Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVIII, No. 6

RADIOLABELING AND EFFICIENT SYNTHESIS OF TRITIATED 2-CHLORO-N^6-(3-IODOBENZYL)ADENOSINE-5'-*N*-METHYLURONAMIDE, A POTENT, SELECTIVE A_3 ADENOSINE RECEPTOR AGONIST

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SUMMARY

We recently reported that 2-substitution of N^6-benzyladenosine-5'-uronamides greatly enhances selectivity of agonists for rat A_3 adenosine receptors (*J. Med. Chem.* **1994**, *37*, 3614-3621). Specifically, 2-Chloro-N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (2-Cl-IB-MECA), which displayed a K_i value of 0.33 nM, is the most selective for A_3 receptors yet reported with selectivity versus A_1 and A_{2a} receptors of 2500- and 1400-fold, respectively. In order to obtain pharmacological tools for the study of A_3 adenosine receptors, two routes for radiolabeling of 2-Cl-IB-MECA through incorporation of tritium at the 5'-methylamido group were compared. One route formed a 2',3'-protected nucleoside 5'-carboxylic acid (**9**), which was condensed with methylamine and deprotected. The more efficient synthesis started from D-ribose and provided 2-Cl-IB-MECA (**12**) in six steps with an overall yield of 5.6 %. Tritium was introduced in the penultimate step by heating N^6-(3-iodobenzyl)-2-chloro-2',3'-di-*O*-acetyl-5'-(methoxycarbonyl)adenosine (**17**) with [^{3}H]methylamine in methanol at 60 °C for 2 h. The specific activity of [^{3}H]2-Cl-IB-MECA was 29 Ci/mmol with a radiochemical purity of 99%.

Key Words: Adenosine Derivatives, Radioligands, Adenosine Receptors, Tritium, Nucleosides

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CCC 0362–4803/96/060547–14

Received 12 December 1995
Revised 10 January 1996

INTRODUCTION

The A_1, the A_{2a}, the A_{2b}, and the A_3 adenosine receptors have been cloned from several species, including the rat, dog, mouse, sheep, and human (1). The A_3 adenosine receptor is the subtype most recently discovered, using the polymerase chain reaction (PCR) from cDNA libraries from rat brain and testes by sequence homology with other G-protein-coupled receptors (2). Activation of A_3 receptors is associated with hypotensive (3), histamine-releasing (4), and possibly cardioprotective (5) effects. Activation of A_3 receptors inhibits adenylyl cyclase in transfected CHO cells (2) and stimulates phosphatidylinositol metabolism in antigen-exposed mast cells (4).

Recently, we reported the structure-activity relationships of 2-substituted-N^6-benzyladenosine-5'-uronamides for A_3 adenosine receptors (6). Combination of two modifications of 5'-methyluronamide and N^6-(3-iodobenzyl) resulted in the moderately selective (50-fold selective for A_3 vs. either A_1 or A_{2a} receptors) and highly potent A_3 agonist N^6-(3-iodobenzyl)adenosine-5'-*N*-methyluronamide (IB-MECA, K_i = 1.1 nM) (7). Substitution by chloro, methylthio, or methylamino at the 2-position of N^6-(3-iodobenzyl)adenosine-5'-uronamides greatly augmented selectivity for A_3 adenosine receptors versus A_1 and A_2 receptors in rat (6). It was found that 2-chloro-N^6-(3-iodobenzyl)adenosine-5'-methyluronamide (2-Cl-IB-MECA) was a highly potent (K_i = 0.33 nM) and highly selective (2500- and 1400-fold vs. A_1 and A_{2a}, respectively) A_3 agonist (IC_{50} = 66.8 ± 9.0 nM for adenylyl cyclase inhibition). As a result of this selectivity profile the therapeutic potential of this molecule was apparent (8), hence a radiolabeled form of this molecule was eagerly sought as a pharmacological tool.

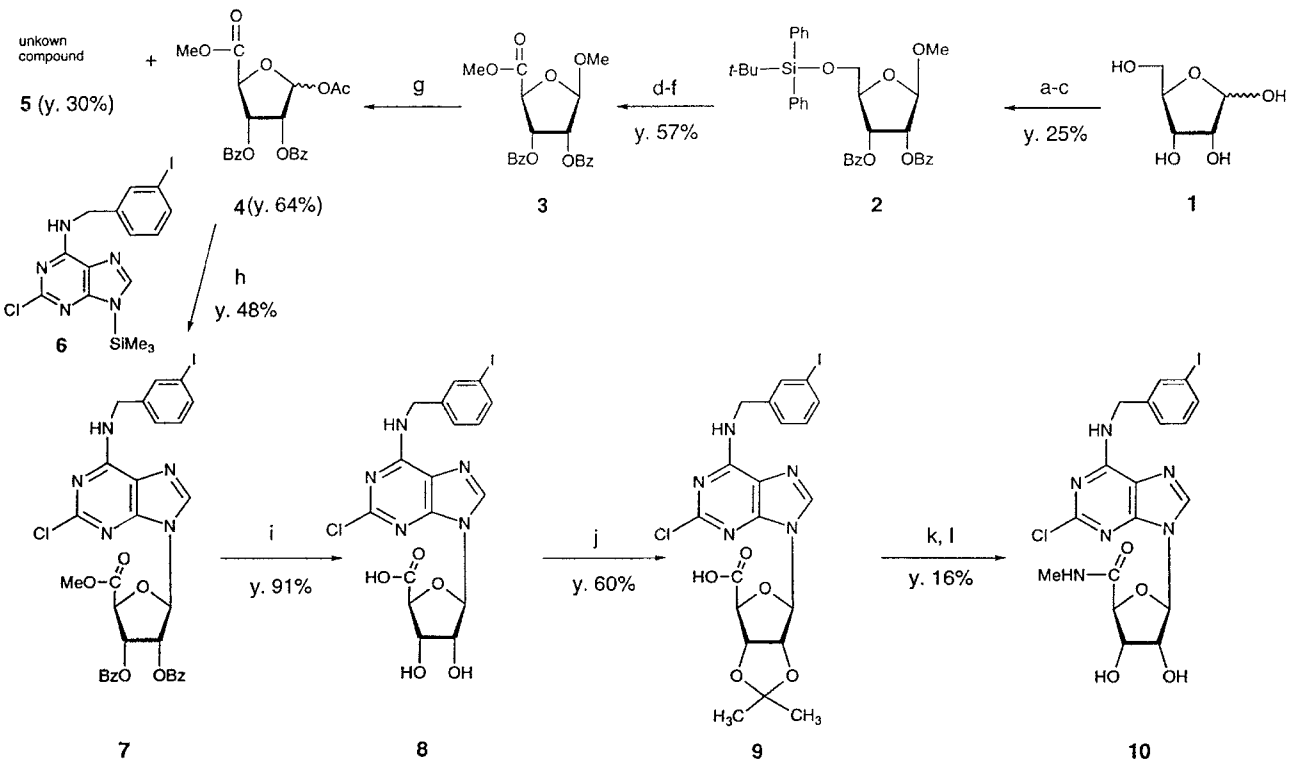
In this study we report a new synthetic route to 2-Cl-IB-MECA. This relatively efficient synthesis was carried out on a larger scale than the original synthesis and demonstrated to bevery suitable for introduction of a tritium label (contained in readily available methylamine) in the penultimate step.

RESULTS AND DISCUSSION

The usual method of tritiation of adenosine derivatives for the purpose of receptor characterization (9) has been the catalytic exchange with tritium oxide or replacement of halo groups. In Cl-IB-MECA, the two halo atoms are necessary for the biological profile, so an alternate route was needed. Thus, formation of the 5'-amide bond was targeted as the means of introducing a radioisotope. For the introduction of [^{3}H] at 5'-methyl position, the intermediate **3**, synthesized from D-ribose in 14% overall yield in our previous study (6) was utilized as a starting material (scheme I). Upon treatment of 1-methyl glycoside **3** with acetic anhydride and conc. sulfuric acid in glacial acetic acid 1-acetate **4** and unknown compound **5**, which were separated in silica gel column chromatography, were obtained in 64 % and 30 % yield, respectively. The 1-acetate **4** was then condensed with silylated 2-chloro-*N*^{6}-(3-iodobenzyl)adenine, **6**, under modified Vorbrüggen conditions (10) to give compound **7**. Base treatment of compound **7** produced diol acid **8**. The 2',3'-dihydroxyl groups of compound **8** were protected as the isopropylidene derivative using *p*-toluenesulfonic acid and 2,2-dimethoxypropane in acetone-DMF to yield compound **9** in 60 % yield. The resulting acid was coupled with [^{3}H]-methylamine in the presence of *N,N-bis*[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (BOP) and triethylamine (11). Deisopropylidenation of the product yielded the desired compound **10** in an overall 16 % yield. Thionyl chloride was reagent first tried for coupling acid **9** with methylamine, however it produced a dark reaction mixture, and no product could be observed by TLC. Since this coupling step failed to give a satisfactory yield and the whole scheme was too long, another route was sought.

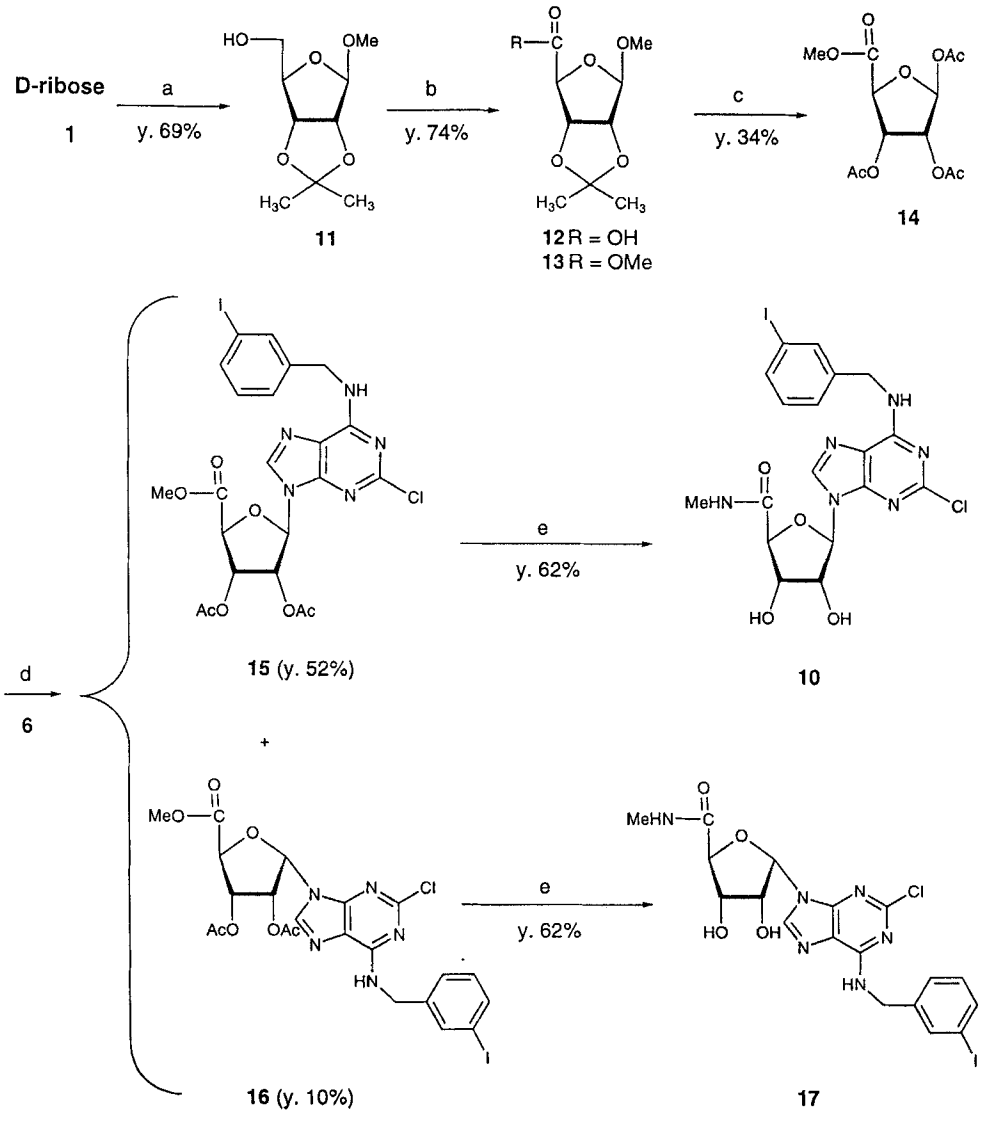
An improved synthesis starting from the same starting material, D-ribose (scheme II). 1-Methyl-2,3-*O*-isopropylidene-β-D-ribofuranoside (**11**) was synthesized in one step according to literature procedure (12) and the primary alcohol of **11** was oxidized to acid **12** and esterified using ruthenium (IV) oxide and EDAC and methanol, respectively. Isopropylidene methyl glycoside **13** was converted to 1,2,3-tri-*O*-acetate **14** by

Scheme I



Reagents: (a) H_2SO_4, MeOH. (b) *tert*-$BuPh_2SiCl$, DMAP, DMF, room temperature. (c) Bz_2O, Pyr. (d) *n*-Bu_4NF, THF. (e) RuO_2, $NaIO_4$, $CHCl_3$-CH_3CN-H_2O (2:2:3). (f) EDAC, DMAP, MeOH. (g) H_2SO_4, Ac_2O, AcOH. (h) TMSOTf, $ClCH_2CH_2Cl$. (i) KOH, MeOH. (j) acetone, *p*-TsOH, 2,2-dimethoxypropane, DMF. (k) N,N-*bis*[2-oxo-3-oxazolidinyl]phosphorodiamidic Cl^-, $MeNH_2$, Et_3N, CH_2Cl_2. l) 88%HCO_2H.

Scheme II



Reagents: (a) HCl, 2,2-dimethoxypropane, MeOH. (b) i. RuO_2, $NaIO_4$, $CHCl_3$-CH_3CN-H_2O (2:2:3). ii. EDAC, DMAP, MeOH. (c) H_2SO_4, Ac_2O, AcOH. (d) TMSOTf, $ClCH_2CH_2Cl$. (e) CH_3NH_2, THF.

concentrated sulfuric acid, acetic anhydride, and glacial acetic acid in 34 % yield. The acetate **14** was then condensed with silylated 2-chloro-N^6-(3-iodobenzyl)adenine using TMS triflate (4) Lewis acid catalyst to yield β-anomer **15** and α-anomer **16** in 52 % and

10 % yields, respectively. After the two isomers were separated using silica gel column chromatography, compound **15** was treated with methylamine in THF overnight at 50 °C in a sealed tube to give 2-chloro-IB-MECA **10** in 62 % yield. The α-isomer **17** was obtained according to the same method. When the reaction temperature was high (at 90 °C) 2-methylamino-IB-MECA was formed. Similarly, tritium was introduced in compound **15** using [^{3}H]-methylamine in methanol by heating for 6 h at 60 °C.

In conclusion, [^{3}H]-2-chloro-IB-MECA was synthesized either in 12 steps of overall 0.28 % yield (scheme I) via the 2',3'-dibenzoyl derivative or in 6 steps of overall 5.6 % yield starting from D-ribose (scheme II) via the 2',3'-diacetyl derivative. Thus, although scheme I provided an anomerically pure coupling approach, the preferred synthetic route was scheme II, which necessitated separation of the α and β- isomers chromatographically. Scheme II was a practical route to both the radioactive 2-chloro-IB-MECA and larger quantities of the unlabeled material needed for pharmacological studies. The problem of low reactivity of the 5'-COOH group towards activation was solved using aminolysis of the methyl ester with excess methylamine, which was found to be suitable even for the radiochemical synthesis.

Experimental Section

New compounds were characterized (and resonances assigned) by 300 MHz proton nuclear magnetic resonance spectroscopy using a Varian GEMINI-300 FT-NMR spectrometer. Unless noted, chemical shifts are expressed as ppm downfield from tetramethylsilane. Synthetic intermediates were characterized by chemical ionization mass spectrometry (NH_3) on a JEOL SX102 mass spectrometer. In the EI mode accurate mass was determined using a VG7070F mass spectrometer. C, H, and N analyses were carried out by Atlantic Microlabs (Norcross, GA), and ±0.4% was acceptable.

Methyl 1-O-acetyl-2,3-di-O-benzoyl-β-D-ribofuronate and methyl 1-O-acetyl-2,3-di-O-benzoyl-α-D-ribofuronate (4). A mixture of methyl 2,3-di-*O*-benzoyl-1-methoxy-β-D-ribofuronate (**3**, 0.5 g, 1.25 mmol), concentrated sulfuric acid (0.366 mL,

6.87 mmol), and acetic anhydride (1.237 mL, 13.1 mmol) in glacial acetic acid (6.22 mL) was stirred for 18 h at room temperature. The reaction mixture was adjusted to pH 4 with saturated $NaHCO_3$ solution. After methylene chloride (100 mL) was added, two layers were shaken, separated and aqueous layer was extracted with methylene chloride (2 x 50 mL). Combined organic layer and extracts were washed with saturated $NaHCO_3$, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness to give a crude mixture, which was purified on a silica gel column chromatography (hexanes-ethyl acetate, 3:1) to give compound **4** [R_f = 0.35 (hexanes-ethyl acetate, 3:1), 0.343 g, 64 %) as a thick syrup and compound **5** [R_f = 0.33 (hexanes-ethyl acetate, 3:1), 0.108 g] as a colorless solid. **4**: ^{1}H NMR ($CDCl_3$) δ 2.12 and 2.17 [2 x s, 3 H (1:1.7), OAc], 3.82 and 3.87 [2 x s, 3 H (1.7:1), OMe], 4.87 (d, *J* = 6.2 Hz, 0.63 H), 4.89 (s, 0.37 H), 5.62 (t, *J* = 5.1 Hz, 0.37 H), 5.75 (d, *J* = 4.8 Hz, 0.63 H), 5.94 (m, 0.37 H), 6.01 (pseudo t, *J* = 5.5 and 5.4 Hz, 0.63 H), 6.45 (s, 0.63 H), 6.77 (d, *J* = 4.6 Hz, 0.37 H), 7.28-8.10 (m, 10 H, Bz). Anal. Calcd for $C_{22}H_{20}O_9 \cdot 0.15C_6H_{14}$: C, 62.32; H, 5.05. Found: C, 62.55; H, 4.74. mass (CI NH_3) m/z 446 ($M^+ + 1 + NH_3$). Unknown compound (**5**): ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3 H, OAc), 3.55 (s, 3 H, OMe), 5.55 (d, *J* = 2.6 Hz, 1 H), 6.00 (dd, *J* = 8.7 and 3.5 Hz, 1 H), 6.04 (dd, *J* = 8.7 and 2.9 Hz, 1 H), 7.13 (s, 1 H), 7.45-8.05 (m, 10 h, Bz). mass (CI NH_3) m/z 548 ($M^+ + 1 + NH_3$).

Methyl 1-[2-chloro-*N*^{6}-(3-iodobenzyl)-adenin-9-yl]-2,3-di-*O*-benzoyl-β-D-ribofuronate (7). A mixture of 2-chloro-*N*^{6}-(3-iodobenzyl)adenine (162 mg, 0.42 mmol), ammonium sulfate (catalytic amount), and HMDS (15 mL) was refluxed for 3 h under N_2. After evaporation of the volatiles *in vacuo* with exclusion of moisture, the residue was dissolved in dry 1,2-dichloroethane (3 mL) and a solution of compound **4** (150 mg, 0.35 mmol) in dry 1,2-dichloroethane (6 mL) and TMS triflate (0.082 mL, 0.42 mmol) were added. The reaction mixture was stirred for 5 min at room temperature and refluxed for 48 h at 70 °C and for 48 h at 90 °C under N_2. After workup as usual, the residue was purified on a silica gel column chromatography (hexanes-ethyl acetate, 1:1) to yield compound **7** [R_f = 0.10 (hexanes-ethyl acetate, 1:1), 125 mg, 48 %] as thick syrup.

^{1}H NMR ($CDCl_3$) δ 3.93 (s, 3 H, OMe), 4.77 (br s, 2 H, CH_2), 4.99 (s, 1 H), 6.01 (pseudo t, *J* = 6.6 and 5.1 Hz, 1 H), 6.12 (dd, *J* = 5.0 and 2.3 Hz, 1 H), 6.29 (br s, 1 H, NH), 6.69 (d, *J* = 6.4 Hz, 1 H), 7.08 (pseudo t, *J* = 7.7 and 7.5 Hz, 1 H, H-16), 7.34-7.73 (m, 11 H, Ar), 7.93 (d, *J* = 7.6 Hz, 1 H, Ar), 8.05 (d, *J* = 7.5 Hz, 1 H, Ar), 8.48 (s, 1 H, H-8). Anal. Calcd for $C_{32}H_{25}N_5O_7Cl_1I_1 \cdot 0.15C_6H_{14} \cdot$ 0.3EtOAc: C, 53.43; H, 4.43; N, 8.37. Found: C, 53.16; H, 4.16; N, 8.02.

1-[2-Chloro-*N*^{6}-(3-iodobenzyl)-adenin-9-yl]-β-D-ribofuronic acid (8). A mixture of compound **7** (64.2 mg, 0.085 mmol) and potassium hydroxide (20 mg, 0.36 mmol) in methanol (0.3 mL) was stirred for 4.5 h at room temperature. After neutralization with glacial acetic acid, the solvent was removed by rotary evaporation. The residue was triturated with absolute ethanol and a solid was collected by suction and dried to give compound **8** (41 mg, 90.6 %). ^{1}H NMR (DMSO-d_6) δ 4.05 (d, *J* = 4.0 Hz, 1 H, H-3'), 4.09 (s, 1 H, H-4'), 4.32 (m, 2 H, H-2' and OH, exchangeable with D_2O), 4.60 (m, 2 H, CH_2), 5.12 and 5.17 (each: br s, 1 H, OH, exchangeable with D_2O), 5.90 (d, *J* = 6.9 Hz, 1 H, H-1'), 7.13 (pseudo t, *J* = 8.0 and 7.7 Hz, 1 H, H-16), 7.36 (d, *J* = 7.5 Hz, 1 H, H-17), 7.60 (d, *J* = 7.6 Hz, 1 H, H-15), 7.75 (s, 1 H, H-13), 8.88 (br s, 1 H, NH), 9.48 (s, 1 H, H-8). Mass (Cl NH_3) m/z 532 (M^+ + 1). Anal. Calcd for $C_{17}H_{15}N_5O_5Cl_1I_1 \cdot 5H_2O \cdot 2MeOH$: C, 33.27; H, 4.76; N, 10.21. Found: C, 33.01; H, 3.30; N, 10.18.

1-[2-Chloro-*N*^{6}-(3-iodobenzyl)-adenin-9-yl]-2,3-*O*-isopropylidene-β-D-ribofuronic acid (9). A mixture of compound **8** (39 mg, 0.07 mmol), 2,2-dimethoxypropane (0.5 mL), and *p*-toluenesulfonic acid (80 mg, 0.42 mmol) in acetone (2.5 mL) and DMF (2.5 mL) was stirred for 3 days at room temperature. The reaction mixture was neutralized with triethylamine and concentrated to dryness *in vacuo*. The residue was dissolved in chloroform and insolubles were removed by filtration. The filtrate was concentrated and purified by preparative TLC (chloroform-methanol-acetic acid, 85:10:5) to give compound **9** [R_f = 0.79 (chloroform-methanol-acetic acid, 85:10:5), 25.3 mg, 60%] as a solid. ^{1}H NMR (DMSO-d_6) δ 1.32 and 1.52 (each: s, 3 H,

isopropylidene), 4.50 (m, 1 H), 4.60 (m, 2 H), 5.10 (br s, 2 H), 6.16 (br s, 1 H, H-1'), 7.13 (t, J = 7.7 Hz, 1 H, H-16), 7.36 (d, J = 7.6 Hz, 1 H, H-17), 7.61 (d, J = 7.7 Hz, 1 H, H-15), 7.74 (s, 1 H, H-13), 7.95 (s, 1 H, H-8), 8.82 (br s, 1 H, exchangeable with D_2O, NH), 11.90 (br s, 1 H, exchangeable with D_2O, COOH). High resolution mass in FAB^+ mode m/z Calcd. for $C_{20}H_{19}N_5O_5Cl_1I_1$: 572.0198. Found: 572.0203.

Methyl 1-[2-chloro-N^6-(3-iodobenzyl)-adenin-9-yl]-β-D-ribofuronamide (10).

N,N-Bis-[2-oxo-3-oxazolidinyl]phosphinic chloride (2 mg, 0.0078 mmol) was added to a solution of compound **9** (4.5 mg, 0.0078 mmol) and triethylamine (0.0011 mL, 0.0078 mmol) in dry dichloroethane (1 mL). The suspension was stirred for 30 min under cooling in an ice-bath. 2.0 M Methylamine in THF (0.004 mL, 0.002 mmol) was added and triethylamine (0.001 mL, 0.0078 mmol) was added slowly. The reaction mixture was stirred for 2 h in an ice-bath and water (2 mL) was added to quench the reaction. Two layers were separated, and the aqueous layer was extracted with chloroform (2 x 5 mL). Combined organic layer and extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, concentrated, and dried *in vacuo* to give crude mixture of methyl 1-[2-chloro-N^6-(3-iodobenzyl)-adenin-9-yl]-2,3-*O*-isopropyli-dene-β-D-ribofuronamide. A mixture of crude isopropylidene compound and 88% formic acid (2 mL) was stirred for 4.5 h at room temperature. The reaction mixture was concentrated to dryness and purified by preparative TLC (chloroform-methanol, 10:1) to give compound **10** (0.706 mg, 16.5 %). ^1H NMR spectrum was identical to the authentic sample (6). UV (MeOH) λ_{max} 272.5 nm.

Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (11). A solution of D-ribose (Aldrich Chemical Co., 50 g, 0.33 mol) in acetone (1 L), 2,2-dimethoxypropane (100 mL), and methanol (200 mL) was treated with methanol (20 mL) saturated with hydrogen chloride. The solution was kept at 0 °C for 15 min and stirred for 2 days at room temperature. The yellow solution was neutralized with pyridine and concentrated under vacuum. The residue was partitioned in water (500 mL) and ether (200 mL), and aqueous layer was extracted with ether (2 x 200 mL). The organic layer and extracts

were combined and dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue syrup was distilled to give compound **11** (46.857 g, 69 %) at 0.067 mmHg/88 °C or at 0.15 mmHg/101 °C. ^{1}H NMR (DMSO-d_6) δ 1.25 and 1.37 (each: s, 3 H, isopropylidene), 3.21 (s, 3 H, OMe), 3.32 (d, J = 10.4 Hz, 1 H, H-5a), 3.39 (dd, J = 11.0 and 6.0 Hz, 1 H, H-5b), 4.00 (m, 1 H, H-4), 4.53 (d, J = 5.7 Hz, 1 H, H-3), 4.67 (d, J = 5.9 Hz, 1 H, H-2), 4.77 (br s, 1 H, exchangeable with D_2O, OH), 4.87 (s, 1 H, H-1). mass (CI NH_3) m/z 222 (M^+ + 1 + NH_3, base).

Methyl 1-*O*-methyl-2,3-*O*-isopropylidene-β-D-ribofuronate (13). A mixture of compound **11** (11.22 g, 55 mmol), sodium periodate, (58.8 g, 275 mmol), ruthenium oxide (II) (0.1 g, 0.75 mmol) in acetonitrile-chloroform-water (2:2:3, 175 mL) was stirred for 5 h at room temperature. After the solid was filtered off, the filtrate was separated, and aqueous layer was extracted with chloroform (2 x 30 mL). The organic layer and extracts were combined and washed with brine (100 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was dried *in vacuo* overnight to give crude compound **12** which was used for next reaction without purification. m.p. 130-131 °C. ^{1}H NMR ($CDCl_3$) δ 1.26 and 1.42 (each: s, 3 H, isopropylidene), 3.38 (s, 3 H, OMe), 4.52 (d, J = 5.5 Hz, 1 H), 4.62 (s, 1 H), 5.01 (s, 1 H), 5.12 (d, J = 5.7 Hz, 1 H). mass (CH NH_3) m/z 236 (M^+ + 1 + NH_3, base).

A solution of compound **12**, 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDAC, Sigma Chemical Co., 15 g, 78 mmol), and dimethylaminopyridine (DMAP, 0.67 g, 5.5 mmol) in methanol (250 mL) was stirred for 23 h at room temperature. After the reaction mixture was concentrated to dryness, the residue was dissolved in ethyl acetate (200 mL) and water (50 mL). Two layers were separated after shaking, and the aqueous layer was extracted with ethyl acetate (2 x 50 mL). The organic layer and extracts were combined and washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The residue was purified on a silica gel column chromatography (hexanes-ethyl acetate, 1:1) to give compound **13** (9.48 g, 74 % from **11**) as a colorless liquid. ^{1}H NMR ($CDCl_3$) δ 1.25 and 1.41 (each: s, 3 H, isopropylidene),

3.32 (s, 3 H, 1-OMe), 3.69 (s, 3 H, 5-OMe), 4.47 (d, J = 5.7 Hz, 1 H), 4.53 (s, 1 H), 4.95 (s, 1 H), 5.15 (d, J = 5.8 Hz, 1 H). mass (CH NH_3) m/z 250 (M^+ + 1 + NH_3, base).

Methyl 1,2,3-tri-*O*-acetyl-β-D-ribofuronate (14). A mixture of compound **13** (9 g, 38.7 mmol), conc. sulfuric acid (11.4 mL, 213 mmol), acetic anhydride (38.4 mL, 407 mmol), and glacial acetic acid (193 mL) was stirred for 21.5 h at room temperature. The reaction mixture was poured into ice-water (500 mL) and extracted with chloroform (2 x 300 mL). Extracts were combined and dried over anhydrous $MgSO_4$, filtered, concentrated, and dried *in vacuo* overnight to give crude compound **14** (3.70 g, 32.5 %) as a colorless liquid. This crude product was used for the next reaction without purification. ^{1}H NMR ($CDCl_3$) δ 2.07, 2.11, 2.14 (each: s, 3 H, OAc), 3.79 (s, 3 H, OMe), 4.62 (d, J = 6.6 Hz, 1 H), 5.37 (d, J = 5.0 Hz, 1 H), 5.65 (pseudo t, J = 6.2 and 5.1 Hz, 1 H), 6.21 (s, 1 H, H-1). mass (CI NH_3) m/z 322 (M^+ + 1 + NH_3).

Methyl 1-[2-chloro-N^6-(3-iodobenzyl)-adenin-9-yl]-2,3-di-*O*-β-D-ribofuronate (15) and **Methyl 1-[2-chloro-N^6-(3-iodobenzyl)-adenin-9-yl]-2,3-di-*O*-α-D-ribofuronate (16).** A mixture of 2-chloro-N^6-(3-iodobenzyl)adenine (1.1 g, 2.85 mmol), ammonium sulfate (catalytic amount), and hexamethyldisilazane (20 mL) was refluxed for 1 h under nitrogen. After the clear solution was concentrated to dryness with exclusion of moisture, the residue was dissolved in dry 1,2-dichloroethane (12 mL), and a solution of crude compound **14** (0.73 g) in 1,2-dichloroethane and TMS triflate (0.5 mL, 2.59 mmol) were added. The slightly yellow solution was stirred for 2 h at room temperature and refluxed for 48 h under a nitrogen atmosphere. Saturated $NaHCO_3$ (20 mL) and methylene chloride (20 mL) were added, and the reaction mixture was stirred for 15 min. Two layers were separated, and the aqueous layer was extracted with methylene chloride (3 x 30 mL). The organic layer and extracts were combined, washed with saturated $NaHCO_3$ (30 mL), brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to dryness. The foamy residue was purified by silica gel column chromatography (hexanes-ethyl acetate, 1:1) to give compounds **15** [R_f = 0.2 (hexanes-ethyl acetate, 1:1), 0.805 g, 52 %] and **16** [R_f = 0.14 (hexanes-ethyl acetate, 1:1), 0.157 g,

10 %] as foams. **15**: ^{1}H NMR ($CDCl_3$) δ 1.98 and 2.14 (each: s, 3 H, OAc), 3.80 (s, 3 H, OMe), 4.67 (s, 1 H, H-4'), 4.70 (br s, 2 H, C$\underline{H}_2$Ph), 5.65 (m, 2 H, H-2', H-3'), 6.12 (br s, 1 H, N^{6}H), 6.36 (d, J = 5.9 Hz, 1 H, H-1'), 7.02 (t, J = 7.8 Hz, 1 H, H-16), 7.28 (d, J = 7.6 Hz, 1 H, H-15), 7.57 (d, J = 7.8 Hz, 1 H, H-17), 7.67 (s, 1 H, H-13), 8.31 (s, 1 H, H-8). UV (MeOH) λ_{max} 272.5 nm. mass (EI) m/z 629 (m^+), 385 (m^+ - sugar), 245 (m^+ - base). **16**: ^{1}H NMR ($CDCl_3$) δ 2.05 and 2.12 (each: s, 3 H, OAc), 3.79 (s, 3 H, OMe), 4.71 (br s, 2 H, C$\underline{H}_2$Ph), 4.93 (d, J = 2.9 Hz, 1 H, H-4'), 5.52 (t, J = 2.7 Hz, 1 H, H-2'), 5.65 (t, J = 2.3 Hz, 1 H, H-2'), 6.05 (br s, 1 H, N^{6}H), 6.28 (d, J = 2.3 Hz, 1 H, H-1'), 7.02 (t, J = 7.7 Hz, 1 H, H-16), 7.28 (d, J = 7.5 Hz, 1 H, H-15), 7.58 (d, J = 7.3 Hz, 1 H, H-17), 7.67 (s, 1 H, H-13), 7.84 (s, 1 H, H-8). UV (MeOH) λ_{max} 272.5 nm. mass (EI) m/z 629 (m^+), 385 (m^+ - sugar), 245 (m^+ - base).

Methyl 1-[2-chloro-*N*^{6}-(3-iodobenzyl)-adenin-9-yl]-β-D-ribofuron-amide (10). A solution of compound **15** (30 mg, 0.047 mmol) and 2.0 M methylamine in THF (1 mL) was heated for 20 h at 50 °C in a sealed bottle. After the reaction mixture was concentrated to dryness, the residue was purified on a preparative silica gel TLC plate (chloroform-methanol, 20:1) to give compound **10** (16 mg, 62 %) as a colorless solid. The ^{1}H NMR spectra of this was identical to that of authentic sample (1).

Methyl 1-[2-chloro-*N*^{6}-(3-iodobenzyl)-adenin-9-yl]-α-D-ribofuron-amide (17). A solution of compound **16** (27 mg, 0.043 mmol) and 2.0 M methylamine in THF (1 mL) was heated for 20 h at 50 °C in a sealed bottle. After the reaction mixture was concentrated to dryness, the residue was purified on a preparative silica gel TLC plate (chloroform-methanol, 20:1) to give compound **17** (14.5 mg, 62 %) as a colorless solid. mp 121-122 °C. ^{1}H NMR (DMSO-d_6) δ 2.62 (d, J = 4.6 Hz, 3 H, NHMe), 4.26 and 4.34 (each: s, 1 H, H-2', H-3'), 4.61 (br s, 3 H, C$\underline{H}_2$Ph, H-4'), 5.86 (d, J = 4.6 Hz, 1 H, exchangeable with D_2O, OH), 5.95 (d, J = 4.3 Hz, 1 H, exchangeable with D_2O, OH), 6.10 (s, 1 H, H-1'), 7.13 (t, J = 7.7 Hz, 1 H, H-16), 7.36 (d, J = 7.6 Hz, 1 H, H-17), 7.61 (d, J = 8.0 Hz, 1 H, H-15), 7.74 (s, 1 H, H-13), 8.05 (br s, 1 H, exchangeable with D_2O, NH), 8.38 (s, 1 H, H-8), 8.93 (br s, 1 H, NH). mass (CI NH_3) m/z 545 (m^+ + 1).

Preparation of 2-Chloro-IB-[^{3}H]MECA.

A saturated solution of potassium hydroxide in methanol (2 mL) was added to a flask containing [^{3}H]-methylamine hydrochloride (1.28 Ci, 0.044 mmol). This solution was then short path distilled into a flask containing compound **15** (4.3 mg, 0.0068 mmol). The reaction flask was heated at 60 °C for 2 h, left at ambient temperature overnight, then heated again at 60 °C for a further 4 h. The reaction mixture was rotary evaporated to dryness. Methanol (10 mL) was added and the solution rotary evaporated to dryness to remove any remaining labile tritium. The residues were dissolved in ethanol (10 mL). A count of this solution showed a crude yield of 38 mCi.

HPLC purification: The crude active solution was rotary evaporated to approximately 0.5 mL, diluted with 0.5 mL of water and purified by injection on the following system.

Column:	Hypersil BDS-C_{18} (250 x 4.6 mm)
Eluent A:	ethanol:water (4:6)
Eluent B:	ethanol:water (1:1)
Gradient:	0%B to 50%B over 30 minutes
Flow:	1 mL/min.
UV:	270 nm

The active peak (retention time 48-52 min) was collected. As this material was contaminated with UV absorbing impurities the above HPLC was repeated. Yield after the second HPLC: 9.8 mCi.

Analysis: HPLC analysis on a Hypersil MOS (150 x 4.6 mm) column with a 30 min gradient from water to methanol showed material with a radiochemical purity of 99% and co-eluting with the supplied inactive material. Mass spectrometry gave a spectrum that was consistent with the required structure and a specific activity of 29 Ci/mmol.

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