Conjugate Addition of Alkyl Mercaptans and Thioacetic Acid to β-Chloro-α,β-didehydro-α-amino Acids: Studies toward the Synthesis of β,β-Bis(alkylthio)-α-amino Acids

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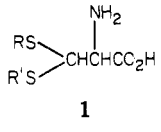
The conjugate addition of mercaptans to β-chloro-α,β-didehydroamino acids has been studied as a method for the preparation of β,β-bis(alkylthio)-α-amino acids. The addition of thioacetic acid was observed to yield β,β-bis(acetylthio)-DL-alanine derivatives. The addition of other mercaptans, such as benzyl mercaptan or appropriate cysteine derivatives, gave only the monosubstitution product, a β-(alkylthio)-α,β-didehydro-α-amino acid; addition of a second molecule of the mercaptan failed to occur. Studies were carried out on the addition of mercaptans to β,β-dichloro-α,β-didehydro-α-amino acid derivatives. By this method the unsymmetrical α,β-bis(alkylthio)dehydroamino acid 14 was prepared, though in low yield and of undetermined configuration.

β,β-Bis(alkylthio)-α-amino acids (1) are rare in nature. Echinomycin and other members of the quinomycin group of depsipeptide antibiotics[1] contain a β-(methylthio)lanthionine unit, 2. Our interest in the total synthesis of the quinomycin antibiotics, as has been accomplished in our laboratories for the related triostins,[2] has led to a study for preparation of β,β-bis(alkylthio)-α-amino acids.



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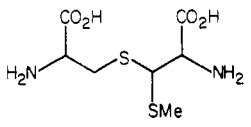

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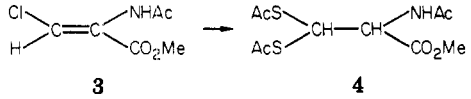
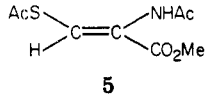
One approach we have investigated involved the use of α,β-didehydroamino acids as precursors to the corresponding β,β-dimercapto derivatives. Limited reports[3] have appeared in the literature involving preparation of β,β-dimercaptoamino acids from dehydroamino acids. In all cases, these have involved conjugate addition of a thiol to a β-halo- or β-(alkylthio)-α,β-didehydroamino acid or related oxazolone.

Our initial studies provided a convenient preparation of *N*-acetyl-β,β-bis(acetylthio)-DL-alanine methyl ester (4).



3 4



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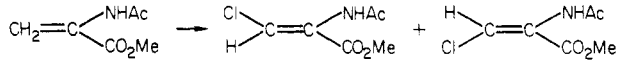
This was accomplished in 71% yield by treatment of (*Z*)-*N*-acetyl-β-chlorodehydroalanine methyl ester (3) with an excess of thioacetic acid in acetonitrile containing 1 equiv of sodium bicarbonate and a small amount of 1,4-diazabicyclo[2.2.2]octane (Dabco). Reaction of 3 with 1 equiv of thioacetic acid furnished the acetylthio derivative (*Z*)-*N*-acetyl-β-(acetylthio)dehydroalanine methyl ester (5) in 40% yield. As expected, treatment of 5 with thioacetic acid gave, in 70% yield, the β,β-bis(acetylthio) derivative 4.

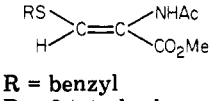
Preparation of the chloro compound 3 was effected as previously reported, except that methylene chloride was used as the reaction solvent,[4] by treatment of *N*-acetyldehydroalanine methyl ester (6) with 1 equiv of chlorine



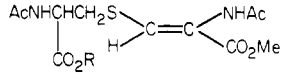
6 3 7

and subsequent elimination with Dabco. This reaction provided an 80:20 mixture of geometrical isomers 3 and 7, from which the *Z* isomer 3 was isolated in 28–41% yields. The *E* isomer 7 was not isolated but was obtained from the mother liquors in 25% yield as an approximate 1:1 mixture of *E* and *Z* isomers. Use of 1,5-diazabicyclo-[5.4.0]undec-5-ene (DBU) or potassium *tert*-butoxide as the base gave elimination product having an *E*/*Z* ratio of 90:10. Reaction of thioacetic acid with the above 1:1 mixture of *E* and *Z* isomers gave a mixture of the corresponding (*E*)- and (*Z*)-β-(acetylthio)-*N*-acetyldehydroalanine methyl esters, which upon column chromatography provided the *E* isomer in 12% yield.

Reaction of the β-chlorodehydroalanine 3 with 1 equiv of benzyl mercaptan gave (*Z*)-*N*-acetyl-β-(benzylthio)-dehydroalanine methyl ester (8). Likewise, condensation



8, R = benzyl
9, R = 2-tetrahydropyranyl



10, R = H
11, R = Me

of 3 with 2-tetrahydropyranthiol, *N*-acetyl-L-cysteine, or *N*-acetyl-L-cysteine methyl ester furnished the corresponding *Z* isomers 9–11, respectively. However, all attempts to add a second molecule of benzyl mercaptan or thioacetic acid to 8, under either basic or radical conditions, failed and led only to recovered reactant. Likewise, the addition of thioacetic acid to 10 was unsuccessful. These results are contrary both to literature results[3] and to our experience with the β-(acetylthio)dehydroalanine 5, which readily undergoes conjugate addition of a second acetylthio unit. Attempts to effect addition of benzyl mercaptan or

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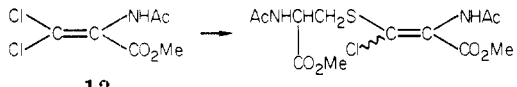
N-acetyl-L-cysteine methyl ester to the β-(acetylthio)-dehydroalanine **5** yielded a mixture of products. The major products formed appeared to be the S-acylated derivatives of the reactant thiols formed by *S*-acyl interchange.

The above substitution reactions of the β-halo group by mercaptans proceed with retention of configuration as has been observed in most cases involving addition–elimination reactions of vinyl halides.[5] Stereochemistry of the geometrical isomers obtained in this study was established by use of our previously reported observation that the position of the vinylic proton in the ^{1}H NMR spectrum of the *Z* isomer recorded in $CDCl_3$ is at higher field than the vinylic proton of the corresponding *E* isomer;[6] a pronounced downfield shift of the vinylic proton in TFA relative to its position in $CDCl_3$ also is observed for the *Z* isomer.

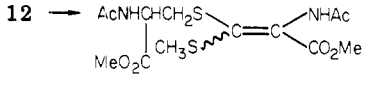
We also have studied the addition–elimination reaction of mercaptans to the β,β-dichloro-α,β-didehydroalanine **12**.



12 **13**

Compound **12** was prepared in 54% yield by the addition of chlorine to the β-chlorodehydroalanine **3**, followed by treatment with Dabco to effect elimination. Reaction of **12** with *N*-acetyl-L-cysteine methyl ester in acetonitrile with catalysis by DBU gave **13** as a mixture of geometrical isomers in 40–60% yield based upon recovered **12**, from which the major isomer was isolated in a yield of 22%. We have not assigned stereochemistry to the isomers of **13**, as criteria for doing so on tetrasubstituted dehydroamino acids such as these have not been developed.

Treatment of the major isomer of **13** with methyl mercaptan in the presence of base invariably gave a mixture of products. In one instance involving the reaction of sodium methyl sulfide with **13**, the β,β-dithiodehydroalanine **14** was isolated in 8% yield by column chroma-



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tography. Due to the low yields realized in the above approach from the β,β-dichloro derivative **12**, further study of these reactions has not been undertaken.

Experimental Section

A Thomas-Hoover capillary melting point apparatus was used to determine melting points. All melting points and boiling points are uncorrected. The solvents were removed under reduced pressure (water aspirator) with a Buchler rotary evaporator. A Beckman Model 20A infrared spectrophotometer was used to record the infrared spectra of compounds as potassium bromide pellets, chloroform-deposited films, or neat liquids. Proton magnetic resonance spectra were recorded on Varian XL-100-12 or Varian EM-360 spectrometers. The format of the data reported is as follows: chemical shifts (multiplicity, integral intensity, source). Elemental analyses were performed by M-H-W Laboratories. Thin-layer chromatography (TLC) was performed on Quantum Industries silica gel MW6F 1 in. × 3 in. plates or Brinkmann precoated silica gel plates. Spots were detected in iodine vapor and/or UV light. The TLC solvents used were as follows: A, chloroform–methanol–acetic acid (85:10:5); B, chloroform–acetic acid (95:5); C, chloroform–methanol–acetic acid (10:5:1); or as noted. Solvents were often dried before use over Linde 3A molecular sieves (MS-3A). Methylene chloride was purified by being washed with 10% sodium carbonate and dried over MS-3A.

β,β-Bis(acetylthio)-*N*-acetyl-D,L-alanine Methyl Ester (4). A. A solution of 5.33 g (0.03 mol) of (*Z*)-β-chloro-*N*-acetyldehydroalanine methyl ester (**3**) in 100 mL of acetonitrile was treated with 5 mL of thioacetic acid, 2.5 g (0.03 mol) of sodium bicarbonate, and several specks of Dabco. After the mixture was stirred overnight at room temperature, the solvent was evaporated in vacuo and the residue was diluted with $CHCl_3$. The $CHCl_3$ phase was washed with water, dried over $MgSO_4$, filtered, and evaporated in vacuo. Crystallization from methylene chloride–petroleum ether (bp 30–60 °C), after treatment with Norit, gave 6.2 g (71%) of **4**: mp 94–96 °C (an alternate crystalline modification of **4** has a melting point of 113–114 °C); ^{1}H NMR ($CDCl_3$) δ 2.07 (s, 3 H, NAc), 2.37 (s, 6 H, SAc), 3.80 (s, 3 H, Me ester), 5.17 (m, 1 H, α-CH), 5.60 (d, 1 H, β-CH), 6.57 (br, 1 H, NH), R_f(A) 0.75, R_f(B) 0.36, R_f(C) 0.60. Anal. ($C_{10}H_{15}NO_5S_2$) C, H, N, S.

B. To a solution of 0.5 g (2.3 mmol) of (*Z*)-β-(acetylthio)-*N*-acetyldehydroalanine methyl ester (**5**) in 30 mL of acetonitrile were added a small spatula of Dabco and 1 mL of thioacetic acid. After the mixture was stirred for 4 h at room temperature, 1 mL of 4 N HCl in dioxane was added and the solvent removed in vacuo. After the above workup, crystallization from methylene chloride–petroleum ether (bp 30–60 °C) gave 0.47 g (70%) of **4**, mp 94–96 °C.

(*Z*)- and (*E*)-β-Chloro-*N*-acetyldehydroalanine Methyl Esters (3 and 7). Treatment of *N*-acetyldehydroalanine methyl ester (**6**;[7] 42.7 g, 0.30 mol) with chlorine in CCl_4 as described[4] gave crude α,β-dichloro compound, which after removal of solvent was taken up in 300 mL of methylene chloride. To the solution was added 33.7 g (0.30 mol) of Dabco or 45.0 g (0.30 mol) of DBU. The reaction was also carried out by dissolution of the residue in 200 mL of *tert*-butyl alcohol and treatment with 33.7 g (0.30 mol) of potassium *tert*-butoxide. After the mixture was stirred for 20 min at room temperature, the solvent was removed in vacuo, and the residue was dissolved in water. The aqueous solution was acidified with 1 N HCl, extracted with ether, saturated with sodium chloride, and extracted with ether. The ether extracts were dried over $MgSO_4$, filtered, and evaporated in vacuo. ^{1}H NMR analysis of the crude reaction mixture indicated a 80:20 *Z*/*E* mixture (Dabco) or a 90:10 *Z*/*E* mixture (DBU or *t*-BuOK) of **3** and **7**. Crystallization from ether gave 15.1 g (28%) of (*Z*)-**3** [mp 96–96.5 °C (lit.[4] mp 96–97 °C)] and 13.6 g (25%) of an approximately 1:1 mixture of *Z*/*E* isomers: ^{1}H NMR ($CDCl_3$) δ 7.00 (*Z* isomer, s, 1 H, vinyl), 7.43 (*E* isomer, s, 1 H, vinyl). A partial separation of the *E* isomer could be effected by column chromatography on silica gel (Sigma Type I) and elution with solvent B; however, this was generally not done, and the 1:1 mixture of *Z*/*E* isomers **3** and **7** was used for further reactions. As reported previously,[4] we have obtained **3** in 41% yield using a procedure slightly modified from that given above.

(*Z*)-*N*-Acetyl-β-(acetylthio)-α,β-didehydroalanine Methyl Ester ((*Z*)-5). A solution of (*Z*)-β-chloro derivative **3** (8.88 g, 50 mmol) in 100 mL of acetonitrile (dried over 3A molecular sieves) was stirred with 5.60 g (50 mmol) of Dabco and 3.80 g (50 mmol) of thioacetic acid at room temperature for 1.25 h. The reaction mixture was filtered and the precipitate washed with ethyl acetate. The combined filtrate and washings were removed in vacuo to yield a yellow paste. This material was triturated with ethyl acetate (2 × 100 mL). The combined ethyl acetate phases were washed once with 50 mL each of 1 N HCl, saturated $NaHCO_3$, and water. After the ethyl acetate phases were dried over $MgSO_4$, the solvent was removed in vacuo to yield 7.2 g of crude product. After trituration with 1:1 ether–petroleum ether (bp 30–60 °C) (2 × 50 mL), the residue was recrystallized from $CHCl_3$ to give 2.40 g (22%) of **5**, mp 141–142.5 °C. A second crop (2.51 g) was obtained (mp 136–138 °C) which upon recrystallization gave 1.98 g (18%) of **5**: mp 138–140 °C; R_f(A) 0.68, R_f(B) 0.36; ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3 H, *N*-acetyl), 2.45 (s, 3 H, *S*-acetyl), 3.87 (s, 3 H, methyl ester), 7.53 (br s, 1 H, NH), 7.93 (s, 1 H, vinyl proton) (position of vinyl proton in TFA, 8.40);UV (MeOH) λ_{max} 283 nm (ε 15 149).

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(*E*)-*N*-Acetyl-β-(acetylthio)-α,β-didehydroalanine Methyl Ester ((*E*)-5). A solution of an approximate 45:55 mixture of *E* and *Z* isomers of the β-chloro derivatives **7** and **3** (8.88 g, 50 mmol) in 100 mL of acetonitrile was treated as above with equimolar amounts of Dabco and thioacetic acid at room temperature for 1.5 h. The solvent was removed in vacuo and the residue triturated with ethyl acetate (2 × 50 mL). The combined ethyl acetate phases were washed twice with water, once with 10% Na_2CO_3, and once with water and dried over $MgSO_4$. Treatment with Norit and removal of the drying agent gave 4.75 g of a brown oil, which was placed upon a dry column of silica gel and eluted with chloroform–acetic acid (95:5). The *E* isomer, (*E*)-**5**, was obtained as a solid (1.31 g, 12%), which upon recrystallization from $CHCl_3$–petroleum ether (bp 30–60 °C) gave crystalline product: 0.54 g (5%); mp 104–106 °C; R_f(A) 0.70, R_f(B) 0.44; ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 3 H, *N*-acetyl), 2.48 (s, 3 H, *S*-acetyl), 3.95 (s, 3 H, methyl ester), 8.80 (s, 1 H, vinyl) (position of vinyl proton in TFA, 8.80), 8.86 (br s, 1 H, NH). Anal. ($C_8H_{11}NO_4S$) C, H, N.

(*Z*)-*N*-Acetyl-β-(benzylthio)-α,β-didehydroalanine Methyl Ester (8). A 4-g (22.5 mmol) sample of (*Z*)-β-chloro compound **3** was dissolved in 200 mL of dry acetonitrile followed by the addition of triethylamine (2.75 g, 27.2 mmol) and benzyl mercaptan (2.8 g, 22.5 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was removed in vacuo, the residue was covered with 300 mL of ethyl acetate, and the precipitated hydrochloride salts were removed by filtration. The organic phase was washed once with water and dried over $MgSO_4$. Filtration and removal of solvent in vacuo gave a light yellow solid, which was recrystallized from ethyl acetate–diethyl ether; several crops of crystals were obtained, which were combined and recrystallized as before to yield 3.81 g (64%) of product: mp 102.5–104 °C; R_f(A) 0.72; ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3 H, acetyl), 3.70 (s, 3 H, methyl ester), 3.98 (s, 2 H, benzyl), 7.30 (s, 5 H, phenyl), 7.35 (br, 1 H, NH), 7.43 (s, 1 H, vinyl) (position of vinyl proton in TFA, 8.10); UV (MeOH) λ_{max} 285 nm (ε 14 336). Anal. ($C_{13}H_{15}NO_3S$) C, H, N, S.

(*Z*)-*N*-Acetyl-β-(2-tetrahydropyranylthio)-α,β-didehydroalanine Methyl Ester (9). β-Chlorodehydroalanine **3** (3.55 g, 20 mmol) was dissolved in 50 mL of acetonitrile, and 2.36 g (20 mmol) of 2-tetrahydropyranthiol[8] was added followed by 2.24 g (20 mmol) of Dabco. The reaction mixture was stirred at room temperature for 2 h, acidified with 2 N HCl in THF, and filtered through Celite, and the Celite pad was washed with ethyl acetate. The solvent was removed in vacuo, and the residue was taken up in 125 mL of ethyl acetate and dried over $MgSO_4$. Filtration and removal of solvent in vacuo gave a yellow oil that was dissolved in diethyl ether and cooled to give 2.6 g of a white solid, mp 85–93 °C. Recrystallization from diethyl ether–methylene chloride (approximately 6:1) gave, in various crops, 2.75 g (53%) of product: mp 94–95.5 °C; R_f(A) 0.63, R_f(B) 0.40; ^{1}H NMR ($CDCl_3$) δ 1.73 (br m, 6 H, tetrahydropyranyl methylenes), 2.15 (s, 3 H, *N*-acetyl), 3.82 (s) superimposed upon multiplet at 3.90 (5 H, methyl ester, CH_2O), 5.26 (m, 1 H, CHS), 7.10 (br, 1 H, NH), 7.70 (s, 1 H, vinyl) (position of vinyl proton in TFA, 8.26); an analytical sample was prepared by recrystallization from ethyl acetate–petroleum ether (bp 30–60 °C); mp 97–98.5 °C. Anal. ($C_{11}H_{17}NO_4S$) C, H, N, S.

(*Z*)-*N*-Acetyl-β-[(*N*-acetyl-L-alan-β-yl)thio]-α,β-didehydroalanine Methyl Ester (10). A 5-g (28.2 mmol) sample of β-chloro derivative **3** in 100 mL of acetonitrile was treated with Dabco (6.33 g, 56.4 mmol) and *N*-acetyl-L-cysteine (4.58 g, 28.1 mmol) with stirring at room temperature for 3 h. The reaction mixture was treated with 23 mL of 4 N HCl in dioxane and filtered through Celite, and the Celite pad was washed with 200 mL of acetonitrile. The solvent from the filtrate was removed in vacuo, and the residue was dissolved in 200 mL of cold methanol. Diethyl ether (100 mL) was added, and the small amount of solid that precipitated was removed by filtration. Removal of the solvent in vacuo gave 7.0 g of a yellow oil, which upon dissolution in acetone deposited white crystals (4.36 g) in various crops. Recrystallization from acetone–diethyl ether gave 3.46 g (40%) of **10**: mp 179.5–180 °C; R_f(A) 0.42, R_f(C) 0.32; ^{1}H NMR (TFA) δ 2.16 (s, 6 H, *N*-acetyls), 3.40 (d, 2 H, CH_2S), 3.73 (s, 3 H, methyl ester), 4.93 (m, 1 H, α-H), 7.73 (br, 1 H, NH), 7.83 (s, 1 H, vinyl), 8.22 (br, 1 H, NH). Anal. ($C_{11}H_{16}N_2O_6S$) C, H, N, S.

(*Z*)-*N*-Acetyl-β-[(*N*-acetyl-L-alan-β-yl)thio]-α,β-didehydroalanine Dimethyl Ester (11). Compound **3** (5.0 g, 28 mmol) in 100 mL of acetonitrile was caused to react with *N*-acetyl-L-cysteine methyl ester (4.9 g, 28 mmol) and Dabco (3.1 g, 28 mmol) at room temperature for 2.75 h. During the course of the reaction the pH, as measured with litmus paper, was kept basic by addition of small quantities of Dabco. Upon completion of the reaction, the mixture was acidified with 1 N HCl, and the solvent was removed in vacuo. To the residue was added 50 mL of saturated NaCl solution, followed by extraction with ethyl acetate (4 × 50 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated to yield 7.74 g (80%) of a light yellow foam. The foam, which could not be induced to crystallize, had an NMR spectrum consistent with that of product **11**; this material showed two minor spots on TLC analysis in two solvent systems, ethyl acetate and ethyl acetate–acetone (6:4), but was not further purified: ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 6 H, *N*-acetyls), 3.40 (d, 2 H, CH_2S), 3.88 (s, 6 H, methyl esters), 4.93 (m, 1 H, α-H), 7.47 (s, 1 H, vinyl) (position of vinyl proton in TFA, 8.07), 7.56 (br, 1 H, NH), 8.12 (br, 1 H, NH).

β,β-Dichloro-*N*-acetyldehydroalanine Methyl Ester (12). To a solution of 44.5 g (0.25 mol) of β-chloro-*N*-acetyldehydroalanine methyl ester (**3**) as a mixture of *Z* and *E* isomers in 350 mL of methylene chloride was added chlorine gas until a permanent yellow solution was obtained. After the mixture was stirred for 20 min at room temperature, the solvent was evaporated in vacuo. The yellow residue was dissolved in 300 mL of methylene chloride and 28.0 g (0.25 mol) of Dabco was slowly added with stirring at room temperature. After the addition was completed, the reaction was stirred for 10 min at room temperature and then heated at reflux for 10 min. The reaction was cooled, washed with water, dried over $MgSO_4$, and filtered. The solvent was evaporated in vacuo and the residue crystallized from water (a hot, water-insoluble gum was removed by filtration) to give 29.0 g (54%) of **12**: mp 102.5–103.5 °C; R_f(B) 0.34, R_f(C) 0.79; ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3 H, *N*-acetyl), 3.90 (s, 3 H, methyl ester), 7.83 (br, 1 H, NH). Anal. ($C_6H_7Cl_2NO_3$) C, H, N.

***N*-Acetyl-β-chloro-β-[(*N*-acetyl-L-alan-β-yl)thio]-α,β-didehydroalanine Dimethyl Ester (13).** To a solution of 2.12 g (10.0 mmol) of **12** in 50 mL of acetonitrile were added 1.77 g (10.0 mmol) of *N*-acetyl-L-cysteine methyl ester and 1.6 g (10.0 mmol) of 96% DBU. The mixture was heated at reflux for 3 h, after which 2 mL of 1 N HCl was added, and the solvent was removed in vacuo. The residue was dissolved in water and extracted with chloroform, and the $CHCl_3$ extracts were dried over $MgSO_4$, filtered, and evaporated in vacuo. Column chromatography on silica gel 60 (E. Merck) 230–400 mesh and elution with ethyl acetate gave 0.5 g of recovered **12**, 0.63 g (18%) of the major isomer of **13**, 0.81 g (23%) of a mixture of the major and minor isomers of **13**, and several other unidentified fractions. Recrystallization of the major isomer from EtOAc–petroleum ether (bp 30–60 °C) gave 0.3 g (9%) of **13** (major isomer): mp 134.5–135.5 °C; R_f 0.84 (in acetone), R_f 0.41 (in ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.13 and 2.20 (2 s, 6 H, *N*-acetyls), 3.13 (m, 2 H, CH_2S), 3.87 and 3.93 (2 s, 6 H, methyl esters), 4.93 (m, 1 H, α-H), 6.90 (br, 1 H, NH), 8.83 (br, 1 H, NH). Anal. ($C_{12}H_{17}ClN_2O_6S$) C, H, N, S. The ^{1}H NMR ($CDCl_3$) of the minor isomer is as follows: δ 2.03 and 2.17 (s, 6 H, *N*-acetyl), 3.40 (br d, 2 H, CH_2S), 3.83 and 3.93 (2 s, 6 H, methyl esters), 4.97 (m, 1 H, α-H), 7.40 (br d, 1 H, NH), 8.17 (br, 1 H, NH).

***N*-Acetyl-β-(methylthio)-β-[(*N*-acetyl-L-alan-β-yl)thio]-α,β-didehydroalanine Dimethyl Ester (14).** To a solution of sodium metal (70 mg, 3.0 mmol) in 5 mL of methanol were added 50 mL of acetonitrile and 20 mg (4.2 mmol) of methyl mercaptan. After the mixture was stirred a few minutes at room temperature, 1.0 g (2.8 mmol) of the major isomer of **13** was added. After this mixture was stirred for 70 h at room temperature, 1 N HCl was added, and the solvent was evaporated in vacuo. The residue was diluted with ethyl acetate, the mixture was dried over $MgSO_4$ and filtered, and the solvent was evaporated in vacuo. TLC analysis in ethyl acetate showed several components present in the reaction mixture. Column chromatography of the mixture on silica gel 60 (E. Merck, 230–400 mesh) and elution with EtOAc gave 80 mg

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(8%) of the major isomer of 14: mp 126–127 °C; R_f 0.63 (in ethyl acetate–acetone, 6:4); ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3 H, SCH_3), 2.20 and 2.27 (2 s, 6 H, *N*-acetyls), 3.13 (m, 2 H, CH_2S), 3.83 and 3.93 (2 s, 6 H, methyl esters), 4.87 (m, 1 H, α-H), 6.67 (br, 1 H, NH), 8.87 (br, 1 H, NH). Anal. ($C_{13}H_{20}N_2O_6S_2$) C, H, N.

In a subsequent fraction eluted from the column, 20 mg (2%) of an oil was obtained that appeared to be an isomer of the above compound: R_f 0.57 (in ethyl acetate–acetone, 6:4); ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 3 H, SCH_3), 2.17 and 2.33 (2 s, 6 H, *N*-acetyls), 3.40 (br s, 2 H, CH_2S), 3.83 and 3.97 (2 s, 6 H, methyl esters), 4.97 (m, 1 H, α-H), 7.63 (br, 1 H, NH), 7.97 (br, 1 H, NH).

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Registry No. **3**, 60084-47-1; **4**, 73972-24-4; *E*-**5**, 73972-25-5; *Z*-**5**, 73972-26-6; **6**, 35356-70-8; **7**, 73972-27-7; **8**, 73972-28-8; **9**, 73972-29-9; **10**, 73972-30-2; **11**, 73972-31-3; **12**, 60389-02-8; *E*-**13**, 73972-32-4; *Z*-**13**, 73972-33-5; *E*-**14**, 73972-34-6; *Z*-**14**, 73972-35-7; thioacetic acid, 507-09-5; benzyl mercaptan, 100-53-8; 2-tetrahydropyranthiol, 40446-64-8; *N*-acetyl-L-cysteine, 616-91-1; *N*-acetyl-L-cysteine methyl ester, 7652-46-2; methyl mercaptan, 74-93-1.

Stereospecific Alkylation of the Schiff Base Ester of Alanine with 2-Substituted-(*E*)- and -(*Z*)-vinyl Bromides. An Efficient Synthesis of 2-Methyl-(*E*)-3,4-didehydroglutamic Acid, a Potent Substrate-Induced Irreversible Inhibitor of L-Glutamate-1-decarboxylase

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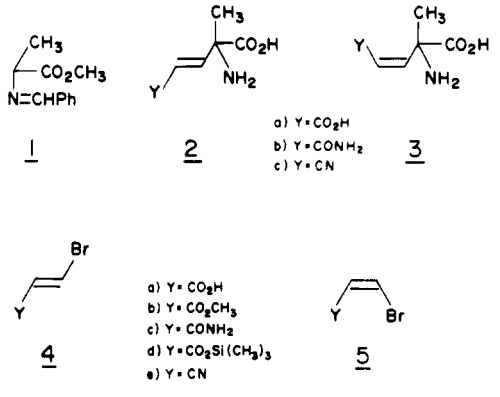
The nucleophilic vinylic substitution by the enolate of methyl *N*-benzylidenealanate (**1**) of (*E*)- and (*Z*)-vinyl bromides **4** and **5** has been examined as an approach to the synthesis of the *E* and *Z* isomers of 2-methyl-3,4-didehydroglutamic acid. Under aprotic conditions, the nucleophilic displacement has been found to proceed stereospecifically with retention of configuration of the double bond to afford in good yield the corresponding (*E*)- and (*Z*)-substituted products. The configuration of the substitution products has been assigned from the analysis of their ^{1}H NMR spectra on the basis of the value of the coupling constant of the vicinal vinylic protons. Subsequent removal of the protecting groups from the substitution products **6** in the *E* series gives the corresponding 2-methyl-(*E*)-3,4-didehydroglutamic acid derivatives **2** in good yield. The *Z* isomers **3** prove to be very unstable and have not been isolated.

Enzyme-activated irreversible inhibitors are highly specific enzyme inactivators.[1] Their specificity results from their binding affinity and also from their effectiveness to serve as substrates for the target enzymes. The availability of such inhibitors for enzymes involved in metabolic pathways of bioactive molecules such as neurotransmitters has proven extremely useful to elucidate the physiological role of these molecules.[2] We recently demonstrated that 2-(*R*,*S*)-2-methyl-(*E*)-3,4-didehydroglutamic acid (**2a**) is a potent enzyme-activated irreversible inhibitor of chick-embryo brain L-glutamate-1-decarboxylase (E.C.4.1.1.15),[3] the enzyme catalyzing the formation of the inhibitory neurotransmitter γ-aminobutyric acid.[4] We report now an efficient and stereoselective synthesis of (*E*)-3,4-didehydro-2-methylglutamate derivatives **2**, as well as the attempted preparation of the corresponding *Z* isomers **3**.

The major synthetic challenge in the structural features of **2** and **3** lies in the presence of a 1,2-disubstituted double bond in a β,γ position relative to an α-amino acid functionality. Of the few methods available for the preparation of β,γ-unsaturated-α-amino acids,[5] none are suitable for the stereoselective formation of the double bond. Earlier studies from this laboratory demonstrated the utility of the enolates derived from Schiff base alkyl esters of α-amino acids as general synthons for the preparation of α-substituted-α-amino acids.[6] Of particular interest is the fact that these enolates add quantitatively in a Michael



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