JOC Article
pubs.acs.org/joc

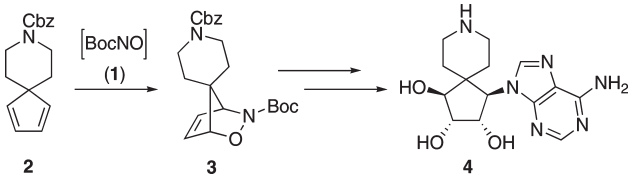
Diastereoselective Synthesis of a Spironoraristeromycin Using an Acylnitroso Diels−Alder Reaction†

Weimin Lin,‡ Kristopher G. Virga,‡ Kyung-Hee Kim,‡ Jaroslav Zajicek,‡ David Mendel,§ and Marvin J. Miller*,‡

‡Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, and §Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285

mmiller1@nd.edu

Received May 2, 2009



The *tert*-butyl *N*-hydroxycarbamate-derived nitroso reagent **1** reacted with *N*-Cbz-protected spirocyclic diene **2** to provide spirocycloadduct **3**. Here we describe the efficient conversion of **3** into the novel carbocyclic nucleoside spironoraristeromycin **4**.

Introduction

Synthetic and naturally occurring carbocyclic nucleosides (Figure 1) frequently exhibit potent antiviral and/or anticancer activities.[1] Aristeromycin and neplanocin A, isolated from *Streptomyces citricolor*[2] and *Ampullariella regularis*,[3] possess strong antiviral activity due to their potent inhibition of the cellular enzyme *S*-adenosyl homocysteine hydrolase.[4] Neplanocin A has also demonstrated anticancer activity, with particular selectivity for leukemia.[5] Carbovir was discovered and subsequently shown to possess significant in vitro activity as an inhibitor of HIV reverse transcriptase.[6] The cyclopropylamino derivative of carbovir, abacavir (Ziagen, 1592U89), is an FDA-approved anti-HIV therapeutic agent.[7] Other important members of the carbocyclic nucleoside class include modification of the carbon ring per se. Cyclopentenyl cytosine contains an unsaturated ring; noraristeromycin places the 5′ hydroxyl equivalent directly on the cyclopentane ring. Such ring modifications produce bioactive molecules. Cyclopentenyl cytosine exhibits both antiviral[8] and anticancer activities.[9] Noraristeromycin possesses improved antiviral activity and less cytoxicity relative to aristeromycin;[10] thus, it may be fruitful to assess whether increased potency and lower cytotoxicity track with other truncated carbocyclic nucleosides. In the search for safe and effective new carbocyclic nucleoside drugs, an efficient, divergent synthetic route is needed to readily incorporate various modifications.

Numerous synthetic approaches to carbocyclic nucleosides and analogues are known.[11] Three routes frequently used to form the key carbocycle−nucleobase bond (Scheme 1) are as follows: (a) nucleophilic displacement of

† We dedicate this paper to Prof. Jeremiah P. Freeman on the occasion of his 80th birthday and with deep gratitude for his 25 years of service to organic chemistry as secretary of Organic Syntheses.

(1) (a) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319. (b) Jeong, L.-S.; Lee, J.-A. *Antiviral Chem. Chemother.* **2004**, *15*, 235. (c) Schneller, S. W. *Curr. Top. Med. Chem.* **2002**, *2*, 1087. (d) Rodriguez, J. B.; Comin, M. J. *Mini-Rev. Med. Chem.* **2003**, *3*, 95.

(2) Kishi, T.; Muroi, M.; Kusaka, T.; Nishikawa, M.; Kamiya, M.; Mizuno, K. *Chem. Pharm. Bull.* **1972**, *20*, 940.

(3) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. *J. Antibiot.* **1981**, *34*, 359.

(4) (a) De Clercq, E. *Biochem. Pharmacol.* **1987**, *36*, 2567. (b) Cools, M.; De Clercq, E. *Biochem. Pharmacol.* **1989**, *38*, 1061. (c) Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521.

(5) Niitsu, N.; Yamamoto-Yamaguchi, Y.; Anatani, Y.; Shuto, S.; Matsuda, A.; Umeda, M.; Honma, Y. *Exp. Hematol.* **1997**, *25*, 1296.

(6) (a) Vince, R.; Hua, M. *J. Med. Chem.* **1990**, *33*, 17. (b) Orr, D. C.; Figueiredo, H. T.; Mo, C.-L.; Penn, C. R.; Cameron, J. M. *J. Biol. Chem.* **1992**, *276*, 4177.

(7) (a) Foster, R .H.; Failds, D. *Drugs* **1998**, *55*, 729. (b) Melroy, J.; Nair, V. *Curr. Pharm. Des.* **2005**, *11*, 3847. (c) Yuen, G. J.; Weller, S.; Pakes, G. E. *Clin. Pharm.* **2008**, *47*, 351.

(8) Lim, B. B.; Marquez, V. E.; Dobyns, K. A.; Cooney, D. A.; De Clercq, E. *Nucleosides Nucleotides* **1992**, *11*, 1123.

(9) (a) Agbaria, R.; Kelly, J. A.; Ackman, J.; Viola, J. J.; Ram, Z.; Oldfield, E.; Johns, D. G. *Oncol. Res.* **1997**, *9*, 111. (b) Schimmel, K. J. M.; Gelderblom, H.; Guchelaar, H. J. *Curr. Cancer Drug Targets* **2007**, *7*, 504.

(10) Patil, S. D.; Schneller, S. W.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 3372.

(11) (a) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229. (b) Casu, F.; Chiacchio, M. A.; Romeo, R.; Gumina, G. *Curr. Org. Chem.* **2007**, *11*, 999. (c) Chiacchio, U.; Saita, M. G.; Crispino, L.; Gumina, G.; Mangiafico, S.; Pistara, V.; Romeo, G.; Piperno, A.; De Clercq, E. *Tetrahedron* **2006**, *62*, 1171.

DOI: 10.1021/jo900877b Published on Web 07/14/2009

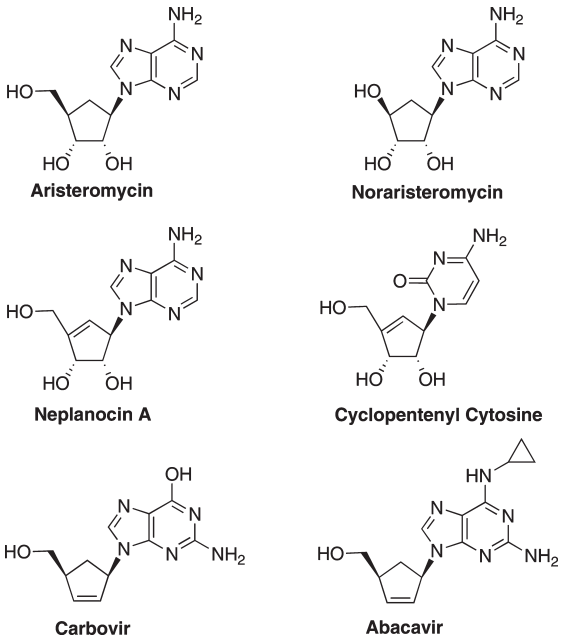
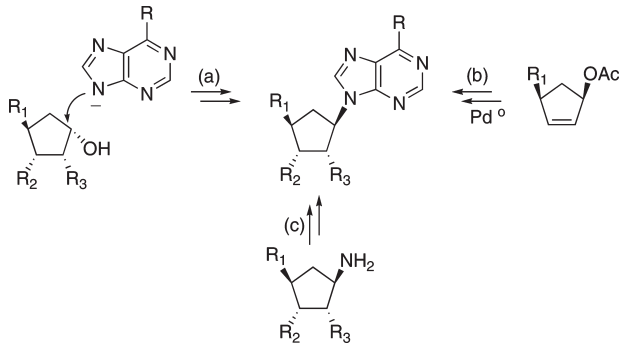


FIGURE 1. Representative carbocyclic nucleosides and analogues.

an appropriately substituted hydroxycyclopentane by a nucleobase analogue with net inversion of stereochemistry,[12] (b) use of a Pd-catalyzed allylic alkyation of an allylic cyclopentene skeleton with net retention of configuration,[13] and (c) elaboration of the heterocyclic nucleobase from an aminocyclopentane.[14] Replacing the furanose oxygen of the corresponding nucleoside with a methylene unit renders the resulting carbocyclic nucleosides resistant to cleavage by phosphorylases and hydrolases.[15] However, the related conformational changes have the potential to affect bioactivity.[16] To avoid this disadvantage and to enhance the activity of carbocyclic nucleosides, a number of research groups have reported syntheses of conformationally restricted analogues, including the incorporation of a spirocycle in the five-membered ring.[17] We recently reported a convenient synthetic approach to simple spirocarbocyclic nucleoside analogues using iminonitroso Diels–Alder reactions.[18] Here we describe a diastereoselective synthesis of a spironoraristeromycin using acylnitroso Diels–Alder chemistry.

SCHEME 1



Results and Discussion

The acylnitroso Diels–Alder reaction diene partner, spirocycle **2**, can be prepared on a 20 mmol scale in 64% overall yield from cyclopentadiene.[18] Though many methods for the preparation of acyl nitroso reagents have been reported,[19] oxidative conditions using sodium periodate[20] were adopted for the ability to perform well on scale. To install orthogonal protecting groups and thus facilitate efficient synthesis of the desired product, *tert*-butyl hydroxycarbamate was selected as the acylnitroso precursor for condensation with Cbz-protected spirocyclic diene **2** (Scheme 2). The key coupling product, racemic spirocycloadduct **3**, was generated in 70% average yield in small-scale reactions but on larger scale (~40 mmol) was prone to lower yields as heavy precipitation impeded proper mixing. To overcome this issue, a few modifications were made to the reaction and workup procedures: (1) use of an overhead stirring device, (2) use of a saturated aqueous sodium periodate solution followed by the addition of water or methanol as the mixture became viscous, and (3) trituration of the crude reaction mixture with ether to afford a 61% yield of pure compound **3**. Direct dihydroxylation of compound **3** using *N*-methylmorpholine oxide and osmium tetraoxide in THF[21] failed to provide the desired diol derivative **5**. Thus, we chose to cleave the N–O bond before attempting the dihydroxylation. N–O reduction was accomplished using molybdenum hexacarbonyl and sodium borohydride[22] at 80 °C over 8 h to give alcohol **6** in high yield.

Compound **6** was then acetylated to provide compound **7** in 90% yield. Compound **7** is a useful and compact molecular scaffold for diversification as it contains three differentially protected heteroatom functionalities in addition to the alkene group. For purposes of this disclosure, the primary amine and alkene functions of **7** were exploited to prepare a novel spiro-noraristeromycin bearing a secondary amine.

(12) (a) Slusarchyk, A.; Young, M. G.; Bisacchi, G. S.; Hockstein, D. R.; Zahler, R. *Tetrahedron Lett.* **1989**, *30*, 6453. (b) Benner, S. *Tetrahedron Lett.* **1991**, *32*, 7029. (c) Mulvihill, M. J.; Miller, M. J. *Tetrahedron* **1998**, *54*, 6605. (d) Bremond, P.; Audran, G.; Aubin, Y.; Monti, H. *Synlett* **2007**, *7*, 1124. (e) Gosselin, G.; Griffe, L.; Meillon, J.-C.; Storer, R. *Tetrahedron* **2006**, *62*, 906.

(13) (a) Trost, B. M.; Kou, G.-H.; Benneche, T. *J. Am. Chem. Soc.* **1988**, *110*, 621. (b) Dyatkina, N.; Costisella, B.; Thiel, F.; Janta-Lipinski, M. *Tetrahedron Lett.* **1994**, *35*, 1961. (c) Kitade, Y.; Ando, T.; Yamaguchi, T.; Hori, A.; Nakanishi, M.; Ueno, Y. *Bioorg. Med. Chem.* **2006**, *14*, 5578. (d) Jung, M. E.; Rhee, H. J. *J. Org. Chem.* **1994**, *59*, 4719.

(14) (a) Patil, S. D.; Schneller, S. W. *J. Heterocycl. Chem.* **1991**, *28*, 823. (b) Bergmeier, S. C.; Cobas, A. A.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 2369.

(15) Marquez, V. E.; Lim, M.-I. *Med. Res. Rev.* **1986**, *6*, 1.

(16) (a) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205. (b) Choi, Y.; Moon, H.-R.; Yoshimura, Y.; Marquez, V. E. *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 547. (c) Marquez, V. E.; Hughes, S. H.; Sei, S.; Agbaria, R. *Antiviral Res.* **2006**, *71*, 268.

(17) (a) Roy, A.; Achari, B.; Mandal, S. B. *Tetrahedron Lett.* **2006**, *47*, 3875. (b) Fischer, R.; Hyrosova, E.; Fisera, L.; Hametner, C.; Cyranski, M. K. *J. Org. Chem.* **2004**, *69*, 7442. (c) Hartung, R. E.; Paquette, L. A. *Synthesis* **2005**, *19*, 3209. (d) Kittaka, A.; Asakura, T.; Kuze, T.; Tanaka, H.; Yamada, N.; Nakamura, K. T.; Miyasaka, T. *J. Org. Chem.* **1999**, *64*, 7081. (e) Ravindra Babu, B.; Keinicke, L.; Petersen, M.; Nielsen, C.; Wengel, J. *Org. Biomol. Chem.* **2003**, *1*, 3514.

(18) Lin, W.-M.; Gupta, A.; Kim, K. H.; Mendel, D.; Miller, M. J. *Org. Lett.* **2009**, *11*, 449.

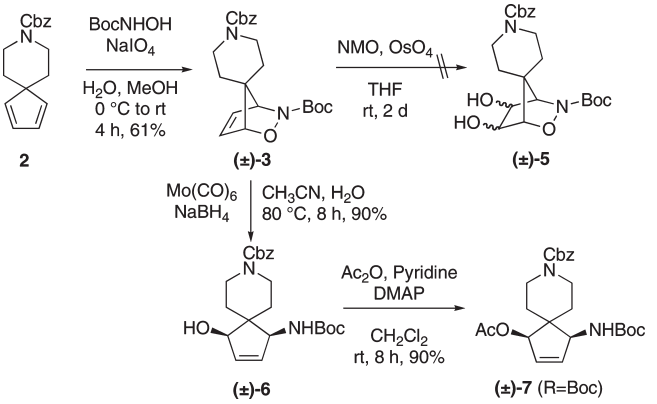
(19) (a) Adamo, M. F. A.; Bruschi, S. *Targets Heterocycl. Syst.* **2007**, *11*, 396. (b) Iwasa, S.; Fakhruddin, A.; Nishiyama, H. *Mini-Rev. Org. Chem* **2005**, *2*, 157.

(20) Bodnar, B. S.; Miller, M. J. *J. Org. Chem.* **2007**, *72*, 3929.

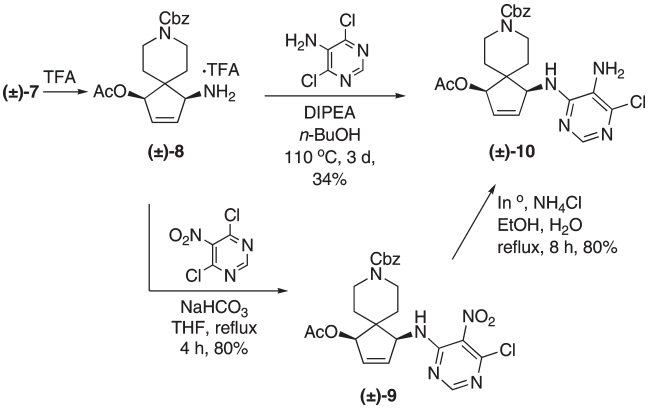
(21) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *23*, 1973.

(22) Jiang, M. X.-W.; Jin, B.-H.; Cage, J. L.; Priour, A.; Savela, G.; Miller, M. J. *J. Org. Chem.* **2006**, *71*, 4164.

SCHEME 2



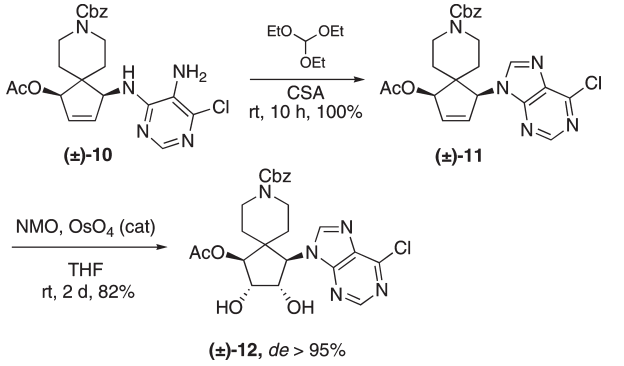
SCHEME 3



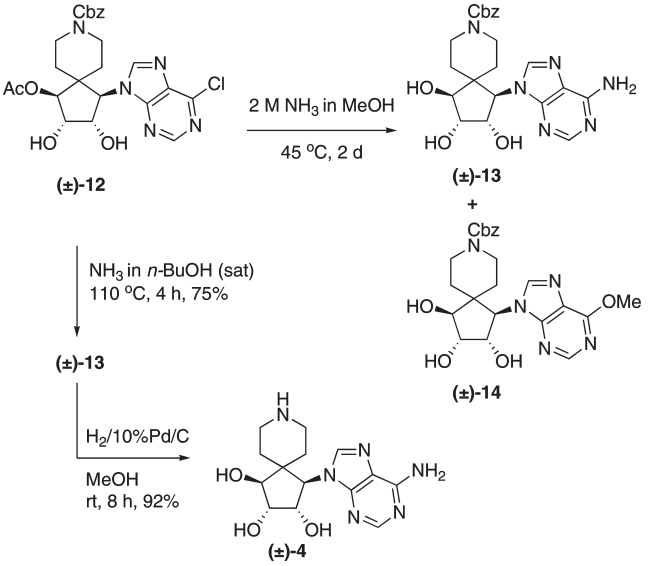
Conversion of **7** to spiro-noraristeromycin **4** is depicted in Schemes 3,4−5. The Boc group was selectively removed from **7** in quantitative yield to afford primary amine **8**. Initial attempts to condense compound **8** with 4,6-dichloro-5-aminopyrimidine under various reported conditions[23] were ineffective, presumably due to steric hindrance of the spirocyclic ring neighboring the amine nucleophile. However, use of DIPEA as base along with the addition of 50 mol % DMAP and extended reaction in a sealed tube at 110 °C afforded key intermediate **10** in 34% yield. Alternatively, use of 4,6-dichloro-5-nitropyrimidine as the electrophile for the S_NAr reaction gave **9** in 80% yield.[24] Subsequent indium-mediated nitro group reduction of **9** produced **10** in 64% overall yield for the two-step sequence.[25]

The next stage in the synthesis involved formation of the 6-chloropurine intermediate **11** (Scheme 4) with triethyl orthoformate as the purine C8 carbon source. Initial condensations with catalytic amounts of (±)-camphorsulfonic acid (CSA) yielded only partial conversion. However, addition of a full equivalent of CSA provided condensation product **11** in quantitative yield. Dihydroxylation of compound **11** using *N*-methylmorpholine oxide and osmium

SCHEME 4



SCHEME 5



tetraoxide required 2 days for completion to afford an 82% yield of compound **12** as a single diastereomer.

A detailed NMR study was performed to determine the facial selectivity for dihydroxylation of compound **11**. Based on an MM2 model of compound **12** using Chem-3D (Figure 2), the dihedral angle for H(1)−C−C−H(2) is nearly 90°, whereas the H(1)−C−C−H(2) dihedral angle was calculated to be 34.5° for the other, all syn, diastereomer from dihydroxylation on the same face as the C(1) and C(2) substituents. Thus, consistent with a very small coupling constant between H(1) and H(2), the structure of **12** was assigned as shown, based on the Karplus equation.[26] The single peak for H(1) that appears in the ^{1}H NMR spectrum of compound **12** reflected the anti relationship for H(1) and H(2). To confirm this result, compound **12** was subjected to a 2D-ROSEY study that showed a strong NOE response between H(1) and H(4). However, no NOE response between H(1) and H(3) was observed, indicating that H(1) lies on the opposite face of the carbocyclic ring relative to H(3).

Ammonolysis of compound **12** was then carried out using 2.0 M methanolic ammonia in a sealed tube (Scheme 5). This reaction procedure also removed the acetyl protecting group to produce triol **13**. However, adenine derivative **13** was

(23) (a) Menichincheri, M.; Bassini, D. F.; Gude, M.; Angiolini, M. *Tetrahedron Lett*. **2003**, *44*, 519. (b) Yang, W.; Wang, Y.; Corte, J. R. *Org. Lett*. **2003**, *5*, 3131. (c) Klapars, A.; Antilla, J. C.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc*. **2001**, *123*, 7727.

(24) Baraldi, P. G.; Broceta, A. U.; Pineda de las Infantas, M. J.; Mochun, J. J. D.; Espinosa, A.; Romagnoli, R. *Tetrahedron* **2002**, *58*, 7607.

(25) Banik, B. K.; Banik, I.; Becker, F. F. *Org. Synth*. **2005**, *81*, 188.

(26) Karplus, M. *J. Am. Chem. Soc*. **1963**, *85*, 2870.

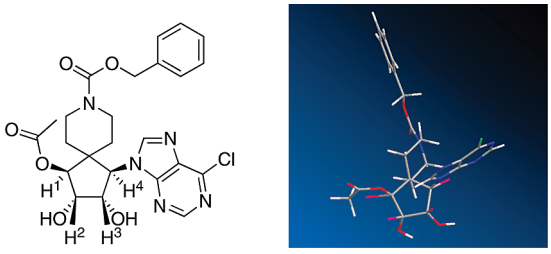


FIGURE 2. Chem 3D-MM2 model of compound **12**.

obtained in only 33% yield due to competitive formation of the 6-methoxypurine derivative **14** (47%) by methanolysis. A modified ammonolysis of compound **12** was then carried out using saturated ammonia/*n*-butanol in a sealed tube at 110 °C to provide **13** in 75% yield. Hydrogenolysis of the Cbz group in **13** afforded sprionoraristereomycin **4** in 92% yield.

Conclusion

Acylnitroso Diels−Alder reaction with *N*-Cbz-protected spirocyclic diene **2** provided the corresponding cyclcoadduct **3** as a useful scaffold for further derivatization. The synthesis of spironoraristeromycin reported here represents one of many potential applications of this chemistry. Biological studies of the spironoraristeromycin **4** will be reported in due course.

Experimental Section

Benzyl 8-Azaspiro[4.5]deca-1,3-diene-8-carboxylate (2). A 250-mL oven-dried, round-bottomed flask equipped with a septum with a flow of nitrogen through a needle and a stir bar was charged with sodium hydride (2.4 g, 60 mmol, 60% in the mineral oil) and anhydrous DMSO (25 mL). The solution was warmed to 75 °C for 45 min to give a gray suspension. The solution was then cooled to 0 °C, and anhydrous THF (50 mL) was added followed by dropwise addition of freshly distilled cyclopentadiene (3.8 mL, 45 mmol, in 20 mL of anhydrous THF). A large amount of white precipitate formed, and vigorous stirring was required. After the mixture was stirred for 15 min, benzyl bis(2-chloroethyl)carbamate (5.0 g, 18 mmol) was added and the reaction mixture was stirred at room temperature for 8 h. The solution was quenched with saturated NH_4Cl solution (50 mL) and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (50 mL) and brine (50 mL) and dried over anhydrous Na_2SO_4. The resulting liquid was filtered and concentrated under reduced pressure. The residue was purified on silica (hexanes/EtOAc = 15:1, R_f = 0.2) to yield 3.1 g (64%) of **2** as a light yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), 6.48 (m, 2H), 6.31 (m, 2H), 5.16 (s, 2H), 3.64 (m, 4H), 1.55 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.3, 142.2, 136.8, 129.5, 128.4, 127.8, 127.6, 66.9, 55.1, 48.8, 43.3, 39.4, 32.2, 30.7; HRMS calcd for $C_{17}H_{20}NO_2$ $(M + H)^+$ 270.1494, found 270.1505.

(±)-1′-Benzyl 3-*tert*-Butyl 2-oxa-3-azaspiro[bicyclo[2.2.1]-hept[5]ene-7,4′-piperidine]-1′,3-dicarboxylate (3). A 500-mL round-bottomed flask was charged with 200 mL of methanol, *tert*-butyl hydroxycarbamate (7.4 g, 56 mmol), and compound **2** (10.0 g, 37 mmol). The solution was cooled to 0 °C. A solution of sodium periodate (12.0 g, 56 mmol) in water (50 mL) was added to the reaction mixture dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature with stirring until TLC analysis indicated consumption of the starting diene **2** (∼4 h). The resulting mixture was filtered and then concentrated to about 50−75 mL. The concentrate was extracted with dichloromethane (3 × 50 mL), and the combined organic layers were washed with saturated $NaHCO_3$ solution (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give a brown oil. The oil was triturated with ether and concentrated to give a yellowish solid. The solid was washed thoroughly with ether to give 9.0 g (61%) of **3** as a white solid: mp: 157−158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (m, 5H), 6.27 (br, 2H), 5.04 (s, 2H), 4.66 (s, 1H), 4.46 (s, 1H), 3.41 (dd, J = 5.7, 5.8 Hz, 2H), 3.29 (dd, J = 5.7, 5.8 Hz, 2H), 1.74 (m, 2H), 1.39 (m, 11H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.1, 136.6, 131.4, 128.3, 127.9, 127.8, 86.5, 82.2, 67.0, 60.1, 41.9, 41.4, 28.0; IR (neat, cm^{-1}) 2925, 1694, 1682, 1455; HRMS calcd for $C_{22}H_{29}N_2O_5$ $(M + H)^+$ 401.2076, found 401.2081.

(±)-(1*S,4*R**)-Benzyl 1-(*tert*-Butoxycarbonylamino)-4-hydroxy-8-azaspiro[4.5]dec-2-ene-8-carboxylate (6).** A 500-mL round-bottomed flask was charged with 200 mL of acetonitrile, 50 mL of water, and spirocycloadduct **3** (9.0 g, 22 mmol). To this was added molybdenum hexacarbonyl (2.4 g, 9.0 mmol), and the reaction mixture was warmed to 40 °C. Sodium borohydride (2.6 g, 68 mmol) was added to the mixture in small portions with stirring; mild to vigorous effervescence was observed. The resulting mixture was then heated at 80 °C for 8 h and then allowed to cool to room temperature, filtered through Celite, concentrated to about 50 mL, and extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ (30 mL) and then brine (30 mL), dried over anhydrous Na_2SO_4, and filtered. The organic layer was then concentrated under reduced pressure and the crude residue purified by flash chromatography (hexanes/EtOAc = 3:1, R_f = 0.1) to give 8.0 g (90%) of **6** as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 5H), 6.03 (m, 1H), 5.87 (m, 1H), 5.11 (s, 3H), 4.94 (s, 1H), 4.16 (m, 1H), 4.02 (m, 1H), 3.51 (m, 4H), 1.80−1.40 (m, 13H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.6, 136.8, 135.0, 128.4, 127.9, 127.8, 79.8, 67.0, 62.2, 45.7, 41.2, 35.6, 28.3, 26.8. IR (neat, cm^{-1}): 3348 (br), 2925, 1694, 1455; HRMS calcd for $C_{22}H_{31}N_2O_5$ $(M + H)^+$ 403.2233, found 403.2220.

(±)-1-Acetoxy-4-(*tert*-butoxycarbonylamino)-8 azaspiro[4.5]-dec-2-ene-8-carboxylate (7). A 50-mL oven-dried round-bottomed flask, equipped with a septum and nitrogen inlet and stir bar, was charged with anhydrous dichloromethane (20 mL), compound **6** (1.6 g, 4.0 mmol), pyridine (2.0 mL, 22 mmol), acetic anhydride (2.0 mL, 22 mmol), and DMAP (0.24 g, 2.0 mmol). The resulting mixture was stirred at room temperature for 8 h. The organic solution was then washed with 0.1 N HCl (2 × 20 mL), brine (20 mL), saturated $NaHCO_3$ solution (2 × 20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, and filtered. The organic layer was concentrated under reduced pressure to give a light yellow solid. The solid was washed with hexanes and filtered to give 1.6 g (90%) of **7** as a white solid: mp 88−89 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (m, 5H), 6.01 (m, 1H), 5.94 (m, 1H), 5.22 (m, 1H), 5.08 (m, 2H), 4.42 (m, 2H), 3.55 (m, 2H), 3.41 (m, 2H), 2.02 (s, 3H), 1.61 (m, 2H), 1.48 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.1, 155.0, 136.9, 136.6, 131.4, 128.3, 127.8, 127.6, 81.8, 79.8, 66.9, 60.5, 44.7, 40.8, 35.2, 28.2, 26.4, 20.9; IR (neat, cm^{-1}) 2923, 1687, 1585, 1455; HRMS calcd for $C_{24}H_{33}N_2O_6$ $(M + H)^+$ 445.2339, found 445.2349.

(±)-(1*R,4*S**)-Benzyl 1-Acetoxy-4-(6-chloro-5-nitropyrimidin-4-ylamino)-8-azaspiro[4.5]dec-2-ene-8-carboxylate (9).** A 25-mL oven-dried, round-bottomed flask equipped with a septum with a flow of nitrogen through a needle and a stir bar was charged with sodium bicarbonate (0.64 g, 6.0 mmol), compound **8** as the TFA salt (1.4 g, 3.0 mmol), 4,6-dichloro-5-nitropyrimidine (0.58 g, 3.0 mmol), and 8 mL of anhydrous THF in the indicated order. The mixture was refluxed for 4 h, allowed to cool to room temperature, filtered, and concentrated to give 1.2 g

(80%) of **9** as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.34 (m, 5H), 6.22 (m, 1H), 6.15 (m, 1H), 5.37 (m, 1H), 5.28 (m, 1H), 5.08 (s, 3H), 3.66−3.33 (m, 4H), 2.12 (m, 4H), 1.61 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 158.2, 155.1, 136.2, 134.0, 128.5, 128.0, 127.9, 80.8, 67.2, 61.2, 45.9, 40.8, 28.4, 20.9; IR (neat, cm^{-1}) 2923, 1694, 1538; HRMS calcd for $C_{23}H_{25}{}^{35}ClN_5O_6$ $(M + H)^+$ 502.1493, found 502.1501.

(±)-(1*R,4*S**)-Benzyl 1-Acetoxy-4-(5-amino-6-chloropyrimidin-4-ylamino)-8-azaspiro[4.5]dec-2-ene-8-carboxylate (10). Method A.** A 25-mL round-bottomed flask was charged with 8 mL of ethanol, 4 mL of 15% aqueous NH_4Cl solution, and compound **9** (0.80 g, 1.6 mmol). To this was added indium metal (0.74 g, 6.4 mmol), and the reaction mixture was refluxed for 8 h. The mixture was allowed to cool to room temperature, diluted with 10 mL of water, and filtered through Celite. The filtrate was extracted with ethyl acetate (5 × 10 mL). The combined organic extract was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash chromatography (hexanes/EtOAc = 1:1, R_f = 0.1) to give 0.60 g (80% yield) of **10** as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.31 (m, 5H), 6.11 (m, 1H), 6.06 (m, 1H), 5.34 (m, 1H), 5.17 (m, 1H), 5.10 (s, 2H), 4.83 (m, 1H), 3.71 (m, 1H), 3.57 (m, 1H), 3.39 (m, 3H), 3.27 (m, 1H), 2.06 (s, 3H), 1.64 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 155.1, 154.4, 149.7, 144.1, 137.1, 136.6, 136.0, 132.0, 128.4, 127.8, 81.8, 67.1, 60.9, 45.4, 40.9, 35.3, 29.1, 26.8, 21.1; IR (neat, cm^{-1}) 2926, 1694, 1574; HRMS calcd for $C_{23}H_{27}{}^{35}ClN_5O_4$ $(M + H)^+$ 472.1752, found 472.1750.

Method B. In a 15 mL sealed tube, compound **8** as the TFA salt (0.95 g, 2.0 mmol) was dissolved in *n*-BuOH (8 mL). To the solution were then added diisopropylethylamine (2.5 mL, 14 mmol), DMAP (0.12 g, 1.0 mmol), and 5-amino-4,6-dichloropyrimidine (0.98 g, 6.0 mmol). The tube was sealed and heated to 110 °C for 3 d with stirring. The reaction mixture turned a cloudy brown color. It was then allowed to cool to room temperature. The tube was opened, and the reaction mixture was then poured into a 100 mL round-bottomed flask and concentrated in vacuo while being heated in a water bath (60 °C). The crude residue was purified by flash chromatography (hexanes/EtOAc = 1:1, R_f = 0.1) to give 0.32 g (34%) of **10** as a yellow oil.

(±)-(1*R,4*S**)-Benzyl 1-Acetoxy-4-(6-chloro-9*H*-purin-9-yl)-8-azaspiro[4.5]dec-2-ene-8-carboxylate (11).** A 25-mL round-bottomed flask was charged with 7 mL of triethyl orthoformate, (±)-camphorsulfonic acid (CSA) (0.16 g, 0.68 mmol), and compound **10** (0.32 g, 0.68 mmol). The reaction mixture was stirred for 10 h at room temperature, at which time TLC indicated the absence of any starting material. The reaction mixture was quenched with saturated $NaHCO_3$ solution (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic layers were combined, washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The organic layer was then concentrated under reduced pressure and purified by flash chromatography (hexanes/EtOAc = 3:1, R_f = 0.25) to give 0.33 g of **11** as a light yellow oil in quantitative yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.12 (s, 1H), 7.30 (bs, 5H), 6.46 (dd, J = 2.4, 5.5 Hz, 1H), 6.22 (dd, J = 2.6, 5.7 Hz, 1H), 5.60 (s, 1H), 5.48 (s, 1H), 5.07 (s, 2H), 3.90 (m, 1H), 3.51 (m, 1H), 3.38 (m, 1H), 2.98 (m, 1H), 2.09 (s, 3H), 1.86 (m, 1H), 1.71 (m, 1H), 1.26 (m, 1H), 1.08 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.8, 154.9, 152.2, 151.8, 151.3, 144.5, 136.0, 133.6, 131.5, 128.5, 128.1, 127.9, 80.0, 67.2, 65.0, 46.3, 40.7, 35.3, 26.5, 20.9; IR (neat, cm^{-1}) 2911, 1694, 1563, 1436; HRMS (FAB) calcd for $C_{24}H_{25}{}^{35}ClN_5O_4$ $(M + H)^+$ 482.1595, found 482.1582.

(±)-(1*S,2*S**,3*S**,4*R**)-Benzyl 1-Acetoxy-4-(6-chloro-9*H*-purin-9-yl)-2,3-dihydroxy-8-azaspiro[4.5]decane-8-carboxylate (12).** A 10-mL oven-dried, round-bottomed flask equipped with a septum with a flow of nitrogen through a needle and a stir bar was charged with 4 mL of THF, compound **11** (0.32 g, 0.66 mmol), *N*-methymorpholine *N*-oxide (0.16 g, 1.4 mmol), and osmium tetraoxide (0.6 mL, 0.06 mmol, 2.5 wt % in *tert*-butyl alcohol). The solution was allowed to stir at room temperature for 2 d. After TLC analysis (hexanes/EtOAc = 3:1, R_f = 0.25) indicated that the starting material was consumed, the reaction was quenched with 10% of $Na_2S_2O_5$ solution (5 mL) and allowed to stir for an additional 30 min. The solution was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous sodium sulfate, and filtered. The solution was concentrated under reduced pressure, and the residue was chromatographed on silica (hexanes/EtOAc = 1:1, R_f = 0.1) to yield 0.28 g (82%) of **12** as a yellow oil: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.74 (s, 1H), 8.13 (s, 1H), 7.29 (bs, 5H), 5.15 (s, 1H), 5.06 (m, 1H), 5.00 (m, 2H), 4.82 (d, J = 10.8 Hz, 1H), 4.21 (dd, J = 2.4, 6.6 Hz, 1H), 3.97 (m, 1H), 3.57 (m, 1H), 3.11 (m, 1H), 2.81 (m, 1H), 2.17 (s, 3H), 2.04 (m, 2H), 1.71 (m, 1H), 0.78 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.2, 155.0, 152.6, 152.2, 151.4, 144.7, 144.6, 131.5, 128.4, 128.1, 127.9, 80.3, 74.1, 67.3, 43.2, 40.3, 21.1. IR (neat, cm^{-1}): 3363 (br), 2924, 1674, 1478; HRMS (FAB) calcd for $C_{24}H_{27}{}^{35}ClN_5O_6$ $(M + H)^+$ 516.1650, found 516.1646.

1-(6-Aminopurin-9-yl)-2,3,4-trihydroxy-8-azaspiro[4.5]decane-8-carboxylic Acid Benzyl Ester (13) (Method C) and 2,3,4-Trihydroxy-4-(6-methoxypurin-9-yl)-8-azaspiro[4.5]decane-8-carboxylic Acid Benzyl Ester (14). In a 15 mL sealed tube was dissolved compound **12** (0.28 g, 0.54 mmol) in 2.0 M methanolic ammonia (8 mL). The tube was sealed and heated to 45 °C for 2 d with stirring. The reaction mixture was allowed to cool to room temperature, poured into a 100 mL round-bottomed flask, and concentrated under reduced pressure. TLC indicated the formation of two major products, which were purified by flash chromatography (0−40% DCM/MeOH) to give compounds **13** (80 mg, 33% yield) and **14** (0.12 g, 47%). Compound **13**: ^{1}H NMR (600 MHz, CD_3OD) δ 8.32 (s, 1H), 8.27 (s, 1H), 7.30 (m, 5H), 5.05 (m, 1H), 5.03 (m, 1H), 4.74 (m, 1H), 4.08 (m, 2H), 3.92 (m, 1H), 3.33 (m, 4H), 2.02 (m, 2H), 1.81 (m, 1H), 0.55 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ 157.4, 156.9, 153.8, 142.1, 138.2, 129.5, 129.0, 79.1, 77.8, 75.2, 68.1, 45.4, 42.4; IR (neat, cm^{-1}) 2935, 1661, 1442; HRMS (FAB) calcd for $C_{22}H_{27}N_6O_5$ $(M + H)^+$ 455.1965, found 455.1982.

Compound **14**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (s, 1H) 8.07 (br, 1H), 7.26 (m, 5H), 5.08 (m, 1H), 4.96 (m, 3H), 4.64 (m, 1H), 4.23 (m, 1H), 4.13 (m, 1H), 4.07 (s, 3H), 3.80 (m, 1H), 3.34 (m, 1H), 3.15 (m, 1H), 3.00 (m, 1H), 2.07 (m, 1H), 1.84 (m, 1H), 1.60 (m, 1H), 0.60 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.8, 155.2, 152.0, 142.7, 136.3, 128.4, 128.0, 127.7, 78.0, 74.5, 67.1, 54.5, 40.9, 36.4, 29.7, 28.3; HRMS (FAB) calcd for $C_{23}H_{28}N_5O_6$ $(M + H)^+$ 470.2040, found 470.2043

(±)-(1*R,2*S**,3*R**,4*S**)-Benzyl 1-(6-Amino-9*H*-purin-9-yl)-2,3,4-trihydroxy-8-azaspiro[4.5]decane-8-carboxylate (13) (Method D).** In a 15 mL sealed tube, compound **12** (0.10 g, 0.20 mmol) was dissolved in a saturated ammonia in *n*-BuOH solution (3 mL). The tube was sealed and heated to 110 °C for 4 h with stirring. The reaction mixture turned brown and was allowed to cool to room temperature. The tube was opened, and the reaction mixture was then poured into a 25-mL round-bottomed flask and concentrated under reduced pressure with gentle heating in a water bath (60 °C). The crude residue was purified by flash chromatography (EtOAc, R_f = 0.2) to give 68 mg (75%) of **13** as a white solid: mp 208−210 °C.

(±)-(1*S,2*R**,3*S**,4*R**)-4-(6-Amino-9*H*-purin-9-yl)-8-azaspiro[4.5]decane-2,3,4-triol (4).** A 10-mL oven-dried, two-necked, round-bottomed flask equipped with a septum with a flow of nitrogen through a needle and a stir bar was charged with methanol (4 mL), compound **13** (40 mg, 0.088 mmol), and

10% Pd on carbon (15 mg). The vessel was purged with hydrogen using a balloon, and the nitrogen was removed. The suspension was allowed to stir at room temperature for 8 h. The suspension was purged with argon gas, filtered, and concentrated under reduced pressure to yield 26 mg (92%) of **4** as a white solid: mp 255−257 °C; ^{1}H NMR (500 MHz, CD_3OD) δ 8.23 (s, 1H), 8.16 (s, 1H), 4.90 (dd, J = 4.5, 11.5 Hz, 1H), 4.70 (d, J = 10.0 Hz, 1H), 4.00 (m, 2H), 3.08 (m, 2H), 2.87 (m, 2H), 2.11 (m, 2H), 1.90 (m, 1H), 0.77 (m, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 164.9, 157.3, 153.9, 151.9, 142.1, 120.1, 79.1, 77.8, 69.6, 44.5, 43.1, 37.0, 36.0, 31.7, 27.8; IR (neat, cm^{-1}) 2995, 1645, 1485; HRMS (FAB) calcd for $C_{14}H_{21}N_6O_3$ $(M + H)^+$ 321.1675, found 321.1661.

Acknowledgment. We thank Eli Lilly and Company and the NIH (R01 GM68012) for support of this research. We acknowledge Nonka Sevova for mass spectroscopy.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for **2**−**4**, **6**, **7**, and **9**−**14**. *g*HSQC, *g*COSY, and ROSEY NMR spectra for **12**. This material is available free of charge via the Internet at http://pubs.acs.org.