A Practical Access to [1-^{13}C]1-Deoxy-D-xylulose and its Derivatives

Hiroshi Okumoto,* Hiroko Katto

Department of Chemistry and Bioscience, Kurashiki University of Science and the Arts, 2640 Nishinoura, Tsurajima, Kurashiki, 712-8505 Japan
Fax +81(86)4401062; E-mail: okmt@chem.kusa.ac.jp
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Abstract: A practical synthesis of [1-^{13}C]1-deoxy-D-xylulose and its triacetate and 5-phosphate derivatives is described. The present scheme is developed based on the consideration of the cost efficiency of ^{13}C.

Key words: isotope, labeled compound, stereoselective synthesis, carbohydrates, terpenoids, natural products, biosynthesis

Terpenoids are an important class of natural products and are mainly of plant origin. The potential use of terpenoids as medicinal agents and aroma ingredients in both the native and chemically-modified forms is exceedingly high. For decades it was believed that the isoprene unit, the smallest constituent of the terpenes, is produced from isoprenyl diphosphate (IPP) solely through the mevalonic acid (MVA) pathway. Recently, an alternative biosynthetic route was discovered, wherein 1-deoxy-D-xylulose (DOX) 5-phosphate and methyl erythritol (ME) phosphate were indispensable for the formation of IPP. The new pathway has received particular attention, since it was found to play a major role in isoprenoid biosynthesis in many bacteria and all plants. Investigations attempting to delineate the two pathways in plants and plant cell cultures have made use of ^{13}C-labeled compounds such as glucose and MVA in an effort to trace the fate of the ^{13}C-labeled metabolites. A ^{13}C NMR analysis of the labeled metabolites can lead to the successful determination of biosynthetic pathways, both classical and new. In order to obtain more detailed and distinct information on the MVA-independent pathway, there is now a substantial demand for larger quantities of ^{13}C-labeled DOX and ME, since these substrates are specific to the non-mevalonate pathway.[1]

Attention was initially focused on the synthesis of ^{13}C-labeled DOX, rather than ME, due to the high efficiency of incorporation of DOX into the culture cell. Thus, we would like to describe herein a practical and facile synthesis of [1-^{13}C]1-deoxy-D-xylulose (1-^{13}C-DOX), the stable triacetate derivative, and 1-^{13}C-DOX 5-phosphate (1-^{13}C-DOXP).

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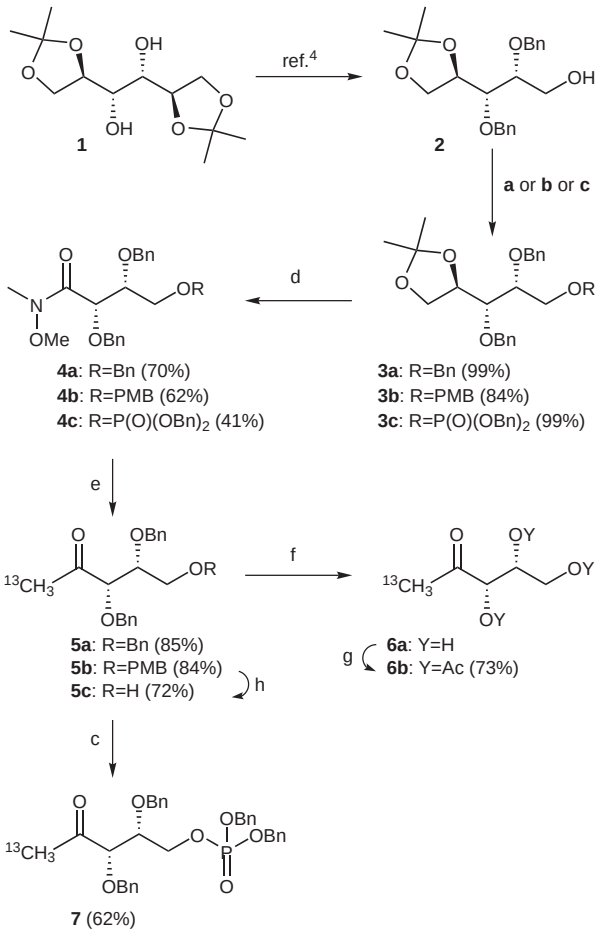
Although several synthetic routes to DOX and DOXP have been previously described,[2] the prohibitive cost of the ^{13}C isotope imposes strict restrictions on its use in synthetic approaches. A consideration of the cost efficiency of ^{13}C isotope usage prompted us to develop a new route utilizing an appropriate intermediate. The synthetic process is outlined in Scheme 1. The synthetic steps following the incorporation of the ^{13}C-labeled group must be as short as possible and a simple operation for isolating the water-soluble final products is required in the last stage. Amide **4** is the key precursor that matched our requirements. The Weinreb's amide is known to react readily with a stoichiometric amount of Grignard reagent to selectively afford the ketone.[3] All hydroxyl groups are protected with a benzyl group to facilitate the quantitative removal and facile isolation of the resulting sugar derivatives by simple operations, i.e. heterogeneous hydrogenation, filtration and concentration.

Commercially available mannitol diacetonide (**1**) was converted to the alcohol **2** according to a published procedure[4] with slight modification. In the route leading to the synthesis of DOX **6a** and its acetate **6b**, the alcohol **2** was protected with BnBr. Transformation of the 1,2-diol acetonide **3a** to the amide **4a** was carried out in the usual manner. Thus, hydrolysis of the acetonide **3a** with 5% HCl in MeOH, and oxidative cleavage of the vicinal diol followed by treatment of the crude aldehyde with $NaClO_2$ yielded carboxylic acid. Condensation with Weinreb's amine proceeded in the presence of DCC and pyridine, giving rise to intermediate **4a**. Incorporation of ^{13}C was achieved using $^{13}CH_3MgI$, prepared from commercially available $^{13}CH_3I$ and Mg, at room temperature to selectively obtain the methyl ketone **5a**. Simple hydrogenation on 10%Pd/C in acetone/H_2O at ambient temperature, followed by filtration and concentration, lead to the isolation of the labeled sugar **6a**. If necessary, purification by column chromatography on silica gel with $CHCl_3$/MeOH can be performed. The stable derivative **6b** was afforded by exposure of **6a** to a mixture of acetic anhydride and pyridine.

For the synthesis of the phosphate **7**, methylation of the amide **4c** was initially performed in a similar way to the preparation of **5a**. The envisioned amide **4c** was prepared by the phosphorylation of the alcohol **2** and the ensuing conversion of the acetonide moiety to the amide. However, the reaction with methyl Grignard reagent prepared from MeI failed to give the desired methyl ketone in spite of several attempts under various experimental condi-



Scheme 1 a) BnBr/NaH/DMF; b) PMBCl/NaH/DMF; c) (1) *i*-$Pr_2NP(OBn)_2$/tetrazole/CH_2Cl_2; (2) H_2O_2; d) (1) 5% HCl/dioxane / MeOH; (2) $NaIO_4$/MeOH/H_2O; (3) $NaClO_2$/*t*-BuOH/H_2O; (4) MeNHOMe-HCl/DCC/pyridine/CH_2Cl_2; e) $^{13}CH_3MgI$/THF; f) H_2, 10% Pd-C/acetone/H_2O; g) Ac_2O/pyridine; h) DDQ/CH_2Cl_2/H_2O

tions. The benzyl phosphate group was found to be reactive toward the MeMgI reagent, where MeMgBr could lead to the formation of the corresponding methyl ketone. Consequently, protection of the primary alcohol **2** with PMBCl was carried out. Conversion of the acetonide **3b** to the amide **4b**[5] proceeded with high yield, and subsequent treatment with $^{13}CH_3MgI$ gave rise to the ^{13}C-labeled ketone **5b** without any problems. The selective deprotection of the PMB group with DDQ produced the alcohol **5c**, which was then subjected to phosphorylation. The benzyl-protected substrate **7** was thus synthesized, and the deprotection of **7** is already reported in the literature[2i] using unlabeled compound.

Given the restricted ^{13}C isotope source, a few further manipulations were inevitable for the preparation of the phosphate after the introduction of the 1-^{13}C-methyl group. However, the final steps do not detract from the overall efficiency of the synthetic route since only routine steps are required.

The synthesis of 1-^{13}C-DOX (**6a**) and its acetate **6b** was achieved in short steps. The last stage, heterogeneous hydrogenation, facilitated the isolation of the water-soluble target molecule. In addition, we can offer a stable derivative, 1-^{13}C-DOX-triacetate (**6b**), for use as a tracing material in the analysis of biosynthetic pathways.

References

(1) (a) Thiel, R.; Adam, K. P. *Phytochemistry* **2002**, *59*, 269. (b) Putra, S. R.; Lois, L. M.; Campos, N.; Boronat, A.; Rohmer, M. *Tetrahedron Lett.* **1998**, *39*, 23; and references cited therein.

(2) For the enzymatic synthesis, see: (a) Querol, J.; Grosdemange-Billiard, C.; Rohmer, M.; Boronat, A.; Imperial, S. *Tetrahedron Lett.* **2002**, *43*, 8265. (b) Hecht, S.; Kis, K.; Eisenreich, W.; Amslinger, S.; Wungsintaweekul, J.; Herz, S.; Rohdich, F.; Bacher, A. *J. Org. Chem.* **2001**, *66*, 3948. (c) Shabat, D.; List, B.; Lerner, R. A.; Barbas, C. F. III. *Tetrahedron Lett.* **1999**, *40*, 1437. (d) For the chemical synthesis, see: Fechter, M. H.; Gaisberger, R.; Griengl, H. *J. Carbohydr. Chem.* **2001**, *20*, 833. (e) Zhao, S.; Petrus, L.; Serianni, A. S. *Org. Lett.* **2001**, *3*, 3819. (f) See also: Hoeffler, J.-F.; Grosdemange-Billiard, C.; Rohmer, M. *Tetrahedron Lett.* **2001**, *42*, 3065. (g) Blagg, B. S.; Poulter, C. D. *J. Org. Chem.* **1999**, *64*, 1508. (h) See further: Jux, A.; Boland, W. *Tetrahedron Lett.* **1999**, *40*, 6913. (i) Thiel, R.; Adam, K. P. *Tetrahedron Lett.* **1999**, *40*, 5307. (j) Tayler, S. V.; Vu, L. D.; Begley, T. P. *J. Org. Chem.* **1998**, *63*, 2375. (k) Giner, J.-L. *Tetrahedron Lett.* **1998**, *39*, 2479. (l) Piel, J.; Boland, W. *Tetrahedron Lett.* **1997**, *38*, 6387. (m) Zeidler, J. G.; Lichtenthaler, H. K.; May, H. U.; Lichtenthaler, F. W. *Z. Naturforsch., C: Biosci.* **1997**, *52*, 15. (n) Kennedy, I. A.; Hemscheidt, T.; Britten, J. F.; Spenser, I. D. *Can. J. Chem.* **1995**, *73*, 1329. (o) Backstrom, A. D.; McMordie, R. A. S.; Begley, T. P. *J. Carbohydr. Chem.* **1995**, *14*, 171. (p) David, S.; Estramareix, B.; Fischer, J.-C.; Therisod, M. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2131. (q) For the synthesis of 1-deoxy-L-xylulose, see tribenzoate: Wolfrom, M. L.; Bennet, R. B. *J. Org. Chem.* **1965**, *30*, 458.

(3) Weinreb, S. M.; Folmer, J. J. In *Encyclopedia of Reagents for Organic Synthesis*, Vol. 3; Paquette, L. A., Ed.; John Wiley and Sons: Chichester, **1995**, 2083.

(4) Chandrasekhar, M.; Kusum, K. L.; Singh, V. K. *Tetrahedron Lett.* **2002**, *43*, 2773.

(5) The selected physical data are shown as follows.
4a: ^1H NMR (500 MHz): δ = 3.09 (s, 3 H), 3.43 (s, 3 H), 3.63 (dd, *J* = 5.5, 10.3 Hz, 1 H), 3.69 (dd, *J* = 5.5, 10.3 Hz, 1 H), 4.06 (dd, *J* = 5.5, 10.7 Hz, 1 H), 4.43–4.51 (m, 4 H), 4.63–4.76 (m, 2 H), 7.25–7.35 (m, 15 H).
4b: ^1H NMR (500 MHz): δ = 3.09 (s, 3 H), 3.44 (s, 3 H), 3.60 (dd, *J* = 5.4, 10.3 Hz, 1 H), 3.66 (dd, *J* = 5.2, 10.3 Hz, 1 H), 3.80 (s, 3 H), 4.04 (dd, *J* = 5.4, 10.7 Hz, 1 H), 4.30–4.40 (m, 2 H), 4.40–4.50 (m, 2 H), 4.60–4.80 (m, 3 H), 6.83 (d, *J* = 8.5 Hz, 2 H), 7.18 (d, *J* = 8.5 Hz, 2 H), 7.25–7.34 (m, 10 H).
5a: $[\alpha]_D^{23}$ –36 (*c* 0.36, $CHCl_3$). ^1H NMR (500 MHz): δ = 2.14 (d, *J* = 128 Hz, $^{13}CH_3$), 3.60 (d, *J* = 6.1 Hz, 2 H), 3.95–3.97 (m, 2 H), 4.39–4.45 (m, 3 H), 4.51 (d, *J* = 11.6 Hz, 1 H), 4.63 (d, *J* = 11.6 Hz, 1 H), 4.67 (d, *J* = 11.9 Hz, 1 H), 7.24–7.34 (m, 15 H).
5b: $[\alpha]_D^{23}$ –41 (*c* 1.0, $CHCl_3$). ^1H NMR (500 MHz): δ = 2.13 (d, *J* = 127 Hz, $^{13}CH_3$), 3.58 (d, *J* = 6.0 Hz, 2 H), 3.80 (s, 3 H), 3.93–3.96 (m, 2 H), 4.34 (d, *J* = 11.3 Hz, 1 H), 4.37 (d, *J* = 11.3 Hz, 1 H), 4.43 (d, *J* = 11.7 Hz, 1 H), 4.50 (d, *J* = 11.7 Hz, 1 H), 4.62 (d, *J* = 11.7 Hz, 1 H), 4.65 (d,

J = 11.7 Hz, 1 H), 6.85 (d, *J* = 8.6 Hz, 2 H), 7.18 (d, *J* = 8.6 Hz, 2 H), 7.20–7.34 (m, 10 H).
6b: $[\alpha]_D^{23}$ +35 (*c* 0.65, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 2.06, 2.08, 2.21 (s, OAc × 3), 2.21 (d, *J* = 129 Hz, $^{13}CH_3$), 4.14 (dd, *J* = 6.6, 11.6 Hz, 1 H), 4.30 (dd, *J* = 5.8, 11.6 Hz, 1 H), 5.25 (d, *J* = 3.0 Hz, 1 H), 5.59 (ddd, *J* = 3.0, 5.8, 6.6 Hz, 1 H).
7: $[\alpha]_D^{23}$ –18 (*c* 0.35, $CHCl_3$). ^{1}H NMR (500 MHz): δ = 2.09 (d, *J* = 128 Hz, $^{13}CH_3$), 3.85 (d, *J* = 3.3 Hz, 1 H), 3.92 (ddd, *J* = 3.3, 5.8, 6.0 Hz, 1 H), 4.06–4.14 (m, 2 H), 4.38 (d, *J* = 11.5 Hz, 1 H), 4.41 (d, *J* = 11.5 Hz, 1 H), 4.53 (d, *J* = 11.5 Hz, 1 H), 4.60 (d, *J* = 11.5 Hz, 1 H), 4.96–5.03 (m, 4 H), 7.18–7.35 (m, 20 H). ^{31}P NMR: δ = –6.4.