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Carbohydrates from glycerol: an enzymatic four-step, one-pot synthesis

Rob Schoevaart, Fred van Rantwijk and Roger A. Sheldon*

Laboratory of Organic Chemistry and Catalysis, Delft University of Technology, Julianalaan 136, 2628 BL Delft, The Netherlands. E-mail: r.a.sheldon@tnw.tudelft.nl

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A novel one-pot procedure, involving a cascade of four enzymatic steps, for the synthesis of carbohydrates from glycerol and an aldehyde is reported.

Dihydroxyacetone phosphate (DHAP)-dependent aldolases catalyze the highly stereoselective synthesis of a wide variety of natural and non-natural carbohydrates[1–3] *via* the aldol reaction of DHAP with an aldehyde acceptor. C–C bond formation can produce four different stereoisomers and it is possible, by an appropriate choice of aldolase, to selectively produce any one of the four possible stereoisomers. For example, the fructose-1,6-bisphosphate aldolase[4] (FruA) used in this study produces an aldol adduct with 3*S*,4*R* stereochemistry. Chemical methods for preparing DHAP are circuitous and/or require expensive reagents.[5] Known enzymatic methods require the use of expensive enzymes (kinases) and regeneration of ATP.[6,7] We reasoned that the use of a phosphatase[8,9] as the phosphorylation catalyst would enable the use of inexpensive inorganic pyrophosphate as the phosphate source.

Our synthetic scheme embodies a cascade of four enzymatic steps: kinetically controlled phosphorylation of glycerol by inorganic pyrophosphate, glycerol phosphate oxidase (GPO)-catalysed aerobic oxidation of L-glycerol-3-phosphate to DHAP coupled with catalase mediated decomposition of hydrogen peroxide,[10] aldol reaction of DHAP with an aldehyde acceptor[11] and, finally, enzymatic dephosphorylation of the aldol adduct (see Scheme 1). The key to its success depends on the judicious use of pH control to switch the activities of the various enzymes on and off during the cascade.†

The phosphatase of choice was phytase[12] from *Aspergillus ficuum*, which is a cheap and readily available industrial enzyme. Its production of DL-glycerol-3-phosphate is pH dependent with a very broad optimum. For quick analysis L-glycerol-3-phosphate is detected by oxidation to DHAP and subsequent coupled assay[13] (equal amounts of the D-isomer are assumed to be formed). At pH 2 phosphorylation [glycerol > 10% (v/v)] was substantial and it decreased above pH 4 to become zero at pH 7. The yield of glycerol-3-phosphate (at pH 4) increased with increasing glycerol concentration, presumably because the competing hydrolysis of pyrophosphate and glycerol-3-phosphate is suppressed at low water concentrations. At 95% glycerol 75 mM L-glycerol-3-phosphate (corresponding to the theoretical yield of 50%) was formed according to the assay, after 24 h reaction time. ^{31}P NMR analysis confirmed that under these conditions the conversion of pyrophosphate into DL-glycerol-3-phosphate was 100%. Glycerol-2-phosphate was absent in the reaction mixture, demonstrating that phytase is completely regiospecific.

The concentration of glycerol was adjusted to 55%, because the activity of glycerol phosphate oxidase (GPO) is low in 95% glycerol. Moreover, the pH was increased to pH 7.5, which corresponds with the optimum of GPO and catalase (and that of the aldolase as well) and renders the phytase inactive, thus preventing undesirable hydrolysis. After quantitative oxidation of L-glycerol-3-phosphate to DHAP, fructose-1,6-bisphosphate aldolase (FruA) from *Staphylococcus carnosus* and butanal[14,15] were added to start the aldol addition (78% conversion after 4 h). Lowering the pH to 4 initiated the dephosphorylation of the butanal–DHAP adduct, affording 5-deoxy-5-ethyl-D-xylulose[14] in 57% yield from L-glycerol-3-phosphate. The addition of extra phosphatase for removal of the phosphate group was not necessary since phytase was still present and active.

The combination of four different enzymes and four enzymatic transformations in one pot provides an attractive procedure for performing aldol reactions with DHAP aldolases starting from the cheap, readily available glycerol and pyrophosphate. Combined with the broad substrate specificity of DHAP aldolases towards acceptor substrates, a wide variety of carbohydrates is readily accessible using this method.

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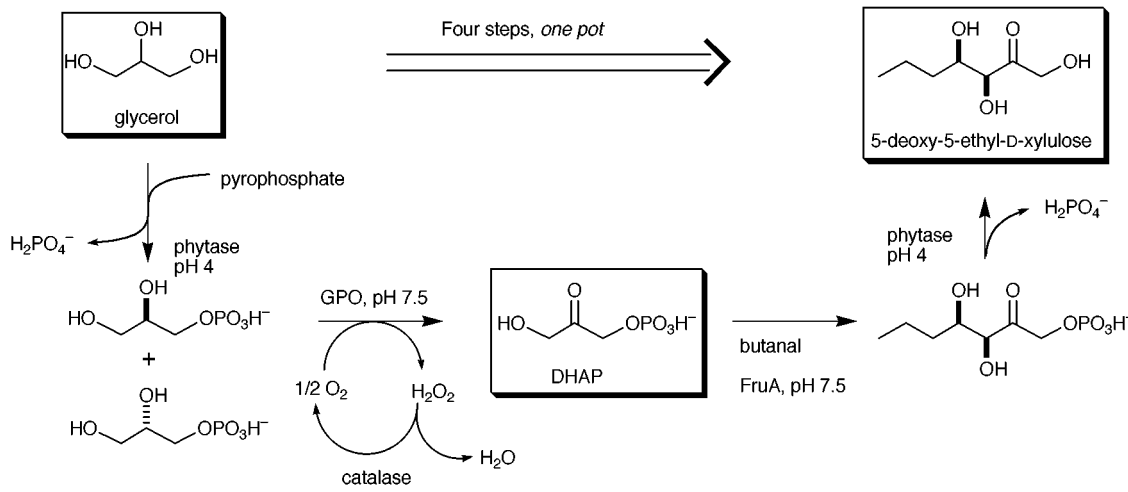

Scheme 1

Notes and references

† Optimal conditions (per ml): phosphorylation was carried out in 95% glycerol with 150 mM pyrophosphate and 1 mg phytase at pH 4.0. After shaking at 37 °C for 24 h the pH was adjusted to 7.5 and the glycerol concentration was reduced to 55% by dilution. GPO (5 units) and catalase (50 units) were added and oxygen was applied for 3 h at room temperature. Then the mixture was shaken for 4 h with FruA (1.25 units) and butanal (100 mM). Lowering the pH to 4, stirring overnight and extraction with EtOAc afforded 5-deoxy-5-ethyl-D-xylulose (57% from L-glycerol-3-phosphate).

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11 Oxidation and aldol reaction can be conducted *in situ*, see ref. 10.
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15 Butanal was used as acceptor because we had already fully characterized the corresponding final product, 5-deoxy-5-ethyl-D-xylulose. (ref. 14).

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