Pergamon

Tetrahedron, Vol. 53, No. 21, pp. 7181-7190, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00421-3

Purification of 9*N*-[(1'*R*,3'*R*)-*trans*-3'-Hydroxycyclopentanyl]adenine HCl: a Combination of Theory and Experiment

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Abstract: This paper describes the preparation of 9*N*-[(1'*R*,3'*R*)-*trans*-3'-hydroxycyclopentanyl]-adenine (**1**), 3*N*-[(1'*R*,3'*R*)-*trans*-3'-hydoxycyclopentanyl]adenine (**2**) and 7*N*-[(1'*R*,3'*R*)-*trans*-3'-hydroxycyclopentanyl]adenine (**3**). Calculations and titrations to predict and determine the basicity of **1**, **2** and **3** are also disclosed. A method was devised to purify a mixture of silyl ethers **4**, **5** and **6** via an acid wash (to remove **5**; as supported by calculations and titrations) followed by recrystallization of **4** (to remove **6**). Subsequent desilylation of **4** gives desired **1**. © 1997 Elsevier Science Ltd.

INTRODUCTION

9*N*-[(1'*R*,3'*R*)-*trans*-3'-Hydroxycyclopentanyl]adenine (**1**•HCl, scheme 1) inhibits the formation of Tumor Necrosis Factor-alpha (TNF-α). Reaction of (1*S*,3*R*)-*cis*-3-*t*-butyldimethylsilyloxycyclopentanyl mesylate (**8**) with preformed sodium adenylate provided regioisomeric adenyl carbocycles 9*N*-[(1'*R*,3'*R*)-*trans*-3'-*t*-butyldimethylsilyloxycyclopentanyl]adenine (**4**), 3*N*-[(1'*R*,3'*R*)-*trans*-3'-*t*-butyldimethylsilyloxycyclopentanyl]adenine (**5**) and 7*N*-[(1'*R*,3'*R*)-*trans*-3'-*t*-butyldimethylsilyloxycyclopentanyl]adenine (**6**) in 65%, 11% and 2% yield, respectively (Scheme 1).[1] After chromatographic separation, treatment of **4**, **5** and **6** with HCl provided HCl salts **1**•HCl, **2**•HCl and **3**•HCl respectively in 96%, 87% and 64% yield. Whereas regioisomeric adenyl carbocycles **4**, **5** and **6** could be separated by chromatography, with the need to prepare kilogram quantities of **1** alternatives to chromatography were required. Literature data[2] on related compounds such as the pKa values for *N*-7 and *N*-3 substituted adenines together with initial observations that the co-products **5** (streaked out of the baseline) and **6**, had much lower R_f values than **4** (tlc), led one to hypothesize that the impurities might be removed via an acid wash. Based on literature data,[2] the pKa of **5** was expected to be higher than **4** or **6**. In order to support the hypothesis, titrations and several quantum calculations were performed on several of these compounds and their protonated forms.

Proton Affinity Calculation.

The calculation of the proton affinity of organic bases is usually done by computing the heats of formation of the base and its corresponding acid as shown in equation 1. If a comparison of proton affinities for two bases is to be done, equation 2 can be evaluated for the two bases and relative proton affinities can be calculated. This can be done for different bases or different atoms in the same base. The heats of formation can

be calculated for the base and its acid in aqueous media using the AM1-SM2 model.[3-6] Similarly calculation of the proton affinity of the organic base in vacuum can be done using equation 3 by computing the corresponding heats of formation.[5,6]

$$CH_3NH_3^+(aq) + Base(aq) \rightleftharpoons CH_3NH_2(aq) + BaseH^+(aq) \quad (1)$$

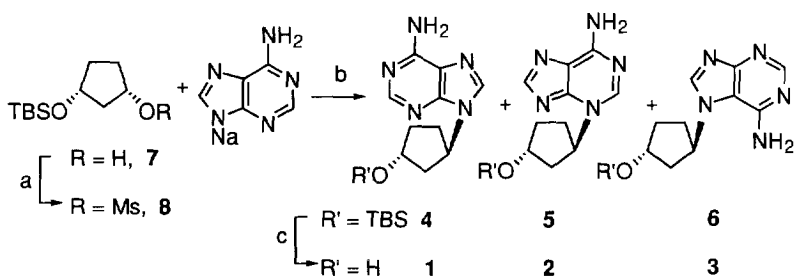
$$\Delta H_{rel} = \Delta H_{BaseH^+} - \Delta H_{Base} \quad (2)$$

$$CH_3NH_3^+(g) + Base(g) \rightleftharpoons CH_3NH_2(g) + BaseH^+(g) \quad (3)$$

The compounds chosen for these calculations were **1**, **2** and **3** since they had fewer atoms than **4**, **5** and **6**; though experimentally the separation was conducted on a mixture of **4**, **5** and **6**. The outcome of the calculations should not be affected by the additional *t*-butyldimethylsilyl group.

To calculate the proton affinities of **1**, **2** and **3** the heats of formation of the corresponding protonated forms is to be computed as well. Since each molecule has more than one possible protonation site, all possible protonation sites for the three molecules were considered. In the case of **1**, there are four possible sites corresponding to the H_2NC-6, the *N*-1, *N*-3 and *N*-7 positions. For **2**, the possible protonation sites again include H_2NC-6, *N*-1, *N*-7 and *N*-9 positions. For **3**, the possible protonation sites again include the H_2NC-6, *N*-1 position, *N*-3 and *N*-7 position. Compounds **1**, **2** and **3** were synthesized via scheme 1 and were subjected to titrimetry.

Scheme 1



a) TBME, MsCl (1.1 eq), Et_3N (1.2 eq), -10 to 0 °C; 95% crude. b) DMA, 65-70 °C; 65% **4**, 11% **5** and 2% **6**. c) 6N HCl, EtOH, rt; for **1**•HCl: 96%; for **2**•HCl: 87%; for **3**•HCl: 64%.

RESULTS AND DISCUSSION

Theoretical

The conformational search of structures **1**, **2** and **3** resulted in single low energy conformations. The lowest energy conformations of **1** after optimization using *ab initio* calculations showed a value of 15.9° for the torsion between the atoms C8-N9-C1'-C5'. The value for the corresponding torsion C8-N9-C1'-O1' in the crystal structure[7] of adenosine is 9.9°. The torsion C8-N9-C1'-C2' in the *ab initio* minimized structure of *N*-1

protonated form of **1** is -59.2° and the torsion between the atoms C8-N9-C1'-O1' in the crystal structure[7] of adenosine hydrochloride is -43.0°. The energies of the various structures calculated via the different quantum methods are listed in Table 1. The *ab initio* -derived, energy differences between the lowest energy form of the protonated products and the free base form of the compound are shown in Figure 1. The ΔH_{rel} calcuated by equation 2 for the three compounds in Figure 1, suggests that it would be easier to protonate compound **2** rather than **3** or **1**. The data in Figure 1 illustrates the relative stability and magnitude of energy change of the free base and the protonated form in vacuum for **1-3**.

The heats of formation for regioisomeric protonated products of **1**, **2** and **3** are shown in Table 1. The data for **1** leads to the conclusion that the preferred (low energy) protonation site is either at *N*-3 or *N*-1. The crystallographic[8] structure of the HCl salt of adenosine shows protonation at the *N*-1 position. Recent calculations in vacuum[9,10] using both semiempirical as well as ab initio methods on adenine suggest protonation at the *N*-3 or *N*-7 site in agreement with the ab initio calculations performed here. Reported studies[11,12] carried out in solution of the HCl salt show that the pyrimidine nitrogens in adenosine to be the most basic; this is in agreement with the continuum solvent model, AM1/SM2 computations.

Table 1: Calculated Energies of the Different Protonated Forms of Adenyl Derivatives **1**, **2**, and **3** and Their Free Bases

Method of Calculation	Free Base of **1**	Proton. at *N*-1	Proton. at *N*-3	Proton. at H_2NC-6	Proton. at *N*-7
AM1	32.34	174.279	173.353	191.442	179.938
AM1/SM2	9.187	105.718	109.757	112.59	112.8371
ab initio	-729.2708	-729.6732	-729.6745	-729.6274	-729.6588
	Free Base of **2**	Proton. at *N*-1	Proton. at H_2NC-6	Proton. at *N*-7	Proton. at *N*-9
AM1	43.916	201.683	191.442	175.225	173.156
AM1/SM2	15.583	120.192	112.589	112.837	113.561
ab initio	-729.2489	-729.6346	-729.6274	-729.6717	-729.6727
	Free Base of **3**	Proton. at *N*-1	Proton. at *N*-3	Proton. at H_2NC-6	Proton. at *N*-9
AM1	39.829	183.704	174.775	202.346	180.555
AM1/SM2	17.196	116.272	112.475	125.64	116.418
ab initio	-729.2575	-729.6578	-729.6782	-729.6139	-729.6705

*Units are in kcal/mol for AM1 and AM1/SM2 and Hartrees for *ab initio* calculations.

The AM1 calculations agree with the *ab initio* calculations. The Mulliken bond orders calculated on the *ab initio* optimized structures as well as on the low energy protonated forms confirm the resonance structure contributing to the stability of the protonated structure of **1**.[13a] The *ab initio* calculations on **2** (in vacuum) predict that *N*-9 or *N*-7 would be preferably protonated. The AM1-SM2 computations favor the H_2NC-6 protonation which is in agreement with solution studies of 3-methyl adenine.[3] Previous experimental data and calculations[4] using AM1-

SM1 and AM1-SM2 on charged forms of dopamine and other charged molecules, show that the conformation with the largest dipole moment is more stable in solution, although the gas phase calculation and data does not show the conformation with the largest dipole to be the most stable. In the case of **2**, H_2NC-6 protonation would produce the largest dipole moment compared with protonation at other sites. This is consistent with the previous

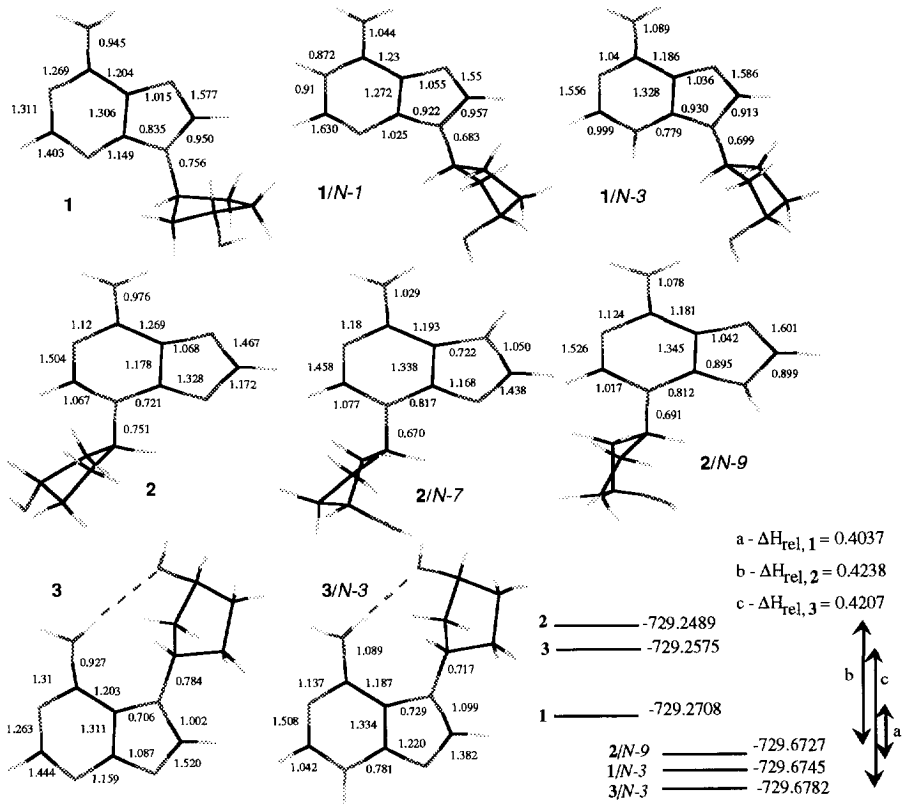


Figure 1: Energy profiles and Mulliken bond orders of the adenine ring for **1**, **2** and **3** with the lowest energy protonated form for each compound. Energy Units are Hartrees.

calculations[4]. The protonation of **2** at *N*-9 and *N*-7 restores "aromaticity" to the pyrimidine ring of adenine as shown by the contributing resonance structures.[13b] The Mulliken bond orders in Figure 1 for this ring increase suggesting increased electron delocalization. The calculations on **3** in vacuum show a hydrogen bond. However, the AM1-SM2 model calculation which has a continuum solvent approximation does not show this hydrogen bond which should be replaced by hydrogen bonds to water (the solvent). The protonation of **3** as calculated by the *ab initio* method occurs at the *N*-3 position.The expected protonation sites are expected to be similar to those of **1**, based on contributing resonance structures[13c] as well as computed energies. Considering that the heats of formation calculated by *ab initio* methods are more accurate than semi-empirical techniques, Figure 1 suggests that **2** should be more easily protonated than **3** which should be more easily protonated than **1**.

Titrimetry

The *N*-3 adenine derivative **2**•HCl has a pKa of 5.7. An equivalence point was observed during both the titration with sodium hydroxide solution as well as the back-titration with hydrochloric acid solution. The pKa of this *N*-3 adenine derivative is similar to other *N*-3 substituted adenines. The pKa of 3-benzyladenine has been reported[2] as 5.1. The *N*-7 and the *N*-9 adenine derivatives **3**•HCl and **1**•HCl have pKa's of 3.8 and 3.9, respectively. The apparent pKa of 7-methyladenine was reported[2] as 3.6 for a 1:1 DMF/H_2O solution. The pKa's for the *N*-7 **3**•HCl and *N*-9 **1**•HCl adenine derivatives were measured from the titration curves for the 0.10 N sodium hydroxide solution. Neither the *N*-7 **3** nor *N*-9 **1** derivative had an observable equivalence point for the 0.04 N hydrochloric acid solution back-titration. Both adenine derivatives are very weak bases; and therefore, at the analyte concentration and acid titrant strength used, *the equivalence point was not observed.*

CONCLUSION

In conclusion, the titrimetic data as well as the theoretical calculations suggest that a separation of **1** from a mixture of **1** and **2** (or derivatives thereof) could be done via an acid wash.[14] Using theoretical calculations one can predict the low-energy protonated forms of the structures, **1**, **2** and **3**. Compound **1** is calculated to preferentially, weakly protonate at the *N*-3 or *N*-1 position. Compound **2** is calculated to preferentially protonate at *N*-9 or *N*-7 in vacuum and at H_2NC-6 position in water in agreement with solution protonation data. Compound **3** is calculated to preferentially, weakly protonate at the *N*-3 position. A mixture of **4** and **5** (*O*-silylated derivative of **1** and **2**) in a 4:1 molar ratio was treated with an acid (0.1M $KHSO_4$). A salt of **5** is formed preferentially and **4** remains soluble in organic solvent and not protonated. Due to the lack of regioselectivity in the alkylation of purine like bases in carbocylic nucleosides as well as nucleosides, we believe that this simple wash method could aid in the purification of such compounds. Also, similar calculations performed on other nucleosides could be useful in predicting products involved in acid-base equilibria, tautomerism, and other processes.[15]

EXPERIMENTAL

Theoretical

The calculations were performed on structures **1**, **2** and **3** even though the acid wash was to be done on the mixture of **4**, **5** and **6**. The three structures are first optimized using the Tripos 52 Force field[16] and subjected to a systematic search[17] around the bond between the ring systems as well as the ring bonds. The cyclopentanyl-adenine bond was subjected to a torsional increment of 30° and the cyclopentanyl ring bonds, increments of 10°. All three structures showed a definitive single low energy conformation with the lowest energy conformation of **3** showing an intramolecular hydrogen bond.

The lowest energy conformations for structures **1**, **2** and **3** were "protonated" at all possible basic sites and both the base form as well as all "protonated" forms were subjected to three sets of calculations. A full geometry optimization was done using the AM1 Hamiltonian and repeated using the AM1-SM2 Hamiltonian and again at the *ab initio* level using the split valence basis set, 3-21G(*) as available in SPARTAN.[18]

Synthesis

General Experimental: Melting points were obtained on a Thomas Hoover melting point apparatus and are uncorrected. Specific rotation values were obtained using Jasco Model DIP 360 Polarimeter. IR spectra were recorded using a Mattson Galaxy Series 5000 FTIR. NMR spectra were recorded on a Varian XL-300, XL-400 or Gemini 300 at 300 or 400 MHz (^{1}H) or at 75 or 100 MHz (^{13}C) and chemical shifts are reported in ppm relative to TMS internal standard. Mass spectra were obtained on a Finnigan MAT TSQ700 spectrometer. GC standard conditions: flow (He) = 30 mL/min; column: HP-5 cross linked, 5% PH ME silicone, 30 m x 0.32 mm; injection port, 200 °C; detector, 275 °C; gradient, 100 °C (10 min) increase 10 °C/min to 200 °C (5 min). HPLC chiral column chiralpak AD (0.46 x 25 cm): Method A; 85/15 pentane/isopropanol, 1 mL/min, 260 psi. Method B; 80/20 methanol/pentane, 1 mL/min, 420 psi. Flash chromatography and plug filtration on silica gel were performed (Merck 60, 230-400 mesh). Tlc was performed on silica gel (Merck 60 F_{254}, 0.25mm).

(1*S*,3*R*)-*cis*-3-*t*-Butyldimethylsilyloxycyclopentanyl Mesylate (8). A solution of alcohol **7**[19-21] (13.4 g, 61.7 mmol) in TBME (80 mL) was cooled to -10 °C and treated sequentially with $MeSO_2Cl$ (5.2 mL, 67.1 mmol, 1.09 eq) and Et_3N (10.4mL, 74.6 mmol, 1.21eq; dropwise over 30 min, -10 to 0 °C). After the addition of Et_3N was complete, the reaction mixture was allowed to warm to rt and stirred for 2 h. The resulting mixture was poured into 1/2 sat'd brine (100 mL), phases separated and the aqueous phase extracted with TBME (1 x 100 mL). The organic phases were combined, dried ($MgSO_4$), filtered and the filtrate evaporated *in vacuo* (20 mmHg, rt) to give 17.2 g, 95% crude yield, of mesylate **8** as an amber oil. Mesylate **8** was used in subsequent reactions without purification. For crude **8**: $[\alpha]_D^{20}$ = -1.0° (c = 0.99, $CHCl_3$); t_R (GC) = 23.1 min; R_f = 0.2 (20% EtOAc/hex; co-spots with sm); ^{1}H NMR ($CDCl_3$) 5.0 (m, 1H), 4.2 (m, 1H), 2.99 (s,3H), 2.3 (m, 1H), 2.0 (m, 3H), 1.8 (m, 2H), 0.88 (s, 9H), 0.046 (s, 6H); IR (KBr) ν_{max} 2955, 2932, 1356, 1256, 1179, 1094, 1063 cm^{-1}; CIMS (CH_4) m/z (% relative intensity) 295 (MH^+,30), 199 (MH^+-HO_3SMe, 100).

9*N*-[(1'*R*,3'*R*)-*trans*-3'-*t*-Butyldimethylsilyloxycyclopentanyl]adenine (4). A slurry of adenine (9.60 g, 71.0 mmol, 1.21 eq) and NaH (60% in oil, 2.86 g, 71.5 mmol, 1.22 eq) in DMA (70 mL) was heated at 70 °C for 2 h. The resulting mixture was allowed to cool to 40 °C then treated with a solution of mesylate **8** (17.2 g, 58.4 mmol) in DMA (70 mL) and heating continued at 70 °C for 4 h. The reaction mixture was allowed to cool (stirred overnight), poured into H_2O (225 mL) and extracted with EtOAc/PhMe (2/1, 3 x 150 mL). The organic phases were combined, dried ($MgSO_4$), filtered and the filtrate evaporated *in vacuo* (20 mmHg, 55 °C) to give a crude reddish solid. Purification by chromatography on SiO_2 (10 x 16 cm, 50% acetone/heptane to acetone to 50% EtOH/acetone) gave 12.6 g (65%) of 9*N*-adenyl isomer **4**, 2.2 g (11%) of 3*N*-adenyl isomer **5**, and 400 mg (2%) of 7*N*-adenyl isomer **6**. Recrystallization of **4** from MeCN (175 mL) provided 10.1 g, 52% yield of 9*N*-isomer **4** with >98% HPLC purity. For **4**: mp 181-182 °C; $[\alpha]_D^{20}$ = -20.8° (c = 1.01, $CHCl_3$); t_R (method A) = 9.5 min; R_f= 0.64 (acetone); ^{1}H NMR ($CDCl_3$) 8.35 (s, 1H), 7.81 (s, 1H), 6.35 [s(broad), 2H], 5.12 (p, 1H, *J* = 7.8 Hz), 4.5 (m, 1H), 2.4 (m, 1H), 2.2 (m, 3H), 2.0 (m, 1H), 1.8 (m, 1H), 0.09 (s,9H), 0.08 (s, 6H); ^{1}H NMR (DMSO-d_6) 8.20 (s, 1H), 8.10 (s, 1H), 7.14 [s(broad), 2H], 5.03 (p, 1H, *J* = 8.2 Hz), 4.6 (m, 1H), 2.3 (m, 2H), 2.2 (m, 1H), 2.1 (m, 1H), 1.9 (m, 1H), 1.6 (m, 1H), 0.87 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (DMSO-d_6) 155.9, 152.1, 149.3, 139.6, 119.2, 72.5, 53.4, 41.5, 34.3, 29.9, 25.8, 17.7, -4.8; IR

(KBr) ν_{max} 3325, 3183, 2955, 2932, 1667, 1605, 1472, 1252, 1061 cm^{-1}; UV (MeOH) λ_{max} 261 nm (ε = 14,800); CIMS (CH_4) m/z (%relative intensity) 334 (MH^+,100). Anal. calcd for $C_{16}H_{27}N_5OSi$ (333.51): C, 57.62; H, 8.16; N,21.00. Found: C, 57.67; H, 8.16; N, 21.23.

3*N*-[(1'*R*,3'*R*)-*trans*-3'-*t*-Butyldimethylsilyloxycyclopentanyl]adenine (5). Isolated from sodium adenate displacement of mesylate **8**. For **5**: mp 209-210 °C; $[\alpha]_D^{20}$ = -22.8° (c = 1.02, $CHCl_3$); t_R (method A) = 7.8 min; R_f = 0.1 (20% EtOH/acetone); ^1H NMR ($CDCl_3$) 8.05 (s, 1H), 8.00 (s, 1H), 5.2 (m, 1H), 4.6 (m, 1H), 2.5 (m, 1H), 2.4 (m, 1H), 2.2 (m, 3H), 1.8 (m, 1H), 0.87 (s, 9H), 0.05 (s, 6H); ^1H NMR (DMSO-d_6) 8.40 (s, 1H), 7.9 [s(broad), 1H], 7.8 [s(broad), 1H], 7.74 (s, 1H), 5.2 (m, 1H), 4.6 (m, 1H), 2.54 (ddd, 1H, *J* = 5.6, 9.0, 12.9 Hz), 2.4 (m, 1H), 2.2 (m, 2H), 2.0 (m, 1H), 1.6 (m, 1H), 0.87 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (DMSO-d_6) 154.9, 152.2, 148.9, 142.5, 120.8, 72.9, 60.3, 40.1, 34.9, 28.6, 25.8, -4.8; IR (KBr) ν_{max} 3312, 3194, 2955, 2932, 2857, 1655, 1620, 1113, 1061 cm^{-1}; UV (MeOH) λ_{max} 275 nm (ε = 12,800); CIMS (CH_4) m/z (% relative intensity) 334 (MH^+,100). Anal. calcd for $C_{16}H_{27}N_5OSi$ (333.51): C, 57.62; H, 8.16; N, 21.00. Found: C, 57.48; H, 8.16; N, 21.26.

7*N*-[(1'*R*,3'*R*)-*trans*-3'-*t*-Butyldimethylsilyloxycyclopentanyl]adenine (6). Isolated from sodium adenate displacement of mesylate **8**. For **6**: mp 224-225 °C; $[\alpha]_D^{20}$ = +10.7° (c = 0.98, $CHCl_3$); t_R (method A) = 9.7 min; R_f = 0.11 (acetone); ^1H NMR ($CDCl_3$) 8.50 (s, 1H), 8.06 (s, 1H), 5.4 [s(broad), 2H], 5.0 (m, 1H), 4.5 (m, 1H), 2.5 (m, 1H), 2.3 (m, 1H), 2.2 (m, 2H), 1.9 (m, 2H), 0.91 (s, 9H), 0.098 (s, 3H), 0.095 (s, 3H); ^{13}C NMR ($CDCl_3$) 161.0, 153.1, 150.6, 141.9, 112.2, 71.7, 57.3, 44.3, 34.1, 31.0, 25.8, 18.0, - 4.76, -4.82; IR (KBr) ν_{max} 3327, 3190, 2955, 2932, 1643, 1599, 1555, 1472, 1385, 1252 cm^{-1}; UV (MeOH) λ_{max} 271 nm (ε = 9,560); CIMS (CH_4) m/z (% relative intensity) 334 (MH^+,100). Anal. calcd for $C_{16}H_{27}N_5OSi$ (333.51): C, 57.62; H, 8.16; N, 21.00. Found: C, 57.64; H,8.15; N, 21.20.

9*N*-[(1'*R*,3'*R*)-*trans*-3'-Hydroxycyclopentanyl]adenine HCl (1•HCl). This silyl ether cleavage is an illustrative example of conditions used for the conversion of **5** and **6** to **2** and **3** respectively.
A slurry of 9*N*-isomer **4** (9.7 g, 29 mmol) in EtOH (45 mL) was treated with 6N HCl (5.8 mL, 35 mmol) and stirred at rt for 4 h. The mixture was diluted with EtOH/hept (1:1, 65 mL) and stored at -20 °C for 3 d (usually overnight). The resulting white solid was filtered and allowed to air dry to provide 7.4 g, 96% yield of **1**. Mp 245-252 °C (decomp); $[\alpha]_D^{20}$ = -13.0° (c = 1.03, MeOH); t_R (method B) = 18.2 min; ^1H NMR (DMSO-d_6) 9.4 [s(broad), 1H], 8.8 [s(broad), 1H], 8.62 (s, 1H), 8.49 (s, 1H), 5.1 (m, 1H), 4.4 (m, 1H), 2.3 (m, 1H), 2.2 (m, 3H), 2.0 (m, 1H), 1.7 (m, 1H); ^{13}C NMR (DMSO-d_6) 150.4, 148.3, 144.6, 142.7, 118.3, 70.2, 54.6, 41.5, 33.5, 30.1; IR (neat) ν_{max} 3412, 3270, 3069, 2971, 2947, 1692, 1406, 1240 cm^{-1}; CIMS (CH_4) m/z (% relative intensity) 220 (M+H, 100). Anal. calcd for $C_{10}H_{14}N_5OCl.0.9\ H_2O$ (271.92): C, 44.17; H, 5.86; N, 25.76. Found: C,44.36; H,5.76; N, 25.63.

3*N*-[(1'*R*,3'*R*)-*trans*-3'-Hydroxycyclopentanyl]adenine HCl (2•HCl). Using conditions reported above, 3*N*-isomer **5** (508 g, 1.53 mmol) provided **2** as a white solid; 319 mg, 87% yield. For **2**: mp >250 °C; $[\alpha]_D^{20}$ = -12.5° (c =0.99, MeOH); t_R(method B) = 18.2 min; ^1H NMR (DMSO-d_6) 9.3 (m, 2H), 8.83 (s, 1H), 8.64 (s, 1H), 5.3 (m, 1H), 4.4 (m, 1H), 2.3 (m, 2H), 2.2 (m, 3H), 1.7 (m, 1H); ^{13}C NMR (DMSO-d_6) 153.3,

147.2, 147.0, 144.0, 110.8, 70.4, 61.0, 40.1, 33.6, 28.7; IR (KBr) ν_{max} 3412, 3289, 3105, 2953, 1658, 1433, 1410 cm^{-1}; UV λ_{max} 276nm (ε = 15,600); CIMS (CH_4) m/z (% relative intensity) 220 (MH^+, 100). Anal. calcd for $C_{10}H_{14}N_5OCl$ (255.71): C, 46.97; H, 5.52; N, 27.39. Found: C,47.23; H,5.54; N, 27.18.

7*N*-[(1'*R*,3'*R*)-*trans*-3'-Hydroxycyclopentanyl]adenine HCl (3•HCl). Using conditions reported above, 7*N*-isomer **6** (100 mg, 0.300 mmol) gave **3**, 49 mg, 64% yield as a white solid. For **3**: mp 243-245 °C; $[\alpha]_D^{20}$ = -10.5° (c = 0.494, MeOH); t_R(method B; 0.8mL/min) = 4.3 min; ^1H NMR (DMSO-d_6) 8.8 (m, 2H), 8.61 (s, 1H), 5.4 (m, 1H), 4.4 (m,1H), 3.8 (m, 2H), 2.4 (m, 1H), 2.2 (m, 2H), 2.0 (m, 1H), 1.9 (m, 1H), 1.7 (m, 1H); ^{13}C NMR (DMSO-d_6) 152.4, 150.7, 146.3, 144.9, 110.6, 69.9, 57.1, 42.1, 33.6, 31.6; IR (KBr) ν_{max} 3314, 3204, 3071, 3036, 1643, 1391, 1244 cm^{-1}; UV λ_{max} 275 nm (ε = 12,200); CIMS (CH_4) m/z (% relative intensity) 220 (MH^+, 100). Anal. calcd for $C_{10}H_{14}N_5OCl.1.5\ H_2O$ (282.55): C, 42.51; H, 6.06; N, 24.79. Found: C,42.83; H,5.99; N, 24.74.

$KHSO_4$ Wash to Remove 5 from a Mixture of 4 and 5

9*N*-Silyl ether **4** (851 mg, 2.56 mmol) and 3*N*-silyl ether **5** (275 mg, 0.826 mmol) were dissolved in EtOAc/hex (16 mL/8mL) and treated with 0.1M $KHSO_4$ (9 mL), stirred for 5 min then filtered. The organic phase was separated, dried ($MgSO_4$), filtered and the filtrate evaporated *in vacuo*. The resulting white solid (718 mg, 84% of 9*N*-silyl ether **4** contained <1% of 3*N*-silyl ether **5** by HPLC (method A).

pKa Determination Using Aqueous Titrimetry

The *N*-3 (**2**), *N*-7 (**3**), and *N*-9 (**1**) hydroxycyclopentyladenine hydrochloride derivatives were titrated as 7-11 mM aqueous solutions with 0.10 N sodium hydroxide. The pH was plotted versus volume of sodium hydroxide added. After the titration had proceeded past the neutralization equivalence point (pH 9-11), each of these basified solutions was then titrated with 0.04 N hydrochloric acid to a pH less than the initial pH of the aqueous solutions for the hydrochloride salts of these adenine derivatives. The titration curves were presented as graphs of the pH plotted versus volume of hydrochloric acid added. The pKa's were measured directly from the titration curves.

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Acknowledgement: The authors thank Dave Borcherding, Steve Horgan, and F. Mark Laskovics for their helpful comments during the conduct of this work. The authors would like to extend their gratitude to Dirk Friedrich for long range HETCOR studies to aid in proving the regiochemistry of adenyl alkylation.

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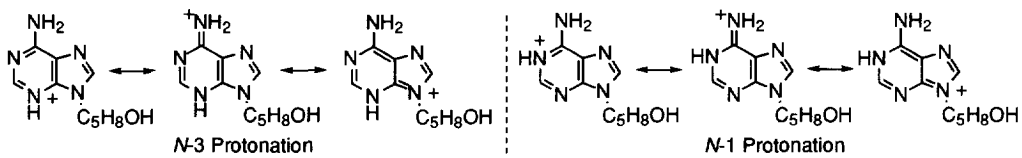
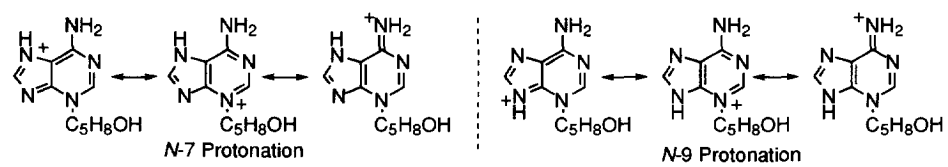
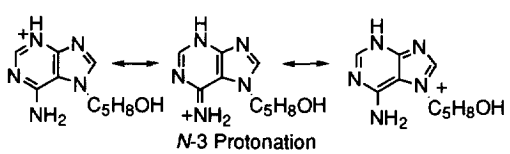
13. a) The resonance structures of the lower energy forms for the protonated forms of **1**.



b) The resonance structures of the lower energy forms for the protonated forms of **2**.



c) The resonance structures of the lower energy forms for the protonated forms of **3**.



14. Although for compounds **4**, **5** and **6** we do not describe the separation of the products on a crude reaction mixture, in the enantiomeric series, we have separated **ent-4**, from **ent-5** and **ent-6** via an acid wash (to remove **ent-5**) and recrystallization from MeCN (to remove **ent-6**) gave pure **ent-4** (50% yield; >98% HPLC).
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19. Cyclopentanol **7** [colorless oil; $[\alpha]_D^{20} = -4.5$ (c= 1.09, $CHCl_3$)] was prepared by double bond hydrogenation of (1*S*,3*R*)-*cis*-3-*t*-Butyldimethylsilyloxycyclopentenol using Ni_2B.[21]
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(*Received in USA* 2 *December* 1996; *revised* 24 *March* 1997; *accepted* 7 *April* 1997)