



Chemical synthesis of polyprenyl sialyl phosphate, a probable biosynthetic intermediate of bacterial polysialic acid

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ABSTRACT

Using reaction of moraprenyl phosphate with the known *N*-acetylsialyl chloride and the novel *N,N*-diacetylsialyl (Neu5Ac₂) chloride α - and β -anomers of polyprenyl sialyl phosphate were synthesized for the first time. The α -selectivity dramatically increased when Neu5Ac₂ chloride was used as the glycosyl donor.

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1. Introduction

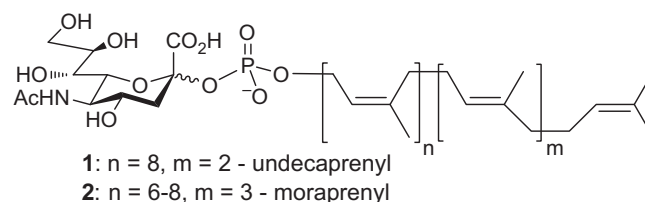
Glycosyl polyprenyl monophosphates have been identified as important intermediates in biosynthesis of carbohydrate chains of various cell surface biopolymers.¹ Although many derivatives of hexoses have been prepared,² polyprenyl sialyl phosphate **1** with undecaprenol chain (Scheme 1) has never been synthesized. This compound was postulated to be a primary glycosyl acceptor in the biosynthesis of bacterial polysialic acid, but its structure was based on indirect evidence, in particular, the stereochemistry is unknown.³ Further progress in elucidating the biological functions of **1** is hampered by absence of methods of its preparation. In this paper we describe the first chemical synthesis of polyprenyl sialyl phosphate **2**, which incorporates a slightly different polyprenyl chain. We used readily available plant moraprenol rather than poorly accessible bacterial undecaprenol since the corresponding glycosyl moraprenyl phosphates are known² to be equally recognized by bacterial enzymes as the parent undecaprenyl derivatives.

2. Results and discussion

An efficient method for the synthesis of polyprenyl glycosyl phosphates is based on reaction of polyprenyl trichloroacetimidate with glycosyl phosphates.² The reaction between Neu5Ac α -glycosyl phosphate α -**3a**^{4,6} and moraprenyl trichloroacetimidate **4**² (Scheme

2) was performed in CH₂Cl₂, the course of the reaction being monitored by ³¹P NMR (δ_P –5.4 (α -**3a**) and δ_P –6.4 (α -**5a**)). The yield of target compound α -**5a**, estimated by integration of the respective signals in the ³¹P NMR spectrum, was only about 5% as the activation of the imidate **4** under the reaction conditions was not effective. Nevertheless, this experiment allowed us to determine the chemical shifts of the α -anomer of moraprenyl sialyl phosphate α -**5a** (see below).

Since the known method for the synthesis of glycosyl polyprenyl phosphates failed in the case of sialic acid we were forced to develop a novel approach to the synthesis of polyprenyl sialyl phosphate **2**. We have recently shown that the *N*-acetylsialyl chloride **7a** could successfully be applied for the synthesis of sialyl phosphate **3a**.^{4–6} The glycosyl chloride **7a** was found to react readily with tetrabutylammonium salts of various types of phosphates.^{4–6} We were interested if polyprenyl phosphate **8** would react with sialyl chloride **7a** with the formation of the desired polyprenyl sialyl phosphate **5a** in one step (Scheme 3). The polyprenyl

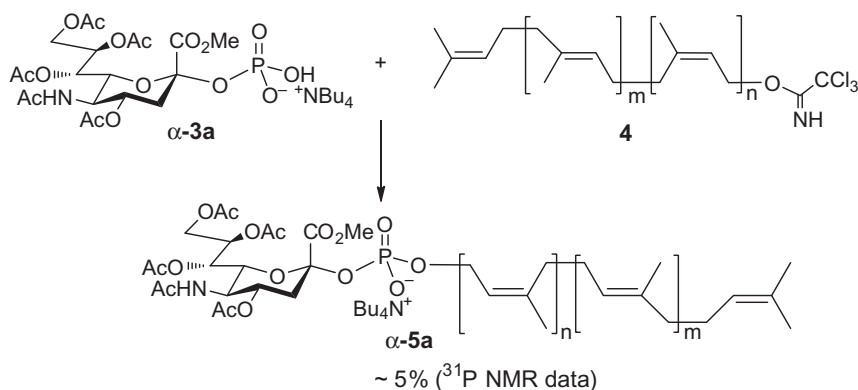


Scheme 1. Polyprenyl sialyl phosphates with different polyprenyl chains.

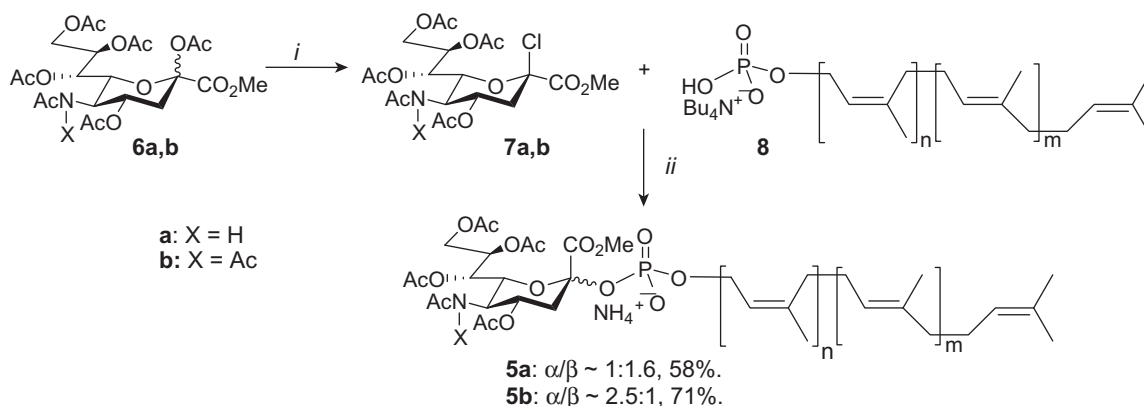
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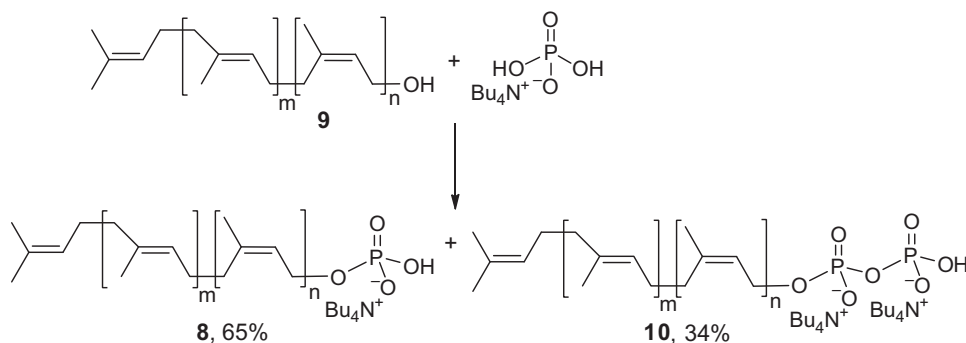
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Scheme 2. Reagents and condition: CH₂Cl₂, 24 h, α-**3a**:**4** = 1:3.3.



Scheme 3. Reagents and conditions: (i) HCl, AcCl, CH₂Cl₂, 4 °C; (ii) CH₃CN–CH₂Cl₂, 3:1, 16 h.



Scheme 4. Reagents and condition: CCl₃CN, CH₂Cl₂, 16 h.

phosphate **8**² was prepared from moraprenol **9** (Scheme 4) and tetra-n-butylammonium dihydrogen phosphate in the presence of CCl₃CN in CH₂Cl₂. The obtained tetra-n-butylammonium polyprenyl phosphate **8** was contaminated with ~34% of tetra-n-butylammonium polyprenyl pyrophosphate **10**. Since we needed to use tetra-n-butylammonium salt, we used polyprenyl phosphate **8** without further purification. As the reaction solvent, we selected a 3:1 MeCN–CH₂Cl₂ mixture, in which sialyl chloride **7a** and polyprenyl phosphate **8** were both soluble. Fourfold excess of phosphate nucleophile was shown to be required to minimize the elimination reaction. As polyprenyl glycosyl phosphates are not stable on the silica gel and are usually purified by anion exchange chromatography, we had to decrease ionic strength of the reaction mixture prior to application onto column. Since the polyprenyl phosphate **8** was sparingly soluble in MeCN, the concentrated reaction

mixture was resuspended in MeCN and the major amount of starting polyprenyl phosphate **8** was separated by centrifugation. The carbohydrate derivatives remained in the supernatant. We have observed all expected signals in ¹³C NMR spectrum of the crude phosphodiester **5a** thus obtained. Two doublets in the anomeric region indicated that we have indeed prepared the anomeric phosphodiesters **5a** (α-**5a**: δ_C 97.3, J_{P,C} = 4.0 Hz (C-2), β-**5a**: δ_C 96.5, J_{P,C} = 5.3 Hz (C-2)). The signals of phosphodiester moiety were also present in ³¹P NMR spectrum of the crude product (**5a**, Bu₄N⁺-salt): δ_P –6.2 (α-**5a**) and δ_P –5.7 (β-**5a**). The signal of α-anomer of phosphodiester α-**5a** almost coincided with the signal of the product with α-configuration prepared earlier by the reaction of α-**3a** and **4** (see above). Purification of phosphodiester **5a** by anion exchange chromatography on DEAE cellulose (elution with concentration gradient of NH₄OAc in MeOH) gave two fractions: the first one

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