TEMPO-Mediated Regiospecific Oxidation of Glucosides to Glucuronides

Rakesh N. Desai, Len F. Blackwell*

Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand
Fax +64(6)3505682; E-mail: L.F.Blackwell@massey.ac.nz

Received 21 August 2003

Abstract: A TEMPO/hypochlorite/bromide oxidant has been used for the conversion of aryl and steroidal glucosides to the corresponding glucuronide conjugates in good (48–74%) yield. An isoflavone glucoside failed to undergo this transformation.

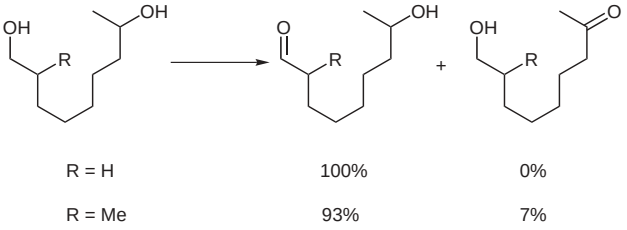
Key words: oxidation, glucuronide, glucoside, regiospecific, TEMPO

Glucuronide conjugates are important metabolites found in urine and are used as biomarkers for health and disease. In our ongoing efforts on the development of the Ovarian Monitor System, glucuronide conjugate concentrations are measured to infer the fertile state of the woman during her menstrual cycle.[1] In order to develop such systems applicable to a range of health issues, we require procedures for the synthesis of steroidal and phytoestrogenic glucuronides.

However, methods for their syntheses are limited and are not entirely reliable. The Koenigs–Knorr reaction[2] is still the most popular coupling protocol for glycoside and glucuronide formation. Reaction between alcohols or phenols and 2-acetoxyglycosyl donors occurs with very high β-glycoside selectivity through neighbouring group participation.[3] However, the presence of the methoxycarbonyl group in glucuronyl donors reduces their reactivity relative to glycosyl donors and reaction is not always observed.[4] In light of this observation, we wished to investigate the possibility of using glucosides as glucuronide equivalents, exchangeable through selective primary alcohol oxidation.

Though many oxidants have been developed for the smooth and mild oxidation of primary and secondary alcohol groups (such as PCC, Swern reagents, Dess–Martin periodinane and TPAP), regioselective oxidation of one alcohol group in the presence of others is still generally not feasible. In particular, selective oxidation of primary hydroxyl groups in the presence of secondary ones is a difficult conversion in organic synthesis.

Recently, Semmelheck[5] has demonstrated the selective oxidation of primary alcohols to aldehydes in the presence of a secondary alcohol, using TEMPO (2,2,6,6-tetramethylpiperidin-1-oxyl radical) (Equation 1).

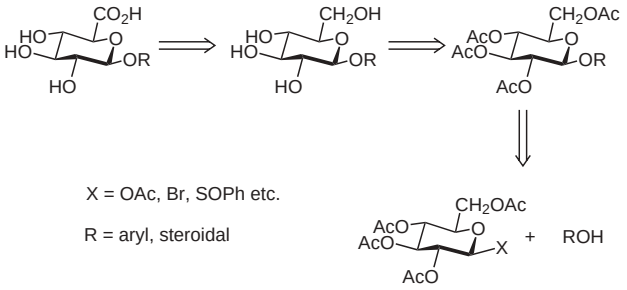


Equation 1 Selective primary alcohol oxidation using TEMPO

The usefulness of dinitrogen tetroxide in preferential oxidation of primary alcohol groups in carbohydrates to uronic acids was first realised by Maurer and Drefahl,[6] and Yackel and Kenyon[7] in 1942. Oxygen has also been used as a selective oxidant, in the presence of a platinum catalyst; however, the yields are often low.[8]

In more recent times, nitrosyl radicals have emerged as useful selective oxidants. Davis[9] was the first to investigate the use of TEMPO in the oxidation of glucosides to glucuronides. Treatment of methyl or octyl glucosides with a TEMPO/hypochlorite/bromide oxidant afforded the desired glucuronides in very good yields. A similar oxidation has been performed on glucosyl azides to afford glucuronyl azides.[10]

In this paper, we wish to report our findings regarding the application of the TEMPO/hypochlorite/bromide oxidant system for the smooth conversion of aryl and steroidal glucosides to their respective glucuronides. Scheme 1 shows our retrosynthetic plan.

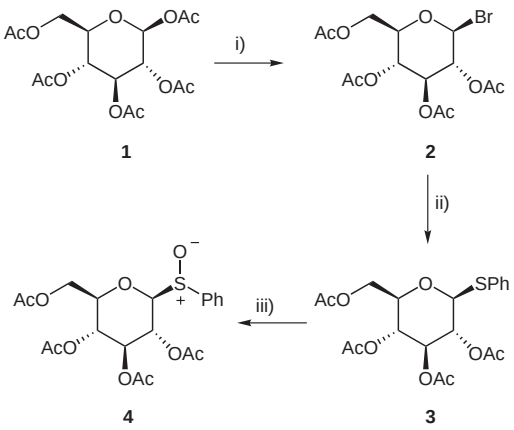


Scheme 1 Retrosynthetic analysis of glucuronide synthesis

In order to establish the procedure, we wished to be able to incorporate both steroidal and aryl aglycones into glucuronide conjugates. In the first instance, *p*-cresol was reacted with β-D-glucose penta-*O*-acetate **1** in the presence of boron trifluoride diethyl etherate. Glucoside **5a**[11] was isolated in moderate (53%) yield by this Fischer–Helferich procedure. Attempts to apply the same procedure to

SYNLETT 2003, No. 13, pp 1981–1984
Advanced online publication: 08.10.2003
DOI: 10.1055/s-2003-42048; Art ID: D21003ST

the sterically hindered and less nucleophilic 2,6-dimethylphenol resulted in failure. The success reported by Kahn and co-workers[12] in the glycosylation of unreactive substrates with a sulfoxide glycosyl donor, prompted us to investigate such a system for coupling with this substrate. The required donor was prepared by the coupling of thiophenol with 1-bromo-1-deoxy-2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (**2**), followed by mono-oxidation to the sulfoxide using *meta*-chloro-peroxobenzoic acid (Scheme 2).



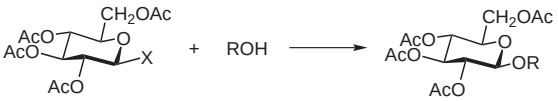
Scheme 2 Glucosyl donor synthesis. *Reagents and conditions*: i) HBr, AcOH, dark, r.t., 16 h, 80%; ii) Bu_4NHSO_4 , Na_2CO_3, PhSH, EtOAc, r.t., 30%, 81%; iii) MCPBA, CH_2Cl_2, –70 °C, 1 h, 81%

In the event, coupling between sulfoxide donor **4** and 2,6-dimethylphenol using triflic anhydride and triethyl phosphite as an acid scavenger promoted the formation of glucoside **5b** in moderate (34%) yield. The mass balance was accounted for by the formation of thioglycoside **3**, formed by deoxygenation with triethyl phosphite.

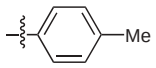
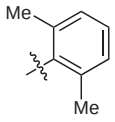
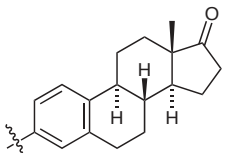
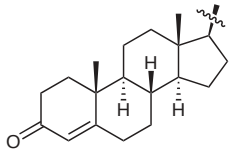
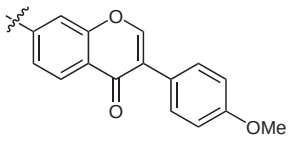
Estrone was sufficiently reactive to couple efficiently with the penta-*O*-acetate **1** and glucoside **5c** was isolated in excellent (88%) yield. Attempts to perform the Fischer–Helferich reaction on testosterone resulted in extensive decomposition of both the steroid and the sugar. However, Koenigs–Knorr coupling with bromo sugar **2**, using cadmium carbonate as a promoter successfully formed the glucoside **5d** in good (65%) yield.

Several attempts were also made to synthesise formononetin glucoside **5e** using either the Fischer–Helferich or Koenigs–Knorr protocols. In all cases, none of the desired product was formed. The insolubility of the substrate in the reaction media was probably the cause. However, the phase-transfer-catalysed (PTC) glycosylation procedure of Wahala[13] worked well and the deprotected glucoside was isolated in moderate (32%, 2 steps, average 57% per step, see Table 2) yield. Unreacted formononetin was present in all runs, but attempts to drive the reaction to completion by extending the reaction time resulted in significant decomposition of the sugar (via hydrolysis and glucal formation) and base-induced ring opening of the chromene ring in formononetin. It was also possible to form the desired product by reacting the bromosugar with the sodium alkoxide (generated in situ) in DMF. However, the isolated yield was inferior to the above PTC procedure. Table 1 summarises these results.

Table 1 Syntheses of Protected Glucosides

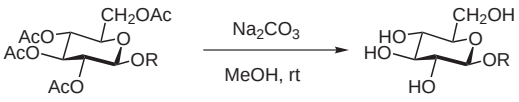


1,**2** or **4** **5**

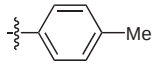
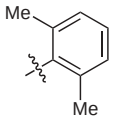
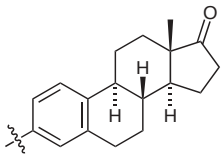
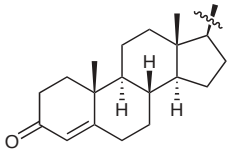
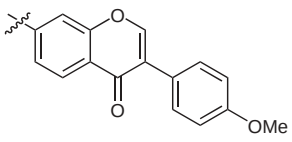
X	R	Isolated yield (%)
β-OAc	 **5a**	53
β-SOPh	 **5b**	34
β-OAc	 **5c**	88
β-Br	 **5d**	65
β-Br	 **5e**	–

With the peracetylated glucosides **5a**–**d** in hand, we turned our attention to their hydrolyses to the free glucopyranosides. Deprotection of the sugar moiety was carried out using a standard Zemplen deacetylation protocol (NaOMe–MeOH) to afford the free glucosides. However, it was found that the deprotection could also be carried out using methanolic Na_2CO_3 at ambient temperature. In the case of formononetin tetra-*O*-acetyl-β-D-glucopyranoside **5e**, it was safer to use this procedure so as to avoid the formation of the 4,5-didehydro glucoside. The milder carbonate salt also reduces the possibility of destructive base-mediated chromene ring opening. Table 2 shows the glucopyranosides **6a**–**e** isolated in moderate to good (57–68%) yield.[14]

Table 2 Formation of Deacetylated Glucosides



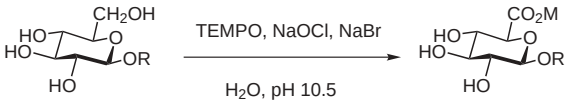
5 **6**

R	Yield (%)
 **6a**	57
 **6b**	68
 **6c**	67
 **6d**	65
 **6e**	32 (2 steps)

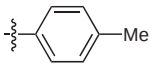
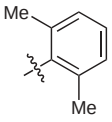
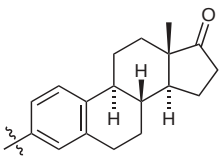
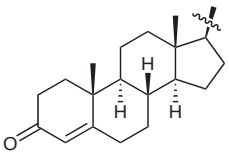
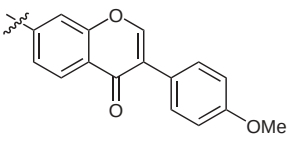
Purification of the crude *p*-cresol, 2,6-dimethylphenol, estrone and testosterone glucosides was conveniently achieved by neutralising the reaction mixture with 1 M HCl or by bringing the pH of the reaction mixture to pH ca 6. The compounds were then purified by reverse phase chromatography on a Waters® C_{18} Sep-Pak column, eluting with water to remove water-soluble inorganic salts followed by 50% aq MeOH to recover the desired glucoside in almost pure form. The purification of the formononetin glucoside required special attention due to the acid and base sensitive nature of the chromene ring. In this case, the crude reaction mixture was carefully neutralised with Amberlite 120, resulting in a pH ca 7. The glycosidic linkage is stable at pH 7 for long periods. The formononetin glucoside was purified initially by eluting with a 50–70% aq MeOH gradient using a non-ionic, neutral, Amberlite XAD-2 stationary phase. MeOH was removed and the product further purified by reverse phase chromatography on a Waters® C_{18} Sep-Pak column eluting with 50% aq MeOH and MeOH.

While there have been a few reports on the use of TEMPO in the selective oxidation of pyranosides, a literature survey revealed only one method involving the use of TEMPO-mediated selective oxidation on various phenyl β-D-glucosides.[15] The simple *p*-cresol glucuronide was conveniently prepared using *tert*-butyl hypochlorite–TEMPO in water in good yield, as reported by Melvin et al. The advantage of this method was the absence of inorganic salts and hence resulted in easy isolation and purification of the glucuronide. However, *t*-BuOCl is extremely unstable at ambient temperature and has only a limited lifetime at 0 °C. It was decided to investigate an alternative TEMPO/NaOCl method as reported by Nooy[16] with some modifications to account for the various glucosides at hand. Table 3 summarises the results.

Table 3 Oxidation of Glucosides to Glucuronides with TEMPO



6 **7**

R	M	Yield (%)
 **7a**	K	74
 **7b**	Na	74
 **7c**	Na	62
 **7d**	Na	48
 **7e**	Na	0

Initially, methyl β-D-glucoside was oxidised using TEMPO–NaBr–NaOCl in water at 0 °C and pH 10.5, controlled by the addition of 0.5 M NaOH using a pH-stat. The reaction was complete within 45 minutes and the

glucuronide was isolated as the sodium salt by filtration through a Waters® Sep-Pak C_{18} column using water, then 67% aqueous MeOH. Glucuronides **7a**–**d** were synthesised in a similar manner and were isolated as their sodium or potassium salts in good (48–74%) yield,[17] except for formononetin glucuronide **7e**. All attempts to prepare this material resulted in failure. Both the highly alkaline conditions required, which are incompatible with the base sensitive chromene ring, and the low solubility of formononetin in the reaction medium, are probable factors.

In conclusion, we have been able to show that TEMPO can be used as a selective oxidant for the conversion of glucosides to glucuronides in good yield. This procedure should compete well with the more traditional Koenigs–Knorr protocol.

Acknowledgement

We would like to thank Dr Jeremy J. Fullbrook for the preparation of this manuscript.

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(10) Gyorgydeak, Z.; Thiem, J. *Carbohydrate Res.* **1995**, *268*, 85.
(11) **Preparation of 5a:** β-D-Glucose penta-*O*-acetate (0.36 g, 0.92 mmol) and boron trifluoride diethyl etherate (0.13 ml, 0.92 mmol) were added to a solution of *p*-cresol (0.2 g, 1.84 mmol) in CH_2Cl_2 (5 mL) containing molecular sieves (4 Å, activated). The resulting reaction mixture was protected from moisture and stirred at 25–30 °C overnight (16 h). The reaction mixture was diluted with CH_2Cl_2 (40 mL) and washed with aq KOH (2 M, 4 × 25 mL), water (2 × 25 mL) and brine (25 mL). The organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure to afford a crude off-white solid. The crude product was recrystallised from anhyd EtOH to afford **5a** (214 mg, 53%) as colourless crystals; R_f 0.70 (2:1 EtOAc–hexane), mp 110–111 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.11 (d, *J* = 8.6 Hz, 2 H), 6.90 (d, *J* = 8.6 Hz, 2 H), 5.31–5.15 (envelope, 3 H), 5.03 (d, *J* = 7.3 Hz, 1 H), 4.27 (dd, *J* = 11.9, 5.0 Hz, 1 H), 4.18 (dd, *J* = 11.9, 2.3 Hz, 1 H), 3.90–3.79 (m, 1 H), 2.31 (s, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 170.1, 169.2, 169.1, 154.6, 132.7, 129.8, 116.9, 99.4, 72.4, 71.9, 71.1, 68.2, 61.9, 20.6, 20.5.
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(14) **Preparation of 6a:** Compound **5a** (0.25 g, 0.57 mmol) was dissolved in MeOH (10 mL) and aq Na_2CO_3 (2 M, 3.5 mL) was added. The reaction mixture was stirred at ambient temperature for 5 h with monitoring by TLC. The mixture was neutralised with HCl (1 M) and the solvent removed under reduced pressure. The compound was then purified by reverse phase chromatography on a Waters® C_{18} Sep-Pak column by eluting with water and then with 50% aq MeOH. Appropriate fractions were pooled and the solvent removed under reduced pressure to afford **6a** (88 mg, 57%) as a white solid. ^{1}H NMR (400 MHz, D_2O): δ = 7.44 (d, *J* = 8.6 Hz, 2 H), 7.35 (d, *J* = 8.6 Hz, 2 H), 4.25 (d, *J* = 9.8 Hz, 1 H), 4.08 (d, *J* = 3.9 Hz, 1 H), 3.86–3.80 (envelope, 5 H), 2.63 (s, 3 H), ^{13}C NMR (100 MHz, D_2O): δ = 155.8, 131.7, 129.7, 116.6, 101.4, 76.9, 73.8, 70.3, 61.4, 19.7. HRMS–FAB$^+$: *m*/*z* [M] calcd for $C_{13}H_{18}O_6$: 270.1103; found, 270.1090.
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(16) Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Carbohydr. Res.* **1995**, *269*, 89.
(17) **Preparation of 7a:** Glucoside **6a** (200 mg, 0.74 mmol) was dissolved in distilled water (10 mL) and TEMPO (0.005 equiv) and NaBr (0.15 equiv) were added. The solution was cooled to 0 °C and a cold solution of 12–15% hypochlorite in water (previously brought to pH = 10 by addition of 4 M HCl) was added. The pH was controlled at ca 10–10.5 by dropwise addition of KOH (0.5 M) with a syringe. The reaction was complete within 30 min (TLC) during which time the pH was generally stable. The reaction was quenched by addition of EtOH (5 mL) and the mixture neutralised with 1 M HCl. The organic solvent was removed under reduced pressure and the remaining solution was freeze-dried. The crude product was purified using either XAD-2 column or Waters® Sep-Pak column chromatography by eluting with water, 50% aq MeOH and MeOH. Appropriate fractions were pooled and the solvent removed under reduced pressure and the crude product freeze-dried to afford pure **7a** (176 mg, 74%) as a white solid. ^{1}H NMR (400 MHz, D_2O): δ = 7.14 (d, *J* = 8.7 Hz, 2 H), 6.98 (d, *J* = 8.7 Hz, 2 H), 4.96 (d, *J* = 9.2 Hz, 1 H), 3.77 (d, *J* = 4.0 Hz, 1 H), 3.57–3.53 (envelope, 3 H), 2.13 (s, 3 H). ^{13}C NMR (100 MHz, D_2O): δ = 175.9, 154.9, 133.7, 130.7, 117.1, 100.9, 76.5, 73.2, 72.2, 20.1. HRMS–FAB$^+$: *m*/*z* [M + H] calcd for $C_{13}H_{16}O_7K$, 323.0533; found, 323.0521. HRMS–FAB$^+$: *m*/*z* [M + K] calcd for $C_{13}H_{15}O_7K_2$: 361.0092; found, 361.0059. MS (FAB$^+$): *m*/*z* (%) = 361 (10) [M + K], 323 (15) [M + H], 315 (10), 223(40), 131 (100), 39 (27) [K].