Synthesis, Antiviral, Antibacterial and Antitumor Cell Activities of 2'-Deoxy-2'-fluoropuromycin

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A procedure for the synthesis of 2'-deoxy-2'-fluoropuromycin (1b) was developed. Ring opening of the lyxo-epoxide (4) or nucleophilic displacement of the 3'-O-mesy late (5) by an azide ion afforded two azido nucleosides, 6a and 7a. The major product (7a) was reacted with diethylaminosulfur trifluoride (DAST) to give the 2'-fluoronucleoside (8), which was converted to the 3'-aminonucleoside (9) by hydrogenation. Compound 9 was condens ed with an amino acid by the conventional method and subsequently deprotected by acid to give 1b. Compounds 1b, 6b and 7b exhibited no selective antiviral activity against several DNA and RNA viruses. Compound 1b had weak antibacterial activity (minimum inhibitory concentration approximately 25—50 μg/ml) and was cytotoxic to several tumor cell lines (L1210, Molt 4, CEM) at a concentration of about 5 μM. This antitumor cell activity may be attributed to inhibition of protein biosynthesis.

Key words puromycin; fluorinated sugar; dimethylaminosulfur trifluoride; antiviral activity; antibacterial activity; antitumor cell activity

Puromycin (1a), a nucleoside antibiotic isolated from S. alboniger, inhibits protein biosynthesis in vitro and in vivo and exhibits antitumor activity. Puromycin analogs modified at the base, sugar and amino acid moieties have been prepared to investigate the structure-activity relationship. In particular, Daluge and Vincne proved that the 5'-hydroxymethyl group and oxygen of the furanose ring are not necessary for inhibition of protein synthesis. However, the role of the 2'-hydroxyl group of puromycin is not clear, because no 2'-modified analogues, except for 2'-deoxypuromycin, have been reported. Wohlrab et al. reported that 2'-deoxy-2'-fluorocytidine has antiviral activity against herpes simplex virus type 1 (HSV-1). This prompted us to prepare 2'-deoxy-2'-fluoropuromycin (1b). In this paper, the synthesis of 1b via the lyxo-epoxide is described, and the antiviral activity, antibacterial activity and antitumor activity against several tumor cell lines are presented.

Chemical Synthesis

The synthesis of the 3'-azido-2'-fluoro-2',3'-dideoxy analogs of pyrimidine nucleosides has been accomplished, using diethylaminosulfur trifluoride (DAST), from the 3'-azido-3'-deoxyarabinosides. The purine arabinosides were also converted to the corresponding 2'-deoxy-2'-fluororibosides using a similar approach. We adopted this reagent for the synthesis of 8. Thus, compound 2 was converted to 9-(2,3-anhydro-5-O-trityl-β-L-lyxofuranos yl)-6-chloropurine (3) as mentioned in a previous report, then the product 3 was treated with 50% aqueous dimethylamine in N,N-dimethylformamide (DMF) to afford 4. The lyxo-epoxide 4 was subjected to nucleophilic reaction with NaN₃ to give 6a and 7a in 17% and 58% yields, respectively. The 1H-NMR spectrum of the major product 7a revealed that the signals of H2' and H3' appeared at about 4.5—4.6 ppm, and the acetylation of 7a caused a downfield shift of H2' (ca. 0.8 ppm), indicating that the acetyl group was introduced at 2'-OH. This 3'-preference could be explained in terms of the stability of the intermediary anion as follows: attack of the azido ion at the 3'-carbon resulted in the formation of a 2'-alkoxide ion, which is thought to be stabilized by the electron-withdrawing base moiety. In the case of the 3'-alkoxide ion, the base exerts little stabilizing effect. Since yields were not satisfactory, an alternative route to 7a was explored. Compound 2 was treated with 6 eq of 50% aqueous dimethylamine in DMF at 4°C overnight to afford the 3'-O-mesy late 5. This product was treated with NaN₃ in a similar manner to that described for 4 to give 6a and 7a in 21% and 78% yields, respectively. The mechanism could be explained in terms of a two-step reaction. Initial attack of the azido ion at 2'-OH resulted in ring closure to form the intermediary lyxo-epoxide 4, which was then attacked by the nucleophile. The azido- nucleosides (6b, 7b) were obtained by the hydrolysis of the corresponding 5'-O-tritylated compounds, 6a and 7a. Synthesis of the 2'-fluoride 8 was carried out by the reaction of 7a with DAST in the presence of pyridine in 72% yield. The 1H-NMR spectrum of 8 indicated that the 2'-fluorine caused a downfield shift and a large H2'-

puromycin (1a) : R=OH
2'-deoxy-2'-fluoropuromycin (1b) : R=F

Fig. 1

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C-F geminal coupling (52.3 Hz) of the 2'-proton. The configuration of 8 was identified as the riboside structure from the nuclear Overhauser effect (NOE) observed between H2' and H3' on the two-dimensional NOE (NOESY) spectrum. Castillon et al. reported that neighboring group participation by the azido group occurred in the DAST reaction on the pyranose ring. In contrast, no participation by the 3'-azido group was observed in the nucleophilic displacement of 7. The 2'-fluoride 8 was hydrogenated under H2 gas using 5% Pd-C as a catalyst to give the 3'-aminonucleoside 9. Condensation of 9 with N-tert-butoxycarbonyl-p-methoxyphenyl-L-alanine was performed in the presence of N-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in dry DMF to give the protected puromycin analog 10. Finally, compound 10 was hydrolyzed with 80% CH3COOH to afford the trifluoroacetate of 2'-deoxy-2'-fluoropuromycin (1b), from which free 1b was obtained. The structures of both the trifluoroacetate and free 1b were identified from the spectroscopic data in combination with elemental analyses.

**Biological Activity**

Puromycin is a broad-spectrum antibiotic with antitumor activity. That fact prompted us to explore the antitumor activity of its 2'-deoxy-2'-fluoro analog 1b towards murine leukemia cells (L1210/0) and human T-lymphocyte cells (Molt/4, CEM/0). For the biological evaluation of 1b, the trifluoroacetate was chosen because of its solubility in water. Although the azidonucleosides (6b and 7b) displayed no antitumor activity, the puromycin analog 1b demonstrated appreciable antitumor activity against tumor cell lines (L1210, Molt 4, CEM) and this was also evident from its toxicity for Vero cells (minimum...
Table 1. Antibacterial Activity of Compound 1b

<table>
<thead>
<tr>
<th>Minimum inhibitory concentration</th>
<th>&gt; 50 μg/ml</th>
<th>50 μg/ml</th>
<th>25 μg/ml</th>
<th>12.5 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus faecalis</td>
<td>Candida albicans</td>
<td>Candida tropicalis</td>
<td>Bacillus cereus</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>Micrococcus flavus</td>
<td>Micrococcus lysodeikticus</td>
<td>Sarcina lutea</td>
<td>Serratia marcescens</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC₅₀ (μg/ml)</td>
<td>L1210/0</td>
<td>Mol4/C8</td>
<td>CEM/0</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>5.00 ± 3.40</td>
<td>7.55 ± 0.68</td>
<td>5.63 ± 1.40</td>
<td></td>
</tr>
<tr>
<td>6b</td>
<td>340 ± 139</td>
<td>&gt; 500</td>
<td>238 ± 55</td>
<td></td>
</tr>
<tr>
<td>7b</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td></td>
</tr>
</tbody>
</table>

a) 50% Inhibitory concentration, or concentration required to inhibit cell growth by 50%.

cytotoxic concentration, 40 μg/ml). Compound 1b also showed weak antibacterial activity and its minimum inhibitory concentration (MIC) was 12.5 μg/ml for Staphylococcus aureus and 25—50 μg/ml for most other bacteria examined. This activity is almost equal to that of puromycin (MIC for S. aureus, 25 μg/ml). This is the first example of a 2'-modified puromycin showing marked biological activity. It is likely that the cytotoxicity and antibacterial activity of 1b are caused by the inhibition of protein biosynthesis. Duluge and Vince reported that the 5'-hydroxymethyl group and oxygen of the furanose ring are not necessary for the inhibition of protein synthesis, but the 2'-hydroxyl group seems more important, since it is located close to the amino acid group. Koizumi et al. demonstrated that the 2'-deoxy analog of puromycin has severely reduced activity. The difference between 1b and 2'-deoxypuromycin could be explained as follows: it is known that the electronegativity of substituents influences the conformation of the carbohydrate moiety of 2'-substituted nucleosides. For example, introduction of fluoro at the 2'-carbon position favors the 3'-endo conformation, which resembles a ribonucleoside. In the 1H-NMR spectrum of 1b, H1' appears as a doublet with J1'1',f = 19.4 Hz and J1',2 = 0 Hz in contrast to J1',2 = 6.1 Hz for 2'-deoxypuromycin. This tendency is in good agreement with what is seen in the adenosine series, in which J1',2 values are 3.1 Hz for 2'-deoxy-2'-fluoroadenosine and 7.7 Hz for 2'-deoxyadenosine. As a consequence, 1b may be assumed to occur in the 3'-endo form, and this conformation would be necessary for binding to the ribosome. In contrast, 2'-deoxypuromycin should assume a different conformation that is unfavorable for binding to the ribosome. However, it is not excluded that dipole-dipole interaction of 2'-substituents may affect the ribosome-binding activity.

The antiviral effects of 1b and azidonucleosides 6b, 7b were assayed according to previously established proce-

dures. These compounds displayed no activity against several DNA viruses, such as herpes simplex virus type 1 and type 2, and vaccinia virus. No antiviral activity was observed against several RNA viruses, such as vesicular stomatitis, Coxsackie, polio, parainfluenza-3, reo-1, Sindbis and Semliki forest virus. In an antiviral experiment compound 1b showed some cytotoxicity towards cell cultures (minimum cytotoxic concentrations for Vero cells, HeLa cells and E,SM cells were 40 μg/ml).
azido-2-deoxy-5-O-trityl-β-D-xylorafuranoS)-N9,N11-dimethyldihamine (6a) (190 mg, 17%). UV 380 nm: 273.5, 250 nm: 380, 280 nm: 268; 293.5, 280 nm: 250 nm: 273.5. 1H-NMR (CDCl3) δ: 8.18, 7.80 (1 each, s, H2, H8); 7.15–7.47 (15H, m, 5-O(C6H5)2), 5.59 (1H, d, J = 1.95 Hz, H1), 4.53 (1H, d, J = 1.95 Hz, H2), 4.10–4.28 (2H, m, H3 and H4), 3.45–3.64 (8H, m, N(CH3)2 and H5), 3.11 (1H, s, SO4CH3), MS m/z: 562 (M+). The second fraction was evaporated to afford 95-azido-2-deoxy-5-O-trityl-β-D-arabinofuranosyl)-N9,N11-dimethyldihamine (7a) (666 mg, 58%) as a caramel. UV 380 nm: 273.5, 250 nm: 380, 280 nm: 268; 293.5, 280 nm: 250 nm: 273.5. 1H-NMR (CDCl3) δ: 8.29 (1H, s, H2), 7.98 (1H, s, H8), 7.22–7.36 (15H, m, 5-O(C6H5)2), 6.08 (1H, d, J = 5.1 Hz, H1), 4.49–4.62 (2H, m, H2 and H3), 3.87 (1H, m, H4), 3.53 (6H, br, N(CH3)3), 3.43 (2H, m, H5). MS m/z: 562 (M+). The third fraction was a solution of 5 (632 mg, 1.03 mmol) and NaN3 (325 mg, 5 eq) in DMF (30 ml) was stirred at 100 °C for 1 h, then cooled. Usual work-up of the solution and Silica gel chromatography similar to that as mentioned above gave 2-azidonucleoside 6a (122 mg, 21%) and 3'-azidonucleoside 7a (456 mg, 78%), which were identical with the samples obtained as described above.

O-Acetate of 7a Acetic anhydride (0.5 ml) was added to a solution of 7a (25 mg, 0.045 mmol) in pyridine (1 ml) and the mixture was kept at 180 °C for 1 h at room temperature. A usual work-up of the mixture gave 9-2-azido-3-azido-2-deoxy-5-O-trityl-β-D-arabinofuranosyl)-N9,N11-dimethyldihamine as a caramel (30 mg, quantitative). UV 380 nm: 273; 250 nm: 380, 280 nm: 268; 293.5, 280 nm: 250 nm: 273.5. 1H-NMR (CDCl3) δ: 8.28, 7.95 (1 each, s, H2, H8), 7.20–7.49 (15H, m, 5-O(C6H5)2), 6.47 (1H, d, J = 9.1 Hz, H1), 5.35 (1H, t, J = 3.9 Hz, H2), 4.54 (1H, d, J = 5.2, 3.9 Hz, H3), 3.41–3.57 (11H, m, N(CH3)2 and H5), 1.82 (2H, s, 2-OC6H5). MS m/z: 604 (M+).

2-Azido-2-deoxy-5-O-β-D-xylorafuranoS)-N9,N11-dimethyldihamine (6b) A solution of 6a (200 mg, 0.36 mmol) in 80% aqueous C4H9COOH (3 ml) was stirred at room temperature for 10 min and evaporated in vacuo to give a residue, which was evaporated azetrotopically with water (3 ml) twice. The solid thus obtained was dissolved in a small amount of CHCl3 and chromatographed on a column of Silica gel (2.0 x 20 cm) using 0–7.7% EtOH in CHCl3 (520 ml) to afford a caramel (81.2 mg, 71%). UV 380 nm: 274; 250 nm: 380, 280 nm: 268; 293.5, 280 nm: 250 nm: 274. 1H-NMR (CDCl3) δ: 8.27, 8.73 (1 each, s, H2, H8), 6.05 (1H, br, 3-OH), 5.50 (1H, d, J = 6.2 Hz, H1), 5.45 (1H, br, 5-OH), 4.81 (1H, t, J = 6.2 Hz, H2), 4.54 (1H, m, H3), 4.32 (1H, dt, J = 6.4, 2.7 Hz, H4), 3.9–4.1 (2H, m, H5), 3.55 (6H, br, N(CH3)3). MS m/z: 320 (M+), 192 (dimethyldihamine+CHO)+, 164 (dimethyldihamine)+. Anal. Calc. for C23H29N3O5. 0.5H2O: C, 43.77; H, 5.20; N, 34.03. Found: C, 43.98; H, 5.01; N, 33.91.

2-Azido-3-deoxy-5-O-arabinofuranosyl)-N9,N11-dimethyldihamine (7b) Compound 7a (200 mg, 0.36 mmol) was subjected to a similar reaction to obtain 7b as white crystallines (78.2 mg, 69%), mp 199–202 °C. UV 380 nm: 274; 250 nm: 380, 280 nm: 268; 293.5, 280 nm: 250 nm: 274. 1H-NMR (CDCl3) δ: 8.33 (1H, s, H2), 7.92 (1H, s, H8), 2.23 (1H, m, J = 10.4 Hz, H1), 1.76 (1H, d, J = 6.0 Hz, 2-OH), 2.55 (1H, br, 5-OH), 4.51 (1H, t, J = 4.5 Hz, H2), 4.30 (1H, t, J = 7.7 Hz, H3), 3.74 (1H, m, H4), 3.62 (2H, m, H5), 3.4 (6H, br, N(CH3)3). MS m/z: 320 (M+), 278 (M+–N3)+, 192 (dimethyldihamine+CHO)+, 164 (dimethyldihamine)+. Anal. Calc. for C23H25N3O5. 0.5H2O: C, 44.81; H, 4.46; N, 31.89. Triethylamine (0.1 ml) was added to a solution of the trifluoroacetate of 1b (78 mg, 0.067 mmol) in EtOH (3 ml) and the solution was evaporated to dryness. Recrystallization of the residue from a small amount of water gave white crystals (48.4 mg, 95%), mp 128–130 °C. Anal. Calc. for C12H22FN3O3·H2O: C, 53.76; H, 6.15; N, 19.94. Found: C, 53.91; H, 5.93; N, 19.51.

Acknowledgments

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References and Notes

12) *N*-tert-Butoxy carbonyl-p-methoxyphenyl-L-alanine was prepared by treatment of Boc-L-tyrosine with methyl iodide followed by alkaline hydrolysis.