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SYNTHESIS OF CYTIDINE DIPHOSPHATE-D-QUINOVOSE

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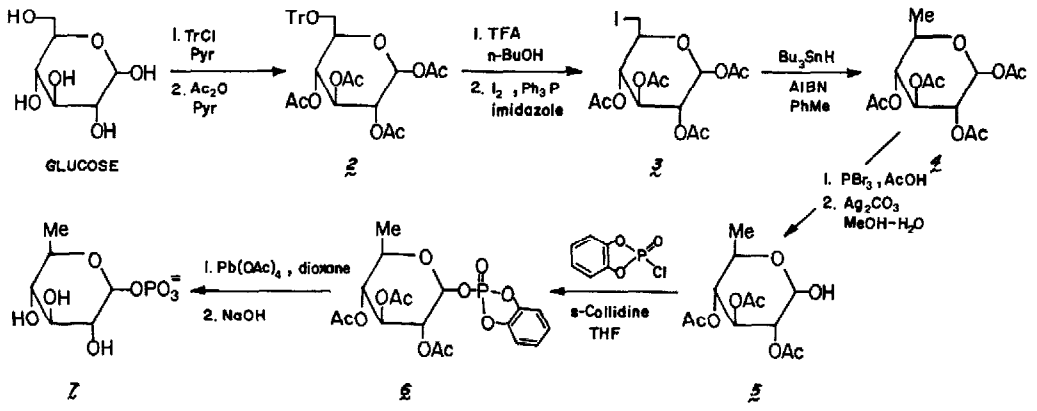
ABSTRACT: The title molecule, cytidine-D-quinovose (CDP-6-deoxy-D-glucose) **1**, was synthesized by two different methods from the key intermediate quinovose-1-phosphate **7** which was prepared from glucose.

A great number of nucleoside diphosphate sugars with diverse biological activities have been found ubiquitously in nature.[1] One of their primary functions *in vivo* is to serve as donors of the glycosyl moiety in the biosynthesis of oligo- and polysaccharides.[2] Since the free energy of hydrolysis of the nucleotidyl glycosides is sufficiently negative (ca. 7×10^3 kcal/mole), consequent transglycosylation is essentially irreversible.[3] The biochemical synthesis of nucleoside diphosphate sugars is catalyzed by the corresponding nucleoside pyrophosphorylase. In an attempt to study this enzymatic process at the molecular level, we have recently isolated an enzyme of this class, cytidine diphosphate-D-glucose pyrophosphorylase, from *Yersinia pseudotuberculosis*. This enzyme catalyzes the conversion of glucose-l-phosphate to CDP-D-glucose in the presence of cytidine triphosphate.[4] To aid in defining the catalytic properties of this enzyme in detail, it has been necessary for us to prepare several CDP-D-glucose analogues containing unusual sugar moieties. Although many nucleotidyl glycosides bearing different bases and different sugar components are known, they are, in most cases, present in minute quantities. It requires no special insight to perceive that the isolation of these molecules from natural sources can be laborious. Thus, an approach utilizing the existing arsenal of organic chemistry to make the target molecules seems perspicacious. Moreover, the need for radioactive derivatives and structural analogues of these molecules may also be addressed by chemical synthesis. In this paper we highlight our efforts in this area by presenting the synthesis of CDP-D-quinovose (CDP-6-deoxy-D-glucose) which has never before been chemically prepared. Since the development of a general and efficient preparation sequence for this class of molecules was the primary goal of this work, several different synthetic routes were attempted and compared *vis a vis* one another.

The first phase of the synthesis focused on the preparation of quinovose-1-phosphate **7** from glucose. The importance of phosphate esters in biological reactions has led to the development of many chemical methods for the conversion of an alcohol into its corresponding monophosphate ester.[5] However, phosphorylation of a hemiacetal is more perplexing than that of a normal alcohol, and none of the phosphorylation procedures described in the literature stand out as being the most generally useful. Furthermore, quinovose-1-phosphate, like the corresponding fucose and rhamnose derivatives, is more vulnerable to acid hydrolysis than most common glycosyl phosphates.[6,7] Thus, preparation of this molecule can be expected to be more difficult. As shown in Scheme 1, the C-6 deoxygenation of glucose to make the quinovose skeleton was accomplished in a series of standard reactions initiated by monotritylation at C-6 with tritylchloride in pyridine and then peracetylation with acetic anhydride in the same solvent.[8] Compound **2** isolated from this one pot reaction was predominantly the C-1 beta isomer (52% overall yield). Removal of the triphenylmethyl group from **2** with trifluoroacetic acid in *n*-butanol[9] was

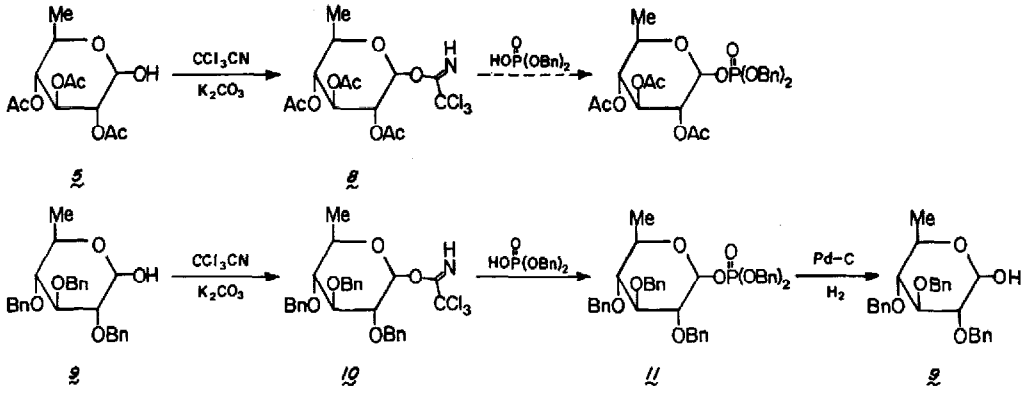
SCHEME I



carefully executed so as to prevent acetyl group migration from C-4 to C-6 (48%). This was followed by C-6 iodination with I_2/triphenylphosphine/imidazole (71%),[10] and subsequent reduction of **3** with tributyltin hydride to produce **4** (92%).[11] The free sugar **5** was obtained from **4** by C-1 bromination with PBr_3 in acetic acid followed by hydrolysis of the resulting acetobromo-sugar with silver carbonate.[12] The overall yield of the last two steps was 82%.

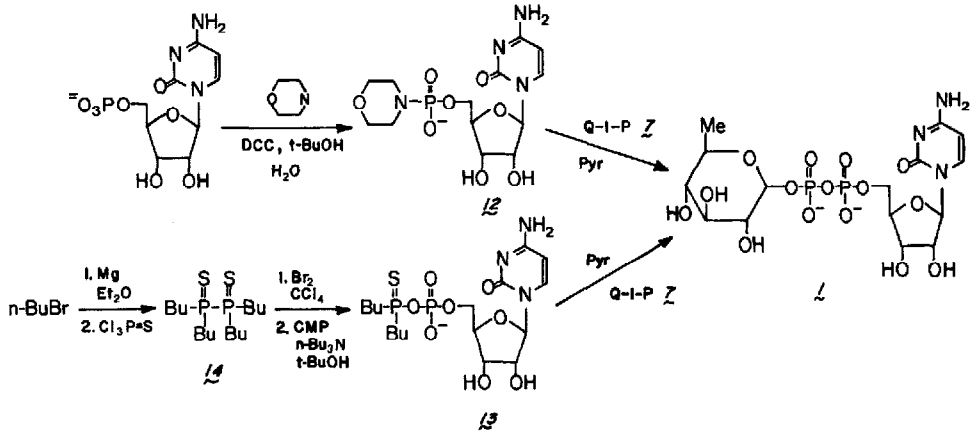
Phosphorylation of **5** ($C_{1\alpha}$:$C_{1\beta}$=2:1) was attempted by two different methods. The first route relied on using 1,2-phenylene phosphorochloridate as the monophosphorylating agent which is known to react rapidly and quantitatively with stoichiometric amounts of alcohols in the presence of a suitable base.[13] As shown in Scheme 1, compound **5**, when incubated with 1,2-phenylene phosphorochloridate and *s*-collidine, gave rise to phosphodiester **6** ($C_{1\alpha}$:$C_{1\beta}$=3:1). Compound **6** was then transformed to the desired quinovose-1-phosphate **7** by treatment with lead tetraacetate in dioxane, followed by alkaline hydrolysis. Product **7** was purified by Dowex-1 (formate) ion-exchange chromatography using a linear gradient of 0.05-0.3 M TEAB buffer (pH 7.5) for the elution.[7] This preparative sequence was rather tedious and also gave a low yield (23% from **5**). Therefore, an alternative approach calling for the synthesis of an activated glycosylimidate intermediate **8** prior to phosphorylation was attempted. This key intermediate **8** was prepared from **5** by the method of Schmidt et al[14] with trichloroacetonitrile and K_2CO_3. The resulting glycosyl trichloroacetimidate **8** was mainly the beta isomer ($C_{1\alpha}$:$C_{1\beta}$=1:2.5, 82% yield). However, compound **8**, upon the addition of dibenzyl phosphate, underwent a series of intramolecular acetoxyl migrations and resulted in the formation of an array of 1-acetyl glycoside derivatives. In order to obviate this undesired neighboring group participation, a benzyl ether derivatized analogue **10** prepared from **9**[15] was subjected to this reaction sequence. As shown in Scheme 2, compound **10** ($C_{1\alpha}$:$C_{1\beta}$=1:3), upon the addition of dibenzyl phosphate, transfered its glycosyl moiety directly to the phosphoric acid diester.[16] The stereochemical course of this displacement is well-established and shown to proceed via inversion. Thus, the alpha isomer of the glycosyl phosphate **11** was obtained preferentially from the β-imidate **10** as expected (54% yield from **9**). Unfortunately, the benzyl ether protecting groups were vulnerable to hydrogenolysis (10% Pd-C, THF/EtOAc= 1:1) only in the presence of a catalytic amount of acid. Since the nascent glycosyl phosphate was acid labile, compound **9** was isolated as the proximate product of this reaction. Therefore, phosphorylation by way of a 1,2-phenylene phosphodieser intermediate **6** appeared to be the only alternative in the present case.

SCHEME 2



Two forms of activated CMP, CMP-morpholidate **12** and cytidine 5'-phosphoric di-*n*-butylphosphinothioic anhydride **13**, were used to synthesize CDP-D-quinovose **1** from quinovose-1-phosphate **7**. Since traces of water interfered with the formation of the phosphodiester by hydrolyzing the phosphorylating reagent, the reaction mixture was vigorously dried by repeatedly evaporating to dryness in vaccuo from pyridine. The cytidine 5'-phosphoromorpholidate **12** was prepared from cytidine 5'-phosphate in a mixture of morpholine, dicyclohexyl-

SCHEME 3



carbodimide in *t*-butanol and water (82% yield).[17] The directly obtained 4-morpholine N,N'-dicyclohexyl-carboxamidine salt of **12** was then reacted with **7** in dry pyridine at room temperature. The course of the reaction was monitored by HPLC equipped with a Whatman Partisil 10 SAX column which was eluted with a linear gradient of 0.03-0.12 M potassium phosphate buffer (pH 6.6).[18] The retention times of CMP and CDP-D-quinovose were 7.6 and 5.8 min, respectively, at a flow rate of 1 mL/min. The reaction was stopped when product formation reached its plateau (ca 2-3 days). The resulting CDP-D-quinovose **1** was purified on a Dowex-1 (formate) column eluted with a linear gradient of 0-0.4 M LiCl. Fractions containing **1** (checked by HPLC) were combined, lyophilized, and desalted on a column of Bio-Gel P-2. The overall yield was 20%.

In accordance with a method of Nunez et al,[7] CDP-D-quinovose could also be synthesized by coupling **7** and **13** together with silver carbonate in dry pyridine at room temperature. As shown in Scheme 3, the phosphinothioic anhydride **13** was prepared in *t*-butanol from CMP and di-*n*-butylphosphinothionyl bromide **14** in the presence of tri-*n*-butylamine at 40 °C. Compound **14** was derived from *n*-butylbromide via a Grignard reaction with trichlorothiophosphine.[19] The ^{31}P-NMR of compound **13** showed two doublets at -10 and +103 ppm with coupling constants of 34 Hz. The use of freshly prepared phosphinothioic anhydride **13** was essential in this synthesis. The CDP-D-quinovose product was assayed and purified as previously described, with an overall yield of less than 10%. Coupling of cytidine phosphodichloridate and tris-(tributylammonium)quinovose-1-phosphate based on a modified procedure of Ruth and Cheng[18] was also attempted. Unfortunately, no product could be detected despite numerous trials. Thus, the classical phosphomorpholidate procedure still appears to be the method of choice to synthesize sugar nucleotides.

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FOOTNOTES AND REFERENCES:

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15. Compound **9** was prepared from methyl α-D-glucopyranoside via a sequence of C-6 tritylation (TrCl, DMF, Et_3N, DMAP; 84%), perbenzylation (BnBr, NaH, Bu_4NI, THF; 90%), detritylation (HCl, MeOH; 83%), iodination (I_2, Ph_3P, imidazole; 92%), reduction (LAH, THF; 98%), and hydrolysis (aq HCl; 85%).
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