

RING-OPENING OF 3- β -D-RIBOFURANOSYL-3,7,8,9-TETRAHYDROPYRIMIDO [1,2-*i*]PURIN-8-OL AND PREPARATION OF 2-THIO- AND 2-AZA-ADENOSINE DERIVATIVES

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□ The adduct 3- β -D-ribofuranosyl-3,7,8,9-tetrahydropyrimido[1,2-*i*]purin-8-ol (**2**), obtained from adenosine and epichlorohydrin, underwent ring fission at basic conditions. The initial ring-opening took place at C2 of the pyrimidine unit resulting in 2-(5-amino-1- β -D-ribofuranosyl-imidazol-4-yl)-1,4,5,6-tetrahydropyrimidin-5-ol (**3**). Also the tetrahydropyrimidine ring of **3** could be opened resulting in 5-amino-1-(β -D-ribofuranosyl)-imidazole-4-(*N*-3-amino-2-hydroxyl-propyl)-carboxamide (**4**). In hot acid conditions, **2** was both deglycosylated and ring-opened yielding 2-(5-amino-imidazol-4-yl)-1,4,5,6-tetrahydropyrimidin-5-ol (**7**) as the final product. When reacting **3** with CS₂ or HNO₂ ring-closure took place and 3- β -D-ribofuranosyl-3,4,7,8,9-pentahydropyrimido[1,2-*i*]purin-8-ol-5-thione (**5**), and 3- β -D-ribofuranosyl-imidazo[4,5-*e*]-3,7,8,9-tetrahydropyrimido[1,2-*c*][1,2,3]triazine-8-ol (**6**), respectively, were obtained. Also, the pyrimidine ring of the epichlorohydrin adduct with adenine, 10-imino-5,6-dihydro-4*H*,10*H*-pyrimido[1,2,3-*cd*]purin-5-ol (**10**), underwent ring fission and the product was identified as 3-hydroxy-1,2,3,4-tetrahydroimidazo[1,5-*a*]pyrimidine-8-carboximidamide (**11**).

Keywords Adenosine; ring opening; adduct; epichlorohydrin

INTRODUCTION

The ring-opening of 1,*N*⁶-etheno adenine derivatives has been studied by Baker and Joseph,^[1] Montgomery,^[2] Tsou,^[3] and others.^[4] They used the reaction for the preparation of 2-aza-1,*N*⁶-etheno-adenosine from 1,*N*⁶-etheno-adenosine. Since then, adenine ring opening reaction has been extensively studied and used for various synthetical purposes.^[5,16] For example, Koomen describes aminolytic ring opening,^[6] and a way to use

Received 23 June 2008; accepted 20 August 2008.

Financial support for this work was obtained from Magnus Ehrnrooth Foundation, Finland.

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the fact that amine ring opening is reversible to avoid permanent ring opening at basic conditions.^[7] Adenine ring opening also occurs in the biosynthesis of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), the source of imidazole-moieties for purines and histidine in the cell. A chemical simulation of the biochemical reaction path has been developed by Ranganathan.^[8] Ring opening is also one of the possible fates of the 1,*N*⁶-ethenoadenine adduct in DNA.^[9]

The ring-opening mechanism has the first step in common with the Dimroth rearrangement, and was originally observed in 1888 by Rathke, and in 1909 Dimroth provided a mechanistic explanation for the reaction.

To our knowledge, the ring-opening of 1,*N*⁶-hydroxypropanoadenosines has not been studied previously. In the current work, we have explored the ring-opening and utilized the reaction for the preparation of 2-thio and 2-azaadenosine derivatives.

RESULTS AND DISCUSSION

The preparation of **2** has earlier been described by Solomon^[10] and Sund and Kronberg.^[11] In the current work, the epichlorohydrin reaction was performed at pH 3.2 to avoid further attack by epichlorohydrin on **2**.^[11] When **2** is held at pH 9 and 60°C for a few days, ring opening occurs at C2, giving compound **3**. The symmetry of the formed tetrahydropyrimidine ring was evident from the ¹H NMR spectrum where the two axial H10 protons exhibited equivalent shifts. This was also found to be the case of the equatorial protons. Also, the H2 signal has disappeared, and C6 ($\delta = 152.3$ ppm) shows correlations to H10a and H10b.

When **2** is held at pH 12 and 60°C for 24 hours, the initial ring-opening is followed by a second ring-fission, which involves the hydroxypropano-ring, giving **4**. The ¹H NMR spectrum of the imidazole derivative shows the presence of an amide proton signal at $\delta = 7.46$ ppm and separate signals for H10 ($\delta = 3.25$ and 3.21 ppm) and H12 ($\delta = 2.65$ and 2.54 ppm), that is, the propano-moiety is no longer symmetric. Comparison of the ¹³C NMR spectrum to that of a similar compound, 5-amino-1-(β -D-2'-deoxyribofuranosyl)imidazole-[4-¹⁵N]-carboxamide), also supports this interpretation.^[19] The mechanism for the hydrolysis is thought to occur by an attack of a hydroxide ion on C6 with subsequent ring-opening between C6 and one of the tetrahydropyrimidine nitrogens.^[12]

It was also found that treatment of **2** with 20% H₃PO₄ at 90°C gives the aglycon **7**. Compound **7** could also be obtained by deglycosylation at low pH of deoxy-**3**, the 2'-deoxyribose analogue of **3** (which was obtained in the same way as **3**). Acidic ring-opening of ethenoadenosine and 1-methyladenosine has been previously described in the literature.^[13]

When **3** was treated with CS₂ or HNO₂, ring-closure took place and 2-thio- and 2-azaadenosine derivatives were obtained. In the product **5**, the symmetry of the propano-moiety was lost and a new carbon signal at $\delta = 175.6$ ppm was observed. The carbon signal was assigned to C2 based on a HMBC-correlation to H10a ($\delta = 4.97$ ppm) in the propano-moiety. The ¹³C NMR shift of C2 is consistent with that of a thioamide (compared to 1,3-dimethylthiourea and 2-imidazolidinethione; SDBSWeb at <http://www.aist.go.jp/RIODB/SDBS/>, National Institute of Advanced Industrial Science and Technology). The broad peak in the ¹H NMR spectrum at $\delta = 9.16$ ppm could be attributed to the N3-thioamide proton.

The product from ring-closure by HNO₂ is compound **6**. High-resolution MS confirms the presence of six nitrogens in the structure. The ¹H NMR spectrum shows separate signals for H10 ($\delta = 4.30$ and 4.20 ppm) and H12 ($\delta = 3.49$ and 3.33 ppm).

In the reaction of epichlorohydrin and adenine at neutral conditions, **8** and **9** are formed.^[14] In the current work, it was found that **10** can be obtained by ring-closure of **8** and **9** at the same conditions. The product of ring-opening of **10** at neutral conditions is **11**. In the NMR spectra of the compound, the carbon signal of C2 is missing and the signals of C4 and C8 shows correlations to protons in the propane moiety. However, there is also the possibility that C8 is eliminated by ring opening, as this would also fit 2D NMR data. This possibility could be excluded since the ¹³C NMR shifts of **11** is very similar to shifts reported for analogous compounds^[15,16] and since the UV spectrum of **11** have roughly the same features as the spectrum of aglycon **7**, indicating similar conjugation in both compounds.

CONCLUSIONS

The initial steps of both Dimroth rearrangement and all ring openings presented in this paper (and even deaminations of adenosine analogs), is an amidine hydrolysis. In the unmodified purine core of adenosine and adenine, the amidine group at N7-C8-N9 is the target for the hydrolysis and the final outcome is opening of the imidazole ring.^[17] But when one of the N1- or N3-nitrogens of the pyrimidine ring is alkylated, as in the case of **2**, the electrophilic amidine-group at N1-C2-N3 becomes the preferred target for the hydrolysis and opening of the pyrimidine ring becomes possible. Generally, the amidine hydrolysis is initiated by a hydroxide attack on the protonated starting material. As it is known that l-hydroxyisoguanine also undergoes ring opening, the electrophilic target may also be a hydroxycarbamide function.^[18]

In this work, several adenosine and adenine analogs were made by ring opening of epichlorohydrin adducts **2** and **10**. In the case of compound

3, the ring can be reclosed by CS₂ or HNO₂, yielding 2-thio- and 2-azaadenosine derivatives.

MATERIALS AND METHODS

(Caution: Epichlorohydrin has been found to be carcinogenic in mice and rats and has been classified as a probable human carcinogen.)

Chemicals

Distilled water purified with a Millipore system (Simplicity 185, USA) was used in the reactions and for all chromatography. The epichlorohydrin was racemic. Adenosine (Adenine-9- β -D-ribofuranoside) was purchased from Fluka AG (Switzerland). Deoxyadenosine (Adenine-9- β -D-2'-deoxyribofuranoside) was purchased from Sigma-Aldrich Chemie (Germany).

Chromatographic Methods

Analytical HPLC was performed on an Agilent 1100 series liquid chromatograph equipped with a quaternary pump, a diode array detector (used wavelength range: 190 to 400 nm) and the Chemstation software. The column employed was a Hypersil BDS-C18, 5 μ m, 4.0 \times 125 mm. It was eluted isocratically for 2 minutes with 2% acetonitrile in 10 mM ammonium acetate (pH 7) and then with a gradient from 2 to 20% over the course of 18 minutes at a flow rate of 0.5 mL/minute. The UV spectra were recorded while the compounds eluted from the column.

Semipreparative HPLC was performed on a Varian 5000 liquid chromatograph equipped with a UV detector working at 254 nm or on the Agilent 1100 system (see above) equipped with an Agilent fraction collector.

Spectroscopic and Spectrometric Methods

The mass spectrometry analyses were performed on an Agilent 1100 Series LC/MSD SL Trap system (Agilent Technologies, Finland), which was equipped with an API electrospray interface and operated in the positive ion mode. Nitrogen was used as nebulizer gas (40 psi) and as drying gas (12 L/minute). The drying gas was heated to 350°C. The capillary exit offset had a value of 118.0 V and skim 1 was set at 40.0 V. In the scan mode, the maximum ion accumulation time was 10 ms and the target (ion) value was 20,000. Scanning from m/z 100 to 500 was applied for recording of the full scan mass spectra. Collision induced dissociation (CID) experiments coupled with multiple tandem mass spectrometry (MSⁿ) employed helium as collision gas. The fragmentation amplitude was 1.0 V. An Agilent 254 nm wavelength UV detector was also coupled to the system,

and the LC conditions were identical to the analytical HPLC described above, with the exception of the pump, which was of binary type. The high-resolution mass spectrum for compound **6** was recorded on a LC/MSD TOF mass spectrometer (Agilent G1969, USA). The system was operated in the positive ion mode, and it was tuned and calibrated using the automated functions, CheckTune and Calibrate, using Agilent TOF Electrospray Calibrant Solution (G1969–85001). The pressure of the nebulizer gas was 35 psig and the drying gas flow was 12 L/minute. The drying gas was heated to 300°C. Scanning from m/z 100 to 1000 was applied for recording of the full scan mass spectra. The fragmentor voltage was 150 V, skimmer voltage was 60 V and Oct RF was 250 V. The infusion rate was 10 μ L/minute, and masses were measured as an average of 11 measurements, with reference masses at 121.050873 and 922.009798. The high resolution mass spectra for all other compounds were recorded on an Agilent 6220 Accurate-Mass TOF mass spectrometer using ESI ionization in conjunction with HPLC, and operated in positive ion mode. The Sciex software from Perkin-Elmer was utilized. To prepare NMR samples, the solutions of the pure compounds were evaporated to dryness, then residual water was removed by coevaporation twice with 5 mL acetonitrile. DMSO-*d*₆, 800 μ L, was added and the solutions were filtered through a coarse-porosity sintered glass filter into NMR tubes. The NMR spectra were recorded on a Bruker Avance 600 spectrometer operating at 600.13 MHz for ¹H and 150.90 MHz for ¹³C (Bruker Biospin Corporation). The solvent was DMSO-*d*₆, residual non-deuterated solvent was used as internal reference for ¹H NMR (at 2.50 ppm). For ¹³C NMR the solvent signal was used as reference (at 39.5 ppm). The ¹H NMR signal assignments were based on chemical shifts and ¹H-¹H and ¹³C-¹H correlation data. Assignment of carbon signals was based on chemical shifts and ¹³C-¹H correlations.

Calculation of Yields

Product yields were determined in the following way: Quantitative ¹H NMR was performed, using residual undeuterated solvent as an internal standard (the concentration of undeuterated solvent was determined for the solvent lot used). HPLC standard solutions were prepared by diluting aliquots from the NMR samples. The standards and the reaction mixtures were analyzed by HPLC, and the peak areas in standards and reaction mixtures were compared at 254 nm.

*3-β-D-ribofuranosyl-3,7,8,9-tetrahydropyrimido[1,2-*i*]purin-8-ol (2)*

Adenosine, (4 mmol, 1070 mg) was dissolved in 0.5 M potassium phosphate buffer (50 ml) at pH 3.2 (pH was adjusted with phosphoric acid). Epichlorohydrin, (3.2 ml, 35 mmol), was added and the mixture was stirred and held at 37°C for 2 days. Excess epichlorohydrin was removed by rotary

evaporation, giving an oily and crystalline residue, which was suspended in water (15 ml) and pH adjusted to 6.5 with 6 M sodium hydroxide solution. The mixture was again rotary evaporated, and coevaporated three times with dry methanol (20 ml). To separate phosphate salts, the residue was washed three times with dry methanol (15 ml, each time vigorously shaken), and the washings combined and evaporated to an oil. Dry acetone (20 ml) was added (causing precipitation) and the mixture vigorously stirred until the precipitate was evenly distributed. The mixture was centrifuged and the supernatant was discarded (containing epichlorohydrin hydrolysates). The precipitate was washed with acetone in this way three times. The white precipitate was dissolved in dry methanol (3 ml), and purified on a short silica column, eluted with a solvent gradient from acetone to methanol to water, all eluents contained 10 mM TFA. The product still contained some epichlorohydrin hydrolysis products, but was usable for the next step. Yield 50% (of pure product, excluding contaminations). MS(ESI): m/z 324 (100%, $M+H^+$).

2-(5-amino-1- β -D-ribofuranosyl-imidazol-4-yl)-1,4,5,6-tetrahydropyrimidin-5-ol (3)

A solution of compound **2** (622 mg, 2 mmol) in water (15 ml) was held at pH 9 and 60°C for 2 days. The pH was adjusted with 6 M NaOH each hour in the beginning of the reaction, then twice daily. The yield before purification was 26%. The product was purified by repeated semipreparative HPLC runs on the Varian LC, the column was a Thermo Hypersil Keystone (BDS Hypersil C18), 250 \times 10 mm, 5 μ m particle size, the buffer was 10 mM ammonium acetate at pH 7 with an acetonitrile gradient. The flow rate was 3 ml/minutes. UV spectrum: λ_{\max} 224, 225 (sh), 283 nm, λ_{\min} 249 nm, $\epsilon = 5000 \text{ M}^{-1}\text{cm}^{-1}$. ^1H NMR (DMSO-*d*6): $\delta = 7.52$ (s, 1 H, H8); 5.54 (d, J = 5.4 Hz, 1 H, H1'); 4.24 (t, J = 5.5 Hz, 1 H, H2'); 4.05 (t, J = 4.2 Hz, 1 H, H3'); 3.99 (m, 1 H, H11); 3.90 (m, J = 2.6 Hz, 1 H, H4'); 3.59 (qd, J = 11.5 and 2.6 Hz, 2 H, H5a' and H5b'); 3.39 (br d, J = 12.3 Hz, 2 H, 2 \times H10a); 3.21 (br d, J = 12.3 Hz, 2 H, 2 \times H10b). ^{13}C NMR (DMSO-*d*6): = 152.3 (C6); 141.8 (C4); 131.3 (C8); 107.4 (C5); 87.8 (C1'); 73.4 (C2'); 70.1 (C3'); 57.7 (C11); 85.7 (C4'); 60.9 (C5'); 44.81 and 44.82 (C10). MS(ESI): m/z 314 (100%, $M+H^+$). MS² of m/z 314: 182 (100%, $M+H^+$ -ribosyl+H). MS³ of m/z 314 \rightarrow 182: 127 (100%), 110 (66%), 139 (33%). HRMS(ESI) m/z 314.1460 ($M+H^+$, calcd. for $\text{C}_{12}\text{H}_{19}\text{N}_5\text{O}_5+H^+$ 314.1459).

5-amino-1-(β -D-ribofuranosyl)-imidazole-4-(N-3-amino-2-hydroxyl-propyl)-carboxamide (4)

A solution of unpurified compound **2** (196 mg, 0.6 mmol) in 0.085 M phosphate buffer (9 ml) was held at pH 12 and 70°C for 25 hours. The yield before purification was 24%. The mixture was neutralized with phosphoric acid and purified by repeated semipreparative HPLC runs on the Varian

LC with a Hypersil C18 column (250 × 10 mm). The buffer was 10 mM phosphate at pH 7 with an acetonitrile gradient, and the flow rate was 3 ml/minutes. UV spectrum: λ_{\max} 268 nm, λ_{\min} 221 nm, $\epsilon = 8600 \text{ M}^{-1}\text{cm}^{-1}$. ^1H NMR (DMSO-*d*₆): $\delta = 7.46$ (t, $J = 6 \text{ Hz}$, 1 H, amide NH); 7.34 (s, 1 H, H8); 5.48 (d, $J = 5.6 \text{ Hz}$, 1 H, H1'); 4.26 (m, 1 H, H2'); 4.05 (m, 1 H, H3'); 3.89 (m, 1 H, H4'); 3.61 (d, $J = 5.1 \text{ Hz}$, H11); 3.58 (s, H5a' and H5b'); 3.25 (m, 1 H, H10a); 3.21 (m, 1 H, H10b); 2.65 (m, $J = 10.5 \text{ Hz}$, 1 H, H12a); 2.54 (m, H12b). ^{13}C NMR (DMSO-*d*₆): $\delta = 164.8$ (C6); 142.6 (C4); 128.7 (C8); 112.4 (C5); 87.6 (C1'); 85.4 (C4'); 73.0 (C2'); 70.1 (C3'); 69.1 (C11); 61.0 (C5'); 44.0 (C12); 41.5 (C10). MS(ESI): m/z 332 (100%, M+H⁺). MS² of m/z 332: 200 (100%, M+H⁺-ribosyl+H), 183 (34%), 314 (18%), 182 (16%). MS³ of m/z 332→200: 183 (100%), 182 (61%). HRMS(ESI) m/z 332.1562 (M+H⁺, calcd. for C₁₂H₂₁N₅O₆+H⁺ 332.1565).

2-(5-amino-imidazol-4-yl)-1,4,5,6-tetrahydropyrimidin-5-ol (7)

Method A: A solution of **2** (50 mg, 0.28 mmol) in 20% H₃PO₄ (5.1 ml) was held at 90°C for one day, the yield of **7** before purification was 64%. Five ml water was added, and pH was adjusted to 9.6 with 7 ml of 6 M NaOH. The volume was reduced to 12 ml by rotary evaporation, the solution was allowed to stand in a fridge overnight and filtered the following day. The filtrate was evaporated to 5 ml and filtered again, and flashed on a silica column, eluted with a solvent gradient from methanol to water, all eluents contained 0.01 M pyridine. UV spectrum: λ_{\max} 202, 222, 283 nm, λ_{\min} 214, 247 nm, $\epsilon = 4900 \text{ M}^{-1}\text{cm}^{-1}$. MS(ESI): m/z 182 (100%, M+H⁺). MS² of m/z 182: 127 (100%), 110 (86%), 139 (45%).

Method B (used for identification): The pH of a water solution of deoxy-**3** (obtained by a method similar to that for **3**) was adjusted to 1.8 with H₃PO₄, and the solution was then held at 70°C for 8 hours. The aglycon was analyzed by LC-MS and UV-spectroscopy, and the compound was found to be identical to that obtained by method A.

3-β-D-ribofuranosyl-3,4,7,8,9-pentahydropyrimido[1,2-*i*]purin-8-ol-5-thione (5)

To a solution of purified compound **3** (10 mg, 0.032 mmol) in DMSO (1 ml) was added K₂CO₃ (125 mg) and a solution of CS₂ (100 μl) in DMSO. After one day at ambient temperature, 17 mM phosphate buffer (15 ml) at pH 4.7 was added and the solution was washed twice with dichloromethane (5 ml) and rotary evaporated to 5 ml. The product was purified by repeated semipreparative HPLC runs on the Agilent 1100 system with a Thermo Hypersil BDS C18 column (250 × 10 mm). The buffer was 2 mM ammonium acetate at pH 7 with an acetonitrile gradient, and the flow rate was 2 ml/minutes. The yield after purification was 33%. UV spectrum: λ_{\max} 208, 237, 296 nm, λ_{\min} 224, 258 nm, $\epsilon = 4000 \text{ M}^{-1}\text{cm}^{-1}$. ^1H NMR (DMSO-*d*₆): δ

= 9.16 (br, 1 H, NH); 8.20 (s, 1 H, H8); 5.76 (d, $J = 6.1$ Hz, 1H, H1'); 5.47 (d, $J = 2.5$ Hz, 1 H, OH); 5.45 (d, $J = 6.0$ Hz, 1 H, OH); 5.24 (dd, $J = 6.4$ and 5.1, 1 H, OH5'); 5.17 (d, $J = 3.4$ Hz, 1 H, OH); 4.97 (dt, $J = 14.3$ and 3.2 Hz, 1 H, H10a); 4.50 (t, $J = 5.5$ Hz, 1 H, H2'); 4.32 (quin, $J = 3.1$ Hz, 1 H, H11); 4.11 (dd, $J = 4.3$ and 3.3 Hz, 1 H, H3'); 3.93 (q, $J = 3.3$ Hz, 1 H, H4'); 3.86 (d, $J = 14.3$ Hz, 1 H, H10b); 3.64 (dd, $J = 12.1$ and 3.5 Hz, 1 H, H5a'); 3.53 (dd, $J = 12.2$ and 3.2 Hz, 1 H, H5b'); 3.45 (dd, $J = 12.8$ and 1.7 Hz, 1H, H12a); 3.27 (dt, $J = 13.2$ and 2.9 Hz, 1 H, H12b). ^{13}C NMR (DMSO-*d*6): $\delta = 175.6$ (C2); 148.3 (C6); 147.5 (C4); 139.2 (C8); 112.8 (C5); 86.9 (C1'); 53.1 (C10); 73.6 (C2'); 58.3 (C11); 70.5 (C3'); 85.8 (C4'); 61.5 (C5'); 44.3 (C12). MS(ESI): m/z 356 (100%, M+H⁺). MS² of m/z 356: 224 (100%, M+H⁺-ribosyl+H). MS³ of m/z 356→224: 180, (100%), 170 (25%), 169 (18%), 151 (17%), 206 (16%), 152 (11%). HRMS(ESI) m/z 356.1027 (M+H⁺, calcd. for C₁₃H₁₇N₅O₅S+H⁺ 356.1023).

3-β-D-ribofuranosyl-imidazo[4,5-e]-3,7,8,9-tetrahydropyrimido[1,2-c][1,2,3]triazine-8-ol (6)

To a solution of purified compound **3** (8 mg, 0.026 mmol) in water (7 ml), cooled with an ice bath, was added 12 M HCl (1.7 ml) and then a cooled solution of KNO₂ (170 mg) in water (1.5 ml). The reaction was held on the ice bath for an hour, and then allowed to slowly reach ambient temperature. The mixture was then neutralized with Na₂HPO₄ and NaOH, filtered and purified by repeated semipreparative HPLC runs on the Agilent 1100 system with a Thermo Hypersil BDS C18 column (250 × 10 mm). The buffer was 2 mM ammonium acetate at pH 7 with an acetonitrile gradient, and the flow rate was 2 ml/minutes. The yield after purification was 31%. UV spectrum: λ_{max} 230, 258 (sh), 307 nm, λ_{min} 211, 283 nm, $\epsilon = 16000 \text{ M}^{-1}\text{cm}^{-1}$. ^1H NMR (DMSO-*d*6): $\delta = 8.42$ (s, 1 H, H8); 5.92 (dd, $J = 5.5$ Hz, 1 H, H1'); 4.49 (dt, $J = 5.2$ Hz, 1 H, H2'); 4.30 (d, $J = 12.9$ Hz, 1 H, H10a); 4.20 (1 H, H10b); 4.18 (1 H, H11); 4.13 (t, $J = 4.2$ Hz, 1 H, H3'); 3.96 (q, $J = 4.0$ Hz, 1 H, H4'); 3.66 (dd, $J = 12.0$ and 4.2 Hz, 1 H, H5a'); 3.55 (dt, $J = 11.9$ and 3.6 Hz, 1 H, H5b'); 3.49 (d, $J = 16.2$ Hz, 1 H, H12a); 3.33 (dt, $J = 16.3$ and 3.1 Hz, 1 H, H12b). ^{13}C NMR (DMSO-*d*6): $\delta = 141.1$ (C8); 138.6 (C4); 125.7 (C5); 88.2 (C1'); 85.8 (C4'); 74.4 (C2'); 70.1 (C3'); 61.1 (C5'); 60.2 (C11); 55.4 (C12); 49.8 (C10). MS(ESI): m/z 325 (100%, M+H⁺). MS² of m/z 325: 193 (100%, M+H⁺-ribosyl+H). MS³ of m/z 356→193: 147 (100%), 120 (59%), 93 (17%), 137 (10%). HRMS(ESI) m/z 325.1256 (M+H⁺, calcd. for C₁₂H₁₆N₆O₅+H⁺ 325.1255).

General Procedure for Compounds 8, 9, and 10

To a solution of adenine (283.4 mg, 2.1 mmol) in 0.1 M phosphate buffer (160 ml) at pH 7 was added epichlorohydrin (1 ml, 13 mmol). The stirred reaction was allowed to proceed for one day in room temperature,

then the mixture was washed with toluene (20 ml), evaporated to 12 ml and filtered. The filtrate was purified by repeated semipreparative HPLC runs on the Varian 5000 chromatograph with a Hypersil C18 column (250 × 10 mm). The mobile phase was 5 mM phosphate buffer at pH 7 with an acetonitrile gradient, and the flow rate was 3 ml/minutes. The fractions were analyzed by LC-MS, and NMR spectra were recorded for those fractions containing **8**, **9**, and **10**.

1-(6-amino-3H-purin-3-yl)-3-chloropropan-2-ol (8)

UV spectrum: λ_{\max} 213, 276 nm, λ_{\min} 246 nm. ^1H NMR (DMSO-*d*6): δ = 8.21 (s, 1 H, H2); 8.03 (br, 1 H); 7.89 (br, 1 H); 7.76 (s, 1 H, H8); 4.54 (dd, J = 13.4 and 3.1 Hz, 1 H, H10a); 4.27 (m, 1 H, H11); 4.16 (dd, J = 13.4 and 8.8 Hz, 1 H, H10b); 3.73 (dd, J = 11.2 and 4.8 Hz, 1 H, H12a); 3.65 (dd, J = 11.2 and 5.4 Hz, 1 H, H12b). ^{13}C NMR (DMSO-*d*6): δ = 155.1 (C6); 152.2 (C8); 149.7 (C4); 144.3 (C2); 120.3 (C5); 67.4 (H11); 52.9 (C10); 47.3 (C12). MS(ESI): m/z 228 (100%, M+H⁺), 230 (32%, M+H⁺).

1-(6-amino-9H-purin-9-yl)-3-chloropropan-2-ol (9)

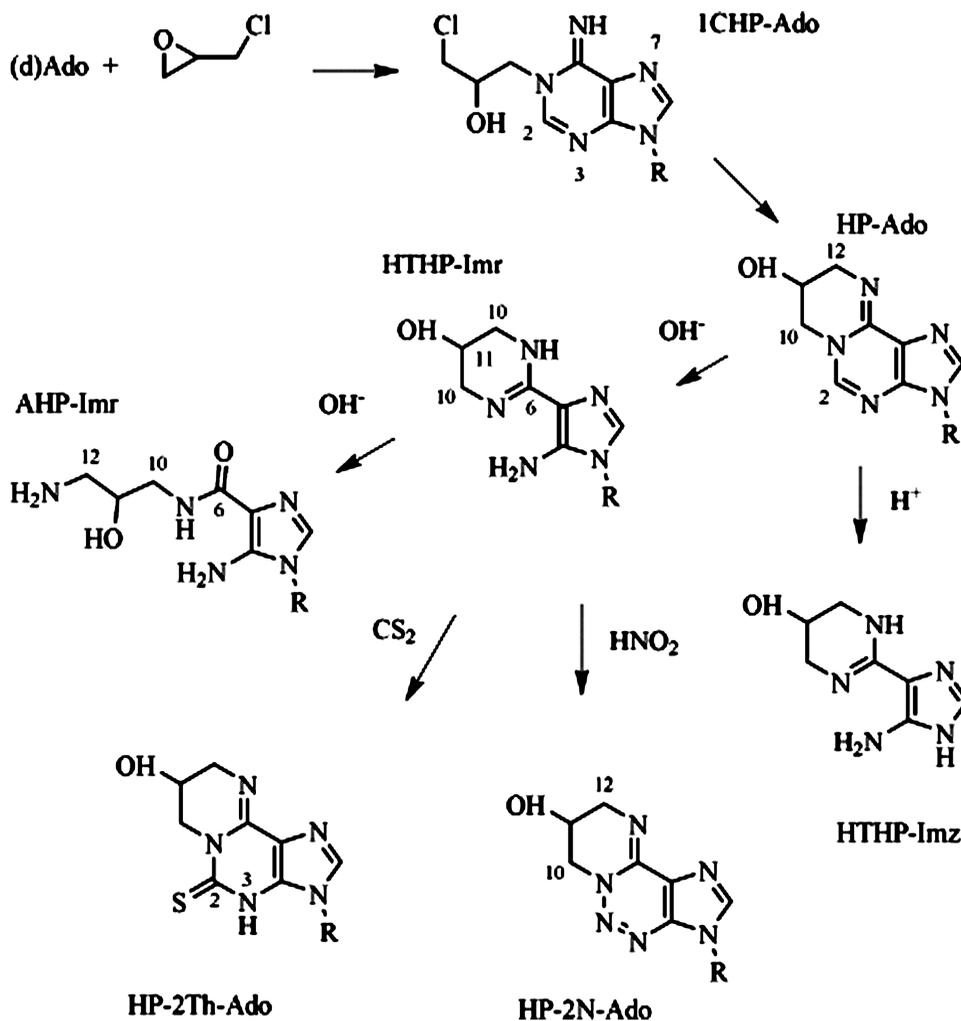
UV spectrum: λ_{\max} 207, 262 nm, λ_{\min} 228 nm. ^1H NMR (DMSO-*d*6): δ = 8.14 (s, 1 H, H2); 8.05 (s, 1 H, H8); 7.20 (br, 2 H, NH₂); 5.68 (br, 1 H, OH); 4.31 (m, 1 H, H10a); 4.12 (m, 1 H, H11); 4.11 (m, 1 H, H10b); 3.66 (dd, J = 11.3 and 4.4 Hz, 1 H, H12a); 3.58 (dd, J = 11.3 and 4.8 Hz, 1 H, H12b). ^{13}C NMR (DMSO-*d*6): δ = 155.9 (C6); 152.3 (C2); 149.6 (C4); 141.5 (C8); 118.6 (C5); 68.4 (C11); 47.2 (C12); 46.5 (C10). MS(ESI): m/z 228 (100%, M+H⁺), 230 (32%, M+H⁺).

10-imino-5,6-dihydro-4H,10H-pyrimido[1,2,3-*cd*]purin-5-ol (10)

UV spectrum: λ_{\max} 209, 268 nm, λ_{\min} 241 nm. ^1H NMR (DMSO-*d*6): δ = 8.35 (s, 1 H, H2); 8.30 (s, 1 H, H8); 4.47 (quin, J = 2.5 Hz, 1 H, H11); 4.34 (dt, J = 13.5 and 3.0 Hz, 1 H, H10a); 4.26 (d, J = 13.3 Hz, 1 H, H10b); 3.57 (dd, J = 13.9 and 1.7 Hz, 1 H, H12a); 3.44 (dt, J = 13.7 and 3.0 Hz, 1 H, H12b). ^{13}C NMR (DMSO-*d*6): δ = 148.2 (C4); 147.7 (C2); 143.6 (C8); 118.0 (C5); 57.6 (C11); 53.1 (C10); 45.5 (C12); C6 not observed. MS(ESI): m/z 192 (100%, M+H⁺). MS² of m/z 192: 148 (100%), 107 (21%). HRMS(ESI) m/z 192.0884 (M+H⁺, calcd. for C₈H₉N₅O+H⁺ 192.0880).

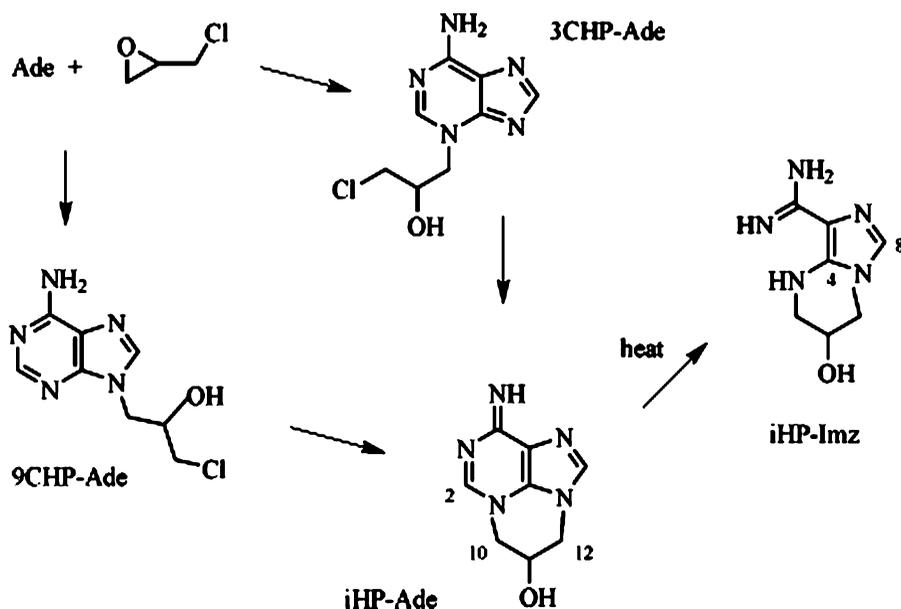
3-hydroxy-1,2,3,4-tetrahydroimidazo[1,5-*a*]pyrimidine-8-carboximidamide (11)

Fractions containing **8**, **9** and **10** were pooled and held at 70°C for five days, then evaporated to 10 ml, filtered, and the filtrate was purified by repeated semipreparative HPLC runs on the Varian 5000 chromatograph with a Thermo Hypersil BDS C18 column (250 × 10 mm). The buffer was 1 mM ammonium acetate at pH 7 with an acetonitrile gradient, and the



SCHEME 1 Structures and reactions of adenosine adducts. R = ribose. Purine numbering is retained for all compounds with the exception of IUPAC names.

flow rate was 3 ml/minutes. Yield 6%. UV spectrum: λ_{max} 207, 243 (sh), 297 nm, λ_{min} 256 nm, $\epsilon = 1200 \text{ M}^{-1}\text{cm}^{-1}$. ^1H NMR (DMSO-*d*₆): $\delta = 7.36$ (s, 1 H, H8); 4.13 (quin, $J = 3.2$ Hz, 1 H, H11); 3.99 (dd, $J = 12.7$ and 3.0 Hz, 1 H, H12a); 3.89 (dq, $J = 12.7$ and 3.8 Hz, 1 H, H12b); 3.31 (dd, $J = 12.6$ and 1.7 Hz, H10a); 3.24 (ddd, $J = 12.8$, 4.4 and 1.5 Hz, H10b). ^{13}C NMR (DMSO-*d*₆): $\delta = 158.0$ (C6); 141.3 (C4); 132.7 (C8); 105.1 (C5); 58.5 (C11); 47.6 (C12); 44.7 (C10). MS(ESI): m/z 182 (100%, $\text{M}+\text{H}^+$). MS^2 of m/z 182: 165 (100%). MS^3 of m/z 182 \rightarrow 165: 121 (100%), 147 (99%). HRMS(ESI) m/z 182.1043 ($\text{M}+\text{H}^+$, calcd. for $\text{C}_7\text{H}_{11}\text{N}_5\text{O}+\text{H}^+$ 182.1036).



SCHEME 2 Structures and reactions of adenine adducts.

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