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Thiophosphate and thiophosphonate analogues of glucose-1-phosphate: synthesis and enzymatic activity with a thymidylyltransferase

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ABSTRACT

Synthetic methods were investigated for the preparation of *O* and *S*-glucosyl thiophosphates and glucosyl 1C-thiophosphonate. Four protected glucosyl thiophosphate compounds were synthesized and characterized as precursors to glucose 1-thiophosphate. The effect of various reaction conditions and the nature of the carbohydrate and thiophosphate protecting groups and how they impact both the yields and α/β diastereoselectivity of the glucosyl thiophosphate products were explored. A novel isomerization from an *O*-linked to *S*-linked glucosyl thiophosphate was observed. α-D-Glucose-1C-thiophosphonate was synthesized and evaluated as a substrate for the thymidylyltransferase, Cps2L. Tandem mass spectrometric analysis determined the position of sulfur in the sugar nucleotide product.

1. Introduction

Glycosyl phosphates are natural phosphates of importance in biological systems as key metabolic intermediates and as bacterial cell wall constituents.[1,2] Thiophosphates are analogues of natural phosphates in which the P–O bond has been replaced with a phosphorus–sulfur (P–S) bond. The sulfur atom within thiophosphates is capable of mimicking both the binding and nucleophilic properties of the oxygen atom of natural phosphates.[3,4] Many classes of enzymes utilize phosphate or pyrophosphate as substrates or recognition elements. Thiophosphates have provided significant insight into interactions of these enzymes, including determining the stereochemical outcome of phosphoryl transfer at phosphorus.[5] Despite the extensive literature associated with enzymes transferring phosphates, aspects of the mechanism of enzymatic phosphoryl transfer remain contentious, with metal fluoride complexes providing new insights for those enzymes forming covalent phosphoryl-enzyme intermediates.[6] Phosphonothioates are non-hydrolysable derivatives of phosphates with the structure $RPS(OH)_2$ and have been used to probe receptor signaling. Prestwich and coworkers reported the synthesis and evaluation of several phosphonothioate analogues as antagonists of lysophosphatic acid (LPA) receptors.[7] Sugar phosphorylases are a class of enzymes that use glycosyl phosphates as substrates to furnish oligosaccharides. These enzymes offer complementary approaches to that of chemical synthesis in the preparation of oligosaccharides due to the regio- and stereoselectivities of the glycosidic linkage formed.[8]

Glycosyl phosphates including β- and α-GlcNAc-1-phosphate, β- and α-glucose 1-phosphate, have various roles in biological pathways, making their thiophosphate or thiophosphonate analogues attractive synthetic targets with which to probe enzyme function. β-D-Glucose 1-phosphate is a substrate for β-phosphoglucomutase and is suspected to be a precursor in cell wall biosynthesis in *Lactococcus lactis*.[9,10] α-D-Glucose 1-phosphate is a substrate for nucleotidylyltransferase enzymes in a number of biosynthetic pathways, including the incorporation of L-rhamnose into the cell wall of harmful pathogens including *Streptococcus pneumoniae*, *Pseudomonas aeruginosa,* and *Mycobacterium tuberculosis*.[11–13]

Glycosyl thiophosphates have been synthesized in both *O* and *S*-glycosylated forms. *O*-glycosyl thiophosphates have been previously synthesized as glycosyl donors for the stereoselective production of glycosides. Hui and coworkers reported the synthesis of acetyl and benzyl-protected glycosyl dimethylthiophosphates in 51% and 86% yields, respectively.[14] They generated several diastereotopic mixtures of fully protected glycosyl thiophosphates with varying α/β selectivity, depending on the nature of the glycosyl protecting groups. More recently, Piekutowska and Pakulski generated a series of benzoyl protected mannose, galactose, and glucose *S*-glycosyl thiophosphates by reacting anomeric thiocyanates with *O*-alkyl or *O*-trimethylsilyl phosphites.[15] However, the global deprotection of these glycosyl thiophosphates was not reported.

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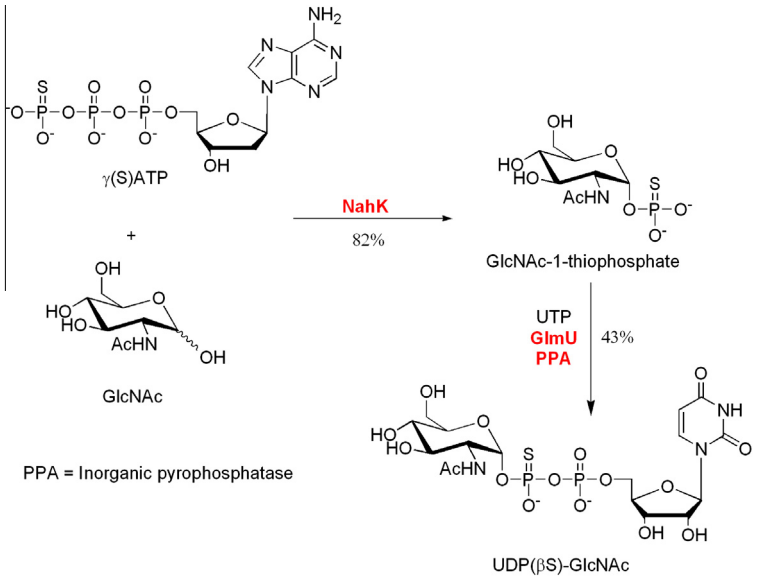
The only example of a free sugar glycosyl thiophosphate was reported by Wang and coworkers in 2011 using enzymatic approaches.[16] Using NahK (EC 2.7.1.162), an *N*-acetylhexosamine 1-kinase, they were able to produce GlcNAc-1-thiophosphate on a milligram scale from GlcNAc and commercially available γ(S)ATP. The GlcNAc-1-P uridylyltransferase GlmU (EC 2.3.1.157) was then utilized to produce UDP(βS)-GlcNAc in a respectable yield (Scheme 1).

No synthetic route currently exists to access fully deprotected *O*-glycosyl and *S*-glycosyl sugar 1-thiophosphates or glucosyl 1C-thiophosphonate. We report the synthesis of a series of differentially protected *O*-glucosyl and *S*-glucosyl thiophosphates. We looked at the influence of reaction conditions and protecting groups on the diastereotopic ratio of products and explored common deprotection strategies toward the production of *O*- and *S*-glucose 1-thiophosphate. The thiophosphonate analogue, Glucose 1C-thiophosphonate, was synthesized and enzymatically transformed into a sugar nucleotide analogue of deoxythymidine diphosphate-α-D-glucose (dTDP-Glc) using thymidylyltransferase Cps2L.[17] This compound represents the first example of a thiophosphonate containing sugar nucleotide analogue.

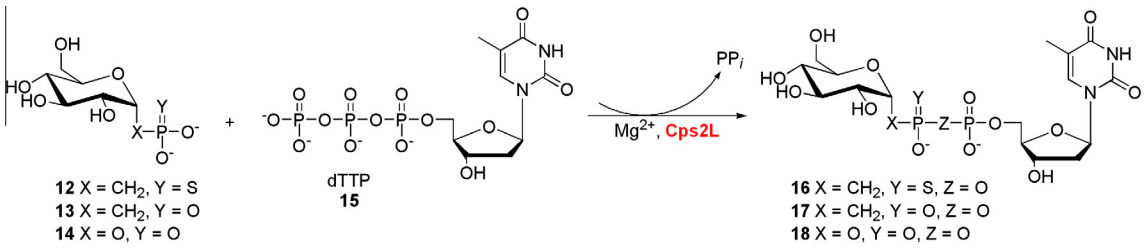
2. Results and discussion

The formation of glucosylated thiophosphates was explored using acetyl (**1**) or benzyl (**2**) protected glucopyranose as the starting sugar together with diethylchlorothiophosphate (**3**) using the conditions described by Hui[14] or diphenylchlorothiophosphate (**4**) using the conditions described[18] for preparation of glycosyl phosphates (Scheme 3). Table 1 summarizes the various reaction conditions and isolated yields for α/β-D-glucopyranosyl thiophosphate synthesis.

The reaction solvent and base were investigated in an attempt to enhance yield and selectivity. When diethylchlorothiophosphate (**3**) was used as the electrophile (entries 1–3), both *n*-butyllithium (*n*-BuLi) and lithium diisopropylamide (LDA) were comparably effective bases in tetrahydrofuran (THF), however 4-dimethylaminopyridine (DMAP) in dichloromethane (CH_2Cl_2) was ineffective in promoting glycosyl coupling. The yields for **5** and **6** (entries 1–2) were comparable with analogous methyl protected glycosyl thiophosphates reported by Hui (51–86%).[14] For ease of preparation, *n*-BuLi was chosen as the base of choice for the production of **5** and **6**. When diphenyl chlorothiophosphate (**4**) was used as the electrophile (entries 4–5), either *n*-BuLi/THF or DMAP/CH_2Cl_2 conditions proved to be sufficient for the production of **7a**. The complete structures of **7a** and **7b** can be found in Scheme 4. The reaction conditions used affected both the diastereotopic ratio of α/β products as well as the overall yield. When *n*-BuLi/THF conditions were used the resultant α/β ratio of **7a** was found to be ~6:1 with a 16% overall yield (entry 5). When DMAP/CH_2Cl_2 conditions were used, only the α isomer was obtained with a 24% overall yield (entry 4). The yields of **7a/7b** (entry 4) were significantly less than analogous glycosyl phosphates synthesized using DMAP/CH_2Cl_2 conditions reported previously (55–82%).[18] While reduced coupling efficiency can be partially attributed to the steric bulk of the phenyl protecting groups of **4** relative to the ethyl protected **3**, the driving factor in the reduced yields is believed to be the excellent leaving group capacity of the diphenylthiophosphate



Scheme 1. Enzymatic synthesis of GlcNAc-1TP and UDP(βS)-GlcNAc.[16]



Scheme 2. Reactions catalyzed by Cps2L.