Phosphorylation of Disaccharides with Inorganic *cyclo*-Triphosphate in Aqueous Solution

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The phosphorylation of disaccharides by inorganic *cyclo*-triphosphate (P_{3m}) with a six-membered ring was examined in aqueous solution. In the phosphorylation of cellobiose, lactose, and α,α-trehalose with P_{3m}, β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl 1-triphosphate, β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl 1-triphosphate, and 3-*O*-triphospho-α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside were synthesized with maximum yields of 28%, 35%, and 20%, respectively. In the reactions of maltose and sucrose with P_{3m}, two phosphorylated products were obtained in yields of 42% and 58%, respectively. The main phosphorylated products were assigned to α-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl 1-triphosphate and β-D-fructofuranosyl-(2→1)-2-*O*-triphospho-α-D-glucopyranoside by heteronuclear multiple bond correlation (HMBC) NMR. The phosphorylation mechanism of disaccharides with P_{3m} is discussed.

Key words phosphorylation; disaccharide; sodium *cyclo*-triphosphate; multinuclear NMR; heteronuclear multiple bond correlation (HMBC) NMR; HPLC

Sucrose, maltose, and lactose are important disaccharides and cheap and valuable sources of renewable materials for industrial applications.[1] For example, sucrose can be converted to ethanol,[2] which is widely used in various fields of industry and the source of C1 chemistry. Recently, natural disaccharides and trisaccharides have attracted enormous attention.[3,4] A large number of oligosaccharides have been conjugated for new DNA-binding antitumor agents,[3] and specific ligands for cell-surface recognition[4] have been produced by chemical and enzymatic syntheses.

We demonstrated that the reaction of D-glucose with *cyclo*-triphosphate (P_{3m}) afforded β-D-glucopyranosyl 1-triphosphate in good yield in a one-step process without protection of hydroxyl groups.[5,6] Although D-glucose exists as an equilibrium mixture of α and β anomers under the reaction conditions, only β-D-glucopyranosyl 1-triphosphate was obtained stereoselectively. This method would be applicable to the phosphorylation of other monosaccharides.[7,8] The phosphorylated products of oligo- and polysaccharides are expected to act as chiral selectors,[9] masking agents of metal ions,[10] and material for drug delivery systems (DDS).[11] Although this stereoselective phosphorylation is effective for monosaccharides, the existence of 1-OH is essential, which is not feasible in polysaccharides. It is more significant to apply this stereoselective phosphorylation reaction to disaccharides to open the way for the phoshorylation of oligo- and polysaccharides.

In the present study, the reaction of cellobiose (**1**), lactose (**2**), maltose (**3**), sucrose (**4**), and α,α-trehalose (**5**) with P_{3m} was studied to develop selective phosphorylation of disaccharides in aqueous solution.

Results and Discussion

Phosphorylation of Cellobiose (1), Lactose (2), and Maltose (3) with P_{3m} Reducing disaccharides used in the present study are shown in Chart 1. Phosphorylation was carried out essentially according to the previous method.[6] Figure 1 shows the HPLC profiles for the reaction solution of cellobiose (**1**) ($1.0\,mol\cdot dm^{-3}$) and P_{3m} ($0.5\,mol\cdot dm^{-3}$) incubated at pH 12 at room temperature. The peak of the main product (**6**) appeared at a retention time of about 17 min. The yield of **6** increased with reaction time to reach the maximum yield of 28% after 78 h and then decreased gradually (Fig. 2).

To identify product (**6**) in the phosphorylation of **1** with P_{3m}, **6** was isolated using anion-exchange resin (see Experimental). ^{31}P- and ^{1}H-NMR spectra were measured to confirm the reaction product. The ^{31}P-NMR data (Table 1) of **6** show two doublets (−11.8, −5.51 ppm) and one doublet of doublets (−20.6 ppm) characteristic of triphosphate derivatives.[6—8,12] Figure 3A shows the ^{31}P-NMR spectrum of the P_α region in **6**. The spectrum had one doublet of doublets at −11.8 ppm, which became a doublet on ^{1}H-decoupling, indicating the characteristic peak of P_α in triphosphate deriva-

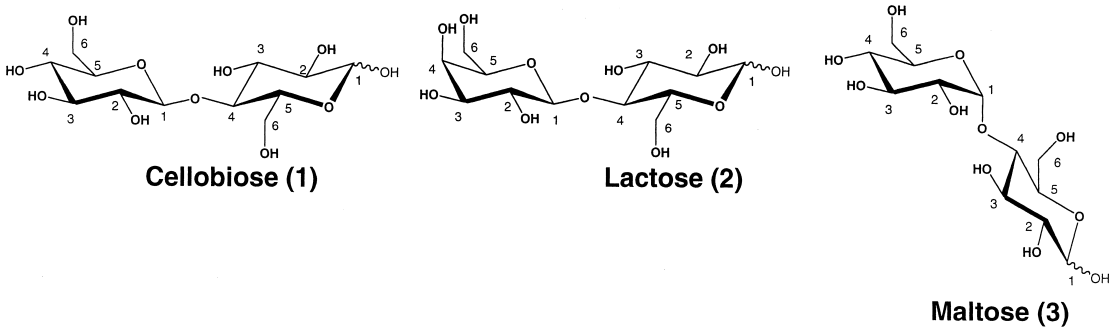

Chart 1

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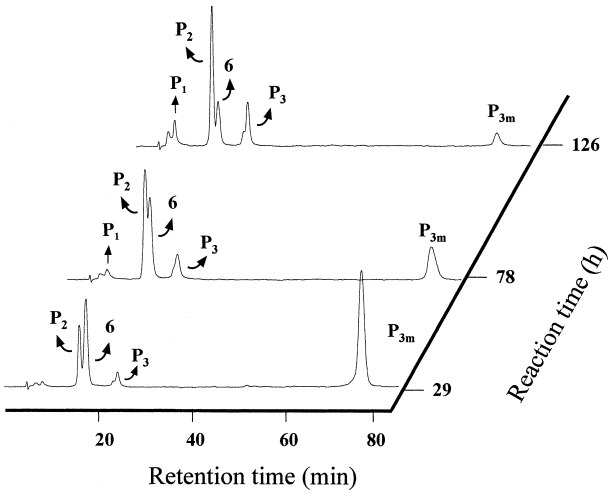


Fig. 1. HPLC Profiles for the Reaction Solution of **1** and P_{3m}

Cellobiose (**1**): P_{3m}=1.0 mol·dm^{-3}: 0.5 mol·dm^{-3}, pH 12, room temperature. P_1, monophosphate; P_2, diphosphate; P_3, triphosphate; P_{3m}, *cyclo*-triphosphate; **6**, product (*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl 1-triphosphate).

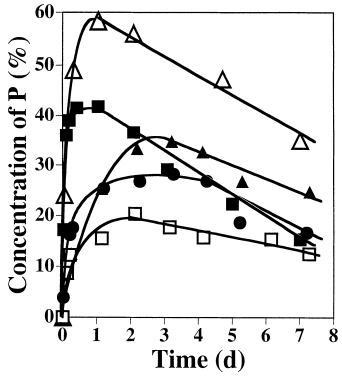
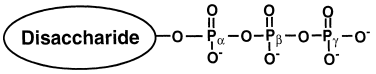


Fig. 2. Changes in the Amounts Obtained by HPLC Measurement of the Phosphorylated Products in the Reaction of Disaccharides with P_{3m} at pH 12, Room Temperature

●, Cellobiose (**1**); ▲, lactose (**2**); ■, maltose (**3**); △, sucrose (**4**); □, *α*,*α*-trehalose (**5**). **1**, **2**: P_{3m}=1.0 mol·dm^{-3}: 0.5 mol·dm^{-3}; **3**, **4**, **5**: P_{3m}=2.0 mol·dm^{-3}: 0.5 mol·dm^{-3}.

Table 1. ^{31}P-NMR Data of Disaccharide Triphosphates



Compound	*δ* (ppm)			*J* (Hz)				
	P_α	P_β	P_γ	P_α, P_β	P_β, P_γ	P_α, H-1	P_α, H-2	P_α, H-3
6	−11.8	−20.6	−5.51	18.4	20.0	8.2		
7	−11.6	−20.2	−5.57	18.3	19.0	8.6		
8	−12.1	−21.3	−5.64	18.8	20.0	8.5		
9	−10.8	−21.0	−5.56	17.7	20.0		9.2	
10	−11.0	−21.2	−6.41	17.8	20.0		9.4	
11	−11.2	−21.6	−6.65	18.8	20.6			10.0
12	−10.8	−21.4	−6.35	18.5	19.6			8.8

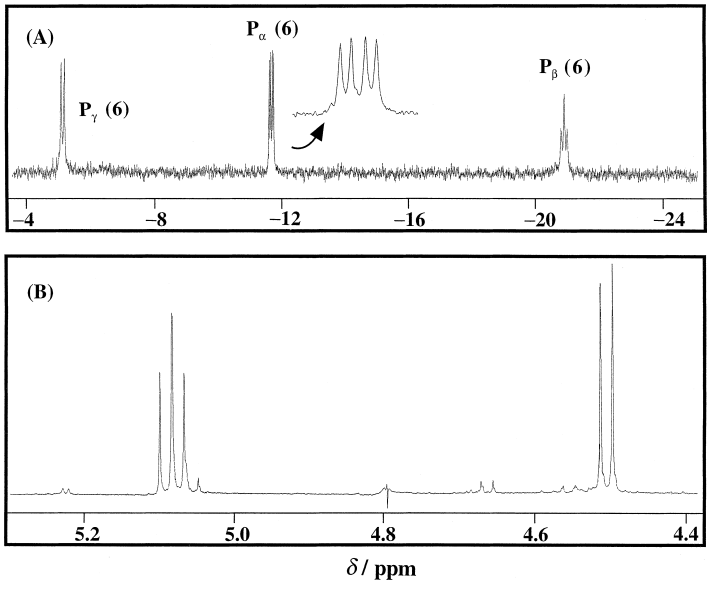
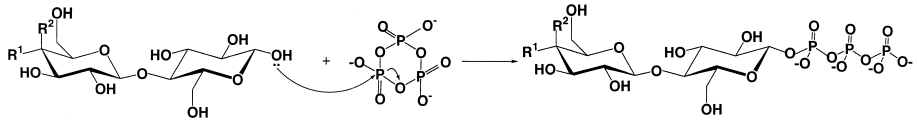


Fig. 3. ^{31}P-NMR Spectrum of the P_α Region (A), and ^{1}H-NMR Spectrum of the H-1 Region (B) of **6**

Table 2. ^{1}H-NMR Chemical Shifts and Coupling Constants of Disaccharide Triphosphates

	Residue	δ (ppm)				J (Hz)				
		H-1	H-2	H-3	H-4	P,H-1	P,H-2	1,2	2,3	3,4
6	β-D-Glucopyranosyl	4.50	3.32	3.51	3.42			8.0	9.5	9.0
	β-D-Glucopyranosyl-1-triphosphate	5.08	3.42	3.68	3.68	8.5		8.0	9.0	9.0
7	β-D-Galactopyranosyl	4.44	3.55	3.66	3.92			7.5	10.0	3.5
	β-D-Glucopyranosyl-1-triphosphate	5.08	3.42	3.67	3.67	8.5		8.5	9.0	9.5
8	α-D-Glucopyranosyl	5.38	3.52	3.67	3.37			3.8	10.0	9.0
	β-D-Glucopyranosyl-1-triphosphate	5.04	3.37	3.81	3.64	8.5		8.5	9.5	9.5
9a	2-*O*-Triphospho-α-D-glucopyranosyl	5.76	4.14	3.76	3.49		9.2	4.0	9.1	9.0
	β-D-Glucopyranose	4.65	3.19	3.85	3.69			8.6	9.0	9.3
9b	2-*O*-Triphospho-α-D-glucopyranosyl	5.73	4.14	3.76	3.49		9.2	4.0	9.1	9.0
	α-D-Glucopyranose	5.18	3.49	4.04	3.69			3.8	9.4	9.3



1: R^1=OH, R^2=H
2: R^1=H, R^2=OH

6: R^1=OH, R^2=H
7: R^1=H, R^2=OH

Chart 2

tives[12] of D-aldose. The other doublet at −5.51 ppm is assigned to the phosphorus atom (P_γ) of **6**. The doublet of doublets at −20.6 ppm is characteristic of the middle-group phosphorus atom. Therefore it is assigned to the middle phosphorus atom (P_β) of **6**.

Figure 3B shows the ^{1}H-NMR spectrum in the region of H-1. ^{1}H-COSY spectra showed a correlation between 3.42 ppm (H-2 on the reducing unit of **6**) and 5.08 ppm. The doublet of doublets at 5.08 ppm is therefore assigned to H-1 on the reducing unit of **6**.[6—8] The doublet at 4.50 ppm is due to H-1 on the nonreducing unit of **6**.[13] The ^{31}P–^{1}H heteronuclear multiple bond correlation (HMBC) 2D-NMR experiment showed a correlation of P_α at −11.8 ppm and the H-1 signal at 5.08 ppm. The downfield shift from 4.65 ppm (H-1 on the reducing unit of **1**) to 5.08 ppm is a result of phosphorylation.[7] The $^3J_{1,2}$ value (Table 2) of the H-1 on the reducing unit is close to that of β-D-glucopyranose.[14] The $^3J_{P_\alpha,H\text{-}1}$ value was consistent with that obtained from the ^{31}P-NMR data (Table 1) and with the data of β-D-glucopyranosyl 1-phosphate,[15] β-D-glucopyranosyl 1-triphosphate,[6—8] and β-D-aldose 1-phosphate.[15] Therefore the phosphorylated product (**6**) in the reaction of **1** with P_{3m} was confirmed to be β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl 1-triphosphate.

The phosphorylation of lactose (**2**) with P_{3m} was carried out under the same conditions as for **1**. The yield of product (**7**) increased with the reaction time to reach the maximum yield of 35% after 77 h and then decreased gradually (Fig. 2). The identification of **7** was performed in a similar way as that of **6** (Tables 1, 2). The results suggested **7** to be β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl 1-triphosphate. In the reaction of **1** or **2** with P_{3m}, the 1-OH group of a reducing unit was selectively phosphorylated, similar to D-glucose[6] and most monosaccharides.[7,8] Therefore **1** and **2** stereoselectively react with P_{3m} to form **6** and **7**, respectively (Chart 2).

The reaction of maltose (**3**) (2.0 mol·dm^{-3}) with P_{3m} (0.5 mol·dm^{-3}) was carried out at pH 12 at room temperature.

Table 3. Yields in the Phosphorylation of **3** and **4**

Reaction conditions			Yield (%)
Mixing ratio	pH	Time (d)	
P_{3m} : **3**			
1 : 4 (0.5 M : 2.0 M)	12	1	42
	10	18	37
1 : 1 (0.5 M : 0.5 M)	12	3	22
P_{3m} : **4**			
1 : 4 (0.5 M : 2.0 M)	12	2	58
	10	86	43
1 : 1 (0.5 M : 0.5 M)	12	3	40

The yield of the phosphorylated product was 42% (Fig. 2) after 25 h. As can be seen from Fig. 2, the rate of decomposition of the phosphorylated product of **3** was more rapid than those of **6** and **7**. The yield was comparable with that of D-glucose and much higher than those of **6** and **7**. Table 3 summarizes the yields of the phosphorylated product of **3** under various reaction conditions. The yields increased with the increase in the initial concentration of **3**. The maximum yield and its time of occurrence suggested that the optimum conditions for the phosphorylation of **3** with P_{3m} are pH 12 and a molar ratio of P_{3m} : **3** = 1 : 4. In the phosphorylation of **3**, the yield of the product was 42%. This indicates that the high reactivity of **3** is attributable to its higher solubility in water. As shown in Fig. 2, the triphosphate derivative of **3** was gradually hydrolyzed to give a monophosphate derivative of **3**, inorganic diphosphate, and monophosphate.

The ^{31}P-NMR spectra with ^{1}H-decoupling of the product shown in Fig. 4 had the characteristic signals due to two triphosphate esters (**8**, **9**), although the HPLC profile showed a single peak due to the reaction product at the retention time of 19 min. Under these reaction conditions, the yields of **8** and **9** were determined to be 19% and 17%, respectively,

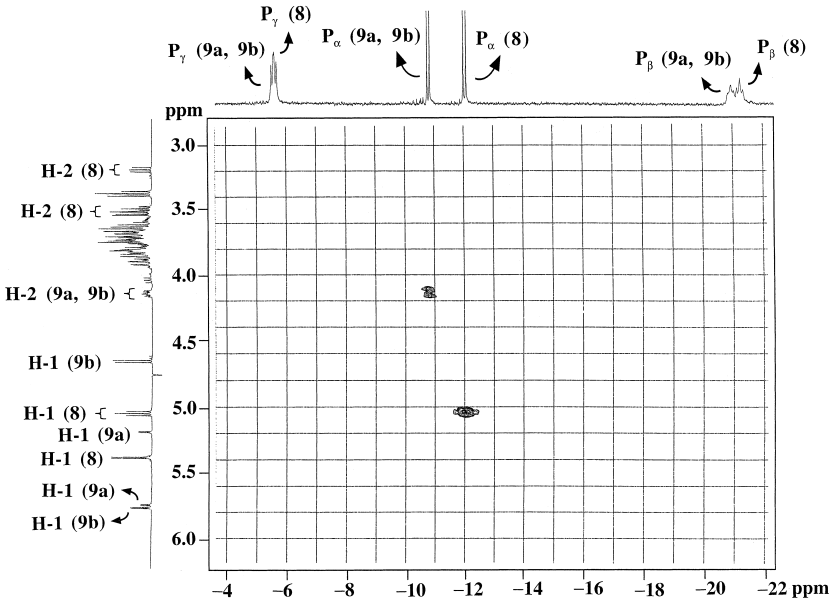


Fig. 4. ^{31}P–^{1}H 2D HMBC-NMR Spectra of **8**, **9a**, and **9b**

Maltose (**3**): P_{3m}=2.0 mol·dm^{-3}: 0.5 mol·dm^{-3}, pH 12, room temperature, after 2 h.

from the integration of ^{31}P-NMR signals. The yield of **8** increased with the reaction time and reached 28% after 25 h, while that of **9** decreased with time and was 14% after 25 h. Figure 4 shows the ^{31}P–^{1}H 2D HMBC correlation spectrum of products **8** and **9**. The peaks at −12.1, −21.3, and −5.64 ppm of the ^{31}P-NMR spectrum were assigned to **8**. A correlation of P_α at −12.1 ppm of product **8** and H-1 signal at 5.04 ppm was observed. The doublet of doublets at 5.04 ppm could be assigned to H-1 on the reducing unit of **8**. The doublet at 5.38 ppm is due to H-1 on the nonreducing unit of **8**.[13] This assignment was confirmed by ^{1}H-COSY spectra, indicating a correlation between 3.37 ppm (H-2 on the reducing unit of **8**[13]) and 5.04 ppm. The $^{3}J_{1,2}$ value (Table 2) of H-1 of the reducing unit is close to that of *β*-D-glucopyranose.[14] The $^{3}J_{P_\alpha,H\text{-}1}$ value was consistent with that obtained from the ^{31}P-NMR data (Table 1) and with data on *β*-D-glucopyranosyl 1-phosphate,[15] *β*-D-glucopyranosyl 1-triphosphate,[6—8] and *β*-D-aldose 1-phosphate.[15] Therefore product **8** was verified to be *α*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranose 1-triphosphate. The other NMR data in Tables 1 and 2 were consistent with this assignment.

The other peaks at −10.8, −21.0, and −5.56 ppm in the ^{31}P-NMR spectrum were due to product **9**. A correlation between P_α at −10.8 ppm of **9** and the ^{1}H signal at 4.14 ppm is shown in Fig. 4. The doublet of doublets of doublets at 4.14 ppm was assigned to H-2 on the nonreducing unit of **9** based on ^{1}H-COSY spectra. ^{1}H-COSY spectra showed two correlations between H-2 (4.14 ppm) and H-1 (5.73, 5.76 ppm), suggesting the coexistence of *α* and *β* anomers of **9**. Therefore product **9** was found to be an anomeric mixture (**9a**, **b**). As can be seen from Table 2, **9** is considered to be 2-*O*-triphospho-*α*-D-glucopyranosyl-(1→4)-D-glucopyranose. The results of previous studies[6—8] indicated that the phosphorylation products of D-aldohexoses and D-aldopentoses with P_{3m} are exclusively 1-triphosphate esters. However, the phosphorylation of the nonreducing unit of **3** proceeded exceptionally. Therefore **3** reacts with P_{3m} to form **8**, **9a**, and **9b**. Unfortunately, products **8** and **9** could not be separated.

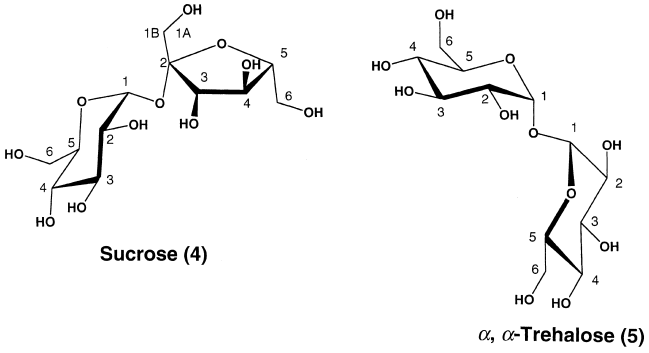


Chart 3

Phosphorylation of Sucrose (4) and *α*,*α*-Trehalose (5) with P_{3m} Chart 3 shows the nonreducing disaccharides used in the present study. Figure 2 shows the reaction time dependence of the yield of the phosphorylation product in the reaction of **4** with P_{3m}. Table 3 summarizes the yields of the phosphorylated product of **4** under various reaction conditions. The yield increased with the increase in the initial concentration of **4**. The maximum yield and its time of occurrence suggested that the optimum conditions for the phosphorylation of **4** with P_{3m} are pH 12 and a molar ratio of P_{3m} : **4** = 1 : 4. In the phosphorylation of **4**, the yield was 58%, although **4** has no anomeric OH. The triphosphate derivative of **4** was gradually hydrolyzed to form a monophosphate derivative of **4**, inorganic diphosphate, and monophosphate.

The ^{31}P-NMR spectra with ^{1}H-nondecoupling of the phosphorylation product of **4** with P_{3m} indicated that the products are two triphosphate esters (**10**, **11**), although the HPLC profile showed a single peak due to the reaction product at the retention time of 19 min. Under these reaction conditions, the yields of **10** and **11** were determined to be 35% and 14%, respectively, by comparing the integration areas of the ^{31}P-NMR signals of two triphosphate esters. The ratio of the

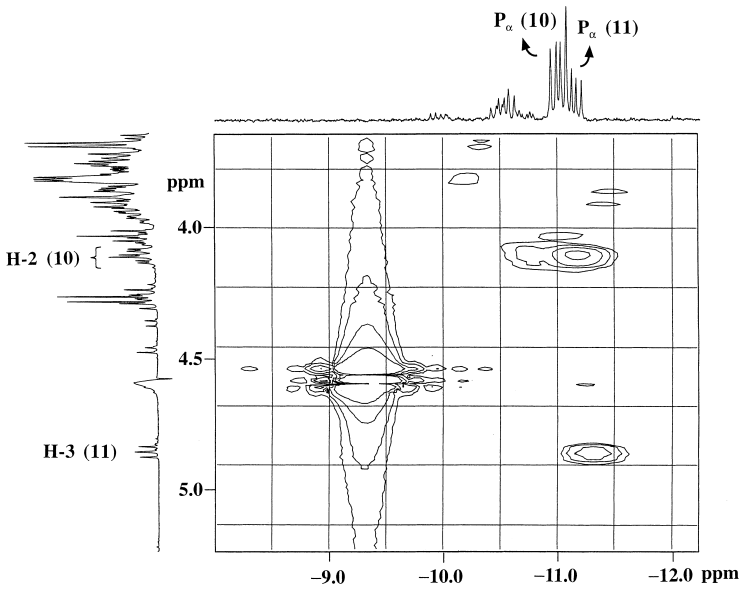


Fig. 5. ^{31}P–^{1}H 2D HMBC-NMR Spectra of **10** and **11**

Sucrose (**4**): P_{3m}=2.0 mol·dm^{-3}: 0.5 mol·dm^{-3}, pH 12, room temperature, after 9 h.

Table 4. ^{1}H-NMR Chemical Shifts and Coupling Constants of Disaccharide Triphosphates

	Residue	δ (ppm)					J (Hz)						
		H-1A	H-1B	H-2	H-3	H-4	P,2	P,3	1A,1B	1,2	2,3	3,4	4,5
10	2-*O*-Triphospho-α-D-glucopyranosyl	5.52		4.08	3.83	3.52	9.3			3.5	9.5	9.5	10.0
	β-D-Fructofuranoside	3.71	3.68		4.20	4.00			12.8			8.5	8.5
11	α-D-Glucopyranosyl	5.40		3.44	3.82	3.35				3.5	10.0	9.5	10.0
	3-*O*-Triphospho-β-D-fructofuranoside	3.71	3.68		4.78	4.26		9.5	12.8			8.5	8.5
12	3-*O*-Triphospho-α-D-glucopyranosyl	5.06		3.57	3.94	3.80		8.6		4.0	9.4	10.0	9.6
	α-D-Glucopyranoside	5.05		3.46	3.73	3.30				3.5	10.0	9.5	9.5

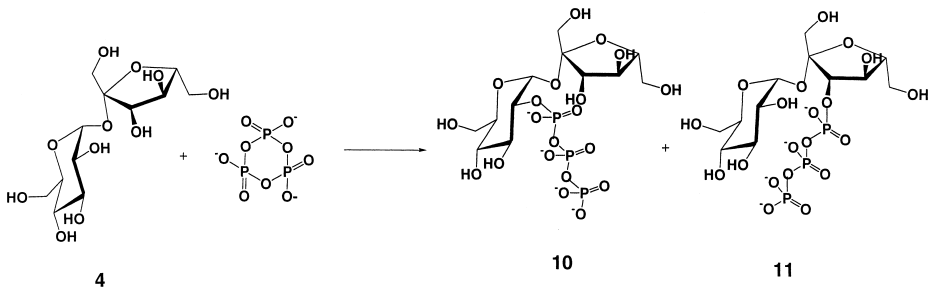


Chart 4

yield of **10** and **11** was constant, that is, 2.6 under various reaction conditions. Unfortunately, products **10** and **11** could not be separated.

Compound **10** showed ^{31}P-NMR signals at −11.0, −21.2, and −6.41 ppm. Also, the ^{31}P–^{1}H 2D-NMR experiment (Fig. 5) showed the correlation of P_α at −11.0 ppm and the H-2 signal at 4.08 ppm. The doublet of doublets of doublets at 4.08 ppm was assigned to H-2 of the α-D-glucopyranosyl group based on the ^{1}H-COSY spectra. The downfield shift from 3.56 ppm[16] (H-2 of the α-D-glucopyranosyl group) to 4.08 ppm is the result of phosphorylation. The other ^{1}H-NMR signals of **10** are shown in Table 4. From the results of NMR, the main product **10** was confirmed to be β-D-fructofranosyl-(2→1)-2-*O*-triphospho-α-D-glucopyranoside.

Figure 5 shows the correlation between P_α at −11.2 ppm due to product **11** and the H-3 at 4.78 ppm. The doublet of doublets at 4.78 ppm was assigned to H-3 of the β-D-fructofranosyl group by ^{1}H-COSY experiments. The other ^{1}H-NMR signals of **11** were assigned as shown in Table 4. Therefore the product **11** was found to be 3-*O*-triphospho-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside. The reaction of **4** with P_{3m} proceeded at 2-OH of the α-D-glucopyranosyl residue and 3-OH of the β-D-fructofuranosyl residue (Chart 4).

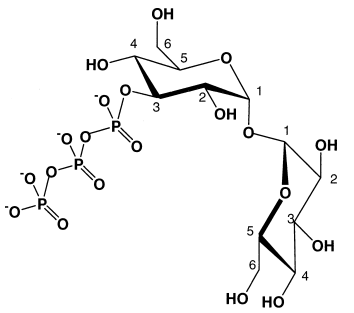
In the reaction of α,α-trehalose (**5**) with P_{3m}, the yield of the phosphorylated product (**12**) of **5** increased with the reac-

tion time to reach 20% after 51 h, as shown in Fig. 2. The yield of **12** remained constant after 7 d without hydrolysis to a monophosphate derivative. The product **12** was verified to be 3-*O*-triphospho-α-D-glucopyranosyl-(1→1)-α-D-glucopyranoside (Tables 1, 4) from the results of NMR.

Mechanism of the Reaction of Dissacharides with P_{3m} In the phosphorylation of reducing disaccharides with P_{3m}, β-anomers of 1-triphosphates were obtained with the yields of more than 28%. On the other hand, nonreducing disaccharides were also phosphorylated in spite of the lack of a 1-OH group. The total yield of products **10** and **11** in the reaction of **4** with P_{3m} was 58%, comparable with that of the product in the reaction of D-glucose with P_{3m}.[6)]

The phosphorylation mechanism of **1** or **2** with P_{3m} is proposed to be as follows. At pH 12, P_{3m} is easily attacked by nucleophilic reagents such as ammonia,[17)] alcohol,[18)] nucleoside,[19)] amino acid,[20)] and amino alcohol.[21)] Thus in the present study, the lone electron pair on the anomeric hydroxyl group of reducing β-D-glucopyranose nucleophilically attacked a phosphorus atom on P_{3m} to open its six-membered ring. The absence of an attack by α-D-glucopyranose might have been due to the kinetic anomeric effect.[22,23)] In addition, substitution at equatorially oriented hydroxyl groups favors β-substitution.

The reaction of **3** or **4** with P_{3m} produced several triphosphate derivatives (**8**—**11**). Figure 6 shows the possible phosphorylation mechanism at the nonreducing unit of **3**. The existence of an intramolecular hydrogen bond between 2-OH of the nonreducing unit and 3-OH of the reducing unit is known in α1→4-linked oligosaccharides such as maltose, maltotriose, and cyclodextrins.[24)] The distance between 2-OH of the nonreducing unit and 3-OH of the reducing unit allows the approach of P_{3m}. The steps of phosphorylation involve: 1) the formation of a hydrogen bond between the 3-OH of the reducing unit and an oxygen atom of P_{3m}; 2) nucleophilic attack of a phosphorus atom of P_{3m} by the lone electron pair on the oxygen atom of 2-OH of the nonreducing unit; and 3) the formation of an intermediate and the P–O bond cleavage of P_{3m}. The hydrogen bond between the 3-OH of the reducing unit and an oxygen atom of P_{3m} will accelerate the attack by the oxygen atom of 2-OH against the phosphorous of P_{3m}. The same reaction mechanism was recognized in the phosphorylation of nucleotides[25)] or phenols[26)] with P_{3m}. Only the lone electron pair on the oxygen atom of 2-OH of the nonreducing unit could attack the phosphorus atom of P_{3m} to produce 2-*O*-triphospho-α-D-glucopyranosyl-(1→4)-D-glucopyranose (**9a**, **b**). The lone electron pair on the oxygen atom of 3-OH of the reducing unit could not attack P_{3m}.



12

Chart 5

In the reaction of **4** with P_{3m}, the phosphorylated products were β-D-fructofranosyl-(2→1)-2-*O*-triphospho-α-D-glucopyranoside (**10**) and 3-*O*-triphospho-β-D-fructofuranosyl-(2→1)-α-D-glucopyranoside (**11**). Because the distance between 2-OH of the α-D-glucopyranosyl group and 3-OH of the β-D-fructofuranosyl group allows the approach of P_{3m}, the phosphorylation reaction would be accelerated. Both the lone electron pair on the oxygen atom of 2-OH of the α-D-glucopyranosyl group and 3-OH of the β-D-fructofuranosyl group could attack the phosphorus atom of P_{3m} to give **10** and **11**, respectively.

In the case of **5**, the phosphorylation of 3-OH of the α-D-glucopyranosyl residue proceeded stereoselectively to form **12**, although its yield was quite low. This is because neither an intramolecular hydrogen bond nor an anomeric carbon was present.

In conclusion, the phosphorylation of disaccharides with 1-OH of the reducing unit was found to be possible similar to

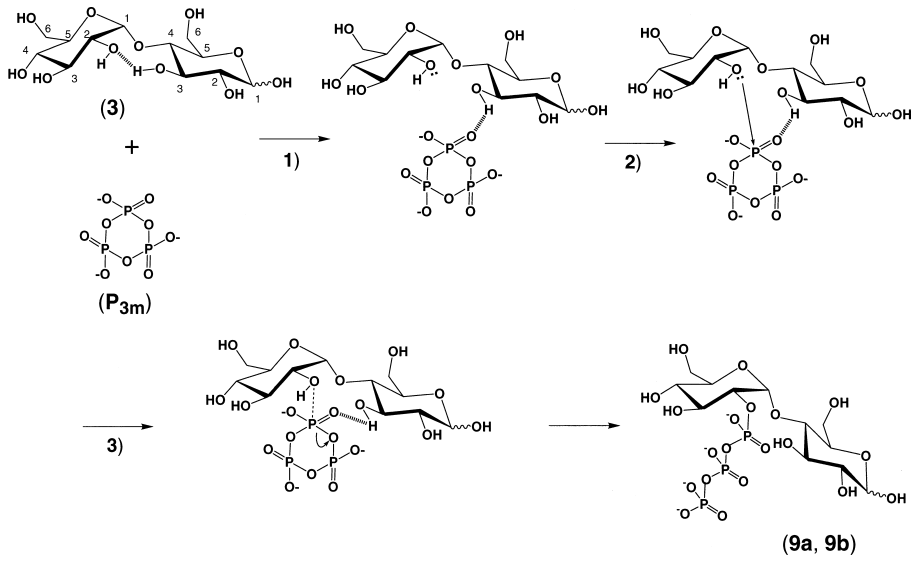


Fig. 6. Phosphorylation Mechanism of Nonreducing Unit of Maltose (**3**) with P_{3m}

D-glucose. Furthermore, the phosphorylation of nonreducing disaccharide with no 1-OH group present was possible for maltose and sucrose. The yields of **9** and **10** were 20% and 41%, respectively. These results open a new path for the phosphorylation of oligosaccharides and polysaccharides in a one-step process in aqueous solution.

Experimental

Materials and Methods P_{3m}, $Na_3P_3O_9 \cdot 6H_2O$, was prepared by the procedure described in previous papers.[5,27] Cellobiose (**1**) and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Other chemicals were purchased from Wako Chemical (Osaka, Japan).

^{1}H-NMR spectra were measured with a Varian Gemini 300 spectrometer. Samples were dissolved in D_2O (99.9%). DSS was used as an external reference for ^{1}H-NMR spectra. ^{31}P-NMR spectra with and without broad-band decoupling and ^{31}P–^{1}H 2D HMBC spectra were obtained with a Varian INOVA-500 spectrometer. Eighty-five percent H_3PO_4 was used as an external standard.

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 postcolumn reaction. A column (150×6.0 mm i.d.) packed with a polystyrene-based anion-exchanger (TSK gel, SAX, 5 μm, TOSOH, Japan) was used for the analysis of phosphate. The flow rate was 1.0 ml·min^{-1}, and the column temperature was maintained at 40 °C. A convex gradient elution technique using 0.12 and 0.45 mol·dm^{-3} of potassium chloride aqueous solution was employed for the separation of phosphate. Sugar phosphate esters, diphosphate (P_2), triphosphate (P_3), and *cyclo*-triphosphate (P_{3m}) were hydrolyzed to monophosphate (P_1) by 6 mol·dm^{-3} sulfuric acid at 140 °C, and the resulting monophosphate was allowed to react with the chromogenic reagent (molybdenum(V)–molybdenum(VI) reagent) to form a phosphorus-molybdenum heteropoly blue complex. The absorbance of the complex was measured at 830 nm.

Procedure for Syntheses The reactions of maltose (**3**), sucrose (**4**), and α,α-trehalose (**5**) (2.0 mol·dm^{-3}) with P_{3m} (0.5 mol·dm^{-3}) were carried out at pH 12 at room temperature. Because of the lower solubility of **1** and **2**, their phosphorylation (1.0 mol·dm^{-3}) with P_{3m} (0.5 mol·dm^{-3}) was carried at pH 12 at room temperature. The yields and the structures of the products were determined by HPLC and NMR measurements.

Isolation of Phosphorylated Products The isolation of the phosphorylated products of cellobiose (**1**), lactose (**2**), maltose (**3**), sucrose (**4**), and α,α-trehalose (**5**) was accomplished by anion-exchange chromatography with a 2×80 cm column filled with Dowex 1-X2 resin (100—200 mesh, chloride form). Elution was carried out with 0.3 mol·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). For the purpose of desalting, an aqueous solution of the concentrate was passed over the PD-10 column (Amersham Biosciences, NJ, U.S.A.).

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