General Synthesis of 3,4-Dinitrophenyl α -Glycopyranosides

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Abstract: A generalised simple method for the synthesis of 3,4-dinitrophenyl α -glycosides is described. The nucleophilic aromatic substitution of 1-fluoro-3,4-dinitrobenzene can be achieved with a suite of partially protected sugar hemiacetals, under basic conditions.

Synthetic phenyl glycosides are useful activated substrates for use in glycobiology, in particular for the study of glycoside hydrolases (glycosidases). Applications include the use as an indicator of activity in biological assays, where the activity of the enzyme being studied can be monitored in real time, due to the phenol giving a colourimetric output (at a suitable pH).^[1] The best known and utilised compound class for this application are the 4-nitrophenyl glycosides (pNP-glycosides), due to their ease of synthesis as well as the pK_a of the leaving group (7.18^[2]). Due to the general stability of these compounds, assays can be conducted under different conditions such as varying pH and also in the presence of inhibitors which allows for insight into the overall activity of the glycosidase and potency of these compounds, respectively.

Figure 1. Cleavage of 3,4-dinitrophenyl α -glycosides by a glycoside hydrolase.

An additional utility of phenyl glycosides, due to the diversity of phenyl groups that can be installed with varying pK_a values, is that detailed kinetic analysis of glycosidases can be performed. These types of studies have been greatly aided by the use of these compounds due to the nature of the catalytic mechanism(s) of glycosidases which, in general, utilise a proton transfer process from enzyme to substrate^[3] and so the reaction rate observed is affected by the pK_a of the particular phenyl glycoside being used. Overall these types of studies allow for great insight into the catalytic mechanism and in some cases the active site of these enzymes.

The majority of phenyl glycosides used in kinetic studies tend to have leaving groups with pK_a values for the phenol >7 , and with only a few with pK_a values <7 , one of the most common of which is the 2,4-dinitrophenyl glycoside-type (pK_a 3.96^[2]). The common synthetic methods for the formation of phenyl β -glycosides are the Koenigs-Knorr reaction,^[4] and via glycosyl

imidates^[5] or the Helferich method^[6] for α -glycosides. In terms of phenols with a low pK_a , these reactions tend to form the desired glycoside in a low yield, due to their low nucleophilicity. This may indeed also be a reason as to why kinetic studies of glycosidases use a smaller sample of phenyl glycosides with low pK_a values. Another common substrate used are the 3,4-dinitrophenyl glycosides, which have a slightly higher pK_a (5.36^[2]) than 2,4-dinitrophenyl glycosides giving an additional data point in kinetic analysis and due to this reduced reactivity, may also be advantageous if more reactive glycosides such as the 2,4-dinitrophenyl type are not synthetically accessible (Figure 1). Some 3,4-dinitrophenyl glycosides have been used in detailed studies of a range of α -^[2, 7] and β -glycosidases^[8] Notably the 3,4-dinitrophenyl 2-acetamido-2-deoxy- β -D-glucopyranoside was used to assay for urinary NAGase, which has an optimum pH of ~ 5 .^[8c] This allowed for a direct continuous-based assay to be utilised. Despite 3,4-dinitrophenyl β -glycosides having been widely used in the literature for studying β -glycosidases, the α -anomer is less common. This is possibly due to the limited synthetic methods trialled. We therefore sought a simple general synthesis to prepare molecules of this type that could be applied to a range of carbohydrate-based molecules.

Scheme 1. Proposed general synthesis of 3,4-dinitrophenyl α -glycopyranosides.

Previous syntheses of 3,4-dinitrophenyl α -glycosides have included glycosylation of the relevant anomeric acetate with 3,4-dinitrophenol catalysed by $BF_3 \cdot OEt_2$ ^[7b] or CF_3SO_3H ,^[7d] or via chemoenzymatic synthesis.^[7c] However, these procedures had limited scope and gave varying yields. A methodology that drew our attention was in the synthesis of 2,4-dinitrophenyl glycosides introduced by Van Boom *et al.*,^[9] where a partially protected sugar hemiacetal is condensed with the reactive 1-fluoro-2,4-dinitrobenzene (Sanger's reagent), under basic conditions. This reaction relies on the aromatic molecule being able to undergo nucleophilic aromatic substitution and primarily yields the desired β -glycoside (the α -glycoside could also be obtained through an additional anomerisation step). This methodology was more succinctly applied to the synthesis of 2,4-dinitrophenyl α -glycosides by Chen and Withers,^[10] who postulated that initially using a stronger base would catalyse the formation of the β -glycoside and the anomerisation to the α -glycoside in one-pot. This was successfully applied to the synthesis of a range of 2,4-dinitrophenyl α -D-glycopyranosides (using K_2CO_3 in DMF^[10]). Based on previous observations that 1-fluoro-3,4-dinitrobenzene **1** undergoes nucleophilic displacement reactions^[11] similar to 1-fluoro-2,4-dinitrobenzene we felt that there was an excellent chance that 3,4-dinitrophenyl

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Supporting information for this article is given via a link at the end of the document.

Table 1. Optimisation of the reaction conditions, utilising the synthesis of 3,4-dinitrophenyl 2,3,4,6-tetra-O-acetyl- α -D-glucopyranoside **2** as the model reaction.

Entry	Base	Solvent	α : β ratio ^[a]	Yield % ^[b]
1	K ₂ CO ₃	DMF	1:0.27	44
2	K ₂ CO ₃	DMSO	1:0.26	53
3	K ₂ CO ₃	ACN	1:1.1	39
4	DBU	DMF	1:0.22	37
5	DBU	DMSO	1:0.15	26
6	DBU	ACN	1:0.33	41
7	Cs ₂ CO ₃	DMF	1:0.28	46
8	Cs ₂ CO ₃	DMSO	1:0.23	57
9	Cs ₂ CO ₃	ACN	1:0.86	40
10	K ₃ PO ₄	DMF	1:0.28	43
11	K ₃ PO ₄	DMSO	1:0.22	34
12	K ₃ PO ₄	ACN	1:1.1	33

[a] Ratio of yield between 3,4-dinitrophenyl α -glucopyranoside and β -glucopyranoside (determined using ¹H NMR). [b] Yield of desired α -glucoside.

α -glycosides could also be prepared directly using a hemiacetal, and a suitable base (Scheme 1).

Initially, using the conditions of K₂CO₃ in DMF^[10] resulted in low yields (44% and 30% for the glucose **2** and galactose **3** based derivatives, respectively), likely due to the poorer reactivity of **1**. Therefore, a range of bases and solvents were trialled to improve the yield and thus the ease of access to these useful synthetic substrates. Initially a range of conditions were tested using the condensation of the hemiacetal **4** with **1** (Table 1) as a model. Of the bases and solvents tested the best yielding combination was that of Cs₂CO₃ in DMSO which had a useful yield as well as a reduced ratio of unwanted β -glycoside to **2** (Entry 8, Table 1). With the optimised conditions obtained the scope of the reaction conditions were tested across a range of hemiacetals **5–15** (Table 2). The desired 3,4-dinitrophenyl α -glycosides **3**, **16–22** were pleasingly obtained in good yields (Entries 1–9, Table 2) noting that for the hemiacetal **13** the expected axial product was obtained as the major product, corresponding to the β -glycoside **23** (Entry 10, Table 2). Disappointingly when these conditions were used to prepare the *N*-acetylglucosamine derivative **24**, the desired product was not observed and the isolated material was a complex mixture of compounds. Chen and Withers^[10] when trying to prepare the corresponding 2,4-dinitrophenyl α -glycoside observed the formation of di-(2,4-dinitrophenyl) moieties **25** and **26** (Figure 2),

which reduced their overall yield of the desired compound. To reduce the formation of **25** and **26** they decreased the equivalents of 1-fluoro-2,4-dinitrobenzene used (1.3 to 1) and also noted that DMSO exacerbated undesired product formation. Thus the reaction was attempted with 1 equivalent of **1** to hemiacetal **14**, in the presence of Cs₂CO₃ in DMF. Pleasingly the desired glycoside **24** was obtained, although in a low yield. In addition, the *N*-acetylgalactosamine derivative **27** was also obtained in this fashion. To try and improve the yield of **24**, the reaction was attempted with K₂CO₃ in DMF, which were the next best conditions without utilising DMSO (Entry 1, Table 1). However, this reaction resulted in no formation of the desired product **24**, and interestingly the major product obtained was not consistent with either of the diarylated species observed by Chen and Withers.^[10] Indeed upon analysis of the material it was found to be the aniline **28** (Figure 2, confirmed by 2D NMR and comparison with similar compounds in the literature^[12]). A possible explanation for the formation of **28** is a combination of the nitrogen atom of the 2-acetamido group acting as a nucleophile to attack **1** and acetyl migration to the C-1 hemiacetal. To probe the role of the nitrogen atom in the formation of **28** we took 2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranose and treated it with **1** under the same conditions that led to the isolation of **28**. After an extended period (48 h) no reaction was observed, which is suggestive that in the reaction of **14** to give **28** that the acetyl migration probably occurs first and *N*-arylation does not occur without the presence of the hemiacetal.

The isolated protected 3,4-dinitrophenyl α -glycosides **2**, **3**, **16–24** and **27** can now be deprotected using the methods of Ballardie *et al.*^[13] which utilises methanolic HCl or acetyl chloride to the methanol solution^[10] which have commonly been used for deacetylation of phenyl glycosides with a low leaving group pK_a.

Overall, the described methodology can be applied to obtain 3,4-dinitrophenyl α -glycosides with ease from readily accessed partially protected hemiacetals and 1-fluoro-3,4-dinitrobenzene **1**. These glycosides can then be used in the extensive study of interesting and important glycosidases in chemical, biochemical and industrial applications.

Figure 2. Major products **25** and **26** observed by Chen and Withers.^[10] Major product of reaction between hemiacetal **14** and **1** in the presence of K₂CO₃ in DMF.

Supporting Information Summary

The Supplementary information contains experimental procedures and spectroscopic data for all synthesised compounds, and ¹H and ¹³C NMR spectra for novel compounds.

Table 2. Scope of reaction with different sugar moieties.

Entry	Substrate	Product	Yield %	Entry	Substrate	Product	Yield %
1[a]	4	2	57	7[a]	10	20	69
2[a]	5	3	48	8[a]	11	21	65
3[a]	6	16	63	9[a]	12	22	71
4[a]	7	17	56	10[a]	13	23	52
5[a]	8	18	51	11[b]	14	24	33
6[a]	9	19	65	12[b]	15	27	22

[a] Reaction conditions: Hemiacetal (0.5 mmol), 1-fluoro-3,4-dinitrobenzene **1** (1.3 eq.) and Cs₂CO₃ (2.5 eq.) in DMSO (4.5 ml) at room temperature. [b] Reaction conditions: Hemiacetal (0.5 mmol), 3,4-dinitrofluorobenzene (1 eq.) and Cs₂CO₃ (2.5 eq.) in DMF (4.5 ml) at room temperature.

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General Synthesis of 3,4-Dinitrophenyl- α -glycopyranosides

Chromogenic substrates have been used extensively to study glycoside hydrolases, therefore simple and general methods to access these useful materials desired. A simple synthetic methodology to access a relatively underused aryl glycoside is presented.