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Purine (*N*)-Methanocarba Nucleoside Derivatives Lacking an Exocyclic Amine as Selective A_3 Adenosine Receptor Agonists

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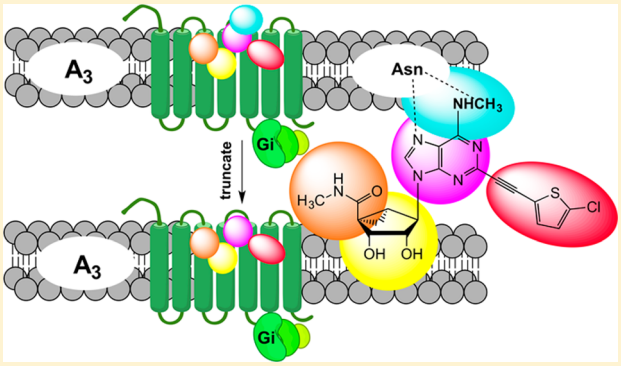
Supporting Information

ABSTRACT: Purine (*N*)-methanocarba-5′-*N*-alkyluronamidoriboside A_3 adenosine receptor (A_3AR) agonists lacking an exocyclic amine resulted from an unexpected reaction during a Sonogashira coupling and subsequent aminolysis. Because the initial C6-Me and C6-styryl derivatives had unexpectedly high A_3AR affinity, other rigid nucleoside analogues lacking an exocyclic amine were prepared. Of these, the C6-Me-(2-phenylethynyl) and C2-(5-chlorothienylethynyl) analogues were particularly potent, with human A_3AR K_i values of 6 and 42 nM, respectively. Additionally, the C2-(5-chlorothienyl)-6-H analogue was potent and selective at A_3AR (MRS7220, K_i 60 nM) and also completely reversed mouse sciatic nerve mechanoallodynia (in vivo, 3 μmol/kg, po). The lack of a C6 H-bond donor while maintaining A_3AR affinity and efficacy could be rationalized by homology modeling and docking of these hypermodified nucleosides. The modeling suggests that a suitable combination of stabilizing features can partially compensate for the lack of an exocyclic amine, an otherwise important contributor to recognition in the A_3AR binding site.



INTRODUCTION

There is an expanding effort to develop selective adenosine receptor (AR) agonists and antagonists for clinical use in inflammation, pain, ischemia, cancer, and other conditions.[1−3] Generally, insight into ligand affinity for the four ARs (A_1, A_{2A}, A_{2B}, and A_3) has come from screening, mutagenesis and structure-based studies,[4−7] with the latter currently relying on homology with the known X-ray structures of ligand-bound A_{2A}ARs.[8−10] Structure-based optimization of known ligands and the discovery of novel chemotypes for the ARs have also been reported.[11] In general, these studies suggest that recognition of AR pharmacophores, especially as applied to adenosine derivatives, depends on a set of interactions with typically conserved amino acid residues. For example, the adenine ring engages in aromatic $\pi-\pi$ stacking with a conserved Phe residue in EL2 (168 in the human (h) A_3AR), and the 5′-*N*-alkyluronamide of potent AR agonists such as **1** (nonselective) typically H-bonds with a conserved Thr/Ser 3.36 (using standard notation[12]) in transmembrane helix (TM) 3. The N^6 hydrogen, as H-bond donor, and N^7, as H-bond acceptor, form a bidentate coordination with Asn (6.55). This latter interaction is the reason that 7-deaza adenosine derivatives are nearly inactive as AR agonists.[13,14] The removal of H-bonding groups on the adenosine pharmacophore that interacts through these conserved recognition points often reduces agonist potency and/or efficacy across the four AR subtypes.

Although changes to the primary pharmacophore may disrupt binding completely, subtle changes in these conserved drug−receptor interactions can lead to subtype specificity. For example, substituting the ribose oxygen for sulfur reduced A_1 and increased A_{2A}AR affinity of 2-chloroadenosine.[15] Conversely, replacing the flexible ribose conformation by a rigid Northern (*N*)-methanocarba moiety decreased A_{2A} and increased A_1 and A_3 AR affinity.[7] Similarly, N^6-3-halobenzyl and 5′-*N*-methyluronamide moieties, along with combinations thereof, are particularly important for enhancing A_3AR selectivity.[7] There are also derivatives lacking an exocyclic NH, such as C6-phenylpurine (nonriboside) derivatives that

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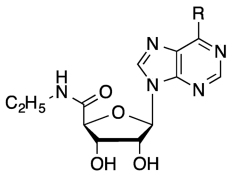
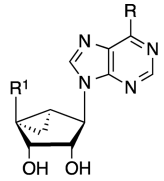
bind to the A_1AR as antagonists[16] and various purine-9-riboside derivatives that bind to the A_3AR.[17−19] For example, the N^6-dimethyl **2** and inosine **3** analogues of the nonselective, potent agonist 5′-*N*-ethylcarboxamidoadenosine **1** maintain moderate A_3AR binding affinity (Chart 1). Among nucleosides having a ring-constrained (*N*)-methanocarba (bicyclo[3.1.0]hexane) modification that maintains an A_3AR-preferred conformation, several inosines, **4**, **5**, and thioinosine **6** derivatives show moderate binding affinity.[17−19] Thus, compounds **2** and **6** lack a H-bond donor at the C6 position yet have μM A_3AR binding affinity.

Chart 1. Reported Examples of C6-Modified Ribose-Containing (1−3)[17] and (*N*)-Methanocarba (4−6) Nucleosides[18,19] as AR Ligands[a]





1 R = NH_2 (0.113, r)
2 R = $N(CH_3)_2$ (2.26, r)
3 R = OH (5.00, r)

4 R = OH, R^1 = CH_2OH (20.5±2.1, h)
5 R = OH, R^1 = $CONHCH_3$ (28.8, r)
6 R = $SCH_2C_6H_5$-4-NO_2, R^1 = CH_2OH (1.97±0.10, h)

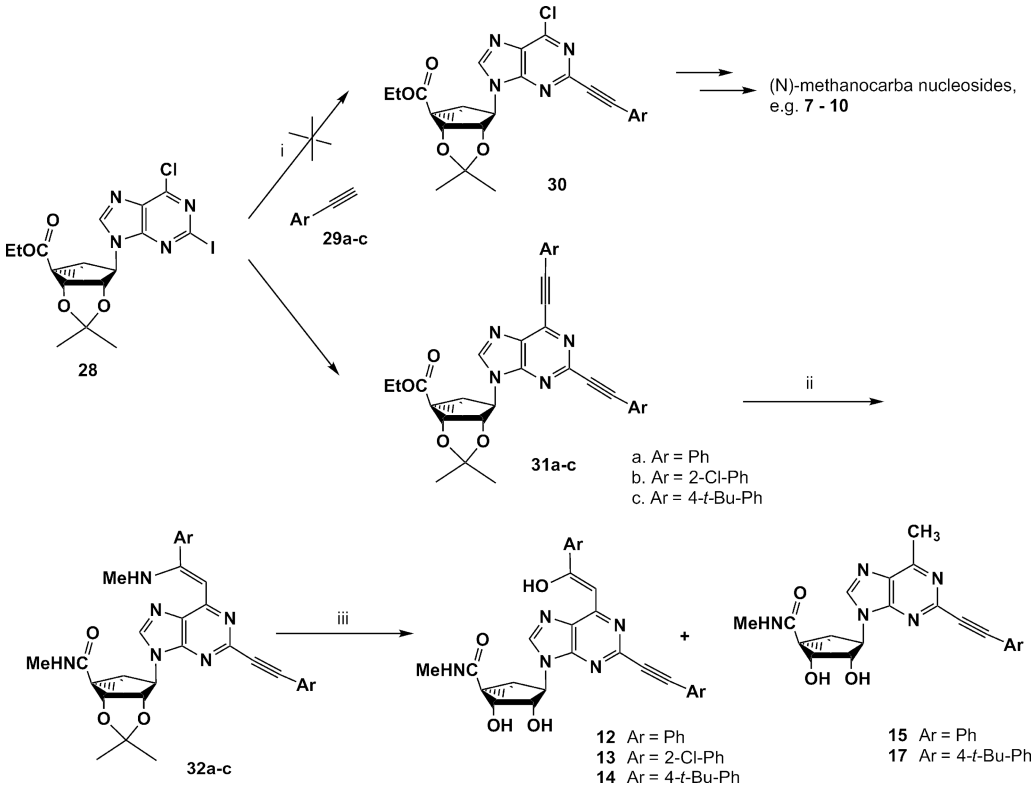
[a]The binding K_i values (μM) at the rat (r) A_3AR or hA_3AR are shown in parentheses. Values for **4** and **6** were measured for the present study using the same methods as in Table 1.

Here, we considered whether other analogues lacking the H-bond donor at N^6 behave as potent A_3AR-selective agonists. Using structural modification of known A_3AR agonists and assays for binding, function, and in vivo efficacy, we characterize such motifs. Also, we use molecular modeling based on an agonist-bound A_{2A}AR crystal structure to explore the binding of this redefined pharmacophore. The presence of other stabilizing interactions in these hypermodifed analogues appears to compensate for the lack of an exocyclic NH. We show that this new variety of ligands not only enlarges the class of high affinity and specific A_3AR ligands, which is highly desirable clinically but also gives new insight into the specificity attributes of the A_3AR pharmacophore.

RESULTS

Chemical Synthesis. The opportunity to explore the structure−activity relationship (SAR) of C6-methylated and other C6-alkylated adenosine derivatives arose from a side reaction and an unanticipated fragmentation product that occurred during the attempted reaction of a 6-chloro-2-iodo intermediate **28**.[20] The attempted Sonogashira coupling of **28** was sought as an alternate route to synthesize C2-arylethynyl (*N*)-methanocarba nucleosides, e.g., 6-amino derivatives **7**−**10** (Scheme 1), which we reported previously to be highly specific A_3AR agonists.[6] During the Sonogashira reaction of compound **28** with phenylacetylene, instead of the desired monosubstituted product **30**, only disubstituted product **31a** was obtained. To convert the 5′-ester group of compound **31a** to an amide derivative, it was stirred with 40% methylamine

Scheme 1. Attempted Synthesis of 6-Chloro (*N*)-Methanocarba Intermediate 30 as a Possible Precursor of Selective A_3AR Agonists and the Redirected Route to A_3AR Agonist Series Containing at the C6 Position Either a Substituted Styryl 12−14 or a Methyl Group 15, 17 (Affinities in Table 1)[a]



[a](i) $PdCl_2(Ph_3P)_2$, CuI, Et_3N, DMF, rt; (ii) 40% $MeNH_2$, MeOH, rt; (iii) 10% TFA, MeOH, 70 °C.

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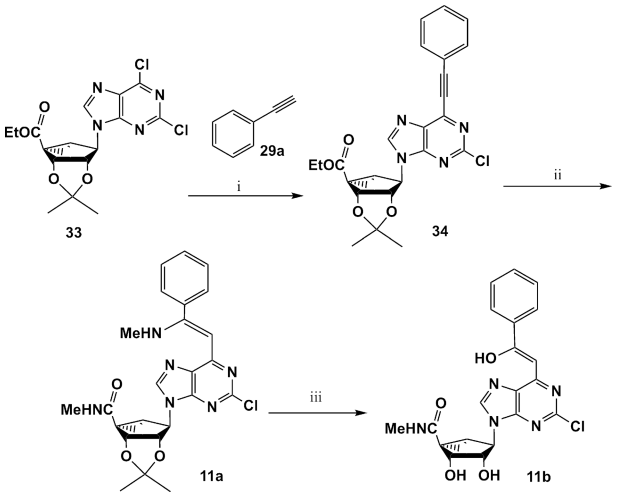
solution in methanol at room temperature overnight. It was interesting to observe that a hydroamination reaction[21,22] had occurred at the C6-phenylacetylene group in addition to amidation to give compound **32a**. We have never observed a similar hydroamination product when a phenylacetylene group is present at the C2 position.[6,7] The structure of this product **32a** was thoroughly characterized by various NMR studies (Figures S3−S5, Tables S1−S3, Supporting Information (SI)). Similar products were also observed during a reaction of compound **28** with 2-chloro-phenylacetylene and 4-*t*-butyl-phenylacetylene followed by amination to give the C2-aminostyryl derivatives **32b** and **32c**. To prove chemically that the hydroamination reaction occurred exclusively at the C6 phenylacetylene group, compound **34** was synthesized by a Sonogashira reaction of 2,6-dichloro derivative **33** with phenylacetylene (Scheme 2). Aminolysis of compound **34** with methylamine solution provided compound **11a**. Attempted removal of the isopropylidene group of **32a** with 10% TFA in methanol at 70 °C provided a hydrolyzed enol derivative **12** and the unexpected fragmented product **15**. Enamines are known to be hydrolyzed to ketones under acidic conditions.[23] Both products were extensively characterized by various NMR studies, and also a plausible mechanism for the formation of fragmented 6-Me product **15** is presented in Scheme S1 (SI). We propose that under acidic conditions, methanol may attack the protonated keto tautomer equivalent of the enol group followed by a fragmentation that leaves a 6-Me group on the purine base. Similarly, hydrolysis of **32b−c** and **11a** provided enol derivatives **13**, **14**, and **11b**. However, only a C6 fragmented product, e.g., **17**, was observed in the hydrolysis reaction of 4-*t*-Bu-phenylethynyl derivative **32c**. No fragmentation products were detected upon hydrolysis of 2-chlorophenylethynyl **32b** and 2-chloro **11a** derivatives.

Scheme 2. Application of the Redirected Route from Scheme 1 to the Preparation of C6-Substituted 2-Cl (*N*)-Methanocarba Derivative 11[a]

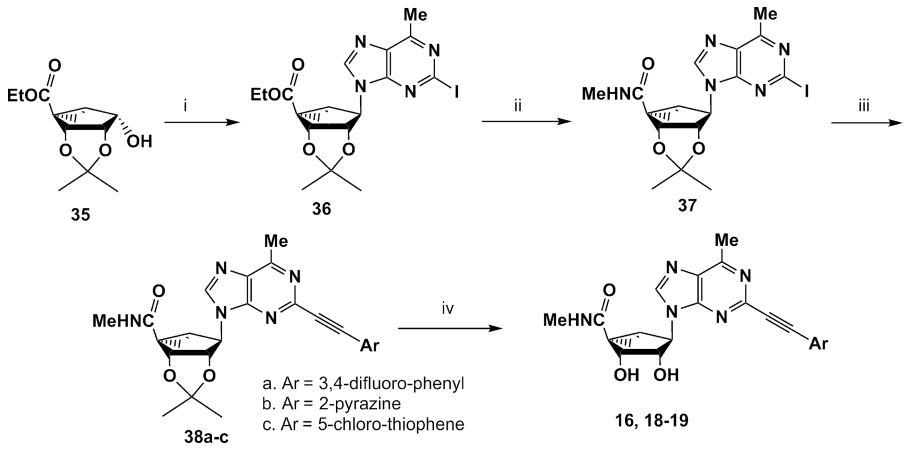


[a]Although many 2-Cl derivatives are potent A_3AR agonists,[1] this compound only weakly bound to the receptor (Table 1). (i) phenylacetylene, $PdCl_2(Ph_3P)_2$, CuI, Et_3N, DMF, rt; (ii) 40% $MeNH_2$, MeOH, rt; (iii) 10% TFA, MeOH, 70 °C.

C6-Me derivatives (**16**, **18**, and **19**) having different C2-arylethynyl groups were synthesized by an alternate route, which preinstalled a 6-Me group on the nucleobase (Scheme 3). The nucleobase intermediate 2-iodo-6-methyl purine **55** was prepared from a 9-protected 2-amino-6-methyl purine **52** as shown in Scheme S2 (SI). Similarly, C6-H derivatives were prepared from an intermediate 6-iodopurine (Scheme 4). However, it was observed that during attempted conversion of 5′-ester **39** to an amide, $MeNH_2$ also replaced the iodo group at C2 position to give compound **40**, which upon acid hydrolysis provided compound **23**. To avoid this side reaction at the C2 position, a Sonogashira coupling was first performed on compound **39** with different arylalkynes to give **41a−c**. Amidation of esters **41a** and **41b** with a methylamine solution followed by acid hydrolysis yielded C6-H derivatives **20** and **21**, respectively. In contrast, the same reactions for the pyrazine derivative **41c** yielded a hydroamination product **43**, which upon acid hydrolysis gave the enol derivative **24**.

For comparison, we also prepared one C6-methoxy (*N*)-methanocarba nucleoside **22**, based on inosine, containing an extended C2 substituent. In the first route, we have synthesized a C6-OMe derivative **44**; however, the attempted conversion of the ester to an amide by treatment of **44** with methylamine solution gave a C6-NHMe substituted derivative **45**[20] (Scheme 5). To avoid this side reaction, an alternate route featuring

Scheme 3. Synthesis of C6-CH_3 (*N*)-Methanocarba Derivatives 16, 18, and 19[a]



[a]Reagents and conditions: (i) 2-iodo-6-methyl purine **54**, Ph_3P, DIAD, THF, rt; (ii) 40% $MeNH_2$, MeOH, rt; (iii) aryl alkynes, $PdCl_2(Ph_3P)_2$, CuI, Et_3N, DMF; (iv) 10% TFA, MeOH, H_2O, 70 °C.

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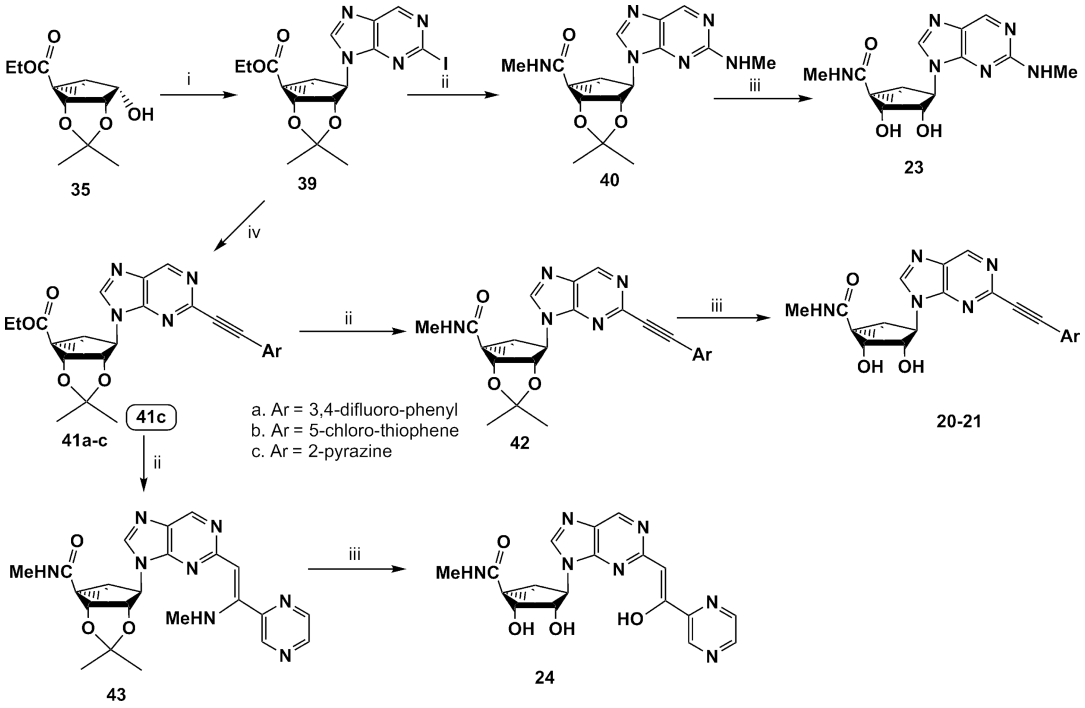
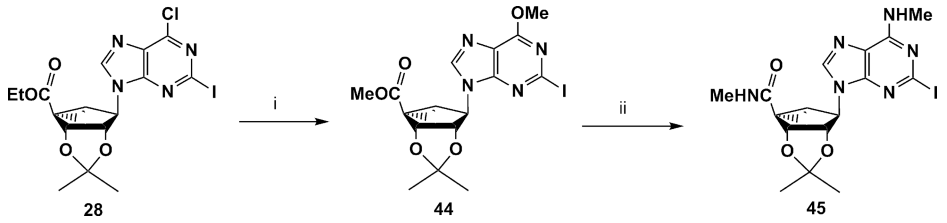
Scheme 4. Synthesis of C6-H (*N*)-Methanocarba Derivatives 20, 21, 23, and 24[a]



[a]Reagents and conditions: (i) 2-iodo-purine, Ph_3P, DIAD, THF, rt; (ii) 40% $MeNH_2$, MeOH, rt; (iii) 10% TFA, MeOH, H_2O, 70 °C; (iv) aryl alkynes, $PdCl_2(Ph_3P)_2$, CuI, Et_3N, DMF.

Scheme 5. Unproductive Synthetic Route to (*N*)-Methanocarba-inosine Derivative 22[a]



[a]Reagents and conditions: (i) MeONa, MeOH, rt; rt; (ii) 40% $MeNH_2$, MeOH, rt.

oxidation of a 5′-CH_2OH and $MeNH_2$ coupling was designed (Scheme 6). Compound **46**[24] was converted to C6-OMe derivative **47**, which upon TBDPS deprotection and PDC oxidation gave the acid derivative **49**. Coupling of $MeNH_2$ with compound **49** in the presence of HATU gave the desired precursor **50**, with no detectable C6-NHMe side products. Sonogashira coupling of compound **50** with 2-chloro-5-ethynylthiophene followed by acid hydrolysis afforded the C6-OMe derivative **22**.

Pharmacological Activity. Table 1 lists the AR affinities for the various synthesized purine nucleoside analogues lacking a C6-exocyclic amino group and their related N^6-substituted adenosine derivatives. Standard radioligand (**25**–**27**) binding assays were performed on human (h)A_1, A_{2A}, and A_3 ARs using reported methods,[7,20] and IC_{50} values were transformed to K_i as described.[25,26] The fortuitously synthesized initial C6-methyl derivative **15** had unexpectedly high binding at the hA_3AR (K_i 6.01 nM). This motivated us to explore other accessible C6-alkyl or alkenyl derivatives containing either a 2-Cl (**11b**) or 2-arylethynyl (**15**–**19**) group. None of these compounds bound significantly to the hA_1 or A_{2A} ARs and were therefore selective for the A_3 AR. The 2-chloro analogue **11b** bound only weakly to the hA_3AR (K_i 1.14 μM). Many other 2-chloro nucleosides are known to have potent A_3AR agonist activity,[1] which suggests that the presence of a rigid extension at the C2 position, e.g., the 6-styryl derivatives **12** and **13**, enhances binding to the A_3AR in this series lacking an exocyclic NH.

A C2 modified, 5-chlorothienylethynyl substituent was associated with higher affinity in C6-modified analogues than most other aryl groups except an unsubstituted phenyl, as in **15**. The 5-chlorothienylethynyl group in C6-Me (**19**) and C6-H (**21**) analogues produced K_i values of 42 and 60 nM, respectively, and were both highly A_3AR selective, with hA_1 and A_{2A}ARs K_i values extrapolated to ≫10 μM. Other aryl groups, specifically substituted phenyl rings, did not achieve such high affinity. The rank order of decreasing hA_3AR affinity was: **15** > **19** > **16** > **17** ≥ **18**. The least potent C6-Me analogues, compounds **17** (K_i 305 nM, hA_3AR) and **18** (K_i 343 nM) contained a bulky *p*-*t*-Bu-phenyl group or a pyrazine group, respectively. The disubstituted 6-styryl derivatives **12**–**14** were of intermediate affinity at the hA_3AR, ranging from K_i ∼ 80–500 nM. It is interesting that the 4-*t*-Bu group was highly detrimental to hA_3AR affinity in the case of C6-Me but not with a larger C6 substituent.

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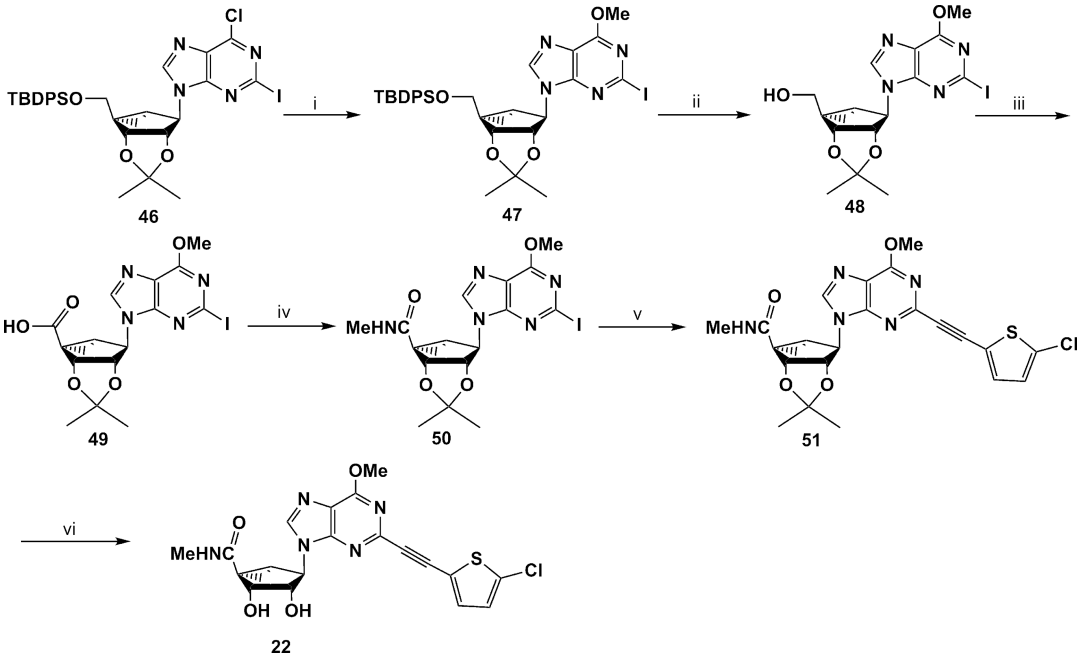
Scheme 6. Synthesis of (*N*)-Methanocarba-inosine Derivative 22[a]



[a]Reagents and conditions: (i) MeONa, MeOH, rt; (ii) TBAF, THF, rt; (iii) PDC, DMF, 40 °C; (iv) $MeNH_2$, HATU, DIPEA, DMF; (v) 2-chloro-5-ethynylthiophene, $PdCl_2(Ph_3P)_2$, CuI, Et_3N, DMF; (vi) Dowex50, MeOH, H_2O, 70 °C.

Selected nucleosides were tested in a binding assay at the mouse (m) A_3AR expressed in HEK293 cells using reported methods.[6] The K_i values of **12** and **15** were 136 ± 9 and 158 ± 10 nM, respectively, which suggested that human vs mouse species differences are greater for the C6-methyl analogue than with a larger group at that position. Other analogues were weaker in binding at the mA_3AR, with K_i values: **19**, 722 ± 35, and **21**, 396 ± 29 nM.

Selected high affinity ligands (**15**, **19**, and **21**) were examined in a functional activity at hA_3AR, e.g., the ability to inhibit production of cyclic AMP[27] via the hA_3AR expressed in CHO cells. All three compounds activated the hA_3AR as full agonists with a similar rank order of potency as in the binding results; the EC_{50} values (nM) were: **15**, 3.16 ± 0.72; **19**, 12.5 ± 2.8; **21**, 26.9 ± 8.4 (Figure 1A). Compounds **12** and **15** were also tested in a functional assay at the mA_3AR expressed in HEK293 cells, e.g., the ability to inhibit production of cyclic AMP. Figure 1B shows that these compounds were also full agonists for this receptor, with efficacy comparable to the reference compound 2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine **55**. Both **12** and **15** were quite potent in activating the mA_3AR, with EC_{50} values of 4.86 and 20.2 nM, respectively.

On the basis of the potent in vitro A_3AR activity of these congeners, selected compounds were tested in vivo using previously reported methods[28] for the ability to reduce chronic neuropathic pain following oral administration in the mouse chronic constriction injury (CCI) model[29] (Figure 2). 2-Phenylethynyl analogues **12** and **15**, differing in the nature of the C6 group, were efficacious in reducing pain at the point of peak pain, day 7, although neither reached 100% reversal of the pain and the duration of action was less than that observed for the corresponding C6-NHMe analogue, i.e., **9**.[6] The 2-(5-chlorothienyl)ethynyl compounds **19** and **21** were also compared in the mouse CCI model. Here, the 6-H analogue **21** was clearly more efficacious and longer lasting (at least 5 h) than the corresponding 6-CH_3 analogue **19** and other compounds tested in vivo. The absence of a C6 substitution in **21** evidently contributes to its prolonged activity in vivo. The absence of an exocyclic amine slightly improved the physicochemical parameter tPSA, which might be related to the increased in vivo efficacy. The tPSA value of **21** is 110 Å^2, compared to 122 Å^2 for compound **10**, suggesting better drug-like qualities and bioavailability. On the other hand, the cLogP of **21** is 1.27, compared to 2.17 for **10**, which may be advantageous for solubility.

Off-target activities of compounds **15**, **19**, and **21** were evaluated at various receptors by the Psychoactive Drug Screening Program (PDSP).[30] Results (SI) indicated only a few off-target interactions in the μM range. Compound **15** showed no significant binding inhibition at the diverse receptors, but at 10 μM it enhanced human dopamine transporter (hDAT) binding of [^{3}H]methyl (1*R*,2*S*,3*S*)-3-(4-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate by roughly 200% (Figure S1, SI), similar to other (*N*)-methanocarba-adenosine derivatives.[31] This unusual activity of this chemical series was shown by Janowsky et al.[31] to correlate with an allosteric enhancement of the affinity of the tropane radioligand binding to DAT. Compound **19** showed only one such off-target interaction (K_i at δ opioid receptor 5.8 μM, 68% inhibition), and compound **21** showed no off-target interactions.

Molecular Modeling. Docking simulations were carried out to explore the environment of receptor-bound C6 substituted purine nucleosides. Selected compounds (K_i < 100 nM) were docked into the putative TM binding site of a previously reported homology model of the hA_3AR,[7,32] based on a hybrid A_{2A}AR-β_2 adrenergic receptor template.

The docking poses were selected by taking into account optimal interaction geometries with the residues surrounding the binding site and by inspecting electrostatic and van der Waals contributions of computed per residue interaction scores, denoted IS_{ele} and IS_{vdW}, respectively. The "interaction score

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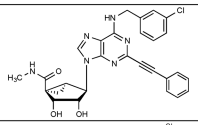
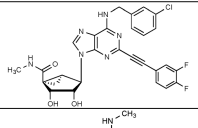
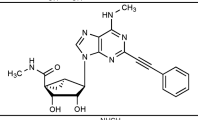
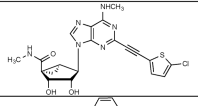
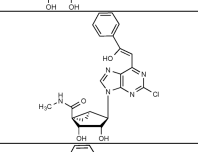
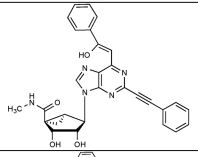
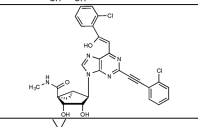
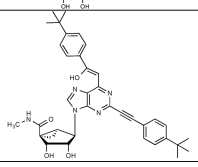
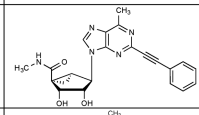
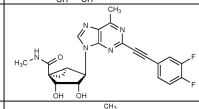
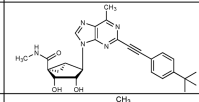
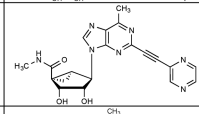
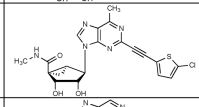
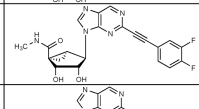
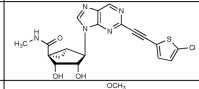
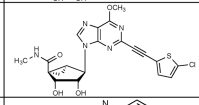
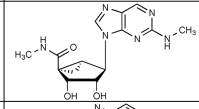
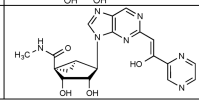
Table 1. Structures and Binding Affinities at Three ARs of Reference Compounds (7−10)[23,26] and Newly Synthesized Nucleoside Derivatives (11−24)[a]

Compound	Structure	hA_1AR K_i (nM) or % inhibition	$hA_{2A}AR$ % inhibition	hA_3AR K_i (nM)
7[b]		*20% ± 3%*	*27% ± 3%,*	1.34 ± 0.30
8[b]		*6% ± 4%*	*41% ± 10%,*	3.49 ± 1.84
9[b]		*18% ± 8%*	*14% ± 7%*	0.85 ± 0.22
10[b]		*6% ± 1%*	*24% ± 13%*	0.70 ± 0.11
11b		*30% ± 12%*	*24% ± 8%*	1140 ± 170
12		*16% ± 5%*	*16% ± 7%*	78.5 ± 19.8
13		*28% ± 15%*	*21% ± 1%*	515 ± 107
14		*24% ± 9%*	*19% ± 9%*	94.3 ± 30.9
15		*15% ± 6%*	*34% ± 7%*	6.01 ± 1.60
16		5430±750	*14% ± 2%*	98.5 ± 32.5
17		*16% ± 10%*	*15% ± 11%*	305 ± 39
18		*15% ± 9%*	*17% ± 7%*	343 ± 127
19		*<10%*	*12% ± 5%*	42.2 ± 17.3
20		*17% ± 9%*	*12% ± 3%*	124 ± 23
21		*<10%*	*18% ± 10%*	60 ± 19
22		796±404	*27% ± 8%*	684 ± 285
23		*17% ± 1%*	*<10%*	604 ± 261
24		*14% ± 2%*	*20% ± 5%*	1480 ± 560

[a]Binding[7,20,32] in membranes prepared from CHO or HEK293 ($hA_{2A}AR$ and mA_3AR) cells stably expressing one of three hAR subtypes. The binding affinity for hA_1, A_{2A}, and A_3ARs was expressed as K_i values (n = 3−4) measured using agonist radioligands [^{3}H]N^6-*R*-phenylisopropyladenosine **25**, [^{3}H]2-[*p*-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamido-adenosine **26**, or [^{125}I]N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyl-uronamide **27**, respectively. A percent in italics refers to inhibition of binding at 10 μM. Nonspecific binding was determined using adenosine-5′-*N*-methyluronamide **1** (10 μM at hARs, 100 μM at mA_3AR). The concentrations of radioligands and their K_D values at the corresponding hARs in parentheses used to calculate K_i values of competing ligands were all in nM: **25**, 1.0 (1.5); **26**, 10 (16.2); **27**, 0.2 (1.22). [b]Data from Tosh et al.[6,7]

maps" (ISMs) arising from the latter analysis (Figure S2 (SI)) identify a common binding mode for derivatives **15**, **16**, **19**, and **21**, involving residues located mainly in TM3, extracellular loop (EL) 2, TM6, and TM7. On the other side, the C6-styryl derivatives **12** and **14** interact with residues belonging to EL2, EL3, and TM7, thus implying that their placement in the binding site is shifted toward the extracellular side of the receptor (data not shown).

As an example of the binding mode exhibited by the majority of the considered purine nucleosides, Figure 3 shows the docking pose of compound **15** (K_i = 6.0 nM). The ligand resides in the upper region of the TM bundle (see also SI, Video S1) with the C2 terminal cyclic group pointing toward the extracellular environment, and this mode features several interactions typical for AR agonists. The planar bicyclic core establishes an aromatic π−π stacking interaction with Phe168 (EL2), whereas the purine N7 engages the side chain of Asn250 (6.55) acting as H-bond donor. A tight hydrogen bond network with Thr94 (3.36), Ser271 (7.42), and His272 (7.43) anchors the methanocarba region of the compound in the binding pocket. In addition to these conserved recognition points, the ISMs (Figure S2B (SI)) report several other residues involved in favorable contacts with the ligand, including Leu91 (3.32), Ile92 (3.33), Val169 (EL2), Trp243 (6.48), Leu246 (6.51), and Ile268 (7.31).

DISCUSSION

Previously, removal of the exocyclic NH of adenosine derivatives was not considered a feasible approach to the design of new, selective AR agonists. In an early, pioneering SAR paper by Bruns,[13] purine-9-riboside **56** (nebularine) and 6-methylpurine-9-riboside (structures not shown) were described as weak AR agonists at 1 mM with only 15% and 6%, respectively, of the efficacy of 10 μM adenosine at a receptor in

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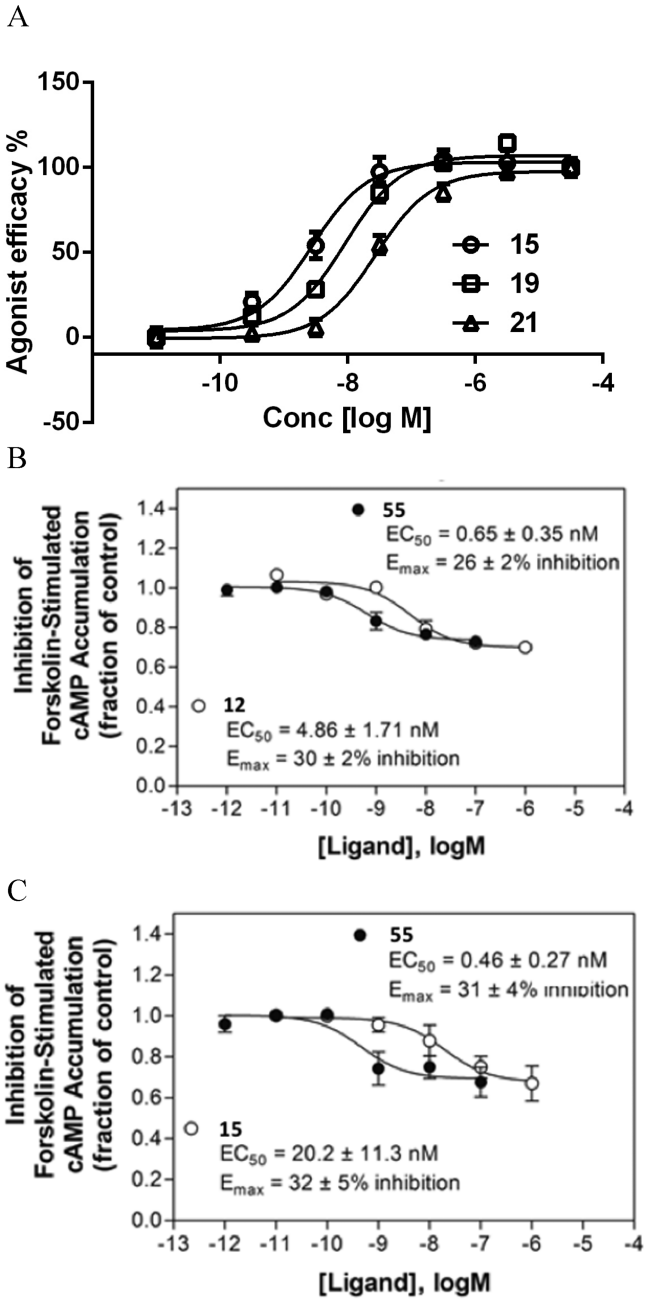

Figure 1. Functional agonism at the hA_3AR (A) and the mA_3AR (B) of nucleosides lacking an exocyclic NH, which remain selective A_3AR ligands. (A) Compounds **15**, **19**, and **21** proved to be potent, full agonists at the hA_3AR (% values relative to inhibition of forskolin-stimulated cyclic AMP accumulation by adenosine-5′-*N*-methyluronamide **1** at 10 μM). Compounds **12** (B) and **15** (C) in an assay of inhibition of forskolin-stimulated cyclic AMP accumulation with HEK293 cells expressing the mA_3AR, as described.[7] Concentration–effect curves with reference full agonist 2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarboxamidoadenosine **55** are included. Data are the mean ± SEM, n = 4–7.

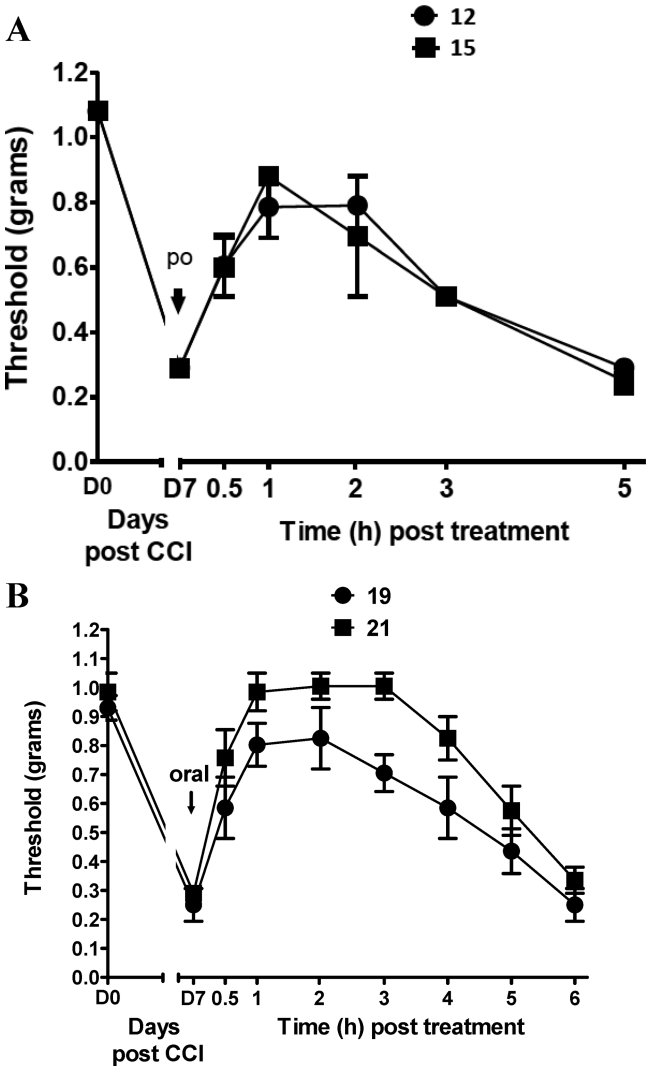

Figure 2. Time course of protection hind paw mechanoallodynia of the sciatic nerve in the CCI mouse model (po administration on day 7, 3 μmol/kg). The vehicle was 10% DMSO in 0.5% methylcellulose, which when administered alone had no effect on PWT. There was no effect on the contralateral paw. (A) CCI results (n = 3) for compounds **12** (●) and **15** (■). Data are the mean ± SEM. For comparison, compound **8** at the same dose provided 100% and 23.7 ± 10.8% protection against mechanoallodynia in the same model at 1 and 3 h, respectively.[7] (B) CCI results (n = 2) for compounds **19** (●) and **21** (■).

fibroblasts that was later identified as the human $A_{2B}AR$. **56** was 40-fold less potent than adenosine in activation of the canine coronary artery $A_{2A}AR$, and the exocyclic NH was deemed essential for AR activation.[33] Compound **56** is also a weak inhibitor of adenosine deaminase; however, we are not concerned about that off-target activity with respect to the potent A_3AR agonists in this study because 5′-*N*-alkyluronamido and other modifications of adenosine preclude interaction with that enzyme.[34,35] Purine-9-riboside analogues lacking an exocyclic amine have also been explored as anticancer and anti-infective agents through activities unrelated to ARs.[37,38] We did not prepare the corresponding hypermodified 9-ribosides for direct comparison with (*N*)-methanocarba analogues in Table 1.

Many adenosine derivatives containing a monosubstituted N^6 group, in combination with other substitutions, have been reported as potent A_3AR agonists.[7,36] We revisited these two previously rejected modifications of adenosine for AR agonists, C6-H and C6-Me, using highly optimized A_3AR agonists as lead structures. In this compound series, the loss of the exocyclic NH still preserved moderate affinity and high selectivity for the A_3AR. Moreover, these purine analogues maintained an ability to fully activate the G_i-coupled human

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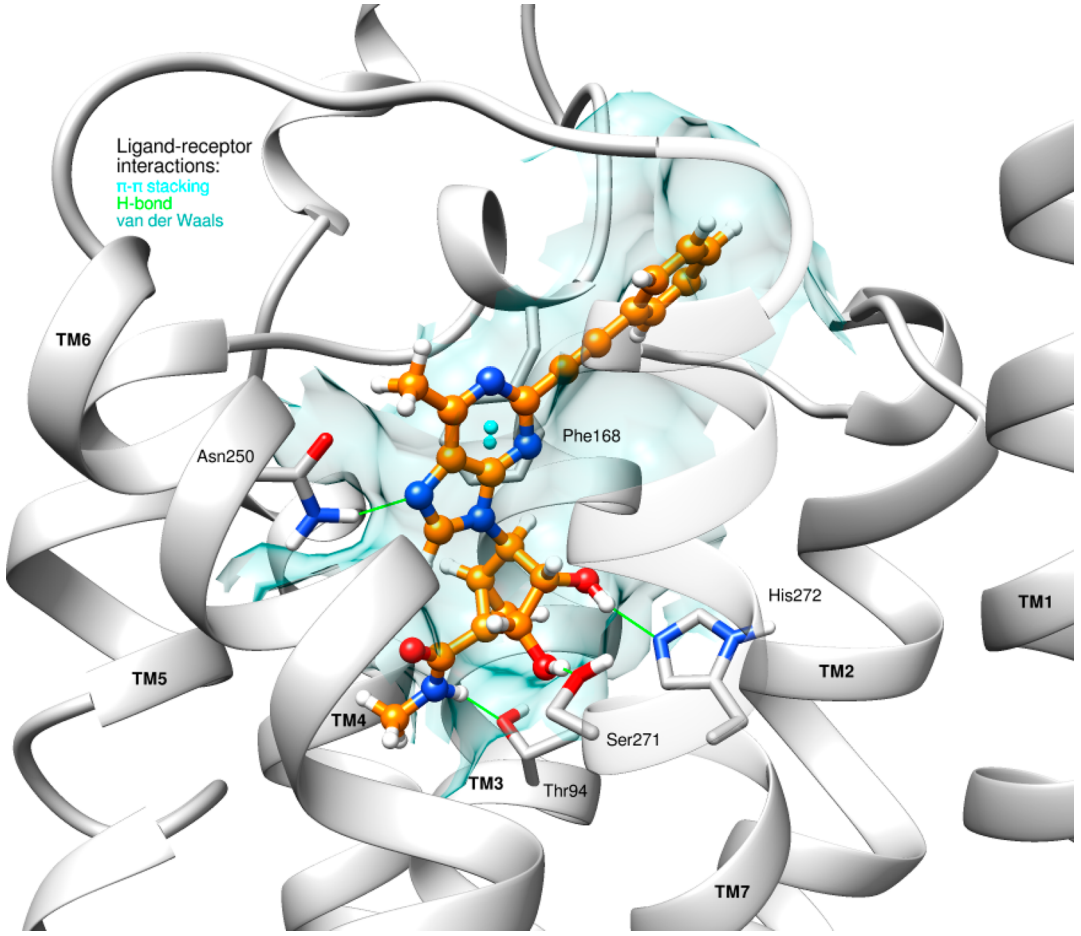

Figure 3. Hypothetical binding mode of C6-methyl (*N*)-methanocarba derivative **15** (orange carbon atoms, ball and stick representation), a potent and selective agonist, obtained after docking simulations at the hA_3AR. Side chains of residues important for ligand recognition are reported as sticks (gray carbon atoms). H-bonds are pictured as green solid lines, whereas $\pi-\pi$ stacking interactions as cyan dashed lines with the centroids of the aromatic rings displayed as cyan spheres. Nonpolar hydrogen atoms are omitted.

and mouse A_3ARs and to protect against neuropathic pain in the mouse, a characteristic A_3AR effect.

The correlation of activation of the A_3AR with relief from chronic neuropathic pain has been established in various rodent models.[3,28] Selected compounds evaluated in functional assays and in the CCI pain model in mice had A_3AR selectively and activity comparable to nucleosides containing an exocyclic amine. Activation of the A_1AR is also known to reduce neuropathic pain,[39] but except for two relatively weak analogues, 6-Me **16** and 6-MeO **22**, these derivatives have no appreciable affinity for the A_1AR. Therefore, we conclude that the antinociceptive activity of orally administered **12**, **15**, **19**, and **21** was due to activation of the A_3AR, with **21** completely reversing mechanoallodynia of the mouse sciatic nerve. With both peripheral and central mechanisms contributing to the antinociceptive effects,[3] a novel class of AR agonists that lacks the exocyclic amine might have different patterns of distribution in vivo including conceivably greater entry into the brain due to the loss of an H-bonding group, which could affect the net protection against pain. Thus, these modifications might serve as a means of improving the bioavailability in A_3AR agonists by altering physicochemical properties.

With the elucidation of the structures of antagonist-bound and agonist-bound A_{2A} adenosine receptors (ARs),[8−10] the design of selective AR ligands is increasingly structure-based.[4−6] The fact that otherwise optimized nucleoside analogues lacking the exocyclic NH remain efficacious in activating the A_3AR can be analyzed structurally. Homology modeling of the A_3AR and other GPCRs based on a closely related templates is a useful component in the design of novel ligands.[40] Our homology model of the A_3AR based on the X-ray structure of an agonist-bound A_{2A}AR has aided in understanding the recognition of nucleoside agonists at this subtype.[7] Previously, the exocyclic amine (specifically an NH group) was considered an important recognition element for nucleosides binding to ARs in general, as it H-bonds to the conserved Asn (6.55). We have discovered that other structural features of the ligand can partially compensate for the lack of this important contributor and also increase A_3AR specificity.

The C6-truncated or C6-methyl or styryl compounds prepared in this study display a wide range of A_3AR affinities. Certain 6-methyl analogues were particularly potent, with K_i values of 6 nM (**15**) to ∼50 nM (**19**, **21**), and other 6-methyl analogues bound in the μM range. Unsubstituted phenyl and 5-chlorothienylethynyl groups at the C2 position appeared to promote higher A_3AR affinity compared to other aryl groups. The loss of energetic stabilization provided by binding of an exocyclic NH of conventional A_3AR agonists can be compensated by other groups at different locations on the nucleoside, such as the extended C2 substituent, the rigid bicyclic ring, or the 5′-methylamide. All of these groups contribute to A_3AR affinity and selectivity and help to anchor

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the ligand. For example, the C2 extended analogue **12** was 15-fold more potent at the hA_3AR than the corresponding C2−Cl derivative **11b**. The inspection of the ligand−receptor interactions suggests that the above-mentioned groups are engaged in hydrophobic contacts with several residues (mainly leucine side chains located in TM3 and TM7) surrounding the binding cavity. These ancillary interactions ensure an optimal orientation of the hydrogen-bonding groups toward the conserved recognition points. From the present set of ligands, we have no evidence that selective A_1AR or $A_{2A}AR$ agonists lacking an exocyclic NH can be designed.

It was already observed that H-bonding groups located on the ribose moiety are more closely related to the activation of the A_3AR, i.e., the "message" portion of the molecule, and the adenine constitutes the "address" portion of the nucleoside.[41] The present findings reinforce that generalized division of function in that full agonism is observed in the absence of the NH.

CONCLUSION

In conclusion, this study is the first demonstration that removal of an H-bond donor group at C6 of purine nucleosides is still compatible with binding and activation of an AR subtype. This set of novel A_3AR agonists arose from an unexpected series of reactions on the adenosine precursors that left a methyl or styryl group at the C6 position of adenine. After we discovered the biological utility of such truncated purine derivatives, we found synthetic approaches to enlarge the SAR beyond the accidental analogues. It is surprising that the adenine 6-NH group, which is traditionally considered essential for the recognition of nucleosides at the various ARs, is not universally essential. Importantly, these hypermodified nucleosides have lower polar surface area than the equivalent adenine analogues, which should be advantageous for bioavailability. Moreover, the C6-truncated and C6-C compounds are selective agonists of the A_3AR that display considerable in vivo activity against chronic neuropathic pain in a mouse model.

EXPERIMENTAL PROCEDURES

Materials and Instrumentation. All reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO). Routine ^1H NMR spectra were obtained at 298 K with a Bruker AVIII 400 MHz or AV 500 MHz spectrometer using $CDCl_3$, CD_3OD, and DMSO as solvents. Reported chemical shifts (δ, ppm) are referenced to tetramethylsilane (0.00) for $CDCl_3$, methanol (3.30) for CD_3OD, and water (3.30) for DMSO, unless otherwise noted. Confirmation of the product structures was obtained by mass spectrometry and standard 1D and 2D NMR methods including COSY, TOCSY, HSQC, and HMBC. TLC analysis was carried out on glass sheets precoated with silica gel F254 (0.2 mm) from Aldrich. The purity of final nucleoside derivatives was checked using a Hewlett−Packard 1100 HPLC equipped with a Zorbax SB-Aq 5 μm analytical column (50 mm × 4.6 mm; Agilent Technologies Inc., Palo Alto, CA). Mobile phase: linear gradient solvent system, 5 mM TBAP (tetrabutylammonium dihydrogen phosphate)-CH_3CN from 80:20 to 0:100 in 13 min; the flow rate was 0.5 mL/min. Peaks were detected by UV absorption with a diode array detector at 230, 254, and 280 nm. All derivatives tested for biological activity showed >95% purity by HPLC analysis (detection at 254 nm). Low-resolution mass spectrometry was performed with a JEOL SX102 spectrometer with 6 kV Xe atoms following desorption from a glycerol matrix or on an Agilent LC/MS 1100 MSD, with a Waters (Milford, MA) Atlantis C18 column. High resolution mass spectroscopic (HRMS) measurements were performed on a proteomics optimized Q-TOF-2 (Micromass-Waters) using external calibration with polyalanine, unless noted. Observed mass accuracies are those expected based on known performance of the instrument as well as trends in masses of standard compounds observed at intervals during the series of measurements. Reported masses are observed masses uncorrected for this time-dependent drift in mass accuracy. All of the monosubsituted alkyne intermediates were purchased from Sigma-Aldrich (St. Louis, MO), Small Molecules, Inc. (Hoboken, NJ), Anichem (North Brunswick, NJ), PharmaBlock, Inc. (Sunnyvale, CA), Frontier Scientific (Logan, UT), and Tractus (Perrineville, NJ). tPSA and cLogP were calculated using ChemDraw Professional V. 15.0 (PerkinElmer, Boston, MA).

Chemical Synthesis. *(3aR,3bS,4aS,5R,5aS)-5-(2-Chloro-6-((Z)-2-(methylamino)-2-phenylvinyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**11a**).* Methylamine solution (40%, 2 mL) was added to a solution of compound **34** (24.5 mg, 0.051 mmol) in methanol (2.5 mL) and the mixture stirred at room temperature for 24 h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 40:1) to give the compound **11a** (16 mg, 65%) as a yellowish syrup. ^1H NMR ($CDCl_3$, 400 MHz) (δH, H-multiplicity, *J* Hz, H-integral) (9.97, br s, 1H), (7.78, s, 1H), (7.46, s, 5H), (6.94, d 4.4, 1H), (5.76, s, 1H), (5.70, d 6.8, 1H), (4.83−4.80, m, 2H), (3.01, d 5.2, 3H), (2.95, d 4.8, 1H), (2.08−2.04, m, 1H), (1.72−1.68, m, 1H), (1.57, s, 3H), (1.34−1.30, m, 4H). HRMS calculated for $C_{25}H_{28}N_6O_3Cl$ $(M + H)^+$, 495.1906; found, 495.1907.

*(1S,2R,3S,4R,5S)-4-(2-Chloro-6-((Z)-2-hydroxy-2-phenylvinyl)-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**11b**).* A solution of compound **11a** (10 mg, 0.02 mmol) in methanol (2 mL) and 10% trifluoromethanesulfonic acid (1.5 mL) was heated at 70 °C overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 25:1) to give the compound **11b** (6.8 mg, 77%) as a colorless syrup. ^1H NMR (CD_3OD, 400 MHz) (8.43, s, 1H), (7.98, dd 7.2:1.6, 2H), (7.52−7.50, m, 3H), (6.87, s, 1H), (5.13, d 6.4, 1H), (4.93, s, 1H), (4.08, d 6.8, 1H), (2.90, s, 3H), (2.14−2.11, m, 1H), (1.85, t 4.8, 1H), (1.43−1.39, m, 1H). HRMS calculated for $C_{21}H_{21}N_5O_4Cl$ $(M + H)^+$, 442.1277; found, 442.1279.

*(1S,2R,3S,4R,5S)-2,3-Dihydroxy-4-(6-((Z)-2-hydroxy-2-phenylvinyl)-2-(phenylethynyl)-9H-purin-9-yl)-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**12**).* A solution of compound **32a** (58 mg, 0.103 mmol) in methanol (5 mL) and 10% trifluoromethanesulfonic acid (3.5 mL) was heated at 70 °C overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the compound **12** (31 mg, 59%) as a colorless syrup. Column was further eluted with (CH_2Cl_2:MeOH = 15:1) to give the C6-methyl compound **15** (6.2 mg, 15%) as colorless syrup. NMR (CD_3OD, 500 MHz) (δH:δC, H-multiplicity, *J* Hz, H-integral).

*Compound **12**.* ^1H NMR (CD_3OD, 500 MHz) (1.42:15.5, ddd 9.1:4.9:1.7, 1H), (1.89:15.5, t 5.0, 1H), (2.16:28.9, ddd 9.1:4.7:1.5, 1H), (2.85:27.2, s, 3H), (4.12:77.9, dt 6.6:1.3, 1H), (5.0:64.1, s, 1H), (5.12:73.4, dd 6.6:1.3, 1H), (7.4−7.5:129.9, 130.1, 131.4, 132.4, m, 6H), (7.72:133.6, m, 2H), (8.0:127.6, m, 2H), (8.43:144.6, s, 1H); δCq 40.1, 87.4, 89.3, 122.5, 127.6, 137.4, 143.4, 149.7, 156.1, 157.8, 174.9. HRMS calculated for $C_{29}H_{26}N_5O_4$ $(M + H)^+$, 508.1985; found, 508.1991.

*Compound **15**.* ^1H NMR (CD_3OD, 500 MHz) (1.40:15.5, ddd 9.1:5.1:1.7, 1H), (1.87:15.5, t 5.1, 1H), (2.14:28.8, ddd 9.1:4.8:1.4, 1H), (2.82:19.3, s, 3H), (2.84:27.3, s, 3H), (4.10:77.9, dt 6.6:1.1, 1H), (4.99:64.2, s, 1H), (5.14:73.5, dd 6.6:1.3, 1H), (7.43−7.49:130.0, 131.1, m, 3H), (7.68:133.5, ~ dd 7.8:1.6, 2H), (8.5:146.5, s, 1H); δCq 39.9, 87.7, 89.3, 123.0, 147.2, 151.8, 160.8, 175.0. HRMS calculated for $C_{22}H_{22}N_5O_3$ $(M + H)^+$, 404.1723; found, 404.1719.

*(1S,2R,3S,4R,5S)-4-(6-((Z)-2-(2-Chlorophenyl)-2-hydroxyvinyl)-2-(2-chlorophenyl)ethynyl)-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**13**).* A solution of compound **32b** (33 mg, 0.052 mmol) in methanol (3 mL) and 10% trifluoromethanesulfonic acid (2.5 mL) was heated at 70 °C overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 25:1) to give the compound **13** (22 mg, 75%) as a colorless syrup and no C6-

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methyl product was identified in this reaction. ^{1}H NMR (CD_3OD, 400 MHz) (8.43, s, 1H), (7.79, d 6.0, 1H), (7.67, d 6.8, 1H), (7.60, d 7.2, 1H), (7.53−7.49, m, 2H), (7.48−7.42, m, 3H), (6.47, s, 1H), (5.13, d 5.6, 1H), (5.00, s, 1H), (4.13, d 6.4, 1H), (2.84, s, 3H), (2.18−2.14, m, 1H), (1.88, t 5.2, 1H), (1.44−1.40, m, 1H). HRMS calculated for $C_{29}H_{24}N_5O_4Cl_2$ (M + H)$^+$, 576.1205; found, 576.1208.

*(1S,2R,3S,4R,5S)-4-(6-((Z)-2-(4-(tert-Butyl)phenyl)-2-hydroxyvinyl)-2-(4-(tert-butyl) phenyl)ethynyl)-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**14**).* A solution of compound **32c** (24 mg, 0.035 mmol) in methanol (2.5 mL) and 10% trifluoromethanesulfonic acid (2.5 mL) was heated at 70 °C overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the compound **14** (12 mg, 55%) as a colorless syrup. Column was further eluted with (CH_2Cl_2:MeOH = 15:1) to give the C6-methyl compound **17** (2.8 mg, 17%) as colorless syrup.

*Compound **14**.* ^{1}H NMR (CD_3OD, 400 MHz) (8.36, s, 1H), (7.92, d 8.8, 1H), (7.63, d 8.8, 1H), (7.55−7.52, m, 4H), (6.75, s, 1H), (5.10, d 6.6, 1H), (4.95, s, 1H), (4.11, d 6.8, 1H), (2.85, s, 3H), (2.17−2.13, m, 1H), (1.88, t 5.2, 1H), (1.38, s, 18H). HRMS calculated for $C_{37}H_{42}N_5O_4$ (M + H)$^+$, 620.3237; found, 620.3232.

*Compound **17**.* ^{1}H NMR (CD_3OD, 400 MHz) (8.51, s, 1H), (7.62, d 8.4, 1H), (7.52, d 8.4, 1H), (5.15, d 6.4, 1H), (5.00, s, 1H), (4.11, d 6.8, 1H), (2.86, s, 3H), (2.83, s, 3H), (2.16−2.13, m, 1H), (1.88, t 5.2, 1H), (1.44−1.40, m, 1H), (1.37, s, 9H). HRMS calculated for $C_{26}H_{30}N_5O_3$ (M + H)$^+$, 460.2349; found, 460.2341.

*(1S,2R,3S,4R,5S)-4-(2-((3,4-Difluorophenyl)ethynyl)-6-methyl-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**16**).* A solution of compound **38a** (20 mg, 0.041 mmol) in methanol (2 mL) and 10% trifluoromethanesulfonic acid (2 mL) was heated at 70 °C for 5 h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 20:1) to give the compound **16** (16 mg, 89%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.52, s, 1H), (7.64−7.61, m, 1H), (7.55−7.50, m, 1H), (7.43−7.36, m, 1H), (5.14, d 6.8, 1H), (5.00, s, 1H), (4.11, d 5.6, 1H), (2.84, s, 3H), (2.83, s, 3H), (2.16−2.12, m, 1H), (1.88, t 5.2, 1H), (1.43−1.39, m, 1H). HRMS calculated for $C_{22}H_{20}N_5O_3F_2$ (M + H)$^+$, 440.1534; found, 440.1530.

*(1S,2R,3S,4R,5S)-2,3-Dihydroxy-N-methyl-4-(6-methyl-2-(pyrazin-2-ylethynyl)-9H-purin-9-yl)bicyclo[3.1.0]hexane-1-carboxamide (**18**).* Compound **18** (91%) was prepared from compound **38b** following the same method as for compound **16**. ^{1}H NMR (CD_3OD, 400 MHz) (8.95, s, 1H), (8.71, d 2.4, 1H), (8.66, d 2.4, 1H), (8.57, s, 1H), (5.20, d 6.8, 1H), (5.01, s, 1H), (4.13, d 5.6, 1H), (2.86, s, 3H), (2.85, s, 3H), (2.16−2.12, m, 1H), (1.86, t 5.2, 1H), (1.44−1.40, m, 1H). HRMS calculated for $C_{20}H_{20}N_7O_3$ (M + H)$^+$, 406.1628; found, 406.1621.

*(1S,2R,3S,4R,5S)-4-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-methyl-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**19**).* Compound **19** (93%) was prepared from compound **38c** following the same method as for compound **16**. ^{1}H NMR (CD_3OD, 400 MHz) (8.52, s, 1H), (7.36, d 4.0, 1H), (7.05, d 4.0, 1H), (5.12, d 6.4, 1H), (4.99, s, 1H), (4.10, d 5.6, 1H), (2.88, s, 3H), (2.81, s, 3H), (2.16−2.12, m, 1H), (1.87, t 5.2, 1H), (1.43−1.39, m, 1H). HRMS calculated for $C_{20}H_{19}N_5O_3ClS$ (M + H)$^+$, 444.0897; found, 444.0899.

*(1S,2R,3S,4R,5S)-4-(2-((3,4-Difluorophenyl)ethynyl)-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**20**).* A solution of compound **42a** (25 mg, 0.053 mmol) in methanol (3 mL) and 10% trifluoromethanesulfonic acid (2.5 mL) was heated at 70 °C for 5 h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 20:1) to give the compound **20** (19 mg, 85%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (9.10, s, 1H), (8.60, s, 1H), (7.64, t 8.4, 1H), (7.54−7.51, m, 1H), (7.43−7.36, m, 1H), (5.15, d 6.4, 1H), (5.03, s, 1H), (4.12, d 6.4, 1H), (2.84, s, 3H), (2.17−2.14, m, 1H), (1.89, t 5.2, 1H), (1.44−1.40, m, 1H). HRMS calculated for $C_{21}H_{18}N_5O_3F_2$ (M + H)$^+$, 426.1378; found, 426.1385.

*(1S,2R,3S,4R,5S)-4-(2-((5-Chlorothiophen-2-yl)ethynyl)-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**21**).* Compound **21** (83%) was prepared from compound **42b** following the same method for compound **20**. ^{1}H NMR (CD_3OD, 400 MHz) (9.09, s, 1H), (8.59, s, 1H), (7.38, d 4.0, 1H), (7.05, d 4.0, 1H), (5.13, d 6.4, 1H), (5.02, s, 1H), (4.11, d 6.4, 1H), (2.88, s, 3H), (2.17−2.14, m, 1H), (1.88, t 4.8, 1H), (1.44−1.40, m, 1H). HRMS calculated for $C_{19}H_{17}N_5O_3SCl$ (M + H)$^+$, 430.0741; found, 430.0734.

*(1S,2R,3S,4R,5S)-4-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-methoxy-9H-purin-9-yl)-2,3-dihydroxy-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**22**).* Dowex 50 (H^+ form, 18 mg) was added to a solution of compound **51** (18 mg, 0.036 mmol) in MeOH (1 mL)-water (0.6 mL) and the mixture heated at 70 °C for 2.5 h. Reaction mixture was filtered, the filtrate was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 25:1) to give the compound **22** (14 mg, 88%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.34, s, 1H), (7.35, d 4.0, 1H), (7.04, d 4.0, 1H), (5.09, d 6.2, 1H), (4.96, s, 1H), (4.21, s, 3H), (4.07, d 7.4 Hz 1H), (2.87, s, 3H), (2.15−2.11, m, 1H), (1.87, t 5.2, 1H), (1.42−1.38, m, 1H). HRMS calculated for $C_{20}H_{19}N_5O_4SCl$ (M + H)$^+$, 460.0846; found, 460.0852.

*(1S,2R,3S,4R,5S)-2,3-Dihydroxy-N-methyl-4-(2-(methylamino)-9H-purin-9-yl)bicycle[3.1.0]hexane-1-carboxamide (**23**).* A solution of compound **40** (145 mg, 0.40 mmol) in methanol (4 mL) and 10% trifluoromethanesulfonic acid (4 mL) was heated at 70 °C for 5 h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 8:1) to give the compound **23** (106 mg, 83%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.61, s, 1H), (8.16, s, 1H), (5.18, d 6.8, 1H), (4.8, s, 1H), (4.15, d 6.2, 1H), (2.99, s, 3H), (2.83, s, 3H), (2.13−2.10, m, 1H), (1.78, d 5.2, 1H), (1.38−1.34, m, 1H). HRMS calculated for $C_{14}H_{19}N_6O_3$ (M + H)$^+$, 319.1513; found, 319.1511.

*(1S,2R,3S,4R,5S)-2,3-Dihydroxy-4-(2-((Z)-2-hydroxy-2-(pyrazin-2-yl)vinyl)-9H-purin-9-yl)-N-methylbicyclo[3.1.0]hexane-1-carboxamide (**24**).* Compound **24** (72%) was prepared from compound **43** following the same method for compound **20**. ^{1}H NMR (CD_3OD, 400 MHz) (8.12, d 1.2, 1H), (9.08, s, 1H), (8.68, d 2.4, 1H), (8.62, d 2.4, 1H), (8.46, s, 1H), (7.01, s, 1H), (5.26, d 6.4, 1H), (4.98, s, 1H), (4.13, d 6.4, 1H), (3.0, s, 3H), (2.16−2.13, m, 1H), (1.85, t 5.2, 1H), (1.44−1.40, m, 1H). HRMS calculated for $C_{19}H_{20}N_7O_4$ (M + H)$^+$, 410.1577; found, 410.1576.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2,6-Bis(phenylethynyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]-dioxole-3b(3aH)-carboxylate (**31a**).* $PdCl_2(PPh_3)_2$ (28 mg, 0.4 mmol), CuI (3.8 mg, 0.02 mmol), phenylacetylene (0.13 mL, 1.2 mmol), and triethylamine (0.28 mL, 2.0 mmol) was added to a solution of compound **28** (101 mg, 0.2 mmol) in anhydrous DMF (4 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound **31a** (91.8 mg, 84.2%) as a brownish glassy solid. ^{1}H NMR ($CDCl_3$, 400 MHz) (8.14, s, 1H), (7.71−7.75, m, 4H), (7.44−7.41, m, 6H), (5.95, d 7.2, 1H), (5.08, s, 1H), (4.81, d 7.2, 1H), (4.25−4.22, m, 2H), (2.32−2.29, m, 1H), (1.82−1.78, m, 1H), (1.64−1.60, m, 4H), (1.33, s, 3H), (1.22, t 7.2, 3H). HRMS calculated for $C_{33}H_{29}N_4O_4$ (M + H)$^+$, 545.2189; found, 545.2197.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2,6-Bis((2-chlorophenyl)ethynyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta-[1,2-d][1,3]dioxole-3b(3aH)-carboxylate (**31b**).* Compound **31b** (82%) was prepared from compound **28** following the same method for compound **31a**. ^{1}H NMR (CD_3OD, 400 MHz) (8.65, s, 1H), (7.91−7.56, m, 2H), (7.53−7.51, m, 2H), (7.50−7.40, m, 4H), (5.98, d 7.2, 1H), (5.18, s, 1H), (5.01, d 7.2, 1H), (4.21−4.10, m, 2H), (2.43−2.39, m, 1H), (1.74−1.70, m, 1H), (1.60−1.56, m, 4H), (1.31, s, 3H), (1.15, t 7.2, 3H). HRMS calculated for $C_{33}H_{27}N_4O_4Cl_2$ (M + H)$^+$, 613.1409; found, 613.1400.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2,6-Bis((4-(tert-butyl)phenyl)-ethynyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]-cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxylate (**31c**).* Compound **31c** (85%) was prepared from compound **28** following the same method for compound **31a**. ^{1}H NMR (CD_3OD, 400 MHz) (8.61, s, 1H), (7.72, dd 11.2:2.8, 4H), (7.54, dd 11.2:2.8, 4H), (5.97, d 6.8, 1H), (5.16, s, 1H), (4.96, d 7.2, 1H), (4.27−4.17, m, 2H), (2.43−2.39, m, 1H), (1.75−1.71, m, 1H), (1.60−1.56, m, 4H), (1.38, s, 18H),

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(1.32, s, 3H), (1.18, d 7.2, 3H). HRMS calculated for $C_{41}H_{45}N_4O_4$ (M + H)$^+$, 657.3441; found, 657.3446.

*(3aR,3bS,4aS,5R,5aS)-N,2,2-Trimethyl-5-(6-((Z)-2-(methylamino)-2-phenylvinyl)-2-(phenylethynyl)-9H-purin-9-yl)-tetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**32a**).* Methylamine solution (40%, 5 mL) was added to a solution of compound **31a** (97 mg, 0.173 mmol) in methanol (6 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 40:1) to give the compound **32a** (67.4 mg, 68%) as a yellowish syrup. NMR (CD_3OD, δC:49.2), (500 MHz) (δH:δC, H- multiplicity, *J* Hz, H-integral) (1.30:24.7, s, 3H), (1.42:17.7, t 5.5, 1H), (1.54:26.5, s, 3H), (1.54:17.7, ddd, 1H), (2.18:36.9, ddd 9.4:5.5:1.5, 1H), (2.76:27.5, s, 3H), (2.97:32.2, s, 3H), (4.89:90.7, ddd 7.1:1.7:0.4, 1H), (4.95:61.9, s, 1H), (5.67:89.3, s, 1H), (5.81:83.1, dd 7.1:1.1, 1H), (7.43−7.51:129.4, 129.8, 130.0, 130.6, 130.7, m, 8H), (7.69−7.73:133.6, m, 2H), (8.22:143.4, s, 1H); NH in $CDCl_3$ (7.38, q), (10.25, q); δCq 42.8, 85.6, 90.4, 114.0, 123.6, 128.0, 138.2, 146.8, 149.4, 158.6, 164.7, 174.4. HRMS calculated for $C_{33}H_{33}N_6O_3$ (M + H)$^+$, 561.2614; found, 561.2612.

*(3aR,3bS,4aS,5R,5aS)-5-(6-((Z)-2-(2-Chlorophenyl)-2-(methylamino)vinyl)-2-((2-chloro phenyl)ethynyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta [1,2-d][1,3]-dioxole-3b(3aH)-carboxamide (**32b**).* Compound **32b** (71%) was prepared from compound **31b** following the same method for compound **32a**. ^{1}H NMR ($CDCl_3$, 400 MHz) (10.41, br s, 1H), (7.86, s, 1H), (7.72, d 6.0, 1H), (7.53, d 6.8, 1H), (7.48, d 6.0, 1H), (7.42−7.38, m, 5H), (5.83, d 6.8, 1H), (5.69, s, 1H), (4.91, t 7.2, 1H), (4.86, s, 1H), (2.91, d 5.2, 3H), (2.83, d 4.8, 3H), (2.04−1.97, m, 1H), (1.72−1.69, m, 1H), (1.58, s, 3H), (1.32−1.24, m, 4H). HRMS calculated for $C_{33}H_{31}N_6O_3Cl_2$ (M + H)$^+$, 629.1829; found, 629.1835.

*(3aR,3bS,4aS,5R,5aS)-5-(6-((Z)-2-(4-(tert-Butyl)phenyl)-2-(methylamino)vinyl)-2-(4-(tert-butyl)phenyl)ethynyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclo-propa[3,4]cyclopenta [1,2-d]-[1,3]dioxole-3b(3aH)-carboxamide (**32c**).* Compound **32c** (70%) was prepared from compound **31c** following the same method for compound **32a**. ^{1}H NMR ($CDCl_3$, 400 MHz) (10.30, br s, 1H), (7.84, s, 1H), (7.62, d 8.4, 2H), (7.47−7.44, m, 8H), (5.82, d 7.2, 1H), (5.80, s, 1H), (4.89, d 6.0, 1H), (4.85, s, 1H), (3.04, d 5.2, 3H), (2.86, d 4.8, 3H), (2.01−1.97, m, 1H), (1.72−1.68, m, 1H), (1.58, s, 3H), (1.37, s, 18H), (1.31−1.24, m, 4H). HRMS calculated for $C_{41}H_{49}N_6O_3$ (M + H)$^+$, 673.3866; found, 673.3876.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2-Chloro-6-(phenylethynyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxylate (**34**).* $PdCl_2(PPh_3)_2$ (13.8 mg, 0.02 mmol), CuI (1.9 mg, 0.01 mmol), phenylacetylene (64 μL, 0.58 mmol), and triethylamine (0.13 mL, 0.98 mmol) was added to a solution of compound **33** (40.6 mg, 0.098 mmol) in anhydrous DMF (1.5 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 2:1) to give the compound **34** (40.4 mg, 86%) as a colorless glassy syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.59, s, 1H), (7.80, d 8.0, 2H), (7.53−7.50, m, 3H), (5.88, d 7.2, 1H), (5.13, s, 1H) (4.28−4.25, m, 2H), (2.40−2.36, m, 1H), (1.73−1.70, m, 1H), (1.59−1.55, m, 4H), (1.34, t 7.2, 3H), (1.30, s, 3H). HRMS calculated for $C_{25}H_{24}N_4O_4Cl$ (M + H)$^+$, 479.1481; found, 479.1482.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-methyl-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]-dioxole-3b(3aH)-carboxylate (**36**).* DIAD (0.23 mL, 1.2 mmol) was added to a solution of triphenylphosphine (0.326 g, 1.242 mmol) and 2-iodo-6-methylpurine **54** (0.234 g, 0.9 mmol) in dry THF (4 mL) at 0 °C, and after addition it was stirred at room temperature for 10 min. A solution of compound **35** (0.145 g, 0.6 mmol) in THF (2 mL) was added to the reaction mixture and stirred overnight at room temperature. Solvent was evaporated, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound **36** (0.243 g, 84%) as a colorless foamy solid. ^{1}H NMR (CD_3OD, 400 MHz) (8.36, s, 1H), (5.85, d 6.4, 1H), (5.06, s, 1H), (4.88, d 6.8, 1H), (4.34−4.29, m, 2H), (2.74, s, 3H), (2.32−2.28, m, 1H), (1.68−1.64, m, 1H), (1.55−1.53, m, 4H), (1.34, t 7.2, 3H), (1.29, s, 3H). HRMS calculated for $C_{18}H_{22}N_4O_4I$ (M + H)$^+$, 485.0686; found, 485.0684.

*(3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-methyl-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**37**).* Methylamine solution (40%, 5 mL) was added to a solution of compound **36** (288 mg, 0.595 mmol) in methanol (5 mL) and the mixture stirred at room temperature for 24 h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 35:1) to give the compound **37** (184 mg, 66%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.38, s, 1H), (5.75, d 7.2, 1H), (5.05, s, 1H), (4.90, d 6.8, 1H), (2.91, s, 3H), (2.74, s, 3H), (2.21−2.17, m, 1H), (1.55−1.51, m, 4H), (1.43, t 5.2, 1H), (1.30, s, 3H). HRMS calculated for $C_{17}H_{21}N_5O_3I$ (M + H)$^+$, 470.0689; found, 470.0694.

*(3aR,3bS,4aS,5R,5aS)-5-(2-((3,4-Difluorophenyl)ethynyl)-6-methyl-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]-cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**38a**).* $PdCl_2(PPh_3)_2$ (6.37 mg, 0.01 mmol), (CuI (1 mg, 0.005 mmol), (3,4-difluoro-phenylacetylene (32 μL, 0.27 mmol), and triethylamine (63 μL, 0.45 mmol) was added to a solution of compound **37** (21 mg, 0.045 mmol) in anhydrous DMF (1.2 mL), and stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound **38a** (20 mg, 92%) as a yellowish syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.54, s, 1H), (7.75−7.70, m, 1H), (7.61−7.57, m, 1H), (7.43−7.37, m, 1H), (5.86, d 6.8, 1H), (5.13, s, 1H), (4.93, d 7.2, 1H), (2.83, s, 3H), (2.78, s, 3H), (2.24−2.19, m, 1H), (1.58−1.55, m, 4H), (1.47, t 5.2, 1H), (1.31, s, 3H). HRMS calculated for $C_{25}H_{24}N_5O_3F_2$ (M + H)$^+$, 480.1847; found, 480.1849.

*(3aR,3bS,4aS,5R,5aS)-N,2,2-Trimethyl-5-(6-methyl-2-(pyrazin-2-ylethynyl)-9H-purin-9-yl)tetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**38b**).* Compound **38b** (86%) was prepared from compound **37** following the same method for compound **38a**. ^{1}H NMR (CD_3OD, 400 MHz) (9.01, d 1.2, 1H), (8.73−8.72, m, 1H), (8.68, d 2.4, 1H), (8.57, s, 1H), (5.93, d 7.2, 1H), (5.14, s, 1H), (4.97, d 7.2, 1H), (2.84, s, 3H), (2.80, s, 3H), (2.23−2.17, m, 1H), (1.61−1.56, m, 4H), (1.47, t 5.2, 1H), (1.32, s, 3H). HRMS calculated for $C_{23}H_{24}N_7O_3$ (M + H)$^+$, 446.1941; found, 446.1942.

*(3aR,3bS,4aS,5R,5aS)-5-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-methyl-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]-cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**38c**).* Compound **38c** (89%) was prepared from compound **37** following the same method for compound **38a**. ^{1}H NMR (CD_3OD, 400 MHz) (8.53, s, 1H), (7.43, d 4.0, 1H), (7.06, d 4.0, 1H), (5.84, d 7.2, 1H), (5.1, s, 1H), (4.92, d 6.8, 1H), (2.83, s, 3H), (2.81, s, 3H), (2.22−2.18, m, 1H), (1.59−1.56, m, 4H), (1.46, d 5.2, 1H), (1.34, s, 3H). HRMS calculated for $C_{23}H_{23}N_5O_3ClS$ (M + H)$^+$, 484.1210; found, 484.1204.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxylate (**39**).* DIAD (0.196 mL, 1.0 mmol) was added to a solution of triphenylphosphine (0.261 g, 1.0 mmol) and 2-iodopurine (0.184 g, 0.75 mmol) in dry THF (4 mL) at 0 °C, and after addition it was stirred at room temperature for 10 min. A solution of compound **35** (0.121 g, 0.5 mmol) in THF (2 mL) was added to the reaction mixture and stirred overnight at room temperature. Solvent was evaporated, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 2:1) to give the compound **39** (0.181 g, 72%) as a colorless foamy solid. ^{1}H NMR (CD_3OD, 400 MHz) (8.83, s, 1H), (8.44, s, 1H), (5.85, d 7.2, 1H), (5.09, s, 1H), (4.89, d 7.2, 1H), (4.36−4.30, m, 2H), (2.35−2.31, m, 1H), (1.69−1.65, m, 1H), (1.56−1.54, m, 4H), (1.34, t 7.2, 3H), (1.29, s, 3H). HRMS calculated for $C_{17}H_{20}N_4O_4I$ (M + H)$^+$, 471.0524; found, 471.0523.

*(3aR,3bS,4aS,5R,5aS)-N,2,2-Trimethyl-5-(2-(methylamino)-9H-purin-9-yl)tetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**40**).* Methylamine solution (40%, 4 mL) was added to a solution of compound **39** (460 mg, 0.97 mmol) in methanol (4 mL) and the mixture stirred at room temperature for 24

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h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the compound **40** (224 mg, 64%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.56, s, 1H), (8.05, s, 1H), (5.80, d 7.2, 1H), (4.96−4.94, m, 2H), (2.96, s, 3H), (2.77, s, 3H), (2.25−2.22, m, 1H), (1.55, s, 3H), (1.52−1.48, m, 1H), (1.42, t 5.2, 1H), (1.31, s, 3H). HRMS calculated for $C_{17}H_{23}N_6O_3$ (M + H$^+$, 359.2002; found, 359.2001.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2-((3,4-Difluorophenyl)ethynyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta-[1,2-d][1,3]dioxole-3b(3aH)-carboxylate (**41a**).* Compound **41a** (84%) was prepared from compound **39** following the same method for compound **38a**. ^{1}H NMR (CD_3OD, 400 MHz) (9.08, s, 1H), (8.59, s, 1H), (7.76−7.21, m, 1H), (7.68−7.57, m, 3H), (7.44−7.37, m, 1H), (5.96, d 7.2, 1H), (5.17, s, 1H), (4.90, d 7.2, 1H), (4.25−4.14, m, 2H), (2.41−2.37, m, 1H), (1.76−1.73, m, 1H), (1.61, t 5.6, 1H), (1.56, s, 3H), (1.30, s, 3H), (1.18, t 6.8, 3H). HRMS calculated for $C_{25}H_{23}N_4O_4F_2$ (M + H)$^+$, 481.1687; found, 481.1689.

*Ethyl (3aR,3bS,4aS,5R,5aS)-5-(2-((5-Chlorothiophen-2-yl)-ethynyl)-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]-cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxylate (**41b**).* Compound **41b** (87%) was prepared from compound **39** following the same method for compound **38a**. ^{1}H NMR (CD_3OD, 400 MHz) (9.07, s, 1H), (8.58, s, 1H), (7.54, d 4.0, 1H), (7.05, d 4.0, 1H), (5.93, d 7.2, 1H), (5.16, s, 1H), (4.91, d 7.2, 1H), (4.30−4.20, m, 2H), (2.40−2.36, m, 1H), (1.76−1.72, m, 1H), (1.59, t 5.2, 1H), (1.55, s, 3H), (1.29, s, 3H), (1.23, t 7.2, 3H). HRMS calculated for $C_{23}H_{22}N_4O_4SCl$ (M + H)$^+$, 485.1050; found, 485.1042.

*Ethyl (3aR,3bS,4aS,5R,5aS)-2,2-Dimethyl-5-(2-(pyrazin-2-ylethyn-yl)-9H-purin-9-yl)tetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]-dioxole-3b(3aH)-carboxylate (**41c**).* Compound **41c** (82%) was prepared from compound **39** following the same method for compound **38a**. ^{1}H NMR (CD_3OD, 400 MHz) (9.14, s, 1H), (9.06, s, 1H), (8.71, d 6.8, 1H), (8.66, d 2.8, 1H), (8.62, s, 1H), (5.97, d 7.2, 1H), (5.18, s, 1H), (4.92, d 7.2, 1H), (4.28−4.22, m, 2H), (2.42−2.38, m, 1H), (1.76−1.72, m, 1H), (1.59, t 5.2, 1H), (1.56, s, 3H), (1.30, s, 3H), (1.20, t 7.2, 3H). HRMS calculated for $C_{23}H_{23}N_6O_4$ (M + H)$^+$, 447.1781; found, 447.1775.

*(3aR,3bS,4aS,5R,5aS)-5-(2-((3,4-Difluorophenyl)ethynyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta-[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**42a**).* Compound **42a** (68%) was prepared from compound **39** following the same method for compound **37**. ^{1}H NMR (CD_3OD, 400 MHz) (9.10, s, 1H), (8.60, s, 1H), (7.75−7.70, m, 1H), (7.61−7.58, m, 1H), (7.43−7.37, m, 1H), (5.87, d 7.2, 1H), (5.16, s, 1H), (4.94, d 6.4, 1H), (2.78, s, 3H), (2.26−2.22, m, 1H), (1.60−1.57, m, 4H), (1.49, t 5.2, 1H), (1.32, s, 3H). HRMS calculated for $C_{24}H_{22}N_5O_3F_2$ (M + H)$^+$, 466.1691; found, 466.1689.

*(3aR,3bS,4aS,5R,5aS)-5-(2-((5-Chlorothiophen-2-yl)ethynyl)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta-[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**42b**).* Compound **42b** (66%) was prepared from compound **39** following the same method for compound **37**. ^{1}H NMR (CD_3OD, 400 MHz) (9.08, s, 1H), (8.60, s, 1H), (7.44, d 4.0, 1H), (7.06, d 4.0, 1H), (5.85, d 7.2, 1H), (5.15, s, 1H), (4.93, d 7.2, 1H), (2.84, s, 3H), (2.25−2.21, m, 1H), (1.60−1.57, m, 4H), (1.48, t 5.2, 1H), (1.31, s, 3H). HRMS calculated for $C_{22}H_{21}N_5O_3SCl$ (M + H)$^+$, 470.1054; found, 470.1047.

*(3aR,3bS,4aS,5R,5aS)-N,2,2-Trimethyl-5-(2-((Z)-2-(methylamino)-2-(pyrazin-2-yl)vinyl)-9H-purin-9-yl)tetrahydrocyclopropa[3,4]-cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**43**).* Methylamine solution (40%, 1.5 mL) was added to a solution of compound **41c** (21 mg, 0.044 mmol) in methanol (2 mL) and the mixture stirred at room temperature for 24 h. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 25:1) to give the compound **43** (12.8 mg, 62%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.98, s, 1H), (8.91, d 1.6, 1H), (8.73, s, 1H), (8.68, d 2.4, 1H), (8.36, s, 1H), (5.75, d 7.2, 1H), (5.59, s, 1H), (5.07, s, 1H), (4.92, d 7.2, 1H), (2.92, s, 3H), (2.71, d 2.8, 3H), (2.34−2.28, m, 1H), (1.62−1.56, m, 4H), (1.47, d 5.2, 1H), (1.29, s, 3H). HRMS calculated for $C_{23}H_{27}N_8O_3$ (M + H)$^+$, 463.2206; found, 463.2208.

*Methyl (3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-methoxy-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]-dioxole-3b(3aH)-carboxylate (**44**).* Sodium methoxide (24 mg, 0.44 mmol) was added to a solution of compound **28** (45 mg, 0.09 mmol) in methanol (2 mL) and the mixture stirred at room temperature overnight. The reaction mixture was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 1:1) to give the compound **44** (11 mg, 25%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) (8.21, s, 1H), (5.85, d 6.8, 1H), (5.04, s, 1H), (4.88, d 6.8, 1H), (4.15, s, 3H), (3.85, s, 3H), (2.31−2.27, m, 1H), (1.68−1.65, m, 1H), (1.57−1.51, m, 4H), (**1.29**, m, 3H). HRMS calculated for $C_{17}H_{20}N_4O_5I$ (M + H)$^+$, 487.0478; found, 487.0482.

*(3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-(methylamino)-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]-dioxole-3b(3aH)-carboxamide (**45**).* 40% Methylamine solution (0.5 mL) was added to a solution of compound **44** (11 mg, 0.022 mmol) in methanol (0.5 mL) and the mixture stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 35:1) to give the compound **45** (7.4 mg, 68%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (7.95, s, 1H), (5.72, d 7.2, 1H), (4.93, s, 1H), (4.84, d 7.2, 1H), (3.05, br s, 3H), (2.90, s, 3H), (2.15−2.11, m, 1H), (1.54−1.49, m, 4H), (1.39, t 5.2, 1H), (1.30, s, 3H). HRMS calculated for $C_{17}H_{22}N_6O_3I$ (M + H)$^+$, 485.0798; found, 485.0798.

*9-(3aR,3bR,4aS,5R,5aS)-3b-(((tert-Butyldiphenylsilyl)oxy)methyl)-2,2-dimethylhexahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]-dioxol-5-yl)-2-iodo-6-methoxy-9H-purine (**47**).* Sodium methoxide (77.8 mg, 1.44 mmol) was added to a solution of compound **46** (202 mg, 0.28 mmol) in methanol (5 mL) and the mixture stirred at room temperature for 1.5 h. The reaction mixture was evaporated under vacuum, and the residue was partitioned with ethyl acetate and water. Combined organic layer was dried, filtered, and evaporated, and the residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 2:1) to give the compound **47** (192 mg, 96%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.26, s, 1H), (7.67−7.59, m, 4H), (7.40−7.23, m, 6H), (5.28, d 6.8, 1H), (4.97, s, 1H), (4.78, d 6.8, 1H), (4.16, s, 3H), (4.14, d 10.8, 1H), (4.02, d 10.8, 1H), (1.57−1.53, m, 4H), (1.25, s, 3H), (1.10−1.05, m, 11H). HRMS calculated for $C_{32}H_{38}N_4O_4SiI$ (M + H)$^+$, 697.1707; found, 697.1710.

*((3aR,3bR,4aS,5R,5aS)-5-(2-Iodo-6-methoxy-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxol-3b-(3aH)-yl)methanol (**48**).* Tetrabutylammonium fluoride (0.41 mL, 1 M solution in THF) was added to a solution of compound **47** (192 mg, 0.27 mmol) in dry THF (4 mL) and the mixture stirred at room temperature for 1 h. Solvent was evaporated, and residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the compound **48** (115 mg, 91%) as a syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.38, s, 1H), (5.35, d 7.2, 1H), (5.02, s, 1H), (4.75, d 6.8, 1H), (4.16, s, 3H), (3.92, d 11.6, 1H), (3.79, d 11.6, 1H), (1.70−1.66, m, 1H), (1.53, s, 3H), (1.27, s, 3H), (1.15, t 5.2, 1H), (1.03−0.99, m, 1H). HRMS calculated for $C_{16}H_{20}N_4O_4I$ (M + H)$^+$, 459.0529; found, 459.0526.

*(3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-methoxy-9H-purin-9-yl)-2,2-dimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxylic Acid (**49**).* PDC (567 mg, 1.5 mmol) was added to a solution of compound **48** (115 mg, 0.25 mmol) in dry DMF (2 mL) and the mixture heated at 40 °C overnight. After completion of starting material, water (10 mL) was added into the reaction mixture and extracted with ethyl acetate (3 × 10 mL). Combined organic layer was washed with brine (15 mL), dried, filtered, and evaporated, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 20:1) to give the compound **49** (91 mg, 72%) as a colorless syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.20, s, 1H), (5.85, d 7.2, 1H), (5.04, s, 1H), (4.85, d 6.8, 1H), (4.15, s, 3H), (2.28−2.24, m, 1H), (1.70−1.66, m, 1H), (1.54−1.52, m, 4H), (1.29, s, 3H). HRMS calculated for $C_{16}H_{18}N_4O_5I$ (M + H)$^+$, 474.0322; found, 474.0321.

*(3aR,3bS,4aS,5R,5aS)-5-(2-Iodo-6-methoxy-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**50**).* $MeNH_2$ (53 μL, 2 M solution in THF) and DIPEA (22 μL, 0.12 mmol) were added to a solution of

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compound **49** (46 mg, 0.09 mmol) and HATU (48.15 mg, 0.12 mmol) in dry DMF (1.5 mL). The reaction mixture was stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 60:1) to give the compound **50** (31 mg, 67%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) (8.22, s, 1H), (5.75, d 7.2, 1H), (5.02, s, 1H), (4.87, d 6.8, 1H), (4.15, s, 3H), (2.90, d 3.6, 3H)), (2.20−2.15, m, 1H), (1.55−1.51, m, 4H), (1.42, t 5.2, 1H), (1.31, s, 3H). HRMS calculated for $C_{17}H_{21}N_5O_4I$ $(M + H)^+$, 486.0638; found, 486.0644.

*(3aR,3bS,4aS,5R,5aS)-5-(2-((5-Chlorothiophen-2-yl)ethynyl)-6-methoxy-9H-purin-9-yl)-N,2,2-trimethyltetrahydrocyclopropa[3,4]-cyclopenta[1,2-d][1,3]dioxole-3b(3aH)-carboxamide (**51**).* $PdCl_2(PPh_3)_2$ (8.96 mg, 0.01 mmol), (CuI (1 mg, 0.006 mmol), (2-chloro-5-ethynylthiophene (54.6 mg, 0.38 mmol), and triethylamine (90 μL, 0.45 mmol) was added to a solution of compound **50** (31 mg, 0.063 mmol) in anhydrous DMF (1.2 mL) and stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 40:1) to give the compound **51** (24 mg, 77%) as a yellowish syrup. ^{1}H NMR (CD_3OD, 400 MHz) (8.37, s, 1H), (7.42, d 4.0, 1H), (7.05, d 4.0, 1H), (5.82, d 6.4, 1H), (5.09, s, 1H), (4.90, d 7.0, 1H), (4.20, s, 3H), (2.82, d 4.4, 3H), (2.21−2.17, m, 1H), (1.58−1.55, m, 4H), (1.45, t 5.2, 1H), (1.31, s, 3H). HRMS calculated for $C_{23}H_{23}N_5O_4SCl$ $(M + H)^+$, 500.1159; found, 500.1150.

*2-Iodo-9-(4-methoxybenzyl)-6-methyl-9H-purine (**53**).* CuI (558 mg, 3.04 mmol), iodine (704 mg, 2.77 mmol), CH_2I_2 (2.23 mL, 27.7 mmol), and isoamyl nitrite (1.12 mL, 8.33 mmol) were added to a solution of compound **52** (747 mg, 2.77 mmol) in dry THF (30 mL) and refluxed at 80 °C for 1.5 h. Water was added into the reaction mixture, and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with saturated sodium bisulfite solution followed by brine, dried, filtered, and evaporated under vacuum. The residue was purified on flash silica gel column chromatography (hexane:ethyl acetate = 2:1) to give the compound **53** (736 mg, 58%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) (8.35, s, 1H), (8.34, d 8.8, 2H), (6.91, d 8.8, 2H), (4.87, s, 2H), (3.78, s, 3H), (2.73, s, 3H). HRMS calculated for $C_{14}H_{14}N_4OI$ $(M + H)^+$, 460.0846; found, 460.0852.

*2-Iodo-6-methyl-9H-purine (**54**).* A solution of compound **53** (1.00 g, 2.17 mmol) in TFA (14 mL)−CH_2I_2 (2 mL) was heated at 50 °C overnight. Solvent was evaporated, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 30:1) to give the compound **54** (519 mg, 92%) as a colorless powder. ^{1}H NMR (CD_3OD, 400 MHz) (8.40, s, 1H), (2.75, s, 3H). HRMS calculated for $C_6H_6N_4I$ $(M + H)^+$, 260.9637; found, 260.9628.

Human AR Binding and A_3AR Activation. Radioligand binding was performed as described (footnote a of Table 1),[32] and agonism at the hA_3AR was measured as described[7] in CHO cells expressing the hA_3AR.

Mouse A_3AR Binding and Activation. Binding of agonist radioligand (**27**) at the mA_3AR was performed as described,[32] and agonism at the mA_3AR was measured as described[7] in HEK293 cells expressing the mA_3AR.

Chronic Neuropathic Pain Model. As in our previous reports,[6,32] adenosine agonists were dissolved in vehicle and administered by oral gavage (po, 3 μmol/kg, ∼ 0.2 mL, n = 3) to mice (Harlan, Indianapolis, IN, USA); on day 7, the time peak pain was reached following ligation of the sciatic nerve, as described by Bennett and colleagues.[29] The vehicle consisted of 10% DMSO in 0.5% methylcellulose, diluted from a 5 mM stock solution in DMSO). Methylcellulose (lot no. 021M0067 V) was obtained from Sigma Viscosity 400 cP and prepared in sterile distilled water (UPS). The PWT (g) of the ipsilateral hind paw was measured as a function of time following drug administration. This time course allowed the assessment of duration of action and indirectly indicated sufficient bioavailability when protection was observed. All in vivo experiments were performed by methods described[25] and in accordance with the International Association for the Study of Pain and the National Institutes of Health guidelines on laboratory animal welfare and the recommendations by Saint Louis University Institutional Animal Care and Use Committee. All experiments were conducted with the experimenters blinded to treatment conditions.

Molecular Modeling. *hA_3AR Homology Model.* In this study, we used a previously published hA_3AR homology model[7] based upon a hybrid template structure and built by means of the homology modeling tool implemented in the MOE suite.[42] In particular, the agonist-bound $hA_{2A}AR$ crystal structure (PDB code 3QAK)[9] was selected as a template for the entire A_3AR structure except for the extracellular terminus of TM2 (residues from Val63 to Ser73) and EL1 (residues from Leu74 to Tyr81). The X-ray structure of the $h\beta_2$ adrenergic receptor in complex with the Gs protein (PDB code 3SN6),[43] after superimposition with the $hA_{2A}AR$ crystal structure, was set as template for the extracellular terminus of TM2. No structural templates were used to model the EL1. Details of the modeling procedure have been previously described.[7,32]

Molecular Docking of (N)-Methanocarba C6 Substituted Purine Nucleoside Derivatives. The ligands were built with the build panel implemented in the Schrödinger suite and prepared for docking with LigPrep.[44,45] Molecular docking of the ligands at the hA_3AR model was performed by means of the Glide[46] package part of the Schrödinger suite. The docking site was defined centering a 20 Å × 20 Å × 20 Å box on key residues of the hA_3AR binding pocket, namely Phe168 (EL2), Asn250 (6.55), Trp243 (6.48), and His272 (7.43). Docking of ligands was carried out in the rigid binding site using the XP (extra precision) procedure with post-docking refinement of the obtained poses. The top ranked conformations of each ligand were subjected to visual inspection and analysis of protein−ligand interactions to select the final binding pose.

Analysis of Ligand−Receptor Interactions. Per residue electrostatic and van der Waals interaction scores (hereby denoted as IS_{ele} and IS_{vdW}, respectively) were computed as implemented in Glide.[46] By means of in-house bash scripts and Gnuplot 4.6, the scores were converted into heat-like maps (interaction scores maps, ISMs), highlighting key residues involved in the binding along with a quantitative estimate of the occurring interaction scores reported in kcal/mol and rendered with a color code (the more intense and blue/green-shifted the color, the better the interaction).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01998.

NMR analysis and mass spectra of selected synthesized compounds, results of PDSP screening, and supplementary chemical schemes (PDF)
Molecular formula strings (CSV)
Video related to Figure 3 (AVI)
3D coordinates of the modeled hA_3AR complex with **15** (PDB)
3D coordinates of the modeled hA_3AR complex with **21** (PDB)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AR, adenosine receptor; cAMP, adenosine 3′,5′-cyclic monophosphate; CCI, chronic constriction injury; CHO, Chinese hamster ovary; DIPEA, diisopropylethylamine; DMEM, Dulbecco's Modified Eagle Medium; DMF, *N*,*N*-dimethylformamide; EL, extracellular loop; GPCR, G protein-coupled receptor; HATU; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; HEK, human embryonic kidney; HMBC, heteronuclear multiple bond correlation; HRMS, high resolution mass spectroscopy; IS_{ele}, per residue interaction score, electrostatic; IS_{vdW}, per residue interaction score, van der Waals; NMR, nuclear magnetic resonance; PBS, phosphate buffered saline; PDC, pyridinium dichromate; PDSP, Psychoactive Drug Screening Program; PWT, paw withdrawal threshold; RMS, root-mean-square; SAR, structure−affinity relationship; TBAP, tetrabutylammonium dihydrogen phosphate; TBDPS, *tert*-butyldiphenylsilyl; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TM, transmembrane helix; tPSA, total polar surface area; MW, molecular weight

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