Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 340 (2005) 1766–1772
Carbohydrate RESEARCH

Regioselective phosphorylation of branched cyclodextrins with *cyclo*-mono-μ-imidotriphosphate

Hideko Inoue, Toyohiro Kawashita, Hirokazu Nakayama and Mitsutomo Tsuhako*

Department of Functional Molecular Chemistry, Kobe Pharmaceutical University, Kobe 658-8558, Japan

Received 26 May 2005; accepted 3 June 2005
Available online 1 July 2005

Abstract—The phosphorylation of the branched cyclodextrins, mono-6-*O*-(α-D-glucopyranosyl)cyclomaltohexaose, mono-6-*O*-(α-D-maltosyl)cyclomaltohexaose, mono-6-*O*-(α-D-glucopyranosyl)cyclomaltoheptaose, and mono-6-*O*-(α-D-maltosyl)cyclomaltoheptaose, in aqueous solution by sodium *cyclo*-mono-μ-imidotriphosphate (cMITP) was examined. In these reactions, only the 2-OH group of a single α-D-glucopyranosyl residue of the cyclodextrin ring was phosphorylated, in a maximum yield of 67%. A possible mechanism for the phosphorylation is discussed.

Keywords: Phosphorylation; Branched cyclodextrin; *cyclo*-Mono-μ-imidotriphosphate

1. Introduction

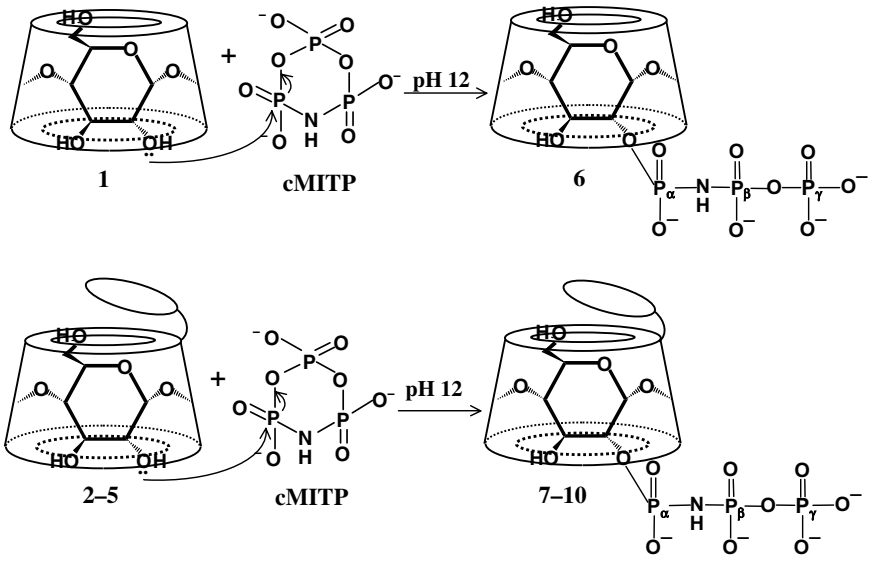
Sodium *cyclo*-triphosphate, $Na_3P_3O_9$ (P_{3m}), is a simple and efficient phosphorylating agent. Previously, one of us reported that amino acids,[1,2] nucleosides,[3] nucleotides,[4] and carbohydrates[5] are readily phosphorylated by P_{3m} in aqueous solution under mild conditions. We have extended these investigations to the phosphorylation of other bioorganic compounds.[6–10] The phosphoryl- ated products of oligo- and polysaccharides are expected to be useful as chiral selectors,[11] masking agents for metal ions,[12] and materials for drug delivery system (DDS).[13] Recently, we demonstrated that cyclomaltohexaose (α-CD), cyclomaltooctaose (γ-CD), and branched cyclodextrins could be readily phosphorylated by P_{3m} in aqueous solution.[14,15] β-Cyclodextrin (β-CD) forms inclusion complexes with a variety of different guest compounds[16] and phosphorylated analogs are expected to have applications in many fields. However, β-CD could not be phosphorylated by P_{3m}, because of its low solubility in water. Substitution of 6-OH group of β-CD by glucosyl or maltosyl residue improves its solubility considerably. Therefore, the phosphorylation of branched β-cyclodextrins by P_{3m} was possible. Unfortunately, the yields were low (ca. 30%) and the products were easily decomposed to monophosphate derivatives.[14,15]

We have therefore explored the use of *cyclo*-mono-μ-imidotriphosphate (cMITP) for phosphorylation of β-CD and related derivatives. The structure of cMITP is shown in Scheme 1. The six-membered ring is composed of both P–NH–P and P–O–P linkages in contrast with *cyclo*-triphosphate (P_{3m}), which contains only P–O–P linkages. Compared with the P–O–P linkage, the P–NH–P linkage is stable and difficult to hydrolyze.[17] Therefore, the product formed by cMITP is expected to be more stable than that formed by P_{3m}.

In the present study, we first studied the reaction of cyclomaltohexaose (α-CD; **1**) with cMITP in aqueous solution. We then investigated phosphorylations of mono-6-*O*-(α-D-glucopyranosyl)cyclomaltohexaose (mono-G_1-α-CD; **2**), mono-6-*O*-(α-D-maltosyl)cyclomaltohexaose (mono-G_2-α-CD; **3**), mono-6-*O*-(α-D-glucopyranosyl)cyclomaltoheptaose (mono-G_1-β-CD; **4**), and mono-6-*O*-(α-D-maltosyl)cyclomaltoheptaose (mono-G_2-β-CD; **5**) by cMITP for use in the preparation of new DDS hosts.

* Corresponding author. Tel.: +81 784417552; fax: +81 78441753; e-mail: i-hideko@kobepharma-u.ac.jp

0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.06.001



Scheme 1.

2. Results and discussion

The yields of the product **6** in the reaction of **1** and cMITP were examined under various reaction conditions. HPLC analyses served as a tool for evaluating the yields of products. At a molar ratio of 1:1 (**1**/cMITP = 0.15 M:0.15 M) and 23 °C, the yield of the product **6** decreased gradually with a decrease in pH, for example, 42% at pH 12 and 12% at pH 10. No product was obtained at pH 7. To study the effect of temperature, the reaction was carried out with a 1:1 molar ratio of **1** and cMITP at pH 12; the yields of **6** were 45% at 10 °C, 42% at 23 °C, and 40% at 40 °C. The yield of the product increased with the increase of the initial concentration of **1**. At pH 12 and 40 °C, the yields of **6** were 40% at a molar ratio of 1:1 and 63% at 2:1 (**1**/cMITP = 0.3 M:0.15 M). Thus, the maximum yield of the product was not attained under the condition of excess cMITP (**1**/cMITP = 0.15 M:0.3 M). This is in contrast with the reaction of α-CD and P_{3m} where the maximum yield was attained under the condition of excess P_{3m}.[15] The recommended conditions for the phosphorylation of **1** with cMITP is a molar ratio of **1**/cMITP = 2:1, pH 12, and 40 °C.

To determine the structure of **6**, ^{31}P NMR spectra were measured. As can be seen in Figure 1, the characteristic peaks (0.8, −6.1, and −10.8 ppm) for the presumed monoimidotriphosphate derivative of **1** were observed. Table 1 shows ^{31}P NMR chemical shifts and coupling constants of phosphorylated α-CD, monoimidotriphosphate, and triphosphate. A previous work indicated that the phosphorylation products of aminoalcohols[18] and D-glucosamine[8] with P_{3m} are *N*-(ω-hydroxyalkyl)triphosphoramidates and β-D-glucosamine

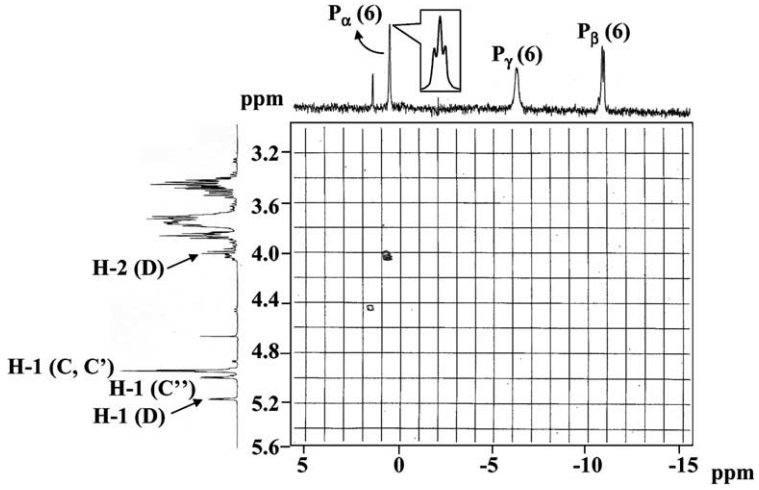


Figure 1. ^{31}P–^{1}H 2D HMBC spectrum of **6**. α-CD (**1**)/cMITP = 0.15 M:0.15 M, pH 12, and 23 °C, after 13 days.

Table 1. ^{31}P NMR chemical shifts and coupling constants of monoimidotriphosphate ester of **1** (**6**), triphosphate ester of **1**, monoimidotriphosphate, and triphosphate

	δ (ppm)			J (Hz)		
	P_α	P_β	P_γ	P_α, P_β	P_β, P_γ	$P_{\alpha,H-2}$
Monoimidotriphosphate ester of **1** (**6**)	0.8	−10.8	−6.1	6.0	20.0	6.0
Triphosphate ester of **1**[15]	−10.8	−20.9	−5.9	18.2	20.2	8.8
Monoimidotriphosphate[18]	−0.4	−10.0	−6.1	6.1	20.8	
Triphosphate[8]	−4.0	−18.2	−4.0	18.8	19.6	

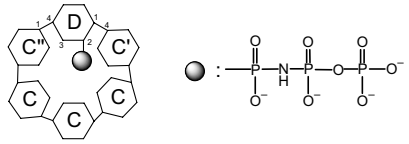
2-triphosphate, which contain a –NH–P_α–O– bond. On the other hand, the phosphorylation product of methylamine with cMITP is diphosphoramidophosphonomethylamine with a –NH–P_α–NH– bond.[19] These products show a characteristic P_α signal at around 0 ppm in their ^{31}P NMR spectrum. Compared with the triphosphate ester having an –O–P_α–O– bond, a downfield shift in P_α of these products was observed. The ^{1}H coupled–^{31}P NMR spectrum (Fig. 1) showed a doublet of doublets at 0.8 ppm, which became a doublet in the ^{1}H-decoupled spectrum, indicating the characteristic peak of P_α similar to those of triphosphate derivatives.[8,18] The other doublet at −6.1 ppm and the doublet of doublets at −10.8 ppm did not change with or without ^{1}H-decoupling. Because of the same chemical shifts and coupling constants of monoimidotriphosphate, the doublet at −6.1 ppm and the doublet of doublets at −10.8 ppm were assigned to P_γ and P_β of **6**, respectively. Compared with triphosphate ester of α-CD, the chemical shifts of P_α and P_β of **6** indicated significant downfield shifts whereas there was no shift for P_γ. Also, the value of J_{P_α,P_β} of **6** was one-third of J_{P_α,P_β} of the triphosphate ester of α-CD whereas the value of J_{P_β,P_γ} of **6** was the same as that of the triphosphate ester of α-CD. These results suggest the existence of an –O–P_α–NH–P_β– bond. Therefore, the product **6** was verified to be diphosphoramidophosphono-α-CD with an NH group between P_α and P_β.

To confirm the site of phosphorylation, ^{1}H NMR and ^{31}P–^{1}H heteronuclear multiple bond correlation (HMBC) 2D NMR spectra (Fig. 1) were measured. A correlation between P_α at 0.8 ppm and the ^{1}H signal at 4.05 ppm was observed. The ^{1}H–^{1}H COSY spectrum showed a correlation between H-2 (4.05 ppm) and H-1 (5.14 ppm), indicating that the doublet of doublets of doublets at 4.05 ppm was the signal arising from H-2. As shown in the H-1 region of the ^{1}H NMR spectrum (Fig. 1), there are three types of glucopyranosyl residues in **6**. From the comparison of peak areas of the three H-1 signals, only one of the six glucose units of α-CD was verified to be phosphorylated. The phosphorylated glucopyranosyl residue was named as residue D, and the other glucopyranosyl residues were named as shown in Table 2. Similar to the triphosphate ester of α-CD, downfield shifts of the H-1 and H-2 signals of phosphorylated glucopyranosyl residue were observed.[15]

The reaction of **1** with cMITP gave only one monoimidotriphosphate and did not give bis- or different imidotriphosphate derivatives of **1**. Because of electric repulsion between **6** and cMITP, it would be difficult for **6** to react further with cMITP. The ESI mass spectrum of **6** showed a molecular ion peak (*m*/*z* 604.8) for the dipotassium salt ($[C_{36}H_{62}NO_{38}P_3]^{2-}$). There were no peaks for bis- or other imidotriphosphate esters of α-CD. Therefore, the 2-OH of a single glucopyranosyl residue in α-CD was selectively phosphorylated.

^{1}H NMR chemical shifts and coupling constants determined by ^{1}H, COSY, TOCSY, and ROESY NMR spectroscopy are shown in Table 2. The doublet at 4.98 ppm was assigned to H-1 of residue C″. The

Table 2. ^{1}H NMR chemical shifts and coupling constants of **6**



Compound	Residue	δ (ppm)				J (Hz)			
		H-1	H-2	H-3	H-4	P_α, H-2	H-1, H-2	H-2, H-3	H-3, H-4
6	C	4.94	3.48	3.84	3.54		3.8	9.6	9.1
	C′	4.94	3.48	3.84	3.54		3.8	9.6	9.3
	C″	4.98	3.42	3.86	3.42		3.5	9.5	9.3
	D	5.14	4.05	3.98	3.55	6.0	3.5	9.5	9.4
α-CD[15]	C	5.05	3.62	3.99	3.57		3.5	10.0	8.7

doublet at 4.94 ppm was assigned to H-1 of residues C and C′.[20] The ROESY spectrum showed that residues C′ and C″ are linked to 1-OH and 4-OH of the residue D, respectively. The $^{3}J_{P_\alpha}$,H-2 value was 6.0 Hz, which is consistent with that obtained from the ^{31}P NMR data (Table 1). Therefore, the phosphorylated product in the reaction of **1** with cMITP was confirmed to be 2-*O*-diphosphoramidophosphonocyclomaltohexaose (**6**) as shown in Scheme 1.

β-Cyclodextrin is expected to find applications in many fields because of its ability to form inclusion compounds with many other compounds. However, β-CD could not be phosphorylated by cMITP because of its low solubility in water. Branched cyclodextrin derivatives show higher solubility in water due to the presence of the additional carbohydrate residue bonded to the cyclodextrin ring, usually through a (1→6)-glycosidic linkage. Therefore, phosphorylation of mono-G_1-α-CD (**2**), mono-G_2-α-CD (**3**), mono-G_1-β-CD (**4**), or mono-G_2-β-CD (**5**) with cMITP was carried out under various conditions.

Each branched CD, **2**–**5**, showed a single product, **7**–**10**. Table 3 shows the yields of the monoimidotriphosphate esters **7**–**10**. The maximum yields of **7**–**10** were 82%, 87%, 67%, and 75%, respectively. Compared with **1**, the higher yields were due to the higher solubility of the branched CDs in water. The pH of reaction solutions was fixed at 12 as in the case of **1**. At 40 °C, the yields of **7**–**10** were the same at the molar ratio of **2**–**5**/cMITP = 3:1 and 5:1, but the reaction rate of **2**–**5** decreased on increasing the molar ratio of **2**–**5**. At a molar ratio of 3:1, the yields of **7**–**10** were almost the same between 10 and 40 °C, and decreased at 60 °C. Therefore, the recommended condition for the phosphorylation of **2**–**5** with cMITP is a molar ratio of **2**–**5**/cMITP = 3:1 and 40 °C.

Table 3. Yields of the products **7**–**10** in the reactions of **2**–**5** with cMITP

Concentration (M)	Product	*T* (°C)	Time (day)	Yield (%)
Mono-G_1-α-CD (**2**)/cMITP	**7**			
0.45:0.15		10	30	82
0.45:0.15		23	22	82
0.45:0.15		40	5	78
0.45:0.15		60	1	57
0.45:0.30		40	7	64
0.45:0.09		40	7	80
Mono-G_2-α-CD (**3**)/cMITP	**8**			
0.45:0.15		10	28	80
0.45:0.15		23	16	87
0.45:0.15		40	3	79
0.45:0.15		60	2	56
0.45:0.30		40	7	73
0.45:0.09		40	9	80
Mono-G_1-β-CD (**4**)/cMITP	**9**			
0.45:0.15		10	52	67
0.45:0.15		23	11	66
0.45:0.15		40	4	61
0.45:0.15		60	2	59
0.45:0.30		40	6	49
0.45:0.09		40	10	65
Mono-G_2-β-CD (**5**)/cMITP	**10**			
0.45:0.15		10	43	71
0.45:0.15		23	22	75
0.45:0.15		40	8	73
0.45:0.15		60	2	58
0.45:0.30		40	5	52
0.45:0.09		40	5	67

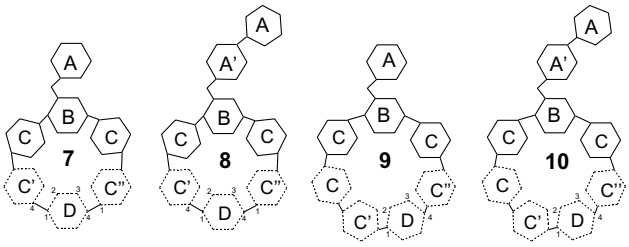
Table 4. ^{31}P NMR chemical shifts and coupling constants of the products **7**–**10**

Product	δ (ppm)			*J* (Hz)		
	P_α	P_β	P_γ	P_α, P_β	P_β, P_γ	P_α, H-2
7	0.6	−10.9	−6.9	6.7	20.4	6.7
8	0.5	−10.9	−7.2	7.0	21.2	7.3
9	0.2	−11.3	−9.9	6.5	21.2	6.8
10	0.2	−11.3	−9.8	6.5	20.3	7.5

The identification of reaction product **10** was performed as was described for **6**. The ^{31}P NMR spectrum (Table 4) showed three peaks at 0.2, −11.3, and −9.8 ppm. In the ^{31}P–^{1}H HMBC 2D NMR spectrum of **10**, a correlation between P_α at 0.2 ppm and the ^{1}H signal at 4.05 ppm was observed. The ^{1}H–^{1}H COSY spectrum showed a correlation between the doublet of doublets of doublets at 4.05 ppm and the H-1 signal at 5.15 ppm. Therefore, these results indicate that the 2-OH of the glucopyranosyl residue of cyclodextrin **5** was phosphorylated.

To determine the number of phosphorylated 2-OH groups, the H-1 signals of **10** were investigated. A comparison of the peak areas of the five H-1 signals, showed that only one of the nine glucopyranosyl residues of **5** was phosphorylated by cMITP. The COSY, ROESY, and TOCSY NMR spectra were measured, and the data are summarized in Table 5. Figure 2 shows four possible structures of **10** as determined by NMR spectroscopy. Similar to the triphosphate ester of mono-G_2-β-CD,[15] the phosphorylation did not occur in the branched glucopyranosyl residue but instead occurred at one of the residues of the β-CD ring. The branched glucopyranosyl residue is located above the entrance of the CD cavity.[21] Thus, for steric reasons, it would be difficult for cMITP to react at the 2-OH of the branched glucopyranosyl residue. In addition, residue B and its neighbors were not phosphorylated as shown in Figure 2. Unfortunately, it was impossible to confirm the phosphorylated glucopyranosyl residue D. The ESI mass spectra of **10** showed its molecular ion peak for the dipotassium salt; *m/z* 848.0, calculated 848.6 for $[C_{54}H_{93}NO_{54}P_3]^{2-}$. There was no peaks of bis- or other imidotriphosphate esters of **5**. Therefore, the 2-OH in the one of glucopyranosyl residues of the β-CD ring, was regioselectively phosphorylated. Product **10** was

Table 5. ^{1}H NMR chemical shifts and coupling constants of **7**–**10**



Compound	Residue	δ (ppm)				J (Hz)			
		H-1	H-2	H-3	H-4	P_α, H-2	H-1	H-2, H-3	H-3, H-4
7	A	4.81	3.38	3.61	3.30		3.8	9.5	9.5
	B	4.91	3.53	3.79	3.37		3.0	9.6	9.9
	C	4.91	3.50	3.86	3.46		3.1	9.7	9.4
	C′	4.91	3.50	3.86	3.51		3.1	9.7	10.2
	C″	4.96	3.40	3.84	3.42		3.1	9.9	9.5
	D	5.12	4.05	3.97	3.56	6.7	3.0	9.9	9.9
8	A	5.24	3.44	3.57	3.28		3.3	9.4	9.2
	A′	4.80	3.44	3.87	3.42		3.3	10.0	9.6
	B	4.93	3.57	3.84	3.56		3.1	9.5	9.5
	C	4.92	3.47	3.84	3.46		3.3	9.6	9.6
	C′	4.92	3.47	3.84	3.51		3.3	9.7	9.2
	C″	4.97	3.44	3.87	3.42		3.0	9.8	10.0
	D	5.12	4.02	3.96	3.56	7.3	3.1	10.2	10.2
9	A	4.82	3.41	3.61	3.30		3.5	9.4	9.1
	B	4.94	3.55	3.75	3.49		3.4	10.0	9.5
	C	4.94	3.50	3.84	3.49		3.3	9.6	9.7
	C′	4.94	3.50	3.84	3.55		3.4	9.6	9.4
	C″	4.99	3.43	3.84	3.47		3.1	9.7	9.1
	D	5.16	4.07	3.98	3.57	6.8	3.8	9.7	9.5
10	A	5.24	3.44	3.56	3.28		4.0	9.8	9.8
	A′	4.80	3.46	3.84	3.43		3.6	9.9	9.8
	B	4.94	3.54	3.74	3.47		4.0	9.5	9.0
	C	4.94	3.52	3.84	3.49		3.8	9.5	9.6
	C′	4.94	3.50	3.84	3.54		3.9	9.6	9.4
	C″	4.98	3.44	3.86	3.42		3.6	9.7	9.4
	D	5.15	4.05	3.99	3.55	7.5	4.0	9.1	9.4

: possible phosphorylated residue.

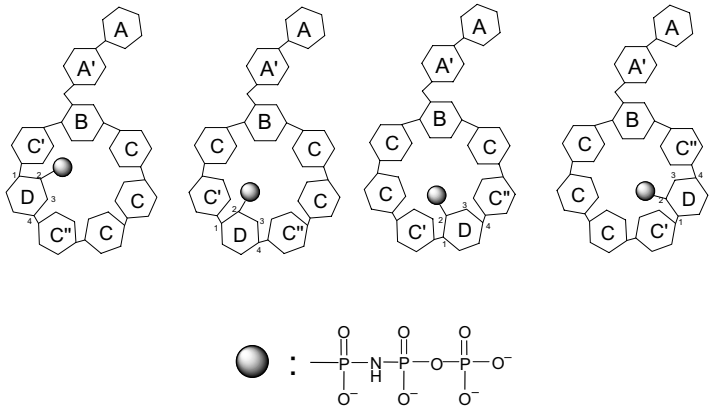


Figure 2. Possible structures of **10**.

verified to be 6-*O*-(α-D-maltosyl)-2^{I}-*O*-diphosphoramidophosphonocyclomaltoheptaose (Scheme 1).

Products **7**, **8**, and **9** were identified similarly. Tables 4 and 5 list the data of ^{31}P and ^{1}H NMR of **7**–**9**. The phosphorylation products of **2**, **3**, and **4** were verified to be 6-*O*-(α-D-glucopyranosyl)-2^{I}-*O*-diphosphoramidophosphonocyclomaltohexaose (**7**), 6-*O*-(α-D-maltosyl)-2^{I}-*O*-diphosphoramidophosphonocyclomaltohexaose (**8**), and 6-*O*-(α-D-glucosyl)-2^{I}-*O*-diphosphoramidophosphonocyclomaltoheptaose (**9**). Therefore, **2**–**5** are regioselectively phosphorylated with cMITP in aqueous solution to form **7**–**10**, respectively (Scheme 1).

Thus, similar to the phosphorylation with P_{3m},[15] the reaction of **1**–**5** with cMITP, leads to phosphorylation of the 2-OH group of a single glucopyranosyl group on the cyclodextrin ring. The reactivity of the hydroxyl groups at C-2, C-3, and C-6 in cyclodextrins strongly depends on their acidity and the 2-OH has the highest acidity (pK_a 12.2) in a α-D-glucopyranosyl ring.[22,23] The 2-OH on the branched glucopyranosyl or maltosyl groups was not phosphorylated, probably due to the steric hindrance.[21]

The phosphorylation mechanism of **1**–**5** with cMITP likely involves nucleophilic attack of the 2-OH group of the α-D-glucopyranosyl group onto the phosphorus atom of an –O–P–NH– moiety in cMITP to open the six-membered ring. Although cMITP has two –O–P–NH– linkages and one –O–P–O– linkage, only one –O–P–NH– linkage reacts selectively.

In conclusion, the phosphorylation of branched cyclodextrins was found to be similar to that of α-CD. The phosphorylation of branched cyclodextrins proceeded regioselectively at the 2-OH group of a glucopyranosyl group of the cyclodextrin ring. These results open a new path for the phosphorylation of CDs and polysaccharides in a one-step process in aqueous solution.

3. Experimental

3.1. General

cyclo-Mono-μ-imidotriphosphate, $Na_3P_3O_8NH$ (cMITP), was prepared according to the previous paper.[24] Branched CDs (**2**–**5**) were purchased from Bio Research of Yokohama (Yokohama, Japan). Sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was purchased from Sigma Chemical Co. (St. Louis, USA). Other reagents were purchased from Wako Chemicals (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 broadband decoupling and ^{31}P–^{1}H HMBC, ^{1}H–^{1}H COSY, TOCSY, and ROESY NMR spectra were obtained on a Varian Unity INOVA 500 spectrometer. H_3PO_4 (85%) 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 post-column 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 and the column temperature was maintained at 40 °C. A convex gradient elution technique using 0.2 and 0.45 M aqueous potassium chloride was employed for the analysis of phosphate. Sugar phosphate esters, diphosphate (P_2), triphosphate (P_3), and *cyclo*-mono-μ-imidotriphosphate (cMITP) were hydrolyzed to monophosphate (P_1) by 6 M 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.

MS measurement was performed by using an API3000 (Applied Biosystems) electrospray ionization (ESI) for ion production. The mass spectrometer was operated in a negative ion mode.

3.2. 2^{I}-*O*-Diphosphoramidophosphonocyclomaltohexaose

Cyclomaltohexaose (**1**) (0.73 g) and cMITP (0.2422 g) were dissolved in H_2O (10 mL) and the solution was adjusted to pH 12 and 23 °C. After 13 days, the solution was adjusted to pH 7 by the addition of 1 M HCl. The yield of the phosphorylated product **6** was 32% by HPLC. The separation of **6** from the reaction solution was accomplished by anion-exchange chromatography with a 2 × 80 cm column filled with Dowex 1-X2 resin (100–200 mesh, Cl-form). Elution was carried out with distilled water until no further cyclodextrin appeared. Elution was then carried out with aqueous 0.3 M KCl and each 50 mL fraction was analyzed by HPLC. The fractionated solution containing only **6** was concentrated at −113 °C in vacuo (freeze-drying). For the purpose of desalting, an aqueous solution of the concentrate was passed in the PD-10 column (Amersham Biosciences, NJ, USA). Each 0.5 mL fraction was monitored by HPLC, and the fractionated solution containing only **6** was freeze-dried. The final yield and purity of **6** was 0.1 mmol and 96%, respectively. The ESI mass spectrum of **6** showed a peak at a *m*/*z* corresponding to a molecular ion of 2^{I}-*O*-diphosphoramidophosphonocyclomaltohexaose (*m*/*z* 604.8, calculated 604.9 for the dipotassium salt, $[C_{36}H_{62}NO_{38}P_3]^{2-}$).

3.3. Syntheses of 7–10

The reactions of branched cyclodextrins (**2**–**5**) (0.45 M, 5 mL) with cMITP (0.15 M, 5 mL) were carried out at

pH 12 and 23 °C. The isolation procedure of products **7**–**10** was the same as described for **6**. The yield and purity of **7**–**10** were **7** (0.08 mmol, 100%), **8** (0.09 mmol, 100%), **9** (0.07 mmol, 94%), and **10** (0.09 mmol, 94%), respectively. The ESI mass spectra of **7**–**10** showed their molecular ions for the dipotassium salt; **7**: *m*/*z* 685.8, calculated 686.5 for $[C_{42}H_{73}NO_{43}P_3]^{2-}$, **8**: *m*/*z* 767.0, calculated 767.5 for $[C_{48}H_{83}NO_{48}P_3]^{2-}$, **9**: *m*/*z* 767.0, calculated 767.5 for $[C_{48}H_{83}NO_{48}P_3]^{2-}$, **10**: *m*/*z* 848.0, calculated 848.6 for $[C_{54}H_{93}NO_{54}P_3]^{2-}$.

Acknowledgments

The authors thank Assistant Professor M. Sugiura of Kobe Pharmaceutical University for the measurement of ^{31}P, ^{1}H, ^{31}P–^{1}H HMBC, ^{1}H–^{1}H COSY, TOCSY, and ROESY NMR spectra. This work was supported by the Science Research Promotion Fund from the Japan Private School Promotion Foundation.

References

1. Tsuhako, M.; Fujita, N.; Nakahama, A.; Matsuo, T.; Kobayashi, M.; Ohashi, S. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1968–1972.
2. Inoue, H.; Baba, Y.; Furukawa, T.; Maeda, Y.; Tsuhako, M. *Chem. Pharm. Bull.* **1993**, *41*, 1895–1899.
3. Tsuhako, M.; Fujimoto, M.; Ohashi, S.; Nariai, H.; Motooka, I. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 3274–3280.
4. Tsuhako, M.; Kunitomi, R.; Baba, Y.; Miyajima, T. *Bull. Chem. Soc. Jpn.* **1991**, *64*, 490–497.
5. Tsuhako, M.; Sueyoshi, C.; Baba, Y.; Miyajima, T.; Ohashi, S.; Nariai, H.; Motooka, I. *Chem. Lett.* **1987**, 1431–1434.
6. Inoue, H.; Watanabe, M.; Nakayama, H.; Tsuhako, M. *Chem. Pharm. Bull.* **1998**, *46*, 681–683.
7. Inoue, H.; Nakayama, H.; Tsuhako, M. *Carbohydr. Res.* **2000**, *324*, 10–16.
8. Inoue, H.; Watanabe, M.; Nakayama, H.; Tsuhako, M. *Chem. Pharm. Bull.* **2000**, *48*, 802–807.
9. Inoue, H.; Tone, N.; Nakayama, H.; Tsuhako, M. *Chem. Pharm. Bull.* **2002**, *50*, 1453–1459.
10. Inoue, H.; Nakayama, H.; Tsuhako, M. *Phosphorus Res. Bull.* **2003**, *16*, 11–18.
11. Tanaka, Y.; Terabe, S. *J. Chromatogr. A* **1997**, *781*, 151–160.
12. Inoue, H.; Baba, Y.; Tsuhako, M. *Chem. Pharm. Bull.* **1995**, *43*, 677–678.
13. Cho, A.; Ochoa Lara, K. L.; Yatsimirsky, A. K.; Eliseev, A. V. *Org. Lett.* **2000**, *2*, 1741–1743.
14. Inoue, H.; Baba, Y.; Tsuhako, M. *Phosphorus Res. Bull.* **1993**, *3*, 61–66.
15. Inoue, H.; Tone, N.; Nakayama, H.; Tsuhako, M. *Carbohydr. Res.* **2003**, *338*, 2067–2074.
16. Hedges, A. R. *Chem. Rev.* **1998**, *98*, 2035–2044.
17. Polland, F. H.; Nickless, G.; Warrender, R. W. *J. Chromatogr.* **1962**, *9*, 493–505.
18. Inoue, H.; Nakayama, H.; Tsuhako, M. *Phosphorus Res. Bull.* **2001**, *12*, 65–72.
19. Inoue, H.; Nakayama, H.; Tsuhako, M. *Phosphorus Res. Bull.* **2004**, *17*, 170–173.
20. Yamamoto, Y.; Inoue, Y.; Chûjô, R. *Carbohydr. Res.* **1987**, *166*, 156–161.
21. Inoue, Y.; Kanda, Y.; Yamamoto, Y. *Carbohydr. Res.* **1992**, *226*, 197–208.
22. Gelb, R. I.; Schwartz, L. M.; Bradshaw, J. J.; Laufer, D. A. *Bioorg. Chem.* **1980**, *9*, 299–304.
23. Gelb, R. I.; Schwartz, L. M.; Laufer, D. A. *Bioorg. Chem.* **1982**, *11*, 274–280.
24. Sakurai, M.; Watanabe, M. *J. Mater. Sci.* **1994**, *29*, 4897–4900.