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A one-step phosphorylation of D-aldohexoses and D-aldopentoses with inorganic *cyclo*-triphosphate

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Abstract

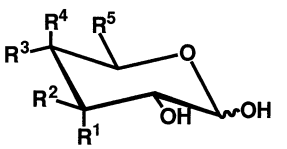
The phosphorylation reaction by inorganic *cyclo*-triphosphate (P_{3m}) having a six-membered ring was examined for D-aldohexoses and D-aldopentoses in aqueous solution. Similar to the process for D-glucose, D-galactose, D-xylose or D-allose were phosphorylated with P_{3m} to give stereoselectively β-D-galactopyranosyl 1-triphosphate, β-D-xylopyranosyl 1-triphosphate or β-D-allopyranosyl 1-triphosphate with maximum yields of 31.3, 32.5 or 32.1%, respectively. On the other hand, in the reaction of D-ribose, D-lyxose, D-mannose or D-arabinose with P_{3m}, the yields of β-D-ribopyranosyl 1-triphosphate, α-D-lyxopyranosyl 1-triphosphate, α-D-mannopyranosyl 1-triphosphate or α-D-arabinopyranosyl 1-triphosphate were 8.0, 16.5, 9.6 or 14.1%, respectively. The phosphorylation mechanism of D-aldopyranoses with P_{3m} was also discussed.

Keywords: Phosphorylation; β-D-Aldopyranosyl 1-triphosphate; *cyclo*-Triphosphate

1. Introduction

In 1937, Cori et al. [1] demonstrated the synthesis of α-D-aldose 1-phosphate by the reaction of trisilver phosphate with α-D-aldose. Since then, other synthetic methods for aldohexopyranosyl phosphates have been reported using α-D-acetylaldose [2,3] and per-*O*-acetyl-α-D-aldosyl bromides [4,5] as starting materials. However, these methods require multistep reactions because of the necessity of protection of hydroxyl groups and anomer-exchange reactions.

Recently, we demonstrated that the reaction of D-glucose (**3**) with *cyclo*-triphosphate (P_{3m}) afforded β-D-glucopyranosyl 1-triphosphate (**11**) in good yield in a one-step process [6,7] without protection of the hydroxyl groups. Although **3** exists as the equilibrium mixture of α and β anomers under the reaction conditions, only **11** was stereoselectively obtained [7]. It is more important to apply this stereoselective phosphorylation reaction to other monosaccharides in order possibly to shed light upon the reaction mechanism.



	R^1	R^2	R^3	R^4	R^5
1 (Gal)	H	OH	H	OH	CH_2OH
2 (Xyl)	H	OH	OH	H	H
3 (Glc)	H	OH	OH	H	CH_2OH
4 (Rib)	OH	H	OH	H	H
5 (All)	OH	H	OH	H	CH_2OH

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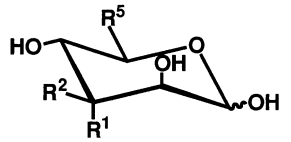


	R^1	R^2	R^5
6 (Lyx)	H	OH	H
7(Man)	H	OH	CH_2OH
8 (Ara)	OH	H	H

In the present study the reaction of D-galactose (**1**), D-xylose (**2**), D-ribose (**4**), D-allose (**5**), D-lyxose (**6**), D-mannose (**7**) or D-arabinose (**8**) with P_{3m} was studied to develop a selective phosphorylation of D-aldoses in aqueous solution.

2. Results and discussion

The syntheses of aldose triphosphates (**9**, **10** and **12**–**16**) were carried out essentially according to the previous method [7]. Fig. 1 shows the high-performance liquid chromatography (HPLC) profiles of the reaction solution of D-galactose (**1**) and P_{3m} incubated at pH 12 and room temperature. A peak of the main product appeared at a retention time of about 44 min. The product was predicted to be a triphosphate derivative of **1**, based on its retention time. The yield of the product increased with the passage of reaction time to reach a maximum of 31.3% after 24 h, then decreased gradually (Fig. 2). The triphosphate derivatives of **1** and P_{3m} were gradually hydrolyzed to form a monophosphate derivative of **1**, diphosphate, and monophosphate.

^1H, ^{13}C and ^{31}P NMR spectra were measured to confirm the reaction product. ^{31}P NMR spectral data (Table 1) for the reaction product of **1** with P_{3m} show a characteristic two doublets and one doublet of doublets for the presumed triphosphate derivative [7,8] of **1**, as predicted from HPLC measurements. Fig. 3(A) shows the ^1H NMR spectrum in the H-1 region. The doublet of doublets at 4.85 ppm is assigned to H-1 of the reaction product [9]. The $^3J_{H\text{-}1,\ H\text{-}2}$ value is close to that of β-D-galactopyranose [9,10]. The $^3J_{P\alpha,\ H\text{-}1}$ value is consistent with that obtained from the ^{31}P NMR data (Table 1), and with data for β-D-galactopyranosyl 1-phosphate [11], β-D-glucopyranosyl 1-triphosphate (**11**) [7] and β-D-aldose 1-phosphate [11]. The ^{13}C NMR spectrum of the reaction product of **1** with P_{3m}

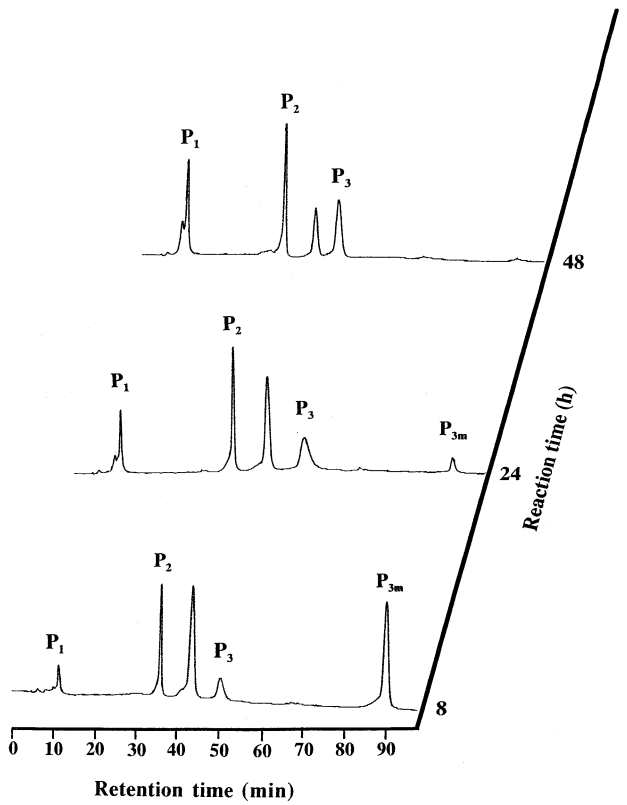


Fig. 1. HPLC profiles for the reaction solution of D-galactose (**1**) and P_{3m}. D-galactose (**1**):P_{3m} = 1.5 mol dm^{-3}:0.5 mol dm^{-3}, pH 12 and room temperature. P_1, monophosphate; P_2, diphosphate; P_3, triphosphate; P_{3m}, *cyclo*-triphosphate.

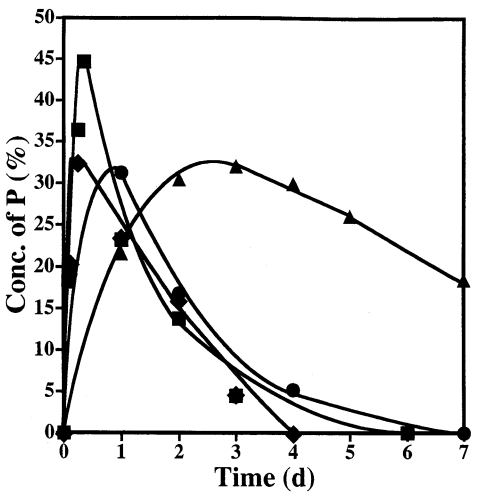


Fig. 2. Changes of the amounts, obtained by HPLC measurement, of the phosphorylated products in the reaction of D-galactose (**1**, ●), D-xylose (**2**, ◆), D-glucose (**3**, ■) or D-allose (**5**, ▲) with P_{3m} at pH 12 and room temperature.

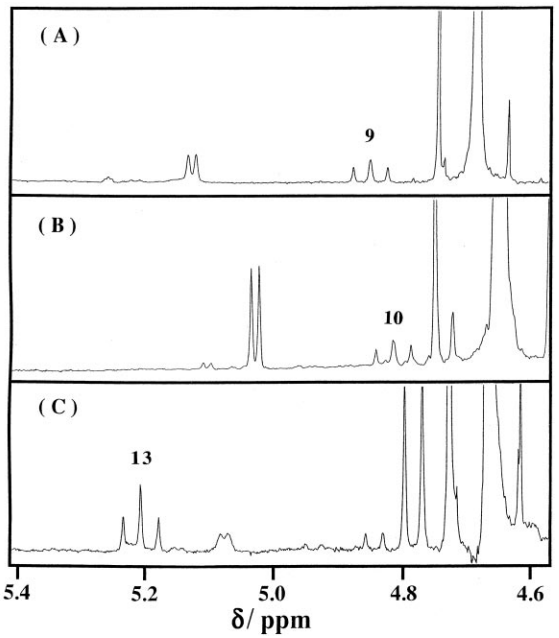


Fig. 3. The 300 MHz ^{1}H NMR spectra in the H-1 of (A) the main product formed by the reaction of D-galactose (**1**) and P_{3m} at pH 12 for 2 days; (B) D-xylose (**2**) at pH 12 for 1 day; (C) D-allose (**5**) at pH 12 for 2 days. The big signals at about 4.7 ppm are due to the residual H in water.

also supports the formation of β-D-galactopyranosyl 1-triphosphate (**9**). Table 3 lists the ^{13}C NMR chemical shift values and coupling constants of the reaction product. The $^2J_{P\alpha,\ C\text{-}1}$ and $^3J_{P\alpha,\ C\text{-}2}$ values for **9** are close to those of **11** and β-D-galactopyranosyl 1-phosphate [11]. Furthermore, the chemical shift values of **9** are close to those of β-D-galactopyranosyl 1-phosphate [11] and β-D-galactopyranose [12]. Therefore, compound **1** stereoselectively reacts with P_{3m} to form **9** (Scheme 1).

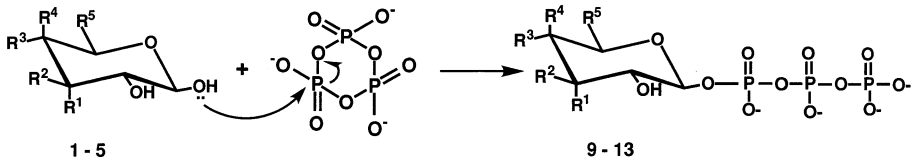
The phosphorylation of D-xylose (**2**) or D-allose (**5**) with P_{3m} was carried out under the same conditions of pH and temperature. The yields for **2** and **5** were 32.5 and 32.1%, respectively (Fig. 2). As can be seen from Fig. 2, yields of above 30% can be attained for **1**–**3** and **5**, although the rate of formation is very different for these compounds. The identification of reaction products for **2** and **5** was performed in a similar way as for **9** (Tables 1–3) by comparison with the NMR data of **2** and **5** [9,10,12,13]. The phosphorylation products of **2** and **5** with P_{3m} were verified to be β-D-xylopyranosyl 1-triphosphate (**10**) and β-D-allopyranosyl 1-triphosphate (**13**). Therefore, **1**, **2** or **5** stereoselectively reacts with P_{3m} to form **9**, **10** or **13**, respectively (Scheme 1).

Fig. 4 shows the reaction-time dependence of the yield of the main phosphorylation product in the reaction of **4**, **6**, **7** or **8** with P_{3m}. The yields for these four aldoses were less than 17%. Furthermore, the other phosphorylated products were also observed for **4** and **6**–**8**, suggesting no selectivity, although the by-products could not be determined due to low yield. In order to identify the main product in the phosphorylation of **4**, **6**, **7** or **8** with P_{3m}, the product was isolated by using anion-exchange resin (see Section 3). ^{31}P NMR data shown in Table 1 and ^{13}C NMR data in Table 3 indicate that the products are D-aldopyranosyl 1-triphosphate derivatives (**12**, **14**, **15** and **16**) by comparison with ^{13}C NMR data of D-aldoses [9,10,12,13] and D-aldose 1-phosphate [11]. Because the differences in chemical shifts between the α and β anomers are small in these compounds, one must be careful to specify the anomer. ^{1}H NMR data of H-1 in **15** and **16** (Table 2) are

Table 1
^{31}P NMR data of D-aldose 1-triphosphates

Compound	δ (ppm)			J (Hz)		
	P_α	P_β	P_γ	P_α, P_β	P_β, P_γ	P_α, H-1
9	−11.5	−20.1	−4.6	17.8	20.2	7.9
10	−11.7	−19.9	−4.6	17.7	19.0	8.6
11	−11.7	−20.1	−4.6	17.7	19.6	8.0
12 [a]	−12.1	−20.3	−5.6	19.0	19.0	7.9
13	−11.3	−20.1	−4.5	17.6	19.6	7.9
14 [a]	−12.4	−20.2	−5.8	19.0	20.2	7.9
15 [a]	−12.5	−20.3	−5.4	18.8	19.0	7.9
16 [a]	−11.6	−20.2	−6.2	17.9	19.4	8.2

[a] After isolation.



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	R^1	R^2	R^3	R^4	R^5
1,9	H	OH	H	OH	CH_2OH
2,10	H	OH	OH	H	H
3,11	H	OH	OH	H	CH_2OH
4,12	OH	H	OH	H	H
5,13	OH	H	OH	H	CH_2OH

Scheme 1.

Table 2
^{1}H NMR data of D-aldose 1-triphosphates

Compound	δ (ppm)	J (Hz)	
	H-1	H-1, H-2	H-1, P_α
9	4.85	8.2	8.0
10	4.82	7.6	8.4
11 [a]	4.90	8.1	8.1
12 [b]	5.23	3.5	8.0
13	5.21	8.2	7.9
14 [b]	5.21	3.0	8.0
15 [b]	5.29	1.9	7.6
16 [b]	4.68	8.0	8.0

[a] See Ref. [7].
[b] After isolation.

δ 5.29 (dd, $J_{1,2}$ 1.9 Hz) and 4.68 (dd, $J_{1,2}$ 8.0 Hz), suggesting the α anomers as evidenced from the ^{1}H NMR data of the α anomer in **7** (δ 5.00, $J_{1,2}$ 1.9 Hz, H-1) and **8** (δ 4.37, $J_{1,2}$ 7.8 Hz, H-1) [9,10,13]. In the case of the products **12** and **14**, their $^3J_{1,2}$ values are neither equal to the value of the α nor the β anomer, probably due to mutarotation. The specific rotations of **12** and **14** were measured to conclude that the phosphorylated products of **4** and **6** are β-D-ribopyranosyl 1-triphosphate (**12**) and α-D-lyxopyranosyl 1-triphosphate (**14**), respectively (Schemes 1 and 2).

Tables 4 and 5 show the yield of product and the population of anomers of the reactant used in this study. From the results, it is suggested that the main anomer is phosphorylated, although the reactivity of each anomer is different. In the case of **1**–**5**, where the β anomer is predominant in reactants, the phosphorylation proceeds stereoselectively and results in a yield of more than 30% except for **4**, whereas **6**–**8**, where the α anomer is predominant in the reactants, show low reactivity and no stereoselectivity.

A mechanism of phosphorylation of **1**, **2**, **3**, and **5** with P_{3m} is formulated as follows. At pH 12, P_{3m} is easily attacked by nucleophilic reagents such as ammonia [14], alcohol [15],

Table 3
^{13}C NMR chemical shifts and coupling constants of D-aldose 1-triphosphates

Compound	δ (ppm)						J (Hz)	
	C-1	C-2	C-3	C-4	C-5	C-6	P_α, C-1	P_α, C-2
9	100.8	73.7	74.7	71.1	78.4	63.7	6.1	8.2
10	100.9	75.8	77.6	71.5	68.0		5.9	8.2
11 [a]	100.2	76.1	77.7	72.0	79.0	63.3	5.6	8.2
12 [b]	99.2	72.2	70.0	68.2	66.8		5.9	8.3
13	98.4	73.2	74.0	69.1	76.8	63.9	6.7	8.1
14 [b]	99.0	72.2	72.3	68.8	65.8		5.6	9.1
15 [b]	98.8	72.5	72.0	68.9	76.2	63.1	5.7	9.2
16 [b]	100.9	73.5	74.2	70.4	69.2		5.6	7.8

[a] See Ref. [7].
[b] After isolation.

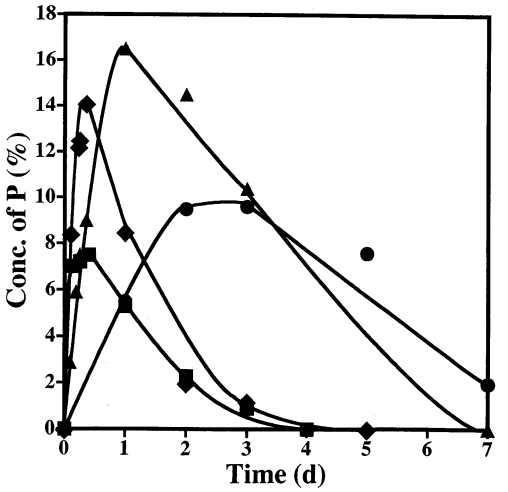


Fig. 4. Changes in the amounts of the phosphorylated products in the reaction of D-ribose (**4**, ■), D-lyxose (**6**, ▲), D-mannose (**7**, ●) or D-arabinose (**8**, ◆) with P_{3m} at pH 12 and room temperature.

nucleoside [16], and amino acid [17]. In the present study, the lone electron pair on the anomeric hydroxyl group of β-D-aldopyranose nucleophilically attacks a phosphorus atom on P_{3m} to open its six-membered ring. No attack of α-D-aldopyranose might be due to the steric hindrance of the cis conformation in hydroxyl groups at C-1 and C-2. In **1**, **2**, **3** and **5**, the trans conformation of the hydroxyl groups at C-1 and C-2 is retained to form **9**, **10**, **11** and **13** stereoselectively. Furthermore, the high population of β anomer under the reaction conditions [18] induces the yield of more than 30%.

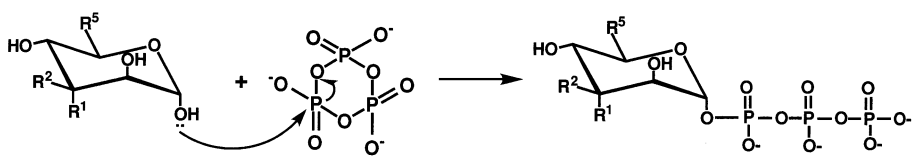
The reactions of **4**, **6**, **7** and **8** with P_{3m} produce their triphosphate derivatives in low yield and with poor stereoselectivity. In spite of the trans conformation of hydroxyl groups at C-1 and C-2 in the α forms of **6**–**8**, their reactivities with P_{3m} are low, probably due to the axial arrangement of their hydroxyl groups at C-2 [19]. In the case of the β form of **4**, a high yield is expected because the situation is similar to that for **1**, **2** and **5**. However, this is not the case, probably because of a low population of the β form of **4** under equilibrium conditions and the existence of furanose forms [18].

The reactivities of D-aldoses strongly depend on the configuration of the hydroxyl groups at C-1 and C-2 and the proportion of their anomers. The reactions of D-aldoses and P_{3m} proceed without difficulty when the hydroxyl groups at C-1 and C-2 are in an equatorial position.

3. Experimental

Chemicals.—Sodium *cyclo*-triphosphate (P_{3m}), $Na_3P_3O_9{\cdot}6H_2O$, was prepared using the procedure described in previous papers [6,20]. D-Galactose (**1**), D-xylose (**2**), D-allose (**5**), D-lyxose (**6**) and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were purchased from Wako Chemicals (Osaka, Japan).

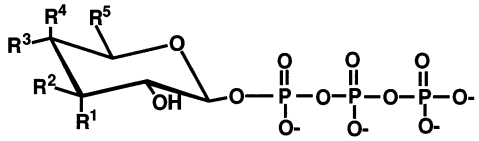
Preparative procedures.—The reactions of D-aldohexoses, except for D-galactose (**1**), or D-aldopentoses (2.5 mol dm^{-3}) with P_{3m} (0.5 mol dm^{-3}) were carried out at pH 12 and room temperature (rt). Because of the lower solubility of **1**, its phosphorylation (1.5 mol dm^{-3}) with P_{3m} (0.5 mol dm^{-3}) was carried at pH 12 and at rt. The yields and the structures of the products were determined respectively by HPLC and NMR spectroscopic measure



	R^1	R^2	R^5
6,14	H	OH	H
7,15	H	OH	CH_2OH
8,16	OH	H	H

Scheme 2.

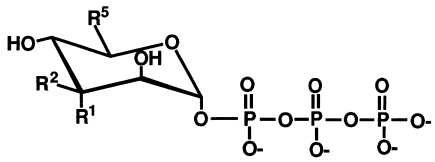
Table 4
Yields of the phosphorylated products and anomer ratio of pyranose forms in reactants



Products	Substituents					Yield (%)	Ratio [a]	
	R^1	R^2	R^3	R^4	R^5		α (%)	β (%)
9	H	OH	H	OH	CH_2OH	31.3	30 [b]	64 [b]
10	H	OH	OH	H	H	32.5	36.5 [b]	63
11	H	OH	OH	H	CH_2OH	44.7 [c]	38	62
12	OH	H	OH	H	H	8.0	21.5 [b]	58.5 [b]
13	OH	H	OH	H	CH_2OH	32.1	14 [b]	77.5 [b]

[a] See Ref. [18].
[b] Furanose form also exists.
[c] See Ref. [7].

Table 5
Yields of the phosphorylated products and anomer ratio of pyranose forms in reactants



Products	Substituents			Yield (%)	Ratio [a]	
	R^1	R^2	R^5		α (%)	β (%)
14	H	OH	H	16.5	70 [b]	28 [b]
15	H	OH	CH_2OH	9.6	65.5	34.5
16	OH	H	H	14.1	60 [b]	35.5 [b]

[a] See Ref. [18].
[b] Furanose form also exists.

ments of the reaction solution. In the case of D-ribose (**4**), D-lyxose (**6**), D-mannose (**7**) and D-arabinose (**8**), the isolation was performed to identify the products because of their low yields.

NMR measurement.—^{13}C NMR spectra with broad-band decoupling and ^1H NMR spectra were measured with Varian Gemini 300 and 200 spectrometers. Samples were dissolved in D_2O (99.9%). DSS was used as an external reference for ^{13}C and ^1H NMR spectra. ^{31}P NMR spectra with and without broad-band decoupling were obtained with a Varian VXR-500 spectrometer. As an external standard, 85% H_3PO_4 was used.

HPLC measurement.—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^{-1}, and the column temperature was maintained at 40 °C. A convex gradient-elution technique using 0.12 and 0.45 mol dm^{-3} of potassium chloride aqueous solutions was employed for the analysis of phosphate. Sugar phosphate esters, diphosphate (P_2), triphosphate (P_3) and

cyclo-triphosphate (P_{3m}) were hydrolyzed to monophosphate (P_1) by 6 mol dm^{-3} H_2SO_4 at 140 °C, and the resulting monophosphate was allowed to react with the chromogenic reagent to form a phosphorus–molybdenum heteropoly blue complex. The absorbance of the blue complex at 830 nm was measured by the spectrophotometer.

Isolation of the phosphorylated products.— The separation of the phosphorylated products of D-ribose (**4**), D-lyxose (**6**), D-mannose (**7**) and D-arabinose (**8**) from the reaction solution was accomplished by anion-exchange chromatography with a 2×80 cm column filled with Dowex 1-X 2 resin (100–200 mesh, Cl^- form). Elution was carried out with 0.3 mol dm^{-3} KCl aqueous solution, and each 100 mL fraction was measured by HPLC. The solution fractionated was concentrated at −113 °C in vacuo (freeze-drying). After freeze-drying, an appropriate aliquot was dissolved in D_2O for HPLC, and for ^1H, ^{13}C and ^{31}P NMR measurements.

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