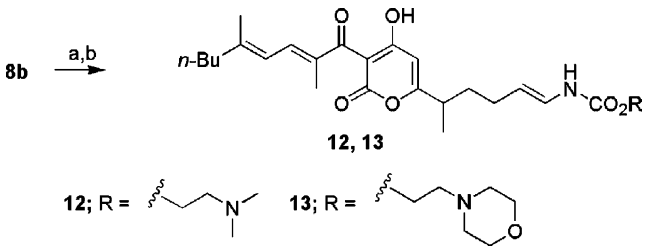
the addition of 1-iodo-3-(*tert*-butyldimethylsilyloxy)propane, **3**,[5] afforded the regioselective alkylation in excellent yield.

Silyl deprotection followed by Dess–Martin periodinane oxidation and Horner–Emmons–Wadsworth homologation afforded **4a** as a 9:1 mixture of geometrical isomers (*E*:*Z*), which were separated by chromatography. Addition of freshly distilled $TiCl_4$ to **4a** at −78 °C followed by addition of DIPEA generated the titanium enolate, which was condensed with *E*-3-methyl-2-heptenal **5**.[9] Although TLC indicated complete condensation to the β-hydroxy ketone **7a** within 30 min, the dehydration step was unreliable and we were never able to isolate the desired dehydrated product **8a** in more than 15–35% yield together with a significant amount of β-hydroxy ketone **7a**. Fortunately, **7a**, could be converted to dienone **8a** in two steps to improve the overall yield. Specifically, treatment of **7a** with methanesulfonyl chloride gave rise to corresponding β-chloro compound that eliminated at elevated temperature in the presence of DBU to produce **8a**. Basic hydrolysis of **8a** produced the corresponding acid, which was converted to the enecarbamate in two steps by a Curtius rearrangement as reported by Panek co-workers.[5] The elevated temperature necessary to generate the isocyanate apparently causes isomerization of the dienone moiety of the molecule. The final products were isolated as a 3:1 mixture of geometrical isomers. Therefore, a final HPLC purification was necessary to produce pure **1a**.[10]

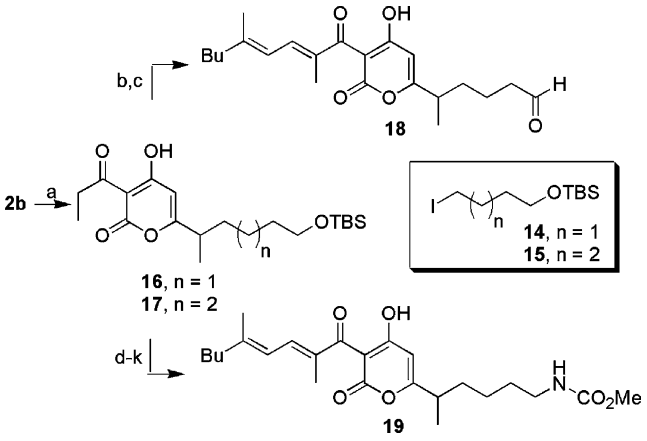
Several other myxopyronin analogs were prepared through the same reaction sequence utilizing either different pyrones (**1a–c**) in the alkylation step or aldehydes (**5** or **6**) in the aldol condensation step. Racemic myxopyronin B (**1b**) and its geometrical isomer **1e** (see Table 1) was produced from 6-ethyl-4-hydroxy-3-propionyl-2-pyrone (**2b**).[5,7] Compound **1d**, lacking the δ-methyl group, was produced from **2b** and *trans*-2-heptenal **6** and finally compound **1c** was produced from 3-acetyl-6-ethyl-4-hydroxy-2-pyrone (**2c**), which was prepared from 6-ethyl-4-hydroxy-2-pyrone[11] and acetyl chloride in the presence of TFA.[8] Compound **10**, the *cis*-enecarbamate isomer of myxopyronin B, was prepared from the *cis* product of the Wittig reaction (see **4b**) via the same protocol. Hydrolysis of intermediate **4b**, furnished the corresponding enecarbamate **9** after the Curtius reaction. O-Methylation of myxopyronin B with MeI produced **11**.

Two analogs, in which a solubilizing group was attached to the enecarbamate, were prepared from **8b** as shown in Scheme 2.

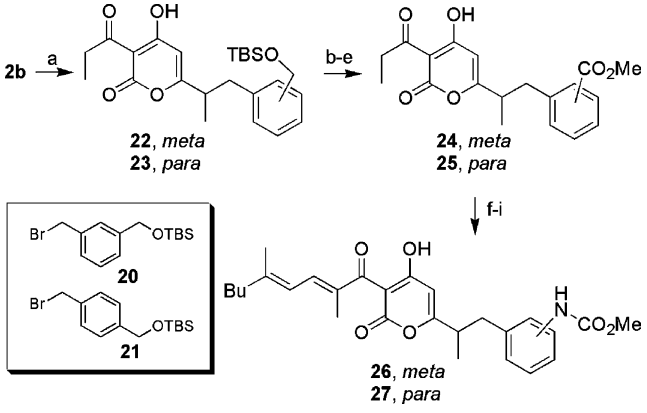
Further modifications to the enecarbamate of myxopyronin B are depicted in Schemes 3 and 4. Alkylation of **2b** with 1-iodo-4-(*tert*-butyldimethylsilyloxy)butane (**14**)[12] furnished **16**, which was converted to the dienone by the Ti(IV) mediated aldol condensation with concurrent silyl deprotection. The resulting alcohol was subsequently oxidized to aldehyde **18**. The saturated carbamate analog **19** was prepared from **2b** and 1-iodo-5-(*tert*-butyldimethylsilyloxy)pentane (**15**)[13] to furnish **17**, which was carried forward as described above to produce **19**.



Scheme 2. Reagents and conditions: (a) 1 M LiOH/THF (1:3), 23 °C, 12 h, 95%; (b) ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (10 equiv), H_2O, 0 °C, 90 min; (c) toluene, 2 h, reflux; ROH (20 equiv), 18 h, 70 °C, 50–65% two steps.

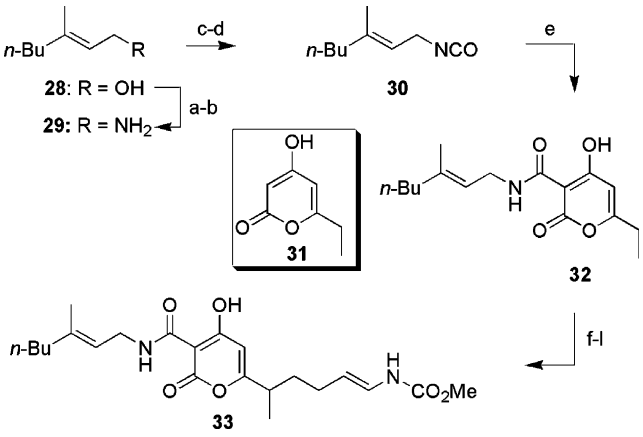


Scheme 3. Reagents and conditions: (a) LDA (3.2 equiv), HMPA (3.2 equiv), THF, −78 °C, 1 h; then **14** or **15** (1.1 equiv), −78 °C, 1 h, 65–76%; (b) **16** (1 equiv), $TiCl_4$ (4.0 equiv), CH_2Cl_2, −78 °C, 30 min; then DIPEA (5.0 equiv), −78 °C, 3 h; then **5** (3.0 equiv), CH_2Cl_2, −78 °C, 48 h; −78 to 23 °C, 1 h, 20%; (c) Dess–Martin periodinane (2.0 equiv), $NaHCO_3$ (5.0 equiv), CH_2Cl_2, 23 °C, 2 h, 80%; (d) AcOH/THF/H_2O (3:1:1), 23 °C, 12 h, 90%; (e) Dess–Martin periodinane (2.0 equiv), $NaHCO_3$ (5.0 equiv), CH_2Cl_2, 23 °C, 2 h, 85%; (f) $NaClO_2$ (2 equiv), NaH_2PO_4 (3 equiv), 2-methyl-2-butene (20 equiv), THF/*t*-BuOH/H_2O (2:2:1), 23 °C, 2 h; (g) $TMSCHN_2$ (1.3 equiv), benzene/MeOH (3:1), 23 °C, 1 h, 65% two steps; (h) **17** (1 equiv), $TiCl_4$ (4.0 equiv), CH_2Cl_2, −78 °C, 30 min; then DIPEA (5.0 equiv), −78 °C, 3 h; then **5** (3.0 equiv), CH_2Cl_2, −78 °C, 48 h; −78 to 23 °C, 1 h, 15%; (i) 1 M LiOH/THF (1:3), 23 °C, 12 h, 70%; (j) ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (10 equiv), H_2O, 0 °C, 90 min; (k) toluene, 2 h, reflux; then MeOH, 18 h, 70 °C, 65% two steps.



Scheme 4. Reagents and conditions: (a) LDA (3.2 equiv), HMPA (3.2 equiv), THF, −78 °C, 1 h; then **20** or **21** (1.1 equiv), −78 °C, 1 h, 40–55%; (b) AcOH/THF/H_2O (3:1:1), 23 °C, 12 h, 75–86%; (c) Dess–Martin periodinane (2.0 equiv), $NaHCO_3$ (5.0 equiv), CH_2Cl_2, 23 °C, 2 h, 92–95%; (d) $NaClO_2$ (2 equiv), NaH_2PO_4 (3 equiv), 2-methyl-2-butene (20 equiv), THF/*t*-BuOH/H_2O (2:2:1), 23 °C, 2 h; (e) $TMSCHN_2$ (1.3 equiv), benzene/MeOH (3:1), 23 °C, 1 h, 60–65% two steps; (f) $TiCl_4$ (4.0 equiv), CH_2Cl_2, −78 °C, 30 min; then DIPEA (5.0 equiv), −78 °C, 3 h; then **5** (3.0 equiv), CH_2Cl_2, −78 °C, 48 h; −78 to 23 °C, 1 h, 15–20%; (g) 1 M LiOH/THF (1:3), 23 °C, 12 h; (h) ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (10 equiv), H_2O, 0 °C, 90 min; (i) toluene, 2 h, reflux; then MeOH, 18 h, 70 °C, 52–64% two steps.

To introduce rigidity and chemical stability to the enecarbamate, two analogs, **26** and **27**, in which the double bond was incorporated into an aromatic system were



Scheme 5. Reagents and conditions: (a) phthalimide (1.3 equiv), Ph_3P (1.3 equiv), DEAD (1.3 equiv), THF, 23 °C, 6 h, 86%; (b) $MeNH_2$ (8.0 equiv), EtOH, reflux, 7 h; (c) HCl (1.1 equiv), MeOH/acetone (1:14), 0 °C, 5 min, 99%; (d) $COCl_2$ (10.0 equiv), toluene, reflux, 3 h, 85%; (e) **31** (1.0 equiv), **30** (1.0 equiv), Et_3N (1.0 equiv), xylene, reflux, 4 h, 76%; (f) LDA (3.2 equiv), HMPA (3.2 equiv), THF, −78 °C, 1 h; then **3** (1.1 equiv), −78 °C, 1 h; (g) AcOH/THF/H_2O (3:1:1), 23 °C, 12 h; (h) Dess–Martin periodinane (2.0 equiv), $NaHCO_3$ (5.0 equiv), CH_2Cl_2, 23 °C, 2 h; (i) trimethyl phosphonoacetate (2.6 equiv), NaH (2.5 equiv), THF, 23 °C, 15 min; then aldehyde (1.0 equiv), THF, 23 °C, 3 h; (j) 1 M LiOH/THF (1:3), 23 °C, 12 h; (k) ethyl chloroformate (2.2 equiv), DIPEA (2.4 equiv), acetone, 0 °C, 90 min; then NaN_3 (10 equiv), H_2O, 0 °C, 90 min; (l) toluene, 2 h, reflux; then MeOH, 18 h, 70 °C, 16% seven steps.

prepared (Scheme 4). The alkylation reagents **20**[14] and **21** were prepared in two steps from 1,3- and 1,4-bis(hydroxymethyl)benzene,[15] respectively. Hence, treatment of 1,3- and 1,4-bis(hydroxymethyl)benzene, with NaH, followed by addition of TBSCl furnished the mono-protected compounds in good yields, which were treated with CBr_4 and PPh_3 to produce **20** and **21**, respectively.

To investigate the importance of the dienone moiety for RNAP-activity and furthermore remove a potential Michael acceptor moiety from the molecule, the β−γ unsaturated amide **33** was prepared (Scheme 5). Amine **29** was prepared from alcohol **28**,[9] following a protocol developed by Sen and Roach,[16] and further converted to isocyanate **30** under standard conditions.[17] Condensation between 6-ethyl-4-hydroxy-2-pyrone **31**[11] and isocyanate **30** proceeded in refluxing xylene[18] to produce **32** in good yield, which was converted to the final enecarbamate **33** in seven steps as described above.

The in vitro inhibitory activity (IC_{50}) against RNAP (*E. coli*) of the myxopyronin analogs was evaluated in a nucleotide coupled NADPH/pyrophosphate release assay (Table 1) based upon a scheme described previously.[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 and displayed no toxicity up to 40 μM (~18 μg/mL). The results are compiled in Table 1.

Changes to the dienone region of myxopyronin B revealed several interesting features required for the activity of this class of compounds (Table 1, entries 2–5 and 16). The geometrical isomer of myxopyronin B, **1e**, showed a threefold decrease in activity in the RNAP enzymatic assay and a 10-fold decrease in potency in the growth inhibition test.

Although compound **1d**, lacking the methyl δ to the carbonyl group, showed similar activity against RNAP in the biochemical assay and toward *E. coli* (Tol C), the activity against *S. aureus* decreased significantly. Removing the methyl adjacent to the carbonyl group (**1c**) resulted in an almost complete loss of antibacterial activity. Replacement of the dienone with an amide functionality as in **33** or complete absence of the diene moiety, as in **9**, resulted in a loss of antibacterial potency, as well as the enzymatic activity. The enecarbamate component of myxopyronin B was even more sensitive toward changes (Table 1, entries 6–14). Extending the carbamate with solubilizing groups (**12** and **13**), replacement of the carbamate with an ester (**8b**) and the incorporation of the carbamate alkene into an aromatic moiety (**26**–**27**) reduced the enzymatic activity and resulted in a complete loss of potency against all three bacterial strains. Aldehyde **18**, which is a potential degradation product of myxopyronin B, lacked potency and was therefore dismissed as an RNAP inhibitor.

The *cis* analog, **10**, displayed comparable IC_{50} value as the parent *trans* compound with a small decrease in activity toward *E. coli* and *S. aureus*. The saturated carbamate analog **19** displayed comparable activity against RNAP, but a significant decrease in MIC was observed. Methylation of the pyrone skeleton (**11**) resulted in fivefold decrease toward RNAP and complete loss of cellular activity, indicating the importance of the free hydroxyl group for biological activity.

The desmethyl analog, **1a**, is the most active analog synthesized in this series. The activity against RNAP was improved threefold, without any increase in the cellular potency. This result shows that it is possible to remove the methyl group and hence the chirality from this class of compounds without diminishing the biological activity. Thus, the preparation of the next generation of myxopyronin B analogs, was designed without the chiral center. The synthesis and biological evaluation on the second-generation analogs will be reported elsewhere.

In conclusion, the SAR generated from this study showed that minor structural changes to myxopyronin B led to loss of biological activity. At the present time natural product within this class do not represent viable lead candidates for antibacterial therapy until a better understanding of the exact mode of action is determined.

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9. (a) Aldehyde **5** was prepared from methyl 2-butynoate immediately prior to addition, in a three-step reaction sequence: (i) *n*-BuLi, CuI, methyl 2-butynoate; (ii) $LiAlH_4$; (iii) TPAP, NMO. See: Anderson, R. J.; Corbin, V. L.; Cotterrell, G.; Cox, G. R.; Henrick, C. A.; Schaub, F.; Siddall, J. B. *J. Am. Chem. Soc.* **1975**, *97*, 1197–1204; (b) Goura, K.; Nishino, T.; Koyama, T.; Seto, S. *J. Am. Chem. Soc.* **1970**, *92*, 1197–1204.
10. Satisfactory spectroscopic data were obtained for all new compounds and all final analogs were purified by reverse phase HPLC and characterized by ^1H NMR and LC–MS. HPLC ($MeOH/H_2O/AcOH$ = 70:30:4, flow rate = 40 mL/min) t_r = 2.6 min (**1a**, minor), t_r = 3.9 min (**1a**, major).
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NADPH formation typically was monitored by fluorescence (excitation wavelength, ~340 nm; emission wavelength, ~440 nm) rather than absorbance at 340 nm as in the original assay to improve sensitivity. Although compounds with strong absorbance at 340 nm can in principle influence the assay, we found it to be acceptable for all compounds discussed herein. Inhibitory potencies of selected compounds were also evaluated by effects on incorporation of radiolabeled nucleotide into RNA, and comparable results were obtained.