Synthesis of the Ezomycin Nucleoside Disaccharide

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Supporting Information

Experimental procedures and spectroscopic characterization for new compounds

Apparatus and Reagents. IR spectra were recorded by using a Perkin-Elmer 727B spectrophotometer; selected absorbances are reported in cm^{-1}. ^{1}H NMR spectra were obtained with a Varian Associates VXR-200, VXR-300 or an XL-400 instrument by using deuteriochloroform solutions unless otherwise specified. Chemical shifts are reported in parts per million downfield from tetramethylsilane and coupling constants (*J* values) are given in hertz. ^{13}C NMR spectra were obtained at 50.103 MHz or 75.0 MHz on the aforementioned Varian Associates VXR-200 or VXR-300 instrument by using deuteriochloroform solutions unless otherwise specified. Fast atom bombardment mass spectra (FAB-MS) were obtained on a VG Analytical Model 7070 EQ spectrometer by

using a dithiothreitol-dithioerythritol matrix. The collision induced dissociation spectra (see **1**) were recorded on a triple quadrupole instrument TSQ700 (Finnegan).

Precoated silica gel plates (Merck 60) were used for analytical thin-layer chromatography. E Merck silica gel (230-400 mesh) was used for column chromatography. HPLC chromatography (analytical and preparatory) was performed on a Laboratory Data Control instrument. Tetrahydrofuran (THF) and dichloromethane were distilled from calcium hydride. N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), triethylamine, pentane, and benzene were purchased as anhydrous reagents from Aldrich chemical company. All reactions except those in aqueous solutions were performed under a static argon atmosphere.

3-Azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*gluco*pyranose (2). A solution of 10 g (38.42 mmol) of 1,2:5,6-di-*O*-isopropylidene-α-D-*allo*furanose in 100 mL of dry 1,2-dichloroethane was cooled to –40 °C and treated dropwise with a solution of 7.80 mL (46.10 mmol) of trifluoromethanesulfonic anhydride in 20 mL of dry 1,2-dichloroethane. The reaction was allowed to stir for 1 h, during which time the temperature was allowed to warm to 20 °C. The reaction mixture was diluted with 400 mL of dichloromethane and washed with cold 0.5 N HCl (3 x 250 mL) followed by brine. The organic layer was dried over sodium sulfate and concentrated and the crude triflate was used as such for the next step.

The crude triflate was dissolved in 85 mL of dry DMF, cooled to –20 °C and then treated with 4.70 g (96.05 mmol) of lithium azide. The reaction was allowed to warm to room temperature over 16 h, whereupon TLC analysis confirmed disappearance of starting material. The reaction mixture was concentrated under high vacuum to an oil,

which was dissolved in 400 mL of ethyl acetate and 200 mL of water. The organic layer was washed with brine. The aqueous layer was back-extracted with 100 mL of ethyl acetate. The organic layers were combined, dried over sodium sulfate, and then concentrated to a residue, which was chromatographed with 3:1 hexane / ethyl acetate as the eluant to give 10.5 g (96%) of azide **2** as a colorless oil: ^{1}H NMR (400 MHz) 5.86 (d, *J* = 2.9, H-1), 4.62 (d, *J* = 3.9, H-2), 4.22-4.28 (m, H-5) 4.14 (dd, *J* = 8.79, 7.82, H-6), 4.08-4.11 (m, H-3, H-4), 3.99 (dd, *J* = 8.79, 4.88, H-6), 1.51 [s, C(C$\underline{H}_3$)$_2$], 144 [s, C(C$\underline{H}_3$)$_2$], 1.37 [s, C(C$\underline{H}_3$)$_2$], 1.33 [s, C(C$\underline{H}_3$)$_2$]; ^{13}C NMR (75 MHz) 112.5, 109.7, 105.2, 83.6, 80.7, 73.2, 67.8, 66.5, 27.1, 26.9, 26.4, 25.4; IR (film) 2110, 1384; HR-FAB-MS m/z 270.1087 (calcd for M^+–CH_3 270.1090)

3-Azido-6-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-α-D-*gluco*furanose (**3**). A 500 mL three-neck round bottom flask fitted with a reflux condenser was charged with 5.0 g (17.5 mmol) of azide **2** followed by 185 mL of 70 % aqueous acetic acid. The resulting solution was stirred at 55 °C. TLC analysis after 1 h indicated completion of the reaction. The reaction mixture was concentrated under high vacuum to a colorless oil, which was dissolved in 300 mL of ethyl acetate and 100 mL of water. The organic layer was washed with 2% ammonium bicarbonate (2 x 125 mL). The aqueous layers were back-extracted with 150 mL of ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate and then concentrated to give the crude diol, which was chromatographed with 1:3 hexane / ethyl acetate as the eluant to give 3.75 g (87%) of the diol as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) 5.80 (d, *J* = 3.91, H-1), 4.97 (d, *J* = 5.86, CH-O$\underline{H}$), 4.67 (d, *J* = 3.91, H-2), 4.58 (t, *J* = 5.86, $CH_2O\underline{H}$), 4.16 (d, *J* = 2.93, H-3), 4.03 (dd, *J* = 8.79, 2.93, H-4), 3.55-3.59 (m, H-5, H-6), 3.36-3.38 (m, H-6),

1.40 [s, C(C<u>H</u>$_3$)$_2$], 1.26 [s, C(C<u>H</u>$_3$)$_2$]; ^{13}C NMR (75 MHz) 112.5, 105.1, 83.4, 79.1, 69.8, 66.5, 64.5, 26.7, 26.4; IR (film) 2109, 1385; HR-FAB-MS m/z 245.1018 (calcd for MH^+ 245.1018).

A 500 mL three-neck round bottom flask fitted with a reflux condenser was charged with 2.45 g (10.0 mmol) of the 5,6-diol, 100 mL of dry methanol, and 2.56 g (10.3 mmol) of dibutyltin oxide. The mixture was heated at gentle reflux until a clear solution was obtained. The reaction mixture was cooled to ~40 °C and then concentrated to an oily residue, which was further azeotropically dried by using 50 mL of toluene. The resulting oily residue was dissolved in 100 mL of dry toluene and was then treated sequentially with 3.70 g (10.0 mmol) of tetra-*n*-butylammonium iodide and 3.57 mL (30.0 mmol) of benzyl bromide. The resulting solution was heated at 95 °C for 2 h. TLC analysis of the reaction mixture at this point indicated complete disappearance of the starting material. The reaction mixture was cooled to room temperature and concentrated under high vacuum. The residue thus obtained was dissolved in 400 mL of ethyl acetate, which was washed with 2 x 100 mL of 3% aqueous ammonium bicarbonate followed by 100 mL of brine. The organic layer was dried over sodium sulfate, concentrated, and then chromatographed with 5:2 hexane / ethyl acetate as the eluant to give 3.02 g (90%) of the benzyl ether **3** as a light yellow oil: ^{1}H NMR (400 MHz, DMSO-d$_6$) 7.34-7.35 (m, 5 Ph-H), 5.81 (d, *J* = 3.7, H-1), 5.26 (d, *J* = 6.12, CH-O<u>H</u>), 4.69 (d, *J* = 3.56, H-2), 4.54 and 4.50 (ABq, *J* = 12.4, PhC<u>H</u>$_2$), 4.14 (d, *J* = 9.26, 2.99, H-4), 3.60-3.68 (m, H-5), 3.60 (dd, *J* = 11.3, 2.04, H-6), 3.46 (dd, *J* = 11.3, 5.7, H-6'), 1.40 [s, C(C<u>H</u>$_3$)$_2$], 1.26 [s, C(C<u>H</u>$_3$)$_2$]; ^{13}C NMR (75 MHz) 137.9, 128.7, 128.1, 128.0, 112.5, 105.2, 83.5, 79.2, 73.7, 72.1,

68.7, 66.7, 26.9, 26.5; IR (film) 3600, 2111, 1086; HR-FAB-MS m/z 336.1566 (calcd for MH^+ 336.1559).

3-Azido-6-*O*-benzyl-3-deoxy-1,2-*O*-isopropylidene-α-D-glucopyranose (4). A solution of 3.00 g (8.95 mmol) of **3** in 60 mL of 90% aqueous trifluoroacetic acid was stirred at room temperature for 1 h. TLC analysis at this time indicated completion of the reaction. The reaction mixture was concentrated under high vacuum to a colorless oil, which was dissolved in 300 mL of ethyl acetate and washed with 2 x 100 mL of aqueous ammonium bicarbonate. The organic layer was washed with brine. The aqueous layers were combined and back-extracted with 150 mL of ethyl acetate. The organic layers were combined, dried over sodium sulfate, and then concentrated to give a residue, which was chromatographed with 7:2 ethyl acetate / hexane as the eluant to give 2.35 g (89%) of the diol as a pale yellow oil that was used as such for the next step without characterization.

A solution of 2.69 g (9.11 mmol) of the diol in 85 mL of 2,2-dimethoxypropane was added to a 250 mL round bottom flask containing 1.73 g (9.11 mmol) of p-toluenesulfonic acid monohydrate (previsouly azeotropically dried with 60 mL of dry acetonitrile) at room temperature. The resulting amber colored solution was stirred for 3 h. TLC analysis at this point indicated formation of the product along with some unreacted starting material. There was no further change in the TLC even after prolonged stirring. Therefore, work up was done after 3 h of reaction time by partitioning the reaction mixture between 300 mL of ethyl acetate and 100 mL of water. The organic layer was washed with 75 mL of saturated sodium bicarbonate. The aqueous layers were combined and back-extracted with 100 mL of ethyl acetate. The combined organic layer was washed with brine, dried over sodium sulfate, and then concentrated to a residue that

was chromatographed with 2:1 hexane / ethyl acetate as the eluant to give 2.20 g (72%) of acetonide **4** as an amber oil: ^{1}H NMR (400 MHz, DMSO-d_6) 7.28-7.36 (m, 5 PhH), 5.76 (d, *J* = 6.89, OH), 5.51 (d, *J* = 4.66, H-1), 4.51 (s, $PhCH_2$), 3.93 (dd, *J* = 4.65, 6.46, H-2), 3.72-3.80 (m, H-3), 3.58-3.68 (m, H-5, two H-6), 3.39-3.46 (m, H-4), 1.48 [s, $C(CH_3)_2$], 1.30 [s, $C(CH_3)_2$]; ^{13}C NMR (75 MHz, $CDCl_3$) 137.7, 128.8, 128.2, 128.1, 109.5, 97.3, 75.2, 74.0, 71.8, 70.2, 69.3, 65.6, 27.7, 26.8; IR (film) 3499, 2111, 1252, 1088; HR-FAB-MS m/z 358.1381 (calcd for MNa^+ 358.1381).

6-*O*-Benzyl-3,4-dideoxy-1,2-*O*-isopropylidene-3-trifluoroacetamido-α-D-glucopyranose (5). A solution of 2.10 g (6.26 mmol) of the azide **4** in 55 mL of THF was stirred with 2.5 g of Lindlar catalyst at room temperature under hydrogen atmosphere for 2.5 h. TLC analysis at this point indicated formation of a very polar spot, presumably the amine, and complete disappearance of starting material. The catalyst was removed by filtration through a Celite pad, and the filter cake was washed with 80 mL of THF. The filtrate was concentrated at room temperature to give the crude amine, which was used without purification for the next step.

The crude amine was dissolved in 20 mL of dichloromethane, cooled to 0 °C, and then treated with 4.0 mL (49.46 mmol) of pyridine followed by 2.7 mL (19.12 mmol) of trifluoroacetic anhydride. The reaction was allowed to warm to room temperature, and then was stirred for 10 h. TLC analysis at this point indicated complete consumption of the amine. Concentration of the reaction mixture gave an oily residue that was partitioned between 300 mL of ethyl acetate and 100 mL of water. The organic layer was washed sequentially with 75 mL of 1.0 N aqueous hydrochloric acid, 75 mL of saturated aqueous sodium bicarbonate, and 100 mL of brine, then dried over sodium sulfate and concentrated

to a residue, which was chromatographed with 3:1 hexane / ethyl acetate as the eluant to give 2.03 g (80%) of trifluoroacetamido product as a pale yellow oil: ^{1}H NMR (300 MHz, DMSO-d_6) 9.42 (d, *J* = 8.42, NH), 7.24-7.37 (m, 5 Ph-H), 5.54 (d, *J* = 4.03, H-1), 5.44 (d, *J* = 6.96, O<u>H</u>), 4.50 (s, PhC<u>H</u>$_2$), 3.93-4.03 (m, H-2, H-3), 3.76-3.81 (m, H-5), 3.58-3.69 (m, two H-6), 3.43-3.52 (m, H-4), 1.47 [s, C(CH$_3$)$_2$], 1.28 [s, C(CH$_3$)$_2$]; ^{13}C NMR (75 MHz, DMSO-d_6) 158.2, 139.1, 128.9, 128.1, 118.6, 108.3, 97.2, 75.3, 74.0, 73.0, 69.9, 66.0, 56.7, 42.4, 41.0, 39.3, 28.2, 27.5; IR (film) 3400, 1789, 1727; HR-FAB-MS m/z 428.1297 (calcd for MNa$^+$ 428.1297).

A solution of 540 mg (1.33 mmol) of the hydroxy trifluoroacetamide in 13 mL of dry 1,2-dichloroethane was treated with 1.0 g (5.61 mmol) of 1,1-thiocarbonyl diimidazole and heated at reflux for 6 h. The reaction mixture was cooled to room temperature, then diluted with 100 mL of dichloromethane and 50 mL of water. The organic layer was washed with 50 mL of cold 0.5 N HCl, 50 mL of saturated aqueous sodium bicarbonate and then 50 mL of brine. The organic layer was dried over sodium sulfate and then concentrated to give a residue, which was chromatographed with 1:1 hexane / ethyl acetate as the eluant to give 585 mg (85%) of thioester as a foamy solid: ^{1}H NMR (200 MHz) 8.31 (s, Im-H-5), 7.61 (d, Im-H-2, *J* = 1.40), 7.29-7.38 (m, 5 Ph-H), 7.22 (d, *J* = 8.83, N<u>H</u>), 7.04 (d, *J* = 1.74, Im-H-3), 5.83-5.86 (m, H-4), 5.72 (d, *J* = 5.42, H-1), 4.74-4.69 (m, H-3), 4.70 and 4.56 (ABq, *J* = 11.85, PhC<u>H</u>$_2$), 4.34-4.39 (m, H-2), 4.22 (dt, *J* = 5.62, 2.46, H-5), 3.82 and 3.74 (each ddd, *J* = 10.2, 2.4, H-6), 1.61 [s, C(CH$_3$)$_2$], 1.37 [s, C(CH$_3$)$_2$]; ^{13}C NMR (75 MHz) 182.7, 157.0, 156.0, 136.9, 131.1, 128.6, 128.3, 128.1, 118.2, 110.6, 96.6, 75.4, 73.9, 70.8, 69.3, 68.5, 47.2, 25.9, 25.1; IR (KBr) 3301, 1724, 1214, 1169; HR-FAB-MS m/z 516.1395 (calcd for MH$^+$ 516.1395)

To a gently refluxing solution of 0.50 mL (1.86 mmol) of tri-*n*-butyltinhydride and 45 mg (0.27 mmol) of AIBN in 12 mL of dry toluene was added a solution of 603 mg (1.17 mmol) of the thioester in 13 mL of toluene over a 5 min period. TLC analysis after 25 min indicated completion of reaction. The reaction mixture was cooled to room temperature and concentrated to give an oily residue that was chromatographed with 4:1 hexane / ethyl acetate as the eluant to give 320 mg (70%) of deoxysugar **5** as a foamy solid: ^{1}H NMR (200MHz) 8.13 (d, J = 4.80, NH), 7.31-7.41 (m, 5 Ph-H), 5.66 (d, J = 5.53, H-1), 4.73 and 4.55 (ABq, J = 12.40, CH_2Ph), 4.27-4.30 (m, H-2, H-5), 4.19-4.22 (m, H-3), 3.69 (dd, J = 9.93, 1.98, H-6), 3.37 (dd, J = 10.00, 2.18, H-6'), 2.34-2.41 (m, H-4_{eq}), 1.73-1.78 (m, H-4_{ax}), 1.55 [s, $C(CH_3)_2$], 1.35 [s, $C(CH_3)_2$]; ^{13}C NMR (100 MHz) 156.9, 156.5, 136.7, 128.6, 128.4, 128.1, 127.8, 117.2, 108.9, 97.3, 73.6, 71.6, 69.8, 63.6, 44.4, 26.1, 25.5, 22.9; IR (KBr) 3299, 1725, 1216, 1166; HR-FAB-MS m/z 388.1354 (calcd for MH^+ 388.1371)

Phenyl 6-*O*-Benzyl-3,4-dideoxy-2-*O*-(2,2-dimethylpropionyl)-1-thio-3-trifluoroacetamido-α-D-glucopyranoside (6). A solution of 290 mg (0.74 mmol) of acetonide **5** in 4 mL of 80% aqueous acetic acid was heated at 60 °C for 3 h. TLC analysis indicated complete disappearance of starting material. The reaction mixture was concentrated and azeotropically dried with toluene.

The resulting diol was dissolved in 8 mL of benzene and treated with 0.30 mL of triethylamine followed by 0.80 mL of pivaloyl chloride. The reaction mixture was stirred at room temperature for 16 h, then partitioned between 75 mL of ethyl acetate and 50 mL of water. The organic layer was washed sequentially with 30 mL of water, 45 mL of saturated aqueous sodium bicarbonate, and 45 mL of brine, dried over sodium sulfate,

concentrated, and then chromatographed with 5:1 hexane / ethyl acetate as the eluant to give 328 mg (85%) of dipivaloate as a sticky white solid: ^{1}H NMR (400 MHz) 7.20-7.30 (m, 5 H, Ph-H), 6.76 (d, *J* = 8.22, NH), 5.68 (d, *J* = 7.12, H-1), 4.85 and 4.83 (dd, *J* = 10.50, 7.13, H-2), 4.48 (t, *J* = 12.60, PhCH$_2$), 4.17-4.26 (m, H-3), 3.80-3.85 (m, H-5), 3.51-3.45 (dt, *J* = 10.29, 5.04, 4.69, two H-6), 2.19 and 2.16 (ddd, *J* = 10.27, 4.84, 1.88, H-4$_{eq}$), 1.58 (t, *J* = 7.35, 5.16, H-4$_{ax}$), 1.11 [s, C(CH$_3$)$_2$], 1.07 [s, C(CH$_3$)$_2$]; ^{13}C NMR (100 MHz) 179.2, 176.4, 156.8, 137.7, 128.4, 127.8, 127.7, 116.9, 114.1, 92.2, 73.4, 72.7, 71.1, 50.7, 38.9, 38.7, 33.4, 26.9; IR ($CHCl_3$) 3414, 1731, 1195, 1079; HR-FAB-MS m/z 540.2201 (calcd for MNa^+ 540.2185).

A solution of 240 mg (0.46 mmol) of the dipivaloate and 0.20 mL (1.95 mmol) of thiophenol in 8 mL of dichloromethane was cooled to 0 °C and treated with 0.19 mL (1.54 mmol) of boron trifluoride etherate. The cooling bath was removed and the reaction mixture was stirred at room temperature. TLC analysis after 5 h indicated completion of the reaction. The reaction mixture was diluted with 100 mL of dichloromethane and 75 mL of water. The organic layer was washed with 75 mL of saturated aqueous sodium bicarbonate and then with 50 mL of brine, dried over sodium sulfate, concentrated, and then chromatographed with 5:1 hexane / ethyl acetate as the eluant to give 205 mg (84%) of thioglycoside **6** as a white crystalline solid, mp 109-110 °C, whose spectroscopic properties (^{1}H NMR, ^{13}C NMR, FAB-MS) matched those of authentic material as prepared previously by the published route.

1-[3,7-Anhydro-5-azido-5-deoxy-2-*O*-(2,2-dimethylpropionyl)-6,8-*O*-(phenylmethylene)-D-*erythro*-α-D-*allo*-octofuranosyl]- 1H,3H-5-iodo-pyrimidine-2,4-dione (9). To a solution of 310 mg (1.21 mmol) of *O*,*O*′-bis(trimethylsilyl)uracil in

6.5 mL of dry 1,2-dichloroethane was added 130 mg (0.58 mmol) of N-iodosuccinimide, and the solution was stirred for 5 min. A solution of 130 mg of the thioglycoside **7** in 6 mL of dry 1,2-dichloroethane followed by 0.048 mL (0.54 mmol) of trifluoromethanesulfonic acid was added, and the reaction mixture was stirred at room temperature for 1 h. TLC analysis at this point indicated only traces of product in the reaction mixture. An additional 102 mg (0.45 mmol) of N-iodosuccinimide followed by 0.014 mL (0.16 mmol) of trifluoromethanesulfonic acid was added, and stirring was continued at room temperature. TLC analysis after 1.5 h indicated complete consumption of starting material and formation of two lower R_f products. The reaction mixture was diluted with 75 mL of dichloromethane, quenched with 5 mL of 10 % saturated sodium thiosulfate, and stirred until it turned to pale yellow color. It was further diluted with 25 mL of dichloromethane and 25 mL of saturated aqueous sodium bicarbonate. The organic layer was washed with 50 mL of brine, dried over anhydrous sodium sulfate, concentrated, and chromatographed with 1:1 hexane / ethyl acetate as the eluant to give 105 mg (65%) of iodinated nucleoside **9** and 19 mg (8 %) of nucleoside **8** as foamy white solids: (For the iodide **9**) ^{1}H NMR (300 MHz) 9.19 (br s, NH), 8.06 (s, H-6), 7.36-7.44 (m, 5 Ph-H), 5.82 (s, H-1'), 5.52 (s, C<u>H</u>Ph), 5.47 (d, *J* = 5.1, H-2'), 4.38-4.47 (m, H-4', H-5'), 4.29 (d, *J* = 12.5, H-8'), 4.18 (dd, *J* = 3.1, 0.9, H-6'), 4.00-4.09 (m, H-3', H-8'), 3.75 (s, H-7'), 1.26 [s, C(C<u>H</u>$_3$)$_3$]; ^{13}C NMR (75 MHz) 177.1, 160.0, 149.5, 144.7, 137.4, 129.7, 128.5, 126.4, 101.9, 90.8, 75.0, 73.1, 71.9, 69.4, 69.1, 68.9, 60.3, 39.3, 27.4; IR (film) 3380, 2110, 1722, 1696, 1265, 1136; HR-FAB-MS m/z 640.0903 (calcd for MH^+ 640.0904). (For **8**) ^{1}H NMR (400 MHz) 8.39 (br s, NH), 7.27-7.37 (m, 5 Ph-H, H-6), 5.80 (s, H-1'), 5.74 (d, *J* = 8.14, H-5), 5.46 (s, C<u>H</u>Ph), 4.29-4.38 (m, H-4',

H-5'), 4.23 (d, *J* = 12.8, H-8'), 4.09 (dt, *J* = 3.1, 1.2, H-6'), 3.97-4.02 (m, H-3', H-8'), 3.66 (s, H-7'), 1.20 (s, C(C<u>H</u>$_3$)$_3$]; ^{13}C NMR (100 MHz) 177.0, 162.0, 149.4, 139.7, 137.2, 129.4, 128.3, 127.0, 126.1, 103.1, 101.7, 90.6, 74.8, 74.5, 72.9, 71.9, 69.2, 68.7, 59.8, 38.9, 27.0; IR (film) 3387, 2109, 1723, 1698, 1269, 1119; HR-FAB-MS m/z 514.1950 (calcd for MH$^+$ 514.1938).

1-[3,7-Anhydro-5-azido-8-*O*-benzyl-5-deoxy-2-*O*-(2,2-dimethylpropionyl)- D-*erythro*-α-D-*allo*-octofuranosyl]- 1H-3-(benzyloxymethyl)-5-iodo-pyrimidine-2,4-dione (10). A solution of 123 mg (0.19 mmol) of 5-iodo nucleoside **9** in dry THF was cooled to 0 °C, treated with 0.063 mL (0.22 mmol) of 2-*tert*-tutylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BEMP, commercially available from Fluka) and then stirred for 5 min. It was then treated with 0.035 mL (0.234 mmol) of benzyl-(chloromethyl) ether (94%, purchased from Acros) and stirring was continued for 45 min. The reaction mixture was partitioned between 75 mL of dichloromethane and 50 mL of water. The organic layer was washed with 35 mL of saturated sodium bicarbonate followed by 50 mL of brine, dried over anhydrous sodium sulfate, concentrated, and then chromatographed with 2:1 hexane / ethyl acetate as the eluant to give 138 mg (95 %) of the N-3-protected nucleoside as a foamy white solid: ^{1}H NMR (300 MHz) 7.97 (s, H-6), 7.27-7.43 (m, 10 Ph-H), 5.80 (s, H-1'), 5.53 (m, C<u>H</u>Ph, NC<u>H</u>$_2$O), 5.42 (d, *J* = 4.8, H-2'), 4.69 (br s, C<u>H</u>$_2$Ph), 4.39-4.48 (m, H-4', H-5'), 4.31 (d, *J* = 12.1, H-8'), 4.19 (m, H-6'), 4.08 (dd, *J* = 12.8, 1.8, H-8'), 3.94 (dd, *J* = 9.9, 4.8, H-3'), 3.75 (br s, H-7'), 1.27 [s, C(C<u>H</u>$_3$)$_3$]; ^{13}C NMR (75 MHz) 176.9, 159.7, 150.1, 143.3, 138.0, 137.4, 129.7, 128.6, 128.5, 128.0, 127.8, 126.4, 101.9, 91.1, 75.1, 73.0, 72.8, 72.3, 71.9, 69.4, 69.1, 68.6, 60.4,

39.2, 27.5; IR (film) 2110, 1717, 1669, 1276, 1119; HR-FAB-MS m/z 760.1480 (calcd for MH^+ 760.1479).

A suspension of 70 mg (0.09 mmol) of the N-3-protected nucleoside (from above), 85 mg (1.12 mmol) of 85 % sodium cyanoborohydride, 6 mL of THF, and 85 mg of activated 3 Å molecular sieves was treated dropwise with a solution (purchased from Aldrich) of 1 M anhydrous HCl in ether (~4.5 mL over 3 h) until TLC analysis of the reaction mixture indicated complete disappearance of the starting material. The reaction was diluted with 45 mL of dichloromethane and 10 mL of water and stirred for 5 min, then filtered through a cotton plug. The aqueous layer was washed with an additional 20 mL of dichloromethane, and the combined organic extract was washed with 20 mL of saturated aqueous sodium bicarbonate, and then dried over anhydrous sodium sulfate. The crude product was concentrated and chromatographed with 2:1 hexane / ethyl acetate as the eluant to give 60 mg (85%) of the benzyl ether **10** as a colorless oil: ^{1}H NMR (400 MHz) 7.98 (s, H-6), 7.26-7.36 (m, 10 Ph-H), 5.77 (s, H-1'), 5.53 (s, $NC\underline{H}_2O$), 5.36 (d, J = 4.8, H-2'), 4.69 (s, $NCH_2OC\underline{H}_2Ph$), 4.58 and 4.52 (ABq, J = 11.8, $OC\underline{H}_2Ph$), 4.39-4.47 (m, H-4', H-5'), 4.25 (s, H-6'), 4.11 (d, J = 3.3, $O\underline{H}$), 3.80-3.88 (m, H-3', H-7', 2 H-8'); ^{13}C NMR (75 MHz) 176.0, 159.0, 149.9, 143.0, 137.8, 136.6, 128.6, 128.3, 127.7, 127.6, 90.7, 74.7, 74.6, 74.1, 72.8, 72.6, 71.9, 71.2, 60.9, 38.9, 27.1; IR (film) 3400, 2107, 1749, 1722, 1668, 1144; HR-FAB-MS m/z 784.1444 (calcd for MNa^+ 784.1456).

Nucleoside Disaccharide 11: Glycosylation of Nucleoside 10. A solution of 57.7 mg (0.076 mmol) of nucleoside **10** and 144 mg (0.274 mmol) of the thioglycoside **6** in 6 mL of 1,2-dichloroethane containing 136 mg of activated 3 Å molecular sieves was charged with 65.5 mg (0.291 mmol) of N-iodosuccinimide and stirred at room temperature

for 7 min and then treated with 4.20 μL (0.047 mmol) of trifluoromethanesulfonic acid. TLC analysis after 20 min indicated formation of a lower R_f product. The reaction mixture was allowed to stir for another 20 min, diluted with 50 mL of dichloromethane, quenched with 5 mL of 10 % saturated sodium thiosulfate, and then stirred until it turned to a pale yellow color. It was partitioned between 25 mL of dichloromethane and 25 mL of saturated sodium bicarbonate. The organic layer was washed with 50 mL of brine, dried over anhydrous sodium sulfate, concentrated, and then chromatographed with 5:2 hexane / ethyl acetate as the eluant to give 69 mg (78 %) of disaccharide **11** as a foamy white solid: ^{1}H NMR (400 MHz) 7.90 (s, H-6), 7.26-7.39 (m, 15 Ph-H), 6.54 (d, *J* = 7.7, NH), 5.74 (s, H-1'), 5.53 (s, NCH$_2$O), 5.35 (d, *J* = 5.1, H-2'), 4.69 (m, H-3'', NCH_2OCH_2Ph), 4.61 (d, *J* = 7.7, H-1''), 4.51 (m, OCH_2Ph), 4.45 (br s, OCH_2Ph), 4.30 (t, *J* = 3.3, H-5'), 4.17-4.23 (m, H-3''), 4.13 (dd, *J* = 10.30, 3.4, H-4'), 4.03 (t, *J* = 4.9; H-7'), 3.96 (m, H-6'), 3.83 (dd, *J* = 10.3, 5.1, H-3'), 3.75-3.79 (m, H-5''), 3.69 (dd, *J* = 10.6, 5.1, H-8'), 3.42-3.55 (m, H-8', 2 H-6''), 2.22 (br dd, *J* = 12.8, 5.1, H-4eq''), 1.59 (q, *J* = 12.5, H-4ax''), 1.22 [br s, two $C(CH_3)_3$]; ^{13}C NMR (75 MHz) 211.17, 178.9, 176.4, 159.6, 150.1, 142.9, 138.4, 137.9, 137.7, 128.8, 128.6, 128.6, 128.2, 127.9, 127.9, 127.8, 127.6, 99.9, 90.7, 78.4, 77.5, 76.6, 75.1, 74.2, 73.8, 73.6, 72.8, 72.3, 71.8, 71.5, 69.2, 68.7, 59.4, 51.2, 39.3, 33.7, 27.3, 27.3; IR (film) 3412, 2109, 1729, 1669, 1277, 1166; HR-FAB-MS 1199.3020 (calcd for MNa^+ 1199.3062).

N-Benzoylurea 12. A solution of 19 mg (0.016 mmol) of the nucleoside disaccharide **11** in 9 mL of ethyl acetate was stirred with 65 mg of 10% palladium-on-carbon at room temperature under a hydrogen atmosphere for 2 h. TLC analysis at this point indicated formation of a slower spot and complete disappearance of starting

material. The catalyst was removed by filtration through a Celite pad, and the filter cake was washed with 25 mL of ethyl acetate. The combined filtrate was concentrated to give a reddish brown solid product, which was chromatographed with 2:1 hexane / ethyl acetate as the eluant to give 13.5 mg (80%) of the de-iodinated nucleoside as a white solid: ^{1}H NMR (400 MHz) 7.28-7.38 (m, 15 Ph-H, H-6), 6.57 (d, *J* = 8.1, NH), 5.83 (d, *J* = 7.7, H-5), 5.80 (s, H-1'), 5.49 (s, NCH_2O), 5.34 (d, *J* = 5.1, H-2'), 4.68-4.73 (m, H-2'', NCH_2OCH_2Ph), 4.61 (d, *J* = 7.7, H-1''), 4.51 (s, OCH_2Ph), 4.45 (s, OCH_2Ph), 4.30 (t, *J* = 3.3, H-5'), 4.18-4.25 (m, H-3''), 4.10 (dd, *J* = 10.3, 3.3, H-4'), 4.02 (t, *J* = 5.1, H-7'), 3.96 (m, H-6'), 3.88 (dd, *J* = 9.9, 5.1, H-3'), 3.75-3.78 (m, H-5''), 3.69 (dd, *J* = 10.6, 5.1, H-8'), 3.54 (dd, *J* = 10.6, 6.2, H-8'), 3.43-3.50 (m, two H-6''), 2.24 (br d, *J* = 12, H-4_{eq}''], 1.51-1.65 (m, obscured by H_2O peak, H-4ax''), 1.23 [s, $C(CH_3)_3$], 1.21 [s, $C(CH_3)_3$]; ^{13}C NMR (75 MHz) 211.7, 179.0, 176.6, 162.4, 157.0, 162.4, 150.6, 138.4, 138.0, 137.9, 137.7, 128.7, 128.6, 128.5, 128.2, 127.9, 127.9, 127.6, 113.8, 103.0, 99.8, 90.7, 77.5, 76.5, 74.7, 74.1, 73.8, 73.6, 73.1, 72.6, 72.3, 72.0, 71.8, 71.5, 70.7, 69.2, 59.1, 39.3, 39.1, 33.7, 27.3, 27.3, 21.2; IR (film) 2107, 1729, 1679, 1602, 1163; HR-FAB-MS 1051.4305 (calcd for MH^+ 1051.4276).

A solution of 10 mg (0.0095 mmol) of the de-iodinated nucleoside in 8 mL of THF was stirred with 50 mg of Lindlar catalyst at room temperature under a hydrogen atmosphere for 2 h. TLC analysis at this point indicated formation of a very polar spot, presumably the amine, and complete disappearance of starting material. The catalyst was removed by filtration through a Celite pad, and the filter cake was washed with 30 mL of THF. The combined filtrate was concentrated at room temperature to give the amine, which was used without purification for the next step.

A solution of the amine in 2 mL of dichloromethane was treated with 0.41 mL (0.022 mmol) of a 0.053 M solution of benzoylisocyanate at room temperature. TLC analysis after 2 h indicated completion of the reaction. The reaction mixture was concentrated and the crude product was chromatographed with 1:1 hexane / ethyl acetate as the eluant to give 7.5 mg (67 %) of the N-benzoylurea **12** as a white solid: ^{1}H NMR (300 MHz) 9.20 (d, *J* = 5.49, N<u>H</u>CONHCOPh), 8.42 (br s, N<u>H</u>COPh), 7.75 (d, *J* = 7.69, 2 Ar-H_o), 7.62 (t, *J* = 7.69, Ar-H_p), 7.47 (t, *J* = 7.69, 2 Ar-H_m), 7.36 (d, *J* = 8.06, H-6), 7.15-7.29 (m, 15 Ph-H's), 6.47 (d, *J* = 8.79, N<u>H</u>COCF$_3$), 5.77 (s, H-1'), 5.73 (d, *J* = 8.06, H-5), 5.42 (s, NC<u>H</u>$_2$O), 5.36 (d, *J* = 5.13, H-2'), 4.61-4.70 (m, NCH$_2$OC<u>H</u>$_2$Ph, H-1'', H-2''), 4.45-4.58 (m, H-5'), 4.43 (s, OC<u>H</u>$_2$Ph), 4.37 (s, OC<u>H</u>$_2$Ph), 4.10-4.14 (m, H-3'', H-4', H-6'), 3.96-3.97 (m, H-7'), 3.80 (dd, *J* = 5.13, 10.6, H-3'), 3.66 (dd, *J* = 10.6, 4.03, H-8'), 3.60 (m H-5''), 3.52 (dd, *J* = 10.6, 6.2, H-8'), 3.32-3.43 (m, two H-6''), 2.13 (ddd, *J* = 13.2, 5.5, H-4eq''), 1.49 (q, obscured by H_2O peak, H-4ax''), 1.17 [br s, two C(C<u>H</u>$_3$)$_3$]; ^{13}C NMR (75 MHz) 211.0, 179.0, 176.7, 168.6, 162.5, 150.7, 138.4, 138.0, 137.9, 134.3, 129.4, 128.7, 128.6, 128.5, 128.1, 127.9, 127.8, 127.7, 105.0, 103.0, 99.9, 90.9, 77.5, 77.1, 73.8, 73.7, 73.0, 72.6, 71.9, 71.5, 69.9, 51.1, 49.5, 39.3, 39.1, 30.4, 27.4, 27.3, 27.3; IR (film) 3400, 1723, 1665, 1269; HR-FAB-MS 1194.4471 (calcd for MH$^+$ 1194.4510).

Protected 4-Desamino-4-oxo-ezomycin A_2 (13). A solution of 9 mg (0.0077 mmol) of **12** in 7 mL of 15:1 ethyl acetate / ethanol was stirred with 27 mg of 20% palladium-hydroxide-on-carbon at room temperature under a hydrogen atmosphere for 2 h. TLC analysis at this point indicated formation of a very polar spot and complete disappearance of starting material. The catalyst was removed by filtration through a Celite pad, and the filter cake was washed with 25 mL of ethanol. The combined filtrate

was concentrated and then chromatographed with 97:3 ethyl acetate / methanol as the eluant to give 5.6 mg (84 %) of the diol as a white solid: ^{1}H NMR (300 MHz) 7.95-7.93 (m, 3 NH), 7.72 (d, *J* = 8.05, H-6), 7.62-7.66 (m, 2 Ar-H_o), 7.51-7.55 (m, Ar-H_p, two Ar-H_m), 5.77 (s, H-1'), 5.68 (d, *J* = 8.05, H-5), 5.51 (d, *J* = 5.57, H-2'), 4.78-4.83 (m, H-1'', H-2''), 4.58 (br t, *J* = 3.71, H-5'), 4.33-4.41 (m, H-3''), 4.24-4.25 (m, H-6'), 4.18 (dd, *J* = 4.33, 11.14, H-4'), 4.03 (dd, *J* = 4.95, 10.52, H-3'), 3.86 (t, *J* = 4.95, 10.71, H-7'), 3.80 (dd, *J* = 6.81, 11.76, H-8'), 3.74-3.80 (m, partially overlapped with H-8', H-5''), 3.53-3.64 (m, H-8', two H-6''), 1.88 (ddd, *J* = 1.99, 4.95, 11.76, H-4eq''), 1.64, (q, *J* = 12.38, H-4ax''), 1.24 [br s, C(CH$_3$)$_3$], 1.22 [br s, C(CH$_3$)$_3$]; ^{13}C NMR (75 MHz, DMSO-D_6) 201.7, 175.5, 175.2, 168.6, 162.3, 152.8, 149.3, 140.6, 132.4, 131.6, 127.9, 127.6, 117.1, 103.7, 101.4, 99.4, 88.9, 73.3, 72.4, 71.5, 64.7, 62.6, 59.6, 37.7, 31.5, 26.1; IR (film) 3412, 1727, 1700, 1544, 1160; HR-FAB-MS 872.3139 (calcd for MH^+ 872.3177).

A solution of 5.0 mg (0.0057 mmol) of the diol in 1.5 mL of 1:1 MeCN / H_2O was treated sequentially with 19.5 mg (0.0605 mmol) of iodobenzenediacetate, 1.7 mg (0.0109 mmol) of TEMPO, and 3.2 mg (0.0381 mmol) of sodium bicarbonate, and the reaction mixture was stirred at room temperature for 4 h. HPLC-MS analysis of the reaction mixture at this point indicated complete consumption of the starting material. Formation of the desired diacid along with the intermediate aldehyde / acid was determined by the detection of the corresponding molecular ions in the HPLC-MS analysis. The reaction mixture was diluted with 2 mL of water and lyophilized to give an off-white solid.

The solid obtained was dissolved in 3 mL of 1:1 *t*-BuOH / water and treated sequentially with 13 mg (0.094 mmol) of NaH_2PO_4, 45 μL (0.425 mmol) of 2-methyl-2-butene, and 10.3 mg (0.114 mmol) of $NaClO_2$, and then the reaction mixture was stirred

for 1 h. HPLC analysis indicated complete conversion of the intermediate aldehyde / acid to the desired diacid product. The reaction mixture was diluted with 2 mL of water and lyophilized to give an off-white solid. The crude product was dissolved in 800 µL of 10% MeCN / 90% H_2O / 0.1% TFA solution and applied to a Monitor C-18 column (1.0 X 10 cm, Column Engineering, Ontario, Canada). The column was eluted at 5 mL / min with a linear gradient of 10% MeCN / 90% H_2O / 0.1% TFA to 50% MeCN / 50% H_2O / 0.1% TFA over 30 min with detection at 215 nm. The fractions containing the product were combined and concentrated to give 3.0 mg (58%) of protected nucleoside disaccharide **13** as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) 11.49 (d, J = 1.95, N<u>H</u>CONHCOPh), 11.04 (s, N<u>H</u>COPh), 9.54 (d, J = 8.79, N<u>H</u>COCF_3), 9.11 (br s, uracil-N<u>H</u>), 7.99 (d, J = 6.84, two Ar-H_o), 7.66 (d, J = 7.81, H-6), 7.64 (t, obscured, Ar-H_p), 7.54 (t, J = 8.79, two Ar-H_m), 5.73 (s, H-1'), 5.62 (d, J = 7.81, H-5), 5.51 (d, J = 4.88, H-2'), 4.85 (d, J = 7.81, H-1''), 4.69 (t, J = 9.77, H-2''), 4.47-4.52 (m, H-3''), 4.37-4.46 (m, H-5', H-6', H-7'), 4.33 (d, J = 11.72, H-4'), 3.95-4.04 (m, H-3', H-5''), 1.98-2.00 (m, H-4eq''), 1.83 (q, J = 12.69, H-4ax''), 1.20 [s, C(C<u>H</u>$_3$)$_3$], 1.15 [br s, C(C<u>H</u>$_3$)$_3$]; IR (film) 3459, 1699, 1634, 1161; HR-FAB-MS 900.2761 (calcd for MH^+ 900.2762).

4-Desamino-4-oxo-ezomycin-A_2 (1). A solution of 3.0 mg (0.0033 mmol) of the diacid in 1.5 mL of 7 N ammonia in methanol (purchased from Fluka) was heated in a sealed tube at 55 °C for 20 h. The solution was cooled to room temperature and concentrated on the rotary evaporator. HPLC analysis of the crude product at this point indicated complete consumption of the starting material, with formation of a very polar product. The crude product was dissolved in 800 µl of 10% MeCN / 90% H_2O / 0.1% TFA solution and applied to a 5µ Spherisorb ODSI column (4.6 x 25 cm, Phase

Separations). The column was eluted at 5 mL / min with a linear gradient of 0% MeCN / H_2O / 0.1% TFA to 25% MeCN / 75% H_2O / 0.1% TFA over 20 min with detection at 261 nm. The fractions obtained were combined and concentrated to give 1.2 mg (65%) of 4-desamino-4-oxo-ezomycin-A_2 (**1**) as a colorless film: ^{1}H NMR (400 MHz, D_2O) 7.58 (d, *J* = 8.1, H-6), 5.86 (d, *J* = 8.4, H-5), 5.76 (s, H-1'), 4.79-4.82 (m, H-5', H-6'), 4.71 (d, *J* = 6.6, H-1''), 4.51 (d, *J* = 4.9, H-2'), 4.44 (br s, H-7'), 4.35 (dd, *J* = 10.9, 4.3, H-4'), 4.11 (br d, *J* = 12.1, H-5''), 3.79 (dd, *J* = 10.9, 4.9, H-3'), 3.42-3.49 (m, H-2'', H-3''), 2.42 (br d, *J* = 12.1, H-4eq''), 1.74 (br q, *J* = 12.1, H-4ax''); UV λ_{max} (D_2O) 261 nm; HR-MS (collision induced dissociated mass spectrum; the sample was infused from a nanospray capillary into a Finnegan electrospray source) 532.12 (calcd for MH^+ 532.15); also observed were the fragment ions for the ezoaminuroic acid unit MH^+ 160.06 (calcd for $C_6H_9NO_4H^+$ 160.06) and the ezomycin octosyl acid unit MH^+ 373.04 (calcd for $C_{13}H_{16}N_4O_9H^+$ 373.10). The Table on the following page shows the ^{1}H NMR spectrum of **1** in comparison to that of ezomycin A_2.

Table. ^{1}H-NMR comparison of ezomycin A_2 and 4-desamino-4-oxo-ezomycin A_2 (1)

compound ⇒	**ezomycin A_2**	**4-Desamino-4-oxo-ezomycin A_2 (1)**
Solvent ⇒	**2% ND_3 in D_2O (100 MHz)**	**D_2O (400 MHz)**
Proton ⇓	**Multiplicity, chemical shift (coupling constant)**	**Multiplicity, chemical shift (coupling constant)**
H-5	d, 6.13 (8.0)	d, 5.86 (8.4)
H-6	d, 7.69 (8.0)	d, 7.58 (8.1)
H-1'	s, 5.88	s, 5.76
H-2'	m, ~4.4 - 4.6	d, 4.51 (4.9)
H-3'	dd, 3.91 (11.0, 5.0)	dd, 3.79 (10.9, 4.9)
H-4'	m, ~4.4 - 4.6	dd, 4.35 (10.9, 4.3)
H-5'	m, ~4.4 - 4.6	m, 4.79 - 4.82
H-6'	br t, 4.98	m, 4.79 - 4.82
H-7'	m, ~4.4 - 4.6	br s, 4.44
H-1''	d, 4.74 (6.5)	d, 4.71 (6.6)
H-2''	m, 3.2	m, 3.42 - 3.49
H-3''	m, 3.2	m, 3.42 - 3.49
H-4eq''	br d, 2.33 (11.0)	br d, 2.42 (12.1)
H-4ax''	br q, 1.55 (11.0)	br q, 1.73 (12.1)
H-5''	br d, 4.18	br d, 4.11 (12.1)