Bioorganic & Medicinal Chemistry 17 (2009) 5347–5352

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Novel L-adenosine analogs as cardioprotective agents

Harinath Kasiganesan [a], Gary L. Wright [a], Maria Assunta Chiacchio [b], Giuseppe Gumina [c,*]

[a] Department of Pharmaceutical Sciences, College of Pharmacy, Medical University of South Carolina, 280 Calhoun Street, PO Box 250140, Charleston, SC 29425, USA
[b] Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy
[c] Department of Pharmaceutical Sciences, South University School of Pharmacy, 709 Mall Boulevard, Savannah, GA 31406, USA

ARTICLE INFO

Article history:
Received 20 October 2008
Revised 2 December 2008
Accepted 7 December 2008
Available online 14 December 2008

Keywords:
L-Nucleoside
Adenosine
Cardioprotection

ABSTRACT

Two L-nucleosides, L-3′-amino-3′-deoxy-N^6-dimethyladenosine (L-3′-ADMdA) **1**, previously synthesized in our laboratory, and the novel L-3′-amino-3′-deoxy-N^6-methyladenosine-5′-*N*-methyluronamide (L-3′-AM-MECA) **2** were evaluated in an ischemia/reperfusion model on Langendorff perfused mouse heart. L-3′-ADMdA **1** was found to enhance functional recovery from ischemia (32.2 ± 3.7 cm H_2O/s % rate pressure product, compared to 21.3 ± 1.4 for the control and 30.7 ± 3.4 for adenosine) and increase the time to onset of ischemic contracture (14.5 ± 0.9 min, compared to 10.5 ± 1.0 min for the control and 13.6 ± 0.6 min for adenosine) comparable to adenosine. Consistent with the functional recovery data, decreased infarction area was seen in the case of **1** (19.1 ± 8.4, compared to 40.5 ± 7.2% for the control and 11.5 ± 2.1% for adenosine). In contrast, L-3′-AM-MECA **2** did not show significant functional recovery, increased onset of contracture, nor decreased infarction area compared to control. Unlike adenosine, neither **1** nor **2** induced cardiac standstill in mouse heart.

1. Introduction

Adenosine is an important mediator of numerous biological functions both in the nervous system and peripheral tissues. It exerts its action by interacting with at least four different receptor (AR) subtypes (A_1, A_{2a}, A_{2b} and A_3).[1] Several studies on the action of adenosine in different tissues showed the potential benefits of AR ligands (agonists or antagonists) for the treatment of several diseases. Thus, cardioprotective action has been demonstrated for A_1 AR,[2] A_{2A} AR[3] and A_3 AR[2b,4,5] agonists, whereas A_1 AR[6] and A_{2a} AR[7] antagonists have shown potential anti-Alzheimer properties. A_{2a} AR antagonists are actively studied as anti-Parkinson agents,[8] and have also been found protective against free radical neuronal damage.[9] A_3 AR antagonists are under consideration as agents for the treatment of glaucoma.[4,10] A selective A_1 AR agonist showed neuroprotective effect in a rat model of Huntington's disease.[11] Finally, recent studies indicate that A_3 AR may be targets for cancer therapy and chemoprotection.[12] Such a range of potential therapeutical applications and the need of fully understanding the pharmacological properties of each AR subtype have prompted numerous efforts to discover more potent and selective ligands to each receptor subtype. Among the structural modifications reported in literature, L-nucleosides have been rarely considered, probably following early reports of little or no interactions of the L-enantiomers of adenosine, 2-chloroadenosine, 2-azidoadenosine and *N*-ethylcarboxamidoadenosine (NECA) with A_2 AR in different animal tissues.[13] L-Adenosine, a plant hormone is also inactive on animal enzymes such as *S*-adenosyl-L-homocysteine hydrolase,[14] and does not interact with mammalian nucleoside transporters.[15] In the past, however, favorable features of L-nucleosides, such as low cellular toxicity[16] and high metabolic stability,[16,17] have been exploited in the design of successful antiviral and promising anticancer agents. The favorable features of L-nucleosides and the potential cardioprotective activity of adenosine analogs prompted us to evaluate L-3′-amino-3′-deoxy-N^6-dimethyladenosine (L-3′-ADMdA) **1** (Fig. 1), recently synthesized in our laboratory,[18] in Langendorff perfused hearts as well as in a metabolic essay on a myoblast cell line responsive to A_1/A_3 stimulation. At the same time, in order to evaluate the effect of the favorable 5′-carboxamide replacement, we prepared and evaluated the novel analog L-3′-amino-3′-deoxy-N^6-methyladenosine-5′-*N*-methyluronamide (L-3′-AM-MECA) **2**, enantiomer of compound **3**, a simplified and A_3 AR-selective analog of the A_1/A_3 agonist IB-MECA (Fig. 1).

2. Results and discussion

2.1. Chemistry

L-3′-ADMdA **1** was synthesized from L-xylose in 11 steps as previously reported.[18] L-3′-AM-MECA **2** was prepared from the common intermediate **4** (Scheme 1), prepared in seven steps from L-xylose as previously described.[18] Protected L-3-amino-3-deoxyribose **4** was protected as the *tert*-butyldiphenylsilyl ether **5**, which was sub-

* Corresponding author.
E-mail address: gumina@musc.edu (G. Gumina).

0968-0896/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2008.12.011

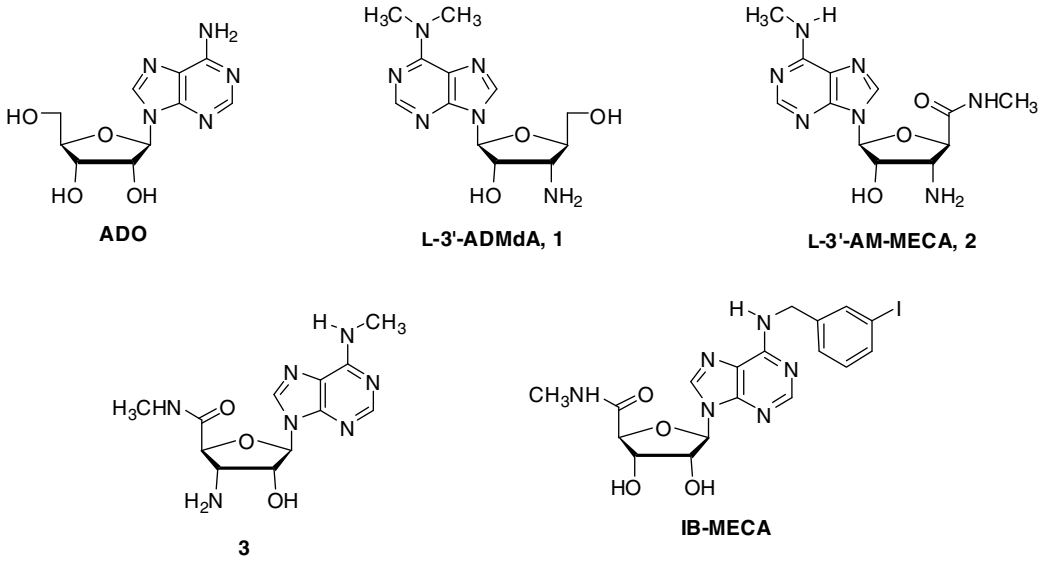
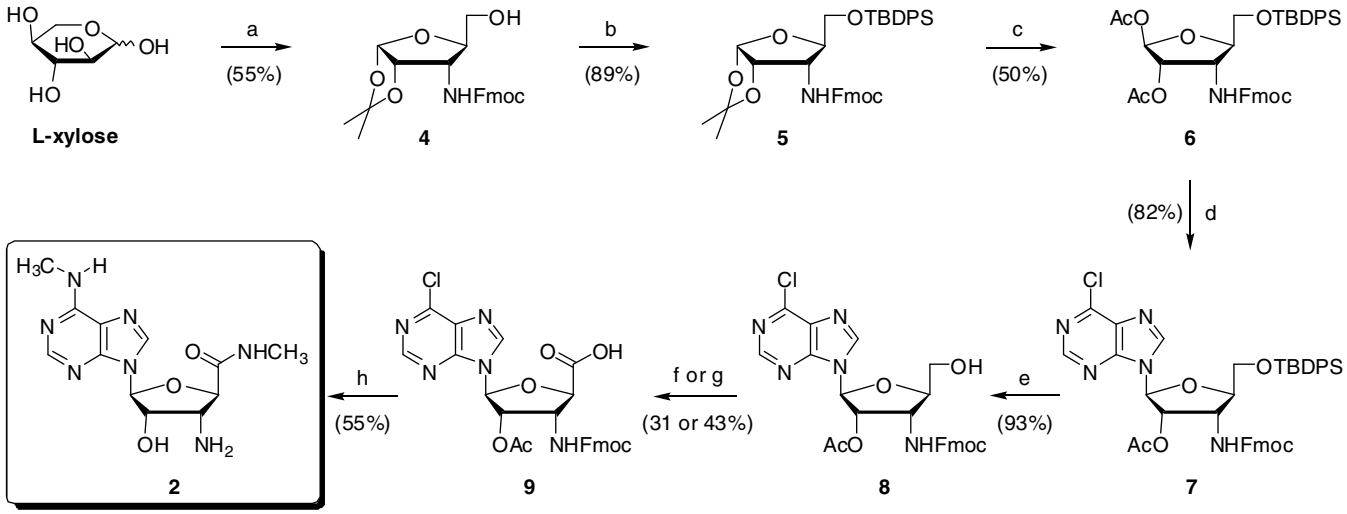


Figure 1. Adenosine (ADO), L-3′-ADMdA **1**, L-3′-AM-MECA **2**, the selective A_3AR agonist **3**, and IB-MECA.



Scheme 1. Synthesis of L-3′-AM-MECA **2**. Reagents and conditions: (a) seven steps, Ref. 18; (b) TBDPSCl, Py, CH_2Cl_2, 0 °C to rt, 24 h; (c) AcOH, H_2SO_4, rt, overnight, then Ac_2O, Py, rt, 4 h; (d) silylated 6-chloropurine, TMSOTf, MeCN, 0 °C to rt, overnight; (e) $Et_3N{\cdot}3HF$, THF, rt, 24 h; (f) (diacetoxyiodo)benzene, TEMPO 1:1 MeCN/H_2O, rt, 48 h; (g) $NaIO_4$, $RuCl_3{\cdot}H_2O$, H_2O/MeCN/CCl_4, rt, 24 h; (h) EtOC(O)Cl, Et_3N, DMF, 0 °C, 10 min, then 40% $MeNH_2/H_2O$, 0 °C to rt, 48 h.

ject to acetolysis to give acetate **6**, almost exclusively as the β-anomer, as expected on the basis of the participation of the 2-acetate and supported by the lack of coupling between H-1 and H-2 in the ^{1}H NMR spectrum. The ^{1}H NMR of the crude reaction mixture showed other minor signals in the anomeric region, but none was of significant intensity to attempt purification or spectroscopic characterization. Acetate **6** was coupled to 6-chloropurine in Vorbrüggen conditions to give protected nucleoside **7**. Also in this case, ^{1}H NMR of the crude reaction mixture showed only one isomer. The expected β-stereochemistry could be easily confirmed by desilylation of **7** to **8** and conversion of the latter to **1** according to our reported procedure.[18] Oxidation of the primary alcohol functionality of **8** was attempted using two methods. Using (diacetoxyiodo)benzene as the oxidizer and TEMPO as initiator we were able to obtain carboxylic acid **9** in moderate yield. The ruthenium-mediated method afforded lower yields and a product contaminated with a dark impurity, presumably from the catalyst, that could not be purified chromatographically. Conversion of **9** to the mixed anhydride with ethyl chloroformate and in situ treatment with 40% aqueous methylamine afforded the synthesis of the desired amide **2**. The conversion of **9** to **2** is the result of four different reactions: amide formation, Fmoc deprotection, hydrolysis of the 2′-*O*-acetate and replacement of the 6-chloride. Similarly to what we observed during the synthesis of **1**,[18] we noticed that these reactions proceed at different rates, that is, Fmoc deprotection and amide formation are very fast and complete within a few minutes, whereas acetate hydrolysis usually requires 2–4 h, and 6-substitution is the slowest process, requiring several hours to complete. These observations are supported by qualitative tlc and UV data, consistent with the intermediate formation of the 6-chloropurine analog of **2**.

2.2. Cardioprotection in murine isolated perfused heart

The cardioprotective effect of **1** and **2** was evaluated on the well established Langendorff mouse isolated perfused heart model.[19] Hearts derived from Swiss Webster mice were subject to 40 min of global ischemia and 60 min of reperfusion. Compounds were perfused for 5 min prior to ischemia to evaluate potential

protective effects (Fig. 2). Left ventricular developed pressure (LVDP) was continuously monitored during the ischemia-reperfusion (IR) protocol and the % functional recovery was expressed as the final rate pressure product (RPP)/initial RPP * 100. To assess necrosis after the IR injury, hearts were evaluated by perfusing them with a 1% TTC solution, which stains viable tissue red. High resolution scans were made of transverse sections of heart and the infarction area (i.e., not stained red) was determined and expressed as a % of total tissue area. The cardioprotective effects of adenosine were reflected in: significantly less evidence of necrosis as assessed with TTC staining (Fig. 2A); a delayed time until onset of ischemic contracture (Fig. 2B); and most importantly, significantly increased recovery of function upon reperfusion after 40 min of ischemia (Fig. 2C).

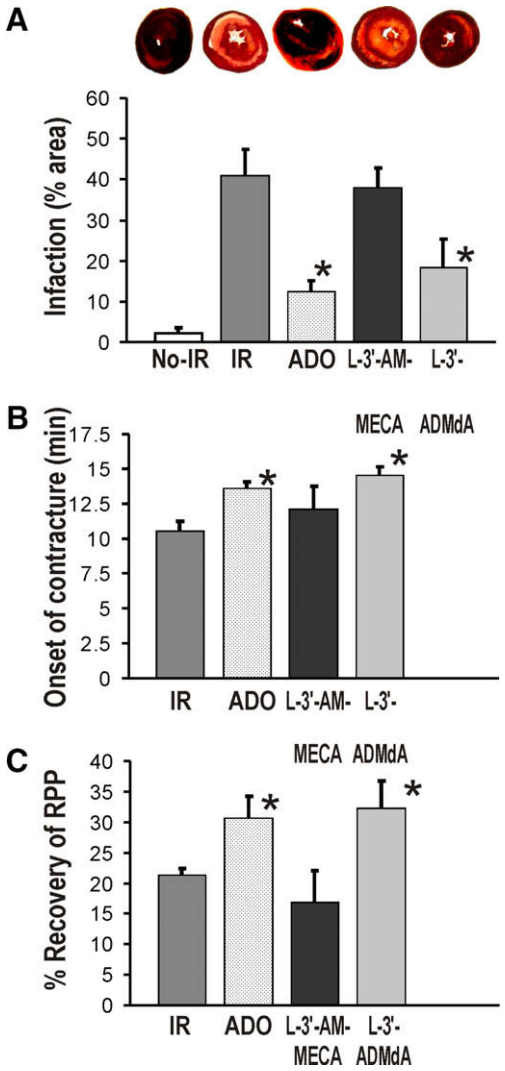


Figure 2. Effects of L-3′-AM-MECA and L-3′-ADMdA on mouse isolated perfused hearts. **A**. Infarction area following ischemia-reperfusion (IR). Heart tissue was stained with TTC, which is reduced to the bright red triphenyl formazane by viable cells. Red areas indicate living tissue, while colorless or pale yellow areas indicate necrotic tissue. Data is expressed as the percentage of area that lacks significant TTC staining (19.1 ± 8.4 for **1**, compared to 40.5 ± 7.2% for the control and 11.5 ± 2.1% for adenosine). **B**. Time until onset of contracture often correlates with the kinetics of ischemic ATP depletion, and ultimately the damage sustained by the myocardium (14.5 ± 0.9 min for **1**, compared to 10.5 ± 1.0 min for the control and 13.6 ± 0.6 min for adenosine). **C**. Functional recovery of contractility, expressed in % recovery of RPP (32.2 ± 3.7 cm H_2O/s for **1**, compared to 21.3 ± 1.4 for the control and 30.7 ± 3.4 for adenosine). * Statistically different from control (IR).

Consistent with it behaving as adenosine receptor agonist, L-3′-ADMdA **1** decreased evidence of cardiac tissue necrosis (Fig. 2A), significantly prolonged the time to ischemic contracture (Fig. 2B) and improved functional recovery (Fig. 2C). In contrast, treatment of hearts with L-3′-AM-MECA **2** provided no salubrious effects and did not significantly alter any of these parameters when compared to controls (Fig. 2). Adenosine (50 μM) produced a transient arrest of contractile activity during its perfusion as previously reported.[20] Interestingly, unlike adenosine, neither analog caused a cessation of heart beating (i.e., cardiac standstill) during their perfusion.

In summary, we have described a novel L-nucleoside (L-3′-ADMdA, **1**) endowed with cardioprotective effect. In view of its nature of adenosine analog, we speculate that the activity of **1** may be due to agonistic action on A_1 and/or A_3 AR subtypes, but further studies will be needed to confirm this speculation. Notably, L-L-3′-ADMdA is the first L-nucleoside that shows biological activity besides antiviral or anticancer effect. Furthermore, since the first step in the activation of known antiviral and antitumor L-nucleosides is the interaction with cellular or viral kinases, L-3′-ADMdA is one of a few examples of L-nucleosides that interact with animal enzymes other than kinases.[21] This discovery opens a new niche in the search for AR ligands. The nature of L-nucleosides is likely to endow potential candidates with favorable features such as lower toxicity and higher metabolic stability than their D-counterparts. Our synthetic procedures are versatile, thanks to the different reactivity of the functional groups in **8** and **9**, which can allow the synthesis of analogs with different substitution patterns and a full structure-activity relationship study. Such studies are currently in progress.

3. Experimental

All the reactions were carried out under a positive pressure of argon and monitored by TLC on Uniplates (silica gel) purchased from Analtech Co. All the reagents and anhydrous solvents were purchased from various commercial sources and used without further purification except where noted. Chromatographic purifications were performed on flash silica gel (particle size 40–63 μm) purchased from Silicycle or TLC grade silica gel (particle size 5–15 μm) purchased from Sorbent Technologies. All solvents for chromatographic purifications were HPLC grade. Melting points were determined on a Barnstead Mel-Temp and are uncorrected. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometer using Me_4Si as an internal standard and signals are represented as s (singlet), d (doublet), t (triplet), m (multiplet), or combinations of the above. Mass spectra were obtained on a Finnigan 'LCQ' Liquid Chromatograph Ion Trap Mass Spectrometer. UV spectra were obtained on a BECKMAN DU-650 spectrophotometer. Optical rotations were measured on a Rudolph Research Analytical Autopol IV digital polarimeter. Elemental analyses were performed by Atlantic Microlabs Inc. Norcross, GA.

3.1. 5-*O*-*tert*-Butyldiphenylsilyl-3-deoxy-3-fluorenylmethylcarbonylamino-1,2-*O*-(1-methylethylidene)-α-L-ribofuranose (5)

tert-Butyldiphenylchlorosilane (2.4 mL, 9.38 mmol) was added to an ice-cold solution of **4** (2.61 g, 6.34 mmol) and anhydrous pyridine (0.80 mL, 9.89 mmol) in anhydrous dichloromethane (50 mL), and the resulting solution was allowed to warm up to rt and stirred for 24 h. Since the reaction did not proceed to completion, more *tert*-butyldiphenylsilyl chloride (2.4 mL, 9.38 mmol) and pyridine (0.80 mL, 9.89 mmol) were added, and stirring was continued at rt for 24 h more. The resulting solution was washed with a 0.1 N solution of HCl (2 × 10 mL), water (10 mL), sodium bicarbonate saturated solution (10 mL), water (10 mL) and brine (10 mL). The organic layer was then dried over magnesium sulfate, filtered and concentrated to a crude that was purified by TLC-grade silica

gel flash chromatography (1:9 to 1:4 ethyl acetate/hexanes) to give **5** as a white solid (3.65 g, 89%). **5**: R_f 0.16 (1:4 ethyl acetate/hexanes); mp 61–63 °C; $[\alpha]_D^{25}$ −36.94 (*c* 0.32, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) *δ 7.80–7.76* (m, 2H), 7.71–7.69 (m, 4H), 7.63–7.57 (m, 2H), 7.42–7.30 (m, 10H), 5.86 (d, *J* = 2.9 Hz, 1H), 5.08 (d, *J* = 9.6 Hz, 1H), 4.65–4.63 (m, 1H), 4.41 (d, *J* = 6.8 Hz, 2H), 4.34–4.28 (m, 1H), 4.22 (t, *J* = 6.6 Hz, 1H), 3.92–3.88 (m, 1H), 3.87–3.83 (m, 1H), 3.79–3.73 (m, 1H), 2.18 (s, 3H), 2.05 (s, 3H), 1.04 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 155.7, 143.8, 143.7, 141.2, 135.6, 133.2, 133.1, 129.6, 127.7, 127.6, 127.0, 125.0, 119.9, 112.3, 104.2, 80.3, 78.9, 66.9, 62.4, 53.3, 47.1, 26.7, 26.5, 26.3, 19.1; Mass ($[M+Na]^+$) 672. Anal. Calcd for $C_{39}H_{43}NO_6Si$: C, 72.08; H, 6.67; N, 2.16. Found: C, 71.80; H, 6.79; N, 2.10.

3.2. 5-*O*-*tert* -Butyldiphenylsilyl-3-deoxy-1,2-diacetyl-3-fluorenylmethylcarbonylamino-β-L-ribofuranose (6)

Sulfuric acid (60 μL, 1.13 mmol) was added to a stirring mixture of **5** (3.65 g, 5.62 mmol) in acetic acid (50 mL), and the reaction was stirred at rt overnight. Acetic anhydride (7.2 mL, 76.17 mmol) was then added, followed by pyridine (1.7 mL, 21.02 mmol), and the mixture was stirred at rt for 4 h. Volatiles were evaporated in vacuo, and the residue was dissolved in dichloromethane (100 mL), washed with a saturated solution of sodium bicarbonate (20 mL), water (20 mL) and brine (20 mL). The organic solution was dried over magnesium sulfate, filtered and concentrated under reduced pressure to a crude that was purified by tlc-grade silica gel flash chromatography (1:4 to 3:7 ethyl acetate/hexanes) to give **6** as a white solid (1.94 g, 50%). R_f 0.46 (3:7 ethyl acetate/hexanes); mp 70–72 °C; $[\alpha]_D^{26}$ −24.43 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) *δ 7.79–7.76* (m, 2H), 7.68–7.66 (m, 4H), 7.59–7.55 (m, 2H), 7.44–7.28 (m, 10H), 6.12 (s, 1H), 5.17–5.15 (m, 1H), 4.81–4.79 (m, 1H), 4.55–4.42 (m, 3H), 4.22 (t, *J* = 6.6 Hz, 1H), 4.00–3.97 (m, 1H), 3.86 (dd, *J* = 11.3, 3.0, 1H), 3.71 (dd, *J* = 11.3, 4.1, 1H), 2.14 (s, 3H), 1.90 (s, 3H), 1.09 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 170.5, 169.5, 155.5, 143.7, 143.6, 141.3, 135.6, 135.5, 135.2, 130.0, 127.7, 127.7, 127.0, 124.8, 120.0, 97.7, 83.1, 75.7, 66.9, 63.3, 50.7, 47.1, 26.8, 22.9, 20.7, 19.0; Mass ($[M+Na]^+$) 716, ($[2M+Na]^+$) 1409. Anal. Calcd for $C_{40}H_{43}NO_8Si$: C, 69.24; H, 6.25; N, 2.13. Found: C, 69.00; H, 6.24; N, 2.05.

3.3. 9-(2-*O*-Acetyl-5-*O*-*tert* -butyldiphenylsilyl-3-deoxy-3-fluorenylmethylcarbonylamino-β-L-ribofuranosyl)-6-chloropurine (7)

A mixture of 6-chloropurine (0.67 g, 4.33 mmol) and ammonium sulfate (30 mg, 0.23 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (30 mL) was refluxed for 3 h, then the solvent was removed in vacuo at 35–40 °C. A solution of **6** (1.88 g, 2.71 mmol) in anhydrous acetonitrile (30 mL) was added to the residual solid. The resulting solution was cooled to 0 °C and trimethylsilyl triflate (0.78 mL, 4.31 mmol) was added, and the reaction was stirred at rt overnight. The resulting solution was diluted to 100 mL with dichloromethane and slowly added to an ice-cold saturated solution of sodium bicarbonate (200 mL). The organic layer was separated and the aqueous phase was extracted with dichloromethane (2 × 100 mL). The combined organic extracts were washed with water (50 mL), brine (50 mL), dried over magnesium sulfate, filtered and concentrated to a crude that was purified by tlc-grade silica gel flash chromatography (1:49 methanol/dichloromethane) to give **7** as a white solid (1.75 g, 82%). R_f 0.14 (1:49 methanol/dichloromethane); mp 91–93 °C (dec.); $[\alpha]_D^{25}$ −17.83 (*c* 0.42, $CHCl_3$); UV (MeOH) λ_{max} 264.0, 299.0; ^1H NMR ($CDCl_3$, 400 MHz) *δ* 8.70 (s, 1H), 8.34 (s, 1H), 7.81–7.78 (m, 2H), 7.68–7.57 (m, 6H), 7.44–7.36 (m, 4H), 7.35–7.29 (m, 4H), 7.26–7.22 (m, 2H), 6.15 (s, 1H), 5.67–5.64 (m, 1H), 5.12–5.06 (m, 1H), 4.99–4.95 (m, 1H), 4.55–4.45 (m, 2H), 4.25–4.21 (m, 1H), 4.16–4.12 (m, 1H), 4.03–3.99 (m, 1H), 3.88–3.83 (m, 1H), 2.15 (s, 3H), 1.03 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 169.4, 152.0, 151.1, 150.8, 143.8, 143.5, 143.4, 141.2, 135.5, 135.3, 132.5, 132.2, 132.0, 129.7, 127.6, 127.5, 126.9, 124.7, 124.6, 119.9, 88.0, 83.2, 75.4, 66.8, 62.8, 50.9, 47.0, 26.6, 20.5, 19.0; Mass ($[M+H]^+$) 788, ($[M+Na]^+$) 810, ($[2M+Na]^+$) 1597. Anal. Calcd for $C_{43}H_{42}ClN_5O_6Si$: C, 65.51; H, 5.37; N, 8.88. Found: C, 65.24; H, 5.54; N, 8.56.

3.4. 9-(2-*O*-Acetyl-3-deoxy-3-fluorenylmethylcarbonylamino-β-L-ribofuranosyl)-6-chloropurine (8)

Triethylamine trihydrofluoride (1.6 mL, 9.82 mmol) was added to a stirring solution of **7** (1.54 g, 1.95 mmol) in anhydrous tetrahydrofuran (20 mL) at rt, and the resulting solution was stirred at rt for 24 h, then tlc-grade silica gel was added and volatiles were removed under reduced pressure. The residue was loaded on a tlc-grade silica gel column packed with dichloromethane and eluted with a gradient of dichloromethane to 1:19 methanol/dichloromethane to give **8** as a white solid, containing minor impurities (1.00 g, 93%). A small sample was purified by preparative silica gel tlc (1:19 methanol/dichloromethane) to give pure **8** as a white solid. R_f 0.16 (1:19 methanol/dichloromethane); mp 196–197 °C; $[\alpha]_D^{22}$ +35.78 (*c* 0.34, $CHCl_3$); UV (MeOH) λ_{max} 264.0, 299.5, 288.5; ^1H NMR ($CDCl_3$, 400 MHz) *δ* 8.77 (s, 1H), 8.42 (s, 1H), 7.79–7.75 (m, 2H), 7.59–7.57 (m, 2H), 7.44–7.38 (m, 2H), 7.35–7.29 (m, 2H), 6.22 (d, *J* = 2.2 Hz, 1H), 5.54–5.52 (m, 1H), 5.20 (d, *J* = 8.0 Hz, 1H), 4.87–4.82 (m, 1H), 4.60–4.49 (m, 2H), 4.29–4.19 (m, 3 H), 4.00 (d, *J* = 12.0 Hz, 1H), 3.76 (d, *J* = 12.0 Hz, 1H), 2.11 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 169.5, 156.1, 151.8, 151.3, 150.5, 144.5, 143.4, 141.2, 132.2, 127.7, 126.9, 124.6, 119.9, 88.7, 84.3, 75.5, 66.7, 61.1, 50.5, 47.0, 20.5; Mass ($[M+H]^+$) 550, ($[M+Na]^+$) 572. Anal. Calcd for $C_{27}H_{24}ClN_5O_6$: C, 58.97; H, 4.40; N, 12.73. Found: C, 59.05; H, 4.62; N, 12.33.

3.5. 1-(6-Chloro-9H-purin-9-yl)-2-*O* -acetyl-1,3-dideoxy-3-fluorenylmethylcarbonylamino-β-L-ribofuranoic acid (9)

Method A: (Diacetoxyiodo)benzene (770 mg, 2.39 mmol) was added to a suspension of **8** (600 mg, 1.09 mmol) and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) (43 mg, 0.28 mmol) in 1:1 acetonitrile/water (50 mL), and the resulting mixture was stirred at rt for 48 h, then diluted with water (100 mL) and extracted with dichloromethane (3 × 150 mL). The combined organic extracts were washed with brine (30 mL), dried over sodium sulfate, filtered and concentrated to a crude that was purified by tlc-grade silica gel flash chromatography. Elution with dichloromethane to 1:49 methanol/dichloromethane allowed to recover unreacted **8** (140 mg, 25%); subsequent elution with 1:19 to 2:23 methanol/dichloromethane gave **9** as a yellow solid, containing minor impurities (190 mg, 31%). A small amount of pure **9** was obtain by preparative silica gel tlc (3:17 methanol/dichloromethane, washed with 2:23 methanol/dichloromethane) as a white solid. R_f 0.44 (3:17 methanol/dichloromethane); mp 198–200 °C (dec.); $[\alpha]_D^{22}$ −11.43 (*c* 0.30, $CHCl_3$); UV (MeOH) λ_{max} 264.0, 299.0, 288.5; ^1H NMR (DMSO-d_6, 400 MHz) *δ* 9.87 (br s, 1H), 8.80 (s, 1H), 8.20 (d, *J* = 8.8 Hz, 1H), 7.89 (d, 2H, *J* = 7.4 Hz), 7.75–7.72 (m, 3H), 7.42 (t, 2H, *J* = 7.4 Hz), 7.34 (d, 2H, *J* = 7.4 Hz), 6.49 (d, *J* = 4.5 Hz, 1H), 5.60 (t, 1H, *J* = 4.9 Hz), 4.55–4.49 (m, 1H), 4.36–4.29 (m, 2H), 4.24–4.16 (m, 2H), 1.96 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 173.3, 169.3, 155.9, 151.8, 151.5, 149.1, 146.8, 143.9, 143.8, 140.7, 131.3, 127.7, 127.2, 127.1, 125.4, 125.3, 120.2, 120.1, 86.3, 83.5, 75.7, 65.9, 55.4, 46.6, 20.4; Mass ($[M+H]^+$) 564. Anal. Calcd for $C_{27}H_{22}ClN_5O_7$: C, 57.50; H, 3.93; N, 12.42. Found: C, 57.28; H, 3.79; N, 9.42. *Method B: Ruthenium(III)* chloride monohydrate (7.5 mg, 0.04 mmol) was added to a vigorously stirred biphasic heterogeneous mixture of **8** (90 mg, 0.16 mmol) and sodium

periodate (145 mg, 0.68 mmol) in water (6 mL), acetonitrile (4 mL) and carbon tetrachloride (4 mL) at rt, and the resulting mixture was stirred at rt for 72 h. Solvents were then evaporated under reduced pressure, the residue was dissolved in methanol, tlc-grade silica gel was added, and the solvent was removed under reduced pressure. The residue was loaded on a very thin pad of tlc-grade silica gel column packed with dichloromethane and eluted with a gradient of dichloromethane to 1:19 methanol/dichloromethane to give **9** as a dark yellow oil (40 mg, 43%). Although this product looked clean by tlc, repeated attempts to purify it by preparative silica gel tlc did not result in the isolation of a solid.

3.6. L-3′-Deoxy-N^6-methyl-3′-methylaminoadenosine-5′-methylcarboxamide (2)

Triethylamine was added to a solution of **9** (200 mg, 0.36 mmol) and ethyl chloroformate (40 μL, 0.42 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) at 0 °C, and the resulting mixture was stirred at 0 °C for 10 min, then treated with a 40% methylamine solution in water (5 mL). The mixture was allowed to warm up to rt, then stirred for 48 h. Solvents were evaporated in vacuo and the residue was purified by flash silica gel chromatography (dichloromethane to 1:13 methanol/dichloromethane) to give an orange solid that was further purified by preparative silica gel tlc: R_f 0.20 (3:17 methanol/dichloromethane) to give pure **2** as an off-white solid (60 mg, 55%). Mp 165–167 °C (dec.); UV (MeOH) λ_{max} 265.5; ^{1}H NMR (CD_3OD, 400 MHz) δ 8.34 (s, 1H), 8.30 (br s, 1H), 6.07 (d, J = 4.1 Hz, 1H), 4.62 (dd, J = 5.3, 4.1 Hz, 1H), 4.34 (d, J = 5.7 Hz, 1H), 3.80 (t, J = 5.7 Hz, 1H, D_2O exchangeable), 3.11 (br s, 3H), 2.83 (s, 3H), 2.40–2.38 (m, 1H), 1.26 (br s, 4H, D_2O exchangeable); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.9, 156.8, 153.9, 149.0, 121.4, 92.1, 83.6, 74.6, 50.0, 26.3, 25.6; Mass ($[M+H]^+$) 308. Anal. Calcd for $C_{12}H_{17}N_7O_3$: C, 46.90; H, 5.58; N, 31.91. Found: C, 47.07; H, 5.98; N, 31.68.

3.7. Langendorff perfused heart preparation

Hearts were isolated from 10 weeks old male Swiss Webster white mice and perfused with Krebs buffer containing (mM): NaCl, 118; $NaHCO_3$, 25; KCl, 4.7; Mg_2SO_4, 1.2; KH_2PO_4, 1.2; glucose, 11; $CaCl_2$, 1.8, as previously described in detail.[22] Buffer was equilibrated with 95% O_2, 5% CO_2 at water-jacketed reservoirs maintained at 37 °C. After thoracotomy, hearts were excised into ice-cold perfusion buffer, the aorta was cannulated (20 gauge PVC cannula) and perfused at 100 cm H_2O pressure. A fluid filled silicon balloon was inserted into the left ventricle via the mitral valve. The balloon was attached to a pressure transducer connected to a Power Lab data acquisition system for the recording of left ventricular pressure (ADInstruments, Colorado, USA). Hearts were immersed in perfusate at 37 °C and balloons inflated to an end diastolic pressure of ~10 cm H_2O. Hearts were stabilized for 20 min and baseline functions like left ventricular developed pressure (LVDP) and heart rate (HR) and coronary flow were measured. Hearts showing baseline values of LVDP lower than 80 cm H_2O, spontaneous heart rates lower than 300 BPM and/or coronary flow greater than 5 mL/min were disqualified from the study according to previously published criteria.[22a] Hearts were untreated or subject to preconditioning stimuli either with adenosine or with synthetic analogs **1** or **2** for a 5 min of perfusion. After the baseline period, all the untreated and treated hearts underwent 40 min global ischemia followed by 60 min reperfusion. The parameters of LVDP and HR were reassessed after reperfusion.

3.8. Triphenyltetrazolium chloride staining

Hearts were perfused with 1% triphenyltetrazolium chloride (TTC) immediately after completion of the experiment and incubated for 15 min at 37 °C. Then the hearts were weighed and sliced into ~1 mm transverse sections and scanned using Microteck film scanner (Miroteck International Inc, New York, USA). The area of infarcted (unstained) and viable (stained) tissue was measured using Adobe Photoshop image analysis software. The ratio of infarct area to total cross sectional area of the ventricle from each slice was determined (percent infarction).

3.9. Statistical analysis

Baseline data and post-ischemic functional recoveries in different experimental groups were analyzed by one-way ANOVA with Bonferroni's Correction. A P value of <0.05 was considered significant. All values are presented as mean ± SEM.

Acknowledgment

This research was supported by institutional funds from the Medical University of South Carolina.

References

1. Fredholm, B. B.; Ijzerman, A. P.; Jacobson, K. A.; Klotz, K.-N.; Linden, . *Pharmacol. Rev.* **2001**, *53*, 527.
2. (a) Donato, M.; Gelpi, R. J. *Mol. Cell Biochem.* **2003**, *251*, 153; (b) de Jonge, R.; de Jong, J. W.; Giacometti, D.; Bradamante, S. *Eur. J. Pharmacol.* **2001**, *414*, 55; (c) de Jong, J. W.; de Jonge, R.; Keijzer, E.; Bradamante, S. *Pharmacol. Ther.* **2000**, *87*, 141.
3. (a) Glover, D. K.; Riou, L. M.; Ruiz, M.; Sullivan, G. W.; Linden, J.; Rieger, J. M.; Macdonald, T. L.; Watson, D. D.; Beller, G. A. *Am. J. Physiol. Heart Circ. Physiol.* **2005**, *288*, H1851; (b) Hein, T. W.; Wang, W.; Zoghi, B.; Muthuchamy, M.; Kuo, L. *J. Mol. Cell Cardiol.* **2001**, *33*, 271; (c) Jordan, J. E.; Zhao, Z. Q.; Sato, H.; Taft, S.; Vinten-Johansen, J. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 301.
4. Müller, C. E. *Curr. Top. Med. Chem.* **2003**, *3*, 445.
5. (a) DeNinno, M. P.; Masamune, H.; Chenard, L. K.; DiRico, K. J.; Eller, C.; Etienne, J. B.; Tickner, J. E.; Kennedy, S. P.; Knight, D. R.; Kong, J.; Oleynek, J. J.; Tracey, W. R.; Hill, R. J. *J. Med. Chem.* **2003**, *46*, 353; (b) Tracey, W. R.; Magee, W. P.; Oleynek, J. J.; Hill, R. J.; Smith, A. H.; Flynn, D. M.; Knight, D. R. *Am. J. Physiol. Heart Circ. Physiol.* **2003**, *285*, H2780; (c) Tracey, W. R.; Magee, W.; Masamune, H.; Kennedy, S. P.; Knight, D. R.; Buchholz, R. A.; Hill, R. J. *Cardiovasc. Res.* **1997**, *33*, 410.
6. Angulo, E.; Casadó, V.; Mallol, J.; Canela, E. I.; Viñals, F.; Ferrer, I.; Lluis, C.; Franco, R. *Brain Pathol.* **2003**, *13*, 440.
7. Dall'Igna, O. P.; Porciúncula, L. O.; Souza, D. O.; Cunha, R. A.; Lara, D. R. *Br. J. Pharmacol.* **2003**, *138*, 1207.
8. (a) Pinna, A.; Volpini, R.; Cristalli, G.; Morelli, M. *Eur. J. Pharmacol.* **2005**, *512*, 157; (b) Vu, C. B.; Pan, D.; Peng, B.; Kumaravel, G.; Smits, G.; Jin, X.; Phadke, D.; Engber, T.; Huang, C.; Reilly, J.; Tam, S.; Grant, D.; Hetu, G.; Petter, R. C. *J. Med. Chem.* **2005**, *48*, 2009; (c) Vu, C. B.; Peng, B.; Kumaravel, G.; Smits, G.; Jin, X.; Phadke, D.; Engber, T.; Huang, C.; Reilly, J.; Tam, S.; Grant, D.; Hetu, G.; Chen, L.; Zhang, J.; Petter, R. C. *J. Med. Chem.* **2004**, *47*, 4291; (d) Dall'Igna, O. P.; Souza, D. O.; Lara, D. R. *Ann. Pharmacother.* **2004**, *38*, 717; (e) Chase, T. N.; Bibbiani, F.; Bara-Jimenez, W.; Dimitrova, T.; Oh-Lee, J. D. *Neurology* **2003**, *61*, S107; (f) Schwarzschild, M. A.; Xu, K.; Oztas, E.; Petzer, J. P.; Castagnoli, K.; Castagnoli, N., Jr.; Chen, J. –F. *Neurology* **2003**, *61*, S55; (g) Chen, J. F.; Xu, K.; Petzer, J. P.; Staal, R.; Xu, Y. H.; Beilstein, M.; Sonsalla, P. K.; Castagnoli, K.; Castagnoli, N., Jr.; Schwarzschild, M. A. *J. Neurosci.* **2001**, *21*, 143.
9. Behan, W. M. H.; Stone, T. W. *Br. J. Pharmacol.* **2002**, *135*, 1435.
10. Okamura, T.; Kurogi, Y.; Hashimoto, K.; Sato, S.; Nishikawa, H.; Kiryu, K.; Nagao, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3775.
11. Blum, D.; Gall, D.; Galas, M. –C.; d'Alcantara, P.; Bantubungi, K.; Schiffmann, S. N. *J. Neurosci.* **2002**, *22*, 9122.
12. (a) Fishman, P.; Bar-Yehuda, S.; Madi, L.; Cohn, I. *Anticancer Drugs* **2002**, *13*, 437; (b) Fishman, P.; Bar-Yehuda, S.; Barer, F.; Madi, L.; Multani, A. S.; Pathak, S. *Exp. Cell Res.* **2001**, *269*, 230.
13. (a) Mustafa, S. J.; Askar, A. O. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 49; (b) Burnstock, G.; Hills, J. M.; Hoyle, C. H. *Br. J. Pharmacol.* **1984**, *81*, 533; (c) Brown, C.; Burnstock, G.; Cusack, N. J.; Meghji, P.; Moody, C. J. *Br. J. Pharmacol.* **1982**, *75*, 101; (d) Cusack, N. J.; Planker, M. *Br. J. Pharmacol.* **1979**, *67*, 153; (e) Cusack, N. J.; Jickman, M. E.; Born, G. V. *Proc. Royal Soc. London B Bio. Sci.* **1979**, *206*, 139.
14. Wang, M.; Zhang, J.; Andrei, D.; Kuczera, K.; Borchardt, R. T.; Wnuk, S. F. *J. Med. Chem.* **2005**, *48*, 3649.
15. (a) Al Safarjalani, O. N.; Naguib, F. N. M.; el Kouni, M. H. *Antimicrob. Agents Chemother.* **2003**, *47*, 3247; (b) Carter, N. S.; Mamoun, C. B.; Liu, W.; Silva, E. O.; Landfear, S. M.; Goldberg, D. E.; Ullman, B. *J. Biol. Chem.* **2000**, *275*, 10683.
16. (a) Gumina, G.; Chong, Y.; Choo, H.; Song, G. –Y.; Chu, C. K. *Curr. Top. Med. Chem.* **2002**, *2*, 1065; (b) Gumina, G.; Song, G. –Y.; Chu, C. K. *FEMS Microbiol. Lett.* **2001**, *202*, 9.
17. Jurovcik, M.; Holy, A.; Sorm, F. *FEBS Lett.* **1971**, *18*, 274.

18. Gilbert, C. L. K.; Lisek, C. R.; White, R. L.; Gumina, G. *Tetrahedron* **2005**, *61*, 8339.
19. (a) Tikh, E. I.; Fenton, R. A.; Dobson, J. G., Jr. *Am. J. Physiol. Heart Circ. Physiol.* **2006**, *290*, H348; (b) Peart, J.; Headrick, J. P. *Cardiovasc. Res.* **2003**, *58*, 589; (c) Sutherland, F. J.; Baker, K. E.; Shattock, M. J.; Hearse, D. J. *Clin. Exp. Pharmacol. Physiol.* **2003**, *30*, 867; (d) Sutherland, F. J.; Baker, K. E.; Shattock, M. J.; Hearse, D. J. *Clin. Exp. Pharmacol. Physiol.* **2003**, *30*, 879.
20. (a) Canyon, S. J.; Dobson, G. P. *J. Thorac. Cardiovasc. Surg.* **2005**, *130*, 371; (b) Nussbaum, E. S.; Sebring, L. A.; Ostanny, I.; Nelson, W. B. *Neurosurgery* **2000**, *47*, 240; (c) De Giovanni, J. V.; Edgar, R. A.; Cranston, A. *Heart* **1998**, *80*, 330; (d) Shryock, J. C.; Belardinelli, L. *Am. J. Cardiol.* **1997**, *79*, 2.
21. The other example known to us is the recent report by Schramm and co-workers describing the inhibitory activity of L-Immucillin-H and L-DADMe-Immucillin-H toward purine nucleoside phosphorylase (a) Rinaldo-Matthis, A.; Murkin, A. S.; Ramagopal, U. A.; Clinch, K.; Mee, S. P. H.; Evans, G. B.; Tyler, P. C.; Furneaux, R. H.; Almo, S. C.; Schramm, V. L. *J. Am. Chem. Soc.* **2008**, *130*, 842; (b) Clinch, K.; Evans, G. B.; Fleet, G. W.; Furneaux, R. H.; Johnson, S. W.; Lenz, D. H.; Mee, S. P. H.; Rands, P. R.; Schramm, V. L.; Taylor Ringia, E. A.; Tyler, P. C. *Org. Biomol. Chem.* **2006**, *4*, 1131.
22. (a) Sutherland, F. J.; Baker, K. E.; Shattock, M. J.; Hearse, D. J. *Clin. Exp. Pharmacol. Physiol.* **2003**, *30*, 867; (b) Sutherland, F. J.; Baker, K. E.; Shattock, M. J.; Hearse, D. J. *Clin. Exp. Pharmacol. Physiol.* **2003**, *30*, 879.