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Preparation and characterization of novel 4-bromo-3,4-dimethyl-1-phenyl-2-phospholene 1-oxide and the analogous phosphorus heterocycles or phospha sugars

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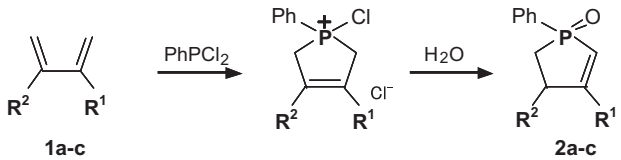
ABSTRACT

4-Bromo-3,4-dimethyl-1-phenyl-2-phospholene 1-oxide (**3c**) was first synthesized from 3,4-dimethyl-1-phenyl-2-phospholene 1-oxide (**2c**) by a bromo-radical substitution reaction occurred at C-4 position by *N*-bromosuccinimide and 2,2′-azobisisobutyronitrile. The novel phospha sugar analogue **3c** exerted high anti-proliferative effect on U937 cells evaluated by MTT in vitro methods and was much more efficient than that of Gleevec®, which is known as a molecule targeting chemotherapeutical agent. The substitution of 2-phospholenes at C-3 and C-4 position with methyl groups as well as 4-bromo substituent suggests a good anti-proliferative effect.

Phospha sugars have a phosphorus atom instead of the oxygen atom in the hemiacetal ring of the normal sugars and they construct one new category of the pseudo sugars.[1–4] Well known typical pseudo sugars are *carba*-, *aza*-, and *thia*-sugars,[5–7] with a carbon, a nitrogen, and a sulfur atom instead of the oxygen atom, respectively, in the hemiacetal ring of the normal sugars, which are known to exist in nature and are also prepared by synthetic sugar chemistry. Many of them exert important biological activities, therefore, a lot of studies on them have actively been performed. Recently bioactivities of phospha sugars were found, especially, deoxybromophospha sugars exert important biological activities and have quite efficient anti-cancer activities for leukemia cells.[8] Since then researches on synthesis and evaluation of phospha sugars are rapidly progressing to develop new type of molecular targeting chemotherapeutic anti-tumor agents and the structure and activity relationship and the mechanism of the action against tumor cells are gradually been elucidated.[9,10]

In this paper, we will deal with the research on novel phospha sugar analogues or 2-phospholene derivatives **2** and 4-bromo-2-phospholene derivatives **3** which were prepared from 2-phospholene derivatives, being substituted on the 3- and/or 4-positions by alkyl group(s) and/or 4-position by a bromo group as well as no substituents on the position, and the evaluation of the biological activity for leukemia cells by using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) in vitro methods.

Synthesis of 1-phenyl-2-phospholene 1-oxides **2a–c**,[11,12] being used as the precursor of a pentofuranose-type phospha sugar, was carried out by applying a known [4+2] cycloaddition reaction (McCormac reaction)[13] of 1,3-dienes **1a–c** with phenylphosphonous dichloride and the successive hydrolysis reaction (Scheme 1). On the other hand, synthesis of 3-ethyl-1-phenyl-2-phospholene 1-oxide (**2d**) was carried out by an alkylation reaction of the methyl group of 3-methyl-1-phenyl-2-phospholene 1-oxide (**2b**) by using n-buthyllithium and iodomethane (Scheme 2).



Scheme 1. Preparation of 1-phenyl-2-phospholene 1-oxides **2a–c**: (a) $R^1 = R^2 = H$; (b) $R^1 = CH_3$, $R^2 = H$; (c) $R^1 = R^2 = CH_3$.

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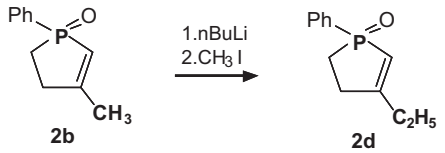

Scheme 2. Preparation of 3-ethyl-1-phenyl-2-phospholene 1-oxide (**2d**).

Scheme 3. Preparation of 4-bromo-2-phospholene 1-oxides **3a**–**d**: (a) $R^1 = R^2 = H$; (b) $R^1 = CH_3$, $R^2 = H$; (c) $R^1 = R^2 = CH_3$; (d) $R^1 = C_2H_5$, $R^2 = H$.

4-Bromo-2-phospholene derivatives **3a**–**d**[11,14] were prepared from 2-phospholene compounds **2a**–**d** by the bromo-radical substitution reaction with *N*-bromosuccinimide (NBS) and 2,2′-azobisisobutyronitrile (AIBN) (Scheme 3). The allylic radical bromination was occurred by the radical species, produced by thermal decomposition of 2,2′-azobisisobutyronitrile and NBS, which attacked at the C-4 position of 2-phospholene 1-oxides **2**. These results are summarized in Table 1.[14]

The prepared 2-phospholene 1-oxides **2** and 4-bromo-2-phospholene 1-oxides **3** were bio-assayed by MTT in vitro method against leukemia cells of U937 cell lines for the first time. The results against U937 cells are shown in Figures 1 and 2. U937 cells of leukemia cell lines were incubated with 2-phospholene 1-oxides **2** or 4-bromo-2-phospholene 1-oxides **3** at the indicated concentrations (0–1000 μM) at 37 °C for 48 h. The cell proliferation or inhibition was measured as the function of the absorbance at 560 nm visible light and evaluated by MTT in vitro assay.[15]

4-Bromo-3,4-dimethyl-2-phospholene 1-oxides **3c** strongly suppressed the cell proliferation of U937 cells in a dose-dependent manner and the intensity of the absorbance at 560 nm decreased. The decrease of the absorbance means the death of the leukemia cells and clearly indicates that **3c** possesses the growth inhibitory effect on U937 cells.The results obtained by MTT in vitro evalua-

Table 1
Preparation of 2-phospholene 1-oxides **2** and 4-bromo-2-phospholene 1-oxides **3**

Entry	Substituent		2-Phospholene 1-oxide **2**			4-Bromo-2-phospholene 1-oxide **3**		
	R^1	R^2	Compound	Conditions	Yield (%)	Compound	Conditions	Yield (%)
1	H	H	**2a**	$CHCl_3$, rt, 3 weeks	22	**3a**	$CHCl_3$, reflux, 6 h	26
2	CH_3	H	**2b**	$CHCl_3$, rt, 2 weeks	68	**3b**	$CHCl_3$, reflux, 6 h	65
3	CH_3	CH_3	**2c**	$CHCl_3$, rt, 2 weeks	30	**3c**	$CHCl_3$, reflux, 6 h	31
4	C_2H_5	H	**2d**	THF, −78 °C, 24 h	34	**3d**	$CHCl_3$, reflux, 6 h	44

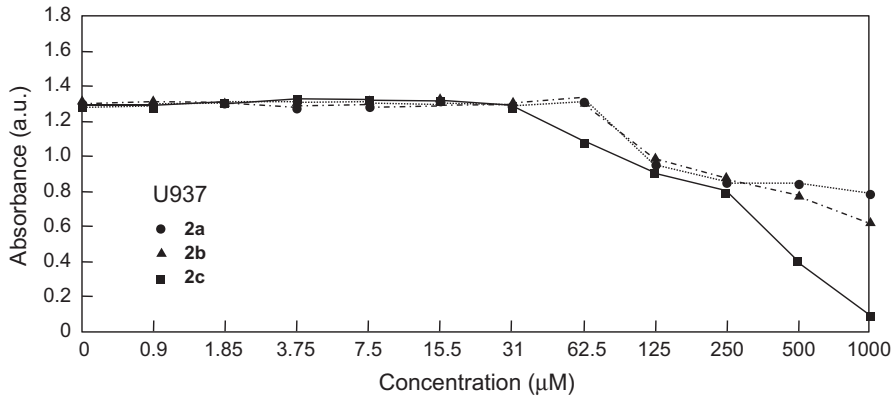

Figure 1. Evaluation by MTT in vitro assay for inhibition from cell proliferation of U937 cells by treatment with 2-phospholene 1-oxides **2a**–**2c** at 37 °C for 48 h.

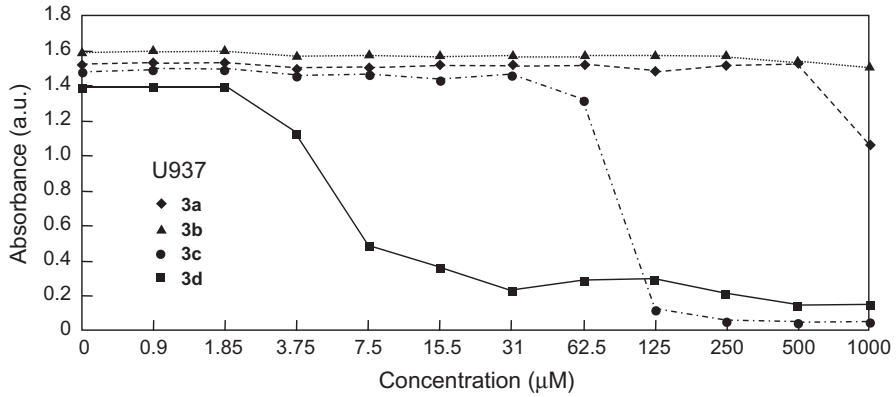

Figure 2. Evaluation by MTT in vitro assay for inhibition from cell proliferation of U937 cells by treatment with 4-bromo-2-phospholene 1-oxides **3** at 37 °C for 48 h.

Table 2
Substituent effect of R^1 and/or R^2 at 3- and/or 4-positions of 1-phenyl-2-phospholene 1-oxides (**2**) on anti-proliferative effect by alkyl group of C-3 position

Compound **2**	Substituents at 3- and/or 4-position		IC_{50} (μM)
	R^1	R^2	
2a	H	H	>1000
2b	CH_3	H	900
2c	CH_3	CH_3	350

Table 3
Substituent effect of R^1 and/or R^2 at 3- and 4-positions of 4-bromo-1-phenyl-2-phospholene 1-oxides (**3**) on anti-proliferative effect by alkyl group of C-3 position

Compound **3**	Substituents at 3- and/or 4-position		IC_{50} (μM)
	R^1	R^2	
3a	H	H	>1000
3b	CH_3	H	87
3c	CH_3	CH_3	5.6
3d	CH_2CH_3	H	>1000

tion at the half of the absorbance intensity (IC_{50}) against U937 cells are shown in Tables 2 and 3. Tables 2 and 3 show that 4-bromo substituent remarkably enhances the anti-leukemia activity. Table 2 shows that 3,4-dimethyl-2-phospholene 1-oxide (**2c**) has a higher activity than 3-methyl-2-phospholene 1-oxide (**2b**). Table 3 shows that 3,4-dimethyl-4-bromo-2-phospholene 1-oxide (**3c**) has the highest activity among **3** and 3-methyl derivative **3b** is much more active than **3a** and **3d**, whose R^1 is H and C_2H_5, respectively. The IC_{50} value of **3c** was 5.6 μM. The observed anti-proliferative effect of 4-bromo-3,4-dimethyl-1-phenyl-2-phospholene 1-oxide **3c** on U937 cells is much more efficient than that of Gleevec® (IC_{50} = 500 μM), which is clinically used as a molecule targeting chemotherapeutical agent.[9,10,16]In conclusion, unsaturated phospha sugars and unsaturated deoxybromo phospha sugars or 2-phospholenes **2a**–**2c** and **3a**–**3d** were prepared. The novel phospha sugar analogues **3b** and **3c** exerted anti-proliferative effect against U937 leukemia cells evaluated by MTT in vitro methods. Especially 4-bromo-3,4-dimethyl-1-phenyl-2-phospholene 1-oxide **3c**, whose 3- and 4-positions were substituted with methyl groups, possesses quite higher anti-cancer activity than Gleevec® (clinically being used against).

Acknowledgements

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11. *General procedures and methods*: TLC (Silica gel: Wako Chromato Sheet and/or Merk Kieselgel 60; Eluent: $CHCl_3$/MeOH = 20:1, in R_f value); Melting point apparatus (Gallenkamp, in °C); 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}); ^1H NMR (JEOL JNM-AL300 (300 MHz); solvent: $CDCl_3$, in δ (ppm)); and HPLC (JASCO and GL Science HPLC apparatuses, in t_R) were used for analyzing the products.
12. Preparation of 2-phospholenes **2**: *Synthesis of 3,4-dimethyl-1-phenyl-2-phospholene 1-oxide* (**2c**): 2,3-dimethyl-1,3-butadiene 10 ml (90 mmol; 1.0 equiv) and phenylphosphonous dichloride 16 ml (120 mmol; 1.30 equiv) were mixed and reacted at room temperature for 2 weeks. The formed solid 3,4-dimethyl-1-phenyl-2-phospholenium dichloride was dissolved in chloroform (200 ml) and hydrolyzed at 0 °C by addition of ice. The reaction mixture was neutralized with sodium hydrogencarbonate and then filtered and extracted with chloroform (20 ml × 3). The chloroform extract was washed with water (50 ml), saturated sodium hydrogencarbonate solution (50 ml), and saturated sodium chloride solution (50 ml). Drying over the chloroform extract with anhydrous sodium sulfate followed by filtration, evaporation of the solvent in vacuo, and distillation under the reduced pressure afforded 3,4-dimethyl-1-phenyl-2-phospholene 1-oxide (**2c**, 5.6 g) in 30% yield. Bp: 130–132 °C/0.12 mmHg; R_f = 0.30 ($CHCl_3$/MeOH = 20:1); ^1H NMR ($CDCl_3$, 300 MHz) δ = 1.24–1.37 (dd, 3H, C4–*CH*$_3$), 1.80 (s, 3H, C3–*CH*$_3$), 2.07–2.17 (ss, 1H, C4), 2.67–2.93 (m, 2H, C5), 5.89–5.97 (dd, 1H, C2), 7.48–7.75 (m, 5H, Ph) MALDI-TOF mass: *m*/*z* 205.7 (MH^+, 50), 207.7 (MH^+, 100); HPLC (Wakosil 5SIL, $CHCl_3$/MeOH = 20:1, flow rate 0.5 mL/min, λ = 254 nm); t_R = 11.04 min.
Similarly, 1-phenyl-2-phospholene 1-oxide (**2a**) and 3-methyl-1-phenyl-2-phospholene 1-oxide (**2b**) were prepared.
1-Phenyl-2-phospholene 1-oxide (**2a**): Registry number: 703-03-7; R_f = 0.38 ($CHCl_3$/MeOH = 20:1); bp 145–152 °C (0.08 mmHg); ^1H NMR ($CDCl_3$, 300 MHz) δ = 2.12–2.21 (m, 2H, C5), 2.78–2.94 (dd, 2H, C4), 6.25–6.36 (dt, 1H, C3), 7.05–7.23 (dt, 1H, C2), 7.47–7.50 (m, 3H, *m*, *p*-Ph), 7.64–7.71 (m, 2H, *o*-Ph); MALDI-TOF mass: *m*/*z* 179.6 (MH^+, 100); HPLC (Wakosil 5SIL, $CHCl_3$/MeOH = 20:1, flow rate 0.5 mL/min, λ = 254 nm); t_R = 10.88 min.
3-Methyl-1-phenyl-2-phospholene 1-oxide (**2b**): Registry number: 707-61-9; R_f = 0.32 ($CHCl_3$/MeOH = 20:1); bp 148–161 °C (0.10 mmHg); ^1H NMR ($CDCl_3$, 300 MHz) δ = 2.08 (s, 1H, –CH_3), 2.17–2.29 (m, 2H, C5), 2.59–2.83 (dd, 2H, C4), 5.90–5.99 (dd, 1H, C2), 7.43–7.71 (m, 5H, Ph); MALDI-TOF mass *m*/*e* 193.7 (MH^+, 100); HPLC (Wakosil 5SIL, $CHCl_3$/MeOH = 20:1, flow rate 0.5 mL/min, λ = 254 nm); t_R = 10.06 min.
3-Ethyl-1-phenyl-2-phospholene 1-oxide (**2d**): Under Ar atmosphere, to tetrahydrofuran dehydrate (THF; 10 ml) solution of iodomethane (0.26 mL, 4.0 mmol, 2.0 equiv) was added *n*-hexane solution (1.6 mol/l; 2.55 ml, 4.0 mmol, 2.0 equiv) at −78 °C. After 2 h, **2b** (384 mg, 2.0 mmol, 1.0 equiv) in THF was added, and then the reaction mixture was stirred at −78 °C for 24 h. After the completion of the reaction, the reaction mixture was allowed to stand at room temperature and worked up by washing with 10% hydrochloric acid (3 ml) and saturated ammonium chloride solution (3 ml), and then dried over with anhydrous sodium sulfate and rotary evaporated. Purification of the product through silica gel column ($CHCl_3$/MeOH = 30:1–15:1) afforded 3-ethyl-1-phenyl-2-phospholene 1-oxide (**3**; 205 mg, 1.00 mmol) in 50% yield. Formula: $C_{12}H_{15}OP$; Exact Mass: 206.09; Registry number: 848484-65-1; R_f = 0.29 ($CHCl_3$/MeOH = 20:1); ^1H NMR ($CDCl_3$, 300 MHz) δ = 2.13 (t, 3H, –CH_2CH_3), 2.24–2.26 (m, 2H, C5), 2.38 (m, 2H, –CH_2CH_3), 2.71–2.86 (m, 2H, C4), 5.93–6.01 (dd, 1H, C2), 7.53–7.74 (m, 5H, Ph); MALDI-TOF mass: *m*/*e* 207 (MH^+, 100).
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14. Preparation of 4-bromo-2-phospholenes **3**: *4-Bromo-3,4-dimethyl-1-phenyl-2-phospholene 1-oxide* (**3c**): To a chloroform (3 ml) solution of 3,4-dimethyl-1-phenyl-2-phospholene 1-oxide (**2c**; 206.1 mg, 1.00 mmol, 1.0 equiv) and *N*-bromosuccinimide (NBS, 213.6 mg, 1.20 mmol, 1.2 equiv) was added dropwise a chloroform (3 ml) solution of 2,2′-azobisisobutyronitrile (AIBN, 24.6 mg, 0.15 mmol, 0.15 equiv) at 60 °C and the reaction mixture was refluxed for 6 h under Ar atmosphere. The reaction mixture was neutralized with saturated $NaHCO_3$ aqueous solution (10 ml), washed with water (10 ml) and saturated NaCl solution (10 ml), and dried over with anhydrous sodium sulfate. The solvent of the filtrate was evaporated under a reduced pressure to give an oily residual material. The residue was purified by column chromatography on silica gel by using chloroform and methanol (20:1) as the eluent to give 4-bromo-3,4-dimethyl-1-phenyl-2-phospholene 1-oxide (**3c**; 0.089 g, 0.314 mmol) in 31% yield; TLC (Silica gel:Wako Chromato Sheet and/or Merk Kieselgel 60; Eluent: $CHCl_3$/MeOH = 20:1), R_f = 0.59; MS (MALDI-TOF-MS: GL Science (Voyager-DE Porimerix); Matrix: α-Cyano-4-hydroxycinnamic acid (*m*/*z*)), 285.3 ($M-H^+$); ^1H NMR (JEOL JNM-AL300 (300 MHz); solvent: $CDCl_3$, δ (ppm)) = 1.73 (s, 3H, C3–CH_3), 1.90–2.31 (m, 2H, C5), 2.21 (s, 3H, C4–CH_3), 5.97–6.05 (dd, 1H, C-2), 7.48–7.79 (m, 5H, Ph).
Similar procedure for **3c** afforded **3a**, **3b**, and **3d** as follows:
4-Bromo-1-phenyl-2-phospholene 1-oxide (**3a**): Yield, 26 %; R_f = 0.49 ($CHCl_3$/MeOH = 20:1); MS (*m*/*z*), 285.3($M-H^+$); ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm) = 2.12–2.21(m, 2H, C5), 5.30–5.35 (t, 1H, C4), 6.26–6.37 (dt, 1H, C3), 7.06–7.24 (dt, 1H, C2) 7.48–7.71 (m, 5H, Ph).

4-Bromo-3-methyl-1-phenyl-2-phospholene 1-oxide (**3b**): Yield, 65 %; R_f = 0.42 ($CHCl_3$/MeOH = 20:1); MS (*m*/*z*), 284.0($M-H^+$); ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm) = 2.22 (s, 1H, C3–CH_3), 2.65–3.10 (m, 2H, C5), 4.96–5.17 (dd, 1H, C4), 5.91–6.38 (dd, 1H, C-2), 7.49–7.87(m, 5H, Ph).

4-Bromo-3-ethyl-1-phenyl-2-phospholene 1-oxide (**3d**): Yield, 44%; R_f = 0.25 ($CHCl_3$/MeOH = 15:1); MS (*m*/*z*), 285.3($M-H^+$); ^1H NMR ($CDCl_3$, 300 MHz), δ (ppm) = 1.55–1.69 (t, 3H, C3–CH_2CH_3), 1.86–1.98 (m, 2H, C5), 2.14–2.18 (q, 2H, C3–CH_2CH_3), 4.94 (s, 1H, C-4), 6.12–6.20 (dd, 1H, C-2), 7.74–7.99 (m, 5H, Ph).

15. *MTT in vivo evaluation*: *Reagent and solvent for the in vitro MTT evaluation*: 2-Phospholene 1-oxides **2** and 4-bromo-2-phospholene 1-oxides **3**, the reagents being evaluated by the in vitro MTT method, were dissolved in dimethylsulfoxide (DMSO) (Sigma Chemical Company, St. Louis, MO, USA) as the solvent, and were diluted into appropriate concentration with DMSO in culture medium immediately before use. The final concentrations of **2** and **3** 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 U937 cells at 37 °C for 48 h.

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