Electronic Supplementary Information (ESI)

One-pot, two-step cascade synthesis of naturally rare L-*erythro* (3*S*,4*S*) ketoses by coupling thermostable transaminase and transketolase

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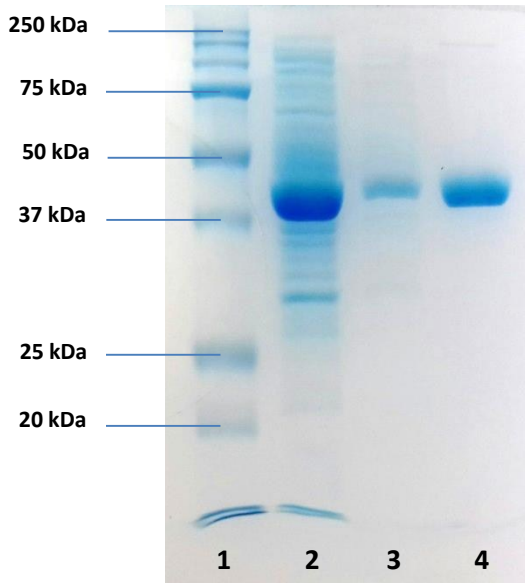
Sequence of serine-glyoxylate L-α-transaminase from *Thermosinus carboxydivoran*s (TA_{tca})

MLQKPYLMVPGPTAVPERVLQAMHRPVINHRGPQYEALFRDVSDRLKTVFKTKQDVLTYPAAGTGMMEAAVVNILSPGDHV
LVVSIGVFGDRFAEIAAKFGAVVEKLDFAWGEAAAPRVLAERLAGDKEGRIKAVFLTHNETSTGVTNDVQALAAACKGHPALVV
VDAVSSLGAMDLAMDEWGLDVVITGSQKALMLPPGLGFMALSERAWAACAQSTMPKFYWDAQAVKKALAKGQNPYTPPV
SLLFGLAEALRLIEEEGLDNIFARHRTLRAALRAGVRAMGLGLLADDKVASPGVTAVLPPTGIEAKKIQKTMRERFGITLAGGQKK
LENQIFRIGHLGYVAQTDILVTLAALEMTLALLGHKVELGAGVRAAQEILMEG

SDS-PAGE analysis of the wild-type TA_{tca}



SDS-PAGE analysis of the wild-type TA_{tca}. Lane **1**: All Blue Precision Plus ProteinTM Standards (BioRad), lane **2**: crude extract wild-type TA_{tca} (40 µg), lane **3**: crude extract wild-type TA_{tca} (5 µg), lane **4**: wild-type TA_{tca} purified by IMAC (5 µg).

Kinetic parameters relative to TK cofactors, thiamine diphosphate (ThDP) and $MgCl_2$ for TK_{gst} and other TK sources

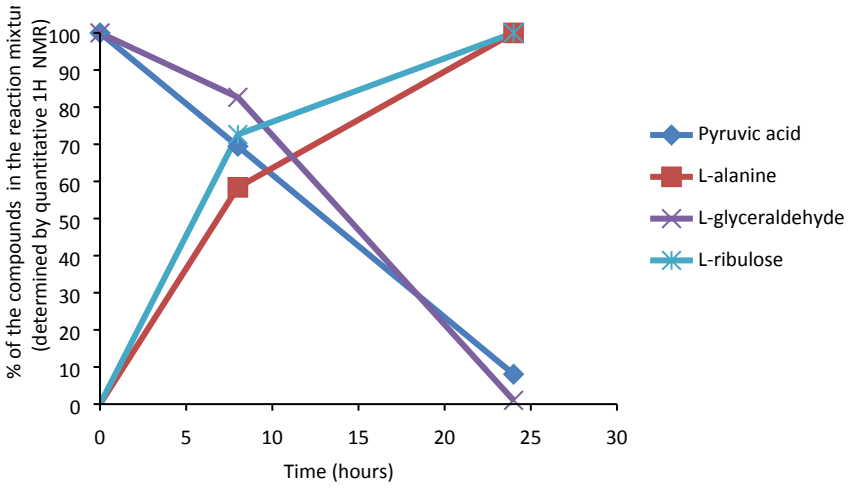
	K_M (µM)			V_{max} ($U.mg^{-1}$)		V_{max}/K_M ($U.mg^{-1}.\mu M$)
Cofactors	TK_{gst}	TK_{sce}	TK_{human}	TK_{gst}	TK_{sce}	TK_{gst}
ThDP[a]	1.8±0.0002	0.6±0.1[c]	0.074[e]	22.7±2.567	-	12.6
$MgCl_2$[b]	1.4±0.002	400[d]		25.2±4.639	-	18

[a][Mg^{2+}] = 1 mM ; [b][ThDP] = 0.1 mM ; [c] C.Wikner U. Nilsson, L.Meshalkina, C. Udekwu, Y. Lindqvist and G. Schneider, Biochemistry, 1997, **36**, 15643-15649 ; [d] Y. Kobori, D. C. Myles and G. M. Whitesides, *J. Org. Chem.,* 1992, **57**, 5899-5907; [e]L. E. Meshalkina, , O. N. Solovjeva, Y. A. Khodak, V. L. Drutsa, G. A. Kochetov, *Biochemistry, .* 2010, **75**, 873–880.

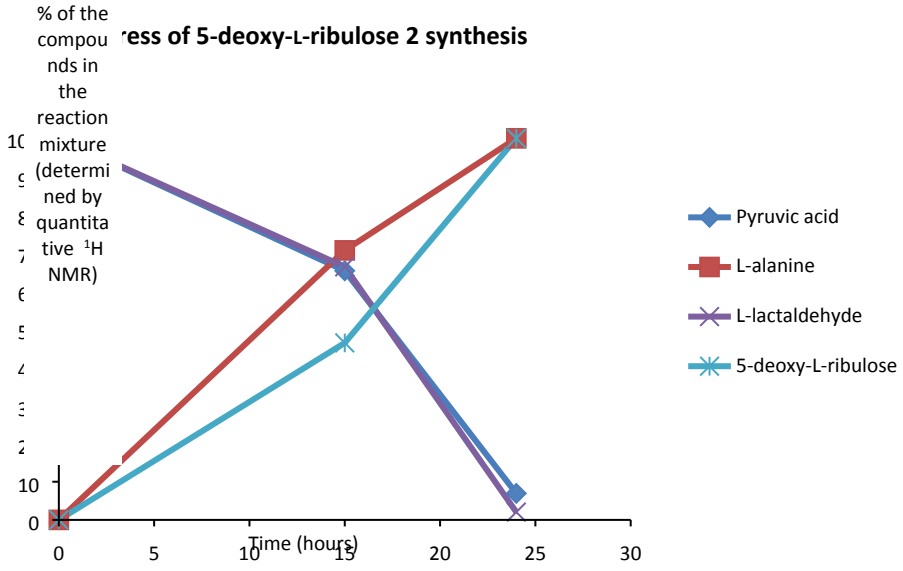
Progress of preparative scale enzymatic synthesis by *in situ* ^{1}H NMR measurements

Reactions were monitored by using quantitative *in situ* ^{1}H NMR relative to 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP-d4) as internal standard**.** Aliquots of reaction mixtures (450 µL) were mixed with 50 µL of TSP-d4 (50 mM, 8.5 mg.mL^{-1} of D_2O).

- **Progress of L-ribulose 1 synthesis**

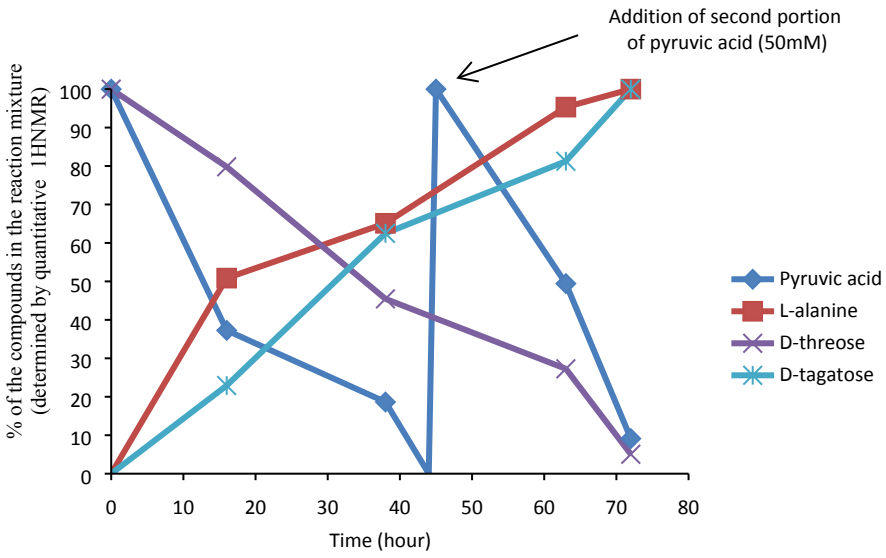


Progress of preparative scale enzymatic synthesis of L-ribulose 1. Reaction conditions: ThDP (0.1 mM), $MgCl_2{\cdot}6H_2O$ (1 mM), PLP (0.2 mM) and pyruvic acid (50 mM) were dissolved in H_2O and the pH was adjusted to 7 with 0.1 M NaOH. To this stirred solution was added TK_{gst} (6 mg) and TA_{tca} (4.8 mg) and the mixture was stirred for 20 min at 60°C. In another flask, L-glyceraldehyde and L-serine (150 mM) were mixed and the pH adjusted to 7 with 0.1 M NaOH. After preincubation (20 min.) of enzymes, cofactors and pyruvic acid, L-serine and L-glyceraldehyde were added and the mixture was stirred at 60°C. The final volume was 20 mL. The pH was maintained at 7 by adding 0.1M HCl using a pH stat (Radiometer Analytical).



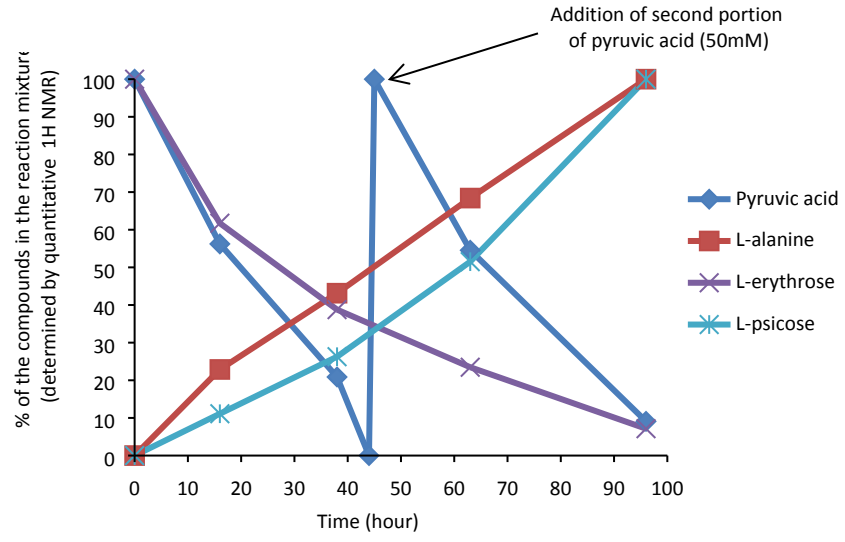
Progress of preparative scale enzymatic synthesis of 5-deoxy-L-ribulose 2. Reaction conditions: ThDP (0.1 mM), $MgCl_2{\cdot}6H_2O$ (1 mM), PLP (0.2 mM) and pyruvic acid (50 mM) were dissolved in H_2O and the pH was adjusted to 7 with 0.1 M NaOH. To this stirred solution was added TK_{gst} (6 mg) and TA_{tca} (4.8 mg) and the mixture was stirred for 20 min at 60°C. In another flask, L-lactaldehyde (50 mM) and L-serine (150 mM) were mixed and the pH adjusted to 7 with 0.1 M NaOH. After preincubation (20 min.) of enzymes, cofactors and pyruvic acid, L-serine and L-lactaldehyde were added and the mixture was stirred at 60°C. The pH was maintained at 7 by adding 0.1M HCl using a pH stat (Radiometer Analytical).

● **Progress of D-tagatose 3 synthesis**



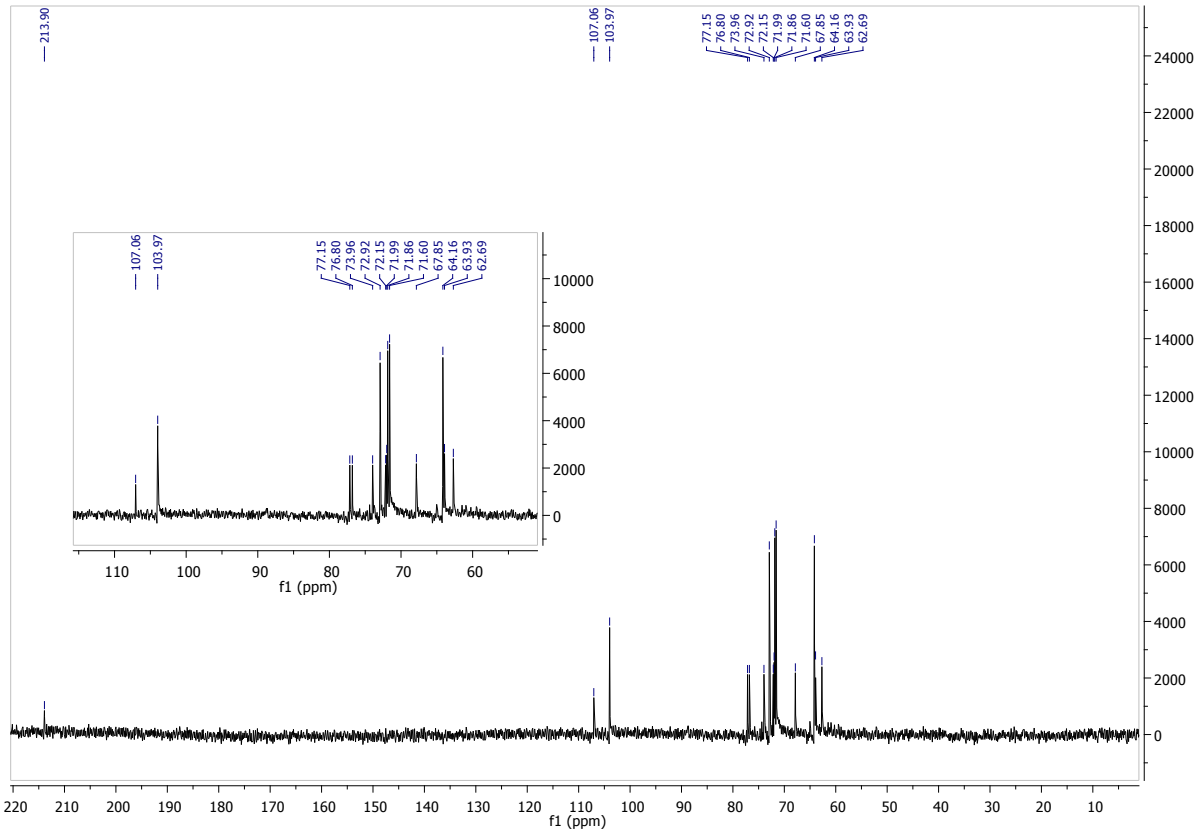
Progress of preparative scale enzymatic synthesis of D-tagatose 3. Reaction conditions: ThDP (0.1 mM), $MgCl_2 \cdot 6H_2O$ (1 mM), PLP (0.2 mM) and pyruvic acid (50 mM) were dissolved in H_2O and the pH was adjusted to 7 with 0.1 M NaOH. To this stirred solution was added TK_{gst} (10 mg) and TA_{tca} (4.8 mg) and the mixture was stirred for 20 min at 60°C. In another flask, D-threose and L-serine (150 mM) were mixed and the pH adjusted to 7 with 0.1 M NaOH. After preincubation (20 min.) of enzymes, cofactors and pyruvic acid, L-serine and D-threose were added and the mixture was stirred at 60°C. The final volume was 20 mL. The pH was maintained at 7 by adding 0.1M HCl using a pH stat (Radiometer Analytical). After complete disappearance of pyruvic acid (48h), a second portion (50 mM) was added at the reaction mixture.

● **Progress of L-psicose 4 synthesis**



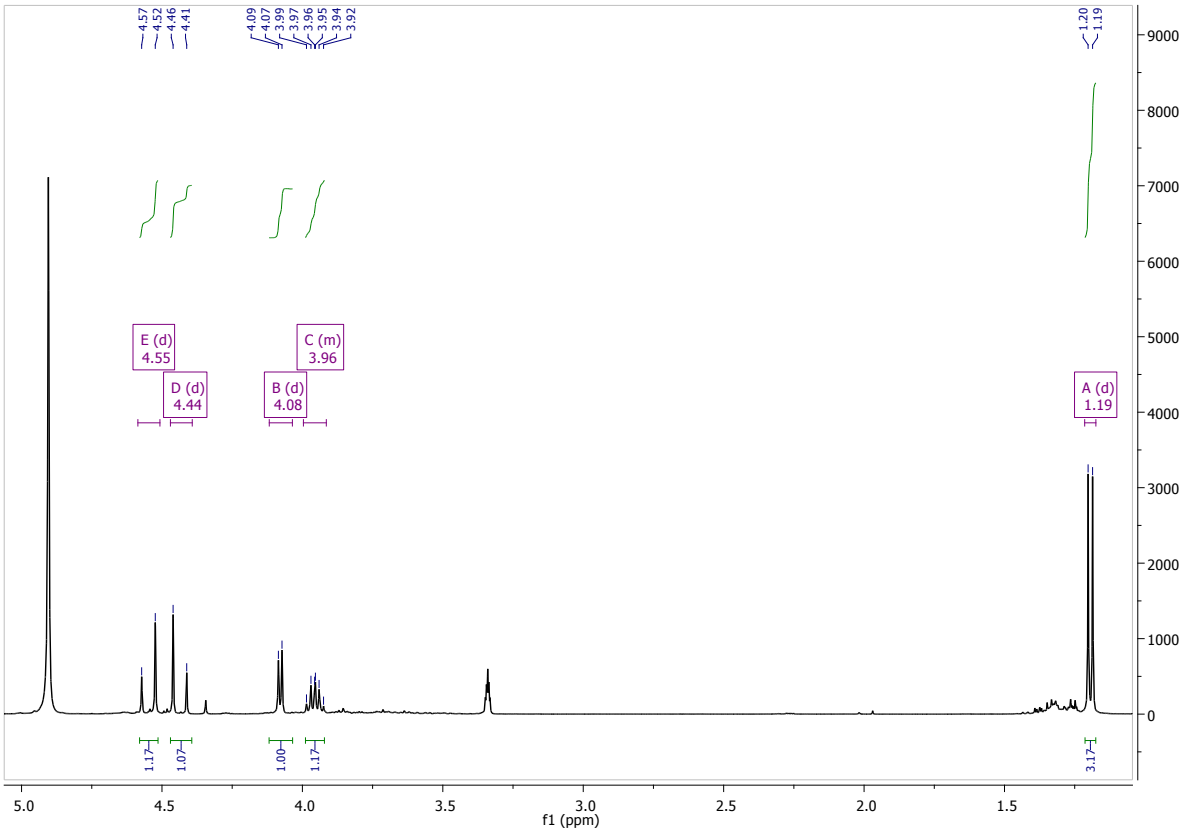
Progress of preparative scale enzymatic synthesis of L-psicose 4. Reaction conditions: ThDP (0.1 mM), $MgCl_2 \cdot 6H_2O$ (1 mM), PLP (0.2 mM) and pyruvic acid (50 mM) were dissolved in H_2O and the pH was adjusted to 7 with 0.1 M NaOH. To this stirred solution was added TK_{gst} (10 mg) and TA_{tca} (4.8 mg) and the mixture was stirred for 20 min at 60°C. In another flask, L-erythrose and L-serine (150 mM) were mixed and the pH adjusted to 7 with 0.1 M NaOH. After preincubation (20 min.) of enzymes, cofactors and pyruvic acid, L-serine and L-erythrose were added and the mixture was stirred at 60°C. The final volume was 20 mL. The pH was maintained at 7 by adding 0.1M HCl using a pH stat (Radiometer Analytical). After complete disappearance of pyruvic acid (48h), a second portion (50 mM) was added at the reaction mixture.

^{13}C NMR spectra of (3*S*,4*S*)-1,3,4,5-tetrahydroxypentan-2-one (L-ribulose) 1



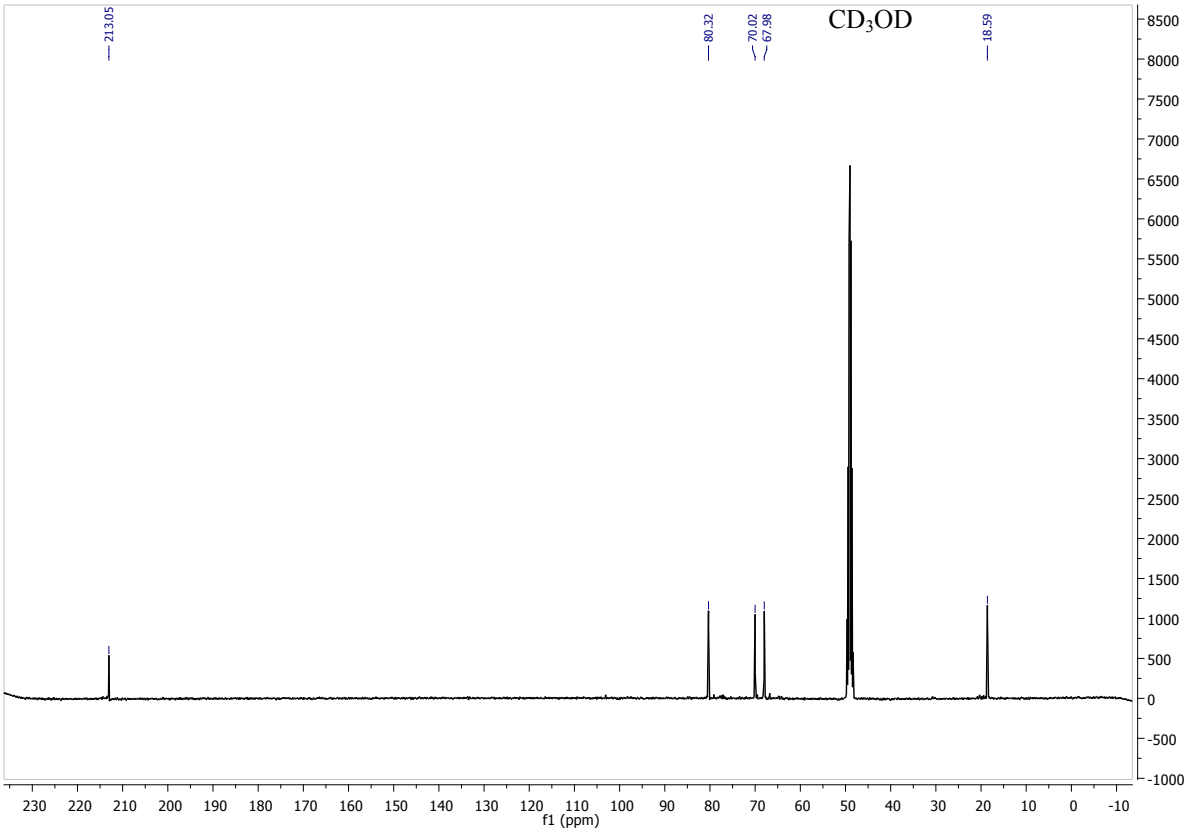
^{13}C NMR spectrum for L-ribulose equilibrated in D_2O at 20°C

^{1}H NMR spectra of (3*S*,4*S*)-1,3,4-trihydroxypentan-2-one (5-deoxy- L-ribulose) 2



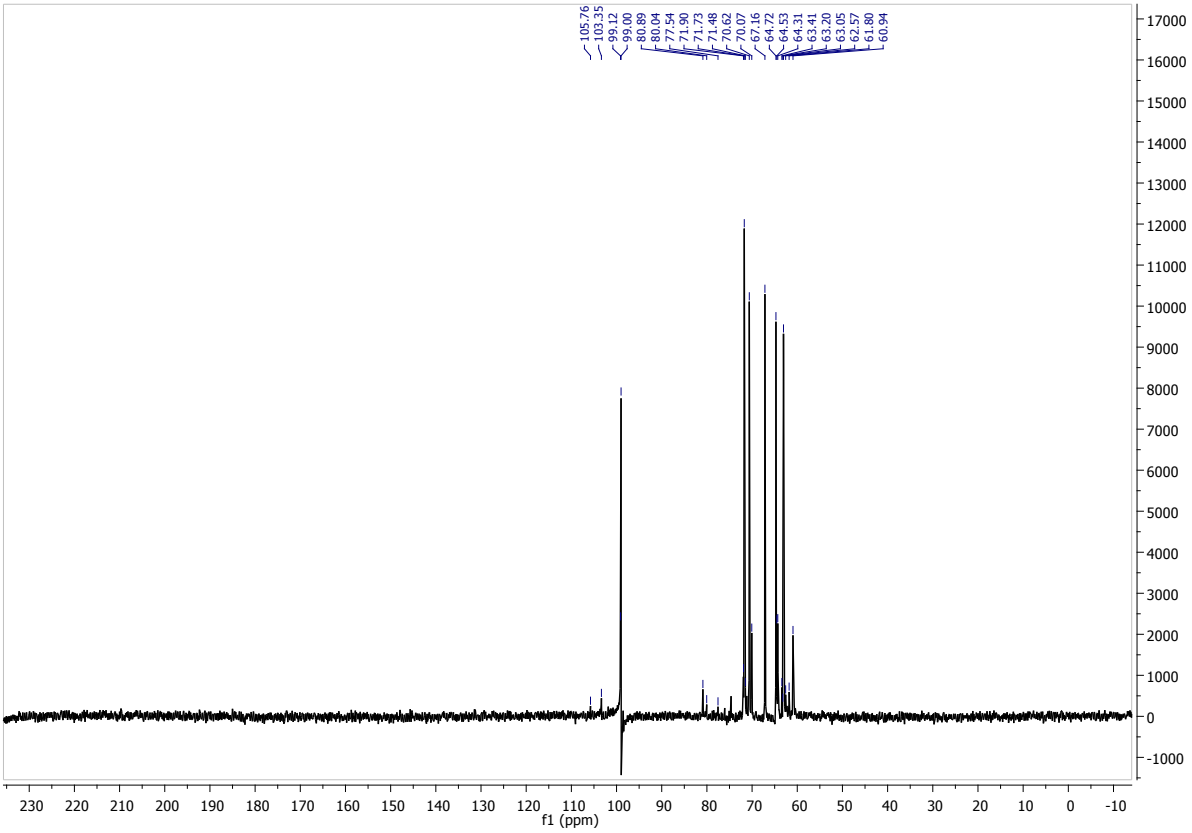
^{1}H NMR spectrum for 5-deoxy-L-ribulose equilibrated in CD_3OD at 20°C

^{13}C NMR spectra of (3*S*,4*S*)-1,3,4-trihydroxypentan-2-one (5-deoxy-L-ribulose) 2



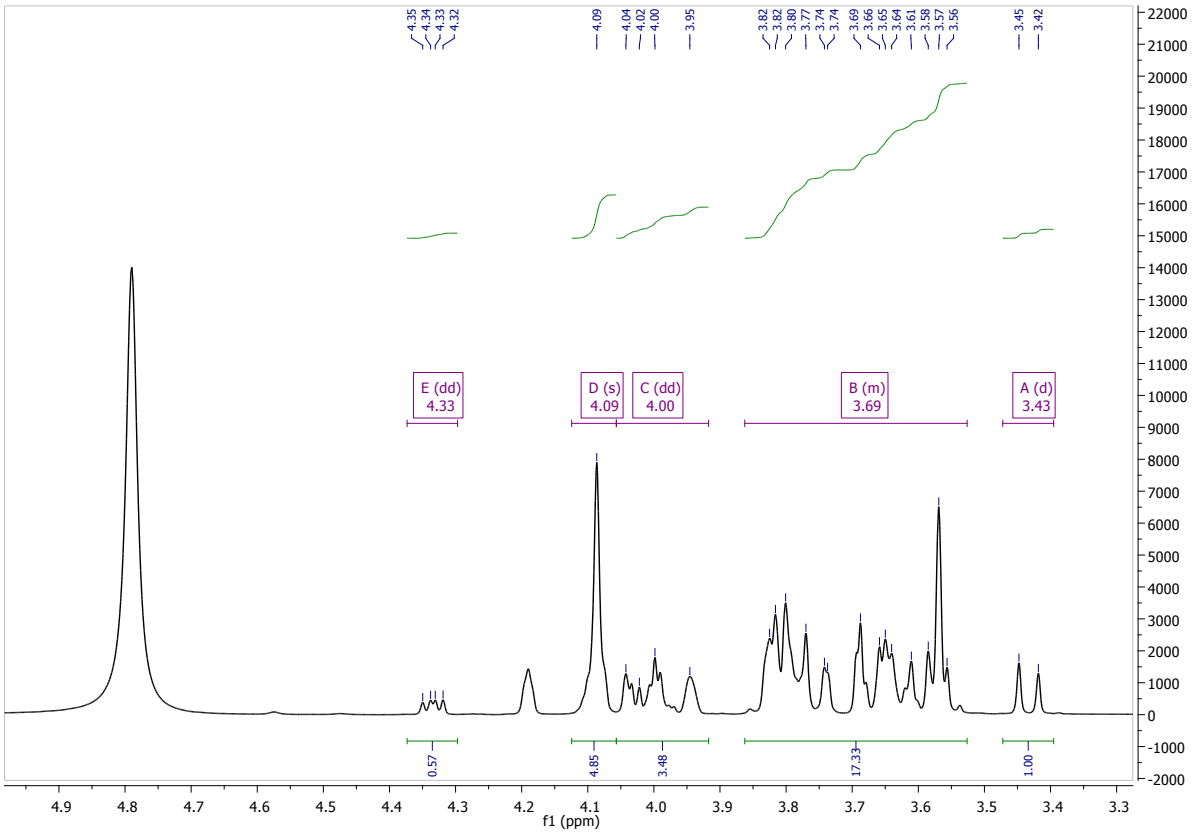
^{13}C NMR spectrum for 5-deoxy-L-ribulose equilibrated in CD_3OD at 20°C

^{13}C NMR spectra of (3*S*,4*S*,5*R*)-1,3,4,5,6-pentahydroxyhexan-2-one (D-tagatose) 3



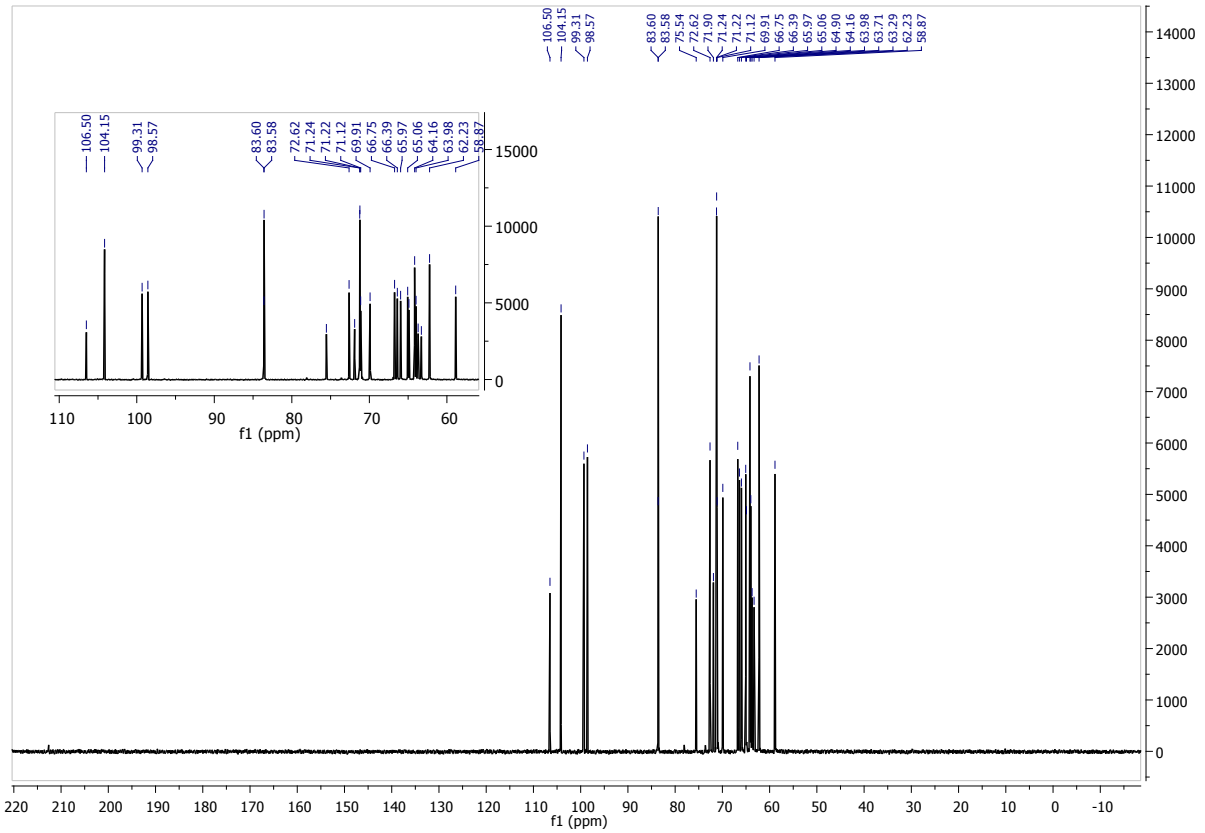
^{13}C NMR spectrum for D-tagatose equilibrated in D_2O at 20°C

^{1}H NMR spectra of (3*S*,4*S*,5*S*)-1,3,4,5,6-pentahydroxyhexan-2-one (L-psicose) 4



^{1}H NMR spectrum for L-psicose equilibrated in D_2O at 20°C

^{13}C NMR spectra of (3*S*,4*S*,5*S*)-1,3,4,5,6-pentahydroxyhexan-2-one (L-psicose) 4

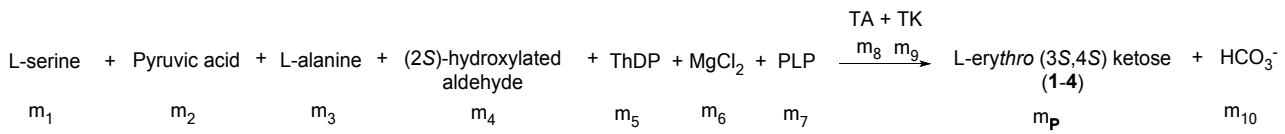


^{13}C NMR spectrum for L-psicose equilibrated in D_2O at 20°C

Calculation of *E* factors

$$E_{factor} = m_{waste} / m_{product}$$

- **cascade process with *in situ* HPA generation catalyzed by TA coupled with TK :**



$$E_{cascade} = m_{waste}\,[(m_1+m_2+m_3+m_4+m_5+m_6+m_7+m_8+m_9+m_{10}) - m_P] / m_{product}\,(m_P)$$

- Product **1** :

m_{waste} (g) = [(0.31+ 0.11 + 0.089 + 0.09 + 0.00092 + 0.004 + 0.001 + 0.0048 + 0.006 + 0.061) – 0.084] = 0.592

$m_{product1}$ (g) = 0.084

$E_{product\ 1}$ = 7.04

- Product **2** :

m_{waste} (g) = [(0.31+ 0.11 + 0.089 + 0.09 + 0.00092 + 0.0040 + 0.0010+ 0.0048 + 0.006 + 0.061) – 0.131] = 0.545

$m_{product\ 2}$ (g) = 0.131

$E_{product\ 2}$ = 4.16

- Product **3** :

m_{waste} (g) = [(0.31+ 0.22 + 0.089 + 0.09 + 0.00092 + 0.0040 + 0.0010+ 0.0048 + 0.010 + 0.061) – 0.093] = 0.697

$m_{product\ 3}$ (g) = 0.093

$E_{product\ 3}$ = 7.49

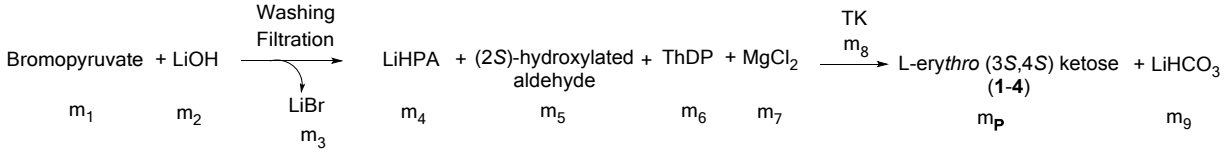
- Product **4** :

m_{waste} (g) = [(0.31+ 0.22 + 0.089 + 0.09 + 0.00092 + 0.0040 + 0.0010+ 0.0048 + 0.010 + 0.061) – 0.092] = 0.698

$m_{product\ 4}$ (g) = 0.092

$E_{product\ 4}$ = 7.58

- **sequential process with chemical Li-HPA (obtained from bromopyruvate) used as TK donor**



$$E_{sequential} = m_{waste}\,[(m_1+m_2+m_3+m_4+m_5+m_6+m_7+m_8+m_9) - m_P] \,/\, m_{product}\,(m_P)$$

- Product **1** :

m_{waste} (g) = [(0.4 + 0.24 + 0.2 + 0.132 + 0.09 + 0.022 + 0.0365 + 0,005 + 0.073) – 0.016] = 1,182

$m_{product\mathbf{1}}$ (g) = 0.016

$\boldsymbol{E_{product\ 1}}$ **= 73.87**

- Product **2** :

m_{waste} (g) = [(0.4 + 0.24 + 0.2 + 0.132 + 0.09+ + 0.022 + + 0.0365 + + 0,005 + 0.073) – 0.022] = 1,176

$m_{product}$ 2 (g) = 0.022

$\boldsymbol{E_{product\ 2}}$ **= 53.45**