408 (5), 391 (59), 349 (56), 348 (54), 307 (34), 306 (30), 289 (57), 247 (39), 229 (31), 228 (33), 205 (17), 186 (37), 43 (100); exact mass calcd for $C_{18}H_{27}O_{11}P$ (M) 450.1291, found 450.1272.

Fraction C [$R_f$ 0.56 (C)] gave **11b** as colorless needles: 6.7 mg (1.8%); mp 228–229 °C (from AcOEt–hexane); for 500-MHz $^1$H NMR data, see Table I; MS, $m/z$ 450 (0.55, $M^+$), 408 (13), 391 (13), 366 (21), 349 (100), 348 (39), 307 (44), 306 (41), 289 (44), 247 (43), 229 (42), 228 (69), 204 (24), 187 (28), 186 (57); exact mass calcd for $C_{18}H_{27}O_{11}P$ (M) 450.1291, found 450.1280.

Fraction D [$R_f$ 0.53 (C)] gave **11c** as a colorless syrup: 16.7 mg (4.6%); for 500-MHz $^1$H NMR data, see Table I; MS, $m/z$ 450 (0.22, $M^+$), 408 (4.1), 366 (19), 349 (33), 307 (33), 306 (26), 289 (27), 288 (30), 247 (37), 228 (47), 205 (22), 186 (45), 43 (100); exact mass calcd for $C_{18}H_{27}O_{11}P$ (M) 450.1291, found 450.1261.

Fraction E [$R_f$ 0.50 (C)] gave **11d** as a colorless syrup: 26.1 mg (7.2%); for 500-MHz $^1$H NMR data, see Table I; MS, $m/z$ 451 (3.3, M + 1), 450 (0.68, $M^+$), 435 (7), 408 (7), 391 (10), 366 (41), 349 (52), 337 (78), 307 (59), 306 (58), 289 (37), 261 (27), 247 (56), 228 (80), 186 (56), 163 (100), 141 (27), 122 (26); exact mass calcd for $C_{18}H_{27}O_{11}P$ (M) 450.1291, found 450.1266.

Besides these separated products, an unseparable mixture of **11a–d** (ca. 20 mg) was recovered as the intermediate fractions.

# Total Synthesis of Cyclobutane Amino Acids from *Atelia herbert smithii*†

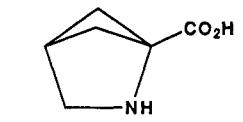
Philip Hughes*,‡ and Jon Clardy

*Baker Laboratory of Chemistry, Cornell University, Ithaca, New York 14853*

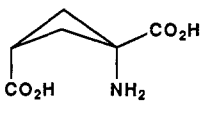
*Received May 17, 1988*

The syntheses of two amino acids from the seeds of the legume *Atelia herbert smithii*, 2,4-methanoproline and 2,4-methanoglutamic acid, are described. The synthesis of a third amino acid, *cis*-1-amino-3-(hydroxymethyl)cyclobutanecarboxylic acid and subsequent comparison with a natural sample show that it too is a component of the seeds. The synthesis of a fourth possible seed component, 2,4-methanopyroglutamic acid, is also described.

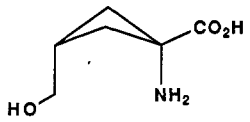
The seeds of the legume *Atelia herbert smithii*, found only in the Santa Rosa National Park in Costa Rica, are ignored by over 100 normal seed predators. This led Bell and co-workers to the isolation and structural solution by X-ray crystallography of two new amino acids: 2,4-methanoproline (**1**) and 2,4-methanoglutamic acid (**2**).[1] A minor ninhydrin-reacting component was also detected though its structure was not defined. The structure was postulated to be the hydroxy amino acid **3**.[2] A fourth compound, 2,4-methanopyroglutamic acid (**4**), although not isolated, was proposed as a possible seed component or an intermediate in the biosynthesis of the other three amino acids. The strain inherent in such an azabicyclo[2.1.1]-hexane lactam should make acid **4** a good acylating agent, and such activity may give rise to the observed seed avoidance.



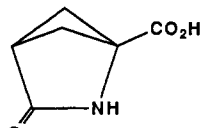

1




2




3



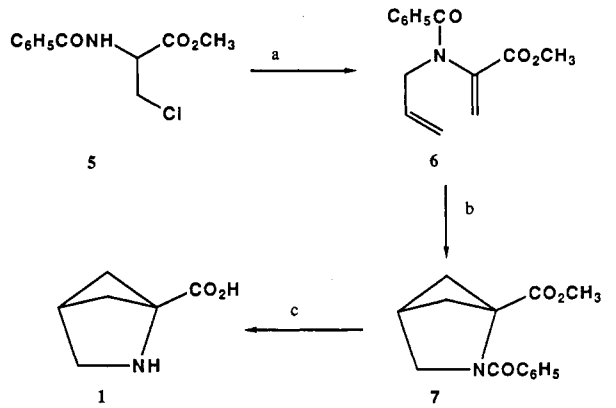

4

Besides their possible function as antifeedants, amino acids **1** and **2** are achiral proline and glutamic acid analogues, respectively. To facilitate investigation of their natural roles and to allow for other possible uses, we report here efficient syntheses of compounds **1–4**.[3]

## Results

Our approach to the synthesis of the bicyclic amino acid **1** was suggested by the work of Liu and Hammond on the photochemistry of myrcene.[4] Acetophenone-sensitized photocyclization of myrcene leads cleanly to a bicyclo-[2.1.1]hexane. The analogous azahexadiene photoprecursor for the synthesis of the desired azabicyclo[2.1.1]hexane was synthesized in a straightforward manner (Scheme I).

**Scheme I**[a]




[a] (a) THF/KO-*t*-Bu, $CH_2$=$CHCH_2Br$; (b) $h\nu$, acetophenone, Pyrex; (c) 6 N HCl.

Serine was converted by known methods to the crystalline methyl 2-benzamido-3-chloropropionate (**5**) (83%).[5] The photoprecursor, azahexadiene **6**, was then prepared in one pot from chloride **5** by sequential dehydrohalogenation and amide allylation. Addition of chloride **5** to potassium *tert*-butoxide (2.2 equiv) in THF at −78 °C followed by allyl bromide (12 equiv) gave, after being warmed to room temperature and stirred for 4 h, the photoprecursor **6** (94%). Ether extraction followed by concentration gave product of sufficient purity for the next

† Taken from the thesis of P. Hughes, Cornell University, 1983.

‡ Present address: Box CN 8000, Wyeth-Ayerst Research, Princeton, NJ 08543-8000.

(1) Bell, E. A.; Querishi, M. Y.; Pryce, R. J.; Jansen, D. H.; Lemke, P.; Clardy, J. *J. Am. Chem. Soc.* **1980**, *102*, 1409–1412.

(2) Personal communication from R. Pryce.

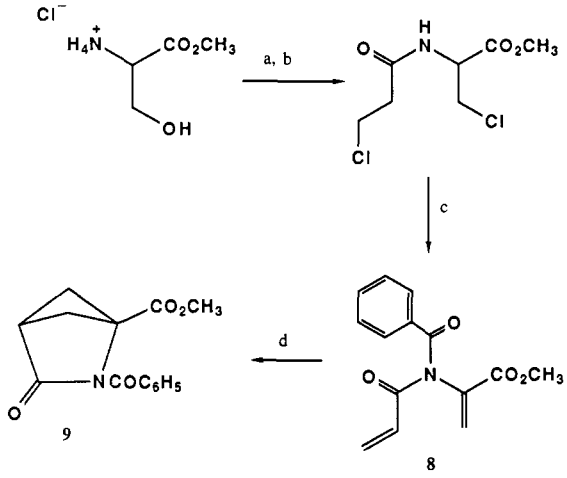
(3) The synthesis of 2,4-methanoproline (**1**) was previously reported without experimental detail. (a) Hughes, P.; Martin, M.; Clardy, J. *Tetrahedron Lett.* **1980**, *21*, 4579. (b) Pirrung, M. *Tetrahedron Lett.* **1980**, *21*, 4577. A synthesis of 2,4-methanoglutamic acid was recently reported: Gaoni, Y. *Tetrahedron Lett.* **1988**, *29*, 1591.

(4) Liu, R. S. H.; Hammond, G. S. *J. Am. Chem. Soc.* **1964**, *86*, 1892.

(5) Painter, E. P. *J. Am. Chem. Soc.* **1947**, *69*, 229–232.

0022-3263/88/1953-4793$01.50/0 

**Scheme II**[a]



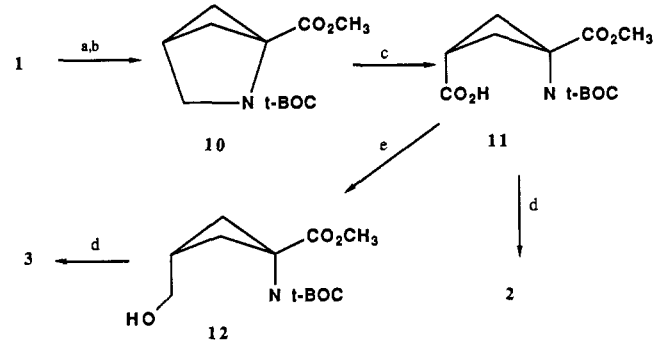

[a] (a) $H_2O/NaHCO_3/ClCH_2CH_2COCl$; (b) $SOCl_2/CH_2Cl_2$; (c) KO-*t*-Bu/THF/$C_6H_5COCl$; (d) *hν*, acetophenone, Pyrex.

reaction. A 2% solution of hexadiene **6** in benzene containing 0.2% acetophenone was irradiated through Pyrex with a medium-pressure Hanovia lamp for 12 h. Concentration and chromatography to remove the acetophenone gave the desired photoadduct **7** (87%). Hydrolysis of photoadduct **7** (6 N HCl, 100 °C, 15 h) gave, after an ether wash to remove benzoic acid and concentration of the aqueous layer, a quantitative yield of 2,4-methanoproline (**1**) as its hydrochloride salt. Passing the hydrochloride salt through a weak anion exchange resin (Amberlite IR-45A) gave the free amino acid **1**. Recrystallization from ethanol/water gave large cubes, which were spectroscopically indistinguishable from natural 2,4-methanoproline (**1**).[6]

Synthesis of the remaining amino acids could be accomplished by either photocyclization of a more highly oxidized hexadiene than **6** or subsequent oxidation of methanoproline (**1**). Initial attempts centered on the synthesis and photocyclization of hexadiene **8**. Unfortunately, hexadiene **8**, synthesized from serine (32% overall yield, Scheme II), proved to be highly susceptible to polymerization, especially under photocyclization conditions. By use of concentrations of 0.2% in hexadiene **8**, the isolated yield of the bicyclic product **9** was less than 3%. Photocyclization using high dilution conditions, achieved by slow addition of hexadiene **8** to a large volume of solvent, gave better yields of **9** (40%). However, this was only practical for the synthesis of small amounts of product. Attention was therefore turned to oxidation of methanoproline (**1**).

We felt that the best approach to oxidation of 2,4-methanoproline would be to acylate methanoproline and use any of the known amide to imide oxidation methods. The oxidation of proline to glutamic acid using ruthenium tetraoxide reported by Yoshifuji[7] provided the ideal model reaction. The appropriate oxidation precursor **10** (Scheme III) was prepared from 2,4-methanoproline (**1**) by acylation with di-*tert*-butyl dicarbonate (*t*-$BOC_2O$) followed by

**Scheme III**[a]




[a] (a) $((CH_3)_3COCO)_2O$/NaOH; (b) $CH_2N_2/Et_2O$; (c) $RuO_4$; (d) $HCl/H_2O$; (e) $BH_3$/THF.

diazomethane (80%). Oxidation with ruthenium tetraoxide using Sharpless conditions[8] gave acid **11** (66%), arising from the in situ hydrolysis of the desired oxidation product. Unfortunately, the reaction, though clean, generally took from 3 to 4 weeks to reach completion. This rate seemed to be independent of the *N*-acyl group. When cyclohexylcarbonyl was used instead of *t*-BOC, the reaction still took about 3 weeks to reach completion, although the imide remained intact. In contrast, the oxidation of proline to glutamic acid using the same acyl group is reported by Yoshifuji[7] to occur in 24 h.

The oxidation product **11** was hydrolyzed (6 N HCl, 100 °C, 15 h) (Scheme III) to give 2,4-methanoglutamic acid (**2**). The product, which crystallizes from the reaction mixture on cooling, was obtained in quantitative yield from **11** as its hydrochloride salt. The free amino acid was obtained by ion-exchange chromatography and recrystallized from water to give a product spectroscopically indistinguishable from natural 2,4-methanoglutamic acid (**2**).

The hydroxy amino acid **3** was formed via selective reduction (Scheme III) of the free carboxylic acid of oxidation product **11**. Treatment of **11** with borane (2.0 molar equiv, THF, 0 °C) gave the desired alcohol **12**. Acid hydrolysis (1 N HCl, 80 °C, 3 h) of **12** followed by anion-exchange chromatography gave the free hydroxy amino acid **3** (95% from **11**). The product was recrystallized from aqueous ethanol.

The only available sample of the natural hydroxymethyl amino acid **3** occurred as a minor impurity in the natural 2,4-methanoproline (**1**) sample. Comparisons were made of the synthetic hydroxy amino acid **3** with the minor impurity. Both had identical $R_f$'s on TLC in two different solvent systems and gave strong ninhydrin stains. Retention times on an ion-exchange amino acid analyzer (Technicon) were also identical. The mass spectrum of synthetic **3** gave a base peak at $m/z$ 87 and a parent ion at $m/z$ 146. These two peaks and others account well for the anomalous peaks[6] seen in the mass spectrum of our natural sample of 2,4-methanoproline (**1**). Most importantly, the 300-MHz $^1H$ NMR spectrum of the natural sample of **1** showed an impurity of about 15% with peaks corresponding exactly to the $^1H$ NMR spectrum of the synthetic hydroxy amino acid **3**.

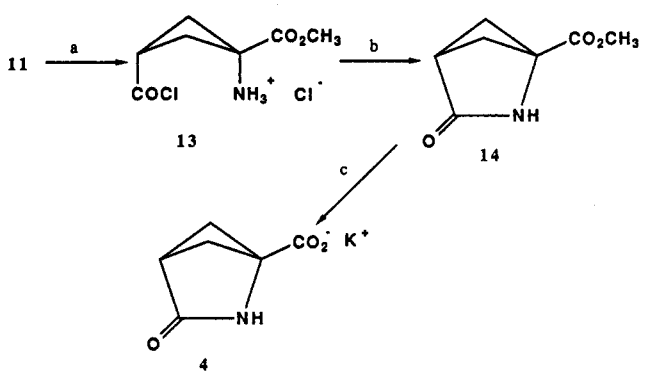
The bicyclic lactam, 2,4-methanopyroglutamic acid (**4**), was formed by reclosing the ring hydrolyzing during the ruthenium tetraoxide oxidation. Treatment of carbamate **11** (Scheme IV) with thionyl chloride (10 equiv, $CH_2Cl_2$,

(6) The mass spectrum of our synthetic material was markedly different from that of the natural product sample. The synthetic material showed no peaks at $m/z$ 146 or 87, whereas the natural sample showed both with $m/z$ 87 being the base peak. Also, the synthetic material did not give a positive ninhydrin reaction whereas the natural sample did. We were later able to show that these inconsistences were caused by a minor impurity in our natural sample corresponding to the amino acid of proposed structure **3**, as discussed below.

(7) Yoshifuji, S.; Matsumoto, H.; Tanaka, K.; Nitta, Y. *Tetrahedron Lett.* **1981**, *22*, 2963–2964.

(8) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938.

**Scheme IV**[a]




[a] (a) $SOCl_2/CH_2Cl_2$; (b) $NEt_3/CH_2Cl_2$; (c) $K_2CO_3/CH_3OH/H_2O$.

10 h) formed the acid chloride. The released HCl decomposes the *t*-BOC to give an intermediate proposed as the acid chloride amine hydrochloride salt **13**. A small amount of HCl gas was then bubbled through the reaction mixture to ensure *t*-BOC decomposition. The solvent and excess thionyl chloride were removed by vacuum, and the residue was added as a methylene chloride slurry in portions to a rapidly stirring solution of triethylamine in methylene chloride. The product, ester lactam **14**, was obtained after chromatography (Sephadex LH-20, $CH_3OH$) as a crystalline solid (80%).

The lactam **14** was saponified to the potassium salt of the desired bicyclic lactam, 2,4-methanopyroglutamic acid (**4**), by treatment with potassium carbonate in aqueous methanol. After concentration of the reaction mixture, $^1H$ NMR analysis of the residue in $D_2O$ indicated clean (>95%) formation of a new product similar to that of **14** but lacking the methyl signal. Attempts to remove the excess carbonate from the potassium salt of lactam **4** by gel filtration (Bio-gel P-2, $H_2O$) led to the formation of some 2,4-methanoglutamic acid (**2**), though some of the carbonate free lactam was obtained. The $^1H$ NMR spectrum of this material showed no change had occurred on chromatography, and the IR spectra of this material showed carbonyl stretches at 1720 and 1600 $cm^{-1}$, indicative of the lactam and the acid salt. Attempts to form the free acid of lactam **4** either by neutralization or ion-exchange chromatography gave only 2,4-methanoglutamic acid (**2**). In a weakly basic $D_2O$ solution at 2 °C, the lactam ring slowly opened over a period of weeks to cleanly give 2,4-methanoglutamic acid (**2**). The lactam reacted instantaneously with aqueous ammonium sulfate to give 2,4-methanoglutamine.

## Discussion

The goal of this work was to develop efficient syntheses of the four amino acids **1–4** so that they would be accessible for biological testing as free amino acids or as replacements for normal amino acids in peptides. Because of interest in the use of methanoproline (**1**) as a proline analogue, theoretical and experimental studies on its conformation in peptide fragments have been done.[9] A second goal was to prove the structural assignment of amino acid **3**. The third goal was to test the chemical viability of lactam **4** as a seed component. The synthesis of methanoproline (**1**) is quite efficient: 80% from known chloride **5** and 68% from serine. The syntheses of the other three target compounds are also efficient, being greater than 25% overall for each from serine. Unfortunately, one step, the ruthenium tetraoxide oxidation is quite slow though the yield is acceptable. The synthesis of (hydroxymethyl)-cyclobutane amino acid **3** and its subsequent comparison with the natural sample show that the proposed structure of **3** was correct. It is now apparent that lactam **4** has only a transient existence in neutral to acidic media and is a good acylating agent. Its lability also suggest that if it does play a role in long-term avoidance of the seeds of *A. herbert smithii*, it must be packaged in the seeds in a protective environment.

(9) (a) Montelione, G. T.; Hughes, P.; Clardy, J.; Scheraga, H. A. J. *J. Am. Chem. Soc.* **1986**, *108*, 6765–6773. (b) Talluri, S.; Montelione, G. T.; van Duyne, G.; Clardy, J.; Scheraga, H. A. J. *J. Am. Chem. Soc.* **1987**, *109*, 4473–4477. (c) Piela, L.; Némethy, G.; Scheraga, H. A. J. *J. Am. Chem. Soc.* **1987**, *109*, 4477–4485.

## Experimental Section

Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 299-B spectrophotometer. $^1H$ NMR spectra were recorded on a Varian EM-390 or a Bruker WM-300 at 90 and 300 MHz, respectively. $^{13}C$ NMR spectra were recorded on a JEOL FX-90Q at 22.49 MHz. Mass spectra were recorded on an AEI MS-902 spectrometer with a VG Micromass 2040 data reduction system. Microanalyses were done by Galbraith Laboratories Inc.

**Methyl *N*-Allyl-2-benzamidopropenoate (6).** Potassium *tert*-butoxide (2.0 g, 17.8 mmol) in THF (125 mL) at −78 °C under nitrogen was treated rapidly with a THF solution (50 mL) of methyl 3-chloro-2-benzamidopropanoate (**5**) (2.0 g, 8.28 mmol) followed by allyl bromide (14 mL, 19.6 g, 162 mmol). The reaction mixture was allowed to warm to room temperature, stirred for 4 h, and then partitioned between ether (200 mL) and water (100 mL). The ether layer was washed with brine, dried ($MgSO_4$), and concentrated to give 1.92 g (94%) of diene **6** as an oil pure enough for the photolysis reaction: IR (neat) 1739, 1669–1626 $cm^{-1}$; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 3.90 (3 H, s), 4.26 (2 H, d, $J$ = 12 Hz, additional small splitting), 5.08 (1 H, br s), 5.23 (1 H, d, $J$ = 12 Hz, additional small splitting), 5.63 (1 H, s), 6.02 (1 H, s), 5.60–6.2 (1 H, m), 7.23–7.67 (5 H, br m); $^{13}C$ NMR (22.49 MHz, $CDCl_3$) $\delta$ 51.73 (t), 52.27 (q), 117.94 (t), 122.05 (t), 127.77 (d), 127.95 (d), 130.16 (d), 132.72 (d), 135.52 (s), 140.23 (s), 164.18 (s), 170.68 (s); mass spectrum (EI), $m/z$ 245 (12%, $M^+$), 105 (100%, $C_6H_5CO$); HRMS, exact mass calcd for $C_{14}H_{15}NO_3$ 245.105, found 245.105.

**Methyl *N*-Benzoyl-1-azabicyclo[2.1.1]hexane-5-carboxylate (7).** Diene **6** (2.0 g, 8.2 mmol) was dissolved in benzene (100 mL) containing acetophenone (200 mg) and with a constant nitrogen purge was irradiated through Pyrex with a 450-W medium-pressure Hanovia lamp at room temperature for 12 h. The reaction mixture was concentrated, and the residue was chromatographed (silica gel, 3/2, hexane/ethyl acetate) to give 1.74 g (87%) of the photo adduct **7**: mp 124.5–125 °C; IR (KBr) 1754, 1629 $cm^{-1}$; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 1.80 (2 H, d, $J$ = 6 Hz, additional small splitting), 2.10–2.30 (2 H, br m), 2.73–2.87 (1 H, br m), 3.57 (2 H, br s), 7.4–7.93 (5 H, br m); $^{13}C$ NMR (22.49 MHz, $CDCl_3$) $\delta$ 35.23 (d), 41.60 (t), 51.91 (q), 54.89 (t), 69.85 (s), 128.13 (d), 128.25 (d), 131.23 (d), 134.20 (s), 168.71 (s), 173.66 (s); mass spectrum (EI), $m/z$ 245 (4.7%, $M^+$), 105 (100%, $C_6H_5CO$); HRMS, exact mass calcd for $C_{14}H_{15}NO_3$ 245.105, found 245.104.

**2,4-Methanoproline (1).** Methyl *N*-benzoyl-2,4-methanoproline (**7**) (1 g, 4.08 mmol) was added to 6 N HCl (50 mL) and heated at reflux for 15 h. The reaction mixture was concentrated to give 725 mg (98%) of methanoproline (**1**) as a crystalline hydrate hydrochloride salt. The crystals were dissolved in water and passed through a weak anion exchange column (Amberlite IR-45A, $OH^-$ form). The eluant was freeze-dried and recrystallized from aqueous ethanol to give the free methanoproline (**1**): mp 195 °C dehydration, 226–227 °C dec; IR (KBr) 1330, 1400, 1660, 3450 $cm^{-1}$; $R_f$ 0.25, 4/1/1, *n*-BuOH/$CH_3CO_2H$/$H_2O$, visualized by (1) 2% vanillin in ethanol, (2) 0.1 N KOH in 95% ethanol, (3) heat: $^1H$ NMR (300 MHz, $D_2O$–TFA) $\delta$ 1.46 (2 H, m, $J$ = 2.2, 5.9, 14.7 Hz), 2.07 (2 H, br m), 2.60 (1 H, m), 4.38 (2 H, br s); $^{13}C$ NMR (22.49, $D_2O$–TFA) $\delta$ 37.72 (d), 41.12 (t), 50.30 (t), 71.99 s), 169.36 (s); crystal lattice parameters: Pbca 10.162, 11.562, 11.887; mass spectrum (EI), $m/z$ 128 (6.2%, $M^+$ + 1), 127 (3.25%, $M^+$),

126 (7.6%, $M^+ - 1$), 109 (80%, $M^+ - H_2O$), 81 (100%, $M^+ - H_2O - CO$); mass spectrum (CI, methane) 128 (100%, $M^+ + 1$), 110 (62%, $M^+ - H_2O + 1$), 82 (50%, $M^+ - H_2O - CO + 1$); HRMS, exact mass calcd for $C_6H_9NO_2$ 127.0633, found 127.0624.

**Methyl *N*-(*tert*-Butyloxycarbonyl)-2,4-methanoproline (10).** 2,4-Methanoproline hydrate (1) (4.0 g, 2.7 mmol), sodium hydroxide (1.32 g, 33.1 mmol), and di-*tert*-butyl dicarbonate (8.8 g, 40.5 mmol) were dissolved in *tert*-butyl alcohol and water (100 mL each) and stirred at room temperature for 24 h. The *tert*-butyl alcohol was removed in vacuo, and the aqueous solution was extracted with ether (100 mL). The aqueous layer was then acidified to pH 2.0 and continuously extracted with methylene chloride. The methylene chloride solution was dried ($MgSO_4$) and treated with diazomethane in ether. After the excess diazomethane (acetic acid) was quenched, the reaction mixture was concentrated and distilled ((Kugelrohr, 80 °C (0.1mm Hg)) to give 5.5 g (83%) of **10** as a clear oil: IR (neat) 1750, 1704 $cm^{-1}$; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 1.39 (9 H, s), 1.46–1.67 (2 H, br dd), 2.04–2.08 (2 H, br m), 2.69–2.72 (1 H, br m), 3.42 (1 H, br s), 3.74 (3 H, s); $^{13}C$ NMR (22.49 MHz, $CDCl_3$) $\delta$ 28.02 (q), 34.45 (d), 42.44 (t), 51.61 (q), 52.21 (t), 69.79 (s), 80.34 (s), 157.15 (s), 168.83 (s); mass spectrum (EI), $m/z$ 141 (21%, $M^+ - C_4H_8 - CO_2$), 109 (42%, $M^+ - t$-BOC $- CH_3OH$), 57 ($C_4H_9$); mass spectrum (CI methane), $m/z$ 242 (11%, $M^+ + 1$), 186 (100%, $M^+ + 1 - C_4H_8$), 142 (95.5%, $M^+ - t$-BOC). Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.80. Found: C, 59.65; H, 7.91; N, 6.03.

**Methyl *N*-(*tert*-Butyloxycarbonyl)-2,4-methanoglutamic Acid Monoester (11).** The protected 2,4-methanoproline **10** (5.84 g, 24.2 mmol), sodium *m*-periodate (21.77 g, 101.8 mmol), and ruthenium dioxide dihydrate (40 mg, 2.42 mmol) were dissolved in a 3/2/2 mixture of water, acetonitrile, and carbon tetrachloride (170 mL total), stoppered, and stirred vigorously. Loss of starting material was monitored by GC (OV-101, 150–210 °C, 10 °C/min). After 3 weeks no starting material was detected. The reaction was quenched with 2-propanol, and the two-phase mixture was filtered through Celite followed by an ethyl acetate wash. The organic solvents were removed under vacuum, and the remaining aqueous slurry was extracted with ethyl acetate (2 × 250 mL). The product was then extracted into aqueous sodium bicarbonate (3 g of $NaHCO_3$ in 100 mL). The aqueous layer was titrated with 1 N HCl to pH 2.5 and extracted into fresh ethyl acetate (2 × 200 mL). The ethyl acetate was then washed with brine, dried ($MgSO_4$), and passed through a Florisil column (15 × 2.5 cm). The eluant was concentrated to give 4.21 g (66%) of the protected methanoglutamic acid **11** as white crystals: mp 144–147 °C; IR (KBr) 3324 (br), 3180 (v br), 1730, 1725, 1675, 1520 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 1.40 (9 H, br s), 2.4–2.6 (0.72 H, v br m), 2.6–2.9 (1.28 H, v br m), 2.8–2.9 (2 H, br t), 3.15–3.3 (1 H, v br m), 3.80 (3 H, br s), 5.35–5.5 (0.45 H, br s), 6.3–6.53 (0.25 H, br s); $^{13}C$ NMR (22.49 MHz, $CDCl_3$) $\delta$ 28.16 (q), 31.47 (br d), 35.11 (br t), 52.69 (q), 54.53 (s), 80.99 (br s), 173.96 (s), 178.90 (s); mass spectrum (EI), $m/z$ 172 (3.6%, $M^+ - t$-BOC), 145 (8%, $M^+ - C_4H_7 - CH_2CHCO_2H$), 101 (40.7%, 145 $- CO_2$), 57 ($C_4H_7$); mass spectrum (CI, methane), $m/z$ 274 (3%, $M^+ + 1$), 174 (80.4%, $M^+ + 1 - C_4H_8 - CO_2$). Anal. Calcd for $C_{12}H_{19}NO_6$: C, 52.74; H, 7.01; N, 5.13. Found: C, 52.80; H, 6.91; N, 5.14.

**2-Methanoglutamic Acid (2).** Methyl *N*-(*tert*-butyloxycarbonyl)-2,4-methanoglutamic acid mono ester (**11**) (1 g, 3.66 mmol) was added to 6 N HCl (50 mL) and heated at reflux for 15 h. The reaction mixture was cooled slowly to room temperature and set aside for 1 day. The product, large cubelike crystals of the methanoglutamic acid (**2**) hydrate hydrochloride salt, was collected by filtration by using a Teflon filter to give 750 mg (96%). To obtain the free acid of 2,4-methanoglutamic acid (**2**), the crystals were dissolved in a minimal amount of 1 N sodium hydroxide solution and added to a weak anion-exchange column (Amberlite IR-45A, $OH^-$ form). The column was rinsed with water until the sodium hydroxide had eluted (pH 7), and then the 2,4-methanoglutamic acid was eluted with 1 N acetic acid. The acid wash was done quickly to avoid crystallization of the product in the column. The eluant was concentrated, and the crystalline residue was recrystallized from hot water to give the free 2,4-methanoglutamic acid (**2**): mp 170 °C dehydration, 245–250 °C dec; IR (KBr) 1575, 1675, 2400–3100 (v br), 3440, 3510 $cm^{-1}$; $^1H$ NMR (300 MHz, $D_2O$–NaOD) $\delta$ 1.86 (2 H, pseudo-dt, $J$ = 2.6, 9.6 Hz), 2.4 (2 H, pseudo-dt, $J$ = 2.6, 9.6 Hz), 2.66 (1 H, pseudo-quint, $J$ = 9.1 Hz); $^{13}C$ NMR (22.49 MHz, $D_2O$–NaOD) $\delta$ 33.64 (d), 40.10 (t), 56.14 (s), 184.67 (s), 185.88 (s); mass spectrum (EI), $m/z$ 160 (12.2%, $M^+ + 1$), 142 (5%, $M^+ + 1 - H_2O$), 114 (11.3%, $M^+ - H_2O - CO$), 87 (100%, $NH_2C(CH_2)CO_2H$; HRMS, exact mass calcd for $C_6H_9NO_4$ ($M^+ + 1$ peak) 160.0610, found 160.0614.

**Methyl *cis*-1-[(*tert*-Butyloxycarbonyl)amino]-3-(hydroxymethyl)cyclobutanecarboxylate (12).** The oxidation product **11** (250 mg, 0.92 mmol) in THF (12 mL) at 0 °C was treated with borane (2.2 mL, 0.83 M in THF, 1.83 mmol) and stirred for 15 min. The reaction mixture was then slowly treated with brine (25 mL) and extracted with ethyl acetate (125 mL). The orgnic layer was dried ($MgSO_4$), filtered, and concentrated to give 216 mg (91%) of the desired alcohol **12** as a crystalline solid: mp 74–75 °C; IR (KBr) 1700, 3300–3500 $cm^{-1}$; $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 3.4 (9 H, s), 2.2 (2 H, br m), 2.6 (2 H, br m), 2.9 (1 H, br m), 3.4 (2 H, br d), 3.71 (3 H, s); $^{13}C$ NMR (22.49 MHz, $CDCl_3$) $\delta$ 28.2 (q), 29.15 (d), 29.75 (t), 52.45 (q), 54.71 (s), 65.44 (t), 79.98 (s), 154.83 (s), 175.79 (s); mass spectrum (EI), $m/z$ 203 (7.4%, $M^+ - C_4H_8$), 145 (21%, $M^+ - NCO_2$-*t*-Bu), 101 (40%, $CO_2$ - *t*-Bu), 57 (100%, $CCH_3)_3$); mass spectrum (CI, isobutane), $m/z$ 260 (0.9%, $M^+ + 1$), 160 (100%, $M^+ + 1 - C_4H_8 - CO_2$). Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.48; H, 8.02; N, 5.34.

***cis*-1-Amino-3-(hydroxymethyl)cyclobutanecarboxylic Acid (3).** Protected hydroxy amino acid **12** (150 mg, 0.58 mmol) was added to 1 N HCl and heated at 80 °C for 3 h. The reaction mixture was cooled to room temperature and concentrated to give 112 mg (97%) of crystalline hydroxy amino acid **3** as its hydrochloride hydrate. The crystals were dissolved in water and passed down a weak anion-exchange column (Amberlite IR-45A, $OH^-$ form). The eluant was freeze-dried and recrystallized from aqueous ethanol to give the free hydroxy amino acid **3**: mp 185 °C dehydration, 250–260 °C dec; IR (KBr) 1400, 1525, 2500–3100 (v br), 3400 (br); $^1H$ NMR (300 MHz, $D_2O$) $\delta$ 1.91 (2 H, m $J$ = 10 Hz, additional small coupling), 2.43 (2 H, m, $J$ = 10 Hz, additional small coupling), 2.47 (1 H, m, $J$ = 5.7 Hz, additional small coupling), 3.41 (2 H, d, $J$ = 5.7 Hz); $^{13}C$ NMR (22.49 MHz, $D_iO$) $\delta$ 30.28 (d), 33.53 (t), 55.9 (s), 65.91 (t), 177.7 (s); mass spectrum (EI), $m/z$ 146 (3%, $M^+ + 1$), 100 (3.4%, $M^+ + 1 - H_2O - CO$), 87 (100%, $NH_2C(CH_2)CO_2H$); HRMS, exact mass calcd for $C_6H_{11}NO_3$ 145.0739, found 145.0751; exact mass calcd for $C_6H_{11}NO_3$ ($M^+ + 1$) 146.0817, found 146.0816.

**Methyl 2,4-Methanopyroglutamate (14).** The oxidation product **11** (1 g, 3.66 mmol) was dissolved in methylene chloride (50 mL) containing thionyl chloride (4.35 g, 36.6 mmol) and stirred. After 12 h, the reaction mixture was saturated with HCl gas, stirred an additional hour, and concentrated to remove the solvent and the unreacted thionyl chloride. The solid residue was then slurried in fresh methylene chloride (15 mL) and added in small portions (2–3 mL each) to a rapidly stirred solution of triethylamine (1.48 g, 14.6 mmol) in methylene chloride (15 mL). The mixture was then concentrated and chromatographed on LH-20 (2.5 × 95 cm) in methanol. The fractions were analyzed by TLC (silica gel, 1/1, hexanes/ethyl acetate, detection by $Cl_2$–starch iodide, $R_f$ 0.15). The active fractions were concentrated to give 450 mg (80%) of crystalline product **14**: mp 76.5–77.5 °C; IR ($CCl_4$) 1765, 1750 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 2.60–2.62 (2 H, br dd), 2.78–2.83 (3 H, m), 3.78 (3 H, s), 5.75–5.9 (1 H, br s); $^{13}C$ NMR (22.49 MHz, $CDCl_3$) $\delta$ 46.19 (d), 52.57 (q), 55.49 (t), 64.78 (s), 167.22 (s), 182.48 (s); mass spectrum (EI), $m/z$ 155 (5.7%, $M^+$), 127 (21.6%, $M^+ - CO$), 123 (13%, $M^+ - CH_3OH$), 112 (19.5%, $M^+ - OCNH$), 96 (49%, $M^+ - CO_2CH_3$), 68 (100%, $C_3H_2NO$); HRMS, exact mass calcd for $C_7H_9NO_3$ 155.0582, found 155.0576.

**2,4-Methanopyroglutamic Acid (4).** Methyl 2,4-methanopyroglutamate (**14**) (50 mg, 0.32 mmol) was dissolved in 80% aqueous methanol (5 mL), treated with potassium carbonate (50 mg, 0.36 mmol), and stirred for 2 h. The mixture was concentrated and then dissolved in $D_2O$ for NMR analysis. $^1H$ NMR and $^{13}C$ NMR analyses showed clean conversion (>95%) to a single new product. $^1H$ NMR (300 MHz, $D_2O$–$K_2CO_3$) $\delta$ 2.44 (2 H, m, $J$ = 1.7, 4.2 Hz), 2.57 (1 H, t, $J$ = 2.5 Hz), 2.63 (2 H, m, $J$ = 1.7, 2.5, 4.2 Hz); $^{13}C$ NMR (300 MHz, $D_2O$–$K_2CO_3$) $\delta$ 46.42 (d), 57.45 (t), 69.01 (s), 175.32 (s), 189.44 (s). The product was chromatographed on Bio-gel P-2 in water to remove the excess potassium and carbonate. The fractions containing clean product were combined and freeze-dried: IR (KBr) 1340, 1440, 1720, 3100–3600 $cm^{-1}$.