Synthesis and Biological Activity of a New Series of N^6-Arylcarbamoyl, 2-(Ar)alkynyl-N^6-arylcarbamoyl, and N^6-Carboxamido Derivatives of Adenosine-5′-*N*-ethyluronamide as A_1 and A_3 Adenosine Receptor Agonists

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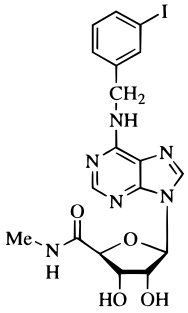
A new series of 1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide-bearing *N*-arylureas or *N*-arylcarboxamido groups at the purine 6 position and *N*-arylureas combined with halogens or alkynyl chains at the 2 position have been synthesized and tested for affinity at A_1 and A_{2A} adenosine receptors in rat brain membranes and at cloned rat A_3 receptors expressed in CHO cells. The derivatives contained the 5′ substituent found in the potent, nonselective agonist 1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide (NECA). While the carboxamido derivatives (**9**−**13**) showed affinity for A_1 receptors, the urea derivatives (**30**−**45**) showed different degrees of affinity and selectivity for the A_3 adenosine receptor subtype. In particular the derivative bearing a *p*-sulfonamidophenyl-urea at the 6 position, **31** showed a high affinity (K_i = 9 nM) and selectivity for the A_3 receptors compared to that of the reference compound 1-[6-[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide (IB-MECA). Furthermore, the importance of the stereochemistry in the interaction of these ligands at the rat A_3 adenosine receptors has been evaluated by introducing a chiral chain at the 6 position. The introduction of halogens or alkynyl chains at the purine 2 position of selected ureas did not give the expected enhancement of potency at A_{2A} and/or A_3 receptors but rather showed a dramatic reduction of A_{2A} affinity, resulting in compounds with good A_{2A}/A_3 selectivity. For example, the 2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-6-(4-methoxyphenylurea) derivative **61** showed the capability to bind simultaneously to A_1 and A_3 receptor subtypes, excluding the A_{2A} receptor. Compound **31** was shown to be an agonist, 9-fold more potent than NECA, at A_3 receptors in rat RBL-2H3 mast cell membranes through stimulation of binding of [^{35}S]GTP-γ-S.

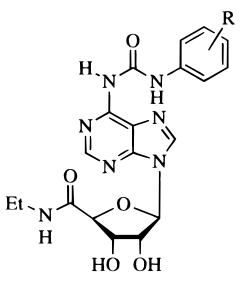
Introduction

Four subtypes of adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) have been cloned[1−4] and characterized pharmacologically with the use of potent agonists and antagonists. The A_1 and A_2 receptor subtypes are linked to inhibition and stimulation, respectively, of adenylyl cyclase.[5−7] A_3 receptors are coupled both to the stimulation of phospholipase C, as shown in rat RBL-2H3 mast cells[8] and in rat brain slices,[9] and to the inhibition of adenylyl cyclase.[4]

Many selective agents have been developed for the A_1[10−15] and A_{2A}[16−19] receptor subtypes. Some of these seem promising as potential therapeutic agents in the treatment of Parkinson's disease,[20] cognitive deficits,[21] schizophrenia,[22] epilepsy,[23a] and renal failure.[23b] Selective and/or high affinity agonists and antagonists for the A_{2B} receptor have not yet been reported. Since the cloning of the A_3 receptor from a rat brain cDNA library,[4] recent efforts have been made in order to develop selective agonist ligands[24] as well as some selective antagonists.[25−28] The selective agonists, 1-[6-[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide (IB-MECA) **1**, shows a K_i value of 1.1 ± 0.3 nM at rat A_3 receptors and a 50-fold selectivity vs either A_1 or A_{2A} receptors.[29] The related agonist, [^{125}I]1-[6-[[(4-amino-3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuron-



1, IB-MECA



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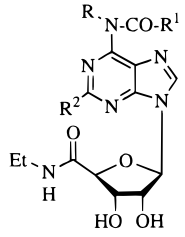
amide ([^{125}I]AB-MECA),[30] has become a useful radioligand for the screening of new derivatives interacting with cloned A_3 receptors.

The A_3 receptor mediates many processes such as inflammation,[31] hypotension,[32] and mast cell degranulation.[33] Also, the A_3 receptor apparently has a role in the central nervous system. IB-MECA **1** induces behavioral depression[34] and, upon chronic administration, protects against cerebral ischemia.[35] Furthermore, at high concentrations A_3 selective agonists were also found to induce apoptosis in HL-60 human leukemia cells.[36]

From the point of view of structure−activity relationships, the ribose moiety of adenosine has to be maintained unchanged, with the exception of the 5′ position, at which alkyluronamide groups are well tolerated.[37] Furthermore, some substitutions at the purine 6 and/or 2 positions modulates affinity and selectivity of adenosine agonists for the different receptor subtypes.[24] Alkyl or aryl derivatives at the 6 position of the purine nucleus are A_1 selective, and many derivatives at the 2 position bearing *C*, *N*, or *O* substituents are A_{2A} selective. 6-Benzyl analogues also containing the 5′-uronamide modification, such as **1**, have been found to be potent and selective A_3 agonists.[38] We have recently reported a series of adenosine 5′-uronamide derivatives, substituted at the purine 6 position, of general formula **2**, which have shown high affinity at adenosine A_3 receptors. In particular, NECA derivatives containing at the 6-positon the 3-chloro-, 2-chloro-, or 4-methoxyphenylcarbamoyl moieties have shown affinity for A_3 adenosine receptors with 4−7 nM K_i values. However, while the selectivity with respect to A_{2A} receptors was high (A_{2A}/A_3 from 96 to 510), the discrimination between A_1 and A_3 receptors was very low (A_1/A_3 from 4- to 10-fold).[39]

A new series of carboxamido and urea derivatives at the 6 position of NECA (**9**−**13**, **30**−**45**, and **52**−**62**) have

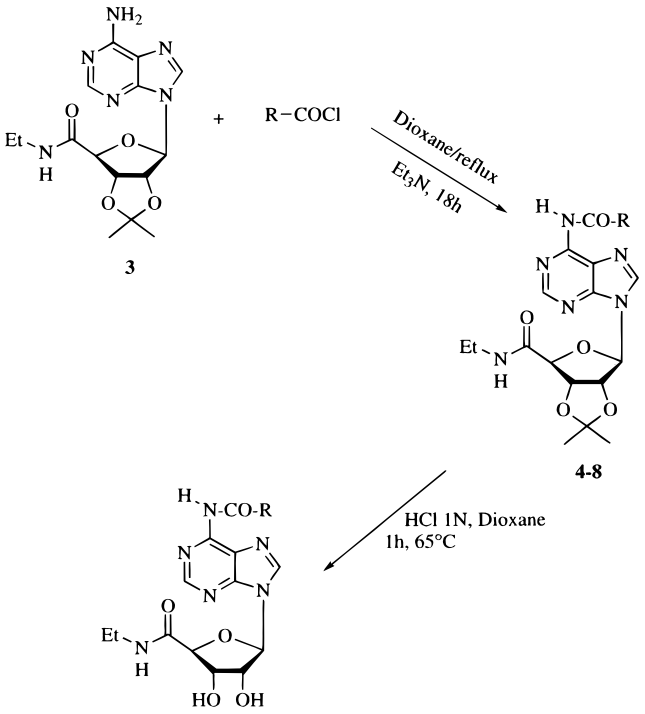


9-13, 30-45, 52-62

R = H, aryl-NH-CO, heteroaryl-NH-CO
R^1 = aryl, aralkyl, aryl-NH, heteroaryl-NH
R^2 = H, halogen, (Ar)alkynyl

been prepared to further explore the structural basis for the selectivity for A_3 vs A_1 receptors. In particular, we were interested in evaluating the influence of the length of the acyl chain at the adenine *N* position on the receptor affinity. We prepared some amide derivatives of NECA (**9**−**13**), which are structurally related to our previously reported series of urea derivatives.[39] Also, some new urea derivatives at purine 6 position of NECA (**30**−**45**) were synthesized to evaluate the physicochemical parameters involved in the interaction with A_3 adenosine receptors. Furthermore, we introduced a chiral center in compounds **33** and **34** to evaluate the stereoselectivity of binding of the substituent at 6 position.

Scheme 1



3 **4-8** **9-13**

R = aryl, aralkyl

On the basis of the observation that 2-(1-hexyn-1-yl)-1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-*β*-D-ribofuranuronamide (HENECA), a potent A_{2A} adenosine agonist,[16,40] also showed high affinity at rat A_3 receptors,[41] we combined ureido moieties at the purine 6 position, that were favorable in A_3 receptor binding, with the substitution at the 2 position. Using results from a previous work,[39] we introduced the A_3 receptor affinity enhancing 4-methoxyphenyl-and 3-chlorophenyl-urea groups at the purine 6 position, while substituting the 2 position with halogens (Cl, I) (**52**−**55**) and alkynyl groups (**56**−**62**).

Results and Discussion

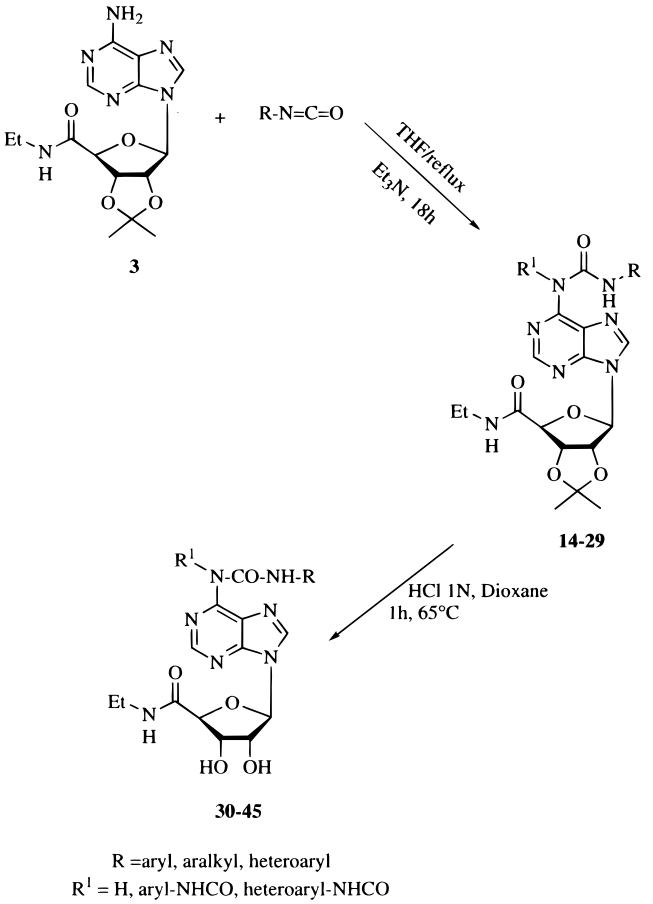
Chemistry. The preparation of *N*-acyl derivatives of NECA, compounds **9**−**13**, was performed following the general synthetic strategy depicted in Scheme 1.

The amino group at the 6 position of NECA is not very reactive; therefore, it was necessary to protect the hydroxyl groups of the ribose moiety during selective acylation. Reaction of 2′,3′-*O*-isopropylidene-protected NECA (**3**) with the appropriate acyl chloride in the presence of a catalytic amount of triethylamine at reflux afforded the adducts **4**−**8** in a good yield, which were in turn converted into the desired compounds (**9**−**13**) by deprotection in aqueous 1 N HCl and dioxane at 65 °C. In a similar manner, derivatives **30**−**45** were prepared by nucleophilic reaction of the appropriate isocyanate with **3** and then by deprotection of the ribosyl moiety (Scheme 2).

When not commercially available, the isocyanate was prepared by reacting the corresponding substituted anilines using trichloromethylchloroformate, as described previously.[42]

Using 4-nitrophenyl isocyanate or 5-chloropyridin-2-yl isocyanate under the conditions mentioned above, the

Scheme 2



bis adducts **44** and **45** were the only products isolated from the reaction mixture.

The 2-chloro- and the 2-iodo-6-arylcarbamoyl derivatives of NECA **52**−**55** were synthesized from 2′,3′-*O*-isopropylidene-2-chloro-NECA (**46**)[43] and 2′,3′-*O*-isopropylidene-2-iodo-NECA (**47**),[44] respectively (Scheme 3).

The reaction of **46** or **47** in dry THF with the appropriate isocyanate to afford the protected nucleosides **48**−**51** was carried out in a steel bomb at 110 °C for 24 h. The nucleosides were deprotected in 1 N HCl and dioxane at 65 °C to give the corresponding compounds **52**−**55**. The synthesis of the (ar)alkynyl derivatives **56**−**62** was carried out by a modification of the palladium-catalyzed cross-coupling reaction.[40]

The 2-iodo derivatives **54** and **55** in dry acetonitrile, DMF, and triethylamine reacted with cuprous iodide, bis(triphenylphosphine)palladium dichloride, and the appropriate terminal alkyne, at room temperature for several hours under an atmosphere of N_2, to produce the alkynyl derivatives **56**−**58** and **59**−**62**, respectively (Scheme 4).

Biological Activity. All the synthesized compounds (**9**−**13**, **30**−**45**, and **52**−**62**) were tested in radioligand binding assays for affinity at the rat brain A_1, A_{2A}, and A_3 receptors, and the results are summarized in Table 1.[45]

In our previous studies, we demonstrated that the 6-substituted adenosine uronamides are full agonists in inhibiting adenylate cyclase via rat A_3 receptors.[39]

From the binding assays, it appeared that the presence of an amide vs urea functionality at the 6 position (**9**−**13**) was generally detrimental in terms of affinity at rat A_3 receptors, whereas several of these derivatives (**11** and **13**) showed roughly 1 order of magnitude increase in affinity for adenosine A_{2A} receptor. Affinity at A_1 receptors was highly variable, ranging from 5.9 nM (biphenyl derivative, **9**) to 1.2 μM. For example, the *o*-chlorobenzoyl derivative (**12**) was 100-fold less potent at A_3 receptors and was 10-fold less potent at A_1 receptors than the corresponding urea derivative.[39] The *p*-methoxybenzoyl derivative (**11**) was 127-fold less potent at A_3 receptors than the corresponding *p*-methoxyphenyl urea derivative.[39] These results suggested that a small modification in the chain at the 6 position could modulate the affinity for A_1 or A_3 receptors. In the series of urea-derivatives (**30**−**45**), a substituted-phenyl (**31**, **32**, and **37**) or substituted-benzyl (**30**, **33**, and **34**) group led to higher affinity and selectivity at A_3 adenosine receptors. In fact, derivatives **31**, **32**, and **37** showed a high affinity (9.7−107 nM) at A_3 receptors with varying degrees of A_1/A_3 selectivity. In particular, compound **31** was less active than IB-MECA (9.7 nM vs 1.1 nM) but showed selectivity for A_3 vs either A_1 or A_{2A} receptors comparable to the reference compound. In addition, it seemed necessary to have a free amino group on the sulfonamido moiety, since its substitution with some heteroaryl groups (e.g., compounds **41**−**43**) led to derivatives with modest affinity at rat A_3 adenosine receptors and low selectivity with respect to A_1 and A_{2A} receptors.

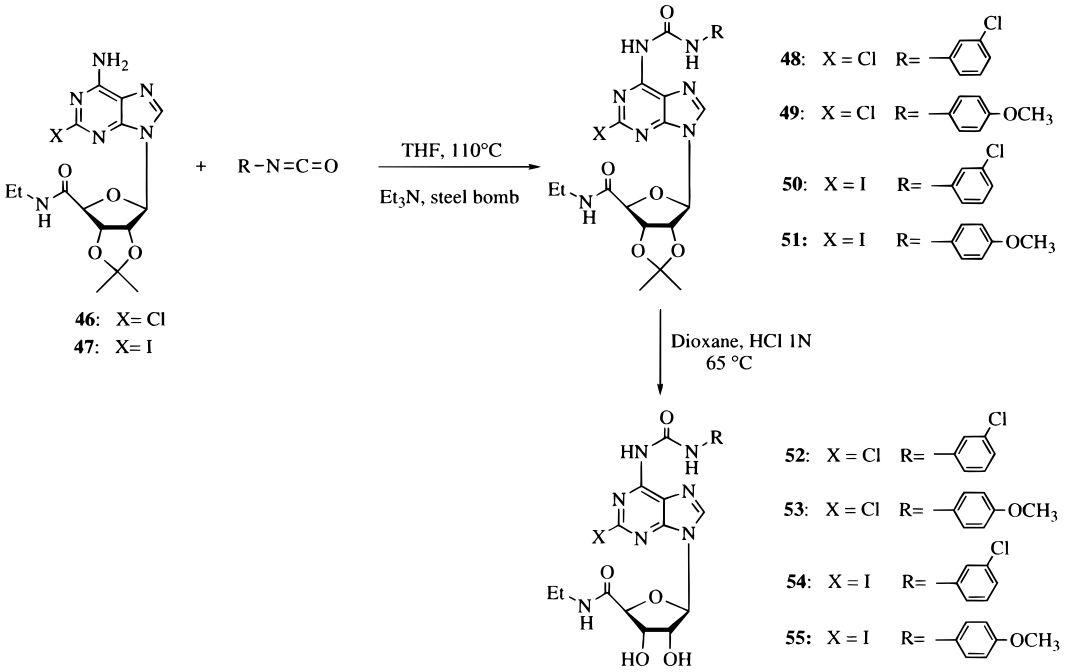
The data demonstrate the importance of a phenylurea moiety at the purine 6 position for an interaction with the A_3 adenosine receptor subtype.[46] When the phenyl ring was replaced with various heterocycles (**35**, **36**, and **40**) both affinity and selectivity for A_3 receptors decreased.

Moreover, the disubstitution at the 6 position led to derivatives (**44**−**45**) with diminished activity, in particular at A_{2A} and A_3 receptors. This could be explained by the absence of one hydrogen atom at N^6, which could act as a hydrogen bond donor, already noted as a requirement for binding at the A_1 and A_{2A} receptor subtypes.[47]

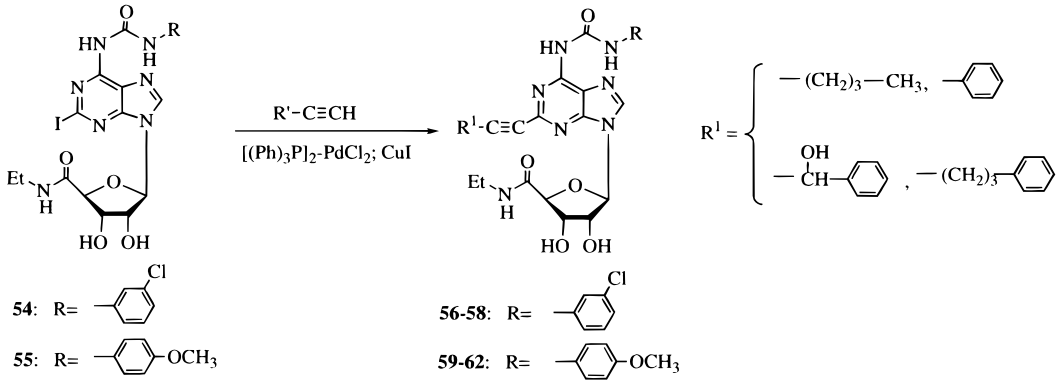
The benzylic series (**30**, **33**, and **34**) showed a SAR pattern comparable to the phenylic series. In fact, the unsubstituted compound **30** showed affinity and selectivity at A_3 receptors very similar to those of the previously reported phenyl derivative.[39] Interestingly, comparing the two diastereomers **33** and **34** in which substituents at the purine 6 position are of opposite configuration, the *R*-isomer (**33**) was more potent than the *S*-isomer (**34**) at the rat A_{2A} and A_3 adenosine receptor subtypes. The binding stereoselectivity of the N^6 substituent confirms the importance of the stereochemistry at this subregion of the adenosine receptor binding site, similar to that already observed for the A_1 agonists *R*-PIA and *S*-PIA.[48] A similar observation has been made for the C^2 subregion of the A_{2A} subtype, both with geometric isomers of 2-alkenyl-NECA[49] and with diastereomers of 2-alkynyl derivatives of NECA [*R*- and *S*-PHPNECA].[50]

The data also demonstrate the importance of chain length at the 6 position and suggest that phenyl or benzyl chains are the preferred substituents for the interaction with the A_3 adenosine receptor subtype.

Scheme 3



Scheme 4



Longer chains, with respect to the phenylic or benzylic moieties, seemed not to improve the affinity at rat A_3 receptors. Thus, compounds, **38** and **39**, bearing 2-phenylethyl and 3,4-dimethoxy-2-phenylethyl groups at the purine 6 position, respectively, showed submicromolar affinity but low selectivity at rat A_3 receptors.

These results and our previous work[39] seem to indicate that the lipophilicity of the para substituents of a phenyl ring plays a significant role in A_3 affinity. A quantitative correlation was not found, but compounds **31** (π $SO_2NH_2 = -1.82$), **32** (π $COCH_3 = -0.55$), and the previously reported 6-phenyl urea derivative substituted with a methoxy group at the para position (π $OCH_3 = -0.02$), bearing the least lipophilic substituents, show some of the most favorable K_i values within the series.[51]

Species differences, which are pronounced for antagonists but not for agonists, in binding of ligands to A_3 receptors have been noted. The most interesting derivative in the present study (**31**) showed affinity at human A_3 adenosine receptors (56.1 ± 9.1 nM) comparable to that at rat A_3 receptors, but there was 6-fold more potency at rat receptors. This finding confirms, as already observed, significant differences between rat and human A_3 adenosine receptor subtypes.[28]

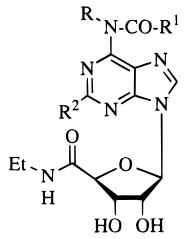
A functional assay indicated that compound **31** acted as a full agonist at rat A_3 receptors. The assay consisted of agonist-induced stimulation of binding of a guanine nucleotide to membranes of rat RBL-2H3 mast cells, which contain a high density of A_3 receptors.[30] Figure 1 shows that the potent A_3 agonists 1-[6-[[(4-amino-3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide (I-AB-MECA) and 2-chloro-1-[6-[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide (Cl-IB-MECA) increased binding of [^{35}S]GTP-γ-S in a dose-dependent manner and with 9-fold greater potency than NECA, as expected for A_3 receptors.

A correlation between binding affinity and potency in this functional assay has been demonstrated.[52]

Compound **31** was as efficacious as these two potent agonists but approximately 1 order of magnitude less potent, with an EC_{50} value of 1.15 μM.

The introduction of halogens at the 2 position of selected urea derivatives at the purine 6 position led to compounds **52**−**55**, which show an A_3 affinity ranging from 17 nM (**53**) to 315 nM (**54**). For example, the presence of a chlorine atom in the 2 position reduced the affinity and selectivity with respect to the corresponding 2-unsubstituted urea derivatives[39] (**52**, K_i at

Table 1. Affinities of Synthesized Derivatives (**9**−**13**, **30**−**45**, and **52**−**62**) in Radioligand Binding Assays at Rat Brain A_1, A_{2A}, and A_3 Adenosine Receptors



9-13, 30-45, 52-62

compd	R	R^1	R^2	K_1 (nM) or % inhibition $K_i(A_1)^a$	$K_i(A_{2A})^b$	$K_i(A_3)^c$	A_1/A_3	A_{2A}/A_3
IB-MECA, **1**				54 ± 5	56 ± 8	1.1 ± 0.3	49	51
Cl-IB-MECA				820 ± 570	470 ± 365	0.33 ± 0.08	2500	1400
NECA[d]				6.3	10.3	113	0.071	0.091
9	H	4-biphenyl	H	5.92 ± 1.04	3580 ± 850	979 ± 22	0.0060	3.7
10	H	2,4-Cl-Ph-CH_2	H	16.0 ± 3.3	26.6 ± 8.8	167 ± 7	0.095	0.16
11	H	4-CH_3O-Ph	H	86.4 ± 14.2	237 ± 46	837 ± 142	0.10	0.28
12	H	2-Cl-Ph	H	1170 ± 220	1960 ± 300	754 ± 132	1.6	2.6
13	H	Ph	H	252 ± 52	670 ± 211	824 ± 126	0.30	0.81
30	H	$PhCH_2NH$	H	171 ± 15	1793 ± 230	38.3 ± 6.4	4.5	47
31	H	4-SO_2NH_2-PhNH	H	453 ± 141	1180 ± 360	9.73 ± 0.75	47	120
32	H	4-CH_3CO-PhNH	H	72.7 ± 11.8	1050 ± 270	20.9 ± 7.51	3.5	50
33	H	*(R)*-α-phenylethyl-NH	H	433 ± 171	279 ± 171	16.3 ± 3.7	27	17
34	H	*(S)*-α-phenylethyl-NH	H	537 ± 33	2970 ± 930	319 ± 134	1.7	9.3
35	H	5-Me-isoxazol-3-yl-NH	H	146 ± 39	884 ± 232	532 ± 240	0.27	1.7
36	H	1,3,4-thiadiazol-2-yl-NH	H	208 ± 42	917 ± 254	5550 ± 2800	0.037	0.16
37	H	4-*n*-C_3H_7O-PhNH	H	247 ± 43	255 ± 85	107 ± 34	2.3	2.4
38	H	Ph-CH_2CH_2NH	H	129 ± 44	2650 ± 640	149 ± 26	0.86	18
39	H	3,4-MeO-Ph-CH_2CH_2NH	H	1770 ± 490	1570 ± 220	411 ± 178	4.3	3.8
40	H	fur-2-yl-CH_2NH	H	419 ± 22	1860 ± 370	713 ± 91	0.58	2.6
41	H	4-(pyridin-2-yl-$NHSO_2$)PhNH	H	292 ± 76	740 ± 283	54.1 ± 10.8	5.4	14
42	H	4-(5-Me-isoxazol-3-yl-$NHSO_2$)PhNH	H	901 ± 62	977 ± 425	155 ± 5	5.8	6.3
43	H	4-(pyrimidin-2-yl-$NHSO_2$)PhNH	H	725 ± 141	16.8 ± 3.1	405 ± 12	1.7	0.041
44	4-NO_2-Ph-NH-CO	4-NO_2-Ph-NH	H	89.1 ± 8.8	2530 ± 200	168 ± 42	0.53	15
45	5-Cl-pyridin-2-yl-NH-CO	5-Cl-pyridin-2-yl-NH	H	761 ± 187	799 ± 191	5700 ± 100	0.13	0.14
52	H	3-Cl-Ph-NH	Cl	90.0 ± 23.7	557 ± 112	77.6 ± 25	1.2	4.3
53	H	4-MeO-Ph-NH	Cl	22.0 ± 5.5	59.0 ± 5.3	17.1 ± 8.6	1.2	3.5
54	H	3-Cl-Ph-NH	I	103 ± 23	10 600 ± 3700	315 ± 63	0.33	34
55	H	4-MeO-Ph-NH	I	29.4 ± 12.1	260	251 ± 95	0.12	1.0
56	H	3-Cl-Ph-NH	$nC_4H_9-C\equiv C$	2040 ± 540	7130 ± 2570	581 ± 82	3.5	12
57	H	3-Cl-Ph-NH	$Ph-C\equiv C$	1330 ± 490	48700 ± 1400	611 ± 100	2.1	80
58	H	3-Cl-Ph-NH	$PhCH(OH)-C\equiv C$	814 ± 113	12 ± 3% (10^{-4})	696 ± 149	1.2	>140
59	H	4-MeO-Ph-NH	$nC_4H_9-C\equiv C$	428 ± 124	2330 ± 480	211 ± 104	2.0	11
60	H	4-MeO-Ph-NH	$Ph-C\equiv C$	4790 ± 760	29 ± 3% (10^{-4})	154 ± 46	31	>4000
61	H	4-MeO-Ph-NH	$PhCH(OH)-C\equiv C$	75.1 ± 20.6	12 ± 6% (10^{-4})	324 ± 65	0.23	>300
62	H	4-MeO-Ph-NH	$Ph(CH_2)_3-C\equiv C$	14400 ± 4000	1670 ± 420	89.5 ± 19	160	19

[a] Displacement of specific [^{3}H]R−PIA binding (A_1) in rat brain membranes, expressed as K_i ± SEM in nM (n = 3−6). [b] Displacement of specific [^{3}H]CGS 21680 binding (A_{2A}) in rat striatal membranes expressed as K_i ± SEM in nM (n = 3−6). [c] Displacement of specific [^{125}I]AB-MECA binding at rat A_3 receptors expressed in CHO cells, expressed as K_i ± SEM in nM (n = 3−6). [d] Values at A_1 and A_{2A} receptors are taken from Bruns et al.[45] K_i values at A_1 receptors are vs specific binding of [^{3}H]-N^6-cyclohexyladenosine ([^{3}H]-CHA). K_i values at A_{2A} receptors are vs specific binding of [^{3}H]-NECA in the presence of 50 nM CPA in rat striatal membranes.

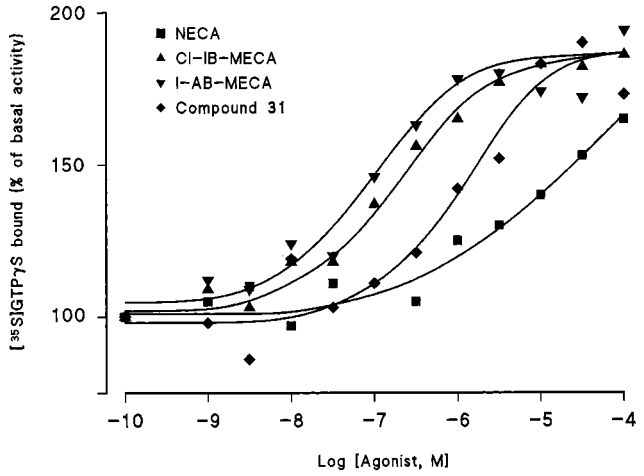


Figure 1. Effect of the adenosine agonists NECA, compound **31**, Cl-IB-MECA, and I-AB-MECA on the binding of [^{35}S]GTP-γ-S to membranes of rat RBL-2H3 mast cells. EC_{50} values were NECA (10.6 μM), compound **31** (1.15 μM), Cl-IB-MECA (174 nM), and I-AB-MECA (86.8 nM).

A_3 = 77.6 nM vs 4.4 nM; **53**, K_i at A_3 = 17 nM vs 6.6 nM), unlike data for various other 6-substituted A_1[53] and A_3[29] receptor agonists. Similarly, the presence of an iodine atom further decreased affinity and selectivity (**54**, K_i at A_3 = 315 nM; **55**, K_i at A_3 = 251 nM).

The introduction of an alkynyl chains at the 2 position of these 5′-carboxamide adenosine derivatives was expected to enhance the potency at A_3 receptors, consistent with the high affinity demonstrated by HENECA (K_i at A_3 = 25.6 nM).[41] Surprisingly, the 2-alkynyl-substituted N^6-urea 5′-carboxamide derivatives (**56**−**62**), in fact, showed a dramatic reduction of A_{2A} affinity, indicating the interdependence of substitution at the purine 2 and 6 positions. The triple substitution resulted in compounds with extremely high A_3 vs A_{2A} receptor selectivity, **60** > **61** > **58** > **57** (in order of selectivity). Thus, the most potent compounds in this group, showing high selectivity versus the A_{2A} subtype and which also showed a comparable affinity for A_1 and A_3 receptors, the 2-(3-hydroxy-3-phenyl-1-propyn-1-yl) derivative **61**, could be an interesting candidate for the simultaneous stimulation of both A_1 and A_3 subtypes. This could be interesting since an interaction between these two subtypes has been demonstrated in the rat hippocampus, in the presynaptic control of release of glutamic acid.[54] Since both the A_1 and A_3 receptors are coupled to the inhibition of adenylate cyclase, it may prove useful to have such pharmacological probes of mixed A_1 and A_3 selectivity such that they would exert a dual, simultaneous action on the same second messenger system. Conversely, compound **62** showed very high selectivity for the A_3 versus the A_1 subtype.

Conclusions

The present study provides useful information concerning the structural requirements necessary for recognition by the A_3 adenosine receptor. It appears that a phenyl- or benzylcarbamoyl moiety at the purine 6 position seems favorable for A_3 adenosine receptor affinity and selectivity, while an arylcarboxamido group enhances A_1 affinity and selectivity. In particular, in the urea series, compound **31** was shown to be less potent but with approximately the same degree of A_3 selectivity vs that of either A_1 or A_{2A} with respect to the reference compound IB-MECA.

Furthermore, a simultaneous substitution at the 6 and 2 positions does not improve affinity and selectivity for A_3. However, the 2-(3-hydroxy-3-phenyl-1-propyn-1-yl) derivative **61** showed the capability to bind simultaneously to A_1 and A_3 receptor subtypes, excluding the A_{2A} receptor.

Experimental Section

Chemistry. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates), and products were visualized with iodine or aqueous potassium permanganate. Infrared spectra (IR) were measured on a Perkin-Elmer 257 instrument. ^{1}H NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solutions with a Bruker AC 200 spectrometer, peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and J values are given in Hz. Light petroleum ether refers to the fractions boiling at 40−60 °C. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatography was performed with Merck 60−200 mesh silica gel. All products reported showed IR and ^{1}H NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous magnesium sulfate. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara, and were within ±0.4% of the theoretical values for C, H, and N.

General Procedure for the Preparation of 1-Deoxy-1-[6-[[(substituted)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (4−8). 2′,3′-*O*-Isopropylidene-NECA **3** (0.43 mmol) was dissolved in freshly distilled dioxane (4 mL), and the appropriate acyl chloride (1.3 equiv) and a catalytic amount of triethylamine (2−3 drops) were added. The mixture was refluxed under argon for 15 h. The solvent was then removed under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2−EtOAc 20%) to afford the desired compound **4**−**8**.

1-Deoxy-1-[6-[[(4-biphenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (4): yield 80%; pale yellow foam; IR (neat) cm^{-1} 3445, 1720, 1640, 1575; ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, J = 7), 1.39 (s, 3H), 1.62 (s, 3H), 3.02−3.11 (m, 2H), 4.71 (d, 1H, J = 2), 5.39−5.46 (m, 2H), 6.19 (d, 1H, J = 2), 6.66 (t, 1H, J = 2), 7.40−7.74 (m, 7H), 8.09−8.16 (m, 3H), 8.74 (s, 1H), 9.44 (bs, 1H). Anal. ($C_{28}H_{28}N_6O_5$) C, H, N.

1-Deoxy-1-[6-[[(2,4-dichlorobenzyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (5): yield 67%; white foam; IR (neat) cm^{-1} 3440, 1730, 1625, 1565, 1210; ^{1}H NMR ($CDCl_3$) δ 0.82 (t, 3H, J = 7), 1.17 (s, 3H), 1.26 (s, 3H), 2.99−3.13 (m, 2H), 4.32 (s, 2H), 4.72 (d, 1H, J = 1.8), 5.38−5.48 (m, 2H), 6.16 (d, 1H, J = 1.8), 6.56 (t, 1H, J = 2), 7.20−7.32 (m, 3H), 7.41 (s, 1H), 8.20 (s, 1H), 8.67 (s, 1H). Anal. ($C_{23}H_{24}Cl_2N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[(4-methoxyphenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (6): yield 85%; white solid, mp 105−107 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3445, 1715, 1640, 1555, 1210; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J = 7), 1.40 (s, 3H), 1.63 (s, 3H), 3.03−3.12 (m, 2H), 3.90 (s, 3H), 4.72 (d, 1H, J = 2), 5.41−5.49 (m, 2H), 6.16 (d, 1H, J = 2), 6.61 (t, 1H, J = 2), 7.01 (d, 2H, J = 9), 8.02 (s, 2H, J = 9), 8.10 (s, 1H), 8.74 (s, 1H), 9.09 (bs, 1H). Anal. ($C_{23}H_{26}N_6O_6$) C, H, N.

1-Deoxy-1-[6-[[(2-chlorophenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (7): yield 72%; white foam; IR (neat) cm^{-1} 3435, 1720, 1620, 1550, 1230; ^{1}H NMR ($CDCl_3$) δ 0.83 (t, 3H, J = 7), 1.39 (s, 3H), 1.62 (s, 3H), 3.00−3.07 (m, 2H), 4.71 (d, 1H, J = 2), 5.41−5.44 (m, 2H), 6.18 (d, 1H, J = 2), 6.67 (t, 1H, J = 2), 7.30−7.44 (m, 3H), 7.71−7.76 (m, 1H), 8.16 (s, 1H), 8.69 (s, 1H), 10.24 (bs, 1H). Anal. ($C_{22}H_{23}$ Cl N_7O_5) C, H, N.

1-Deoxy-1-[6-[[(phenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (8): yield 90%; pale yellow oil; IR (neat) cm^{-1} 3425, 1730, 1640, 1555, 1240; ^1H N:MR ($CDCl_3$) δ 0.79 (t, 3H, J = 7), 1.39 (s, 3H), 1.61 (s, 3H), 2.91−3.03 (m, 2H), 4.71 (d, 1H, J = 2), 5.45−5.52 (m, 2H), 6.18 (d, 1H, J = 2), 6.40 (t, 1H, J = 2), 7.27−7.53 (m, 3H), 7.83−7.87 (m, 2H), 8.19 (s, 1H), 8.63 (s, 1H), 9.18 (bs, 1H). Anal. ($C_{22}H_{24}N_6O_5$) C, H, N.

General Procedure for the Preparation of 1-Deoxy-1-[6-[[[(substituted)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (14−29). 2′,3′-*O*-Isopropylidene-NECA **3** (0.43 mmol) was dissolved in freshly distilled THF (4 mL), and then the appropriate isocyanate (1.3 equiv) and a catalytic amount of triethylamine (two drops) were added. The mixture was refluxed under argon for 18 h. Next the solvent was removed under reduced pressure, and the residue was purified by flash chromatography (CH_2Cl_2−EtOAc 20%) to afford the desired compound **14**−**29**.

1-Deoxy-1-[6-[[[(benzyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (14): yield 82%; white solid, mp 109−111 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3440, 1740, 1610, 1550, 1210; ^1H NMR ($CDCl_3$) δ 0.76 (t, 3H, J = 7), 1.21 (s, 3H), 1.63 (s, 3H), 2.96−3.03 (m, 2H), 4.64 (d, 2H, J = 6), 4.71 (d, 1H, J = 1.8), 5.44−5.48 (m, 2H), 6.16 (d, 1H, J = 2), 6.52 (t, 1H, J = 2), 7.26−7.38 (m, 5H), 8.14 (s, 1H), 8.45 (s, 1H), 8.46 (s, 1H), 9.79 (bs, 1H). Anal. ($C_{26}H_{31}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(4-sulfonamidophenyl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (15): yield 60%; white solid, mp 207 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3450−3250, 1730, 1610, 1565, 1360, 1230; ^1H NMR ($CDCl_3$) δ 0.57 (t, 3H, J = 7), 1.35 (s, 3H), 1.54 (s, 3H), 2.96−3.03 (m, 2H), 4.60 (d, 1H, J = 2), 5.47−5.50 (m, 2H), 6.47 (d, 1H, J = 2), 6.60 (t, 1H, J = 2), 7.26 (d, 2H, J = 9), 7.74 (d, 2H, J = 9), 8.60 (s, 1H), 8.64 (s, 1H), 9.22 (s, 1H), 10.47 (bs, 1H), 12.05 (s, 1H). Anal. ($C_{22}H_{26}N_8O_7S$) C, H, N.

1-Deoxy-1-[6-[[[(4-acetylphenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (16): yield 77%; white solid, mp 204 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3430, 1740, 1730, 1630, 1545, 1240; ^1H NMR ($CDCl_3$) δ 0.83 (t, 3H, J = 7), 1.42 (s, 3H), 1.64 (s, 3H), 2.61 (s, 3H), 2.99−3.08 (m, 2H), 4.74 (d, 1H, J = 2), 5.47−5.55 (m, 2H), 6.21 (d, 1H, J = 2), 6.47 (t, 1H, J = 2), 7.74 (d, 2H, J = 9), 7.98 (d, 2H, J = 9), 8.21 (s, 1H), 8.63 (s, 1H), 8.67 (bs, 1H), 12.01 (s, 1H). Anal. ($C_{21}H_{27}N_7O_6$) C, H, N.

1-Deoxy-1-[6-[[[(*R*)-1-phenylethyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (17): yield 65%; white solid, mp 110−111 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3430, 1730, 1620, 1555, 1230; ^1H NMR ($CDCl_3$) δ 0.68 (t, 3H, J = 7), 1.41 (s, 3H), 1.63 (s, 3H), 1.64 (d, 3H, J = 7), 2.87−2.96 (m, 2H), 4.71 (d, 1H, J = 1.8), 5.16 (m, 1H), 5.48−5.51 (m, 2H), 6.16 (d, 1H, J = 2), 6.52 (bs, 1H), 7.25−7.40 (m, 5H), 8.15 (s, 1H), 8.51 (s, 1H), 9.84 (bs, 1H), 10.94 (s, 1H). Anal. ($C_{23}H_{29}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(*S*)-1-phenylethyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (18): yield 82%; white solid, mp 107−108 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3445, 1725, 1615, 1560, 1230; ^1H NMR ($CDCl_3$) δ 0.85 (t, 3H, J = 7), 1.40 (s, 3H), 1.63 (s, 3H), 1.64 (d, 3H, J = 7), 3.00−3.09 (m, 2H), 4.72 (d, 1H, J = 1.8), 5.17 (m, 1H), 5.39−5.50 (m, 2H), 6.14 (d, 1H, J = 2), 6.57 (bs, 1H), 7.26−7.45 (m, 5H), 8.12 (s, 1H), 8.51 (s, 1H), 9.81 (bs, 1H), 10.87 (s, 1H). Anal. ($C_{23}H_{29}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(5-methyl-isoxazol-3-yl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (19): yield 83%; pale yellow solid, mp 120 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3450, 1720, 1620, 1550, 1215; ^1H NMR ($CDCl_3$) δ 0.82 (t, 3H, J = 7), 1.20 (s 3H), 1.41 (s, 3H), 2.44 (s, 3H), 3.00−3.07 (m, 2H), 4.74 (d, 1H, J = 2), 5.46−5.50 (m, 2H), 6.21 (d, 1H, J = 2), 6.55 (t, 1H, J = 2), 6.70 (s, 1H), 8.37 (s, 1H), 8.63 (s, 1H), 9.39 (bs, 1H), 12.34 (bs, 1H). Anal. ($C_{20}H_{24}N_8O_6$) C, H, N.

1-Deoxy-1-[6-[[[(1,3,4-thiadiazol-2-yl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (20): yield 70%; yellow solid, mp 148 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3430, 1730, 1615, 1560, 1240; ^1H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 7), 1.44 (s 3H), 1.67 (s, 3H), 3.07−3.14 (m, 2H), 4.78 (d, 1H, J = 1.8), 5.47−5.51 (m, 2H), 6.24 (d, 1H, J = 1.8), 6.52 (t, 1H, J = 2), 8.36 (s, 1H), 8.75 (s, 1H), 8.91 (bs, 1H), 9.40 (bs, 1H), 11.72 (bs, 1H). Anal. ($C_{18}H_{21}N_9O_5S$) C, H, N.

1-Deoxy-1-[6-[[[(4-*n*-propyloxy-phenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (21): yield 70%; pale yellow foam; IR (neat) cm^{-1} 3440, 1725, 1620, 1555, 1220; ^1H NMR ($CDCl_3$) δ 0.71 (t, 3H, J = 7), 0.90 (t, 3H, J = 7), 1.40 (s 3H), 1.62 (s, 3H), 1.81−2.03 (m, 2H), 2.95−3.01 (m, 2H), 3.90 (t, 2H, J = 7), 4.71 (d, 1H, J = 1.8), 5.46−5.51 (m, 2H), 6.19 (d, 1H, J = 1.8), 6.51 (t, 1H, J = 2), 6.89 (d, 2H, J = 9), 7.48 (d, 2H, J = 9), 8.25 (s, 1H), 8.56 (s, 1H), 8.80 (bs, 1H), 11.48 (s, 1H). Anal. ($C_{22}H_{25}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(2-phenylethyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (22): yield 60%; pale yellow solid, mp 88−90 °C (EtOAc−light petroleum); IR (KBr) cm^{-1} 3440, 1730, 1615, 1550, 1225; ^1H NMR ($CDCl_3$) δ 0.70 (t, 3H, J = 7), 1.40 (s 3H), 1.62 (s, 3H), 2.94−3.23 (m, 4H), 3.67 (t, 2H, J = 6.8), 4.71 (d, 1H, J = 1.8), 5.46−5.51 (m, 2H), 6.18 (d, 1H, J = 1.8), 6.49 (t, 1H, J = 6), 7.24−7.32 (m, 5H), 8.25 (s, 1H), 8.30 (s, 1H), 9.48 (bs, 1H), 11.48 (s, 1H). Anal. ($C_{24}H_{29}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(3,4-dimethoxy-2-phenylethyl)amino]-carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (23): yield 55%; yellow solid, 85−87 °C mp (EtOAc−light petroleum); IR (KBr) cm^{-1} 3440, 1724, 1623, 1554, 1225; ^1H NMR ($CDCl_3$) δ 0.73 (t, 3H, J = 7), 1.23 (t, 2H, J = 8), 1.41 (s 3H), 1.62 (s, 3H), 2.95−3.07 (m, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 4.71 (d, 1H, J = 1.8), 5.47−5.51 (m, 2H), 6.17 (d, 1H, J = 1.8), 6.51 (t, 1H, J = 2), 6.60 (s, 1H), 6.62 (s, 2H), 8.23 (s, 1H), 8.36 (s, 1H), 8.71 (s, 1H), 9.46 (bs, 1H). Anal. ($C_{26}H_{33}N_7O_7$) C, H, N.

1-Deoxy-1-[6-[[[(fur-2-yl-methyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (24): yield 58%; yellow solid mp 90−93 °C (EtOAc−light petroleum); IR (KBr) cm^{-1} 3445, 1727, 1620, 1550, 1222; ^1H NMR ($CDCl_3$) δ 0.72 (t, 3H, J = 7), 1.40 (s, 3H), 1.62 (s, 3H), 2.84−2.99 (m, 2H), 4.6 (d, 2H, J = 6), 4.71 (d, 1H, J = 1.8), 5.45−5.55 (m, 2H), 6.19 (d, 1H, J = 1.8), 6.30−6.36 (m, 2H), 6.60 (s, 1H), 6.5 (t, 1H, J = 6), 7.38 (d, 1H, J = 2), 8.32 (s, 1H), 8.49 (s, 1H), 9.00 (s, 1H), 9.84 (bs, 1H). Anal. ($C_{21}H_{25}N_7O_6$) C, H, N.

1-Deoxy-1-[6-[[[(4-(pyridin-2-yl-aminosulfonyl)phenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (25): yield 48%; yellow solid mp 168−170 °C (EtOAc−light petroleum); IR (KBr) cm^{-1} 3450, 1730, 1625, 1550, 1350, 1220; ^1H NMR ($CDCl_3$) δ 0.71 (t, 3H, J = 7), 1.41 (s, 3H), 1.60 (s, 3H), 2.80−2.95 (m, 2H), 4.73 (d, 1H, J = 1.8), 5.40−5.60 (m, 2H), 6.19 (d, 1H, J = 1.8), 6.65 (t, 1H, J = 6), 7.15−7.25 (m, 1H), 7.45 (d, 2H, J = 9), 7.55−7.75 (m, 3H), 7.92 (d, 2H, J = 9), 8.27 (s, 1H), 8.50 (s, 1H), 8.99 (s, 1H), 9.80 (bs, 1H), 11.02 (bs, 1H). Anal. ($C_{27}H_{29}N_9O_7S$) C, H, N.

1-Deoxy-1-[6-[[[(4-(5-methyl-isoxazol-3-yl-aminosulfonyl)phenyl)amino] carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (26): yield 56%; yellow solid mp 157−159 °C (EtOAc−light petroleum); IR (KBr) cm^{-1} 3455, 1725, 1630, 1557, 1355, 1224; ^1H NMR (DMSO-d_6) δ 0.79 (t, 3H, J = 7), 1.40 (s, 3H), 1.62 (s, 3H), 2.30 (s, 3H), 2.91−3.04 (m, 2H), 4.68 (d, 1H, J = 1.8), 5.40−5.48 (m, 2H), 6.11 (d, 1H, J = 1.8), 6.39 (s, 1H), 7.06 (t, 1H, J = 6), 7.63 (d, 2H, J = 9), 7.85 (d, 2H, J = 9), 8.44 (s, 1H), 8.59 (s, 1H), 9.07 (s, 1H), 9.62 (bs, 1H), 12.11 (bs, 1H). Anal. ($C_{26}H_{29}N_9O_8S$) C, H, N.

1-Deoxy-1-[6-[[[(4-(pyrimidin-2-yl-aminosulfonyl)-phenyl)amino] carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (27): yield 53%; yellow solid mp 157−159 °C (EtOAc−light petro-

leum); IR (KBr) cm^{-1} 3450, 1722, 1633, 1552, 1350, 1220; ^1H NMR (DMSO-d_6) δ 0.57 (t, 3H, $J = 7$), 1.35 (s, 3H), 1.54 (s, 3H), 2.71−2.80 (m, 2H), 4.59 (d, 1H, $J = 1.8$), 5.40−5.46 (m, 2H), 6.46 (d, 1H, $J = 1.8$), 7.03 (t, 1H, $J = 6$), 7.56 (m, 1H), 7.60 (d, 2H, $J = 9$), 7.85 (d, 2H, $J = 9$), 8.48 (s, 1H), 8.51 (s, 1H), 8.60 (d, 2H, $J = 7$), 10.45 (bs, 1H), 11.71 (bs, 1H), 12.04 (s, 1H). Anal. ($C_{26}H_{28}N_{10}O_7S$) C, H, N.

1-Deoxy-1-[6,6-[[[bis-(4-nitrophenyl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (28): yield 65%; yellow solid, mp 261 °C (CH_2Cl_2−Et_2O); IR (KBr) cm^{-1} 3430, 1730, 1620, 1555, 1350, 1210; ^1H NMR (DMSO-d_6) δ 0.56 (t, 3H, $J = 7$), 1.34 (s, 3H), 1.54 (s, 3H), 2.67−2.83 (m, 2H), 4.61 (d, 1H, $J = 1.8$), 5.47−5.50 (m, 2H), 6.47 (d, 1H, $J = 1.8$), 7.61 (t, 1H, $J = 2$), 7.71 (d, 2H, $J = 9$), 7.91 (d, 2H, $J = 9$), 8.22 (d, 2H, $J = 9$), 8.24 (s, 1H), 8.64 (d, 2H, $J = 9$), 9.72 (s, 1H), 10.63 (bs, 1H), 12.24 (s, 1H). Anal. ($C_{29}H_{28}N_{10}O_{10}$) C, H, N.

1-Deoxy-1-[6,6-[[[bis-(5-chloro-pyridin-2-yl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-*β*-D-ribofuranuronamide (29): yield 60%; yellow foam; IR (neat) cm^{-1} 3440, 1710, 1630, 1540, 1220; ^1H NMR (DMSO-d_6) δ 0.58 (t, 3H, $J = 7$), 1.35 (s 3H), 1.54 (s, 3H), 2.71−2.85 (m, 2H), 4.60 (d, 1H, $J = 2$), 5.47−5.51 (m, 2H), 6.47 (d, 1H, $J = 2$), 7.52 (t, 1H, $J = 2$), 7.79−7.92 (m, 4H), 8.34−8.38 (m, 2H), 8.60 (s, 1H), 8.65 (s, 1H), 10.66 (bs, 1H), 12.22 (bs, 1H). Anal. ($C_{27}H_{26}Cl_2N_8O_6$) C, H, N.

General Procedure for the Preparation of 1-Deoxy-1-[6-[[(substituted)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (9−13) and 1-Deoxy-1-[6-[[[(substituted)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (30−45). A solution of the isopropylidene derivative, **4**−**8** or **14**−**29**, (0.084 mmol) in aqueous 1 N HCl (5 mL) and dioxane (5 mL) was stirred at 65 °C for 1 h. The solvent was then removed under reduced pressure, and the residue was crystallized from ethanol to afford the desired compound, **9**−**13** or **30**−**45**.

1-Deoxy-1-[6-[[(4-biphenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (9): yield 65%; white solid, mp 221 °C (EtOH); IR (KBr) cm^{-1} 3500−3100, 1720, 1615, 1550; ^1H NMR (DMSO-d_6) δ 1.07 (t, 3H, $J = 7$), 3.17−3.24 (m, 2H), 4.23−4.25 (m, 1H), 4.36 (d, 1H, $J = 2$), 4.71−4.73 (m, 1H), 5.70 (d, 1H, $J = 8$), 5.79 (d, 1H, $J = 4$), 6.13 (d, 1H, $J = 8$), 7.43−7.53 (m, 3H), 7.78−7.89 (m, 4H), 8.16 (d, 2H, $J = 11$), 8.46 (t, 1H, $J = 4$), 8.80 (s, 1H), 8.82 (s, 1H), 11.34 (bs, 1H). Anal. ($C_{25}H_{24}N_6O_5$) C, H, N.

1-Deoxy-1-[6-[[(2,4-dichlorobenzyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (10): yield 55%; off white solid, mp 132−134 °C (EtOH); IR (KBr) cm^{-1} 3450−3050, 1730, 1635, 1545, 1230; ^1H NMR (DMSO-d_6) δ 1.08 (t, 3H, $J = 7$), 3.18−3.25 (m, 2H), 3.34 (s, 2H), 4.12−4.15 (m, 1H), 4.30 (s, 1H), 4.49−4.62 (m, 2H), 5.57 (d, 1H, $J = 8$), 5.77 (d, 1H, $J = 4$), 5.95 (d, 1H, $J = 8$), 7.44−7.51 (m, 3H), 8.19 (s, 1H), 8.39 (s, 1H), 8.95 (bs, 1H). Anal. ($C_{20}H_{20}Cl_2N_6O_5$) C, H, N.

1-Deoxy-1-[6-[[(4-methoxyphenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (11): yield 80%; white solid, mp 167−169 °C (EtOH); IR (KBr) cm^{-1} 3550−3150, 1710, 1640, 1530, 1270; ^1H NMR (DMSO-d_6) δ 1.07 (t, 3H, $J = 7$), 3.15−3.23 (m, 2H), 3.86 (s, 3H), 4.20−4.24 (m, 1H), 4.35 (d, 1H, $J = 2$), 4.70−4.75 (m, 1H), 5.69 (d, 1H, $J = 7$), 5.77 (d, 1H, $J = 4$), 6.11 (d, 1H, $J = 7$), 7.08 (d, 2H, $J = 9$), 8.04 (d, 2H, $J = 9$), 8.44 (bs, 1H), 8.77 (s, 1H), 8.78 (s, 1H), 11.12 (s, 1H). Anal. ($C_{20}H_{22}N_6O_6$) C, H, N.

1-Deoxy-1-[6-[[(2-chlorophenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (12): yield 78%; white solid, mp 179−180 °C (EtOH); IR (KBr) cm^{-1} 3510−3050, 1720, 1660, 1510, 1250; ^1H NMR (DMSO-d_6) δ 1.06 (t, 3H, $J = 7$), 3.15−3.22 (m, 2H), 4.22 (bs, 1H), 4.34 (s, 1H), 4.68−4.70 (m, 1H), 5.66 (d, 1H, $J = 7$), 5.76 (d, 1H, $J = 4$), 6.10 (d, 1H, $J = 7$), 7.43−7.61 (m, 4H), 8.44 (bs, 1H), 8.67 (s, 1H), 8.82 (bs, 1H), 11.53 (bs, 1H). Anal. ($C_{19}H_{19}N_6O_5$) C, H, N.

1-Deoxy-1-[6-[[(phenyl)carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (13): yield 67%; white solid, mp 157−159 °C (EtOH); IR (KBr) cm^{-1} 3500−3000, 1715, 1630, 1535, 1260; ^1H NMR (DMSO-d_6) δ 1.03 (t, 3H, $J = 7$), 3.16−3.19 (m, 2H), 3.74 (bs, 2H), 4.22 (bs, 1H), 4.36 (s, 1H), 4.51−4.63 (m, 1H), 6.13 (d, 1H, $J = 6.8$), 7.48−7.59 (m, 3H), 8.04−8.11 (m, 2H), 8.52 (bs, 1H), 8.79 (s, 1H), 8.83 (s, 1H), 11.22 (s, 1H). Anal. ($C_{19}H_{20}N_6O_5$) C, H, N.

1-Deoxy-1-[6-[[[(benzyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (30): yield 70%;white solid, mp 184−186 °C (EtOH); IR (KBr) cm^{-1} 3500−3100, 1675, 1620, 1565, 1520, 1310; ^1H NMR (DMSO-d_6) δ 1.06 (t, 3H, $J = 7$), 3.10−3.20 (m, 2H), 4.05 (bs, 2H), 4.17−4.20 (m, 1H), 4.36 (s, 1H), 4.47−4.50 (m, 2H), 4.55−4.65 (m, 1H), 6.05−6.10 (m, 1H), 7.17−7.40 (m, 5H), 8.30−8.40 (m, 1H), 8.62 (s, 1H), 8.86 (s, 1H), 9.50−9.60 (m, 1H), 10.30 (bs, 1H). Anal. ($C_{20}H_{24}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(4-sulfonamidophenyl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (31): yield 63%; white solid, mp 183−185 °C (EtOH); IR (KBr) cm^{-1} 3550−3050, 1715, 1630, 1545, 1370, 1250; ^1H NMR (DMSO-d_6) δ 1.07 (t, 3H, $J = 7$), 3.16−3.26 (m, 2H), 4.21 (bs, 1H), 4.36 (s, 1H), 4.63−4.69 (m, 1H), 5.62 (d, 1H, $J = 6.8$), 5.78 (d, 1H, $J = 4$), 6.11 (d, 1H, $J = 6.8$), 7.31 (bs, 2H), 7.81 (s, 4H), 8.49 (bs, 1H), 8.75 (s, 1H), 8.89 (s, 1H), 9.69 (bs, 1H), 11.92 (bs, 1H). Anal. ($C_{19}H_{22}N_8O_7S$) C, H, N.

1-Deoxy-1-[6-[[[(4-acetylphenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (32): yield 83%; white solid, mp 187 °C (EtOH); IR (KBr) cm^{-1} 3550−3100, 1740, 1720, 1630, 1525, 1215; ^1H NMR (DMSO-d_6) δ 1.08 (t, 3H, $J = 7$), 2.55 (s, 3H), 3.16−3.23 (m, 2H), 4.18−4.20 (m, 1H), 4.21 (d, 1H, $J = 2$), 4.45 (bs, 2H), 4.64−4.69 (m, 1H), 6.11 (d, 1H, $J = 7$), 7.77 (d, 2H, $J = 9$), 7.98 (d, 2H, $J = 9$), 8.49 (bs, 1H), 8.76 (s, 1H), 8.88 (s, 1H), 10.62 (bs, 1H), 11.94 (bs, 1H). Anal. ($C_{21}H_{23}N_7O_6$) C, H, N.

1-Deoxy-1-[6-[[[(*R*)-1-phenylethyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (33): yield 65%; white solid, mp 153−155 °C (EtOH); IR (KBr) cm^{-1} 3550−3100, 1720, 1635, 1535, 1240; ^1H NMR (DMSO-d_6) δ 1.05 (t, 3H, $J = 7$), 1.51 (d, 3H, $J = 8$), 3.14−3.21 (m, 2H), 3.35−3.41 (m, 1H), 4.20 (s, 1H), 4.36 (s, 1H), 4.93−5.00 (m, 3H), 6.09 (d, 1H, $J = 6.8$), 7.25−7.39 (m, 5H), 8.48 (bs, 1H), 8.65 (s, 1H), 8.89 (s, 1H), 9.51 (bs, 1H), 10.38 (bs, 1H). Anal. ($C_{21}H_{25}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(*S*)-1-phenylethyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (34): yield 72%; white solid, mp 151 °C (EtOH); IR (KBr) cm^{-1} 3550−3100, 1725, 1630, 1525, 1240; ^1H NMR (DMSO-d_6) δ 1.06 (t, 3H, $J = 7$), 1.49 (d, 3H, $J = 8$), 3.15−3.21 (m, 2H), 3.28−3.39 (m, 1H), 4.00 (bs, 2H), 4.35 (s, 1H), 4.61−4.66 (m, 1H), 4.94−5.01 (m, 1H), 6.07 (d, 1H, $J = 6.8$), 7.24−7.39 (m, 5H), 8.48 (bs, 1H), 8.64 (s, 1H), 8.83 (s, 1H), 9.57 (bs, 1H), 10.12 (bs, 1H). Anal. ($C_{21}H_{25}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(5-methyl-isoxazol-3-yl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (35): yield 58%; white solid, mp 197 °C (EtOH); IR (KBr) cm^{-1} 3550−3050, 1715, 1620, 1535, 1240; ^1H NMR (DMSO-d_6) δ 1.06 (t, 3H, $J = 7$), 2.40 (s 3H), 3.12−3.25 (m, 2H), 4.21−4.23 (m, 1H), 4.36 (d, 1H, $J = 2$), 4.64−4.70 (m, 1H), 5.68 (m, 2H), 6.09 (d, 1H, $J = 6.8$), 6.67 (s, 1H), 8.46 (t, 1H, $J = 6$), 8.74 (s, 1H), 8.84 (s, 1H), 10.75 (bs, 1H), 12.19 (bs, 1H). Anal. ($C_{17}H_{20}N_8O_6$) C, H, N.

1-Deoxy-1-[6-[[[(1,3,4-thiadiazol-2-yl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (36): yield 65%; white solid, mp 197 °C (EtOH); IR (KBr) cm^{-1} 3450−300, 1720, 1635, 1515, 1240; ^1H NMR (DMSO-d_6) δ 1.06 (t, 3H, $J = 7$), 3.15−3.22 (m, 2H), 3.72 (bs, 2H), 4.21−4.24 (m, 1H), 4.36 (d, 1H, $J = 2$), 4.65−4.70 (m, 1H), 6.12 (d, 1H, $J = 6.8$), 8.45 (t, 1H, $J = 7$), 8.80 (s, 1H), 8.89 (s, 1H), 9.19 (s, 1H), 10.35 (bs, 1H), 11.38 (bs, 1H). Anal. ($C_{15}H_{17}N_4O_5S$) C, H, N.

1-Deoxy-1-[6-[[[(4-*n*-propyloxy-phenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (37): yield 66%; white solid, mp 270 °C (EtOH); IR (KBr) cm^{-1} 3450−3050, 1720, 1630, 1535, 1240; ^1H NMR (DMSO-d_6) δ 0.95 (t, 3H, $J = 7$), 1.09 (t, 3H, $J = 7$), 1.65−1.75 (m, 2H), 3.06−3.23 (m, 2H), 3.67 (bs, 2H), 3.89 (t, 2H, $J = 7$),

4.18−4.20 (m, 1H), 4.33 (s, 1H), 4.51−4.61 (m, 1H), 6.05 (d, 1H, J = 6.8), 6.91 (d, 2H, J = 9), 7.52 (d, 2H, J = 9), 7.92 (t, 1H, J = 7), 8.22 (s, 1H), 8.52 (s, 1H), 9.65 (bs, 1H), 11.52 (bs, 1H). Anal. ($C_{22}H_{27}N_7O_6$) C, H, N.

1-Deoxy-1-[6-[[[(2-phenylethyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (38): yield 82%; light brown solid, mp 166 °C (EtOH); IR (KBr) cm^{-1} 3450−3000, 1725, 1610, 1560, 1210; ^1H NMR (DMSO-d_6) δ 1.04 (t, 3H, J = 7), 2.84−2.95 (m, 2H), 3.01−3.23 (m, 2H), 4.15−4.20 (m, 1H), 4.47 (s, 1H), 4.54−4.79 (m, 3H), 5.39−5.48 (m, 2H), 6.21 (d, 1H, J = 1.8), 6.53 (t, 1H, J = 6), 7.31−7.44 (m, 5H), 8.32 (s, 1H), 8.41 (s, 1H), 9.55 (bs, 1H), 11.18 (s, 1H). Anal. ($C_{21}H_{25}N_7O_5$) C, H, N.

1-Deoxy-1-[6-[[[(3,4-dimethoxy-2-phenylethyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (39): yield 68%; light brown solid, mp 120−122 °C (EtOH); IR (KBr) cm^{-1} 3540−3060, 1734, 1633, 1500, 1215; ^1H NMR (DMSO-d_6) δ 1.11 (t, 3H, J = 7), 1.23 (t, 2H, J = 8), 2.88−3.01 (m, 2H), 3.78 (s, 3H), 3.81 (s, 3H), 4.18−4.23 (m, 1H), 4.42 (s, 1H), 4.54−4.59 (m, 1H), 4.87−5.02 (bs, 2H), 5.47−5.51 (m, 2H), 6.15 (d, 1H, J = 1.8), 6.59 (t, 1H, J = 2), 6.65 (s, 1H), 6.71 (s, 2H), 8.31 (s, 1H), 8.47 (s, 1H), 9.05 (s, 1H), 10.46 (bs, 1H). Anal. ($C_{23}H_{29}N_7O_7$) C, H, N.

1-Deoxy-1-[6-[[[(fur-2-yl-methyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (40): yield 79%; brown solid mp 189−191 °C (EtOH); IR (KBr) cm^{-1} 3500−3087, 1731, 1630, 1550, 1335, 1221; ^1H NMR (DMSO-d_6) δ 1.06 (t, 3H, J = 7), 2.94−3.05 (m, 2H), 4.20−4.24 (m, 1H), 4.38 (s, 1H), 4.56 (s, 2H, J = 6), 4.61−4.67 (m, 1H), 4.96−5.04 (bs, 2H), 6.17 (d, 1H, J = 1.8), 6.35−6.46 (m, 1H), 6.62 (s, 1H), 6.56 (t, 1H, J = 6), 7.48 (d, 1H, J = 2), 8.38 (s, 1H), 8.54 (s, 1H), 9.08 (s, 1H), 9.94 (bs, 1H). Anal. ($C_{18}H_{21}N_7O_6$) C, H, N.

1-Deoxy-1-[6-[[[(4-(pyridin-2-yl-aminosulfonyl)phenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (41): yield 88%; brown solid mp 163 °C (EtOH); IR (KBr) cm^{-1} 3500−3050, 1734, 1633, 1552, 1345, 1213; ^1H NMR (DMSO-d_6) δ 1.05 (t, 3H, J = 7), 2.99−3.18 (m, 2H), 4.23−4.26 (m, 1H), 4.42 (s, 1H), 4.54−4.62 (m, 1H), 4.91−5.15 (bs, 2H), 6.16 (d, 1H, J = 1.8), 6.71 (t, 1H, J = 6), 7.18−7.31 (m, 1H), 7.57 (d, 2H, J = 9), 7.63−7.71 (m, 3H), 7.95 (d, 2H, J = 9), 8.37 (s, 1H), 8.78 (s, 1H), 9.05 (s, 1H), 10.15 (bs, 1H), 12.45 (bs, 1H). Anal. ($C_{24}H_{25}N_9O_7S$) C, H, N.

1-Deoxy-1-[6-[[[(4-(5-methyl-isoxazol-3-yl-aminosulfonyl)phenyl)amino] carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (42): yield 77%; pale yellow solid mp 184 °C (EtOH); IR (KBr) cm^{-1} 3550−3150, 1730, 1638, 1550, 1350, 1210; ^1H NMR (DMSO-d_6) δ 1.08 (t, 3H, J = 7), 2.30 (s, 3H), 3.17−3.20 (m, 2H), 4.22−4.23 (m, 1H), 4.36 (s, 1H), 4.40−4.95 (m, 3H), 6.11 (d, 1H, J = 1.8), 6.15 (s, 1H), 6.68 (t, 1H, J = 6), 7.37 (d, 2H, J = 9), 7.84 (d, 2H, J = 9), 8.42 (bs, 1H), 8.73 (s, 1H), 8.85 (s, 1H), 9.63 (bs, 1H), 11.37 (bs, 1H), 12.02 (s, 1H). Anal. ($C_{23}H_{25}N_9O_8S$) C, H, N.

Deoxy-1-[6-[[[(4-(pyrimidin-2-yl-aminosulfonyl)-phenyl)amino] carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (43): yield 80%; brown solid mp 184 °C (EtOH); IR (KBr) cm^{-1} 3550−3100, 1720, 1630, 1555, 1355, 1200; ^1H NMR (DMSO-d_6) δ 1.08 (t, 3H, J = 7), 3.17−3.23 (m, 2H), 4.22−4.25 (m, 1H), 4.37 (s, 1H), 4.64−4.70 (m, 1H), 4.90−5.12 (bs, 2H), 6.11 (d, 1H, J = 1.8), 6.60 (t, 1H, J = 6), 7.03−7.08 (m, 1H), 7.81 (d, 2H, J = 9), 7.98 (d, 2H, J = 9), 8.51 (d, 2H, J = 6), 8.74 (s, 1H), 8.89 (s, 1H), 9.55 (bs, 1H), 11.19 (bs, 1H), 11.90 (s, 1H). Anal. ($C_{23}H_{24}N_{10}O_7S$) C, H, N.

1-Deoxy-1-[6,6-[[[bis-(4-nitrophenyl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (44): yield 52%; white solid, mp 213 °C (EtOH); IR (KBr) cm^{-1} 3500−3050, 1720, 1630, 1545, 1340, 1220; ^1H NMR (DMSO-d_6) δ 1.07 (t, 3H, J = 7), 3.12−3.23 (m, 2H), 4.21−4.23 (m, 1H), 4.35 (d, 1H, J = 2), 4.64−4.69 (m, 1H), 5.69 (bs, 2H), 6.09 (d, 1H, J = 6.8), 7.68 (d, 2H, J = 9), 7.91 (d, 2H, J = 9), 8.18−8.29 (m, 4H), 8.83 (s, 1H), 9.69 (s, 1H), 10.68 (bs, 1H), 12.26 (bs, 1H). Anal. ($C_{26}H_{29}N_{10}O_{10}$) C, H, N.

1-Deoxy-1-[6,6-[[[bis-(5-chloro-pyridin-2-yl)amino]-carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (45): yield 68%; white solid, mp 220 °C (EtOH); IR (KBr) cm^{-1} 3550−3100, 1725, 1630, 1525, 1250; ^1H NMR (DMSO-d_6) δ 1.06 (t, 3H, J = 7), 3.15−3.22 (m, 2H), 4.22−4.24 (m, 1H), 4.36 (d, 2H, J = 2), 4.64−4.69 (m, 1H), 5.95 (bs, 2H), 6.10 (d, 1H, J = 6.8), 7.80−8.06 (m, 6H), 8.39 (t, 1H, J = 7), 8.76 (s, 1H), 8.93 (s, 1H), 10.39 (bs, 1H), 12.11 (bs, 1H). Anal. ($C_{24}H_{22}Cl_2N_8O_6$) C, H, N.

General Procedure for the Synthesis of 2-Chloro- and 2-Iodo-1-deoxy-1-[6-[[[(substituted)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (48−51). The appropriate commercially available isocyanate (2.1 mmol) was added to a solution of 2′,3′-*O*-isopropylidene-2-chloro-NECA (**46**)[43] or to 2′,3′-*O*-isopropylidene-2-iodo-NECA (**47**)[44] (0.42 mmol) and a catalytic amount of triethylamine in THF (10 mL), and the mixture was heated at 110 °C in a steel bomb for 24 h. After the mixture was concentrated in vacuo, the residue was flash chromatographed on a silica gel column eluting with $CHCl_3$−MeOH (99:1) to give the desired protected nucleosides **48**−**51**.

2-Chloro-1-deoxy-1-[6-[[[(3-chlorophenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (48): yield 33%; white solid, mp 98−100 °C dec (CH_3CN−EtOH); IR (KBr) cm^{-1} 3400−3050, 1685, 1650, 1520, 1235; ^1H NMR (DMSO-d_6) δ 0.66 (t, 3H, J = 7.2 Hz), 1.37 (s, 3H), 1.56 (s, 3H), 2.74−2.92 (br m, 2H), 4.61 (s, 1H), 5.42 (s, 2H), 6.42 (s, 1H), 7.17 (d, 1H, J = 7.0 Hz), 7.32−7.42 (m, 2H),7.60−7.70 (m, 1H), 7.79 (s, 1H), 8.59 (s, 1H), 10.64 (s, 1H), 10.79 (s, 1H). Anal. ($C_{22}H_{23}Cl_2N_7O_5$) C, H, N.

2-Chloro-1-deoxy-1-[6-[[[(4-methoxyphenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (49): yield 35%; vitreous solid; IR (KBr) cm^{-1} 3450−3050, 1680, 1650, 1510, 1230; ^1H NMR (DMSO-d_6) δ 0.67 (t, 3H, J = 7.0 Hz), 1.38 (s, 3H), 1.56 (s, 3H), 2.76−2.94 (m, 2H), 3.76 (s, 3H), 4.61 (s, 1H), 5.42 (s, 2H), 6.41 (s, 1H), 6.93−7.03 (m, 2H), 7.41−7.53 (m, 2H), 7.65−7.75 (m, 1H), 8.61 (s, 1H), 10.43 (s, 1H), 11.11 (s, 1H). Anal. ($C_{23}H_{26}ClN_7O_6$) C, H, N.

2-Iodo-1-deoxy-1-[6-[[[(3-chlorophenyl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (50): yield 62%; white solid, mp 127−130 °C (CH_3CN−EtOH); IR (KBr) cm^{-1} 3450−3050, 1690, 1650, 1520, 1240; ^1H NMR (DMSO-d_6) δ 0.66 (t, 3H, J = 7.2 Hz), 1.37 (s, 3H), 1.56 (s, 3H), 2.74−2.92 (br m, 2H), 4.61(s, 1H), 5.42 (s, 2H), 6.42 (s, 1H), 7.17 (d, 1H, J = 7.0 Hz), 7.32−7.42 (m, 2H), 7.56−7.66 (m, 1H), 7.79 (s, 1H), 8.50 (s, 1H), 10.64 (s, 1H), 11.33 (s, 1H). Anal. ($C_{22}H_{23}ClIN_7O_5$) C, H, N.

2-Iodo-1-deoxy-1-[6-[[[(4-methoxyphenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (51): yield 70%; white solid, mp 127−130 °C (CH_3CN−EtOH); IR (KBr) cm^{-1} 3450−3025, 1680, 1660, 1510, 1230; ^1H NMR (DMSO-d_6) δ 0.67 (t, 3H, J = 7.0 Hz), 1.38 (s, 3H), 1.56 (s, 3H), 2.76−2.94 (m, 2H), 3.76 (s, 3H), 4.61 (s, 1H), 5.42 (s, 2H), 6.41 (s, 1H), 6.93−7.03 (m, 2H), 7.41−7.53 (m, 2H), 7.54−7.65 (m, 1H), 8.49 (s, 1H), 10.43 (s, 1H), 11.11 (s, 1H). Anal. ($C_{23}H_{26}IN_7O_6$) C, H, N.

General Procedure for the Synthesis of 2-Chloro- and 2-Iodo-1-deoxy-1-[6-[[[(substituted)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (52−55). To a solution of 2′,3′-*O*-isopropylidene-2-chloro-6-ureas (**48**−**49**) or 2′,3′−O-isopropylidene-2-iodo-6-ureas (**50**−**51**) (0.96 mmol) in dioxane (50 mL) was added 1 N HCl (50 mL), and the mixture was heated at 65 °C for 1 h. After the mixture was concentrated in vacuo, the residue was flash chromatographed on a silica gel column eluting with $CHCl_3$−MeOH (95:5) to give the desired compounds **52**−**55**.

2-Chloro-1-deoxy-1-[6-[[[(3-chlorophenyl)amino]-carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (52): yield 66%; white solid, mp 150−153 °C dec (EtOH); IR (KBr) cm^{-1} 3500−3000, 1690, 1640, 1530, 1240; ^1H NMR (DMSO-d_6) δ 1.07 (t, 3H, J = 7.2 Hz), 3.13−3.31 (m, 2H), 4.25 (s, 1H,), 4.38 (d,1H, J = 2.1 Hz), 4.67 (pq, 1H), 6.05

(d, 1H, J = 6.7 Hz), 7.17 (d, 1H, J = 5.2 Hz), 7.35−7.44 (m, 2H), 8.82 (s, 1H), 8.26−8.33 (m, 1H), 8.87 (s, 1H), 10.80 (s, 1H), 11.33 (s, 1H). Anal. ($C_{19}H_{19}Cl_2N_7O_5$) C, H, N.

2-Chloro-1-deoxy-1-[6-[[[(4-methoxyphenyl)amino]-carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (53): yield 67%; white solid, mp 158−160 °C dec (EtOH); IR (KBr) cm^{-1} 3400−3050, 1680, 1660, 1520, 1240; ^{1}H NMR (DMSO-d_6) δ 1.02−1.14 (m, 3H), 3.12−3.30 (m, 2H), 3.75 (s, 3H), 4.20−4.30 (m, 1H), 4.37 (s,1H), 4.61−4.72 (m, 1H), 6.04 (d, 1H, J = 6.8 Hz), 6.97 (d, 2H, J = 8.9 Hz), 7.47 (d, 2H, J = 8.9 Hz), 8.22−8.35 (m, 1H), 8.85 (s, 1H), 10.51 (s, 1H), 10.62 (s, 1H). Anal. ($C_{20}H_{22}ClN_7O_6$) C, H, N.

2-Iodo-1-deoxy-1-[6-[[[(3-chlorophenyl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (54): yield 85%; white solid, mp 202−204 °C dec (EtOH); IR (KBr) cm^{-1} 3500−3000, 1700, 1640, 1530, 1240; ^{1}H NMR (DMSO-d_6) δ 1.07 (t, 3H, J = 7.2 Hz), 3.13−3.31 (m, 2H), 4.25 (s, 1H), 4.38 (d, 1H, J = 2.1 Hz), 4.67 (pq, 1H), 6.05 (d, 1H, J = 6.7 Hz), 7.17 (d, 1H, J = 5.2 Hz), 7.35−7.44 (m, 2H), 8.82 (s, 1H), 8.13−8.23 (m, 1H), 8.79 (s, 1H), 10.66 (s, 1H), 11.33 (s, 1H). Anal. ($C_{19}H_{19}ClIN_7O_5$) C, H, N.

2-Iodo-1-deoxy-1-[6-[[[(4-methoxyphenyl)amino]carbonyl]-amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (55): yield 89%; white solid, mp 214−218 °C dec (EtOH); IR (KBr) cm^{-1} 3375−3050, 1680, 1650, 1510, 1240; ^{1}H NMR (DMSO-d_6) δ 1.01−1.12 (m, 3H), 3.12−3.32 (m, 2H), 3.76 (s, 3H), 4.20−4.28 (m, 1H), 4.36 (s,1H), 4.60−4.73 (m, 1H), 6.04 (d, 1H, J = 6.6 Hz), 6.93−7.04 (m, 2H), 7.44−7.56 (m, 2H), 8.11−8.21 (m, 1H), 8.76 (s, 1H), 10.45 (s, 1H), 11.09 (s, 1H). Anal. ($C_{20}H_{22}IN_7O_6$) C, H, N.

General Procedure for the Synthesis of 2-(Ar)alkynyl-1-deoxy-1-[6-[[[(substituted)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (56−62). Cuprous iodide (0.12 mg), bis(triphenylphosphine)palladium dichloride (1.7 mg) and the appropriate terminal alkyne (0.6 mmol) were added to a solution of 2-iodo-6-ureas (**54** or **55**) (0.12 mmol) in a mixture of dry acetonitrile (4 mL), DMF (4 mL), and triethylamine (0.3 mL), and the mixture was stirred at room temperature for 16 h in an atmosphere of N_2. The solvent was removed under vacuum, and the residue was chromatographed on silica gel column, eluting with a suitable mixture of solvents (see below) to give the desired alkynyl derivatives **56**−**62**.

2-(1-Hexyn-1-yl)-1-deoxy-1-[6-[[[(3-chlorophenyl)amino]-carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (56): chromatography solvent, $CHCl_3$−MeOH (92:8); yield 75%; white solid, mp 196−198 °C dec (EtOH); IR (KBr) cm^{-1} 3500−3150, 2235, 1680, 1660, 1545, 1260; ^{1}H NMR (DMSO-d_6) δ 0.91−1.0 (m, 3H), 1.02−1.13 (m, 3H), 1.40−1.71 (m, 4H), 2.52−2.60 (m, 2H), 3.19−3.34 (m, 2H), 4.18−4.26 (m, 1H), 4.37 (d, 1H, J = 2.2 Hz), 4.60−4.70 (m, 1H), 6.07 (d, 1H, J = 7.1 Hz), 7.11−7.22 (m, 1H), 7.41 (d, 2H, J = 5.1 Hz), 7.74 (s, 1H), 8.40−8.50 (m, 1H), 8.84 (s, 1H), 10.56 (s, 1H), 11.80 (s, 1H). Anal. ($C_{25}H_{28}ClN_7O_5$) C, H, N.

2-(Phenylethynyl)-1-deoxy-1-[6-[[[(3-chlorophenyl)-amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (57): chromatography solvent, $CHCl_3$−MeOH (92:8); yield 59%; white solid, mp 210−214 °C dec (EtOH); IR (KBr) cm^{-1} 3450−3050, 2210, 1680, 1670, 1550, 1270; ^{1}H NMR (DMSO-d_6) δ 1.02−1.13 (m, 3H), 3.17−3.34 (m, 2H), 4.20−4.29 (m, 1H), 4.39 (s, 1H), 4.63−4.74 (m, 1H), 6.13 (d, 1H, J = 7.0 Hz), 7.17 (d, 1H, J = 7.2 Hz), 7.36−7.59 (m, 5H), 7.69−7.79 (m, 3H), 8.35−8.44 (m, 1H), 8.92 (s, 1H), 10.61 (s, 1H), 11.65 (s, 1H). Anal. ($C_{27}H_{24}ClN_7O_5$) C, H, N.

2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-1-deoxy-1-[6-[[[(3-chlorophenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (58): chromatography solvent, $CHCl_3$-cC_6H_{12}−CH_3CN-MeOH (73:10:5:12); yield 43%; yellow solid, mp 214−216 °C dec (EtOH); IR (KBr) cm^{-1} 3450−3050, 2325, 1700, 1640, 1540, 1250; ^{1}H NMR (DMSO-d_6) δ 0.96−1.14 (m, 3H), 3.04−3.30 (m, 2H), 4.30−4.45 (m, 2H), 4.78−4.90 (m, 1H), 6.19 (d, 1H, J = 6.6 Hz), 7.12−8.34 (m, 11H), 8.94 (s, 1H), 10.51 (s, 1H), 11.47 (s, 1H). Anal. ($C_{28}H_{26}ClN_7O_6$) C, H, N.

2-(1-Hexyn-1-yl)-1-deoxy-1-[6-[[[(4-methoxyphenyl)-amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (59): chromatography solvent, $CHCl_3$−C_6H_6−MeOH (81:10:9); yield 71%; white solid, mp 175−178 °C dec (EtOH); IR (KBr) cm^{-1} 3500−3000, 2240, 1660, 1600, 1520, 1240; ^{1}H NMR (DMSO-d_6) δ 0.91−1.02 (m, 3H), 1.04−1.17 (m, 3H), 1.43−1.70 (m, 4H), 2.50−2.61 (m, 2H), 3.18−3.32 (m, 2H), 3.77 (s, 3H), 4.18−4.28 (m, 1H), 4.37 (d, 1H, J = 1.7 Hz), 4.66 (pq, 1H), 6.07 (d, 1H, J = 7.3 Hz), 6.96 (d, 2H, J = 8.9 Hz), 7.48 (d, 2H, J = 9.1 Hz), 8.40−8.51 (m, 1H), 8.82 (s, 1H), 10.31 (s, 1H), 11.49 (s, 1H). Anal. ($C_{26}H_{31}N_7O_6$) C, H, N.

2-(Phenylethynyl)-1-deoxy-1-[6-[[[(4-methoxyphenyl)-amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (60): chromatography solvent, $CHCl_3$−C_6H_6−MeOH (80:10:10); yield 60%; white solid, mp 204−206 °C dec (EtOH); IR (KBr) cm^{-1} 3400−3050, 2205, 1675, 1660, 1510, 1240; ^{1}H NMR (DMSO-d_6) δ 0.98−1.14 (m, 3H), 3.15−3.34 (m, 2H), 3.77 (s, 3H), 4.20−4.30 (m, 1H), 4.39 (s, 1H), 4.65−4.75 (m, 1H), 6.12 (d, 1H, J = 6.9 Hz), 6.99 (d, 2H, J = 8.8 Hz), 7.44−7.62 (m, 5H), 7.64−7.78 (m, 2H), 8.34−8.47 (m, 1H), 8.90 (s, 1H), 10.36 (s, 1H), 11.29 (s, 1H). Anal. ($C_{28}H_{27}N_7O_6$) C, H, N.

2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-1-deoxy-1-[6-[[[(4-methoxyphenyl)amino]carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (61): chromatography solvent, $CHCl_3$−C_6H_6−MeOH (80:10:10); yield 40%; yellow solid, mp 218−220 °C dec (EtOH); IR (NaCl) cm^{-1} 3450−3050, 2300, 1680, 1650, 1510, 1240; ^{1}H NMR (DMSO-d_6) δ 0.90−1.10 (m, 3H), 3.13−3.23 (m, 2H), 3.77 (s, 3H), 4.30−4.46 (m, 2H), 4.76−4.89 (m, 1H), 6.19 (d, 1H, J = 6.7 Hz), 6.88−7.02 (m, 2H), 7.43−7.83 (m, 5H), 8.03−8.30 (m, 4H), 8.92 (s, 1H), 10.30 (s, 1H), 11.32 (s, 1H). Anal. ($C_{29}H_{29}N_7O_7$) C, H, N.

2-(5-Phenyl-1-pentyn-1-yl)-1-deoxy-1-[6-[[[(4-methoxyphenyl)amino] carbonyl]amino]-9H-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (62): chromatography solvent, $CHCl_3$−MeOH (94:6); yield 77%; white solid, mp 210−212 °C dec (EtOH); IR (NaCl) cm^{-1} 3450−3050, 2240, 1680, 1660, 1510, 1240; ^{1}H NMR (DMSO-d_6) δ 1.02−1.16 (m, 3H), 1.86−2.04 (m, 2H), 2.49−2.64 (m, 2H), 2.73−2.88 (m, 2H), 3.16−3.32 (m, 2H), 3.71 (s, 3H), 4.18−4.29 (m, 1H), 4.37 (s, 1H), 4.66 (pq, 1H), 6.07 (d, 1H, J = 7.0 Hz), 6.84 (d, 2H, J = 8.1 Hz), 7.17−7.38 (m, 5H), 7.41−7.54 (m, 2H), 8.39−8.52 (m, 1H), 8.83 (s, 1H), 10.32 (s, 1H), 11.50 (s, 1H). Anal. ($C_{31}H_{33}N_7O_6$) C, H, N.

Methods for Receptor Binding Assays. Procedures for preparation of rat brain membranes and CHO cell membranes were reported previously.[30,38,55] For binding experiments, membrane homogenates were frozen and stored at −20 °C for ≤2 months. Adenosine deaminase (ADA) was from Boehringer Mannheim (Indianapolis, IN). [^{3}H]*R*-PIA was from Amersham (Arlington Heights, IL), and [^{3}H]CGS 21680 was from DuPont NEN (Boston, MA). [^{125}I]-AB-MECA was prepared as described by Olah et al.[30]

Binding of [^{125}I]-AB-MECA to CHO cells stably transfected with the rat A_3 receptor clone or to HEK-293 cells stably expressing the human A_3 receptor was performed essentially as described.[28,30,38,55] Assays were performed in 50 mM Tris/10 mM $MgCl_2$/1 mM EDTA buffer (adjusted to pH 8.26 at 5 °C) in glass tubes containing 100 μL of the membrane suspension, 50 μL of [^{125}I]-AB-MECA (final concentration 0.3 nM), and 50 μL of inhibitor. Inhibitors were routinely dissolved in DMSO. Concentrations of DMSO in incubations never exceeded 1%, at which concentration [^{125}I]-AB-MECA binding was not affected. Incubations were carried out in duplicate for 1 h at 37 °C and were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). Tubes were washed three times with 3 mL of buffer. Radioactivity was determined in a Beckman gamma 5500B γ-counter. Nonspecific binding was determined in the presence of 200 μM NECA. K_i values were calculated according to Cheng−Prusoff[56] assuming a K_d for [^{125}I]-AB-MECA of 1.48 nM.

Binding of [^{3}H]*R*-PIA to A_1 receptors from rat cortical membranes and of [^{3}H]CGS 21680 to A_{2A} receptors from rat striatal membranes was performed as described previously.[57] Adenosine deaminase (2 units/mL) was present during the preparation of brain membranes. Additional deaminase was not added during incubation with the radioligand.

Binding of [^{35}S]GTP-γ-S. The binding of [^{35}S]GTP-γ-S (Amersham, Chicago, IL, specific activity 1275 Ci/mmol) was carried out using rat RBL-2H3 mast cell membranes by the general method of Lorenzen et al.[58] Membranes (approximately 5 μg) were suspended in a buffer containing 50 mM Tris (pH 7.4), 3 units/mL adenosine deaminase, 1 mM EDTA, 1 mM DTT, 10 μM GDP, 100 mM NaCl, and 10 mM $MgCl_2$. [^{35}S]GTP-γ-S was added to a final concentration of 0.1 nM in a total volume of 125 μL, and the mixture was incubated for 2 h at 30 °C. Nonspecific binding was determined in the presence of 10 μM GTP-γ-S (Sigma, St. Louis, MO). Incubation of the reaction mixture was terminated by filtration over GF/B glass filter using a Brandell cell harvester and washed twice with the same buffer.

Abbreviations: [^{125}I]AB-MECA, [^{125}I]1-[6-[[(4-amino-3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide; HENECA that 2-(1-hexyn-1-yl)-1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide; CHO cells, Chinese hamster ovary cells; CGS 21680, 2-[4-[(2-carboxyethyl)phenyl]ethyl-amino]1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide; Cl-IB-MECA, 2-Chloro-1-[6-[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide; *R*-PIA, 1-[6-[[(phenylisopropyl]amino]-9H-purin-9-yl]-1-deoxy-β-D-ribofuranose; DMSO, dimethysulfoxide; EDTA, ethylenediaminotetraacetate, I-AB-MECA, 1-[6-[[(4-amino-3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide; IB-MECA, 1-[6-[[(3-iodophenyl)methyl]amino]-9H-purin-9-yl]-1-deoxy-*N*-methyl-β-D-ribofuranuronamide; K_i, equilibrium inhibition constant; NECA, 1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide; PHPNECA, 2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-1-(6-amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide; THF, tetrahydrofuran; Tris, tris(hydroxymethyl)aminomethane.

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