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Elucidation of the 2-*C*-methyl-D-erythritol 4-phosphate pathway for isoprenoid biosynthesis: straightforward syntheses of enantiopure 1-deoxy-D-xylulose from pentose derivatives

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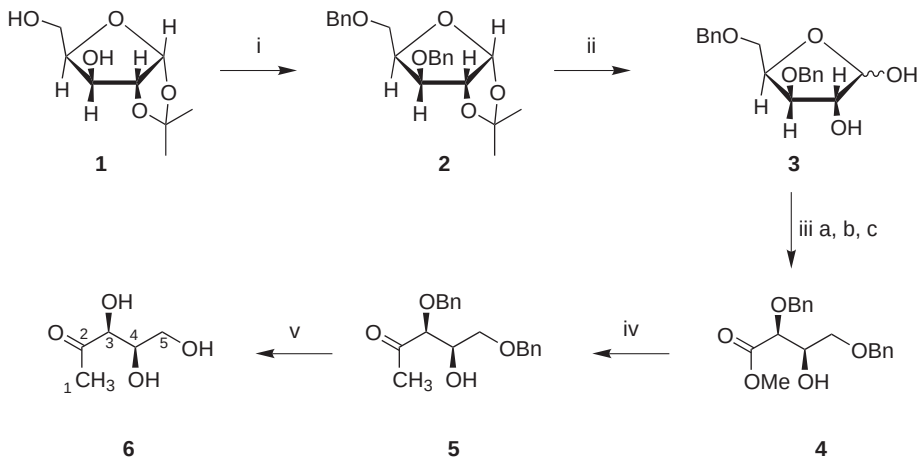
Abstract—Optically pure 1-deoxy-D-xylulose, a key metabolite for feeding experiments in the methylerythritol phosphate pathway for isoprenoid biosynthesis, is conveniently synthesised from 1,2-*O*-isopropylidene-α-D-xylofuranose or from D-arabinose. This renders labelling with hydrogen isotopes possible. © 2001 Elsevier Science Ltd. All rights reserved.

1-Deoxy-D-xylulose 5-phosphate is the first C_5 intermediate in the mevalonate independent methylerythritol phosphate (MEP) pathway for isoprenoid biosynthesis, occurring in bacteria and plant plastids.[1] Although not directly an isoprenoid precursor, the corresponding free pentulose is easily incorporated into most isoprenoids synthesised by this pathway. Unlabelled deoxyxylulose is required for supplementation of mutants with a disrupted deoxyxylulose phosphate synthase gene (*dxs*), which are used for the identification of the unknown steps of the pathway. Deuterium-labelled isotopomers of isoprenoid precursors, such as deoxyxylulose or methylerythritol, were required for the detection of the branching in the MEP pathway leading in *Escherichia coli* separately to IPP and DMAPP from an unknown common intermediate.[2] Therefore, the interest in deoxyxylulose increased rapidly and several synthetic approaches, often based on D-tartrate derivatives as starting material, were reported during the last 5 years and proved useful for the synthesis of deuterium-labelled isotopomers.[2a,3] The reported deuterium con-



Scheme 1. Chemical synthesis of 1-deoxy-D-xylulose from 1,2-isopropylidene-α-D-xylofuranose: (i) NaH, Bu_4NI, 18-crown-6, BnBr, THF (97%); (ii) 0.25 M HCl, dioxane/water (3:1), 90°C (78%); (iii) (a) $NaIO_4$, dioxane/water (2:1); (b) $AgNO_3$, dioxane/water (4:1), 2 M KOH; (c) CH_2N_2, ether (90%); (iv) CH_3Li, THF, −100°C (72%); (v) H_2, Pd/C, MeOH (98%).

Keywords: 1-deoxy-D-xylulose; methylerythritol phosphate; biosynthesis; isoprenoids; 2-*C*-methyl-D-erythritol phosphate; terpenoids.

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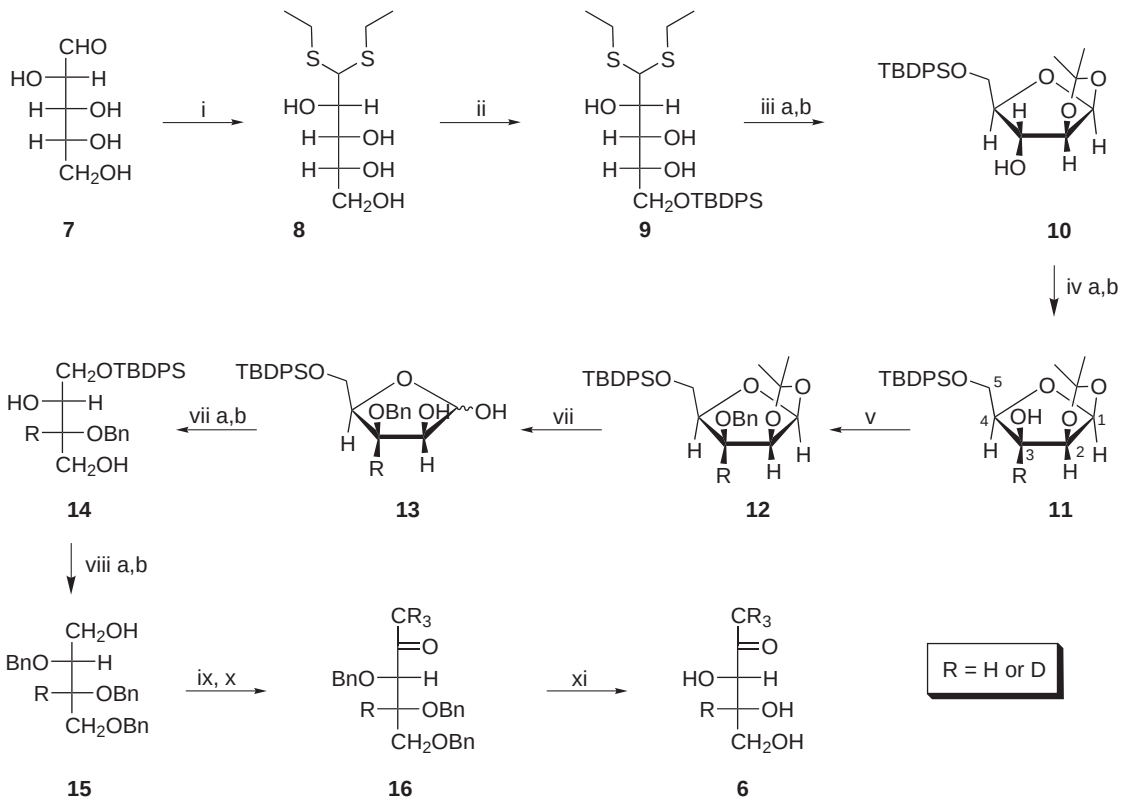
tent was however not always quantitative, or the deprotection steps often resulted in significant unexpected deuterium losses. Only two syntheses of deoxyxylulose have been described starting from a carbohydrate derivative.[4] In this contribution, two syntheses of enantiopure 1-deoxy-D-xylulose are proposed, including the possibility of deuterium-labelling at C-1 and/or C-4.

1-Deoxy-D-xylulose **6** was easily synthesised from commercially available 1,2-*O*-isopropylidene-α-D-xylofuranose **1**, which presents the required configuration of the two asymmetric carbons of 1-deoxy-D-xylulose **6** (Scheme 1). Benzylation of the two hydroxy groups of the 1,2-*O*-isopropylidene-α-D-xylofuranose **1** followed by hydrolysis of the acetonide in acidic conditions afforded the 3,5-*O*-dibenzyl-D-xylofuranose **3**. The free sugar was successively oxidised with $NaIO_4$ and $AgNO_3$–KOH to give 2,4-*O*-dibenzyl-D-*threo*-trihydroxybutanoic acid, which was subsequently esterified with diazomethane.[5] Addition of methyllithium to this ester **4**,[6] followed by a catalytic hydrogenation over palladium on charcoal gave 1-deoxy-D-xylulose **6**.

This synthesis of enantiopure 1-deoxy-D-xylulose is very efficient (seven steps, but the isolation of intermediates was not always necessary) with a 48% overall yield. This approach allows the ^2H or ^{13}C labelling on the C-1 methyl group, but could not be used to prepare the enantiopure 1-deoxy-D-xylulose with ^2H labelling at C-4 required for the detection of the branching in the MEP pathway.[2] Another synthetic route was therefore developed, starting from D-arabinose (Scheme 2).

The initial step of this alternative synthesis was the conversion of arabinose **7** onto its thioacetal.[7] Treatment of the arabinose thioacetal **8** with *t*-butyldiphenylsilyl chloride in dimethylformamide in the presence of imidazole resulted in a highly selective protection of the primary hydroxy group to afford the silyl ether **9** in 94% yield. Deprotection of the thioacetal moiety in **9** by treatment with a mercury(II) derivative in acetone was followed by the formation of an acetonide in the presence of a catalytic amount of acid to give the arabinofuranose derivative **10** with only the C-3 hydroxyl group unprotected.[8] The configuration was inverted at C-3 by Swern oxidation[9] to the intermediate ketone, followed by highly stereoselective reduction with sodium borohydride to yield the xylofuranoside **11**.[10] The presence of the 1,2-*O*-iso-



Scheme 2. Chemical synthesis of 1-deoxy-D-xylulose from D-arabinose: (i) EtSH, HCl (70%); (ii) TBDPSCl, imidazole, DMF, 0°C (94%); (iii) (a) HgO, $HgCl_2$, acetone; (b) $CuSO_4$, acetone, H^+ (84%, two steps); (iv) (a) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78°C to −35°C; (b) $NaBH_4$ or $NaBD_4$, EtOH (71%, two steps); (v) NaH, Bu_4NI, 18-crown-6, BnBr, THF (80%); (vi) 80% AcOH, H_2O (83%); (vii) (a) $NaIO_4$, H_2O, MeOH; (b) $NaBH_4$, EtOH (97%, two steps); (viii) (a) NaH, Bu_4NI, 18-crown-6, BnBr, THF; (b) Bu_4NF, THF (74%, two steps); (ix) $(COCl)_2$, DMSO, TEA, THF, −78°C to −35°C and CH_3MgCl or CD_3MgI, −10°C (98%); (x) $(COCl)_2$, DMSO, TEA, CH_2Cl_2, −78°C to −35°C (91%); (xi) H_2, Pd/C, MeOH (98%).

propylidene group on the β-face of the furanoside directed the reduction of the oxo group by hydride (or deuteride) from the less hindered α-face to afford the xylofuranose **11** with the required configuration. Confirmation of the structure of **11** was achieved by NOESY NMR experiments on derivative **12** after benzylation of the secondary hydroxy group. The NOEs between H-1 and H-2 confirmed the anomeric β configuration. The position of the proton at C-3 on the α-face of the furanose ring was supported on the one hand by strong NOEs between H-2, H-4 and the H-3, and on the other hand by medium NOEs between H-3 and H-1. The acetonide protection of **12** was hydrolysed with 80% aqueous acetic acid to yield a mixture of the two anomers **13** in 83% yield.[11] Smooth oxidative cleavage occurred on treatment of diol **13** with sodium metaperiodate in aqueous methanol to give an aldehyde in almost quantitative yield. This aldehyde was immediately reduced with sodium borohydride to afford 2-*O*-benzyl-4-*O*-(*t*-butyldiphenylsilyl)-D-threitol **14**.

Benzylation of this diol **14** and deprotection of the silyl ether yielded the 1,2,3-*O*-tribenzyl-D-threitol **15**. One-pot oxidation and nucleophilic addition of methylmagnesium chloride was achieved using the Swern–Ireland procedure.[12] A Swern oxidation of the resulting mixture of diastereomeric alcohols afforded 3,4,5-*O*-tribenzyl-1-deoxy-D-xylulose **16**. Quantitative deprotection of **16** was achieved by hydrogenation over 10% Pd/C in methanol at room temperature and atmospheric pressure to afford 1-deoxy-D-xylulose **6** in 16% overall yield. This second synthetic route allowed deuterium labelling at C-1 and/or C-4. The deuterium content was around 83–93% at C-4, according to ^{1}H and ^{13}C NMR of the tribenzyl derivative **16** of deoxyxylulose, depending on the sodium borodeuteride batch (98% expected isotopic abundance). In contrast, no proton was detected at C-1 when trideuterated methylmagnesium iodide (isotopic abundance>99%) was used.

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References

1. (a) Lichtenthaler, H. K.; Rohmer, M.; Schwender, J. *Physiol. Plant*. **1997**, *101*, 643–652; (b) Eisenreich, W.; Schwarz, M.; Cartayrade, A.; Arigoni, D.; Zenk, M. H.; Bacher, A. *Chem. Biol*. **1998**, *5*, R221–R223; (c) Rohmer, M. *Nat. Prod. Rep*. **1999**, *16*, 565–574.
2. (a) Giner, J.-L.; Jaun, B.; Arigoni, D. *Chem. Commun*. **1998**, 1857–1858; (b) Charon, L.; Hoeffler, J.-F.; Pale-Grosdemange, C.; Lois, L.-M.; Campos, N.; Boronat, A.; Rohmer, M. *Biochem. J*. **2000**, *346*, 737–742; (c) Hahn, F. M.; Hurburt, A. P.; Poulter, C. D. *J. Bacteriol*. **1999**, *181*, 4499–4504; (d) Rodriguez-Concepcion, M.; Campos, N.; Lois, L.-M.; Maldonado, C.; Hoeffler, J.-F.; Grosdemange-Billiard, C.; Rohmer, M.; Boronat, A. *FEBS Lett*. **2000**, *473*, 328–332; (e) Rieder, C.; Jaun, B.; Arigoni, D. *Helv. Chim. Acta* **2000**, *83*, 2504–2513.
3. (a) Kennedy, I. A.; Hemscheidt, T.; Britten, J. F.; Spenser, P. A. *Can. J. Chem*. **1995**, *73*, 1329–1337; (b) Backstrom, A. D.; McMordie, R. A. S.; Begley, T. P. *J. Carbohydr. Chem*. **1995**, *14*, 171–175; (c) Piel, J.; Boland, W. *Tetrahedron Lett*. **1997**, *38*, 6387–6390; (d) Taylor, S. V.; Vu, L. D.; Begley, T. P.; Schörken, U.; Grolle, S.; Sprenger, G. A.; Bringer-Meyer, S.; Sahm, H. *J. Org. Chem*. **1998**, *63*, 2375–2377; (e) Giner, J.-L. *Tetrahedron Lett*. **1998**, *39*, 2479–2482; (f) Blagg, B. S. J.; Poulter, C. D. *J. Org. Chem*. **1999**, *64*, 1508–1511.
4. (a) David, S.; Estramareix, B.; Fischer, J.-L.; Thérisod, M. *J. Chem. Soc., Perkin Trans. 1* **1982**, 2131–2137; (b) Zeidler, J. G.; Lichtenthaler, H. K.; May, H. U.; Lichtenthaler, F. W. *Z. Naturforsch*. **1997**, *52c*, 15–23.
5. Takahashi, Y.; Ueda, C.; Tsuchiya, T.; Kobayashi, Y. *Carbohydr. Res*. **1993**, *249*, 57–76.
6. Denmark, S. E.; Stavenger, R. A. *J. Org. Chem*. **1998**, *63*, 9524–9527.
7. White, J. D.; Jensen, M. S. *J. Am. Chem. Soc*. **1995**, *117*, 6224–6233.
8. Vázquez-Tato, M. P.; Seijas, J. A.; Fleet, G. W. J.; Mathews, C. J.; Hemmings, P. R.; Brown, D. *Tetrahedron* **1995**, *51*, 959–974.
9. Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem*. **1978**, *43*, 2480–2482.
10. (a) Hoeffler, J.-F.; Pale-Grosdemange, C.; Rohmer, M. *Tetrahedron* **2000**, *56*, 1485–1489; (b) Parr, I. B.; Horenstein, B. A. *J. Org. Chem*. **1997**, *62*, 7489–7494.
11. Roberts, M. S.; Shoberu, K. A. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2625–2632.
12. Ireland, R. E.; Norbeck, D. W. *J. Org. Chem*. **1985**, *50*, 2198–2200.