Organic Phosphorus Compounds. IV. Asymmetric 4-(Benzothiazol-2-yl)benzylphosphonates as Potent Calcium Antagonistic Vasodilators[1)]

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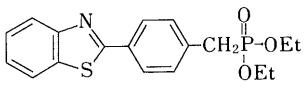
Various asymmetric or cyclic ester derivatives of the phosphonic acid moiety in the calcium antagonist fostedil (KB-944) were synthesized. The coronary vasodilator activity of these compounds was assessed by Langendorff's method. Among them, the ethyl isopropyl ester 12 showed the most potent activity, which was comparable to that of fostedil.

Keywords fostedil; coronary vasodilator; calcium antagonist; phosphonic acid; phosphonate; benzothiazole

Fostedil (**1**) is a novel calcium antagonist developed by us[2)] and whose structure is totally different from those of convention calcium antagonists. In our earlier work,[2,3)] the presence of the dialkoxyphosphinoylmethyl moiety was proved to play an important role in coronary vasodilator action of fostedil.

The structure activity relationship of 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-pyridine-3,5-dicarboxylate (abbreviated as DNPD hereafter), which is the basic structure of the representative calcium antagonist nifedipine was reported by Mayer and co-workers.[4)] Carboxylic groups at the 3,5-position of DNPD were modified in various ways and asymmetric esters were shown to have stronger and longer-lasting vasodilator activity than symmetric esters.[4)]

In this paper, we report the synthesis and the coronary vasodilator activity of a series of asymmetric phosphonates (**10**—**30**), as well as the coronary vasodilator activity of cyclic phosphonates (**33**—**35**). We also investigated whether the structure–activity relationship of DNPD applied to the phosphonates as well.



fostedil (**1**)

Fig. 1

Chemistry

The asymmetric esters **17**, **21**, **22** and **24** were prepared

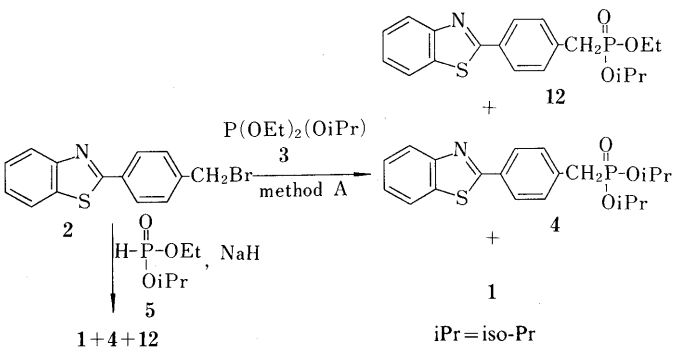


Chart 1

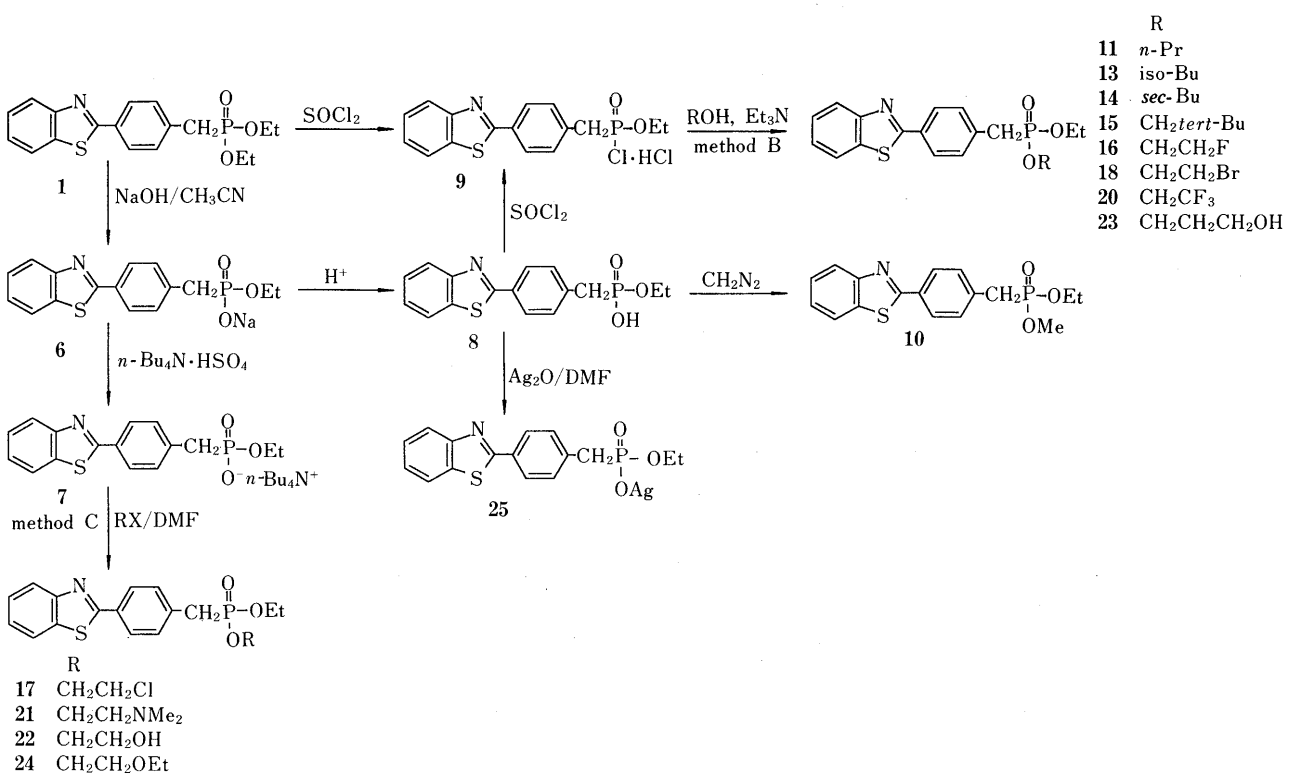


Chart 2

from the reaction of the ammonium salt **7** and various halides as shown in Chart 2 (method C). Fostedil[2] (**1**) was treated with NaOH in CH_3CN to give the sodium monophosphonate **6**, which was converted to the tetra-*n*-butylammonium salt **7** by means of the ion-pair extraction method. Then, the ammonium salt **7** was allowed to react with various halides in dimethylformamide (DMF), and the asymmetric phosphonates were obtained in moderate yield (26—50%).

Compound **11**, **13**—**16**, **18**, **20**, **23** were successfully synthesized from the phosphonochloridate **9** and alcohols as shown in Chart 2 (method B). We found that the chloridate **9**, which has generally been prepared from the monoester **8**, could be directly obtained from fostedil simply by refluxing fostedil with an excess of thionyl chloride. The phosphonochloridate thus obtained was then allowed to react with various alcohols in tetrahydrofuran (THF) in the presence of triethylamine, and corresponding asymmetric phosphonates were obtained in yields of 24—80%.

We also examined other conventional methods for synthesis of asymmetric phosphonates. However, no other method investigated in this study was effective. For example, the monoester **8** was converted to a silver salt with $AgNO_3$ and treated with methyl iodide in DMF, but no reaction occurred. This is probably due to the very low solubility of the silver salt in DMF. Synthesis of the ethyl isopropyl ester **12** was attempted by the reaction of the benzyl bromide[2] **2** with diethyl isopropyl phosphite[5] **3** (Michaelis–Arbuzov reaction) or reaction of **2** with ethyl isopropyl phosphite[6] **5** (Michaelis–Becker reaction). These reactions gave mixtures of the ethyl isopropyl ester **12**, diisopropylester **4** and diethylester **1**, and separation of these mixtures was rather difficult.

The synthetic pathway for the nitrate ester derivatives is shown in Chart 3 (method D). The hydroxyalkylester was nitrated with concentrated nitric acid in the presence of anhydrous acetic acid to give the nitrate esters **26**, **27** and **28**, together with the acetate esters **29** and **30**. The nitrate esters could easily be separated from the acetate esters by silica gel column chromatography.

Chart 4 shows the synthetic pathway for the cyclic esters. The propanediol cyclic ester **33** was synthesized from the benzyl bromide[2] **2** and ethyl propanediol cyclic phosphite[8] **31** by means of the Michaelis–Arbuzov reaction, but the ring-opened compound **19** was formed as a by-product. The cyclic esters **34** and **35** were synthesized from **2** by treatment with the alkyl hydrogen cyclic phosphite sodium salt **32** in benzene (method E).

Pharmacological Results and Discussion

The coronary vasodilator action of asymmetric esters was examined in isolated guinea-pig hearts (Langendorff's method).[7] Table I shows the coronary vasodilator effects of the asymmetric esters at a constant concentration of 10 μg/heart. The phosphonic acid monoester **8** exhibited only a weak action. In the case of the asymmetric alkylesters **10**—**13** and **15**, the greater the carbon number of the alkyl group, the stronger the action was. In particular, the iso-Bu ester **13** showed a stronger activity than that of fostedil. The halogen-containing fostedil derivatives **16**, **17** and **18** were found to be similar to fostedil in vasodilator activity regardless of the type of introduced halogen. Replacement of the ethyl group of fostedil by a tri-

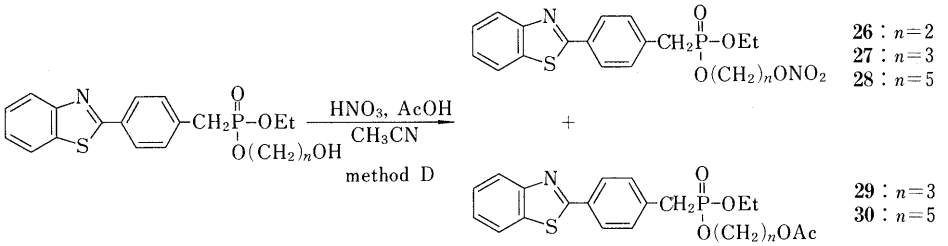


Chart 3

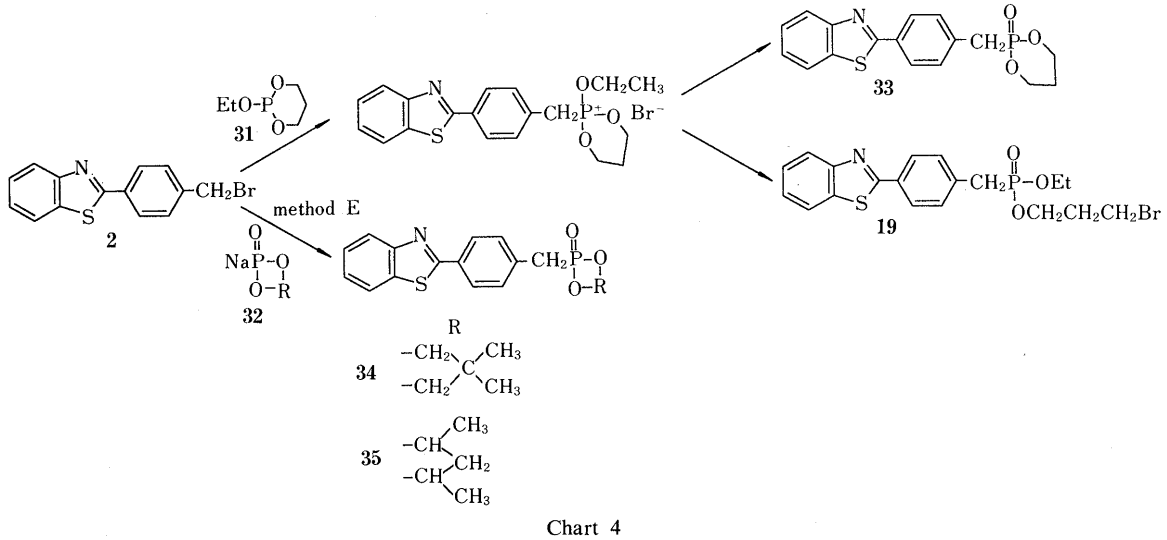
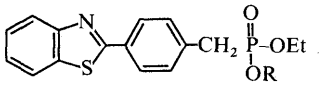


Chart 4

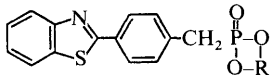
TABLE I. Effect of the Asymmetric Phosphonates on Coronary Blood Flow



Compd. No.	R	Method of prpn.	Yield (%)	mp (°C)	Recrystn. solvent	Formula	Max increase in coronary flow, (%)	
							Isolated heart (10 μg/heart)[a]	Dog, i.v. (0.1 mg/kg)[b]
8	H	F[c]	65	197—198	Benzene	$C_{16}H_{16}NO_3PS$	23.8± 5.2	ND
10	Me	F	76	82—83	*n*-Hexane	$C_{17}H_{18}NO_3PS$	54.9±16.6	ND
11	*n*-Pr	B	80	99—100	Cyclohexane	$C_{19}H_{22}NO_3PS$	44.0±12.5	ND
12	iso-Pr	A	25	95—97	Cyclohexane	$C_{19}H_{22}NO_3PS$	77.5± 6.0	79
13	iso-Bu	B	72	94—95	*n*-Hexane	$C_{20}H_{24}NO_3PS$	93.5± 7.5	58
14	*sec*-Bu	B	38	88—92	*n*-Hexane	$C_{20}H_{24}NO_3PS$	ND	59
15	CH_2*tert*-Bu	B	25	102—103	*n*-Hexane	$C_{21}H_{26}NO_3PS$	83.9±10.9	32
16	CH_2CH_2F	B	79	88—89	Cyclohexane	$C_{18}H_{19}FNO_3PS$	71.1± 7.5	70
17	CH_2CH_2Cl	C	39	95—96	Cyclohexane	$C_{18}H_{19}ClNO_3PS$	79.1± 7.8	66
18	CH_2CH_2Br	B	40	104—106	Cyclohexane	$C_{18}H_{19}BrNO_3PS$	88.7± 9.8	51
19	$(CH_2)_3Br$	B	24	77—78	*n*-Hexane	$C_{19}H_{21}BrNO_3PS$	67.1± 5.8	ND
20	CH_2CF_3	B	58	143—144	Cyclohexane	$C_{18}H_{17}F_3NO_3PS$	59.1± 3.5	Inactive
21	$CH_2CH_2NMe_2$	C	35	38—43	*n*-Hexane	$C_{20}H_{25}N_2O_3PS$	48.8± 6.6	ND
22	CH_2CH_2OH	C	50	115—116	Cyclohexane	$C_{18}H_{20}NO_4PS$	34.4± 5.9	ND
23	$(CH_2)_3OH$	B	36	60—62	*n*-Hexane	$C_{19}H_{22}NO_4PS$	ND	38
24	CH_2CH_2OEt	C	26	50—51	*n*-Hexane	$C_{20}H_{24}NO_4PS$	40.1± 5.5	ND
26	$CH_2CH_2ONO_2$	D	60	81—82	*n*-Hexane	$C_{18}H_{19}N_2O_6PS$	ND	53
27	$(CH_2)_3ONO_2$	D	38	68—70	*n*-Hexane	$C_{19}H_{21}N_2O_6PS$	ND	45
28	$(CH_2)_5ONO_2$	D	27	73—75	*n*-Hexane	$C_{21}H_{25}N_2O_6PS$	ND	35
29	$(CH_2)_3OAc$	D	54	49—50	*n*-Hexane	$C_{21}H_{24}NO_5PS$	ND	43
30	$(CH_2)_5OAc$	D	8	69—71	*n*-Hexane	$C_{23}H_{28}NO_5PS$	ND	48
Fostedil	(R=Et)						79.9± 8.6	82

a) Langendorff's method in isolated guinea pig heart. See the text. The test compound dissolved in propylene glycol to a concentration of 100 μg/ml was infused at a rate of 0.1 ml/min. The results are presented as the mean±SE for five experiments. *b*) The test compound was dissolved in 0.9% saline which contained 20% polyethyleneglycol-400 (PEG-400) and 20% ethanol and injected into the femoral vein at a dose of 0.1 mg/kg. The results are presented as the average of three experiments. *c*) See experimental section.

TABLE II. Effect of the Cyclic Phosphonates on Coronary Blood Flow



Compd. No.	R	Method of prpn.	Yield (%)	mp (°C)	Recrystn. solvent	Formula	Max increase in coronary flow, (%)	
							Isolated heart (10 μg/heart)[a]	Dog, i.v.
33	$-(CH_2)_3-$	A	15	216—217	AcOEt	$C_{17}H_{16}NO_3PS$	59.5±13.2	ND
34	$-CH_2C(CH_3)_2CH_2-$	E	30	216—217	Benzene	$C_{19}H_{20}NO_3PS$	37.0± 4.9	ND
35	$-CH(CH_3)CH_2CH(CH_3)-$	E	77	262—264	$CHCl_3$–ether	$C_{19}H_{20}NO_3PS$	34.1±15.3	ND

a) Langendorff's method in isolated guinea pig heart. See the text. The test compound dissolved in propylene glycol to a concentration of 100 μg/ml was infused at a rate of 0.1 ml/min. The results are presented as the mean±S.E. for five experiments.

fluoroethyl group (compound **20**) resulted in a reduced activity. Introduction of a dimethylamino group (compound **21**), a hydroxy group (compound **22**) or an ethoxy group (compound **24**) into the ethyl group of fostedil also reduced the activity.

The coronary vasodilator effects of the cyclic esters are given in Table II. The cyclic esters were found to show weaker coronary vasodilator action than fostedil. From the comparison of the vasodilator activity of **33** with that of **34** or **35**, it is evident that introduction of an alkyl group into the ester ring causes a decrease in coronary vasodilator activity.

In the next step, the asymmetric esters were administered to dogs intravenously at a dose of 0.1 mg/kg and the coronary vasodilator effects were examined. The results are shown in Table I. The coronary vasodilator action in isolated guinea-pig hearts and that in dogs (i.v.) were not necessarily parallel for the compounds tested here. For example, compound **13** was most active in the isolated guinea-pig heart assay system, but exhibited only a moderate activity in dog, and its action was weaker than that of fostedil. Species difference may cause this lack of parallelism, but it is also conceivable that interactions of the compounds with blood constituents such as albumin may have affected the result in the case of intravenous injection in the dogs.

Among the asymmetric or cyclic esters, the ethyl isopropyl ester **12** exhibited the strongest coronary vasodilator activity when injected into dog, and it was nearly equipotent to fostedil.

Although Mayer and co-workers found that asymmetric esters exhibited stronger coronary vasodilator activity than symmetric esters,[4] the above results indicate that, in the case of the phosphonates, conversion of a symmetric ester to an asymmetric one does not lead to an increase in the coronary vasodilator activity.

Experimental

Melting points were taken on a capillary melting point apparatus (Yamato MR-21) and are uncorrected. The structures of all compounds were supported by their infrared spectrum (IR) (Shimazu IR-440) and 60 and 100 MHz proton nuclear magnetic resonance (^{1}H-NMR) (Hitachi R-24A and Nihon Denshi PS-100) spectra. All compounds were analyzed for C, H and N and the results were within 0.4% of the calculated theoretical values. No attempt was made to maximize the yields.

Ethyl Isopropyl 4-(Benzothiazol-2-yl)benzylphosphonate (12), Method A A mixture of 6.0 g (0.020 mol) of 2-(4-bromomethylphenyl)-benzothiazole[2] (**2**) and 4.6 g (0.026 mol) of diethyl isopropyl phosphite[5] (**3**) was stirred at 130—140 °C for 15 min under a slow nitrogen gas stream. The reaction mixture was allowed to cool to room temperature. The resulting solid, which contained 3 components, was washed with *n*-hexane and chromatographed on silica gel, eluting with benzene. The main fraction was obtained as a mixture of components. Each pure fraction was evaporated and the residue was recrystallized from cyclohexane. The products were identified as the diethylester[2] **1** (0.6 g), diisopropyl ester[2] **4** (0.4 g) and ethyl isopropyl ester **12** (1.8 g, 25%) by NMR. **12** was obtained as colorless needles: mp 96.5—98.0 °C. *Anal.* Calcd for $C_{19}H_{22}NO_3PS$: C, 60.79; H, 5.91; N, 3.73. Found: C, 60.75; H, 6.01; N, 3.84. ^{1}H-NMR ($CDCl_3$) δ: 1.13—1.35 (9H, m), 3.0 (2H, d, J=22 Hz), 3.72—4.21 (2H, m), 4.31—4.85 (1H, m), 7.0—7.44 (4H, m), 7.64—8.09 (4H, m).

Ethyl Hydrogen 4-(benzothiazol-2-yl)benzylphosphonate (8) A solution of sodium hydroxide (0.13 g, 0.0031 mol) in EtOH (5 ml) was added to a stirred solution of diethyl 4-(benzothiazol-2-yl)benzylphosphonate[2] (**1**) (1.0 g, 0.0027 mol) in EtOH (5 ml) at room temperature. The reaction mixture was heated at reflux for 3 h and then allowed to cool to room temperature. The precipitated solid was collected and dissolved in water (80 ml); a small quantity of insoluble material was filtered off. The pH of the filtrate was adjusted to 1.5 by the addition of 1 N HCl. The resulting solid was collected by filtration and washed with water to give a colorless crystalline powder. Recrystallization of the powder from benzene yielded 0.60 g (65%) of **8** as colorless needles: mp 197.0—198.0 °C. *Anal.* Calcd for $C_{16}H_{16}NO_3PS$: C, 57.65; H, 4.84; N, 4.20. Found: C, 57.76; H, 4.75; N, 4.10. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (3H, t, J=8 Hz), 2.45—2.57 (1H, br), 3.20 (2H, d, J=21 Hz), 3.72—4.20 (2H, m), 7.30—7.67 (4H, m), 7.82—8.22 (4H, m).

2-Chloroethyl Ethyl 4-(Benzothiazol-2-yl)benzylphosphonate (17), Method C Tetra-*n*-butylammonium hydrogen sulfate (9.1 g, 0.027 mol) was added to a solution of the monophosphonate **8** (9.0 g, 0.027 mol) in 10% aqueous NaOH (150 ml). The mixture was stirred at room temperature for 30 min and extracted with CH_2Cl_2 (60 ml × 3). The organic layer was dried over $MgSO_4$, and evaporated. The residue was washed with cyclohexane to give a pale yellow oil. The residual oil was dissolved in DMF (40 ml), then 1-bromo-2-chloroethane (3.9 g, 0.0273 mol) was added at room temperature, and the whole was stirred at 50 °C for 4 h. The reaction mixture was poured into 100 ml of water and extracted with CH_2Cl_2 (80 ml × 3). The organic phase was washed with water, dried over $MgSO_4$, and evaporated. The residual solid was chromatographed on silica gel with benzene and ethyl acetate as eluents to give 4.2 g (39%) of **17** as a colorless powder. Recrystallization from cyclohexane gave **17** as colorless needles: mp 94.5—95.5 °C. *Anal.* Calcd for $C_{18}H_{19}ClNO_3PS$: C, 54.62; H, 4.84, N, 3.54. Found: C, 54.36; H, 4.98; N, 3.32. ^{1}H-NMR($CDCl_3$) δ: 1.28 (3H, t, J=8 Hz), 3.19 (2H, d, J=22 Hz), 3.35—3.60 (2H, m), 3.77—4.25 (4H, m), 7.20—7.45 (4H, m), 7.67—8.00 (4H, m).

Ethyl Methyl 4-(Benzothiazol-2-yl)benzylphosphonate (10) A solution of the monophosphonate **8** (2.3 g, 0.0069 mol) in methanol (150 ml) was cooled to 0 °C and an excess of an ethereal solution of diazomethane was added. The mixture was stirred at 0—10 °C for 2 h. After additional stirring at room temperature for 1 h, the solution was evaporated and residual solid was recrystallized from *n*-hexane to give 1.8 g (76%) of **10** as colorless needles: mp 82.0—83.0 °C. *Anal.* Calcd for $C_{17}H_{18}NO_3PS$: C, 58.78; H, 5.22; N, 4.03. Found: C, 58.93; H, 5.28; N, 3.95. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7 Hz), 3.05 (2H, d, J=22 Hz), 3.57 (3H, d, J=11 Hz), 3.95 (2H, m), 7.15—7.50 (4H, m), 7.65—8.05 (4H, m).

Isobutyl Ethyl 4-(Benzothiazol-2-yl)benzylphosphonate (13), Method B A mixture of diethyl 4-(benzothiazol-2-yl)benzylphosphonate (**1**) (3.6 g, 0.01 mol), $SOCl_2$ (25 g), and a catalytic quantity of DMF was heated at reflux for 3 h. Evaporation of the excess $SOCl_2$ gave the hydrochloride salt of the phosphonochloridate **9** as a colorless powder (4.0 g, quant.). Et_3N (4.0 g, 0.04 mol) was added dropwise to a stirred suspension of **9** in THF (50 ml) at 0—5 °C. A solution of isobutyl alcohol (3.0 g, 0.04 mol) in THF (5 ml) was added dropwise with stirring. The ice bath was removed and the reaction mixture was stirred for 4 h. After partial evaporation of THF, the residue was diluted with water (100 ml) and extracted with ether (100 ml × 3). The organic layer was washed with water, dried over $MgSO_4$, and evaporated to yield an orange solid, which was purified *via* column chromatography on silica gel with AcOEt to give 2.8 g (72%) of **13**: mp 94.0—95.0 °C. *Anal.* Calcd for $C_{20}H_{24}NO_3PS$: C, 61.68; H, 6.21; N, 3.60. Found: C, 61.54; H, 6.09; N, 3.62. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (6H, d, J=8 Hz), 1.28 (3H, t, J=8 Hz), 1.68—2.08 (1H, m), 3.24 (2H, d, J=22 Hz), 3.76 (2H, t, J=8 Hz), 3.92—4.20 (2H, m), 7.28—7.56 (4H, m), 7.84—8.12 (4H, m).

Ethyl 3-Hydroxypropyl 4-(Benzothiazol-2-yl)benzylphosphonate (23) A suspension of the chloridate **9** (6.1 g, 0.015 mol) in THF (50 ml) was stirred and cooled at 0—5 °C during the dropwise addition of triethylamine (4.6 g, 0.06 mol). To the resultant solution, a solution of trimethyleneglycol (3.4 g, 0.045 mol) in THF (15 ml) was added dropwise with stirring. Stirring was continued at room temperature for 2 h, then the THF was evaporated off. The resulting solid was chromatographed on silica gel with chloroform/AcOEt (15 : 1) to give 2.1 g (36%) of **23**: mp 60.0—62.0 °C. *Anal.* Calcd for $C_{19}H_{22}NO_4PS$: C, 58.30; H, 5.67; N, 3.58. Found: C, 57.78; H, 6.02; N, 5.59. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=8 Hz), 1.70—1.93 (2H, m), 2.88—3.04 (1H, br), 3.26 (2H, d, J=21 Hz), 3.56—3.76 (2H, m), 3.94—4.26 (4H, m), 7.24—7.56 (4H, m), 7.82—8.04 (4H, m).

Ethyl 3-Nitroxypropyl 4-(Benzothiazol-2-yl)benzylphosphonate (27), Method D A solution of concentrated HNO_3 (d=1.52, 0.67 g) and acetic anhydride (1.1 g, 0.011 mol) in CH_3CN (7 ml) was gradually added to a solution of **23** in CH_3CN (30 ml) at 0—5 °C. The reaction mixture was stirred at room temperature for 96 h, then neutralized with 10% $NaHCO_3$ solution. The resulting solution was extracted with AcOEt (50 ml × 2) and the organic layer was dried over $MgSO_4$, then concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, AcOEt) to give **27** in 38% yield (0.62 g), mp 68—70 °C. *Anal.* Calcd for $C_{19}H_{21}N_2O_6PS$: C, 52.29; H, 4.85; N, 6.42. Found: C, 52.32; H, 5.05; N, 6.41. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=8 Hz), 1.88—2.12 (2H, m), 3.24 (2H, d, J=22 Hz), 3.92—4.20 (4H, m), 4.47 (2H, t, J=6 Hz), 7.24—7.58 (4H, m), 7.86—8.12 (4H, m). Ethyl 3-acetoxypropyl 4-(benzothiazol-2-yl)benzylphosphonate (**29**) (0.90 g, 54%) was obtained as a by-product: mp 49—50 °C. *Anal.* Calcd for $C_{21}H_{24}NO_5PS$: C, 58.19; H, 5.58; N, 3.23. Found: C, 58.02; H, 5.48; N, 3.21. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=8 Hz), 1.95 (2H, t, J=7 Hz), 2.05 (3H, s), 3.26 (2H, d, J=22 Hz), 3.92—4.22 (6H, m), 7.26—7.56 (4H, m), 7.84—8.08 (4H, m).

4-(Benzothiazol-2-yl)benzylphosphonic Acid 1,3-Propanediol Cyclic Ester (33) A mixture of 2-(4-bromomethylphenyl)benzothiazole[2] (**2**) (5.6 g, 0.018 mol) and ethyl propanediol cyclic phosphite[8] (**31**) (4.0 g, 0.027 mol) was stirred at 140 °C for 20 min under a slow nitrogen gas flow. The reaction mixture was allowed to cool to room temperature, and addition of a small amount of benzene gave a colorless precipitate. The solid was collected by filtration and was recrystallized from ethyl acetate to yield 0.95 g (15%) of **33**: mp 216.0—217.0 °C. *Anal.* Calcd for $C_{17}H_{16}NO_3PS$: C, 59.12; H, 4.67; N, 4.05. Found: C, 58.87; H, 4.67; N, 4.05. ^{1}H-NMR ($CDCl_3$) δ: 1.60—2.20 (2H, m), 3.45 (2H, d, J=21 Hz), 3.90—4.55 (4H, m), 7.25—7.60 (4H, m), 7.75—8.05 (4H, m).

4-(Benzothiazol-2-yl)benzylphosphonic Acid 2,4-Pentanediol Cyclic Ester (35), Method E A solution of 2,4-pentanediol hydrogen cyclicphosphite[9] (2.5 g, 0.017 mol) in dry benzene (20 ml) was treated with 50% NaH (0.8 g, 0.016 mol) in dry benzene (20 ml). After the evolution of hydrogen gas had ceased, 2-(4-bromomethylphenyl)benzothiazole (**2**) (5.0 g, 0.016 mol) was added and the mixture was stirred at room temperature for 1 h, and then under reflux for 1 h. After cooling to room temperature, the resulting solid was collected by filtration and recrystallized from ether–chloroform mixture to give **35** (4.6 g, 77%): mp 261.5—263.5 °C. *Anal.* Calcd for $C_{19}H_{20}NO_3PS$: C, 61.11; H, 5.40; N, 3.75. Found: C, 60.87; H, 5.33; N,

3.67. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (6H, dd, $J=2$ Hz, 6 Hz), 1.58—1.95 (2H, m), 3.30 (2H, d, $J=21$ Hz), 4.20—4.35 (2H, m), 7.28—7.65 (4H, m), 7.70—8.05 (4H, m).

Effect on Coronary Flow in the Isolated Guinea Pig Heart Male guinea pigs of 400 to 500 g body weight were killed and promptly thoracotomized. After cannulation of the ascending aorta, the heart was enucleated. The isolated heart was then perfused with Krebs–Henseleit fluid which was oxygenated with a gaseous mixture of 95% O_2 and 5% CO_2, at 34 ± 1 °C under a perfusion pressure of 60 cm H_2O by the method of Langendorff.[7] The test compound dissolved in propylene glycol to a concentration of 100 μg/ml was infused at a rate of 0.1 ml/min. The coronary flow was measured with a square wave electromagnetic flow mether (Nihon Kohden, MF-26) with an extracorporal probe (Nihon Kohden FE) set at the top of the cannula, and recorded with a multipurpose polygraph (Nihon Kohden, RM-85). The coronary flows before and after infusion were measured and the percentage change in coronary flow was obtained.

Effect on Coronary Arterial Blood Flow in Anesthetized Dogs Dogs were anesthetized with sodium pentobarbital (35 mg/kg, i.v.) and thoracotomized at the 5th left intercostal space under artificial respiration. The blood flow of the circumflex branch of the left coronary artery and that of the carotid artery were measured with an electromagnetic flow meter (Nihon Kohden, MF-26). The test compounds were dissolved in 0.9% saline which contained 20% polyethyleneglycol-400 (PEG-400) and 20% ethanol and injected into the femoral vein at a dose of 0.1 mg/kg.

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