m/e (relative intensity) 374 (M^+, 2), 233 (75), 145 (25), 131 (25), 109 (100), 105 (50), 93 (38), 91 (75); calcd for $C_{22}H_{30}O_3S$ m/e 374.1916, found m/e 374.1930.

For eudesmane **6b**: mp 105 °C; IR (KBr) 1728, 1702, 1583 cm^{-1}; NMR δ 7.57–7.13 (m, 5 H), 3.78–3.50 (m, 1 H), 3.59 (s, 3 H), 3.20–2.80 (m, 2 H), 1.22 (s, 3 H), 0.96 (d, 3 H, J = 7 Hz), 0.84 (d, 3 H, J = 7 Hz); mass spectrum, m/e (relative intensity) 374 (M^+, 28), 233 (100), 205 (15), 109 (13), 107 (17), 93 (24), 91 (15); calcd for $C_{22}H_{30}O_3S$ m/e 374.1916, found m/e 374.1931.

For eudesmane **7**: mp 175–176 °C; IR (KBr) 3380, 1725, 1690, 1586 cm^{-1}; NMR δ 7.50–7.15 (m, 5 H), 3.64 (s, 3 H), 3.11 (s, 1 H), 3.05–2.80 (m, 1 H), 1.47 (s, 3 H), 0.96 (d, 3 H, J = 6.8 Hz), 0.84 (d, 3 H, J = 6.8 Hz); mass spectrum, m/e (relative intensity) 390 (M^+, 15), 221 (100), 110 (35), 109 (75), 95 (30), 91 (35), 69 (50); calcd for $C_{22}H_{30}O_4S$ m/e 390.1865, found m/e 390.1894. The total overall yield was 61% of the mixture of the four isomers **5**, **6a**, **6b**, and **7** in the ratio of 23:10:49:18, respectively.

X-ray Analysis. Single crystals of the eudesmane **7** were prepared by slow crystallization from methanol. The crystals were orthorhombic, space group $P2_12_12_1$, with a = 10.148 (2) Å, b = 14.023 (2) Å, c = 14.721 (2) Å, and d_{calcd} = 1.238 g cm^{-3} for Z = 4 ($C_{22}H_{30}O_4S$, mol wt 390.54). A crystal measuring approximately 0.30 × 0.30 × 0.40 mm was used for data collection. The intensity data were measured on a Hilger-Watts diffractometer (Ni-filtered Cu K radiation, θ–2θ scans, pulse-height discrimination) and were corrected for absorption (μ = 15.3 cm^{-1}). A total of 1629 reflections were measured for $\theta < 57°$, of which 1579 were considered to be observed [$I > 2.5\sigma(I)$]. The structure was solved by a multiple-solution procedure[6] and was refined by full-matrix least-squares methods. Three reflections which were strongly affected by extinction were excluded from the final refinement and difference map. In the final refinement anisotropic thermal parameters were used for the nonhydrogen atoms, and isotropic temperature factors were used for the hydrogen atoms. The hydrogen atoms were included in the structure factor calculations, but their parameters were not refined. The final discrepancy indices are R = 0.032 and R_w = 0.042 for the remaining 1576 observed reflections. The final difference map has no peaks greater than ±0.2 e $Å^{-3}$. The absolute configuration of **7** depicted in Figure 1 was determined by the anomalous scattering of the sulfur atoms and was established by refining both enantiomers. The final weighted R values were 0.0415 for the configuration shown and 0.442 for its antipode.[4]

(6) Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. A* **1971**, *A27*, 368.

Epimerization of the Eudesmane 6a. To a solution of sodium (0.10 g) in methanol (5 mL) was added the eudesmane **6a** (0.020 g, 0.053 mmol), and the subsequent mixture was refluxed for 20 h. The methanol was removed under reduced pressure, and the residue was diluted with water and acidified. This aqueous mixture was then extracted with ether, and the ether extracts were dried (anhydrous $MgSO_4$) and evaporated. The crude residue was then treated with ethereal CH_2N_2, and the resulting product was shown to be identical with **6b** by ^1H NMR.

Similar treatment of **6b** resulted only in recovered **6b**. None of the isomeric **6a** was found.

Acknowledgment. We thank Dr. B. Kane of Glidden-Durkee for a generous sample of *trans*-piperitol, Mr. Scott Bram and Ms. Nada Soueidan for technical assistance, Temple University for the award of a University Fellowship to J.F.C., the Biomedical Research Support Program (Grant No. 7115), and the National Science Foundation (Grant No. CHE-76-05757) for partial support.

Registry No. 4a, 74878-02-7; **5**, 77462-50-1; **6a**, 77462-51-2; **6b**, 77519-46-1; **7**, 77462-52-3.

Supplementary Material Available: Tables II–VI containing final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles and torsion angles, respectively, for **7** (6 pages). Ordering information is given on any current masthead page.

N-[(Carbobenzyl)oxy]-Δ^E-phenylalanine Ethyl Ester

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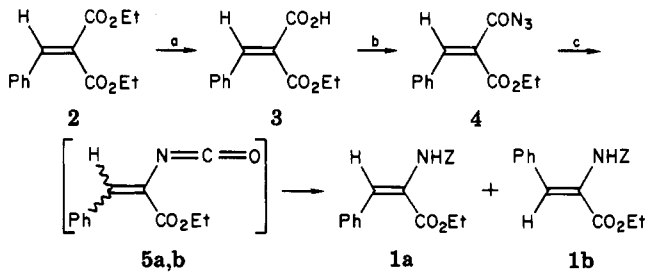
The synthesis of the title compound (**1a**) has been accomplished by rearrangement of a vinylic isocyanate, and its configuration was confirmed by X-ray crystallography. The configurational stability of **1a** to both acids and bases was investigated, and the NMR "rules" developed by other workers in the dehydro amino acid field were checked. It was concluded that an (*E*)-dehydrophenylalanine residue will probably not survive the standard methods of peptide synthesis.

In most of the recent work directed toward the synthesis of biologically important dehydropeptides,[1] only the thermodynamically stable isomer (*Z* configuration) of the dehydro amino acid residue has been incorporated. Since the configuration of the double bond in the dehydro residue may control the conformations of several proximal amino acid residues and will afford information on conformations acceptable to the bioreceptor, it is important that we be able to incorporate *both* double bond isomers into bioactive peptides. To this end we have synthesized (*Z*)-Δ^E-PheOEt (**1a**) by a new method and studied its thermal and chemical stability to reagents used in peptide synthesis.

(1) M. L. English and C. H. Stammer, *Biochem. Biophys. Res. Commun.*, **83**, 1464 (1978); M. L. English and C. H. Stammer, *ibid.*, **85**, 780 (1978); C. H. Stammer and S. Konno, *Int. J. Pept. Protein Res.*, **12**, 222 (1978).

Scheme I[a]



[a] (a) 1 equiv of KOH/ethanol; H_3O^+; (b) $[(CH_3)_2CH]_2NC_2H_5$, EtOCOCl, acetone, −5 °C, NaN_3/H_2O; (c) $PhCH_2OH$, 100 °C.

Scheme I shows the synthesis of (*Z*)-Δ^E-PheOEt (**1a**) from the known[2] monoacid **3**. This compound has the

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Table I. Azide 4 Rearrangement

entry	temp, °C	catalyst	time	% 1a[a]	% 1b[a]
1	100	none	10 min	43	57
2	60	none	3 h	30	70
3	rt[c]	none	20 h	41	59
4	rt[c]	DMAP[b]	30 min	0	100

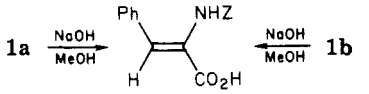
[a] Percent composition of the two isomers was determined by integration of the methyl groups of the ester after excess benzyl alcohol was removed (see Experimental Section). [b] 4-(Dimethylamino)pyridine, 0.1 equiv. [c] Room temperature.

presumed *Z* configuration shown, since it is obtained by a sterically controlled hydrolysis[3a] of the diester[3b] **2**. The *E* configuration of **1a** then follows from the rearrangement of azide **4**, which occurs with retention of configuration.[4] Careful control of the temperature during the azide rearrangement and urethane formation is necessary to maximize the yield of the *E* isomer **1a**. We found that **1a** can be isomerized to **1b** on heating in $CDCl_3$ at 54 °C; thus we can not be sure whether the *Z* isomer formed during the preparation of **1a** originates by rearrangement of **1a** or the isocyanate **5a**. Since it is known that isocyanates react with carboxylic acids,[5] attempts were made to convert the isocyanate **5** directly into a peptide by this reaction. When **4** was rearranged in the presence of (*Z*)-GlyOH, only a trace of the dipeptide (*Z*)-Gly-Δ^Z-PheOEt was found, with none of the desired *E* isomer being found. The bulk of the products obtained apparently resulted from oligomerization of the isocyanate. The addition of various catalysts (DBU, TEA, DMAP, BF_3, $SnCl_4$) to this reaction mixture did not improve the yield of desired product.

The azide **4** readily loses nitrogen at room temperature when it comes in contact with solid surfaces. Both anhydrous $MgSO_4$ and Na_2SO_4 promote evolution of gas, as do ground-glass joints and scratches in the glassware. The infrared spectrum of crude **4** shows both azide and isocyanate absorptions. The isocyanate **5** is best generated by adding a toluene solution of **4** to hot toluene (100 °C) and quickly cooling the solution to 0 °C. It is readily apparent from the NMR spectrum of the crude product that a mixture of *E* and *Z* isomers is formed in approximately equal amounts, since there are two vinyl proton singlets (7.17 and 6.98 ppm), two quartets (CH_2CH_3, 4.38 and 4.18 ppm), and two triplets (CH_2CH_3, 1.37 and 1.08 ppm) observed in the spectrum. A $CDCl_3$ solution of this mixture remained unchanged after 5 days; however, addition of a small amount of DBU rapidly decomposed the isocyanates. The mixture of these isocyanates failed to ring close when refluxed in xylene with a catalytic amount of iodine after 2 weeks, although the ring closure of styryl isocyanates to isocarbostyryls by this means has been reported.[4a]

As can be seen from Table I, optimal formation of **1a** occurs at 100 °C with a reaction time of 10 min. Prolonged heating with smaller amounts of benzyl alcohol drastically reduced the yield of **1a** (10–15% by NMR). Since **1a** was slowly isomerized at 54 °C in $CDCl_3$, the smaller amount of **1a** in entry 2 is accounted for. Within experimental error, the yields reported in entries 1 and 3 are equal, thus reflecting the relative amounts of the two isomers. As can be seen from entry 4, the presence of DMAP in the reaction mixture greatly accelerates the formation of **1b**. We found that both **1a** and **1b** are stable to DMAP for several days at room temperature, so that DMAP clearly catalyzed the isomerization of the (*E*)-isocyanate **5a** to **5b**. Several other basic catalysts, triethylamine, Dabco, and *N*-methylmorpholine, failed to catalyze this reaction while DBU led to an extremely complex mixture.

Scheme II



6b

Attempts to hydrolyze the ester function of **1a** with 1.1 equiv of 3 N potassium hydroxide in aqueous *p*-dioxane gave only the (*Z*)-acid **6b** (Scheme II). Hydrolysis was complete only after 24 h. Treatment of either isomer with 3.25 equiv of 4 N sodium hydroxide in methanol afforded only the (*Z*)-acid **6b** after 1.5 h. This result was unexpected since it has been shown that basic hydrolysis of the *E* and *Z* isomers of *N*-benzoyldehydrophenylalanine ethyl ester afforded the corresponding *E* and *Z* acids.[11] TLC analysis of the hydrolysis reaction mixture indicated the total absence of **1a** after 20 min. We can only speculate that the greater electron-donating ability of the benzyloxy function (vs. phenyl) allows for more electron delocalization throughout the enamine system where it is rapidly protonated under the protic conditions, affording **1b**. This sensitivity to base will make the direct incorporation of the *E* isomer of dehydrophenylalanine into a peptide chain difficult if not impossible. It should be noted that the best yields of the Δ^Z-acid were obtained when the *Z* ester was hydrolyzed in a *large excess* of base as described in the Experimental Section.

Since the isomers **1a** and **1b** were synthesized by a means not previously used, we chose to verify the configuration of **1a** by X-ray crystallography (Figure 1). A crystal of (*Z*)-Δ^E-PheOEt ($C_{19}H_{19}NO_4$) was mounted on a Syntex $P2_1$ automated diffractometer. Cell dimensions [a = 15.021 (5) Å, b = 13.087 (3) Å, c = 8.941 (3) Å, β = 90.30 (3)°] were determined during normal crystal alignment procedures. Data were collected [2390 observations of which 1080 were classed as observed; $I > 3\sigma(I)$] by using Cu Kα radiation (λ = 1.5418 Å). The structure was solved in space group $P2_1/c$ [volume 1757.6 Å^3, d_{calcd} = 1.23 (2) g cm^{-3} for Z = 4, mol wt 325.2, d_{obsd} = 1.30 g cm^{-3}] by using direct methods[6] and refined[7] to a final R_1 = 8.8%. During refinement, disorder in the position of C(12) became apparent. The disorder was approximated by two 50% occupancy positions, C(121) and C(122). Hydrogen positions were calculated and included in the final refinements, but their positions were not refined. Hydrogens attached to C(12) and C(13) were not included. Charge delocalization is evident along the bonds of the atom series C(1)–C(2)–N(1)–C(3)–O(2) with bond lengths 1.349 (4), 1.379 (4), 1.360 (4), and 1.324 (4) Å. The dihedral angle between the plane of the benzene ring and that including atoms H-

(2) A. Nabeya, F. B. Culp, and J. A. Moore, *J. Org. Chem.*, **35**, 2015 (1970).

(3) (a) For a similar example, see: *Chem. Abstr.*, **63**, 149796 (1965); (b) "Organic Syntheses", Collect. Vol. III, Wiley, New York, 1955, p 377.

(4) (a) G. J. Mikol and J. H. Boyer, *J. Org. Chem.*, **37**, 724 (1972); (b) L. E. Overman, G. F. Taylor, C. B. Petty, and P. J. Jessup, *ibid.*, **43**, 2164 (1978).

(5) J. H. Sanders and K. C. Frisch, "Polyurethanes: Chemistry and Technology", Interscience, New York, 1963, Part 1, p 186; C. Naegeli and A. Tyabji, *Helv. Chim. Acta*, **17**, 931 (1934); **18**, 142 (1935); W. Dieckmann and F. Breest, *Chem. Ber.*, **39**, 3052 (1906); S. Motoki, T. Suito, and H. Kagami, *Bull. Chem. Soc. Jpn.*, **47**, 775 (1974).

(6) Solved by J. P. Main, M. M. Woolfson, L. Lessinger, G. Germain, and J. P. Declercq, "MULTAN 74. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data", Universities of York, England, and Louvain, Belgium, 1974.

(7) J. M. Stewart, Technical Report TR-446, Computer Science Center, University of Maryland, College Park, MD, 1976.

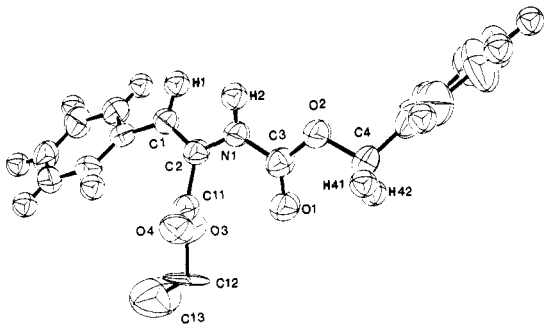
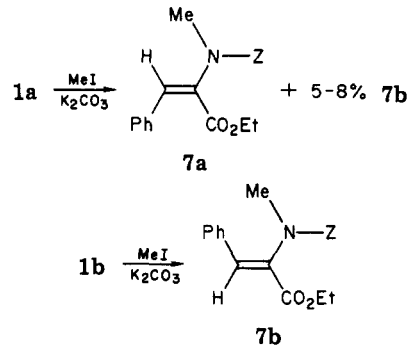


Figure 1.

Scheme III



(1)–C(1)–C(2)–N(1) is 38.02°, indicating very little conjugation in this region. Atoms N(1), C(3), O(2), and O(1) are planar (deviation 0.004), and their plane has dihedral angle 77.8° to the plane of C(2), C(11), O(3), and O(4) (deviation 0.001). Consideration of the packing of molecules in the cell reveals an intermolecular hydrogen bond between the hydrogen attached to N(1) and O(4) of a neighboring molecule.

In recent years some empirical spectroscopic rules have been developed by workers in the field of dehydro amino acid chemistry for the determination of the configuration of these compounds. Olsen et al.[8] have observed a correlation between configuration and change in chemical shift ($\Delta\delta$) of the vinyl proton when measured in $CDCl_3$ and trifluoroacetic acid (TFA). The vinyl proton of the *Z* isomer shows a $\Delta\delta$ of 0.34–0.54 ppm *downfield*, while the vinyl proton of the corresponding *E* isomer experiences an *upfield* shift of 0.18–0.32 ppm. We observed an upfield shift of 0.20 ppm for **1a** (*E* isomer) following this solvent change, but the vinyl proton of **1b** (*Z* isomer) remained concealed under the aromatic proton envelope in both spectra, and no shift data could be obtained; i.e., Olsen's rule was partially confirmed.

Rich et al.[9] have reported that upon N-methylation of the isomers of Boc-ΔAib-OMe, the vinyl proton of the *Z* isomer experienced a large (+0.70 ppm) upfield shift, while the vinyl proton of the *E* isomer underwent a small (–0.03 ppm) *downfield* shift. These results were revised by Olsen and co-workers,[8] who reported that the original configurational assignments made by Rich were incorrect, and, thus, it was actually the *E* isomer vinyl proton which underwent an *upfield* shift. Accordingly, we prepared the *N*-methyl derivatives of **1a** and **1b**, **7a** and **7b**, respectively, by the method of Rich (Scheme III). We observed a large (–0.92 ppm) *upfield* shift of the vinyl proton for **1a**, the *E* isomer, while the vinyl proton of **7b**, the *Z* isomer, remained concealed within the aromatic envelope. These results are in full agreement with those of Olsen and may indicate that there is a consistent relationship between configuration and the $\Delta\delta$ of the vinyl proton upon N-methylation of both aliphatic and aromatic dehydro amino acid derivatives. Alternatively, we did observe that the *N*-methyl protons of the methylated *Z* isomer appeared at 0.28 ppm upfield of those protons in the methylated *E* compounds. This difference may occur because the methyl group of the *Z* isomer is very close to the face of the benzene ring in some conformations, whereas this is not possible in the *E* configuration. The chemical shifts of these methyl protons might, therefore, be used to assign at least the configurations of dehydro *aromatic* amino acids.

Table II. Base-Catalyzed Isomerization of 1a (~25 °C)

base[a]	% 1b		
	3 h	16 h	41 h
DMAP	0.0	0.0	2.8
Dabco	4.8	10.8	27.8
TEA	3.0	3.3	7.3
DIPEA	0.0	0.0	0.0
DBU[b]	9.2	13.2	21.8
NMM	0.0	0.0	0.0
PYR	0.0	0.0	0.0

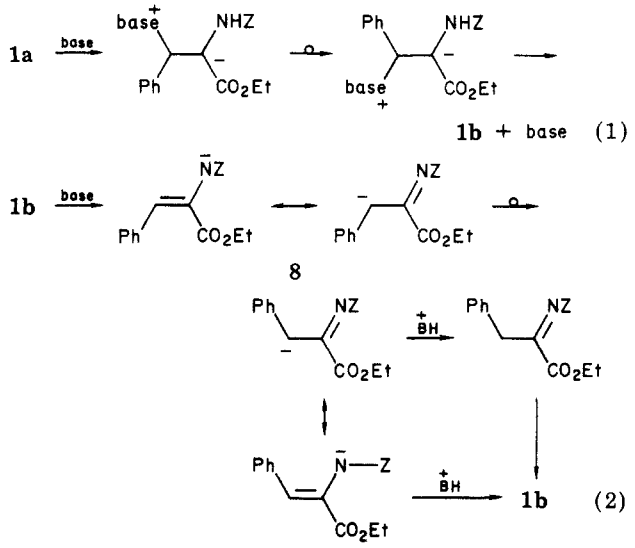
[a] DMAP = 4-(dimethylamino)pyridine, Dabco = 1,4-diazabicyclo[2.2.2]octane, TEA = triethylamine, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, NMR = *N*-methylmorpholine, PYR = pyridine, DIPEA = diisopropylethylamine. [b] DBU caused the formation of 5.2% of an unknown compound after 41 h; **1b** gave the same compound when treated with DBU.

Prokof'ev and Karpeiskaya[10] recently reported the use of the $^3J_{C_\delta,H_\beta}$, vicinal coupling constant between the carbonyl carbon and the vinyl proton in the proton-coupled ^{13}C NMR spectrum to distinguish between the *E* and *Z* isomers of unsaturated azlactones and the corresponding carboxylic acids. They found 12.5- and 10-Hz coupling constants for the *E* isomers and 5.5- and 5-Hz coupling constants for the *Z* isomers of the azlactones and acids, respectively. In the decoupled spectrum, the carbonyl carbon of **1a** had a chemical shift of 164.48 ppm and that of **1b** one of 165.28 ppm. In the proton-coupled spectra, we observed a $^3J_{CO_2Et,H_\beta}$ of 15 Hz for **1a** and 3 Hz for **1b**, thus confirming those author's results. These coupling constants could, therefore, be used successfully to determine the configuration of dehydro amino acid derivatives.

Since both Carter,[11a] Rao,[11b] and Boyd[11c] found that unsaturated azlactones having the *E* configuration were unstable in pyridine solutions, we investigated the stability of **1a** to several tertiary amines. Equimolar amounts of **1a** and the base were dissolved in $CDCl_3$, and the isomerization was followed by both NMR and TLC. Product composition was determined by integration of the benzyl protons of the (benzyloxy)carbonyl group (δ 5.11 for **1a** and δ 5.17 for **1b**). As can be seen in Table II, the isomerization rate is dependent on the base employed. From this study, the use of either *N*-methylmorpholine or diisopropylethylamine in coupling reactions is recommended when a Δ^E-amino acid is involved.

The *E* isomer (**1a**) also proved to be isomerized by trifluoroacetic acid (TFA) and hydrogen chloride. A solution of 1 in neat TFA was isomerized to the extent of 18% in

(8) A. Srinivasan, K. D. Richards, and R. K. Olsen, *Tetrahedron Lett.*, 891 (1976).
(9) D. H. Rich, J. Tam, P. Mathiaparanam, and J. Grant, *Synth. Commun.*, 402 (1975).
(10) E. P. Prokof'ev and E. I. Karpeiskaya, *Tetrahedron Lett.*, 737 (1979).
(11) (a) H. E. Carter and W. C. Risser, *J. Biol. Chem.*, **139**, 255 (1941); (b) Y. S. Rao and R. Filler, *Synthesis*, 749 (1975), and references cited therein; (c) G. V. Boyd and P. H. Wright *J. Chem. Soc., Perkin Trans. 1*, 909 (1972).



40 min. After 1 h, a solution of **1a** in 3.9 N HCl in ethyl acetate was totally isomerized.

There are two possible mechanisms which might be written for the isomerization of **1a** by bases: (1) a Michael-type addition of the base to the β-carbon atom followed by rotation about the newly formed single bond and elimination of the base (eq 1) or (2) abstraction of the amide proton by the base to form the delocalized anion **8** which can rotate about the C_β–C_α bond (eq 2). It is known that treatment of the *E* isomers of azlactones with pyridine at room temperature rapidly isomerizes them to the *Z* isomers[11a-c] and that these same *E* isomers prefer to add Grignard reagents to the β-carbon.[12] Amide anions are thought to be intermediates in the base-catalyzed N-alkylation of amides with alkyl halides,[13] so both mechanisms are quite possible. To establish which of the mechanisms applies in the case at hand, we studied the stability of the *N*-methyl derivative **7a** to DBU. After 48 h there was no detectable amount of isomerization, and after 4 weeks less than 10% of the *E* isomer had isomerized. This result does not completely exclude the addition mechanism, but it indicates that the deprotonation mechanism (eq 2) is the more likely isomerization route. This is further supported by the observation that 5–8% of **7b** was obtained from the methylation of **1a**. From this we can conclude that the N-methylation of **1a** occurs at a faster rate than does the isomerization of the anion formed under the methylation conditions (K_2CO_3, DMF). Pertinent to this, we studied the isomerization of the anion of **1a** under both kinetic (aprotic) and thermodynamic (protic) conditions. The kinetic anion was prepared by the slow addition of a solution of **1a** in DMF to a suspension of 1.3 equiv of NaH in DMF. Aliquots were removed, quenched with acetic acid, and analyzed by NMR and TLC. Over a 22-h period, *none* of the *Z* isomer (**1b**) was formed. The thermodynamic control experiment was performed by the slow addition of a solution of **1a** in DMF to a suspension of 0.2 equiv of NaH and DMF. Analysis, performed as above, showed a slow isomerization of the anion occurring over a 24-h period accompanied by the formation of several other products. This result is in good agreement with the work of Knorr and Löw[14] on the isomerization of anils to *sec*-enamines and corroborates the mechanism presented in eq 2.

It seems clear from this work that a Δ^E-Phe residue will be difficult to incorporate into a peptide sequence because of its sensitivity to the acidic and basic conditions often encountered during peptide synthesis. Other routes to Δ^E-Phe peptides are presently being examined.

Experimental Section

All melting points were determined on a Thomas-Hoover capillary melting point apparatus. Infrared spectra were recorded on a Perkin-Elmer Model 297 infrared spectrophotometer with polystyrene as the standard. The ^{1}H NMR spectra were recorded on a Varian EM-390 spectrometer using tetramethylsilane as the internal standard. The spectra were recorded in the lock mode and the chemical shifts reported downfield from Me_4Si.

Reagent grade acetone was dried and stored over Na_2CO_3. Diisopropylethylamine was distilled from and stored over CaO. Reagent grade toluene was dried and stored over $MgSO_4$. Ethyl chloroformate (Aldrich) was used as received. Benzyl alcohol was distilled prior to use and DMF was distilled from CaH_2 and stored over 4-Å molecular sieves. Methyl iodide was distilled and stored over copper wire. K_2CO_3 was ground to a fine powder and heated for 2 days at 120 °C prior to use. The diethyl ether and petroleum ether used in recrystallizations were stored over sodium ribbon, and tetrahydrofuran was distilled from potassium metal prior to use; *p*-dioxane was used as received. Silica gel 60 was purchased from E. Merck Co. Elemental analyses were performed by Atlantic Microlabs.

Hydrogen Ethyl (*Z*)-Benzalmalonate (3). To a solution of 139.0 g (0.560 mol) of diethyl benzalmalonate[3b] dissolved in 300 mL of *p*-dioxane was added a solution of 34.4 g of 90% KOH (0.552 mol) in 100 mL of water. The reaction mixture was stirred overnight. Following removal of the solvents in vacuo, the gellish residue was partitioned between 100 mL of water and 50 mL of diethyl ether. The ethereal layer was separated and the aqueous layer extracted twice with 30-mL portions of diethyl ether. The aqueous solution was chilled in an ice–water bath and acidified with concentrated HCl to pH 1 (pH paper). The resulting oily aqueous suspension was extracted with three 100-mL portions of diethyl ether. The ethereal extracts were combined and dried with $MgSO_4$. Crystallization occurred after 200 mL of petroleum ether was added to afford 41.6 g of **3**: mp 87–90 °C;[15] IR (KBr disc) 3400–2700 (br, OH), 1725 (sharp, ester C=O), 1645 (sharp, acid C=O), 1610 (sharp, C=O), 1400 (br, C=C), 1240 cm^{-1} (br, C=O); NMR ($CDCl_3$) δ 1.29 (t, 3 H, J = 7 Hz, CH_3CH_2), 4.38 (q, 2 H, J = 7.3 Hz, CH_3CH_2), 7.51 (m, 5 H, C_6H_5), 7.99 (s, 1 H, vinyl H), 11.07 (s, 1 H, COOH, exchanged with D_2O). An additional 46.5 g of **3** was obtained from the mother liquor (mp 86–90 °C) for a combined yield of 73%.

(*Z*)-Δ^E-PheOEt (1a) and (*Z*)-Δ^Z-PheOEt (1b). To 5.00 g (22.7 mmol) of **3** dissolved in 30 mL of dry acetone was added 4.35 mL (25.0 mmol) of diisopropylethylamine. The solution was chilled to −5 °C (ice/salt/water bath), and a solution of 2.40 mL (25.0 mmol) of ethyl chloroformate and 10 mL of dry acetone was added dropwise, while maintaining the temperature at −5 °C. After 30 min, a solution of 3.25 g (50.0 mmol) of sodium azide and 15 mL of water was added dropwise, while maintaining the temperature at or below 5 °C. The mixture was stirred an additional 30 min, after which it was poured into an ice-cold mixture of 75 mL of toluene and 25 mL of water. The aqueous layer was extracted twice with 50-mL portions of toluene. The organic layers were combined and dried with $MgSO_4$, and the toluene was removed in vacuo. The IR of the resulting oil indicated the presence of both azide **4** (2130 cm^{-1}) and the isocyanate **5** (2200 cm^{-1}). The oil was dissolved in 15 mL of dry toluene and added quickly to 30.0 g of benzyl alcohol at 100 °C. The solution was heated for an additional 5 min and then cooled immediately in an ice bath. The toluene was removed in vacuo. The oil was dissolved in 40 mL of petroleum ether and 15 mL of diethyl ether. Extraction with 300 mL of water caused separation into three layers. The lower aqueous layer was removed, and ether was added to effect solution of the two remaining layers. Water (300 mL) was added, and the procedure was repeated four more times, after which TLC analysis indicated the absence of benzyl alcohol. The organic phase was dried with $MgSO_4$ and concentrated in vacuo to give

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5.84 g of a yellow oil. Passage through 220 g of silica gel 60 with ether/petroleum ether (1:10) as eluent afforded 2.16 of oily **1a** and 1.90 g of oily **1b**, both of which solidified upon standing. Recrystallization of crude **1a** from 20 mL of ether and 30 mL of petroleum ether afforded 1.82 g of pure **1a**: mp 61–62 °C; NMR ($CDCl_3$) δ 0.96 (t, 3 H, CH_3CH_2), 4.02 (q, 2 H, CH_3CH_2), 5.11 (s, 2 H, CH_2), 7.00 (s, 1 H, NH), 7.20 (s, 5 H, Phe aromatic), 7.30 (s, 5 H, aromatic), 7.57 (s, 1 H, vinyl proton); IR (KBr) 3340 (NH), 1720 (urethane and ester carbonyls), 1655 (C=C), 1530 (amide II), 1250 and 1230 cm^{-1} (CO). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.88; N, 4.30. Found: C, 70.16; H, 5.90; N, 4.30. From the mother liquor was obtained an additional 0.11 g for a combined yield of 26%. Recrystallization of crude **1b** from 20 mL of ether and 30 mL of petroleum ether afforded 1.39 g of pure **1b**: mp 58–59 °C;[16] NMR ($CDCl_3$) δ 1.32 (t, 3 H, CH_3CH_2), 4.32 (q, 2 H, CH_3CH_2), 5.17 (s, 2 H, CH_2), 6.41 (s, 1 H, NH), 7.42–7.73 (m, 11 H, Ar H and CH=C); IR (KBr) 3310 (NH), 1730 (urethane C=O), 1700 (ester C=O), 1650 (C=C), 1500 (amide II), 1280 and 1230 cm^{-1} (CO). Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.88; N, 4.30. Found: C, 70.08; H, 5.89; N, 4.32. From the mother liquor was obtained an additional 0.39 g for a combined yield of 24.1%.

(*Z*)-*N*-Me-Δ^E-PheOEt (7a). To a solution of 200 mg (0.615 mmol) of **1a** dissolved in 2 mL of dry DMF were added 0.20 mL (5.2 equiv) of methyl iodide and 292 mg of anhydrous K_2CO_3. The reaction mixture was stirred for 24 h, diluted with 20 mL of dry $CHCl_3$, filtered, and concentrated in vacuo to a yellow oil. Elution through 30 g of silica gel 60 with ether/petroleum ether (1:10) as eluent afforded 122 mg (61%) of **6a** as a colorless oil: NMR ($CDCl_3$) δ 0.93 (br t, 3 H, CH_3CH_2), 3.30 (s, 3 H, CH_3), 3.91 (br, q, 2 H, CH_3CH_2), 5.12 (s, 2 H, CH_2), 6.68 (s, 1 H, vinyl proton), 7.24 and 7.30 (2 s, 10 H, Ar H); IR (NaCl plates) 1730 (urethane C=O), 1715 (ester C=O), 1645 (C=C), 1450 (NCH_3), 1220 and 1160 cm^{-1} (CO). Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.68; H, 6.29; N, 4.06.

(*Z*)-*N*-Me-Δ^Z-PheOEt (7b). By use of the procedure given for **7a**, the crude oily product was crystallized from 20 mL of ether/petroleum ether (1:1) to afford 154 mg (74%) of **7b**: mp 78–79 °C; NMR ($CDCl_3$) δ 1.67 (t, 3 H, CH_3CH_2), 3.02 (s, 3 H, NCH_3), 4.13 (q, 2 H, CH_3CH_2), 5.07 (s, 2 H, CH_2), 7.17 (s) and 7.3 (m, 11 H, Ar H and vinyl proton); IR (KBr) 1720 (urethane C=O), 1710 (ester C=O), 1645 (C=C), 1450 (NMe), 1275 and 1205 cm^{-1} (CO). An analytical sample was recrystallized from diethyl ether and petroleum ether; mp 77–78 °C. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.59; H, 6.31; N, 4.07.

(*Z*)-Δ^Z-PheOH (6b). Method A. To 528 g (1.62 mmol) of **1b** dissolved in 5 mL of methanol was added 3.0 mL of 4 N NaOH, and the reaction mixture was stirred 1 h. The solvent was removed in vacuo, and the residue was dissolved in 15 mL of water and extracted (2 × 10 mL) with diethyl ether. The aqueous solution was acidified to pH 3 (pH paper) with solid citric acid and extracted (3 × 20 mL) with ethyl acetate, and the organic phase was dried with anhydrous $MgSO_4$. The solvent was removed in vacuo to afford a white solid which was recrystallized from 50 mL of ethyl acetate/petroleum ether (2:3) to afford 338 mg of pure **6b**: mp 157–159 °C;[17] NMR ($CDCl_3$) δ 5.07 (s, 2 H, CH_2), 6.53 (s, 1 H, NH), 7.10–7.60 (m, 11 H, Ar H and vinyl proton), 9.70 (s, 1 H, CO_2H); IR (KBr) 3290 (NH), 2940 (OH), 1690 (acid and urethane C=O), 1650 (C=C), 1510 (amide II), 1250 cm^{-1} (CO). Anal. Calcd for $C_{17}H_{15}NO_4$: C, 68.68; H, 5.08; N, 4.71. Found: C, 68.76; H, 5.13; N, 4.68. From the mother liquor was obtained an additional 79.0 mg for a combined yield of 86.5%.

Method B. Hydrolysis of 1b with 3.25 Equiv of NaOH. To 200 mg (0.615 mmol) of **1b** dissolved in 2.0 mL of methanol was added 0.50 mL of 4 N NaOH. The reaction mixture was stirred for 1.5 h and the methanol removed in vacuo. The gellike residue was dissolved in 20 mL of water and extracted twice with 15-mL portions of ether. The aqueous layer was acidified to pH 2 (pH paper) with solid citric acid and extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried (anhydrous $MgSO_4$) and evaporated to dryness in vacuo. The colorless oil obtained (170 mg) solidified upon standing. Recrystallization from 4 mL of ethyl acetate and 4 mL of hexanes afforded 74.5 mg of pure **7b**, mp 157–158 °C. An additional 44.2 mg was obtained from the mother liquor for a total yield of 65%. Recrystallization from benzene–cyclohexane gave a melting point of 155.5–156.5 °C. This product was identical (IR, NMR, TLC) with that obtained by method A.

Method C. Hydrolysis of 1a with 3.25 Equiv of NaOH. Compound **1a** (200 mg, 0.615 mmol) was treated as described above. The crude colorless oil obtained was purified by preparative TLC using Whatman PK6F plates with chloroform–methanol–acetic acid (25:5:1) as eluent. Recrystallization from benzene–cyclohexane afforded 80.2 mg of pure **7b**, mp 154–155 °C. An additional 25.6 mg was obtained from the mother liquor for a total yield of 58%. This product was identical (IR, NMR, TLC) with that obtained by method A, and a mixture melting point was not depressed.

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Synthesis of Dehydrothyroliberin, Δ^ZPhe^2-TRF

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The first use of the N-chlorination–dehydrochlorination method in the synthesis of a dehydrodipeptide is reported in the preparation of the title compound.

Due to the biological importance of thyroliberin (TRF, 1) numerous analogues have been synthesized and their bioactivities examined. Of particular interest among these is the Phe^2 analogue (2) which has about 10% the potency

pGlu-His-ProNH$_2$ (1) pGlu-Phe-ProNH$_2$ (2)

pGlu-Δ^2Phe-ProNH$_2$ (3)

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