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Synthesis and Purine Receptor Affinity of 6-Oxopurine Nucleosides and Nucleotides Containing (N)-Methanocarba-pseudoribose Rings

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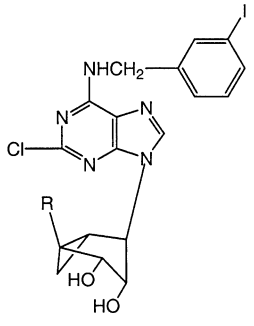
Abstract—6-Oxopurine derivatives containing a northern (N) methanocarba modification (i.e., fused cyclopropane and cyclopentane rings in place of the ribose) were synthesized and the adenosine receptor affinity measured. Guanine or hypoxanthine was coupled at the 7-position, or 1,3-dibutylxanthine was coupled at the 9-position. The pseudoribose ring was also substituted at the 5′-position with an *N*-methyluronamide or with phosphate groups. Published by Elsevier Science Ltd.

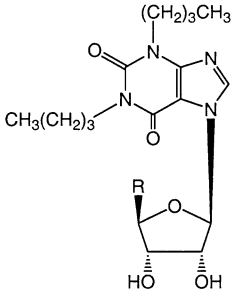
We recently examined conformational requirements of the ribose moiety in adenosine derivatives acting as agonists or partial agonists at P2 receptors and at subtypes of adenosine receptors (A_1, A_{2A}, and A_3 ARs).[1–3] Using a methanocarba modification originally introduced by Marquez and co-workers,[4] the ribose-like moiety of these derivatives was constrained to either a northern [(N), 2′-*exo*] or southern [(S), 2′-*endo*] conformation through fused cyclopropane and cyclopentane rings. Such analogues helped to define the role of ribofuranosyl puckering in stabilizing the active AR-bound conformation, and thereby allowed identification of the (N)-methanocarba conformation as the isomer which preserved affinity at the A_3 and to a lesser extent, A_1 AR. Thus, the (N)-methanocarba analogues, **1** and **2**, were highly potent as full agonist (**2**)[3] or partial agonist (**1**) at the human A_3 AR.[2] These analogues contained substituent groups known to enhance A_3 affinity in the ribose series [i.e., N^6-(3-iodobenzyl) and 2-chloro]. The affinity of adenosine methanocarba analogues at the A_{2A} AR was greatly decreased for the (N)-methanocarba isomer and nearly absent for the (S)-methanocarba isomer.

In this study, we combined the (N)-methanocarba modification with known purine receptor ligands containing a 6-oxo-substitution. Among the nucleoside analogues synthesized were derivatives of hypoxanthine, guanosine, and xanthine. For inosine[5,6] and xanthine-7-riboside[7] analogues, a 5′-methyluronamide modification (nucleoside numbering) known to enhance the A_3 AR affinity was included.[3,5] In comparison, xanthine-7-riboside derivatives **3** and **4** bound to and activated the rat A_3 AR with partial or full efficacy, respectively,[7,8] and were used as reference compounds. In other analogues, phosphate groups were introduced at the 5′-position with the aim of targeting P2 receptors,[9] G proteins (in the case of a guanosine 5′-triphosphate analogue)[10] or enzymes involved in purine metabolism (such as IMP dehydrogenase).[11]



1, R = CH_2OH MRS 1760

2, R = $CONHCH_3$ MRS 1898



3, R = CH_2OH DBXR

4, R = $CONHCH_3$ DBXRM

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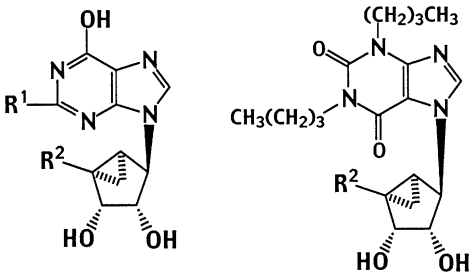
Table 1. Affinities of (N)-methanocarba analogues of 6-oxpurine ribosides in radioligand binding assays at rat A_1,[a] rat A_{2A},[b] and human A_3 receptors,[c] unless noted, and in other biochemical assay systems[d]



5-9 **10-12**

Compound	R^1	R^2	K_i (μM) or % displacement at 100 μM		
			rA_1[a]	rA_{2A}[b]	hA_3[c]
5 MRS 1957	H	CH_2OH	<10%	<10%	30±10%
6 MRS 1997	H	$CONHCH_3$	<10%	<10%	28.8±5.2
7a MRS 2397[d]	H	CH_2O–phosphate			11±1%
7b MRS 2398[d]	H	CH_2O–phosphate]$_2$ dimer			21.6±0.4
8 MRS 1941	NH_2	CH_2OH	<10%	<10%	20.5±2.1
9 MRS 2351[d]	NH_2	CH_2O–triphosphate			<10%
10 MRS 1971	—	CH_2OH	2.97±0.15	<10%	4.35±0.40
11 MRS 1998	—	$CONHCH_3$	24.4±4.8	<10%	1.86±0.23
12 MRS 2396	—	CH_2O–triphosphate			<10%

[a]Displacement of specific [^{3}H]*R*-PIA binding to A_1 receptors in rat brain membranes, expressed as K_i±SEM or % (n=3–5).
[b]Displacement of specific [^{3}H]CGS 21680 binding to A_{2A} receptors in rat striatal membranes, expressed as K_i±SEM or % (n=3–6).
[c]Displacement of specific [^{125}I]AB-MECA binding at human A_3 receptors expressed in HEK-293 cells, in membranes, expressed as K_i±SEM or % (n=3–5).
[d]Selected compounds were examined in HL-60 cells for effects on calcium mobilization. Compounds **7a** and **7b** (100 μM) were inactive in raising intracellular calcium, while compound **9** (100 μM) caused a rise in intracellular calcium equivalent to 53±18% of the effect of 1 μM UTP (n=3).

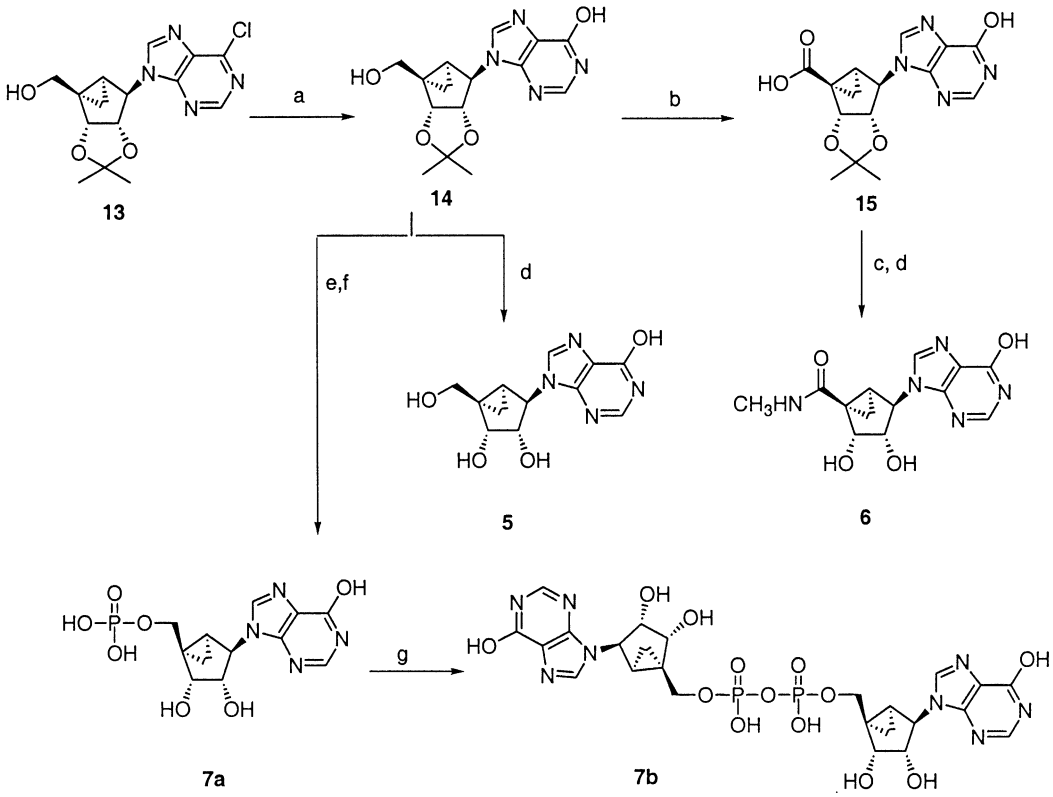
The structures of (N)-methanocarba purine analogues (**5**–**12**) synthesized for testing in binding and other biochemical assays are shown in Table 1. We introduced the (N)-methanocarba modification of known potent adenosine agonists having equivalents of either 5′-hydroxymethyl (**5**, **8**, and **10**) or 5′-uronamide groups (**6** and **11**). These 6-oxopurine bases were coupled at either the 7- (**10**–**12**) or 9-position (**5**–**9**) to the pseudoribose moiety, according to well-characterized alkylation patterns of purine bases.[7,12] A 5′-monophosphate derivative, **7a**, and several 5′-triphosphate derivatives were included (**9** and **12**).

The synthetic methods used to prepare the new analogues are outlined in Schemes 1–3.[13–15] 6-Monosubstituted derivatives were prepared from the 6-chloropurine derivative[3] as a common intermediate. Refluxing **13** in the presence of 2-mercaptoethanol and NaOMe in MeOH gave the protected inosine derivative, **14**, which was converted into triol **5** in acidic conditions or to the 5′-uronamide, **6**, via a 5′-carboxylic acid, **15**. Treatment of **14** with $RuCl_3$, $NaIO_4$, followed by C_{18} column chromatography gave **15**, which condensed with $MeNH_2$ in the presence of BOP-Cl [*N,N*-bis(2-oxo-3-oxazolidinyl)-phosphinic chloride] to yield **6**. Use of $RuCl_3$ instead of RuO_2 produced a cleaner reaction. The 5′-monophosphate derivative **7a** was prepared by reacting **14** with di-*t*-butyl-*N,N*-diethylphosphoramidite in the presence of tetrazole and then with mCPBA at −78 °C, followed by the treatment with Dowex resin. Attempted synthesis of the corresponding triphosphate by treating with 1,1′-carbonyldiimidazole followed by tetrabutylammonium pyrophosphate failed, and instead a dimer, **7b**, was obtained.

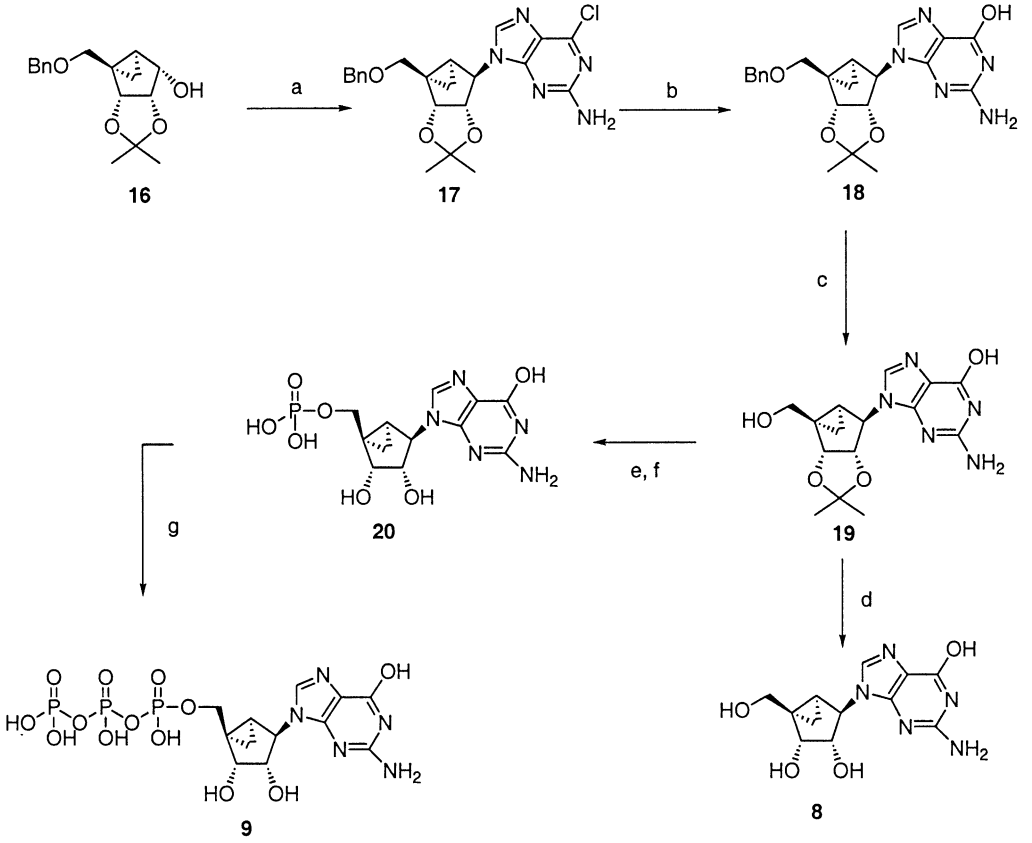
Guanosine derivative **8** was prepared by a Mitsunobu reaction of **16** and 2-amino-6-chloropurine to furnish **17**, which was heated with 2 N NaOH in dioxane at 100 °C to yield the guanosine derivative, **18**. Debenzylation at the 5′ position using Pd to give compound **19**, followed by TFA deprotection provided the guanosine derivative, **8**. Compound **19** was converted into monophosphate **20** by the phosphoramidite method. This monophosphate was then converted to phosphorimidazolidate by treatment with 1,1′-carbonyldiimidazole followed by 5% Et_3N/MeOH, and the phosphorimidazolidate reacted with tetrabutylammonium pyrophosphate to provide the triphosphate, **9b**.

Xanthine derivatives were prepared using a Mitsunobu coupling of **16** and 1,3-dibutylxanthine to furnish **21**, which was deprotected with BCl_3 to yield the xanthine methanocarba nucleoside, **10**. Compound **21** was debenzylated to furnish **22**, which was converted to the monophosphate **25** and to the triphosphate **12** by the above phosphorylation procedures. Compound **22** was oxidized using RuO_2, $NaIO_4$, K_2CO_3 to furnish the acid **23**, which was condensed with $MeNH_2$ using BOP-Cl to yield intermediate **24** and finally the deprotected uronamide, **11**.

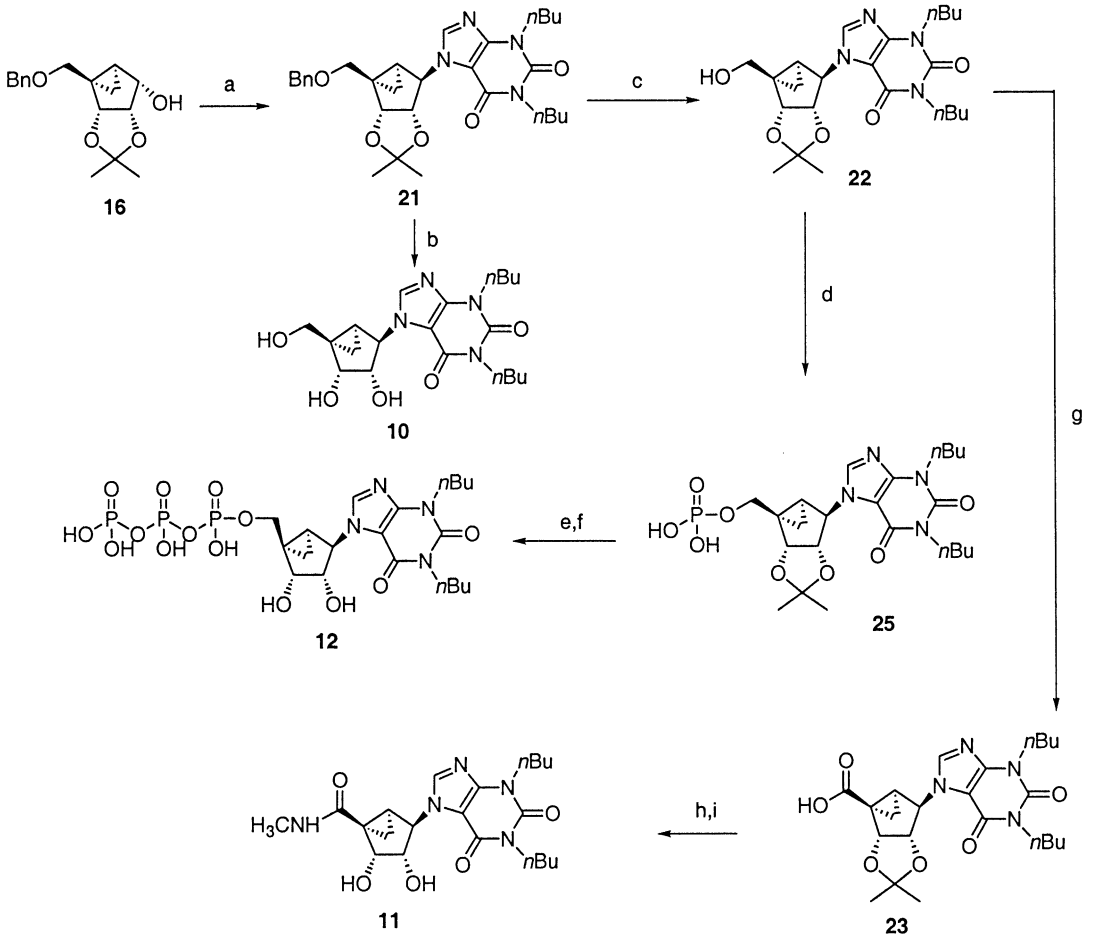
The nucleoside analogues were evaluated in binding at three subtypes of ARs. While inosine itself displayed a measurable affinity at the A_3 AR and was even suggested



Scheme 1. (a) $HSCH_2CH_2OH$, NaOMe, MeOH, reflux; (b) $RuCl_3$, $NaIO_4$, $CH_3CN/CHCl_3/H_2O = 2:2:3$; (c) BOP-Cl, Et_3N, $MeNH_2$ (2 M/THF), $CH_2Cl_2/DMF = 1:1$; (d) CF_3CO_2H, wet MeOH, 60 °C; (e) (i) 1*H*-tetrazole, di-*t*-butyl *N*,*N*-diethylphosphoramidite, THF; (ii) mCPBA, CH_2Cl_2; (f) DOWEX 50×8–200, $MeOH/H_2O = 2:1$; (g) (i) 1,1′-carbonyldiimidazole, DMF; (ii) 5% Et_3N/MeOH; (iii) tetrabutylammonium pyrophosphate.



Scheme 2. (a) 2-Amino-6-chloropurine, DIAD, Ph_3P, THF; (b) 2 N NaOH, dioxane; (c) Pd black, 10% HCO_2H in MeOH; (d) TFA/$MeOH/H_2O$; (e) (i) tetrazole, di-*t*-butyl-*N*,*N*-diethylphosphoramidite, THF; (ii) mCPBA, CH_2Cl_2; (f) TFA/$MeOH/H_2O$ (g) (i) 1,1′-carbonyldiimidazole, DMF; (ii) 5% Et_3N/MeOH; (iii) tetrabutylammonium pyrophosphate.



Scheme 3. (a) 1,3-Dibutylxanthine, DEAD, Ph_3P, THF; (b) BCl_3, CH_2Cl_2; (c) Pd black, 10% HCO_2H in MeOH; (d) (i) tetrazole, di-*t*-butyl-*N*,*N*-diethylphosphoramidite, THF; (ii) mCPBA, CH_2Cl_2; (e) DOWEX 50×8–200, MeOH/H_2O = 1:1; (f) (i) 1,1′-carbonyldiimidazole, DMF; (ii) 5% Et_3N/MeOH; (iii) tetrabutylammonium pyrophosphate; (g) RuO_2, $NaIO_4$, K_2CO_3, $H_2O/CH_3CN/CHCl_3$; (h) CH_3NH_2·HCl, Et_3N, BOP-Cl; (i) BCl_3/CH_2Cl_2.

to be an endogenous ligand for this AR,[6] the corresponding (N)-methanocarba analogue, **5**, bound weakly. An effort to enhance the affinity in the inosine series through the introduction of 5′-methyluronamide group slightly increased the affinity of **6** (K_i 29 μM). The inosine-5′-monophosphate analogue, **7a**, was inactive at the adenosine A_3 AR. Curiously, the dimer **7b** displaced binding at the A_3 AR with a K_i value of 22 μM. Thus, this was an example of dimerization of an inactive ligand to increase the affinity, perhaps through cross-linking two sites on a receptor or by bridging two proximal receptor molecules. It is unusual for such a highly anionic molecule to bind to an AR, which may be the result of an enhancing effect of the (N)-methanocarba ring on A_3 affinity.[2] It will also be interesting to evaluate **7b** at P2X receptors, at which diinosine polyphosphates are potent antagonists.[16]

A receptor for guanosine was proposed,[17] based on neuroprotective effects observed for this nucleoside. However, the guanosine analogues here were examined only for interaction with ARs. The (N)-methanocarba analogue, **8**, bound weakly, but selectively to the A_3 AR with a K_i value of 21 μM. The corresponding triphosphate, **9**, of interest for its potential interaction with G proteins, was inactive in binding to the A_3 AR.

The (N)-methanocarba analogue of 1,3-dibutylxanthine-7-riboside, **10**, was equipotent at A_1 and A_3 ARs and inactive at the A_{2A} AR. If proven to be an agonist at both AR subtypes, this profile of selectivity would be desirable for a cardioprotective agent.[18] Introduction of the 5′-*N*-methyluronamide group, in **11**, decreased A_1 affinity by 8-fold and increased A_1 affinity by 2-fold. A comparison of compounds **3** and **4**[7] with the corresponding (N)-methanocarba analogues, **10** and **11**, respectively, showed that rat A_1 affinities were the same, and rat A_3 affinities of the ribosides were roughly similar to K_i values at the human A_3 AR found in this study. The xanthine-riboside-5′-triphosphate analogue, **12**, was inactive at the A_3 AR.

The phosphate-containing analogues were tested for effects on intracellular calcium concentration in HL-60 leukemia cells, known to express several P2 receptor subtypes.[19] Thus, a P2 receptor agonist would be expected to cause a rapid rise in intracellular calcium. Compounds **7a** and **7b** were essentially inactive at a concentration of 100 μM, either at stimulating calcium accumulation or inhibiting the rise in calcium produced by 1 μM UTP (acting at $P2Y_2$ receptors). Compound **9** (100 μM) stimulated a rise in intracellular calcium equivalent to 53% of the rise produced

by 1 μM UTP, suggesting that it may activate a P2Y receptor.

In conclusion, we have identified non-adenosine analogues containing the (N)-methanocarba modification which bound weakly, and in some cases selectively, at the human A_3 AR. Since selectivity has been achieved in these simple derivatives, this could provide the basis for further development of more potent ligands. The phosphate analogues must also be evaluated at a range of enzymatic and receptor activities. The full evaluation of the biological activity of the new analogues is in progress.

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13. Procedures in Scheme 1. A solution of **13** (40 mg, 0.119 mmol), 2-mercaptoethanol (0.03 mL, 0.475 mmol), 0.5 N NaOMe/MeOH (0.95 mL, 0.475 mmol) in MeOH (1 mL) was heated at 90 °C for 3 h and the reaction mixture was neutralized by glacial AcOH. The resulting mixture was concentrated and purified on preparative TLC ($CHCl_3$/MeOH = 6:1) to provide the hypoxanthine derivative **14** (37 mg, 98%). ^{1}H NMR (CD_3OD) δ 8.06 (s, 1H), 7.96 (s, 1H), 5.34 (d, J = 7.1 Hz, 1H), 5.04 (s, 1H), 4.69 (d, J = 6.9 Hz, 1H), 3.97 (d, J = 11.5 Hz, 1H), 3.52 (d, J = 11.5 Hz, 1H), 1.72 (m, 1H), 1.51 (s, 3H), 1.24 (s, 3H), 1.16 (m, 1H), 0.97 (m, 1H). FAB m/z 319 (MH^+). **15**: A mixture of **14** (37 mg, 0.116 mmol), $RuCl_3$ (10 mg, 0.048 mmol), $NaIO_4$ (181 mg, 0.847 mmol) in $CH_3CN/CHCl_3/H_2O$ = 2:2:3 was vigorously stirred for 6 h at rt. Solvents were removed and the residue was subjected to reverse phase C_{18} column chromatography using H_2O/MeOH (30:1–7:1) as eluents to afford the acid **15** (20.6 mg, 53.4%). ^{1}H NMR (D_2O) δ 8.15 (s, 1H), 8.11 (s, 1H), 5.74 (d, J = 7.4 Hz, 1H), 5.03 (s, 1H), 4.87 (d, J = 7.1 Hz, 1H), 1.22 (m, 1H), 1.61 (m), 1.55 (s, 3H), 1.34 (m, 1H), 1.28 (s, 3H). FAB m/z 333 (MH^+). **5** (1*R*,2*R*,3*S*,4*R*,5*S*)-4-[6-oxo-9*H*-purin-9-yl]-1-(hydroxymethyl)bicyclo-[3.1.0]hexane-2,3-diol): ^{1}H NMR (CD_3OD) δ 8.51 (s, 1H), 8.06 (s, 1H), 7.91 (s, 1H), 4.82 (s, 1H), 4.76 (d, J = 6.6 Hz, 1H), 4.24 (d, J = 11.8 Hz, 1H), 4.88 (d, J = 6.8 Hz, 1H), 1.60 (m, 1H), 1.53 (m, 1H), 0.74 (m, 1H). FAB m/z 279 (MH^+). HR-MS (FAB) calcd 279.1093, found 279.1084. Acetonide intermediate of **6**: BOP-Cl (26.5 mg, 0.104 mmol) was added to a solution of **15** (11.5 mg, 0.035 mmol) and Et_3N (0.015 mL, 0.104 mmol) in CH_2Cl_2/DMF (2 mL). To the suspension 2.0 M $MeNH_2$/THF (0.03 mL, 0.06 mmol) was added and the resulting mixture was stirred for 24 h at rt. Water was added and the solvent was removed. The residue was purified on silica gel ($CHCl_3$/MeOH = 6:1) to give the protected amide (8.1 mg, 68.6%). ^{1}H NMR (CD_3OD) δ 8.11 (s, 1H), 8.01 (s, 1H), 5.74 (d, J = 6.8 Hz, 1H), 5.03 (s, 1H), 2.79 (s, 3H), 2.17 (m, 1H), 1.55 (m, 1H), 1.52 (s, 3H), 1.42 (m, 1H), 1.27 (s, 3H). FAB m/z 346 (MH^+). **6**: ^{1}H NMR (D_2O) δ 8.18 (s, 1H), 8.10 (s, 1H), 5.08 (d, J = 6.3 Hz, 1H), 4.96 (s, 1H), 4.17 (d, J = 6.6 Hz, 1H), 2.85 (s, 3H), 2.26 (m, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.51 (m, 1H), 1.51 (m, 1H). FAB m/z 306 (MH^+). HR-MS (FAB) calcd 306.1202, found 306.1211. **7a** *t*-butyl intermediate. ^{1}H NMR (CD_3OD) δ 8.45 (s, 1H), 8.20 (s, 1H), 4.92 (s, 1H), 4.44 (dd, J = 5.2, 12 Hz, 1H), 4.04 (d, J = 6.3, 1H, 2H), 4.70 (dd, J = 5.7, 11.1 Hz, 1H), 1.86 (m, 1H), 1.49 (m, 1H), 0.97 (t, J = 6.7 Hz, 1H), 1.34–1.25 (m) 1.09 (m, 1H). FAB m/z 510 (MH^+). **7a**: ^{1}H NMR (D_2O) δ 8.18 (s, 1H), 8.10 (s, 1H), 5.08 (d, J = 6.3 Hz, 1H), 4.96 (s, 1H), 4.17 (d, J = 6.6 Hz, 1H), 2.85 (s, 3H), 2.26 (m, 1H), 1.80 (t, J = 5.2 Hz, 1H), 1.51 (m, 1H), 1.51 (m, 1H). ^{31}P NMR (D_2O) δ 0.51. FAB m/z 357 ($(M-1)^-$). HR-MS (FAB^-) calcd 357.0600, found 357.0607. **7b**: ^{1}H NMR (D_2O) δ 8.17 (s, 1H), 8.05 (s, 1H), 4.60 (d, J = 10.7 Hz, 1H), 3.93 (d, J = 6.6 Hz, 1H), 3.80 (d, J = 11.0, 1H), 1.88 (s, 1H), 1.53 (t, J = 4.7 Hz, 1H), 0.97 (t, J = 7.1 Hz, 1H). ^{31}P NMR (D_2O) δ −10.96. FAB m/z 697 ($(M-1)^-$). HR-MS (FAB^-) calcd 697.1173, found 697.1166.
14. Procedures in Scheme 2. To a mixture of **16** (0.2 g, 0.69 mmol), 2-amino-6-chloropurine (0.234 g, 1.38 mmol) and triphenylphosphine (0.361 g, 1.38 mmol) was added DIAD (diisopropyl azodicarboxylate, 0.27 mL, 1.38 mmol) dropwise at 0 °C. The mixture was warmed up to rt and stirred for 6 h. The solvent was evaporated under vacuum and the residue was purified by flash chromatography using 6:4 petroleum ether/ethyl acetate to furnish **17** as a solid (0.2 g, 67%). ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.36 (s, 5H), 5.29 (d, J = 7.15 Hz, 1H), 5.18 (bs, 2H), 4.96 (s, 1H), 4.69–4.53 (ABq, 2H), 4.48 (d, J = 7.15 Hz, 1H), 3.92 (d, J = 10.16 Hz, 1H), 3.28 (d, J = 10.16 Hz, 1H), 1.65–1.60 (m, 1H), 1.55 (s, 3H), 1.28–1.20 (m, 1H), 1.24 (s, 3H), 0.96–0.88 (m, 1H). **18**: To a solution of **17** (0.1 g, 0.23 mmol) in dioxane (2 mL) was added 2 N NaOH (8 mL) and heated at 90 °C for 3 h. The mixture was concentrated to dryness under vacuum and purified by flash chromatography using 10% MeOH in $CHCl_3$ to furnish **18** as a solid (0.08 g, 82%). ^{1}H NMR ($CDCl_3$) δ 7.96 (s, 1H), 7.37 (s, 5H), 6.29 (bs, 2H), 5.27 (d, J = 6.87 Hz, 1H), 4.89 (s, 1H), 4.67–4.50 (m, 3H), 3.92 (d, J = 10.16 Hz, 1H), 3.32 (d, J = 10.16 Hz, 1H), 1.70–1.60 (m, 1H), 1.55 (s, 3H), 1.25 (s, 4H), 0.96–0.86 (m, 1H). **19**: To a solution of **18** (0.06 g, 0.14 mmol) in 5% HCOOH/MeOH (5 mL) was added Pd black (0.06 g) and stirred at rt for 8 h. The reaction mixture was filtered through Celite and concentrated and purified by flash chromatography using 10% MeOH/$CHCl_3$ to furnish **19** as a solid (0.04 g, 86%). ^{1}H NMR (D_2O) δ 789 (s, 1H), 7.83 (s, 1H), 5.28 (d, J = 6.87 Hz, 1H), 4.77 (s, 1H), 4.59 (d, J = 6.87 Hz, 1H), 3.90 (d, J = 10.54 Hz, 1H), 3.44 (d, J = 10.54 Hz, 1H), 1.64–1.54 (m, 1H), 1.43 (s, 3H), 1.18 (s, 3H), 1.10–1.00 (m, 1H), 0.94–0.82 (m, 1H). **8**: Substrate **19** (0.02 g, 0.06 mmol) was dissolved in 5% TFA/MeOH (3 mL) and H_2O (1 mL) and stirred at rt for 6 h. The reaction mixture was concentrated to

dryness under vacuum and purified by preparative TLC using 15% MeOH/$CHCl_3$ to furnish **8** (0.012 g, 68%). ^{1}H NMR (D_2O) δ 9.20 (s, 1H), 4.69 (d, J = 6.32 Hz, 1H), 4.25 (d, J = 11.54 Hz, 1H), 3.92 (d, J = 6.32 Hz, 1H), 3.23 (d, J = 11.54 Hz, 1H), 1.66–1.48 (m, 2H), 0.82–0.70 (m, 1H). **20**: To a solution of **19** (0.02 g, 0.06 mmol) in anhydrous THF (2 mL) was added tetrazole (0.013 g, 0.18 mmol) and di-*t*-butyl-*N*-*N*-diethylphosphoramidite (0.025 mL, 0.09 mmol) and stirred at rt for 12 h. The reaction mixture was cooled to −78 °C and was added a solution of mCPBA (0.018 g) and warmed to rt. Methanol (2 mL) was added to the reaction mixture and concentrated under vacuum and purified by preparative TLC using 10% MeOH/$CHCl_3$ to furnish 0.022 g product. This compound (0.02 g, 0.038 mmol) was dissolved in 5% TFA/MeOH (3 mL) and H_2O (1 mL) and stirred at rt for 6 h. The reaction mixture was concentrated to dryness under vacuum to furnish 0.013 g of **20**, which was used directly for the further reaction. ^{1}H NMR (D_2O) δ 8.15 (s, 1H), 4.71 (s, 1H), 4.43 (dd, J = 4.94, 11.26 Hz, 1H), 4.02 (d, J = 6.59 Hz, 1H), 3.70 (dd, J = 4.94, 11.26 Hz, 1H), 1.88–1.78 (m, 1H), 1.54–1.42 (m, 1H), 1.12–0.92 (m, 1H). ^{31}P NMR (D_2O) δ 0.87. **9**: To a suspension of monophosphate (0.008 g, 0.02 mmol) in DMF (1 mL) was added 1,1′-carbonyldiimidazole (0.016 g, 0.1 mmol) and stirred for 12 h. A reaction mixture was treated with a solution of 5% Et_3N/MeOH and stirred for 4 h. This mixture was concentrated to dryness under vacuum and were added tributylammonium pyrophosphate (0.055 g, 0.012 mmol) and DMF (1 mL) and stirred for 3 days. Triethylammonium bicarbonate buffer (3 mL) was added to the reaction mixture followed by water (2 mL) and lyophilized. The crude material obtained was purified on a Sephadex column using 0.5 M ammonium bicarbonate and water to furnish 3.5 mg of **9**. ^{1}H NMR (D_2O) δ 8.12 (s, 1H), 4.69 (s, 1H), 4.54–4.44 (m, 1H), 4.07 (d, J = 6.32 Hz, 1H), 3.92–3.82 (m, 1H), 1.84–1.78 (m, 1H), 1.46–1.38 (m, 1H), 1.02–0.94 (m, 1H). ^{31}P NMR (D_2O) δ −8.29, −10.69, −22.26.

15. Procedures in Scheme 3: To a mixture of **16** (0.1 g, 0.34 mmol), 1,3-dibutylxanthine (0.18 g, 0.68 mmol) and triphenylphosphine (0.178 g, 0.68 mmol) was added DEAD (diethyl azodicarboxylate, 0.1 mL, 0.68 mmol) dropwise at 0 °C. The mixture was warmed up to rt and stirred for 6 h. The solvent was evaporated under vacuum and the residue was purified by flash chromatography using 4:1 petroleum ether/ethyl acetate to furnish **21** (0.16 g, 88%). ^{1}H NMR ($CDCl_3$) δ 8.25 (s, 1H), 7.40–7.28 (m, 5H), 5.55 (s, 1H), 5.21 (d, J = 7.14 Hz, 1H), 4.64 (d, J = 12.4 Hz, 1H), 4.58 (d, J = 12.4 Hz, 1H), 4.49 (d, J = 7.14 Hz, 1H), 4.16–3.84 (m, 5H), 3.16 (d, J = 9.89 Hz, 1H), 1.82–1.54 (m, 4H), 1.52 (s, 3H), 1.48–1.28 (m, 6H), 1.23 (s, 3H), 1.02–0.84 (m, 7H). **10**: To a solution of **21** (0.025 g, 0.047 mmol) in anhydrous CH_2Cl_2 (1 mL) was added 1 M BCl_3/CH_2Cl_2 (0.23 mL, 0.23 mmol) at 0 °C and the mixture warmed to rt and stirred for 15 min. MeOH (1 mL) was added to the reaction mixture and concentrated under vacuum. **10** was purified by preparative TLC using 10% MeOH/$CHCl_3$ (12 mg, 63%). ^{1}H NMR (CD_3OD) δ 8.40 (s, 1H), 5.09 (s, 1H), 4.55 (d, J = 6.32 Hz, 1H), 4.17 (d, J = 11.54 Hz, 1H), 4.06–3.82 (m, 5H), 3.18 (d, J = 11.54 Hz, 1H), 1.70–1.40 (m, 5H), 1.40–1.18 (m, 5H), 0.88 (t, J = 7.14 Hz, 6H), 0.72–0.62 (m, 1H). **22**: To a solution of **21** (0.07 g, 0.13 mmol) in 5% HCOOH/MeOH (10 mL) was added Pd black (0.07 g) and stirred at rt for 8 h. The reaction mixture was filtered through Celite and concentrated and purified by flash chromatography using 5% MeOH/$CHCl_3$ to furnish **22** as a solid (0.05 g, 86%). ^{1}H NMR ($CDCl_3$) δ 7.88 (s, 1H), 5.46 (d, J = 7.14 Hz, 1H), 4.96 (s, 1H), 4.78 (d, J = 7.14 Hz 1H), 4.25–3.99 (m, 5H), 3.26 (d, J = 11.54 Hz, 1H), 1.77–1.58 m, 5H), 1.53 (s, 3H), 1.44–1.30 (m, 5H), 1.27 (s, 3H), 1.10–0.89 (m, 7H). **25**: To a solution of **22** (0.026 g, 0.058 mmol) in anhydrous THF (2 mL) was added tetrazole (0.012 g, 0.18 mmol) and di-*t*-butyl-*N*,*N*-diethylphosphoramidite (0.024 mL, 0.09 mmol) and stirred at rt for 12 h. The reaction mixture was cooled to −78 °C and was added a solution of mCPBA (0.017 g) and warmed to rt. Methanol (2 mL) was added to the reaction mixture and concentrated under vacuum and purified by preparative TLC using 10% MeOH/$CHCl_3$ to furnish 0.022 g product **25**. ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 5.48 (s, 1H), 5.20 (d, J = 6.87 Hz, 1H), 4.54 (d, J = 6.87 Hz, 1H), 4.31–3.96 (m, 6H), 1.82–1.23 (m, 34H), 1.10–0.89 (m, 7H). Compound **25** was treated with DOWEX in MeOH/H_2O at 90 °C for 2 h and filtered and concentrated to furnish 0.011 g of monophosphate. To a suspension of this monophosphate (0.011 g, 0.023 mmol) in DMF (1 mL) was added 1,1′-carbonyldiimidazole (0.019 g, 0.12 mmol) and stirred for 12 h. The reaction mixture was treated with a solution of 5% Et_3N/MeOH and stirred for 4 h. This mixture was concentrated to dryness under vacuum, treated with tributylammonium pyrophosphate (0.063 g, 0.014 mmol) and DMF (1 mL) and stirred for 3 days. Triethylammonium bicarbonate buffer (3 mL) was added to the reaction mixture followed by water (2 mL). After lyophilization, the crude material obtained was purified on a Sephadex colum using 0.5 M ammonium bicarbonate and water to furnish 3.5 mg of **12**. ^{1}H NMR (D_2O) δ 8.20 (s, 1H), 5.18 (s, 1H), 4.80–4.73 (m, 2H), 4.09–3.77 (m, 6H), 1.80–1.22 (m, 10H), 1.04–0.84 (m, 7H). **23**: A mixture of **22** (0.03 g, 0.067 mmol), RuO_2 (0.18 g, 1.34 mmol), $NaIO_4$ (0.286 g, 1.34 mmol), K_2CO_3 (0.184 g, 1.34 mmol) in CH_3CN/$CHCl_3$/H_2O = 2:2:3 (10 mL) was stirred at rt vigorously for 20 h. Reaction mixture was filtered through Celite. Organic layer was separated and the aqueous layer was acidified with HCl and extracted with EtOAc, dried and concentrated under vacuum to furnish 0.012 g product. ^{1}H NMR ($CDCl_3$) δ 7.64 (s, 1H), 5.69 (d, J = 7.14 Hz, 1H), 5.30 (s, 1H), 4.74 (d, J = 7.14 Hz, 1H), 4.09 (t, J = 7.42 Hz, 2H), 4.00 (t, J = 7.42 Hz, 2H), 1.85–1.59 (m, 6H), 1.55 (s, 3H), 1.44–1.33 (m, 4H), 1.28 (s, 3H), 1.02–0.86 (m, 7H). **11**: A mixture of **23** (0.01 g, 0.022 mmol), BOP-Cl (0.011 g, 0.044 mmol), CH_3NH_2·HCl (0.006 g, 0.088 mmol), Et_3N (0.012 mL, 0.088 mmol) in THF (2 mL) was stirred at rt for 8 h. Solvent was removed under vacuum and the crude material was purified by preparative TLC using 10% MeOH/$CHCl_3$. The pure material was treated with 1 M BCl_3/CH_2Cl_2 (0.1 mL, 0.1 mmol) at 0 °C for 10 min. Methanol was added to the reaction mixture and the solvent was evaporated and the crude material was purified by preparative TLC using 15% MeOH/$CHCl_3$ to furnish 0.002 g product **11**. ^{1}H NMR ($CDCl_3$) δ 8.29 (s, 1H), 5.14 (s, 1H), 4.87 (s, 1H), 4.72 (s, 1H), 4.18–3.94 (m, 4H), 3.67 (s, 3H), 1.84–1.22 (m, 10H), 1.00–0.82 (m, 7H).

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