(18) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).
(19) I. L. Webb, "Enzyme and Metabolic Inhibitors", Vol. I, Academic Press, New York, N.Y., 1963.
(20) H. Suzuki in "Electronic Absorption Spectra and Geometry of Organic Molecules", Academic Press, New York, N.Y., 1967, p 269.
(21) G. B. Robertson, *Nature (London)*, **191**, 593 (1961).

Potential Inhibitors of Collagen Biosynthesis. 5,5-Difluoro-DL-lysine and 5,5-Dimethyl-DL-lysine and Their Activation by Lysyl-tRNA Ligase

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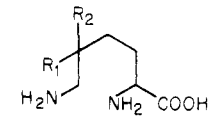
The synthesis of lysine analogues wherein blocking groups are substituted at position 5, the site of hydroxylation by peptidyl lysine hydroxylase, is described. Thus, 5,5-difluorolysine (1) and 5,5-dimethyllysine (2) were synthesized via a four- and six-step sequence, respectively, starting from ketone precursors. The propensity for these lysine analogues to be incorporated into procollagen protein in vivo was assessed by their ability to stimulate the lysine-dependent ATP–PP_i exchange reaction in the presence of lysyl-tRNA ligase in vitro. The difluoro analogue 1 stimulated exchange, but at a K_m (1.3×10^{-3} M) 1000 times greater than that for lysine itself. The dimethyl analogue 2 did not stimulate exchange, but at high concentrations was a competitive inhibitor of lysine, with an apparent K_i of 1.6×10^{-2} M. Thus, electronegative and/or bulky substituents at the 5 position of lysine cannot be tolerated by lysyl-tRNA ligase, and this position must be kept free in lysine analogues specifically designed to block collagen biosynthesis.

A number of reports linking aberrant collagen production with pathologic defects found in a variety of disease states[1] and in tumor growth[2] suggest that agents that can selectively control collagen biosynthesis might be of therapeutic utility. Recent advances in our knowledge of collagen biosynthesis[3] indicate the importance of lysyl and hydroxylysyl residues in collagen for the formation of intra- and intermolecular cross-links, such cross-links being vital to the structural integrity and metabolic stability of collagen. Cross-link formation involves the oxidative deamination of the ϵ-amino groups of lysyl or hydroxylysyl residues in collagen to reactive aldehydes by the enzyme, lysyl oxidase. These aldehydes serve as focal centers for cross-linking via aldol condensation or aldimine formation.

The biosynthesis of hydroxylysine,[4] specific for collagen and one other protein, viz., the Clq component of complement,[5] involves the introduction of a hydroxyl group at the 5 position of lysyl residues in procollagen, a collagen precursor, by an α-ketoglutarate-dependent mixed function oxidase, viz., peptidyl lysine hydroxylase (PLH, E.C. 1.14.11.4; lysine, 2-oxoglutarate dioxygenase[6]). This 5-hydroxyl group on lysyl residues is also a position for polysaccharide attachment. The uniqueness of this hydroxylation step to collagen and the essential role of hydroxylysine in cross-link formation suggest that lysine analogues, wherein hydroxylation and/or cross-link formation can be blocked, might selectively inhibit collagen biosynthesis.

Certain synthetic lysine analogues have been reported to substitute for L-lysine in proteins, viz., 4-thialysine,[7] 4-azalysine,[8] 4-selenolysine,[9] and *trans*-4,5-dehydrolysine.[10] Incorporation of the latter in a collagen synthesizing system has been reported to generate abnormal procollagens which were poorly secreted and thus were subject to ready degradation.[11] Therapeutic attempts to limit collagen production in patients with progressive systemic scleroderma by administration of agents that inhibit cross-linking have met with only limited success due to adverse side reactions. Such agents, e.g., β-aminoproprionitrile[12] or penicillamine,[13] act by either preventing aldehyde formation or by entrapping the reactive aldehydes necessary for cross-link formation.

5,5-Difluoro-DL-lysine (1) and 5,5-dimethyl-DL-lysine (2),

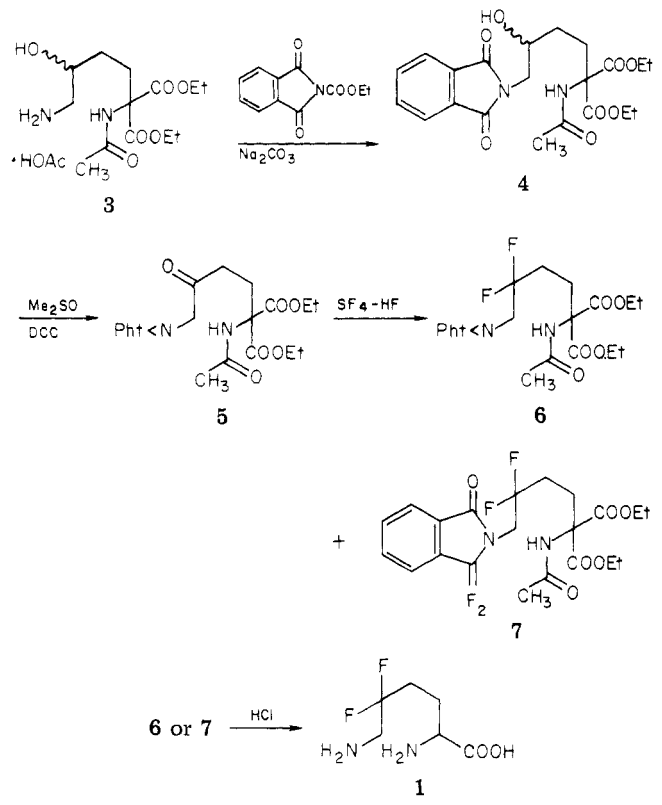


1, $R_1 = R_2 = F$
2, $R_1 = R_2 = CH_3$

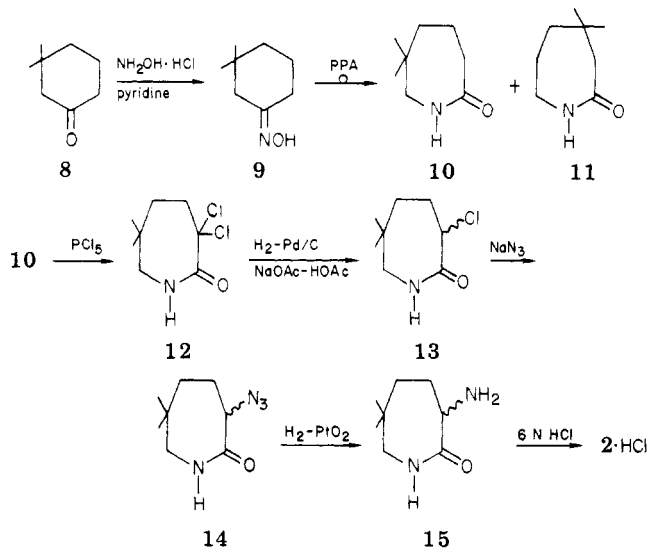
the title compounds projected for synthesis, represent lysine analogues bearing geminal blocking groups at the 5 position. This geminal substitution was designed to serve two purposes, viz., (1) to block hydroxylation at this site by PLH and (2) to prevent the aldol condensation of the aldehyde generated by oxidative deamination of the ϵ-amino group by virtue of α,α-disubstitution on the newly generated aldehyde group. Inhibition of collagen biosynthesis by 1 and 2 can therefore be envisioned to take place by formation of abnormal procollagen which would have decreased propensity for extracellular secretion and/or cross-link formation.

Chemistry. The synthesis of 1 (Scheme I) started with the substituted aminomalonic ester derivative 3 described by Zyl et al.[14] Protection of the ω-amino group of 3 was achieved by phthaloylation with *N*-carbethoxyphthalimide[15] to give 4. Oxidation of the unprotected secondary alcohol function of 4 with Me_2SO–DCC in the presence of pyridinium trifluoroacetate[16] gave a product whose elemental analyses and infrared and NMR spectra were consistent with the expected structure 5. Fluorination of 5 with SF_4–HF[17] gave a mixture of products which was resolved into three components by preparative TLC. They were, in the order of their increasing R_f values, the unreacted ketone 5 and two other compounds whose elemental analyses pointed to the desired difluorinated product 6 and a tetrafluorinated product 7. Comparison of the IR spectra of 6 and 7 with 5 suggested that only the ketone function in 6 was fluorinated, whereas in 7 both the

Scheme I



Scheme II



ketone group as well as one of the phthalimide carbonyls had been fluorinated. Thus, the phthalimide carbonyl absorption at 1778 and 1715 cm^{-1} present in **5** and retained in **6** was absent in **7**, while the ketone carbonyl absorption at 1740 cm^{-1} was absent in both **6** and **7**.

Corroborative evidence for these structural assignments was adduced by comparison of the NMR spectra of **6** and **7** with that of **5**. The symmetrical A_2B_2 pattern for the four aromatic protons exhibited by **5** was retained in the spectrum of **6**. A more complex pattern for these aromatic protons was displayed by **7** as would be expected by their now unsymmetrical environment. In addition, the singlet due to the ϵ-methylene group at δ 4.40, present in **5**, was replaced by triplets (J = 13 Hz) in **6** (δ 4.07) indicating the presence of an adjacent CF_2 group. Hydrolysis of either **6** or **7** in 6 N HCl gave the desired 5,5-difluoro-DL-lysine (**1**).

The synthesis of 5,5-dimethyl-DL-lysine (**2**) followed Scheme II. Thus, 3,3-dimethylcyclohexanone (**8**), prepared by catalytic reduction of 3-chloro-5,5-dimethyl-2-cyclohexenone,[18,19] was converted to its oxime **9**, which on Beckman rearrangement in polyphosphoric acid (PPA) yielded a mixture of isomeric lactams, viz., 6,6-dimethylhexahydro-2*H*-azepin-2-one (**10**) and 4,4-dimethylhexahydro-2*H*-azepin-2-one (**11**). Fractional crystallization[20] of the mixture from hexane gave pure **11**, mp 140–141 °C, and mother liquor residues greatly enriched in **10**. Repeated chromatography of the latter on neutral alumina followed by crystallization gave **10**, mp 81–82 °C, in 35% yield. Comparison of the NMR spectra of **10** and **11** indicated that **10** was essentially free of **11**.[21] Reaction of **10** with PCl_5 gave the dichloro lactam **12**, which was converted to the monochloro lactam **13** by hydrogenolysis. Nucleophilic displacement of the chloro group in **13** with NaN_3 led to the 2-azido derivative **14**. Catalytic hydrogenation of **14** gave the amino lactam **15**. Subsequent hydrolysis of **15** in 6 N HCl gave the desired 5,5-dimethyl-DL-lysine (**2**), isolated as the hydrochloride.

As was the case with the substituted prolines,[22] the methane chemical ionization mass spectra (CI–MS) of the two 5,5-disubstituted lysine analogues **1** and **2** fully corroborated the structures of these amino acids. The fragmentation pathway for 5,5-dimethyl-DL-lysine (**2**) was consistent with and predictable from the known fragmentation of L-lysine itself,[23] viz., the presence of a prominent quasimolecular ion (MH^+) and major fragment ions due to the loss of ammonia ($MH^+ - NH_3$) followed by a further loss of the elements of $COOH_2$ from the latter. A more complex CI–MS was observed for 5,5-difluoro-DL-lysine due to the superimposition of the loss of HF on this basic fragmentation pattern. In addition to the $(MH - 29)^+$ and $(MH - 64)^+$ ions, fragment ions due to loss of the elements of HF appeared at m/e 163, 146, 143, and 97.

The basic dissociation constants (pK_a) of the ϵ-amino group for 5,5-dimethyllysine (**2**) were similar to that for L-lysine itself (Table I), while the presence of the electronegative *gem*-difluoro group β to this amino group in **1** reduced the pK_a by 3.7 units. Substitution of sulfur for the methylene group at position 4 of lysine as in 4-thialysine dropped the pK_a of this ϵ-amino group only slightly, viz., to 9.52.[24]

5,5-Difluoro-DL-lysine was eluted ahead of L-lysine, while 5,5-dimethyl-DL-lysine was eluted after L-lysine from a standard cation-exchange column programmed to elute basic amino acids from biological fluids (Figure 1, see paragraph at end of paper regarding supplementary material).

Biological Results. The anticipated biological action of the lysine analogues **1** and **2** depends on their ability

Table I. Comparison of pK_a and Kinetic Parameters in the ATP-PP_i Exchange Reaction of Lysine and Related Analogues

Compd	pK_a (NH_2^+)[a]	K_m (or K_i),[c] M	V_{max}, nmol/min/mg
L-Lysine	10.61 ± 0.06	2.33×10^{-6} ± 0.39 5×10^{-7}[d]	35.7
2	10.19 ± 0.03	(1.62×10^{-2})	
4-Thialysine	9.52[b]	1.4×10^{-6}[d]	
1	6.88 ± 0.06	1.31×10^{-3} ± 0.06	25.7

[a] Determined by the method of Albert and Serjeant.[33] [b] Reported by Hermann and Lemke.[24] [c] For Lineweaver-Burk plots, see Supplementary Material. [d] Reported by Stern and Mehler.[7]

Table II. Effect of 5,5-Difluoro-DL-lysine on Peptidyl Lysine Hydroxylase Activity in L-929 Fibroblasts

5,5-Difluoro-DL-lysine,[a] mM	Release of ^3H as ^3HOH from [4,5-^3H]lysylprotocollagen,[b] cpm/mg of protein
0.00	1228
0.01	1220
0.05	1266
0.10	1229
0.20	1274
1.0	1073
2.0	1082
5.0	920

[a] Media contained 0.3 mM L-lysine. [b] Determined by the method of Miller.[32]

to be accepted by the aminoacyl ligase specific for L-lysine followed by incorporation into procollagen proteins. Therefore, the ability of these synthetic lysine analogues to stimulate the amino acid dependent ATP–PP_i exchange reaction in the presence of lysyl-tRNA ligase,[7] the first step in this sequence, was determined and compared to L-lysine itself.

5,5-Difluoro-DL-lysine (1) stimulated exchange in the presence of the activating enzyme, but at a K_m which was 1000-fold higher than L-lysine and with a V_{max} which was 72% that of L-lysine. In contrast, 2 was not a substrate at concentrations as high as 25 mM. However, at these high concentrations 2 was a competitive inhibitor of lysine with an apparent K_i of 1.6×10^{-2} M (Table I).

The presence of 1 at concentrations as high as 5.0 mM in a collagen secreting L-929 fibroblast system had no effect on the rate of incorporation of [^3H]lysine or [^{14}C]proline into proteins. Under the same conditions 1 appeared to be slightly inhibitory toward LPH at 5.0 mM (Table II) but had no effect on PPH activity (data not shown).[25] 1 and 2, when tested for antitumor activity against P-388 lymphocytic leukemia cells in suspension culture,[26] did not significantly inhibit their growth even at concentrations as high as 0.55 mM. Under the same test conditions 4-thialysine (0.5 mM) inhibited cell growth by 93% and *N*-hydroxyurea (0.15 mM) by 87%.

Discussion

The substrate structural requirements for lysyl-tRNA ligase have been examined by several investigators.[7,10] This enzyme is capable of tolerating minor alterations in the side chain of this trifunctional amino acid, e.g., insertion of a nitrogen atom or unsaturation into the chain, provided that the α- and ω-amino groups are placed a certain minimal distance apart. The strategic replacement of H by F in position 5 of lysine as in 1 was not expected to introduce large steric changes in the molecule, based on the atomic radius of F and the shortness of the C–F bond.[27] Thus, it was anticipated that 1 would be activated by lysyl-tRNA ligase and incorporated into collagen.

However, the presence of the *gem*-difluoro group β to the ϵ-amino group in 1 drastically decreased the pK_a of this amino group and altered the ability of 1 to act as a substrate in the ATP–PP_i exchange reaction. The inability of 5,5-dimethyllysine (2) to act as a substrate in the exchange reaction indicates that the exclusion of 5-hydroxylysine[28] as a substrate must be due solely to steric considerations, the pK_a of the ϵ-amino group being only minimally affected by methyl or hydroxyl substitution at this position. Moreover, replacement of $-CH_2-$ by $-S-$ at position 4 of lysine yields an analogue wherein the pK_a of the ϵ-amino group is only slightly reduced (by one pK_a unit), and thialysine is only slightly less active than L-lysine in the lysine-dependent ATP–PP_i exchange reaction. However, in the second esterification step with lysyl-tRNA ligase, a higher specificity prevails, and the transfer of thialysine to lysyl-tRNA from thialysyladenylate occurs at a K_m value 1000 times higher than the K_m for L-lysine.[7]

These results suggest that lysyl-tRNA ligase is unable to tolerate electronegative or bulky groups at the 5 position of lysine. It can be anticipated, therefore, that analogues of lysine which do not possess activation rates comparable to L-lysine would have a low probability of being esterified by tRNA and being incorporated into collagenous proteins. These conclusions are supported by the inability of 1 to prevent the incorporation of [^{14}C]proline or [^3H]lysine into protein at concentrations as high as 5.0 mM by a collagen secreting L-929 fibroblast system.[25]

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus or in a Mel-Temp apparatus and are corrected to reference standards. All solvent evaporations were carried out in vacuo on a mechanical rotating evaporator using water aspiration. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn., or by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. Spectrometers used were IR, Beckman IR-10; NMR, Varian A-60D; EI–MS, Hitachi Perkin-Elmer RMU-6 (ionization energy, 70 eV; ion source temperatures as indicated). CI–MS were provided by Dr. Roger Foltz, Battelle Columbus Laboratories, Columbus, Ohio, using an AEI-MS-902 mass spectrometer equipped with an SRIC Model CIS-2 combined CI–EI ion source. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter, and pK_a values were determined using a Beckman Century SS expanded scale pH meter equipped with a Beckman Futura combination electrode and a Mettler digital buret.

Acetylamino[4-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-3-hydroxybutyl]propanedioic Acid Diethyl Ester (4). The acetate salt 3 was prepared as described by Zyl et al.[14] To a vigorously stirred solution of Na_2CO_3 (2.92 g, 55 mmol) and *N*-carbethoxyphthalimide[15] (12.05 g, 55 mmol) in 50 mL of H_2O was added 18.2 g of 3 (50 mmol) in 30 mL of H_2O. After stirring for 2 h, the reaction mixture was extracted twice with $CHCl_3$. The combined extracts were washed (H_2O), dried (Na_2SO_4), and evaporated to dryness. Recrystallization of the residue from EtOAc–Et_2O gave 13.3 g (61.7%) of white crystals: mp 138–141 °C; IR (KBr) 3400 (OH), 1780, 1720 (phthalimide C=O), 1750 (ester C=O), 1675 cm^{-1} (amide C=O). Anal. ($C_{21}H_{26}N_2O_8$) C, H, N.

Acetylamino[4-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-3-oxobutyl]propanedioic Acid Diethyl Ester (5). To 434 mg (1.0 mmol) of 4 in 3 mL of anhydrous $(CH_3)_2SO$ and 3 mL of anhydrous benzene was added 0.08 mL of anhydrous pyridine followed by 0.04 mL of 99% trifluoroacetic acid.[16] Dicyclohexylcarbodimide (0.62 g, 3.0 mmol) was added and the reaction mixture stirred for 20 h at room temperature. The reaction mixture was then poured into ice water and extracted with $CHCl_3$ (3×75 mL). The $CHCl_3$ extracts were dried (Na_2SO_4) and concentrated in vacuo, and residual $(CH_3)_2SO$ was removed by lyophilization. The residual solids were extracted into dioxane and filtered. Evaporation of the dioxane gave 0.34 g (79%) of white solids. An analytical sample was recrystallized from EtOAc–Et_2O: mp 180–182 °C; IR (KBr) 3250 (NH), 1778, 1715 (phthalimide C=O), 1760 (sh, ester C=O), 1740 (ketone C=O), 1665 cm^{-1} (amide C=O). Anal. ($C_{21}H_{24}N_2O_8$) C, H, N.

Acetylamino[4-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)-3,3-difluorobutyl]propanedioic Acid Diethyl Ester (6) and Acetylamino[4-(1,3-dihydro-1,1-difluoro-3-oxo-2*H*-isoindol-2-yl)-3,3-difluorobutyl]propanedioic Acid Diethyl Ester (7). The ketone 5 (500 mg, 1.16 mmol) and 315 μL of H_2O were sealed in a 40-mL monel Parr reaction bomb equipped with both a gas inlet and outlet valve. The system was evacuated at 12–15 mmHg for 10–15 min (H_2O aspirator) while cooling in a dry ice–acetone bath. The outlet valve was closed and gaseous SF_4 allowed to fill the evacuated bomb through the inlet valve for 15 min. Based on weight difference, 37.0 g (0.342 mol) of SF_4 was added. The bomb was shaken for 6 h at room temperature and cooled in a dry ice–acetone bath and the contents vented

slowly through 5 N NaOH. Residual SF_4 and HF were removed by evacuating the system briefly. The clear residual glass was dissolved in $CHCl_3$ and taken to dryness in vacuo. The solids were dissolved in $CHCl_3$ and charged on a 2 × 31 cm column of silica gel (30–70 mesh). Elution with $CHCl_3$ gave a mixture which was separated into four bands by preparative TLC on silica gel PF_{254} using EtOAc–hexane (1:1) as the solvent. Each band was extracted with $CHCl_3$–MeOH (9:1) and the silica gel removed by centrifugation of the extract through spin thimbles (Reeve Angel). The band with lowest R_f contained **5** (0.02 g, 0.4%), the next band **6** (0.32 g, 60.8%), the third **7** (0.10 g, 18.1%), and the fourth, 0.03 g of an unidentified compound. The combined yield of **6** and **7** was 78.9%. An analytical sample of **6** was recrystallized from EtOAc–petroleum ether (bp 30–60 °C): mp 108–110 °C; IR (KBr) 3230 (NH), 1780, 1718 (phthalimide C=O), 1760 (ester C=O), 1640 cm^{-1} (amide C=O); NMR δ (from Me_4Si, $DCCl_3$) 1.28 (t, 6 H, OCH_2CH_3), 1.84 (t, 2 H, $-CH_2-$), 2.08 (s, 3 H, $-COCH_3$), 2.67 (m, 2 H, $-CF_2CH_2-$), 4.07 (t, 2 H, $-NCH_2CF_2-$), 4.30 (q, 4 H, $-OCH_2CH_3$), 6.80 (s, 1 H, NH), 7.92 (m, 4 H, aromatic). Anal. ($C_{21}H_{24}N_2O_7F_2$) C, H, N, F. An analytical sample of **7** was recrystallized from Et_2O–hexane: mp 108–110 °C; IR (KBr) 3240 (NH), 1765 (ester C=O), 1750, 1740 (fluorophthalimide C=O), 1650 cm^{-1} (amide C=O); NMR δ (from Me_4Si, $DCCl_3$) 1.27 (t, 6 H, OCH_2CH_3), 1.78 (m, 2 H, $-CH_2-$), 2.05 (s, 3 H, $-COCH_3$), 2.65 (m, 2 H, $-CF_2CH_2$), 3.94 (t, 2 H, $-NCH_2CF_2-$), 4.27 (q, 4 H, $-OCH_2CH_3$), 6.75 (s, 1 H, NH), 7.75 (s, 4 H, aromatic). Anal. ($C_{21}H_{24}N_2O_6F_4$) C, H, N, F.

5,5-Difluoro-DL-lysine (1). A suspension of **7** (0.76 g, 1.6 mmol) in 6 N HCl in sealed reactor tubes was hydrolyzed at 100 °C for 12 h in a micro Carius furnace. After cooling, the clear reaction mixture was extracted with $CHCl_3$ and the aqueous layer evaporated to dryness in vacuo. The residue was dissolved in H_2O, adjusted to pH 1, and charged on a 2 × 13 cm column of Dowex 50W X4 (NH_4^+). The column was washed with distilled H_2O until the effluent no longer contained Cl^-. The difluorolysine **1** was eluted with 1 N NH_3. Recrystallization of **1** from aqueous EtOH gave 0.24 g (83%) of product: mp 228–231 °C dec; IR (KBr) 3390, 3300, 2750–2680, 1660–1585, 1415, and 1350 cm^{-1}. Hydrolysis of **6** in the same manner gave the identical product **1**. An analytical sample was recrystallized from H_2O: mp 227–230 °C dec; CI–MS (methane) m/e (rel intensity) 183 (78, MH^+), 166 (8, $MH^+ - NH_3$), 163 (8, $MH^+ - HF$), 146 (16, $MH^+ - NH_3 - HF$), 143 (54, $MH^+ - 2HF$), 137 (22, $MH^+ - HCO_2H$), 120 (100, $MH^+ - NH_3 - HCO_2H$). Anal. ($C_6H_{12}N_2O_2F_2$) C, H, N, F.

3,3-Dimethylcyclohexanone (8). A solution of 3-chloro-5,5-dimethyl-2-cyclohexenone[18] (7.93 g, 0.050 mol) and sodium acetate (18 g) in 50 mL of HOAc was hydrogenated over 2.0 g of 1% Pd/C at 3.3 kg/cm^2 (47 lb/in.^{2}) for 1.5 h. After removal of the catalyst, the filtrate was adjusted to pH 7 and subjected to continuous extraction with $CHCl_3$ in a liquid–liquid extractor for 18 h. The $CHCl_3$ layer was dried (Na_2SO_4) and evaporated on the steam bath. Distillation of the residual oil gave 4.98 g of **8** (76% yield), bp 65–67 °C (13–14 mmHg) [reported[19] 75.5 °C (25 mmHg)].

4,4-Dimethylhexahydro-2*H*-azepin-2-one (11) and 6,6-Dimethylhexahydro-2*H*-azepin-2-one (10). 3,3-Dimethylcyclohexan-2-one (**8**) (60.0 g, 0.475 mol) was converted to its oxime and the oxime was subjected to Beckman rearrangement by procedures previously described.[29] The yield of isomeric lactams was 57.45 g (86%). This crude mixture was digested in 150 mL of boiling hexane (three times). The hexane-insoluble residue was recrystallized from CH_2Cl_2–hexane to give 12.4 g of the 4,4-dimethyl isomer **11**, mp 140–141 °C (sublimes >100 °C). The hexane extracts (400 mL) were evaporated to dryness and subjected to fractional crystallization[20] using hexane as the crystallizing solvent. Using this procedure, an additional 7.5 g of the 4,4-dimethyl isomer, mp 139–141 °C, was obtained. The solids obtained from the fractional crystallization mother liquors above were dissolved in hexane (700 mL) and passed through a 3.8 × 41 cm column of neutral alumina (Merck Darmstadt, activity grade I). The early hexane eluates contained the pure 6,6-dimethyl isomer **10**, mp 80–82 °C. Further elution with hexane followed by chloroform yielded a mixture of isomers, mp 60–68 °C, and final elution with $CHCl_3$–MeOH (1:1) yielded fractions enriched with the 4,4-dimethyl isomer **11**. Digestion of the latter with hexane yielded additional quantities of the pure **11**. The mother liquors were evaporated, redissolved in hot hexane, and rechromatographed (twice) on a smaller (2.8 × 40 cm) column using the elution sequence above to yield additional quantities of the pure isomers. By this procedure 20.3 g (30%) of the recrystallized 6,6-dimethyl isomer **10** was obtained: mp 81–82 °C; NMR δ (from Me_4Si, $DCCl_3$) 0.90 (s, 6 H), 1.54 (m, 4 H), 2.37 (m, 2 H), 2.89 (d, 2 H); NMR δ ($DCCl_3$–D_2O) 2.9 (d, 2 H). Anal. ($C_8H_{15}NO$) C, H, N. The recrystallized 4,4-dimethyl isomer **11** was obtained in 33% yield (22.4 g): mp 140–141 °C (sublimes >100 °C); NMR δ (from Me_4Si, $DCCl_3$) 1.03 (s, 6 H), 1.6 (m, 4 H), 2.37 (s, 2 H), 3.17 (m, 2 H); NMR δ ($DCCl_3$–D_2O) 3.14 (t, 2 H, J = 4 Hz). Anal. ($C_8H_{15}NO$) C, H, N.

3,3-Dichloro-6,6-dimethylhexahydro-2*H*-azepin-2-one (12). **10** (22.7 g, 0.16 mol) was chlorinated using procedures described previously[29] to give 29.3 g (87%) of white crystals: mp 129–129.5 °C; IR (CH_2Cl_2) 3410 (NH), 1690 cm^{-1} (amide C=O). Anal. ($C_8H_{13}NOCl_2$) C, H, N.

3-Chloro-6,6-dimethylhexahydro-2*H*-azepin-2-one (13). **12** (21.01 g, 0.10 mol) was hydrogenolyzed using Pd/C as a catalyst[29] to give 9.75 g (56%) of white crystals of **13**: mp 101.5–103 °C; NMR δ (from Me_4Si, $DCCl_3$) 6.7 (NH, 1 H) 4.47 (m, 1 H, $-CH_2CHCl-$), 3.02 (q, 1 H, $-CH_2NH-$), 2.93 (q, 1 H, $-CH_2NH-$), 1.78 (m, 4 H), 0.95 (s, 6 H); IR (CH_2Cl_2) 3420 (NH), 1675 cm^{-1} (amide C=O). Anal. ($C_8H_{14}NOCl$) C, H, N.

3-Azido-6,6-dimethylhexahydro-2*H*-azepin-2-one (14). A mixture of **13** (14.15 g, 0.08 mol), sodium azide (6.00 g, 0.092 mol), ethanol (8 mL), and H_2O (18 mL) was heated under reflux for 20 h. The solvent was removed in vacuo and the residue was extracted into CH_2Cl_2 (3 × 25 mL). The CH_2Cl_2 extract was dried over Na_2SO_4, decolorized with charcoal, and concentrated in vacuo. Addition of hexane gave 10.5 g (72%) of **14** as colorless plates. An analytical sample which was recrystallized from ether and dried in vacuo at 50 °C for 4 h melted at 100–101 °C; IR (CH_2Cl_2) 3410 (NH), 2100 (azide), 1680 cm^{-1} (amide C=O). Anal. ($C_8H_{14}N_4O$) C, H, N.

3-Amino-6,6-dimethylhexahydro-2*H*-azepin-2-one (15). To a solution of **14** (10.5 g, 0.058 mol) in ethanol (100 mL) was added platinium oxide (0.4 g) and the mixture hydrogenated at 3.5 kg/cm^2 (50 psi) starting pressure. When H_2 uptake ceased, the catalyst was removed by filtration and the solvent evaporated in vacuo. The residue was recrystallized from THF–ether to give 7.72 g (86%) of white crystalline **15**. An analytical sample recrystallized from ether gave colorless needles: mp 116–118 °C; IR (CH_2Cl_2) 3430 (NH), 1675 cm^{-1} (amide C=O). Anal. ($C_8H_{16}N_2O$) C, H, N.

5,5-Dimethyl-DL-lysine Monohydrochloride (2). The azidolactam **14** (2.72 g, 0.015 mol) was reduced as described above. The filtrate was evaporated in vacuo, and the residue was dissolved in 6 N HCl (20 mL) and heated under reflux for 24 h. After decolorization with charcoal the solution was evaporated to dryness. The residue was dissolved in 95% ethanol (30 mL) and pyridine (1.20 g, 0.015 mol) was added. After 18 h at room temperature, the precipitate was filtered. The solids were dissolved in H_2O and decolorized with charcoal. Concentration of the filtrate to 2 mL and dilution with hot ethanol (10 mL) gave the monohydrochloride of **2** as colorless needles (1.27 g, 40.2%): mp 281.8–282.6 °C; CI–MS (methane) m/e (rel intensity) 175 (29, MH^+), 158 (33, $MH^+ - NH_3$), 157 (10, $MH^+ - H_2O$), 129 (3, $MH^+ - COOH_2$), 112 (100, $MH^+ - NH_3 - COOH_2$); CI–MS (isobutane) m/e (rel intensity) 175 (100, MH^+), 157 (15, $MH^+ - H_2O$). Anal. ($C_8H_{19}N_2O_2Cl$) C, H, N.

Ion-Exchange Chromatography (Figure 1, Supplementary Material). A Beckman Spinco Model 120 amino acid analyzer was used with PA-35 cation-exchange resin, column size 0.9 × 23 cm, flow rate 75 mL/h. A mixture containing 0.5 μmol of each of the amino acids was applied to the column; L-lysine and L-ornithine served as markers.

In Vitro Enzyme Inhibition and Growth Inhibition Studies. Lysyl-tRNA ligase, isolated from *Escherichia coli* B,[7] had a specific activity of 1.0 unit/mg of protein. One unit of enzyme activity is equivalent to the incorporation of 1 μmol of [^{32}P]pyrophosphate into ATP per milligram of protein in 15 min under the assay conditions described.[7] K_m values for L-lysine and 5,5-difluorolysine were determined using the Lineweaver–Burk relationship. Inhibition of L-lysine stimulated exchange was determined at fixed inhibitor concentrations: 1.0 mM for 5,5-

dimethyllysine (based on the L isomer). Apparent K_i values were determined from the slopes of the double reciprocal plots of L-lysine in the presence and absence of inhibitor (concentrations given in terms of the L isomer) using the values determined for K_m/V_m for L-lysine.[30]

The effect of varying concentrations of 1 on the incorporation of [^{3}H]lysine and [^{14}C]proline into TCA precipitable proteins of L-929 fibroblast cells was determined according to Kerwar et al.[31] LPH activity was assayed by the method of Miller.[32] Growth inhibition of P-388 lymphoid leukemia cells in tissue culture was evaluated using varying concentrations of 1 and 2 according to published procedures.[26]

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Supplementary Material Available: Figure 1, ion-exchange chromatography of 1 and 2, and Figures 2 and 3, Lineweaver–Burk plots of the ATP-PP_i exchange reaction with lysyl-tRNA ligase and 5,5-difluoro-DL-lysine and 5,5-dimethyl-DL-lysine (3 pages). Ordering information is given in any current masthead page.

References and Notes

(1) (a) K. I. Kivirikko and L. Risteli, *Med. Biol.*, **54**, 159 (1976); (b) M. Rojkind and D. Kershenobich, "Progress in Liver Diseases", Vol. V, H. Popper and F. Schaffner, Ed., Grune and Stratton, New York, N.Y., 1976, pp 294–310; (c) H. Popper and S. Udenfriend, *Am. J. Med.*, **49**, 707 (1970); (d) J. Uitto and J. R. Lichtenstein, *J. Invest. Dermatol.*, **66**, 59 (1976); (e) G. C. Fuller, A. L. Matoney, D. O. Fisher, N. Fausto, and G. J. Cardinale, *Atherosclerosis*, **24**, 483 (1976); (f) J. Uitto and D. J. Prockop, "Inflammation and Anti-inflammatory Therapy: Proceedings of the Symposium", G. Katona and J. Blengio, Ed., Halsted Press, New York, N.Y., 1975, pp 129–132; (g) A. Ooshima, G. C. Fuller, G. J. Cardinale, S. Spector, and S. Udenfriend, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3019 (1974).

(2) (a) K. R. Curtroneo, N. A. Guzman, and A. G. Liebelt, *Cancer Res.*, **32**, 2828 (1972); (b) J. Zimmerberg, O. Greengard, and E. W. Knox, *ibid.*, **35**, 1009 (1975); (c) B. D. Smith, G. R. Martin, E. J. Miller, A. Dorfman, and R. Swarm, *Arch. Biochem. Biophys.*, **166**, 181 (1975); (d) M. Gordon, S. Zyborie, B. Gardner, J. Patti, and H. Gray, *Cancer*, **29**, 509 (1972).

(3) (a) G. N. Ramachandran and A. H. Reddi, "Biochemistry of Collagen", Plenum Press, New York, N.Y., 1976; (b) A. J. Bailey and S. P. Robins, *Sci. Prog. (Oxford)*, **63**, 419 (1976); (c) M. L. Tanzer, *Science*, **180**, 561 (1973); (d) E. J. Miller and P. B. Robertson, *Biochem. Biophys. Res. Commun.*, **54**, 432 (1973); (e) A. J. Bailey, S. P. Robins, and G. Balian, *Nature (London)*, **251**, 105 (1974); (f) P. Bornstein, *Annu. Rev. Biochem.*, **43**, 567 (1974).

(4) (a) F. M. Sinex and D. D. VanSlyke, *J. Biol. Chem.*, **216**, 245 (1955); (b) F. M. Sinex, D. D. VanSlyke, and D. R. Christman, *ibid.*, **234**, 918 (1959).

(5) K. Yonemasu, R. M. Stround, W. Neidermeier, and W. T. Butler, *Biochem. Biophys. Res. Commun.*, **43**, 1388 (1971).

(6) "Enzyme Nomenclature (1972)", American Elsevier, New York, N.Y., p 110.

(7) R. Stern and A. H. Mehler, *Biochem. Z.*, **342**, 400 (1965).

(8) J. Kolc, *Collect. Czech. Chem. Commun.*, **34**, 630 (1969).

(9) C. DeMarco, V. Busiello, M. DiGirolamo, and D. Cavallini, *Biochem. Biophys. Acta*, **454**, 298 (1976).

(10) E. M. Lansford Jr., N. M. Lee, and W. Shive, *Arch. Biochem. Biophys.*, **119**, 272 (1967).

(11) P. J. Christner and J. Rosenbloom, *J. Biol. Chem.*, **246**, 7551 (1971).

(12) H. R. Keiser and A. Sjoerdsma, *Clin. Pharmacol. Ther.*, **8**, 593 (1967).

(13) (a) E. D. Harris, Jr., and A. Sjoerdsma, *Lancet*, **2**, 996 (1966); (b) G. Asboe-Hansen, *Acta Dermatoyener (Stockholm)*, **55**, 461 (1975); (c) R. C. Siegel, *J. Biol. Chem.*, **252**, 254 (1977).

(14) G. VanZyl, E. E. VanTamelen, and G. D. Zuidema, *J. Am. Chem. Soc.*, **73**, 1765 (1951).

(15) G. H. L. Nefkens, G. K. Tesser, and R. J. F. Nivard, *Recl. Trav. Chim. Pays-Bas*, **79**, 688 (1960).

(16) K. E. Pfitzner and J. G. Moffat, *J. Am. Chem. Soc.*, **87**, 5670 (1965).

(17) (a) M. S. Raasch, *J. Org. Chem.*, **27**, 1406 (1962); (b) W. R. Hasek, W. C. Smith, and V. A. Engelhardt, *J. Am. Chem. Soc.*, **82**, 543 (1960); (c) D. G. Martin, *Ann. N.Y. Acad. Sci.*, **145**, 161 (1967).

(18) A. W. Crossley and H. R. LeSueur, *J. Chem. Soc.*, 117 (1903).

(19) A. W. Crossley and N. Renouf, *J. Chem. Soc.*, 80 (1907).

(20) A. A. Morton, "Laboratory Technique in Organic Chemistry", McGraw-Hill, New York, N.Y., 1938, p 162.

(21) Although our NMR data for pure **10** and **11** agree with that recently reported by T. Duong, R. H. Prager, J. M. Tippett, A. D. Ward, and D. I. B. Kerr [*Aust. J. Chem.*, **29**, 2667 (1976)], our melting points are not in agreement. Our isomeric compounds were deemed pure by GLC and NMR criteria; indeed, mixtures were analyzed by quantitative NMR measurements.

(22) F. N. Shirota, H. T. Nagasawa, and J. A. Elberling, *J. Med. Chem.*, **20**, 1176 (1977).

(23) G. W. A. Milne, T. Axenrod, and H. M. Fales, *J. Am. Chem. Soc.*, **92**, 5170 (1970).

(24) P. Hermann and K. Lemke, *Hoppe-Seyler's Z. Physiol. Chem.*, **349**, 390 (1968).

(25) S. S. Kerwar, personal communication.

(26) R. G. Almquist and R. Vince, *J. Med. Chem.*, **16**, 1396 (1973).

(27) L. Pauling, "The Nature of the Chemical Bond", 3rd ed, Cornell University Press, Ithaca, N.Y., 1960, pp 93, 260.

(28) A. H. Mehler, personal communication.

(29) H. T. Nagasawa, J. A. Elberling, P. S. Fraser, and N. S. Mizuno, *J. Med. Chem.*, **14**, 501 (1971).

(30) J. L. Webb, "Enzyme and Metabolic Inhibitors", Vol. I, Academic Press, New York, N.Y., 1963, pp 150–153.

(31) S. S. Kerwar, R. J. M. Marcel, and R. A. Salvador, *Arch. Biochem. Biophys.*, **172**, 685 (1976).

(32) R. L. Miller, *Anal. Biochem.*, **45**, 202 (1972).

(33) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases", Wiley, New York, N.Y., 1962, pp 1–68.