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for

A Fragment-Based Approach to Understanding Inhibition of 1-Deoxy-D-Xylulose-5-Phosphate Reductoisomerase

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Experimental Details

All reagents and solvents were obtained from Sigma-Aldrich, Clariant Group and Fisher Scientific chemical companies and not further purified unless otherwise stated. All anhydrous solvents were purchased from Fluka and were transferred under dried N_2 gas. All reactions were carried out under N_2 atmosphere unless otherwise stated. ^1H and ^{13}C NMR spectra were obtained using JEOL Δ-400 spectrometer operating at 400 MHz and 100 MHz respectively. ^{31}P NMR spectra were obtained using ecp 300 spectrometer operating at 121 MHz. Chemical shifts for ^1H are quoted in ppm relative to TMS (at 0 ppm) and CD_3OD (at 3.30 ppm). Chemical shifts for ^{13}C NMR are quoted in ppm relative to $CDCl_3$ (at 77.10 ppm) and CD_3OD (at 47.73 ppm). ^{31}P NMR experiments were proton-decoupled. IR spectra were obtained using a Perkin Elmer 1600 FTIR spectrometer, under neat conditions. Melting points were obtained using an electrothermal melting point apparatus. Mass spectra were obtained in the indicated mode using a Fisons Autospec instrument. Optical rotation were obtained using a Bellingham+Stanley Ltd

ADP220 polarimeter with a 1 mL, 0.25 dm cell. Flash chromatography were performed according to the method of Still using Merck silica gel 60 (0.035-0.070 mm).[1] Tlc analysis was performed using Aldrich glass backed 0.25 mm silica plates (F_{254}) developed with phosphomolybdic acid.

(3-Aminopropyl)phosphonic acid hydrochloride salt 8: (3-Aminopropyl)phosphonic acid diethyl ester **12** (227 mg, 1.16 mmol) and concentrated HCl (10 mL) were stirred for 22 h at 110-120°C and then water was added. This aqueous solution was washed with EtOAc (3 times), evaporated and dried in vacuo for several hours to give the title compound **8** (155 mg, 76%) as a slightly brown solid: R_f = 0.10 (butanol/acetic acid/water 4:4:1); m.p. 160-165°C; IR (neat): n = 985, 949, 929 (P = O and P-OH) cm^{-1}; ^1H NMR (400 MHz; CD_3OD): d = 3.12 (t, ^3J(H,H) = 7.7 and 7.3 Hz, 2H, H-3), 2.06-1.95 (m, 2H, H-1 or H-2), 1.87-1.79 (m, 2H, H-1 or H-2) ppm; ^{13}C NMR (100 MHz, CD_3OD): d = 24.69 (C-2 or C-3), 23.31 (C-2 or C-3), 19.77 (d, ^1J(C,P) = 3.85 Hz, C-1) ppm; ^{31}P NMR (121 MHz, CD_3OD): d = 28.26 ppm.

Dipotassium (3-acetamido-propyl)-phosphonate 9: (3-Acetamidopropyl)phosphonic acid diethyl ester **13** (280 mg, 1.18 mmol) was dissolved in dry dichloromethane (5 mL). Trimethylbromosilane (0.778 mL, 5.90 mmol) was added and the reaction mixture was stirred for 23 h at RT. The volatiles were then removed in vacuo. The residue was treated with aqueous KOH (0.5M, 10 mL) for 1h and then evaporated under reduced pressure. Purification by flash chromatography on silica gel (dichloromethane/MeOH 80/10, 80/20, 50/50 then pure MeOH) followed by several filtrations through Celite gave the title compound **9** (155 mg, 60%) as a colourless amorphous solid: R_f=0.20 (butanol/acetic acid/water 4:4:1); m.p. 90-93°C; IR (neat): n = 3251 (NH), 1619 (CO), 1140 (P=O), 1017, 888 (P-O) cm^{-1}; ^1H NMR (400 MHz; D_2O): d = 3.43/3.38 (t, ^3J(H,H)=7.3/7.3 Hz, 2H, H-3), 2.13 (s, 3H, C**H**$_3$ acetyl), 1.89-1.71 (m, 2H, H-2 or H-1), 1.62-1.51 (m, 2H, H-1 or H-2) ppm; ^{13}C NMR (75 MHz, D_2O): d = 174.13 (CO acetyl), 49.75/46.71 (d, ^3J(C,P)=20.15/19.58 Hz, C-3), 25.9/24.21 (d, ^2J(C,P)=19.58/20.73 Hz, C-2 or C-1), 22.37/21.39 (d, ^1J(C,P)=8.06/7.49 Hz, C-1 or C-2), 20.81 (**C**H_3 acetyl) ppm; ^{31}P NMR (121 MHz, CD_3OD): d=28.79/28.06 ppm; MS (ESI): m/z (%): 257.97 $[M]H^+$; HRMS (ESI): calcd for $C_5H_{11}K_2NO_4P$: 257.96999 $[M]H^+$; found 257.96943.

(3-Azidopropyl)phosphonic acid diethyl ester 11: (3-Bromopropyl)phosphonic acid diethyl ester **10**[2] (3.184 g, 12.3 mmol) was dissolved in EtOH (5 mL) and a solution of sodium azide (4.01 g, 61.7 mmol) in water (15 mL) was added. The reaction mixture was heated at reflux for 16 h and then EtOH was evaporated. The resulting aqueous solution was extracted with CH_2Cl_2 (3 times). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated in vacuo to give the crude product (1.535 g). Purification by flash chromatography on silica gel (EtOAc) gave the title compound **11** (1.38 g, 51%) as a slightly yellow oil: R_f = 0.50 (ethyl acetate); IR (neat): n = 2094 (N_3), 1239 (P = O), 1018, 999 (P-O-alkyl) cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): d = 4.18-4.04 (m, 4H, 2C**H$_2$**CH_3), 3.39 (t, ^3J(H,H) = 6.8 and 6.4 Hz, 2H, H-3), 1.95-1.77 (m, 4H, H-2 and H-1), 1.33 (t, ^3J(H,H) = 7.3 and 6.8 Hz, 6H, $2CH_2C$**H$_3$**) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): d = 61.23 (d, ^2J(C,P) = 6.2 Hz, 2**C**H_2CH_3), 51.11 (d, ^3J(C,P) = 16.1 Hz, C-3), 23.25 (C-2), 22.09 (d, ^1J(C,P) = 4.6 Hz, C-1), 16.05 (d, ^3J(C,P) = 5.4 Hz, CH_2**C**H_3), 16.00 (d, ^3J(C,P) = 3.8 Hz, CH_2**C**H_3) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): d = 31.46 ppm; MS (CI): *m/z* (%): 222 (80%) [M]H^+, 179 (45%) $[M\text{-}N_3]^+$, 138 (100%) $[HPO(OEt)_2]H^+$, 84 (28%) $[M\text{-}PO(OEt)_2]^+$; HRMS (CI): calcd for $C_7H_{17}N_3O_3P$: 222.100755 $[M]H^+$; found 222.100571.

(3-Aminopropyl)phosphonic acid diethyl ester 12: (3-Azidopropyl)phosphonic acid diethyl ester **11** (1.00 g, 3.88 mmol) was dissolved in EtOH 95 (5 mL). Pd/C 10% (100 mg, 10% in weight) was added and saturated with hydrogen. The reaction mixture was stirred for 17 h at RT and filtered through Celite. The Celite was washed with EtOH 95 and the solvent was removed in vacuo to give the crude product (834 mg). Purification by flash chromatography on silica gel (EtOAc/MeOH 4/1 and then 1/1) followed by a filtration Celite gave the title compound **12** (591 mg, 78%) as a slightly yellow oil: R_f = 0.22 (ethyl acetate/MeOH 95:5); IR (neat): n = 3428 (NH_2), 1212 (P = O), 1017, 958 (P-O-alkyl) cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): d = 4.16-4.03 (m, 4H, 2C**H$_2$**CH_3), 2.67 (t, ^3J(H,H) = 6.8 and 6.3 Hz, 2H, H-3), 1.83-1.71 (m, 4H, H-2 and H-1), 1.32 (t, ^3J(H,H) = 7.3 and 6.8 Hz, 6H, $2CH_2C$**H$_3$**) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): d = 61.29 (d, ^2J(C,P) = 6.2 Hz, 2**C**H_2CH_3), 49.66 (d, ^3J(C,P) = 16.9 Hz, C-3), 24.07 (C-2), 22.98 (d, ^1J(C,P) = 5.4 Hz, C-1), 16.34 (d, ^3J(C,P) = 6.2 Hz, $2CH_2$**C**H_3) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): d = 30.08 ppm; MS (CI): *m/z* (%): 194 (70%) $[M\text{-}H]^+$, 179 (40%) $[M\text{-}NH_3]H^+$; HRMS (CI): calcd for $C_7H_{19}NO_3P$: 196.110257 $[M]H^+$; found 196.109779.

(3-Acetamidopropyl)phosphonic acid diethyl ester 13: (3-Aminopropyl)phosphonic acid diethyl ester **12** (640 mg, 3.28 mmol) was dissolved in acetic anhydride (2.7 mL). Et_3N (0.457 mL, 3.28 mmol) was added and the reaction mixture was stirred for 2 h at RT, then filtered through Celite. The Celite was washed with CH_2Cl_2/MeOH and the solvents were removed in vacuo to give the title compound **13** as a yellow oil (703 mg, 90%): R_f=0.59 (CH_2Cl_2/MeOH 8:2); IR (neat): n = 3457 (NH), 1635 (CO amide), 1238 (P=O), 1015, 955 (P-O-alkyl) cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): d = 4.19-4.03 (m, 4H, 2C**H**$_2$CH$_3$), 3.41-3.35 (m, 2H, H-3), 2.12/2.02 (s, 3H, C**H**$_3$ acetyl), 1.93-1.69 (m, 4H, H-2 and H-1), 1.34/1.33 (t, ^3J(H,H)=7.0/7.3 Hz, 6H, 2CH$_2$C**H**$_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): d = 172.61/170.23 (CO acetyl), 61.46/61.25 (d, ^2J(C,P)=6.92/6.92 Hz, 2**C**H$_2$CH$_3$), 48.33/45.34 (d, ^3J(C,P)=15.4/18.5 Hz, C-3), 24.22/21.80 (d, ^2J(C,P)=29.2/30.0 Hz, C-2 or C-1), 21.54 (d, ^1J(C,P)=4.6 Hz, C-1 or C-2), 20.99/20.52 (**C**H$_3$ acetyl), 20.39 (d, ^1J(C,P)=4.6 Hz, C-1 or C-2), 16.07/16.01 (d, ^3J(C,P)=2.3/3.1 Hz, 2CH$_2$**C**H$_3$) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): d = 32.12/31.24 ppm; MS (EI): *m/z* (%): 237 (14%) $[M]^+$, 208 (80%) $[M\text{-}Et]^+$.

(±)-4-Hydroxymethylphosphate-3-*N*-benzyloxy-2-oxazolidinone (±)-14: (±)-4-Hydroxymethyldibenzylphosphate-3-*N*-benzyloxy-2-oxazolidinone (**33**, 315 mg, 0.65 mmol) was dissolved in MeOH (10 mL). Pd/C 10% (90 mg, 30% in weight) was added and saturated with hydrogen. The reaction mixture was stirred for 3 days at RT under hydrogen atmosphere and then filtered through Celite. The Celite was washed with MeOH and the solvent was removed in vacuo to give a crude product (166 mg). Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH 95:5 and then 80:20) gave the title compound (±)-1**4** (71 mg, 51%) as a slightly orange solid: R_f = 0.25 (butanol/acetic acid/water, 4:1:1); IR (neat): n = 1715 (C = O), 999, 946 (P = O and P-O-H) cm^{-1}; ^1H NMR (400 MHz; CD_3OD): d = 4.45 (br t, ^2J(H,H) **=** 8.8 Hz, ^3J(H,H) = 8.4 Hz, 1H, H-5), 4.29-4.23 (m, 2H, H-5 and CC**H**$_2$OP), 4.11-4.03 (m, 2H, H-4 and CC**H**$_2$OP) ppm; ^{13}C NMR (100 MHz, CD_3OD): d = 159.8 (CO), 63.6 (C-5), 62.7 (d, ^2J(H,H) = 4.6 Hz, C**C**H$_2$OP), 58.6 (d, ^3J(H,H) = 7.7 Hz, C-4) ppm; ^{31}P NMR (61 MHz, $CDCl_3$): d = 0.90 ppm; MS (EI): *m/z* (%): 213 (60%) $[M]^+$; HRMS (EI): calcd for $C_4H_8NO_7P$: 213.00384 $[M]^+$; found 213.1409.

(*S*)-(+)-4-Hydroxymethyldibenzylphosphate-2-oxazolidinone (*S*)-19: (*R*)-(+)-4-Hydroxymethyl-2-oxazolidinone[3] ((*R*)-**18**, 1.15 g, 9.81 mmol) and *N*-methylanilinium trifluoroacetate (3.25 g, 14.7 mmol) were dissolved in dry CH_2Cl_2 (15 mL). *N*,*N*-diisopropyl dibenzylphosphoramidite (46%, 10.8 g, 14.4 mmol) was added using a syringe and this reaction mixture was stirred for 35 min at RT and then cooled to 0°C using an ice bath. *m*CPBA (50-55%, 8.46 g, 24.5 mmol) was then added slowly in portions. The reaction mixture was stirred for 4.5 h at RT, then quenched with an aqueous saturated solution of $NaHCO_3$. The aqueous and organic layers were separated and the aqueous solution was extracted with CH_2Cl_2 (3 times). The organic layers were combined and washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a crude product (14.13 g). Purification by flash chromatography on silica gel (EtOAc) gave the title compound (*S*)-**19** (1.906 g, 50%) as a slightly yellow solid: R_f = 0.30 (ethyl acetate); m.p. 75-78°C; $[\alpha]_D$ = +16.6 (c = 1.00 in $CHCl_3$); IR (neat): ν = 3290 (N-H), 1720 (C = O), 1250 (P = O), 999 (P-O-alkyl) cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ = 7.35 (br s, 10H, 2Ph), 6.09 (br s, 1H, N-H), 5.09-4.98 (m, 4H, 2POC**H$_2$**Ph), 4.33 (fake t, ^2J(H,H) = 8.8 Hz, ^3J(H,H) = 8.4 Hz, 1H, H-5), 4.01 (dd, ^2J(H,H) = 8.8 Hz, ^3J(H,H) = 4.4 Hz, 1H, H-5), 3.93-3.83 (m, 3H, H-4 and CC**H$_2$**OP) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.9 (CO), 135.6 (C-Ph *ipso*), 135.5 (C-Ph *ipso*), 128.9 (2C-Ph), 128.8 (4C-Ph), 128.2 (2C-Ph), 128.2 (2C-Ph), 69.9 (d, ^2J(C,P) = 4.6 Hz, Ph**C**H$_2$OP), 69.9 (d, ^2J(C,P) = 4.6 Hz, Ph**C**H$_2$OP), 68.0 (d, ^2J(C,P) = 6.2 Hz, C**C**H$_2$OP), 66.2 (C-5), 51.9 (d, ^3J(C,P) = 7.7 Hz, C-4) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): δ = -0.27 ppm; MS (CI): *m/z* (%): 378 (50%) $[M]H^+$, 181 (90%), 91 (100%) $[PhCH_2]^+$; HRMS (CI): calcd for $C_{18}H_{21}NO_6P$: 378.110651 $[M]H^+$; found 378.110405.

(*R*)-(-)-4-Hydroxymethyldibenzylphosphate-2-oxazolidinone (*R*)-19: The same procedure was used to transform (*S*)-(-)-4-hydroxymethyl-2-oxazolidinone[3] ((*S*)-**18**, 398 mg, 3.40 mmol) into the title compound (354 mg, 18%) as a slightly yellow solid with identical analytical data except: m.p. 77-80°C; $[\alpha]_D$ = -16.0 (c = 1.00 in $CHCl_3$); elemental analysis calcd (%) for $C_{18}H_{20}NO_6P$: C 57.30, H 5.34, N 3.71; found: C 56.92, H 5.21, N 3.69.

(*S*)-(+)-4-Hydroxymethylphosphate-2-oxazolidinone (*S*)-21. (*S*)-(+)-4-Hydroxymethyldibenzylphosphate-2-oxazolidinone ((*S*)-**19**, 570 mg, 1.51 mmol) was dissolved in 95%

EtOH (10 mL). Pd/C 10% (100 mg, 17.5% in weight) was added and the reaction flask saturated with hydrogen. The reaction mixture was stirred for 4 h at RT and then filtered through Celite. The Celite was washed with 95% EtOH and the solvent was removed in vacuo to give the title compound (*S*)-**21** (295 mg, 99%) as a slightly brown oil: R_f = 0.60 (butanol/acetic acid/water 4:1:1); $[\alpha]_D$ = +15.9 (c = 1.46 in MeOH); IR (neat): ν = 3283 (N-H), 1710 (C = O), 1003, 941 (P = O and P-O-H) cm^{-1}; ^1H NMR (400 MHz; CD_3OD): δ = 4.49 (dd, ^2J(H,H) = 9.2 Hz, ^3J(H,H) = 8.8 Hz, 1H, H-5), 4.27 (dd ^2J(H,H) = 9.2 Hz, ^3J(H,H) = 4.8 Hz, 1H, H-5), 4.15-4.06 (m, 1H, H-4), 4.02-3.89 (m, 2H, CC**H$_2$**OP) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 160.8 (CO), 66.8 (C-5), 66.7 (d, ^2J(C,P) = 4.6 Hz, C**C**H$_2$OP), 52.2 (d, ^3J(C,P) = 7.7 Hz, C-4) ppm; ^{31}P NMR (121 MHz, CD_3OD): δ = 0.70 ppm; MS (FAB): *m/z* (%): 220 (95%) $[M]Na^+$, 198 (100%) $[M]H^+$, 179 (10%) $[M\text{-}H_2O]^+$.

(*R*)-(-)-4-Hydroxymethylphosphate-2-oxazolidinone (*R*)-21: The same procedure was used to transform (*R*)-(-)-4-hydroxymethyldibenzylphosphate-2-oxazolidinone ((*R*)-**19**, 218 mg, 5.78 mmol) into the title compound (*R*)-**21** (197 mg, 100%) as a slightly brown oil with identical analytical data except: $[\alpha]_D$ = -13.8 (c = 1.45 in MeOH).

(*S*)-(+)-5-Hydroxymethyldibenzylphosphate-2-oxazolidinone (*S*)-26: (*S*)-(+)-5-Hydroxymethyl-2-oxazolidinone[17] ((*S*)-**25**, 509 mg, 4.35 mmol) and *N*-methylanilinium trifluoroacetate (1.44 g, 6.51 mmol) were dissolved in dry CH_2Cl_2 (10 mL). *N*,*N*-diisopropyl dibenzylphosphoramidite (59%, 3.81 g, 6.51 mmol) was added using a syringe and this reaction mixture was stirred for 4 h at RT and then cooled to 0°C using an ice bath. *m*CPBA (50-55% max., 7.50 g, 21.7 mmol) was then added slowly in portions. The reaction mixture was stirred for 21 h at RT and then quenched with an aqueous saturated solution of $NaHCO_3$. The aqueous and organic layers were separated and the aqueous solution was extracted with CH_2Cl_2 (3 times). The organic layers were combined and washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a crude product. Purification by flash chromatography on silica gel (EtOAc) gives the title compound (*S*)-**26** (470 mg, 29%) as a slightly brown solid: R_f = 0.22 (ethyl acetate); m.p. 92°C; $[\alpha]_D$ = +24.2 (c = 1.15 in $CHCl_3$); IR (neat): ν = 3250 (N-H), 1745 (C = O), 1235 (P = O), 1014 (P-O-alkyl) cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): δ = 7.35 (br s, 10H, 2Ph), 5.91 (br s, 1H, N-H), 5.07 (dd, ^2J(H,H) = 11.7 Hz, ^3J(H,H) = 8.8 Hz, 2H, POC**H$_2$**Ph), 5.03 (dd, ^2J(H,H) = 11.7 Hz, ^3J(H,H) = 8.8 Hz, 2H,

POC**H**$_2$Ph), 4.71-4.65 (m, 1H, H-5), 4.09 (ddd, ^2J(H,H) = 11.4 Hz, ^3J(H,H) = 6.6, 4.4 Hz, 1H, CC**H**$_2$OP), 4.02 (ddd, ^2J(H,H) = 11.4 Hz, ^3J(H,H) = 7.0, 4.4 Hz, 1H, CC**H**$_2$OP), 3.55 (fake t, ^2J(H,H) = ^3J(H,H) = 8.8 Hz, 1H, H-4), 3.32 (dd, ^2J(H,H) = 8.8 Hz, ^3J(H,H) = 6.2 Hz, 1H, H-4) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): d = 159.0 (CO), 135.7 (d, ^3J(C,P) = 3.08 Hz, C-Ph *ipso*), 135.6 (d, ^3J(C,P) = 3.08 Hz, C-Ph *ipso*), 128.8 (2C-Ph), 128.8 (4C-Ph), 128.2 (2C-Ph), 128.2 (2C-Ph), 74.0 (d, ^3J(C,P) = 7.68 Hz, C-5), 69.9 (d, ^2J(C,P) = 5.4 Hz, 2Ph**C**H$_2$OP), 66.9 (d, ^2J(C,P) = 4.6 Hz, C**C**H$_2$OP), 42.0 (C-4) ppm; ^{31}P NMR (121 MHz, $CDCl_3$): d = -0.43 ppm; MS (CI): *m/z* (%): 378 (4%) $[M]H^+$, 181 (4%), 91 (100%) $[PhCH_2]^+$; elemental analysis calcd (%) for $C_{18}H_{20}NO_6P$: C 57.30, H 5.34, N 3.71; found: C 56.97, H 5.65, N 3.83.

(*R*)-(-)-5-Hydroxymethyldibenzylphosphate-2-oxazolidinone (*R*)-26: The same procedure was used to transform (*R*)-(-)-5-hydroxymethyl-2-oxazolidinone[17] (**25**, 923 mg, 7.89 mmol) into the title compound (*R*)-**26** (1.271 g, 43%) as a yellow solid with identical analytical data except: m.p. 89-90°C; $[a]_D$ = -40.4 (c = 1.09 in $CHCl_3$) elemental analysis calcd (%) for $C_{18}H_{20}NO_6P$: C 57.30, H 5.34, N 3.71; found: C 57.71, H 5.40, N 3.80.

(*S*)-(+)-5-Hydroxymethylphosphate-2-oxazolidinone (*S*)-28: (*S*)-(+)-5-Hydroxymethyldibenzylphosphate-2-oxazolidinone (*S*)-**26** (379 mg, 1.00 mmol) was dissolved in 95% EtOH (6 mL). Pd/C 10% (70 mg, 18.5% in weight) was added and the reaction flask was saturated with hydrogen. The reaction mixture was stirred for 2 h at RT under hydrogen atmosphere and then filtered through Celite. The Celite was washed with 95% EtOH and the solvent was removed in vacuo to give the titled compound (*S*)-**28** (199 mg, 100%) as a white solid: R_f = 0.75 (butanol/acetic acid/water 4:1:1); m.p.: 122-125°C; $[a]_D$ = +27.6 (c = 1.11 in MeOH); IR (neat): n = 3295 (N-H), 1633 (C = O), 1018, 960 (P = O and P-O-H) cm^{-1}; ^{1}H NMR (400 MHz; CD_3OD): d = 4.86 (br s, 1H, H-5), 4.17-4.03 (m, 2H, CC**H**$_2$OP), 3.68 (dd, ^2J(H,H) = ^3J(H,H) = 8.8 Hz, 1H, H-4), 3.48 (dd, ^2J(H,H) = 8.8 Hz, ^3J(H,H) = 7.0 Hz, 1H, H-4) ppm; ^{13}C NMR (100 MHz, CD_3OD): d = 160.6 (CO), 75.0 (d, ^3J(C,P) = 8.5 Hz, C-5), 66.1 (d, ^2J(C,P) = 4.6 Hz, C**C**H$_2$OP), 41.5 (C-4) ppm; ^{31}P NMR (121 MHz, CD_3OD): d = 0.45 ppm; MS (EI): *m/z* (%): 197 (0.75%) $[M]^+$; elemental analysis calcd (%) for $C_4H_8NO_6P$: C 24.38, H 4.09, N 7.11; found: C 24.79, H 4.10, N 6.74.

(*R*)-(-)-5-Hydroxymethylphosphate-2-oxazolidinone ((*R*)-28: Using the same procedure (*R*)-(-)-5-hydroxymethyldibenzylphosphate-2-oxazolidinone ((*R*)-**26**, 172 mg, 0.46 mmol) was transformed into the title compound (*R*)-**28** (90 mg, 100%) as a white solid with identical analytical data except: m.p. 115-119°C; $[\alpha]_D$ = -17.0 (c = 0.94 in MeOH).

1,3-Dihydroxy-2-*O*-benzyloximepropane 30: 1,3-Dihydroxyacetone dimer (**29**, 7.94 g, 44.1 mmol) was dissolved in distilled water (250 mL). *O*-benzylhydroxylamine hydrochloride (14.42 g, 90.3 mmol) was added and the reaction mixture was stirred for 23 h at RT and then extracted with CH_2Cl_2 (8 times). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent was removed in vacuo to give the titl compound **30** (17.14 g, 100%) as a white oily solid. The product was characterised and used without any further purification: R_f = 0.59 (ethyl acetate); IR (neat): ν = 3337 (O-H) cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$): δ = 7.33 (br s, 5H, 2Ph), 5.08 (s, 2H, OC**H$_2$**Ph), 4.52 (s, 2H, C**H$_2$**OH), 4.31 (s, 2H, C**H$_2$**OH), 2.87 (br s, 1H, OH), 2.81 (br s, 1H, OH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.9 (C = N), 135.4 (C-Ph *ipso*), 128.5 (2C-Ph), 128.2 (2C-Ph), 128.1 (C-Ph *para*), 76.5 (Ph**C**H$_2$O), 61.7 (**C**H$_2$OH), 58.6 (**C**H$_2$OH) ppm; MS (CI): *m/z* (%): 196 (8%) $[M]H^+$; HRMS (CI): calcd for $C_{10}H_{14}NO_3$: 196.097368 $[M]H^+$; found 196.097195.

1,3-Dihydroxy-2-*O*-benzylhydroxylaminepropane 31: 1,3-Dihydroxy-2-*O*-benzyloximepropane (**30**, 6.83 g, 35.0 mmol) was dissolved in methanol (60 mL) and cooled to 0°C using an ice bath. Sodium cyanoborohydride (5.62 g, 89.4 mmol) was added. A few drops of trifluoroacetic acid (TFA) were added before removing the ice bath. The reaction mixture was stirred for 18 h at RT with addition of few drops of TFA and then concentrated in vacuo. The slurry was dissolved in CH_2Cl_2 and an aqueous saturated solution of $NaHCO_3$ was added. The aqueous and organic layers were separated and the aqueous layer was extracted with CH_2Cl_2 (6 times). The organic layers were combined and dried over $MgSO_4$, filtered and the solvent was removed in vacuo to give the titled compound **31** (5.781 g, 84%) as a colorless oil that solidified upon standing to give a white solid: R_f = 0.26 (ethyl acetate); m.p. 38-41°C; IR (neat): ν = 3308, 3237, 3196 (O-H, N-H and O-H free) cm^{-1}; ^{1}H NMR (400 MHz; CD_3OD): δ = 7.36-7.24 (m, 5H, Ph), 4.69 (s, 2H, OC**H$_2$**Ph), 3.63 (dd, ^3J(H,H) = 11.0, 5.5 Hz, 2H, C**H$_2$**OH), 3.60 (dd, ^3J(H,H) = 11.0, 5.9 Hz, 2H, C**H$_2$**OH), 3.04 (q, ^3J(H,H) = 5.9 Hz, 1H, C**H**NH) ppm; ^{13}C NMR (100 MHz, CD_3OD): δ = 137.9 (C-Ph *ipso*), 128.1 (2C-Ph), 128.0 (2C-Ph), 127.5

(C-Ph *para*), 127.1 (Ph**C**H_2O), 63.8 (C-N), 59.7 (2CH_2OH) ppm; MS (CI): *m/z* (%): 198 (90%) $[M]H^+$, 107 (45%) $[M\text{-}Bn+H]^+$, 91 (100%) $[PhCH_2]^+$; elemental analysis calcd (%) for $C_{20}H_{30}N_2O_6$: C 60.90, H 7.67, N 7.10; found: C 60.87, H 7.89, N 7.15.

(±)-4-Hydroxymethyl-3-*N*-benzyloxy-2-oxazolidinone 32: 1,3-Dihydroxy-2-*O*-benzyl-hydroxylaminepropane (**31**, 3.78 g, 19.2 mmol) was dissolved in dry CH_2Cl_2 (60 mL) in a two-necked flask equipped with a dropping funnel and cooled to 0°C using an ice bath. *N*,*N*-diisopropylethylamine (6.69 mL, 38.4 mmol) was added dropwise using a syringe. The reaction mixture was stirred for 15 min at 0°C. A solution of triphosgene (1.71 g, 5.76 mmol) in dry CH_2Cl_2 (50 mL) was then added *via* the dropping funnel dropwise over a period of 1 h. The reaction mixture was stirred for an additional 1 h at 0°C and then the solvent was removed in vacuo. The crude material was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 98:2 and then 95:5) to give a product (5.72 g) which is recrystallised from CH_2Cl_2/Petroleum ether 40-60°C to give the title compound **32** (2.03 g, 47%) as small colorless crystals: R_f = 0.50 (ethyl acetate); m.p. 92-95°C; IR (neat): ν = 3414 (O-H), 1747 (C = O) cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$): δ = 7.48-7.40 (m, 5H, Ph), 5.04 (d, ^2J(H,H) = 11.4 Hz, 1H, OC**H**$_2$Ph), 4.98 (d, ^2J(H,H) = 11.7 Hz, 1H, OC**H**$_2$Ph), 4.24 (dd, ^2J(H,H) = 8.42 Hz, ^3J(H,H) = 8.06 Hz, 1H, H-5), 4.20 (dd, ^2J(H,H) = 8.4 Hz, ^3J(H,H) = 7.3 Hz, 1H, H-5), 3.66-3.61 (m, 1H, H-4), 3.57 (dd, ^2J(H,H) = 12.5 Hz, ^3J(H,H) = 3.3 Hz, 2H, CC**H$_2$**OH), 3.38 (dd, ^2J(H,H) = 12.5 Hz, ^3J(H,H) = 2.6 Hz, 2H, CC**H$_2$**OH), 1.20 (br s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.7 (CO), 135.7 (C-PH *ipso*), 130.0 (2C-Ph), 129.3 (C-Ph *para*), 128.8 (2C-Ph), 77.8 (Ph**C**H_2O), 63.4 (C-5), 59.6 (C-4), 59.3 (CH_2OH) ppm; MS (CI): *m/z* (%): 224 (40%) $[M]H^+$, 181 (40%), 134 (23%) $[M\text{-}Bn+H]H^+$, 91 (100%) $[PhCH_2]^+$; elemental analysis calcd (%) for $C_{11}H_{13}NO_4$: C 59.19, H 5.87, N 6.27; found: C 59.29, H 5.82, N 6.17.

(±)-4-Hydroxymethyldibenzylphosphate-3-*N*-benzyloxy-2-oxazolidinone 33: (±)-4-Hydroxymethyl-3-*N*-benzyloxy-2-oxazolidinone (**32**, 226 mg, 1.01 mmol) and *N*-methylanilinium trifluoroacetate (447 mg, 2.02 mmol) were dissolved in dry CH_2Cl_2 (10 mL). *N*,*N*-diisopropyl dibenzylphosphoramidite (59%, 1.18 g, 2.02 mmol) was added by using a syringe and this solution was stirred 5 h at RT and then cooled to 0°C using an ice bath. *m*CPBA (50-55% max., 697 mg, 2.02 mmol) was added slowly in portions. The reaction mixture was stirred for 17 h at RT and quenched with an aqueous saturated

solution of $NaHCO_3$. The aqueous and organic layers were separated and the aqueous solution was extracted with CH_2Cl_2 (3 times). The organics layers were combined and dried over $MgSO_4$, filtered and concentrated in vacuo to give a crude product. Purification by flash chromatography on silica gel (hexane and then hexane/EtOAc 8:2) gives the title compound **33** (301 mg, 62%) as a slightly brown solid: R_f = 0.56 (ethyl acetate); IR (neat): n = 1776 (C = O), 1272 (P = O), 994 (P-O-alkyl) cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$): d = 7.45-7.30 (m, 15H, 3Ph), 5.06-4.96 (m, 4H, 2POC**H**$_2$Ph), 4.94 (d, ^2J(H,H) = 11.0 Hz, 1H, NOC**H**$_2$Ph), 4.91 (d, ^2J(H,H) = 11.0 Hz, 1H, NOC**H**$_2$Ph), 4.24-4.13 (m, 1H, H-5), 4.05-3.98 (m, 2H, H-5 and CC**H**$_2$OP), 3.85 (ddd, ^2J(H,H) = 11.0 Hz, ^3J(H,H) = 5.9 Hz, ^2J(H,P) = 2.6 Hz, 1H, CC**H**$_2$OP), 3.65-3.68 (m, 1H, H-4); ^{13}C NMR (100 MHz, $CDCl_3$): d = 158.31 (CO), 135.6 (C-Ph *ipso* BnOP), 135.5 (C-Ph *ipso* BnOP), 135.1, (C-Ph *ipso* BnON), 129.5 (2C-Ph), 128.9 (C-Ph), 128.8 (C-Ph), 128.7 (C-Ph), 128.7 (4C-Ph), 128.6 (2C-Ph), 128.1 (4C-Ph), 78.9 (Ph**C**H$_2$ON), 69.8 (d, ^2J(H,H) = 6.2 Hz, 2Ph**C**H$_2$OP), 64.0 (d, ^2J(H,H) = 5.4 Hz, C**C**H$_2$OP), 63.5 (C-5), 57.3 (d, ^3J(H,H) = 8.5 Hz, C-4) ppm; ^{31}P NMR (61 MHz, $CDCl_3$): d = -0.51 ppm; MS (CI): *m/z* (%): 484 (60%) $[M]H^+$, 304 (30%) $[M\text{-}2Bn+2H]H^+$, 214 (36%) $[M\text{-}3Bn+3H]H^+$, 181 (68%), 91 (100%) $[PhCH_2]^+$.

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