Supporting Material for Yang, J. et al. "Studies on Substrate Specificity of Escherichia coli Galactokinase"

Methyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-•-D-galactopyranoside (**16**) Methyl 2,3,6-tri-*O*-benzoyl-•-D-glucopyranoside **15** (3.9 g, 7.7 mmol) was dissolved in 100 mL of dichloromethane. To this solution, 2 mL of anhydrous pyridine was added, the mixture was cooled to –10 °C, then triflic anhydride (1.7 mL, 10.1 mmol) was added through a syringe, followed by stirring for 30 min at to –10 °C and 2 hrs at room temperature. The reaction mixture was diluted with 300 mL EtOAc, washed with 5% HCl, water, $NaHCO_3$ solution, brine, dried and concentrated. The resulting residue was dissolved in 30 mL anhydrous DMF and to this solution, NaN_3 (2.2 g, mmol) was added. The reaction mixture was stirred at room temprature overnight and subsequently diluted with EtOAc, washed with water (3 × 40 mL), dried and concentrated. Purification via column chromatography gave 3.1 g of product, yield 77% for two steps. ^{1}H-NMR ($CDCl_3$): 8.10-7.97 (m, 6H), 7.6-7.33 (m, 9H), 5.99 (dd, *J* = 3.6, 10.38 Hz, 1H), 5.63 (dd, *J* = 3.2, 8.4 Hz, 1H), 5.20 (d, *J* = 3.6 Hz, 1H), 4.61 (dd, *J* = 7.2, 11.6 Hz, 1H), 4.53 (dd, *J* = 5.4; 11.2 Hz, 1H), 4.44 (m, 1H), 4.38 (m, 1H), 3.42 (s, 3H); ^{13}C-NMR ($CDCl_3$): 165.93, 165.76, 165.55, 133.52, 133.23, 129.74, 129.57, 139.40, 129.06, 128.53, 128.41, 128.30, 97.33, 70.10, 69.00, 66.23, 63.40, 61.49, 55.47.

4-azido-4-deoxy-D-galactose (9) Compound **16** (2.2 g, 4.14 mmol) was dissolved in 30 mL anhydrous MeOH, treated with MeONa (1 mL, 30% wt) solution and then the mixture was stirred overnight at room temperature. The mixture was subsequently neutralized with DOWEX 50WX8-100 ion-exchange resin, the filtrate was concentrated and dissolved in 18 mL of 2N H_2SO_4. The mixture was stirred for 6 hrs at 98 °C followed by the addition of barium carbonate until pH = 7. The precipitated barium sulfate was removed by filtration, the filtrate was concentrated and purified by silica gel chromatography ($CHCl_3$: MeOH= 8 : 1) to give 490 mg of product (57.7%). ^{1}H-NMR (D_2O): 4.57 (d, *J* = 7.2 Hz, 1H), 3.49 (dd, *J* = 7.2, 9.8 Hz, 1H), 3.93 (dd, *J* =9.8, 3.9 Hz, 1H), 4.00 (d, *J* = 3.9 Hz, 1H), 3.74 (m, 1H), 3.76 (m, 2H); ^{13}C-NMR (D_2O): 96.74, 72.09, 73.46, 62.94, 73.77, 61.24. MS: calculated for $C_6H_{11}N_3O_5$ 205.1, found m/z 203.9 [M-H]$^-$.

1,2:3,4-Di-*O*-isopropylidene-•-D-galactopyranose (17) To a suspension of D-galactopyranose **1** (4 g, 22 mmol) and $CuSO_4$ anhydrous (8 g, 50 mmol) in acetone (100 mL) was added concentrated H_2SO_4 (0.5 mL) and this mixture was stirred at room temperature for 40 hr. The reaction mixture was filtered and washed with acetone. The filtrate was treated with saturated $NaHCO_3$ (25 mL) and then evaporated. The residue was extracted with $CHCl_3$ (4 × 40 mL) and the combined $CHCl_3$ extracts were dried over Na_2SO_4 and evaporated to a syrup. This syrup was purified via silica chromatography using a $CHCl_3$:acetone gradient (90:10 to 85:15) to give 4.5 g (78%) of purified product. ^{1}H-NMR ($CDCl_3$): 5.57 (d, *J* = 5.1 Hz, 1H), 4.34 (dd, *J* = 5.1, 2.35 Hz, 1H), 4.62 (dd, *J* = 2.35, 7.9 Hz, 1H), 4.28 (d, *J* = 7.9 Hz, 1H), 3.87 (m, 1H), 3.85 (m, 1H), 3.76 (m, 1H), 1.46 (s, 3H), 1.54 (s, 3H), 1.34 (s, 6H); ^{13}C-NMR ($CDCl_3$): 96.52, 70.79, 70.98, 71.79, 68.33, 62.51, 109.68, 26.15, 26.24, 108.90, 24.52, 25.15.

1,2:3,4-Di-*O*-isopropylidene-6-*p*-toluenesulfonyl-6-deoxy-•-D-galactopyranose (18) A solution of **17** (3.8 g, 14.6 mmol) in pyridine (26 mL) was cooled to 0 °C and *p*-toluenesulfonyl chloride (20 g, 10.5 mmol) was added. The reaction was warmed to room temperature and stirred for 20 hr. The reaction mixture was then cooled and H_2O (5 mL) was added. The resulting clear solution was poured into an ice-water mixture and, after standing for 1 hr, the crystals were collected by filtration, washed with H_2O (40 mL), dissolved in toluene, and co-evaporated with toluene until no pyridine remained. The addition of ethanol (4 mL) resulted in re-crystallization of 4.6 g of **18** (76%). ^{1}H-NMR ($CDCl_3$): 5.46 (d, *J* = 5.0 Hz, 1H), 4.29 (dd, *J* = 5.0, 2.5 Hz, 1H), 4.59 (dd, *J* = 2.5, 7.96 Hz, 1H), 4.20 (d, *J* = 7.96 Hz, 1H), 4.05 (m, 1H), 4.09 (m, 1H), 4.20 (m, 1H), 1.32 (s, 3H), 1.50 (s, 3H), 1.28 (s, 3H), 1.35 (s, 3H), 7.81 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 2.44 (s, 3H); ^{13}C-NMR ($CDCl_3$): 96.33, 70.61, 70.72, 70.57, 66.07, 68.39, 109.78, 25.11, 26.18, 109.15, 24.55, 26.01, 144.95, 128.33, 129.95, 133.04, 21.83.

1,2:3,4-Di-*O*-isopropylidene-6-azido-6-deoxy-•-D-galactopyranose (19) Sodium azide (2.4 g, 37 mmol) was added to a solution of **18** (3.9 g, 9.4 mmol) in DMF (75 mL) and heated at 130°C for 16 hr. The mixture was cooled and filtered, the filtrate was concentrated to 25 mL, and H_2O (30 mL) was added. The aqueous solution was extracted with $CHCl_3$ (4 × 30 mL), and the combined $CHCl_3$ extracts were dried over Na_2SO_4 and evaporated to a syrup. This syrup was purified via silica chromatography with toluene:EtOAc gradient (85:15 to 60:40) to give 2.5 g (92%) of purified **19**. ^{1}H-NMR ($CDCl_3$): 5.50 (d, *J* = 5.1 Hz, 1H), 4.29 (dd, *J* = 5.1, 2.5 Hz, 1H), 4.58 (dd, *J* = 2.5, 7.9 Hz, 1H), 4.15 (dd, *J* = 7.9, 1.9 Hz, 1H), 3.87 (m, 1H), 3.31 (m, 1H), 3.46 (m, 1H), 1.29 (s, 3H), 1.50 (s, 3H), 1.30 (s, 3H), 1.41 (s, 3H); ^{13}C-NMR ($CDCl_3$): 96.40, 70.44, 70.86, 71.24, 67.08, 50.72, 109.63, 24.92, 26.06, 108.82, 24.46, 25.99.

6-azido-6-deoxy-D-galactopyranose (12) Compound **19** (1.85 g, 6.5 mmol) was hydrolyzed in 80% trifluoro-acetic acid (50 mL) at room temperature for 1 hr. The mixture was evaporated under reduced pressure and azeotroped with toluene (3 × 30 mL) to give 1.2 g (90%) of **12** as a white powder, which was further subjected to re-crystallization from ethanol (20 mL). ^{1}H-NMR (D_2O): 5.28 (d, *J* = 3.7 Hz, 1H), 3.81 (dd, *J* = 3.7, 10.3 Hz, 1H), 3.87 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.97 (dd, *J* = 3.2, 0.8 Hz, 1H), 4.21 (m, 1H), 3.52 (m, 2H); ^{13}C-NMR (D_2O): 92.61, 68.42, 69.27, 69.88, 69.17, 51.15. MS: calculated for $C_6H_{11}N_3O_5$ 205.1, found m/z 206 $[M+H]^+$.

6-amino-6-deoxy-D-galactopyranose (13) A solution of **12** (0.51 g, 2.5 mmol) in methanol (15 mL) was hydrogenated in the presence of 10% palladium on carbon (102 mg) for 6 hr. The mixture was filtered and evaporated under reduced pressure to give 0.4 g (90%) of **13** as a light yellow powder. ^{1}H-NMR (D_2O): 5.30 (d, J = 3.5 Hz, 1H), 3.81 (dd, J = 3.5, 10.3 Hz, 1H), 3.87 (dd, J = 10.3, 3.2 Hz, 1H), 3.99 (d, J = 3.2 Hz, 1H), 4.27 (m, 1H), 3.35 (m, 1H), 3.26 (m, 1H); ^{13}C-NMR (D_2O): 92.58, 68.27, 69.13, 70.06, 66.45, 40.58. MS: calculated for $C_6H_{13}NO_5$ 179.1, found m/z 180.0 $[M+H]^+$.

Benzyl 4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-••-galactopyranoside (21) The mixture of compound **20** (2.36 g, 6.6 mmol) and dibutyltin oxide (1.64 g, 6.6 mmol) in 50 mL toluene was azeotropically refluxed overnight and concentrated to ~10 mL. To the cooled mixture, PMBCl (1.82 mL, 13.2 mmol) was added and heated at 90 °C for 8h. After the removal of the solvent, the colorless crude was purified via silica gel column chromatography (ethyl acetate : benzene =1 : 4) to give a white pure solid **21** (2.63 g, 84%). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.60-6.80 (m, 14H), 5.47 (s, 1H), 4.98 (d, J = 12.3 Hz, 1H), 4.71-4.60 (m, 3H), 4.39 (d, J = 8.4 Hz), 4.34 (dd, J = 12.1, 1.6 Hz, 1H), 4.14-3.99 (m, 3H), 3.79 (s, 3H), 3.46 (dd, J = 9.6, 3.6 Hz, 1H), 3.34(d, J = 1.8 Hz, 1H), 2.45 (d, J = 1.8 Hz, 1H); ^{13}C-NMR ($CDCl_3$): 159.6, 138.0, 137.4, 130.3, 129.7, 129.2, 128.7, 128.4, 128.1, 126.7, 114.1, 101.9, 101.4, 79.0, 73.4, 71.4, 70.8, 70.3, 69.5, 67.0, 55.5.

Benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(4-methoxybenzyl)-••-galactopyranoside (22) NaH (0.44 g, 60%, 11.0 mmol) was slowly added into the DMF solution (30 mL) of **21** (2,63g, 5.5 mmol) at 0 °C. The mixture was stirred for 30 min and then BnBr (1.4 mL, 11 mmol) was added and the mixture stirred overnight at ambient temperature. The reaction mixture was carefully quenched by small amount of water, dichloromethane (100 mL) was added and the mixture was washed with water (3 x 50 mL). The organic solution was dried over $MgSO_4$ and concentrated in *vacuo*. To the crude product was added a mixed solvent (MeCN : H_2O = 9 : 1; 80 mL total) and CAN (7.2g, 13.2 mmol) was added to the mixture in small aliquots over 30 min. The reaction mixture was stirred for 4 h at 0° C, dichloromethane (100 mL) was added and the mixture washed with water (3 x 50 mL). The obtained crude product was purified via silica gel chromatography (EtOAc : benzene = 1 : 4) to give **22** as a white solid (2.24 g, 91%). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.58-7.26 (m, 15H), 5.57 (s, 1H), 5.04 (d, J = 12.2 Hz, 1H), 5.01 (d, J = 11.1 Hz, 1H), 4.74 (d, J = 11.1 Hz, 1H), 4.69 (d, J = 11.7 Hz, 1H), 4.52 (d, J = 7.6 Hz, 1H), 4.37 (dd, J = 1.3, 12.4 Hz, 1H), 4.20 (dd, J = 2.5, 1.0 Hz, 1H), 4.08 (dd, J = 1.7, 12.4 Hz), 3.77-3.69 (m, 2H), 3.42 (d, J = 1.0 Hz, 1H), 2.57 (d, J = 7.1Hz, 1H); ^{13}C-NMR ($CDCl_3$): 138.8, 137.8, 137.7, 129.4, 128.7, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 126.7, 102.6, 101.7, 79.6, 75.8, 75.2, 72.7, 71.2, 69.4, 66.8.

Benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-••-D-*xylo*-3-hexulopyranoside (23) To the solution of compound **22** (2.24 g, 5.0 mmol) in dichloromethane (120 mL), Dess-Martin oxidant (2.54g, 6.0 mmol) was added. The reaction was stirred at room temperature and reaction progress monitored by TLC. Upon completion, the mixture was filtered through celite and concentrated in *vacuo*. The obtained residue was purified via silica gel column chromatography (EtOAc : benzene = 1 : 9) to give **23** as a colorless oil (2.05 g, 92%). ^{1}H-NMR (500 MHz, $CDCl_3$): 7.56-7.24 (m, 15H), 5.60 (s, 1H), 5.03 (d, J = 11.5 Hz, 1H), 4.85 (d, J = 11.2 Hz, 1H), 4.74 (d, J = 11.6 Hz, 1H), 4.73 (d, J = 12.2 Hz, 1H), 4.70 (d, J = 7.6 Hz, 1H), 4.60 (d, J = 8.1 Hz, 1H), 4.47 (dd, J = 1.5, 12.7 Hz, 1H), 4.44 (d, J = 1.0 Hz, 1H), 4.16 (dd, J = 1.5, 12.7 Hz, 1H), 3.53-3.54 (m, 1H); ^{13}C-NMR ($CDCl_3$): 199.7, 137.7, 137.2, 129.6, 128.7, 128.6, 128.6, 128.5, 128.5, 128.2, 128.1, 128.0, 126.5, 102.9, 101.2, 82.9, 81.6, 74.1, 71.5, 68.7, 66.3.

Benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*C*-methyl-••-D-galactopyranoside (24) The solution of compound **23** (491 mg, 1.1 mmol) in THF (15 mL) was cooled in the diethyl ether-dry ice bath. The methyl Grignard reagent solution (1.0 M, 1.3 mL, 1.3 mmol) was added dropwise via an air-tight syringe. The reaction was kept at this temperature for 2h and slowly warmed to room temperature overnight. The reaction was carefully quenched with H_2O and dichloromethane (30 mL) was added. The organic solution was washed with 0.1N HCl and H_2O twice, dried over $MgSO_4$ and evaporated to give a mixture of two diastereoisomers. The main isomer (**24**) was isolated via silica gel column chromatography as a white solid (425 mg, 84%. TLC: ethyl acetate : hexanes = 4:6, *Rf* = 0.31) ^{1}H-NMR (500 MHz, $CDCl_3$): 7.60-7.26 (m, 15H), 5.57 (s, 1H), 5.03 (d, J = 12.2 Hz, 1H), 4.92 (d, J = 11.4 Hz, 1H), 4.82 (d, J = 11.4 Hz, 1H), 4.68(d, J =12.2 Hz, 1H), 4.60 (d, J = 8.1 Hz, 1H), 4.39 (d, J = 12.2Hz, 1H), 4.06 (d, J =11.4 Hz, 1H), 3.82 (s, 1H), 3.67 (d, J = 8.1 Hz, 1H), 3.64 (s, 1H), 3.10-2.70 (br, 1H), 1.35 (s, 3H); ^{13}C-NMR ($CDCl_3$): 139.2, 138.0, 137.8, 129.5, 128.6, 128.5, 128.4, 128.0, 128.0, 127.9, 127.6, 126.7, 102.0, 101.8, 82.2, 81.0, 75.7, 73.8, 71.4, 69.5, 65.7, 18.7. This main isomer was further proved to be compound **24** by NOE data. When the methyl group (• 1.35) was irradiated, NOE signals were observed for the following peaks: H-5 (• 3.64), H-4 (• 3.82) and H-1 (• 4.60). When H-4 (• 3.82) was irradiated, the following peaks had NOE responses: CH_3 (• 1.35) and the allylic-H of the benzylidene group (• 5.57).

3-Methyl-D-Galactose (6) Compound **24** (372 mg, 0.81 mmol), a catalytic amount of 10% Pd/C and 10 mL methanol were combined and the debenzylation process carried out under H_2 atmosphere (40 psi) overnight. Filtration and solvent evaporation led to **6** as a pale yellow compound. ^{1}H-NMR (500 MHz, CD_3OD): 4.56-4.48 and 4.14-4.08 (m, 1H), 3.84-3.34

(m, 5H), 1.40-1.14 (m, 3H); ^{13}C-NMR (CD_3OD): 96.2, 74.9, 74.4, 74.0, 73.7, 73.7, 17.8; ESI-MS: calculated for $C_7H_{14}O_6Na$ 217.1, found m/z 217.1 $[M+Na]^+$.

Saturation plots of V_0 vs. [Galactose] and V_0 vs. [ATP]. The inset is a double reciprocal plot. In the reaction system, [galactose] = 1-6mM; [ATP] = 0.5-10mM; [$MgCl_2$] = 5mM; [GalK] = 5.6 μM.