2-Triazole-Substituted Adenosines: A New Class of Selective A_3 Adenosine Receptor Agonists, Partial Agonists, and Antagonists

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"Click chemistry" was explored to synthesize two series of 2-(1,2,3-triazolyl)adenosine derivatives (**1**−**14**). Binding affinity at the human A_1, A_{2A}, and A_3 ARs (adenosine receptors) and relative efficacy at the A_3AR were determined. Some triazol-1-yl analogues showed A_3AR affinity in the low nanomolar range, a high ratio of A_3/A_{2A} selectivity, and a moderate-to-high A_3/A_1 ratio. The 1,2,3-triazol-4-yl regiomers typically showed decreased A_3AR affinity. Sterically demanding groups at the adenine C2 position tended to reduce relative A_3AR efficacy. Thus, several 5′-OH derivatives appeared to be selective A_3AR antagonists, i.e., **10**, with 260-fold binding selectivity in comparison to the A_1AR and displaying a characteristic docking mode in an A_3AR model. The corresponding 5′-ethyluronamide analogues generally showed increased A_3AR affinity and behaved as full agonists, i.e., **17**, with 910-fold A_3/A_1 selectivity. Thus, N^6-substituted 2-(1,2,3-triazolyl)-adenosine analogues constitute a novel class of highly potent and selective nucleoside-based A_3AR antagonists, partial agonists, and agonists.

Introduction

Adenosine receptors (AR) are G-protein-coupled receptors and consist of four subtypes classified as A_1, A_{2A}, A_{2B}, and A_3. Among the four AR subtypes, the A_3AR is the most recently identified.[1] The distribution of A_3AR is species-dependent, and in humans this subtype occurs in the lungs, liver, heart, kidneys, and brain.[2−4] Activation of this receptor subtype inhibits adenylyl cyclase activity, increases phosphatidylinositol-specific phospholipase C activity, and stimulates Ca^{2+} mobilization.[3] Adenosine A_3 receptor stimulation induces cardioprotection through the activation of K_{ATP} channels[4] and is also involved in neuroprotection, suggesting the possibility of using A_3AR agonists to treat cardial and cerebral ischemia.[5] A_3AR agonists also exhibit systemic anticancer and chemoprotective effects.[6] A_3AR modulators have been proposed as antiinflammatory and antiasthmatic drugs.[7,8] Selective A_3AR antagonists promise to be useful in the regulation of cell growth[8,9] and as cerebroprotective agents.[10,11] They also seem to enhance anticancer treatment by counteracting P-glycoprotein efflux in multidrug resistance.[12] A_3AR antagonists are also proposed as potential therapeutics for the treatment of glaucoma; application of A_3-AR antagonists externally to the eye substantially lowers intraocular pressure in mice and monkeys.[13−15]

Although diverse in structure, most AR antagonists share some common structural features. In general, they are planar, aromatic, or π-electron-rich and nitrogen-containing heterocycles. Additionally, most AR antagonists lack the ribose moiety, which seems essential for agonist activity.[16] Various heterocyclic classes have been identified as promising leads for A_3AR antagonists, among them 1,4-dihydropyridines, pyridines, deazaadenines, pyrazolopyrimidines, adenines, and 1,2,4-triazolo[4,3-*a*]quinoxalin-1-ones.[4,7,17,18]

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However, the A_3AR, more than other AR subtypes, is amenable to the design of nucleoside-based antagonists. The efficacy of nucleoside derivatives in activation of the A_3AR is particularly sensitive to molecular substitution of the ligand.[19] A wide range of adenosine derivatives have been shown to antagonize this receptor, including the highly potent A_1AR agonist 2-chloro-N^6-cyclopentyladenosine. N^6-Benzyl groups are associated with reduced A_3AR efficacy, leading to partial agonists and antagonists. However, many of the nucleosides so far demonstrated to be antagonists of the A_3AR are not highly subtype-selective.[20] Nucleoside-based A_3AR antagonists maintaining an intact ribose moiety were reported by Volpini et al.,[21] with a series of 8-alkynyladenosine derivatives that exhibited A_3AR selectivity, but suffered from weak A_3AR affinity. A spirolactam derivative, in which the 5′-alkyluronamide group was cyclized onto the 4′ carbon, was found to potently and selectively antagonize the A_3AR.[15,19] An advantage of nucleoside-based A_3AR antagonists over other heterocyclic antagonists is the ability to achieve high affinity at murine species.

Recently, researchers from CV Therapeutics described a series of 2-pyrazolyladenosine analogues.[22] Several representative compounds containing an N^6-methyl substituent proved to display high affinity and selectivity for the A_3AR. This study confirms the former finding[23] that introduction of a methyl group into the N^6 position increases the affinity for the human A_3AR and enhances the selectivity versus A_1 and A_{2A}ARs. On the basis of these results, we explored the versatile "click chemistry" approach[24] to synthesize two series of N^6-methyl-2-(1,2,3-triazolyl)adenosine derivatives and evaluated their affinity, selectivity, and efficacy at the A_3AR. Although a number of 1,2,3-triazole nucleoside derivatives have been described,[25] most involve base replacement with 1,2,3-triazole or introduction of 1,2,3-triazole at C8 or at the sugar moiety.

Results and Discussion

Chemistry. The synthesis of the 1,2,3-triazol-1-yladenosine derivatives is depicted in Scheme 1. 2-Iodo-N^6-methyladenosine **22**[23] was prepared by reacting **21**[26] with 2.0 M CH_3NH_2 in THF.

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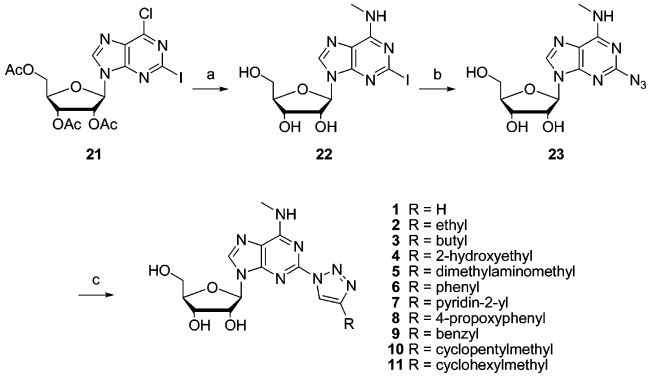
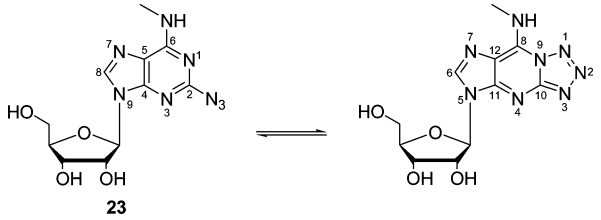
Scheme 1. Synthesis of 1,2,3-Triazol-1-yl Analogues of N^6-Methyladenosine **1**−**11**[a]



[a] Reagents and conditions: (a) CH_3NH_2 in THF, 2 days; (b) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, L-proline, Na_2CO_3, NaN_3, H_2O/^{t}BuOH 1:1, 60 °C; (c) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, alkyne, H_2O/^{t}BuOH 3:1, room temp.

Scheme 2. Azido/Tetrazole Tautomerism of a 2-Substituted Adenosine Derivative **23**

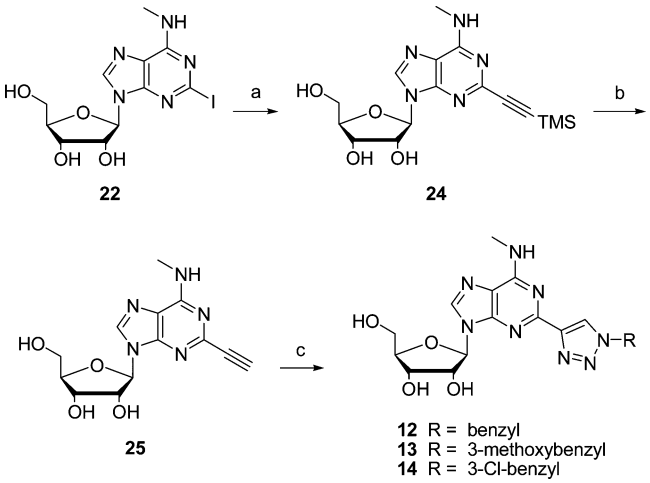


Since the reaction conditions[27] for a Cu(I)-catalyzed nucleophilic substitution are very similar to those used in the "click" variant of Huisgen's 1,3-dipolar cycloaddition, we initially attempted to perform a one-pot conversion of **22** to the desired 1,4-disubstituted 1,2,3-triazoles. The 2-azido derivative **23** was isolated as the main reaction product, and only a minor amount of the appropriate triazole was formed. This event forced us to perform the reaction in two steps. First the azido intermediate **23** was prepared in 66% yield from **22**. ^{1}H and ^{13}C NMR in DMSO-d_6 proved the presence of a tautomeric fused tetrazole form (17%) of the 2-azidoadenosine derivative **23**, due to a spontaneous cyclization (Scheme 2). Such azido/tetrazole tautomerism has been previously reported for 2-azidoadenine and for 2-azidoadenosine.[28,29] Next we applied a Cu(I)-catalyzed 1,3-cycloaddition reaction of azide **23** with the appropriate alkyne to generate the triazole analogues **1**−**11** (Scheme 1).[30] Generally, the use of a water/butanol mixture as a solvent for the 1,3-cycloaddition allowed simple isolation of the desired compounds, which precipitated from the reaction medium.

Similarly, the 1,2,3-triazol-4-yl analogues **12**−**14** (Scheme 3) were prepared by a Cu^+-catalyzed Huisgen 1,3-dipolar cycloaddition reaction of 2-ethynyl-N^6-methyladenosine (**25**) with the appropriate azide.

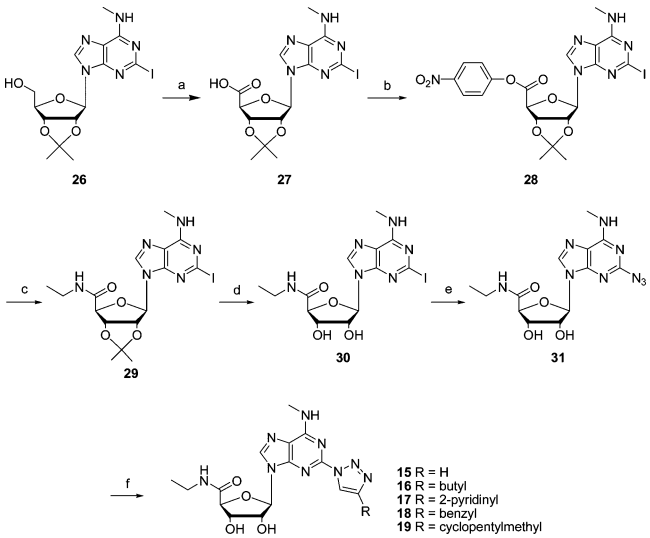
The synthesis of the 5′-*N*-ethylcarbamoyl 2-(1,2,3)-triazol-1-yladenosine analogues **15**−**19** was carried out starting from 2-iodo-9-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)-N^6-methyladenine (**26**). After permanganate oxidation, carboxylic acid **27** was converted into its *p*-nitrophenyl ester **28**, which upon treatment with ethylamine gave uronamide **29**. Deprotection with 80% trifluoroacetic acid yielded 5′-ethylcarbamoyl-N^6-methyl-2-iodoadenosine (**30**).[30] The conversion of this 2-iodo derivative into the azido intermediate **31** was performed in 79% yield. The presence of a tautomeric fused tetrazole form (20%) of the 2-azidoadenosine derivative **31**, due to a spontaneous cyclization, was here also observed in the NMR spectrum. Finally we applied the Cu(I)-catalyzed 1,3-cycloaddition reaction of azide

Scheme 3. Synthesis of 1,2,3-Triazol-4-yl Analogues of N^6-Methyladenosine **12**−**14**[a]



[a] Reagents and conditions: (a) trimethylsilylacetylene, CuI, $(Ph_3P)_3PdCl_2$, DMF; (b) 7 N NH_3 in MeOH, 0 °C; (c) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, alkyne, H_2O/^{t}BuOH 3:1, room temp.

Scheme 4. Synthesis of 1,2,3-Triazol-1-yl Analogues of N^6-Methyladenosine-5′-*N*-ethyluronamide **15**−**19**[a]



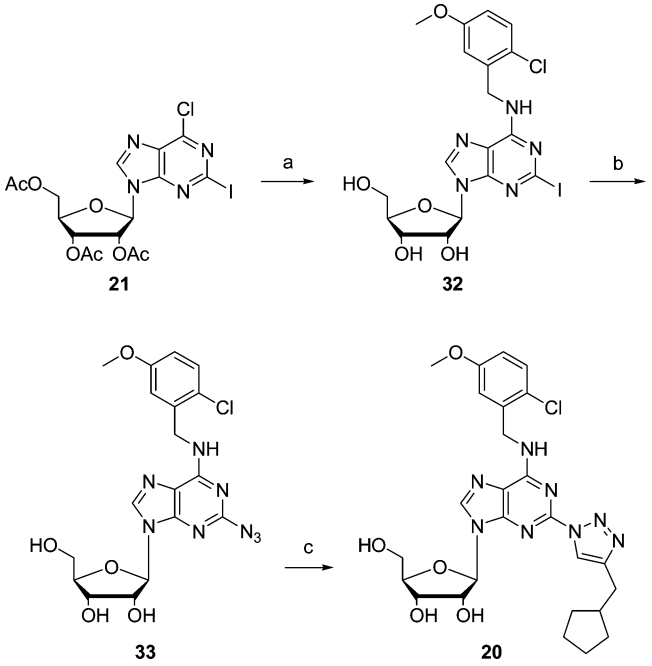
[a] Reagents and conditions: (a) $KMnO_4$, KOH, room temp, 20 h; (b) *p*-nitrophenol, EDCI, DMF, room temp; (c) ethylamine; (d) 80% TFA/H_2O; (e) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, L-proline, Na_2CO_3, NaN_3, H_2O/^{t}BuOH 1:1, 60 °C; (f) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, alkyne, H_2O/^{t}BuOH 1:1, room temp.

31 with the appropriate alkyne to generate the triazole analogues **15**−**19** (Scheme 4).

2-Azido-N^6-(5-chloro-2-methoxybenzyl)-2-(4-cyclopentylmethyl-1,2,3-triazol-1-yl)-9-(β-D-ribofuranosyl)adenine (**20**) was prepared in three steps starting from intermediate **21**, as depicted in Scheme 5.

Biological Evaluation. The binding affinities of the newly synthesized adenosine derivatives were measured at the hA_1, hA_{2A}, and hA_3ARs expressed in CHO (Chinese hamster ovary) cells as previously described,[20] and their relative efficacy in the activation of the A_3AR was determined (Table 1). The binding affinity of more potent compounds at the ARs was evaluated with full competition curves, while the weaker compounds at the hA_1 and hA_{2A} ARs were measured at a fixed concentration of 10 μM. Several compounds showed affinity for the A_3AR in the low nanomolar range, a very high ratio of A_3/A_{2A} selectivity, and a moderate-to-high A_3/A_1 selectivity ratio. A functional

Scheme 5. Synthesis of Compound **20**, 2-(4-Cyclopentylmethyl-1,2,3-triazol-1-yl)-N^6-(2-chloro-5-methoxybenzyl)adenosine[a]



[a] Reagents and conditions: (a) 2-chloro-5-methoxybenzylammonium chloride, Et_3N, EtOH, reflux; (b) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, L-proline, Na_2CO_3, NaN_3, H_2O/^{t}BuOH 1:1, 60 °C; (c) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, alkyne, H_2O/^{t}BuOH 1:1, room temp.

assay of A_3AR activation consisted of the ability of a single, high concentration of the nucleoside (10 μM) to inhibit forskolin-stimulated adenylyl cyclase measured by the method of Nordstet and Fredholm,[32] in comparison to the full agonist NECA (10 μM). Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine) was also a full agonist (100%) in this assay.[19] The range of efficacies observed depended on the nature of the groups at the 2 and N^6 positions.

The 2-azido precursor **23** showed high binding affinity at the A_3AR (K_i = 10.8 nM) and modest selectivity in comparison to the A_1AR. The 1,2,3-triazol-1-yl derivatives obtained by 1,3-dipolar cycloaddition of azide **23** with acetylene (**1**), butyne (**2**), and hexyne (**3**) maintained high affinity for the A_3AR and increased selectivity. They displayed K_i values of 10.4, 13.8, and 11.7 nM, respectively. Also, aromatic triazole substituents (**6**, **7**, **9**) resulted in similar K_i values of about 10 nM and even greater selectivity. Introducing nitrogen or oxygen including substituents at position 4 of the 1,2,3-triazole ring (**4**, **5**, and **8**) reduced the A_3AR affinity. Among the investigated analogues, the 4-cyclopentylmethyl derivative **10** exhibited the highest affinity for the A_3AR (K_i = 1.3 nM) and 260-fold selectivity in comparison to the A_1AR. Replacement of the cyclopentyl ring with a phenyl (**9**) or cyclohexyl (**11**) moiety adversely affected A_3AR affinity. Remarkably, the 1,2,3-triazol-4-yl regiomers (**12**−**14**) showed decreased affinity for the A_3AR in comparison to similar 1,2,3-triazol-1-yl regiomers. In particular, a comparison of homologous compounds **12** and **9** indicated a 6-fold loss of affinity at the A_3AR for the 4-yl isomer, approximately the same affinity at the A_1AR, and no significant measurable gain in affinity at the A_{2A}AR.

Replacement of the ribose 4′-hydroxymethyl moiety of the 2-azido derivative **23** by a 5′-*N*-ethyluronamide did not appreciably affect affinity at any of the AR subtypes. However, a similar substitution in the 2-(1,2,3-triazol-1-yl)-substituted series provided a modest (2- to 5-fold) increase in A_3AR affinity and small or no changes in A_1AR affinity, as demonstrated for the unsubstituted, 4-butyl, 4-pyridin-2-yl, and 4-benzyl substituted 1,2,3-triazol-1-yl combinations (**15**, **16**, **17**, and **18**, in comparison to **1**, **3**, **7**, and **9**, respectively). Curiously, one 5′-uronamide, compound **19**, exhibited decreased A_3AR affinity (K_i = 11.5 nM) compared to its potent 5′-OH analogue **10** (K_i = 1.3 nM).

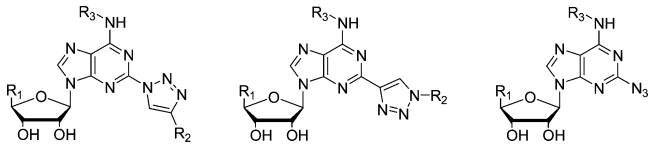
Replacement of the N^6-methyl substituent of the 2-azido precursor **23** by a sterically demanding 2-chloro-5-methoxybenzyl group yielded **33**, which manifested very high A_3AR affinity (K_i = 1.4 nM). A similar replacement of the N^6-methyl group of an analogue **10**, also having a bulky 2-position substituent, to yield **20** reduced A_3AR affinity but not appreciably. This was in accordance with previous observations that a simultaneous substitution at the 6 and 2 positions did not improve A_3AR affinity.[22,33] Thus, the effects of substitution at the 2 and N^6 positions were not independent; however, it was possible to retain considerable A_3AR selectivity (46-fold in compound **20**). This was not representative of findings in a previous study in which double substitution greatly diminished the affinity and selectivity at the human A_3AR.[22]

Whereas some previously synthesized 2-substituted adenosine derivatives[22,23] displayed selective A_3AR agonist activity, all 2-triazol-1-yl-N^6-methyladenosine analogues synthesized with an unmodified ribose moiety (**1**−**11** and **20**) behaved as antagonists or weak partial agonists. Similar findings were reported for 2-ester derivatives of adenosine, in which a combination of 2 and N^6 substitution reduced efficacy.[34] Direct ring substitution at the 4-position of the 1,2,3-triazole with alkyl or aryl groups resulted in weak partial agonists (**1**, **2**, **4**−**8**), but subtle changes of structure resulted in a loss of efficacy, e.g., the 4-butyl derivative **3**. 2-Triazol-1-yl-N^6-methyladenosine analogues with a methylene spacer between the 1,2,3-triazole moiety and a ring system yielded full A_3AR antagonists (**9**−**11**, **20**), since they bound to the receptor but did not activate it. The 2-triazol-4-yl-N^6-methyladenosine derivatives (**12**−**14**) also behaved as full A_3AR antagonists. Thus, the 5′-OH derivatives **3**, **9**−**14**, and **20** appeared to be A_3AR antagonists with the following order of decreasing selectivity for the A_3AR in comparison to the A_1AR: 4-cyclopentylmethyl-N^6-methyl **10** (260-fold) > 4-butyl-N^6-methyl **3** (72-fold), 4-cyclohexylmethyl-N^6-methyl **11** (67-fold) > 4-cyclopentylmethyl-N^6-(5-chloro-2-methoxybenzyl) **20**.

Interestingly, the 5′-*N*-ethyluronamide modification was able to reestablish the A_3AR agonist activity in analogues with sterically bulky substitution at the 2 position. This is consistent with previous findings that similar 5′-uronamides overcome the efficacy-reducing activity of substitution at the adenine 2 and N^6 positions but not at the ribose 3′ position.[19,35,36] Indeed, all 5′-*N*-ethyluronamide analogues studied here (**15**−**19**) proved to be full agonists at the A_3AR. Among them are highly selective A_3AR agonists, N^6-methyladenosine-5′-*N*-ethyluronamide 2-(1,2,3-triazol-1-yl) derivatives: pyridin-2-yl **17** (910-fold) > unsubstituted **15** (280-fold) > benzyl **18** (180-fold). The 2-azido-N^6-methyl precursors **23** and **31** also showed full agonist activity, whereas azide **33** having a bulky N^6 group showed partial agonist activity.

Selected potent agonists in this series were measured in a functional assay of the human A_{2B}AR. At 10 μM, compounds **3**−**7**, **15**, and **16** did not significantly stimulate adenylyl cyclase in human A_{2B}AR-expressing CHO cells (<10% of the effect of 10 μM NECA, as a full agonist). Compounds **2**, **8**−**14**, **17**, **19**, and **23** at 10 μM stimulated adenylyl cyclase by <50%. Compounds **18** and **31** produced approximately 50% stimulation at 10 μM. Thus, selectivity for the A_3AR was demonstrated;

Table 1. Binding Affinities of Adenosine Derivatives at Human A_1, A_{2A}, and A_3ARs Expressed in CHO Cells and Relative Efficacy at the A_3AR[a]



1-11, 15-20 **12-14** **23, 31, 33**

				K_i (nM) or % inhibition (in parentheses) at 10 μM			% efficacy[b]
	R_1	R_2	R_3	hA_1	hA_{2A}	hA_3	hA_3
1	CH_2OH	H	CH_3	1000 ± 30	(13 ± 3)	10.4 ± 0.2	41 ± 6
2	CH_2OH	ethyl	CH_3	2920 ± 910	(18)	13.8 ± 3.3	23[c]
3	CH_2OH	butyl	CH_3	848 ± 76	(23)	11.7 ± 3.1	3 ± 8
4	CH_2OH	2-hydroxyethyl	CH_3	1270 ± 260	(14)	45.0 ± 4.4	25[c]
5	CH_2OH	dimethylaminomethyl	CH_3	3800 ± 600	(6)	117 ± 25	8[c]
6	CH_2OH	phenyl	CH_3	(36)	(5)	14.9 ± 1.7	14 ± 8
7	CH_2OH	pyridin-2-yl	CH_3	1970 ± 210	(40)	10.3 ± 1.5	11 ± 4
8	CH_2OH	4-propoxyphenyl	CH_3	(49)	(14)	25.2 ± 2.6	31[c]
9	CH_2OH	benzyl	CH_3	589 ± 55	(20)	9.5 ± 0.7	−1 ± 5
10[d]	CH_2OH	cyclopentylmethyl	CH_3	335 ± 13	(39)	1.3 ± 0.4	−5 ± 7
11	CH_2OH	cyclohexylmethyl	CH_3	1430 ± 60	(16 ± 1)	21.3 ± 8.1	2 ± 5
12	CH_2OH	benzyl	CH_3	770 ± 210	(21 ± 5)	53.9 ± 6.6	2 ± 3
13	CH_2OH	3-methoxybenzyl	CH_3	957 ± 65	(43 ± 10)	86.1 ± 3.8	−1 ± 3
14	CH_2OH	3-Cl-benzyl	CH_3	956 ± 6	(39 ± 10)	81.1 ± 5.0	0 ± 5
15[d]	C_2H_5NHCO	H	CH_3	590 ± 70	(18 ± 3)	2.1 ± 0.1	102 ± 5
16	C_2H_5NHCO	butyl	CH_3	750 ± 110	(43 ± 1)	5.6 ± 0.2	89 ± 3
17[d]	C_2H_5NHCO	pyridin-2-yl	CH_3	1640 ± 90	(45 ± 12)	1.8 ± 0.6	90 ± 7
18[d]	C_2H_5NHCO	benzyl	CH_3	510 ± 50	(33 ± 2)	2.8 ± 1.3	86 ± 5
19	C_2H_5NHCO	cyclopentylmethyl	CH_3	1250 ± 150	(36 ± 7)	11.5 ± 1.4	83[c]
20	CH_2OH	cyclopentylmethyl	2-Cl−5-MeO−Bn	830 ± 40	6000	18 ± 11	−6 ± 3
23	CH_2OH		CH_3	230 ± 10	(23)	10.8 ± 3.1	84 ± 9
31	C_2H_5NHCO		CH_3	429 ± 55	(18 ± 3)	11.4 ± 4.2	112[c]
33	CH_2OH		2-Cl−5-MeO−Bn	60 ± 10	1800 ± 500	1.4 ± 0.1	44 ± 5

[a] All binding experiments were performed using cells stably transfected with cDNA encoding one of the human ARs. Binding at human A_1, A_{2A}, and A_3ARs in this study was carried out as described in Experimental Section using [^{3}H]CCPA, [^{3}H]CGS 21680, or [^{125}I]IAB-MECA as a radioligand. Values from the present study are expressed as K_i values (mean ± SEM, $n = 3$, unless otherwise noted) or as percent displacement of radioligand. [b] % activation at 10 μM, relative to cyclic AMP inhibitory effect of 10 μM NECA (=100%). Cl-IB-MECA was also a full agonist (100%) in this assay. [c] $n = 2$. [d] **10**, LC 153; **15**, LC 260; **17**, LC 257; **18**, LC 259.

these nucleosides that activate the A_3AR at low nanomolar concentrations activated the A_{2B}AR only at substantial micromolar concentrations.

On the basis of previous findings, it is predicted that only the analogues containing the substituted N^6-benzyl group (5-chloro-2-methoxy), i.e., full agonist **20** and partial agonist **33**, would be expected to bind in the nanomolar range to the rat A_3AR. Small alkyl groups at the N^6 position, such as methyl and ethyl, although conducive to high affinity at the human A_3AR, led to negligible affinity at the rat homologue of the receptor. Selected compounds were measured in binding to the rat A_3AR expressed in CHO cell membranes using [^{125}I]I-AB-MECA. The K_i values determined were as follows: compounds **5**, **7**, and **8**, $K_i > 10$ μM; compound **9**, $K_i = 1.79$ μM; compound **10**, $K_i = 0.312$ μM.

Molecular Modeling. To explain the structural basis for the high binding affinity of the nucleoside 2-(4-cyclopentylmethyl-1,2,3-triazol-1-yl)-N^6-methyl-9-(β-D-ribofuranosyl)adenine **10** at hA_3AR, we performed a computational study of ligand docking in a previously derived A_3AR model based on the high-resolution structure of bovine rhodopsin.[37,38] Various bound conformations of the C2-substituent and χ_1 angles for the adenine ring were generated for an energetically favorable binding location and orientation, and the resulting conformations were compared energetically in the putative binding site.

The result of docking **10** in the putative binding site of the A_3AR is shown in Figure 1A. The purine ring was surrounded by a hydrophobic pocket, defined by L91 (3.33) and L246 (6.51). In addition, the H-bonds formed between the exocyclic amine and the hydroxyl group of S247 (6.52) and between the purine N^1 atom and the side chain of N250 (6.55). The 2′-OH group of the ribose moiety formed a H-bond with the side chain of Q167 (EL2), and the 3′-OH group formed an intramolecular H-bond with the 5′-OH group. Unlike the previously reported docking models of N^6-substituted adenosines,[37] here the 5′-OH group H-bonded with the side chain of H272 (7.43) and the backbone carbonyl group of S271 (7.42). The cyclopentyl moiety interacted with aliphatic hydrophobic residues, M177 (5.38) and V178 (5.39), through a hydrophobic interaction and were situated in proximity to F168 (EL2) F182 (5.43), consistent with the optimized binding affinity of compound **10**.

A comparison of the docking modes of Cl-IB-MECA and compound **10** in the putative binding domain showed considerable overlap of the ribose rings and of the adenine moieties, although in compound **10** both were situated a little closer to extracellular loop 2 (Figure 1B). Previously, it was noted that 5′-uronamide analogues, typically of derivatives having bulky N^6-subsitituents, generally gain affinity in comparison to the corresponding 5′-hydroxyl analogue. Here, the 5′-uronamide analogue **19** (agonist) of the most potent 5′-hydroxyl analogue **10** (antagonist) displayed a lower binding affinity, which could be explained by the shift of the ribose position in adenosine analogue having a bulky 2-(4-cyclopentylmethyl-1,2,3-triazol-1-yl) substituent in comparison to those having N^6 bulky substituents. The binding of the cyclopentylmethyl group in **10** was directed more toward the upper part of TM5, partially overlapping with the binding site of the 3-iodophenyl ring in Cl-IB-MECA. Curiously, other closely related triazolo derivatives displayed a higher potency of the 5′-uronamide analogues; thus, compound **10** must bind to the receptor in a very distinct

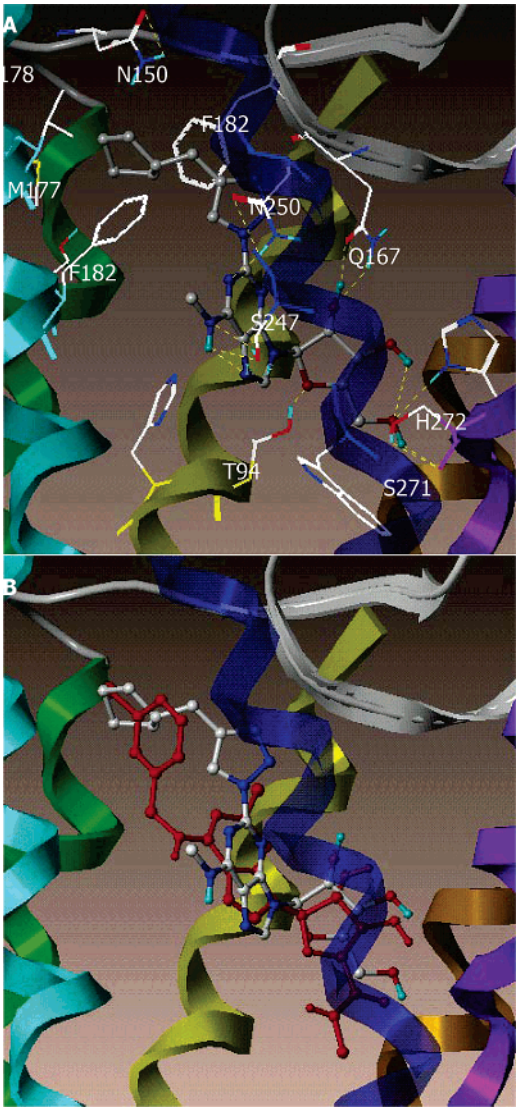


Figure 1. (A) Docking complexes of compound **10**, 2-(4-cyclopentylmethyl-1,2,3-triazol-1-yl)-N^6-methyladenosine. (B) Superimposition of Cl-IB-MECA in red and compound **10** in color by atom type. Residues that were within 5 Å to the ligand in this putative binding site were L91 (3.33), T94 (3.36), H95 (3.37), Q167 (EL2), F168 (EL2), M172 (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), C251 (6.56), I268 (7.39), S271 (7.42), and H272 (7.43). The ligand is represented by a ball-and-stick model. The H-bonds are indicated with yellow dots. By use of the MOLCAD ribbon surface program, the A_3AR is shown in a ribbon model with different colors for each TM (TM1, red; TM2, orange; TM3, yellow; TM4, green; TM5, cyan; TM6, blue; TM7, purple; H8, violet).

manner. There was a subtle difference in orientation between the 2-cyclopentyl group and bulkier groups like benzyl (**9**) or cyclohexylmethyl (**11**), which were associated with unfavorable van der Waals interactions and resulted in a decrease of binding affinity of 7- and 16-fold, respectively. In addition, the same preferred χ_1 angles of the energetically favorable bound conformation, common to Cl-IB-MECA and compound **10**, were consistent with the empirical finding that the combination of bulky N^6 and C2 substituents was unfavorable for A_3AR selectivity because of competitive interaction of these bulky substituents. Thus, the modeling has demonstrated that the human A_3AR preference of 2-(4-cyclopentylmethyl-1,2,3-triazol-1-yl) derivatives in the 5′-OH series might be explained by optimal van der Waals interactions.

Conclusions

Several 2-(1,2,3-triazol-1-yl)-N^6-methyl-substituted adenosine derivatives described in the present study displayed A_3AR affinities in the low nanomolar range, showed very high A_3/A_{2A}, and a moderate to high A_3/A_1 selectivity. Contrary to what we expected, the 2-triazole analogues with an unmodified ribose moiety (**1**−**14**) showed antagonist or weak partial agonist activity at the A_3AR. A 2-(4-cyclopentylmethyl-(1,2,3-triazol-1-yl))-N^6-methyl derivative **10** was 260-fold selective in binding in comparison to the A_1AR. The binding of the 4-cyclopentylmethyl group in **10**, in distinction to the binding of closely related bulky groups pendent on the triazole ring, was directed more toward the upper part of TM5 partially overlapping with the binding site of the 3-iodophenyl ring in Cl-IB-MECA. The 5′-*N*-ethyluronamide modification was dominant over the efficacy reducing effects at the 2 position and was capable of fully re-establishing the A_3AR agonist activity, resulting in highly potent and selective A_3AR agonists **15**−**19**. The most selective agonist derivative was compound **17**, 9-(5-ethylcarbamoyl-β-D-ribofuranosyl)-N^6-methyl-2-(4-pyridin-2-yl-1,2,3-triazol-1-yl)adenine, which was 910-fold selective in binding to the A_3AR in comparison to the A_1AR. The retention of high human A_3AR affinity in compound **20** was not typical of previous findings that double bulky substitution at the 2 and N^6 positions tended to reduce A_3AR affinity markedly. Thus, the 2-triazol-1-yl-N^6-methyladenosine analogues **1**−**11** constitute a novel class of highly potent and selective nucleoside-based A_3AR partial agonists and antagonists (all of which maintain an intact ribose in the molecular structure) and agonists. Since the reported analogues show excellent affinity for the A_3AR and span the full intrinsic activity range, they might be useful as pharmacological tools or as leads for further optimization.

Experimental Section

All reagents were from standard commercial sources and of analytic grade, except for the benzylic azides, which were prepared by treating the corresponding benzylic bromides with NaN_3 in DMF. Precoated Merck silica gel F254 plates were used for TLC, and spots were examined under UV light at 254 nm and further visualized by sulfuric acid−anisaldehyde spray. Column chromatography was performed on ICN silica gel (63−200 μm, 60 Å, ICN Biochemicals, Eschwege, Germany). NMR spectra were obtained with a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent signals, which in the case of DMSO-d_6 were 2.54 ppm for ^{1}H and 40.5 ppm for ^{13}C. Structural assignment was confirmed with COSY and DEPT. All signals assigned to hydroxyl groups were exchangeable with D_2O. Exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qToF 2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL/min. For the compounds that precipitated from the reaction medium, the yields were calculated from the amount obtained after filtration and are lower than the actual yields, since in most cases a considerable amount remained in solution.

***N*^{6}-Methyl-9-(β-D-ribofuranosyl)-2-(1,2,3-triazol-1-yl)adenine (1).** In a pressure tube was added **23** (165 mg, 0.51 mmol), trimethylsilylacetylene (292 μL, 2.05 mmol), and 4 mL of DMF. The mixture was stirred at 105 °C for 15 h. After solvent evaporation, the yellowish residue was dissolved in 2 mL of a 1.0 M solution of tetrabutylammonium fluoride in THF and stirred for 5 h. The reaction was monitored by NMR. After evaporation of the solvent, the residue was dissolved in ethyl acetate. Water was added, and the triazole product was precipitated in the water layer. After overnight cooling and filtration, the precipitate was further purified on a silica gel column (CH_2Cl_2/MeOH, 92:8) and yielded

compound **1** as a white solid (82 mg, 46%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.06 (d, 3H, J = 4.5 Hz, N^6-CH_3), 3.54−3.61 (m, 1H, H-5′A), 3.65−3.72 (m, 1H, H-5′B), 3.96 (dd, 1H, J = 3.8 and 7.9 Hz, H-4′), 4.20 (dd, 1H, J = 4.7 and 8.2 Hz, H-3′), 4.65 (app q, J = 5.9 Hz, H-2′), 4.98 (t, 1H, J = 5.6 Hz, 5′-OH), 5.23 (d, 1H, J = 5.0 Hz, 3′-OH), 5.48 (d, 1H, J = 6.2 Hz, 2′-OH), 5.95 (d, 1H, J = 5.9 Hz, H-1′), 7.92 (d, 1H, J = 1.2 Hz, H-4″), 8.37 (d, 1H, J = 4.6 Hz, N^6-H), 8.46 (s, 1H, H-8), 8.82 (br s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.84 (N^6-CH_3), 62.19 (C-5′), 71.13 (C-3′), 74.30 (C-2′), 86.42 (C-4′), 88.01 (C-1′), 119.75 (C-5), 124.67 (C-5″), 134.25 (C-4″), 141.13 (C-8), 149.56 and 149.85 (C-2 and C-4), 156.082 (C-6). HRMS (ESI-MS) $C_{13}H_{17}N_8O_4$ [M + H]$^+$: 349.1367 found; 349.1372 calcd. Anal. ($C_{13}H_{16}N_8O_4 \cdot {}^1/_2H_2O$) C, H, N.

2-(4-Ethyl-1,2,3-triazol-1-yl)-*N*^{6}-methyl-9-(β-D-ribofuranosyl)-adenine (2). Compound **23** (70 mg, 0.217 mmol), sodium ascorbate (8.6 mg, 0.043 mmol), and $CuSO_4 \cdot 5H_2O$ (2.2 mg, 0.009 mmol) were suspended in 20 mL of H_2O/^{t}BuOH (3:1). The mixture was saturated with 1-butyne and stirred for 4 days at room temperature in a Parr apparatus. Purification on a preparative TLC plate (CH_2Cl_2/MeOH, 90:10) resulted in compound **2** as a white solid in 40% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.27 (t, 3H, J = 7.62 Hz, CH_3), 2.75 (q, 2H, J = 7.6 Hz, CH_2), 3.05 (d, 3H, J = 4.4 Hz, N^6-CH_3), 3.51−3.59 (m, 1H, H-5′A), 3.61−3.70 (m, 1H, H-5′B), 3.94 (dd, 1H, J = 4.0 and 7.6 Hz, H-4′), 4.15 (dd, 1H, J = 4.4 and 7.9 Hz, H-3′), 4.60 (app q, J = 5.6 Hz, H-2′), 4.97 (t, 1H, J = 5.6 Hz, 5′-OH), 5.22 (d, 1H, J = 4.7 Hz, 3′-OH), 5.47 (d, 1H, J = 6.2 Hz, 2′-OH), 5.93 (d, 1H, J = 6.2 Hz, H-1′), 8.34 (d, 1H, J = 4.4 Hz, N^6-H), 8.45 (s, 1H, H-8), 8.55 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 14.32 (CH_3), 19.08 (CH_2), 27.84 (N^6-CH_3), 62.13 (C-5′), 71.15 (C-3′), 74.34 (C-2′), 86.43 (C-4′), 87.84 (C-1′), 119.57 (C-5), 120.962 (C-5″), 140.92 (C-8), 149.31, 149.63, 149.88 (C-2, C-4, and C-4″), 156.05 (C-6). HRMS (ESI-MS) $C_{15}H_{21}N_8O_4$ [M + H]$^+$: 377.1682 found; 377.1685 calcd. Anal. ($C_{15}H_{20}N_8O_4$) C, H, N.

General Procedure for the Synthesis of 4″-Substituted 2-(1,2,3-Triazol-1-yl)adenosine Derivatives 3−11. Compound **23** (70 mg, 0.217 mmol), sodium ascorbate (8.6 mg, 0.043 mmol), and $CuSO_4 \cdot 5H_2O$ (2.2 mg, 0.009 mmol) were suspended in 2 mL of H_2O/^{t}BuOH (3:1). The appropriate alkyne (2 equiv) was subsequently added, and the mixture was stirred overnight at room temperature. The 2-triazol-1-yl compounds (generally) precipitated from this reaction medium and were isolated by filtration with water.

2-(4-Butyl-1,2,3-triazol-1-yl)-*N*^{6}-methyl-9-(β-D-ribofuranosyl)-adenine (3). The reaction of **23** (70 mg, 0.217 mmol) with 1-hexyne (50 μL, 0.435 mmol) gave compound **3** in 59% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.93 (t, 3H, J = 7.3 Hz, CH_3), 1.38 (m, 2H, CH_2), 1.66 (m, 2H, CH_2), 2.71 (t, 2H, J = 7.2 Hz, C4″-CH_2), 3.05 (d, 3H, J = 4.0 Hz, N^6-CH_3), 3.52−3.62 (m, 1H, H-5′A), 3.62−3.72 (m, 1H, H-5′B), 3.95 (dd, 1H, J = 3.6 and 7.2 Hz, H-4′), 4.18 (dd, 1H, J = 4.8 and 8.1 Hz, H-3′), 4.62 (app q, 1H, J = 5.7 Hz, H-2′), 5.01 (t, 1H, J = 5.2 Hz, 5′-OH), 5.29 (d, 1H, J = 4.0 Hz, 3′-OH), 5.54 (d, 1H, J = 5.6 Hz, 2′-OH), 5.94 (d, 1H, J = 5.9 Hz, H-1′), 8.36 (d, 1H, J = 4.1 Hz, N^6-H), 8.45 (s, 1H, H-8), 8.56 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 14.37 (CH_3), 22.37 (CH_2), 25.21 (CH_2), 27.83 (N^6-CH_3), 31.68 (CH_2), 62.14 (C-5′), 71.15 (C-3′), 74.36 (C-2′), 86.43 (C-4′), 87.88 (C-1′), 119.56 (C-5), 121.33 (C-5″), 140.96 (C-8), 147.88 and 149.90 (C-2, C-4 and C-4″), 156.07 (C-6). HRMS (ESI-MS) $C_{17}H_{25}N_8O_4$ [M + H]$^+$: 405.1992 found, 405.1998 calcd. Anal. ($C_{17}H_{24}N_8O_4$) C, H, N.

2-[4-(2-Hydroxyethyl)-1,2,3-triazol-1-yl]-*N*^{6}-methyl-9-(β-D-ribofuranosyl)adenine (4). The reaction of **23** (70 mg, 0.217 mmol) with 3-butyn-1-ol (33 μL, 0.435 mmol) afforded compound **4** without precipitation. After solvent evaporation, the mixture was purified on a silica gel column (90:10 CH_2Cl_2/MeOH + 1% 7 N NH_3 in MeOH), yielding compound **11** as a white solid in 68% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.85 (t, 2H, J = 6.9 Hz, CH_2), 3.03 (d, 3H, J = 4.7 Hz, N^6-CH_3), 3.52−3.59 (m, 1H, H-5′A), 3.63−3.72 (m, 3H, H-5′B and CH_2), 3.94 (dd, 1H, J = 3.5 and 7.3 Hz, H-4′), 4.16 (dd, 1H, J = 4.8 and 8.1 Hz, H-3′), 4.59 (app q, 1H, J = 5.4 Hz, H-2′), 4.74 (t, 1H, J = 5.8 Hz, CH_2−OH), 4.97 (app t, 1H, J = 5.6 Hz, 5′-OH), 5.24 (d, 1H, J = 5.0 Hz, 3′-OH), 5.50 (d, 1H, J = 6.2 Hz, 2′-OH), 5.93 (d, 1H, J = 6.2 Hz, H-1′), 8.31 (d, 1H, J = 4.7 Hz, N^6-H), 8.43 (s, 1H, H-8), 8.54 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.83 (N^6-CH_3), 29.70 (CH_2), 60.85 (CH_2−OH), 62.13 (C-5′), 71.15 (C-3′), 74.35 (C-2′), 86.43 (C-4′), 87.81 (C-1′), 119.56 (C-5), 122.02 (C-5″), 140.49 (C-8), 145.49, 149.88, and 149.65 (C-2, C-4, and C-4″), 156.05 (C-6). HRMS (ESI-MS) $C_{15}H_{21}N_8O_5$ [M + H]$^+$: 393.1630 found, 393.1634 calcd. Anal. ($C_{15}H_{20}N_8O_5 \cdot H_2O$) C, H, N.

2-(4-Dimethylaminomethyl-1,2,3-triazol-1-yl)-*N*^{6}-methyl-9-(β-D-ribofuranosyl)adenine (5). The reaction of **23** (70 mg, 0.217 mmol) with 1-dimethylamino-2-propyne (47 μL, 0.435 mmol) gave compound **5** without precipitation. The volatiles were removed under reduced pressure, and the residue was purified on a silica gel column (80:20 CH_2Cl_2/MeOH + 1% 7 N NH_3 in MeOH). Compound **5** was obtained as a white solid in 67% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.20 (s, 6H, 2 × CH_3), 3.05 (d, 3H, J = 4.4 Hz, N^6-CH_3), 3.54−3.62 (m, 3H, H-5′A and CH_2), 3.65−3.72 (m, 1H, H-5′B), 3.95 (dd, 1H, J = 4.1 and 7.6 Hz, H-4′), 4.18 (dd, 1H, J = 5.0 and 8.2 Hz, H-3′), 4.62 (app q, 1H, J = 5.4 Hz, H-2′), 4.98 (t, 1H, J = 5.6 Hz, 5′-OH), 5.23 (d, 1H, J = 5.0 Hz, 3′-OH), 5.49 (d, 1H, J = 6.2 Hz, 2′-OH), 5.95 (d, 1H, J = 6.2 Hz, H-1′), 8.38 (d, 1H, J = 4.4 Hz, N^6-H), 8.45 (s, 1H, H-8), 8.64 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.85 (N^6-CH_3), 45.18 ($N(CH_3)_2$), 53.85 (CH_2), 62.10 (C-5′), 71.12 (C-3′), 74.38 (C-2′), 86.41 (C-4′), 87.84 (C-1′), 119.64 (C-5), 123.328 (C-5″), 140.97 (C-8), 144.41, 149.59, 149.79 (C-2, C-4, and C-4″), 156.05 (C-6). HRMS (ESI-MS) $C_{16}H_{24}N_9O_4$ [M + H]$^+$: 406.1944 found, 406.1951 calcd. Anal. ($C_{16}H_{23}N_9O_4$) C, H, N. N calcd, 31.09; found, 30.41.

***N*^{6}-Methyl-2-(4-phenyl-1,2,3-triazol-1-yl)-9-(β-D-ribofuranosyl)adenine (6).** The reaction of **23** (70 mg, 0.217 mmol) with phenylacetylene (48 μL, 0.435 mmol) yielded compound **6** (54%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.11 (d, 3H, J = 4.5 Hz, N^6-CH_3), 3.55−3.63 (m, 1H, H-5′A), 3.67−3.74 (m, 1H, H-5′B), 3.97 (dd, J = 3.6 and 7.2 Hz, H-4′), 4.21 (dd, J = 4.8 and 8.1 Hz, H-3′), 4.66 (app q, J = 5.4 Hz, H-2′), 5.03 (app t, 1H, J = 5.6 Hz, 5′-OH), 5.29 (d, 1H, J = 5.0 Hz, 3′-OH), 5.54 (d, 1H, J = 6.2 Hz, 2′-OH), 5.99 (d, 1H, J = 5.9 Hz, H-1′), 7.4 (t, 1H, J = 7.3 Hz, Ph), 7.50 (t, 2H, J = 7.5 Hz, Ph), 8.06 (d, 2H, J = 7.3 Hz, Ph), 8.44 (d, 1H, J = 4.5 Hz, N^6-H), 8.49 (s, 1H, H-8), 9.31 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.92 (N^6-CH_3), 62.16 (C-5′), 71.17 (C-3′), 74,39 (C-2′), 86.48 (C-4′), 87.94 (C-1′), 119.85 (C-5), 120.68 (C-5″), 126.27, 128.95, 129.61, and 130.85 (Ph), 141.082 (C-8), 147.02 and 149.77 (C-2, C-4, and C-4″), 156.10 (C-6). HRMS (ESI-MS) $C_{19}H_{21}N_8O_4$ [M + H]$^+$: 425.1689 found, 425.1685 calcd. Anal. ($C_{19}H_{20}N_8O_4$) C, H, N.

***N*^{6}-Methyl-2-[4-pyridin-2-yl-1,2,3-triazol-1-yl]-9-(β-D-ribofuranosyl)adenine (7).** The reaction of **23** (70 mg, 0.217 mmol) with 2-ethynylpyridine (44 μL, 0.435 mmol) afforded compound **7** as a white solid in 55% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.09 (d, 3H, J = 4.1 Hz, N^6-CH_3), 3.57−3.66 (m, 1H, H-5′A), 3.67−3.76 (m, 1H, H-5′B), 3.97 (dd, 1H, J = 3.9 and 7.5 Hz, H-4′), 4.19 (dd, 1H, J = 4.8 and 8.1 Hz, H-3′), 4.64 (app q, 1H, J = 6.0 Hz, H-2′), 4.99 (t, 1H, J = 5.6 Hz, 5′-OH), 5.26 (d, 1H, J = 5.0 Hz, 3′-OH), 5.54 (d, 1H, J = 6.2 Hz, 2′-OH), 5.99 (d, 1H, J = 5.9 Hz, H-1′), 7.42 (m, 1H, pyridin-2-yl) , 7.96 (m, 1H, pyridin-2-yl), 8.16 (d, 1H, J = 7.3 Hz, pyridin-2-yl), 8.42 (d, 1H, J = 4.1 Hz, 1H, N^6-H), 8.49 (s, 1H, H-8), 8.68 (d, 1H, J = 4.1 Hz, pyridin-2-yl), 9.16 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.92 (N^6-CH_3), 62.13 (C-5′), 71.14 (C-3′), 74.49 (C-2′), 86.47 (C-4′), 88.16 (C-1′), 119.92 (C-5), 120.53 (C-5″), 121.07 (pyridin-2-yl), 122.25 (pyridin-2-yl), 137.79 (pyridin-2-yl), 141.28 (C-8), 148.47, 150.45 and 150.56 (C-2, C-4, C-4″, and pyridin-2-yl), 156.28 (C-6). HRMS (ESI-MS) $C_{18}H_{19}N_9O_4Na$ [M + Na]$^+$: 448.1458 found, 448.1457 calcd. Anal. ($C_{18}H_{19}N_9O_4$) C, H, N.

***N*^{6}-Methyl-2-[4-(4-propoxyphenyl)-1,2,3-triazol-1-yl]-9-(β-D-ribofuranosyl)adenine (8).** The reaction of **23** (70 mg, 0.217 mmol) with 1-eth-1-ynyl-4-propoxybenzene (57 μL, 0.435 mmol) afforded compound **8** as a white solid in 36% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.99 (t, 3H, J = 7.3 Hz, CH_3), 1.71−1.78 (m, 2H,

CH_2), 3.10 (d, 3H, $J = 4.1$ Hz, N^6-CH_3), 3.54−3.62 (m, 1H, H-5′A), 3.64−3.72 (m, 1H, H-5′B), 3.98 (m, 3H, H-4′ and CH_2), 4.18 (dd, 1H, $J = 4.8$ and 8.1 Hz, H-3′), 4.64 (app q, 1H, $J = 5.4$ Hz, H-2′), 4.99 (t, 1H, $J = 5$, 7 Hz, 5′-OH), 5.25 (d, 1H, $J = 4.1$ Hz, 3′-OH), 5.50 (d, 1H, $J = 5.9$ Hz, 2′-OH), 5.97 (d, 1H, $J = 6.2$ Hz, H-1′), 7.03 (d, 2H, $J = 8.8$ Hz, Ph), 7.94 (d, 2H, $J = 8.8$ Hz, Ph), 8.36 (d, 1H, $J = 4.1$ Hz, 1H, N^6-H), 8.45 (s, 1H, H-8), 9.15 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 11.08 (CH_3), 22.73 (CH_2), 27.90 (N^6-CH_3), 62.16 (C-5′), 69.71 (OCH_2), 71.18 (C-3′), 74.38 (C-2′), 86.46 (C-4′), 87.91 (C-1′), 115.50 (Ph), 119.60 (C-5), 123.28 (C-5″), 127.66 (Ph), 140.01 (C-8), 147.01, 149.82, 150.45 (C-4, C-2, and C-4″), 156.10 (C-6). HRMS (ESI-MS) $C_{22}H_{27}N_8O_5$ $[M + H]^+$: 483.2109 found, 483.2104 calcd. Anal. ($C_{22}H_{26}N_8O_5$) C, H, N.

N^6-Methyl-2-(4-benzyl-1,2,3-triazol-1-yl)-9-(β-D-ribofuranosyl)adenine (9). The reaction of **23** (70 mg, 0.217 mmol) with 3-phenyl-1-propyne (54 μL, 0.435 mmol) gave compound **9** as a white solid in 43% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03 (d, 3H, $J = 3.8$ Hz, N^6-CH_3), 3.53−3.62 (m, 1H, H-5′A), 3.64−3.71 (m, 1H, H-5′B), 3.95 (dd, 1H, $J = 3.7$ and 7.2 Hz, H-4′), 4.11 (s, 2H, CH_2), 4.17 (dd, 1H, $J = 4.8$ and 8.1 Hz, H-3′), 4.61 (app q, 1H, $J = 5.7$ Hz, H-2′), 4.97 (app t, 1H, $J = 5.3$ Hz, 5′-OH), 5.22 (d, 1H, $J = 4.7$ Hz, 3′-OH), 5.47 (d, 1H, $J = 5.9$ Hz, 2′-OH), 5.94 (d, 1H, $J = 5.9$ Hz, H-1′), 7.23 (m, 1H, Ph), 7.32 (d, 4H, $J = 4.4$ Hz, Ph), 8.34 (d, 1H, $J = 3.8$ Hz, N^6-H), 8.45 (s, 1H, H-8), 8.59 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 25.32 (N^6-CH_3), 32.51 (CH_2), 62.11 (C-5′), 71.13 (C-3′), 74.43 (C-2′), 86.41 (C-4′), 87.96 (C-1′), 121.42 (C-5), 121.97 (C-5″), 126.91, 129.12, 129.21, and 140.02 (Ph), 140.88 (C-8), 147.42, 149.58, and 149.81 (C-4, C-2, and C-4″), 156.09 (C-6). HRMS (ESI-MS) $C_{20}H_{23}N_8O_4$ $[M + H]^+$: 439.1846 found, 439.1842 calcd. Anal. ($C_{20}H_{22}N_8O_4$) C, H, N.

2-(4-Cyclopentylmethyl-1,2,3-triazol-1-yl)-N^6-methyl-9-(β-D-ribofuranosyl)adenine (10). The reaction of **23** (70 mg, 0.217 mmol) with 3-cyclopentyl-1-propyne (57 μL, 0.435 mmol) yielded compound **10** (32%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.23−1.28 (m, 2H, cyclopentyl), 1.48−1.62 (m, 4H, cyclopentyl), 1.71−1.75 (m, 2H, cyclopentyl), 2.19−2.25 (m, 1H, cyclopentyl), 2.72 (d, 2H, $J = 7.3$ Hz, CH_2-cyclopentyl), 3.05 (d, 3H, $J = 3.9$ Hz, N^6-CH_3), 3.53−3.61 (m, 1H, H-5′A), 3.63−3.72 (m, 1H, H-5′B), 3.96 (dd, 1H, $J = 3.6$ and 7.2 Hz, H-4′), 4.19 (dd, 1H, $J = 4.8$ and 8.1 Hz, H-3′), 4.62 (app q, 1H, $J = 5.9$ Hz, H-2′), 4.98 (t, 1H, $J = 5.3$ Hz, 5′-OH), 5.23 (d, 1H, $J = 4.7$ Hz, 3′-OH), 5.49 (d, 1H, $J = 6.5$ Hz, 2′-OH), 5.95 (d, 1H, $J = 5.9$ Hz, H-1′), 8.34 (d, 1H, $J = 3.9$ Hz, N^6-H), 8.45 (s, 1H, H-8), 8.55 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 25.37 (cyclopentyl), 27.83 (N^6-CH_3), 31.56 (CH_2), 32.55 (cyclopentyl), 62.15 (C-5′), 71.15 (C-3′), 74.34 (C-2′), 86.43 (C-4′), 87.87 (C-1′), 119.60 (C-5), 121.58 (C-5″), 140.92 (C-8), 147.33, 149.89 (C-4, C-2, and C4″), 156.07 (C-6). HRMS (ESI-MS) $C_{19}H_{27}N_8O_4$ $[M + H]^+$: 431.2153 found, 431.2155 calcd. Anal. ($C_{19}H_{26}N_8O_4$) C, H, N.

2-(4-Cyclohexylmethyl-1,2,3-triazol-1-yl)-N^6-methyl-9-(β-D-ribofuranosyl)adenine (11). The reaction of **23** (70 mg, 0.217 mmol) with cyclohexyl-1-propyne (63 μL, 0.435 mmol) gave compound **11** in 82% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86−1.28 (br m, 6H, cyclohexyl), 1.54−1.72 (br m, 5H, cylcohexyl), 2.58 (d, 2H, $J = 6.9$ Hz, CH_2), 3.03 (d, 3H, $J = 3.9$ Hz, N^6-CH_3), 3.51−3.59 (m, 1H, H-5′A), 3.63−3.70 (m, 1H, H-5′B), 3.94 (dd, 1H, $J = 4.2$ and 7.5 Hz, H-4′), 4.16 (dd, 1H, $J = 4.8$ and 8.1 Hz, H-3′), 4.59 (app q, 1H, $J = 6.3$ Hz, H-2′), 4.97 (t, 1H, $J = 5.5$ Hz, 5′-OH), 5.22 (d, 1H, $J = 4.8$ Hz, 3′-OH), 5.47 (d, 1H, $J = 6.3$ Hz, 2′-OH), 5.93 (d, 1H, $J = 6.0$ Hz, H-1′), 8.30 (d, 1H, $J = 3.9$ Hz, N^6-H), 8.43 (s, 1H, H-8), 8.51 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 26.31 (cyclohexyl), 26.66 (cyclohexyl), 27.84 (N^6-CH_3), 33.16 (cyclohexyl), 38.22 (CH_2), 62.14 (C-5′), 71.15 (C-3′), 74.35 (C-2′), 86.44 (C-4′), 87.86 (C-1′), 119.60 (C-5), 121.93 (C-5″), 140.92 (C-8), 146.45, 149.92 (C-4, C-2, and C-4″), 153.51 (C-6). HRMS (ESI-MS) $C_{20}H_{29}N_8O_4$ $[M + H]^+$: 445.2305 found, 445.2311 calcd. Anal. ($C_{20}H_{28}N_8O_4$) C, H, N.

General Procedure for the Synthesis of 4″-Substituted 2-(1,2,3-Triazol-4-yl)adenosine Derivatives 12−14. Compound **25** (100 mg, 0.32 mmol), sodium ascorbate (13 mg, 0.06 mmol mmol), and $CuSO_4 \cdot 5H_2O$ (3 mg, 0. 013 mmol) were suspended in 30 mL of H_2O/^{t}BuOH (3:1). The appropriate azide (2 equiv) was subsequently added, and the mixture was stirred overnight at room temperature. The 2-triazol-4-yl compounds (generally) precipitated from this reaction medium and were isolated by filtration with water.

2-(1-Benzyl-1,2,3-triazol-4-yl)-N^6-methyl-9-(β-D-ribofuranosyl)adenine (12). The reaction of **25** (100 mg, 0.32 mmol) with 85 mg (0.64 mmol) of benzylazide gave compound **12** in 78% yield (110 mg). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03 (br s, 3H, N^6-CH_3), 3.52−3.58 (m, 1H, H-5′A), 3.60−3.66 (m, 1H, H-5′B), 3.92 (app d, H-4′, $J = 2.9$ Hz, H-4′), 4.15 (dd, 1H, $J = 4.7$ and 7.6 Hz, H-3′), 4.60 (app q, 1H, $J = 5.9$ Hz, H-2′), 5.08 (t, 1H, $J = 5.4$ Hz, 5′-OH), 5.19 (d, 1H, $J = 3.5$ Hz, 3′-OH), 5.44 (d, 1H, $J = 5.6$ Hz, 2′-OH), 5.68 (s, 2H, CH_2), 5.97 (d, 6.2 Hz, H-1′), 7.38 (br s, 5H, Ph), 7.82 (br s, 1H, N^6-H), 8.36 (s, 1H, H-8), 8.66 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.54 (N^6-CH_3), 53.62 (CH_2), 62.29 (C-5′), 71.30 (C-3′), 74.30 (C-2′), 86.41 (C-4′), 87.77 (C-1′), 119.52 (C-5), 126.41 (C-5″), 128.63, 128.86, 129.48 and 136.71 (Ph), 140.27 (C-8), 148.22, 153.56, 153.72 (C-2, C-4, and C-4′), 155.69 (C-6). HRMS (ESI-MS) $C_{20}H_{23}N_8O_4$ $[M + H]^+$: 439.1834 found, 439.1842 calcd. Anal. ($C_{20}H_{22}N_8O_4$) C, H, N.

2-[1-(3-Methoxybenzyl)-1,2,3-triazol-4-yl)-N^6-methyl-9-(β-D-ribofuranosyl)adenine (13). The reaction of **25** (100 mg, 0.32 mmol) with 104 mg (0.64 mmol) of 3-methoxybenzylazide gave compound **13** in 80% yield (120 mg). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.03 (br s, 3H, N^6-CH_3), 3.52−3.60 (m, 1H, H-5′A), 3.64−3.72 (m, 1H, H-5′B), 3.95 (app d, H-4′, $J = 2.9$ Hz, H-4′), 4.15 (dd, 1H, $J = 4.7$ and 7.6 Hz, H-3′), 4.60 (app q, 1H, $J = 6.4$ Hz, H-2′), 5.07 (t, 1H, $J = 5.1$ Hz, 5′-OH), 5.21 (d, 1H, $J = 4.2$ Hz, 3′-OH), 5.46 (d, 1H, $J = 6.3$ Hz, 2′-OH), 5.62 (s, 2H, CH_2), 5.95 (d, 1H, $J = 6.6$ Hz, H-1′), 6.91 (m, 3H, Ph), 7.29 (t, 1H, $J = 7.9$ Hz, Ph), 7.79 (br s, 1H, N^6-H), 8.34 (s, 1H, H-8), 8.63 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.59 (N^6-CH_3), 53.45 (CH_2), 55.82 (OCH_3), 62.30 (C-5′), 71.33 (C-3′), 74.25 (C-2′), 86.42 (C-4′), 87.70 (C-1′), 114.44 and 114.20 (Ph), 119.51 (C-5), 120.72 (Ph), 130.65 (C-5″), 138.21 (C-8), 148.22, 153.56, 153.64 (C-2, C-4, and C-4′), 155.69 (C-6), 160.14 (Ph). HRMS (ESI-MS) $C_{21}H_{25}N_8O_5$ $[M + H]^+$: 469.1938 found, 469.1947 calcd. Anal. ($C_{21}H_{24}N_8O_5$) C, H, N.

2-[1-(3-Chlorobenzyl)-1,2,3-triazol-4-yl)-N^6-methyl-9-(β-D-ribofuranosyl)adenine (14). The reaction of **25** (100 mg, 0.32 mmol) with 107 mg (0.64 mmol) of 3-chlorobenzylazide gave compound **14** in 73% yield (110 mg). ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.04 (br s, 3H, N^6-CH_3), 3.53−3.60 (m, 1H, H-5′A), 3.64−3.71 (m, 1H, H-5′B), 3.96 (app d, H-4′, $J = 2.9$ Hz, H-4′), 4.17 (dd, 1H, $J = 4.7$ and 7.6 Hz, H-3′), 4.64 (app q, 1H, $J = 5.8$ Hz, H-2′), 5.09 (t, 1H, $J = 5.3$ Hz, 5′-OH), 5.20 (d, 1H, $J = 4.4$ Hz, 3′-OH), 5.45 (d, 1H, $J = 6.2$ Hz, 2′-OH), 5.97 (d, 1H, $J = 6.2$ Hz, H-1′), 7.31−7.36 (m, 1H, Ph), 4.41−7.47 (m, 3H, Ph), 7.83 (br s, 1H, N^6-H), 8.37 (s, 1H, H-8), 8.72 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.54 (N^6-CH_3), 52.80 (CH_2), 62.31 (C-5′), 71.33 (C-3′), 74.27 (C-2′), 86.43 (C-4′), 87.69 (C-1′), 119.56 (C-5), 126.63 (C-5″), 127.53, 128.53, 128.84, 131.42, 133.98, and 139.15 (Ph), 140.36 (C-8), 148.22, 150.03, and 153.43 (C-2, C-4, and C-4′), 155.78 (C-6). HRMS (ESI-MS) $C_{20}H_{22}N_8O_4Cl$ $[M + H]^+$: 473.1452 found, 473.1452 calcd. Anal. ($C_{20}H_{21}N_8O_4Cl$) C, H, N.

9-(5-Ethylcarbamoyl-β-D-ribofuranosyl)-N^6-methyl-2-(1,2,3-triazol-1-yl)adenine (15). In a pressure tube was added **31** (110 mg, 0.30 mmol), trimethylsilylacetylene (259 μL, 1.81 mmol), and 4 mL of DMF. The mixture was stirred at 105 °C for 15 h. Solvent evaporation yielded a yellowish solid that was dissolved 6 mL of a 1.0 solution of tetrabutylammonium fluoride in THF and stirred for 5 h. After solvent evaporation, the residue was dissolved in ethyl acetate. Water was added, and the triazole precipitated in the water layer. After overnight cooling and filtration, the precipitate was further purified on a silica gel column (CH_2Cl_2/MeOH, 93:7) and yielded compound **15** as a white solid (49 mg, 42%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.90 (t, 3H, CH_3), 3.05−3.21 (m, 2H, N-CH_2), 3.05 (d, 3H, $J = 4.2$ Hz, N^6-CH_3), 4.26 (m, 1H, H-3′), 4.33 (d, 1H, $J = 2.1$ Hz, H-4′), 5.61 (d, 1H, $J = 6.2$ Hz, 3′-OH),

5.71 (d, 1H, J = 4.7 Hz, 2′-OH), 6.04 (d, 1H, J = 7.2 Hz, H-1′), 7.91 (d, 1H, J = 1.2 Hz, H-4″), 8.07 (t, 1H, J = 5.3 Hz, *N*HCO), 8.41 (d, 1H, J = 4.2 Hz, N^6-H), 8.54 (s, 1H, H-8), 8.82 (br s, 1H, H-5″). ^{13}CMR (300 MHz, DMSO-d_6): δ 15.16 (CH_3), 27.85 (N^6-CH_3), 34.07 (CH_2), 73.64 (C-2′ and C-3′), 84.98 (C-4′), 88.02 (C-1′), 119.91 (C-5), 124.70 (C-5″), 134.29 (C-4″), 141.59 (C-8), 149.69 and 149.90 (C-2 and C-4), 156.14 (C-6), 169.73 (C═O). HRMS (ESI-MS) $C_{15}H_{20}N_9O_4$ [M + H]$^+$: 390.1676 found, 390.1683 calcd. Anal. ($C_{15}H_{19}N_9O_4$) C, H, N.

General Procedure for the Synthesis of 4″-Substituted 2-(1,2,3-Triazol-1-yl)adenosine Derivatives 16−18. To a mixture of **31** (100 mg, 0.28 mmol), CuI (5 mg, 0.03 mmol), and triethylamine (40 μL, 0.28 mmol) in water/acetonitrile (1:1), the appropriate alkyne (2 equiv) was added. The mixture was stirred for 5 days at room temperature. The reaction was monitored by ^{1}H NMR. The product was precipitated with water and cooled overnight. After filtration, the yellowish solid was purified on a silica gel column (CH_2Cl_2/MeOH, 90:10) to obtain the 1,2,3-triazol-1-yladenosine derivative as a white solid.

2-(4-Butyl-1,2,3-triazol-1-yl)-9-(5-ethylcarbamoyl-β-D-ribofuranosyl)-N^6-methyladenine (16). The reaction of compound **31** (100 mg, 0.28 mmol) with 1-hexyne (64μL, 0.56 mmol) gave 65 mg (53%) of compound **16** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88−0.95 (m, 6H, 2 × CH_3), 1.31−1.43 (m, 2H, CH_2), 1.61−1.71 (m, 2H, CH_2), 2.72 (t, 2H, J = 7.8 Hz, C4″-CH_2), 3.05−3.22 (m, 2H, *N*-CH_2), 3.05 (d, 3H, J = 4.1 Hz, N^6-CH_3), 4.25 (m, 1H, H-3′), 4.33 (d, 1H, J = 2.1 Hz, H-4′), 4.73 (m, 1H, H-2′), 5.59 (d, 1H, J = 6.3 Hz, 3′-OH), 5.69 (d, 1H, J = 4.5 Hz, 2′-OH), 6.04 (d, 1H, J = 6.9 Hz, H-1′), 8.09 (t, 1H, J = 5.4 Hz), 8.37 (d, 1H, J = 4.1 Hz, N^6-H), 8.53 (s, 1H, H-8), 8.56 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): 14.36 (CH_3), 15.17 (CH_3), 22.35 (CH_2), 25.21 (CH_2), 27.84 (N^6-CH_3), 31.70 (CH_2), 34.07 (CH_2), 73.60 and 73.64 (C-2′ and C-3′), 84.99 (C-4″), 87.91 (C-1′), 119.79 (C-5), 121.45 (C-5″), 141.49 (C-8), 147.97, 149.72, and 149.93 (C-2, C-4, and C-4″), 156.11 (C-6), 169.73 (C═O). HRMS (ESI-MS) $C_{19}H_{28}N_9O_4$ [M + H]$^+$: 446.2256 found, 446.2264 calcd. Anal. ($C_{19}H_{27}N_9O_4$) C, H, N.

9-(5-Ethylcarbamoyl-β-D-ribofuranosyl)-N^6-methyl-2-(4-pyridin-2-yl-1,2,3-triazol-1-yl)adenine (17). The reaction of compound **31** (50 mg, 0.14 mmol) with 2-ethynylpyridine (56 μL, 0.56 mmol) gave 35 mg (54%) of compound **17** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.93 (t, 3H, J = 7.2 Hz, CH_3), 3.09−3.21 (m, 2H, *N*-CH_2), 3.10 (d, 3H, J = 4.2 Hz, N^6-CH_3), 4.25 (m, 1H, H-3′), 4.35 (d, 1H, J = 2.1 Hz, H-4′), 4.74 (m, 1H, H-2′), 5.68 (d, 1H, J = 6.6 Hz, 2′-OH), 5.75 (d, 1H, J = 4.5 Hz, 3′-OH), 6.08 (d, 1H, J = 6.6 Hz, H-1′), 7.41 (m, 1H, pyridin-2-yl), 7.96 (m, 1H, pyridin-2-yl), 8.10 (t, 1H, J = 6.0 Hz, *N*HCO), 8.16 (d, 1H, J = 8.1 Hz, pyridin-2-yl), 8.48 (d, 1H, J = 4.1 Hz, 1H, N^6-H), 8.60 (s, 1H, H-8), 8.67 (d, H, J = 5.1 Hz, pyridin-2-yl), 9.17 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 15.18 (CH_3), 27.94 (N^6-CH_3), 34.11 (*N*-CH_2), 73.69 and 73.78 (C-2′ and C-3′), 84.95 (C-4′), 87.98 (C-1′), 120.04 (C-5), 120.65 (C-5″), 122.20 and 124.11 (pyridin-2-yl), 138.027 (pyridin-2-yl), 141.64 (C-8), 147.95, 149,64, 149.67, and 149.98 (C-2, C-4, C-4″, and pyridin-2-yl), 150.49 (pyridin-2-yl), 156.13 (C-6), 169.76 (C═O). HRMS (ESI-MS) $C_{20}H_{23}N_{10}O_4$ [M + H]$^+$: 467.1899 found, 467.1903 calcd. Anal. ($C_{20}H_{22}N_{10}O_4$) C, H, N. N calcd, 30.03; found, 29.55.

9-(5-Ethylcarbamoyl-β-D-ribofuranosyl)-N^6-methyl-2-(4-benzyl-1,2,3-triazol-1-yl)adenine (18). The reaction of compound **31** (70 mg, 0.19 mmol) with 3-phenyl-1-propyne (49μL, 0.56 mmol) gave 35 mg (38%) of compound **18** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, 3H, J = 7.5 Hz, CH_3), 3.03−3.20 (m, 2H, *N*-CH_2), 3.03 (d, 3H, 4.5 Hz, N^6-CH_3), 4.11 (s, 2H, CH−Ph), 4.24 (m, 1H, H-3′), 4.32 (d, 1H, J = 2.1 Hz, H4′), 4.72 (m, 1H, H-2′), 5.59 (d, 1H, J = 6.6 Hz, 3′-OH), 5.69 (d, 1H, J = 4.5 Hz, 2′-OH), 6.03 (d, 1H, J = 6.9 Hz, H1′), 7.22 (m, 1H, Ph), 7.32 (d, 4H, J = 4.2 Hz, Ph), 8.06 (t, 1H, J = 5.7 Hz, NHCO), 8.38 (d, 1H, J = 4.1 Hz, N^6-H), 8.54 (s, 1H, H-8), 8.60 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 15.16 (CH_3), 27.84 (N^6-CH_3), 31.72 (CH_2), 34.07 (N-CH_2), 73.64 (C-2′ and C-3′), 84.98 (C-4′), 87.87 (C-1′), 119.91 (C-5), 122.11 (C-5″), 126.93, 129.13, 129.22, and 140.07 (Ph), 140.48 (C-8), 147.05, 149.87 (C-4, C-2, and C-4″), 156.12 (C-6), 169.71 (C═O). HRMS (ESI-MS) $C_{22}H_{26}N_9O_4$ [M + H]$^+$: 480.2098 found, 480.2107 calcd. Anal. ($C_{22}H_{25}N_9O_4$) C, H, N.

2-(4-Cyclopentylmethyl-1,2,3-triazol-1-yl)-9-(5-ethylcarbamoyl-β-D-ribofuranosyl)-N^6-methyladenine (19). Compound **31** (60 mg, 0.17 mmol), sodium ascorbate (13 mg, 0.066 mmol) and $CuSO_4$·$5H_2O$ (3.5 mg, 0.013 mmol) were suspended in 4 mL of ^{t}BuOH/H_2O (1:1). 3-Cyclopentyl-1-propyne (58 μL, 0.44 mmol) was subsequently added, and the mixture was stirred for 2 days at room temperature. The 2-triazol-1-yl compound precipitated from the reaction medium. Water was added, and the mixture was cooled overnight. The precipitate was filtered off and washed with water and hexane to obtain **19** as a white solid in 33% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.89 (t, 3H, CH_3), 1.19−1.28 (m, 2H, cyclopentyl), 1.45−1.75 (m, 6H, cyclopentyl), 2.15−2.25 (m, 1H, CH, cyclopentyl), 2.72 (d, 2H, J = 7.2 Hz, CH_2), 3.06 (d, 3H, J = 4.5 Hz, N^6-CH_3), 3.09−3.22 (m, 2H, *N*-CH_2), 4.24 (dt, 1H, J = 1.5 and 4.8 Hz, H-3′), 4.33 (d, 1H, J = 2.1 Hz, H-4′), 4.71−7.76 (app q, 1H, J = 6.6 Hz, H-2′), 5.61 (d, 1H, J = 6.3 Hz, 2′-OH), 5.71 (d, 1H, J = 4.8 Hz, 3′-OH), 6.03 (d, 1H, J = 6.9 Hz, H-1′), 8.10 (t, 1H, J = 5.7 Hz, *N*HCO), 8.40 (d, 1H, J = 5.1 Hz, N^6-H), 8.54 (s, 1H, H-8), 8.56 (s, 1H, H-5″). ^{13}C NMR (300 MHz, DMSO-d_6): δ 15.17 (CH_3), 25.36 (cyclopentyl), 27.85 (N^6-CH_3), 31.53 (cyclopentyl), 31.51 (CH_2), 34.07 (CH_2), 73.63 and 73.56 (C-2′ and C-3′), 84.99 (C-4′), 87.93 (C-1′), 119.77 (C-5), 121.69 (C-5″), 141.51 (C-8), 147.48 and 149.92 (C-2, C-4, and C-4″), 156.10 (C-6), 169.74 (C═O). HRMS (ESI-MS) $C_{21}H_{30}N_9O_4$ [M + H]$^+$: 472.2415 found, 472.2420 calcd. Anal. ($C_{21}H_{29}N_9O_4$) C, H, N.

N^6-(5-Chloro-2-methoxybenzyl)-2-(4-cyclopentylmethyl-1,2,3-triazol-1-yl)-9-(β-D-ribofuranosyl)adenine (20). Compound **33** (100 mg, 0.22 mmol), sodium ascorbate (17 mg, 0.086 mmol), and $CuSO_4$·$5H_2O$ (3.5 mg, 0.017 mmol) were suspended in 4 mL of H_2O/^{t}BuOH (1:1). 3-Cyclopentyl-1-propyne (29 μL, 0.44 mmol) was subsequently added, and the mixture was stirred for 2 days at room temperature. The 2-triazol-1-yl compound precipitated from the reaction medium. Water was added, and the mixture was cooled overnight. The precipitate was filtered off and washed with water and hexane to obtain **20** as a white solid in 59% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.23−1.28 (m, 2H, cyclopentyl), 1.48−1.61 (m, 4H, cyclopentyl), 1.66−1.73 (m, 2H, cyclopentyl), 2.15−2.25 (m, 1H, cyclopentyl), 2.72 (d, 2H, J = 7.2 Hz, CH_2-cyclopentyl), 3.56−3.61 (m, 1H, H-5′A), 3.66−3.71 (m, 1H, H-5′B), 3.86 (s, 3H, OCH_3), 3.97 (m, 1H, H-4′), 4.20 (m, 1H, H-3′), 4.64 (m, 1H, H-2′), 4.73 (br s, 2H, N^6-CH_2), 4.96 (t, 1H, J = 6.0 Hz, 5′-OH), 5.22 (d, 1H, J = 4.8 Hz, 3′-OH), 5.48 (d, 1H, J = 5.7 Hz, 2′-OH), 5.95 (d, 1H, J = 6.3 Hz, H-1′), 7.04 (d, 1H, J = 9.0 Hz, Ph), 7.25−7.29 (m, 2H, Ph), 8.40 (s, 1H, H-8), 8.81 (s, 1H, H-5″), 8.81 (br s, 1H, N^6-H). ^{13}C NMR (300 MHz, DMSO-d_6): δ 25.36 (cyclopentyl), 31.54 (CH_2), 32.54 (cyclopentyl), 38.86 (CH_2), 56.57 (OCH_3), 62.13 (C-5′), 71.15 (C-3′), 74.37 (C-2′), 86.46 (C-4′), 88.07 (C-1′), 113.15 (Ph), 119.58 (C-5), 121.37 (C-5″), 124.66, 128.23, and 129.93 (Ph), 141.37 (C-8), 147.40, 149.66, and 150.12 (C-2, C-4, and C-4″), 155.51 (Ph), 156.33 (C-6). HRMS (ESI-MS) $C_{26}H_{32}N_8O_5Cl$ [M + H]$^+$: 571.2184 found, 571.2184 calcd. Anal. ($C_{26}H_{31}N_8O_5Cl$) C, H, N.

2-Azido-N^6-methyl-9-(β-D-ribofuranosyl)adenine (23). Sodium ascorbate (19.4 mg, 0.098 mmol) and $CuSO_4$·$5H_2O$ (12.2 mg, 0.049 mmol) were added to a mixture of **22** (200 mg, 0.491 mmol), sodium azide (38,3 mg, 0.589 mmol), L-proline (11,3 mg, 0.098 mmol), and sodium carbonate (10.4 mg, 0.098 mmol) in 10 mL of H_2O/^{t}BuOH (1:1). The mixture was stirred overnight at 65 °C and was monitored by ^{1}H NMR. Then 50 mL of dilute NH_4OH was added and the crude mixture extracted with ethyl acetate (3 × 60 mL). The organic layer was washed with brine (60 mL), dried over $MgSO_4$, and purified on a silica gel column (CH_2Cl_2/MeOH, 95:5) to afford compound **23** as a slightly yellow solid in 66% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.91 (d, 3H, J = 4.4 Hz, N^6-CH_3), 3.48−3.55 (m, 1H, H-5′A), 3.58−3.66 (m, 1H, H-5′B), 3.90 (dd, 1H, J = 3.8 and 7.3 Hz, H-4′), 4.10 (dd, 1H, J = 4.7 and 9.7 Hz,

H3′), 4.53 (app q, 1H, J = 5.9 Hz, H-2′), 5.04 (dd, 1H, J = 5.2 and 6.2 Hz, 5′-OH), 5.19 (d, 1H, J = 5.0 Hz, 3′OH), 5.43 (d, 1H, J = 6.2 Hz, 2′-OH), 5.78 (d, 1H, J = 6.2 Hz, H-1′), 8.12 (d, J = 4.4 Hz, N^6-H), 8.27 (s, 1H, H-8). Small peaks from 1/6 tetrazole tautomeric form: δ 3.15 (d, 3H, J = 5.0 Hz, N^6-CH_3), 3.95 (d, H-4′), 4.15 (d, H-3′), 5.51 (d, 2′-OH), 5.94 (d, H-1′), 8.51 (s, H-8). ^{13}C NMR (300 MHz, DMSO-d_6): δ 27.54 (N^6-CH_3), 62.21 (C-5′), 71.17 (C-3′), 74.12 (C-2′), 86.35 (C-4′), 88.01 (C-1′), 118.07 (C-5), 139.99 (C-8), 156.06 and 156.20 (C-2 and C-6). Small peaks from 1/6 tetrazole tautomeric form: δ 31.89 (N^6-CH_3), 61.79 (C-5′), 70.77 (C-3′), 74.37 (C-4′), 112.30 (C-12), 142.91 (C-11), 147.60 (C-6). HRMS (ESI-MS) $C_{11}H_{15}N_8O_4$ [M + H]$^+$: 323.1208 found, 323.1216 calcd. Anal. ($C_{11}H_{14}N_8O_4$) C, H, N.

N^6-Methyl-9-(β-D-ribofuranosyl)-2-[2-trimethylsilylethyn-1-yl]adenine (24). Compound **22** (500 mg, 1.23 mmol), CuI (12 mg, 0.062 mmol), and $(Ph_3P)_3PCl_2$ were dissolved in 9 mL of DMF. Triethylamine (205 μL, 1.47 mmol) and trimethylsilylacetylene (210 mg, 1.47 mmol) were added, and the reaction mixture was stirred overnight. After solvent evaporation, the residue was dissolved in CH_2Cl_2 and filtered through a pad of Celite. Purification on a silica gel column (CH_2Cl_2/MeOH, 95:5) yielded 305 mg (66%) of compound **23**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.00 (9H, s, $(CH_3)_3Si$), 2.7 (3H, N^6-CH_3), 3.26−3.34 (m, 1H, H-5′A), 3.37−3.44 (m, 1H, H-5′B), 3.68 (dd, 1H, J = 3.5 Hz, H-4′), 3.86 (dd, 1H, J = 3.4 and 8.2 Hz, H-3′), 4.23 (app q, 1H, J = 5.9 Hz, H-2′), 4.87 (t, 1H, J = 5.0, 5′-OH), 4.94 (d, 1H, J = 4.99 Hz, 3′-OH), 5.21 (d, 1H, J = 6.2 Hz, 2′-OH), 5.63 (d, 1H, J = 6.2 Hz, H-1′), 7.69 (br s, 1H, N^6-H), 8.19 (s, 1H, H-8). HRMS (ESI-MS) $C_{16}H_{24}N_5O_4Si$: [M + H]$^+$: 378.1586 found; 378.1597 calcd.

2-Ethynyl-N^6-methyl-9-(β-D-ribofuranosyl)purine (25). An amount of 300 mg (0.8 mmol) of compound **24** was dissolved in 7 N ammonia in methanol and stirred for 2 h at 0 °C. After solvent evaporation, the residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 95:5) to obtain 160 mg (65%) of derivative **25**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.91 (s, 3H, N^6-CH_3), 3.48−3.56 (m, 1H, H-5′A), 3.61−3.68 (m, 1H, H-5′B), 3.92 (m, 1H, H-4′), 4.02 (s, 1H, C≡CH), 4.11 (dd, 1H, J = 5.0 and 8.2 Hz, H-3′), 4.53 (app q, 1H, J = 5.9 Hz, H-2′), 5.15 (m, 2H, 3′-OH and 5′-OH), 5.44 (d, 1H, J = 6.15 Hz, 2′-OH), 5.84 (d, 1H, J = 6.16 Hz, H-1′), 7.95 (br s, 1H, N^6-H), 8.40 (s, 1H, H-8). HRMS (ESI-MS) $C_{13}H_{16}N_5O_4$ [M + H]$^+$: 306.1197 found; 306.1202 calcd.

1-Deoxy-1-(6-methylamino-2-iodo-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-β-D-ribufuranuronic Acid (27). To a stirred solution of 3.8 g (8.5 mmol) of **26** in 560 mL of H_2O were added 1.4 g of KOH and, dropwise, a solution of 4.03 g (25.5 mmol) of $KMnO_4$ in 110 mL of H_2O. The mixture was stirred in the dark for 20 h, cooled to 0 °C, and quenched with 30 mL of 7% H_2O_2. The mixture was filtered through Celite. The filtrate was concentrated in vacuo and then acidified to pH 4 with 3 N HCl. The resulting precipitate was filtered off and successively washed with water and ether to give 2.98 g (76%) of **27** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.36 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 2.89 (d, 3H, J = 3.3 Hz, N^6-CH_3), 4.68 (d, 1H, J = 1.8 Hz, H-4′), 5.40 (d, 1H, J = 6.0 Hz, H-2′), 5.47 (dd, 1H, J = 6.0 and 1.8 Hz, H-3′), 6.28 (s, 1H, H-1′), 8.08 (d, 1H, J = 3.3 Hz, N^6-H), 8.16 (s, 1H, H-8). HRMS (ESI-MS) $C_{14}H_{16}N_5O_5I$ [M + H]$^+$: 462.0273 found; 462.0276 calcd.

9-(5-Ethylcarbamoyl-β-D-ribofuranosyl)-2-iodo-N^6-methyladenine (30). *p*-Nitrophenol (402 mg, 2.89 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (506 mg, 2.65 mmol) were added to a solution of **27** (1.11 g, 2.41 mmol) in 10 mL of dry DMF. The reaction mixture was stirred for 3 h at room temperature and cooled to 0 °C, and 1.6 mL (24.1 mmol) of ethylamine was added. The solution turned yellow immediately and was further stirred for 1 h at room temperature. After evaporation of the volatiles, the residue was partitioned between ethyl acetate (3 × 100 mL) and H_2O (100 mL). The organic layer was washed with brine (100 mL), dried over $MgSO_4$, and concentrated to dryness. The residue was dissolved in 80% aqueous TFA (20 mL) and stirred for 2 h at room temperature. The mixture was concentrated in vacuo, coevaporated several times with EtOH, and purified by silica gel chromatography (CH_2Cl_2/MeOH, 96:4). Compound **30** was obtained as a white solid in 78% yield (840 mg). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.05 (t, 3H, J = 7.2 Hz, CH_3), 2.91 (d, 3H, J = 4.4 Hz, N^6-CH_3), 3.19−3.29 (m, 2H, N-CH_2), 4.16 (dt, 1H, J = 2.1 and 4.4 Hz, H-3′), 4.31 (d, 1H, J = 2.1 Hz, H-4′), 4.58 (app q, 1H, J = 5.6 Hz, H-2′), 5.59 (d, 1H, J = 6.5 Hz, 2′-OH), 5.71 (d, 1H, J = 4.7 Hz, 3′-OH), 5.92 (d, 1H, J = 7.1 Hz, H-1′), 8.12 (t, 1H, J = 5.5 Hz, *N*HCO), 8.19 (d, 1H, J = 4.4 Hz, N^6-H), 8.38 (s, 1H, H-8). HRMS (ESI-MS) $C_{13}H_{18}N_6O_4I$ [M + H]$^+$: 449.0429 found, 449.0436 calcd.

2-Azido-9-(5-ethylcarbamoyl-β-D-ribofuranosyl)-N^6-methyladenine (31). Sodium ascorbate (69 mg, 0.34 mmol) and $CuSO_4$·$5H_2O$ (5.6 mg, 0.17 mmol) were added to a mixture of **30** (780 mg, 1.74 mmol), sodium azide (226 mg, 3.48 mmol), L-proline (40 mg, 0.35 mmol), and sodium carbonate (37 mg, 0.35 mmol) in 20 mL of H_2O/^{t}BuOH (1:1). The mixture was stirred for 2 days at 65 °C and monitored by ^{1}H NMR. An amount of 100 mL of dilute NH_4OH was added, and the crude mixture was extracted with ethyl acetate (5 × 150 mL) and washed with brine (150 mL). The organic layer was dried over $MgSO_4$ and purified on a silica gel column (CH_2Cl_2/MeOH, 96:4) to afford compound **31** as a white solid in 79% yield (500 mg). ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.06 (t, 3H, J = 7.2 Hz, CH_3), 2.96 (d, 3H, J = 4.0 Hz, N^6-CH_3), 3.16−3.29 (m, 2H, N-CH_2), 4.14 (dt, 1H, J = 1.8 and 4.8 Hz, H-3′), 4.29 (d, 1h, J = 1.8, H-4′), 4.53−4.59 (app q, 1H, J = 6.3 Hz, H-2′), 5.52 (d, 1H, J = 6.3 Hz, 2′-OH), 5.68 (d, 1H, J = 4.8 Hz, 3′-OH), 5.90 (d, 1H, J = 7.2 Hz, H-1′), 8.20 (d, 1H, J = 4.0 Hz, N^6-H), 8.35 (s, 1H, H-8), 8.49 (t, 1H, J = 6.0 Hz, *N*HCO). Small peaks from 1/5 tetrazole tautomeric form: δ 1.08−1.12 (t, 3H, J = 7.2 Hz, CH_3), 4.34 (d, 1H, J = 2.1 Hz, H-4′), 4.67−4.73 (app q, 1H, J = 7.2 Hz, H-2′), 5.54 (d, 1H, J = 4.5 Hz, 2′-OH) 6.00 (d, 1H, J = 7.2 Hz, H-1′). ^{13}C NMR (300 MHz, DMSO-d_6): δ 15.48 (CH_3), 27.52 (N^6-CH_3), 33.94 (N-CH_2), 73.06 and 73.75 (C-2′ and C-3′), 85.15 (C-4′), 88.09 (C-1′), 118.36 (C-5), 140.69 (C-8), 156.032 and 156.143 (C-2 and C-4), 169.79 (C-6). HRMS (ESI-MS) $C_{13}H_{18}N_9O_4$ [M + H]$^+$: 364.1473 found; 364.1481 calcd. Anal. ($C_{13}H_{17}N_9O_4$) C, H, N.

N^6-(5-Chloro-2-methoxybenzyl)-2-iodo-9-(β-D-ribofuranosyl)-adenine (32). Compound **21** (1 g, 1.86 mmol) was dissolved in EtOH (30 mL). 5-Chloro-2-methoxybenzylammonium chloride (580 mg, 2.79 mmol) and Et_3N (392 μL, 2.79 mmol) were added, and the solution was refluxed overnight. The mixture was concentrated to dryness, dissolved in 7 N NH_3 in methanol, and stirred at room temperature for 2 h to deprotect the 2′-hydroxyl group. The volatiles were removed under reduced pressure, and the residue was purified by silica gel column (CH_2Cl_2/MeOH, 97:3). The product, compound **32**, was realized in 80% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.51−3.58 (m, 1H, H-5′A), 3.63−3.68 (m, 1H, H-5′B), 3.85 (s, 3H, OCH_3), 3.94 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.52−4.59 (m, 3H, N^6-CH_2 and H-2′), 5.03 (t, 1H, J = 5.6 Hz, 5′-OH), 5.21 (d, 1H, J = 5.0 Hz, 3′-OH), 5.48 (d, 1H, J = 5.8 Hz, 2′-OH), 5.83 (d, 1H, J = 6.2 Hz, H-1′), 7.03 (d, 1H, J = 8.8 Hz, Ph), 7.16 (d, 1H, J = 2.7 Hz, Ph), 7.29 (dd, 1H, J = 2.7 and 8.8 Hz, Ph), 8.35 (s, 1H, H-8), 8.62 (br s, 1H, N^6-H). HRMS (ESI-MS) $C_{18}H_{20}N_5O_5$-ICl [M + H]$^+$: 548.0204 found; 548.0199 calcd.

2-Azido-N^6-(5-chloro-2-methoxybenzyl)-9-(β-D-ribofuranosyl)adenine (33). Sodium ascorbate (14 mg, 0.073 mmol) and $CuSO_4$·$5H_2O$ (9 mg, 0.037 mmol) were added to a mixture of **32** (200 mg, 0.365 mmol), sodium azide (47 mg, 0.73 mmol), L-proline (8 mg, 0.073 mmol), and sodium carbonate (8 mg, 0.073 mmol) in 4 mL of H_2O/^{t}BuOH (1:1). The mixture was stirred for 2 days at 65 °C and monitored by ^{1}H NMR. An amount of 10 mL of dilute NH_4-OH was added, and the crude mixture was extracted with ethyl acetate (5 × 15 mL) and washed with brine (15 mL). The organic layer was dried over $MgSO_4$ and purified on a silica gel column (CH_2Cl_2/MeOH, 97:3) to afford compound **33** as a white solid in 82% yield. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.51−3.56 (m, 1H, H5′-A), 3.60−3.66 (m, 1H, H5′-B), 3.83 (s, 3H, OCH_3), 3.93 (m, 1H, H 4′), 4.13 (m, 1H, H-3′), 4.56−4.62 (m, 3H, N^6-CH_2 and H-2′), 5.03 (t, 1H, J = 5.4 Hz, 5′-OH), 5.17 (d, 1H, J = 4.8 Hz, 3′-OH), 5.41 (d, 1H, J = 6.3 Hz, 2′-OH), 5.81 (d, 1H, J = 6.0 Hz,

H-1′), 7.02 (d, 1H, $J = 8.7$ Hz, Ph), 7.15 (d, 1H, $J = 3.0$ Hz, Ph), 7.28 (dd, 1H, $J = 2.7$ and 8.7 Hz, Ph), 8.34 (s, 1H, H-8), 8.63 (br s, 1H, N^6-H). ^{13}C NMR (300 MHz, DMSO-d_6): δ 38.59 (CH_2), 56.49 (OCH_3), 62.19 (C-5′), 71.15 (C-3′), 74.13 (C-2′), 86.36 (C-4′), 88.13 (C-1′), 113.02 (Ph), 119.58 (C-5), 124.29 (Ph), 128.10 (Ph), 129.93 (Ph), 140.43 (C-8), 150.69 (C-4), 155.51 (Ph and C-2), 156.33 (C-6). HRMS (ESI-MS) $C_{18}H_{20}N_8O_5Cl$ $[M + H]^+$: 463.1248 found, 463.1245 calcd. Anal. ($C_{18}H_{19}N_8O_5Cl$) C, H, N.

Cell Culture and Membrane Preparation. CHO cells expressing recombinant human ARs or the rat A_3AR were cultured in DMEM (Dulbecco's modified Eagle's medium) and F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 μmol/mL glutamine. After harvest and homogenization, the cells were centrifuged at 500*g* for 10 min. The pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$ and 1 mM EDTA. 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 resulting pellets were resuspended in buffer containing 3 units/mL of adenosine deaminase, and the suspension was stored at −80 °C prior to the binding experiments. The protein concentration was measured using the Bradford assay.[39]

Radioligand Binding Studies. For the A_3AR binding experiments, the procedures were similar to those previously described.[19] Briefly, each tube contained 100 μL of membrane suspension, 50 μL of [^{125}I]I-AB-MECA ([^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide, final concentration of 0.5 nM), and 50 μL of increasing concentrations of compounds in Tris-HCl buffer (50 mM, pH 7.4) containing 10 mM $MgCl_2$ and 1 mM EDTA. Nonspecific binding was determined using 10 μM NECA (adenosine-5′-*N*-ethyluronamide). 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 (Brandel, Gaithersburg, MD). Filters were washed three times with ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter. The binding of [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) to the recombinant hA_1AR and the binding of [^{3}H]CGS21680 (2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine) to the recombinant hA_{2A}AR was performed as previously described.[20,44]

Cyclic AMP Accumulation Assay. Intracellular levels of 3′,5′-cyclic AMP were measured by the competitive protein binding method.[32] CHO cells expressing recombinant human[40] ARs were harvested by trypsinization. After resuspension in the medium, cells were plated in 24-well plates in 0.5 mL of medium/well. After 24 h the medium was removed and cells were washed three times with 1 mL/well of DMEM containing 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, pH 7.4. Cells were then treated with agonists and/or test compounds in the presence of rolipram (10 μM) and adenosine deaminase (3 units/mL) and incubated at 37 °C. For the A_3AR, after 45 min forskolin (10 μM) was added to the medium, and incubation was continued for an additional 15 min. The reaction was terminated upon removal of the medium, and the cells were lysed with 200 μL/well 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 μL of the cell lysate, and 30 μL of 0.1 M HCl. Bound radioactivity was separated by rapid filtration through Whatman GF/C filters under reduced pressure and washed once with cold buffer. Bound radioactivity was subsequently measured by scintillation spectrometry. Calculation of the relative maximal efficacy at the A_3AR was determined at a fixed concentration of the nucleoside analogue (10 μM) and expressed as a relative percent of the effect of 10 μM NECA determined in each experiment, which typically reached ~50% inhibition of the forskolin stimulated cyclase.

Molecular Modeling. All calculations were performed on a Silicon Graphics Octane2 workstation (600 MHz IP30 processor, MIPS R14000). Compound **10**, 2-(4-cyclopentylmethyl-1,2,3-triazole)-N^6-methyladenosine, was constructed with the use of the Sketch Molecule of SYBYL 7.1 (Tripos Inc., 1699 South Hanley Road, St. Louis, MO 63144). A grid search was performed in which flexible bonds were rotated by 0° and 180° for t1 ($C_5-C_6-N^6-C_{Me}$) at the N^6 position, t2 (4′O−4′C−5′C−5′OH) at the 5′-position, and t3 ($N_3-C_2-N_1'-N_2'$) and by 60°, 180°, and −60° for t4 ($N_3'-C_4'-C_{Me}-C_{Cyc}$) and t5 ($C_4'-C_{Me}-C_{Cyc}-C_{Cyc}$) at the C2 position. The low-energy conformers from the grid search were reoptimized, removing all torsional constraints. Merck molecular force field (MMFF)[41] and charges were applied with the use of distance-dependent dielectric constants and the conjugate gradient method until the gradient reached 0.05 kcal·mol^{-1}·Å^{-1}. After the low-energy conformers from the result of the grid search were clustered, the representative ones from all groups were reoptimized by semiempirical molecular orbital calculations with the PM3 method in the MOPAC 6.0 package.[42]

A human A_3AR model (PDB code 1OEA) constructed by homology to the X-ray structure of bovine rhodopsin with 2.8 Å resolution (PDB code 1F88)[38] was used for the docking study. All atom types were assigned by the Amber7_FF99 force field.[43] Amber charges for protein and MMFF charges for ligand were calculated. The starting geometry of the ligand conformation was chosen from the human A_3AR complex model with Cl-IB-MECA,[19] which was already validated by point mutation. The ribose binding position was fixed, using an atom-by-atom fitting method for the carbon atoms of the ribose ring. To determine the binding region of the 2-(4-cyclopentylmethyl-1,2,3-triazole) moiety at the adenine 2 position, the flexible bond defining a χ_1 ($O-C_{1'}-N_9-C_4$) angle was searched while docked within the putative binding cavity through various low-energy conformers with diverse t1−t5 angles, rotating by −60°, −110°, and −160°, assuming an anti conformation. Several conformations without any steric bump were selected for further optimization. The initial structures of all complexes were optimized using the Amber force field with a fixed dielectric constant of 4.0 and a terminating gradient of 0.05 kcal·mol^{-1}·Å^{-1}.

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Supporting Information Available: Elemental analysis data for compounds **1**−**20**, **23**, **31**, and **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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