Electronic Supplementary Material (ESI) for Green Chemistry


# Efficient biocatalytic processes for highly valuable terminally phosphorylated C5 to C9 D-ketoses

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## Supplementary Information

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# 1 General remarks

**Material and methods**: Dihydroxyacetone **1**, glycolaldehyde and 1-hydroxybutan-2-one **3** were purchased from Sigma-Aldrich. Hydroxyacetone **2** was purchased from Fluka (purity 90%) and purified by silica gel chromatography. D-fructose-6-phosphate dipotassium salt, D,L-glyceraldehyde 3-phosphate diethyl acetal barium salt and phosphoriboisomerase (RPI) from spinach were purchased from Sigma-Aldrich. FSA was produced as previously reported.[1] A desalting step by gel filtration is necessary to remove glycylglycine (Gly-Gly) buffer. Glycerol dehydrogenase was obtained as described by A.K. Samland[2] and glycolaldehyde phosphate was obtained as described[3].

Nuclear magnetic resonance (NMR) spectra were measured in deuterated solvent ($D_2O$) on a Bruker AC-400 spectrometer, operating at 400 MHz for $^{1}H$ and 100 MHz for $^{13}C$ nuclei. Residual solvent signals were used as internal reference. Chemical shifts (δ) are reported in ppm, coupling constant values (*J*) are given in hertz. Acyclic sugar phosphates were recorded as their barium salt in $D_2O$, adding the minimum amount of 12N HCl solution to fully dissolve the compound. Sugar phosphates able to cyclise were recorded in $D_2O$ after barium to ammonium prior exchange, in order to avoid chemical shifts changes due to pH for easier comparison with literature data.

Optical rotations were measured on a Jasco DIP-370 polarimeter, using a 10 cm quartz cell. Values for $[\alpha]^{T}_{D}$ were obtained with the D-ray of sodium at the indicated temperature T, using solutions of concentration (*c*) in units of g/100 mL. High resolution electrospray ionization mass spectra (ESI-HRMS) were recorded on a micro q-tof Micromass (3000 V) with an internal lock mass ($H_3PO_4$) and an external lock mass (Leu-enkephalin).

# 2 Kinetic measurements

## 2.1 General considerations

In kinetic assays, one unit (U) of FSA is defined as the amount of enzyme able to cleave 1 $\mu$mol of D-fructose-6-phosphate (D-F6P) to afford D-glyceraldehyde-3-phosphate (D-G3P) and dihydroxyacetone (DHA) per minute at pH 8.5 (glycylglycine 50 mM buffer) and 25°C.

In addition one unit (U) of RPI is defined as the amount of enzyme able to transform 1 $\mu$mol of D-ribose-5-phosphate (D-R5P) per minute whereas one unit (U) of RPE is defined as the amount of enzyme able to transform 1 $\mu$mol of D-ribulose-5-phosphate (D-Ru5P) per minute, both at pH 7.5 and 25°C.

## 2.2 FSA kinetic parameters for glycolaldehyde phosphate 4, hydroxyacetone 2 being the donor substrate

The reaction consisted in the formation of 1-deoxy-D-xylulose-5-phosphate **6** and measurement of **2** consumption was done with glycerol dehydrogenase. To a solution of **4** (concentrations from 50 to 300 mM) and 70 mM of **2** in 50 mM Gly-Gly buffer pH 8 at 25 °C, wt-FSA (2 U) was added, final volume being 1 mL. Aliquots were withdrawn at different times (t=0, 5, 10 and 15 minutes) and the remaining amount of **2** was determined using GDH enzyme (10 U) in the presence of NADH (0.7 mM). One mmol of NADH oxidized was equivalent to 1 mmol of remaining **2**. All these experiments were done in triplicate.

## 2.3 FSA deactivation by glycolaldehyde phosphate

FSA deactivation by glycolaldehyde phosphate was evaluated compared to a control consisting of FSA without any substrate. Thus a solution of 100 mM of glycolaldehyde phosphate was incubated with 10 U of FSA in a final volume of 1 mL. Aliquots were withdrawn after 7 and 28 hours and FSA activity was measured using the already published assay (F6P cleavage).[4]

## 2.4 Ribose-5-phosphate isomerase (RPI) and Ribulose-5-phosphate epimerase (RPE) activity detection

Four samples were prepared each containing 500 µL of a solution of **8** (50 mg in 1 mL Gly-Gly buffer pH 8, 50 mM). FSA A129S purified by a 70°C heat treatment during 20 minutes (50 U) was added to the first one whereas the second one was loaded with FSA A129S purified by a 70°C heat treatment during 30 minutes (50 U). The third one was loaded with His-tagged FSA A129S purified by IMAC (50 U). The last one was the control since it didn't contain any FSA.
After 12 hours $^{13}C$ NMR spectra was recorded and analysed.

## 2.5 Ribose-5-phosphate isomerase (RPI) activity estimation[5]

10 mg (50 U) of FSA A129S (purified by a 70°C heat treatment during 20 or 30 minutes) were incubated in 500 µL of $D_2O$ with 5 µL of DMF (internal reference) and 500 µL of a solution of **8** (50 mg in 1 mL of $D_2O$). $^1H$ NMR spectra were recorded upon time and disappearance of **8** was quantified at t=0, 90 and 330 minutes following the decrease of the H-1 signals of the α and β forms, in comparison to the signal of the internal reference. This experiment was duplicated.

### 2.6 Ribulose-5-phosphate epimerase (RPE) activity estimation[5]

The experimental conditions were the same as mentioned above but the solution of **8** was pre-incubated with 5 units of a commercially available phosphoriboisomerase for 20 minutes (till the equilibrium between **8** and **12** was reached). Appearance of **5** was followed by $^1$H NMR upon time at t=0 and 20 minutes following the increase of the H-1 signal in comparison to the signal of the internal reference. This experiment was triplicated. We noted that this signal was detected as a singlet due to a deuterium exchange.

## 3 General procedure 1 for FSA biocatalysed one pot / one step reactions

The reactions were carried out mixing glycolaldehyde phosphate **4** (700 mM solution in water, pH 7.5) or D-ribose-5-phosphate **8** and **1**, **2** or **3** (2 eq.) in a final water volume corresponding to a 100 mM concentration of **4** or 50 mM of **8**. The reactions were initiated upon addition of FSA A129S or wt-FSA partially purified by heat treatment (45 minutes at 70°C) and allowed to gentle stir (100-200 rpm) at room temperature for 24 hours. The final compound was isolated as its barium salt as follows. The reaction mixture was first adjusted to pH 3 with a 1N HCl solution and then to pH 6 with a 1N NaOH solution. The suspension obtained was centrifuged and 2 equivalents of $BaCl_2$, $2H_2O$ were added to the supernatant. 6 volumes of ethanol were poured and the mixture allowed cooling at 4°C for at least 1 hour. The suspension was centrifuged and the precipitate was washed twice with ethanol and acetone, and dried under vacuum. For the reactions achieved with **8** as acceptor substrate, barium counter ion of the final ketose was exchanged to ammonium using a Dowex 50WX8 ammonium form ion exchange resin.

## 4 General procedure 2 for FSA A129S biocatalysed one pot / four steps reaction

The reaction was carried out mixing **4** (700 mM solution in water, pH 7.5) and **1** (2.1 eq.) in a final water volume corresponding to a 50 mM concentration of **4**. The reaction was initiated upon addition of 150 U of FSA A129S partially purified by heat treatment (30 minutes at 70°C) followed by a PD10 desalting column to remove Gly-Gly buffer. The resulting mixture was then allowed to gently stir (100-200 rpm) at room temperature for 60 hours. The final compound was isolated as its barium salt as follows. The reaction mixture was first adjusted to pH 3 with a 1N HCl solution and then to pH 6 with a 1N NaOH solution. The suspension obtained was centrifuged and 2 equivalents of $BaCl_2$, $2H_2O$ were added to the supernatant. 6 volumes of ethanol were poured and the mixture allowed cooling at 4°C for at least 1 hour. The suspension was centrifuged and the precipitate was washed twice with

ethanol and acetone, and dried under vacuum. Barium counter ion from the final ketose was exchanged to ammonium using a Dowex 50WX8 ammonium form ion exchange resin.

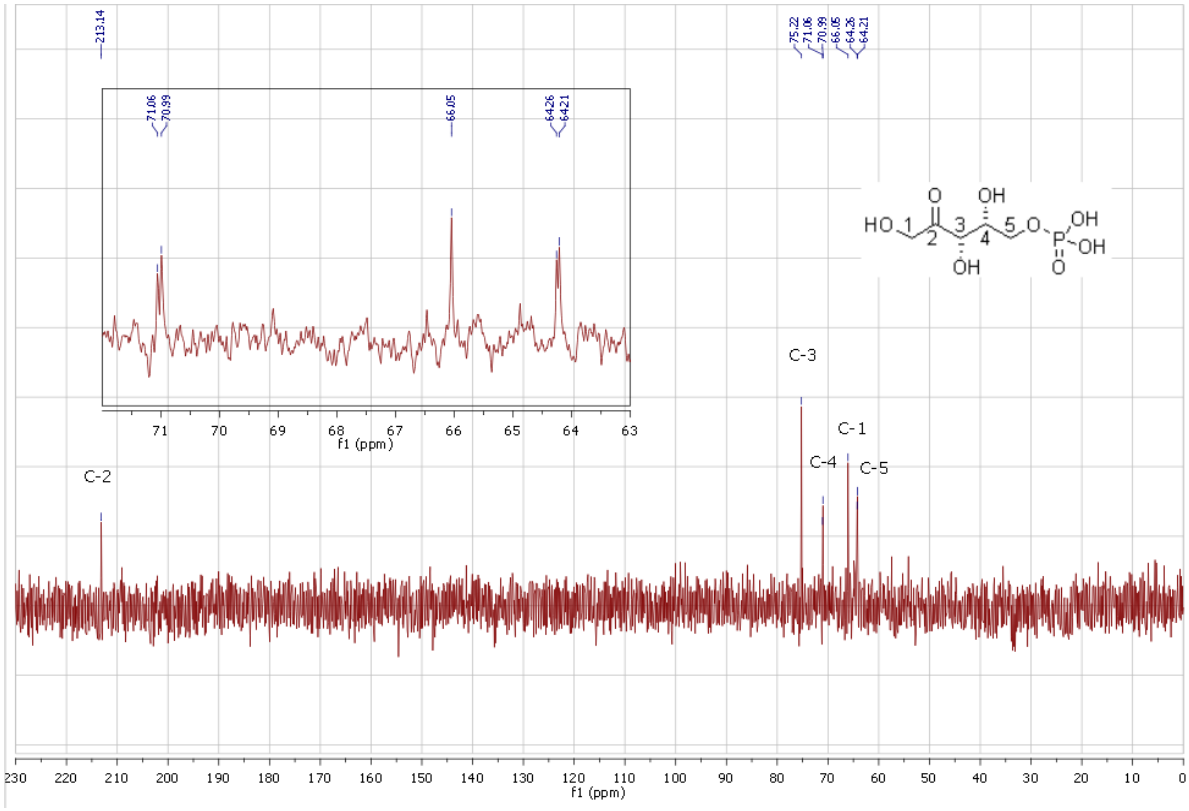
## 5 Synthesis and analytical data

### 5.1 D-xylulose-5-phosphate 5

According to general procedure 1, 400 µL of a 700 mM **4** solution in water was added to 52 mg of **1** in 2.4 mL of water. The reaction was initiated by adding 175U of FSA A129S. After workup and purification, 92 mg of compound **5** was obtained as its barium salt (89% yield).

$^{1}$H NMR (400 MHz, $D_2O$+HCl) δ 4.73 (d, 1H, *J*=19.4 Hz, H-1a), 4.57 (d, 1H, *J*=15.3 Hz, H-1b), 4.59 (d, 1H, *J*=1.8 Hz, H-3), 4.24 (td, 1H, *J*=2.1, 6.4 Hz, H-4), 3.90-3.93 (m, 2H, H-5).

$^{13}$C NMR (100 MHz, $D_2O$+HCl) δ 213.14 (C-2), 75.22 (C-3), 71.03 (d, *J*=6.6 Hz, C-4), 66.05 (C-1), 64.23 (d, *J*=4.8Hz, C-5); $^{1}$H and $^{13}$C NMR were identical to those from the literature[6].




### 5.2 1-deoxy-D-xylulose-5-phosphate 6

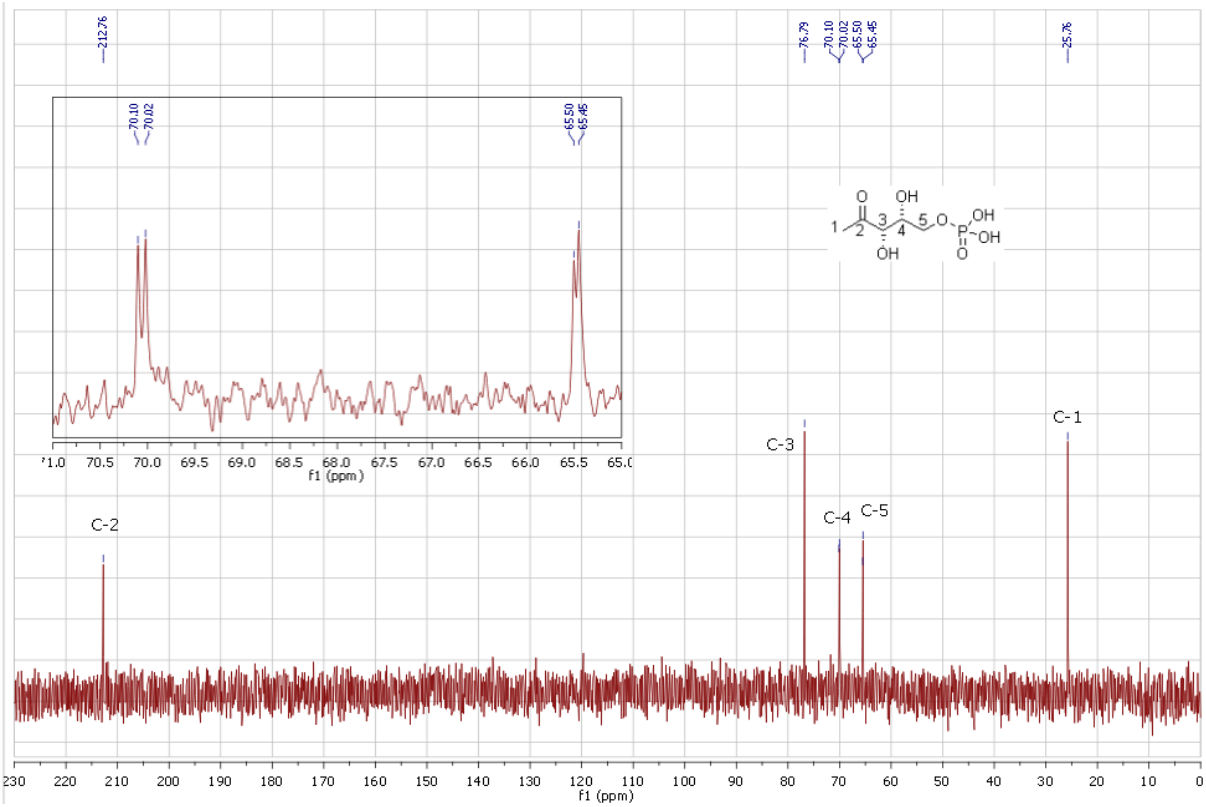
According to general procedure 1, 2.6 mL of a 700 mM **4** solution in water was added to 185 µL of **2** in 15.4 mL of water. The reaction was initiated by adding 80U of wt-FSA. After workup and purification, 385 mg of compound **6** was obtained as its barium salt (85% yield).

$^{1}$H NMR (400 MHz, $D_2O$+HCl) δ 4.22 (d, 1H, *J*=1.8 Hz, H-3), 4.16-4.09 (td, 1H, *J*=1.8, 6.7 Hz, H-4), 3.72 (m, 2H, H-5), 2.05 (s, 3H, H-1).

$^{13}$C NMR (100 MHz, $D_2O$+HCl) δ 212.76 (C-2), 76.79 (C-3), 70.06 (d, *J*=8.0 Hz, C-4), 65.48 (d, *J*=5.2 Hz, C-5), 25.76 (C-1).

HRMS (ESI$^-$); calculated for $[C_5H_{10}O_7P]$: 213.0242, found: 213.0168

$[\alpha]_D^{25}$ = +15.5 (*c* =2, HCl 1N).




## 5.3 (2*S*,3*R*) 2,3-dihydroxy-4-oxohexyl phosphate 7

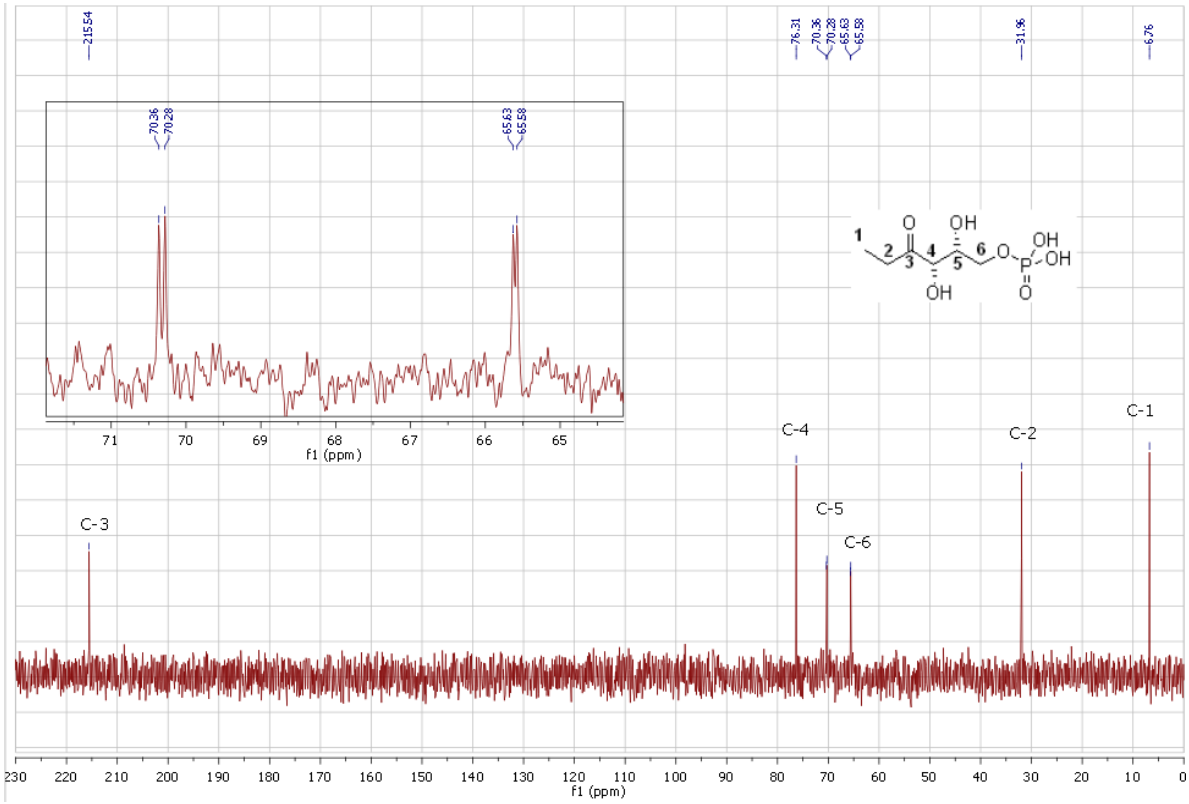
According to general procedure 1, 2.6 mL of a 700 mM **4** solution in water was added to 230 µL of **3** in 15.4 mL of water. The reaction was initiated by adding 80U of wt-FSA. After workup and purification, 336 mg of compound **7** was obtained as its barium salt (77% yield).

$^{1}$H NMR (400 MHz, $D_2O$+HCl) δ 4.34 (d, 1H, *J*=2.0 Hz, H-4), 4.31 (td, 1H, *J*=2.0, 6.9 Hz, H-5), 3.95 (m, 1H, H-6), 2.66 (m, 2H, H-2), 1.03 (t, 3H, *J*=7.2 Hz, H-1).

$^{13}$C NMR (100 MHz, $D_2O$+HCl) δ 215.54 (C-3), 76.31 (C-4), 70.32 (d, *J*=8.2 Hz, C-5), 65.60 (d, *J*=5.0 Hz, C-6), 31.91 (C-2), 6.76 (C-1).

HRMS (ESI$^-$); calculated for $[C_6H_{12}O_7P]$: 227.0399, found: 227.0328

$[\alpha]_D^{25}$ = +14.40 (*c* =2, HCl 1N)




## 5.4 D-*glycero*-D-*altro*-octulose-8-phosphate 9

According to general procedure 1, 50 mg of D-ribose-5-phosphate disodium salt hydrate (**8**) was added to 33 mg of **1** in 3.4 mL of water. The reaction was initiated by adding 150U of FSA A129S. After workup and purification, 58 mg of compound **9** was obtained as its ammonium salt (90% yield).

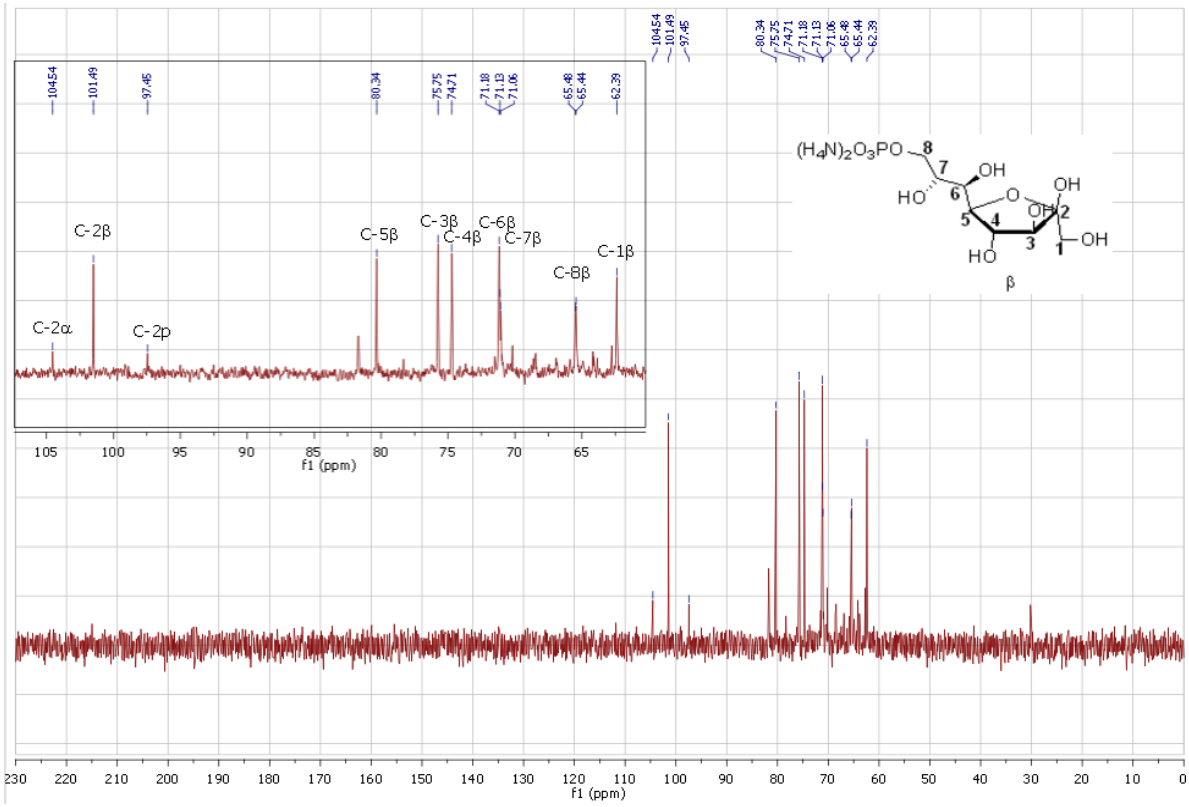
According to general procedure 2, 235 µL of a 700 mM glycolaldehyde phosphate solution in water was added to 32 mg of 1 in 3.4 mL of water. The reaction was initiated by adding 150U of FSA A129S. After workup and purification, 38.5 mg of compound **9** was obtained as its ammonium salt (66% yield).

Due to overlapping and too weak signals for minority forms, only the resonances of the β structure could be fully assigned (α furanose, β furanose and linear forms were respectively noted as α, β and l. Pyranoses forms were noted p).

$^{1}$H NMR (400 MHz, $D_2O$) δ 4.32 (t, 1H, *J*=7.8 Hz, H-4β), 4.05 (d, 1H, *J*=7.9 Hz, 3β), 4.00 (dd, 1H, *J*=3.8, 7.1 Hz, H-5β), 3.93-3.91 (m, 2H, H-8β), 3.87-3.82 (m, 2H, H-6β, H–7β), 3.54 (d, 1H, *J*=12.2 Hz, H-1aβ), 3.51 (d, 1H , *J*=12.1Hz, H-1bβ).

$^{13}$C NMR (100 MHz, $D_2O$) δ 104.54 (C-2α), 101.49 (C-2β), 97.45 (C-2p), 80.34 (C-5β), 75.75 (C-3β), 74.71 (C-4β), 71.18 (C-6β), 71.09 (d, *J*=6.9Hz, C-7β), 65.46 (d, *J*=4.8Hz, 8β), 62.39 (C-1β). $^{13}$C NMR spectrum was identical to that from the literature[7].

HRMS (ESI$^-$); calculated for [$C_8H_{17}O_{11}$P-H]: 319.0430, found: 319.0416




## 5.5 1-deoxy-D-*glycero*-D-*altro*-octulose-8-phosphate 10

According to general procedure 1, 50 mg of **8** was added to 25 µL of **2** in 3.4 mL of water. The reaction was initiated by adding 90U of wt-FSA. After workup and purification, 47 mg of compound **10** was obtained as its ammonium salt (77% yield).

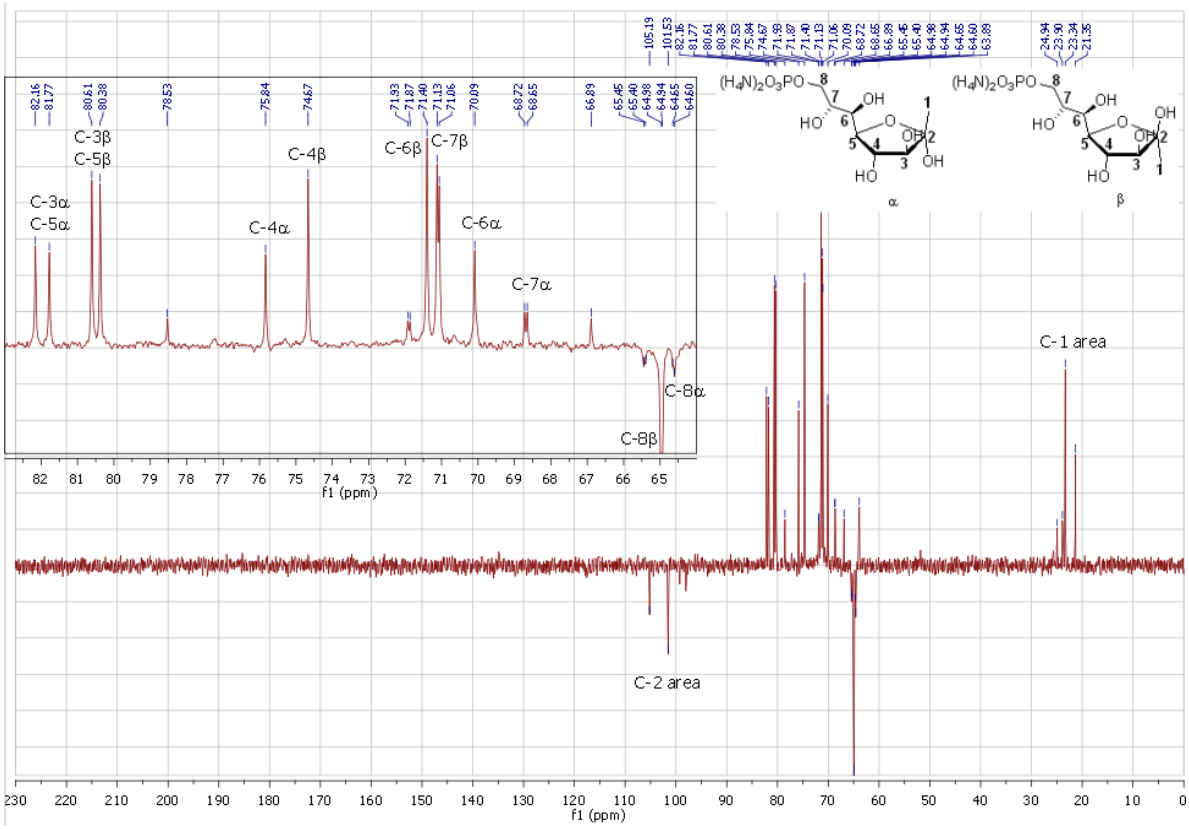
Due to overlapping and too weak signals for minority forms (two pyranose forms), only the resonances of α and β structures could be fully assigned (α furanose, β furanose were respectively noted as α, β. Pyranoses forms were noted p).

$^{1}$H NMR (400 MHz, $D_2O$) δ 4.31-3.62 (m, 7H, H-3, H-4, H-5, H-6, H-7, H-8 (pα, pβ, α, β)), 1.46 (s, 3H, H-1pα or H-1pβ), 1.44 (s, 3H, H-1β), 1.40 (s, 3H, H–1α), 1.35 (s, 3H, H-1pβ or pα).

$^{13}$C NMR (100 MHz, $D_2O$) δ 105.19 (C-2α), 101.53 (C-2β), 99.23 (C-2p), 98.03 (C-2p), 82.15 (C-3α or C-5α), 81.77 (C-3α or C-5α), 80.60 (C-3β or C-5β), 80.37 (C-3β or C-5β), 75.83 (C-4α), 74.66 (C-4β), 71.40 (C-6β), 71.07 (d, *J*=6.2Hz, C-7β), 70.08 (C-6α), 68.67 (d, *J*=6.9Hz, C-7α), 64.96 (d, *J*=4.2Hz, C-8β), 64.62 (d, *J*=4.6Hz, C-8α), 24.93 (C-1pα or pβ), 23.90 (C-1pβ or pα), 23.33 (C-1β), 21.35 (C-1α).

HRMS (ESI$^-$); calculated for [$C_8H_{17}O_{10}P$-H]: 303.0481, found: 303.0492

$[\alpha]_D^{25}$ = -6.5 (*c* =2.2, HCl 0.1N)




## 5.6 (2*R*,3*R*,4*R*,5*R*,6*S*)-2,3,4,5,6-pentahydroxy-7-oxononyl phosphate 11

According to general procedure 1, 50 mg of **8** was added to 32.6 µL of **3** in 3.4 mL of water. The reaction was initiated by adding 90U of wt-FSA. After workup and purification, 50.5 mg of compound **11** was obtained as its ammonium salt (79% yield).

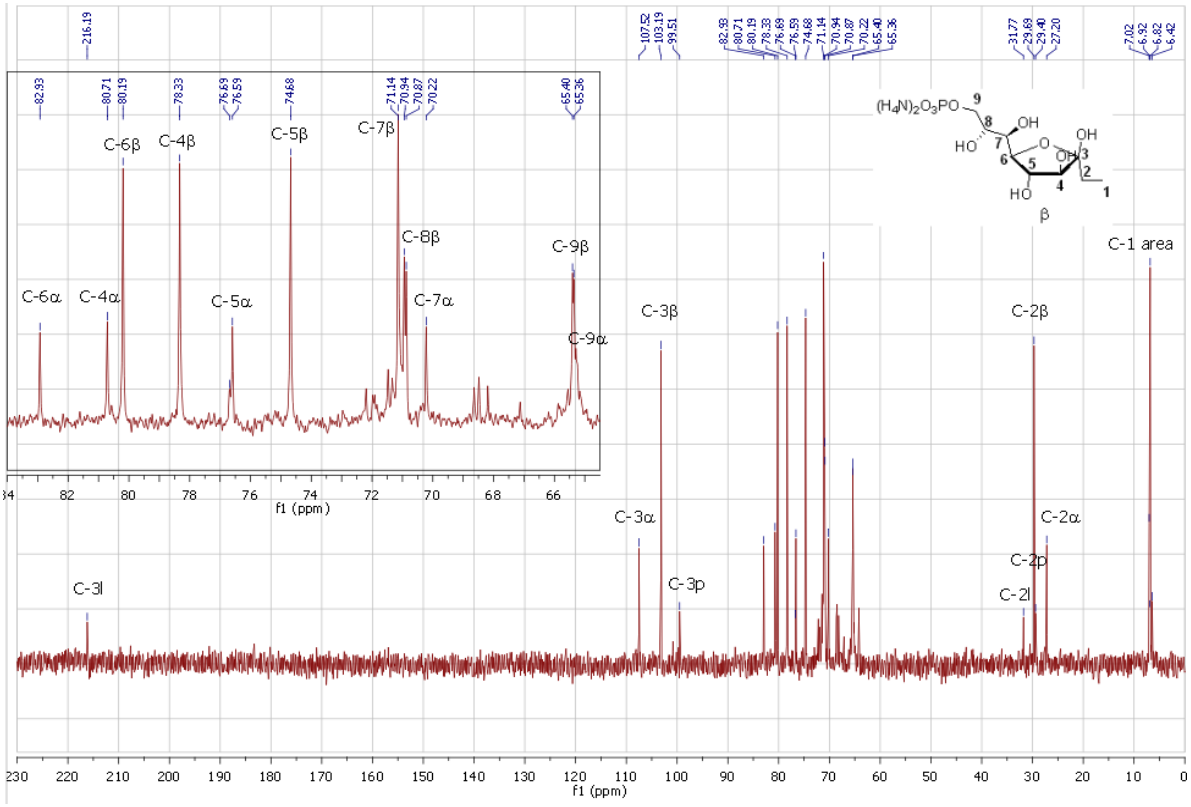
Due to overlapping and too weak signals for minority forms, only the resonances of β structure could be totally assigned (α furanose, β furanose were respectively noted as α, β. Pyranoses and linear forms were noted p and l respectively).

$^{1}$H NMR (400 MHz, $D_2O$) δ 3.80-4.31 (m, 7H, H-4, H-5, H-6, H-7, H-8, H-9 (l, p, α, β)), 1.69-1.72 (m, 2H, H-2 (l, p, α, β)), 0.88-1.00 (m, 3H, H-1 (l, p, α, β)).

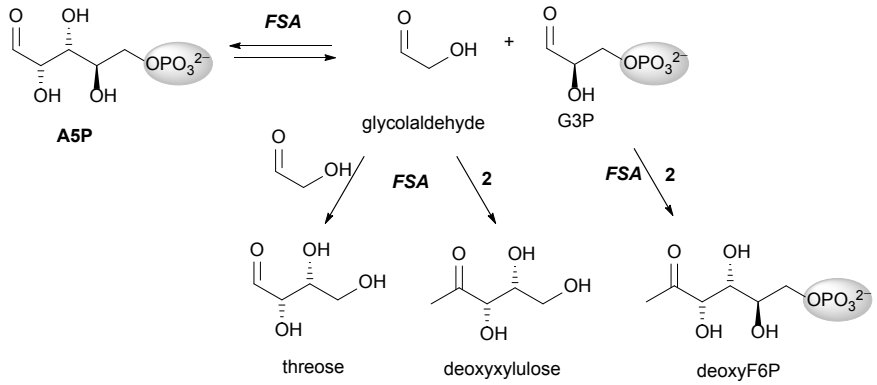
$^{13}$C NMR (100 MHz, $D_2O$) δ 216.19 (C-3l), 107.52 (C-3α), 103.19 (C-3β), 99.51 (C-3p), 82.93 (C-6α), 80.71 (C-4α), 80.19 (C-6β), 78.33 (C-4β), 76.59 (C-5α), 74.68 (C-5β), 71.14 (C-7β), 70.90 (d, *J*=6.6 Hz, C-8β), 70.22 (C-7α), 65.38 (d, *J*=4.5 Hz, C-9β), 65.27 (d, *J*=4.4 Hz, C-9α), 31.77 (C-2l), 29.69 (C-2β), 29.40 (C-2p), 27.20 (C-2α), 7.02 (C-1α), 6.92 (C-1l), 6.82 (C-1β), 6.42 (C-1p).

HRMS (ESI$^-$); calculated for $[C_9H_{19}O_{10}P\text{-}H]$: 317.0638, found: 317.0629

$[\alpha]_D^{25}$ = -51.2 (*c* =2, HCl 0.1N)




## 6 Scheme illustrating arabinose-5-phosphate conversion by FSA wild-type




Arabinose-5-phosphate (A5P), C-2 epimer of **8**, was not acceptor substrate of FSA wild-type. Indeed when using 1 equivalent of A5P per equivalent of **2**, the tlc analysis of reaction mixture aliquots between 15 min to 24h invariably showed formation of deoxyfructose-6-phosphate (dF6P) and deoxyxylulose along with D-threose. This was due to the ability of FSA to cleave A5P into D-G3P and glycolaldehyde via a retroaldol reaction. Thereby dF6P resulted from the aldol addition of **2** to D-G3P, whereas glycolaldehyde either reacted in a self-aldolisation reaction to D-threose or reacted as acceptor with **2** as donor to furnish deoxyxylulose.

## 7 References

[1] J. A. Castillo, C. Guérard-Hélaine, M. Gutiérrez, X. Garrabou, M. Sancelme, M. Schürmann, T. Inoue, V. Hélaine, F. Charmantray, T. Gefflaut, L. Hecquet, J. Joglar, P. Clapés, G. A. Sprenger and M. Lemaire, *Adv. Synth. Catal.* 2010, **352**, 1039-1046.
[2] S. Schneider, M. Gutierrez, T. Sandalova, G. Schneider, P. Clapès, G. A. Sprenger and A. K. Samland *ChemBioChem*, 2010**, 11**, 5, 681-690.
[3] M. Awada and P. C. Dedon, *Chem. Res. Toxicol.* 2001, **14**, 1247-1253.
[4] M. Schurmann, G.A. Sprenger *J. Biol Chem.,* 2001, **14**, 11055-61
[5] T. Wood, *Anal. Biochem.* 1970, **33**, 297-306.
[6] F. T. Zimmermann, A. Schneider, U. Schörken, G. A. Sprenger and W.-D. Fessner, *Tetrahedron: Asymmetry*, 1999, **10**, 1643-1646.
[7] M. Kapuscinski, F. P. Franke, I. Flanigan, J. K. MacLeod and J. F. Williams, *Carb. Res.*, 1985, **140**, 69-79.