Table V. Crystal Data for **9e**

formula	$C_{23}H_{20}O$
crystal system	triclinic
space group	$P\bar{1}$
a, Å	10.034 (2)
b, Å	12.474 (2)
c, Å	7.604 (3)
α, deg	107.55 (2)
β, deg	106.00 (2)
γ, deg	78.75 (2)
V, Å^3	865.8 (8)
Z	2
density calcd, g/cm^3	1.198
crystallizing solvent	hexane–ethyl acetate
crystal habit	tabular (white)
crystal dimensions, mm	0.2 × 0.5 × 0.6
μ, cm^{-1}	0.67
transmission factor range	not applied
extinction	not applied
2θ limit, deg (octants)	50.5 ($\pm h \pm k + l$)
intensities (unique, R_i)	3436 (3033, 0.015)
intensities > $1.96\sigma(I)$	1809
R (observed intensities)	0.048
R_w [for $w = 1/\sigma^2(F_o) + pF_o^2$]	0.055 (p = 0.020)
max density in ΔF map, e/Å^3	0.16

least-squares fit to the automatically centered settings for 25 reflections ($2\theta > 20°$). Three reference reflections monitored during the experiment showed no significant variation. Intensity data were corrected for Lorentz–polarization effects. Crystal data are listed in Table V. Space group assignment was suggested by cell geometry and average values of the normalized structure factors; the choice was confirmed by successful refinement.

The structure was solved by direct methods (SHELX[55]); correct positions for all non-hydrogen atoms were deduced from E maps. Difference Fourier electron density maps revealed positions for all hydrogen atoms, and the final least squares refinement cycle (SHELX) included independent parameters for all positions, anisotropic thermal coefficients for all nonhydrogen atoms, and isotropic thermal parameters for hydrogen atoms. The final difference Fourier map had no significant features. Atomic scattering factors, mass attenuation coefficients, and anomalous dispersion corrections were taken from ref 56.

Acknowledgment. We are grateful for support of this research through a grant from the National Institutes of Health (Grant PHS 2R37 DK 15556). High-field NMR spectra and high-resolution mass spectra were obtained on instruments supported by grants from the National Institutes of Health (Grants RR 02299 and GM 27029, respectively). We are thankful to Kathryn E. Carlson for performing the estrogen receptor binding assays.

Supplementary Material Available: Atomic numbering scheme, tables of atomic coordinates, thermal parameters, bond lengths, and bond angles for compound **9e** (4 pages). Ordering information is given on any current masthead page.

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Synthesis of Acyclic and Dehydroaspartic Acid Analogues of Ac-Asp-Glu-OH and Their Inhibition of Rat Brain N-Acetylated α-Linked Acidic Dipeptidase (NAALA Dipeptidase)

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The following structural and conformationally constrained analogues of Ac-Asp-Glu-OH (**1**) were synthesized: Ac-Glu-Glu-OH (**2**), Ac-D-Asp-Glu-OH (**3**), Ac-Glu-Asp-OH (**4**), Ac-Asp-Asp-OH (**5**), Ac-Asp-3-aminohexanedioic acid (**6**), Ac-3-amino-3-(carboxymethyl)propanoyl-Glu-OH (**7**), *N*-succinyl-Glu-OH (**8**), *N*-maleyl-Glu-OH (**9**), *N*-fumaryl-Glu-OH (**10**), and Ac-Δ^ZAsp-Glu-OH (**11**). These analogues were evaluated for their ability to inhibit the hydrolysis of Ac-Asp-[3,4-^3H]-Glu-OH by N-acetylated α-linked acidic dipeptidase (NAALA dipeptidase) in order to gain some insight into the structural requirements for the inhibition of this enzyme. Analogues **4**–**6** and **9** were very weak inhibitors of NAALA dipeptidase (K_i > 40 μM), while **2**, **3**, and **7** with K_i values ranging from 3.2–8.5 μM showed intermediate inhibitory activity. The most active inhibitors of NAALA dipeptidase were compounds 8, 10, and 11 with K_i values of 0.9, 0.4, and 1.4 μM, respectively. These results suggest that the relative spacing between the side chain carboxyl and the α-carboxyl group of the C-terminal residue may be important for binding to the active site of the enzyme. They also indicate that the χ_1 torsional angle for the aspartyl residue is in the vicinity of 0°.

Localization[1-3] and release studies[4,5] suggest a role for the acidic dipeptide Ac-Asp-Glu-OH (**1**) in synaptic processes. Binding studies[6] which demonstrated that Ac-Asp-Glu-OH was capable of displacing radiolabeled glutamic acid from synaptic plasma membranes and electrophysiological studies[7-13] that reported that Ac-Asp-Glu-OH exhibited excitatory effects when injected into a rat brain led many researchers to suggest a neurotransmitter role for **1**. The identification of an N-acetylated α-linked acidic dipeptidase (NAALA dipeptidase) capable of degrading Ac-Asp-Glu-OH into Ac-Asp-OH and Glu-OH

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Table I. Physical Properties of the Dipeptide Intermediates 12–19

no.	compound	yield, %	mp, °C	$[\alpha]_D$, deg (c 1.0, solvent)	formula[a]
12	Boc-Glu(OBzl)-Glu(OBzl)-OBzl	85	71–72	−4.3 ($CHCl_3$)	$C_{36}H_{42}N_2O_9$
13	Boc-D-Asp(OBzl)-Glu(OBzl)-OBzl	92	102–103	+5.5 ($CHCl_3$)	$C_{35}H_{40}N_2O_9$
14	Boc-Glu(OBzl)-Asp(OBzl)-OBzl	86	80–81	+17.7 ($CHCl_3$)	$C_{35}H_{40}N_2O_9$
15	Boc-Asp(OBzl)-Asp(OBzl)-OBzl	83	96–98	−13.6 (MeOH)	$C_{34}H_{38}N_2O_9$
16	Ac-Glu(OBzl)-Glu(OBzl)-OBzl	74	115–116	−22.4 (MeOH)	$C_{33}H_{36}N_2O_8$
17	Ac-D-Asp(OBzl)-Glu(OBzl)-OBzl	67	94–96	+6.5 (MeOH)	$C_{32}H_{34}N_2O_8$
18	Ac-Glu(OBzl)-Asp(OBzl)-OBzl	73	94–95	−13.6 (MeOH)	$C_{32}H_{34}N_2O_8$
19	Ac-Asp(OBzl)-Asp(OBzl)-OBzl	79	114–115	−16.8 (MeOH)	$C_{31}H_{32}N_2O_8 \cdot H_2O$

[a] C, H, N analyses were within ±0.4% of the theoretical values.

provided further support for this theory of Ac-Asp-Glu-OH action.[14]

NAALA dipeptidase is a membrane bound, chloride activated, metallopeptidase which is mainly localized in the central nervous system (CNS), the kidney being the only non-neural tissue containing detectable amounts of this enzyme.[14,15] The observed subcellular localization of NAALA dipeptidase in synaptic plasma membranes and the ontogenetic studies[15] that show that its appearance in the brain parallels synaptic development indicates that this enzyme may have some involvement in synaptic processes. The identification of NAALA dipeptidase, however, has cast doubt upon the previous binding and electrophysiology studies that failed to control the degradation of Ac-Asp-Glu-OH by this enzyme.[16] Furthermore, recent developments in the study of Ac-Asp-Glu-OH have made it more difficult to definitively assign it a neurotransmitter role.[17] Nevertheless, Ac-Asp-Glu-OH's high concentration but uneven distribution in the CNS[2,18,19] and its localization in nerve tissue suggests a role for this acidic peptide in synaptic transmission. In addition to neurotransmission, other possible actions of Ac-Asp-Glu-OH might be to serve as an inactive storage form for the active transmitter glutamate or to provide a slow release mechanism for glutamate in the synaptic cleft through the action of NAALA dipeptidase. It is also possible that Ac-Asp-Glu-OH could be involved in nerve cell metabolism in the cell terminus and thereby play a secondary role in synaptic transmission. In addition, certain discrepancies in the regional and ontogenetic profiles of Ac-Asp-Glu-OH and NAALA dipeptidase[15] suggest that the relationship between these two may not be an exclusive one and indeed may reflect a role for NAALA dipeptidase on other acidic peptides in vivo.

It is clear that further study of the role of Ac-Asp-Glu-OH in the CNS is warranted. Future studies, however, must carefully control the activity of NAALA dipeptidase so that the effects due to Ac-Asp-Glu-OH can be distinguished from those due to glutamic acid. An important and useful means of delineating the functional roles of Ac-Asp-Glu-OH and NAALA dipeptidase would be through the use of inhibitors of NAALA dipeptidase. Early studies which made use of commercially available compounds have identified a number of inhibitors of NAALA dipeptidase.[14] In order to characterize further the enzyme's active site and to develop potentially useful inhibitors of this enzyme for pharmacological studies, we have synthesized several structural and conformationally constrained analogues of Ac-Asp-Glu-OH, compounds **2–11** (Chart I), and have evaluated the ability of these analogues to inhibit the hydrolysis of Ac-Asp-[3,4-^{3}H]-Glu-OH by NAALA dipeptidase.

We have followed two approaches to determine the relative spatial disposition of the three carboxyl moieties when binding to the enzyme. In one approach, we synthesized a series of flexible acyclic analogues of Ac-Asp-Glu-OH, compounds **2–7**, in which the relative distance between the three carboxyl groups has been varied. In the second approach, the maleyl, fumaryl, and *N*-acetyl-α,β-dehydroaspartyl (Ac-ΔAsp-OH) residues have been substituted for the Ac-Asp-OH residue in Ac-Asp-Glu-OH to give analogues **9–11**, respectively, in order to define the conformational relationship between the aspartyl carboxyl group and the two carboxyl groups of the glutamic acid

Scheme I

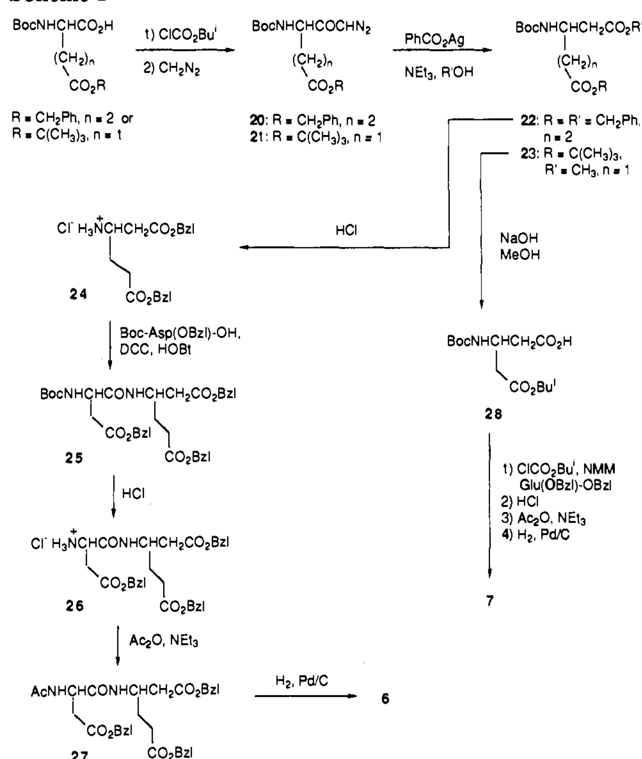

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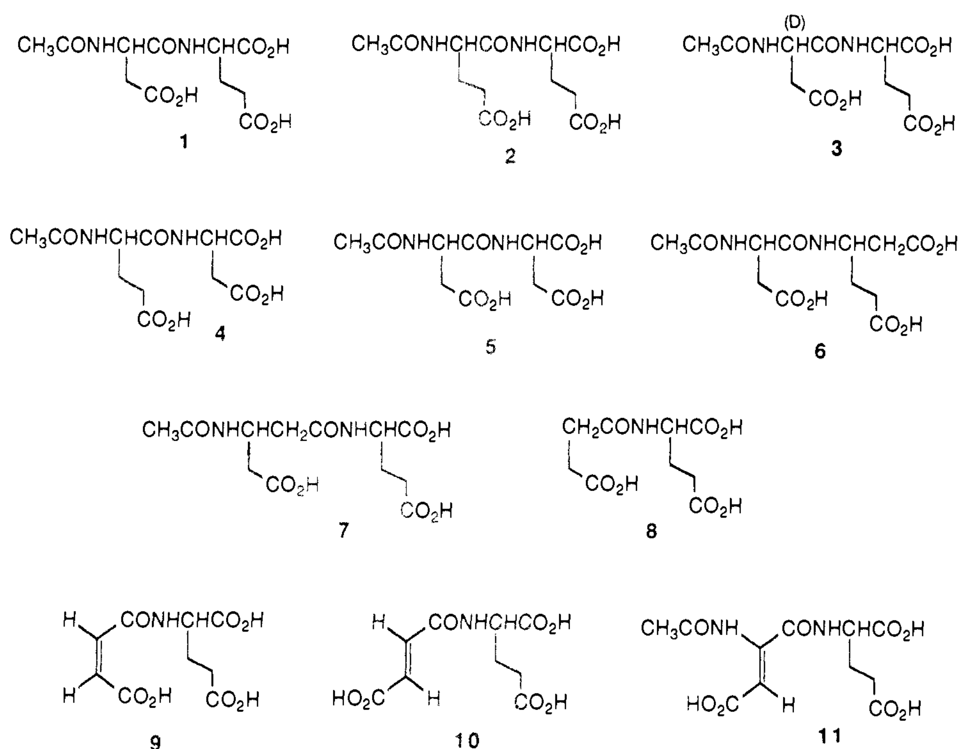
Chart I



residue. Analogue 8 was synthesized in order to evaluate the importance of the acetamido moiety in binding to the enzyme.

Results and Discussion

Chemical Syntheses. The synthesis of the Ac-Asp-Glu-OH analogues **2–5** was carried out in a manner whereby either Boc-Glu(OBzl)-OH, Boc-D-Asp(OBzl)-OH, or Boc-Asp(OBzl)-OH was coupled by the mixed anhydride method[20] with either Glu(OBzl)-OBzl or Asp(OBzl)-OBzl. With this general methodology the fully protected dipeptides **12–15** listed in Table I were made. The *N-tert*-butoxycarbonyl protecting group of each of these dipeptides was removed with HCl in dioxane and the resulting deprotected species then acetylated with Ac_2O in the presence of NEt_3 to provide the carboxyl protected acetylated dipeptides **16–19** (Table I). Removal of the benzylcarboxyl protecting groups from **16–19** by hydrogenolysis gave the acetylated dipeptides **2–5**.

The synthesis of the two analogues **6** and **7** are outlined in Scheme I. For analogue **6** the β-amino acid derivative **24** was required. This material was synthesized by first treating the mixed anhydride formed from Boc-Glu-(OBzl)-OH and isobutyl chloroformate with diazomethane to give diazo ketone **20**. This material was dissolved in benzyl alcohol and then treated with silver benzoate in NEt_3 in a manner analogous to that used by us previously[21] to give the β-amino acid derivative **22**. Removal of the *N-tert*-butoxycarbonyl protecting group from **22** with HCl gave **24** which was coupled to Boc-Asp(OBzl)-OH with dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) to provide dipeptide **25**. This material was converted to **6** by the same set of procedures that were used to convert **12–15** to **2–5**.

As in the case of **6**, the synthesis of **7** required a β-amino acid derivative. In this case, however, the β-amino acid derivative that was needed was compound **28**. This material was synthesized by first reacting the mixed anhydride between Boc-Asp(OBu^t)-OH and isobutyl chloroformate with diazomethane to give diazoketone **21**. Treatment of a methanolic solution of this material with silver benzoate in NEt_3 in the same manner as that used by us previously[21] yielded the β-amino acid derivative **23**. This was converted to **28** by the selective hydrolysis of the methyl ester. Compound **28** was subsequently converted to **7** by the same set of procedures that were used to make **2–5**.

N-Succinyl-L-glutamic acid (**8**) was obtained by first treating Glu(OBzl)-OBzl with succinic anhydride in acetone to give *N*-succinyl-Glu(OBzl)-OBzl (**29**) and then subjecting this material to hydrogenolysis. Our initial attempt to synthesize *N*-maleyl-L-glutamic acid (**9**) involved the addition of Glu(OBu^t)-OBu^t to maleic anhydride to give *N*-maleyl-Glu(OBu^t)-OBu^t. All attempts, however, at removing the *tert*-butyl esters of this material under a variety of conditions resulted only in inseparable mixtures of products. Subsequently, **9** was prepared by fusing Glu-OH with maleic anhydride at 100 °C, a procedure adapted from that used by Werbin and Spoerri[22] to synthesize *N*-maleylglycine.

N-Fumaryl-L-glutamic acid (**10**) was synthesized by first coupling monomethyl fumarate with Glu(OBu^t)-OBu^t with the aid of DCC/HOBT to give *N*-(β-methylfumaryl)-Glu-(OBu^t)-OBu^t (**30**), followed by treatment of **30** with CF_3CO_2H and then NaOH to remove the *tert*-butyl and methyl esters, respectively.

The method of Schmidt et al.,[23] who have prepared a number of dehydroamino acids and dehydropeptides by the condensation of aldehydes with *N*-acyl-2-[bis(alkyloxy)phosphinyl]glycine esters in a Wittig–Horner type olefination, was employed to obtain dehydropeptide **11**. Initially, we attempted to synthesize **11** by the pathway outlined in Scheme II. In this pathway trimethyl *N*-Z-

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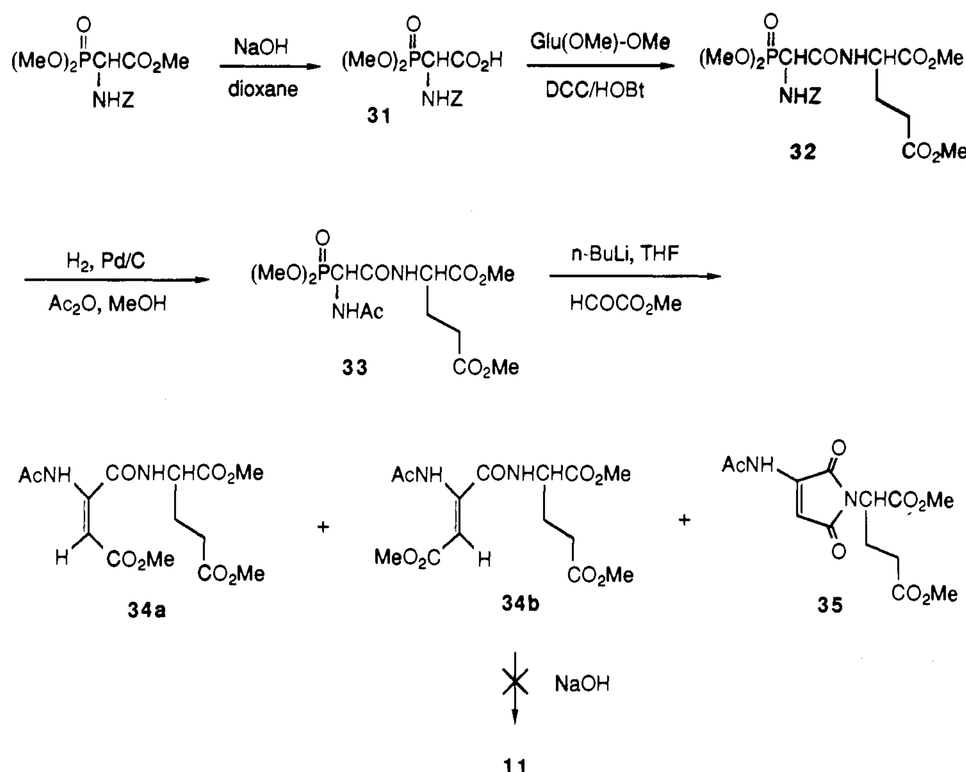
Scheme II



phosphonoglycinate was treated with NaOH to give the free acid **31**. This material was coupled to Glu(OMe)-OMe with DCC/HOBt to give **32** as a 9:11 mixture of diastereoisomers. The *N*-benzyloxycarbonyl group of **32** was exchanged for the acetyl group by subjecting the mixture of diastereoisomers to hydrogenolysis in the presence of Ac_2O. The condensation of methyl glyoxylate with **33** gave the expected *E* and *Z* dehydropeptides, **34a** and **34b**, respectively along with imide **35** in a ratio of 5:14:1 as determined by ^1H NMR. The formation of **35** was not entirely unexpected, since acid- or base-catalyzed imide formation in aspartyl containing peptides has been well-documented.[24] In fact, one would expect the *E*-dehydroaspartyl residue to have an enhanced propensity for imide formation since the α,β-double bond holds the β-carboxyl group in close proximity to the α-carboxamide nitrogen. This ready formation of **35** from **34a** was also seen in a two-dimensional thin-layer silica gel chromatography experiment. When EtOAc was used as the developing solvent, a portion of **34a** was readily converted to the imide. Attempts to hydrolyze **34b** in order to obtain the desired deprotected dehydropeptide **11** resulted only in the formation of a complex mixture of products, however.

An alternate route to **11** was successfully developed, whereby the *tert*-butyl group was used as the carboxyl protecting group (Scheme III). In this route, **31** was coupled to Glu(OBu^t)-OBu^t with the water-soluble carbodiimide 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and HOBt to give the phosphonodipeptide **36** as a mixture of diastereoisomers. Catalytic hydrogenolysis of this mixture in the presence of Ac_2O gave the N-acetylated dipeptide **37**. The *E* and *Z* isomers of Ac-ΔAsp(OBu^t)-Glu(OBu^t)-OBu^t (**39a** and **39b**, respectively) were formed by condensing the ylide of **37**, which was generated with Bu^{t}OK in CH_2Cl_2, with *tert*-butyl glyoxylate (**38**). The isolated ratio of **39a** and **39b** was 2:3.

The *Z* isomer **39b** could be readily distinguished from the *E* isomer **39a** by ^1H NMR, since it had been shown[25,26] that for *N*-acyldehydroaspartic acid derivatives the chemical shift of the *E* isomer olefinic hydrogen appears downfield of that of the *Z* isomer olefinic hydrogen. For **39a** the olefinic hydrogen is observed at 7.65 ppm, while for **39b** it appears at 5.39 ppm. Similar chemical-shift differences were also observed between **34a** and **34b**. Furthermore, in dehydroaspartic acid derivatives the signal for the enamine NH of the *Z* isomer is much farther downfield than that of the *E* isomer. In the case of **39a** and **39b** they appear at 8.87 and 10.20 ppm, respectively. The presence of a strong intramolecular hydrogen bond between the enamine NH and the β-carboxyl group of the Δ^ZAsp residue has been invoked to explain the chemical shift of the enamine NH in the *Z* isomer.[27] By contrast, the signal for the Glu NH of the *E* isomer **39a** is located much farther downfield than the Glu NH signal of the *Z* isomer **39b**.

Deprotection of the *Z* isomer **39b** with CF_3CO_2H afforded the desired dehydropeptide Ac-Δ^ZAsp-Glu-OH (**11**). To our knowledge this is the first reported synthesis of a dehydroaspartyl-containing peptide in which the β-carboxyl group is not protected.[28] When the *E* isomer **39a** was treated in a similar manner, ^1H NMR indicated that the dehydropeptide Ac-Δ^EAsp-Glu-OH (**40**) was generated.

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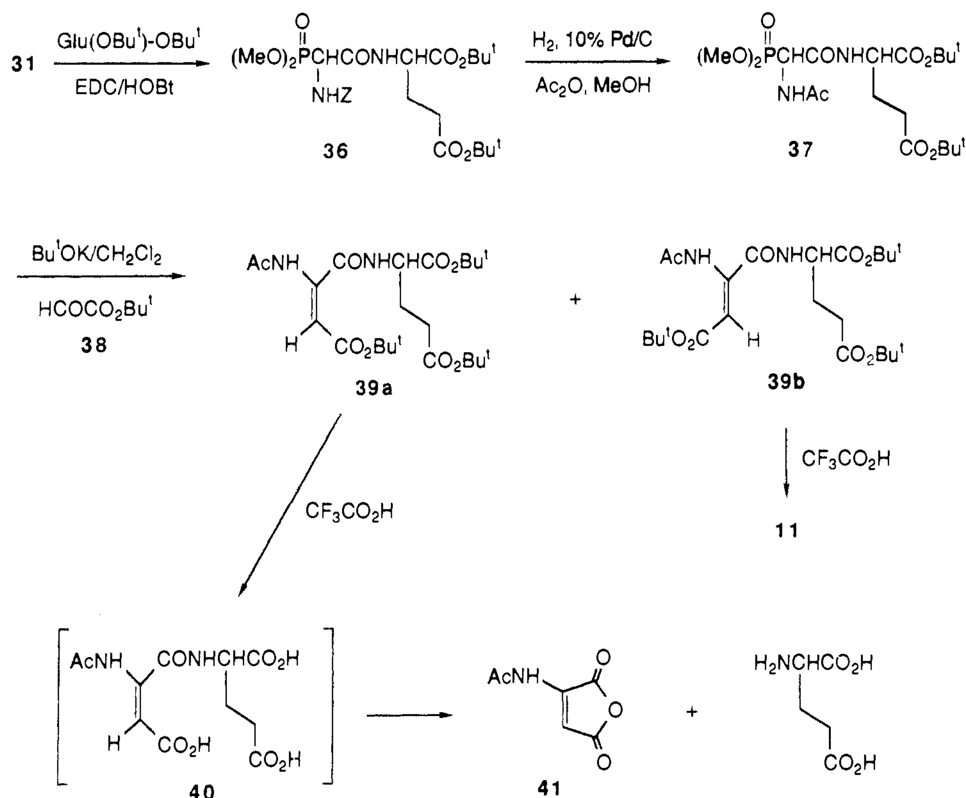
Scheme III



However, when attempts were made to isolate this material only the decomposition products of **40**, *N*-acetyldehydroaspartic anhydride (**41**) and glutamic acid could be isolated.

Enzyme Inhibition Studies. The "buffy coat" membrane preparation used in this study as the source of the NAALA dipeptidase activity was obtained from rat forebrains in a manner analogous to that previously reported in the literature.[14] The NAALA dipeptidase activity of these membrane preparations possessed a Michaelis constant for Ac-Asp-Glu-OH hydrolysis equal to 1.0 μM and a V_{max} value of 300 pmol min^{-1} mg protein^{-1}. For each compound **2–11**, the inhibitor concentration (IC_{50}) which caused a 50% inhibition of Ac-Asp-Glu-OH hydrolysis at a substrate concentration of 1.0 μM was determined. The results obtained are summarized in Table II. For those compounds with an IC_{50} value less than 100 μM the K_i for the inhibition of Ac-Asp-Glu-OH hydrolysis was also determined. All of these compounds demonstrated competitive inhibition. These results are also presented in Table II.

Consistent with earlier studies,[14] those compounds that contained replacements for the C-terminal glutamic acid residue, compounds **4–6**, were considerably less potent than those that contained this residue. The low potencies of Ac-Glu-Asp-OH (**4**) and Ac-Asp-Asp-OH (**5**) suggest that the length of the amino acid side chain on the C-terminal residue is important to binding. Although this may be a factor in the low potency of these compounds, the low inhibitory activity of **6**, which contains the same carboxyl side chain as that found in glutamic acid but, in addition, has the peptide backbone extended by one methylene unit, suggests that the relative spacing between the side chain carboxyl and the α-carboxyl group of the C-terminal residue may be important for binding to the active site of the enzyme. That it is simply not the extension of the peptide backbone that gives rise to the low activity of **6** is shown by the much greater inhibitory activity of **7**, an analogue that also has an extended peptide backbone.

Table II. Inhibition of Ac-Asp-[3,4-^{3}H]-Glu-OH Hydrolysis by **2–11**[a]

no.	compound	IC_{50} (μM)	K_i (μM)
2	Ac-Glu-Glu-OH	9.0 ± 1.8	3.5
3	Ac-D-Asp-Glu-OH	15.0 ± 3.0	8.5
4	Ac-Glu-Asp-OH	193.0 ± 11.0	ND[b]
5	Ac-Asp-Asp-OH	250.0 ± 25.0	ND
6	Ac-Asp-NHCH($CH_2CH_2CO_2H$)CH_2CO_2H	200.0 ± 56.0	ND
7	Ac-NHCH(CH_2CO_2H)CH_2CO-Glu-OH	8.0 ± 1.8	3.2
8	N-Succinyl-Glu-OH	2.5 ± 0.1	0.9
9	N-Maleyl-Glu-OH	83.0 ± 1.4	41.0
10	N-Fumaryl-Glu-OH	0.8 ± 0.1	0.4
11	Ac-Δ^ZAsp-Glu-OH	2.2 ± 0.3	1.4

[a] The IC_{50} values listed are mean values of three experiments ± SEM. All data conform to a hypothetical hyperbolic binding curve with a Hill coefficient of 1. K_i values were determined as described in the text for all compounds with observed IC_{50} values less than 100 μM and under the conditions used in this study, the K_i is approximately equal to one-half the observed IC_{50} value. All compounds for which a K_i was determined demonstrated competitive inhibition. [b] ND = not determined.

A previous study[14] on the inhibition of Ac-Asp-[3,4-^{3}H]-Glu-OH hydrolysis by NAALA dipeptidase has shown that the removal of the acetyl group from Ac-Asp-Glu-OH results in a 4-fold increase in the IC_{50} value. This loss of potency could be due to the loss of an acetyl binding interaction or an unfavorable interaction of the resulting positively charged N-terminus of Asp-Glu-OH at the active site. In order to test these possibilities **8** was synthesized. This analogue is a good inhibitor of NAALA dipeptidase. Its K_i value of 0.9 μM indicates that this compound has an affinity to NAALA dipeptidase similar to that of Ac-Asp-Glu-OH. This suggests that the acetamido moiety is not an important requirement for binding to the enzyme and that the lower affinity of Asp-Glu-OH is probably due to an unfavorable interaction between the positively charged N-terminal amino group and the enzyme.

Compounds **9–11** were synthesized in order to define the conformational relationship between the aspartyl carboxyl group and the two carboxyl groups of the glutamic acid residue. As the results in Table II show maleyl-Glu-OH (**9**) is a poor inhibitor of NAALA dipeptidase. It is some

100 times less active than the fumaryl derivative **10**, which with a K_i value of 0.4 μM is the most potent inhibitor in the series of analogues examined in this study. This analogue also has about 2.5 times greater affinity to NAALA dipeptidase than Ac-Asp-Glu-OH itself. In the fumaryl derivative, the carboxyl moiety that corresponds to the β-carboxyl group of the Asp residue is rigidly held in a extended conformation with respect to the Glu carboxyl moieties, whereas a more folded conformation can be adopted in the case of **9**. These results suggest that when Ac-Asp-Glu-OH interacts with NAALA dipeptidase the side chain of the Asp residue has a χ_1 torsional angle of approximately 0°.

We see that in the case of Ac-Δ^ZAsp-Glu-OH (**11**) the addition of the acetamido residue actually results in a compound that has less affinity for NAALA dipeptidase than either **10** or Ac-Asp-Glu-OH. Like **10**, **11** has its χ_1 torsional angle restricted to 0°. Thus, the three carboxyl groups of **11** should be able to achieve the same relative spatial arrangement as the three carboxyl groups in **10**, indicating that it is the acetamido group that decreases the binding of **11** to NAALA dipeptidase. An explanation for this may be that the α,β-double bond also restricts the Φ torsional angle, in addition to the χ_1 torsional angle, due to adverse steric interactions between the acetamido moiety and the rigidly fixed β-carboxyl group. In *Z* dehydropeptides such as **11**, the Φ torsional angle should be restricted to the following range $90° > \Phi < -90°$.[29] This restriction on the Φ torsional angle may very well place the acetamido group in a conformation that is detrimental to its interaction with NAALA dipeptidase.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus 6406-K and are uncorrected. Specific rotations were measured with a Rudolph Research Autopol III polarimeter at 589 nm (Na D line). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results were within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded on either a JEOL FX-90 MHz, an IBM 200 MHz, or a Nicolet Zeta 300 MHz spectrometer. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) in $CDCl_3$ or DMSO-d_6 and to sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (TSP) in D_2O. ^{13}C NMR was performed on either the JEOL FX-90 instrument at 22.5 MHz, an IBM instrument at 50 MHz, or a Nicolet Zeta instrument at 75 MHz. When DMSO-d_6 was used as solvent, it served as the internal standard at δ 39.5. When D_2O was used dioxane (δ 64.5) was added as the external standard. FAB, CI, and EI mass spectra were obtained on a VG 7070E-HF, a Finnigan 4000, and a AEI-MS-30 mass spectrometer, respectively. Column chromatography was performed with Silica Woelm (32–63 μm) from ICN Nutritional Biochemicals. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GF Uniplates. Visualization was achieved with either UV, I_2, or ninhydrin spray. Ac-Asp-[3,4-^{3}H]-Glu-OH (sp act = 54.7 Ci/mmol) was obtained from DuPont/NEN Products, Boston, MA.

General Procedure for the Synthesis of Dipeptides 12–15. Either Boc-Glu(OBzl)-OH, Boc-D-Asp(OBzl)-OH or Boc-Asp-(OBzl)-OH (2 mmol) and *N*-methylmorpholine (0.22 mL, 2 mmol) were dissolved in 10 mL of THF. This solution was cooled to −25 °C after which time a solution of isobutyl chloroformate (0.26 mL, 2 mmol) in THF (2 mL) was added slowly. The mixture was stirred for 2 min and then treated with a solution of either Glu(OBzl)-OBzl·*p*TsOH or Asp(OBzl)-OBzl·*p*TsOH (2 mmol) and NEt_3 (0.56 mL, 4 mmol) in 2 mL of THF. After the mixture was stirred for 45 min at room temperature, the THF was removed in vacuo. The residue was partitioned between 10% citric acid and EtOAc. The EtOAc layer was washed with 10% citric acid, 1 M $NaHCO_3$, and saturated NaCl solution before being dried over $MgSO_4$. Removal of EtOAc under reduced pressure and trituration of the resulting residue with hexane or recrystallization from EtOAc/hexane yielded the fully protected dipeptides **12–15**. The physical properties of these dipeptides are listed in Table I.

General Procedure for the Synthesis of Dipeptides 16–19. Each (2.3 mmol) of the *N*-*tert*-butoxycarbonyl protected dipeptides **12–15** was treated with 3 mL of 4 N HCl in dioxane for 2 h at room temperature. The excess HCl and dioxane were removed in vacuo, and the deprotected dipeptide was dissolved in a mixture of H_2O (15 mL) and dioxane (15 mL). This solution was cooled to 4 °C, and NEt_3 (0.23 g, 2.3 mmol) and Ac_2O (0.23, 2.3 mmol) were added sequentially. The solution was stirred at 4 °C for 1 h and then at room temperature for 3 h after which time it was reduced in volume by one-half. Water (20 mL) was added to this mixture and this was then extracted with EtOAc (2 × 30 mL). The combined EtOAc extracts were washed with 1 N HCl, 1 M $NaHCO_3$, and saturated NaCl solution and then dried ($MgSO_4$). Removal of EtOAc under reduced pressure afforded the acetylated dipeptides **16–19**. The physical properties of these dipeptides are listed in Table I.

General Procedure for the Synthesis of Dipeptides 2–5. Each of the protected dipeptides **16–19** (0.85 mmol) was dissolved in 10 mL of DMF. This solution was added to a Parr bottle containing 60 mg of 10% Pd/C. The mixture was placed on a Parr hydrogenator at 40 psi H_2 for 2 h. The mixture was filtered through a pad of Celite with the aid of MeOH. Removal of the solvents in vacuo yielded the crude product which was purified on a Bio-Rad AG1-X8 (100–200 mesh) anion exchange column containing 10 g of resin that had been equilibrated with 0.1 N formic acid. The product was eluted with a gradient of 0.1–1 N formic acid. Lyophilization of the appropriate fractions afforded the desired acetylated acidic dipeptide. The chemical and physical properties of the acetylated acidic dipeptides prepared in this manner are listed below.

Ac-Glu-Glu-OH (2): $[\alpha]_D$ −23.9° (*c* 1.0, MeOH); NMR (300 MHz, D_2O) δ 2.03 (s, 3 H, $COCH_3$), 1.93–2.73 (m, 4 H, Glu β-CH_2), 2.46–2.54 (m, 4 H, Glu γ-CH_2), 4.33–4.43 (m, 2 H, Glu α-CH); FAB-MS *m/z* 319 $(MH)^+$. Anal. ($C_{12}H_{18}N_2O_8$) C, H, N.

Ac-D-Asp-Glu-OH (3): mp 132–134 °C; $[\alpha]_D$ +38.8° (*c* 1.0, MeOH); TLC, R_f (*n*-BuOH/AcOH/H_2O/EtOAc, 1:1:1:1) = 0.60, (*n*-BuOH/pyridine/AcOH/H_2O, 15:10:3:12) = 0.32; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.78–1.91 (m with s at 1.84, 5 H, Glu β-CH_2 and $COCH_3$), 2.18–2.27 (m, 2 H, Glu γ-CH_2), 2.44 (dd, J = 8.0 and 16.3 Hz, 1 H, Asp β-CH_2), 2.62 (dd, J = 8.0 and 16.3 Hz, 1 H, Asp β-CH_2), 4.06–4.13 (m 1 H, Glu α-CH), 4.53–4.60 (m, 1 H, Asp α-CH), 7.83 (d, 1 H, CONH), 8.19 (d, 1 H, CONH); ^{13}C NMR (22.5 MHz, D_2O) δ 22.4, 26.4, 29.8, 37.6, 49.4, 51.3, 169.2, 170.5, 171.4, 172.7, 173.6; FAB-MS *m/z* 305 $(MH)^+$. Anal. ($C_{11}H_{16}N_2O_8$) C, H, N; C: calcd, 43.42; found, 42.80.

Ac-Glu-Asp-OH (4): $[\alpha]_D$ −15.3° (*c* 1.5, MeOH); ^{1}H NMR (300 MHz, D_2O) δ 1.90–2.20 (m with s at 2.03, 5 H, Glu β-CH_2 and $COCH_3$), 2.47–2.52 (m, 2 H, Glu γ-CH_2), 2.85–3.01 (m, 2, H, Asp β-CH_2), 4.34–4.39 (m, 1 H, Glu α-CH), 4.67–4.71 (m, 1 H, Asp α-CH); ^{13}C NMR (22.5 MHz, D_2O) δ 24.6, 29.2, 33.0, 39.2, 53.0, 55.8, 170.0, 175.8, 177.0, 177.7, 180.1; FAB-MS *m/z* 305 $(MH)^+$. Anal. ($C_{11}H_{16}N_2O_8$) C, H, N.

Ac-Asp-Asp-OH (5): $[\alpha]_D$ −18.9° (*c* 0.9, MeOH); ^{1}H NMR (D_2O) δ 2.03 (s, 3 H, $COCH_3$), 2.74–2.95 (m, 4 H, Asp β-CH_2), 4.61–4.65 (m, 1 H, Asp α-CH), 4.70–4.77 (m, 1 H, Asp α-CH); ^{13}C NMR (22.5 MHz, D_2O) δ 24.7, 38.5, 38.8, 52.9, 53.0, 175.0, 177.1, 177.2, 177.5; FAB-MS *m/z* 291 $(MH)^+$. Anal. ($C_{10}H_{14}N_2O_8$) C, H, N.

Benzyl 4-[(*tert*-Butoxycarbonyl)amino]-5-oxo-6-diazohexanoate (20). To a solution of Boc-Glu(OBzl)-OH (6.74 g, 20 mmol) and *N*-methylmorpholine (2.02 g, 20 mmol) in THF (80 mL) at −30 °C was added isobutyl chloroformate (2.73 g, 20 mmol). After the addition of isobutyl chloroformate was complete, Et_2O (70 mL), which had been cooled to −70 °C, was added. The mixture was transferred to a filter addition funnel connected to a receiving flask containing a Et_2O solution of diazomethane (80 mL) at −20 °C. The yellow solution was allowed to warm up to room temperature where it was stirred for 19 h. The solvents and excess diazomethane were removed in vacuo, and the yellow oil that remained was dissolved in Et_2O (100 mL). This solution was

(29) Nitz, T. H.; Shimohigashi, Y.; Costa, T.; Chen, H.-C.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1986**, *27*, 522.

washed with distilled H_2O, 1 M $NaHCO_3$, and saturated NaCl solution. After the Et_2O solution was dried over $MgSO_4$, the solvent was removed, and the yellow oil that remained was subjected to flash chromatography (300 g of silica gel) with EtOAc/hexane (7:13) as the eluting solvent. The fractions containing the desired product were stripped of solvent, and the residue that was obtained crystallized upon standing. These crystals were collected with the aid of petroleum ether (bp 30–60 °C) to give 5.28 g (73%) of **20** as light yellow crystals: mp 69–70 °C; $[\alpha]_D$ −12.5° (*c* 1.0, CH_2Cl_2); ^1H NMR (90 MHz, $CDCl_3$) δ 1.39 (s, 9 H, $C(CH_3)_3$), 1.75–2.10 (m, 2 H, CH_2), 2.36 (bt, 2 H, CH_2CO), 4.18–4.22 (m, 1 H, NCHCO), 5.04 (s, 2 H, CH_2Ph), 5.20 (b, 1 H, NH), 5.40 (s, 1 H, CHN_2), 7.27 (s, 5 H, Ph Hs); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 27.7, 28.3, 30.1, 53.8, 56.7, 66.4, 80.1, 128.2, 128.5, 135.8, 155.3, 172.5, 193.1. Anal. ($C_{18}H_{23}N_3O_5$) C, H, N.

***tert*-Butyl 3-[(*tert*-Butoxycarbonyl)amino]-4-oxo-5-diazopentanoate (21).** This material was prepared from Boc-Asp(OBu^t)-OH (1.2 g, 4.25 mmol) in a manner analogous to that described above for **20**. The product was purified by flash chromatography (50 g of silica gel) with EtOAc/CH_2Cl_2 (1:4) as the eluting solvent and was obtained as a yellow oil in a 71% yield: $[\alpha]_D$ −55.8° (*c* 1.06, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 18 H, $C(CH_3)_3$), 2.56 (bd, 1 H, CH_2CO), 2.84 (bd, 1 H, CH_2CO), 4.35–4.5 (m, 1 H, NCHCO), 5.60 (bd, 2 H, CHN_2 and NH). Anal. ($C_{14}H_{24}N_3O_5$) C, H, N.

Dibenzyl 3-[(*tert*-Butoxycarbonyl)amino]hexanedioate (22). To a solution of **20** (5.06 g, 14 mmol) in benzyl alcohol (120 mL) was added in a dropwise manner a solution of silver benzoate (0.42 g, 1.82 mmol) in NEt_3 (50 mL). The dark reaction mixture was stirred at room temperature for 16 h after which time it was decolorized with activated charcoal. The yellow filtrate was washed with saturated NaCl solution and then dried with $MgSO_4$. This material was subjected to vacuum distillation to remove the excess benzyl alcohol. The solid residue that remained after the distillation was purified by flash chromatography (250 g of silica gel) with a solvent system consisting of EtOAc/CH_2Cl_2 (1:4). This provided 3.16 g (51%) of the desired product. An analytical sample was obtained by recrystallizing the material from EtOH: mp 78–79 °C; $[\alpha]_D$ −15.5° (*c* 1.0, CH_2Cl_2); ^1H NMR (90 MHz, $CDCl_3$) δ 1.41 (s, 9 H, $C(CH_3)_3$), 1.82–1.97 (m, 2 H, CH_2), 2.40 (d, *J* = 7.2 Hz, 2 H, CH_2CO), 2.58 (d, *J* = 5.5 Hz, 2 H, CH_2CO), 3.92–4.02 (m, 1 H, NCH), 5.10 (s, 4 H, CH_2Ph), 7.33 (s, 10 H, Ph Hs). Anal. ($C_{25}H_{31}NO_6$) C, H, N.

***tert*-Butyl Methyl 3-[(*tert*-Butoxycarbonyl)amino]pentanedioate (23).** To a solution of **21** (0.93 g, 2.97 mmol) in MeOH (12.5 mL) was added dropwise a solution of silver benzoate (0.1 g, 0.42 mmol) in NEt_3 (9.2 mL). The reaction and its workup was carried out in the same manner as that described above for **22**. The crude material was purified by flash chromatography (25 g of silica gel) with 10% EtOAc in CH_2Cl_2 as the eluting solvent. The pure product (0.37 g, 39%) was obtained as a light yellow oil: ^1H NMR (200 MHz, $CDCl_3$) δ 1.34 (s, 18 H, $C(CH_3)_3$), 2.38–2.55 (m, CH_2CO), 3.59 (s, 3 H, OCH_3), 4.16 (m, 1 H, NCH), 5.29 (bd, 1 H, NH). Anal. ($C_{15}H_{27}NO_6$) C, H, N.

Dibenzyl 3-Aminohexanedioate Hydrochloride (24). Compound **22** (2.63 g, 5.95 mmol) was treated with 20 mL of 4 N HCl in dioxane. The solution was stirred at room temperature for 8 h, after which time the dioxane and excess HCl were removed in vacuo. The residue was dried over KOH under vacuum for 17 h and then isolated by trituration with Et_2O to give 2.1 g (93%) of product: mp 131–132 °C; $[\alpha]_D$ +8.5° (*c* 1.0, CH_2Cl_2). Anal. ($C_{20}H_{25}NO_4Cl$) C, H, N.

Dibenzyl 3-[*N*-[Boc-L-Asp(OBzl)]amino]hexanedioate (25). To a solution of **24** (2.0 g, 5.28 mmol) and *N*-methylmorpholine (0.53 g, 5.3 mmol) in CH_2Cl_2 (30 mL) at 4 °C was added successively Boc-Asp(OBzl)-OH (1.71 g, 5.28 mmol), 1-hydroxybenzotriazole (1.07 g, 7.9 mmol), and dicyclohexylcarbodiimide (1.25 g, 6.07 mmol). The reaction mixture was stirred in an ice bath for 2 h and then at room temperature for 28 h. The white precipitate was removed by filtration and the filtrate was washed successively with 10% citric acid, 1 M $NaHCO_3$, and saturated NaCl solution before being dried over $MgSO_4$. Purification of the product by flash chromatography (180 g of silica gel) with 20% EtOAc in CH_2Cl_2 as the eluting solvent system provided 2.3 g (67%) of **25** as a white powder: mp 100–101 °C; $[\alpha]_D$ −11.6° (*c* 1.0, CH_2Cl_2). Anal. ($C_{36}H_{42}N_2O_9$) C, H, N.

Dibenzyl 3-[*N*-[L-Asp(OBzl)]amino]hexanedioate Hydrochloride (26). Compound **25** (2.1 g, 3.25 mmol) was deprotected with 4 N HCl in dioxane (10 mL) and isolated in the same manner as that described above for **24**. A yield of 1.89 g (99%) was obtained: mp 133–135 °C; $[\alpha]_D$ +4.4° (*c* 1.0, $CHCl_3$). Anal. ($C_{31}H_{35}N_2O_7Cl$) C, H, N.

Dibenzyl 3-[*N*-[Ac-Asp(OBzl)]amino]hexanedioate (27). This material was prepared from **26** (1.78 g, 3 mmol) in the same manner as that described above for the preparation of the acetylated dipeptides **16–19**. The product was purified by flash chromatography (80 g of silica gel) with 5% MeOH in CH_2Cl_2 as the eluting solvent. The yield of **27** obtained was 1.61 g (89.7%): mp 112–113 °C; $[\alpha]_D$ −13.7° (*c* 1.0, CH_2Cl_2); ^1H NMR (200 MHz, $CDCl_3$) δ 1.81–1.92 (m, 2 H, CH_2), 1.98 (s, 3 H, CH_3CO), 2.39 (t, *J* = 7.3 Hz, 2 H, CH_2CH_2CO), 2.53 (d, *J* = 5.5 Hz, 2 H, $CHCH_2CO$), 2.62 (dd, *J* = 6.2 and 17.2 Hz, 1 H, Asp β-CH_2), 3.02 (dd, *J* = 4.2 and 17.2 Hz, 1 H, Asp β-CH_2), 4.22–4.25 (m, 1 H, NCH), 4.70–4.79 (m, 1 H, Asp α-CH), 5.06–5.09 (m, 6 H, CH_2Ph), 6.69 (d, *J* = 8.4 Hz, 1 H, NH), 7.12 (d, *J* = 9 Hz, 1 H, NH), 7.32–7.38 (m, 15 H, Ph Hs); ^{13}C NMR (50 MHz, $CDCl_3$) δ 23.18, 28.98, 30.91, 35.93, 38.80, 46.22, 49.34, 66.53, 66.64, 66.92, 128.40, 128.44, 128.66, 135.43, 135.62, 135.92, 169.97, 170.2, 171.06, 171.93, 173.22. Anal. ($C_{33}H_{36}N_2O_8$) C, H, N.

3-[*N*-(Acetyl-L-aspartyl)amino]hexanedioic Acid (6). A solution of **27** (1 g, 1.7 mmol) in MeOH (80 mL) was added to a Parr bottle that contained 0.1 g of 10% Pd/C. The mixture was placed on a Parr hydrogenator at 40 psi H_2 for 17 h. The mixture was then filtered and the MeOH removed in vacuo to give the desired product as a hygroscopic foam in a yield of 0.53 g (98%): $[\alpha]_D$ −34.1° (*c* 1.08, MeOH); ^1H NMR (200 MHz, D_2O) δ 1.66–1.79 (m, 2 H, CH_2), 1.95 (s, 3 H, CH_3CO), 2.34 (t, 2 H, CH_2CH_2CO), 2.47–2.57 (m, 2 H, CH_2CO), 2.72–2.76 (m, 2 H, Asp β-CH_2), 4.13–4.20 (m, 1 H, CH), 4.49–4.56 (t, 1 H, Asp α-CH); ^{13}C NMR (50 MHz, D_2O) δ 24.42, 31.48, 32.96, 38.38, 41.68, 48.90, 53.16, 174.86, 176.68, 176.76, 177.80, 180.33. Anal. ($C_{12}H_{18}N_2O_8$) C, H, N.

3-[(*tert*-Butoxycarbonyl)amino]pentanedioic Acid 5-*tert*-Butyl Ester (28). To a solution of **23** (0.37 g, 1.17 mmol) in 15 mL of MeOH at 4 °C was added 2.2 mL of 1 N NaOH. The reaction mixture was stirred at room temperature for 4.5 h after which time the MeOH was removed by evaporation under reduced pressure. The residue was dissolved in H_2O (10 mL), and this solution was washed with EtOAc (10 mL). The aqueous layer was acidified with 10% citric acid and then extracted with EtOAc (2 × 15 mL). The combined EtOAc extracts were dried (Na_2SO_4) and then stripped of solvent to give 0.34 g (96%) of the product as an oil. This material was characterized as the dicyclohexylammonium salt: mp 179–180 °C. Anal. ($C_{26}H_{48}N_2O_6$) C, H, N.

[3-(*N*-Acetylamino)-3-(carboxymethyl)propanoyl]-L-glutamic Acid (7). The protected amino acid **28** (0.15 g, 0.49 mmol) was coupled with Glu(OBzl)-OBzl·TsOH (0.247 g, 0.49 mmol) by the same general method as that used to synthesize **12–15**. The resulting dipeptide was purified by flash chromatography (13 g of silica gel) with 3% MeOH in CH_2Cl_2 as the eluting solvent. A yield of 0.22 g (73%) was obtained. This material was N-deprotected and then N-acylated by the same general procedure as that described above for the synthesis of **16–19**. Hydrogenolysis of the resulting material in the same manner as that described above for **6** gave 60 mg (53%) of the desired product as a foam: $[\alpha]_D$ −10.3° (*c* 0.8, MeOH); ^1H NMR (200 MHz, D_2O) δ 1.93 (s, 3 H, CH_3CO), 2.09–2.12 (m, 2 H, Glu β-CH_2), 2.43 (t, *J* = 7.2 Hz, 2 H, Glu γ-CH_2), 2.47–2.53 (dd, *J* = 4.4 and 8.2 Hz, 2 H, $CHCH_2CON$), 2.59 (d, *J* = 5.6 Hz, 2 H, $CHCH_2CO_2H$), 4.29–4.36 (m, 1 H, Glu α-CH), 4.50 (m, 1 H, CH); ^{13}C NMR (50 MHz, D_2O) δ 24.63, 28.54, 32.73, 41.21, 42.63, 46.69, 54.83, 175.44, 176.06, 177.55, 177.62, 179.78; FAB-MS *m/z* 319 $(MH)^+$. Anal. ($C_{12}H_{18}N_2O_8$) C, H, N.

Dibenzyl *N*-Succinyl-L-glutamate (29). Glu(OBzl)-OBzl (1.97 g, 6.03 mmol) was dissolved in 5 mL of acetone. To this solution were added sequentially succinic anhydride (0.77 g, 7.7 mmol) and NEt_3 (0.61 g, 6.03 mmol). The mixture was stoppered and stirred for 21 h. The acetone was evaporated and the residue was dissolved in EtOAc. This solution was washed with 10% citric acid and saturated NaCl and then dried over anhydrous $MgSO_4$. The EtOAc was evaporated, and the residue that remained was crystallized as its dicyclohexylammonium salt. Recrystallization

of the salt and the subsequent release of the free acid with 10% citric acid gave a colorless oil. Upon cooling, the oil solidified to give 1.81 g (70%) of **29** as a white solid: mp 66.5 °C; $[\alpha]_D$ −13.9° (*c* 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93–2.29 (m, 2 H, Glu β-CH_2), 2.29–2.50 (m, 4 H, $COCH_2CH_2CO$), 2.59–2.75 (m, 2 H, Glu γ-CH_2), 4.59–4.72 (m, 1 H, α-CH), 5.08 (s, 2 H, CH_2Ph), 5.15 (s, 2 H, CH_2Ph), 6.61 (d, *J* = 7.8 Hz, 1 H, NH), 7.32 (m, 10 H, Ph Hs); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 27.27 (CH_2), 29.32 (CH_2), 30.29 (CH_2), 30.63 (CH_2), 52.04 (Glu α-C), 67.44 (CH_2Ph), 127.37, 128.24, 128.58, 135.85 (Ph Cs), 171.54 (C=O), 171.83 (C=O), 172.66 (C=O), 175.78 (C=O). Anal. ($C_{23}H_{25}NO_7$) C, H, N.

***N*-Succinyl-L-glutamic Acid (8).** *N*-Succinyl-Glu(OBzl)-OBzl (**29**, 0.48 g, 1.12 mmol) was placed in 5 mL of MeOH and to this ammonium formate (2.4 g, 38 mmol) and 10% Pd/C (0.48 g) was added. The mixture was mixed with a vortex mixer for 25 min. The mixture was filtered, and the filtrate was lyophilized to give **8** as a white solid. The solid was purified by ion-exchange chromatography on an AG-1-X8 (formate form) anion exchange column to give 0.082 g (30%) of pure **8**: mp 73–76 °C; $[\alpha]_D$ −14.9° (*c* 1.0, H_2O); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.64–2.02 (m, 2 H, Glu β-CH_2), 2.21–2.30 (m, 2 H, CH_2CON), 2.34–2.50 (m, 4 H, Glu γ-CH_2 and CH_2COO), 4.08–4.33 (m, 1 H, α-CH), 8.11 (d, *J* = 7.85 Hz, 1 H, NH); ^{13}C NMR (22.5 MHz, DMSO-d_6) δ 26.52 (Glu β-C), 29.06 (CH_2), 29.82 (CH_2), 30.04 (CH_2), 51.17 (Glu α-C), 171.14 (C=O), 173.20 (C=O), 173.60 (C=O); FAB MS *m/z* 248 $(MH)^+$. Anal. ($C_9H_{13}NO_7$) C, H, N.

***N*-Maleyl-L-glutamic Acid (9).** Maleic anhydride (0.6 g, 6.1 mmol) and Glu-OH (3 g, 20 mmol) were ground together and mixed well by using a motar and pestle. The solid mixture was placed in a 50-mL round-bottom flask and heated in an oil bath at 100 °C for 2.5 h. During this time the mixture tends to aggregate, thus the aggregate was broken up from time to time. At the end of this period the mixture was repeatedly extracted with $CHCl_3$ to remove the unreacted maleic anhydride. The mixture was then extracted with acetone. The acetone fraction was evaporated and the resulting residue was dissolved in water and lyophilized to give 0.53 g (35%) of **9** as an ivory colored solid: mp 161–162 °C; $[\alpha]_D$ −10.8° (*c* 0.59, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 1.70–2.10 (m, 2 H, Glu β-CH_2), 2.30 (distorted t, 2 H, Glu γ-CH_2), 4.30 (ABq, *J* = 5.1 Hz, 1 H, α-CH), 6.2 (ABq, *J* = 12.2 Hz, 2 H, CH=CH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.39 (Glu β-C), 30.10 (Glu γ-C), 51.73 (α-C), 130.54 (*C*=C), 132.66 (C=*C*), 165.14 (C=O), 166.22 (C=O), 172.57 (C=O), 173.73 (C=O); FAB-MS *m/z* 246 $(MH)^+$, 244 $(M - H)^-$. Anal. ($C_9H_{11}NO_7$) C, H, N.

Di-*tert*-butyl *N*-(β-Methylfumaryl)-L-glutamate (30). Glu(OBu^t)-OBu^t·HCl (2 g, 6.8 mmol), monomethyl fumarate (0.9 g, 6.9 mmol), HOBt (1.8 g, 13.3 mmol), and *N*-methylmorpholine (1.48 g, 14.6 mmol) were placed in 20 mL of dry THF and cooled to 0 °C. While this mixture was stirred, DCC (1.4 g, 6.8 mmol) was added to the reaction. The reaction was stirred for 1 h at 0 °C and then overnight at room temperature. At the end of this period the *N*,*N*-dicyclohexylurea that formed was separated by filtration and the solvent evaporated from the filtrate in vacuo. The resulting residue was dissolved in EtOAc and this solution was washed with 1 N $NaHCO_3$, 10 % citric acid, H_2O, and saturated NaCl solution. The resulting solution was dried over anhydrous $MgSO_4$ and evaporated to give an oil. The oil was purified by flash chromatography (EtOAc/petroleum ether 30–60 °C, 1:4) to give a colorless oil, which upon trituration with EtOAc/petroleum ether (bp 30–60 °C) gave 1.64 g (65%) of **30** as a crystalline solid: mp 68 °C, $[\alpha]_D$ +11.9° (*c* 0.62, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.42 (s, 9 H, $C(CH_3)_3$), 1.46 (s, 9 H, $C(CH_3)_3$), 1.94–2.17 (m, 2 H, Glu β-CH_2), 2.19–2.36 (m, 2 H, Glu γ-CH_2), 3.79 (s, 3 H, OCH_3), 4.50–4.60 (m, 1 H, α-CH), 6.87 (ABq, *J* = 15.4 Hz, 2 H, CH=CH), 6.70 (d, *J* = 7.76 Hz, 1 H, Glu NH); ^{13}C NMR (22.5 MHz, $CDCl_3$), δ 27.22 (Glu β-C) 27.66 ($C(CH_3)_3$), 31.22 (Glu γ-C), 51.65 (Glu α-C), 52.28 (OCH_3), 80.27 ($C(CH_3)_3$), 81.97 ($C(CH_3)_3$), 129.66 (*C*=C), 136.19 (C=*C*), 163.15 (C=O), 165.64 (C=O), 170.42 (C=O), 171.59 (C=O). Anal. ($C_{19}H_{29}NO_7$) C, H, N.

***N*-Fumaryl-L-glutamic Acid (10).** Compound **30** (0.48 g, 1.3 mmol) was dissolved in CH_2Cl_2 (3 mL). While this solution was stirred well, CF_3CO_2H (3 mL) was added dropwise. Stirring was continued until all the starting material was consumed as indicated by TLC (~3 h). After an additional 30 min of stirring, the mixture was evaporated to dryness under reduced pressure and then dried further under high vacuum. The resulting material was dissolved in distilled water (7 mL) and cooled in an ice bath. To this solution 1.3 mL of 2 N NaOH was added while the solution was stirred. This reaction was continued for 1 h at 0 °C and then allowed to warm up to room temperature where it was stirred for another 30 min. Throughout this period the pH was maintained at around 13 by the addition of 2 N NaOH when necessary. The reaction mixture was neutralized with 2 N formic acid and lyophilized to give a white solid. This was dissolved in a minimum amount of water and run through a cation exchange column (AG-50W-X8) with H_2O as the eluting solvent. The fraction containing the desired material was lyophilized to give 0.23 g (72%) of **10** as a white solid: mp 179–180 °C; $[\alpha]_D$ −20.8° (*c* 0.5, H_2O); ^{1}H NMR (200 MHz, D_2O) δ 1.91–2.30 (m, 2 H, Glu β-CH_2), 2.43–2.50 (m, 2 H, Glu γ-CH_2), 4.46–4.53 (m, 1 H, α-CH), 6.84 (ABq, *J* = 15.6 Hz, 2 H, CH=CH); ^{13}C NMR (50 MHz, DMSO-d_6) δ 26.31 (Glu β-C), 30.01 (Glu γ-C), 51.51 (Glu α-C), 130.17 (*C*=C), 136.40 (C=*C*), 163.24 (C=O), 166.36 (C=O), 172.78 (C=O), 173.55 (C=O); FAB-MS *m/z* 246 $(MH)^+$, 244 $(M - H)^-$. Anal. ($C_9H_{11}NO_7$) C, H, N.

(±)-2-[(Benzyloxycarbonyl)amino]-2-(dimethoxyphosphinyl)acetic Acid (31). (±)-Methyl 2-[(benzyloxycarbonyl)amino]-2-(dimethoxyphosphinyl)acetate (Fluka, 5 g, 15 mmol) was dissolved in dioxane (12 mL). To this solution 2 N NaOH (7.5 mL) was added with stirring. The mixture was stirred until hydrolysis was shown to be complete by TLC (~40 min). The dioxane was evaporated in vacuo, and the remaining aqueous layer was washed with EtOAc (20 mL). The aqueous layer was acidified with 5 N HCl and extracted with EtOAc (2 × 100 mL). The combined extracts were dried over Na_2SO_4, and the EtOAc was evaporated in vacuo. Drying the residue under high vacuum gave 4.6 g (96.5 %) of **31** as a white solid. This material was recrystallized from EtOAc: mp 87 °C [lit.[23] mp 87–88 °C]; ^{1}H NMR (90 MHz, $CDCl_3$) δ 3.76 (d, *J* = 10.55 Hz, 3 H, OCH_3), 3.84 (d, *J* = 11.4 Hz, 3 H, OCH_3), 4.99 (dd, *J* = 9.2 and 22.9 Hz, 1 H, α-CH), 5.13 (s, 2 H, $PhCH_2$), 5.98 (d, *J* = 9.23 Hz, 1 H, NH), 7.33 (s, 5 H, Ph Hs).

Dimethyl *N*-[2-[(Benzyloxycarbonyl)amino]-2-(dimethoxyphosphinyl)acetyl]-L-glutamate (32). Glu(OMe)-OMe·HCl (1.6 g, 7.56 mmol) and **31** (2.27 g, 7.15 mmol) were dissolved in THF (15 mL) in a round-bottomed flask equipped with a stir bar. To this were added *N*-methylmorpholine (1.51 g, 14.9 mmol) and HOBt (2.00 g, 14.8 mmol), and the mixture was cooled in an ice bath. To this mixture was added DCC (1.95 g, 9.4 mmol), and the mixture was stirred at 0 °C for 1 h and overnight at room temperature. At the end of this period, the precipitated *N*,*N*-dicyclohexylurea was removed by filtration and the THF was removed from the filtrate by evaporation under reduced pressure. The remaining mixture was placed in EtOAc, washed with 10% citric acid, 1 M $NaHCO_3$, and saturated NaCl, and then dried over anhydrous $MgSO_4$. Evaporation of EtOAc and drying under high vacuo gave an oil. Purification by silica gel column chromatography (EtOAc) gave 2.39 g (70.5%) of **32** as a 9:11 mixture of diastereoisomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.93–2.34 (m, 2 H, Glu β-CH_2), 2.34–2.46 (m, 2 H, Glu γ-CH_2), 3.63 and 3.64 (s, 3 H, Glu OCH_3), 3.72 (s, 3 H, Glu OCH_3), 3.65–3.74 (m, 6 H, $P(OCH_3)_2$), 4.56–4.69 (m, 1 H, Glu α-CH), 4.80–4.95 (m, 1 H, PCH), 5.11 (s, 2 H, $PhCH_2$), 5.65–5.82 (m, 1 H, NH), 7.22 (d, *J* = 8.31 Hz, 0.55 H, Glu NH), 7.47 (d, *J* = 7.45 Hz, 0.45 H, Glu NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.06, 27.21 (Glu β-C), 29.63, 29.96 (Glu γ-C), 50.52, 51.78, 52.05, 52.35, 52.53, 52.60, 53.70, 53.84, 53.96, 54.09, 54.28, 54.35 (OCH_3 and α-Cs), 67.55 ($PhCH_2$), 128.10, 128.28, 128.55, 136.02 (Ph Cs), 155.98 (urethane C=O), 164.54, 164.92, 164.61, 165.01 (C=O), 171.39, 171.47, 172.93 (C=O). Anal. ($C_{19}H_{27}N_2O_{10}P$) C, H, N, P.

Dimethyl *N*-[2-(Acetylamino)-2-(dimethoxyphosphinyl)acetyl]-L-glutamate (33). The mixture of diastereoisomers of **32** (1.25 g, 2.6 mmol) was dissolved in MeOH (6 mL). To this solution, 10% Pd/C (70 mg) and Ac_2O (0.66 mL) were added, and the mixture was hydrogenolyzed for 5 h under a H_2 pressure of 44 psi. The resulting mixture was filtered through two layers of filter paper and MeOH was evaporated from the filtrate. The residue was dried under high vacuum to give 0.99 g (99.6 %) of **33** as an oil. A portion of this was purified by medium-pressure silica gel column chromatography ($CHCl_3$/

MeOH, 30:1) for analytical studies: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, J = 7.8 Hz, 0.45 H, Glu NH), 7.39 (d, J = 7.96 Hz, 0.55 H, Glu NH), 6.87 (distorted t, 1 H, NH), 5.25 (dd, J = 8.47 and 20.32 Hz, 0.55 H, α-CH), 5.24 (dd, J = 8.52 and 20.72 Hz, 0.45 H, α-CH), 4.52–4.62 (m, 1 H, Glu α-CH), 3.76–3.84 (m, 6 H, $POCH_3$), 3.70, 3.71 (s, 3 H, Glu OCH_3), 3.63 (s, 3 H, Glu OCH_3), 2.33–2.44 (m, 2 H, γ-CH_2), 2.04, 2.049, 2.051 (s, 3 H, CH_3CO), 1.92–2.33 (m, 2 H, βCH_2).

(*E*)- and (*Z*)-Dimethyl *N*-(*N*-Acetyl-β-methyldehydroaspartyl)-L-glutamate (34a and 34b). The diastereoisomeric mixture **33** was dissolved in THF (3.5 mL) and placed in a round-bottomed flask containing a magnetic stir bar and fitted with a rubber septum. The system was flushed with Ar and cooled to −78 °C. While the solution was stirred, *n*-BuLi was gradually dripped in. The reaction mixture was stirred for 1 h, at which time methyl glyoxylate dissolved in THF was added dropwise to the reaction mixture. The reaction mixture was stirred for 6 h at −78 °C and then for 2.5 h at room temperature. At the end of this period, the reaction was quenched with H_2O and the THF evaporated under reduced pressure. The aqueous residue was extracted twice with EtOAc, and the combined EtOAc extracts were washed with saturated NaCl and dried over anhydrous Na_2SO_4. EtOAc was evaporated under reduced pressure and the resulting yellow oil was shown by ^{1}H NMR to contain **34a**, **34b**, and **35** in a ratio of 5:14:1. This material was subjected to silica gel column chromatography. Imide **35** was eluted first with a solvent system of EtOAc/hexane (1:3). A mixture of the *E* and *Z* isomers, **34a** and **34b**, respectively, was eluted with EtOAc/hexane/MeOH (24:71:5) and gave after evaporation and drying under high vacuo 94 mg (80.5%) of an oil. The isomers were separated by silica gel column chromatography (EtOAc/hexane, 1:1) to give **34a** and **34b** as oils.

Ac-Δ^EAsp(OMe)-Glu(OMe)-OMe (34a): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.99–2.42 (m, 2 H, β-CH_2), 2.17 (s, 3 H, CH_3CO), 2.42–2.52 (m, 2 H, γ-CH_2), 3.69 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 4.59–4.76 (m, 1 H, α-CH), 7.79 (s, 1 H, C=CH), 8.93 (s, 1 H, ΔAsp NH), 11.56 (d, J = Hz, 1 H, Glu NH).

Ac-Δ^ZAsp(OMe)-Glu(OMe)-OMe (34b): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (s, 3 H, CH_3CO), 1.89–2.41 (m, 2 H, Glu β-CH_2), 2.41–2.64 (m, 2 H, Glu γ-CH_2), 4.65–4.80 (m, 1 H, Glu α-CH), 5.46 (s, 1 H C=CH), 6.66 (d, J = 7.58 Hz, 1 H, Glu NH), 10.17 (s, 1 H, ΔAsp NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.79 (CH_3CO), 27.27 (Glu β-C), 29.88 (Glu γ-C), 51.90 (OCH_3), 51.92 (OCH_3), 52.04 (OCH_3), 52.78 (Glu α-C), 100.32 (C=CH), 147.22 (C=*C*N), 163.53 (C=O), 168.03 (C=O), 168.47 (C=O), 171.88 (C=O), 173.52 (C=O); FAB-MS *m/s* 345 (MH)$^+$.

Ac-Dehydroaspartimidyl-Glu(OMe)-OMe (35): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27–2.59 (m, 4 H, Glu β- and γ-CH_2), 2.25 (s, 3 H, CH_3CO), 3.63 (s, 3 H, OCH_3), 3.70 (s, 3 H, OCH_3), 4.64–4.75 (m, 1 H, α-CH), 6.57 (s, 1 H, C=CH), 8.30 (s, 1 H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.96 (CH_3CO), 24.20 (Glu β-C), 30.57 (Glu γ-C), 51.15 (OCH_3), 51.86 (OCH_3), 52.92 (Glu α-C), 104.87 (C=CH), 138.42 (C=*C*N), 167.41 (C=O), 169.13 (C=O), 171.09 (C=O), 172.63 (C=O); FAB-MS *m/z* 313 (MH)$^+$, 311 (M − H)$^-$.

Di-*tert*-butyl *N*-[2-[(Benzyloxycarbonyl)amino]-2-(dimethoxyphosphinyl)acetyl]-L-glutamate (36). L-Glu(OBu^t)-OBu^t·HCl (5.39 g, 18 mmol) and **31** (5.39 g, 17 mmol) were dissolved in THF (25 mL) in a round-bottom flask equipped with a stir bar. To this solution were added *N*-methylmorpholine (3.64 g, 36 mmol) and HOBt (2.3 g, 17 mmol). The mixture was cooled in an ice bath after which time EDC (3.44 g, 18 mmol) was added. The mixture was stirred at 0° C for 1 h and then overnight at room temperature. At the end of this period the THF was removed under reduced pressure. The residue was placed in EtOAc, and this mixture was washed with 10% citric acid, 1 M $NaHCO_3$, and saturated NaCl and dried over anhydrous $MgSO_4$. Evaporation of the EtOAc followed by drying under high vacuum gave an oil, which was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to give 4.94 g (52%) of **36** as a mixture of diastereoisomers: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.36, 1.37 (s, 18 H, $(CH_3)_3$), 1.40 (s, 9 H, $(CH_3)_3$), 1.83–2.31 (m, 4 H, Glu β- and γ-CH_2), 3.68–3.80 (m, 6 H, $P(OCH_3)_2$), 4.41–4.52 (m, 1 H, Glu α-CH), 4.79–4.97 (m, 1 H, α-CH), 5.06 (s, 2 H, $PhCH_2$), 5.90–5.98 (m, 1 H, NH), 7.28 (s, 5 H, Ph), 7.41 (d, J = 7.36 Hz, 0.4 H, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 27.45, 27.58 (Glu β-C), 27.87, 27.99 ($C(CH_3)_3$), 30.99, 31.29 (Glu γ-C), 50.98, 52.52, 52.85, 53.56, 53.66, 53.87, 53.96, 54.12, 54.18 ($P(OCH_3)_3$ and α-Cs), 67.31 ($PhCH_2$), 80.39, 80.49 ($C(CH_3)_3$), 82.13, 82.25 ($C(CH_3)_3$), 127.97, 128.10, 128.41, 136.02 (Ph Cs), 155.88 (urethane C=O), 164.35, 164.40, 164.63, 164.69 (C=O), 170.10, 170.31, 171.77 (C=O). Anal. ($C_{25}H_{39}N_2O_{10}P$) C, H, N, P.

Di-*tert*-butyl *N*-[2-(Acetylamino)-2-(dimethoxyphosphinyl)acetyl]-L-glutamate (37). The benzyloxycarbonyl protected mixture of diastereoisomers **36** (4.7 g, 8.41 mmol) was dissolved in MeOH (7 mL). To this solution were added 10% Pd/C (0.7 g) and Ac_2O (6.6 mL). The mixture was hydrogenolyzed for 3 h under a H_2 pressure of 44 psi and then filtered. The MeOH was evaporated, and the residue obtained was dried under high vacuum to give 3.9 g (99%) of **37** as an oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.44 (s, 18 H, $C(CH_3)_3$), 2.08 (s, 3 H, CH_3CO), 1.88–2.48 (m, 4 H, Glu β- and γ-CH_2), 3.77 (d, J = 10.99 Hz, 3 H, OCH_3), 3.83 (d, J = 10.99 Hz, 3 H, OCH_3), 4.24–4.60 (m, 1 H, Glu α-CH), 5.20 (dd, J = 8.79 and 19.35 Hz, 1 H, α-CH), 5.80–6.12 (m, 1 H, Glu NH), 6.79 (d, J = 8.79 Hz, 1 H, NH).

***tert*-Butyl Glyoxylate (38).** A three-necked flask was equipped with a reflux condenser, a pressure equalizing dropping funnel, and a mechanical stirrer. *tert*-Butyl alcohol (67 g, 0.9 mol), which was predried over Na and distilled, was placed in the flask. To this alcohol *N,N*-dimethylaniline (69 g, 0.57 mol) and dry Et_2O (200 mL) were added. While the above mixture was stirred fumaryl chloride (43 g, 0.28 mol) in Et_2O (30 mL) was added gradually via the dropping funnel. The temperature of the reaction mixture was maintained below 30 °C during this addition process. After all of the fumaryl chloride had been added the mixture was refluxed for 2.5 h. At the end of this period the Et_2O solution was extracted with 6 N H_2SO_4. The aqueous layer was washed with Et_2O, and the two Et_2O layers were combined and washed with saturated Na_2CO_3 and saturated NaCl. The Et_2O was evaporated to give a black solid. This material was recrystallized from acetone to give 31.3 g (49%) of di-*tert*-butyl fumarate as a white solid: mp 71–71.5 °C [lit.[30] mp 69–70 °C]; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47 (s, 18 H, $C(CH_3)_3$), 6.65 (s, 2 H, CH=CH).

The above fumarate ester (31.3 g, 0.14 mol) was converted to **38** by using a method previously reported by Jung et al.[31] for the synthesis of other types of glyoxylate esters. The ester was dissolved in dry CH_2Cl_2 and placed in a gas-bubbler bottle. The solution was cooled to −78 °C and ozone was bubbled through the solution for 3 h. At the end of this period the solution was purged with oxygen for 0.5 h until the blue color of the ozone was gone. The resulting solution was placed in a dropping funnel and dripped into a three-necked flask, which was equipped with a reflux condenser, a stir bar, and a nitrogen inlet adapter containing dimethyl sulfide (10 g, 0.16 mol) under N_2. During this process the mixture starts to reflux on its own. The mixture was allowed to stir overnight under N_2. The dimethyl sulfide and CH_2Cl_2 were distilled off at atmospheric pressure (heated at 67 °C in an oil bath). The remaining liquid was fractionally vacuum distilled under a N_2 atmosphere with use of a 5-cm vigreux column to give 10 g (30%) of **38**: bp 34 °C (11 mmHg); phenylhydrazone derivative mp 187–188 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.57 (s, 9 H, $C(CH_3)_3$), 9.30 (s, 1 H, CHO); IR ν_{max} (film) 1717.2 (aldehyde C=O), 1750.7 (ester C=O) cm^{-1}; CI-MS *m/z* (CH_4) 130 M$^+$.

(*E*)- and (*Z*)-Di-*tert*-butyl *N*-(*N*-Acetyl-β-*tert*-butyldehydroaspartyl)-L-glutamate (39). Potassium *tert*-butoxide (0.06 g, 0.5 mmol) was placed in a 25-mL round-bottom flask and cooled in a dry ice–isopropyl alcohol bath. To this mixture was added CH_2Cl_2 (1 mL). While this solution was stirred well Wittig regent **37** (0.24 g, 0.5 mmol) in CH_2Cl_2 (4 mL) was added slowly. To this mixture was added **38** (0.26 g, 2 mmol) in CH_2Cl_2 (1 mL) dropwise. The bath was allowed to warm up slowly to −10 °C (∼2 h) at which time the bath was removed and the stirring continued for an additional 2 h. The CH_2Cl_2 was evaporated and the residue mixed with EtOAc. This mixture was extracted with H_2O, washed with saturated NH_4Cl solution, and then dried over $MgSO_4$. Evaporation of EtOAc gave a yellow oil which was purified by medium-pressure C18 reverse-phase column chroma-

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tography ($MeOH/H_2O$, 2:1) to give 0.13 g (55%) of **39** as a mixture of *E* and *Z* isomers (**39a** and **39b**, respectively). The isomers were separated by silica gel column chromatography (EtOAc/hexane, 1:2) to give 0.04 g (17%) of the E isomer **39a** and 0.06 g (25%) of the Z isomer **39b** as white solids.

Ac-Δ^EAsp(OBu^t)-Glu(OBu^t)-OBu^t (39a): mp 124–125 °C $[\alpha]_D$ +11.7° (*c* 0.15, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9 H, $C(CH_3)_3$), 1.47 (s, 9 H, $C(CH_3)_3$), 1.48 (s, 9 H, $C(CH_3)_3$), 2.16 (s, 3 H, CH_3CO), 1.96–2.29 (m, 2 H, Glu β-CH_2), 2.29–2.39 (m, 2 H, Glu γ-CH_2), 4.43–4.55 (m, 1 H, Glu α-CH), 7.65 (s, 1 H, =CH), 8.87 (s, 1 H, ΔAsp NH), 11.28 (d, *J* = 6.1 Hz, 1 H, Glu NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.77 (CH_3CO), 27.41 (Glu β-C), 28.24 ($C(CH_3)_3$), 28.29 ($C(CH_3)_3$), 31.67 (Glu γ-C), 54.00 (Glu α-C), 80.83 ($C(CH_3)_3$), 82.37 ($C(CH_3)_3$), 82.60 ($C(CH_3)_3$), 108.41 (C=CH), 138.98 (C=CN), 161.19 (C=O), 168.89 (C=O), 169.65 (C=O), 170.18 (C=O), 171.95 (C=O).

Ac-Δ^ZAsp(OBu^t)-Glu(OBu^t)-OBu^t (39b): mp 116–117 °C; $[\alpha]_D$ +11.5° (*c* 0.31, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 1.44 (s, 9 H, $C(CH_3)_3$), 1.48 (s, 18 H, $C(CH_3)_3$), 2.15 (s, 3 H, CH_3CO), 1.95–2.32 (m, 2 H, Glu β-CH_2), 2.32–2.48 (m, 2 H, Glu γ-CH_2), 4.44–4.64 (m, 1 H, Glu α-CH), 5.39 (s, 1 H, =CH), 6.60 (d, *J* = 7.46 Hz, 1 H, Glu NH), 10.20 (s, 1 H, ΔAsp NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.60 (CH_3CO), 27.30 (Glu β-C), 27.89 ($C(CH_3)_3$), 27.97 ($C(CH_3)_3$), 28.01 ($C(CH_3)_3$), 31.15 (Glu γ-C), 52.41 (Glu α-C), 80.54 ($C(CH_3)_3$), 81.69 ($C(CH_3)_3$), 82.46 ($C(CH_3)_3$), 102.35 (C=CH), 146.46 (C=CN), 163.61 (C=O), 167.47 (C=O), 167.76 (C=O), 170.61 (C=O), 172.36 (C=O). Anal. ($C_{23}H_{37}N_2O_8$) C, H, N.

(*Z*)-*N*-(*N*-Acetyldehydroaspartyl)-L-glutamic Acid (11). Ac-Δ^ZAsp(OBu^t)-Glu(OBu^t)-OBu^t (**39b**, 0.23 g, 0.5 mmol) was dissolved in dry CH_2Cl_2 (2 mL). While this solution was stirred CF_3CO_2H (2 mL) was added. The reaction was stirred overnight after which time the CH_2Cl_2 and CF_3COOH were evaporated in vacuo. The residue obtained was dried under high vacuum to give a pink solid. This material was partially purified by passing it through a C18 column (CH_3CN/H_2O, 9:1) to give a yellow colored solid, which was decolorized by dissolving the solid in water and then passing the solution through a pad of activated charcoal. Lyophilization of the filtrate gave 0.14 g (93%) of **11** as a white solid: mp 138 °C dec; $[\alpha]_D$ −7.3° (*c* 1.0, H_2O); ^1H NMR (200 MHz, DMSO-d_6) δ 2.06 (s, 3 H, CH_3CO), 1.70–2.16 (m, 2 H, Glu β-CH_2), 2.31–2.39 (m, 2 H, Glu γ-CH_2), 4.16–4.36 (m, 1 H, Glu α-CH), 5.33 (s, 1 H, =CH), 8.59 (d, *J* = 8.01 Hz, 1 H, Glu NH), 10.15 (s, 1 H, ΔAsp NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 23.27 (CH_3CO), 26.26 (Glu β-C), 30.11 (Glu γ-C), 51.46 (Glu α-C), 104.00 (C=CH), 145.5 (C=CN), 163.91 (C=O), 167.64 (C=O), 168.9 (C=O), 173.06 (C=O), 174.09 (C=O); FAB-MS *m/z* 303 $(MH)^+$. Anal. ($C_{11}H_{14}N_2O_8$) C, H, N.

(*E*)-*N*-(*N*-Acetyldehydroaspartyl)-L-glutamic Acid (40). Ac-Δ^EAsp(OBu^t)-Glu(OBu^t)-OBu^t (**39a**, 0.23 g, 0.5 mmol) was dissolved in dry CH_2Cl_2 (2 mL). While this solution was stirred CF_3CO_2H (2 mL) was added. The reaction was stirred overnight after which time the CH_2Cl_2 and CF_3CO_2H were evaporated in vacuo. The residue obtained was dried under high vacuum to give a pink solid. A small portion of this solid was dissolved in pyridine-d_5, and ^1H NMR analysis suggested that it was the desired Ac-Δ^EAsp-Glu-OH (**40**): ^1H NMR (200 MHz, pyridine-d_5) δ 2.09 (s, 3 H, CH_3CO), 2.5–3.00 (m, 2 H, Glu β-CH_2), 3.00–3.25 (m, 2 H, Glu γ-CH_2), 5.25–5.40 (m, 1 H, Glu α-CH), 7.86 (s, 1 H, =CH), 10.73 (d, *J* = 7.48 Hz, 1 H, Glu NH), 11.05 (s, 1 H, ΔAsp NH).

The remaining portion of the solid was dissolved in acetone in an attempt to recrystallize it. After the mixture stood in acetone overnight a white precipitate separated from the solution. This solid was shown to be glutamic acid on the basis of TLC analysis, melting point, and ^1H NMR. When Et_2O was added to the remaining acetone solution a crystalline solid precipitated. ^1H NMR and mass spectral analysis showed this material to be *N*-acetyldehydroaspartic anhydride (**41**): ^1H NMR (200 MHz, acetone-d_6) δ 2.32 (s, 3 H, CH_3CO), 6.77 (s, 1 H, =CH), 10.30 (s, 1 H, ΔAsp NH); EI-MS *m/z* 155 (M^+). This degradation process could be accelerated upon warming the acetone solution to 35 °C.

"Buffy Coat" Membrane Preparation. Rat forebrains were used for all experiments. Preparation of synaptosomes was essentially identical with that employed in previous studies on NAALA dipeptidase.[14,15] All procedures were carried out at 4 °C unless otherwise indicated. Sprague-Dawley rats (45–90 days) were killed by cervical dislocation followed by decapitation. Forebrains were removed, placed in 0.32 M sucrose solution and homogenized (6 strokes, 600 rpm). This mixture was then centrifuged at 800*g* for 10 min to remove debris. The supernatant was centrifuged at 20000*g* for 20 min to pellet the synaptosomes. The pellet was resuspended in 15 mL of ice cold distilled H_2O to lyse the synaptosomes, then centrifuged at 8000*g* for 20 min. The loose material on the surface of the pellet, the "buffy coat", which contained the synaptic membranes was removed by trituration of the pellet with H_2O. The supernatant containing the buffy coat material was then subjected to centrifugation at 35000*g* for 10 min. This pellet was resuspended by sonication in 20 mL of 0.1 M Hepes buffer (pH 7.4) then incubated for 30 min at 37 °C. The solution was again centrifuged at 35000*g* for 10 min, and resuspended by sonication in 0.1 M Hepes buffer (pH 7.4). Following two more washes with this same procedure, the membranes were suspended by sonication in 0.1 M Hepes buffer (pH 7.4) to a final protein concentration of 1–2 mg/mL as determined by Lowry assay[32] with bovine serum albumin as the standard. The membrane preparation was frozen until needed in the peptidase assay described below.

Peptidase Inhibition Assay. Inhibition assays were performed in reaction mixtures containing 50 mM Hepes buffer (pH 7.4), 32 mM NaCl, 2 mM $CoCl_2$, 50.0 μg/mL membrane protein, and varied concentrations of Ac-Asp-[3,4-^{3}H]Glu-OH (1.0 μM for IC_{50} determinations and 0.25, 0.5, 1.0, 2.0, and 5.0 μM for K_i determinations) in a volume of 1–1.5 mL.

Assays were initiated by the addition of membrane protein and terminated at 0, 10, 20, 30, and 40 min by removing 200-μL aliquots and mixing these with 200 μL of cold 0.1 M phosphate buffer (pH = 7.5). A portion (100 μL) of this mixture was applied to a 7-cm minicolumn prepared in a 14.5-cm Pasteur pipet using de-fined AG1 × 8 Dowex anion exchange resin (200–400 mesh, formate form) equilibrated with 0.5 M formic acid. Elution with 1.8 mL of 0.5 M formic acid yielded greater than 95% retention of the Ac-Asp-[3,4-^{3}H]Glu-OH substrate and 80% elution of the [3,4-^{3}H]Glu product. The eluates were diluted with 10 mL of Ecolume scintillation fluid, and the radioactivity was determined by scintillation spectrometry. Activity of the aliquot taken at 0 min was subtracted from the activity of the 10-, 20-, 30-, and 40-min aliquots to correct for coelution of substrate with product.

The inhibitor concentration which caused a 50% inhibition of the rate of Ac-Asp-[3,4-^{3}H]Glu-OH hydrolysis (IC_{50}) at a substrate concentration of 1.0 μM was determined with inhibitor concentrations approximately equal to 2- and 4-fold above and 2- and 4-fold below the concentration estimated to produce a 50% inhibition. For each concentration of inhibitor, velocities were calculated from the linear portion of an activity vs time plot (at least 30 min). The percent inhibition was then determined and plotted against the log of the concentration of inhibitor present in the reaction mixture. The IC_{50} was calculated from this curve by interpolating the inhibitor concentration producing 50% inhibition of Ac-Asp-[3,4-^{3}H]Glu-OH hydrolysis.

The K_i for inhibition of Ac-Asp-[3,4-^{3}H]Glu-OH hydrolysis was determined for those compounds with an IC_{50} value of less than 100 μM. Reaction velocities were determined for inhibitor concentrations equal to the IC_{50} value, at 2- and 4-fold above and at 2- and 4-fold below the IC_{50} value, and at 0 μM for Ac-Asp-[3,4-^{3}H]Glu-OH concentrations of 0.25, 0.5, 1.0, 2.0, and 5.0 μM. A plot of $1/v$ vs 1/[Ac-Asp-[3,4-^{3}H]Glu-OH] was made for each of the five inhibitor concentrations. The slopes of these lines, which are equal to Km_{app}/V_{max}, were then replotted against the concentration of inhibitor. The slope of the replot is equal to $K_m/(V_{max}K_i)$ where K_i is the inhibition constant.[33] The K_i was calculated by dividing the slope of the double reciprocal plot of 0 μM inhibitor by the slope of the replot.

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of Minnesota. The technical assistance of Kathy Norman in the preparation of the Ac-Asp-Glu-OH analogues **2–5** is gratefully acknowledged.

Registry No. **2**, 28809-18-9; **3**, 128135-23-9; **4**, 99624-53-0; **5**, 104870-25-9; **6**, 128164-09-0; **7**, 128135-24-0; **8**, 33981-72-5; **9**, 128135-25-1; **10**, 128135-26-2; **11**, 128135-27-3; **12**, 89092-61-5; **13**, 128164-22-7; **14**, 99429-23-9; **15**, 104870-31-7; **16**, 128135-28-4; **17**, 128135-29-5; **18**, 128135-30-8; **19**, 104870-33-9; **20**, 128135-31-9; **21**, 128135-32-0; **22**, 128135-33-1; **23**, 128135-34-2; **24**, 128135-35-3; **25**, 128135-36-4; **26**, 128135-37-5; **27**, 128135-38-6; **28**, 128135-39-7; **29**, 128135-40-0; **30**, 128135-41-1; **31**, 100945-16-2; **32** (diastereomer 1), 128135-42-2; **32** (diastereomer 2), 128135-43-3; **33** (diastereomer 1), 128135-52-4; **33** (diastereomer 2), 128135-53-5; **34a**, 128135-44-4; **34b**, 128135-51-3; **35**, 128135-45-5; **36** (diastereomer-1), 128164-23-8; **36** (diastereomer-2), 128164-24-9; **37** (diastereomer-1), 128135-49-9; **37** (diastereomer-2), 128135-50-2; **38**, 7633-32-1; **39a**, 128164-25-0; **39b**, 128135-48-8; **40**, 128135-46-6; **41**, 128135-47-7; (*E*)-*t*-BuOCOCH=CHCOOBu-*t*, 7633-38-7; OHCCOOMe, 922-68-9; (±)-$(MeO)_2P(O)CH(NHZ)COOMe$, 100945-15-1; H-Glu-(OMe)-OMe·HCl, 23150-65-4; H-Glu(OBu-*t*)-OBu-*t*·HCl, 32677-01-3; H-Glu-OH, 56-86-0; H-Glu(OBzl)-OBzl, 2768-50-5; H-Glu-(OBzl)-OBzl·TsOH, 2791-84-6; (*E*)-MeOCOCH=CHCOOH, 2756-87-8; BOC-Asp(OBu-*t*)-OH, 1676-90-0; BOC-Glu(OBzl)-OH, 13574-13-5; BOC-D-Asp(OBzl)-OH, 51186-58-4; H-Asp(OBzl)-OBzl·TsOH, 2886-33-1; BOC-Asp(OBzl)-OH, 7536-58-5; succinic anhydride, 108-30-5; maleic anhydride, 108-31-6; NAALA depeptidase, 111070-04-3.

Penta- and Hexadienoic Acid Derivatives: A Novel Series of 5-Lipoxygenase Inhibitors

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The synthesis of a series of pentadienoic and hexadienoic acid derivatives is reported. These compounds were tested as inhibitors of 5-lipoxygenase (5 LO) and cyclooxygenase (CO) in vitro and as inhibitors of arachidonic acid (AA) induced ear edema in mice in vivo. Their potency is compared with that of the standard inhibitors nafazatrom, BW 755C, NDGA, KME4, quercetine, and L 652,243. The most potent compound in vivo, diethyl 2-hydroxy-5-(ethylthio)-2(*Z*),4(*Z*)-hexadienedioate (**20**) inhibited AA-induced ear edema when administered topically or orally, with an ED_{50} value of 0.01 mg/ear and 20 mg/kg, respectively. Among the standard compounds tested, L 652, 243 was the most active compound in this test with an ED_{50} value of 0.01 mg/ear and 1 mg/kg po, but unlike this compound, **20** is a selective inhibitor of 5-LO (IC_{50} = 2 μM) without any significant activity against CO (IC_{50} > 50 μM). Most of the other compounds in this series are also selective 5-LO inhibitors.

The arachidonic acid (AA) cascade is known to generate numerous mediators involved in specific diseases including inflammation and immediate and delayed hypersensitivities. Inhibition of the cyclooxygenase (CO) pathway may explain, in part, the therapeutic properties of nonsteroidal antiinflammatory drugs. Since the sulfidopeptidal leukotrienes are potent bronchoconstrictors and LTB_4 is a potent chemotactic factor on human leukocytes, orally active, specific inhibitors of 5-lipoxygenase (5-LO) may be of interest as potential therapeutic agents.

It is generally believed that 5-LO contains a catalytically important iron atom, as seems to be the case for other LO enzymes.[1] It is assumed that the first step of the reaction involves a reduction of the enzymatic Fe^{III} to Fe^{II} at the same time as the *pro-S* hydrogen atom of C_7 in AA is abstracted to give a delocalized radical which reacts with molecular oxygen[1] (Scheme I).

This biochemical background led several groups to develop rational strategies to design potential 5-LO inhibitors: structural analogues of AA[2] or of 15-HETE,[3] antioxidant compounds,[4] and Fe^{III} chelating agents such as hydroxamic acid derivatives.[5,6]

We report herein the synthesis and some biological activities of dienoic acid derivatives, structurally unrelated to any previous 5-LO inhibitors. Most of these compounds have been shown to inhibit 5-LO in vitro and some of them are active in vivo in an acute inflammation animal model.

Chemistry

The synthetic pathway for the preparation of the compounds listed in Tables I–IV is shown in Scheme II. Compound **1** as the perchlorate salt had been previously described.[7] We synthesized vinamidinium tetrafluoroborates **1–4** by reaction of the appropriate enaminones with

Scheme I

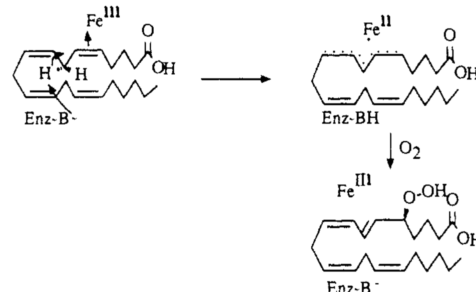

Table I. Vinamidinium Salts 1–5

compd	W	Z	mp, °C	% yield
1	BF_4^-	$CO_2C_2H_5$	102	58
2	BF_4^-	$CON(CH_3)_2$	170	79
3	BF_4^-	COC_6H_5	140	51
4	BF_4^-	C_6H_5	136	93
5	Cl^- [a]	H	190[b]	51

[a] Reference 15. [b] Literature mp 187–189 °C.

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