ORGANIC LETTERS

2012
Vol. 14, No. 16
4226–4229

Protecting-Group-Free Synthesis of Glycosyl 1—Phosphates

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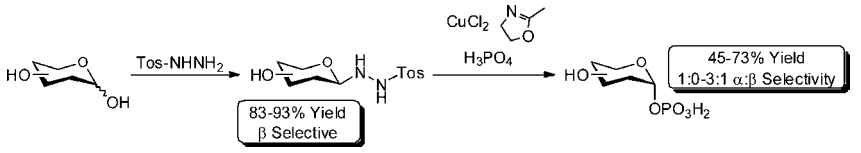
Received July 10, 2012

ABSTRACT



Glycosyl 1-phosphates enriched in the α-anomer are obtained without the use of protecting groups in two steps starting from the free hemiacetal. Condensation of free hemiacetals with toluenesulfonylhydrazide yields a range of glycosylsulfonohydrazide donors which can be oxidized using cupric chloride in the presence of phosphoric acid and the coordinating additive 2-methyl-2-oxazoline to give useful yields of the fully deprotected glycosyl 1-phosphates.

Glycosyl 1-phosphates are central intermediates in carbohydrate metabolism and the biosynthesis of oligosaccharides.[1] To access these useful intermediates a variety of protocols, both chemical and enzymatic, have been developed. Enzymatically, glycosyl 1-phosphates are often prepared by glycosyl 1-phosphate kinases although routes *via* phosphoglycomutases have also been explored.[2] Many monosaccharide-1-phosphate kinases have been cloned, expressed, and characterized for use in the scalable synthesis of glycosyl 1-phosphates.[3,4] Corresponding sugar nucleotides may also be obtained if a nucleotidyltransferase and inorganic pyrophosphatase are employed in addition to the glycosyl kinase in a one-pot protocol.[4] This methodology has been harnessed by recent work in glycorandomization which uses non-native glycosyl 1-phosphates as substrates for nucleotidyltransferase enzymes modified to have increased substrate promiscuity *via* directed evolution.[5] The resultant non-native sugar nucleotides have been shown to be effective glycosyl donors for glycosylation of a variety of natural products.[6]

Although major advances have been made in the development of enzymatic protocols, chemical approaches to glycosyl 1-phosphates remain attractive as they are more broad in their substrate scope.[7] The majority of these syntheses rely on hydroxyl protecting groups and as a consequence are multistep procedures.[7] For example, a fully protected glycosyl donor such as a glycosyl trichloroacetimidate, thioglycoside, glycosyl acetate, 1,2 orthoester, glycosyl halide, pentenoyl glysoside, glycal, or oxosulfonium glycoside can be used to glycosylate phosphate or a phosphoester.[8] Other methodologies couple unprotected hemiacetals to protected electrophilic phosphorus(III) sources such as chlorophosphates and phosphoramidates, which require a separate oxidation step to generate the corresponding phosphate.[9] The resultant glycosyl 1-phosphate may then be converted to its corresponding

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10.1021/ol3019083
Published on Web 07/30/2012

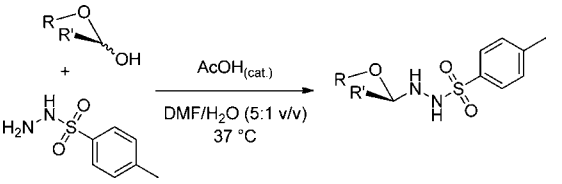
nucleotide sugar *via* exposure to a nucleoside phosphoromorpholidate, phosphoimidazolide, or another appropriate electrophilic phosphorus(V) source.[7,10] Although these methods can provide access to a desired deprotected glycosyl 1-phosphate, the number of steps required and difficult purifications limit their efficiency. These synthetic issues culminate in limited commercial availability and prohibitive prices for many glycosyl 1-phosphates.

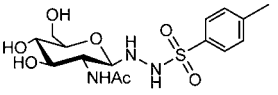
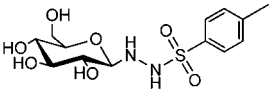
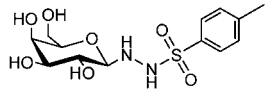
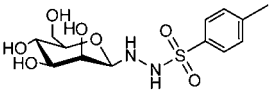
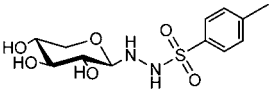
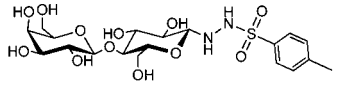
Hanessian et al. have elegantly circumvented the use of protecting groups in glycosyl 1-phosphate synthesis by exploiting methoxypyridine (MOP) glycosyl donors.[11] Although the phosphorylation step in this methodology is protecting-group-free, synthesis of each MOP donor from free sugar requires a multistep protocol that involves protecting groups. We herein report the first example of a two-step, protecting-group-free protocol for the synthesis of glycosyl 1-phosphates from hemiacetals.

We have previously reported the use of *N*′-glycosyltoluenesulfonohydrazides (GSHs) as glycosyl donors for the protecting-group-free synthesis of *O*-glycosides and glycosyl azides of *N*-acetyl-D-glucosamine (GlcNAc).[12] These GSH donors are easily prepared under mild conditions in one step from free sugars and can be activated using a source of electrophilic halogen such as *N*-bromosuccinimide (NBS). Due to the robustness of *O*-glycosylation using these donors, we speculated that they could be used to synthesize glycosyl 1-phosphates, if anhydrous phosphoric acid was used as the acceptor.

A range of GSH donors were easily accessed *via* acid-catalyzed condensation of free hemiacetals with *p*-toluenesulfonyl hydrazide (Table 1). This protocol does not require chromatographic purification; the donors were isolated by precipitation in high purity and excellent yield. Additionally, only β-anomers of GSH donors were formed under these conditions, further simplifying isolation and chacterization. The exclusive formation of the β-mannosylsulfonohydrazide (**4**) was confirmed by NOE experiments which showed similar enhancements between H1, H3, and H5.

Table 1. Acid-Catalyzed Condensation of *p*-Toluenesulfonylhydrazide with Various Carbohydrates



	Product	yield [a]
1		93%
2		90%
3		84%
4		84%
5		83%
6		90%

[a] Isolated yields.

Initial investigation into the phosphorylation of GSH donor **1** employed the conditions that were successful for *O*-glycosidations: NBS in DMF and anhydrous phosphoric acid as the acceptor.[12] No conversion of donor **1** to the corresponding glycosyl phosphate **7** was observed under these conditions (Table 2). We hypothesized that the poor nucleophilicity of triprotic phosphoric acid was preventing formation of the glycosyl 1-phosphate. Attempts to improve the nucleophilicity of the phosphate using less acidic conditions failed to improve the reaction conversion (Table 2).

Next, Lewis acidic metal ion oxidants, which may coordinate the phosphoric acid and activate the GSH donor, were explored. Several transition metal salts were screened as oxidizing agents of GSHs, by qualitatively monitoring the reaction for gas evolution (N_2) (Table 2). Of the metal salts screened only cupric chloride ($CuCl_2$) was an effective oxidant. This is consistent with previous reports of Cu(II)-mediated oxidation of anomeric hydrazines.[13]

Although the desired glycosyl phosphate **7** was formed in the presence of $CuCl_2$, the conversion was low and a substantial amount of the hydrolysis product (GlcNAc) was observed. Since all glycosylations were performed

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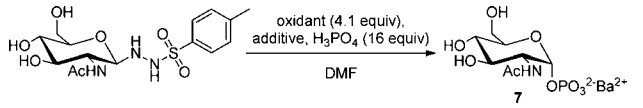
(12) Gudmundsdottir, A. V.; Nitz, M. *Org. Lett.* **2008**, *10*, 3461–3463.

(13) Hann, R. M.; Hudson, C. S. *J. Am. Chem. Soc.* **1934**, *56*, 957–958.

Table 2. Optimization of Protecting-Group-Free Phosphorylation[a]



oxidant[b]	additive[c]	**7** (GlcNAc)[d]
NBS	none	0% (0%)
NBS	diisopropylethylamine (7.8 equiv)	0% (0%)
$Cu(OTf)_2$	none	no reaction
$CuCl_2$	none	22% (66%)
$CuCl_2$	imidazole (4.1 equiv)	33% (66%)
$CuCl_2$	2-methyl-2-oxazoline (4.1 equiv)	66% (33%)
$CuCl_2$	2-methyl-2-oxazoline (1.0 equiv)	3% (26%)
$CuCl_2$	2-methyl-2-oxazoline (8.2 equiv)	0% (0%)
$CuCl_2$	2-methyl-2-oxazoline (4.1 equiv)[e]	91% (9%)

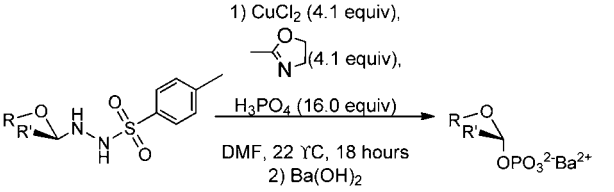
[a] Reaction time was 2 h except where otherwise indicated. [b] The concentration of **1** in the reaction was held at 48 mM. [c] The number of equivalents of additive is relative to compound **1**. [d] Qualitative NMR yield based on percent area of integration of all resonances in the anomeric region of the spectrum (5.5–4.0 ppm). [e] Reaction time was 18 h.

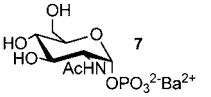
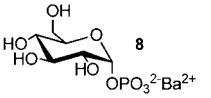
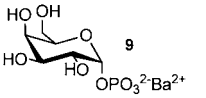
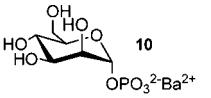
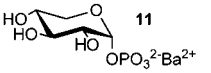
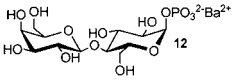
under anhydrous conditions the hydrolysis was thought to arise from reaction with solvent. This process is presumably facilitated by $CuCl_2$, as little hydrolysis was observed in the presence of NBS. In efforts to reduce the extent of hydrolysis, various coordinating and noncoordinating additives were investigated (Tables 2 and S1). Of the additives screened, imidazole and 2-methyl-2-oxazoline were the most promising, as they promoted GSH donor oxidation by Cu(II). 2-Methyl-2-oxazoline proved to be a superior additive, as less donor hydrolysis was observed under reaction conditions. Optimization of the number of equivalents of this additive produced interesting results, with less than or more than 1 equiv per Cu(II) ion promoting side-product formation. When 1 equiv of additive was included the desired α-glycosyl 1-phosphate was formed in a 2:1 ratio with hydrolyzed donor, and no other carbohydrate related products were observed. Increasing the duration of reaction from 2 to 18 h led to glycosyl 1-phosphate **7** as the major carbohydrate product. The time dependence of the reaction suggests that an unstable intermediate is formed, which is slowly converted to the glycosyl 1-phosphate.

The optimized reaction conditions proved versatile and, when applied to donors **1**–**6**, gave the desired α-glycosyl 1-phosphates as the major product (Table 3). The mannose GSH donor (**4**) provided exclusively the α-anomer of the corresponding glycosyl phosphate **10**. A particularly attractive attribute of this protocol is its ability to provide good yields of higher order glycosyl 1-phosphates, such as disaccharide **12**.

A general protocol was developed for the isolation of the glycosyl 1-phosphate barium salts that does not require chromatography. An initial precipitation of the crude product from the reaction mixture with dichloromethane

Table 3. Copper-Mediated Phosphorylation of Various *p*-Toluenesulfonohydrazide Glycosyl Donors[a]



donor	glycosyl-1-phosphate	isolated yield (α:β)
1	 **7**	60% (6.7:1)
2	 **8**	64% (3.5:1)
3	 **9**	62% (7:1)
4	 **10**	45% (1:0)
5	 **11**	51% (5:1)
6	 **12**	73% (3:1)

[a] Minor noncarbohydrate impurities and trace amounts of corresponding free hemiacetals were present in all isolated glycosyl 1-phosphates. Compounds **7**, **10**, **11**, and **12** were further purified using Sephadex A-25 anion exchange resin with NH_4HCO_3 as the eluent. This polishing purification resulted in a 25–62% loss of yield relative to those shown in the table.

removes the majority of noncarbohydrate impurities. The resulting precipitate is dissolved in a solution of dry DMF/ethanol and subsequently neutralized with barium hydroxide to remove excess phosphate and cupric/cuprous chloride *via* formation of water insoluble barium phosphate and copper hydroxide species respectively. The barium salts of the phosphate sugars are water-soluble and can be isolated after lyophilization of the aqueous solution.[14] The isolated yields reported in Table 3 reflect the isolated product that contains small amounts of free hemiacetal and minor impurities (see Supporting Information (SI)). The barium salts of the phosphate sugars can be converted into biologically useful ammonium salts after a polishing purification step with anion exchange chromatography which yields analytically pure samples (see SI).

The present study demonstrates that GSH donors efficiently provide a wide variety of glycosyl 1-phosphates enriched in the α-anomer in yields comparable to previous literature reports (Table 3). Investigations into

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bolstering the diastereoselectivity of this protocol and the mechanism of the transformation are currently underway.

Acknowledgment. Funding from the Natural Science & Engineering Research Council of Canada, the Canadian Foundation for Innovation (Project #19119), and the Ontario Research Fund for funding of the Centre for Spectroscopic Investigation of Complex Organic Molecules and Polymers is gratefully acknowledged. We thank Dr. T. Burrow, Dr. D. C. Burns, and Mr. D. Pichugin for assistance in obtaining NMR spectral data.

Supporting Information Available. Full experimental details and NMR spectra data are available. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.