Phosphorylation of D-Glucose Derivatives with Inorganic Cyclo-Triphosphate: Substituent Effect at the 5-CH₂OH or 2-OH Group

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The phosphorylation of several D-glucose derivatives has been achieved using inorganic sodium cyclo-triphosphate hexahydrate (P_{3m}), $Na_3P_3O_9 \cdot 6H_2O$, in aqueous solution. In the phosphorylation of D-glucuronic acid, 6-phosphoryl-D-glucose and D-xylose, β -D-glucuronic acid 1-triphosphate, 6-phosphoryl- β -D-glucose 1-triphosphate and β -D-xylose 1-triphosphate were synthesized stereoselectively with maximum yields of 43.5, 32.8 and 41.9%, respectively. In the case of D-glucosamine and 2-deoxy-D-glucose, the main phosphorylated products were assigned to β -D-glucosamine 1-triphosphate and 2-deoxy- β -D-glucose 1-triphosphate by 1H -, ^{13}C - and ^{31}P -NMR, and the yields were 13.9 and 13.4%, respectively.

Key words phosphorylation; β -D-glucose 1-triphosphate; cyclo-triphosphate hexahydrate; multinuclear NMR; HPLC

Sodium cyclo-triphosphate (P_{3m}), which is a simple inorganic condensed phosphate having a six-membered ring, is a very attractive phosphorylation reagent for synthesizing biologically important compounds in one step in aqueous solution. The reaction proceeds well for organic compounds having an amino or a hydroxyl group. Feldmann demonstrated the phosphorylation of ammonia¹⁾ and alcohols²⁾ with P_{3m} . Later on, the phosphorylation reactions of amino acids,^{3,4)} alkyldiamine,⁵⁾ nucleosides,⁶⁾ sugar,^{7,8)} phenols,⁹⁾ and cellulose¹⁰⁾ were examined in detail. Recently, we have reported that D-glucose (1) reacts with P_{3m} to stereoselectively form β -D-glucose 1-triphosphate (5)⁸⁾ in aqueous solution, and that the optimum condition for the phosphorylation is a molar ratio of P_{3m} and 1 = 1 : 5, pH 12, and room temperature.⁷⁾ In order to clarify the mechanism of the stereoselective reaction at 1-OH of 1, the phosphorylation of D-glucose derivatives in which the neighbor of the reaction site, that is, the CH₂OH group at C-5 or OH group at C-2, is exchanged by other function groups, was examined in detail.

Results and Discussion

Phosphorylation of D-Glucuronic Acid (2), 6-Phosphoryl-D-glucose (3) and D-Xylose (4) with P_{3m} D-Glucose (1) and its derivatives used in the present study are shown in Chart 1. The phosphorylation was carried out essentially according to the previous method.⁸⁾ Figure 1 shows HPLC profiles for the reaction mixture of D-glucuronic acid (2) ($1.25 \text{ mol} \cdot \text{dm}^{-3}$) and P_{3m} ($0.25 \text{ mol} \cdot \text{dm}^{-3}$) incubated at room temperature and pH 12. A peak of the main phosphorylated product (6) appeared at the retention time of about 50 min, predicting that the product (6) is a triphosphate derivative of 2. A peak at the retention time of about 21 min (reaction time of 72 h) was assigned as a by-product. The other chromatographic peaks were assigned to P_{3m} and its hydrolytic products, *i.e.*, monophosphate (P_1), diphosphate (P_2), and triphosphate (P_3), respectively.

phate (P_3), respectively.

Figure 2 shows changes in the amounts of the products in the reaction of 2 ($1.25 \text{ mol} \cdot \text{m}^{-3}$) and P_{3m} ($0.25 \text{ mol} \cdot \text{m}^{-3}$) at pH 12 and room temperature. The yield of 6 increased with the passage of reaction time, reaching about 30% after 8 h, then it decreased gradually. This decrease is due to the hydrolysis of 6 to form a monophosphate derivative (by-product). At the same time, P_{3m} was gradually hydrolyzed to P_2 and P_1 . Therefore, P_2 increased monotonously (Fig. 2). Table

Chart 1

Fig. 1. HPLC Profiles for the Reaction Mixture of 2 and P_{3m}

2: $P_{3m} = 1.25 \text{ mol} \cdot \text{dm}^{-3}$; $0.25 \text{ mol} \cdot \text{dm}^{-3}$, pH 12, and room temperature. P_1 , monophosphate; P_2 , diphosphate; P_3 , triphosphate; P_{3m} , cyclo-triphosphate.

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1 summarizes the yields of the product **6** in the reactions of **2** with P_{3m} under various mixing ratios. The yield of **6** increased with the increase of initial concentration of **2**. The maximum yield and its time suggest that the optimum condition for the phosphorylation of **2** with P_{3m} is pH 12 and a molar ratio of $P_{3m} : 2 = 1 : 7$.

To identify the main product (**6**), ^{31}P -, 1H - and ^{13}C -NMR spectra were measured. Figure 3(A) shows the ^{31}P -NMR spectrum of the P_α region in the reaction product of **2** with P_{3m} . The spectrum showed one doublet of doublets at -11.4 ppm, which became doublet on 1H -decoupling, indicating the characteristic peak of P_α in triphosphate derivatives^{9,11)} of D-aldose.

Fig. 2. Changes in the Amounts of the Products in the Reaction of **2** ($1.25 \text{ mol} \cdot \text{dm}^{-3}$) and P_{3m} ($0.25 \text{ mol} \cdot \text{dm}^{-3}$) at pH 12 and Room Temperature
 $\blacktriangle$, **6**; $\blacksquare$, by-product; $\triangle$, P_1 ; $\square$, P_2 ; $\square$, P_3 ; $\circ$, P_{3m} .

Table 1. Yields in the Phosphorylation of D-Glucose Derivatives

Reaction conditions		Yield (%) (product)
Mixing ratio	Time (h)	
$P_{3m} : 2$		
$= 1 : 10$ (0.25 M : 2.5 M)	3.0	43.4 (6)
$= 1 : 7$ (0.25 M : 1.75 M)	4.5	43.5 (6)
$= 1 : 5$ (0.25 M : 1.25 M)	6.5	29.9 (6)
$P_{3m} : 3$		
$= 1 : 10$ (0.2 M : 2.0 M)	6.0	31.8 (7)
$= 1 : 7$ (0.2 M : 1.4 M)	20.5	32.8 (7)
$= 1 : 5$ (0.2 M : 1.0 M)	23.5	28.6 (7)
$P_{3m} : 4$		
$= 1 : 10$ (0.5 M : 5.0 M)	6.5	41.9 (8)
$= 1 : 7$ (0.5 M : 3.5 M)	8.5	38.1 (8)
$= 1 : 5$ (0.5 M : 2.5 M)	30.0	31.3 (8)

Figure 3(B) shows 1H -NMR spectra in the region of H-1 of the reaction mixture. The doublet of doublets at 4.92 ppm was assigned to H-1 of **6**. The $^3J_{1,2}$ value of **6** is 7.8 Hz, which is typical for β -D-glucose 1-triphosphate (**5**),⁸⁾ β -D-aldose 1-monophosphates¹²⁾ and β -D-glucuronic acid.¹³⁾ The $^3J_{P_\alpha H-1}$ value from 1H -NMR is consistent with that deduced from ^{31}P -NMR data and also with data of **5**⁸⁾ and β -D-aldose 1-phosphates.¹²⁾ Thus, these results suggest that **6** is β -D-glucuronic acid 1-triphosphate.

^{13}C -NMR chemical shift values and coupling constants of **6** also confirmed this identification. The chemical shift values of **6** are close to those of the β -anomer of **2**,¹³⁾ although δ in C-1 shows a down-field shift as a result of the phosphorylation. This tendency is observed in β -D-aldose 1-phosphates.¹²⁾ Also, the $^2J_{P_\alpha C-1}$ and $^3J_{P_\alpha C-2}$ values of **6** were close to those of **5**⁸⁾ and β -D-aldose 1-phosphates.¹²⁾ Therefore, **2** reacts with P_{3m} to form β -D-glucuronic acid 1-triphosphate (**6**) stereoselectively, as shown in Chart 2.

The reactions of 6-phosphoryl-D-glucose (**3**) and D-xylose (**4**) with P_{3m} were carried out under the same reaction condition as **2**. The results of HPLC indicated that the products are their triphosphate derivatives. The experiment at different mixing ratios showed that maximum yields of **7** and **8** were

Fig. 3. ^{31}P -NMR Spectrum of the P_α Region (A), and 1H -NMR Spectrum of the H-1 Region (B) in the Reaction Mixture

$2 : P_{3m} = 1.25 \text{ mol} \cdot \text{dm}^{-3} : 0.25 \text{ mol} \cdot \text{dm}^{-3}$, pH 12, and room temperature, after 6.5 h.

Chart 2

32.8 and 41.9% at molar ratios of 1 (P_{3m}): 7 (**3**) and 1 (P_{3m}): 10 (**4**), respectively (Table 1).

Similar to **6**, ^{31}P -, ^1H - and ^{13}C -NMR spectra of **7** and **8** suggest them to be β -D-aldose 1-triphosphates.^{12,14,15} Therefore, **3** and **4** stereoselectively react with P_{3m} to form 6-phosphoryl- β -D-glucose 1-triphosphate (**7**) and β -D-xylose 1-triphosphate (**8**). The phosphorylation of D-glucose derivatives, in which the 5- CH_2OH group is substituted with other functional groups, *i.e.*, COOH , $\text{CH}_2\text{OPO}_3\text{H}_2$ and H , proceeds by a mechanism similar to that of **1** (Chart 2).

Phosphorylation of D-Glucosamine (9), 2-Deoxy-D-glucose (10) and 2-Fluoro-2-deoxy-D-glucose (11) with P_{3m}
Chart 3 shows D-glucose derivatives substituted at 2-OH. Figure 4 shows HPLC profiles for the reaction mixture of D-glucosamine (**9**) ($1.25 \text{ mol} \cdot \text{dm}^{-3}$) and P_{3m} ($0.25 \text{ mol} \cdot \text{dm}^{-3}$) incubated at room temperature and pH 12. Two peaks due to the phosphorylated products (**12** and **13**) appeared at retention times of about 23 and 32 min, respectively. The amount of **12** increased with the passage of reaction time and reached 11.6% after 3 d; the maximum amount remained more than 7 d. The formation rate of **13** was slow compared with that of **12** and the yield was about 5.0% at 5 d. The reaction of **9** with P_{3m} was carried out under various reaction conditions. The yields of **12** and **13** did not increase by an increase of the initial concentration of **9** (Table 2).

To identify the phosphorylated products **12** and **13**, ^{31}P - and ^1H -NMR spectra for the reaction mixture were measured because of the difficulty of isolation due to low yields. Figure 5 shows the ^{31}P -NMR spectra for the reaction mixture of **9** and P_{3m} . A proton-decoupling spectrum showed characteristic signals assigned to two triphosphate derivatives of **9** in addition to the signals of P_2 (-4.3 ppm) and P_3 (-4.0 and -18.2 ppm). At this reaction condition, the yields of **12** and **13** were 10.3 and 2.2%, respectively, indicating that the signal of **12** would mainly be observed. When the pH of the reaction mixture was changed to 3 (acidic), the peaks at 0.62, -4.2 and -19.6 ppm disappeared, and the peak at 32 min in

Chart 3

9 (GlcN) : $R^2 = \text{NH}_2$
10 (2dGlc) : $R^2 = \text{H}$
11 (2F2dGlc) : $R^2 = \text{F}$

Fig. 4. HPLC Profiles for the Reaction Mixture of **9** and P_{3m}
9: $P_{3m} = 1.25 \text{ mol} \cdot \text{dm}^{-3}$; $0.25 \text{ mol} \cdot \text{dm}^{-3}$, pH 12, and room temperature.

Table 2. Yields in the Phosphorylation of D-Glucose Derivatives

Reaction conditions		Yield (%) (product)
Mixing ratio	Time (h)	
P_{3m} : 9		
= 1 : 7 (0.25 M : 1.75 M)	48	13.9 (12)
	48	4.1 (13)
= 1 : 5 (0.25 M : 1.25 M)	72	11.6 (12)
	120	5.0 (13)
P_{3m} : 10		
= 1 : 5 (0.2 M : 1.0 M)	144	7.9 (14)
= 1 : 4 (0.5 M : 2.0 M)	24	13.4 (14)
P_{3m} : 11		
= 1 : 5 (0.02 M : 0.1 M)	—	0

Fig. 5. ^{31}P -NMR Spectra for the Reaction Mixture of **9** ($1.25 \text{ mol} \cdot \text{dm}^{-3}$) and P_{3m} ($0.25 \text{ mol} \cdot \text{dm}^{-3}$) Incubated at pH 12 and Room Temperature for 1 d
 The big signal at 0 ppm on ^1H -decoupling spectrum is an external reference (H_3PO_4).

HPLC profile disappeared as well. Therefore, the peaks at -4.1 , -11.3 , and -19.7 ppm were assigned to **12**. The doublet at -11.3 ppm was assigned to the signal of the P_α atom in **12** because the proton coupling spectrum shows that the doublet was split into a doublet of doublets ($^3J_{P_\alpha H-1}=8.5$ Hz). The triplet at -19.7 ppm and the doublet at -4.1 ppm were assigned to the middle (P_β in **12**) and end phosphorus (P_γ in **12**) atoms, respectively.^{9,1f)}

Figure 6 illustrates ^1H -NMR spectra in the region of H-1 of the reaction mixture at 14 h (A), 1 d (B) and 3 d (C). At 14 h (A), a signal due to **12** was observed because the amounts of **12** and **13** were 7.7 and 1.8%, respectively. As

Fig. 6. The 300 MHz ^1H -NMR Spectra in the Region of H-1 of the Main Product in the Reaction of D-Glucosamine (**9**) ($1.25 \text{ mol} \cdot \text{dm}^{-3}$) with P_{3m} ($0.5 \text{ mol} \cdot \text{dm}^{-3}$) at pH 12 for 14 h (A), 1 d (B) and 3 d (C)

the reaction time elapsed, the signal due to **13** increased gradually. In Figs. 6(A) and (B), the doublet of doublets at about 5.27 ppm is assigned to H-1 of β -D-glucosamine 1-triphosphate (**12**). The $^3J_{1,2}$ value of **12** is 8.5 Hz, which is equal to that of β -D-glucosamine.¹⁶⁾ The triplet is due to the accidental coincidence of $^3J_{1,2}$ and $^3J_{P_\alpha H-1}$. The latter is consistent with $^3J_{P_\alpha H-1}$ deduced from ^{31}P -NMR (Fig. 5). Furthermore, the ^{31}P - ^1H 2D-NMR (heteronuclear multiple bond correlation spectroscopy (HMBC)) experiment also showed a correlation of P_α at -11.3 ppm and the ^1H signal at 5.27 ppm, confirming the above conclusion. Therefore, the main phosphorylated product in the reaction of **9** with P_{3m} was proved to be β -D-glucosamine 1-triphosphate (**12**).

The previous works indicated that the phosphorylation products of alkylamines¹⁷⁾ and alkyldiamine⁵⁾ with P_{3m} are alkylamido- and alkyimidotriphosphates with a P-N bond. These products show a characteristic P_α signal⁴⁾ of ^{31}P -NMR at around 0 ppm, and are easily recycled to P_{3m} under an acidic condition. From these facts, **13**, which shows ^{31}P -NMR signals at 0.62, -19.6 and -4.2 ppm, is considered to be β -D-glucosamine 2-triphosphate with a P-N bond. From the previous work,¹⁸⁾ the amino group is predicted to be more easily phosphorylated with P_{3m} than the OH group. However, this is not the case in the phosphorylation of **9** with P_{3m} , probably because the 1-OH group in **9** has an aldehyde character and shows higher reactivity than the 2-NH₂ group (Chart 4).

The reactions of 2-deoxy-D-glucose (**10**) and 2-fluoro-2-deoxy-D-glucose (**11**) with P_{3m} were carried out with mixing ratios of 5:1 and 4:1 at pH 12 and room temperature. The maximum yield of the main phosphorylated product (**14**) of **10** was 13.4%, whereas **11** did not react with P_{3m} at all (Table 2). A ^{31}P - ^1H 2D HMBC correlation experiment showed correlation between P_α at -12.4 ppm and the ^1H signal at 5.25 ppm, indicating that the main product is confirmed to be 2-deoxy- β -D-glucose 1-triphosphate (**14**), as shown in Chart 5.

Mechanism of the Reaction of D-Glucose Derivatives with P_{3m} In the phosphorylation of D-glucose derivatives (**2**–**4**) substituted at the 5-CH₂OH of **1** with P_{3m} , β -D-al-dopyranose 1-triphosphates (**6**–**8**), they were stereoselectively synthesized with yields of more than 32%. On the other hand, the reactions of D-glucose derivatives (**9** and **10**) substituted at 2-OH of **1** with P_{3m} produced their triphosphate derivatives in low yields with poor selectivity. 2-Fluoro-2-

Chart 4

Chart 5

Table 3. Population of Anomers in H₂O at Room Temperature

Monosaccharide	α (%)	β (%)
1	36	64
2	35	65
3	35	65
4	37	63
9	31	69
10	35	65
11	33	67

a) See ref. 20.

deoxy-D-glucose (11) did not react with P_{3m} at all.

The stereoselectivity of the reactions of D-glucose derivatives substituted at 5-CH₂OH with P_{3m} will be explained by the following mechanism. At pH 12, P_{3m} is easily attacked by nucleophilic reagents such as amine,^{5,17)} alcohol,²⁾ amino acid,^{3,4)} and nucleoside.⁶⁾ In the present study, thus, the lone electron pair on the anomeric hydroxyl group of β -D-aldoose nucleophilically attacks a phosphorus atom on P_{3m} to open its six-membered ring. It is noteworthy that the formation of hydrogen bonding between the hydroxyl group at C-2 and the oxygen atom on P_{3m} would make the attack of cyclo-triphosphate easier. No attack of α -D-aldoose must be due to the steric hinderance of the *cis* conformation of the hydroxyl groups at C-1 and C-2, or to the 1,3-diaxial interaction¹⁹⁾ between H-3 and the triphosphate group. Table 3 shows the population²⁰⁾ of each anomer of D-aldoose used in this study, as estimated from ¹H-NMR. From these results, it is suggested that the main anomer, that is, the energetically stable β -anomer, is selectively phosphorylated. Furthermore, the population of the β -anomer increases in the reaction condition (pH 12), inducing the yields of more than 32%.

No noteworthy difference in the reactivity of the hydroxyl group at C-1 of D-glucose derivatives, that is, CH₂OH, COOH, CH₂OPO₃H₂ and H groups at C-5, was observed, although the polarities of these functional groups are different. This is probably because the bond between O-5 and C-1 is not so strong under anomer equilibrium, thus, the effect of these functional groups on the phosphorylation of β -D-aldoose is small.

The reactions of D-glucose derivatives substituted at 2-OH with P_{3m} produced their triphosphate derivatives with low yield and poor selectivity. In spite of the *trans* conformation of the hydroxyl group at C-1 and the amino group at C-2 in β -D-glucosamine, its reactivity with P_{3m} is low. P_{3m} is known to react with ammonia,^{1,21)} alkylamine,¹⁷⁾ 1,6-hexanediamine,²²⁾ and amino acids^{3,4)} to yield new compounds with a P-N bond in their molecules. In the case of alkylamines, the yields are more than 80%. Furthermore, ¹³C-NMR showed that **9** decomposes gradually in an alkaline solution. Therefore, the reactivity at 1-OH in **9** is low, with poor stereoselectivity. In the cases of 2-deoxy- β -D-glucose and 2-fluoro-2-deoxy- β -D-glucose, high yields and stereoselectivity are expected because the population of their β -anomers is nearly equal to those of **1**, **2**, **3** and **4** (Table 3). However, 2-deoxy- β -D-glucose and 2-fluoro-2-deoxy- β -D-glucose showed low yield. Although the reason for this is not clear at present, the OH group at C-2 will be essential to obtaining a stereoselective phosphorylated product. The substituent effect in 2-OH in the phosphorylation of D-glucose derivatives with P_{3m} is

noteworthy.

In conclusion, the phosphorylation of D-glucose derivatives (**2**–**4**) substituted at 5-CH₂OH with P_{3m} proceeds stereoselectively, with a yield of more than 32%, whereas the phosphorylation of D-glucose derivatives (**9** and **10**) substituted at 2-OH with P_{3m} proceeds with low yield and poor selectivity.

Experimental

Materials and Methods Sodium cyclo-triphosphate (P_{3m}), Na₃P₃O₆·6H₂O, was prepared as described in a previous paper.³⁾ 6-Phosphoryl-D-glucose (**3**), D-xylose (**4**), 2-fluoro-2-deoxy-D-glucose (**11**), and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were purchased from Sigma (St. Louis, U.S.A.). Unless otherwise stated, guaranteed grade reagents from Wako Chemical Industries, Ltd. (Osaka, Japan), were used. ¹³C-NMR spectra with broad band ¹H-decoupling and ¹H-NMR spectra were measured with Varian Gemini 300 and 200 spectrometers. Samples were dissolved in D₂O (99.9%) to avoid the effect of ¹H in water. DSS was used as an external standard for ¹³C and ¹H-NMR spectra. ³¹P-NMR spectra with and without broad band ¹H-decoupling and ³¹P-¹H 2D HMBC spectra were obtained with a Varian VXR-500 spectrometer. As an external standard, 85% H₃PO₄ was used. HPLC analysis was carried out with a JASCO GULLIVER HPLC system (Tokyo, Japan), coupled with a JASCO DU-4F flow injection system to detect phosphate by a post-column reaction. A column (150×6.0 mm i.d.) packed with a polystyrene-based anion-exchanger (TSK gel, SAX, 5 μ m, TOSOH, Japan), was used for the analysis of phosphate. The column temperature was maintained at 40 °C. A convex gradient elution technique using 0.17 and 0.45 mol·dm⁻³ potassium chloride solutions was employed for the analysis of phosphate. Determination of phosphorus was carried out by spectrophotometry of a phosphorus-molybdenum heteropoly blue complex at 830 nm. The flow rate of the eluent was 1.0 ml·min⁻¹.

The Procedure for the Syntheses of β -D-Glucuronic Acid 1-Triphosphate (6**), 6-Phosphoryl- β -D-glucose 1-Triphosphate (**7**), and β -D-Xylose 1-Triphosphate (**8**)** The reaction of D-glucuronic acid (**2**) (1.25–2.5 mol·dm⁻³) with P_{3m} (0.25 mol·dm⁻³) was carried out at pH 12 and room temperature. The yields and the structures of the products were determined by HPLC and NMR measurements. Because the reaction was performed under a P_{3m} deficient condition, that is, an aldose rich condition, the yield was calculated based on the amount of P_{3m}. Similar procedures were used for the syntheses of **7** and **8**.

Compounds **6** and **8** were isolated as crystalline barium salts. The reactions of **2** and **4** (2.5 mol·dm⁻³) with P_{3m} (0.25 mol·dm⁻³) were carried out at pH 12 and room temperature. After 3 h, the reaction mixture was neutralized using *ca.* 5 mol·dm⁻³ HCl. The addition of 1 mol·dm⁻³ BaCl₂ aqueous solution precipitated barium diphosphate, Ba₂P₂O₇, which was separated by filtration. When 1 mol·dm⁻³ BaCl₂ aqueous solution was added to the filtrate, **6** and **8** precipitated out. They were separated from the solution, then washed with distilled water and ethanol.

β -D-Glucuronic Acid 1-Triphosphate (6**):** ¹H-NMR (D₂O) δ : 5.04 (1H, dd, *J*_{1,2}=7.8 Hz, *J*_{p,H-1}=8.6 Hz, H-1), 3.39 (1H, dd, *J*_{1,2}=7.8 Hz, *J*_{2,3}=7.5 Hz, H-2), 3.52 (1H, dd, *J*_{2,3}=7.5 Hz, *J*_{3,4}=9.0 Hz, H-3), 3.56 (1H, dd, *J*_{3,4}=9.0 Hz, *J*_{4,5}=9.4 Hz, H-4), 3.79 (1H, d, *J*_{4,5}=9.4 Hz, H-5). ¹³C-NMR (D₂O) δ : 99.8 (1C, d, *J*_{p,C-1}=5.6 Hz, C-1), 75.7 (1C, d, *J*_{p,C-2}=8.5 Hz, C-2), 78.2 (1C, s, C-3), 73.9 (1C, s, C-4), 77.4 (1C, s, C-5), 178.1 (1C, s, C-6). ³¹P-NMR (D₂O) δ : -11.4 (1P, dd, *J*_{p,p_β}=18.2 Hz, *J*_{p,H-1}=8.6 Hz, *P_α*), -20.5 (1P, dd, *J*_{p,p_β}=18.2 Hz, *J*_{p,p_γ}=20.8 Hz, *P_β), -4.8 (1P, d, *J*_{p,p_γ}=20.8 Hz, *P_γ).**

6-Phosphoryl- β -D-glucose 1-Triphosphate (7**):** ¹H-NMR (D₂O) δ : 4.91 (1H, dd, *J*_{1,2}=8.2 Hz, *J*_{p,H-1}=8.6 Hz, H-1). ¹³C-NMR (D₂O) δ : 100.4 (1C, d, *J*_{p,C-1}=5.6 Hz, C-1), 76.2 (1C, d, *J*_{p,C-2}=7.8 Hz, C-2), 77.0 (1C, s, C-3), 71.2 (1C, s, C-4), 78.3 (1C, s, C-5), 67.0 (1C, s, C-6). ³¹P-NMR (D₂O) δ : -11.6 (1P, dd, *J*_{p,p_β}=17.7 Hz, *J*_{p,H-1}=8.6 Hz, *P_α*), -20.0 (1P, dd, *J*_{p,p_β}=17.7 Hz, *J*_{p,p_γ}=19.4 Hz, *P_β), -4.7 (1P, d, *J*_{p,p_γ}=19.4 Hz, *P_γ).**

β -D-Xylose 1-Triphosphate (8**):** ¹H-NMR (D₂O) δ : 4.85 (1H, dd, *J*_{1,2}=7.5 Hz, *J*_{p,H-1}=8.6 Hz, H-1), 3.23 (1H, dd, *J*_{1,2}=7.5 Hz, *J*_{2,3}=9.0 Hz, H-2), 3.37 (1H, dd, *J*_{2,3}=9.0 Hz, *J*_{3,4}=9.0 Hz, H-3), 3.50 (1H, ddd, *J*_{3,4}=9.0 Hz, *J*_{4,5}=5.3 Hz, *J*_{4,5'}=10.2 Hz, H-4), 3.84 (1H, dd, *J*_{4,5}=5.3 Hz, *J*_{5,5'}=11.5 Hz, H-5), 3.25 (1H, dd, *J*_{4,5'}=10.2 Hz, *J*_{5,5'}=11.5 Hz, H-5'). ¹³C-NMR (D₂O) δ : 100.9 (1C, d, *J*_{p,C-1}=5.9 Hz, C-1), 75.8 (1C, d, *J*_{p,C-2}=8.2 Hz, C-2), 77.6 (1C, s, C-3), 71.5 (1C, s, C-4), 68.0 (1C, s, C-5). ³¹P-NMR (D₂O) δ : -11.7 (1P, dd, *J*_{p,p_β}=17.7 Hz, *J*_{p,H-1}=8.6 Hz, *P_α*), -19.9 (1P, dd, *J*_{p,p_β}=17.7 Hz, *J*_{p,p_γ}=19.0 Hz, *P_β), -4.6 (1P, d, *J*_{p,p_γ}=19.0 Hz, *P_γ).**

The Procedure for the Syntheses of β -D-Glucosamine 1-Triphosphate

(12), β -D-Glucosamine 2-Triphosphate (13), and 2-Deoxy- β -D-glucose 1-Triphosphate (14) The reaction of D-glucosamine ($1.25\text{--}1.75\text{ mol}\cdot\text{dm}^{-3}$) with P_{3m} ($0.25\text{ mol}\cdot\text{dm}^{-3}$) was carried out at pH 12 and room temperature. The yields and structures of the products were determined by the above mentioned procedures. The separation of **12** from the reaction mixture was accomplished by anion-exchange chromatography with a $2\times 80\text{ cm}$ column filled with Dowex 1-X2 resin (100—200 mesh, chloride form). Elution was carried out with $0.3\text{ mol}\cdot\text{dm}^{-3}$ KCl aqueous solution, and each 100 ml fraction was measured by HPLC. The solution fractionated was concentrated at -113°C *in vacuo* (freeze-drying). An aliquot of the obtained product was dissolved in D_2O for HPLC, and 1H -, ^{13}C - and ^{31}P -NMR measurements. In the case of **13** and **14**, the isolation could not be performed because of low yield.

β -D-Glucosamine 1-Triphosphate (**12**): 1H -NMR (D_2O) δ : 5.27 (1H, dd, $J_{1,2}=8.5\text{ Hz}$, $J_{p,H-1}=8.5\text{ Hz}$, H-1), 3.09 (1H, dd, $J_{1,2}=8.5\text{ Hz}$, $J_{2,3}=11.0\text{ Hz}$, H-2), 3.67 (1H, dd, $J_{2,3}=11.0\text{ Hz}$, $J_{3,4}=9.5\text{ Hz}$, H-3), 3.39 (1H, dd, $J_{3,4}=9.5\text{ Hz}$, $J_{4,5}=9.5\text{ Hz}$, H-4), 3.53 (1H, ddd, $J_{4,5}=9.5\text{ Hz}$, $J_{5,6}=2.0\text{ Hz}$, $J_{5,6'}=6.0\text{ Hz}$, H-5), 3.82 (1H, dd, $J_{5,6}=2.0\text{ Hz}$, $J_{6,6'}=11.5\text{ Hz}$, H-6), 3.64 (1H, dd, $J_{5,6'}=6.0\text{ Hz}$, $J_{6,6'}=11.5\text{ Hz}$, H-6'). ^{13}C -NMR (D_2O) δ : 96.7 (1C, d, $J_{p,C-1}=5.2\text{ Hz}$, C-1), 58.6 (1C, d, $J_{p,C-2}=9.1\text{ Hz}$, C-2), 74.0 (1C, s, C-3), 71.8 (1C, s, C-4), 79.3 (1C, s, C-5), 63.0 (1C, s, C-6). ^{31}P -NMR (D_2O) δ : -11.3 (1P, dd, $J_{p,\alpha P_\beta}=17.1\text{ Hz}$, $J_{p,H-1}=8.5\text{ Hz}$, P_α), -19.7 (1P, dd, $J_{p,\alpha P_\beta}=17.1\text{ Hz}$, $J_{p,\beta P_\gamma}=18.4\text{ Hz}$, P_β), -4.1 (1P, d, $J_{p,\beta P_\gamma}=18.4\text{ Hz}$, P_γ).

β -D-Glucosamine 2-Triphosphate (**13**): 1H -NMR (D_2O) δ : 5.27 (1H, d, $J_{1,2}=8.6\text{ Hz}$, H-1). ^{31}P -NMR (D_2O) δ : 0.62 (1P, d, $J_{p,\alpha P_\beta}=19.0\text{ Hz}$, P_α), -19.6 (1P, dd, $J_{p,\alpha P_\beta}=19.0\text{ Hz}$, $J_{p,\beta P_\gamma}=17.2\text{ Hz}$, P_β), -4.2 (1P, d, $J_{p,\beta P_\gamma}=17.2\text{ Hz}$, P_γ).

2-Deoxy- β -D-glucose 1-Triphosphate (**14**): 1H -NMR (D_2O) δ : 5.25 (H-1). ^{31}P -NMR (D_2O) δ : -12.4 (1P, dd, $J_{p,\alpha P_\beta}=17.6\text{ Hz}$, $J_{p,H-1}=8.0\text{ Hz}$, P_α), -20.2 (1P, dd, $J_{p,\alpha P_\beta}=17.6\text{ Hz}$, $J_{p,\beta P_\gamma}=18.8\text{ Hz}$, P_β), -4.5 (1P, d, $J_{p,\beta P_\gamma}=18.8\text{ Hz}$, P_γ).

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