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**PAPER**

# Regio- and stereoselective synthesis of truncated 3′-aminocarbanucleosides and their binding affinity at the $A_3$ adenosine receptor

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The stereoselective synthesis of truncated 3′-aminocarbanucleosides **4a–d** *via* a stereo- and regioselective conversion of a diol **9** to bromoacetate **11a** and their binding affinity towards the human $A_3$ adenosine receptor are described.

## Introduction

Adenosine binds to four subtypes ($A_1$, $A_{2A}$, $A_{2B}$, and $A_3$) of adenosine receptors (ARs) located on cell membranes and regulates many physiological functions by modulating cell signalling.[1] Second messengers, such as inositol trisphosphate ($IP_3$), diacylglycerol (DAG), and cyclic AMP, are essential for cell signalling and are controlled by ARs. Thus, ARs have been promising targets for the treatment of several diseases associated with these signalling pathways.[2] For example, $A_3$ AR agonists have therapeutic potential against cancer,[3] cerebral ischemia,[4] and myocardial ischemia,[5] while $A_3$ AR antagonists display anti-inflammatory,[6] anti-asthma,[7] and anti-glaucoma activities.[8]

The locked methanocarba nucleosides **1**,[9] with fixed Northern conformations were reported to be potent and selective $A_3$ AR agonists. However, their truncated analogues **2**[10] were transformed to potent and species-independent $A_3$ AR antagonists due to the absence of 5′-uronamide, whose NH serves as a key hydrogen-bonding donor essential for receptor activation. On the other hand, 3′-deoxy-3′-aminoadenosine derivatives **3**[11] have been discovered to be potent and selective $A_3$ AR agonists. The 3′-amino group of **3** improved its selectivity for other subtypes and its aqueous solubility. Based on these findings, we designed compounds **4** (Fig. 1), which combine the properties of truncated nucleosides and 3′-amino nucleosides to develop potent and selective $A_3$ AR antagonists. The 3′-amino group was introduced by the regioselective opening of the acylinium ion with bromide, followed by treating the bromide with sodium azide. Herein, we report the stereo- and regioselective synthesis of truncated 3′-aminonucleosides **4** starting from D-ribose and their binding affinity towards the human $A_3$ AR.

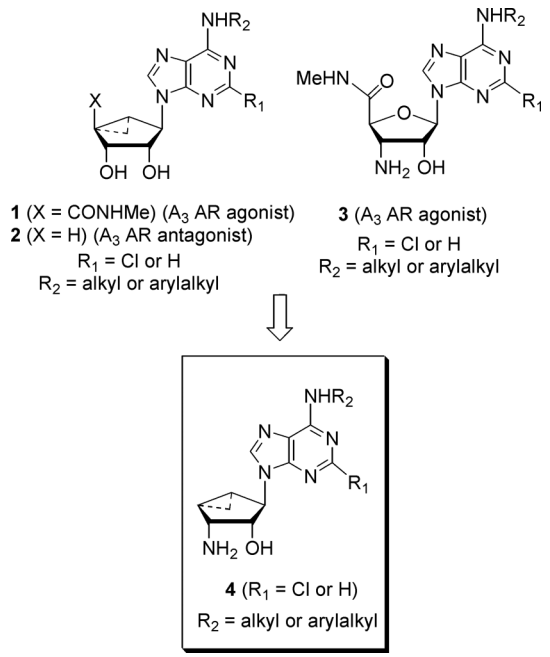


**Fig. 1** Rationale for the design of the target nucleosides.

## Results and discussion

Synthesis of the desired nucleosides was achieved by first synthesising the glycosyl donor and then condensing it with purine bases.

Scheme 1 illustrates the synthesis of the key precursor **9** for the introduction of a 3′-amino group. D-Ribose was easily converted to cyclopentenone **5** according to our previously published procedure.[12] Treatment of cyclopentenone **5** with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ gave the α-allylic alcohol **6**[12] as a single diastereomer, resulting from the attack of the hydride to the less hindered convex face of the ketone.[13] The stereoselective cyclopropanation of the α-allylic alcohol **6** with diethyl zinc and methylene iodide afforded the cyclopropyl-fused alcohol **7** as a single diastereomer due to the directing effect of the allylic

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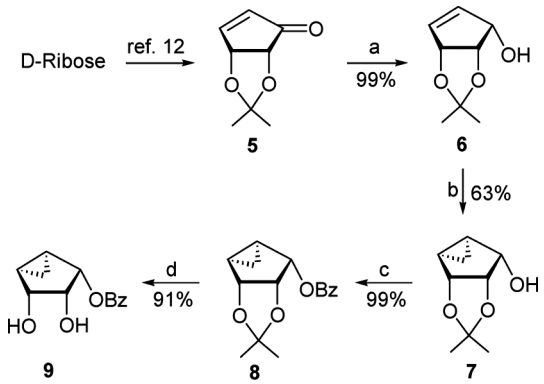
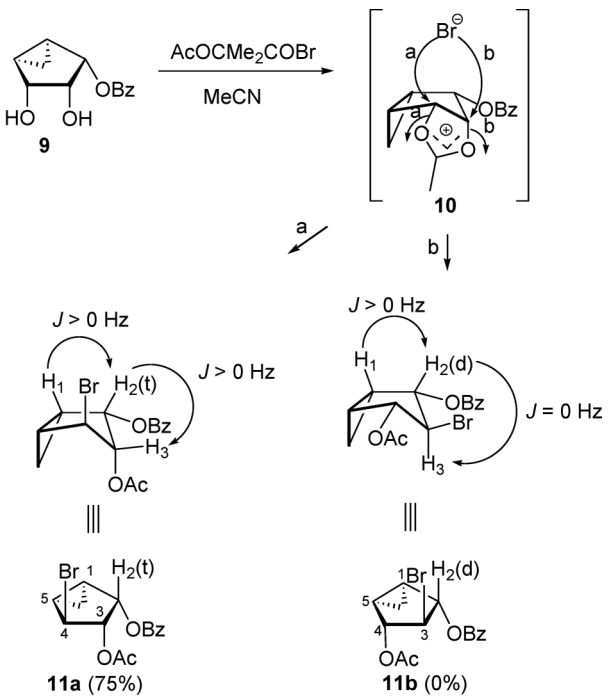



***Reagents and Conditions***: a) $NaBH_4$, $CeCl_3$ $7H_2O$, 0 °C, 40 min; b) $Et_2Zn$, $CH_2I_2$, 0 °C to rt, 3 h; c) BzCl, pyridine, rt, 3.5 h; d) 50% TFA, rt, overnight.

**Scheme 1**

hydroxyl group.[13,14] Benzoylation of **7**, followed by hydrolysis of the isopropylidene group in benzoate **8** with 50% trifluoroacetic acid (TFA) yielded the diol **9**, which is a key precursor to azidation.

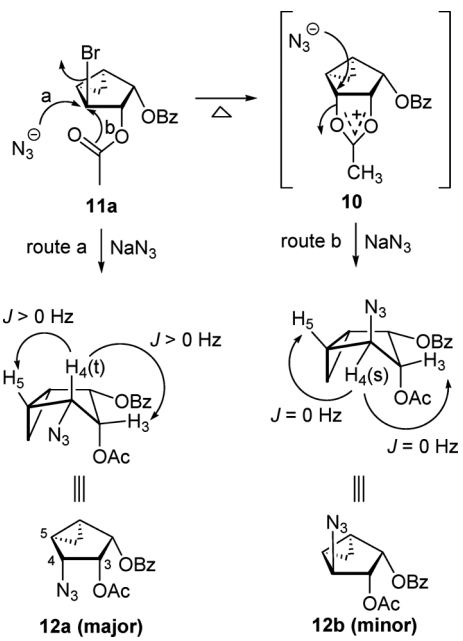
Treatment of the diol **9** with 2-acetoxyisobutyryl bromide[15] gave bromoacetate **11a** as a single regioisomer (75%) without the formation of its regioisomer **11b** (Scheme 2). In this reaction, after the diol **9** was first converted to an acylinium ion **10**, the bromide attacked the C4 position of **10** in the pseudoaxial direction (route a), which was more highly favoured than the same reaction in the pseudoequatorial direction (route b), yielding the desired regioisomer **11a** exclusively. Two regioisomers, **11a** and **11b**, were unambiguously assigned by the diagnostic coupling constants typical of the boat conformation of the bicyclo[3.1.0]hexane system, which has been extensively confirmed by X-ray crystallography and NMR analysis.[16] In the case of compound **11a**, the coupling constant of the $J_{H2,H3}$ was greater than zero because of the *cis* relationship, while, in the case of **11b**, the corresponding coupling constant was zero because a $H_2$–C–C–$H_3$ dihedral angle with a *trans* relationships is close to 90°.[16] Thus, H-2 in **11a** should be split into a pseudo triplet or doublet of doublets, but H-2 in **11b** should appear as a doublet. Indeed, $^1$H NMR spectroscopy of **11a** indicated that H-2 showed a pseudo triplet at 5.95 ppm, confirming the structure of **11a**. Furthermore, irradiation of the H-2 pseudo triplet at $\delta$ 5.95 caused the H-3 doublet at $\delta$ 5.60 to coalesce into a singlet. This situation could not be the case for the alternative regioisomer **11b**. It should be noted that this type of assignment works only in this system and could not be applied to the flexible, carbocyclic counterparts.




$J_{H2,H3}$ = 6.8 Hz; the signal for the H3 (doublet) becomes a singlet when the H2 (triplet) is irradiated. The signal for the H4 (at 4.28 ppm) is a singlet.

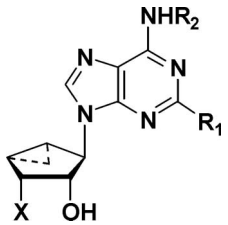
**Scheme 2**

The desired product **11a** was treated with $NaN_3$ in DMF at 100 °C to give the azido derivative **12a** (44%), but its stereoisomer **12b** (11%) was also obtained as a minor isomer (Scheme 3). As illustrated in Scheme 3, the $S_N2$ reaction of the bromide **11a** with sodium azide yielded the desired azido compound **12a** as a major isomer, but **11a** also underwent an intramolecular $S_N2$ reaction with heating, forming the intermediate **10**, which was attacked by sodium azide at the C4 position (Scheme 2) to give **12b** as a minor isomer. The structures of **12a** and **12b** were also confirmed by the diagnostic coupling constants typical of the boat conformation of the bicyclo[3.1.0]hexane system, as shown in Scheme 3.[16] In the case of **12a**, H-4 was split as a pseudo triplet at 4.12 ppm because of two dihedral angles ($H_3$–C–C–$H_4$ and $H_4$–C–C–$H_5$) with *cis* relationships, while, in the case of **12b**, H-4 was split as a singlet at 3.93 ppm because of two dihedral angles ($H_3$–C–C–$H_4$ and $H_4$–C–C–$H_5$) with *trans* relationships.[16]




**Scheme 3**

**Table 1** Binding affinities of the known $A_3$AR antagonist **2** and 3′-amino derivatives **4a–d** at three subtypes of hARs

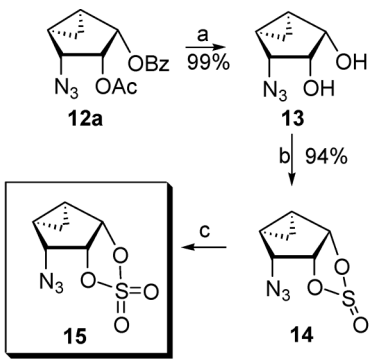



| Compound (X, $R_1$, $R_2$) | Affinity ($K_i$, nM ± SEM, or % inhibition)[a] | | |
|---|---|---|---|
| | $hA_1$ | $hA_{2A}$ | $hA_3$ |
| **2** (X = OH, $R_1$ = Cl, $R_2$ = 3-iodobenzyl)[b] | 3040 ± 610 | 1080 ±310 | 1.44 ± 0.60 |
| **4a** (X = $NH_2$, $R_1$ = Cl, $R_2$ = 3-iodobenzyl) | 15 ± 8% | 12% ± 5% | 24.7% ± 0.6% |
| **4b** (X = $NH_2$, $R_1$ = Cl, $R_2$ = methyl) | 8 ± 6% | 5% ± 2% | 42.3% ± 7.4% |
| **4c** (X = $NH_2$, $R_1$ = H, $R_2$ = 3-iodobenzyl) | 10% | 24 ± 3% | 5460 ± 1280 |
| **4d** (X = $NH_2$, $R_1$ = H, $R_2$ = methyl) | 3 ± 2% | 5 ± 2% | 27.2% ± 5.3% |

[a] All binding experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the appropriate hAR ($A_1$AR and $A_3$AR in CHO cells and $A_{2A}$AR in HEK-293 cells). Binding was carried out using 1 nM [$^3$H]R-PIA, 10 nM [$^3$H]CGS21680, or 0.5 nM [$^{125}$I]I-AB-MECA as radioligands for $A_1$, $A_{2A}$, and $A_3$ARs, respectively. Values are expressed as mean ± sem, n = 3-4 (outliers eliminated) and normalized against a non-specific binder, 5′-*N*-ethylcarboxamidoadenosine (NECA, 10 μM). Values expressed as a percentage refer to percent inhibition of specific radioligand binding at 10 μM, with nonspecific binding defined using 10 μM NECA. [b] Data from ref. 10.



Removal of the protecting groups of **12a** with NaOMe afforded the diol **13** (Scheme 4). Treatment of the diol **13** with thionyl chloride followed by oxidation of the resulting cyclic sulfiphite **14** with $RuCl_3$ and $NaIO_4$ gave the cyclic sulphate **15** as an epoxide surrogate.[17]




***Reagents and Conditions***: a) NaOMe, MeOH, rt, 1 h; b) $SOCl_2$, $Et_3N$, MC, 0 °C, 30 min; c) $RuCl_3$-$H_2O$, $NaIO_4$, $CCl_4/CH_3CN/H_2O$=1/1/1.5, rt, 10 min.

**Scheme 4**

The glycosyl donor **15** was transformed to the 3′-deoxy-3′-aminoadenosine derivatives **4a–d** as illustrated in Scheme 5. Condensation of **15** with 2,6-dichlorpurine and 6-chloropurine gave the azido derivatives **16** and **17**, respectively, after acid-catalysed hydrolysis of the sulphates.[18] The ring-opening reaction proceeded with complete regioselectivity. Proton NMR spectroscopy of **16** confirmed the structure unambiguously by virtue of the diagnostic coupling constants typical of the boat conformation of the bicyclo[3.1.0]hexane system (Scheme 3). Indeed, irradiation of the H-2′ triplet at $\delta$ 4.68 caused the H-3′ doublet at $\delta$ 4.20 to coalesce into a singlet. Treatment of **16** and **17** with 3-iodobenzylamine produced **18a** and **18c**, respectively. Treatment of **16** and **17** with methylamine produced **18b** and **18d**, respectively. Conversion of the 3′-azido group to the 3′-amino group was achieved by treating **18a–d** with $Ph_3P$ and $NH_4OH$ in THF-$H_2O$ to give **4a–d**, respectively.[11]

Binding assays were performed using standard radioligands and membrane preparations from Chinese hamster ovary (CHO) cells stably expressing the human $A_1$ or $A_3$AR or from human embryonic kidney cells (HEK-293) expressing human $A_{2A}$AR.[10] As shown in Table 1, all synthesised 3′-amino derivatives displayed much lower binding affinities towards the human $A_3$ AR than the reference $A_3$ AR antagonist **2**. This result was unexpected because the 3′-amino group can serve as the same hydrogen bonding donor as the 3′-hydroxyl group, and some 3′-amino nucleosides are reported to be potent $A_3$AR agonists.[11] Only compound **4c** showed moderate binding affinity for the human $A_3$ AR.

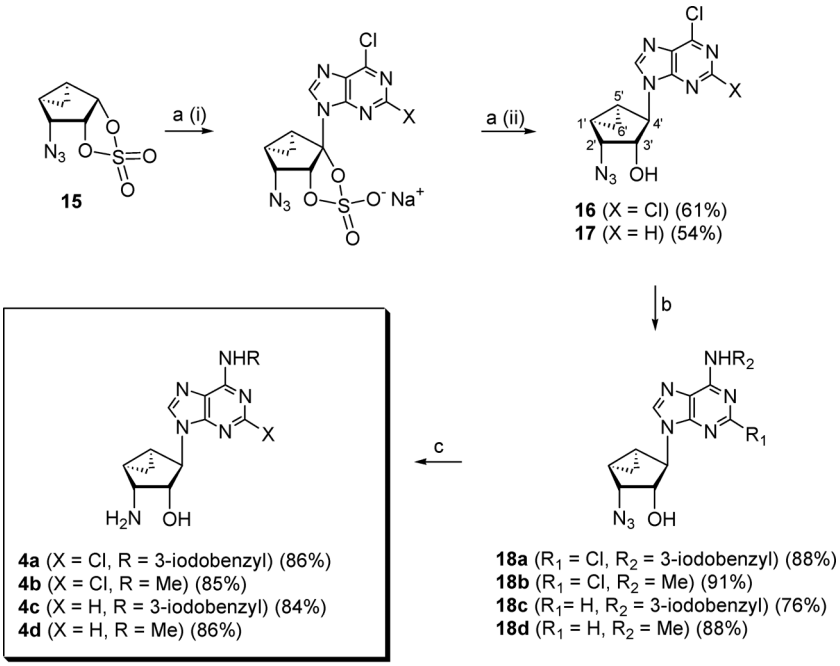
## Conclusions

In summary, we have achieved stereoselective synthesis of truncated 3′-aminocarbanucleosides, which are fixed in an (*N*) conformation. Their binding affinities towards human ARs were also determined. The key step for the introduction of the 3′-amino group was to stereo- and regioselectively convert the diol **9** to the bromoacetate **11a** *via* an acylinium ion **10**. Finally, we observed that azidation of the bromoacetate **11a** with sodium azide proceeded *via* an acylinium ion **10**, in addition to direct a $S_N2$ reaction.

## Experimental section

### General methods

$^1$H and $^{13}$C NMR Spectra ($CDCl_3$ or $CD_3OD$) were recorded on 400 and 100 MHz NMR, respectively. The $^1$H NMR data are




***Reagents and Conditions***: a) i. 2,6-dichloropurine or 6-chloropurine, NaH, rt, 4 h; ii. 35% $H_2SO_4$, rt, overnight; b) 3-iodobenzylamine or methylamine, $Et_3N$, EtOH, rt, overnight; c) $PPh_3$, $NH_4OH$, THF, rt, overnight.

**Scheme 5**



reported as peak multiplicities: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, br s for broad singlet and m for multiplet. Coupling constants are reported in hertz. The chemical shifts are reported as parts per million (δ) relative to the solvent peak. Column chromatography was performed on silica gel 60 (230–400 mesh). All the anhydrous solvents were distilled over $CaH_2$, $P_2O_5$ or sodium/benzophenone prior to the reaction.

### (+)-(1*R*,2*S*,3*S*,4*R*,5*S*)-3,4-*O*-Isopropylidene bicycle[3.1.0]hexa-2-ol (7)

Diethylzinc (64 mL, 64.07 mmol, 1.0 M solution in hexane) and diiodomethane (10.34 mL, 128.14 mmol) were added to a solution of **6** (5.00 g, 32.03 mmol) in methylene chloride (68 mL), cooled in an ice bath, and the reaction mixture was stirred at room temperature for 3 h. The mixture was quenched with a cold, aqueous ammonium chloride solution and extracted with methylene chloride. The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane : ethyl acetate, 4 : 1) to give **7** (3.20 g, 63%) as a colourless syrup: MS (ESI$^+$) Found: 193.0836 [M+Na]$^+$ for $C_9H_{14}O_3Na$, calculated: 193.0844, $[\alpha]_D^{27.1}$ 42.7 (*c* 3.72, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 0.56–0.62 (m, 1 H), 0.94 (dd, $J$ = 4.0, 8.0 Hz, 1 H), 1.25 (s, 3 H), 1.50 (s, 3 H), 1.57–1.64 (m, 1 H), 1.78–1.84 (m, 1 H), 2.43 (d, $J$ = 4.0 Hz, 1 H, $D_2O$ exchangeable), 4.43 (pseudo t, $J$ = 8.0 Hz, 1 H), 4.46–4.52 (m, 1 H), 4.84 (pseudo t, $J$ = 4.0 Hz, 1 H); $^{13}$C NMR ($CDCl_3$) $\delta$ 6.7, 21.4, 24.7, 26.2, 28.3, 71.5, 79.2, 81.1, 113.0; Anal. calcd for $C_9H_{14}O_3$: C, 63.51; H, 8.29. Found: C, 63.53; H, 8.21.

### (+)-(1*R*,2*S*,3*S*,4*R*,5*S*)-Benzoic acid 3,4-*O*-Isopropylidene bicycle[3.1.0]hexa-2-yl-ester (8)

Benzoyl chloride (6.94 mL, 59.83 mmol) was added to a solution of **7** (3.16 g, 19.94 mmol) in pyridine (20 mL), and the reaction mixture was stirred at room temperature for 3.5 h. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane : ethyl acetate, 3 : 1) to give **8** (5.51 g, 99%) as a colourless syrup: MS (ESI$^+$) Found: 297.1090 [M+Na]$^+$ for $C_{16}H_{18}O_4Na$, calculated: 297.1098, $[\alpha]_D^{26.8}$ 43.80 (*c* 4.37, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 0.71–0.77 (m, 1 H), 1.24 (s, 3 H), 1.31 (dd, $J$ = 4.0, 8.0 Hz, 1 H), 1.47 (s, 3 H), 1.66–1.72 (m, 1 H), 1.89–1.95 (m, 1 H), 4.57 (pseudo t, $J$ = 8.0 Hz, 1 H), 4.95 (pseudo t, $J$ = 8.0 Hz, 1 H), 5.46 (pseudo t, $J$ = 8.0 Hz, 1 H), 7.45–7.40 (m, 2 H), 7.55–7.51 (m, 1 H), 8.07–8.10 (m, *2* H); $^{13}$C NMR ($CDCl_3$) $\delta$ 7.5, 21.2, 24.7, 26.3, 74.3, 78.5, 80.9, 113.2, 128.4, 129.9, 130.4, 133.0, 166.5; Anal. calcd for $C_{16}H_{18}O_4$: C, 70.06; H, 6.61. Found: C, 70.08; H, 6.21.

### (–)-(1*R*,2*S*,3*S*,4*R*,5*S*)-Benzoic acid 3,4-dihydroxy-bicyclo[3.1.0]hex-2-yl ester (9)

A solution of **8** (384.2 mg, 1.46 mmol) and 50% TFA (8 mL) was stirred at room temperature overnight. The solvent was evaporated and coevaporated with toluene to give a colourless syrup. The syrup was purified by silica gel column chromatography (methylene chloride : methanol, 19 : 1) to give **9** (311.2 mg, 91%) as a white solid: mp 149.7–256.8 °C; MS (ESI$^+$) Found: 491.1653 [2 M+Na]$^+$ for $C_{13}H_{14}O_4Na$, calculated: 491.1638, $[\alpha]_D^{27.1}$ –0.68 (*c* 2.79, $CHCl_3$); $^1$H NMR ($CDCl_3$) $\delta$ 0.52–0.58 (m, 1 H), 1.29 (dd, $J$ = 4.0, 8.0 Hz, 1 H), 1.71–1.83 (m, 2H), 2.73 (s, 1 H, $D_2O$

exchangeable), 2.75 (d, $J$ = 3.2 Hz, 1 H, $D_2O$ exchangeable), 4.20 (dd, $J$ = 8.0, 12.0 Hz, 1 H), 4.46–4.51 (m, 1 H), 5.38 (pseudo t, $J$ = 4.0 Hz, 1 H), 7.41–7.45 (m, 2 H), 7.58–7.53 (m, 1 H), 8.05–8.08 (m, 2 H); $^{13}$C NMR ($CDCl_3$) $\delta$ 5.6, 19.9, 22.6, 68.7, 71.9, 76.1, 128.6, 129.9, 130.2, 133.4, 166.7; Anal. calcd for $C_{13}H_{14}O_4$: C, 66.66; H, 6.02. Found: C, 66.56; H, 6.24.

### (+)-(1*R*,2*S*,3*S*,4*S*,5*S*)-Benzoic acid 3-acetoxy-4-bromo-bicyclo[3.1.0]hex-2-yl ester (11a)

A solution of 2-acetoxyisobutyryl bromide (1.86 mL, 12.81 mmol) in anhydrous acetonitrile (10 mL) was added dropwise to a suspension of **9** (1.00 g, 4.27 mmol) in anhydrous acetonitrile (43 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 3.5 h. A saturated sodium bicarbonate solution was added dropwise at 0 °C until the pH of the solution reached 8. The solution was then neutralised with acetic acid to pH 5. The mixture was extracted with ethyl acetate and washed with sodium bicarbonate (x2) and brine. The organic layer was dried ($MgSO_4$), filtered, and evaporated. After evaporation, the residue was purified by silica gel column chromatography (hexane : ethyl acetate, 10 : 1) to give **11a** (1.08 g, 75%) as a colourless syrup: MS (ESI$^+$) Found: 361.0040 [M+Na]$^+$ for $C_{15}H_{15}BrO_4Na$, calculated: 361.0048, $[\alpha]_D^{27.1}$ 0.18 ($c$ 15.1, $CHCl_3$); IR (KBr) 2101.9 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.72–0.78 (m, 1 H), 1.21–1.25 (m, 1 H), 1.94–1.96 (m, 1 H), 2.0 (s, 3 H), 2.11–2.15 (m, 1 H), 4.28 (s, 1 H), 5.60 (d, $J$ = 6.8 Hz, 1 H), 5.95 (pseudo t, $J$ = 6.7 Hz, 1 H), 7.40–7.44 (m, 2 H), 7.53–7.57 (m, 1 H), 7.96–7.99 (m, 2 H); $^{13}$C NMR ($CDCl_3$) $\delta$ 10.6, 20.9, 25.1, 51.9, 73.7, 76.5, 128.5, 129.6, 133.2, 165.8, 168.8; Anal. calcd for $C_{15}H_{15}BrO_4$: C, 53.12; H, 4.46. Found: C, 53.52; H, 4.21.

### (+)-(1*R*,2*S*,3*S*,4*R*,5*S*)-Benzoic acid 3-acetoxy-4-azido-bicyclo[3.1.0]hex-2-yl ester (12a) and (+)-(1*R*,2*S*,3*S*,4*S*5*S*)-Benzoic acid 3-acetoxy-4-azido-bicyclo[3.1.0]hex-2-yl ester (12b)

Sodium azide (225.6 mg, 3.47 mmol) was added to a suspension of **11a** (235.4 mg, 0.69 mmol) in DMF (8 mL) at room temperature, and the reaction mixture was stirred at 100 °C for 3 h. The solvent was evaporated and the residue was quenched with water and extracted with ether. The organic layer was dried ($MgSO_4$), filtered, and evaporated. After evaporation, the residue was purified by silica gel column chromatography (hexane : ethyl acetate, 9 : 1) to give **12a** (92.7 mg, 44%) as a colourless syrup and **12b** (23.2 mg, 11%) as a colourless syrup.

**Compound 12a.** MS (ESI$^+$) Found: 324.0949 [M+Na]$^+$ for $C_{15}H_{15}N_3O_4Na$, calculated: 324.0957, $[\alpha]_D^{26.5}$ 0.33 ($c$ 3.29, $CHCl_3$); IR (KBr) 2101.3 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.76–0.81 (m, 1 H), 1.49–1.53 (m, 1 H), 1.84–1.85 (m, 1 H), 1.89–1.94 (m, 1 H), 2.08 (s, 3 H), 4.12 (pseudo t, $J$ = 4.0 Hz, 1 H), 5.47 (pseudo t, $J$ = 8.0 Hz, 1 H), 5.63 (pseudo t, $J$ = 4.0 Hz, 1 H), 7.41–7.46 (m, 2 H), 7.54–7.58 (m, 1 H), 7.98–8.01 (m, 2 H); $^{13}$C NMR ($CDCl_3$) $\delta$ 6.3, 18.7, 19.2, 20.7, 61.0, 69.4, 73.6, 128.6, 129.7, 133.36, 165.87, 169.41; Anal. calcd for $C_{15}H_{15}N_3O_4$: C, 59.79; H, 5.02; N, 13.95. Found: C, 60.12; H, 5.21; N, 13.56.

**Compound 12b.** MS (ESI+) $m/z$ 301.1057 [M]$^+$; $[\alpha]_D^{26.5}$; 0.30° ($c$ 1.1, $CHCl_3$); IR (KBr) 2101.3 cm$^{-1}$;$^1$H NMR ($CDCl_3$) $\delta$ 0.77–0.83 (m, 1 H), 1.07–1.11 (m, 1 H), 1.60–1.65 (m, 1 H), 2.0 (s, 3H), 2.01–2.06 (m, 1 H), 3.93 (s, 1 H), 5.26 (d, $J$ = 8.0 Hz, 1 H), 5.71–5.74 (m, 1 H), 7.42–7.42 (m, 2 H), 7.55–7.59 (m, 1 H), 7.97– 8.01 (m, 2 H); Anal. calcd for $C_{15}H_{15}N_3O_4$: C, 59.79; H, 5.02; N, 13.95. Found: C, 59.92; H, 5.20; N, 13.55.

### (–)-(1*R*,2*S*,3*S*,4*R*,5*S*)-4-Azido-bicyclo[3.1.0]hexane-2,3-diol (13)

Sodium methoxide (0.2 g, 3.78 mmol) was added to a solution of **12a** (0.38 g, 1.26 mmol) in methanol (20 mL), and the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was partitioned between ethyl acetate and water. The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (methylene chloride:methanol, 10 : 1) to give **13** (0.20 g, 99%) as a colourless syrup: $[\alpha]_D^{26.7}$ –0.15 ($c$ 2.68, MeOH); IR (KBr) 2101.2 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 0.37–0.42 (m, 1 H), 1.32 (dd, $J$ = 4.0, 8.0 Hz, 1 H), 1.44–1.48 (m, 1 H), 1.55–1.59 (m, 1 H), 3.62 (pseudo t, $J$ = 4.0 Hz, 1 H), 3.73–3.77 (m, 1 H), 4.14–4.19 (m, 1 H), 4.37 (d, $J$ = 8.0 Hz 1 H, $D_2O$ exchangeable), 5.19 (d, $J$ = 4.0 Hz, 1 H, $D_2O$ exchangeable); $^{13}$C NMR (MeOH-$d_4$) $\delta$ 6.5, 19.7, 21.7, 63.5, 71.5, 73.4; Anal. calcd for $C_6H_9N_3O_2$: C, 46.45; H, 5.85; N, 27.08. Found: C, 46.42; H, 5.88; N, 27.56.

### (–)-(1*R*,2*S*,3*S*,4*R*,5*S*) 4-Azido-bicyclo[3.1.0]hexane-2,3-*O*-sulphite (14)

Triethylamine (0.18 mL, 0.54 mmol) and thionyl chloride (0.04 mL, 0.54 mmol) were added to a solution of **13** (55.2 mg, 0.36 mmol) in methylene chloride (3 mL) at 0 °C, and the reaction mixture was stirred at 0 °C for 10 min. The reaction mixture was partitioned between methylene chloride and water. The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane : ethyl acetate, 2 : 1) to give **14** (66.6 mg, 94%) as a colourless syrup: $[\alpha]_D^{27.1}$ –2.33 ($c$ 3.31, $CHCl_3$); IR (KBr) 2104.7 cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 0.87–0.93 (m, 1 H), 1.0–1.03 (m, 1 H), 1.83–1.89 (m, 1 H), 1.96–2.03 (m, 1 H), 4.30 (pseudo t, $J$ = 4.0 Hz, 1 H), 5.21–5.25 (m, 1 H), 5.65 (pseudo t, $J$ = 8.0 Hz, 1 H); $^{13}$C NMR ($CDCl_3$) $\delta$ 8.3, 20.4, 25.3, 60.4, 82.2, 87.5; Anal. calcd for $C_6H_7N_3O_3S$: C, 35.82; H, 3.51; N, 20.88. Found: C, 35.53; H, 3.22; N, 20.98.

### (–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Azido-4-(2,6-dichloro-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (16)

Sodium metaperiodate (2.04 g, 6.5 mmol) and ruthenium chloride trihydrate (71.9 mg) were added to a solution of **14** (0.68 g, 3.40 mmol) in carbon tetrachloride, acetonitrile, and water (1 : 1 : 1.5, 17.5 mL), and the reaction mixture was stirred at room temperature for 10 min. The reaction mixture was partitioned between methylene chloride and water and the organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane : ethyl acetate, 1 : 1) to give **15** as a colourless syrup, which was immediately used for the next reaction.

A suspension of 2,6-dichloropurine (0.96 g, 5.1 mmol), sodium hydride (0.2 g, 5.1 mmol, 60% dispersion in mineral oil), and 18-crown-6 (1.35 g, 5.1 mmol) in tetrahydrofuran (10 mL) was stirred at 80 °C for 3 h. To this solution, a solution of **15** in tetrahydrofuran (10 mL) was added, and the mixture was stirred at room temperature for 4 h. The mixture was cooled to 0 °C, and 30% sulphuric acid was added carefully to lower the pH to 2. The reaction mixture was stirred at room temperature overnight

and then neutralised with a sodium bicarbonate solution. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified by silica gel column chromatography (methylene chloride : methanol, 30 : 1) to give **16** (0.68 g, 61%) as a colourless syrup: MS (ESI$^+$) Found: 326.0320 $[M+H]^+$ for $C_{11}H_{10}Cl_2N_7O$, calculated: 326.0347, $[\alpha]_D^{26.2}$ –7.68 (*c* 1.15, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 274.5 nm; IR (KBr) 2104.4 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.00–1.06 (m, 1 H), 1.24–1.28 (m, 1 H), 1.41–1.44 (m, 1 H), 1.73–1.78 (m, 1 H), 2.18–2.24 (m, 1 H), 2.63 (br s, 1 H, $D_2O$ exchangeable), 4.20 (d, $J$ = 6.4 Hz, 1 H), 4.68 (pseudo t, $J$ = 5.6 Hz, 1 H), 4.97 (s, 1 H), 8.17 (s, 1 H); $^{13}C$ NMR (MeOH-$d_4$) $\delta$ 9.1, 19.1, 22.3, 63.9, 65.4, 78.6, 132.2, 147.4, 152.1, 153.8, 155.3; Anal. calcd for $C_{11}H_9Cl_2N_7O$: C, 40.51; H, 2.78; N, 30.06. Found: C, 40.63; H, 2.44; N, 30.16.

### (–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Azido-4-(6-chloro-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (17)

Compound **15** (288.0 mg, 1.44 mmol) was condensed with 6-chloropurine to give compound **17** (228.1 mg, 54%) as a foam according to a procedure similar to that was used to prepare **16**: MS (ESI$^+$) Found: 292.0706 $[M+H]^+$ for $C_{11}H_{11}ClN_7O$, calculated: 292.0734, $[\alpha]_D^{26.4}$ –1.65 (*c* 1.03, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 264.0 nm; IR (KBr) 2110.76 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 1.00–1.06 (m, 1 H), 1.43–1.46 (m, 1 H), 1.77–1.81 (m, 1 H), 2.17–2.23 (m, 1 H), 2.99 (pseudo t, $J$ = 2.8 Hz, 1 H, $D_2O$ exchangeable), 4.23 (d, $J$ = 6.6 Hz, 1 H), 4.69 (pseudo t, $J$ = 5.6 Hz, 1 H), 5.01 (s, 1 H), 8.20 (s, 1 H), 8.76 (s, 1 H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 8.9, 19.8, 21.9, 63.8, 64.1, 77.9, 132.3, 143.8, 151.4, 151.7, 152.3; Anal. calcd for $C_{11}H_{10}ClN_7O$: C, 45.29; H, 3.46; N, 33.61. Found: C, 45.69; H, 3.24; N, 33.56.

### General Procedure for the Synthesis of 18a–d

An appropriate amount of amine (1.5 equiv.) was added to a solution of **16** and **17** in EtOH (5 mL) at room temperature, and the mixture was stirred at room temperature for a time period ranging from 20 min to 1 d and then evaporated. The residue was purified by flash silica gel column chromatography (hexane : ethyl acetate, 1 : 1) to give **18a,18b,18c**, and **18d**, respectively.

### (–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Azido-4-[2-chloro-6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo[3.1.0] hexan-3-ol (18a)

Yield: 89%; white foam; MS (ESI$^+$) Found: 523.0238 $[M+H]^+$ for $C_{18}H_{17}ClIN_8O$, calculated: 523.0266, $[\alpha]_D^{26.0}$ –7.21 (*c* 2.62, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 272.0 nm; IR (KBr) 2105.6 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.93–0.98 (m, 1 H), 1.43–1.46 (m, 1 H), 1.66–1.70 (m, 1 H), 2.05–2.11 (m, 1 H), 4.18 (d, $J$ = 6.8 Hz, 1 H), 4.36 (t, $J$ = 6.0 Hz, 1 H), 4.68 (s, 1 H, $D_2O$ exchangeable), 4.77 (s, 2 H), 4.84 (s, 1 H), 7.0 (br s, 1 H, $D_2O$ exchangeable), 7.04 (pseudo t, $J$ = 8.0 Hz, 1 H), 7.32 (d, $J$ = 7.6 Hz, 1 H), 7.58 (dt, $J$ = 1.2, 9.2 Hz, 1 H), 7.70 (pseudo t, $J$ = 1.2 Hz, 1 H), 7.72 (s, 1 H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 8.7, 14.4, 19.1, 21.4, 43.9, 60.6, 63.2, 77.5, 94.7, 118.9, 127.3, 130.6, 136.8, 138.5, 140.6, 149.9, 154.8, 155.2, 171.4; Anal. calcd for $C_{18}H_{16}ClIN_8O$: C, 41.36; H, 3.09; N, 21.44. Found: C, 41.76; H, 3.24; N, 21.57.

### (–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Azido-4-(2-chloro-6-methylamino-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (18b)

Yield: 91%; white solid; mp 228.1–228.9 °C; MS (ESI$^+$) Found: 321.0974 $[M+H]^+$ for $C_{12}H_{14}ClN_8O$, calculated: 321.1001, $[\alpha]_D^{27.2}$ –0.34 (*c* 5.8, MeOH); UV (MeOH) $\lambda_{max}$ 272.0 nm; IR (KBr) 2105.38 $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$) $\delta$ 0.90–0.96 (m, 1 H), 1.54–1.57 (m, 1 H), 1.72–1.77 (m, 1 H), 2.06–2.12 (m, 1 H), 3.09 (s, 3 H), 4.04 (d, $J$ = 6.4 Hz, 1 H), 4.15 (pseudo t, $J$ = 5.2 Hz, 1 H), 4.79 (s, 1 H), 7.98 (s, 1 H); $^{13}C$ NMR (MeOH-$d_4$) $\delta$ 8.7, 18.6, 21.7, 27.8, 63.1, 63.8, 77.9, 119.5, 139.2, 149.8, 155.3, 156.8; Anal. calcd for $C_{12}H_{13}ClN_8O$: C, 44.94; H, 4.09; N, 34.94. Found: C, 44.79; H, 4.24; N, 35.10.

### (–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Azido-4-[6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo[3.1.0]hexan-3-ol (18c)

Yield: 76%; white foam; MS (ESI$^+$) Found: 489.0640 $[M+H]^+$ for $C_{18}H_{18}IN_8O$, calculated: 489.0667; $[\alpha]_D^{27.0}$ –1.08 (*c* 1.98, $CHCl_3$); UV ($CHCl_3$) $\lambda_{max}$ 267.5 nm; IR (KBr) 2104.3 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 0.99 (dd, $J$ = 7.6, 14.0 Hz, 1 H), 1.54–1.59 (m, 1 H), 1.72–1.76 (m, 1 H), 2.07–2.13 (m, 1 H), 2.30 (br s, 1 H, $D_2O$ exchangeable), 4.16 (d, $J$ = 5.6 Hz, 1 H), 4.32 (dd, $J$ = 5.6, 11.2 Hz, 1 H), 4.83 (s, 2 H), 4.92 (s, 1 H), 6.65 (s, 1 H, $D_2O$ exchangeable), 7.04 (pseudo t, $J$ = 8.0 Hz, 1 H), 7.32 (d, $J$ = 7.6 Hz, 1 H), 7.59 (d, $J$ = 8.0 Hz, 1 H), 7.71 (s, 1 H), 7.78 (s, 1 H), 8.39 (s, 1 H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 8.8, 18.9, 21.2, 24.1, 29.9, 43.8, 62.9, 63.2, 94.8, 120.3, 127.4, 130.6, 136.6, 136.7, 138.1, 141.2, 153.4, 154.9; Anal. calcd for $C_{18}H_{17}IN_8O$: C, 44.28; H, 3.51; N, 22.95. Found: C, 44.45; H, 3.29; N, 22.58.

### (–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Azido-4-(6-methylamino-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (18d)

Yield: 88%; white foam; MS (ESI$^+$) Found: 287.1365 $[M+H]^+$ for $C_{12}H_{15}N_8O$, calculated: 287.1393, $[\alpha]_D^{27.1}$ –17.60 (*c* 2.21, MeOH); UV ($CHCl_3$) $\lambda_{max}$ 269.5 nm; IR (KBr) 2104.9 $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$) $\delta$ 0.90–0.96 (m, 1 H), 1.49–1.52 (m, 1 H), 1.74–1.79 (m, 1 H), 2.06–2.12 (m, 1 H), 3.11 (s, 3 H), 4.10 (dd, $J$ = 0.8, 6.4 Hz, 1 H), 4.22 (pseudo t, $J$ = 5.2 Hz, 1 H), 4.86 (s, 1 H), 8.14 (s, 1 H), 8.26 (s, 1 H); $^{13}C$ NMR (MeOH-$d_4$) $\delta$ 8.9, 19.3, 21.9, 27.9, 63.8, 64.2, 78.8, 120.9, 139.9, 153.8, 156.8; Anal. calcd for $C_{12}H_{14}N_8O$: C, 50.34; H, 4.93; N, 39.14. Found: C, 50.67; H, 4.94; N, 38.99.

### General Procedure for the Synthesis of 4a–d

Triphenylphosphine was added to a solution of **18a–d** in THF (1 mL), and the solution was cooled to 0 °C. After 30 min, an ammonium hydroxide solution (0.1 mL) and water (0.02 mL) were added, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and 1 *N* HCl was added to adjust to pH 1. The resultant solution was then partitioned between diethyl ether and water. The aqueous layer was further neutralised with sodium bicarbonate. After complete removal of water, the solid obtained was filtered and washed with a methylene chloride : methanol (6 : 1) solution. The residue was recrystallised using methylene chloride and methanol to give pure **4a–d** as a white solid.

**(–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Amino-4-[2-chloro-6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo [3.1.0]hexan-3-ol (4a)**

Yield: 86%; mp 255.8–262.2 °C; MS (FAB) Found: 497.0354 for $C_{18}H_{19}ClIN_6O$, calculated: 497.7460, $[\alpha]_D^{26.9}$ −7.5 (*c* 0.32, AcOH); UV (MeOH) $\lambda_{max}$ 272.0 nm; $^1$H NMR (AcOH-$d_4$) $\delta$ 0.87–0.94 (m, 1 H), 1.24–1.31 (m, 1 H), 1.84–1.89 (m, 1 H), 2.17–2.21 (m, 1 H), 4.40 (d, *J* = 6.4 Hz, 1 H), 4.60 (pseudo t, *J* = 5.2 Hz, 1 H), 4.75 (s, 2 H), 5.0 (s, 1 H), 7.07 (pseudo t, *J* = 7.6 Hz, 1 H), 7.40 (d, *J* = 7.6 Hz, 1 H), 7.60 (d, *J* = 8.0 Hz, 1 H), 7.81 (s, 1 H), 8.22 (s, 1 H); $^{13}$C NMR (AcOH-$d_4$) $\delta$ 8.0, 21.8, 21.9, 30.6, 44.2, 55.1, 64.8, 75.4, 94.8, 118.3, 128.2, 131.2, 137.9, 140.7, 142.3, 150.5, 155.8, 156.0; Anal. calcd for $C_{18}H_{18}ClIN_6O$: C, 43.52; H, 3.65; N, 16.92. Found: C, 43.12; H, 3.88; N, 16.58.

**(–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Amino-4-(2-chloro-6-methylamino-purin-9-yl)-bicyclo[3.1.0] hexan-3-ol (4b)**

Yield: 85%; mp 249.2–259.6 °C; MS (ESI$^+$) Found: 295.1069 [M+H]$^+$ for $C_{12}H_{16}ClN_6O$, calculated: 295.1096, $[\alpha]_D^{26.3}$ −2.0 (*c* 1.3, AcOH); UV (MeOH) $\lambda_{max}$ 272.0 nm; $^1$H NMR (AcOH-$d_4$) $\delta$ 0.93–0.88 (m, 1 H), 1.28–1.30 (m, 1 H), 1.84–1.88 (m, 1 H), 2.17–2.20 (m, 1 H), 2.54 (s, 3 H), 4.39 (d, *J* = 8 Hz, 1 H), 4.59 (pseudo t, *J* = 4 Hz, 1 H), 4.98 (s, 1 H), 8.19 (s, 1 H); $^{13}$C NMR (AcOH-$d_4$) $\delta$ 8.0, 21.9, 27.7, 55.0, 64.7, 75.4, 118.3, 139.9, 140.1, 149.8, 156.2, 156.5; Anal. calcd for $C_{12}H_{15}ClN_6O$: C, 48.90; H, 5.13; N, 28.51. Found: C, 48.79; H, 5.24; N, 28.58.

**(–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Amino-4-[6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo[3.1.0]hexan-3-ol (4c)**

Yield: 84%; mp 239.9–244.7 °C; MS (ESI$^+$) Found: 463.0723 [M+H]$^+$ for $C_{18}H_{20}IN_6O$, calculated: 463.07651, $[\alpha]_D^{26.3}$ −1.90 (*c* 1.38, AcOH); UV (MeOH) $\lambda_{max}$ 268.0 nm; $^1$H NMR (AcOH-$d_4$) $\delta$ 1.0–0.90 (m, 1 H), 1.60–1.80 (m, 1 H), 1.98–2.0 (m, 1 H), 2.20–2.25 (m, 1 H), 4.36 (d, *J* = 8.0 Hz, 1 H), 4.51 (pseudo t, *J* = 8.0 Hz 1 H), 4.81 (s, 2 H), 5.08 (s, 1 H), 7.06 (pseudo t, *J* = 8.0 Hz, 1 H), 7.39 (d, *J* = 8.0 Hz, 1 H), 7.60 (d, *J* = 8.0 Hz, 1 H), 7.79 (s, 1 H), 8.34 (s, 1 H), 8.46 (s, 1 H); $^{13}$C NMR (AcOH-$d_4$) $\delta$ 8.1, 20.0, 21.7, 44.3, 54.8, 64.4, 75.3, 94.8, 119.4, 127.7, 127.8, 131.3, 137.5, 140.2, 142.1, 148.4, 154.0, 155.2; Anal. calcd for $C_{18}H_{19}IN_6O$: C, 46.77; H, 4.14; N, 18.18. Found: C, 46.77; H, 4.24; N, 18.11.

**(–)-(1*S*,2*R*,3*S*,4*R*,5*R*)-2-Amino-4-(6-methylamino-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (4d)**

Yield: 86%; mp 238.7–242.4 °C; MS (ESI$^+$) Found: 261.1460 [M+H]$^+$ for $C_{12}H_{17}N_6O$, calculated: 261.1488, $[\alpha]_D^{26.1}$ −2.30 (*c* 1.43, MeOH); UV (MeOH) $\lambda_{max}$ 267.0 nm; $^1$H NMR (AcOH-$d_4$) $\delta$ 0.66–0.72 (m, 1 H), 1.23 (dd, *J* = 4.0, 8.0 Hz, 1 H), 1.66–1.71 (m, 1 H), 1.89–2.0 (m, 1 H), 2.87 (s, 3 H), 3.73–3.75 (m, 1 H), 3.78–3.80 (m, 1 H), 4.90 (s, 1 H), 8.17 (s, 1 H), 8.27 (s, 1 H); $^{13}$C NMR (AcOH-$d_4$) $\delta$ 7.3, 19.9, 25.4, 55.0, 64.7, 77.1, 139.8, 153.8; Anal. calcd for $C_{12}H_{16}N_6O$: C, 55.37; H, 6.20; N, 32.29. Found: C, 55.38; H, 6.21; N, 32.11.

## Pharmacology

### Cell culture and membrane preparation

Chinese hamster ovary (CHO) cells stably expressing either the recombinant $hA_1$ or $hA_3AR$ and human embryonic kidney (HEK)-293 cells stably expressing the human $A_{2A}AR$ were cultured in DMEM and F12 (1 : 1) supplemented with 10% foetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 μmol mL$^{-1}$ glutamine. In addition, 800 μg mL$^{-1}$ Geneticin and 500 μg mL$^{-1}$ hygromycin were added to the $A_{2A}$ media and the $A_1$ and $A_3$ media, respectively. After harvesting the cells, they were homogenised for 10 s with an electric homogeniser, pipetted into 1-mL vials, and stored at −80 °C until the binding experiments were conducted. The concentration of protein was determined using a BCA Protein Assay Kit from Pierce Biotechnology (Rockford, IL).[19]

### Radioligand membrane binding studies

Radioligand binding assays with $A_1$, $A_{2A}$, and $A_3$ARs were performed according to the procedures described previously.[20–22] Each tube contained 100 μL of membrane suspension (20 μg of protein), 50 μL of a stock solution of agonist radioligand, and 50 μL of increasing concentrations of the test ligands in Tris-HCl buffer (50 mM, pH 7.5) containing 10 mM $MgCl_2$. Nonspecific binding was determined using a final concentration of 10 μM NECA, a non-specific agonist, diluted with the buffer.

The mixtures were incubated at 25 °C for 60 min. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburg, MD). The filters were washed three times with 5 mL of 50 mM ice-cold Tris-HCl buffer (pH 7.5). The radioactive agonists [$^3$H]R-PIA and [$^3$H]CGS21680 were used for the $A_1$ and $A_{2A}$AR assays, respectively, while [$^{125}$I]AB-MECA was used for the $A_3$AR assay. All of the filters were washed three times with Tris-HCl, pH 7.5. Filters for $A_1$ and $A_{2A}$AR binding were placed in scintillation vials containing 5 mL of Hydrofluor scintillation buffer and counted using a PerkinElmer Tricarb 2810TR Liquid Scintillation Analyzer. Filters for $A_3$AR binding were counted using a PerkinElmer Cobra II γ-counter.

### Data analysis

Binding and functional parameters were calculated using the Prism 5.0 software (GraphPAD, San Diego, CA, USA). $IC_{50}$ values obtained from competition curves were converted to $K_i$ values using the Cheng-Prusoff equation.[23] Data were expressed as mean ± standard error.

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