Synthesis of Dehydroalanine Peptides from β-Chloroalanine Peptide Derivatives

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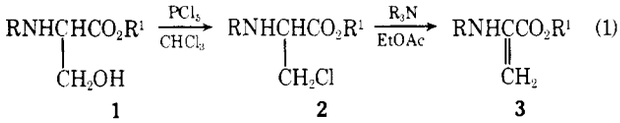
A series of N-protected di- and tripeptides containing the dehydroalanine unit has been prepared from the corresponding serine-containing peptides by a sequence of chlorination, using phosphorus pentachloride in chloroform, followed by base-catalyzed elimination. In two instances where *N*-benzyloxycarbonyl-L-phenylalanine was the N-terminal unit of a tripeptide, oxazoline hydrochlorides were obtained in the chlorination step. The chlorinated peptides also were obtained by direct incorporation of a β-chloro-L-alanine residue into the peptide by use of the carbodiimide method.

The dehydroalanine moiety has been and is of current interest in peptide chemistry. Dehydroamino acids are important constituents of the peptide antibiotics nisin,[1,2] subtilin,[2] thiostrepton,[3a] tentoxin,[3b] and alternariolide,[3c] for which the activity of the former two antibiotics has been proposed[4] to be related to the presence of unsaturated amino acids. Studies have been made to convert existing amino acids to dehydroalanine units with subsequent cleavage at the positions of the unsaturated residues.[5] The conversion of a serine to a dehydroalanine unit was used in a study[6] of the active site in chymotrypsin.

Dehydroalanine derivatives have been prepared by condensation of pyruvic acid with amides[7] and alkyl-α-halonitriles.[8] Azlactones, prepared by treatment of an *N*-acylglycine with an aromatic aldehyde, yielded dehydroamino acids upon hydrolysis;[9] more recently, oxidation of azlactones has led to the formation of dehydroamino acids.[10] Elimination reactions involving sulfonium[11] and sulfinyl[12] derivatives of cysteine and *O*-tosyl derivatives of serine[13] have found general use for preparation of dehydroalanine and peptides containing this unsaturated unit. Application of elimination reactions on β-chloroalanine derivatives for preparation of dehydroalanines has been reported.[14]

We were interested in the preparation of di- and tripeptides containing a dehydroalanine residue. We have found that reaction of serine-containing peptide derivatives with phosphorus pentachloride in chloroform readily effected chlorination of serine to yield a β-chloroalanine unit. Subsequent elimination, using a tertiary amine as base, gave the unsaturated peptide derivative in good yield (eq 1, Table I). An al-



(1)

ternative, but related, method is to introduce directly into the peptide the β-chloroalanine unit,[15] either as *N*-benzyloxycarbonyl-β-chloro-L-alanine or β-chloro-L-alanine methyl ester, followed by elimination.

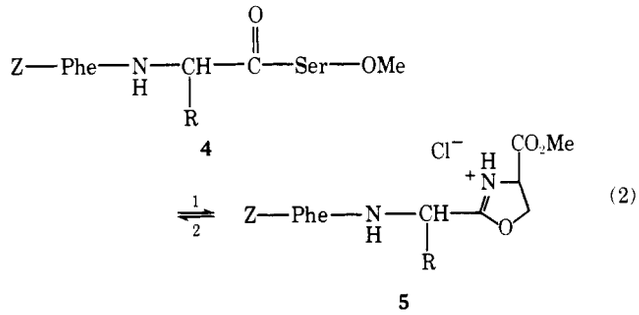
The conversion of alcohols to alkyl halides by use of phosphorus halides is a common reaction; indeed, serine and threonine ester hydrochlorides have been reported[16] to give the corresponding β-chloro derivatives upon treatment with phosphorus pentachloride. From our experience, the advantages of the above method are manifest in the consistent yields and clean nature of the reactions. We have successfully applied the method on protected serine as on di- and tripeptide derivatives containing serine or β-chloroalanine in various positions of the peptide. The amino protective groups used were benzyloxycarbonyl and, in two instances, trifluoroacetyl, while methyl and ethyl esters were employed as carboxyl protective groups. We have observed that dipeptides having a *N*-benzyloxycarbonyldehydroalanine unit in the N-terminal position are prone to undergo polymerization. Triethylamine effected elimination when the β-chloroalanine residue was at the C-terminal position in the peptide; when the above residue was at an internal or N-terminal position, it was necessary to employ 1,4-diazabicyclo[2.2.2]octane (Dabco) as base. The dehydroalanine group was readily characterized by the presence of vinyl proton absorptions in the NMR spectrum of the olefinic products.

The di- and tripeptides containing serine employed in this study were prepared by coupling the appropriate *N*-benzyloxycarbonyl-L-amino acid or dipeptide, using the carbodiimide procedure, with L-serine methyl ester or a peptide containing L-serine; new compounds prepared are listed in Table II.

The peptides containing β-chloroalanine (Table III) were prepared by chlorination of the seryl hydroxyl group using PCl_5 in chloroform or by the direct incorporation of β-chloro-L-alanine into the peptide. Chlorination of serine methyl ester appears[15,17] to occur without racemization. In this study, chlorination of Z-Gly-Ser-OMe gave product with a specific rotation of −7.0° (lit. −7.5°), while the specific rotations for Z-Gly-Phe-βClAla-OMe, prepared by chlorination of the tripeptide or by introduction of H-βClAla-OMe, were the same. Thus, racemization in the chlorination step does not appear to be occurring to a significant extent.

Comparison of the NMR spectra of the serine peptides with that of the corresponding β-chloroalanine derivatives in trifluoroacetic acid showed, upon chlorination, a downfield shift of approximately 0.3 ppm for the seryl α hydrogen and an upfield shift of similar magnitude for the seryl β hydrogens (see Tables II and III).

Chlorination of Z-Phe-Gly-Ser-OMe and Z-Phe-Ala-Ser-OMe gave products that were shown to be oxazoline hydrochlorides[19] (eq 2). In these two instances, the products precipitated from the reaction solution in contrast to the homogeneous nature of those reactions that yielded the desired β-chloroalanine derivatives. The oxazolines gave a positive



(2)

R = H or Me

1 PCl_5, $CHCl_3$
2 Et_3N, H_2O

R = H or Me

Table I. Preparation of Dehydroalanine Derivatives from β-Chloroalanine Derivatives via Elimination[a]

Reactant	Registry no.	Product[b,c]	Registry no.	Yield, %	Recrystn solvent[d]	Mp, °C	NMR, δ[e]	[α]D (concn)[f]
Z-βClAla-OMe	62107-38-4	Z-ΔAla-OH[g]	39692-63-2	68	A	108–109	5.92, 6.35[h]	
TFA-βClAla-OMe	62076-45-3	TFA-ΔAla-OMe	58137-35-2	91		Oil	6.12, 6.67[h]	
TFA-Gly-βClAla-OMe	62085-24-9	TFA-Gly-ΔAla-OMe	62076-54-4	83		Oil	5.96, 6.55[h]	
Z-Gly-βClAla-OMe	14131-96-5	Z-Gly-ΔAla-OH[i]	62076-55-5	55	B	188–189	6.46, 6.74	
Z-Ala-βClAla-OMe	19525-94-1	Z-Ala-ΔAla-OH	62076-56-6	52	C	118–119	6.40, 6.70	−17.9 (2.2)
Z-Phe-βClAla-OMe	62076-46-4	Z-Phe-ΔAla-OH	62076-57-7	49	A	149–150	6.36, 6.65	−8.4 (2.3)
Z-Val-βClAla-OMe	62076-47-5	Z-Val-ΔAla-OMe	62076-58-8	90	A	116–117	5.92, 6.62[h]	+3.6 (2.1)
Z-βClAla-Gly-OEt	7625-66-3	Z-ΔAla-Gly-OEt[j]	55477-82-2	63	E	76–78	5.25, 6.13[h]	
Z-βClAla-Ala-OEt	62076-48-6	Z-ΔAla-Ala-OEt	62076-59-9	80		Oil[k]	5.25, 6.10[h]	
Z-βClAla-Leu-OMe	62076-49-7	Z-ΔAla-Leu-OH	62076-60-2	40		Oil	5.32, 6.10[h]	
Z-Gly-Gly-βClAla-OMe	62076-50-0	Z-Gly-Gly-ΔAla-OMe	62076-61-3	86	D	156–157	6.36, 6.64	
Z-Gly-Phe-βClAla-OMe	62076-51-1	Z-Gly-Phe-ΔAla-OMe	62076-62-4	90	A	159–160	6.31, 6.58	−9.0 (2.2)
Z-Phe-Gly-βClAla-OMe	62076-52-2	Z-Phe-Gly-ΔAla-OMe	62076-63-5	60	A	114–115	6.37, 6.61	−23.1 (1.3)
Z-Gly-βClAla-Gly-OEt	7678-33-3	2-Gly-ΔAla-Gly-OEt[l]	36935-08-7	50	F	129–131	5.45, 6.47[h]	
Z-Phe-βClAla-Gly-OEt	62076-53-3	Z-Phe-ΔAla-Gly-OEt	62076-64-6	91		Oil	5.43, 6.45[h]	

[a] See Experimental Section for general reaction conditions. [b] Acceptable analytical data (±0.4% for C, H, N) were obtained for all new compounds except the oils listed (entries 2, 3, 9, and 10). Satisfactory NMR and TLC data were obtained on all compounds. Literature references are given for known compounds. [c] The acids listed were obtained by alkaline hydrolysis (1 N NaOH in EtOH) of the corresponding ester. [d] A = EtOAc–petroleum ether (bp 30–60 °C), B = EtOH, C = benzene–petroleum ether, D = EtOAc, E = ethyl ether–petroleum ether, F = $CHCl_3$–petroleum ether. [e] Chemical shift values for vinyl protons recorded in trifluoroacetic acid with an internal Me_4Si standard, unless noted otherwise. [f] Optical rotations recorded in DMF at 24 °C. [g] Reference 13a. [h] Recorded in $CDCl_3$. [i] Reference 28. [j] Reference 13a. [k] Oil polymerized upon attempted purification. [l] Reference 13b.

Table II. Data on Serine-Containing Peptides[a,b]

Registry no.	Peptide	Yield, %	Recrystn solvent[c]	Mp, °C	NMR, δ[d]	[α]D (concn)[e]
34078-88-1	Z-Val-Ser-OMe	57	A	162–163	4.90, 4.22[f]	+10.4 (1.5)
62076-41-9	Z-Phe-Gly-Ser-OMe	41	B	136–137	4.91, 4.24	−18.3 (1.7)
62076-42-0	Z-Phe-Ala-Ser-OMe	37	B	176–177	4.93, 4.26	+7.5 (2.5)
62076-43-1	Z-Gly-Gly-Ser-OMe	58	B	69–70	4.90, 4.25	+1.8 (1.4)
62076-44-2	TFA-Gly-Ser-OMe	80	C	136–137	4.95, 4.31[f]	

[a] Only new compounds are listed in this table. References to known compounds used in this study are given in the Experimental Section. Acceptable analytical data (±0.4% for C, H, N) were obtained on all new compounds except for entry 5 above. Satisfactory NMR and TLC data were obtained on all compounds. [b] See Experimental Section for general reaction conditions. [c] A = EtOAc–petroleum ether (bp 30–60 °C), B = EtOAc, C = diethyl ether. [d] Chemical shift values for α-methine and β-methylene protons, respectively, in trifluoroacetic acid with internal Me_4Si, unless noted otherwise. [e] Measured in DMF at 24 °C. [f] Chemical shift values in $CDCl_3$.

silver nitrate test for ionic chlorine, while treatment with triethylamine followed by aqueous workup gave the original serine-containing tripeptide.[19c] Chlorination of Z-Gly-Gly-Ser-OMe and Z-Gly-Phe-Ser-OMe proceeded normally to yield the desired β-chloroalanine tripeptides. The reasons for obtaining oxazoline derivatives when phenylalanine is at the N-terminal position are not clear. The desired tripeptide, Z-Phe-Gly-β-ClAla-OMe, was obtained by coupling the appropriate protected dipeptide with β-chloroalanine methyl ester using *N,N'*-dicyclohexylcarbodiimide. The above chlorotripeptide underwent elimination in the normal fashion to yield the corresponding dehydroalanine tripeptide derivative.

Experimental Section

All new compounds reported in this paper gave satisfactory analytical data for C, H, and N to within ±0.4% of the calculated values, except as noted in tables. NMR spectra were obtained for all compounds with Varian EM 360 or XL-100-12 spectrometers; partial data are given in the tables. Recrystallized products were shown to be homogeneous by TLC on Brinkmann silica gel F_{254} plates developed in chloroform–methanol–acetic acid (85:10:5). Evaporations in vacuo were carried out with a Buchler rotary evaporator. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich. The *N*-benzyloxycarbonyl-L-amino acids and L-serine methyl ester used were from commercial sources.

General Procedure for Preparation of Di- and Tripeptides Containing Serine. New compounds prepared in this work are listed in Table II. Known compounds employed are listed below with literature references: Z-Gly-Phe-OH,[20] Z-Phe-Gly-OH,[20] Z-Phe-Ala-OH,[21] Z-Gly-Gly-OH,[22] Z-Gly-Ser-OMe,[23,24] Z-Ala-Ser-OMe,[24] Z-Phe-Ser-OMe,[13a] Z-Gly-Phe-Ser-OMe,[25] TFA-Gly-OH,[26] TFA-Ser-OMe.[27]

The *N*-benzyloxycarbonyl or *N*-trifluoroacetylamino acid or dipeptide (5–15 mmol) and 1 equiv of L-serine methyl ester hydrochloride in chloroform were treated with 1 equiv of triethylamine and *N,N'*-dicyclohexylcarbodiimide. The reaction mixture was stirred at room temperature for 8–12 h. The dicyclohexylurea was removed by filtration and the filtrate was washed with dilute HCl, 5% $NaHCO_3$, and water. The organic phase was dried over Na_2SO_4. Evaporation in vacuo gave the peptide product which was recrystallized from the appropriate solvent (see Table II or appropriate literature reference). TFA-Gly-Ser-OMe proved to be water soluble; therefore, the above washing procedures were omitted.

General Procedure for Chlorination of Serine Peptide Derivatives. The β-chloroalanine peptide derivatives were prepared

Table III. Data for β-Chloroalanine Peptide Derivatives

Product[a]	Yield %[b]	Solvent[c]	Mp, °C	NMR, δ[d]	$[\alpha]_D$ (concn)[e]
Z-Gly-βClAla-OMe[f]	81	A	123–125	5.12, 3.96	
Z-Ala-βClAla-OMe	76	A	131–133	5.14, 3.96	+1.7 (1.5)
Z-Phe-βClAla-OMe	73	B	127–129	4.88, 3.97[g]	−11.1 (1.4)
Z-Val-βClAla-OMe	55	B	151–152	5.01, 3.97	+7.6 (1.5)
Z-βClAla-Gly-OEt	(78)	C	131–132.5	4.73, 4.05[i,g]	
Z-βClAla-Ala-OEt	50 (87)	D	149–150.5	4.78, 4.08[g]	−4.1 (1.5)
Z-βClAla-Leu-OMe	(88)	B	87–88.5	4.63, 3.86[g]	−7.3 (1.5)
Z-Gly-Gly-βClAla-OMe	78 (37)	B	147–148	5.01, 3.91	−6.6 (1.8)
Z-Gly-Phe-βClAla-OMe	63 (63)	B	140–141	5.08, 3.95	−5.5 (1.4)
Z-Phe-Gly-βClAla-OMe	0 (51)	B	148–149	5.08, 4.00	−12.1 (2.3)
Z-Gly-βClAla-Gly-OEt[h]	(68)	C	116–119	5.02, 4.00[i,g]	
Z-Phe-βClAla-Gly-OEt	(50)	E	184–186	5.00, 4.00[i]	−6.7 (1.4)
TFA-βClAla-OMe	37		Oil	4.96, 4.00[g]	
TFA-Gly-βClAla-OMe	77	A	133–134	5.23, 4.04[g]	

[a] See Experimental Section for general reaction conditions. Acceptable analytical, NMR, and TLC data were obtained on all compounds except for the last two entries. [b] Numbers in parentheses refer to yields obtained by DCC condensation with appropriate β-chloroalanine moiety. [c] A = benzene–petroleum ether (bp 30–60 °C), B = EtOAc–petroleum ether, C = EtOAc, D = $CHCl_3$–petroleum ether, E = $CHCl_3$–EtOH–petroleum ether. [d] Chemical shift values in trifluoroacetic acid for α and β protons, respectively, unless otherwise noted. Values given correspond to center of multiplet for each set of protons. [e] Recorded in DMF at 24 °C. [f] Reference 18. [g] Chemical shift values in $CDCl_3$. [h] Reference 15. [i] Approximate value as multiplet superimposed upon peaks due to glycyl methylene and ethyl ester methylene.

from the corresponding serine derivatives (0.2–20 mmol) by treatment with 1 equiv of PCl_5, added in portions, to a solution of the peptide in $CHCl_3$. The reaction mixture was stirred at room temperature for 12–16 h, washed with water, and dried over sodium sulfate. Evaporation in vacuo yielded the product as an oil which normally solidified upon standing. The solid product was recrystallized from the appropriate solvent listed in Table III.

Preparation of Di- and Tripeptides Containing β-Chloroalanine by Carbodiimide Method. The tripeptides, Z-Phe-Gly-βClAla-OMe, Z-Gly-Gly-βClAla-OMe, and Z-Gly-Phe-βClAla-OMe, were prepared by condensation of the appropriate *N*-benzyloxycarbonyldipeptide (5 mmol) with 1 equiv of β-chloro-L-alanine methyl ester hydrochloride[28] in 30 mL of chloroform–dimethylformamide (2:1) containing 1 equiv each of triethylamine and *N,N'*-dicyclohexylcarbodiimide. After 8 h, the urea was removed by filtration. The filtrate was diluted with chloroform, washed successively with dilute HCl, 5% $NaHCO_3$, and water, and dried over Na_2SO_4. Evaporation of the solvent in vacuo gave the tripeptide (see Table III).

The dipeptides (entries 5, 6, and 7, Table III) were prepared by the above procedure by condensation of *N*-benzyloxycarbonyl-β-chloro-L-alanine[15] with the appropriate amino acid ester in chloroform. The tripeptides (entries 11 and 12, Table III) were prepared in a similar fashion by condensation of the appropriate *N*-benzyloxycarbonylamino acid with β-chloro-L-alanylglycine ethyl ester hydrobromide.[15]

General Procedure for Preparation of Peptides Containing Dehydroalanine. The β-chloroalanine peptide derivative (1–5 mmol) in EtOAc was treated with 1 equiv of triethylamine or Dabco and the reaction mixture was stirred for 8–12 h at room temperature. The precipitated solid was removed by filtration and the filtrate was washed with water and dried over Na_2SO_4. The solvent was removed in vacuo to yield the dehydroalanine derivative (see Table I).

For those cases in Table I for which the acid is listed rather than the methyl ester, the ester was hydrolyzed according to the procedure of Photaki[13a] in a mixture of alcohol and 1 N sodium hydroxide.

Formation of Oxazoline Derivatives upon Chlorination. To a suspension of Z-Phe-Gly-Ser-OMe (**4**, R = H) (0.91 g, 2.0 mmol) in 10 mL of $CHCl_3$ was added PCl_5 (0.42 g, 2.0 mmol). A slightly exothermic reaction soon subsided whereupon a white solid was deposited. The mixture was stirred for 6 h and the solid was removed by filtration, washed with ether, and dried. Recrystallization of the solid from acetone yielded 0.75 g (75%) of oxazoline **5** (R = H), mp 159–160 °C, $[\alpha]^{24}_D$ −18.7° (*c* 1.0, DMF). This material gave an immediate precipitate with silver nitrate solution.

To a suspension of **5** (500 mg, 1 mmol) in 5 mL of ethyl acetate was added 1 equiv of triethylamine and the resulting mixture was stirred overnight at room temperature. The solid material was removed by filtration and the filtrate was washed with water, dried over Na_2SO_4, and removed in vacuo to yield 250 mg of a solid, mp 135–137 °C. This material was shown to be Z-Phe-Gly-Ser-OMe by comparison of melting point, NMR spectra, and TLC data.

In a similar manner, Z-Phe-Ala-Ser-OMe (**4**, R = Me) gave the oxazoline **5** (R = Me), mp 172–174 °C from acetone–ether, $[\alpha]^{24}_D$ −6.2° (*c* 2.0, DMF), in 81% yield. **5**, upon treatment with triethylamine followed by aqueous workup, gave **4** (R = Me).

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Registry No.—Z-Gly-Phe-OH, 1170-76-9; Z-Phe-Gly-OH, 13122-99-1; Z-Phe-Ala-OH, 21881-18-5; Z-Gly-Gly-OH, 2566-19-0; Z-Gly-Ser-O-Me, 10239-27-7; Z-Ala-Ser-O-Me, 19542-34-8; Z-Phe-Ser-O-Me, 860-55-9; Z-Gly-Phe-Ser-O-Me, 23828-12-8; TFA-Gly-OH, 383-70-0; TFA-Ser-O-Me, 1604-45-1; Z-Val-OH, 1149-26-4; L-Ser-O-Me-HCl, 5680-80-8; β-chloro-L-alanine methyl ester HCl, 17136-54-8; *N*-benzyloxycarbonyl-β-chloro-L-alanine, 7625-65-2; oxazoline **5** (R = H), 62076-65-7; oxazoline **5** (R = Me), 62078-95-9.

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Conversion of Threonine Derivatives to Dehydroamino Acids by Elimination of β-Chloro and *O*-Tosyl Derivatives

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DL-Threonine methyl ester was converted by chlorination with phosphorus pentachloride followed by N-acylation to *erythro*-α-acylamino-β-chloro-DL-butyric acid methyl esters, which upon elimination with Dabco as base yielded 2-acylaminocrotonates as a mixture of *E* and *Z* isomers. Elimination with DBU as base furnished predominantly the *E* isomer. The *Z* isomers formed from the above erythro compounds were shown to arise by isomerization of the corresponding *E* isomer. In comparison, *N*-acyl-*O*-tosyl-DL-threonine methyl esters (threo configuration) yielded only the *Z* isomer upon elimination. *N*-Tosylthreonine derivatives, regardless of configuration, undergo elimination to give *N*-tosylaminocrotonates of *Z* configuration. Evidence is given that aziridines are intermediates in the formation of olefin from *N*-tosylthreonine derivatives.

Dehydroamino acids are constituents of certain peptide antibiotics.[1] Considerable attention[2] has been given recently to the preparation of dehydroamino acids, particularly of the dehydroalanine unit, which unit is generally derived from serine or cysteine derivatives. In this paper, we report studies on elimination reactions of threonine to give 2-acylaminocrotonate derivatives.

Threonine derivatives have been converted to 2-acylaminocrotonates by dehydration[3] and tosylate elimination.[4] Other methods not directly involving threonine for preparation of 2-acylaminocrotonates have been by elimination reactions of sulfonium salts,[2b] sulfoxides,[2c] *N*-chloro-α-amino acid esters,[2f] α-(*N*-acylhydroxyamino) acid esters,[2g] and by amide condensation with α-keto esters.[2h] We recently have prepared peptides containing dehydroalanine by conversion of serine to a β-chloroalanine unit with subsequent elimination.[2i] In this paper, we report application of this method, and also elimination reactions of tosylate derivatives, for preparation of dehydroamino acids from derivatives of threonine.

DL-Threonine methyl ester hydrochloride (**1**) was transformed to *erythro*-β-chloro-DL-α-aminobutyric acid methyl ester hydrochloride (**2**)[5] by chlorination with phosphorus pentachloride, a reaction known to occur with inversion of configuration.[5] Acylation of **2** gave the *N*-acyl-*erythro*-β-chloro-DL-α-aminobutyrates **4**.

Treatment of the *erythro*-β-chlorobutyrates **4** with 1,4-diazabicyclo[2.2.2]octane (Dabco) in ethyl acetate effected elimination to yield the 2-acylaminocrotonates **5**. In all cases, a mixture of geometrical isomers was obtained, though the relative amounts of the *E* and *Z* isomers varied depending upon the *N*-acyl group (Table I). The proportion of geometrical isomers formed and assignment of configuration were determined by NMR spectroscopy[6] (see Table IV). The *E* isomer would be the product expected to be formed from the *erythro*-β-chlorobutyrates if a trans E_2 elimination was occurring. The *Z* isomer formed in these reactions likely arises from isomerization of the *E* isomer. Evidence for this was obtained by treatment of an 87:13 *E*:*Z* mixture of **5a** under the conditions of elimination for an additional 16 h, whereupon NMR analysis showed the composition to be 75:25 *E*:*Z*. Likewise, **4c** and **4e** each gave predominantly the *Z* isomer upon elimination, a result consistent with enhanced *E* to *Z* isomerization due to the electron-withdrawing effects of the *N*-benzyloxycarbonyl and trifluoroacetyl groups, respectively. Poisel and Schmidt[2f] have reported that, under acidic conditions, the *Z* isomer of 2-acylaminocrotonates is the thermodynamically controlled product, a result consistent with our observations.