(±)-N^9-(2-(Hydroxymethyl)spiro[3.3]hept-6-yl)adenine. The First Biologically Active Saturated Analogue of Adenallene with Axial Dissymmetry

Bryan C. N. M. Jones,† John C. Drach,⊥ Thomas H. Corbett,‡ David Kessel,§ and Jiri Zemlicka*,†

Department of Chemistry, Developmental Therapeutics Program, Michigan Cancer Foundation, Departments of Internal Medicine and Pharmacology, Wayne State University School of Medicine, Detroit, Michigan 48201, and Department of Biologic and Materials Science, School of Dentistry, University of Michigan, Ann Arbor, Michigan 48019

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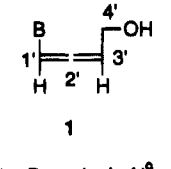
Synthesis of the title analogue **2** is described. Fecht's acid (**3**) was esterified with *N,N*-dimethylformamide dimethyl acetal to give monoester **4** along with diester **5**. Compound **4** was transformed to ester amide **6** by the reaction with isobutyl chloroformate and triethylamine followed by ammonolysis. Hoffman rearrangement of **6** effected by lead tetraacetate in *tert*-butyl alcohol led to the *N*-*tert*-butoxycarbonyl ester **7**. The latter was reduced with $Ca(BH_4)_2$ to give the protected amino alcohol **8**. Removal of the *N*-*tert*-butoxycarbonyl group with 2 M HCl in methanol afforded the hydrochloride of amino alcohol **9**. Reaction of **9** with 5-amino-4,6-dichloropyrimidine and triethylamine gave the pyrimidine derivative **10** which, in turn, was cyclized to 6-chloropurine **11a**. Ammonolysis of the latter intermediate afforded the title analogue **2**. The ^1H NMR spectrum of Fecht's acid (**3**) in CD_3COCD_3 showed that four methylene protons were magnetically nonequivalent (two quartets) whereas the other four were equivalent, forming a single doublet. Compound **2** inhibited the replication of human cytomegalovirus (IC_{50} 32 μM) and growth of murine leukemia L1210 cells (IC_{50} 30 μM). Zone assays showed inhibition of the following tumor cultures at 0.5 mg/disk: murine leukemia P388, mouse tumors PO3, C38, and M17/Adr as well as human tumors MCF-7 and CX-1.

Adenallene (**1a**) is an effective anti-HIV agent of both theoretical and practical significance.[1,2] The axial dissymmetry of **1a** coupled with a system of cummulated (allenic) double bonds makes it a unique nucleoside analogue.[3] In fact, compound **1a** and its cytosine counterpart cytallene **1b** belong to a small group of allenes with a significant biological potential.[4] Studies of structure–activity relationships have shown that the allenic function of **1a** and **1b** is of key importance for their antiretroviral effect. Thus, saturation of one or both double bonds of **1a** invariably led to a total loss of anti-HIV activity.[2] However, such changes eliminated both allenic system and axial dissymmetry of the respective analogues. Although allenes **1a** and **1b** are considered analogues of antiretroviral 2′,3′-dideoxyribonucleosides,[5] the exact role of allenic system for their biological effect has not been elucidated. It is possible that in addition to the favorable molecular shape other effects such as hydrogen bonding[3] involving the allenic function can play a part in binding of **1a** or **1b** to the relevant receptor or enzyme sites. Thus, intra- and intermolecular hydrogen bonding to the sp-hybridized carbon atom was invoked to explain the optical properties of tetrasubstituted allenic alcohols.[6,7] It is therefore of interest to investigate analogues with an axial dissymmetry similar to adenallene (**1a**) or cytallene (**1b**) but lacking the cummulated double bonds and, therefore, the capability of functioning as acceptors of hydrogen bond. The synthesis and biological activity of the first such analogue, compound **2**, is the subject of this report.



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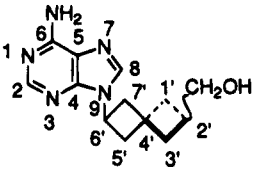

2

1a: B = adenin-N^9-yl

1b: B = cytosin-N^1-yl

Synthesis. Fecht's acid[8,9] (spiro[3.3]heptane-2,6-dicarboxylic acid, **3**) offered a convenient starting material for the synthesis of analogue **2**. Desymmetrization of **3** achieved previously only with the aid of enzymes[10,11] was a key element in the synthesis. It was tempting to employ a recently described procedure for selective mo-

* Address correspondence to this author at the Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201-1379. Telephone: (313) 833–0715 ext 312. Fax: (313) 831–8714. e-mail: jiriz@mcf.roc.wayne.edu.

† Michigan Cancer Foundation.

⊥ University of Michigan.

‡ Department of Internal Medicine.

§ Department of Pharmacology.

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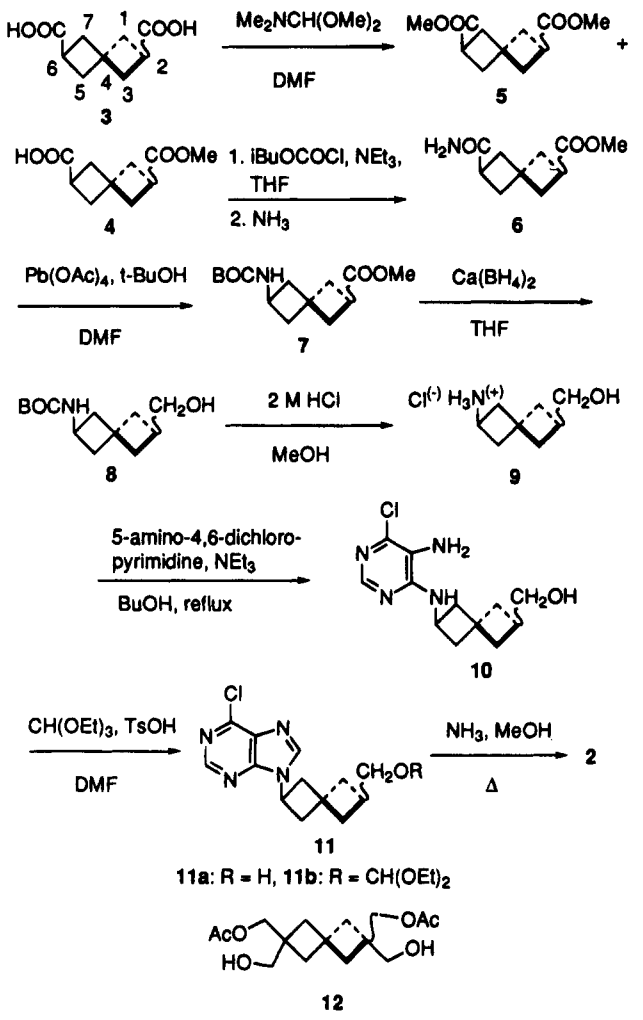
Scheme 1



noesterification[12] of terephthalic acid with *N,N*-dimethylformamide dimethyl acetal on an analytical scale. Reaction of acid **3** (1 mmol) in DMF with a 3-fold molar excess of *N,N*-dimethylformamide dimethyl acetal in DMF gave monoester **4** (52%) in addition to diester **5** (17%, Scheme 1). When the scale was expanded to 16 mmol of **3**, the extent of formation of diester **5** (37%) approximately equaled that of monoester **4** (34%). At any rate, a sufficient amount of **4** was generated to proceed with the synthetic sequence. The carboxy group of monoester **4** was activated with isobutyl chloroformate[13] and NEt_3 in tetrahydrofuran (THF). Subsequent treatment with NH_3 gave amido ester **6** in 72% yield. Hoffman rearrangement of **6** was effected with $Pb(OAc)_4$ in *t*-BuOH to give BOC ester **7** (62%). The latter was reduced with $Ca(BH_4)_2$ in THF, affording *N*-protected amino alcohol **8** in 68% yield. Finally, an acid-catalyzed methanolysis of **8** furnished the key intermediate—amino alcohol **9** as a hydrochloride. The adenine ring was constructed stepwise.[13] First, reaction of **9** with 5-amino-4,6-dichloropyrimidine in BuOH under reflux using NEt_3 as a base gave intermediate **10** in 69% yield. The latter was, in turn, transformed to 6-chloropurine derivative **11a** (79%) by triethyl orthoformate and TsOH. Ammonolysis in methanol at 100 °C in an autoclave then gave the title analogue **2** in 86% yield.

NMR Spectra. As expected, the NMR spectra of unsymmetrical 2,6-spiroheptanes **2,4,6,7** and **9** indicated a lack of symmetry (seven peaks in ^{13}C NMR). More interesting are the ^1H NMR spectra of starting Fecht's acid (**3**). Previous studies[14] of diester **5** were performed at 100–300 MHz in nonpolar solvents, but only the ^1H NMR spectrum at 220 MHz in naphthalene at 95 °C was amenable to a straightforward interpretation. We have found that the spectrum of diacid **3** in CD_3COCD_3 at 500 MHz is much simplified. Thus, the H_2 and H_6 appear as an expected quintet[9,14] at ∂ 2.95 with $J = 8–8.5$ Hz. Two different kinds of methylene groups are readily discernible, but the coupling patterns strongly differ from those reported[14] for diester **5**. A pair of CH_2 functions appears as a single doublet at ∂ 2.14 ($J = 7.5$ Hz) whereas another pair forms two partially overlapped quartets between ∂ 2.30 and 2.20 (Figure 1, part A). This pattern indicates that protons of the CH_2 groups at ∂ 2.14 are magnetically equivalent but those occurring as a cluster of four doublets are not. Double resonance experiments are in accord with this conclusion. Thus, irradiation of the doublet at ∂ 2.14 transformed the quintet at ∂ 2.95 to a triplet (Figure 1, part B), and the four remaining doublets became sharper. Likewise, irradiation of the quintet at ∂ 2.95 led to a collapse of the doublet at ∂ 2.14 to a singlet whereas the remaining two quartets were transformed to two doublets of $J = 12$ Hz, reflecting the geminal coupling (Figure 1, part C). It is possible that the signal pattern is influenced by the proximity of the carboxy or carbalkoxy function to the relevant methylene groups in the molecule. Thus, compound **12**, which also exhibits axial dissymmetry but lacks a properly positioned carbonyl group, showed all CH_2 protons of the spiro[3.3]-heptane system as a singlet.[10]

Biological Activity. Compound **2** is resistant to adenosine deaminase from calf intestine. It also displayed no antiretroviral activity in an assay employing ATH8 cells[1] infected with HIV-1 at 100 μM, but it was moderately cytotoxic with IC_{50} ca. 100 μM. This result may indicate that the π-electron system of adenallene (**1a**) is important for anti-HIV activity. However, the distance between the heterocyclic base and hydroxymethyl group of **2** (2′-CH_2–N_9 5.77 Å) is longer than that in adenallene (**1a**) (C_4′–N_9 4.46 Å), which can also influence the biological activity. Compound **2** inhibited the replication of human cytomegalovirus (HCMV) in human foreskin fibroblast (HFF) culture with IC_{50} 32 μM and the growth of murine leukemia L1210 cells (IC_{50} 30 μM). It was inactive against herpes simplex virus (HSV-1) in BSC-21 cells. Zone assays showed inhibition of the following tumor cultures at 0.5 mg/disk: murine leukemia P388, mouse tumors PO3, C38, and M17/Adr as well as human tumors MCF-7 and CX-1.

Resistance toward adenosine deaminase may be favorable for biological activity of analogue **2**. In addition, compound **2** is racemic and, therefore, one would expect that only one of the enantiomers will possess the biological activity. This would put **2** into the realm of analogues of therapeutical interest.[15]

These results indicate that biologically active nucleoside analogues based on the principle of axial dissymmetry can be derived from saturated nonallenic systems.

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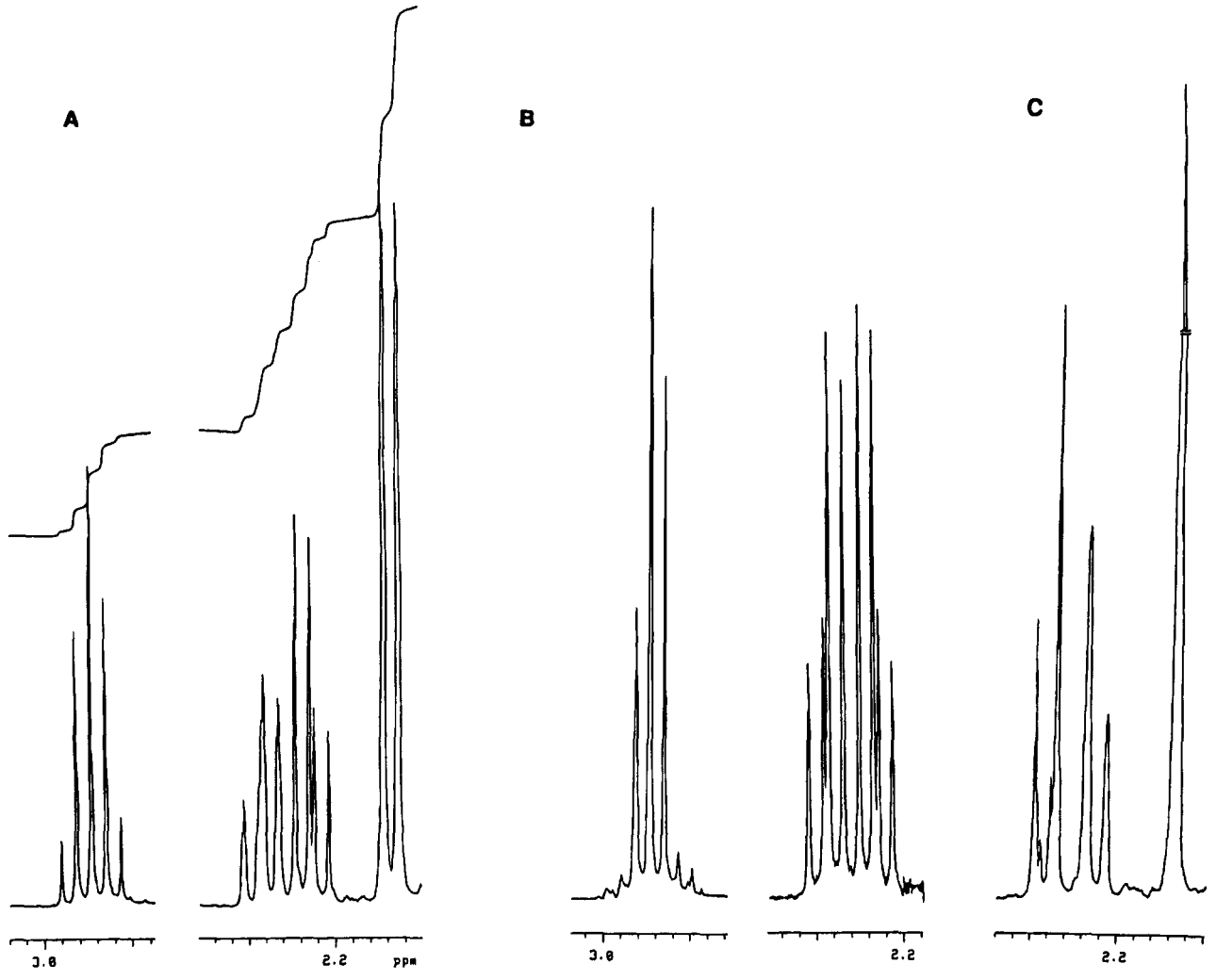

Figure 1. Decoupling patterns of spiro[3.3]heptane protons in Fecht's acid (**3**). Panel A. ^{1}H NMR spectrum (500 MHz, acetone-d_6) of spiro[3.3]heptane portion of **3**. Panel B. Irradiation of the CH_2 protons at ∂ 2.14. Note that quintet at ∂ 2.95 was transformed to a triplet. Panel C. Irradiation of the H_2 and H_6 protons at ∂ 2.95. Note that the original system of two quartets and one doublet (see panels A and B) collapsed to two doublets and one singlet.

In addition, analogue **2** is the first spiro[3.3]heptane derivative exhibiting antiviral and antitumor activity.

Experimental Section

General Methods. See refs 3 and 8. ^{1}H and ^{13}C NMR spectra were determined at 300 and 75.48 MHz, respectively, unless stated otherwise. Chromatography was performed in solvent system S_1, CH_2Cl_2–MeOH (95:5), S_2, hexanes–acetone (7:3), and preparative TLC with 20 × 20 cm 2 mm thick silica gel GF (Uniplate, Analtech, Newark, DE). The spots were detected by charring after spraying with 10% $HClO_4$ in 30% MeOH or spraying with 1% ninhydrin in EtOH (compounds **7**, **8**, **9**) or by UV light (compounds **2**, **10**, **11**).

Fecht's Acid (3). Compound **3** was prepared as described.[8] ^{1}H NMR (acetone-d_6, 500 MHz): ∂ 2.95, 2.30, 2.28, 2.24, 2.22, 2.14. Details of the spectrum are given in Figure 1. ^{13}C NMR (acetone-d_6, 75.47 MHz): 176.48, 38.56, 38.09, 37.30, 33.02.

(±)-2-(Carbomethoxyspiro[3.3]heptane-6-carboxylic Acid (4). Fecht's acid (**3**, 3.00 g, 16.3 mmol) was dissolved in DMF (250 mL). *N,N*-Dimethylformamide dimethyl acetal (5.2 mL, 39.1 mmol) was added slowly with stirring at room temperature. The stirring was continued for 16 h, and the progress of the reaction was monitored by TLC ($XS_{11}S_2$). Water (5 mL) was then added, and the solution was evaporated (oil pump). The residue was partitioned between saturated aqueous $NaHCO_3$ (200 mL) and CH_2Cl_2 (2 × 100 mL). The organic phase was washed with water (50 mL), it was dried ($MgSO_4$) and evaporated to give crude diester **5** as a brown syrup (1.27 g, 37%). The aqueous portion was acidified with HCl and extracted with CH_2Cl_2 (4 × 100 mL). The extract was dried (Na_2SO_4) and evaporated to give an oil which was kept under high vacuum overnight and then at −10 °C to give monoester **4** as a white solid (1.08 g, 34%), mp 49–51 °C. ^{1}H NMR ($CDCl_3$): ∂ 3.59, 2.94, 2.50, 2.18. ^{13}C NMR: 181.41, 175.73, 51.71, 37.82, 37.75, 37.36, 37.15, 36.68, 32.65, 32.60. Anal. Calcd for $C_{10}H_{14}O_4$: C, 60.59; H 7.12. Found: C, 60.52; H 7.10.

(±)-2-Carbomethoxyspiro[3.3]heptane-6-carboxamide (6). A solution of monoester **4** (1.00 g, 5.1 mmol) in THF (50 mL) was cooled to 0 °C under N_2. Triethylamine (0.85 mL, 6.1 mmol) followed by isobutyl chloroformate (0.79 ml, 6.1 mmol) was added with stirring, which was continued for 40 min. THF saturated with NH_3 at 0 °C (50 mL) was added, the mixture was stirred at 0 °C for 30 min, at room temperature for 1 h, and then it was stored at −10 °C overnight. The resultant solution was filtered, and the filtrate was evaporated. A solid residue (0.97 g) was partitioned between $CHCl_3$ (20 mL) and saturated aqueous $NaHCO_3$ (2 × 15 mL). The organic phase was washed with water (15 mL), dried (Na_2SO_4), and filtered, and the filtrate was evaporated to give a white solid (**6**, 0.72 g, 72%), mp 148–150 °C. IR (KBr): 3350 cm^{-1} (s, NH_2), 1760 (s, CO, ester), 1675 (s, CO, amide). ^{1}H NMR ($CDCl_3$, 500 MHz): ∂ 5.74, 5.41, 3.65, 2.98, 2.89, 2.25. ^{13}C NMR (120.75 MHz): 177.13, 175.77, 51.68, 37.93, 37.87, 37.41, 37.17, 36.31, 33.83, 32.67. EI-MS (rel intensity): 198 (M + H, 4.1), 197 (M, 7.1), 196 (M − H, 0.3), 166 (22.6), 138 (14.8), 124 (11.6), 111 (100.0), 93 (31.3), 72 (89.3), 55 (45.1). HRMS:

M calcd 197.1052, found 197.1049. Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.90; H, 7.67; N, 7.10. Found: C, 60.75; H, 7.67; N 7.13.

(±)-2-Carbomethoxy-6-[(*N*-*tert*-butoxycarbonyl)amino]spiro[3.3]heptane (7). Compound **6** (0.7 g, 3.6 mmol) was dissolved in a mixture of *t*-BuOH (50 mL) and DMF (25 mL). Lead tetraacetate (7.87 g, 17.75 mmol) was added at room temperature. The solution became brown immediately. The mixture was refluxed for 40 min. It was allowed to cool to room temperature and then evaporated. The residue was chromatographed on a silica gel column (solvent system S_1) to furnish product **7** (0.59 g, 62%), mp 76–78 °C. IR (KBr): 3300 cm^{-1} (s, NH), 1740 (bs, C=O). ^1H NMR ($CDCl_3$): ∂ 4.63, 3.95, 3.63, 2.98 , 2.45, 2.27, 2.08, 1.39. ^{13}C NMR: 175.62, 155.01, 79.18, 51.61, 43.63, 42.96, 41.01, 37.53, 36.62, 33.47, 33.07, 28.29. EI-MS (rel intensity): 196 (M − Me_3CO, 5.6), 182 (6.1), 143 (15.6), 127 (7.3), 114 (15.3), 93 (15.5), 87 (16.7), 68 (6.0), 57 (Me_3C, 100.0). HRMS: M − Me_3CO calcd 196.0974, found 196.0977. CI-MS (rel intensity) 270 (M + H, 1.2), 254 (M − CH_3, 4.8), 214 (100.0, MH − $Me_2C{=}CH_2$), 196 (3.1), 182 (6.1), 143 (15.6), 127 (7.3), 114 (15.6), 93 (15.5), 87 (16.7), 68 (6.0), 57 (Me_3C, 100.0). Anal. Calcd for $C_{14}H_{23}NO_4$: C, 62.43; H, 8.61; N, 5.20. Found: C, 62.53%; H, 8.65; N, 5.21.

(±)-2-(Hydroxymethyl)-6-[(*N*-*tert*-butoxycarbonyl)amino]spiro[3.3]heptane (8). A mixture of powdered anhydrous $CaCl_2$ (0.64 g, 5.8 mmol) and $NaBH_4$ (0.44 g, 11.5 mmol) was stirred in THF (60 mL) for 1 h at room temperature. A solution of ester **7** (0.5 g, 1.86 mmol) in THF (10 mL) was added, and the resultant mixture was refluxed for 22 h. The solids were filtered off, the solvent was evaporated, and the residue was chromatographed on a silica gel column in solvent system S_1 to give a gum, which was transformed on drying in vacuo for 24 h to a white solid **8** (0.31 g, 68%), mp 61–63 °C. ^1H NMR ($CDCl_3$): ∂ 4.75, 3.92, 3.47, 2.31, 2.07, 1.92, 1.70, 1.36. ^{13}C NMR: 155.94, 79.10, 66.98, 44.08, 43.72, 41.17, 36.95, 36.21, 33.34, 31.98, 28.31. HRMS: calcd for M − $Me_2C{=}CH_2$ 185.1052, found 185.1047. CI-MS (rel intensity): 242 (M + H, 0.9), 226 (1.0), 213 (0.9), 186 (100.0, M + H − $Me_2C{=}CH_2$), 143 (6.6), 124 (1.0), 107 (8.0), 93 (1.1), 87 (3.8), 69 (0.9). Anal. Calcd for $C_{13}H_{23}NO_3$: C, 64.70; H, 9.61; N, 5.80. Found: C, 64.82; H, 9.40; N, 5.70.

(±)-6-Amino-2-(hydroxymethyl)spiro[3.3]heptane Hydrochloride (9). A solution of the protected amino alcohol **8** (0.25 g, 1.0 mmol) in 2 M HCl in methanol (30 mL, 60 mmol) was stirred for 8 h at room temperature. The solution was evaporated, and the resultant gum was left under high vacuum for 24 h to give a white solid which was recrystallized from methanol/ether to give compound **9** (0.14 g, 79%), mp 128–130 °C. ^1H NMR (D_2O): ∂ 3.64, 3.46, 2.44, 2.32, 2.06, 1.74. ^{13}C NMR: 66.09, 41.02, 39.57, 39.13, 36.16, 35.79, 33.23, 30.70. EI-MS (rel intensity): 142 (M + H, 5.0), 113 (19.4), 93 (18.0), 83 (100.0), 68 (73.0). HRMS: calcd for M + H 142.1232, found 142.1227. Anal. Calcd for $C_8H_{16}ClNO$: C, 54.08; H, 9.08; N, 7.88. Found: C, 54.21; H, 8.82; N, 7.66.

(±)-*N*^{9}-(2-(Hydroxymethyl)spiro[3.3]hept-6-yl)adenine (2). A mixture of amino alcohol hydrochloride **9** (0.10 g, 0.56 mmol), 5-amino-4,6-dichloropyrimidine (0.18 g, 1.13 mmol), and NEt_3 (0.62 mL, 4.48 mmol) in BuOH (20 mL) was refluxed for 18 h. The solution was cooled and evaporated in vacuo, and the residue was flash chromatographed on a silica gel column in solvent S_1 to give 5-amino-4-chloro-6-((4-(hydroxymethyl)spiro[3.3]hept-6-yl)amino)pyrimidine (**10**, 0.11 g, 69%) as a yellow solid homogeneous on TLC (S_1). UV max (EtOH) 298 nm (ε 9250), 275 (ε 7400), 206 (ε 21 250). FAB-MS (rel intensity): 271 and 269 (M + H, 0.4 and 1.0), 241 and 239 (11.3 and 33.4), 102 (100.0).

A mixture of compound **10** (0.1 g, 0.37 mmol), $TsOH{\cdot}H_2O$ (0.14 g, 0.73 mmol,) and triethyl orthoformate (10 mL) in DMF (5 mL) was stirred for 16 h at room temperature. Triethylamine (0.10 mL, 0.73 mmol) was added, and the solution was evaporated to a brown syrup. A faster moving product (probably compound **11b**) observed on analytical TLC (S_1) disappeared after preparative separation in the same solvent system. The latter afforded 6-chloro-*N*^{9}-(2-(hydroxymethyl)-spiro[3.3]hept-6-yl)purine (**11a**, 81 mg, 79%) as a yellow solid: UV max (EtOH) 266 nm (ε 8650), 208 (ε 23 200). FAB-MS (rel intensity): 281, 279 (M + H, 34.4, 100.0).

A stream of NH_3 was introduced at −70 °C into a solution of compound **9** (75 mg, 0.27 mmol) in 20% NH_3 in methanol (30 mL) which was placed in a stainless steel bomb. The bomb was heated at 100 °C (bath temperature) for 16 h. After cooling, the contents were evaporated to give a brown syrup which was purified by preparative TLC (S_1) to give compound **2** (60 mg, 86%), mp 186–188 °C. HPLC (Synchropak RP-P, H_2O, 0.5 mL/min, 261 nm, reversed phase): t_R 6.80 (100%). UV max (EtOH) 261 nm (ε 14 600), 209 (ε 19 500). IR (KBr): 3350 cm^{-1} (ms, NH), 3180 (bms, OH). ^1H NMR (DMSO-d_6): ∂ 8.21, 8.07, 7.16, 4.77, 4.45, 3.30, 2.55, 2.36, 2.25, 2.13. ^{13}C NMR 156.34, 152.61, 149.75, 139.84, 119.46, 65.51, 44.34, 42.72, 42.44, 36.86, 36.12, 33.71, 32.35. FAB-MS (rel intensity): 260 (M + H, 20.5), 259 (M, 100.0), 161 (9.0), 136 (45.1), 91 (24.2), 57 (13.4). Anal. Calcd for $C_{13}H_{17}N_5O$: C, 60.21; H, 6.61; N, 27.01. Found: C, 59.98; H, 6.45; N, 26.88.

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