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SUBTITLE: Synthesis of Pentacoordinate Phosphorus Compounds, "Bait and Switch" Compounds, and Soman Simulants as Haptens for Production of Catalytic Antibodies Capable of Catalyzing Phosphoryl Transfer Reactions of Organic Phosphorus Agents

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The subject of catalytic antibodies has attracted a great deal of attention because of their capability of catalyzing a variety of chemical transformations.[1-4] These antibodies can be generated against almost any target molecule of interest, however, the design of the hapten is the most critical part. The four stereoisomers of soman differ in toxicity, rate of inhibition of cholinesterases, response to reactivators and rate of detoxification in the body.[5] From the viewpoint of neutralizing soman by use of an antibody it is of value to synthesize antibodies against each pure stereoisomer. This can be accomplished using a soman simulant.
We report a detailed study on the synthesis, isolation and characterization of the four pure enantiomers of [(S or R)-4-amino-2,2-dimethyl-2-butyl](methyl)methylphosphonates which were conjugated to protein carriers for use in the development of potentially therapeutic antibodies that are cross-reactive with soman.

A further goal is to determine whether monoclonal antibodies generated against each pure simulant stereoisomer behave selectively in catalyzing ester hydrolysis

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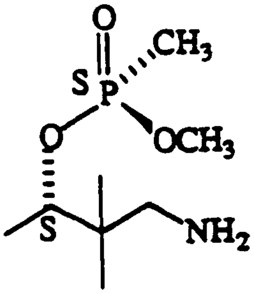
CHAPTER I: STEREOSELECTIVE SYNTHESIS OF SOMAN SIMULANTS AS HAPTENS FOR RAISING ANTIBODIES FOR CHEMICAL DEFENSE.

A. Introduction

The subject of catalytic antibodies has attracted a great deal of attention because of their capability of catalyzing a variety of chemical transformations.[1-4] These antibodies can be generated against almost any target molecule of interest, however, the design of the hapten is the most critical part. The four stereoisomers of soman differ in toxicity, rate of inhibition of cholinesterases, response to reactivators and rate of detoxification in the body.[5] From the viewpoint of neutralizing soman by use of an antibody it is of value to synthesize antibodies against each pure stereoisomer. This can be accomplished using a soman simulant.

We report a detailed study on the synthesis, isolation and characterization of the four pure enantiomers of [(S or R)-4-amino-2,2-dimethyl-2-butyl](methyl)methylphosphonates (1a-c), which were conjugated to protein carriers for use in the development of potentially therapeutic antibodies that are cross-reactive with soman.



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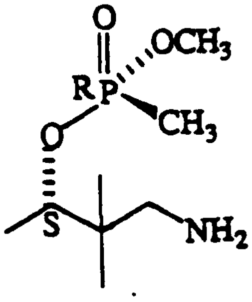

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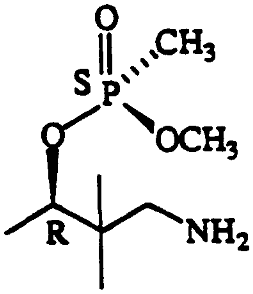

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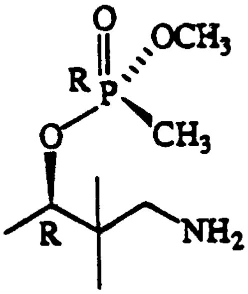
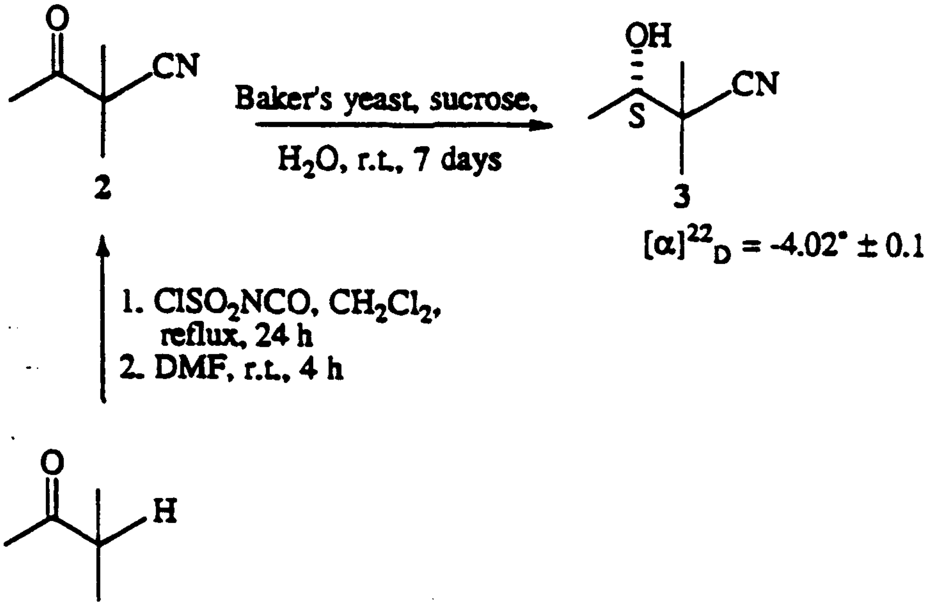


1d

A further goal is to determine whether monoclonal antibodies generated against each pure simulant stereoisomer behave selectively in catalyzing ester hydrolysis.

B. Results and Discussion

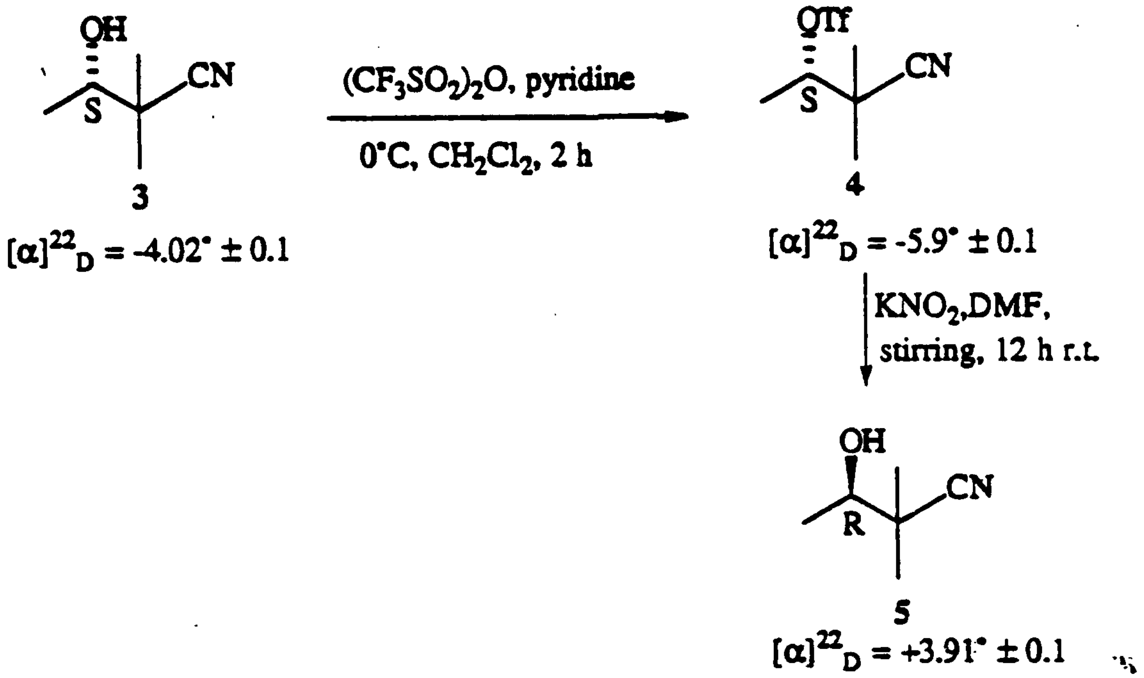
The stereochemical center at carbon is the easiest to control. The use of Baker's yeast as chiral reducing agent of the carbonyl group of a variety of ketones is known to proceed with high optical purities. In our case, reduction of 2,2-dimethyl-3-oxobutanenitrile[6] 2 with Baker's yeast gives exclusively (as shown by shift reagent studies) the S-alcohol 3 in 65% yield.[7,8,9]



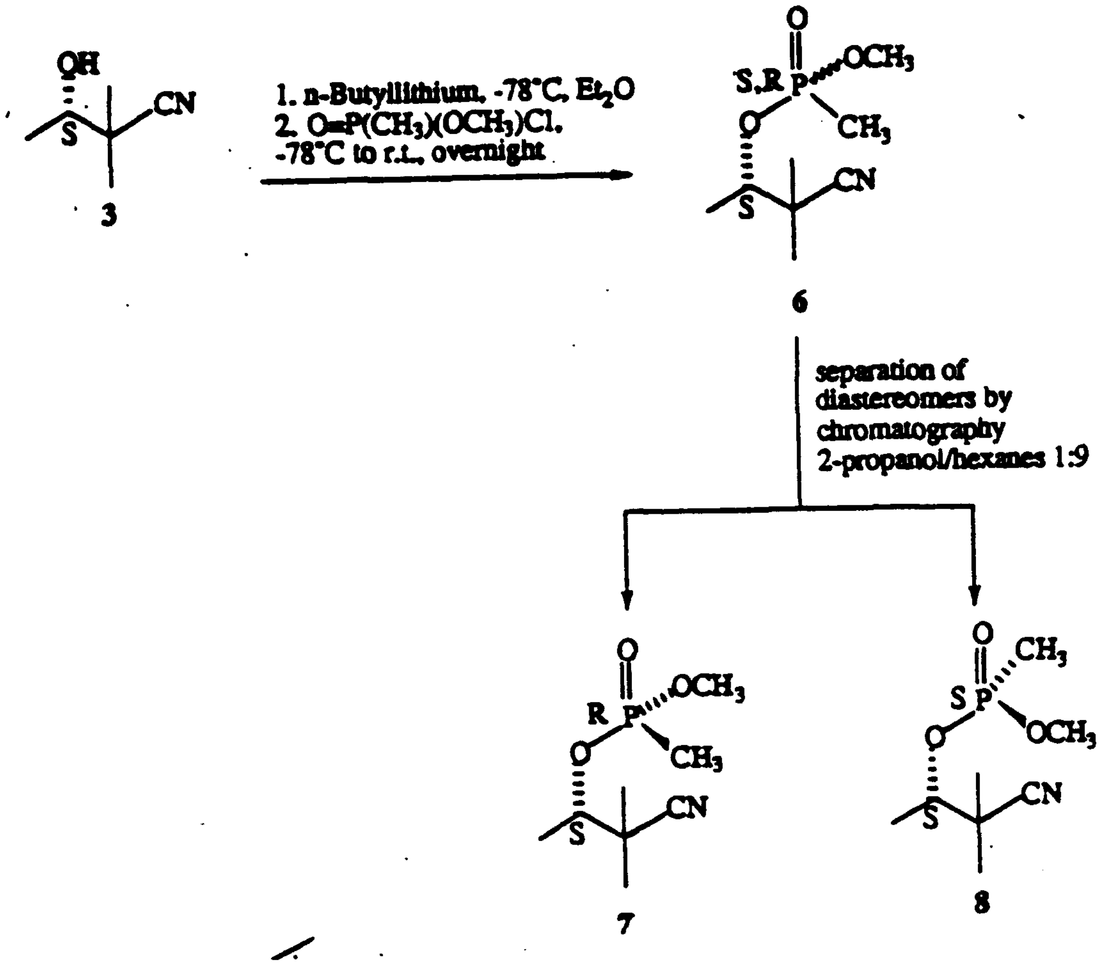
Scheme I: Yeast reduction of 2,2-dimethyl-3-oxo-butanenitrile (2).

The C_R-derivative is obtained by inversion of configuration of the S-cyanoalcohol: reaction of the (S)-cyanoalcohol with triflic anhydride at 0°C yields triflate 4 which upon stirring at room temperature for 12 h with potassium nitrite in DMF gives the (R)-2,2-dimethyl-3-hydroxybutanenitrile 5.

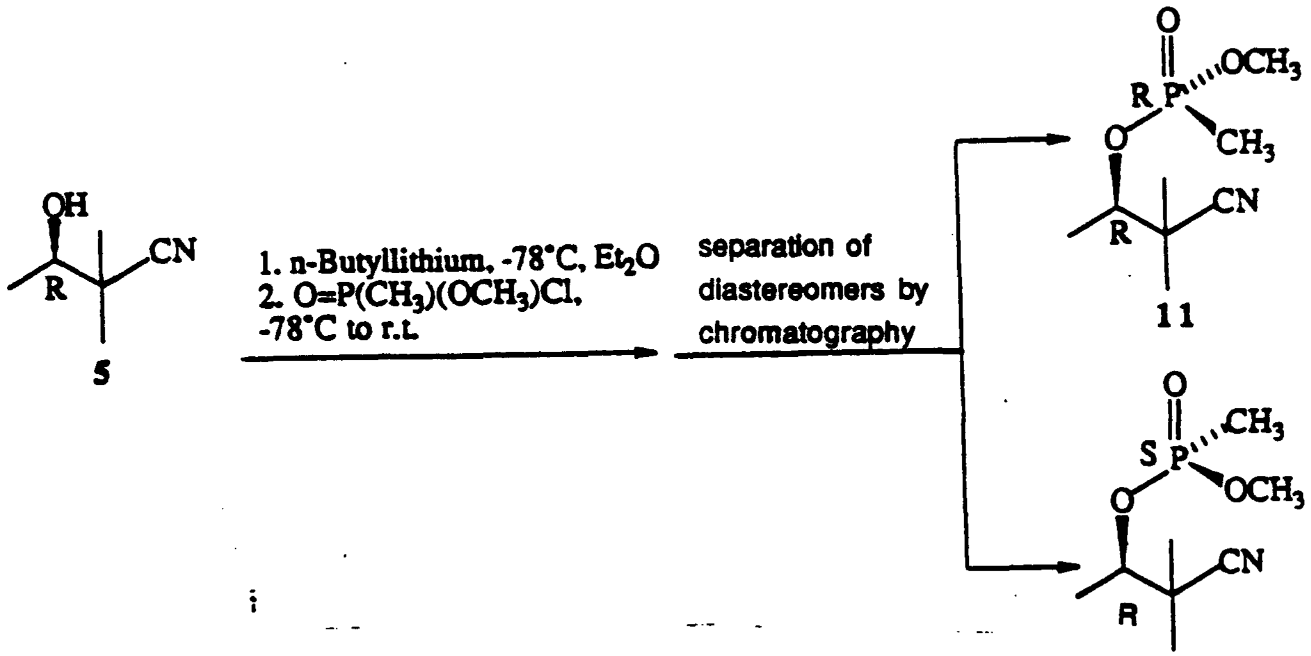
When the (S)-alcohol (3) is reacted with $CH_3P(O)(OCH_3)Cl$,[10] a mixture of diastereomeric C_SP_S, P_R cyanophosphonates **6** is obtained as an oil. The 400 MHz ^1H-NMR spectrum shows two doublets for $P\text{-}OCH_3$ and $P\text{-}CH_3$ respectively in a 1:1 ratio. Separation of the C_SP_R and C_SP_S diastereomers is then achieved by flash chromatography over silica gel with 2-propanol-hexanes as eluent. The ^1H NMR spectrum of one diastereomer isolated from column chromatography as an oil shows only one doublet for $P\text{-}OCH_3$ and one doublet for $P\text{-}CH_3$. Similar result was obtained when the (R)-alcohol (5) was treated with $CH_3P(O)(OCH_3)Cl$ (Scheme IV).



Scheme II: Synthesis of (R)-2,2-dimethyl-3-hyddroxybutanenitrile.

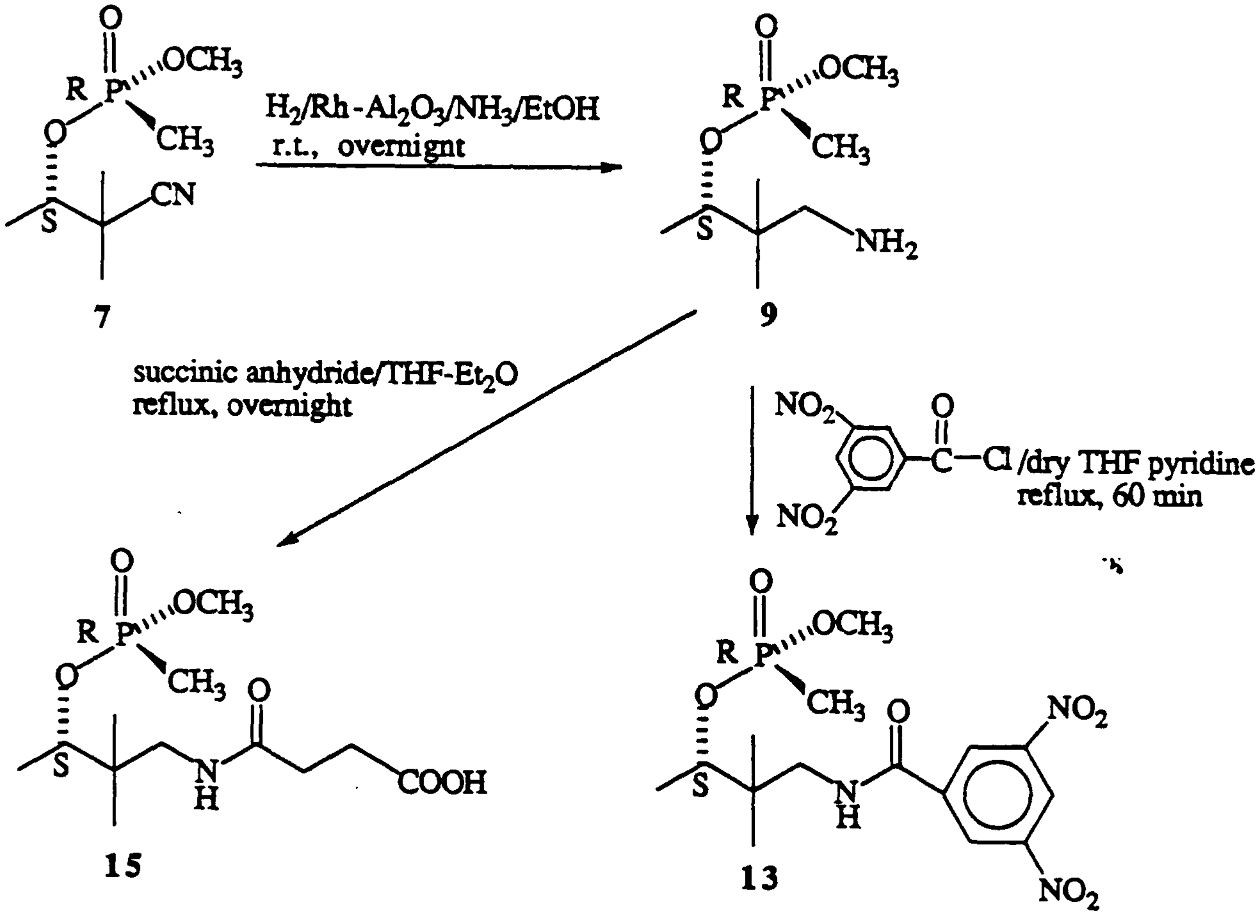


Scheme III: Reaction of (S)-alcohol with $CH_3P(O)(OCH_3)Cl$



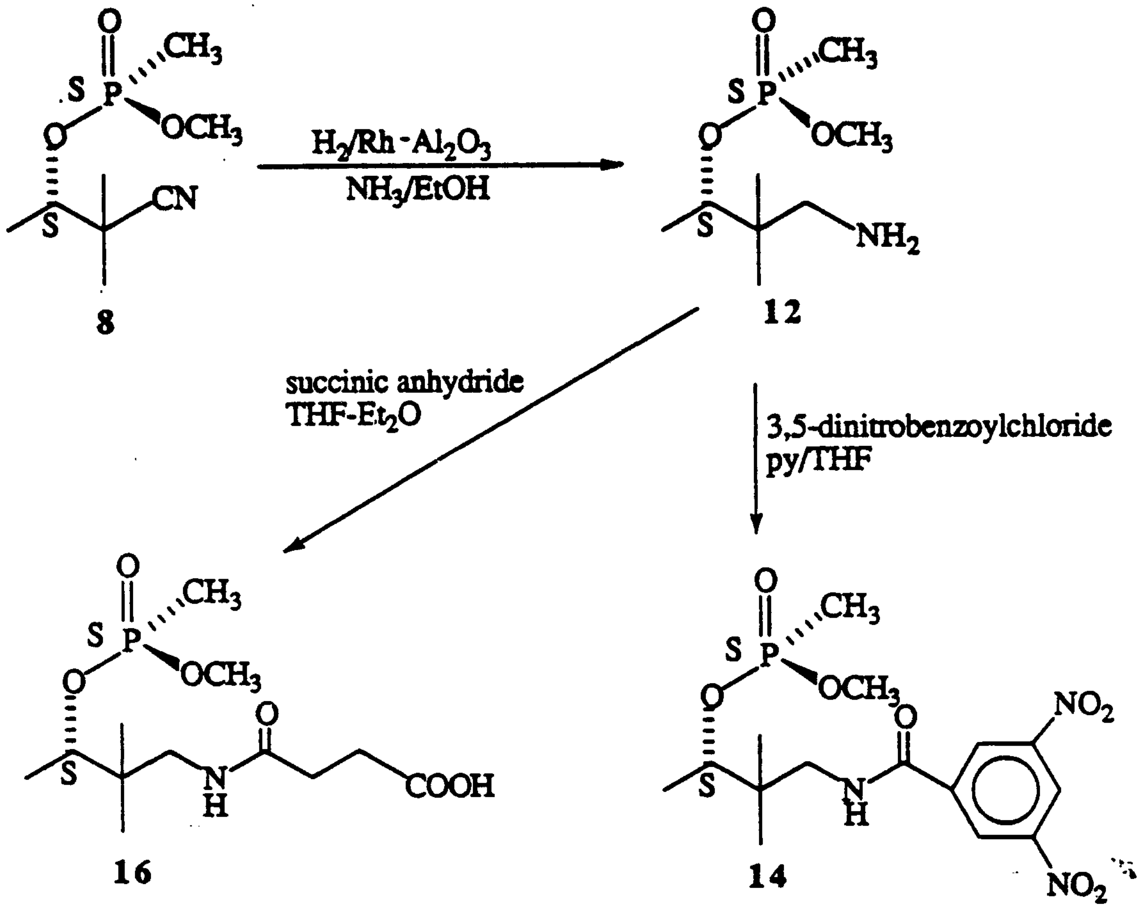
Scheme IV: Reaction of (R)-alcohol with $CH_3P(O)(OCH_3)Cl$.

In order to determine the configuration at phosphorus, the cyano group of the pure diastereomer 7 is hydrogenated[11] over rhodium on alumina to give aminophosphonate **1b**, which is benzoylated with 3,5-dinitrobenzoyl chloride to give the crystalline 3,5-dinitrobenzoyl derivative **13**.



Scheme V: Synthesis of phosponate with linkers from aminophosphonate 1b.

(Scheme VI).



The 3,5-dinitrobenzoyl derivative 13 is purified by chromatography (acetone/petroleum ether 1:3) and recrystallized from chloroform/ether. The X-ray structure shows the R-configuration at phosphorus (Figure I).

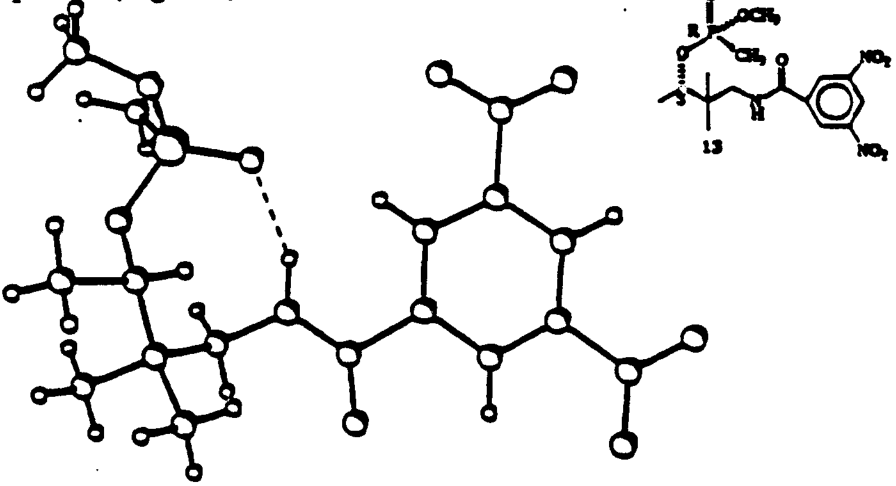


Figure I: X-ray structure of C_SP_R-3,5-dinitrobezamide derivative 13.

The other diastereomer C_SP_S cyanophosphonate **8** is obtained in the last fractions of the chromatography. This again is hydrogenated and benzoylated to give **14**. The X-ray structure of the crystals shows S-configuration at phosphorus (Figure II). The succinamido derivatives of the C_RP_S and C_RP_R derivatives were synthesized in a similar method.

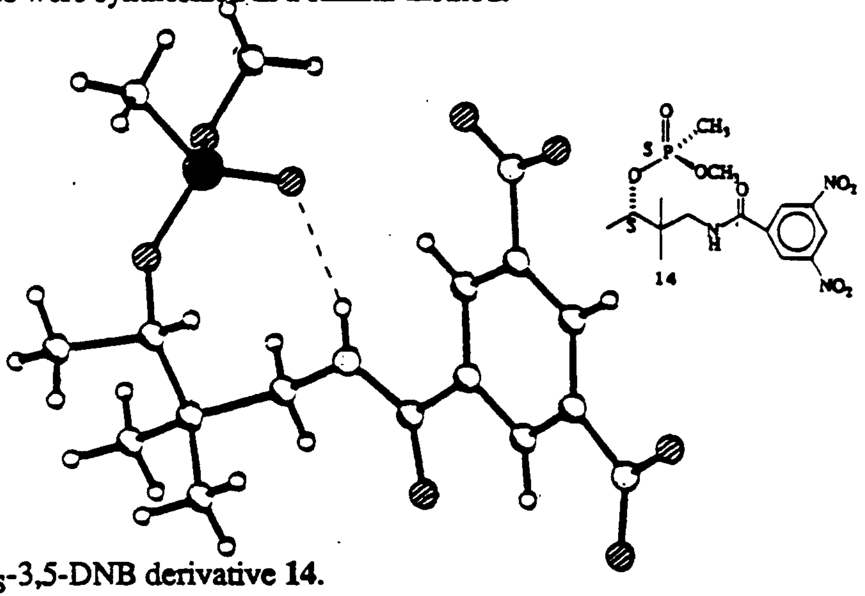
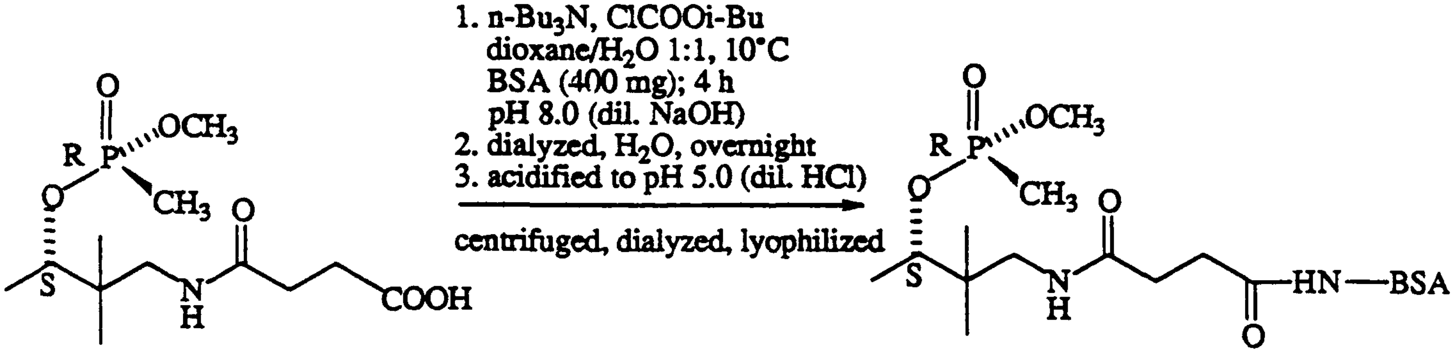


Figure II: X-ray structure of C_SP_S-3,5-DNB derivative **14**.

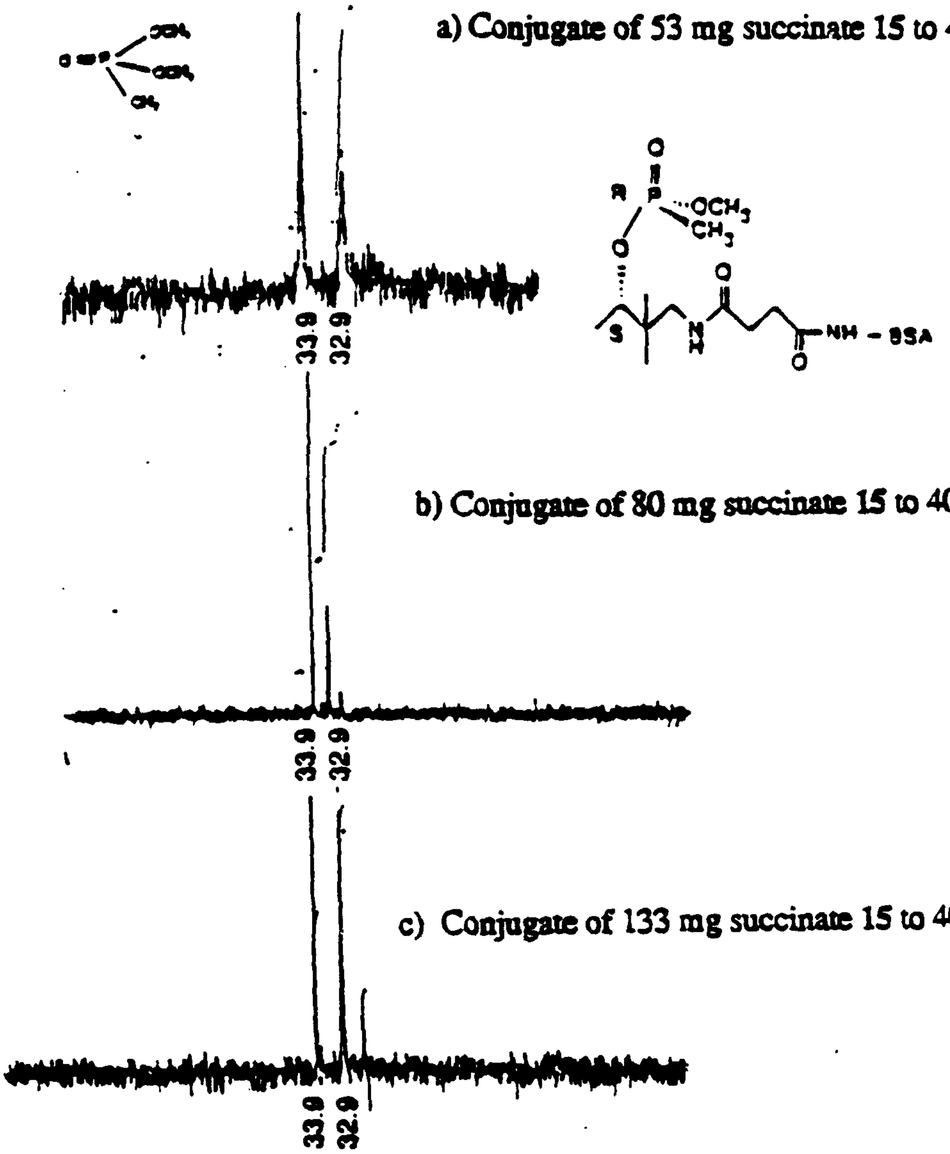
For the conjugation of the aminophosphonate to proteins we used the succinyl group as a spacer. When aminophosphonate **1b** is refluxed with succinic anhydride in THF/ether overnight, the succinate **15** is obtained as an oil in 76% yield. (Scheme V) This succinate can be conjugated to the carrier proteins HSA and BSA. The coupling reaction of the succinate takes place with the free amino group of the lysine residues in the proteins. We chose the mixed anhydride method[12] for the coupling reaction (Scheme VI).



15 **17**

Scheme 6: Conjugation of C_SP_R succinate **15** to BSA

After exhaustive dialyses and lyophilization of the protein conjugate the ^{31}P NMR technique is used to determine the amount of conjugated succinate with dimethoxy methyl phosphonate as a reference. Each diastereomer is conjugated to one particular protein at three different hapten/carrier ratios. Figure III shows the phosphorus NMR spectrum of the C_SP_R-BSA-conjugate in DMSO. As seen from the integration, an increasing amount of hapten relatively to the amount of protein used also increases the hapten/carrier ratio.



a) Conjugate of 53 mg succinate 15 to 400 mg BSA

b) Conjugate of 80 mg succinate 15 to 400 mg BSA

c) Conjugate of 133 mg succinate 15 to 400 mg BSA

Figure III: Phosphorus NMR spectra of C_SP_R-BSA-conjugates 17 (DMSO)-d_6) with dimethyl methylphosphonate as standard.

In conclusion, we have developed a convenient method for the synthesis of the four pure enantiomers of (4-amino-2,2-dimethyl-2-butyl)(methyl)methylphosphonates. Yeast reduction of the α-cyano ketone is used to introduce S-chirality at carbon, whereas the R-configuration at carbon is obtained by inversion of configuration. The diastereomeric cyano phosphonates are separated by chromatography. Conjugates of the four enantiomers to BSA and HSA have been prepared.

C. References

1. J. Jacobs, P. E. Schultz, R. Sugasaware, M. Powell, *J. Am. Chem. Soc.*, **109**, 2574 (1987).
2. S. J. Pollack, J. W. Jacobs, P.E. Schultz, *Science*, **234**, 1570 (1986).
3. A. D. Napper, S. J. Benkovic, R. A. Lerner, *Science*, **244**, 437 (1989).
4. K. D. Janda, s. J. Benkovic, R. A. Lerner, *Science*, **244**, 437 (1989).
5. H. P. Benschop, C. A. G. Konings, L. P. A. de Jong, *J. Am. Chem. Soc.*, **103**, 4260 (1981).
6. J. K. Rasmussen, A. Hassner, *Synthesis*, 682 (1973).
7. R. W. Hoffman, W. Ladner, K. Steinbach, W. Massa, R. Schmidt, E. Snatzhe, *Chem. Ber.*, 114, 2786 (1981).
8. B. Seuring, D. Seebach, *Helv. Chim. Acta, 60,* 1175 (1975).
9. M.F. Zuger, M.J. Brenne, *Helv. Chem. Acta, 67,* 1843 (1984).
10. T. M. Balthazor, R. A. Flores, *J. Org. Chem.*, **45**, 529 (1980).
11. M. Freifelder *J. Am. Chem. Soc.*, **82**, 2386 (1960).
12. B. F. Erlanger, F. Borek, S. M. Beiser, S. Liebermann *J. Biol. Chem.*, **228**, 713 (1958); B.F. Erlanger in "Methods in Enzymlolgy" Vol 70, *Academic Press,* New York, 1980, p. 85.

D.Experimental

General

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained with a Unican SP 1000 IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 NMR spectrometer (400 and 100 MHz, respectively, in ppm relative to tetramethylsilane, ^{31}P NMR spectra were recorded on a Bruker AM-200 NMR spectrometer (with 85% H_3PO_4 as external standard in ppm). Optical rotation was determined on a Perkin-Elmer 241 Polarimeter.

(S)-2,2-Dimethyl-3-hydroxybutanenitrile (3)

A 5 liter, two-necked, round-bottomed flask equipped with a mechanical stirrer was charged with 3.2 L of tap water, 600g sucrose and 600g Baker's yeast (Red Star Baker's Yeast; Universal Food Corporation). The mixture was stirred at room temperature for 1-2 h, during which time fermentation took place. Then 2,2-dimethyl-3-oxobutanenitrile 2 (33.4 g, 0.3 mole) was added and stirring was continued at room temperature for 7 days. Additional sucrose was added for the next 5 days, 100g each day. The reaction mixture was filtered over a thin layer of Celite. The aqueous filtrate was extracted with diethyl ether (5 x 1000 mL) and the combined ether extracts were dried ($MgSO_4$), and evaporated. The residue was distilled in vacuum to give 24.4 g (72%) of (S)-(-)-2,2-dimethyl-3-hydroxybutanenitrile (3) as a colorless liquid, bp 55°C/0.05mm; $[\alpha]^{22}{}_D$ - 4.02°(c 0.04, $CHCl_3$); IR(neat, cm^{-1}): 3500 (OH), 2240 (CN). ^{1}H NMR (400 MHz), ($CDCl_3$); δ 1.31 (s, 3H, C-CH_3), 1.32 (d, J =6.3 Hz, 3H, HC-C$\underline{H}_3$), 1.37 (s, 3H, C-CH_3), 3.65 (q, J =6.3 Hz, 1H, H_3C-C$\underline{H}$) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ 19.08, 23.20, 73.47, 123.88 ppm.

(R)-(+)-2,2-Dimethyl-3-hydroxybutanenitrile (5).

a) Synthesis of triflate $\underline{4}$:

A solution of S-cyanoalcohol 3 (1.13 g., 0.01 mole) and pyridine (0.985 g., 0.012 mole) in dry methylene chloride (20 mL) was added dropwise at 0°C to a solution of trifluoromethanesulfonic anhydride (3.38 g, 0.012 mole) in methylene chloride (50 mL). After stirring for 30 min. at 0°C, the isoluble salt was removed by filtration. The resulting solution was washed with water (3 x 50 mL) and a saturated solution of sodium bicarbonate (3 x 50mL), dried ($MgSO_4$), and concentrated in vacuum to yield 2.3 g (95%) triflate 4; $[\alpha]^{22}{}_D$ - 5.99° (c 0.085, $CHCl_3$); IR (neat, cm^{-1}) 2170 (CN); ^1H NMR ($CDCl_3$, 400 MHz)δ 1.45 (s, 3H, C-CH_3), 1.50 (s, 3H, C-CH_3), 1.70 (d, J =6.8Hz, 3H, HC-$C\underline{H}_3$), 4.82 (q, J=6.8 Hz, 1H, H_3C-$C\underline{H}$)ppm.

b) Synthesis of R-alcohol 5: A mixture of triflate 4 (2.45 g. 0.01 mole), potassium nitrite (8.5g, 0.1mole) and dimethyl formamide (50mL) was stirred at room temperature for 12 h, then diluted with water (400mL) and extracted with diethyl ether (4 x 100 mL). The combined ether extracts were washed with aqueous sodium bicarbonate solution, and dried ($MgSO_4$). The solvent was removed in vacuum and the crude product was purified by chromatography with hexanes/ethyl acetate 3:1 as an eluent. 0.96 g (85%) R-cyano alcohol 5 was obtained as a colorless liquid; $[\alpha]^{22}{}_D$ +3.91° (c=0.042, $CHCl_3$). For IR and ^1H NMR, see S-alcohol 3.

[(S)-(-)-(3-cyano-3-methyl)-2-butyl](methyl)methylphosphonate (6).

To a stirred solution of(S)-(-)-2,2-dimethyl-3-hydroxybutanenitrile $\underline{3}$ (10.0 g, 88.5 mmol) in anhydrous diethyl ether (125 mL) was added dropwise 2.5M $\underline{n}$-butyllithium in hexane (49 mL) at -78°C under nitrogen atmosphere. After stirring at -78°C for 30 min, methyl-(methoxy)phosphonylchloride (10.0g, 106 mmol) in anhydrous diethyl ether (50mL) was added dropwise with stirring at the same temperature. After complete addition, the reaction mixture was allowed to come to room temperature, and stirring was continued overnight at room temperature. The reaction mixture was quenched with water (75mL), the organic layer was separated and the water phase was extracted with diethyl ether (5 x 75 mL). The combined organic phases were dried ($MgSO_4$), and evaporated. Flash chromatography over silica gel with 2-propanolhexanes 1:4 as eluent afforded 11g (65%) of a slightly yellow oil. Analysis of its ^1H NMR spectrum (400 MHz) indicated a 1:1 mixture of the C_SP_R and C_SP_S cyanophosphonates 7 and 8. Alternatively, the product was distilled, bp 70°C/0.05 mm. IR (neat, cm^{-1}) 2260 (CN), 1270 (P=O). MS m/z 206 (M^++1). Separation of the diastereomers was achieved by flash chromatography over silica eluting with 2-propanol/hexanes 1:9. C_SP_R cyanophosphonate 7 was collected in the first fractions (analyzed by ^1H-NMR), the C_SP_R diastereomer 8 was collected in the last fractions.

C_SP_R - cyanophosphonate (7)

^1H NMR (100 MHz, $CDCl_3$) δ 1.34 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.47 (d, J=6.26 Hz, 3H, HC-C<u>H</u>$_3$), 1.53 (d, J=17.63 Hz, 3H, P-CH_3), 3.72 (d, J=11.2 Hz, 3H, P-OCH_3), 4.40 (m, 1H, H_3C-C<u>H</u>) ppm. ^{13}C NMR (400 MHz, $CDCl_3$) δ 10.87 (d, $J_{C,P}$=147.1, Hz, P-CH_3), 17.88, 22.88, 23.47, 38.27, 52.12 (d, $J_{C,P}$ =6.0Hz, P-O-C), 76.23 (d, $J_{C,P}$ =6.7Hz, P-O-C), 122.66 (CN) ppm. ^{31}P NMR (81 MHz, $CDCl_3$), δ +32.57 ppm.

C_SP_S - cyanophosphonate (8)

^1H NMR (400 MHz, $CDCl_3$) δ 1.34 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.45 (d, J=6.26 Hz, 3Hz, HC-C<u>H</u>$_3$), 1.52 (d, J=17.62 Hz, 3Hz, P-OCH_3), 3.76 (d, J=11.1 Hz, 3H, P-OCH_3), 4.39 (m, 1H, H_3C-C<u>H</u>). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 11.76 (d, $J_{C,P}$ = 145.9 Hz, P-CH_3), 17.58, 22.82, 23.25, 38.14, 52.35 (d, $J_{C,P}$ = 6.6Hz, P-OCH_3), 76.43 (d, $J_{C,P}$ = 6.6 Hz, P-O-C), 122.58 (CN) ppm. ^{31}P NMR (81 MHz, $CDCl_3$)δ +31.64 ppm.

R-alcoholmethoxymethylphosphonates

These were obtained according to the procedure described above by reaction of R-cyano alcohol **5** with methylmethoxyphosphonyl chloride and separation by flash chromatography. The NMR spectrra of C_RP_S cyano phosphonate **10** were identical with the spectra of C_SP_R derivative **7**, and the spectra of C_RP_R-cyanophosphonate **11** were identical with the spectra of C_SP_S derivative **8**.

[(S)-(4-amino-2,2-dimethyl)-2-butyl](methyl)-(R)-methylphosphonate (9); typical procedure

Cyanophosphonate 7 (492 mg, 2.4 moles) was dissolved in a saturated solution of ammonia in ethanol (70 mL) and 5% rhodium-on-alumina (0.2g) was added. The mixture was shaken in a Parr apparatus under hydrogen at 40 lb pressure overnight. The solution was filtered to remove the catalyst, and the filtrate was evaporated in vacuum. Aminophosphonate 9 was obtained in 98% (491 mg) as colorless oil.

IR (neat, cm^{-1}) 3480, 3380 (NH_2), 1250 (P=O). MS m/z 210 (M^+ +1). Anal. Calc. for $C_8H_{20}NO_3P$ (209.22): C, 45.92, H, 9.64, N, 6.70 P, 14.80. Found: C, 46.28, H, 9.31, N, 9.35, P, 15.01.

C_SP_R-aminophosphonate (9)

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.27 (d, J=6.83Hz, 3H, HC-$C\underline{H}_3$), 1.47 (d, J=18.69Hz, 3H, P-CH_3), 2.49 (d, J=14.0 Hz, 1H, N-CH), 2.73 (d, J=14.0 Hz, 1H, N-CH), 3.4 (broad, NH_2), 3.71 (d, J=11.88 Hz, 3H, P-OCH_3), 4.49-4.52 (m, 1H, H_3C-$C\underline{H}$)ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ=10.72 (d, $J_{C,P}$=152.93 Hz, P-CH_3), 16.67, 20.00. 21.34, 39.04 (d, $J_{C,P}$=6.40 Hz, C-O-P), 50.16, 52.05, (d, $J_{C,P}$=5.30 Hz, C-O-P). ^{31}P-NMR (8 1 MHz, $CDCl_3$) δ=32.26 ppm.

C_SP_S-amino phosphonate (12)

^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 1.29 (d, 3H, J=6.50 Hz, $HC\text{-}C\underline{H}_3$), 1.48 (d, J+17.64 Hz, 3H, $P\text{-}CH_3$), 1.5 (broad, NH), 2.45 (d, J=13.40 Hz, 1H, N-CH), 2.74 (d, J=13.40 Hz, 1H, N-CH), 3.75 (d, J=11.17 Hz, 3H, $P\text{-}OCH_3$), 4.44-4.50 (m, 1H, $H_3C\text{-}C\underline{H}$)ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.97 (d, $J_{C,P}$=151.03 Hz, $P\text{-}CH_3$), 16.56, 19.93, 21.39, 39.132 (d, $J_{C,P}$=5.33, C-O-P), 50.16, 52.15 (d, $J_{C,P}$=5.41 Hz, C-O-P)ppm. ^{31}P NMR (81 MHz, $CDCl_3$) δ =31.51 ppm.

3,5-Dinitrobenzamide derivatives (13) and (14); typical procedure

To a solution of amino phosphonate **11** (80 mg, 0.38 mmole) in dry pyridine (1 mL) and dry tetrahedrofuran (20 mL) was added 3,5-dinitrobenzoyl chloride (106 mg, 0.46 mmole). The mixture was heated under reflux for 60 min. Tetrahedrofuran was evaporated and methylen chloride (20 mL) was added. The mixture was poured into ice water (30 mL), the methylene chloride phase was separated and the aqueous phase was extracted with methylene chloride (10 mL). The combined organic phases were washed with dil. sodium carbonate solution (10 mL) and water (10 mL), dried ($MgSO_4$), and evaporated. Column chromatography of the crude product over silica gel with acetone/petroleum ether 1:3 as eluent gave slightly yellow crystals of 3,5-dinitrobenzamide derivative **13**. The product was recrystalized from chloroform/ether, yield 99mg (65%); mp 152-153°C. IR (KBr, cm^{-1}): 3360, 3140, (NH), 1690 (CO), 1560, 1350 (NO_2).

C_SP_R-dinitrobenzamide derivative (13)

^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.31 (d, J=6.41 Hz, $HC\text{-}C\underline{H}_3$), 1.59 (d, J=17.57 Hz, 3H, $P\text{-}CH_3$), 3.00 (dd, J=13.70 Hz, J=3.75 Hz, 1H, N-C-H), 3.88 (dd, J=13.49 Hz, J=9.06 Hz, 1H, N-C-H), 3.92 (d, J=11.00 Hz, 3H, $P\text{-}OCH_3$), 4.51-4.55 (m, 1H, $H_3C\text{-}C\underline{H}$), 9.14-9.15 and 9.31-9.32 (3H, Ar-H), 9.5 (broad, 1H, NH)ppm.

C_SP_S-dinitrobenzamide derivative (14)

µπ 176–177°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.30 (d, J=6.43 Hz, 3H, HC-C$\underline{H}_3$), 1.63 (d, J=17.25 H3, 3H, P-CH_3), 3.10 (dd, J=13.58 Hz, J=3.68 Hz, 1H, N-C-H), 3.81 (d, J=11.22 Hz, 3H, P-OCH_3), 3.91 (dd, J=13.56 Hz, J=9.28 Hz, 1H, N-C-H), 4.44-4.50 (m, 1H, H_3C-C$\underline{H}$), 9.14-9.15 and 9.24-9.26 (3H, Ar-H), 9.37 (broad, 1H, NH) ppm.

Succinamido derivatives (15) and (16); typical procedure

Amino phosphate **11** (450 mg, 2.1 mmol) and succinic anhydride (200 mg, 2.1 mmol) were refluxed overnight in diethyl ether/tetrahedrofuran 1:1 (60mL). The solution was cooled to room temperature and evaporated. To the residue was added water (10 mL) and saturated sodium bicarbonate solution (~30 mL) until basic. The aqueous phase was extracted with methylene chloride (40 mL) and acidified with dil. HO. This aqueous solution was saturated with sodium chloride and extracted with methylene chloride (5 x 75 mL). The combined organic extracts were dried ($MgSO_4$), and evaporated to give succinamido derivative **15** as a colorless oil in 76% yield (494 mg). IR (neat, cm^{-1}) 3350, 3100 (NH), 3000-2500 (OH), 1750 (CO), 1700, 1580 (CO), 1250 (P=O).

C_SP_R succinamido derivative (15)

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.27 (d, J=6.42 HZ, 3H, H_3C-C$\underline{H}$), 1.53 (d, J=17.62 Hz, 3H, P-CH_3), 2.57-2.61 and 2.65-2.69 (m, 4H, C_2H_4), 2.82 (dd, J=13.75 Hz, J=4.12 Hz, 1H, N-C-H), 3.55 (dd, J=13.77 Hz, J=9.00 Hz, 1H, N-C-H), 3.76 (d, J=11.12 Hz, 3H, P-OCH_3), 4.45-4.49 (m, 1H, H_3C-C$\underline{H}$), 7.43 (broad, 1H, NH)ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.36 (d, $J_{C,P}$ =146.77 Hz, P-CH_3), 16.28, 19.23, 21.82, 30.47, 31.06, 39.24, 46.60, 53.00 (d, $J_{C,P}$ = 5.49 Hz, C-O-P), 76.15 (d, J =7.64 Hz, C-O-P), 173.15 (CO), 174.95 (CO)ppm. ^{31}P NMR (81

MHz, $CDCl_3$)δ +34.98ppm.

C_SP_S succinamido derivative (16)

^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.26 (d, J= 6.39 Hz, 3H, H_3C-C$\underline{H}$), 1.55 (d, J= 17.35 Hz, 3Hz, P-CH_3), 2.57-2.63 and 2.66-2.71 (m, 4H, C_2H_4), 2.89 (dd, J= 14.02 Hz, J= 4.1 Hz, 1H, N-C-H), 3.56 (dd, J= 13.74 Hz, J= 9.00 Hz, N-C-H), 3.75 (d, J= 11.25 Hz, 3H, P-OCH_3), 4.40-4.44 (m, 1H, H_3C-C$\underline{H}$), 7.33 (broad, 1H, NH)ppm. ^{13}C-NMR ($CDCl_3$, 100 MHz)δ=11.44 (d, $J_{C,P}$= 143.17 Hz, P-CH_3), 16.28, 19.37, 21.86, 30.28, 31.10, 39.40 (d, $J_{C,P}$=4.02 Hz, C-O-P), 465.36, 52.25 (d, $J_{C,P}$= 6.52 Hz, P-O-C), 77.18 (d, $J_{C,P}$= 6.33 Hz, C-O-P), 172.88 (CO), 175.14 (CO)ppm. ^{31}P NMR (81 MHz, $CDCL_3$)δ=33.83ppm.

Conjugation of succinamido derivatives and to the proteins BSA, HSA and KLH at three different hapten-to carrier ratios. Typical procedure; C_SP_R-BSA-conjugate (17)

Succinamido phosphonate **15** (53 mg, 0.17 mmol) and tri-$\underline{n}$-butylamine (0.027 g, 0.035 mL, 0.17 mmol) were dissolved in dioxane (3 mL). The solution was cooled to 10-15°C, and isobutyl chlorocarbonate (0.023 mL, 0.17 mmol) was added. The reaction was allowed to proceed for 30 min at 10-15°C. Then the mixture was added in one portion to a stirred, cooled solution of BSA (400 mg, 0.0057 mmol) in water/dioxane 1:1 (10 mL) which had been adjuncted to pH=8.5-9.0 by dil. NaOH solution. The pH of the mixture dropped to ~ 7.0 and gas evolution was visible. A few more drops of dil. NaOH solution was added, raising the pH to 8.5. After 1 hour, the pH of the mixture was checked again. If necessary, more NaOH was added so that the pH remained at 8.0-8.5. Stirring and cooling were continued for 4 h The solution was dialyzed against distilled water for 2 days and then brought to pH=5.0 with dil. HCl. The resulting precipitate was kept at 4°C overnight and then collected by centrifugation. It was redissolved by bringing the pH to 6.0 with $NaHCO_3$ solution and dialyzed overnight against dist. water. Zyophilization gave 170 mg of the conjugate.

CHAPTER II: A NEW SYNTHETIC ROUTE TO UNSYMMETRICALLY SUBSTITUTED PENTACOORDINATED PHOSPHORUS. HYDROLYTICALLY STABLE CHIRAL MONOCYCLIC OXYPHOSPHORANES

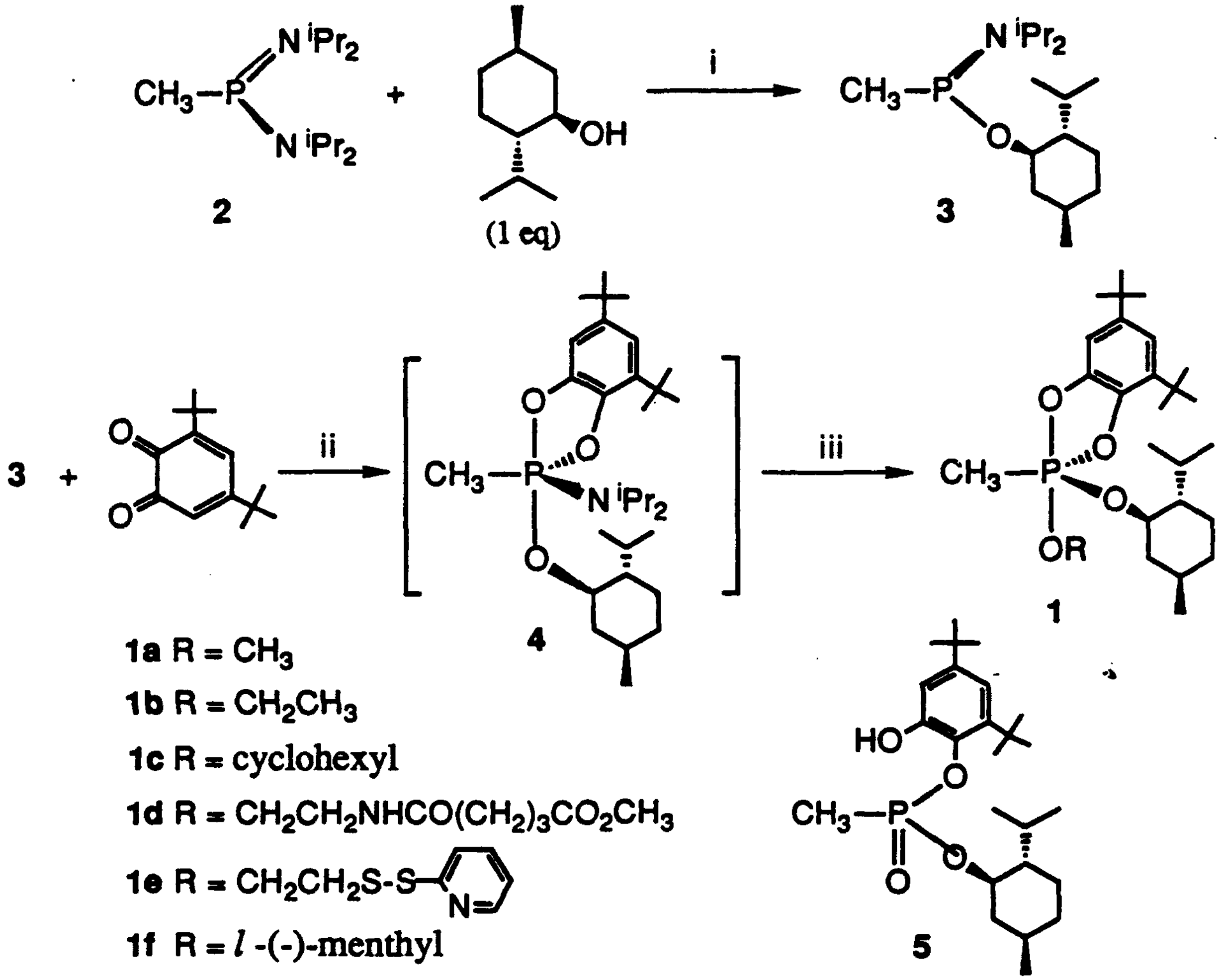
A. Introduction

Pentacoordinated phosphorus (phosphoranes) are of interest as models for the intermediate or transition state for phosphoryl transfer occurring in the hydrolysis of phosphates and phosphonates.[1a-d] Such intermediates have been proposed for an increasing number of reactions catalyzed by phosphoryl- and nucleotidyl-transfer enzymes.[1c, 2] Monocyclic oxyphosphoranes represent an important stable model system for such intermediates. The stereochemical course of phosphoryl-transfer reactions has been discussed in terms of structure, stereochemistry and pseudorotational processes observed for model phosphoranes.[1b] However, the monocyclic phosphoranes synthesized to date are invariably "symmetric" phosphoranes in the sense that at least two or three identical alkoxy substituents are bound to phosphorus. The proposed intermediates or transition states in phosphoryl-transfer reactions frequently involve "unsymmetric" oxyphosphoranes possessing five different substituents bound to phosphorus. No general synthetic method for such unsymmetric phosphoranes has been reported.

B. Synthesis and Discussion

We now describe a novel synthetic route to the unsymmetric methylphosphoranes **1a-e** having one *l*-menthoxy group and various other alkoxy groups bound to phosphorus. The key synthetic step in this process is the direct displacement of the *N, N*-diisopropylamino group in the intermediate **4** by alcohols (**Scheme I**). Methylphosphoranes **1a-e** were obtained in high yield after purification *via* column chromatography under basic conditions (**Table I**).

Scheme I [a]



1a R = CH_3
1b R = CH_2CH_3
1c R = cyclohexyl
1d R = $CH_2CH_2NHCO(CH_2)_3CO_2CH_3$
1e R = CH_2CH_2S-S-
1f R = *l*-(-)-menthyl

[a] Reagents and conditions: (i) *1H*-tetrazole (5 mol%), dry CH_2Cl_2, 25°C, 40 h; (ii) dry CH_2Cl_2, 0°C, 2 h; (iii) ROH (1 eq), dry CH_2Cl_2, 25°C, overnight.

The diastereomeric mono-substituted aminophosphine 3[3,4] was allowed to react with 3,5-di-*tert*-butyl-1,2-benzoquinone[6] to afford the key intermediate aminophosphorane 4. The formation of diastereomeric 4 was monitored by ^1H and ^{31}P NMR;[7] without isolation, 4 was allowed to react directly with the appropriate alcohol at room temperature to yield 1a-f.[8] Importantly, no substitution of the *l*-menthoxy group in 4 was observed under these conditions.[9]

Unsymmetric phosphoranes **1a-e** displayed two signals of approximately equal intensity in ^{31}P NMR; two doublets were also observed in both ^{1}H and ^{13}C NMR for the methyl bound to phosphorus. On the other hand, the symmetric phosphorane **1f** prepared by the same method gave only one signal in ^{31}P NMR and one doublet for the corresponding methyl in ^{1}H and ^{13}C NMR (**Table I**).

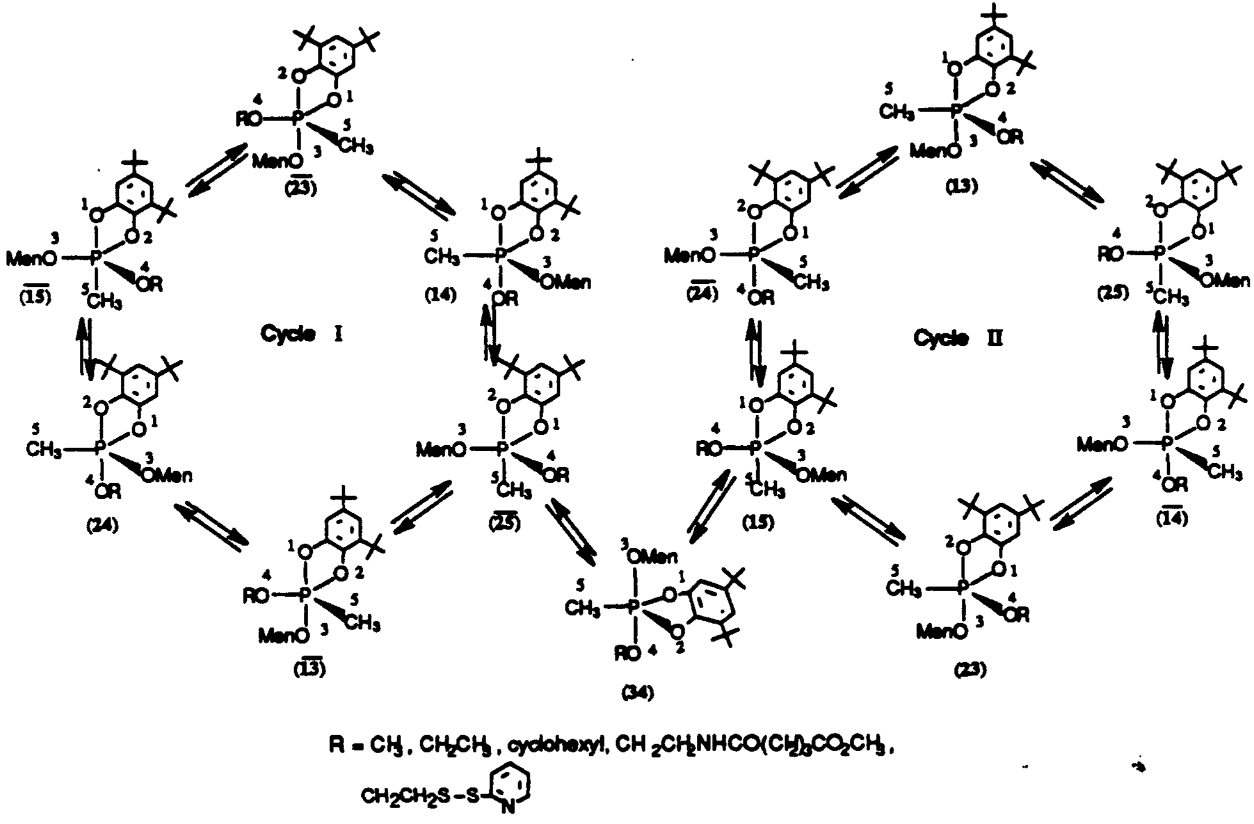
Table I. Isolated Yield and ^{31}P, ^{1}H and ^{13}C NMR of Phosphoranes 1

Phosphoranes	Yield[a] [%]	$\delta^{31}P$[b] [ppm]	$\delta^{1}H$ [ppm] for CH_3-P[c] (coupling const., J_{HCP})	$\delta^{13}C$ [ppm] for CH_3-P[c] (coupling const., J_{CP})
1a	88	-20.24	1.81 (d, 17.7 Hz)	20.8 (d, 193 Hz)
		-21.56	1.87 (d, 17.6 Hz)	21.8 (d, 192 Hz)
1b	94	-20.21	1.82 (d, 17.7 Hz)	21.1 (d, 193 Hz)
		-21.43	1.87 (d, 17.6 Hz)	22.1 (d, 192 Hz)
1c	86	-19.66	1.81 (d, 17.8 Hz)	21.7 (d, 194 Hz)
		-20.78	1.87 (d, 17.7 Hz)	22.5 (d, 193 Hz)
1d	90[d]	-20.53	1.84 (d, 17.8 Hz)	21.0 (d, 193 Hz)
		-21.95	1.89 (d, 17.7 Hz)	22.0 (d, 192 Hz)
1e	100	-20.48	1.82 (d, 17.8 Hz)	20.9 (d, 193 Hz)
		-21.91	1.88 (d, 17.7 Hz)	21.9 (d, 192 Hz)
1f	95	-20.21	1.84 (d, 17.8 Hz)	22.8 (d, 199 Hz)

[a] Phosphoranes **1** were purified by flash column chromatography on silica gel (hexane - NEt_3, 9 : 1) unless otherwise specified. [b]Solvent is $CDCl_3$. Chemical shifts downfield of the reference (85% H_3PO_4 as an external standard) are indicated as positive. [c]Methyl group bound to phosphorus atom. Solvent is $CDCl_3$. [d]Hexane - AcOEt - NEt_3 (5 : 1 : 0.6) was used as solvent for chromatography.

Variable temperature ^{31}P NMR showed that the two signals persisted for **1a** at 119 °C ($CDCl_2CDCl_2$) and the signal of **1f** remained a single peak even at -93 °C (acetone-d_6). These spectral data may be understood in terms of **Scheme II** which depicts two sets of trigonal bipyramidal pseudorotatomers.

Scheme II



Structures within Cycle I are mutually diastereomeric and rapidly interconverting. They are insulated from the diastereomerically related set in Cycle II because the linkage between the two is the strained diequatorial (34) stereoisomer. Furthermore, **1f** is a structurally symmetrical (but not a stereochemically symmetrical) analog. Accordingly, each pseudorotatomer of one cycle for **1f** becomes identical to the corresponding rotamer of the second cycle, with the result that only one pseudorotatomer cycle exists.

Surprisingly, **1a-f** are hydrolytically stable in the absence of acid; phosphorane **1b**, for example, remained unchanged in $CDCl_3$ for at least 2 weeks even in the presence of water or 0.1 *N* NaOH at room temperature. However, **1a-f** are extremely labile to aqueous acids;

compound **1a** or **1b** reacted with 0.1 *N* HCl immediately to give a 1:1 mixture of diastereomeric phosphonate **5** quantitatively (**Scheme I**).

Finally, this work represents a first step toward the development of stable transition state analogs for phosphoryl-transfer reactions. Analogs **1d** and **1e** possess the requisite stability and necessary linker group required of a hapten for antibody production and this aspect of the work will be reported separately.

C. References and footnotes

1. (a) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70-78. (b) Buchwald, S. L.; Pliura, D. H.; Knowles, J. R. *J. Am. Chem. Soc.* **1984**, *106*, 4916-4922. (c) Holmes, R. R.; "Pentacoordinated Phosphorus"; American Chemical Society : Washington, D C, 1980, vol. I and II. (d) Taira, K.; Lai, K.; Gorenstein, D. G. *Tetrahedron* **1986**, *42*, 229-238.
2. (a) Knowles, J. R. *Ann. Rev. Biochem.* **1980**, *49*, 877-919. (b) Wlodawer, A.; Miller, M.; Sjölin, L. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 3628-3631.
3. Compound **3** was prepared from diaminophosphine **2**[4] using 5 mol % of *1H*-tetrazole as a catalyst: bp 96-100°C at 0.08 mmHg, 88 %. ^{31}P NMR of **3** gave two signals (δ +112.4 and +121.6 ppm relative to 85% H_3PO_4 as an external standard), indicating that **3** exists as a mixture of two diastereomers.
4. Diaminophosphine **2** was synthesized by methylation of bis(*N, N*-diisopropylamino)chlorophosphine[5] with CH_3Li (Et_2O, -78°C to 25°C, 16 h): bp 70-74°C at 0.07 mmHg, 87 %; ^{31}P NMR δ +40.9 ppm.
5. King, R. B.; Sundaram, P. M. *J. Org. Chem.* **1984**, *49*, 1784-1789.
6. (a) Ramirez, F.; Desai, N. B. *J. Am. Chem. Soc.* **1963**, *85*, 3252-3258. (b) Abdou, W. M.; Mahran, M. R. H.; Hafez, T. S.; Sidky, M. M. *Phosphorus and Sulfur*, **1986**, *27*, 345-353.
7. Two signals were observed in ^{31}P NMR (δ -15.4 and -15.7 ppm) and two doublets were observed for CH_3-P in ^1H NMR (δ 1.74 and 1.84 ppm, J_{HP} = 16.2 and 16.4 Hz, respectively), indicating that **4** was composed of two diastereomers.
8. The aminophosphorane **4** reacted rapidly with water to give *N, N*-diisopropylamine and the diastereomeric phosphonate **5** quantitatively; ^{31}P NMR +32.2 (48 %) and +32.9 (52 %) ppm.
9. In our alternative approach to **1** from **3** *via* unsymmetrical alkoxy(*l*-menthoxy)(methyl)phosphines, substitution of both the *l*-menthoxy group and the *N, N*-

diisopropylamino group of 3 by an alcohol (*i.e.* cyclohexanol) was observed to give a mixture (1 : 2 : 1) of the three possible dialkoxy(methyl)phosphines.

D. Experimental

General

^1H and ^{13}C NMR spectra were measured on a Bruker AM 400 (400 MHz for ^1H) spectrometer. ^{31}P NMR spectra were recorded on a Bruker WP200SY (81.0 MHz for ^{31}P) spectrometer with 85% H_3PO_4 as an external standard. Chemical shifts downfield of the reference are indicated as positive. Deuteriochloroform dried over molecular sieves 4A was used as solvent for all NMR experiments with residual chloroform as an internal standard for ^1H and ^{13}C NMR (δ_H 7.27 and δ_C 77.01 ppm). IR spectra were measured on an IBM IR/32 spectrometer. Mass spectra were obtained by chemical ionization (CI) technique with a Finnigan MAT 90 mass spectrometer. A Perkin-Elmer 241 polarimeter was used for measuring optical rotations. Dichloromethane was distilled over P_2O_5 and stored over molecular sieves 4A. Diethyl ether, tetrahydrofuran (THF) were distilled from sodium metal immediately before use. Hexane and pentane were distilled over CaH_2 and stored over molecular sieves 4A. The products were isolated by short-path still under an argon atmosphere or by flash column chromatography on silica gel (Silica Gel 40 μm Flash Chromatography Packing, J. T. Baker Inc.).

Preparation of Bis(*N*,*N*-diisopropylamino)(methyl)phosphine (2)

A 1.4 *M* solution of CH_3Li in dry Et_2O (100 mL, 0.14 mole) was added dropwise to a cold suspension of bis(*N*, *N*-diisopropylamino)chlorophosphine[5] (35.5 g, 0.13 mole) in dry Et_2O (600 mL) at -78°C. After the addition was completed, the mixture was stirred at room temperature for 16 h. The reaction mixture was filtered and the colorless precipitate (LiCl) was washed with dry Et_2O. The combined filtrate and washings were concentrated; the residual pale yellow liquid was distilled to afford 2 as a colorless liquid: 28.4 g (87 %), bp 70-74°C at 0.07

mmHg; ^{1}H NMR δ 1.09 and 1.18 (2 x d, J = 6.65 and 6.72 Hz, 24H, 4 x iPr), 1.21 (d, 3H, J_{HP} = 10.2 Hz, CH_3P), 3.40 (m, 4H, 4 x N$\underline{CH}(CH_3)_2$); ^{31}P NMR δ +40.8; MS *m/z* (relative intensity) 246 ([M]$^+$, 100 %), 231 (20) and 146 (87).

Preparation of *N,N*-diisopropylamino(*l*-menthoxy)phosphine (3)

To a solution of 2 (4.64 g, 18.8 mmol) in dry CH_2Cl_2 (70 mL) were added *l*-(-)-menthol (3.00 g, 19.2 mmol) and *N, N*-diisopropylamine salt of *1H*-tetrazole (161 mg, 0.94 mmol) successively at 0°C. The mixture was stirred at room temperature for 40 h. The reaction mixture was evaporated to give a colorless oil with a small amount of solid (tetrazole salt). Dry pentane (30 mL) was added to the residue and filtered. The filtrate was evaporated and the residual oil was distilled to afford 3 as a colorless liquid: 4.99 g (88 %), bp 96-100°C at 0.08 mmHg; ^{1}H NMR δ 0.74 and 0.81 (2 x d, J = 6.92 and 6.93 Hz, 3H, $\underline{CH_3}CH$), 0.89 (m, 6H, $CH(\underline{CH_3})_2$), 0.8-1.0 (m, 4H), 1.10 (d, J = 6.73 Hz, 6H, $NCH(\underline{CH_3})_2$), 1.18 and 1.20 (2 x d, J = 6.64 and 6.38 Hz, 6H, $NCH(\underline{CH_3})_2$), 1.21 and 1.22 (2 x d, J_{HP} = 6.95 and 6.51 Hz, 3H, CH_3P), 1.4 (m 1H), 1.6 (m, 2H), 2.0-2.3 (m, 2H), 3.39 and 3.51 (2 x m, 1H, OCH), 3.53 (m, 2H, $N[\underline{CH}(CH_3)_2]_2$); ^{31}P NMR δ +112.5 (58 %) and +121.7 (42 %), diastereomeric mixture.

Preparation of 2-(methoxyglutaryl)aminoethanol

N, N-Dicyclohexylcarbodiimide (1.6 g, 7.5 mmol) and 1-hydroxybenzotriazole hydrate (1.9 g, 13.7 mmol) were added to a cold solution of glutaric acid monomethyl ester (1.0 g, 6.8 mmol) in dry THF (40 mL) with ice-cooling. The mixture was stirred at 0°C for 10 min, then at room temperature for 1 h. To this solution was added a solution of 2-aminoethanol (0.46 g, 7.5 mmol) in dry THF (10 mL) at room temperature. The mixture was stirred at room temperature overnight. The reaction mixture was filtered and the filtrate was evaporated to give colorless oil. Ethyl acetate (25 mL) was added to the residue and the solution was kept at -20°C overnight to remove the residual *N, N*-dicyclohexylurea. The mixture was filtered and the filtrate was extracted with water (3 x 20 mL). The combined aqueous extracts were saturated with NaCl and extracted with CH_2Cl_2 (7 x 20 mL). The combined extracts were dried over Na_2SO_4 and evaporated to afford 2-(methoxyglutaryl)aminoethanol as a colorless oil: 490 mg (38 %); ^{1}H NMR 1.98 (m, 2H, CH_2), 2.28, (t, J = 7.2 Hz, 2H, $C\underline{H}_2CONH$), 2.40 (t, J = 7.1 Hz, 2H, $C\underline{H}_2CO_2CH_3$), 3.0 (br s, 1H, OH), 3.42 (q, J = 5.5 Hz, 2H, $C\underline{H}_2NH$), 3.68 (s, 3H, OCH_3), 3.72 (t, J = 5.0 Hz, 2H, $C\underline{H}_2OH$), 6.15 (br s, 1H, CONH).

Preparation of 2-(2-pyridyldithio)ethanol

A solution of freshly distilled 2-mercaptoethanol (121 mg, 1.55 mmol) in 99.5% ethanol (2 mL) was added dropwise to a solution of 2, 2'-dithiodipyridine (682 mg, 3.09 mmol) in ethanol (8 mL) at room temperature. The mixture was stirred at room temperature overnight under an argon atmosphere. The reaction mixture was evaporated to give a yellow oil. Purification by flash column chromatography on silica gel (hexane - AcOEt, 1 : 1) gave 2-(2-pyridyldithio)ethanol as a colorless oil: 240 mg (83 %); ^{1}H NMR δ 2.97 (t, J = 5.1 Hz, 2H, CH_2S), 3.81 (t, J = 5.1 Hz, 2H, $C\underline{H}_2OH$), 5.7 (br s, 1H, OH), 7.16 (m, 1H, 5'-H), 7.41 (d, J = 8.0 Hz, 1H, 3'-H), 7.60 (td, J = 7.9 and 1.8 Hz, 1H, 4'-H), 8.53 (d, J = 4.3 Hz, 6'-H).

Preparation of 2-*l*-menthoxy-2-methoxy-2-methyl-1,3-dioxa(4,6-di-*tert*-butyl)-2-benzophosphole (1a); typical procedure

A solution of 3 (821 mg, 2.72 mmol) in dry CH_2Cl_2 (30 mL) was added dropwise to a solution of 3,5-di-*tert*-butyl-1,2-benzoquinone (630 mg, 2.86 mmol) in dry CH_2Cl_2 (20 mL) at 0°C. The mixture was stirred at room temperature for 2 h. The dark red color of the benzoquinone disappeared and the mixture became pale green in color after 2 h. The formation of the aminophosphorane 4 was confirmed by ^{1}H and ^{31}P NMR: ^{1}H NMR δ 0.74-0.95 (m, 9H, CH_3 and CH(C$\underline{H}_3$)$_2$), 1.0-1.4 (m, 5H), 1.15 and 1.17 (2 x d, *J* = 6.89 and 7.74 Hz, 6H, $N(CH_3)_2$), 1.22 and 1.23 (2 x d, *J* = 6.75 and 6.48 Hz, 6H, $N(CH_3)_2$), 1.29, 1.30, 1.38 and 1.39 (4 x s, 18H, *t*Bu), 1.6-1.7 (m, 3H), 1.74 and 1.84 (2 x d, J_{HP} = 16.21 and 16.37 Hz, 3H, CH_3P), 2.15-2.70 (m, 2H), 3.81 (m, 2H, N[C$\underline{H}$(CH_3)$_2$]$_2$), 3.87 and 4.04 (2 x m, 1H, OCH), 6.68-6.75 (m, 2H, aromatic protons); ^{31}P NMR δ -15.38 (38 %) and -15.70 (62 %), diastereomeric mixture. The aminophosphorane 4 was unstable and allowed to react with alcohols without isolation. Methanol (87.3 mg, 2.72 mmol) was added directly to the solution of 4 at 0°C. The mixture was stirred at room temperature overnight. The reaction mixture was evaporated and the residual oil was purified by flash column chromatography on silica gel (hexane - NEt_3, 9 : 1) to afford the phosphorane 1a as a colorless sticky oil: 1.09 g (88.1 %); optical rotations (*c* 1.05, CH_2Cl_2), $[\alpha]^{25}_{D}$ -47.6°, $[\alpha]^{25}_{578}$ -49.6°, $[\alpha]^{25}_{546}$ -54.6°, $[\alpha]^{25}_{436}$ -93.2°, $[\alpha]^{25}_{365}$ -148.1°; ^{1}H NMR δ 0.12 and 0.80 (2 x d, *J* = 6.90 and 7.08 Hz, 3H, CH_3), 0.68, 0.92, 0.949 and 0.954 (4 x d, *J* = 6.51, 6.97, 7.02 and 6.51 Hz, 6H, (C$\underline{H}_3$)$_2$CH), 0.75-1.0 (m, 2H), 1.1-2.3 (m, 7H), 1.28 and 1.40, 1.30 and 1.40 (4 x s, 18H, 2 x *t*Bu), 1.81 and 1.87 (2 x d, J_{HP}= 17.68 and 17.64 Hz, 3H, CH_3P), 3.55 and 3.57 (2 x d, J_{HP} = 11.58 and 11.43 Hz, 3H, CH_3O-P), 4.06 and 4.31 (2 x m, 1H, OCH), 6.76-6.81 (m, 2H, aromatic protons); ^{31}P NMR δ -20.24 (47 %) and -21.56 (53 %), diastereomeric mixture; ^{13}C NMR δ 14.9 and 16.1 (C_1), 20.8 and 21.8 (2 x d, $^1J_{CP}$ = 193 and 192 Hz, PCH_3), 20.9 and 21.2 (C_2), 21.9 and 22.2 (C_3), 22.6 and 23.2 (C_4), 25.3 and 26.1 (C_5), 29.7 and 29.8 (C_6), 31.5 (C_7), 31.7 (C_8), 34.1 (C_{11}), 34.2 (C_{10}), 34.7 (C_9), 43.0 and 43.1 (C_{12}), 48.1 and 48.4 (2 x d, $^3J_{CP}$ = 6.6 and 9.0 Hz, C_{13}), 51.8 and 52.1 (2 x d, $^2J_{CP}$ = 10.7 and 10.5 Hz, CH_3O-P), 77.7 and 77.8 (2 x d, $^2J_{CP}$ = 11.4 and 11.2 Hz, C_{14}), 104.9 and 105.0 (2 x d, $^3J_{CP}$ = 10.8 and 10.5 Hz, C_{15}), 113.81 and 113.84 (C_{16}), 132.07 and 132.08 (2 x d, $^3J_{CP}$ = 6.2 and 9.0 Hz, C_{17}), 139.8 (C_{18}), 143.1 and 144.4 (C_{19}), 145.1 and 145.3 (C_{20}); MS *m/z* (relative intensity) 453 ($[M+1]^+$, 100 %), 437 (3), 421 (17), 314 (8), 297 (64), 283 (17); IR (film) 2959, 2870, 1593, 1487, 1454, 1428, 1362, 1302, 1239, 1075, 1034, 1001, 907, 868, 853, 723 and 693 cm^{-1}. **All of the phosphoranes 1a-f were unstable on silica gel, but stabilized by adding NEt$_3$ (10 % v/v) to the solvent.**

2-Ethoxy-2-*l*-menthoxy-2-methyl-1,3-dioxa(4,6-di-*tert*-butyl)-2-benzophosphole (1b)

Yield 94 %, colorless sticky oil; optical rotations (*c* 1.14, CH$_2$Cl$_2$) $[\alpha]^{25}{}_{D}$ -43.8°, $[\alpha]^{25}{}_{578}$ -45.7°, $[\alpha]^{25}{}_{546}$ -51.3°, $[\alpha]^{25}{}_{436}$ -87.0° and $[\alpha]^{25}{}_{365}$ -137.9°; ^{1}H NMR δ 0.16 and 0.80 (2 x d, *J* = 6.90 and 7.04 Hz, 3H, CH$_3$), 0.69, 0.91, 0.94 and 0.95 (4 x d, *J* = 6.53, 6.95, 7.03 and 6.46 Hz, 6H, (C$\underline{H}_3$)$_2$CH), 0.75-1.1 (m, 2H), 1.23 (t, *J* = 7.04 Hz, 3H, C$\underline{H}_3$CH$_2$), 1.1-2.3 (m, 7H), 1.28 and 1.39, 1.29 and 1.39 (4 x s, 18H, 2 x *t*Bu), 1.82 and 1.87 (2 x d, $^2J_{HP}$ = 17.69 and 17.63 Hz, 3H, CH$_3$P), 3.8-4.0 (m, 2H, OC$\underline{H}_2$CH$_3$), 4.06 and 4.29 (2 x m, 1H, OCH), 6.75-6.80 (m, 2H, aromatic protons); ^{31}P NMR δ -20.21 (54 %) and -21.43 (46 %), diastereomeric mixture; ^{13}C NMR δ 14.6 and 16.2 (C$_1$), 16.80 and 16.82 (2 x d, $^3J_{CP}$ = 7.32 Hz, $\underline{C}$H$_3$CH$_2$O-P), 20.9 and 21.2 (C$_2$), 21.1 and 22.1 (2 x d, $^1J_{CP}$ = 193 and 192 Hz, PCH$_3$), 21.9 and 22.2 (C$_3$), 22.7 and 23.3 (C$_4$), 25.3 and 26.1 (C$_5$), 29.66 and 29.73 (C$_6$), 31.48 and 31.50 (C$_7$), 31.7 (C$_8$), 34.1 and 34.2 (C$_{11}$), 34.2 (C$_9$), 34.7 (C$_{10}$), 43.1 and 43.2 (C$_{12}$), 48.3 and 48.6 (2 x d, $^3J_{CP}$ = 7.4 and 9.7 Hz, C$_{13}$), 59.8 and 60.0 (2 x d, $^2J_{CP}$ = 10.7 and 10.5 Hz, P-OCH$_2$), 77.57 and 77.64 (2 x d, $^2J_{CP}$ = 12.4 and 11.5 Hz, C$_{14}$), 104.9 and 105.0 (2 x d, $^3J_{CP}$ = 11.3 and 10.4 Hz, C$_{15}$), 113.6 and 113.7 (C$_{16}$), 131.95 and 131.99 (2 x d, $^3J_{CP}$ = 10.2 and 8.4 Hz, C$_{17}$), 139.9 (C$_{18}$), 142.9 and 143.2 (C$_{19}$), 145.2 and 145.4 (C$_{20}$); MS *m/z* (relative intensity) 467 ([M+1]$^+$, 100 %), 421 (11), 329 (23), 311 (91), 283 (31); IR (film) 2957, 2869, 1487, 1428, 1071, 1034, 1001, 934, 907, 852, 733 and 700 cm^{-1}.

2-Cyclohexyloxy-2-*l*-menthoxy-2-methyl-1,3-dioxa(4,6-di-*tert*-butyl)-2-benzophosphole (1c)

Yield 86 %, colorless sticky oil; optical rotations (*c* 1.43, CH$_2$Cl$_2$) $[\alpha]^{25}{}_{D}$ -40.0°, $[\alpha]^{25}{}_{578}$ -

41.9°, $[\alpha]^{25}_{546}$ -46.4°, $[\alpha]^{25}_{436}$ -78.1° and $[\alpha]^{25}_{365}$ -121.7°; ^1H NMR δ 0.14 and 0.80 (2 x d, *J* = 6.91 and 7.08 Hz, 3H, CH_3), 0.70, 0.90, 0.94 and 0.95 (4 x d, *J* = 6.54, 6.96, 7.02 and 6.54 Hz, 6H, $(C\underline{H}_3)_2CH$), 0.75-1.0 (m, 3H), 1.1-2.3 (m, 16H), 1.28 and 1.38, 1.29 and 1.39 (4 x s, 18H, 2 x *t*Bu), 1.81 and 1.87 (2 x d, $^2J_{HP}$ = 17.78 and 17.74 Hz, 3H, CH_3P), 3.93-4.06 and 4.19-4.27 (m, 2H, 2 x OCH), 6.73-6.80 (m, 2H, aromatic protons); ^{31}P NMR δ -19.64 (56 %) and -20.76 (45 %), diastereomeric mixture; ^{13}C NMR δ 14.6 and 16.3 (C_1), 20.9 and 21.4 (C_2), 21.7 and 22.5 (2 x d, $^1J_{CP}$ = 194 and 193 Hz, PCH_3), 22.0 and 22.2 (C_3), 22.7 and 23.3 (C_4), 24.3, 24.4, 24.6 and 25.8 (cyclohexyl), 25.4 and 26.0 (C_5), 29.55 and 29.62 (C_6), 31.50 and 31.52 (C_7), 31.73 (C_8), 34.0, 34.1, 34.4 and 34.5 (cyclohexyl), 34.2 (C_9), 34.3 (C_{11}), 34.7 (C_{10}), 43.3 (C_{12}), 48.68 and 48.74 (2 x d, $^3J_{CP}$ = 7.4 and 8.8 Hz, C_{13}), 72.5 and 72.8 (2 x d, $^2J_{CP}$ = 11.2 and 11.4 Hz, P-OCH), 77.2 and 77.5 (2 x d, $^2J_{CP}$ = 12.9 and 12.0 Hz, C_{14}), 104.8 (d, $^3J_{CP}$ = 10.2 Hz, C_{15}), 113.2 and 113.4 (C_{16}), 132.0 and 132.1 (2 x d, $^3J_{CP}$ = 10.1 and 10.2 Hz, C_{17}), 139.6 and 140.0 (C_{18}), 142.8 and 143.5 (C_{19}), 145.5 and 146.1 (C_{20}); MS *m/z* (relative intensity) 521 ($[M+1]^+$, 100 %), 438 (1), 421 (11), 383 (13), 365 (30) and 283 (39); IR (film) 2955, 2867, 1485, 1453, 1428, 1069, 1032, 986, 941, 905, 851 and 777 cm^{-1}.

2-*l*-Menthoxy-2-[2-(methoxyglutaryl)aminoethoxy]-2-methyl-1,3-dioxa(4,6-di-*tert*-butyl)-2-benzophosphole (1d)

Purified by flash column chromatography on silica gel (hexane - AcOEt - NEt_3, 5 : 1 : 0.6), yield 90 %, colorless sticky oil; optical rotations (*c* 0.472, CH_2Cl_2) $[\alpha]^{25}_D$ -31.4°, $[\alpha]^{25}_{578}$ -30.9°, $[\alpha]^{25}_{546}$ -33.1°, $[\alpha]^{25}_{436}$ -56.8° and $[\alpha]^{25}_{365}$ -90.3°; ^1H NMR δ 0.06 and 0.77 (2 x d, *J* = 6.90 and 7.0 Hz, 3H, CH_3), 0.65, 0.93, 0.95 and 0.96 (4 x d, *J* = 6.48, 7.00, 7.13 and 6.48 Hz, 6H, $(C\underline{H}_3)_2CH$), 1.27 and 1.37, 1.29 and 1.37 (4 x s, 18H, 2 x *t*Bu), 0.8-2.2 (m, 9H), 1.84 and 1.89 (2 x d, $^2J_{HP}$ = 17.76 and 17.70 Hz, 3H, CH_3P), 2.01 (m, 2H, CH_2), 2.27 (m, 2H, $C\underline{H}_2CONH$), 2.42 (t, *J* = 7.24 Hz, 2H, $C\underline{H}_2CO_2CH_3$), 3.44 (q, *J* =

5.12 Hz, 2H, C$\underline{H}_2$NH), 3.67 (s, 3H, OCH$_3$), 3.83-3.96 (m, 2H, OC$\underline{H}_2$CH$_2$), 4.08 and 4.32 (2 x m, 1H, OCH), 6.0 (br s, 1H, NH), 6.75-6.80 (m, 2H, aromatic protons); ^{31}P NMR -20.53 (52 %) and -21.95 (48 %), diastereomeric mixture.; ^{13}C NMR δ 14.3 and 16.2 (C$_1$), 20.8 and 20.9 (CH$_2$), 20.9 and 21.1 (C$_2$), 21.0 and 22.0 (2 x d, $^1J_{CP}$ = 193 and 192 Hz, CH$_3$P), 21.8 and 22.1 (C$_3$), 22.6 and 23.2 (C$_4$), 25.4 and 26.3 (C$_5$), 29.6 and 29.7 (C$_6$), 31.4 (C$_7$), 31.6 (C$_8$), 33.1 ($\underline{C}$H$_2$CONH), 34.0 (C$_{11}$), 34.1 (C$_9$), 34.7 (C$_{10}$), 35.5 and 35.6 ($\underline{C}$H$_2$CO$_2$CH$_3$), 40.6 and 40.7 (2 x d, $^3J_{CP}$ = 6.6 and 6.5 Hz, CH$_2$NH), 43.1 (C$_{12}$), 48.2 and 48.5 (2 x d, $^3J_{CP}$ = 7.3 and 10.0 Hz, C$_{13}$), 51.5 (CH$_3$O), 62.6 and 62.9 (2 x d, $^2J_{CP}$ = 10.2 and 10.7 Hz, P-OCH$_2$), 78.0 and 78.2 (2 x d, $^2J_{CP}$ = 12.0 and 11.8 Hz, C$_{14}$), 105.0 and 105.1 (2 x d, $^3J_{CP}$ = 10 and 11.5 Hz, C$_{15}$), 114.2 (C$_{16}$), 132.1 and 132.2 (2 x d, $^3J_{CP}$ = 8.3 and 8 Hz, C$_{17}$), 139.8 (C$_{18}$), 143.2 and 143.5 (C$_{19}$), 144.7 and 144.9 (C$_{20}$), 171.9 (CONH), 173.5 ($\underline{C}$O$_2$CH$_3$); MS *m/z* (relative intensity) 610 ([M]$^+$, 8.4 %), 454 (1.3), 439 (5.8), 421 (69), 283 (100), 190 (21), 172 (52) and 140 (5).; IR (film) 3303 (br, amide NH), 2957, 2870, 1742 (C=O, ester), 1647 (C=O, amide), 1487, 1452, 1428, 1239, 1084, 1032, 1001, 909, 853, 731 and 696 cm^{-1}.

2-*l*-Menthoxy-2-methyl-2-[2-(2-pyridyldithio)ethoxy]-1.3-dioxa(4,6-di-*tert*-butyl)-2-benzophosphole (1e)

Yield 100 %, colorless sticky oil; optical rotations (*c* 0.93, CH$_2$Cl$_2$) $[\alpha]^{25}_D$ -18.5°, $[\alpha]^{25}_{578}$ -19.4°, $[\alpha]^{25}_{546}$ -20.1°, $[\alpha]^{25}_{436}$ -32.6 and $[\alpha]^{25}_{365}$ -45.9°; ^{1}H NMR δ 0.083 and 0.78 (2 x d, *J* = 6.88 and 7.03 Hz, 3H, C$\underline{H}_3$CH), 0.64, 0.89, 0.91 and 0.94 (4 x d, *J* = 6.49, 7.15, 7.21 and 6.48 Hz, 6H, (C$\underline{H}_3$)$_2$CH), 0.7-1.0 (m, 2H), 1.1-2.3 (m, 7H), 1.27 and 1.36, 1.28 and 1.36 (4 x s, 18H, 2 x *t*Bu), 1.82 and 1.88 (2 x d, $^2J_{HP}$ = 17.79 and 17.72 Hz, 3H, CH$_3$P), 3.03 (m, 2H, CH$_2$S), 4.02-4.12 and 4.20-4.31 (m, 3H, P-OCH$_2$ and P-OCH), 6.76 (m, 2H, aromatic protons), 7.08 (m, 1H, pyridyl 5'-H), 7.65 (m, 1H, pyridyl 4'-H), 7.82 (m, 1H, pyridyl 3'-H), 8.47 (m, 1H, pyridyl 6'-H); ^{31}P NMR δ -20.48 (57 %) and -21.91 (43

%), diastereomeric mixture; ^{13}C NMR δ 14.4 and 16.1 (C_1), 20.7 and 21.1 (C_2), 20.9 and 21.9 (2 x d, $^{1}J_{CP}$ = 193 and 192 Hz, CH_3P), 21.8 and 22.1 (C_3), 22.5 and 23.2 (C_4), 25.4 and 26.1 (C_5), 29.6 and 29.7 (C_6), 31.4 (C_7), 31.6 (C_8), 33.98 (C_{11}), 34.03 (C_9), 34.6 (C_{10}), 39.9 and 40.1 (2 x d, $^{3}J_{CP}$ = 6.50 and 7.03 Hz, CH_2S), 42.91 and 42.94 (C_{12}), 48.0 and 48.2 (2 x d, $^{3}J_{CP}$ = 7.15 and 9.85 Hz, C_{13}), 62.0 and 62.3 (2 x d,$^{2}J_{CP}$ = 10.6 and 11.1 Hz, P-OCH_2), 77.9 and 78.0 (2 x d, $^{2}J_{CP}$ = 11.3 and 10.8 Hz, C_{14}), 104.9 and 105.1 (2 x d, $^{3}J_{CP}$ = 11.2 and 10.5 Hz, C_{15}), 113.8 and 113.9 (C_{16}), 119.26 and 119.31 (5'-pyridyl), 120.3 (3'-pyridyl), 131.9 and 132.0 (2 x d, $^{3}J_{CP}$ = 9.46 Hz, C_{17}), 136.80 and 136 83 (4'-pyridyl), 139.71 (C_{18}), 143.1 and 143.2 (C_{19}), 144.9 and 145.0 (C_{20}), 149.4 (6'-pyridyl), 160.7 and 160.8 (2'-pyridyl); MS *m/z* (relative intensity) 607 ($[M]^+$, 8.1 %), 469 (10), 421 (50), 283 (100), 188 (17) and 170 (37); IR (film) 2957, 2870, 1487, 1417, 1426, 1067, 1026, 1001, 939, 907, 853, 760 and 693 cm^{-1}.

2,2-Di-*l*-menthoxy-2-methyl-1,3-dioxa(4,6-di-*tert*-butyl)-2-benzophosphole (1f)

Yield 95 %, colorless sticky oil; optical rotations (*c* 1.13, CH_2Cl_2) $[\alpha]^{25}_{D}$ -68.2°, $[\alpha]^{25}_{578}$ -71.0°, $[\alpha]^{25}_{546}$ -79.7°, $[\alpha]^{25}_{436}$ -134.8° and $[\alpha]^{25}_{365}$ -213.8°; ^{1}H NMR δ 0.46 and 0.86 (2 x d, *J* = 6.87 and 7.05 Hz, 3H, CH_3CH), 0.76, 0.90, 0.93 and 0.94 (4 x d, *J* = 6.54, 6.92, 7.03 and 6.45 Hz, 6H, $(\underline{CH_3})_2CH$), 0.8-1.35 (m, 9H, ring protons), 1.28 and 1.38 (2 x s, 18H, 2 x *t*Bu), 1.5-1.7 (m, 6H, ring protons), 1.86 (d, $^{2}J_{HP}$ = 17.82 Hz, 3H, CH_3P), 2.15-2.44 (m, 3H, ring protons), 3.89 and 4.16 (2 x m, 2H, OCH), 6.8 (m, 2H, aromatic protons; ^{31}P NMR δ -20.2, single peak; ^{13}C NMR δ 15.9 and 16.2 (C_1), 21.3 and 21.5 (C_2), 22.2 and 22.3 (C_3), 22.8 (d, $^{1}J_{CP}$ = 199 Hz, CH_3P), 22.9 and 23.1 (C_4), 25.1 and 25.5 (C_5), 29.7 (C_6), 31.6 and 31.7 (C_7), 31.74 (C_8), 34.1 (C_9), 34.45 and 34.47 (C_{11}), 34.6 (C_{10}), 43.3 and 43.6 (C_{12}), 48.9 and 49.3 (2 x d, $^{3}J_{CP}$ = 7.74 and 7.43 Hz, C_{13}), 75.6 and 76.3 (2 x d, $^{2}J_{CP}$ = 12.73 and 12.65 Hz, C_{14}), 105.1 (d, $^{3}J_{CP}$ = 11.9 Hz, C_{15}), 114.2 (C_{16}), 131.5 (d, $^{3}J_{CP}$ = 7.8 Hz, C_{17}), 141.1 (C_{18}), 142.0 (C_{19}), 144.5 (C_{20}); MS *m/z* (relative intensity) 577 ([M]$^+$, 100 %), 439 (14), 421 (26), 300 (13), 283 (7.7) and 267 (4.0); IR (film) 2957, 2870, 1489, 1456, 1428, 1051, 1019, 995, 907, 853 and 774 cm^{-1}.

Stability test of phosphorane (1b)

Phosphorane **1b** (16.3 mg, 0.035 mmol) was dissolved in $CDCl_3$ (500 µΛ). To this solution was added (i) D_2O (100 µΛ) or (ii) 0.1 N NaOH in D_2O (100 µΛ), or (iii) 0.1 N HCl in D_2O (100 µΛ) at room temperature. The mixture was shaken and allowed to stand at room temperature for an appropriate period during which the reaction was monitored with ^1H and ^{31}P NMR. There was no change in ^1H and ^{31}P NMR for 15 and 18 days in the experiment (i) and (ii), respectively. In the experiment (iii), however, the phosphorane **1b** decomposed completely to a diastereomeric mixture of phosphonate **5** after 8 min : $\delta^{31}P$ +32.3 (52 %) and 32.9 (48 %) ppm.

Preparation of phosphonate (5) from (1a)

The phosphorane **1a** (100 mg, 0.22 mmol) was dissolved in a mixture of Et_2O (3 mL) and THF (0.5 mL). To this solution was added one drop of 2*N* HCl and the mixture was stirred at room temperature for 5 min. The reaction was completed in 5 min (TLC, hexane - NEt_3, 9 : 1). The reaction mixture was evaporated to give colorless solid: 91 mg (94 %), mp 125-140°C, ^1H NMR δ 0.48 and 0.85 (2 x d, J = 6.90 and 6.98 Hz, 3H, $C\underline{H}_3CH$), 0.76, 0.77, 0.93 and 0.94 (4 x d, J = 6.54, 7.06, 6.50 and 7.00 Hz, 6H, $(C\underline{H}_3)_2CH$), 0.8-2.2 (m, 9H, ring protons), 1.28, 1.29, 1.417 and 1.423 (4 x s, 18H, *t*Bu), 1.68 and 1.70 (2 x d, $^2J_{HP}$ = 17.7 and 17.6 Hz, 3H, $CH_3P{=}O$), 4.36 (m, 1H, P(O)-OCH), 6.92 (m, 1H, aromatic protons), 7.12 (m, 1H, aromatic protons), 8.01 and 8.21 (2 x s, 1H, OH); ^{31}P NMR δ +32.3 (52 %) and 32.9 (48 %), diastereomeric mixture; ^{13}C NMR δ 11.1 and 12.4 (2 x d, $^1J_{CP}$ = 146 and 145 Hz, CH_3P), 14.9 and 15.6 (C_1), 20.86 and 20.88 (C_2), 21.8 and 21.9 (C_3), 22.6 and 22.9 (C_4), 25.2 and 26.1 (C_5), 29.7 (C_6), 31.4 (C_7), 31.5 (C_8), 33.89 and 33.91

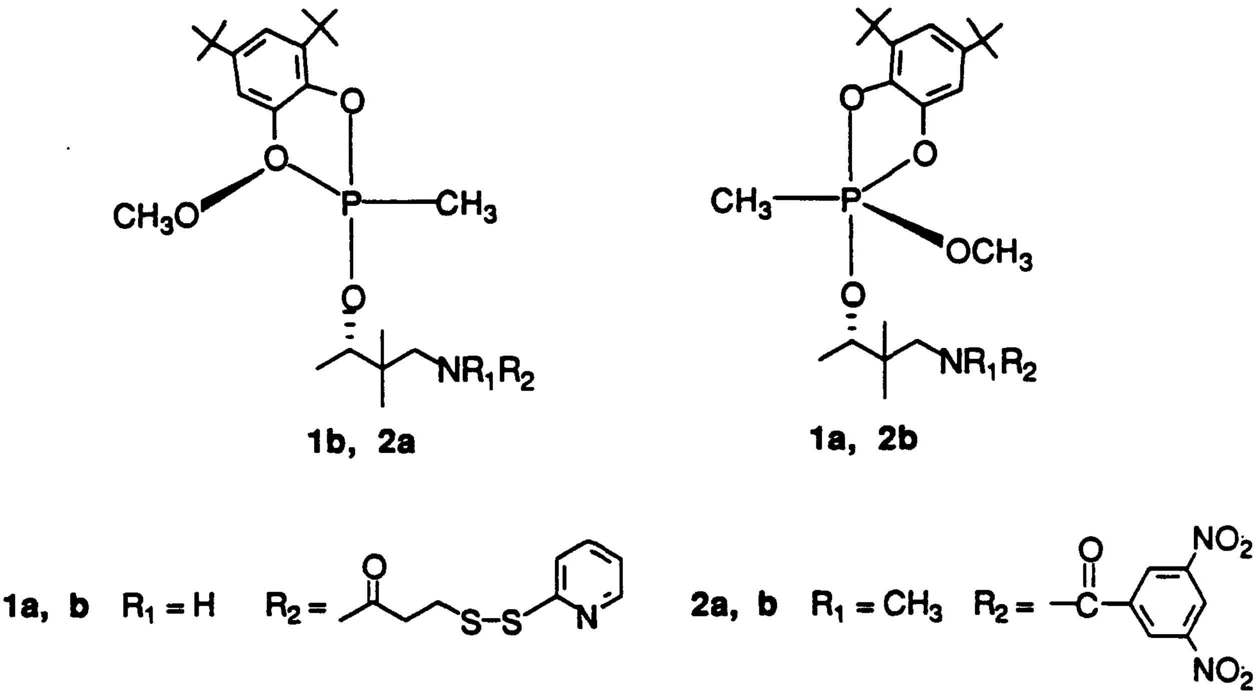
(C_{11}), 34.23 and 34.27 (C_9), 35.35 and 35.39 (C_{10}), 42.8 and 43.0 (C_{12}), 48.3 and 48.4 (C_{13}), 79.2 and 79.6 (2 x d, $^2J_{CP}$ = 8.0 and 7.3 Hz, C_{14}), 116.4 and 116.7 (2 x d, $^4J_{CP}$ = 4.12 and 3.82 Hz, C_{15}), 120.4 and 120.5 (C_{16}), 138.86 and 138.92 (2 x d, $^3J_{CP}$ = 9.05 and 9.76 Hz, C_{17}), 139.2 (C_{18}), 142.18 and 142.24 (C_{19}), 144.1 and 144.3 (2 x d, $^1J_{CP}$ = 2.62 Hz, C_{20}); MS *m/z* (relative intensity) 438 ($[M]^+$, 100), 300 (10), 283 (7.2) and 267 (4.2); IR (film) 3370 (br, OH), 1493, 1420, 1314, 1300, 1175 and 1157 (P=O), 1016, 994, 920, 874 and 750 cm^{-1}.

CHAPTER III: ISOLATION AND CHARACTERIZATION OF STEREOISOMERS ON PENTACOORDINATED PHOSPHORUS. STRUCTURAL STUDY AND PSEUDOROTATIONAL PROCESS OF UNSYMMETRICALLY SUBSTITUTED CHIRAL MONOCYCLIC OXYPHOSPHORANES

A. Introduction

Pentacoordinated phosphorus (phosphoranes) have attracted much attention as models for the intermediate or transition state in nonenzymatic and enzymatic phosphoryl-transfer reactions.[1] The stereochemical course and product distribution of phosphoryl-transfer reaction have been discussed in terms of structure, stereochemistry, and pseudorotational processes of the proposed pentacoordinated intermediate.[2,1e] Such intermediates or transition states often involve a chiral pentacoordinated phosphorus if it is substituted unsymmetrically. Accordingly, the isolation and characterization of stereoisomers on pentacoordinated phosphorus offer a promising approach for the study of the stereochemistry of phosphoryl-transfer reaction as well as for the study of stereochemical nonrigidity of phosphoranes.However, the isolation of chiral phosphoranes is quite rare owing to its nonrigid character or the low energy barrier required for the interconversion from one form to another.[3] We now report the isolation and characterization of diastereomerically related stable pseudorotatomers of chiral monocyclic oxyphosphoranes **1** and **2** having five different substituents bound to phosphorus (Scheme I)..

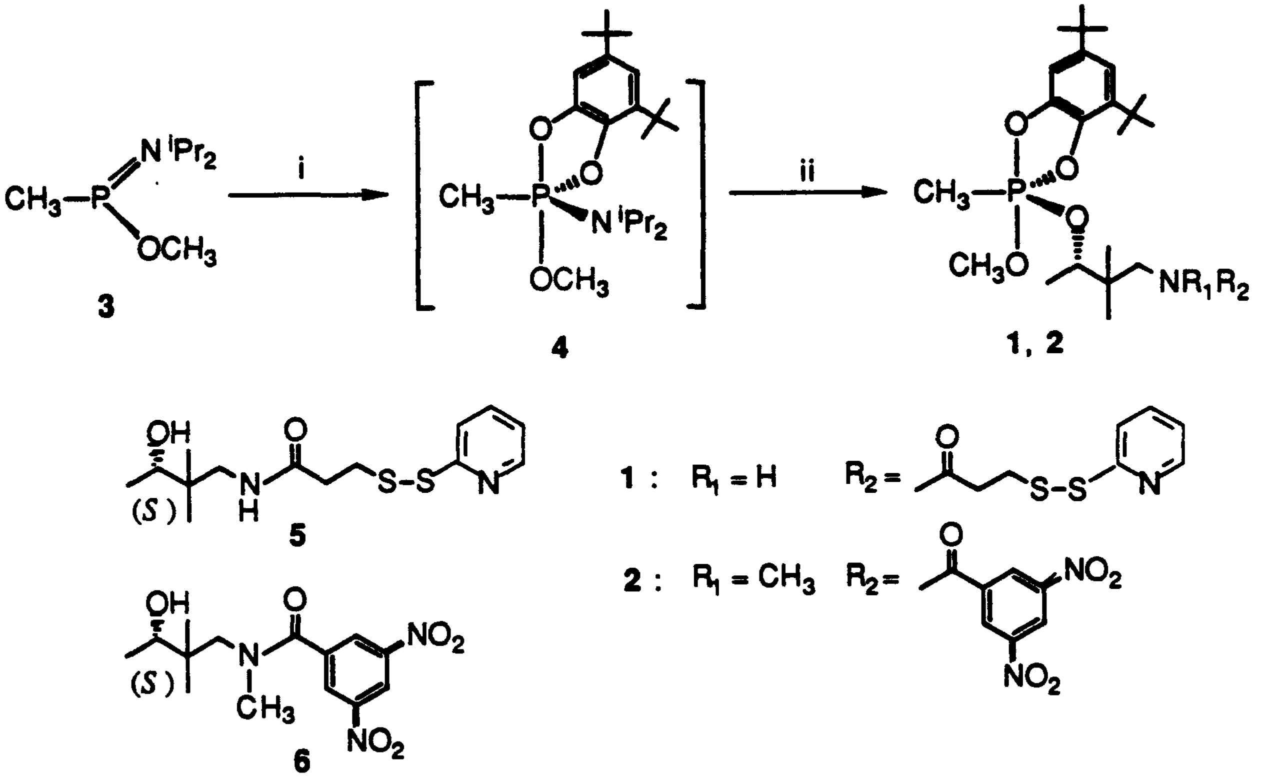
Scheme I



B. Results and Discussion

The phosphoranes 1 and 2 were synthesized according to the method reported previously.[4] The substitution of *N*, *N*-diisopropylamino group in the key intermediate 4 by the alcohols 5 or 6 proceeded only in the presence of 1*H*-tetrazole as a catalyst,[5] giving the oxyphosphoranes 1 and 2 in 78 and 91% yield, respectively. Each compound gave two spots on TLC as well as two signals of approximately equal intensity in ^{31}P NMR and two sets of signals for ^{1}H and ^{13}C NMR, indicating that 1 and 2 were composed of two diastereomerically related isomers with different configuration around phosphorus **1a, 1b** and **2a, 2b**, respectively. These diastereomers were separated by silica gel column chromatography under basic conditions (**1a**, **1b**, and **2b**) or fractional crystallization (**2a**) (**Table I**).

Scheme Ia



[a] Reagents and conditions: (i) 3,5-di-*tert*-butyl-1,2-benzoquinone (1 equiv.), dry CH_2Cl_2, 0°C to 25°C, 2h; (ii) alcohols **5** or **6** (1 equiv.), 1*H*-tetrazole (10 mol%), dry CH_2Cl_2, 0°C to 25°C, 1h.

Table I. ^{31}P, ^{1}H and ^{13}C NMR data and specific rotations for phosphoranes **1a,b** and **2a,b**[a]

Phosphoranes	$\delta^{31}P^{b}$ [ppm]	$\delta^{1}H$ [ppm] for CH_3-P^{c} (coupling const. [Hz])	$\delta^{13}C$ [ppm] for CH_3-P^{c} (coupling const. [Hz])	$[\alpha]^{25}_{D}$ (deg.)
1a	-20.36	1.85 (d, 17.3)	20.8 (d, 188.2)	+5.56[d]
1b	-20.39	1.81 (d, 17.4)	20.5 (d, 191.0)	+11.9[e]
2a	-20.70	1.88 (d, 17.5)	21.4 (d, 190.8)	+9.40[f]
2b	-20.17	1.85 (d, 17.6)	20.9 (d, 193.0)	+2.94[g]

[a] Phosphorane **1a**, **1b** and **2b** were purified by flash column chromatography on silica gel (hexane-AcOEt-NEt_3, 5 : 2 : 0.7 and hexane-CH_2Cl_2-NEt_3, 9 : 1 : 1, respectively). Phosphorane **2a** was crystallized from hexane-NEt_3, 9 : 1. [b] Solvent is $CDCl_3$. Chemical shifts downfield of the reference (85% H_3PO_4 as an external standard) are indicated as positive. [c] Methyl group bound to phosphorus. Solvent is $CDCl_3$. [d] *c* 0.9, $CHCl_3$. [e] *c* 1.03, $CHCl_3$. [f] *c* 1.0, CH_2Cl_2. [g] *c* 1.02, CH_2Cl_2.

The absolute configuration of the crystalline phosphorane **2a** was determined by X-ray diffraction analysis (Fig). The distances and angles involving the pentacoordinated phosphorus atom are listed in **Table II**.

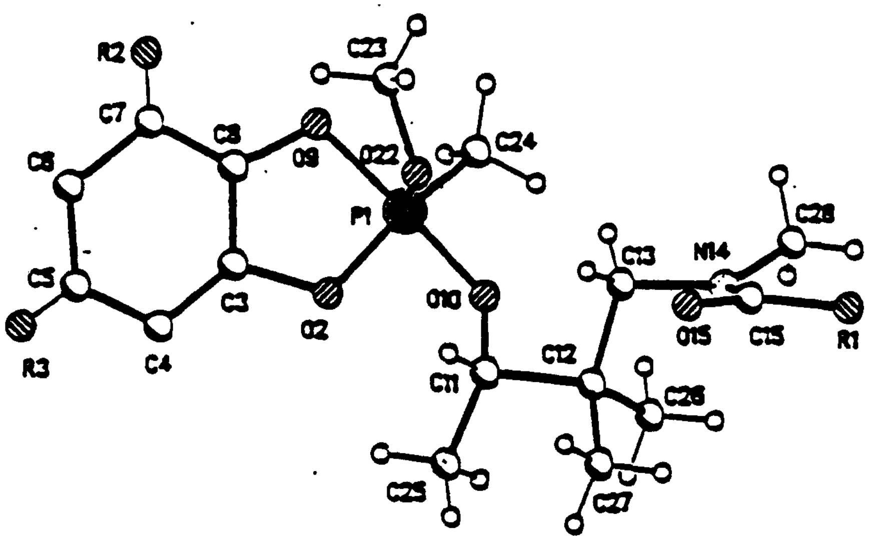
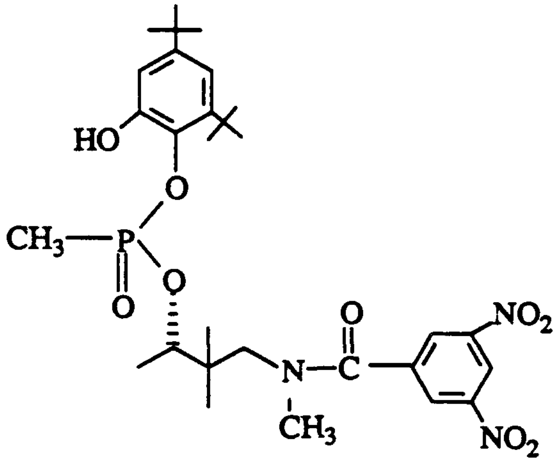


Table II. Phosphorus bond length (Å) and angles (°)

P(1)-O(2)	1.627(9)	P(1)-O(9)	1.792(9)
P(1)-O(10)	1.648(8)	P(1)-O(22)	1.545(8)
P(1)-C(24)	1.772(12)		
O(9)-P(1)-C(24)	85.2(5)	O(2)-P(1)-O(9)	87.2(4)
O(2)-P(1)-C(24)	89.8(4)	O(10)-P(1)-C(24)	90.3(5)
O(9)-P(1)-O(22)	94.1(4)	O(10)-P(1)-O(22)	94.5(4)
O(2)-P(1)-O(22)	115.5(4)	O)22)-P(1)-C(24)	116.2(5)
O(2)-P(1)-C(24)	128.2(5)	O(9)-P(1)-O(10)	171.3(4)

The coordination is quite close to a perfectly regular trigonal bipyramid, with atoms O9 and O10 lying on the trigonal axis, and all angles falling within 5° of the ideal values (90, 120, and 180°). By knowing the absolute configuration of C(11) as (*S*),[6] the absolute configuration of chiral pentacoordinated phosphorus was determined unambiguously. Another notable feature of these phosphoranes is that the configuration around phosphorus is interconvertible between the two diastereomeric form **2a** and **2b** by heating, although the configuration is stable enough to allow the isolation of each forms at room temperature. The kinetics of the interconversion was first order; **2a** was heated at 90°C to give a 1:1 mixture of **2a** and **2b** with a first-order rate constant of 2.40 x 10^{-2} [min^{-1}].[7] The activation energy for this process was calculated to be 27.0 [kcal mol^{-1}]. This value is one of the highest energy barrier found for pseudorotational process of phosphoranes.[8]

As a dynamic aspect of chiral phosphoranes, the acid-catalyzed hydrolysis of **1** and **2** was examined.[9] Diastereomer **2a** reacted with 0.1N HCl immediately to give a 1:1 mixture of two diastereomeric phosphonates **7a** and **7b**. The other diastereomer **2b**, however, gave **7a** and **7b** in unequal amount (73 : 37), suggesting that the chirality on pentacoordinated phosphorus can affect the stereochemical course of the hydrolysis.[10]



7a, 7b

The stereochemistry of the hydrolysis of chiral pentacoordinated phosphorus will give insight into the mechanism of the hydrolysis of chiral phosphates or phosphonates such as soman, a well known neurotoxic agent. This aspect of the work is currently going on and will be reported elsewhere.

C. References and footnotes

1. (a) Westheimer, F.H. *Acc. Chem. Res.* **1968**, *1*, 70-78. (b) Buchwald, S.L.; Pliura, D.H.; Knowles, J.R. *J. Am. Chem. Soc.* **1984**, *106*, 4916-4922. (c) Knowles, J.R. *Ann. Rev. Biochem.* **1980**, *49*, 877-919. (d) van Ool, P.J.J.M.; Buck, H.M. *Recl. Trav. Chim. Pays-Bas* **1984**, *103*, 119-122. (e) Holmes, R.R.; "Pentacoordinated Phosphorus"; American Chemical Society: Washington, D C, **1980**, vol. II, p. 87 and references cited therein.
2. (a) Yu, J.H.; Arif, A.M.; Bentrude, W.G. *J. Am. Chem. Soc.* **1990**, *112*, 7451-7461. (b) Deiters, J.A.; Holmes, R.R. *J. Am. Chem. Soc.* **1983**, *105*, 609-615.
3. Chiral spirophosphoranes have been synthesized and one of the stereoisomers of them has been isolated by fractional crystallization accompanied by a second-order asymmetric transformation: Klaebe, A.; Carrelhas, A.; Brazier, J.-F.; Houalla, D.; Wolf, R. *Phosphorus and Sulfur* **1977**, *3*, 61-76. Klaebe, A.; Brazier, J.F.; Carrelhas, A.C.; Garrigues, B.; Marre, M.R.; Contreras, R. *Tetrahedron* **1982**, *38*, 2111-2122 and references cited therein.
4. Moriarty, R.M.; Hiratake, J.; Liu, K. *J. Am. Chem. Soc.* **1990**, *112*, 8575-8577.
5. Without catalyst, no reaction was observed after 2 h; however, the reaction was completed in 30 min at room temperature in the presence of 0.1 equiv. of 1*H*-tetrazole.
6. The alcohol **5** and **6** were synthesized from (*S*)-3-hydroxy-2,2-dimethylbutanenitrile prepared by yeast reduction of 2,2-dimethyl-3-oxobutanenitrile. The stereochemistry of the yeast reduction of unsymmetric ketones is well established:
7. A solution of **2a** (15 mg) in CDCl$_3$ (600 μL) was placed in a sealed tube and heated at 90°C for a certain period of time; the conversion of the reaction was determined with ^{31}P NMR.
8. (a) Holmes, R.R. "Pentacoordinated Structures" in Progress in Inorganic Chemistry, ed. by Lippard, S.J., John Wiley & Sons, New York, **1984**, vol. 32, p. 119. (b) Holmes, R. R. "Pentacoordinated Phosphorus"; American Chemical Society: Washington, DC, **1980**, vol. I, p. 101-233.
9. The phosphoranes **1** and **2** are hydrolytically stable in neutral and basic conditions, but extremely labile to aqueous acids: they remained unchanged for at least 3 days in the presence of water or 0.1*N* NaOH at room temperature. Also see reference 4.
10. Similar results were obtained for the acid hydrolysis of **1**: the diastereomer **1a** gave a 63 : 36 mixture of the corresponding diastereomeric phosphonates, whereas **1b** afforded a 1 : 1 mixture.

D. Experimental

General

^1H and ^{13}C NMR spectra were measured on a Bruker AM 400 (400 MHz for ^1H) spectrometer. ^{31}P NMR spectra were recorded on a Bruker WP200SY (81.0 MHz for ^{31}P) spectrometer with 85% H_3PO_4 as an external standard. Chemical shifts downfield of the reference are indicated as positive in ^{31}P NMR. Deuteriochloroform dried over molecular sieves 4Å was used as solvent for all NMR experiments with residual chloroform as an internal standard for ^1H and ^{13}C NMR (δ_H 7.27 and δ_C 77.01 ppm). IR spectra were measured on an IBM IR/32 spectrometer. Mass spectra were obtained by chemical ionization (CI) or fast atom bombardment (FAB) techniques with a Finnigan MAT 90 mass spectrometer. A Perkin-Elmer 241 polarimeter was used for measuring optical rotations. Dichloromethane was distilled over P_2O_5 and stored over molecular sieves 4Å. Diethyl ether and tetrahydrofuran (THF) were dried by distillation from sodium metal immediately before use. Dry hexane for recrystallization was distilled over CaH_2 and stored over molecular sieves 4Å. Triethylamine was distilled from KOH in the presence of phthalic anhydride. Dimethylformamide (DMF) was vacuum distilled and stored over molecular sieves 4Å. The products were isolated by short-path distillation under an argon atmosphere or by flash column chromatography on silica gel (Silica Gel 40 µm Flash Chromatography Packing, J. T. Baker Inc.).

Preparation of (*S*)-3,3-dimethyl-4-{*N*-[3-(2-pyridyldithio)]propionyl}amino-2-butanol (5)

A solution of *N*-succinimidyl-3-(2-pyridyldithio)propionate (1.49 g, 4.77 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise to a cold solution of the aminoalcohol **10** (533 mg, 4.55 mmol) and triethylamine (690 mg, 6.82 mmol) in dry CH_2Cl_2 (20 mL) with ice-cooling. The mixture was stirred at 0°C for 30 min, then at room temperature for 1 h. The reaction mixture was then diluted with CH_2Cl_2 (40 mL) and washed with sat. $NaHCO_3$ (2 x 15 mL) and sat.

NaCl (1 x 15 mL), successively. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum to give a light yellow oil. Purification by flash column chromatography (hexane-AcOEt, 1 : 3) gave **5** as a colorless oil: 1.5 g (100%); ^1H NMR δ 0.87 (2 x s, 6H, $(CH_3)_2C$), 1.10 (d, *J* = 6.45 Hz, 3H, $C\underline{H}_3CH$), 2.64-2.68 (m, 3H, $C\underline{H}_2CO$ and $C\underline{H}_2NH$), 3.09 (t, *J* = 6.55 Hz, 2H, CH_2S), 3.50 (m, 1H, $C\underline{H}OH$), 3.76 (dd, *J* = 13.96 and 8.16Hz, 1H, $C\underline{H}_2NH$), 4.07 (d, *J* = 3.97 Hz, 1H, OH), 7.13 (m, 1H, 5'-H), 7.22 (br s, 1H, NH), 7.60-7.67 (m, 2H, 3'- and 4'-H), and 8.45 (m, 1H, 6'-H); ^{13}C NMR δ 16.70 and 18.80 ($(\underline{C}H_3)_2C$), 23.44 ($\underline{C}H_3CH$), 35.16 and 35.78 ($\underline{C}H_2CO$ and $\underline{C}H_2S$), 38.89 ($(CH_3)_2\underline{C}$), 48.78 (CH_2N), 70.30 (CHOH), 120.5 and 121.1 (3'- and 5'-C), 137.1 (4'-C), 149.5 (6'-C), 159.3 (2'-C), and 172.4 (C=O); MS m/z (relative intensity) 315 ($[M+1]^+$, 100%); IR (film) 3500-3300 (br, OH and amide NH str.), 2971, 1647 (amide C=O), 1563 (NH), 1574, 1447, 1418, 1117 and 762 cm^{-1}.

Preparation of (*S*)-4-(*N*-3,5-dinitrobenzoyl-*N*-methyl)amino-3,3-dimethyl-2-butanol (6)

The amide **11** (2.85 g, 9.16 mmol) and iodomethane (6.50 g, 45.8 mmol) were dissolved in dry DMF (30 mL). Powdered NaOH (732 mg, 18.3 mmol) was added to the solution; the mixture turned dark purple in color immediately. The mixture was then heated at 100-105°C in a sealed tube for 15 min. The mixture became light orange in color and the reaction was found to be completed (TLC, hexane-AcOEt, 1:1). The reaction mixture was poured into 0.5*N* HCl (150 mL), and the product was liberated as yellow solid. The solid was collected and recrystallized from hot hexane-AcOEt (1 : 7) to afford **6** as slightly yellow crystals: 2.49 g (83%), mp 144.5 - 146°C; optical rotations (*c* 2.03, $CHCl_3$) $[\alpha]^{27}_D$ -39.7°, $[\alpha]^{27}_{578}$ -41.5°, $[\alpha]^{27}_{546}$ -48.2°, and $[\alpha]^{27}_{436}$ -91.9°; ^1H NMR δ 0.97 and 1.05 (2 x s, 3H, $(CH_3)_2C$), 1.16 (d, *J* = 6.43 Hz, 3H, CH_3), 2.80 (d, *J* = 14.0 Hz, 1H, CH_2N), 3.12 (s, 3H, NCH_3), 3.57 (m, 1H, CH), 4.11 (d, *J* = 4.32 Hz, 1H, OH), 4.26 (d, *J* = 14.0 Hz, 1H, CH_2N), 8.59 (d,

J = 2.05 Hz, 2H, 2'-H and 6'-H); and 9.09 (t, J = 2.05 Hz, 4'-H); ^{13}C NMR 16.53 and 19.22 ($(\underline{C}H_3)_2C$), 24.49 ($\underline{C}H_3CH$), 40.97 ($\underline{C}(CH_3)_2$), 41.69 (NCH_3), 58.00 (CH_2N), 69.79 (CH), 119.6 (4'-C), 127.1 (2'-C and 6'-C), 139.5 (1'-C), 148.5 (3'-C and 5'-C), and 168.6 (C=O); MS m/z (relative intensity) 326 ($[M+1]^+$, 53%) and 308 (100); IR (KBr) 3404 (OH), 3104, 2975, 2880, 1620 (amide C=O), 1545 (NO_2), 1414, 1388, 1362, 1348 (NO_2), 1316, 1119, 1111, 916, 729, and 654 cm^{-1}.

Preparation of *N,N*-diisopropylamino(methoxy)(methyl)phosphine (3)

An ethereal solution of methyllithium (1.4 M, 18.0 mL, 25.2 mmol) was added dropwise to a solution of chloro(*N,N*-diisopropylamino)(methoxy)phosphine (4.98 g, 25.2 mmol) in dry diethyl ether (25 mL) at -78°C under an argon protection. After the addition, the mixture was stirred for 30 min at -78°C, then stirred overnight at room temperature. Colorless powder (LiCl) was precipitated after 1 h. The reaction mixture was filtered under an argon protection and evaporated below 30°C under reduced pressure. Distillation of the residual oil gave the phosphine 3 as a colorless liquid with disagreeable odor: 4.02 g (90%), bp 39-43°C at 5 mmHg; ^{1}H NMR δ 1.09 and 1.19 (2 x d, J = 6.78 and 6.71 Hz, 12H, $N[CH(C\underline{H}_3)_2]_2$), 1.18 (d, $^2J_{HP}$ = 8.20 Hz, 3H, CH_3P), 3.39 (d, $^3J_{HP}$ = 13.5 Hz, 3H, CH_3OP), and 3.50 (m, 2H, $N[C\underline{H}(CH_3)_2]_2$); ^{13}C NMR δ 17.8 (d, $^1J_{CP}$ = 11.47 Hz, CH_3P), 24.1 and 24.7 (2 x d, $^3J_{CP}$ = 7.44 and 6.44 Hz, 2 x $NCH(\underline{C}H_3)_2$), 44.0 (d, $^2J_{CP}$ = 9.15 Hz, $N[\underline{C}H(CH_3)_2]_2$), and 53.9 (d, $^2J_{CP}$ = 17.30 Hz, CH_3OP); ^{31}P NMR δ +125.0; MS m/z (relative intensity) 178 ($[M+1]^+$, 100%), 162 (53), and 146 (66); IR (film) 2967, 2930, 1362, 1184, 1125, 1051, 972, 870, and 695 cm^{-1}.

Preparation of (3,5-Di-*tert*-butyl-1,2-phenylenedioxy){(*S*)-3,3-dimethyl-4-[3-(2-pyridyldithio)propionyl]amino-2-butoxy}(methoxy)(methyl)phosphorane (1)

A solution of the phosphine 3 (388 mg, 2.19 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a solution of 3,5-di-*tert*-butyl-1,2-benzoquinone (506 mg, 2.30 mmol) in dry CH_2Cl_2 (35 mL) at 0°C under an argon atmosphere. The mixture was stirred at 0°C for 30 min and then stirre [illegible] oom temperature for 1.5 h. The formation of the aminophosphorane 4 was checked by TLC (hexane - NEt_3, 9 : 1, $R_f = 0.9$). A solution of the alcohol 5 (688.2 mg, 2.19 mmol) in dry CH_2Cl_2 (10 mL) was added to the mixture at 0°C. The crystals of 1*H*-tetrazole (15.4 mg, 0.22 mmol) was added to the mixture successively. The mixture was stirred at 0°C for 0.5 h, then at room temperature for 1 h. The solvent was removed under reduced pressure and the residual oil was purified by flash column chromatography (hexane - AcOEt - NEt_3, 5 : 2 : 0.7) to give the phosphorane 1 as a colorless oil (diastereomer **1a**, 220 mg; diastereomer **1b**, 233 mg; overall isolated yield of 1, 78%).

Diastereomer 1a: optical rotations (*c* 0.9, $CHCl_3$) $[\alpha]^{26.5}_{D}$ +5.56°, $[\alpha]^{26.5}_{578}$ +5.78°, $[\alpha]^{26.5}_{546}$ +6.11°, $[\alpha]^{26.5}_{436}$ +8.00°, and $[\alpha]^{26.5}_{365}$ +7.22°; ^1H NMR δ 0.84 and 0.87 (2 x s, 6H, $(CH_3)_2C$), 1.29 and 1.39 (2 x s, 18H, 2 x $C(CH_3)_3$), 1.34 (d, J = 6.29 Hz, 3H, $C\underline{H}_3CH$), 1.85 (d, $^2J_{HP}$ = 17.34 Hz, 3H, CH_3P), 2.35 (t, J = 7.07 Hz, 2H, CH_2CO), 3.02 (m, 3H, $C\underline{H}_2S$ and $C\underline{H}_2NH$), 3.36 (dd, J = 13.7 and 8.06 Hz, 1H, $C\underline{H}_2NH$), 3.62 (d, $^3J_{HP}$ = 11.66 Hz, 3H, CH_3OP), 4.24 (m, 1H, $CH_3C\underline{H}$), 5.95 (br m, 1H, NH), 6.84 (m, 2H, 4'- and 6'-H), 7.07 (m, 1H, 5"-H), 7.60-7.70 (m, 2H, 3"- and 4"-H), and 8.45 (m, 1H, 6"-H); ^{13}C NMR δ 16.56 and 21.03 ($(\underline{C}H_3)_2C$), 20.75 (d, $^1J_{CP}$ = 188.2 Hz, CH_3P), 22.81 ($\underline{C}H_3CH$), 29.68 and 31.73 (2 x $C(\underline{C}H_3)_3$), 34.19 and 34.79 (2 x $\underline{C}(CH_3)_3$), 34.22 and 35.48 (CH_2CH_2S), 38.76 (d, $^3J_{CP}$ = 6.40 Hz, $(CH_3)_2\underline{C}$), 46.25 (CH_2N), 52.67 (d, $^2J_{CP}$ = 10.15 Hz, CH_3OP), 79.64 (d, $^2J_{CP}$ = 12.37 Hz, $CH_3\underline{C}H$), 105.3 (d, $^3J_{CP}$ = 11.17 Hz, 6'-C), 114.5 (4'-C), 119.7 and 120.7 (3"- and 5"-C), 132.5 (3'-C), 137.0 (4"-C), 139.5 (5'-C), 143.6 (1'-C), 144.7 (2'-C), 149.6 (6"-C), 160.0 (2"-C), and 170.6 (C=O); ^{31}P NMR δ -20.36; MS (CI) m/z (relative intensity) 611 ([M+1]$^+$, 0.8%), 297 (100); IR (film) 3308 (NH), 2963, 1655 (amide C=O), 1574, 1420, 1302, 1076, 994, 855, 760, 718, and 691 cm^{-1}.

Diastereomer 1b: optical rotations (*c* 1.03, $CHCl_3$) $[\alpha]^{26.5}_D$ +11.9°, $[\alpha]^{26.5}_{578}$ +12.4°, $[\alpha]^{26.5}_{546}$ +14.4°, $[\alpha]^{26.5}_{436}$ +25.2°, and $[\alpha]^{26.5}_{365}$ +45.1°; ^{1}H NMR δ 0.92 and 0.96 (2 x s, 6H, $(CH_3)_2C$), 0.99 (d, *J* = 6.36 Hz, 3H, $C\underline{H}_3CH$), 1.29 and 1.39 (2 x s, 18H, 2 x $C(CH_3)_3$), 1.81 (d, $^2J_{HP}$ = 17.43 Hz, 3H, CH_3P), 2.64 and 2.65 (2 x t, *J* = 7.16 and 6.87 Hz, 2H, CH_2CO), 3.12 (t, *J* = 6.99 Hz, 2H, CH_2S), 3.22 (dd, *J* = 13.6 and4.55 Hz, 1H, $C\underline{H}_2NH$), 3.37 (dd, *J* = 13.77 and 7.08 Hz, 1H, $C\underline{H}_2NH$), 3.58 (d, $^3J_{HP}$ = 11.74 Hz, 3H, CH_3OP), 4.43 (m, 1H, $CH_3C\underline{H}$), 6.69 (br s, 1H, NH), 6.79 (m, 2H, 4'- and 6'-H), 7.10 (m, 1H, 5"-H), 7.62-7.72 (m, 2H, 3"- and 4"-H), and 8.46 (m, 1H, 6"-H); ^{13}C NMR δ 16.76 and 21.00 ($(\underline{C}H_3)_2C$), 20.52 (d, $^1J_{CP}$ = 190.9 Hz, CH_3P), 23.10 ($\underline{C}H_3CH$), 29.71 and 31.71 (2 x $C(\underline{C}H_3)_3$), 34.18 and 34.75 (2 x $\underline{C}(CH_3)_3$), 34.37 and 35.88 (CH_2CH_2S), 38.38 (d, $^3J_{CP}$ = 8.85 Hz, $(CH_3)_2\underline{C}$), 46.70 (CH_2N), 52.06 (d, $^2J_{CP}$ = 10.46 Hz, $CH_3O\underline{P}$), 80.24 (d, $^2J_{CP}$ = 12.47 Hz, $CH_3\underline{C}H$), 104.94 (d, $^3J_{CP}$ = 10.26 Hz, 6'-C), 114.1 (4'-C), 119.9 and 120.7 (3"- and 5"-C), 132.4 (d, $^3J_{CP}$ = 9.15 Hz, 3'-C), 137.0 (4"-C), 139.1 (5'-C), 143.8 (1'-C), 145.0 (2'-C), 149.6 (6"-C), 159.9 (2"-C), and 170.6 (C=O); ^{31}P NMR δ -20.39; IR (film) 3301 (NH), 2959, 2870, 1650 (amide C=O), 1447, 1420, 1302, 1076, 994, 760, and 718 cm^{-1}.

Preparation of (3,5-di-*tert*-butyl-1,2-phenylenedioxy)[(*S*)-3,3-dimethyl-4-(*N*-3,5-dinitrobenzoyl-*N*-methyl)amino-2-butoxy](methoxy)(methyl)-phosphorane (2)

A solution of the phosphine 4 (569 mg, 3.21 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a solution of 3,5-di-*tert*-butyl-1,2-benzoquinone (707 mg, 3.21 mmol) in dry CH_2Cl_2 (60 mL) at 0°C. The mixture was stirred at 0°C for 30 min and then stirred at room temperature for 1 h. The formation of aminophosphorane 5 was checked with TLC (hexane-

NEt$_3$, 9 : 1; R$_f$ = 0.9). The crystals of the alcohol **7** (1.04 g, 3.21 mmol) and 1*H*-tetrazole (22.5 mg, 0.32 mmol) were added to the solution at 0°C. The mixture was stirred at 0°C for 2 h, then at room temperature for 2 h. The reaction mixture was evaporated and the residual oil was purified by flash column chromatography (hexane-CH$_2$Cl$_2$-NEt$_3$, 5 : 1 : 0.6) to give a diastereomeric mixture of the phosphorane **2** as an amorphous solid (foam): 1.80 g (91 %); ^{31}P NMR -20.70 (50%) and -20.18 (50%) ppm.

The diastereomer 2a was isolated from the mixture by fractional crystallization as follows: the diastereomeric mixture (1.55 g) was dissolved in a mixture of dry hexane-NEt$_3$ (9 : 1, 15 mL) at room temperature. The solution was allowed to stand at room temperature overnight to afford **2a** as colorless fine crystals: 577 mg (63%); mp 124.5 - 127.5°C (subject to epimerization), $[\alpha]^{25}_{D}$ +9.4° (*c* 1.0, CH$_2$Cl$_2$); ^{1}H NMR δ 0.96 and 1.02 (2 x s, 6H, (CH$_3$)$_2$C), 1.25 and 1.40 (2 x s, 18H, 2 x C(CH$_3$)$_3$), 1.45 (d, J = 6.30 Hz, 3H, C$\underline{H}_3$CH), 1.88 (d, $^2J_{HP}$ = 17.48 Hz, 3H, CH$_3$P), 2.84 (s, 3H, NCH$_3$), 3.33 (d, J = 13.7 Hz, 1H, CH$_2$N), 3.68 (d, $^3J_{HP}$ = 11.73 Hz, 3H, CH$_3$O-P), 3.78 (d, J = 13.7 Hz, 1H, CH$_2$N), 4.29 (m, 1H, CH), 6.79 (d, J = 4.24 Hz, 2H, 4'- and 6'-H), 8.51 (d, J = 2.0 Hz, 2H, 2"- and 6"-H), 9.07 (d, J = 2.0 Hz, 1H, 4"-H); ^{13}C NMR δ 16.64 (($\underline{C}$H$_3$)$_2$C), 21.43 (d, $^1J_{CP}$ = 190.8 Hz, CH$_3$P), 21.98 (($\underline{C}$H$_3$)$_2$C), 23.06 ($\underline{C}$H$_3$CH), 29.68 and 31.66 (2 x C($\underline{C}$H$_3$)$_3$), 34.15 and 34.70 (2 x $\underline{C}$(CH$_3$)$_3$), 41.13 (NCH$_3$), 41.30 (d, $^3J_{CP}$ = 6.35 Hz, (CH$_3$)$_2$$\underline{C}$), 52.60 (d, $^2J_{CP}$ = 11.03 Hz, CH$_3$OP), 55.15 (CH$_2$N), 79.87 (d, $^2J_{CP}$ = 11.76 Hz, CH-OP), 105.0 (d, $^3J_{CP}$ = 11.26 Hz, 6'-C), 114.3 (4'-C), 119.4 (4"-C), 127.1 (2"- and 6"-C), 132.1 (d, $^3J_{CP}$ = 9.96 Hz, 3'-C), 139.8 (5'-C), 140.3 (1"-C), 143.2 (1'-C), 144.8 (2'-C), 148.5 (3"- and 5"-C), and 167.6 (C=O); ^{31}P NMR δ -20.70; MS (FAB) m/z (relative intensity) 621 ([M]$^+$, 8.6%), 299 (100); IR (film) 3106, 2963, 1649 (C=O), 1547 (NO$_2$), 1485, 1426, 1345 (NO$_2$), 1304, 1078, 994, 855, 722, and 691 cm^{-1}. The mothor liquor of the crystallization of **2a** was concentrated and the residual oil was purified by flash column chromatography (hexane-CH$_2$Cl$_2$-NEt$_3$, 9 : 1 : 1) to give the phosphorane **2b** as an amorphous solid (foam):

255 mg (28%), $[\alpha]^{25}_{D}$ +2.94° (c 1.02, CH_2Cl_2); ^1H NMR δ 1.12 (s, 6H, $(CH_3)_2C$), 1.25-1.32 (m, 3H, $C\underline{H}_3CH$), 1.29 and 1.40 (2 x s, 18H, 2 x $C(CH_3)_3$), 1.85 (d, $^2J_{HP}$ = 17.60 Hz, 3H, CH_3P), 3.13 (s, 3H, NCH_3), 3.59 (d, $^3J_{HP}$ = 11.78 Hz, 3H, CH_3OP), 3.62 and 3.75 (2 x d, J = 13.5 Hz, 2H, CH_2N), 4.53 (m, 1H, CH), 6.80 (d, J = 5.80 Hz, 2H, 4'- and 6'-H), 8.62 (d, J = 2.0 Hz, 2H, 2"- and 6"-H), and 9.10 (d, J = 2.0 Hz, 1H, 4"-H); ^{13}C NMR 16.64 ($(\underline{C}H_3)_2C$), 20.88 (d, $^1J_{CP}$ = 193.0 Hz, CH_3P), 22.02 ($(\underline{C}H_3)_2C$), 23.26 ($\underline{C}H_3CH$), 29.69 and 31.69 (2 x $C(\underline{C}H_3)_3$), 34.16 and 34.71 (2 x $\underline{C}(CH_3)_3$), 41.28 (d, $^3J_{CP}$ = 8.93 Hz, $(CH_3)_2\underline{C}$), 41.51 (NCH_3), 51.88 (d, $^2J_{CP}$ = 10.56 Hz, CH_3OP), 55.43 (CH_2N), 79.51 (d, $^2J_{CP}$ = 12.36 Hz, CH-OP), 105.0 (d, $^3J_{CP}$ = 10.76 Hz, 6'-C), 114.1 (4'-C), 119.4 (4"-C), 127.2 (2"- and 6"-C), 132.2 (d, $^3J_{CP}$ = 10.06 Hz, 3'-C), 139.6 (5'-C), 140.4 [illegible]C), 143.3 (1'-C), 144.9 (2'-C), 148.5 (3"- and 5"-C), and 167.7 (C=O); ^{31}P NMR δ -20.17; IR (film) 3105, 2963, 1649 (C=O), 1547 (NO_2), 1487, 1428, 1345 (NO_2), 1306, 1078 (P-O), 994 (P-O), 903, 855, 723 and 693 cm^{-1}.

Preparation of phosphonates 8a and 8b from (2)

A diastereomeric mixture of the phosphorane **2** (270 mg, 0.43 mmol) was dissolved in $CHCl_3$ (20 mL) and two drops of 1.2N HCl was added to the solution. The mixture was stirred vigorously at room temperature for 30 min. The reaction was monitored with TLC (hexane-CH_2Cl_2-NEt_3, 7 : 1 : 0.8) and found to be completed in 30 min. The reaction mixture was neutralized by adding $NaHCO_3$ powder and filtered. The filtrate was dried over anhydrous Na_2SO_4 and evaporated to yield a yellow oil. The residual oil was purified by flash column chromatography on silica gel (hexane-AcOEt, 1 : 1) to give diastereomeric **8a** and **8b** as yellow amorphous solid (foam).

Phosphonate (8a)

Yield 118 mg; ^{1}H NMR δ 1.10 (br s, 6H, $(CH_3)_2C$), 1.19 (d, J = 6.53 Hz, 3H, $C\underline{H}_3CH$), 1.27 and 1.39 (2 x s, 18H, 2 x $C(CH_3)_3$), 1.76 (d, $^2J_{HP}$ = 17.67 Hz, 3H, CH_3P), 3.10 (s, 3H, NCH_3), 3.59 and 3.65 (2 x d, J = 13.8 Hz, 2H, CH_2N), 4.59 (m, 1H, CH), 6.93 and 7.12 (2 x s, 2H, 4'- and 6'-H), 7.93 (s, 1H, OH), 8.6 (m, 2H, 2"- and 6"-H), and 9.1 (m, 1H, 4"-H); ^{13}C NMR 10.85 (d, $^1J_{CP}$ = 145.2 Hz, CH_3P), 16.55 and 21.83 ($(\underline{C}H_3)_2C$), 23.06 ($\underline{C}H_3CH$), 29.55 and 31.44 (2 x $C(\underline{C}H_3)_3$), 34.28 and 35.37 (2 x $\underline{C}(CH_3)_3$), 40.97 (d, $^3J_{CP}$ = 6.46 Hz, $(CH_3)_2\underline{C}$), 41.47 (NCH_3), 55.26 (CH_2N), 80.44 (d, $^2J_{CP}$ = 8.17 Hz, CH-OP), 116.4 (d, $^4J_{CP}$ = ca. 4 Hz, 6'-C), 119.5 (4'-C), 120.6 (4"-C), 127.2 (2"- and 6"-C), 138.8 (d, $^3J_{CP}$ = 9.36 Hz, 3'-C), 139.3 (5'-C), 140.1 (1"-C), 142.4 (1'-C), 144.2 (d, $^2J_{CP}$ = ca. 3 Hz, 2'-C), 148.5 (3"- and 5"-C), and 167.8 (C=O); ^{31}P NMR δ +33.85; MS (CI) m/z (relative intensity) 608 ($[M+1]^+$, 22%), 308 (100), and 283 (8.2); IR (film) ca. 3200 (br, OH), 3106, 2963, 1649 (C=O), 1547 (NO_2), 1491, 1345 (NO_2), 1314, 1252, 984, 914 and 731 cm^{-1}.

Phosphonate (8b)

Yield 91mg, $[\alpha]^{25}_D$ +4.80° (c 1.00, CH_2Cl_2); ^{1}H NMR δ 1.01 and 1.02 (2 x s, 6H, $(CH_3)_2C$), 1.26 and 1.35 (2 x s, 18H, 2 x $C(CH_3)_3$), 1.47 (d, J = 6.4 Hz, 3H, $C\underline{H}_3CH$), 1.73 (d, $^2J_{HP}$ = 17.50 Hz, 3H, CH_3P), 2.91 (s, 3H, NCH_3), 3.45 and 3.51 (2 x d, J = 13.8 Hz, 2H, CH_2N), 4.61 (dq, $^3J_{HP}$ = 6.64 and J = 6.6 Hz, 1H, CH), 6.97 and 7.10 (2 x s, 2H, 4'- and 6'-H), 7.97 (s, 1H, OH), 8.52 (m, 2H, 2"- and 6"-H) and 9.05 (m, 1H, 4"-H); ^{13}C NMR δ 11.94 (d, $^1J_{CP}$ = 142.7 Hz, CH_3P), 16.79 and 21.00 ($(\underline{C}H_3)_2C$), 22.89 ($\underline{C}H_3CH$), 29.52 and 31.44 (2 x $C(\underline{C}H_3)_3$), 34.29 and 35.34 (2 x $\underline{C}(CH_3)_3$), 41.00 (d, $^3J_{CP}$ = 6.07 Hz, $(CH_3)_2\underline{C}$), 41.25 (NCH_3), 55.14 (CH_2N), 80.45 (d, $^2J_{CP}$ = 7.40 Hz, CH), 116.4 (d, $^4J_{CP}$ = 3.32 Hz, 6'-C), 119.5 (4'-C), 120.1 (4"-C), 127.2 (2"- and 6"-C), 138.7 (d, $^3J_{CP}$ = 9.26 Hz, 3'-C), 139.1 (5'-C), 140.0 (1"-C), 142.4 (1'-C), 144.0 (d, $^2J_{CP}$ = 3.42 Hz, 2'-C), 148.4 (3"- and 5"-C), and 167.9 (C=O); ^{31}P NMR δ +32.41; MS (CI) m/z (relative intensity) 608 ($[M+1]^+$, 12%), 308 (100) and 283 (20); IR (film) ca. 3200 (br, OH), 3106, 2961, 1649 (C=O), 1547 (NO_2), 1491, 1360, 1345 (NO_2), 1312, 1254, 988, and 914 cm^{-1}.

Single-crystal X-ray diffraction analysis of (2a)

Single crystals of **2a** were obtained by recrystallization from hexane-AcOEt-NEt_3, 3 : 1 : 0.4. $C_{30}H_{44}N_3O_9P$, F.W. = 621.7, monoclinic space group $P2_1$, $a = 6.277(2)$, $b = 9.487(5)$, $c = 28.006(7)$ Å, $\beta = 95.65(2)^\circ$, $V = 1659.5(1.0)$ Å^3, $Z = 2$, $\rho_{calc} = 1.244$ mg mm^{-3}, λ (CuKα) = 1.54184 Å, $\mu = 1.165$ mm^{-1}, $F(000) = 664$, $T = 295^\circ$K. A clear colorless 0.03 x 0.20 x 0.40 mm crystal, in the shape of a triangular wedge, was used for data collection on an automated Siemens R3m/V diffractometer equipped with an incident beam monochromator. Lattice parameters were determined from 25 centered reflections within $26 \leq 2\theta \leq 49^\circ$. The data collection range of hkl was: $0 \leq h \leq 6$, $0 \leq k \leq 10$, $-26 \leq l \leq 25$, with $[(\sin\theta)/\lambda]_{max} = 0.531$. Three standards, monitored after every 97 reflections, exhibited random variations with devs. up to ±4.0% during the data collection. A set of 2298 reflections was collected in the $\theta/2\theta$ scan mode, with scan width $[2\theta(K_{\alpha1}) - 0.8]$ to $[2\theta(K_{\alpha2}) + 0.8]^\circ$ and ω scan rate (a function of count rate) from 5.0°/min. to 20.0°/min. There were 2011 unique reflections, and 1565 were observed with $F_O > 3\sigma(F_O)$. The structure was solved and refined with the aid of the SHELXTL system of programs [Sheldrick, G.M. (1980). *SHELXTL 80. An Integrated System for Solving, Refining, and Displaying Crystal Structures from Diffraction*]. The full-matrix least-squares refinement varied 389 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms, and one common isotropic thermal parameters for the hydrogen atoms. H atoms were included but not refined, using a riding model [coordinate shifts of C applied to attached H atoms]. The C-H distances were set to 0.96 Å, and the H angles idealized to 109.5 or 120°, as appropriate. Final residuals were $R = 0.065$ and $wR = 0.058$ with final difference map excursions of 0.23 and -0.28 eÅ^{-3}.

Epimerization of phosphorane (2a)

A solution of the phosphorane **2a** in dry $CDCl_3$ (15 mg in 0.6 mL) was placed in NMR sample tubes and sealed. The tubes were heated at 89.5°C by inserting them into a silicon oil bath equilibrated at 90.0°C. After a certain period of time, each sample tube was taken out and cooled immediately with icewater. The concentration of the diastereomer **2b** which was formed by epimerization was determined by ^{31}P NMR at room temperature. The concentration of **2b** at equilibrium was determined after heating the samples for 3 h at 89.5°C to allow the reaction to reach the equilibrium. The equilibrium constant

$(K_e = [\mathbf{2b}]_e / ([\mathbf{2a}]_e + [\mathbf{2b}]_e))$ at 89.5°C was found to be 49.9 ± 0.86 %.

Table. Epimerization of phosphrane at 89.5°C[a]

t (min)	[**2b**] (%)	$[\mathbf{2b}]_e - [\mathbf{2b}]$ (%)	$[\mathbf{2b}]_e/[\mathbf{2a}]_0 \ln([\mathbf{2b}]_e/([\mathbf{2b}]_e-[\mathbf{2b}]))$
1	5.4	44.5	0.057
2	6.2	43.7	0.066
4	13.1	36.8	0.015
8	17.0	32.9	0.208
12	22.7	27.2	0.302
16	27.0	22.9	0.389
22	33.9	16.0	0.568
34	39.5	10.4	0.783
65	47.9	2.0	1.611

[a] Concentration is given in relative value (%). $[\mathbf{2b}]_e$ is the concentration of **2b** at equilibrium, 49.9 (%). $[\mathbf{2a}]_0$ is the initial concentration of **2a**, 100%

CHAPTER IV: SYNTHESIS OF ACID-STABLE PENTACOORDINATED PHOSPHORUS COMPOUND AS HAPTENS FOR PRODUCTIONS OF CATALYTIC ANTIBODIES

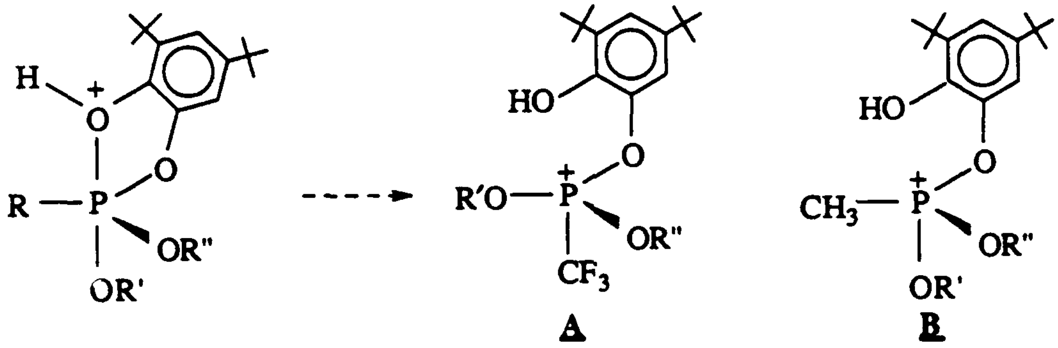
A. Introduction

Last year we successfully developed a novel synthetic route to a class of pentacoordinated phosphorus compounds (phosphoranes) with five different substituents bound to phosphorus. (All the phosphoranes we synthesized were found to be hydrolytically stable in natural to basic aqueous media, but they were extremely sensitive to aqueous acid.) We then applied this synthesis to a crystalline diastereomer. The absolute configuration of this crystalline stereoisomer has been determined by X-ray diffraction analysis; the coordination around phosphorus is quite close to perfectly regular trigonal bipyramid.

This year, we emphasized our research on design and synthesizing more stable haptens for raising catalytic antibodies. These include the following two aspects: (i) pentacoordinate phosphorus compounds with trifluoromethyl groups bound to the phosphorus center, and (ii) the bifunctional (also named "bait and switch") compounds. This chapter and the following chapter will report in detail how the two strategies actually worked.

One of the most challenging tasks in the catalytic antibody field is to synthesize water-stable transition state analogs of phosphoryl transfer reactions.[1a-c] For a long time pentacoordinated phosphorus compounds attracted attention as models for the intermediates or transition states in nonenzymatic and enzymatic phosphoryl-transfer reactions.[2] Recently we described the development of a novel synthetic method for unsymmetrically substituted monocyclic oxyphosphoranes[1a] and the application of this synthesis to single stereoisomers, including a crystalline trigonal bipyramid structure.[1b] All the phosphoranes synthesized were stable in neutral and in basic aqueous media but showed a common instability toward aqueous acidic conditions. One approach to synthesize water-stable phosphoranes under all pH range is to introduce a trifluoromethyl group directly attached to phosphorus. This expectation is based upon (i) the relative instability of intermediate A compared to B (Scheme I) which was proposed as the first step in acid conversion of a phosphorane to a phosphonate;[1b] (ii) the CF_3 preferentially occupies the axial position which makes other alkoxy groups difficult to leave.[3]

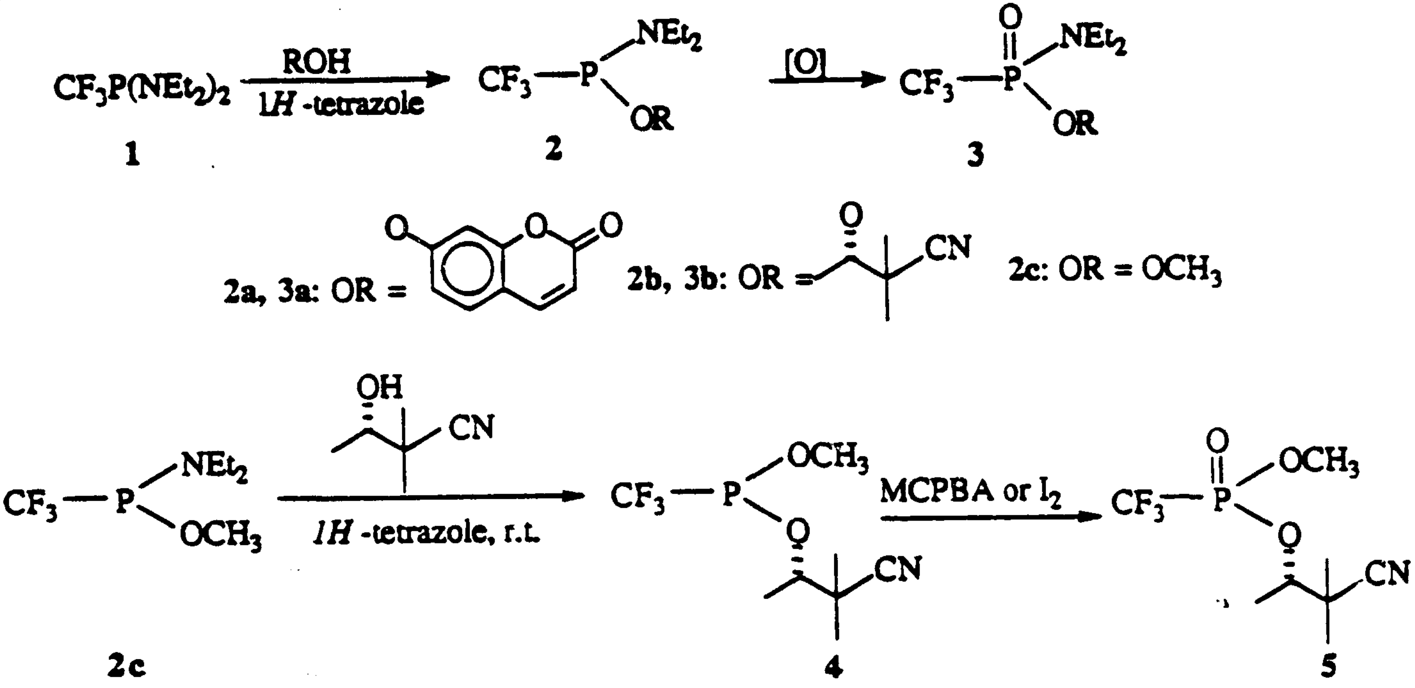
Scheme I



Up to date, the syntheses of most trifluoromethyl phosphorus species were limited to CF_3PI_2 as the common starting material[4a-c] and no general synthetic method is known.[5] CF_3PI_2 had to be synthesized from high-temperature (220°C), high-pressure (autoclave) reactions of CF_3I (gas), elemental phosphorus and iodine and subsequent isolation via repeat vacuum line fractionation.

B. Synthesis and Discussion

We now report the facile syntheses of trifluoromethylated phosphonamidites, phosphonites, phosphonamidates, phosphonates and phosphoranes with good yield at 0°C to room temperature, under ambient pressure and without involving autoclave or vacuum line. Each class of compounds outlined above may be synthesized by a "one-pot" reaction. Trifluoromethylphosphonamidites **2a-c** were easily obtained from bis(diethylamino)trifluoromethylphosphine **1**[6] on treatment of 1 equivalent mole of the corresponding alcohol in the presence of 1 equivalent 1*H*-tetrazole at 0°C to room temperature (**Scheme II**).

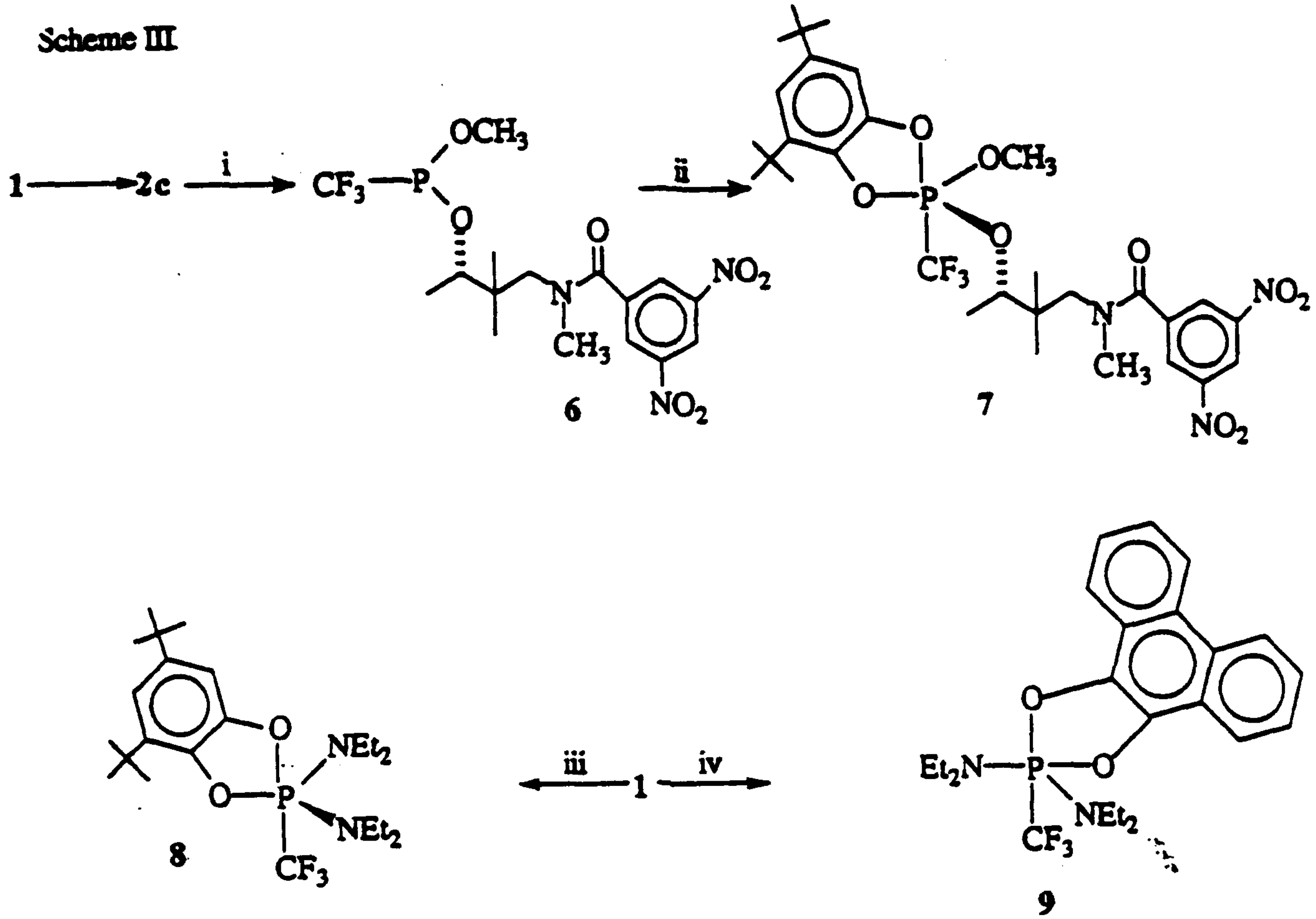


The rate of the reactions seemed to depend on steric bulkiness of the alcohols used. The smaller the alcohol, the faster the reaction. Phosphonamidite **2c** was prepared in good yield in ca. 30 min., while **2b** was synthesized in 6 hours. Aromatic alcohol reacted faster than any aliphatic one as evidenced by the fast **2a** formation (in ca. 10 min). Phosphonamidite **2a** was a colorless crystalline compound which was stable in air. **2a** and **2b** gave correct elemental analysis or high resolution MS data and were characterized by ^1H, ^{13}C, ^{31}P NMR, IR and MS. Further oxidation of **2a** and **2b** with MCPBA or I_2 gave trifluoromethylphosphonamidates **3a,b** in quantitative ^{31}P NMR yield (93 and 78% isolated yields, respectively). Interestingly, the two diastereomers of **3b** can be separated easily by flash-chromatography on silica gel to give two pure forms of the novel optically active trifluoromethylphosphonamidites.

diesters of trifluoromethylphosphonites which were the precursors of the targeted phosphoranes. When the crude reaction mixture of **2c** from phosphine **1** was treated with one equivalent of (*S*)-3-hydroxy-2,2-dimethylbutanenitrile and one equivalent of 1*H*-tetrazole, the displacement of the diethylamino group of **2c** by alcohol underwent smoothly to give phosphonite **4** with high ^{31}P NMR yield (Scheme II). Without isolation[7] phsophonite **4** was oxidized with I_2 or MCPBA to yield the unsymmetrically substituted diester of trifluoromethylpohosphonate 5 (75% isolated yield) which belonged to a class of reported anticholinergic agents[8] that were prepared through a series of tough reactions that involved autoclave, vacuum line fractionation, gaseous chlorine and heating.[9] It is important to note that the two diethylamino groups in phosphine **1** were displaced by alcohols in a controllable stepwise manner, in contrast to its CH_3-phosphonamidite analogs in which case a random substitution of both amino and alkoxy groups occurred to produce a mixture of three possible dialkoxy methylphosphines.[10] This is probably because the highly electronegative CF_3 group withdraws electron density from the phosphorus atom to reduce the reactivity of the phosphonamidite toward acid-catalyzed substitution on phosphorus, thereby increasing the selectivity.

The novel trifluoromethylphosphoranes having five different substituents around phosphorus were synthesized by oxidative addition of *ortho*-quinones to the unsymmetrically substituted diesters of trifluoromethyl-phosphines. In the one pot reaction starting from **1** (Scheme III), the diester of trifluoromethylphosphonite **6** (a mixture of two diastereomers) was synthesized in a stepwise manner described above. Without isolation, 3,5-di-*tert*-butyl-1,2-benzoquinone was added to the reaction mixture to give a diastereomeric mixture of trifluoromethylphosphoranes **7(a,b)**. Similarly, symmetric analogs of trifluoromethylphosphoranes **8** and **9** were prepared in 83 and 90% isolated yield, respectively.

Scheme III



a = Reagents and conditions:
(i) *1H*-tetrazole (1 equiv.) and (S)-4-(N-3,5-dinitrobenzoyl-N-methyl)amino-3,3-dimethyl-2-butanol; (ii) 3,5-di-*tert* -butyl-1,2-benzoquinone
(iii) 3,5-di-*tert* -butyl-1,2-benzoquinone,(1.1 equiv.); (iv) phenanthrenequinone (1.1 equiv.)

Phosphoranes **7, 8, 9** were stable on silica gel and were isolated into pure forms chromatographically without preneutralizing silica gel with triethylamine as required in the isolation of the corresponding methylphosphoranes.[1a-b] Hydrolytic stability of 7 examined by ^{31}P NMR in the presence of acidic, neutral and basic aqueous solutions showed that, as we expected, trifluoromethylphosphorane 7 was stable in all pH range for at least 12 h, even in acidic condition, while the corresponding methylphosphoranes decomposed instantaneously.[1a-b]

Diastereomeric mixture of phosphorane **7** showed two spots on TLC and were carefully separated into two stereoisomers by silica gel chromatography (hexane-EtOAc, 5:1). The optical rotation data of the resulted optically active phosphoranes **7a** and **7b** are shown in Table I.

TABLE I: yields, ^{31}P NMR and specific rotation data

Compound	Yield[a] %	δ (^{31}P)[c] ppm	Coupling const. J_{PCF} (Hz)	optical rotation $\alpha^{22.5}_D$(°)
1	56	75.3	(q) 92.0	
2a	80	109.5	(q) 92.1	
2b	85	112.0	(q) 89.6	
		113.6	(q) 87.5	
2c	95[b]	117.5	(q) 84.7	
3a	93	2.67	(q) 118.7	
3b	78	4.92	(q) 113.9	-9.6[d] (diastereomer A)
		4.80	(q) 113.4	-2.1[e] (diastereomer B)
4	80[b]	134.6	(q) 80.0	
		136.9	(q) 83.2	
5	75	-1.15	(q) 126.9	
		-1.09	(q) 127.9	
6	64[b]	137.1	(q) 74.8	
		139.0	(q) 80.5	
7	47	-56.53	(br.q) 97.5	-18.1[f] (diastereomer **7a**)
		-56.53	(br.q) 97.5	-3.5[g] (diastereomer **7b**)
8	85	-31.7	(q) 63.4	
9	90	-28.0	(q) 64.3	

a. Isolated yields unless indicated. b. ^{31}P NMR yields. Two δ values indicate two diastereomers. c. Solvent is $CDCl_3$. Chemical shifts downfield of the reference (85% H_3PO_4 as an external standard) are indicated as positive. d. $c = 1.2$, $CHCl_3$. e. $c = 1.09$, $CHCl_3$; f. $c = 0.84$, $CHCl_3$; g. $c = 1.02$, $CHCl_3$.

The configuration of the crystalline phosphorane 9 was determined by X-ray diffraction analysis (Scheme IV). The distance and angles involving the phosphorus atom are listed in Table II.

Scheme IV:

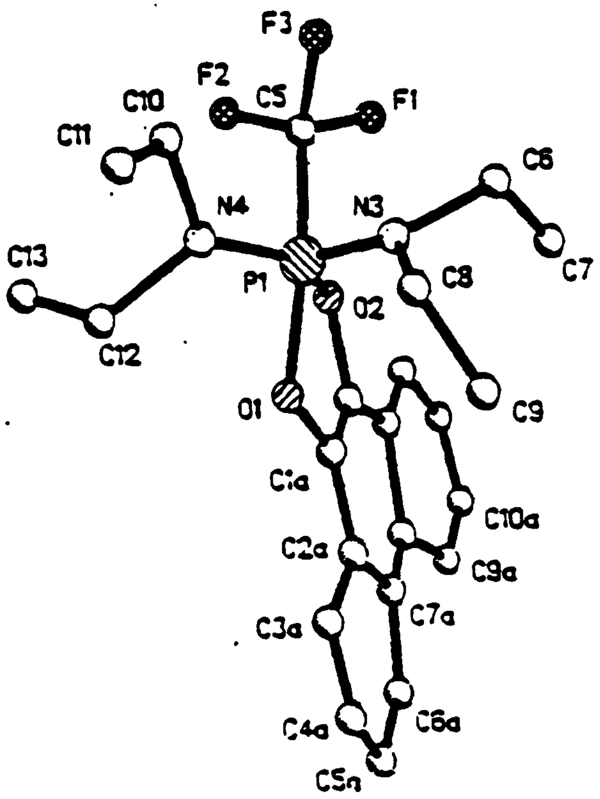


TABLE II: Phosphorus bond lengths (Å) and angles (deg)

P(1) - O(1)	1.760 (3)	P(1) - N(3)	1.627 (4)
P(1) - O(2)	1.652 (3)	P(1) - N (4)	1.652 (4)
P(1) -C(5)	1.976 (6)		
O(1) - P(1) - O(2)	88.5 (2)	O(1) - P(1) - N (3)	94.0 (2)
O(2) - P(1) - N(3)	118.5 (2)	O(1) - P(1) - N (4)	89.0 (2)
O(2) -P(1) - N(4)	126.7 (2)	N(3) - P(1) - N (4)	114.8 (2)
O(1) -P(1) - C(5)	167.7 (2)	O(2) -P(1) - C(5)	79.8 (2)
N(3) - P(1) - C(5)	95.0 (2)	N(4) - P(1) - C(5)	94.8 (2)

It seems reasonable to expect that trifluoromethylphosphoranes would be transition state inhibitors for some phosphoryl-transfer enzymes, due to their trigonal bipyramid configurations around phosphorus.[11]

C. References

1. (a) Moriarty, R. M.; Hiratake, J.; Liu, K. *J. Am. Chem. Soc.* **1990**, *112*, 8575-8577.
   (b) Moriarty, R. M.; Hiratake, J.; Liu, K.; Wendler, A.; Awathi, A. K.; Gilardi, R. *J. Am. Chem. Soc.* **1991**, *113*, 9374-9376.
   (c) Chen, J. L.; Janda, K. *J. Am. Chem. Soc.* **1992**, *114*, 2257-2258.
2. (a) Westheimer, F.H. *Acc. Chem. Res.* **1968**, *1*, 70-78. (b) Buchwald, S.L.; Pliura, D.H.; Knowles, J.R. *J. Am. Chem. Soc.* **1984**, *106*, 4916-4922. (c) Knowles, J.R. *Ann. Rev. Biochem.* **1980**, *49*, 877-919. (d) van Ool, P.J.J.M.; Buck, H.M. *Recl. Trav. Chim. Pays-Bas* **1984**, *103*, 119-122. (e) Holmes, R.R.; "Pentacoordinated Phosphorus"; American Chemical Society: Washington, D. C., **1980**, vol. II, p. 87 and references cited therein. f) Taira, K.; Lai, K.; Gorenstein, D. G. *Tetrahedron* **1986**, *48*, No. 1, 229-238.
3. Holmes, R. R. *Pentacoordinated Phosphorus*; American Chemical Society: Washington, DC, 1980; Vol. I.
4. (a) Bennett, Emeleus, and Haszeldine. *J. Chem. Soc.* **1953**, 1565-1571. (b) Burg, A.; Mahler, W.; Bilbo, A.; Haber, C.; and Herring, D. *J. Am. Chem. Soc.* **1956**, 247.
   (c) Cavell, R. G.; Pirakitigoon, S.; and Griend, L. V. *Inorg. Chem.* **1983**, *22*, 1378-1389.
5. Burton, D. J.; Flynn, R. M. *Synthesis* **1979**, 615.
6. Volbach, W.; Ruppert, I. *Tet. Lett.* **1983**, 5509-5512.
7. Phosphonite **4** was extremely sensitive to oxidation.
8. Brestkin, A. P.; Golovanov, A. V.; Lavrent'ev, A. I.; Maslennikov, I. G.; Yanson, N. A. *Ukr. Biokhim. Zh.* **1988**, *60(6)*, 38-42.
9. Maslennikov, I. G.; Lavrent'ev, A. N.; Lyubimova, M. V.; Shvedova, Y. I.; Lebedev, V. B. *Zh. Obshch. Khim.* **1983**, *53*, No. 12, 2681-2684.
10. Thesis of Kaiming Liu, 1992, Univ. of Illinois at Chicago.
11. Robert, G. C. K.; Dennis, E. A.; Meadows, D. H.; Cohen, J. S.; and Jardetky, O. *Proc. Natl. Acad. Sci. USA* **1969**, *62*, 1151.

D. Experimental

Preparation of bis(diethylamino)trifluoromethylphosphine (1)

A modified procedure of Volbach and Ruppert[5] was used. Phosphorus trichloride (2.77 g, 20.2 mmol) was added dropwise with stirring to hexaethylphosphorus triamide (25 g, 101 mmol) in a 100 mL Pyrex pressure tube under argon at 0°C. Stirring was continued for 20 min at 0°C and 30 min at room temperature. Then bromotrifluoromethane (9.8 g, 66 mmol) was charged into this pressure tube immersed in liquid N_2. The tube was sealed with a teflon screw stopper and placed behind a safety shield. The mixture was stirred at room temperature overnight (a light green clear solution resulted). Excess CF_3Br was evaporated and the product was extracted with dry petroleum ether (5 x 20 mL). The solvent was removed under reduced pressure and the product was distilled under an argon atmosphere (b.p. 40-45°C/1.6mmHg) to yield **1** as colorless liquid(15.3 g, 62%); ^1H NMR δ 3.15(m, 4H, 2 x $C\underline{H}_2CH_3$), 1.05 (t, J=7.10 Hz, 6H, 2 x $CH_2C\underline{H}_3$); ^{31}P NMR δ 75.3 (q, J_{PCF}=92.0 Hz); ^{13}C NMR δ 14.5 (s, 2 x CH_3), 44.0 (d, J_{CNP}= 19.52 Hz, 2 x CH_2), 129.74 (dq, J_{CF}= 326.50 Hz, J_{CP}= 128.12 Hz, CF_3).

Preparation of 7-coumaryloxy(diethylamino)(trifluoromethyl)phosphine (2a)

A solution of phosphine (0.91 g, 3.7 mmol) in dry CH_3CN (10 mL) was added to a cloudy suspension of 7-hydroxycoumarin (0.60 g, 3.7 mmol) in dry CH_3CN (10 mL) with vigorous stirring at room temperature under an argon atmosphere. Then *1H*-tetrazole (0.26 g, 3.7 mmol) was added in one portion. The reaction was completed (96%) in 5 min as evidenced by ^{31}P NMR. A mixture of hexane and EtOAc (6:1) was added to precipitate out the tetrazole salt. The reaction mixture was filtered and the solvent was removed under reduced pressure. The resulting solid was recrystallized from hexane-EtOAc (3:2) to give **2a** as a colorless crystalline product; mp 88.2-88.9°C; ^1H NMR δ 1.13 (t, J= 7.13 Hz, 6H, 2 x CH_3), 3.28

(m, 4H, 2 x CH_2), 6.42 (d, J = 9.58 Hz, 1H, 2'-H), 7.23 (m, 2H, 6'- and 8'-H), 7.50 (d, J =8.43 Hz, 1H, 5'-H) and 7.69 (d, J = 9.52 Hz, 1H, 3'-H); ^{31}P NMR δ 109.5 (q, J_{PCF} = 92.1 Hz); ^{13}C NMR δ 127.1 (dq, J_{CF}= 324.23 Hz, J_{CP}= 24.14 Hz, CF_3), 42.2 (d, J_{CNP}=19.52 Hz, CH_2), 14.5 (CH_3), and 7-hydroxycoumarin carbons: 106.9 (s), 107.0 (s), 114.6 (d, J=11.27 Hz), 116.4 (d, J=8.75 Hz), 129.0 (s), 143 (s), 155.2 (s), 158.1 (d, J=10.86 Hz), 160.5 (s, C=O); MS(CI) m/z (relative intensity): 334 ($[M+1]^+$, 100%); IR (KBr) 2982, 1732, 1615, 1503, 1399, 1385, 1273, 1233, 1211, 1178, 1154, 1113, 1024, 990, 860 and 835cm^{-1}. Anal. calcd for $C_{14}H_{15}F_3NO_3P$: C, 50.46; H, 4.54; N, 4.16. Found: C, 50.65; H, 4.46; N, 4.16.

Preparation of (S)-3-cyano-3-methyl-2-butoxy(diethylamino) trifluoro-methyl)phosphine (2b)

A solution of 3-hydroxy-2,2-dimethylbutanenitrile (2.36 g, 9.7 mmol) in dry CH_2Cl_2 (5 mL) was added to a solution of bis(diethylamino) trifluoromethylphosphinel (2.37 g, 9.70 mmol) in dry CH_2Cl_2 (15 mL)at 0°C under an argon atmosphere. Then *1H*-tetrazole (0.68 g, 9.7 mmol) was added in one portion. The mixture was stirred at 0°C for 20 min, and stirring was continued at room temperature. The conversion reached 95% after 1h as monitored with ^{31}P NMR. The solvent was then removed under reduced pressure and dry Et_2O was added to precipitate out the tetrazole salt. After filtration, Et_2O was evaporated under reduced pressure to afford a diastereomeric mixture of **2b** in 95% yield (pure by ^1H and ^{31}P NMR). If further purification is desired, the product can be distilled by a short path distillation apparatus to afford the product as a colorless liquid: 2.4 g, 85% yield; bp 60-65°C/0.25 mmHg. ^1H NMR δ 1.2 [t, 6H, J=7.12 Hz ($\underline{H}$... $(C\underline{H}_3CH_2)_2N$], 1.3-1.4 (m, 9H, 3x$C\underline{H}_3$), 3.2 (m, 2H, NCH_2), 3.9 (m, 1H, $C\underline{H}CH_3$); ^{31}P NMR δ 111.96 (q, J_{PCF}=89.56 Hz, diastereomer A) and 113.55 (q, J_{PCF}=87.48 Hz, diastereomer B); IR (film) 2984, 2142, 2240(CN),1383(P=O), 1280, 1230, 1201(C-F), 1184, 1113, 1075, 1030, 1011, 986, 941, 785, and 554 cm^{-1}.

Preparation of diethylamino(methoxy)(trifluoromethyl)phosphine (2c), (S)-3-cyano-3-methyl-2-butoxy(methoxy)(trifluoromethyl)phosphine (4), S)-3-cyano-3-methyl-2-butyl methyl trifluoromethylphosphonate (5)

Dry methanol (0.29 g, 8.9 mmol) was added to a solution of **1** (2.2 g, 8.9 mmol) in dry CH_2Cl_2 (10 mL) with stirring at 0°C under an argon atmosphere. Then *1H*-tetrazole was added to the reaction mixture in one portion. The mixture was stirred at 0°C for 20 min, then stirring was continued at room temperature. The reaction was complete in 30 min to give **2c** in ca 95% yield: ^{31}P NMR δ 117.5 (q, J_{PCF}=84.70 Hz). The phosphonamidite **2c** was not very stable in the air and was pure enough for further transformations. Thus a solution of (*S*)-3-hydroxy-2,2-dimethylbutanenitrile (1 g, 8.9 mmol) in dry CH_2Cl_2 (3 mL) was added to the crude solution of **2c** (1.8 g, 8.9 mmol) in dry CH_2Cl_2 at room temperature under an argon atmosphere. *1H*-tetrazole (0.63 g, 8.9 mmol) was added in one portion. The reaction was complete after 12 h at room temperature to yield the phosphonite **4**: ^{31}P NMR: δ 134.6 (q, J_{PCF}=80.76 Hz, diastereomer A), and 136.9 (q, J_{PCF}=83.19 Hz, diastereomer B). The yield was ca. 80% (^{31}P NMR). The phosphonite **4** is very sensitive to air and need not be isolated for our purpose. Thus,a solution of *m*-chloroperbenzoic acid (MCPBA) (2.2 g, 12.8 mmol) in dry CH_2Cl_2 (10 mL) was added to this crude solution of **4** (ca.1.7 g, 7.1 mmol) in dry CH_2Cl_2 at room temperature. The reaction was complete in ca. 5 min as evidenced by ^{31}P NMR. The reaction mixture was diluted with CH_2Cl_2 (200 mL) and the solution was washed successively with Na_2SO_3, sat. $NaHCO_3$, sat. NaCl and dried over $MgSO_4$. After filtration, the filtrate was evaporated and the residual liquid was purified by column chromatography (hexane-EtOAc, 3:1, R_f=0.35) to give phosphonate **5** as a colorless liquid: 1.7 g, 75% yield. ^{1}H NMR δ 1.40 and 1.42 (2 x s, 6H, $(CH_3)_2C$), 1.54 and 1.62 (2 x d, $J_{\text{diastereomer B}}$=6.33 Hz, $J_{\text{diastereomer A}}$=6.31 Hz, 3H, $C\underline{H}_3CH$), 4.00 and 4.02 (2 x d, $J_{\text{diastereomer B}}$=6.33 Hz, $J_{\text{HCOP(diastereomer A)}}$=10.92 Hz, 3H, CH_3O), 4.55 and 4.65 (2 x m, 1H, $C\underline{H}CH_3$); ^{31}P NMR δ -1.15 (q, J_{PCF}=126.85 Hz, diastereomer A) and -1.09 (q, J_{PCF}=127.92 Hz, diastereomer B); IR (film) 3488,. 2990, 2240(CN), 1462, 1389, 1300, 1237, 1144, 1069, 1017, and 826 cm^{-1}. MS(CI) *m/z* (relative intensity) 260 ($[M+1]^+$, 100%).

Preparation of 7-coumaryloxy diethyl(trifluoromethyl)phosphonamidate (3a)

A solution of MCPBA (216 mg, 1.3 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a solution of **2a** (208 mg; 0.62 mmol) in dry CH_2Cl_2 (10 mL) at room temperature. After 10 min, TLC showed the complete disappearance of starting material. The reaction mixture was diluted with CH_2Cl_2 (150 mL) and the solution was washed successively with 20% Na_2SO_3, sat. $NaHCO_3$, brine and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield **3a** as a colorless solid: 202 mg, 93% yield. The product was pure enough (^1H NMR). Analytical sample was purified by recrystallization from hexane-EtOAc, (5:1) to give colorless crystalline product: mp 102.0-102.5°C; ^1H NMR δ 1.17 (t, *J*=7.14 Hz, 6H, 2 x CH_3), 3.31 (m, 4H, CH_2), 6.41(d, J=9.57Hz, 1-H, 2'-H), 7.25 (m, 2H, 6'- and 8'-H), 7.49 (d, *J*=8.37Hz, 1H, 5'-H), and 7.68 (d, *J*=9.57Hz, 1H, 3'-H); ^{31}P NMR δ 2.67 (q, J_{PCF}= 118.73 Hz); MS (CI) *m/z* (relative intensity) 350 ($[M+1]^+$, 100%); IR (KBr) 1742 (vs C=O),1617, 1402, 1279 (s, P=O), 1266, 1228, 1200, 1127, (vs, C-F), 994, 970, 910, and 864 cm^{-1}. Anal. calcd for $C_{14}H_{15}F_3NO_4P$: C, 48.14; H, 4.33; N, 4.02; F, 16.32; P, 8.87. Found: C, 48.05; H, 4.34; N, 4.05; F, 16.08; P, 8.93.

Preparation of (S)-3-cyano-3-methyl-2-butyl diethyl(trifluoromethyl) phosphonamidate (3b)

A solution of MCPBA (2.5 g, 14.6 mmol) in dry CH_2Cl_2 (10 mL) was added to a solution of **2b** (2.07 g, 7.3 mmol) in dry CH_2Cl_2 (20 mL) at room temperature under an argon atmosphere. The reaction was completed in 10 min as monitored by ^{31}P NMR (100% yield). The reaction mixture was diluted with CH_2Cl_2 (300mL) and the solution was washed successively with Na_2SO_3, sat. $NaHCO_3$, sat. NaCl and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure to give a diastereomeric mixture of **3b** as a colorless liquid. The crude product was purified by column chromatography (hexane-EtOAc, 2:1, Rf= 0.47) to give **3b**: 2.0 g 78% yield. The two diastereomers (A and B) were separated by column chromatography (hexane-EtOAC, 2:1 R_f(A) = 0.50; R_f(B) = 0.41). Diastereomer A (oil) (upper spot on TLC): optical rotations (*c* 1.2, $CHCl_3$) $[\alpha]^{22.5}_{D}$ - 9.6°, $[\alpha]^{22.5}_{578}$ - 10.3°, $[\alpha]^{22.5}_{546}$ -10.6°, $[\alpha]^{22.5}_{436}$ -17.0°, and $[\alpha]^{22.5}_{365}$ -24.3°C); ^{1}H NMR δ 1.18 (t, *J*=7.13 Hz, 6H, $N(CH_2C\underline{H}_3)_2$), 1.46 and 1.38 (2 x s, 6H, $C(CH_3)_2$), 1.53 (d, *J*=6.35 Hz, 3H, $CHC\underline{H}_3$), 3.25 (m, 4H, 2 x CH_2), and 4.60 (m, 1H, $C\underline{H}CH_3$); ^{13}C NMR δ 14.01 [s, $N(CH_2\underline{C}H_3)_2$], 17.39 (s, $\underline{C}H_3CH$), 23.14 and 23.94 [2 x s, $C(\underline{C}H_3)_2$], 38.70 [d, $^{3}J_{CP}$=4.92 Hz, $\underline{C}(CH_3)_2$], 39.31 [d, J_{CNP}=4.10 Hz, $N(\underline{C}H_2CH_3)_2$], 79.34 (d, J_{COP}=6.31 Hz, $\underline{C}HCH_3$), 122.14 (s, CN) and 122.65 (dq, J_{CP}=252.46 Hz, J_{CF}=313.12 Hz, CF_3); ^{31}P NMR δ 4.92 (q, J_{PCF}=113.98 Hz); MS(CI)*m/z* 301 ([M+1]$^{+}$, 100%); IR (film) 2241 (w, C≡N), 1388 (m, P=O), 1281, 1202 (vs, C-F), 1169 (m, C-F), 1129 (vs, C-F), 1034, 1013, and 984 cm^{-1}. HRMS(EI) calcd for $C_{11}H_{20}F_3N_2O_2P$ (M^{+}), 300,1215. Found: 300.1208.

Diastereomer B (oil) (lower spot on TLC): optical rotations (*c* 1.09, $CHCl_3$) $[\alpha]^{22.5}_{D}$ - 2.1°, $[\alpha]^{22.5}_{578}$ - 2.8°, $[\alpha]^{22.5}_{546}$-2.5°; $[\alpha]^{22.5}_{436}$ - 6.0°, and $[\alpha]^{22.5}_{365}$ - 11.6°: ^{1}H NMR δ 1.15 [t, *J*=7.12 Hz, 3H, $N(CH_2C\underline{H}_3)_2$], 1.35 and 1.43 [2 x s, 6H, $C(C\underline{H}_3)_2$], 1.58 (d, *J*=6.50 Hz, 3H, $CHC\underline{H}_3$), 3.22 (m, 4H, CH_2), and 4.49 (m, 1H, $C\underline{H}CH_3$). ^{13}C NMR δ 14.01 [s, $N(CH_2\underline{C}H_3)_2$], 18.74 (s, $\underline{C}H_3CH$), 23.43 and 23.71 [2 x s, $C(\underline{C}H_3)_2$], 38.67 [d, $^{3}J_{CP}$=8.85 Hz, $\underline{C}(CH_3)_2$], 39.46 [d, J_{CNP}=4.83 Hz, $N(\underline{C}H_2HC_3)_2$], 79.45 (d, J_{COP}=6.24 Hz, $\underline{C}HCH_3$), 121.92 (s, CN), and 121.62 (dq, J_{CP}=253.51 Hz, J_{CF}=313.23 Hz, CF_3); ^{31}P NMR δ 4.80 (q, J_{PCF}=113.44 Hz); MS(CI) *m/z* 301 ([M+1]$^{+}$, 100%); IR (film) 2241 (w, C≡N), 1388 (m, P=O), 1281, 1202 (vs, C-F), 1169 (m, C-F) and 1129 (vs, C-F) cm^{-1}.

Preparation of (3,5-di-tert-butyl-1,2-phenylenedioxy)[bis (diethylamino)] (trifluoromethyl)phosphorane (6)

3,5-di-*tert*-Butyl-1,2-benzoquinone (332 mg, 1.7 mmol) was added to a solution of 1 (424 mg, 1.7 mmol) in dry CH_2Cl_2 (10 mL) in one portion at room temperature under an argon atmosphere. Stirring was continued at room temperature overnight to yield **6** (92% by ^{31}P NMR). The solvent was removed under reduced pressure and the product was isolated by column chromatography (hexane-EtOAc, 10:1, R_f=0.9) as a sticky oil: 684 mg, 85% yield. ^1H NMR δ 1.06 (t, J=7.02 Hz, 12H, 4 x CH_3), 1.30 and 1.39 [2 x s, 18H, 2 x $C(CH_3)_3$], 3.10 (m, 8H, 4 x CH_2), and 6.75 (m, 2H, aromatic protons). ^{13}C NMR δ 13.76 [d, $^3J_{CP}$=2.3 Hz, 2 x $(CH_2\underline{C}H_3)$], 29.79 and 32.01 (2 x s, 2 x $C(\underline{C}H_3)_3$), 34.29 and 35.06 [2 x s, 2 x $(CH_3)_3\underline{C}$], 44.08 (d, J_{CNP}=19.32 Hz, 2 x $\underline{C}H_2$), 104.9 (d, J_{COP}=12.47 Hz, 6'-C), 113.6 (s, 4'-C), 129.1 (dq, J_{CP}=207.2 Hz, J_{CF}=336.8 Hz, CF_3), 132.9 (d $^3J_{CP}$=10.97 Hz, 3'-C), 138.2 (s, 5'-C), 144 (s, 1'-C), and 145.8 (s, 2'-C); ^{31}P NMR δ -31.70 (q, J_{PCF}=63.35 Hz); MS(CI) *m/z* (relative intensity) 465 ($[M+1]^+$, 82%), 392 (100%); IR (film) 2967, 2874, 1485, 1470, 1427, 1383, 1362, 1239, 1204 (C-F), 1161 (C-F), 1086, 1022, 999 (C-F) and 857 cm^{-1}.

[(S)-4-[N-(3,5-dinitrobenzoyl)-N-methyl]amino-3,3-dimethyl-2-butoxy(methoxy)-(trifluoromethyl)phosphine (7) and 3,5-di-*tert*-1,2-phenylenedioxy[(S)-3,3-dimethyl-4-{N-(3,5-dinitrobenzoyl)-N-methyl}-amino-2-butoxy] (methoxy) trifluoromethylphosphorane (8a,b)

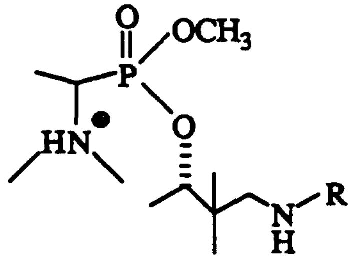
Dry methanol (38 mg, 1.17 mmol) was added to a solution of **1** (287 mg, 1.17 mmol) in dry CH_2Cl_2 (10 mL) at 0°C under an argon atmosphere. Then *1H*-tetrazole (82 mg, 1.17 mmol) was added in one portion at 0°C under argon. The reaction mixture was stirred for 20 min at 0°C and then 30 min at room temperature. To this reaction mixture was then added a solution of (*S*)-4-[*N*-(3,5-dinitrobenzoyl)-*N*-methyl]amino-3,3-dimethyl-2-butanol (382 mg; 1.17 mmol) in dry CH_2Cl_2 (5 mL) under argon, and *1H*-tetrazole (83 mg; 1.17 mmol) was added at room temperature. The reaction was complete (^{31}P NMR) after stirring for 24 h to give compound **7** in 64% yield: ^{31}P NMR δ 137.1 (J_{PCF}=74.79 Hz, diastereomer A) and 139.0 (J_{PCF}=80.51 Hz, diasteroemer B). Then 3,5-di-*tert*-butyl-1,2-benzoquinone (258 mg, 1.17

mmol) was added to this crude reaction mixture in one portion at room temperature under an argon atmosphere. The reaction mixture was stirred at room temperature for 3 h to give phosphorane **8** with 55% yield as detected by ^{31}P NMR. Two close spots were shown on thin layer chromatography (TLC) for the two diastereomers. The product was purified and the two diastereomers were separated by silica gel column chromatography (hexane-EtOAc, 5:1, R_f(A)=0.39, R_f(B)=0.33) to give **8** in an overall (diastereomer **a** + **b**) isolated yield of 47% (511 mg). Diastereomer **8a** (oil), 85 mg: optical rotations (*c*, 0.84, $CHCl_3$) $[\alpha]^{22.5}_D$,-18.1°; $[\alpha]^{22.5}_{578}$, -18.7°; $[\alpha]^{22.5}_{546}$, -21.5°; $[\alpha]^{22.5}_{436}$, -37.9°; ^1H NMR δ 0.97 and 1.03 [2 x s, 6H, $(CH_3)_2C$], 1.28 and 1.41 [2 x s, 18H, 2 x $C(CH_3)_3$], 1.52 (d, *J*=6.31 Hz, 3H, $C\underline{H}_3CH$), 2.89 (s, 3H, NCH_3), 3.35 (d, *J*=13.74 Hz, 1H, CH_2N), 3.73 (d, *J*=15.09 Hz, 1H, CH_2N), 3.83 (d, $^3J_{HP}$=14.72 Hz, 3H, CH_3OP), 4.29 (m, 1H, CH), 6.94 (m, 2H, 4'- and 6'-H), 8.51 (br s, 2H, 2"-and 6"-H), and 9.07 (br s, 1H, 4"-H); ^{31}P NMR δ 56.53 (q, J_{PCF}=97.53 Hz). MS (CI) *m/z* (relative intensity) 674.4 ($[M-1]^+$, 0.4%), 308.1 (100); IR (film) 2963, 1651 (s, C=O), 1547 (s, NO_2), 1487, 1422, 1362, 1345, (s, NO_2), 1221, 1123 (s, C-F), 1063, 1015, 997, 868 and 588 cm^{-1}. Anal. calcd for $C_{30}H_{41}F_3N_3O_9P$: C, 53.33; H, 6.12; N, 6.22; F, 8.44; P, 4.58. Found: C, 53.61; H, 6.46; N, 6.46; F, 8.22; P, 4.78. Diastereomer **8b** (oil), 22 mg: optical rotations (*c* 1.02, $CHCl_3$) $[\alpha]^{22.5}_D$, -3.5°; $[\alpha]^{22.5}_{578}$, -3.3°; $[\alpha]^{22.5}_{546}$, -3.7°; and $[\alpha]^{22.5}_{436}$, -8.5°; ^1H NMR δ 1.13 [br s, 6H, $(CH_3)_2C$], 1.32 and 1.41 [2 x s, 18H, 2 x $C(CH_3)_3$], 1.34 (d, overlaped, ca. 3H, $C\underline{H}_3CH$), 3.13 (s, 3H, NCH_3), 3.61 (d, *J*=13.64 Hz, 1H, CH_2N), 3.68 (d, *J*=14.88 Hz, 1H, CH_2N), 3.81 (d, $^3J_{HP}$=14.65 Hz, 3H, CH_3OP), 4.58 (m, 1H, CH), 6.95 (m, 2H, 4'-and 6'-H), 8.62 (br s, 2H, 2"-and 6"-H), and 9.11 (br s, 1H, 4"-H); ^{31}P NMR δ ca. 56.53 (q, J_{PCF}=97.53 Hz); IR (film) 2963, 1651 (s, C=O), 1547 (s, NO_2), 1487, 1423, 1362, 1345, (s, NO_2), 1221, 1123 (s, C-F), 1063, 1015, 997, 986, 868, and 588 cm^{-1}. Anal. calcd for $C_{30}H_{41}F_3N_3O_9P$: C, 53.33; H, 6.12; N, 6.22. Found: C, 53.78; H, 6.28; N, 6.47.

CHAPTER V: SYNTHESIS OF BIFUNCTIONAL "BAIT AND SWITCH" COMPOUNDS AS HAPTENS FOR PRODUCTION OF CATALYTIC ANTIBODIES

A. Introduction

α-Aminophosphonates possess antibacterial activity and their inhibition to alanine racemases has been investigated.[1] In the past three decades, a variety of symmetrically (two identical alkoxyl groups) substituted α-aminophosphonates have been synthesized.[2,3,4] However, no general synthetic method exists for unsymmetrically substituted α-aminophosphonates, especially those with liable moieties. The author reports here a general synthetic pathway to unsymmetrically substituted α-aminophosphonates **1(a-c)**. These phosphonates serve as potential "bait and switch"[5] haptens for generation of monoclonal antibodies[6,7] catalyzing phosphoryl-transfer reactions.[8,9] The α-amino group in compound **1**, under physiological pH, becomes protonated, which would complimentarily induce the formation of a negatively charged amino acid moiety (in the antibody binding site) which would serve as a general base catalyst for the phosphoryl center of substrates. The secondary ester part of **1** mimics the cholinergic agent Soman and has the necessary linker group for protein conjugation. The extra α-methyl group would accommodate a water molecule in the antibody binding site to assist the hydrolysis of phosphonate substrates or phosphoryl enzyme complexes.

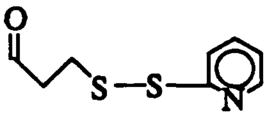


1

1a: R = 3,5 dinitrobenzoyl

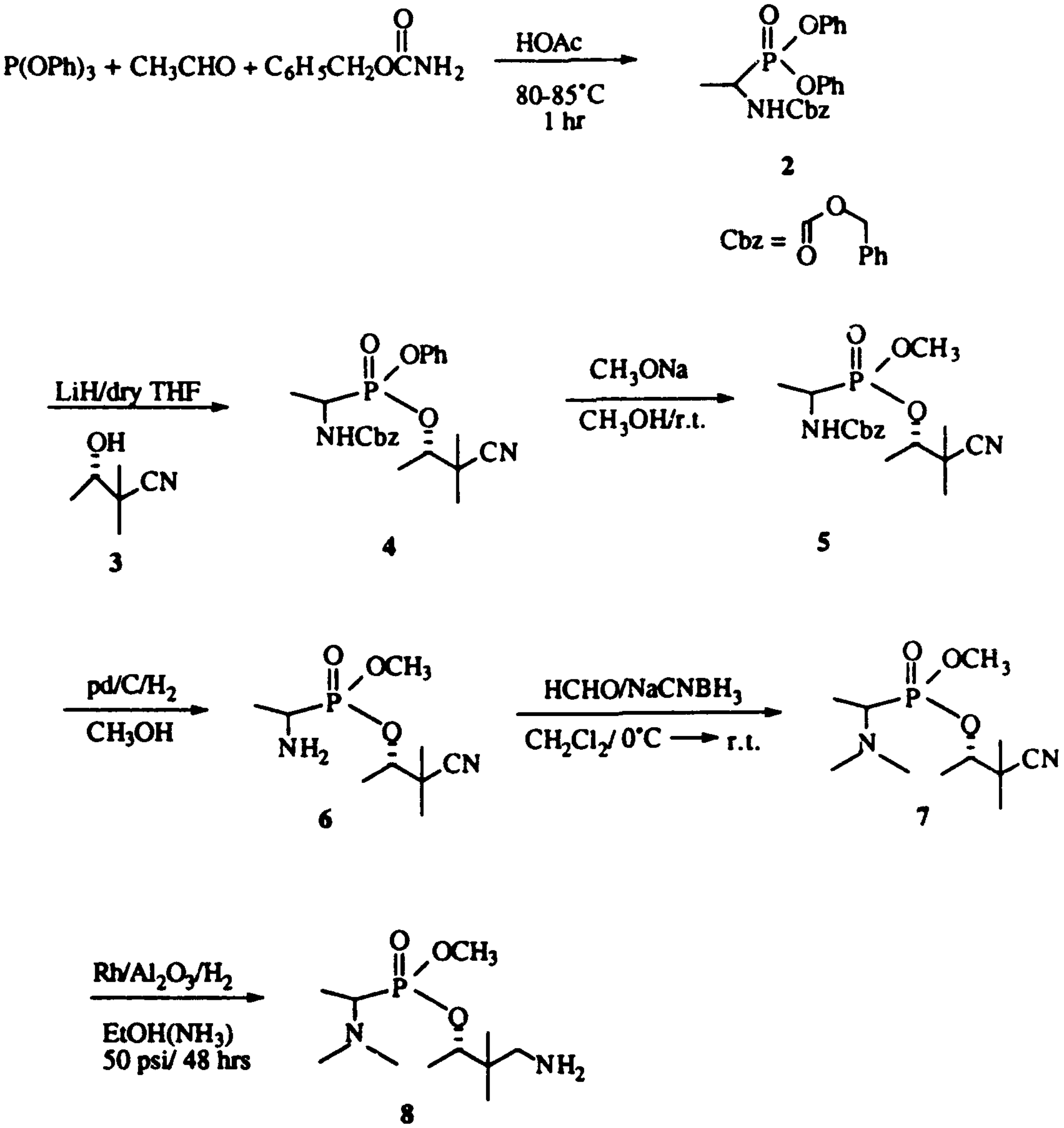
1b: R = 4-nitrobenzoyl

1c: R =

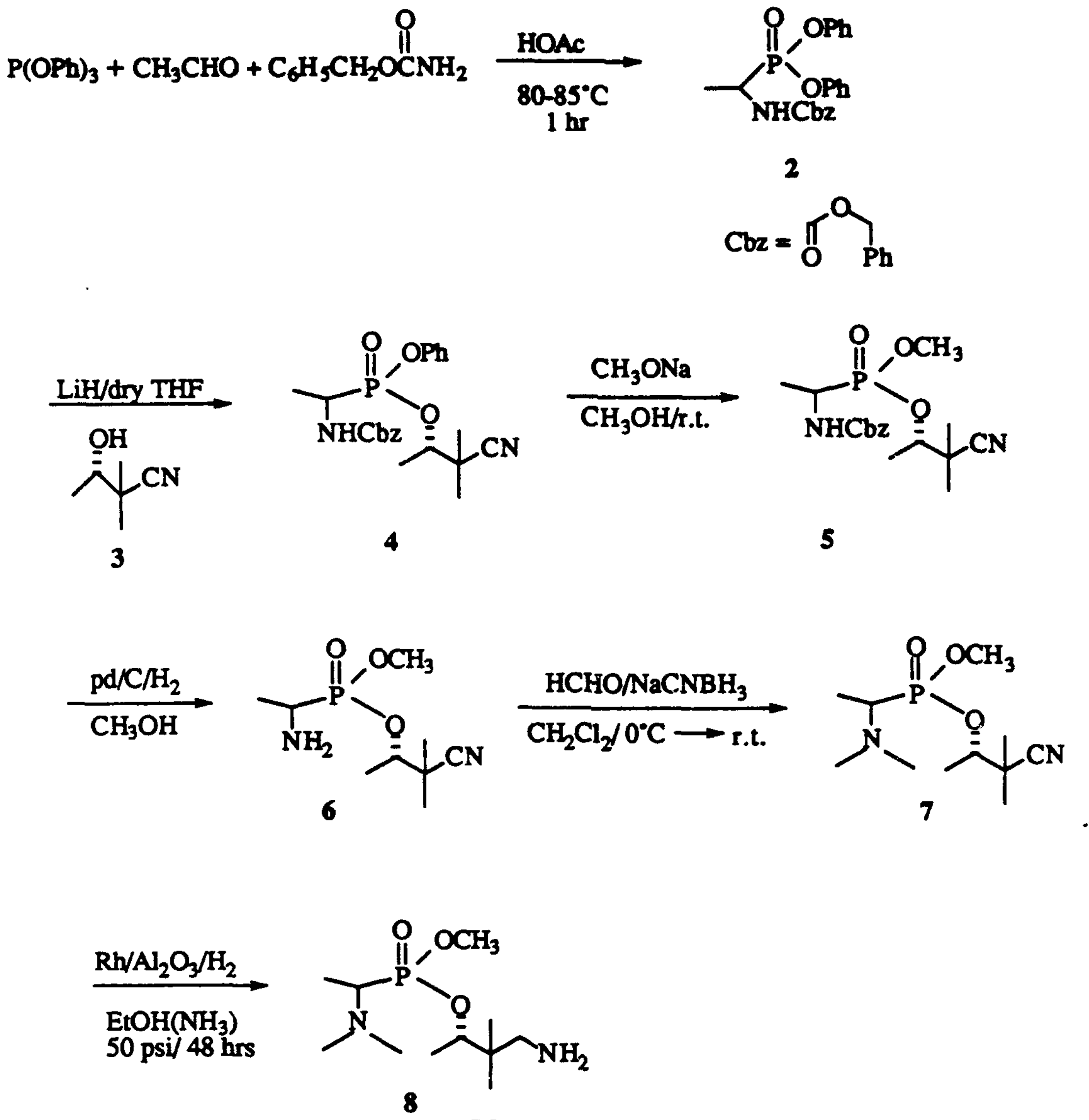


B. Results and Discussion

The synthesis started with diphenyl N-carbobenzyloxy(1-aminoethyl)phosphonate **2** derived from the reaction of acetaldehyde, benzyl carbamate and triphenylphosphite.[3] (Scheme I) Transesterification product **4** was obtained by treatment of phosphonate **2** with LiH and one equivalent of the cyanoalcohol **8** in dry THF in 43% isolated yield. Lithium ion probably acted as Lewis acid to chelate both the phosphoryl oxygen atom and the phenoxy group to assist the reaction.

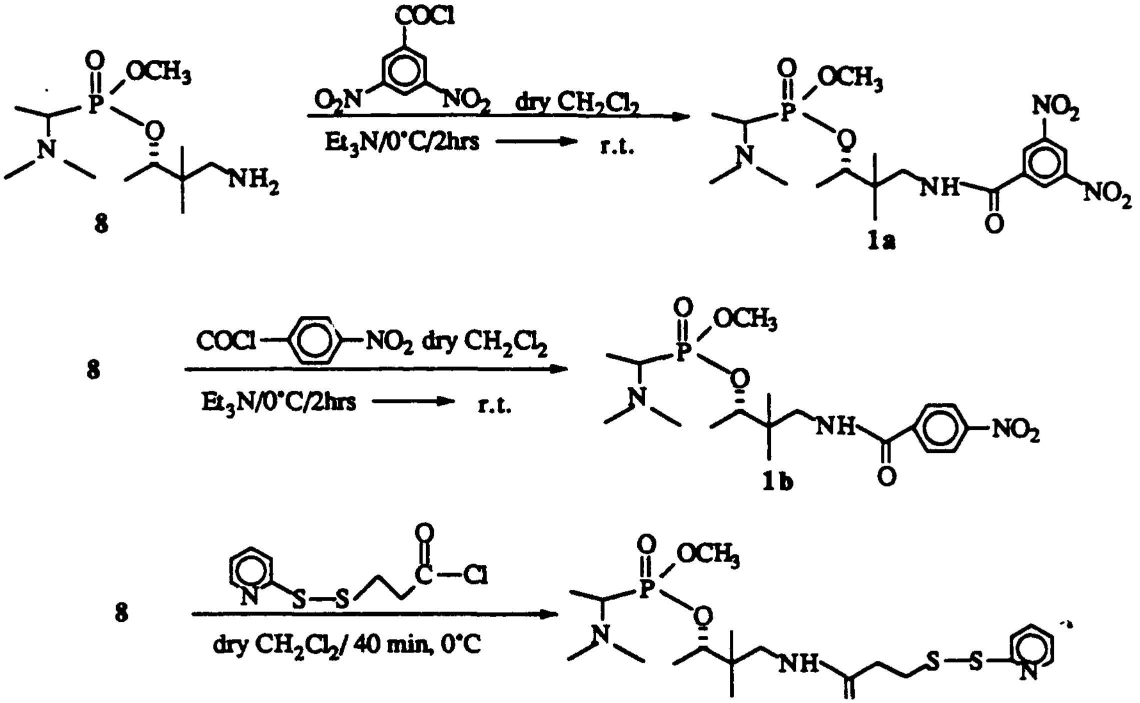


SCHEME I



SCHEME I

The displacement reaction was readily controlled stoichiometrically without the formation of the di-substituted product. The second phenoxy group was smoothly displaced by one equivalent of $NaOCH_3$ at room temperature. The reaction was clean and without the displacement of the other alkoxy substituent. The Cbz group was then selectively cleaved by hydrogenation under 1 atm pressure of hydrogen in the presence of Pd-C. The reaction proceeded neatly to afford 6 in ca. 99% yield and no reduction of the cyano group was detected (^1H NMR). Reductive amination of phosphonate 6 with formaldehyde and $NaBH_3CN$ gave the tertiary aminophosphonate **7** in 41% isolated yield after silica gel column chromatography. Rh catalyzed hydrogenation completely reduced the cyano group to give the terminal amine for attaching the necessary linker group. (Scheme II)



SCHEME II

Phosphonates **1 (a,b)** were readily obtained by treatment of intermediate **8** with the corresponding benzoyl chloride in 41% and 55% yield, respectively. Hapten **1c** having the disulfide pyridyl linker group was synthesized from **8** in 40% isolated yield by treatment of (pyridyl disulfide)propionyl chloride in the presence of triethyl amine. Phosphonates **1(a,b,c)** have three chiral centers and therefore consist of four stereoisomers. The typical ^1H NMR and ^{31}P NMR data and yields for all the phosphonates synthesized are summarized in the following.

TABLE
ISOLATED YIELD AND ^{31}P, ^{1}H NMR OF PHOSPHONATES

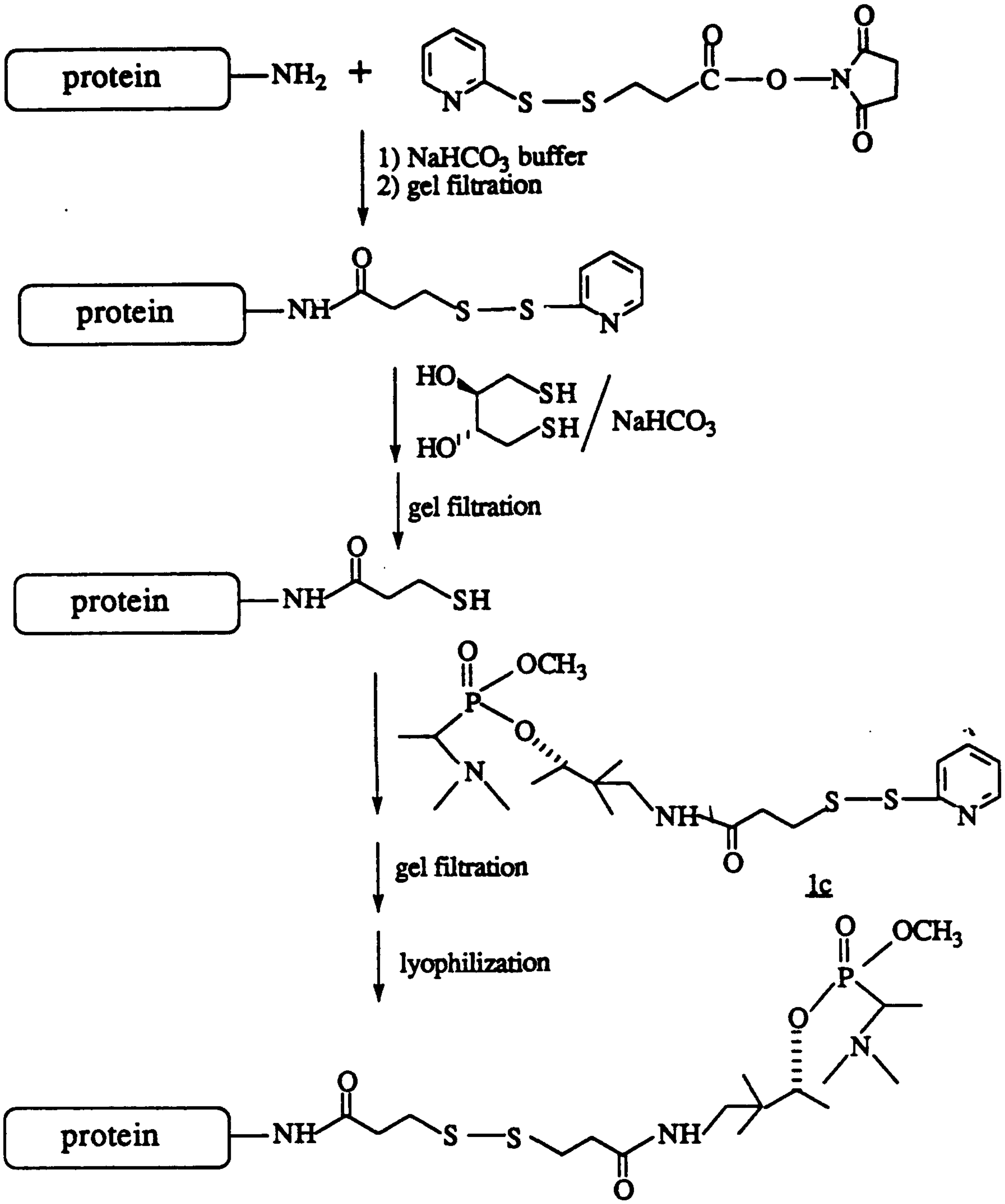
PHOSPHONATES	YIELD[a] [%]	δ^{31}P[c] [ppm]	δ^{1}H[ppm] for P-OCH$_3$[d] (coupling const., J_{HCP})
1a	41[b]	+ 27.84, + 30.04 + 32.69, + 33.57	3.78-3.99 (3 x d, J_1=10.63 Hz, J_2=10.34 Hz, J_3=10.24 Hz)
1b	55	+ 29.73, + 31.91 + 32.19	3.77-3.91 (4 x d, J_1=10.54 Hz, J_2=9.10 Hz, J_3=10.39 Hz, Hz)
1c	40	+ 29.43, + 29.79 + 30.94, + 31.12	3.73-3.88 (4 x d, J_1=10.52 Hz, Δ_2=10.50 Hz, J_3=10.33 Hz, J_4=10.26 Hz)
2	46	+ 20.89	
4	59	+ 21.77, + 22.13 + 22.39, + 22.89	
5	76	+ 26.54, + 27.02 + 27.68, + 28.67	3.75-3.81 (2 x d, J_1=16.27 Hz, J_2= 16.29 Hz)
6	99	+ 29.12, + 29.38	3.86-3.94 (4 x d, J_1=10.81 Hz, J_2=10.82 Hz, J_3=10.33 Hz,J_4=10.32 Hz)
7	47	+ 30.60, + 30.98 + 31.67, + 32.10 J_4=10.39 Hz)	3.73-3.85 (4 x d, J_1=10.60 Hz, J_2=10.37 Hz, J_3=10.44 Hz,
8	97	+ 32.95, + 33.96 + 34.64, + 35.07	3.39-3.50 (3 x d, J_1=9.94 Hz, J_2=10.41 Hz, J_3=9.94 Hz)

a. Purified by flash column chromatography on silica gel unless otherwise specified.

b. Purified by recrystallization.

c. CDCl$_3$ as solvent.

d. CDCl$_3$ as solvent.



PROTEIN: BSA, HSA, PTG

Scheme III: Protein Conjugation by N-succinimidyl 3-(2-pyridyldithio) propionate

The reaction solution containing the conjugated protein was repeatedly passed through gel filtration column (PD-10 column) to ensure the complete removal of the nonconjugated phosphorus compounds. Three kinds of protein conjugates with phosphonate **1c** were finally isolated by lyophilization. the loose protein powder showed excellent solubility in water or NaHCO3 buffer. The immunization and monoclonal antibody protocol are actively being pursued by the scientists in the Army.

D. References

1. Badet, B.; Inagaki, K.; Soda, K.; and Walsh, C.I., *Biochemistry* **1986**, *25*, 3275-82.
2. Fields, E.K., *J. Am. Chem. Soc.*, **1952**, *74*, 1528-31.
3. Oleksyszyn, J., Subotkowska, L., Wastalerz, P., *Synthesis Communications*, **1979**, 985-6.
4. Oleksyszyn, J. and Tyka, R., *Tetrahedron Letters*, **1977**, *32*, 2823-4.
5. a) Janda, K.D., Weinhouse, M.I., Danon, T., Pacelli, K.A., and Schloeder, D.M., *J. Am. Chem. Soc.*, **1991**, *113*, 5427-34.
   b) Janda, K.D., Weinhouse, M.I., Schloeder, D.M., and Lerner, R.A., *J. Am. Chem. Soc.*, **1990**, *112*, 1274-1275.
6. Shokat, K.M., Ko, M.K., Scanlan, T.S., Kochersperger, L., Yokovich, S., Thaisrivongs, S., and Schultz, P.G., *Angew. Chem. Int. Ed. Engl.*, **1990**, *29*, 1296-1303.
7. Lerner, R.A., Benkovic, S.J., and Schultz, P.G., *Science*, **1991**, *252*, 660-667.
8. Moriarty, R.M., Hiratake, J., and Liu, K., *J. Am. Chem. Soc.*, **1990**, *112*, 8575-8577.
9. Moriarty, R.M., Hiratake, J., Liu, K., Wendler, A., Awasthi, A.K., and Gilardi, R., *J. Am. Chem. Soc.*, **1991**, *113*, 9374-9376.

E. Experimental

General

Melting points were determined using a Thomas Hoover Unimelt capillary tube melting point apparatus and were uncorrected. All ^1H NMR spectra were recorded on a Bruker AM-400 spectrometer at 400 MHz. All ^{31}P NMR spectra were recorded on a Bruker WP200SY spectrometer at 81.0 MHz. Chemical shifts downfield of the reference (H_3PO_4) are indicated as positive. Abbreviations used are s (singlet), d (doublet), dd (doublet of doublets), q (quartet), and m (multiplet). $CDCl_3$ solution dried over molecular sieves 4Å was used as solvent for all NMR experiments with residual chloroform as an internal standard for ^1H NMR and ^{13}C NMR. IR spectra were measured on an IBM IR/30 and IR/32 spectrometer. Mass spectra were obtained by chemical ionization (CI) and electrical ionization (EI) technique with a Finnigan MAT 90 mass spectrometer. Unless otherwise noted, reactions were carried out in oven-dried glassware. Reagent and solvent transfers were made with oven-dried syringes and needles. Methylene chloride was distilled from calcium hydride and stored over molecular sieves 4Å. Tetrahydrofuran (THF) were distilled from sodium metal/benzophenone immediately before used. All reagents were purchased from Aldrich Chemical Co. All chromatography solvents were obtained commercially and used as received. Reactions were monitored by analytical thin-layer chromatographic (TLC) methods with the use of E. Merck silica gel 60F glass plates (0.25 mm). The products were isolated by short-path distillation under an argon atmosphere or by flash column chromatography with the use of E. Merck silica gel 60Å (230-400 mesh).

Preparation of diphenyl 1-(N-benzyloxycarbonyl)amino ethylphosphonate (2)

To a mixture of triphenyl phosphite (39.5 g, 0.13 mol), acetaldehyde (8.39 g, 0.19 mol), benzyl carbamate (19.19 g, 0.13 mol) was introduced glacial acetic acid (19.1 mL). The resulting mixture was stirred for 1.5 h until the exothermic reaction subsided. The mixture was then heated at 80-85°C for 1 h. After the reaction was completed, the resulting volatile products were removed under reduced pressure to give a brown oily residue, which was redissolved in methanol (150 mL) and left for crystallization at -10°C overnight. The white crystalline ester 2 was collected by filtration and recrystallized twice by dissolution in a minimum amount of hot chloroform and adding a 4-fold volume of methanol to yield phosphonate 2 as granular white crystals: 22.4 g (43%); mp 115-116°C (Lit. mp 115-117°C, Oleksyszyn, J., *Synthesis Communication*, 1979, 985); TLC (ethyl acetate : hexane, 1:2), single component (Rf=0.44) under UV light and after visualization with PMA (phosphomolybdic acid); ^{1}H NMR ($CDCl_3$) δ 1.55 (dd, 3H, J_{PH}=17.95 Hz, CH_3), 4.56-4.64 (m, 1H, CH), 5.14 (s, 2H, CH_2), 5.57 (d, 1H, NH, J_{HH}=9.72 Hz), and 7.10-7.35 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 16.36 (CH_3), 43.53 and 45.12 (CH), 67.52 (CH_2Ph), 120.71, 120.82, 125.53, 125.66, 128.43, 128.52, 128.82, 129.97, 130.09, 136.41, 150.41, 150.63, and 155.82 (C=O); ^{31}P NMR ($CDCl_3$) δ +18.78; IR (KBr) 3267, 3059, 1720 (C=O), 1591, 1541, 1491, 1456, 1307, 1280 (P=O), 11248, 1196, 1161, 1109, 1020, 945 cm^{-1}; MS *m/z* (relative intensity) 412 ($[M+1]^+$, 100%), 318 (10), 224 (0.9) and 14 (10).

Preparation of phenyl-(S)-α-cyano-α-methyl-butyl-(N-benzyloxycarbonyl)-α-amino ethylphosphonate (4)

To an oven-dried, argon-flushed 250 mL round-bottom flask containing lithium hydride (0.27 g, 22.2 mmol) in dry THF (100 mL) at room temperature was slowly introduced a solution of (*S*)-2,2-dimethyl-3-hydroxybutanenitrile (1.3 g, 11.1 mmol) in dry THF (50 mL) by double-ended needle. After addition was complete, the reaction mixture was refluxed for 2 h. The reaction mixture was then transferred by double-ended needle into a 1000 mL round-bottom flask containing a solution of diphenyl 1-(*N*-benzyloxycarbonyl)-α-amino ethylphosphonate 2 (4.6 g, 11.1 mmol) in dry THF (300 mL). After addition was complete, the reaction mixture was refluxed overnight under argon. Solvent (THF) was removed at reduced pressure and water (20 mL) was added. The resulting mixture was extracted with methylene chloride (4×100 mL), and the combined extracts were washed with saturated ammonium chloride (3×50 mL), water (3×50 mL), saturated sodium chloride (3×50 mL), and dried over anhydrous magnesium sulfate. Methylene chloride was removed under reduced pressure to give a light-yellow oil. Flash column chromatography on silica gel (ethyl acetate - hexane, 1:3) gave compound **4** as a colorless, viscous oil: 3.42 g (79%); TLC (ethyl acetate - hexane, 1:2), single component (Rf=0.46) under UV light and after visualization with PMA; ^{1}H MNR ($CDCl_3$) δ 1.23, 1.24, 1.28, and 1.29 (4×d, J_1=8.57 and 8.88 Hz, J_2=6.27 and 6.59 Hz, 3H, $OCH(C\underline{H}_3)_2$), 1.34 (s, 3H, $C(CH_3)$), 1.39 (s, 3H, $C(CH_3)$), 1.50-1.56 (2×d, J=17.7 and 17.7 Hz, 3H, $NCH(C\underline{H}_3)$), 4.34-4.54 (m, 1H, OCH), 5.08 (s, 2H, CH_2Ph), 5.22-5.33 (m, 1H, $NC\underline{H}(CH_3)$), 7.16-7.36 (m, 10H, aromatic protons); ^{31}P NMR ($CDCl_3$) δ +21.78, +22.14, +22.39, and +22.88, diastereomeric mixture; IR(film) 3262 (NH), 3038, 2986, 2239 (CN), 1724 (C=O), 1454, 981 (POC), 846, 806, and 767 cm^{-1}; MS (CI) *m/z* (relative intensity) 431 ($[M+1]^+$, 100%), 337 (22), 224 (0.6), and 134 (10). HRMS (FAB) calcd for $C_{22}H_{27}N_2O_5P$ 431.1735, found 431.1734.

Preparation of methyl-(S)-α-cyano-α-methyl-butyl-1-(N-benzyloxy-carbonyl)-α-amino ethylphosphonate (5)

To a solution of **4** (4.1 g, 9.6 mmol) in anhydrous methanol (50 mL) was added dropwise a solution of sodium methoxide (0.51 g, 9.6 mmol) in anhydrous methanol (200 mL) at room temperature. The reaction mixture was stirred overnight under argon. TLC analysis (ethyl acetate - hexane, 1:2) was used to monitor the disappearance of the starting material. Rotary evaporation of the resulting solution yielded a pale-yellow sticky oil which was redissolved in chloroform (200 mL), followed by washing with saturated ammonium chloride (3×50 mL), saturated sodium chloride (3×50 mL), and then dried over anhydrous magnesium sulfate. The solvent was evaporated and the residual oil was purified by flash column chromatography on silica gel (ethyl acetate - hexane, 1:4) to afford the unsymmetrical substituted phosphonate **5** as a colorless sticky oil: 2.7 g (81%); TLC (ethyl acetate : hexane, 1:2), single component (Rf=0.39) under UV light and after visualization with PMA; ^{1}H NMR (CDCl$_3$) δ 1.29 (s, 3H, C(CH$_3$)), 1.35 (s, 3H, C(CH$_3$)), 1.31, 1.38, and 1.41 (3×d, *J*=3.2, 3.53, 3.15 Hz, 3H, OCH(C$\underline{H}_3$)), 1.34, 1.45, and 1.46 (3×d, *J*=11.70, 11.54, 10.91 Hz, 3H, NH(C$\underline{H}_3$)), 3.72, 3.73, 3.77, and 3.791 (4×d, J_{HCOP}=10.5, 10.7, 10.5, 10.2 Hz, 3H, POCH$_3$), 4.18-4.30 (m, 1H, HOC$\underline{H}$), 4.34-4.4 (m, 1H, NCH), 5.11 (s, 2H, CH$_2$Ph), 5.20 (m, 1H, NH), 7.33-7.34 (m, 5H, aromatic proton); ^{31}P NMR (CDCl$_3$) δ +26.43, +26.91, +27.53, and +28.46, four diastereomeric mixture; IR(film) 3248 (NH), 2986, 2941, 2239 (CN), 1720 (C=O), 1537, 1456, 1383, 1250 (P=O), 1169, 1109, 1012, 848 (P-O), 740, and 698 cm^{-1}; MS *m/z* (relative intensity) 369 ([M+1]$^+$, 100%), 274 (8), 261 (10), 224 (5), 178 (10), and 166 (27). HRMS (FAB) calcd for $C_{17}H_{25}N_2O_5P$ 369.1560, found 369.1579.

Preparation of methyl-(S)-α-cyano-α-methyl-butyl-1-α-amino ethyl-phosphonate (6)

To an oven-dried, hydrogen-flushed, 500 mL round-bottom flask containing 10% palladium on activated carbon (ca. 0.8 g) and unsymmetrical substituted α-aminophosphonate **5** at room temperature was slowly introduced anhydrous methanol (300 mL). The resulting reaction mixture was hydrogenated overnight under atmospheric pressure. TLC analysis (ethyl acetate - hexane, 1:1) was used to monitor the disappearance of starting material. Upon completion of the reaction, the reaction mixture was filtered through Celite and washed with methanol. The methanol washing was evaporated to give white foamy solid with quantitative yield. The formation of α-amino phosphonate **6** was confirmed by IR, ^{1}H NMR and ^{31}P NMR; TLC (acetone : hexane, 1:1), single component (Rf=0.31) after visualization with PMA; ^{1}H NMR (CDCl$_3$) δ 1.36-1.51 (m, 12H, 2×CH$_3$ and OCH(C$\underline{H}_3$)$_2$, NCH(C$\underline{H}_3$)$_2$), 3.61, 3.62, 3.63, and 3.65 (m, 1H, OCH(C$\underline{H}_3$)$_2$), 3.86-3.94 (4×d, 3H, J_{HCOP}=10.57, 10.58, 10.25, 10.28

Hz, $POCH_3$), 4.49-4.51 (m, 1H, NCH), 4.60-4.79 (m, 1H, NH); ^{31}P NMR ($CDCl_3$) δ +28.23, +28.92, +29.72, +30.60, and 31.05, diastereomeric mixture; IR ($CHCl_3$) 3375 (NH_2), 3308, 2984, 2237 (CN), 1599, 1394, 1383, 1238, 1047, 864 ($POCH_3$), and 792 cm^{-1}; HRMS (FAB) calcd for $C_9H_{19}N_2O_5P$ 235.1211, found 235.1207. The α-amino phosphonate **6** was unstable and was subjected to reductive amination without further purification.

Preparation of methyl-(S)-α-cyano-α-methyl-butyl-1-(N,N-dimethyl)-α-amino ethylphosphonate (7)

To a stirred solution of α-aminophosphonate **6** (1.5 g, 6.2 mmol) and 37% aqueous formaldehyde (2.5 g, 30.8 mmol) in acetonitrile (20 mL) was added *portionwise sodium* cyanoborohydride (0.8 g, 12.3 mmol) at room temperature. A vigorous exothermic reaction ensued, and a brown residue separated. The reaction mixture was stirred for 30 min, and then glacial acetic acid was added dropwise until the solution tested neutral on wet pH paper. Stirring was continued for an additional 50 min while glacial acetic acid was being added occasionally to maintain the pH near neutrality. The reaction solvent was removed by rotary evaporator, and 2N KOH (25 mL) was added to the residue. The resulting mixture was extracted with ethyl acetate (3×25 mL). The combined extracts were washed with 0.5 N KOH (2×30 mL) and followed extraction by 1N HCl (5×20 mL). The acid extracts were combined and neutralized with solid potassium hydroxide and then extracted with ethyl acetate (3×30 mL) The combined ethyl acetate extracts were dried over potassium carbonate and evaporated *in vacuo* to give a pale-yellow sticky oil. Purification by flash column chromatography on silica gel (acetone - hexane, 1:3) gave methyl-(*S*)-α-cyano-α-methyl butyl-1-(*N,N*-dimethyl)-α-amino ethylphosphonate **7** as a colorless sticky oil: 0.9 g (74%); TLC (acetone - hexane, 1:1), single component (Rf=0.37) after visualization with PMA; ^1H NMR ($CDCl_3$) δ 1.17, 1.19, 1.22, and 1.24 (4×d, *J*=1.67, 1.82, 1.82, 1.82 Hz, 3H, $OCH(C\underline{H}_3)$), 1.28-1.37 (m, 6H,

$C(CH_3)_2$), 1.40 and 1.43 (2×d, *J*=6.37 and 6.26 Hz, 3H, $NCH(C\underline{H}_3)$), , 2.40 and 2.41 (2×s, 6H, $N(CH_3)_2$), 2.89-3.08 (m, 1H, $OC\underline{H}(CH_3)$), 3.68, 3.73, 3.76, and 3.78 (4×d, J_{HCOP}=10.69, 10.57, 10.47, 10.34 Hz, 3H, $POCH_3$), 4.34-4.47 (m, 1H, $NC\underline{H}(CH_3)$) ; ^{13}CNMR ($CDCl_3$) δ 8.34, 8.54 and 8.67 ($C(\underline{C}H_3)_2$), 17.35 and 17.74 ($OC(\underline{C}H_3)$), 22.89 and 23.13 ($NC(\underline{C}H_3)$), 38.11 ($\underline{C}(CH_3)_2$), 41.90 and 42.02 ($N\underline{C}(CH_3)$), 52.21, 53.51, 55.31 and 55.46 ($N(\underline{C}H_3)_2$), 58.37 and 58.52 ($O\underline{C}(CH_3)$), 75.25, 75.40, 75.91, 76.07, 76.47 ($POCH_3$), 122.50, 128.60, and 130.76 (CN); ^{31}P NMR ($CDCl_3$) δ +32.95, +33.96, +34.64, +35.07, diastereomeric mixture; IR (film) 3478 ($N(CH_3)$), 2945, 2876, 2833, 2239 (CN) 1462, 1338, 1240 (P=O), 1184, 1045 ($POCH_3$), 976, and 771 cm^{-1}; MS *m/z* (relative intensity) 263 ($[M+1]^+$, 100%), 247 (3). HRMS (FAB) calcd for $C_{11}H_{23}N_2O_3P$ 263.1512, found 263.1524.

Preparation of methyl-(S)-amino-1,2,2-trimethyl-propyl-1-(N,N-dimethyl) α-amino ethylphosphonate (8)

To an oven-dried, argon-flushed hydrogenation bottle containing freshed rhodium on alumina powder (250 mg) at room temperature was introduced a solution of 7 (780 mg, 3.0 mmol) in absolute ethyl alcohol (130 mL) saturated with ammonium (NH_3). The resulting reaction mixture was hydrogenated under 50 psi at room temperature for 48 h. The reaction mixture was filtered through Celite under argon and the filtrate was evaporated by rotary evaporator to afford **8** as a colorless viscous oil: 769 mg (98%); TLC (acetone - hexane, 1:1), single component (Rf=0.18) after visualization with PMA; ^{1}H NMR ($CDCl_3$) δ 0.57-1.16 (m, 12H, 2×CH_3, $NCH(C\underline{H}_3)$ and $OCH(C\underline{H}_3)$), 2.06 (s, 6H, $N(CH_3)_2$), 2.19-2.76 (m, 2H, NH_2 and 1H, $OC\underline{H}(CH_3)$), 3.40, 3.45, and 3.49 (3×d, 3H, J_{HCOP}=9.94, 10.41, 9.94 Hz, $POCH_3$), 4.10-4.21 (m, 1H, $NC\underline{H}(CH_3)$), 4.94 (s, 2H, CH_2N); ^{13}C NMR 100 MHz ($CDCl_3$) δ 7.23, 8.28 and 8.70 ($\underline{C}(CH_3)_2$), 16.34, 16.77, 16.90($NC(\underline{C}H_3)$), 19.41, 19.82, 19.99 ($NC(\underline{C}H_3)$), 21.67, 21.82, 21.98, 22.58 ($O\underline{C}(CH_3)_2$), 38.15, 38.20, 38.38, 38.43, 38.51, 38.58, 38.63

(N$\underline{C}$(CH_3)), 41.80, 42.00, 42.06 (O$\underline{C}$(CH_3)), 48.97, 49.15 (CNH_2), 52.20, 52.26, 52.50, 53.65, 53.71, 55.52, 56.08, 56.19, 57.09, 57.74 ($N(CH_3)_2$), 76.01, 76.09, 77.35, 77.67, 77.76, 77.86, 77.99 ($POCH_3$); ^{31}P NMR ($CDCl_3$) δ +33.01, +34.06, +34.69, and +35.14, diastereomeric mixture ; IR (film) 3418 (NH_2), 2968, 2876, 2799, 1574, 1452, 1394, 1234 (P=O), 1180, 1047, 974 and 877 ($POCH_3$) cm^{-1}; Compound **8** was unstable and was used without purification.

Preparation of {3,3-dimethyl-4-[3-(3,5-dinitro)benzoyl]amino-2-butoxy}(methyl) [(1-dimethyl)amino] ethylphosphonate (1a)

To an oven-dried, argon-flushed 100 mL round-bottom flask containing a solution of α-aminophosphonate **8** (0.6 g, 2.3 mmol) and distilled triethylamine (0.5 mL, 3.4 mmol) in dry methylene chloride (30 mL) was added dropwise a solution of 3,5-dinitrobenzoyl chloride (0.6 g, 2.5 mmol) in dry methylene chloride (10 mL) by double-ended needle at 0°C under argon. The reaction mixture was stirred at 0°C for 1 h, and then at room temperature for 3 hours under argon atmosphere, during which time the color of the mixture changed from yellow to purple with the formation of a precipitate. The reaction mixture was filtered and the filtrate was sequentially washed with saturated sodium bicarbonate (2×20 mL), saturated sodium chloride (2×20 mL), and was then dried over anhydrous sodium sulfate. The solvent was evaporated and the yellow residual oil was purified by flash column chromatography on silica gel (acetone - hexane, 1:4) to afford **1a** as a yellow viscous oil: 430 mg (47%); TLC (acetone - hexane, 1:1), single component (Rf=0.42) under UV light and after visualization with PMA; ^1H NMR ($CDCl_3$) δ 0.95-1.00 (m, 6H, $C(CH_3)_2$), 1.28-1.37 (m, 6H, $NC(CH_3)$ and $OC(CH_3)$), 2.43 and 2.50 (2×s, 6H, $N(CH_3)_2$), 2.99-3.21 (m, 2H, $N(CH_2)$), 3.83-3.93 (m, 1H, OCH), 3.78, 3.96, and 3.99 (3×d, J_{HCOP}=10.63, 10.35, 10.24 Hz, 3H, $POCH_3$), 4.63-4.56 (m, 1H, NCH), 8.98-9.69 (m, 3H, aromatic proton); ^{31}P NMR ($CDCl_3$) δ +27.84, +30.04, +32.69, and +33.57 diastereomeric mixture; IR (film) 3055 ($N(CH_3)_2$), 2988, 1674 (C=O), 1545, 1423, 1346, 1265 (P=O), 1174, 1116, 1076($POCH_3$), 1051, 983, 897, and 713 cm^{-1}; MS

(CI) *m/z* (relative intensity) 461 ([M+1]$^+$, 100%), 431 (5). HRMS (FAB) calcd for $C_{18}H_{29}N_4O_8P$ 461.1801; found: 461.1791.

Preparation of {3,3-dimethyl-4-[3-(4-nitro)benzoyl]amino-2-butoxy} (methyl) [(1-dimethyl)amino]ethylphosphonate (1b)

To a stirred solution of α-aminophosphonate **8** (400 mg, 1.5 mmol) and distilled triethylamine (310 μL, 2.2 mmol) in dry methylene chloride (30 mL) was introduced dropwise a solution of 4-nitrobenzoyl chloride (330 mg, 1.8 mmol) in dry methylene chloride (5 mL) by double-ended needle at 0°C under argon. After the addition was completed, the reaction mixture was stirred at 0°C for 30 min; then the reaction system was warmed up to room temperature. TLC analysis (acetone : hexane, 1:1) was used to monitor the disappearance of the starting material. The reaction mixture was diluted with methylene chloride (30 mL) and sequentially washed with saturated sodium bicarbonate (2×30 mL), saturated sodium chloride (2×30 mL), and was then dried over anhydrous sodium sulfate. The methylene chloride was removed on a rotary evaporator to give a yellow oily residue which was purified by flash column chromatography on silica gel (acetone : hexane, 1:3) to afford **1b** as a yellow sticky oil: 410 mg (55%); TLC (acetone : hexane, 1:1), single yellow-colored component (Rf=0.52) under UV light and after visualization with PMA; ^1H NMR ($CDCl_3$) δ 0.95-0.98 (m, 6H, $C(CH_3)_2$), 1.26-1.34 (m, 6H, $NC(CH_3)$ and $OC(CH_3)$), 1.65 (m, 1H, NH), 2.38-2.45 (m, 6H, $N(CH_3)_2$), 2.98-3.17 (m, 3H, NCH_2 and OCH), 3.78, 3.82, 3.85, and 3.90 (4×d, J_{HCOP}=10.54, 9.10, 10.39, 10.26 Hz, 3H, $POCH_3$), 4.43-4.56 (m, 1H, NCH), 8.17-8.23 (m, 2H, aromatic proton), 8.27-8.30 (m, 1H, aromatic proton), 8.72-9.12 (m, 1H, aromatic proton); ^{31}P NMR ($CDCl_3$) δ +29.73, +31.91 and +32.19, diastereomeric mixture; IR (film) 3294 ($N(CH_3)_2$), 2974 (NH), 1670 (C=O), 1603, 1489, 1464, 1346, 1221 (P=O), 1174, 1076 ($POCH_3$), 1053, 997, 980, 819, 756, and 713 cm^{-1}; MS (CI) *m/z* (relative intensity) 416 ([M+1]$^+$, 86%), 289 (4), 277 (11), 249 (100), and 111 (3); HRMS (FAB) calcd for $C_{18}H_{30}N_3O_6P$: 415.1872; found:

415.1874.

Preparation of {3,3-dimethyl-4-[3-(2-pyridyldithio)propionyl]amino-2-butoxyl}(methyl)[(1-dimethyl)amino]ethylphosphonate (1c)

To a solution of 3-(2-pyridyldithio)propionyl chloride (0.39 g, 1.68 mmol) in dry methylene chloride (5 mL) was added dropwise a solution of α-aminophosphonate **8** (450 mg, 1.7 mmol) and distilled triethylamine (350 μL, 2.5 mmol) in dry methylene chloride (20 mL) by double-ended needle at 0°C. Upon completion of addition, the resulting reaction mixture was stirred for 30 min at 0°C (the precipitate disappeared) and was stirred for an additional 12 hours at room temperature under argon. The reaction mixture was diluted with methylene chloride and was sequentially washed with saturated ammonium chloride (2×20 mL), saturated sodium bicarbonate (2×20 mL), saturated sodium chloride (2×20 mL) and was dried over anhydrous magnesium sulfate. The solvent was removed by rotary evaporator to yield a yellow sticky oil which was purified by flash column chromatography on silica gel (acetone - methylene chloride, 10:1) to afford **1c** as a pale-yellow viscous oil: 278 mg (59%); TLC (acetone), single component (Rf=0.5) under UV light and after visualization with PMA; ^{1}H MNR ($CDCl_3$) δ 0.68-0.72 (m, 6H, $C(CH_3)_2$), 1.03-1.32 (m, 6H, $NCH(C\underline{H}_3)$ and $OCH(C\underline{H}_3)$), 2.21 and 2.23 (2xs, 6H, $N(CH_3)_2$), 2.47 (q, J_1=6.51 Hz, J_2=10.08 Hz, 2H, SCH_2), 2.92 (q, J_1=6.38 Hz, J_2=10.42 Hz, 2H, $OCCH_2$), 2.60-2.88 (m, 2H, NCH_2), 3.26-3.41 (m, 1H, $OC\underline{H}(CH_3)$), 3.57, 3.61, 3.64, and 3.68 (4×d, J_{HCOP}=10.52, 10.58, 10.28, 10.26 Hz, 3H, $POCH_3$), 4.18-4.24 (m, 1H, $NC\underline{H}(CH_3)$), 6.89-6.93 (m, 1H), 7.45-7.49 (m, 1H,), 7.52-7.56 (m, 1H), 8.25-8.26 (m, 1H); ^{31}P NMR 80.1MHz ($CDCl_3$) δ +29.43, +29.79, +30.94, and 31.12, diastereomeric mixture; IR (film) 3302 ($N(CH_3)_2$), 2978, 2876, 1672 (C=O), 1446 (SS), 1417, 1394, 1369, 1225 (P=O), 1145, 1047 ($POCH_3$), 976, 819, 756, and 719 cm^{-1}; MS (CI) *m/z* (relative intensity) 464 ($[M+1]^+$, 100%); HRMS (FAB) calcd for $C_{19}H_{34}N_3O_4PS_2$: 464.1806; found: 464.1804.

A list of personnel receiving the support of this contract:
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2. Jun Hiratake
3. Kaiming Liu
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