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Synthesis of Sugar Nucleotides by Application of Phosphoramidites

Henrik Gold,[‡,†] Pieter van Delft,[‡,†] Nico Meeuwenoord,[†] Jeroen D. C. Codée,[†] Dmitri V. Filippov,[†] Gerrit Eggink,[§] Herman S. Overkleeft,[†] and Gijs A. van der Marel*[,†]

Leiden Institute of Chemistry, Bioorganic Chemistry, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA, Leiden, The Netherlands, and Agrotechnology and Food Sciences Group, Wageningen University and Research Centre, P.O. Box 17, 6700 AA Wageningen, The Netherlands

marel_g@chem.leidenuniv.nl

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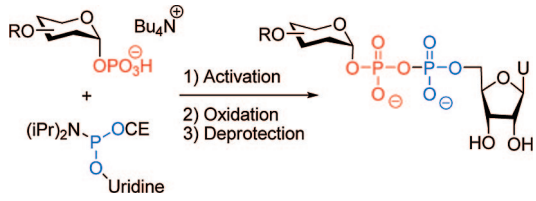

A new method for the construction of pyrophosphates is reported based on the coupling of a sugar phosphate and a nucleoside phosphoramidite. The *in situ* formed phosphate−phosphite intermediate was subsequently oxidized with *t*BuOOH. Three UDP-*N*-acetylglucosamine derivatives were prepared using this one-pot procedure in good yields.

Oligosaccharides and glycoconjugates play important roles in a variety of biological processes. The biosynthesis of these ubiquitous and structurally diverse biopolymers is catalyzed by glycosyltransferases.[1] These enzymes transfer a monosaccharide residue in a regio- and stereoselective manner from a specific sugar nucleotide donor to acceptors such as (oligo)saccharides, lipids, or proteins. On the basis of sequence similarities, 91 families of glycosyltransferases can be discerned.[2] While eukaryotic glycosyltransferases employ a limited number of sugar nucleotides as glycosyl donors (UDP-Glc, UDP-GlcNAc, UDP-GlcUA, UDP-Gal, UDP-GalNAc, GDP-Fuc, GDP-Man, and CMP-Neu5Ac), the structures of nucleoside diphosphate sugars in prokaryotes are both numerous and diverse.[3] Advances made in engineering of glycosyltransferases show promise that these enzymes may be used for the synthesis of relevant oligosaccharides.[4] Glycosyltransferases that incorporate non-natural monosaccharides or accept non-natural substrates for glycosylation may become a powerful tool for the synthesis of non-naturally occurring bioconjugates. Furthermore, synthesis of labeled or orthogonally tagged sugar nucleotides will aid the efforts in glycomics.[5] Thus, suitable procedures are required that make sufficient quantities of nucleoside diphosphate sugars available, in which the sugar moiety may be a natural or a modified monosaccharide.

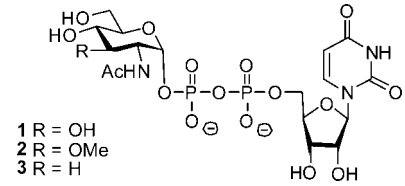

FIGURE 1. UDP−GlcNAc and analogues.

In this paper we present a new method for the preparation of nucleoside diphosphate sugars by the synthesis of UDP-*N*-acetylglucosamine (UDP-GlcNAc, **1**) and the unnatural congeners **2** and **3** (Figure 1). The modified sugar nucleotides **2** and **3** were selected by their virtue of being potential chain terminators in enzyme mediated polymerization reactions. For instance, hyaluronic acid (HA), a negatively charged and high molecular weight polymer, can be assembled using hyaluronan synthase (HAS), UDP-GlcNAc, (**1**) and UDP-glucuronic acid (UDP-GlcA).[6] Incorporation of an *N*-acetylglucosamine in which the C3−OH function is capped with a methyl functionality (**2**) or removed (**3**) would prohibit chain elongation at that position. Thus, it is expected that the length of the HA oligomer can be tuned by the use of **2** or **3** as additional substrates in the HAS reaction. Recently the group of Fairbanks reported the potential of 3-OH modified UDP-glucose derivatives as a new class of inhibitors of the biosynthesis of pathogenic oligosaccharides.[7]

A key step in the synthesis of nucleoside diphosphate sugars is the introduction of the pyrophosphate function between the 5′-OH position of a nucleoside and the anomeric hydroxyl of a saccharide unit. A variety of procedures has been reported that can roughly be classified in two main categories. The first one comprises the reaction of a protected glycosyl donor such as a halogenide with the tetrabutylammonium salt of a nucleoside 5′-diphosphate.[8] This approach is often hampered by a low anomeric diastereoselectivity, although there are some exceptions.[8a] In the second category, the pyrophosphate moiety can be introduced by coupling of a monosaccharide-1-phosphate with an activated nucleoside 5′-monophosphate.[9] The anomeric configuration of the final product can be controlled by stereo-

[†] Leiden Institute of Chemistry.
[‡] H.G. and P.v.D contributed equally to this work.
[§] Wageningen University and Research Centre.

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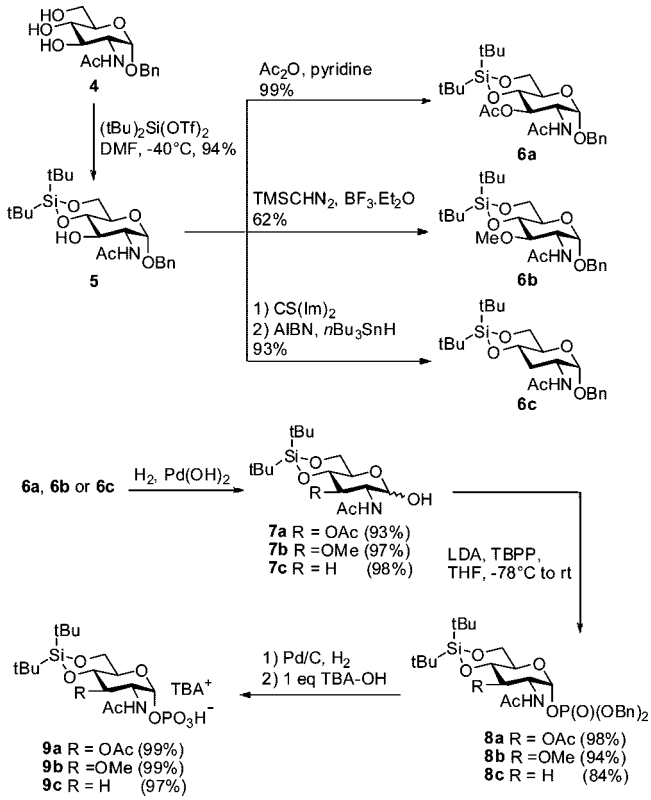
SCHEME 1. Synthesis of Sugar Phosphates 9a−9c

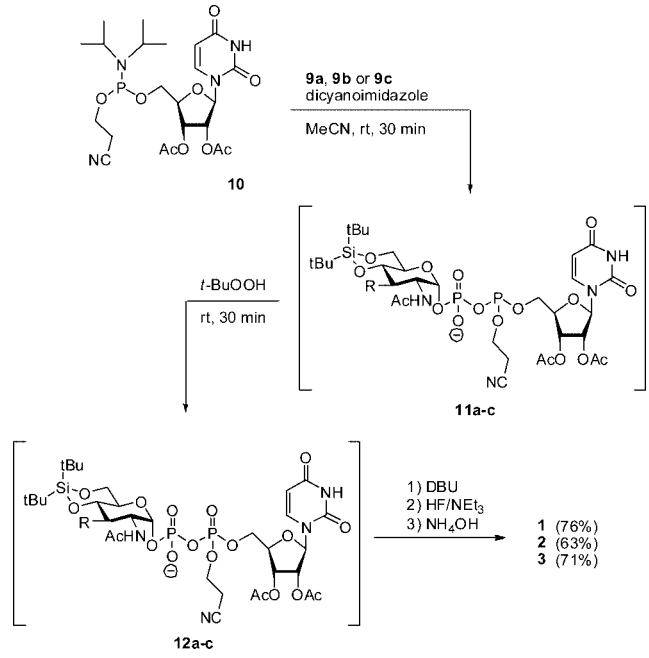


selective synthesis of the sugar 1-phosphate. The original method of Khorana and Moffat using nucleoside phosphoromorpholidates as activated phosphates has been most extensively pursued.[9a,b] Improvements of this method in terms of reaction time and yield have been reported by the utilization of a catalyst, such as tetrazole.[10] Recently, *cyclo*-saligenyl nucleosyl phosphotriesters were presented as active esters to attain nucleoside diphosphate glucopyranoses.[11]

The extensive use of phosphoramidite chemistry in synthesis of (modified) DNA/RNA fragments[12] encouraged us to explore the implementation of this chemistry in the formation of pyrophosphate moieties. UDP-GlcNAc **1** was selected as a first target to explore whether the reaction of an activated phosphoramidite with a phosphate and subsequent oxidation would lead to the desired pyrophosphate functionality.

The tetrabutylammonium salt of GlcNAc-α-1-phosphate **9a** (Scheme 1) was synthesized, starting from the known benzyl

SCHEME 2. One-Pot Reaction for the Synthesis of Sugar Nucleotides 1−3



2-acetamido-2-deoxy-α-D-glucoside (**4**)[13] in 84% total yield over 5 steps. The C4−OH and C6−OH were masked using a di-*tert*-butylsilylene group, which greatly enhances solubility of GlcNAc derivatives. The remaining hydroxyl of **5** was acetylated (**6a**) after which the anomeric benzyl group was removed with $Pd(OH)_2$ and hydrogen gas to produce hemiacetal **7a**. The anomeric phosphate was stereoselectively introduced by treatment of **7a** with LDA at −78 °C, followed by addition of tetrabenzylpyrophosphate (TBPP) to give the α-dibenzylphosphotriester **8a**. Hydrogenolysis of the benzyl groups in **8a** and addition of one equivalent of tetrabutylammonium hydroxide yielded GlcNAc-α-1-phosphate **9a**.

Uridine phosphoramidite **10**[14] (Scheme 2) was easily synthesized in four steps from commercially available uridine, in 93% total yield. The cyanoethyl group was selected as a phosphite protection because of its easy removal under anhydrous, basic conditions.

With sugar phosphate **9a** and phosphoramidite **10** in hand, the pyrophosphate formation was examined. The reaction progress was monitored by ^{31}P NMR spectroscopy (CD_3CN, 162 MHz). The activator dicyanoimidazole[15] and phosphate monoester **9a** ($\delta = -1.2$ ppm) were simultaneously added to a solution of phosphoramidite **10** ($\delta = 149.5$, 149.4 ppm) in CD_3CN. Within 30 min at room temperature, complete disappearance of amidite **10** and formation of two diastereomeric phosphate-phosphite intermediates **11a** [$\delta = 130.3$ (d, $J = 5.8$ Hz), 128.3 (d, $J = 4.5$ Hz), −10.9 (d, $J = 4.5$ Hz), −11.0 (d, $J = 5.8$ Hz) ppm] was observed. Subsequent oxidation with anhydrous *tert*-butylperoxide[16] gave the diastereomeric cyanoethyl protected pyrophosphate **12a** [$\delta = -10.7$ (d, $J = 16.2$ Hz), −11.4 (d, $J = 17.8$ Hz), −13.5 (d, $J = 17.8$ Hz), −13.6

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(16) Anhydrous conditions were used to prevent possible hydrolysis of the labile phosphite−phosphate anhydride **11**.

(d, $J = 16.2$ Hz) ppm]. The protecting groups were immediately removed by a three step procedure. First the cyanoethyl group was removed by treatment with anhydrous DBU, followed by addition of HF/Et_3N to deblock the di-*tert*-butylsilylene group and ammonium hydroxide to unmask the remaining alcohol functions. Purification of the obtained crude product by ion exchange chromatography gave pure UDP-GlcNAc **1** [$\delta = -10.8$ (d, $J = 21.1$ Hz), -12.5 (d, $J = 21.1$ Hz) ppm] in 76% yield.

Having established the value of cyanoethyl phosphoramidite **10** in the formation of UDP-GlcNAc **1**, the synthesis of the non-natural UDP-GlcNAc derivatives **2** and **3** was undertaken. The requisite methylated and deoxygenated GlcNAc-α-1-phosphate derivatives **9b** and **9c** were obtained from **5** as depicted in Scheme 1. The methyl group at the C3−OH of **5** was selectively introduced by treatment with TMS-diazomethane and $BF_3 \cdot OEt_2$ to give fully protected GlcNAc **6b**, without unwanted N-methylation. Alternatively, compound **5** was subjected to the Barton−McCombie procedure. Thus, after formation of the xanthate ester and radical reduction with AIBN and tri*n*-butyltin hydride, the deoxygenated compound **6c** was obtained in 93% yield. The modified GlcNAc derivatives **6b** and **6c** were then transformed into the corresponding α-phosphates **9b** and **9c** by the same sequence of reactions as described for the conversion of **6a** into **9a** (Scheme 1). Dicyanoimidazole mediated reaction of **9b** and **9c** with uridine phosphoramidite **10**, followed by oxidation and the outlined deprotection sequence, smoothly provided the modified UDP-GlcNAc derivates **2** and **3** in 63% and 71% yield respectively.

In conclusion, we have developed an efficient, robust and rapid procedure for the synthesis of sugar nucleotides, using easily accessible nucleoside phosphoramidites and sugar phosphates. Phosphoramidites were shown to be powerful phosphorylating agents in the formation of the pyrophosphate moiety. The procedure presents an attractive alternative to existing methods and is a valuable asset for future research in glycobiology. Current research is directed at the extension of the methodology to other nucleophiles and nucleobases.

Experimental Section

General Experimental Procedure for Reaction of a Sugar Phosphate (9a−c) and Nucleoside Phosphoramidite (10). Sugar phosphate **9a−c** (0.12 mmol, 1.2 equiv) and dicyanoimidazole (0.2 mmol, 2 equiv) were dissolved in 2 mL anhydrous MeCN and added to a solution of phosphoramidite **10** (0.1 mmol, 1 equiv) in anhydrous MeCN (1.5 mL). The reaction mixture was stirred at room temperature for 30 min after which *t*-BuOOH (0.4 mmol, 4 equiv) was added. After 30 min of reaction time DBU (0.5 mmol, 5 equiv) was added and the reaction was stirred for an additional 30 min. The reaction mixture was stirred with triethylamine (1.6 mmol, 16 equiv) and $NEt_3 \cdot (HF)_3$ (0.8 mmol, 8 equiv) for four hours, followed by addition of NH_4OH 25% (40 mmol, 40 equiv). The reaction was stirred for 12 h and then diluted with 4 mL MilliQ. The mixture was washed with 4 mL of DCM and the organic phase was extracted with 4 mL MilliQ. The combined water layers were concentrated in vacuo, redissolved in MilliQ and centrifuged. The supernatant was applied to a strong anion exchange column and eluted with a gradient of ammonium acetate (0.05−0.5 M). The fractions containing the product were collected and concentrated under reduced pressure. Repeated lyophilization and filtration over Amberlite Na^+-form produced the desired sugar nucleotides **1**, **2**, and **3** in 63−76% yield based on phosphoramidite **10**.

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Supporting Information Available: Detailed experimental procedures, including full characterization of unknown compounds and copies of ^1H, ^{13}C, and ^{31}P spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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