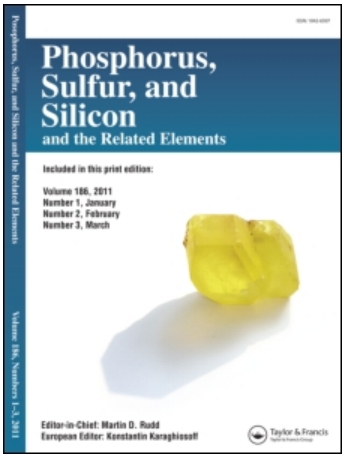

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STEREOSPECIFIC INTRAMOLECULAR CYCLIZATION FOR ASYMMETRIC SYNTHESIS OF (*RP*)- AND (*SP*)-ENANTIOMERS OF 2-OCTYL- AND 2-PHENYL-4*H*-1,3,2-BENZODIOXAPHOSPHORIN 2-OXIDES

Shao-Yong Wu[a]; John E. Casida[a]
[a] Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, California, USA

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STEREOSPECIFIC INTRAMOLECULAR CYCLIZATION FOR ASYMMETRIC SYNTHESIS OF (*Rp*)- AND (*Sp*)-ENANTIOMERS OF 2-OCTYL- AND 2-PHENYL-4*H*-1,3,2-BENZODIOXAPHOSPHORIN 2-OXIDES

SHAO-YONG WU and JOHN E. CASIDA†

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720-3112, USA

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Our earlier studies established that: 1) 2-octyl- and 2-phenyl-4*H*-1,3,2-benzodioxaphosphorin 2-oxides (octyl- and phenyl-BDPOs) induce delayed neuropathy in adult hens at 3 and 200 mg/kg, respectively; 2) phenyl-BDPO also significantly potentiates the acute toxicity of the insecticide malathion to mice; 3) the (*Rp*) and (*Sp*) enantiomers of these compounds, resolved on a mg scale by chiral HPLC, differ in potency by 92- and 137-fold, respectively, as inhibitors of neuropathy target esterase. In order to further study the stereospecificity in their toxic effects, the individual enantiomers of octyl- and phenyl-BDPOs are prepared here by a general method of asymmetric synthesis from the corresponding TLC-resolved diastereomeric precursors (*Rp*)- and (*Sp*)-methyl *N*-[2-hydroxymethylphenoxy(octyl or phenyl)phosphonyl] *L*-prolinates through acid-catalyzed intramolecular cyclization. This occurs under mild conditions in high yields and involves inversion of configuration at phosphorus. HPLC with the CHIRALCEL OC column established their absolute configurations, based on a previously-published assignment, and 97–100% *e.e.* for (*Rp*)- and (*Sp*)-octyl- and phenyl-BDPOs.

Key words: Asymmetric synthesis, 1,3,2-benzodioxaphosphorin 2-oxide, cyclization, NTE inhibitors, *L*-prolinates, stereochemistry.

INTRODUCTION

2-Substituted-4*H*-1,3,2-benzodioxaphosphorin 2-oxides (2-substituted-BDPOs) are known to have a variety of biological activities. Methoxy-BDPO, the active metabolite of the insecticide salithion, is a potent inhibitor of acetylcholinesterase[1] while octyl- and phenyl-BDPOs show high and moderate activities against neuropathy target esterase (NTE) and induce delayed neuropathy in hens at 3 and 200 mg/kg, respectively.[2,3] Phenyl-BDPO also significantly potentiates the acute toxicity of the insecticide malathion to mice[3] by inhibiting the major detoxifying enzyme, carboxylesterase. Due to the chirality of phosphorus in 2-substituted-BDPOs, individual enantiomers have been prepared by either asymmetric synthesis through *L*-prolinate derivatives or by enantiomeric separation with HPLC using chiral columns.[4–6] The stereospecificities differ between each pair of BDPO enantiomers examined, with a magnitude depending on the 2-substituent and type of activity.[5] Although (*Sp*)-octyl- and (*Sp*)-phenyl-BDPOs were 92- and 137-fold more active

†To whom correspondence should be addressed.

NTE inhibitors, respectively, than the corresponding (*Rp*) enantiomers,[5] the stereoselectivity in inducing delayed neuropathy remains unknown.

Enantiomers of 2-alkyl-or 2-aryl-BDPOs, including octyl- and phenyl-BDPOs, have been obtained in only limited quantity in the past by chiral HPLC separation due to the lack of a general method of asymmetric synthesis. This might be achieved via *L*-prolinate derivatives which have been widely used to synthesize chiral phosphorus esters, including several organophosphorus insecticides, by a two-step procedure: 1) resolve the diastereomeric *N*-phosphonyl *L*-prolinates; 2) displace the *L*-prolinate moiety with an alkoxy group by stereospecific acid-catalyzed alcoholysis with inversion of configuration at phosphorus.[7-11] So far, such alcoholysis only involved intermolecular displacement in which alcohols were used in great excess (in most cases as solvents) and refluxing with H_2SO_4 was required. The reactivity and stereochemistry to remove the *L*-prolinate moiety by an intramolecular alcoholic hydroxyl group are of interest in the sense of understanding the reaction mechanism and synthesizing chiral cyclic phosphorus esters.

This investigation develops synthetic pathways using *L*-prolinate derivatives for asymmetric synthesis of the (*Rp*)- and (*Sp*)-enantiomers of octyl- and phenyl-BDPOs in 97–100% *e.e.* through stereospecific intramolecular cyclization. The methods described here are used under mild conditions with high yields and should also be applicable to synthesize analogous BDPOs and other cyclic phosphorus esters.

RESULTS AND DISCUSSION

*Synthesis of Octylphosphonic Dichloride (***4a***) (Figure 1)*

Triethyl phosphite (**1**) was heated with equimolar 1-bromooctane to give diethyl octylphosphonate (**2**) which was hydrolyzed in concentrated hydrochloric acid. Octylphosphonic acid (**3**) was then chlorinated with phosphorus pentachloride to obtain **4a**.

*Synthesis of (Rp)- and (Sp)-2-Octyl- and 2-Phenyl-4H-1,3,2-benzodioxaphosphorin 2-Oxides [(Rp)- and (Sp)-***8a, 8b***] (Figure 2)*

Octyl- or phenylphosphonic dichloride (**4a** or **4b**) was reacted with equivalent methyl *L*-prolinate (**5**) in the presence of triethylamine to form methyl *N*-[chloro(octyl or phenyl)phosphonyl] *L*-prolinate (**6a** or **6b**). The chloride in **6a** or **6b** was replaced by the 2-hydroxymethylphenoxy group through the reaction with 2-hydroxybenzyl alcohol (saligenin). The product methyl *N*-[2-hydroxymethylphenoxy(octyl or phenyl)phosphonyl] *L*-prolinate (**7a** or **7b**) consisted of two diastereomers which were resolved into individual (*Rp*) and (*Sp*) isomers by silica gel TLC with multiple

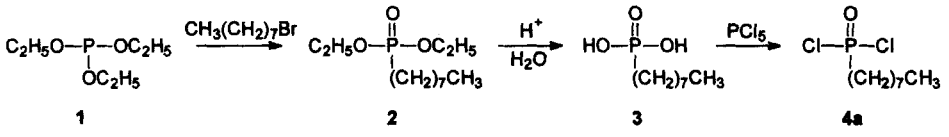


FIGURE 1 Synthesis of octylphosphonic dichloride (**4a**).

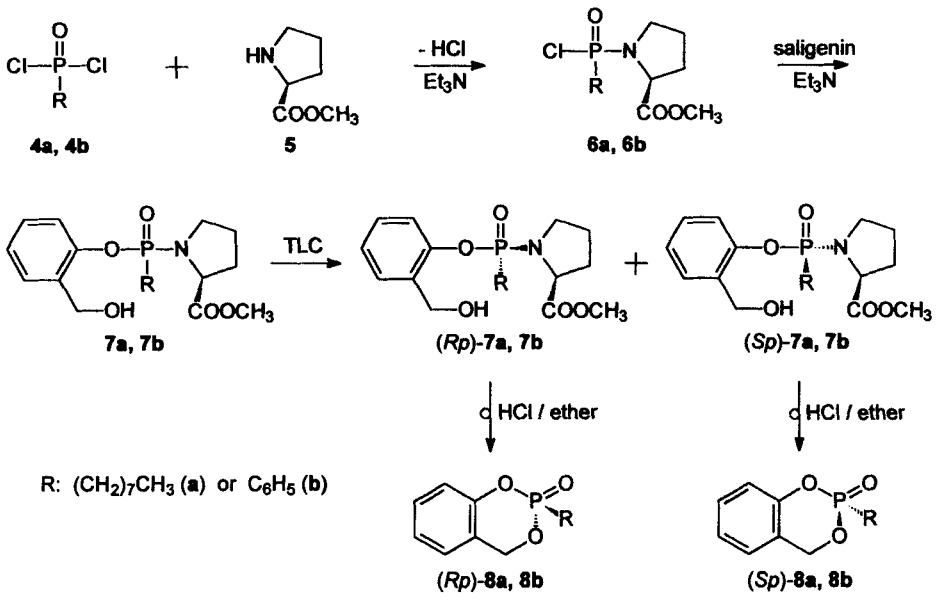


FIGURE 2 Synthesis of (*Rp*)- and (*Sp*)-2-octyl- and 2-phenyl-4*H*-1,3,2-benzodioxaphosphorin 2-oxides [(*Rp*)- and (*Sp*)-**8a, 8b**].

TABLE I

Spectroscopic and chromatographic characterization

Compd.	Config.	NMR, ppm		EI-MS, *m/z*		R_f[1] or R_t[2] (min)	*d.e.* or *e.e.* (%)	Yield (%)
		^{31}P	^{1}H (CH_2O)	Base ion	M^+ (int.)			
(Rp)- and (Sp)-methyl N-[2-hydroxymethylphenoxy(octyl and phenyl)phosphonyl] L-prolinates								
7a	*Rp*	31.66	4.42 (*d*), 4.78 (*d*)	70	411 (4)	0.42	100	17
	Sp	30.87	4.54 (*d*), 4.62 (*d*)	70	411 (3)	0.39	100	29
7b	*Rp*	17.15	4.52 (*d*), 4.78 (*d*)	70	375 (7)	0.36	100	33
	Sp	17.48	4.58 (*d*), 4.68 (*d*)	70	375 (4)	0.32	100	45
(Rp)- and (Sp)-2-octyl- and phenyl-4H-1,3,2-benzodioxaphosphorin 2-oxides								
8a	*Rp*	25.54	5.08 (2*d*), 5.45 (2*d*)	184	282 (32)	7.3	97	89
	Sp	25.53	5.08 (2*d*), 5.45 (2*d*)	184	282 (37)	8.7	99	91
8b	*Rp*	10.57	5.18 (2*d*), 5.55 (2*d*)	246	246 (100)	15.7	100	86
	Sp	10.59	5.18 (2*d*), 5.54 (2*d*)	246	246 (100)	18.0	99	97

[1] POLYGRAM SIL G/UV_{254}, silica gel, 40 x 80 mm, ether-hexane (6:1), 3 developments.
[2] CHIRALCEL OC column, hexane-isopropanol (7:3), flow rate 1.0 ml/min.

developments. Treatment of (*Rp*)- and (*Sp*)-**7a** or **7b** with 1.0 M HCl-ether at ambient temperature gave the corresponding (*Rp*)- and (*Sp*)-**8a** or **8b**, respectively, in 86–97% yield within 30 min.

Spectroscopic and Chromatographic Characterization of (Rp) and (Sp) Stereoisomers of **7a, 7b** *and* **8a, 8b** *(Table I)*

The (*Rp*) and (*Sp*) diastereomers of **7a** and **7b** showed characteristic chemical shifts in their ^{31}P and ^{1}H NMR spectra. In addition to the protons in the CH_2O group, those in the methoxy, prolinate ring and aromatic moiety of (*Rp*)- and (*Sp*)-**7a** and **7b** also resonated at different ppm. These differences were used to determine the optical purities, and the four resolved diastereomers appeared to be in 100% *d.e.* Mass spectra of all **7a** and **7b** diastereomers gave weak M^+ peaks and the same mass of 70 m/z for the base ion, which was considered to be the pyrrolidine cation split from the prolinate moiety. (*Rp*)-**7a** and **7b** had higher TLC R_f values than their corresponding (*Sp*) diastereomers, but the difference was small and needed to be enlarged by multiple developments.

The NMR and EI-MS data of individual enantiomers of **8a** and **8b** were consistent with those of their racemates described before.[2] High resolution mass spectra (FAB) of both (*Rp*)- and (*Sp*)-**8a** confirmed their identity. Elemental analysis of racemic **8b** was performed before.[12] HPLC analysis with the CHIRALCEL OC column indicated that the optical purities of these synthesized BDPO enantiomers were 97–100% *e.e.*

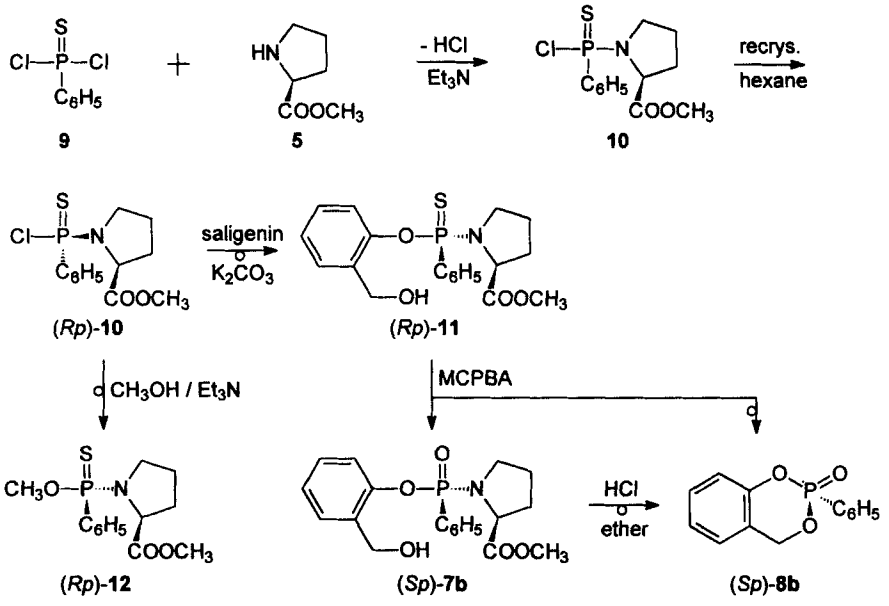


FIGURE 3 Synthesis of (*Rp*)-methyl *N*-[chloro(phenyl)thiophosphonyl] *L*-prolinate [(*Rp*)-**10**] and its stereospecific conversion to (*Sp*)-2-phenyl-4*H*-1,3,2-benzodioxaphosphorin 2-oxide [(*Sp*)-**8b**] through intermediates (*Rp*)-methyl *N*-[2-hydroxymethylphenoxy(phenyl)thiophosphonyl] *L*-prolinate [(*Rp*)-**11**] and (*Sp*)-methyl *N*-[2-hydroxymethylphenoxy(phenyl)phosphonyl] *L*-prolinate [(*Sp*)-**7b**].

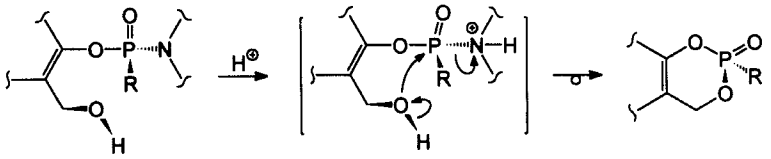
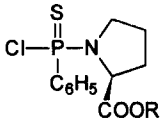


FIGURE 4 Stereochemistry of the intramolecular cyclization.

TABLE II

Comparison of diastereomeric methyl and ethyl *N*-[chloro(phenyl)thiophosphonyl] *L*-prolinates

	R = CH_3		R = C_2H_5[1]	
	(*Rp*)	(*Sp*)	(*Rp*)	(*Sp*)
ratio in formation	79	21	90	10
specific rotation $[\alpha]_D$	+18.4°		+24.3°	-52.1°
melting point, °C	118-120		53-54	
polarity on silica gel				
parent compound	less	more	less	more
methoxy derivative	less	more	less	more
^{1}H-NMR of methoxy derivative, CH_3OP	3.84	3.72	3.75	3.65

[1] From references 13 and 14.

Stereochemistry (Figures 3 and 4, Table II)

The absolute configurations of the enantiomers of **8a** and **8b** were assigned according to a previously-established generalization,[5] for alkyl- and aryl-BDPOs under the present HPLC conditions, that the first-eluted enantiomers have the (*Rp*) and the more retained ones have the (*Sp*) configuration.

Phosphorus stereochemistry in the resolved **7a** and **7b** diastereomers was established on the basis of the experimental results obtained from a key intermediate (*Rp*)-methyl *N*-[chloro(phenyl)thiophosphonyl] *L*-prolinate [(*Rp*)-**10**]. Diastereomeric mixture **10** was prepared from phenylphosphonothioic dichloride (**9**) and methyl *L*-prolinate (**5**) under similar conditions to those used for synthesis of its oxon analog **6b**. Recrystallization of **10** from hexane afforded a single diastereomer whose configuration at phosphorus was assigned as (*Rp*) in comparison with the known ethyl analogs (*Rp*)- and (*Sp*)-ethyl *N*-[chloro(phenyl)thiophosphonyl] *L*-prolinate in terms of the *Rp*/*Sp* ratio in formation, specific rotation, crystallizability, polarity of the parent compounds and their methoxy derivatives on silica gel, and the ^{1}H NMR of the methoxy derivatives (Table II).[13,14] When treated with saligenin and K_2CO_3, (*Rp*)-**10** was converted to (*Rp*)-methyl *N*-[2-hydroxymethylphenoxy-(phenyl)thiophosphonyl] *L*-prolinate [(*Rp*)-**11**] with inversion of configuration at

phosphorus.[15-18] (*Rp*)-Methyl *N*-[methoxy(phenyl)thiophosphonyl] *L*-prolinate [(*Rp*-**12**] was also synthesized from (*Rp*)-**10** via the same stereochemical mechanism. Oxidation of (*Rp*)-**11** by *m*-chloroperbenzoic acid (MCPBA) gave a major product (*Sp*)-**7b** with retention of stereochemistry at phosphorus[19] and a minor product (*Sp*)-**8b**, which was also obtained by treating (*Sp*)-**7b** with HCl in ether. Clearly, the last intramolecular cyclization occurred with inversion (Figure 4). Based on these results, the phosphorus configuration of all the other **7a** and **7b** diastereomers was assigned from both the stereochemical mechanism of the cyclization and the configuration of their corresponding products **8a** and **8b** determined by HPLC analysis as already described.

EXPERIMENTAL

Spectroscopy. NMR spectra were recorded with a Bruker AM-300 Spectrometer at 300 MHz (^{1}H) or 121.5 MHz (^{31}P) for solutions in $CDCl_3$. Chemical shifts are referenced to internal tetramethylsilane for ^{1}H and external trimethyl phosphate in $CDCl_3$ for ^{31}P. They are reported in ppm on the δ scale with positive shifts downfield from the reference and coupling constants in Hz. Diastereomeric excess was determined by integration of the ^{1}H and ^{31}P resonances. Mass spectrometry (MS) with a Hewlett-Packard 5985B instrument utilized a direct insertion probe and electron impact (EI, 70 eV, 200°C). The data are presented as m/z and intensity (%). High resolution mass spectra (FAB) were recorded using a Fisons VG 70S instrument.

Chromatography. Thin-layer chromatography (TLC) was carried out on POLYGRAM SIL G/UV$_{254}$ silica gel plates (40 × 80 mm) for analytical purposes and on silica gel F_{254} chromatoplates (200 × 200 mm, gel thickness: 1-mm) for diastereomer separations. R_f values were obtained by using the analytical plates developed three times with ether-hexane (6:1) as the mobile phase. Column chromatography utilized silica gel 60 (0.040–0.063 mm). HPLC was performed at a flow rate of 1 mL/min with the CHIRALCEL OC column (Daicel, Tokyo, Japan; 4.6 mm i.d. × 25 cm) developed with 70/30 (v/v) hexane/isopropanol for enantiomeric analysis, and retention times (R_t) are given in min. Quantitation was by integration of peak areas monitored at 254 nm.

O,O-Diethyl octylphosphonate (**2**). A mixture of 1-bromooctane (114 g, 0.59 mol) and triethyl phosphite (**1**, 98 g, 0.59 mol) was stirred at 110°C for 24 h; bromoethane (~30 mL, a by-product) was condensed into a separate container during the reaction. ^{31}P NMR indicated that the starting material **1** was completely transformed into two phosphorus-containing products: diethyl octylphosphonate and diethyl ethylphosphonate in the ratio of 4:1. The desired product **2** was obtained as a colorless liquid by distillation at reduced pressure. B.p. 121–123°C/0.6 mm Hg. Yield: 117 g, 79%. ^{1}H: 0.88 (3H, t, J = 6.3, $CCCH_3$), 1.32 [16H, m, $P(OCCH_3)_2$, $PCC(CH_2)_5$], 1.67 [4H, m, $P(CH_2)_2$], 4.08 [4H, m, $P(OCH_2C)_2$]. ^{31}P: 30.36.

Octylphosphonic acid (**3**). The diethyl ester **2** (76 g, 0.3 mol) was added to concentrated hydrochloric acid (150 mL) and the mixture refluxed for 24 h. After cooling to room temperature, the product **3** was extracted with ether (50 mL × 3), dried over Na_2SO_4, and then purified by recrystallization from hexane. Yield: 50 g, 86%. M.p. 95–97°C. ^{1}H: 0.88 (3H, t, J = 6.5, CH_3), 1.32 [10H, m, $PCC(CH_2)_5$], 1.71 [4H, m, $P(CH_2)_2$]. ^{31}P: 34.50.

Octylphosphonic dichloride (**4a**). To a mixture of octylphosphonic acid (**3**, 7.4 g, 0.038 mol) and toluene (60 mL), PCl_5 (20 g, 0.096 mol) was added slowly and the reaction continued at 110°C for 3 h. The solvent and by-products, HCl and $POCl_3$, were removed by evaporation at reduced pressure, and the residue showed only one ^{31}P signal at 48.60. This product was used directly in the synthesis of **6**.

*(Rp)- and (Sp)-Methyl N-[2-hydroxymethylphenoxy(octyl or phenyl)phosphonyl] L-prolinates [(Rp)- and (Sp)-***7a** *or* **7b**]. To a solution of **4a** or **4b** (1 mmol) in methylene chloride (20 mL) cooled to −60°C by a dry ice-acetone bath, methyl *L*-prolinate hydrochloride (1 mmol) and triethylamine (2 mmol) were added. After stirring at −60°C to room temperature for 1 h, the **6a** or **6b** produced was not isolated but instead was treated directly with 2-hydroxybenzyl alcohol (1 mmol) and triethylamine (1 mmol)

and stirred for an additional 2 h. Work-up involved filtration and evaporation to remove triethylamine hydrochloride and the solvent. The crude product was purified on a silica gel column eluted with hexane/ether to give the diastereomeric mixture **7a** or **7b** which was separated into individual diastereomers by preparative TLC developed 6 times in ether/hexane (5:1) for **7a** and 6 times in ether for **7b**. (*Rp*)-**7a**: yield 48 mg, 17%, 100% *d.e.*, R_f 0.42. ^{1}H: 0.89 (3H, t, J = 6.5, CCH_3), 1.37 [10H, m, $PCC(CH_2)_5$], 1.7–2.2 [8H, m, $NC(CH_2)_2$, $P(CH_2)_2$], 3.41 (2H, m, NCH_2), 3.68 (3H, s, OCH_3), 4.27 (1H, m, NCH), 4.42, 4.78 (2H, 2d, J = 11.9, $PhCH_2$), 6.9–7.5 (4H, m, aromatic). ^{31}P: 31.66. EI-MS: 411 (M^+, 4%), 334 (4%), 283 (13%), 260 (8%), 128 (9%), 70 (100%). (*Sp*)-**7a**: yield 81 mg, 29%, 100% *d.e.*, R_f 0.39. ^{1}H: 0.88 (3H, t, J = 6.1, CCH_3), 1.31 [10H, m, $PCC(CH_2)_5$], 1.6–2.2 [8H, m, $NC(CH_2)_2$, $P(CH_2)_2$], 3.42 (2H, m, NCH_2), 3.53 (3H, s, OCH_3), 4.33 (1H, m, NCH), 4.54, 4.62 (2H, 2d, J = 12.0, $PhCH_2$), 7.1–7.5 (4H, m, aromatic). ^{31}P: 30.87. EI-MS: 411 (M^+, 3%), 334 (2%), 283 (11%), 260 (13%), 128 (12%), 70 (100%). (*Rp*)-**7b**: yield 80 mg, 33%, 100% *d.e.*, R_f 0.36. ^{1}H: 1.89–2.18 [4H, m, $NC(CH_2)_2$], 3.21, 3.39 (2H, 2m, NCH_2), 3.66 (3H, s, OCH_3), 4.32 (1H, m, NCH), 4.52, 4.78 (2H, 2d, J = 12.2, $PhCH_2$), 7.06, 7.22, 7.53, 8.05 (9H, 4m, aromatic). ^{31}P: 17.15. EI-MS: 375 (M^+, 7%), 334 (3%), 247 (43%), 155 (19%), 107 (30%), 70 (100%). (*Sp*)-**7b**: yield 110 mg, 45%, 100% *d.e.*, R_f: 0.32. ^{1}H: 1.84–2.24 [4H, m, $NC(CH_2)_2$], 3.21, 3.38 (2H, 2m, NCH_2), 3.51 (3H, s, OCH_3), 4.53, (1H, m, NCH), 4.58, 4.68 (2H, 2d, J = 12.1, $PhCH_2$), 6.97, 7.16, 7.51, 7.93 (9H, 4m, aromatic). ^{31}P: 17.48. EI-MS: 375 (M^+, 4%), 334 (4%), 247 (34%), 155 (20%), 107 (28%), 70 (100%).

(Rp)- and (Sp)-2-Octyl- or 2-phenyl-4H-1,3,2-benzodioxaphosphorin 2-oxides [*(Rp)- and (Sp)*-**8a** *or* **8b**]. (*Rp*)-**7a** (48 mg) was mixed with 5 mL of 1.0 M HCl-ether solution and stirred for 30 min. Purification by silica gel column (hexane/ether) gave 29 mg of (*Rp*)-**8a**. Yield: 89%, 97% *e.e.*, R_t 7.3. Similarly, (*Sp*)-**8a**, (*Rp*)-**8b** and (*Sp*)-**8b** were synthesized from (*Sp*)-**7a**, (*Rp*)-**7b** and (*Sp*)-**7b**, respectively. (*Sp*)-**8a**: yield 91%, 99% *e.e.*, R_t 8.7. (*Rp*)-**8b**: yield 86%, 100% *e.e.*, R_t 15.7. (*Sp*)-**8b**: yield 97%, 99% *e.e.*, R_t 18.0. The NMR and EI-MS data for these enantiomers were consistent with those of their corresponding racemates reported before.[2] HRMS (FAB) found 283.1465 for (*Rp*)-**8a**, 283.1468 for (*Sp*)-**8a**, $C_{15}H_{23}O_3PH^+$ requires 283.1463.

(Rp)-Methyl N-[chloro(phenyl)thiophosphonyl] L-prolinate [*(Rp)*-**10**]. Methyl *L*-prolinate hydrochloride (5 g, 0.03 mol) and triethylamine (6.1 g, 0.06 mol) were added successively to a solution of phenylphosphonothioic dichloride (6.4 g, 0.03 mol) in methylene chloride (50 mL) at −60°C, and the dry ice-acetone cooling bath was then removed and the reaction continued for 2 h before mixing with ether (50 mL) to precipitate the triethylamine hydrochloride which was removed by filtration. Purification with the silica gel column (hexane/ether) resulted in a mixture of (*Rp*)- and (*Sp*)-**10** in a ratio of 79:21, respectively. Yield: 7 g, 77%. Recrystallization from hexane gave 4.5 g of (*Rp*)-**10** in 100% *d.e.* M.p. 118–120°C, $[\alpha]_D$ + 18.4° (c = 0.91, CCl_4, 23°C, Perkin-Elmer Model 243 polarimeter). ^{1}H: 2.04, 2.27 [4H, 2m, $NC(CH_2)_2$], 3.12, 3.24 (2H, m, NCH_2), 3.77 (3H, s, OCH_3), 4.82 (1H, m, NCH), 7.51, 8.05 (5H, 2m, aromatic). ^{31}P: 78.15. The NMR data for (*Sp*)-**10** were obtained by subtracting those of (*Rp*)-**10** from the spectra of the (*Rp*) and (*Sp*) mixture. ^{1}H: 2.05, 2.23 [4H, 2m, $NC(CH_2)_2$], 3.24, 3.52 (2H, 2m, NCH_2), 3.60 (3H, s, OCH_3), 4.49 (1H, m, NCH), 7.52, 7.96 (5H, 2m, aromatic). ^{31}P: 76.73.

(Rp)-Methyl N-[2-hydroxymethylphenoxy(phenyl)thiophosphonyl] L-prolinate [*(Rp)*-**11**. (*Rp*)-**10** (0.5 g, 1.65 mmol) was mixed with equimolar 2-hydroxybenzyl alcohol and K_2CO_3 in acetone (10 mL) and the mixture refluxed overnight. After filtration and evaporation, the residue was purified by silica gel column with hexane and ether (10:1) as eluent to give (*Rp*)-**11**. Yield: 0.4 g, 62%, 92% *d.e.* ^{1}H [(*Rp*)-**11**]: 1.85, 2.01, 2.18 [4H, 3m, $NC(CH_2)_2$], 3.12, 3.45 (2H, m, NCH_2), 3.59 (3H, s, OCH_3), 4.67 (1H, m, NCH), 4.63, 4.77 (2H, 2d, J = 12.4, $PhCH_2$), 7.16, 7.49, 7.98 (9H, 3m, aromatic). ^{31}P: 73.47 [(*Rp*)-**11**, major], 73.16 [(*Sp*)-**11**, minor].

*Synthesis of (Sp)-***8b** *from (Rp)***11**. A solution of (*Rp*)-**11** (0.4 g, 1 mmol) in tetrahydrofuran (5 mL) was mixed with MCPBA (0.5 g, 3 mmol) at 0°C, and the mixture stirred at room temperature overnight. Purification by TLC (ether) gave two products: (*Sp*)-**7b** (0.1 g, 26%, 100% *d.e.*) and (*Sp*)-**8b** (0.02 g, 8%, 98% *e.e.*). The diastereomer (*Sp*)-**7b** was transformed into (*Sp*)-**8b** by treating with HCl-ether, as described above. Yield: 91%, 98% *e.e.* The structures and stereochemistries of (*Sp*)-**7b** and (*Sp*)-**8b** were confirmed by comparing their NMR and HPLC data with those of the same stereoisomers synthesized as in Figure 2.

(Rp)-Methyl N-[methoxy(phenyl)thiophosphonyl] L-prolinate [*(Rp)*-**12**]. A solution of (*Rp*)-**10** (0.3 g, 1 mmol) and triethylamine (0.1 g, 1 mmol) in methanol (10 mL) was refluxed for 3 h, followed by evaporation and purification with the silica gel column (hexane/ether) to give (*Rp*)-**12**. Yield: 0.25 g, 84%, 100% *d.e.* ^{1}H: 1.81, 1.96, 2.16 [4H, 3m, $NC(CH_2)_2$], 2.91, 3.19 (2H, 2m, NCH_2), 3.71 (3H, s,

$COCH_3$), 3.84 (3H, d, J = 13.7, $POCH_3$), 4.59 (1H, m, NCH), 7.43, 7.78 (5H, 2m, aromatic). ^{31}P: 73.91. Similarly, diastereomeric mixture (*RpSp*)-**10** produced (*RpSp*)-**12** in 80% yield. The NMR data for (*Sp*)-**12** were obtained by comparing the corresponding spectra of (*RpSp*)-**12** and (*Rp*)-**12**. ^1H: 1.82, 1.97, 2.18 [4H, 3m, $NC(CH_2)_2$], 2.92, 3.24 (2H, 2m, NCH_2), 3.69 (3H, s, $COCH_3$), 3.72 (3H, d, J = 13.9, $POCH_3$), 4.54 (1H, m, NCH), 7.45, 7.92 (5H, 2m, aromatic). ^{31}P: 74.17.

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REFERENCES

1. M. Eto, "Organophosphorus Pesticides: Organic and Biological Chemistry," CRC Press, Cleveland, OH, p. 253, 1974.
2. S.-Y. Wu and J. E. Casida, *Chem. Res. Toxicol.*, **5,** 680 (1992).
3. J. E. Casida, R. L. Baron, M. Eto and J. L. Engel, *Biochem. Pharmacol.*, **12,** 73 (1963).
4. S.-Y. Wu, A. Hirashima, M. Eto, K. Yanagi, E. Nishioka and K. Moriguchi, *Agric. Biol. Chem.*, **53,** 157 (1989).
5. S.-Y. Wu and J. E. Casida, *Chem. Res. Toxicol.*, **7,** 77 (1994).
6. S.-Y. Wu and J. E. Casida, *Phosphorus, Sulfur, and Silicon*, **88,** 129 (1994).
7. T. Koizumi, Y. Kobayashi, H. Amitani and E. Yoshii, *J. Org. Chem.*, **42,** 3459 (1977).
8. Y. Kobayashi, T. Koizumi and E. Yoshii, *Chem. Pharm. Bull.*, **27,** 1641 (1979).
9. H. Leader and J. E. Casida, *J. Agric. Food Chem.*, **30,** 546 (1982).
10. A. Hirashima, H. Leader, I. Holden and J. E. Casida, *J. Agric. Food Chem.*, **32,** 1302 (1984).
11. A. Miyazaki, T. Nakamura, M. Kawaradani and S. Marumo, *J. Agric. Food Chem.*, **36,** 835 (1988).
12. M. Eto and Y. Oshima, *Agric. Biol. Chem.*, **26,** 452 (1962).
13. T. Koizumi, H. Takagi and E. Yoshii, *Chem. Lett.*, 1403 (1980).
14. H. Takagi, Master Thesis, Toyama Med. Pharm. Univ., Japan, p. 49, 1980.
15. J. Michalski and M. Mikołajczyk, *Tetrahedron*, **22,** 3055 (1966).
16. M. Mikołajczyk, *Tetrahedron*, **23,** 1543 (1967).
17. J. Michalski, M. Mikołajczyk and J. Omelańczuk, *Tetrahedron Lett.*, 3565 (1968).
18. J. Michalski, M. Mikołajczyk, B. Młotkowska and J. Omelańczuk, *Tetrahedron*, **25,** 1743 (1969).
19. A. W. Herriott, *J. Am. Chem. Soc.*, **93,** 3304 (1971).