Synthesis and Antimicrobial Activity of 2'-Deoxypuromycin

Fumiaki Koizumi, Takayuki Oritani and Kyohei Yamashita

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University,
Aoba-ku, Sendai 981, Japan

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2'-Deoxypuromycin (2) was synthesized to learn the effect of the 2'-hydroxyl group on the biological activity. Acylated xylose 3 was condensed with silylated 6-chloropurine to give β-D-xylofuranosyl-6-chloropurine derivative 4, whose 6-dimethylation, 2'-deoxygenation and deprotection afforded 2'-deoxy-β-D-xylofuranosyl purine analog 7. The latter was converted to 2'-deoxypuromycin (2) in 8 steps. 2'-Deoxy analog 2 showed only weak antimicrobial activity compared with that of puromycin (1).

An aminoacylnucleoside antibiotic puromycin (1), which was isolated from a culture broth of Streptomyces alboniger by Porter et al.,\(^1\) has been found to inhibit protein biosynthesis as a 3'-end mimic of aminoacyl-t-RNA.\(^2\) Many structural analogs of 1 have been synthesized in order to lower its toxicity and enhance its biological activities as an antimicrobial,\(^3\)\(^4\) antitrypanosoma\(^5\)\(^6\) and antitumor agent,\(^3\)\(^4\)\(^7\)\(^8\) while puromycin has been used as a biological tool in an investigation of the mechanism for the peptide-elongation reaction.\(^2\) Nathans et al. have also clarified that there are some structural requirements for the puromycin reaction.\(^9\) The rigid configuration of aminonucleoside\(^10\) and aromatic amino acid moieties\(^11\) are required for biological activity. However, the methyl substituent on the dimethylamino group,\(^12\) the hydroxymethyl group,\(^4\) oxygen in the furanosyl ring,\(^13\) the 5'-hydroxyl group,\(^6\) and the methoxyl group in the amino acid moiety\(^11\) might be unnecessary for puromycin-like activity. The effect of the 2'-hydroxyl group of 1 on its biological activity is still obscure.\(^14\) In this paper we describe the synthesis of

[Fig. 1]
2'-deoxypuromycin (2) to clarify its antimicrobial activity.

Generally for nucleoside synthesis, the lack of a 2-hydroxyl group has given an anomeric mixture of 2'-deoxynucleosides, except for a few examples. From such a point of view, 2'-deoxypuromycin (2) was synthesized as outlined in Figure 1.

Acylated xylose 316) was condensed with silylated 6-chloropurine and SnCl₄17) and then converted to β-D-xylofuranosylpurine derivative 4. The glycosidation position of the purine base was assigned by ¹H-NMR.18) 4 was heated in 50% aq. HNMe₂/THF to afford 6-dimethylamino purine derivative 5. 2'-Deoxyamination of 5 was accomplished by phenyloxythiocarbonylation19) and successive reduction with n-Bu₃SnH and AIBN (a,a'-azobisobutyronitrile) in toluene to yield 2'-deoxy-derivative 6. Deprotection of 6 with methanolic ammonia gave 2'-deoxy-β-D-xylofuranosyl purine derivative 7. The 5'-hydroxyl group of 7 was protected as pivalate 8, whose 3'-hydroxyl group was mesylated and then converted to a-azide 9 in the SN₂ manner. When the 5'-hydroxyl group was protected as a trityl ether, by steric hindrance of the β-face, a-azide 9 was obtained in only a low yield (<40%; 2 steps). Saponification of 9 with sodium methoxide gave 6-dimethylamino-9-(3'-azido-2',3'-dideoxyribosyl)purine 10. Firstly, the azide group was hydrogenated to an amino group and acylated by the conventional method for peptide synthesis (DCC-7N-hydroxysuccinimide).12) However, the reduction proceeded in a low yield, and acylation of the amino group proceeded slowly. Secondly, we applied the Staudinger reaction to form a peptide bond.20) Treatment of 10 with triphenylphosphine in toluene resulted in the formation of an iminophosphorane, which was reacted with N-benzyloxy carbonyl-p-methoxy-L-phenylalanine8) to give aminoacyl derivative 11. This protocol has been reported by Zaloom and co-workers,20) and from our studies, it was found to be useful for directly synthesizing an aminoacylnucleoside from an azide intermediate. Hydrogenolysis of 11 with 10% Pd on charcoal gave the desired 2'-deoxypuromycin (2).

2'-Deoxypuromycin (2) and puromycin (1) were tested for antimicrobial activity. The minimum inhibitory concentration in a broth of 2 and 1 was as follows (µg/ml): Staphylococcus aureus 6243, >100 and 25; Bacillus subtilis var. niger IFO 3108, >100 and 50; Escherichia coli 6038, >100 and 25.

2'-Deoxypuromycin (2) lost its strong antimicrobial activity, this result being explainable by the fact that 1 is known to exist in an N-(3'-endo) conformation, whereas the 2'-deoxy nucleosides are more likely to exist in an S-(2'-endo) conformation.2) This conformation was supported by ¹H-NMR measurements. The coupling constants of the proton signals of 1, 2 and some intermediates are shown in Table I. Especially, the difference in ¹J₂,₃,₃ values of the proton signals of 1 and some 2'-deoxynucleosides could suggest the puckering of the ribose ring.21) It could be supposed that such puckering of the ribose ring would cause a change in orientation of the aminoacyl moiety of puromycin (1) and influence the recognition by a peptidyl transferase and its biological activity.

### Table I. Coupling Constants for Proton Signals in Puromycin and Some 2'-Deoxynucleosides

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<tr>
<th></th>
<th>Puromycin</th>
<th>2</th>
<th>10</th>
<th>11</th>
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<tr>
<td>1'-2'β</td>
<td>2.7a</td>
<td>6.1b</td>
<td>5.4a</td>
<td>6.2a</td>
</tr>
<tr>
<td>1'-2'α</td>
<td>—</td>
<td>7.9</td>
<td>9.3</td>
<td>8.2</td>
</tr>
<tr>
<td>2'α-3'</td>
<td>—</td>
<td>7.6</td>
<td>6.1</td>
<td>7.8</td>
</tr>
<tr>
<td>2'β-3'</td>
<td>5.8</td>
<td>2.9</td>
<td>04a</td>
<td>2.3</td>
</tr>
<tr>
<td>2'α-2'β</td>
<td>—</td>
<td>13.4</td>
<td>13.7</td>
<td>13.9</td>
</tr>
<tr>
<td>3'-4'</td>
<td>8.0</td>
<td>2.9</td>
<td>6.8</td>
<td>6.2</td>
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<tr>
<td>b ¹H-NMR (270 MHz, CDCl₃+D₂O).</td>
<td></td>
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<tr>
<td>c ¹H-NMR (100 MHz, CDCl₃).</td>
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<tr>
<td>d The signal of H-2' was observed as dd (J = 5.4 and 13.7 Hz).</td>
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### Experimental

All melting points (mp) are uncorrected. IR spectra were...
recorded on a JASCO IR-810 infrared spectrometer. 1H-NMR spectra were measured on a JEOL JNM FX-100 (100 MHz)/GSX-270 (270 MHz) spectrometer with TMS as an internal standard. High-resolution mass spectra were obtained with a JEOL JMX-HX110 mass spectrometer, while ultraviolet spectra were recorded on a Hitachi 124 spectrophotometer. Optical rotation values were measured with a JASCO DIP-4 digital polarimeter, and thin-layer chromatography was performed on silica gel (Merck 60 PF254, 0.75 mm in thickness).

6-Chloro-9-(2'0-acetyl-3',5'-di-O-benzoyl-D-xylolofuranosyl)purine (4). To a stirred suspension of 6-chloropurine (250 mg) in anhydrous acetonitrile (250 ml) were successively added hexamethyldisilazane (HMDS, 6.92 ml, 29.8 mmole) and trimethylchlorosilane (TMS-Cl, 3.78 ml, 29.8 mmole) and SnCl4 (a 4.96 M solution in CH2Cl2, 9 ml, 44.6 mmole). The mixture was stirred, and the temperature was raised to 60°C. After evaporating the solvent, the residue was treated with CH2Cl2 and water. The organic layer was washed with dil. HCl, brine, and then with CH2Cl2. The combined organic phase was separated, and the aqueous phase was extracted with CH2Cl2 (400 ml), poured into a cold sat. NaHCO3 solution, and dried over MgSO4. After evaporating the solvent, the residue was chromatographed on a silica gel column (CH2Cl2-EtOAc=7:1) of the residue afforded 4 (10.86 g, 54%) as a viscous syrup, [a]D +65.1° (c=0.67, CHC13).

6-Dimethylamino-9-(2'-deoxy-3',5'-di-O-benzoyl-D-xylolofuranosyl)purine (5). To a stirred solution of 4 (28.03 g, 52.2 mmole) in THF (60 ml) was added dropwise 50% aq. HNMe2 (100 ml) at 90°C (bath temp.) over 1.5 min. The mixture was heated under reflux for 1.5 min and then cooled to room temperature. After evaporating the solvent, the residue was treated with CH2Cl2 and water. The aqueous phase was reextracted. The organic layer was combined, and washed successively with dil. HCl, brine and a sat. NaHCO3 solution, and dried over MgSO4. After evaporating the solvent, the residue was chromatographed on silica gel (CH2Cl2-MeOH=30:1) to give 5 (19.76 g, 75%) as a viscous syrup, [a]D 23 +47.1° (c=0.52, CHCl3). IR vmax (film) cm−1: 3300, 1720, 1595. 1H-NMR (CDCl3, 100 MHz) δ: 3.50 (6H, s, H-2'), 8.27 (1H, s, H-8). Anal. Found: C, 60.95; H, 4.90; N, 13.62. Calcd. for C26H25N5O6 0.5H2O: C, 60.93; H, 4.90; N, 13.67.

6-Dimethylamino-9-(2'-deoxy-3',5'-di-O-benzoyl-D-xylolofuranosyl)purine (6). To a stirred solution of 5 (19.76 g, 39.2 mmole) and 4-dimethylaminopyridine (7.18 g, 58.8 mmole) in anhydrous acetonitrile (300 ml) was slowly added phenoxysiocarbonyl chloride22 (8.13 ml, 58.8 mmole) with ice-cooling. The reaction mixture was stirred for 24 hr at room temperature, before the solvent was evaporated. The residue was then treated with CH3Cl2 (400 ml) and water (100 ml). The organic layer was washed with dil. HCl, sat. NaHCO3 and brine, and dried over MgSO4. After evaporating the solvent, the residue was chromatographed on silica gel (CH2Cl2-MeOH=60:1) to give 19.41 g (82%) of crude 6-dimethylamino-9-(3',5'-di-O-benzoyl-2'-O-phenoxythiocarbonyl-D-xylolofuranosyl)purine. 1H-NMR (CDCl3, 100 MHz) δ: 3.52 (6H, s, 6-NMe2), 4.71–5.00 (3H, m, H-2', 4'), 5.74 (1H, d, J2',3'=4.0 Hz, H-3'), 6.43–6.52 (2H, m, H-1' and H-2'), 7.1–8.06 (15H, m, Ph), 8.06 (1H, s, H-2').

In this reaction, a considerable amount of a polar by-product was produced (ca. 20%). This compound was assigned to be the N-7 isomer. XH-NMR (CDCl3, 100 MHz) δ: 6.71 (1H, d, J2',3'=1.5 Hz, H-1'), 8.83 (1H, s, H-2'), 8.91 (1H, s, H-8). However, no further investigation of this compound was made.
temperature for 24 hr and warmed to ca. 50°C, before being evaporated to dryness. The residue was recrystallized from ethanol to give pure 7 (1.11 g, 73%) as a white needle, mp 210–211°C. \([\delta]_D^{22}= -22.7° (c=0.52, H_2O).\) UV \(\lambda_{max}\) (EtOH) 275 nm (\(\varepsilon=2.17 \times 10^4\)). IR \(\nu_{max}\) (KBr) cm\(^{-1}\): 3350, 3200, 1610. 1\(^H\)-NMR (CDCl\(_3\), 100 MHz) \(\delta\): 2.25 (1H, dd, \(J_{1',2'}=2.2\) Hz, \(J_{2',2''}=13.2\) Hz, H-2', H-2''), 2.79 (1H, ddd, \(J_{1',2'}=8.5\) Hz, \(J_{2',2''}=5.2\) Hz, H-2', H-2''), 3.45 (6H, br.s, 6-NMe\(_2\)), 3.69 (2H, m, H-5', H-5''), 3.86 (1H, m, H-4'), 4.33 (1H, m, H-3'), 4.70 (1H, t, \(J_{2',2''}=5.5\) Hz, 5'-OH, disappeared by D\(_2\)O exchange), 7.83 (1H, s, H-2), 8.32 (1H, s, H-8). Anal. Found: C, 51.60; H, 6.14; N, 25.08%.

6-Dimethylamino-9-(2'-deoxy-3'-O-mesyl-5'-O-pivalyl-\(\beta\)-D-xylofuranosyl)purine (8). To a stirred solution of 7 (1.50 g, 6-NMe\(_2\)), 4.43 (3H, m, H-4', H-5' and H-5''), 5.44 (1H, m, H-3'), 6.91 (1H, d, \(J_{2',2''}=5.6\) Hz, 5'-OH, disappeared by D\(_2\)O exchange), 8.28 (1H, s, H-8). Anal. Found: C, 56.17; H, 6.14; N, 25.08%. Calcd. for C\(_{17}\)H\(_{25}\)N\(_5\)O\(_4\): C, 56.18; H, 6.93; N, 19.23.}

To the vacuum-dried crude methanesulfonate were added DMF (25 ml) and Na\(_2\)SO\(_4\) (1.77 g, 27.2 mmol). The suspension was stirred for 5 hr at 120°C under an N\(_2\) atmosphere. The mixture was filtered, and the residue washed with CHCl\(_3\), and then the filtrate was evaporated to dryness. The residue was dissolved in CHCl\(_3\), washed with sat. NaHCO\(_3\), dried over MgSO\(_4\), and evaporated. The residue was finally chromatographed on silica gel (CH\(_2\)Cl\(_2\)-MeOH = 60 : 1) to give 9 (0.89 g, 84%) as a syrup, \([\delta]_D^{22}= -6.9° (c=0.51, CHCl\(_3\)).\) UV \(\lambda_{max}\) (EtOH) 275 nm (\(\varepsilon=2.39 \times 10^4\)). IR \(\nu_{max}\) (film) cm\(^{-1}\): 1730, 1600, 1340, 1180. 9-(2'-deoxy-3'-O-mesyl-5'-O-pivalyl-\(\beta\)-D-ribofuranosyl)purine (9). To a stirred solution of 8 (6-NMe\(_2\)), 4.07 (1H, m, H-4'), 4.22 (1H, dd, \(J_{1',2'}=6.2\) Hz, H-1'), 7.83 (1H, s, H-2'), 8.32 (1H, s, H-8). Anal. Found: C, 51.96; H, 5.99; N, 28.44. Calcd. for C\(_{12}\)H\(_{17}\)N\(_5\)O\(_3\): C, 51.39; H, 5.99; N, 25.08%.

6-Dimethylamino-9-(2'-deoxy-5''-O-pivaloyl-\(\beta\)-D-xylofuranosyl)purine (10). A mixture of 9 (620.7 mg, 1.57 mmol) with ice-cooling. The mixture was stirred at room temperature for 20 hr. The solution was neutralized with CO\(_2\) gas, and evaporated under reduced pressure. The residual solid was passed through a short silica gel column (CH\(_2\)Cl\(_2\)-MeOH = 15 : 1) to remove the inorganic salt. The residue was purified by PTLC (CH\(_2\)Cl\(_2\)-MeOH = 20 : 1) to give 10 (493.2 mg, 99%) as a white solid, mp 80.5–81°C (from CHCl\(_3\)-n-hexane). UV \(\lambda_{max}\) (EtOH) 274 nm (\(\varepsilon=2.38 \times 10^4\)). IR \(\nu_{max}\) (film) cm\(^{-1}\): 1730, 1600, 1340, 1180. 1\(^H\)-NMR (CDCl\(_3\), 100 MHz) \(\delta\): 2.33 (1H, dd, \(J_{2',2''}=6.7\) Hz, H-2', H-2''), 2.90 (1H, d, \(J_{2',2''}=6.1\) Hz, H-2', H-2''), 2.95 (2H, m, H-2', 2''), 3.00 (3H, s, mesyl), 3.52 (6H, br.s, 6-NMe\(_2\)), 5.44 (1H, m, H-3'), 6.51 (1H, dd, \(J_{1',2''}=5.4\) Hz, \(J_{1',2''}=5.4\) Hz, H-1'), 8.06 (1H, s, H-2), 8.33 (1H, s, H-8).
Synthesis of 2'-Deoxypuromycin

\[ \text{cm}^{-1}: \ 1710, 1660, 1600. \]
\[ \text{H-NMR (CDCl}_3, 270 \text{ MHz):} \ 2.10 (1H, symmetrical m, H-2'), 2.92 (1H, dd, J_{\alpha,\beta} = 8.4 Hz, J_{2',3'} = 7.8 Hz, H-2'a), 3.05 (1H, ddd, J_{r,2'a} = 8.2 Hz, J_{2'a,3'} = 13.3 Hz, H-3'), 3.78 (3H, s, OMe), 3.91 (2H, m, H-4' and H-5''), 4.33 (1H, dd, H-\alpha), 4.54 (1H, ddd, J_{2',3'} = 2.3 Hz, J_{2',3'} = 7.8 Hz, H-3'), 5.10 (2H, s, PhCH\_2), 5.45 (1H, m, \alpha-NH), 5.94 (1H, dd, J_{r,2} = 6.2 Hz, H-1'), by D\_2O exchange), 6.15 (1H, m, 3'-NH, by D\_2O exchange), 6.87 (2H, d, J = 8.6 Hz, anisyl), 7.14 (2H, d, J = 8.6 Hz, anisyl), 7.33 (5H, s, Ph), 7.33 (1H, m, 5'-OH, by D\_2O exchange), 7.74 (1H, s, H-2), 8.24 (1H, s, H-8). \]
\[ \text{HR-FAB-MS m/z:} \ 590.2763 (MH\_+, 590.2725 \text{ calcd. for C}_{30}\text{H}_{36}\text{N}_7\text{O}_6). \]

6-Dimethylamino-9-[(3'-(p-methoxyphenyl-L-alanyl-amino)-2',3'-dideoxy-D-ribofuranosyl]purine (2'-deoxy-puromycin) (2). A suspension of 11 (50.3mg, 85\( \mu \)mol) and 10% Pd on charcoal in 1,4-dioxane-ethanol (1 : 1, 5 ml) was stirred under hydrogen at ordinary pressure and room temperature for 18 hr. After filtration through Celite, the filtrate was evaporated. The residue was purified with PTLC (CH\_2Cl\_2-MeOH=8:1) to give 2 (24.7mg, 63%) as a solid, mp 153.5-155°C (from 99% EtOH). \[ \text{[\alpha]_D} = -25.5 (c=0.20, 1,4-dioxane). \]
\[ \text{UV } \lambda_{\text{max}} (\text{EtOH}) 275nm (e=3.28 \times 10^4). \]
\[ \text{IR } \nu_{\text{max}} (\text{film}) \text{ cm}^{-1}: \ 3300, 1670, 1600. \]
\[ \text{H-NMR (CDC}_3, 270 \text{ MHz):} \ 2.05 (2H, br.s, \alpha-NH\_2), 2.29 (1H, ddd, J_{2',3'} = 6.1 Hz, J_{2',3'} = 13.4 Hz, H-2'), 2.81 (1H, dd, J_{\alpha,\beta} = 8.1 Hz, J_{\alpha,\beta} = 13.9 Hz, H-3'), 3.01-3.14 (2H, m, H-2'a and H-5''), 3.53 (6H, br.s, 6-NMe\_2), 3.61 (1H, dd, J_{\alpha,\beta} = 4.4 Hz, J_{\alpha,\beta} = 8.1 Hz, H-\alpha), 3.78 (1H, dd, J_{2'a,3'} = 2.2 Hz, H-5', hidden under the signal of OMe), 3.79 (3H, s, OMe), 3.96 (1H, dd, J_{\alpha,\beta} = 1.7 Hz, J_{\alpha,\beta} = 12.7 Hz, H-5'), 4.11 (1H, m, H-4'), 4.65 (1H, dd, J_{2'a,3'} = 7.6 Hz, J_{2'a,3'} = 2.9 Hz, H-3'), 6.17 (1H, dd, J_{\alpha,\beta} = 7.9 Hz, H-1'), 6.85 (2H, d, J = 8.6 Hz, anisyl), 7.13 (2H, d, J = 8.6 Hz, anisyl), 7.69 (1H, m, 3'-NH), 7.85 (1H, s, H-2), 8.27 (1H, s, H-8), 8.27 (1H, m, 5'-OH, disappeared by D\_2O exchange) by D\_2O exchange. \]
\[ \text{HR-FAB-MS m/z:} \ 590.2763 (MH\_+, 590.2725 \text{ calcd. for C}_{30}\text{H}_{36}\text{N}_7\text{O}_6). \]

References


16) 1,2-Di-O-acetyl-3,5-di-O-benzoyl-D-xylofuranose (3) was prepared from D-(+)-xylose by following the reported method for 1,2,3,5-tetra-Oacetyl-D-xylofuranose; A. Magnani and Y. Mikuriya, Carbohydr. Res., 25, 158 (1973).


