Supplementary Information:

Mechanistic divergence of two closely related aldol-like enzyme-catalysed reactions

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Experimental for the preparation of the aldose phosphate substrate analogues

Synthesis of D-threose 4-phosphate from D-diethyltartrate

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D-Diethyl tartrate (8.6mL, 0.05 mol) was dissolved in dry CH₂Cl₂ (100mL) and BnBr (17.82mL, 150mmol), Ag₂O (24.7 g, 106mmol), and KI (1.66mg, 10mmol) were added. The reaction mixture was refluxed under argon for 1 hour and filtered over a celite pad. The filtrate was then washed with water, dried and the solvent was removed in vacuo. The residual oil was purified by flash chromatography on silica gel to give the title compound as a colorless oil (18.9g, 97%).

Rf (hexane:EtOAc 1:1) = 0.89.

¹H NMR (400 MHz, CDCl₃) δ 7.3 (m, 10H), 4.89 (d, J = 9.6 Hz, 2H), 4.48 (d, J = 9.6 Hz, 2H), 4.42 (s, 2H), 4.23 (dt, J = 8.5, 5.7 Hz, 2H), 4.10 (dt, J = 8.5, 5.7 Hz, 2H), 1.21 (t, J = 5.7 Hz, 6H) ppm. ¹³C NMR (125 MHz, CDCl₃) δ 14.1, 61.3, 73.2, 78.4, 128.0, 128.3, 128.4, 137.0, 169.2 ppm.

Synthesis of (2R,3R)-2,3-bis(phenylmethoxy)-1,4-butanediol

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LiAlH₄, Et₂O, reflux

76%
Lithium aluminium hydride (LAH, 0.83g, 21.85mmol) was suspended in dry ether (20mL) and added dropwise over 1 hour to dibenzyl-D-diethyl (2S,3S)-bis(benzyloxy)tartrate (3.95g, 0.3mmol) dissolved in dry ether (20mL) under N₂ at 0°C. The reaction mixture was then refluxed for 4 hours. Excess LAH was destroyed by sequential addition of H₂O (1mL), 15% NaOH (1mL), and extra 1mL of H₂O at 0°C. The reaction mixture was filtered through Celite, and the celite pad was washed with ether. Removal of the solvent in vacuo yielded an oil. Flash chromatography on silica gel (130g, 1:1 EtOAc/hexane) gave the title compound (2.34 g, 76 %) as a white solid.

R_F (hexane:EtoAc 1:1) = 0.14.

1H NMR (500 MHz, CDCl₃) δ 7.4 (m, 10H), 4.65 (s, 4H), 3.85 (d, J = 11.6 Hz, 2H), 3.75 (d, J = 12 Hz 2H), 3.73 (m, 2H), 2.44 (br s, 2H).

Synthesis of phosphoric acid (2R,3R)-2,3-bis-benzyloxy-4-hydroxy-butyl ester diphenyl ester

Imidazole (0.82g, 12mmol) was added to diol (2.4g, 8.1mmol) dissolved in dry CH₂Cl₂ (30mL) under N₂ at 0°C. After the imidazole had completely dissolved, diphenylchlorophosphate (0.43mL, 2.1 mmol) was added and the reaction was stirred for 4 hours under N₂. The reaction mixture was quenched by adding H₂O (5mL) and 10% HCl (5mL) to the reaction mixture. The organic layer was removed and the aqueous layer was further extracted with CH₂Cl₂. Concentration of the combined organic in vacuo extracts gave an oil. Flash chromatography gave the monoester as a colorless oil (1.2 g, 48 %).

R_F (hexane:EtoAc, 1:1) = 0.54.

m/z (+ve FAB) cal. 535.18857 found. 535.18912.

1H NMR (400 MHz, CDCl₃): δ 7.3 (m, 20H), 4.66 (d, J = 11.6 Hz, 1H), 4.57 (m, 4H), 4.25 (ddd, J = 11.0, 6.6, 7.1 Hz, 1H), 3.90 (m, 1H), 3.74 (m, 1H), 3.63 (m, 2H) 2.90 (br s, 1H) ppm. 13C NMR (100 MHz, CDCl₃) δ 61.0, 68.7 (d, J = 6.5 Hz), 72.9, 73.3, 77.8 (d, J = 7.3 Hz), 78.4 ppm.
To a stirred solution of the alcohol (0.3g, 0.56mmol) in dry CH₂Cl₂ (40mL) was added Dess-Martin periodinane⁵ (0.36g, 0.84mmol) at room temperature. The reaction mixture was refluxed for 4 hours, and then stirred at room temperature overnight under argon. Excess periodinane was quenched with ether (45mL), the mixture was washed with saturated aqueous Na₂S₂O₃ (40mL), and saturated aqueous NaHCO₃ (40mL), water (40mL) and Et₂O (200mL). The resulting mixture was stirred at room temperature (1h) and then extracted with ether (3 x 80mL). The combined extracts were washed with water and brine and concentrated in vacuo. The residual oil was purified by flash chromatography on silica gel to give the title compound (0.26g, 87%) as a light yellow syrup.

Rₐ (hexane:EtOAc 1:1) = 0.75.

m/z (+ve FAB) cal. 533.17292 found. 533.17337.

1H NMR (400 MHz, CDCl₃) δ 9.63 (s, 1H), 7.3 (m, 2OH), 4.69, (d, J = 11.8 Hz, 1H), 4.59 (d, J = 11.6 Hz, 1H), 4.51 (d, J = 11.6 Hz, 1H), 4.47 (d, J = 11.6, 1H), 4.42, (m, 2H), 4.05 (m, 1H), 3.89 (d, J = 3.8 Hz, 1H) ppm. 13C NMR (100 MHz, CDCl₃) δ 66.3, 73.5, 73.7, 77.1, 81.93, 120.1 – 150.4 (ArH), 202.1 ppm.

Trimethyl orthoformate (0.124mL, 1.13mmol), and H₂SO₄ (0.98µL, 18.39µmol) were added to a solution of (2R,3S)-2,3-bis-benzyloxy-4,4-dimethoxy-butyl ester diphenyl ester (60.2mg, 0.133mmol) in dry methanol (1mL). The reaction was stirred overnight at room temperature under argon. The reaction mixture was diluted with ether (10mL), H₂O (5mL), and saturated NaHCO₃ (3mL). The aqueous layer was extracted with Et₂O (3 x 2mL). The organic extracts were combined and concentrated in vacuo. The residual oil was purified by flash chromatography on silica gel to give the title compound (49.7mg, 76 %) as a colorless oil.

Rₐ (hexane:EtOAc 2:1) = 0.49.
m/z (+ve FAB) cal. 601.19673 found 601.19715.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 4.74 (d, $J = 11.4$, 1H), 4.61 (d, $J = 11.4$ Hz, 1H), 4.51 (d, $J = 11.4$ Hz, 2H), 4.46 (d, $J = 6.1$ Hz, 1H), 4.30 (m, 2H), 3.88 (ddd, $J = 5.8$, 5.8, 3.5, 1H), 3.5 (d, $J = 6.1$, 3.5Hz, 1H), 3.43 (s, 3H), 3.25 (s, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 54.8, 56.3, 68.1 (d, $J = 6.6$ Hz) 71.7, 73.76, 73.8, 74.5, 77.2 (d, $J = 8.4$ Hz), 77.9, 105.16 ppm.

**Synthesis of D-threose 4-phosphate (T4P)**

Protected T4P (49.7mg, 85.8 µmol) was dissolved in methanol (2mL) and PtO$_2$ (19.5 mg, 86 µmol) was added. The mixture was stirred at room temperature overnight under H$_2$ (atmospheric pressure). The reaction mixture was filtered over Celite and the solvent was removed in vacuo to give the acetal (21mg, 100%). The acetal (21mg, 85.6 µmol) was then dissolved in D$_2$O and stirred for two days. The progress of the reaction was followed by $^1$H NMR. When deprotection was complete the yield of T4P (43.7 µmol, 51%) was determined using the DAH7PS assay by following the loss of PEP at 232 nm (with T4P limiting).
m/z (-ve ESI) cal. 199.0008 (M-H–) found 199.0009.

**Synthesis of 2-deoxyE4P from β-hydroxy-γ-butyrolactone**

**Synthesis of (S)-4-(benzyloxy)-dihydrofuran-2(3H)-one**

β-Hydroxy-γ-butyrolactone (0.3229g, 3.16mmol) was dissolved in 5mL of dry CH$_2$Cl$_2$ and cooled to 0°C under N$_2$. BnBr (564µL, 4.74mmol), Ag$_2$O (1.0985g, 4.74mmol), and KI (0.0670g, 0.40mmol) were added and the solution stirred and allowed to warm to room temperature. After 5 1/2 hours, the reaction mixture was filtered through celite and washed with saturated NaHCO$_3$ (25mL), H$_2$O (25mL),
and sat NaCl (25mL). The combined aqueous extracts were washed with CH₂Cl₂ (10mL x 3). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. The product was then purified using flash chromatography, giving 383mg (63%) of the product, a colourless oil.

Rf=0.53 (1:1 Hexane:EtOAc).
m/z (+ve FAB) cal 192.0786 found 192.0781.

¹H NMR (CDCl₃, 400MHz) δ 7.40-7.27 (m, 5H), 4.54 (d, J=11.9Hz, 1H), 4.51 (d, J=11.9Hz, 1H), 4.42-4.37 (m, 3H), 2.74-2.62 (m, 2H) ppm. ¹³C NMR (CDCl₃, 100MHz) δ 175.8, 137.3, 129.0, 128.6, 128.1, 74.2, 73.5, 71.6, 35.3 ppm.

Synthesis of (S)-3-(benzyloxy)-4-hydroxybutanal

(S)-4-(benzyloxy)-dihydrofuran-2(3H)-one (300mg, 1.56mmol) was dissolved in dry CH₂Cl₂ (25mL) and cooled to –78°C under N₂. DIBAL in CH₂Cl₂ (2.18mL of 1M solution) was added dropwise, and the solution stirred at -78°C. Once the starting material had all disappeared, the reaction was quenched by adding 3mL of 10% HCl and allowed to warm to room temperature. The reaction mixture was diluted with H₂O (20mL) and CH₂Cl₂ (40mL). The organic extract was then removed and washed with Rochelle’s salt (50mL), H₂O (50mL), and sat NaCl (20mL). The combined aqueous extracts were washed with CH₂Cl₂ (20mL). The combined organic extracts were dried (MgSO₄) and concentrated in vacuo. The product was purified by flash chromatography to give 230mg (76%) of colourless oil.

Rf=0.47 (1:1 Hexane:EtOAc).
m/z (+ve FAB) cal 193.0865, found 193.0865

¹H NMR (CDCl₃, 400MHz) δ 7.32 (m, 10H), 5.85 (m, 1H), 5.41 (m, 1H), 4.24 (m, 4H), 4.10 (m, 3H), 4.08 (m, 3H), 4.05 (m, 1H), 4.03 (m, 2H), 2.01 (m, 2H), 1.99 (m, 3H), 1.98 (m, 4H), 0.84 (m, 2H) ppm. ¹³C NMR (CDCl₃, 100MHz) δ 207.6, 141.4, 138.3, 137.7, 129.0, 128.9, 128.8, 128.6, 128.4, 128.3, 128.1, 128.0, 127.3, 99.3, 99.0, 78.8, 78.4, 73.5, 72.8, 71.8, 71.6, 71.5, 67.6, 65.6, 40.8, 40.0, 38.7, 31.3.
Dimethylpropanediol (0.1007g, 0.97mmol) and TMSCl (245µL, 1.94mmol) were dissolved in dry CH₂Cl₂ (10mL) and cooled to 0°C under N₂ for 15 minutes. The lactol (120mg, 0.64mmol) in 5mL CH₂Cl₂ was then added to the reaction mixture and stirred for four hours. The mixture was then washed with sat NaHCO₃ (20mL x 3) and the combined aqueous extracts washed with CH₂Cl₂ (20mL x 3). The organic extracts were dried (MgSO₄) and concentrated in vacuo. The product was then purified using flash chromatography to yield 85mg of the title compound (47%), as well as 8mg (4%) of the five-membered ring. Rf=0.52 (1:1 Hexane:EtOAc).

m/z (+ve FAB) cal 280.1675 found 280.1676

₁H NMR (CDCl₃, 400MHz) δ 7.33-7.23 (m, 5H), 4.59-4.51(m, 3H), 3.73-3.69 (m, 2H), 3.57-3.53 (m, 3H), 3.40-3.34 (m, 2H), 2.01-1.97 (m, 2H), 1.86-1.81(m, 1H), 1.14 (s, 3H), 0.68 (s, 3H) ppm. ¹³C NMR (CDCl₃, 100MHz) δ 128.9 (CH), 128.3 (CH), 128.2, (CH), 100.1 (CH), 77.7 (CH₂), 77.6 (CH₂), 76.2 (CH), 72.0 (CH₂), 64.8 (CH₂), 36.9 (CH₂), 23.5 (CH₃), 22.2 (CH₃) ppm.

Phosphorylation of (S)-2-(benzyloxy)-3-(5,5-dimethyl-1,3-dioxan-2-yl)propan-1-ol

(S)-2-(benzyloxy)-3-(5,5-dimethyl-1,3-dioxan-2-yl)propan-1-ol (97mg, 0.34mmol) was dissolved in dry CH₂Cl₂ (4mL) and cooled to 0°C under N₂. Imidazole (88mg, 0.42mmol) and diphenylchlorophosphate (32µL, 1.4mmol) were added, and the reaction mixture stirred for five hours under N₂ while being allowed to warm slowly to room temperature. H₂O (5mL) and CH₂Cl₂ (10mL)
were then added to the reaction mixture, and it was transferred to a separating funnel. 10% HCl (3mL) was added and the aqueous layer extracted with CH₂Cl₂ (10mL x 3). The combined organic layers were then washed with sat NaHCO₃ (10mL), H₂O (10mL), and sat NaCl (10mL). The combined organic extracts were then dried (MgSO₄) and the solvent removed to give 87mg (51%) of product. Rᵢ=0.48 (2:1 Hex:EtOAc).

¹H NMR (CDCl₃, 400MHz) δ 7.29-7.13 (m, 15H), 4.57 (d, J=11.5Hz 1H), 4.52-4.50 (m, 1H), 4.45 (d, J=11.5Hz 1H), 4.39-4.30 (m, 1H), 3.90-3.86 (m, 1H), 3.54-3.50 (m, 2H), 3.34 (d, J=11.3Hz, 2H), 3.28 (d, J=11.3Hz, 2H), 1.92-1.89 (m, 1H), 1.84-1.64 (m, 1H), 1.12 (s, 3H), 0.67 (s, 3H) ppm. ¹³C NMR (CDCl₃, 100MHz) δ 130.2, 128.7, 128.3, 128.1, 125.7, 120.6, 120.5, 99.6 (CH), 77.8(CH₂), 77.6 (CH₂), 74.2(CH), 72.6(CH₂), 71.0(CH₂), 37.0 (CH₂), 30.5 (CH), 23.4 (CH₃), 22.2 (CH₃) ppm.

Synthesis of (S)-3-(5,5-dimethyl-1,3-dioxan-2-yl)-2-hydroxypropyl phosphate

The protected analogue (33mg, 0.06mmol) was dissolved in MeOH (1mL) and PtO₂ added (15mg, 0.06mmol). The mixture was stirred at room temperature overnight under H₂ (atmospheric pressure). The PtO₂ was filtered out and the pH adjusted to 9.5 using Et₃N. The product was then lyophilized, giving 18.1mg (83%) of the triethylamine salt.

¹H NMR (D₂O, 400MHz) δ 4.67-4.63 (m, 1H), 3.84-3.79 (m, 1H), 3.68-3.65 (m, 1H), 3.61-3.57 (m, 1H), 3.52-3.44 (m, 5H), 1.69-1.66 (dd, J=6Hz, 6Hz, 2H), 0.98 (s, 3H), 0.69 (s, 3H) ppm. ¹³C NMR (D₂O, 100MHz) δ 100.0, 77.2, 68.8, 67.1, 37.6, 29.9, 22.3, 20.9ppm.

Synthesis of (S)-2-deoxyE4P

(S)-3-(5,5-dimethyl-1,3-dioxan-2-yl)-2-hydroxypropyl phosphate (24mg, 0.05mmol) was dissolved in H₂O (1.5mL) and freshly activated Dowex® 50W-H⁺ form was added to give a pH of 2. The solution was then heated to 40°C and stirred overnight. The Dowex® 50W resin was then filtered out and the pH
adjusted to 6.8 using NaOH. Charcoal was used to decolourise the solution, which was then filtered and used directly in the enzyme assays.

References