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The Improved Synthesis of β-D-Glucuronides using TEMPO and t-Butyl Hypochlorite

Fiona Melvin,[a] Alan McNeill,[b] Peter J. F. Henderson[a] and Richard B. Herbert[a*]

a: Centre for Biomolecular Sciences, University of Leeds, Leeds LS2 9JT;
b: Isotope Chemistry Laboratories, Sanofi Research, Willowburn Avenue, Alnwick, Northumberland NE66 2JH, UK

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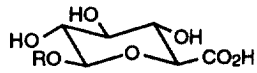
Abstract: TEMPO/t-BuOCl is used to oxidise β-D-glucosides to β-D-glucuronides in high yield as a pivotal step in the preparation of labelled glucuronides from labelled glucose samples.

1-O-β-D-Glucuronides **1** are important natural compounds, often being the conjugates by which a xenobiotic or drug is excreted from the human body. The drug connection provides a pharmaceutical imperative for the synthesis of these compounds.[1] *Escherichia coli*, in the human gut, uses glucuronides as a carbon source, assimilating them through the cell wall by agency of the membrane protein GusB, one of a family of important bacterial transport proteins.[2]



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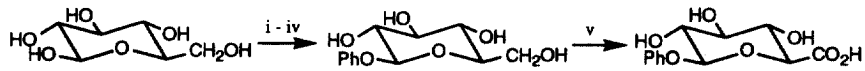
We are applying novel NMR methods[3] to determine the 3-D structure of the binding site of GusB using glucuronides that are labelled with stable isotopes in the sugar moiety. Starting with ^{13}C-labelled glucose (which is readily available commercially) we have developed a high yielding route to various labelled β-D-glucuronides involving, as a pivotal step, the oxidation of β-D-glucosides by tetramethylpiperidinyl-1-oxy (TEMPO), and t-butyl hypochlorite. The route is readily applicable to the preparation of glucuronides from other glucose samples labelled with stable or radioactive isotopes.



Scheme I i Ac_2O/Pyr; ii HBr in AcOH; iii PhOH/Ag Imidazolate/$ZnCl_2$/Mol. Sieves; iv NaOMe in MeOH; v TEMPO/t-BuOCl/NaOH

The conversion of glucose into phenyl-β-D-glucoside is well documented; the route that gave us the highest yield on a 500 mg scale is illustrated in Scheme I (i-iv).[4] Selective oxidation of the primary hydroxyl group in glucosides generally may be achieved catalytically (Pt/O_2) but the yield can be low[5]. In our hands the oxidation of phenyl-β-D-glucoside failed. Oxidation of glucosides under alkaline conditions (pH 10-10.5) using TEMPO is also selective for the primary alcohol function and affords the glucuronide as product.[6] In the normal

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experimental procedure TEMPO is used with an oxidising agent such as sodium hypochlorite, which generates the active oxidising species, the oxyammonium salt. Inevitably the water soluble, acid-unstable glucuronide is isolated in the presence of substantial amounts of inorganic salts and we consequently found it impossible to purify phenyl glucuronide prepared in this way on the scale applied.

The simple expedient of using t-butyl hypochlorite[7] (see Scheme 1) avoided this problem and afforded a range of glucuronides isolated as their sodium salts in generally excellent yield (phenyl-β-D-glucuronide, also [1-^{13}C]- and [6-^{13}C]-labelled material, 78-96%; 2'-fluorophenyl-β-D-glucuronide, 97%; 4'-nitrophenyl-β-D-glucuronide, 64%; methyl-β-D-glucuronide, 34%). With water insoluble alcohols a two-phase system is recommended.[8] The TEMPO oxidation of octyl-β-D-glucoside under two-phase conditions[9] failed in our hands where the TEMPO/t-BuOCl method[10] gave a quantitative yield.

Esterification (CH_2N_2 under acid conditions) of the product of the oxidation reaction of phenyl-β-D-glucoside gave a methyl ester identical to that isolated from the esterification of a commercial sample of phenyl-β-D-glucuronide (overall yield 74%). The oxidation product of methyl-β-D-glucose was similarly treated to yield the methyl methyl-β-D-glucuronate in 71% overall yield.

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10. Typical oxidation procedure: The β-D-glucoside (100mg) was dissolved in distilled water (up to 10ml) and TEMPO (~1mg) added. The pH was adjusted to 10-10.5 (pH meter) by dropwise addition of aqueous NaOH (2M). Bu^{t}OCl (2 equivalents) was added to the solution with stirring. After approximately 0.5h the pH was generally stable ; TLC analysis (50:50 $CHCl_3$/MeOH) confirmed the reaction was complete. The reaction was quenched by addition of EtOH (5ml) and the pH was adjusted to 5 by dropwise addition of dilute aqueous H_2SO_4. The aqueous solution was washed with $CHCl_3$ (2X10ml) and taken to dryness *in vacuo*. After drying in a desiccator overnight the solid was extracted with dry MeOH (20ml). The methanolic extract was evaporated *in vacuo* to leave the sodium salt of the glucuronide. Purity was confirmed by ^{1}H, ^{13}C NMR and microanalysis.