Glycosyltransferase-catalysed Stereoselective Glycosidation of Monosaccharide-based Glycosidase Inhibitors: a New Approach to the Synthesis of Sequence-specific Glycosidase Inhibitors

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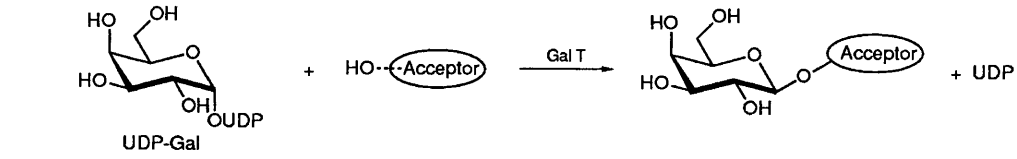
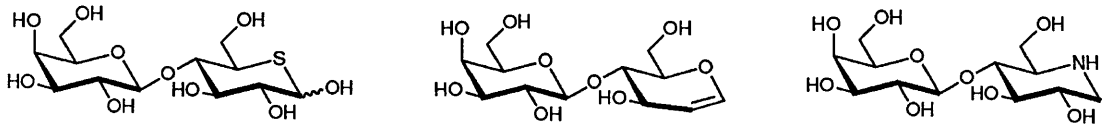
β-1,4-Galactosyltransferase was used as catalyst for galactosidation of 5-thioglucose, glucal and 1-deoxynojirimycin to form the corresponding β-1,4-galactosides as potential sequence-specific glycosidase inhibitors.

A number of procedures are available for the effective synthesis of monosaccharide-based glycosidase inhibitors.[1] Stereoselective glycosidation of these glycosidase inhibitors for the preparation of sequence-specific glycosidase inhibitors, however, still represents a significant problem; different protection and deprotection strategies are often required for the synthesis.[2] We report here the use of β-1,4-galactosyltransferase (GalT, EC 2.4.1.22)[3] for stereoselective galactosidation of three glucosidase inhibitors,[4] including 5-thioglucose, glucal and 1-deoxynojirimycin (DNJ), to form the corresponding β-1,4-galactosides, **1**, **2** and **3**. 5-Thioglucose was indicated to be a substrate for GalT but no isolation and characterization of the product **3** was reported. Compound **2** was prepared previously by a chemical method.[2d]





1 2 3

In a representative procedure for the synthesis of **1**, 5-thioglucose (0.1 g), GalT (5U), UDP-glucose (0.35 g), α-lactalbumin (0.1 mg cm^{-3}) and UDP-glucose epimerase (10U) were dissolved in 10 cm^3 of 50 mmol dm^{-3} sodium cacodylate (pH 7.0) containing 5 mmol dm^{-3} of $MnCl_2$. The reaction mixture was incubated at 37 °C for 2 days. The product was isolated *via* a Dowex 1 formate column followed by gel filtration (Bio Gel P-2) to give 90 mg of **1** in 50% yield.[5] For the synthesis of **2** and **3**, longer reaction times (4 days) were required and each was obtained in 20–40% yield.† The lower yields in the latter syntheses were due to the incomplete reaction; no by-product was observed in the reactions. The assignment of the glycosidic linkage was based upon ^1H and ^{13}C NMR data. A downfield shift for the 4-H proton and C-4 of the acceptor moiety relative to the starting substrate was observed.

These syntheses demonstrate that glycosyltransferases can be used as catalysts for stereoselective glycosidation of various glycosidase inhibitors. With an appropriate choice of glycosyltransferase, a number of sequence-specific glycosidase inhibitors perhaps can be prepared based on the illustrated methodology. The major advantage of this approach is that glycosyltransferases are highly specific with regard to the glycosidic linkage and no protection–deprotection steps are required. Procedures for the regeneration of sugar nucleotides are also available for large-scale processes.[5] Given that glycosyltransferases are relatively stable[5,6] and readily available through cloning techniques,[7] a number of novel and unprotected oligosaccharide structures may become accessible *via* glycosyltransferase reactions. Work is in progress to explore the unusual catalytic properties of other glycosyltransferases.

This work was supported by the NIH.

Received, 15th April 1991; Com. 1/01735G

† For **1**: ^1H NMR (D_2O, 500 MHz) δ 4.95 (d, $J_{1,2}$ 3 Hz, H-1α 5ThioGlc), 4.51 (d, $J_{1,2}$ 8 Hz, H-1 Gal), 4.05 (dd, $J_{5,6}$ 5, $J_{6,6}$ 12 Hz, H-6′ 5ThioGlc), 3.83–3.9 (H-4 Gal, H-4 5ThioGlc, H-6 5ThioGlc), 3.8 (dd, $J_{2,3}$ 9.6 Hz, H-2 5ThioGlc), 3.65–3.75 (H-5 Gal, H-6 Gal, H-6′ Gal, H-3 5ThioGlc), 3.62 (dd, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3 Gal), 3.53 (dd, H-2 Gal), 3.32 (ddd, $J_{4,5}$ 10.5, $J_{5,6}$ 2.5, $J_{5,6}$ 5 Hz, H-5 5-ThioGlc); ^{13}C NMR (125 MHz, D_2O) δ for Gal 103.2 (C-1), 71.7 (C-2), 72.7 (C-3), 69.0 (C-4), 75.8 (C-5), 61.5 (C-6); for ThioGlc 73.3 (C-1), 75.7 (C-2), 73.0 (C-3), 82.3 (C-4), 42.5 (C-5), 59.7 (C-6); HRMS (M + Cs^+) calc. 490.9988, found *m/z* 491.0022. For **2**, ^1H NMR (500 MHz, D_2O) δ 6.4 (dd, $J_{1,2}$ 6, $J_{1,3}$ 1.6 Hz, H-1 Glucal), 4.79 (dd, $J_{2,3}$ 2.6 Hz, H-2 Glucal), 4.49 (d, $J_{1,2}$ 7.8 Hz, H-1 Gal), 4.35 (br dt, $J_{2,3}$ 2.6, $J_{3,4}$ 6.5 Hz, H-3 Glucal), 3.99 (br dt, $J_{4,5}$ 9.3, $J_{5,6}$ = $J_{5,6'}$ = 3.7 Hz, H-5 Glucal), 3.85–3.9 (H-4 Gal, H-6 Glucal, H-6′ Glucal), 3.82 (dd, H-4 Glucal), 3.68–3.75 (H-5 Gal, H-6 Gal, H-6′ Gal), 3.63 (dd, $J_{2,3}$ 10.0, $J_{3,4}$ 3.4 Hz, H-3 Gal), 3.5 (dd, $J_{1,2}$ 8.6 Hz, H-2 Gal); ^{13}C NMR (125 MHz, D_2O) δ for Gal 103.9 (C-1), 71.9 (C-2), 73.5 (C-3), 69.5 (C-4), 76.3 (C-5), 62.0 (C-6); for glucal 144.9 (C-1), 102.7 (C-2), 68.3 (C-3), 78.4 (C-4), 77.7 (C-5), 60.6 (C-6); HRMS (M + Cs^+) calc. 441.0162, found *m/z* 441.0121. For **3**, ^1H NMR (D_2O, 500 MHz) δ 4.3 (d, $J_{1,2}$ 7.5 Hz, H-1 Gal), 3.76 (dd, $J_{5,6}$ 3.0 Hz, $J_{6,6'}$ 12.5 Hz, H-6′ DNJ), 3.74 (br d, *J* 3 Hz, H-4 Gal), 3.7 (dd, $J_{5,6}$ 5.0 Hz, H-6 DNJ), 3.52–3.65 (m, H-5 Gal, H-6 Gal, H-6′ Gal, H-2 DNJ, H-4 DNJ), 3.5 (dd, $J_{2,3}$ 10.5, $J_{3,4}$ 3.5 Hz, H-3 Gal), 3.39 (t, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3 DNJ), 3.38 (dd, H-2 Gal), 3.13 (dd, $J_{1eq,2}$ 5.0, $J_{1eq,1ax}$ 12.5 Hz, H-1*eq* DNJ), 2.85–2.90 (m, H-5 DNJ), 2.56 (br t, *J* 12 Hz, H-1*ax* DNJ); ^{13}C NMR (125 Hz, D_2O), δ for Gal 103.7 (C-1), 71.7 (C-2), 73.2 (C-3), 69.2 (C-4), 76.3 (C-5), 61.8 (C-6); for DNJ 47.4 (C-1), 69.04 (C-2), 76.2 (C-3), 78.9 (C-4), 59.4 (C-5), 60.0 (C-6). HRMS (M + Cs^+) calc. 458.0427, found *m/z* 458.0444.

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