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Highly Efficient and Versatile Synthesis of Isotopically Labelled 1-Deoxy-D-xylulose

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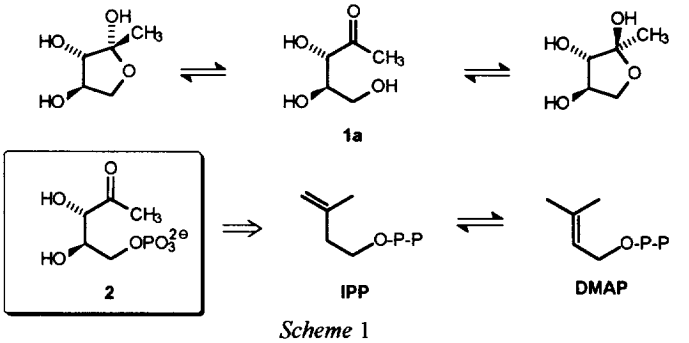
Abstract: An efficient and versatile synthesis of 1-deoxy-[4,4-$^2H_2$]-D-xylulose **1b** (= 1-deoxy-[4,4-$^2H_2$]-D-*threo*pentulose) from dimethyl 2,3-*O*-isopropylidene-D-tartrate in 31% overall yield is described. The synthetic protocol allows a flexible adaptation to other labelling patterns and isotopes. Labelled 1-deoxy-xylulose is of high value as a metabolic probe in biosynthetic studies towards terpenoids and prenylated compounds following the Rohmer pathway. © 1997 Published by Elsevier Science Ltd.

1-Deoxy-D-xylulose **1a**, first isolated in 1976 from *Streptomyces hygroscopicus*,[1] and its 5-phosphate **2** represent important intermediates within several biochemical pathways of pro- and eucaryotes. In bacteria, for example *E. coli*, **1a** is incorporated into the thiazole nucleus of thiamin (vitamin B_1)[2] and from there into pyridoxine (vitamin B_6).[3] After the recent discovery of a novel, mevalonate-independent pathway towards terpenoids by Rohmer et al., **1a** has gained much attention as an alternate precursor of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAP), the two principal building blocks of all terpenoids.[4-8]



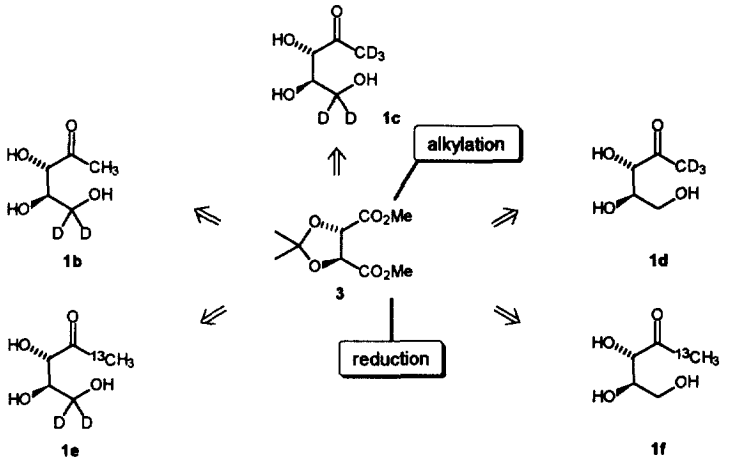
Scheme 1

The formation of IPP according to the novel pathway proceeds via an aldol-type condensation of a C_2-unit, derived from pyruvate decarboxylation with glyceraldehyde-3-phosphate and yields 1-deoxy-D-xylulose **1a** as a central intermediate. Further processing and a transposition step create the branched skeleton of IPP.[6] Recent studies based on feeding experiments with labelled glucose and/or labelled **1a** established that both, the classical mevalonate-based pathway and the novel route may be operative simultaneously in different plant organs.[5,9,10] To gain insight into the subcellular localisation of the two pathways and to unravel into which compound classes the IPP and DMAP, derived from the different precursors are channelled, efficient and flexible syntheses of **1a** isotopically labelled at various positions are required. Owing to the late and specific position in the sequence towards IPP, administration of isotopomers of **1a** is expected to give more precise results than the use of general precursors like labelled glucose, glycerinealdehyde-3-phosphate and pyruvate. Various synthetic approaches towards labelled **1a** have been reported in the literature in connection with studies on the bacterial biosynthesis of thiamin and pyridoxine. Thus, 1-deoxy-D-xylulose **1a** has been

prepared from threonamide,[11] deuterated derivatives of acetaldehyde,[2] D-arabinose,[2] xylitol[12] and triethyl [1,2-$^{13}C_2$]phosphonoacetate,[13] respectively. A recent approach introduced a single deuterium atom into a readily available derivative of 2,3-*O*-isopropylidene-β-D-xylulose.[14] The major drawbacks of most of these approaches is either the early introduction of isotopes, rendering the synthesis unflexible and costly, or the large number of steps resulting in low overall yields.

We report here a novel four step sequence starting from the commercially available dimethyl 2,3-*O*-isopropylidene-D-tartrate **3** which allows a highly flexible and economic transformation into differently labelled 1-deoxy-D-xyluloses **1b-f** on a large scale.

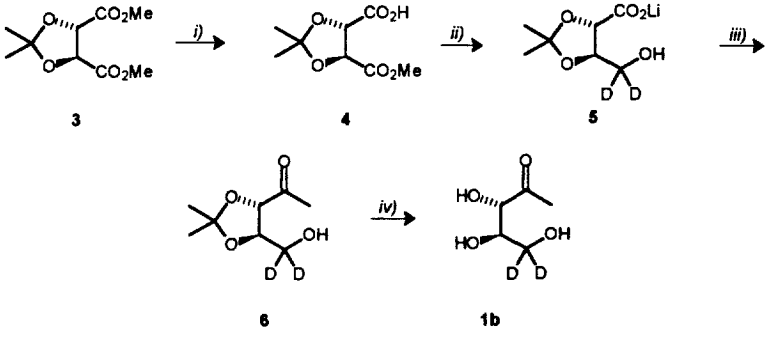
Scheme 2



As depicted in Scheme 2, the use of different combinations of labelled and unlabelled alkylation- and reducing reagents following the same synthetic protocol (cf. *Scheme 3*) can provide five different deuterium- and/or ^{13}C-labelled isotopomers of **1a**. Here, the synthesis of the dideuterated 1-deoxy-D-xylulose **1b** is described as an example.

To render C-1 and C-4 of the C_2-symmetric diester **3** discernible for further manipulations, one of the two ester groups was saponified yielding **4** as a single diastereoisomer. Unlike the less effective chemical saponifi-

Scheme 3



i) PLE, pH 8, 25 °C, 4h. *ii)* LiBEt$_3$D/THF, 0 °C, 1 h. *iii)* MeLi/THF, 0 °C, 2 h. *iv)* 2 N HCl/H$_2$O/CH$_3$CN, 25 °C, 24 h

cation of **1b** (63 % yield),[15] the biocatalytic hydrolysis of **1b** with pig liver esterase (PLE)[16] under controlled conditions (pH = 8.0) afforded the half ester **4** in 81% yield. Subsequent reduction of the ester group of **4** by LiBEt$_3$D yielded the lithio salt of the isopropylidene-protected dideutero threonate **5**. Owing to the *trans*-

configuration of the substituents at C-2 and C-3 lactonisation during acidic work-up does not occur. The resulting lithio salt can be used after thorough drying for the subsequent alkylation without further purification. Thus, treatment of the carboxylate **5** with 11.8 equivalents of methyl lithium, followed by a non-protic work-up with CO_2 provided the protected dideuterated xylulose **6** in 62 % overall yield. First attempts to minimize the amount of dimethylated by-product by quenching the reaction mixture with chlorotrimethylsilane[17] led to considerable decomposition and deprotection of the compound. This difficulty was finally overcome by rapid bubbling of dry CO_2 into the reaction mixture which converted methyl lithium into lithio acetate without liberating the ketone **6** from the dimetallated acetal-type intermediate. The removal of the isopropylidene group was achieved in 73% yield with 2 N HCl using a solvent mixture of H_2O and CH_3CN.[13] The overall yield from commercial **3** to the deuterium labelled sugar **1b** is 37 % and illustrates the high efficiency of the sequence. Moreover, since isopropylidene-protected D- and L-diethyl tartrate are readily available starting materials, the above synthesis not only provides isotopomers of D-**1a** but also labelled xyluloses of the L-series without changing the protocol.

First experiments with **1a** and jasmonic acid pre-treated plants[18] (Lima bean, *Phaseolus lunatus*) resulted in very high incorporation rates of **1a** into volatile mono- sesqui-, and diterpenoid derived volatiles. Details will be given elsewhere.

Experimental

General: Reactions were performed under argon; solvents were dried according to standard methods. For analytical instrumentation and chromatographic procedures see ref. 19. Optical rotations $[\alpha]_D$ were recorded at 25 °C in 1 ml cells, using a Perkin-Elmer 241 polarimeter.

Monomethyl 2,3-*O*-isopropylidene-D-tartrate (4)

A mixture of dimethyl 2,3-*O*-isopropylidene-D-tartrate **3** (2.5 g, 11.4 mmol), PLE (Fluka) (1 mg, 134 U) and 0.1 M sodium phosphate buffer, pH 8, (6 ml) was shaken at rt. The pH was maintained at 8.0 by continuous addition of 0.2 M NaOH from a peristaltic pump controlled by a pH-Stat. After consumption of the calculated amount of NaOH (57 ml, 11.4 mmol; ca. 4 h) the mixture was extracted with Et_2O (2 x 15 ml) to remove the educt. Then, the aq. layer was saturated with NaCl, the pH adjusted to 3.5 by adding 2 N HCl, and the solution was extracted with EtOAc (6 x 30 ml). After each extraction the aq. phase was readjusted to pH 3.5. The combined organic layers were dried (Na_2SO_4). Following removal of the solvent *i.v.* the product was purified by column chromatography (silica gel, petroleum ether/ethyl acetate/formic acid, 30:70:1). Finally, the eluent was washed with brine to remove formic acid. Evaporation of the solvent *i.v.* afforded the half-ester **(4)** as a colorless oil. (1.885 g; 81 %).

Lithio 2,3-*O*-isopropylidene-[4,4-$^2H_2$]-D-threonate (5)

A well-stirred, chilled solution of **4** (195 mg, 0.95 mmol) in dry THF (2 ml) was gradually treated within 10 min with $LiBEt_3D$ in Et_2O (1 M solution, 3.1 ml; 3.1 mmol). Stirring was continued for 1 h at 0°, and water was slowly added until the evolution of hydrogen ceased. More water (10 ml) was added, and the THF was removed *i.v.* Then, the aq. solution was acidified with 2 N HCl to destroy the Bet_3 complexes. Following neutralization with 0.2 N LiOH, water and the volatile BEt_3 were removed *i.v.* Thorough drying at 60 °C under reduced pressure afforded crude **5** as a white solid, consisting of **5** and inorganic salts. The crude solid was used without purification for alkylation. ^1H NMR (D_2O, 400 MHz): δ 1.38 (3H, s), 1.41 (3H, s), 4.12 (1H, d, *J*=8 Hz), 4.16 (1H, d, *J*=8 Hz). ^{13}C NMR (D_2O, 400 MHz): δ 27.7, 28.5, 79.0, 82.1, 113.2, 180.0.

3,4-*O*-Isopropylidene -1-deoxy-[5,5-$^2H_2$]-D -xylulose (6)

To well-stirred, chilled suspension of crude **5** (prepared from 0.195 mg of **4**) in dry THF (15.0 ml) a solution of methyl lithium in ether (5%-soln, 8.15 ml, 12.5 mmol) was added within 15 sec. Stirring was continued for

2 h at this temperature. Then, dry CO_2 was quickly sparkled at rt. through the mixture to destroy any remaining methyl lithium. The resulting thick, milky-white suspension was slowly poured into conc. aq. NH_4Cl (200 ml) while the pH was maintained ≈ 7.0 by occasional addition of 2 N HCl. The solution was saturated with NaCl and extracted with EtOAc (3 x 100 ml). The combined organic layers were dried (Na_2SO_4), the solvent evaporated, and the product was purified by column chromatography on Florisil® using hexane/ethyl acetate (1:1) for elution. Colourless oil (103.6 mg, 62 % overall from **4**). ^1H NMR ($CDCl_3$, 400 MHz): δ 1.43 (3H, s), 1.48 (3H, s), 2.18 (1OH, s), 2.32 (3H, s), 4.09 (1H, d, *J*=7.9 Hz), 4.26 (1H, d, *J*=7.9 Hz). ^{13}C NMR ($CDCl_3$ 100 MHz): δ 26.1, 26.7, 26.8, 77.9, 81.6, 110.7, 209.3. IR (KBr, film) 3472*br.*, 2988, 2937, 2213, 2104, 1716, 1382, 1215, 1091, 975, 879, 812 cm^{-1}. MS, *m/z* 161 ($M^{+\bullet}$, -15)(1), 143(1), 133(15), 115(1), 99(8), 85(7), 75(3), 63(3), 59(74), 47(5), 43(100). $[\alpha]_D^{20}$ -20.7 (c=1.83, CH_3OH). HRMS, *m/z* calcd for $C_7H_9D_2O_4$ ($M^{+\bullet}$-15): 161.0782, found: 161.0782.

1-Deoxy-[5,5-$^2H_2$]- D-xylulose (1b)

A solution of **6** (0.10 g, 0.56 mmol) in a mixture of 2 N HCl, H_2O and CH_3CN (15.0 ml, 3:6:90) was stirred at rt. for 24 h. The mixture was neutralized with 0.2 N NaOH, the solvent removed i.v., and the labelled sugar was purified by column chromatography (SiO_2, ethyl acetate/methanol, 95:5). (56.0 mg, 73 %).
^1H NMR (D_2O, 400 MHz): δ 1.35, 1.39 (anomers), 2.20 (open chain) (total 3H, 3s in the ratio 11:13:76), 3.52-3.78 (2H, m). ^{13}C NMR (D_2O, 100 MHz) δ 215.9, 74.3, 80.0, 28.5. IR (KBr, film) 3384*br.*, 2118, 1716, 1360, 120, 985 cm^{-1}. $[\alpha]_D^{21}$ 34.8 (*c* 1, H_2O) ($[\alpha]_D^{21}$ 34.0 (*c* 1, H_2O)).[2] Anal. calcd. for $C_5H_{10}O_4$: C, 44.77; H, 7.51. found: C,44.75; H,7.50.

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