

Synthesis of Pyridinium Dinitrobenzyl Sulfates and Potassium (Dinitrobenzyl β -D-Glucopyranosid)Uronates

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Sulfates and glucuronides of 2,4-dinitrobenzyl alcohol **1a** and 2,6-dinitrobenzyl alcohol **1b**, which are major or putative metabolites of 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT), were synthesized from **1a** and **1b** by reaction with pyridinium sulfonate and methyl (2,3,4-tri-*O*-acetyl- α -D-glucopyranosyl)uronate bromide **3**, respectively, as their pyridinium salts (**2a**, **2b**) and potassium salts (**6a**, **6b**). These conjugates are important for the study of the carcinogenicity of 2,4-DNT and 2,6-DNT.

Key words dinitrobenzyl sulfate; dinitrobenzyl glucuronide; dinitrotoluene; carcinogenicity

The glucuronides of dinitrobenzyl alcohols (**1a**, **1b**), which are major urinary and biliary metabolites in rats dosed with 2,4-dinitrotoluene (2,4-DNT)¹⁾ and 2,6-dinitrotoluene (2,6-DNT),²⁾ have been postulated to be precursors of mutagenic metabolites of 2,4-DNT³⁾ and 2,6-DNT.⁴⁾ In addition, the glucuronide of **1b** has been shown to be an intermediary metabolite responsible for the carcinogenicity of 2,6-DNT.⁵⁾ However, these glucuronides have not been synthesized chemically. Moreover, metabolites having a hydroxyl group are known to undergo sulfation in the metabolic process.⁶⁾

It seems likely that the secondary metabolism of these conjugates is essential for investigation of the toxic action

of 2,4-DNT and 2,6-DNT. In this note, we report the synthesis of pyridinium dinitrobenzyl sulfates (**2a**, **2b**) and potassium (dinitrobenzyl β -D-glucopyranosid)uronates (**6a**, **6b**).

Synthesis of Pyridinium Dinitrobenzyl Sulfates (2a, 2b) 2,4-Dinitrobenzyl sulfate and 2,6-dinitrobenzyl sulfate, as free sulfates, were synthesized from **1a** and **1b**, respectively, by sulfonation with chlorosulfonic acid in methylene chloride, but they were highly hygroscopic. Thus, the sulfates of **1a** and **1b** were synthesized as their pyridinium salts (**2a**, **2b**), as shown in Chart 1. Pyridinium 2,4-dinitrobenzyl sulfate **2a** and its isomer **2b** were synthesized from **1a** and **1b** by sulfonation with pyridinium

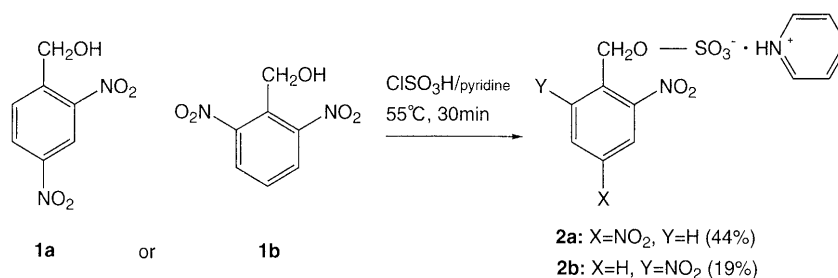


Chart 1

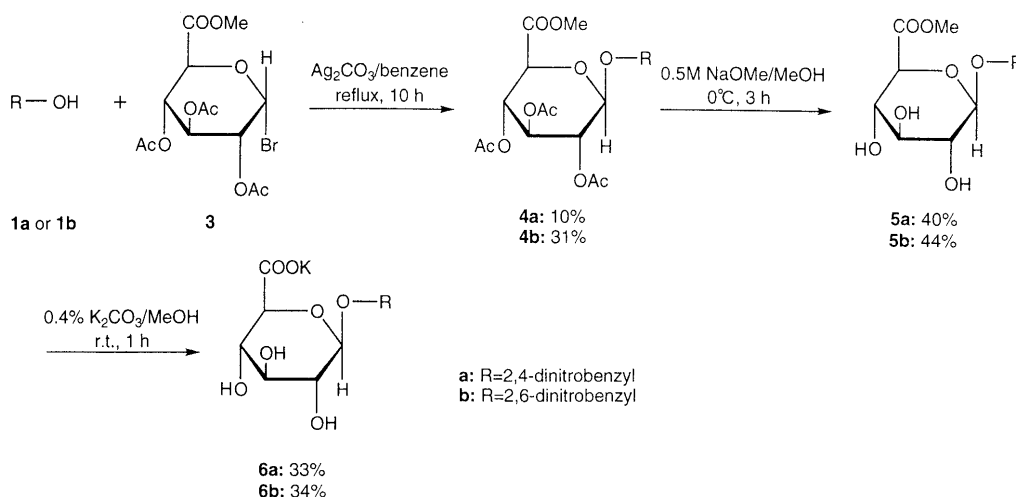


Chart 2

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sulfonate in pyridine, in yields of 44 and 19%, respectively. The signals of the pyridinium protons appeared as doublets and triplets at δ 8.00–8.88 in the $^1\text{H-NMR}$ spectra. Strong peaks due to the sulfates appeared at 1232 and 1238 cm^{-1} , respectively, in the IR spectra.

Synthesis of Potassium (Dinitrobenzyl β -D-Glucopyranosid)uronates (6a, 6b) Potassium (2,4-dinitrobenzyl β -D-glucopyranosid)uronate **6a** and its isomer **6b** were synthesized *via* the route shown in Chart 2. The reaction of **1a** and **1b** with methyl (2,3,4-tri-*O*-acetyl- α -D-glucopyranosyl)uronate bromide **3** according to Bollenback's procedure⁷⁾ in the presence of silver carbonate gave **4a** and **4b**, in yields of 10 and 31%, respectively. Kanaoka *et al.*⁸⁾ have shown that 18 β -glycyrrhetyl glucuronide is obtained directly by hydrolysis of the condensation product of glycyrrhetic acid and **3** with 5% KOH in MeOH. However, since the treatment of **4a** and **4b** with the methanolic 5% KOH gave **1a** and **1b**, the deprotection of **4a** and **4b** was performed stepwise. The solvolysis of **4a** and **4b** with sodium methoxide afforded **5a** and **5b** in yields of 40 and 44%, respectively. Subsequently, the hydrolysis of **5a** and **5b** with potassium carbonate gave **6a** and **6b** in 33 and 34% yields, respectively. The signals of the anomeric protons of **6a** and **6b** appeared as doublets at δ 4.38 ($J=7.5\text{ Hz}$) and δ 4.29 ($J=7.7\text{ Hz}$), respectively. β -Configuration of the glucuronide linkages of **6a** and **6b** was supported by the optical rotation and the fact that they were hydrolyzable with β -glucuronidase.

In conclusion, the chemical synthesis of these conjugates may be useful for studies on the active species related to the carcinogenicity of 2,4-DNT and 2,6-DNT.

Experimental

All melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded with a JASCO FT/IR-7000 spectrometer, and UV spectra with a Hitachi 150-20 spectrometer. Optical rotations were recorded with a JASCO DIP-4 digital polarimeter. $^1\text{H-NMR}$ spectra were recorded with a Varian Unity-5000 spectrometer, with tetramethylsilane as an internal standard. MS were recorded with a JEOL JMS-D300 spectrometer. Wakogel C-200 (silica gel) and Merck Kieselgel 60F₂₅₄ (silica gel, aluminum sheet) were used for column chromatography and thin layer chromatography (TLC), respectively. Enzymic hydrolysis was carried out as follows: glucuronides (**6a** and **6b**, each 1.3 μmol) were incubated at 37°C for 10 h in 0.2 M sodium acetate buffer (pH 5) with β -glucuronidase (1000 U); dinitrobenzyl alcohols (**1a**, **1b**) liberated were detected by TLC (CHCl_3 : MeOH = 9:1) and HPLC (ODS-80TM column, MeCN: H_2O = 1:1). Compounds **1a**,⁹⁾ **1b**¹⁰⁾ and **3**⁷⁾ were prepared by methods described previously.

Pyridinium 2,4-Dinitrobenzyl Sulfate (2a) A solution of 2,4-dinitrobenzyl alcohol **1a** (3.0 g, 15.2 mmol) in dry pyridine (3 ml) was added to pyridinium sulfonate, which was prepared from dry pyridine (18 ml) and ClSO_3H (5.3 g, 45.5 mmol),¹¹⁾ and the mixture was kept at 55°C for 30 min, then evaporated *in vacuo*. The residue was recrystallized from MeOH to give pure **2a** (pale brownish plates, mp 134–135°C, 2.4 g, 44%). UV λ_{max} (EtOH) nm (ϵ): 245 (18900). IR (KBr) cm^{-1} : 1531 (NO_2), 1232 (sulfate). $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ : 5.27 (s, 2H, methylene), 8.00 (t, 2H, $J=6.7\text{ Hz}$, pyridinium 3,5-H), 8.07 (d, 1H, $J=9.2\text{ Hz}$, aromatic 6-H), 8.51 (t, 1H, $J=7.9\text{ Hz}$, pyridinium 4-H), 8.61 (dd, 1H, $J=2.4, 8.5\text{ Hz}$, aromatic 5-H), 8.78 (d, 1H, $J=2.4\text{ Hz}$, aromatic 3-H), 8.88 (d, 2H, $J=4.9\text{ Hz}$, pyridinium 2,6-H). MS (FAB) m/z : 277 ($\text{M}-\text{C}_5\text{H}_6\text{N}^+$). Anal. Calcd for $\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_8\text{S}$: C, 40.34; H, 3.10; N, 11.76. Found: C, 40.39; H, 3.15; N, 11.85.

Pyridinium 2,6-Dinitrobenzyl Sulfate (2b) 2,6-Dinitrobenzyl alcohol **1b** (2.0 g, 10.0 mmol) was treated with pyridinium sulfonate in the same manner as described for the synthesis of **2a**. The residue obtained was recrystallized from MeOH to give pure **2b** (pale yellowish powder, mp

136–137°C, 0.7 g, 19%). UV λ_{max} (EtOH) nm (ϵ): 231 (13400). IR (KBr) cm^{-1} : 1537 (NO_2), 1238 (sulfate). $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ : 5.14 (s, 2H, methylene), 7.78 (t, 2H, $J=8.1\text{ Hz}$, aromatic 4-H), 8.01 (t, 2H, $J=7.1\text{ Hz}$, pyridinium 3,5-H), 8.20 (d, 2H, $J=8.3\text{ Hz}$, aromatic 3,5-H), 8.53 (t, 1H, $J=7.8\text{ Hz}$, pyridinium 4-H), 8.90 (d, 2H, $J=4.9\text{ Hz}$, pyridinium 2,6-H). MS (FAB) m/z : 277 ($\text{M}-\text{C}_5\text{H}_6\text{N}^+$). Anal. Calcd for $\text{C}_{12}\text{H}_{11}\text{N}_3\text{O}_8\text{S}$: C, 40.34; H, 3.10; N, 11.76. Found: C, 40.21; H, 3.12; N, 11.55.

Methyl (2,4-Dinitrobenzyl 2,3,4-tri-*O*-acetyl- β -D-Glucopyranosid)uronate (4a) Compound **1a** (5.0 g, 25.3 mmol) was dissolved in dry benzene (150 ml). To the boiling solution, a solution of **3** (15.0 g, 37.8 mmol) in dry benzene (150 ml) and freshly prepared Ag_2CO_3 (1.0 g) were added little by little over 10 h. Benzene was distilled off gradually, and stirring was continued. The mixture was filtered, and the filtrate was concentrated to a syrup. The syrup was purified by column chromatography (CHCl_3 : hexane 1:4) to give **4a** (colorless needles, mp 159–160°C, 1.3 g, 10%). UV λ_{max} (EtOH) nm (ϵ): 206 (10200), 242 (14700). IR (KBr) cm^{-1} : 1750 (ester), 1543 (NO_2). $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ : 1.98, 2.00, 2.05 (s, 3H each, CH_3CO), 3.64 (s, 3H, OCH_3), 4.50 (d, 1H, $J=9.8\text{ Hz}$, glucuronate 5-H), 4.95 (dd, 1H, $J=7.9, 9.6\text{ Hz}$, glucuronate 2-H), 5.00 (dd, 1H, $J=9.6, 9.8\text{ Hz}$, glucuronate 4-H), 5.06 (d, 1H, $J=7.9\text{ Hz}$, glucuronate 1-H), 5.19 (abq, 2H, $J=15.6\text{ Hz}$, methylene), 5.38 (t, 1H, $J=9.6\text{ Hz}$, glucuronate 3-H), 7.90 (d, 1H, $J=8.8\text{ Hz}$, aromatic 6-H), 8.66 (dd, 1H, $J=2.4, 8.5\text{ Hz}$, aromatic 5-H), 8.79 (d, 1H, $J=2.4\text{ Hz}$, aromatic 3-H). MS (FAB) m/z : 514 (M^+). Anal. Calcd for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_{14}$: C, 46.70; H, 4.31; N, 5.45. Found: C, 46.83; H, 4.30; N, 5.43.

Methyl (2,6-Dinitrobenzyl 2,3,4-tri-*O*-acetyl- β -D-Glucopyranosid)uronate (4b) 2,6-Dinitrobenzyl alcohol **1b** (5.0 g, 25.3 mmol) was reacted with **3** (15.0 g, 37.8 mmol) in the same manner as the synthesis of **4a**. The resulting syrup was purified by column chromatography (CHCl_3 : hexane = 1:3) to give **4b** (colorless needles, mp 162–164°C, 4.0 g, 31%). UV λ_{max} (EtOH) nm (ϵ): 205 (13600), 230 (11300). IR (KBr) cm^{-1} : 1750 (ester), 1537 (NO_2). $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ : 1.94, 1.97, 1.99 (s, 3H each, CH_3CO), 3.64 (s, 3H, OCH_3), 4.43 (d, 1H, $J=9.9\text{ Hz}$, glucuronate 5-H), 4.72 (dd, 1H, $J=8.2, 9.3\text{ Hz}$, glucuronate 2-H), 4.88–4.95 (m, 2H, glucuronate 1,4-H), 5.03 (abq, 2H, $J=13.2\text{ Hz}$, methylene), 5.31 (t, 1H, $J=9.3\text{ Hz}$, glucuronate 3-H), 7.87 (t, 1H, $J=8.2\text{ Hz}$, aromatic 4-H), 8.26 (d, 2H, $J=8.2\text{ Hz}$, aromatic 3,5-H). MS (FAB) m/z : 514 (M^+). Anal. Calcd for $\text{C}_{20}\text{H}_{22}\text{N}_2\text{O}_{14}$: C, 46.70; H, 4.31; N, 5.45. Found: C, 46.47; H, 4.40; N, 5.53.

Methyl (2,4-Dinitrobenzyl β -D-Glucopyranosid)uronate (5a) A solution of **4a** (1.0 g, 1.95 mmol) in dry MeOH (200 ml) was treated with 0.5 M NaOMe (4 ml) and the solution was stirred for 3 h in an ice bath. The mixture was neutralized with Dowex 50W $\times 8$ (H^+ form), filtered and concentrated. The residue was recrystallized from EtOAc to give pure **5a** (colorless needles, mp 183–185°C, 0.3 g, 40%). UV λ_{max} (EtOH) nm (ϵ): 206 (8800), 243 (13500). IR (KBr) cm^{-1} : 3400 (OH), 1734 (CO), 1535 (NO_2). $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ : 3.16–3.25 (m, 3H, glucuronate 2,3,4-H), 3.67 (s, 3H, OCH_3), 3.82 (d, 1H, $J=9.8\text{ Hz}$, glucuronate 5-H), 4.49 (d, 1H, $J=7.5\text{ Hz}$, glucuronate 1-H), 5.17 (abq, 2H, $J=17.1\text{ Hz}$, methylene), 5.25 (d, 1H, $J=8.8\text{ Hz}$, aromatic 1-H), 8.29 (d, 1H, $J=8.8\text{ Hz}$, aromatic 6-H), 8.58 (dd, 1H, $J=2.4\text{ Hz}$, aromatic 5-H), 8.80 (d, 1H, $J=2.4\text{ Hz}$, aromatic 3-H). MS (FAB) m/z : 388 (M^+). Anal. Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_{11}$: C, 43.31; H, 4.15; N, 7.21. Found: C, 43.51; H, 4.21; N, 7.07.

Methyl (2,6-Dinitrobenzyl β -D-Glucopyranosid)uronate (5b) Compound **4b** (1.0 g, 1.95 mmol) was treated with 0.5 M NaOMe (200 ml) in the same manner as described for the synthesis of **5a**. The resulting oily residue was purified by column chromatography (EtOAc: CHCl_3 = 10:3) to give **5b** (pale yellowish foam, 0.33 g, 44%). UV λ_{max} (EtOH) nm (ϵ): 206 (12700), 229 (10100). IR (KBr) cm^{-1} : 3408 (OH), 1746 (CO), 1535 (NO_2). $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ : 2.86–3.25 (m, 3H, glucuronate 2,3,4-H), 3.62 (s, 3H, OCH_3), 3.65 (d, 1H, $J=9.6\text{ Hz}$, glucuronate 5-H), 4.29 (d, 1H, $J=7.7\text{ Hz}$, glucuronate 1-H), 5.00 (abq, 2H, $J=13.5\text{ Hz}$, methylene), 5.07 (d, 1H, $J=5.3\text{ Hz}$, OH), 5.12 (d, 1H, $J=5.6\text{ Hz}$, OH), 5.30 (d, 1H, $J=6.0\text{ Hz}$, OH), 7.81 (t, $J=8.2\text{ Hz}$, aromatic 4-H), 8.20 (d, 2H, $J=8.1\text{ Hz}$, aromatic 3,5-H). MS (FAB) m/z : 388 (M^+). Anal. Calcd for $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_{11}$: C, 43.31; H, 4.15; N, 7.21. Found: C, 43.09; H, 4.28; N, 7.03.

Potassium (2,4-Dinitrobenzyl β -D-Glucopyranosid)uronate (6a) A solution of **5a** (1.0 g, 2.58 mmol) in MeOH (200 ml) was treated with 0.4% K_2CO_3 (100 ml) in portions in an ice bath. The mixture was stirred at room temperature for 1 h, neutralized with Dowex 50W $\times 8$ (H^+ form),

filtered, and evaporated *in vacuo*. The residue was recrystallized from MeOH–acetone–H₂O (10:1:0.1) to give **6b** (colorless needles, decomp. 184–190 °C, 0.36 g, 33%). $[\alpha]_D^{20} -56.6^\circ$ ($c=0.1$, H₂O). UV $\lambda_{\max}$ (H₂O) nm (ϵ): 249 (41900). IR (KBr) cm^{-1} : 3320 (OH), 1605 (COOK), 1541 (NO₂). ¹H-NMR (DMSO-*d*₆, 500 MHz) δ : 3.14–3.26 (m, 4H, glucuronate 2,3,4,5-H), 4.38 (d, 1H, $J=7.5$ Hz, glucuronate 1-H), 5.18 (abq, 2H, $J=17.0$ Hz, methylene), 8.34 (d, 1H, $J=8.5$ Hz, aromatic 6-H), 8.59 (dd, 1H, $J=2.4$, 8.6 Hz, aromatic 5-H), 8.80 (d, 1H, $J=2.4$ Hz, aromatic 3-H). High-resolution MS m/z : Calcd for C₁₃H₁₄N₂O₁₁K: 413.0235. Found: 413.0234. Anal. Calcd for C₁₃H₁₃N₂O₁₁K·0.5H₂O: C, 37.06; H, 3.35; N, 6.65. Found: C, 37.34; H, 3.30; N, 6.63.

Potassium (2,6-Dinitrobenzyl β -D-Glucopyranosid)uronate (6b) Compound **5b** (1.0 g, 2.58 mmol) was treated with 0.4% K₂CO₃ (100 ml) in MeOH (200 ml) in the same manner as described for the synthesis of **5a**. After neutralization with Dowex 50W $\times 8$ (H⁺ form), the mixture was filtered and concentrated *in vacuo*. The residue was recrystallized from MeOH–acetone–H₂O (10:1:0.1) to give **6b** (colorless needles, decomp. 184–190 °C, 0.37 g, 34%). $[\alpha]_D^{20} -106^\circ$ ($c=0.1$, H₂O). UV $\lambda_{\max}$ (H₂O) nm (ϵ): 236 (10600). IR (KBr) cm^{-1} : 3400 (OH), 1618 (COOK), 1541 (NO₂). ¹H-NMR (DMSO-*d*₆, 500 MHz) δ : 2.84–3.24 (m, 4H, glucuronate 2,3,4,5-H), 4.14 (d, 1H, $J=7.7$ Hz, glucuronate 1-H), 4.98 (abq, 2H, $J=13.0$ Hz, methylene), 7.83 (t, 1H, $J=8.1$ Hz, aromatic 4-H), 8.23 (d, 2H, $J=8.1$ Hz, aromatic 3,5-H). High-resolution MS m/z : Calcd for C₁₃H₁₄N₂O₁₁K: 413.0235. Found: 413.0230. Anal. Calcd for C₁₃H₁₃N₂O₁₁K·0.5H₂O: C, 37.06; H, 3.35; N, 6.65. Found: C, 37.34; H, 3.30; N, 6.63.

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