Phosphorylation of D-Glucose Derivatives with Inorganic Monoimido-*cyclo*-triphosphate

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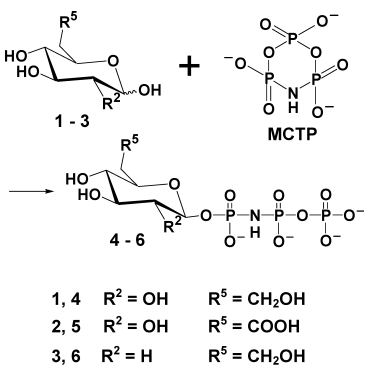
Phosphorylation of several D-glucose derivatives has been achieved using inorganic monoimido-*cyclo*-triphosphate (MCTP, $Na_3P_3O_8NH$) in aqueous solution. In the phosphorylation of D-glucose, D-glucuronic acid, 2-deoxy-D-glucose and D-galactose, 1-*O*-diphosphoramidophosphono-β-D-glucose, 1-*O*-diphosphoramidophosphono-β-D-glucuronic acid, 1-*O*-diphosphoramidophosphono-2-deoxy-β-D-glucose, and 1-*O*-diphosphoramidophosphono-β-D-galactose were stereoselectively synthesized with yields of 54, 32, 37 and 46%, respectively. In the case of methyl α-D-glucoside, the phosphorylated products were methyl 3-*O*-diphosphoramidophosphono-α-D-glucoside and methyl 4-*O*-diphosphoramidophosphono-α-D-glucoside, and in the case of methyl β-D-glucoside the products were methyl 2-*O*-diphosphoramidophosphono-β-D-glucoside, methyl 3-*O*-diphosphoramidophosphono-β-D-glucoside, and methyl 4-*O*-diphosphoramidophosphono-β-D-glucoside. For D-mannose and D-allose, several phosphorylated products were obtained and the main products were 1-*O*-diphosphoramidophosphono-β-D-aldoses.

Key words phosphorylation; monoimido-*cyclo*-triphosphate; multinuclear NMR; HPLC

Sodium *cyclo*-triphosphate, $Na_3P_3O_9$ (P_{3m}), is a simple and efficient phosphorylating agent. Previously, we reported that alkylamines,[1] aminoalcohols,[2] and carbohydrates[3—8] are readily phosphorylated with P_{3m} to give their triphosphate derivatives. In particular, the reaction of D-glucose with P_{3m} in aqueous solution afforded 1-*O*-triphospho-β-D-glucose stereoselectively in good yield (47%) in a one-step process without protection of hydroxyl groups.[3—5] This reaction was found to be applicable to other monosaccharides and oligosaccharides.[6—8] Unfortunately, the products formed from carbohydrates are easily decomposed to monophosphate derivatives.

Imido-*cyclo*-triphosphates, $Na_3P_3O_{9-n}(NH)_n$, have been known since 1962.[9] Compared with the P–O–P linkage, the P–NH–P linkage is stable and difficult to hydrolyze.[9] We therefore explored the use of monoimido-*cyclo*-triphosphate (MCTP) for the phosphorylation of biologically important compounds. The structure of MCTP is shown in Chart 1: the six-membered ring is composed of one P–NH–P and two P–O–P linkages. We recently demonstrated that the phosphorylation of methylamine[10] and amino acids[11] proceeded with MCTP. More recently, we reported that cyclodextrins reacted with MCTP to form 2-*O*-diphosphoramidophosphonocyclodextrins regioselectively in aqueous solution.[12] Organic compounds containing an amino or a hydroxyl group were easily phosphorylated by MCTP.



1, 4 R^2 = OH R^5 = CH_2OH
2, 5 R^2 = OH R^5 = COOH
3, 6 R^2 = H R^5 = CH_2OH

Chart 1

Kamasaka *et al.*[13] have reported that phosphoryl oligosaccharides had the ability to form a soluble complex with calcium and had an inhibitory effect on the formation of calciumphosphate precipitate. Moreover, phosphoryl oligosaccharides effectively enhanced remineralization of enamel and dentin lesion. From the point of chelating of calcium by them, triphosphoryl saccharides lead to an advantageous food ingredient as a soluble calcium. This ability would be improved by diphosphoramidophosphate ester because it was not hydrolyzed to the imidophosphate ester and diphosphate.

In the present work, we first studied the reaction of D-glucose with MCTP in aqueous solution, and then investigated the phosphorylation of D-glucose derivatives by MCTP in order to develop a one-step synthesis of phosphorylated monosaccharides.

Results and Discussion

Phosphorylation of D-Glucose (1), D-Glucuronic Acid (2) and 2-Deoxy-D-glucose (3) with MCTP D-Glucose (**1**) and its derivatives used in the present study are shown in Table 1. Phosphorylation was carried out essentially according to the previously described method.[10—12] Figure 1 shows HPLC profiles for the reaction mixture of D-glucose (**1**) (1.5 M) and MCTP (0.3 M) incubated at pH 12 and 25 °C. A peak attributed to the phosphorylated product (**4**) appeared at a retention time of about 20 min. The other chromatographic peaks were assigned to MCTP and its hydrolytic product (monoimidotriphosphate, MTP), respectively.

To identify the product **4**, ^{31}P- and ^{1}H-NMR spectra were measured. Figure 2 shows ^{31}P-NMR spectra of **4**, showing the characteristic peaks (0.1, −5.3, −10.8 ppm) of the monoimidotriphosphate derivative of **1**. A previous study indicated that the phosphorylation products of cyclodextrins[12] with MCTP are diphosphoramidophosphonocyclodextrins with an $-O-P_{\alpha}-NH-P_{\beta}-$ bond. These products show a characteristic P_{α} signal at around 0 ppm in their ^{31}P-NMR spec-

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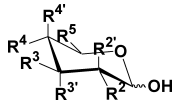
Table 1. Structures of D-Glucose, D-Glucose Derivatives and Epimers of D-Glucose Used in This Work



Compounds	Substituents						
	R^2	$R^{2'}$	R^3	$R^{3'}$	R^4	$R^{4'}$	R^5
D-Glucose (**1**)	OH	H	OH	H	OH	H	CH_2OH
D-Glucuronic acid (**2**)	OH	H	OH	H	OH	H	COOH
2-Deoxy-D-glucose (**3**)	H	H	OH	H	OH	H	CH_2OH
D-Galactose (**14**)	OH	H	OH	H	H	OH	CH_2OH
D-Allose (**15**)	OH	H	H	OH	OH	H	CH_2OH
D-Mannose (**16**)	H	OH	OH	H	OH	H	CH_2OH

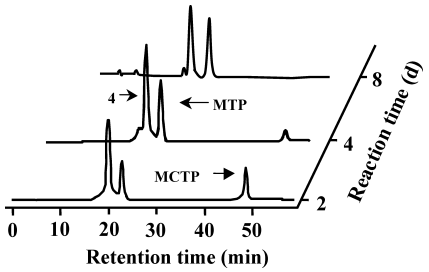


Fig. 1. HPLC Profiles for the Reaction Mixture of **1** and MCTP

MCTP : D-Glucose (**1**) = 0.3 M : 1.5 M, pH 12, and 25 °C.

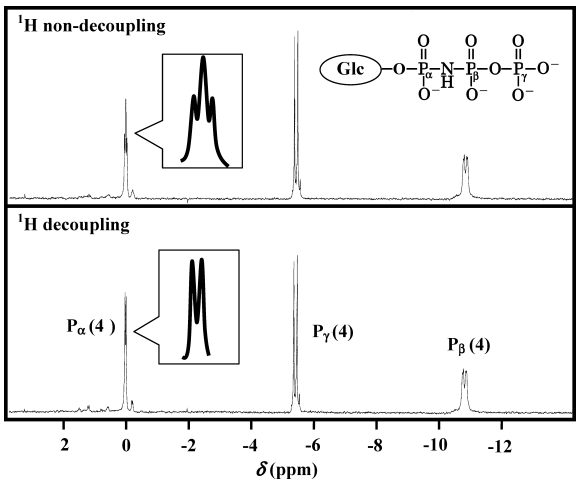


Fig. 2. ^{31}P-NMR Spectra of **4**

MCTP : D-Glucose (**1**) = 0.3 M : 1.5 M, pH 12, and 25 °C, after 2 d.

tra. The ^{1}H non-decoupled ^{31}P-NMR spectrum of **4** showed a doublet of doublets at 0.1 ppm, which became a doublet in the ^{1}H decoupled spectrum, which is the characteristic peak of P_α similar to those of monoimidotriphosphate derivatives.[10—12] The other doublet at −5.3 ppm and the doublet of doublets at −10.8 ppm did not change with ^{1}H decoupling. The chemical shifts of the middle phosphorus atom (P_β) and the end phosphorus atom (P_γ) of monoimidotriphosphate derivatives are about −10.0 and −6.0 ppm, respectively.[10—12] Therefore, the doublet at −5.3 ppm and the doublet of doublets at −10.8 ppm were assigned to P_γ and P_β of **4**, respectively. Compared with the triphosphate ester of D-glucose, the chemical shifts of P_α and P_β of **4** showed downfield shifts,

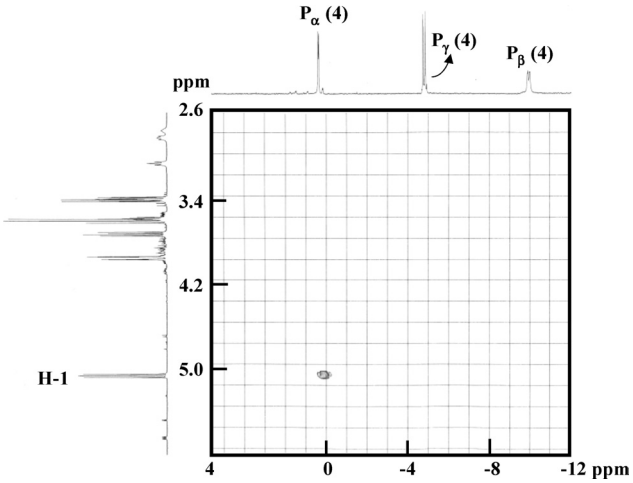


Fig. 3. ^{31}P–^{1}H 2D HMBC NMR Spectrum of **4**

MCTP : D-Glucose (**1**) = 0.3 M : 1.5 M, pH 12, and 25 °C, after 2 d.

whereas there was no shift for P_γ. Also, the value of J_{P_α,P_β} of **4** was one-third of J_{P_α,P_β} for the triphosphate ester of D-glucose,[3] and the value of J_{P_β,P_γ} of **4** was the same as that of the triphosphate ester of D-glucose.[3] These results suggest the existence of an –O–P_α–NH–P_β– bond in the phosphorylated product **4**. Therefore, **4** was confirmed to be a diphosphoramidophosphono-D-glucose.

Figure 3 shows the ^{31}P–^{1}H heteronuclear multiple bond correlation spectroscopy (HMBC) 2D NMR spectrum of **4**. The ^{31}P–^{1}H 2D HMBC NMR experiment showed a correlation between P_α at 0.1 ppm and the ^{1}H signal at 5.02 ppm. The doublet of doublets at 5.02 ppm was assigned to H-1 of **4**.[3] The $^3J_{1,2}$ value of **4** is 7.5 Hz, which is typical for *β*-D-glucose 1-triphosphate[3] and *β*-D-aldose 1-monophosphates.[14] The $^3J_{P_\alpha,H\text{-}1}$ value (9.0 Hz) from ^{1}H-NMR is consistent with that deduced from ^{31}P-NMR data and also with data of *β*-D-glucose 1-triphosphate[3] and *β*-D-aldose 1-phosphates.[14] From these results, **4** was confirmed to be 1-*O*-diphosphoramidophosphono-*β*-D-glucose. This shows that **1** reacts with MCTP to form 1-*O*-diphosphoramidophosphono-*β*-D-glucose (**4**) stereoselectively, as shown in Chart 1.

Table 2 summarizes the yields of **4** obtained from the reaction of **1** with MCTP under various conditions. Considering the yield and reaction time, the optimal conditions for phosphorylation of **1** with MCTP are pH 12, 25 °C, and a molar ratio of MCTP : **1** = 1 (0.3 M) : 10 (3.0 M).

Table 2. Yields of 1-*O*-Diphosphoramidophosphono-*β*-D-glucose (**4**)

Conc. (M)		Temp. (°C)	pH	Time (d)	Yield (%)
MCTP	D-Glucose				
0.3	3.0	10	12	12	54
		25	12	2	49
		40	12	1	32
		25	10	31	51
		40	10	15	33
		25	7	—	—
0.3	1.5	10	12	14	45
		25	12	4	54
		40	12	1	28
0.3	0.3	25	12	6	17

—: no reaction.

The reactions of D-glucuronic acid (**2**) and 2-deoxy-D-glucose (**3**) with MCTP were carried out under the same reaction conditions as **1**. HPLC analysis indicated that the products were the monoimidotriphosphate derivatives. The yields of the products, **5** and **6**, increased with reaction time, reaching a maximum of 32% (after 7 d) and 37% (after 27 d), respectively. Compared with the reaction of **1** (54% after 4 d) with MCTP, those of **2** and **3** were low in yield and time-consuming.

^{31}P- and ^{31}P–^{1}H 2D HMBC NMR spectra of **5** and **6** suggest that they are 1-*O*-diphosphoramidophosphono-*β*-D-aldoses (Chart 1). This shows that **2** and **3** stereoselectively react with MCTP to form 1-*O*-diphosphoramidophosphono-*β*-D-glucuronic acid (**5**) and 1-*O*-diphosphoramidophosphono-2-deoxy-*β*-D-glucose (**6**).

Phosphorylation of Methyl *α*-D-Glucoside (7) and Methyl *β*-D-Glucoside (8) with MCTP As mentioned above, *β*-anomers of **1**, **2** and **3** were stereoselectively phosphorylated by MCTP at the 1-OH position, similarly to the reaction with P_{3m}. In order to determine the possibility of reaction at another site using MCTP, phosphorylation of methyl *α*-D-glucoside (**7**) and methyl *β*-D-glucoside (**8**) were studied. Figure 4 shows the amounts of products obtained over various periods in the reactions of **7** and **8** with MCTP at pH 12 and 25 °C. Although the HPLC profile of the reaction of **7** with MCTP showed a single peak attributable to the reaction product, ^{31}P-NMR spectra showed two imidotriphosphate esters, **9** and **10**, which could not be separated by HPLC. The total amounts of **9** and **10** increased with reaction time, with the yield reaching 18% after 21 d. The yield remained constant after 60 d at pH 12 without hydrolysis of the imidotriphosphate ester. In the reaction of **8** with MCTP, three phosphorylated products, **11**, **12** and **13**, were observed in the HPLC profile. The maximum yields of **11**, **12** and **13** were 14, 20 and 7%, respectively. The yields of **11**—**13** remained constant after 44 d without hydrolysis of the imidotriphosphate ester.

To identify **9** and **10**, ^{31}P- and ^{1}H-NMR spectra were measured. Figure 5 shows the ^{31}P–^{1}H 2D HMBC correlation spectrum. In the ^{31}P-NMR spectrum, the peak at 2.1 ppm was assigned to P_α of **9**, and the peak at 1.2 ppm to P_α of **10**. The doublet at −5.3 ppm and the doublet of doublets at −10.5 ppm were assigned to P_γ and P_β, respectively. The spectrum showed a correlation between P_α at 2.1 ppm and the ^{1}H signal at 4.26 ppm. The doublet of doublets of doublets at

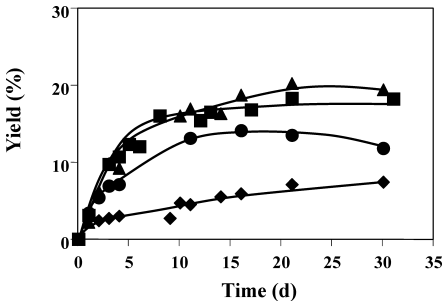


Fig. 4. Changes of the Amounts of Reaction Products in the Reaction of Methyl *α*-D-Glucoside (1.5 M) or Methyl *β*-D-Glucoside (1.5 M) with MCTP (0.3 M) at pH 12 and 25 °C

■: **9**+**10**, ●: **11**, ▲: **12**, ◆: **13**.

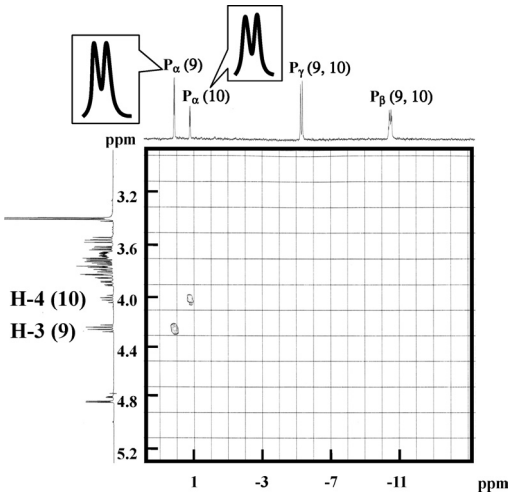


Fig. 5. ^{31}P–^{1}H 2D HMBC NMR Spectrum of **9** and **10**

MCTP : Methyl *α*-D-glucoside (**7**)=0.3 M : 1.5 M, pH 12 and 25 °C.

4.26 ppm was assigned to H-3 of **7** based on the ^{1}H–^{1}H COSY spectrum. The downfield shift from 3.56 ppm (H-3 of **7**) to 4.26 ppm is the result of phosphorylation. The other ^{1}H-NMR signals of **9** were assigned by ^{1}H–^{1}H COSY and ^{1}H–^{1}H TOCSY NMR. In this way, the main product **9** was confirmed to be methyl 3-*O*-diphosphoramidophosphono-*α*-D-glucoside (**9**).

Figure 5 also shows the correlation between P_α at 1.2 ppm (due to **10**) and the H-4 at 4.03 ppm. The doublet of doublets of doublets at 4.03 ppm was assigned to H-4 of **10** by ^{1}H–^{1}H COSY experiments. The other ^{1}H-NMR signals of **10** were assigned as shown in Experimental section. In this way, product **10** was determined as methyl 4-*O*-diphosphoramidophosphono-*α*-D-glucoside. In the reaction of **8** with MCTP, the phosphorylated products **11**, **12** and **13** were verified to be methyl 2-*O*-diphosphoramidophosphono-*β*-D-glucoside (**11**), methyl 3-*O*-diphosphoramidophosphono-*β*-D-glucoside (**12**), and methyl 4-*O*-diphosphoramidophosphono-*β*-D-glucoside (**13**), respectively, from the results of ^{31}P-, ^{1}H-, ^{31}P–^{1}H 2D HMBC, ^{1}H–^{1}H COSY and ^{1}H–^{1}H TOCSY NMR.

Phosphorylation of D-Galactose (14), D-Allose (15) and D-Mannose (16) with MCTP Phosphorylation of D-glucose epimers with MCTP was carried out to investigate the effect of the orientation of the hydroxyl groups at C-1, C-2, C-3 and C-4 in the phosphorylation reaction. The reaction of D-galactose (**14**) with MCTP showed a product **17** by HPLC

with the maximum yield of 46%. In contrast, D-allose (**15**) and D-mannose (**16**) gave three (**18**—**20**) and four products (**21**—**24**), respectively. In HPLC profiles of the reaction solution of **15** and MCTP, three peaks due to the phosphorylated products **18**, **19** and **20** appeared at retention times of about 21, 25 and 30 min with maximum yields of 27, 51 and 7%, respectively. In the reaction of **16** and MCTP, four peaks due to the products, **21**, **22**, **23** and **24**, appeared at retention times of about 14, 15, 25 and 27 min with yields of 22, 10, 21 and 8%, respectively. From HPLC retention times and NMR data, all of the products (**17**—**24**) were identified as monoimidotriphosphate derivatives. To identify the phosphorylated products **17**—**24**, ^{31}P-, ^{1}H-, ^{31}P–^{1}H 2D HMBC, ^{1}H–^{1}H COSY and ^{1}H–^{1}H TOCSY NMR spectra were measured. The stereoselectivity of 1-OH of **17**, **18**, **19** and **20** were determined from their $J_{1,2}$ values of α- and β-anomers,[15] and that of **21** was determined from chemical shift of α- and β-anomers by ^{13}C-NMR spectra.[4,14,15]

Similarly to **4**—**6**, **14** reacted stereoselectively with MCTP to form 1-*O*-diphosphoramidophosphono-β-D-galactose (**17**). In the reaction of **15** and MCTP, phosphorylation proceeded at the 3-OH in addition to the 1-OH. The products **18**, **19** and **20** were verified to be 1-*O*-diphosphoramidophosphono-α-D-allose, 1-*O*-diphosphoramidophosphono-β-D-allose and 3-*O*-diphosphoramidophosphono-β-D-allose, respectively. However, the main products in the phosphorylation of **16** with MCTP were 1-*O*-diphosphoramidophosphono-β-D-mannose (**21**) and 1-*O*-diphosphoramidophosphono-β-D-glucose (**23**). C-2 Epimerization of D-mannose in basic solution occurred easily to form D-glucose.[16] Because of their low yields, **22** and **24** could not be identified. Thus it was confirmed that in the reactions of D-glucose epimers **14**—**16** with MCTP, the main products were 1-*O*-diphosphoramidophosphono-β-D-aldoses.

Reaction Mechanism of D-Glucose Derivatives with MCTP The stereoselectivity of the reaction of **1**, **2** or **3** with MCTP may be explained with reference to the following mechanism. At pH 12, MCTP is easily attacked by nucleophilic reagents such as amines,[10] amino acids[11] and cyclodextrins.[12] In the present study, the lone electron pair on the anomeric hydroxyl group of β-D-aldose nucleophilically attacks a phosphorus atom of MCTP, cleaving its six-membered ring. It is noteworthy that the existence of hydrogen bonding between the hydroxyl group at C-2 and the oxygen atom of MCTP would make attack of MCTP easier. The fact that α-D-aldose does not attack MCTP is probably due to the steric hindrance of the *cis* conformation of the hydroxyl groups at C-1 and C-2, or to the 1,3-diaxial interaction between H-3 and the monoimidotriphosphate group.

Figure 6 shows the yields of phosphorylated esters in the reactions of D-glucose (2.5 M) with P_{3m} (0.5 M) and D-glucose (1.5 M) with MCTP (0.3 M) at pH 12 and 25 °C. The maximum yield of the phosphorylated product (1-*O*-triphospho-β-D-glucose) of D-glucose with P_{3m} was 42% after 2 d, and the product was easily hydrolyzed to the monophosphate ester. The yield of product **4** in the reaction of D-glucose with MCTP increased with reaction time, reaching 54% after 4 d. The yield remained constant after 30 d without hydrolysis of the imidotriphosphate ester, unlike the reaction with P_{3m}. It was concluded that the stability of the phosphorylated monosaccharide was improved by use of MCTP.

Table 3 shows the yields obtained in the phosphorylation of D-glucose derivatives at pH 12 and 25 °C. The yields of **5** and **6** were 32% in 7 d, and 37% in 27 d, respectively. Because of electrostatic repulsion between MCTP and the carboxyl group of **2**, it should be more difficult to react MCTP with **2** than with **1**. The hydrogen bond between the 2-OH of **1** and an oxygen atom of MCTP accelerates attack by the oxygen atom of 1-OH against the phosphorus of MCTP, as mentioned above. Compound **3** cannot form a hydrogen bond similar to that found in **1**, because it does not contain a 2-OH group. Compared with the reaction of **1** with MCTP, those of **2** and **3** were, therefore, low in yield and time-consuming. In

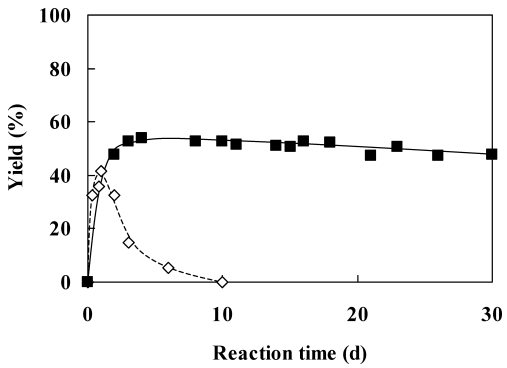


Fig. 6. Yields of Phoshorylated Esters in the Reaction of D-Glucose (2.5 M) with P_{3m} (0.5 M) or D-Glucose (1.5 M) with MCTP (0.3 M) at pH 12 and 25 °C

■: MCTP, ◇: P_{3m}.

Table 3. Yields in the Phosphorylation of D-Glucose Derivatives at pH 12 and 25 °C

Reactant	Product	Phosphorylated OH site	Time (d)	Yield (%)
D-Glucose (**1**)	**4**	β1	4	54
D-Glucuronic acid (**2**)	**5**	β1	7	32
2-Deoxy-β-D-glucose (**3**)	**6**	β1	27	37
Methyl α-D-glucoside (**7**)	**9**	α3	21	18 (**9**+**10**)
	10	α4		
Methyl β-D-glucoside (**8**)	**11**	β2	16	14
	12	β3	21	20
	13	β4	21	7
D-Galactose (**14**)	**17**	β1	6	46
D-Allose (**5**)	**18**	α1	8	27
	19	β1	8	51
	20	β3	2	7
D-Mannose (**16**)	**21**	β1	10	22

contrast, the reactions with P_{3m} of D-glucose derivatives substituted at 2-OH produced the triphosphate derivatives in low yield (14%).[5] The reaction of 2-deoxy-D-glucose with MCTP resulted in the formation of the monoimidotriphosphate derivative with a higher yield than with P_{3m}, although the reason for this is unclear.

The reaction of **7** with MCTP proceeded at the 3-OH and 4-OH of **7**. The fact that attack by the 2-OH of **7** did not occur is probably due to steric hindrance by the bulky methoxy group at C-1, or strain of the glucose unit for the 1,3-diaxial interaction[17] between H-3 and H-5. Therefore, the total yield of **9** and **10** was lower than that of the phosphorylation product of **8** (Table 3).

The reactions of **14**, **15** and **16** with MCTP produced the corresponding monoimidotriphosphate esters, and the main products were 1-*O*-diphosphoramidophosphono-*β*-D-aldoses, with maximum yields of 46, 51 and 22%, respectively. Similarly to **4**—**6**, **14** reacts stereoselectively with MCTP to form 1-*O*-diphosphoramidophosphono-*β*-D-galactose. A previous work showed that the yields of the reaction products of D-galactose, D-allose or D-mannose with P_{3m} were 31, 32 and 10%, respectively.[4] Compared with the products obtained by reaction with P_{3m}, the yields of the products by reaction with MCTP (Table 3) were higher.

Conclusion

In the reactions of D-glucose (**1**), D-glucuronic acid (**2**) and 2-deoxy-D-glucose (**3**) with MCTP, 1-*O*-diphosphoramidophosphono-*β*-D-aldoses (**4**—**6**) were stereoselectively synthesized with yields of more than 32%. The reactions of D-glucose epimers **14**—**16** with MCTP produced the corresponding monoimidotriphosphate derivatives with poor selectivity, except for D-galactose (**14**). The main products of the reactions of **14**—**16** were 1-*O*-diphosphoramidophosphono-*β*-D-aldoses. In the reactions of methyl *α*-D-glucoside (**7**) and methyl *β*-D-glucoside (**8**) with MCTP, the 2-OH, 3-OH or 4-OH of the glucose unit was phosphorylated, although **7** and **8** have no 1-OH. These results suggest that the phosphorylation of oligo- and polysaccharides by MCTP is a promising area for development.

Experimental

Materials and Methods Monoimido-*cyclo*-triphosphate, $Na_3P_3O_8NH$ (MCTP), was prepared according to a previous paper.[18] D-Galactose (**14**) and sodium 2,2-dimethy-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.

^{1}H-NMR spectra were measured with Varian Gemini 300 spectrometer. Samples were dissolved in D_2O (99.9%) to avoid the effect of ^{1}H in water. DSS was used as an external standard for ^{1}H-NMR spectra. ^{31}P-NMR spectra with and without broad band ^{1}H-decoupling and ^{31}P–^{1}H 2D HMBC spectra were obtained with a Varian INOVA-500 spectrometer. As an external standard, 85% H_3PO_4 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.[19] 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 column temperature was maintained at 40 °C. A convex gradient elution technique using 0.2 and 0.45 M 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 Products The reaction of D-glucose (**1**) (0.3—3.0 M) with MCTP (0.3 M) was carried out at pH 12 and 25 °C. The pH of the mixed solution was adjusted by adding 6 M sodium hydroxide solution. The separation of **4** from the reaction mixture 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 M KCl aqueous solution, and each 50 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 ^{1}H- and ^{31}P-NMR measurements. Similar procedures were used for the syntheses of **5**, **6**, **9**—**13** and **17**—**24**. The product **23** was assigned to the same product of **1**. Because of low yield, **22** and **24** could not be identified.

1-*O*-Diphosphoramidophosphono-*β*-D-glucose (**4**): ^{1}H-NMR (D_2O) δ: 5.02 (1H, dd, $J_{1,2}$=7.5 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, H-1), 3.34 (1H, dd, $J_{1,2}$=7.5 Hz, $J_{2,3}$=10.0 Hz, H-2), 3.55 (1H, dd, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.5 Hz, H-3), 3.35 (1H, dd, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, H-4), 3.53 (1H, ddd, $J_{4,5}$=9.5 Hz, $J_{5,6}$=2.5 Hz, $J_{5,6'}$=6.5 Hz, H-5), 3.90 (1H, dd, $J_{5,6}$=2.5 Hz, $J_{6,6'}$=12.5 Hz, H-6), 3.67 (1H, dd, $J_{5,6'}$=6.5 Hz, $J_{6,6'}$=12.5 Hz, H-6′). ^{31}P-NMR (D_2O) δ: 0.1 (1P, dd, $J_{P_\alpha P_\beta}$=6.7 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, P_α), −10.8 (1P, dd, $J_{P_\alpha P_\beta}$=6.7 Hz, $J_{P_\beta P_\gamma}$=21.2 Hz, P_β), −5.3 (1P, d, $J_{P_\beta P_\gamma}$=21.2 Hz, P_γ).

1-*O*-Diphosphoramidophosphono-*β*-D-glucuronic acid (**5**): ^{1}H-NMR (D_2O) δ: 5.05 (1H, dd, $J_{1,2}$=8.0 Hz, $J_{P_\alpha H\text{-}1}$=9.2 Hz, H-1), 3.41 (1H, dd, $J_{1,2}$=8.0 Hz, $J_{2,3}$=9.2 Hz, H-2), 3.60 (1H, dd, $J_{2,3}$=9.2 Hz, $J_{3,4}$=9.2 Hz, H-3), 3.54 (1H, dd, $J_{3,4}$=9.2 Hz, $J_{4,5}$=9.2 Hz, H-4), 3.82 (1H, d, $J_{4,5}$=9.2 Hz, H-5). ^{31}P-NMR (D_2O) δ: 0.2 (1P, dd, $J_{P_\alpha P_\beta}$=6.7 Hz, $J_{P_\alpha H\text{-}1}$=9.2 Hz, P_α), −10.6 (1P, dd, $J_{P_\alpha P_\beta}$=6.7 Hz, $J_{P_\beta P_\gamma}$=20.0 Hz, P_β), −5.6 (1P, d, $J_{P_\beta P_\gamma}$=20.0 Hz, P_γ).

1-*O*-Diphosphoramidophosphono-2-deoxy-*β*-D-glucose (**6**): ^{1}H-NMR (D_2O) δ: 5.30 (1H, ddd, $J_{1,2}$=2.0 Hz, $J_{1,2'}$=9.5 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, H-1), 2.43 (1H, ddd, $J_{1,2}$=2.0 Hz, $J_{2,2'}$=12.2 Hz, $J_{2,3}$=5.0 Hz, H-2), 1.60 (1H, ddd, $J_{1,2'}$=9.5 Hz, $J_{2,2'}$=12.2 Hz, $J_{2',3}$=12.2 Hz, H-2′), 3.78 (1H, ddd, $J_{2,3}$=5.0 Hz, $J_{2',3}$=12.2 Hz, $J_{3,4}$=9.5 Hz, H-3), 3.42 (1H, dd, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, H-4), 3.48 (1H, ddd, $J_{4,5}$=9.5 Hz, $J_{5,6}$=2.5 Hz, $J_{5,6'}$=6.5 Hz, H-5), 3.91 (1H, dd, $J_{5,6}$=2.5 Hz, $J_{6,6'}$=12.5 Hz, H-6), 3.70 (1H, dd, $J_{5,6'}$=6.5 Hz, $J_{6,6'}$=12.5 Hz, H-6′). ^{31}P-NMR (D_2O) δ: −0.8 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, P_α), −11.4 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\beta P_\gamma}$=20.2 Hz, P_β), −5.6 (1P, d, $J_{P_\beta P_\gamma}$=20.2 Hz, P_γ).

Methyl 3-*O*-Diphosphoramidophosphono-*α*-D-glucoside (**9**): ^{1}H-NMR (D_2O) δ: 4.84 (1H, d, $J_{1,2}$=4.0 Hz, H-1), 3.73 (1H, dd, $J_{1,2}$=4.0 Hz, $J_{2,3}$=9.5 Hz, H-2), 4.26 (1H, ddd, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.5 Hz, $J_{P_\alpha H\text{-}3}$=9.0 Hz, H-3), 3.58 (1H, dd, $J_{3,4}$=9.5 Hz, $J_{4,5}$=8.5 Hz, H-4), 3.68 (1H, ddd, $J_{4,5}$=8.5 Hz, $J_{5,6}$2.0 Hz, $J_{5,6'}$=5.0 Hz, H-5), 3.85 (1H, dd, $J_{5,6}$=2.0 Hz, $J_{6,6'}$=12.5 Hz, H-6), 3.77 (1H, dd, $J_{5,6'}$=5.0 Hz, $J_{6,6'}$=12.5 Hz, H-6′), 3.42 (3H, s, –OCH_3). ^{31}P-NMR (D_2O) δ: 2.1 (1P, dd, $J_{P_\alpha P_\beta}$=7.5 Hz, $J_{P_\alpha H\text{-}3}$=9.0 Hz, P_α), −10.5 (1P, dd, $J_{P_\alpha P_\beta}$=7.5 Hz, $J_{P_\beta P_\gamma}$=20.6 Hz, P_β), −5.3 (1P, d, $J_{P_\beta P_\gamma}$=20.6 Hz, P_γ).

Methyl 4-*O*-Diphosphoramidophosphono-*α*-D-glucoside (**10**): ^{1}H-NMR (D_2O) δ: 4.80 (1H, d, $J_{1,2}$=4.0 Hz, H-1), 3.64 (1H, dd, $J_{1,2}$=4.0 Hz, $J_{2,3}$=10.0 Hz, H-2), 3.80 (1H, dd, $J_{2,3}$=10.0 Hz, $J_{3,4}$=9.0 Hz, H-3), 4.03 (1H, ddd, $J_{3,4}$=9.0 Hz, $J_{4,5}$=9.0 Hz, $J_{P_\alpha H\text{-}4}$=8.5 Hz, H-4), 3.69 (1H, ddd, $J_{4,5}$=9.0 Hz, $J_{5,6}$=4.0 Hz, $J_{5,6'}$=2.0 Hz, H-5), 3.91 (1H, dd, $J_{5,6}$=4.0 Hz, $J_{6,6'}$=13.0 Hz, H-6), 3.79 (1H, dd, $J_{5,6'}$=2.0 Hz, $J_{6,6'}$=13.0 Hz, H-6′), 3.41 (3H, s, –OCH_3). ^{31}P-NMR (D_2O) δ: 1.2 (1P, dd, $J_{P_\alpha P_\beta}$=7.9 Hz, $J_{P_\alpha H\text{-}4}$=8.5 Hz, P_α), −10.5 (1P, dd, $J_{P_\alpha P_\beta}$=7.9 Hz, $J_{P_\beta P_\gamma}$=20.6 Hz, P_β), −5.3 (1P, d, $J_{P_\beta P_\gamma}$=20.6 Hz, P_γ).

Methyl 2-*O*-Diphosphoramidophosphono-*β*-D-glucoside (**11**): ^{1}H-NMR (D_2O) δ: 4.43 (1H, d, $J_{1,2}$=8.0 Hz, H-1), 3.82 (1H, ddd, $J_{1,2}$=8.0 Hz, $J_{2,3}$=9.5 Hz, $J_{P_\alpha H\text{-}2}$=10.0 Hz, H-2), 3.66 (1H, dd, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.5 Hz, H-3), 3.40 (1H, dd, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, H-4), 3.44 (1H, ddd, $J_{4,5}$=9.5 Hz, $J_{5,6}$=2.5 Hz, $J_{5,6'}$=6.0 Hz, H-5), 3.88 (1H, dd, $J_{5,6}$=2.5 Hz, $J_{6,6'}$=12.5 Hz, H-6), 3.68 (1H, dd, $J_{5,6'}$=6.0 Hz, $J_{6,6'}$=12.5 Hz, H-6′), 3.52 (3H, s, –OCH_3). ^{31}P-NMR (D_2O) δ: 0.3 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\alpha H\text{-}2}$=10.0 Hz, P_α), −10.2 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\beta P_\gamma}$=20.8 Hz, P_β), −5.5 (1P, d, $J_{P_\beta P_\gamma}$=20.8 Hz, P_γ).

Methyl 3-*O*-Diphosphoramidophosphono-*β*-D-glucoside (**12**): ^{1}H-NMR (D_2O) δ: 4.44 (1H, d, $J_{1,2}$=8.0 Hz, H-1), 3.39 (1H, dd, $J_{1,2}$=8.0 Hz, $J_{2,3}$=9.0 Hz, H-2), 4.17 (1H, ddd, $J_{2,3}$=9.0 Hz, $J_{3,4}$=9.0 Hz, $J_{P_\alpha H\text{-}3}$=8.5 Hz, H-3), 3.52 (1H, m, H-4), 3.71 (1H, m, H-5), 3.89 (1H, dd, $J_{5,6}$=2.0 Hz, $J_{6,6'}$=12.0 Hz, H-6), 3.52 (1H, m, H-6′), 3.54 (3H, s, –OCH_3). ^{31}P-NMR (D_2O) δ: 2.0 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\alpha H\text{-}3}$=8.5 Hz, P_α), −10.6 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\beta P_\gamma}$=21.2 Hz, P_β), −5.3 (1P, d, $J_{P_\beta P_\gamma}$=21.2 Hz, P_γ).

Methyl 4-*O*-Diphosphoramidophosphono-*β*-D-glucoside (**13**): ^{1}H-NMR (D_2O) δ: 4.38 (1H, d, $J_{1,2}$=8.0 Hz, H-1), 3.30 (1H, dd, $J_{1,2}$=8.0 Hz, $J_{2,3}$=9.5 Hz, H-2), 3.67 (1H, dd, $J_{2,3}$=9.5 Hz, $J_{3,4}$=9.5 Hz, H-3), 3.98 (1H, ddd, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, $J_{P_\alpha H\text{-}4}$=8.5 Hz, H-4), 3.50 (1H, ddd,

$J_{4,5}$=9.5 Hz, $J_{5,6}$=2.5 Hz, $J_{5,6'}$=4.5 Hz, H-5), 3.86 (1H, dd, $J_{5,6}$=2.5 Hz, $J_{6,6'}$=13.0 Hz, H-6), 3.82 (1H, dd, $J_{5,6'}$=4.5 Hz, $J_{6,6'}$=13.0 Hz, H-6′), 3.54 (3H, s, –OCH_3). ^{31}P-NMR (D_2O) δ: 1.1 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\alpha H\text{-}4}$=8.5 Hz, P_α), −10.4 (1P, dd, $J_{P_\alpha P_\beta}$=7.3 Hz, $J_{P_\beta P_\gamma}$=20.6 Hz, P_β), −5.3 (1P, d, $J_{P_\beta P_\gamma}$=20.6 Hz, P_γ).

1-*O*-Diphosphoramidophosphono-β-D-galactose (**17**): ^{1}H-NMR (D_2O) δ: 4.95 (1H, dd, $J_{1,2}$=8.0 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, H-1), 3.57 (1H, dd, $J_{1,2}$=8.0 Hz, $J_{2,3}$=10.0 Hz, H-2), 3.69 (1H, dd, $J_{2,3}$=10.0 Hz, $J_{3,4}$=3.5 Hz, H-3), 3.88 (1H, dd, $J_{3,4}$=3.5 Hz, $J_{4,5}$=1.0 Hz, H-4), 3.75 (1H, ddd, $J_{4,5}$=1.0 Hz, $J_{5,6}$=8.0 Hz, $J_{5,6'}$=2.2 Hz, H-5), 3.79 (1H, dd, $J_{5,6}$=8.0 Hz, $J_{6,6'}$=11.5 Hz, H-6), 3.70 (1H, dd, $J_{5,6'}$=2.2 Hz, $J_{6,6'}$=11.5 Hz, H-6′). ^{31}P-NMR (D_2O) δ: 0.2 (1P, dd, $J_{P_\alpha P_\beta}$=6.8 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, P_α), −10.6 (1P, dd, $J_{P_\alpha P_\beta}$=6.8 Hz, $J_{P_\beta P_\gamma}$=21.4 Hz, P_β), −5.6 (1P, d, $J_{P_\beta P_\gamma}$=21.4 Hz, P_γ).

1-*O*-Diphosphoramidophosphono-α-D-allose (**18**): ^{1}H-NMR (D_2O) δ: 5.60 (1H, dd, $J_{1,2}$=4.0 Hz, $J_{P_\alpha H\text{-}1}$=8.5 Hz, H-1), 3.34 (1H, ddd, $J_{1,2}$=4.0 Hz, $J_{2,3}$=3.5 Hz, $J_{P_\alpha H\text{-}2}$=1.0 Hz, H-2), 3.75 (1H, dd, $J_{2,3}$=3.5 Hz, $J_{3,4}$=3.5 Hz, H-3), 3.64 (1H, dd, $J_{3,4}$=3.5 Hz, $J_{4,5}$=10.0 Hz, H-4), 4.06 (1H, ddd, $J_{4,5}$=10.0 Hz, $J_{5,6}$=2.5 Hz, $J_{5,6'}$=5.5 Hz, H-5), 3.92 (1H, dd, $J_{5,6}$=2.5 Hz, $J_{6,6'}$=12.0 Hz, H-6), 3.75 (1H, dd, $J_{5,6'}$=5.5 Hz, $J_{6,6'}$=12.0 Hz, H-6′). ^{31}P-NMR (D_2O) δ: −0.2 (1P, ddd, $J_{P_\alpha P_\beta}$=6.1 Hz, $J_{P_\alpha H\text{-}1}$=8.5 Hz, $J_{P_\alpha H\text{-}2}$=1.0 Hz, P_α), −10.8 (1P, dd, $J_{P_\alpha P_\beta}$=6.1 Hz, $J_{P_\beta P_\gamma}$=20.8 Hz, P_β), −6.1 (1P, d, $J_{P_\beta P_\gamma}$=20.8 Hz, P_γ).

1-*O*-Diphosphoramidophosphono-β-D-allose (**19**): ^{1}H-NMR (D_2O) δ: 5.28 (1H, dd, $J_{1,2}$=8.5 Hz, $J_{P_\alpha H\text{-}1}$=8.5 Hz, H-1), 3.53 (1H, dd, $J_{1,2}$=8.5 Hz, $J_{2,3}$=3.0 Hz, H-2), 4.18 (1H, dd, $J_{2,3}$=3.0 Hz, $J_{3,4}$=3.0 Hz, H-3), 3.61 (1H, dd, $J_{3,4}$=3.0 Hz, $J_{4,5}$=10.5 Hz, H-4), 3.88 (1H, ddd, $J_{4,5}$=10.5 Hz, $J_{5,6}$=2.5 Hz, $J_{5,6'}$=7.0 Hz, H-5), 3.89 (1H, dd, $J_{5,6}$=2.5 Hz, $J_{6,6'}$=12.5 Hz, H-6), 3.67 (1H, dd, $J_{5,6'}$=7.0 Hz, $J_{6,6'}$=12.5 Hz, H-6′). ^{31}P-NMR (D_2O) δ: 0.2 (1P, dd, $J_{P_\alpha P_\beta}$=6.7 Hz, $J_{P_\alpha H\text{-}1}$=8.5 Hz, P_α), −10.8 (1P, dd, $J_{P_\alpha P_\beta}$=6.7 Hz, $J_{P_\beta P_\gamma}$=20.8 Hz, P_β), −6.1 (1P, d, $J_{P_\beta P_\gamma}$=20.8 Hz, P_γ).

3-*O*-Diphosphoramidophosphono-β-D-allose (**20**): ^{1}H-NMR (D_2O) δ: 4.97 (1H, d, $J_{1,2}$=8.5 Hz, H-1), 3.40 (1H, ddd, $J_{1,2}$=8.5 Hz, $J_{2,3}$=2.5 Hz, $J_{P_\alpha H\text{-}2}$=1.0 Hz, H-2), 4.83 (1H, ddd, $J_{2,3}$=2.5 Hz, $J_{3,4}$=2.5 Hz, $J_{P_\alpha H\text{-}3}$=8.5 Hz, H-3), 3.64 (1H, ddd, $J_{3,4}$=2.5 Hz, $J_{4,5}$=10.0 Hz, $J_{P_\alpha H\text{-}4}$=1.0 Hz, H-4), 3.87 (1H, m, H-5), 3.87 (1H, m, H-6), 3.70 (1H, dd, $J_{5,6'}$=6.5 Hz, $J_{6,6'}$=12.5 Hz, H-6′). ^{31}P-NMR (D_2O) δ: 2.8 (1P, dddd, $J_{P_\alpha P_\beta}$=6.1 Hz, $J_{P_\alpha H\text{-}2}$=1.0 Hz, $J_{P_\alpha H\text{-}3}$=8.5 Hz, $J_{P_\alpha H\text{-}4}$=1.0 Hz, P_α), −10.8 (1P, dd, $J_{P_\alpha P_\beta}$=6.1 Hz, $J_{P_\beta P_\gamma}$=20.8 Hz, P_β), −6.2 (1P, d, $J_{P_\beta P_\gamma}$=20.8 Hz, P_γ).

1-*O*-Diphosphoramidophosphono-β-D-mannose (**21**): ^{1}H-NMR (D_2O) δ: 5.18 (1H, dd, $J_{1,2}$=1.0 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, H-1), 4.10 (1H, dd, $J_{1,2}$=1.0 Hz, $J_{2,3}$=3.5 Hz, H-2), 3.67 (1H, dd, $J_{2,3}$=3.5 Hz, $J_{3,4}$=9.5 Hz, H-3), 3.48 (1H, dd, $J_{3,4}$=9.5 Hz, $J_{4,5}$=9.5 Hz, H-4), 3.41 (1H, ddd, $J_{4,5}$=9.5 Hz, $J_{5,6}$=2.0 Hz, $J_{5,6'}$=7.0 Hz, H-5), 3.86 (1H, dd, $J_{5,6}$=2.0 Hz, $J_{6,6'}$=12.5 Hz, H-6), 3.65 (1H, dd, $J_{5,6'}$=7.0 Hz, $J_{6,6'}$=12.5 Hz, H-6′). ^{13}C-NMR (D_2O) δ: 97.8 (1C, d, $J_{P_\alpha C\text{-}1}$=4.3 Hz, C-1), 73.3 (1C, d, $J_{P_\alpha C\text{-}2}$=5.7 Hz, C-2), 75.0 (1C, s, C-3), 69.3 (1C, s, C-4), 79.3 (1C, s, C-5), 63.7 (1C, s, C-6). ^{31}P-NMR (D_2O) δ: −0.7 (1P, dd, $J_{P_\alpha P_\beta}$=6.9 Hz, $J_{P_\alpha H\text{-}1}$=9.0 Hz, P_α), −11.2 (1P, dd, $J_{P_\alpha P_\beta}$=6.9 Hz, $J_{P_\beta P_\gamma}$=20.8 Hz, P_β), −5.5 (1P, d, $J_{P_\beta P_\gamma}$=20.8 Hz, P_γ).

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