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Chemical synthesis of uridine 5′-diphospho-α-D-xylopyranose

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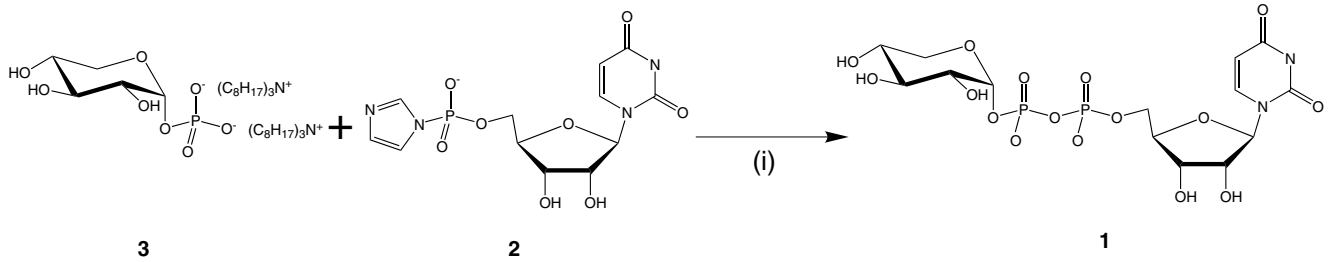
Abstract—Uridine 5′-diphospho-α-D-xylopyranose, which donates D-xylose during glycoconjugate biosynthesis, was chemically synthesized from α-D-xylose 1-phosphate and uridine 5′-monophosphoimidazolide.

1. Introduction

D-Xylose is widely distributed in nature and is a component of various glycoconjugates, such as proteoglycans[1] and *O*-glucose type glycans[2] of animal cells; polysaccharides of plant cell walls;[3] *N*-glycans[4] of plants and some invertebrates; and capsular polysaccharides[5] of fungi. Uridine 5′-diphospho-α-D-xylopyranose **1** is a substrate for xylosyltransferase, which transfers the D-xylose to glycoconjugates. Biosynthesis of **1** is mediated by UDP-glucuronic acid decarboxylase with UDP-α-D-glucuronic acid as the substrate.[6] Compound **1** was enzymatically prepared using UDP-glucuronic acid decarboxylase in vitro.[6] By regioselective attack of the 1,2-anhydro sugar (D-xylal) by the nucleoside diphosphate moiety, UDP-D-xylose was chemically synthesized;[7] however, it was an α/β anomeric mixture (α/β = 5:3). Preparation of pure **1** is critical for glycoconjugate biosynthetic studies and for structural, functional, and kinetic studies of xylosyltransferases. Here, we report the chemical synthesis of **1**, using the phosphoimidazolidate activation method.[8]

2. Results and discussion

Compound **1** was prepared from the activated compound, uridine 5′-monophosphate imidazole **2** and the tri-*n*-octylammonium salt of D-xylopyranose 1-phosphate **3** (Scheme 1). Compound **2** was prepared by reaction of the UMP free acid **5** and *N*,*N*′-carbonyldiimidazole[9] (Scheme 2). Compound **3**[10] was used for the coupling reaction with **2** because **3** is soluble in pyridine, whereas other salts (ammonium, monocyclohexylammonium, pyridinium, and triethylamine) of α-D-xylopyranose 1-phosphate are not. The reaction[11] was monitored by TLC. The best yield occurred after a reaction time of 5 days. The product was purified first using Bio-gel P2 gel-filtration chromatography and then Wako-Sil-II $5C_{30}$ reverse-phase HPLC. The yield was 34–36%.



3 2 1

Scheme 1. Reagents and conditions. (i) DMF:pyridine (35:8, v/v), 5 days.

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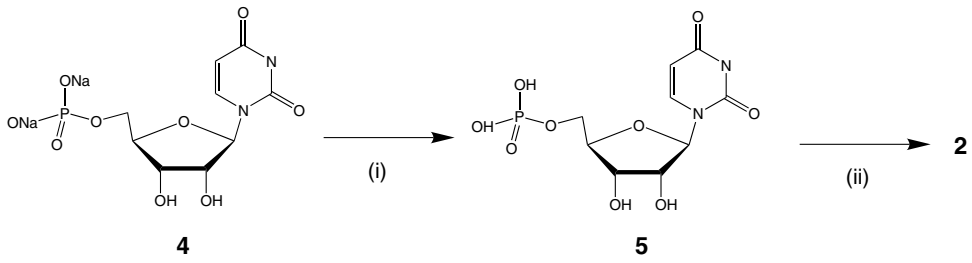

Scheme 2. Reagents and conditions. (i) Dowex H^+; (ii) *N*,*N*′-carbonyldiimidazole, DMF, 25 °C, 15 h.

The structure of **1** was confirmed using ^1H NMR spectroscopy and mass spectrometry. The anomeric configuration of **1** is α, because $J_{1'',2''}$ is 3.3 Hz. The ^1H NMR spectrum of **1** is essentially superimposable with that of enzymatically prepared **1**.[6b] The mass spectrum signal for **1** ($[M+H]^+$, 537.0) corresponds to that calculated for uridine diphosphopentose ($[M+H]^+$, 537.3).

Compound **1** is a substrate for β-D-glucoside α-1,3-xylosyltransferase,[12] which is involved in biosynthesis of the Xylα1-3Xylα1-3Glcβ1-*O*-Ser conjugate of the epidermal growth factor-like domain of coagulation factor VII and IX.[2] When 2-[(2-pyridyl)amino]ethyl β-D-glucopyranoside (Glcβ-R) was used as the acceptor in an α 1,3-xylosyltransferase assay with **1** as the D-xylose donor, Xylα1-3Glcβ-R was generated (Fig. 1).[13]

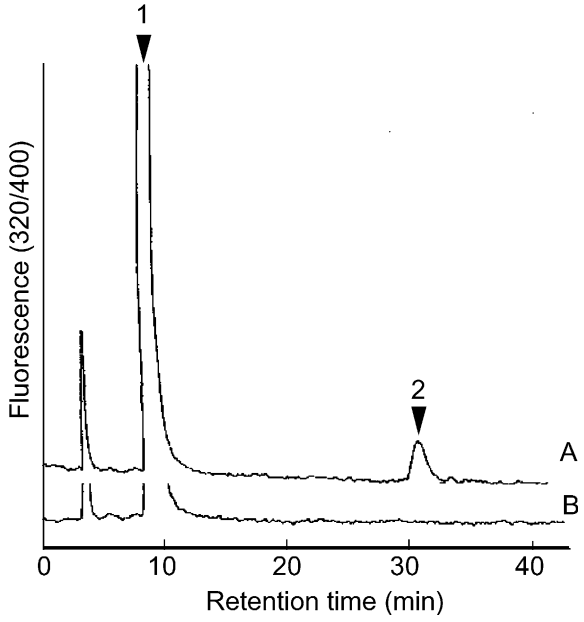

Figure 1. β-D-Glucoside:α 1,3-xylosyltransferase assay using **1** synthesized chemically as described herein. (A) A 12-h incubation in the presence of enzyme. (B) A 12-h incubation mixture in the absence of enzyme. Arrowheads indicate the elution positions of 1,2-[(2-pyridyl)amino]ethyl β-D-glucopyranoside (Glcβ-R) and 2, Xylα1-3Glcβ-R.

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9. The UMP disodium salt **4** (90 mg, 244 μmol) was dissolved in water (3 cm^3). Dowex 50W-X2 (H^+ form) was added to bring the pH of the solution to 1. The mixture was stirred at ambient temperature for 1 h and then poured into a glass column (0.5 × 10 cm). The resin was washed with 5-column volumes of water. The appropriate fractions were pooled and lyophilized. *N*,*N*′-carbonyldiimidazole (75 mg, 463 μmol) and **5** were dissolved in DMF (4.5 cm^3) and the solution stirred at room temperature for 15 h under nitrogen (Scheme 2). The reaction was monitored using TLC silica gel 60 F_{254} coated glass plates (1.5 × 3.5 cm) with chloroform-methanol–0.5 M ammonium bicarbonate (5:5:1, v/v/v) as the mobile solvent and visualized using UV light at 310 nm. Methanol (0.03 cm^3) was added to destroy excess *N*,*N*′-carbonyldiimidazole and the mixture was stirred for an additional 30 min. The mixture was concentrated and azeotroped with pyridine (2 cm^3) three times in vacuo to remove water and the residue used as **2**.
10. The di(monocyclohexylammonium) salt of α-D-xylose 1-phosphate (80 mg, 187 μmol) was dissolved in water (1.5 cm^3) and chromatographed over Dowex 50W-X2 (pyridinium form; 0.5 × 10 cm column). The resin was washed with water (5 cm^3) and the eluent concentrated to 3 cm^3. Pyridine (9 cm^3) and tri-*n*-octylamine (0.09 cm^3) were added to the concentrate, producing **3**. The solution was concentrated and azeotroped with pyridine (1 cm^3) three times.
11. Compounds **2** and **3** were dissolved in a mixture of DMF–pyridine (35:8, v/v, 4.3 cm^3) and the solution stirred at room temperature for 5 days. The reaction was monitored using TLC silica gel 60 F_{254} coated glass plates (1.5 × 3.5 cm) with 2-propanol–1 M ammonium bicarbonate (2:1, v/v) as the mobile solvent and visualized using UV

light at 310 nm. The reaction was stopped by addition of water (4.3 cm^3). Then, 100 mM ammonium bicarbonate (2 cm^3) was added and excess tri-*n*-octylamine removed by extraction with diethylether (4.3 cm^3). The sample was concentrated and applied to Bio-gel P2 (2×160 cm column) and eluted with 250 mM ammonium bicarbonate. Product **1** was detected by measuring the absorbance at 260 nm. The appropriate fractions were concentrated and further purified using Wako Sil-II5C_{30}-AR reverse-phase HPLC (0.46×25 cm column) with 50 mM ammonium acetate buffer, pH 4.5, as the eluent and a flow rate of 1 mL/min. The fractions, containing **1**, were pooled and desalted. ^{1}H NMR (600 MHz, D_2O, Me_4Si): δ 3.34 (dt, 1H, H-2″), 3.44 (td, 1H, H-4″), 3.53 (t, 1H, H-3″), 3.58 (m, 2H, H-5″), 4.04 (m, 2H, H-5′), 4.11 (m, 1H, H-3′) 4.20 (d, 2H, H-2′nd H-4′), 5.37 (dd, 1H, H-1″), 5.81, (d, 1H, H-1′), 5.79 (d, 1H, H-5), 7.79 (d, 1H, H-6) ppm; $J_{1'',2''}$ = 3.4 Hz, $J_{1'',P}$ = 7.0 Hz, $J_{2'',3''}$ = 9.6 Hz, $J_{2'',P}$ = 3.4 Hz, $J_{3'',4''}$ = 9.3 Hz, $J_{1',2'}$ = 4.1 Hz, $J_{5,6}$ = 8.3 Hz; MS (MALDI-TOF): m/z = 537.0 ($[M+H]^+$).

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13. A bovine liver microsomal fraction, 7 mM Glcβ-R, and 13 mM **1** were incubated in 20 mM HEPES–NaOH, pH 7.2, 150 mM NaCl, 0.1% TritonX-100, 20 mM $MnCl_2$ at 37 °C for 12 h. Then, the mixture was chromatographed over WakoSil-II 5C_{18}-HG (0.6×15 cm column). The product was eluted with 50 mM ammonium acetate, pH 4.5 at a flow rate of 2 mL/min. The elution was monitored by measuring the fluorescence at 400 nm (excitation at 320 nm). Structural analysis of the product, Xylα1-3Glcβ-R, has been described.[12]