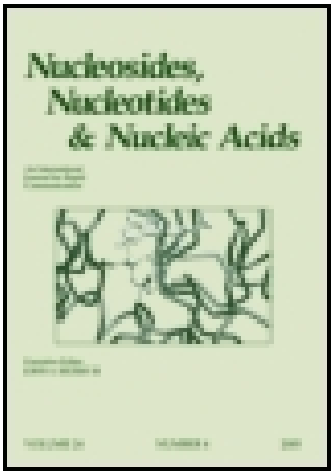

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Facile Synthesis of α-Anomeric Pyrimidine Nucleosides

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NOTE

FACILE SYNTHESIS OF α-ANOMERIC PYRIMIDINE NUCLEOSIDES

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Abstract: 2-Amino-α-D-ribofurano[1',2':4,5]-2-oxazoline reacted with α-bromoacrylonitrile yielding 2,2'-anhydro-1-α-D-ribofuranosylcytosine which was converted to α-cytidine and α-2'-deoxycytidine. Reaction of 2-amino-α-D-ribofurano[1',2':4,5]-2-oxazoline and ethyl α-(bromomethyl)acrylate afforded 2,2'-anhydro-1-α-D-ribofuranosylthymine, a precursor of α-thymidine and α-ribothymidine.

Oligonucleotide analogs consisting of α-nucleotide units have been shown to possess strong nuclease resistance and to form stable parallel duplexes with the complementary DNA or RNA single strands.[1] Thus, α-oligonucleotide analogs are considered as one of the appropriate antisense agents. α-Anomeric nucleosides, starting materials for the α-oligonucleotides, have been prepared either by coupling of protected nucleobases and sugar derivatives[2] or anomerization of natural β-nucleosides.[3] These approaches encounter problems of selectivity of α- or β-anomer formation, although α-anomer was preferentially formed under some conditions.[2c, 2d, 2e] Appropriate protecting groups in the base and sugar moieties are also required in the reaction. We have explored a simple and stereoselective synthetic method for α-nucleosides by modifying the oxazoline method which was originally developed by Sanchez and Orgel.[4] In this paper we describe the facile synthesis of α-cytidine, α-2'-deoxycytidine and α-thymidine derivative using easily accessible reagents.

The starting material, 2-amino-α-D-ribofurano[1',2':4,5]-2-oxazoline, (**1**) was prepared from D-ribose and cyanamide.[4] The conventional synthetic method for the

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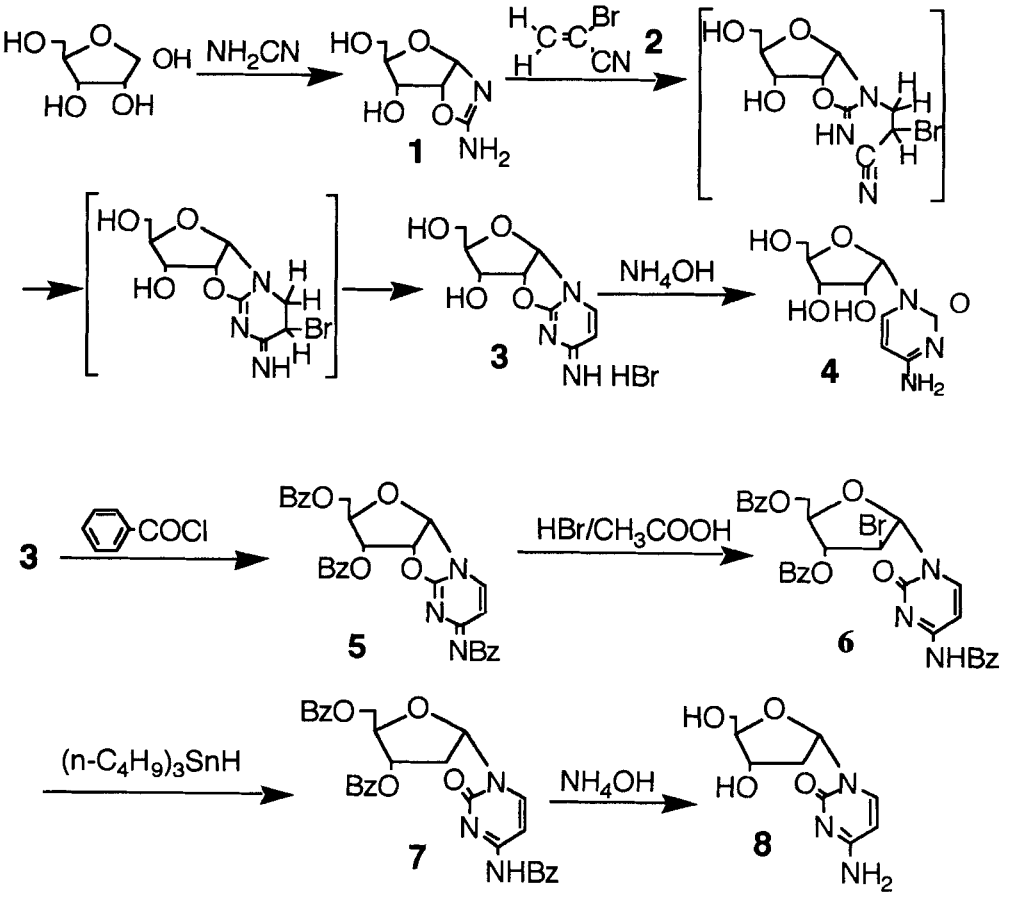
This paper is dedicated to Professor Morio Ikehara on the occasion of his 70th birthday.

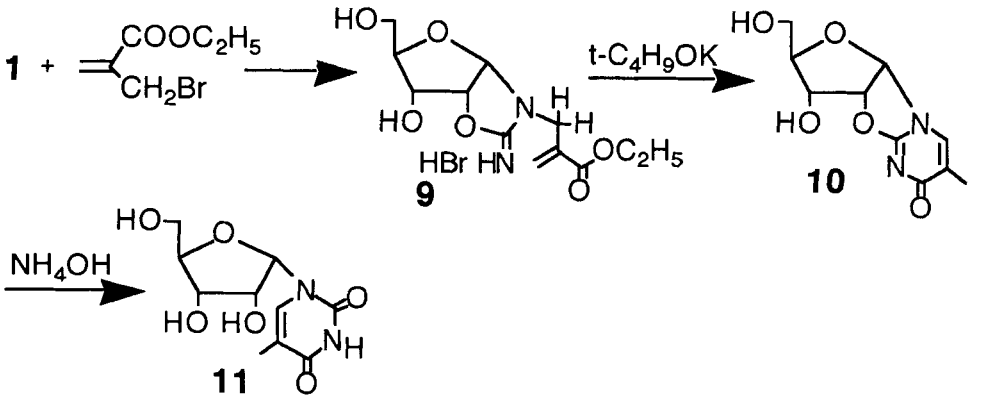
cytosine nucleosides from the aminooxazoline derivatives uses propiolonitrile[4] or cis-β-trimethylammoniumacrylonitrile tosylate.[5] As the former reagent is hazardous and both are not easy to prepare from low-cost materials, we tried α-bromoacrylonitrile (**2**) as a substitute. α-Bromoacrylonitrile was prepared from acrylonitrile and bromine easily. The reaction of **1** with **2** in methanol at 70°C gave 2,2'-anhydro-1-α-D-ribofuranosylcytosine (**3**) in 76 % yield. In this reaction, α-bromoacrylonitrile likely condenses with **1** to afford an intermediate adduct which undergoes cyclization and elimination of hydrogen bromide to furnish **3**. A similar adduct formation followed by cyclization has been reported in the synthesis of 2,2'-anhydro-1-β-D-arabinofuranosyl-5,6-dihydrouracil from methyl acrylate and 2-amino-β-D-arabinofurano[1',2':4,5]-2-oxazoline.[6] Hydrolysis of **3** with aqueous ammonia occurred with retention of 2'-oxygen configuration giving α-cytidine (**4**).

The conversion of **3** to α-2'-bromo-2'-deoxycytidine with HBr/CF_3COOH was unsuccessful, probably because the equilibrium **3** α-2'-bromo-2'-deoxycytidine is favorable for **3**. To shift the equilibrium to the formation of the 2'-bromo derivative, we conducted benzoylation of the 4-imino group of **3**. The pyridine solution of **3** was treated with benzoyl chloride in the presence of triethylamine and dimethylaminopyridine at 50°C for 4 d yielding $O^{3'},O^{5'},N^4$-tribenzoyl-2,2'-anhydro-1-α-D-ribofuranosylcytosine (**5**). Conversion of **5** to $O^{3'},O^{5'},N^4$-tribenzoyl-α-2'-bromo-2'-deoxycytidine (**6**) was achieved with 25 % HBr/CH_3COOH. Reduction of **6** with tributyltin hydride in benzene under initiation by 2,2'-azobis(isobutyronitrile) afforded $O^{3'},O^{5'},N^4$-tribenzoyl-α-2'-deoxycytidine (**7**) in 14% yield. The observed low yield of **7** is partly due to the unexpected removal of the N-benzoyl group of **7** during the reduction. Treatment of **7** with 25% NH_4OH solution in dioxane at room temperature gave α-2'-deoxycytidine (**8**) quantitatively.

Synthesis of α-thymidine derivatives has also been established by the modified oxazoline method as shown in Scheme 2. The key starting material, ethyl α-(bromomethyl)acrylate, was prepared in 60-80% yield from either triethylphosphonoacetate and formaldehyde or ethyl acrylate and formaldehyde, followed by bromination with hydrogen bromide.[7] Addition of ethyl α-(bromomethyl)acrylate to **1** gave the adduct (**9**), 1-(2-carboethoxyallyl)-α-ribofurano[1',2':4,5]-2-oxazoliminium bromide, in high yield. Potassium *t*-butoxide-mediated cyclization of **9** gave 2,2'-anhydro-1-α-D-ribofuranosylthymine (**10**) in 34 % yield, along with 10 % yield of α-ribothymidine (**11**). The compound **11** was formed by hydrolysis of **10** during the workup and silica gel column chromatography.



Scheme 1



Scheme 2

Upon treatment with NH_4OH solution, **10** was converted to **11** completely. Conversion of **10** to α-thymidine may also be achieved in the same procedure described for the conversion of 2,2'-anhydro-1-β-D-arabinofuranosyluracil to 2'-β-deoxyuridine.[8]

In conclusion, a facile and completely stereoselective synthesis of α-anomers of pyrimidine nucleosides has been established starting from **1** and low cost materials.

Experimental

General. Melting points were taken with a Yanagimoto MP-500D apparatus and uncorrected. ^1H NMR spectra were taken with a Varian Gemini-200 spectrometer. UV spectra were measured with a Hitachi 3200 apparatus. IR spectra were recorded on a Perkin Elmer FT-IR 1600. Mass spectra were kindly taken by a staff at the Research Institute of Kirin Brewery Co. with a Hitachi M-80-B instrument in FD mode. High performance liquid chromatography (HPLC) analyses were carried out on a JASCO 800 apparatus using a Wakosil C18 column. All reaction solvents were dried and distilled in the usual manner. 2-Amino-α-D-ribofurano[1',2':4,5]-2-oxazoline (**1**) was prepared from D-ribose and cyanamide according to the procedure by Shannahoff and Sanchez[4] or by Hessler.[5] Ethyl α-(bromomethyl)acrylate was prepared from triethyl phosphonoacetate or from ethyl acrylate by the published method.[7]

α-Bromoacrylonitrile (2). This material was prepared by the modification of the method described by Yokota and Hirabayashi.[9] Bromine (52.7 g, 0.33 mol) and 0.1 g of iodine in chloroform (40 ml) was added gradually to a solution of acrylonitrile (16.6 g, 0.31 mol) in chloroform (20 ml) with stirring in an ice bath, and the mixture was stirred overnight at room temperature. Triethylamine (31.5 g, 0.31 mol) in dichloromethane (100 ml) was then added to the reaction mixture with stirring, and the stirring was continued for 6 h at room temperature. Water was poured into the reaction mixture, and the organic layer was washed with 0.1 M HCl and then saturated sodium chloride solution, dried over anhydrous sodium sulfate and distilled under reduced pressure to yield **2** (23.2 g, 56 %). bp 46°C/44 mmHg (lit.[9] 28°C/23 mmHg).

2,2'-Anhydro-1-α-D-ribofuranosylcytosine hydrobromide (3). To a solution of **1** (9.6 g, 55 mmol) in dry methanol (400 ml) was added **2** (8.09 g, 61.3 mmol) gradually and stirred for 2 d at 70 °C . Acetic acid (15 ml) was added to the reaction mixture in an ice bath. After removal of the solvent *in vacuo*, the residue was applied to a Dowex 50W-X8 (H^+ form) column and eluted with water, 1 M HCl and 3 M HCl. The appropriate fraction was evaporated to dryness yielding **3** as a white foam

(14.0 g, 76%). mp 192-195°C(dec.). Anal Calcd for $C_9H_{11}O_4N_3$ HBr1.5H_2O: C, 32.53; H, 4.55; N, 12.65. Found: C, 32.65; H, 5.00; N, 13.02. ^{1}H-NMR (D_2O) δ: 8.13 (d, 1 H, J = 7.5 Hz, H-6), 6.63(d, 1 H, J = 7.5 Hz, H-5), 6.54(d, 1 H, J = 5.3 Hz, H-1'), 5.60(t, 1 H, J = 5.3 Hz, H-2'). Mass (FD)*m/z* 226 [M+H]. UV(H_2O) λmax 232(ε 9,500), 262 nm(ε 10,300) [lit.[4] λmax 232(ε 9,700), 262(ε 10,800)]

α-cytidine (4). A solution of **3** (110 mg, 0.33 mmol) in 0.5 M ammonium hydroxide (10 ml) was kept overnight. HPLC of an aliquot of the reaction mixture showed that **3** was converted to **4** completely. After removal of the solvent *in vacuo*, the residue was applied to a Dowex 50W-X8 column (H^+ form) and eluted with water and 2 M HCl. The appropriate fraction was evaporated to dryness yielding **4** (51 mg, 48%) as a hydrochloride. mp 135-137°C. Anal Calcd for $C_9H_{13}O_5N_3$ HCl 2.5H_2O: C, 33.29; H, 5.90; N, 12.94. Found: C, 33.25; H, 5.49; N, 13.11. ^{1}H-NMR (D_2O) δ: 8.04(d, 1 H, H-6), 6.26(d, 1 H, H-6), 6.10(d, 1 H, H-5), 5.13(t, 1 H, H-2'), 5.01(d, 1 H, H-3'), 4.63(t, 1 H, H-4'), 3.77(m, 2 H, H-5'). UV(H_2O) λmax 286 nm (ε 11,900).

α-2'-deoxycytidine (8). To a mixture of **3** (4.83 g, 14.5 mmol) in dry pyridine (150 ml), benzoyl chloride (20.0 ml, 172 mmol), triethylamine (2.5 ml) and dimethylaminopyridine (250 mg) were added with stirring, and the reaction mixture was kept at 50°C for 4 d with stirring. After cooling to 4°C, saturated sodium hydrogen carbonate solution was added, and the solution was extracted with ethyl acetate. The organic layer was washed with saturated sodium hydrogen carbonate solution, 0.1 M HCl and finally saturated sodium chloride solution, dried over anhydrous magnesium sulfate, and evaporated to dryness to give $O^{3'},O^{5'},N^4$-tribenzoyl-2,2'-anhydro-1-α-D-ribofuranosylcytosine (**5**) as a white foam (2.85 g, 37 %). ^{1}H-NMR ($CDCl_3$) δ: 7.3-8.2(m, 16 H, 3 x C_6H_5, H-6), 6.62(d, 1 H, H-5), 6.14(s, 1 H, H-1'), 5.71(s, 1 H, H-2'). Mass (FD) *m/z* 537[M]

A mixture of **5** (380 mg, 0.71 mmol) in 25 % HBr solution in acetic acid (15 ml) was kept overnight at 40°C with stirring. After cooling, water was added to precipitate solids which were recrystallized from ethanol-water yielding $O^{3'},O^{5'},N^4$-tribenzoyl-α-2'-bromo-2'-deoxycytidine (**6**) (353 mg, 73 %). mp 94-96°C. Anal Calcd for $C_{30}H_{24}O_7N_3Br$ CH_3COOH: C, 56.65; H, 4.16; N, 6.19. Found: C, 56.28; H, 4.01; N, 5.84. ^{1}H-NMR ($CDCl_3$) δ: 7.35-8.15 (m, 18 H, 3 x C_6H_5, H-5, H-6 and NH), 6.23(s, 1 H, H-1'), 5.70(s, 1 H, H-2'), 5.04(t, 1 H, H-4'), 4.94(s, 1 H, H-3'), 4.81(q, 2 H, H-5').

To a mixture of **6** (253 mg, 0.38 mmol) and 2,2'-azobis(isobutyronitrile) (48 mg) in benzene was added 0.5 M tributyltin hydride in benzene (2.0 ml, 1.0 mmol) with stirring, and the solution was stirred at 70°C for 6 h. Removal of the solvent and chromatography of the residue on silica gel with ethyl acetate-benzene (1:1) as an eluant provided $O^{3'},O^{5'},N^4$-tribenzoyl-α-2'-deoxycytidine (**7**) as a white solid (29 mg, 14 %). mp 77°C. ^{1}H-NMR ($CDCl_3$) δ: 8.13-7.35(m, 17 H, 3 x C_6H_5, H-5 and H-6), 6.36(d, 1 H, H-1'), 5.67(d, 1 H, H-3'), 4.98(t, 1 H, H-4'), 4.61(d, 2 H, H-5'), 3.05(m, 1 H, H-2'), 2.74(d, 1 H, H-2'). Mass(FD) *m/z* 540[M+H].

A solution of **7** (54 mg, 0.1 mmol) in dioxane (2 ml) and 25% NH_4OH solution (2 ml) was kept over night at 50°C. HPLC analysis of the reaction mixture showed that **7** was completely converted to **8.** The solution was evaporated *in vacuo* and the residue was passed through a Dowex 1-X8 (OH^- form) column with H_2O as an eluant. The appropriate fraction was evaporated to dryness to give **8** as a white solid (19 mg, 84 %). An analytical sample was recrystallized from ethanol to afford the purified **8**. mp 196.5-197.5°C (lit.[10] 193-194°C). ^{1}H-NMR (D_2O) δ: 7.87(d, 1 H, H-6), 6.15(dd, 1H, H-1'), 6.03(d, 1 H, H-5), 4.41-4.38(m, 2 H, H-3' and H-4'), 3.79-3.65(m, 2 H, H-5'), 2.89-2.65(m, 1 H, H-2'), 2.20-2.12(m, 1 H, H-2'). UV(EtOH) λmax 273 nm (ε 7,500).

1-(2-Carboethoxyallyl)-α-D-ribofurano[1',2':4,5]-2-oxazoliminium bromide (9). A mixture of **1** (1.00 g, 6.66 mmol) and ethyl α-(bromomethyl) acrylate (1.30 ml, 9.69 mmol) in dimethylacetamide (6 ml) was stirred overnight at room temperature. The reaction mixture was poured into the solution of dichloromethane-n-hexane (1:1 V/V, 40 ml) to precipitate an oily residue, which was evaporated to dryness yielding **9** as a white foam (2.50 g, 88 %). mp 116-120°C. Anal Calcd for $C_{12}H_{19}O_6N_2Br$: C, 39.25; H, 5.22; N, 7.63. Found: C, 39.01; H, 5.61; N, 7.90. ^{1}H-NMR (D_2O) : 6.51, 6.11(2s, 2 H, CH_2=), 5.98(d, 1 H, H-1'), 5.40(t, 1 H, H-2'), 4.53, 4.38(2d, 2 H, NCH_2), 4.27(m, 1 H, H-3'), 4.27(q, 2 H, OCH_2CH_3), 3.97-3.64 (m, 3 H, H-4', H-5'), 1.31(t, 3 H, OCH_2CH_3). Mass(FD) *m/z* 287[M-Br].

2,2'-Anhydro-1-α-D-ribofuranosylthymine (10) and α-ribothymidine (11). A mixture of **9** (1.25 g, 2.87 mmol) and potassium t-butoxide (prepared from 248 mg potassium metal and t-butanol, 6.34 mmol) in t-butanol (20 ml) was stirred for 3 d at 30°C. The reaction mixture was passed through a short silica gel column using methanol as an eluant to remove inorganic salts. The eluate was evaporated under reduced pressure and subjected to silica gel column chromatography using 15 %

methanol-dichloromethane as an eluant. The fraction containing **10** was evaporated to dryness and recrystallized from aqueous ethanol giving purified **10** (240 mg, 34%). mp 230-235°C(dec.). Anal Calcd for $C_{10}H_{12}O_5N_2 1/2H_2O$: C,48.26; H, 5.26; N, 11.27. Found: C, 48.17; H, 5.30; N, 11. 10. ^{1}H-NMR (D_2O) : 7.80(s, 1 H, H-6), 6.42(d, 1 H, H-1'), 4.38(dd, 1 H, H-3'), 3.96(dd, 1 H, H-5'), 3.85(m, 1 H, H-4'), 3.77(dd, 1 H, H-5'), 1.98(s, 3 H, CH_3). Mass(FD) *m/z* 241[M+H]. UV(H_2O) λmax 252.5 nm (ε 4,500), 224.5 nm (ε 3,500).

The fraction containing **11** was evaporated to dryness and recrystallized from acetonitrile giving purified **11**(79 mg, 10 %). mp 174-175°C (lit.[11] 174-175°C). ^{1}H-NMR (D_2O) :7.63(d, 1 H, H-6), 6.16(d, 1 H, H-1'), 4.47(t, 1 H, H-2'), 4.24-4.38(m, 2 H, H-3', H-4'), 3.92(dd, 1 H, H-5'), 3.72(dd, 1 H, H-5'), 1.92(s, 3 H, CH_3). Mass(FD) *m/z* 258 [M]. UV(H_2O) λmax 269 nm (ε 8,200).

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