Published in final edited form as:

Nucleosides Nucleotides Nucleic Acids. 2008 April ; 27(4): 408–420. doi:10.1080/15257770801944436.

Synthesis of 3'-Acetamidoadenosine Derivatives as Potential A₃ Adenosine Receptor Agonists

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Abstract

On the basis of high binding affinity of 3'-aminoadenosine derivatives **2b** at the human A₃ adenosine receptor (AR), 3'-acetamidoadenosine derivatives **3a–e** were synthesized from 1,2:5,6-di-*O*-isopropylidene- β -D-glucose via stereoselective hydroboration as a key step. Although all synthesized compounds were totally devoid of binding affinity at the human A₃AR, our results revealed that 3'-position of adenosine can only be tolerated with small size of a hydrogen bonding donor like hydroxyl or amino group in the binding site of human A₃AR.

Keywords

3'-acetamidoadenosines; human A₃ adenosine receptor; hydrogen bonding donor; hydroboration-oxidation; steric effects

INTRODUCTION

Adenosine is an endogenous chemical messenger, regulating many physiological functions through four subtypes (A₁, A_{2A}, A_{2B}, and A₃) of adenosine receptors (ARs).[1] Among these, A₃ adenosine receptor is the most recently identified and known to be involved in cell signaling. [2] Activation of A₃AR stimulates phospholipase C (PLC), increasing levels of inositol triphosphate (IP₃) and diacylglycerol (DAG), but inhibits adenylate cyclase, lowering the level of cAMP.[2] Because IP₃, DAG, and cAMP serve as second messengers, A₃AR is closely related to signal transduction of the cell. Thus, control of A₃AR may modulate several diseases related to cell signaling, making it promising therapeutic target.[3] A₃AR agonists have potentials to be used for the treatment of cancer, cardiac ischemia, and cerebral ischemia, while A₃AR antagonists can be developed as anti-asthma, anti-glaucoma, anti-inflammatory agents. [3]

Based on the structure of adenosine, a natural messenger, a number of nucleoside derivatives have been synthesized as A₃AR agonists.[3] Among these, *N*⁶-(3-iodobenzyl)-5-*N*-methylcarboxamidoadenosine (IB-MECA, **1a**) and its 2-chloro analogue (Cl-IB-MECA, **1b**)

are representatives of A₃AR agonists, showing high binding affinity to A₃AR and high selectivity to A₁, A_{2A}, and A_{2B} ARs and are developed as anticancer agents (Figure 1).[4,5]

On the basis of high binding affinity and selectivity of **1a** and **1b**, 3'-fluoro analogue **2a** in bioisosteric relationship with **1a** and **1b** as a hydrogen bonding acceptor was synthesized to determine if 3'-hydroxyl group serves as a hydrogen bonding acceptor or donor.[6] From this study, it was concluded that compound **2a** displayed significantly decreased binding affinity to A₃AR, indicating 3'-hydroxyl group acted as a hydrogen bonding donor. However, 3'-amino analogue **2b** which are also in bioisosteric relationship with **1a** and **1b** as a hydrogen bonding donor exhibited high binding affinity to A₃AR comparable to **1a** and **1b**, indicating that 3'-hydroxyl group serves as a hydrogen bonding donor.[7] Since 3'-amino group served as a hydrogen bonding donor, Jeong *et al.*[8] synthesized 3'-ureido derivatives **2c** being capable of multiple hydrogen bonding, but they were totally devoid of binding affinities at the all subtypes of ARs, probably due to intramolecular hydrogen bondings between 3'-ureido group and 2'-hydroxyl group. Based on these findings, we synthesized 3'-acetamido derivatives **3a–e** which can serve as the same hydrogen bonding donor like 3'-amino group, but are expected to possess better pharmacokinetic profiles than basic 3'-amino derivatives **2b**. Herein, we report the synthesis of 3'-acetamidoadenosine derivatives and their binding affinities at the ARs.

RESULTS AND DISCUSSION

The synthetic strategy for the synthesis of the final nucleosides **3a–e** was first to synthesize the glycosyl donor **14** and then to condense with 2,6-dichloropurine. Synthesis of the glycosyl donor **14** from 1,2;5,6-di-*O*-isopropylidene- α -D-glucose is shown in Scheme 1. Swern oxidation of 1,2;5,6-di-*O*-isopropylidene- α -D-glucose followed by Wittig reaction with methyl triphenylphosphonium bromide in the presence of potassium *t*-butoxide afforded 3-methylene derivative **4**. [9] Hydroboration-oxidation of **4** gave 3-*C*-hydroxymethyl derivative **5** as a single stereoisomer, which was formed by the steric effect by 1,2-isopropylidene group. Benzyl protection of **5** followed by selective hydrolysis of 5,6-isopropylidene group of the resulting benzylated derivative **6** afforded diol **7**. [10] Oxidative cleavage of diol **7** with sodium metaperiodate followed by reduction of the resulting aldehyde with sodium borohydride gave **8** which was benzoylated to yield **9**. Hydrolysis of **9** with 4 *N* HCl in MeOH afforded methyl glycoside **10**, in which 2-hydroxyl group was protected with benzoyl group to give **11**. Removal of the benzyl group of **11** using catalytic hydrogenation gave **12**. Treatment of **12** with ruthenium chloride and sodium metaperiodate followed by treating with MeOH in the presence of DCC gave methylester **13** which was converted to the glycosyl donor **14** by treating with acetic acid, acetic anhydride, and sulfuric acids.

Condensation of acetate **14** with silylated 2,6-dichloropurine in the presence of TMSOTf as Lewis acid gave the protected nucleoside **15** as a single diastereomer due to the neighboring group effect by 2'-benzoyl group (Scheme 2). Treatment of **15** with various alkyl, arylalkyl, or cycloalkyl amines yielded *N*⁶-substituted derivatives which were reacted with methyl amine to give the final nucleosides **3a–e**.

The final nucleosides **3a–e** were subjected to competitive radioligand binding assays.[11] A₃AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human A₃AR using [¹²⁵I]-AB-MECA (1.0 nM) as radioligand, while binding experiments at A₁ and A_{2A} ARs were carried out using [³H]CPX (0.5 nM, recombinant human A₁ AR) or [³H]ZM241385 CPX (2 nM, recombinant human A_{2A} AR) as radioligand.[9] All tested compounds were totally devoid of binding affinities at the all subtypes of ARs, indicating that 3'-acetamido group, could alter ring puckering of sugar moiety by lack of Gauche effect between 3'-OH and ring oxygen or might cause steric repulsion at the binding site of the

A₃AR, instead of forming strong hydrogen bonding with A₃AR, despite of its hydrogen bonding donor ability.

In conclusion, we have accomplished the synthesis of 3'-acetamidoadenosine derivatives, starting from 1,2:5,6-di-*O*-isopropylidene- α -D-glucose via stereoselective hydroboration as a key step. Unfortunately, all synthesized compounds did not show any significant binding affinity at the human A₃AR, probably due to the steric effects of 3'-acetamido group. Our findings indicate that 3'-position of adenosine can only be tolerated with small size of a hydrogen bonding donor like hydroxyl or amino group in the binding site of human A₃AR. Molecular modeling study of these synthesized compounds at human A₃AR is in progress and will be reported elsewhere.

Experimental section

General Methods

NMR data were recorded on a 300 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard, and the chemical shifts are reported in ppm (δ). Coupling constants are reported in hertz. The abbreviations used are as follows: s (singlet), d (doublet), m (multiplet), dd (doublet of doublet), br s (broad singlet). TLC and preparative TLC were carried out on Merck precoated plates (Kieselgel 60F₂₅₄). Silica gel for chromatography was Merck Kieselgel 60. All anhydrous solvents were distilled over CaH₂ or Na/benzophenone prior to use.

3-Deoxy-1,2;5,6-di-*O*-isopropylidene-3-methylene- α -D-glucofuranose (4)

To a solution of DMSO (3.4 mL, 48.0 mmol) in anhydrous CH₂Cl₂ (150 mL), was added oxalyl chloride (2.18 mL, 25.0 mmol) dropwise carefully at -78 °C. After 10 min of stirring, to the reaction mixture was added a solution of di-acetone- α -D-glucose (5 g, 19.2 mmol) in anhydrous CH₂Cl₂ (50 mL) with cannula at -78 °C. The mixture was stirred for 10 min at the same temperature followed by treatment of TEA (17 mL), and the yellow slurry was formed immediately and the mixture was stirred for 30 min at room temperature. The reaction mixture was poured into water and extracted with methylene chloride. The organic layer was washed with brine, dried (MgSO₄), filtered, and evaporated under reduced pressure to give the ketone (4.6 g, 93%).

To a solution of methyl triphenyl phosphonium bromide (12.7 g, 35.7 mmol) in anhydrous THF (150 mL) was added 2M solution of potassium tertiary butoxide (17.9 mL) in THF at 0 °C and the mixture was stirred at room temperature for 20 min and then cooled to 0 °C. To the reaction mixture was added a solution of ketone (4.6 g, 17.8 mmol) in anhydrous THF (30 mL). After 1 hr of stirring at room temperature, the mixture was partitioned between EtOAc and water. The aqueous layer was washed 3 times by EtOAc, the combined organic layer was dried over MgSO₄, filtered and evaporated. The resulting residue was purified by silica gel column chromatography (hexane : ethyl acetate = 2:1) to give compound **4** (3.92 g, 86%) as a colorless oil, whose spectral data were identical with those of authentic sample.[9]

3-Deoxy-C-hydroxymethyl-O-1,2;5,6-di-*O*-isopropylidene- α -D-allofuranose (5)

To a solution of **1** (323 mg, 1.26 mmol) in anhydrous THF (10 mL), 2M solution of BH₃-SMe₂ complex in THF (3.78 mL) was dropped at 0 °C. The mixture was stirred for 3 hr at room temperature followed by consecutive addition of THF : H₂O (1:1, 1.8 mL), 2N NaOH (5.3 mL), 30% H₂O₂ (3.2 mL) at 0 °C and then stirred for 2 hr at room temperature. The mixture was extracted with EtOAc, and the organic layer was washed with brine, dried, filtered, and evaporated. Column chromatography (hexane : ethyl acetate = 1:1) afforded **5** (283 mg, 82%) as a colorless oil, whose spectral data were identical with those of authentic sample [9]

3-C-Benzylloxymethyl-3-deoxy-1,2;5,6-di-O-isopropylidene- α -D-allofuranose (6).[10]

To a solution of **5** (601 mg, 2.19 mmol) in anhydrous THF (22 mL) were added 60% suspension of NaH in mineral oil (80 mg, 3.29 mmol) and *n*-Bu₄NI (catalytic amount). The mixture was stirred for 20 min at room temperature, and then BnBr (0.4 mL, 3.29 mmol) was added at 0 °C. The reaction mixture was stirred for 18 hr at room temperature and quenched with H₂O, poured into EtOAc. The organic layer was washed with brine, dried (MgSO₄), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 10:1) to give **6** (596 mg, 75%): $[\alpha]_D^{25}$ 56.81° (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 7.36~7.26 (m, 5H), 5.78 (d, 1H, *J* = 3.7 Hz), 4.77 (dd, 1H), 4.56 (s, 2H) 4.17~3.71 (m, 6H), 2.27~2.18 (m, 1H), 1.50 (s, 3H), 1.36 (s, 3H), 1.34 (s, 3H), 1.33 (s, 3H); MS (FAB) *m/z* 363 (M-H).

3-C-Benzylloxymethyl-3-deoxy-1,2-O-isopropylidene- α -D-allofuranose (7).[10]

To a solution of **6** (2.02 g, 5.54 mmol) in a 1:1 mixture of THF and H₂O (25 mL) was added 4N HCl (0.5 mL). After being stirred at 50 °C for 24 hr, the reaction mixture was neutralized by addition of triethylamine. The mixture was poured into EtOAc, and the organic layer was washed with brine, dried (MgSO₄), filtered and evaporated. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 1:2) to give diol **7** (1.17 g, 65%): $[\alpha]_D^{25}$ 25.44° (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 7.41~7.31 (m, 5H), 5.79 (d, 1H, *J* = 3.7 Hz), 4.71~4.52 (m, 4H), 4.05~3.55 (m, 6H), 2.32~2.28 (m, 1H), 2.22~2.13 (m, 1H), 1.50 (s, 3H), 1.29 (s, 3H); IR (neat) 3397 cm⁻¹; MS (FAB) *m/z* 325 (M+H).

3-C-Benzylloxymethyl-3-deoxy-1,2-O-isopropylidene- α -D-ribofuranose (8)

To a solution of **7** (178 mg, 0.60 mmol) in MeOH (3 mL) was added NaIO₄ (140 mg, 0.66 mmol) in H₂O (3 mL) at 0 °C. After 30 min of stirring, NaBH₄ (32 mg, 0.89 mmol) was added at 0 °C, and the resulting mixture was stirred for 30 min at room temperature. After evaporating to remove MeOH, the mixture was poured into EtOAc. The organic layer was washed with brine, dried over MgSO₄, filtered and evaporated. Column chromatography (hexane : ethyl acetate = 1:1) afforded **8** (137 mg, 85%): $[\alpha]_D^{25}$ 35.36° (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 7.38~7.26 (m, 5H), 5.81 (d, 1H, *J* = 3.5 Hz), 4.71 (m, 1H), 4.55 (s, 2H), 2.68~2.63 (m, 1H), 4.10~3.57 (m, 5H), 2.68~2.64 (m, 1H), 2.31~2.22 (m, 1H), 1.5 (s, 3H), 1.3 (s, 3H); IR (neat) 3460 cm⁻¹; MS (FAB) *m/z* 293 (M-H).

5-O-Benzoyl-3-C-benzylloxymethyl-3-deoxy-1,2-O-isopropylidene- α -D-ribofuranose (9)

To a solution of **8** (1.18 g, 4.00 mmol) in anhydrous pyridine (20 mL) was added benzoyl chloride (1.65 mL, 12 mmol) at 0 °C. The mixture was stirred at room temperature for 2 hr, quenched with a cold saturated NaHCO₃ solution, and extracted with CH₂Cl₂ three times. The combined extracts were dried over MgSO₄, and evaporated to dryness in vacuo. The residue was dissolved in 5 mL of toluene and the evaporation was repeated to remove pyridine. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 8:1) to give **9** (1.48 g, 93%): $[\alpha]_D^{25}$ 35.62° (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 8.10~7.28 (m, 10H), 5.86 (d, 1H, *J* = 30.0 Hz), 5.87 (d, 1H, *J* = 3.7 Hz), 4.69~4.77 (m, 2H), 4.54 (s, 2H), 4.22~4.39 (m, 2H), 3.82~3.88 (dd, 1H, *J* = 7.0 and 6.8 Hz), 2.30~2.40 (m, 1H), 1.64 (s, 3H), 1.38 (s, 3H); IR (neat) 1721 cm⁻¹; MS (FAB) *m/z* 399 (M+H).

Methyl-5-O-benzoyl-3'-C-benzylloxymethyl-3-deoxy- α -D-ribofuranoside (10)

To a solution of **9** (535 mg, 1.34 mmol) in MeOH (13 mL) was added AcCl (0.1 mL) dropwise at 0 °C. The reaction mixture was stirred for 30 hr, quenched with TEA until being basic condition, and then evaporated to remove MeOH. The mixture was partitioned between EtOAc and water. The organic layer was dried over MgSO₄, filtered and evaporated under reduced pressure. Column chromatography (hexane : ethyl acetate = 3:1) afforded **10** (432 mg, 86%):

$[\alpha]_D^{25} -13.40^\circ$ (c 1.0, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ 8.07~8.04 (m, 2H), 7.60~7.53 (m, 1H), 7.46~7.41 (m, 2H), 7.47~7.32 (m, 7H), 4.83 (s, 1H), 4.61~4.46 (m, 4H), 4.37~4.28 (m, 2H), 3.86~3.77 (m, 2H), 3.26 (s, 3H), 3.02~3.00 (d, 1H, $J = 21.6$ Hz), 2.63~2.55 (m, 1H); IR (neat) 3440, 1720 cm^{-1} ; MS (FAB) m/z 373 (M+H).

Methyl-2,5-di-O-benzoyl-3-C-benzoyloxymethyl-3-deoxy- β -D-ribofuranoside (11)

To a solution of **10** (1.11 g, 3.10 mmol) in anhydrous pyridine (15 mL) was added benzoyl chloride (0.58 mL, 4.7 mmol) at 0°C . The mixture was stirred at room temperature for 5 hr, quenched with a cold saturated NaHCO_3 solution. The mixture was poured into CH_2Cl_2 , and then organic layer was dried, filtered and evaporated. The residue was dissolved in toluene and evaporated repeatedly to remove pyridine. Column chromatography (hexane : ethyl acetate = 10:1) afforded **11** (1.16 g, 82%): $[\alpha]_D^{25} 40.89^\circ$ (c 1.0, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ 8.13~8.10 (m, 2H), 7.99~7.95 (m, 2H), 7.61~7.55 (m, 2H), 7.48~7.41 (m, 4H), 7.28~7.18 (m, 3H), 5.49 (d, 1H, $J = 4.7$ Hz), 4.98 (s, 1H), 4.68~4.63 (dd, 1H, $J = 8.6$ and 14.3 Hz), 4.52~4.36 (m, 4H), 3.83~3.78 (m, 1H), 3.68~3.60 (m, 1H), 3.36 (s, 3H), 3.03~2.94 (m, 1H); IR (neat) 1722 cm^{-1} ; MS (FAB) m/z 499 (M+Na).

Methyl-2,5-di-O-benzoyl-3-deoxy-3-C-hydroxymethyl- β -D-ribofuranose (12)

To a solution of **11** (1.26 g, 2.64 mmol) in EtOAc (25 mL) was added Pd/C. After stirring for 5 hr with hydrogenation, the solution was filtered through a pad of Celite, concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 2:1) to give **12** (970 mg, 95%): $[\alpha]_D^{25} -2.47^\circ$ (c 1.0, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ 8.12~8.04 (m, 4H), 7.65~7.56 (m, 2H), 7.55~7.43 (m, 4H), 5.47 (d, 2H, $J = 4.4$ Hz), 5.06 (s, 1H), 4.63~4.56 (m, 1H), 4.50~4.32 (m, 2H), 3.85~3.71 (m, 2H), 3.39 (s, 3H), 2.95~2.86 (m, 1H), 2.56~2.51 (m, 1H), 2.17 (s, 1H); IR (neat) 3497, 1721 cm^{-1} ; MS (FAB) m/z 409 (M+Na).

Methyl-2,5-di-O-benzoyl-3-deoxy-3-C-methoxycarbonyl- β -D-ribofuranate (13)

A mixture of **12** (1.23 g, 3.19 mmol), NaIO_4 (2.73 g, 12.75 mmol) and RuCl_3 (0.189 g, 0.701 mL) in $\text{CCl}_4/\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1:1:1, 9 mL) was stirred at room temperature for 2 hr. The reaction mixture was evaporated to give the residue, which was dissolved in 5 mL of toluene. The evaporation was repeated to remove water. The residue was dissolved in anhydrous CH_2Cl_2 and to this solution were added DMAP (78 mg, 0.64 mmol), DCC (1M in CH_2Cl_2 , 4.15 mL, 4.15 mmol) and MeOH (0.17 mL, 4.15 mmol). The mixture was stirred at room temperature for 12 hr, evaporated, and the residue was purified by silica gel column chromatography (hexane : ethyl acetate = 5:1) to give **13** (837 mg, 64%): $[\alpha]_D^{25} 28.17^\circ$ (c 1.0, CHCl_3); $^1\text{H NMR}$ (CDCl_3 , 300 MHz) δ 8.11~8.08 (m, 2H), 8.00~7.98 (m, 2H), 7.62~7.56 (m, 2H), 7.49~7.43 (m, 4H), 5.60 (d, 1H, $J = 4.8$ Hz), 5.14~5.08 (m, 1H), 5.03 (s, 1H), 4.66~4.44 (m, 2H), 3.69~3.59 (m, 4H), 3.36 (s, 3H); IR (neat) 1723 cm^{-1} ; MS (FAB) m/z 415 (M+H).

1-O-Acetyl-2,5-di-O-benzoyl-3-deoxy-3-C-methoxycarbonyl- α,β -D-ribofuranate (14)

To a stirred solution of **13** (430 mg, 1.04 mmol) in AcOH (5 mL) and Ac_2O (1.2 mL) was added conc. H_2SO_4 dropwise at room temperature. The reaction mixture was stirred for 30 min, poured into a cold saturated NaHCO_3 solution, and extracted with CH_2Cl_2 three times. The combined extracts were dried over MgSO_4 , filtered, and evaporated. The residue was purified by column chromatography (hexane : ethyl acetate = 8:1) to afford **14**, which was used directly for the next step.

2,6-Dichloro-9-(2,5-di-O-benzoyl-3-deoxy-3-C-methyl-O-carbonyl- β -D-ribofuranosyl) purine (15)

To a solution of silylated 2,6-dichloropurine, prepared by refluxing 2,6-dichloropurine (125 mg, 0.613 mmol) and ammonium sulfate (catalytic amount) in HMDS (2 mL), in anhydrous

$\text{ClCH}_2\text{CH}_2\text{Cl}$ (2 mL) was added a solution of **14** (208 mg, 0.472 mmol) in anhydrous $\text{ClCH}_2\text{CH}_2\text{Cl}$ (3 mL) followed by addition of TMSOTf (0.11 mL, 0.613 mmol) at 0 °C, and the mixture was stirred at room temperature for 20 min and then at 50 °C for 12 hr. The mixture was quenched with saturated NaHCO_3 solution, filtered through a Celite pad, and poured into CH_2Cl_2 . The organic layer was washed with brine, dried, filtered, and evaporated. Column chromatography (hexane : ethyl acetate = 4:1) afforded **12** (228 mg, 85%): UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ 18.06° (c 1.0, CHCl_3); ^1H NMR (CDCl_3 , 300 MHz) δ 8.20 (s, 1H), 8.01~7.98 (m, 2H), 7.89~7.86 (m, 2H), 7.68~7.38 (m, 6H), 6.30~6.24 (m, 1H), 6.13 (d, 1H, J = 1.3 Hz), 5.16~5.11 (m, 1H), 4.88~4.83 (m, 1H), 4.69~4.64 (m, 1H), 4.40~4.35 (dd, 1H, J = 3.7 and 16.5 Hz), 3.76 (s, 3H); IR (neat) 1726 cm^{-1} ; MS (FAB) m/z 571 (M+H); Anal. Calcd for C, 54.65; H, 3.53; N, 9.81. Found: C, 54.72; H, 3.48; N, 9.96.

2-Chloro-N⁶-(3-iodobenzyl)-9-(3-C-methylcarbamoyl-3-deoxy- β -D-ribofuranosyl) adenine (**3a**)

To a solution of **15** (76 mg, 0.133 mmol) in EtOH (3 mL) was added 3-iodobenzylamine (62 mg, 0.266 mmol), and then the mixture was stirred at 50 °C for 5 hr, evaporated, and extracted with EtOAc three times. The combined extracts were dried over MgSO_4 , filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 2:1) to give an iodobenzyl derivative (82 mg, 81%): UV (MeOH) λ_{max} 272 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ -7.48° (c 1.0, CHCl_3); ^1H NMR (CDCl_3 , 300 MHz) δ 8.02~7.93 (m, 4H), 7.80~7.61 (m, 4H), 7.55~7.26 (m, 6H), 7.11~7.06 (m, 1H), 6.25 (d, 2H, J = 6.2 Hz), 6.05 (s, 1H), 5.11~5.07 (m, 1H), 4.85~4.51 (m, 5H), 3.73 (s, 3H); IR (neat) 3362, 1727 cm^{-1} ; MS (FAB) m/z 790 (M+Na). A mixture of protected nucleoside (18 mg, 0.023 mmol) and MeNH_2 (2M in THF, 5 mL) was stirred at room temperature for 24 hr. The mixture was evaporated and the residue was purified by silica gel column chromatography (methylene chloride : methanol = 30:1) to give **3a** (6 mg, 45%): mp 222.3~222.9 °C; UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ -15.56° (c 1.0, THF); ^1H NMR ($\text{DMSO}-d_6$, 400 MHz) δ 8.50 (s, 1H), 7.74 (s, 1H), 7.61 (d, 1H, J = 8.0 Hz), 7.36 (d, 1H, J = 7.6 Hz), 7.15~7.11 (dd, 1H, J = 7.6 and 8.0 Hz), 5.93 (d, 1H, J = 5.2 Hz), 5.90 (d, 1H, J = 2.8 Hz), 5.16~5.11 (m, 1H), 4.73~4.56 (m, 3H), 4.49~4.46 (m, 2H), 3.82~3.76 (m, 1H), 3.59~3.50 (m, 2H), 3.15~3.11 (dd, 1H, J = 6.0 and 2.0 Hz), 2.59 (d, 3H, J = 4.8 Hz); IR (neat) 3331, 1613 cm^{-1} ; MS (FAB) m/z 559 (M+H); Anal. Calcd for $\text{C}_{19}\text{H}_{20}\text{ClIN}_6\text{O}_4$: C, 40.84; H, 3.61; N, 15.04. Found: C, 40.60; H, 3.56; N, 14.86.

2-Chloro-N⁶-methyl-9-(3-C-methylcarbamoyl-3-deoxy- β -D-ribofuranosyl) adenine (**3b**)

To a solution of **15** (33 mg, 0.058 mmol) in anhydrous THF (2 mL) was added anhydrous 0.642M $\text{MeNH}_2 \cdot \text{AcOH}$ (2.16 mL, 1.39 mmol), and then the reaction mixture was stirred overnight. Solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane : ethyl acetate = 1:1) to afford a methyl derivative (31.5 mg, 96%): UV (MeOH) λ_{max} 268 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ 4.80° (c 1.0, CHCl_3); ^1H NMR (CDCl_3 , 300 MHz) δ 8.04~7.86 (m, 4H), 7.79 (s, 1H), 7.66~7.37 (m, 6H), 6.27 (d, 1H, J = 6.2 Hz), 6.05 (d, 1H, J = 1.3 Hz), 5.13~5.07 (m, 1H), 4.91~4.77 (m, 1H), 4.71~4.63 (m, 1H), 4.58~4.53 (m, 1H), 3.73 (s, 3H), 3.16 (s, 3H); IR (neat) 2925, 1725 cm^{-1} ; MS (FAB) m/z 566 (M+H). A solution of protected nucleoside (113 mg, 0.200 mmol) in 40% MeNH_2 : THF (1:1, 4 mL) was stirred overnight at room temperature. Solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography (methylene chloride : methanol = 20:1) and then purified again by prep. TLC (methylene chloride : methanol = 20:1, five times) to provide **3b** (16 mg, 23%): mp 227.1~229.9 °C; UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ -16.49° (c 0.5, THF); ^1H NMR (CD_3OD , 400 MHz) δ 8.34 (s, 1H), 6.00~5.95 (dd, 1H, J = 3.6 and 14.0 Hz), 4.83~4.79 (m, 1H), 4.71~4.63 (m, 1H), 4.00~3.97 (dd, 1H, J = 2.4 and 12.4 Hz), 3.71~3.67 (dd, 2H, J = 2.4 and 12.6 Hz), 3.07 (s, 3H), 2.76 (s, 3H); IR (neat) 3431, 2934, 1631 cm^{-1} ; MS (FAB) m/z 357 (M+); Anal. Calcd for $\text{C}_{13}\text{H}_{17}\text{ClIN}_6\text{O}_4$: C, 43.77; H, 4.80; N, 23.56. Found: C, 43.94; H, 4.94; N, 23.92.

2-Chloro-N⁶-(2-methylbenzyl)-9-(3-C-methylcarbamoyl-3-deoxy-β-D-ribofuranosyl) adenine (3c)

To a stirred solution of **15** (127 mg, 0.222 mmol) in THF (4 mL), 2-methylbenzylamine (29.6 mg, 0.244 mmol) and TEA (0.28 mL, 2.00 mmol) were added, and the mixture was stirred at room temperature for 4 hr. Solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane : ethyl acetate = 3:1 to 2:1) to afford a 2-methylbenzyl derivative (110 mg, 75%): UV (MeOH) λ_{max} 272 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ -7.63° (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 8.02~7.93 (m, 4H), 7.73 (s, 1H), 7.66~7.60 (m, 1H), 7.54~7.31 (m, 6H), 7.23~7.18 (m, 3H), 6.26 (d, 1H, *J* = 6.2 Hz), 6.11 (s, 1H), 6.04 (d, 1H, *J* = 1.0 Hz), 5.12~5.06 (m, 1H), 4.85~4.80 (m, 3H), 4.69~4.63 (dd, 1H, *J* = 4.4 and 12.3 Hz), 4.57~4.52 (m, 1H), 3.73 (s, 3H), 2.38 (s, 3H); IR (neat) 3431, 2924, 1727 cm⁻¹; MS (FAB) *m/z* 657 (M+H); Anal. Calcd for C₃₄H₃₀ClN₅O₇: C, 62.24; H, 4.61; N, 10.67. Found: C, 62.43; H, 4.58; N, 10.66. A solution of protected nucleoside (58 mg, 0.0884 mmol) in 40% MeNH₂ : THF (1:1, 2 mL) was stirred at room temperature for 1 hr. Solvents were removed under reduced pressure and the residue was purified by prep. TLC (methylene chloride : methanol = 20:1, twice) to give **3c** (10 mg, 25%): mp 245.3~246.1 °C; UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ -11.63° (c 1.0, THF); ¹H NMR (CD₃OD, 300 MHz) δ 8.36 (s, 1H), 7.33 (d, 1H, *J* = 5.9 Hz), 7.18~7.14 (m, 2H), 6.92 (d, 1H, *J* = 0.8 Hz), 6.01 (d, 1H, *J* = 3.5 Hz), 4.82~4.79 (m, 1H), 4.74 (s, 2H), 4.65~4.63 (m, 1H), 4.01~3.96 (dd, 1H, *J* = 2.2, 2.4 and 12.5 Hz), 3.71~3.66 (dd, 2H, *J* = 2.6 and 12.6 Hz), 2.75 (s, 3H), 2.39 (s, 3H); IR (neat) 3421, 2937, 1739 cm⁻¹; MS (FAB) *m/z* 447 (M+H); Anal. Calcd for C₂₀H₂₃ClN₆O₄: C, 53.75; H, 5.19; N, 18.81. Found: C, 53.85; H, 5.12; N, 18.92.

2-Chloro-N⁶-cyclopropyl-9-(3-C-methylcarbamoyl-3-deoxy-β-D-ribofuranosyl) adenine (3d)

To a stirred solution of **15** (36 mg, 0.0630 mmol) in THF (2 mL), a solution of AcOH (23 mg, 0.383 mmol) in THF (1 mL) and cyclopropyl amine (22 mg, 0.385 mmol) were added, and the mixture was stirred at room temperature for 2 hr. Solvents were removed under reduced pressure and the residue was purified by silica gel chromatography (hexane : ethyl acetate = 4:1 to 3:1) to give a cyclopropyl derivative (36 mg, 97%): UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ 1.72° (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 8.01~7.93 (m, 4H), 7.80 (s, 1H), 7.66~7.39 (m, 6H), 6.26~6.04 (m, 2H), 6.05~6.00 (m, 1H), 5.11~5.07 (m, 1H), 4.85~4.80 (dd, 1H, *J* = 3.1 and 12.3 Hz), 4.68~4.62 (dd, 1H, *J* = 4.4, 4.6 and 12.4 Hz), 4.58~4.52 (m, 1H), 3.73 (s, 3H), 3.09 (s, 1H), 0.95~0.93 (m, 2H), 0.67~0.62 (m, 2H); IR (neat) 3355, 2952, 1727 cm⁻¹; MS (FAB) *m/z* 593 (M+H); Anal. Calcd for C₂₉H₂₆ClN₅O₇: C, 58.84; H, 4.43; N, 11.83. Found: C, 58.40; H, 4.62; N, 11.52. A solution of protected nucleoside (73 mg, 0.123 mmol) in 40% MeNH₂ : THF (1:1, 2 mL) was stirred at room temperature for 4 hr. Solvents were removed under reduced pressure and the residue was purified by prep. TLC (methylene chloride : methanol = 20:1, three times) to give **3d** (15 mg, 32%): UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ -15.97° (c 1.0, THF); ¹H NMR (CD₃OD, 300 MHz) δ 8.37 (s, 1H), 6.01 (d, 1H, *J* = 3.5 Hz), 4.83~4.78 (m, 1H), 4.66~4.62 (m, 1H), 4.01~3.96 (dd, 1H, *J* = 2.2, 2.4 and 12.5 Hz), 3.72~3.67 (dd, 1H, *J* = 2.6, 2.7 and 12.5 Hz), 3.34 (d, 1H, *J* = 2.0 Hz), 2.76 (s, 3H), 0.91~0.85 (m, 2H), 0.66~0.61 (m, 2H); MS (FAB) *m/z* 383 (M+H); Anal. Calcd for C₁₅H₁₉ClN₆O₄: C, 47.06; H, 5.00; N, 21.95. Found: C, 47.35; H, 5.18; N, 22.37.

2-Chloro-N⁶-cyclopentyl-9-(3-C-methylcarbamoyl-3-deoxy-β-D-ribofuranosyl) adenine (3e)

To a stirred solution of **15** (125 mg, 0.219 mmol) in THF (4 mL), a mixture of AcOH (79 mg, 1.32 mmol) and cyclopentyl amine (112 mg, 1.32 mmol) was added, and the reaction mixture was stirred for 4 hr at room temperature. Solvents were removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane : ethyl acetate = 3:1 to 2:1) to give a cyclopentyl derivative (101 mg, 75%): UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25}$ -0.26° (c 1.0, CHCl₃); ¹H NMR (CDCl₃, 300 MHz) δ 8.02~7.93 (m, 4H), 7.77 (s, 1H),

7.65~7.39 (m, 6H), 6.26 (d, 1H, $J = 5.9$ Hz), 6.04 (d, 1H, $J = 1.1$ Hz), 5.92 (s, 1H), 5.12~5.06 (m, 1H), 4.85~4.80 (dd, 1H, $J = 2.9$ and 12.3 Hz), 4.68~4.54 (m, 3H), 3.73 (s, 3H), 2.13 (m, 2H), 1.77~1.69 (m, 4H), 1.56~1.52 (m, 2H); IR (neat) 3355, 2955, 1726 cm^{-1} ; MS (FAB) m/z 621 (M+H); Anal. Calcd for $\text{C}_{31}\text{H}_{30}\text{ClN}_5\text{O}_7$: C, 60.05; H, 4.88; N, 11.29. Found: C, 60.36; H, 5.01; N, 10.85. A solution of protected nucleoside (128 mg, 0.206 mmol) in 40% MeNH_2 : THF(1:1, 4 mL) was stirred at room temperature for 3 hr. Solvents were removed under reduced pressure and the residue purified by prep. TLC (methylene chloride : methanol = 20:1, twice) to give **3e** (19 mg, 22%): mp 245.0~245.6 $^{\circ}\text{C}$; UV (MeOH) λ_{max} 273 nm (pH 7); $[\alpha]_{\text{D}}^{25} -17.66^{\circ}$ (c 0.8, THF); ^1H NMR (CD_3OD , 400 MHz) δ 8.34 (s, 1H), 5.99 (d, 1H, $J = 2.7$ Hz), 4.80~4.79 (m, 1H), 4.63~4.62 (m, 1H), 4.50 (s, 1H), 3.99~3.96 (dd, 1H, $J = 1.6$ and 10.0 Hz), 3.70~3.67 (dd, 1H, $J = 1.9$, 2.0 and 10.0 Hz), 3.32 (s, 1H), 2.75 (s, 3H), 2.10~2.00 (m, 2H), 1.79 (m, 2H), 1.76~1.67 (m, 2H), 1.63~1.58 (m, 2H); IR (neat) 3430, 2955, 1640 cm^{-1} ; MS (FAB) m/z 411 (M+H); Anal. Calcd for $\text{C}_{17}\text{H}_{23}\text{ClN}_6\text{O}_4$: C, 49.70; H, 5.64; N, 20.45. Found: C, 49.56; H, 5.92; N, 20.71.

Acknowledgment

This work was supported by the National Core Research Center (NCRC) program (No. R15-2006-020) of the Ministry of Science and Technology (MOST) and the Korea Science and Engineering Foundation (KOSEF).

References

1. Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev* 2001;53:527–552. [PubMed: 11734617]
2. Zhou QY, Li C, Olah ME, Johnson RA, Stiles GL, Civelli O. Molecular cloning and characterization of an adenosine receptor: The A_3 adenosine receptor. *Proc. Natl. Acad. Sci. USA* 1992;89:7432–7436. [PubMed: 1323836]
3. Jacobson KA, Gao Z-G. Adenosine receptors as therapeutic targets. *Nature Rev. Drug Disc* 2006;5:247–264. and references cited therein.
4. Gao Z-G, Blaustein JB, Gross AS, Melman N, Jacobson KA. N^6 -Substituted adenosine derivatives: selectivity, efficacy, and species differences at A_3 adenosine receptors. *Biochem. Pharmacol* 2003;65:1675–1684. [PubMed: 12754103]
5. Kim HO, Ji X-d, Siddiqi SM, Olah ME, Stiles GL, Jacobson KA. 2-Substitution of N^6 -benzyladenosine-5'-uronamides enhances selectivity for A_3 adenosine receptors. *J. Med. Chem* 1994;37:3614–3621. [PubMed: 7932588]
6. Lim MH, Kim HO, Moon HR, Lee SJ, Chun MW, Gao Z-G, Melman N, Jacobson KA, Kim JH, Jeong LS. Design, synthesis and binding affinity of 3'-fluoro analogues of Cl-IB-MECA as adenosine A_3 receptor ligands. *Bioorg. Med. Chem. Lett* 2003;13:817–820. [PubMed: 12617898]
7. DeNinno MP, Masamune H, Chenard LK, DiRico K, Eller C, Etienne JB, Tickner JE, Kennedy SP, Knight DR, Kong J, Oleynek JJ, Tracey WR, Hill RJ. 3'-Aminoadenosine-5'-uronamides: Discovery of the First Highly Selective Agonist at the Human Adenosine A_3 Receptor. *J. Med. Chem* 2003;46:353–355. [PubMed: 12540233]
8. Jeong LS, Kim MJ, Kim HO, Gao Z-G, Kim S-K, Jacobson KA, Chun MW. Design and synthesis of 3'-ureidoadenosine-5'-uronamides: effects of the 3'-ureido group on binding to the A_3 adenosine receptor. *Bioorg. Med. Chem. Lett* 2004;14:4851–4854. [PubMed: 15341938]
9. (a) Acton EM, Goerner RN, Uh HS, Ryan KJ, Henry DW. Improved antitumor effects in 3'-branched homologues of 2'-deoxythioguanosine. Synthesis and evaluation of thioguanine nucleosides of 2,3-dideoxy-3-(hydroxymethyl)-d-erythro-pentofuranose. *J. Med. Chem* 1979;22:518–5525. [PubMed: 582321] (b) Mazur A, Tropp BE, Engel R. Isosteres of natural phosphates. 11. Synthesis of a phosphonic acid analogue of an oligonucleotide. *Tetrahedron* 1984;40:3949–3956.
10. (a) Björnsne M, Classon B, Kers I, Samuelsson B, Kvarnström I. Synthesis of 2',3'-dideoxy-3',4'-dihydroxymethyl substituted pyrimidine nucleoside analogues. *Bioorg. Med. Chem. Lett* 1995;5:43–46. (b) Kim MJ, Kim HO, Kim H-D, Kim JH, Jeong LS, Chun MW. Synthesis and biological

evaluation of thymine nucleosides fused with 3',4'-tetrahydrofuran ring. *Bioorg. Med. Chem. Lett* 2003;13:3499–3501. [PubMed: 14505657]

11. Gao Z-G, Jeong LS, Moon HR, Kim HO, Choi WJ, Shin DH, Elhalem E, Comin MJ, Melaman N, Mamedova L, Gross AS, Rodriguez JB, Jacobson KA. Structural determinants of efficacy at A3 adenosine receptors: modification of the ribose moiety. *Biochem. Pharmacol* 2004;67:893–901. [PubMed: 15104242]

Figure 1.
 The rationale for the design of the target nucleosides

Scheme 1.

Reagents and conditions: (a) DMSO, Oxalyl Chloride, MC; (b) $\text{Ph}_3\text{PCH}_3\text{Br}$, *t*-BuOK, THF; (c) i. Borane Methyl Sulfide, THF, ii. H_2O , 2N NaOH, H_2O_2 ; (d) NaH, TBAI, BnBr, THF; (e) 4N HCl, THF/ H_2O ; (f) i. NaIO_4 THF/ H_2O , ii. NaBH_4 ; (g) BzCl , MC; (h) 0.1% AcCl , MeOH; (i) BzCl , pyridine; (j) H_2 Pd-C; (k) i. NaIO_4 , RuCl_3 , MeCN/ CCl_4 / H_2O , ii. DCC, MeOH, 4-aminopyridine, MC; (l) $\text{AcOH}/\text{Ac}_2\text{O}/\text{c-H}_2\text{SO}_4$.

Scheme 2.

Reagents and conditions: (a) silylated 2,6-dichloropurine, TMSOTf, DCE; (b) various amines; (c) MeNH₂.