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Exploring human adenosine A_3 receptor complementarity and activity for adenosine analogues modified in the ribose and purine moiety

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Abstract—In this paper we investigated the influence on affinity, selectivity and intrinsic activity upon modification of the adenosine agonist scaffold at the 3′- and 5′-positions of the ribofuranosyl moiety and the 2- and N^6-positions of the purine base. This resulted in the synthesis of various analogues, that is, **3**–**12** and **24**–**33**, with good hA_3AR selectivity and moderate-to-high affinities (as in **32**, K_i = 27 nM). Interesting was the ability to tune the intrinsic activity depending on the substituent introduced at the 3′-position.

1. Introduction

G protein-coupled receptors (GPCRs) with their typical seven-helix transmembrane (7TM) domains, constitute a large group of integral membrane proteins. Interacting with structurally diverse extracellular signals, GPCRs provide a molecular link for activation (or inhibition) of intracellular processes via given signal transduction pathways[1] and represent the most prominent family of validated drug targets.[2]

The regulatory actions of adenosine are mediated by four subtypes of GPCRs called adenosine receptors (ARs) that are ubiquitously expressed in the body and can be distinguished as A_1, A_{2A}, A_{2B} and A_3 receptors.[3] Activation of the A_3AR subtype, which is distributed in various organs (lung, liver, kidney, heart and brain),[4] has been shown to mediate adenylate cyclase inhibition[5] and phospholipase C[6] and D[7] stimulation. All four AR subtypes have been characterized on a pharmacological level as well as on a molecular level.[8] ARs from different species show a high degree of amino acid sequence homology (82–93%) with the only exception being the A_3AR subtype, which only exhibits 74% primary sequence homology between rat and human or sheep.[9,10] To be of therapeutic value, synthetic AR ligands need to be highly selective for a given receptor subtype and tissue targeted. Although several agonists have been synthesized that are selective for the known ARs subtypes,[3,11] so far the only AR agonist approved for clinical use is adenosine itself. In this paper we focus on the human (h)A_3AR subtype, the most recently identified member of the AR family.[12–14]

The A_3AR, plays a crucial role in some of the physiological effects of adenosine.[15,16] In addition to cardio-[17–19] and cerebroprotective effects,[20,21] hA_3AR agonists may be therapeutically useful for the treatment of stroke,[22] inflammation[23] and in cancer therapy.[24] While full agonists maximally stimulate the receptor, partial agonists show reduced intrinsic activity, may exhibit fewer side effects[25,26] and may induce less receptor down-regulation and desensitization than full agonists.[15] Partial A_3AR agonists can act as cardioprotective agents.[27] Selective antagonists for the A_3AR promise to be useful

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in the regulation of cell growth[28,29] and as anti-asthmatic,[30] cerebroprotective[20,31] and anti-inflammatory agents.[32]

Since its discovery in 1991,[12] the development of agonists of the A_3AR has been an active area of research. Many variations have been made on the adenosine scaffold in view of potent and selective A_3AR binding.[11,15,33] Generally, substitution at the 2′- and 8-positions has affinity- and efficacy-lowering effects.[11,15] Known A_3AR-selective alterations relevant to the work presented in this paper are N^6-modifications, such as 3-iodobenzyl (IB)[11,15,33,34] or 5-chloro-2-methoxybenzyl (CMB),[35] and smaller substituents like a Cl or CN at the 2-position[33–35] of the purine moiety. Both the 2- and N^6-purine modifications have been described with and without the 5′-methylcarbamoyl (MEC)[33] insertion in the nucleoside sugar moiety. Combinations of these groups are often additive in their potency enhancement and resulted in potent and moderately selective A_3AR agonists, such as Cl-IB-MECA and IB-MECA,[36] which are still used as reference tools for pharmacological study of the A_3AR.

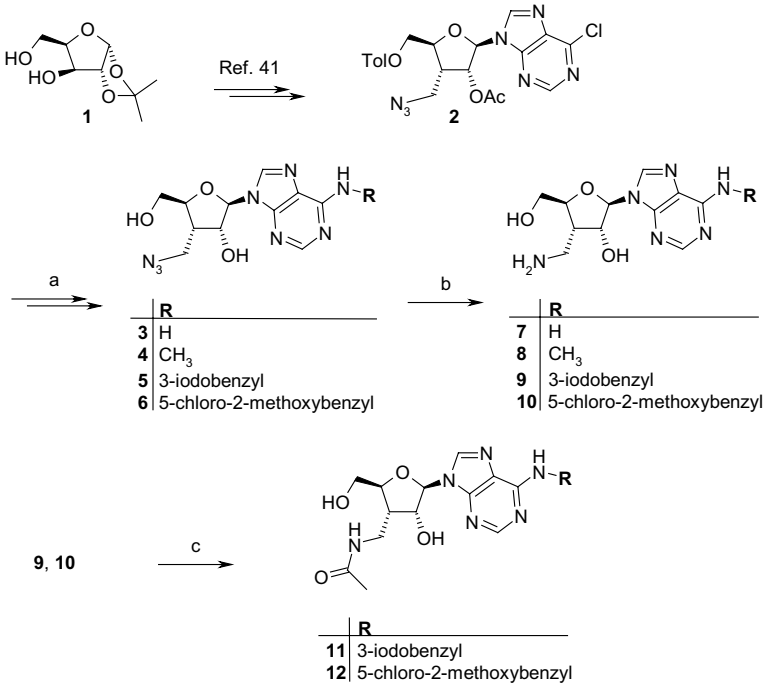
Investigated to a lesser extent are 3′-modifications of the ribofuranosyl moiety.[37,38] We and others have shown that a 3′-amino substitution opens perspectives towards influencing the hA_3AR selectivity.[39,40] This stimulated us to investigate the effect on both affinity and efficacy of this 3′-amino modification when combined with the above mentioned variations at the 5′-, 2- and N^6-positions (derivatives **27**, **29** and **32**). By introducing an α-oriented methylene spacer (the so-called branching) between the 3′-carbon of the ribofuranosyl moiety and the amine functional group (derivatives **7**–**10**, **28** and **33**), we aimed to modulate the hydrogen bond-donating effects of the 3′-amine, known to be crucial from our neoceptor work.[39,41] With this work we wanted to provide more insight into the effect of 3′, 5′-, 2- and N^6-positional variations of the adenosine nucleoside scaffold on hA_3AR affinity and to investigate the impact of such a combined substitution pattern on the hA_3AR efficacy.

2. Results and discussion

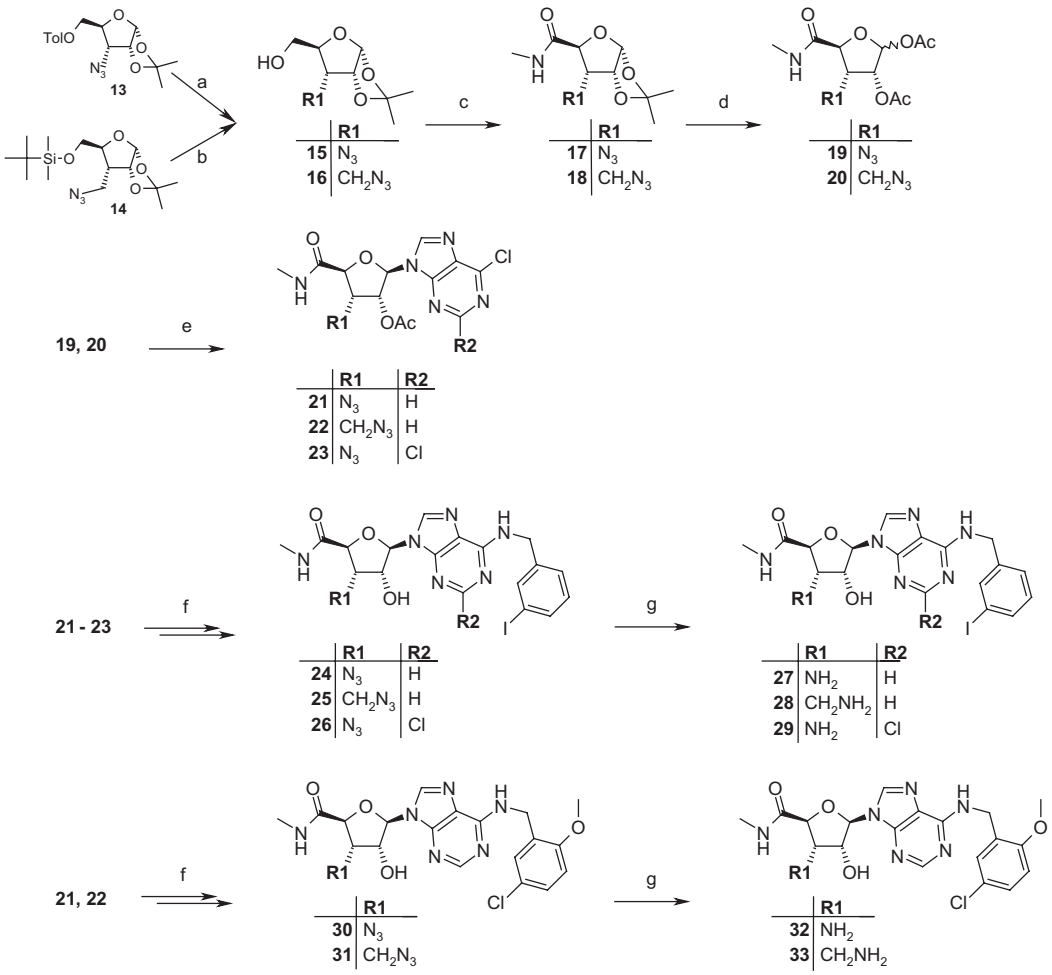
2.1. Chemistry

The synthesis of the simple 3′-branched N^6-modified adenosine analogues is depicted in Scheme 1. Starting from the commercially available 1,2-*O*-isopropylidene-D-xylofuranose (**1**) the 3′-*C*-azidomethyl synthon **2** was prepared according to our recently reported procedure.[41] Displacement of the 6-chloro atom with ammonia, methylamine, 3-iodobenzylamine or 5-chloro-2-methoxybenzylamine,[42] followed by deprotection with methanolic ammonia produced the 3′-*C*-azidomethyl nucleosides **3**–**6**. This chloropurine coupling strategy was found to be superior to the coupling with the N^6-modified purines.[39] Triphenylphosphine reduction of the azido moiety gave the 3′-*C*-aminomethyl nucleosides **7**, **8**, **9**[41] and **10**. Amidation of **9**, **10** was performed using an acyl chloride under Schotten–Baumann conditions and furnished derivatives **11** and **12**.

For the synthesis of the modified analogues in Scheme 2, the 3-α-azido (**15**[43,44]) and 3-*C*-α-azidomethyl (**16**[45]) sugars were obtained by simple 5′-deprotection of the previously described intermediates **13**[39] and **14**.[41]



Scheme 1. Reagents and conditions: (a) (i) amine HCl (or ammonia for **3**), Et_3N, EtOH, reflux; (ii) 7 N NH_3 in MeOH, rt; (b) Ph_3P, NH_4OH, pyridine, rt; (c) CH_3COCl, 50% aqueous NaOAc, THF, rt.



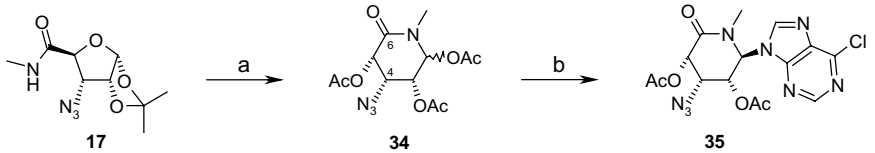
Scheme 2. Reagents and conditions: (a) 0.1 N $NaOCH_3$, MeOH, rt; (b) TBAF, THF, rt; (c) (i) $NaIO_4$, $RuCl_3$, $CHCl_3$–CH_3CN–H_2O (2:2:3), rt; (ii) dry MeOH, EDC, DMAP, rt; (iii) 2 M CH_3NH_2 in THF, 55 °C; (d) H_2SO_4, Ac_2O, AcOH, rt; (e) silylated 6-chloropurine or 2,6-dichloropurine, TMSOTf, dry 1,2-dichloroethane, reflux; (f) (i) amine HCl, Et_3N, EtOH, reflux (ii) 7 N NH_3 in MeOH, rt; (g) Ph_3P, NH_4OH, pyridine, rt.

Periodate oxidation[46] followed by esterification of the carboxylic acid and subsequent treatment with methylamine in a pressure tube[47] afforded the ribofuronamides **17**[40] and **18**. A one-pot deprotection–acetylation strategy afforded the peracylated sugar moieties **19** and **20** modified at the 3′,5′-positions.

As pointed out in Scheme 3, deprotection of **17** with 70% acetic acid and subsequent acetylation, using an acetic anhydride–pyridine (1:2) mixture, resulted in the rearrangement formation of compound **35** (via **34**). Vorbrüggen coupling[48] of **19** and **20** (Scheme 2) with silylated 6-chloropurine and 2,6-dichloropurine[49] quantitatively yielded the key synthons **21**–**23**. Selective displacement of the 6-chloro atom of **21**–**23** with 3-iodobenzylamine and 5-chloro-2-methoxybenzylamine, followed by deprotection with methanolic ammonia produced the 3′-azido (**24**, **26** and **30**) and 3′-*C*-azidomethyl (**25** and **31**) nucleosides. Triphenylphosphine reduction of the azido moieties smoothly furnished the respective amino nucleosides **27**–**29**, **32** and **33**.

2.2. Biological activity

Modifications of the adenosine scaffold known to increase hA_3AR binding affinity and selectivity among adenosine agonists include: a 5′-uronamide moiety (as in **24**–**33**) and substitutions at the 2- (as in **26** and **29**) and N^6-positions (as in **4**–**6** and **8**–**12** and **24**–**33**). In this paper we investigated the influence on hA_3AR affinity



Scheme 3. Reagents and conditions: (a) (i) 70% HOAc, 60 °C; (ii) Ac_2O–pyridine (1:2), rt; (b) silylated 6-chloropurine, TMSOTf, dry 1,2-dichloroethane, reflux.

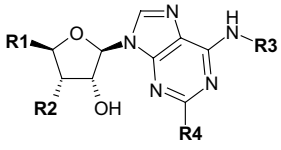
and intrinsic activity of combining these 5′-, 2- and N^6-modifications with the amino(methyl) substitution at the 3′-position that we[39,41] and others[40] recently reported.

Generally, substitution of the 2′- and 3′-hydroxyl groups of the ribofuranose moiety of AR agonists has been avoided. It has been demonstrated that modification of the 2′-position, compared to the 3′-position, had a negative impact on both potency and intrinsic activity.[37,38,50] However, a 3′-amino modification was recently shown to be beneficial for hA_3AR selectivity depending on the overall substitution pattern of the adenosine nucleoside.[40] This prompted us to investigate the boundaries of this 3′-amino substitution by insertion of a methylene spacer between the ribofuranose ring and the amine functional group.

2.2.1. Affinity and selectivity. Looking at the analogues that exhibited <1 μM affinities (Table 1), it was clear that the 5′-uronamide modification for both the direct and branched-chain amine improved the overall affinity. All compounds evaluated showed very good selectivity for the hA_3AR subtype. In the simple 3′-amino series the most potent compound **32** (K_i = 27 nM) showed a 300-fold selectivity over the A_1AR, compared to the 22-fold selectivity for its N^6-iodobenzyl substituted analogue **27**. Introduction of a chloro atom at the 2-position resulted in the selective and moderately potent (K_i = 132 nM) partial agonist **29**. All branched-chain analogues on the other hand had a good hA_3AR selectivity profile, but displayed weak binding characteristics, for example, analogue **33** with a K_i of 557 nM having the highest affinity in this series. Introduction of the methylene spacer also affected intrinsic activity (see Section 2.2.2). In both the 3′-amino and 3′-aminomethyl series the affinity of the azido precursors was lower. This affinity difference was striking especially for the N^6-iodobenzyl substituted analogues: **24** (K_i = 2260 nM) vs **27** (K_i = 137 nM) and **26** (K_i = 4270 nM) vs **29** (K_i = 132 nM).

Focusing on the N^6-substituents, known to be important for hA_3AR selectivity,[36] we observed a difference between the simple 3′-amino and branched-chain 3′-aminomethyl series, depending on the modification at the 5′-position. In the 5′-hydroxy 3′-amino series, 3′-amino-

Table 1. Binding affinity (A_1R, $A_{2A}R$ or A_3R) or functional activation ($A_{2B}R$ and A_3R) of the adenosine derivatives at human adenosine receptors, n = 3, unless noted



Compound	R1	R2	R3	R4	hA_1AR[a]	$hA_{2A}AR$[b]	$hA_{2B}AR$[c]	hA_3AR[d]	cAMP hA_3AR[g]
[h]	CH_2OH	NH_2	H	H	14%	6%	—	442,000 ± 121,000	53 ± 5
3	CH_2OH	CH_2N_3	H	H	25%	38%	—	53 ± 1%	1.1 ± 0.9
7	CH_2OH	CH_2NH_2	H	H	39%	33%	—	43 ± 1%	23 ± 8
4	CH_2OH	CH_2N_3	CH_3	H	69%	51%	—	20,600[e]	2.7 ± 1.6
8	CH_2OH	CH_2NH_2	CH_3	H	42%	22%	—	48 ± 3%	48 ± 6
[h]	CH_2OH	NH_2	3-IB	H	93%	70%	—	870 ± 180	—
5	CH_2OH	CH_2N_3	3-IB	H	27%	28%	—	61 ± 1%	6.8 ± 1.7
9	CH_2OH	CH_2NH_2	3-IB	H	72%	22%	—	8700[e]	9.2 ± 2.1
11	CH_2OH	CH_2NHAc	3-IB	H	38%	0%	—	31,500[e]	20 ± 5
10	CH_2OH	CH_2NH_2	CMB	H	61%	20%	0%	13,800 ± 1400	18 ± 3
12	CH_2OH	CH_2NHAc	CMB	H	—	—	—	4%[f]	1.4 ± 0.2
24	MeUron	N_3	3-IB	H	64%	32%	0%	2260 ± 480	0
27	MeUron	NH_2	3-IB	H	3080 ± 380	67%	20%	137 ± 41	37 ± 5
25	MeUron	CH_2N_3	3-IB	H	24%	0%	0%	23 ± 1%	23 ± 4
28	MeUron	CH_2NH_2	3-IB	H	52%	6%	0%	1690 ± 330	17 ± 2
26	MeUron	N_3	3-IB	Cl	70%	29%	0%	4270 ± 1400	0
29	MeUron	NH_2	3-IB	Cl	73%	50%	0%	132 ± 60	30 ± 6
30	MeUron	N_3	CMB	H	66%	34%	13%	376 ± 83	0
32	MeUron	NH_2	CMB	H	8190 ± 750	49%	7%	27 ± 11	51 ± 4
31	MeUron	CH_2N_3	CMB	H	54%	43%	19%	755 ± 40	0
33	MeUron	CH_2NH_2	CMB	H	52%	2%	2%	557 ± 164	30 ± 6

[a] K_i (nM) or % inhibition of binding at 100 μM ([^{3}H]R-PIA, 2.0 nM) in CHO cells expressing hA_1AR.
[b] % Inhibition of binding at100 μM ([^{3}H]CGS21680, 15 nM) in HEK-293 cells expressing $hA_{2A}AR$.
[c] % activation (cAMP assay) at100 μM in CHO cells expressing $hA_{2B}AR$ (NECA is 100%).
[d] K_i (nM) or % inhibition at 100 μM ([^{125}I]I-AB-MECA, 0.5 nM) in CHO cells expressing hA_3AR, unless noted.
[e] n = 1.
[f] At 10 μM.
[g] % Inhibition at 100 μM of forskolin-stimulated cAMP production at 10 μM, in CHO cells expressing the hA_3AR, as a percentage of the response of the full agonist Cl-IB-MECA (n = 2).
[h] Affinities previously reported in Ref. 39.

N^6-iodobenzyladenosine (K_i = 870 nM) showed a significant 500-fold potency enhancement compared to 3′-aminoadenosine (K_i = 442 μM). In the 5′-hydroxy branched-chain series (as in **3**–**12**), however, contrary to what we recently reported for simple N^6-substituted adenosine analogues,[35] N^6-(5-chloro-2-methoxybenzyl) substitution (as in **10**, K_i = 13.8 μM) did not improve affinity over the N^6-iodobenzyl modification (as in **9**, K_i = 8.7 μM). The reduction of hA_3AR affinity upon acetamide formation (as in analogues **11** and **12**) indicated that introduction of a 3′-branching was the sterically maximal allowed modification.

In the 5′-uronamide series, both for the 3′-amino and branched-chain 3′-aminomethyl analogues, the influence of N^6-substitution on hA_3AR binding was consistent with our previous findings,[35] that is, N^6-iodobenzyl (as in **27**, **28** and **29** with K_i = 137 nM, 1.7 μM and 132 nM, respectively), was less affinity-enhancing than N^6-(5-chloro-2-methoxybenzyl) (as in **32** and **33** with K_i = 27 and 557 nM, respectively).

2.2.2. Intrinsic activity. The results of the cyclic AMP-assay (Table 1) indicated that all analogues were strong partial agonists at best. In the simple 5′-hydroxy 3′-amino series, 3′-aminoadenosine and 3′-amino-N^6-iodobenzyladenosine are known full agonists.[39] The 5′-uronamide analogues **27** and **32** were partial agonists, contrary to what was reported earlier.[40]

In the 3′-branched-chain series, comparison of the 5′-hydroxy derivatives **5**, **9** and **10** with the 5′-uronamides **25**, **28** and **33**, demonstrated a moderate influence of the 5′-methyluronamide modification on intrinsic activity. Thus, in general, introduction of a 3′-branching reduced the efficacy and, contrary to efficacy-reducing substitutions at the N^6- and 2-positions,[33,38] this effect could only partially be overcome by uronamide modification of the 5′-position.

A modification known to have contradictory effects on A_3AR binding and intrinsic activity is the introduction of a chlorine at the 2-position.[33] Comparing **27** with the 2-chloro substituted **29**, this modification did not alter the affinity nor the efficacy in this series.

An overall conclusion is that, contrary to the 3′-aminomethyl modification, the simple 3′-amino is better tolerated in terms of affinity and efficacy, resulting at best in (strong) partial agonists with moderate binding properties (as in analogues **27**, **29** and **32**) and low hA_3AR affinity antagonists for the branched-chain derivatives, with exception of **33**. In both series, the azido precursors (as in compounds **3**–**5**, **24**–**26, 30** and **31**) were full antagonists.

The 3′-amino modification has hydrogen bond donor properties, like the 3′-hydroxyl group,[33,39] whereas for the 3′-azido, like the 3′-F,[37] this hydrogen bond pattern is no longer possible, resulting in a drop of efficacy.[38] For the branched-chain series, this difference in efficacy between the 3′-azidomethyl and 3′-aminomethyl analogues was less clear, which is most likely due to the steric impact of this modification.

3. Conclusion

From a pharmacological point of view the modulation of hA_3AR activity by selective agonists, partial agonists and antagonists is very important. In this paper we investigated the influence on affinity, selectivity and intrinsic activity of combined modifications at the 3′- and 5′-positions of the ribofuranosyl moiety with purine modifications at the 2- and N^6-positions. Various synthetic analogues, that is, **3**–**12** and **24**–**33**, displayed good hA_3AR selectivity and moderate-to-high affinities. More interesting, however, was the ability to tune the efficacy depending on the substituent introduced at the 3′-position. A 3′-amino function (as in **27**, **29** and **32**) resulted in (strong) partial agonist activity, whereas the azide precursors (as in **24**, **26** and **30**) converted these analogues into antagonists. Introduction of a methylene spacer between these functionalities and the ribofuranose ring (as in **3**–**12**, **25**, **28**, **31** and **33**) had an overall efficacy- and affinity-lowering effect.

The (branched-chain) amino and azido modifications at the 3′-position presented herein, open interesting perspectives towards tuning the efficacy and selectivity for the A_3AR, starting from the adenosine nucleoside agonist scaffold. The analogues reported in this paper also represent valuable tools for the further exploration of the neoceptor concept, that is, investigation of molecular complementarity at mutant A_{2A}[41] and A_3[39] adenosine receptors.

4. Experimental part

4.1. Synthesis

^1H NMR spectra were obtained with a Varian 300 MHz spectrometer. The solvent signal of $CDCl_3$ (7.26 ppm) and DMSO-d_6 (2.50 ppm) were used as a secondary reference. Assignment of all ^1H-resonances was confirmed by 2D ^1H–^1H COSY experiments. All signals assigned to amino and 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, UK) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol–water (1:1) mixture at 3 μL/min. Elemental analyses were performed at the University of Konstanz, Germany, and are within ±0.4% of theoretical values unless otherwise specified.

4.2. General procedure for the synthesis of the N^6-substituted nucleosides 3–6, 24–26, 30 and 31 from the chloropurine derivatives 2, 21, 22 and 23

An amount of the appropriate chloropurine and the appropriate amine salt (or ammonia in the case of **3**) (1.5 equiv), were dissolved in EtOH (15 mL/mmol chloropurine) containing Et_3N (1.25 equiv). The reaction mixture was refluxed overnight and evaporated to dryness. The residue was dissolved in 7 N NH_3 in MeOH (ca. 30 mL), stirred for 24 h at room temperature and

evaporated in vacuo. Precipitation from MeOH and subsequent filtration for **3**–**6** or purification by silica gel chromatography (CH_2Cl_2–MeOH) for **24**–**26**, **30** and **31** furnished the desired product as a white pure solid.

4.3. 9-(3-*C*-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-adenine (3)

Compound **2** (300 mg, 0.62 mmol) yielded 80 mg (42%) of **3**: ^{1}H NMR (DMSO-d_6) δ 2.58–2.67 (m, 1H, H-3′), 3.44 (dd, 1H, J = 5.7 Hz and −12.4 Hz, 3′-CH_b), 3.53 (ddd, 1H, J = 3.8 Hz and 5.9 Hz and −12.2 Hz, H-5B′), 3.65 (dd, 1H, J = 8.2 Hz, 3′-CH_a), 3.73 (ddd, 1H, J = 3.0 Hz and 5.1 Hz, H-5A′), 3.99 (dt, 1H, J = 3.2 Hz and 8.6 Hz, H-4′), 4.54–4.58 (m, 1H, H-2′), 5.22 (t, 1H, J = 5.5 Hz, 5′-OH), 5.91 (d, 1H, J = 2.1 Hz, H-1′), 6.04 (d, 1H, J = 4.6 Hz, 2′-OH), 7.29 (s, 2H, 6-NH_2), 8.13 and 8.39 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for $C_{11}H_{15}N_8O_3$ $[M+H]^+$: 307.1267, found 307.1270. Anal. ($C_{11}H_{14}N_8O_3 \cdot 1/4H_2O$) C, H, N.

4.4. 9-(3-*C*-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-methyladenine (4)

Compound **2** (400 mg, 0.82 mmol) yielded 180 mg (68%) of **4**: ^{1}H NMR (DMSO-d_6) δ 2.58–2.68 (m, 1H, H-3′), 2.93 (br s, 3H, N^6–CH_3), 3.44 (dd, 1H, J = 5.6 Hz and −12.31 Hz, 3′-CH_b), 3.53 (ddd, 1H, J = 3.7 Hz, 5.7 Hz and −12.2 Hz, H-5B′), 3.64 (dd, 1H, J = 7.9 Hz, 3′-CH_a), 3.73 (ddd, 1H, J = 2.9 Hz and 5.3 Hz, H-5A′), 3.99 (dt, 1H, J = 3.2 Hz and 8.8 Hz, H-4′), 4.55 (dt, 1H, J = 2.1 Hz and 5.2 Hz, H-2′), 5.23 (t, 1H, J = 5.4 Hz, 5′-OH), 5.92 (d, 1H, J = 2.3 Hz, H-1′), 6.05 (d, 1H, J = 5.0 Hz, 2′-OH), 7.78 (s, 1H, N^6–H), 8.22 and 8.39 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for $C_{12}H_{17}N_8O_3$ $[M+H]^+$: 321.1423, found 321.1429. Anal. ($C_{12}H_{16}N_8O_3 \cdot 2/3H_2O$) C, H, N.

4.5. 9-(3-*C*-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-(3-iodobenzyl)adenine (5)

Compound **2** (340 mg, 0.69 mmol) yielded 227 mg (62%) of **5**: ^{1}H NMR (DMSO-d_6) δ 2.59–2.68 (m, 1H, H-3′), 3.44 (dd, 1H, J = 5.6 Hz and −12.3 Hz, 3′-CH_b), 3.54 (ddd, 1H, J = 3.7 Hz, 5.6 Hz and −12.3 Hz, H-5B′), 3.64 (dd, 1H, J = 8.2 Hz, 3′-CH_a), 3.73 (ddd, 1H, J = 2.9 Hz and 5.3 Hz, H-5A′), 4.00 (dt, 1H, J = 3.2 Hz and 8.6 Hz, H-4′), 4.55–4.59 (m, 1H, H-2′), 4.64 (br s, 2H, N^6–CH_2–Ar), 5.20 (t, 1H, J = 5.6 Hz, 5′-OH), 5.93 (d, 1H, J = 2.1 Hz, H-1′), 6.05 (d, 1H, J = 5.0 Hz, 2′-OH), 7.05 (t, 1H, J = 7.8 Hz, Ar–H-5), 7.34 (d, 1H, J = 7.9 Hz, Ar–H-6), 7.56 (d, 1H, J = 8.2 Hz, Ar–H-4), 7.70 (s, 1H, Ar–H-2), 8.20 and 8.44 (2s, 2H, H-2 and H-8), 8.46 (br s, 1H, N^6–H); exact mass (ESI-MS) calculated for $C_{18}H_{20}IN_8O_3$ $[M+H]^+$: 523.0704, found 523.0698. Anal. ($C_{18}H_{19}IN_8O_3$) C, H, N.

4.6. 9-(3-*C*-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-(5-chloro-2-methoxybenzyl) adenine (6)

Compound **2** (300 mg, 0.62 mmol) yielded 100 mg (35%) of **6**: ^{1}H NMR (DMSO-d_6) δ 2.61–2.70 (m, 1H, H-3′), 3.45 (dd, 1H, J = 5.7 Hz and −12.3 Hz, 3′-CH_b), 3.55 (ddd, 1H, J = 3.7 Hz, 5.4 Hz and −12.2 Hz, H-5B′), 3.65 (dd, 1H, J = 8.1 Hz, 3′-CH_a), 3.75 (ddd, 1H, J = 2.9 Hz and 5.0 Hz, H-5A′), 3.83 (s, 3H, Ar–OCH_3), 4.01 (dt, 1H, J = 3.2 Hz and 8.7 Hz, H-4′), 4.63 (m, 3H, H-2′ and N^6–CH_2–Ar), 5.16 (t, 1H, J = 5.4 Hz, 5′-OH), 5.95 (d, 1H, J = 2.1 Hz, H-1′), 6.01 (d, 1H, J = 5.0 Hz, 2′-OH), 7.01 (d, 1H, J = 8.8 Hz, Ar–H-3), 7.08 (br s, 1H, Ar–H-6), 7.24 (dd, 1H, J = 2.6 and 8.8 Hz, Ar–H-4), 8.19 (br s, 2H, H-2 and N^6–H), 8.44 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{19}H_{22}ClN_8O_4$ $[M+H]^+$: 461.1452, found 461.1460. Anal. ($C_{19}H_{21}ClN_8O_4$) C, H, N.

4.7. 9-[3-Azido-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl) adenine (24)

Compound **21** (300 mg, 0.79 mmol) yielded 250 mg (60%) of **24**; spectroscopic data of this compound in accordance with those reported in Ref. 40; Anal. ($C_{18}H_{18}IN_9O_3 \cdot 3/2\ H_2O$) C, H, N.

4.8. 9-[3-*C*-Azidomethyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl)adenine (25)

Compound **22** (300 mg, 0.75 mmol) yielded 360 mg (73%) of **25**: ^{1}H NMR (DMSO-d_6) δ 2.63 (d, 3H, J = 4.4 Hz, CH_3–N), 2.75–2.84 (m, 1H, H-3′), 3.50 (dd, 1H, J = 5.9 Hz and −12.3 Hz, 3′-CH_b), 3.71 (dd, 1H, J = 7.9 Hz, 3′-CH_a), 4.33 (d, 1H, J = 8.5 Hz, H-4′), 4.63 (m, 3H, H-2′ and N^6–CH_2–Ar), 6.03 (d, 1H, J = 2.1 Hz, H-1′), 6.15 (d, 1H, J = 4.7 Hz, 2′-OH), 7.09 (t, 1H, J = 7.8 Hz, Ar–H-5), 7.35 (d, 1H, J = 7.6 Hz, Ar–H-6), 7.57 (d, 1H, J = 7.9 Hz, Ar–H-4), 7.71 (s, 1H, Ar–H-2), 8.24 (m, 2H, H-2 and NH–CO), 8.43 (br s, 1H, N^6–H), 8.55 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{19}H_{21}IN_9O_3$ $[M+H]^+$: 550.0813, found 550.0803. Anal. ($C_{19}H_{20}IN_9O_3 \cdot 3/2H_2O$) C, H, N.

4.9. 2-Chloro-9-[3-azido-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl)adenine (26)

Compound **23** (200 mg, 0.48 mmol) yielded 210 mg (77%) of **26**: ^{1}H NMR (DMSO-d_6) δ 2.69 (d, 3H, J = 4.1 Hz, CH_3–N), 4.35 (d, 1H, J = 2.9 Hz, H-4′), 4.47 (t, 1H, J = 4.1 Hz, H-3′), 4.60 (br s, 2H, N^6–CH_2–Ar), 4.92 (app d, 1H, J = 4.9 Hz, H-2′), 5.92 (d, 1H, J = 6.2 Hz, H-1′), 6.31 (d, 1H, J = 4.7 Hz, 2′-OH), 7.12 (t, 1H, J = 7.8 Hz, Ar–H-5), 7.35 (d, 1H, J = 7.3 Hz, Ar–H-6), 7.59 (d, 1H, J = 7.9 Hz, Ar–H-4), 7.74 (s, 1H, Ar–H-2), 8.25 (d, 1H, J = 4.1 Hz, NH–CO), 8.49 (s, 1H, H-8), 8.99 (br s, 1H, N^6–H); exact mass (ESI-MS) calculated for $C_{18}H_{17}ClIN_9O_3Na$ $[M+Na]^+$: 592.0087, found 592.0092. Anal. ($C_{18}H_{17}ClIN_9O_3 \cdot 1/2H_2O$) C, H, N.

4.10. 9-[3-Azido-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(5-chloro-2-methoxybenzyl)adenine (30)

Compound **21** (300 mg, 0.79 mmol) yielded 251 mg (67%) of **30**: ^{1}H NMR (DMSO-d_6) δ 2.68 (d, 3H, J = 4.7 Hz, CH_3–N), 3.83 (s, 3H, Ar–OCH_3), 4.34 (d, 1H, J = 3.2 Hz, H-4′), 4.49 (dd, 1H, J = 3.2 Hz and

5.3 Hz, H-3′), 4.65 (br s, 2H, N^6–CH_2–Ar), 4.97 (q, 1H, J = 5.6 Hz and 11.1 Hz, H-2′), 6.00 (d, 1H, J = 6.5 Hz, H-1′), 6.29 (d, 1H, J = 5.3 Hz, 2′-OH), 7.00 (d, 1H, J = 9.08 Hz, Ar–H-3), 7.08 (br s, 1H, Ar–H-6), 7.24 (dd, 1H, J = 2.8 Hz and 8.6 Hz, Ar–H-4), 8.25 (s, 1H, H-2), 8.35 (br s, 1H, N^6–H), 8.47 (s, 1H, H-8), 8.62 (d, 1H, J = 4.7 Hz, NH–CO); exact mass (ESI-MS) calculated for $C_{19}H_{21}ClN_9O_4$ $[M+H]^+$: 474.1404, found 474.1400. Anal. ($C_{19}H_{20}ClN_9O_4 \cdot 1/2H_2O$) C, H, N.

4.11. 9-[3-Azidomethyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(5-chloro-2-methoxybenzyl)adenine (31)

Compound **22** (300 mg, 0.75 mmol) yielded 300 mg (82%) of **31**: ^{1}H NMR (DMSO-d_6) δ 2.63 (d, 3H, J = 4.4 Hz, CH_3–N), 2.76–2.85 (m, 1H, H-3′), 3.50 (dd, 1H, J = 5.9 Hz and −12.4 Hz, 3′-CH_b), 3.71 (dd, 1H, J = 7.8 Hz, 3′-CH_a), 3.83 (s, 3H, Ar–OCH_3), 4.32 (d, 1H, J = 8.8 Hz, H-4′), 4.60 (br s, 3H, N^6–CH_2–Ar and H-2′), 6.04 (s, 1H, H-1′), 6.21 (d, 1H, J = 4.7 Hz, 2′-OH), 7.00 (d, 1H, J = 8.8 Hz, Ar–H-3), 7.05 (br s, 1H, Ar–H-6), 7.24 (dd, 1H, J = 2.6 Hz and 8.5 Hz, Ar–H-4), 8.21 (s, 1H, H-2), 8.30 (d, 1H, J = 4.7 Hz, NH–CO), 8.36 (br s, 1H, N^6–H), 8.60 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{20}H_{23}ClN_9O_4$ $[M+H]^+$: 488.15661, found 488.1559. Anal. ($C_{20}H_{22}ClN_9O_4 \cdot 1/2H_2O$) C, H, N.

4.12. General procedure for the synthesis of the amino nucleosides 7–10, 27–29, 32 and 33 from their azido precursors 3–6, 24–26, 30 and 31

The azido nucleoside was dissolved in dry pyridine (8 mL/mmol) and PhP_3 (1.6 equiv) was added to the solution. After stirring at room temperature for 1.5 h, concentrated NH_4OH (3 mL/mmol) was added. The reaction mixture was stirred for another 2 h, evaporated to dryness and purified by silica gel chromatography (CH_2Cl_2–MeOH).

4.13. 9-(3-*C*-Aminomethyl-3-deoxy-β-D-ribofuranosyl)-adenine (7)

Compound **3** (60 mg, 0.20 mmol) furnished 35 mg (64%) of **7** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.31–2.40 (m, 1H, H-3′), 2.65 (dd, 1H, J = 6.2 Hz and −12.6 Hz, 3′-CH_b), 2.89 (dd, 1H, J = 7.3 Hz, 3′-CH_a), 3.56 (dd, 1H, J = 3.2 Hz and −11.9 Hz, H-5B′), 3.68 (dd, 1H, J = 4.0 Hz, H-5A′), 3.98 (dt, 1H, J = 3.7 Hz and 9.4 Hz, H-4′), 4.50 (dd, 1H, J = 1.5 Hz and 5.3 Hz, H-2′), 5.88 (d, 1H, J = 1.5 Hz, H-1′), 7.27 (s, 2H, 6-NH_2), 8.12 and 8.36 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for $C_{11}H_{17}N_6O_3$ $[M+H]^+$: 281.1362, found 281.1370. Anal. ($C_{11}H_{16}N_6O_3$) C, H, N.

4.14. 9-(3-*C*-Aminomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-methyladenine (8)

Compound **4** (100 mg, 0.31 mmol) furnished 64 mg (70%) of **8** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.26–2.35 (m, 1H, H-3′), 2.62 (dd, 1H, J = 6.5 Hz and −12.6 Hz, 3′-CH_b), 2.87 (dd, 1H, J = 7.0 Hz, 3′-CH_a), 2.93 (br s, 3H, N^6–CH_3), 3.56 (dd, 1H, J = 3.5 Hz and −11.9 Hz, H-5B′), 3.67 (dd, 1H, J = 4.3 Hz, H-5A′), 3.99 (dt, 1H, J = 3.8 Hz and 9.1 Hz, H-4′), 4.50 (dd, 1H, J = 1.6 Hz and 5.1 Hz, H-2′), 5.88 (d, 1H, J = 1.8 Hz, H-1′), 7.75 (s, 1H, N^6–H), 8.21 and 8.34 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for $C_{12}H_{19}N_6O_3$ $[M+H]^+$: 295.1518, found 295.1513. Anal. ($C_{12}H_{18}N_6O_3 \cdot 3/2H_2O$) C, H, N.

4.15. 9-(3-*C*-Aminomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-(3-iodobenzyl)adenine (9)

Compound **5** (200 mg, 0.38 mmol) furnished 131 mg (69%) of **9** as a white solid: spectroscopic data of this compound in accordance with those reported in Ref. 41; Anal. ($C_{18}H_{21}IN_6O_3$) C, H, N.

4.16. 9-(3-*C*-Aminomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-(5-chloro-2-methoxybenzyl) adenine (10)

Compound **6** (90 mg, 0.17 mmol) furnished 54 mg (64%) of **10** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.29–2.38 (m, 1H, H-3′), 2.65 (dd, 1H, J = 6.3 Hz and −12.5 Hz, 3′-CH_b), 2.89 (dd, 1H, J = 7.0 Hz, 3′-CH_a), 3.57 (dd, 1H, J = 3.5 Hz and −11.9 Hz, H-5B′), 3.69 (dd, 1H, J = 4.1 Hz, H-5A′), 3.83 (s, 3H, Ar–OCH_3), 4.01 (dt, 1H, J = 3.9 Hz and 9.0 Hz, H-4′), 4.55 (dd, 1H, J = 1.2 Hz and 5.0 Hz, H-2′), 4.65 (br s, 2H, N^6–CH_2–Ar), 5.91 (d, 1H, J = 1.5 Hz, H-1′), 7.00 (d, 1H, J = 8.8 Hz, Ar–H-3), 7.07 (d, 1H, J = 2.3 Hz, Ar–H-6), 7.24 (dd, 1H, J = 2.6 Hz and 8.8 Hz, Ar–H-4), 8.18 (br s, 2H, H-2 and N^6–H), 8.40 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{19}H_{24}ClN_6O_4$ $[M+H]^+$: 435.1547, found 435.1546. Anal. ($C_{19}H_{23}ClN_6O_4 \cdot 1/4H_2O$) C, H, N.

4.17. 9-[3-Amino-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl) adenine (27)

Compound **24** (176 mg, 0.33 mmol) furnished 30 mg (18%) of **27** as a white solid: spectroscopic data of this compound in accordance with those reported in Ref. 40; Anal. ($C_{18}H_{20}IN_7O_3 \cdot 1/2H_2O$) C, H, N.

4.18. 9-[3-*C*-Aminomethyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl)adenine (28)

Compound **25** (100 mg, 0.18 mmol) furnished 45 mg (48%) of **28** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.41–2.47 (m, 1H, H-3′), 2.64 (d, 3H, J = 4.7 Hz, CH_3–N), 2.76 (dd, 1H, J = 5.3 Hz and −12.3 Hz, 3′-CH_b), 2.90 (dd, 1H, J = 7.6 Hz, 3′-CH_a), 4.34 (d, 1H, J = 8.8 Hz, H-4′), 4.55 (dd, 1H, J = 2.1 Hz and 5.3 Hz, H-2′), 4.66 (br s, 2H, N^6–CH_2–Ar), 6.00 (d, 1H, J = 2.4 Hz, H-1′), 7.09 (t, 1H, J = 7.8 Hz, Ar–H-5), 7.35 (d, 1H, J = 7.9 Hz, Ar–H-6), 7.56 (d, 1H, J = 7.6 Hz, Ar–H-4), 7.71 (s, 1H, Ar–H-2), 8.22 (s, 1H, H-2), 8.29 (d, 1H, J=4.7 Hz, NH–CO), 8.42 (br s, 1H, N^6–H), 8.62 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{19}H_{23}IN_7O_3$ $[M+H]^+$: 524.0908, found 524.0912. Anal. ($C_{19}H_{22}IN_7O_3 \cdot 3/4H_2O$) C, H, N.

4.19. 2-Chloro-9-[3-amino-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(3-iodobenzyl)adenine (29)

Compound **26** (200 mg, 0.35 mmol) furnished 140 mg (74%) of **29** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.67 (d, 3H, J = 4.7 Hz, CH_3–N), 3.54 (t, 1H, J = 5.6 Hz, H-3′), 4.11 (d, 1H, J = 5.9 Hz, H-4′), 4.31 (t, 1H, J = 4.1 Hz, H-2′), 4.59 (br s, 2H, N^6–CH_2–Ar), 5.95 (d, 1H, J = 3.5 Hz, H-1′), 7.11 (t, 1H, J = 7.8 Hz, Ar–H-5), 7.35 (d, 1H, J = 7.6 Hz, Ar–H-6), 7.59 (d, 1H, J = 7.6 Hz, Ar–H-4), 7.73 (s, 1H, Ar–H-2), 8.15 (d, 1H, J = 4.1 Hz, NH–CO), 8.61 (s, 1H, H-8), 8.93 (br s, 1H, N^6–H); exact mass (ESI-MS) calculated for $C_{18}H_{20}ClIN_7O_3$ $[M+H]^+$: 544.0362, found 544.0366. Anal. ($C_{18}H_{19}ClIN_7O_3$) C, H, N.

4.20. 9-[3-Amino-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(5-chloro-2-methoxybenzyl)adenine (32)

Compound **30** (176 mg, 0.33 mmol) furnished 29 mg (20%) of **32** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.67 (d, 3H, J = 4.7 Hz, CH_3–N), 3.57 (t, 1H, J = 5.4 Hz, H-3′), 3.83 (s, 3H, Ar–OCH_3), 4.12 (d, 1H, J = 5.9 Hz, H-4′), 4.38 (t, 1H, J = 4.5 Hz, H-2′), 4.65 (br s, 2H, N^6–CH_2–Ar), 6.02 (d, 1H, J = 4.1 Hz, H-1′), 7.00 (d, 1H, J = 8.8 Hz, Ar–H-3), 7.08 (br s, 1H, Ar–H-6), 7.20 (dd, 1H, J = 2.6 Hz and 8.8 Hz, Ar–H-4), 8.23 (s, 1H, H-2), 8.29 (br s, 1H, N^6–H), 8.44 (d, 1H, J = 5.0 Hz, NH–CO), 8.57 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{19}H_{23}ClN_7O_4$ $[M+H]^+$: 448.1499, found 448.1483. Anal. ($C_{19}H_{22}ClN_7O_4$·3/2H_2O) C, H, N.

4.21. 9-[3-Aminomethyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N^6-(5-chloro-2-methoxybenzyl)adenine (33)

Compound **31** (150 mg, 0.31 mmol) furnished 100 mg (70%) of **33** as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.40–2.48 (m, 1H, H-3′), 2.64 (d, 3H, J = 4.7 Hz, CH_3–N), 2.76 (dd, 1H, J = 5.1 Hz and −12.8 Hz, 3′-CH_b), 2.90 (dd, 1H, J = 7.9 Hz, 3′-CH_a), 3.83 (s, 3H, Ar–OCH_3), 4.34 (d, 1H, J = 9.1 Hz, H-4′), 4.55 (dd, 1H, J = 1.6 Hz and 5.1 Hz, H-2′), 4.62 (br s, 2H, N^6–CH_2–Ar), 6.01 (d, 1H, J = 1.8 Hz, H-1′), 7.00 (d, 1H, J = 8.8 Hz, Ar–H-3), 7.04 (s, 1H, Ar–H-6), 7.24 (dd, 1H, J = 2.6 Hz and 8.8 Hz, Ar–H-4), 8.20 (s, 1H, H-2), 8.35 (br d, 2H, J = 4.7 Hz, NH–CO and N^6–H), 8.68 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{20}H_{25}ClN_7O_4$ $[M+H]^+$: 462.1652, found 462.1657. Anal. ($C_{20}H_{24}ClN_7O_4$·3/2H_2O) C, H, N.

4.22. Procedure for the amidation of 9 and 10 under Schotten–Baumann conditions

To a solution of the appropriate amine in THF (10 mL/mmol) were added 50% aqueous NaOAc solution (10 mL/mmol) and an acetyl chloride (0.9 equiv). After completion of reaction (6 h), THF and brine were added. The organic phase was separated, washed with water, dried over $MgSO_4$ and concentrated in vacuo. Precipitation from MeOH and subsequent filtration furnished the product as a white solid in 60–63% yield.

4.23. 9-(3-Acetamidomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-(3-iodobenzyl)adenine (11)

Compound **9** (70 mg, 0.14 mmol) yielded 48 mg (63%) of **11**: ^{1}H NMR (DMSO-d_6) δ 1.77 (s, 3H, CH_3), 2.46 (m, 1H, H-3′), 3.08–3.16 (m, 1H, 3′-CH_b), 3.24–3.30 (m, 1H, 3′-CH_a), 3.54 (ddd, 1H, J = 3.4 Hz, 5.3 Hz and −12.5 Hz, H-5B′), 3.77 (ddd, 1H, J = 2.6 Hz and 5.0 Hz, H-5A′), 3.97 (dt, 1H, J = 2.8 Hz and 9.4 Hz, H-4′), 4.41 (t, 1H, J = 4.0 Hz, H-2′), 4.63 (br s, 2H, N^6–CH_2–Ar), 5.22 (t, 1H, J = 5.3 Hz, 5′-OH), 5.85 (d, 1H, J = 4.4 Hz, H-1′), 5.93 (d, 1H, J = 1.2 Hz, 2′-OH), 7.09 (t, 1H, J = 7.8 Hz, Ar–H-5), 7.34 (d, 1H, J = 7.6 Hz, Ar–H-6), 7.56 (d, 1H, J = 7.9 Hz, Ar–H-4), 7.70 (s, 1H, Ar–H-2), 7.89 (t, 1H, J = 5.4 Hz, 3′-C–NH), 8.20 (s, 1H, H-2), 8.47 (2s, 2H, H-8 and N^6–H); exact mass (ESI-MS) calculated for $C_{20}H_{24}$ IN_6O_4 $[M+H]^+$: 539.0905, found 539.0890. Anal. ($C_{20}H_{23}IN_6O_4$·3/2H_2O) C, H, N.

4.24. 9-(3-Acetamidomethyl-3-deoxy-β-D-ribofuranosyl)-N^6-(5-chloro-2-methoxybenzyl) adenine (12)

Compound **10** (10 mg, 0.023 mmol) yielded 6.6 mg (60%) of **11**: ^{1}H NMR (DMSO-d_6) δ 1.79 (s, 3H, CH_3), 3.10-3.19 (m, 1H, H-3′), 3.27–3.36 (m, 1H, 3′-CH_b), 3.51–3.60 (m, 1H, 3′-CH_a), 3.77–3.84 (m, 5H, H-5B′, H-5A′ and Ar–OCH_3), 4.00 (m, 1H, H-4′), 4.45 (app s, 1H, H-2′), 4.65 (br s, 2H, N^6–CH_2–Ar), 5.22 (t, 1H, J = 5.1 Hz, 5′-OH), 5.87 (d, 1H, J = 4.4 Hz, H-1′), 5.95 (d, 1H, J = 1.5 Hz, 2′-OH), 7.02 (d, 1H, J = 8.8 Hz, Ar–H-3), 7.07 (s, 1H, Ar–H-6), 7.25 (dd, 1H, J = 2.8 Hz and 8.7 Hz, Ar–H-4), 7.90 (t, 1H, J = 5.3 Hz, 3′-C–NH), 8.20 (s, 1H, H-2), 8.26 (br s, 1H, N^6–H), 8.49 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{21}H_{26}ClN_6O_5$ $[M+H]^+$: 477.1653, found 477.1641. Anal. ($C_{21}H_{25}ClN_6O_5$·1/2H_2O) C, H, N.

4.25. Methyl 3-azido-3-deoxy-1,2-isopropylidene-α-D-ribofuronamide (17)[40]

A biphasic mixture of water (46 mL), $CHCl_3$ (31 mL) and acetonitrile (31 mL) containing compound **15**[43,44] (3.3 g, 15.33 mmol), $RuCl_3$ hydrate (160 mg, 0.77 mmol) and $NaIO_4$ (13.45 g, 62.87 mmol) was vigorously stirred for 4.5 h at room temperature. The reaction mixture was then diluted with water (100 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic phase was dried over $MgSO_4$, filtrated and evaporated. A dark green oily residue was triturated with diethyl ether to precipitate the ruthenium salts, that were removed by filtration through celite. The filtrate was concentrated in vacuo, leaving 2.7 g (76.8%) 3-azido-3-deoxy-1,2-isopropylidene-α-D-ribofuronic acid as a lightly coloured oil that was used without further purification. A mixture of 3-azido-3-deoxy-1,2-isopropylidene-α-D-ribofuronic acid (2.5 g, 11.78 mmol), EDC (5.2 mL, 29.5 mmol) and DMAP (145 mg, 1.2 mmol) in anhydrous methanol (50 mL) was stirred at room temperature for 24 h. The reaction mixture was concentrated to dryness and the residue was dissolved in CH_2Cl_2 (200 mL) and water (100 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 200 mL) and the combined organic phase

was dried over $MgSO_4$, filtered and evaporated. The residue was dissolved in 2 M methylamine in THF (20 mL) and was heated for 24 h at 55 °C in a sealed tube. After cooling, the reaction mixture was concentrated to dryness and purified by silica gel chromatography (pentane–EtOAc) to give **17** (1.2 g, 32.5%) as a transparent oil. Spectroscopic data of this compound in accordance with those reported in Ref. 40.

4.26. Methyl 3-azidomethyl-3-deoxy-1,2-isopropylidene-α-D-ribofuronamide (18)

Compound **18** (1.3 g, 32%) was prepared from **16**[45] (3.6 g, 15.7 mmol) in analogy to the procedure described for **17**: ^{1}H NMR ($CDCl_3$) δ 1.34 and 1.49 (2s, 6H, $2\times CH_3$), 2.15–2.25 (m, 1H, H-3), 2.80 (d, 3H, J = 5.0 Hz, CH_3–N), 3.64 (dd, 1H, J = 11.1 Hz and −12.1 Hz, 3–CH_b), 3.87 (dd, 1H, J = 4.3 Hz, 3–CH_a), 4.15 (d, 1H, J = 10.6 Hz, H-4), 4.73 (t, 1H, J = 3.8 Hz, H-2), 5.85 (d, 1H, J = 5.5 Hz, H-1), 6.50 (br s, 1H, N*H*–CO); exact mass (ESI-MS) calculated for $C_{10}H_{17}N_4O_4$ $[M+H]^+$: 257.1249, found 257.1253.

4.27. 9-[2-*O*-Acetyl-3-*C*-azido-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranose]-6-chloropurine (21)

A mixture of **17** (1.2 g, 5 mmol), concentrated sulfuric acid (1.47 mL, 27.5 mmol) and acetic anhydride (4.95 mL, 52.4 mmol) in glacial acid (25 mL) was stirred for 18 h at room temperature. After cooling in an ice bath, saturated $NaHCO_3$ solution (50 mL) and CH_2Cl_2 (50 mL) were slowly added and the mixture was stirred for another 10 min. After separation the aqueous phase was extracted with CH_2Cl_2 (3×100 mL). The combined organic phase was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, filtered and evaporated to dryness to yield 787 mg (55%) of the crude methyl 3-azido-3-deoxy-1,2-diacetyl-α-D-ribofuronamide (**19**) as a yellowish foam: ^{1}H NMR (DMSO-d_6) δ 2.01 and 2.10 (2s, 6H, $2\times CH_3$), 2.79 (d, 3H, J = 5.0 Hz, CH_3–N), 4.33–4.43 (m, 2H, H-3 and H-4), 5.24 (dd, 1H, J = 0.8 Hz and 5.0 Hz, H-2), 6.07 (br s, 1H, H-1), 6.53 (d, 1H, J = 4.7 Hz, N*H*–CO). Compound **19** (0.78 g, 2.72 mmol) was used to prepare the title compound (987 mg, 95%) in analogy to the procedure described for **2**. Spectroscopic data of this compound in accordance with those reported in Ref. 40.

4.28. 9-[2-*O*-Acetyl-3-*C*-azidomethyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranose]-6-chloropurine (22)

From **18** (1.3 g, 5 mmol), in analogy to the procedure described for **19**, the methyl 3-azidomethyl-3-deoxy-1,2-diacetyl-α-D-ribofuronamide (0.6 g, 40%) was prepared: ^{1}H NMR ($CDCl_3$) δ 2.06 and 2.13 (2s, 6H, $2\times CH_3$), 2.74–2.77 (m, 1H, H-3), 2.81 (d, 3H, J = 5.0 Hz, CH_3–N), 3.55 (dd, 1H, J = 10.5 Hz and −12.3 Hz, 3-CH_b), 3.91 (dd, 1H, J = 4.4 Hz, 3-CH_a), 4.28 (d, 1H, J = 9.7 Hz, H-4), 5.23 (d, 1H, J = 4.7 Hz, H-2), 6.12 (s, 1H, H-1), 6.41 (d, 1H, J = 4.1 Hz, N*H*–CO). This acetylated ribofuronamide (0.6 g, 2 mmol) was used to prepare the title compound (750 mg, 95%) in analogy to the procedure described for **2**. ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, CH_3), 2.83 (d, 3H, J = 5.0 Hz, CH_3–N), 3.38–3.48 (m, 1H, H-3′), 3.73 (dd, 1H, J = 9.1 Hz and −12.6 Hz, 3′-CH_b), 3.87 (dd, 1H, J = 4.4 Hz, 3′-CH_a), 4.48 (d, 1H, J = 9.4 Hz, H-4′), 5.62 (dd, 1H, J = 2.5 Hz and 6.6 Hz, H-2′), 6.09 (d, 1H, J = 2.3 Hz, H-1′), 6.98 (d, 1H, J = 4.4 Hz, N*H*–CO), 8.22 and 8.75 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for $C_{14}H_{16}ClN_8O_4$ $[M+H]^+$: 395.0982, found 395.0982.

4.29. 9-[2-*O*-Acetyl-3-*C*-azido-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranose]-2,6- dichloropurine (23)

Compound **19** (200 mg, 0.69 mmol) was coupled with silylated 2,6-dichloropurine in analogy to the procedure described for **2**, to yield the the title compound **23** (200 mg, 70%): ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3), 2.63 (d, 3H, J = 4.7 Hz, CH_3–N), 4.52 (d, 1H, J = 5.6 Hz, H-4′), 4.93 (t, 1H, J = 5.6 Hz, H-3′), 5.96 (t, 1H, J = 4.7 Hz, H-2′), 6.37 (d, 1H, J = 3.8 Hz, H-1′), 8.16 (d, 1H, J = 4.4 Hz, N*H*–CO), 8.99 (s, 1H, H-8); exact mass (ESI-MS) calculated for $C_{13}H_{12}Cl_2N_8O_4$ $[M+H]^+$: 437.0256, found 437.0252.

4.30. Acetic acid 5-acetoxy-4-azido-2-(6-chloro-purin-9-yl)-1-methyl-6-oxo-piperidin-3-yl ester (35)

Compound **17** (0.75 g, 3.0 mmol) was dissolved in 70% HOAc (30 mL). The solution was kept at 60 °C and after 48 h the reaction mixture was evaporated to dryness. The residue was dissolved in a acetic anhydride–pyridine (2:1) mixture (50 mL). After 3 h, the mixture was partitioned between CH_2Cl_2 (100 mL) and 7% $NaHCO_3$ (150 mL). The aqueous layer was washed with CH_2Cl_2 (150 mL) and the combined organic layers were dried with $MgSO_4$, filtered and evaporated in vacuo. Treatment with a hexane–EtOAc mixture allowed precipitation of 0.6 g (61%) of acetic acid 3,5-diacetoxy-4-azido-6-oxo-piperidin-2-yl ester (**34**) as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.11, 2.16 and 2.34 (3s, 9H, CH_3), 2.91 (s, 3H, N–CH_3), 4.35 (t, 1H, J = 3.4 Hz, H-4), 5.24 (dd, 1H, J = 3.8 Hz and 4.7 Hz, H-3), 5.57 (d, 1H, J = 2.9 Hz, H-5), 6.09 (d, 1H, J = 5.0 Hz, H-2). Compound **34** (0.58 g, 1.8 mmol) was used to prepare the title compound (710 mg, 93%) in analogy to the procedure described for **2**. ^{1}H NMR ($CDCl_3$) δ 1.99 and 2.30 (2s, 6H, $2\times CH_3$), 2.66 (s, 3H, N–CH_3), 4.51 (t, 1H, J = 2.8 Hz, H-3′), 5.82 (d, 1H, J = 7.9 Hz, H-5′), 5.97 (dd, 1H, J = 2.6 Hz and 7.9 Hz, H-4′), 6.01 (d, 1H, J = 2.9 Hz, H-2′), 8.24 and 8.79 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for $C_{15}H_{16}ClN_8O_5$ $[M+H]^+$: 423.0932, found 423.0934.

4.31. Elemental analysis

This is given in Table 2.

4.32. Biological assays

4.32.1. Cell culture and membrane preparation. CHO cells expressing recombinant human and rat A_3ARs were cultured in DMEM (Dulbecco's modified Eagle's medium) and F12 (1:1) supplemented with 10% fetal

Table 2. Elemental analysis of evaluated derivatives

Compound	Calculated, %			Found, %		
	C	H	N	C	H	N
3	42.51	4.70	36.05	42.86	4.60	35.84
4	43.37	5.26	33.72	43.62	4.99	33.55
5	41.39	3.67	21.45	41.40	4.00	21.11
6	49.52	4.59	24.31	49.60	4.73	24.11
7	47.14	5.75	29.98	47.05	5.88	29.64
8	44.85	6.59	26.15	44.95	6.58	26.35
9[41]	43.56	4.26	16.93	43.90	4.58	16.60
10	51.94	5.39	19.13	52.07	5.31	19.13
11	42.49	4.64	14.86	42.26	4.42	14.53
12	51.91	5.39	17.29	51.78	5.03	17.11
24[40]	**38.45**	3.76	22.42	**38.94**	3.51	22.11
25	39.60	4.02	21.87	39.68	3.87	21.78
26	37.36	3.13	21.78	37.62	2.94	21.56
27[40]	41.71	4.08	18.92	41.82	4.25	18.59
28	42.51	4.41	18.26	42.83	4.34	17.90
29	39.76	3.52	18.03	39.47	3.34	17.66
30	47.26	4.38	26.11	46.94	4.09	26.44
31	48.34	4.67	25.37	48.52	4.44	29.97
32	48.05	5.31	20.65	48.27	5.18	20.73
33	49.13	5.57	20.05	48.86	5.19	19.90

bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 2 μmol/mL glutamine and 800 μg/mL geneticin. 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 8.0) 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 20,000*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 rat A_3AR was expressed recombinantly via transfection in CHO cells, and the procedure was the same as for the human subtype. The protein concentration was measured using the Bradford assay.[51]

4.32.2. Binding assay. For the A_3AR binding experiments, the procedures used were similar to those previously described.[33] Briefly, each tube contained 100 μL of membrane suspension, 50 μL of [^{125}I]I-AB-MECA (final concentration 0.5 nM) and 50 μL of increasing concentrations of compounds in Tris–HCl buffer (50 mM, pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA. Non-specific binding was determined using 10 μM NECA (5′-*N*-ethyluronamidoadenosine). 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]RPIA to the recombinant hA_1AR and the binding of [^{3}H]CGS21680 to the recombinant hA_{2A}AR was performed as previously described.[34,52]

4.32.3. Cyclic AMP accumulation assay. Intracellular cyclic AMP levels were measured with a competitive protein binding method.[53] CHO cells expressing recombinant human[4] and rat[13] ARs were harvested by trypsinization. After resuspension in the medium, cells were plated in 24-well plates in 0.5 mL 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 A_{2A} and A_{2B}ARs, incubation was carried out for 1 h. For A_1 and A_3ARs, 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_2HPO4/EDTA buffer (K_2HPO4, 150 mM; EDTA, 10 mM), 20 μL of the cell lysate, and 30 μL 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 liquid scintillation spectrometry.

4.33. Statistical analysis

Binding and functional parameters were estimated with GraphPAD Prism software (GraphPAD, San Diego, CA). IC_{50} values obtained from competition curves were converted to K_i values using the Cheng–Prusoff equation.[54] Data were expressed as mean ± standard error.

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