orated and the residue was dissolved in a mixture of MeOH (15 mL), THF (10 mL), and aqueous sodium bicarbonate 5% (10 mL). After the mixture was stirred at room temperature for 15 min, the product was extracted with CH_2Cl_2, dried, and concentrated. Chromatography of the residue (SiO_2, CH_2Cl_2) gave 0.062 g (29%) of the bis(anhydro) derivative **29**: mp 250–252 °C; NMR δ 2.78 (s, 3 H, CH_3), 7.84 (m, 2 H, Ar), 8.32 (dd, 1 H, J = 8, 1.5 Hz, H-8), 8.46 (m, 2 H, Ar), 8.54 (d, 1 H, J = 8 Hz, H-7), 8.99 (dd, 1 H, J = 1.5 Hz, H-10), 14.93 (s, 1 H, OH), 15.08 (s, 1 H, OH); IR 1690 ($COCH_3$), 1630 (H-bonded quinone), 1590 cm^{-1} (Ar); UV–vis λ_{max} (CH_3CN) 267 (log ε 3.57), 4.54 (sh, 2.72), 478 (2.87), 510 nm (2.77); MS, m/e 332 (M, 100), 289 (22), 261 (15), 233 (19); anal. calcd for $C_{20}H_{12}O_5$; 332.0681; found, 332.0682.

Continued elution with CH_2Cl_2–EtOH (1%) gave 0.115 g (48%) of **24** and 0.022 g (9%) of **25**, identical (TLC, MS, NMR) with samples obtained in method A.

Method C. A sample of tetraacetate **28** (20 mg) was hydrolyzed in 10 mL of MeOH–H_2O (4:2) with 0.5 mL of HCl (12%). The resulting solution was refluxed under nitrogen for 3 days. The mixture was poured into H_2O and extracted with CH_2Cl_2. The organic phase was dried and concentrated, giving 11.3 mg (85%) of a crude mixture of **24** and **25** (2:1, respectively), containing less than 3% of aromatic **29**. Epimerization as before gave 44% of (±)-4-demethoxydaunomycinone (**24**) and 9% of the 7-epi isomer (**25**), after chromatography.

Method D. To 0.10 g of bromide **22** in 5 mL of trifluoroacetic acid was added 0.130 g of silver trifluoroacetate. The mixture was stirred for 2 h at room temperature. The solvent was removed and the residue was dissolved in CH_2Cl_2 and filtered. To the solution was added 0.20 g of $AlCl_3$. This mixture was stirred for 15 min at room temperature; at this point the methylene chloride was shaken with dilute HCl, and the layers were separated and dried. The methylene chloride was removed in vacuo and the residue was dissolved in 20 mL of THF and to this was added 5 mL of 10% NaOH. This solution was stirred for 2 h; at this point the solution was neutralized with 10% HCl and extracted with methylene chloride. The layers were separated and the methylene chloride was removed in vacuo. Epimerization of the residue was accomplished with 10 mL of trifluoroacetic acid and by stirring for 2 h at room temperature. The solvent was removed in vacuo and the residue was chromatographed (SiO_2, CH_2Cl_2/MeOH(3%)), giving 5.7 mg (8%) of the epi isomer **25** and 28.7 mg (40%) of the cis isomer **24**.

Acknowledgment. We thank Adria Laboratories, Columbus, OH, for a grant in support for this work.

Registry No. 4, 77422-62-9; **5**, 84498-97-5; **6**, 76811-56-8; **7** (isomer 1), 84498-98-6; **7** (isomer 2), 84498-99-7; *cis*-**10**, 84499-00-3; *trans*-**10**, 84499-01-4; **11**, 84499-02-5; *cis*-**12**, 84499-03-6; *trans*-**12**, 84499-04-7; (±)-**16**, 65529-77-3; **16**, 63229-48-1; **16** (hydrazone, isomer 1), 84499-05-8; **16** (hydrazone, isomer 2), 84499-06-9; **17**, 84198-22-1; **18**, 69813-88-3; **19**, 33628-86-3; **20**, 84499-07-0; **21**, 84499-08-1; **22**, 84499-09-2; **24**, 58924-49-5; **25**, 65877-42-1; **26**, 70071-85-1; **27**, 71571-55-6; **28** (isomer 1), 84499-10-5; **28** (isomer 2), 84499-11-6; **29**, 84499-12-7.

Cyclic Phosphonic–Carboxylic Imides and Anhydrides as Reactive Intermediates. 1. Rearrangement and Solvolysis of *N*-(Amino(methyl)phosphinyl)-L-phenylalanine Derivatives

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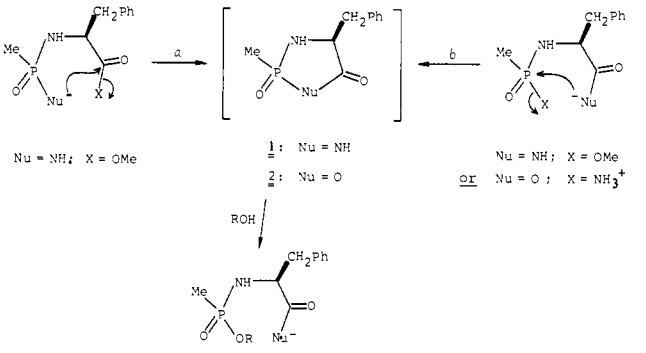
Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received August 5, 1982*

Abstract: Structural, kinetic, and stereochemical evidence indicates the involvement of cyclic intermediates of the structure Me-P(O)-L-Phe-X, **1** (X = NH) and **2** (X = O), in the anomalous reactions of derivatives of *N*-(amino(methyl)phosphinyl)-L-phenylalanine. Cyclization of Me-PO(NH_2)-L-Phe-OMe (**7**) and Me-PO(OMe)-L-Phe-NH_2 (**12**) gives **1**, while cyclization of Me-PO(NH_2)-L-Phe (**10**) gives **2**. The transient intermediates **1** and **2** are not observed, but rapidly undergo ring opening at phosphorus by a solvent molecule. Evidence for the intermediacy of **1** and **2** includes the transfer of NH_2 from phosphorus to the phenylalanine carbonyl in the base-catalyzed solvolysis of **7**, large rate accelerations in the base-catalyzed solvolysis of **12** and the acid-catalyzed solvolysis of **10**, and the stereochemical outcome of the latter reaction: the configuration at phosphorus is retained, as predicted by a cyclization–ring opening mechanism. The S_P diastereomer of **2**, in which the methyl and benzyl groups are cis related in the ring, is formed 2–3 times faster than the R_P isomer.

In connection with our investigations of phosphonamidate dipeptide analogues as inhibitors of carboxypeptidase A,[1] we required the phosphonic diamide **10**. During the course of our synthetic work, we observed a number of anomalous reactions, for example the extreme hydrolytic sensitivity of **10** in comparison to the carboxylic ester derivative **7**, and the ready transformation of **7** into the carboxamide **9** under alkaline conditions (see Scheme III). These reactions appeared to involve transient intermediates **1** and **2** (Scheme I). Such intermediates arise either by cyclization of a phosphorus-bound nucleophile on the acyl carbon (path a) or by cyclization of a carbon-bound nucleophile on the phosphorus center (path b). In hydroxylic solvents, the intermediates **1** and **2** undergo rapid cleavage, exclusively at phosphorus, to give overall nucleophile transfer and solvolysis (path a) or catalyzed solvolysis (path b).

Scheme I



A number of previous studies have implicated five-membered ring phosphoric–carboxylic and phosphonic–carboxylic anhydride

(1) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654.

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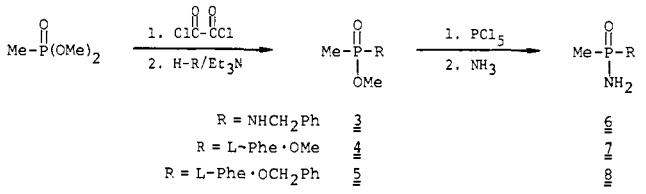
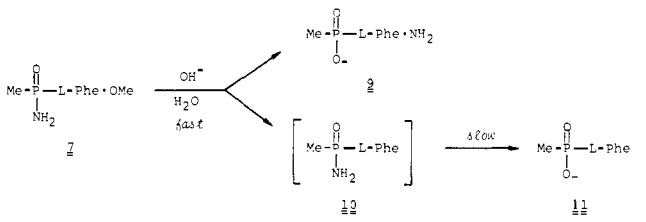
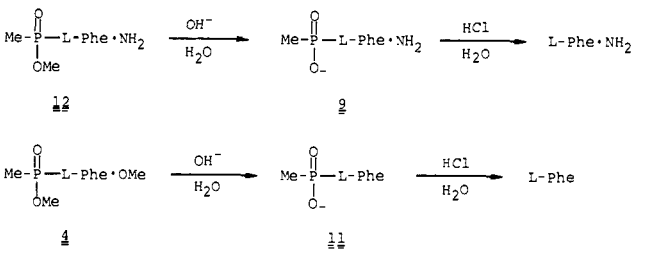
Scheme II



Scheme III



Scheme IV



and imide intermediates in intramolecular catalysis. For example, dramatic rate accelerations have been observed both for carboxyl catalysis of phosphorus ester hydrolysis[2-5] and for phosphonate catalysis of carboxylic ester hydrolysis.[6] Base-induced cyclizations which lead to imide intermediates related to **1** have been observed for *N*-phosphonyl (path a) and *N*-phosphoryl (path b) amino acid derivatives as well.[7] These studies have been confined to kinetic and structural investigations, however. The incorporation of a chiral amino acid into our substrates provided us with the opportunity to study the stereochemical consequences of reactions such as these. In this paper, we present both kinetic and stereochemical evidence for the intermediacy of **1** and **2** in the reactions of *N*-(amino(methyl)phosphinyl)-L-phenylalanine derivatives. In the following paper, we discuss the kinetic and stereochemical consequences of intermediates such as **2** in the reactions of *N*-(hydroxy(methyl)phosphinothioyl)-L-phenylalanine derivatives.

Results

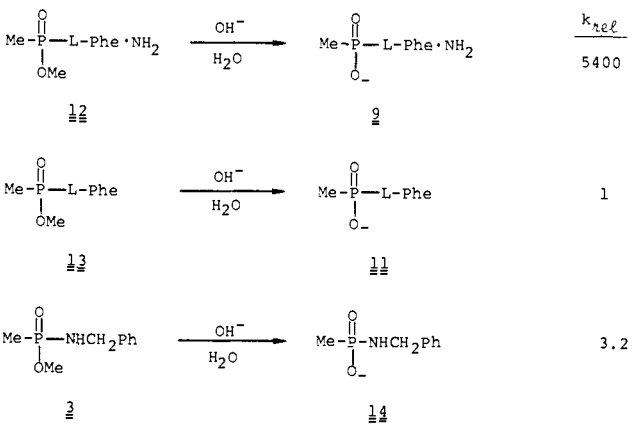
The phosphonic diamides **6–8** were prepared in a two-step sequence from dimethyl methylphosphonate (Scheme II).

Whereas the *N*-benzyl diamide **6** is recovered unchanged after 15 min in 2 M NaOH at 21 °C, under similar conditions the diamide methyl ester **7** undergoes immediate conversion to a 1:1 mixture of the carboxamide **9** and the diamide monoanion (Scheme III). The presence of **10** was indicated by its ^1H and ^{31}P NMR chemical shifts, which are similar to those of the starting material **7**, and by the observed doubling of NMR absorbances due to the presence of diastereomers. Unfortunately, **10** could not be isolated; its hydrolysis to give the dianion **11** is slow in the alkaline hydrolysis mixture but becomes rapid when excess base is neutralized. The rearrangement product **9** and solvolysis product **11** were isolated and characterized by their degradation to phenylalanine amide and phenylalanine, respectively, and by independent synthesis from the corresponding methyl ester **12** and the dimethyl ester **4**, respectively (Scheme IV). The ^{31}P NMR chemical shifts of **9** and **11** are similar and considerably upfield from those of diamides **7** and **10**. Moreover, lack of diastereomeric doubling of peaks in the ^1H, ^{13}C, or ^{31}P NMR spectra provided further support for the structures assigned to **9** and **11**.

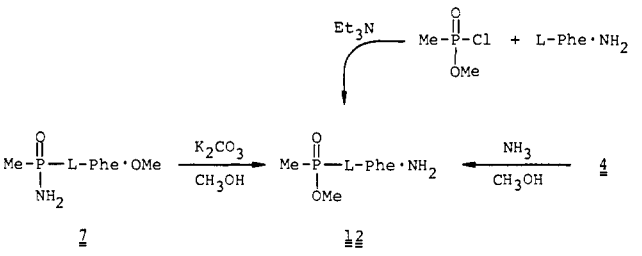
The hydrolysis of **12** to give **9** is also unusually rapid: 5400 times faster than cleavage of the corresponding phenylalanine derivative **13** to give **11** and 1700 times faster than for the simple phosphonamidate **3** (Scheme V)

When the reaction mixture from hydrolysis of diamide methyl ester **7** (Scheme III) is diluted into buffer solution immediately after the initial reaction to give carboxamide **9** and diamide monoanion **10**, hydrolysis of the diastereomers of **10** to give **11**

Scheme V



Scheme VI



Scheme VII

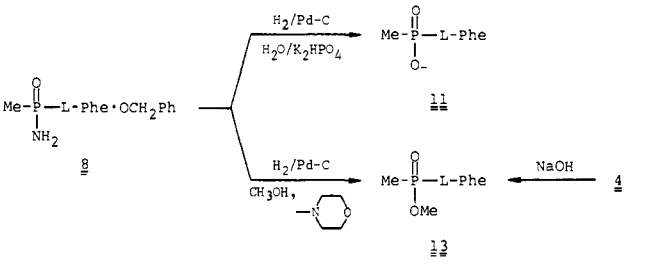


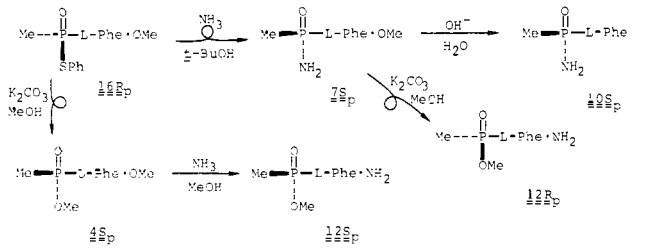
Table I. Rate of Decomposition in Aqueous Solution

conditions[a]	$k_{app} \times 10^4$, s^{-1} 10S[b]	10R[b]	7
0.003 M NaOH	3 ± 1	9 ± 2	
buffer, pH 10.5	10 ± 8	22 ± 11	0.048
buffer, pH 10.0	17 ± 7	55 ± 9	
buffer, pH 9.5	167	>444	
buffer, pH 8.5			0.013
buffer, pH 7.5	>200	>200	0.0015
buffer, pH 6.0			<0.000 16
buffer, pH 4.0			0.058

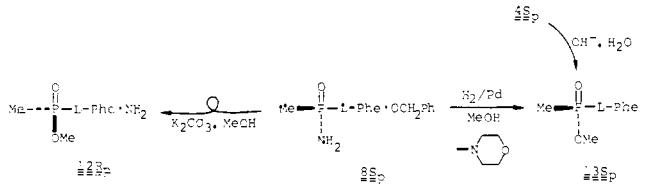
[a] 10% D_2O by volume, 22 °C. [b] Assignment of the absolute configuration of the diastereomeric diamides **10** rests on their correlation with $4R_P$ and $4S_P$ (see text and ref 9).

(2) Blackburn, G. M.; Brown, M. J. *J. Am. Chem. Soc.* **1969**, *91*, 525.
(3) (a) Khan, S. A.; Kirby, A. J.; Wakselman, M.; Horning, D. P.; Lawlor, J. M. *J. Chem. Soc. B* **1970**, 1182. (b) Cosmatos, A.; Photaki, I.; Zervas, L. *Chem. Ber.* **1961**, *94*, 2644.
(4) (a) Schray, K. J.; Benkovic, S. J. *J. Am. Chem. Soc.* **1971**, *93*, 2522. (b) Clark, V. M.; Kirby, A. J. *Ibid.* **1963**, *85*, 3705.
(5) Sampson, E. J.; Fedor, J.; Benkovic, P. A.; Benkovic, S. J. *J. Org. Chem.* **1973**, *38*, 1301.
(6) Shames, S. L.; Byers, L. D. *J. Am. Chem. Soc.* **1981**, *103*, 6177.
(7) Mulliez, M. *Tetrahedron Lett.* **1974**, 2351.

Scheme VIII



Scheme IX



can be observed by ^{31}P NMR spectroscopy. The pseudo-first-order rate constants for this process are presented in Table I, along with the rate of decomposition of starting methyl ester **7**.

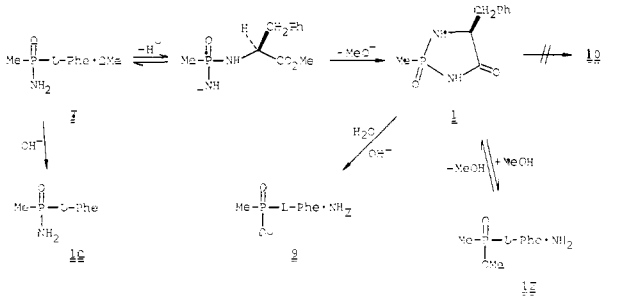
The rearrangement pathway can be observed independently of the solvolysis pathway by treating the starting diamide **7** with potassium carbonate in methanol (Scheme VI). Complete conversion to the phenylalanine amide derivative **12** is accomplished in 14 h at 21 °C. The product **12** was characterized by independent synthesis from both phenylalanine amide and the dimethyl ester **4**.

The solvolysis pathway can be observed independently of the rearrangement pathway by generating diamide **10** directly from the corresponding benzyl ester **8** (Scheme VII). When the hydrogenolysis is carried out in water at pH 7, only the hydrolysis product **11** is obtained. In methanol with *N*-methylmorpholine added to prevent acidification, only the methyl ester **13** is obtained. The product **13** was characterized by independent synthesis from the dimethyl ester **4**.

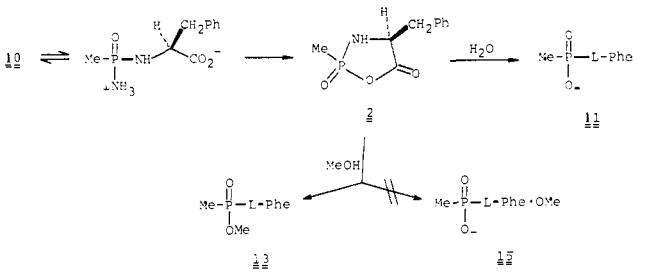
In all of the reactions discussed thus far, the starting materials and products were 1:1 mixtures of the R_P and S_P diastereomers. In order to determine the stereochemical course of these transformations, diastereomerically enriched samples were prepared. The phenylthio ester **16** (Scheme VIII) is obtained from methyl ester **4** by treatment with PCl_5 followed by thiophenol and triethylamine. After separation of diastereomers by preparative TLC, methanolysis of $\mathbf{16}R_P$[8] gives dimethyl ester $\mathbf{4}S_P$,[9] while ammonolysis gives diamide $\mathbf{7}S_P$. All attempts to convert dimethyl ester $\mathbf{4}S_P$ to diamide methyl ester **7** by treatment with PCl_5 and ammonia (as shown in Scheme II) led to a 1:1 mixture of isomers $\mathbf{7}R_P$ and $\mathbf{7}S_P$. Hydrolysis of $\mathbf{7}S_P$ with aqueous base gives initially a 1:1 mixture of the rearranged product **9** and the simple hydrolysis product $\mathbf{10}S_P$. On the other hand, rearrangement of diamide $\mathbf{7}S_P$ in methanol gives methyl ester carboxamide $\mathbf{12}R_P$. Ammonolysis of dimethyl ester $\mathbf{4}S_P$ gives the isomeric carboxamide $\mathbf{12}S_P$. Since displacement of thiophenolate from $\mathbf{16}R_P$ presumably occurs with inversion of configuration and ammonolysis of dimethyl ester $\mathbf{4}S_P$ does not affect the configuration at phosphorus, the rearrangement of diamide **7** to carboxamide **12** must occur with inversion of configuration at phosphorus.

The diastereomeric diamide benzyl esters $\mathbf{8}S_P$ and $\mathbf{8}R_P$ were separated by preparative high-pressure liquid chromatography (Scheme IX). Comparison of the ^1H and ^{31}P NMR chemical shifts of methyl ester **7** and benzyl ester **8** shows a strong correspondence of $\mathbf{7}S_P$ with $\mathbf{8}S_P$ and of $\mathbf{7}R_P$ with $\mathbf{8}R_P$, suggesting that

Scheme X



Scheme XI



$\mathbf{8}S_P$ is, in fact, the S_P diastereomer. Furthermore, rearrangement of $\mathbf{8}S_P$ in methanol[10] gives the same methyl carboxamide $\mathbf{12}R_P$, obtained by rearrangement of methyl ester $\mathbf{7}S_P$. Hydrogenolysis of benzyl ester $\mathbf{8}S_P$ in methanol gives methyl ester $\mathbf{13}S_P$, the same isomer obtained by partial saponification of dimethyl ester $\mathbf{4}S_P$. Since the conversion of dimethyl ester $\mathbf{4}S_P$ to monoanion $\mathbf{13}S_P$ or carboxamide $\mathbf{12}S_P$ does not affect the configuration at phosphorus, the hydrogenolysis/methanolysis of $\mathbf{8}S_P$ must proceed with retention of configuration at phosphorus to give $\mathbf{13}S_P$.

Discussion

The conversion of diamide **7** both to carboxamide **9** in aqueous base and to methyl ester carboxamide **12** using catalytic methoxide in methanol require the intermediacy of cyclic imide **1** (Scheme X). The same rearrangement has been observed by Mulliez in a closely related system and proposed as the basis for a method for peptide synthesis.[7] A mechanism involving solvolytic loss of ammonia followed by ammonolysis of the methyl ester can be ruled out because the *N*-benzyl amide **6** does not lose ammonia in aqueous hydroxide and because ammonolysis is much slower than the observed rapid conversion of diamide **7** to carboxamide **9**.

Likewise, the large rate acceleration for hydrolysis of methyl ester carboxamide **12** (Scheme V) also requires the intermediacy of **1**. Ring opening of **1** must occur exclusively at phosphorus, since neither diamide **10**, resulting from ring opening at the carbonyl center, nor its hydrolysis product **11** are observed in this reaction (Scheme X). These results also are precedented in the work of Mulliez.[7,11] It follows that in the hydrolysis of methyl ester **7**, the monoanion **10** must arise from direct saponification of the methyl ester of **7**.

The rapid solvolytic loss of ammonia from diamide monoanion **10** in comparison to the corresponding methyl ester **7** (Table I) requires the intermediacy of the cyclic anhydride **2** (Scheme XI). The rate acceleration ($>1.3 \times 10^5$ at pH 7.5) cannot simply be due to a more favorable protonation on nitrogen due to the proximity of the free carboxylate. Protonation of disodium succinate, for example, is only 13 times more favorable than protonation of sodium methyl succinate.[12] The pH dependence of solvolysis (Table I) suggests that protonation on the unsubstituted amide precedes or accompanies cyclization. Ring opening by solvent occurs exclusively at phosphorus, as evidenced by the absence of carboxylic methyl ester **15** when solvolysis occurs in

(8) For all isolated neutral compounds discussed in this paper, the S_P isomer elutes first (is less polar) in silica gel chromatography (ethanol/chloroform eluant). For all compounds that were obtained in diastereomerically enriched form, the S_P isomer resonates at higher field in the ^{31}P NMR spectrum.

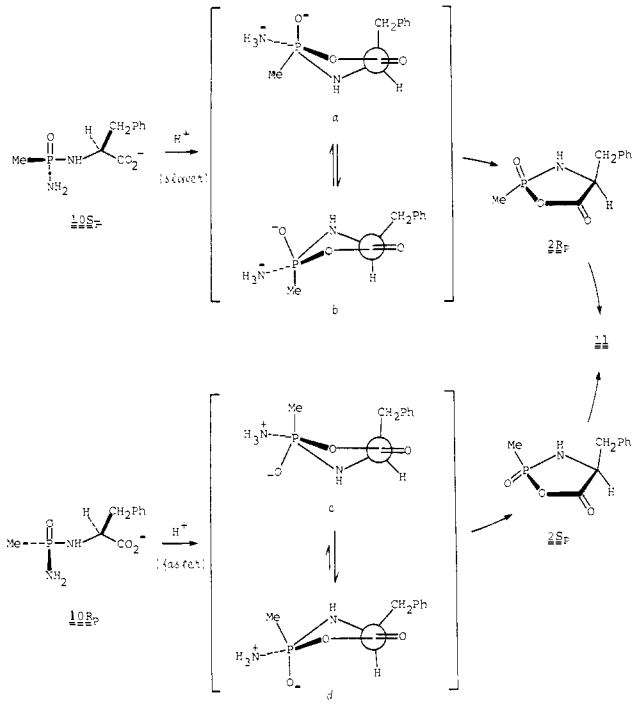
(9) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1983**, *105* (following paper in this issue); the absolute configuration at phosphorus of compounds **4***R* and **4***S* is proven in this paper.

(10) Rearrangement of **8** occurs primarily by ester exchange to **7**, followed by rearrangement to **12**.

(11) Mulliez, M.; Wakselman, M. *Phosphorus Sulfur* **1980**, *8*, 41.

(12) Speakman, J. C. *J. Chem. Soc.* **1943**, 271.

Scheme XII. TBP Intermediates a–d Are Shown in a Newman Projection Looking Down the C(O)–C_α Bond



methanol. Although **15** has not been prepared independently, analogous compounds are stable over a wide pH range.[7]

While many studies have investigated intramolecular catalysis in solvolysis reactions at phosphorus,[2-7] none has addressed the stereochemical consequences of such a mechanism. The incorporation of L-phenylalanine in the molecule permits facile separation of isomers differing in configuration at phosphorus, as well as providing a simple method of stereochemical analysis of the reaction products based on the ^{1}H NMR chemical shift of the *P*-methyl group. The critical prediction of a solvolysis mechanism proceeding through the cyclic intermediate **2** (Scheme XI) is that displacement of ammonia by methanol to give methyl ester monoanion **13** from diamide **10** should occur with two consecutive inversions at phosphorus to give overall retention of configuration. In contrast, direct solvolysis of diamide **10** would presumably proceed with inversion. The observation that solvolysis occurs with ≥98% retention of configuration (Scheme IX) provides the strongest evidence for the intermediacy of the cyclic anhydride **2**. The observation that rearrangement of diamide methyl ester **7** to methyl ester carboxamide **12** occurs with inversion of configuration (Scheme VIII) is also consistent with the proposed mechanism (Scheme X).

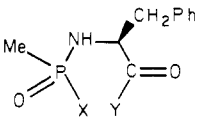
The fact that the diamide monoanion **10**R_P is hydrolyzed 2–3 times faster than the other diastereomer, **10**S_P (Table I), warrants an examination of the interaction of the phosphorus chiral center and the amino acid chiral center in the transition state (Scheme XII). Since the cyclic intermediate **2** does not accumulate in concentrations high enough to be detected by NMR spectroscopy, ring closure must be rate determining and the "cis" intermediate **2**S_P must be the more easily formed. Although Dreiding models suggest that a single planar ring conformation is favored for **2**, with **2**S_P being the less thermodynamically stable isomer, there are numerous examples of kinetic preference for formation of the less stable isomer in cyclizations that lead to five- and six-membered ring derivatives of methylphosphonic acid.[13] Furthermore, Dreiding models of the trigonal-bipyramid (TBP) intermediates which lead to **2**R_P and **2**S_P suggest that the TBP leading to the "cis" intermediate **2**S_P is lower in energy. (The puckered ring of the TBP intermediates results from the O–P–N angle of 90°.)

(13) Hall, C. R.; Inch, T. D. *Tetrahedron* **1980**, *36*, 2059 and references cited therein.

On simple steric grounds the TBP derived from **10**R_P (c and d in Scheme XII) should exist entirely in the conformation d with both alkyl groups pseudoequatorially oriented, and TBP(d) should be lower in energy than either of the conformers (a and b) derived from **10**S_P, which have one pseudoaxial alkyl group. To the extent that the transition state for cyclization is similar to the TBP, these interactions account for the small difference (0.4–0.6 kcal/mol in activation energy.

Conclusions

Although we have not observed cyclic intermediates **1** and **2** spectroscopically, the results presented above confirm their importance in a number of reactions of β-phosphono–carboxylic acid derivatives in which one center is activated toward substitution while the other is nucleophilic. Deprotonation may activate a nucleophilic center (e.g., diamide **7** and carboxamide **12**), or



$$\mathbf{7} \xrightarrow{-H^+} X = NH^-;\ Y = OMe$$

$$\mathbf{12} \xrightarrow{-H^+} X = OMe;\ Y = NH^-$$

$$\mathbf{10} \xrightarrow{H^+} X = NH_3^+;\ Y = O^-$$

$$\mathbf{13} \equiv X = OMe;\ Y = O^-$$

protonation may activate a center toward substitution (e.g., diamide monoanion **10**). Methyl esters are not highly activated and require a strong nucleophile (e.g., methyl ester **13** is relatively stable). Cyclization is followed immediately by solvolytic ring opening at phosphorus to give, overall, a dramatically enhanced rate of solvolysis accompanied (in cases where phosphorus bears the nucleophilic center) by transfer of a heteroatom from phosphorus to the acyl carbon.

Experimental Section

General Information. Chloroform was rendered ethanol free by shaking with concentrated H_2SO_4 and water, drying over $CaCl_2$, and distilling from P_2O_5. ^{1}H NMR spectra were measured with the indicated resonant frequency and solvent; data are presented as follows: chemical shift in ppm on the δ scale relative to internal tetramethylsilane (multiplicity, integrated intensity, coupling constants in hertz). ^{1}H NMR spectra measured in D_2O are referenced to $(CH_3)_3Si(CD_2)_2CO_2Na$ as 0 ppm or CH_3OD as 3.36 ppm; the reference in CD_3OD was residual CHD_2OD as 3.30 ppm. ^{13}C NMR spectra were acquired at the indicated resonant frequency; chemical shifts are reported in ppm on the δ scale, referenced to $CDCl_3$ solvent as 77.0 ppm or to dioxane as 66.5 ppm in D_2O solvent. ^{31}P NMR spectra were measured at 72.9 MHz; chemical shifts are reported in ppm relative to external 85% H_3PO_4 (downfield positive). ^{31}P NMR spectra are referenced to either external 2 M H_3PO_4 (sealed capillary) as 0.55 ppm or to 0.05% internal trimethyl phosphate as 3.09, 3.78, 3.49, or 3.95 ppm in $CDCl_3$, D_2O, CD_3OD, or CD_3COCD_3 solvents, respectively. The following buffer solutions were employed: pH 10.5 Et_3N/HCl 0.10 M; pH 10.0 Na_2CO_3/$NaHCO_3$ 0.050 M; pH 9.5 H_3BO_3/NaOH 0.10 M; pH 8.5 Tris/HCl 0.10 M; pH 7.5 Tris/HCl 0.10 M.

Methyl *N*-Benzylmethylphosphonamidate (3). A solution of 29.2 g (0.23 mol) of oxalyl chloride in 10 mL of dry ether was added dropwise to a solution of 12.4 g (0.10 mol) of dimethyl methylphosphonate in 125 mL of dry ether with stirring under a nitrogen atmosphere. After 72 h at 21 °C, the solvent was removed at reduced pressure and the remaining oxalyl chloride was removed by two cycles of dilution with 125 mL of chloroform followed by concentration at reduced pressure. The crude methyl methylphosphonochloridate was dissolved in 100 mL of chloroform and cooled to 0 °C, and a solution of 10.7 g (0.10 mol) of benzylamine and 11.2 g (0.11 mol) of triethylamine in 25 mL of chloroform was added. After 18 h at 21 °C, the reaction mixture was washed with 150 mL of 2 M H_2SO_4 and dried ($MgSO_4$), and the solvent was removed at reduced pressure. Crystallization of the residue from hot diisopropyl ether and recrystallization gave 8.8 g (44% yield) of water-soluble colorless crystals: mp 45–47 °C; 250-MHz ^{1}H NMR ($CDCl_3$) δ 1.47 (d, 3, J = 16.6), 3.64 (d, 3, J = 11.2), 2.85 (br s, 1), 4.11 (AB of ABXP, 2, $J_{AX} \approx 7.1$, $J_{AP} \approx 9.2$, $J_{BX} \approx 7.4$, $J_{BP} \approx 9.7$, $\Delta\delta \approx 0.01$ ppm), 7.2–7.4 (m, 5); 25.1-MHz ^{13}C NMR ($CDCl_3$) δ 12.5 (d, J_{CP} = 132.1), 44.7, 50.0 (d, J_{CP} = 6.4), 127.1, 128.5, 139.8 (d, J_{CP} = 4.9); ^{31}P NMR ($CDCl_3$)

34.75. Anal. ($C_9H_{14}NO_2P$) C, H, N, P.

***N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine Methyl Ester (4).** The dimethyl ester **4** was prepared by an analogous procedure to that described above for **3**, using 1.24 g (10 mmol) of dimethyl methylphosphonate and 12 mmol of L-phenylalanine methyl ester. The crude product was crystallized from 75 mL of benzene/hexane (1:4 ratio) to give 1.22 g (45% yield) of white crystals: mp 82–89 °C; 250-MHz ^1H NMR see below for $4R_P$ and $4S_P$; ^{13}C NMR ($CDCl_3$) δ 12.7 (d, J_{CP} = 134), 13.0 (d, J_{CP} = 134), 40.4 (d, J_{CP} = 4.9), 40.6 (d, J_{CP} = 4.7), 49.8 (d, J_{CP} = 6.3), 51.8, 55.0, 55.1, 126.8, 128.3, 129.3, 136.3, 136.4, 173.3; ^{31}P NMR see below; HPLC (silica gel, ethanol/chloroform, 1:200), retention time in column volumes: 13.3 ($4S_P$) and 13.8 ($4R_P$). Anal. ($C_{12}H_{18}NO_4P$) C, H, N, P.

***N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine Methyl Ester ($4R_P$) from $16S_P$, and $4S_P$ from $16R_P$.** *N*-(Methyl(phenylthio)phosphinyl)-L-phenylalanine methyl ester $16S_P$ (8.3:1 ratio of diastereomers, 4 mg, 0.012 mmol) was stirred with 1.5 mg (0.011 mmol) of K_2CO_3 in 0.6 mL of dry methanol for 22 h at 21 °C. The reaction mixture was applied directly to a preparative TLC plate (silica gel), eluting with ethanol/chloroform (1:9) to obtain 2.4 mg (77% yield) of $4R_P$ (7.3:1 ratio of diastereomers) as a crystalline solid: 250-MHz ^1H NMR (CD_3OD) δ 1.26 (d, 3, J = 17.1), 2.81 and 3.18 (AB of ABXP, 2, J_{AB} = 13.6, $J_{AX} \approx$ 9.8, $J_{BX} \approx$ 4.8, $J_{BP} \approx$ 2.6), 3.23 (d, 3, J = 11.4), 3.73 (s, 3), 4.08 (ddd, X of ABXP, J_{PH} = 9.4, J_{HH} = 4.9, 9.4), 7.15–7.35 (m, 5); ^{31}P NMR ($CDCl_3$) δ 34.39.

Under the same conditions, $16R_P$ (4 mg, 13.4:1 ratio of diastereomers) gave 2.5 mg (80% yield) of $4S_P$ (8.8:1 ratio of diastereomers) as a crystalline solid: 250-MHz ^1H NMR (CD_3OD) δ 1.02 (d, 3, J = 17.2), 2.78 and 3.16 (AB of ABXP, 2, J_{AB} = 13.5, $J_{AX} \approx$ 9.9, $J_{BX} \approx$ 4.8, $J_{BP} \approx$ 2.4), 3.42 (d, 3, J = 11.6), 3.73 (s, 3), 4.09 (ddd, X of ABXP, J_{HP} = 9.9, J_{HH} = 4.7, 9.9), 7.2–7.35 (m, 5); ^{31}P NMR ($CDCl_3$) δ 33.57.

***N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine Benzyl Ester (5).** The benzyl ester **5** was prepared by a procedure analogous to that described above for **3**, using 0.37 g (3.0 mmol) of dimethyl methylphosphonate, 1.37 g (3.2 mmol) of L-phenylalanine benzyl ester *p*-tosylate, and 0.89 mL (6.4 mmol) of triethylamine. The crude product was applied to a column of silica gel and eluted with ethanol/chloroform (3:97) to give 0.65 g (62% yield) of a viscous oil which solidified upon standing: 250-MHz ^1H NMR ($CDCl_3$) δ 1.16 and 1.17 (two d, 1:1 ratio of diastereomers, 3, J = 16.8 and 16.9), 2.88 and 2.92 (two A of ABX, 1, J_{AB} = 13.6, $J_{AX} \approx$ 10.2 and 10.0), 3.10 (B of ABX, 1, $J_{BX} \approx$ 5.3), 3.32 and 3.37 (two d, 3, J = 11.3 and 11.4), 4.15 (X of ABX, m, 1), 5.14 (br s, 2), 7.1–7.3 (m, 5), 7.33 (br s, 5); 63.1-MHz ^{13}C NMR ($CDCl_3$) δ 12.6 (d, J_{CP} = 133.1), 13.0 (d, J_{CP} = 134.1), 40.3 (d, J_{CP} = 5.3), 40.5 (d, J_{CP} = 5.3), 49.8 (d, J_{CP} = 6.8), 55.1, 55.2, 66.9, 126.8, 128.3, 128.4, 129.3, 134.9, 136.1, 136.2, 172.7; ^{31}P NMR ($CDCl_3$) δ 34.25, 34.42. An analytical sample was obtained by bulb-to-bulb distillation (225 °C (0.15 torr)). Anal. ($C_{18}H_{22}NO_4P$) C, H, N, P.

***N*-Benzylmethylphosphonic Diamide (6).** Methyl *N*-benzylmethylphosphonamidate (**3**; 1.06 g, 5.32 mmol) was stirred with 1.11 g (5.32 mmol) of PCl_5 in 20 mL of chloroform under a nitrogen atmosphere for 5 h at 21 °C. After removal of solvent at reduced pressure, the remaining traces of $POCl_3$ were removed by dilution with 35 mL of chloroform followed by concentration at reduced pressure. Anhydrous ammonia was bubbled through a solution of the crude amidochloridate in 35 mL of chloroform for 12 min at 21 °C, ammonium chloride was removed by filtration, and the filtrate was concentrated at reduced pressure. Column chromatography on silica gel, eluting with a step gradient of ethanol/chloroform (1:9, 1:4, 1:3), gave 0.27 g (27% yield) of a white solid: 250-MHz ^1H NMR ($CDCl_3$) δ 1.51 (d, 3, J = 15.4), 2.6 (br s, 2), 2.75 (br s, 1), 4.18 (dd, 2, J = 9.8, 7.0), 7.2–7.4 (m, 5); 25.1-MHz ^{13}C NMR (D_2O) δ 14.4 (d, J_{CP} = 112.2), 43.6, 127.2, 127.3, 128.6, 140.5; ^{31}P NMR ($CDCl_3$) δ 30.6; (D_2O) δ 36.2; mass spectrum, m/z 185 (M + 1), 184 (M^+), 167, 149, 137, 125, 111, 106 (base), 91, 79; exact mass calcd for $C_8H_{13}N_2OP$, 184.0765; found, 184.0768.

***N*-(Methyl(phenylthio)phosphinyl)-L-phenylalanine Methyl Ester (16).** *N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine methyl ester (62 mg, 0.23 mmol) was converted to the crude amidochloridate as described above for the preparation of **6**. This was dissolved in 2 mL of chloroform and treated with 0.10 mL (0.69 mmol) of triethylamine and 0.07 mL (0.69 mmol) of thiophenol. After the mixture was stirred for 1 h at 21 °C, the solvent was evaporated and the residue was purified by preparative TLC (silica gel, ethanol/chloroform, 1:9). The band corresponding to product was arbitrarily divided into two equal parts, the higher R_f part yielding 22 mg (28% yield) of $16S_P$ (8.3:1 ratio of diastereomers), and the lower R_f part yielding 21 mg (27% yield) of $16R_P$ (13.4:1 ratio), both as colorless oils.

$16S_P$: 250-MHz ^1H NMR ($CDCl_3$) δ 1.60 (d, 3, J = 14.7), 3.09 (AB of ABX, 2, J_{AB} = 14.2, $J_{AX} \approx$ 6, $J_{BX} \approx$ 5), 3.43 (br dd, 1, J = 9.5, 14.0), 3.70 (s, 3), 4.45 (dddd, X of ABX, J_{HP} = 11.4 J_{HH} = 9.7, 5.7, 5.7), 7.1–7.15 (m, 2), 7.2–7.4 (m, 6), 7.5–7.6 (m, 2); 63.1-MHz ^{13}C NMR ($CDCl_3$) δ 19.38 (d, J_{CP} = 98.7), 40.79 (d, J_{CP} = 3.4), 52.13, 53.83, 126.8, 127.8, 128.2, 128.7 (d, J_{CP} = 2.5), 129.2, 129.4, 134.9 (d, J_{CP} = 2.8), 135.6, 172.6 (d, J_{CP} = 4.8); ^{31}P NMR ($CDCl_3$) δ 44.88.

$16R_P$: 250-MHz ^1H NMR ($CDCl_3$) δ 1.50 (d, 3, J = 14.6), 3.04 (AB of ABX, J_{AB} = 14.1, J_{AX} = 6, J_{BX} = 6), 3.36 (br t, 1, J = 10.5), 3.67 (s, 3), 4.29 (dddd, X of ABX, 1, J_{HP} = 10.8, J_{HH} = 6.0, 6.0, 10.8), 7.1–7.15 (m, 2), 7.2–7.4 (m, 6), 7.5–7.6 (m, 2); 63.1-MHz ^{13}C NMR ($CDCl_3$) δ 19.24 (d, J_{CP} = 96.7), 40.58 (d, J_{CP} = 4.7), 52.15, 54.78, 127.0, 127.6 (d, J_{CP} = 5.8), 128.4, 128.6, 129.1, 129.4, 134.6 (d, J_{CP} = 4.1), 135.7, 172.7 (d, J_{CP} = 4.0); ^{31}P NMR ($CDCl_3$) δ 45.75. Anal. ($C_{17}H_{20}NO_3PS$) C, H, N, P, S.

***N*-(Amino(methyl)phosphinyl)-L-phenylalanine Methyl Ester (7).** *N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine methyl ester (**4**; 0.39 g, 1.44 mmol) was treated with PCl_5 and ammonia as described above for the preparation of **6**, and the crude product was purified by column chromatography on silica gel, eluting with a step gradient of ethanol/chloroform (1:9, 1:4), to give 161 mg (43% yield) of a colorless oil which solidified on standing. Recrystallization from benzene/hexane afforded white crystals: mp 73–75 °C; 250-MHz ^1H NMR see below for $7R_P$ and $7S_P$; ^{31}P NMR see below; 25.1-MHz ^{13}C NMR ($CDCl_3$) δ 15.4 (d, J_{CP} = 116.1), 15.7 (d, J_{CP} = 116.1), 40.6 (d, J_{CP} = 5.9), 51.8, 55.0, 126.7, 128.3, 129.4, 136.5, 173.7; mass spectrum, m/z 256 (M^+), 224, 197, 165, 120, 91, 78; exact mass calcd for $C_{11}H_{17}N_2O_3P$, 256.0977; found, 256.0972; HPLC (silica gel, ethanol/chloroform, 4:96) retention time in column volumes, 10.6 ($7S_P$) and 11.5 ($7R_P$).

***N*-(Amino(methyl)phosphinyl)-L-phenylalanine Methyl Ester (7) by Ammonolysis of Phenylthio Ester 16.** A solution of 36 mg (0.103 mmol) of (S_P)-*N*-(methyl(phenylthio)phosphinyl)-L-phenylalanine methyl ester, $16S_P$ (15:1 ratio of diastereomers), in 7 mL of *tert*-butyl alcohol was saturated with gaseous ammonia at 21 °C and capped tightly for 66 h. Evaporation of solvent and preparative TLC (silica gel, ethanol/chloroform, 1:4) gave 6.4 mg (24% yield) of $7R_P$ (6.2:1 ratio of diastereomers): 250-MHz ^1H NMR (CD_3OD) δ 1.21 (d, 3, J = 15.9), 2.91 and 3.05 (AB of ABX, 2, J_{AB} = 13.2, $J_{AX} \approx$ 8.0, $J_{BX} \approx$ 6.1), 3.68 (s, 3), 4.08 (ddd, X of ABX, 1, J_{HP} = 9.6, J_{HH} = 6.0, 8.0), 7.15–7.35 (m, 5); ^{31}P NMR ($CDCl_3$) δ 28.74; (D_2O) δ 35.46.

In the same manner, 29.5 mg (0.084 mmol) of $16R_P$ (22:1 ratio of diastereomers) gave 7.9 mg (37% yield) of $7S_P$ (21:1 ratio): 250-MHz ^1H NMR (CD_3OD) δ 1.14 (d, 3, J = 15.9), 2.90 and 3.07 (AB of ABX, 2, J_{AB} = 13.5, $J_{AX} \approx$ 8.3, $J_{BX} \approx$ 5.9), 3.68 (s, 3), 4.09 (ddd, X of ABX, 1, J_{HP} = 9.5, J_{HH} = 6.1, 8.1), 7.15–7.35 (m, 5); ^{31}P NMR ($CDCl_3$) δ 28.34; (D_2O) δ 35.31.

***N*-(Amino(methyl)phosphinyl)-L-phenylalanine Benzyl Ester (8).** *N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine benzyl ester (**5**; 0.30 g, 0.86 mmol) was treated with PCl_5 and ammonia as described above for the preparation of **6**, and the crude product was purified by column chromatography on silica gel, eluting with ethanol/chloroform (1:5) to give 0.22 g (77% yield) of a viscous oil which solidified upon standing. Recrystallization from benzene/hexane gave white crystals: mp 114 °C; 250-MHz ^1H NMR see below for $8R_P$ and $8S_P$; 63.1-MHz ^{13}C NMR ($CDCl_3$) δ 15.4 (d, J_{CP} = 116.5), 15.7 (d, J_{CP} = 117.0), 40.5 (d, J_{CP} = 5.2), 54.97, 55.03, 66.9, 126.7, 128.3, 128.4, 129.4, 135.01, 135.06, 136.3, 136.4, 173.2; ^{31}P NMR see below; mass spectrum m/z 332 (M^+), 241, 224, 197, 133, 108, 91, 79; exact mass calcd for $C_{17}H_{21}N_2O_3P$, 332.1290; found, 332.1280; HPLC (silica gel, ethanol/chloroform, 2:98) retention time in column volumes, 16.2 ($8S_P$) and 17.7 ($8R_P$). Anal. ($C_{17}H_{21}N_2O_3P$) C, H, N.

The diastereomeric diamide benzyl esters $8R_P$ and $8S_P$ were separated by preparative HPLC (silica gel, ethanol/chloroform, 2:98) to obtain $8R_P$ (5.2:1 ratio of diastereomers) and $8S_P$ (>200:1 ratio).

$8R_P$: 250-MHz ^1H NMR (CD_3OD) δ 1.21 (d, 3, J = 15.9), 2.93 and 3.04 (AB of ABX, 2, J_{AB} = 13.2, $J_{AX} \approx$ 7.6, $J_{BX} \approx$ 6.5), 4.12 (ddd, X of ABX, 1, J_{HP} = 9.7, J_{HH} = 7.0, 7.0), 5.11 (s, 2), 7.15–7.4 (m, 10); ^{31}P NMR ($CDCl_3$) δ 28.76.

$8S_P$: 250-MHz ^1H NMR (CD_3OD) δ 1.15 (d, 3, J = 15.9), 2.93 and 3.06 (AB of ABX, 2, J_{AB} = 13.3, $J_{AX} \approx$ 7.7, $J_{BX} \approx$ 6.2), 4.13 (ddd, X of ABX, 1, J_{HP} = 9.7, J_{HH} = 7.3, 7.3), 5.11 (s, 2), 7.15–7.35 (m, 10), ^{31}P NMR ($CDCl_3$) δ 28.31.

Treatment of *N*-Benzylmethylphosphonic Diamide (6) with Aqueous Base. A solution of 13 mg (0.068 mmol) of **6** in 0.10 mL (0.20 mmol) of 2.0 M NaOH showed no reaction by TLC after 15 min at 21 °C. Anion-exchange chromatography (Bio-Rad AG1-X2, HCO_3^- form, 8.7 mL resin), eluting with 200 mL of a linear gradient (0–2.5 M) of triethylammonium bicarbonate gave a single UV-absorbing fraction which emerged in the first 16 mL of eluant.

Treatment of *N*-(Amino(methyl)phosphinyl)-L-phenylalanine Methyl Ester (7) with Aqueous Base. A solution of 131 mg (0.51 mmol) of **7** in 0.52 mL (0.99 mmol) of 1.9 M NaOH was stirred for 15 min at 21 °C, applied to an anion-exchange column (Bio-Rad AG1-X2, HCO_3^-

form, 40 mL resin) and eluted with 400 mL of a linear gradient (0–1.25 M) of triethylammonium bicarbonate to give two fractions of equal UV intensity at 257 nm.

The first fraction to elute, containing *N*-(hydroxy(methyl)phosphinyl)-L-phenylalanine amide, triethylammonium salt (**9**), was treated with 0.17 mL (0.17 mmol) of 1.0 M NaOH and 3.4 mL of 0.1 M Tris/HCl buffer (pH 8.0) and lyophilized to give a white solid: 250-MHz ^{1}H NMR (D_2O) δ 0.87 (d, 3, J = 15.6), 2.92 and 3.11 (AB of ABX, 2, J_{AB} = 13.6, $J_{AX} \approx 8.0$, $J_{BX} \approx 5.7$), 3.83 (ddd, X of ABX, 1, J_{HP} = 9.7, J_{HH} = 5.6, 8.0), 7.28–7.46 (m, 5); 25.1-MHz ^{13}C NMR (D_2O) δ 14.1 (d, J_{CP} = 123.7), 39.6 (d, J_{CP} = 5.4), 56.7, 126.9, 128.7, 129.6, 137.4, 179.8; ^{31}P NMR (D_2O) δ 25.68; UV λ_{max} 258 nm. This product was hydrolyzed with 1.0 mL of 2.0 M HCl for 10 min at 21 °C, neutralized with 2.0 mL of 1.0 M NaOH, and applied to a cation-exchange column (Bio-Rad AG50W-X8, H^+ form, 28 mL resin). Elution with 60 mL of water followed by 60 mL of 4 M ammonia gave (after lyophilization of the ammonia fraction) 25 mg of a white solid, identical by TLC, ninhydrin color, melting point, and ^{1}H NMR spectroscopy with an authentic sample of L-phenylalanine amide.

The second fraction to elute, containing *N*-(hydroxy(methyl)phosphinyl)-L-phenylalanine, bis(triethylammonium) salt (**11**), was treated with 0.14 mL of 1.0 M NaOH and 2.8 mL of 0.1 M Tris/HCl buffer (pH 8.0) and lyophilized to give a white solid: 250-MHz ^{1}H NMR (D_2O) δ 1.10 (d, 3, J = 15.4), 2.92 and 2.94 (AB of ABX, 2, J_{AB} = 13.3, $J_{AX} \approx 7.0$, $J_{BX} \approx 6.6$), 3.75 (ddd, X of ABX, 1, J_{HP} = 8.9, J_{HH} = 6.5, 6.5), 7.25–7.45 (m, 5); 25.1-MHz ^{13}C NMR (D_2O) δ 14.5 (d, J_{CP} = 122.3), 41.3 (d, J_{CP} = 4.8), 58.3, 126.4, 128.3, 129.7, 138.5, 181.4; ^{31}P NMR (D_2O) δ 26.07; UV δ_{max} 258 nm. This product was acid-hydrolyzed, and the amino acid fraction was isolated as above to give a material which was identical by TLC and ninhydrin color with an authentic sample of L-phenylalanine.

Hydrolysis of *N*-(Amino(methyl)phosphinyl)-L-phenylalanine Methyl Ester (7), Monitored by ^{1}H and ^{31}P NMR Spectroscopy. A solution of 17 mg (0.066 mmol) of **7** in 0.75 mL of D_2O was treated with 0.040 mL (0.071 mmol) of 1.78 M NaOD in D_2O, and the 250-MHz ^{1}H NMR spectrum was recorded at intervals. After 2 min at 21 °C, the starting material **7** (δ 1.23 (d, J = 15.8, CH_3P of **7**S_P) and 1.33 (d, J = 15.8, CH_3P of **7**R_P), 1:1 ratio) was nearly gone and two products were present in 1:1 ratio: *N*-(amino(methyl)phosphinyl)-L-phenylalanine, sodium salt (**10**; δ 1.26 (d, J = 15.7, CH_3P of **10**R_P) and 1.28 (d, J = 15.7, CH_3P of **10**S_P), 1:1 ratio), and *N*-(hydroxy(methyl)phosphinyl)-L-phenylalanine amide, sodium salt (**9**; δ 0.87 (d, J = 15.6, CH_3P)). The mole fraction of **9** remained constant while **10** was slowly decomposed to *N*-(hydroxy(methyl)phosphinyl)-L-phenylalanine, disodium salt (**11**; δ 1.10 (d, J = 15.4, CH_3P)), with a pseudo-first-order rate constant of 4×10^{-5} s^{-1}. Addition of 10 μL (0.57 μmol) of 0.057 M KH_2PO_4 in D_2O increased the rate to 1×10^{-4} s^{-1} and an additional 75 μL (4.25 μmol) of KH_2PO_4 gave a rate of 6×10^{-3} s^{-1}. After the decomposition of **10** was complete, TLC of the reaction mixture showed approximately equal quantities of **9** and **11**. When the same reaction was monitored by ^{31}P NMR spectroscopy, similar results were obtained, allowing the following assignments: **10**R_P, δ 35.62; **10**S_P, δ 35.26. The above stereochemical assignments were made by monitoring the hydrolysis of 1.2 mg of **7**S_P (3.4:1 ratio of diastereomers) by ^{1}H NMR spectroscopy and by monitoring the hydrolysis of 0.6 mg of **7**S_P (22:1 ratio of diasteromers) by ^{31}P NMR spectroscopy. In both cases, the initial reaction product was a 1:1 mixture of **10** (diastereomer ratio same as that of the starting material **7**) and **9**.

Stability of *N*-(Amino(methyl)phosphinyl)-L-phenylalanine Methyl Ester (7) as a Function of pH. A 0.45-mL aliquot of a solution of 288 mg (1.12 mmol) of **7** in 4.6 mL of water was diluted with 1.00 mL of buffer solution and 0.16 mL of a 1% solution of trimethyl phosphate in D_2O and the ^{1}H-decoupled ^{31}P NMR spectrum was recorded at intervals at 22 °C. First-order rate constants for decomposition, based on integrated intensities or computed signal-to-noise ratios, are presented in Table I. The products of decomposition (δ 25.1, 25.8, 26.0, 26.2) were not isolated or identified. The ^{31}P NMR chemical shifts of **7**R_P and **7**S_P were invariant over the pH range studied.

Stability of *N*-(Amino(methyl)phosphinyl)-L-phenylalanine, Sodium Salt (10), as a Function of pH. A 20-mg (0.078 mmol) sample of *N*-(amino(methyl)phosphinyl)-L-phenylalanine methyl ester (**7**) was dissolved in 0.36 mL of water and 75 μL (0.078 mmol) of 1.04 M NaOH was added. After 3 min at 21 °C, 1.00 mL of buffer solution and 0.16 mL of a 1% solution of trimethyl phosphate in D_2O were added and the ^{1}H-decoupled ^{31}P NMR spectrum was recorded at intervals at 22 °C. First-order rate constants for the conversion of **10** to **11**, obtained from peak heights or signal-to-noise ratios, are presented in Table I.

***N*-(Hydroxy(methyl)phosphinyl)-L-phenylalanine, Disodium Salt (11).** *N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine methyl ester (**4**; 60 mg, 0.22 mmol) was added to 0.33 mL (0.66 mmol) of 2.0 M NaOH. The reaction mixture was stirred at 21 °C for 24 h, applied to an anion-exchange column (Bio-Rad AG1-X2, HCO_3^- form, 10 mL resin), and eluted with 300 mL of a linear gradient (0–2.5 M) of triethylammonium bicarbonate. The single UV-absorbing fraction (0.24 mmol, using ϵ_{257} 155, the extinction coefficient of **4** in ethanol) was treated with 0.35 mL of 1.0 M NaOH and lyophilized to give a white solid, identical by ^{1}H NMR spectroscopy and gradient elution position with the second fraction obtained from the hydrolysis of **7** (see above).

***N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine Amide (12). A. From L-Phenylalanine Amide.** The crude methyl methylphosphonochloridate, prepared from 130 mg (1.05 mmol) of dimethyl methylphosphonate as described above for the preparation of **3**, was dissolved in 2.0 mL of chloroform and 0.27 mL (1.94 mmol) of triethylamine, and 205 mg (1.25 mmol) of L-phenylalanine amide was added at 21 °C. After 18 h, the reaction mixture was concentrated at reduced pressure, and the residue was dissolved in 8 mL of water, filtered, and applied to a cation-exchange column (Bio-Rad AG50W-X8, NH_4^+ form, 30 mL resin bed), eluting with water. The UV-active fraction was lyophilized and the residue was purified by preparative TLC (ethanol/chloroform 1:3, silica gel) to give 128 mg (40% yield) of a white solid. Recrystallization from benzene/methanol gave white filaments: mp 164–166 °C; 250-MHz ^{1}H NMR see below for **12**R_P and **12**S_P; 25.1-MHz ^{13}C NMR (D_2O) δ 11.0 (d, J_{CP} = 130.7), 11.3 (d, J_{CP} = 131.1), 39.6 (d, J_{CP} = 4.8), 50.9 (d, J_{CP} = 6.9), 51.0 (d, J_{CP} = 6.9), 55.9, 56.0, 127.0, 128.7, 129.5, 137.0, 137.1, 177.6; ^{31}P NMR see below; mass spectrum, m/z 256 (M^+), 224, 212, 180, 165, 120, 93, 91; exact mass calcd for $C_{11}H_{17}N_2O_3P$, 256.0977; found, 256.0976; HPLC (silica gel, ethanol/chloroform, 4:96) retention time in column volumes, 12.7 (**12**S_P) and 14.9 (**12**R_P).

B. By Ammonolysis of 4. A solution of 51 mg (0.19 mmol) of *N*-(methoxy(methyl)phosphinyl)-L-phenylalanine methyl ester (**4**) in 4 mL of dry methanol was saturated with ammonia gas at 0 °C, capped tightly, and kept at 21 °C for 24 h. Evaporation of solvent gave 50 mg of a white solid, identical by ^{1}H NMR Spectroscopy, and TLC with a sample of **12** obtained from L-phenylalanine amide. Ammonolysis of **4**S_P (8:1 ratio) gave **12**S_P (7.6:1 ratio): 250-MHz ^{1}H NMR (CD_3OD) δ 1.05 (d, 3, J = 16.9), 2.75 and 3.12 (AB of ABXP, 2, J_{AB} = 13.6, $J_{AX} \approx 9.8$, $J_{BX} \approx 4.8$, $J_{BP} \approx 2.4$), 3.35 (d, 3, J = 11.5), 3.91 (ddd, X of ABXP, 1, J_{HP} = 9.9, J_{HH} = 4.7, 9.9), 7.15–7.35 (m, 5); ^{31}P NMR (CD_3OD) δ 37.24.

Ammonolysis of **4**R_P (7.3:1 ratio) gave **12**R_P (4.8:1 ratio): 250-MHz ^{1}H NMR (CD_3OD) δ 1.12 (d, 3, J = 16.9), 2.76 and 3.11 (AB of ABXP, 2, J_{AB} = 13.5, $J_{AX} \approx 9.7$, $J_{BX} \approx 4.8$, $J_{BP} \approx 2.4$), 3.32 (d, 3, J = 11.8), 3.87 (ddd, X of ABXP, 1, J_{HP} = 9.6, J_{HH} = 4.8, 9.6), 7.2–7.35 (m, 5); ^{31}P NMR (CD_3OD) δ 37.44.

C. By Rearrangement of *N*-(Amino(methyl)phosphinyl)-L-phenylalanine Methyl Ester (7). A solution of 10.3 mg (0.040 mmol) of **7** in 0.25 mL of dry methanol was stirred with 1.9 mg (0.014 mmol) of anhydrous K_2CO_3 for 19 h at 21 °C, and the reaction mixture was applied directly to a preparative TLC plate (silica gel). Elution with ethanol/chloroform (1:4), extraction of the major band with absolute ethanol and evaporation of solvent gave 8.4 mg (82% yield) of a white solid, identical by ^{1}H NMR spectroscopy and TLC with a sample of **12** prepared from L-phenylalanine amide. When the rearrangement was carried out in CD_3OD and observed by ^{1}H NMR spectroscopy, the conversion of **7** to **12** (d_3) was quantitative and complete in 14 h. Under the same conditions, *N*-(amino(methyl)phosphinyl)-L-phenylalanine benzyl ester (**8**) was also converted to **12**; **8**S_P (>200:1 ratio of diastereomers) gave **12**R_P (12:1 ratio); **8**R_P (5.2:1 ratio) gave **12**S_P (4.7:1 ratio); and **7**S_P (21:1 ratio) gave **12**R_P (11:1 ratio).

***N*-(Hydroxy(methyl)phosphinyl)-L-phenylalanine Amide, Sodium Salt (9), by Hydrolysis of 12.** A solution of 42 mg (0.16 mmol) of *N*-(methoxy(methyl)phosphinyl)-L-phenylalanine amide (**12**) in 0.28 mL (0.25 mmol) of 0.89 M NaOH was stirred for 1 h, neutralized with 24 mg (0.18 mmol) of KH_2PO_4, applied to an anion-exchange column (Bio-Rad AG1-X2, HCO_3^- form, 9 mL resin bed), and eluted with 100 mL of a linear gradient (0–1.25 M) of triethylammonium bicarbonate. The single UV-active product (0.13 mmol based on UV absorbance, using ϵ_{257} 155) was eluted in the same position of the gradient as the first fraction obtained in the hydrolysis of *N*-(amino(methyl)phosphinyl)-L-phenylalanine methyl ester **7** (see above). Treatment with 0.10 mL (0.10 mmol) of 1.0 M NaOH and lyophilization gave 50 mg of a white solid, identical by ^{1}H and ^{13}C NMR spectroscopy with the sample of **9** which was obtained from the hydrolysis of **7**.

Hydrolysis of Methyl Methylphosphonamidates: Kinetic Study. A. Hydrolysis of Methyl *N*-Benzylmethylphosphonamidate (3) To Give 14. A solution of 5.7 mg (0.029 mmol) of **3** in 0.76 mL of D_2O was treated with 0.100 mL (0.20 mmol) of 2.0 M NaOD in D_2O and the 250-MHz ^{1}H NMR spectrum was recorded at intervals. The hydrolysis of **3** (δ 1.52 (d, 3, J = 16.2), 3.61 (d, 3, J = 11.9), 4.13 (d, 2, J = 11.7), 7.4 (m, 5)) proceeded at an initial rate of 2.6×10^{-4} M^{-1} S^{-1} to give **14**: 250-MHz ^{1}H NMR (D_2O) δ 1.24 (d, 3, J = 15.4), 4.03 (d, 2, J = 8.9), 7.4 (m, 5);

^{31}P NMR (acetone-d_3, no decoupling) δ 26.6 (tq, J_{PH} = 9.1, 15.5).

B. Hydrolysis of *N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine, Sodium Salt (13), To Give 11. *N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine methyl ester **4** (4.9 mg, 0.018 mmol) was combined with 0.30 mL of D_2O and 10 μL (0.020 mmol) of 2.0 M NaOD in D_2O. After 15 min at 21 °C, the reaction mixture was diluted with 0.39 mL of D_2O and the ^1H NMR spectrum showed the presence of only methanol and **13**. A 0.10-mL (0.20 mmol) aliquot of 2.0 M NaOD in D_2O was added and the 250-MHz ^1H NMR spectrum was recorded at intervals. The initial rate of hydrolysis of **13** to give **11** was 8.1×10^{-5} M^{-1} s^{-1}.

C. Hydrolysis of *N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine Amide (12) To Give 9. A 4.9-mg (0.019 mmol) sample of **12** was dissolved in 0.64 mL of D_2O, 10 μL (0.020 mmol) of 2 M NaOD in D_2O was added, and the 90-MHz ^1H NMR spectrum was recorded at 1-min intervals. The hydrolysis of **12** proceeded at a rate of 0.44 M^{-1} s^{-1} to give **9**.

***N*-(Methoxy(methyl)phosphinyl)-L-phenylalanine, Sodium Salt (13).** A solution of 8.1 mg (0.030 mmol) of *N*-(methoxy(methyl)phosphinyl)-L-phenylalanine methyl ester (**4**) in 2.1 mL of acetone/water (11:1) was treated with 35 μL (0.036 mmol) of 1.03 M NaOH. After 1.5 h at 21 °C, the solvent was removed at reduced pressure to give an oily residue: 250-MHz ^1H NMR see below for **13**R_P and **13**S_P. 63.1-MHz ^{13}C NMR (CD_3OD) δ 10.28 (d, J_{CP} = 133.1), 10.61 (d, J_{CP} = 133.0), 40.46 (d, J_{CP} = 8.1), 40.58 (d, J_{CP} = 7.4), 48.46 (d, J_{CP} = 6.7), 48.87 (d, J_{CP} = 6.7), 57.8, 58.1, 125.4, 127.3, 129.0, 138.4, 138.53, 178.36, 178.53.

Under the same conditions, saponification of **4**R_P (18:1 ratio of diastereomers) gave **13**R_P (12:1 ratio): 250-MHz ^1H NMR (CD_3OD) δ 0.91 (d, 3, J = 17.0), 2.69 and 3.13 (AB of ABXP, 2, J_{AB} = 13.3, $J_{AX} \approx 9.7$, $J_{BX} \approx J_{BP} \approx 3.2$), 3.43 (d, 3, J = 11.4), 3.64 (ddd, X of ABXP, 1, J_{HP} = 9.8, J_{HH} = 3.8, 9.8), 7.1–7.35 (m, 5); ^{31}P NMR (CD_3OD) δ 38.72.

Saponification of **4**S_P (5.9:1 ratio of diastereomers) gave **13**S_P (5.7:1 ratio): 250-MHz ^1H NMR (CD_3OD) δ 1.22 (d, 3, J = 16.8), 2.70 and 3.13 (AB of ABXP, 2, J_{AB} = 13.4, $J_{AX} \approx 9.6$, $J_{BX} \approx J_{BP} \approx 3.2$), 3.11 (d, 3, J = 11.3), 3.67 (ddd, X of ABXP, 1, J_{HP} = 9.6, J_{HH} = 4.1, 9.6), 7.1–7.35 (m, 5); ^{31}P NMR (CD_3OD) δ 37.78.

Hydrogenolysis of *N*-(Amino(methyl)phosphinyl)-L-phenylalanine Benzyl Ester (8). A. In H_2O/Acetone. A 35-μL aliquot of a solution of 21 mg (0.12 mmol) of K_2HPO_4 in 0.35 mL of H_2O/acetone (2:1) was added to 1.4 mg (0.0042 mmol) of **8** and 0.4 mg of 5% palladium on charcoal in a small vial. Hydrogen gas (1 atm) was introduced through a cannula and the reaction mixture was shaken for 45 min at 21 °C. After dilution with 0.5 mL of D_2O and filtration, only acetone and *N*-(hydroxy(methyl)phosphinyl)-L-phenylalanine, dipotassium salt (**11**), were observed by 250-MHz ^1H NMR spectroscopy.

B. In Methanol. A 35-μL aliquot of a solution of 12.7 mg (0.126 mmol) of *N*-methylmorpholine in 0.35 mL of anhydrous methanol was added to 2.0 mg (6.0 μmol) of **8** and 0.4 mg of 5% palladium on charcoal in a small vial. Hydrogen gas (1 atm) was introduced through a cannula and the reaction mixture was shaken at 21 °C for 30 min. After dilution with 0.4 mL of methanol-d_4 and filtration, only toluene, *N*-methylmorpholine, and *N*-(methoxy(methyl)phosphinyl)-L-phenylalanine, ammonium salt (**13**), were observed by 250-MHz ^1H NMR spectroscopy. Addition of 5 μL (10 μmol) of 2.0 M NaOD in D_2O followed by evaporation of the solvent gave a material identical by 250-MHz ^1H NMR spectroscopy in both methanol-d_4 and D_2O with the sodium salt **13** obtained by partial saponification of **4** (see above). Under the same conditions, hydrogenolysis of **8**S_P (>200:1 ratio of diastereomers) gave **13**S_P (>57:1 ratio) and hydrogenolysis of **8**R_P (5.2:1 ratio) gave **13**R_P (5.2:1 ratio).

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Registry No. 1, 80556-17-8; **2**, 84558-47-4; **3**, 84558-48-5; **4** (isomer 1), 84558-46-3; **4** (isomer 2), 84558-49-6; **5** (isomer 1), 84558-49-6; **6**, 84558-51-0; **7** (isomer 1), 84558-52-1; **7** (isomer 2), 84558-53-2; **8** (isomer 1), 84621-23-8; **8** (isomer 2), 84621-24-9; **9**·Et_3N, 84621-25-0; **9**·Na, 84621-26-1; **10** (isomer 1), 84621-27-2; **10** (isomer 2), 84621-28-3; **11**·2(Et_3N), 84558-54-3; **11**·2Na, 84558-45-2; **12** (isomer 1), 84680-11-5; **12** (isomer 2), 84680-12-6; **13** (isomer 1), 84621-29-4; **13** (isomer 2), 84621-30-7; **14**, 84558-55-4; **16** (isomer 1), 84558-56-5; **16** (isomer 2), 84558-57-6; dimethyl methylphosphonate, 756-79-6; L-phenylalanine methyl ester, 2577-90-4; L-phenylalanine benzyl ester *p*-tosylate, 1738-78-9; methyl methylphosphonochloridate, 1066-52-0; L-phenylalanine amide, 5241-58-7; benzylamine, 100-46-9; **5** (isomer 2), 84558-58-7.

Cyclic Phosphonic–Carboxylic Imides and Anhydrides as Reactive Intermediates. 2. Solvolysis of *N*-(Hydroxy(methyl)phosphinothioyl)-L-phenylalanine Derivatives

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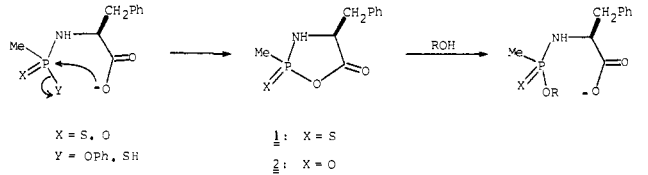
Abstract: Kinetic and stereochemical evidence is presented for the intermediacy of cyclic anhydrides Me-P(X)-L-Phe·O, **1** (X = S) and **2** (X = O), respectively, in the displacement of phenoxide from the phenyl ester **12** and H_2S from the dianion **10**. These substitution reactions both proceed with retention of configuration at phosphorus, indicating a clean, double inversion mechanism. In contrast, cleavage of the related methyl ester **11** takes place by direct attack of hydroxide on phosphorus in a simple inversion process. Analysis of the rates of appearance and disappearance of **12** during the course of alkaline hydrolysis of the diester precursor **5** indicates that the cyclic anhydride **1** is formed reversibly and that the relative rates at which it reacts with hydroxide, phenoxide, and water are 1.4×10^4, 7.6×10^3, and 1, respectively, at 22 °C.

In a continuation of our investigation of phosphonamidate peptide analogues as inhibitors of carboxypeptidase A,[1] we needed the diastereomers of phosphonamidothioates **10**R_P and **10**S_P (Scheme IV) in pure form. The hydrolytic behavior of these compounds and their precursors implicated the cyclic anhydrides **1** and **2** (Scheme I) as intermediates in a number of transformations, in analogy with our observations with the oxo derivatives described in the preceding paper.[2] For example, the diastereomers

Scheme I



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