Synthesis and structural analysis of 3-methoxy-1,2,4-tri-O-acetyl-5-deoxy-5C-(isopropylphosphinyl)-D-pentopyranose and immobilization of its conformation by β-cyclodextrin

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Abstract: The title compound of a heterosaccharide (pseudo sugar) containing a phosphorus atom in the hemiacetal ring (phospha sugar) was synthesized. The conformation and the configuration of the phospha sugar configuration at the anomeric carbon were then characterized by ^{1}H NMR. The analyzed conformation of the title compound was an equilibrium mixture of $^4C_1$ and $^1C_4$ in $CDCl_3$ and DMSO-d_6. When the phospha sugar included in β-cyclodextrin in water, the conformation of the equilibrium mixture was immobilized in the $^1C_4$ conformation alone.

Key Words: Phospha sugar, Synthesis and structure analysis, Immobilization of Conformation, β-Cyclodextrin

Introduction

Various sugar analogs with the oxygen atom in the hemiacetal ring replaced by a heteroatom are of extensive biochemical interest.[1-7] Here, we report the novel synthesis of 3-methoxy-1,2,4-*tri*-*O*-acetyl-5-deoxy-5*C*-(isopropylphosphinyl)-D-pentopyranose as a phospha sugar derivative, along with the detailed studies of its conformation and configuration. Hanaya et al. have reported the synthesis of phospha sugar derivatives, and the detailed structural analyses of their conformation by ^{1}H NMR were reported.[8-9] We have reported that the conformation of these phospha sugar derivatives in solution (as determined by NMR analysis) is the same as that in the solid state (as determined by X-ray crystallographic analysis).[10] In this paper we will deal with the first case of immobilization of the conformation by inclusion with β-cyclodextrin.

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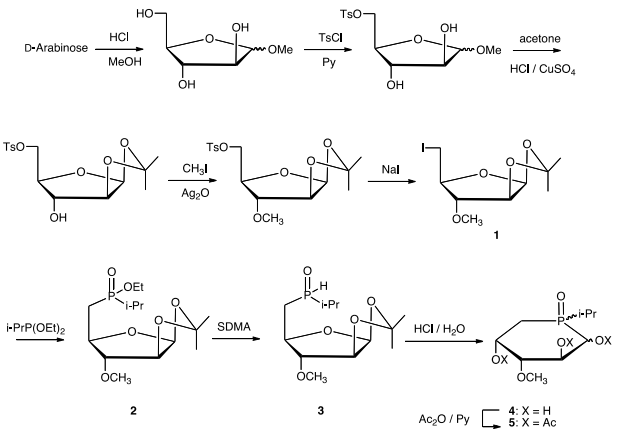
Results and Discussion

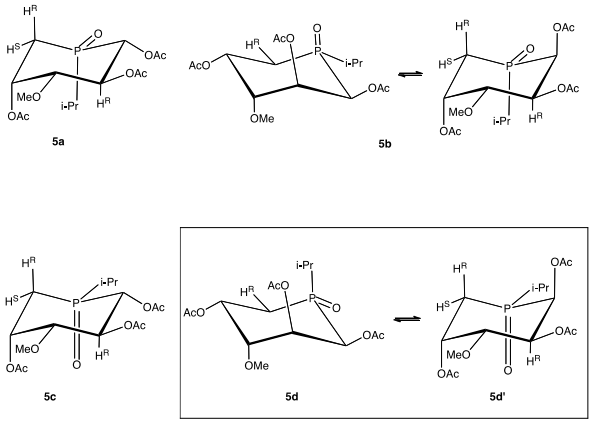
Phospha sugar of the title compound **5** was synthesized from D-arabinose as the starting material. The synthesis process is shown in **Scheme 1**.



Scheme 1. Route of synthesis of phospha-sugar derivative **5** from D-arabinose.

Iodine compound (6-deoxy-6-iodo compound) **1** was derived from D-arabinose by using a general synthetic process. Phosphinate **2** was easily obtained by heating a mixture of compound **1** and excess amount of diethyl isopropylphosphonite at 150 °C. Compound **3** was synthesized by treating compound **2** with sodium dihydrobis(2-methoxyethoxy) aluminate (SDMA). The subsequent opening of furanoid ring of **3** to synthesize the

pyranoid ring were processed under acidic conditions as described in the report.[10] Phospha sugar derivatives **5** of the title compound were obtained in high yield by the acetylation of compound **4**. Diastereomers of phospha sugar **5a**, **5b**, **5c** and **5d** were separated from the mixture of reaction products by flash chromatography. The chemical yields of the diastereomers **5a**, **5b**, **5c**, and **5d** synthesized from compound **1** were 2.0%, 23.5%, 4.0%, and 13.1%, respectively.



Scheme 2. Diastereoisomers of phospha-sugar **5** and their conformations.

The detailed structures of diastereomers **5a**, **5b**, **5c**, and **5d** were determined by their 600 and 400 MHz ^{1}H NMR spectra. The results of the NMR spectra analyzed are summarized in **Table 1**. Structure elucidation was performed carefully to determine the conformations of phospha sugar derivatives **5a** to **5d**. The magnitude relationship between the coupling constants $J_{2,P}$ (17.8–20.8 Hz for **5d** and **5b** vs. 2.9–8.5 Hz for **5a** and **5c**) and $J_{4,P}$ (7.0–9.5Hz for **5b** and **5c** vs. 20–30 Hz for **5a**, **5c** and **5d**) corresponded to the value of the dihedral angle H-C-C-P. As a result, compounds **5a** and **5c** retained the $^1C_4$ conformation, and compound **5b** and **5c** were equilibrium mixture of $^1C_4$ and $^4C_1$ conformation in $CDCl_3$. The moderate magnitudes of $J_{5R,P}$ (8.8-11.0 Hz) support the *anti* orientation of H^R-5-C5-P=O for **5b** and **5d**, and the small long range coupling constants shown $J_{3,5R}$ (0.5-1.2 Hz) for **5b** and **5c**. In a pyranose-type phospha sugar derivative, the coupling constant $J_{2,P}$ required to support the value of the dihedral angle is $J_{2,P}$ = 5.54–5.81 Hz, when P = O and H-2 are axial to each other and $J_{2,P}$ = 22.7–25.3 Hz, when P = O is axial and H-2 is equatorial.[9] Therefore, compounds **5b** and **5c** should present as equilibrium mixtures of $^1C_4$ and $^4C_1$. The orientation of P = O was determined by the chemical shift value (δppm) for H-4 and H^S-5 of compounds **5a** to **5d**. That is, H-2, which is 1,3-diaxial to the orientation of P = O, shifted to the down field. Thus, in $CDCl_3$, compounds **5a** and **5c** retained the $^1C_4$ conformation, and compounds **5b** and **5d** retained equilibrium mixtures of the $^1C_4$ and the $^4C_1$ conformations. In DMSO-d_6,[9, 11-14] the equilibrium mixtures of **5b** and **5d** shifted slightly to the $^1C_4$ enriched conformation (Scheme 2).

Single-crystal X-ray structural analysis showed that the equilibrium mixture of $^1C_4$ and $^4C_1$ was maintained at room temperature in the solid state. This is because the atoms constituting the phospha sugar derivative were disordered at room temperature.[10] The equilibrium mixtures **5d** and **5d'** were included in β-cyclodextrin to immobilize the equilibrium mixture of $^1C_4$ and $^4C_1$ into either the $^1C_4$ or $^4C_1$ conformation. β-Cyclodextrin is widely used in supramolecular chemistry and in the food and chemical industries. Inclusion of a phospha sugar derivative in cyclodextrin may have an interesting effect on the use of the latter in drug delivery.

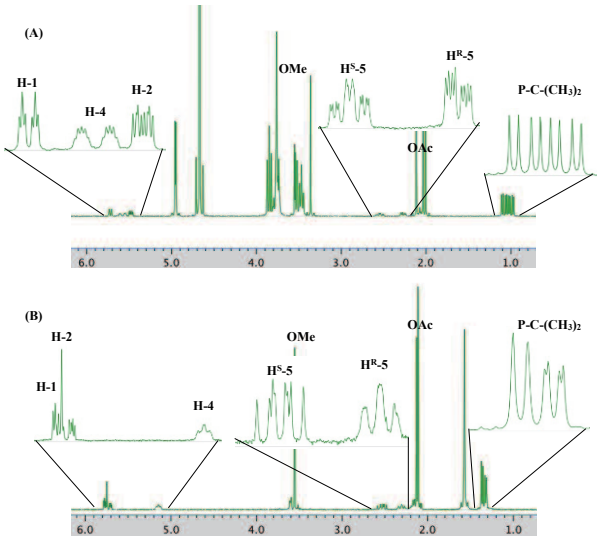


Fig. 1. ^{1}H NMR spectra (400 MHz) of a solution containing (**A**) compound **5d'** (6.0 mM) and β–cyclodextrin (13.1 mM) in D_2O, (**B**) the equilibrium mixture **5d** and **5d'** in $CDCl_3$.

Table 1. ^{1}H and ^{31}P NMR parameters for compounds **5a**, **5b**, **5c**, and **5d**.

Compound	^{1}H NMR Chemical shifts (δ, ppm)													
	H-1	H-2	H-3	H-4	H^{R}-5	H^{S}-5	Ac-1, 2, 4[c]			MeO-3	HC-P	Me-C-P		^{31}P
5a[a]	5.50	5.39	3.50	5.40	2.14	2.51	2.12	2.115	2.10	3.42	2.11	1.33	1.24	45.7
5a[b]	5.58	5.19	3.72	5.47	2.31	2.48	2.065	2.05	2.00	3.27	2.10	1.23	1.09	
5b[a]	5.51	5.58	3.75	5.67	2.26	2.29	2.16	2.15	2.09	3.52	2.02	1.25	1.20	41.2
5b[b]	5.39	5.31	3.76	5.48	2.20	2.31	2.09	2.05	2.02	3.41	2.05	1.095	1.07	
5c[a]	5.37	5.74	3.37	5.38	1.87	2.62	2.17	2.16	2.08	3.44	2.00	1.24	1.235	42.2
5c[a]	5.45	5.54	3.66	5.39	2.04	2.36	2.05	2.005	2.00	3.30	1.94	1.035	1.01	
5d[a]	5.75	5.73	3.60	5.13	2.30	2.53	2.13	2.11	2.105	3.55	2.17	1.34	1.335	41.7
5d[b]	5.44	5.425	3.71	5.32	2.20	2.34	2.13	2.02	2.02	3.39	2.02	1.12	1.04	
5d[e]	5.72	5.48	3.73	5.58	2.27	2.55	2.12	2.04	2.01	3.37	2.05	1.08	1.00	

Compound	^{1}H NMR Coupling constants (Hz)																	
																i-Pr		
	$J_{1,2}$	$J_{1,P}$	$J_{1,5R}$	$J_{1,5S}$	$J_{2,3}$	$J_{2,P}$	$J_{3,4}$	$J_{3,5R}$	$J_{4,5R}$	$J_{4,5S}$	$J_{4,P}$	$J_{5R,5S}$	$J_{5R,P}$	$J_{5S,P}$	$2J_{H,P}$	$3J_{H,H}$	$3J_{H,P}$	
5a[a]	8.4	9.9	0.5	0	7.6	8.4	2.7	0	3.2	7.8	21.5	14.9	d	9.0	d	7.1	15.6	17.1
5a[b]	10.7	11.3	0	0	9.3	4.2	2.9	0	2.9	5.6	30.6	15.6	2.2	11.0	2.2	7.1	15.0	16.9
5b[a]	3.4	0.5	0	0.5	6.1	17.8	2.4	1.0	4.4	10.5	9.5	14.4	9.5	5.6	9.5	7.1	16.4	16.9
5b[b]	3.2	3.0	0	0.8	7.3	13.0	2.5	0.5	3.4	9.3	15.6	14.9	d	6.8	d	7.1	16.1	16.8
5c[a]	8.3	1.0	0.5	0	7.8	8.5	2.2	0	2.7	7.6	19.5	15.1	8.8	16.4	8.8	7.1	17.0	16.4
5c[b]	10.5	2.4	0	0	9.8	2.9	2.7	0	2.7	6.4	27.1	15.3	11.0	15.3	11.0	7.1	11.0	10.5
5d[a]	3.7	12.9	0	0.5	5.9	20.8	2.4	1.2	2.4	11.5	7.0	14.7	d	16.3	d	7.1	14.8	17.1
5d[b]	3.2	11.1	0	1.5	8.5	8.5	2.7	0.5	3.2	7.6	18.1	15.1	d	15.1	d	7.1	16.6	15.9
5d[e]	3.0	10.6	0	2.0	9.8	4.6	2.6	0	3.2	6.0	24.2	16.2	d	15.2	d	7.1	17.8	17.4

a) In $CDCl_3$. b) In DMSO-d_6. c) The assignment of acetyl groups may have to be interchanged. d) Uncertainly because of the overlapping with other signals. e) Inclusion complex with β-cyclodextrin.

When crystals of the equilibrium mixtures **5d** and **5d'** were added to a solution in which ß-cyclodextrin was dissolved in D_2O and the suspension was ultrasonicated for 5 min to become completely clear solution. The ^1H NMR spectra of the **5dd' /** ß-cyclodextrin inclusion complex were shown in **Fig. 1**.

In **Fig. 1 (A)**, the protons on the carbon atom composing the pyranoid ring of equilibrium mixtures **5d** and **5d'** included in ß-cyclodextrin are much more clearly identified than the spectra of **5d** and **5d'** in $CDCl_3$. For example, the H-1 and H-2 signals of equilibrium mixtures **5d** and **5d'** overlapped at δ5.75 to 5.73 ppm (in $CDCl_3$) but were separated into δ5.72 and δ5.48 ppm by inclusion complex with the ß-cyclodextrin. There were significant changes in the value of $J_{1,5S}$ (long-range coupling) for equilibrium mixtures **5d** and **5d'** ($CDCl_3$) from 0.5 to 2.0 Hz (for **5d'**); in the value of $J_{4,P}$ from 7.0 to 24.2 Hz (for **5d'**); and in the value of $J_{2,P}$ from 20.8 to 4.6 Hz (for **5d'**). Because of the clear change in the coupling constants, equilibrium mixtures **5d** and **5d'** completely shifted to the $^1C_4$ conformation of **5d'** due to the inclusion in ß-cyclodextrin. This indicate that compound **5d'** contained at a nearly ideal $^1C_4$ conformation in the molecules of ß-cyclodextrin by these coupling constants. However, compounds **5a**, **5b**, and **5c** were not included in ß-cyclodextrin.

Experimental

Synthetic procedures for compound **5** from **1** as the starting material were shown in a previous paper.[1] ^1H NMR spectra were measured in $CDCl_3$ (TMS as the internal standard) on a Varian Unity Inova AS600 (600 MHz) and JEOL ECX-400 (400 MHz). Melting points were measured with a micro melting point apparatus (Yamato MP-21 Co., Ltd., Japan) and are uncorrected. Column chromatography was performed by flash column chromatography on silica gel (silica gel : KANTO Co., LTD, 20-40μm). The reactions were monitored by TLC on Kieselgel 60 F254 (Merck) with detection by H_2SO_4. Optical rotations were determined with a digital polarimeter DIP-4 (JASCO Ltd.).

5-Deoxy-5-*C*-(ethoxyisopropylphosphinyl)-1,2-*O*-isopropylidene-3-*O*-methyl-ß-D-arabinofuranose (2): 5-Deoxy-5-iodo-1,2-*O*-isopropylidene-3-*O*-methyl-ß-D-arabinofuranose (**1**) (2.514 g) was treated with diethyl isopropylphosphonite as described above, to give syrupy **2** (1.831 g, 67.2 %); Rf = 0.40 (10:1 EtOAc-MeOH); $[\alpha]_D$ = +33.7° (c, 2.67, $CHCl_3$); ^1H NMR ($CDCl_3$): 1.0-1.6 (m, 15H, iPr-P, $OCMe_2$ P-OCMe), 1.7-2.3 (m, 2H, H-5,5'), 3.38, 3.40 (2s, 3H,OMe-3), 3.6-4.4 (m, 4H, H-3,4, P-O-CH_2-), 4.53 (d, $J_{1,2}$=4.1Hz, 1H, H-2), 5.82 (d, $J_{1,2}$=4.1Hz, 1H, H-1).

5-Deoxy-5-*C*-(isopropylphosphinyl)-1,2-*O*-isopropylidene-3-*O*-methyl-ß-D-arabinofuranose (3): Compound **2** (1.73 g) was treated with SDMA (3.62 g, in 70 % toluene) as described above to give syrupy **3** (966 mg, 63.9 %); Rf = 0.46-0.54 (10:3 EtOAc-MeOH); $[\alpha]_D$ =+41.5° (c, 2.41, $CHCl_3$); ^1H NMR($CDCl_3$): 1.0-1.6 (m, 13H, iPr, CMe_2), 2.0-2.6 (m, 2H, H-5,5'), 3.4,3.47 (2s, 3H, Ome-3), 3.84 (d, $J_{3,4}$=2.42Hz, 1H, H-3), 4.4-4.6 (m, 1H, H-4), 4.62 (d, $J_{1,2}$=3.95Hz, 1H, H-2), 5.93 (2d, $J_{1,2}$=3.95Hz, 1H, H-1), 6.8 (broad d, $J_{P\text{-}H}$=456Hz, 1H, P-H).

Hydrolysis of compound 3 and 1,2,4-*tri*-*O*-acethyl-5-deoxy-5-*C*-(isopropylphosphinyl)-*O*-methyl-D-arabino-pyranoses (5): Compound **3** (913 mg) in water (30 mL) was treated with HCl (0.3 mL) as described above to give syrupy **4**, which was treated with acetic anhydride (2 mL) in dry pyridine (5 mL), to afford syrupy mixture **5**. The mixture was separated by medium pressure column chromatography on silica gel, to give **5a**—**5d**. 5-*C*-[(*S*)-isopropylphosphinyl]-α-D-arabino-pyranose derivative (**5a**) (23 mg, 2.0 % from **1**), mp 178-179° (crystallized from EtOAc-hexane); $[\alpha]_D$ = -27. 8° (c, 1.53, $CHCl_3$). Anal. Calcd for $C_{13}H_{23}O_8P$: C, 49.45; H, 6.92. Found: C, 49.50; H, 6.82.5-C-[(*S*)-isopropylphosphin-yl]-ß-D-arabinopyranose derivative (**5b**) (269 mg, 23.5 % from **1**), mp 123.5-124° (crystallized from EtOAc-hexane); $[\alpha]_D$ = -12.4° (c, 2.42, $CHCl_3$). Anal. Calcd for $C_{13}H_{23}O_8P$: C, 49.45; H, 6.92. Found: C, 49.00; H, 7.01. 5-C-[(*R*)-isopropylphosphinyl]-α-D-arabinopyranose derivative (**5c**) (46 mg, 4.0 % from **1**), mp 203-204° (crystallized from EtOAc-n-hexane); $[\alpha]_D$ = -20. 6° (c, 1.58, $CHCl_3$). Anal. Calcd for $C_{13}H_{23}O_8P$: C, 49.45; H, 6.92. Found: C, 48.21; H, 6.91. 5-C-[(*R*)-isopropylphosphinyl]-ß-D-arabino-pyranose derivative (**5d**): 150 mg (13.1 % from **1**), mp

186-187° (crystallized from EtOAc-n-hexane), $[\alpha]_D$ = -27.6° (c, 1.90, $CHCl_3$). Anal. Calcd for $C_{13}H_{23}O_8P$: C, 49.45; H, 6.92. Found: C, 49.62; H, 7.00.

Acknowledgement

This work financial support from National Institute of Technology, Numazu College.

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