Synthesis of 2'-Amino-2'-deoxy-5-fluorocytidine

R. A. Sharma*, A. Bloch and M. Bobek

Grace Cancer Drug Center, Roswell Park Memorial Institute,
Buffalo, New York 14263

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Acetylation of 2'-deoxy-5-fluoro-2'-trifluoroacetamidouridine with acetic anhydride in pyridine, followed by treatment with phosphorus pentasulfide in refluxing dioxane afforded 3',5'-di-*O*-acetyl-2'-deoxy-5-fluoro-2'-trifluorothioacetamido-4-thiouridine (**3**). Treatment of **3** with methanolic sodium methoxide furnished 2'-deoxy-2'-trifluorothioacetamido-4-thiouridine (**4**), whereas its treatment with methanolic ammonia gave 2'-amino-2'-deoxy-5-fluorocytidine (**5**). An alternative approach for the preparation of this compound proceeding from 2'-trifluoroacetamidocytidine was unsuccessful, since the use of acetic anhydride in pyridine led to the replacement of the trifluoroacetyl function by an acetyl group, yielding an intermediate unsuitable for obtaining the target compound.

The title compound was inactive at 1×10^{-4} *M* concentration against HeLa and leukemia L1210 cells *in vitro*, but inhibited the *in vitro* growth of *E. coli* cells at a concentration of 1×10^{-7} *M*. It was also found to be a substrate for CR/dCR deaminase partially purified from human liver, with a Km of 128 μM.

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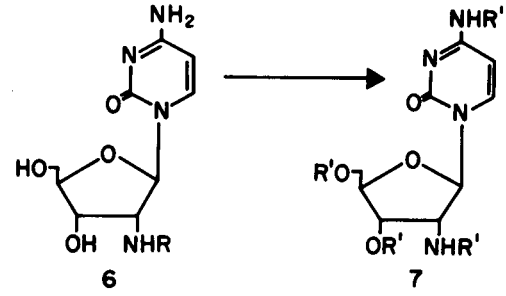
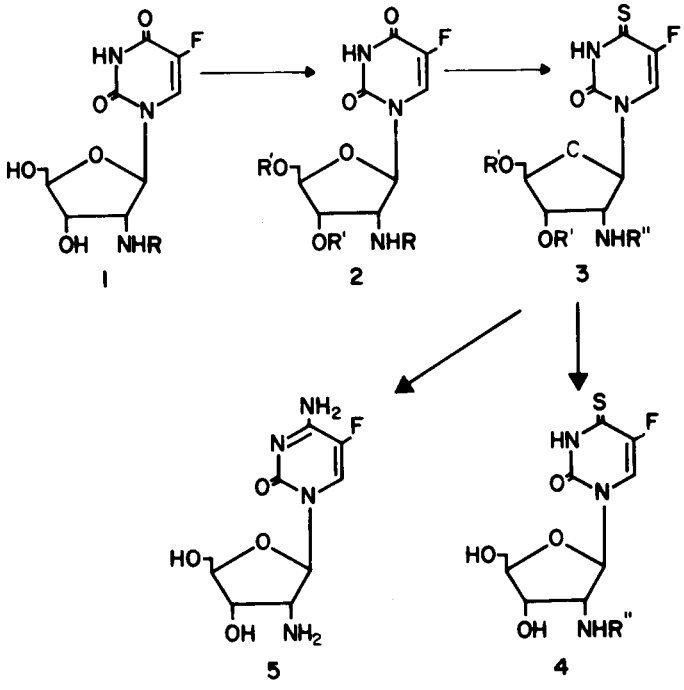
Introduction.

Analogs of the natural nucleosides containing amino groups in the carbohydrate moiety have the potential for diverse biological activity. Thus, puromycin (1), 2'-amino-2'-deoxyguanosine (2), and 2'-amino-2'-deoxyarabinofuranosylcytosine (3) were significantly inhibitory to tumor cells *in vitro*. Since 5-fluorocytidine and 2'-deoxy-5-fluorocytidine have demonstrated pronounced antitumor activity, albeit at the cost of considerable toxicity (4), the preparation and biological evaluation of 2'-amino-2'-deoxy-5-fluorocytidine was considered worthwhile.



R=$COCF_3$; R'=$COCH_3$; R''=$CSCF_3$



Chemistry.

Acetylation of 2'-deoxy-5-fluoro-2'-trifluoroacetamidouridine (**1**) with acetic anhydride in pyridine furnished compound **2** in good yield (5). Upon treatment of **2** with phosphorus pentasulfide in refluxing dioxane, 3',5'-di-*O*-acetyl-2'-deoxy-5-fluoro-2'-trifluorothioacetamido-4-thiouridine (**3**) was obtained. Reaction of **3** with methanolic sodium methoxide provided 2'-deoxy-5-fluoro-2'-trifluorothioacetamido-4-thiouridine (**4**), whereas treatment of **3** with methanolic ammonia at 70-80° gave 2'-amino-2'-deoxy-5-fluorocytidine (**5**). The structure of **5** was confirmed by ^1H and ^{19}F magnetic resonance spectroscopy.

The synthesis of compound **5** had been initially projected to proceed from 2'-deoxy-2'-trifluoroacetamidocytidine (**6**). To improve the solubility of this intermediate at the conditions required for fluorination (5), acetylation of **6** with acetic anhydride in pyridine was carried out. Unlike acetylation of the corresponding uridine derivative, the reaction with the cytidine derivative **6**, led to the displacement of the trifluoroacetyl group with an acetyl func-

Table 1

Effect of 2'-Amino-2'-deoxy-5-fluorocytidine and Related Compounds on the *in vitro* Cell Growth of L-1210 and *E. coli* K_{12} Cells

Compound	Molar Concentrations for 50% Growth Inhibition of L-1210	*E.coli* K_{12}
2'-Amino-2'-deoxy-5-fluorocytidine	$> 10^{-4}$	1×10^{-7}
2'-Deoxy-5-fluoro-2'-trifluorothio-acetamido-4-thiouridine	9×10^{-5}	3×10^{-6}
2'-Deoxy-2'-trifluoroacetamido-cytidine	7×10^{-5}	8×10^{-7}

tion, providing the fully acetylated product **7**. Since removal of the acetyl group from an amino function requires relatively harsh conditions, the projected synthesis was abandoned in favor of the one described above.

Biological Activity.

Whereas the title compound **5** and its related analogs **4** and **6** were either inactive or only marginally active against L-1210 cells, they were markedly effective in inhibiting the *in vitro* growth of *E. coli* K_{12} cells at concentrations ranging from 3×10^{-7} to 1×10^{-7} *M*. The reasons for this difference in activity in the two cell systems remains undetermined. 2'-Amino-2'-deoxy-5-fluorocytidine served as a substrate for cytidine deaminase partially purified from human liver, the Km being 128 μM as compared to a Km of 67 μM for cytidine.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Ultraviolet spectra were measured on a Cary Model 14 spectrophotometer, and nmr spectra on a Varian XL-100 spectrometer using TMS as internal standard. The presence of solvent in the analytical sample was verified by nmr spectroscopy. Thin-layer chromatography was performed on silica gel N-HR/UV_{254} precoated plastic sheets (Brinkman), and column chromatography on silica gel (60-200 mesh), J. T. Baker No. 3405. Elemental analyses were performed by Robertson Laboratory, Florham Park, New Jersey.

3',5'-Di-*O*-acetyl-2'-deoxy-5-fluoro-2'-trifluoroacetamidouridine (**2**).

A solution of **1** (4.8 g, 13.45 mmoles), acetic anhydride (25 ml) and pyridine (15 ml) in anhydrous methylene chloride (150 ml) was stirred overnight at room temperature. The mixture was evaporated to dryness, methanol (100 ml) was added to decompose the remaining acetic anhydride, and the solvent removed by evaporation. Toluene (100 ml) was added and the solvent evaporated. The residue was dissolved in 500 ml of ethyl acetate, washed twice with saturated aqueous sodium bicarbonate (200 ml) and then with water, and dried (sodium sulfate). After evaporation of the solvent the product **2** was obtained as crystals (single spot on tlc) in 5.4 g (91%) yield. The analytical sample was prepared by recrystallization from ethanol, mp 76-81°; nmr (deuteriochloroform): δ 2.21 and 2.24 (s, 6H, $OCOCH_3$), 4.26-5.32 (m, 5H, sugar protons), 6.36 (dd, 1H, $J_{1',2'} = 8.8$ Hz, $J_{1',5F} = 1.9$ Hz, H-1'), 7.68 (d, 1H, $J_{6,5F} = 6$ Hz, H-6), 7.94 (d, 1H, $J_{2',NH} = 9$ Hz, $NHCOCF_3$), 10.26 (d, 1H, $J_{N^3H,5F} = 4.5$ Hz, N^3H).

Anal. Calcd. for $C_{15}H_{15}N_3F_4O_8$: C, 40.81; H, 3.40; N, 9.52, F, 17.23. Found: C, 40.65; H, 3.64; N, 9.26; F, 17.01.

3',5'-Di-*O*-acetyl-2'-deoxy-5-fluoro-2'-trifluorothioacetamido-4-thiouridine (**3**).

Compound **2** (4.47 g, 10.14 mmoles) was dissolved in 200 ml of dioxane (dried over molecular sieves) and the solution was refluxed for 1.5 hours with stirring, in the presence of phosphorus pentasulfide (5 g, 22.52 mmoles). The mixture was evaporated to dryness, and the residue triturated in 200 ml of ethyl acetate, stirred with 5 g of silica gel and filtered. The filtrate was successively extracted with water (200 ml), dried (sodium sulfate), and evaporated. The residue was dissolved in a small amount of ethyl acetate and purified on a dry silica gel column, using benzene-ethyl acetate (7:3) as the eluent, yield 3.44 g (72%), mp 159-160° (benzene-ether); uv (methanol): λ max 335 nm (18,082), 281 (12,181), sh at 240; λ min 303 (8850); nmr (deuteriochloroform): δ 2.12 (s, 6H, $OCOCH_3$), 4.28-5.50 (m, 5H, sugar protons), 6.52 (dd, 1H, $J_{1',2'} = 8.5$ Hz, $J_{1',5F} = 1.6$ Hz, H-1'), 7.60 (d, 1H, $J_{5F,6} = 4$ Hz, H-6), 8.86 (d, 1H, $J_{2',NH} = 8$ Hz, $NHCSCF_3$), 10.62 (d, 1H, $J_{N^3H,5F} = 4$ Hz, N^3H).

Anal. Calcd. for $C_{15}H_{15}F_4N_3S_2O_6$: C, 38.05; H, 3.17; N, 8.88; F, 16.06; S, 13.53. Found: C, 38.07; H, 3.32; N, 8.83; F, 16.35; S, 13.76.

2'-Deoxy-5-fluoro-2'-trifluorothioacetamido-4-thiouridine (**4**).

A solution of **3** (0.5 g, 1.06 mmoles) and sodium methoxide (prepared from 0.15 g of sodium) in 100 ml of methanol was stirred at room temperature for 1 hour. The mixture was neutralized with Dowex 50 (H^+) resin, filtered and evaporated. The product **4** was obtained, chromatographically pure, in 0.4 g (97%) yield. Recrystallization from toluene-ethanol mixture gave 0.384 g (93%) of compound **4**, mp 177-179°; uv (methanol): λ max 337 nm (18,009), 282 (11,423), sh at 243; λ min 303 (8438); nmr (DMSO-d_6): δ 3.84-5.26 (m, sugar protons), 6.32 (dd, 1H, $J_{1',2'} = 8$ Hz, $J_{1',5F} = 1.8$ Hz, H-1'), 8.28 (d, 1H, $J_{6,5F} = 4.5$ Hz, H-6), 9.48 (d, 1H, $J_{2',NH} = 8$ Hz, $NHCSCF_3$), 12.58 (bs, 1H, N^3H).

Anal. Calcd. for $C_{11}H_{11}N_3S_2F_4O_4$: C, 33.93; H, 2.83; N, 10.80; F, 19.54; S, 16.45. Found: C, 34.22; H, 3.11; N, 10.58; F, 20.07; S, 16.42.

2'-Amino-2'-deoxy-5-fluorocytidine (**5**).

A solution of **3** (1.5 g, 3.17 mmoles) in 300 ml of anhydrous methanol, presaturated with ammonia at 0-4°, was heated in a steel vessel at 70-80° for 12 hours. After cooling, the mixture was filtered and the solid was washed with 150 ml of methanol. The solvent was evaporated, the residue treated with 100 ml of ethanol and the solution brought to dryness. The residue was dissolved in 150 ml of methanol, a few drops of acetic acid were added to bring the *p*H to about 6 and the solution evaporated to dryness. The residue was dissolved in 30 ml of a water-methanol mixture (1:1) and was poured onto a 3 × 24 cm Dowex AG50W-X8 column, which was washed successively with 1 liter of water, 500 ml of methanol and with 20% ammonium hydroxide-methanol. The fractions absorbing at 280 nm were combined and evaporated, yielding a syrup. The syrup was dissolved in 200 ml of methanol, boiled with charcoal for 10 minutes and filtered with the aid of celite. The filtrate was concentrated (100 ml) by evaporation and was treated with ethanolic hydrogen chloride to *p*H 4. The mixture was then evaporated to dryness, co-evaporated successively with methanol and ethanol, and the residue dissolved in a water-ethanol mixture (3:7), from which a crystalline material (two crops 0.313 and 0.147 g) were obtained at room temperature. Evaporation of the filtrate gave additional 0.29 g of the crystalline product. The total yield of **5** was in 0.75 g (79%); mp 226-227°; uv (methanol): λ max 280 nm (6700), 241 (7768); λ min 263 (5692), 223 (5811); nmr (DMSO-d_6): δ 3.26-5.0 (m, sugar protons), 5.76 (dd, 1H, $J_{1',2'} = 6.6$ Hz, $J_{1',5F} = 1.90$ Hz, H-1'), 7.44 (bs, 2H, NH_2), 8.06 (d, 1H, $J_{6,5F} = 7.5$ Hz, H-6).

Anal. Calcd. for $C_9H_{13}FN_4O_4 \cdot HCl$: C, 36.42; H, 4.72; N, 18.88; F, 6.40. Found: C, 36.22; H, 4.87; N, 18.80; F, 6.08.

2'-Deoxy-2'-trifluoroacetamidocytidine (**6**).

To a cooled (0-4°) suspension of 2'-amino-2'-deoxycytidine (6) 2.0 g in

200 ml of dry methylene chloride was added dropwise, 10 ml of trifluoroacetic anhydride in 50 ml of methylene chloride. The mixture was stirred at room temperature for 24 hours, evaporated to dryness and co-evaporated with ethyl acetate-methanol (1:1). The residue was successively dissolved in methanol, neutralized with methanolic ammonia, evaporated to dryness and co-evaporated with ethanol. The product was purified on a dry silica gel column, eluted successively with 500 ml of chloroform, 500 ml of chloroform-methanol (9:1) and chloroform-methanol (8:2). The appropriate fractions were combined and evaporated to a small volume at which point the product **6** crystallized. The crystals were filtered and washed with ethanol. The filtrate was evaporated and the residue recrystallized from ethanol. The compound **6** was obtained in 2.6 g (93%) yield, mp 208-209° dec; nmr (DMSO-d_6): δ 5.82 (d, 1H, H-5), 6.06 (d, 1H, $J_{1',2'}$ = 6.5 Hz, H-1'), 7.78 (d, 1H, H-6). The remaining protons were also accounted for.

Anal. Calcd. for $C_{11}H_{13}F_3N_4O_5$ 1.75 H_2O: C, 35.97; H, 4.21; N, 14.89; F, 15.35. Found: C, 35.72; H, 4.46; N, 15.15; F, 15.42.

3',5'-Di-*O*-acetyl-2'-deoxy-2'-acetamido-N^4-acetylcytidine (**7**).

To a suspension of 2.0 g of **6** in 100 ml of methylene chloride was added 10 ml of acetic anhydride and 20 ml of pyridine, and the mixture was stirred at room temperature overnight. Methanol (50 ml) was added to the mixture and evaporated. The residue was co-evaporated twice with 100 ml each of toluene and was dissolved in methylene chloride. The solution was washed with aqueous sodium bicarbonate, water and dried (sodium sulfate). The mixture was purified on a dry silica gel column, washing first with 300 ml of methylene chloride, and then with methylene chloride-methanol (15:1). The fractions were combined and evaporated, and the residue was recrystallized from ethanol-ether to afford 0.45 g (19%) of **7**, mp 120-121°; nmr (deuteriochloroform): δ 1.98, 2.18, 2.28 (3s, 9H, $OCOCH_3$), 4.0-4.66, 5.28 (m, 5, H-2', 3', 4', 5'), 6.38 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 6.74 (d, 1H, 2'-$NHCOCH_3$), 7.60 (d, 1H, H-5), 8.04 (d, 1H, H-6), 9.47 (b, 1H, exocyclic $NHCOCH_3$).

Anal. Calcd. for $C_{17}H_{22}N_4O_8 \cdot C_2H_5OH$: C, 50.0; H, 6.14; N, 12.28. Found: C, 49.73; H, 6.09; N, 12.05.

Biological Procedures.

The techniques used for assaying the effect of the compounds on cell growth have been reported (7,8). Cytidine deaminase from human liver was obtained by established procedures (9,10).

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