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A new synthesis and an antiviral assessment of the 4′-fluoro derivative of 4′-deoxy-5′-noraristeromycin

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ABSTRACT

A synthetic route to (1*S*,2*S*,3*R*,5*S*)-3-(6-amino-9*H*-purin-9-yl)-5-fluorocyclopentane-1,2-diol (that is, the 4′-fluoro derivative of 4′-deoxy-5′-noraristeromycin, **3**) is described via a fluorinated cyclopentanol, which is in contrast to existing schemes where fluorination occurred once the purine ring was present. Compound **3** was assayed versus a number of viruses. A favorable response was observed towards measles (IC_{50} of 1.2 μg/mL in the neutral red assay and 14 μg/mL by the visual assay) but this was accompanied by cytotoxicity in the CV-1 host cells (21–36 μg/mL). Among the viruses unaffected by **3** were human cytomegalovirus and the poxviruses (vaccinia and cowpox), which are three viruses that were inhibited by the 4′,4′-difluoro analog of **3** (that is, **2**).

1. Introduction

To build upon the framework of the antiviral agent 5′-noraristeromycin (**1**) (Fig. 1)[1] we recently reported[2] the effects of the difluoro congener **2** towards human cytomegalovirus and the orthopox viruses vaccinia and cowpox. To ascertain if these latter activities also reside in the monofluoro analog **3**, a new synthesis of it[3,4] was developed and an antiviral analysis conducted.

2. Chemistry

The previous routes to **3**[3,4] introduced the fluoro substituent onto a preformed carbocyclic nucleoside precursor. In the present case, it was desired to have access to a fluorinated cyclopentanol that would lend itself to condensation with a variety of heterocyclic bases, including adenine or its precursors. Thus, for this purpose, **12** (Scheme 1) became the initial target compound.

To begin, the protected ketone **5**,[5] available from (+)-(1*R*,4*S*)-4-hydroxy-2-cyclopenten-1-yl acetate, **4**[6]), which is a common building block in our carbocyclic nucleoside research,[2] was reduced (Luche conditions)[7] to **6**. This was followed by a *p*-methoxybenzyl protection (to **7**) and then a desilylation to **8**. Oxidation of **8** (Parikh–Doering procedure)[8] produced **9**. Reduction of **9** to **10** with subsequent fluorination (**11**) and deprotection provided the sought **12**. Subjecting **12** to Mitsunobu conditions[9] with 6-chloropurine followed by amination yielded **13**. Subsequent, acidic deisopropylidenation resulted in **3**.

The structure of **3** was confirmed by NMR analysis (ge-NOESY, ge-COSY, and ge-HMBC): (1) for the fluoro stereochemistry at the C-4′ center (Fig. 1), there was a strong NOE between H-4′ and H-1′ and H-5′ (α) and between H-2′ and H′-5′ (β) and (2) for the N-9 cyclopentyl substitution site, HMBC assessment found a three-bond coupling between H-1′ and purine C-4 (150.2) and C-8 (140.2).

3. Antiviral results

Compound **3** was subjected to broad antiviral analysis.[10,11] A favorable response was observed towards measles (IC_{50} of 1.2 μg/mL in the neutral red assay and 14 μg/mL by the visual assay) but this was accompanied by cytotoxicity in the CV-1 host cells (21–36 μg/mL). Among the viruses unaffected by **3** were human cytomegalovirus and the poxviruses (vaccinia and cowpox), which are three viruses inhibited by the difluoro analog of **3** (that is, **2**).[2]

4. Conclusion

The synthetic route described herein offers advantages over the two previously described linear routes[3,4] by (i) following a convergent pathway that lends itself to compound library development with various heterocyclic bases and (ii) by introducing the requisite fluorine atom at a stage where its stereochemistry is assured (prior to any influence by the purine base). These results are significant as structural variations of **3** are pursued to explore its potential in the treatment of measles without associated cytotoxicity.

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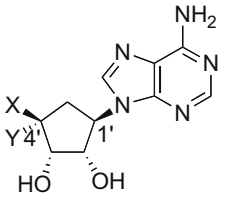
5. Experimental

5.1. General remarks

Melting points were recorded on a Meltemp II melting point apparatus and are uncorrected. Atlantic Microlab, Inc., Norcross, GA performed the combustion analyses. The NMR spectra were recorded on Bruker AC 250 and Avance spectrometers and are referenced to internal tetramethylsilane (TMS) at 0.0 ppm. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Reactions were monitored by thin-layer chromatography (TLC) using 0.5-mm Whatman Diamond Silica Gel 60-F_{254} precoated plates with visualization by irradiation with a Mineralight UVGL-25 lamp. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

5.1.1. (3a*S*,4*S*,6*S*,6a*S*)-2,2-Dimethyl-6-(*tert*-butyldimethylsilyl)-tetrahydro-3a*H*-cyclopenta[*d*][1,3]-4-ol (6)

To a solution of **5**[5] (0.35 g, 1.22 mmol) in MeOH (10 mL) was added $CeCl_3{\cdot}7H_2O$ (0.45 g, 1.22 mmol). This mixture was cooled to 0 °C and then $NaBH_4$ (60 mg, 1.58 mmol) was added portionwise. The resultant mixture was stirred for 30 min at 0 °C, then warmed to rt followed by stirring for 1 h. The reaction was quenched with satd NH_4Cl solution (5 mL). The solvent was removed under reduce pressure and the residue poured into H_2O with subsequent extraction using EtOAc (3 × 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexanes, 3:1) to give **6** as a colorless oil (0.34 g, 97%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.56 (t, J = 5.44 Hz, 1H), 4.34–4.26 (m, 2H), 4.01 (dd, J = 3.58 Hz, J = 0.46 Hz, 1H), 2.25 (d, J = 10.44 Hz, 1H), 1.89–1.93 (m, 1H), 1.68–1.75 (m, 1H), 1.46 (s, 3H), 1.34 (s, 3H), 0.86 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 111.34, 85.83, 78.61, 73.46, 72.00, 39.35, 26.19, 25.91, 24.43, 18.17, −4.69, −4.76. Anal. Calcd for $C_{14}H_{28}O_4Si$: C, 58.29; H, 9.78. Found: C, 58.34; H, 9.85.



1, X=OH;Y=H
2, X=Y=F
3, X=F;Y=H

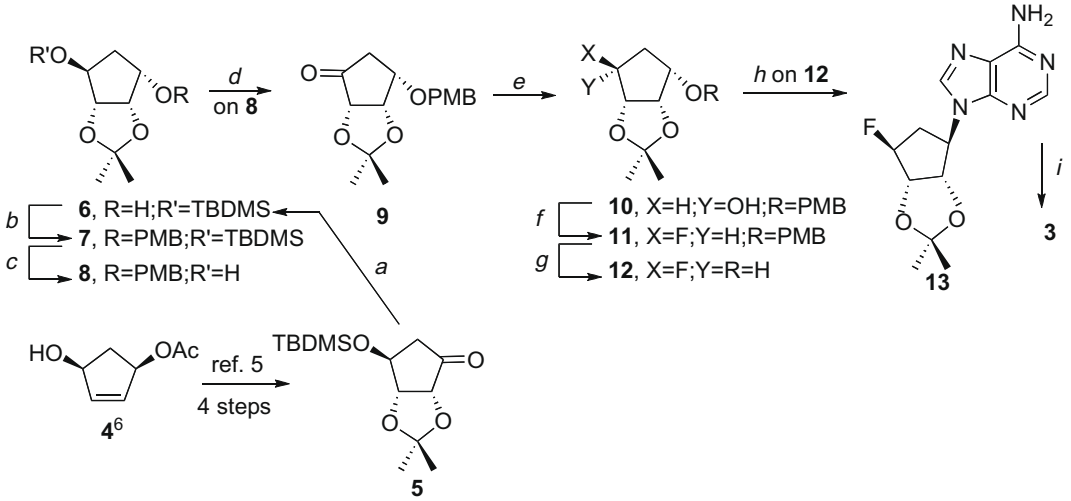
Figure 1.

5.1.2. *tert*-Butyl((3a*S*,4*S*,6*S*,6a*S*)-6-(4-methoxybenzyloxy)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yloxy)-dimethylsilane (7)

Compound **6** (7.56 g, 26.2 mmol) was dissolved in dry DMF (100 mL). After cooling the solution to 0 °C, NaH (1.26 g, 60% in mineral oil, 31.4 mmol) was added portionwise. The solution was stirred at 0 °C for 30 min and then 4-methoxybenzyl chloride (4.3 mL, 31.4 mmol) was added in one portion. After warming the solution to rt, it was stirred for 3 h. The solvent was removed under reduced pressure and the residue quenched with H_2O followed by extraction with EtOAc (3 × 100 mL). The organic layers were combined, dried (Na_2SO_4), concentrated under reduced pressure and the residue subjected to silica gel column chromatography (EtOAc/hexanes, 1:10) to give **7** as a colorless oil (9.0 g, 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, J = 8.4 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 4.61 (m, 1H), 4.45–4.58 (m, 2H), 4.25 (dd, J = 5.6 Hz, J = 1.6 Hz, 1H), 4.02–4.05 (m, 1H), 3.94 (d, J = 4.0 Hz, 1H), 3.79 (s, 3H), 1.90–1.94 (m, 1H), 1.75–1.76 (m, 1H), 1.48 (s, 3H), 1.30 (s, 3H), 0.83 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 159.24, 130.47, 129.72, 113.72, 110.98, 85.74, 77.76, 77.50, 73.42, 71.55, 55.29, 35.73, 26.13, 25.71, 24.07, 17.93, −4.89. Anal. Calcd for $C_{22}H_{36}O_5Si$: C, 64.67; H, 8.88. Found: C, 64.99; H, 8.64.

5.1.3. (3a*R*,4*S*,6*S*,6a*S*)-6-(4-Methoxybenzyloxy)-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (8)

To **7** (9.0 g, 22.0 mmol) dissolved in THF (100 mL) was added tetrabutylammonium fluoride (33 mL, 1.0 M in THF, 33 mmol). This mixture was stirred at rt for 1 h and then quenched with H_2O. Following extraction with EtOAc (3 × 300 mL), the combined organic layers were dried (Na_2SO_4), concentrated under reduced



Scheme 1. Reagents and conditions: (a) $CeCl_3{\cdot}7H_2O$, $NaBH_4$, MeOH, 0 °C to rt, 97%; (b) NaH, PMBCl, DMF, 0 °C, 84%; (c) TBAF, THF, rt, 89%; (d) pyridine·SO_3, DMSO, DIEPA, CH_2Cl_2, 0 °C, 82%; (e) $LiAlH_4$, THF, 0 °C, 81%; (f) DAST, pyridine, CH_2Cl_2, 0 °C to rt, 70%; (g) DDQ, CH_2Cl_2/H_2O, rt, 86%; (h) (i) TPP, 6-chloropurine, DIAD, THF, 0 °C to rt, 30% (by proton NMR); (ii) NH_3/MeOH, 120 °C, 85%; (i) 0.5 M HCl/MeOH, rt, 93%.

pressure, and subjected to silica gel column chromatography (EtOAc/hexanes, 1:5–1:1) to give **8** as a colorless oil (5.8 g, 89%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 4.52–4.66 (m, 3H), 4.32 (dd, J_1 = 5.6 Hz, J_2 = 1.2 Hz, 1H), 4.04–4.09 (m, 2H), 3.80 (s, 3H), 2.00–2.07(m, 1H), 1.83–1.88 (m, 1H), 1.58 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.42, 159.46, 130.59, 129.76, 113.98, 111.38, 85.51, 77.96, 73.46, 71.74, 55.48, 35.82, 26.36, 24.29. Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.03; H, 7.62.

5.1.4. (3a*S*,6*S*,6a*S*)-6-(4-Methoxybenzyloxy)-2,2-dimethyldihydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4(5*H*)-one (9)

Compound **8** (5.8 g, 19.7 mmol) was dissolved in dry CH_2Cl_2 (200 mL) and then DMSO (10 mL) and DIPEA (6.95 mL, 39.4 mmol) were added. The solution was cooled to 0 °C and Py·SO_3 complex (6.25 g, 39.4 mmol) was added portionwise. The solution was stirred at 0 °C for 1 h. This mixture was quenched with ice cold H_2O (200 mL). The organic layer was separated, washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexanes, 1:5–1:1) to give **9** as a colorless oil (4.7 g, 82%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, J = 8.8 Hz, 2H), 6.89 (d, J = 8.8 Hz, 2H), 4.80 (t, J = 4.2 Hz, 1H), 4.60–4.68 (m, 2H), 4.18 (dt, J = 4.8 Hz, J = 1.2 Hz, 1H), 4.05–4.11 (m, 1H), 3.81 (s, 3H), 2.68–2.76 (m, 1H), 2.47–2.53 (m, 1H), 1.48 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.05, 159.62, 129.77, 129.25, 113.99, 113.52, 80.51, 77.69, 71.45, 70.08, 55.31, 39.88, 26.90, 25.24. Anal. Calcd for $C_{16}H_{20}O_5$: C, 65.74; H, 6.90. Found: C, 65.60; H, 6.91.

5.1.5. (3a*R*,4*R*,6*S*,6a*S*)-6-(4-Methoxybenzyloxy)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-ol (10)

Following dissolving **9** (0.27 g, 0.92 mmol) in dry THF (20 mL), $LiAlH_4$ (52.3 mg, 1.38 mmol) was added portionwise at 0 °C. The mixture was then stirred at 0 °C for 3 h and then quenched with H_2O with subsequent filtering through Celite. The filtrate was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), and concentrated under reduced pressure to give **10** as a colorless oil (0.22 g, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.8 Hz, 2H), 4.52–4.59 (m, 3H), 4.40 (t, J = 5.6 Hz, 1H), 3.80 (s, 3H), 3.69–3.78 (m, 1H), 3.45–3.51 (m, 1H), 2.41 (d, J = 10.8 Hz, 1H), 2.10–2.15 (m, 1H), 1.72–1.80 (m, 1H), 1.56 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.54, 130.19, 129.73, 114.01, 111.53, 78.48, 78.06, 73.76, 71.36, 68.58, 55.47, 34.76, 25.97, 24.42. Anal. Calcd for $C_{16}H_{22}O_5$: C, 65.29; H, 7.53. Found: C, 65.13; H, 7.53.

5.1.6. (3a*S*,4*S*,6*S*,6a*S*)-6-Fluoro-4-(4-methoxybenzyloxy)-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole (11)

At 0 °C, pyridine (2.5 mL, 31.2 mmol) was added to **10** (4.7 g, 15.9 mmol) that had been dissolved in dry CH_2Cl_2 (100 mL). Retaining the reaction temperature at 0 °C, to this DAST (4.1 mL, 31.2 mmol) was added by means of a syringe. The mixture was warmed to rt, then refluxed for 2 days under N_2. The mixture was quenched with saturated $NaHCO_3$ solution (100 mol) and the organic layer separated, dried (Na_2SO_4), concentrated under reduced pressure, and subjected to silica gel column chromatography (EtOAc/hexanes, 1:5) to give **11** as a colorless oil (3.3 g, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 4.47–4.81 (m, 5H), 4.00–4.05 (m, 1H), 3.81 (s, 3H), 2.04–2.14 (m, 2H), 1.48 (s, 3H), 1.32 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.60, 130.21, 129.81, 114.16, 111.88, 94.40 (d, J = 174.5 Hz), 82.82(d, J = 33.4 Hz), 77.83, 77.13, 71.90, 55.50, 33.70 (d, J = 20.1 Hz), 26.33, 24.27. Anal. Calcd for $C_{16}H_{21}FO_4$: C, 64.85; H, 7.14. Found: C, 64.72; H, 7.19.

5.1.7. 9-((3a*S*,4*R*,6*S*,6a*S*)-6-Fluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl)-9*H*-purin-6-amine (13)

Compound **11** (0.98 g, 3.31 mol) was dissolved in CH_2Cl_2/H_2O mixture (100 mol CH_2Cl_2, 5 mol H_2O). To this DDQ (0.9 g, 3.96 mol) was added. The resultant mixture was stirred at rt for 1 h. Saturated $NaHCO_3$ (20 mol) was added to quench the reaction. The organic layer was separated, washed with brine, dried (Na_2SO_4), concentrated under reduced pressure, and subjected to silica gel column chromatography (EtOAc/hexanes, 1:5) to give (3a*S*,4*S*,6*S*,6a*S*)-6-fluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*]-[1,3]dioxol-4-ol (**12**) as a white solid (0.5 g, 86%), mp 51–52 °C: ^{1}H NMR (400 MHz, $CDCl_3/D_2O$) δ 4.68 (dd, J = 46.0 Hz, J = 3.6 Hz, 1H), 4.56–4.60 (m, 2H), 4.26–4.32 (m, 1H), 2.26–2.32 (td, J = 15.2 Hz, J = 5.6 Hz, 1H), 1.83 (dddd, J = 44.4 Hz, J = 14.4 Hz, J = 10.8 Hz, J = 3.6 Hz, 1H), 1.47 (s, 3H), 1.35 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 111.73, 93.81 (d, J = 172.3 Hz), 82.48 (d, J = 33.2 Hz), 78.21, 71.43, 36.89 (d, J = 20.8 Hz), 25.93, 24.14.

After dissolving **12** (0.73 g, 4.14 mol) in dry THF (50 mol), TPP (1.30 g, 4.9 mol) and 6-chloropurine (0.76 g, 4.9 mol) were added. This mixture was cooled to 0 °C and then allowed to warm to rt followed by heating and stirring at 50 °C overnight. The solvent was removed under reduced pressure and the residue purified by silica gel column chromatography to give 6-chloro-9-[(3a*S*,4*R*,6*S*,6a*S*)-6-fluoro-2,2-dimethyltetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxol-4-yl]-9*H*-purine (0.4 g, 30%, estimated from ^{1}H NMR) that was contaminated with di*iso*propyl hydrazine-1,2-dicarboxylate.

In a high pressure reaction vessel, the crude material from the previous step (0.5 g, 1.6 mmol) was dissolved in dry MeOH (100 mL). This solution was cooled to 0 °C and saturated with NH_3. The vessel was sealed and heated to 120 °C overnight. The solvent was removed under reduced pressure, and the residue purified by silica gel column chromatography to give **13** as white solid (0.4 g, 85%), mp 158–160 °C: ^{1}H NMR (400 MHz, DMSO) δ 8.16 (s, 1H), 8.10 (s, 1H), 7.25 (br s, 2H), 4.8–5.2 (m, 4H), 2.55–2.80 (m, 2H), 1.45 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (62.8 MHz, DMSO) δ 156.01, 152.45, 149.43, 139.10 (d, J = 6.5 Hz), 118.81, 111.44, 96.96 (d, J = 177.6 Hz), 83.52 (d, J = 23.1 Hz), 83.22, 58.65, 35.14 (d, J = 20.3 Hz), 26.26, 24.18. Anal. Calcd for $C_{13}H_{16}FN_5O_2$: C, 53.24; H, 5.50; N, 23.88. Found: C, 53.05; H, 5.47; N, 23.74.

5.1.8. (1*S*,2*S*,3*R*,5*S*)-3-(6-Amino-9*H*-purin-9-yl)-5-fluorocyclopentane-1,2-diol (3)

Compound **13** (0.4 g, 1.36 mol) was dissolved in 0.5 M Hal in Mesh (100 mol). The mixture was stirred at rat overnight. The resultant mixture was neutralized with Ambulate IRA-67 ion exchange resin and then filtered, concentrated under reduced pressure to a residue that was purified by silica gel column chromatography (EtOAc/MeOH/NH_3–H_2O = 3:1:0.2) to give **3** as a white solid (0.32 g, 93%) mp 256–257 °C (dec.): ^{1}H NMR (250 MHz, DMSO) δ 8.16 (s, 1H), 8.12 (s, 1H), 7.22 (s, 2H), 5.36 (d, J = 4.2 Hz, 1H), 5.25 (d, J = 6.5 Hz, 1H), 4.59–4.97 (m, 2H), 4.58–4.63 (m, 1H), 4.04 (dm, J = 12 Hz, 1H), 2.68–2.76 (m, 1H), 2.20–2.34 (m, 1H); ^{13}C NMR (62.8 MHz, DMSO) δ 156.05, 152.20, 149.69, 140.27, 119.40, 95.14 (d, J = 177.7 Hz), 73.78 (d, J = 10.5 Hz), 73.46, 57.86 (d, J = 3.1 Hz), 33.31 (d, J = 22.6 Hz). Anal. Calcd for $C_{10}H_{12}FN_5O_2$: C, 47.43; H, 4.78; N, 27.66. Found: C, 47.28; H, 4.86; N, 27.36.

5.2. Antiviral assays

The viruses evaluated and the procedures used are in Refs. 10,11.

Acknowledgments

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10. Viruses subjected to **3** were herpes simplex virus 1 and 2, herpes simplex virus 1 (TK^-), human cytomegalovirus, vaccinia virus, cowpox virus, respiratory syncytial virus, Punta Toro virus, hepatitis C, coxsackie B4, vesicular stomatitis, parainfluenza-3, reovirus-1, and Sindbis, measles, influenza A (H1N1, H3N2) influenza B, feline corona, rhinovirus, and adenovirus.[11]
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