A New and Simple Method of Resolution. Preparation of 3-Fluoro-D-alanine-*2-d*

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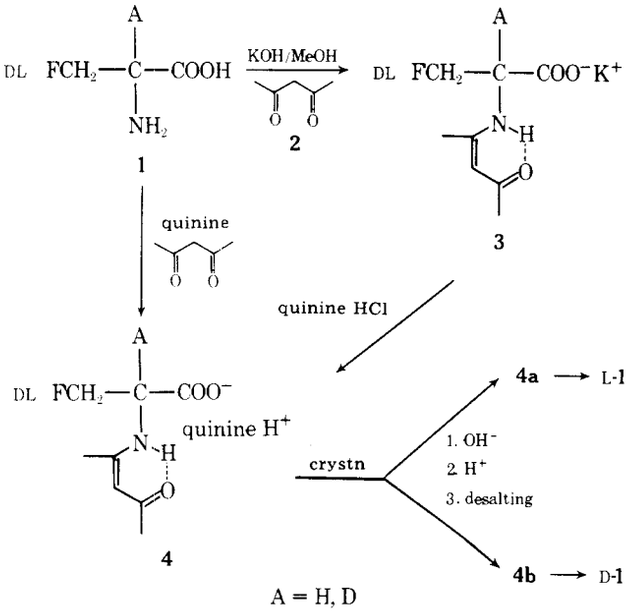
A simple method for resolving 3-fluoro-DL-alanine-*2-d* and its protio analogue is described. The highly acid labile *N*-(1-methyl-2-acetylvinyl)amino acids were prepared as the quinine salts and the diastereomers were separated by crystallization.

It was recently reported[1] that the combination of 3-fluoro-D-alanine-*2-d* (**1**) and a derivative of the antibiotic cycloserine is a potent gram-positive and gram-negative antibacterial agent. We report here a new method of resolution for DL-**1**, which is also applicable to its protio analogue,[5] alanine itself, and presumably many other amino acids.

β-Diketones have been previously used for protecting α-amino acids as their enamines during peptide synthesis.[2,3] Prior to our report these derivatives were synthesized as their potassium salt[2,4] or the more crystalline dicyclohexylamine salt.[3] We explored the possibility of using an optically active base to form their crystalline diastereomers, and found that the quinine salt crystallized easily in good yield and excellent purity. Further advantages of this resolution method include the one-step derivatization–salt formation and the ease of removing the protecting group.

Conversion of 3-fluoro-DL-alanine (**1**)[5,6] to the quinine *N*-(1-methyl-2-acetylvinyl)-3-fluoro-DL-alaninate (**4**) is accomplished by warming **1** with quinine and 2,4-pentanedione

Scheme I



in methanol. The L isomer **4a** crystallizes from the reaction mixture in high (≈99%) optical purity.

The quinine salt **4** can also be made by treatment of the potassium salt **3** with 1 equiv of quinine HCl. From the resolved **4a** or **4b** the quinine is separated by extraction with chloroform of the basified solution. The masking group is readily cleaved from the resolved substrate by mild acid hydrolysis and separated by extraction. In the final step ion exchange is employed for the removal of inorganics. The L-**1** or D-**1** is eluted with 0.5 N ammonium hydroxide and isolated by crystallization after reducing the volume of eluate in vacuo.

The scope of the method has not been explored. We have also resolved alanine by this procedure, and Southard's work[3b] describing the preparation of numerous amino acid enamine derivatives suggests that this method should be generally applicable.

In the Experimental Section are detailed procedures for direct quinine salt formation with 3-fluoroalanine-*2-d* and alanine itself, and the K-salt method with 3-fluoroalanine.

Experimental Section

All optical rotations were determined on a Carl Zeiss photoelectric precision polarimeter Model LEP A1 as a 6% solution in 1 N hydrochloric acid at 25 °C unless otherwise specified. NMR spectra were obtained with a Varian T-60 spectrometer and sodium 3-(trimethylsilyl)propanesulfonate as the internal standard and interpreted by Dr. Alan W. Douglas. Microanalyses were done through the courtesy of Mr. J. P. Gilbert and associates. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected.

Resolution of 3-Fluoro-DL-alanine-*2-d* (1). Racemic 3-fluoroalanine-*2-d*[6] (100 g, 0.935 mol), 2,4-pentanedione (103 g, 1.03 mol), and quinine (320 g, 0.99 mol) in 1870 ml of anhydrous methanol were treated under reflux in a nitrogen atmosphere for 1 h. After cooling and stirring for 1 h at 20 °C the quinine salt of *N*-(1-methyl-2-acetylvinyl)-3-fluoro-L-alanine-*2-d* (**4a**) CH_3OH solvate was collected, washed with cold CH_3OH, and dried in vacuo at room temperature to give 176 g, $[\alpha]^{25}D$ −89.6° (*c* 2.0, 95% EtOH), mp 143–144 °C dec.

Anal. Calcd for $C_{28}H_{36}FN_3O_5 \cdot CH_3OH$: C, 63.83; H, 7.39; F, 3.48; N, 7.70. Found: C, 63.78; H, 7.30; F, 3.69; N, 7.90.

Removal of the solvent from the filtrate and washes, followed by crystallization of the residue from 500 ml of EtOAc, gave after 16 h at 0 °C a second crop of 55.2 g. The yield of analytically pure first and second crops was 96.6%. The anhydrous form was obtained by drying a sample at 50 °C for 2 h.

Anal. Calcd for $C_{28}H_{36}FN_3O_5$: C, 65.48; H, 7.07; F, 3.70; N, 8.18. Found: C, 65.66; H, 7.46; F, 3.77; N, 8.23.

The residue (**4b**) from the second crop was dissolved in 360 ml of water and basified by adding 450 ml of 1.2 N (0.54 mol) sodium hydroxide with stirring at 10–15 °C. The liberated quinine was removed by chloroform extraction and the aqueous phase was acidified and stirred with 500 ml of 2 N hydrochloric acid at 15–20 °C. After 15 min, the protecting group was removed as evidenced by the dissolution of the precipitated *N*-(1-methyl-2-acetylvinyl)-3-fluoro-D-alanine-*2-d*. After extraction with chloroform to remove 2,4-pentanedione, the aqueous phase was clarified with charcoal and the filtrate was percolated through 1.1 l. of Dowex 50W × 4 (H^+ form). The column was washed free of Cl^- with water, then the product was eluted with 0.5 N ammonium hydroxide, collecting and concentrating in vacuo the ninhydrin-positive fractions until the product crystallized (≈150 ml). The mixture was cooled to 5 °C and aged for several hours, and the crystalline 3-fluoro-D-alanine-*2-d* was collected, washed with cold water, and vacuum dried at 40 °C to give 28.5 g (57%), mp 174–175 °C, $[\alpha]^{25}D$ −10.4°.

Anal. Calcd for $C_3H_6NO_2F$: C, 33.65; H, 5.65; N, 13.08; F, 17.74. Found: C, 33.41; H, 5.74; N, 12.96; F, 17.53.

3-Fluoro-L-alanine-*2-d* was separated from the crystalline quinine *N*-(1-methyl-2-acetylvinyl)-3-fluoro-L-alaninate-*2-d* (231.5 g) as described above for its enantiomer. In this manner 35.5 g (68%) of 3-fluoro-L-alanine-*2-d* was obtained, mp 174–175 °C, $[\alpha]^{25}D$ +10.3°.

Anal. Calcd for $C_3H_6NO_2F$: C, 33.65; H, 5.65; N, 13.08; F, 17.74. Found: C, 33.45; H, 5.78; N, 13.01; F, 17.77.

Potassium *N*-(1-Methyl-2-acetylvinyl)-3-fluoro-DL-alaninate

(3). To a suspension of 10.71 g (100 mmol) of 3-fluoro-DL-alanine in 40 ml of 90% methanol was added a solution of 5.61 g (100 mmol) of potassium hydroxide in 20 ml of 90% MeOH followed by the addition of a solution of 10.1 g (100 mmol) of 2,4-pentanedione in 60 ml of methanol. The mixture was refluxed for 20 min and concentrated in vacuo. Recrystallization from 2-propanol (300 ml) yielded 17.6 g (78%) of product.

Anal. Calcd for $C_8H_{11}NO_3FK$: C, 42.27; H, 4.87; N, 6.16; F, 8.35. Found: C, 42.30; H, 4.88; N, 6.23; F, 8.11.

^1H NMR (D_2O): δ 1.97 (s, 3, CH_3), 2.02 (s, 3, CH_3), 4.4 (m, 1, CH, $J_{H-F} \approx 31$ Hz), 4.8 (m, 2, CH_2F, $J_{H-F} \approx 46$ Hz).

Resolution of 3-fluoro-DL-alanine was performed starting from potassium *N*-(1-methyl-2-acetylvinyl)-3-fluoro-DL-alaninate.

To a solution of 22.72 g (100 mmol) of potassium *N*-(1-methyl-2-acetylvinyl)-3-fluoro-DL-alaninate (3) in 200 ml of methanol was added 40.5 g (102.5 mmol) of quinine hydrochloride dihydrate. The mixture was heated under reflux for a period of 1 h. Application of the same method as described above for the separation and isolation gave 3-fluoro-D-alanine, $[\alpha]^{25}D$ −10.4°, and 3-fluoro-L-alanine, $[\alpha]^{25}D$ +10.4°, in 54.2 and 64% yield, respectively.

Resolution of DL-Alanine. Application of the same method gave quinine *N*-(1-methyl-2-acetylvinyl)-L-alaninate in 93% yield, mp 142–143 °C, $[\alpha]^{25}D$ −73.8°.

Anal. Calcd for $C_{28}H_{37}N_3O_5 \cdot \frac{1}{2}H_2O$: C, 66.64; H, 7.59; N, 8.33. Found: C, 66.45; H, 7.80; N, 8.10.

L-Alanine was obtained from the crystalline enamine–quinine salt as described above for the fluoroalanine in 76% yield, $[\alpha]^{25}D$ +12.8° (*c* 5%, 5 N HCl) (lit.[7] +13°).

Registry No.—DL-**1**, 16652-37-2; D-**1**, 35455-20-0; L-**1**, 35455-21-1; **2**, 123-54-6; **3**, 60526-14-9; **4a**, 60526-16-1; **4b**, 60526-18-3; quinine, 130-95-0; potassium hydroxide, 1310-58-3; DL-alanine, 302-72-7; quinine *N*-(1-methyl-2-acetylvinyl)-L-alaninate, 60526-20-7; L-alanine, 56-41-7.

References and Notes

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Some Novel, Acid-Labile Amine Protecting Groups[1]

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Under certain circumstances the lability of the *t*-Boc protecting group to 50% aqueous acetic acid is a shortcoming during the synthesis of large polypeptides in solution. The somewhat more stable 1-methylcyclobutyloxycarbonyl protecting group has been found to overcome this problem, but still is sufficiently acid labile to be useful as a temporary protecting group. Selected small ring carbamates exhibiting varying degrees of acid lability have also been prepared and evaluated. During cleavage in trifluoroacetic acid partial isomerization of the protecting group was observed in the case of *N*-cyclopropylcarbinyloxycarbonyl phenylalanine. The significance of this observation for the design of protecting groups is discussed. The effect of added nucleophile on the rate of protecting group removal for three selected N-protected phenylalanine derivatives has also been studied, and the implications of this effect in peptide synthesis are noted.

In the synthesis[2] of some large peptide fragments of ribonuclease S-protein using *tert*-butyloxycarbonyl (*t*-Boc) for temporary protection of α-amino nitrogen, substantial undesired loss of this protecting group was occasionally encountered during purification by gel filtration in 50% aqueous acetic acid. For example, after the purification of the synthetic N-terminal *t*-Boc eicosapeptide ribonuclease fragment 21–40 the loss of about 3–7% of the protecting group was demonstrated.[3] This result is consistent with the reported[4] half-life of *t*-Boc glycine ethyl ester of 10 days in 60% aqueous acetic acid at 22–25 °C, even if one considers that N-terminal *t*-Boc peptides are more stable to acid, presumably due to the fact that the terminal urethane group in N-carbamoylated peptides is less basic than that of the N-protected amino acid.[5]

To avoid undesired loss of the *t*-Boc group we undertook the search for an acid-labile protecting moiety more stable than *t*-Boc in 50% acetic acid, yet readily and completely removable by relatively mild acid treatment. At the same time we sought to avoid both introduction of a new asymmetric center and significant reduction of the solubility of the protected peptides by a new protecting group.

Some years ago Blaha and Rudinger[6] demonstrated a direct correlation of alkylcarbamate stability with the rates of ethanolysis of the corresponding *p*-toluenesulfonates. We were forced to adopt a more empirical approach, owing mainly to the lack of solvolytic rate data on derivatives of *tert*-butyl alcohol required to establish a relationship to the *t*-Boc group. Using published solvolytic data[7–11] as a rough guide to the selection of synthetic targets, we prepared and studied several derivatives of L-phenylalanine (see Chart I) in the hope that they would comprise a series of graded stability.

The alkoxycarbonyl amino acids were prepared by conversion of the corresponding alcohols into chloroformates by reaction with phosgene. The chloroformates were allowed to react with either phenylalanine in a dilute solution of sodium bicarbonate or with phenylalanine methyl ester in chloroform solution in the presence of triethylamine.

Rates of removal of the various protecting groups were compared by measuring the liberation of amino acid or dipeptide ester in either trifluoroacetic acid or formic acid. Trifluoroacetic acid was appropriate for study of the more stable protecting groups, formic acid for study of the more labile groups (see Table I).

The data suggested that the 1-methylcyclobutyloxycar-