Synthesis of isoaristeromycin from D-glucose

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Isoaristeromycin 1, an analogue of the nucleoside antibiotic aristeromycin, has been synthesized by the application of intermolecular nitrene cycloaddition (INC) reaction on a D-glucose derived substrate.

The significant bioactivities displayed by many carbocyclic nucleosides have directed considerable attention towards the synthesis of these nucleosides1-5. Recently, we have reported the use of intramolecular nitrene cycloaddition (INC) reaction on D-glucose derived substrates to synthesize functionalized carbocycles of different ring sizes6 and consequently nucleosides with a carbocycle or heterocycle fused to the ribose ring7 as also carbocyclic nucleosides8-10. We report herein the usefulness of the methodology to allow an efficient synthesis of the trihydroxy aminopentane 13 and isoaristeromycin 1, an analogue of aristeromycin which is a naturally occurring antitumor agent.

Results and Discussion
INC reaction between C-5 olefin and C-1 nitrene: Synthesis of isoxazolidinocarbocycles 8 and 9

The olefinic compound 6 was prepared from 1,2:5,6-di-O-isopropylidene-D-glucofuranose 2 in good yield through a sequence of reactions, viz. tosylation with tosyl chloride/py, removal of 5,6-0-isopropylidene function with aqueous HOAc, vicinal diol cleavage with sodium metaperiodate in aqueous EtOH and Wittig reaction using triphenyl phosphonium methyl iodide and n-butyl lithium in THF (Scheme I). Presence of the tosyl group in 3 was confirmed by its 1H NMR spectrum exhibiting two doublets (J = 8 Hz) at δ 7.36 and 7.88 for the aromatic protons. Absence of the 5,6-O-isopropylidene group in 4 was indicated by the disappearance of two singlets (3H each) around δ 1.16 and 1.20 for two methyl groups. The characteristic peak at 1740 cm⁻¹ in the IR spectrum of 5 indicated the presence of an aldehyde functionality. The vinylic CH proton signal in the 1H NMR spectrum of 6 appeared at δ 5.63 (1H) as doublet of a doublet doublet (J = 17.3, 10.4, 6.5 Hz) and that for the vinylic CH₂ protons at δ 5.17 (d, J = 10.4 Hz) and 5.30 (d, J = 17.3 Hz), confirming its structure. Presence of a doublet signal at -δ 5.60 (J = 4 Hz), characteristic for the anomeric proton in 1,2-O-isopropylidene derivatives, furnished clear indication for the protecting group in the compounds 3-6.

Removal of 1,2-O-isopropylidene group from 6, affording the mixture of anomers 7, was confirmed from the 1H NMR spectrum of the product which showed the disappearance of the pair of singlets expected for the methyl groups. Subsequently, INC reactions of the N-benzyl nitrene of the masked aldehyde and the olefin (derived from 6) afforded the isoxazolidines 8 (75% yield) and 9 (7% yield), when carried out in benzene. However, upon changing the solvent from benzene to ethanol, the same reaction afforded only 8. The structure and stereochemistry of the isoxazolidines 8 and 9, however, could not be ascertained from the 1H NMR spectrum until the isoxazolidine ring of 8 was cleaved and the resulting product was converted to the tetraacetylated carbocycle 11 (Scheme II).

Though the INC reaction could have afforded either a six-membered or a five-membered isoxazolidine (or a mixture of the two) depending
Scheme I

(a) TsCl/py, rt, 12 h; (b) H₂O-HOAc (2:3), 55°C, 7 h; (c) NaIO₄ (1.3 equiv.), EtOH, rt, 30 min.;
(d) Ph₃P/CH₃I (1.9 equiv.), n-BuLi (1.6M), -60°C;
(e) CH₃CN-H₂SO₄-H₂O, 60-65°C, 2.5 h;
(f) PhCH₂NHOH (1.2 equiv.), dry EtOH, rt, 24 h.

Scheme II

(a) Pd/C (10%), cyclohexene, EtOH, reflux, N₂; (b) Ac₂O/Py, rt, 12 h.

upon the mode of cyclization, the structure 8 was assigned from the location of a triplet at δ 70.0 in the ¹³C NMR spectrum indicating the presence of -CH₂O- linkage. The stereochemistry of C-2, C-3 and C-4 were, however, the same as the corresponding carbons in D-glucose, since these centres were not disturbed during the reaction sequence. Though the cis ring juncture is energetically favored in case of bicyclo[3.3.0]octane system¹, the relative disposition of H-1 and H-5 (ring juncture) could not be settled from the ¹H NMR spectrum due to signal overlap. However, reductive opening of the isoxazolidine ring of 8 followed by acetylation afforded 11, in the NMR spectrum of which the signal at δ 4.42 (td, J = 10 and 7 Hz) changed on D₂O exchange into a clear triplet of J = 10 Hz (with disappearance of the doublet of J = 7 Hz at δ 6.12), showing that it must be due to H-1 with both J₁₂ and J₁₅ = 10 Hz. The other signals could thereafter be assigned using chemical shift considerations and coupling constant values. Further, attempted vicinal cleavage of the derived amino-alcohol 10 with NaIO₄ proved unsuccessful, indicating that C₁-NH₂ and C₂-OH must be trans; since H-1, H-5 are cis, they must be in α-face. Therefore, the ring juncture protons in 9 will certainly be in β-face. Inspection of a Dreiding model revealed that the compound 11 is likely to assume an envelope conformation with C₂ at the tip; the 1-NHAc, 2-OAc and 3-OTs groups assume the energetically preferred equatorial conformation and the other substituents are in isoclinal position. The coupling constants are then in agreement with the observed dihedral angles.

Synthesis of the carbocyclic nucleoside isooristeromycin 1

Reductive elimination of the tosyl group of 8 (Scheme III) by LiAlH₄ in THF afforded the isoxazolidinocarbocycle 12, the ¹H NMR of which indicated the absence of two doublets in the aromatic region. Cleavage of the isoxazolidine ring of 12 employing transfer hydrogenation (Pd-C, 10%, cyclohexene)¹² furnished aminocyclopentane 13 (91% yield), the key precursor of the nucleoside 16. Reaction between aminotriol 13 and 5-amino-4,6-dichloropyrimidine in refluxing n-BuOH yielded 14 which was cyclized to the chloronucleoside derivative 15 with triethyl orthoformate/p-toluenesulphonic acid in DMF. Finally the compound 15 was converted to the target isooristeromycin 16 by heating with a solution of ammonia in MeOH (80% yield). The presence of nucleoside base in 14 was confirmed by the presence (in its NMR) of a 1H singlet in the aromatic region; on the other hand, two singlets (1H each) were observed for H-2 and H-8 in the NMR of...
Experimental Section

Melting points were taken in open capillaries and are uncorrected. IR spectra were measured on a JASCO 700 spectrophotometer. $^1$H and $^{13}$C NMR spectra were measured either on a JEOL FX-100 or on a Bruker AM 300 L spectrometer mostly using TMS as internal standard. Mass spectra were obtained using a JEOL AX-500 spectrometer operating at 70 eV for EIMS. Optical rotations were measured in a JASCO DIP 360 polarimeter. Reagents and solvents were of analytical grade or were purified by standard procedures prior to use. Column chromatography was performed with silica gel (60-120 mesh; SRL, India). Dowex1-OH$^{-}$ and Dowex 50W-H$^{+}$ resins were used for ion exchange column chromatography. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F$_{254}$ precoated plates.

1, 2 : 5, 6 - Di - O - isopropyldene-3-O-tosyl-α-D-glucofuranose 3. To a stirred solution of 2 (10.0 g, 38.5 mmole) in dry pyridine (50 mL) was added tosyl chloride (9.5 g, 49.87 mmole) and the solution was stirred for 25 hr at 80-85°C. Excess of pyridine was removed under reduced pressure, the residue was extracted with CHCl$_3$ (3 x 100 mL). CHCl$_3$ extracts were washed with saturated solution of NaHCO$_3$, dried (Na$_2$SO$_4$) and evaporated to give a crude solid material. The crude product was subjected to column chromatography (silica gel, CHCl$_3$/pet.ether 4:1) to afford 3 (14.0 g, 87.9%) as a white solid, m.p. 120-21°C; $[\alpha]_D^20$ -49.5° (c 0.82, CHCl$_3$); IR (KBr): 2942, 1598, 1372, 1258, 1173, 1076, 1020, 861, 746, 668 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 100 MHz): δ 1.16 (s, 3H), 1.20 (s, 3H), 1.32 (s, 3H), 1.48 (s, 3H), 2.46 (s, 3H), 3.84-4.28 (m, 4H), 4.84 (s, 1H and d, 1H), 5.96 (d, 1H, J = 3 Hz), 7.36 (d, 2H, J = 8 Hz), 7.88 (d, 2H, J = 8 Hz); EIMS, m/z: 399 (M$^+$-15), 155, 113. Anal. Calcd for C$_{19}$H$_{26}$S$_{8}$O$_{9}$: C, 55.06; H, 6.32. Found C, 55.03; H, 6.38%.

1,2-O-Isopropyldene-3β-O-tosyl-α-D-glucofuranose 4. Compound 3 (2.0 g, 4.83 mmole) dissolved in a mixture of H$_2$O-CH$_3$OH (2:3) (25 mL) and MeOH (5 drops) was heated at 60°C for 1 hr until TLC showed complete disappearance of the starting material. The solvent was evaporated in a rotary evaporator and the last traces of CH$_3$OH removed by toluene co-evaporation (3 x 20 mL). The residue was then chromatographed over silica gel. Elution with CHCl$_3$-MeOH (99 : 1) mixture gave the dihydroxy compound 4 (1.78 g, 98.5%) as a gummy solid. IR (neat): 3528, 2966, 1723, 1598, 1373, 1217, 1175, 1090, 1021, 953, 884, 845, 815 cm$^{-1}$; EIMS, m/z: 374 (M$^+$), 359 (M$^+$-15), 155, 113.
5-β-Formyl-4-β-O-tosyl-2α, 3α-O-isopropylidene-
3,4,5,6-tetrahydrofuran 5. To an ice-cold solution of 4 (1.82 g, 4.87 mmole) in aq. EtOH (50%, 30 mL) was added dropwise a solution (10 mL) of NaIO₄ (1.3 g, 6.07 mmole) in water. After stirring at room temperature for 1 hr, it was filtered, the solvent was evaporated and the product was extracted with CHCl₃ (3 x 50 mL). The CHCl₃ solution was dried (Na₂SO₄), evaporated and the crude product 5 (1.63 g) was dried under vacuum. It was used without further purification for the next step. IR (neat): 2948, 1749, 1605, 1531, 1494, 1364, 1175, 1019, 867, 815, 728 cm⁻¹; ¹H NMR (CDCl₃, 100 MHz): δ 2.46 (s, 3H), 3.02 (brs, 2H), 4.32 (m, 1H), 4.60-5.00 (m, 2H), 5.08-5.90 (m, 4H), 7.38 (d, 2H, J = 8 Hz), 7.82 (d, 2H, J = 8Hz).

(3αR, 4R, 5S, 6αR)-1-Benzyl-4α, 5β, 6α-tetrahydroxycyclopent[c] isoxazole 5-tosylate 8, and (3αS, 4R, 5S, 6αS)-1-benzyl-4α, 5β, 6α-tetrahydroxycyclopent[c] isoxazole 5-tosylate 9. To a solution of the lactol 7 (340 mg, 1.13 mmole) in dry benzene (25 mL) was added N-benzyl hydroxylamine (172 mg, 1.4 mmole) and the mixture was stirred for 24 hr at room temperature. The solvent was evaporated and the product was extracted with CHCl₃ (3 x 25 mL). The combined extract was washed with water, dried (Na₂SO₄) and evaporated to give a solid crude material. The mixture was purified by column chromatography over silica gel. Petroleum ether-CHCl₃ (1:1) eluents afforded two colourless crystalline compounds 8 (267 mg, 58%) and 9 (30 mg, 7%). When the reaction was carried out in dry ethanol solvent, only one product 8 (338 mg, 74%) was obtained. 8: m.p. 142-143°C; [α]D₂⁰ + 12.0° (c 1.12, MeOH); IR (KBr): 3492, 2876, 1599, 1496, 1454, 1363, 1190, 1067, 1035, 964, 899, 846, 810, 723, 661 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): δ 2.42 (s, 3H), 2.92 (m, 1H), 3.37 (t-like, 1H), 3.50 (brs, 1H), 3.68 (d, 1H, J = 13 Hz), 3.78 (dd, 1H, J = 1.8, 9.2 Hz), 4.02 (m, 4H), 4.27 (t, 1H, J = 8 Hz), 7.30 (m, 7H), 7.81 (d, 2H, J = 8 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 21.7 (q), 49.2 (d), 59.4 (t), 70.0 (t), 70.8 (d), 73.6 (d), 76.6 (d), 89.2 (d), 127.6 (2xd), 128.2 (2xd), 128.5 (2xd), 129.0 (2xd), 130.0 (2xd), 132.0 (s), 136.2 (s), 145.4 (s); EIMS, m/z: 405 (M⁺), 233, 161, 93, 29, 8. 9: m.p. 122-123°C; [α]D₂⁰ -4.3° (c 0.23, CHCl₃); IR (KBr): 3350, 2872, 1600, 1546, 1357, 1174, 1090, 1046, 996, 872, 810, 727, 673 cm⁻¹; ¹H NMR (CDCl₃, 300 MHz): 2.45 (s, 3H), 3.38 (q, 1H, J = 7 Hz), 3.50-3.60 (m, 2H), 3.67 (t, 1H, J = 7.5 Hz), 3.77 (d, 1H, J = 12.8 Hz), 3.82 (m, 1H), 4.00 (dd, 1H, J = 7, 9.5 Hz), 4.12 (d, 1H, J = 12.8 Hz), 4.17 (t, 1H, J = 8 Hz), 4.25 (t, 1H, J = 8 Hz), 4.45 (d, 1H, J = 9.5 Hz), 7.26-7.36 (m, 7H), 7.83 (d, 2H, J = 8 Hz); ¹³C NMR (CDCl₃, 75 MHz): δ 21.7 (q), 47.7 (d), 59.2 (t), 64.3 (t), 66.8 (d), 70.7 (d), 71.2 (d), 89.7 (d), 128.0 (d), 128.2 (2xd), 128.6 (2xd), 129.0 (2xd), 129.9 (2xd), 130.0 (2xd), 132.0 (s), 136.2 (s), 145.4 (s); EIMS, m/z: 405 (M⁺), 233, 161, 93, 29, 8.
To a solution of 8 (200 mg, 0.49 mmole) in dry EtOH (15 mL) was added Pd/C (10%, 200 mg) and cyclohexene (1.5 mL), and the mixture was heated at reflux under N₂ for 4 hr. The catalyst was filtered off, the solvent was evaporated in vacuo to afford 10 (150 mg) which was used in the next step without further purification. IR (Neat): 3416, 1600, 1516, 1348, 1175, 1124, 1038, 858, 815 cm⁻¹.

(1R, 2S, 3S, 4R, 5R)-2-(Acetoxymethyl)cyclopentane-2, 3, 4-triol 14. To a solution of the amino compound 10 (150 mg, 0.47 mmole) in dry pyridine (6 mL) was added acetic anhydride (2 mL) and a catalytic amount of DMAP, and the reaction mixture was stirred at room temperature for 24 hr. The solvent was evaporated in vacuo. The last trace of pyridine were removed by azeotropic distillation with toluene and the crude residue was purified by column chromatography over silica gel. Elution with chloroform-methanol (49:1) afforded a solid which was recrystallized from ether to furnish 11 (80 mg, 35%), m.p. 107-08°C; [α]D⁰_{25} -12.4° (c 0.29, MeOH); IR (KBr): 3532, 2992, 1746, 1656, 1562, 1496, 1425, 1367, 1260, 1103, 1038, 992, 860, 815, 669 cm⁻¹; 1H NMR (CDCl₃, 100 MHz): δ 1.96 (s, 6H), 2.01 (s, 3H), 2.12 (s, 3H), 2.46 (s, 3H), 2.60 (m, 1H), 4.12 (dd, 1H, J = 4, 12 Hz), 4.28 (dd, 1H, J = 4, 12 Hz), 4.48 (td, 1H, J = 7, 10 Hz), 4.96 (dd, 1H, J = 5, 8 Hz), 5.12 (t, 1H, J = 5 Hz), 5.32 (dd, 1H, J = 7, 10 Hz), 6.12 (brd, 1H, J = 7 Hz), 7.38 (d, 2H, J = 8 Hz), 7.82 (d, 2H, J = 8 Hz); EI MS, m/z: 365 (M⁺-120), 205, 151, 91. Anal. Calcd for C₁₅H₂₀N₂O₇: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.35; H, 7.38; N, 5.68%.

(3aR, 4S, 6R, 6aR)-1-Benzyl-4, 6-dihydroxycyclopent[e]isoxazole 12. To a solution of 8 (640 mg, 1.58 mmole) in dry THF (50 mL) was added LAH (250 mg, 6.58 mmole) and the mixture was heated at reflux under N₂ for 4 hr. Excess LAH was decomposed by dropwise addition of ice-cold water, the mixture was filtered through celite, and the solvent was evaporated in vacuo to give a crude residue which was purified by column chromatography on silica gel with methanol-chloroform (1:24) as the eluting solvent to afford 12 (275 mg, 74%), m.p. 117-18°C; IR (KBr): 3362, 2906, 1443, 1350, 1254, 1100, 1052, 975, 746, 694 cm⁻¹; 1H NMR (CDCl₃, 100 MHz): δ 1.88 (td, 1H, J = 1, 16 Hz), 2.12 (td, 1H, J = 4, 16 Hz), 2.60 (brs, 2H), 3.12-3.68 (m, 3H), 3.80-4.32 (m, 5H), 7.36 (m, 5H); EI MS, m/z: 235 (M⁺), 92.

(1R, 2R, 4S, 5R)-2, 4-Dihydroxy-5-(hydroxymethyl)cyclopentylamine 13. To a solution of isoxazolidine carbocycle 12 (550 mg, 2.34 mmole) in dry EtOH (45 mL) was added Pd/C (10%, 825 mg) and cyclohexene (4.5 mL), and the mixture was heated at reflux under N₂ for 4 hr. The Pd/C was filtered off, the solvent was evaporated in vacuo, and the crude amino compound 13 (335 mg) was used in the next step without further purification.

(1R, 3R, 4S)-2-(5-Amino-6-chloropyrimidine-4-yl) amino]-3-(hydroxymethyl)cyclopentane-1, 4-diol 15. To a solution of crude amine 13 (335 mg, 2.28 mmole) in dry n-BuOH (25 mL) was added 5-amino-4, 6-dichloropyrimidine (600 mg, 3.55 mmole, 1.6 equiv.) and Et₃N (1.5 mL), and the mixture was then heated at reflux for 18 hr under N₂. The solvent was evaporated, and the residue was extracted with H₂O (3 x 30 mL). The aqueous part was washed with CHCl₃ (2 x 30 mL) [to remove free pyrimidine base] and evaporated to a thick oil, which under vacuum over solid KOH, was turned into a brownish foamy solid 14 (475 mg), m.p. 131-32°C; [α]D⁰_{25} +41.7° (c 0.35, MeOH); IR (KBr): 3284, 1498, 1430, 1394, 1357, 1281, 990 cm⁻¹; 1H NMR (D₂O, 100 MHz): δ 1.62 (dt, 1H, J = 8, 12 Hz), 2.32-2.72 (m, 2H), 3.50 (dd, 1H, J = 4, 12 Hz), 3.64 (dd, 1H, J = 6, 12 Hz), 4.18 (brq, 2H, J = 7 Hz), 4.48 (brt, 1H, J = 8 Hz), 7.88 (s, 1H); FABMS, m/z: 275 (M⁺+1, for Cl⁺), 277(M⁺+1, for Cl⁺).
chloronucleoside 15 (218 mg, 44%), m.p. 119-20°C; $[\alpha]_D^{27} = -27.8^\circ$ (c 0.51, MeOH); IR (KBr): 3424, 2988, 1655, 1593, 1473, 1397, 1187, 1121, 1033, 951, 817, 680 cm$^{-1}$; $^1$H NMR (D$_2$O + acetone, 100 MHz): $\delta$ 1.80 (td, 1H, $J = 9, 13$ Hz), 2.40-2.90 (m, 2H), 3.32 (d, 2H, $J = 5$ Hz), 4.26 (m, 1H), 4.70-5.20 (m, 2H), 8.38 (s, 1H), 8.45 (s, 1H); FABMS, m/z: 285 (M$^+$+1, for Ce$^{3+}$), 287 (M$^+$+1, for Ce$^{3+}$). Anal. Calcd for C$_{11}$H$_{13}$CIN$_4$O$_3$: C, 46.41; H, 4.60; N, 19.68. Found: C, 46.32; H, 4.61; N, 19.23%.

(1R, 2R, 3R, 4S)-2-(Adenin-9-yl)-3-(hydroxymethyl)cyclopentane-1, 4-diol 1. Chloroadenosine derivative 15 (100 mg, 0.35 mmole) in dry methanolic ammonia (7 mL) was heated at 100°C for 48 hr in a sealed tube. The tube was cooled and opened up, and the reaction mixture was warmed on a water bath to remove excess ammonia. The solvent was removed under vacuum to obtain a foam which was purified by flash column chromatography over silica gel (mesh size 230-400) using 5% MeOH in CHCl$_3$ as eluent to furnish 1 (76 mg, 81.6%). m.p. 155-56°C; $[\alpha]_D^{28} = -24.9^\circ$ (c 0.42, MeOH); IR (KBr): 3352, 1641, 1411, 1087 cm$^{-1}$; $^1$H NMR (D$_2$O, 100 MHz): $\delta$ 1.50-1.94 (m, 1H, overlapped by solvent signal), 2.42-2.86 (m, 2H), 3.32 (d, 2H, $J = 6$ Hz), 4.28 (brq, 1H, $J = 6$ Hz), 4.78-5.14 (m, 2H), 8.20 (s, 1H), 8.24 (s, 1H); $^{13}$C NMR (D$_2$O, 25 MHz): $\delta$ 40.8, 50.4, 60.0, 63.1, 71.1, 72.6, 119.1, 142.1, 150.4, 153.1, 156.0; FABMS, m/z: 266 (M$^+$+1), 240, 213. Anal. Calcd for C$_{11}$H$_{13}$N$_2$O$_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.65; H, 5.70; N, 26.12%.

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References