And as there is the possibility of the simultaneous determination with SAs for several other synthetic antibacterials containing ethopabate, this method, extended and complemented a little more, is considered to be used as the official method.

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Synthesis of Fosfazinomycin Derivatives

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In the previous papers, we reported synthesis of Fosfazinomycin A and B.[1,2] Aminophosphonic acids and their derivatives have attracted attention because of their antibiotic, herbicidal, pesticidal, anticancer and enzyme inhibitory activities, and particulary their structural similarity to the biologically important amino acids. Since 2-aminophosphonic acid (2-AEPn) was isolated from sheep rumen in 1959 by Horiguchi and his coworkers,[3] many aminophosphonic acids and their derivatives have been discovered in living organism. Aminophosphonic acids are also discovered in mammalian tissues like human muscale, sheep liver, and ox brain.[4-10] Its concentration in human tissues was higher in heart and skeletal muscale than in liver and brain. Recently, incorporated with synthetic derivatives, biological activity of aminophosphonopeptides were widely investigated. Among various disciplines neurochemistry and neuropharmacology are the most brisk areas concerning the activity of aminophosphonic acid.[11] Fosfazinomycin, formerly called AM 630, is a new antifungal substance isolated from the fermentation broth of *Steptomyces lavendofoliae*.[12-15]

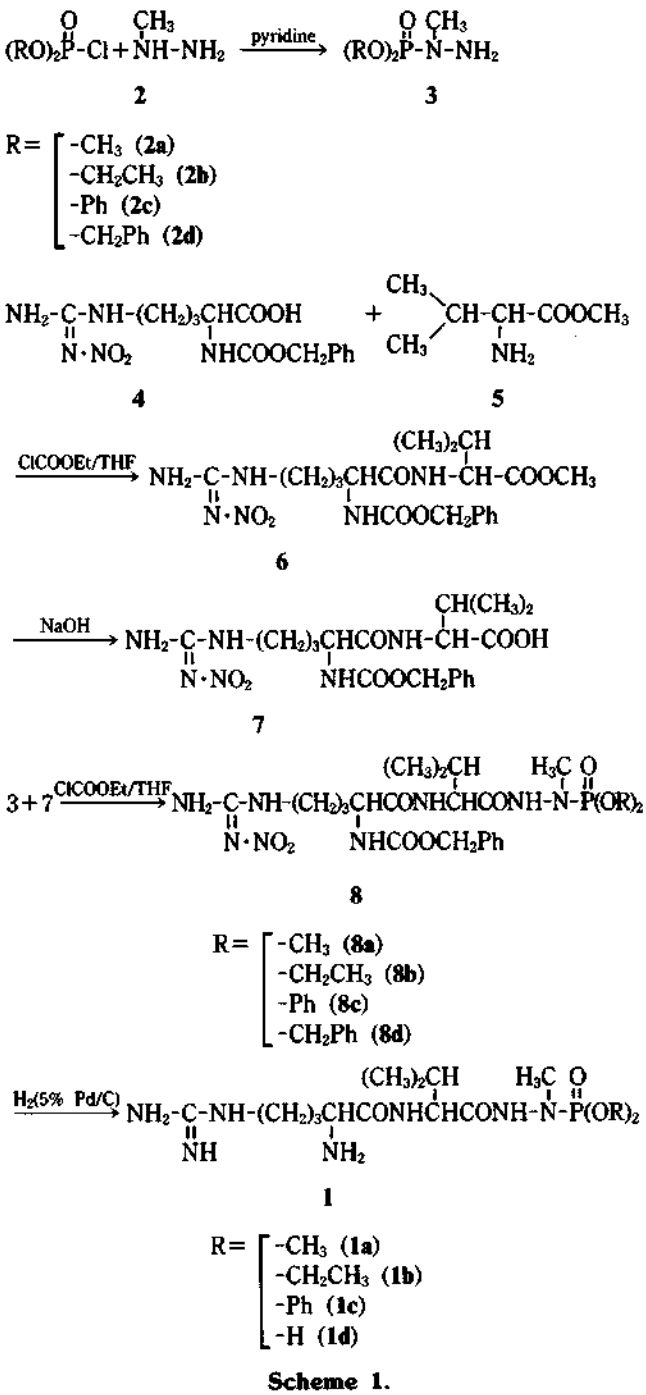
In this paper, the authors wish to report the synthesis of Fosfazinomycin derivatives **1(a)-1(d)**, dialkyl arginylvalyl-*N*-methylhydrazinophosphate, as shown in Scheme 1. Dialkyl chlorophosphate (**2**) was reacted with methylhydrazine to give dialkyl *N*-methylhydrazinophosphate (**3**). The peptide **6** was prepared by coupling of *N*-carbobenzyloxy-*N*-nitroarginine (**4**) with methyl valinate (**5**). *N*-Carbobenzyloxy-*N*-nitroarginylvaline (**7**) was reacted with dialkyl *N*-methylhydrazinophosphate (**3**) to give a coupled product of dialkyl *N*-carbobenzyloxy-*N*-nitroarginylvalyl-*N*-methylhydrazinophosphate **8(a)-8(d)**. The deprotection of **8** by hydrogenation yielded Fosfazinomycin derivatives **1(a)-1(d)**. In conclusion, Fosfazinomycin derivatives **1(a)-1(d)**, a new kind of phosphorus compunds, were synthesized efficiently in 5 steps with 23-29% overall yields.

Experimental Section

All reactions were carried out with the precaution for rigorous exclusion of air and moisture. The solvents, ether and THF, were purified by refluxing for several hours in the presence of sodium metal and benzophnone followed by distillation under nitrogen prior to use. Melting points were measured by Mettler F61 melting point apparatus. IR spectra were recorded with Beckmann acculab TMI spectrometer, and proton NMR spectra were taken on Varian EM-360 (80 MHz) spectrometer with TMS as an internal standard. Low pressure hydrogenation was carried out with Parr instrument hydrogenator.

Dimethyl *N*-methylhydrazinophosphate (3a). To a solution of dimethyl chlorophosphate (**2a**) (4.64 g, 0.032 mol) in dry THF (20 m*l*), a solution of methylhydrazine (1.68 m*l*, 0.032 mol) and pyridine (2.6 m*l*) was added slowly under nitrogen at −78℃. The mixture was stirred for 4 hr at −78 ℃ and 8 hr at room temperature. A few m*l* of H_2O was added to the solution, and the resulting mixture was extracted with ether and ethyl acetate successively. The combined organic layers were dried over anhydrous magnesium sulfate, and the solvent was removed *in vacuo*. The crude product was chromatographed on a silica gel column using ethyl acetate and hexane (1 : 1, v/v) as an eluent to give an oily product in 66% yield.

Anal. Calc. for $C_3H_{11}N_2O_3P$; C, 23.38; H, 7.14; N, 18.19;



Scheme 1.

O, 31.17; P, 20.11. Found: C, 22.41; H, 7.13; N, 18.91; O, 30.41; P, 21.14. ^{1}H-NMR ($CDCl_3$): δ 2.87 (d, 3H, $J_{H\text{-}P}$=6 Hz, -NC$\underline{H}_3$), 3.9 (d, 6H, $J_{H\text{-}P}$=7 Hz, -OC$\underline{H}_3$); IR (neat): 3370 (NH_2), 1200 (P=O), 990 cm^{-1} (P-O-C). (**3b**) ^{1}H-NMR ($CDCl_3$): δ 1.4 (m, 6H, -C$\underline{H}_3$), 4.2 (m, 4H, -C$\underline{H}_2$CH$_3$), (**3c**) ^{1}H-NMR ($CDCl_3$): δ 7.2 (s, 10H, -C$_6\underline{H}_5$), (**3d**) ^{1}H-NMR ($CDCl_3$): δ 5.0 (d, 4H, $J_{H\text{-}P}$=8 Hz, -C$\underline{H}_2$C$_6$H$_5$), 7.35 (s, 10H, -CH$_2$C$_6\underline{H}_5$).

Methyl *N*-carbobenzyloxy-*N*-nitroarginylvalinate (6). To a solution of *N*-carbobenzyloxy-*N*-nitro-arginine (**4**)[2] (16.25 g, 0.046 mol) in dry THF (46.5 m*l*), triethylamine (6.35 m*l*) and ethyl chloroformate (4.425 m*l*, 0.046 mol) were added carefully through a dropping funnel over 20 min under nitrogen at 0℃. After stirring for 30 min, a solution of methyl valinate (**5**) (6.03 g, 0.046 mol) in DMF (50 m*l*) and THF (23 m*l*) was added carefully through a dropping funnel during 40 min and resulting mixture was stirred for 24 hr at 0℃. After the solvent was removed *in vacuo*, ethyl acetate (50 m*l*) was added. This mixture was washed with 1N HCl, water, and saturated aqueous solution of $NaHCO_3$ successively, then the organic layer was dried over anhydrous sodium sulfate. After the solvent was removed *in vacuo*, the crude product recrystallized with methanol and *n*-hexane. A white crystal of methyl *N*-carbobenzyloxy-*N*-nitroarginylvalinate was obtained in 69% yield.

Anal. Calc. for $C_{20}H_{30}N_6O_7$; C, 51.50; H, 6.43; N, 18.03; O, 24.03. Found: C, 50.91; H, 6.41; N, 18.66; O, 24.02. mp. 153℃; ^{1}H-NMR ($CDCl_3$): δ 1.1 (m, 6H, -CH(C$\underline{H}_3$)$_2$), 1.85 (m, 4H, -NHCH$_2$C$\underline{H}_2$C$\underline{H}_2$CH-), 2.3 (m, 1H, -C$\underline{H}$(CH$_3$)$_2$), 3.3 (m, 3H, -NHC$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}$-), 3.5 (d, 1H, J=5 Hz, -NHC$\underline{H}$-), 3.7 (s, 3H, -OC$\underline{H}_3$), 5.25 (s, 2H, -OC$\underline{H}_2$C$_6$H$_5$), 7.2 (s, 5H, -OCH$_2$C$_6\underline{H}_5$), IR (KBr): 3500-3300 (amino group), 1750 (C=O), 1680-1630 cm^{-1} (-CONH-).

***N*-Carbobenzyloxy-*N*-nitroarginylvaline (7).** To a solution of methyl *N*-carbobenzyloxy-*N*-nitroarginylvalinate (**6**) (0.49 g, 1 mmol) in acetone (3 m*l*) and water (3 m*l*), 1N NaOH solution (1.1 m*l*) was added carefully through a dropping funnel over 10 min and stirred for 1.5 hr at room temperature. After the solvent was removed *in vacuo*, the crude product was washed with ether and chloroform successively and acdified with concentrated HCl. The oily product was extracted with ethyl acetate. After the solvent was removed *in vacuo*, the crude product recrystallized with ethanol and *n*-hexane to give a white crystal in 87% yield.

Anal. Calc. for $C_{19}H_{28}N_6O_7$; C, 50.44; H, 6.19; N, 18.58; O, 24.78. Found: C, 51.23; H, 6.02; N, 18.63; O, 24.12. mp. 179℃; ^{1}H-NMR ($CDCl_3$): δ 0.9 (m, 6H, -CH(C$\underline{H}_3$)$_2$), 1.75 (m, 4H, -NHCH$_2$C$\underline{H}_2$C$\underline{H}_2$CH-), 2.15 (m, 1H, -C$\underline{H}$(CH$_3$)$_2$), 3.25 (m, 3H, -NHC$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}$-), 3.4 (d, 1H, J=5 Hz, -NHC$\underline{H}$-), 5.2 (s, 2H, -OC$\underline{H}_2$C$_6$H$_5$), 7.15 (s, 5H, -OCH$_2$C$_6\underline{H}_5$), 11.25 (s, 1H, -COO$\underline{H}$): IR (KBr): 3500 (amino group), 3000 (COOH), 1700 (C=O), 1620-1600 cm^{-1} (amide).

Diethyl *N*-carbobenzyloxy-*N*-nitroarginylvalyl-*N*-methylhydrazinophosphate (8b). To a solution of *N*-carbobenzyloxy-*N*-nitroarginylvaline (**7**) (0.84 g, 1.86 mmol) in dry THF (3 m*l*) and triethylamine (0.26 m*l*), ethyl chloroformate (0.37 m*l*) was added carefully through the dropping funnel at 0℃. After stirring for 30 min, a solution of diethyl *N*-methylhydrazinophosphate (**3b**) (0.34 g, 1.87 mmol) in anhydrous THF (2.4 m*l*) and triethylamine (0.26 m*l*) was added slowly at 0℃. After stirring for 12 hr at 5℃, the resulting mixture was extracted with ether nd ethyl acetate successively. After the solvent was removed *in vacuo*, the residue was purified by silica gel column eluting with ethyl acetate and hexane (1 : 1, v/v) to give an oily product in 75% yield.

Anal. Calc. for $C_{28}H_{41}N_8O_9P$; C, 50.60; H, 6.18; N, 16.87; O, 21.69; P, 4.66. Found: C, 50.33; H, 6.14; N, 17.09; O, 21.63; P, 4.81. ^{1}H-NMR ($CDCl_3$): δ 0.95 (m, 6H, -CH(C$\underline{H}_3$)$_2$), 1.3 (m, 6H, -OCH$_2$C$\underline{H}_3$), 1.7 (m, 4H, -NHCH$_2$C$\underline{H}_2$C$\underline{H}_2$CH-), 2.9 (d, 3H, $J_{H\text{-}P}$=6 Hz, -NC$\underline{H}_3$), 3.25 (m, 3H, -NHC$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}$-), 4.1 (m, 4H, -OC$\underline{H}_2$CH$_3$), 5.3 (s, 2H, -OC$\underline{H}_2$C$_6$H$_5$), 7.3 (s, 5H, -OCH$_2$C$_6\underline{H}_5$); IR (neat): 3550 (amino group), 1650 (amide), 1220 (P=O), 1000 cm^{-1} (P-O-C). (**8a**) 3.8 (d, 6H, $J_{H\text{-}P}$=7 Hz, -OC$\underline{H}_3$), (**8c**) 7.15 (s, 10H, -C$_6\underline{H}_5$), (**8d**) 4.9 (d, 4H, $J_{H\text{-}P}$=8 Hz, -C$\underline{H}_2$C$_6$H$_5$), 7.25 (s, 10H, -CH$_2$C$_6\underline{H}_5$).

Diphenyl arginylvalyl-*N*-methylhydrazinophosphate (1c). In a 250 ml Parr low pressure hydrogenation apparatus, diphenyl *N*-carbobenzyloxy-*N*-nitroarginylvalyl-*N*-methylhydrazinophosphate (8c) (0.57 g, 0.8 mmol) in methanol (20 ml) and 5% palladium on charcoal (0.4 g) were added. Hydrogenation was accomplished under 15 psig of hydrogen pressure. After shaking for 4 hr at room temperature, the mixture was filtered through Celite and the solvent was removed *in vacuo*. The resulted yellow-green oil was chromatographed on silica gel column using ethyl acetate and hexane (2 : 1, v/v) as an eluent, to give a white crystal in 82% yield.

Anal. Calc. for $C_{20}H_{36}N_7O_5P$; C, 49.49; H, 7.42; N, 20.21; O, 16.50; P, 6.39. Found: C, 49.05; H, 7.39; N, 20.65; O, 16.44; P, 6.47. mp. 94℃ (methanol/*n*-hexane); ^{1}H-NMR ($CDCl_3$): δ 1.0 (m, 6H, -CH(CH($C\underline{H}_3$)$_2$), 1.75 (m, 4H, -NHCH$_2$C$\underline{H}_2$C$\underline{H}_2$CH-), 2.1 (m, 1H, -C$\underline{H}$(CH$_3$)$_2$), 2.87 (d, 3H, J=6 Hz, -NC$\underline{H}_3$), 3.2 (m, 3H, -NHC$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}$-), 3.3 (d, 1H, J=5 Hz, -C$\underline{H}$(CH$_3$)$_2$), 7.15 (s, 5H, -OC$_6\underline{H}_5$); IR (KBr): 3500-3200 (Guano group), 1650 (amide), 1250 (P=O), 980 cm^{-1} (P-O-C). (**1a**) mp. 86℃ (ethyl acetate/*n*-hexane); ^{1}H-NMR ($CDCl_3$): δ 3.8 (d, 6H $J_{H\text{-}P}$=7 Hz, -OC$\underline{H}_3$); IR (KBr): 3600-3500 (Guano group), 1680 (amide), 1170 (P=O), 980 cm^{-1} (P-O-C). (**1b**) mp. 89℃ (ethyl acetate/*n*-hexane); ^{1}H-NMR ($CDCl_3$): δ 1.35 (m, 6H, -OCH$_2$C$\underline{H}_3$), 4.3 (m, 4H, -OC$\underline{H}_2$CH$_3$); IR (KBr): 3500-3300 (Guano group), 1660 (amide), 1200 (P=O), 990 cm^{-1} (P-O-C). (**1d**) mp. 185℃ (ethanol/H_2O); IR (KBr): 3550-3300 (Guano group), 1690 (amide), 1150 (P=O), 1000 cm^{-1} (P-O-C).

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Theoretical Studies on the Photochemical Reaction of Psoralen Derivatives

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Psoralens are a family of naturally occurring or synthetic substances which are used in association with UV-A irradiation for treatment of several skin diseases. Psoralen derivatives have been used for many years in the experiments of vitiligo an psoriasis.[1,2] These compounds have also been studied very thoroughly in model systems and with model compounds, and their in *vivo* funtioning is not well understood yet.[3-5]

Various biological targets are likely to be involved in the therapeutic effects observed with such compounds. Their antiproliferative effects are usually related to their ability to undergo photocycloaddition reactions with pyrimidine bases of DNA, and particularly with thymine, leading to the formation of both monofunctional and bifunctional adducts.[6] The molecule which is being much studied at the moment is 3-carbethoxypsoralen (3-CPs).[7] This, unlike 8-MOP, only forms monoadducts and is supposed to be less toxic from the point of view of structure-activity relationship, that is, it is possibly less likely to cause skin cancer.

A mechanistic model for the photochemical reaction in which carcinogenophore contributes to the stabilization of the psoralen derivatives is postulated in our work.

The energy of the triplet state determind from the PM3 calculation appears to be significantly higher in the case of pyridopsoralens (PyPs) than for 3,4-benzopsoralen(3,4-BPs) and 3-CPs, increasing in the order:

4′,5′-BPs<3,4-BPs<3-CPs<PyPs

In the photoreactive psoralen derivatives, the intermolecular π-π interaction is not restricted to the excited states. The electron contour maps in the main molecular planes of excited psoralen derivatives are given in Figure 1.

The pyrone 3,4 and furan 4′,5′-double bonds are necessary