Synthesis of Ethyl (1*S*,2*R*,3*S*,4*S*,5*S*)-2,3-*O*-(Isopropylidene)-4-Hydroxy-Bicyclo[3.1.0]Hexane-Carboxylate from L-Ribose: A Versatile Chiral Synthon for Preparation of Adenosine and P2 Receptor Ligands

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SYNTHESIS OF ETHYL (1*S*,2*R*,3*S*,4*S*,5*S*)-2,3-*O*-(ISOPROPYLIDENE)-4-HYDROXY-BICYCLO[3.1.0]HEXANE-CARBOXYLATE FROM L-RIBOSE: A VERSATILE CHIRAL SYNTHON FOR PREPARATION OF ADENOSINE AND P2 RECEPTOR LIGANDS

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□ *Substitution of the ribose moiety of various nucleosides and nucleotides with the (N)-methanocarba ring system increases the potency and selectivity as ligands at certain subtypes of adenosine and P2 receptors. We have prepared a key intermediate in the synthesis of these derivatives, ethyl (1S,2R,3S,4S,5S)-2,3-O-(isopropylidene)-4-hydroxybicyclo[3.1.0]hexane-carboxylate (15), starting from L-ribose (8) as a readily available, enantiopure building block. L-ribose was converted to the corresponding 5′-iodo derivative (9), which was cleaved reductively with Zn. Improvements were made in subsequent steps corresponding to a published route to biologically important (N)-methanocarba 5′-uronamido nucleosides, and new steps were added to prepare related 5′-nucleotides.*

Keywords G protein–coupled receptor; Nucleosides; Nucleotides; Purines; Pyrimidines; Iodination

INTRODUCTION

In solution, the conformation of the ribose ring of nucleosides and nucleotides exists in a rapid, dynamic two-state equilibrium between a (N)

(1a)   R = 3-Cl, MRS3558

(1b)   R = 2,5-di-OCH$_3$, MRS3602

(2)   R = Cl, n = 1, MRS2339

(3)   R = SCH$_3$, n = 2, MRS2365
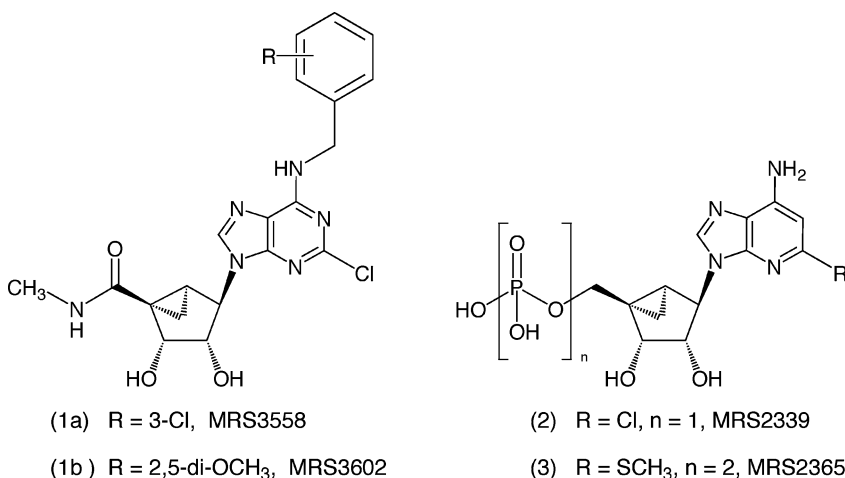
CHART 1 Structures of biologically-important (N)-methanocarba nucleoside derivatives **1a, b** that act as adenosine A$_3$ receptor ligands and nucleotides **2, 3** that act as P2 receptor ligands.

(Northern, 2′-exo) or (S) (Southern, 2′-endo) pucker conformation.[1,2] It is expected that the target receptors for these small molecules may prefer one of these conformations. It follows logically that when the nucleoside furanose ring is locked in either a (N) or (S) conformation an enhancement of potency and selectivity towards the target receptors may occur. Recently, we have successfully executed this approach by replacing the ribose moiety with the (N)-methanocarba ring system in nucleosides/nucleotides (Chart 1), and demonstrated enhanced selectivity and potency for therapeutically important A$_3$ adenosine receptors (ARs, e.g., **1**) and P2 nucleotide receptors (e.g., **2, 3**).[3–6]

Both purine and pyrimidine nucleotides have been synthesized bearing the (N)-methanocarba modification of ribose.[7] Our previously reported synthetic route for the above (N)-methanocarba adenine nucleosides is depicted in Scheme 1.[3] By following this synthetic approach and by employing the methanocarba sugar moiety as key synthon we have recently reported a highly A$_3$AR selective series of (N)-methanocarba 5′-*N*-methyluronamide derivatives, prominent among them were MRS3558 **1a**
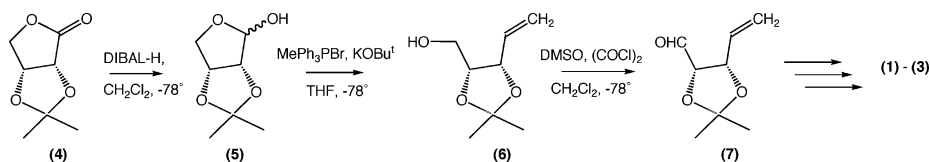
SCHEME 1 Previously reported initial steps in the synthesis of (N)-methanocarba nucleoside and nucleotide derivatives **1–3**.

and MRS73602 **1b**.[4] In particular, MRS3558 has a K_i value in binding to the human A_3AR of 0.3 nM with at least 900-fold selectivity for the A_3AR in comparison to other subtypes, that is, A_1, A_{2A}, and A_{2B} ARs. Recently, Matot and coworkers have shown that MRS3558 is effective in attenuating reperfusion lung injury in cats.[8] This result suggests that A_3AR agonists have potential as an effective therapy for ischemia/reperfusion-induced lung injury. In addition, Fishman and coworkers have studied extensively A_3AR agonists as potential inhibitors in a model of adjuvant-induced arthritis in rats.[9] P2X and P2Y receptors are effectively targeted using related nucleotide derivatives, for example, MRS2339 **2** and MRS2365 **3**, respectively.[10,11] Clearly, the potential therapeutic use of (N)-methanocarba nucleoside and nucleotides that act as receptor ligands demands their easy access in multigram amounts for further biological and clinical studies.

We previously reported a new synthetic route for the above (N)-methanocarba nucleosides starting from 2,3-*O*-isopropylidene-D-erythronolactone **4** as depicted in Scheme 1.[3] However, this scheme includes three low temperature steps, an inconsistent, low-yielding Wittig reaction to provide **6** and a subsequent tedious Swern oxidation step to provide **7**. The low and inconsistent yields in the Wittig reaction on large scale may be attributed to the quality of potassium *t*-butoxide and also an excess of Wittig salt in the reaction. In addition, the starting material 2, 3-*O*-isopropylidene-D-erythronolactone **4** is expensive and not readily available. In this communication we delineate a modified procedure for preparing (N)-methanocarba nucleosides, in particular **1a**, on a reasonably large scale. Toward this endeavor we evaluated L-ribose as a less expensive and readily available starting material, using reactions similar to those previously reported using D-ribose.[12,13] Synthesis of **7** from L-ribose avoided problems of the Wittig reaction and Swern oxidation encountered in the previous route. Improvements also were made in steps subsequent to **7** corresponding to our published route to biologically important (N)-methanocarba nucleosides and nucleotides.

METHODS

Chemical Synthesis

^{1}H NMR spectra (300 MHz) were obtained with a Varian Gemini 300 spectrometer (Varian, Inc., Palo Alto, CA, USA). ^{31}P NMR spectra were recorded at room temperature with a Varian XL 300 spectrometer (121.42 MHz); orthophosphoric acid (85%) was used as an external standard. Purity of compounds was checked using a Hewlett-Packard 1100 HPLC equipped with a Luna 5 μm RP-C18(2) analytical column (250 mm × 4.6 mm; Phenomenex, Torrance, CA, USA). System A parameters consisted of the following: linear gradient solvent system of H_2O/CH_3CN from 95/5

to 20/80 in 20 minutes; flow rate of 1 mL/minute. System B parameters consisted of the following: linear gradient solvent system of 5 mm TBAP/CH$_3$CN from 80/20 to 20/80 in 20 minutes followed by isocratic for 2 minutes; flow rate of 1 mL/minute. Peaks were detected by UV absorption with a diode array detector.

Materials

All reagents were from standard commercial sources and of analytical grade.L-ribose was purchased from V-Labs, Inc. (Covington, LA, USA).

Ethyl (4S,5S)-3-[2,2-dimethyl-5-vinyl(1,3-dioxolan-4-yl)]-3-oxopropanoate (10). Concentrated HCl (5 mL) was added to a suspension of L-ribose **8** (150 g, 1 mol) in acetone (400 mL) and methanol (400 mL) at room temperature. The resulting reaction mixture was refluxed for 3 hours and cooled to room temperature. It was then neutralized with pyridine and concentrated in vacuo. The residue was partitioned between water (300 mL) and ethyl acetate (300 mL three times). The combined organic phase was washed with a saturated copper sulfate solution followed by brine, dried over sodium sulfate, and concentrated. The crude residue was used in the next step without further treatment.

The above crude methyl ethers (164 g) were dissolved in toluene (750 mL) and the solution was treated with imidazole (85 g, 1.25 mol), triphenylphosphine (243 g, 0.93 mol), and acetonitrile (200 mL). The resulting reaction mixture was heated to 70°C, and iodine (253 g, 1 mol) was added carefully in small portions till the reaction mixture maintained a dark brown appearance. The reaction temperature was held constant at 70°C for 1 hour. The reaction mixture was filtered, and the residue was washed with toluene (500 mL). The combined organic layer was washed with 5% sodium thiosulfate solution, water, and brine and then concentrated. The crude residue was first treated with a mixture of hexanes and ethyl acetate (3:1). Solid triphenylphosphine oxide precipitated, and the mixture was filtered and the filtrate concentrated. This process was repeated to remove most of the triphenylphosphine oxide. A further purification of the concentrated filtrate by flash column chromatography (silica gel, ethyl acetate:hexanes, 1:10) afforded the iodo derivative **9** as the mixture of anomers (216 g, colorless oil, 69% in two steps). $\alpha_D^{25} + 3.1°$ (c = 2.7, EtOH).

Powdered Zn (9.75 g, 150 mmol) was added to the solution of the iodides **9** (31.4 g, 100 mmol) in isopropanol (100 mL). To this resulting reaction slurry, acetic acid (10.5 g, 175 mmol) was slowly added in 30 minutes (cold water bath was used to control the reaction temperature), and the reaction mixture was maintained at 30°C and stirred for an additional 2 hours. The solution was decanted and concentrated under reduced

pressure. The residue obtained was partitioned with ethyl acetate (200 mL), and water (100 mL) and stirred for 30 minutes. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layer was washed with brine, dried and concentrated to afford crude aldehyde **7** as a colorless oil (12.8 g), which was carried forward for the next step.

Crude aldehyde **7** in dichloromethane (25 mL) was treated with anhydrous $SnCl_2$ (1.9 g, 10 mmol) and ethyldiazoacetate (9.3 g, 82 mmol) in dichloromethane (80 mL) was added drop wise over a period of 1 hour at 0°C. The mixture was then allowed to stir at room temperature for 1 hour. The reaction mixture was filtered through a pad of Celite and concentrated. The desired keto ester was purified by column chromatography (silica gel, hexanes: ethyl acetate, 95:5) to afford **10**, (16.3 g, 68% based on the iodide derivative) as an oil. ^{1}H NMR ($CDCl_3$) δ 11.92 (s, 0.1H, D_2O exchangeable, enolic O*H*), 5.93–6.08 (m, 1H), 5.39–5.56 (m, 2H), 4.46–4.72 (m, 1H), 4.13–4.38 (m, 3H), 3.65 (AB q, 2H, $J = 17.4$ Hz), 1.64–1.57 (m, 6H, 2), 1.34–1.41 (m, 3H); HRMS $(M - 1)^-$: calculated 241.1076, found 241.1084.

*Ethyl (4*S*,5*S*)-3-[2,2-dimethyl-5-vinyl(1,3-dioxolan-4-yl)]-2-diazo-3-oxopropanoate (11).* Neat triethylamine (28.3 g, 280 mmol) was added dropwise to a solution of keto ester **10** (34.0 g, 140 mmol) and tosyl azide (27.6 g, 140 mmol) in acetonitrile (150 mL) at 0°C. The reaction mixture was stirred for 30 minutes at 0°C and then a further 30 minutes at room temperature followed by evaporation. The residue was dissolved in chloroform (100 ml) and the resulting precipitate was removed by filtration. The filtrate was diluted with hexane (100 mL), filtered again, and evaporated. The residue was purified by column chromatography (silica gel, 5% to 10% ethyl acetate-hexane) to afford the title compound as pale yellow oil (36.0 g, 133 mmol, 95%). ^{1}H NMR ($CDCl_3$) δ 6.01 (ddd, 1H, $J = 16.3, 10.4, 7.4$ Hz), 5.70 (d, 1H, $J = 7.6$ Hz), 5.60 (d, 1H, $J = 17.1$ Hz), 5.41 (d, 1H, $J = 10.2$ Hz), 4.97 (t, 1H, $J = 7.5$ Hz), 4.30 (q, 2H, $J = 7.1$ Hz), 1.65 (s, 3H), 1.42 (s, 3H), 1.32 (t, 3H, $J = 7.1$ Hz); FAB MS *m/z* (relative intensity) 269 (MH$^+$, 86). $\alpha_D^{25} + 6.1°$ (c = 1.1).

*Ethyl (1*S*,3*S*,4*S*,5*S*)-3,4-O-isopropylidene-2-oxobicyclo[3.1.0]hexanecarboxylate (12).* A solution of diazo compound **11** (26.9 g, 100 mmol) in dry toluene (100 mL) was added through a dropping funnel over a period of 4 hours to a refluxing mixture of toluene (25 mL) and CuI (3.8 g, 20 mmol). The reaction mixture was refluxed for 1 hour. The reaction mixture was cooled to room temperature, concentrated and purified by column chromatography (silica gel; hexanes:ethyl acetate, 70:30) to provide bicyclic compound **12** as a colorless oil (12.24 g, 51%) and compound **13** as a pale yellow oil (3.36 g, 14%).

12: ^{1}H NMR ($CDCl_3$) δ 5.13 (ddd, 1H, $J = 9.3, 5.6, 1.2$ Hz), 4.37 (d, 1H, $J = 8.3$ Hz), 4.21 (q, 2H, $J = 7.3$ Hz), 2.75 (dt, 1H, $J = 8.7, 5.4$ Hz),

2.10 (dd, 1H, $J = 8.6, 5.1$ Hz), 1.81(t, 1H, $J \sim 5.6$ Hz), 1.52 (s, 3H), 1.31(s, 3H), 1.29 (t, 3H, $J = 7.1$ Hz); HRMS (M + 1)$^+$: calculated 241.1076, found 241.1092; $\alpha_D^{25} + 9.5°$ (c = 1.1, EtOH). **13**: ^{1}H NMR (CDCl$_3$) δ 4.87 (d, 1H, $J = 5.1$ Hz), 4.48 (dd, 1H, $J = 4.6, 1.6$ Hz), 4.31 (dq, 2H, $J = 7.1, 1.5$ Hz), 2.92 (dd, 1H, $J = 8.7, 5.8$ Hz), 2.31 (ddd, 1H, $J = 8.8, 5.6, 1.7$ Hz), 1.56 (s, 3H), 1.41 (s, 3H), 1.32 (t, 3H, $J = 4.1$ Hz), 182 (t, 1H, $J = 5.1$ Hz); HRMS (M + 1)$^+$: calculated 241.1076, found 241.1082; $\alpha_D^{25} + 4.9°$ (c = 0.65, EtOH).

Ethyl   (1S,2R,3S,4S,5S)-3,4-O-isopropylidene-2-hydroxybicyclo[3.1.0]hexane-carboxylate (14).   NaBH$_4$ (0.38 g, 10 mmol) was added to a stirred solution of **12** (2.4 g, 10 mmol) in ethanol (30 mL) at room temperature, and stirring was continued for an additional 1 hour. The reaction mixture was then treated with acetone (4 mL) and concentrated to dryness. The residue was purified by column chromatography (silica gel; hexanes:ethyl acetate, 70:30) to give compound **14** (1.68 g, 69%) as a white solid. **14:** m.p. 111°C (cyclohexane); ^{1}H NMR (CDCl$_3$) δ 5.05 (t, 1H, $J = 7.5$ Hz), 4.91 (t, 1H, $J = 6$ Hz), 4.66 (t, 1H, $J = 7$ Hz), 4.14–4.30, (m, 2H), 2.46 (d, 1H, $J = 12$ Hz, OH), 2.16–2.29(m, 1H), 1.38–1.54(m, 5H), 1.31 (s, 3H), 1.24 (t, 3H, $J = 6.5$ Hz). HRMS (M + 1)$^+$: calculated 243.1232, found 243.1233.

Ethyl   (1S,2R,3S,4S,5S)-2,3-O-(isopropylidene)-4-hydroxybicyclo[3.1.0]hexane-carboxylate (15).   A solution of **14** (0.96 g, 4.0 mmol) and trifluoroacetic acid (0.68 g, 6 mmol) in acetone (20 mL) was stirred at 25°C for 6 hours. The reaction mixture was then concentrated under reduced pressure. The residue was dissolved in CHCl$_3$ (50 mL) and washed with 10% sodium bicarbonate solution. The organic layer was concentrated to furnish a mixture of isomerized alcohols **14** and **15** in a 6:4 ratio based on NMR. This crude mixture was further purified by crystallization from cyclohexane (20 mL), in which alcohol **14** separates out as crystals. The mother liquor was concentrated to afford the requisite **15** (0.340 g, 35%) as a colorless solid with purity >80%. The analytical sample of alcohol **15** can be obtained by repeated recrystallization from cyclohexane. **15**: m.p. 83°C (cyclohex-ane). ^{1}H NMR (CDCl$_3$) δ 5.35 (d, 1H, $J = 5.8$ Hz), 4.38–4.56 (m, 2H), 4.04–4.19 (m, 2H), 2.36–2.41 (m, 2H), 1.41–1.62 (m, 5H), 1.33 (s, 3H), 1.13 (t, 2H, $J = 3.2$ Hz); HRMS (M + 1)$^+$: calculated 243.1232, found 241.1245.

Ethyl   (1′S,2′R,3′S,4′R,5′S)-4′-(2,6-dichloropurin-9-yl]-2′,3′-O-(isopropylidene)-bicyclo[3.1.0]hexanecarboxylate (16).   A mixture of triphenyl phosphine (1.04 g, 4.0 mmol) and 2,6-dichloropurine (0.756 g, 4.0 mmol) in dry THF (5 mL) was treated with diisopropyl azodicarboxylate (0.80 g, 4.0 mmol) at room temperature. After stirring for 20 minutes, a solution of the methanocarba sugar **15** (0.48 g, 2.0 mmol) in THF (5 mL) was added and the mixture was stirred an additional 8 hours. Concentration and purification of the residue by repeated column chromatography (silica gel;

ethyl acetate:hexanes, 3:1) provided provided pure **16** (0.346 g, 42%) as a colorless amorphous solid.

16: ^{1}H NMR (CDCl$_3$) δ 8.06 (s, 1H), 5.81 (d, 1H, 6.7 Hz), 4.92 (s, 1H), 4.68 (d, 1H, $J = 5.5$ Hz), 4.05–4.36 (m, 2H), 2.14–2.2 (m, 1H), 1.75–1.82 (m, 1H), 1.52–1.62 (m, 4H), 1.11–1.35 (m, 6H); HRMS (M + 1)$^+$: calculated 413.0783, found 413.0780; $\alpha_D{}^{25} + 2.6°$ (c = 1.4, EtOH).

(1′S,2′R,3′S,4′S,5′S)-4′-[6-(3-Chlorobenzylamino)-2-chloropurin-9-yl]-2′,3′-O-isopropylidenebicyclo[3.1.0]hexane-1′-carboxylic Acid Ethyl Ester (17). 3-Chloro-benzylamine (0.705 g, 5.0 mmol) was added to a solution of **16** (0.413 g, 1.0 mmol) and triethylamine (2 mL) in methanol (5 mL). The mixture was stirred at room temperature for 3 hours, and then concentrated in vacuo to dryness. The residue was purified by column chromatography (silica gel; chloroform:methanol, 10:1) to give **17** as a pale yellow oil (0.316 g, 61%). ^{1}H NMR (CDCl$_3$) δ 1.18–1.37 (m, 3H), 1.41–1.85 (m, 8H), 2.18–2.29 (m, 1H), 4.05–4.41 (m, 2H), 4.78–4.87 (m, 3H), 5.39 (d, $J = 4.8$ Hz, 1H), 5.82 (d, $J = 7.6$ Hz, 1H), 6.24 (br s, 1H), 7.21–7.42 (m, 4H), 7.68 (s, 1H). HRMS (M + 1)$^+$: calculated 518.1362, found 518.1357; $\alpha_D{}^{25} + 3.0°$ (c = 0.4, EtOH).

(1′S,2′R,3′S,4′S,5′S)-4′-[6-(3-Chlorobenzylamino)-2-chloropurin-9-yl]-2′,3′-di-hydroxybicyclo[3.1.0]hexane-1′-carboxylic Acid Methylamide (1a). Compound **17** (0.518 g, 1 mmol) was dissolved in methanol (5 mL) and treated with an aqueous solution of methylamine (2 mL, 40%). This mixture was stirred at room temperature for 12 hours, then evaporated to dryness. This crude intermediate was treated directly with a solution of trifluoroacetic acid in MeOH (15 mL, 10%) and H$_2$O (0.5 mL), and the mixture was heated at 70°C for 3 hours. The solution was cooled, and the solvent was removed to dryness by coevaporation with acetonitrile in vacuo. The white residue was purified by flash column chromatography (silica gel, chloroform:methanol, 8:2) to give the final product **1a** as a white powder (0.250 g, 54%). M.p. 122°C (decomp). ^{1}H NMR (CDCl$_3$) δ 1.18–1.39 (m, 1H), 1.46–1.63 (m, 1H), 2.04–2.09 (m, 1H), 2.83 (d, $J = 4.8$ Hz, 1H), 3.87–4.08 (m, 2H), 4.78–5.05 (m, 5H), 6.73 (br s, 1H), 7.04 (br s, 1H), 7.13–7.31 (m, 4H), 7.75(s, 1H). HRMS (M + 1)$^+$: calculated 463.1052, found 463.1053. HPLC (system A) 14.8 minutes (99%), (system B) 12.5 minutes (99%). $\alpha_D{}^{25} + 1.2°$ (c = 1.0, EtOH).

(1′S,2′R,3′S,4′R,5′S)-4-(2,6-dichloro-purin-9-yl)-1-[hydroxymethyl]bicyclo-[3.1.0]-hexane-2,3-(O-isopropylidine) (18). A stirred solution of ester **16** (0.207 g, 0.5 mmol) in CH$_2$Cl$_2$ (10 mL) cooled to −78°C was treated dropwise with DIBAL-H (1.5 mL, 1.5 M solution in toluene) and stirred at that temperature for 1 hour. Methanol (1.5 mL) and 1 M cold sulfuric acid (10 mL) were added carefully to adjust the reaction mixture to pH 4–5. The reaction mixture was filtered through a pad of Celite, and the filtrate was separated. The aqueous phase was extracted with dichloromethane, and the

combined organic layer was evaporated and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, $CHCl_3$:MeOH, 10:1) to give alcohol **18** (0.103 g, 56%) as a solid. ^{1}H NMR ($CDCl_3$) δ 1.05–1.08 (m, 1H), 1.21–138 (m, 7H), 1.70–1.74 (m, 1H), 3.51(d, $J = 12$ Hz, 1H), 4.21–4.27 (m, 1H), 4.63 (d, $J = 7.5$ Hz, 1H), 4.96 (s, 1H), 5.51 (d, $J = 12$ Hz, 1H), 8.32 (s, 1H). HRMS $(M + 1)^+$: calculated 371.0678, found 371.0669.

(1′S,2′R,3′S,4′R,5′S)-4-(6-amino-2-chloro-purin-9-yl)-1-[hydroxymethyl]bicyclo-[3.1.0]hexane-2,3-(O-isopropylidine) (19). Compound **18** (0.186 g, 0.5 mmol) was treated with 2 M ammonia in isopropyl alcohol (2 mL) and stirred for 8 hours. The resulting reaction mixture was concentrated and the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH, 80:20) to afford **19** (0.08 g, 46%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1H), 5.89 (bs, 2H), 5.56 (d, 1H, $J = 6.4$ Hz), 4.76 (s, 1H), 4.66 (d, 1H, $J = 7.4$ Hz), 4.26 (d, 1H, $J = 11.5$ Hz), 3.37 (d, 1H, $J = 11.5$ Hz), 1.78–1.70 (m, 1H), 1.55 (s, 3H), 1.26 (s, 3H), 1.14–1.20 (m, 1H), 0.96–1.20 (m, 1H). HRMS $(M + 1)^+$: calculated 352.1176, found 353.1163.

*(1′S,2′R,3′S,4′R,5′S)-4-(6-Amino-2-chloro-9H-purin-9-yl)-1-[(di-*tert*-butyl phosphate)methyl]bicyclo[3.1.0]hexane-2,3-(O-isopropylidene) (20).* Neat di-*t*-butyl *N*,*N*-diethylphosphoramidite (0.075 g, 0.3 mmol) was added to a stirred solution of **19** (0.035 g, 0.1 mmol) in anhydrous THF (2 mL) at room temperature followed by addition of tetrazole (0.063 g, 0.9 mmol). After 1 hour the reaction mixture was cooled to $-78°C$, and *m*-chloroperbenzoic acid (0.050 g, 77%) was added. The reaction mixture was warmed to 0°C and stirred for 15 minutes and then treated with triethylamine (0.5 mL). Purification was accomplished by preparative thin-layer chromatography using 4:1 chloroform:methanol to give **20** as a solid (0.025 g, 45%). ^{1}H NMR ($CDCl_3$): δ ^{1}H NMR ($CDCl_3$) δ 7.93 (s, 1H), 5.98 (bs, 2H), 5.35 (d, 1H, $J = 6.1$ Hz), 4.94 (d, 1H, $J = 5.7$ Hz), 4.65 (d, 1H, $J = 6.1$ Hz), 4.30–4.45 (m, 2H), 1.76–1.84 (m, 1H), 1.22–1.61 (m, 25H), 1.37–1.42 (m, 1H), 1.13–1.19 (m, 1H). HRMS $(M + 1 + Na)^+$: calculated 566.1911, found 566.1916. $\alpha_D^{25} + 0.6°$ (c = 0.15, EtOH).

(1′S,2′R,3′S,4′R,5′S)-4-(6-Amino-2-chloro-9H-purin-9-yl)-1-[phosphoryloxy-methyl]bicyclo[3.1.0]hexane-2,3-diol (2). To a solution containing **20** (0.028 g, 0.05 mmol) in MeOH (3 mL) and water (3 mL) was added Dowex-50 resin (0.050 g). The mixture was stirred for 3 hours at 70°C and the resin removed by filtration. The filtration was then treated with triethylammonium bicarbonate buffer (1 mL, 1 M) and concentrated under reduced pressure. The residue was purified by ion-exchange column chromatography using Sephadex-DEAE A-25 resin with linear gradient (0.01–0.5 M) of 0.5 M ammonium bicarbonate as mobile phase to give **2** (0.005 g, 25%) as a white solid. ^{1}H NMR (D_2O) δ 8.44 (s, 1H), 4.72 (s, 1H), 4.42–4.52 (m, 1H), 4.00 (d, 1H, $J = 6.6$ Hz), 3.62–3.76 (m, 1H), 1.84–1.96 (m, 1H), 1.53–1.62 (m, 1H), 0.96–1.05 (m, 1H). ^{31}P NMR (D_2O) 0.677.
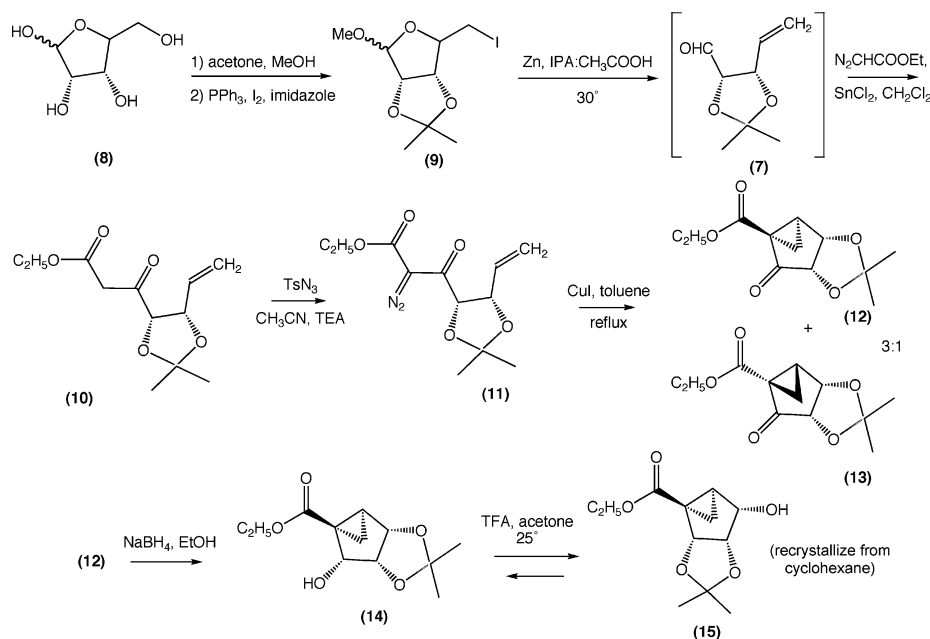
SCHEME 2 Improved synthesis of a key (N)-methanocarba bicyclic pseudosugar intermediate **15** starting from L-ribose **8**.

HRMS $(M-1)^+$: calculated 390.0357, found 390.0359. HPLC (system B) 8.2 minutes (98%). For optical rotation measurements a crystalline salt with triethylamine was crystallized. M.p. 215°C (decomp); $\alpha_D{}^{25} + 0°$ (c = 0.16, 80% EtOH).

RESULTS AND DISCUSSION

The key step in our published route to (N)-methanocarba nucleosides involves intramolecular cyclopropanation of the diazo derivative **11** to afford the requisite bicyclo[3.1.0]hexan-2-one derivative **12** as the major isomer (Scheme 2). Isomerization of the isopropylidene group in **14** provided the key (N)-methanocarba bicyclic pseudosugar intermediate, ethyl (1*S*,2*R*,3*S*,4*S*,5*S*)-2,3-O-(isopropylidene)-4-hydroxybicyclo[3.1.0]-hexane-carboxylate **15** which was converted into requisite (N)-methanocarba nucleosides by following a standard set of reactions in good yields (Scheme 3).

We explored the use of commercially available L-ribose **8** as an enantiopure building block for the construction of **15**. Accordingly, **8** was converted into a 5:1 mixture of iodides **9**, which were isolated as a colorless oil in 69% overall yield. An important attribute of this iodide preparation was the trituration of the crude product with hexanes/ethyl acetate (3:1)
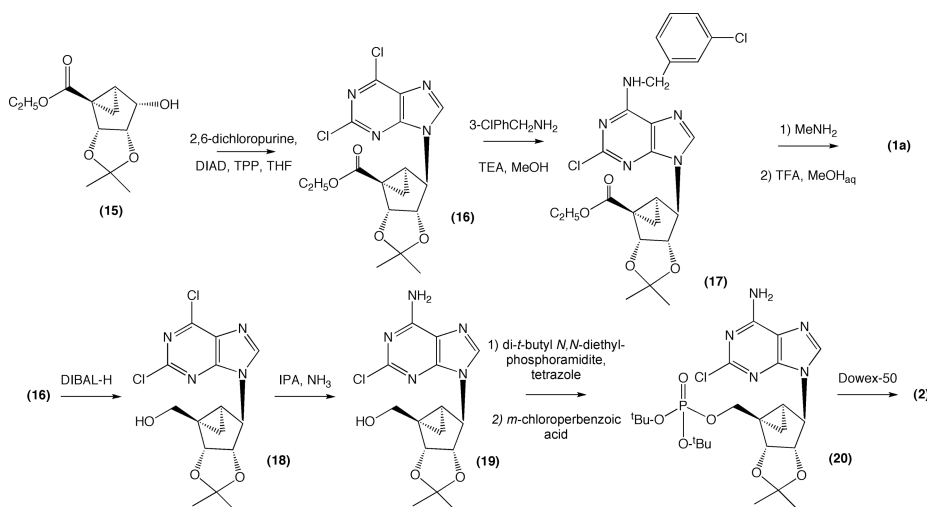
SCHEME 3 Synthesis of the (N)-methanocarba nucleoside derivative MRS3558 **1a** and nucleotide derivative MRS2339 **2**.

to precipitate the triphenylphosphine oxide byproduct thereby allowing facile removal by filtration. The mixture of 5′-iodoribose derivatives was initially cleaved reductively with Zn in refluxing methanol according to a literature procedure.[12,13] The purification of the crude aldehyde **7** was attempted by using distillation under reduced pressure,[13] which resulted in polymerization of the product. To overcome this problem the aldehyde was generated in situ and was reacted further without purification. We also employed isopropyl alcohol as a co-solvent along with methanol in this reaction. It is presumed that the formation of the undesired methyl hemiacetal was minimized to almost negligible levels due to the presence of a large excess of isopropyl alcohol. At this stage the reaction mixture was simply filtered through Celite to remove an excess of Zn, and the alcoholic layer containing **7** was concentrated to dryness. The residue was redissolved in dichloromethane and reacted with ethyl diazoacetate to afford the keto ester **10** in 42% yield based on the iodoribose mixture **9**. Although we obtained some improvement in yields using this reaction protocol we continued to probe this reaction sequence further. We observed that the resulting aldehyde was very unstable under the present reaction conditions, that is, refluxing in *i*-PrOH/MeOH. So, our aim was to carry out this reaction sequence at lower temperature to avoid the decomposition of the aldehyde. Accordingly, when the same reaction was carried out in the presence of acetic acid we observed that the Zn-mediated reduction proceeded smoothly at room temperature. We believe that this acid-catalysed reduction occurs upon protonation of the methoxy group followed by the rate-determining reductive elimination of the "*MeOH*" moiety to afford the

aldehyde. We also observed that instead of simply filtering to remove the Zn, an aqueous workup at this stage was necessary to remove the unwanted ZnI from the reaction mixture. The resulting aldehyde was subjected to further reaction with a tin-mediated ethyl diazoacetate addition reaction to afford **10** in 68% yield based on the iodoribose mixture **8**. It is pertinent to mention here that longer reaction times with ethyl diazoacetate led to formation of unwanted side products that made the column chromatographic separation of **10** difficult as well as lowering the overall yields. It is to be noted here that the combined yield of **10** by this modified acid catalyzed protocol is 47% based on L-ribose, while our earlier approach had only a 19% overall yield based on expensive **2**, 3-*O*-isopropylidene-D-erythronolactone.

The keto ester **10** was reacted with tosyl azide to afford the diazo derivative **11** in 95% yield. The byproduct *p*-toluenesulfonamide generated in this reaction separates out easily when the residue was dissolved in chloroform. The diazo derivative **11** then underwent a thermally-induced intramolecular cyclopropanation to give bicyclo[3.1.0]hexan-2-one derivatives **12** and **13** in a combined 48% yield as per our earlier procedure. However we observed that the newly formed sterically strained α-keto cyclopropyl derivatives **12** and **13** were unstable under these reaction conditions. Based on this observation the slow addition (over 4 hours) of the diazo derivative **11** was employed during this reaction. This modification has indeed resulted in considerable improvement in the yields, and a favorable diastereoisomeric ratio (4:1) for the desired isomer **12** (51%) was obtained. The small amount of the starting diazo compound **11** was also recovered from this reaction. This bicyclo derivative **12** was isolated chromatographically and reduced stereoselectively with NaBH$_4$ to give alcohol **14** as a single product in 69% yield. Alcohol **14** was then subjected to trifluoroacetic acid-catalyzed equilibration at room temperature to produce the requisite isomeric acetonide **15**. The isolation of **15** was achieved by crystallization from cyclohexane, in which the mother liquor was rich in the requisite isomer **15** with purity >80%. The crystalline isomer **14** could be recycled by re-subjecting it to the above equilibration procedure. Pure **15** could be obtained by repeated recrystallizations from cyclohexane. However, for most applications it was practical to use **15** at ~80% purity in subsequent reactions.

Our initial studies towards nucleophilic substitution of either the 1′-mesylate or 1′-triflate derivative of **15,** with the sodium salt of purines under a variety of conditions failed presumably due to steric reasons. The best result was obtained when alcohol **15** was subjected to a Mitsunobu coupling reaction using 2,6-dichloropurine as the base to afford the condensed product **16** (Scheme 3). However, we observed that this condensed product was contaminated with byproducts arising from the Mitsunobu

reaction. Repeated purification (at least three times) with silica gel column chromatography afforded the pure compound **16** in 42% yield. We observed that a slightly impure product could be carried forward in reaction with 3-chlorobenzylamine to provide **17** in 61% yield after silica gel column chromatography. Finally, **17** was treated with excess methylamine followed by acetonide deprotection with 10% TFA to produce **1a** (MRS 3558) in 54% yield over two steps whose spectral properties matched those of the identical compound reported in the literature.[4]

This modified procedure starting from L-ribose was also used to prepare MRS2339 **2**, a (N)-methanocarba derivative of 2-chloroadenosine 5′-monophosphate. Recently, Liang and coworkers have shown that compound **2** acts as a P2X receptor agonist and when administered in a mouse model of heart failure it reduces cardiac myocyte hypertrophy and enhances survival.[10] For its preparation we first reduced the ester functionality in **16** using diisobutylaluminum hydride (DIBAL-H) at −78°C, to afford the alcohol **18** in 56% yield. Initially, the chloro group at the 6-position was replaced with an amino functionality by exposure to methanolic ammonia, however the reaction was also accompanied by the formation of the 6-methoxy substituted derivative. This problem was overcome by treating **18** with 2 M ammonia in isopropyl alcohol to afford the 6-amino derivative **19** in moderate yields. (N)-Methanocarba nucleoside **19** was phosphorylated by first reacting with di-*t*-butyl *N,N*-diethylphosphoramidite and tetrazole followed by treatment with *m*-chloroperbenzoic acid to afford the di-*t*-butyl phosphate derivative **20** in 45% yield. Both *t*-butyl groups and the acetonide group in **20** were deprotected simultaneously by using Dowex-50 resin in the acid form, to afford the crude (N)-methanocarba nucleotide **2**. The final purification of the resulting monophosphate was accomplished by using Sephadex ion exchange column chromatography to afford **2** as the ammonium salt in 25% yield. The spectral properties of **2** were identical to those reported previously.[14]

In conclusion, we have demonstrated the feasibility of employing readily available L-ribose as a precursor in the preparation of (N)-methanocarba nucleosides and nucleotides of biological interest. In this regard the modified synthesis bypasses the pitfalls experienced in our earlier approach thereby allowing a highly efficient and convergent synthesis of purine receptor ligands MRS 3558 and MRS 2339. In addition to certain subtypes of adenosine and P2 receptors, the (N)-methanocarba nucleosides have been explored and found to interact effectively with adenosine deaminase,[15] and the ENT1 nucleoside transporter.[16] Furthermore, nucleosides bearing the (N)-methanocarba modification have been shown to be substrates for cellular and viral DNA polymerases.[7,17] At present, use of this modified synthetic procedure to synthesize (N)-methanocarba nucleosides with potential antiviral activity is underway in our laboratories.

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