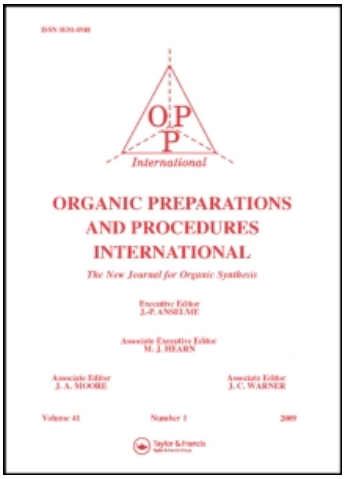


Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

ACTIVE-SITE-DIRECTED ENZYME-ACTIVATED N-NITROSOAMIDE INHIBITORS OF TRYPSIN

Yulong Chen[a]; Emil H. White[b]
[a] Department of Biological and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA [b] Department of Chemistry, The Johns Hopkins University, Baltimore, MD

To cite this Article Chen, Yulong and White, Emil H.(1996) 'ACTIVE-SITE-DIRECTED ENZYME-ACTIVATED N-NITROSOAMIDE INHIBITORS OF TRYPSIN', Organic Preparations and Procedures International, 28: 1, 65 — 76
To link to this Article: DOI: 10.1080/00304949609355908
URL: http://dx.doi.org/10.1080/00304949609355908

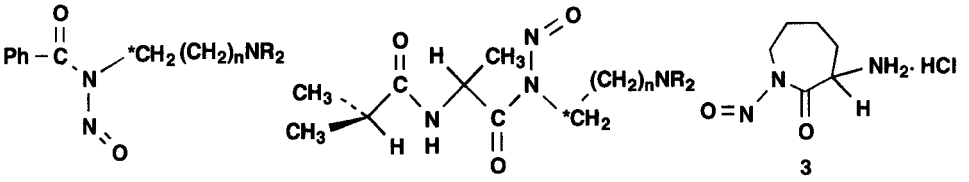
ORGANIC PREPARATIONS AND PROCEDURES INT., 28 (1), 65-76 (1996)

ACTIVE-SITE-DIRECTED ENZYME-ACTIVATED N-NITROSOAMIDE INHIBITORS OF TRYPSIN

Yulong Chen† and Emil H. White*

Department of Chemistry
The Johns Hopkins University, Baltimore, MD 21218

This article covers the syntheses of active-site-directed enzyme-activated N-nitrosoamides (**1-3**), which have been found to be highly active inhibitors of trypsin.[1] The preparation of the N-nitroso modified amide and peptide inhibitors for trypsin posed two major problems: (1) the necessity of nitrosating an amide group in the presence of the more basic primary amino function. This problem was largely solved by keeping the amino group in its protonated form; nevertheless, carefully defined conditions were necessary since the acids required for protonation can also denitrosate the nitrosoamide group; (2) the presence in some of the inhibitors of two amide groups only one of which



1a: R = CH_3, n = 2 (HNO_3 salt)
1b: R = H, n = 3 (hydrochloride)
1c: R = CH_3, n = 3

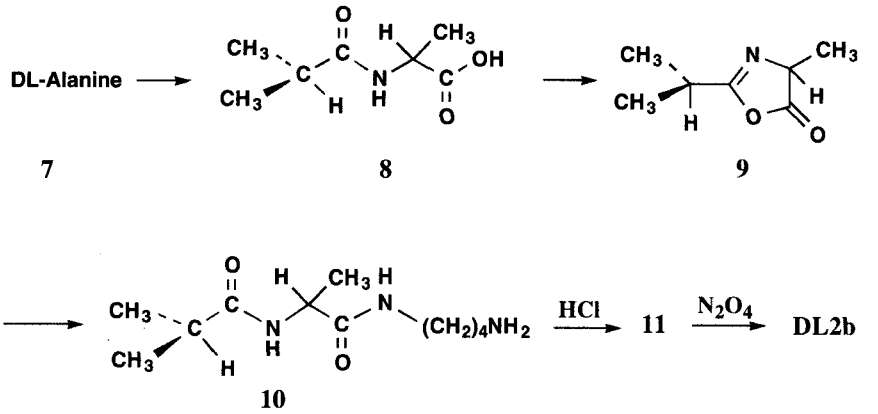
D2a: R = CH_3 n = 2 (HNO_3 salt)
DL2b: R = H, n = 3 (hydrochloride)
D2b:
D2c: R = CH_3, n = 3 (HNO_3 salt)
* = $^{13}CH_2$ or $^{12}CH_2$

was to be nitrosated. This problem was addressed by limiting the amount of nitrosating agent used, by controlling the reaction temperature and time, and by design, in that congestion was built into one of the amide linkages to sterically inhibit access by the nitrosating agent.[2] In related studies, selective nitrosations of ureas have been reported recently.[3] Inhibitors **1a**, **1b**, and **1c** were prepared by nitrosation[4] of N-(3-dimethylaminopropyl)benzamide (**4**), N-(4-aminobutyl)-benzamide (**5**), and N-(4-dimethylaminobutyl)benzamide (**6**), respectively; the amides were obtained by benzoylation of the corresponding amines with benzoyl chloride.

Inhibitor **DL2b** was prepared as illustrated in Scheme 1. N-Isobutyryl-DL-alanine (**8**) was prepared from DL-alanine **7** and isobutyryl chloride.[5] Compound **8** was cyclized through action by

ethyl chlorocarbonate to produce oxazolone **9**, which was then reacted with N,N'-bis(trimethylsilyl)-1,4-diaminobutane to produce, after hydrolysis, the inverse lysine analog **10**. The hydrochloride salt (**11**) was then nitrosated to yield N-nitrosoamide **DL2b**.

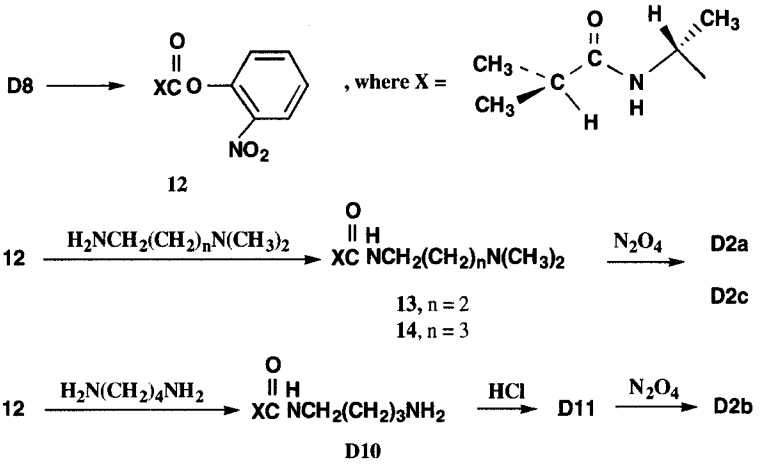
Because the oxazolone route leads to extensive racemization, **D2a**, **D2b**, and **D2c** were prepared by a different route (Scheme 2), beginning with the coupling[6] of the 2-nitrophenyl ester **12** of isobutyryl-D-alanine (**D8**) with 3-dimethylaminopropylamine, 4-dimethylaminobutylamine, and 1,4-diaminobutane, respectively, to produce aminoamides **13**, **14**, and **D10**. Compounds **D2a** and **D2c** were prepared from their precursor amides by the standard nitrosation method,[4] and the hydrochloride salt, **D11**, was nitrosated to give **D2b**. To determine the optical purity of **D2b**, compound **D8**, prepared through the decomposition of compound **D2b** in 5% sodium bicarbonate solution, was converted into the methyl ester (**15**) with diazomethane (eq 1). The methyl ester (**15**) contained 83% of the D-isomer based on analysis of NMR spectra measured in the presence of the chiral shift reagent, tris[1,7,7-trimethyl-3-(trifluoroacetyl)bicyclo[2.2.1]heptan-2-onato-O, O']europium(III), $Eu(tfc)_3$.[7]



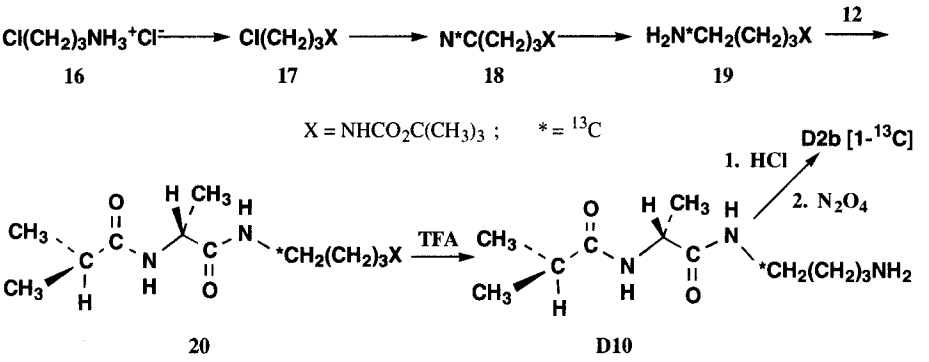
Scheme 1. Synthesis of DL2b.

For the preparation of ^{13}C-labeled **D2b** (Scheme 3), 3-chloropropylamine hydrochloride (**16**) was treated with di-*tert*-butyl dicarbonate in a biphasic medium of sodium bicarbonate solution and chloroform[8] to produce carbamate **17**. A nucleophilic substitution of the 3-chloro group by ^{13}C-enriched potassium cyanide in acetonitrile in the presence of 18-crown-6 ether yielded the ^{13}C-labeled cyanocarbamate **18**. Compound **18** was reduced with sodium borohydride/cobaltous chloride hexahydrate in methanol[9] to the aminocarbamate (**19** [4-^{13}C]); condensation of the 2-nitrophenyl ester **12** with **19** [4-^{13}C] produced the alaninamide derivative **20** [1-^{13}C]. Following removal of the t-Boc protecting group, the amide, **D10** [1-^{13}C] , was nitrosated with N_2O_4 to produce N-nitrosoamide **D2b** [1-^{13}C].

$$\mathbf{D2b} \xrightarrow[(2)\ H_3O^+]{(1)\ OH^-} \mathbf{D8} \xrightarrow{CH_2N_2} \underset{\mathbf{15}}{\mathbf{(CH_3)_2CHCONHCH(CH_3)CO_2CH_3}} \qquad (1)$$



Scheme 2. Synthesis of the Optically Active Inhibitors (D2a, D2b, and D2c)



Scheme 3. Synthesis of ^{13}C-Labeled D2b.

Inhibitor **3** was prepared by the nitrosation of its precursor amide, DL-α-amino-ε-caprolactam hydrochloride (**21**), in the presence of glacial acetic acid.

The methods developed for the syntheses of ^{13}C-labeled inhibitors **1c**[10] and **D2b** would appear to be general approaches to the preparation of ^{13}C-labeled diamines and polyamines; the latter have important roles in biological functions such as the stimulation of chromatin transcription[11] and binding to DNA.[12]

EXPERIMENTAL SECTION

^{1}H NMR and ^{13}C NMR spectra were recorded on Varian XL-400 and Bruker AMX-300 spectrometers; chemical shifts of ^{1}H NMR spectra were reported in parts per million downfield from internal tetramethylsilane; chemical shifts of ^{13}C NMR spectra were reported using chemical shifts of solvents as secondary references (δ DMSO-d_6 = 39.5 ppm; δ $CDCl_3$ = 77 ppm). Polarimetric measurements were made on a Perkin-Elmer 141 polarimeter at the sodium D line (589 nm). Mass spectroscopic

measurements were made on a VG 70-S Gas Chromatograph-Mass spectrometer. Melting points of chemicals were measured on a Hoover Mel-Temp without further correction. UV-VIS spectra were measured on Beckman 25 and Varian 635 UV-VIS spectrophotometers. HPLC separations were carried out on a Waters Associates Liquid Chromatograph consisting of an ALC 202 Liquid Chromatograph, 6000 pumps, a 660 solvent programmer, and a U6K sample injector. The pHs were determined with a Beckman Model 4500 digital pH meter. The syntheses of N-(4-dimethylaminobutyl)-benzamide (**6** [1-^{13}C])[10], N-(4-dimethylaminobutyl)-N-nitrosobenzamide (**1c** [1-^{13}C])[10], and N-(4-aminobutyl)-N-nitroso-N'-isobutyryl-D-alaninamide hydrochloride (**D2b**)[1] appear elsewhere.

The nitrosoamides reported in this manuscript are particularly unstable because of the presence of a basic neighboring group, *or* the presence of an acidic neighboring group, and they could not be purified to organic chemical standards, although the physical data reported indicate that most are pure at time zero. For some of the intermediates, physical data including ^{13}C NMR spectra are presented to indicate purity; further confidence is gained since "chains" of intermediates were used, the chemistry of interconversions further supporting the structures.

N-(3-Dimethylaminopropyl)benzamide (4).- To a stirred solution of 3-dimethylamino-propylamine (1.00 g, 9.79 mmol) in 1 N NaOH (20 mL) in a cold water bath was slowly added benzoyl chloride (1.37 mL, 11.8 mmol) in ether (5.0 mL), and the reaction mixture was allowed to reach room temperature. TLC after 30 min showed that the reaction was almost complete. After stirring for an additional day, the mixture was saturated with sodium chloride, and extracted with CH_2Cl_2 (4 x 15 mL). The extracts were dried over Na_2SO_4, filtered, and evaporated and the residue was dried *in vacuo* to give a pale yellow oil (1.27 g, 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.46 (broad s, 1H), 7.78 (d, m, J = 6.6 Hz, 2H), 7.49-7.38 (m, 3H), 3.55 (q, J = 5.8 Hz, 2H), 2.48 (t, J = 6.0 Hz, 2H), 2.28 (s, 6H), 1.75 (quintet, J = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.0, 134.7, 130.9, 128.3, 126.7, 59.3, 45.4, 40.5, 25.4; IR (CH_2Cl_2), 1653, 1604, 1579, 1526, 1487, 1469, 1461, 1448, 1431, 1369, 1345 cm^{-1}.

N-(3-Dimethylaminopropyl)-N-nitrosobenzamide (HNO_3 salt) (1a) (Note: *All work with N-nitrosoamides should be carried out in a good fume hood and protective gloves should be used*).- Using the general nitrosation method,[4] N_2O_4 (66 mL of gas, 2.3 mmol) was introduced over a stirred solution of N-(3-dimethylaminopropyl)benzamide (**4**) (200 mg, 0.97 mmol) in CH_2Cl_2 (4 mL) at -78° (N_2O_4 was directed to the walls of the flask). The reaction was allowed to warm to 0° and it was kept at that temperature for 45 min. The solvent and excess N_2O_4 were evaporated *in vacuo* to give a yellow oil [85% product in the form of the nitrate salt and 15% starting material (based on the integrals of methylene protons adjacent to the amide nitrogen: δ 4.02 for the N-nitroso compound and δ 3.55 for the starting amide)]. ^{1}H NMR for **1a** (300 MHz, $CDCl_3$): δ 10.3 (b s, 1H), 7.76 (d, J = 6.3 Hz, 2H), 7.61 (t, J = 5.7 Hz, 1H), 7.51 (t, J = 5.7 Hz, 2H), 4.02 (t, J = 5.3 Hz, 2H), 3.15-3.10 (m, 2H), 2.90 (d, J = 2.4 Hz, 6H), 1.99 (quintet, J = 5.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0 (C=O), 132.9 (C-4') 130.8 (C-2', 6'), 128.3 (C-3', 5'), 127.3 (C-1'), 55.8 (CH_2-3), 43.3 ($2CH_3$), 36.5[-N(NO)-CH_2-] 24.4 (-CH_2-2); IR (CH_2Cl_2): 2500, 1703, 1602 cm^{-1}.

N-(4-Aminobutyl)benzamide (5) and its hydrochloride were prepared from 1,4-diaminobutane and benzoyl chloride.[13] Compound **5** was obtained as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.50

(t, J = 6.5 Hz, 1H), 7.85 (d, t, J_1 = 6.6, J_2 =1.6 Hz, 2H), 7.49 (t, t, J_1 = 6.9 Hz, J_2 = 2.0 Hz, 1H), 7.45 (t, d, J_1 = 6.7 Hz, J_2 = 2.0 Hz, 2H), 3.24 (quartet, J = 6.9 Hz, 2H), 3.24 (broad overlapping s, H_2O + NH_2), 2.54 (t, J = 6.9 Hz, 2H), 1.55 (quintet, J = 6.9 Hz, 2H), 1.37 (quintet, J = 6.9 Hz, 2H). The free aminoamide was dissolved in 1N HCl (4.32 mL) and the solution was lyophilized to give a yellowish solid (1.14 g, very sensitive to moisture). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.61 (t, J = 6.5 Hz, 1H), 8.02 (broad s, 3H), 7.87 (d, J = 7.0 Hz, 2H), 7.52 (t, J = 7.3 Hz, 1H), 7.46 (t, J = 7.6 Hz, 2H), 3.28 (q, J = 6.0 Hz, 2H), 2.80 (m, 2H), 1.59 (m, 4H); TLC on silica gel ($CH_3OH/NH_3{\cdot}H_2O/H_2O$, 8:1:1 by vol.): R_f 0.38.

N-(4-Aminobutyl)-N-nitrosobenzamide Hydrochloride (1b).- N-(4-Aminobutyl)benzamide (**5**) hydrochloride (100 mg, 0.34 mmol) was ground into a fine powder and dried *in vacuo* for 1 hr. The product was mixed with 20 mL of acetic anhydride. The reaction flask was sealed with a rubber septum and attached to a balloon containing nitrogen. The suspension was stirred vigorously and then cooled to 0°. Dinitrogen tetraoxide (34 mL of gas, 1.4 mmol) was introduced slowly under the surface of the reaction mixture during a period of 5 min; a clear yellow solution resulted. After 30 min, the excess N_2O_4 and the solvent were removed quickly below 0° *in vacuo*; the residue was dried for 2 hrs at ~10^{-2} torr to give a yellow solid which was washed with ethyl acetate (2 x 15 mL), then dried *in vacuo* at ~10^{-2} torr for 8 hrs to give spectrally pure **1b** hydrochloride (64 mg, 57%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75 (d, t, J_1 = 7.0 Hz, J_2 = 1.7 Hz, 2H), 7.66 (t, d, J_1 = 8.6 Hz, J_2 = 1.6 Hz, 1H), 7.56 (t, d, J_1 = 7.4 Hz, J_2 = 1.3 Hz, 2H), 3.90 (t, J = 6.3 Hz, 2H), 3.39 (t, J = 21.7 Hz, -NH_3^+), 2.78 (broad s, 2H), 1.49 (m, 4H); UV-VIS (acetonitrile): λ_{max} 425 nm (ε 89), 408 (94), 247 (8835).

N-(4-Dimethylaminobutyl)benzamide (6) was prepared from 4-dimethylaminobutylamine (0.161 g, 1.38 mmol), triethylamine (0.961 mL, 6.91 mmol), and benzoyl chloride (160 μL, 1.38 mmol) in anhydrous ethyl ether (22 mL) under argon.[13] The amide **6** was obtained as a pale oil (0.18 g, 60%). The physical data are the same as those of **6** [1-^{13}C].[10] In addition, ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.6, 135.1, 131.1, 128.4, 126.9, 59.3, 45.3, 40.1, 27.5, 25.6.

A similar run gave a product purer than 99% in the initial evaporation; it was used directly in the next reaction.

N-(4-Dimethylaminobutyl)-N-nitrosobenzamide (1c) was prepared from (4-dimethylaminobutyl)benzamide (**6**) (13.4 mg, 0.0614 mmol) and gaseous N_2O_4 (6.85 mL, 0.26 mmol) in methylene chloride (5 mL) containing suspended anhydrous sodium acetate (35.3 mg, 0.43 mmol) at 0°.[4] The nitrosoamide **1c** was dried *in vacuo* to give a yellow oil (13.4 mg, 88%): ^{1}H NMR showed that the product contained *ca.* 5% of the starting material. The pure N-nitrosobenzamide (**1c**) was obtained by chromatography on silica gel deactivated with 10% water, using hexane, methylene chloride, acetonitrile, and acetonitrile containing 3% triethylamine as the eluants. The physical data were essentially the same as those of **1c**[1-^{13}C].[10]

N-Isobutyryl-D-alanine (D8).- The synthesis was based on the published method.[5] Recrystallization of the product from ethyl acetate, and subsequently from ethanol and hexane, gave large crystals of compound **D8**, mp 147.5 - 148.5°. $[\alpha]_D^{25}$ + 45.0° (c 1.00 g/100 mL, glacial acetic acid); ^{1}H NMR (400

MHz, DMSO-d_6): δ 7.999 (d, J = 7.4 Hz, 1H), 4.165 (quintet, J = 7.3 Hz, 1H), 2.404 (septet, J = 6.9 Hz, 1H), 1.242 (d, J = 7.3 Hz, 3H), 0.985 (d, J = 6.8 Hz, 6H).

N-Isobutyryl-DL-alanine (DL8) was prepared by the method used for the D-enantiomer. Recrystallization of the white solid from boiling methanol and water gave large, diamond shaped crystals of compound **DL8** (6.21g, 20%), mp 128-130° (lit.[5] 129-130°).

2-Nitrophenyl N-isobutyryl-D-alaninate (12) was prepared from N-isobutyryl-D-alanine (3.00 g, 18.8 mmol) and 2-nitrophenol (2.62 g, 18.8 mmol) in dry ethyl acetate (40 mL) (procedure of reference 6) using dicyclohexyl carbodiimide (4.03 g, 19.5 mmol) in dry ethyl acetate (20 mL) with vigorous stirring at 0°. The crude ester **12** was recrystalized from ethyl acetate (7 mL) and hexane (33 mL) to give needle-like crystals. The crystals were washed with hexane (40 mL) and dried *in vacuo* to give ester **12** (1.96 g, 51%), mp 87-88°. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (d, J = 6.4 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 7.85 (t, J = 7.9 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 4.48 (quintet, J = 6.6 Hz, 1H), 2.47 (septet, J = 6.8 Hz, 1H), 1.46 (d, J = 7.3 Hz, 3H), 1.02 (d, J = 6.9 Hz, 6H); TLC on silica gel (ethyl acetate/hexane/methanol, 67:32:1 by vol.): R_f 0.59; $[\alpha]_D^{25}$ + 21.6° (c 0.99 g/100 mL, ethyl acetate).

N-(3-Dimethylaminopropyl)-N'-isobutyryl-D-alaninamide (13).- To a solution of 2-nitrophenyl N-isobutyryl-D-alaninate (**12**) (100 mg, 0.357 mmol) in dry ether (5.0 mL) under nitrogen was introduced a solution of 3-dimethylaminopropylamine (0.045 mL, 0.357 mmol) and triethylamine (0.199 mL, 1.41 mmol) in dry ether (1.5 mL) during a period of 3 min. The reaction mixture was stirred vigorously for 12 hrs at 25°. The yellow reaction mixture was diluted with ether to 15 mL and the solution was extracted with 0.17 N HCl (2 x 8 mL). The aqueous extracts were made alkaline to pH 11-12 with 1 N NaOH and the solution was extracted with CH_2Cl_2 (3 x 30 mL). The organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated and the residue was dried *in vacuo* for 4 hrs at room temperature and 1 hr at 45° to give a pale yellow solid (70.2 mg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73 (s, 1H), 6.59 (d, J = 6.9 Hz, 1H), 4.46 (quintet, J = 7.1 Hz, 1H), 3.32 (m, 2H), 2.38 (septet, J = 6.9 Hz, 1H), 2.34 (t, J = 6.8 Hz, 2H), 2.22 (s, 6H), 1.66 (quintet, J = 6.5 Hz, 2H), 1.34 (d, J = 6.9 Hz, 3H), 1.16 (d, J = 6.9 Hz, 3H), 1.14 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.6, 172.2, 58.3, 48.5, 45.3, 39.1, 35.3, 26.1, 19.5, 19.3, 18.8; IR (KBr): 3271, 3077, 2965, 2814, 2763, 1638, 1549, 1249, 1100, 1041 cm^{-1}.

N-(3-Dimethylaminopropyl)-N-nitroso-N'-isobutyryl-D-alaninamide (D2a) was prepared from N-(3-dimethylaminopropyl)-N'-isobutyryl-D-alaninamide (**13**) and gaseous N_2O_4 by the procedure used for **1a**. A yellow oil was obtained (the reaction mixture contained *ca.* 50% of the starting material amide **13**). A further attempt to nitrosate the mixture led to formation of dinitroso derivative: integral ratio from 5.88 ppm (quartet, dinitroso)/5.76 ppm (quintet, mononitroso) ~1/2. Purification was not pursued for this compound and therefore **D2a** was not used for inhibition studies[1] [Note: The NMR data for **D2a** and its dinitroso derivative are very similar to those for the purified **D2c** and its dinitroso counterpart (*vide infra*)].

N-(4-Aminobutyl)-N'-isobutyryl-D-alaninamide Hydrochloride (D11)[1] was obtained as a pale

solid, mp 202-205°; $[\alpha]_D^{25}$ + 8.7° (c 1.00 g/100 mL, glacial acetic acid); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.06 (broad s, 3H), 7.98 (m, 2H), 4.21 (quintet, *J* = 7.2 Hz, 1H), 3.05 (m, 2H), 2.75 (m, 2H), 2.46 (septet, *J* = 6.8 Hz, 1H), 1.55 (m, 2H), 1.45 (m, 2H), 1.18 (d, *J* = 7.2 Hz, 3H), 0.977 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.8, 172.4, 48.0, 38.4, 37.7, 33.5, 25.9, 24.3, 19.5, 19.3, 18.4; IR (KBr): 3293, 2968, 1956 (broad), 1633 (amide) 1548 (amide), 1512, 1446, 1374, 1234, 1169, 1094, 694 cm^{-1}; TLC on silica gel ($CH_3OH/NH_3{\cdot}H_2O/H_2O$, 18:1:1 by vol.): R_f 0.46. Caution: recrystallization from an acetonitrile-ethanol mixture at 70° gave largely racemic product, $[\alpha]_D^{25}$ 0° (c 0.40 g/100 mL, ethanol); $[\alpha]_D^{25}$ + 1° (c 0.40 g/100 mL, methanol).

N-(4-Aminobutyl)-N-nitroso-N'-isobutyryl-D-alaninamide Hydrochloride (D2b) was prepared according to the reported procedure.[1] Reverse phase HPLC (μ Bondapak C18 column, CH_3CN) was used to purify the material; pure **D2b** gave R_f (HPLC, CH_3CN) 7.0 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.49 (d, *J* = 5.9 Hz, 1H), 7.59 (broad s, 3H), 5.33 (quintet, *J* = 6.7 Hz, 1H), 3.72 (t, *J* = 6.4 Hz, 2H), 2.74 (m, 2H), 2.47 (m, 1H), 1.40 (d, *J* = 7.3, 3H), 1.36 (m, 4H), 1.00 (d, *J* = 6.9 Hz, 6H); UV (CH_3CN): λ_{max} 244 nm (ε 7505); VIS (CH_3CN): λ_{max} 425 nm (ε 84), 408 (88).

N-Isobutyryl-D-alanine Methyl Ester (15).- Diazomethane in 30 mL of ether, prepared from Diazald[14] (200 mg, 0.65 mmol), was added in 6 portions to a stirred solution of N-isobutyryl-D-alanine (**D8**) (100 mg, 0.63 mmol) in methanol (0.5 mL) and ether (10 mL). After 25 mL of the diazomethane had been added, the reaction solution became yellowish. A further 5 mL of the diazomethane solution was added, then glacial acetic acid (0.4 mL) (to destroy the excess diazomethane). The solution was evaporated to an oil, which was dissolved in 10 mL of ethyl ether. The solution was washed with 5% sodium bicarbonate (2 x 5 mL); the organic phase was separated, dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give white crystals (86.3 mg, 79%). The pure ester **15** was obtained by sublimation at about 45° and ~10^{-2} torr, m.p. 55.5-56.5° (Lit.[2] 55.5 - 57.0°). TLC on silica gel (hexane/ether/ethyl acetate, 5:4:1 by vol.): R_f 0.11; ^{1}H NMR (400 MHz, $CDCl_3$ + CCl_4, 1:2, v/v): δ 5.99 (broad s, 1H), 4.55 (quintet, *J* = 7.2 Hz, 1H), 3.76 (s, 3H), 2.37 (septet, *J* = 6.9 Hz, 1H), 1.39 (d, *J* = 7.1 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 6H).

Determination of the Optical Purity of N-(4-Aminobutyl)-N-nitroso-N'-isobutyryl-D-alaninamide Hydrochloride (D2b).- To 0.4 mmol of **D2b** was added 4.0 mL of 5% sodium bicarbonate solution. The yellow color disappeared immediately and gas was released from the reaction. The resulting solution was stirred for 2 hrs and extracted with ethyl ether (10 mL). The organic phase was discarded; the aqueous phase was adjusted to pH ~1 with 1 N HCl and extracted with ethyl ether (10 mL) and ethyl acetate (10 mL). The extracts were pooled and the organic solvents were removed. The residue contained a majority of the isobutyrylalanine from the hydrolysis, as determined by the ^{1}H NMR spectrum. The isobutyryl-D-alanine in the residue was converted to its methyl ester **15** and the ester was purified by sublimation as described in the previous entry. With the use of a chiral shift reagent,[7] Eu(tfc)$_3$, at a 1/1 molar ratio to sample in 0.6 mL of $CDCl_3/CCl_4$ (1/2, v/v), it was found that this sample of methyl ester **15** contained a minimum of 83% of the D-enantiomer based on the integral ratio of the α-proton of the D-enantiomer (13.05 ppm, broad s) to the α-proton of the L-enan-

tiomer (12.65 ppm, broad s) (the reference sample of **D15** described in the previous section gave under the same conditions a single peak at 13.05 ppm).

***tert*-Butyl N-(3-Chloropropyl)carbamate (17)** was prepared based on the published method.[8] To a suspension of 3-chloropropylamine hydrochloride (**16**) (1.30 g, 10.0 mmol) in chloroform (20 mL) was added a solution of sodium bicarbonate (0.840 g, 10.0 mmol) and sodium chloride (2.0 g) in 20 mL of water. To this two phase mixture was added di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol) in chloroform (4 mL) and the reaction mixture was refluxed with vigorous stirring for 90 min. The reaction mixture was cooled and the organic layer was separated. The aqueous layer was extracted with 20 mL of chloroform. The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to give compound **17** as a colorless oil (1.98 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.60 (broad s, 1H), 3.58 (t, J = 6.4 Hz, 2H), 3.27 (q, J = 6.5 Hz, 2H), 1.96 (quintet, J = 6.3 Hz, 2H), 1.44 (s, 9H); IR (CH_2Cl_2): 3451, 1708, 1506, 1278, 1249, 1167 cm^{-1}; TLC on silica gel (EtOAc/hexane/MeOH, 8:1:1 by vol.): R_f 0.78.

***tert*-Butyl N-(3-Cyanopropyl)carbamate (18)**.- To a 10-mL flask containing dry, powdered potassium cyanide (0.200 g, 3.1 mmol) was added a solution of *tert*-butyl N-(3-chloropropyl)carbamate (**17**) (0.600 g, 3.1 mmol) and 18-crown-6 (70 mg, 0.26 mmol) in acetonitrile (5 mL).[15] The reaction flask was equipped with a condenser fitted with a drying tube ($CaSO_4$-NaOH). After the reaction mixture was refluxed for 14 hrs, it was filtered, the filtrate was concentrated, and the residue was diluted with water (5 mL) and extracted with CH_2Cl_2 (12 x 10 mL). The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness *in vacuo* to give a crude product (90% mol product + 10% 18-crown-6; 0.52 g, 91% yield). Distilling the product from the 18-crown-6 under vacuum failed since both co-distilled. The crude product (0.391 g) in ether (10 mL) was extracted with water (3 x 5 mL). The ether layer was separated, dried over anhydrous sodium sulfate, and evaporated to give compound **18** as a colorless oil (0.32 g; 74%); spectral data were consistent with those for **18** [^{13}C].

***tert*-Butyl N-(3-Cyano[^{13}C]propyl)carbamate (18 [^{13}C])** was prepared from potassium cyanide (96.55 pure, 99.9% carbon-13) and compound **17** using the procedure described for the natural abundance compound **18**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.70 (broad s, 1H), 3.24 (q, J = 6.3 Hz, 2H), 2.40 (d, t, J_{CHCN} = 9.7 Hz, J_{CHCH} = 7.2 Hz, 2H), 1.86 (quintet, J = 6.9 Hz, 2H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.0, 119.0 (^{13}CN), 78.4, 39.2, 28.4, 26.0, 14.5; IR ($CHCl_3$): 3457 (NH-), 2981, 2936, 2873, 2196 (-^{13}CN), 1711 (-OCONH-), 1506 (-OCONH-), 1250, 1165, 909 cm^{-1}; TLC on silica gel (EtOAc/hexane/MeOH, 67:32:1 by vol.): R_f 0.48.

***tert*-Butyl N-(4-Aminobutyl)carbamate (19)**.- To a solution of *tert*-butyl N-(3-cyanopropyl)carbamate (**18**) (200 mg, 1.1 mmol) and cobaltous chloride hexahydrate (520 mg, 2.2 mmol) in methanol (6 mL) at 0° was added, portionwise, sodium borohydride (420 mg, 11 mmol)[9] for 30 min with vigorous stirring. After additional stirring at 0° for 1 hr, the black reaction mixture was acidified (pH 4-5) with 1 N HCl. The mixture was filtered through a Celite cake. The pink aqueous filtrate was extracted with ether (2 x 15 mL), and the aqueous solution was made alkaline with aqueous ammonia. The latter was extracted

with chloroform (3 x 20 mL), and the extracts were dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to yield compound **19** as a colorless oil (0.153 mg, 74%). NMR data were consistent with those for **19** [4-^{13}C]; IR ($CHCl_3$): 3455 (NH), 2976, 2935, 2865, 1711(-OCONH-), 1507 (-OCONH-), 1246, 1169, 906 cm^{-1}; TLC on silica gel ($CH_3OH/NH_3{\cdot}H_2O/H_2O$, 8:1:1 by vol.): R_f 0.35.

***tert*-Butyl N'-(4-Aminobutyl[4-^{13}C])carbamate (19 [4-^{13}C])** was prepared in the same way as the natural abundance compound **19**. The crude sample (0.50 g) was chromatographed (silica gel column; $CH_3OH/NH_3{\cdot}H_2O/H_2O$, 18/1/1 by vol.) to give pure compound **19** [4-^{13}C] (178 mg) and also an unseparated mixture (~200 mg). For the pure product, ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.64 (broad s, 1H); 3.13 (q, J = 6.1 Hz, 2H), 2.71 (d, t, J_{CH} = 135 Hz, J_{CHCH} = 6.7 Hz, 2H), 1.90 (broad s, NH_2), 1.58 -1.47 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 157.0, 78.7, 41.8 (-$^{13}CH_2$-NH_2), 40.2, 28.2, 27.3, 27.1.

N-(4-*tert*-Butylcarbamylbutyl[1-^{13}C]-N'-isobutyryl-D-alaninamide (20 [1-^{13}C]) was prepared from 2-nitrophenyl N-isobutyryl-D-alaninate (**12**), compound **19** [4-^{13}C] (0.13 g, 0.68 mmol), and triethylamine (0.75 mL, 5.4 mmol) in dry ether (20 mL) using the procedure described for compound **D11**. Compound **20** [1-^{13}C] was obtained as a white solid (0.217 g, 97%), m.p. 139-140°. $[\alpha]_D^{25}$ + 3.8° (c 0.25 g/100 mL, EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.57 (broad s, 1H), 6.19 (broad d, J = 6.0 Hz, 1H), 4.61 (broad s, 1H), 4.45 (quintet, J = 7.2 Hz, 1H), 3.26 (d, m, J_{CH} = 138 Hz, 2H), 3.12 (m, 2H), 2.39 (septet, J = 6.9 Hz, 1H), 1.55 (m, 4H), 1.44 (s, 9H), 1.35 (d, J = 6.9 Hz, 3H), 1.16 (d, J = 6.9 Hz, 3H), 1.14 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 39.1 (-CONH-$^{13}CH_2$-); IR (KBr): 3361, 3294, 2967, 2930, 2871, 1681 (-CONH), 1638 (-CONH-), 1529 (broad), 1172 cm^{-1}; TLC on silica gel (EtOAc/hexane/MeOH, 67:32:1 by vol.): R_f 0.11.

N-(4-Aminobutyl)[1-^{13}C]-N'-isobutyryl-D-alaninamide (D10 [1-^{13}C]).- To compound **20** [1-^{13}C] (163 mg, 0.49 mmol) was added a mixture of trifluoroacetic acid and methylene chloride (10 mL, 1:1, v/v) and the solution was stirred vigorously at room temperature for 1 hr. The reaction solution was evaporated and the oily residue was dissolved in methylene chloride (30 mL); the solution was then extracted with 1 N NaOH saturated with sodium chloride (15 mL). The organic layer was separated and the aqueous layer was extracted with methylene chloride (3 x 30 mL). The organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give **D10** [1-^{13}C] as a sticky yellow oil (0.112 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.69 (broad s, 1H), 6.17 (d, J = 6.0 Hz, 1H), 4.41 (quintet, J = 7.2 Hz, 1H), 3.33 (d, m, J_{CH} = 145 Hz, 2H), 2.76 (t, J = 6.5 Hz, 2H), 2.38 (septet, J = 6.8 Hz, 1H), 1.67 (broad s, NH_2 + H_2O), 1.55 (m, 4H), 1.35 (d, J = 6.9 Hz, 3H), 1.16 (d, J = 6.9 Hz, 3H), 1.14 (d, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 39.2 (-CONH-$^{13}\underline{C}H_2$-). The oil was dissolved in a mixture of chloroform and ether (5 mL, 3/2, v/v) and the solution was saturated with HCl gas until no further precipitate formed. The mixture was evaporated to dryness. The sticky material was redissolved in methanol (5.0 mL) and evaporated to give a pale yellow solid **D11** [1-^{13}C].

N-(4-Aminobutyl[1-^{13}C])-N-nitroso-N'-isobutyryl-D-alaninamide Hydrochloride (D2b [1-^{13}C]) was prepared from **D11** [1-^{13}C] using the procedure described for natural abundance **D2b**. ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.6 [-CON(NO)$^{13}CH_2$]; TLC on silica gel (n-butanol/HOAc/H_2O, 4:1:1

by vol.): R_f 0.12. A yield of 57% was obtained as determined through the absorbance at 425 nm (ε = 84; measured utilizing natural abundance **D2b**). The compound was used directly without further purification.

2-Isopropyl-4-methyl-2-oxazolin-5-one (9).- To a stirred solution of N-isobutyrylalanine (1.00 g, 6.28 mmol) and triethylamine (0.875 mL, 6.28 mmol) in dry methylene chloride (40 mL) at 0° in a flask equipped with a rubber septum was added ethyl chlorocarbonate (0.600 mL, 6.28 mmol) according to the literature procedure[16]. After the addition was complete, the reaction mixture was allowed to warm to room temperature and kept at that temperature for 12 hrs; ^{1}H NMR spectra indicated that the reaction was complete. The volatile species including the oxazolone derivative were distilled at 25° and ~10^{-2} torr and trapped with liquid nitrogen. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.195 (q, d, J_1 = 7.4 Hz, J_2 = 1.7 Hz, 1H), 2.750 (septet, d, J_1 = 6.9 Hz, J_2 = 1.7 Hz, 1H), 1.474 (d, J = 7.5 Hz, 3H), 1.275 (d, J = 7.0 Hz, 6H). Further purification was not pursued because solvent and ethanol did not interfere with the next reaction and also because the oxazolone partially decomposed during attempted distillation under normal pressure.

N-(4-Aminobutyl)-N'-isobutyryl-DL-alaninamide Hydrochloride (11).- The distilled oxazolone **9** from the run described above was transferred to a 100-mL r.b. flask and cooled to 0°. The flask was flushed with N_2 and stirring was commenced; N,N'-*bis*-(trimethylsilyl)-1,4-butanediamine (1.78 mL, 6.3 mmol)[17] was added dropwise. A small amount of precipitate was formed. The reaction mixture was stored at 25° under nitrogen for 12 hrs (more precipitate formed). The precipitate was removed by filtration and the filtrate was extracted with water (30 mL). The aqueous phase was lyophilized to give a white solid (0.513 g), which was heated to 55-60° at ~10^{-2} torr for 1.5 hrs to produce a white residue (0.400 g). Sublimation of this material was carried out at 102-103° at ~10^{-2} torr to give the pure aminoamide product (**10**) (0.351 g, 24%), mp 144-151°. ^{1}H NMR spectra (400 MHz, DMSO-d_6) showed that *ca.* 50% of amino groups were probably transformed to a carbamic acid (formed from atmospheric carbon dioxide) with signals for -$\underline{CH_2}NH_2$ at 2.57 ppm and -$\underline{CH_2}$NHCOOH at 3.03 ppm; the latter overlaps the signal for -CONH$\underline{CH_2}$- at 3.03 ppm; TLC on alumina ($CH_3OH/NH_3{\cdot}H_2O/H_2O$, 8:1:1 by vol.): R_f 0.83; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (m, 2H); 4.20 (quintet, J = 7.3 Hz, 1H), 3.39 (broad s, -NH_2 + $2H_2O$), 3.03 (m, 3H: 2 from -CONH$\underline{CH_2}$- + 1 from -$\underline{CH_2}$NHCOOH), 2.57 (t, J = 6.8 Hz, 1H), 2.43 (septet, J = 6.9 Hz, 1H), 1.37 (m, 4H), 1.16 (d, J = 7.5 Hz, 3H), 0.972 (d, J = 6.8 Hz, 6H). The neutral aminoamide derivative was dissolved in 0.1 N HCl and the white hydrochloride salt was obtained by the removal of the solvent and excess HCl at ~10^{-2} torr. Recrystallization from a methanol and acetonitrile mixture gave pale white crystals (87% recovery), mp 213-213.5° (dec.). NMR data were consistent with those for **D11**.

Anal Calcd for $C_{11}H_{24}ClN_3O_2$: C, 49.71; H, 9.10. Found: C, 49.29; H, 9.27

N-(4-Aminobutyl)-N-nitroso-N'-isobutyryl-DL-alaninamide Hydrochloride (DL2b) was prepared from its precursor amide **11** by the method described for the D-enantiomer.

N-(4-Dimethylaminobutyl)-N'-isobutyryl-D-alaninamide (14) was prepared from 2-nitrophenyl N-isobutyryl-D-alaninate (**12**) (200 mg, 0.714 mmol), 4-dimethylaminobutylamine (82.5 mg, 0.714

mmol), and triethylamine (4 eq) in 13 mL of dry ethyl acetate by the procedure used for the synthesis of compound **13**. A white solid **14** (144 mg, 79%) was obtained, mp 108-108.5°; $[\alpha]_D^{25}$ +7.0° (c 0.4 g/100 mL, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 7.54 (broad s, 1H), 6.23 (d, *J* = 7.1 Hz, 1H), 4.38 (quintet, *J* = 7.1 Hz, 1H), 3.25 (q, *J* = 6.0 Hz, 2H), 2.37 (septet, *J* = 6.9 Hz, 1H), 2.28 (t, *J* = 6.4 Hz, 2H), 2.23 (s, 6H), 1.56 (m, 4H), 1.35 (d, *J* = 6.9 Hz, 3H), 1.16 (d, *J* = 6.9 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 3H); M^+/z Calcd for $C_{13}H_{27}O_2N_3$: 257.2103; Found: 257.2106.

Anal Calcd for $C_{13}H_{27}O_2N_3 \cdot 1/2\ H_2O$: C, 59.62; H, 10.58; N, 16.04. Found: C, 59.77; H, 10.41; N, 15.98

N-(4-Dimethylaminobutyl)-N-nitroso-N'-isobutyryl-D-alaninamide (D2c).- N-(4-Dimethylaminobutyl)-N'-isobutyryl-D-alaninamide (**14**) (35 mg, 0.14 mmol) in methylene chloride (25 mL) was nitrosated with dinitrogen tetraoxide (23 mL gas, 0.82 mmol) using the procedure described for compound **1c**. Compound **D2c** was obtained as a yellow oil in the form of the nitrate salt (17.1 mg, 36%). ^1H NMR (300 MHz, $CDCl_3$): δ 6.34 (d, *J* = 6.5 Hz, 1H), 5.62 (quintet, *J* = 7.0 Hz, 1H), 3.82 (m, 3H), 2.94 (q, *J* = 8.1 Hz, 2H), 2.76 (s, 6H), 2.49 (septet, *J* = 6.9 Hz, 1H), 1.70 (quintet, *J* = 6.7 Hz, 2H), 1.54 (d, *J* = 7.1 Hz, 3H), 1.50 (quintet, *J* = 7.1 Hz, 2H), 1.18 (d, *J* = 6.9 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 3H). Note: higher concentrations of reagents (e.g., 5 times higher than those used above) led to formation of the dinitroso compound, N-(4-dimethylaminobutyl)-N,N'-dinitroso-N'-isobutyryl-D-alaninamide, which was easily separated on polyamide TLC plates (15 x 15 cm, 50 μ ; Cheng Chin Trading Co. Ltd). The purified dinitroso compound was obtained as a yellow oil. TLC on polyamide plates (ether/hexane, 33:67, v/v): R_f 0.84 ; ^1H NMR (300 MHz, $CDCl_3$): δ 5.88 (q, *J* = 6.9 Hz, 1H), 3.76 (septet, *J* = 6.9 Hz, 1H), 3.71 (m, 2H), 2.20 (m+s, 8 H), 1.43 (d, *J* = 6.9 Hz, 3H), 1.33 (m, 4H), 1.26 (d, *J* = 7.0 Hz, 3H), 1.23 (d, *J* = 7.0 Hz, 3H).

DL-α-Amino-ε-caprolactam Hydrochloride (21), prepared from the lactam,[18] was obtained as a white powder, mp 293-294° (Lit.[18] mp >270°). ^1H NMR (400 MHz, DMSO-d_6): δ 8.150 (t, *J* = 6.4 Hz, 1H), 7.950 (broad s, 3H), 4.063 (d, *J* = 11.6 Hz, 1H), 3.186 (d, t, J_1 = 10.8 Hz, J_2 = 4.2 Hz, 1H), 3.082 (m, 1H), 1.913 (m, 2H), 1.759 (m, 1H), 1.623 (d, q, J_1 = 10.8 Hz, J_2 = 4.2 Hz, 1H), 1.501 (q, *J* = 11.6 Hz, 1H), 1.213 (q, *J* = 12.3 Hz, 1H). The IR data matched those of reference 18.

DL-α-Amino-ε-N-nitrosocaprolactam Hydrochloride (3).- Dinitrogen tetraoxide (90 μl liq., 1.41 mmol) was introduced under the surface of a stirred solution of DL-α-amino-ε-caprolactam hydrochloride (77.2 mg, 0.467 mmol) in 6 mL of glacial acetic acid. After 18 hrs, the reaction mixture was lyophilized to give a yellow, sticky solid (85.1 mg). IR (KBr): 3284, 2940, 1738, 1636, 1383, 1241 cm^{-1} (Lit.[19] values for N-nitrosovalerolactam are: 2900, 1750, 1520 cm^{-1}). Based on the IR absorbances at 1738 and 1636 cm^{-1}, the solid was a 1/1 mixture of nitrosolactam **3** and the starting lactam.

Acknowledgement.- This work was supported by Grant GM21450 from the Institute of General Medical Science of the U. S. Public Health Service.

REFERENCES

† Present Address: Department of Biological and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, USA.

1. E. H. White and Y. Chen, *Biochemistry,* **34**, 15123 (1995).

2. E. H. White, L. W. Jelinski, I. R. Politzer, B. R. Branchini, and D. F. Roswell, *J. Am. Chem. Soc.*, **103**, 4231 (1981).

3. J. E. Saavedra and D. W. Farnsworth, *Org. Prep. Proced. Int.,* **24**, 655 (1992).

4. E. H. White and C. A. Aufdermarsh, Jr., *J. Am. Chem. Soc.*, **83**, 1174 (1961).

5. D. G. Doherty and E. A. Popenoe, Jr., *J. Biol. Chem.,* **189**, 447 (1951).

6. M. Bondansky and A. Bondanszky, *The Practice of Peptide Synthesis*; p.118, Spring-Verlag, New York, NY, 1984.

7. H. L. Goering, J. N. Eikenberry, G. S. Koermer and C. J. Lattimer, *J. Am. Chem. Soc.*, **96**, 1493 (1974).

8. D. S. Tarbell, Y. Yamamoto and B. M. Pope, *Proc. Nat. Acad. Sci.*, **69**, 730 (1972).

9. T. Harayama, M. Ohtani, M. Oki and Y. Inubushi, *Chem. Pharm. Bull. Jpn,* **23**, 1511 (1975).

10. E. H. White and Y. Chen, *J. Label. Compds. Radiopharm.*, **XXXIII**, 97 (1993).

11. D. A. Pierce and N. Fausto, *Biochemistry*, **17**, 102 (1978).

12. A. E. Pegg, *Cancer Research*, **48**, 759-774 (1988).

13. J. Kahovec and J. Stamberg, *Coll. Czech. Chem. Commun.*, **42**, 1838 (1977).

14. L. F. Fieser and M. Fieser, *"Reagents for Organic Synthesis"* Vol. 1, p. 191, John Wiley and Sons, Inc., New York, NY, 1967.

15. F. L. Cook, C. W. Bowers and C. L. Liotta, *J. Org. Chem.*, **39**, 3416 (1974).

16. H. Takagaki, N. Yasuda, M. Asaoka and H. Takei, *Chemistry. Lett.*, 183 (1979).

17. M. Taddei and F. Tempesti, *Synth. Commun.*, **15**, 1019 (1985)

18. S. Terashima, M. Wagatsuma and S. Yamada, *Tetrahedron*, **29**, 1487 (1973).

19. E. H. White, D. F. Roswell, I. R. Politzer and B. K. Branchini, *Methods Enzymol.,* **46**, 216 (1977).

(Received February 2, 1995; in revised form November 20, 1995)