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Synthesis and Evaluation of *N*^{6}-Substituted Apioadenosines as Potential Adenosine A_3 Receptor Modulators

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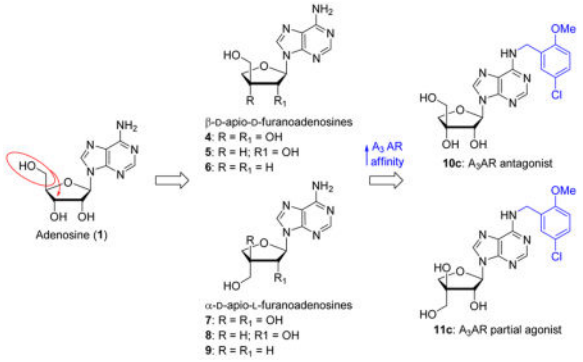
Abstract

Adenosine receptors (ARs) trigger signal transduction pathways inside the cell when activated by extracellular adenosine. Selective modulation of the A_3AR subtype may be beneficial in controlling diseases such as colorectal cancer and rheumatoid arthritis. Here, we report the synthesis and evaluation of β-D-apio-D-furano- and α-D-apio-L-furanoadenosines and derivatives thereof. Introduction of a 2-methoxy-5-chlorobenzyl group at N^6 of β-D-apio-D-furanoadenosine afforded an A_3AR antagonist (**10c**, K_i = 0.98 μM), while a similar modification of an α-D-apio-L-furanoadenosine gave rise to a partial agonist (**11c**, K_i = 3.07 μM). The structural basis for this difference was examined by docking to an A_3AR model; the antagonist lacked a crucial interaction with Thr94.



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Keywords

G protein-coupled receptor; apionucleosides; Adenosine A_3 receptor

INTRODUCTION

Transmembrane receptors coupled to G-proteins (heterotrimeric guanine nucleotide binding proteins) constitute a large receptor family, referred to as G protein-coupled receptors (GPCRs) or seven-transmembrane domain (7TM) receptors. Triggered by messenger molecules or signals outside the cell, GPCRs activate different signal transduction pathways inside the cell and, ultimately, cellular responses. The extracellular messengers range from photons to biogenic amines and other neurotransmitters to proteins. GPCRs are ubiquitous and involved in processes varying from directed chemotaxis[1] of small organisms (e.g. searching food for survival) to triggering apoptosis (programmed cell death) in large animals. They are the targets of almost 50% of marketed active pharmaceutical ingredients.[2]

GPCRs activated by extracellular adenosine are classified as adenosine receptors (ARs), which are divided in four different subtypes, i.e. A_1, A_{2A}, A_{2B} and A_3 ARs.[3,4] The amino acid sequence similarity between the human (h) A_3AR and hA_1, hA_{2A}, hA_{2B} AR is 54 %, 48 % and 44 %, respectively.[5] The ARs use different signaling pathways; the A_1 AR and A_3 AR are preferentially coupled to Gi-proteins, and upon activation lead to adenylate cyclase inhibition, while the A_{2A} AR and A_{2B} AR are preferentially coupled to Gs-proteins and lead to adenylate cyclase activation. In some cells (e.g., mast cells), the A_{2B}AR is dually coupled to Gs and Gq and consequently also mobilizes calcium and activates phospholipase C and MAPK. [3,6]

Besides adenosine (**1**) itself, which is used clinically for the treatment of supraventricular tachycardia and in myocardial perfusion imaging,[7] only one AR-specific agent, the A_{2A}AR agonist regedenoson, has so far been approved by the FDA. However, a relatively large group of AR ligands is currently under clinical evaluation.[8]

With the exception of compound **2**, which was reported in the mid-1980s as being inactive at A_1 and A_2ARs,[9] and the recently reported carbocyclic analogue **3**,[10] a weak A_3AR agonist, 4′-hydroxymethyl transposed nucleosides have not been investigated as AR ligands.

This led us to employ a new and convenient method for the synthesis of apionucleosides from 1,2-*O*-isopropylidene-α-L-threose,[11] for the construction of suitably modified 9-(3-*C*hydroxymethyl-β-D-erythrofuranosyl)adenines (aka β-D-apio-D-furanoadenosines) (**4**-**6**) and 9-(3-*C*-hydroxymethyl-α-L-threofuranosyl)adenines (aka α-D-apio-L-furanoadenosines) (**7**-**9**) as potential A_3AR modulators (Figure 1).

On the one hand, we envisaged to substitute the N^6 position of β-D-apio-D-furanoadenosine **4** with substituted benzyl groups known to enhance A_3AR affinity;[12] on the other hand we planned to substitute the well-known ethylcarboxamide moiety in place of the 4′-CH_2OH group.[13] We decided to introduce a benzyl moiety at the N^6 position and an N-

alkylcarboxamide moiety at the 5′ position because this combination was shown to be conducive to selectivity at the A_3AR. For example, N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine and its 2-chloro analogue (Cl-IB-MECA) display K_i values at A_3AR of ~1 nM and ≥50-fold selectivity for this subtype. [14] Subsequently, an N^6-(3-chloro-5-methoxybenzyl) substitution was shown to be beneficial for A_3AR selectivity,[12] and we incorporated this moiety in the current target compounds.

However, synthetic problems in introducing an ethylcarboxamide moiety motivated us to introduce a *N*-methyl or *N*-ethylcarbamoyloxymethyl group instead. Analogue modifications were carried out on α-D-apio-L-furanoadenosine **7**.

RESULTS AND DISCUSSION

Chemistry

The β-D-apio-D-furanoadenosines **4-6** and the α-D-apio-L-furanoadenosines **7-9** were prepared by microwave assisted synthesis as described in ref. [11].

The L-*threo* analogue **7** was used as a model substrate for N^6-derivatisation reactions. Treatment of **7** with the appropriate benzyl bromide first afforded the N^1-benzyl derivative, which was isolated in the case of the 3-chlorobenzyl derivative **16** (Scheme 1). Upon prolonged heating with ammonia solution, the N^1-benzyl intermediates were converted to the desired N^6-benzylated compounds **11a-11c** via Dimroth rearrangement.[14] Although in many cases this method suffered from low yields, it allowed gaining fast access to the target molecules. Using similar conditions β-D-apio-D-furanoadenosine **4** was benzylated at N^6 affording the desired derivatives in low yield after purification by RP-HPLC or preparative TLC. In the case of **10c**, using excess of the benzyl bromide led to degradation of the starting material and afforded bis-(2-methoxy-5-chlorobenzyl)adenine as observed by HRMS. By limiting the amount of this benzyl bromide to one equivalent, **10c** could be obtained in 21% yield.

Selective modification of the 5′-position requires a suitable protecting strategy. First attempts were made to per-silylate the hydroxyl groups of **7** (Scheme 2). Under mild conditions the tertiary hydroxyl group failed to react, while raising the temperature to 100 °C in DMF led to exclusive formation of the N^6-imide **17**. Since exploration of different solvents and scavenging bases did not allow the preparation of compound **18**, the latter was obtained by treatment of **17** with ammonia in methanol.[15] Two attempts to selectively remove the TBDMS group from the primary hydroxyl of **18** resulted in complex reaction mixtures, from which isomers **20** and **21** proved inseparable by flash chromatography.[16]

The aforementioned problems led us to synthesize the bis-silylated products **22** and **23**, from which the primary OH group could be selectively deprotected to provide **24** and **25** in excellent yields. Introduction of the desired 3′-carboxamide was tested on intermediate **25**. Conversion of the primary hydroxyl group of **25** to the corresponding carboxylic acid via a TEMPO-BAIB oxidation gave the corresponding TEMPO ester (Entry 1, Table 1).[17] This may be due to the high catalyst loading in the reaction, since slow conversion of the aldehyde intermediate forced us to add extra amounts of reagents. Efforts to convert the

TEMPO-ester to the ethylamide failed. Under conditions of Jones oxidation (CrO_3 + dil. H_2SO_4) the starting material degraded. Oxidation using $RuCl_3$-$NaIO_4$,[18] followed by amide coupling, provided **27** and **28**, albeit in very low yield. Removal of the TBDMS groups in **28** gave the desired 3′-ethylcarboxamide **15**. Attempted ruthenium chloride oxidation of **24** resulted in decarboxylation and further oxidation to the ketone as the only product (Entry 2, Table 1). On the other hand, oxidation of **20** (as a mixture with **21**) did not proceed beyond the aldehyde stage, thus weakening the prospects of synthesizing the corresponding alkylcarboxamide via the carboxylic acid route. Sequential treatment of **24** and **25** with carbonyldiimidazole and ethylamine afforded the 5′-*O* ethylcarbamate derivatives **26** and **27**,[19] which were deprotected with NH_4F in warm methanol to give the desired 5′-*O*-ethylcarbamate apionucleosides **13** and **14** in excellent yields.

Given the problems encountered for the oxidation of α-D-apio-L-furanoadenosine (**24**), the corresponding D-furano epimer was only converted to carbamate **12** (Scheme 3). Silylation and desilylation of **4** rendered the 2′-*O*-monosilylated species **30**, which upon treatment with carbonyldiimidazole and liq. methylamine gave **31**. Deprotection of the TBDMS group furnished 5′-*O*-methylcarbamoyl-β-D-apio-D-furanoadenosine **12**.

Pharmacological Evaluation

For the apio-type adenosine derivatives **4-16**, we measured the binding affinities at the hA_1, hA_{2A} and hA_3AR. The results are reported in Table 2. The ability of each of these adenosine derivatives to compete for radioligand binding at each of these hARs in cell membranes was evaluated at a fixed concentration of 10 μM. While most of the analogues inhibited binding to a negligible degree, it was feasible to determine full inhibition curves for compounds **10c** and **11c** at the A_3AR (Figure 2). These two compounds display a modest degree of binding selectivity at the A_3AR, but only with μM affinity for both. They were then examined in a functional assay,[12] i.e. inhibition of forskolin-stimulated cyclic AMP accumulation in an hA_3AR-expressing CHO cell line. Compared to Cl-IB-MECA as reference full agonist, compound **11c** was shown to be a partial agonist of the hA_3AR, while compound **10c** was inactive. The IC_{50} values for **11c** and Cl-IB-MECA were 1560 ± 470 nM (50.9 ± 5.8% maximal efficacy) and 1.21 ± 0.35 nM (100% efficacy), respectively.

Docking studies performed at a hA_3AR model

A hybrid homology model of the hA_3AR based on an agonist-bound hA_{2A}AR structure [20] (and modified with the position of TM2 based on the active state of the β_2 adrenergic receptor) was built and used for docking of the nucleosides as described. [21],[22] The side chain of Asn250 (6.55, using Ballesteros-Weinstein notation [23]) in the hA_3AR homology model strongly interacts with Cl-IB-MECA through two H-bonds involving the 6-amino group and the N^7 atom of the adenine ring (Figure 3). Moreover, the adenine ring is anchored inside the binding site by a π–π stacking interaction with Phe168 (EL2) and strong hydrophobic contacts with Leu246 (6.51) and Ile268 (7.39). The N^6-3-iodobenzyl ring is accommodated in a hydrophobic pocket delimited by TM5, TM6 and EL2 to form strong hydrophobic interactions with Val169 (EL2), Met174 (5.35) and Ile253 (6.58). Finally, the 3′- and 2′-hydroxyl groups of the ribose ring form H-bonds with Ser271 (7.42) and His272 (7.43), respectively, while the 5′-*N*-methyluronamido moiety forms a H-bond with Thr94

(3.36). These latter interactions involving the ribose moiety are particular to agonist binding, as shown by the comparison of the reported crystallographic structures of the $hA_{2A}AR$ in complex with agonists and antagonists, and are proposed to be important for the activation process in the AR family.

It has been previously shown that the 4′-truncation of nucleoside derivatives that are potent A_3AR ligands leads to compounds with good affinity for the A_3 receptor but with decreased efficacy, i.e. behaving as partial agonist or antagonist of this subtype. [24] Our docking of nucleoside analogues in the hA_3AR homology model indicated that the absence of a hydroxymethyl or uronamide substituent at the 4′-position prevented these compounds from interacting with Thr94 (3.36). This residue is a conserved recognition point in agonist binding, and therefore the lack of this interaction was considered as a reason for the low efficacy profile of 4′-truncated nucleosides.

Both compounds **10c** and **11c** show docking poses at the hA_3AR well superimposable to the binding mode of Cl-IB-MECA with conserved interactions stabilizing the adenine core and the N^6 substituent. Differences can be observed in the interactions formed by the ribose moiety; in fact, both compounds form only two of the three H-bonds predicted for binding of the full agonist Cl-IB-MECA. In particular, compound **10c** (Figure 4) forms two H-bonds with Ser271 (7.42) and His272 (7.43), but it cannot reach Thr94 in TM3, while compound **11c** (Figure 5) forms H-bonds with Thr94 (3.36) and Ser271 (7.42) and not with His272 (7.43). Therefore, compound **10c** show a binding mode similar to the one observed for other 4′-truncated nucleosides [22] and the missing interaction with Thr94 (3.36) is consistent with its lack of receptor activation, indicative of antagonist behavior. However, the ability of partial agonist **11c** to bridge between TM3 and TM7 likely correlates with the ability to induce the conformational changes required for receptor activation, such as an inward movement of TM7.[3] Thus, the preferred modeled binding modes of **10c** and **11c** differ in the crucial interaction with Thr94, which seems to be associated with residual efficacy at the hA_3AR in ribose-modified nucleosides.

CONCLUSIONS

In summary, we have synthesized a small series of modified apioadenosines as potential A_3AR ligands. The N^6 substituted 9-(3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenines showed weak but selective binding affinity to the A_3AR. A docking study allowed us to rationalize the lack of intrinsic efficacy of antagonist β-D-apio-D-furanoadenosine **10c** and the partial agonist activity of α-D-apio-L-furanoadenosine **11c** based on hydrogen bonding interaction with a key Thr residue. Synthetic problems in introducing a 3′-ethylcarboxamide moiety, motivated us to introduce a ((ethylcarbamoyl)oxy)methyl group instead. None of these 3′-altered derivatives showed highly potent binding affinity for the A_3AR. Overall it may be concluded that substitution of an apiofuranose for a ribofuranose moiety is detrimental for binding to the ARs, but the addition of a favorable N^6 substituent can partially compensate for this loss of binding affinity. Thus, structural changes in the sugar moiety, such as the configuration at the 3′ carbon, have major effects on the ability to activate the receptor, as well as binding recognition.

EXPERIMENTAL SECTION

Chemical Synthesis

All reagents were from standard commercial sources and of analytic grade. Dry solvents were obtained directly from commercial sources and stored on molecular sieves. Moisture sensitive reactions were carried out under argon atmosphere. Precoated Merck silica gel F254 plates were used for TLC, and spots were examined under ultraviolet light at 254 nm and further visualized by sulphuric acid-anisaldehyde spray. Column chromatography was performed on silica gel (200–400 mesh, 60 Å, Biosolve, Valkenswaard, The Netherlands). RP-HPLC was performed using Waters XBridge OBD™ Prep C18 5μm column @ 17.5 mL/min flow rate. NMR spectra were determined using a Varian Mercury 300 MHz spectrometer. Chemical shifts are given in ppm (δ) relative to the residual solvent signals or TMS as internal standard. Exact mass measurements were performed on a Waters LCT Premier XETM Time of flight (TOF) mass spectrometer equipped with a standard electrospray ionization (ESI) and modular LockSpray TM interface. Samples were infused in a CH_3CN/water (1:1) mixture at 10 μL/min. NMR signals of sugar protons and carbons are indicated with a prime, and signals of base protons and carbons are given without a prime.

1′-[*N*^{1}-(3-Chlorobenzyl)-adenin-9-yl]-α-D-apio-L-furanose bromide (16)—To a solution of α-D-apio-L-furanoadenosine **7** (25 mg, 0.094 mmol) in anh. DMF (1 mL) was added 3-chlorobenzyl bromide (50 μL, 0.374 mmol), and the mixture was stirred at 50 °C for 48 h. The solvent was evaporated under reduced pressure, and the residue was purified first by column chromatography (8–12% MeOH in CH_2Cl_2) and then with preparative thin layer chromatography (15% MeOH in CH_2Cl_2, R_f: 0.2) to afford the title compound **16** as a white solid (16 mg, 44%). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.75 (d, *J* = 11.7 Hz, 1H, 5′-H), 3.85 (d, *J* = 11.7 Hz, 1H, 5′-H), 4.18 (d, *J* = 9.7 Hz, 1H, 4′-H), 4.25 (d, *J* = 9.4 Hz, 1H, 4′-H), 4.42 (d, *J* = 0.9 Hz,1H, 2′-H), 5.63 (s, 2H, PhC*H*$_2$), 6.15 (d, *J* = 1.5 Hz, 1H, 1′-H), 7.14 – 7.24 (m, 1H, Ph-H), 7.33 - 7.46 (m, 3H, Ph-H's), 8.55 (s, 1H, 8-H), 8.68 (s, 1H, 2-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 53.40 (Ph*C*H$_2$), 63.43 (5′-C), 78.14 (4′-C), 81.97 (2′-C), 82.77 (3′-C), 94.30 (1′-C), 120.91 (5-C), 126.28, 128.22 (*Ph* 2,6-Cs), 130.06, 131.98 (*Ph* 3,4-Cs), 136.26 (*Ph* 5-C), 136.52 (*Ph* 1-C), 144.79 (8-C), 148.09 (4-C), 148.42 (2-C), 152.29 (6-C). ESI-HRMS for $[C_{17}H_{18}ClN_5O_4 + H]^+$ calcd, 392.1126; found, 392.1139.

1′-[*N*^{6}-(3-Chlorobenzyl)-adenin-9-yl]-α-D-apio-L-furanose (11a)—To a solution of α-D-apio-L-furanoadenosine **7** (25 mg, 0.094 mmol) in anh. DMF (1 mL) was added 3-chlorobenzyl bromide (50μL, 0.374 mmol), and the mixture was stirred at 50 °C for 48 h. Ammonium hydroxide (25%, 3.0 mL) was added and the mixture stirred at 50 °C for 24 h (alternatively for **11a**, at 90 °C for 3h). The volatiles were evaporated under reduced pressure and the residue purified by column chromatography (3–6% MeOH in CH_2Cl_2) to afford desired product **11a** (20 mg, 56%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.62 (d, *J* = 5.6 Hz, 2H, 5′-H), 3.99 (d, *J* = 9.1 Hz, 1H, 4′-H), 4.05 (d, *J* = 9.1 Hz, 1H, 4′-H), 4.40 (dd, *J* = 5.0, 2.9 Hz, 1H, 2′-H), 4.64 (t, *J* = 5.6 Hz, 1H, 5′-OH), 4.71 (br.s, 2H, PhC*H*$_2$), 5.32 (s, 1H, 3′-OH), 5.86 (d, *J* = 5.3 Hz, 1H, 2′-OH), 5.92 (d, *J* = 2.9 Hz, 1H, 1′-H), 7.23 – 7.37 (m, 3H, *Ph*-H's), 7.39 (s, 1H, *Ph*-H), 8.23 (s, 1H, 2-H), 8.35 (s, 1H, 8-H), 8.44

(br.s, 1H, NH). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 44.68 (Ph*C*H$_2$), 63.74 (5′-C), 77.69 (4′-C), 82.33 (2′-C), 82.73 (3′-C), 94.27 (1′-C), 120.82 (5-C), 126.96, 128.34, 128.56, 131.16 (*Ph* 2,3,4,6-C's), 135.50 (*Ph* 5-C), 141.44 (8-C), 143.08 (*Ph* 1-C), 149.50 (4-C), 153.79 (2-C), 156.08 (6-C). ESI-HRMS for $[C_{17}H_{18}ClN_5O_4 + H]^+$ calcd, 392.1126; found, 392.1105.

1′-[*N*^{6}-(3-Iodobenzyl)-adenin-9-yl]-α-D-apio-L-furanose (11b)—Following the protocol for the synthesis of compound **11a**, the title compound **11b** (33 mg, 36%) was obtained from α-D-apio-L-furanoadenosine **7** (50 mg, 0.19 mmol) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.76 (d, *J* = 11.6 Hz, 1H, 5′-H), 3.84 (d, *J* = 11.6 Hz, 1H, 5′-H), 4.12 (d, *J* = 9.5 Hz, 1H, 4′-H), 4.18 (d, *J* = 9.5 Hz, 1H, 4′-H), 4.37 (d, *J* = 1.5 Hz, 1H, 2′-H), 4.78 (br.s, 2H, PhC*H*$_2$), 6.02 (d, *J* = 1.8 Hz, 1H, 1′-H), 7.07 (t, *J* = 7.8 Hz, 1H, *Ph* 5-H), 7.37 (d, *J* = 7.7 Hz, 1H, *Ph* 2-H), 7.58 (d, *J* = 7.9 Hz, 1H, *Ph* 4-H), 7.74 (s, 1H, *Ph* 6-H), 8.26 (s, 1H, 2-H), 8.29 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 44.45 (Ph*C*H$_2$), 63.60 (5′-C), 77.54 (4′-C), 82.19 (2′-C), 82.59 (3′-C), 94.13 (1′-C), 94.96 (*Ph* 5-C), 120.68 (5-C), 127.84(*Ph* 2-C), 131.37 (*Ph* 3-C), 137.33 (*Ph* 4-C), 137.51 (*Ph* 6-C), 141.30 (8-C), 143.11 (*Ph* 1-C), 149.46 (4-C), 153.66 (2-C), 155.91 (6-C). ESI-HRMS for $[C_{17}H_{18}IN_5O_4 + H]^+$ calcd, 484.0482; found, 484.0470.

1′-[*N*^{6}-(2-Methoxy-5-chlorobenzyl)adenin-9-yl]-α-D-apio-L-furanose (11c)—Following the protocol used for the synthesis of compound **11a**, α-D-apio-L-furanoadenosine **7** (50 mg, 0.19 mmol) was converted to the title compound **11c** (15 mg, 20%), which was obtained as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.75 (d, *J* = 11.4 Hz, 1H, 5′-H), 3.84 (d, *J* = 11.7 Hz, 1H, 5′-H), 3.87 (s, 3H, Ph-OC*H*$_3$), 4.12 (d, *J* = 9.3 Hz, 1H, 4′-H), 4.18 (d, *J* = 9.9 Hz, 1H, 4′-H), 4.37 (d, *J* = 1.2 Hz, 1H, 2′-H), 4.77 (br.s, 2H, PhC*H*$_2$), 6.02 (d, *J* = 1.5 Hz, 1H, 1′-H), 6.94 (d, *J* = 8.7 Hz, 1H, *Ph* 5-H), 7.21 (dd, *J* = 8.7, 2.7 Hz, 1H, *Ph* 4-H), 7.25 (d, *J* = 2.4 Hz, 1H, *Ph* 6-H), 8.26 (s, 1H, 2-H), 8.29 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 40.30 (Ph*C*H$_2$), 56.27 (PhO*C*H$_3$), 63.60 (5′-C), 77.56 (4′-C), 82.17 (3′-C), 82.60 (2′-C), 94.15 (1′-H), 112.83 (*Ph* 3-C), 120.66 (5-C), 126.30 (*Ph* 5-C), 129.07 (*Ph* 6-C), 129.09 (*Ph* 4-C), 130.00 (*Ph* 1-C), 141.24 (8-C), 149.20 (4-C), 153.68 (2-C), 155.99 (6-C), 157.57 (*Ph* 2-C). ESI-HRMS for $[C_{18}H_{20}ClN_5O_5 + H]^+$ calcd, 422.1231; found, 422.1236.

1′-[*N*^{6}-(3-Chlorobenzyl)-adenin-9-yl]-β-D-apio-D-furanose (10a)—Following the protocol used for the synthesis of compound **11a**, β-D-apio-D-furanoadenosine **4** (100 mg, 0.38 mmol) was converted to the title compound, which was first purified by silica-gel column chromatography and then by RP-HPLC (H_2O/AcCN, 90/10→70/30 in 18 min, R_t = 12.5 min) to obtain **10a** as white solid (16 mg, 11%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.46 (q, *J* = 11.0 Hz, 2H, 5′-H), 3.77 (d, *J* = 9.3 Hz, 1H, 4′-H), 4.32 (d, *J* = 9.1 Hz, 1H, 4′-H), 4.72 (br. s, 2H, PhC*H*$_2$), 4.82 (d, *J* = 7.5 Hz, 1H, 2′-H), 4.87 (br.s, 1H, 3′-OH), 5.43 (br.s, 1H, 2′-OH), 5.90 (d, *J* = 7.7 Hz, 1H, 1′-H), 7.23 – 7.43 (m, 4H, *Ph*-H's), 8.23 (s, 1H, 2-H), 8.38 (s, 1H, 8-H), 8.44 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 42.41 (Ph*C*H$_2$), 62.38 (5′-C), 73.42 (2′-C), 74.59 (4′-C), 78.28 (3′-C), 87.81 (1′-C), 119.70 (5-C) 125.80, 126.58, 126.88, 130.14, 132.87 (*Ph*-C's), 140.21 (8-C), 142.78 (*Ph*-C), 149.18 (4-C), 152.56 (2-C), 154.33 (6-C). ESI-HRMS for $[C_{17}H_{18}ClN_5O_4 + H]^+$ calcd, 392.1120; found, 392.1127.

1′-[*N*^{6}-(3-Iodobenzyl)-adenin-9-yl]-β-D-apio-D-furanose (10b)—Following the protocol used for the synthesis of compound **11a**, β-D-apio-D-furanoadenosine **4** (100 mg, 0.38 mmol) was converted to the title compound **10b**, which was first purified by silica-gel column chromatography and then isolated as a white solid following trituration with methanol (15 mg, 8%).^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.37 – 3.57 (m, 2H, 5′-CH_2), 3.76 (d, *J* = 9.2 Hz, 1H, 4′-H), 4.32 (d, *J* = 9.2 Hz, 1H, 4′-H), 4.67 (br. s, 2H, PhCH_2), 4.76 – 4.82 (m, 1H, 2′-H), 4.84 (s, 1H, 3′-OH), 4.90 (t, *J* = 5.5 Hz, 1H, 5′-OH), 5.40 (d, *J* = 6.9 Hz, 1H, 2′-H), 5.89 (d, *J* = 7.5 Hz, 1H, 1′-H), 7.11(t, *J* = 7.6 Hz, 1H, *Ph* 5-H), 7.36 (d, *J* = 7.6 Hz, 1H, *Ph*-H) 7.58 (d, *J* = 7.8 Hz, 1H, *Ph*-H) 7.72 (s, 1H, *Ph* 2-H), 8.22 (s, 1H, 2-H), 8.38 (s, 1H, 8-H), 8.41 (br.s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 42.24 (Ph*C*H$_2$), 62.37 (5′-C), 73.39 (2′-C), 74.57 (4′-C), 78.26 (3′-C), 87.80 (1′-C), 94.71 (*Ph*-C), 119.72 (5-C), 126.59, 130.47, 135.32, 135.68 (*Ph*-C's), 140.17 (8-C) 142.88 (*Ph*-C), 149.13 (4-C), 152.54 (2-C), 154.31 (6-C). ESI-HRMS for [$C_{17}H_{18}IN_5O_4$ + H]$^+$ calcd, 484.0476; found, 484.0490.

1′-[*N*^{6}-(2-Methoxy-5-chlorobenzyl)-adenin-9-yl]-β-D-apio-D-furanose (10c)—To a solution of β-D-apio-D-furanoadenosine **4** (60 mg, 0.23 mmol) in anh. DMF (2 mL) was added 2-methoxy-5-chlorobenzyl bromide (58 mg, 0.25 mmol), and the mixture was stirred at rt for 24 h. Solvent was evaporated in vacuo. The residue was treated with ammonium hydroxide (25%, 10 mL) and the mixture stirred at 55 °C for 24 h. The volatiles were evaporated under reduced pressure and the residue purified by silica-gel column chromatography (3–6% MeOH in CH_2Cl_2). The isolated residue was further purified by preparative thin layer chromatography to afford desired product **10c** (20 mg, 21%) as a homogeneous white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 3.59 – 3.74 (q, *J* = 11.4 Hz, 2H, 5′-H), 3.87 (s, 3H, PhOCH_3), 3.98 (d, *J* = 9.6 Hz, 1H, 4′-H), 4.49 (d, *J* = 9.6 Hz, 1H, 4′-H), 4.77 (br.s, 2H, PhCH_2), 4.85 (d, *J* = 7.1 Hz, 1H, 2′-H), 6.01 (d, *J* = 7.1 Hz, 1H, 1′-H), 6.93 (d, *J* = 8.7 Hz, 1H, *Ph* 5-H), 7.20 (dd, *J* = 8.7, 2.7 Hz, 1H, *Ph* 4-H), 7.25 (d, *J* = 2.5 Hz, 1H, *Ph* 2-H), 8.25 (s, 2H, 2-H & 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 40.25 (Ph*C*H$_2$), 56.25 (PhO*C*H$_3$), 64.42 (5′-C), 75.99 (2′-C), 76.42 (4′-C), 79.85 (3′-C), 90.47 (1′-C), 112.79 (*Ph*-C) 120.99 (5-C), 126.27, 129.03, 129.05, 129.96 (*Ph*-C's), 141.13 (8-C), 150.25 (4-C), 153.97 (2-C), 156.01 (6-C), 157.52 (*Ph*-C). ESI-HRMS for [$C_{18}H_{20}ClN_5O_5$ + H]$^+$ calcd, 422.1226; found, 422.1235.

1′-[*N*^{6}-(*N,N*-Dimethylformamidine)-adenin-9-yl]-2′,3′,5′-*O*-tris(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (17)—To a solution of α-D-apio-L-furanoadenosine **7** (150 mg, 0.56 mmol) in anh. DMF (5 mL) was added imidazole (458 mg, 6.73 mmol) and TBDMSCl (845 mg, 5.6 mmol). The mixture was heated to 100 °C for 3 days and cooled, and a saturated aqueous NH_4Cl solution was added. The product was extracted with EtOAc (3 × 20 mL), and the combined organic layers were dried over anh. Na_2SO_4. The residue obtained after evaporation of the organic layers was subjected to column chromatography (15–30% EtOAc in hexanes) to afford **17** (300 mg, 80 %) as a colorless glassy solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.38 (s, 3H, $SiCH_3$), −0.07 (s, 3H, $SiCH_3$), 0.00 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$), 0.18 (s, 3H, $SiCH_3$), 0.78 (s, 9H, $C(CH_3)_3$), 0.93 (s, 9H, $C(CH_3)_3$), 0.96 (s, 9H, $C(CH_3)_3$), 3.21 (s, 3H, NCH_3), 3.27 (s, 3H, NCH_3), 3.71 (d, *J* = 10.5 Hz, 1H, 4′-H), 3.95 (d,

J = 10.5 Hz, 1H, 4′-H), 4.25 (d, *J* = 8.5 Hz, 1H, 5′-H), 4.34 (d, *J* = 8.8 Hz, 1H, 5′-H), 5.16 (d, *J* = 6.2 Hz, 1H, 2′-H), 5.75 (d, *J* = 6.2 Hz, 1H, 1′-H), 7.94 (s, 1H, 8-H), 8.54 (s, 1H, 2-H), 8.97 (s, 1H, N^6CH). ESI-HRMS for $[C_{31}H_{60}N_6O_4Si_3 + H]^+$ calcd, 665.4062; found, 665.4075.

1′-(Adenin-9-yl)-2′,3′,5′-*O*-tri(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (18)—The N^6-dimethyformamidine derivative **17** (300 mg, 0.45 mmol) was dissolved in a 7N solution of ammonia in MeOH and stirred at rt for 18 h. The mixture was evaporated and the residue purified by column chromatography (20–40% EtOAc in hexanes) to afford **18** (200 mg, 73%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.34 (s, 3H, $SiCH_3$), −0.04 (s, 3H, $SiCH_3$), 0.01 (s, 3H, $SiCH_3$), 0.09 (s, 3H, $SiCH_3$), 0.14 (s, 3H, $SiCH_3$), 0.15 (s, 3H, $SiCH_3$), 0.17 (s, 3H, $SiCH_3$), 0.79 (s, 9H, $C(CH_3)_3$), 0.92 (s, 9H, $C(CH_3)_3$), 0.97 (s, 9H, $C(CH_3)_3$), 3.71 (d, *J* = 10.5 Hz, 1H, 4′-H), 3.96 (d, *J* = 10.8 Hz, 1H, 4′-H), 4.25 (d, *J* = 8.8 Hz, 1H, 5′-H), 4.32 (d, *J* = 8.5 Hz, 1H, 5′-H), 5.13 (d, *J* = 6.2 Hz, 1H, 2′-H), 5.61 (s, 2H, NH_2), 5.73 (d, *J* = 6.2 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H) 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.29, −5.27, −5.19, −4.65, −2.85, −2.70 ($SiCH_3$), 17.97, 18.41, 18.75 ($C(CH_3)_3$), 25.66, 26.04, 26.19 ($C(CH_3)_3$), 64.94 (5′-C), 74.45 (4′-C), 83.16 (2′ & 3′-C), 90.42 (1′-C), 120.57 (5-C), 140.12 (8-C), 150.07 (4-C), 153.22 (2-C), 155.55 (6-C). ESI-HRMS for $[C_{28}H_{55}N_5O_4Si_3 + H]^+$ calcd, 610.3640; found, 610.3651.

Method A: A solution of compound **18** (180 mg, 0.3 mmol) in anh. THF (4.25 mL) was placed in a Teflon flask under an inert atmosphere and cooled to 0 °C. In a separate polypropylene flask, anh. THF (1.25 mL) and anh. pyridine (0.5 mL) were placed under inert atmosphere and the mixture cooled to 0 °C. A solution of 70% HF in pyridine (0.5 mL) was added dropwise to the latter mixture. 1.7 mL of the resulting chilled THF-pyridine-HF.pyridine (2.5:1:1) mixture was added dropwise to the solution of **18**, and the mixture was stirred at 0 °C for 1 h. The ice-bath was removed and reaction continued at rt for 24h. The reaction mixture was poured to an ice-cold solution of $NaHCO_3$ (1.5 g in 8 mL) with vigorous stirring. The product was extracted with EtOAc (3 × 20 mL), washed with brine and dried over anh. Na_2SO_4. The residue obtained after evaporation was subjected to flash column chromatography (2–5% MeOH in CH_2Cl_2) to obtain following compounds as white foams: Starting material **18** (70 mg, 39%), compound **19** (33 mg, 29%) and a mixture of compounds **20** and **21** (~2:1, 37 mg, 25%).

Method B: To a cooled (0 °C) solution of compound **18** (70 mg, 0.12 mmol) in anh. THF (1 mL) was added a solution of trichloroacetic acid (507 mg, 3.1 mmol) in water (0.27 mL), and the mixture was stirred for 20 min at 0 °C, and for 24 h at rt. The reaction mixture was cooled and neutralized with a cold sat. $NaHCO_3$ solution. The products were extracted with EtOAc (3 × 30 mL), the combined organic phase was dried over anh. Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by column chromatography to afford following compounds: Starting material **18** (20 mg, 29%), compound **19** (10 mg, 23%) and a mixture of compounds **20** and **21** (~2:1, 17 mg, 30%).

1′-(Adenin-9-yl)-3′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (19)—^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.08 (s, 6H, $(SiCH_3)_2$), 0.90 (s, 9H, $C(CH_3)_3$), 3.74 (d, *J*

= 10.5 Hz, 1H, 4′-H), 3.84 (d, *J* = 10.3 Hz, 1H, 4′-H), 3.99 (d, *J* = 9.1 Hz, 1H, 5′-H), 4.06 (d, *J* = 8.8 Hz, 1H, 5′-H), 4.41 (dd, *J* = 5.3, 3.22 Hz, 1H, 2′-H), 5.32 (s, 1H, 5′-OH), 5.88 (d, *J* = 5.6 Hz, 1H, 2′-OH), 5.90 (d, *J* = 3.2 Hz, 1H, 1′-H), 7.26 (br. s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.30 (s, 1H, 8-H). ^{1}H NMR (75 MHz, DMSO-d_6) δ ppm −5.48, −5.37 ($SiCH_3$), 18.05 ($C(CH_3)_3$), 25.79 ($C(CH_3)_3$), 64.25 (5′-C), 75.07 (4′-C), 79.93 (2′-C), 80.13 (3′-C), 90.57 (1′-C), 118.76 (5-C), 139.62 (8-C), 148.99 (4-C), 152.39 (2-C), 155.90 (6-C). ESI-HRMS for $[C_{16}H_{27}N_5O_4Si + H]^+$ calcd, 382.1911; found, 382.1903.

1′-(Adenin-9-yl)-2′,3′-*O*-di(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (20)—^{1}H NMR (300 MHz, DMSO-d_6) δ ppm −0.18, −0.05, 0.08 (s, 4×3H, $SiCH_3$), 0.78, 0.90 (s, 2×9H, $C(CH_3)_3$), 3.67 (d, *J* = 10.3 Hz, 1H, 5′-H), 3.82 (d, *J* = 10.3 Hz, 1H, 5′-H), 4.01 (d, *J* = 8.8 Hz, 1H, 4′-H), 4.11 (d, *J* = 8.8 Hz, 1H, 4′-H), 4.68 (d, *J* = 4.4 Hz, 1H, 2′-H), 5.33 (s, 1H, 5′-OH), 5.89 (d, *J* = 4.4 Hz, 1H, 1′-H), 7.27 (br. s., 2H, NH_2), 8.15 (s, 1H, 2-H), 8.29 (s, 1H, 8-H). ESI-HRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2775.

1′-(Adenin-9-yl)-3′,5′-*O*-di(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (21)—^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.10, 0.10, 0.12, 0.13 (s, 4×3H, $SiCH_3$), 0.87, 0.92 (s, 2×6H, $C(CH_3)_3$), 3.75 (d, *J* = 10.8 Hz, 1H, 5′-H), 3.87 (d, *J* = 11.1 Hz, 1H, 5′-H), 4.06 (d, *J* = 8.2 Hz, 1H, 4′-H), 4.19 (d, *J* = 8.2 Hz, 1H, 4′-H), 5.00 (t, *J* = 5.1 Hz, 1H, 2′-H), 5.76 (d, *J* = 5.9 Hz, 1H, 1′-H), 5.84 (d, *J* = 5.0 Hz, 1H, 2′-OH), 7.27 (br. s., 2H, NH_2), 8.12 (s, 1H, 2-H), 8.24 (s, 1H, 8-H). ESIHRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2775.

1′-(Adenin-9-yl)-2′,5′-*O*-di(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (22)—To a solution of α-D-apio-L-furanoadenosine **7** (150 mg, 0.56 mmol) in anh. DMF (2 mL) was added imidazole (155 mg, 2.28 mmol), followed by TBDMSCl (253 mg, 1.68 mmol) and the mixture was stirred at rt for 18 h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over anh. Na_2SO_4, filtered and evaporated. The residue was purified by column chromatography using 3–5% MeOH in CH_2Cl_2 to afford **22** (230 mg, 83%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H, $SiCH_3$), 0.10 (s, 6H, $SiCH_3$), 0.12 (s, 3H, $SiCH_3$), 0.91 (s, 18H, $C(CH_3)_3$), 3.75 (d, *J* = 10.0 Hz, 1H, 4′-H), 3.92 (d, *J* = 10.3 Hz, 1H, 4′-H), 4.08 (d, *J* = 9.1 Hz, 1H, 5′-H), 4.21 (d, *J* = 9.4 Hz, 1H, 5′-H), 4.45 (d, *J* = 1.5 Hz, 1H, 2′-H), 5.80 (brs, 2H, NH), 5.95 (d, *J* = 1.8 Hz, 1H, 1′-H), 8.13 (s, 1H, 8-H) 8.32 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.51, −5.31, −5.22, −4.55 ($SiCH_3$), 17.87, 18.37 ($C(CH_3)_3$), 25.66, 25.91 ($C(CH_3)_3$), 63.15 (4′-C), 76.68 (5′-C), 81.43 (3′-C), 81.53 (2′-C), 92.75 (1′-C), 119.81 (5-C), 140.04 (8-C), 149.27 (4-C), 152.68 (2-C), 155.31 (6-C). ESI-HRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2783.

1′-(Adenin-9-yl)-2′,5′-*O*-di(*tert*-butyldimethylsilyl)-3′-deoxy-α-D-apio-L-furanose (23)—To a solution of L-furano-3′-deoxy-D-apioadenosine **8** (170 mg, 0.67 mmol) in anh. DMF (2 mL) was added imidazole (230 mg, 3.38 mmol), followed by TBDMSCl (305 mg, 2.03 mmol) and the mixture was stirred at rt for 18h. The reaction mixture was diluted with water (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried over anh. Na_2SO_4, filtered and evaporated. The residue

was purified by column chromatography using 3–5% MeOH in CH_2Cl_2 to afford **23** (242 mg, 75%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.05 (s, 6H, $Si(CH_3)_2$), 0.07 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.88 (s, 9H, $C(CH_3)_3$), 0.90 (s, 9H, $C(CH_3)_3$), 2.56 (qt, *J* = 7.8, 5.7 Hz, 1H, 3′-H), 3.75 (dd, *J* = 10.0, 7.9 Hz, 1H, 5′-H), 3.87 (dd, *J* = 10.1, 6.0 Hz, 1H, 5′-H), 4.08 (t, *J* = 8.4 Hz, 1H, 4′-H), 4.45 (dd, *J* = 8.2, 7.6 Hz, 1H, 4′-H), 4.85 (dd, *J* = 5.0, 2.05 Hz, 1H, 2′-H), 5.55 (br.s, 2H, NH_2), 5.90 (d, *J* = 2.1 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.47, −5.39, −5.28, −4.70 ($SiCH_3$), 17.99, 18.26 ($C(CH_3)_3$), 25.69, 25.87 ($C(CH_3)_3$), 44.77 (3′-C), 60.02 (5′-C), 71.91 (4′-C), 76.02 (2′-C), 92.47 (1′-C), 120.45 (5-C), 138.77 (8-C), 149.35 (4-C), 152.95 (2-C), 155.24 (6-C). ESI-HRMS for [$C_{22}H_{41}N_5O_3Si_2$] calcd, 480.2826; found, 480.2828.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-α-D-apio-L-furanose (24)— Following the desilylation method B (which was slightly modified in that the reaction mixture was stirred for 2h at rt) **22** (210 mg, 0.42 mmol) was converted to **24** (140 mg, 87%), which was obtained as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm −0.17 (s, 3H, $SiCH_3$), −0.04 (s, 3H, $SiCH_3$), 0.79 (s, 9H, $C(CH_3)_3$), 3.55 (dd, *J* = 11.1, 5.0 Hz, 1H, 5′-H), 3.64 (dd, *J* = 11.1, 5.3 Hz, 1H, 5′-H), 4.02 (d, *J* = 8.8 Hz, 1H, 4′-H), 4.08 (d, *J* = 8.8 Hz, 1H, 4′-H), 4.66 (d, *J* = 4.1 Hz, 1H, 2′-H), 4.74 (t, *J* = 5.3 Hz, 1H, 5′-OH), 5.35 (s, 1H, 3′-OH), 5.87 (d, *J* = 4.1 Hz, 1H, 1′-H), 7.27 (brs, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.32 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm −5.30 ($Si(CH_3)_2$), 17.49 ($C(CH_3)_3$), 25.37 ($C(CH_3)_3$), 61.85 (5′-C), 74.59 (4′-C), 79.92 (3′-C) 81.13 (2′-C) 89.86 (1′-C), 118.84 (5-C), 139.70 (8-C), 149.00 (4-C), 152.42 (2-C), 155.90 (6-C). ESIHRMS for [$C_{16}H_{27}N_5O_4Si$ +H]$^+$ calcd, 382.1911; found, 382.1908.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-α-D-apio-L-furanose (25)—Following the desilylation method A (which was slightly modified in that the reaction mixture was stirred for 1h at rt) starting material **23** (240 mg, 0.5 mmol) was converted to **25** (150 mg, 82%), obtained as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.08 (s, 3H, $SiCH_3$), 0.11 (s, 3H, $SiCH_3$), 0.91 (s, 9H, $C(CH_3)_3$), 2.14 (br. s., 1H, 5′-OH), 2.66 (tq, *J* = 7.9, 5.7 Hz, 1H, 3′-H), 3.89 (d, *J* = 5.6 Hz, 2H, 5′-H), 4.19 (t, *J* = 8.4 Hz, 1H, 4′-H), 4.46 (dd, *J* = 8.5, 7.9 Hz, 1H, 4′-H), 5.07 (dd, *J* = 5.7, 2.5 Hz, 1H, 2′-H), 5.56 (br. s., 2H, NH_2), 5.89 (d, *J* = 2.4 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.22, −4.72 ($SiCH_3$), 17.94 ($C(CH_3)_3$), 25.70 ($C(CH_3)_3$), 43.69 (3′-C), 59.79 (5′-C), 70.79 (4′-C), 77.09 (2′-C), 92.38 (1′-C), 120.45 (5-C), 138.92 (8-C), 149.38 (4-C), 153.04 (2-C), 155.29 (6-C). ESI-HRMS for [$C_{16}H_{27}N_5O_3Si$ + H]$^+$ calcd, 366.1961; found, 366.1956.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (26)—To a solution of compound **24** (30 mg, 0.078 mmol) in anh. THF (2 mL) was added CDI (23 mg, 0.156 mmol) and the mixture was stirred at rt for 3 h. Cold ethylamine (0.3 mL) was added to the reaction mixture and stirring continued at rt for 16 h. Volatiles were evaporated, and the residue was suspended in CH_2Cl_2 and washed with sat. NH_4Cl, water and brine. The organic layer was dried over anh. Na_2SO_4, filtered, volatiles evaporated. The residue was purified by column chromatography (1–3% MeOH in CH_2Cl_2) to afford the title compound **26** (17 mg, 52%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$)

δ ppm 0.01 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.91 (s, 9H, $C(CH_3)_3$), 1.14 (t, *J* = 7.2 Hz, 3H, NCH_2CH_3), 3.24 (qd, *J* = 7.3, 1.2 Hz, 2H, NCH_2CH_3) 4.06 (d, *J* = 9.4 Hz, 1H, 4′-H), 4.17 (d, *J* = 9.4 Hz, 1H, 4′-H), 4.32 (d, *J* = 11.4 Hz, 1H, 5′-H), 4.41 (d, *J* = 11.4 Hz, 1H, 5′-H), 4.56 (s, 1H, 2′-H), 4.84 (t, *J* = 5.1 Hz, 1H, CONH), 5.75 (s, 1H, 1′-H), 5.83 (br. s., 2H, NH_2), 7.95 (s, 1H, 8-H), 8.32 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.22 ($SiCH_3$), −4.44 ($SiCH_3$), 15.17 (NCH_2CH_3), 17.81 ($C(CH_3)_3$), 25.61 ($C(CH_3)_3$), 35.99 (NCH_2CH_3), 65.30 (5′-C), 76.41 (4′-C), 80.48 (3′-C), 82.81 (1′-C), 94.41 (1′-C), 120.43 (5-C), 140.41 (8-C), 148.43 (4-C), 152.50 (2-C), 155.70 (6-C), 156.44 (CO). ESI-HRMS for $[C_{19}H_{32}N_6O_5Si + H]^+$ calcd, 453.2282; found, 453.2220.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (27)—Following the same protocol as described for **26**, 30 mg (0.082 mmol) of **25** was converted to **27** (35 mg, 98%), obtained as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 0.12 (s, 3H, $SiCH_3$), 0.18 (s, 3H, $SiCH_3$), 0.92 (s, 9H, $C(CH_3)_3$), 1.12 (t, *J* = 7.2 Hz, 3H, NCH_2CH_3) 2.61 – 2.80 (m, 1H, 3′-H), 3.20 (quin, *J* = 6.6 Hz, 2H, NCH_2CH_3), 4.05 (t, *J* = 8.8 Hz, 1H, 4′-H), 4.14 – 4.34 (m, 2H, 5′-H), 4.44 (t, *J* = 8.1 Hz, 1H, 4′-H), 4.62 (t, *J* = 4.8 Hz, 1H, NH), 4.87 (d, *J* = 4.1 Hz, 1H, 2′-H), 5.65 (br.s, 2H, NH_2), 5.87 – 5.96 (d, *J* = 1.2 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.29, −4.59 ($Si(CH_3)_2$), 15.25 (NCH_2CH_3), 18.00 ($C(CH_3)_3$), 25.72 ($C(CH_3)_3$), 35.89 (NCH_2CH_3), 41.75 (3′-C), 61.03 (5′-C), 71.26 (4′-C), 75.99 (2′-C), 92.67 (1′-C), 120.44 (5-C), 138.51 (8-C), 149.24 (4-C), 153.00 (2-C), 155.30 (6-C), 155.97 (CO). ESI-HRMS for $[C_{19}H_{32}N_6O_4Si]$ calcd, 437.2333; found, 437.2338.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-5′-(*N*-ethyl)-carboxamido-α-D-apio-L-furanose (28)—To a suspension of compound **25** (30 mg, 0.082 mmol) in a 1:1:1.5 mixture of acetonitrile-CCl_4-water (2 mL) was added $NaIO_4$ (75 mg, 0.33 mmol), followed by $RuCl_3$ (12 mg). The reaction mixture was stirred at rt for 7h and the solvents evaporated under vacuum. The residue was suspended in MeOH (5 mL), subjected to centrifugation and the clear supernatant liquid was decanted and evaporated. The residue was dried under high vacuum, and suspended in anh. THF (1.5 mL). To this suspension was added DMAP (23 mg, 0.19 mmol) and CDI (27 mg, 0.17 mmol), and the mixture was stirred at rt for 2–3 h. Cold ethylamine (0.3 mL) was added and stirring was continued at rt for 18h, after which volatiles in the reaction mixture were evaporated and the residue purified by column chromatography. The products were further purified by preparative thin layer chromatography (10% MeOH in CH_2Cl_2) to afford the title compound **28** (R_f: 0.4, 3 mg, 9%), as well as the carbamate **27** (R_f: 0.55, 3 mg, 8%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.01 (s, 3H, $SiCH_3$), 0.06 (s, 3H, $SiCH_3$), 0.87 (s, 9H, $C(CH_3)_3$), 1.18 (t, *J* = 7.3 Hz, 3H, NCH_2CH_3), 3.14 – 3.23 (m, 1H, 3′-H), 3.27 – 3.44 (m, 2H, NCH_2CH_3), 4.51 (dd, *J* = 8.8, 6.7 Hz, 1H, 4′-H), 4.57 (dd, *J* = 8.6, 7.2 Hz, 1H, 4′-H), 5.33 (dd, *J* = 5.9, 3.2 Hz, 1H, 2′-H), 5.64 (br. s., 2H, 6-NH_2), 5.91 (d, *J* = 3.5 Hz, 1H, 1′-H), 6.09 (t, *J* = 4.5 Hz, 1H, 5′-NH), 7.86 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ESI-HRMS for $[C_{18}H_{30}N_6O_3Si + H]^+$ calcd, 407.2227; found, 407.2222.

1′-(Adenin-9-yl)-3′-deoxy-5′-(*N*-ethyl)-carboxamido-α-D-apio-L-furanose (15)—To a solution of compound **28** (3 mg, 0.007 mmol) in MeOH (0.5 mL) in a polypropylene

vessel was added NH_4F (6 mg, 0.14 mmol), and the reaction mixture was stirred at 50 °C for 48 h. After addition of CH_2Cl_2 (1.5 mL), the reaction mixture was filtered through a celite pad. The filtrate was concentrated and the residue purified by column chromatography (5–7 % MeOH in CH_2Cl_2) to afford the title compound **15** (1.5 mg, 70 %) as a white solid after lyophilization. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.02 (t, J = 7.2 Hz, 3H, NCH_2CH_3), 3.04 – 3.15 (m, 2H, NCH_2CH_3), 4.23 (t, J = 8.2 Hz, 1H, 4′-H), 4.35 (t, J = 8.1 Hz, 1H, 4′-H), 4.77 (br. s., 1H, 2′-H), 5.88 (d, J = 3.5 Hz, 1H, 2′-OH), 5.95 (d, J = 2.6 Hz, 1H, 1′-H), 7.26 (s, 2H, 6-NH_2), 7.89 (t, J = 5.4 Hz, 1H, 5′-NH), 8.15 (s, 1H, 2-H), 8.23 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 14.64 (NCH_2CH_3), 33.43 (NCH_2CH_3), 47.11 (3′-C), 69.12 (4′-C), 75.17 (2′-C), 90.38 (1′-C), 119.19 (5-C), 138.83 (8-C), 148.91 (4-C), 152.56 (2-C), 156.01 (6-C), 168.19 (CO). ESI-HRMS $[C_{12}H_{16}N_6O_3 + H]^+$ calcd, 293.1362; found, 293.1360.

1′-(Adenin-9-yl)-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (13)—Following the protocol used for the synthesis of **15**, compound **26** (20 mg, 0.044 mmol) was converted to the title compound **13** (14 mg, 94%), obtained as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.11 (t, J = 7.2 Hz, 3H, NCH_2CH_3), 3.14 (q, J = 7.0 Hz, 2H, NCH_2CH_3), 4.13 (d, J = 9.4 Hz, 1H, 4′-H), 4.20 (d, J = 9.4 Hz, 1H, 4′-H), 4.26 (d, J = 11.4 Hz, 1H, 5′-H), 4.33 (d, J = 11.1 Hz, 1H, 5′-H), 4.37 (d, J = 1.2 Hz, 1H, 2′-H), 6.04 (d, J = 1.8 Hz, 1H, 1′-H), 8.21 (s, 1H, 2-H), 8.31 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 15.33 (NCH_2CH_3), 36.70 (NCH_2CH_3), 66.37 (5′-C), 77.43 (4′-C), 81.34 (3′-C), 81.93 (2′-C), 93.89 (1′-C), 120.30 (5-C), 141.53 (8-C), 150.00 (4-C) 153.69 (2-C), 157.35 (6-C), 158.83 (CO). ESI-HRMS for $[C_{13}H_{18}N_6O_5 + H]^+$ calcd, 339.1417; found, 339.1409.

1′-(Adenin-9-yl)-3′-deoxy-5′-*O*-(*N*-ethylcarbamoyl)-α-D-apio-L-furanose (14)—Following the protocol used for the synthesis of **15**, compound **27** (35 mg, 0.08 mmol) was converted to the title compound **14** (23 mg, 90%), obtained as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.00 (t, J = 7.2 Hz, 3H, NCH_2CH_3), 2.73 – 2.87 (m, 1H, 3′-H), 2.93 – 3.05 (m, 2H, NCH_2CH_3), 3.85 (t, J = 8.4 Hz, 1H, 4′-H), 4.03 (dd, J = 10.6, 8.3 Hz, 1H, 5′-H), 4.23 (dd, J = 10.9, 6.2 Hz, 1H, 5′-H), 4.37 (t, J = 7.8 Hz, 1H, 4′-H), 4.66 (t, J = 3.6 Hz, 1H, 2′-H), 5.86 (d, J = 4.8 Hz, 1H, 2′-OH), 5.92 (d, J = 2.1 Hz, 1H, 1′-H), 7.10 (t, J = 5.4 Hz, 1H, NH), 7.27 (br.s, 2H, NH_2), 8.15 (s, 1H, 2-H), 8.23 (s, 1H, 8-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 15.01 (NCH_2CH_3), 35.00 (NCH_2CH_3), 41.19 (3′-C), 60.76 (5′-C), 70.59 (4′-C), 74.24 (2′-C), 91.29 (1′-C), 119.24 (5-C), 139.05 (8-C), 148.73 (4-C), 152.55 (2-C), 155.99 (CO), 156.02 (6-C). ESI-HRMS for $[C_{13}H_{18}N_6O_4 + H]^+$ calcd, 323.1468; found, 323.1472.

1′-(Adenin-9-yl)-2′,5′-*O*-di(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (29)—Following the reaction protocol used for the synthesis of compound **22**, β-D-apio-D-furano adenosine **4** (150 mg, 0.56 mmol) was converted to the title compound **29** (207 mg, 74%) as white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.47, −0.07, 0.10, 0.12 (s's, 4 × 3H, $SiCH_3$), 0.81, 0.97 (s's, 9H, $C(CH_3)_3$), 3.01 (s, 1H, 3′-OH), 3.50 (d, J = 10.3 Hz, 1H, 5′-H), 3.59 (d, J = 10.3 Hz, 1H, 5′-H), 4.02 (d, J = 9.4 Hz, 1H, 4′-H), 4.50 (dd, J = 9.4, 1.03 Hz, 1H, 4′-H), 5.41 (d, J = 6.6 Hz, 1H, 2′-H), 5.65 (br.s, 2H, NH_2), 5.89 (d, J = 6.8 Hz, 1H, 1′-H), 7.86 (s, 1H, 2-H), 8.34 (s, 1H, 8-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.68, −5.47,

−5.22, −5.02 (Si*C*H_3), 17.76, 18.13 (*C*(CH_3)$_3$) 25.54, 25.73 (C(*C*H_3)$_3$), 62.13 (5′-C), 73.95 (2′-C), 74.11 (4′-C), 78.79 (3′-C), 89.89 (1′-C), 120.43 (5-C), 140.03 (8-C), 150.11 (4-C), 153.23 (2-C), 155.46 (6-C). ESI-HRMS for $[C_{22}H_{41}N_5O_4Si_2 + H]^+$ calcd, 496.2775; found, 496.2781.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-β-D-apio-D-furanose (30)— Following the desilylation method B (which was slightly modified in that the reaction mixture was stirred for 3 h at rt), compound **29** (200 mg, 0.4 mmol) was converted to **30** (90 mg, 35%), which was obtained as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.33, −0.03 (s's, 2 × 3H, SiCH_3), 0.84 (s, 9H, C(CH_3)$_3$), 3.28 (s, 1H, 3′-OH), 3.78 (s, 2H, 5′-H), 4.04 (d, *J* = 9.7 Hz, 1H, 4′-H), 4.45 (d, *J* = 9.7 Hz, 1H, 4′-H), 5.25 (d, *J* = 5.3 Hz, 1H, 2′-H), 5.71 (br.s, 2H, NH_2), 5.78 (d, *J* = 5.3 Hz, 1H, 1′-H), 7.87 (s, 1H, 8-H), 8.36 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −4.91, 0.15 (Si*C*H_3), 17.96 (*C*(CH_3)$_3$), 25.69 (C(*C*H_3)$_3$), 65.64 (5′-C), 75.56 (2′-C), 75.74 (4′-C), 78.33 (3′-C), 91.67 (1′-C), 120.72 (5-C), 140.57 (8-C), 149.76 (4-C), 153.35 (2-C), 155.78 (6-C). ESI-HRMS for $[C_{16}H_{27}N_5O_4Si + H]^+$ calcd, 382.1911; found, 382.1910.

1′-(Adenin-9-yl)-2′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*N*-methylcarbamoyl)-β-D-apio-Dfuranose (31)—Following the same protocol as described for **26** (which was slightly modified in that the liq. methylamine was reacted instead of liq. ethylamine), 85 mg (0.22 mmol) of **30** was converted to **31** (70 mg, 72%), obtained as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm −0.42, −0.05 (s's, 3H, SiCH_3), 0.83 (s, 9H, C(CH_3)$_3$), 2.85 (d, *J* = 4.9 Hz, 3H, NCH_3), 3.26 (br.s, 1H, 3′-OH), 4.09 (d, *J* = 9.8 Hz, 1H, 4′-H), 4.18 (d, *J* = 11.4 Hz, 1H, 5′-H), 4.28 (d, *J* = 11.4 Hz, 1H, 5′-H), 4.46 (d, *J* = 9.8 Hz, 1H, 4′-H), 4.90 (br.s, 1H, NH), 5.32 (d, *J* = 6.1 Hz, 1H, 2′-H), 5.81 (d, *J* = 6.1 Hz, 1H, 1′-H), 5.89 (br.m, 2H, NH_2), 7.86 (s, 1H, 8-H), 8.35 (s, 1H, 2-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm −5.14, −5.09 ($SiCH_3$), 17.76 (*C*(CH_3)$_3$), 25.52 (C(*C*H_3)$_3$), 27.71 (NCH_3), 65.43 (5′-C), 74.99 (2′-C), 75.13 (4′-C), 90.60 (1′-C), 120.60 (5-C), 140.49 (8-C), 149.86 (4-C), 153.17 (2-C), 155.65 (6-C), 156.60 (C=O). ESI-HRMS for $[C_{18}H_{30}N_6O_5Si + H]^+$ calcd, 439.2125; found, 439.2128.

1′-(Adenin-9-yl)-5′-*O*-(*N*-methylcarbamoyl)-β-D-apio-D-furanose (12)— Following the protocol used for the synthesis of **15**, compound **31** (60 mg, 0.13 mmol) was converted to the title compound **12** (40 mg, 90%), obtained as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ ppm 2.73 (s, 3H, NCH_3), 3.99 (d, *J* = 9.6 Hz, 1H, 4′-H), 4.15 – 4.31 (q, *J* = 11.2 Hz, 2H, 5′-H), 4.46 (d, *J* = 9.8 Hz, 1H, 4′-H), 4.91 (d, *J* = 7.1 Hz, 1H, 2′-H), 5.99 (d, *J* = 7.1 Hz, 1H, 1′-H), 8.21 (s, 1H, 2-H), 8.28 (s, 1H, 8-H). ^{13}C NMR (75 MHz, CD_3OD) δ ppm 27.53 (NCH_3), 66.69 (5′-C), 76.15 (2′-C), 76.43 (4′-C), 78.55 (3′-C), 90.40 (1′-C), 120.72 (5-C), 141.79 (8-C), 150.86 (4-C), 153.92 (2-C), 157.33 (6-C), 159.21 (C=O). ESI-HRMS for $[C_{12}H_{16}N_6O_5 + H]^+$ calcd, 325.1255; found, 325.1260.

Pharmacological assay procedures

Cyclic AMP accumulation assay—Intracellular cAMP levels were measured with a competitive protein binding method.[25] Chinese hamster ovary (CHO) cells that expressed the recombinant hA_3AR were harvested by trypsinization. After centrifugation and

resuspension in medium, cells were plated in 24-well plates in 0.5 mL medium. After 24 h, the medium was removed, and cells were washed three times with 1 mL DMEM, containing 50 mM N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid (HEPES), 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). Forskolin (10 μM) was added to the medium after 45 min. After the addition of forskolin, the incubation was continued an additional 15 min. The reaction was terminated upon removal of the supernatant, and cells were lysed upon the addition of 200 μL of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at −20°C. For determination of cAMP production, protein kinase A (PKA) was incubated with [^{3}H]cAMP (2 nM) in K_2HPO_4/EDTA buffer (K_2HPO_4, 150 mM; EDTA, 10 mM), 20 μL of the cell lysate, and 30 μL 0.1 M HCl or 50 μL of cAMP solution (0–16 pmol/200 μL for standard curve). Bound radioactivity was separated by rapid filtration through Whatman GF/C filters and washed once with cold buffer. Bound radioactivity was measured by liquid scintillation spectrometry.

Binding assays—Binding assays were performed using CHO cells stably expressing the recombinant hA_1AR or hA_3AR or HEK-293 cells stably expressing the $hA_{2A}AR$.[24] Binding and functional parameters were calculated using Prism 5.0 software (GraphPAD, San Diego, CA, USA). IC_{50} values obtained from competition curves were converted to K_i values using the Cheng-Prusoff equation.[26] Data were expressed as mean ± standard error of the mean.

Molecular Modeling Methods

A homology model of the hA_3AR was built based on a hybrid structural template using the homology modeling tool implemented in the MOE suite,[27] as previously reported.[21,28] In particular, an agonist-bound structure of the closely related $hA_{2A}AR$ (PDB code 3QAK)[20] was used as a template for most of the hA_3AR structure except for the extracellular terminus of TM2 (residues from Val63 to Ser73) and EL1 (residues from Leu74 to Tyr81). The extracellular portion of TM2 was based on the X-ray structure of the $h\beta_2$ adrenergic receptor in complex with the Gs protein (PDB code 3SN6).[29] Molecular docking of apio-adenosine derivatives (prepared for docking using the build panel and the LigPrep panel implemented in the Schrödinger suite)[30] at the hA_3AR hybrid model was performed by means of the Glide[31] module of the Schrödinger suite. A Glide Grid was centered on the centroid of some key residues of the binding pocket of adenosine receptors, namely Phe (EL2), Asn (6.55), Trp (6.48) and His (7.43). The Glide Grid was built using an inner box (ligand diameter midpoint box) of 10 Å × 10 Å × 10 Å and an outer box that extended 20 Å in each direction from the inner one. Docking of compounds was performed in the rigid binding site using the XP (extra precision) procedure. The top scoring docking poses for each ligand were subjected to visual inspection and analysis of protein-ligand interactions to select the proposed binding conformations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AR	adenosine receptor
BAIB	bis-acetoxyiodobenzene
cAMP	adenosine 3′,5′-cyclic phosphate
CDI	carbonyldiimidazole
Cl-IB-MECA	2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine
CHO	Chinese hamster ovary
DMF	*N*,*N*-dimethylformamide
DMEM	Dulbecco's modified Eagle's medium
EDTA	ethylenediaminetetraacetic acid
GPCR	G protein-coupled receptor
HEK	human embryonic kidney
I-AB-MECA	N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyl-uronamide
NECA	5′-*N*-ethylcarboxamidoadenosine
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRMS	high resolution mass spectroscopy
NMR	nuclear magnetic resonance
***R*-PIA**	N^6-R-phenylisopropyladenosine
TBDMS	*tert*-butyldimethylsilyl
TCA	trichloroacetic acid
TEMPO	(2,2,6,6-tetramethylpyperidin-1yl)-oxyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TM	transmembrane helical domain

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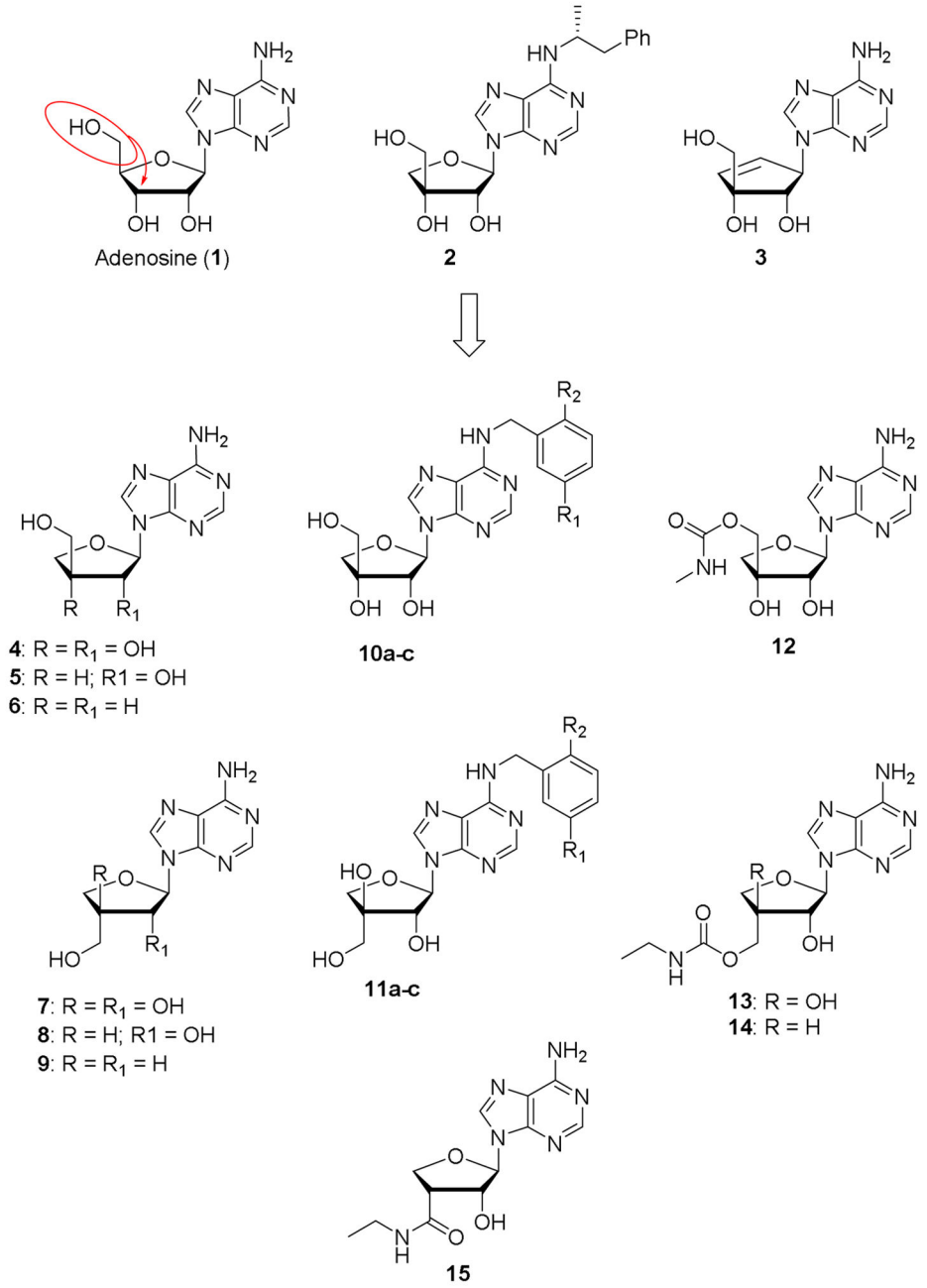


Figure 1.

Apio-type nucleosides previously tested for modulation of ARs (**1**-**3**) and target analogues of the present study (**4**-**15**).

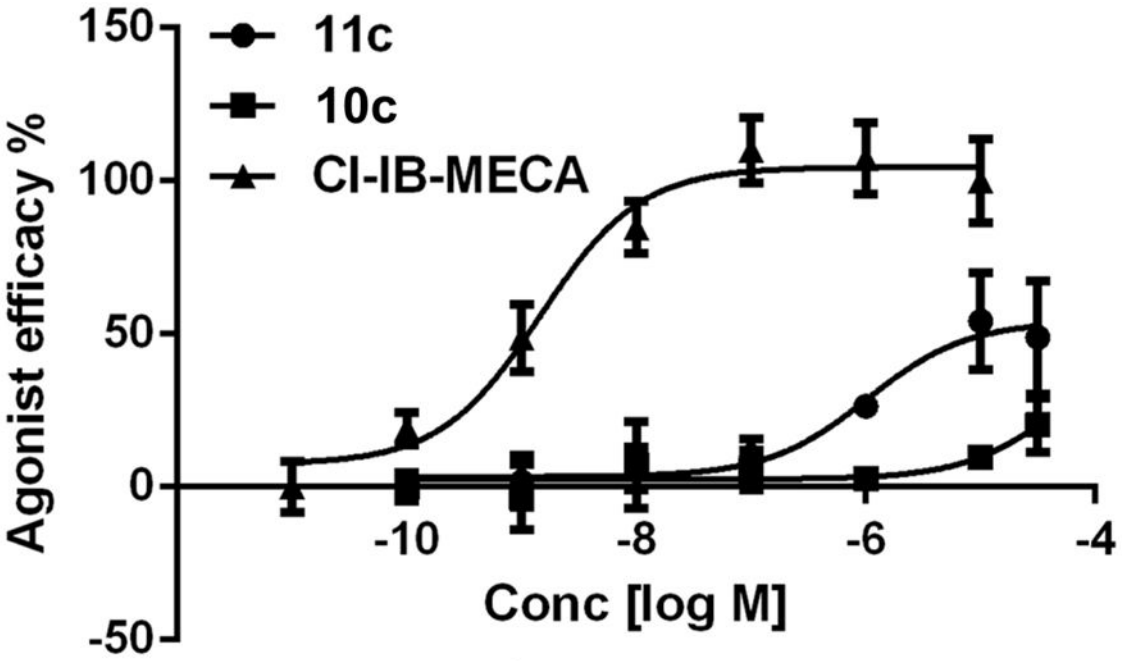


Figure 2.

Effect of compounds **10c** and **11c** on forskolin-induced stimulation of cAMP production at the hA_3AR expressed in CHO cells, compared to Cl-IB-MECA as reference full agonist (= 100%). Representative curves from 3 determinations are shown.

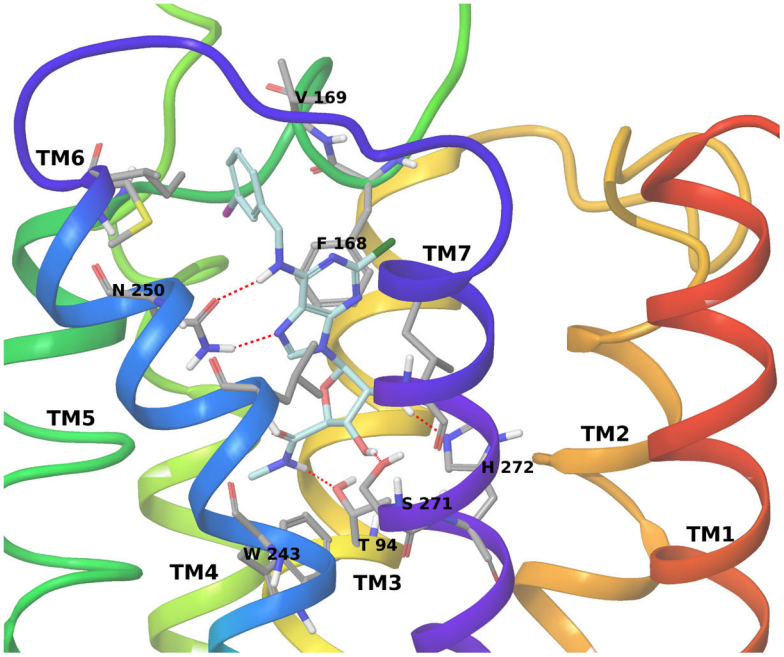


Figure 3.
Binding of A_3AR full agonist Cl-IB-MECA

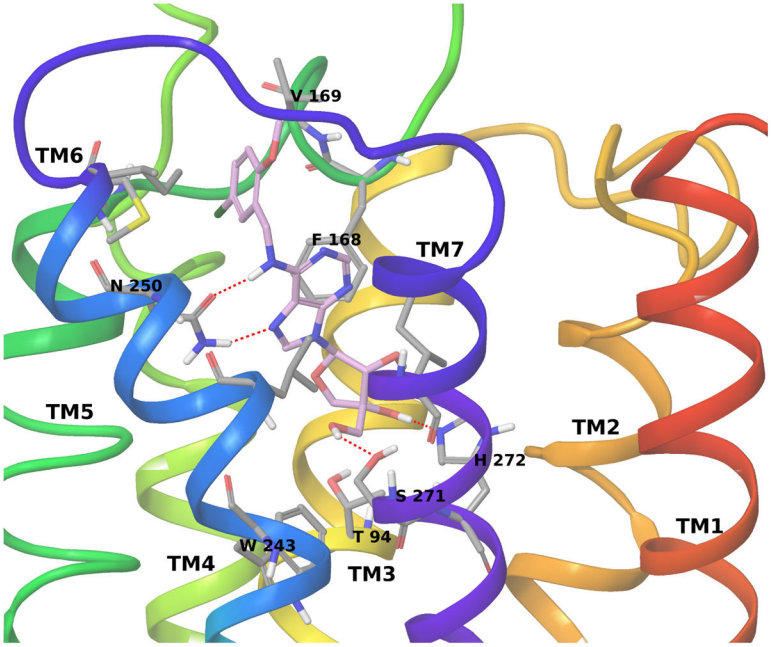


Figure 4.
Binding of A_3AR antagonist **10c**

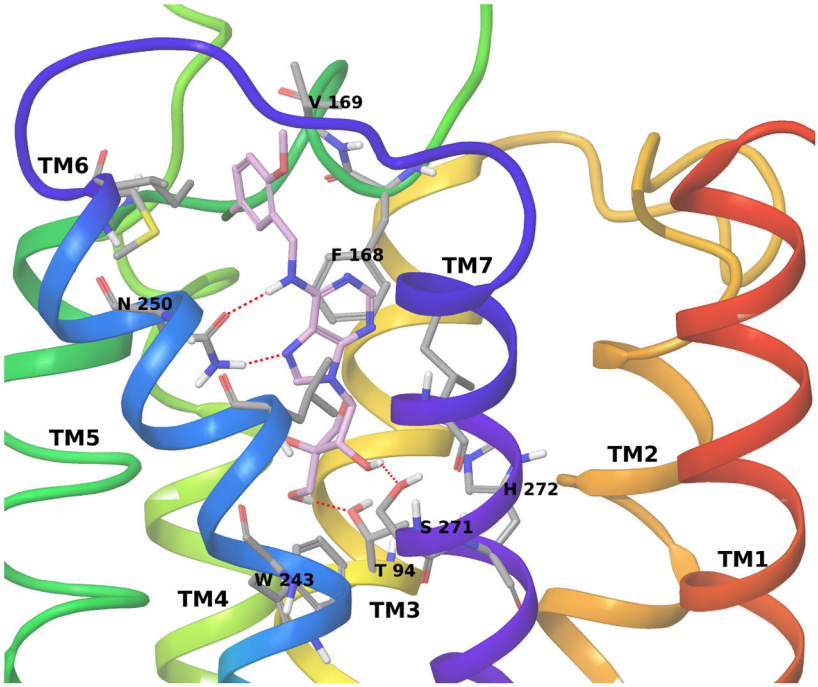
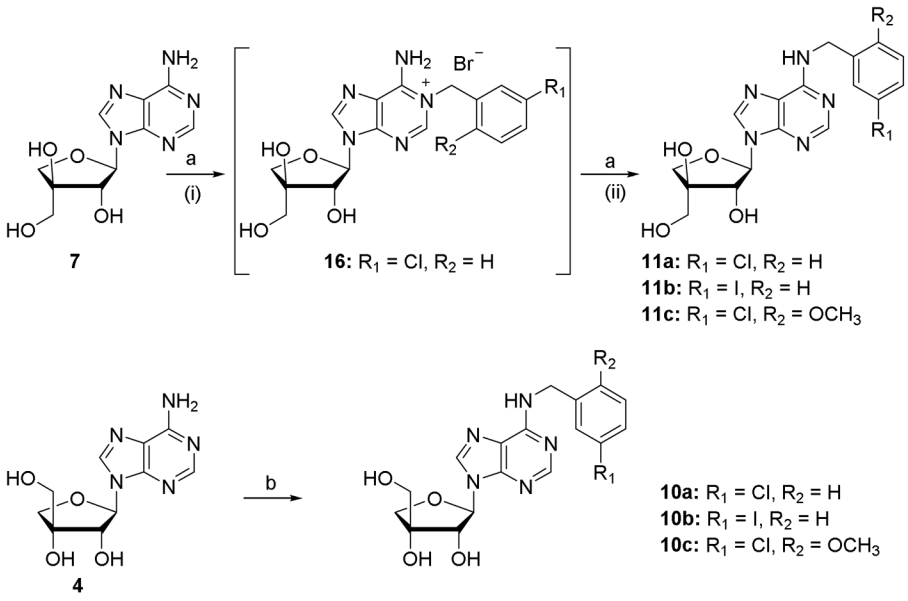
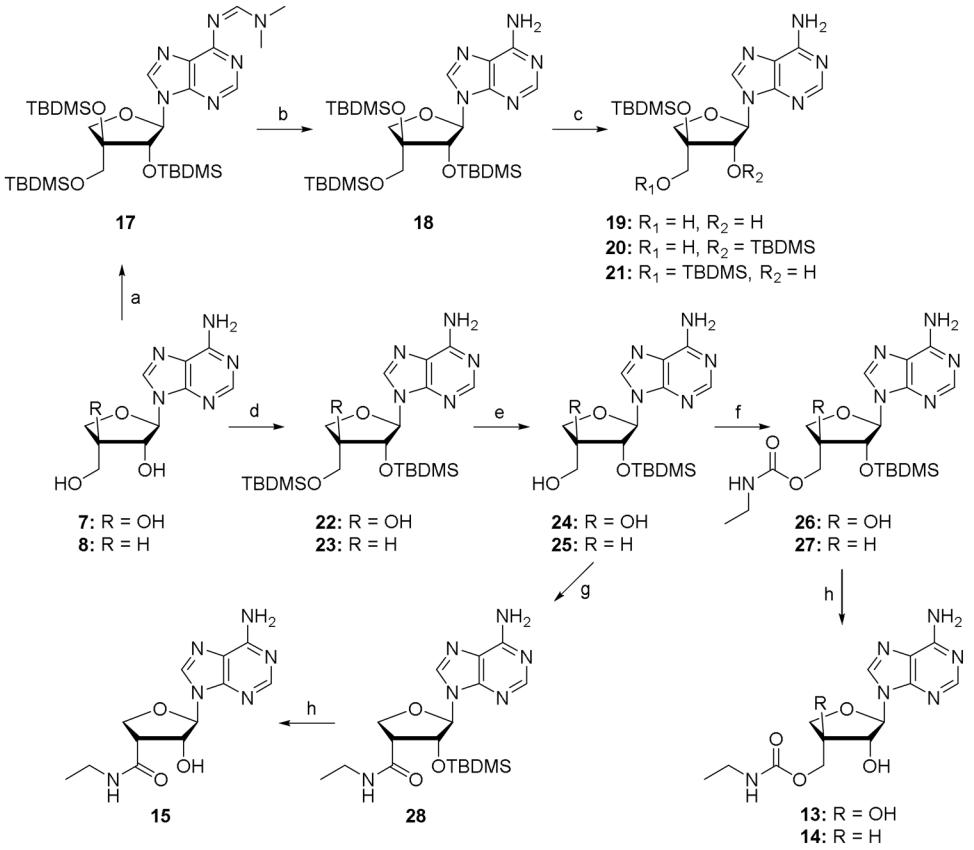


Figure 5.
Binding of A_3AR partial agonist **11c**



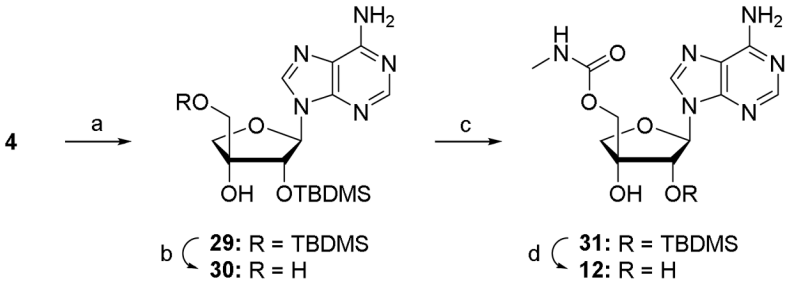
Scheme 1.

Synthesis of N^6 substituted apioadenosines. *Reagents and conditions:* (a) (i) appropriate benzyl bromide, DMF, 50 °C, 48 h; (ii) 25% NH_4OH, 50 °C, 48 h or 90 °C, 3 h, 20–56% over two steps; (b) (i) appropriate benzyl bromide, DMF, rt, 48 h; (ii) 25% NH_4OH, 50 °C, 24 h, 8–21% over two steps.



Scheme 2.

Synthesis of sugar modified α-D-apio-L-furanoadenosines. *Reagents and conditions*: (a) TBDMSCl, imidazole, DMF, 100 °C, 3 days, 80%; (b) 7N NH_3 in MeOH, rt, 18 h, 73%; (c) HF.pyridine, pyridine, THF, rt or TCA-H_2O, THF, rt; (d) TBDMSCl, imidazole, DMF, rt, 18 h, 75–83%; (e) **22**, TCA-H_2O, THF, 0°C, 1 h, rt, 1 h, 87%; **23**, HF.pyridine, pyridine, THF, 0°C, 1 h, rt, 1 h, 82%; (f) (i) CDI, THF, rt, 3h; (ii) $EtNH_2$, rt, 16 h, 55–95%; (g) (i) $RuCl_3$, $NaIO_4$, AcCN-CCl_4-H_2O, rt, 7 h; (ii) CDI, THF, rt, 3 h; (iii) $EtNH_2$, rt, 18 h, 9% over three steps; (h) NH_4F, MeOH, 50 °C, 48 h, 70–94%.



Scheme 3.

Synthesis of 5′-*O*-methylcarbamoyl-β-D-apio-D-furanoadenosine. *Reagents and conditions:* (a) TBDMSCl, imidazole, DMF, rt, 18 h, 74%; (b) TCA-H_2O, THF, 0°C, 1 h, rt, 3 h, 35%; (c) (i) CDI, THF, rt, 3 h; (ii) $MeNH_2$, rt, 16 h, 72%; (d) NH_4F, MeOH, 50 °C, 48 h, 90%.

Table 1

Products from oxidation reactions

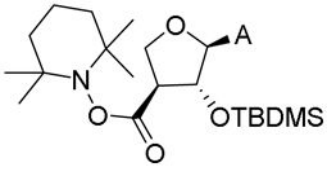
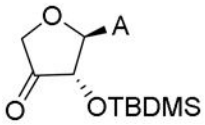
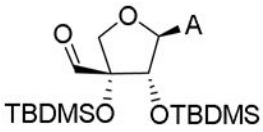
Entry	Reactant	Condition	Product	HRMS
1	**25**	TEMPO (0.7 eq), BAIB (4 eq), CH_3CN-H_2O, rt, 16h		$[M+H]^+$: 519.3119
2	**24**	$RuCl_3$ (0.7 eq), $NaIO_4$ (4 eq), CH_3CN-CCl_4-H_2O, rt, 7h		$[M+H]^+$: 350.1656 $[M+H_3O]^+$: 368.1762
3	**20** (+**21**)	$RuCl_3$ (0.7 eq), $NaIO_4$ (4 eq), CH_3CN-CCl_4-H_2O, 50 °C, 2days		$[M+H]^+$: 494.2609 $[M+H_3O]^+$: 512.2742

Table 2

Percent inhibition of radioligand binding or binding affinities of apioadenosine derivatives at three hAR subtypes.[a]

Compound	A_1 AR, % inhibition	A_{2A} AR, % inhibition	A_3 AR, % inhibition or K_i (nM)
4	*6 ± 4%*	*19±8%*	*35 ± 4%*
5	*6 ± 5%*	*19±10%*	*32 ± 2%*
6	*10 ± 7%*	*15±12%*	*31 ± 2%*
7	*0%* [b]	*64%* [b]	*10%*
8	*74%* [b]	*76%* [b]	*7%*
9	*0%* [b]	ND	*7%*
10a	*10 ± 6%*	*10±8%*	*43 ± 5%*
10b	*27 ± 9%*	*17±11%*	*48 ± 6%*
10c	*12 ± 3%*	*17±6%*	978 ± 175
11a	*19 ± 1%*	*15 ± 5%*	*44 ± 1%*
11b	*35 ± 5%*	*22 ± 2%*	*45 ± 1%*
11c	*13 ± 5%*	*14 ± 5%*	3070 ± 750
12	*12 ± 4%*	*10±8%*	*26 ± 6%*
13	*9 ± 5%*	*12 ± 4%*	*6 ± 2%*
14	*7 ± 2%*	*2 ± 2%*	*8 ± 1%*
15	*8 ± 7%*	*9 ± 5%*	*10 ± 2%*
16	*6 ± 5%*	*12 ± 7%*	*3 ± 2%*

[a] Binding in membranes of CHO or HEK293 (A_2AR only) cells stably expressing one of three hAR subtypes. Percent refers to inhibition of binding at 10 μM, unless noted, using agonists [^{3}H]*N*^{6}-[(*R*)-1-methyl-2-phenylethyl]adenosine, [^{3}H]2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine, or [^{125}I]*N*^{6}-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide, respectively. Values not in italics refer to the binding affinity at hA_3ARs expressed as a K_i value. Values are mean ± S.E.M.

[b] Percent refers to inhibition of binding at 50 μM. ND, not determined.