PREPARATION AND CONFORMATION OF NOVEL 1,3-OXAZACYCLOPHOSPHAMIDE DERIVATIVES OF SUGARS, AND REACTION OF 1,3-OXAZACYCLOPHOSPHAMIDIC CHLORIDES WITH AMINO ACIDS

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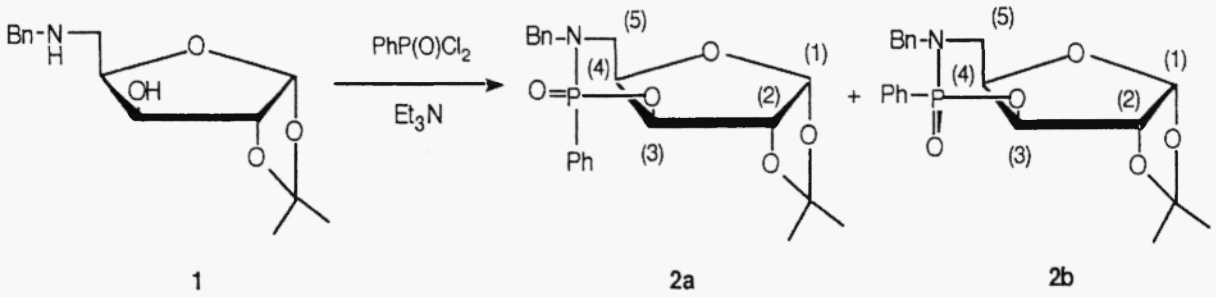
Abstract: Novel 1,3-oxazacyclophosphamide derivatives bearing a sugar structure were prepared by the treatment of the amino sugars derived from D-xylose with phenylphosphonic dichloride as well as with phosphoryl chlolide. The conformations of the cyclophosphamides prepared were determined by ^{1}H-NMR and MOPAC (PM3) method.

Cyclophosphamide derivatives, which are widely used as one of alkylation type anti-cancer agents (1), have previously been prepared via cyclo-condensation of amino alcohols derived from amino acids with substituted phosphoramidodichloridate (2). In our previous paper some amino sugars were used as the starting materials to synthesize cyclophosphamides of sugars (3), however, the conformation of cyclophosphamides derived from amino sugars had not been discussed. The results of experiments concerning the synthesis and conformation of some novel cyclophosphamides are discussed in the present paper.

D-Xylose was employed as a sugar starting material in this study, and the sugar was converted into the corresponding amino sugar derivative according to the previous paper (3). Amino sugar **1** was converted into cyclophosphamide dervatives **2a** and **2b** by a treatment with phenylphosphonic dichloride in the presence of triethylamine (Scheme 1). To a tetrahydrofuran (3.0 ml) solution of 5-benzylamino-5-deoxy-1,2-*O*-isopropylidene-α-D-xylofuranose **1** (100 mg, 0.36 mmol) and triethylamine (80 mg, 0.80 mmol) was added phenylphosphonic dichloride (70 mg, 0.36 mmol) at room temperature. and the mixture was stirred over night. The reaction mixture was filtrated to remove triethylammonium chloride and the filtrate was concentrated *in vacuo*. Products were separated easily by thin layer chromatography on alumina (eluent: ethyl acetate) to afford syrupy diastereomers **2a** (45.9 % yield) and **2b** (48.2 % yield).



1 2a 2b

Scheme 1

The ratio of 1,3-oxazacyclophosphamide derivatives **2a** and **2b** was 1 / 1 on ^{31}P-NMR (4). ^{1}H-NMR Spectraldata for these derivatives shown in Table 1 as well as HPLC analysis clearly showed that the diastereomers formed were separated completely each other by TLC. Stereochemistries at the phosphorus of 1,3-oxazacyclophosphamides **2a** and **2b** were assigned by ^{1}H-NMR, where the chemical shift of C(3)H of diastereomer **2b** (δ , 4.93 ppm) was observed at lower magnetic field than that of diastereomer **2a** (δ , 4.60 ppm) by 1,3-deshielding effect of the P=O group (5). The conformations of these cyclophosphamide derivatives were investigated based on torsion angles estimated by vicinal coupling constants of $J_{POC(3)H}$, $J_{PNC(5)H}$, $J_{PNC(5)H'}$, and $J_{C(3)HC(4)H}$ (Table 1) with Karplus like curve (6).

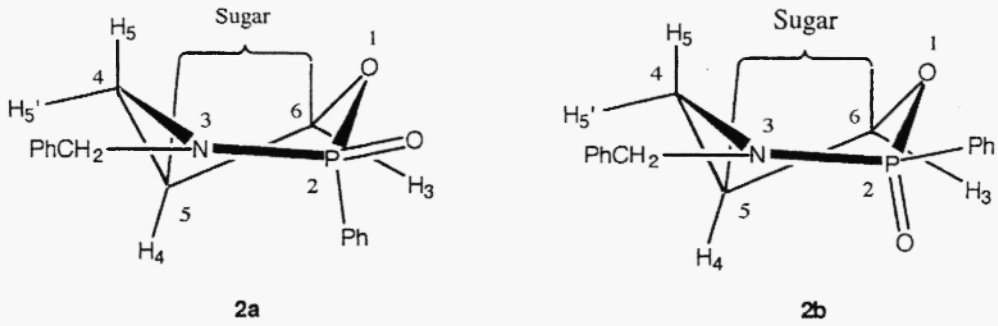
The precise structure of these compounds were confirmed on the basis of the 270 MHz ^{1}H-NMR spectra. The assignments of all signals were completely performed by employing first-order analysis with the aid of a decoupling technique. The splitting patterns of the C(3)H (dd with $J_{POC(3)H}$ = 3.0 Hz and $J_{C(3)HC(4)H}$ = 2 4 Hz ($J_{C(2)HC(3)H}$ was almost nearly 0 Hz)), the C(4)H (ddd with $J_{C(3)HC(4)H}$ = 2.4 Hz, $J_{C(4)HC(5)H}$ =2.7, and $J_{C(4)HC(5)H'}$ = 2.2 Hz), C(5)H (ddd with $J_{PNC(5)H}$ = 15.2 Hz, $J_{C(4)HC(5)H}$ = 2.7, and $J_{C(5)HC(5)H'}$ =15.1 Hz), and C(5)H' signals (ddd with $J_{PNC(5)H'}$ = 2.7 Hz, $J_{C(4)HC(5)H'}$ = 2.2 Hz, and $J_{C(5)HC(5)H'}$ = 15.1 Hz) of diastereomer **2a** were analyzed, therefore, the torsion angles of P-O-C(3)-H, P-N-C(5)-H, and P-N-C(5)-H' being ca. 116° , 129° , and 56° , respectively (from MOPAC (PM3) calculation, the three torsion angles were 131.6° , 168.0° , and 76.9° , respectively), were estimated with Karplus like curves. The difference of values between torsion angles obtained from ^{1}H-NMR spectra and MOPAC (PM3) calculation may reflect the difference of solvation of the molecule in the systems, i.e., in $CDCl_3$ solution and in vacuum, respectively, (7) and the torsion angles imply that the formation of a *twist boat* conformer is most feasible under thermodynamic control. Based on these analyses *twist boat* conformation ($^{O}S_5$) is favorable for **2a** as well as for **2b** in $CDCl_3$ as shown in Figure 1, and these spectral data also suggest the eqatorial preference for the N-benzyl group in the six membered ring, because the chemical shifts and coupling constants of P-

Table 1: ^{1}H-NMR Chemical shifts and coupling constants for diastereomers **2a** and **2b** in $CDCl_3$.[a, b]

Diastereomer	Chemical shift (δ , ppm)									
	C(1)H	C(2)H	C(3)H	C(4)H	C(5)H	C(5')H	N-CH-H'	N-CH-H'	CH_3	CH_3
2a	6.15	4.82	4.60	4.23	3.44	3.25	4.54	4.33	1.45	1.34
2b	5.99	4.66	4.93	4.44	3.44	3.29	4.14	3.93	1.48	1.31

Diastereomer	Coupling constant (Hz) [c]										
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{P,3}$	$J_{4,5}$	$J_{4,5'}$	$J_{5,5'}$	$J_{P,5}$	$J_{P,5'}$	$J_{NCH,NCH'}$	$J_{P,NCH}$	$J_{P,NCH'}$
2a	4.1	~0	2.4	3.0	2.7	2.2	15.1	15.2	2.7	14.9	8.1	5.4
2b	3.8	~0	2.5	2.4	5.4	3.8	14.9	15.4	3.8	14.6	9.7	5.7

a) Numbering of carbon atoms are in accordance with carbohydrate nomenclature. b) Aromatic protons are omitted. c) $J_{1,2}$ Means the coupling constant between C(1)H and C(2)H represented as $J_{C(1)HC(2)H}$ in the text.



2a **2b**

Figure 1: The most stable *twist boat* conformations ($^OS_5$) for diastereomers **2a** and **2b** in $CDCl_3$. The numbers consist with 1,3-oxazacyclophosphamide nomenclature.

N-C(5)-H and P-N-C(5)-H' were suffered from 1,3-deshielding and torsion angle effects. It is well known generally that a *boat* conformation is less stable compared with a *chair* one, however, the present conformer prefers *twist boat* to *chair* form probably because the oxygen atom in six membered ring of cyclophosphamides **2a** and **2b** does not exert a repulsive interaction with C(5)H. Estimation of stabilities for the conformations of structures **2a** and **2b** using MOPAC (PM3) was performed and the most stable conformation reconfirmed was shown as computational graphics in Figure 2. Here, the conformations established by torsion angles based on ^{1}H-NMR and MOPAC (PM3) and by heats of formation estimated by MOPAC (PM3) agreed precisely. MOPAC (PM3) calculation does not include *d*-orbitals, however, the results obtained by the present study seem to be satisfactory enough for the present system. From these calculated results, the heats of formations were -1.21 and -1.20 KJ / mol for *twist boat* conformers **2a** and **2b**, respectively.

Highly stereoselective syntheses of 1,3-oxazacyclophosphamidic chloride **3** and of other related compounds from amino alcohol **1** were reported (3, 9), and an attempted method for novel phosphamide derivatives using chloride **3** was performed as shown in Scheme 2. The chloride on phosphorus atom of the formed cyclophosphamide ring was first replaced by nitrogen nucleophiles such as L-(-)-phenylglycine ethyl ester. A mixture of L(-)-phenylglycine

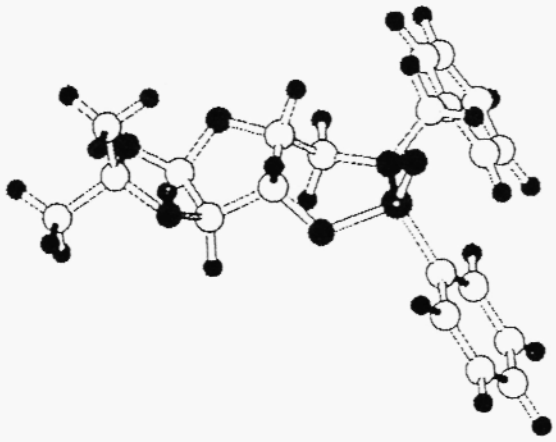
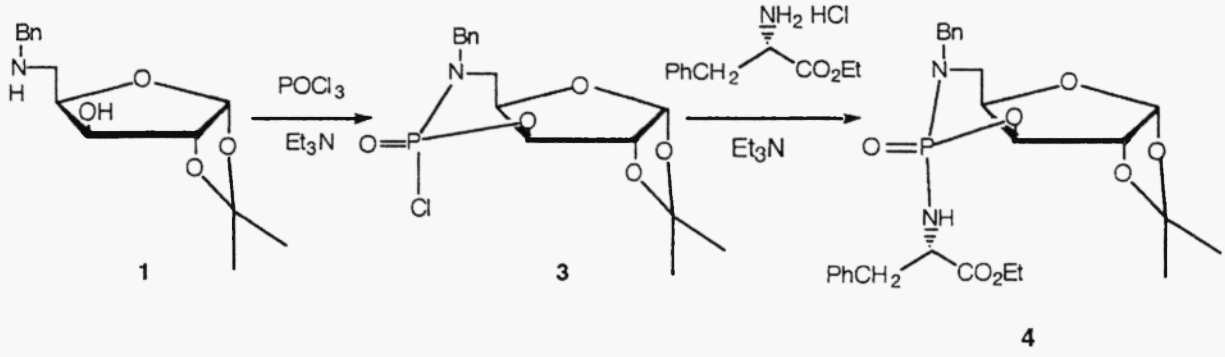


Figure 2: Molecular model of diastereomer **2a**. The structure was fully optimized using the BFGS gradient-minimization routine with energies computed using MOPAC version 6.0 which has been optimized to run in parallel mode on a Silicon Graphics (8).



Scheme 2

ethyl ester hydrochloric acid salt (30 mg, 0.12 mmol), cyclophosphamidic chloride **3** (40 mg, 0.12 mmol), and triethylamine (30 mg, 0.27 mmol) in tetrahydrofuran was stirred for 6 h at room temperature. Work-up and purification of the product by flash chromatography on silica gel afforded cyclophosphamide derivative **4** in 89 % yield. The stereochemistry at phosphorus for compound **4** was determined by ^{1}H-NMR chemical shift of C(3)H signal (δ = 4.21 ppm), which showed that the streochemistry at phosphorus of cyclophosphamidic chloride **3** was (R_P) (10). Thus, configuration at phosphorus of compound **4** might be (R_P) because substitution of the halide ion in chiral 1,3-oxazacyclophosphamidic chloride ring system was known to proceed with complete retention of configuration at phosphorus (11). Studies on the peptide derivatives of these new class of 1,3-oxazacyclophosphamides are now in progress.

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References and Notes

(1) K. Umezawa, *Gendai Kagaku*, 18(1988).

(2) T. Sato, H. Ueda, K. Nakagawa, and N. Bodor, *J. Org. Chem.*, 48, 98 (1983).

(3) M. Yamashita, C. Takahashi, and K. Seo, *Heterocycles*, 36, 651 (1993).

(4) ^{31}P-NMR Chemical shifts of diastereomers **2a** and **2b** were δ = 19.13 and 17.38 ppm, respectively.

(5) (a) D. G. Gorenstein, "Phosphorus-31 P NMR: Principles and Applications, "Academic Press, Orlando, FL., 1984.(b) L. D. Quin and J. G. Verkade, "Phosphorus-31 NMR Spectroscopy in Stereochemical Analysis," Vol

8, VCH Publishers, Inc. Deerfield Beach, FL., 1987. (c) R. Engel; Handbook of Organophosphorus Chemistry, Marcel Dekker, Inc, New York, 1992. (d) D. B. Cooper, J. M. Harrison, and T. D. Inch, *Tetrahedron Lett.*, 2697 (1974). (e) D. B. Cooper, C. R. Hall, J. M. Harrison, and T. D. Inch, *J. Chem. Soc.*, 1967 (1977). (f) J. Devillers, M. Cornus, J. Russell, J. Navech, and F. Mathis, *Org. Mang. Reson.*, 8, 399 (1976).

(6) W. Kung, R. E. Marsh, and M. Kainosho, *J. Am. Chem. Soc.*, 99, 5471 (1977).

(7) Solvent dependence of conformations were elucidated based on PM3 of MOPAC (Stewart) calculation; M. Yamashita, S. Kumagai, and T. Oshikawa, unpublished results.

(8) Calculations were performed using MOPAC version 6.0, and the energies for structures **2a** and **2b** have been minimized by running in MOLGRAPH pakcage (Daikin Industries,Ltd.).

(9) For oxazacyclophosphamidic chloride **3**; ^{31}P-NMR ($CDCl_3$), δ = 6.99 ppm. All spectra of ^{31}P-NMR were measured at 36.10 MHz (JEOL, EX-90), and chemical shift values were given with 85% phosphoric acid (δ , 0.0 ppm) as the external standard with proton decoupling.

(10) (a) V. L. Boyd, V. L. Himes, J. K. Stalick, A. D. Mighell, and H. V. Secar, *J. Med. Chem.*, 23, 372 (1980). (b) W. G. Bentrude, R. O. Day, J. M. Holmes, G. S. Quin, W. N. Stezer, A. E. Sopchik, and R. R. Holmes, *J. Am. Chem. Soc.*, 106, 106 (1984). (c) G. S. Bajwa, S. Chandrasekaran, J. H. Hargis, A. E. Sopchik, D. Blatter, and W. G. Bentrude, *bid*, 104, 6385 (1982).

(11) For compound **4**; ^{31}P-NMR ($CDCl_3$), δ = 8.45 ppm. ^{1}H-NMR ($CDCl_3$), δ = 1.22 (t, 3H, J_{HH} = 7.3 Hz, $CO_2CH_2CH_3$), 1.31 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.92 (br, 1H, NH), 2.88 (dt, 1H, J_{HH} = 7.0 Hz, J_{PNCH} = 15.7 Hz, N-CH), 3.21-3.35 (m, 4H, C(5)-H, C(5)-H', and $PhCH_2CH$), 3.90 (d, 1H, J_{HH} = 6.8 Hz, PhCHH-N), 3.92 (d, 1H, J_{HH} = 6.8 Hz, PhCHH-N), 4.14 (q, 2H, J_{HH} = 7.3 Hz, $CO_2CH_2CH_3$), 4.21 (ddd, 1H, J_{HH} = 1.9 Hz, J_{HH} = 2.2 Hz, and J_{POCH} = 1.9 Hz, C_3-H), 4.41 (d, 1H, J_{HH} = 1.9 Hz, C_4-H), 4.77 (dd, 1H, J_{HH} = 2.2 Hz and J_{HH} = 3.8 Hz, C_2-H), 5.93 (d, 1H, J_{HH} = 3.8 Hz, C_1-H), 7.16-7.38 (m, 10H, aroma).

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