scraped from an agar surface and added to the vegetative media. Mycelia between 9 and 12 days old proved most viable for production of the metabolite. The vegetative cultures were grown on a rotary shaker at 28 °C for 45 h. The vegetative mycelia thus prepared served as inocula for the productive media prepared above. Dextrose solutions were added separately. Cultures were grown on a rotary shaker for 120 h.

Isolation of Daunomycinone. The culture broth from the shake flasks was centrifuged with Celite at 0 °C and the solids were extracted several times with a 3:1 mixture of acetone/0.5 N HCl. The aqueous acetone solution was neutralized and the acetone removed in vacuo. The aqueous suspension remaining was immediately hydrolyzed with 0.5 N HCl (1 h; 90 °C) and the resultant suspension extracted with chloroform.

The chloroform extracts were pooled, concentrated, and chromatographed (silica gel; 1% methanol/chloroform) to yield 25–30 mg of daunomycinone from 1 L of broth. The daunomycinone thus obtained was not purified further.

Preparation of Sodium [^{13}C]Acetate Solutions and Pulsing Conditions. In 18 mL of distilled water was dissolved 1.8 g of sodium [^{13}C]acetate (1-^{13}C, 2-^{13}C, and 1,2-^{13}C for the three experiments). The solutions were sterilized and added daily to growing cultures of *S. peucetius*. Additions commenced 45 h after inoculation and continued at 12-h intervals through the 108th hour of the growth period. Each pulse consisted of a 0.5-mL aliquot of the solution and this ensured that the concentration of acetate would be <200 mg/L.

Daunomycinone Tetraacetate. The chromatographed daunomycinone (32 mg; 0.08 mmol) was dissolved in 1.0 mL (12.4 mmol) of pyridine. Acetic anhydride (1.5 mL; 15.0 mmol) was then added and the mixture was stirred at 60 °C for 3 h. The solution was quenched in ice water and extracted with chloroform. The crude product, a mixture of tri- and tetraacetates, was chromatographed on silica gel (5% methanol/benzene) to yield 31.1 mg (0.055 mmol; 68%) of the tetraacetate. This was used for the ^{13}C NMR experiments without additional purification; NMR (100 MHz; $CDCl_3$) δ 2.01 (6 H, s), 2.22 (3 H, s), 2.42 (3 H, s), 2.48 (3 H, s), 3.10 (1 H, AB quartet, J = 18 Hz), 3.96 (3 H, s), 6.34 (1 H, bd), 7.24 (1 H, dd, J = 7.5, 2 Hz), 7.59 (1 H, t, J = 7.5 Hz), 7.70 (1 H, dd, J = 7.5, 2 Hz) (The multiplet expected for H-8 is obscured by the resonances in the 2.0–2.6-ppm range, but shows in the integration.); IR (KBr) 1781, 1746, 1681, 1590, 1375, 1242, 1192, 1077, 1019 cm^{-1}; UV-vis λ_{max}(MeOH) 375 (ϵ 5960), 252 (ϵ 30 200); MS *m/e* 488 (2), 362 (50), 60 (61), 44 (42).

Registry No.—**1a,** 20830-81-3; **2a,** 476-56-2; **2b,** 18713-46-7; daunomycinone, 21794-55-8; daunomycinone tetraacetate, 32384-96-6.

References and Notes

(1) A. DiMarco, F. Arcamone, and F. Zunino in "Antibiotics", Vol. III, W. Corcoran and F. E. Hahn, Ed., Springer-Verlag, New York, N.Y., 1975, p 101.
(2) H. Seto, L. W. Cary, and M. Tanabe, *J. Chem. Soc., Chem. Commun.*, 867 (1973).
(3) A preliminary communication has appeared: R. C. Paulick, M. L. Casey, and H. W. Whitlock, *J. Am. Chem. Soc.*, **98,** 3370 (1976).
(4) S. Gatenbeck, *Acta Chem. Scand.*, **14,** 296 (1960).
(5) A preliminary communication has appeared: R. C. Paulick, M. L. Casey, D. H. Hillenbrand, and H. W. Whitlock, *J. Am. Chem. Soc.*, **97,** 5303 (1975).
(6) M. L. Casey, R. C. Paulick, and H. W. Whitlock, *J. Am. Chem. Soc.*, **98,** 2636 (1976).
(7) (a) O. A. Gansow, A. R. Burke, and W. D. Vernon, *J. Am. Chem. Soc.*, **94,** 2550 (1972); (b) S. Barcza and N. Engstrom, *ibid.*, **94,** 1762 (1972); (c) O. A. Gansow, A. R. Burke, and G. N. LaMar, *J. Chem. Soc., Chem. Commun.*, 456 (1972); (d) G. N. LaMar, *J. Am. Chem. Soc.*, **93,** 1040 (1971).
(8) B. H. Howard and H. Raistrick, *Biochem. J.*, **44,** 227 (1949).
(9) O. J. O. Sopp, *Videnskapsselsk. Skr., 1,* **11,** 161 (1912).
(10) A. Grein, C. Spalla, A. DiMarco, and G. Canevazzi, *G. Microbiol.*, 109 (1963).
(11) (a) F. Arcamone, G. Franeschi, P. Orezzi, G. Cassinelli, W. Barbieri, and R. Mondelli, *J. Am. Chem. Soc.*, **86,** 5334 (1964); (b) F. Arcamore, G. Cassinelli, P. Orezzi, G. Franceschi, and R. Mondelli, *ibid.*, **86,** 5335 (1964). (c) F. Arcamone, G. Franceschi, P. Orezzi, and S. Penco, *Tetrahedron Lett.*, 3349 (1968); (d) F. Arcamone, G. Cassinelli, G. Franceschi, and P. Orezzi, *ibid.*, 3353 (1968); (e) R. H. Iwamoto, P. Lim, and N. S. Bhacca, *ibid.*, 3891 (1968); (f) M. G. Brazhnikova, V. B. Zbarsky, D. Tresselt, and K. Eckardt, *J. Antibiot.*, **24,** 469 (1976).
(12) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972.
(13) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, N.Y., 1972.
(14) Spectra of daunomycinone tetraacetate were taken on the XL-100 spectrometer with a resolution of 1.38 Hz/point.
(15) W. D. Ollis, I. O. Sutherland, R. C. Codner, J. J. Gordon, and G. A. Miller, *Proc. Chem. Soc., London,* 347 (1960).
(16) "American Type Culture Collection Catalog of Strains", 11th ed, 1974, medium 325.
(17) C. W. Hesseltine, R. G. Benedict, and T. G. Pridham, *Ann. N.Y. Acad. Sci.*, **60,** 136 (1954).

Synthesis and Resolution of 3-Fluoro-D,L-alanine-*2-d:* A Selective Deuteration via Reductive Amination with Sodium Borodeuteride

Ulf-H. Dolling,* Alan W. Douglas, Edward J. J. Grabowski, Erwin F. Schoenewaldt, Paul Sohar, and Meyer Sletzinger

Merck Sharp & Dohme Research Laboratories, Merck & Co., Inc., Rahway, New Jersey 07065

Received November 1, 1977

3-Fluoro-D,L-alanine-2-*d* (**11**) is synthesized in aqueous (protio) ammonia from lithium fluoropyruvate (**3**) via reductive amination with sodium borodeuteride with complete retention of isotopic purity. Fluoropyruvate salts (**5**) equilibrate in 13, 6.5, and 4 M aqueous ammonia to 95:5, 85:15, and 80:20 mixtures of 3-fluoro-2,2-diaminopropionate (**8**) and 3-fluoro-2-amino-2-hydroxypropionate (**6**), respectively. The reduction of these mixtures with sodium borodeuteride to **11** and 3-fluoro-2-hydroxypropionic-2-*d* acid (**10**), a side product, is studied in detail. A mechanistic scheme is proposed in which the rate-limiting step for the formation of **10** is the reequilibration of **8** to **5,** and for the formation of **11** it is the reduction of 3-fluoro-2-iminopropionate (**7**) with sodium borodeuteride. The yield of **11** is maximized with respect to an efficient use of sodium borodeuteride. Racemic **11** is resolved via the *N*-carbobenzoxy derivative with quinine and by a continuous resolution via preferential crystallization of the benzenesulfonate salt.

3-Fluoro-D-alanine-2-*d* in combination with the 2,4-pentanedione enamine of cycloserine, sodium salt, constitutes a novel, uniquely synergistic, bactericidal antimicrobial with an unusually broad spectrum.[1] The first synthesis of 3-fluoro-D,L-alanine by fluorination of 2-phenyl-4-chloromethylene-5-azlactone, followed by hydrolysis, hydrogenation, and saponification was reported by Yuan et al.[2] Later, Lettré and Wölcke obtained the racemic amino acid by α-bromination and subsequent ammonolysis of 3-fluoropropionic acid.[3] However, neither approach can introduce deuterium selectively into the α position. Photofluorination of D-alanine-2-*d* with CF_3OF in liquid HF at −78 °C produced the first 3-flu-

0022-3263/78/1943-1634$01.00/0

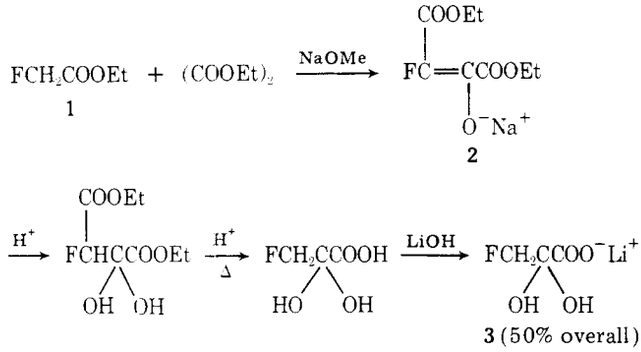
oro-D-alanine-2-*d*.[4] This process has been the only known synthesis, and its limitations in the production of larger quantities are obvious. We wish to report the reductive amination of fluoropyruvate salts in aqueous ammonia to 3-fluoro-D,L-alanine-2-*d* and the resolution of the racemate.

Although the reduction of Schiff bases with sodium borohydride,[5] sodium cyanohydroborate ($NaBH_3CN$),[6] and sulfurated borohydrides[7] is well established, this is, to our knowledge, the first report of the use of sodium borodeuteride in protio aqueous ammonia for the synthesis of isotopically pure α-deuterio amino acids. In this instance, the fluorine presents an additional synthetic hazard since the instability of the fluorine bond in Schiff base derivatives of fluoropyruvic acid to chemical and electrolytic reductions has been reported.[8] The synthesis of α-amino acids from α-keto acids in alcoholic or aqueous ammonia by catalytic hydrogenation was reported by Knoop and Oesterlin.[9] However, the synthesis of alanine from pyruvic acid failed.[9] We found that fluoropyruvic acid under similar conditions substantially defluorinated and produced alanine.

Results and Discussion

The synthesis of 3-fluoro-D-alanine-2-*d* comprises four steps: (1) synthesis of lithium fluoropyruvate, (2) its equilibration with aqueous ammonia, (3) reduction with $NaBD_4$, and (4) resolution of 3-fluoro-D,L-alanine-2-*d*.

1. Synthesis of Lithium Fluoropyruvate. Fluoropyruvic acid was prepared according to the literature procedures[10] from ethyl fluoroacetate (**1**). After hydrolysis of the intermediate ethyl ethoxallylfluoroacetate sodium salt (**2**), fluoropyruvate is conveniently isolated by precipitation of the highly insoluble lithium fluoropyruvate monohydrate (**3**). This isolation procedure avoids the low yield distillation of the acid.[10] Impurities such as oxalic acid and chloropyruvic acid can be removed by precipitation as the calcium salt and by an aqueous bicarbonate wash, respectively.[11,12] However, their removal is not essential for the subsequent steps.

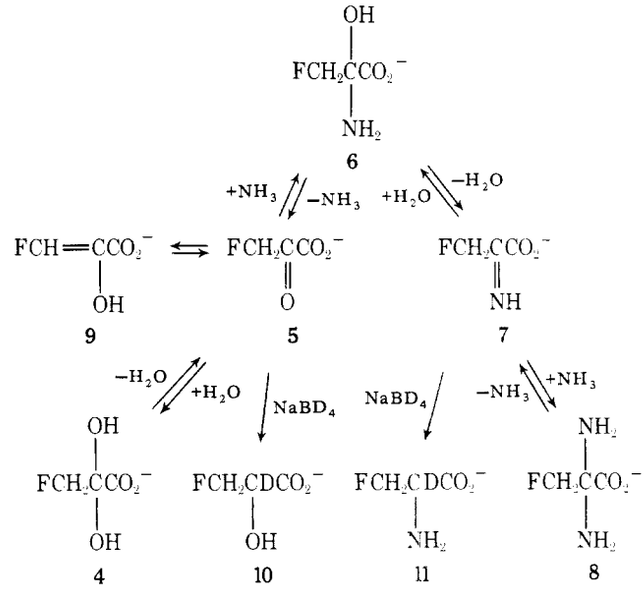


1 2 3 (50% overall)

2. Equilibration of Fluoropyruvate with Aqueous Ammonia. The proton NMR spectrum of lithium fluoropyruvate in D_2O shows two FCH_2 groups: one adjacent to an sp^3 carbon at 4.43 ppm (downfield from DSS; d, J_{HF} = 47 Hz), consistent with the *gem*-diol **4** (Scheme I), and the second adjacent to an sp^2 carbon at 5.45 ppm (d, J_{HF} = 46 Hz), consistent with the keto species **5**. The ratio of **4** to **5** in D_2O is 7:1. This ratio is pH dependent, with lower pH values favoring the keto form **5**. The spectrum of sodium fluoropyruvate in D_2O shows essentially only the diol species **4**. This is in contrast to pyruvate which shows a *gem*-diol to keto ratio of 1:19 in water at pH 7.[13] In the solid state, neither lithium pyruvate monohydrate nor sodium (or lithium) fluoropyruvate monohydrate shows a carbonyl frequency in the infrared spectrum (KBr).

A priori, a complex equilibrium mixture of fluoropyruvate in aqueous ammonia can be expected. As shown in Scheme I, diol **4** can equilibrate by the loss and addition of H_2O or NH_3

Scheme I. Equilibration and Reduction of Fluoropyruvate in Aqueous Ammonia



with the ketone **5**, aminal **6**, imine **7**, and diamine **8** species. However, when fluoropyruvate is dissolved in 13 M ammonium hydroxide, initially only one species is observed at 4.47 ppm (J_{HF} = 46 Hz). With time the intensity of the doublet decreases and only one new doublet arises at 4.42 ppm (J_{HF} = 46 Hz).

These two equilibrating species were identified by ^{13}C NMR. The proton-decoupled ^{13}C NMR spectrum of sodium fluoropyruvate in D_2O shows the C_3 carbon absorption at 86.1 ppm ($^1J_{CF}$ = 171 Hz), C_2 at 93.5 ppm ($^2J_{CF}$ = 22 Hz), and C_1 (carboxyl) at 176.1 ppm ($^3J_{CF}$ = 2 Hz). During the equilibration in 13 M ammonium hydroxide, two sets of signals are observed: that of an initial species with C_3 at 87.6 ppm ($^1J_{CF}$ = 172 Hz), C_2 at 83.5 ppm ($^2J_{CF}$ = 19 Hz), and C_1 (carboxyl) at 176.8 ppm ($^3J_{CF}$ = 3 Hz); and that of the new species with C_3 at 88.9 ppm ($^1J_{CF}$ = 173 Hz), C_2 at 70.1 ppm ($^2J_{CF}$ = 19 Hz), and C_1 (carboxyl) at 178.6 ppm ($^3J_{CF}$ = 3 Hz). The large chemical shift differences between the diol C_2 and the equilibrating initial and final C_2 carbon types suggest the equilibrating species to be the aminal **6** and the diamine **8**.[14] *gem*-Diol **4** was not detected.

To determine the structure of the intermediate unequivocally, sodium fluoropyruvate was equilibrated in 95% enriched ammonium-^{15}N hydroxide (12 M). The predicted diamine intermediate **8** is expected to show the $^{13}C_2$ doublet split into triplets by two neighboring ^{15}N's, whereas the aminal **6** would show the $^{13}C_2$ doublet split only into a doublet. The ^{13}C spectrum of the equilibrated solution shows the C_2 carbon of the dominant species at 70.1 ppm split by ^{19}F into a doublet and by ^{15}N into a triplet ($^2J_{CF}$ = 19 Hz, $^1J_{C-^{15}N}$ = 5 Hz). The C_3 carbon at 88.9 ppm shows similar splitting ($^1J_{CF}$ = 173 Hz, $^2J_{C-^{15}N}$ = 3 Hz). These data clearly identify the initial and final equilibrating species as the aminal **6** and the diamine **8**, respectively.

Concomitant with the above equilibration of **6** to **8** is the slow enolization to **9**. During the equilibration of a 0.59 M solution of lithium fluoropyruvate in 26% ND_3/D_2O for 90 min at 37 °C, the β protons slowly exchanged and the signal intensity (vs. an internal standard) decreased by approximately 35%. Subsequent reduction, isolation, and demonstration of deuterium incorporation into the β position verified the exchange and excluded decomposition as the cause of the decrease in signal intensity.

This enolization is considerably slower than that of the chloropyruvate. The high stability of the fluoropyruvate to

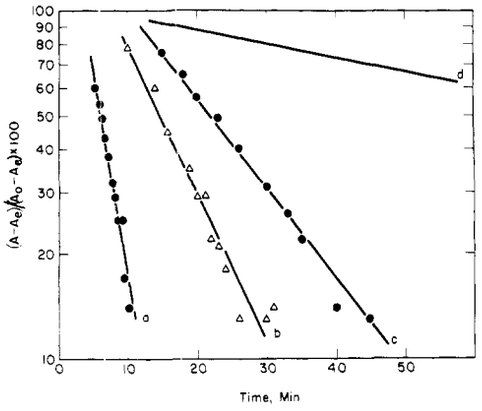


Figure 1. Semilog plot of disappearance of **6** (A = concentration at time t, A_e = concentration at equilibrium, and initial concentration, 0.59 M) in aqueous ammonia. (a) 13 M NH_3, 37 °C, 1.0 equiv of NH_4Cl; slope = −0.1264, $t_{1/2}$ = 2.4 min. (b) 6.5 M NH_3, 37 °C; slope = −0.0423, $t_{1/2}$ = 7.1 min. (c) 13 M NH_3, 37 °C; slope = −0.0259, $t_{1/2}$ = 11.6 min. (d) 13 M NH_3, 20 °C; slope = −0.00385, $t_{1/2}$ = 78 min (line extrapolated from measurements taken between 60 and 220 min).

these reaction conditions is in contrast to the instability of the chloro and bromo analogues.[12]

The equilibration of **6** and **8** in the aqueous ammonia system can be followed quantitatively by proton NMR spectroscopy. Treatment of the rate data as an equilibrium (**6** ⇄ **8**) shows that the equilibration follows pseudo-first-order kinetics. Semilog plots of $(A - A_e)/(A_0 - A_e)$ (A = concentration of **6** at time t, A_e = concentration at equilibrium, and A_0 = initial concentration) vs. time give straight lines (see Figure 1). The data are summarized in Table I. The lithium and sodium salts of **4** give identical results. At 37 °C in 13 M ammonia the half-life is 11.6 min and the equilibrium ratio of diamine **8** to aminal **6** at t = infinity is 95:5. Lowering the temperature to 20 °C increases the half-life to 78 min but does not alter the final equilibrium ratio. In 6.5 M ammonia the half-life decreases to 7.1 min and the final equilibrium ratio is lowered to 85:15. However, the use of the free acid (generation of NH_4^+) or addition of NH_4Cl catalyzes the equilibration substantially. The presence of 1 equiv of ammonium chloride decreases the half-life of **6** from 11.6 to 2.4 min at 37 °C. The ammonium ion acts as an acid catalyst and enhances the addition of NH_3.

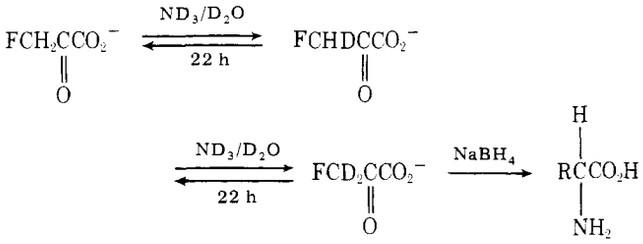
3. Reduction with $NaBD_4$: (a) Isotopic Purity. Reduction of fluoropyruvic acid with sodium borodeuteride in liquid ammonia provided **11** in 64% yield but with only 80% isotopic purity. When the sodium fluoropyruvate was equilibrated and reduced in aqueous ammonia with NH_4Cl catalysis, **11** had 96% isotopic purity, which represented a small but significant scrambling of the deuterium label. The scrambling was attributed to the presence of the NH_4^+ ions which, acting as an acid catalyst, exchanged the deuterium of $NaBD_4$ prior to the reduction. The difference in isotopic purity of both runs is enhanced by the considerably slower reduction in liquid ammonia, thereby providing ample time for exchange. In a control experiment, sodium borodeuteride, 2 N in 13 M aqueous ammonia at ambient temperature, slowly exchanged deuterium with the solvent system as observed by a small increase in the proton NMR absorptions of $NaBH_4$ after 70 h. However, the rate of exchange was so small that it had no significant effect on the faster reduction. Attempts to measure the rate of exchange in the presence of ammonium chloride were hampered by the rapid decomposition of sodium borodeuteride. $NaBD_3CN$ has also been reported to exchange with the solvent system in a more acidic medium.[6]

Equilibration of lithium or sodium fluoropyruvate in aqueous ammonia in the absence of added ammonium ions followed by borodeuteride reduction afforded **11** with no dilution of the deuterium at the α-carbon. No deuterium was detected at the β-carbon. Conversely, equilibration of lithium fluoropyruvate with ND_3/D_2O followed by borohydride reduction yielded 3-fluoroalanine with extensive deuteration on the β-carbon (by slow enolization and exchange of the fluoropyruvate and/or its imine), but with no detectable deuterium on the α-carbon.

Table I. Equilibration of 0.59 N $FCH_2C(OH)_2CO_2X$ in Aqueous Ammonia

X	Registry no.	$[NH_3]$, M	Temp, °C	Half-life, min	Ratio 8/6 ($t = \infty$)
Li		13	20	78	95:5
Li		13	37	11.2	95:5
Li[a]		13	37	2.4	95:5
Na	65120-54-9	13	37	11.6	95:5
Li	60079-40-5	6.5	37	7.1	85:15
Li		4.0	37	[b]	80:20

[a] Addition of 1 equiv of NH_4Cl. [b] Not measured.



R = FCH_2 (32%), FCHD (40%), and FCD_2 (28%)

(b) Kinetics. The species **5** and **7** (Scheme I) in the equilibrated solution can be reduced by sodium borodeuteride to 3-fluoro-2-hydroxypropionic-2-*d* acid (**10**) and 3-fluoro-D,L-alanine-2-*d* (**11**), respectively. It is apparent from Scheme I that the ratio of **10** to **11** in the final product mixture may depend on the relative rates of reduction of **5** to **10** and **7** to **11** and/or on the rate of reequilibration of **8**, **7**, and **5**. To gain some insight into the reaction and to maximize the yield and the efficient use of $NaBD_4$ the reduction was examined with respect to the concentrations of $NaBD_4$, ammonia, diamine **8**, and added salts (catalytic effects). The reaction progress was conveniently followed by proton NMR spectroscopy. The only components observed after the addition of $NaBD_4$ to an equilibrated solution were **8**, **10**, and **11** (the latter two presumably as their boron complexes). A graph of typical results is presented in Figure 2.

Effect of $NaBD_4$ Concentration. The initially present **6** was always reduced to **10** within the time period needed to take the first spectrum, whereas the disappearance of **8** was slower and could be measured. Formation of **11** increased markedly with higher concentrations of $NaBD_4$ (Figure 3; Table II, runs 1, 2, and 3), whereas the rate of formation of **10** decreased (Figure 4). If, for example, only 1 equiv of $NaBD_4$ was used, the slow reduction permitted large amounts of **8** to reequilibrate to **5** prior to reduction. These results are consistent with the following scheme: a rapid preequilibrium exists between **8** and **7**. Subsequently, the rate-limiting step for the formation of **11** is the bimolecular reduction of **7** to **11**, whereas the rate of formation of **10** is limited by the pseudo-first-order conversion of **7** to **5**.

Effect of Ammonia Concentration. In 26 M aqueous ammonia (under pressure) no reduction with $NaBH_4$ was observed. If the ammonia concentration was reduced from 13 to 6.5 M, the initial ratio of **8** to **6** was less favorable but a substantially higher reduction rate of **7** to **11** allowed less of **7** to reequilibrate to **5**, even though the rate of equilibration

Table II. Reduction of Fluoropyruvate with $NaBD_4$ in Aqueous NH_3 at 37 °C[a]

Run	$[FCH_2COCO_2Li \cdot H_2O]$, M[c]	$NaBD_4$, equiv	$[NH_3]$, M	NMR yield, %[b] 11	10	8
1	0.59	1.14	13	26	51	23
2	0.59	2.28	13	45	55	0
3	0.59	4.55	13	76	24	0
4	0.59	1.14	6.5	36	45	19
5	0.59[d]	1.14	6.5	40	41	19
6	0.59	2.28	6.5	63	37	0
7	0.67[e]	2.0	6.5	69	31	0
8	0.67[e,f]	2.0	6.5	72	28	0
9	0.67[e,g]	2.0	6.5	71	29	0
10	0.59[h]	1.7	4.0	65	35	0
11	0.295	2.28	6.5	43	57	0

[a] Fluoropyruvate was completely equilibrated in solution prior to reduction. [b] Relative yields, normalized to 100%; **11, 10, and 8** were the only detectable reaction components; isolated yields were usually within 5% of the NMR yields. [c] Based on the assayed purity of fluoropyruvate. [d] LiCl added (1.14 equiv). [e] Sodium fluoropyruvate. [f] NaCl added (2.0 equiv). [g] LiCl added (2.0 equiv). [h] LiCl added (2.3 equiv).

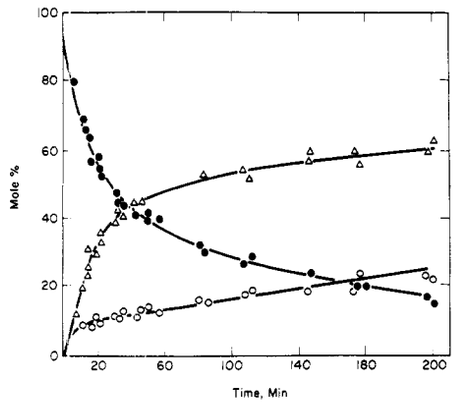


Figure 2. Reduction of an equilibrated solution of **3** in 13 M ammonia at 37 °C with 4.55 equiv of $NaBD_4$: ● = **8**, △ = **11**, and ○ = **10**.

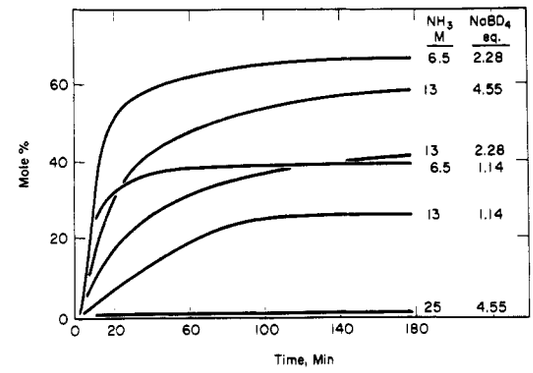


Figure 3. Formation of **11** during the reduction of equilibrated solutions of **3** with $NaBD_4$ at 37 °C. The reaction in 25 M ammonia was run under pressure with 4.55 equiv of $NaBH_4$.

increased slightly (vide supra). The net effect was a higher yield of **11** than in 13 M ammonia (Table II, runs 1, 2, and 4, 6). The reduction of imines and ketones is pH dependent,[5a] and therefore the decrease in the ammonia concentration from 13 to 6.5 M, which lowered the pH by approximately one unit, increased the imine reduction rate but apparently had a smaller effect on the reequilibration of **7** to **5**.

Effect of Concentration of 8, Salt, and Temperature. Most reduction studies were run at a standard concentration of 0.59 M fluoropyruvate (Table II). As expected from the above discussion, a decrease in concentration of **4** to 0.3 M (6.5 M NH_3, 2.3 equiv of $NaBD_4$) decreased the yield of **11** from 63 to 43% (Table II, runs 6 and 11), concomitant with an increase in the yield of **10**. On the other hand, with increasing concentrations a more rapid polymerization and/or decomposition of the equilibrating fluoropyruvate solution was noticed. Thus, solutions of 1 M concentrations rapidly turned dark. Addition of lithium chloride or sodium chloride (sodium fluoropyruvate as starting material) had little effect on the yield (runs 7, 8, and 9). This is in agreement with previous reports on aqueous $NaBH_4$ reductions.[15] Reactions in the presence of ammonium ions (NH_4Cl) were not studied further because of the marked increase in $NaBD_4$ decomposition and loss of the deuterium label. The effect of temperature on the reduction was briefly examined. At 20 °C both the reduction of **7** to **11** and the reequilibration of **7** to **5** (Table I) in 13 M ammonia are slower than at 37 °C.

(c) Comparison between $NaBD_4$ and $NaBH_4$. All of the considerations described for borodeuteride apply to borohydride, but the reaction rates are more favorable for the amino

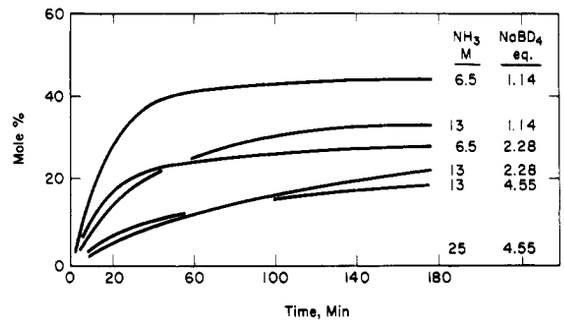


Figure 4. Formation of **10** during the reduction of equilibrated solutions of **3** with $NaBD_4$ at 37 °C. The reaction in 25 M ammonia was run under pressure with 4.55 equiv of $NaBH_4$, and **10** was not detectable.

acid synthesis with borohydride. The rate of hydrolysis of $NaBD_4$ in aqueous systems is greater than that of $NaBH_4$,[16] while the rate of reduction of the imine **7** is slower with $NaBD_4$. The slower reduction provides more time for decomposition of $NaBD_4$ and more time for reequilibration to **5**. The comparative result shown in Figure 5 is striking.

The implication for the synthesis of the protio analogue is clear: a large excess of $NaBH_4$ will essentially quench the equilibrium mixture. Thus, an equilibrated solution (13 N NH_3, 0.67 M fluoropyruvate) was reduced with 5.5 equiv of borohydride to an isolated yield of 88% of 3-fluoro-D,L-alanine.

The high cost of sodium borodeuteride does not permit the

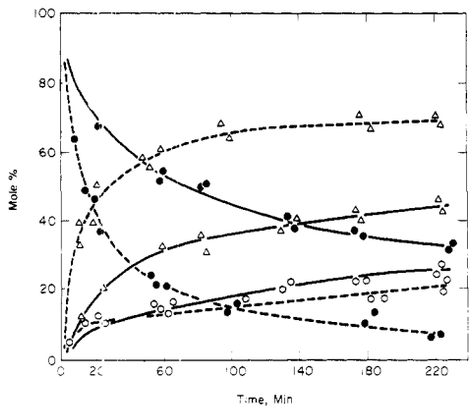


Figure 5. Comparison between $NaBD_4$ (–) and $NaBH_4$ (- - -) reductions (2.28 equiv) of equilibrated 0.59 M solutions of **3** in 13 M NH_3 at 37 °C: ● = **8**, △ = 3-fluoroalanine, and ○ = 3-fluorolactic acid (H and D).

luxury of the high excess of reducing agent as described above. Considering the data obtained from the equilibration and reduction studies, the following procedure uses $NaBD_4$ efficiently: lithium fluoropyruvate is equilibrated, at 0.67 M, with 13 M NH_4OH at 37 °C for 1.5 h (obtaining 95% conversion to **8**). The solution is cooled to 10 °C (effectively freezing the equilibrium) and treated with 1.7 equiv of $NaBD_4$. Under vacuum and with vigorous N_2 sparging, the ammonia is purged from the reaction mixture and the solution is allowed to warm from 10 to 30 °C over a 3-h period. In the ammonia-poor system the reduction proceeds rapidly, even at these temperatures. A crude yield of 70% is obtained, and after recrystallization 56% (overall) of pure **11** is obtained. The only impurities in the crude material were small amounts of alanine and serine.

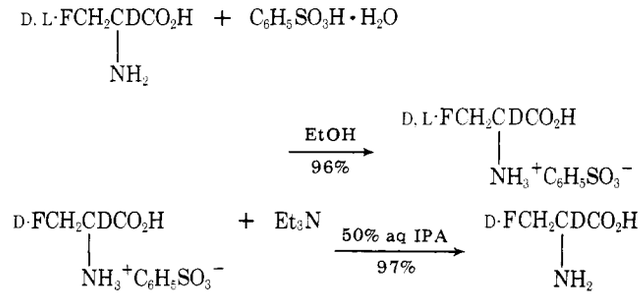
4. Resolution. Two resolutions of 3-fluoro-D,L-alanine-*2-d* were developed:[17] (a) a chemical resolution of carbobenzoxy-3-fluoroalanine-*2-d* with quinine, and (b) a continuous resolution via preferential crystallization of the benzenesulfonate salts of D- and L-3-fluoroalanine-*2-d*.[18,19]

(a) Quinine Resolution. 3-Fluoro-D,L-alanine-*2-d* was acylated under Schotten–Baumann conditions to the *N*-carbobenzoxy derivative (*N*-Cbz).[20] The *N*-Cbz-3-fluoro-D-alanine-*2-d* quinine salt crystallized from ethanol, ethyl acetate, or acetonitrile in almost complete optical purity. One recrystallization usually brought the optical purity to 99%. The L salt was crystallized from the mother liquors by the addition of acetone. After liberation of the acid, catalytic hydrogenolysis (5% Pd/C, EtOH) generated the 3-fluoro-D-alanine-*2-d* with complete retention of the label in an overall yield of 50% of theory.

(b) Continuous Resolution. A basic prerequisite for a continuous resolution by preferential crystallization is that the D,L form be a racemic mixture and not a racemic compound. The D or L isomer, therefore, will not dissolve in solutions saturated with respect to the racemic material. The first resolution based on this principle was reported by Gernez in 1866.[21] Resolutions of optical isomers by crystallization have been reviewed.[22]

3-Fluoro-D,L-alanine-*2-d* itself is a racemic compound and therefore not suitable for a continuous resolution. The benzenesulfonate salt of the D,L amino acid, however, has been found to be a racemic mixture by x-ray crystallography.[23]

The racemic salt was readily prepared from the amino acid and an excess of benzenesulfonic acid in 95% ethanol. The salt was crystallized by concentration and replacement of solvent by 1-propanol. After the resolution the amino acid was crystallized from 50% aqueous 2-propanol (IPA) by neutralization with triethylamine. Solubility and stability studies suggested 1-propanol as the solvent of choice with a solubility of 40 mg/mL at room temperature and a 16.8% supersaturation value obtainable by saturating at 28 °C and crystallizing at 23 °C.[23]



The optical purity of the resolved benzenesulfonate salt was usually 99+%. The insolubility of the optically pure salt in a solution saturated with respect to D,L permits a highly efficient purification by "slurry" in a calculated volume of 1-propanol. Occasionally, optically impure products were restored to optical purity by overnight slurrying, with recoveries of 93–99% of the theoretically obtainable pure isomer. The isomeric purity of regenerated 3-fluoro-D-alanine-*2-d* was 99.995% as measured by an enzymatic assay.[24] On a 13-kg scale the yield of optically pure 3-fluoro-D-alanine-*2-d* from benzenesulfonate salt was 85% of theory.

Experimental Section

Proton NMR spectra were measured on a Varian A-60 spectrometer, and chemical shifts are reported in parts per million downfield from 3-(trimethylsilyl)propanesulfonic acid sodium salt (TSP) or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Carbon-13 spectra were obtained with a Varian CFT-20 or XL-100 instrument, and chemical shifts were measured with dioxane as an internal standard set at 67.4 ppm relative to Me_4Si.

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Optical rotations were obtained on a Carl Zeiss photoelectric precision polarimeter (LEP A1). The rotations of $[\alpha]_D$ were obtained by extrapolation: $[\alpha]_D = [\alpha]_{546}/[1 + 1.3727([\alpha]_{546} - [\alpha]_{578})/[\alpha]_{578}]$. Mass spectra were recorded with a LKB-900 instrument and interpreted by Jack L. Smith. The isotopic purity of 3-fluoroalanine-*2-d* at C_2 and C_3 was determined by comparison of the intensity ratio *I m/e* 134:135:136 (M − 117 = M − $COOSi(CH_3)_3$) with that of *I m/e* 218:219 (M − CH_2F, − CHDF, − CD_2F) using unlabelled 3-fluoroalanine as a reference. Amino acid analyses were carried out on a Spinco-Beckman automatic amino acid analyzer. Sodium fluoropyruvate monohydrate was purchased from Calbiochem. $NaBD_4$ (98.5–99% D), ND_3/D_2O, and $^{15}NH_3$ were obtained from Merck Sharp and Dohme Canada Ltd. Ethyl fluoroacetate[25] was purchased from Fike Chemical, Inc. TLC analyses were carried out on Quanta Q1F silica gel plates with *n*-BuOH/HCO_2H/H_2O (78:14:8) (alanine, R_f 0.27; fluoroalanine, R_f 0.16; serine, R_f 0.10).

Lithium Fluoropyruvate Hydrate (3). A 2-L flask equipped with a stirrer, thermometer, N_2 inlet, dropping funnel, and condenser was charged with 440 mL of anhydrous ethyl ether and 54 g (1 mol) of fresh sodium methoxide in a N_2 atmosphere. To the well-stirred suspension was added 204 mL (1.5 mol) of freshly distilled diethyl oxalate dropwise over 0.5 h. A slight exotherm (T = 34 °C) was noted, but no cooling was required. Ethyl fluoroacetate[25] (106.1 g, 1 mol) was added to the almost clear yellow solution over a period of 2.5 h. A precipitate of the ethyl ethoxallylfluoroacetate sodium salt appeared near the midpoint of the addition. After aging overnight the mixture was filtered, and the product was washed with 2 × 800 mL of ether and 200 mL of hexane and dried in the air, yield 138 g (60.5%).

A mixture of 400 mL of ethyl ether and 240 mL of 5 N HCl was chilled to −15 to −20 °C in a flask equipped with good mechanical stirring, a powder funnel, thermometer, and N_2 inlet. With good stirring under a N_2 atmosphere, 138 g of sodium enolate, free of lumps, was added in a steady stream such that the temperature did not rise above −15 °C. (Too strenuous cooling may freeze the aqueous layer in the flask.) When addition was complete, the mixture was warmed to room temperature, diluted with 250 mL of water, and heated at atmospheric pressure to distill out the ether. Heating and distillation was continued until the flask temperature reached 102–105 °C. After

refluxing for 4 h, the resulting solution was cooled to room temperature, stirred with 6 g of Darco G-60, and filtered through (acid prewashed) Supercel using a minimum of water as wash. The filtrate was neutralized (ice cooling, pH meter) by addition of solid $LiOH \cdot H_2O$ (ca. 47 g) to a final pH of 6.0–6.5. The slurry was aged at 0–5 °C overnight and filtered, and the filter cake was washed with a minimum amount of cold water and then with 2 × 200 mL of methanol and 2 × 200 mL of acetone. The product was air-dried, yield 56 g (70%). The lithium fluoropyruvate monohydrate thus obtained was approximately 80% pure, the major impurities being lithium oxalate and lithium chloropyruvate. The purity was assayed by NMR spectroscopy in 3.5 N HCl with DSS as an internal standard.

Fluoropyruvic Acid. Lithium fluoropyruvate monohydrate (16.5 g, 0.127 mol) was dissolved in 700 mL of water, passed through 100 mL of Dowex W50-X8 (H^+), and eluted with water. The acidic fractions were concentrated on a rotary evaporator (20 mm; bath temperature, 30–40 °C) to an oil. The oil was dissolved in 400 mL of ether/CH_2Cl_2 (1:1). After concentration to a semisolid, the flushing was repeated to yield 15.3 g (91%) of a waxy solid containing 19.6% water as measured by Karl Fischer analysis. Anhydrous fluoropyruvic acid (which is extremely hygroscopic) may be obtained by freeze-drying of an aqueous solution of the acid, but this operation is accompanied by substantial losses due to sublimation.

3-Fluoro-D,L-alanine-2-d. Lithium fluoropyruvate hydrate (26 g, 0.2 mol) was added with good agitation to 300 mL of 13 M aqueous ammonia (0.67 N in pyruvate) at room temperature. The slurry was equilibrated at 37 °C for 1.5 h to a clear yellow solution, cooled to 10 °C, and treated with 3.57 g (0.085 mol, 1.7 equiv) of $NaBD_4$. Under vacuum and with vigorous N_2 sparging, the ammonia was purged from the reaction mixture as the bath was allowed to warm from 10 to 30 °C over a 3-h period. Residual ammonia was removed by concentration on a rotary evaporator. The solution was chilled in an ice bath, stirred, and acidified by the addition of ca. 165 mL of 2.5 N HCl (0.41 mol). The acidified solution was stirred at room temperature with 1.3 g of Darco G-60 for 15 min, filtered, and passed through a column of 400 mL of Dowex 50W-X8 (H^+). The column was washed with water until the eluate was neutral. The initial fractions contained **10**,[26] which was identified by its NMR spectrum. The amino acid **11** was eluted with 0.5 N NH_4OH. The ninhydrin-active fractions were combined and freeze-dried to give 15 g (70%) of crude **11** containing small amounts of deuterated alanine (<0.5%) and serine (1.1%). The product recrystallized from 70 mL of H_2O at 60 °C by the addition of preheated 2-propanol (ca. 50 mL), aging in an ice bath for 2 h, and filtering, and it was washed with 2 × 20 mL of cold 90% aqueous 2-propanol, 2 × 10 mL of 2-propanol, and hexane. The product was dried to a constant weight in vacuo at 50–60 °C, yield 12.0 g (80% recovery). The ^1H and ^{19}F NMR spectra were identical with the reported spectra of 3-fluoro-D-alanine-2-d.[4a] The mass spectrum showed an isotopic purity of 99%. No deuterium was detectable at C_3. Anal. Calcd for $C_3H_5DFNO_2$: C, 33.34; H, 5.60; N, 12.96; F, 17.58. Found: C, 33.55; H, 5.68; N, 13.09; F, 17.64.

NMR Studies: (a) Equilibrations. Aqueous ammonia of the desired concentration was pipetted onto the weighed amounts of fluoropyruvate and salts. As soon as the solid was dissolved, the solution was filtered through a glass wool plug into an NMR tube. This procedure usually took about 2–3 min, and the first spectrum was obtained within 5 min. The reaction rates were measured by peak heights normalized relative to an internal standard (TSP).

(b) Reductions. Fully equilibrated stock solutions of fluoropyruvate and ammonia were prepared. Aliquots were pipetted onto $NaBD_4$ (or $NaBH_4$) and, where desired, salts. Further preparation and measurements were carried out as above (a).

Reduction in Liquid Ammonia. Fluoropyruvic acid (0.34 g, 3.2 mmol) was added to 40 mL of liquid ammonia in a glass-lined bomb. To the colorless solution 70 mg (1.67 mmol) of $NaBD_4$ was added. After shaking for 40 h at room temperature, the ammonia was evaporated with a slow stream of nitrogen. The residue was taken up in 10 mL of MeOH and concentrated to dryness. The solid was dissolved in 4 mL of 1 N HCl containing 10 g of ice. After dilution to 50 mL, **11** was isolated by ion exchange on Dowex 50W-X8 as described above. Freeze-drying yielded 0.22 g (64%) of **11**, which contained by mass spectral analysis 20% of the protio analogue.

Reduction in ND_3/D_2O with $NaBH_4$. Lithium fluoropyruvate monohydrate (35 mg, 0.269 mmol) was dissolved in 0.50 mL of 26% ND_3/D_2O (TSP as an internal standard). The equilibration was followed by NMR spectroscopy (37 °C). During the first 1.5 h the signal intensity (relative to TSP) of the β protons decreased by 35% due to exchange. The solution was allowed to stand at room temperature for 20 h. The mixture was reduced with 7.8 mg (0.206 mmol, 3 equiv) of $NaBH_4$ at 37 °C. After the reduction was completed (20 min by NMR spectroscopy), the solution was acidified with DCl and diluted with H_2O to ~30 mL. 3-Fluoroalanine was again isolated by ion exchange and freeze-drying, yield 19 mg (66%). Mass spectral analysis showed no deuterium in the α position but 40% mono- and 28% dideuteration in the β position.

3-Fluoro-D,L-alanine-^{15}N. Ammonia-^{15}N (100 mL, 4.46 mmol, 95.9 atom % ^{15}N) was condensed at liquid nitrogen temperature, 0.37 mL of H_2O was added, and the solid was allowed to warm to 0 °C. In this solution 32 mg (0.22 mmol) of sodium fluoropyruvate monohydrate was dissolved and equilibrated. The intermediate diamine **8** was observed by ^{13}C NMR spectroscopy (see Results and Discussion). After reduction with 5 equiv of $NaBH_4$ and usual work up (see above), 8 mg (34%) of 3-fluoro-D,L-alanine-^{15}N was isolated and identified by its ^{13}C NMR spectrum: carboxyl C_1 at 171.4 ppm ($^3J_{CF}$ = 6.0 Hz), C_2 at 55.8 ppm ($^2J_{CF}$ = 19.8 Hz, $J_{C-^{15}N}$ = 6.4 Hz), and C_3 at 82.9 ppm ($^1J_{CF}$ = 169 Hz).

***N*-Carbobenzoxy-3-fluoro-D,L-alanine-2-d.**[20] A 500-mL three-neck round-bottom flask fitted with a mechanical stirrer, pH probe, and two dropping funnels, one for benzyl chloroformate and the other for 2.5 N NaOH, was charged with 10.7 g (0.10 mol) of **11** in 107 mL of H_2O. The reaction mixture was cooled to 0–5 °C, and pH was adjusted to 10.5–11.0 with approximately 30 mL of 2.5 N NaOH, and 33.2 g (0.198 mol) of benzyl chloroformate was added over a period of 1 h, keeping the pH between 10.5–11.0 by the addition of NaOH (60 mL was required). After the pH drift ceased, the aqueous solution was extracted with 2 × 100 mL of ethyl acetate, acidified to pH 2 in the cold with 2.5 N HCl (39.3 mL), and extracted with 3 × 100 mL of ethyl acetate. The combined ethyl acetate solutions were dried over $MgSO_4$, filtered, and concentrated to dryness. The crude material was dissolved in 3 L of CCl_4 at reflux. After concentration to 250 mL, the crystallized product was filtered off, washed with CCl_4, and dried overnight at 0.1 mm over P_2O_5: yield 23.4 g (97%); mp 112–113 °C. The NMR spectrum was identical with that of an authentic sample.[20]

Resolution with Quinine. *N*-Cbz-3-fluoro-D,L-alanine-2-d (3.62 g, 15 mmol) and quinine (4.87 g, 15 mmol) were dissolved in 6 mL of warm ethanol[27] and allowed to crystallize slowly overnight. The crystals were filtered, washed with cold ethanol, and dried at 65 °C (0.1 mm) to give 2.65 g (62%) with mp 144–145 °C. This material was dissolved in 3 mL of hot ethanol, concentrated slightly, and allowed to crystallize (ice cooling). After filtration, washing, and drying, 2.32 g (55%), mp 150–151 °C, was isolated. The quinine salt was distributed between 20 mL of 1 N HCl and 30 mL of ethyl acetate; the organic phase was washed with 3 × 15 mL of 0.5 N HCl. The organic solution was dried over $MgSO_4$ and concentrated to dryness. Recrystallization from 12 mL of wet CCl_4 yielded 912 mg (50%) of the *N*-Cbz derivative with $[\alpha]^{20}_D$ −4.35° (*c* 11, EtOH). The reference compound prepared from authentic 3-fluoro-D-alanine-2-d[20] showed a rotation of $[\alpha]^{20}_D$ −4.46° (*c* 11, EtOH).

Hydrogenolysis as described below on a larger scale yielded 390 mg (96%) of 3-fluoro-D-alanine-2-d with a rotation of $[\alpha]^{20}_D$ −10.16° (*c* 6, 1.0 N HCl). A reference sample[20] had, under identical conditions, a rotation of $[\alpha]^{20}_D$ −10.4° (lit. $[\alpha]^{20}_D$ −10.0°,[4a] $[\alpha]^{25}_D$ −10.4°[17]). The ^1H NMR spectrum agreed with the reported one.[4a] Anal. Calcd for $C_3H_5DFNO_2$: C, 33.34; N, 12.96; F, 17.58. Found: C, 33.21; N, 12.75; F, 17.85.

Hydrogenolysis of *N*-Carbobenzoxy-3-fluoro-D-alanine-2-d. Resolved *N*-Cbz-3-fluoro-D-alanine-2-d (22.34 g, 0.092 mol) was hydrogenated for 3 h in 150 mL of MeOH over 2 g of 10% Pd/C at 40 psig and ambient temperature. The mixture was diluted with 250 mL of H_2O and partially concentrated in vacuo. After filtration the filtrate was evaporated to dryness. The white crystalline mass was triturated with 2-propanol, filtered off, washed with hexane, and air-dried: yield 9.31 g (93.5%); $[\alpha]^{20}_D$ −10.36° (*c* 6, 1.0 N HCl). The product gave satisfactory analyses.

3-Fluoro-D,L-alanine-2-d Benzenesulfonate. To a solution of benzenesulfonic acid hydrate (363 g, 2.06 mol, 110% of theory) in 3 L of 95% ethanol was added **11** (201.87 g, 1.87 mol). An additional 1.7 L of 95% ethanol was added as needed to effect solution at room temperature. The clear brownish solution was decolorized by stirring with 53 g of activated charcoal for 1 h. After filtration (Supercel) and washing with 95% ethanol, the pale yellow filtrate was concentrated in vacuo at an internal temperature of ≤25 °C to a heavy crystalline slurry of a total volume of 900 mL. The slurry was stirred as 900 mL of ethyl ether was added over 0.5 h. After stirring for an additional hour, the mixture was filtered and washed with 500 mL of 3:1 ether/ethanol and then with 500 mL of ether. The filtrate and washings were removed, and the cake was further washed liberally with hexane. This first crop, after air-drying, weighed 420.35 g (84.5%).

The mother liquor and washings were combined and concentrated in vacuo (internal temperature ≤25 °C) to a volume of ca. 250 mL.

1-Propanol (250 mL) was added, and the mixture was reconcentrated to 250 mL. This flushing (to remove H_2O) was repeated. To the heavy crystalline slurry 250 mL of ethyl ether was added. The mixture was filtered, and the cake was washed with 400 mL of 3:1 ether/1-propanol, then with 250-mL of ethyl ether, and then liberally with hexane. Air-drying provided an additional 57.07 g (11.5%; total yield 96%) of product: ^1H NMR (D_2O) 7.7 (m, 5 H), 4.99 ppm (ABX pattern, J_{HF} = 46 Hz, 2 H). Anal. Calcd for $C_9H_{11}DNFSO_5$: C, 40.60; H, 4.54; N, 5.26; F, 7.14; S, 12.04. Found: C, 40.74; h, 4.63; N, 5.05; F, 7.42; S, 12.03.

Continuous Resolution.[28] A stirred saturated solution (1-propanol) of 3-fluoro-D,L-alanine-*2-d* benzenesulfonate salt with excess suspended salt was maintained at 28 °C in a dissolver. The solution was drawn through filter candles by a pump and pushed through a gas disengagement device, a pressure relief valve, a flow meter, a pressure gauge, and a heat exchanger to lower the temperature to that of the crystallizer (e.g., 23 °C). The solution was jetted into the L-seeded column, which was maintained as a fluidized bed. The supernatant liquid was rewarmed in a heat exchanger, passed through a filter, recooled to the crystallizer temperature, jetted into the D-seeded column, and recycled to the dissolver through a prewarmer.

At the end of the crystal growth period, the L and D isomers were filtered, washed with a minimum of 1-propanol and hexane, and dried at RT (20 mm). In a typical run with a temperature differential of 5 °C, corresponding to 16.8% supersaturation, a flow rate of 400–600 mL/min, a growth period of 8.4 h, and 100 g of seed of each isomer (80–140 mesh), a net yield of 323 g of D isomer and 330 g of L isomer was obtained. ^1H NMR (D or L isomer) (37% DCl) 7.7 ppm (m, 5 H), 5.1 (J_{HF} = 46 Hz, 2 H). Isotopic purity by mass spectral analysis: D isomer, 98.6% D; L isomer, 98.6% D. Optical purity ($CuSO_4$ buffer; see below): D isomer, $[\alpha]^{20}_{436}$ +68.4°; L isomer, $[\alpha]^{20}_{436}$ −68.7°. Anal. Calcd for $C_9H_{11}DNFSO_5$: C, 40.60; H, 4.54; N, 5.26; F, 7.14; S, 12.04. Found: (D isomer) C, 40.67; H, 4.67; N, 5.49; F, 7.41; S, 12.08; ash, none. Found: (L isomer) C, 40.42; H, 4.54; N, 5.25; F, 6.88; S, 12.22; ash, none.

3-Fluoro-D-alanine-*2-d*: Reversal of the Benzenesulfonate. The benzenesulfonate salt of 3-fluoro-D-alanine-*2-d* (10.0 g, 37.6 mol) was dissolved with stirring in 30 mL of 50% aqueous 2-propanol at room temperature. The clear colorless solution was cooled with stirring in an ice bath and treated dropwise with triethylamine (3.81 g, 37.6 mol). The slurry was stirred in the cold for 0.5 h, filtered, and washed with 50 mL of cold 2-propanol/water (9:1), 50 mL of 2-propanol, and then liberally with hexane. The product was vacuum-dried at room temperature, yield 3.84 g (97%). The ^1H and ^{19}F NMR spectra were identical with those reported previously.[4a] Isotopic purity by mass spectral analysis: 98.8% D. Optical purity: $[\alpha]^{20}_D$ −10.4° (*c* 6, 1.0 N HCl). Spinco analysis: 9.36 μmol/mg; no other amino acids were detected. Titration (NaOH) 100%, equivalent weight 108.1. Anal. Calcd for $C_3H_5DFNO_2$: C, 33.34; H, 5.60; N, 12.96. Found: C, 33.28; H, 5.56; N, 12.84.

3-Fluoro-L-alanine *2-d* was regenerated by the same procedure. The ^1H and ^{19}F NMR spectra were identical to those of the D isomer. Isotopic purity by mass spectra analysis: 99.0% D. Optical purity: $[\alpha]^{20}_D$ +10.4° (*c* 6, 1.0 N HCl). Spinco analysis: 9.34 μmol/mg; trace of alanine and ammonia, but no other amino acids were detected. Titration (NaOH) 99.9%, equivalent weight 108.0. Anal. Calcd for $C_3H_5DFNO_2$: C, 33.34; H, 5.60; N, 12.96. Found: C, 33.42; H, 5.65; N, 12.74.

Purification of 3-Fluoro-D-alanine-*2-d* Benzenesulfonate via 1-Propanol Slurry. The slurry was based on a solubility of 36 mg for the racemic salt in 1 mL of 1-propanol at 23 °C. The effectiveness of the method was demonstrated by the slurry of an optically impure sample. A slurry of 66.9 g (optical purity 64.6%) of 3-fluoro-D-alanine-*2-d* benzenesulfonate in 707 mL (calcd 658 mL) of 1-propanol was stirred overnight at 23 °C. After filtration, washing with a minimum of 1-propanol and hexane, and drying, 42.7 g of material was recovered (98.8% of theory): $[\alpha]_{365}$ +122.5°; enzymatic assay, isomeric purity >99.995%.

The optical rotations were measured in copper sulfate/acetic acid/sodium acetate buffer solutions (4.0 g of anhydrous NaOAc, 10.0 mL of glacial acetic acid, and 13.5 g of $CuSO_4$ in 100 mL of aqueous solution). The specific rotations for the optically pure compound 11, $[\alpha]_{365}$ +310.8° (*c* 1), $[\alpha]_{405}$ +217.5°, and $[\alpha]_{436}$ +174.0°, and the benzenesulfonate salt of 11 (24.75 mg/mL of solution), $[\alpha]_{365}$ +122.5°, $[\alpha]_{405}$ +85.6°, and $[\alpha]_{436}$ +68.5°, are within a reproducibility of ±0.5.

Acknowledgment. We thank Robert A. Reamer of our NMR laboratory group for skillfully and patiently obtaining most of the NMR reaction-rate measurements.

Registry No.—10, 65120-55-0; D,L-**11,** 59189-03-6; diethyl oxalate, 95-92-1; ethyl fluoroacetate, 459-72-3; ethyl ethoxallylfluoroacetate Na salt, 7582-61-8; fluoropyruvic acid, 433-48-7; *N*-Cbz-3-fluoro-D,L-alanine-*2-d*, 65120-56-1; benzyl chloroformate, 201-53-1; quinine, 130-95-0; *N*-Cbz-3-fluoro-D-alanine-*2-d* quinine salt, 65120-58-3; *N*-Cbz-3-fluoro-D-alanine-*2-d*, 65120-57-2; 3-fluoro-D,L-alanine-*2-d* benzenesulfonate, 59189-04-7; 3-fluoro-D-alanine-*2-d* benzenesulfonate, 59189-07-0; 3-fluoro-L-alanine-*2-d* benzenesulfonate, 59189-06-9; 3-fluoro-D-alanine-*2-d*, 35523-45-6; 3-fluoro-L-alanine-*2-d*, 59189-05-8; **5,** 59769-04-9; **6,** 65120-59-4; **8,** 65120-60-7; 3-fluoro-D,L-alanine-^{15}N, 65149-95-3.

References and Notes

(1) (a) F. M. Kahan and H. Kropp, Abstracts, International Conference on Antimicrobiol Agents and Chemotherapy, Washington, D.C., Sept 1975, No. 100; (b) H. Kropp, F. M. Kahan, and H. B. Woodruff, *ibid.*, No. 101; (c) J. Kollonitsch, L. Barash, N. P. Jensen, F. M. Kahan, S. Marburg, L. Perkins, S. M. Miller, and T. Y. Shen, *ibid.*, No. 102; (d) F. M. Kahan, H. Kropp, H. R. Onishi, and D. P. Jacobus, *ibid.*, No. 103.

(2) C. Y. Yuan, C. N. Chang, and Y. F. Yeh, *Yao Hsueh Hsueh Pao,* **7,** 237 (1959); *Chem. Abstr.,* **54,** 12096 (1960).

(3) (a) H. Lettré and U. Wölcke, *Justus Liebigs Ann. Chem.,* **708,** 75 (1967); (b) H. Gershon, M. W. McNeil, and E. D. Bergmann, *J. Med. Chem.,* **16,** 1407 (1973).

(4) (a) J. Kollonitsch, L. Barash, F. M. Kahan, and H. Kropp, *Nature (London),* **243,** 346 (1973); J. Kollonitsch and L. Barash, *J. Am. Chem. Soc.,* **98,** 5591 (1976); J. Kollonitsch and F. M. Kahan, U.S. Patent 4 028 405. (b) Recently two further syntheses were developed: J. Kollonitsch, S. Marburg, and L. M. Perkins, *J. Org. Chem.,* **40,** 3808 (1975); **41,** 3107 (1976).

(5) (a) E. Schenker in "Newer Methods of Preparative Organic Chemistry", Vol. IV, Verlag Chemie, Weinheim/Bergstr., Germany, 1968. (b) K. Harada and J. Or-Hashi, *Bull. Chem. Soc. Jpn.,* **43,** 960 (1970). (c) Japanese Patent Publication 6884, 1963.

(6) R. F. Borch, M. D. Bernstein, and M. D. Durst, *J. Am. Chem. Soc.,* **93,** 2897 (1971).

(7) J. M. Lalancette and J. R. Brindle, *Can. J. Chem.,* **48,** 735 (1970).

(8) *Carbon Fluorine Compd: Chem., Biochem., Biol. Act., Ciba Found. Symp., 1971 (1972),* 157–159 (1972).

(9) F. Knoop and H. Oesterlin, *Hoppe-Seyler's Z. Physiol. Chem.,* **148,** 294 (1925); **170,** 186 (1927).

(10) (a) I. Blank, J. Mager, and E. D. Bergmann, *J. Chem. Soc.,* 2190 (1955); (b) P. V. Nair and H. Busch, *J. Org. Chem.,* **23,** 137 (1958).

(11) We are indebted to R. C. Zerfing for identification of chloropyruvic acid in the product mixture and for development of the oxalate removal procedure.

(12) (a) D. B. Epinson and E. Chargaff, *J. Biol. Chem.,* **164,** 417, 433 (1946); (b) M. Garino, A. Cereseto, M. Berni, and M. Brambilla, *Gazz. Chim. Ital.,* **60,** 582 (1930); (c) H. Busch and P. V. Nair, *J. Biol. Chem.,* **229,** 377 (1957).

(13) M. Becker, *Ber. Bunsenges. Phys. Chem.,* **68,** 669 (1964).

(14) J. B. Stothers, "Carbon-13 NMR Spectroscopy, Organic Chemical Monographs", Vol. 24, Academic Press, New York and London, 1972, Chapter 5.

(15) H. O. House, "Modern Synthetic Reactions", W. A. Benjamin, Menlo Park, Calif., 1972, p 51ff.

(16) R. E. Davis, E. Bromels, and C. L. Kilby, *J. Am. Chem. Soc.,* **84,** 885 (1962); J. A. Gardiner and J. W. Collat, *Ibid.,* **87,** 1692 (1965).

(17) Dr. G. Gal et al. of these laboratories applied an elegant new method for the resolution of amino acids successfully to **11:** G. Gal, J. M. Chemerda, D. F. Reinhold, and R. M. Purick, *J. Org. Chem.,* **42,** 142 (1977).

(18) Dr. D. F. Reinhold suggested this resolution. U.S. Patent pending.

(19) S. Yamada, m. Yamamoto, and I. Chibata, *J. Org. Chem.,* **38,** 4408 (1973).

(20) We are indebted to Dr. J. Kollonitsch not only for providing us with pure samples of D- and L-3-fluoroalanine-*2-d* but also for many helpful discussions, including experimental details for a high-yielding preparation of the *N*-Cbz derivative.

(21) D. Gernez, *C. R. Hebd. Seances Acad. Sci.,* **63,** 843 (1866); *Justus Liebigs Ann. Chem.,* **143,** 376 (1867).

(22) R. M. Secor, *Chem. Rev.,* **63,** 297 (1963); G. B. Kauffman and R. D. Myers, *J. Chem. Educ.,* **52,** 777 (1975). For applications, see K. H. Krieger, J. Lago, and J. A. Wantuck, U. S. Patent 3 405 159, and M. Midler, Jr., U.S. Patents 3 510 266 and 3 892 539.

(23) Solubility and stability studies were carried out by Dr. G. B. Smith. X ray analyses were performed by Dr. J. A. McCauley.

(24) The enzymatic assay was developed by F. M. Kahan and H. Kropp, who kindly supplied us with the experimental details.

(25) *Caution!* Ethyl fluoroacetate is very toxic. The LD_{50} for man is 2–3 mg/kg; it may be absorbed through the skin and by inhalation. All operations should be conducted with gloves in a well-ventilated hood.

(26) L. K. Gottwald and E. Kun, *J. Org. Chem.,* **30,** 877 (1965).

(27) Ethyl acetate works equally well. The other isomer can be crystallized from the mother liquors, after removal of solvent, with acetone.

(28) We gratefully acknowledge the valuable assistance of Dr. M. Midler who provided many helpful suggestions (and various equipment parts) and of Dr. H. Kao for his participation with us in the apparatus design.