α-Functionalized Phosphonylphosphinates: Synthesis and Evaluation as Transcarbamoylase Inhibitors

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Diverse α-methyl-substituted phosphonylphosphinates (P−C−P−C−X) are accessible from a protected, pentafluorophenylsulfonated phosphonylphosphinate via nucleophilic displacement. The utility of this route is demonstrated with several nitrogen nucleophiles. The resulting amine and amino acid phosphonylphosphinate derivatives were evaluated as inhibitors of *Streptococcus faecalis* ornithine transcarbamoylase (OTC). Compared with the structurally related phosphonoacetyl-L-ornithine (L-PALO), a known inhibitor of OTCs from various sources, the phosphonylphosphinates are surprisingly poor inhibitors, binding several orders of magnitude less tightly to the enzyme. These results suggest that the tetrahedral intermediate formed in the normal transcarbamoylase reaction is poorly mimicked by a tetrahedral and anionic phosphonate, either because of directly unfavorable interactions with a hydrogen-bond acceptor within the active site or because transition-state analogues are unable to induce the protein conformation changes that normally accompany reaction.

Introduction

Phosphonates and phosphinates are useful moieties for probing biological function and for creating high-affinity enzyme inhibitors. Methylenebis(phosphonate), for example, is a nonhydrolyzable surrogate for pyrophosphate that has been successfully incorporated into inhibitors of several enzymes that process pyrophosphorylated substrates, including squalene synthase,[1] prenyl transferase,[2] glutamine synthetase,[3] and a nucleoside phosphorylase.[4] Phosphonates have been similarly employed as potent kinase inhibitors[5] and, because of their anionic character and tetrahedral geometry, as transition-state analogues for acyl-transfer reactions[6] and as haptens for eliciting catalytic antibodies.[7,8]

Pyrophosphates   Phosphonylphosphinates   Reaction with phosphoanhydride
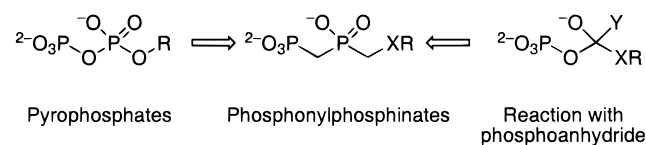
By analogy, α-functionalized phosphonylphosphinates, which combine both motifs in a single molecule, are potentially interesting as inhibitors of enzymatic reactions involving phosphoanhydrides. Compounds such as **1**, for instance, could be considered as "expanded transition-state analogues"[9] for the reaction of L-ornithine with carbamoyl phosphate to give L-citrulline and inorganic phosphate (Scheme 1). This reaction, which is catalyzed by the enzyme ornithine transcarbamoylase (OTC), is a key step in the biosynthesis of arginine and also important in the detoxification of ammonia in terrestrial vertebrates.[10] It occurs via reactive interme-

Scheme 1
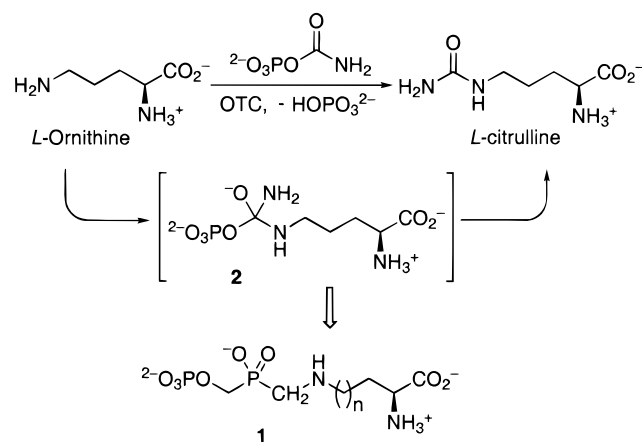
diate **2** which is formed when the δ-amino group of ornithine attacks the carbonyl center of carbamoyl phosphate. Compound **1** mimics the transition state leading to this high-energy species: Its terminal phosphonate corresponds to the phosphate leaving group, the phosphinate moiety mimics the tetrahedral, anionic center undergoing attack, and the additional methylene group inserted between the phosphinate phosphorus and the nitrogen simulates the extended developing bond between the incoming nucleophile and the carbonyl center. Requirements with respect to the nucleophile in this reaction can be explored by variation in the amino acid side chain of **1**.

Compounds such as **1** are not directly accessible by existing methodologies for preparing substituted phosphonylphosphinates.[11] Procedures involving alkylation of phosphonylphosphonites with alkyl halides at elevated temperatures[2] or of phosphonylphosphinyl dianions at low temperatures[12] have been described, but α-functionalized phosphonylphosphinates (P−C−P−

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Scheme 2
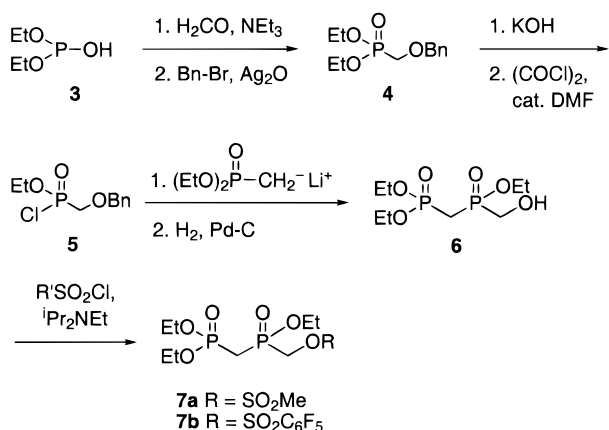
C−X compounds with variable X) cannot be prepared by these methods. Addition of the carbanion derived from diethyl methylphosphonate to an appropriately derivatized and activated phosphonate ester to form the central P−CH$_2$−P moiety would be an alternative approach,[1,13,14] but this strategy cannot be used for compounds such as **1** containing sensitive functionality in the side chain. Here we report an efficient and general route to α-functionalized phosphonylphosphinates via nucleophilic substitution on an appropriately activated derivative of a phosphonomethyl(hydroxymethyl)phosphinate. The scope of this approach is illustrated with several nitrogen nucleophiles. The resulting compounds were tested as inhibitors of OTC.

Results

Synthesis of Phosphonylphosphinates. The synthesis of α-functionalized phosphonylphosphinates is complicated by the base sensitivity of methylphosphonates bearing electron-withdrawing α-substituents[15,16] and by the very low reactivity at the phosphinyl methyl carbon.[17] We anticipated, however, that α-functionalized phosphonylphosphinates might be readily derived from **6** (Scheme 2). Activation of the alcohol as a suitably tuned sulfonate ester, followed by nucleophilic displacement, would yield the desired compounds. Such an approach would have the significant advantage that multiple derivatives bearing different side chains could be prepared from a common intermediate.

Alcohol **6** was synthesized in gram quantities starting from diethyl phosphite in a six-step sequence (Scheme 2). Addition of formaldehyde to diethyl phosphite, followed by the protection of the hydroxymethyl group with benzyl bromide/silver(I) oxide, yielded **4**. In our hands, this sequence of reactions was found to be superior to previous routes to **4**.[18,19] Partial saponification with potassium hydroxide in ethanol/water, followed by treatment with oxalyl chloride and catalytic amounts of DMF, yielded acid chloride **5**. The latter was easily coupled with lithiated diethyl methylphosphonate. Deprotection of the resulting adduct by catalytic hydrogenation gave alcohol **6** in an overall yield of 30%.

Although diethyl hydroxymethylphosphonate can be triflated in good yield by various methods (unpublished results),[20] all attempts to triflate **6** failed. Mesylate **7a**, in contrast, is readily formed. Unfortunately, its reactivity is so low that even with sodium azide in DMF at 65

°C no reaction occurs within 48 h, and **7a** is quantitatively recovered. Pentafluorophenylsulfonates are known to solvolyze at rates intermediate between the triflate and the mesylate,[21−23] but they have not been previously exploited in synthesis, presumably because low yields in their preparation have been claimed as a major drawback.[24] We have found, however, that the pentafluorophenylsulfonate **7b** can be prepared in even higher yield than the corresponding mesylate. Moreover, compound **7b** is crystalline, is stable in air, and can be stored at −78 °C for over a year without decomposition. Even at 0 °C, only slow decomposition is observed. Nevertheless, its reactivity is sufficient to allow introduction of a variety of amine nucleophiles under mild conditions (Scheme 3).

Pentafluorophenylsulfonate **7b** was coupled in good yield (72−98%) with an N^δ-unprotected ornithine derivative (**8**), the corresponding α,γ-aminobutyric acid **14**, and azide (Scheme 3). Subsequent deprotection yielded compounds **11** and **17**. The product of the azide reaction (**18**) was reduced by catalytic hydrogenation and deprotected using neat TMSBr to give amine **20** in good yield.

Compound **8** was synthesized by protection of the α-amino group of commercially available H-L-Orn(Z)-OtBu with Boc$_2$O and subsequent catalytic hydrogenation of the Z group in EtOH/HOAc.[25,26] Compound **14** was synthesized starting from Boc-L-Asp(OH)-OtBu (Scheme 4). Selective reduction of the side-chain carboxylic group with borane and subsequent treatment with MesCl yielded the mesylate **12** as a stable compound. Substitution of the mesylate with azide and final hydrogenation gave **14**.

Surprisingly, the protected phosphonylphosphinate amino acid adducts **9** and **15** are less stable than the pentafluorophenylsulfonate **7b** and could only be stored for a few weeks at −78 °C. These derivatives were deprotected in a two-step procedure, involving initial treatment with bromotrimethylsilane (TMSBr) in the presence of the acid scavenger hexamethyldisilazane (HMDS) to remove all the protecting groups except the Boc moiety, followed by removal of the Boc group in aqueous 3.2 M HCl (Scheme 3). HMDS could not be replaced with allyltrimethylsilane or collidine, and attempts to remove all protecting groups in a single step using TMSBr or TMSI in the absence of HMDS resulted in the cleavage of the amino acid side chain from the methyl(phosphonomethyl)phosphinate unit. Decomposition was not observed when the terminal amine was protected with an isobutyloxycarbonyl group, which is stable against TMSBr even in the absence of an acid scavenger, suggesting that the free amine facilitates the cleavage reaction. Phosphonate/phosphinate esters are deprotected more slowly than the primary amine with TMS halides in the absence of an acid scavenger, and it is known that functionalized phosphinate esters with an electrophilic group in the α-position are more sensitive to fragmentation than their corresponding acids.[16] The final amino acid adducts **11** and **17** are stable in aqueous solution between pH 1 and 12. Amine **20** also proved stable for months.

Inhibition of Ornithine Transcarbamoylase. Phosphonylphosphinates **11**, **17**, and **20** were evaluated as inhibitors of OTC from *Streptococcus faecalis*[27−29] using a standard colorimetric assay.[30] For comparison, inhibi-

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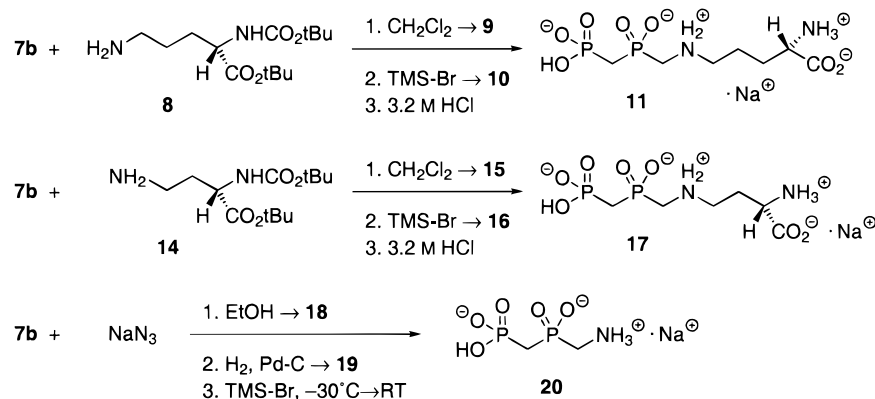
Scheme 4
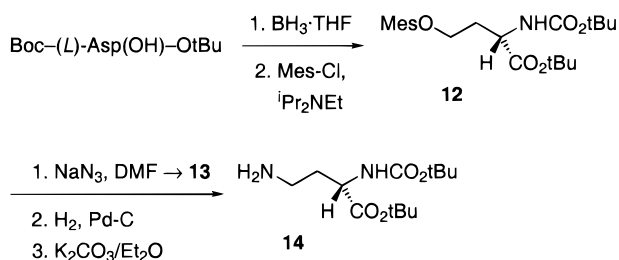
tion constants for phosphate, pyrophosphate, methylenebis(phosphonate), and the known OTC inhibitor L-PALO[31,32] (**21**) were also determined. All reactions were performed in either maleate (pH 6) or Tris (pH 7−8) buffers, which are known to be compatible with the assay.[27,33] The concentration of ornithine was 4.8 mM; at this concentration the enzyme should be nearly saturated (K_m = ca. 740 μM[27,34]) but not subject to substrate inhibition (which occurs at concentrations of ornithine greater than 6 mM[28,35]). The concentration of carbamoyl phosphate was varied between 70 and 500 μM to bracket its K_m value (ca. 200 μM in the absence of inhibitor[27,28]).
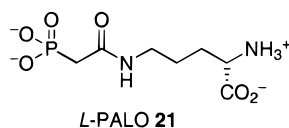
L-PALO **21**

Compounds **11** and **20**, like L-PALO (**21**) and pyrophosphate, were found to be competitive inhibitors with respect to carbamoyl phosphate (Figure 1), whereas **17** displays noncompetitive behavior. Inhibition constants were determined from Dixon plots ($1/v$ vs [I]) at several pH values and are summarized in Table 1. Compared to L-PALO, the phosphonylphosphinates prepared in this study are relatively weak inhibitors of OTC. The tightest binder, amine **20**, is recognized ca. 10^3-fold less well than L-PALO. The ornithine derivative **11** is a somewhat weaker inhibitor still, comparable to pyrophosphate[36] but substantially better than the corresponding N^α-Boc-protected derivative **10**, phosphonylphosphinate **17**, phosphate,[36] and methylenebis(phosphonate).

Discussion

This study provides a general route to diverse phosphonylphosphinates (P−C−P−C−X) and establishes the synthetic utility of the pentafluorophenylsulfonate moi-
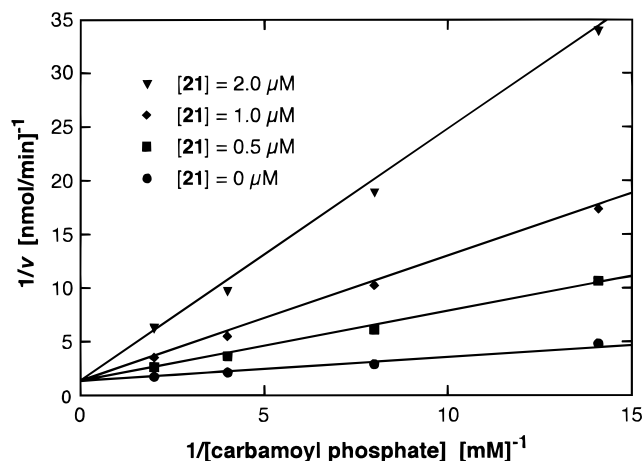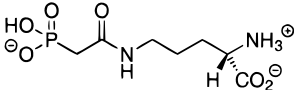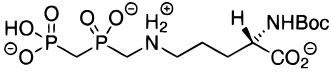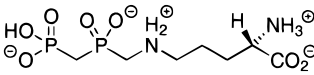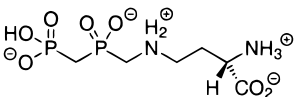

Figure 1. Lineweaver−Burk plots for the inhibition of OTC from *S. faecalis* with various concentrations of L-PALO (**21**): 4.8 mM ornithine, 10 ng/mL OTC, 50 mM Tris·HCl, 0.5 mM EDTA, pH 8.0, 37 °C.

ety as a leaving group of intermediate reactivity. In contrast to previously described methodologies, which do not permit ready introduction of side chains after the formation of the phosphonylphosphinate unit, a wide variety of α-functionalized phosphonylphosphinates can be prepared via displacement of the pentafluorophenylsulfonate in **7b** by an appropriate nucleophile. A major advantage of this approach is its compatibility with side chains containing acid- or base-sensitive groups. Moreover, the 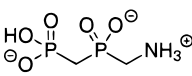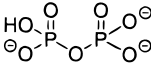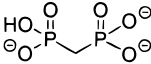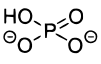current approach is convergent inasmuch as **7b** serves as a common intermediate for the introduction of different side chains (Scheme 3). Orthogonality problems between the side-chain protecting groups and the phosphonylphosphinate moiety are also avoided since the side chain is introduced at the end of the synthesis via a very mild coupling step. By using acid-labile protecting groups in the side chain, as in the examples presented here, the final deprotection can be accomplished in a single pot using mild TMS-Br/HMDS or neat TMS-Br.

With the exception of compound **20**, which lacks the amino acid side chain, the α-functionalized phosphonylphosphinates are surprisingly poor inhibitors of *S. faecalis* OTC (Table 1). Even **20** is orders of magnitude weaker than L-PALO, a previously described inhibitor of rat liver OTC[31,32,37] that also binds to the *Streptococcus* enzyme with submicromolar affinity (Table 1). Given the structural similarities between L-PALO, **11**, and **17**,

Table 1. Inhibition of *S. faecalis* OTC at 37 °C

| Compound	Structure	K_i [µM] at pH 8.0[a]	K_i [µM] at pH 7.0[b]	K_i [µM] at pH 6.0[c]
L-PALO (**21**)		0.20	0.10	0.13
10		2,000	1,000	>20,000
11		1,900	750	660
17		3,200 (nc)	2,300 (nc)	>20,000
20		800	265	44
Pyrophosphate		2,000	890	240
Methylene-disphosphonate		9,000	>20,000	>20,000
Phosphate		6,000	12,000	>20,000

[a] 50 mM Tris·HCl, 0.5 mM EDTA, pH 8.0. [b] 50 mM Tris·HCl, 0.5 mM EDTA, pH 7.0. [c] 50 mM maleate, 0.5 mM EDTA, pH 6.0. nc, noncompetitive inhibition.

such weak inhibition was unexpected and suggests that the amino acid side chains in the phosphonylphosphinates make few productive interactions with the OTC active site. This conclusion is supported by the similarity in K_i values determined for **11** and pyrophosphate, another known competitive inhibitor of OTCs,[29] and by the fact that protection of the primary amine of **11** with a Boc group has a relatively minor effect on binding, except at pH 6 where the differences between the inhibitors are more pronounced but the enzyme is substantially less active.[27,38]

Compound **11** is longer than *L*-PALO by a methylene group, and this could conceivably account for the observed differences in affinity, but **17**, which lacks this group, is an even poorer inhibitor. In fact, *L*-PALO and **17** differ only in replacement of the neutral, planar amide with a zwitterionic, α-amino-substituted, tetrahedral phosphinate. It is possible that OTC, as suggested for the related enzyme aspartate transcarbamoylase,[39,40] functions via a "closed transition state",[41] accessible only by a protein conformational change induced by ground-state analogues containing a trigonal carbonyl group.[42] Alternatively, and perhaps more

likely, the additional oxygen at the tetrahedral phosphinate center may make unfavorable contacts within the active site. A crystal structure of the *S. faecalis* enzyme is not available, but the complex between *L*-PALO and an *E. coli* OTC was recently solved at 2.8-Å resolution.[43] This structure suggests that Gln136 might stabilize tetrahedral intermediate **2** by hydrogen bonding to the amine derived from carbamoyl phosphate via the carbonyl group in its side-chain amide. An analogous interaction (with Gln171) has been postulated for the human enzyme,[44] and if a similar situation prevails in *S. faecalis* OTC, replacement of the amine in the inhibitor with a phosphinate oxygen would be unfavorable.

Covalent inactivation of OTC by phaseolotoxin (**22**)[45,46] (*Harg* ≡ homoarginine) shows that potent inhibition can be achieved with molecules containing tetrahedral phosphorus. Future attempts to develop more potent transition-state analogues for OTC might therefore

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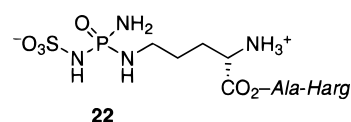
profitably focus on replacing the phosphinate moiety in **11** and **17** with a phosphinic amide or sulfonamide. Such compounds would be valuable for studies of the enzyme and as haptens for the generation of catalytic antibodies with OTC activity.

Experimental Section

General Experimental Conditions. Carbamoyl phosphate and ornithine were obtained from Sigma, H-L-Orn(Z)-OtBu and Boc-L-Asp(OH)-OtBu from Bachem, and all other chemicals from Aldrich. N^5-(Phosphonoacetyl)-L-ornithine was synthesized by a modified literature procedure (see Supporting Information).[47,48] Ornithine transcarbamoylase from *S. faecalis* was purchased from Sigma and showed a specific activity of 156 nmol of citrulline formed/mg of enzyme/min at 37 °C in 50 mM Tris·HCl at pH 8.5. THF was freshly distilled from potassium, benzene was distilled from sodium, and pyridine was distilled from calcium hydride. Dichloromethane was distilled from phosphorus pentoxide and checked for the absence of chloride before use. All reactions were performed under a dry argon atmosphere with the exception of those that used water as solvent. Reaction progress was monitored by thin-layer chromatography (TLC) or HPLC. TLC analyses were performed with 0.25-mm silica gel plates on glass support containing F-254 indicator or with octadecyl-modified silica on glass support and visualized using UV light (254 nm), alkaline potassium permanganate, or ninhydrin/nBuOH/HOAc. Flash chromatography was performed on silica 60 from Merck, Darmstadt. HPLC analyses were performed on LiChrospher 100 CH18/2 (5 μm) and LiChrosorb RP 18 (10 μm) with water/MeCN (containing 0.005% TFA) and monitored at 220 nm. Melting points are uncorrected.

Diethyl Hydroxymethylphosphonate. Diethyl phosphite (15 mL, 120 mmol), triethylamine (16 mL, 120 mmol), and paraformaldehyde (4.0 g, 130 mmol) were mixed in a reaction vessel. The vessel was tightly sealed under vacuum and then heated to 90 °C for 12 h. All volatile components were subsequently removed under reduced pressure, and the remaining semisolid was dissolved in 40 mL of dichloromethane. The solution was extracted with saturated NH_4Cl, K_2CO_3, and again with NH_4Cl and dried with $MgSO_4$ and the solvent removed under reduced pressure. Compound diethyl hydroxymethylphosphonate was obtained as a pale-yellow oil (11.7 g, 7.0 mmol, 60%). All spectroscopic data agreed with previously published data.[20,49]

Diethyl Benzyloxymethylphosphonate (4). A solution of diethyl hydroxymethylphosphonate (14 g, 82 mmol) in 50 mL of absolute dichloromethane was treated successively with silver(I) oxide (10 g, 50 mmol) and benzyl bromide (14 mL, 115 mmol). The reaction was stirred in the dark at room temperature for 48 h. After filtration and removal of solvent in vacuo, benzyl bromide was distilled off at 50 °C and 0.1 mbar. Flash chromatography on silica with ethyl acetate as eluent afforded 14 g (54 mmol, 66%) of **4** as a pale-yellow oil. All data agreed with the previously published data.[18,50]

Ethyl Hydrogen Benzyloxymethylphosphonate. A solution of **4** (4.0 g, 15 mmol) in ethanol (125 mL) and 1 M aqueous potassium hydroxide (85 mL) was heated to 75 °C for 2.5 h. The solution was cooled to 0 °C and then brought to pH 7.0 with 3.2 M HCl. The ethanol was removed under reduced pressure. The remaining solution was diluted with 400 mL of CH_2Cl_2 and acidified with 3.2 M HCl to pH 1.0. The aqueous phase was separated and extracted again with CH_2Cl_2. The combined organic extracts were washed with 50 mL of saturated NaCl and dried over $MgSO_4$. Removal of the solvent under reduced pressure yielded ethyl hydrogen benzyloxymethylphosphonate as a pale-yellow oil (3.5 g, 15 mmol, >98%). All data agreed with previously published data.[50]

Ethyl Benzyloxymethyl(chloro)phosphonate (5). Oxalyl chloride (3.8 mL, 43 mmol) was added slowly to a solution of ethyl hydrogen benzyloxymethylphosphonate (4.0 g, 17.3 mmol) and DMF (10 μL) in 50 mL of anhydrous benzene. After stirring for 2.5 h at room temperature, the solvent was

removed in vacuo and the residue codistilled twice with anhydrous benzene. The yellow oil (3.7 g) was >90% pure by NMR and used without further purification. ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.42 (t, $J = 14$ Hz, 3H, OEt), 4.01 (d, $J = 2.5$ Hz, 2H, CH_2OBn), 4.27−4.38 (m, 2H, OEt), 4.73 (s, 2H, CH_2Ph), 7.32−7.40 (m, 5H, Ph). ^{13}C NMR (CDCl$_3$, 125.7 MHz): δ 16.3 (s, OCH$_2$CH$_3$), 64.1 (d, $J = 10$ Hz, OCH$_2$CH$_3$), 68.5 (d, $J = 143$ Hz, CH$_2$Bn), 74.9 (d, $J = 10$ Hz, CH$_2$Ph), 128.1 (s, C-2′), 128.3 (s, C-4′), 128.5 (s, C-3′), 136.8 (s, C-1′). ^{31}P NMR (CDCl$_3$, 161.9 MHz): δ 35.23 (s, P).

Ethyl Benzyloxymethyl[(diethoxyphosphoryl)methyl]-phosphinate. *n*-Butyllithium (2.0 M in cyclohexane, 7.5 mL, 15 mmol) was slowly added to a solution of diethyl methylphosphonate (2.2 mL, 15 mmol) in 60 mL of absolute THF at −78 °C. After 15 min a white suspension formed, and 3.7 g (∼15 mmol) of **5** in 30 mL of absolute THF was added over a period of 30 min, after which the suspension clarified. The reaction was stirred 1 h at −78 °C and 1 h at 0 °C and then quenched by the addition of 10% HCl and diethyl ether. The pH was adjusted to ca. 1, and after separation the aqueous phase was extracted with dichloromethane. The combined organic phases were extracted with saturated NaHCO$_3$ and dried with MgSO$_4$, and the solvent was removed in vacuo. Flash chromatography on silica gel with EtOAc/5% MeOH yielded 4.0 g of ethyl benzyloxymethyl[(diethoxyphosphoryl)-methyl]phosphinate (11 mmol, 73% based on **5**) as a pale-yellow oil. ^{1}H NMR (CDCl$_3$, 250 MHz): δ 1.20−1.40 (m, 9H, OEt), 2.40−2.60 (m, 2H, P-CH_2-P), 3.80−3.95 (m, 2H, CH_2-OBn), 4.0−4.25 (m, 6H, OEt), 4.55−4.70 (m, 2H, CH_2Ph), 7.25−7.40 (m, 5H, Ph). ^{13}C NMR (CDCl$_3$, 100.6 MHz): δ 16.3 (d, $J = 6$ Hz, P^{2}OCH$_2$CH$_3$), 16.4 (d, $J = 6$ Hz, P^{1}OCH$_2$CH$_3$), 25.2 (dd, $J = 135$ Hz, 83 Hz, P-CH$_2$-P), 64.6 (d, $J = 6.5$ Hz, P^{1}OCH$_2$CH$_3$), 62.5 (d, $J = 6.2$ Hz, P^{2}OCH$_2$CH$_3$), 62.7 (d, $J = 6.2$ Hz, P^{2}OCH$_2$CH$_3$), 65.6 (d, $J = 119$ Hz, CH$_2$OBn), 75.2 (d, $J = 13.5$ Hz, CH$_2$Ph), 128.1 (s, C-2′), 128.1 (s, C-4′), 128.4 (s, C-3′), 136.8 (s, C-1′). ^{31}P NMR (CDCl$_3$, 161.9 MHz): δ 20.38 (d, $J = 4.5$ Hz, P-1), 41.82 (d, $J = 4.5$ Hz, 1P, P-2). HRMS (FAB, NBA): C$_{15}$H$_{27}$O$_6$P$_2$ (M + H$^+$) calcd 365.1283, found 365.1275.

Ethyl [(Diethoxyphosphoryl)methyl]hydroxymethylphosphinate (6). A solution of ethyl benzyloxymethyl-[(diethoxyphosphoryl)methyl]phosphinate (4.0 g, 11 mmol) in 300 mL of ethanol was treated with 0.4 g of palladium on carbon (5%, contained 50% H$_2$O) and then hydrogenated at ambient pressure for 12 h. After separation from the catalyst and evaporation under reduced pressure, 3.0 g (11 mmol, >98%) of **6** was obtained as a colorless, clear resin. ^{1}H NMR (CDCl$_3$, 200 MHz): δ 1.33−1.40 (m, 9H, OEt), 2.47−2.65 (m, 2H, P−CH_2−P), 4.03−4.07 (m, 2H, CH_2OH), 4.09−4.24 (m, 6H, OEt). ^{13}C NMR (CDCl$_3$, 100.6 MHz): δ 16.1 (d, $J = 5.5$ Hz, P^{2}OCH$_2$CH$_3$), 16.2 (d, $J = 7$ Hz, P^{2}OCH$_2$CH$_3$), 16.3 (s, $J = 6$ Hz, P^{1}OCH$_2$CH$_3$), 25.3 (dd, $J = 135$ Hz, 78 Hz, P-CH$_2$−P), 59.0 (d, $J = 112$ Hz, CH$_2$OH), 61.5 (d, $J = 6.5$ Hz, P^{1}OCH$_2$CH$_3$), 62.7 (d, $J = 6.5$ Hz, P^{2}OCH$_2$CH$_3$), 62.8 (d, $J = 6$ Hz, P^{2}OCH$_2$-CH$_3$). ^{31}P NMR (CDCl$_3$, 161.9 MHz): δ 21.68 (d, $J = 36.8$ Hz, P-1), 44.23 (d, $J = 44.4$ Hz, 1P, P-2). HRMS (FAB, NBA/NaI): C$_8$H$_{20}$O$_6$P$_2$Na (M + Na$^+$) calcd 297.0633, found 297.0638.

[(Diethoxyphosphoryl)methyl(ethoxy)phosphoryl]-methyl Methanesulfonate (7a). A solution of **6** (260 mg, 1 mmol) in 20 mL of absolute pyridine was treated with methanesulfonyl chloride (180 μL, 2.3 mmol, dissolved in 7 mL of pyridine) at 0 °C over a period of 30 min. The solution was kept at this temperature for 24 h, then poured into ice water, extracted three times with CH$_2$Cl$_2$, and dried with Na$_2$SO$_4$ and the solvent removed in vacuo. Flash chromatography on silica with CH$_2$Cl$_2$/MeOH/acetone (30:2:2) afforded 179 mg (53%) of **7a** as a colorless oil. ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.35 (t, $J = 6$ Hz, 9H, OEt), 2.50 (td, $J = 19$ Hz, 6 Hz, 2H, P−CH_2−P), 3.12 (s, 3H, SO$_2$Me), 4.15 (m, 6H, OEt), 4.59 (m, 2H, CH_2OMes).

[(Diethoxyphosphoryl)methyl(ethoxy)phosphoryl]-methyl 2,3,4,5,6-Pentafluorobenzenesulfonate (7b). A solution of **6** (1.0 g, 3.6 mmol) and *N*-ethyldiisopropylamine (1.0 mL, 7.8 mmol) in 30 mL of absolute CH$_2$Cl$_2$ was cooled to

−78 °C and slowly treated with a solution of pentafluorobenzenesulfonyl chloride (800 µL, 5.4 mmol) in 10 mL of absolute CH_2Cl_2. After 3 h at −78 °C the solution was slowly warmed to −15 °C and then kept for 12 h. The solvent was evaporated at reduced pressure and the yellow oil loaded immediately on a silica column, preequilibrated with CH_2Cl_2/MeOH/acetone (30:2:2). The column was rapidly eluted with the same solvent, and the fractions containing product (R_f 0.6) were combined. Removal of the solvent in vacuo yielded **7b** (1.5 g, 3.0 mmol, 84%) as pale yellow-crystals. Mp: 65 °C. ^{1}H NMR (CDCl₃, 500 MHz): δ 1.34 (t, J = 7 Hz, 3H, P[2]OCH₂C*H₃*), 1.35 (t, J = 7 Hz, 3H, P[2]OCH₂C*H₃*), 1.39 (t, J = 7 Hz, 3H, P[1]OCH₂C*H₃*), 2.51 (m, 2H, P-CH₂-P), 4.17 (m, 6H, OC*H₂*CH₃), 4.66 (m, 2H, C*H₂*OPf). ^{13}C NMR (CDCl₃, 125.7 MHz): δ 16.0 (d, J = 6 Hz, P[2]OCH₂*CH₃*), 16.1 (d, J = 6 Hz, P[2]OCH₂*CH₃*), 16.2 (d, J = 5 Hz, P[1]OCH₂*CH₃*), 25.4 (dd, J = 90 Hz, 45 Hz, P-*CH₂*-P), 62.3 (d, J = 6 Hz, P[2]O*CH₂*CH₃), 62.5 (d, J = 7.5 Hz, P[2]O*CH₂*CH₃), 63.2 (d, J = 6 Hz, P[1]O*CH₂*CH₃), 64.7 (d, J = 113 Hz, *CH₂*OPf), 111.0 (m, C[1′]-SO₃), 137.9 (dm, J = 239 Hz, C[3′]-F), 145.3 (dm, J = 264 Hz, C[2′]-F, C[4′]-F). ^{31}P NMR (CDCl₃, 161.9 MHz): δ 18.07 (s, P-1), 35.29 (s, P-2). ^{19}F NMR (CDCl₃, 376.3 MHz): δ −154.5 (m, 2F, 2-F), −138.9 (m, 1F, 4-F), −130.2 (m, 3-F). HRMS (FAB, NBA/CsI): $C_{14}H_{19}O_8F_5P_2SCs$ (M + Cs⁺) calcd 636.9250, found 636.9263.

N**[α]*-tert**-Butoxycarbonyl-**N**[δ]*-{[(diethoxyphosphoryl)-methyl(ethoxy)phosphoryl]methyl}-L-ornithine **tert**-Butyl Ester (9).** Pentaflate **7b** (500 mg, 1.0 mmol) was dissolved in 35 mL of absolute CH_2Cl_2 and cooled to 0 °C. Then **8** (721 mg, 2.5 mmol), in 15 mL of absolute CH_2Cl_2, was added in one batch and the solution immediately heated to 40 °C for 3 h. After cooling to ambient temperature, the solution was diluted with 200 mL of Et₂O and extracted with 20 mL of saturated K₂CO₃. After back-extraction of the aqueous layer, the combined organic phases were dried over MgSO₄ and evaporated under reduced pressure and the yellow oil was chromatographed on silica with CH_2Cl_2/MeOH/acetone (30:2:2) as eluent. **9** (392 mg, 0.72 mmol, 72%) was obtained as a clear, colorless resin. ^{1}H NMR (500 MHz, CDCl₃): δ 1.32 (m, 9H, OEt), 1.41 (s, 9.5H, NH−CO₂C*Me₃*, γ-CH₂), 1.43 (s, 9.5H, CH−CO₂C*Me₃*, γ-CH₂), 1.52 (m, 1H, γ-CH₂), 1.63 (m, 1H, β-CH₂), 1.75 (m, 1H, β-CH₂), 2.51 (m, 2H, P−C*H₂*−P), 2.68 (m, 2H, δ-CH₂), 3.6 (m, 2H, P−C*H₂*−N), 4.15 (m, 7H, OEt, α-CH), 5.23 (m, 1H, NH). ^{13}C NMR (CDCl₃, 125.7 MHz): δ 16.2 (m, P[2]*CH₂*CH₃), 16.4 (m, P[1]*CH₂*CH₃), 25.3 (s, β-CH₂), 25.9 (dd, J = 252 Hz, 180 Hz, P-*CH₂*-P), 27.9 (s, CH−CO₂C*Me₃*), 28.2 (s, NH−CO₂C*Me₃*), 30.3 (s, γ-CH₂), 47.6 (d, J = 114 Hz, P-*CH₂*N), 50.6 (d, J = 109 Hz, α-CH), 53.7 (s, δ-CH₂), 61.2 (m, P[1]O*CH₂*CH₃), 62.4 (m, P[2]O*CH₂*CH₃), 79.3 (NH−CO₂*C*Me₃), 81.5 (s, CH−CO₂*C*Me₃), 155.9 (s, NH-*CO₂*tBu), 172.5 (s, CH-*CO₂*tBu). ^{31}P NMR (161.9 MHz, CDCl₃): δ 20.84 (d, J = 2.5 Hz, P-1), 44.84 (dd, J = 12 Hz, 3 Hz, P-2). HRMS (FAB, NBA/CsI): $C_{22}H_{46}N_2O_9P_2$ + Cs⁺ (M + Cs⁺) calcd 677.1730, found 677.1746. [α]$_D^{25}$ = +1.64 (c = 1.1, CH_2Cl_2), [α]$_{436}^{25}$ = +4.73 (c = 1.1, CH_2Cl_2).

N**[α]*-tert**-Butoxycarbonyl-**N**[δ]*-{[dihydroxyphosphoryl-methyl(hydroxy)phosphoryl]methyl}-L-ornithine Sodium Salt (10).** Hexamethyldisilazane (210 µL, 1.0 mmol) was added to a solution of **9** (50 mg, 97 µmol) in 5 mL of absolute dichloromethane, and the clear solution was cooled to 0 °C. After addition of bromotrimethylsilane (130 µL, 1.0 mmol) the solution was slowly warmed to room temperature and then stirred for 12 h. After a second addition of both reagents and stirring for an additional 24 h, all volatile components were removed under reduced pressure. Water (4 mL) was added and the pH adjusted to 8.0 with 2 M NaOH at 0 °C. Chromatography on RP-18 silica with water as eluent and subsequent lyophilization yielded **10** as a white powder (34 mg, 75 µmol, 77%). ^{1}H NMR (500 MHz, D₂O, pH 5.0): δ 1.49 (s, 9H, tBu), 1.75−2.02 (m, 4H, β-CH₂, γ-CH₂), 2.13 (t, J = 19 Hz, 2H, P−C*H₂*−P), 3.11 (m, 4H, P−C*H₂*−N, δ-CH₂), 4.00 (t, J = 6.5 Hz, α-CH₂). ^{13}C NMR (125.7 MHz, D₂O, pH 5.0) δ 22.51 (s, β-CH₂), 29.77 (s, tBu), 29.85 (s, β-CH₂), 36.26 (dd, J = 85 Hz, 32 Hz, P-*CH₂*-P), 49.80 (d, J = 92 Hz, P-*CH₂*N), 50.65 (d, J = 7.5 Hz, δ-CH₂), 55.39 (s, α-CH₂), 88.39 (s, tBu), 131.22 (s,

n-*C*O₂tBu), 171.96 (s, CO₂⁻). ^{31}P NMR (121.4 MHz, D₂O, pH 5.0): δ 16.9 (s, P-1), 23.0 (s, P-2).

***N**[δ]*-{[Dihydroxyphosphorylmethyl(hydroxy)phos-phoryl]methyl}-L-ornithine Sodium Salt (11).** Compound **10** (34 mg, 75 µmol) was dissolved in 2 mL of 3.2 M HCl at 0 °C and lyophilized after 30 min. The white powder was dissolved in water, and the pH was adjusted to 8.0 with 2 M NaOH. Chromatography on RP-18 silica with water/methanol (1:1) and subsequent lyophilization yielded 30 mg (75 µmol, >98%) of **11** as a white, hygroscopic powder. ^{1}H NMR (500 MHz, D₂O, pH 6.0): δ 1.70−2.05 (m, 4H, γ-CH₂, β-CH₂), 2.13 (t, J = 37 Hz, 2H, P−C*H₂*−P), 3.09 (m, 2H, δ-CH₂), 3.11 (t, J = 11 Hz, 2H, P−C*H₂*−N), 3.78 (J = 6 Hz, t, α-C*H*). ^{13}C NMR (125.7 MHz, D₂O, pH 8.0) δ 24.51 (s, β-CH₂), 30.19 (s, γ-CH₂), 36.4 (dd, J = 116 Hz, 85 Hz, P-*CH₂*-P), 41.63 (s, δ-CH₂), 49.9 (d, J = 91 Hz, P-*CH₂*N), 51.02 (s, α-CH), 176.9 (s, *C*O₂). ^{31}P NMR (161.9 MHz, D₂O, pH 8.0): δ 16.6 (s, P-1), 23.0 (s, P-2). HRMS: (ESI) $[C_7H_{15}N_2O_7P_2]^{3-}$·2H⁺ (M[3−] + 2H⁺) calcd 303.0511, found 303.0519; $[C_7H_{15}N_2O_7P_2]^{3-}$·2Na⁺ (M[3−] + 2Na⁺) calcd 347.0150, found 347.0159.

N**[α]*-tert**-Butoxycarbonyl-L-ornithine **tert**-Butyl Ester (8).** Compound **8** was prepared in >98% yield from H-L-Orn-(Z)-OtBu by protection with Boc₂O in NaOH/THF, subsequent catalytic hydrogenation in EtOH/HOAc with Pd/C as catalyst, and final extraction with saturated aqueous K₂CO₃/Et₂O. ^{1}H NMR, ^{13}C NMR, and melting point were consistent with previously published data.[26,51] HRMS (FAB, NBA): $C_{14}H_{29}N_2O_4$ (M + H⁺) calcd 289.2127, found 289.2122. [α]$_D^{25}$ +1.85 (c = 1, CH_2Cl_2); [α]$_{436}^{25}$ = +5.45 (c = 1, CH_2Cl_2).

tert**-Butyl 2(S)-tert**-Butoxycarbonylamino-4-methyl-sulfonyloxybutyrate (Boc-L-Hse(Mes)-OtBu) (12).** To a solution of 2(S)-2-*tert*-butoxycarbonylamino-4-hydroxybutyric acid *tert*-butyl ester[52] (250 mg, 0.9 mmol, prepared from Boc-L-Asp(OH)-OtBu by borane reduction in THF) in 5 mL of CH_2Cl_2 was added 360 µL (2.7 mmol) of N-ethyldiisopropylamine and 78 µL (1.0 mmol) of methanesulfonyl chloride at 0 °C. The resulting solution was stirred for 20 min and then washed with saturated aqueous NH₄Cl. The aqueous layer was back-extracted three times with CH_2Cl_2 and dried over MgSO₄, and the combined organic phases were evaporated in vacuo. Flash chromatography on silica with EtOAc/hexane (1:1) afforded 229 mg (640 mmol, 71%) of **12** as white needles. Mp: 85−87 °C. ^{1}H NMR (400 MHz, CDCl₃): δ 1.45 (s, 9H, OtBu), 1.48 (s, 9H, Boc), 2.03−2.33 (m, 2H, β-CH₂), 3.03 (s, 3H, OMes), 4.25−4.33 (m, 3H, α-CH, γ-CH₂), 5.17 (s, 1H, NH). ^{13}C NMR (100.6 MHz, CDCl₃): δ 27.97 (s, OtBu), 28.30 (s, Boc), 32.22 (s, β-CH₂), 37.30 (s, OMes), 50.95 (s, α-CH), 66.18 (s, γ-CH₂), 80.13 (s, NHCO₂*tBu*), 82.78 (s, CHCO₂*tBu*), 155.39 (s, NHCO₂*tBu*), 170.80 (s, CH*CO₂*tBu). HRMS (FAB, NBA + PEGMEE): $C_{14}H_{28}NO_7S$ (M + H⁺) calcd 354.1586, found 354.1584. [α]$_D^{25}$ +11.6 (c = 1.03, CHCl₃).

tert**-Butyl 4-Azido-2(S)-tert**-butoxycarbonylaminobu-tyrate (13).** A solution of 229 mg (0.65 mmol) of mesylate **12** and 211 mg (3.2 mmol) of sodium azide in 10 mL of dimethylformamide was stirred at 40 °C for 12 h. The solvent was removed in vacuo, the residue dissolved in water/CH_2Cl_2, and after separation of the layers the aqueous layer extracted three times with CH_2Cl_2. The organic layers were combined and dried over MgSO₄, and evaporation of the solvent afforded 138 mg (0.46 mmol, 71%) of **13** as a clear, colorless oil. ^{1}H NMR (400 MHz, CDCl₃): δ 1.45 (s, 9H, tBu), 1.48 (s, 9H, tBu), 1.8−2.1 (m, 2H, β-CH₂), 3.39 (t, J = 6.5 Hz, 2H, γ-CH₂), 4.25 (d, J = 4 Hz, 1H, α-CH), 5.15 (s, 1H, NH). ^{13}C NMR (100.6 MHz, CDCl₃): δ 27.97 (s, tBu), 28.32 (s, tBu), 32.10 (s, β-CH₂), 47.85 (s, γ-CH₂), 51.91 (s, α-CH), 79.96 (s, Boc), 82.49 (s, CO₂*tBu*), 155.34 (s, Boc), 171.04 (s, *C*O₂tBu). HRMS (FAB, NBA): $C_{13}H_{25}N_4O_4$ [M + H⁺] calcd 301.1876, found 301.1879. [α]$_D^{25}$ +20.1 (c = 1.17, CHCl₃).

tert**-Butyl 4-Amino-2(S)-tert**-butoxycarbonylaminobu-tyrate (14).** A solution of **13** (90 mg, 0.30 mmol) in 10 mL of ethanol and 17 µL (0.30 mmol) of acetic acid was treated with 21 mg of palladium on carbon (5%) and hydrogenated at ambient pressure for 1 h. Separation from the catalyst and evaporation of the solvent at reduced pressure afforded 103

mg (0.30 mmol, >98%) of **14** as a clear, colorless resin. Compound **14** was characterized as the acetate salt. ^{1}H NMR (200 MHz, CDCl$_3$): δ 1.42 (s, 9H, tBu), 1.45 (s, 9H, tBu), 1.8–2.1 (m, 2H, β-CH$_2$), 1.94 (s, 3H, OAc), 2.9–3.1 (m, 2H, γ-CH$_2$), 4.15 (d, J = 4 Hz, 1H, α-CH$_2$), 5.15 (s, 1H, NH), 6.5 (s, 3H, NH$_3$). ^{13}C NMR (100.6 MHz, CDCl$_3$): δ 22.8 (s, OAc), 28.9 (s, 2 tBu), 33.03 (s, β-CH$_2$), 36.9 (s, γ-CH$_2$), 51.7 (s, α-CH), 80.3 (s, tBu), 82.6 (s, tBu), 156.1 (s, OAc), 171.2 (s, N-*C*O$_2$tBu), 177.1 (s, CH-*C*O$_2$tBu). HRMS (FAB, NBA): C$_{13}$H$_{27}$N$_2$O$_4$ (M + H$^+$) calcd 275.1971, found 275.1971.; [α]$_D^{25}$ −2.6 (c = 4.2, CHCl$_3$).

tert-Butyl 2(S)-*tert*-Butoxycarbonylamino-4-({[(dimethoxyphosphoryl)methyl(ethoxy)phosphoryl]methyl}-amino)butyrate (15). Compound **15** was synthesized from **14** (as the free base by extraction with K$_2$CO$_3$/Et$_2$O) and **7b** in the manner described for **9**. **15** was obtained as a clear, colorless resin. ^{1}H NMR (500 MHz, CDCl$_3$): δ 1.35 (td, J = 7 Hz, 2.5 Hz, 3H, OEt), 1.44 (s, 9H, OtBu), 1.47 (s, 9H, OtBu), 1.77–2.04 (m, 2H, β-CH$_2$), 2.59 (m, 2H, P–CH$_2$–P), 2.78 (t, J = 7 Hz, 2H, γ-CH$_2$), 3.11 (d, J = 11 Hz, 2H, P–CH$_2$N), 3.83 (m, 6H, OMe), 4.18 (m, 2H, OEt), 4.23 (m, 1H, α-CH), 5.20 (dd, J = 11 Hz, 8 Hz, N*H*Boc). ^{13}C NMR (125.7 MHz, CDCl$_3$): δ 16.6 (s, OCH$_2$*C*H$_3$), 24.6 (dd, J = 78 Hz, 134 Hz, P–CH$_2$–P), 28.1 (s, OtBu), 28.4 (s, OtBu), 32.3 (d, J = 27 Hz, β-CH$_2$), 47.4 (s, γ-CH$_2$), 47.9 (d, J = 109 Hz, P–CH$_2$N), 52.4 (d, J = 8 Hz, OMe), 53.0 (d, J = 7 Hz, OMe), 53.1 (dd, J = 16 Hz, 7 Hz, α-CH), 61.6 (d, J = 7 Hz, O*C*H$_2$CH$_3$), 79.6 (s, N*C*O$_2$*C*Me$_3$), 81.8 (s, O*C*O$_2$*C*Me$_3$), 155.6 (s, N*C*O), 171.9 (s, O*C*O). ^{31}P NMR (121.4 MHz, CDCl$_3$): δ 24.0 (s, P-1), 45.0 (d, J = 50 Hz, P-2). HRMS (FAB): C$_{19}$H$_{41}$N$_2$O$_9$P$_2$ (M + H$^+$) calcd 503.2287, found 503.2299. [α]$_D^{25}$ +5.75 (c = 0.4, CHCl$_3$).

Sodium 2(S)-*tert*-Butoxycarbonylamino-4-({[dihydroxyphosphorylmethyl(hydroxy)phosphoryl]methyl}-amino)butyrate (16). Compound **15** was deprotected to give **16** as described for **10**. ^{1}H NMR (300 MHz, D$_2$O): δ 1.55 (s, 9H, tBu), 2.32 (m, 2H, P–CH$_2$–P, β-CH$_2$), 3.25 (d, J = 11 Hz, 2H, P–CH$_2$N), 3.37 (m, 2H, γ-CH$_2$), 4.19 (m, 1H, α-CH). ^{31}P NMR (121.4 MHz, D$_2$O): δ 16.31 (s, P-1), 22.65 (s, P-2).

Sodium 2(S)-Amino-4-({[dihydroxyphosphorylmethyl(hydroxy)phosphoryl]methyl}amino)butyrate (17). Compound **16** was deprotected to **17** using the same method as that described for **11**. ^{1}H NMR (500 MHz, D$_2$O): δ 2.20–2.30 (m, 4H, P–C*H*$_2$–P, β-CH$_2$), 3.21 (dd, J = 11 Hz, 3.5 Hz, 2H, P–C*H*$_2$N), 3.24–3.33 (m, 2H, γ-CH$_2$), 3.84 (m, 1H, α-CH). ^{13}C NMR (125.7 MHz, D$_2$O): δ 29.76 (s, β-CH$_2$), 35.22 (dd, J = 121 Hz, 85 Hz, P-*C*H$_2$–P), 39.16 (s, γ-*C*H), 48.36 (d, J = 8 Hz, α-CH$_2$), 49.76 (d, J = 93 Hz, P-*C*H$_2$N), 175.74 (s, *C*O$_2$). ^{31}P NMR (121.4 MHz, CDCl$_3$): δ 15.9 (s, P-1), 23.1 (s, P-2). HRMS (ESI): [C$_6$H$_{13}$N$_2$O$_7$P$_2$]$^{3-}$·3H$^+$ (M^{3-} + 2H$^+$) calcd 289.0355, found 289.0366.

Ethyl Azidomethyl[(diethoxyphosphoryl)methyl]-phosphinate (18). A solution of **7b** (300 mg, 0.60 mmol) in 12 mL of absolute ethanol was treated with sodium azide (200 mg, 3.0 mmol), and the suspension stirred at ambient temperature for 24 h. After filtration, the solvent was removed in vacuo and the residue suspended in dry Et$_2$O and refiltered. Removal of solvent under reduced pressure yielded 179 mg (0.60 mmol, >98%) of **18** as a clear, colorless resin. ^{1}H NMR (400 MHz, CDCl$_3$): δ 1.31–1.40 (m, 9H, OEt), 3.72 (dd, J = 5 Hz, 4.5 Hz, 2H, P–C*H*$_2$–P), 4.20 (m, 2H, P–C*H*$_2$N$_3$), 4.25–4.17 (m, 6H, OEt). ^{13}C NMR (100.6 MHz, CDCl$_3$): δ 16.3 (d, J = 7 Hz, P(OEt)$_2$), 16.5 (d, J = 6 Hz, P(CH$_2$N$_3$)OEt), 26.0 (dd, J = 83 Hz, 52 Hz, P-*C*H$_2$–P), 47.8 (d, J = 107 Hz, P-*C*H$_2$N$_3$), 62.2 (d, J = 7 Hz, OEt), 62.7 (d, J = 6 Hz, OEt), 63.0 (d, J = 7 Hz, OEt). ^{31}P NMR (161.9 MHz, CDCl$_3$): δ 19.3 (s, P-1), 40.1 (s, P-2). HRMS (FAB, NBA/NaI): C$_8$H$_{19}$N$_3$O$_5$P$_2$Na (M + Na$^+$) calcd 322.0698, found 322.0692.

Ethyl Aminomethyl[(diethoxyphosphoryl)methyl]-phosphinate (19). A solution of **18** (179 mg, 0.60 mmol) in 60 mL of ethanol was treated with 50 mg of palladium on carbon (5%) and hydrogenated at ambient pressure for 17 h. After separation from the catalyst and removal of the solvent under reduced pressure, 164 mg (0.60 mmol, >98%) of **19** was obtained as a clear, colorless resin. ^{1}H NMR (CDCl$_3$, 500 MHz): δ 1.37 (m, 9H, OEt), 2.53 (dd, J = 16.5 Hz, 4 Hz, 2H,

P–C*H*$_2$–P), 3.21 (d, J = 8 Hz, 2H, P–C*H*$_2$–NH$_2$), 4.22–4.15 (m, 6H, OEt). ^{13}C NMR (CDCl$_3$, 125.7 MHz): δ 16.2 (d, J = 5 Hz, P(OEt)$_2$), 16.4 (d, J = 5 Hz, P(CH$_2$NH$_2$)O*E*t), 25.3 (dd, J = 77 Hz, 59 Hz, P-*C*H$_2$–P), 40.9 (d, J = 106 Hz, *C*H$_2$NH$_2$), 61.1 (d, J = 5 Hz, P(CH$_2$NH$_2$)O*E*t), 62.4 (d, J = 5 Hz, OEt) 62.7 (d, J = 5 Hz, OEt). ^{31}P NMR (CDCl$_3$, 161.9 MHz): δ 20.9 (s, P-1), 45.6 (s, P-2). HRMS (NBA/NaI): C$_8$H$_{22}$NO$_5$P$_2$ (M + H$^+$) calcd 274.0973, found 274.0977.

Sodium Aminomethyl(dihydroxyphosphorylmethyl)-phosphinate (20). Compound **19** (50 mg, 183 μmol) was treated with 240 μL (1.8 mmol) of bromotrimethylsilane at −40 °C. After 1 h, the solution was slowly warmed to 0 °C and stirred for 60 h. Workup and purification in the same way as described for **10** yielded 27 mg (69%) of **20** as a white powder. ^{1}H NMR (D$_2$O, pH 12.0, 400 MHz): δ 1.80 (t, J = 18 Hz, 2H, P–C*H*$_2$–P), 3.47 (m, 2H, P–C*H*$_2$NH$_2$). ^{13}C NMR (D$_2$O, pH 6.0, 75.4 MHz): δ 35.0 (dd, J = 83 Hz, 39 Hz, P–CH$_2$–P), 41.7 (d, J = 95 Hz, P–CH$_2$N). ^{31}P NMR (D$_2$O, pH 12.0, 161.9 MHz): δ 12.6 (d, J = 4 Hz, P-1), 39.2 (d, J = 4 Hz, P-2); (D$_2$O, pH 6, 121.4 MHz): 16.1 (s, P-1), 25.0 (s, P-2); (D$_2$O, pH 1, 121.4 MHz): 19.5 (s, P-1), 26.2 (s, broad, P-2). MS (ESI): C$_2$H$_8$NO$_5$P$_2$ (M^{3-}·2H$^+$): 188.

Inhibition Studies with Ornithine Transcarbamoylase. Ornithine (96 mM, adjusted to pH 8.5 with 2 M KOH), and carbamoyl phosphate (96 mM), and OTC (2 μg/mL) were frozen in aliquots and thawed immediately prior to use. A chromogenic assay described by Pastra-Landis[30] was used. For the color reagent, antipyrin (10 mL, 250 mg in 50 mL of 50% H$_2$SO$_4$) and 2,3-butanedione monoxime (5 mL, 200 mg in 25 mL of 5% HOAc, freshly prepared before use) were mixed just before use and stored on ice. The assays were initiated by the addition of carbamoyl phosphate, quenched by the addition of color reagent, and then stored in the dark for 12 h at room temperature. The samples were then heated 24 min to 45 °C under illumination by a 60-W bulb at a distance of 70 cm. Then the absorption was measured at 466 nm. Blank samples contained all reagents with the exception of OTC, and controls contained all reagents but no inhibitor.

The concentration of the different inhibitors was varied stepwise between 0.5 μM and 2.5 mM. Typically, the appropriate inhibitor, 4.8 mM ornithine, 10 ng/mL OTC, and 71–500 μM carbamoyl phosphate in a total volume of 0.1 mL were incubated at 37.0 °C for 8 min and then quenched by the addition of 0.1 mL of color reagent; 50 mM Tris·HCl, 0.5 mM EDTA (pH 8.0 and 7.0), and 50 mM maleate, 0.5 mM EDTA (pH 6.0) were used as buffers. Lineweaver–Burk plots (1/v vs 1/[carbamoyl phosphate]) were used to assess competitive behavior, and Dixon plots of 1/v vs [I] were used to determine the K_i values.[53] K_m values for carbamoyl phosphate at the different pH values in the buffers used were taken from Kurtin et al.:[27] 180 μM (pH 8.0), 111 μM (pH 7.0), and 30 μM (pH 6.0).

Supporting Information Available: Preparation and characterization of ethyl chloromethyl[(diethoxyphosphoryl)-methyl]phosphinate, N^α-isobutoxycarbonyl-L-ornithine *tert*-butyl ester, and N^α-isobutoxycarbonyl-N^δ-{[(diethoxyphosphoryl)methyl(ethoxy)phosphoryl]methyl}-L-ornithine *tert*-butyl ester; modified procedures for the preparation of dibenzyl methylphosphonate and (dibenzyloxyphosphoryl)acetic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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