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2,3,6-Trideoxy sugar nucleotides: synthesis and stability

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ABSTRACT

The synthesis and characterization of highly challenging 2,3,6-trideoxy sugar nucleotides were described for the first time. The study of their hydrolysis kinetics in aqueous buffers provided insight into their application as glycosyl donors.

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Introduction

Glycosyltransferases play key roles in important cellular processes such as cell wall biosynthesis in bacterial pathogens and signal transduction, and carcinogenesis in human.[1–3] Glycosylated natural products produced by microorganisms, such as erythromycin and vancomycin, represent an indispensable source of biomedically useful molecules.[1–3] The biosynthesis of these glycoconjugates is mediated by glycosylation reactions catalyzed by specific glycosyltransferases which require activated sugar donors in the form of sugar nucleotides as substrates.[2–4] A variety of sugars appended to natural product scaffolds are of crucial importance in tuning the therapeutic properties of these complex molecules.[1–3] Interestingly, it was found that glycosyltransferases are rather promiscuous with respect to sugar nucleotide substrates, which can be utilized to produce diversified compounds.[5] Unfortunately, this effort, as well as the study of glycoconjugate biosynthesis, were hindered by the limited availability of required unusual sugar nucleotides.

Sugar nucleotides present a synthetic challenge due to a number of complications, including susceptibility to hydrolytic cleavage, low solubility in organic solvents, and the presence of polar and charged groups.[6–9] The deoxy-sugar nucleotides are especially difficult to synthesize because the removal of electron-withdrawing hydroxyls makes the positively charged oxocarbenium, which is the intermediate of hydrolytic cleavage, more stable, thus the sugar nucleotides and their synthetic intermediates are more susceptible to hydrolysis.[10,11] The stability of sugar nucleotides decreases with the decreasing number of hydroxyls on sugar ring.[12] In the past, although the synthesis of 2-deoxy sugar nucleotides[13] and a stable isostere with C-glycosidic phosphonate linker[14] have been reported using both chemical and enzymatic methods, the synthesis of 2,3,6-trideoxy sugar nucleotides has never been explored despite of their existence in a large number of natural products including L-rhodinose in urdamycin B and L-amicetose in tetrocarcin A (Fig. 1).[15,16] Although they have significant value in the elu-

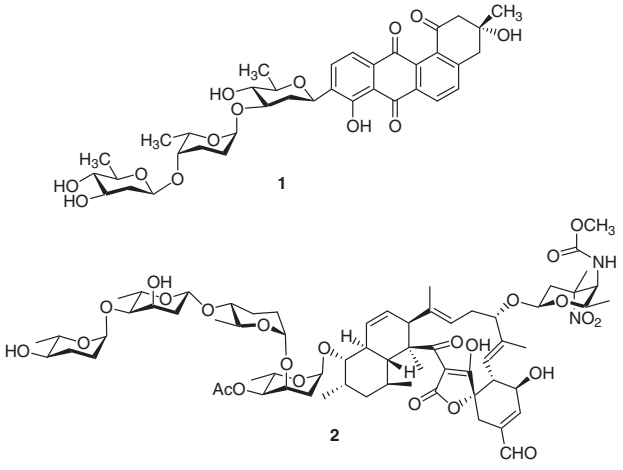

Figure 1. Examples of natural products containing 2,3,6-trideoxy sugars. Urdamycin B (1) has L-rhodinose and tetrocarcin A (2) has L-amicetose.

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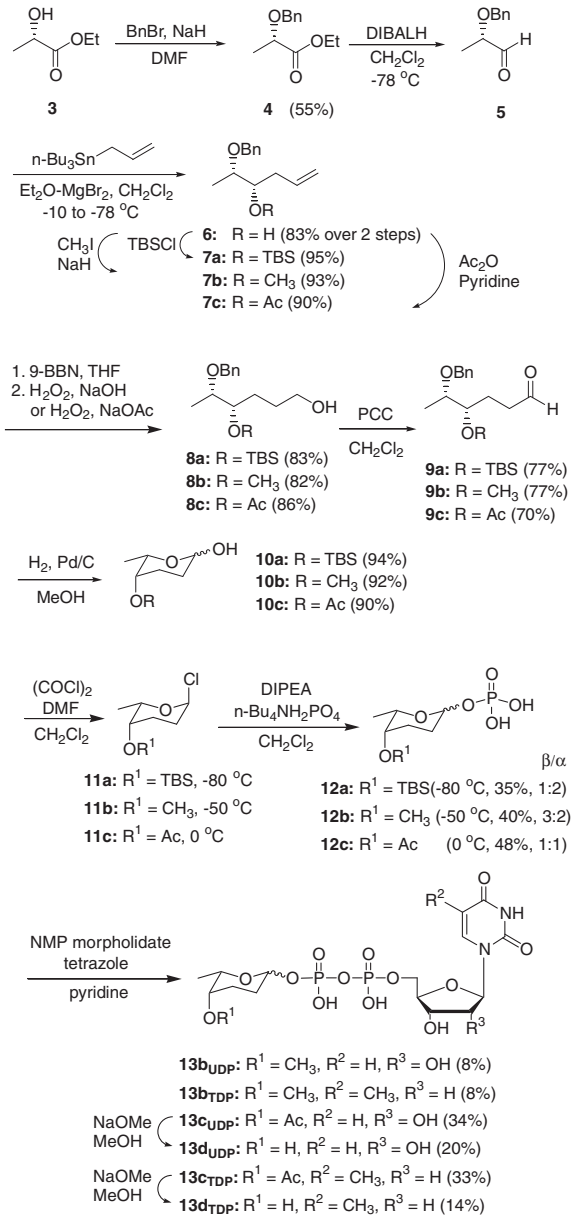
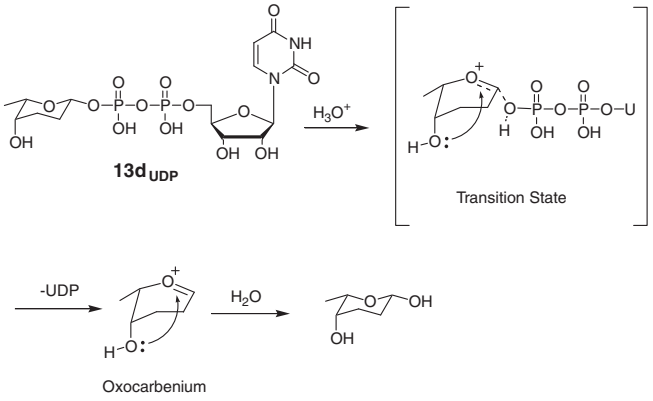
cidation of biosynthetic mechanisms and discovery of new medicinal molecules, the knowledge of the synthetic feasibility and the stability of 2,3,6-trideoxy sugar nucleotides were nonexistent.

Here we undertook the synthesis of NDP-L-rhodinose as representatives of 2,3,6-trideoxy sugar nucleotides and the study of their stability in aqueous buffers. The 4-OH-, as well as the 4-OCH_3- and 4-OAc-NDP-L-rhodinose (**13b**–**d**) were successfully synthesized and characterized, while the 4-OTBS-L-rhodinose monophosphate (**12a**) was too labile to get through the final pyrophosphate bond formation (Scheme 1). This is the first time the synthesis and stability study of 2,3,6-trideoxy sugar nucleotides were reported. It was found that the NDP was the primary hydrolytic cleavage product (Scheme 2) and the rate of cleavage is determined by a through-bond inductive effect as well as a through-space electronic effect. And the kinetics study of the hydrolysis provided a basis for the selection of enzymatic reaction conditions and guidelines for the synthesis and storage of 2,3,6-trideoxy sugar nucleotides.



Scheme 1. Synthesis of NDP-L-rhodinose.



Scheme 2. Hydrolytic reaction of 2,3,6-trideoxy sugar nucleotide.

Results and discussion

Firstly, the 4-OTBS-, 4-OCH_3-, and 4-OAc-L-rhodinose (**10a**–**c**) were synthesized from (*S*)-ethyl lactate (**3**) using methods adapted from Schlessinger et al. (Scheme 1).[17] After protection with benzyl group, ester **4** was reduced to aldehyde **5** with DIBALH. The chelation controlled stereoselective addition with tri-*n*-butylallylstannane in the presence of $MgBr_2$–Et_2O gave olefin **6**, which in turn was converted to its TBS, methyl, or acetyl derivatives **7a**–**c** under conditions of TBSCl, CH_3I/NaH, or Ac_2O/pyridine. Hydroboration of olefin **7a**–**c** with 9-BBN followed by oxidation with H_2O_2–NaOH (**7a** and **b**) or H_2O_2–NaOAc (**7c**) gave the corresponding alcohols **8a**–**c**. After oxidation with PCC, the resulting aldehydes **9a**–**c** were converted to 4-OTBS-, 4-OCH_3-, and 4-OAc-L-rhodinose (**10a**–**c**) under Pd/C catalyzed hydrogenation. It was necessary to use anhydrous methanol as the solvent to suppress the hydrolysis of acetyl group on **10c**.

The glycosyl monophosphates **12a**–**c** were obtained via the glycosyl chloride intermediates **11a**–**c** using the method adapted from Kahne et al. (Scheme 1).[7] The stability of the glycosyl chlorides decreases when the electron-withdrawing ability of the sugar ring substituents decreases due to the stabilization of the oxocarbenium intermediate.[10,11] Indeed, chloride **11c** with an electron-withdrawing 4-OAc substituent was easily generated at 0 °C, while chloride **11a** with an electron-donating 4-OTBS substituent was only successfully prepared under temperatures as low as −80 °C. Consistently, chloride **11b** with a moderate electron-donating 4-OCH_3 was prepared at −50 °C. Decomposition of **11a** and **b** was observed when temperature was raised to 0 °C. Subsequent phosphorylation with tetrabutylammonium dihydrogen phosphate ($Bu_4NH_2PO_4$) in the presence of DIPEA in dichloromethane gave monophosphates **12a**–**c**. In this case, the steric effect instead of electronic effect determined the β/α ratio. Among the three substituted chlorides, **11a** with the bulkiest 4-OTBS substitution gave the lowest β/α ratio of 1:2 presumably because the steric presence of OTBS hindered the S_N2 trajectory thus disfavoring the formation of the corresponding β-anomer. At the same time, the 4-OCH_3 substituted chloride **11b** which is the least sterically hindered gave the highest β/α ratio of 3:2. Phosphates **12b** and **c** were successfully converted to their UDP and TDP derivatives using the method developed by Wong et al.[18] Since the β-anomer is the bioactive configuration in most cases, the isolation and characterization of pure β-anomer were emphasized on purification. Acetylated sugar nucleotides **13c$_{UDP}$** and **13c$_{TDP}$** were converted to NDP-L-rhodinoses (**13d$_{UDP}$**, **13d$_{TDP}$**) by removal of acetyl group under NaOMe/MeOH conditions. Thus, six 2,3,6-tridexoy sugar nucleotides **13b$_{UDP}$**, **13b$_{TDP}$**, **13c$_{UDP}$**, **13c$_{TDP}$**, **13d$_{UDP}$**, and **13d$_{TDP}$** were successfully synthesized. Although they are susceptible to hydrolysis in

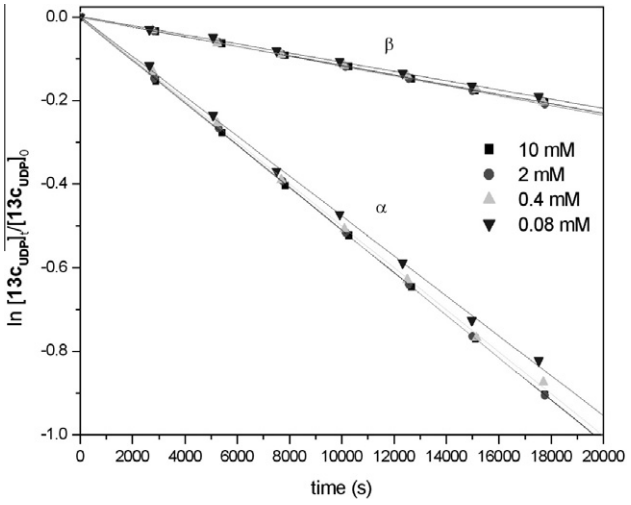


Figure 2. Kinetics of the hydrolysis of **13c$_{UDP}$** α- and β-anomers at different concentrations (0.08–10 mM, pH 8.0, T = 30 °C) proved first order reaction (R >0.999 in all cases). $k_\alpha = 4.99 \times 10^{-5}\ s^{-1}$ and $t_{1/2}$ = 232 min $k_\beta = 1.14 \times 10^{-5}\ s^{-1}$ and $t_{1/2}$ = 1011 min.

aqueous solution, they can be stored at −20 °C without decomposition over a few months as lyophilized powder.

Considering the susceptibility of 2,3,6-trideoxy sugar nucleotides toward hydrolytic cleavage, appropriate selection of the buffer conditions is critical for carrying out successful enzymatic reactions. Thus, we took UDP-L-rhodinose derivatives **13b$_{UDP}$** (4-OCH_3), **13c$_{UDP}$** (4-OAc), and **13d$_{UDP}$** (4-OH) as representatives to study their stability in aqueous buffers. First the hydrolysis reaction was demonstrated to be first order by the linearity of the kinetic curve in Figure 2 (Fig. S1) and the product was found by HPLC and ^{31}P NMR to be UDP instead of UMP (Scheme 2, Fig. S2), which is due to the absence of neighboring group participation from the 2-position.[19,20] The stability decreases in the order of **13c$_{UDP}$**-β > **13c$_{UDP}$**-α > **13b$_{UDP}$**-β >> **13d$_{UDP}$**-β (Fig. 3). The β-anomer is more stable than the α-anomer which is consistent with a kinetic anomeric effect.[21] These sugar nucleotides are most stable at basic

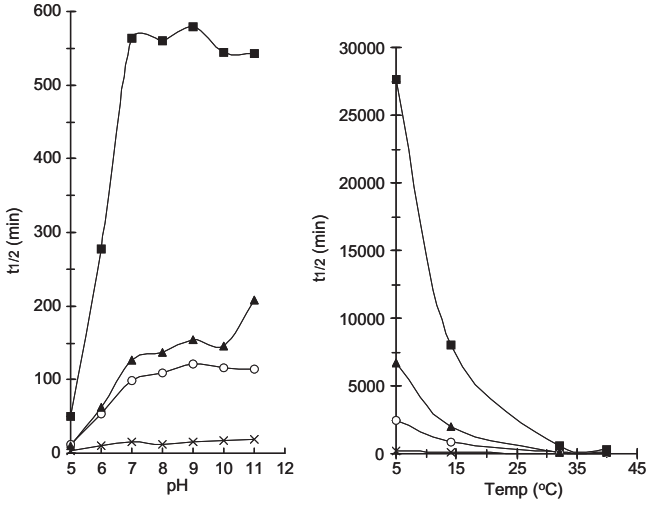


Figure 3. (Left) Effect of pH on the half life ($t_{1/2}$) of sugar nucleotides (30 °C); (right) Effect of temperature on the half life ($t_{1/2}$) of sugar nucleotides (pH 7.0). **13b$_{UDP}$**-β (○); **13c$_{UDP}$**-β (■); **13c$_{UDP}$**-α (▲); **13d$_{UDP}$**-β (×).

pH ⩾ 7.0 and their half life ($t_{1/2}$) increased 30- to 130-fold when temperature was lowered from 40 to 5 °C. The 4-OAc^- substituted **13c$_{UDP}$** is stabilized by the electron-withdrawing acetyl group. It is intriguing to observe the unusually fast hydrolysis of 4-OH-L-rhodinose nucleotide **13d$_{UDP}$** that has a half life of 15 min at pH 7.0 and 30 °C (Fig. S3). It can be rationalized by the through-space electronic effect of the axial 4-OH that donates electron density to the positively charged ring oxocarbenium,[21] thus stabilizing the transition state (Scheme 2).

In summary, using L-rhodinose as an example, the highly interesting but elusive 2,3,6-trideoxy sugar nucleotides were synthesized and characterized for the first time. The stability of its 4-OH, 4-OAc, and 4-OCH_3 derivatives in aqueous buffers was investigated and their stability increased with the elevation of pH and decreased when temperature was raised. The 4-OH derivative showed significant instability in aqueous buffer presumably due to the through space participation of the 4-OH electrons in stabilizing the oxocarbenium intermediate.

Acknowledgments

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Supplementary data

Supplementary data associated (experimental procedures, characterization of new compounds, and kinetics experiments) with this Letter can be found, in the online version, at doi:10.1016/j.tetlet.2011.08.132.

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