# (*N*)-Methanocarba 2,$N^6$-Disubstituted Adenine Nucleosides as Highly Potent and Selective $A_3$ Adenosine Receptor Agonists

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A series of ring-constrained (*N*)-methanocarba-5′-uronamide 2,$N^6$-disubstituted adenine nucleosides have been synthesized via Mitsunobu condensation of the nucleobase precursor with a pseudosugar ring containing a 5′-ester functionality. Following appropriate functionalization of the adenine ring, the ester group was converted to the 5′-*N*-methylamide. The compounds, mainly 2-chloro-substituted derivatives, were tested in both binding and functional assays at human adenosine receptors (ARs), and many were found to be highly potent and selective $A_3$-AR agonists. Selected compounds were compared in binding to the rat $A_3$AR to assess their viability for testing in rat disease models. The $N^6$-(3-chlorobenzyl) and $N^6$-(3-bromobenzyl) analogues displayed $K_i$ values at the human $A_3$AR of 0.29 and 0.38 nM, respectively. Other subnanomolar affinities were observed for the following $N^6$ derivatives: 2,5-dichlorobenzyl, 5-iodo-2-methoxybenzyl, *trans*-2-phenyl-1-cyclopropyl, and 2,2-diphenylethyl. Selectivity for the human $A_3$AR in comparison to the $A_1$AR was the following (fold): the $N^6$-(2,2-diphenylethyl) analogue **34** (1900), the $N^6$-(2,5-dimethoxybenzyl) analogue **26** (1200), the $N^6$-(2,5-dichlorobenzyl) and $N^6$-(2-phenyl-1-cyclopropyl) analogues **20** and **33** (1000), and the $N^6$-(3-substituted benzyl) analogues **17**, **18**, **28**, and **29** (700−900). Typically, even greater selectivity ratios were obtained in comparison with the $A_{2A}$ and $A_{2B}$ARs. The (*N*)-methanocarba-5′-uronamide analogues were full agonists at the $A_3$AR, as indicated by the inhibition of forskolin-stimluated adenylate cyclase at a concentration of 10 $\mu$M. The $N^6$-(2,2-diphenylethyl) derivative was an $A_3$AR agonist in the (*N*)-methanocarba-5′-uronamide series, although it was an antagonist in the ribose series. Thus, many of the previously known groups that enhance $A_3$AR affinity in the 9-riboside series, including those that reduce intrinsic efficacy, may be adapted to the (*N*)-methanocarba nucleoside series of full agonists.

## Introduction

There are four subtypes of adenosine receptors (ARs): $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$.[1] Each of these subtypes is associated with specific experimental therapeutic modalities.[2] Selective adenosine receptor antagonists and agonists are under development for treating such diverse conditions including inflammation, cardiac ischemia, stroke, asthma, diabetes, cardiac arrhythmias, and other disorders.[3−7] Agonists selective for the $A_3$AR are potentially useful for the treatment of stroke, neurodegenerative diseases, myocardial infarction, and cancer.[4,5,8−10] In colon carcinoma, a cytostatic effect of an $A_3$AR agonist appears to be related to its downstream activation of the Wnt pathway.[10] $A_3$AR antagonists are potentially useful for the treatment of glaucoma.[11]

Prototypical high-affinity ligands for the $A_3$AR include the $N^6$-(3-iodobenzyl)adenosine analogues **1**−**5** (Chart 1).[12] The moderately $A_3$AR-selective nucleoside IB-MECA **2a** ($N^6$-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine) is a full agonist,[13] which is currently in phase 2 clinical trials for the treatment of colorectal cancer and rheumatoid arthritis.[10] Also, chronically administered **2a** was neuroprotective in a model of ischemic brain damage in gerbils.[5] The corresponding 2-chloro derivative Cl-IB-MECA **2b** was the first highly selective $A_3$AR agonist[14] and has been shown to be cardioprotective in a variety of in vitro and in vivo models.[4,15,16] In an SAR study of adenosine derivatives,[13] the $N^6$-(3-iodobenzyl) substituent was found to enhance the selectivity for the $A_3$AR. Also, the 2-chloro modification has been found to enhance potency in a variety of AR agonists. When present as the sole modifications of adenosine, as in the 9-riboside derivatives **1a** and **1b**, they reduced the intrinsic efficacy at the $A_3$AR to the extent that 2-chloro-9-riboside **1b** was an antagonist of this subtype. Rigidity introduced in the ribose moiety also tends to reduce $A_3$AR efficacy, such as the (*N*)-methanocarba ring system, a bridged carbocyclic ring system present in the partial agonists **3** and **4** and in the full agonist **5**.[17,18] Structural features that reduced efficacy included $N^6$-benzyl groups, small moieties at the 2-position, and sterically constrained moieties in the ribose region. For each of these efficacy-diminishing factors, the 5′-uronamide group tended to restore the observed loss of $A_3$AR efficacy, as observed in the full agonism displayed by **2** and **5**. There is still a need for the development of more highly selective $A_3$AR agonists than the widely used $A_3$AR agonists **2a** and **2b**, for

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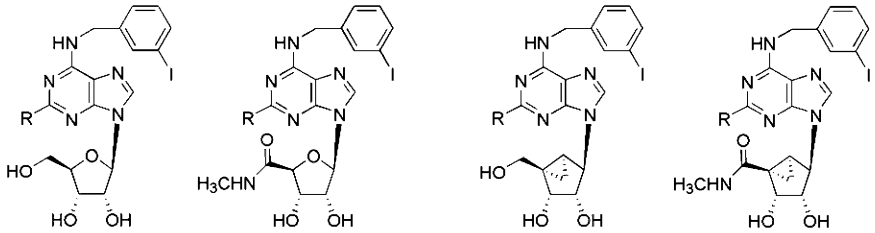
**Chart 1.** Chemical Structures of Adenosine Derivatives Used in the Present Study and Containing a 9-Ribose Moiety (**1**), a 9-Ribose-5′-*N*-methyluronamide Moiety (**2**), or an (*N*)-Methanocarba Moiety (**3**−**5**)




which some literature reports have indicated limited pharmacological selectivity.[19,25]

The present study characterizes the SAR (structure−activity relationship) of a broad class of highly potent and selective $A_3$AR agonists based on compound **5**. The principal design element of these agonists is a modified ribose moiety. The analogues contain the (*N*)-methanocarba (bicyclo[3.1.0]hexane) ring system, which is a rigid ribose substitute lacking the ether oxygen. This ring system maintains the 2′-*exo*-(*N*) ring-twist conformation of the ribose-like ring (pseudosugar moiety), which has been demonstrated to be favored in $A_3$AR binding (more so than at other AR subtypes).[20] These agonists also contain a flexible 5′-uronamide group, necessary to ensure full activation of the $A_3$AR.[12] We utilized a new synthetic route for the synthesis of these derivatives[21,22] that allows for a more versatile substitution of the adenine moiety than the previous synthetic route.[23] In addition, we incorporated $N^6$-substituents that have been shown previously to favor $A_3$AR binding affinity (mainly $N^6$-benzyl and $N^6$-phenylethyl[24] groups) and included new variations thereof. These derivatives were characterized biologically at the four human AR subtypes expressed in Chinese hamster ovary (CHO) cells[25] in binding and functional assays to assess their selectivity within the same species. The results indicate that we achieved the design of many new sterically constrained and selective $A_3$AR agonists with nanomolar and subnanomolar affinities. Several of these high-affinity agonists were docked in the putative binding site of a rhodopsin[26]-based model of the human $A_3$-AR.[18,24,27]

## Results

**Chemical Synthesis.** The novel derivatives **15** and **17**−**36** were synthesized as shown in Schemes 1−3. Most analogues contained a 2-chloro substituent, although 2-amino **15**, 2-iodo **35**, and 2-alkylthio **36** groups were also included.

The general synthetic route follows the method of Joshi et al.[21,22] The protected (*N*)-methanocarba ring system **37** containing a carbonyl group at the 5′-position was prepared in eight steps from isopropylidene erythronolactone **36** (Scheme 1). Compound **37** and the nucleobase precursor, 2,6-dichloropurine, were condensed using a Mitsunobu coupling.[28] Substitution of the 6-chloro of **38** was then carried out by treating with an excess of the appropriate amine, such as a substituted benzylamine, to provide the series of protected nucleoside 5′-esters **39**−**54**. Following appropriate substitution at the $N^6$-position, the 5′-esters were treated with an excess of methylamine, and the isopropylidene group was removed from the 2′- and 3′-hydroxyl groups upon acid treatment to provide the target compounds **16**−**33**.

The various benzylamine derivatives used in this study, when not commercially available, were synthesized by the methods shown in Scheme 2. 2,5-Dichlorobenzylamine **64** and 2-chloro-5-iodobenzylamine **65** were prepared from the corresponding benzyl bromides. Compound **60** was prepared from 2-chloro-5-iodotoluene by bromination with *N*-bromosuccinimide in $CCl_4$.[29] **60** and **61** were treated with potassium phthalimide in *N*,*N*-dimethylformamide at 60 °C to obtain compounds **62** and **63** as white solids in 95% yield. Deprotection with hydrazine gave **64** and **65** in 80% yield.[30]

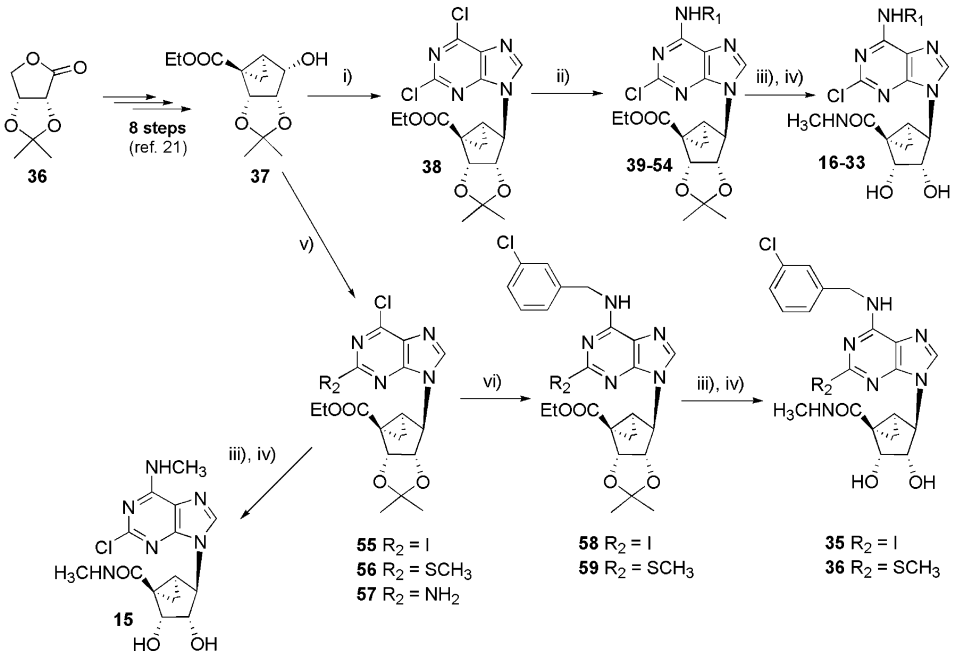
5-Iodo-2-methoxybenzylamine **68** was prepared starting from 5-iodosalicylaldehyde **66**. Accordingly, compound **66** was methylated with methyl iodide in the presence of $K_2CO_3$ in *N*,*N*-dimethylformamide, and subsequently, the formyl moiety was transformed to methylamino by reductive amination with $NaCNBH_4$ in the presence of ammonium acetate.[31]

Compounds **72** and **73** were synthesized through a Sonogashira-type reaction in high yield using CuI and $(Ph_3P)PdCl_2$ as catalysts.[32] For the preparation of both 5-chloro-2-benzyloxybenzylamine **76** and 5-chloro-2-(aminocarbonylmethyloxy)benzylamine **80**, 5-chloro-2-hydroxybenzamide **74** was used as starting material. Compound **74** was treated with benzyl bromide in the presence of $K_2CO_3$ in *N*,*N*-dimethylformamide to obtain 5-chloro-2-benzyloxybenzamide **75** in 98% yield. Finally, the amide **75** was reduced to the corresponding amine **76** with $LiAlH_4$ in tetrahydrofuran.

Reduction of compound **74** with $LiAlH_4$ in tetrahydrofuran gave 5-chloro-2-hydroxybenzylamine **77** in 88% yield. This preparation showed an improvement over the reported procedure, based on the hydrogenation of 5-chloro-2-hydroxybenzaldehyde oxime.[33] The amine **77** was protected as a *tert*-butyl carbamate **78**, and the hydroxyl group was alkylated with 2-bromoacetamide in the presence of $K_2CO_3$ in *N*,*N*-dimethylformamide. The subsequent deprotection of the amino group with 15% trifluoroacetic acid in dichloromethane gave the final product **80** as a white solid.

Substitution at the 2-position by groups other than chloro was possible and accomplished by condensing the appropriate 2-substituted nucleobases with the (*N*)-methanocarba sugar moiety **37** to provide the protected nucleoside esters **55** and **56** (Scheme 1). Successive treatment with 3-chlorobenzylamine and methylamine followed by subsequent acid hydrolysis afforded the

**Scheme 1**[a]




[a] Reagents and conditions: (i) 2,6-dichloropurine, DIAD, TPP, THF, room temp; (ii) $RNH_2$, TEA, MeOH; (iii) 40% aqueous $MeNH_2$, MeOH; (iv) 10% $CF_3COOH$ in MeOH, $H_2O$, 70 °C; (v) 6-chloro-2-iodopurine or 6-chloro-2-methylthiopurine or 6-chloro-2-aminopurine, DIAD, TPP, THF, room temp; (vi) 3-chlorobenzylamine, TEA, MeOH.

2-iodo and 2-thiomethyl analogues **35** and **36**. Alternatively, the 2-amino-substituted analogue was obtained by first condensing the 2-amino-substituted nucleobase with **37** followed by the direct interaction with excess methylamine and subsequent hydrolysis to afford **15**.

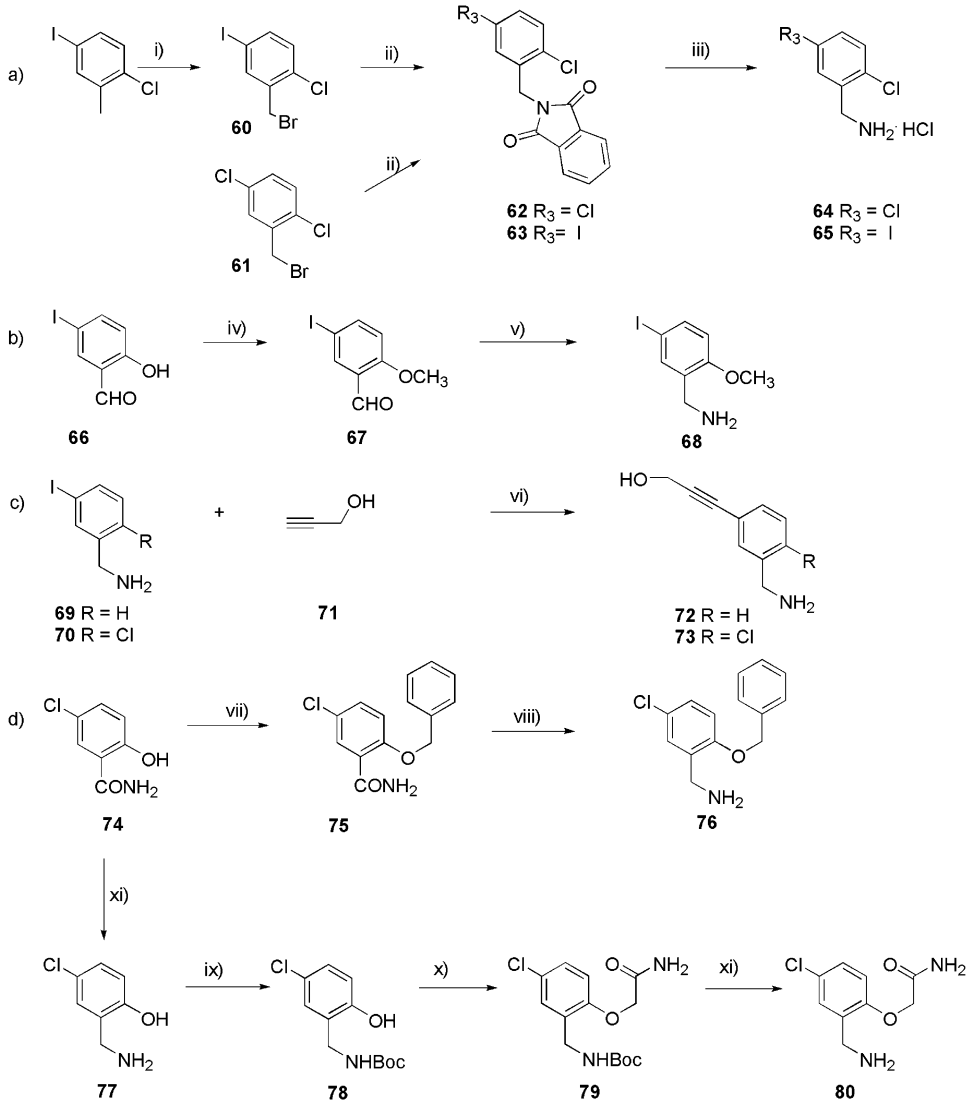
The requisite 6-chloro-2-iodopurine was prepared by a literature procedure,[35] while 6-chloro-2-methylthiopurine was prepared as delineated in Scheme 3. Accordingly, 6-chloro-2-methylthiopurin-9-yl-methyl 2,2-dimethylpropionate[34] **81** was diazotized with *tert*-butyl nitrite, and the resulting diazo intermediate was trapped with an excess of methyl disulfide to afford **82**, which upon hydrolysis of the pivaloylmethyloxy protecting group with aqueous NaOH gave the requisite 2-methylthiopurine **83**.

**Biological Activity.** The AR binding affinities of both newly synthesized (**15** and **17**−**36**) and known (**1**−**14** and **16**) nucleoside derivatives (Table 1) were investigated. The known adenosine agonist derivatives included 9-ribosides **1**, **6**−**12**, (*N*)-methanocarba derivatives **3**−**5**, and 9-riboside 5′-*N*-methyluronamide analogues **2a**, **2b**, **14**, and **16**. Binding affinity at the human $A_3$-AR, and in selected cases the rat $A_3$AR, was studied using the radioiodinated agonist [$^{125}$I]$N^6$-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide,[36] and binding at the human $A_1$AR and $A_{2A}$AR utilized the selective agonists [$^3$H]*R*-$N^6$-[phenylisopropyl]adenosine and [$^3$H]2-[*p*-(carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine, respectively. Functional assays of the human $A_{2B}$AR and $A_3$AR consisted of measuring cyclic AMP production in intact CHO cells expressing the ARs1.[25] The functional effect of $A_3$AR was inhibition of forskolin-stimulated cAMP production, and the $A_{2B}$AR effect was stimulation of basal cAMP production.

Cristalli and co-workers[37] and other investigators[18,23,38] have established that $N^6$-methylation of adenosine analogues tends to increase the human $A_3$AR affinity and selectivity. In the (*N*)-methanocarba series, the $N^6$-methyl-2-chloro derivative **14** was 3-fold more potent in $A_3$AR binding in the present study than reported previously.[23] The replacement of 2-chloro with a 2-amino group to provide **15** increased the human $A_3$AR affinity slightly. Compound **15** was 24-fold more potent in $A_3$-AR binding than the 9-riboside equivalent **7**.[18]

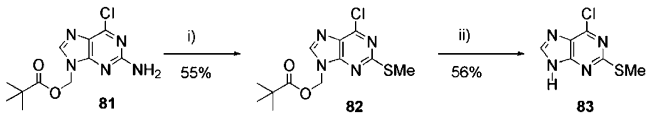
The 3-iodobenzyl group has long been known to enhance affinity and/or selectivity at the $A_3$AR.[13,14] In the (*N*)-methanocarba series, compound **16**, reported previously,[12,23] was confirmed to be selective for the human $A_3$AR (300-fold in comparison to the human $A_1$-AR). Addition of the 2-chloro group resulted in the previously reported agonist **5** having slightly enhanced $A_3$AR affinity, as was seen also for the pairs of compounds **1a** and **1b** and compounds **2a** and **2b**. Substitution at the 3-position with other halo atoms further increased human $A_3$AR affinity (unlike the ribose 5′-uronamide case, in which 3-iodo was optimal[13]). The optimal human $A_3$AR affinity in this series was achieved with the 3-chloro substitution in **18** with a $K_i$ value of 0.29 nM. Compound **18** was selective for the human $A_3$-AR in comparison to the human $A_1$AR and $A_{2A}$AR by 890- and 7800-fold, respectively. The $A_3$AR selectivity of **18** in comparison to the $A_{2B}$AR was >10000-fold. There was a trend toward an increase of human $A_3$AR affinity and a decrease of affinity at the other AR subtypes with decreasing size of the halo atom except for the 3-fluoro analogue, which displayed a $K_i$ value at the $A_3$AR of 2.4 nM. The affinity of the 3-halobenzyl analogues at the rat $A_3$AR was less variable than at the corresponding human receptor, and compounds **5** and **17**−**19** all displayed $K_i$ values within the range 0.8−1.6 nM. Dihalo substitution of the benzyl ring in the 2,5-dichloro analogue **20** and the 2-chloro-5-iodobenzyl analogue **21** maintained subnanomolar affinity at the human $A_3$AR. The 5-chloro substitution combined with

**Scheme 2**[a]




[a] Reagents and conditions: (i) NBS, benzoyl peroxide in dry $CCl_4$, reflux, 3 h; (ii) potassium phthalimide, in dry DMF at 80 °C, 3 h; (iii) $NH_2NH_2$, in EtOH, reflux, 24 h; (iv) $CH_3I$, $K_2CO_3$ in DMF at room temp overnight; (v) $AcONH_4$, $NaCNBH_3$ in dry MeOH, 48 h; (vi) CuI, $(PPh_3)_2PdCl_2$, in dry $Et_2NH$; (vii) benzyl bromide, $K_2CO_3$ in DMF at room temp overnight; (viii) $LiAlH_4$, in dry THF, reflux, 3 h; (ix) $(Boc)_2O$, 10% $Et_3N$ in MeOH, 45 °C, 1 h; (x) 2-bromoacetamide, $K_2CO_3$ in DMF at room temp overnight; (xi) 15% TFA in $CH_2Cl_2$, 45 min.

**Scheme 3**[a]



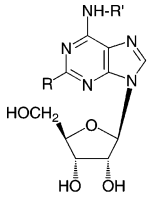

[a] Reagents and conditions: (i) *tert*-butyl nitrite, methyl disulfide, acetonitrile, room temp; (ii) aqueous NaOH, *i*-PrOH, THF.

an ether group at the 2-position (i.e., methyl ether **22**, aminocarbonylmethyl ether **23**, or benzyl ether **24**) also maintained moderately high affinity at the $A_3$AR. The bulky benzyl ether **24** was 230-fold selective for the human $A_3$AR in comparison to the $A_1$AR. The 5-iodo equivalent of the 5-chloro-2-methoxy derivative **22**, i.e., compound **25**, was 3-fold more potent at the $A_3$AR upon halogen exchange. However, the affinity enhancement observed upon replacing *m*-chloro with *m*-iodo was not general. For example, with the monosubstituted benzyl derivatives compounds **5** and **18**, the 3-chloro analogue **18** was 5-fold more potent than the corresponding 3-iodo **5**. Replacement of this 5-chloro atom in compound **22** with methoxy, e.g., **26**, did not change the $A_3$AR affinity; however, the $A_3$AR selectivity improved.

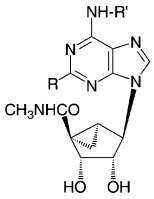
The $N^6$-(2-methylbenzyl) group is present in metrifudil **11**, a nonselective adenosine agonist that previously had been in clinical trials for its hypotensive effect.[39] In the 2-chloro-(*N*)-methanocarba series, this substitution in **27** provided high affinity at the $A_3$AR and selectivity (190-fold in comparison to the $A_1$AR). Placement of the methyl group at the 3-position in **28** enhanced the affinity at the $A_3$AR by 3-fold to give a $K_i$ value of 0.63 nM. Inclusion of a 3-hydroxypropynyl group at the meta position of the benzyl ring in **29** produced high-affinity binding to both the human and rat $A_3$AR, but curiously when a 2-chloro substitution of the benzyl ring was included in **30**, the affinity was significantly (76-fold) reduced.

The high-affinity radioiodinated tracer used in this study for binding to the $A_3$AR, I-AB-MECA, contains an $N^6$-(4-aminobenzyl) group.[32] Compound **31a** in the present series of 2-chloro-(*N*)-methanocarba derivatives contained the same substituent group and displayed a

**Table 1.** Potency of Adenosine Derivatives at Human $A_1AR$, $A_{2A}AR$, $A_{2B}AR$, and $A_3AR$ and Rat $A_3AR$ and Maximal Agonist Effects at Human $A_3ARs$ Expressed in CHO cells[a]




1, 6 - 13




5, 14 - 36

| compd | $N^6$-R′ | C2-R | $K_i(hA_1AR)$,[a] nM or % displacement at 10 $\mu$M | $K_i(hA_{2A}AR)$,[a] nM or % displacement at 10 $\mu$M | % activation ($hA_{2B}AR$)[b] at 10 $\mu$M | $K_i(hA_3AR)$,[a] nM | % activation ($hA_3AR$)[b] at 10 $\mu$M | $K_i(rA_3AR)$, nM |
|---|---|---|---|---|---|---|---|---|
| | | | Riboside Derivatives | | | | | |
| **6**[c] | $CH_3$ | H | 6000 ± 2000 | 17% | 16 | 9.3 ± 0.4 | 96 ± 3 | 6400 ± 1600[d] |
| **7**[c] | $CH_3$ | $NH_2$ | 480 ± 20 | 15% | 11 | 39 ± 2 | 98 ± 3 | >10000 |
| **1a**[c] | 3-iodobenzyl | H | 7.4 ± 1.7 | 140 ± 20 | 58 | 5.8 ± 0.4 | 46 ± 8 | 9.5 ± 1.4 |
| **1b**[c] | 3-iodobenzyl | Cl | 17 ± 2 | 200 ± 30 | 16 | 1.8 ± 0.1 | 0 | 2.7 ± 1.2 |
| **8** | 3-chlorobenzyl | H | 20 ± 3 | 1400 ± 200 | ND[j] | 4.4 ± 1.7[d] | 80 ± 3[d] | 35 ± 20[d] |
| **9**[e] | 5-chloro-2-methyloxybenzyl | H | 9.2 ± 0.5 | 400 ± 10 | 57 | 1.3 ± 0.2 | 53 ± 3 | ND[j] |
| **10**[c] | *trans*−2-phenyl-1-cyclopropyl | H | 120 ± 30 | 2500 ± 700 | 26 | 0.86 ± 0.09 | 101 ± 5 | 400 ± 30 |
| **11**[d] | 2-methylbenzyl | H | 39 ± 8 | 760 ± 110 | ND[j] | 47 ± 11 | 100 ± 3 | 38 ± 15 |
| **12**[d] | 3-pyridylmethyl | H | 42 ± 4 | 2300 ± 120 | ND[j] | 4.5 ± 1.1 | 100 ± 6 | 290 ± 70 |
| **13**[f] | 2,2-diphenylethyl | H | 50 ± 16 | 510 ± 50 | 70 | 3.9 ± 0.7 | 0 | 540 ± 200 |
| | | | 5′-*N*-Methyluronamidoriboside Derivatives | | | | | |
| **2a** | 3-iodobenzyl | H | 51 ± 5 | 2900 ± 600[d] | 0[h] | 1.8 ± 0.7[d] | 100 | 1.1 ± 0.3[i] |
| **2b** | 3-iodobenzyl | Cl | 220 ± 20 | 5400 ± 2500[d] | 0[h] | 1.4 ± 0.3[d] | 100 ± 4 | 0.33 ± 0.08[i] |
| | | | (*N*)-Methanocarba Derivatives | | | | | |
| **3** | 3-iodobenzyl | H | 35 ± 3 | 860 ± 70 | ND[j] | 9.2 ± 0.7[d] | 13 ± 1 | ND[j] |
| **4** | 3-iodobenzyl | Cl | 65 ± 17 | 1600 ± 400 | ND[j] | 1.9 ± 0.7[d] | 3 ± 2 | ND[j] |
| | | | 5′-*N*-Methyluronamido-(*N*)-methanocarba Derivatives | | | | | |
| **14** | $CH_3$ | Cl | 2100 ± 1700 | 6% | ND[j] | 2.2 ± 0.6 | 104 ± 7 | 160 ± 30 |
| **15** | $CH_3$ | $NH_2$ | 1600 ± 200 | 17% | ND[j] | 1.6 ± 0.3 | 117 ± 21 | ND[j] |
| **16** | 3-iodobenzyl | H | 700 ± 270 | 6200 ± 100 | ND[j] | 2.4 ± 0.5[e] | 100[e] | ND[j] |
| **5** | 3-iodobenzyl | Cl | 136 ± 22[g] | 784 ± 97[g] | ND[j] | 1.5 ± 0.2[e] | 100[e] | 1.1 ± 0.1 |
| **17** | 3-bromobenzyl | Cl | 270 ± 70 | 1300 ± 100 | 38 | 0.38 ± 0.11 | 100 ± 11 | 0.76 ± 0.08 |
| **18**[k] | 3-chlorobenzyl | Cl | 260 ± 60 | 2300 ± 100 | 38 | 0.29 ± 0.04 | 103 ± 7 | 1.0 ± 0.10 |
| **19** | 3-fluorobenzyl | Cl | 640 ± 140 | 5100 ± 200 | 28 | 2.4 ± 0.1 | 101 ± 5 | 1.6 ± 0.1 |
| **20**[k] | 2,5-dichloro-benzyl | Cl | 540 ± 70 | 1300 ± 100 | 32 | 0.56 ± 0.06 | 102 ± 3 | ND[j] |
| **21** | 2-chloro-5-iodobenzyl | Cl | 340 ± 20 | 480 ± 20 | 58 | 0.83 ± 0.19 | 105 ± 6 | ND[j] |
| **22** | 5-chloro-2-methoxybenzyl | Cl | 240 ± 50 | 1200 ± 100 | 37 | 1.5 ± 0.0 | 107 ± 15 | ND[j] |
| **23** | 5-chloro-2-(aminocarbonyl-methyloxy)benzyl | Cl | 81 ± 10 | 800 ± 70 | ND[j] | 5.3 ± 0.4 | 106 ± 12 | ND[j] |
| **24** | 5-chloro-2-benzyloxybenzyl | Cl | 1200 ± 200 | 850 ± 160 | 49 | 5.2 ± 1.3 | 110 ± 10 | 11 ± 3 |
| **25** | 5-iodo-2-methoxybenzyl | Cl | 200 ± 20 | 430 ± 30 | 46 | 0.58 ± 0.12 | 101 ± 10 | ND[j] |
| **26**[k] | 2,5-dimethoxybenzyl | Cl | 1600 ± 200 | 52% | 0 | 1.4 ± 0.2 | 107 ± 10 | 0.87 ± 0.24 |
| **27** | 2-methylbenzyl | Cl | 710 ± 300 | 3800 ± 200 | 24 | 3.7 ± 0.6 | 100 ± 3 | ND[j] |
| **28** | 3-methylbenzyl | Cl | 450 ± 80 | 3700 ± 600 | 46 | 0.63 ± 0.18 | 106 ± 3 | ND[j] |
| **29** | 3-(3-hydroxypropynyl)benzyl | Cl | 2600 ± 300 | 56% | 28 | 2.9 ± 0.7 | 102 ± 5 | 1.6 ± 0.6 |
| **30** | 2-chloro-5-(3-hydroxy-propynyl)benzyl | Cl | 14% | 12% | 6 | 217 ± 27 | 108 ± 5 | ND[j] |
| **31a** | 4-aminobenzyl | Cl | 4100 ± 100 | 28% | 22 | 14 ± 3 | 98 ± 10 | ND[j] |
| **31b** | 4-amino-3-iodobenzyl | Cl | 48 ± 2 | 1100 ± 100 | ND[j] | 3.1 ± 0.1 | 103 ± 7 | ND[j] |
| **32** | 3-pyridylmethyl | Cl | 2600 ± 1200 | 33% | 4 | 11 ± 3 | 106 ± 7 | ND[j] |
| **33**[k] | *trans*−2-phenyl-1-cyclopropyl | Cl | 770 ± 50 | 4800 ± 200 | 48 | 0.78 ± 0.06 | 110 ± 7 | ND[j] |
| **34**[k] | 2,2-diphenylethyl | Cl | 1300 ± 100 | 1600 ± 100 | 46 | 0.69 ± 0.02 | 107 ± 9 | 10 ± 4 |
| **35** | 3-chlorobenzyl | I | 2200 ± 600 | 43% | ND[j] | 3.6 ± 0.8 | 107 ± 3 | 3.9 ± 0.4 |
| **36** | 3-chlorobenzyl | $SCH_3$ | 610 ± 40 | 52% | ND[j] | 1.5 ± 0.2 | 100 ± 4 | ND[j] |

[a] All AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human or rat ARs. Percent activation of the human $A_3AR$ was determined at 10 $\mu$M. Binding at human $A_1AR$ and $A_{2A}AR$ in this study was carried out as described in the methods section using [$^3$H]R-PIA or [$^3$H]CGS 21680 as a radioligand. Values from the present study are expressed as the mean ± SEM, $n = 3-5$. [b] Percent activity at 10 $\mu$M, relative to 10 $\mu$M Cl-IB-MECA ($A_3AR$). [c] Data from Ohno et al.[18] [d] Data from Gao et al.[12,17] [e] Data from Lee et al.[23] [f] Data from Tchilibon et al.[24] [g] Data from Jacobson et al.[40] [h] Data from de Zwart et al.[54] [i] Data from Kim et al.[14] [j] ND: not determined. [k] **18**, MRS3558; **20**, MRS3554; **26**, MRS3602; **33**, MRS3490; **34**, MRS3489.

$K_i$ value at the $A_3AR$ of 14 nM. The compound was $A_3$-AR-selective by 300-fold in comparison to the $A_1AR$. Following direct iodination of **31a** to form **31b**, the $A_3$-AR affinity increased by 4-fold; however, the selectivity was greatly reduced.

More diverse aromatic substitution at the 6-position was present in compounds **32**−**34**. $N^6$-(3-Pyridylmethyl)-adenosine **12** displayed a relatively high affinity at the $A_3AR$.[17] Unlike the various $N^6$-benzyl analogues, the 3-pyridylmethyl substitution in the (*N*)-methanocarba analogue **32** resulted in a 2.5-fold reduction in affinity in comparison to the simple 9-riboside **12**. The *trans*-2-phenyl-1-cyclopropyl analogue **33** ($K_i = 0.78$ nM) was equipotent to the 9-riboside **10**, while the 2,2-diphenylethyl analogue **34** ($K_i = 0.69$ nM) was 6-fold more potent than the corresponding 9-riboside **13**. In the case of **34**, applying the (*N*)-methanocarba-5′-uronamide modification converted an $A_3AR$ antagonist **13** into a full agonist.

High selectivity for the $A_3AR$ was achieved for many of the (*N*)-methanocarba analogues. Selectivity for the

$A_3$AR in comparison to the $A_1$AR was the following (fold): the $N^6$-(2,2-diphenylethyl) analogue **34** (1900), the $N^6$-(2,5-dimethoxybenzyl) analogue **26** (1200), the $N^6$-(2,5-dichlorobenzyl) and $N^6$-(2-phenyl-1-cyclopropyl) analogues **20** and **33** (1000), and the $N^6$-(3-substituted benzyl) analogues **17**, **18**, **28**, and **29** (700−900).

The $N^6$-moiety providing the highest affinity at the human $A_3$AR was introduced into several analogues bearing substituents at the 2-position of the adenine ring. Although the 2-chloro group provided some enhancement of affinity over no substituent, the 2-iodo **35** and 2-methylthio **36** analogues were 12- and 5-fold less potent, respectively, than the corresponding 2-chloro analogue **18**. Nevertheless, the selectivity for the human $A_3$AR in comparison to the human $A_1$AR remained high (590- and 390-fold, respectively).

**Molecular Modeling.** To determine an energetically favorable binding region and orientation of compound **18**, the previously reported h$A_3$AR complex with agonist **2b** was used as the starting geometry for the ribose binding position.[12] Different positions of the $N^6$-substituent were examined with the ribose moiety anchored in the putative binding site, and the resulting energies of complex with different conformers were compared. The optimized docking mode of compound **18** is shown in Figure 1A. Residues that were within 5 Å of the ligand in this putative binding site were the same residues for docked $A_3$AR agonists containing a ribose moiety.[12,62]

As shown in Figure 1, the purine ring of **18** was surrounded by a hydrophobic pocket defined by L91 (3.33) and L246 (6.51). In addition, certain orientations placed atoms within H-bonding distance, such as the exocyclic amine acting as donor and the side chain carbonyl group of N250 (6.55) at 2.7 Å, and between the purine $N^3$ atom and the side chain of Q167 (EL2) at 2.8 Å. The Cl atom at the C-2 position was surrounded by hydrophobic residues, L91 (3.33), the methyl group of T87 (3.29), and the aromatic ring of F168 in EL2, and was also proximal to the hydrophilic residue Q167 (EL2). The 2′-OH group of the ribose moiety was within H-bonding distance from the backbone carbonyl group of I268 (7.39) at 2.8 Å and the side chain of H272 (7.43) at 2.5 Å. The 3′-OH group could form an H-bond with the backbone carbonyl group of S271 (7.42) at 2.7 Å. Thus, there were some differences in putative H-bonding of the hydroxyl groups in the (*N*)-methanocarba analogues compared with 9-ribosides such as **2b**, which displayed a putative H-bond between the 3′-OH group and H272 (7.43).[12,14] Also, putative H-bonding was observed between the 5′-amide nitrogen and T94 (3.36) at 2.7 Å and between the 5′-carbonyl group and 3′-hydroxyl group at 2.3 Å. For binding at the $N^6$-position, the benzyl moiety showed an additional hydrophobic interaction with F168 (EL2), and the chloro atom of the benzyl substituent was in proximity to M177 (5.38). This putative hydrophobic interaction was consistent with the high binding affinity of **28**, which has a 3-methyl substituent on the benzyl ring. The receptor docking model suggested that **18** displayed more favorable van der Waals and electrostatic interactions than with the complexes of the $N^6$-5-chloro-2-benzyloxybenzyl derivative **24** and the $N^6$-3-(3-hydroxypropynyl)benzyl analogue **29**.[63,64]

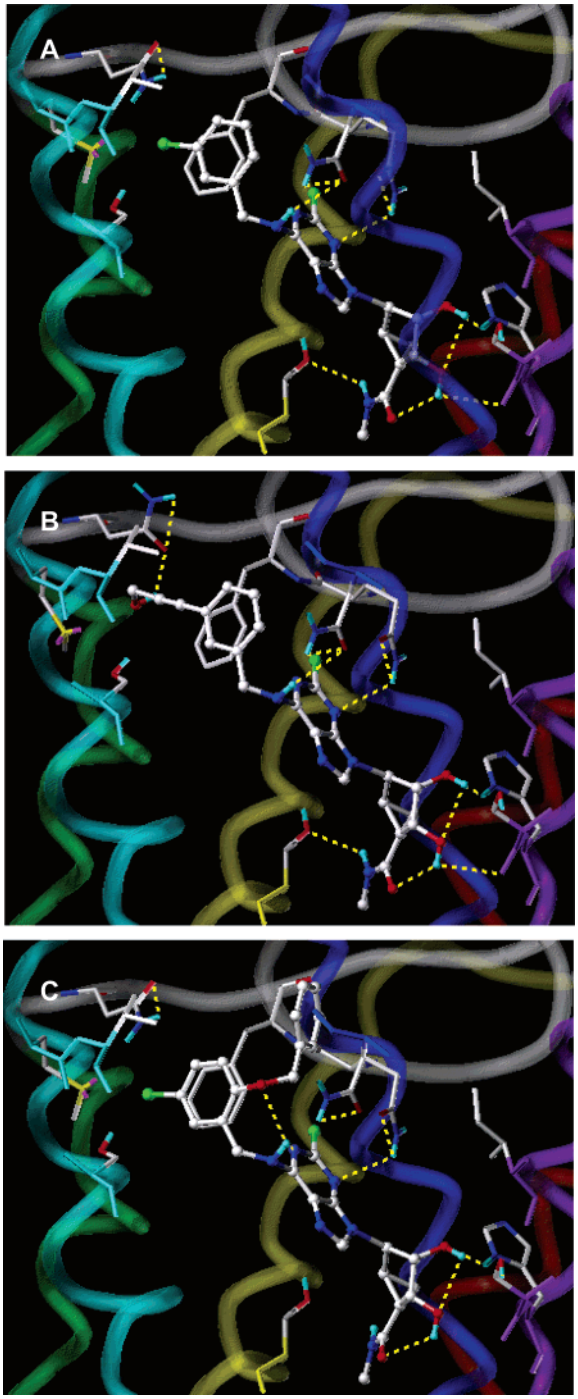



**Figure 1.** Complex of the h$A_3$AR with (A) **18**, (B) **24**, and (C) **29** in the putative binding site. The nucleosides are displayed as ball-and-stick models, and the side chains of h$A_3$AR are shown as stick models. The H-bonding between ligand and h$A_3$AR is displayed in yellow. The $A_3$AR is represented by a tube model with a different color for each TM domain (TM3 in yellow, TM4 in green, TM5 in cyan, TM6 in blue, TM7 in purple). Residues that interacted with ligand in the putative binding site were the following: T94 (3.36), N150 (EL2), Q167 (EL2), F168 (EL2), S181 (5.42), M177 (5.38), V178 (5.39), N250 (6.55), I268 (7.39), S271 (7.42), and H272 (7.43).

## Discussion

Activation of the $A_3$AR is increasingly associated with critical biological processes, such as its action on T-cells,[41] dendritic cells,[42] mast cells,[43−45] natural killer cells,[46] and epithelial cells[47] and in the brain.[48,49] This was accomplished by designing agonist nucleosides, with

an awareness of the structural determinants of full agonism and of the preference for the (*N*)-ribose ring conformation in binding to the $A_3$AR. The goal was to prepare novel, fully efficacious $A_3$AR agonists having high affinity and selectivity. The (*N*)-methanocarba substitution of the ribose ring, resulting in rigidification of the pseudosugar moiety in a 2′-*exo*-(*N*)-envelope conformation,[20] preserves or enhances affinity at the $A_3$-AR to a greater degree than at other AR subtypes. However, the (*N*)-methanocarba substitution alone also appeared to reduce $A_3$AR efficacy; thus, **3** and **4** were partial agonists. Therefore, we have included in these analogues the 5′-uronamido modification, which restores the loss of intrinsic efficacy observed with the (*N*)-methanocarba ring system and with various adenine substitutions (see below),[12] without reducing the associated $A_3$AR selectivity. The *N*-methyl-5′-uronamide was used, since this corresponds to the optimal 5′-substitution observed for the ribose series.[13]

At the $N^6$-position and 2-position of the adenine ring, we have incorporated groups known to be associated with high $A_3$AR potency, for example, substituted $N^6$-benzyl groups.[13] These groups also tend to enhance $A_3$-AR potency in both human and rat, unlike the $N^6$-methyl group, which favors human but not rat $A_3$ARs.[18] The $N^6$-(3-iodobenzyl) group itself may lead to a reduction of efficacy, even in the absence of multiple substitutions of the adenine moiety. In the presence of an $N^6$-substitution, such as 3-iodobenzyl or cyclopentyl, a 2-chloro group in adenosine analogues, in certain cases, leads to further reduced efficacy at the $A_3$AR.[9] The $N^6$-substituted 2-chloroadenosine analogues, however, were full agonists when combined with the 5′-uronamido modification in the present (*N*)-methanocarba series.

The meta position of the $N^6$-benzyl ring in the ribose series is known as a favorable site for substitution. In general, analogues that were substituted at both 5- and 2-positions in the present study were highly potent and selective $A_3$AR agonists. The comparable compounds with or without a 2-chloro group on the benzyl ring were generally similar in $A_3$AR affinity (cf. *m*-iodo analogues **5** and **21** and *m*-chloro analogues **18** and **20**).

A study of novel binary drugs[50] that activate both $A_1$-AR and $A_3$AR has concluded that a linear propynyl group favors $A_3$AR selectivity when placed at the 3-position of the $N^6$-benzyl ring in the ribose series. We wished to test if this finding extended to the (*N*)-methanocarba series. Compound **29** was similar in $A_3$-AR binding affinity ($K_i$ = 2.9 nM) to the other $N^6$-benzyl-substituted analogues. The loss of affinity upon 2-chloro substitution of the same $N^6$-benzyl moiety in **30** ($K_i$ = 220 nM) may be explained in terms of restricted rotation of this ring due to the propynyl group, which might place the 2-chloro atom in an unfavorable position.

Several analogues were included that contain a substituted $N^6$-(2-phenylethyl) moiety, known to produce high $A_3$AR affinity. However, as confirmed in the present structural series, such modifications favor human but not rat $A_3$ARs. The adenosine derivative ($N^6$-(1*S*,2*R*)-(2-phenyl-1-cyclopropyl)adenosine), with a $K_i$ value in binding to the h$A_3$AR of 0.63 nM (38-fold more potent than the 1*R*,2*S* isomer) and high agonist potency,[17,24] constitutes a lead for the development of $N^6$ derivatives of adenosine with high h$A_3$AR affinity. We have utilized this modification in its trans, racemic form (as occurs in analogue **10** in Table 1) in the design of the highly h$A_3$AR-selective agonist **33**. We have not resolved the diastereomers of **33**, but it is likely that the 1*S*,2*R* form is the more potent isomer.[24] The $N^6$-(2,2-diphenylethyl) analogue **34** was a full agonist and the most selective for the h$A_3$AR in the present series. This suggests that the loss of $A_3$AR efficacy seen in related nucleosides[23] can be overcome and that further derivatization of the $N^6$-(2-phenylethyl) moiety might provide useful $A_3$AR agonists.

Other reported $A_3$AR agonists include the 3′-amino-3′-deoxy derivatives reported by DeNinno and co-workers.[51,52] We have examined whether $N^6$ groups found to favor $A_3$AR affinity in that structural series, such as the $N^6$-[5-chloro-2-(aminocarbonylmethoyloxy)benzyl] group in **23**, also led to high affinity within the present series. Indeed, **23** and the 2-benzyloxy ether **24** were highly potent at the human $A_3$AR. Thus, sterically bulky substitution at the 2-position of the benzyl group of the (*N*)-methanocarba nucleosides is tolerated at the $A_3$AR. Furthermore, we provided a molecular model that assigns a putative binding region for this moiety. The 3′-amino-3′-deoxy modification of the ribose reported previously in $A_3$AR ligands was not included in the present study. Other adenosine $A_3$AR agonists are based on 5′-methylthio, 5′-vinyl, and similar groups on the ribose moiety;[38,52] however, these groups were also not included in the present study, in favor of those incorporating the 5′-uronamide group.

In conclusion, many of the previously known substituent groups that enhance $A_3$AR affinity, while in some cases reducing intrinsic efficacy in the 9-riboside series, may be adapted to the (*N*)-methanocarba-5′-uronamide nucleoside series of full agonists. The affinity and selectivity of the agonists in the (*N*)-methanocarba-5′-uronamide series often exceeded those of the corresponding ribosides. According to rhodopsin-based modeling, the binding regions of both the $N^6$-arylalkyl and the ribose (or pseudosugar) moieties were shown to be similar, but not identical, in the 9-riboside and (*N*)-methanocarba-5′-uronamide series. The putative orientation of the $N^6$-benzyl group within the binding site depended on whether bulky substituent groups were present. An additional potential advantage of the (N)-methanocarba nucleosides may be avoiding metabolic transformations to which 9-ribosides would be subject,[20] which is a topic for future studies. It will now be useful to examine these agonists in other pharmacological and disease models[4,10,15] in order to determine the generality of the selectivity for the $A_3$AR and to further validate the therapeutic utility of these selective agonists.

## Experimental Procedures

**Chemical Synthesis. Materials and Instrumentation.** Compound **11** was purchased from Sigma (St. Louis, MO), and compounds **3** and **4** were prepared as reported.[13,14] Compounds **9** and **10** were prepared as reported.[18,24]

Reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO). $^1$H NMR spectra were obtained with a Varian Gemini 300 spectrometer using $CDCl_3$, $CD_3OD$, or DMSO-$d_6$ as solvents. The chemical shifts are expressed as ppm downfield from TMS. All melting points were determined with a Thomas-Hoover apparatus (A. H. Thomas Co.) and are uncorrected.

Purity of compounds was checked using a Hewlett-Packard 1100 HPLC equipped with a Luna 5 $\mu$m RP-C18(2) analytical column (250 mm × 4.6 mm; Phenomenex, Torrance, CA). System A parameters consisted of the following: linear gradient solvent system of $H_2O/CH_3CN$ from 95/5 to 20/80 in 20 min; flow rate of 1 mL/min. System B parameters consisted of the following: linear gradient solvent system of 5 mM TBAP/$CH_3CN$ from 80/20 to 20/80 in 20 min, then isocratic for 2 min; flow rate of 1 mL/min. Peaks were detected by UV absorption with a diode array detector. All derivatives tested for biological activity showed >96% purity in the HPLC systems.

TLC analysis was carried out on aluminum sheets precoated with silica gel $F_{254}$ (0.2 mm) from Aldrich. Low-resolution mass spectrometry was performed with a JEOL SX102 spectrometer with 6 kV Xe atoms following desorption from a glycerol matrix or on LC/MS 1100 Agilent, 1100 MSD, with Waters Atlantis column C18. High-resolution mass measurements were performed on Micromass/Waters LCT Premier Electrospray Time of Flight (TOF) mass spectrometer coupled with a Waters HPLC system.

**Bromomethyl-2-chloro-5-iodobenzene (60).** A mixture of 2-chloro-5-iodotoluene (0.5 g, 1.98 mmol), *N*-bromosuccinimide (0.422 g, 2.35 mmol), and benzoyl peroxide (21.5 mg, 0.089 mmol) in dry $CCl_4$ (5 mL) was stirred and heated to reflux for 3 h. After cooling, the mixture was filtered, and the red filtrate was washed with a saturated solution of sodium thiosulfate (2 × 10 mL). The organic phase was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether) to give **60** as a white solid (361 mg, 55%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.76 (d, $J$ = 2.4 Hz, 1H), 7.56 (dd, $J$ = 8.7, 2.1 Hz, 1H), 7.12 (d, $J$ = 8.4 Hz, 1H), 4.49 (s, 2H). MS (*m/e*) (positive FAB) 332.1 $(M + H)^+$. Mp 94−95 °C.

***N*-(2-Chloro-5-iodobenzyl)phthalimide (63).** Bromomethyl-2-chloro-5-iodobenzene (**60**) (400 mg, 1.2 mmol) and potassium phthalimide (693 mg, 1.5 mmol) were stirred in dry DMF (20 mL) and heated to 80 °C for 3 h. After cooling, the suspension was filtered and concentrated in vacuo, and the residue was partitioned between water (30 mL) and $Et_2O$ (30 mL). The aqueous phase was extracted with ether (20 mL × 3). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated to give **63** (455 mg, 95%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.92−7.86 (m, 2H), 7.78−7.75 (m, 2H), 7.54−7.50 (m, 2H), 7.10 (d, $J$ = 8.4 Hz, 1H), 4.93 (s, 2H). MS (*m/e*) (API-ES) 397.9 $(M)^+$. Mp 134−136 °C.

***N*-(2,5-Dichlorobenzylphthalimide) (62).** Compound **62** was prepared by the same procedure as that for compound **63**. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.93−7.89 (m, 2H), 7.79−7.75 (m, 2H), 7.32 (d, $J$ = 6 Hz, 1H), 7.21−7.16 (m, 2H), 4.96 (s, 2H). MS (*m/e*) (API-ES) 306.12 $(M)^+$. Mp 145−146 °C.

**2-Chloro-5-iodobenzylamine Hydrochloride (65).** Compound **63** (350 mg, 0.88 mmol) was dissolved in dry EtOH (15 mL), and hydrazine (0.1 mL) was added. The stirred mixture was refluxed for 24 h and cooled, and the EtOH was evaporated. The residue was dissolved in $Et_2O$ (3 mL) and treated with HCl/$Et_2O$. The precipitated hydrochloride salt was filtered and triturated with dry $Et_2O$ (3 × 2 mL), obtaining 200 mg of product (yield 75%). $^1$H NMR ($D_2O$, 300 MHz) 7.88 (s, 1H), 7.80 (dd, $J$ = 8.4, 1.8 Hz, 1H), 7.31 ((d, $J$ = 7.8 Hz, 1H), 4.27 (s, 2H). MS (*m/e*) (positive FAB) 268.1 $(M - Cl)^+$. Mp 207−210 °C.

**2,5-Dichlorobenzylamine Hydrochloride (64). 64** was prepared by the same procedure as that for compound **65**. $^1$H NMR ($D_2O$, 300 MHz) $\delta$ 7.59−7.47 (m, 3H), 4.33 (s, 2H). MS (*m/e*) (positive FAB) 177.1. $(M - Cl)^+$. Mp >220 °C.

**2-Methoxy-5-iodobenzaldehyde (67).** 5-Iodosalicylaldehyde (1.0 g, 4.0 mmol) was dissolved in DMF (10 mL), and to the stirred solution $K_2CO_3$ (0.828 g, 6.0 mmol) and $CH_3I$ (1.14 g, 8.0 mmol) were added. The mixture was stirred at room temperature overnight. The suspension was concentrated in vacuo, and the residue was partitioned between water (30 mL) and $Et_2O$ (30 mL). The aqueous phase was separated and extracted with ether (20 mL × 2). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated to give **67** (1.02 g, 98%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 10.34 (s, 1H), 8.09 (s, 1H), 7.82−7.78 (m, 1H), 7.26 (s, 1H), 6.78 (d, $J$ = 8.7 Hz, 1H), 3.91 (s, 3H). MS (*m/e*) (positive FAB) 262.1 $(M + H)^+$. Mp 140−142 °C.

**2-Methoxy-5-iodobenzylamine (68).** To a stirred solution of **67** (500 mg, 1.9 mmol) and ammonium acetate (1.5 g, 19.4 mmol) in dry methanol (6 mL) $NaCNBH_3$ (170 mg, 2.66 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 48 h. Concentrated HCl was added until pH < 2 was attained. The methanol was evaporated, and the resulting white residue was dissolved in water (10 mL) and washed with $Et_2O$ (2 × 10 mL). The aqueous phase was then basified with aqueous KOH (45%), saturated with NaCl, and extracted with $CH_2Cl_2$ (10 mL × 4). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated to give **68** as a yellow oil (200 mg, 40%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.55−7.45 (m, 2H), 6.46−6.58 (m, 1H), 3.82−3.72 (m, 5H), 1.63 (br s, 2H). MS (*m/e*) (positive FAB) 264.1 $(M + H)^+$. Mp 95−97 °C.

**3-(3-Hydroxypropynyl)benzylamine (72).** Cuprous iodide (1.06 mg, 0.0056 mmol) was added to a mixture of $(PPh_3)_2PdCl_2$ (7.84 mg, 0.011 mmol) and 3-iodobenzylamine (262 mg, 1.12 mmol) in dry diethylamine (7 mL) under a nitrogen atmosphere. Then a solution of propargyl alcohol (41.2 $\mu$L, 0.73 mmol) in dry diethylamine (3 mL) was added. The resulting solution was stirred at room temperature for 3 h. The solvent was concentrated in vacuo, and the residue was partitioned between water (20 mL) and $CHCl_3$ (20 mL). The aqueous phase was separated and extracted with $CHCl_3$ (20 mL × 2). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated. The residue was purified by PTLC (chloroform/methanol 9:1) to give **72** (106 mg, 90% yield). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.39−7.26 (m, 4H), 4.48 (s, 2H), 3.85 (s, 2H), 1.73 (br s, 2H). MS (*m/e*) (positive FAB) 162.1 $(M + H)^+$.

**2-Chloro-5-(3-hydroxypropynyl)benzylamine (73).** Compound **73** was prepared by the same procedure as that for compound **72**, using compound **65** as starting material. $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.48 (s, 1H), 7.28−7.25 (m, 3H), 4.48 (s, 2H), 3.92 (s, 2H), 1.92 (br s, 2H). MS (*m/e*) (positive FAB) 196.1 $(M + H)^+$.

**5-Chloro-2-(methoxybenzyl)benzamide (75).** 5-Chloro-2-hydroxybenzamide (1.5 g, 8.7 mmol) was dissolved in DMF (120 mL), and to the stirred solution $K_2CO_3$ (1.38 g, 10 mmol) and benzyl bromide (1.71 g, 10 mmol) were added. The mixture was stirred at room temperature overnight. The suspension was concentrated in vacuo, and the residue was partitioned between water (30 mL) and $Et_2O$ (30 mL). The aqueous phase was separated and extracted with ether (20 mL × 2). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 90/10) to give **75** as a white solid (2.15 g, 95%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.20 (d, $J$ = 3 Hz, 1H), 7.67 (br s, 1H), 7.44−7.38 (m, 6H), 7.99 (d, $J$ = 9 Hz, 1H), 5.85 (br s, 1H), 5.17 (s, 2H). MS (*m/e*) (positive FAB) 262.1 $(M + H)^+$. Mp 110−112 °C.

**5-Chloro-2-(methoxybenzyl)benzylamine Hydrochloride (76).** To a suspension of $LiAlH_4$ (280 mg, 7.37 mmol) in dry tetrahydrofuran (20 mL) under nitrogen atmosphere, a solution of **75** (1.0 g, 3.83 mmol) in THF (10 mL) was added, and the mixture was refluxed for 3 h. After the mixture was cooled, the excess $LiAlH_4$ was destroyed with a saturated solution of sodium sulfate. The mixture was filtered on $MgSO_4$, and the filtrate was concentrated in vacuo. The residue was partitioned between water (30 mL) and $CH_2Cl_2$ (30 mL). The aqueous phase was separated and extracted with $CH_2Cl_2$ (20 mL × 2). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated. The residue was dissolved in $Et_2O$ (5 mL) and treated with HCl/$Et_2O$. The precipitated hydrochloride salt was filtered and triturated with dry $Et_2O$ (3 × 2 mL), obtaining 760 mg of product (yield 70%). $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 7.50−7.33 (m, 7H), 7.15 (d, $J$ = 9 Hz, 1H), 5.22 (s, 2H), 4.13 (s, 2H). MS (*m/e*) (API-ES) 248 $(M - Cl)^+$. Mp 119−121 °C.

**5-Chloro-2-hydroxybenzylamine (77).** To a suspension of $LiAlH_4$ (437 mg, 11.5 mmol) in dry tetrahydrofuran (30 mL) under nitrogen atmosphere, a solution of 5-chloro-2-hydroxybenzamide **74** (1.5 g, 5.7 mmol) in THF (10 mL) was added, and the mixture was refluxed for 3 h. After the mixture was cooled, the excess $LiAlH_4$ was destroyed with a saturated solution of sodium sulfate. The mixture was filtered on $MgSO_4$, and the filtrate was concentrated in vacuo. The residue was partitioned between water (30 mL) and $CH_2Cl_2$ (30 mL). The aqueous phase was separated and extracted with $CH_2Cl_2$ (20 mL × 2). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated. The obtained solid was recrystallized from ethanol, obtaining 787 mg of pure product (yield 88%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.10 (dd, $J$ = 6.6, 2.7 Hz, 1H), 6.94 (d, $J$ = 2.4 Hz, 1H), 6.76 (d, $J$ = 8.7 Hz, 1H), 4.10 (br s, 2H), 1.61 (br s, 2H). MS (*m/e*) (API-ES) 158 $(M+H)^+$. Mp 160−162 °C.

**(5-Chloro-2-hydroxybenzyl)carbamic Acid *tert*-Butyl Ester (78).** Compound **77** (300 mg, 1.9 mmol) was dissolved in a dry solution at 10% of triethylamine in MeOH (12 mL), and a solution of di-*tert*-butyl carbamate (1 M in THF, 3.8 mL, 3.8 mmol) was added. The solution was stirred at 45 °C for 1 h and then concentrated in vacuo. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 90/10) to give **78** as a white solid (350 mg, 80%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 9.02 (br s, 1H), 7.10 (dd, $J$ = 8.4, 2.4 Hz, 1H), 6.94 (d, $J$ = 2.4 Hz, 2H), 6.76 (d, $J$ = 8.7 Hz, 1H), 5.23 (br s, 1H), 4.17 (d, $J$ = 6.9 Hz, 2H), 1.44 (s, 9H). MS (*m/e*) (API-ES) 258 $(M+H)^+$. Mp 124−126 °C.

**(2-Carbamoylmethoxy-5-chlorobenzyl)carbamic Acid *tert*-Butyl Ester (79).** 5-Chloro-2-hydroxybenzyl)carbamic acid *tert*-butyl ester **78** (210 mg, 0.8 mmol) was dissolved in DMF (6 mL), and to the stirred solution $K_2CO_3$ (138 mg, 1 mmol) and 2-bromoacetamide (138 mg, 1 mmol) were added. The mixture was stirred at room temperature overnight. The suspension was concentrated in vacuo, and the residue was partitioned between water (20 mL) and $CH_2Cl_2$ (10 mL). The aqueous phase was separated and extracted with $CH_2Cl_2$ (20 mL × 2). The combined organic phases were dried over $Na_2SO_4$, filtered, and concentrated. The residue was purified by flash chromatography on silica gel (petroleum ether/ethyl acetate 80/20) to give **79** as a white solid (226 mg, 90%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.88 (br s, 1H), 7.27−7.18 (m, 2H), 6.76 (d, $J$ = 8.7 Hz, 1H), 5.54 (br s, 1H), 4.78 ( br s, 1H), 4.47 (s, 1H), 4.36 (d, $J$ = 6.3 Hz), 1.43 (s, 9H). MS (*m/e*) (API-ES) 337.0 $(MNa)^+$. Mp 142−143 °C.

**5-Chloro-2-(aminocarbonylmethyloxy)benzylamine (80).** Compound **79** (200 mg, 0.63 mmol) was treated with a solution at 15% of TFA in $CH_2Cl_2$ at room temperature for 45 min. The solution was concentrated in vacuo, and water (10 mL) was added. The aqueous phase was washed with $Et_2O$, basified with aqueous 2 N NaOH, and extracted with $CHCl_3$ (10 mL × 3), obtaining 130 mg of a white solid (**80**) (yield 96%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.37 (br s, 1H), 7.29−7.22 (m, 2H), 6.82 (d, $J$ = 9.3 Hz, 1H), 5.44 (br s, 1H), 4.60 (s, 2H), 3.89 (s, 2H). MS (*m/e*) (API-ES) 215 $(M+H)^+$. Mp 135−137 °C.

**General Procedure for the Synthesis of Compounds 39−54 and 16−33. (1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Bromobenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (39).** 3-Bromobenzylamine hydrochloride (122 mg, 0.55 mml) was added to a solution of **38** (50 mg, 0.12 mmol) and triethylamine (1 mL) in methanol (3 mL). The mixture was stirred at room temperature for 3 h. Then it was concentrated in vacuo to dryness and the residue was purified by PTLC (chloroform/methanol 15:1) to give **39** (55 mg, 82%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.70 (s, 1H), 7.52 (s, 1H), 7.31−7.18 (m, 2H), 6.18 (br s, 1H), 5.87 (d, $J$ = 7.2 Hz, 1H), 4.85−4.71 (m, 4H), 4.32−4.16 (m, 2H), 2.24−2.19 (m, 1H), 1.75−1.70(m, 1H), 1.55−1.49 (m, 4H), 1.35 (t, $J$ = 7.8, 3H), 1.29 (s, 3H). MS (*m/e*) (positive FAB) 564.1 $(M+H)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Fluorobenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (40).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.65 (s, 1H), 7.34−7.26 (m, 1H), 7.15−6.57 (m, 3H), 6.22 (br s, 1H), 5.87 (d, $J$ = 8.4 Hz, 1H), 4.85 (br s, 3H), 4.72 (d, $J$ = 7.2 Hz, 1H), 4.34−4.17 (m, 2H), 3.48 (d, $J$ = 5.1 Hz, 1H), 2.24−2.18 (m, 1H), 1.75−1.69 (m, 1H), 1.55 (s, 3H), 1.34 (t, $J$ = 7.8, 3H), 1.29 (s, 3H). MS (*m/e*) (ASI-ES) 502.1 $(M+H)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Chlorobenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (41).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.20−1.42 (m, 3H), 1.44−1.83 (m, 8H), 2.20−2.26 (m, 1H), 4.05−4.38 (m, 2H), 4.82−4.94 (m, 3H), 5.38 (d, $J$ = 4.5 Hz, 1H), 5.86 (d, $J$ = 8 Hz, 1H), 6.15 (br s, 1H), 7.21−7.40 (m, 4H), 7.67 (s, 1H). MS (*m/e*) (positive-FAB) 518.1 $(M+1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2,5-Dichlorobenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]-hexane-1′-carboxylic Acid Ethyl Ester (42).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.68 (s, 1H), 7.51 (s, 1H), 7.33 (d, $J$ = 8.7 Hz, 1H), 7.23−7.19 (m, 1H), 6.28 (br s, 1H), 5.57 (d, $J$ = 8.4 Hz, 1H), 4.85 (br s, 3H), 4.7 (d, $J$ = 6 Hz, 1H), 4.32−4.16 (m, 2H), 2.21−2.19 (m, 1H), 1.75−1.70 (m, 1H), 1.55−1.50 (m, 4H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 554.1 $(M+H)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2-Chloro-5-iodobenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]-hexane-1′-carboxylic Acid Ethyl Ester (43).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.85 (s, 1H), 7.67 (s, 1H), 7.57−7.53 (dd, $J$ = 8.1, 1.8 Hz, 1H), 7.12 (d, $J$ = 8.4 Hz, 1H), 6.32 (br s, 1H), 5.58 (d, $J$ = 8.4 Hz, 1H), 4.85 (br s, 3H), 4.73 (d, $J$ = 6 Hz, 1H), 4.33−4.14 (m, 2H), 2.24−2.19 (m, 1H), 1.75−1.70 (m, 1H), 1.55−1.50 (m, 4H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.29 (s, 3H). MS (*m/e*) (positive FAB) 644.1 $(M)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Chloro-2-mehoxybenzylamino)-2-chloropurin-9-yl]-2′,3′-isopropylidenebicyclo[3.1.0]-hexane-1′-carboxylic Acid Ethyl Ester (44).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.11−1.23 (m, 6H), 1.36−1.81 (m, 5H), 1.09−1.23 (m, 1H), 3.86 (s, 3H), 4.08−4.41 (m, 2H), 4.60−4.83 (m, 3 H), 5.30 (s, 1H), 5.85 (d, $J$ = 4.5 Hz, 1H), 6.21−6.29 (m, 1H), 6.81 (d, $J$ = 12 Hz, 1H), 7.21−7.42 (m, 3H), 7.67 (br s, 1H). MS (*m/e*) (positive-FAB) 548.1 $(M+1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Chloro-2-(aminoycarbonylmethyloxy)benzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidene-bicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (45).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.66 (s, 1H), 7.36 (s, 1H), 7.30−7.27 (m, 1H), 7.07−7.05 (m, 1H), 6.81 (d, $J$ = 8.7 Hz, 1H), 6.12 (br s, 1H), 5.85 (d, $J$ = 8.4 Hz, 1H), 5.65 (br s, 1H), 5.01−4.85 (br s, 3H), 4.73 (d, $J$ = 6 Hz, 1H), 4.50 (s, 1H), 4.34−4.21 (m, 2H), 2.23−2.18 (m, 1H), 1.74−1.69 (m, 1H), 1.55−1.50 (m, 4H), 1.34 (t, $J$ = 7.8 Hz, 3H), 1.29 (s, 3H). MS (*m/e*) (API-ES) 591.1 $(M+H)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Chloro-2-benzyloxybenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo-[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (46).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.62 (s, 1H), 7.39−7.30 (m, 6H), 7.18 (dd, $J$ = 8.7, 2.7 Hz, 1H), 6.86 (d, $J$ = 8.7 Hz, 1H), 6.33 (br s, 1H), 5.86 (d, $J$ = 8.4 Hz, 1H), 5.81 (s, 2H), 4.85 (br s, 3H), 4.70 (d, $J$ = 6 Hz, 1H), 4.32−4.19 (m, 2H), 2.23−2.18 (m, 1H), 1.74−1.69 (m, 1H), 1.55−1.50 (m, 4H), 1.34 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 624.2 $(M+H)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Iodo-2-methoxybenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]-hexane-1′-carboxylic Acid Ethyl Ester (47).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.71−7.64 (m, 2H), 7.57−7.53 (m, 2H), 6.21 (br s, 1H), 5.57 (d, $J$ = 8.4 Hz, 1H), 4.84 (s, 2H), 4.75−4.69 (m, 2H), 4.32−4.20 (m, 2H), 3.85 (s, 3H), 2.23−2.18 (m, 1H), 1.74−1.69 (m, 1H), 1.55−1.50 (m, 4H), 1.34 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 640.1 $(M+H)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2,5-Dimethoxybenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]-hexane-1′-carboxylic Acid Ethyl Ester (48).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.62 (s, 1H), 7.03 (s, 1H), 7.79 (s, 2H), 6.39 (br s, 1H), 5.57 (d, $J$ = 8.4 Hz, 1H), 4.83−4.69 (m, 4H), 4.31−4.19 (m, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 2.21−2.17 (m, 1H), 1.73−1.68 (m, 1H), 1.55−1.49 (m, 4H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 544.2 $(M+H)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2-Methylbenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (49).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.58 (s, 1H), 7.32 (d, $J$ = 6.9 Hz, 1H), 7.23−7.16 (m, 2H), 6.07 (br s, 1H), 5.57 (d, $J$ = 8.4 Hz, 1H), 4.83−4.69 (m, 4H), 4.32−4.16 (m, 2H), 2.37 (s, 3H), 2.23−2.18 (m, 1H), 1.74−1.69 (m, 1H), 1.55−1.50 (m, 4H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 498.3 $(M)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Methylbenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (50).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.56 (s, 1H), 7.26−7.09 (m, 4H), 6.27 (br s, 1H), 5.87 (d, $J$ = 8.4 Hz, 1H), 4.83−4.71 (m, 4H), 4.30−4.20 (m, 2H), 2.33 (s, 3H), 2.22−2.17 (m, 1H), 1.74−1.69 (m, 1H), 1.55−1.50 (m, 4H), 1.34 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 498.3 $(M)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-(3-Hydroxypropynyl)benzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo-[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (51).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.56 (s, 1H), 7.38 (s, 1H), 7.34−7.26 (m, 2H), 6.73 (br s, 1H), 5.87 (d, $J$ = 8.4 Hz, 1H), 4.82−4.70 (m, 4H), 4.46 (d, $J$ = 6 Hz, 2H), 4.32−4.20 (m, 2H), 2.41 (br s, 1H), 2.23−2.18 (m, 1H), 1.75−1.70 (m, 1H), 1.55−1.50 (m, 4H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 538.2 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2-Chloro-5-(3-hydroxypropynyl)benzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (52).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.63 (s, 1H), 7.48 (s, 1H), 7.32−7.22 (m, 2H), 6.63 (br s, 1H), 5.87 (d, $J$ = 8.4 Hz, 1H), 4.88−4.79 (m, 3H), 4.71 (d, $J$ = 4.8 Hz, 1H), 4.45 (d, $J$ = 6 Hz, 2H), 4.32−4.19 (m, 2H), 2.35 (br s, 1H), 2.23−2.18 (m, 1H), 1.75−1.68 (m, 2H), 1.55 (s, 3H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 572.1 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(4-Aminobenzylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (53).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.59 (s, 1H), 7.17 (d, $J$ = 8.1 Hz, 2H), 6.65 (d, $J$ = 8.1 Hz, 2H), 6.12 (br s, 1H), 5.87 (d, $J$ = 8.4 Hz, 1H), 4.84 (s, 1H), 4.73−4.58 (m, 3H), 4.33−4.20 (m, 2H), 3.17 (br s, 2H), 2.26−2.18 (m, 1H), 1.75−1.69 (m, 1H), 1.56−1.50 (m, 4H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 499.2 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Pyridylmethylamino)-2-chloropurin-9-yl]-2′,3′-*O*-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (54).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.66 (br s, 1H), 8.54 (br s, 1H), 7.73 (d, $J$ = 6.6 Hz, 1H), 7.67 (s, 1H), 7.34−7.27 (m, 1H) 6.26 (br s, 1H), 5.88 (d, $J$ = 8.4 Hz, 1H), 4.85 (br s, 3H), 4.72 (d, $J$ = 4.8 Hz, 1H), 4.45 (d, $J$ = 6 Hz, 2H), 4.33−4.20 (m, 2H), 2.24−2.18 (m, 1H), 1.75−1.70 (m, 2H), 1.56 (s, 3H), 1.35 (t, $J$ = 7.8 Hz, 3H), 1.28 (s, 3H). MS (*m/e*) (positive FAB) 485.2 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Bromobenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methyl Amide (17).** The ester **39** (45 mg, 0.08 mmol) was dissolved in methanol (3 mL) and treated with an aqueous solution of methylamine (1 mL, 40%). This mixture was stirred at room temperature overnight, then the solvent was evaporated to dryness, and the white residue was purified by PTLC (chloroform/methanol 9:1) to give the uronamide**17** (19.6 mg, 40%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.68 (s, 1H), 7.51 (s, 1H), 7.31−7.19 (m, 2H), 6.83 (br s, 1H), 6.30 (br s, 1H), 5.67 (d, $J$ = 6.9 Hz, 1H), 4.79−4.78 (m, 4H), 2.92 (d, $J$ = 4.5 Hz, 3H), 2.07−2.02 (m, 1H), 1.70−1.65 (m, 2H), 1.55 (s, 3H), 1.27 (s, 3H). MS (*m/e*) (positive FAB) 549.1 $(M + 1)^+$.

This intermediate (18 mg, 0.03 mmol) was treated with a solution of trifluoroacetic acid in MeOH (5 mL, 10%) and $H_2O$ (0.5 mL), and the mixture was heated at 70 °C for 3 h. The solution was cooled, and the solvent was removed to dryness by coevaporation with toluene in vacuo. The white residue was purified by PTLC (chloroform/methanol 9:1) to give the final product **17** (10 mg, 70%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.83 (s, 1H), 7.52−7.50 (m, 1H), 7.43−7.39 (m, 1H), 7.31−7.17 (m, 2H), 6.90 (br s, 1H), 6.63 (br s, 1H), 4.95−4.78 (m, 5H), 4.07 (d, $J$ = 6 Hz, 1H), 2.90 (d, $J$ = 5.1 Hz, 3H), 2.24−2.19 (m, 1H), 1.72 (br s, 2H), 1.36−1.32 (m, 1H). HRMS $(M + 1)^+$: calculated 507.0547, found 507.0571. HPLC (system A) 20.4 min (99%), (system B) 12.9 min (99%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Chlorobenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (18).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.17−1.39 (m, 1H), 1.45−1.61 (m, 1H), 2.01−2.09 (m, 1H), 2.81 (d, $J$ = 4.5 Hz, 1H), 3.91−4.08 (m, 2H), 4.78−5.05 (m, 5H), 6.71 (br s, 1H), 7.04 (br s, 1H), 7.11−7.29 (m, 4H), 7.74 (s, 1H). HRMS $(M + 1)^+$: calcd 463.1029, found 463.1052. HPLC (system A) 14.8 min (99%), (system B) 12.5 min (99%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Fluorobenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (19).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.84 (s, 1H), 7.34−7.26 (m, 1H), 7.15−6.95 (m, 3H), 6.86 (br s, 1H), 6.56 (br s, 1H), 4.88−4.80 (m, 4H), 4.08 (d, $J$ = 6 Hz, 1H), 2.91 (d, $J$ = 5.1 Hz, 3H), 2.27−2.23 (m, 1H), 1.78 (br s, 2H), 1.36−1.32 (m, 1H). HRMS $(M + 1)^+$: calculated 447.1348, found 447.1343. HPLC (system A) 13.6 min (99%), (system B), 16.8 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2,5-Dichlorobenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methyl Amide (20).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.94 (s, 1H), 7.60 (s, 1H), 7.43 (d, $J$ = 8.4 Hz, 1H), 7.34−7.30 (m, 1H), 7.04 (br s, 1H), 6.61 (br s, 1H), 5.12−4.92 (m, 1H), 431−4.22 (m, 1H), 3.03 (d, $J$ = 5.1 Hz, 3H), 2.31−2.27 (m, 1H), 1.54−1.46 (m, 1H). HRMS $(M + 1)^+$: calculated 497.0736, found 497.0797. HPLC (system A) 17.2 min (98%), (system B) 13.8 min (97%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2-Chloro-5-iodobenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (21).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.83 (m, 2H), 7.55 (d, $J$ = 8.4 Hz, 1H), 7.12 (d, $J$ = 8.4 Hz, 1H), 6.86 (br s, 1H), 6.43 (br s, 1H), 4.96−4.80 (m, 3H), 4.13−4.08 (m, 2H), 2.91 (d, $J$ = 5.1 Hz, 3H), 2.24−2.19 (m, 1H), 1.72 (br s, 2H), 1.36−1.32 (m, 1H). HRMS $(M + 1)^+$: calculated 589.0019, found 589.0029. HPLC (system A) 16.6 min (98%), (system B) 14.6 min (97%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Chloro-2-methoxybenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]-hexane-1′-carboxylic Acid Methylamide (22).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.12−1.42 (m, 2H), 2.21−2.27 (m, 1H), 2.91 (d, $J$ = 4.8 Hz, 3H), 3.86 (s, 3H), 4.01−4.22 (m, 2H), 4.65−4.83 (m, 3H), 4.95−5.01 (m, 1H), 6.42 (br s, 1H), 6.81 (d, $J$ = 12 Hz, 1H), 6.95 (br s, 1H), 7.21−7.43 (m, 3H), 7.81 (s, 1H). HRMS $(M + 1)^+$: calculated 493.1158, found 493.1138. HPLC (system A) 15.1 min (99%), (system B) 12.9 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Chloro-2-(aminocarbonylmethyloxy)benzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (23).** $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.04 (s, 1H), 7.38 (s, 1H), 7.25 (d, $J$ = 8.4 Hz, 1H), 6.94 (d, $J$ = 8.4 Hz, 1H), 5.07 (d, $J$ = 6.3 Hz, 1H), 4.59 (s, 2H), 4.99 (d, $J$ = 6.3 Hz, 1H), 2.99−2.68 (m, 5H), 2.05−2.02 (m, 1H), 1.38−1.34 (m, 1H). HPLC (system A) 12.3 min (97%), (system B) 9.1 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Chloro-2-benzyloxybenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]-hexane-1′-carboxylic Acid Methylamide (24).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.80 (s, 1H), 7.41−7.31 (m, 6H), 7.18 (dd, $J$ = 8.7, 2.7 Hz, 1H), 6.90−6.84 (m, 2H), 6.57 (br s, 1H), 5.09 (s, 2H), 4.87−4.77 (m, 5H), 4.07 (d, $J$ = 6 Hz, 1H), 2.90 (d, $J$ = 5.1 Hz, 3H), 2.54−2.21 (m, 1H), 1.92 (br s, 2H), 1.36−1.32 (m, 1H). HRMS $(M + 1)^+$: calculated 569.1471, found 569.1455. HPLC (system A) 17.2 min (98%), (system B) 16.7 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(5-Iodo-2-methoxybenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (25).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.79 (s, 1H), 7.67 (s, 1H), 7.54 (dd, $J$ = 8.7, 2.7 Hz, 1H), 6.94 (br s, 1H), 6.65 (d, $J$ = 8.7 Hz, 1H), 6.48 (br s, 1H), 4.97−4.57 (m, 5H), 4.07 (m, 1H), 3.85 (s, 3H), 2.91 (d, $J$ = 5.1 Hz, 3H), 2.24−2.19 (m, 1H), 1.94−1.85 (br s, 2H), 1.38−1.33 (m, 1H). HRMS $(M + 1)^+$: calculated 585.0514, found 585.0527. HPLC (system A) 18.7 min (98%), (system B) 13.9 min (99%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2,5-Dimethoxybenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (26).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.77 (s, 1H), 6.99−6.97 (m, 2H), 6.78−6.75 (m, 2H), 6.90 (br s, 1H), 6.63 (br s, 1H), 4.91 (d, $J$ = 6.3 Hz, 1H), 4.77−4.74 (m, 3H), 4.05 (d, $J$ = 6 Hz, 1H), 3.82 (s, 3H), 3.75 (s, 3H), 2.90 (d, $J$ = 5.1 Hz, 3H), 2.21−2.15 (m, 3H), 1.36−1.32 (m, 1H). HRMS $(M + 1)^+$: calculated 489.1653, found 489.1635. HPLC (system A) 17.5 min (98%), (system B) 11.3 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2-Methylbenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (27).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.76 (s, 1H), 7.31 (d, $J$ = 6.9 Hz, 1H), 7.22−7.14 (m, 3H), 6.97 (br s, 1H), 6.20 (br s, 1H), 5.07 (br s, 1H), 4.79 (br s, 2H), 4.34 (br s, 1H), 4.11 (d, $J$ = 6 Hz, 1H), 3.14 (br s, 1H), 2.92 (d, $J$ = 5.1 Hz, 3H), 2.37 (s, 3H), 2.19−2.14 (m, 3H), 1.97 (br s, 1H), 1.38−1.33 (m, 1H). HRMS $(M + 1)^+$: calculated 443.1598, found 443.1613. HPLC (system A) 14.3 min (99%), (system B) 12.1 min (99%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Methylbenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (28).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.79 (s, 1H), 7.23−7.09 (m, 4H), 6.93 (br s, 1H), 6.42 (br s, 1H), 4.95 (d, $J$ = 6.3 Hz, 1H), 4.79 4.70 (m, 3H), 4.09 (d, $J$ = 6 Hz, 1H), 2.91 (d, $J$ = 5.1 Hz, 3H), 2.33 (s, 3H), 2.22−2.17 (m, 1H), 1.97 (br s, 2H), 1.37−1.31 (m, 1H). HRMS $(M + 1)^+$: calculated 443.1598, found 443.1596. HPLC (system A) 14.5 min (99%), (system B) 12.0 min (99%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-(3-Hydroxypropynyl)benzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]-hexane-1′-carboxylic Acid Methylamide (29).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.02 (s, 1H), 7.70 (s, 1H), 7.61 (br s, 1H), 7.33−7.22 (m, 2H), 5.62 (br s, 1H), 5.20 (br s, 1H), 5.11−5.03 (m, 1H), 4.94 (br s, 1H), 4.80 (s, 1H), 4.67 (d, $J$ = 6 Hz, 1H), 4.45−4.31 (m, 1H), 3.96 (br s, 1H), 2.97 (d, $J$ = 5.1 Hz, 3H), 2.27−2.24 (m, 1H), 2.07 (br s, 1H), 1.36−1.32 (m, 1H). HRMS $(M + 1)^+$: calculated 483.1548, found 483.1538. HPLC (system A) 16.8 min (97%), (system B) 9.2 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(2-Chloro-5-(3-hydroxypropynyl)benzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (30).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 7.93 (s, 1H), 7.75 (s, 1H), 7.38−7.26 (m, 2H), 5.37−5.26 (m, 1H), 4.80−4.75 (m, 2H), 4.65−4.59 (m, 1H), 4.50−4.41 (m, 2H), 4.02 (br s, 1H), 3.72−3.70 (m, 1H), 3.10−3.07 (m, 1H), 2.99 (d, $J$ = 5.1 Hz, 3H), 2.29−2.23 (m, 1H), 2.01 (br s, 1H), 1.36−1.32 (m, 1H). HRMS $(M + 1)^+$: calculated 517.1158, found 517.1142. HPLC (system A) 17.3 min (98%), (system B) 11.1 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(4-Aminobenzylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (31a).** $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.02 (s, 1H), 7.15 (d. $J$ = 8.1 Hz, 2H), 6.70 (d. $J$ = 8.1 Hz, 2H), 5.09 (d, $J$ = 6.6 Hz, 1H), 4.81 (s, 1H), 4.61 (s, 2H), 4.02 (d, $J$ = 6.6 Hz, 1H), 2.88 (s, 3H), 2.07−2.05 (m, 1H), 1.40−1.35 (m, 1H). HRMS $(M + 1)^+$: calculated 444.1551, found 444.1539. HPLC (system A) 10.8 min (98%), (system B) 7.1 min (98%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Pyridylmethylamino)-2-chloropurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (32).** $^1$H NMR ($CD_3OD$, 300 MHz) $\delta$ 8.57 (s, 1H), 8.37 (s, 1H), 7.99 (s, 1H), 7.85 (d, $J$ = 8.1 Hz, 1H), 7.38−7.34 (m, 1H), 5.05 (d, $J$ = 6.6 Hz, 1H), 4.76 (br s, 3H), 3.97 (d, $J$ = 6.6 Hz, 1H), 2.26 (s, 3H), 2.04−1.99 (m, 1H), 1.35−1.295 (m, 2H). HRMS $(M + 1)^+$: calculated 430.1394, found 430.1403. HPLC (system A) 9.9 min (99%), (system B) 2.7 min (97%).

**6-Chloro-2-methylthiopurin-9-ylmethyl 2,2-dimethylpropionate (82).** To a stirred solution of 2-amino-6-chloropurin-9-ylmethyl 2,2-dimethylpropionate **81** (0.566 g, 2 mmol) in acetonitrile (2 mL) was added methyl disulfide (0.94 g, 10 mmol) and *tert*-butyl nitrite (90%, 1.14 g, 10 mmol), and the resulting reaction mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated in vacuo, and the resulting crude product was subjected to silica gel column chromatography (AcOEt/petroleum ether = 1/10), which furnished **82** (0.364 g, 55%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 8.19 (s, 1H), 6.12 (s, 2H), 2.65 (s, 3H), 1.17 (s, 9H). MS (*m*/*e*) (positive-FAB) 315 $(M + 1)^+$.

**6-Chloro-2-methylthiopurine (83).** To a solution of 6-chloro-2-methylthiopurin-9-ylmethyl 2,2-dimethylpropionate (**82**) (0.314 g, 1 mmol) in *i*-PrOH (10 mL) and THF (25 mL) was added 2 N aqueous NaOH (2 mL), and the resulting reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated under reduced pressure, and the residue obtained was purified by silica gel column chromatography (AcOEt/petroleum ether = 3/7), which afforded **83** (0.112 g, 56%). $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 8.54 (s, 1H), 2.58 (s, 3H). MS (*m*/*e*) (positive-FAB) 200.9 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-Chloro-2-iodopurin-9-yl]-2′,3′-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (55).** To a solution of triphenylphosphine (0.262 g, 1 mmol) and 6-chloro-2-iodopurine (0.175 g, 0.63 mmol) in THF (3 mL) was added DIAD (0.202 g, 1 mmol), and the reaction mixture was stirred for 10 min. A solution of the alcohol **37** (0.121 g, 0.5 mmol) in THF was added to the reaction mixture, and the mixture was further stirred for 10 h. The solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography (AcOEt/petroleum ether = 4/6), which afforded **55** (0.136 g, 54%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.30−136 (m, 4H), 1.57 (s, 6H), 1.75−180 (m, 1H), 2.12−2.22 (m, 1H), 4.1−4.2 (m, 2H), 4.75 (d, $J$ = 14 Hz, 1H), 4.92 (s, 1H), 5.85 (d, $J$ = 15 Hz, 1H), 7.98 (s, 1H). MS (*m*/*e*) (positive-FAB) 505.0 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-Chloro-2-methylthiopurin-9-yl]-2′,3′-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (56).** To a solution of triphenylphosphine (0.262 g, 1 mmol) and 6-chloro-2-methylthiopurine **83** (0.125 g, 0.63 mmol) in THF (3 mL) was added DIAD (0.202 g, 1 mmol), and the reaction mixture was stirred for 10 min. After which time alcohol **37** (0.121 g, 0.5 mmol) in THF was added and the reaction mixture was further stirred for 10 h. The solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography (AcOEt/petroleum ether = 4/6), which afforded **56** (0.112 g, 53%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.25−1.55 (m, 4H), 1.56 (s, 6H), 1.75−1.82 (m, 1H), 2.25−2.32 (m, 1H), 2.64 (s, 3H), 4.18−4.29 (m, 2H), 4.82 (d, $J$ = 12 Hz, 1H), 4.92 (s, 1H), 5.85 (d, $J$ = 11 Hz, 1H), 7.90 (s, 1H). MS (*m*/*e*) (positive-FAB) 425.10 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-Chloro-2-aminopurin-9-yl]-2′,3′-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (57).** To a solution of triphenylphosphine (0.262 g, 1 mmol) and 2-amino-6-chloropurine (0.169 g, 1 mmol) in THF (3 mL) was added DIAD (0.202 g, 1 mmol), and the reaction mixture was stirred for 10 min. After which time, alcohol **37** (0.121 g, 0.5 mmol) in THF was added to the reaction mixture and the mixture was further stirred for 10 h. The solvent was removed in vacuo, and the residue obtained was purified by silica gel column chromatography (AcOEt/petroleum ether = 5/5), which afforded **57** (0.041 g, 21%). $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.20−1.55 (m, 6H), 1.52 (m, 4H), 1.75−1.85 (m, 1H), 2.12−2.24 (m, 1H), 4.11−4.24 (m, 2H), 4.67 (d, $J$ = 11 Hz, 1H), 4.77 (s, 1H), 5.23 (br s, 2H), 5.88 (d, $J$ = 12 Hz, 1H), 7.72 (s, 1H). MS (*m*/*e*) (positive-FAB) 394.2 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Chlorobenzylamino)-2-iodopurin-9-yl]-2′,3′-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (58).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.11−1.22 (m, 4H), 1.23−1.79 (m, 7H), 2.18−2.24 (m, 1H), 4.10−4.21 (m, 2H), 4.66−4.87 (m, 3H), 5.30 (s, 1H), 5.85 (d, $J$ = 4.5 Hz, 1H), 6.05−6.16 (m, 1H), 7.21−7.26 (m, 4H), 7.58 (s, 1H). MS (*m*/*e*) (positive-FAB) 610.1 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Chlorobenzylamino)-2-iodopurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (35).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.15−1.22 (m, 1H), 1.65−1.70 (m, 1H), 2.08−2.13 (m, 1H), 2.94 (d, $J$ = 4.5 Hz, 3H), 4.02−4.23 (m, 2H), 4.61−4.82 (m, 3H), 5.02−5.12 (m, 1H), 6.22−6.31 (m, 1H), 6.61−6.72 (m, 1H),

7.11−7.21 (m, 4H), 7.76 (s, 1H). MS (*m/e*) (positive-FAB) 515.2 $(M + 1)^+$. HPLC (system A) 15.7 min (98%), (system B) 13.5 min (99%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Chlorobenzylamino)-2-methylthiopurin-9-yl]-2′,3′-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (59).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.15−1.40 (m, 7H), 1.42−1.81 (m, 5H), 2.53 (s, 3H), 4.15−4.38 (m, 2H), 4.78−4.92 (m, 4H), 5.84 (d, $J$ = 8.5 Hz), 6.02 (br s, 1H), 7.19−7.40 (m, 4H), 7.56 (s, 1H). MS (*m/e*) (positive-FAB) 555.0 $(M + 1)^+$.

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-(3-Chlorobenzylamino)-2-methylthiopurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (36).** $^1$H NMR ($CDCl_3$, 300 MHz) $\delta$ 1.12−1.18 (m, 1H), 1.62−176 (m, 1H), 2.22−2.29 (m, 1H), 2.51 (s, 3H), 2.90 (d, $J$ = 3.5 Hz, 3H), 4.16 (d, $J$ = 4.5 Hz, 1H), 4.65−4.98 (m, 4H), 6.21−6.32 (m, 1H), 6.71−6.81 (m, 1H), 7.11−7.42 (m, 4H), 7.72 (s, 1H). MS (*m/e*) (positive-FAB) 475.1 (M + 1). HPLC (system A) 15.4 min (98%), (system B) 13.1 min (99%).

**(1′*S*,2′*R*,3′*S*,4′*S*,5′*S*)-4′-[6-Methylamino-2-aminopurin-9-yl]-2′,3′-dihydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (15).** To a stirred solution of compound **57** (0.039 g, 0.1 mmol) in MeOH was added aqueous $CH_3NH_2$ solution (0.5 mL, 40%), and the reaction mixture was stirred for 12 h. It was concentrated to dryness, the residue was dissolved in the mixture containing 10% trifluoroacetic acid/MeOH (4 mL) and $H_2O$ (0.5 mL), and the mixture was heated at 70 °C for 3 h. The solvent was removed, and the residue was dried by coevaporation with dry toluene. The residue was purified using preparative TLC ($CHCl_3$/MeOH, 80:20) to afford **15** (0.011 g, 36%). $^1$H NMR (DMSO-$d_6$, 300 MHz) $\delta$ 1.11−1.23 (m, 1H), 1.45−1.58 (m, 1H), 1.62−1.78 (m, 1H), 2.65 (d, $J$ = 3.5 Hz, 3H), 2.91 (br s, 3H), 3.18 (d, $J$ = 4 hz, 1H), 3.84−3.88 (m, 1H), 4.56 (s, 1H), 4.75 (d, $J$ = 10 Hz, 1H), 5.08 (t, $J$ = 4.5 Hz, 1H), 5.24 (d, $J$ = 6.5 Hz), 5.82 (br s, 2H), 7.1 (br s, 1H), 7.55−7.75 (m, 2H). MS (*m/e*) (positive-FAB) 334.1 (M + 1). HRMS (M + 1): calculated 334.1628, found 334.1634. HPLC (system A) 6.6 min (98%), (system B) 5.0 min (98%).

**Pharmacological Methods.** [$^{125}$I]*N*$^6$-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide (I-AB-MECA, 2000 Ci/mmol), [$^3$H]*R*-PIA (*R*-*N*$^6$-[phenylisopropyl]adenosine, 34 Ci/mmol), [$^3$H]CGS21680 (2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamido-adenosine, 47 Ci/mmol), and [$^3$H]cyclic-AMP (40 Ci/mmol) were from Amersham Pharmacia Biotech (Buckinghamshire, U.K.).

**Cell Culture and Membrane Preparation.** CHO (Chinese hamster ovary) cells expressing the recombinant human $A_3$AR[25] were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 $\mu$g/mL streptomycin, 2 $\mu$mol/mL glutamine, and 800 $\mu$g/mL geneticin. The CHO cells expressing rat $A_3$ARs were cultured in DMEM and F12 (1:1). Cells were harvested by trypsinization. After homogenization and suspension, cells were centrifuged at 500*g* for 10 min, and the pellet was resuspended in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM $MgCl_2$, 1 mM EDTA, and 0.1 mg/mL CHAPS. The suspension was homogenized with an electric homogenizer for 10 s and was then recentrifuged at 20000*g* for 20 min at 4 °C. The resultant pellets were resuspended in buffer in the presence of 3 units/mL adenosine deaminase, and the suspension was stored at −80 °C until the binding experiments. Striatal and forebrain tissues from Wistar rats were homogenized in ice-cold 50 mM Tris-HCl buffer, pH 7.4, using an electric homogenizer. The homogenate was centrifuged at 20000*g* for 10 min at 4 °C, and the pellet was washed in fresh buffer. The final pellet was stored at −80 °C until the binding experiments. The protein concentration was measured using the Bradford assay.[55]

**Binding Assay Using [$^{125}$I]4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide.** Each tube in the competitive binding assay[36] contained 100 $\mu$L of membrane suspension (20 $\mu$g of protein), 50 $\mu$L of [$^{125}$I]4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide (1.0 nM), and 50 $\mu$L of increasing concentrations of the test ligands in Tris-HCl buffer (50 mM, pH 8.0) containing 10 mM $MgCl_2$ and 1 mM EDTA. Nonspecific binding was determined using 10 mM 5′-*N*-ethylcarboxamidoadenosine in the buffer. The mixtures were incubated at 37 °C for 60 min. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure using an MT-24 cell harvester (Brandell, Gaithersburgh, MD). Filters were washed three times with 9 mL of ice-cold buffer. Radioactivity was determined in a Beckman 5500B $\gamma$-counter.

**Cyclic AMP Accumulation Assay.** Intracellular cyclic AMP levels were measured with a competitive protein binding method.[56,57] CHO cells that expressed recombinant human and rat $A_3$ARs were harvested by trypsinization. After centrifugation and resuspension in medium, cells were plated in 24-well plates in 1.0 mL of medium. After 24 h, the medium was removed and cells were washed three times with 1 mL of DMEM containing 50 mM HEPES, pH 7.4. Cells were then treated with agonists and/or test compounds in the presence of rolipram (10 $\mu$M) and adenosine deaminase (3 units/mL). After 45 min forskolin (10 $\mu$M) was added to the medium, and incubation was continued an additional 15 min. The reaction was terminated by removing the supernatant, and cells were lysed upon the addition of 200 $\mu$L of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at −20 °C. For determination of cyclic AMP production, protein kinase A (PKA) was incubated with [$^3$H]cyclic AMP (2 nM) in $K_2HPO_4$/EDTA buffer ($K_2HPO_4$, 150 mM; EDTA, 10 mM), 20 $\mu$L of the cell lysate, and 30 $\mu$L 0.1 M HCl or 50 $\mu$L of cyclic AMP solution (0−16 pmol/200 $\mu$L for standard curve). Bound radioactivity was separated by rapid filtration through Whatman GF/C filters and washed once with cold buffer. Bound radioactivity was measured by liquid scintillation spectrometry.

**Statistical Analysis.** Binding and functional parameters were calculated using Prism 5.0 software (GraphPAD, San Diego, CA). $IC_{50}$ values obtained from competition curves were converted to $K_i$ values using the Cheng−Prusoff equation.[58] Data were expressed as the mean ± standard error.

**Molecular Modeling.** All calculations were performed on a Silicon Graphics Octane workstation (300 MHz MIPS R12000 (IP30) processor). All ligand structures were modified from the lowest energy conformation of Cl-IB-MECA[12] using the "Sketch Molecule" of SYBYL 6.9.[59] In all cases, MMFF force field[60] and charge were applied using distance-dependent dielectric constants and conjugate gradient method until the terminal gradient reached 0.05 kcal mol$^{-1}$ Å$^{-1}$.

A human $A_3$AR model (PDB code: 1o74) constructed by homology to the X-ray structure of bovine rhodopsin with 2.8 Å resolution[26] was used for the docking study. Compounds **18**, **24**, and **29** were docked within the human $A_3$AR model. The atom types of all ligands were manually assigned with the Amber all-atom force field,[61] and their charges were calculated before docking. The starting geometry of ligand conformation was chosen from the human $A_3$AR complex model with Cl-IB-MECA, which was already validated by point mutation.[12] The ribose binding position of this series was fixed, using an atom-by-atom fitting method for the carbon atoms of the ribose ring. To determine the binding region of *N*$^6$-derivatives, the flexible bond of $N_6-C-C_{ar}-C_{ar}$ angle important for the interaction of benzyl substituent was variously searched in the putative binding cavity, rotating by 60° increments. The same values of $C_5-C_6-N_6-C$ (234°) and $C_6-N_6-C-C_{ar}$ (282°) angles from the Cl-IB-MECA complex were used, and the $\chi$ (O4′−C1′−N9−C8) angle was also fixed in an anti conformation. Several starting conformations without any steric bump were selected for further optimization. The initial structures of all complexes were optimized using the Amber force field with fixed dielectric constant of 4.0 and terminating gradient of 0.1 kcal mol$^{-1}$ Å$^{-1}$.

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(62) Residues that were within 5 Å of the ligand in this putative binding site were the following: L91 (3.33), T94 (3.36), H95 (3.37), N150 (EL2), Q167 (EL2), F168 (EL2), S181 (5.42), M177 (5.38), V178 (5.39), F182 (5.43), W243 (6.48), L246 (6.51), S247 (6.52), N250 (6.55), I268 (7.39), S271 (7.42), and H272 (7.43).
(63) Because of intramolecular H-bonding of the benzyloxy and exocyclic amino groups of the 5-chloro-2-benzyloxybenzyl derivative **24**, there was no H-bonding at $N^6$H with N250 (6.55). In addition, no H-bonding at the 3′-OH and 5′-NH groups was shown in its docking complex. Compared to **18**, the binding of the $N^6$-benzyl group in **24** was more shifted toward TM4, and the additional benzyl substituent at the 2-position of its benzyl ring was directed toward the upper regions of TM6 and EL2, which accommodated the bulky substituent. The movement of the $N^6$-benzyl binding region resulted in weak H-bonding interactions at the hydroxyl and 5′-uronamide groups of methanocarba ring, thus decreasing the binding affinity.
(64) The docking complex of the $N^6$-3-(3-hydroxypropynyl)benzyl analogue **29** displayed additional H-bonding of the terminal hydroxyl group with the side chain of N150 in EL2. Thus, the extended hydroxypropynyl group penetrated through a hydrophobic region in proximity to the $N^6$-benzyl ring, and its hydroxyl group entered a hydrophilic region.

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