A VERSATILE SYNTHESIS OF C-5 LABELED ORNITHINES

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SUMMARY

Using diethyl (2-cyanoethyl)acetamidomalonate prepared from either acrylonitrile or 3-tosylpropionitrile and diethyl acetamidomalonate, reduction with PtO_2 and H_2 in ethanol followed by acid hydrolysis affords ornithine in 70-95% yield. [5,5-$^2H_2$]Ornithine and [5-^{13}C,5-^{15}N]ornithine were prepared by these procedures.

Key Words: [5,5-$^2H_2$]Ornithine, [5-^{13}C,5-^{15}N]Ornithine, Michael Addition, ^{2}H-Labeling, ^{13}C-Labeling, ^{15}N-Labeling.

INTRODUCTION

In the course of our biosynthetic studies on the microbial antibiotics streptothricin F (2) and naphthyridinomycin (3), a synthesis of ornithine, 1, was needed that permitted labeling at C-5 with ^{13}C, ^{15}N, and ^{2}H. Of the known syntheses of 1 (4), we found that a modification of the route of Albertson (4g) could be utilized to provide C-5 labeled ornithines in excellent yields.

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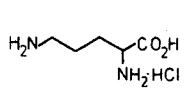
RESULTS AND DISCUSSION

A novel and potentially efficient approach to 1 entailed the nucleophilic displacement by cyanide of a leaving group (X^-) from a homoserine derivative 2 followed by catalytic hydrogenation. To test this, racemic N-[(benzyloxy)carbonyl]-O-tosylhomoserine p-nitrobenzyl ester, 2a, (5) was prepared. However, it proved unreactive with NaCN or KCN in DMSO or DMF, or under the conditions of phase transfer catalysis (6). Benzyl ester 2b (7) as well as bromide 2c and protected lactone 3 were also found to be poor substrates under similar conditions. Although erratic, the best yield (58%) was obtained for nitrile 2d from bromide 2c.



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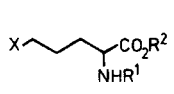
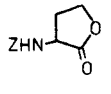


2a: R^1 = Z, R^2 = pNB, X = OTs
2b: R^1 = Z, R^2 = Bn, X = OTs
2c: R^1 = Z, R^2 = pNB, X = Br
2d: R^1 = Z, R^2 = pNB, X = CN



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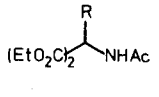
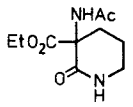
Z = $COOCH_2C_6H_5$, pNB = p-$NO_2C_6H_4CH_2$, Bn = $CH_2C_6H_5$

A substantially higher overall yield was obtained from the Michael Addition of diethyl acetamidomalonate, 4, to acrylonitrile, 5. This route has the added advantage that several syntheses of



4: R = H
6: R = CH_2CH_2CN



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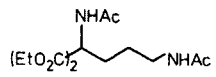

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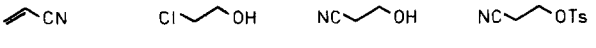
[1-^{13}C]acrylonitrile are available (9). Our initial investigations produced diethyl (2-cyanoethyl)acetamidomalonate, 6, (10) in 96.5% yield, after which hydrogenation over PtO_2 in glacial acetic acid (11) afforded 39.6% of lactam 7. Whereas Raney nickel at high pressure had previously been employed, it was found that reduction

with platinum at one atmosphere was sufficient, and was now used in anticipation of the labeled synthesis to conserve expensive deuterium gas. Reduction in acetic anhydride (12) yielded only 48.3% of the bis-acetamide 8, and hydrolysis then provided a 76.9% yield of ornithine. However, reduction in ethanol (2) afforded a hygroscopic residue, which was hydrolyzed to 1 in 98.4% overall yield. Nitrile 6 was next reduced in ethanol-d_1 with deuterium gas (13). This resulted in a 97.5% yield of [5,5-$^2H_2$]ornithine, 1a, after hydrolysis, with 96.5% enrichment in deuterium at C-5 (14).

Using the route of Naito (9b), reaction of cyanide with 2-chloroethanol, 9, gave nitrile 10 in 65-70% yield, but subsequent dehydration over $MgCO_3$ only produced acrylonitrile in



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poor yield, probably due to the necessarily small-scale reaction employed. However, reaction of the tosylate 11 derived from 10 (15) (65-70% yield), with 4 in liquid ammonia gave 6 in 70% yield after chromatography. It is not clear whether this reaction is an S_N2 displacement of tosylate or a Michael reaction after *in situ* elimination to acrylonitrile, but direct displacement of tosylate failed using diethyl acetamidomalonate and either sodium hydride in benzene or sodium methoxide in methanol.

The successful route, used with sodium [^{13}C,^{15}N]cyanide, afforded nitrile 10a in 66% yield, tosylate 11a in 70% yield, the cyanoethylated adduct 6a in 70% yield, and [5-^{13}C,5-^{15}N]ornithine, 1b, in 99.7% yield.

EXPERIMENTAL

^1H NMR spectra were taken at either 80 or 400.13 MHz on Varian FT-80A and Bruker AM 400 spectrometers, respectively, while ^{13}C NMR spectra were obtained at either 20 or 100.61 MHz on these same instruments. IR spectra were obtained on a Perkin-Elmer 727B infrared spectrophotometer and mass spectra were taken on a Varian MAT CH7 spectrometer with a System Industries 150 data system. Melting points were obtained on a Buchi apparatus and are uncorrected. All solvents were distilled prior to use. All preparative flash chromatography was carried out with E. Merck, 230-400 mesh, 9385 silica gel 60. Thin layer chromatography was done on either EM reagents 5729-6 silica gel 60 F-254 glass-backed, or Eastman 13254 cellulose plastic-backed plates. Ethanol-d_1 and sodium [^{13}C,^{15}N]cyanide were purchased from MSD Isotopes, D_2 gas from Matheson, and 30% DCl in D_2O from Stohler Isotope Chemicals.

p-Nitrobenzyl 2-[N-(Benzyloxy)carbonyl]amino-4-bromobutanoate 2c by the procedure adapted from Hooz and Gilani (16): p-Nitrobenzyl 2-[N-(Benzyloxyl)carbonyl]amino-4-bromobutanoate (5) (998 mg, 2.65 mmol) and carbon tetrabromide (1.766 g, 5.32 mmol) were dissolved in 60 mL dichloromethane (distilled from P_2O_5). To this stirred solution was added a solution of triphenylphosphine (1.407 g, 5.36 mmol) in 20 mL dichloromethane over 0.5 h. After the addtion was complete, the reaction was continued 15 min more. Purification by flash chromatography (7:3 pet ether:ethyl acetate, 3 x 20 cm column) afforded 843 mg (70.5%) of the desired bromide (TLC, pet ether:ethyl acetate=7:3 on silica gel 60 F-254, R_f = 0.32): mp 86-87°C; IR ($CHCl_3$) 3450, 1745, 1730, 1610, 1500, 1340 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz FT) δ 8.21 (d, 2H, J = 8.8 Hz); 7.49 (d, 2H, J = 8.8 Hz), 7.34 (s, 5H), 5.3 (m, 3H), 5.12 (s, 2H), 4.57 (m, 1H), 3.43

(t, 2H, J = 6.8 Hz), 2.37 (m, 2H); ^{13}C NMR ($CDCl_3$, 20 MHz FT) δ 171.19, 142.14, 135.98, 128.53, 128.35, 128.12, 127.87, 127.62, 127.40, 123.88, 67.37, 65.90, 52.96, 35.21, 27.98; mass spectrum [70 eV, M/Z (relative intensity)] 453 (M+2, 2.7); 451 (M^+, 2.9), 91 (100). Anal. Calcd for $C_{19}H_{19}N_2O_6Br$: C, 50.57; H, 4.24; N, 6.20; Br, 17.71. Found: C, 50.56; H, 4.08; N, 6.11; Br, 18.13.

p-Nitrobenzyl 2-[N-(Benzyloxy)carbonyl]amino-4-cyanobutanoate 2d: Dry KCN (32 mg, 0.491 mmol) was placed in 15 mL DMSO (distilled from CaH_2). A condenser was fitted and the suspension heated to 90°C under an N_2 atmosphere with stirring. The apparatus was cooled to room temperature and a 10 mL DMSO solution of 2c (201 mg, 0.445 mmol) slowly added over a period of 1 h. Near the end of the addition, the reaction turned purple in color. After an additional 8 h, the mixture was lyophillized. The lyophillizate was then partitioned between water and chloroform, and the chloroform extracts were combined, dried over anhydrous Na_2SO_4 and concentrated, affording 225 mg of a golden syrup. Flash chromatography (benzene:ethyl acetate=3:1, 1.5 x 30 cm column) gave 102 mg (58%) of 2d, after crystallization from chloroform-pet ether (TLC, benzene:ethyl acetate=3:1 on silica gel 60 F-254, R_f = 0.32): mp 77-78°C; IR ($CHCl_3$) 3450, 2250, 1740, 1720, 1610, 1500, 1340 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz FT) δ 8.15 (d, 2H, J = 8 Hz), 7.43 (d, 2H, J = 8 Hz), 7.3 (s, 5H), 5.51 (d, 1H, J = 7 Hz), 5.24 (s, 2H), 5.09 (s, 2H), 4.45 (m, 1H), 2.25 (m, 4H); ^{13}C NMR ($CDCl_3$, 20 MHz FT) δ 170.56 141.90, 135.87, 128.73, 128.64, 128.54, 128.45, 128.19, 123.96, 123.91, 118.54, 67.50, 66.23, 53.02, 28.34, 13.66; mass spectrum [70 eV, M/Z (relative intensity)] 398 (M+1, 1.05), 273 (34.03), 107 (88.72), 91 (100). Anal. Calcd for $C_{20}H_{19}N_3O_6$: C, 60.44; H, 4.57; N, 10.57. Found: C, 60.51; H, 4.83; N, 10.65.

3-Acetamido-3-carboethoxy-2-piperidone 7: Nitrile 6 (200 mg, 0.740 mmol) was dissolved in 10 mL of glacial acetic acid and hydrogenated over PtO_2 (10 mg) at atmospheric pressure and room temperature. After 3.5 h the catalyst was filtered and washed with water and ethyl acetate. The combined filtrate and washings were concentrated, giving a colorless residue which was taken up in sat. $NaHCO_3$ solution and extracted with dichloromethane. Concentration of the dried (anhydrous $MgSO_4$) organic extracts then afforded a residue which was crystallized from dichloromethane-pet ether, affording 67 mg (39.6%) of the lactam 7 (TLC, butanol:acetic acid:water=4:1:1 on silica gel 60, R_f = 0.54): mp 132-133°C (lit (4g) 136-138.5°C); IR ($CHCl_3$) 3400, 1740, 1680, 1485 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz FT) δ 6.98 (bs, 1H), 6.25 (bs, 1H), 4.28 (q, 2H, J = 7.2 Hz), 3.40 (m, 2H), 2.5-1.6 (m, 4H), 2.03 (s, 3H), 1.31 (t, 3H, J = 7.2 Hz).

Diethyl (3-Acetamidopropyl)acetamidomalonate 8: Nitrile 6 (200 mg, 0.740 mmol) was placed in acetic anhydride (10 mL) containing NaOAc (20 mg, 0.244 mmol) and hydrogenated over PtO_2 (20 mg) at atmospheric pressure and room temperature for a period of 40 h. The catalyst was then removed by filtration and washed with dichloromethane, followed by extraction of the combined filtrate and washings with water. Concentration of the organic layer after drying over anhydrous $MgSO_4$ afforded a residue, which was crystallized from dichloromethane-pet ether to give 113 mg (48.3%) of 8 as white crystals (TLC, 13% methanol-chloroform on silica gel 60, R_f = 0.38) : mp 137-138°C; IR ($CHCl_3$) 3445, 1740, 1675 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz FT) δ 6.75 (bs, 1H), 5.67 (bs, 1H), 4.24 (q, 4H, J = 7.1 Hz), 3.22 (q, 2H, J = 6.1 Hz), 2.3 (m, 2H), 2.03 (s, 3H), 1.95 (s, 3H), 1.28 (m, 2H), 1.25 (t, 6H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz FT) δ 170.10, 169.09, 167.68, 65.98, 62.29, 38.92, 29.59,

23.42, 22.14, 14.29, 13.11; mass spectrum [70 eV, M/Z (relative intensity)] 316 (M^+, 7.5), 142 (100); Anal. Calcd for $C_{14}H_{24}N_2O_6$: C, 53.15; H, 7.65; N, 8.85. Found : C, 52.91; H, 7.43; N, 8.85.

[5-^{13}C,5-^{15}N]Diethyl (2-Cyanoethyl)acetamidomalonate 6a: Anhydrous NH_3 (30 mL) was condensed at -78°C under N_2 atmosphere into a 50 mL three-neck flask equipped with a magnetic stir bar and dry ice-acetone Dewar condenser. Diethyl acetamidomalonate, 4, (209 mg, 0.96 mmol) was added and stirred for 30 min. To this was added [^{13}C,^{15}N]-labeled tosylate 11a (17) (215 mg, 0.95 mmol), and the reaction was stirred at -78°C for 3 h. The reaction was allowed to warm to room temperature, and the resulting colorless residue was stirred with water (10 mL) and extracted with 3 x 10 mL portions of chloroform. The combined organic layers were dried (Na_2SO_4) and the solvent evaporated, affording 195 mg of crude product, contaminated with a small amount of 4. Flash chromatography (ether:chloroform=3:1) then gave 179 mg (70%) of labeled 6a (TLC, acetic acid:ethyl acetate:benzene=1:19:80 on silica gel 60 F-254, R_f = 0.27) : mp 92-94°C (Lit.(4g) 92-94°C); ^{1}H NMR ($CDCl_3$, 400 MHz FT) δ 6.79 (bs, 1H), 4.26 (m, 4H), 2.74 (m, 2H), 2.32 (ddt, 2H, J = 9.6 Hz, $^2J_{CH}$ = 9.6 Hz, $^3J_{NH}$ = 1.7 Hz), 2.05 (s, 3H), 1.25 (t, 6H, J = 7.2 Hz); mass spectrum [70 eV, M/Z (relative intensity)] 273 (M^+, 1.87), 157 (100).

[5,5-$^2H_2$]Ornithine 1a: Nitrile 6 (3.177g, 0.01176 mol) was deuterated at atmospheric pressure and room temperture in ethanol-d_1 (150 mL) containing 38% DCl in D_2O (4.5 mL) and PtO_2 (315 mg) over a period of 2 h. The catalyst was filtered, washed with ethanol and the combined filtrate and washings then concentrated

to a glassy hygroscopic residue. Hydrolysis of this residue in conc. HCl (150 mL) heated at reflux for 4.5 h followed by concentration afforded a syrup which was dissolved in ethanol. The dropwise addition of conc. NH_4OH caused a precipitate to form immediately. Filtration, washing with ethanol, and drying yielded 1.956 g (97.5%) of 1a (TLC, butanol:acetic acid:water=4:1:2 on cellulose, 0.3% ethanolic ninhydrin spray, R_f = 0.62): mp 222°C (dec) (Lit.(4g) 225°C); ^1H NMR (D_2O, 80 MHz FT) δ 3.83 (t, 1H, J = 5.6 Hz), 3.07 (m, 0.07 H), 1.90 (m, 4H).

[5-^{13}C,5-^{15}N]Ornithine 1b: This was prepared using the above procedure except as follows: [^{13}C,^{15}N]-labeled nitrile 6a (114 mg, 0.42 mmol) was hydrogenated over PtO_2 (20 mg) in ethanol (3.5 mL) containing 6 drops of conc. HCl followed by hydrolysis in conc. HCl (4.8 mL). This afforded 71 mg (99.7%) of 1b : ^1H NMR (D_2O, 400 MHz FT) δ 3.80 (t, 1H, J = 6.0 Hz), 3.07 (dt, 2H, J = 3.2 Hz, J_{CH} = 143 Hz), 1.99 - 1.84 (m, 4H).

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REFERENCES

1. Career Development Awardee of the National Cancer Institute (CA00880), 1979-1984.
2. Martinkus K.J., Tann C., Gould S.J.-Tetrahedron 39:3493 (1983)

3. Zmijewski M.J., Jr., Palaniswamy V.A., Gould S.J. (submitted for publication.

4. a. Hannant J., Bruylants A.-Bull. Soc. Chim. Belges. 72:423 (1963)
   b. Itoh T.-Bull. Chem. Soc. Japan 36:25 (1963)
   c. Strojny R.A., White H.C.-J. Org. Chem. 28:1942 (1963)
   d. Saotome K.-Kogyo Kagaku Zasshi 65:1061 (1962)
   e. Neshmeyanov A.N., Freidlina R. Kh., Petrova R.G.-Bull. Acad. Sci. U.S.S.R. Div. Chem. Sci. 459 (1957)
   f. Kato J., Ishihara H., Hiwatashi O.-J. Agr. Chem. Soc. Japan 27:498 (1953)
   g. Albertson N.F., Archer S.-J. Am. Chem. Soc. 67:2043 (1945)
   h. Maeda S., Nozoe M.-Bull. Inst. Phys. Chem. Research 17:661 (1938)

5. Pande C.S., Rudick J., Walter R.-J. Org. Chem. 35:1440 (1970)

6. Phase transfer catalysts examined were 18-crown-6, tricaprylylmethylammonium chloride, or hexadecyltributylphosphonium bromide in benzene.

7. Chang C., Coward J.K.-J. Med. Chem. 19:684 (1976)

8. Flavin M., Slaughter C.-J. Biol. Chem. 235:1103 (1960)

9. a. Kotodziejczyk A., Arendt A.-J. Labelled Compds. 11:385 (1975)
   b. Naito T., Yoshikawa T., Ishikawa F., Isoda S., Omura Y., Nakamura I.-Chem. Pharm. Bull. 13:869 (1965)
   c. Tiedemann, H.-Biochem. Z. 326:511 (1955)

10. Shimo K., Wakamatsu S.-J. Org. Chem. 26:3788 (1961)

11. Ferris A.F., Johnson G.J., Gould F.E., Latourette H.K.-J. Org. Chem. 25:492 (1960)

12. Gould F.E., Johnson G.S., Ferris A.F.-J. Org. Chem. 25:1650 (1960)

13. Rylander P.N.-Catalytic Hydrogenation over Platinum Metals, Academic Press, New York (1967) pp. 203-226

14. [5-$^{2}H_{2}$]Ornithine was later converted to [5-$^{2}H_{2}$]arginine with O-methylisourea tosylate (Turba F., Schuster K.H.-Naturwissenschaften 133:370 (1946)), and then to [2,3,3,5,5-$^{2}H_{5}$]arginine via pyridoxal-catalyzed exchange (LeMaster D.M., Richards R.M.-J. Labelled Compds. and Radiopharm. 19:639 (1982).

15. Sakellarios E.J.-Helv. Chim. Acta 29:1675 (1946)

16. Hooz J., Gilani S.S.H.-Can. J. Chem. 46:86 (1968)

17. The labelled tosylate 11 was prepared from [^{13}C,^{15}N]-3-hydroxypropionitrile by the procedure of Sakellarios (Ref. 15).