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Facile methods for the synthesis of 5-formylcytidine

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Abstract—5′-*O*-Protected 5-methylcytidine **3** was oxidized with $Na_2S_2O_8$ to give a mixture of the corresponding 5-(hydroxymethyl)- and 5-formylcytidine derivatives, **4** and **5**. The hydroxymethyl group of **4** was further oxidized to a formyl group by treatment with ceric(IV) ammonium nitrate (CAN). Alternatively, 2′,3′,5′-*O*-protected 5-(hydroxymethyl)cytidine **10** was directly oxidized with CAN to give the desired 5-formylcytidine derivative **11**. After removal of the protecting groups in each intermediate, 5-formylcytidine (**6**) was obtained in good yield.

The post-transcriptional processing of RNA produces an exceptional number and structural diversity of modified nucleosides.[1,2] Recently, the functional roles of modifications existing in biologically important RNA molecules have been gradually revealed in parallel with continuous discovery of new modified nucleosides.[3–5] Such a new nucleoside, 5-formylcytidine (f^5C), has been found at the first position of the anticodon of $tRNA^{Met}$ from bovine liver mitochondria.[6]

Moriya et al.[6] have synthesized 5-formylcytidine from 5-(hydroxymethyl)cytosine; the oxidation of 5-(hydroxymethyl)cytosine with Ce(IV) gave 5-formylcytosine, which was converted to the corresponding dimethyl acetal and then ribosylated using a Sn(IV) catalyst. Recently, Kasai and co-workers[7] have reported a novel method for the synthesis of 5-formyl-2′-deoxycytidine, which is essentially applicable for the synthesis of 5-formylcytidine, starting from 3′,5′-bis-*O*-*t*-butyldimethyl-silyl-5-iodo-2′-deoxyuridine but through six steps.

On the other hand, it has been reported[8] that a 5-methylcytidine derivative was oxidized with peroxosulfate in a buffer solution to give the desired 5-formylcytidine derivative in low yield together with the formation of 5-(hydroxymethyl)cytidine, 5-(hydroxymethyl)cytosine and 5-methycytosine as hardly separable side products. Moreover, in this case the selective oxidation of the 5-(hydroxymethyl)cytidine derivative to the corresponding 5-formyl derivative has been difficult due to the presence of two primary hydroxyl groups (5-hydroxymethyl and 5′-OH).

This situation led us to investigate the oxidation of 5′-*O*-protected 5-methylcytidine with an expectation that the intermediary 5′-*O*-protected 5-(hydroxymethyl)cytidine would be easily oxidized to the corresponding 5′-*O*-protected 5-formylcytidine, resulting in the improvement of the total yield of the desired 5-formylcytidine derivative. For this purpose we at first synthesized 5-methylcytidine (**5**) as follows: 1-(tri-*O*-benzoyl-β-D-ribofuranosyl)-5-methyl-2,4(1*H*,3*H*)-pyrimidinedione (**1**) was synthesized in 99% yield by the ribosylation of a silylated thymine[9] using Sn(IV) as a catalyst according to the method of Niedballa et al.[10] Compound **1** was treated with 1,2,4-triazole, triethylamine, and phosphorous oxychloride in acetonitrile to give the triazolyl intermediate in 98% yield after chromatographic purification, which was converted to **2** in 95% yield by treatment with a mixture of dioxane and ammonia at room temperature for 24 h and with methanolic ammonia overnight. The physical data of **2**, thus obtained, were in agreement with those in the literature.[11] Treatment of **2** with *t*-butyldiphenylsilyl chloride in the presence of imidazole in DMF gave the 5′-*O*-*t*-butyldiphenylsilylated **3** in 94% yield after chromatographic purification. Oxidation of **3** with sodium peroxosulfate in a buffer solution at 70°C under argon gave 5-(hydroxymethyl)cytidine derivative **4** and 5-formylcytidine derivative **5** in 27 and 20% yields, respectively. The 5-(hydroxymethyl)cytidine derivative **4** was easily converted to **5** by treatment with 1 M ceric(IV) ammonium nitrate (CAN) applying the method of Trahnovski et al.[12] to give **5** in a total yield of 45%. Deprotection of **5** by treatment with tetrabutylammonium fluoride in THF at room temperature gave the desired product, 5-formylcytidine (**6**), in 71% yield (Scheme 1).

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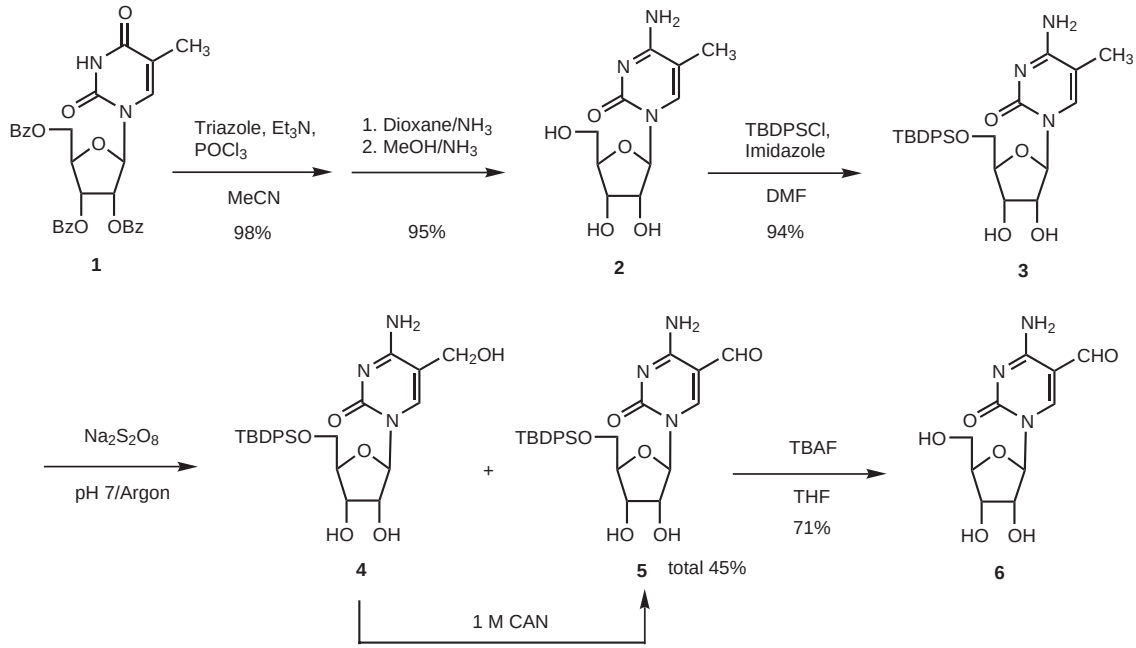
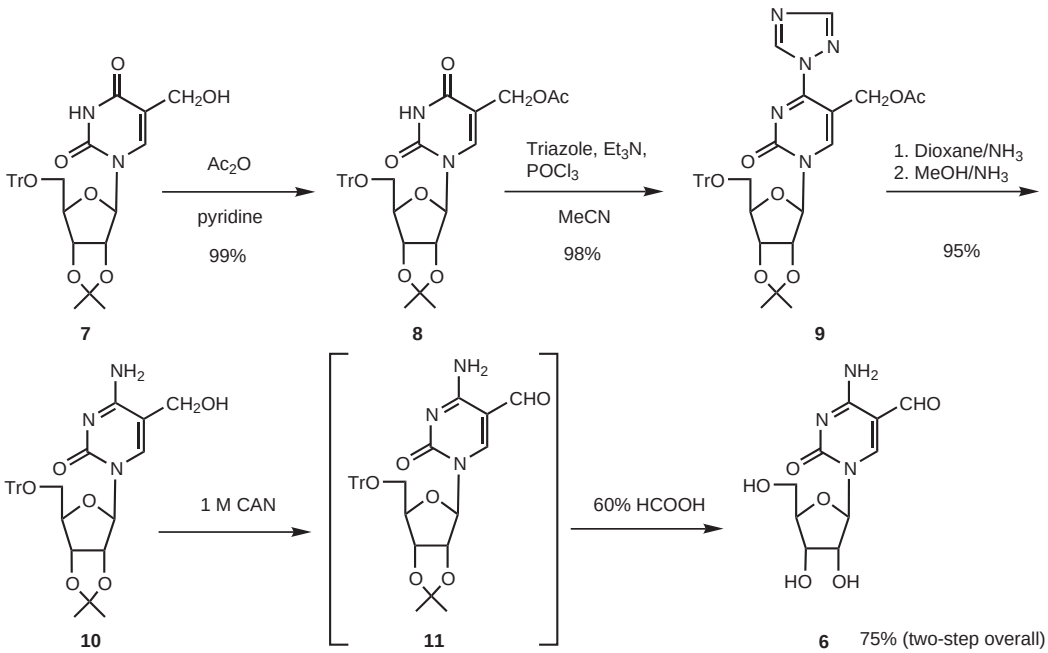


Scheme 1.

In the next stage, in order to develop an alternative, convenient and efficient method for the synthesis of 5-formylcytidine (**6**), we noted the facts that the oxidation of 5-(hydroxymethyl)cytosine with 1 M CAN gave 5-formylcytosine in high yield[6,12] and that **4** could be easily oxidized by CAN to also give **5** in high yield as mentioned above. These facts were considered to indicate that the oxidation of a hydroxymethyl group at the 5-position of a cytosine moiety took place easily. On the basis of this consideration, we tried to apply this oxidation to a protected 5-(hydroxymethyl)cytidine derivative. For this purpose, 2′,3′-*O*-isopropylidene-5′-*O*-trityl-5-(hydroxymethyl)uridine (**7**) was synthesized as a starting material 2′,3′-*O*-isopropylidene-5′-*O*-trityluridine in 70% yield.[13] Compound **7** was acetylated with acetic anhydride in pyridine at room temperature overnight to give the acetylated derivative **8** in 99% yield, which was converted to the triazolyl derivative **9** in 98% yield by a similar method described above.[14] Treatment of **9** with dioxane/NH_3 and then with MeOH/NH_3 gave **10** in 95% yield.[14] The oxidation of **10** with 1 M CAN at 60°C for 1 h followed by



Scheme 2.

deprotection with 60% formic acid at 80°C for 2 h, gave **6** in 75% yield (Scheme 2).

In conclusion, we have demonstrated two facile methods for the synthesis of 5-formylcytidine, which plays a central role in the realization of the higher-ordered structure and finely controlled function of tRNA. In comparison with the previously reported methods, the present ones are more efficient and economical. Physicochemical and biochemical studies of this modified nucleoside are now in progress.

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References

1. Hall, R. H. *The Modified Nucleosides in Nucleic Acids*; Columbia University Press: New York, 1971.
2. Nishimura, S. In *Transfer RNA: Structure, Properties and Recognition*; Schimmel, P.; Söll, D.; Abelson, J. N., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1979; pp. 59–79.
3. Björk, G. R.; Ericsson, J. U.; Gustafsson, C. E. D.; Hagervall, T. G.; Jönsson, Y. H.; Wikström, P. M. *Annu. Rev. Biochem.* **1987**, *56*, 263–287.
4. Björk, G. R. In *Transfer RNA in Protein Synthesis*; Hatfield, D. L.; Lee, B. J.; Pirtle, R. M., Eds.; CRC Press: Boca Raton, FL, 1992; pp. 23–85.
5. In *The RNA World*; Grsteland, R. F.; Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: Plainview, New York, 1993.
6. Moriya, J.; Yokogawa, T.; Wakita, K.; Ueda, T.; Nishikawa, K.; Crain, P. F.; Hashizume, T.; Pomerantz, S. C.; McClosky, J. A.; Kawai, G.; Hayashi, N.; Yokoyama, S.; Watanabe, K. *Biochemistry* **1974**, *33*, 2234–2239.
7. Kamuya, N. M.; Kamiya, H.; Karino, N.; Ueno, H.; Kaji, H.; Matsuda, A.; Kasai, H. *Nucleic Acids Res.* **1999**, *27*, 4385–4390.
8. Itahara, T. *Chem. Lett.* **1991**, 1591–1594.
9. Wittenburg, E. *Z. Chem.* **1964**, *4*, 303.
10. Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, *39*, 3654–3660.
11. Fox., J. J.; Praag, D. V.; Wempen, I.; Doerr, I. L.; Cheong, L.; Knoll, J. E.; Eidinoff, M. L.; Bendich, A.; Brown, G. B. *J. Am. Chem. Soc.* **1959**, *81*, 178–187.
12. Trahanovski, W. S.; Young, B. L.; Brown, C. L. *J. Org. Chem.* **1967**, *32*, 3865–3868.
13. (a) Fromageot, H. P. M.; Grieffin, B. E.; Reese, C. B.; Sulton, J. E. *Tetrahedron* **1967**, *23*, 2315–2331; (b) Leven, P. A.; Tipson, R. S. *J. Biol. Chem.* **1934**, *105*, 385–393; (c) Scheit, K. H. *Chem. Ber.* **1966**, 3884–3891.
14. Selected physical data: **4**: colorless foam: TLC ($CHCl_3$–MeOH, 8:2 v/v): R_f 0.66; ^1H NMR (300 MHz, DMSO-d_6): δ 1.09 (s, 9H, 3×CH_3), 3.48 (s, 2H, CH_2), 3.98 (m, 2H, 5′,5″-H), 4.28 (m, 1H, 4′-H), 4.37 (m, 2H, 2′-H, 3′-H), 5.99 (d, J=1.7 Hz, 1H, 1′-H), 7.38–7.40 (m, 6H, Ar-H), 7.64–7.71 (m, 5H, 6-H, Ar-H). **5**: pale yellow foam: TLC ($CHCl_3$–MeOH, 8:2 v/v): R_f 0.70; ^1H NMR (300 MHz, DMSO-d_6): δ 1.07 (s, 9H, 3×CH_3), 4.09 (m, 2H, 5′,5″-H), 4.21–4.29 (m, 3H, 2′-H, 3′-H, 4′-H), 5.92 (d, J=1.8 Hz, 1H, 1′-H), 7.40–7.45 (m, 6H, Ar-H), 7.63–7.68 (m, 4H, Ar-H), 8.90 (s, 1H, 6-H), 9.13 (s, 1H, CHO). **9**: colorless foam: TLC ($CHCl_3$–MeOH, 9.5:0.5 v/v): R_f 0.80; ^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 2.90 (s, 3H, Ac), 3.45 (s, 2H, CH_2), 3.72–3.81 (m, 2H, 5′,5″-H), 4.31 (m, 1H, 4′-H), 4.98–5.01 (m, 1H, 3′-H), 5.05–5.11 (m, 1H, 2′-H), 5.61 (d, J=1.6 Hz, 1H, 1′-H), 6.98 (m, 6H, Ar-H), 7.25–7.38 (m, 9H, Ar-H), 8.05 (s, 1H, 6-H), 8.30 (s, 1H, H-3 triazole), 9.30 (s, 1H, H-5 triazole). **10**: pale yellow foam: TLC ($CHCl_3$–MeOH, 9:1 v/v): R_f 0.67; ^1H NMR (300 MHz, DMSO-d_6): δ 1.40 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 3.38 (s, 2H, CH_2), 3.75–3.83 (m, 2H, 5′,5″-H), 4.29 (m, 1H, 4′-H), 5.00–5.09 (m, 2H, 2′-H, 3′-H), 5.57 (m, 1H, 1′-H), 7.00–7.09 (m, 6H, Ar-H), 7.30–7.34 (m, 9H, Ar-H), 8.00 (s, 1H, 6-H).