lines with the analogues utilized in this investigation.

The results of our recent study concerning the mechanistic aspects of the inhibition of DNA-unwinding mediated by helicase II suggest that drug modification of DNA slows down the turnover rate of helicase II, which results in an increase of retention time of helicase II on drug-damaged DNA.[22] Likewise, the stalling of helicase II on drug-damaged DNA, particularly (+)-CC-1065- and (+)-AB'C'-modified DNA, was also observed in the primer extension experiment with the combined action of helicase II and polymerase I (see Figure 11). The distance between the drug modification site and termination of primer extension (about 20 nucleotides) appears to represent a critical length of coverage of DNA by helicase II, which is constant from one drug-modified sample to another, and is also about the same length of helical coverage revealed in the nonmodified gapped duplex. A similar observation was made in the previous study.[22]

Conclusively, the results presented in this paper clearly define some of the biochemical consequences of drug-induced winding and helix-stabilizing of DNA molecules on the activity of unwinding enzymes such as helicase II and *E. coli rep* protein. Studies are in progress that use a DNA transcription system in combination with eukaryotic transcriptional factors (e.g., Sp1) to determine the biochemical consequences of structural changes in DNA molecules that result from covalent adduct formation with (+)-CC-1065 and its analogues.

Acknowledgment. This research was supported by the Public Health Service (CA-49751), The Welch Foundation, and the Burroughs Wellcome Fund. We thank Professor Thomas Kodadek, for helicase enzymes and valuable advise, and Mr. David M. Bishop for editorial assistance.

4'-Modified Analogues of Aristeromycin and Neplanocin A: Synthesis and Inhibitory Activity toward *S*-Adenosyl-L-homocysteine Hydrolase

Michael S. Wolfe,[†] Younha Lee,[‡] William J. Bartlett,[†] David R. Borcherding,[†] and Ronald T. Borchardt*[,†,‡]

Departments of Medicinal Chemistry and Biochemistry, The University of Kansas, Lawrence, Kansas 66045.
Received March 8, 1991

The carbocyclic adenosine analogues aristeromycin and neplanocin A both display significant *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase inhibitory activity and broad-spectrum antiviral effects. Since phosphorylation of the 4'-hydroxymethyl substituent has been implicated with the cytotoxicity of these compounds, various analogues modified at this position were synthesized utilizing a key cyclopentenone intermediate **3** which can be derived from several members of the natural chiral pool. Cyclopentenone **3** underwent a highly stereoselective conjugate addition with organocuprate reagents, and the 1,4-adducts so formed were then readily elaborated to the corresponding 4'-modified aristeromycin analogues. Alternatively, quenching the enolate intermediate of the organocuprate conjugate addition with methanesulfinyl chloride followed by pyrolytic syn elimination resulted in the formation of 4'-modified neplanocin A intermediates. Three of the final compounds (**1b**, **1c**, and **1e**) displayed inhibitory activity toward AdoHcy hydrolase in the nanomolar range.

Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (AdoHcy) hydrolase (EC 3.3.1.1) plays an important regulatory role in *S*-adenosyl-L-methionine (AdoMet)-dependent methylation reactions.[1] AdoMet serves as a methyl donor for a variety of biomolecules, including macromolecules such as mRNA,[2] and the byproduct of these methylations, AdoHcy, functions as a feedback inhibitor for the plethora of methyltransferases that catalyze these reactions. Since AdoHcy hydrolase provides the only known mechanism for AdoHcy catabolism in eukaryotes, catalyzing its hydrolysis to adenosine and homocysteine, the action of this enzyme is thought to allow the methylation process to continue at its normal physiological rate.

In recent years, AdoHcy hydrolase has become of interest as a target for antiviral chemotherapy.[3] There are several reasons for this: (1) most plant and animal viruses require a methylated cap structure at the 5'-terminus of their mRNA for viral replication;[4] (2) virus-encoded methyltransferases that are involved in the formation of this methylated cap structure are inhibited by AdoHcy;[5] (3) undermethylation of the viral mRNA cap structure induced by the inhibition of AdoHcy hydrolase has been correlated with the inhibition of viral replication;[6] (4) a close correlation exists between the antiviral potency of adenosine analogues and their inhibitory effects on AdoHcy hydrolase;[7] and (5) a close correlation exists be-

* Correspondence should be sent to author at the following address: Rm 3006 Malott Hall, The University of Kansas, Lawrence, KS 66045.

[†] Department of Medicinal Chemistry.

[‡] Department of Biochemistry.

(1) Cantoni, G. L.; Chiang, P. K. The Role of *S*-Adenosylhomocysteine and *S*-Adenosylhomocysteine Hydrolase in the Control of Biological Methylation. In *Natural Sulfur Compounds*; Cavallini, D.; Gaull, G. E.; Eds.; Plenum Publishing Corp.: New York, 1980; pp 67-80.

(2) Borchardt, R. T. *S*-Adenosyl-L-methionine-dependent Macromolecule Methyltransferases: Potential Targets for the Design of Chemotherapeutic Agents. *J. Med. Chem.* **1980**, *23*, 347-357.

(3) De Clercq, E. *S*-Adenosylhomocysteine Hydrolase Inhibitors as Broad-spectrum Antiviral Agents. *Biochem. Pharmacol.* **1987**, *36*, 2567-2575.

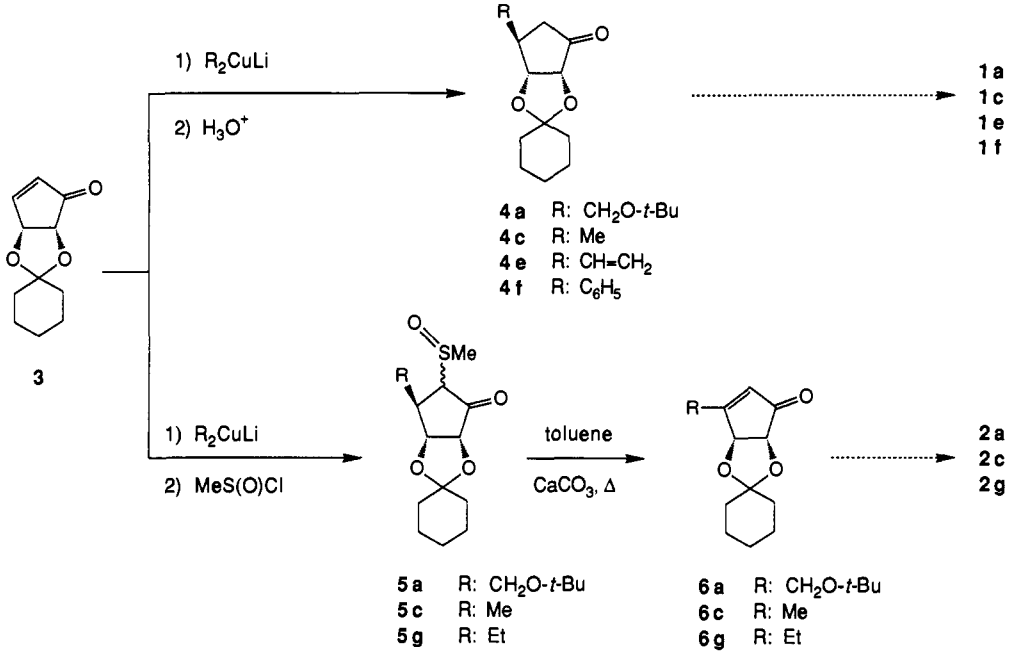
(4) Banerjee, A. K. 5'-Terminal Cap Structure in Eucaryotic Messenger Ribonucleic Acids. *Microbiol. Rev.* **1980**, *44*, 175-205.

(5) Pugh, C. S. G.; Borchardt, R. T. Effects of *S*-Adenosylhomocysteine Analogues on Vaccinia Viral Messenger Ribonucleic Acid Synthesis and Methylation. *Biochemistry* **1982**, *21*, 1535-1541.

(6) Ransohoff, R. M.; Narayan, P.; Ayers, D. F.; Rottman, F. M.; Nilsen, T. W. Priming of Influenza mRNA Transcription is Inhibited in CHO Cells Treated with the Methylation Inhibitor, Neplanocin A. *Antiviral Res.* **1987**, *7*, 317-327.

0022-2623/92/1835-1782$03.00/0

Scheme I. General Synthetic Strategy toward 4'-Modified Analogues of Aristeromycin and Neplanocin A



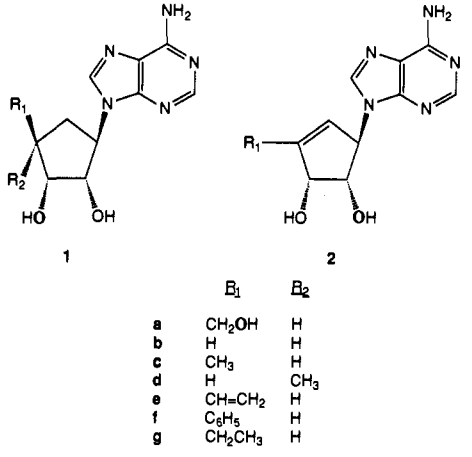


Figure 1. 4'-Modified analogues of aristeromycin and neplanocin A.

tween the antiviral potency of carbocyclic nucleosides and their ability to elevate cellular levels of AdoHcy.[8]

The carbocyclic adenosine analogues aristeromycin (Ari; **1a**)[9,10] and neplanocin A (NpcA; **2a**),[11] depicted in Figure 1, have been reported to be potent inhibitors of AdoHcy hydrolase. These compounds also possess potent antiviral activity; however, their cytotoxicity precludes clinical use.[12-14] Evidence that 5'-phosphorylation leads to at least part of the cytotoxicity of these compounds[15,16] led us to synthesize NpcA analogue **2b** lacking the 4'-hydroxymethyl substituent.[17] The cytotoxicity of this compound was in fact greatly attenuated while AdoHcy hydrolase inhibitory activity and antiviral potency approached that of NpcA.[18-20] Furthermore, the half-life of **2b** in cell culture

(7) Cools, M.; DeClerq, E. Correlation Between the Antiviral Activity of Acyclic and Carbocyclic Adenosine Analogues in Murine L929 Cells and Their Inhibitory Effect on L929 Cell S-Adenosylhomocysteine Hydrolase. *Biochem. Pharmacol.* **1989**, *38*, 1061–1067.

(8) Hasobe, M.; McKee, J. G.; Borchardt, R. T. Relationship Between the Intracellular Concentration of S-Adenosylhomocysteine and Inhibition of Vaccinia Virus Replication and Inhibition of Murine L929 Cell Growth. *Antimicrob. Agents Chemother.* **1989**, *33*, 828–834.

(9) Guranowski, A.; Montgomery, J. A.; Cantoni, G. L.; Chiang, P. K. Adenosine Analogues as Substrates and Inhibitors of S-Adenosylhomocysteine Hydrolase. *Biochemistry* **1981**, *20*, 110–115.

(10) Kim, I.-K.; Zhang, C.-Y.; Chiang, P. K.; Cantoni, G. L. S-Adenosylhomocysteine Hydrolase from Hamster Liver: Purification and Kinetic Properties. *Arch. Biochem. Biophys.* **1983**, *226*, 65–72.

(11) Borchardt, R. T.; Keller, B. T.; Patel-Thrombre, U. Neplanocin A: A Potential Inhibitor of S-Adenosylhomocysteine Hydrolase and of Vaccinia Virus Multiplication in Mouse L929 Cells. *J. Biol. Chem.* **1984**, *259*, 4353–4358.

(12) Hill, D. L.; Straight, S.; Allan, P. W.; Bennett, L. L., Jr. Inhibition of Guanine Metabolism of Mammalian Tumor Cells by the Carbocyclic Analogue of Adenosine. *Mol. Pharmacol.* **1971**, *7*, 375–380.

(13) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. Studies on Neplanocin A, New Antitumor Antibiotic. I. Producing Organism, Isolation and Characterization. *J. Antibiot. (Tokyo)* **1981**, *34*, 359–366.

(14) De Clercq, E. Antiviral and Antimetabolic Activities of the Neplanocins. *Antimicrob. Agents Chemother.* **1985**, *28*, 84–89.

(15) Bennett, L. L., Jr.; Bowdon, B. J.; Allan, P. W.; Rose, L. M. Evidence that the Carbocyclic Analog of Adenosine Has Different Mechanisms of Cytotoxicity to Cells with Adenosine Kinase Activity and to Cells Lacking This Enzyme. *Biochem. Pharmacol.* **1986**, *35*, 4106–4109.

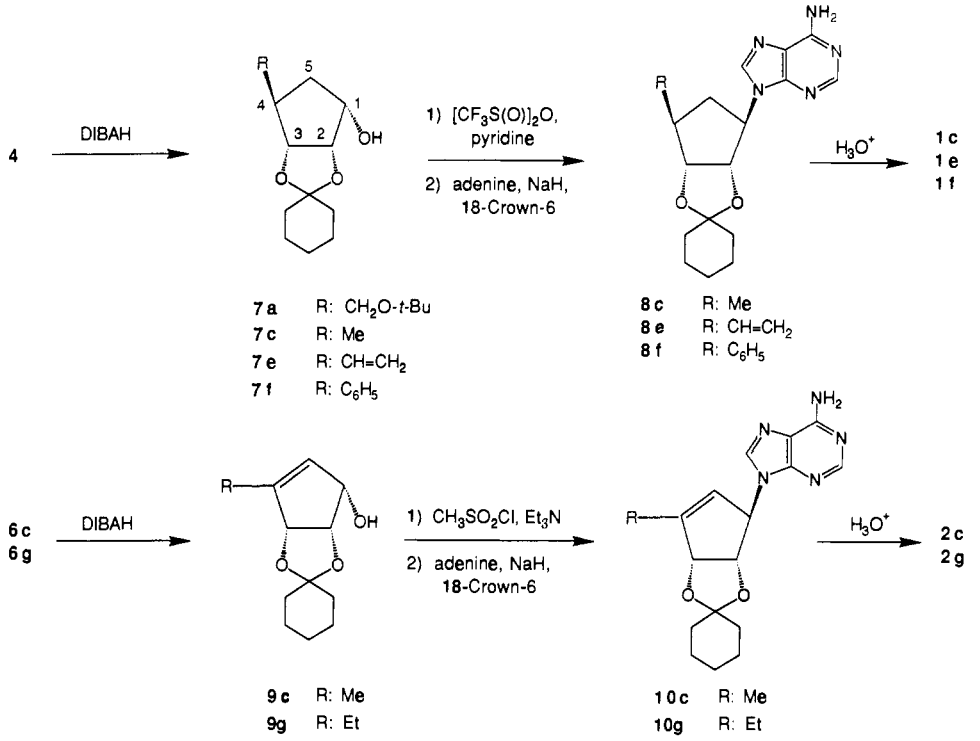
(16) Inaba, M.; Nagashima, K.; Tsukagoshi, S.; Sakurai, Y. Biochemical Mode of Cytotoxic Action of Neplanocin A in L1210 Leukemic Cells. *Cancer Res.* **1986**, *46*, 1063–1067.

(17) Borcherding, D. R.; Scholtz, S. A.; Borchardt, R. T. Synthesis of Analogues of Neplanocin A: Utilization of Optically Active Dihydroxycyclopentenones Derived from Carbohydrates. *J. Org. Chem.* **1987**, *52*, 5457.

(18) Narayanan, S. R.; Keller, B. T.; Borcherding, D. R.; Scholtz, S. A.; Borchardt, R. T. 9-(*trans*-2',*trans*-3'-Dihydroxycyclopent-4'-enyl) Derivatives of Adenosine and 3-Deazaadenosine: Potent Inhibitors of Bovine Liver S-Adenosylhomocysteine Hydrolase. *J. Med. Chem.* **1988**, *31*, 500–506.

(19) Hasobe, M.; McKee, J. G.; Borcherding, D. R.; Borchardt, R. T. 9-(*trans*-2',*trans*-3'-Dihydroxycyclopent-4'-enyl)-Adenine and 3-Deazaadenine: Analogs of Neplanocin A Which Retain Potent Antiviral Activity but Reduced Cytotoxicity. *Antimicrob. Agents Chemother.* **1987**, *31*, 1849–1851.

Scheme II. Synthesis of 4′-Modified Analogues of Aristeromycin and Neplanocin A from Intermediate Ketones



compared to the parent compound was markedly improved since removal of the 5′-hydroxyl group not only eliminated adenosine kinase substrate activity but also prevented transformation by adenosine deaminase.[21] Impressed by these results, we designed 4′-modified Ari analogues **1b–g** and NpcA analogues **2c** and **2g** in attempts to improve AdoHcy hydrolase affinity. We report here the synthesis of these analogues as well as their inhibitory activity on AdoHcy hydrolase.

Chemistry

Our general synthetic strategy toward 4′-modified analogues of Ari and NpcA is presented in Scheme I. We have previously employed this same basic strategy toward the total syntheses of Ari[22] and NpcA[23] themselves. The key steps in the synthesis are those involving organocuprate reagents and intermediate cyclopentenone **3**. Quenching this type of reaction with a proton source yields 3-substituted cyclopentanones that can be carried on to 4′-modified Ari analogues. Alternatively, quenching with methanesulfinyl chloride followed by pyrolytic syn elimination affords 3-substituted cyclopentenones that can likewise be transformed into 4′-modified NpcA analogues. Cyclopentenone **3** in turn can be formed from several members of the natural chiral pool: D-ribonic acid γ-lactone,[17,24] D-gulonic acid γ-lactone,[23] or D-ribose.[25]

Specifically, treatment of **3** with lithium dimethylcuprate followed by acidic quenching resulted in a 67% yield of conjugate addition product **4c**. Likewise, **4e** and **4f** were obtained using appropriate organocuprate reagents. The stereochemical character of these intermediates is implied by analogy with an Ari intermediate (**4a**) formed in a similar manner. In the case of this Ari intermediate, we have conclusively proven that the organocuprate reagent employed added to the less hindered face of **3** as might be expected.[23] Evidence included the very small proton NMR coupling constant between H_3 and H_4 (1 Hz), which was consistent with expectations drawn from molecular models and the Karplus equation, and the fact that this intermediate was successfully converted to Ari. No coupling between H_3 and H_4 was observed in the proton NMR spectra of **4c**, **4e**, and **4f**, suggesting that these conjugate addition products possessed the same stereochemical character as that of **4a**. Further evidence concerning the stereochemistry of **4c** is provided by chemical and spectroscopic data on final products **1c**, **1d**, and **2c** (vide infra).

These ketones were then reduced with diisobutylaluminum hydride (DIBAH; Scheme II) to give diastereomerically pure alcohols **7**. Again, we have conclusively

(20) The potent AdoHcy hydrolase inhibitor 3-deazaNpcA was designed using similar reasoning, and the biological profile of this compound resembles that of **2b**. See: (a) Glazer, R. I.; Knode, M. C.; Tseng, C. K. H.; Haines, D. R.; Marquez, V. E. 3-Deazaneplanocin A: A New Inhibitor of *S*-Adenosylhomocysteine Synthesis and Its Effects in Human Colon Carcinoma Cells. *Biochem. Pharmacol.* **1986**, *35*, 4523–4527 and (b) Tseng, C. K. H.; Marquez, V. E.; Fuller, R. W.; Goldstein, B. M.; Haines, D. R.; McPherson, H.; Parsons, J. L.; Shannon, W. M.; Arnett, G.; Hollingshead, M.; Driscoll, J. S. Synthesis of 3-Deazaneplanocin A, a Powerful Inhibitor of *S*-Adenosylhomocysteine Hydrolase with Potent and Selective In Vitro and In Vivo Antiviral Activities. *J. Med. Chem.* **1989**, *32*, 1442–1446.

(21) Hasobe, M.; McKee, J. G.; Borcherding, D. R.; Keller, B. T.; Borchardt, R. T. Effects of 9-(*trans*-2′,*trans*-3′-Dihydroxycyclopent-4′-enyl)-Adenine and 3-Deazaadenine on the Metabolism of *S*-Adenosylhomocysteine in Mouse L929 Cells. *Mol. Pharmacol.* **1988**, *33*, 713–720.

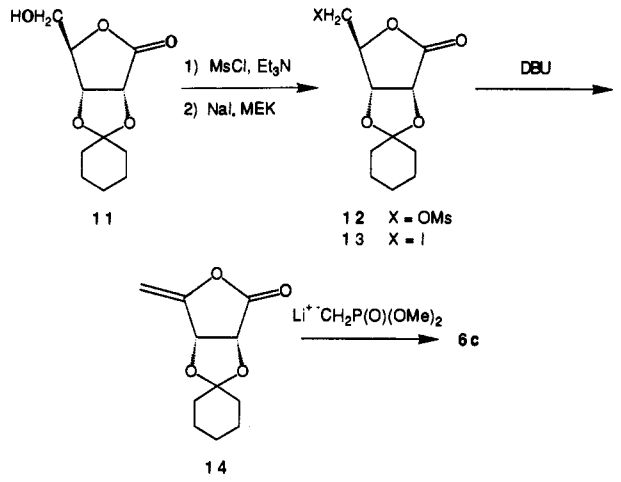
(22) Wolfe, M. S.; Borcherding, D. R.; Borchardt, R. T. A 9-Step Enantiospecific Synthesis of (−)-Aristeromycin from D-Ribonic Acid γ-Lactone. *Tetrahedron Lett.* **1989**, *30*, 1453–1456.

(23) Wolfe, M. S.; Anderson, B. A.; Borcherding, D. R.; Borchardt, R. T. Enantiospecific Syntheses of Aristeromycin and Neplanocin A. *J. Org. Chem.* **1990**, *55*, 4712–4717.

(24) The yield reported in ref 17 for the cyclization reaction leading to cyclopentenone **3** (80%) has not been reproduced either in our laboratory or in a number of other laboratories around the country. Yields for this reaction have been 35–50%, and we cannot account for this discrepancy at present. In our laboratory, better yields have been achieved with a slightly different starting material (see ref 25).

(25) Ali, S. M.; Ramesh, K.; Borchardt, R. T. Efficient Enantioselective Syntheses of Carbocyclic Nucleoside and Prostaglandin Synthons. *Tetrahedron Lett.* **1990**, *31*, 1509–1512.

Scheme III. Alternate Synthesis of **6c**

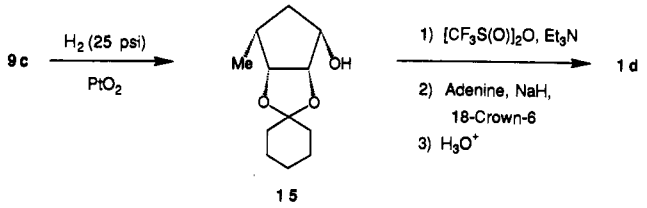


shown that DIBAH reduction of **4a** leads to **7a** with the depicted stereochemistry via proton NMR coupling constants and NOE experiments,[23] and alcohols **7c**, **7e**, and **7f** are believed to possess the same stereochemistry by analogy. Furthermore, NOE experiments on **7f** were consistent with this assignment: preirradiation of all protons believed to be on the same face as the phenyl group (H_1, H_2, H_3, $H_{5\beta}$) led to enhancement of the ortho phenyl protons (0.85%, 0.62%, 2.28%, and 2.05%, respectively), while preirradiation of $H_{5\alpha}$ resulted in no detectable enhancement. Treatment of cyclopentanols **7** with trifluoromethanesulfonic (triflic) anhydride and pyridine followed by displacement of the secondary triflate with sodium adenide in the presence of 18-crown-6 gave protected nucleosides **8** in low to moderate overall yields. Deprotection with dilute HCl then afforded the 4'-modified Ari analogues **1c**, **1e**, and **1f**. Ari analogues **1b** and **1g** were obtained by hydrogenation of **2b** and **1e**, respectively, in the presence of Adam's catalyst.

The reaction of **3** with dimethylcuprate was also quenched with methanesulfinyl chloride (Scheme I),[26] resulting in a 59% yield of β-keto sulfoxide **5c** as a mixture of four diastereomers due to variable chirality at the sulfur atom and at the adjacent α-carbon atom. Pyrolytic syn elimination was induced by refluxing **5c** in toluene along with calcium carbonate to afford cyclopentenone **6c** in 78% yield. The corresponding ethyl-substituted intermediate **6g** was formed in a like manner in 43% overall yield from **3**.

Cyclopentenone intermediate **6c** was also obtained by an alternative method depicted in Scheme III. Protected ribonolactone **11** was first mesylated and displaced with iodide to give the 5'-iodo sugar **13**. After elimination with DBU, the resultant enol lactone **14**[27] was treated with the anion of dimethyl methylphosphonate,[28] and the intramolecular Wittig-type reaction that ensues led to the formation of **6c**. Ketones **6c** and **6g** were then reduced with diisobutylaluminum hydride (Scheme II) to give their corresponding allylic alcohols **9c** and **9g**. These alcohols were then converted to their methanesulfonates (mesylates), which were subsequently displaced with adenide anion and deprotected to give **2c** and **2g**.

Scheme IV. Synthesis of 4'-α-Aristeromycin Analogue **1d**



Toward the synthesis of analogue **1d** (Scheme IV), cyclopentenol **9c** was hydrogenated with Adam's catalyst, affording diastereomerically pure cyclopentanol **15**. This alcohol was then converted to **1d** in the same manner described for the other Ari analogues (i.e., triflation, adenide displacement, and deprotection). Proof for the stereochemical assignments for Ari analogues **1c** and **1d** comes from the catalytic hydrogenation of NpcA analogue **2c**. This reaction produces a 2:3 mixture of **1c** and **1d**, providing conclusive evidence that these two final products are 4'-epimers of one another. Since the catalytic hydrogenation of **9c** could hardly be expected to exclusively result in **4c** (due to the high degree of steric hindrance under the ring), the assignments of **1c** and **1d** are consistent with chemical intuition as well as with the spectroscopic evidence.

Discussion

The NpcA analogue **2b**, lacking the natural product's 4'-hydroxymethyl substituent, has significantly reduced cytotoxicity compared to the parent compound but retains NpcA's broad-spectrum antiviral activity.[18-20] However, the AdoHcy hydrolase inhibitory activity of **2b** is significantly lower than NpcA itself. In order to improve on the inhibitory potency of **2b** toward AdoHcy hydrolase and still maintain selectivity, we designed a number of carbocyclic adenosine compounds modified in the 4'-position. Since Ari and NpcA possess similar structural features and display remarkably parallel biological profiles,[29] we decided to explore 4'-modifications of Ari as well. To this end, we designed and synthesized **1b** (the saturated counterpart of **2b**) along with several 4'-substituted variations on **1b**. One of the reasons for choosing these particular 4'-substituents (Figure 1) was to explore the possibility of the existence of a hydrophobic pocket near the ribose-binding portion of AdoHcy hydrolase's active site; therefore, we have focussed on hydrophobic substituents. Second, the 5'-hydroxyl group is necessary for substrate activity at both adenosine kinase and adenosine deaminase;[30] hence, these analogues should be incapable of forming nucleotide or inosine analogues. Such transformations might otherwise lead to biological inactivation or augmented cytotoxicity. Third, we thought some of these analogues (**1e** and **1f**) might inactivate AdoHcy hydrolyase by taking advantage of its mechanism of action.[31] We have previously deter-

(26) Fujisawa, T.; Noda, A.; Kawara, T.; Sato, T. A Simple Synthesis of α,β-Unsaturated Carbonyl Compounds by Trapping the Michael Addition-Enolate Anion with Methanesulfinyl Chloride. *Chem. Lett.* **1981**, 1159–1160.

(27) After this work was completed, a nearly identical synthesis of an analogue of **14** (isopropylidene protecting group instead of cyclohexylidene) was reported: Belanger, P. and Prasit, P. Carbocycles from Carbohydrates: A Simple Route to an Enantiomerically Pure Prostaglandin Intermediate. *Tetrahedron Lett.* **1988**, *29*, 5521–5524.

(28) The procedure which gave the best results was that reported by Aristoff, P. A.; Johnson, P. D.; Harrison, A. W. Total Synthesis of a Novel Antiulcer Agent Via a Modification of the Intramolecular Wadsworth–Emmons–Wittig Reaction. *J. Am. Chem. Soc.* **1985**, *107*, 7967–7974.

(29) Wolfe, M. S.; Borchardt, R. T. *S*-Adenosyl-L-homocysteine Hydrolase as a Target for Antiviral Chemotherapy. *J. Med. Chem.* **1991**, *34*, 1521–1530.

(30) Bloch, A.; Robins, M. J.; McCarthy, J. R. The Role of the 5'-Hydroxyl Group of Adenosine in Determining Substrate Specificity for Adenosine Deaminase. *J. Med. Chem.* **1967**, *10*, 908–912.

Table I. Inhibitory Activity of 4′-Modified Aristeromycin and Neplanocin A Analogues Versus Bovine Liver S-Adenosyl-L-homocysteine Hydrolase

compd	K_I (nM)	k_2 (min^{-1})	k_2/K_I (nM^{-1} min^{-1})
1a	[a]	[a]	[a]
1b	12	0.109	9.1×10^{-3}
1c	27	0.039	1.4×10^{-3}
1d	5020	0.025	5.0×10^{-6}
1e	22	0.053	2.4×10^{-3}
1f	67600	0.010	1.5×10^{-7}
1g	586	0.083	1.4×10^{-4}
2a	3.8[b]	1.98[b]	5.2×10^{-1}
2b	49	0.043	8.8×10^{-4}
2c	9150	0.090	9.8×10^{-6}
2g	[c]	[c]	[c]

[a] Under the assay conditions employed, Ari (1a) is a reversible inhibitor, consistent with previous reports (see refs 9 and 10). [b] The rate of inactivation by NpcA (2a) under the assay conditions employed for the other analogues was too rapid for accurate values to be obtained; the values above for NpcA are taken from a previous report from our laboratory using different assay conditions (see ref 18). [c] Inactive up to 100 μM.

mined that NpcA and **1b** inactivate AdoHcy hydrolase by a cofactor depletion mechanism.[32,33] These two carbocyclic nucleosides are oxidized in the 3′-position by the tightly bound NAD^+ cofactor of AdoHcy hydrolase, and their 3′-oxidized counterparts have a high affinity for the inactive NADH form of this enzyme that results from this transformation. All of the analogues designed might also be oxidized in their 3′-positions (as is the parent compound, Ari[9]) and inactivate the enzyme, but **1e** and **1f** might go one step further. The normal purpose of this oxidation with the natural substrates AdoHcy or adenosine is to increase the acidity of the 4′-proton, and this in turn facilitates the elimination of homocysteine or water (from AdoHcy or adenosine, respectively). If **1e** or **1f** were likewise oxidized in the enzyme's active site, the vinyl and phenyl groups would increase the acidity of the 4′-hydrogen even more. The resultant shift in the equilibrium of the reaction toward enolate ion formation might then reduce the rate of back-reduction by the enzyme-bound NADH. Finally, we hoped to utilize the AdoHcy hydrolase inhibition data of these analogues to test the accuracy of a computer model of AdoHcy hydrolase generated in our laboratory[34] by computational mutation of lactate dehydrogenase, a functionally related (i.e., NAD^+-binding) protein of known structure.

The synthetic means employed to obtain these analogues appears to be a useful general method for reaching a wide variety of 4′-modified Ari and NpcA compounds. The high degree of stereoselectivity of the organocuprate conjugate addition reactions allows for easy access to 4′-β-Ari analogues. Alternatively, the enolate ions formed in these organocuprate reactions may be trapped with methanesulfinyl chloride, and subsequent pyrolytic syn elimination of the resultant sulfoxides reforms the double bond affording NpcA-intermediate cyclopentenones. Such cyclopentenones can be reduced with DIBAH and then further reduced under catalytic hydrogenation conditions to yield exclusively 4-α-substituted cyclopentanols that can then be elaborated to their corresponding Ari analogues. Hence, the orientation of the 4′-substituent of carbocyclic nucleosides can be easily controlled: the substituent may be α, β, or in the plane of the carbocycle.

Table I lists the K_I, k_2, and k_2/K_I, values for these analogues versus AdoHcy hydrolase. All were time-dependent inactivators (suggesting that 3′-oxidation takes place) with the most potent compounds (**1b**, **1c**, and **1e**) inhibiting the enzyme in the nanomolar range. In fact, these three compounds are better inactivators than the standard analogue **2b**. As mentioned above, both **1e** and **1f** were designed to take advantage of the enzyme's mechanism of action. The spatial constraints in the active site apparently do not allow **1f** to bind due to its large phenyl substituent, but the smaller vinyl group of **1e** is more readily accommodated. That the double bond of the vinyl substituent contributes to the excellent ability of this compound to inactivate AdoHcy hydrolase is illustrated by comparing its k_2/K_I to that of its saturated counterpart **1g**, but further studies must be performed to determine if 4′-proton removal does in fact play an important role in the inactivation of AdoHcy hydrolase by this analogue. The enzymological data on the 4′-methyl-substituted analogues (**1c**, **1d**, and **2c**) point out the importance of substituent orientation with respect to the carbocyclic ring. A β-methyl group is easily accommodated, but an α-methyl group or methyl group in the plane of the carbocycle is not tolerated very well in comparison to their unsubstituted counterparts **1b** and **2b**. The relative inactivity of **2c** and **2g** is particularly interesting in light of the fact that NpcA and **2b** are both potent inhibitors of AdoHcy hydrolase. It is unclear at this point why the methyl and ethyl groups are not tolerated while the hydroxymethyl substituent of NpcA and the hydrogen atom of **2b** are quite acceptable to the enzyme, but our computer model indicates that the 4′-hydroxymethyl group of Ari and NpcA can interact with the NH_3^+ group of a lysine residue in the active site via hydrogen bonding.[34] This interaction may not only contribute to the binding of Ari and NpcA but may also explain the reduced affinity of **2c** and **2g** for the enzyme (i.e., the lipophilic methyl and ethyl groups in the plane of the carbocyclic ring may be oriented too closely to this lysine NH_3^+ group).

As mentioned above, the enzymological data on these compounds were used to test the accuracy of our computer model of rat liver AdoHcy hydrolase. The ability of the inhibitors to bind in the model AdoHcy hydrolase–NAD^+ complex is consistent with their inhibitory effects in vitro. In fact, a linear relationship was found between the relative binding energies of the inhibitors and their log K_I values.[34] This computer model showed retrospectively that it could rationalize the inhibitory activity of four of the Ari and NpcA analogues (**1b**, **1c**, **2b**, **2c**).[34] The model also predicted beforehand the K_I values for **1d** and **1e** fairly accurately,[34] and it is our hope that in the future this computer model will assist us in the design of new and more potent inhibitors of AdoHcy hydrolase.

Currently, we are determining the antiviral activity as well as the cytotoxicity of the analogues reported here. This information, along with measurements of AdoHcy levels in cells treated with these compounds, should help test our hypothesis that AdoHcy levels that prevent viral replication but that are not detrimental to the cell can be attained.[29] With the guidance of our computer model, we

(31) Palmer, J. L.; Abeles, R. H. The Mechanism of Action of S-Adenosylhomocysteinase. *J. Biol. Chem.* **1979**, *254*, 1217–1226.
(32) Paisley, S. D.; Wolfe, M. S.; Borchardt, R. T. Oxidation of Neplanocin A to the Corresponding 3′-Keto-Cyclopentenyl Derivative by Adenosylhomocysteine Hydrolase. *J. Med. Chem.* **1989**, *32*, 1415–1418.
(33) Paisley, S. D.; Hasobe, M.; Borchardt, R. T. Elucidation of the Mechanism By Which 9-(*trans*-2′,*trans*-3′-Dihydroxycyclopent-4′-enyl)-Adenine Inactivates S-Adenosyl-L-homocysteine Hydrolase and Elevates Cellular Levels of S-Adenosylhomocysteine. *Nucleosides Nucleotides* **1989**, *8*, 689–698.
(34) Yeh, J. C.; Borchardt, R. T.; Vedani, A. A Molecular Model for the Active Site of S-Adenosyl-L-homocysteine Hydrolase. *J. Comput.-Aided Mol. Des.* **1991**, *5*, 213–234.

are also working on the design and synthesis of analogues with more hydrophilic 4'-substituents in order to potentially maximize hydrogen bonding interactions and increase the overall affinity for AdoHcy hydrolase.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 1420 spectrophotometer. NMR spectra were obtained on either a Varian XL-300 or a Bruker AM-500 spectrophotometer. All ^{1}H chemical shifts are reported in δ relative to internal standard tetramethylsilane (TMS, δ 0.00). ^{13}C chemical shifts are reported in δ relative to $CDCl_3$ (center of triplet, δ 77.0) or relative to DMSO-d_6 (center of septet, δ 39.5). Mass spectra were recorded on a Ribermag R10-10 quadrupole spectrometer. All samples were ionized by electron impact at 70 eV. Optical rotations were determined using the sodium-D line on a Perkin-Elmer Model 241 polarimeter. Elemental analyses were conducted either at the University of Kansas or by Desert Analytics, Phoenix, AZ. The purity of compounds **1d**, **1e**, and **2c** was established by HPLC using a C-18 reverse-phase column (Econosphere, Alltech, 25 cm × 4.6 mm) in two mobile phase systems. Method A: flow rate of 1.0 mL/min; solvent A, acetonitrile; solvent B, 50 mM sodium phosphate (pH 3.2) containing 10 mM heptanesulfonic acid; program, 10–30% B for 10 min, 50% B for 5 min, and 10% B for 15 min. Method B: flow rate of 1.0 mL/min; solvent A, water; solvent B, acetonitrile; program, 10–50% B for 10 min, 50–90% B for 5 min, 90% B for 5 min, and 100% B for 10 min. Monitoring was done at 254 nm. Silica gel chromatography was accomplished with 70–230 mesh, 60-Å silica gel (Aldrich Chemical Co.). Ion-exchange chromatography was carried out with Dowex 50W (H^+), dry mesh 100–200, 4% cross-linked (Sigma Chemical Co.). All reactions were run under argon atmosphere except where water was used as solvent.

(2*R*,3*R*,4*S*)-2,3-(Cyclohexylidenedioxy)-4-methylcyclopentanone (4c). To a −40 °C suspension of copper(I) iodide (Aldrich Gold Label, 3.43 g, 18 mmol) in 50 mL of ether was added methyllithium (1.2 M in ether) dropwise over the course of 45 min, until the initially formed yellow precipitate had dissolved (25 mL, 30 mmol). The reaction mixture was then warmed to 0 °C, whereupon a solution of **3** (650 mg, 3.6 mmol) in 10 mL of THF/ether (3:2) was added dropwise over 5–10 min. The reaction mixture was then allowed to warm to ambient temperature. After 1 h (including warming time), the mixture was recooled to 0 °C, and 25 mL of 15% acetic acid was added (slowly at first). This mixture was then washed with 50 mL of saturated NH_4Cl solution, 2 × 75 mL of dilute NH_4OH solution, and 50 mL of H_2O. The organic phase was dried over Na_2SO_4, filtered, and concentrated in vacuo, and the residue was passed through silica gel (15 g, ether/hexane, 1:1) to obtain 470 mg (67%) of **4c** as white crystals: mp 30–32 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.03 (d, J = 7 Hz, 3 H), 1.20–1.80 (m, 10 H), 1.96 (d, J = 18 Hz, 1 H), 2.55 (d, J = 7 Hz, 1 H), 2.82 (dd, J = 18, 8 Hz, 1 H), 4.23 (d, J = 5 Hz, 1 H), 4.49 (d, J = 5 Hz, 1 H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 19.1, 23.6, 23.8, 24.9, 31.1, 34.2, 36.5, 41.4, 77.7, 82.6, 112.8, 214.4; IR (neat) 2920, 2850, 1750, 1445, 1400, 1380, 1365, 1330, 1275, 1245, 1230, 1160, 1140, 1110, 1085, 1070, 1035, 960, 940, 925, 905, 845, 825 cm^{-1}; MS (EI) m/e 211 (M + 1), 210 (M^+), 181, 167, 140, 125, 99, 95, 81, 69, 55, 42; $[\alpha]_D$ = −20° (c = 0.705, $CHCl_3$). Anal. ($C_{12}H_{18}O_3$) C, H.

(1*S*,2*S*,3*R*,4*S*)-2,3-(Cyclohexylidenedioxy)-4-methylcyclopentanol (7c). To a −78 °C solution of 681 mg (3.24 mmol) of **4c** in 60 mL of CH_2Cl_2 (stored over 3Å molecular sieves) was added 4.85 mL of 1 M diisobutylaluminum hydride in CH_2Cl_2 (4.85 mmol) over 2 min. After 3.5 h, MeOH (8 mL) was added (dropwise at first), and the reaction mixture was allowed to warm to ambient temperature. Water (15 mL) was then added, and after several minutes, the colloidal suspension was suction filtered, washing with copious quantities of CH_2Cl_2 and H_2O. After removal of the CH_2Cl_2 layer, the aqueous phase was extracted with 2 × 150 mL of additional CH_2Cl_2. The combined organic phases were washed with 200 mL of H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was passed through silica gel (35 g, Et_2O/hexane, 1:1) to obtain 655 mg (95%) of **7c** as a clear, colorless oil: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.93 (dd, J = 3, 8 Hz, 3 H), 1.30–1.75 (m, 11 H), 1.85 (m, 1 H), 2.15 (m, 1 H), 2.50 (d, J = 9 Hz, 1 H, OH), 4.10 (m, 1 H), 4.25 (d, J = 6 Hz, 1 H), 4.50 (dd, J = 6, 6 Hz, 1 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 17.6, 23.5, 23.9, 25.1, 33.7, 35.4, 35.8, 38.2, 71.3, 78.5, 85.6, 111.9; IR (neat) 3480, 2930, 2860, 1450, 1405, 1370, 1285, 1165, 1145, 1105, 1035, 960, 950, 925, 910, 850 cm^{-1}; MS (EI) m/e 212 (M^+), 183, 169, 97, 81, 69, 55, 41; $[\alpha]_D$ = −23° (c = 0.340, $CHCl_3$). Anal. ($C_{12}H_{20}O_3$) C, H.

(1'*R*,2'*S*,3'*R*,4'*S*)-9-[2',3'-(Cyclohexylidenedioxy)-4'-methylcyclopentan-1'-yl]adenine (8c). To a 0 °C solution of **7c** (498 mg, 2.35 mmol) and pyridine (0.210 mL, 204 mg, 2.58 mmol) in 15 mL of CH_2Cl_2 was added trifluoromethanesulfonic anhydride (0.395 mL, 663 mg, 2.35 mmol) dropwise over 8 min. After 40 min, the reaction was quenched with 7 mL of cold H_2O. The mixture was poured into a separatory funnel with an additional 15 mL of CH_2Cl_2. The organic phase was then washed with 2 × 25 mL of cold H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain 880 mg of crude triflate. A suspension of adenine (954 mg, 7.05 mmol), NaH (60% dispersion in mineral oil, 280 mg, 7.0 mmol), and 18-crown-6 (308 mg, 1.17 mmol) in 20 mL of DMF was heated to 70 °C for 4 h and then cooled to room temperature. To this suspension was added the triflate in 5 mL of DMF, and the reaction mixture was allowed to stir overnight. The mixture was suction filtered and extracted between CH_2Cl_2 (75 mL) and saturated NaCl solution (2 × 25 mL). After drying over Na_2SO_4, the organic phase was filtered and concentrated in vacuo. The residue was then passed through silica gel (50 g, CH_2Cl_2/EtOH, 9:1), and then developed on preparative TLC plates (20 cm × 20 cm × 200 μm, EtOAc/hexane, 2:1, developed several times) to obtain 135 mg (17%) of **8c**: mp 80–83 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.25 (d, J = 6 Hz, 3 H), 1.35–1.85 (m, 10 H), 2.15–2.60 (m, 3 H), 4.45 (dd, J = 5, 7 Hz, 1 H), 4.70 (m, 1 H), 5.10 (dd, J = 5, 7 Hz, 1 H), 6.00 (s, 2 H, NH_2), 7.85 (s, 1 H), 8.35 (s, 1 H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 18.0, 23.4, 24.0, 25.0, 34.6, 37.4, 38.8, 38.9, 62.1, 83.3, 85.4, 114.5, 120.5, 139.9, 150.0, 152.7, 155.6; IR (KBr) 3320, 3160, 2925, 2860, 1640, 1595, 1570, 1470, 1415, 1370, 1330, 1300, 1250, 1160, 1090, 1070, 1055, 940, 925, 910, 850, 795, 725, 645 cm^{-1}; MS (EI) m/e 330 (M + 1), 300, 286, 231, 216, 162, 136, 108, 79, 67, 55, 41; $[\alpha]_D$ = −50° (c = 0.402, $CHCl_3$). Anal. ($C_{17}H_{23}N_5O_2$) C, H, N.

(1'*R*,2'*S*,3'*R*,4'*S*)-9-(2',3'-Dihydroxy-4'-methylcyclopentan-1'-yl)adenine (1c). An amount of 128 mg (0.39 mmol) of **8c** was dissolved in 20 mL of 0.3 N HCl. After 18 h, the solvent was removed under reduced pressure, and the residue was passed through Dowex 50W (H^+), first eluting with water and then with dilute NH_4OH to obtain 92 mg (95%) of **1c** as a white solid: mp 172–174 °C; ^{1}H-NMR (300 MHz, DMSO-d_6 + D_2O) δ 1.10 (d, J = 7 Hz, 3 H), 1.60 (m, 1 H), 1.95 (m, 1 H), 2.25 (m, 1 H), 3.60 (dd, J = 5, 6 Hz, 1 H), 4.35 (dd, J = 6, 8 Hz, 1 H), 4.65 (m, 1 H), 8.15 (s, 1 H), 8.25 (s, 1 H); ^{13}C-NMR (75 MHz, DMSO-d_6 + D_2O) δ 19.4, 34.7, 37.8, 60.7, 74.7, 76.5, 119.4, 140.9, 149.9, 152.6, 156.1; IR (KBr) 3330, 3180, 2900, 1660, 1605, 1570, 1485, 1450, 1430, 1335, 1315, 1260, 1220, 1135, 1110, 1080, 830, 795, 720, 660, 630 cm^{-1}; MS (EI) m/e calcd for $C_{11}H_{15}N_5O_2$ 249.1226, found 249.1220; 250 (M + 1), 249 (M^+), 190, 178, 162, 136, 135, 108, 55, 41; $[\alpha]_D$ = −36° (c = 0.994, 0.3 N HCl). Anal. ($C_{11}H_{15}N_5O_2$) C, H, N.

(2*R*,3*R*,4*R*)-2,3-(Cyclohexylidenedioxy)-4-vinylcyclopentanone (4e). To a −5 °C suspension of copper(I) iodide (785 mg, 4.15 mmol) in 25 mL of THF was added 8.25 mL of 1 M vinylmagnesium bromide in THF (8.25 mmol) over 1.5–2.0 min. The suspension was kept at −5 °C for 3 min including addition time, and then cooled to −78 °C. The enone **3** (500 mg, 2.60 mmol) in 10 mL of THF was added dropwise to the reaction mixture. After 1 h, the dry ice/acetone bath was replaced with an ice bath, and the reaction mixture was allowed to come to 0 °C. After 15 min at this temperature, the reaction was quenched with 50 mL of 15% acetic acid (degassed). Workup was the same as for **4c** to obtain 560 mg (98%) of **4e** as a clear, colorless oil: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.30–1.70 (m, 10 H), 2.30 (d, J = 18 Hz, 1 H), 2.85 (dd, J = 18, 9 Hz, 1 H), 3.15 (m, 1 H), 4.20 (d, J = 5 Hz, 1 H), 4.60 (d, J = 5 Hz, 1 H), 5.10 (m, 2 H), 5.85 (m, 1 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 23.6, 23.8, 24.9, 34.3, 36.5, 38.5, 39.7, 77.4, 80.9, 113.0, 116.3, 137.2, 213.4; IR (neat) 2930, 2850, 1755, 1640, 1445, 1430, 1400, 1365, 1335, 1290, 1270, 1250, 1230, 1160, 1105, 1070, 1035, 995, 960, 925, 845, 830, 775 cm^{-1}; MS (EI) m/e 222 (M^+), 193, 179, 140, 107, 97, 81, 67, 55, 41; $[\alpha]_D$ = −192° (c = 0.391,

$CHCl_3$). Anal. ($C_{13}H_{18}O_3$) C, H.

(1*S*,2*S*,3*R*,4*R*)-2,3-(Cyclohexylidenedioxy)-4-vinylcyclopentanol (7e). The same experimental procedure employed in the formation of **7c** was used with 633 mg (2.85 mmol) of **4e** to obtain, after silica gel chromatography, 567 mg (89%) of **7e** as a clear, colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.30–1.75 (m, 10 H), 1.90 (m, 2 H), 2.50 (d, J = 7.5 Hz, 1 H, OH), 2.75 (m, 1 H), 4.05 (m, 1 H), 4.50 (m, 2 H), 5.10 (m, 2 H), 5.75 (m, 1 H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 23.7, 24.1, 25.2, 33.8, 35.9, 36.0, 44.5, 71.0, 78.5, 83.8, 112.2, 115.2, 138.1; IR (neat) 3500 (br), 2930, 2850, 1640, 1450, 1370, 1285, 1165, 1090, 1035, 995, 950, 930, 915, 850, 835 cm^{-1}; MS (EI) m/e 224 (M^+), 195, 181, 109, 91, 81, 67, 55; $[\alpha]_D$ = −9° (c = 0.450, $CHCl_3$). Anal. ($C_{13}H_{20}O_3$) C, H.

(1′*R*,2′*S*,3′*R*,4′*R*)-9-[2′,3′-(Cyclohexylidenedioxy)-4′-vinylcyclopentan-1′-yl]adenosine (8e). The same experimental procedure employed in the synthesis of **8c** was applied to the synthesis of **8e** with 470 mg (2.10 mmol) of **7e**. Compound **8e** was obtained (200 mg; 28%) as a white, crystalline solid: mp 80–82 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.30–1.85 (m, 10 H), 2.45 (m, 2 H), 2.80 (m, 1 H), 4.65 (dd, J = 7, 7 Hz, 1 H), 4.80 (m, 1 H), 5.20 (m, 3 H), 5.95 (m, 1 H), 6.25 (br s, 2 H, NH_2), 7.85 (s, 1 H), 8.35 (s, 1 H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 23.4, 23.9, 25.0, 34.5, 36.5, 37.4, 48.1, 61.7, 82.9, 83.4, 114.6, 115.9, 120.4, 137.7, 139.8, 149.9, 152.7, 155.7; IR (KBr) 3320, 3160, 2925, 2855, 1640, 1595, 1570, 1470, 1415, 1370, 1330, 1300, 1250, 1160, 1110, 1090, 1070, 1055, 920, 850, 800, 725, 650 cm^{-1}; MS (EI) m/e 342 (M + 1), 341 (M^+), 312, 298, 243, 228, 190, 162, 136, 135, 108, 91, 77, 65, 55, 41; $[\alpha]_D$ = −4° (c = 0.220, $CHCl_3$). Anal. ($C_{18}H_{23}N_5O_2$) C, H, N.

(1′*R*,2′*S*,3′*R*,4′*R*)-9-[2′,3′-Dihydroxy-4′-vinylcyclopentan-1′-yl]adenine (1e). The same procedure employed to obtain **1c** was used with 204 mg (0.60 mmol) of **8e** to afford 140 mg (90%) of **1e** as a white powder: mp 210 °C dec; ^{1}H-NMR (300 MHz, DMSO-d_6 + D_2O) δ 1.92 (m, 1 H), 2.27 (m, 1 H), 2.58 (m, 1 H), 3.84 (dd, J = 6, 6 Hz, 1 H), 4.33 (dd, J = 6, 6 Hz, 1 H), 4.69 (m, 1 H), 5.10 (m, 2 H), 6.00 (m, 1 H), 8.15 (s, 1 H), 8.23 (s, 1 H); ^{13}C-NMR (75 MHz, DMSO-d_6 + D_2O) δ 31.6, 47.1, 60.0, 74.3, 74.7, 114.5, 119.3, 140.2, 140.3, 149.5, 152.1, 156.0; MS (EI) m/e calcd for $C_{12}H_{15}N_5O_2$ 261.226, found 261.1221; 261 (M^+), 244, 232, 202, 190, 162, 149, 136, 108, 78, 63, 41; $[\alpha]_D$ = +8° (c = 0.982, 0.3 N HCl); HPLC method A, R_f = 13.18 min; method B, R_f = 14.18 min.

(1′*R*,2′*S*,3′*R*,4′*S*)-9-[2′,3′-Dihydroxy-4′-ethylcyclopentan-1′-yl]adenine (1g). To a solution of **1e** (100 mg, 0.38 mmol) in 60 mL of MeOH was added 10 mg of PtO_2, and the suspension was placed under hydrogen (24 psi) for 15 h. The suspension was then filtered, and the filtrate was concentrated in vacuo to give 101 mg (100%) of **1g** as an off-white solid: mp 191–192 °C; ^{1}H-NMR (300 MHz, DMSO-d_6 + D_2O) δ 0.92 (t, J = 7 Hz, 3 H), 1.40 (m, 1 H), 1.50–1.85 (m, 3 H), 2.25 (m, 1 H), 3.70 (dd, J = 5, 5 Hz, 1 H), 4.35 (dd, J = 9, 6 Hz, 1 H), 4.65 (dd, J = 9, 18, 1 H), 8.10 (s, 1 H), 8.20 (s, 1 H); ^{13}C-NMR (75 MHz, DMSO-d_6 + D_2O) δ 12.5, 27.2, 32.4, 44.9, 60.1, 74.4, 74.5, 119.4, 140.1, 149.9, 152.4, 156.0; IR (KBr) 3330, 3180, 3090, 2960, 2920, 1655, 1605, 1570, 1485, 1450, 1420, 1335, 1315, 1255, 1215, 1120, 1105, 1080, 720, 655 cm^{-1}; MS (EI) m/e calcd for $C_{12}H_{17}N_5O_2$ 263.1382, found 263.1388; 263 (M^+), 234, 216, 190, 178, 162, 136, 78, 63, 45. Anal. ($C_{12}H_{17}N_5O_2 \cdot {}^1/_{10}\ H_2O$) C, H, N.

(2*R*,3*R*,4*R*)-2,3-(Cyclohexylidenedioxy)-4-phenylcyclopentanone (4f). Method A. To a well-stirred suspension of copper(I) iodide (2.95 g, 15.5 mmol, Aldrich Gold Label) in 75 mL of anhydrous ether cooled to −35 °C was added phenyllithium (15.5 mL, 2.0 M in cyclohexane/ether (2:1), 31.0 mmol) dropwise over 10 min. After 30 additional min, the reaction was allowed to warm to 0 °C, whereupon a solution of **3** (1.0 g, 5.15 mmol) in 10 mL of THF was added over several min. After an additional 1 h at 0 °C, the reaction was quenched with 25 mL of 15% acetic acid. The mixture was worked up as described for **4c**. The oily residue was passed through silica gel (50 g, hexane/ether, 2:1) to yield 972 mg (69%) of **10** as a pale yellow oil.

Method B. To a −5 °C suspension of copper(I) iodide (74 mg, 0.39 mmol) in 5 mL of THF was added phenylmagnesium bromide (260 μL, 0.78 mmol, 3 M in ether) over 1.5 min. After another 1.5 min, the reaction mixture was cooled to −78 °C, whereupon a solution of **3** (100 mg, 0.52 mmol) in 2 mL of THF was added dropwise over 5 min. The mixture was stirred at −78 °C for 1 h, then allowed to come to 0 °C. After 20 min, the mixture was quenched with 10 mL of 20% acetic acid and extracted between 75 mL of ether and 50 mL of 6% ammonium hydroxide. The aqueous layer was extracted with 2 × 50 mL of ether, and the combined organic phases were washed with 25 mL of saturated ammonium chloride solution, dried over Na_2SO_4, filtered, and concentrated in vacuo. The oily residue was passed through flash silica gel (35 g, hexane/ether, 4:1) to give 111 mg (79%) of **10** as a pale yellow oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.35–1.75 (m, 10 H), 2.55 (d, J = 20 Hz, 1 H), 3.10 (dd, J = 9, 19 Hz, 1 H), 3.70 (d, J = 8 Hz, 1 H), 4.35 (d, J = 5 Hz, 1 H), 4.70 (d, J = 5 Hz, 1 H), 7.10–7.40 (m, 5 H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 23.6, 23.9, 24.9, 34.3, 36.7, 40.5, 42.7, 77.8, 83.1, 113.2, 126.9, 127.2, 129.1, 141.3, 214.0; IR (neat) 3060, 3030, 2930, 2850, 1755, 1500, 1450, 1370, 1290, 1270, 1235, 1160, 1110, 1035, 985, 945, 930, 910, 850, 830, 770, 700 cm^{-1}; MS (EI) m/e 272 (M^+), 243, 229, 157, 145, 140, 131, 115, 104, 97, 91, 77, 69, 55, 41; $[\alpha]_D$ = −148° (c = 0.194, $CHCl_3$). Anal. ($C_{17}H_{20}O_3$) C, H.

(1*S*,2*S*,3*R*,4*R*)-2,3-(Cyclohexylidenedioxy)-4-phenylcyclopentanol (7f). The reaction was run the same way as described for the synthesis of **7c** using 900 mg (3.31 mmol) of **4f**. The crude product was purified by passage through a silica gel column (hexane/ether, 4:1 followed by 1:1), affording **7f** (670 mg; 74%) as a clear, colorless oil: ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.35–1.85 (m, 10 H), 2.05 (m, 1 H), 2.23 (m, 1 H), 2.67 (d, J = 6 Hz, 1 H, exchanges with D_2O), 3.43 (m, 1 H), 4.20 (m, 1 H), 4.60 (dd, J = 6, 6 Hz, 1 H), 4.70 (dd, J = 3, 6 Hz, 1 H), 7.15–7.40 (m, 5 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 23.5, 24.0, 25.1, 34.0, 36.0, 38.5, 47.1, 70.6, 79.4, 85.8, 113.3, 126.4, 127.2, 128.5, 141.9; IR (neat) 3500, 3050, 3020, 2930, 2850, 1495, 1445, 1370, 1285, 1165, 1100, 1040, 945, 930, 755, 700 cm^{-1}; MS (EI) m/e 274 (M^+), 245, 231, 159, 141, 131, 117, 104, 99, 91, 81, 77, 69, 55, 41; $[\alpha]_D$ = +26° (c = 0.239, $CHCl_3$). Anal. ($C_{17}H_{22}O_3$) C, H.

(1′*R*,2′*S*,3′*R*,4′*R*)-9-[2′,3′-(Cyclohexylidenedioxy)-4′-phenylcyclopentan-1-yl]adenine (8f). The reaction was carried out the same way as for the synthesis of **8c** with 516 mg (1.88 mmol) of **7f**. Compound **8f** (396 mg, 54%) was isolated as a white, crystalline solid: mp 235–237 °C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.35–1.85 (m, 10 H), 2.70 (m, 1 H), 2.90 (dd, J = 12, 25 Hz, 1 H), 3.35 (m, 1 H), 4.85 (m, 2 H), 5.20 (dd, J = 5, 7 Hz, 1 H), 5.60 (br s, 2 H), 7.27 (m, 2 H), 7.37 (m, 3 H), 7.87 (s, 1 H), 8.36 (s, 1 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 23.4, 24.0, 25.0, 34.6, 37.5, 37.7, 49.5, 62.0, 83.0, 85.2, 114.8, 120.4, 126.9, 127.1, 128.7, 140.0, 141.0, 150.0, 152.8, 155.7; IR (KBr) 3290, 3140, 2920, 1665, 1635, 1600, 1565, 1470, 1425, 1365, 1330, 1300, 1240, 1160, 1115, 1095, 930, 700 cm^{-1}; MS (EI) m/e 392 (M + 1), 362, 348, 293, 278, 162, 158, 141, 136, 128, 115, 108, 91, 84, 55; $[\alpha]_D$ = +16° (c = 0.376, $CHCl_3$). Anal. ($C_{22}H_{25}N_5O_2$) C, H, N.

(1′*R*,2′*S*,3′*R*,4′*R*)-9-[2′,3′-Dihydroxy-4′-phenylcyclopentan-1-yl]adenine (1f). The ketal hydrolysis was carried out as described in the synthesis of **1c** with 300 mg (0.77 mmol) of **8f** to yield 200 mg (84%) of **1f** as a white powder: mp 194–195 °C; ^{1}H-NMR (300 MHz, DMSO-d_6 + D_2O) δ 2.25 (dd, J = 12, 24 Hz, 1 H), 2.45 (m, 1 H), 3.10 (m, 1 H), 4.10 (dd, J = 6, 6 Hz, 1 H), 4.47 (dd, J = 6, 6 Hz, 1 H), 4.80 (m, 1 H), 7.25–7.45 (m, 5 H), 8.20 (s, 1 H), 8.35 (s, 1 H); ^{13}C-NMR (75 MHz, DMSO-d_6 + D_2O) δ 34.1, 49.6, 61.0, 74.8, 76.2, 119.5, 126.8, 127.9, 128.9, 141.0, 143.4, 149.9, 152.7, 156.1; IR (KBr) 3400, 3320, 3140, 3020, 2925, 1660, 1605, 1570, 1480, 1415, 1335, 1325, 1250, 1105, 1050, 760, 700, 645 cm^{-1}; MS (EI) m/e calcd for $C_{16}H_{17}N_5O_2$ 311.1382, found 311.1377; 312 (M + 1), 178, 162, 136, 135, 115, 108, 91, 77, 44; $[\alpha]_D$ = +14° (c = 0.20, H_2O). Anal. ($C_{16}H_{17}N_5O_2$) C, H, N.

(1′*R*,2′*S*,3′*R*)-9-[2′,3′-Dihydroxycyclopentan-1′-yl]adenine (1b). To a solution of **2b**[17] (100 mg, 0.425 mmol) in 100 mL of MeOH was added 10 mg of PtO_2, and the suspension placed under hydrogen (25 psi) for 6 h. The catalyst was removed, and the compound was concentrated in vacuo to afford 101 mg (100%) of **2** as an off-white solid: mp 218 °C dec; ^{1}H-NMR (300 MHz, DMSO-d_6 + D_2O) δ 1.6–2.25 (m, 4 H), 3.95 (m, 1 H), 4.25 (dd, 1 H), 4.45 (m, 1 H), 8.40 (s, 1 H), 8.55 (s, 1 H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 25.7, 28.8, 59.5, 70.5, 76.2, 119.5, 140.5, 149.7, 152.0, 156.0; MS (EI) m/e calcd for $C_{10}H_{13}N_5O_2$ 235.1069, found 235.1071; 235 (M^+), 162, 136; $[\alpha]_D$ = −50° (c = 0.80, H_2O). Anal. ($C_{10}H_{13}N_5O_2 \cdot HCl$) C, H, N.

2,3-*O*-Cyclohexylidene-5-*O*-methylsulfonyl-D-ribonic Acid γ-Lactone (12). To a −5 °C solution of 2,3-*O*-cyclohexylidene-D-ribonic acid γ-lactone[35] (15.77 g, 69.1 mmol) and triethylamine

(6.99 g, 69.1 mmol) in 200 mL of CH_2Cl_2 was added dropwise methanesulfonyl chloride (7.92 g, 69.1 mmol) in 50 mL of CH_2Cl_2. The reaction was allowed to come slowly to room temperature and stirred an additional 4 h. The mixture was then recooled to 0 °C, and 50 mL of cold H_2O was slowly added. After stirring several minutes, the layers were separated and the organic phase washed with 2 × 100 mL of H_2O and dried over Na_2SO_4. After filtration and concentration, an orange oil was obtained which solidified upon standing at room temperature for 3 h. This solid was recrystallized from CH_2Cl_2/hexane to obtain 19.1 g (90%) of **12** as an off-white solid. An analytically pure sample was prepared by recrystallizing a small amount of this material from ethyl acetate/hexane: mp 110 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.35–1.71 (m, 10 H), 3.06 (s, 3 H), 4.47 (m, 2 H), 4.78 (d, J = 6 Hz, 1 H), 4.80 (m, 1 H), 4.83 (d, J = 6 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7, 23.8, 24.7, 34.9, 36.3, 37.6, 68.3, 74.7, 76.9, 79.4, 114.8, 173.4; IR (KBr) 2943, 1778, 1363, 1178, 1113, 1068, 1008, 988, 963, 928, 883, 853, 808, 783, 723 cm^{-1}; MS (EI) m/e 306 (M^+), 277, 263, 79, 55; $[\alpha]_D$ = −40° (c = .0430, $CHCl_3$). Anal. ($C_{12}H_{18}O_7S$) C, H.

2,3-*O*-Cyclohexylidene-5-deoxy-5-iodo-D-ribonic Acid γ-Lactone (13). Sodium iodide (607 mg, 4.05 mmol) and **12** (497 mg, 1.62 mmol) were dissolved in 20 mL of methyl ethyl ketone, and the reaction vessel was stoppered with a drying tube. This mixture was stirred at 50 °C for 30 min at which point 20 mL of H_2O was added. The layers were separated, and the aqueous phase was extracted with 2 × 20 mL of ether. The combined organic layers were washed with 30 mL of H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a viscous yellow oil. This yellow oil was passed through silica gel (22 g, hexane/EtOAc; 4:1) to yield 494 mg (90%) of **13** as a clear, colorless oil. This purified oil eventually solidified to a light brown solid which was still spectroscopically pure and gave an acceptable elemental analysis: mp 61–63 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.35–1.72 (m, 10 H), 3.43 (m, 2 H), 4.60 (d, J = 6 Hz, 1 H), 4.64 (dd, J = 4, 4 Hz, 1 H), 4.95 (d, J = 6 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 5.8, 23.7, 23.8, 24.7, 34.9, 36.1, 74.9, 80.0, 81.0, 114.8, 173.2; IR (KBr): 2945, 2925, 1790, 1447, 1418, 1375, 1350, 1285, 1252, 1232, 1215, 1176, 1158, 1108, 1050, 1023 cm^{-1}; MS (EI) m/e 338 (M^+), 309, 295, 98, 69, 55; $[\alpha]_D$ = −21° (c = 2.87, $CHCl_3$). Anal. ($C_{11}H_{15}IO_4$) C, H.

2,3-*O*-Cyclohexylidene-5-deoxy-4,5-didehydro-D-ribonic Acid γ-Lactone (14). A solution of **13** (810 mg, 2.40 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 400 mg, 2.64 mmol) in 15 mL of benzene was stoppered with a drying tube and allowed to stir overnight at room temperature. The reaction was then filtered, washing the white precipitate with dry benzene, and the filtrate was concentrated in vacuo to a clear yellow oil. This oil was quickly passed through a plug of silica gel (5 g in a sintered glass funnel, EtOAc/hexane, 1:1) to give 410 mg (82%) of **14** as a clear colorless oil which by ^{1}H- and ^{13}C-NMR was approximately 95% pure. Due to the instability of this compound, further purification was not possible: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.34–1.71 (m, 10 H), 4.83 (d, J = 3 Hz, 1 H), 4.87 (d, J = 6 Hz, 1 H), 5.03 (d, J = 3 Hz, 1 H), 5.09 (d, J = 6 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.7, 23.8, 24.7, 35.3, 36.2, 74.9, 75.0, 95.1, 115.5, 152.9, 171.5; IR (neat) 2927, 2852, 1818, 1684, 1449, 1369, 1334, 1280, 1229, 1160, 1109, 991, 929, 877, 853 cm^{-1}; MS (EI) m/e 210 (M^+), 181, 167, 153, 97, 81, 69, 55.

(2*R*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-methyl-4-cyclopentenone (6c). Method A. To a −78 °C solution of dimethyl methylphosphonate (0.46 mL, 520 mg, 4.2 mmol) in 25 mL of THF was added dropwise n-butyllithium (2.90 mL, 4.20 mmol). After 1 h, **14** (442 mg, 2.10 mmol) in 10 mL of THF was added slowly dropwise. The mixture was allowed to warm gradually to −10 °C, at which point glacial acetic acid (0.12 mL, 126 mg, 2.10 mmol) in 5 mL of THF was added dropwise. The mixture was then allowed to warm gradually to room temperature, stirred an additional 1 h and quenched with 100 mL of pH 7 phosphate buffer. The mixture was extracted with 5 × 50 mL of ether, and the combined extracts were washed with 2 × 100 mL of H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo. The dark oil thus obtained was passed through silica gel (17 g, hexane/EtOAc, 4:1) to afford 222 mg (51%) of **6c** as a white solid.

Method B. Dimethylcopper was formed as described in the synthesis of **4c** utilizing 513 mg of copper(I) iodide (2.7 mmol) in 25 mL of anhydrous ether and 3.84 mL of methyllithium (1.4 M in ether, 5.4 mmol). Compound **3** (174 mg, 0.9 mmol) was dissolved in 5 mL of THF and added dropwise to the 0 °C dimethylcopper solution. After 3 h, the reaction mixture was transferred to a −78 °C solution of methanesulfinyl chloride[36] (0.430 mL, 590 mg, 7.2 mmol) in 40 mL of ether. The reaction mixture was allowed to warm to 0 °C over 0.5 h at which time 10 mL of 10% aqueous acetic acid was added. The solution was diluted with 100 mL of ether and washed with 2 × 150 mL of saturated NH_4Cl solution with 5% NH_4OH and then with 100 mL of H_2O. The combined aqueous phases were then extracted with 6 × 100 mL of CH_2Cl_2. The CH_2Cl_2 layers were combined, concentrated in vacuo, and passed through silica gel (17 g, CH_2Cl_2/EtOH, 19:1) to obtain 145 mg of β-keto sulfoxides **5c** as a mixture of four diastereomers. These were not further purified, but instead 134 mg was refluxed in 40 mL of toluene with 46 mg $CaCO_3$ for 18 h. After filtration and silica gel chromatography (26 g, ether/cyclohexane, 1:1), 100 mg (46% overall from **1**) of **6c** was obtained as a white solid: mp 80 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.32–2.21 (m, 10 H), 2.22 (s, 3 H), 4.47 (d, J = 5 Hz, 1 H), 5.01 (d, J = 5 Hz, 1 H), 5.89 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.0, 23.7, 23.9, 24.9, 35.9, 37.3, 77.6, 80.7, 116.0, 130.0, 174.7, 202.6; IR (KBr) 2940, 2920, 2830, 1697, 1621, 1375, 1196, 1162, 1100, 921, 834 cm^{-1}; MS (EI) m/e 208 (M^+), 179, 165, 111, 82, 55; $[\alpha]_D$ = −24° (c = 2.33, $CHCl_3$). Anal. ($C_{12}H_{16}O_3$) C, H.

(1*S*,2*S*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-methyl-4-cyclopenten-1-ol (9c). To a −78 °C solution of **6c** (5.00 g, 24.0 mmol) in 250 mL of CH_2Cl_2 was added dropwise diisobutylaluminum hydride (1.0 M in CH_2Cl_2, 36.0 mL, 36.0 mmol). After 6 h at this temperature, the reaction was quenched by the dropwise addition of 25 mL of MeOH and warmed to room temperature. After adding 100 mL of H_2O and stirring vigorously for 15 min, the mixture was suction filtered and the layers separated. The aqueous phase was extracted with 3 × 50 mL of CH_2Cl_2, and the combined organic layers were then washed with 150 mL of H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo. The oily residue was passed through a silica gel column (225 g, hexane/EtOAc, 9:1) to afford 4.80 g (95%) of **9c** as a clear, colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.30–1.70 (m, 10 H), 1.79 (s, 3 H), 2.73 (d, J = 9 Hz, 1 H, exchanged with D_2O), 4.49 (m, 1 H), 4.70 (dd, J = 6, 6 Hz, 1 H), 4.78 (d, J = 6 Hz, 1 H), 5.46 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 23.7, 24.0, 24.9, 36.4, 37.4, 73.4, 77.5, 85.3, 112.8, 130.1, 141.9; IR (neat) 3538, 2933, 2855, 1658, 1445, 1365, 1280, 1171, 1105, 1054, 999, 963, 932, 896 cm^{-1}; MS (EI) m/e 210 (M^+), 181, 167, 95, 56, 42; $[\alpha]_D$ = +3° (c = 1.86, $CHCl_3$). Anal. ($C_{12}H_{18}O_3$) C, H.

(1*S*,2*R*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-methyl-4-cyclopenten-1-ol Methanesulfonate (16). To a −5 °C solution of **7** (356 mg, 1.69 mmol) and triethylamine (188 mg, 1.86 mmol) in 10 mL of CH_2Cl_2 was rapidly added methanesulfonyl chloride (213 mg, 1.86 mmol) in CH_2Cl_2. After 1 h, 15 mL of cold H_2O was added, and the solution was stirred vigorously for several minutes. After separating the layers, the organic phase was washed with 15 mL of cold H_2O, dried over Na_2SO_4, filtered, and concentrated in vacuo to obtain 487 mg (100%) of spectroscopically pure **16** as a clear oil. Due to the instability of this mesylate, no attempt was made to obtain an analytically pure sample: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.30–1.70 (m, 10 H), 1.88 (s, 3 H), 3.15 (s, 3 H), 4.79 (d, J = 5 Hz, 1 H), 4.87 (dd, J = 5 Hz, 1 H), 5.38 (m, 1 H), 5.48 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 23.9, 23.9, 24.9, 36.5, 37.1, 39.0, 76.7, 81.5, 85.2, 113.8, 124.2, 146.6; IR (neat) 2928, 2858, 1661, 1354, 1173, 1445, 1281, 1124, 1096, 1054, 1014, 952, 905, 860, 819, 755 cm^{-1}; MS (EI) m/e 288 (M^+), 259, 245, 193, 95, 55.

(1*R*,2*S*,3*R*)-9-[2',3'-(Cyclohexylidenedioxy)-4'-methylcyclopent-4-enyl]adenine (10c). To a suspension of adenine

(35) Beer, D.; Meuwly, R.; Vasella, A. L-Erythruronic Acid Derivatives as Building Blocks for Nucleoside Analogs. Synthesis of 4'-C-Aryl-D-ribonucleosides. *Helv. Chim. Acta* **1982**, *65*, 2570–2582.

(36) Douglass, I. B.; Norton, R. V. Methanesulfinyl Chloride. *Organic Syntheses*; Baumgarten, H. E., Ed.; John Wiley and Sons, Inc.: New York, 1973; Collect: Vol. V, pp 709–712.

(2.96 g, 21.9 mmol) in 50 mL of DMF was added sodium hydride (80% dispersion in mineral oil, 660 mg, 21.9 mmol) and 18-crown-6 (5.79 g, 21.9 mmol). The mixture was heated to 80 °C for 1.5 h and then allowed to cool back to ambient temperature, at which point **16** (2.1 g, 7.3 mmol) in 10 mL of DMF was added in one portion. After stirring overnight, the reaction mixture was concentrated in vacuo. To the residue was added 100 mL of H_2O, and the aqueous solution was adjusted to pH 11 with 3 M NaOH. This was extracted with 4 × 50 mL of CH_2Cl_2, and the combined extracts were washed with 50 mL of 0.5 M NaOH, dried over K_2CO_3, filtered, and concentrated in vacuo. The resultant residue was passed through silica gel (100 g, CH_2Cl_2/EtOH, 9:1); final purification was achieved by preparative TLC (four 20 × 20 cm × 250 mm plates, CH_2Cl_2/EtOH, 13:1) to obtain 830 mg (35%) of **10c** as a white solid: mp 186 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.34–1.70 (m, 10 H), 1.99 (s, 3 H), 4.66 (d, J = 6 Hz, 1 H), 5.23 (d, J = 6 Hz, 1 H), 5.50–5.60 (br, 4 H, 2 H after D_2O exchange), 7.66 (s, 1 H), 8.39 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 23.9, 24.3, 25.3, 36.1, 37.6, 64.9, 84.2, 86.4, 113.4, 120.4, 122.5, 138.8, 149.2, 150.0, 153.4, 154.0; IR (KBr) 3310, 3160, 2935, 1675, 1600, 1512, 1475, 1415, 1369, 1332, 1303, 1250, 1207, 1164, 1052, 1014 cm^{-1}; MS (EI) m/e 328 (M + 1), 327 (M$^+$), 284, 230, 229, 200, 136, 135, 95, 55, 41; $[\alpha]_D$ = −153° (c = 14.53, $CHCl_3$). Anal. ($C_{17}H_{21}N_5O_2$) C, H, N.

(1*R*,2*S*,3*R*)-9-(2′,3′-Dihydroxy-4′-methyl-4′-cyclopentenyl)adenine (2c). Compound **10c** (532 mg, 1.63 mmol) was dissolved in 0.3 N HCl, and the reaction mixture was stirred overnight. The mixture was concentrated in vacuo to less than 1 mL, and 20 mL of cold EtOH was added. White crystals formed immediately, and the mixture was stored at −20 °C overnight. The crystals were then filtered, washed successively with cold EtOH and ambient-temperature ether, and dried to afford 388 mg (84%) of **2c**·HCl: mp 205 °C dec; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.83 (s, 3 H), 4.28 (m, 1 H), 4.37 (d, J = 6 Hz, 1 H), 4.45–5.35 (br s, 2 H, exchanged with D_2O), 5.43 (m, 1 H), 5.60 (s, 1 H), 8.51 (s, 1 H), 8.55 (s, 1 H), 9.00 (br s, 1 H, exchanged with D_2O), 9.60 (br s, 1 H, exchanged with D_2O); ^{13}C-NMR (75 MHz, DMSO-d_6) δ 14.9, 65.8, 75.2, 76.8, 118.3, 123.8, 142.8, 144.6, 146.5, 148.6, 150.3; IR (Nujol) 3500–3000, 1713, 1685, 1105 cm^{-1}; MS (EI) m/e calcd for $C_{11}H_{13}N_5O_2$ 247.1069, found 247.1081; 248 (M + 1), 136, 135, 108, 94; $[\alpha]_D$ = −151° (c = 2.95, H_2O); HPLC method A, R_f = 10.73 min; method B, R_f = 13.44 min.

(1*S*,2*S*,3*R*,4*R*)-2,3-(Cyclohexylidenedioxy)-4-methylcyclopentanol (15). A solution of **9c** (768 mg, 3.66 mmol) in 250 mL methanol with 80 mg PtO_2 was treated with hydrogen (25 psi) for 16 h. The suspension was filtered and concentrated in vacuo, and the residue was passed through silica gel (35 g, ether/hexane, 1:1) to yield 728 mg (94%) of **15** as a clear, colorless oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.05 (d, J = 7 Hz, 3 H), 1.25–1.70 (m, 12 H), 1.85 (m, 1 H), 2.50 (d, J = 10 Hz, 1 H, OH), 3.85 (m, 1 H), 4.40 (m, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.8, 23.6, 24.0, 25.2, 33.1, 33.7, 35.4, 37.7, 72.3, 78.6, 81.2, 110.8; IR (neat) 3450, 2920, 2850, 1450, 1400, 1370, 1285, 1250, 1230, 1165, 1140, 1110, 1090, 1060, 1030, 1000, 970, 950, 930, 910, 820, 670, 635 cm^{-1}; MS (EI) m/e 212 (M$^+$), 169, 97, 79, 69, 55, 42; $[\alpha]_D$ = +4° (c = 0.810, $CHCl_3$). Anal. ($C_{12}H_{20}O_3$) C, H.

(1′*R*,2′*S*,3′*R*,4′*R*)-9-[2′,3′-(Cyclohexylidenedioxy)-4′-methylcyclopentan-1′-yl]adenine (8d). The triflation and adenine displacement reactions were run as described above for the synthesis of **8c**, using 675 mg (3.18 mmol) of **15** to obtain 1.16 g (quantitative yield) of the crude triflate and 648 mg (62%) of **8d** as a white solid: mp 148–150 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J = 7 Hz, 3 H), 1.30–1.80 (m, 10 H), 1.95 (m, 1 H), 2.20 (m, 1 H), 2.50 (m, 1 H), 4.75 (dd, J = 5, 5 Hz, 1 H), 4.85 (d, J = 7 Hz, 1 H), 5.00 (d, J = 5 Hz, 1 H), 6.35 (s, 2 H, NH_2), 7.70 (s, 1 H), 8.35 (s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.2, 23.6, 24.0, 25.1, 33.6, 36.1, 36.9, 37.2, 61.7, 82.2, 85.2, 111.4, 119.8, 138.9, 149.9, 152.9, 155.7; IR (KBr) 3300, 3140, 2925, 1665, 1645, 1600, 1570, 1475, 1450, 1415, 1370, 1335, 1300, 1250, 1165, 1100, 1075, 1055, 995, 950 cm^{-1}; MS (CI) m/e 330 (M + 1), 286, 231, 216, 136, 108, 81, 79, 55; $[\alpha]_D$ = −20° (c = 0.680, $CHCl_3$). Anal. ($C_{17}H_{23}N_5O_2$) C, H, N.

(1′*R*,2′*S*,3′*R*,4′*R*)-9-(2′,3′-Dihydroxy-4′-methylcyclopentan-1′-yl)adenine (1d). Compound **8d** (648 mg, 1.97 mmol) was dissolved in 75 mL of 0.3 N HCl and stirred at room temperature overnight. By employing the same workup as described for **1c**, 359 mg (73%) of **1d** was obtained as a white powder: mp 238–240 °C dec; ^{1}H-NMR (300 MHz, DMSO-d_6 + D_2O) δ 0.95 (d, J = 7 Hz, 3 H), 1.75–2.10 (m, 2 H), 2.45 (m, 1 H), 3.75 (dd, J = 4, 4 Hz, 1 H), 4.40 (dd, J = 4, 9 Hz, 1 H), 4.70 (m, 1 H), 8.10 (s, 1 H), 8.15 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6 + D_2O) δ 15.3, 33.8, 35.4, 60.6, 74.9, 78.2, 119.7, 141.6, 149.9, 152.8, 156.2; IR (KBr) 3360, 3320, 3110, 2970, 2930, 2890, 1660, 1605, 1565, 1480, 1415, 1410, 1330, 1310, 1245, 1145, 1115, 990, 880, 795, 690, 645 cm^{-1}; $[\alpha]_D$ = −46° (c = 0.668, H_2O); MS (EI) m/e calcd for $C_{11}H_{15}N_5O_2$ 249.1226, found 249.1215; 249 (M$^+$), 232, 214, 190, 178, 162, 136, 135, 108, 67, 55, 41; HPLC method A, R_f = 12.25 min; method B, R_f = 12.45 min.

(2*R*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-ethyl-4-cyclopentenone (6g). To a −5 °C suspension of copper(I) iodide (1.57 g, 8.20 mmol) in 50 mL THF was added 5.50 mL of 3 M vinylmagnesium bromide in ether (16.5 mmol) over 1.5–2.0 min. The suspension was kept at −5 °C for 3 min including addition time and then cooled to −78 °C. The enone **3** (1.00 g, 5.20 mmol) in 10 mL of THF was added dropwise to the reaction mixture. After 1 h, the dry ice/acetone bath was replaced with an ice bath, and the reaction mixture was allowed to come to 0 °C. After 15 min at this temperature, the reaction was recooled to −78 °C and transferred via cannula to a −78 °C solution of methanesulfinyl chloride (2.20 mL, 3.00 g, 3.10 mmol) in 400 mL of THF. The reaction mixture was allowed to warm to 0 °C over 30 min, at which time the reaction was treated as described in the workup of **6c** to obtain 1.15 g of crude sulfoxide diastereomers. The mixture of sulfoxides was then refluxed in toluene (200 mL) in the presence of $CaCO_3$ (1.00 g, 1.00 mmol) for 17 h. Concentration in vacuo afforded a residue which was passed through flash silica gel (CH_2Cl_2/EtOH; 99:1) to provide 495 mg (43% overall from **3**) of pure **6g** as a pale yellow oil: ^{1}H-NMR (500 MHz; $CDCl_3$) δ 1.25 (t, J = 7 Hz, 3 H), 1.30–1.80 (m, 10 H), 2.45 (m, 1 H), 2.65 (m, 1 H), 4.45 (d, J = 6 Hz, 1 H), 5.05 (d, J = 6 Hz, 1 H), 5.90 (s, 1 H); ^{13}C-NMR (75 MHz; $CDCl_3$) δ 10.9, 23.6, 23.8, 24.3, 24.9, 35.8, 37.3, 77.5, 79.8, 115.8, 127.8, 180.1, 202.3; IR (neat) 2930, 2855, 1720, 1615, 1450, 1415, 1370, 1345, 1335, 1290, 1255, 1230, 1195, 1165, 1145, 1110, 1075, 1055, 995, 965, 940, 910, 870, 850, 835 cm^{-1}; MS (EI) 222 (M$^+$), 193, 179, 125, 96, 81, 67, 55, 41; $[\alpha]_D$ = −32.5° (c = 0.962, $CHCl_3$). Anal. ($C_{13}H_{18}O_3$) C, H.

(1*S*,2*S*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-ethyl-4-cyclopenten-1-ol (9g). The same experimental procedure employed in the formation of **7c** was used with 815 mg (3.67 mmol) of **6g** to obtain, after silica gel chromatography, 634 mg (77%) of **9g** as a clear, colorless oil: ^{1}H-NMR (300 MHz; $CDCl_3$) δ 1.10 (t, J = 7 Hz, 3 H), 1.30–1.75 (m, 10 H), 2.10 (m, 1 H), 2.25 (m, 1 H), 2.75 (d, J = 10 Hz, 1 H, exchanges with D_2O), 4.50 (m, 1 H), 4.70 (dd, J = 5, 5, 1 H), 4.85 (d, J = 5, 1 H), 5.45 (s, 1 H); ^{13}C-NMR (75 MHz; $CDCl_3$) δ 11.7, 21.0, 23.8, 24.0, 25.0, 36.5, 37.4, 73.4, 77.4, 84.4, 112.8, 128.0, 148.0; IR (neat) 3540 (br), 2930, 2850, 1645, 1445, 1430, 1390, 1360, 1330, 1275, 1245, 1225, 1160, 1110, 1065, 1040, 980, 945, 930, 900, 870, 845 cm^{-1}; MS (EI) 224 (M$^+$), 195, 181, 126, 109, 95, 81, 69, 55, 41; $[\alpha]_D$ = +1° (c = 0.962, $CHCl_3$). Anal. ($C_{13}H_{20}O_3$) C, H.

(1*S*,2*R*,3*R*)-2,3-(Cyclohexylidenedioxy)-4-ethyl-4-cyclopenten-1-ol Methanesulfonate (17). The same experimental procedure employed in the formation of **16** was used to obtain a quantitative crude yield of **17** as a clear, colorless oil: ^{1}H-NMR (300 MHz; $CDCl_3$) δ 1.15 (t, J = 7 Hz, 3 H), 1.30–1.70 (m, 10 H), 2.15 (m, 1 H), 2.30 (m, 1 H), 3.15 (s, 3 H), 4.85 (m, 2 H), 5.40 (m, 1 H), 5.50 (d, J = 2 Hz, 1 H); ^{13}C-NMR (75 MHz; $CDCl_3$) δ 11.4, 21.4, 23.9 (two superimposed peaks), 24.9, 36.5, 37.1, 39.0, 76.6, 81.4, 84.3, 113.8, 122.3, 152.4; IR (neat) 2960, 2850, 1645, 1445, 1430, 1360, 1280, 1250, 1225, 1170, 1120, 1090, 1050, 1000, 960, 940, 900, 880, 860, 840, 825 cm^{-1}; MS (EI) 302 (M$^+$), 273, 259, 206, 187, 109, 97, 92, 81, 79, 69, 55, 41.

(1′*R*,2′*S*,3′*R*)-9-[2′,3′-(Cyclohexylidenedioxy)-4′-ethylcyclopent-4-enyl]adenine (10g). Compound **9g** was treated as **6g** to afford **10g**: mp 207–208 °C dec; ^{1}H-NMR (300 MHz; $CDCl_3$) δ 1.20 (t, J = 7 Hz, 3 H), 1.30–1.80 (m, 10 H), 2.35 (m, 2 H), 4.65 (d, J = 5, 1 H), 5.30 (d, J = 5, 1 H), 5.50 (s, 1 H), 5.55 (s, 1 H), 6.15 (br s, 2 H, NH_2), 7.65 (s, 1 H), 8.40 (s, 1 H); ^{13}C-NMR (75 MHz; $CDCl_3$) δ 11.6, 21.8, 23.7, 24.0, 25.0, 35.8, 37.4, 64.5, 83.9, 85.3, 113.1, 120.1, 120.4, 138.5, 149.8, 153.1, 154.7, 155.6; IR (KBr) 3280, 3130, 2930, 1680, 1640, 1605, 1570, 1465, 1410, 1360, 1325, 1300, 1205, 1160, 1105, 1040, 730 cm^{-1}; MS (EI) 341 (M$^+$), 298,

243, 134, 109, 55; $[\alpha]_D = -124°$ ($c = 1.08$, $CHCl_3$).

(1′*R*,2′*S*,3′*R*)-9-(2′,3′-Dihydroxy-4′-ethyl-4′-cyclopentenyl)adenine (2g). Ketal **10g** was hydrolyzed as described in the synthesis of 1c: mp 212–214 °C; ^{1}H-NMR (500 MHz; DMSO-d_6 + D_2O) δ 1.05 (t, $J = 7$ Hz, 3 H), 2.15 (m, 2 H), 4.25 (dd, $J = 5, 5, 1$ H), 4.40 (d, $J = 5$, 1 H), 5.30 (s, 1 H), 5.55 (s, 1 H), 8.05 (s, 1 H), 8.10 (s, 1 H); ^{13}C-NMR (125 MHz; DMSO-d_6 + D_2O) δ 11.6, 22.2, 64.9, 74.6, 76.8, 119.3, 123.1, 140.1, 149.9, 151.6, 152.8, 156.1; IR (KBr) 3360, 3320, 3140 (br), 2960, 1650, 1605, 1570, 1480, 1410, 1325, 1305, 1245, 1115, 1050, 860, 800, 690 cm^{-1}; MS (EI) *m/e* calcd for (M + 1) of $C_{12}H_{15}N_5O_2$ 262.1304, found 262.1300; 261 (M^+), 233, 214, 186, 149, 136, 108. Anal. ($C_{12}H_{15}N_5O_2$) C, H, N.

Determination of AdoHcy Hydrolase Inhibition Constants. AdoHcy hydrolase was isolated and purified from bovine liver as previously reported[37] except that Q Sepharose (Pharmacia) was used instead of DE-52 cellulose, and the CM-Sephadex column was not applied. The enzyme activity was determined by incubating 20 nM AdoHcy hydrolase with 0.2 mM adenosine and 5 mM homocysteine for 5 min at 37 °C in 150 mM phosphate buffer (pH 7.6) containing 1 mM EDTA and assaying the AdoHcy produced by HPLC after the reaction was stopped by addition of perchloric acid (final concentration: 0.5 N). Volume of 100 μL of supernatant obtained by centrifugation of the reaction mixture was injected into an HPLC column (C-18 reverse-phase column, Econosphere, Alltech, 25 cm × 4.6 mm) and analyzed with a two-step gradient program at flow rate of 1.0 mL/min [solvent A, acetonitrile; solvent B, 50 mM sodium phosphate (pH 3.2) containing 10 mM heptanesulfonic acid; Program, 5–20% A for 15 min, 20–25% A for 10 min]. The peak area of AdoHcy was monitored at 254 nm to quantitate the AdoHcy. For the determination of inhibition constants, AdoHcy hydrolase was preincubated with various concentrations of inhibitors for various amounts of time, and the remaining enzyme activity was measured. The pseudo-first-order rate of inactivation (k_{obs}) was determined from a plot of the remaining activity versus preincubation time. K_1 and k_2 were obtained from a plot of $1/k_{obs}$ versus 1/[inhibitor] using the equation $1/k_{obs} = K_1/(k_2[I]) + 1/k_2$.

(37) Narayanan, S. R.; Borchardt, R. T. Purification of Bovine Liver *S*-Adenosylhomocysteine Hydrolase by Affinity Chromatography on Blue Dextran-agarose. *Biochim. Biophys. Acta* 1988, *965*, 22–28.

Acknowledgment. We thank Dr. David Vander Velde here at the University of Kansas for assistance with the NOE experiments on intermediate **7f**. This work was supported by a National Institute of Health Predoctoral Training Grant (GM-07775; M.S.W.), a National Science Foundation Graduate Fellowship (W.J.B.), a United States Public Health Service Grant (GM-29332), and Glaxo, Inc.

3-(2-Carboxyindol-3-yl)propionic Acid-Based Antagonists of the *N*-Methyl-D-aspartic Acid Receptor Associated Glycine Binding Site

Francesco G. Salituro,* Boyd L. Harrison, Bruce M. Baron, Philip L. Nyce, Kenneth T. Stewart, John H. Kehne, H. Steven White,† and Ian A. McDonald

Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215, and Anticonvulsant Drug Development Program, Department of Pharmacology & Toxicology, University of Utah, Salt Lake City, Utah 84108.
Received November 14, 1991

A series of substituted 3-(2-carboxyindol-3-yl)propionic acids was synthesized and tested as antagonists for the strychnine-insensitive glycine binding site of the NMDA receptor. Chlorine, and other small electron-withdrawing substituents in the 4- and 6-positions of the indole ring, greatly enhanced binding and selectivity for the glycine site over the glutamate site of the NMDA receptor; one of the most potent compounds is 3-(4,6-dichloro-2-carboxyindol-3-yl)propionic acid (IC_{50} = 170 nM; >2100-fold selective for glycine). The importance of a heteroatom NH and the enhancing effect of the propionic acid side chain were demonstrated and are consistent with previous results which suggest the presence of a pocket on the receptor which can accept an acidic side chain. Substitution of a sulfur at C3 led to the most potent compound 3-[(carboxymethyl)thio]-2-carboxy-4,6-dichloroindole (IC_{50} = 100 nM).

Introduction

The role which the acidic amino acids glutamic acid and aspartic acid play as neurotransmitters in the mammalian central nervous system (CNS) has been intensely studied over the past several years. Several distinct receptor complexes have been defined by the ligands kainic acid (kainate), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and *N*-methyl-D-aspartic acid (NMDA). From a potential therapeutic point of view, the NMDA receptor complex has attracted considerable interest as there is increasing evidence that abnormal stimulation of the receptor may play a role in the neuropathology of disease states such as epilepsy, Huntington's disease, and Alzheimer's disease.[1-3] Furthermore, it is thought that the neurodegeneration which occurs following events of cerbral ischemia is, in part, a result of an overstimulation of the NMDA receptor.

The NMDA receptor complex possesses several allosteric binding sites which alter the cellular response to glutamic acid.[4] This response is presumably initiated by the influx

† University of Utah.

(1) Robinson, M. B.; Coyle, J. T. Glutamate and Related Acyclic Excitatory Neurotransmitters: From Basic Science to Clinical Application. *FASEB J.* 1987, *1*, 446–455.
(2) Meldrum, B. *Neurology and Neurobiology*; Hicks, T. P., Lodge, D., McLennan, H., Eds.; Alan R. Liss, Inc.: New York, 1987; Vol. 24, pp 189–196.
(3) (a) Olney, J. W. Excitatory Amino Acids and Neuropsychiatric Disorders. *Biol. Psychiatry* 1989, *26*, 505–525. (b) Lodge, D. Modulation of *N*-Methyl Aspartate Receptor-Channel Complexes. *Drugs Today* 1989, *25*, 395–411. (c) Wood, P. L.; Rao, T. S.; Iyengar, S.; Lanthorn, T.; Monahan, J.; Cordi, A.; Sun, E.; Vazquez, M.; Gray, N.; Contreras, P. A Review of the *In Vitro* and *In Vivo* Neurochemical Characterization of the NMDA/PCP/Glycine/Ion Channel Receptor Macrocomplex. *Neurochem. Res.* 1990, *15*, 217–230.
(4) Lodge, D.; Jones, M.; Fletcher, E. *The NMDA Receptor*; Watkins, J. C., Collingridge, G. L., Eds.; Oxford University: Oxford, 1989; Chapter 3.

0022-2623/92/1835-1791$03.00/0