A New Synthesis of 2',3'-Dideoxycytidine

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A new synthesis of 2',3'-dideoxycytidine is described and the utility of this synthesis to prepare larger quantities of said nucleoside is shown.

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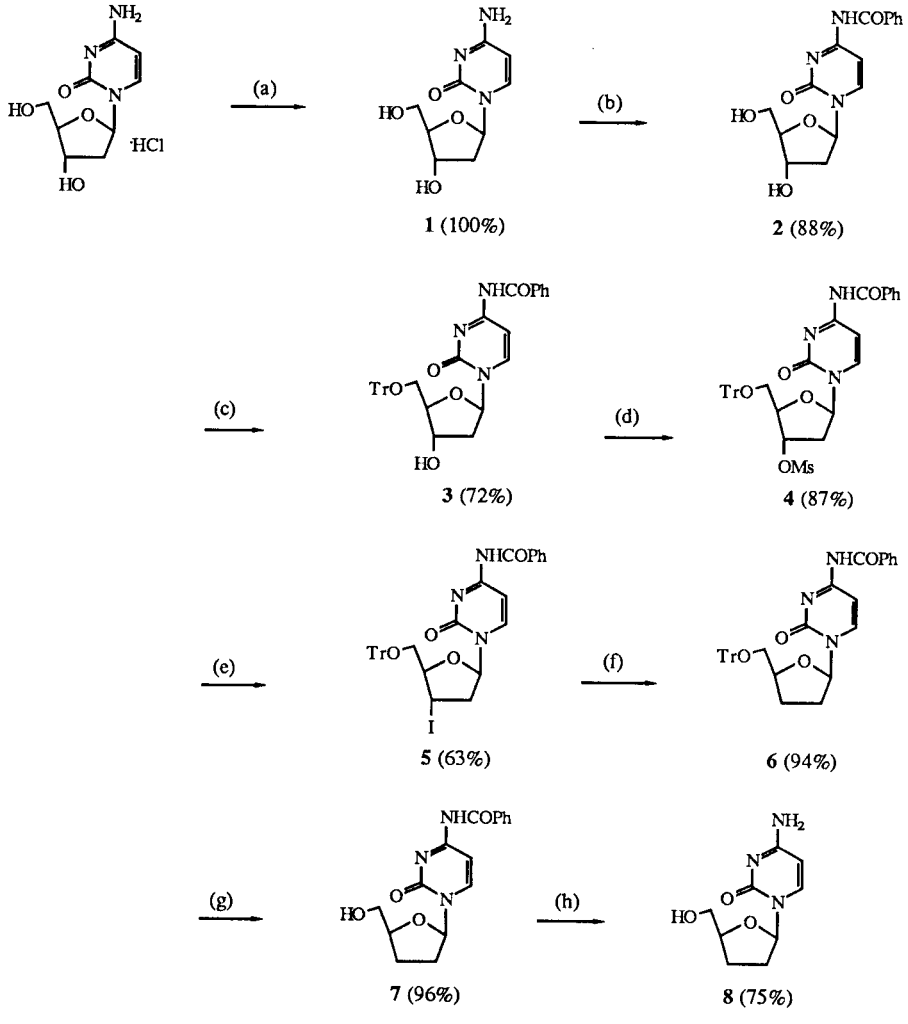
Although successful syntheses of 2',3'-dideoxycytidine have been reported in the literature by Horwitz and co-workers [1], Marumoto *et al.* [2], Samukov [3], Lin [4] and others [5], none of these are suitable for larger scale preparations. The promising initial activity demonstrated by 2',3'-dideoxycytidine against the AIDS-virus has resulted in a requirement for additional, large quantities of this material. Here we wish to report a new synthesis which was utilized to prepare larger (50-100 g) quantities of 2',3'-dideoxycytidine (Scheme I). Commercially available 2'-deoxycytidine (hydrochloride salt) was converted to its free base **1** (100%). Benzoylation of **1** with benzoic anhydride in refluxing ethanol gave *N*-benzoyl-2'-deoxycytidine **2** (88%). Treatment of **2** with trityl chloride

Scheme I



Reagents (a) Et_3N, $CHCl_3$ (b) $(PhCO)_2O$, EtOH (c) TrCl, Py
(d) MsCl, Py (e) LiI, ETOAc (f) H_2/Pd-C, DMF
(g) HCl-EtOH, EtOAc (h) NH_3-MeOH

in pyridine afforded 5'-*O*-trityl compound **3** (72%), which was subsequently treated with methanesulfonyl chloride to afford the completely protected nucleoside **4** (87%). Unlike the literature [6] we were successful in displacing the mesyl group in **4** with iodide using lithium iodide in ethyl acetate to give the 3'-iodo derivative **5** (63%). The stereochemistry of the iodo substituent at the 3'-position in compound **5** was not established conclusively. The assignment was made by analogy with the corresponding uridine structure [7]. Hydrogenolysis of the compound **5** using 10% palladium on carbon catalyst in dimethoxyethane afforded the protected 2',3'-dideoxy nucleoside **6** (94%). Treatment of the nucleoside **6** with ethanolic hydrogen chloride removed the trityl group to give the *N*-benzoyl derivative **7** (96%), which was reacted with methanolic ammonia to give the target 2',3'-dideoxycytidine (75%).

In summary, commercially available 2'-deoxycytidine was converted to the target 2',3'-dideoxycytidine in an overall yield of 23% over eight steps. This represents a convenient synthetic procedure for the preparation of larger quantities of this material. This procedure is also potentially applicable to the preparations of additional dideoxy nucleosides of interest.

EXPERIMENTAL

All chemical reagents were commercially available. 2'-Deoxycytidine hydrochloride was purchased from Calbiochem Behring. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Specific rotations were measured in a 4-dm cell with Perkin-Elmer model 241 polarimeter. Analytical tlc was performed on EM silica gel GF with the solvents indicated. Elemental analyses were performed by Midwest Microlab, Indianapolis, Indiana.

2'-Deoxycytidine (**1**).

Triethylamine (590 mℓ, 4.22 moles) was added to a suspension of 2'-deoxycytidine hydrochloride (1.0 kg, 4.22 moles) in chloroform (10 ℓ). The resulting suspension was stirred vigorously at ambient temperature for 3 hours. The colorless crystalline solid was collected, washed with chloroform (2 ℓ), and air dried to give the title free base **1**, 880 g (100%), mp 185-195°, Lit [8] mp 185-195°.

N^4-Benzoyl-2'-deoxycytidine (**2**).

Benzoic anhydride (590 g, 2.64 moles) was added to a stirred, refluxing solution of **1** (600 g, 2.64 moles) in anhydrous ethanol (10 ℓ). Two further additions of benzoic anhydride (236 g, 1.06 moles x 2) were made at one hour intervals. After the final addition, the solution was heated at reflux for 1 hour and concentrated to 4.5 ℓ under aspirator pressure. The resulting slurry was diluted with ether (7.0 ℓ), stirred with cooling for 1 hour, and filtered. The product was washed successively with ether (2 x 1 ℓ) and petroleum ether (2 x 1 ℓ) and dried to give the crystalline *N*-benzoate **2**, 743 g (85%), mp 230° dec, Lit [9] mp 230° dec.

N^4-Benzoyl-2'-deoxy-5'-*O*-tritylcytidine (**3**).

Trityl chloride (562 g, 2.01 moles) was added to a stirred solution of the N^4-benzoyl derivative **2** (555 g, 1.68 moles) in pyridine (3.9 ℓ). The resulting mixture was stirred at ambient temperature for 70 hours to give a cloudy solution which was filtered (celite). The celite pad was washed with pyridine (250 mℓ) and the filtrate was poured into ice water (14 ℓ). The resulting gummy product, separated from the supernate by decantation, was stirred with water (10 ℓ) until the product solidified. The solid was separated, washed with cold water (3 x 2 ℓ), and then dissolved in methylene chloride (6 ℓ). The solution was washed with water (2 x 3 ℓ) and dried (magnesium sulfate, 400 g). The solvent was evaporated under aspirator pressure and the resulting solid, *ca.* 1160 g, was extracted by trituration with hot reagent alcohol (4.0 ℓ). The extract was cooled and the crystalline product, 620 g, was collected. The mother liquor was evaporated to dryness and the residue was triturated with a mixture of methylene chloride and ether to give additional product, 100 g. The combined solid (720 g) was recrystallized from methylene chloride (3 ℓ) and petroleum ether (6 ℓ) to give crystalline trityl compound **3**, 688 g (72%), mp 222°; $[\alpha]_D^{25}$ +57.9° (c = 0.994, chloroform); tlc, R_f = 0.26 (ethyl acetate).

Anal. Calcd. for $C_{35}H_{31}N_3O_5$ (573.6): C, 73.28; H, 5.45; N, 7.32. Found: C, 73.06; H, 5.46; N, 7.34.

N^4-Benzoyl-2'-deoxy-3'-*O*-mesyl-5'-*O*-tritylcytidine (**4**).

Methanesulfonyl chloride (675 mℓ, 8.72 moles) was added slowly (25 minutes) to a cooled (10-12°) suspension of trityl compound **3** (675 g, 1.178 moles) in pyridine (4.6 ℓ). The resulting solution was stirred at ambient temperature for 1.5 hours and poured in cold water (45 ℓ). The mixture was stirred for 1 hour, filtered, and the solid was washed with water (2 x 3 ℓ). The wet solid was dissolved in methylene chloride (7.0 ℓ) and the solution was washed with water (3 x 3.0 ℓ). After drying (magnesium sulfate, 500 g), the organic phase was evaporated to yield a residue, *ca.* 955 g. The residue was dissolved in reagent alcohol (3.8 ℓ). The solution was cooled. The resulting solid was collected and air-dried to afford the protected nucleoside **4**, 670 g (87%), mp 160° dec, shrinks at 155°; $[\alpha]_D^{25}$ +79.3° (c = 1.01, chloroform); tlc, R_f = 0.36 (ethyl acetate).

Anal. Calcd. for $C_{36}H_{33}N_3O_7S$ (651.7): C, 66.35; H, 5.10; N, 6.45; S, 4.92. Found: C, 66.19; H, 5.32; N, 6.61; S, 4.95.

N^4-Benzoyl-2',3'-dideoxy-3'-iodo-5'-*O*-tritylcytidine (**5**).

The mesylate **4** (508 g, 0.78 mole) was added to a refluxing solution of anhydrous lithium iodide (1016 g, 7.59 moles) in ethyl acetate (6.0 ℓ, dried over potassium carbonate). The resulting solution was heated at reflux for 2.5 hours, cooled to 30°, and poured into ice water (8 ℓ). The two phase system was filtered (celite) to remove the insoluble material. The organic phase was separated and the aqueous phase was extracted with fresh ethyl acetate (2 x 1.5 ℓ). The organic phase and the extract were combined (10 ℓ) and washed successively with aqueous sodium thiosulfate (10%, 2 ℓ), water (2 x 2 ℓ), and dried (magnesium sulfate, 500 g). The solvent was evaporated under aspirator pressure to yield a foam (560 g). The foam (560 g) was slurried in a mixture of reagent alcohol (2.5 ℓ), ether (500 mℓ) and petroleum ether (500 mℓ). The slurry was filtered to yield the crystalline iodide **5**, 335 g (63%), mp 120° (darkens at 160°); $[\alpha]_D^{25}$ + 105.5° (c = 1.01, chloroform); tlc, R_f = 0.60 (ethyl acetate-ether, 1:1).

Anal. Calcd. for $C_{35}H_{30}IN_3O_4$ (683.6): C, 61.49; H, 4.43; I, 18.57; N, 6.15. Found: C, 61.24; H, 4.57; I, 18.34; N, 6.12.

N^4-Benzoyl-2',3'-dideoxy-5'-*O*-tritylcytidine (**6**).

A mixture of the trityl derivative **5** (100 g, 0.146 mole), triethylamine (40 mℓ, 0.278 mole) and 10% Pd/C (20 g) in dimethoxyethane (1.0 ℓ) was hydrogenated at 35 psig for 1.25 hour. The reaction mixture was diluted with methylene chloride (1.0 ℓ) and the mixture was filtered (celite) to remove catalyst. The celite pad was rinsed with additional methylene chloride (120 mℓ). The combined filtrate and rinse was evaporated under aspirator pressure to give a residual solid. Additional trityl derivative **5** (190 g) was hydrogenated as described above (two runs) and the crude solids from all three runs were combined and washed successively with water (1.0 ℓ), aqueous sodium thiosulfate (10%, 1.0 ℓ), and water (1.0 ℓ). The wet solid was dissolved in methylene chloride (4.0 ℓ) and washed with water (3 x 1.0 ℓ). The organic phase was dried (magnesium sulfate, 150 g) and evaporated to yield a foam. The foam was crystallized from a mixture of methylene chloride and petroleum ether (1:1, 1.8 ℓ) to give crystalline, fully-protected 2',3'-dideoxy nucleoside **6**, 223 g (94%), mp 150° dec; $[\alpha]_D^{25}$ + 41.1° (c = 1.0, chloroform); tlc, R_f = 0.45 (ethyl acetate-ether, 1:1).

Anal. Calcd. for $C_{35}H_{31}N_3O_4$ (557.7): C, 75.38; H, 5.60; N, 7.54. Found: C, 75.13; H, 5.53; N, 7.51.

N^4-Benzoyl-2',3'-dideoxycytidine (**7**).

Ethanolic hydrogen chloride (*ca.* 8.5 *M*, 180 mℓ) was added in one portion to a cold suspension (10-15°) of **6** (180 g, 0.322 mole) in reagent grade ethyl acetate (1.8 ℓ). The reaction mixture was stirred for 30 minutes and filtered. The crude product was washed with fresh ethyl acetate (500 mℓ) and suspended in chloroform (1.4 ℓ). The suspension was cooled to 10°. Triethylamine (68 mℓ, 0.48 mole) was added and the resulting clear solution was evaporated under aspirator pressure to yield solid residue. The residue was stirred in cold water (1.0 ℓ) for 30 minutes to dissolve triethylamine hydrochloride and the mixture was filtered. The solid was washed successively with water (200 mℓ) and petroleum ether (2 x 300 mℓ) and dried (0.3 mm Hg) to give crystalline nucleoside **7**, 92 g (90%), mp 285° dec; $[\alpha]_D^{25}$ + 97.0° (c = 0.2, methanol); tlc, R_f = 0.60 (ethyl acetate-ethanol-water, 3:1:1). Similarly, additional trityl derivative **6** (40 g) was processed as above to give additional material (20 g) of the same quality.

Anal. Calcd. for $C_{16}H_{17}N_3O_4$ (315.3): C, 60.94; H, 5.43; N, 13.33. Found: C, 60.73; H, 5.45; N, 13.13.

2',3'-Dideoxycytidine (**8**).

Ammonia was bubbled into a cold suspension of the *N*-benzoyl derivative **7** (91 g, 0.288 mole) in methanol (reagent grade, 1.8 ℓ) until a homogeneous solution (*p*H 12) was obtained. The clear solution was stored at ambient temperature for 22 hours. The methanol was removed under aspirator pressure to give a solid residue which was triturated with hot ethyl acetate (900 mℓ). The mixture was filtered to give a crystalline solid, 59 g. This material was recrystallized from aqueous ethanol (85%, 550 mℓ) to give crystalline title compound **8** (46 g, 75%), mp 223-224°; Lit [1] mp 215-217°; $[\alpha]_D^{25}$ + 75.3° (c = 0.6, water); Lit [1] $[\alpha]_D^{25}$ + 81° (c = 0.64, water); uv (0.1 *N* hydrochloric acid): λ max 281 nm (ε 13,500); (0.1 *N* hydrochloric acid): λ min 242 nm (ε 1,240); (water): λ max 272 nm (ε 9,450); (water): λ min 247 nm (ε 5,090); (0.1 *N* sodium hydroxide): λ max 272 nm (ε 9,070); (0.1 *N* sodium hydroxide): λ min 247 nm (ε 5,470); Lit [1]; (0.1 *N* hydrochloric acid); λ max 281 nm (ε 13,700); (0.1 *N* hydrochloric acid): λ min 242 nm (ε 665); (water): λ max 272 nm (ε 9,650); (water): λ min 239 (ε 5,320), (0.1 *N* sodium hydroxide): λ max 272 nm (ε 9,150), (0.1 *N* sodium hydroxide): λ min 247 nm (ε 4,990).

Anal. Calcd. for $C_9H_{13}N_3O_3$ (211.2): C, 51.18; H, 6.20; N, 19.89; O, 22.73. Found: C, 50.93; H, 6.32; N, 19.79; O, 22.65.

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