

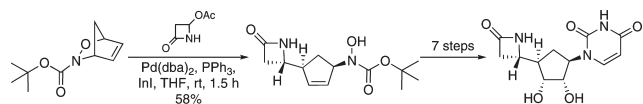
Syntheses of Carbocyclic Uracil Polyoxin C Analogs:
Application of Pd(0)/InI-Allylation of 4-Acetoxy-2-azetidinone[†]

Cara Cesario and Marvin J. Miller*

Department of Chemistry and Biochemistry, University of
Notre Dame, 251 Nieuwland Science Hall, Notre Dame,
Indiana 46556

miller1@nd.edu

Received April 25, 2009



Carbocyclic uracil polyoxin C analogs are prepared from an acylnitroso-derived hetero Diels–Alder cycloadduct in fewer than nine steps. Pd(0)/InI-mediated allylation of 4-acetoxy-2-azetidinone is used to install the β -amino acid side chain at the C-5' position of the carbocycle.

Polyoxins and nikkomycins are peptidyl nucleoside natural products that selectively target fungi while remaining nontoxic to plants, animals and bacteria.¹ Specifically, these molecules are inhibitors of chitin synthase,² an enzyme responsible for the synthesis of the fungal cell wall component chitin. Disrupting chitin biosynthesis compromises the structural integrity of fungal cell walls and results in death of the pathogen.³ Because plant and mammalian cells do not produce chitin, inhibition of chitin synthase only affects fungal cells and is considered a potentially safe and nontoxic approach toward combating fungal infections.

Although polyoxins and nikkomycins demonstrate competitive inhibition of chitin synthase in enzyme assays,⁴ the ionized amino acid side chains significantly impede their cellular transport and has precluded their development as fungicidal agents. Additionally, polyoxins and nikkomycins are less effective in vivo due to their instability toward intracellular proteases.⁵ In efforts to prepare polyoxin and

nikkomycin derivatives with increased potency, several analogs have been synthesized with diverse amino acid side chains,⁶ inverted configurations⁷ and different nucleoside bases.⁸

Additionally, prodrug variants of polyoxins and nikkomycins have been designed to permeate fungal cell membranes and release the active component upon intracellular hydrolysis.⁹ Despite extensive modification of the peptidyl subunits, less effort has focused on structural changes at the central ribose core. By replacing the furanose oxygen with a methylene unit, several classes of carbocyclic nucleosides have displayed increased metabolic stability and improved biological activity.¹⁰ To date, syntheses of carbocyclic derivatives of polyoxins and nikkomycins have been limited.¹¹ Herein, we disclose the synthesis of carbocyclic uracil polyoxin C analogs **1a** and **2a** as potential antifungal agents (Chart 1).

Recently, we reported the diastereoselective allylation of 4-acetoxy-2-azetidinone from a unique allylindium precursor, acylnitroso-derived hetero-Diels–Alder adduct **3**, to incorporate highly functionalized cyclopentenones at the azetidinone C-4 position.¹² Further elaboration of synthon **4** would reveal an unprecedented polyoxin C analog bearing an azetidinone at the C-5' position. Interestingly, several naturally occurring polyoxins (i.e., Polyoxin I)¹³ contain 2-carboxyazetidine side chains and illustrate the structural diversity in this class of molecules. The protected β -amino acid side chain renders polyoxin C analog **1a** as having the potential for improved antifungal activity due to its neutral character. Furthermore, intracellular hydrolysis of target molecule **1a** would provide compound **2a** and reveal the free amino and carboxy groups, two functionalities that are required for inhibition.¹⁴

In a first attempt to synthesize key intermediate **8**, *N*-hydroxycarbamate **4** was reduced to carbamate **5** in the presence of titanocene (III) monochloride¹⁵ generated in situ (Scheme 1). Treatment of compound **5** with TFA and anisole in refluxing CH₂Cl₂ formed the amine salt in quantitative yield after 3 h. The crude material was treated with acyl

[†] We dedicate this paper to Professor Jeremiah P. Freeman on the occasion of his 80th birthday and with thanks for his outstanding service to the profession of organic chemistry through his 25 years as secretary of Organic Syntheses.

(1) For a review of synthetic methods, see: Zhang, D.; Miller, M. J. *Curr. Pharm. Design* **1999**, *5*, 73–99.

(2) Chitin synthase as an antifungal target, see: (a) Ruiz-Herrera, J.; San-Blas, G. *Curr. Drug Targets- Infectious Disorders* **2003**, *3*, 77–91. (b) Behr, J.-B. *Curr. Med. Chem.- Anti-Infective Agents* **2003**, *2*, 173–189.

(3) Ruiz-Herrera, J. *Fungal Cell Wall: Structure, Synthesis and Assembly*; CRC Press: Boca Raton, 1992.

(4) Gooday, G. W. In *The Biochemistry of Cell Walls and Membranes in Fungi*; Goosey, M., Kuhn, P., Trinci, A. P. J., Eds.; Springer-Verlag: Berlin, 1989; pp 61–76.

(5) Naider, F.; Becker, J. M. In *Current Topics in Medical Mycology II*; McGinnis, M. M., Ed.; Springer: Berlin, 1987; pp 170–198.

(6) Shenbagamurthi, P.; Smith, H. A.; Becker, J. M.; Naider, F. *J. Med. Chem.* **1986**, *29*, 802–809.

(7) Emmer, G.; Ryder, N. S.; Grassberger, M. A. *J. Med. Chem.* **1985**, *28*, 278–281.

(8) Azuma, T.; Isono, K.; Crain, P. F.; McCloskey, J. A. *J. Chem. Soc., Chem. Comm.* **1977**, *5*, 159–160.

(9) (a) Krainer, E.; Becker, J. M.; Naider, F. *J. Med. Chem.* **1991**, *34*, 174–180. (b) Naider, F.; Shenbagamurthi, P.; Steinfeld, A. S.; Smith, H. A.; Boney, C.; Becker, J. M. *Antimicrob. Agents Chemother.* **1983**, *24*, 787–796.

(10) (a) Casu, F.; Chiacchio, M. A.; Romeo, R.; Gumina, G. *Curr. Org. Chem.* **2007**, *11*, 999–1016. (b) Jeong, L. S.; Lee, J. A. *Antivir. Chem. Chemother.* **2004**, *15*, 235–250. (c) Rodriguez, J. B.; Comin, M. J. *Mini-Rev. Med. Chem.* **2003**, *3*, 95–114. (d) Marquez, V. E.; Lim, M. I. *Med. Res. Rev.* **1986**, *6*, 1–40.

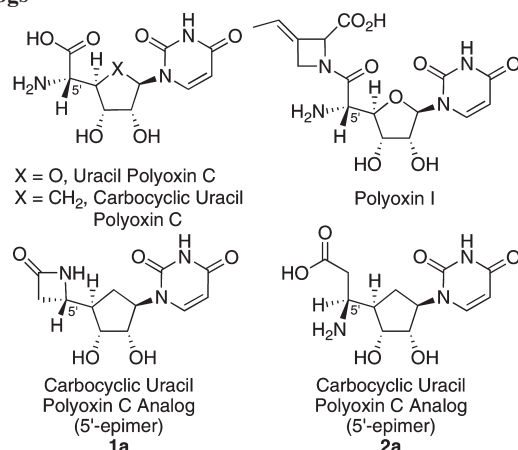
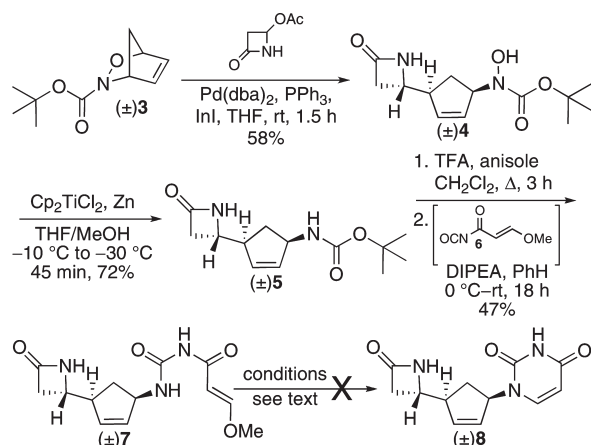
(11) (a) Li, F.; Brogan, J. B.; Gage, J. L.; Zhang, D.; Miller, M. J. *J. Org. Chem.* **2004**, *69*, 4538–4540. (b) Zhang, D.; Miller, M. J. *J. Org. Chem.* **1998**, *63*, 755–759. (c) Ward, S. E.; Holmes, A. B.; Ward, S. E.; McCague, R. *Chem. Comm.* **1997**, *21*, 2085–2086. (d) Aggarwal, V. K.; Monteiro, N. *Org. Biomol. Chem.* **1997**, *17*, 2531–2537. (e) Aggarwal, V. K.; Monteiro, N.; Tarver, G. J.; Lindell, S. D. *J. Org. Chem.* **1996**, *61*, 1192–1193.

(12) Cesario, C.; Miller, M. J. *Org. Lett.* **2009**, *11*, 1293–1295.

(13) Isono, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.* **1969**, *91*, 7490–7505.

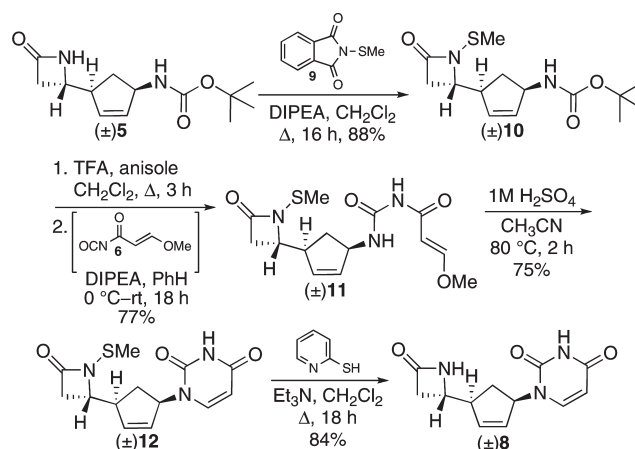
(14) Hori, M.; Kakiki, K.; Misato, T. *Agric. Biol. Chem.* **1974**, *38*, 699–705.

(15) Cesario, C.; Tardibono, L. P.; Miller, M. J. *J. Org. Chem.* **2009**, *74*, 448–451.

CHART 1. Representative Polyoxins and Related Carbocyclic Analogs**SCHEME 1. Initial Synthetic Route to Uracil 8**

isocyanate **6**¹⁶ freshly dissolved in benzene. The reaction was complete within 18 h to afford acyl urea **7** in 47% yield. Cyclization of compound **7** to uracil **8** proved problematic. Starting material **7** was unreactive toward ZnCl₂ and *p*-toluene sulfonic acid at 60 °C. Strong acidic conditions (i.e., 1 M H₂SO₄ or TFA) or strong basic conditions (i.e., NH₄OH) opened the azetidinone ring. The desired product **8** was never observed.

We recognized that the *N*-protio β-lactam required protection to survive cyclization to uracil **8**. As the azetidinone ring of compound **7** is susceptible to opening in the presence of strong acid or base, we required a protecting group that could be installed and removed under neutral conditions. Our group has previously reported *N*-sulfenylation of 2-azetidinones with *N*-methylthiophthalimide.¹⁷ Additionally, the *N*-methylthio group is easily removed under neutral conditions with 2-mercaptopyridine.¹⁸

SCHEME 2. Revised Synthetic Route to Uracil 8

Accordingly, compound **5** was refluxed in CH₂Cl₂ with *N*-methylthiophthalimide **9**¹⁹ to afford protected azetidinone **10** in 88% isolated yield²⁰ (Scheme 2). *N*-Methylthio-β-lactam **10** was refluxed in the presence of TFA and anisole to remove the Boc group. The resultant crude material was treated with a freshly prepared benzene solution of acyl isocyanate **6** to provide acyl urea **11** in an improved 77% yield. With the *N*-methylthio group installed in compound **11**, cyclization to the uracil base readily occurred in 1 M H₂SO₄ and CH₃CN at 80 °C to afford desired product **12**. Removal of the *N*-methylthio group was achieved with 2-mercaptopyridine to afford key carbocycle **8**.

Although we developed an optimized route to intermediate **8**, we anticipated low diastereoselectivity for **1a** in the subsequent dihydroxylation reaction.²¹ As predicted, a 1:3 mixture of *cis*-diols **1a** and **1b**, respectively, was obtained after OsO₄-catalyzed dihydroxylation of cyclopentene **8** (Scheme 3). In an effort to increase the ratio of **1a**, we investigated Woodward–Prévost conditions²² as an alternative method to install the *cis*-diol from the same face as the allylic methine protons in cyclopentene **8**.²³ Although the Woodward–Prévost method has previously been used in the synthesis of carbocyclic nucleosides,²⁴ the strong oxidizing conditions (i.e., I₂ and AgOAc) were not compatible with **8**, yielding a complex product mixture.

(16) For a detailed preparation of acyl isocyanate **6**, refer to experimental section or see: Santana, L.; Teixeira, M.; Uriarte, E. *J. Heterocyclic Chem.* **1999**, *36*, 293–295.

(17) (a) Woulfe, S. R.; Miller, M. J. *J. Org. Chem.* **1986**, *51*, 3133–3139. (b) Woulfe, S. R.; Iwagami, H.; Miller, M. J. *Tetrahedron Lett.* **1985**, *3*, 3891–3894.

(18) Shah, N. V.; Cama, L. D. *Heterocycles* **1987**, *25*, 221–227.

(19) For preparation of *N*-methylthiophthalimide **9**, refer to Supporting Information.

(20) Recently, a class of *N*-alkylthio β-lactams demonstrated anticancer activity. For recent examples of *N*-alkylthio β-lactams as anticancer agents, see: (a) Kuhn, D. J.; Wang, Y.; Minic, V.; Coates, C.; Reddy, Kumar, G.; Daniel, K. G.; Shim, J.-Y.; Chen, D.; Landis-Piwowar, K. R.; Miller, F. R.; Turos, E.; Dou, Q. P. *Front. Biosci.* **2005**, *10*, 1183–1190. (b) Kazi, Hill, R.; Long, T. E.; Kuhn, D. J.; Turos, E.; Dou, Q. P. *Biochem. Pharmacol.* **2004**, *67*, 365–374. (c) Smith, D. M.; Aslamuzzaman, K.; Smith, L.; Long, T. E.; Heldreth, B.; Turos, E.; Dou, Q. P. *Mol. Pharmacol.* **2002**, *61*, 1348–1358.

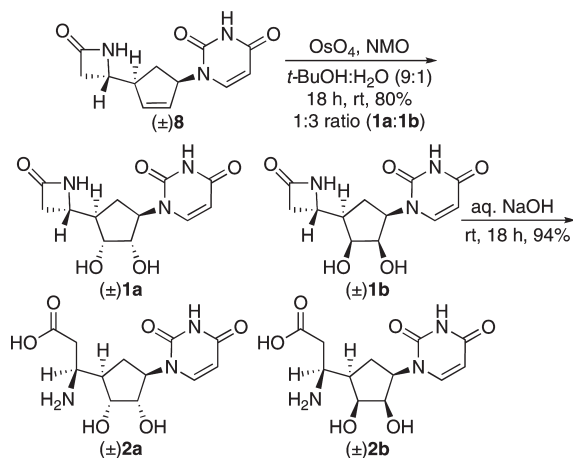
(21) When *J* values from the allylic methine protons and respective *trans*-methylene proton of 1,4-*syn* cyclopentenones are > 5.5 Hz, OsO₄-mediated dihydroxylation occurs from opposite face of the allylic methine protons and *syn*-tetrasubstituted cyclopentenones result. For compound **8**, *J* = 6.8 Hz and all *syn* diastereomer **1b** was obtained as the major product after dihydroxylation. For an explanation of how 1,4-disubstituted cyclopentenone conformations are determined from coupling constants, see: Katagiri, N.; Ito, Y.; Kitano, K.; Toyota, A.; Kaneko, C. *Chem. Pharm. Bull.* **1994**, *42*, 2653–2633.

(22) Woodward, R. B.; Brucher, F. V. Jr. *J. Am. Chem. Soc.* **1958**, *80*, 209–211.

(23) The Woodward–Prévost reaction has been described as a complementary method to OsO₄ dihydroxylation, see: Corey, E. J.; Das, J. *Tetrahedron Lett.* **1982**, *23*, 4217–4220.

(24) Barkley, J. V.; Dhanda, A.; Knutsen, L. J. S.; Nielsen, M. B.; Roberts, S. M.; Varley, D. R. *Chem. Commun.* **1998**, *10*, 1117–1118.

SCHEME 3. Syntheses of Uracil Polyoxin C Analogs



Finally, a mixture of *cis*-diols **1a** and **1b** was treated with an aqueous solution of NaOH to afford carbocyclic homouracil polyoxin C analogs **2a** and **2b**, respectively.

In summary, Pd(0)/InI-mediated allylation chemistry has been applied in the syntheses of azetidinone-derived and carboxylic acid-related carbocyclic nucleosides **1a** and **1b** and β -amino acid related-carbocyclic nucleosides **2a** and **2b**. We are currently screening final compounds **1a-b** and **2a-b** as well as relevant intermediates **4**, **5**, **8**, **10** and **12** for anticancer, antimicrobial, antiviral and antifungal activity. Detailed biological studies will be described subsequently.

Experimental Section

(±)-tert-Butyl-(1R*,4S*)-4-((S*)-4-oxoazetidin-2-yl)cyclopent-2-enylcarbamate 5. A clean flame-dried 250 mL round-bottom flask was evacuated and purged with Ar. A THF solution (50 mL) of Cp_2TiCl_2 (2.55 g, 10.2 mmol) and activated zinc (1.34 g, 20.5 mmol) was stirred at rt under Ar for 45 min. The reaction mixture changed color from dark red to olive green. The reaction mixture was cooled to -30°C and treated dropwise over 3 min with a MeOH solution (40 mL) of compound **4** (1.1 g, 4.1 mmol). The reaction mixture was stirred for 45 min and while maintaining the bath temperature between -10 and -30°C . The reaction mixture was warmed to rt and partitioned between sat. K_2CO_3 (10 mL) and EtOAc (40 mL). The organic layer was removed via pipet and filtered through Whatman Glass Microfiber Filter (Type GF/F) (to remove insoluble titanium salts). The aqueous layer was extracted with EtOAc (4×40 mL), and the organic layer was filtered through Whatman Glass Microfiber Filter (Type GF/F) after each extraction. The combined organic layers were dried over MgSO_4 , again filtered through Whatman Glass Microfiber Filter (Type GF/F), and the filtrate was adsorbed on silica gel. The adsorbed material was purified by silica gel chromatography (2% MeOH/ CH_2Cl_2) to afford **5** as an off white foam (747 mg, 72%). An analytical sample was recrystallized from EtOAc to provide a white powder from which all analytical data was obtained. mp $132\text{--}133^\circ\text{C}$; ^1H NMR (600 MHz, CD_3OD) δ 1.27 (1H, ddd, $J=13.4$ Hz, 6.5 Hz, 6.5 Hz), 1.44 (s, 9H), 2.49 (ddd, 1H, $J=13.4$ Hz, 8.2 Hz, 8.2 Hz), 2.61 (dd, 1H, $J=15.0$ Hz, 2.3 Hz), 2.85–2.89 (m, 1H), 2.94 (dd, 1H, $J=15.0$ Hz, 5.0 Hz), 3.61 (ddd, 1H, $J=6.2$ Hz, 5.0 Hz, 2.4 Hz), 4.60–4.65 (m, 1H), 5.76–5.80 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 28.9, 34.8, 48.7, 49.8, 51.8, 57.8, 80.2, 133.7, 136.6, 158.0, 171.1; IR (thin film, cm^{-1}) 3296, 2978, 2931, 1748, 1694, 1520, 1367; HRMS (FAB) m/z $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_{21}\text{N}_2\text{O}_3^+$, 253.1552; found, 253.1570.

3-Methoxy-2-propenoyl Isocyanate 6¹⁶. A clean flame-dried 100 mL round-bottom flask equipped with a stir bar and condenser was evacuated and purged with Ar. Silver cyanate (3.02 g, 20.2 mmol) was added to the flask and was heated to 50°C (bath temperature) under vacuum for 18 h and then heated to 100°C (bath temperature) for 2 h while the acid chloride was prepared. A clean flame-dried 50 mL round-bottom flask equipped with a stir bar, 4 Å molecular sieves and a condenser was evacuated and purged with Ar. A CH_2Cl_2 (16 mL) suspension of sodium-3-methoxy-2-propenoate (1.0 g, 8.06 mmol) was cooled to 0°C and treated with oxalyl chloride (0.82 mL, 9.67 mmol) followed by DMF (0.06 mL, 0.81 mmol) and the reaction mixture was refluxed for 2 h. The reaction mixture was then cooled to rt and filtered through a glass fritted funnel. The filtrate was transferred to a 50 mL round-bottom flask (previously flamed dried and purged with Ar), concentrated *in vacuo* to yellow solids and the flask was backfilled with Ar. Meanwhile, dry benzene (20 mL) was added to the heated flask containing silver cyanate and the solution was refluxed for 30 min. A benzene solution (20 mL) of the acid chloride was added dropwise to the refluxing solution of silver cyanate. The reaction mixture was refluxed for 1.5 h and then cooled to rt. The solids were filtered through Whatman Glass Microfiber Filter (Type GF/F) and the filtrate was used directly in the preparation of **7** or **11**.

(±)-(E)-3-Methoxy-N-((1R*,4S*)-4-((S*)-4-oxoazetidin-2-yl)cyclopent-2-enylcarbamoyl)acrylamide 7. A clean flame-dried 10 mL round-bottom flask equipped with a stir bar and condenser was evacuated and purged with Ar. A CH_2Cl_2 (2 mL) solution of compound **5** (100 mg, 0.40 mmol) was treated with anisole (0.047 mL, 0.44 mmol) and then TFA (0.15 mL, 1.99 mmol). The reaction mixture was refluxed for 3 h at 60°C (bath temperature). The reaction mixture was concentrated to a brown oil, toluene (1 mL) was added, and the solution was concentrated to an oil. CH_2Cl_2 (2 mL) was added to the resultant residue. The solution was cooled to 0°C and then treated with DIPEA (0.69 mL, 3.96 mmol). The reaction mixture was stirred for 5 min under Ar at 0°C and was then treated with a benzene solution of 3-methoxy-2-propenoyl isocyanate **6** (100 mg, 0.80 mmol, assuming quantitative yield from the previous step). The reaction was stirred for 15 h under Ar as the temperature warmed to rt overnight. The reaction mixture was concentrated to a tan oil and partitioned between CH_2Cl_2 (3 mL) and 10% citric acid (2 mL). The aqueous layer was extracted with CH_2Cl_2 (4×3 mL). The combined organic layers were washed with brine (8 mL), dried over Na_2SO_4 , filtered and concentrated to a tan oil. The residue was purified by silica gel chromatography (100% EtOAc) to afford **7** as a yellow gum (52 mg, 47%). ^1H NMR (600 MHz, CDCl_3) δ 1.41 (ddd, 1H, $J=13.5$ Hz, 5.9 Hz, 5.9 Hz), 2.56 (ddd, 1H, $J=13.5$ Hz, 8.2 Hz, 8.2 Hz), 2.65 (ddd, 1H, $J=14.7$ Hz, 2.6 Hz, 1.2 Hz), 2.91–2.95 (m, 1H), 3.02 (ddd, 1H, $J=15.0$ Hz, 2.6 Hz, 1.2 Hz), 3.67 (ddd, 1H, $J=5.3$ Hz, 5.3 Hz, 2.3 Hz), 3.74 (s, 3H), 4.92–4.97 (m, 1H), 5.20 (d, 1H, $J=12.3$ Hz), 5.81 (ddd, 1H, $J=5.6$ Hz, 3.8 Hz, 2.1 Hz), 5.88 (ddd, 1H, $J=5.6$ Hz, 4.4 Hz, 2.1 Hz), 5.90 (bs, 1H), 7.66 (d, 1H, $J=12.3$ Hz), 8.00 (bs, 1H), 8.61 (bd, 1H, $J=8.2$ Hz); ^{13}C NMR (150 MHz, CDCl_3) δ 33.9, 41.8, 48.9, 50.9, 55.9, 58.5, 97.6, 133.6, 134.6, 153.7, 164.5, 167.8, 167.9; IR (thin film, cm^{-1}) 3258, 2921, 1737, 1678, 1614, 1538; HRMS (FAB) m/z $[\text{M}+\text{H}]^+$: calcd for $\text{C}_{13}\text{H}_{18}\text{N}_3\text{O}_4^+$, 280.1297; found, 280.1303.

(±)-1-((1R*,4S*)-4-((S*)-4-Oxoazetidin-2-yl)cyclopent-2-enyl)pyrimidine-2,4(1H,3H)-dione 8. A CH_2Cl_2 solution (10 mL) of **12** (0.56 g, 1.91 mmol) and 2-mercaptopyridine (0.26 g, 2.32 mmol) in a pressure tube was treated with Et_3N (0.29 mL, 2.29 mmol). The tube was sealed with a Teflon screw cap and the heterogeneous reaction mixture was stirred for 18 h at 60°C (bath temperature). The reaction mixture was cooled to 0°C and the precipitate was filtered and washed with hexanes to afford **8** as an off-white solid (0.40 g, 84%). mp $246\text{--}247^\circ\text{C}$

(dec); ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 1.21 (ddd, 1H, $J=13.8$ Hz, 6.8 Hz, 6.8 Hz), 2.50–2.56 (m, 2H), 2.85 (dd, 1H, $J=5.0$ Hz, 2.1 Hz), 2.86–2.90 (m, 1H), 3.57 (ddd, 1H, $J=8.5$ Hz, 2.6 Hz, 2.6 Hz), 5.46–5.50 (m, 1H), 5.57 (d, 1H, $J=8.2$ Hz), 5.78 (ddd, 1H, $J=5.6$ Hz, 2.1 Hz, 2.1 Hz), 6.01 (ddd, 1H, $J=5.6$ Hz, 2.1 Hz, 2.1 Hz), 7.42 (d, 1H, $J=7.9$ Hz), 7.97 (s, 1H), 11.27 (s, 1H); ^{13}C NMR (150 MHz, $\text{DMSO}-d_6$) δ 32.2, 40.9, 48.4, 48.8, 60.8, 101.6, 131.0, 137.1, 141.7, 150.9, 163.3, 167.0; IR (thin film, cm^{-1}) 1749, 1689, 1614, 1461; HRMS (FAB) m/z $[\text{M} + \text{H}]^+$: calcd for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}_3^+$, 248.1035; found, 248.1026.

(\pm)-1-((1*R**,2*S**,3*R**,4*R**)-2,3-Dihydroxy-4-((*S**)-4-oxoazetidin-2-yl)cyclopentyl)pyrimidine-2,4(1*H*,3*H*)-dione **1a** and 1-((1*R**,2*R**,3*S**,4*R**)-2,3-dihydroxy-4-((*S**)-4-oxoazetidin-2-yl)cyclopentyl)pyrimidine-2,4(1*H*,3*H*)-dione **1b**. A *t*-BuOH:H₂O solution (9:1, 1.8 mL: 0.2 mL) of **8** (50 mg, 0.20 mmol) and *N*-methylmorpholine *N*-oxide (47 mg, 0.40 mol) was treated with a 2.5% (w/w) solution of osmium tetroxide in *t*-BuOH (0.40 mL, 0.04 mmol) and the heterogeneous reaction mixture was stirred at rt for 18 h. After 18 h, the clear yellow reaction mixture was treated with solid $\text{Na}_2\text{S}_2\text{O}_5$ (576 mg, 3.03 mmol) and the mixture was stirred at rt for 30 min. The clear solution was filtered through a Whatman Glass Microfiber Filter (Type GF/F) to remove the brown precipitate. The clear filtrate was concentrated to solids under reduced pressure, toluene (3 mL) was added, and the solution was concentrated to solids. The solids were triturated with MeOH (3 \times 5 mL) and the wet solids were dried under vacuum to afford a 1:8 ratio of **1a**:**1b** as white solids (45 mg, 80%). ^1H NMR of the crude reaction mixture indicated a 1:3 ratio of **1a**:**1b**. Compound **1a**: ^1H NMR (600 MHz, D_2O) δ 1.61 (ddd, 1H, $J=12.3$ Hz, 12.3 Hz, 9.1 Hz), 2.19–2.28 (m, 2H), 2.73 (dd, 1H, $J=15.2$ Hz, 2.4 Hz), 3.11 (dd, 1H, $J=15.3$ Hz, 4.7 Hz), 3.87 (ddd, 1H, $J=6.8$ Hz, 4.7 Hz, 2.1 Hz), 3.94 (dd, 1H, $J=6.2$ Hz, 4.7 Hz), 4.24–4.27 (m, 1H), 4.63–4.68 (m, 1H), 5.86 (d, 1H, $J=7.9$ Hz), 7.66 (d, 1H, $J=7.9$ Hz); ^{13}C NMR (150 MHz, D_2O) δ 26.0, 40.1, 45.9, 49.6, 55.4, 71.2, 73.4, 101.9, 144.4, 152.3, 166.3, 171.9; HRMS (FAB) m/z $[\text{M} + \text{H}]^+$: calcd for $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_5^+$, 282.1090; found, 282.1081. Compound **1b**: ^1H NMR (600 MHz, D_2O) δ 1.95–2.01 (m, 1H), 2.19–2.26 (m, 2H), 2.76 (dd, 1H, $J=15.2$ Hz, 2.3 Hz), 3.13 (dd, 1H, $J=15.3$ Hz, 4.7 Hz), 3.98 (ddd, 1H, $J=6.5$ Hz, 5.0 Hz, 2.4 Hz), 4.24–4.27 (m, 1H), 4.29 (dd, 1H, $J=7.3$ Hz, 4.4 Hz), 4.99–5.04 (m, 1H), 5.82 (d, 1H, $J=8.2$ Hz), 7.90 (d, 1H, $J=7.9$ Hz); ^{13}C NMR (150 MHz, D_2O) δ 28.5, 40.8, 42.4, 48.1, 55.4, 71.2, 72.1, 100.6, 146.1, 152.7, 166.3, 171.9; HRMS (FAB) m/z $[\text{M} + \text{H}]^+$: calcd for $\text{C}_{12}\text{H}_{16}\text{N}_3\text{O}_5^+$, 282.1090; found, 282.1081.

(\pm)-(*S**)-3-Amino-3-((1*R**,2*R**,3*S**,4*R**)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,3-dihydroxycyclopentyl)propanoic Acid **2a** and (\pm)-(*S**)-3-Amino-3-((1*R**,2*S**,3*R**,4*R**)-4-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2,3-dihydroxycyclopentyl)propanoic Acid **2b**. A water solution (0.36 mL) of a 1:8 ratio of **1a**:**1b** (10 mg, 0.036 mmol) was treated with 2 M NaOH (0.089 mL, 0.18 mmol) and was stirred for 18 h at rt. The reaction mixture was adjusted to pH 7 with 1 M HCl. The neutralized reaction mixture was concentrated to solids under reduced pressure. The crude material was adsorbed on Sephadex LH20 and eluted with MeOH to afford a 1:6 ratio of **2a**:**2b** as white solids (10.6 mg, 94%). Compound **2a**: ^1H NMR (600 MHz, D_2O) δ 1.67–1.72 (m, 1H), 2.22–2.29 (m, 2H), 2.50 (dd, 1H, $J=17.5$ Hz, 9.5 Hz), 2.75 (dd, 1H, $J=17.5$ Hz, 4.0 Hz), 3.58 (ddd, 1H, $J=9.2$ Hz, 8.6 Hz, 3.4 Hz), 3.91–3.94 (m, 1H), 4.06–4.08 (m, 1H), 4.56–4.62 (m, 1H), 5.86 (d, 1H, $J=8.0$ Hz), 7.67 (d, 1H, $J=8.0$ Hz); ^{13}C NMR (125 MHz, D_2O) δ 27.0, 37.0, 40.3, 45.3, 51.5, 70.5, 72.8, 102.0, 144.5, 152.5, 166.5, 177.9; HRMS (ESI) m/z $[\text{M} + \text{H}]^+$: calcd for $\text{C}_{12}\text{H}_{18}\text{N}_3\text{O}_6^+$, 300.1196; found, 300.1190. HRMS (ESI) m/z $[\text{M} + \text{Na}]^+$: calcd for $\text{C}_{12}\text{H}_{17}\text{N}_3\text{NaO}_6$, 322.1015; found, 322.1010. Compound **2b**: ^1H NMR (600 MHz, D_2O) δ 1.97 (ddd, 1H, $J=12.6$ Hz, 12.6 Hz, 10.0 Hz), 2.21–2.26 (m, 1H), 2.33 (ddd, 1H, $J=12.6$ Hz, 8.2 Hz, 7.0 Hz), 2.50 (dd, 1H, $J=16.7$ Hz, 8.5 Hz), 2.66 (dd, 1H, $J=16.4$ Hz, 4.7 Hz), 3.72 (ddd, 1H, $J=10.8$ Hz, 7.3 Hz, 4.7 Hz), 4.29–4.32 (m, 2H), 5.08 (ddd, 1H, $J=10.0$ Hz, 8.2 Hz, 8.2 Hz), 5.84 (d, 1H, $J=7.9$ Hz), 7.92 (d, 1H, $J=7.9$ Hz); ^{13}C NMR (150 MHz, D_2O) δ 29.0, 38.6, 40.7, 49.2, 54.8, 70.9, 71.6, 100.9, 146.0, 156.9, 166.3, 178.1; HRMS (ESI) m/z $[\text{M} + \text{H}]^+$: calcd for $\text{C}_{12}\text{H}_{18}\text{N}_3\text{O}_6^+$, 300.1196; found, 300.1190. HRMS (ESI) m/z $[\text{M} + \text{Na}]^+$: calcd for $\text{C}_{12}\text{H}_{17}\text{N}_3\text{NaO}_6$, 322.1015; found, 322.1010.

Acknowledgment. We thank Dr. Jed Fisher for helpful discussions, Dr. Bill Boggess and Nonka Sevova for mass spectroscopic analyses and Dr. Jaroslav Zajicek for NMR assistance. We acknowledge The University of Notre Dame and NIH (GM068012) for support of this work.

Supporting Information Available: General methods and experimental details for the preparation of **9–12**. ^1H and ^{13}C NMR spectra for compounds **1a–b**, **2a–b**, **5**, **7**, **8**, **10**, **11** and **12**. This material is available free of charge via the Internet at <http://pubs.acs.org>.