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Synthesis of (±)-2,3-Methanoproline: A Novel Inhibitor of Ethylene Biosynthesis

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Abstract: The title compound, 2-*aza*-bicyclo[3.1.0]hexane-1-carboxylic acid (**2**) was prepared by treatment of N-benzyloxycarbonyl-2,3-dehydroproline *tert*-butyl ester with diazomethane followed by photolysis of the resulting pyrazoline and deprotection. Its N-acetyl-N'-methyl amide, a peptide mimic, was synthesized and the structure of was confirmed by X-ray diffraction studies. NMR spectroscopy was also used to examine the effect of the cyclopropane ring on its conformation. This 2,3-methanoamino acid (**2**) was found to be a weak inhibitor of ethylene biosynthesis in cucumber cotyledon strips and germinating squash seeds. The data show that **2** probably inhibits the conversion of 1-aminocyclopropanecarboxylic acid to ethylene in these tissues.

The biosynthesis of the plant hormone ethylene has attracted considerable interest in recent years particularly since 1-aminocyclopropanecarboxylic acid (Acc) was found to be the direct precursor of ethylene.[1] Much of the research in this area has concerned the mechanism by which Acc is degraded to ethylene, cyanide, and CO_2 by the ethylene forming enzyme (EFE)[2]. A second area of research has involved the search for potential inhibitors of EFE.[3] Early in these studies, Hoffman and coworkers[4] found that (+)-*allo*-coronamic acid (**1**, (1*R*, 2*S*)-2-ethyl-Acc) was converted into 1-butene at 25% the rate at which Acc yields ethylene, and that this stereoisomer of **1** was processed much more efficiently than any of the other stereoisomers (>40:1).

The many inhibitors of EFE tested have led various workers[3b,4] to propose a model for the active site of EFE (**Figure 1**) in which both the amino and carboxyl functions of Acc are required for binding to occur. The active site is also sterically constrained so that large groups at the 2-position cannot be accommodated.

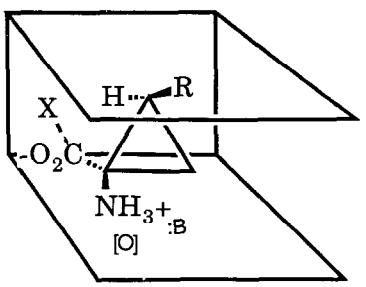
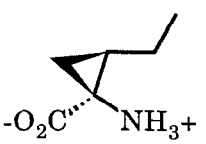


Figure 1



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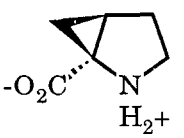


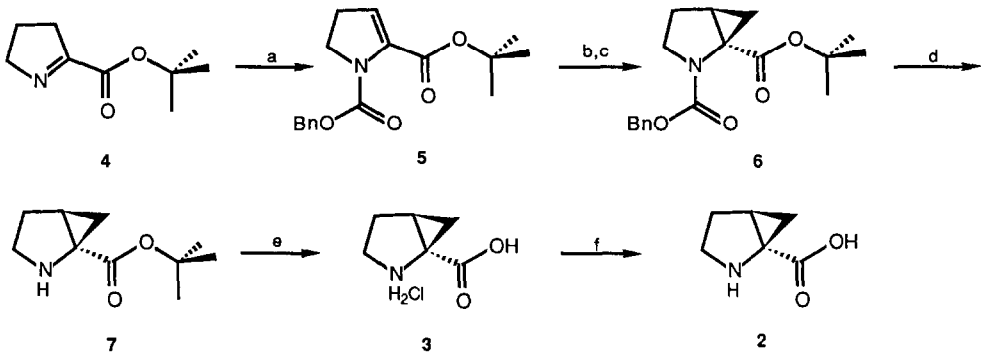
2

Figure 2

Based on this model, it was clear that 2,3-methanoproline (**2**, **Figure 2**) could be accommodated by the active site of EFE. This new cyclopropane amino acid would not only be an analog of proline but also of Acc; i. e., as a ***cyclo-allo***-coronamic acid, which would not be fully processed by EFE because it is a secondary amine. It might, therefore, be a tightly binding competitive or irreversible inhibitor of the enzyme.

Results and Discussion.

The synthesis of **2** was completed as shown (**Scheme 1**). Its hydrochloride salt (**3**) was prepared from the known[5] dehydroproline (**4**) in 31% overall yield. When this imine was treated with excess benzyl chloroformate and pyridine, the N-benzyloxycarbonyl derivative of enamine **5** was isolated and allowed to react with excess diazomethane. The intermediate pyrazoline was not isolated, but was directly photolyzed to give the fully protected cyclopropane **6**. Deprotection of **6** could be accomplished in a single step using trifluoroacetic acid and thioanisole or, more efficiently, in a two-step process involving hydrogenolysis (5% Pd/C) followed by acidolysis (2M HCl). The amino acid zwitterion was then obtained in 73% yield from the hydrochloride using Dowex-1 (acetate form).



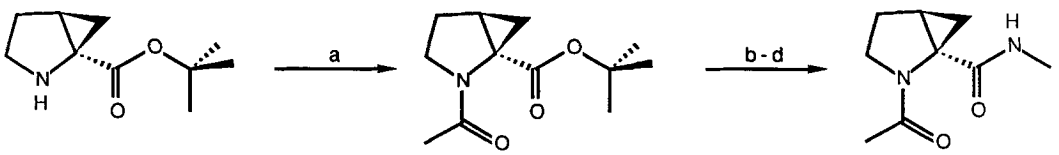
Reagents: a) CbzCl , Pyr ; b) CH_2N_2 ; c) hν ; d) H_2 , 5% Pd/C ; e) HCl; f) Dowex-1

Scheme 1

The crystalline N-acetyl-N'-methylamide derivative **8** was prepared from amino ester **7** by standard methods (**Scheme 2**). X-ray diffraction confirmed the structure of **8** (**Figure 3**) and, by inference, the structure of **2**. This particular derivative was chosen because it is a model peptide and its conformation should give an indication of the conformational behavior of this novel amino acid in peptides. The amide exhibited the small N2-C1-C2-N1 (ψ) dihedral angle which is characteristic of the conformations taken by 2,3-methanoamino acids in the solid state[6]. Furthermore, the ϕ and ψ angles of **8** are quite similar to those obtained by X-ray crystallography of the analogous proline derivative[7]; i.e., in amide **8** , ϕ = 76° and ψ = 7° while in N-acetylproline-N'-methylamide the corresponding values are 76° and 16°. The slightly smaller ψ angle in amide **8** is probably due to conjugation of the carbonyl group with the cyclopropane ring, which is maximized at $\psi = 0°$. These conformational similarities indicate that the cyclopropane containing amino acid may be an effective replacement for proline for purposes of stabilization to enzymolysis with retention of bioactivity.

Crystallography.

A crystal of $C_9H_{14}N_2O_2$ (**8**) was mounted on a Syntex P3 automated diffractometer. Unit cell dimensions (Table I) were determined by least squares refinement of the best angular positions for fifteen independent reflections ($2\theta>15°$) during normal allignment procedures using molybdenum radiation (γ=0.71069Å). Data, (1063 points) were collected at room temperature using a variable scan rate, a θ-2θ scan mode and a scan width of 1.2° below $K\alpha_2$ and 1.2° above $K\alpha_2$ to a maximum 2θ value of 45.0°. Backgrounds were measured at each side of the scan for a combined time equal to the total scan time. The intensities of three standard reflections were remeasured after every 97 reflections and as the intensities of these reflections showed less than 6% variation, corrections for decomposition were deemed unnecessary. Data were corrected for Lorentz, polarization and background effects. After removal of space group forbidden and redundent data, observed data, (423 points) ($I>3.0\sigma(I)$) were used for solution and refinement. The structures were solved for carbon, nitrogen and oxygen positions using direct methods[8]. Least squares refinement[9] converged with anisotropic thermal parameters. Hydrogen atoms were located from a difference Fourier synthesis. These positions were included in the final refinement with isotropic thermal parameters but held invariant. A final difference Fourier revealed no electron density of interpretable level. Scattering factors were taken from Cromer and Mann[10]. The final cycle of refinement - function minimized $\Sigma(|F_o|-|F_c|)^2$, led to final agreement factor, $R=(\Sigma||F_o|-|F_c||/\Sigma|F_o|)$ x 100. Unit weights were used until the final cycles of refinement when weights equal to $1/\sigma F$ were introduced. R_w=10.8%.



Reagents: a) AcCl , NEt_3 b) TFA , CH_2Cl_2 c) $(CH_3)_2CHCH_2OCOCl$,NEt_3 d) $MeNH_3Cl$, NEt_3

Scheme 2

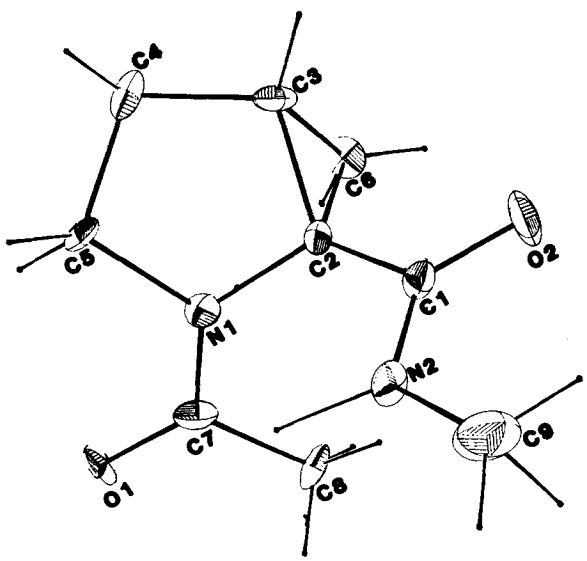


Figure 3

We have also carried out an NMR analysis of amide **8** in order to study its conformational properties in dilute solution and to compare them with the corresponding prolineamide derivative in order to allow interpretation of future peptide spectra containing the new amino acid. Using ^{1}H -^{1}H COSY, ^{1}H -^{13}C COSY, DEPT, steady-state NOE experiments, and a 500 MHz proton spectrum, we have been able to make preliminary assignments in D_2O of the protons and carbon atoms of both the s-*cis* and the s-*trans* conformers of **8** (**Figure 4**). In general, we found that **8** exhibits a somewhat greater preference for the s-*cis* conformation than does the prolineamide. In contrast, a recent study of N-acetyl-2,4-methanoproline-N'-methylamide by Scheraga[11] showed that only the s-*trans* conformer was present in dilute solution. Further NMR studies of amide **8**, now in progress, will be published separately.

Table 1. Ethylene production from plant tissues treated with (±)-2,3-methanoproline (**2**).

	Apple fruit cortex disks		Cucumber cotyledons		Squash seeds		Carrot roots
Concentration (mM)	Post-Climacteric	Pre-Climacteric	Strips	Whole	Germinating	Excised roots	Cortex disks
0.0	61.8[x]	1.61	1.52 a[z]	2.80	4.10 a	12.1	0.231
0.008	64.9	1.69					
0.024	65.0						
0.08	66.6	2.53			3.67 b	11.6	0.243
0.24	64.8						
0.8	61.7	2.04	1.19 b	2.39	2.87 c	13.0	0.252
2.4	61.3						
8.0	62.2	2.41	0.98 c	2.60	2.48 d	14.6	0.268
80			0.75 d	2.76			0.255
LSD 5%	n.s.	n.s.		n.s.		n.s.	n.s.

[x] Ethylene production in nl $(g \cdot hr)^{-1}$

[z] Means followed by the same letter are not significantly different at the 5% level.

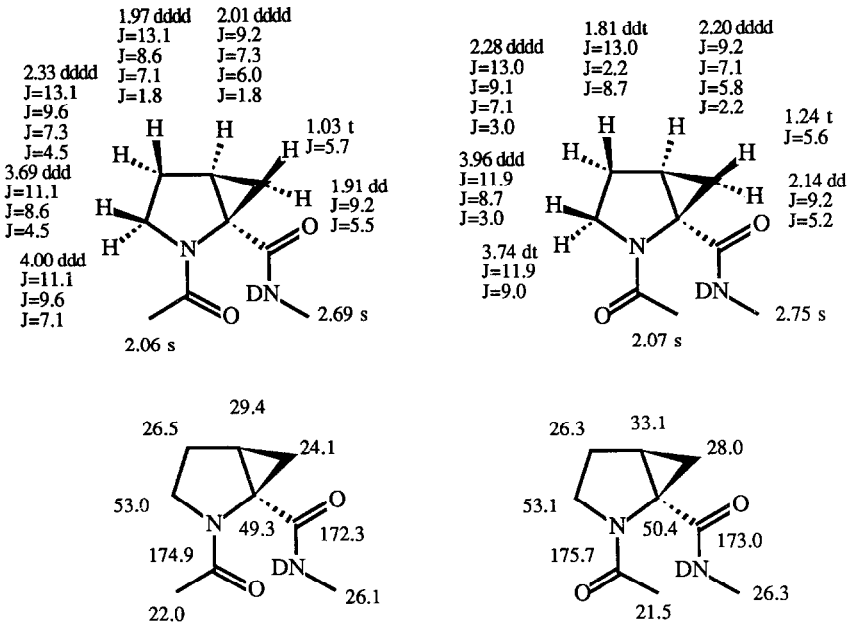


Figure 4

Biology

Application of 40 to 60 µl of 0.8 mM aqueous solutions of 2,3-methanoproline (**2**) to germinating squash seeds and strips of cucumber cotyledons reduced ethylene production by 30% and 20%, respectively. An 8 mM solution of **2** inhibited ethylene production by 36% and 40% in the same respective tissue. Inhibition was roughly loglinear and each 10-fold increase in concentration resulted in an additional 14% reduction in ethylene production from strips of cucumber cotyledons; i. e., 22%, 36%, and 50% reductions for 0.8, 8, and 80 mM solution, respectively.

In contrast, application of from 20 to 60 µl of up to 8 mM **2** had no inhibitory effect on ethylene production by cortex disks of pre- and post-climacteric apple tissue, carrot cortex tissue, whole cucumber cotyledons, or excised squash roots (**Table 1**). Ethylene production from whole cucumber cotyledons was unaffected by 20 µl of the 80 mM solution. Two applications of 20 µl of the 80 mM solution did result in a 28% reduction in ethylene production from pre-climacteric apple cortex disks, but this extremely high concentration was phytotoxic; treated tissue became brown and water soaked (data not shown).

Three other inhibitors of ethylene synthesis were more effective than **2**. Applications of 40 to 60 µl of 0.8, 8.0 and 80 mM solutions of **2** inhibited ethylene production in receptive tissue by an average of 26%, 38% and 50%, respectively, while application of 20 µl of a 10 mM solution of aminoethoxyvinylglycine (AVG) inhibited ethylene production

from tomato pericarp disks by about 97% (11 to 0.3 nL/g·h)[12]. Similarly, 20 μl applications of 5 mM aminooxyacetic acid (AOA) and 100 mM α-aminoisobutyric acid (Aib) solutions inhibited ethylene production by 80% and 95%, respectively. AVG and AOA are thought to interfere with the synthesis of Acc, the immediate precursor of ethylene, while Aib is thought to block the conversion of Acc to ethylene.

Treating cucumber cotyledon strips with 2,3-methanoproline, before application of Acc, indicated that it inhibited ethylene production by affecting the EFE. Ethylene production from the control and treated strips was very low; averaging 0.38 and 0.37 nl/g hr, respectively, and the long incubation period probably resulted in this diminished rate of ethylene production. Addition of 80 μl of 0.5 mM Acc solution stimulated ethylene production over 70-fold in the control tissue, but only 11-fold in tissue treated with 80 μl of 80 mM 2,3-methanoproline solution indicating that it probably inhibited conversion of Acc to ethylene through an effect on EFE. For comparison, the 80 mM solution of **2** required for 50% inhibition can be compared to the 100 mM concentration of Aib that produced a 95% inhibition of ethylene production[9].

Experimental Section

Material and Methods.

(A) Chemistry

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Varian EM-390 continuous wave NMR spectrometer operating at 90 MHz or a Bruker AM-250 Fourier transform NMR at 250 MHz using tetramethylsilane as the internal standard. The ^{13}C NMR spectra were recorded either on a Bruker AM-250 operating at 62.5 MHz or on a JEOL FX 90Q Fourier transform NMR spectrometer operating at 22.5 MHz with $CDCl_3$ or dioxane (for D_2O) as the internal standard.

Anhydrous and HPLC grade solvents were used directly in the synthetic reactions. Other commercial reagents also were used without additional purification. Microanalysis was performed by Atlantic Microlab Inc., Atlanta, Georgia. All reactions were carried out in either a nitrogen or an argon atmosphere.

(B) Biology.

Solution preparation. Aqueous treatment solutions of 2,3-methanoproline were made by diluting 80 and 0.8 mM (i.e., 10,000 and 100 ppm (wt/wt)) stock solutions prepared before each experiment.

Apple cortex tissue. Disks (0.5 x 1 cm diam) were cut with a stainless steel razor blade from cylinders of cortex tissue excised with a cork bore from pre- and post-climacteric apples (Malus domestica Borkh., cv. Golden Delicious). The disks were soaked for 30 min in 500 ml of 0.5 M glycerol, blotted dry, and distributed among 15 x 100 mm plastic petri dishes. Twenty to 60 μl of the test solutions were applied to each disk.

Cucumber cotyledons. Cucumber seedlings (Cucumis sativus L. cv. Poinsett 76) were germinated in vermiculite for about one week at 20°C under fluorescent light. Only fully expanded cotyledons were used. They were excised from the hypocotyl with a razor blade and a 20 µl drop of the test solutions were applied to the base of each cotyledon. Two cotyledons were enclosed in each test tube and each treatment contained 4 replicates. Cotyledons were also cut into 3 mm thick longitudinal slices to increase absorption of the solutions. Sixty µl of the test solutions were applied to about nine strips. Excess solution was removed after 2 hr, and the tissue was weighed and enclosed in 15 mL glass test tubes.

Ethylene forming enzyme activity was assayed using strips of cucumber cotyledons. The strips (0.1 g FW) were treated with 80 µl of water or 80 mM 2,3-methanoproline for 2 hr, blotted dry, and then treated with 80 µl of water or 0.5 mM Acc for 16 hr.

Carrot cortex disks. Disks (0.3 x 1 cm diam) were cut with a stainless steel razor blade from cylinders of cortex tissue excised with a cork bore from carrot roots. The disks were soaked for 30 min in 500 mL deionized water, blotted dry, and distributed among 15 x 100 mm plastic petri dishes. Twenty µl of the test solutions were applied to each disk.

Germinating squash seeds. Squash seeds (Cucurbita pepo L. cv. Table King Acorn) were germinated on moist filter paper for either 3 or 5 days at 20°. Three uniform seeds were placed into 15-ml glass tubes with 40 µl of the test solutions.

Ethylene measurements. After waiting for the solutions to be absorbed by the tissue, the tissue was blotted, weighed, placed in a 16 x 100 mm glass test tube and immediately capped with a rubber serum stopper. After 1 to 6 hr of incubation at 20°C in dim light, 1 mL gas samples of the headspace were taken and analyzed for ethylene by flame ionization gas chromatography as previously described[13]. Each experiment was repeated at least twice and each treatment contained from 3 to 5 replicates in a completely randomized experimental design.

Synthesis.

N-Benzyloxycarbonyl-2,3-dehydroproline-OtBu **5**

To a stirred solution of **4** (16.0 g, 94.8 mmol)[5] and pyridine (12 mL, 150 mmol) in CH_2Cl_2 (100 mL) at -20°C was added benzyl chloroformate (21.4 mL, 150. mmol) dropwise over a 15 min period and the solution was stirred at room temperature overnight. N,N-diethylethylenediamine (14 mL, 100 mmol) was then added dropwise over 15 min to destroy the excess chloroformate and the reaction was stirred an additional 2 h. The solution was then diluted with CH_2Cl_2 (300 mL) and washed with 10% aq citric acid (2 X 300 mL) and 10% aq Na_2CO_3 (300 mL). The organic phase was dried (K_2CO_3), concentrated, and chromatographed on silica gel (230-400 mesh, 65 X 270 mm column, 3 portions, hexanes to 6:4 hexanes/ethyl acetate gradient elution) to afford 22.4 g (78%) of **5** as a colorless oil; ^1H NMR ($CDCl_3$) : δ 7.21 (s, 5H), 5.68 (t, 1H, J = 3 Hz), 5.08 (s, 2H), 3.91 (t, 2H, J = 9 Hz), 2.57 (d of t, 2H, J = 9 Hz, J = 3 Hz), 1.40 (s, 9H) ppm; ^{13}C NMR ($CDCl_3$) : δ 160.8, 153.4, 137.6, 136.0, 128.3, 128.0, 119.1, 81.8, 67.3, 48.6, 28.3, 27.8 ppm.

N-Benzyloxycarbonyl-2,3-methanoproline-OtBu 6

The dehydroamino acid derivative **5** (24.0 g, 79.2 mmol) was divided into three equal portions and each was treated with excess diazomethane generated under nitogen from N-methyl-N-nitroso-*para*-toluenesulphonamide (Diazald, 32 g, 150 mmol) according to the published procedure[14] using a preparative scale apparatus similar to the Aldrich Mini Diazald Apparatus. The resulting diazomethane/ether mixture was collected with a Dry Ice/CCl_4-cooled condenser and added directly to a Dry Ice/CCl_4 cooled solution of **5** in CH_2Cl_2 (100 mL). This solution was then stirred at room temperature for 24 h after which $CaCl_2$ (10-20 g) was added and stirring was continued for 16 h. The $CaCl_2$ was filtered to give a colorless solution of crude pyrazoline which was concentrated and photolyzed for 4 h in CH_2Cl_2 (500 mL) in an ice-water cooled apparatus using a 450 W Hanovia medium-pressure mercury lamp. The crude cyclopropane from these three runs was then chromatographed in two portions on silica gel (230-400 mesh, 65 X 270 mm column, hexanes to 1:1 hexanes/ethyl acetate gradient elution) to afford 16.4 g (65%) of **6** as a colorless oil; ^1H NMR ($CDCl_3$) : δ 7.21 (s, 5H), 5.07 (s, 2H), 3.4-4.1 (m, 2H), 1.6-2.4 (m, 4H), 1.47 (s, 9H), 0.97 (br s, 1H) ppm; ^{13}C NMR ($CDCl_3$) : δ 169.5, 155.7, 136.54, 128.2, 127.5, 80.9, 66.8, 51.4 (br), 48.2, 30.5 (br), 27.7, 26.5, 26.0 (br) ppm.

2,3-Methanoproline CF_3CO_2H salt 2-TFA

The fully protected amino acid **6** (0.5 g, 1.6 mmol) was added at room temperature to a stirred mixture of trifluoroacetic acid (30 mL), thioanisole (3 mL, 25 mmol), and *m*-cresol (3 mL, 28 mmol). The resulting light brown solution was then stirred at room temperature for 4 h. and the mixture was concentrated by rotary evaporation from CCl_4 (3 X 100 mL). The residue was triturated with hexanes (100 mL), washed with ether (100 mL), and dissolved in D_2O (5 mL). The solution was passed through a small reversed-phase silica gel column (5 X 50 mm, 40μ octadecyl silica) followed by an additional 5 mL portion of D_2O. The combined eluate was lyophilized to afford a gummy yellow solid (0.08 g, 40%); the NMR spectrum showed it to be the trifluoroacetate salt of **2** ; ^1H NMR (D_2O) : δ 2.6 - 3.6 (m, 2H), 2.0 - 2.2 (m, 3H), 1.3 - 1.6 (m, 2H) ppm; ^{13}C NMR (D_2O) : δ 174.8, 49.6 (quat), 45.3 (CH_2, J = 148 Hz), 28.6 (CH, J = 182 Hz), 27.0 (CH_2, J = 129 Hz), 16.5 (CH_2, J = 169 Hz) ppm.

2,3-Methanoproline-OtBu 7

Protected amino acid **6** was dissolved in ethyl acetate (300 mL) and the solution was flushed with argon. 5% Pd/C (~0.3 g) was added and hydrogen was bubbled into the stirred suspension until no starting material remained (2 h). The flask was then flushed with nitrogen; the catalyst was removed by filtration and washed with methanol (3 X 100 mL) and the combined filtrates were concentrated. The residue was then Kuglerohr distilled at reduced pressure to give 6.6 g (85%) of **7** as a light yellow oil : bp 90-120°C/0.8 mm; ^1H NMR (C_6D_6) : δ 2.83 (m, 1H), 2.32 (m, 1H), 2.10 (br s, 1H), 1.76 (m, 1H), 1.3-1.65 (m, 3H), 1.36 (s,9H), 0.85 (t, J = 6 Hz) ppm. ^{13}C NMR (C_6D_6) : δ 172.2 (C=O), 80.1 (quat), 50.1 (quat), 44.3 (CH_2), 28.1 (CH_3), 27.9 (CH_2), 27.7 (CH), 16.7 (CH_2) ppm.

2,3-Methanoproline **2**

Distilled **7** (4.4 g, 24 mmol) and anisole (3.0 mL, 28 mmol) were dissolved in 3.0 M HCl in dioxane (40 mL) and the solution was stirred at room temperature overnight during which a white precipitate formed. The suspension was diluted with hexanes (200 mL) and the precpitate was filtered and washed with ether (2 X 100 mL). The solid was dissolved in isopropanol (100 mL) and reprecipitated with ether (800 mL) giving a light orange solid which was chromatographed on reversed-phase silica gel (~ 10 g, 40μ flash chromatography, grade C_{18}, water elution). The aqueous solution was lyophilized and the yellow-white solid was recrystalized from isopropanol/ether and, after the removal of a small amount of highly colored precipitate, the amino acid hydrochloride hydrate (**3**, 3.2 g, 73%) was isolated as granular white crystals; mp 200-215°C; ^{13}C NMR (D_2O) : δ 171.5, 46.7, 43.2, 27.0, 24.6,14.9 ppm; MS : m/e 127 (M^+), 109 (M - H_2O).

Anal. Calcd. for $C_6H_9NO_2 \cdot HCl \cdot H_2O$: C, 39.67%; H, 6.66%; N, 7.74%. Found : C, 39.73%; H, 6.72%; N, 7.68%.

Zwitterion **2** was prepared from **3** by stirring an aqueous solution of **3** with Dowex 1X8-50 (OAc^-,~ 50 g) overnight at room temperature. The resin was removed by filtration, washed with water (3 X 100 mL) and the combined filtrates were lyophilized to afford the desired zwitterion (1.7 g, 53% from **6**) as a white powder : mp 197-203°C; ^1H NMR (D_2O) : δ 3.58 (m, 1H), 3.06 (m, 1H), 2.1-2.5 (m, 3H), 1.3-1.8 (m, 2H) ppm; ^{13}C NMR (D_2O) : δ 173.9, 48.8, 41.2, 24.8, 24.8, 13.2 ppm.

Anal. Calcd. for $C_6H_9NO_2 \cdot 1/4\ H_2O$: C, 54.72%; H, 6.89%; N, 10.68%. Found : C, 54.65%; H, 7.30%; N, 10.55%.

N-Acetyl-2,3-methanoproline-N'-methylamide **8**

To a stirred solution of amino ester **6** (1.0 g, 5.5 mmol) and triethylamine (1.5 mL, 11 mmol) in CH_2Cl_2 (30 mL) at room temperature was added acetyl chloride (0.7 mL, 10 mmol). After 1 h, N,N-diethylethylenediamine (1.0 mL, 7.1 mmol) was added and stirring was continued for an additional hour. The solution was then diluted with CH_2Cl_2 (300 mL), washed with 2% aq $KHSO_4$ (2 X 200 mL) and 10% aq. K_2CO_3 (1 x 200 mL), dried (K_2CO_3), and concentrated to afford 1.0 g (80%) of crude N-acetylester as a light yellow oil; ^1H NMR ($CDCl_3$) : δ 3.4-4.2 (m, 2H), 2.04 (s, 3H), 0.9-2.4 (m, 5H), 1.44 (s, 9H) ppm; ^{13}C NMR ($CDCl_3$) : δ 171.2, 169.2, 81.8, 52.5, 48.8, 35.6, 30.5, 27.9, 26.8, 22.1 ppm.

This material was dissolved in CH_2Cl_2 (30 mL) and anisole (2 mL, 18 mmol) was added. The solution was cooled to 5°C and trifluoroacetic acid (30 mL) was added. The cold bath was removed and the solution was stirred for 1 h, diluted with CCl_4 (100 mL) and concentrated at 35°C. After twice re-evaporation from CCl_4 (200 mL), the residue was dissolved in CH_2Cl_2 (50 mL), the solution was cooled to 5°C, and triethylamine (3.0 mL, 21 mmol) and isobutyl chloroformate (0.8 mL, 6.2 mmol) were added. The solution was stirred for 30 min, after which methylamine hydrochloride (1.0 g, 15 mol) was added and the mixture was stirred at room temperature overnight. The solution was diluted with CH_2Cl_2 (200mL) and extracted with 2% aq $KHSO_4$ (2 X 200 mL). The aqueous solution was then extracted with 10:1 $CHCl_3$/methanol (4 x 150 mL) and the combined extracts were

concentrated to dryness. The residue was reconcentrated from ethyl acetate and chromatographed on silica gel (230-400 mesh, 45 X 240 mm column, 10:1 $CHCl_3$/methanol). The crude product was crystallized from ethyl acetate/hexanes to give 0.2 g (20% from **6**) of amide **7** as analytically pure prisms : mp 150-151°C; ^1H NMR (D_2O, 250 MHz) : δ 3.96 (m, 1H), 3.70 (m, 1H), 2.74 and 2.68 (s, 3H), 2.05 and 2.04 (s, 3H), 1.7-2.4 (m, 4H), 1.23 and 1.02 (t, 1H, J = 6 Hz) ppm; ^{13}C NMR (D_2O) : δ 175.7 (C=O), 174.9 (C=O), 172.9 (C=O), 172.3 (C=O), 53.0 (CH_2), 52.9 (CH_2), 50.4 (quat), 49.3 (quat), 33.1 (CH), 29.4 (CH), 28.0 (CH_2), 26.5 (CH_2), 26.2 (CH_2), 26.2 (CH_3), 26.1 (CH_3), 24.1 (CH_2), 22.0 (CH_3), 21.5 (CH_3) ppm.

Anal. Calcd. for $C_9H_{14}N_2O_2$: C, 59.28%; H, 7.74%; N, 15.43%. Found : C, 59.28%; H, 7.75%; N, 15.37%.

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