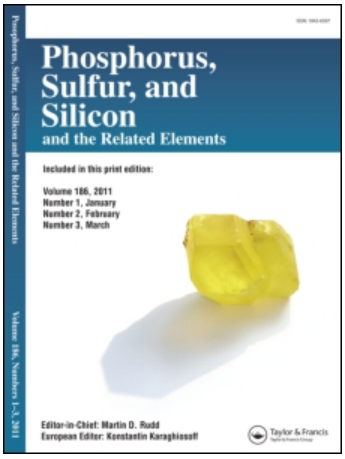

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Preparation and Characterization of Phospha Sugar Analogues, 2,3-Dibromo-3-methyl-1- phenylphospholane 1-Oxide Derivatives, as Novel Anticancer Agents

Manabu Yamada[a]; Kazuhide Asai[b]; Junko Yamashita[a]; Takuya Suyama[b]; Taishi Niimi[b]; Kasthuraiah Maddali[a]; Michio Fujie[ac]; Satoki Nakamura[c]; Mitsuji Yamashita[a]
[a] Department of Nano Materials, Graduate School of Science and Technology, Shizuoka University, Hamamatsu, Japan [b] Department of Materials Science, Graduate School of Engineering, Shizuoka University, Hamamatsu, Japan [c] Department of Internal Medicine III, Hamamatsu University School of Medicine, Hamamatsu, Japan

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PREPARATION AND CHARACTERIZATION OF PHOSPHA SUGAR ANALOGUES, 2,3-DIBROMO-3-METHYL-1-PHENYLPHOSPHOLANE 1-OXIDE DERIVATIVES, AS NOVEL ANTICANCER AGENTS

Manabu Yamada,[1] Kazuhide Asai,[2] Junko Yamashita,[1] Takuya Suyama,[2] Taishi Niimi,[2] Kasthuraiah Maddali,[1] Michio Fujie,[1,3] Satoki Nakamura,[3] and Mitsuji Yamashita[1]

[1]*Department of Nano Materials, Graduate School of Science and Technology, Shizuoka University, Hamamatsu, Japan*
[2]*Department of Materials Science, Graduate School of Engineering, Shizuoka University, Hamamatsu, Japan*
[3]*Department of Internal Medicine III, Hamamatsu University School of Medicine, Hamamatsu, Japan*

The synthesis of new phospha sugar analogues or phosphorus heterocycles and their biological activities as novel anticancer agents are reported in this article. A 1,2-dibromo-1,2-dideoxy phospha sugar derivative, 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (2), was prepared from 1-phenyl-3-methyl-2-phospholene 1-oxide (1), and the yield and ratio of diastereomers 2a to 2d were changed by a catalyst such as manganese(IV) oxide and manganese(II) bromide. The antitumor activities of the mixture of dibromides 2 and the separated diastereomeric components 2a to 2d of the dibromides were evaluated by MTT in vitro method against the human leukemia cell lines of K562 and U937. The results showed that all of the diastereomers 2a to 2d as well as the diastereomer mixture exert excellent anticancer activity, and moreover, among them, diastereomer 2d showed the highest antitumor activity.

Supplemental materials are available for this article. Go to the publisher's online edition of* Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.

Keywords Antileukemia; dibromodideoxyphospha sugars; MTT *in vitro* method; phosphorus heterocycles

INTRODUCTION

Well known typical pseudo sugars are *carba*-, *aza*-, and *thia*-sugars,[1–3] with a carbon, nitrogen, and sulfur atom, respectively, instead of the oxygen atom in the hemiacetal

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Address correspondence to Mitsuji Yamashita, Department of Nano Materials, Graduate School of Science and Technology, Shizuoka University, Johoku 3-5-1, Naka-ku, Hamamatsu 432-8011, Japan. E-mail: tcmyama@ipc.shizuoka.ac.jp

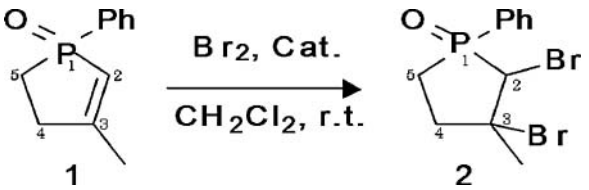
ring of the normal sugars. These pseudo sugars are known to exist in nature and are also prepared through synthetic sugar chemistry, where generally pentoses or hexoses are used as the starting materials for synthesizing pseudo sugars via a series of reactions of protection of specific hydroxyl groups, carbon-hetero atom bond formation, deprotection, and finally heterocycle formation by the reconstruction of the hemiacetal ring of sugars.[4] Pseudo sugars exert important biological activities; therefore, many studies on not only the isolation and synthesis of pseudo sugars but also their characterization and evaluation are actively performed. On the other hand, phospha sugars, one new category of the pseudo sugars that have a phosphorus atom in the hemiacetal ring of sugars, are not yet found in nature and the syntheses of them are rather difficult compared with typical pseudo sugars.[5–8]

We have been searching biologically active phospha sugars, and we have first found new phospha sugar derivatives with antitumor activities against leukemia cell lines by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) *in vitro* evaluation methods.[9] In this article, we will deal with the successful preparation of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**) in high yields by catalysts and the separation of the diastereomers. The evaluation of the biological activity against human leukemia cell lines for dibromides of the mixture **2** and each component **2a**–**2d** against the human leukemia cell lines K562 and U937 are first reported here.

RESULTS AND DISCUSSION

2,3-Dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**) was prepared by an addition reaction of bromine with 3-methyl-1-phenyl-2-phospholene 1-oxide (**1**) (Scheme 1). The addition reaction of bromine to the electron-deficient C=C double bond of 2-phospholene **1** was achieved by a catalyst, i.e., manganese(IV) oxide, manganese(II) bromide, copper(II) bromide, zinc(II) bromide, nickel(II) chloride, under the reaction conditions. The yield of dibromodideoxyphospha sugar **2**, or dibromophospholane **2**, by using manganese(II) bromide catalyst was increased from 33% to 96%, and the results of the catalytic bromine addition are summarized in Table I. The catalyst (Table I, entries 2–8) improved the yield of dibromide **2**. Entries 3–5 imply that the reaction is catalyzed with 0.5 equivalent of manganese(II) bromide within 1 h to give up to 96% yield of the product. The addition reaction of bromine to the double bond of 2-phospholenes in anhydrous media hardly proceeded in the absence of catalyst; however, the reaction in aqueous media is reported to afford 2-bromo-3-hydroxyphospholane derivatives.[10,11]

The addition reaction mechanism of bromine to 2-phospholene **1** by using $MnBr_2$ as a catalyst and the structures of the four diastereomers **2a** to **2d** are shown in Figure 1. The addition of bromine to the C=C double bond is known,[12] and the reaction proceeds via a nucleophilic attack of the bromo anion to the bromonium intermediate. According



1 2

Scheme 1 Synthesis of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**). (The numbering for phospholene and phospholane rings is shown in the structures.)

Table I Preparation of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**) by addition reaction of bromine with 2-phospholene by catalyst

Entry	Catalyst	Conditions[a]	Yield%
1	None	CH_2Cl_2, r.t., 48 h	33
2	MnO_2 (2.0 equiv)	CH_2Cl_2, r.t., 8 h	78
3	$MnBr_2$ (1.0 equiv)	CH_2Cl_2, r.t., 12 h	92
4	$MnBr_2$ (0.5 equiv)	CH_2Cl_2, r.t., 4 h	94
5	$MnBr_2$ (0.5 equiv)	CH_2Cl_2, r.t., 1 h	96
6	$CuBr_2$ (0.5 equiv)	CH_2Cl_2, r.t., 1 h	94
7	$ZnBr_2$ (0.5 equiv)	CH_2Cl_2, r.t., 1 h	86
8	$NiCl_2$ (0.5 equiv)	CH_2Cl_2, r.t., 1 h	61

[a]R.t. = room temperature.

to the reaction mechanism, the addition reaction of bromine with manganese(II) bromide may proceed by nucleophilic attack of $MnBr_3^-$ at either position of C(3) or C(2) of the formed bromonium intermediate, and then the two vicinal bromo substituents should be added in *trans*-fashion. While the *cis*-dibromide may be formed either by the reaction of intermedially formed carbocation[12] on the third position [$C(3)^+$] with bromo anion or the isomerization of the *trans*-fused dibromide via a stable tertiary carbonium intermediate at C(3) position by S_N1 mechanism, which was observed for 3-methylphospholane 1-oxides.[11]

The diastereomeric components of mixture **2** in the product (Table I, entries 2 and 5) were separated by HPLC (column: Wakopak Wakosil Φ 20.0 mm × 250 mm; eluent: $CHCl_3$:MeOH = 30:1; flow rate: 5.0 mL/min) into the four diastereomers **2a** to **2d** (Table II), and the structures of the diastereomers **2a** to **2d** of the fractions with the retention times of 8.1, 9.1, 9.9, and 11.5 min should be assigned to the structures shown in Figure 1 on the bases of the chemical shift values of C(2)-H and C(3)-Me on the 2- and 3-positions, respectively, of the phospholane ring whose proton chemical shifts in ^{1}H NMR spectra are affected by the magnetic anisotropy effect caused by the phenyl group on the phosphorus heterocycle. By this anisotropy effect, the same side of hydrogen on the second position [C(2)H] of the phospholane **2** with the phenyl group was shifted to relatively higher magnetic field. On the other hand, the hydrogen on the opposite side with the phenyl group shifted to relatively lower field. The methyl group on the third position [$C(3)CH_3$] was also shifted by the anisotropy effect to the higher or lower magnetic field depending on the same

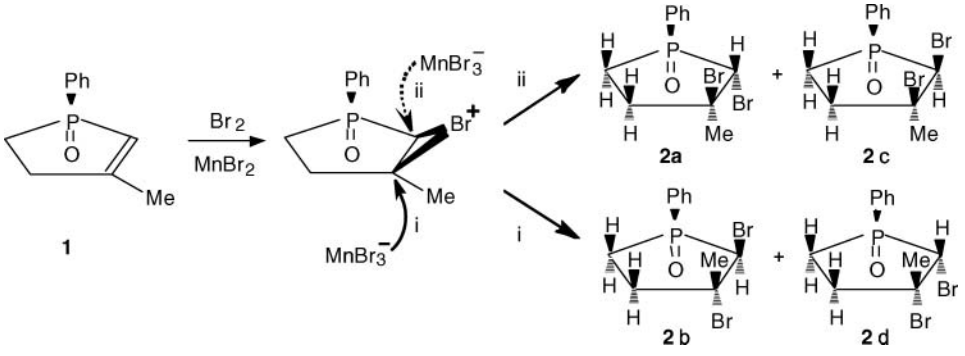


Figure 1 Addition reaction mechanism of bromine to 2-phospholene **1** and structures of diastereomers **2a** to **2d**.

Table II Diastereomers **2a** to **2d** of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**) by MnO_2 and $MnBr_2$ catalyst

Compound	Retention Time of HPLC[a]/ min	^{1}H NMR[b] chemical shift /δ ppm (Coupling constant J_{HP} /Hz)				Ratio of diastereomer[c]/% catalyst	
		C(2)-H	C(3)-Me	C(4,5)-H	P(1)-Ph-H	MnO_2	$MnBr_2$
2a	8.1	4.19(7.8)	1.56	2.20–3.10	7.53–7.86	27	3
2b	9.1	4.51(7.3)	1.55	2.24–2.95	7.51–7.84	32	36
2c	9.9	4.51(5.1)	1.56	2.24–3.13	7.51–7.88	23	6
2d	11.5	4.20(7.2)	1.52	2.22–3.05	7.48–7.84	18	55

[a]The retention time was observed by HPLC analysis (column: Wakopak, Wakosil Φ 4.6 mm × 250 mm; eluent: $CHCl_3$:MeOH = 30:1; flow rate: 0.5 mL/min).

[b]300 MHz (CD_3OD) assigned proton chemical shift in ppm and coupling constant in Hz.

[c]Diastereomer ratio is based on that of HPLC peak area.

or opposite side of the phenyl group, respectively.[13] The largest retention time for **2d** may be supported by the large dipole moment (7.05 D) calculated by MOPAC.

Evaluations of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**; mixture of diastereomers) and the separated four diastereomers **2a** to **2d** as inhibitors on proliferation of leukemia cells were carried out by the *in vitro* MTT assay method.[14] The results against K562 and U937 cells are shown in Figure S1 (Supplemental Materials, available online). The observed antiproliferative effect of dibromide **2** on U937 cells observed was much more efficient than that of Gleevec (imatinib mesylate), which is known as a molecule targeting chemotherapeutic agent.[15]

In conclusion, we have reported the new synthesis of 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**), which was prepared in up to 96% yields, and the ratio of the four diastereomers **2a** to **2d** was varied by the kind of catalyst used. The MTT *in vitro* bioassay method revealed that the prepared dibromophospholane (**2**; mixture of diastereomers **2a** to **2d**), especially diastereomer **2d** among the four diastereomers, have quite efficient anticancer activities for leukemia cells in manners of (i) wide spectra, (ii) high activities, and (iii) high specificities and selectivities. Optimization of the structure–activity relationships is in progress.

EXPERIMENTAL

Instrumentation Details

TLC (Silica gel: Wako Chromato Sheet and/or Merk Kieselgel 60; eluent: $CHCl_3$:MeOH = 20:1, in R*f* value); melting point apparus (Gallenkamp, in °C) and thermal analysis instrument (Shimazu: DTG-60A50AH, TGA & DSC, in °C); HPLC (GL Science: GL-7410 HPLC Pump and GL-7450 UV Detector); MS (MALDI-TOF-MS: GL Science, Voyager-DE Porimerix; Matrix: α-Cyano-4-hydroxycinnamic acid, in *m/z*); IR (JASCO FT/IR 410 (KBr), in cm^{-1}); ^{1}H NMR (JEOL JNM-AL300 (300 MHz) and Hitach R90H (90 MHz); solvent: $CDCl_3$, in δ (ppm) from TMS were used for analyzing the products.

Synthesis of 2,3-Dibromo-3-methyl-1-phenylphospholane 1-oxide (2)

To a CH_2Cl_2 (10 mL) solution of 3-methyl-1-phenyl-2-phospholene 1-oxide (**1**; 0.28 g, 1.5 mmol) and manganese(II) bromide (0.16 g, 0.74 mmol; 0.5 eq.), CH_2Cl_2 (10 mL) solution of bromine (0.40 mL, 9.8 mmol; 7.0 eq.) was added dropwise, and the reaction mixture was stirred for 1 h under Ar atmosphere at room temperature. The reaction was quenched by the addition of saturated sodium sulfite aqueous solution (10 mL). The aqueous mixture was extracted with chloroform (3 × 10 mL). The organic layer was neutralized with saturated $NaHCO_3$ aqueous solution, washed with saturated NaCl solution, and dried with anhydrous sodium sulfate. The solvent of the filtrate was evaporated under reduced pressure to give an oily mixture of product **2**. The mixture was purified by column chromatography on silica gel by using chloroform and methanol (30:1) as the eluent to give 2,3-dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**, 0.49 g) in 96% yield.

R*f*: 0.52; mp: 189.20°C; bp: 280.24°C; MS; (m/z), 353.24 (M-H^+ (Molecular peak-1); IR: 1126 cm^{-1} (P = O), 748 cm^{-1}, 1396 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 300 MHz): δ: 1.67 (s, 3H, CH_3), 2.36–2.46 (m, 2H, C(4)H_2), 2.97–3.02 (m, 2H, C(5)H_2) 4.28–4.31 (m, 1H, C(2)H), 7.51–7.70 (m, 5H, Ph).

The diastereoisomers of product **2** were separated by preparative HPLC (column: Wakopac, Wakosil Φ 20.0 mm × 250 mm (Wako Gel); eluent: chloroform:methanol = 40:1; flow rate: 5.0 mL/min) to give the four diastereomeric components **2a** to **2d** (^{1}H-NMR and HPLC, data are shown in Table II). The MOPAC calculation for dipole moments of **2d** was 7.05 D, which was larger than those of **2a**–**2c** (dipole moments: 5.43, 5.65, and 4.05 D).

In Vitro MTT Assay

2,3-Dibromo-3-methyl-1-phenylphospholane 1-oxide (**2**), being evaluated by the *in vitro* MTT method, was dissolved in dimethylsulfoxide (DMSO) (Sigma Chemical Company, St. Louis, MO, USA) as the solvent, and was diluted into appropriate concentration with DMSO in culture medium immediately before use. The final concentrations of **2** in DMSO in all experiments were less than 0.010%, and all the treatment conditions were compared with vehicle controls. The control experiments for the evaluation were carried out by using DMSO, and the absorption change by DMSO in the MTT method was not observed for K562 and U937 cells at 37°C for 48 h.

Human Tumor Cell Lines and Culture

Chronic myeloid leukemia (K562) and promyeloid leukemia (U937) cells were purchased from American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) 292 mg/L (or 2.0 mmol) L-glutamine, 100 μg/mL streptomycin, and 200 U/mL penicillin (GIBCO-BRL, Gaithersburg, MD, USA). All cells were maintained in a humidified 5% CO_2 atmosphere at 37°C.

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