Supplementary Information:

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

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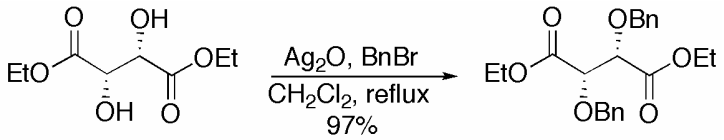
Experimental for the preparation of the aldose phosphate substrate analogues

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

*Synthesis of dibenzyl-D-diethyl (2*S*,3*S*)-bis(benzyloxy)tartrate* [1]

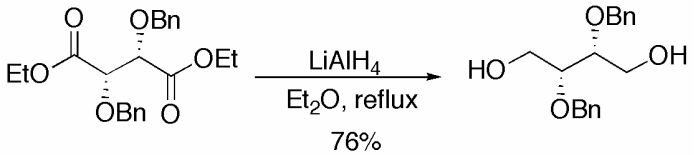


D-Diethyl tartrate (8.6mL, 0.05 mol) was dissolved in dry CH_2Cl_2 (100mL) and BnBr (17.82mL, 150mmol), Ag_2O[2] (24.7 g, 106mmol), and KI (1.66mg, 10mmol) were added. The reaction mixture was refluxed under argon for 1 hour and filtered over a celite pad. The filtrate was then washed with water, dried 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 %).

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

^{1}H NMR (400 MHz, $CDCl_3$) 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. ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 61.3, 73.2, 78.4, 128.0, 128.3, 128.4, 137.0, 169.2 ppm.

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

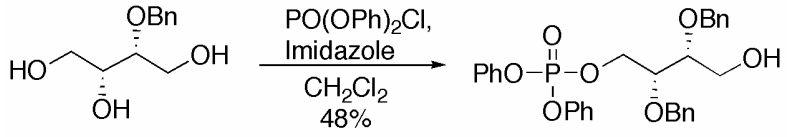


Lithium aluminium hydride (LAH, 0.83g, 21.85mmol) was suspended in dry ether (20mL) and added dropwise over 1 hour to dibenzyl-D-diethyl (2*S*,3*S*)-bis(benzyloxy)tartrate (3.95g, 0.3mmol) dissolved in dry ether (20mL) under N_2 at 0°C. The reaction mixture was then refluxed for 4 hours. Excess LAH was destroyed by sequential addition of H_2O (1mL), 15% NaOH (1mL), and extra 1mL of H_2O 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.

^{1}H NMR (500 MHz, $CDCl_3$) 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 (2*R*,3*R*)-*2,3-bis-benzyloxy-4-hydroxy-butyl ester diphenyl ester*



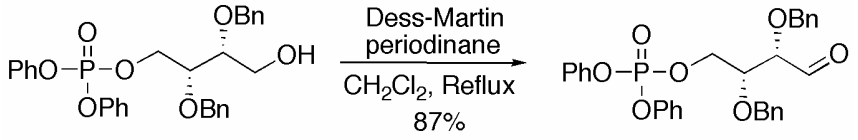
Imidazole (0.82g, 12mmol) was added to diol (2.4g, 8.1mmol) dissolved in dry CH_2Cl_2 (30mL) under N_2 at 0°C. After the imidazole had completely dissolved, diphenylchlorophosphate (0.43mL, 2.1 mmol) was added and the reaction was stirred for 4 hours under N_2. The reaction mixture was quenched by adding H_2O (5mL) and 10% HCl (5mL) to the reaction mixture. The organic layer was removed and the aqueous layer was further extracted with CH_2Cl_2. 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.

^{1}H NMR (400 MHz, $CDCl_3$): 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. ^{13}C NMR (100 MHz, $CDCl_3$) δ 61.0, 68.7 (d, J = 6.5 Hz), 72.9, 73.3, 77.8 (d, J = 7.3 Hz), 78.4 ppm.

*Synthesis of phosphoric acid (2*R*,3*S*)-2,3-bis-benzyloxy-4-oxo-butyl ester diphenyl ester*[4]



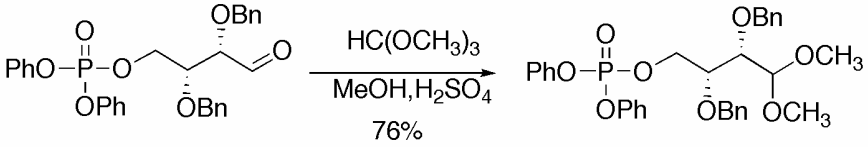
To a stirred solution of the alcohol (0.3g, 0.56mmol) in dry CH_2Cl_2 (40mL) was added Dess-Martin periodinane[5] (0.36g, 0.84mmol) at room temperature. The reaction mixture was refluxed for 4 hours, and then stirred at room temperature overnight under argon. Excess periodinane was quenched with ether (45mL), the mixture was washed with saturated aqueous $Na_2S_2O_3$ (40mL), and saturated aqueous $NaHCO_3$ (40mL), water (40mL) and Et_2O (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.

R_F (hexane:EtOAc 1:1) = 0.75.

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

^{1}H NMR (400 MHz, $CDCl_3$) 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. ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.3, 73.5, 73.7, 77.1, 81.93, 120.1 – 150.4 (ArH), 202.1 ppm.

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



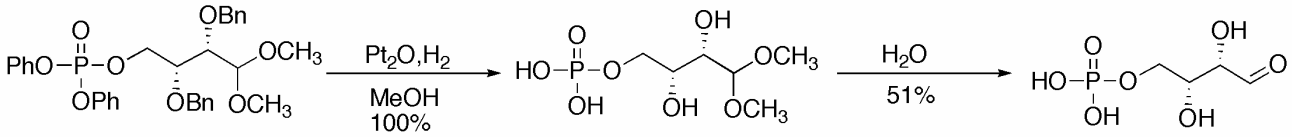
Trimethyl orthoformate (0.124mL, 1.13mmol), and H_2SO_4 (0.98μL, 18.39μmol) were added to a solution of (2*R*,3*S*)-2,3-bis-benzyloxy-4-oxo-butyl ester diphenyl ester (60.2mg, 0.133mmol) in dry methanol (1mL). The reaction was stirred overnight at room temperature under argon. The reaction mixture was diluted with ether (10mL), H_2O (5mL), and saturated $NaHCO_3$ (3mL). The aqueous layer was extracted with Et_2O (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.

R_F (hexane:EtOAc 2:1) = 0.49.

m/z (+ve FAB) cal. 601.19673 found 601.19715.

^{1}H NMR (400 MHz, $CDCl_3$): δ4.74 (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* = 6.1Hz, 1H), 4.30 (m, 2H), 3.88 (ddd, *J* = 5.8, 5.8, 3.5, 1H), 3.5 (dd, *J* = 6.1, 3.5Hz, 1H), 3.43 (s, 3H), 3.25 (s, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 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)

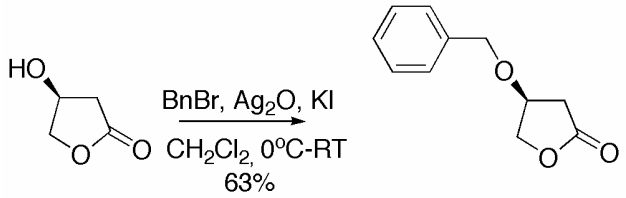


Protected T4P (49.7mg, 85.8μmol) was dissolved in methanol (2mL) and PtO_2 (19.5 mg, 86 μmol) was added. The mixture was stirred at room temperature overnight under H_2 (atmospheric pressure). The reaction mixture was filtered over Celite and the solvent was removed *in vacuo* to give the acetal (21mg, 100%). The acetal (21mg, 85.6μmol) was then dissolved in D_2O and stirred for two days. The progress of the reaction gwas followed by ^{1}H NMR. When deprotection was complete the yield of T4P (43.7μmol, 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_2Cl_2 and cooled to 0°C under N_2. BnBr (564μL, 4.74mmol), Ag_2O[2](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_2O (25mL),

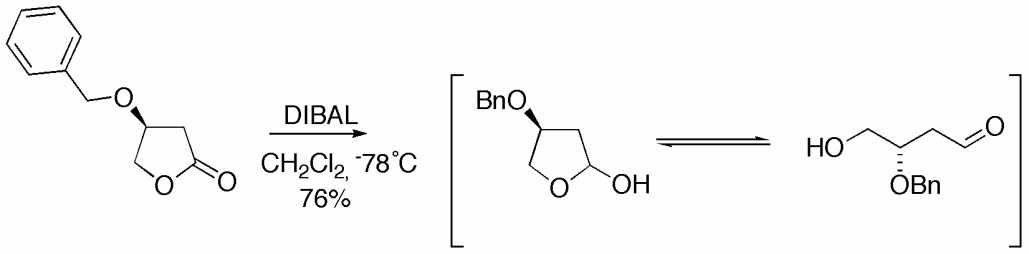
and sat NaCl (25mL). The combined aqueous extracts were washed with CH_2Cl_2 (10mL x 3). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The product was then purified using flash chromatography, giving 383mg (63%) of the product, a colourless oil.

R_F=0.53 (1:1 Hexane:EtOAc).

m/z (+ve FAB) cal 192.0786 found 192.0781.

^{1}H NMR ($CDCl_3$, 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. ^{13}C NMR ($CDCl_3$, 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*



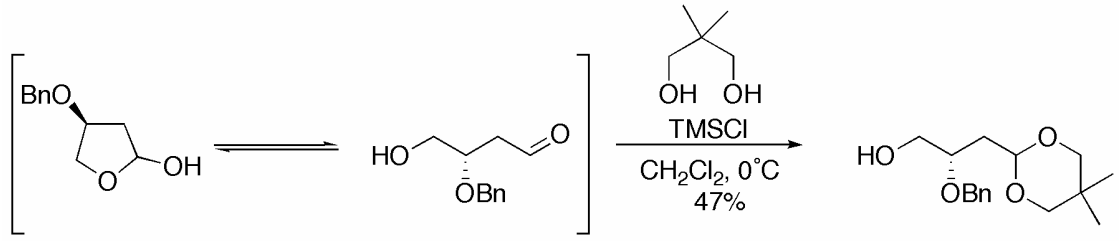
(*S*)-4-(benzyloxy)-dihydrofuran-2(3H)-one (300mg, 1.56mmol) was dissolved in dry CH_2Cl_2 (25mL) and cooled to –78˚C under N_2. DIBAL in CH_2Cl_2 (2.18mL of 1M solution) was added dropwise, and the solution stirred at -78°C. Once the starting material had all disappeared, the reaction was quenched by adding 3mL of 10% HCl and allowed to warm to room temperature. The reaction mixture was diluted with H_2O (20mL) and CH_2Cl_2 (40mL). The organic extract was then removed and washed with Rochelle's salt (50mL), H_2O (50mL), and sat NaCl (20mL). The combined aqueous extracts were washed with CH_2Cl_2 (20mL). The combined organic extracts were dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by flash chromatography to give 230mg (76%) of colourless oil.

R_F=0.47 (1:1 Hexane:EtOAc).

m/z (+ve FAB) cal 193.0865, found 193.0865

^{1}H NMR ($CDCl_3$, 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. ^{13}C NMR ($CDCl_3$, 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.

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



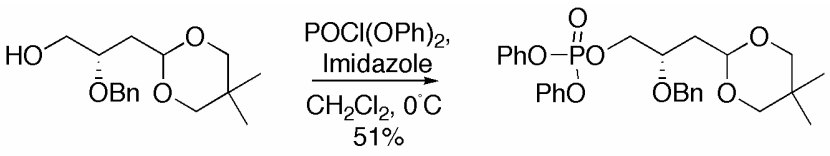
Dimethylpropanediol (0.1007g, 0.97mmol) and TMSCl (245μL, 1.94mmol) were dissolved in dry CH_2Cl_2 (10mL) and cooled to 0˚C under N_2 for 15 minutes. The lactol (120mg, 0.64mmol) in 5mL CH_2Cl_2 was then added to the reaction mixture and stirred for four hours. The mixture was then washed with sat $NaHCO_3$ (20mL x 3) and the combined aqueous extracts washed with CH_2Cl_2 (20mL x 3). The organic extracts were dried ($MgSO_4$) 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

^{1}H NMR ($CDCl_3$,, 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. ^{13}C NMR ($CDCl_3$, 100MHz) δ 128.9 (CH), 128.3 (CH), 128.2, (CH), 100.1 (CH), 77.7 (CH_2), 77.6 (CH_2), 76.2 (CH), 72.0 (CH_2), 64.8 (CH_2), 36.9 (CH_2), 23.5 (CH_3), 22.2 (CH_3) ppm.

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



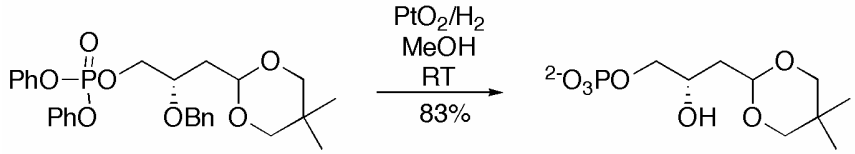
(S)-2-(benzyloxy)-3-(5,5-dimethyl-1,3-dioxan-2-yl)propan-1-ol (97mg, 0.34mmol) was dissolved in dry CH_2Cl_2 (4mL) and cooled to 0˚C under N_2. Imidazole (88mg, 0.42mmol) and diphenylchlorophosphate (32μL, 1.4mmol) were added, and the reaction mixture stirred for five hours under N_2 while being allowed to warm slowly to room temperature. H_2O (5mL) and CH_2Cl_2 (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 CH_2Cl_2 (10mL x 3). The combined organic layers were then washed with sat $NaHCO_3$ (10mL), H_2O (10mL), and sat NaCl (10mL). The combined organic extracts were then dried ($MgSO_4$) and the solvent removed to give 87mg (51%) of product.

R_F=0.48 (2:1 Hex:EtOAc).

^{1}H NMR ($CDCl_3$,, 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. ^{13}C NMR ($CDCl_3$, 100MHz) δ 130.2, 128.7, 128.3, 128.1, 125.7, 120.6, 120.5, 99.6 (CH), 77.8(CH_2), 77.6 (CH_2), 74.2(CH), 72.6(CH_2), 71.0(CH_2), 37.0 (CH_2), 30.5 (CH), 23.4 (CH_3), 22.2 (CH_3) ppm.

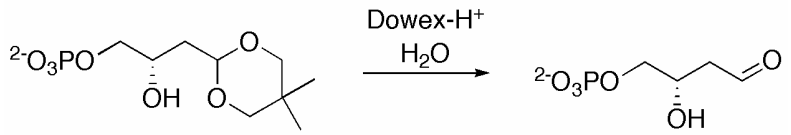
*Synthesis of (*S*)-3-(5,5-dimethyl-1,3-dioxan-2-yl)-2-hydroxypropyl phosphate*



The protected analogue (33mg, 0.06mmol) was dissolved in MeOH (1mL) and PtO_2 added (15mg, 0.06mmol). The mixture was stirred at room temperature overnight under H_2 (atmospheric pressure). The PtO_2 was filtered out and the pH adjusted to 9.5 using Et_3N. The product was then lyophilized, giving 18.1mg (83%) of the triethylamine salt.

^{1}H NMR (D_2O, 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. ^{13}C NMR (D_2O, 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 H_2O (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

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