DIASTEREOSELECTIVE PREPARATION AND STRUCTURE OF NOVEL CYCLOPHOSPHAMIDE DERIVATIVES OF AMINO ACIDS

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Abstract: The reaction of amino alcohol **1** derived from L-phenylalanine being protected the N-terminus by a benzyl group with phosphorus oxychloride afforded stereospecifically a single diastereoisomer of the novel cyclophophamidic chloride **2**, whose successive reaction with some amino acid esters prepared novel cyclophosphamide derivatives **3** having the amino acid residues on the phosphorus atom of the heterocycles. Structures of **2** and **3** were elucidated by X-ray and ^{1}H-NMR analyses.

Introduction

Some bis-(2-chloroethyl)aminocyclophosphamides are widely used as one kind of alkylation type anti-cancer agents (1), however, the bis-(2-chloroethyl)amino group of the cyclophosphamides has an inherent characteristics of toxicity. Therefore, in order to suppress the toxicity and to find out some novel and usuful biological activities studies on preparation of novel cyclophosphamide derivatives were performed (2). The present paper deals with synthesis of some cyclophosphamide derivatives having an amino acid residue on the phosphorus atom instead of the bis-(2-chloroethyl)ethylamino group as well as with the structural analyses by X-ray single crystallography and ^{1}H-NMR spectroscopy.

Results and Discussion

L-Phenylalanine was used as the starting material in this study, because L-phenylalaninol derived from L-phenylalanine might have the known stereochemistry and the cyclocondensation reaction of the amino alcohol with phosphorus oxychloride was expected to give stereospecifically the corresponding single stereoiosmer of cyclophosphamidic chloride (3). Stereospecific preparation of materials are, in general, very important in view of bioactivity, because of the distinct differences between the stereoisomers. L-Phenylalanine (4) was converted stereo-specifically into (*S*)-2-benzylamino-3-phenyl-1-propanol (**1**) by a known method.

To a dry THF solution (30 ml) of amino alcohol **1** (5.0 g, 21 mmol) and triethylamine (6.0 ml, 43 mmol) was added dropwisely a THF solution (30 ml) of phosphorus oxychloride (2.0 ml, 22 mmol) at 0 ℃, and the mixture was stirred for 1d at room temperature. Work-up and purification of the product by recrystailization from ethyl acetate afforded cyclophosphamidic chloride **2A** (4.5 g, 15 mmol) in the pure form in 70% yield giving the single crystalline diastereoisomer. Result of the X-ray single crystallography of compound **2A** confirmed that (i) racemization was not occured during the synthetic steps from L-phenylalanine to compound **2A**; (ii) configuration at phosphorus of compound **2A** was *S* (*Sp*); and (iii) conformation of the cyclophosphamide ring was ^5E (Table 1 and Figure 1). The reaction of **1** with phosphorus oxychloride may proceed through $S_N2(P)$ type substitution involving face attack firstly by the nucleophilic nitrogen atom then by the oxygen atom of the amino alcohol, and then the pseudo rotation around the phosphorus atom may occur (5).

In the intermediary formed pentacoordinate phosphorus species apical orientation of the electron negative group and apical-equatorial orienatlon of the five membered ring stabilize the structure (5). The MOPAC PM3 caluculation to estimate the stability of the plausible diastereoisomeric chlorides **2A** and **2B** revealed that **2A** was more stable than **2B** by 5.5 kJ/mol (6).

Table 1. Selected torsion angles (°), bond angles (°) and bond lengths (Å)for compound **2**.[a)]

Selected dihedral	Angle (°)	Selected bond	Angles (°)	Selected bond	Length (Å)
P(1)-O(1)-C(2)-C(1)	-32.7	P(1)-N(1)-C(1)	112.8	O(1)-C(2)	1.45
P(1)-N(1)-C(1)-C(2)	-14	N(1)-C(1)-C(2)	103.1	P(1)-O(1)	1.583
O(1)-C(**2**)-C(1)-N(1)	28	C(1)-C(**2**)-O(1)	107.7	P(1)-N(1)	1.633
O(1)-P(1)-N(1)-C(1)	-3.6	C(2)-O(1)-P(1)	109.4	N(1)-C(1)	1.47
N(1)-P(1)-O(1)-O(2)	21.7	O(1)-P(1)-N(1)	97.4	C(1)-C(2)	1.54
C(3)-N(1)-P(1)-O(1)	173.0	O(2)-P(1)-Cl(1)	109.8	P(1)-O(2)	1.451
C(3)-N(1)-C(1)-C(2)	169.2	O(2)-P(1)-N(1)	120.6	P(1)-Cl(1)	1.995
C(10)-C(1)-N(1)-P(1)	-133.6	O(2)-P(1)-O(1)	115.1	N(1)-C(3)	1.46
C(10)-C(1)-C(2)-O(1)	148.1	Cl(1)-P(1)-N(1)	107.3	C(1)-C(10)	1.56
O(2)-P(1)-N(1)-C(1)	-128.6	Cl(1)-P(1)-O(1)	105.1	C(3)-C(4)	1.54
O(2)-P(1)-O(1)-C(2)	150.5	C(3)-N(1)-P(1)	125.3	C(10)-C(11)	1.49
Cl(1)-P(1)-N(1)-C(1)	104.8	C(3)-N(1)-C(1)	121.8	C(1)-H(14)	0.76
Cl(1)P(1)-O(1)-C(2)	-88.6	C(10)-C(1)-N(1)	111.6	C(2)-H(11)	1.23
C(4)-C(9)-C(8)-C(7)	0	C(10)-C(1)-C(2)	111.2	C(2)-H(15)	1.14

a) Rigaku AFC7R X-ray diffractometer with four-axis goniometer was used. Crystal data: Empirical formula, $C_{16}H_{17}NO_2ClP$; Formula weight, 321.74; Crystal color, Habit, colorless, prismatic; Crystal dimensions, 020x0.20x0.30 mm; Crystal system, orthorhombic; Lattice type, primitive; No. of reflections used for unit cell determination (2θ range), 24 (36.6-54.1 °); Omega scan peak width at half-height, 0.46° ; Lattice parameters, a=11.297 Å, b=19.404 Å, c=7.252 Å, V=1589.6 Å^3; Space group, $P2_12_12_1$(#9); Z value, 4; D_{calc}, 1.344g/cm^3; F_{000}, 672.00; μ (CuK α), 31.10 cm^{-1}. Intensity measurments: Diffractometer, Rigaku AFC7R; Radiation, CuK α (λ =1.54178 Å), graphite monochormated; Attenuator, Ni foil (factors=1.00, 9.43, 9.43, 9.43); Take-off angle, 6.0° ; Detector aperture, 9.0 mm horizontal, 13.0 mm vertical; Crystal to detector distance 235 mm; Temperature 20.0℃; Scan type, ω-2θ ;Scan rate, 16.0° /min (in ω)-up to 3 scans; Scan width, (1.68 +0.30 tan θ)° ; 2θ_{max} 120.1° ; No. of reflections measured, total 1415; Corrections, Lorentz-polarization, secondary extinction (coefficient: 9.43065e-07). Structure solution and refinement: Structure solution, Direct methods (SHELXS86); Refinement, full-matrix least-squares; Function minimized, $\Sigma w(|Fo|-|Fc|)^2$; Least squares weights, $1/\sigma^2(Fo)=4Fo^2/\sigma^2(Fo^2)$; p-factor, 0.00; Anomalous dispersion, all non-hydrogen atoms; No. observations ($I > 3.00\ \sigma(I)$), 958; No. variables, 259; Reflection/Parameter ratio, 3.70; Residuals: R; Rw , 0.052; 0.039; Goodness of fit indicator, 2.12; Max shift/Error in final cycle, 2.91; Maximum peak in final diff. map, 0.24e$^-$/Å^3; Minimum peak in final diff. map, -0.29e$^-$/Å^3.

Substitution of the chlorine atom on the phosphorus atom of the cyclophosphamidic chloride **2** by L-amino acid (Gly, Ala, Phe, Leu, Asp, and Gly-Leu) methyl and/or benzyl esters was attempted to prepare novel cyclophosphamide derivatives having amino acid residues as shown in Scheme 1. General procedure is as follows: a mixture of cyclophosphamidic chloride **2** (0.84 g, 2.6 mmol), L-alanine benzyl ester hydrochloric acid salt (0.91 g, 2.6 mmol), and triethylamine (0.72 ml, 5.2 mmol) in THF (30 ml) was stirred for 1d at room temperature. Work-up and purification of the product by column chromatography on silica gel afforded cyclophosphamide derivative **3f** (0.91 g, 2.0 mmol) in 76% yield as a single diastereoisomer, whose purity was confirmed by ^{31}P-NMR (100% de). The results are shown in Table 2 and the ^{1}H-NMR (JEOL EX 270 (270 MHz)) spectral data for compound **3b** are shown in Table 3.

The conformation of these cyclophosphamide derivatives was investigated based on dihedral angles estimated by vicinal coupling constants of ^{1}H-NMR with Karplus like curve (7). The results revealed that ^5E is most probable conformation for cyclophosphamide **3b** in $CDCl_3$ as shown in Figure 2.

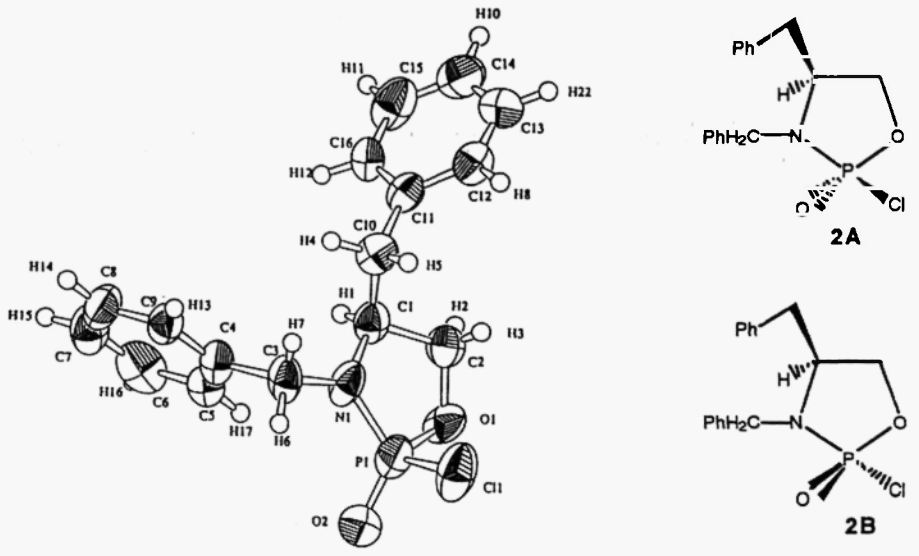
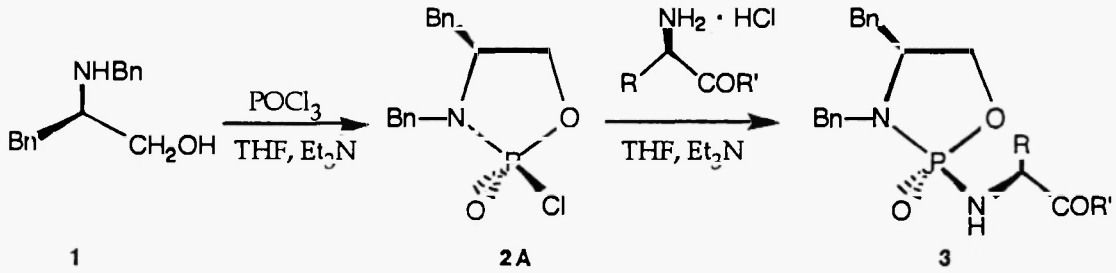


Figure 1. ORTEP Drawing of compound **2A** and diastereoisomeric structures of **2A** and **2B**.



Scheme 1. Preparation of cyclophosphamide derivatives **3** having an amino acid residue.

Table 2. Yields, ^{31}P-NMR data, and eluents of chromatography on silica gel for compounds **3a-j**.

Compd No.	R	R'	Yield (%)	^{31}P-NMR (δ /ppm)[a]	Eluent[b]
3a	H	OMe	32	26.90	A
3b	Me	OMe	42	25.92	A
3c	Bn	OMe	43	25.44	A
3d	$CH_2CH(CH_3)_2$	OMe	49	26.70	B
3e	H	OBn	69	27.20	A
3f	Me	OBn	76	25.34	B
3g	Bn	OBn	54	25.04	A
3h	$CH_2CH(CH_3)_2$	OBn	63	25.73	B
3i	CH_2CO_2Bn	OBn	36	26.11	C
3j	H	$NHCH(CH_2CH(CH_3)_2)CO_2Me$	75	28.66	D

a) ^{31}P-NMR of compound **2A** showed δ =24.211ppm. b) Eluents are as follows: A: ethyl acetate:n-hexane=2:1; B: ethyl acetate:n-hexane=1:1; C: ethyl acetate:methanol=1:10; D: ethyl acetate.

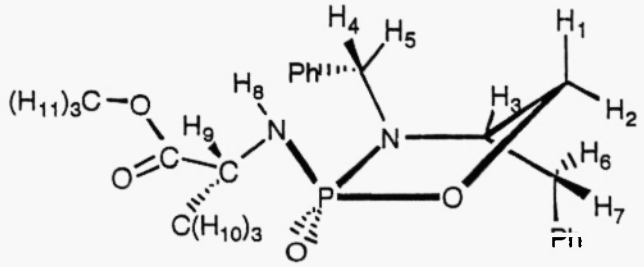


Figure 2. Plausible conformation and configuration of compound **3b**.

The stereochemistry at phosphorus for compound **3b** was further confirmed by ^{1}H-NMR chemical shifts of H_1 (δ =3.98 ppm) and H_2 (δ =3.83 ppm) signals, which might be affected by a little 1,3-shielding effect of the P=O group. These results support that the configuration at phosphorus of cyclophosphamide derivatives **3** is *R*(*Rp*) and agrees well with a knowledge that substitution of the halide ion on phosphorus atom in the chiral 1,3-oxazacyclophosphamidic chloride ring system proceeds with complete retention of configuration at phosphorus (8). Studies on the synthesis, structure, and activity of cyclophosphamides are in progress.

Table 3. ^{1}H-NMR Chemical shifts and coupling constants for compound **3**b in $CDCl_3$.

Chemical shift (δ /ppm)										
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11
3.98	3.83	3.43	4.01	4.37	2.49	2.91	2.85	3.90	1.29	3.68

Coupling constant (Hz)[a)]												
$J_{1,2}$	$J_{1,3}$	$J_{1,P}$	$J_{2,3}$	$J_{2,P}$	$J_{3,6}$	$J_{3,7}$	$J_{3,P}$	$J_{4,5}$	$J_{4,P}$	$J_{6,P}$	$J_{6,7}$	$J_{9,10}$
14.79	9.57	~0	4.59	9.19	8.79	4.05	~0	13.23	4.05	8.91	14.78	6.79

a) $J_{1,2}$ Means the coupling constant between H1 and H2 being represented as H_1 and H_2, respectively, in Figure 2.

Acknowledgement

Financial supports by a Grant-in Aid for Scientific Research (No. 04304049) from the Japanese Ministry of Education, Science, Sports and Culture and by Nippon Soda Co.Ltd. are greatly appreciated.

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Received on January 22, 1997