1600, 1060, 1015, 975, 945; MS, m/e 138 (M^+), 120, 105, 94, 81, 79 (base peak), 67. Anal. Calcd for $C_9H_{14}O$: C, 78.26; H, 10.14. Found: C, 78.09; H, 9.95.

(+)-4-*exo*-Methylbicyclo[3.3.0]oct-2-en-8-*endo*-yl *p*-Toluenesulfonate (11). A solution of (+)-**10** (0.61 g; purity 82% by GLC; 3.6 mmol) and *p*-toluenesulfonyl chloride (0.9 g, 4.7 mmol) in pyridine (15 mL) was kept at 5 °C for 16 h. Extraction with CH_2Cl_2 and H_2O (2 times) and with cold aqueous 10% HCl (3 times), drying of the organic layer with $MgSO_4$, and evaporation of the solvent gave 1.05 g of (+)-**11** (purity 81% by GLC; 85% yield). A sample was chromatographed on Florisil (60–100 mesh; 1:40; ether–pentane, 1:1): $[\alpha]_D$ +38° (c 0.2); ^{1}H NMR δ 0.92 (3 H, d, J = 8 Hz), 1.35 + 1.55 + 1.67 + ca. 1.9 (each 1 H ,m), 2.27 (2 H, m), 2.37 (3 H, s), ca. 3.2 (1 H, m), 4.56 (1 H, s), 5.22 + 5.50 (each 1 H, d, J = 6 Hz), 7.25 + 7.71 (each 2 H, d, J = 8 Hz); IR 1600, 1450, 1355, 910; MS, m/e 120 [M^+ ($C_{16}H_{20}SO_3$) – $C_7H_7SO_3H$; base peak], 105, 93, 77.

Registry No. (–)-**2**, 88195-49-7; (–)-**3**, 88915-72-4; (+)-**4**, 88915-73-5; 8-*exo*-**4**, 88979-72-0; (+)-**5**, 88915-74-6; (+)-**6**, 88915-75-7; **7**, 88915-76-8; (+)-**8**, 88979-71-9; (+)-**9**, 88915-77-9; (+)-**10**, 88915-78-0; (+)-**11**, 88915-79-1.

Synthesis of Peptide Analogues Containing (2-Aminoethyl)phosphonic Acid (Ciliatine)[1]

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Di- and tripeptide analogues containing (2-aminoethyl)phosphonic acid [H–Aep(OH)$_2$], which has been discovered in a wide variety of living organisms, were prepared. As starting materials for incorporating the amino phosphonic acid into a peptide chain, the N-phthalylated diethyl ester [Pht–Aep(OEt)$_2$] and the N-carbobenzyloxylated monoalkyl ester [Cbz–Aep(OR)(OH), R = Me, Et, and Bzl] were used. A phosphonamide bond between the amino phosphonic acid unit and amino acid unit was formed by reaction of Pht–Aep(OEt)Cl with amino acid ethyl ester or coupling reaction between Cbz–Aep(OR)(OH) and amino acid ethyl ester using diphenylphosphoryl azide–triethylamine as a condensing agent. Removal of the protecting groups was studied in connection with the acid-labile phosphonamide bond in the abnormal peptides.

Introduction

Amino phosphonic acids, H_2H–R–PO_3H_2, may be considered to be analogues of amino carboxylic acids and amino sulfonic acids. (2-Aminoethyl)phosphonic acid (ciliatine) [1, H–Aep(OH)$_2$] would be the most interesting compound of the class and was discovered in a wide variety of living organisms ranging from lower protozoans to higher animals including man. Many reports have been concerned with the involvement of **1** in phosphonolipids,[2] while some have shown the presence of **1** in the proteinaceous fractions of sea anemones *Metridium dianthus*[3,4] and *Metridium senile*,[5] the protozoan *Tetrahymena pyriformis*,[6] as well as Chymotrypsin-like proteases from *M. senile*,[7] though the binding mode in the proteins has not been investigated thoroughly.[8]

Parallel with these studies, the synthesis of peptides containing amino phosphonic acids was carried out.[9-17] Biological activities of the artificial peptides appeared to be promising. For instance, L-alanyl-L-(1-aminoethyl)-phosphonic acid was shown to exhibit a very strong antibacterial activity, especially against Gram-negative organisms,[15] and *N*-[[[(carbobenzyloxy)amino]methyl]-hydroxylphosphinyl]-L-phenylalanine was found to function as a potent inhibitor against carboxypeptidase A.[16] Compound **1** was also reported to inhibit bacterial cell wall synthesis.[18]

The synthetic studies, however, dealt exclusively with unnatural (aminomethyl)phosphonic acids and their α-

(1) Abbreviations: Bzl = benzyl; Cbz = carbobenzyloxy; Np = p-nitrophenyl; Pht = phthalyl; H–(aa)–OH = amino acid; DMF = dimethylformamide; THF = tetrahydrofuran; TLC = thin layer chromatography. Derivatives of (2-aminoethyl)phosphonic acid are expressed as the following examples. $C_6H_5CH_2O_2CNHCH_2CH_2P(O)(OR_1)(OR_2)$ = Cbz–Aep(OR$_1$)(OR$_2$); $H_2NCH_2(O)NHCH_2CH_2P(O)(OC_2H_5)NHCH_2CO_2Li$ = H–Gly–Aep(OEt)–Gly(OLi).

(2) Glycerophosphonolipids: Smith, J. D.; Synder, W. R.; Law, J. H. *Biochem. Biophys. Res. Commun.* **1970**, *39*, 1163. Sugita, M.; Hori, T. *J. Biochem.* **1971**, *69*, 1149. Sphingophosphonolipids: Rouser, G.; Kritchefsky, G.; Heller, D.; Lieber, D. *J. Am. Oil Chem. Soc.* **1963**, *40*, 425. Hori, T.; Sugita, M.; Ando, S.; Tsukuda, K.; Shiota, K.; Tsuzuki, M.; Itasaka, O. *J. Biol. Chem.* **1983**, *258*, 2239. Sphingoglycophosphonolipids: Hayashi, A.; Matsuura, F. *Chem. Phys. Lipids* **1978** *22*, 9. Araki, S.; Komai, Y.; Satake, M. *J. Biochem.* **1980**, *87*, 503.

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(8) The amino phosphonic acid content of the proteins varied from sample to sample. Some had significantly high value in comparison to the number of total amino acid residues. These, together with various chemical tests, lend support to the hypothesis that **1** may be bound directly to the protein as constituents within the peptide backbone, directly to the side chains of the amino acid residues such as lysine, or indirectly to the protein via a lipid or a polysaccaride bridge (ref 3–7).

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(12) Hariharan, M.; Motekaitis, R. J.; Martell, A. E. *J. Org. Chem.* **1975**, *40*, 470.

(13) Gilmore, W. F.; McBride, H. A.; *J. Pharm. Sci.* **1974**, *63*, 965. Gilmore, W. F.; McBride, H. A. *Ibid.* **1974**, *63*, 1087.

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

(17) Chambers, J. R.; Isbell, A. F. *J. Org. Chem.* **1964**, *29*, 832; *Ibid.* **1972**, *37*, 4399. Wasielewski, C.; Antczak, K.; Rachon, J. *Pol. J. Chem.* **1978**, *52*, 1315.

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0022-3263/84/1949-1158$01.50/0

methyl and α-phenyl derivatives.[9-16] Further, most of the reports did not mention the conversion of protected peptides into the free forms.

In this paper we wish to describe the synthesis of the di- and tripeptide analogues which have naturally occuring 1 linked to an amino acid residue through a phosphonamide bond (abbreviated hereafter as a P–N bond). The stability of the free peptide analogues will also be discussed briefly in conjunction with the controversy over the presence of such a peptide structure in nature.

Results and Discussion

The amino function of 1 was as reactive to various reagents as that of an amino acid. By contrast, the phosphonic acid function was found to differ in reactivity from the carboxylic acid function. Hence, an amino acid unit could not be attached to the P-terminal of 1 by the usual methods. For instance, the N-protected amino phosphonic acids 3 and 8 hardly condensed with H–Gly–OEt by means of dicyclohexylcarbodiimide (DCC); similar results have been reported.[9,11] Thus, a main problem in the synthesis of the abnormal peptides is how to efficiently form the P–N bond between the amino phosphonic acid and amino acid.

Another problem which made the present study somewhat different from common peptide synthesis arose from the presence of a P–N bond which is labile under acidic conditions. This property requires that reactions of peptide intermediates having a P–N bond be performed under neutral or alkaline conditions and limit the choice of protecting groups of both 1 and the amino acids. In the present study, their amino functions were blocked by phthalylation or carbobenzyloxylation. The phosphonic acid and carboxylic acid functions were protected by esterification.

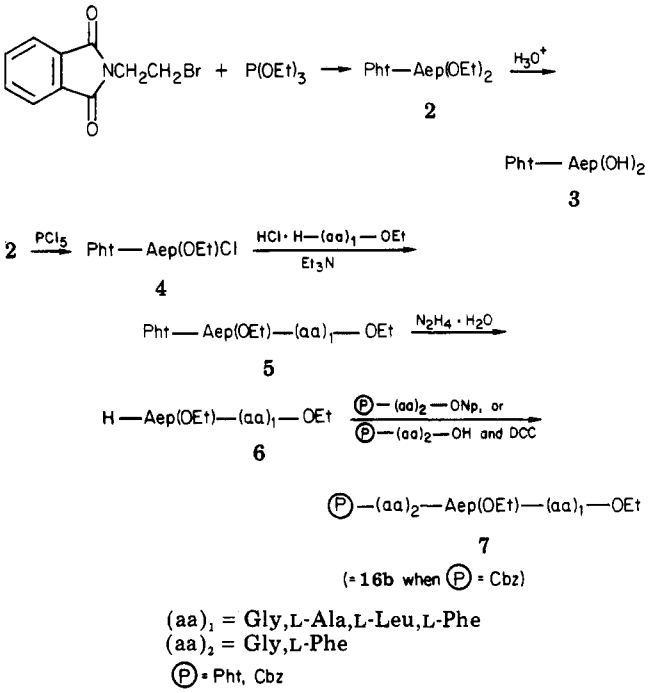
A. Phthalyl Group (Pht) for the Protection of the Amino Function of 1. The N-phthalylated diethyl ester 2 of 1 was chosen as the starting material; the compound was prepared by reaction of *N*-(2-bromoethyl)phthalimide and triethyl phosphite.[19] Taking advantage of the Pht group which was acid stable, 2 was treated with a slight excess of phosphorus pentachloride in benzene to give the phosphono monochloridate 4. Since the chlorination was nearly quantitative on the basis of the NMR spectrum of the reaction mixture, 4 without further purification was subsequently allowed to condense with various amino acid ethyl esters in the presence of triethylamine, affording the corresponding dipeptide analogues 5 in 58–81% yield (Scheme I).

The tripeptide analogues 7 which have an Aep unit in the center were obtained from the above fully protected dipeptides (5) by dephthalylation of 5 with hydrazine to amine 6 and a coupling reaction of 6 with N-protected amino acid *p*-nitrophenyl ester or with N-protected amino acid by means of DCC. The overall yields were 25–30%. The low yields are due to the low recovery of 6 from the dephthalylation reaction mixture and perhaps to a side reaction of the carboxylic acid ester moiety of 5 with hydrazine.

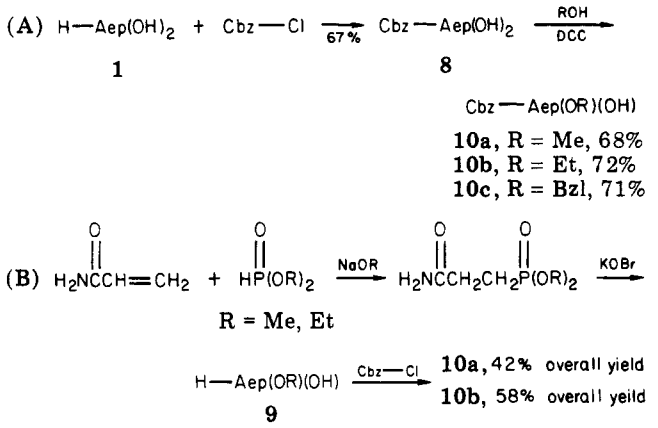
B. Carbobenzyloxy Group (Cbz) for the Protection of the Amino Function of 1. When the amino function of 1 is blocked by carbobenzyloxylation, synthesis of the abnormal peptides may become easier since the Cbz group will be readily unmasked by catalytic hydrogenolysis.

(19) The same Arbuzov reaction was reported previously. The compound 2, however, was not isolated but converted directly into 1 by an acid hydrolyis of the reaction mixture: Kosolapoff, G. M. *J. Am. Chem. Soc.* **1947**, *69*, 2112.

Scheme I



Scheme II

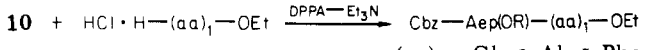


Scheme II illustrates synthetic routes to Cbz–Aep(OR)(OH) (10). These are (A) conversion of 1 with carbobenzoxy chloride (Cbz–Cl) into Cbz–Aep(OH)$_2$ (8) and esterification of 8 with alcohol (ROH, R = Me, Et, Bzl) by means of DCC and (B) the Michael reaction between dialkyl phosphite and acrylamide to give dialkyl (2-carbamylethyl)phosphonate, the Hofmann degradation of the amide moiety with concomitant hydrolysis of the diester moiety to afford alkyl (2-aminoethyl)phosphonate (9), and treatment of 9 with Cbz–Cl. Though route A was convenient, B proved superior from an economic point of view.

In order to prepare dipeptide analogues having a P–N bond, all previous studies adopted a step involving chlorination, e.g., treatment of N-protected amino phosphonic acid esters with $SOCl_2$ or PCl_5 and reaction of the resulting phosphono monochloridate with amino acid esters.[9-13,16] This method, however, turned out to be unfruitful for compound 10, resulting in the formation of a small amount of the dipeptide analogues and a complex mixture of polar compounds.

Consequently, various coupling agents were examined to permit direct condensation of 10 with amino acid esters. It was found that the phosphono dipeptide 11 was not formed by DCC and Woodward's K-reagent,[20] or the yield

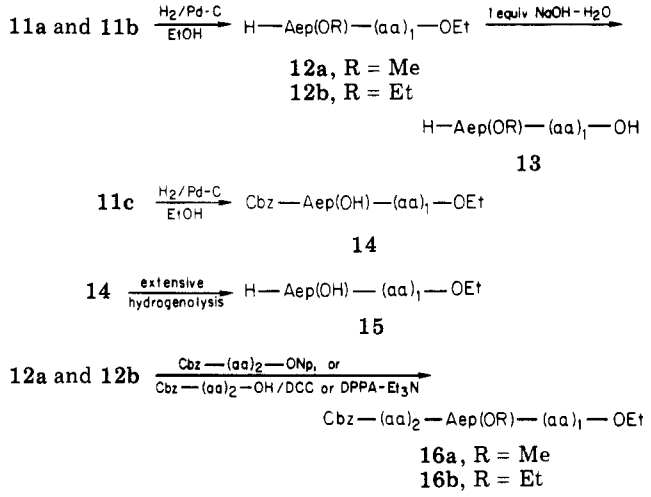
Scheme III[a]



$(aa)_1$ = Gly,L-Ala,L-Phe
11a, R = Me
11b, R = Et
11c, R = Bzl

[a] DPPA is $(C_6H_5O)_2P(=O)N_3$.

Scheme IV



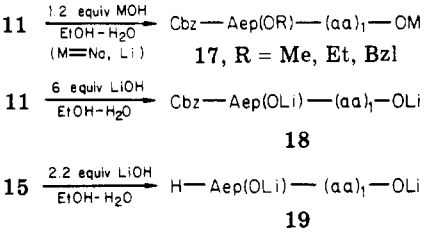
was poor with 2,4,6-triisopropylbenzenesulfonyl chloride[21] and 1-(mesitylsulfonyl)-3-nitro-1,2,4-triazole[22] (TLC yield <10%). In contrast, treatment of a mixture of **10** and amino acid ethyl ester hydrochloride with 20% excess of diphenylphosphoryl azide (DPPA)[23] and 2.2 equiv of triethylamine in THF or DMF at room temperature gave rise to the corresponding dipeptide analogues **11** in fairly good yields (40–65%).[24] These, except those containing a glycine unit, were often isolated as amorphous powders with broad melting ranges, and the NMR spectra suggested that the products were mixtures of two diastereomers due to the presence of asymmetric carbon and phosphorus atoms in the molecules, (Scheme III).

Catalytic hydrogenolysis of **11a** and **11b** using palladium carbon (Pd/C) removed the Cbz group to afford the corresponding dipeptide diesters (**12**) quantitatively. Interestingly, hydrogenolysis of **11c** unmasked the Bzl group preferentially to give **14**, which on continued hydrogenation eventually lost the Cbz group to yield **15**, (Scheme IV).

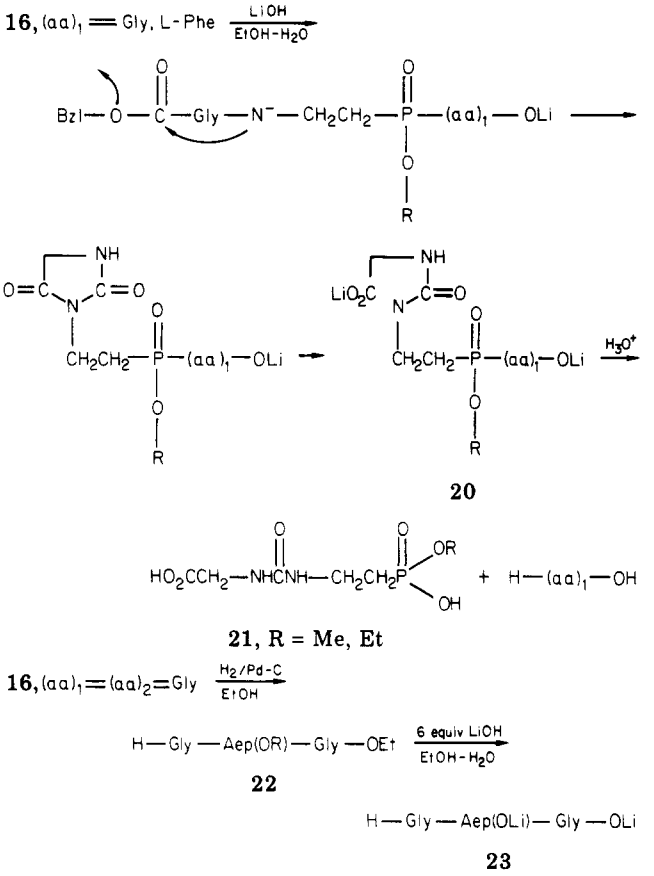
Preparation of tripeptide analogues **16** which have an Aep unit in the middle position was achieved by condensing **12** with Cbz amino acid *p*-nitrophenyl ester or with Pht and Cbz amino acid using DCC or DPPA–triethylamine as coupling agents. The overall yields of **16** from **11** were considerably higher than those of **7** from **5**, e.g., 45–65% vs. 24–30%, respectively.

Thus, though Pht–Aep(OEt)$_2$ (**2**) is useful for high yield conversion to the dipeptide analogues **5**, Cbz–Aep(OR)-(OH) (**10**) was superior for preparation of peptide analogues which contained an Aep unit between the amino acid units. The advantage came with the readily removable Cbz group and the P–N bond formation by DPPA–triethylamine.

Scheme V



Scheme VI



C. Unmasking of Protecting Groups. The *P*-benzyl ester moieties were unmasked by catalytic hydrogenolysis as mentioned previously. However, the *P*-methyl and *P*-ethyl ester moieties could not be cleaved readily. Both *P*-alkyl esters were more resistant to alkaline hydrolysis than the *C*-ethyl ester, though the *P*-methyl ester was hydrolyzed faster than the *P*-ethyl ester. For instance, treatment of **11** and **15** with 20% excess of aqueous lithium or sodium hydroxide at 25 °C for 1 h produced **17** and **19**, respectively, in quantitative yields, while conversion of **11** into **18** required use of a large excess of alkali and long reaction time (~24 h) (Scheme V). Further, it was found that **16** [$(aa)_1$ = Gly,L-Phe, $(aa)_2$ = Gly] furnished a urea derivative (**21**) and H–$(aa)_1$–OH on treatment with excess lithium hydroxide followed by acidification with hydrochloric acid. Conceivably, a hydantoin ring compound was generated in the first stage and then suffered base-catalyzed ring opening according to the pathways depicted in Scheme VI. This result contrasted with the normal hydrolysis of **11a** [$(aa)_1$ = Gly] to **17** under the similar conditions. Thus, prior to alkaline unmasking of the P- and

(20) Hall, P. L.; Perfetti, R. B. *J. Org. Chem.* **1974**, *39*, 111.
(21) Reese, C. B. *Tetrahedron*, **1978**, *34*, 3143. A treatment of a mixture of Cbz–Aep(OH)$_2$ and 1.2 equiv of H–L–Phe(OEt)·HCl with 1.2 equiv of 2,4,6-triisopropylbenzenesulfonyl chloride in dry pyridine (0 °C for 0.5 h and then room temperature for 4 h) gave Cbz–Aep(OH)–L–Phe(OEt) in less than 10% yield (TLC).
(22) Jones, S. S.; Reese, C. B. *J. Am. Chem. Soc.* **1979**, *101*, 7399.
(23) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.
(24) The mechanism of the phosphonamide formation was not clear. Cf. DPPA is capable of condensing carboxylic acid with amine to produce the amide, while action of the reagent on a mixture of carboxylic acid and alcohol furnishes the Curtius rearrangement product (amide) but not the ester. Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.

C-ester moieties of **16**, the Cbz group had to be deblocked by catalytic hydrogenolysis in order to convert **16** into the free form **23**.

Although other methods were investigated to deprotect the *P*-alkyl and *P*-benzyl ester moieties under neutral or weakly alkaline condition, none was successful. Action of thiophenol and sodium methyl mercaptan on **11a** resulted in the formation of **17** even though mercaptans are known to demethylate efficiently trimethyl phosphate in water.[25] Reaction of **11a** with trimethylsilyl bromide, which has been reported to dealkylate alkyl esters of phosphonic acids,[26] caused cleavage of the P–N bond, furnishing glycine ethyl ester chiefly and several unknowns.

D. Stability of Peptide Analogues Containing 1. The partially protected compounds (**12–15, 17, 18, 22**) were stable in aqueous solution when stored at <5 °C and pH 8. By contrast, the free peptide analogues such as **19** and **23** were quite labile, and it was difficult to isolate them in a pure state (see the Experimental Section). For instance, **19** [$(aa)_1$ = Gly] underwent fragmentation into **1** and glycine when its aqueous solution (pH ca. 7) was allowed to stand at room temperature overnight (a half-life of the 15 mM sample was 3–6 h at 25 °C). Compound **23** appeared to decompose substantially during purification by means of gel permeation chromatography. The instability can be attributed to the weak P–N bond and suggests that a peptide containing Aep residue(s) within the backbone may not exist in nature, or, if any, may be obtained as the fragment of the structure of ···–$(aa)_2$–$(aa)_1$–Aep$(OH)_2$ (see also note 8). Biological activities of the abnormal synthetic peptides will be reported later.

Experimental Section

Materials and Chromatographic Systems. Amino acids were commercially available and used without further purification. (2-Aminoethyl)phosphonic acid (**1**) was donated from Sogo-Yakko Co., Ltd., Sagamihara-shi 229, Japan. Column chromatography was carried out using silica gel (Merck, 70–230 mesh). TLC analysis were performed on silica gel (Merck, precoated sheet, 60 F_{254}) and cellulose (Eastman Chromagram sheet), which was impregnated with pH 7 buffer (0.05 M KH_2PO_4–0.2 M NaOH) prior to use. The following procedures are typical. Yields and physical constants including mp, chromatographic, and NMR data are listed in Table I.

Pht–Aep(OEt)$_2$ (2). A mixture of *N*-(2-bromoethyl)phthalimide (20.0 g, 78.7 mmol) and triethyl phosphite (16 mL, 93 mmol) was refluxed in an oil bath (ca. 180 °C) for 2 h with passing nitrogen gas through the solution.[19] Low boiling substances were removed under reduced pressure while the bath temperature was raised gradually from ca. 100 to 160 °C. The resulting residue was diluted with diethyl ether–*n*-hexane and chilled in a freezer to precipitate 13 g of crude product **2**, which was recrystallized from the same solvent, 10.4 g (43%).

Cbz–Aep(OH)$_2$ (8). A solution of **1** (13 g, 0.10 mol) in 2 N sodium hydroxide (100 mL) was stirred vigorously with a toluene solution of carbobenzoxy chloride (21 g, 0.12 mol) at ca. −10 °C for 30 min and then at room temperature for 2 h. After extraction of the excess chloride with diethyl ether, the aqueous solution was acidified to pH 2 with hydrochloric acid to give crystals of the monosodium salt of **8**, which was subsequently converted into the acid form (**8**) by means of a cation exchange resin (H^+ form), 18 g (67%).

Cbz–Aep(OMe)(OH) (10a). Bromine (11 mL, 0.21 mol) was added to 4 N potassium hydroxide (260 mL) at 0 °C. Dimethyl (2-carbaminoethyl)phosphonate[27] (32 g, 0.18 mol) which was dissolved in water (100 mL) was added to the resulting KOBr solution with vigorous sitrring at 0 °C. When the mixture became homogeneous, it was warmed in a water bath of 70 °C with magnetic stirring for 3 h. The reaction mixture was then cooled to 0 °C, neutralized with aqueous hydrobromic acid, and concentrated. The sodium bromide was extracted with ethanol, and the organic solution was concentrated to give **9** (R = Me, 19 g) as a viscous substance, which was then carbobenzyloxylated without further purification as in the preparation of **8**, 20.6 g (42%).

Cbz–Aep(OBzl)(OH) (10c). A mixture of **8** (3.0 g), benzyl alcohol (6.0 g), triethylamine (1.4 g), and DCC (2.9 g) in DMF (50 mL) was heated at 80 °C for 5 h with stirring magnetically. Dicyclohexylurea was removed by suction filtration, and the filtrate was concentrated under reduced pressure. The resulting crude product was recrystallized from acetone, 2.85 g (70%).

Pht–Aep(OEt)–Gly(OEt) [5, $(aa)_1$ = Gly]. A benzene solution (100 mL) of **2** (5.0 g, 16 mmol) and phosphorus pentachloride (3.4 g, 16.3 mmol) was refluxed for 10 h. The reaction mixture was distilled under reduced pressure to remove low boiling substances. The yellow residue, which was chiefly the phosphono monochloridate **4**, was dissolved in freshly distilled chloroform (50 mL) and added dropwise to a cold chloroform solution (ca. 50 mL) of glycine ethyl ester hydrochloride (2.70 g, 19.3 mmol) and dry triethylamine (5.3 mL, 38 mmol). After the addition, the mixture was stirred overnight at room temperature. The solvent was then evaporated to give the residue which was partitioned between chloroform and water. The organic layer was washed with 10% sodium bicarbonate and water and then dried with anhydrous calcium sulfate. Removal of the solvent afforded 5.5 g of the crude product. This material was dissolved in a small amount of diethyl ether and stored in a freezer to give the pure compound as a powder, 4.07 g (69%).

Cbz–Aep(OMe)–Gly(OEt) [11a, $(aa)_1$ = Gly]. A mixture of **10a** (5.5 g, 20 mmol), DPPA (5.2 mL, 24 mmol), triethylamine (6.6 mL, 48 mmol), and glycine ethyl ester hydrochloride (3.4 g, 24 mmol) in DMF (100 mL) was stirred at room temperature for 3 days. The solvent was evaporated under reduced pressure below 50 °C, and the residue was mixed with THF. After removal of the precipitate of triethylammonium chloride with suction filtration, the filtrate was concentrated by a rotary evaporator. The resulting residue in chloroform was washed successively with water, 10% sodium bicarbonate, and water and then dried with anhydrous calcium sulfate. Removal of the solvent gave crude product. This material became crystalline upon dissolving in a small amount of diethyl ether and storing in a freezer, 4.7 g (65%).

Pht–Gly–Aep(OEt)–Gly(OEt) [7, $(aa)_1$ = $(aa)_2$ = Gly]. To a solution of **5** [$(aa)_1$ = Gly, 1.1 g, 3.0 mmol) in ethanol (20 mL) was added 1 N ethanolic hydrazine hydrate (3.4 mmol). After stirring overnight at room temperature, the reaction mixture was filtered with suction to remove diketophthalazine, and concentrated under reduced pressure below 30 °C. The residue was mixed with anhydrous THF to precipitate another crop of the byproduct. Removal of the solvent from the filtrate furnished a yellow oil, which was found to contain chiefly the corresponding amine **6** according to the NMR spectrum. The amine and Pht–Gly–OH (0.5 g, 2.5 mmol) was then dissolved in anhydrous THF (30 mL), and the cooled solution in an ice water bath was mixed with DCC (0.6 g, 3.0 mmol). After stirring at 0–5 °C for 1 h and then at room temperature for 15 h, the reaction mixture was filtered with suction, concentrated, and diluted with chloroform. The organic solution was washed with 10% aqueous sodium bicarbonate and water and then dried with anhydrous calcium sulfate. Removal of the solvent afforded a crude product. This material was purified by a silica gel column chromatography using a mixture of chloroform and ethanol (25:1 v/v) as a solvent, 0.31 g (24%).

Cbz–Gly–Aep(OMe)–Gly(OEt) [16a, $(aa)_1$ = $(aa)_2$ = Gly]. The compound **11a** [$(aa)_1$ = Gly, 1.68 g, 4.51 mmol] was hydrogenated by permitting hydrogen gas to pass through its ethanol solution (20 mL) in the presence of Pd/C (10%, 0.5 g) at room temperature for 1 h. The catalyst was removed by suction filtration, and the filtrate was concentrated, dissolved in dry pyridine, and again concentrated to give **12a** [$(aa)_1$ = Gly] as an oil. (Carbobenzyloxy)glycine (1.06 g, 5.10 mmol) and DCC (1.1 g, 5.3 mmol) were then added to the amine in THF (30 mL). After

(25) Yamauchi, K.; Sugimae, T.; Kinoshita, M. *Tetradedron Lett.* **1977**, 1199.
(26) McKenna, C. E.; Huga, M. T.; Cheung, N. E.; McKenna, M. C. *Tetrahedron Lett.* **1977**, 155. Rabinowitz, R. *J. Org. Chem.* **1963**, *28*, 2975.
(27) Finkelstein, J. *J. Am. Chem. Soc.* **1946**, *68*, 2397.

Table I. Compounds Prepared, Yield %, mp, R_f,[a] and Representative Signals of ^{1}H NMR Spectra[b]

Pht-Aep(OEt)$_2$ (2): 43%; 53-58 °C; δ ($CDCl_3$) 1.30 (t, 3, CH_3), 2.20 (d of t, 2, CH_2P), 4.40 (m, 6, NCH_2 and 2 OCH_2), 7.80 (m, 4, C_6H_4)

Cbz-Aep(OH)$_2$ (8): 67%; 105 °C; δ ($CDCl_3$) 1.9-2.4 (d of t, 2, CH_2P), 3.3-3.7 (m, 2, NCH_2), 5.23 (s, 2, CH_2 of Cbz), 7.55 (s, 5, C_6H_5)

Cbz-Aep(OMe)(OH) (10a): 42%; 95-97 °C; δ ($CDCl_3$) 1.97 (t of t, 2, CH_2P), 3.42 (t of t, 2, NCH_2), 3.62 (d, 3, CH_3), 5.04 (s, 2, CH_2 of Cbz), 7.24 (s, 5, C_6H_5)

Cbz-Aep(OEt)(OH) (10b): 58%; 84-85 °C; δ ($CDCl_3$) 1.27 (t, 3, CH_3), 1.98 (t of t, 2, CH_2P), 3.46 (d of t, 2, NCH_2), 4.06 (d of t, 2, $POCH_2$)

Cbz-Aep(OBzl)(OH) (10c): 70%; 131-133 °C; δ ($CDCl_3$, 60 MHz) 1.95 (d of t, 2, CH_2P), 3.42 (d of t, 2, CH_2CH_2P), 5.02 (d, 2, CH_2 of Bzl), 5.07 (s, 2, CH_2 of Cbz)

Pht-Aep(OEt)-Gly(OEt) (5): 69%; 63-67 °C; δ ($CDCl_3$) 0.97 (t, 3, $COCH_2CH_3$), 1.27 (t, 3, $POCH_2CH_3$), 2.09-2.45 (m, 2, CH_2P), 3.33-ca. 3.8 (m, 2, CH_2CH_2P)

Pht-Aep(OEt)-L-Phe(OEt) (5): 68%; 105-130 °C; δ ($CDCl_3$, 60 MHz) 0.92 (t, 3, $COCH_2CH_3$), 1.35 (t, 3, $POCH_2CH_3$), 1.5-2.3 (m, 2, CH_2P), 4.20 (d of q, 2, $POCH_2$)

Cbz-Aep(OMe)-Gly(OEt) (11a): 65%; 47-55 °C; R_f 0.35 (s, A); δ ($CDCl_3$) 1.25 (t, 3, CH_3), 1.98 (d of t, 3, CH_3), 3.33 (m, 2, CH_2CH_2P), 3.50 (d, 3, $POCH_3$)

Cbz-Aep(OEt)-Gly(OEt) (11b): 74%; 67-69 °C; R_f 0.34 (s, A); δ ($CDCl_3$) 1.25 (t, 3, CH_3), 1.27 (t, 3, CH_3), 1.96 (d of t, 2, CH_2P), 5.08 (s, 2, $CH_2C_6H_5$)

Cbz-Aep(OMe)-L-Phe(OEt) (11a): 43%; 109-111 °C; R_f 0.34 (s, A); δ ($CDCl_3$) 1.25 (t, 3, CH_2CH_3), 1.4-1.9 (m, 2, CH_2P), 4.14 (q, 2, CH_2CH_3), 5.04 (s, 2, $CH_2C_6H_5$)

Cbz-Aep(OMe)-L-Ala(OEt) (11a): 36%; 53-65 °C; R_f 0.32 (s, A); δ ($CDCl_3$) 1.18-1.51 (m, 9, $3CH_3$), 3.61[c] (d, 2, $POCH_2$), 4.15 (q, 2, $COCH_2$), 5.12 (s, 2, $CH_2C_6H_5$)

Cbz-Aep(OEt)-L-Phe(OEt) (11b): 50%; 87-110 °C; R_f 0.34 (s, A); δ ($CDCl_3$) 1.21[c] (t, 3, $POCH_2CH_3$), 1.25 (t, 3, $COCH_2CH_3$), 2.18 (br s, 1, PNH), 4.21 (q, 2, $COCH_2$)

Cbz-Aep(OBzl)-Gly(OEt) (11c): 42%; 58-65 °C; R_f 0.41 (s, A); δ ($CDCl_3$) 1.24 (t, 3, CH_3), 2.00 (d of t, 2, CH_2P), 3.2-3.5 (br q, 2, $PNCH_2$), 3.60 (d of t, 2, CH_2CH_2P)

Cbz-Aep(OBzl)-L-Phe(OEt) (11c): 53%; 68-83 °C; R_f 0.37 (s, A); δ ($CDCl_3$) 1.25[c] (t, 3, CH_3), 1.4-2.0 (m, 2, CH_2P), 4-4.3 (m, 3, $COCH_2$ and PNH), 5.03 (s, 2, CH_2 of Cbz)

Pht-Gly-Aep(OEt)-Gly(OEt) (7): 24%; 168-170 °C; R_f 0.33 (s, B); δ ($CDCl_3$, 60 MHz) 1.20 (t, 3, CH_3), 1.26 (t, 3, CH_3), 4.05 (d, 2, $PNCH_2$), 4.30 (s, 2, Pht-CH_2)

Pht-L-Ala-Aep(OEt)-L-Ala(OEt) (7): 30%; 109-115 °C; R_f 0.25 (s, B); δ ($CDCl_3$) 1.2-1.5 (complex m, 9, $3CH_3$), 1.69 (d, 3, Pht-$NCHCH_3$), ca. 1.4-2.2 (m, 2, CH_2P)

Cbz-Gly-Aep(OMe)-Gly(OEt) (16a): 45%; 81-84 °C; R_f 0.20 (s, A); δ ($CDCl_3$) 1.24 (t, 3, CH_2CH_3), 1.5-2.1 (complex m, 3, CH_2PNH), 3.54 (d, 3, $POCH_3$), 3.79 (d, 2, $O{=}CNHCH_2$)

Cbz-Gly-Aep(OEt)-Gly(OEt) (16b): 61%; 112-113.5 °C; R_f 0.24 (s, A); δ ($CDCl_3$) 1.23 (t, 3, CH_3), 1.25 (t, 3, CH_3), 1.75-2.18 (m, 2, CH_2P), 4.17 (q, 2, OCH_2P)

Cbz-Gly-Aep(OEt)-L-Phe(OEt) (16b): 37%; 35-55 °C; R_f 0.34 (s, A); δ ($CDCl_3$) 1.36-1.82 (m, 2, CH_2P), 4.21 (q, 2, OCH_2), 5.15 (s, 2, $CH_2C_6H_5$), 7.15-7.24 (m, 5, $CHCH_2C_6H_5$)

H-Aep(OEt)-Gly(OH) (13): 70%; 148-150 °C dec; R_f 0.35 (c, C); δ (D_2O) 1.32 (t, 3, CH_3), 2.27 (d of t, 2, CH_2P), 3.33 (d of t, CH_2CH_2P), 3.56 (d, 2, $CH_2C{=}O$)

H-Aep(OH)-Gly(OEt) (15): 78%; 167 °C dec; R_f 0.31 (c, D); δ (D_2O) 1.26 (t, 3, CH_3), 2.00 (d of t, 2, CH_2P), 3.67 (d, 2, NCH_2), 4.17 (q, 2, OCH_2)

Cbz-Aep(OMe)-Gly(ONa) (17): 91%; ca. 200 °C dec; R_f 0.73 (c, C); δ (D_2O) 2.03 (d of t, 2, CH_2P), 3.1-ca. 3.5 (m, 2, CH_2CH_2P), 3.48 (d, 2, $NCH_2C{=}O$)

Cbz-Aep(OLi)-Gly(OLi) (18): 52%; >300 °C; R_f 0.49 (c, C); δ (D_2O) 1.82 (d of t, 2, CH_2P), 3.40 (d of t, 2, CH_2CH_2P), 3.42 (d, 2, CH_2NP)

Cbz-Aep(OLi)-L-Ala(OLi) (18): 49%; >300 °C; R_f 0.56 (c, C); δ (D_2O) 1.27 (d, 3, CH_3), 1.75 (m, 2, CH_2P), 3.33 (m, 2, CH_2CH_2P), 3.60 (m, 1, $CHCH_3$)

Cbz-Aep(OLi)-L-Phe(OLi) (18): 24%; >300 °C; R_f 0.26 (s, E); δ (D_2O) 1.21-1.67 (m, 2, CH_2P), 2.85-3.51 (m, 4, CH_2CH_2P and $CH_2C_6H_5$), 5.09 (s, 2, $CH_2C_6H_5$)

H-Aep(OLi)-Gly(OLi) (19): ~50%; R_f < 0.05 (c, C); δ (D_2O) 1.89 (d of t, 2, CH_2P), 3.18 (d of t, 2, CH_2CH_2P), 3.42 (d, 2, NCH_2CO)

H-Gly-Aep(OLi)-Gly(OLi) (23): ~50%; R_f < 0.05 (s, D); δ (D_2O) 1.86 (d of t, 2, CH_2P), 3.40 (d, 2, $PNHCH_2$), ca. 3.4 (m, 2, CH_2CH_2P), 3.74 (s, 2, NCH_2CO)

20: 79%; δ (D_2O) 1.18 (t, 3, CH_3), 1.67 (d of t, 2, CH_2P), 2.6-3.3 (complex m, 4), 3.67 (s, 2, CH_2N), 3.5-3.9 (m, 4)

21: 60%; 122-123.5 °C; R_f 0.12 (s, D), 0.23 (s, E); δ (D_2O) 1.36 (t, 3, CH_3), 2.21 (d of t, 2, CH_2P), 3.57 (d of t, 2, CH_2CH_2P), 4.09 (s, 2, CH_2N), 4.28 (q of d, 2, OCH_2)

[a] R_f is mobility in TLC. Chromatography supports (silica gel = s, cellulose = c) are described in the Experimental Section. Solvents were: A, chloroform-ethanol = 25-1 v/v; B, chloroform-ethanol = 15-1 v/v; C, *n*-propanol-water = 10-3 10-3 v/v; D, 2-propanol-concentrated ammonia = 4-1 v/v; E, 2-propanol-concentrated ammonia = 5-2 v/v. TLC plates and solvents are indicated in parenthesis in that order. [b] ^{1}H NMR spectra were recorded on a Jeol PS-100 (100 MHz) and a Hitachi-Perkin Elmer R-20 (60 MHz) spectrometer using dilute solutions in $CDCl_3$ and D_2O with tetramethylsilane and sodium [$^2H_4$]-3-(trimethylsilyl)propionate as internal standards, respectively. Unless stated in spectral data, the measurements were done by means of the 100-MHz instrument. [c] Each doublet or triplet peak was split into two peaks, suggesting that the compound may be a mixture of two diastereomers. See supplementary material for more details.

stirring the mixture overnight at room temperature, a byproduct, dicyclohexylurea, was filtered with suction, and the filtrate was concentrated. The residue was subsequently applied to a silica gel column, and the title compound was eluted by using a mixture of chloroform and ethanol (25:1 v/v), 0.86 g (45%).

H-Aep(OEt)-Gly(OH) [13, $(aa)_1$ = Gly]. Hydrogen gas was passed through a magnetically stirred mixture of **11b** [$(aa)_1$ = Gly, 2.0 g, 5.4 mmol] and Pd/C (10%, 0.5 g) in ethanol (30 mL) at atmospheric pressure and 25 °C for 2 h. The catalyst was removed by suction filtration, and the filtrate was mixed with 0.25 N sodium hydroxide and then stirred at room temperature for 1 h. The reaction misture was washed with diethyl ether, acidified to pH ca. 4 with 1 N hydrochloric acid, and concentrated. The resulting residue was treated with gel permeation chromatography (Sephadex G-10, 2.5 cm × 60 cm). The fraction which was positive to ninhydrin test and negative to aqueous silver nitrate was

concentrated below 30 °C to give the title compound, 0.79 g (70%).

Cbz-Aep(OLi)-Gly(OLi) [18, $(aa)_1$ = Gly]. An ethanol solution (30 mL) of 11 [$(aa)_1$ = Gly, R = Me, 2.0 g, 5.6 mmol) was mixed with 2 N lithium hydroxide (17 mL). After 24 h at room temperature, the reaction mixture was neutralized with 1 N hydrochloric acid and concentrated below 30 °C. Upon mixing the residue with anhydrous ethanol, the title compound was precipitated as a powder, which was purified by reprecipitation using water and ethanol as dissolving and precipitating solvents, respectively, 1.96 g (52%).

H-Gly-Aep(OLi)-Gly(OLi) (23). The tripeptide diester **16a** (0.24 g, 0.58 mmol) was hydrogenated using Pd/C (10%, 0.12 g) in ethanol (10 mL) at atmospheric pressure and 25 °C for 1 h. After removal of the catalyst with suction filtration, the filtrate was mixed with 2 N lithium hydroxide (1.7 mL) and allowed to stand at room temperature for 20 h. The hydrolysate was then neutralized with 1 N hydrochloric acid and concentrated to afford the residue, which upon triturating with anhydrous ethanol gave amorphous powder, 0.11 g. The ^1H NMR spectrum agreed with the assigned structure of the title compound, but also indicated the presence of a small amount of impurities including glycine. A gel permeation chromatography (Sephadex G-10) was useless to purify the free peptide analogue, since the compound decomposed considerably during the treatment.

The Dilithium Salt of *N*-(Carboxymethyl)-*N'*-(2-(hydroxyethoxyphosphinyl)ethyl)urea (21). To a cooled ethanolic solution (5 mL) of **16b** [$(aa)_1$ = L-Phe, 0.54 g, 1.05 mmol) was added 1 N lithium hydroxide (3.1 mL). Stirring was continued at room temperature for 5 h. The reaction mixture was then neutralized with 1 N hydrochloric acid and concentrated under reduced pressure below 20 °C. The resulting residue was triturated with anhydrous ethanol to give amorphous powder, which was collected with filtration, washed with ethanol, and dried in a desiccator, 0.39 g. The ^1H NMR spectra and the elemental analysis favored the structure of an intermediate [**20**, $(aa)_1$ = L-Phe, R = Et].

The powder was dissolved in water (5 mL), and the pH of the solution was adjusted to ca. 1 by concentrated hydrochloric acid. After 1 h at room temperature, the solution showed the spots of L-phenylalanine (R_f 0.79) and **21** (R_f 0.28) in cellulose TLC with the solvent D. The solvent was evaporated, and the resulting residue was applied to a cation exchange resin (Amberlite 120B, H^+ form, 1 cm × 20 cm). Elution with ca. pH 2 hydrochloric acid gave **21** in the first 50 mL, which was concentrated and recrystallized from a mixture of diethyl ether and ethanol, 0.16 g (overall yield 60%).

Registry No. **1**, 2041-14-7; **2**, 62514-90-3; **4**, 78157-52-5; **5** [$(aa)_1$ = Gly], 78157-53-6; **5** [$(aa)_1$ = Phe], 82155-09-7; **5** [$(aa)_1$ = Ala], 82155-08-6; **6** [$(aa)_1$ = Gly], 82155-18-8; **6** [$(aa)_1$ = Ala], 88981-16-2; **7** [$(aa)_1$ = $(aa)_2$ = Gly], 88981-17-3; **7** [$(aa)_1$ = $(aa)_2$ = Ala], 88981-18-4; **8**, 88981-19-5; **8**·Na, 88981-20-8; **9** (R = Me), 82155-11-1; **10a** (R = Me), 82155-13-3; **10b** (R = Et), 82155-14-4; **10c** (R = bzl), 88981-21-9; **11a** [$(aa)_1$ = Gly], 82155-15-5; **11a** [$(aa)_1$ = Phe], 82155-17-7; **11a** [$(aa)_1$ = Ala], 82155-16-6; **11b** [$(aa)_1$ = Gly], 82168-78-3; **11b** [$(aa)_1$ = Phe], 82168-79-4; **11c** [$(aa)_1$ = Gly], 88981-22-0; **11c** [$(aa)_1$ = Phe], 88981-23-1; **12a** [$(aa)_1$ = Gly], 88981-24-2; **12b** [$(_aa)_1$ = Gly], 82155-18-8; **13** [$(aa)_1$ = Gly], 82155-22-4; **15** [$(aa)_1$ = Gly], 88981-25-3; **16a** [$(aa)_1$ = $(aa)_2$ = Gly], 88981-26-4; **16b** [$(aa)_1$ = $(aa)_2$ = Gly], 82155-20-2; **16b** [$(aa)_1$ = $(aa)_2$ = Phe], 88981-27-5; **17** [R = Me, $(aa)_1$ = Gly, M = Na], 88981-28-6; **18** [$(aa)_1$ = Gly], 82155-23-5; **18** [$(aa)_1$ = Ala], 82155-24-6; **18** [$(aa)_1$ = Phe], 82155-25-7; **19** [$(aa)_1$ = Gly], 88981-29-7; **20**, 88981-30-0; **21** (R = Et), 88981-31-1; **22** (R = Me), 88981-32-2; **23**, 88981-33-3; H-Gly-OEt·HCl, 623-33-6; H-Phe-OEt·HCl, 3182-93-2; Pht-Gly-OH, 4702-13-0; Pht-Ala-OH, 4192-28-3; *N*-(2-bromoethyl)phthalimide, 574-98-1; triethyl phosphite, 122-52-1; carbobenzoxy chloride, 501-53-1; dimethyl (2-carbaminoethyl)phosphonate, 2526-69-4; (carbobenzyloxy)glycine, 1138-80-3; diethyl (2-carbaminoethyl)phosphonate, 2526-67-2.

Supplementary Material Available: Experimental details, full ^1H NMR data, $[\alpha]^{25}_D$, mobilities in TLC, and elemental analyses (14 pages). Ordering information is given on any current masthead page.

Fluorine-Containing Amino Acids and Their Derivatives. 3.[1] Stereoselective Synthesis and Unusual Conformational Features of *threo*- and *erythro*-3-Fluorophenylalanine

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Reductive amination of *p*-substituted 3-fluorophenylpyruvic acids gave *p*-substituted *erythro*-3-fluorophenylalanines with high stereoselectivity. *threo*-3-Fluorophenylalanine was prepared by enzymatic hydrolysis of the *threo*-3-fluorophenylalanine isopropyl ester. NMR spectroscopic population analysis of rotamers of both erythro and threo diastereomers revealed that stabilization interactions (Coulombic attraction and/or hydrogen bonding) between fluorine and the NH_2 group are very important factors for the selection of stable rotational conformations in solution. Based on this information, an explanation is proposed for the remarkably high erythro selectivity observed in the reductive amination reaction. Single-crystal X-ray analysis of both diastereomers showed that the conformations in the solid state are mainly controlled by steric factors of the functional groups involved.

Introduction of fluorine into pharmacologically active substances can often lead to the development of more potent agonists or antagonists, as has been widely documented.[3] In the amino acid and peptide field also, a

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