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## Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

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### Modified Nucleosides. II.<sup>1</sup> Economical Synthesis of 2',3'-Dideoxycytidine

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**To cite this Article** Marcuccio, Sebastian M. , Elmes, Bryan C. , Holan, George and Middleton, E. John(1992) 'Modified Nucleosides. II.<sup>1</sup> Economical Synthesis of 2',3'-Dideoxycytidine', *Nucleosides, Nucleotides and Nucleic Acids*, 11: 10, 1695 — 1701

**To link to this Article:** DOI: 10.1080/07328319208017816

**URL:** <http://dx.doi.org/10.1080/07328319208017816>

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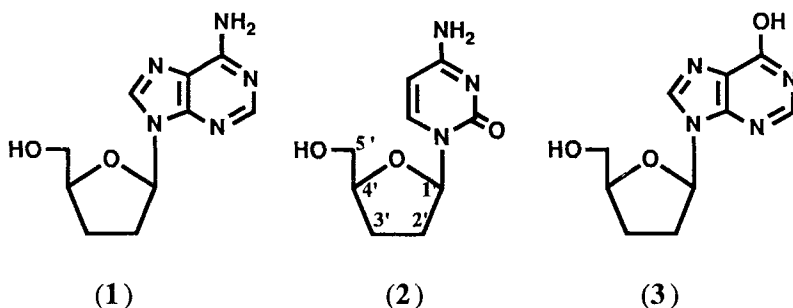
MODIFIED NUCLEOSIDES. II.<sup>1</sup>  
ECONOMICAL SYNTHESIS OF 2',3'-DIDEOXYCYTIDINE

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Abstract: An economical two pot synthesis of 2',3'-dideoxycytidine (**2**) from N<sup>4</sup>-acetylcytidine (**4**) has been developed. The key feature of this sequence is the *in situ* reductive elimination of a mixture of 1-(3-bromo-3-deoxy-2,5-di-O-acetyl-β-D-xylofuranosyl)-N<sup>4</sup>-acetylcytosine (**5**) and 1-(2-bromo-2-deoxy-3,5-di-O-acetyl-β-D-arabinofuranosyl)-N<sup>4</sup>-acetylcytosine (**6**) and subsequent hydrogenation of the resultant olefin over palladised charcoal.

2',3'-Dideoxynucleosides and 2',3'-dideoxy-2',3'-didehydronucleosides have attracted recent interest as potential antiviral agents, particularly against the human immunodeficiency virus (HIV). Currently 2',3'-dideoxyadenosine (**1**) and 2',3'-dideoxycytidine (**2**) (ddC) are undergoing clinical trials<sup>2</sup> in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex, while 2',3'-dideoxyinosine (**3**) has been approved<sup>3</sup> as a treatment for AIDS.



Several methods of synthesis of these dideoxynucleosides have been published recently, generally involving seven or eight steps from ribonucleosides,<sup>2,4</sup> in fewer steps from the more expensive 2'-deoxyribonucleosides,<sup>2,5,6</sup> or by glycosidation of a protected purine or pyrimidine base with 2,3-dideoxyribose derivatives.<sup>7,8</sup> Recent reports of the use of acetyl bromide<sup>9</sup> in the synthesis of dideoxydidehydronucleosides, and an improved synthesis of dideoxycytidine (2) from 2'-deoxycytidine<sup>10</sup> prompt us to disclose our own findings. We wish to report a two pot synthesis of 2',3'-dideoxycytidine (2) in 20% overall yield from N<sup>4</sup>-acetylcytidine (4). The synthesis has been run on a kilogram scale.

Marumoto and Honjo<sup>11</sup> have reported that treatment of 1-(3-bromo-3-deoxy-2,5-di-O-acetyl- $\beta$ -D-xylofuranosyl)-N<sup>4</sup>-acetylcytosine (5) with palladised barium sulfate affords N<sup>4</sup>,O<sup>2'</sup>,O<sup>5'</sup>-triacetyl-3'-deoxycytidine as the major product (51%) together with 2',3'-dideoxycytidine (2) (2% from N<sup>4</sup>-acetylcytidine). Although Starrett et al.,<sup>9</sup> argued that the yield of *trans*-bromoacetate 5 was too poor to use this process, we reasoned that since a pathway existed to the dideoxy compound, alteration of the reaction conditions could result in a higher overall yield of the desired dideoxy compound directly from cytidine. It became apparent in our initial reaction of N<sup>4</sup>-acetylcytidine (4) with acetyl bromide that three products were being formed and that potentially other brominated compounds in addition to the 33% of 1-(3-bromo-3-deoxy-2,5-di-O-acetyl- $\beta$ -D-xylofuranosyl)-N<sup>4</sup>-acetyl cytosine (5) isolated from the reaction by Marumoto and Honjo<sup>11</sup> were available.

NMR examination of the acetyl bromide reaction products indicated that both the 3-bromo compound 5 and the isomeric 2-bromo derivative 6 were being formed in the reaction, together with tetraacetate 8. Since either of the bromo derivatives is a potential source of ddC, we examined alternative procedures for maximizing the overall yield of the brominated products. Acetic anhydride/hydrogen bromide in acetic acid has been

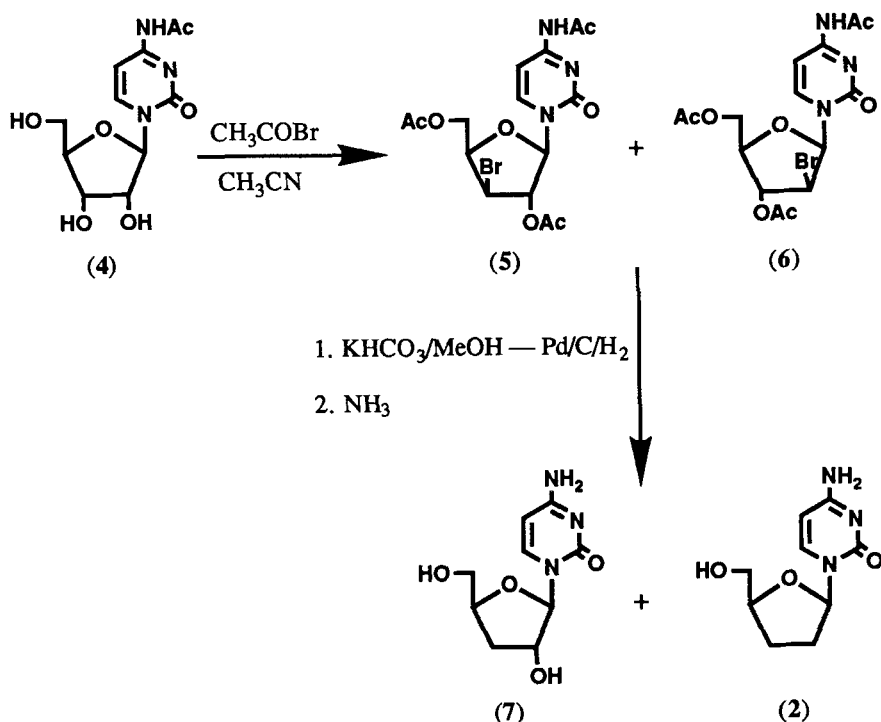
TABLE 1

Reagent	Reaction Temp.	Product % Tetraacetate 8	Product % 3-Bromo 5	Product % 2-Bromo 6	Material recovery %
AcBr/HOAc	50°	86	9	5	88
AcBr/HOAc	0°	83	11	6	56
HBr/HOAc	45°	56	24	14	62
HBr/HOAc	0°	94	4	0	59
Ac <sub>2</sub> O/HBr/HOAc	0°	64	23	13	38
AcBr/CH <sub>3</sub> CN	68°	26	52	22	93

used<sup>12</sup> to generate bromo-acetoxy derivatives of nucleosides, while recently hydrogen bromide in acetic acid was shown to effect the same transformation of activated diols.<sup>13</sup> These procedures led to greater production of N<sup>4</sup>,O<sup>2'</sup>,O<sup>3'</sup>,O<sup>5'</sup>-tetraacetylcytidine (**8**), with variable overall yields, presumably due to cleavage of the sugar moiety under the strongly acidic conditions. Comparative results are shown in Table 1 above.

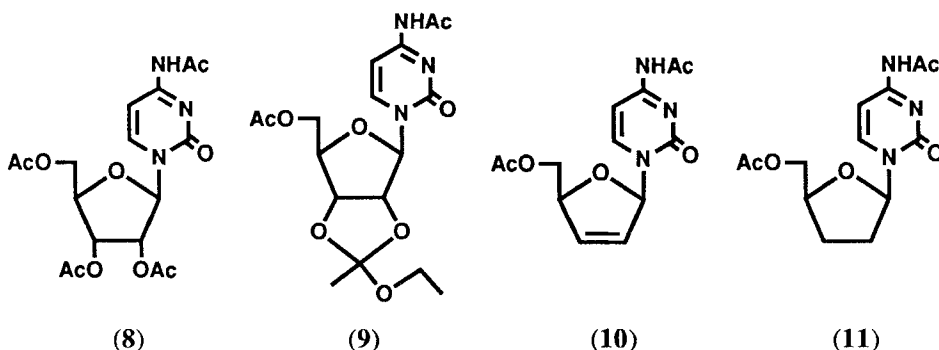
Cytidine was converted to the N<sup>4</sup>-acetyl derivative **4**, which on treatment with acetyl bromide in acetonitrile at 65—70° (optimum internal temperature 68°) gave a mixture of 1-(3-bromo-3-deoxy-2,5-di-O-acetyl-β-D-xylofuranosyl)-N<sup>4</sup>-acetylcytosine (**5**) (52%), 1-(2-bromo-2-deoxy-3,5-di-O-acetyl-β-D-arabinofuranosyl)-N<sup>4</sup>-acetylcytosine (**6**) (22%) and N<sup>4</sup>,O<sup>2'</sup>,O<sup>3'</sup>,O<sup>5'</sup>-tetraacetylcytidine (**8**) (26%). An alternative route *via* the orthoacetate **9**<sup>14</sup> was found to give the same three products, although in different ratios, and as an extra step was involved we found the direct route to be more convenient on a larger scale.

The proposed mechanism for the formation of the dideoxy compound<sup>11,15</sup> involves the reductive elimination of the *trans*-bromoacetate **5** to give N<sup>4</sup>,O<sup>5'</sup>-diacetyl-2',3'-dideoxy-2',3'-didehydrocytidine (**10**) and *in situ* hydrogenation of this olefin to give



SCHEME 1

11. As noted above, 1-(2-bromo-2-deoxy-3,5-di-O-acetyl- $\beta$ -D-arabinofuranosyl)-N<sup>4</sup>-acetylcytosine (**6**) could also participate in this sequence of events. To reduce manipulative losses we used the crude mixture of **5**, **6** and **8**. Trial experiments using various inorganic and organic bases and purified 3-bromo compound **5** in this sequence, followed by deprotection with ammonia, showed that the yield of ddC was sensitive to the nature of the base and solvent. 3'-Deoxycytidine (**7**) was an alternative end product of the reaction, while HPLC indicated some hydrogenolysis occurred in a few instances with cytosine as the byproduct. Highest conversion to ddC was found when the base was potassium hydrogen carbonate and the solvent methanol.



Treatment of a methanolic solution of the bromo-acetylation mixture with hydrogen over palladised charcoal catalyst (Scheme 1) and excess base gave the crude product mixture. Deprotection was achieved by bubbling gaseous ammonia into the same flask and the deoxygenated cytidines were separated by flash chromatography.<sup>16</sup> In this way 2',3'-dideoxycytidine (**2**) (20%), 3'-deoxycytidine (**7**) (23%) and cytidine (13%) were obtained from N<sup>4</sup>-acetylcytidine (**4**) (Scheme 1).

We have recently reported<sup>1</sup> an improved electrochemical synthesis of N<sup>4</sup>,O<sup>5'</sup>-diacetyl-2',3'-dideoxy-2',3'-didehydrocytidine (**10**) from 1-(3-bromo-3-deoxy-2,5-di-O-acetyl- $\beta$ -D-xylofuranosyl)-N<sup>4</sup>-acetylcytosine (**5**) and its hydrogenation and deprotection to ddC (**2**). In our experience the two pot procedure reported above (Scheme 1) and detailed below, using palladised charcoal, is the most convenient for the synthesis of larger quantities of 2',3'-dideoxycytidine (**2**).

#### EXPERIMENTAL

Solvents were HPLC grade (acetonitrile not more than 0.05% water). HPLC was carried out on a Waters system consisting of an M6000A pump, a 15 cm Novapak C<sub>18</sub> column and a Model 481 AZ LC Spectrophotometer. Samples were injected using a Rheodyne

Model 7125 valve, and the spectrophotometer output quantified by a Varian Vista CDS 401 data system. HPLC solvents, of varying polarity according to the mixture being analyzed, were mixtures of acetonitrile and water, buffered with 0.1% potassium dihydrogen phosphate. Flash chromatography<sup>16</sup> was carried out using a Pyrex 1000 ml cylindrical separating funnel, i.d. 70 mm, filled in the straight portion to a height of 160 mm with Merck Silica Gel 60 (0.04 - 0.063 mm) (360 g), with solvent flow regulated by a Fairchild Model 10 pressure regulator on the nitrogen supply. Routine <sup>1</sup>H NMR spectra were recorded on Varian EM360 and EM390 instruments using tetramethylsilane as internal standard; reference <sup>1</sup>H and <sup>13</sup>C spectra were obtained on a Bruker WM 250 machine.

#### **N<sup>4</sup>-Acetylcytidine (4)**

The procedure of Watanabe and Fox<sup>17</sup> was modified by reduction of the solvent volume and continuous addition of the reagent<sup>18</sup> to routinely provide N<sup>4</sup>-acetylcytidine in better than 90% yield. Cytidine (20 g) was suspended in methanol (500 ml) and heated to reflux. Acetic anhydride (80 ml) was added by syringe pump at 40 ml/h, and the reaction was refluxed an additional hour. The reaction mixture did not clarify throughout, but the suspension became noticeably thicker towards the end of the addition. After cooling overnight, the product was filtered off to yield N<sup>4</sup>-acetylcytidine (4) (20.1 g; 85%). Partial evaporation of the mother liquors afforded a second crop of crystals (2.0 g) giving a total yield of 93%.

On a larger scale, cytidine (1.88 kg) in refluxing methanol (45 l) was treated with acetic anhydride (7.7 l, added over 1 h) to give the product 4 (1.92 kg) in 87% yield from the first crop.

#### **Reaction of N<sup>4</sup>-Acetylcytidine with Acetyl bromide.**

In a 1 l flange flask fitted with an oil-sealed mechanical stirrer, pressure-equalizing dropping funnel, thermometer and a guard tube were placed N<sup>4</sup>-acetylcytidine (4) (20 g, 0.07 mole) and acetonitrile (500 ml) and the stirred suspension heated in an oil bath at 65°. The dropping funnel was charged with a solution of acetyl bromide (26 ml, 0.35 mole) in acetonitrile (150 ml), which was added dropwise to the vigorously stirred suspension at such a rate (1.5 h for addition), that the internal temperature did not exceed 70° then heated a further 0.5 h. The suspended material slowly went into solution, all being dissolved by the time half the reagent was added. Solvent and excess reagent were removed on the rotary evaporator, the residue was dissolved in dichloromethane (500 ml), washed twice with cold water (100 ml), the organic layer dried (magnesium sulfate), filtered and the solvent evaporated, affording a tan solid (27.7 g, 93%). HPLC analysis (CH<sub>3</sub>CN - H<sub>2</sub>O 3:7) showed the presence of compounds 5 (52%), 6 (22%), and 8 (26%).

## 2',3'-Dideoxycytidine (2)

The crude bromination mixture above (27.5 g), potassium hydrogen carbonate (23.6 g), 10% palladium on carbon catalyst (5 g) and methanol (600 ml) were placed in a flask fitted with a sealed mechanical stirrer (vigorous stirring is essential) and connected to a balloon of hydrogen and a vacuum outlet. With the contents stirred, the flask was evacuated then filled with hydrogen and stirring continued until **5** and **6** were completely consumed (16 h). The mixture was then saturated with gaseous ammonia and warmed to 50° for 4h, when deprotection was complete. The mixture was cooled in ice, the solids filtered off and washed with methanol, and the combined filtrates evaporated to dryness. The residue was redissolved in methanol (300 ml), chloroform (300 ml) added, and stored in the refrigerator overnight to precipitate inorganic components. The solids were filtered off and the filtrate evaporated onto silica gel (60 g), and applied as a powder to the top of the prepared flash chromatography column. The column<sup>19</sup> was eluted with dichloromethane-methanol-water (85:15:1.5), and fractions 9-13 (500 ml fractions) afforded analytically pure ddC (**2**) (2.97 g, 20 %), whose identity was confirmed by NMR and HPLC comparison with authentic material. Later fractions 16-23 (solvent 80:20:2) gave 3'-deoxycytidine (**7**) (4.0 g, 23%) and then (solvent 50:50:5) cytidine (2.18 g, 13%).

*Note added in Proof:* Further details of USP **4,900,828** (1990) (Ref. 12) have appeared (Manchand, P.S.; Belica, P.S.; Holman, M.J.; Huang, T.-N.; Maehr, H.; Tam, S. Y.-K.; Yang, R.T. *J. Org. Chem.* **1992**, *57*, 3473). Bromination with HBr in acetic acid is described as extremely troublesome on scale-up, confirming our findings, but the authors were not able to achieve our results with acetyl bromide.

**ACKNOWLEDGEMENT:** We would like to thank the Commonwealth AIDS Research Grant Committee for their generous support.

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Received 5/19/92

Accepted 7/24/92