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Exploring human adenosine A3 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, i.e. 3-12 and 24-33, with good hA3AR 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.

Keywords: adenosine receptors; nucleoside analogues; binding; efficacy.

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$_3$AR subtype, which is distributed in various organs (lung, liver, kidney, heart and brain),$^4$ has been shown to mediate
adenylate cyclase inhibition and phospholipase C and D stimulation. All four AR subtypes have been characterised on a pharmacological level as well as on a molecular level. ARs from different species show a high degree of amino acid sequence homology (82-93%) with the only exception being the A3AR subtype, which only exhibits 74% primary sequence homology between rat and human or sheep. 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, so far the only AR agonist approved for clinical use is adenosine itself. In this paper we focus on the human (h)A3AR subtype, the most recently identified member of the AR family.

The A3AR plays a crucial role in some of the physiological effects of adenosine. In addition to cardio- and cerebroprotective effects, hA3AR agonists may be therapeutically useful for the treatment of stroke, inflammation and in cancer therapy. While full agonists maximally stimulate the receptor, partial agonists show reduced intrinsic activity, may exhibit fewer side effects and may induce less receptor down-regulation and desensitisation than full agonists. Partial A3AR agonists can act as cardioprotective agents. Selective antagonists for the A3AR promise to be useful in the regulation of cell growth and as anti-asthmatic, cerebroprotective and anti-inflammatory agents.

Since its discovery in 1991, the development of agonists of the A3AR has been an active area of research. Many variations have been made on the adenosine scaffold in view of potent and selective A3AR binding. Generally, substitution at the 2’- and 8-positions has affinity- and efficacy-lowering effects. Known A3AR-selective alterations relevant to the work presented in this paper are Αδ-modifications, such as 3-iodobenzyl (IB) or 5-chloro-2-methoxybenzyl (CMB), and smaller substituents like a Cl or CN at the 2-position.
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\(_3\)AR agonists, such as Cl-IB-MECA and IB-MECA,\(^{36}\) which are still used as reference tools for pharmacological study of the A\(_3\)AR.

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\(_3\)AR 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 \(\alpha\)-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\(_3\)AR affinity and to investigate the impact of such a combined substitution pattern on the hA\(_3\)AR 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-\(\alpha\)-xylofuranose (1)
the 3'-C-azidomethyl synthon 2 was prepared according to our recently reported procedure. Displacement of the 6-chloro atom with ammonia, methylamine, 3-iodobenzylamine or 5-chloro-2-methoxybenzyl-amine, 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 N6-modified purines. Triphenylphosphine reduction of the azido moiety gave the 3'-C-aminomethyl nucleosides 7, 8, 9 and 10. Amidation of 9, 10 was performed using an acyl chloride under Schotten-Baumann conditions and furnished derivatives 11 and 12.

Scheme 1.

For the synthesis of the modified analogues in Scheme 2, the 3-α-azido (15,43,44) and 3-C-α-azidomethyl (16) sugars were obtained by simple 5'-deprotection of the previously described intermediates 13 and 14. Periodate oxidation followed by esterification of the carboxylic acid and subsequent treatment with methylamine in a pressure tube afforded the ribofuronamides 17 and 18. A one-pot deprotection-acetylation strategy afforded the peracylated sugar moieties 19 and 20 modified at the 3',5'-positions.

Scheme 2.

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 of 19 and 20 (in Scheme 2) with silylated 6-chloropurine and 2,6-dichloropurine 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.

Scheme 3.

2.2. Biological activity

Modifications of the adenosine scaffold known to increase hA3AR 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 N6-positions (as in 4-6 and 8-33). In this paper we
investigated the influence on hA3AR affinity and intrinsic activity of combining these 5’, 2-
and N6-modifications with the amino(methyl) substitution at the 3’-position that we40 and
others40 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
hA3AR 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 (in Table 1), it was clear that the 5’-uronamide modification for both the direct and branched-chain amine (as in 24-33 vs. 3-12) improved the overall affinity. With exception of 5 and 9, all compounds evaluated showed very good selectivity for the hA3AR subtype. In the simple 3’-amino series the most potent compound 32 ($K_i = 27$ nM) showed a 300-fold selectivity over the A1AR, compared to the 22-fold selectivity for its N6-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 hA3AR selectivity profile, but displayed weak binding characteristics, e.g. 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 N6-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 N6-substituents, known to be important for hA3AR 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-N6-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 N6-substituted adenosine analogues,\textsuperscript{35} N6-(5-chloro-2-methoxybenzyl) substitution (as in 10, $K_i = 13.8 \, \mu M$) did not improve affinity over the N6-iodobenzyl modification (as in 9, $K_i = 8.7 \, \mu M$).

The reduction of hA3AR 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 N6-substitution on hA3AR binding was consistent with our previous findings,\textsuperscript{35} i.e. N6-iodobenzyl (as in 27, 28 and 29 with $K_i = 137 \, nM, 1.7 \, \mu M$ and 132 nM respectively) was less affinity-enhancing than N6-(5-chloro-2-methoxybenzyl) (as in 32 and 33 with $K_i = 27 \, nM$ and 557 nM respectively).

### 2.2.2. Intrinsic activity

The results of the cyclic AMP-assay (in 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-N6-iodobenzyladenosine are known full agonists.\textsuperscript{39} The 5'-uronamide analogues 27 and 32 were partial agonists, contrary to what was reported earlier.\textsuperscript{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 N6 and 2-positions,\textsuperscript{33,38} this effect could only partially be overcome by modification of the 5'-position.
A modification known to have contradictory effects on A3AR binding and intrinsic activity is the introduction of a chlorine at the 2-position.\textsuperscript{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 hA3AR 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,\textsuperscript{33,39} whereas for the 3’-azido, like the 3’-F,\textsuperscript{37} this hydrogen bond pattern is no longer possible, resulting in a drop of efficacy.\textsuperscript{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 hA3AR 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\textsuperscript{6}-positions. Various synthetic analogues, i.e. 3-12 and 24-33, displayed good hA3AR 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 A3AR, 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, i.e. investigation of molecular complementarity at mutant A2A41 and A339 adenosine receptors.

4. Experimental Part

4.1. Synthesis

1H NMR spectra were obtained with a Varian 300 MHz spectrometer. The solvent signal of CDCl3 (7.26 ppm) and DMSO-d6 (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 D2O. 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 ionisation (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.

**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 eq.), were dissolved in EtOH (15 mL / mmol chloropurine) containing Et₃N (1.25 eq.). The reaction mixture was refluxed overnight and evaporated to dryness. The residue was dissolved in 7 N NH₃ 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 (CH₂Cl₂-MeOH) for 24-26, 30 and 31 furnished the desired product as a white pure solid.

### 4.2. 9-(3-C-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-adenine (3).

300 mg (0.62 mmol) of 2 yielded 80 mg (42%) of 3: ¹H NMR (DMSO-ᵈₛ) δ 2.58-2.67 (m, 1H, H-3’), 3.44 (dd, 1H, J = 5.7 Hz and -12.4 Hz, 3’-CH₃), 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’-CH₃), 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-NH₂), 8.13 and 8.39 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for C₁₁H₁₅N₈O₃ [M+H]⁺: 307.1267, found 307.1270. Anal. (C₁₁H₁₄N₈O₃·1/₄ H₂O) C,H,N.
4.3. 9-(3-C-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-N6-methyladenine (4).

400 mg (0.82 mmol) of 2 yielded 180 mg (68%) of 4: 1H NMR (DMSO-d6) δ 2.58-2.68 (m, 1H, H-3’), 2.93 (br s, 3H, N6-CH3), 3.44 (dd, 1H, J = 5.6 Hz and -12.31 Hz, 3’-CHb), 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’-CHa), 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, N6-H), 8.22 and 8.39 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for C12H17N8O3 [M+H]+: 321.1423, found 321.1429. Anal. (C12H16N8O3.2/3 H2O) C,H,N.

4.4. 9-(3-C-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-N6-(3-iodobenzyl)adenine (5).

340 mg (0.69 mmol) of 2 yielded 227 mg (62%) of 5: 1H NMR (DMSO-d6) δ 2.59-2.68 (m, 1H, H-3’), 3.44 (dd, 1H, J = 5.6 Hz and -12.3 Hz, 3’-CHb), 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’-CHa), 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, N6-CH2-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, N6-H); exact mass (ESI-MS) calculated for C18H20IN8O3 [M+H]+: 523.0704, found 523.0698. Anal. (C18H19IN8O3) C,H,N.
4.5. 9-(3-C-Azidomethyl-3-deoxy-β-D-ribofuranosyl)-N6-(5-chloro-2-methoxybenzyl) adenine (6).

300 mg (0.62 mmol) of 2 yielded 100 mg (35%) of 6: 1H NMR (DMSO-d6) δ 2.61-2.70 (m, 1H, H-3’), 3.45 (dd, 1H, J = 5.7 Hz and -12.3 Hz, 3’-CH3), 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’-CH3), 3.75 (ddd, 1H, J = 2.9 Hz and 5.0 Hz, H-5A’), 3.83 (s, 3H, Ar-OC6H3), 4.01 (dt, 1H, J = 3.2 Hz and 8.7 Hz, H-4’), 4.63 (m, 3H, H-2’ and N6-CH2-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 N6-H), 8.44 (s, 1H, H-8); exact mass (ESI-MS) calculated for C19H22ClN8O4 [M+H]+: 461.1452, found 461.1460. Anal. (C19H21ClN8O4) C,H,N.

4.6. 9-[3-Azido-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N6-(3-iodobenzyl) adenine (24).

300 mg (0.79 mmol) of 21 yielded 250 mg (60%) of 24; Spectroscopic data of this compound in accordance with those reported in ref. 40; Anal. (C18H18IN9O3.3/2 H2O) C,H,N.

4.7. 9-[3-C-Azidomethyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N6-(3-iodobenzyl) adenine (25).
300 mg (0.75 mmol) of 22 yielded 360 mg (73%) of 25: $^1$H NMR (DMSO-$d_6$) $\delta$ 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$_3$), 3.71 (dd, 1H, J = 7.9 Hz, 3’-CH$_3$), 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 (m, 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$_9$O$_3$ [M+H]$^+$: 550.0813, found 550.0803. Anal. (C$_{19}$H$_{20}$IN$_9$O$_3$.$\frac{3}{2}$ H$_2$O) C,H,N.

4.8. 2-Chloro-9-[3-azido-3-deoxy-5-(methylcarbamoyl)-$\beta$-n-ribofuranosyl]-N$^6$-(3-iodobenzyl)adenine (26).

200 mg (0.48 mmol) of 23 yielded 210 mg (77%) of 26: $^1$H NMR (DMSO-$d_6$) $\delta$ 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 (brs, 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 (brs, 1H, N$^6$-H); exact mass (ESI-MS) calculated for C$_{18}$H$_{17}$ClIN$_9$O$_3$Na [M+Na]$^+$: 592.0087, found 592.0092. Anal. (C$_{18}$H$_{17}$ClIN$_9$O$_3$.$\frac{1}{2}$ H$_2$O) C,H,N.

4.9. 9-[3-Azido-3-deoxy-5-(methylcarbamoyl)-$\beta$-n-ribofuranosyl]-N$^6$-(5-chloro-2-methoxybenzyl)adenine (30).
300 mg (0.79 mmol) of 21 yielded 251 mg (67%) of 30: $^1$H NMR (DMSO-$d_6$) $\delta$ 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$_9$O$_4$ [M+H]$^+$: 474.1404, found 474.1400. Anal. (C$_{19}$H$_{20}$ClN$_9$O$_4$·1/2 H$_2$O) C,H,N.

4.10. 9-[3-Azidomethyl-3-deoxy-5-(methylcarbamoyl)-$\beta$-D-ribofuranosyl]-N$^6$-(5-chloro-2-methoxybenzyl)adenine (31).

300 mg (0.75 mmol) of 22 yielded 300 mg (82%) of 31: $^1$H NMR (DMSO-$d_6$) $\delta$ 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$_2$), 3.71 (dd, 1H, $J$ = 7.8 Hz, 3’-CH$_2$), 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 (brs, 1H, N$^6$-H), 8.60 (s, 1H, H-8); exact mass (ESI-MS) calculated for C$_{20}$H$_{23}$ClN$_9$O$_4$ [M+H]$^+$: 488.15661, found 488.1559. Anal. (C$_{20}$H$_{22}$ClN$_9$O$_4$·1/2 H$_2$O) C,H,N.

**General procedure for the synthesis of the amino nucleosides 7, 8, 9, 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 eq.) was added to the solution. After stirring at
room temperature for 1.5 h, concentrated NH₄OH (3 mL / mmol) was added. The reaction mixture was stirred for another 2 h, evaporated to dryness and purified by silica gel chromatography (CH₂Cl₂-MeOH).

4.11. 9-(3-C-Aminomethyl-3-deoxy-β-D-ribofuranosyl)-adenine (7).

60 mg (0.20 mmol) of 3 furnished 35 mg (64%) of 7 as a white solid: ¹H NMR (DMSO-d₆) δ 2.31-2.40 (m, 1H, H-3’), 2.65 (dd, 1H, J = 6.2 Hz and -12.6 Hz, 3’-CH₆), 2.89 (dd, 1H, J = 7.3 Hz, 3’-CH₆), 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-NH₂), 8.12 and 8.36 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for C₁₁H₁₇N₆O₃ [M+H]⁺: 281.1362, found 281.1370. Anal. (C₁₁H₁₆N₆O₃) C,H,N.

4.12. 9-(3-C-Aminomethyl-3-deoxy-β-D-ribofuranosyl)-N⁶-methyladenine (8).

100 mg (0.31 mmol) of 4 furnished 64 mg (70%) of 8 as a white solid: ¹H NMR (DMSO-d₆) δ 2.26-2.35 (m, 1H, H-3’), 2.62 (dd, 1H, J = 6.5 Hz and -12.6 Hz, 3’-CH₆), 2.87 (dd, 1H, J = 7.0 Hz, 3’-CH₆), 2.93 (br s, 3H, N₆-C₃), 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, N₆-H), 8.21 and 8.34 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for C₁₂H₁₉N₆O₃ [M+H]⁺: 295.1518, found 295.1513. Anal. (C₁₂H₁₈N₆O₃.3/2 H₂O) C,H,N.
4.13. 9-(3-C-Aminomethyl-3-deoxy-β-D-ribofuranosyl)-N6-(3-iodobenzyl)adenine (9).

200 mg (0.38 mmol) of 5 furnished 131 mg (69%) of 9 as a white solid: Spectroscopic data of this compound in accordance with those reported in ref. 41; Anal. (C18H21IN6O3) C, H, N.


90 mg (0.17 mmol) of 6 furnished 54 mg (64%) of 10 as a white solid: 1H NMR (DMSO-d6) δ 2.29-2.38 (m, 1H, H-3’), 2.65 (dd, 1H, J = 6.3 Hz and -12.5 Hz, 3’-CH3), 2.89 (dd, 1H, J = 7.0 Hz, 3’-CH2), 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-OC6H3), 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, N6-CH2-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 N6-H), 8.40 (s, 1H, H-8); exact mass (ESI-MS) calculated for C19H24ClN6O4 [M+H]+: 435.1547, found 435.1546. Anal. (C19H23ClN6O4·1/4 H2O) C, H, N.

4.15. 9-[3-Amino-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N6-(3-iodobenzyl) adenine (27).
176 mg (0.33 mmol) of 24 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\textsubscript{18}H\textsubscript{20}IN\textsubscript{7}O\textsubscript{3}.1\textsubscript{1/2} H\textsubscript{2}O) C\textsubscript{5}H\textsubscript{7}N.

4.16. 9-[3-C-Aminomethyl-3-deoxy-5-(methylcarbamoyl)-\(\beta\)-\(\nu\)-ribofuranosyl]-\(\text{N}^{6}\)-(3-iodobenzyl)adenine (28).

100 mg (0.18 mmol) of 25 furnished 45 mg (48%) of 28 as a white solid: \(^1\)H NMR (DMSO-\(d_6\)) \(\delta 2.41-2.47\) (m, 1H, H-3\textsuperscript{'}), 2.64 (d, 3H, \(J = 4.7\) Hz, \(CH_3\)-N), 2.76 (dd, 1H, \(J = 5.3\) Hz and -12.3 Hz, 3\textsuperscript{'}-\(CH_3\)), 2.90 (dd, 1H, \(J = 7.6\) Hz, 3\textsuperscript{'}-\(CH_3\)), 4.34 (d, 1H, \(J = 8.8\) Hz, H-4\textsuperscript{'}), 4.55 (dd, 1H, \(J = 2.1\) Hz and 5.3 Hz, H-2\textsuperscript{'}), 4.66 (br s, 2H, \(N^6\)-\(CH_2\)-Ar), 6.00 (d, 1H, \(J = 2.4\) Hz, H-1\textsuperscript{'}), 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\textsuperscript{'}), 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\textsubscript{19}H\textsubscript{23}IN\textsubscript{7}O\textsubscript{3} \([\text{M+H}]^+\): 524.0908, found 524.0912. Anal. (C\textsubscript{19}H\textsubscript{22}IN\textsubscript{7}O\textsubscript{3}.3\textsubscript{1/4} H\textsubscript{2}O) C\textsubscript{5}H\textsubscript{7}N.

4.17. 2-Chloro-9-[3-amino-3-deoxy-5-(methylcarbamoyl)-\(\beta\)-\(\nu\)-ribofuranosyl]-\(\text{N}^{6}\)-(3-iodobenzyl)adenine (29).

200 mg (0.35 mmol) of 26 furnished 140 mg (74%) of 29 as a white solid: \(^1\)H NMR (DMSO-\(d_6\)) \(\delta 2.67\) (d, 3H, \(J = 4.7\) Hz, \(CH_3\)-N), 3.54 (t, 1H, \(J = 5.6\) Hz, H-3\textsuperscript{'}), 4.11 (d, 1H, \(J = 5.9\) Hz, H-4\textsuperscript{'}), 4.31 (t, 1H, \(J = 4.1\) Hz, H-2\textsuperscript{'}), 4.59 (brs, 2H, \(N^6\)-\(CH_2\)-Ar), 5.95 (d, 1H, \(J = 3.5\) Hz, H-1\textsuperscript{'}), 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), 7.71 (s, 1H, Ar-H-2), 8.22 (s, 1H, H-2\textsuperscript{'}), 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\textsubscript{19}H\textsubscript{23}IN\textsubscript{7}O\textsubscript{3} \([\text{M+Cl}]^+\): 588.1008, found 588.1013.
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 (bRS, 1H, N6-H); exact mass (ESI-MS) calculated for C18H20ClIN7O3 [M+H]+: 544.0362, found 544.0366. Anal. (C18H19ClIN7O3) C,H,N.

4.18. 9-[(3-Amino-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N6-(5-chloro-2-methoxybenzyl)adenine (32).

176 mg (0.33 mmol) of 30 furnished 29 mg (20%) of 32 as a white solid: 1H NMR (DMSO-d6) δ 2.67 (d, 3H, J = 4.7 Hz, CH3-N), 3.57 (t, 1H, J = 5.4 Hz, H-3’), 3.83 (s, 3H, Ar-OC6H3), 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, N6-CH2-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, N6-H), 8.44 (d, 1H, J = 5.0 Hz, NH-CO), 8.57 (s, 1H, H-8); exact mass (ESI-MS) calculated for C19H23ClN7O4 [M+H]+: 448.1499, found 448.1483. Anal. (C19H22ClN7O4.3/2 H2O) C,H,N.

4.19. 9-[(3-Aminomethyl-3-deoxy-5-(methylcarbamoyl)-β-D-ribofuranosyl]-N6-(5-chloro-2-methoxybenzyl)adenine (33).

150 mg (0.31 mmol) of 31 furnished 100 mg (70%) of 33 as a white solid: 1H NMR (DMSO-d6) δ 2.40-2.48 (m, 1H, H-3’), 2.64 (d, 3H, J = 4.7 Hz, CH3-N), 2.76 (dd, 1H, J = 5.1 Hz and -12.8 Hz, 3’-CHb), 2.90 (dd, 1H, J = 7.9 Hz, 3’-CHa), 3.83 (s, 3H, Ar-OC6H3), 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, N6-CH2-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,
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 eq.). After completion of reaction (6 h), THF and brine were added. The organic phase was separated, washed with water, dried over MgSO4 and concentrated in vacuo. Precipitation from MeOH and subsequent filtration furnished the product as a white solid in 60-63% yield.

4.20. 9-(3-Acetamidomethyl-3-deoxy-β-D-ribofuranosyl)-N6-(3-iodobenzyl)adenine (11).

70 mg (0.14 mmol) of 9 yielded 48 mg (63 %) of 11: 1H NMR (DMSO-d6) δ 1.77 (s, 3H, CH3), 2.46 (m, 1H, H-3’), 3.08-3.16 (m, 1H, 3’-CHβ), 3.24-3.30 (m, 1H, 3’-CHα), 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, N6-CH2-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 N6-H); exact mass (ESI-MS) calculated for C20H24IN6O4 [M+H]+: 539.0905, found 539.0890. Anal. (C20H24IN6O4.3/2 H2O) C,H,N.
4.21. 9-(3-Acetamidomethyl-3-deoxy-β-d-ribofuranosyl)-N6-(5-chloro-2-methoxybenzyl)
adine (12).

10 mg (0.023 mmol) of 10 yielded 6.6 mg (60 %) of 11: 1H NMR (DMSO-d6) δ 1.79 (s, 3H, CH3), 3.10-3.19 (m, 1H, H-3’), 3.27-3.36 (m, 1H, 3’-CH2), 3.51-3.60 (m, 1H, 3’-CH2), 3.77-3.84 (m, 5H, H-5B’, H-5A’ and Ar-OC6H3), 4.00 (m, 1H, H-4’), 4.45 (app s, 1H, H-2’), 4.65 (brs, 2H, N6-CH2-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 (brs, 1H, N6-H), 8.49 (s, 1H, H-8); exact mass (ESI-MS) calculated for C21H26ClN6O5 [M+H]+: 477.1653, found 477.1641. Anal. (C21H25ClN6O5·1/2H2O) C, H, N.

4.22. Methyl 3-azido-3-deoxy-1,2-isopropylidene-α-β-ribofuranamide (17)40.

A biphasic mixture of water (46 mL), CHCl3 (31 mL) and acetonitrile (31 mL) containing compound 1543,44 (3.3 g, 15.33 mmol), RuCl3 hydrate (160 mg, 0.77 mmol) and NaIO4 (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 CH2Cl2 (3 x 200 mL). The combined organic phase was dried over MgSO4, 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-β-ribofuranonic acid as a lightly coloured oil that was used without further purification. A mixture of 3-azido-3-deoxy-1,2-isopropylidene-α-β-ribofuranonic 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 CH₂Cl₂ (200 mL) and water (100 mL). The aqueous phase was extracted with CH₂Cl₂ (3 x 200 mL) and the combined organic phase was dried over MgSO₄, 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.

4.23. Methyl 3-azidomethyl-3-deoxy-1,2-isopropylidene-α-o-ribofuranamide (18).

Compound 18 (1.3 g, 32%) was prepared from 16⁴⁵ (3.6 g, 15.7 mmol) in analogy to the procedure described for 17: ¹H NMR (CDCl₃) δ 1.34 and 1.49 (2s, 6H, 2 x CH₃), 2.15-2.25 (m, 1H, H-3), 2.80 (d, 3H, J = 5.0 Hz, CH₃-N), 3.64 (dd, 1H, J = 11.1 Hz and -12.1 Hz, 3-CH₂), 3.87 (dd, 1H, J = 4.3 Hz, 3-CH₃), 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, NH-CO); exact mass (ESI-MS) calculated for C₁₀H₁₇N₄O₄ [M+H]+: 257.1249, found 257.1253.

4.24. 9-[2-O-Acetyl-3-C-azido-3-deoxy-5-(methylcarbamoyl)-β-o-ribofuranose]-6-chloropurine (21).

A mixture of 17 (1.2 g, 5 mmol), concentrated sulphuric 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 NaHCO₃ solution (50 mL) and CH₂Cl₂ (50 mL) were slowly added and the mixture was stirred for another 10 min. After separation the aqueous phase was extracted with CH₂Cl₂ (3 x 100 mL). The combined organic phase was washed with saturated NaHCO₃ solution and brine, dried over MgSO₄, filtered and evaporated to dryness to yield 787 mg (55%) of the crude methyl 3-azido-3-deoxy-1,2-diacetyl-α-D-ribofuranamide (19) as a yellowish foam: \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 2.01 and 2.10 (2s, 6H, 2 x CH₃), 2.79 (d, 3H, \(J = 5.0\) Hz, CH₃-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, NH-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.

4.25. 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-ribofuranamide (0.6 g, 40%) was prepared: \(^1\)H NMR (CDCl₃) \(\delta\) 2.06 and 2.13 (2s, 6H, 2 x CH₃), 2.74-2.77 (m, 1H, H-3), 2.81 (d, 3H, \(J = 5.0\) Hz, CH₃-N), 3.55 (dd, 1H, \(J = 10.5\) Hz and -12.3 Hz, 3-CH₂), 3.91 (dd, 1H, \(J = 4.4\) Hz, 3-CH₂), 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, NH-CO). This acetylated ribofuranamide (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 (CDCl₃) \(\delta\) 2.20 (s, 3H, CH₃), 2.83 (d, 3H, \(J = 5.0\) Hz, CH₃-N), 3.38-3.48 (m, 1H, H-3’), 3.73 (dd, 1H, \(J = 9.1\) Hz and -12.6 Hz, 3’-CH₂), 3.87 (dd, 1H, \(J = 4.4\) Hz, 3’-CH₂), 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, NH-CO), 8.22 and 8.75 (2s, 2H, H-2 and H-8); exact mass (ESI-MS) calculated for \(\text{C}_{14}\text{H}_{16}\text{ClN}_{8}\text{O}_{4}\) \([\text{M+H}]^+\): 395.0982, found 395.0982.

4.26. 9-[2-O-Acetyl-3-C-azido-3-deoxy-5-(methylcarbamoyl)-\(\beta\)-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 \((\text{CDCl}_3)\) \(\delta\) 2.10 (s, 3H, \(\text{CH}_3\)), 2.63 (d, 3H, \(J = 4.7\) Hz, \(\text{CH}_3\)-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, NH-CO), 8.99 (s, 1H, H-8); exact mass (ESI-MS) calculated for \(\text{C}_{13}\text{H}_{12}\text{Cl}_2\text{N}_8\text{O}_4\) \([\text{M+H}]^+\): 437.0256, found 437.0252.

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

0.75 g (3.0 mmol) of 17 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 \(\text{CH}_2\text{Cl}_2\) (100 mL) and 7% NaHCO\(_3\) (150 mL). The aqueous layer was washed with \(\text{CH}_2\text{Cl}_2\) (150 mL) and the combined organic layers were dried with MgSO\(_4\), filtered and evaporated \textit{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$_3$) $\delta$ 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$_3$) $\delta$ 1.99 and 2.30 (2s, 6H, 2 x 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$_8$O$_5$ [M+H]$^+$: 423.0932, found 423.0934.

4.28. Elemental Analysis

Table 2.

4.29. Biological assays

4.29.1. Cell culture and membrane preparation

CHO cells expressing recombinant human and rat A$_3$ARs were cultured in DMEM (Dulbecco’s modified Eagle’s medium) and F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 µg/mL streptomycin, 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 sec, 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 A3AR 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

4.29.2. Binding assay

For the A3AR binding experiments, the procedures used were similar to those previously described.33 Briefly, each tube contained 100 µL of membrane suspension, 50 µL of [125I]I-AB-MECA (final concentration 0.5 nM), and 50 µL of increasing concentrations of compounds in Tris–HCl buffer (50 mM, pH7.4) containing 10 mM MgCl2, 1mM EDTA. Nonspecific 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 [3H]RPIA to the recombinant hA1AR and the binding of [3H]CGS21680 to the recombinant hA2AR was performed as previously described.34,52

4.29.3. Cyclic AMP accumulation assay
Intracellular cyclic AMP levels were measured with a competitive protein binding method. CHO cells expressing recombinant human and rat 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 A2A and A2BARs, incubation was carried out for 1 h. For A1 and A3ARs, 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 [3H]cyclic AMP (2nM) in K2HPO4/EDTA buffer (K2HPO4, 150mM; EDTA, 10 mM), 20 µL of the cell lysate, and 30 µL 0.1M 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.

4.30. Statistical analysis

Binding and functional parameters were estimated with GraphPAD Prism software (GraphPAD, San Diego, CA). IC50 values obtained from competition curves were converted to Ki values using the Cheng–Prusoff equation. Data were expressed as mean ± standard error.
Acknowledgment

Philippe Van Rompaey is a recipient of an IWT fellowship.

References


40. DeNinno, M. P.; Masumane, H.; Chenard, L. K.; DiRico, K. J.; Eller, C.; Etienne, J. B.;
42, 3247.
49. Van Tilburg, E. W.; van der Klein, P. A. M.; von Frijtag Drabbe Künzel, J.; de Groote,
50. Kim, H. O.; Park, J. G.; Moon, H. R.; Gunaga, P.; Lim, M. H.; Chun, M. W.; Jacobson, K.
52. Hutchison, A. J.; Williams, M.; de-Jesus, R.; Yokoyama, R.; Oei, H. H.; Ghai, G. R.;
Table 1. Binding affinity (A₁R, A₂₅R, or A₃R) or functional activation (A₂B R and A₃R) of the adenosine derivatives at human adenosine receptors, n = 3, unless noted.

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<th>R4</th>
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<th>hA₂₅RB</th>
<th>hA₂BARc</th>
<th>hA₃RAD</th>
<th>cAMP hA₃R²</th>
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Kᵢ (nM) or % inhibition of binding at 100 µM ([³H]R-PIA, 2.0 nM) in CHO cells expressing hA₁AR.

% inhibition of binding at 100 µM ([³H]CGS21680, 15 nM) in HEK-293 cells expressing hA₂AAR.

% activation (cAMP assay) at 100 µM in CHO cells expressing hA₂BAR (NECA is 100%).

Kᵢ (nM) or % inhibition at 100 µM ([¹²⁵I]I-AB-MECA, 0.5 nM) in CHO cells expressing hA₃AR, unless noted.

n = 1.

at 10 µM.

% inhibition at 100 µM of forskolin-stimulated cAMP production at 10 µM, in CHO cells expressing the hA₃AR, as a percentage of the response of the full agonist Cl-IB-MECA (n=2).

Affinities previously reported in ref. 39.
Table 2. Elemental analysis of evaluated derivatives.

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<th>H (%)</th>
<th>N (%)</th>
<th>C (%)</th>
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Schemes

Ref. 41

R

R

R

R

R

R

a

b

c

3-iodobenzyl
5-chloro-2-methoxybenzyl
3-iodobenzyl
5-chloro-2-methoxybenzyl
3-iodobenzyl
5-chloro-2-methoxybenzyl
Legends

Scheme 1."

"Reagents and conditions: (a) (i) amine HCl (or ammonia for 3), Et3N, EtOH, reflux (ii) 7 N NH3 in MeOH, rt; (b) Ph3P, NH4OH, pyridine, rt; (c) CH3COCl, 50% aqueous NaOAc, THF, rt.

Scheme 2."

"Reagents and conditions: (a) 0.1 N NaOCH3, MeOH, rt; (b) TBAF, THF, rt; (c) (i) NaIO4, RuCl3, CHCl3:CH3CN:H2O (2:2:3), rt (ii) dry MeOH, EDC, DMAP, rt (iii) 2 M CH3NH2 in THF, 55°C; (d) H2SO4, Ac2O, AcOH, rt; (e) silylated 6-chloropurine or 2,6-dichloropurine, TMSOTf, dry 1,2-dichloroethane, reflux; (f) (i) amine HCl, Et3N, EtOH, reflux (ii) 7 N NH3 in MeOH, rt; (g) Ph3P, NH4OH, pyridine, rt.

Scheme 3."

"Reagents and conditions: (a) (i) 70% HOAc, 60°C (ii) Ac2O:pyridine (1:2), rt; (b) silylated 6-chloropurine, TMSOTf, dry 1,2-dichloroethane, reflux.