Carbocyclic Nucleosides as Inhibitors of Human Tumor Necrosis Factor-α Production: Effects of the Stereoisomers of (3-Hydroxycyclopentyl)adenines

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A series of four structurally related carbocyclic nucleosides (**6a**, **6b**, **10a**, and **10b**) were synthesized and evaluated for their ability to inhibit tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) production from human primary macrophages. These compounds had little effect on the production of IL-1β and IL-6. It was determined that compound **10a** was the most potent inhibitor of TNF-α production ($IC_{50} = 10\ \mu M$), having 2−5-fold more activity compared to its enantiomer **10b** or its diastereomers **6a** and **6b**. In addition, these compounds were also tested for their ability to protect mice against lethal challenges of lipopolysaccharide (LPS) and D-galactosamine (D-Gal). Compound **10a** showed superior protective effects (100% protection) compared to its enantiomer **10b** or its diastereomers **6a** and **6b** when it was administered to mice which were challenged with 3 times the LD_{100} dose of LPS.

Introduction

Under normal conditions the production of cytokines helps clear viral and bacterial infections and damaged cells from injured tissues. However, there are many cases in which the overproduction of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) can cause multiple-organ diseases and life-threatening shock.[1] One cytokine that is increasingly recognized as a central mediator in a wide spectrum of physiological and immune functions is macrophage-derived TNF-α.[1] The overproduction of TNF-α has been strongly implicated in septic shock, adult respiratory distress syndrome, AIDS, inflammatory bowel disease, bacterial meningitis, malaria, multiple sclerosis, atherosclerosis, and rheumatoid arthritis.[1−3] Thus, agents which can inhibit the production of TNF-α have attracted much attention over the last few years as potential therapies for the treatment of inflammatory diseases.[1]

It has been reported that adenosine (ADO) and certain ADO derivatives are potent regulators of macrophage (Mϕ) functions.[4,5] Adenosine has been shown to inhibit monocyte/macrophage phagocytosis,[6] chemotaxis,[7] and the synthesis and secretion of inflammatory mediators.[8,9] Some, but not all, of these effects are mediated through classical cell-surface ADO receptors that are expressed on mononuclear phagocytes.[9] In our attempt to obtain selective inhibitors of TNF-α production we have designed and synthesized a carbocyclic adenosine analogue **6a** (Scheme 3) which has been shown to selectively inhibit TNF-α production.[8] In the present study we will describe the synthesis and biological activity of (*cis*- and *trans*-3-hydroxycyclopentyl)-adenines which were prepared to better understand the role of the cyclopentanol moiety in the biological activity of this new class of TNF-α inhibitors.

Chemistry

The *cis*-configured carbocyclic nucleosides **6a** and **6b** (Scheme 3) were synthesized from the enantiomerically pure starting materials **2a** and **2b** (Scheme 1). Compound **2a** was prepared from **1** using an enzymatic method,[10] and compound **2b** was obtained commercially. Compounds **2a** and **2b** were converted to the *trans*-substituted mesylates **3a** and **3b** using methanesulfonyl chloride (3 equiv) and triethylamine (3 equiv) at 0 °C in quantitative yield.[11] Compounds **3a** and **3b** were used without further purification and were individually added to stirring solutions containing 3 equiv of sodium adenide in DMF at 60 °C which afforded the chiral, protected nucleosides **4a** and **4b** in 39% and 60% yields, respectively (Scheme 2). The acetate protecting groups of **4a** and **4b** were removed under similar conditions using potassium carbonate in methanol and purified by flash chromatography (eluted with 9:1 methylene chloride/methanol) to give the optically active nucleosides **5a**[11] and **5b** in 67% and 61% yields, respectively. The individual compounds **5a** and **5b** were then reduced with PtO_2 in methanol, and the products were purified by flash chromatography to give **6a**[11] and **6b** in 76% and 80% yields, respectively (Scheme 3). A hydrogenolysis product, 9-adenylcyclopentane, was the major side product (∼20%) in both reductions.

The *trans*-configured carbocyclic nucleosides **10a** and **10b** were prepared from **2a** and **2b** as shown in Scheme 4. The chiral intermediates **2a** and **2b** were reduced using Raney nickel and hydrogen to give **7a** or **7b** in approximately 55% yield. The chiral cyclopentanols **7a** and **7b** were converted to the mesylates **8a** and **8b** using the previously described method and then were individually treated with sodium adenide to give the protected *trans* compounds **9a** and **9b** in 53% and 46% yields, respectively. Compound **10a** was obtained by heating a solution of **9a** in ethanol at reflux for 48 h with the pH adjusted to 2.5 using 6 N HCl in 87% yield.

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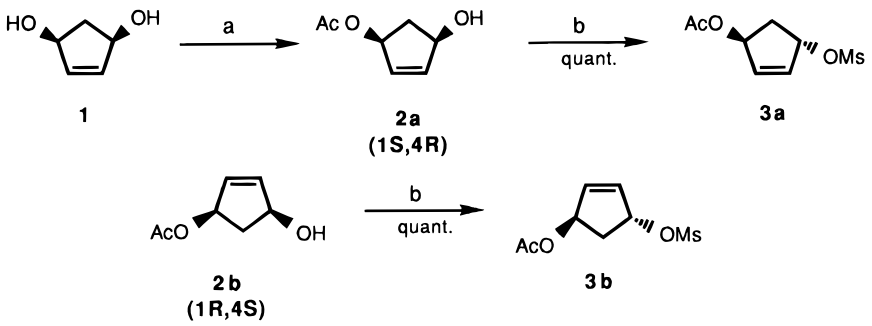
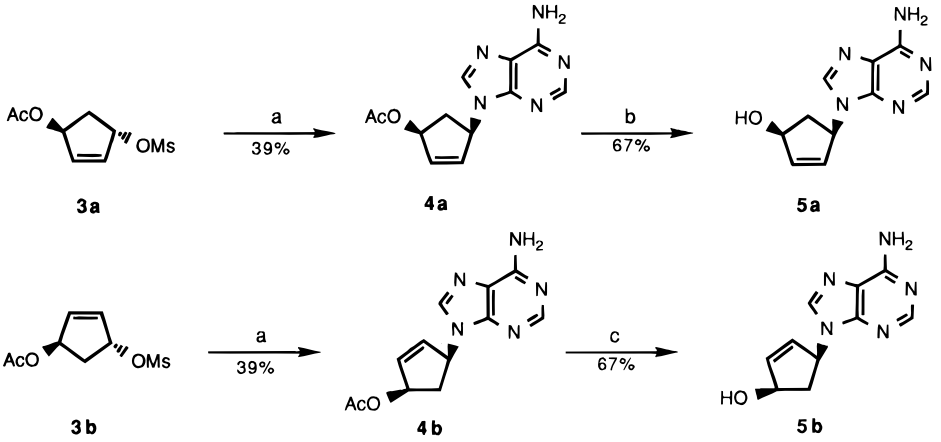
Scheme 1[a]



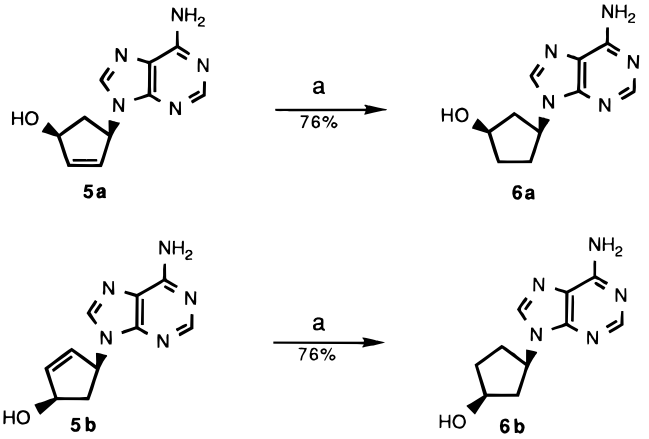
[a] Reagents: (a) ref 16; (b) CH_3SO_2Cl, TEA, CH_2Cl_2.

Scheme 2[a]



[a] Reagents: (a) adenine (3 equiv), NaH, DMF; (b) K_2CO_3, CH_3OH.

Scheme 3[a]



[a] Reagents: (a) PtO_2, H_2, MeOH.

Compound **10b** was obtained by deprotection of **9b** with potassium carbonate in methanol in 77% yield.

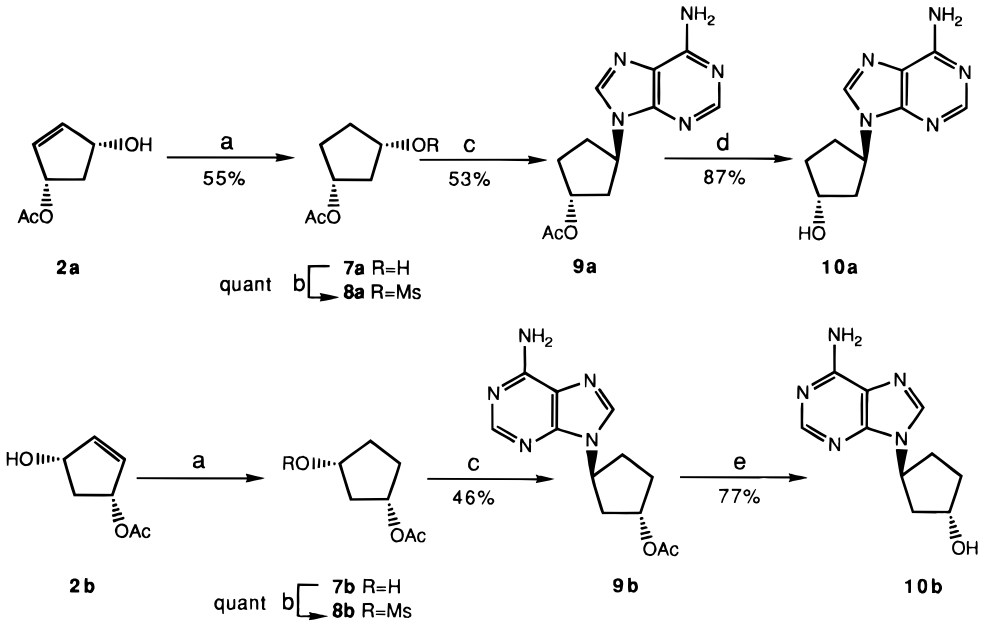
Results and Discussion

Adenosine and ADO-like compounds have been shown to inhibit macrophage-derived TNF-α production.[8,9,12] We have previously reported the design and synthesizis of the carbocyclic adenosine analogue **6a**[11] and its ability to inhibit TNF-α production from mouse thioglycollate-elicited peritoneal Mϕ and to protect D-galactosamine (D-Gal) sensitized mice from a lethal challenge (LD_{100}) of lipopolysaccharide (LPS).[8] In this report we describe the effects of **6a** and its stereoisomeric cyclopentanol analogues (**6b**, **10a**, and **10b**) on the production of TNF-α from primary human Mϕ and the protective effects of these compounds on D-Gal sensitized mice which have received lethal injections of LPS.[13]

Human Mϕ were obtained from healthy volunteers and were incubated with the compounds (ADO, **6a**, **6b**, **10a**, and **10b**) and LPS.[14−17] After incubation for 18 h the culture supernatants were assayed for TNF-α, IL-1β, and IL-6 using human specific ELISA kits. Shown in Table 1 are the inhibitory effects that ADO, **6a**, **6b**, **10a**, and **10b** have on the production of human TNF-α, IL-1β, and IL-6 elicited from LPS-stimulated Mϕ. Only TNF-α was significantly inhibited by the various carbocyclic nucleosides and ADO. ADO and **10a** demonstrated the most potent inhibition of TNF-α production, having IC_{50} values of 6 and 9 μM, respectively. ADO and **10a** showed a 2−5-fold separation between their IC_{50} values and those obtained for compounds **6a**, **6b**, and **10b**. Although **10b** is the enantiomer of **10a,** one can also view **10b** as positional isomer of **10a**. The hydroxyl group on **10a** can be viewed as the equivelant of the 3′-hydroxyl group of adenosine, while the hydroxyl group on **10b** can be considered to reside in the 4′-position. Therefore, compounds **10a** and **10b** are not just simply enantiomers, and this may account for the 2-fold separation in the IC_{50} values for the inhibiton of TNF-α. Compounds **6a** and **6b** can also be considered as positional isomers which may also account for their 2-fold separation in IC_{50} values. The 3′-epimers **10a** and **6b** and the 4′-epimers **10b** and **6b** showed a 2-fold differences in their IC_{50} values with the *cis* compounds being the least active.

These compound were then examined for their protective effects against lethal injections of D-Gal sensitized, LPS-challenged (LD_{100}) CF1 mice.[18−20] In this septic

Scheme 4[a]



[a] Reagents: (a) RaNi, H_2, EtOH; (b) MsCl, TEA, CH_2Cl_2; (c) adenine, NaH, DMF; (d) HCl, EtOH; (e) (i) K_2CO_3, EtOH; (ii) HCl, H_2O.

Table 1. In Vitro and in Vivo Biological Evaluation of Carbocyclic Nucleosides

	IC_{50} (μM)[a]			LPS/D-galactosamine septic shock model % survival[b]	
compd	TNF-α	IL-1β	IL-6	LD_{100}	3 × LD_{100}
control				0	0
ADO	6	>100	>100	0	0
6a	40	>100	>100	88	0
6b	18	>100	>100	50	0
10a	9	76	>100	100	100
10b	16	>100	>100	63	0
PTX	33	>100	>100	66	0

[a] Control: vehicle (PBS). Compound concentrations (dose response): 0.16, 0.8, 4.0, 20.0, and 100.0 μM in PBS. Supernatants were assayed for TNF-α, IL-1β, and IL-6 using ELISA kits. [b] Control: vehicle (PBS). Compound dosaged: 100 mg/kg IP in PBS.

shock model, mice were treated with 100 mg/kg ip of the test compounds (ADO, **6a**, **6b**, **10a**, and **10b**) 1 h prior to challenge with LPS/D-Gal. Pentoxyfylline (PTX) was used in this experiment as a positive control since it has been reported to be effective at preventing LPS/D-Gal-induced septic shock and death in mice.[21] This model has been shown to be a TNF-α driven model since monoclonal anti-TNF-α antibodies can protect D-Gal sensitized mice against the lethal challenge of LPS.[20] In the first experiment, the D-Gal-sensitized mice were challenged with an LD_{100} dose of LPS, and the percent survival was determined after 8 h. All of the control animals died, and the PTX positive control showed 66% protection 8 h postchallenge. ADO showed no protection at 8 h, presumably due to the rapid metabolism of ADO to inosine and/or ATP. Compounds **6a** and **10a** showed 88 and 100% protection, respectively, and were more effective than PTX. Compounds **6b** and **10b** gave protection comparable to PTX.

In another experiment, D-Gal sensitized mice were given 3 times the LD_{100} dose of LPS to determine if any of the compounds had superior potency. Only compound **10a** maintained its ability to protect D-Gal sensitized mice against the lethal challenge of LPS. The increased potency may be due to a longer duration of action. Compound **10a** was determined not to be a substrate for purified calf intestinal adenosine deaminase, and it appeared not to be phosphorylated (data not shown).[22] We have previously shown that the inhibition of TNF-α is mediated through the adenosine A_3 receptor, and improved receptor occupancy for compound **10a** may also be responsible for its superior potency.[16]

It has been demonstrated that the carbocyclic adenosine analogues **6a**, **6b**, **10a**, and **10b** are effective at inhibiting TNF-α production from primary human Mϕ, and this activity can be correlated with the protection seen in the LPS/D-Gal-induced septic shock model. We have previously shown that the inhibition of TNF-α is mediated through the adenosine A_3 receptor which specifically inhibits TNF-α transcription.[12,16] TNF-α gene expression is known to be regulated by the NF-kβ family of transcription factors, and we have also shown that compound **10a** inhibits the activation and translocation of the p50/p65 NF-kβ heterodimer complex from the cytoplasm to the nucleus. Compounds which can inhibit TNF-α production at the transcriptional level offer a unique and novel approach to the development of new therapeutic drugs for the treatment of autoimmune and other inflammatory diseases.[1]

Experimental Section

Except where noted otherwise, reagents and starting materials were obtained from common commercial sources and used as received. "Dry" reaction solvents refer to solvents in Aldrich Sure-Seal bottles. The phrase "concentrated in vacuo" indicates rotary evaporation using a Buchi apparatus at 20-50 °C and 15−30 Torr (KNF Neuberger Model UN 726.12FTP diaphragm pump). Vacuum drying was done at <10 Torr at the temperatures noted. Melting points were determined on a Thomas Hoover Uni-melt capillary melting point apparatus. Melting points and boiling points are reported uncorrected. Thin layer chromatography (TLC) was performed on glass-backed, silica gel 60F-254 plates (EM) coated to a thickness of 0.25 mm. The plates were eluted with solvent systems (v/v) as described and visualized by UV light or a *p*-anisaldehyde solution. Flash chromatography was carried out using EM Science silica gel 60 (40−63 μm) according to the literature

procedure using the solvent systems as described. Infrared (IR) spectra were recorded on a Mattson Galaxy Series 5020 infrared spectrophotometer with samples prepared as indicated, and are reported in wave numbers (cm^{-1}). ^1H NMR spectra were recorded on Varian Gemini 300, Unity 300, Unity 400, or Unity 500 spectrometers with chemical shifts (δ) reported in ppm relative to tetramethylsilane (0.00 ppm). ^{13}C NMR spectra were recorded on the Varian Gemini instrument (75 MHz) with chemical shifts (δ) reported in ppm relative to $CDCl_3$ (77.0 ppm), unless stated otherwise. Mass spectra (MS) were obtained on a Finnigan MAT Model TSQ 700 mass spectrometer system by chemical ionization at 120 eV using methane (CI, 120 eV) unless otherwise indicated, e.g. with electron impact, MS (EI, 70 eV). Ultraviolet (UV) spectra were recorded on a Perkin-Elmer Lambda 4C spectrophotometer and reported in nanometers (nm). The sample was prepared in the solvent indicated at a concentration expressed as mg/mL. Specific rotation [α] was obtained with a JASCO Model DEP 360 polarimeter fitted with a 1 dm cell. Measurements were made at the sodium D line (589 nm) at 20 °C (unless otherwise specified), and the concentration (g/100 mL) and solvent are reported. (1*R*,4*S*)-1-Acetoxy-4-hydroxycyclopent-2-ene was purchased from Fluka. PTX and lipopolysaccharide (LPS) from *Escherichia coli* were purchased from Sigma Chemical Co. (St. Louis, MO). Human recombinant IL-1β (HurIL-1β) was purchased from Upstate Biotechnology Inc. (Lake Placid, NY), and human recombinant tumor necrosis factor-α (HurTNF-α) was purchased from R&D Systems (Minneapolis, MN).

(1*S*,4*R*)-4-Acetoxy-1-(9-adenyl)cyclopent-2-ene (4b). To a 250 mL flask were added (1*R*,4*S*)-1-acetoxy-4-hydroxycyclopent-2-ene (**2b**, 5.0 g, 35.3 mmol), methylene chloride (100 mL), and methanesulfonyl chloride (12.1 g, 105.9 mmol), and the contents were stirred and cooled with an ice bath to 0−5 °C. Neat triethylamine (10.7 g, 105.9 mmol) was added dropwise over a 10 min period, and after the addition was complete the ice bath was removed. The reaction mixture was allowed to come to room temperature and stir for overnight. Progress of the reaction was monitored by thin layer chromatography (EtOAc/hexane (4:1)), and the reaction was judged to be complete ($R_f = 0.75$). The mixture was extracted with water (2 × 25 mL) and brine (1 × 50 mL). The aqueous layers were combined and extracted with methylene chloride (150mL), and the combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give **3b** (7.78 g, 100%) as a yellow oil. This material was used immediately without further purification: ^1H-NMR ($CDCl_3$) δ 6.13 (ddd, 1 H, J = 5, 2, 2 Hz), 6.05 (ddd, 1 H, J = 5, 2, 1 Hz), 5.85 (m, 1 H), 5.08 (m, 1 H), 3.71 (s, 3 H), 2.59 (ddd, 1 H, J = 16, 8, 4 Hz), 2.38 (ddd, 1 H, J = 16, 8, 4 Hz), 2.04 (s, 3 H); ^{13}C-NMR ($CDCl_3$) δ 170.7, 137.5, 133.8, 78.4, 61.3, 41.5, 31.6, 21.0.

Sodium adenide was prepared by adding adenine (14.40 g, 105.9 mmol) to dry DMF (250 mL) and was added to the slurry NaH (60% in mineral oil, 4.24 g, 105.9 mmol), and the reaction mixture was stirred for 3 h at 55−60 °C. The (1*R*,4*R*)-1-acetoxy-4-[(methylsulfonyl)oxy]cyclopent-2-ene (**3b**) (7.78 g, 35.3 mmol) was added, and the reaction mixture was stirred for 48 h at 55−60 °C. The product formation ($R_f = 0.9$) was monitored by TLC with methylene chloride/methanol (4:1). The reaction mixture was cooled to 10 °C (ice bath), and the solid which formed was collected and washed thoroughly with methylene chloride. The filtrate was concentrated in vacuo, and the residue was dissolved in methylene chloride (200 mL) and filtered to removed undissolved solids. The filtrate was extracted successively with brine (2 × 50 mL), and the aqueous layer was extracted with methylene chloride (3 × 100 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo. The residue was purified by flash chromatography eluted with methylene chloride/methanol (19:1) to give 5.5 g (60%) of **4b**: mp 250 °C dec; $[\alpha]_D = -9.4°$ (c = 1.01, MeOH); ^1H-NMR ($CDCl_3$) δ 8.35 (s, 1 H), 7.84 (s, 1 H), 6.32 (m, 1 H), 6.18 (m, 1 H), 6.07 (br s, 2 H), 5.7 (m, 2 H), 3.11 (ddd, 1 H, J = 16, 8, 8 Hz), 2.08 (s, 3 H), 1.95 (ddd, 1 H, J = 16, 2, 2 Hz); ^{13}C-NMR ($CDCl_3$) δ 170.4, 155.6, 153.1, 149.6, 138.6, 135.5, 133.7, 119.5, 77.2, 56.7, 38.7, 21.0; MS (CI, methane) 260 (base, (M + 1), 200, 173, 136, 125. Anal. ($C_{12}H_{13}N_5O_2$) C, H, N.

(1*S*,4*R*)-1-(9-Adenyl)-4-hydroxycyclopent-2-ene (5b). Compound **4b** (5.5 g, 21 mmol) was dissolved in methanol (60 mL), K_2CO_3 (1.0 g) was added, and the reaction mixture was stirred for 2 h at room temperature. The mixture was filtered and concentrated in vacuo. The residue was purified using flash chromatography eluting with 9:1 methylene chloride/methanol. The fractions containing product were combined and concentrated to dryness. The product was dissolved in H_2O, and the pH of the solution was adjusted to 2.5 with 6 N HCl. The solution was lyophilized to give 2.84 mg (61%) of **5b**: mp 204−205 °C; $[\alpha]_D = -80°$ (c = 0.98, MeOH); ^1H-NMR (DMSO-d_6) δ 9.6 (br s, 1 H), 9.0 (br s, 1 H), 8.57 (s, 1 H), 8.49 (s, 1 H), 6.26 (m, 1 H), 6.03 (m, 1 H), 5.54 (m, 1 H), 4.75 (m, 1 H), 2.95 (ddd, 1 H, J = 16, 8, 8, Hz), 1.79 (ddd, 1 H, J = 16, 2, 2, Hz); ^{13}C-NMR (DMSO-d_6) δ 150.5, 147.8, 144.8, 142.2, 140.3, 129.9, 118.0, 73.5, 57.8, 41.3; MS (EI) m/z 217 (M), 188, 173, 135 (base), 108; UV (MeOH) λ = 261 nm, ϵ = 14 500. Anal. ($C_{10}H_{11}N_5O \cdot 0.8HCl$) C, H, N, Cl.

(1*S*,3*R*)-1-(9-Adenyl)-3-hydroxycyclopentane (6b). Compound **5b** (100 mg, 0.39 mmol) was dissolved in methanol (50 mL), PtO_2 (30 mg) was added, and the mixture was hydrogenated at 35 psi of hydrogen for 3 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated in vacuo. The residue was purified by flash chromatography eluted with methylene chloride/methanol (9:1). The fractions containing product were combined and concentrated and then dissolved in water which was adjusted to pH 2.5 with 6 N HCl. The solution was lyophilized to give 76 mg (76%) of compound **6b**: mp 231 °C dec; $[\alpha]_D = -9.0°$ (c = 0.002, MeOH); ^1H-NMR (DMSO-d_6 + D_2O) δ 8.62 (s, 1 H), 8.44 (s, 1 H), 5.03 (p, 1 H, J = 9 Hz), 4.30 (p, 1 H, J = 2 Hz), 2.49 (m, 1 H), 2.26 (m, 1 H), 2.13 (m, 1 H), 1.99−1.8 (m, 3 H); ^{13}C-NMR (DMSO-d_6) δ 150.5, 148.2, 144.9, 142.5, 120.0, 70.3, 53.9, 41.5, 34.0, 31.0; MS (EI) m/z 219 (M), 202, 162, 135 (base) 108. Anal. ($C_{10}H_{14}C_lN_5O \cdot 0.75H_2O$) C, H, N.

(1*R*,3*S*)-1-Acetoxy-3-hydroxycyclopentane (7a). Compound **2a** (36 g, 254 mmol) was added to a suspension of approximately 50 g of Raney nickel in 250 mL of absolute ethanol. The suspension was stirred under 1−4 bar of hydrogen at 15−20 °C for approximately 5 h. The mixture was filtered through a P3 glass filter, and the solid was rinsed with ethanol. The filtrate was concentrated in vacuo, the yellow oil was dissolved in ether (100 mL), 1 g of activated carbon was added, and the mixture was stirred for 5 min and filtered. The filtrate was evaporated to give 33 g of a colorless oil which was distilled bulb-to-bulb (80−90 °C at 0.1 mmHg) to yield 20.0 g (55%) of **7a**: ^1H-NMR ($CDCl_3$) δ 5.14 (m, 1 H), 4.33 (m, 1 H), 2.2 (br s, 1 H), 2.09 (m, 1 H), 2.03 (s, 3 H), 2.0−1.7 (m, 5 H); ^{13}C-NMR ($CDCl_3$) δ 170.8, 75.5, 72.4, 41.7, 33.8, 30.5, 21.2; MS (CI, methane) m/z 145 (M + 1), 127, 101; $[\alpha]_D = -5.43°$ (c = 1.03, MeOH).

(1*S*,3*R*)-1-Acetoxy-3-hydroxycyclopentane (7b). Compound **7b** was prepared in a similar manner as **7a** starting with **2b**: ^1H-NMR ($CDCl_3$) δ 5.14 (m, 1 H), 4.33 (m, 1 H), 2.2 (br s, 1 H), 2.09 (m, 1 H), 2.03 (s, 3 H), 2.0−1.7 (m, 5 H); ^{13}C-NMR ($CDCl_3$) δ 170.8, 75.5, 72.4, 41.7, 33.8, 30.5, 21.2; MS (CI, methane) m/z 145 (M + 1), 127, 101; $[\alpha]_D = +5.30°$ (c = 1.012, MeOH).

(1*R*,3*S*)-1-Acetoxy-3-(methylsulfonoxy)cyclopentane (8a). A solution of (1*R*,3*S*)-1-acetoxy-3-hydroxycyclopentane (**7a**, 49.0 g, 340 mmol) and methanesulfonyl chloride (46.7g, 408 mmol) in methylene chloride (425 mL) was stirred and cooled to 0−5 °C (ice bath). To the solution was added triethylamine (41.3 g, 408 mmol) in 75 mL of methylene chloride over a 10 min period. After the addition was complete the ice bath was removed and the reaction mixture was allowed to come to room temperature and stir for 1 h (TLC; EtOAc/hexane (4:1); $R_f = 0.75$). The mixture was extracted with water (1 × 400 mL, 1 × 200 mL) and brine (1 × 200 mL). The aqueous layers were combined and extracted once with methylene chloride (200 mL), and the combined organic solutions were dried (sodium sulfate) and concentrated in vacuo to give **8a** as a yellow oil (76.4 g, 98%): ^1H-NMR ($CDCl_3$) δ 5.14 (m, 2 H), 3.02 (s, 3 H), 2.39 (dt, 1 H), 2.1−1.95 (m, 8 H);

^{13}C-NMR ($CDCl_3$) δ 170.7, 81.8, 74.0, 39.5, 38.5, 31.7, 30.4, 21.1; IR (neat) 1734, 1352, 1249, 1167, 974, 889; MS (CI, methane) 223 (M + 1), 163, 127 (base), 67; $[\alpha]_D = +5.93°$ (c = 0.97, CH_2Cl_2). Anal. ($C_8H_{14}O_5S$) C, H.

(1*S*,3*R*)-1-Acetoxy-3-(methylsulfonoxy)cyclopentane (8b). Compound **8b** was prepared in a similar manner as **8a** starting with **7b** (1.44 g, 10.0 mmol) to give 2.22 g (100%) of **8b**: ^{1}H-NMR ($CDCl_3$) δ 5.14 (m, 2 H), 3.02 (s, 3 H), 2.39 (dt, 1 H), 2.1−1.95 (m, 8 H); ^{13}C-NMR ($CDCl_3$) 170.7, 81.8, 74.0, 39.5, 38.5, 31.7, 30.4, 21.1; IR (neat) 1734, 1352, 1249, 1167, 974, 889; MS (CI, methane) 223 (M + 1), 163, 127 (base), 67.

(1*R*,3*R*)-3-Acetoxy-1-(9-adenyl)cyclopentane (9a). Adenine (91.9 g, 680 mmol) was suspended in DMF (700 mL), and NaH (60% dispersion in mineral oil, 27.2 g, 680 mmol) was washed with hexane (1 × 200 mL) and was then added to the stirring adenine suspension. The reaction mixture was stirred for 3 h at 55 °C, and **8a** (76 g, 340 mmol) was added to the reaction mixture (rinsed with DMF 2 × 100 mL), the reaction mixture was stirred for 48 h at 55−60 °C (TLC; methylene chloride/methanol (4:1); R_f = 0.9). The reaction mixture was then cooled to 10 °C (ice bath), and the solid which formed was filtered and washed thoroughly with methylene chloride (1.2 L). The filtrate was concentrated in vacuo, and the residue was dissolved in methylene chloride (500 mL) and filtered. The filtrate was extracted with brine (2 × 100 mL), and the aqueous layer was extracted with methylene chloride (3 × 100 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo. The material was dissolved in methylene chloride (300 mL), diluted with hexane (200 mL), seeded, and kept overnight at room temperature. The solid that formed was vacuum-dried overnight to give 32.2 g (36%) of **9a**. An additional 16.9 g of material was obtained from the filtrate after silica gel (500 g) chromatography using methylene chloride/methanol (19:1) to give a total of 49.1 g of **9a**. This material was recrystallized from methylene chloride/hexane to give 47.3 g (53%) of **9a**: mp 148−149 °C; $[\alpha]_D$ = −9.03° (c = 0.997, methanol); ^{1}H-NMR ($CDCl_3$) δ 8.38 (s, 1 H), 7.85 (s, 1 H), 6.75 (br s, 2 H), 5.45 (m, 1 H), 5.10 (p, 1 H), 2.45 (m, 3 H), 2.2−1.85 (m, 5 H); ^{13}C-NMR ($CDCl_3$) δ 170.5, 155.5, 152.8, 150.1, 138.8, 120.2, 74.6, 54.5, 38.9, 31.1, 30.6, 21.2; UV λ_{max} = 261 nm (95% ethanol, ϵ = 14 500); MS (CI, methane) 262 (M, base), 218, 202, 136; IR (KBr) 3311, 3144, 1730, 1666, 1601, 1475, 1419, 1251. Anal. ($C_{12}H_{15}N_5O2\cdot0.2H_2O$) C, H, N.

(1*S*,3*S*)-3-Acetoxy-1-(9-adenyl)cyclopentane (9b). Compound **9b** was prepared in a similar manner as **9a** starting with **8b** (2.2 g, 10.0 mmol), except the crude product was directly chromatographed. For **9b**: yield 1.2 g (46%); mp 148−149 °C; $[\alpha]_D = +7.8°$ (c = 1.007, methanol); ^{1}H-NMR ($CDCl_3$) δ 8.38 (s, 1 H), 7.85 (s, 1 H), 6.75 (br s, 2 H), 5.45 (m, 1 H), 5.10 (p, 1 H), 2.45 (m, 3 H), 2.2−1.85 (m, 5 H); ^{13}C-NMR ($CDCl_3$) δ 170.5, 155.5, 152.8, 150.1, 138.8, 120.2, 74.6, 54.5, 38.9, 31.1, 30.6, 21.2; UV λ_{max} = 261 nm (95% ethanol, ϵ = 14 500); MS (CI, methane) 262 (M + 1, base), 218, 202, 136. Anal. ($C_{12}H_{15}N_5O_2$) C, H, N.

(1*R*,3*R*)-1-(9-Adenyl)cyclopentan-3-ol (10a). Compound **9a** (46.8 g, 177 mmol) was dissolved in ethanol (400 mL), and the pH was adjusted to 2.5 with 6 N HCl (~35 mL). The solution was stirred and heated at reflux for 48 h. The reaction mixture was cooled to 0−5 °C, and a white solid formed. The solid was collected, washed with cold ethanol/hexane (1:1) and hexane, and air-dried overnight to give 29.8 g of **10a**. Concentration of the filtrate gave an additional 12.97 g (in two crops) of **10a**: total yield 42.7 g (87%); mp 246 °C dec; $[\alpha]_D$ = −12.55° (c = 1.01, MeOH); ^{1}H-NMR (DMSO-d_6) δ 9.6 (br s, 1 H), 9.0 (br s, 1 H), 8.7 (s, 1 H), 8.6 (s, 1 H), 5.2 (m, 1 H), 4.4 (m, 1 H), 2.4 (m, 1 H), 2.2 (m, 3 H), 2.0 (m, 1 H), 1.7 (m, 1 H); ^{13}C-NMR (DMSO-d_6) δ 150.4, 148.3, 144.5, 142.7, 118.2, 70.2, 54.7, 41.5, 33.5, 30.1; MS (CI, methane) 220 (M + 1, base), 136; IR (KBr) 3450−2600, 1690, 1064; UV λ_{max} = 261.0 nm (95% ethanol, ϵ = 13 800). Anal. ($C_{10}H_{14}ClN5O\cdot0.5H_20$) C, H, N.

(1*S*,3*S*)-1-(9-Adenyl)cyclopentan-3-ol (10b). Compound 9b (1.2 g, 4.5 mmol) was dissolved in methanol (30 mL), and 5 drops of water was added followed by 1 g (7.3 mmol) of potassium carbonate. The reaction mixture was stirred for 1 h and then filtered and concentrated in vacuo. The residue was purified by flash chromatography eluting with methylene chloride/metanol (4:1). The fractions containing product were combined and concentrated, and the residue was dissolved in water (20 mL). The pH was adjusted to 2.0 with 6 N HCl, and the solution was lypholyzed to give a white powder (0.9 g, 77%): mp 239 °C dec; $[\alpha]_D$ = −11.6° (c = 0.1982, MeOH); ^{1}H-NMR (DMSO-d_6) δ 9.6 (br s, 1 H), 9.0 (br s, 1 H), 8.7 (s, 1 H), 8.6 (s, 1 H), 5.2 (m, 1 H), 4.4 (m, 1 H), 2.4 (m, 1 H), 2.2 (m, 3 H), 2.0 (m, 1 H), 1.7 (m, 1 H); ^{13}C-NMR (DMSO-d_6) δ 150.4, 148.3, 144.5, 142.7, 118.2, 70.2, 54.7, 41.5, 33.5, 30.1; MS (CI, methane) 220 (M, base), 136; UV λ_{max} = 261.0 nm (95% ethanol, ϵ = 13 800). Anal. ($C_{10}H_{14}ClN_5O$) C, H, N.

Cell Preparation and in Vitro Dose−Response. Venous blood was collected from normal volunteers, and peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using LeucoPREP cells separation tubes (Becton Dickinson, Linclon Park, NJ). The monocyte/macrophage populations were obtained by the method described by Kumagai et al.[17] Briefly, PBMC were allowed to adhere to petri dishes (Falcon) previously coated with heat-inactivated FCS. Nonadherent cells were rinsed off after a 1 h incubation period, and adherent monocyte/macrophages populations were removed using RPMI-1640 with 0.2% EDTA and 5% heat-inactivated FCS. As determined by FACS analysis, this procedure routinely increased MO-2+/KC56+ (CD14+/CD45+) cells in the population by 3−5-fold over what was present in the PBMC population. Compounds were dissolved in PBS (0.16, 0.8, 4.0, 20.0, and 100 μM) and added to the monocyte/macrophage cells (10^6/mL) 15 min prior to stimulation with LPS (1 μg/mL) in RPMI-1640 supplemented with 100 units/mL penicillin, 100 μg/mL, 0.18 mM L-glutamine and 10% nonmitogenic heat-inactivated FCS in 24 well tissue culture plates. Cultures were incubated at 37 °C in 5% CO_2 for 18 h. Culture supernatants were then harvested by centrifugation and stored at −70 °C until they were assayed for cytokines. Culture supernatants were assayed for TNF-α, IL-1β, and IL-6 proteins using ELISA kits from Cistron, specific for the protein being assayed. The IC_{50} data was generated using a computerized nonlinear regression analysis as discribed by Baron and Siegel.[23]

Endotoxin Lethality Studies. These studies using CF_1 female mice (Charles Rivers) were conducted in a manner similar to that described by Silverstein.[24] After overnight fasting, mice were pretreated (−1 h) with the test compounds (100 mg/kg in PBS, ip), followed by an ip challenge of D-GalNH_2 (20 mg) and *S. enteritidis* LPS (15−250 ng; DIFCO). The dose of LPS necessary to cause 100% lethality varied slightly between different shipments of mice; therefore, an LD_{100} was determined prior to each shipment for that group of mice. Immediately after challenge, the mice were returned to feed and lethality was recorded 8 h postchallenge.

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