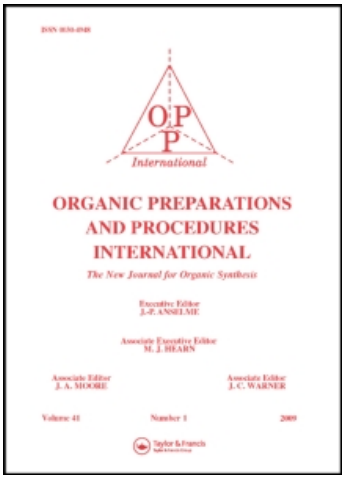

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SYNTHESIS OF BIVALENT ORGANOPHOSPHORUS COMPOUNDS AS ACETYLCHOLINESTERASE INHIBITORS

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SYNTHESIS OF BIVALENT ORGANOPHOSPHORUS COMPOUNDS AS ACETYLCHOLINESTERASE INHIBITORS

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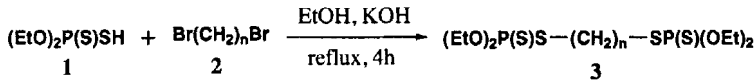
It has been reported that in biological systems, multivalent binding provides a broad range of benefits that are not achievable with the corresponding monovalent interactions.[1] One of classical examples of multivalent interactions, known as the "cluster effect", was described by Lee *et al.*[2] The activity of some multivalent drug molecules were significantly enhanced by their cluster effect.[3-5] Pang *et al.* reported highly potent, selective, and low cost bifunctional acetylcholinesterase (AChE) inhibitors.[6] Since the cluster effect has been applied in drug design, we believe that the theoretical hypothesis can serve as reference and guidance for the discovery of new agrochemicals.

Organophosphorus compounds act as classical acetylcholinesterase(AChE) inhibitors.[7] The unique toxicology of insects provides safety mechanisms for the organophosphorus insecticides, the selectivity of insecticidal nerve poisons being attributable to structural differences in binding subsites (acetylcholinesterase).[8] Based on the geometry of the active-site gorge of AChE, with specific sites at its two extremities, Glu-199, Asp-276 and the hydrophobic residues lining the entrance gorge of AChE,[9] through binding to both catalytic and peripheral sites of the enzyme, a new series of dual binding site AChE inhibitors have been designed and synthesized.[10] The cluster effect, combined with information about the structure of acetylcholinesterase, led us to design and synthesize some bivalent organphosphorus compounds as potential acetylcholinesterase inhibitors. We anticipated that the target compounds would bind to both catalytic and peripheral sites of the enzyme that lead to more stable AChE-organphosphorus complexes.

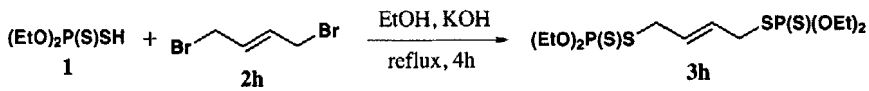
Compounds **3a-h**, consist of two subunits, connected either with a rigid or flexible linker, as shown in *Scheme 1*.

Another series of compounds containing polyethylene glycol linkers were synthesized, as shown in the *Scheme 2*. The oxygen atoms in the linkers may lead to a change in the physicochemical properties of the compounds such as the logP value and the pKa value.

The phthalimide ring can occupy a similar position at the peripheral site of AChE and can remain stacked onto the aromatic ring of Trp279.[11] Thus we synthesized the third series of

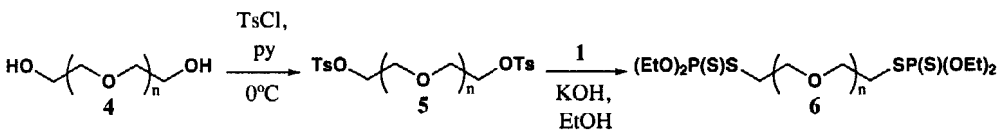


a) n = 4; b) n = 5; c) n = 6; d) n = 7; e) n = 8; f) n = 9; g) n = 10

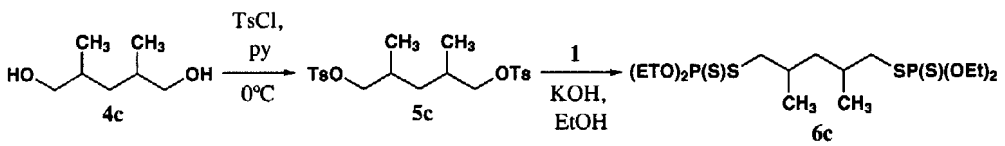


Scheme 1

heterovalent molecules, as shown in the *Scheme 3*. Acetone was chosen as the solvent, and the reaction mixture was refluxed for 10 hrs to give *N*-(ω-bromoalkyl)phthalimides (**8a–g**) and *N*-(ω-bromoalkenyl)phthalimides (**8h**) in good yields (75-93%).

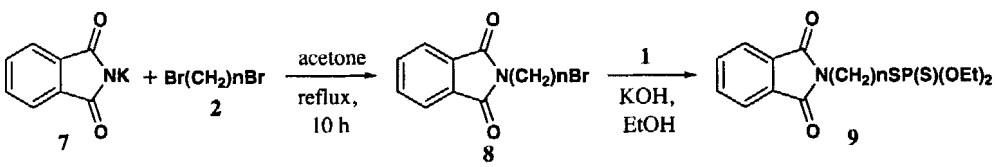


a) n = 1; b) n = 2

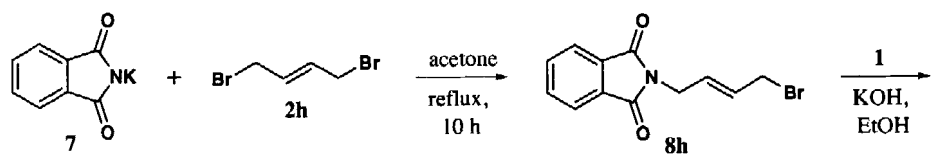


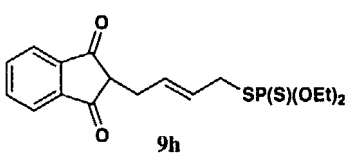
Scheme 2

The insecticidal activity of the new compounds was evaluated by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The preliminary results indicate that compounds **3b**, **3h**, **6a**, **9a**, **9d**, **9e** and **9h** displayed good biological activity to the wheat aphid



a) n = 4; b) n = 5; c) n = 6; d) n = 7; e) n = 8; f) n = 9; g) n = 10





Scheme 3

and housefly. With an *in vitro* assay to screen compounds for ability to inhibit housefly acetylcholinesterase, the compounds **3h**, **9d** and **9e** showed strong activity.

EXPERIMENTAL SECTION

Reagents and solvents were purchased from Beijing Chemical Reagents Company and were used without further purification. Column chromatography was performed on silica gel 200-300 mesh obtained from Qingdao Haiyang Chemical Co., Ltd. Analytical thin-layer chromatography was performed on silica gel plates, and the plots were visualized under UV light at 254 nm or iodine vapor. Elemental analyses (C, H, N, P, S) were performed at the Institute of Chemistry, Chinese Academy of Sciences. Nuclear magnetic resonance spectra were recorded in $CDCl_3$, using a Brucker DPX 300MHz spectrometer (performed at the China Agricultural University), with TMS as an internal standard.

Typical Procedure for the Preparation of Products 3a-h.- To a solution of *O,O*-diethyl hydrogenphosphorodithioate (**1**) (4.2 g, 22.0 mmol) in ethanol (5.0 mL) was added 20% ethanolic potassium hydroxide slowly until the pH of the solution was approximately 7.0. Then 1,4-dibromobutane (**2a**) (2.2 g, 10 mmol) was added to the solution, and the mixture was refluxed for 4 hrs. The reaction mixture was filtered to remove solids, and the filtrate was concentrated under vacuum. The viscous residue was dissolved with 60 mL of ether, then washed with water and the ethereal layer was dried (anhydrous sodium sulfate). The dried solution was concentrated under reduced pressure, and the crude compound was chromatographed on silica gel to give 1,4-di[*S*-(diethoxyphosphinothioyl)mercapto]butane (**3a**) as a colorless liquid. The same procedure was employed to obtain products **3b-h** by using the corresponding α,ω-dibromo compounds **2b-h**.

Typical Procedure for the Preparation of Products 6a-c.- The oligoethyleneglycol ditosylates (**5a-c**) were synthesized following the procedure previously reported.[12] To a solution of *p*-toluenesulfonyl chloride (10.0 g, 52.5 mmol) in pyridine (20.0 mL) was added diethylene glycol (**4a**) (2.7g, 25.4 mmol). The resulting mixture was stirred at 0°C for 1 hr and allowed to warm to room temperature slowly. The precipitate formed was collected, washed with ice water, dried and recrystallized from ethanol to afford diethylene glycol ditosylate (**5a**). The same procedure was followed to afford the products **5b**, **5c** by using the corresponding triethylene glycol (**4b**) and dipropylene glycol (**4c**).

To a solution of *O,O*-diethyl hydrogenphosphorodithioate (**1**) (2.1 g, 11.0 mmol) in ethanol (4.0 mL) was added 20% ethanolic potassium hydroxide slowly until the pH of the solution was approximately 7.0. Then diethylene glycol ditosylate (**5a**) (2.1 g, 5.1 mmol) was added, and the mixture was refluxed for 4 hrs. The reaction mixture was filtered, and the filtrate was concentrated under vacuum. The viscous residue was dissolved with 60 mL of ether, then washed with water and the ethereal layer was dried (anhydrous sodium sulfate). The dried solution was concentrated under reduced pressure, and the crude compound was chromatographed on silica gel to give di(ethylene glycol) di[*S*-(diethoxyphosphinothioyl)] thioether (**6a**). Compound **6b** (62%) was prepared from **1** and **5b**, and **1** and **5c** gave **6c** as described above.

Table 1. Yields and Elemental Analyses of Compounds **3a-3h**, **6a-6c**, **9a-9h**

Cmpd	Yield (%)	bp. (°C)	Elemental Analyses (Found) C	H	N	P	S
3a[a]	44	131-132	33.79 (33.95)	6.62 (6.79)	----	14.52 (14.62)	30.07 (29.88)
3b[a]	82	145-147	35.44 (35.26)	6.86 (6.98)	----	14.06 (14.25)	29.11 (29.17)
3c[a]	29	152-153	36.99 (36.75)	7.09 (7.23)	----	13.63 (13.57)	28.21 (28.31)
3d[b]	67	168-169	38.44 (38.34)	7.31 (7.42)	----	13.22 (13.38)	27.37 (27.25)
3e[b]	67	176-177	39.81 (39.95)	7.52 (7.78)	----	12.83 (12.67)	26.57 (26.39)
3f[b]	54	187-188	41.11 (41.18)	7.71 (7.89)	----	12.47 (12.36)	25.82 (25.72)
3g[b]	87	195-196	42.33 (42.49)	7.89 (7.92)	----	12.13 (12.14)	25.11 (25.08)
3h[c]	87	173-174	33.95 (33.98)	6.17 (6.25)	----	14.59 (14.46)	30.21 (30.24)
6a[b]	80	156-158	32.57 (32.68)	6.38 (6.52)		14.00 (13.92)	28.98 (28.89)
6b[c]	62	172-173	34.56 (34.69)	6.63 (6.75)	----	12.73 (12.69)	26.36 (26.23)
6c[b]	17	181-182	35.73 (35.86)	6.85 (6.96)	----	13.16 (13.09)	27.25 (27.12)
9a[b]	78	151-152	49.60 (49.78)	5.72 (5.89)	3.62 (3.57)	7.99 (7.87)	16.55 (16.43)
9b[c]	73	162-163	50.86 (50.93)	6.03 (6.24)	3.49 (3.47)	7.71 (7.68)	15.97 (15.81)
9c[c]	43	175-176	52.03 (52.19)	6.31 (6.53)	3.37 (3.37)	7.45 (7.29)	15.43 (15.28)
9d[c]	46	183-184	53.13 (53.26)	6.57 (6.69)	3.26 (3.18)	7.21 (7.15)	14.93 (14.89)
9e[c]	47	192-193	54.16 (54.25)	6.82 (6.93)	3.16 (3.25)	6.98 (6.79)	14.46 (14.42)
9f[c]	53	211-212	55.12 (55.29)	7.05 (7.21)	3.06 (2.98)	6.77 (6.64)	14.01 (13.96)
9g[c]	83	223-224	56.03 (56.19)	7.27 (7.46)	2.97 (2.85)	6.57 (6.43)	13.60 (13.54)
9h[c]	86	188-189	49.86 (49.97)	5.23 (5.46)	3.63 (3.63)	8.04 (7.89)	16.64 (16.53)

a) colorless liquid b) pale yellow liquid c) yellow liquid

Typical Procedure for the Synthesis of Compounds 9a-h.- To a solution of 1,3-dibromopropane (**2a**) (30.3 g, 150.1 mmol) in acetone (150.0 mL) was added potassium phthalimide (**7**) (9.3 g, 50.2 mmol). The resulting mixture was refluxed for 10 hrs, and then cooled to room temperature. After filtering off the precipitated potassium bromide, the filtrate was concentrated under reduced pressure to give a viscous yellow oil. *N*-(ω-bromoalkyl)phthalimide (**8a**) was obtained after purification by silica gel column chromatography. The same procedure afforded *N*-(ω-bromoalkyl)phthalimides (**8b-g**) and *N*-(ω-bromoalkenyl)phthalimides (**8h**) by using the corresponding α,ω-dibromo compounds **2b-h**. The intermediate products were used directly in the next reaction.

Table 2. ^{1}H NMR of Compounds **3, 6, 9**

Cmpd	(δ), J (Hz)
3a	1.34-1.39 (m, 12H), 1.77-1.82 (m, 4H), 2.86-2.93 (m, 4H), 4.08-4.25 (m, 8H)
3b	1.34-1.39 (m, 12H), 1.50-1.54 (m, 2H), 1.66-1.73 (m, 4H), 2.82-2.92 (m, 4H), 4.08-4.25 (m, 8H)
3c	1.34-1.44 (m, 16H), 1.66-1.70 (m, 4H), 2.81-2.91 (m, 4H), 4.08-4.25 (m, 8H)
3d	1.33-1.42 (m, 18H), 1.65-1.69 (m, 4H), 2.81-2.91 (m, 4H), 4.08-4.25 (m, 8H)
3e	1.25-1.41 (m, 20H), 1.64-1.69 (m, 4H), 2.80-2.90 (m, 4H), 4.08-4.25 (m, 8H)
3f	1.25-1.39 (m, 22H), 1.61-1.69 (m, 4H), 2.80-2.90 (m, 4H), 4.08-4.25 (m, 8H)
3g	1.28-1.39 (m, 24H), 1.61-1.69 (m, 4H), 2.80-2.90 (m, 4H), 4.08-4.25 (m, 8H)
3h	1.15-1.41 (m, 12H), 3.47-3.54 (m, 4H), 4.06-4.28 (m, 8H), 5.75-5.78 (m, 2H)
6a	1.34-1.39 (m, 12H), 3.00-3.11 (m, 4H), 3.68 (t, 4H, J = 6.5Hz), 4.09-4.26 (m, 8H)
6b	1.34-1.39 (m, 12H), 3.01-3.11 (m, 4H), 3.64 (s, 2H), 3.70 (t, 4H, J = 6.7Hz), 4.09-4.26 (m, 8H)
6c	1.14-1.44 (m, 18H), 2.88-2.97 (m, 2H), 3.46-3.68 (m, 4H), 4.10-4.26 (m, 8H)
9a	1.33-1.38 (m, 6H), 1.70-1.80 (m, 4H), 2.85-2.95 (m, 2H), 3.71 (t, 2H, J = 6.7Hz), 4.07-4.24 (m, 4H), 7.71-7.74 (m, 2H), 7.83-7.86 (m, 2H)
9b	1.33-1.38 (m, 6H), 1.42-1.48 (m, 2H), 1.67-1.75 (m, 4H), 2.80-2.90 (m, 2H), 3.69 (t, 2H, J = 7.2Hz), 4.07-4.24 (m, 4H), 7.70-7.75 (m, 2H), 7.83-7.86 (m, 2H)
9c	1.33-1.46 (m, 10H), 1.62-1.73 (m, 4H), 2.80-2.90 (m, 2H), 3.68 (t, 2H, J = 7.1Hz), 4.07-4.24 (m, 4H), 7.71-7.75 (m, 2H), 7.82-7.86 (m, 2H)
9d	1.34-1.38 (m, 12H), 1.64-1.67 (m, 4H), 2.79-2.89 (m, 2H), 3.68 (t, 2H, J = 7.2Hz), 4.28-4.25 (m, 4H), 7.70-7.73 (m, 2H), 7.83-7.86 (m, 2H)
9e	1.33-1.39 (m, 14H), 1.60-1.67 (m, 4H), 2.79-2.89 (m, 2H), 3.68 (t, 2H, J = 7.2Hz), 4.08-4.25 (m, 4H), 7.70-7.74 (m, 2H), 7.82-7.86 (m, 2H)
9f	1.26-1.39 (m, 16H), 1.60-1.69 (m, 4H), 2.79-2.89 (m, 2H), 3.67 (t, 2H, J = 7.2Hz), 4.08-4.25 (m, 4H), 7.70-7.74 (m, 2H), 7.83-7.86 (m, 2H)
9g	1.26-1.39 (m, 18H), 1.60-1.70 (m, 4H), 2.79-2.90 (m, 2H), 3.67 (t, 2H, J = 7.2Hz), 4.08-4.25 (m, 4H), 7.70-7.74 (m, 2H), 7.81-7.86 (m, 2H)
9h	1.31-1.37 (m, 6H), 3.44-3.52 (m, 2H), 4.05-4.25 (m, 4H), 4.27-4.28 (m, 2H), 5.76-5.80 (m, 2H), 7.72-7.76 (m, 2H), 7.81-7.86 (m, 2H)

The target compounds *N*-(ω-diethoxyphosphinothioyl)phthalimides (**9a-h**) were prepared from **1** and the corresponding *N*-(ω-bromoalkyl)phthalimides (**8a-g**) and *N*-(ω-bromoalkenyl)phthalimides (**8h**) were obtained as described above (see *Table 1*). Data from magnetic resonance spectroscopy are collected in *Table 2*.

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