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Polymeric enzyme mimics: catalytic activity of ribose-containing polymers for a phosphate substrate

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The polymers containing ribose rings: poly(5′-acrylamido-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose) (**11**), poly(5′-acrylamido-5′-deoxy-α-D-ribose) (**12**) and poly(5′-acrylamido-5′-deoxy-1′-*O*-methyl-D-ribose) (**13**) were prepared as enzyme mimics. Polymers **12** and **13** with free *vic*-*cis*-diol groups catalyzed the hydrolysis of phosphodiester (ethyl *p*-nitrophenyl phosphate and *N*-methylpyridinium 4-*tert*-butylcatechol cyclic phosphate) and phosphomonoester substrates with a rate acceleration of 10 ~ 10^3 compared with the uncatalyzed reaction. They also catalyzed the reverse reactions, *i.e.*, the esterification of phosphomonoester to phosphodiester and the phosphorylation of alcohols with phosphate ions. The catalytic activity was attributable to the *vic*-*cis*-diols of riboses on polymer chains, which formed hydrogen bonds with two phosphoryl oxygen atoms of phosphates so as to activate the phosphorus atoms to be attacked by nucleophiles. The catalytic activity was negligible for polymer **11** where *vic*-*cis*-diol groups were blocked with isopropylidene groups. The catalytic activity was attributable to the *vic*-*cis*-diols of riboses on polymer chains, which formed hydrogen bonds with two phosphoryl oxygen atoms of phosphates so as to activate the phosphorus atoms to be attacked by nucleophiles.

Introduction

There has been great interest in the synthesis of enzyme-like polymers.[1–9] The discovery of catalytic nucleic acids such as ribozyme and deoxyribozyme[10,11] especially, has drawn much attention to the investigation of synthetic models that catalyze phosphate ester hydrolysis.

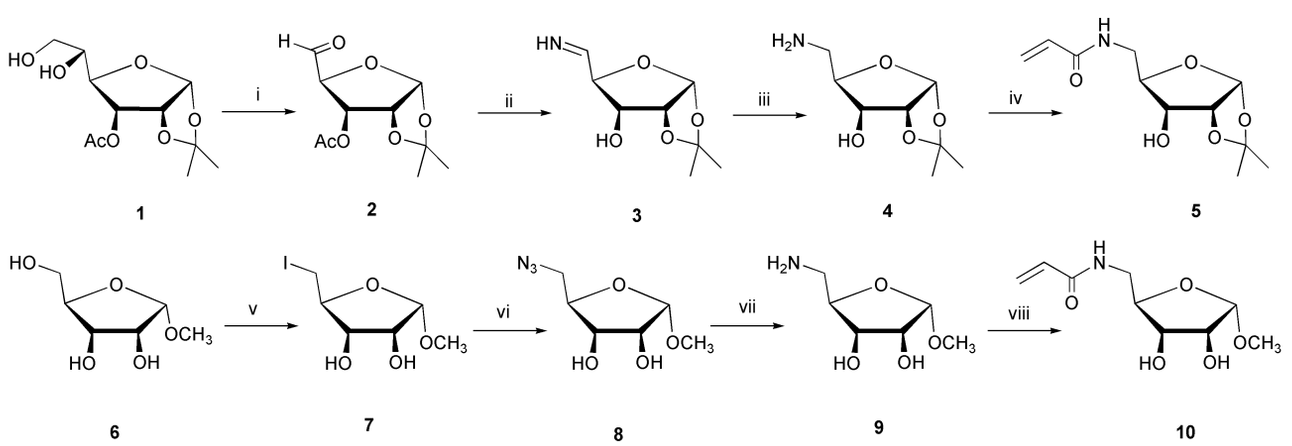
Recently, we reported that the ribose ring capping on β-cyclodextrin, an enzyme model, showed catalytic activity for the hydrolysis of phosphodiester (nuclease) and phosphomonoester (phosphatase), the esterification of phosphomonoester to diester (ligase) and the phosphorylation of alcohols with phosphate ions (phosphorylase).[12] The catalytic activity is attributable to the *vic*-*cis*-diols of riboses, which form hydrogen bonds with two phosphoryl oxygen atoms of the phosphate so as to activate the phosphorus atom for attack by nucleophiles (H_2O or alcohol).[13] These results prompted us to prepare a polymer containing riboses with free *vic*-*cis*-diols, poly(5′-acrylamido-5′-deoxy-α-D-ribose), as an enzyme mimic. Very interestingly, the polymer showed nearly the same catalytic activities (nuclease, ligase, phosphatase, and phosphorylase activities) for the phosphate reactions as the cyclodextrinyl enzyme model. The polymer is believed to form a secondary structure comparable to the cavity of β-cyclodextrin where a substrate is captured inside. The polymer also catalyzed the cleavage of ds DNA and RNA, which was reported in our preceding short communication.[14]

Herein, we report a full account of our studies on the synthesis of ribose-containing polymers, their catalytic activities for phosphate substrates, and the probable reaction mechanism.

Results and discussion

Synthesis of monomers and polymers

Monomer **5**, 5′-acrylamido-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose, was prepared according to Scheme 1. 3′-*O*-Acetyl-1′,2′-*O*-isopropylidenene-5′,6′-diol-α-D-allofuranose (**1**) was



Scheme 1 *Synthesis of monomers:* (i) $NaIO_4$, silica gel, 25 °C, 40 min; (ii) NH_3 in MeOH, 25 °C, 24 h; (iii) Pd/C, 4 atm H_2, 25 °C, 48 h; (iv) acryloyl chloride, 25 °C, 2 h; (v) PPh_3, I_2, Py., dioxane, 25 °C, 24 h; (vi) NaN_3, 110 °C, 24 h; (vii) PPh_3, THF–H_2O, 25 °C, 24 h; (viii) acrylic anhydride, 0 °C, 11 h.

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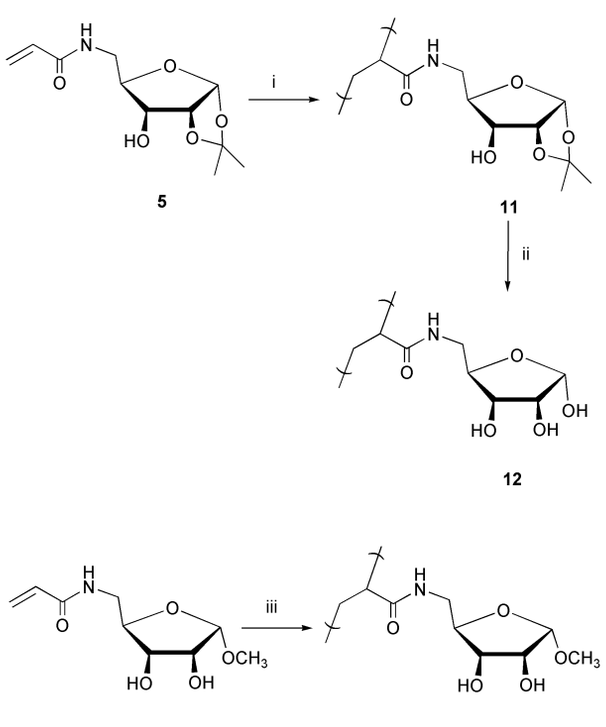
obtained by deprotection of 5′,6′-acetonide groups after the acetylation of 1′,2′:5′,6′-di-*O*-isopropylidene-α-D-allofuranose.[15] Compound **1** was oxidized with the aid of $NaIO_4$ in a suspension of silica gel in methylene chloride to yield 3′-*O*-acetyl-1′,2′-*O*-isopropylidene-α-D-ribo-pentoaldo-1′,4′-furanose (**2**). Treatment of **2** with ammonia saturated in MeOH at room temperature for 24 h gave the imine compound (**3**). 5′-Amino-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose (**4**) was obtained by hydrogenation of **3** with the aid of Pd on charcoal in MeOH by stirring under an H_2 atmosphere at room temperature. The corresponding monomer **5** was obtained by a substitution reaction with acryloyl chloride in anhydrous THF at room temperature.

The synthesis of another monomer, 5′-acrylamido-5′-deoxy-1-*O*-methyl-D-ribose (**10**), was accomplished in four steps starting from 1′-*O*-methyl-D-ribose (**6**) as shown in Scheme 1. Compound **6** was obtained by methylation of α-D-ribose according to the literature.[16] Treatment of **6** with two equivalents of triphenylphosphine and iodine in dioxane at room temperature for 24 h afforded the iodo-ribose compound (**7**), which was reacted with sodium azide in DMF at 110 °C to give 5′-azido-5′-deoxy-1′-*O*-methyl-D-ribose (**8**). Reduction of the azide compound (**8**) with triphenylphosphine in THF–H_2O afforded the corresponding amine (**9**). Monomer **10** was obtained by the reaction of **9** with one equivalent of acrylic anhydride in THF at 0 °C for 11 h.

The polymerization of monomers **5** and **10** was carried out in H_2O by initiation with $K_2S_2O_8$ at 80 °C and at 70 °C to yield poly(5′-acrylamido-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose) (**11**) and poly(5′-acrylamido-5′-deoxy-1′-*O*-methyl-D-ribose) (**13**) respectively (Scheme 2). The polymers were purified by precipitation in acetone. Number-average molecular weights of polymers **11**, **12**, and **13** measured by GPC with poly(ethylene glycol)s as standards in an aqueous 0.1 M $NaNO_3$ solution were 19300, 18000, and 18500, respectively.[14] The ^{1}H NMR spectra of monomer **5** and polymer **11** are shown in Fig. 1. The proton signals of the acryl groups appeared at 6.3–6.8 ppm in Fig. 1a, which are changed to a broad peak at 2.0–2.6 ppm of ethylene groups on the polymer chain in Fig. 1b. Hydrolysis of polymer **11** was accomplished with 1 M HCl at room temperature. After neutralization with a dilute NaOH solution, the desired poly(5′-acrylamido-5′-*O*-deoxy-α-D-ribose) (**12**) was isolated by dialysis through a cellulose membrane with a molecular weight cut off of 1000 and then by freeze-drying.



Scheme 2 *Synthesis of polymers:* (i) $K_2S_2O_8$, H_2O, 10 h, 80 °C; (ii) HCl, 18 h; (iii) $K_2S_2O_8$, H_2O, 15 h, 70 °C.

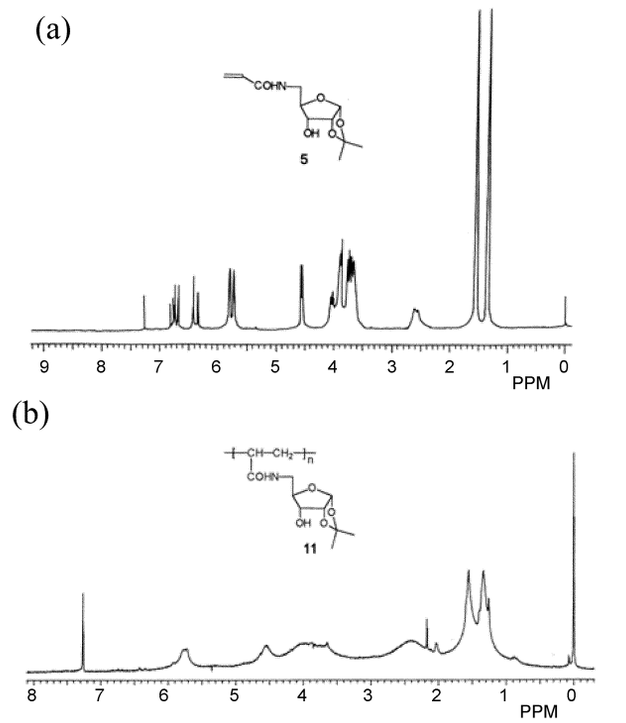


Fig. 1 ^{1}H NMR spectra of (a) monomer **5** in $CDCl_3$ and (b) polymer **11** in D_2O.

Catalytic activity for ethyl *p*-nitrophenyl phosphate (ENPP)

Ethyl *p*-nitrophenyl phosphate (ENPP) was used as the substrate. The hydrolysis reaction of the substrate in the presence of polymer **12** was confirmed by ^{31}P NMR spectroscopy. After reaction for 24 h in Tris-buffer solution under the conditions: [substrate] = 4.67×10^{-4} M and [polymer **12**]† = 5.26×10^{-6} M at pH = 7.4, 50 °C, and ionic strength = 0.02 (KCl), the reaction mixture was examined by ^{31}P NMR spectroscopy. Two peaks at 2.69 ppm for ethyl phosphate and −5.97 ppm for ethyl *p*-nitrophenyl phosphate (substrate) relative to phosphoric acid at 0 ppm were observed in the ^{31}P NMR spectrum. The possibility of transphosphorylation on the hydroxyl groups of the polymer was also investigated. The polymer was separated from the reaction mixture by dialysis through a cellulose membrane with a molecular weight cut off of 1000 and subjected to ^{31}P NMR analysis. Phosphorus atoms were not detected on the polymer, indicating that the hydrolysis reaction took place exclusively.

Hydrolysis rates of the substrate were determined in Tris-buffer (pH = 7.4, ionic strength = 0.02, KCl) at 50 °C in the presence of the monomers and the polymers. The rates were followed by the measurement of the ultraviolet absorption ($\varepsilon_{400\ nm} = 10268$) of the conjugate anion of *p*-nitrophenol evolved. The hydrolysis of a phosphate substrate is often catalyzed by metal ions with various ligands.[17] To exclude this possibility, we used deionized water (resistivity > 18 MΩ cm^{-1}) and Tris-buffer materials crystallized from water–ethanol three times. The concentrations of *p*-nitrophenol produced during the hydrolysis in the presence of monomer **5**, monomer **10**, ribose, or polymers **11**–**13**, at pH 7.4, 50 °C and $\mu = 0.02$, as a function of time are plotted in Fig. 2. Polymers **12** and **13** with free *vic*-*cis*-diols of furanose rings accelerated the hydrolysis, while the catalytic activity was negligible in the presence of polymer **11**, whose *diol* groups were

† Concentration of polymer chains.

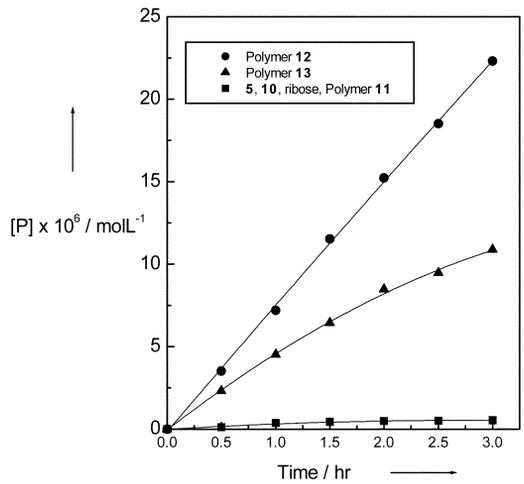


Fig. 2 Concentration of *p*-nitrophenol evolved during hydrolysis of ethyl *p*-nitrophenyl phosphate in the presence of polymers **11**, **12**, **13**, and monomers **5**, **10**, and ribose as a function of time at pH 7.4 (Tris-buffer), 50 °C, μ = 0.02 (KCl). [substrate] = 3.73×10^{-3} M, [polymer] = 5.26×10^{-6} M, [monomer **5**] = [monomer **10**] = [ribose] = 4.67×10^{-4} M (same as the ribose residue concentration of the polymers).

blocked by isopropylidene groups. It is also noteworthy that the monomeric species (**5** and **10**) and ribose showed negligible catalytic activity.

In hydrolysis kinetics, the subtraction of v_u (uncatalyzed reaction) from the measured rate (v_m) is v_c (catalyzed reaction).[18] The initial v_c was obtained at a constant concentration of polymers **12** and **13** by changing the substrate concentrations. Michaelis–Menten kinetics for **12** and **13** were confirmed by plotting the double reciprocal form of Lineweaver and Burk (1/*v vs.* 1/*s*) (Fig. 3), which gave K_m and V_{max}.

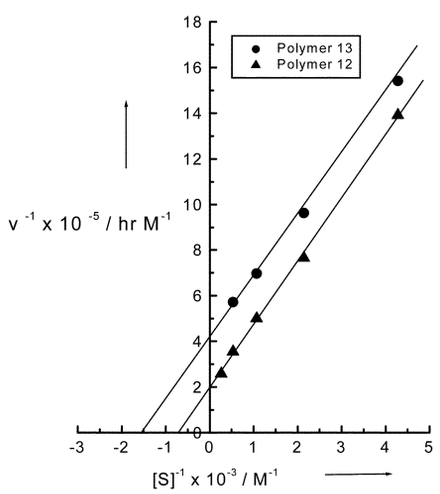


Fig. 3 Reciprocals of the initial rates as a function of reciprocals of the substrate concentrations (1/*v vs.* 1/[S]) in the presence of polymers **12** and **13**.

Since only the polymers showed catalytic activity, we presume that active centers were formed where the substrate was bound, through chain folding. The extent of active center formation will depend on the polymer chain length, *i.e.* molecular weight. Polymers with different molecular weights were obtained by the successive dialysis of polymer **12** through cellulose membrane tubes with different molecular weight cut offs. The four filtrates obtained by dialyzing through the series of tubes (molecular weight cut off = 3500, 8000, 15000, and 25000) and the polymer solution remaining in the last tube were freeze-dried to give five polymer portions with different molecular weights as summarized in Table 1.

Table 1 Fractionation results of polymer **12**

No.[a]	Membrane cut off[b]	M_n[c]
1	3500	2500
2	8000	6400
3	15000	9200
4	25000	17800
		27100[d]

[a] The order of cellulose membrane tubes used for successive dialyses. [b] Molecular weight cut off of the cellulose membrane tube. [c] Number-average molecular weight of the polymer in the filtrate after dialysis. [d] Number-average molecular weight of the polymer in the cellulose membrane tube with a molecular cut off of 25000 after dialysis.

The initial hydrolysis rates of the substrate (ENPP) in the presence of polymer **12** ([polymer] = 5.26×10^{-6} M, [substrate] = 1.86×10^{-3} M) were measured at pH 7.4 (Tris-buffer), 50 °C, and μ = 0.02 (KCl). The rates were dependent on the number-average molecular weights of the polymers. The initial hydrolysis rate increased above an M_n of 17800, and therefore we presumed that one active center formed on each polymer chain with a molecular weight higher than 17800.

Polymer **12** with M_n = 17800 was used for the kinetic study. From Fig. 3, K_m and V_{max} were obtained and k_{cat} was calculated from eqn. (1). Kinetic parameters of the catalysis are summarized in Table 2.

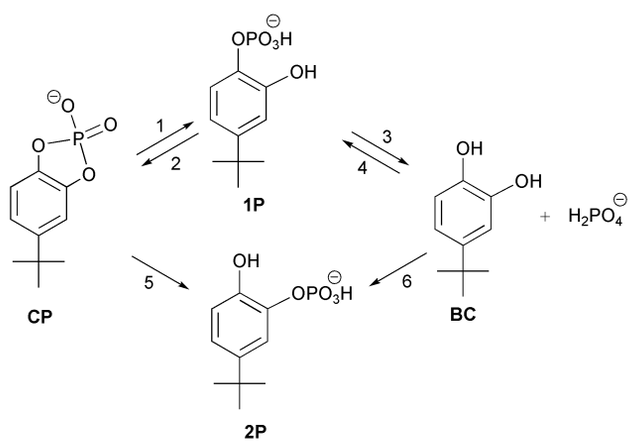
$$k_{cat} = V_{max} / [E]_o \quad (1)$$

The rate constants (k_{cat}) for polymers **12** and **13** were found to be about 10^3 higher than that of the uncatalyzed reaction. Polymer **12** showed higher catalytic activity than **13**, probably because the former contained two *vic*-*cis*-diol groups (1′ 2′ and 2′ 3′) while the latter had one (2′ 3′ OH).

Competitive inhibition was found in the presence of acetate ions and its dissociation constant (K_I) was measured to be 3.49×10^{-4} in the catalysis by polymer **12** (Fig. 4a). Non-competitive inhibition was also observed by addition of K_2HPO_4 ($K_I = 9.89 \times 10^{-4}$ M) (Fig. 4b). Both inhibitions seemed to occur because the *vic*-*cis*-diols were blocked by formation of hydrogen bonds with the inhibitors. The acetate ions seemed to compete with the substrate for forming hydrogen bonds with the polymeric catalyst. The phosphate ion, as a dianion, could form stronger hydrogen bonds with *vic*-*cis*-diols of the polymer catalyst than the substrate, leading to non-competitive inhibition.

Enzyme mimics for nuclease, ligase, phosphatase, and phosphorylase

The catalytic activities of the polymers for the model reactions of *N*-methylpyridinium 4-*tert*-butylcatechol cyclic phosphate (**CP**) were investigated (Scheme 3). The reactions of the



Scheme 3 Reactions of cyclic phosphate substrate **CP**.

Table 2 Kinetic parameters of the catalysis for hydrolysis of ethyl *p*-nitrophenyl phosphate at 50 °C

Polymer no.	k (h^{-1})	K_m (M)	V_{max} (M h^{-1})	k_{cat} (h^{-1})
Blank	9.12×10^{-4}	—	—	—
12	—	1.44×10^{-3}	5.19×10^{-6}	9.43×10^{-1}
13	—	6.14×10^{-4}	2.37×10^{-6}	4.33×10^{-1}

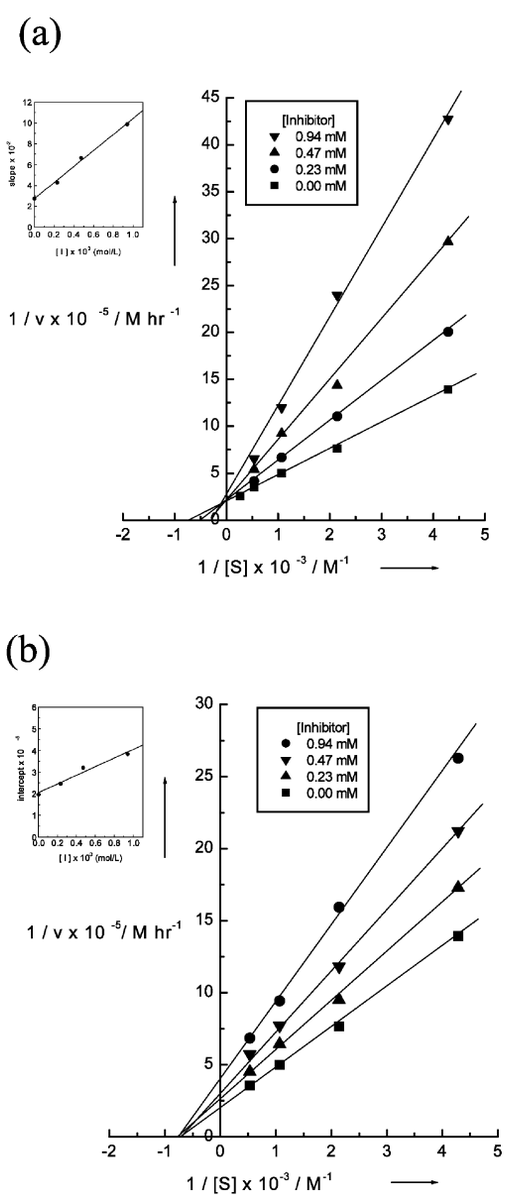


Fig. 4 Reciprocals of the initial rates as a function of reciprocals of the substrate concentrations (1/*v vs.* 1/[S]) measured at different concentrations of inhibitors, (a) sodium acetate or (b) K_2HPO_4, in the presence of polymer **12** in Tris-buffer (pH = 7.4) at ionic strength of 0.02 (KCl) at 50 °C. [polymer **12**] = 5.26×10^{-6} M. K_I determinations are shown in the upper left plots.

phosphate substrate were carried out in Tris-buffer (pH 7.4) at 25 °C and an ionic strength of 0.02 (KCl). The starting concentrations of the substrate and the polymers were 4×10^{-4} M and 2×10^{-5} M respectively. The concentrations of reactants and products were analyzed by liquid chromatography with a UV detector at 285 nm. Under the reaction conditions, the reaction rate was moderate enough to be measured and no significant interference of the polymers was observed in the LC chromatograms.

The hydrolysis rates of **CP** were measured in the buffer solution alone and in the presence of ribose,[19] **11** or **12**. The time-dependent concentration changes of **CP** are shown in Fig. 5a.

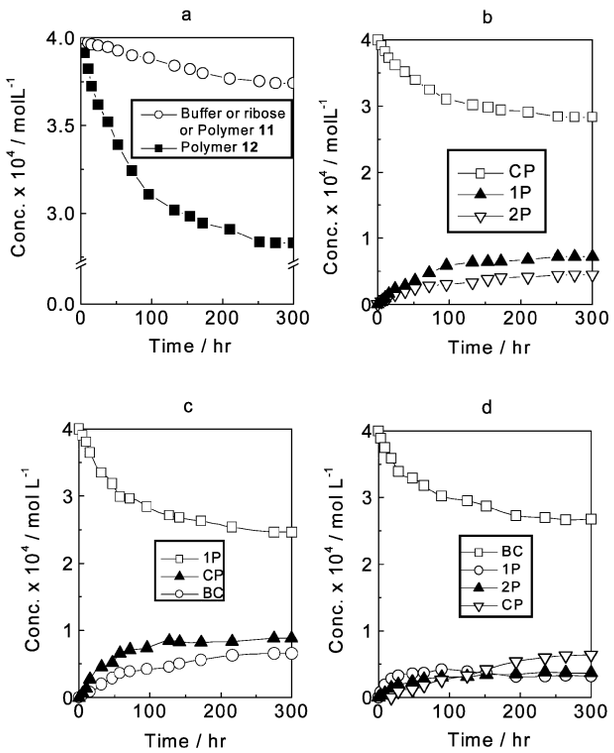


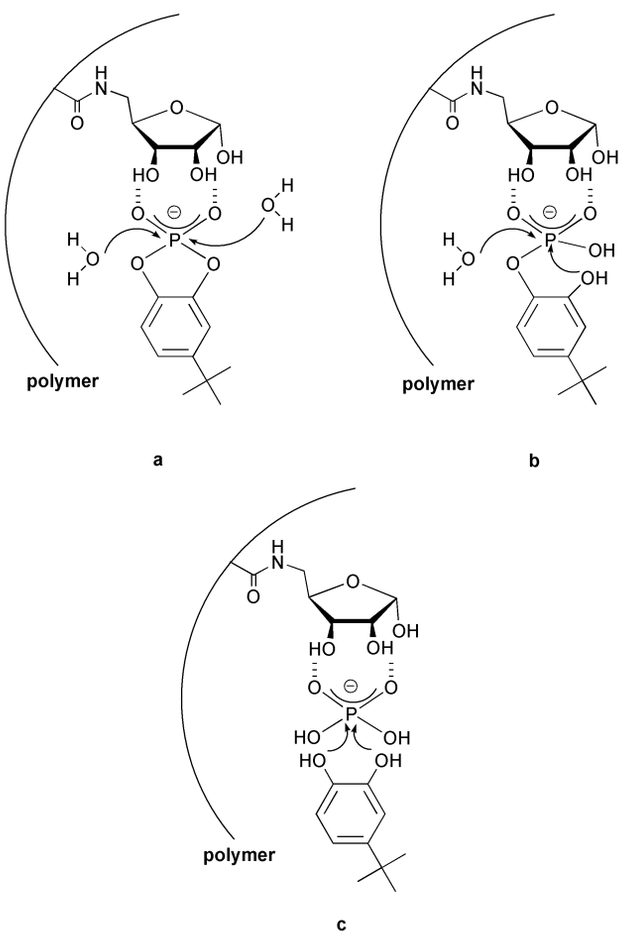
Fig. 5 Concentration changes *vs.* time (a) for the hydrolysis of cyclic phosphate (**CP**) in Tris-buffer solution (○) and in the presence of ribose and **11** (○) and **12** (■), (b) for the reaction of **CP** (□) ⟶ **1P** (▲) + **2P** (▽) catalyzed by **12**, (c) for the reaction of **1P** (□)⟶**CP** (▲) + **BC** (○) catalyzed by **12**, and (d) for the reaction of **BC** (□) + $H_2PO_4^-$ ⟶ **1P** (○) + **2P** (▲) ⟶ **CP** (▽) catalyzed by **12**.

As observed in the reaction of ethyl *p*-nitrophenyl phosphate, only polymer **12** showed catalytic activity for the hydrolysis of **CP**. Ribose and polymer **11** did not accelerate the reaction at all within experimental error. As the reactions were pseudo-first order, the rate constants for the hydrolysis were obtained by plotting logarithmic concentrations of **CP** against time and found to be 3.09×10^{-3} h^{-1} for **12**, which was about 10 times higher than that of the uncatalyzed reaction (3.2×10^{-4} h^{-1}). In the hydrolysis in the presence of **12**, the concentration of **CP** decreased while phosphoric acid mono(4-*t*-butyl 2-hydroxyphenyl) ester (**1P**) and phosphoric acid mono(5-*t*-butyl-2-hydroxyphenyl) ester (**2P**) formed simultaneously as reported by others (Fig. 5b).[20,21] The rate constants of reactions 1 and 5 in Scheme 3 were found to be 1.86 and 1.23×10^{-3} h^{-1} respectively.

1P was stable in the buffer solution. When **1P** was mixed with **12** in Tris-buffer solution at pH 7.4 however, its cyclization to **CP** and hydrolysis to *t*-butylcatechol (**BC**) occurred simultaneously. The concentration changes of the products and the reactants in the cyclization and hydrolysis of **1P** in the presence of **12** are shown in Fig. 5c. The rate constants for reactions 2 and 3 were found to be 3.19 and 1.79×10^{-3} h^{-1} respectively. When **12**, **BC**, and KH_2PO_4 were mixed in the mole ratio of 1 : 10 : 20 at pH 7.4 (Tris-buffer), esterification either to **1P** or to **2P** occurred, which was followed by cyclization to **CP**. In order to lead this reaction to the first order, phosphate ion was added in excess. The concentration changes *vs.* time of the reaction in the presence of **12** are plotted in Fig. 5d. At the beginning of

the reaction, **1P** and **2P** formed, and thereafter **CP**. The rate constants for reactions 4 and 6 were found to be 3.41 and 1.70 × 10^{-3} h^{-3}, respectively.

Based on the experimental results described above and our previous results,[12] the mechanism of catalysis can be postulated as follows: since no activity was found for ribose, the polymer backbone was likely to form an active site to hold a substrate therein by chain folding. It seems possible that the polymer-pendent ribose rings with *vic*-*cis*-diol groups were located inside the active site, where the phosphate substrate was also accommodated. The *vic*-*cis*-diol groups form hydrogen bonds with the two oxygen atoms of the phosphate so as to activate the phosphorus atoms to be attacked by nucleophiles (H_2O or hydroxy groups) as shown in Scheme 4. Either 1′,2′ or 2′,3′-diols of riboses can form hydrogen bonds although only the bonds with 2′,3′- diols are illustrated in Scheme 4. The formation of strong hydrogen bonds was borne out by a theoretical study of the interaction between 3,4-dihydroxytetrahydrofuran and $H_2PO_4^{-1}$.[13]



Scheme 4 Action mechanism of polymer **12** for the catalysis: (a) The phosphorus atom of **CP** is attacked by H_2O either side of the phosphate to form **1P** or **2P**. (b) The hydrolysis and esterification of **1P** occur when H_2O attacks the phosphorus atom to break the P–O ester bond or the 2-OH group of **1P** attacks to eliminate the OH group of the phosphate, respectively. (c) The phosphorylation of **BC** occurs by nucleophilic attack of the 1-OH group of **BC** to the phosphorus atom to eliminate the OH group from the phosphate.

In the case of hydrolysis of **CP** (Scheme 4a), water can attack either side of the activated phosphate to form **1P** or **2P**. The hydrolysis and esterification of **1P** (Scheme 4b) occur when water or the 2-OH group of **1P** attack the phosphorus atom and either break the P–O ester bond or cause the OH group of the phosphate to leave, respectively. The phosphorylation of **BC** (Scheme 4c) occurs by nucleophilic attack of 1-OH or 2-OH of **BC** at the activated phosphorus atom with the OH of the phosphate as a leaving group. The catalytic activities for reactions 1 (and 5), 2, 3, and 4 (and 6) (Scheme 3) correspond to the enzyme activities for nuclease, ligase, phosphatase, and phosphorylase respectively.

In order to investigate whether the reactions described above could occur on the hydroxyl groups of the ribose rings on the polymer, the polymers used for the reactions were separated by dialysis through cellulose membranes with a molecular weight cut off of 1000 and were subjected to ^{31}P NMR analysis. However, P atoms were not detected on the polymers, indicating that the reactions on the hydroxyl groups did not occur.

In conclusion, the polymers containing *vic*-*cis*-diols of riboses catalyzed the hydrolysis of ethyl *p*-nitrophenyl phosphate with a rate acceleration of 10^3 compared with that of the uncatalyzed reaction. They also showed catalytic activity for the hydrolysis of phosphodiester and phosphomonoester, the esterification of phosphomonoester and the phosphorylation of alcohols with phosphate ions. In biological systems there are plenty of biopolymers containing riboses. One of the typical biopolymers containing riboses with *vic*-*cis*-diols is poly(ADP-ribose) formed from NAD^+ in chromatin. Since its discovery in 1966,[22,23] the polymer has been suggested to be involved in numerous biological reactions,[24–27] although its functions are not clear so far. Poly(ADP-ribose) forms during apoptosis[28] and DNA repair,[29,30] where a nuclease will be required. More interestingly, the ribozyme (RNA) also contains *vic*-*cis*-diols of ribose at the 3′-OH termini or at apurinic sites, which might catalyze the cleavage of nucleic acids when the *vic*-*cis*-diols of ribose anchored inside the active center formed by chain folding through base pairing. We hope that this report will contribute to the elucidation of their functions and further investigations are in progress along these lines.

Experimental

Materials and instrumentation

Chemicals were purchased from Sigma-Aldrich. Tris-buffer materials (TRIZMA® Base and TRIZMA® HCl) were recrystallized from deionized water and ethanol three times to exclude metal ions. THF was dried over sodium metal and distilled. Dimethyl formamide was dried over anhydrous $MgSO_4$ and distilled. Pyridine was refluxed over KOH and distilled. Other commercially available reagent chemicals were used without purification. The substrates, ethyl *p*-nitrophenyl phosphate (ENPP),[18] *N*-methyl pyridinium 4-*tert*-butylcatechol cyclic phosphate (**CP**),[20] and 1-phosphate (**1P**)[12] and 2-phosphate (**2P**)[12] of 4-*tert*-butylcatechol were prepared according to the literature.

^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer. IR spectra were obtained with a Nicolet Magna IR-550 spectrophotometer. The reaction rates were determined with a Hitachi (Model 200–20) thermostatted spectrophotometer (±0.1 °C). Measurement of molecular weights was carried out by gel permeation chromatography, Waters 150-Cplus with a RI detector. Elemental analysis was performed at Korea Research Institute of Chemical Technology.

3′-*O*-Acetyl-1′,2′-*O*-isopropylidene-5′,6′-diol-α-D-allofuranose (1)

Compound **1** was synthesized according to the literature.[15]

3′-*O*-Acetyl-1′,2′-*O*-isopropylidene-α-D-ribo-pentoaldo-1′,4′-furanose (2)

A solution of **1** (1 g, 3.83 mmol) in methylene chloride (10 mL) was dropped slowly into a suspension of silica gel (8 g) in methylene chloride (80 mL) containing 10 mL of a 0.65 M $NaIO_4$ aqueous solution. The reaction mixture was stirred for

40 min at room temperature. The solids were removed by filtration and the filtrate was concentrated by evaporation. Compound **2** (0.9 g, 97%) was isolated by column chromatography on silica gel (eluent; ethyl acetate–hexane = 1 : 1, v/v). (Found: C, 51.97; H, 5.93. Calc. for $C_{10}H_{14}O_6$: C, 52.17; H, 6.13%); δ_H (200 MHz, $CDCl_3$): 1.37, 1.57 (ss, 6H, acetonide), 2.16 (s, 3H, acetyl), 4.44 (d, 1H, H^3, J = 8.6 Hz), 4.86 (d, 1H, H^2, J = 2.4 Hz), 4.90 (t, 1H, H^4, J = 4.6 Hz), 5.93 (d, 1H, H^1, J = 3.6 Hz), 9.67 (d, 1H, aldehyde, J = 2.2 Hz); δ_C (50 MHz, $CDCl_3$) 20.35 (CH_3CO_2), 26.54 (CCH_3), 26.62 (CCH_3), 72.07 (C^3), 77.47 (C^2), 80.84 (C^4), 104.75 (CCH_3), 113.91 (C^1), 169.97 (CH_3CO_2), 197.29 (CHO).

5′-Imino-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose (3)

Compound **2** (0.9 g, 3.91 mmol) was dissolved in ammonia saturated in methanol (10 mL) and stirred for 24 h at room temperature. After evaporation of the solvent under reduced pressure, column chromatography on silica gel (eluent; ethyl acetate–hexane = 2 : 1, v/v) gave **3** (0.7 g, 96%). (Found: C, 51.08; H, 6.92; N, 7.24. Calc. for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48%); δ_H (200 MHz, $CDCl_3$): 1.36, 1.57 (ss, 6H, acetonide), 3.89 (d, 1H, H^2, J = 8.8 Hz), 4.03 (s, 1H, H^5), 4.20 (dd, 1H, H^3, J = 5 Hz, J = 5 Hz), 4.54 (t, 1H, H^4, J = 4.3 Hz), 5.78 (d, 1H, H^1, J = 3.6 Hz); δ_C (50 MHz, $CDCl_3$): 26.34 (CCH_3), 26.47 (CCH_3), 70.74 (C^3), 79.24 (C^4), 81.52 (C^2), 104.08 (CCH_3), 112.73 (C^1), 139.81 (NH=CH).

5′-Amino-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose (4)

Compound **3** (0.7 g, 3.74 mmol) was dissolved in methanol (20 mL). After addition of Pd on charcoal (600 mg), the solution was stirred under H_2 atmosphere (4 atm.) for two days at room temperature. After filtration through celite twice and evaporation, the product was isolated by column chromatography on silica gel (eluent; methanol–chloroform = 1 : 7, v/v) (0.5 g, 71%).

(Found: C, 50.84; H, 8.04; N, 7.19. Calc. for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40%); δ_H (200 MHz, $CDCl_3$): 1.36, 1.57 (ss, 6H, acetonide), 2.85, 3.00 (dd, dd, 2H, H^5, J = 5.1, 5.3 Hz, 3.0, 3.6 Hz), 3.85 (m, 2H, $H^{2,3}$), 4.55 (t, 1H, H^4, J = 4.6 Hz), 5.77 (d, 1H, H^1, J = 3.8 Hz); δ_C (50 MHz, $CDCl_3$): 26.36 (CCH_3), 26.62 (CCH_3), 49.13 (CH_2NH_2), 75.38 (C^3), 79.04 (C^4), 87.74 (C^2), 103.91 (CCH_3), 113.10 (C^1).

5′-Acrylamido-5′-deoxy-1′,2′-*O*-isopropylidene-α-D-ribose (5)

Acryloyl chloride (0.4 mL) and **4** (0.3 g, 1.59 mmol) were dissolved in THF (10 mL) and stirred for 2 h at room temperature. After evaporation of the solvent, compound **5** (0.21 g, 54%) was isolated by column chromatography on silica gel (eluent: ethyl acetate). (Found: C, 53.98; H, 6.84; N, 5.47. Calc. for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76%); δ_H (200 MHz, $CDCl_3$): 1.34, 1.56 (ss, 6H, acetonide), 3.56 (m, 2H, H^5), 3.83 (m, 2H, $H^{2,3}$), 4.58 (t, 1H, H^4, J = 4.2 Hz), 5.76 (d, 1H, H^1, J = 4.4 Hz), 6.37 (d, 1H, H^{8c}, J = 14 Hz), 6.71 (d, 1H, H^{8t}, J = 10 Hz), 6.79 (d, 1H, H^7, J = 10 Hz); δ_C (50 MHz, $CDCl_3$): 26.35 (CCH_3), 26.48 (CCH_3), 54.97 (CH_2NH_2), 74.59 (C^3), 79.23 (C^4), 87.94 (C^2), 104.05 (CCH_3), 112.98 (C^1), 125.91 (CH_2=CH), 131.25 (CH_2=CH), 165.17 ($CONH$).

1′-*O*-Methyl-D-ribose (6)

Compound **6** was prepared according to the literature.[16]

5′-Iodo-5′-deoxy-1′-*O*-methyl-D-ribose (7)

To a solution of **6** (5.2 g, 31.6 mmol) in 1,4-dioxane (50 mL), triphenylphosphine (17 g), iodine (17.2 g), and pyridine (6 mL) were added, and the solution was stirred for 24 h at room temperature. After evaporation of the solvent, compound **7** (5.6 g, 61%) was isolated by column chromatography on silica gel (eluent; diethyl ether). (Found: C, 26.17; H, 4.03. Calc. for $C_6H_{11}IO_4$: C, 26.30; H, 4.05%); δ_H (200 MHz, $CDCl_3$): 3.29 (m, 2H, H^5), 3.39 (s, 1H, methoxy), 4.18 (m, 2H, $H^{2,3}$), 4.23 (q, 1H, H^4, J = 6 Hz), 4.87 (s, 1H, H^1); δ_C (50 MHz, $CDCl_3$): 38.03 (CH_2I), 55.28 (OCH_3), 75.45 (C^3), 75.59 (C^4), 82.68 (C^2), 108.13 (C^1).

5′-Azido-5′-deoxy-1′-*O*-methyl-D-ribose (8)

Compound **7** (4 g, 14.6 mmol) and sodium azide (3 g, 46.1 mmol) were dissolved in *N*,*N*-dimethylformamide (70 mL) and the solution was stirred at 110 °C for 24 h. After evaporation of the solvent, the residue was dissolved in H_2O (150 mL) and extracted by diethyl ether 5 times. After drying with anhydrous $MgSO_4$, the solvent was evaporated to give syrupy **8** (2.7 g, 97%). (Found: C, 37.94; H, 5.88; N, 21.92. Calc. for $C_6H_{11}N_3O_4$: C, 38.10; H, 5.86; N, 22.21%); δ_H (200 MHz, $CDCl_3$): 3.37 (s, 3H, methoxy), 3.32, 3.47 (dd, dd, 2H, H^5, J = 6.2 Hz, 3.2 Hz), 3.99 (d, 1H, H^3, J = 5.2 Hz), 4.08 (dd, 1H, H^2, J = 3.2 Hz, 3.2 Hz), 4.18 (q, 1H, H^4, J = 5.2 Hz), 4.83 (s, 1H, H^1); δ_C (50 MHz, $CDCl_3$): 51.12 (CH_2N_3), 55.73 (OCH_3), 75.24 (C^3), 75.03 (C^4), 81.61 (C^2), 107.3 (C^1).

5′-Amino-5′-deoxy-1′-*O*-methyl-D-ribose (9)

Compound **8** (2.7 g, 14.3 mmol) and triphenylphosphine (5.2 g, 19.8 mmol) were dissolved in tetrahydrofuran (50 mL) and the solution was stirred for 24 h at room temperature. To the solution was added 100 mL of H_2O and triphenylphosphine oxide was removed by washing with diethyl ether three times. The solution was concentrated by evaporation and the syrupy residue was dissolved in H_2O. After filtration and evaporation, compound **9** (1.5 g, 64%) was obtained. (Found: C, 43.89; H, 7.99; N, 8.36. Calc. for $C_6H_{13}NO_4$: C, 44.16; H, 8.03; N, 8.58%); δ_H (200 MHz, D_2O): 2.53 (s, 2H, –OH), 2.91 (m, 2H, H^5), 3.37 (s, 3H, methoxy), 3.93 (d, 1H, H^2, J = 4.8 Hz), 3.98 (d, 1H, H^3, J = 4.8 Hz), 4.14 (t, 1H, H^4, J = 5.0 Hz), 4.82 (s, 1H, H^1); δ_C (50 MHz, D_2O): 39.97 (CH_2NH_2), 55.04 (OCH_3), 71.43 (C^3), 74.55 (C^4), 80.54 (C^2), 106.92 (C^1).

5′-Acrylamido-5′-deoxy-1′-*O*-methyl-D-ribose (10)

Compound **9** (0.4 g, 2.45 mmol) and acrylic anhydride (0.247 g, 1.96 mmol) were dissolved in THF (20 mL) and stirred for 20 min at 0 °C and for 11 h at room temperature. After evaporation of the solvent, compound **10** (0.25 g, 47%) was isolated by column chromatography on silica gel (eluent; acetone : CCl_4 = 3 : 3, v/v). (Found: C, 49.51; H, 6.85; N, 6.19. Calc. for $C_9H_{15}NO_5$: C, 49.76; H, 6.96; N, 6.45%); δ_H (200 MHz, [D_6]DMSO): 3.39 (s, 3H, methoxy), 3.58 (t, 2H, H^5, J = 4.2 Hz), 4.01 (d, 1H, H^3, J = 4.6 Hz), 4.12 (m, 2H, $H^{2,4}$), 4.82 (s, 1H, H^1), 5.86 (d, 1H, H^{8c}, J = 10 Hz), 6.19 (m, 2H, $H^{7,8t}$); δ_C (50 MHz, [D_6]DMSO): 41.50 (CH_2NH), 54.19 (OCH_3), 71.63 (C^3), 73.96 (C^4), 80.84 (C^2), 107.71 (C^1), 124.75 (CH_2=CH), 130.48 (CH_2=CH), 164.95 ($CONH$).

Polymerization

Monomer (**5**: 2.47 mmol L^{-1} or **10**: 1.15 mmol L^{-1}) and potassium persulfate (2 mol%) were dissolved in water in a polymerization tube. After three freeze–thaw cycles under N_2, the tube was sealed and placed in a water bath at 80 °C for 15 h. The polymerization solution was precipitated in acetone to give **11** (yield: 84%) or **13** (yield: 68%). Polymer **11**. δ_H (200 MHz, $CDCl_3$): 1.38, 1.59 (ss, 6H, acetonide), 1.39–2.31 (3H, ethylene), 3.89 (4H, $H^{2,3,5}$), 4.55 (1H, H^4), 5.74 (1H, H^1).

Polymer **13**. δ_H (200 MHz, [D_6]DMSO): 1.32–2.32 (3H, ethylene), 3.29 (3H, methyl), 3.82 (3H, $H^{2,3,5}$), 4.29 (1H, H^4), 4.69 (1H, H^1).

Hydrolysis of polymer 11

Polymer **11** (60 mg) was dissolved in 2 M HCl and stirred for 18 h at room temperature. The resulting polymer (**12**) was

isolated by precipitation in acetone and dried (32.4 mg, 65%). δ_H (200 MHz, D_2O): 1.32–2.32 (3H, ethylene), 3.35–4.2 (2H, H^5), 4.07 (1H, H^3), 4.19 (2H, $H^{2,4}$), 5.25 (1H, H^1); δ_C (50 MHz, D_2O): 39.21 (chain $-CH_2-$), 52.04 (C^5), 56.19 (chain $-CH-$), 74.92 (C^3), 76.84 (C^4), 82.97 (C^2), 104.03 (C^1), 179.92 (C=O).

Kinetic measurements for ethyl *p*-nitrophenyl phosphate (ENPP)

The solutions of the catalysts (7.3×10^{-5} M) and the solutions of ethyl *p*-nitrophenyl phosphate (7.0×10^{-3} M) in water buffered with 0.02 M Tris (pH 7.4) were prepared. The ionic strength was adjusted to 0.02 (KCl). The definite portions of the catalyst and the substrate solutions were mixed in a measuring cell. The reference cell was filled with a solution of the same substrate concentration buffered with Tris (pH 7.4) at the ionic strength of 0.02 (KCl). The reaction rates were determined by measuring the absorption (A_t) of the conjugate anion of *p*-nitrophenol (400 nm) as a function of time (t) with a Hitachi (Model 200–20) thermosttated spectrophotometer (±0.1 °C).

Kinetic measurements for *N*-methyl pyridinium 4-*tert*-butylcatechol cyclic phosphate (CP)

Polymer **12** (2×10^{-5} M) and **CP** (4×10^{-4} M) were dissolved in Tris-buffer (pH 7.4) with ionic strength of 0.2 (KCl) at 25 °C. 80 μL of the reaction solution was taken by a microsyringe at definite time intervals and injected into a Waters LC (Conditions: Waters Ultrahydrogel 120 GPC column, UV detector λ = 285 nm, eluent; 0.025 M KCl, flow rate; 0.8 mL min^{-1}). The concentrations of the reactants and products were evaluated by peak areas, which were corrected by the extinction coefficients of the compounds (ε = 2460 for **CP**, 3500 for **1P**, 3490 for **2P** and 2310 for **BC** at 285 nm and pH 7.4).

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