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Fluorescent 1,4-Dihydropyridines: The Malondialdehyde Connection

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Abstract: Under suitable conditions, malondialdehyde is capable of modifying amino acid residues to novel, highly fluorescent 1,4-dihydropyridines. The structures assigned to these compounds are supported by UV, HRMS, high-field NMR, and X-ray crystallographic data. The mechanism of these transformations, which is fully discussed, involves the Michael reaction of alkylidene malondialdehydes with enaminals, both of which are produced as detectable intermediates. These findings may be of significance in explaining some of the biological chemistry of malondialdehyde. The transformation also provides a new approach to the synthesis of a wide range of light stable 4-arylated-1,4-dihydropyridines of potential interest as calcium channel antagonists.

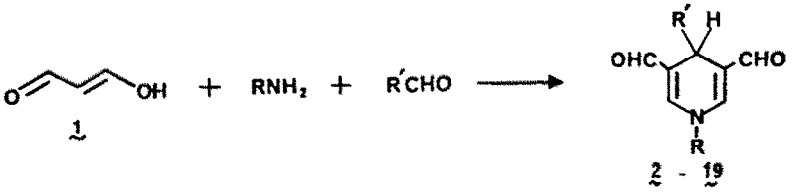
The ubiquitous natural metabolite, malondialdehyde (MDA), is an important carbonyl product of polyunsaturated lipid oxidation.[1-3] The radiolysis of carbohydrates and certain amino acids also produces this dialdehyde.[4,5] Malondialdehyde has long been of interest in food chemistry and its detection by the thiobarbituric acid (TBA) test has been used for the estimation of oxidative rancidity in foods.[1,2,6,7] The chemistry of MDA may be of considerable importance in degenerative processes *in vivo*,[8,9] because of its ability to interact with biological macromolecules.[10-13] For example, MDA is able to modify nucleic acids[14-17] and this is consistent with its observed mutagenicity.[9,10,18] The reactivity of MDA towards proteins to produce fluorescent crosslinked adducts has also been known for some time.[19,20]

Malondialdehyde is readily formed in blood plasma in response to thrombin and other substances that cause blood platelet aggregation.[21,22] It has been shown that hemoglobin A is modified by MDA and that this modified hemoglobin exhibited fluorescence spectra similar to that seen in the overall erythrocytic modification.[23] UV-Visible and fluorescence data on the modified proteins appear to be consistent with the formation of vinylogous amidines,[19,20,24] as well as highly fluorescent heterocyclic systems of unknown structure. This paper reports on model studies of MDA with amino acids and peptides that involve the detection, isolation, and complete characterization of heterocyclic systems of similar UV and fluorescence data as those reported in the aforementioned biological studies.[25] In addition, synthetic ramifications of these model studies are also reported.

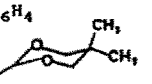
When MDA (1, 3 equiv) was allowed to react with amino acids (e.g. glycine methyl ester, 1 equiv) under aqueous acidic conditions for prolonged periods (> 40 h), the UV spectrum gradually underwent a bathochromic shift. Work-up and chromatographic purification gave low yields of a product which showed a HRMS molecular mass ion at m/z 223.0870. Its UV and

high-field ^1H and ^{13}C NMR data (including delayed decoupling) when taken collectively, suggested that the product was the 4-methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 2. The compound was highly fluorescent, emitting at 454 nm upon excitation at 386 nm with a relative quantum efficiency (Φ) of 0.36.[26] Interestingly, the glycine adduct 3 (i.e. the unprotected form of 2) has a Φ of 0.47 which makes it one of the most fluorescent dihydropyridine systems known. The efficiency of this fluorescence emission is particularly remarkable when compared to the well-known natural 1,4-dihydropyridine system, NADH (Φ= 0.02).

On further investigation, it was discovered that dihydropyridine 2 could be obtained in about 50% yield when MDA (2 equiv) was allowed to react with glycine methyl ester (1 equiv) in the presence of acetaldehyde (1 equiv) at pH 4.3[27] for 7 h. This transformation involving MDA was found to be general and related 1,4-dihydropyridines in about the same yields could be isolated from alanine, serine, methionine, and lysine methyl esters with acetaldehyde, propanal, pentanal, and benzaldehyde (Scheme 1). Studies with lysine were particularly important as a model study for protein modification as the only primary amino group in protein structures apart from the N-terminal α-amino groups is the ϵ-amino group of lysine. The UV and fluorescence spectra for 2 (see expt) are in general typical for dihydropyridines of all of the representative amino acids studied. They can be used for the detection of the formation of these 1,4-dihydropyridines from the modification of peptides by MDA. Two representative examples studied include glutathione and GlyHisLys.



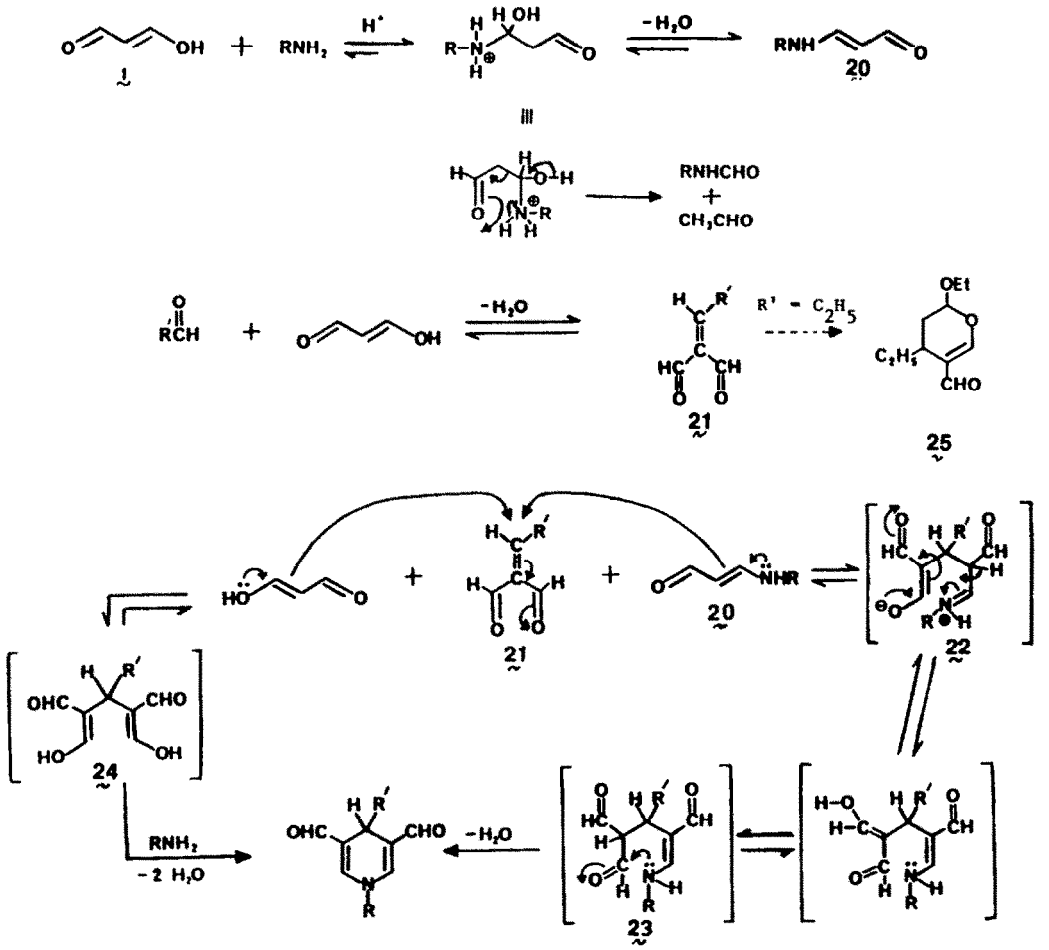
1 — 2 - 19

2	R = $CH_2CO_2CH_3$,	R' = CH_3	11	R = $CHCO_2CH_3$, $CH_2CH_2SCH_3$	R' = CH_3
3	R = CH_2CO_2H,	R' = CH_3			
4	R = $CH_2CO_2CH_3$,	R' = C_2H_5	12	R = Glu·Cys·Gly Side Chain,	R' = CH_3
5	R = CH_2CO_2H,	R' = C_2H_5	13	R = Gly·His·Lys Side Chain,	R' = CH_3
6	R = $CH_2CO_2CH_3$,	R' = C_4H_9	14	R = H, R' = C_2H_5	R' = C_2H_5
7	R = $CH_2CO_2CH_3$,	R' = C_6H_5	15	R = H,	R' = C_6H_5
8	R = $CHCO_2CH_3$, CH_2OH	R' = CH_3	16	R = H,	R' = 2Me-C_6H_4
			17	R = H,	R' = 2F-C_6H_4
9	R = $-(CH_2)_4CHCO_2CH_3$, $NHCOCH_3$	R' = CH_3	18	R = $CH_2CO_2CH_3$	R' = 
10	R = $CHCO_2CH_3$, CH_3	R' = CH_4H_9	19	R = $CH_2CO_2CH_3$,	R' = CH_2CHO

Scheme 1

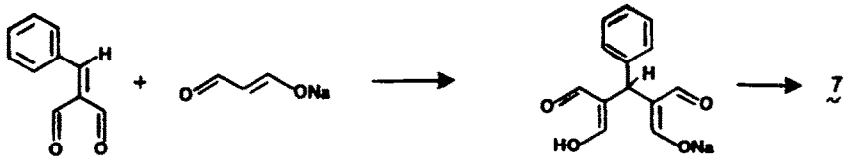
The aforementioned transformation to the dihydropyridines provides a new approach to the synthesis of a variety of N-unsubstituted dihydropyridines by replacement of the amino acid in these reactions with ammonia. For example, when MDA was treated with benzaldehyde and ammonium hydroxide at pH 4.2 at 60 °C for 3 h, 4-phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde (15) was isolated in 26% yield after chromatographic separation and crystallization. The synthesis has generality and may be used for the preparation of a wide variety of such N-unsubstituted compounds.

A plausible mechanism for the formation of the dihydropyridines derived from the amino acids (or ammonia) and MDA is shown in Scheme 2. The reaction apparently proceeds *via* the enaminal **20** and the alkylidene malondialdehyde **21**. The formation of the isolable intermediate **20**, which occurs relatively rapidly, can be clearly seen in the UV spectrum at 280 nm. Intermediate **21** (which can be trapped as the dihydropyran cycloadduct **25**[29]) is the result of an aldol condensation of MDA and an additional aldehyde (explained later), followed by dehydration.[28] This α,β-unsaturated dialdehyde then serves as a Michael acceptor for enaminal **20** to form **22** which can undergo cyclization *via* **23** followed by dehydration to give the 1,4-dihydropyridines.



Scheme 2

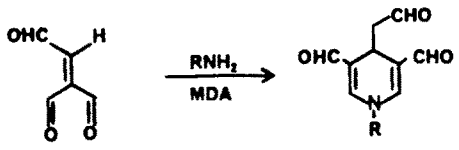
The formation of 1,4-dihydropyridines through the intermediacy of the "bis-MDA" derivative **24** was also examined. For example, benzyl "bis-MDA" (**24**, R'=Ph), a stable compound, can be easily prepared from benzylidene MDA and MDA (Scheme 3). It is smoothly converted to the 1,4-dihydropyridine **7** by reaction with glycine methyl ester. Propyl "bis-MDA" (**24**, R'=Et) gave similar results.



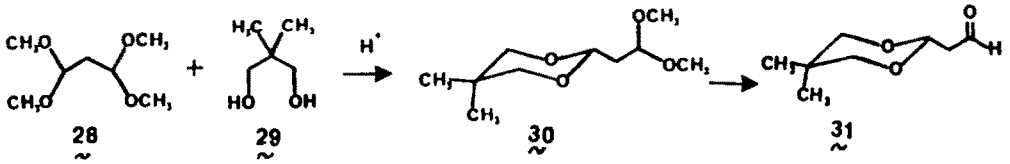
24 (R'=Ph, Na Salt)

Scheme 3

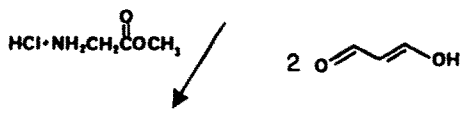
Malondialdehyde interacts with amino acids in the absence of added second aldehyde to give 1,4-dihydropyridines (e.g. **2**, **3**, **9**, etc) and this requires explanation. It is very likely, that in these reactions, the second aldehyde (acetaldehyde) is produced slowly from the thermal cleavage of the amino alcohol (hydrated enaminal) formed from the initial reaction of amino acid and malondialdehyde (Scheme 2). The requirement of the second aldehyde in the formation of 1,4-dihydropyridines raises the question as to why MDA does not itself serve in this role. If MDA behaved as the second aldehyde in this reaction, the alkylidene MDA **26** would form which would result in the 1,4-dihydropyridine system **27**. However, no dihydropyridines with this structure were isolated from any of the reactions studied.

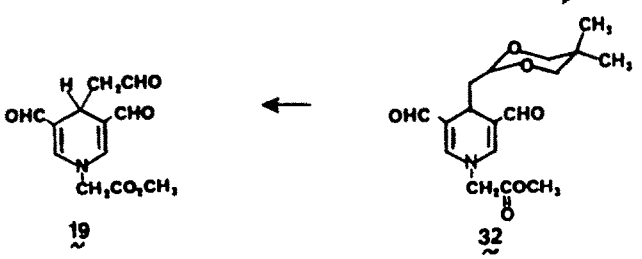


26 **27**



28 **29** **30** **31**





19 **32**

Scheme 4

The possibility that dihydropyridine 2 (and others) were derived by the *in situ* decarbonylation of 27 was also investigated through the unambiguous preparation of an authentic sample of 27 ($R=CH_2CO_2CH_3$, i.e. 19) (Scheme 4). Treatment of 1,1,3,3-tetramethoxypropane 28 with 2,2-dimethyl-1,3-propanediol 29 afforded the mixed bis-acetal of MDA 30 in 40% yield. Selective hydrolysis of 30 with oxalic acid and silica gel in a tetrahydrofuran/dichloroethane/water solution gave the monoacetal of MDA 31 in 79% yield. Utilization of 31 under the standard reaction conditions with MDA and glycine methyl ester afforded the dihydropyridine 32 in 20% yield. Careful hydrolysis of the cyclic acetal moiety was accomplished with pyridinium tosylate/p-toluenesulfonic acid in acetone which gave 19 in 38% yield. However, compound 19 was found to be thermally stable under the conditions used to produce the 1,4-dihydropyridines and even at much higher temperatures.

Finally, it should be mentioned that a number of 4-arylated 1,4-dihydropyridines related to some of the compounds synthesized in this paper are of considerable interest as calcium channel antagonists.[30,31] Some of these compounds are being used clinically in the treatment of various disorders of the cardiovascular system.[32] Two conformational requirements that appear to be important for the biological activity of known 4-arylated 1,4-dihydropyridines are the orthogonal orientation of the phenyl ring and the planarity of the dihydropyridine ring.[33,34] In order to confirm the structures of the dihydropyridines produced in these reactions and to examine the conformational properties of the 4-arylated compounds, we carried out a single crystal X-ray study on compound 15. The results are presented in Fig. 1 and show that the plane of the phenyl ring bisects approximately the dihydropyridine ring even though an ortho substituent is not present (cf. refs. 33,34). In addition, the dihydropyridine ring shows a relatively small deviation from planarity. Calcium channel antagonist activities for this and other dihydropyridines are currently being investigated.

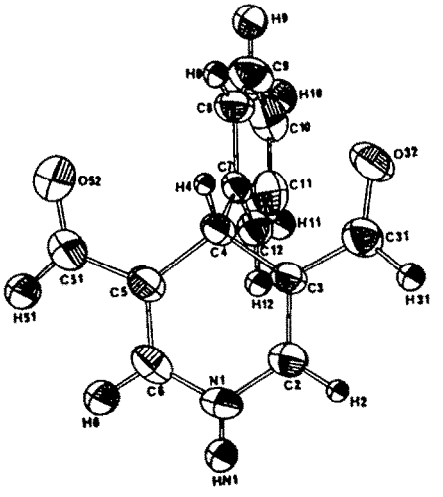


Figure 1. ORTEP plot showing the conformation of 15 determined from single crystal X-ray data.

In summary, it can be stated that MDA is able to modify amino acid residues to highly fluorescent 1,4-dihydropyridines. A mechanistic interpretation of these results has been suggested. These findings may be of significance in understanding the biological chemistry of MDA. Synthetic ramifications of this work includes a new approach to the synthesis of a variety of N-unsubstituted 1,4-dihydropyridines. In contrast to many known 1,4-dihydropyridines, the compounds produced in this study are remarkably light stable. Some of the dihydropyridines synthesized may be useful as fluorescent biological probes of the calcium channel in living systems and the X-ray crystallographic data provide strong structure-activity prediction for this activity.

Experimental Section

Melting points reported are uncorrected and were determined on a Thomas-Hoover melting point apparatus fitted with a microscope. The ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker WM-360 or a JEOL FX-90Q Fourier transform NMR spectrometer. Mass spectra were obtained on a Hewlett-Packard 5985 GC/MS system or a VG Analytical Model ZAB-HF instrument. Ultraviolet spectra were obtained on a Varian-Cary Model 219 ultraviolet-visible spectrophotometer. Fourier transform IR measurements were recorded on an IBM Model 98 instrument. Corrected fluorescence spectra were obtained on an SLM-Aminco SPF-500C spectrofluorimeter interfaced with an IBM personal computer. Relative quantum yields were determined by the method of Guilbault[26] using quinine sulfate (quantum yield 0.70) in 0.1N sulfuric acid as a reference. The X-ray structure was determined on an Enraf-Nonius CAD-4 diffractometer. Lyophilizations were performed on a Virtis Freezemobile 3. Air and moisture sensitive reagents and/or reaction products were handled and transferred, when necessary, in a Labconco glove box under a dry nitrogen atmosphere. Preparative layer chromatography plates for separating reaction mixtures were prepared by coating 20 x 20 cm glass plates with 65 ml each of a slurry prepared from 150 g of E. Merck PF-254 silica gel in 400 ml of water. The plates were air dried for two days, then activated at 135 °C for 4 hours prior to use.

General Procedure for the Formation of 1,4-Dihydropyridines.

(Procedure A). To a 50 ml RBF equipped with a stir bar was added acetate buffer (pH 4.2), sodium MDA (3 equiv),[35] and an amine or amino acid (1 equiv). The pH of this mixture was adjusted to 4.2 with 2M HCl or 2M NaOH. The reaction flask was sealed and stirred in a pre-heated oil bath for several hours. The solution was neutralized with 2M NaOH and the solvent was removed under reduced pressure. The residue was dissolved in a methanol-chloroform mixture and chromatographed on a silica gel (60-230 mesh) column. Fractions with the expected dihydropyridine UV spectrum were collected, pooled, and concentrated. For further purification, the material was chromatographed on silica gel (PF_{254}) preparative layer plates with a specific combination of methanol and chloroform as the developing solvent.

Procedure B. The amine or amino acid (1 equiv), aldehyde (1-2 equiv), and sodium MDA (2 equiv) were dissolved in acetate buffer (pH 4.2) in a 50 ml RBF equipped with a stir bar. The mixture was stirred for several minutes and then the pH was adjusted to 4.2. The reaction flask was sealed and heated in an oil bath for several hours. The reaction mixture was then neutralized with 2M NaOH, and the solvent was removed *in vacuo*. The residue was taken up in a methanol and chloroform mixture and run through a short silica scrubber column to remove NaCl and other polar impurities. Fractions with the appropriate dihydropyridine UV pattern were collected and pooled. The solvent was evaporated and the residue was chromatographed on silica gel plates with methanol-chloroform as the developing solvent.

4-Methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 2 from MDA and Glycine Methyl Ester by Procedure A. Glycine methyl ester hydrochloride (130 mg, 1.04 mmol) and sodium MDA (335 mg, 2.98 mmol) were stirred at 60 °C in 20 ml of water at pH 4.2 for 45 h. The reaction mixture was worked up and chromatographed as described above. The band with R_f 0.30 (5% CH_3OH-$CHCl_3$, 2 elutions) afforded 18 mg (8%) of 2 as a solid, mp 153 °C: UV (H_2O) λ_{max} 236 nm (ε 18,900), 262 nm (ε 7900), 384 nm (ε 8800); Fluorescence data (H_2O) excitation 386 nm, emission 454 nm (Φ = 0.36); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, 3H), 3.84 (m, 4H), 4.20 (s, 2H), 6.64 (s, 2H), 9.30 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.0, 53.0, 54.9, 124.4, 146.1, 168.1, 188.7; mass spectrum, m/z (relative intensity) (30 eV) 224 (M^++1, 1.5), 223 (M^+, 6.4), 208 (100), 149 (15.9); HRMS (EI) calcd for $C_{11}H_{13}O_4N$; 223.0845, found 223.0870.

4-Methyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 2 by Procedure B. Using this procedure with acetaldehyde (2 equiv) as the additional aldehyde, the reaction time was 3 h at 60 °C. Compound **2** was produced in 50% yield.

4-Ethyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 4 from Glycine Methyl Ester by Procedure B. Glycine methyl ester hydrochloride (136 mg, 1.08 mmol), sodium MDA (247 mg, 2.20 mmol) and propionaldehyde (0.08 ml, 1.10 mmol) were stirred in 20 ml of water at pH 4.2 at 55 °C for 4 h. The reaction mixture was worked up and chromatographed to give **4** as a yellow oil, 138 mg (54%). UV (H_2O) λ_{max} 238 nm (ε 19480), 262 nm (ε 8640), 385 nm (ε 8680); Fluorescence data (H_2O) excitation 390 nm, emission 455 nm (Φ = 0.36); ^1H NMR ($(CD_3)_2SO$) δ 0.66 (t, 3 H), 1.33 (m, 2 H), 3.71 (m, 4 H), 4.53 (s, 2 H), 7.35 (s, 2 H), 9.24 (s, 2 H); ^{13}C NMR ($(CD_3)_2SO$) δ 8.6, 25.7, 27.4, 52.2, 53.9, 119.7, 149.5, 169.1, 189.0; mass spectrum, m/z (relative intensity) (30 eV) 237 (M^+, 1.7), 222 (0.6), 208 (100), 179 (5.5), 178 (12.6), 176 (8.6), 164 (1.9), 150 (10.3), 149 (31.1); HRMS (EI) calcd for $C_{10}H_{10}O_4N(M^+-CH_2CH_3)$ 208.0609, found 208.0599.

4-Ethyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 5 from Glycine by Procedure B. Glycine (116 mg, 1.55 mmol), sodium MDA (354 mg, 3.16 mmol) and propionaldehyde (0.15 ml, 2.08 mmol) were stirred in 20 ml of water at pH 4.2 at 50 °C for 3 h. Compound **5** (139 mg, 40%) was obtained as yellow crystals: mp 185-186 °C; UV (H_2O) λ_{max} 238 nm (ε 21,819), 265 nm (ε 8423), 393 nm (ε 9792); Fluorescence data (H_2O) excitation 397 nm, emission 462 nm (Φ = 0.47). ^1H NMR ($(CD_3)_2SO$) δ 0.66 (t, 3 H), 1.34 (m, 2 H), 3.74 (t, 1 H), 4.41 (s, 2 H), 7.35 (s, 2 H), 9.24 (s, 2 H); ^{13}C NMR ($(CD_3)_2SO$) δ 8.7, 25.9, 27.4, 54.1, 119.7, 149.8, 170.0, 189.2; mass spectrum, m/z (relative intensity) (30 eV) 223 (M^+, 2.0), 208 (10.0), 194 (100), 178 (3.8), 176 (14.9), 165 (10.4), 149 (33.3), 136 (33.7), 92 (71.7); HRMS (EI) calcd for $C_{11}H_{13}O_4N$ 223.0845, found 223.0856.

4-Butyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 6 from Glycine Methyl Ester by Procedure B. Glycine methyl ester·HCl (252 mg, 2.01 mmol), sodium MDA (495 mg, 4.40 mmol), and pentanal (333 mg, 3.86 mmol) were stirred in 30 ml of pH 4.2 acetate buffer at 60 °C for 3 h. The reaction mixture was worked up and chromatographed to give **6** as an oil in 43% yield. UV (H_2O) λ_{max} 237 (ε 20,870), 264 (ε 8700), 385 (ε 9500); Fluorescence data (H_2O) excitation 389 nm, emission 464 nm (Φ = 0.28); ^1H NMR ($CDCl_3$) δ 1.56-0.75 (m, 9 H), 3.83 (s, 3 H), 3.97 (t, J = 4.4 Hz, 1 H), 4.33 (s, 2 H), 6.64 (s, 2 H), 9.30 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.7, 22.4, 26.6, 27.1, 33.6, 52.5, 54.5, 121.7, 147.4, 168.1, 188.7; mass spectrum, m/z (relative intensity) (30 eV) 265 (M^+, 0.7), 209 (12.0), 208 ($M^+-C_4H_9$, 100.0) 149 (9.9); HRMS (EI) calcd for $C_{14}H_{19}O_4N$ 265.1314, found 265.1290.

4-Methyl-1,4-dihydropyridine 3,5-dicarboxaldehyde 9 from α-N-Acetyllysine Methyl Ester (Procedure A). Sodium MDA (371 mg, 3.31 mmol) and α-N-acetyllysine-HCl (232 mg, 0.97 mmol) were dissolved in 20 ml of pH 4.2 acetate buffer. The reaction flask was stoppered and heated for 18 h at 60 °C. Work up and chromatography gave **9** as a yellow oil in 11% yield. UV (H_2O) λ_{max} 239 (ε 7,900), 267 (ε 8540), 399 nm (ε 7260); Fluorescence data (H_2O) excitation maxima 398 nm, emission maxima 464 nm (Φ = 0.35); ^1H NMR ($CDCl_3$) δ 1.08 (d, J = 6.8 Hz, 3 H), 1.95-1.34 (m, 6H), 2.02 (s, 3 H), 3.59-3.41 (m, 2 H), 3.74 (s, 3 H), 3.89 (q, 1 H), 4.75-4.50 (m, 1 H), 6.75 (s, 2 H), 9.27 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.0, 22.7, 22.8, 29.1, 31.6, 51.6, 52.1, 54.4, 123.4, 146.3, 170.1, 172.4, 188.7; mass spectrum, m/z (relative intensity) (30 eV) 337 (M^++1, 6.4), 336 (M^+, 28.3), 322 (M^+-CH_2, 11.8), 321 (M^+-CH_3, 79.2), 186 (18.5), 149 (26.0), 144 (63.5), 126 (100.0); HRMS (EI) calcd for $C_{17}H_{24}O_5N_2$ 336.1685, found 336.1696.

Adduct **9** was obtained in 25% yield from the reaction of α-N-acetyllysine methyl ester with acetaldehyde and MDA by Procedure B.

4-Phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde 15 by Procedure B. Ammonium hydroxide (262 mg, 2.16 mmol), sodium MDA (452 mg, 4.03 mmol), and benzaldehyde (437 mg, 4.12 mmol) were dissolved in 25 ml of pH 4.2 acetate buffer. The reaction mixture was sealed and stirred in an oil bath for 3 h at 60 °C. The reaction mixture was worked up and the product was isolated by preparative layer chromatography to give **15** as long needles (26%): mp 240 °C; UV (95% EtOH) λ_{max} 228 (ε 12920), 246 (ε 6640), 276 (sh) (ε 2520), 373 nm (ε 6850); Fluorescence data (H_2O) excitation 383 nm, emission 445 nm (Φ = 0.32); ^1H NMR ($(CD_3)_2SO$) δ 4.76 (s, 1 H), 7.17 (m, 5 H), 7.47 (s, 2 H), 9.25 (s, 2 H), 10.02 (brs, 1 H); ^{13}C NMR ($(CD_3)_2SO$) δ 32.8, 119.8, 125.9, 127.4, 127.8, 144.0, 145.5, 189.0; mass spectrum, m/z (relative intensity) (30 eV) 214 (M^++1, 2.9), 213 (M^+, 16.5), 154 (10.1), 136 ($M^+-C_6H_5$, 100.0) 128 (13.7); HRMS (EI) calcd for $C_{13}H_{11}O_2N$ 213.0790, found 213.0815.

4-(2-Methylphenyl)-1,4-dihydropyridine-3,5-dicarboxaldehyde (16) was prepared by Procedure B as described for 15. Compound **16** was obtained as light yellow crystals in 25% yield: mp 223 °C; UV (95% EtOH) λ_{max} 376 nm (ε 7530), 281.5 nm (ε 2140), 247 nm (ε 2530), 229.5 nm (ε 13120); Fluorescence data (95% EtOH) excitation 385 nm, emission 435 nm (Φ = 0.20); ^{13}C NMR ($(CD_3)_2SO$) δ 19.5, 29.2, 121.8, 125.7, 126.0, 128.3, 128.8, 134.9, 143.9, 145.8, 189.2; ^1H NMR ($(CD_3)_2SO$) δ 2.64 (s, 3 H), 4.83 (s, 1 H), 6.99 (m, 4 H), 7.46 (d, 2 H), 9.16 (s, 2 H), 9.93 (s, 1 H); mass spectrum, m/z (relative intensity) (30 eV) 228 (M^++1,

9.2), 227 (M^+, 45.4), 154 (16.6), 136 (100), 128 (16.6); HRMS (EI) calcd for $C_{14}H_{13}O_2N$ 227.0946, found 227.0952.

4-(2-Fluorophenyl)-1,4-dihydropyridine-3,5-dicarboxaldehyde (17) was prepared by Procedure B as described for 15. Compound 17 was obtained as yellow crystals (22%); mp 220 °C; UV (95% EtOH) λ_{max} 376 nm (ε 3710), 270 (ε 4960), 228 (ε 6870); Fluorescence data (95% EtOH) excitation 385 nm, emission 435 nm (Φ= 0.25); ^{13}C NMR ($(CD_3)_2SO$) δ 27.7, 119.2, 123.8, 127.7, 127.9, 130.5, 132.7, 144.2, 144.4, 188.9; ^1H NMR ($(CD_3)_2SO$) δ 4.94 (s, 1 H), 7.09 (m, 4 H), 7.47 (d, 2 H), 9.18 (s, 2 H), 10.04 (s, 1 H); mass spectrum m/z (relative intensity) (30 eV) 232 (M^++1, 9.2), 231 (M^+, 53.2), 154 (8.9), 136 (100); HRMS (EI) calcd for $C_{13}H_{10}O_2NF$ 231.0696, found 231.0718.

1,4-Dihydropyridine Trialdehyde 19. Sodium MDA (568 mg, 5.07 mmol) was treated with MDA-acetal 31 (398 mg, 2.52 mmol) and glycine methyl ester·HCl in 40 ml of water at pH 4.2 and 50 °C for 6 h (Procedure B). Work up and purification afforded 318 mg (38%) of 32 as yellow crystals: mp 182-184 °C; UV (H_2O) λ_{max} 235 nm (ε20,375), 259 nm (ε 9247), 373 nm (ε 9564); Fluorescence data (H_2O) excitation 385 nm, emission 455 nm (Φ = 0.21); ^1H NMR ($(CD_3)_2SO$) δ 0.64 (s, 3 H), 1.02 (s, 3 H), 1.53 (m, 2 H), 3.26 (d, 2 H, J = 11 Hz), 3.42 (d, 2 H, J = 11 Hz), 3.72 (s, 3 H), 3.76 (m, 1 H), 4.32 (m, 1 H), 4.56 (s, 2 H), 7.32 (s, 2 H), 9.21 (s, 2 H); ^{13}C NMR ($(CD_3)_2SO$) δ 21.4, 22.8, 23.2, 29.4 (two carbons), 52.2, 53.8, 76.0, 100.0, 120.0, 149.1, 169.2, 188.8; mass spectrum, m/z (relative intensity) (30 eV) 337 (M^+, 6.2), 308 (0.1), 279 (0.1), 278 (0.7), 264 (0.1), 235 (1.1), 234 (5.6), 208 (100), 195 (0.7), 179 (2.3), 150 (2.1); HRMS (EI) calcd for $C_{17}H_{23}O_6N$ 337.1525, found 337.1556.

1,4-Dihydropyridine 32 (133 mg, 0.39 mmol) was dissolved in 1:4 water:acetone (15 ml) and treated with pyridinium tosylate (102 mg, 0.41 mmol) and p-toluenesulfonic acid (18 mg, 0.10 mmol) and heated under reflux for 30 h. The reaction mixture was neutralized with a small amount of $NaHCO_3$ and the solvent removed *in vacuo*. The residue was chromatographed on a silica preparative layer plate with 4% methanol/dichloromethane. The band with R_f 0.49 afforded 38 mg (38% yield, 58% conversion) of 19 as a yellow oil: UV (H_2O) λ_{max} 234 nm (ε 18335), 259 nm (ε 7543), 380 nm (ε8108); Fluorescence data (H_2O) excitation 390 nm, emission 455 nm (Φ = 0.32); ^1H NMR ($CDCl_3$) δ 2.65 (dd, 2 H), 3.48 (t, 1 H), 3.85 (s, 3 H), 4.24, (s, 2 H), 6.76 (s, 2 H), 9.29 (s, 2 H), 9.73 (t, 1 H); ^{13}C NMR ($CDCl_3$) δ 23.8, 49.0, 53.1, 54.9, 120.9, 147.7, 168.1, 188.6, 201.2; mass spectrum, m/z (relative intensity) (30 eV) 251 (M^+, 3.2), 222 (3.3), 208 (100), 192 (1.5), 180 (5.8), 179 (4.9), 150 (4.3), 136 (4.8); HRMS (EI) calcd for $C_{12}H_{13}O_5N$ 251.0794, found 251.0774.

Propylidene-MDA and Formation of Diels-Alder Adduct 25 with Ethyl Vinyl Ether. Sodium MDA (231 mg, 2.12 mmol) and propionaldehyde (0.3 ml, 2.08 mmol) were dissolved in 6 ml of deoxygenated water, and allowed to stand at 5 °C under a nitrogen atmosphere for 12 hr. The reaction mixture was warmed to room temperature and 660 mg of $NaH_2PO_4·H_2O$ (5.16 mmol) was added followed by ethyl vinyl ether (0.6 ml, 6.27 mmol) in 2 ml of chloroform. The biphasic reaction solution was stirred vigorously for 3 h, after which $NaHCO_3$ (500 mg) in 5 ml of water was added. The aqueous layer was separated and extracted with chloroform (3 x 20 ml). The organic layer was dried over Na_2SO_4 and chromatographed on a silica gel preparative plate with 8% methanol/chloroform as the solvent. The band with R_f 0.65 afforded 85 mg (22%) of 26 as a pale yellow oil. ^1H NMR ($CDCl_3$) δ 0.90 (t, 3 H), 1.23 (m, 4 H), 1.87 (m, 3 H), 2.49 (br m, 1 H), 3.75 (br m, 2 H), 5.14 (m, 1 H), 7.17 (s, 1 H), 9.22 (s, 1 H); mass spectrum, m/z (relative intensity) (70 eV) 184 (M^+, 2.1) 155 (5.6), 139 (4.6), 138 (9.8), 122 (15.4), 109 (100); HRMS (EI) calcd for $C_{10}H_{16}O_3$ 184.1099, found 184.1098.

Benzyl "bis-MDA" and Its Conversion to 1,4-Dihydropyridine-3,5-dicarboxaldehyde 15. Sodium MDA (210 mg, 1.87 mmol) in 2 ml of water was added to benzylidene MDA[36] (303 mg, 1.89 mmol) in 7 ml of acetone. The reaction mixture was stirred for 2.5 h at room temperature. The solvent was evaporated under reduced pressure and the residue was crystallized (EtOH) to give the benzyl "bis MDA" (24, R'=Ph, Na salt) as yellow crystals (440 mg, 93%): UV (0.1N HCl)λ_{max} 249 nm (ε 13849); UV (0.1N NaOH) λ_{max} 272 nm (ε29277). ^1H NMR (D_2O) δ 5.50 (s, 1 H), 7.24 (m, 5 H), 8.45 (br s, 4 H).

Benzyl "bis-MDA" (1 equiv) was converted to dihydropyridine 7 (35%) by reaction with ammonium acetate (1 equiv) at 60 °C and pH 4.2 for 3 h.

Propyl "bis-MDA" and Its Conversion to 1,4-Dihydropyridine-3,5-dicarboxaldehyde 4. Sodium MDA (187 mg, 1.67 mmol) was dissolved in 1 ml of water to which was added propionaldehyde (0.06 ml, 0.85 mmol) in 7 ml of acetone. The reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was crystallized from ethanol-ether to give the propyl "bis-MDA" (Na salt) (91 mg, 47%) as off-white crystals: UV (0.1N HCl)λ_{max} 248 nm (ε13334); UV (0.1N NaOH) λ_{max} 270 nm (ε 30322); ^1H NMR (D_2O) δ 0.78 (m, 3 H), 1.74 (m, 2 H), 4.47 (t, 1 H), 8.40 (br s, 4 H).

Propyl "bis-MDA" (1 equiv) was converted to the 1,4-dihydropyridine 4 (50%) by heating with glycine methyl ester (1 equiv) at 55 °C and pH 4.2 for 3 h.

Single-Crystal X-ray Structure Determination of 1,4-Dihydropyridine 15. A colorless needle-like crystal, .05 mm(0,1,0)x .10 mm (0,1,-1) x .62 mm (1,0,0) mounted on a glass fiber with [1,0,0] roughly parallel phi rotation axis of Enraf-Nonius CAD-4 diffractometer; graphite monochromator, MoKalpha radiation, alpha(aver)=.71073 A; 295K data collection; omega/two theta scan, 0.6 + .35 tan(theta); background counts, 25% below and above range; peak counting time/background counting time= 2/1; horizontal aperture, 2.4 to 3.0 mm depending on angle; scan speed, 0.5 - 2.5 deg/min depending on intensity; reflections collected to 2 theta(max) = 40. Lorentz and polarization corrections were made but absorption corrections were not (mu= 0.59 cm^{-1}). The three standard reflections used to monitor decay showed a decrease of only 2.1% so reflections were not corrected for decay. A total of 7995 reflections were measured of which 3000 were classed as absent. Net averaged reflections = 1212, of which 661 exceeded 3 sigma. Agreement among equivalent reflections observed is 3.2% based on F, 2.7% based on F*F. Cell dimensions were obtained from 25 reflections used to determine the orientation matrix, a = 7.524(6), b = 14.009(7), c = 20.236(11) A. The cell volume is 2132.95 A^3. For Z = 4, F.W. = 213.25, the calculated density is 1.328 g/cm^3.

The structure was solved by direct methods and refined by full matrix least squares. All hydrogen atoms were located from difference maps, and refined. Anisotropic refinement on all non-hydrogen atoms, but not including hydrogen atom positions = 146 parameters, 661 reflections, gave R = .081, Rw = .120. Anisotropic refinement on all non-hydrogen atoms and isotropic refinement on hydrogen atoms gave R(1) = .022, R(2) = R(w) = .026. The standard deviation of an observation of unit weight = 1.074. Weights used in the refinement are those of Killean and Laurence[37] with P = .01, Q = 0.0. The last parameter shift/error was less than 0.03. The final difference map has a maximum residual electron density of 0.08(2) el/A^3. The rather small ratio of reflections/parameter is justified by (1) the use of averaged data from a full sphere, (2) by the large decrease in the agreement factor on addition of H atoms to the calculation, and (3) the subsequent refinement of H atom positions to reasonable values. All crystallographic calculations were made using the SDP set of programs of Enraf-Nonius Corp. Atom parameters and bond distances and angles are summarized in Tables 1 and 2.

Table 1. Atom parameters for 4-phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde (15).

Atom	x	y	z	B
	x10000	x10000	x100000	
N1	1954(2)	1301(2)	51913(9)	3.86(5)
C2	3549(3)	1380(2)	48798(11)	3.27(5)
C3	5096(3)	1237(2)	51968(9)	2.59(5)
C4	5214(3)	1033(1)	59251(9)	2.57(5)
C5	3369(3)	831(2)	61889(10)	3.01(5)
C6	1904(3)	987(2)	58264(11)	3.67(6)
C31	6679(3)	1278(2)	48049(11)	3.54(5)
C51	3161(3)	460(2)	68457(11)	3.92(6)
C7	6076(2)	1850(2)	62981(9)	2.52(5)
C8	7626(3)	1710(2)	66555(10)	3.44(5)
C9	8418(3)	2458(2)	69848(11)	4.65(6)
C10	7684(3)	3348(2)	69711(11)	4.73(6)
C11	6136(3)	3502(2)	66269(12)	4.19(6)
C12	5340(3)	2754(2)	62939(10)	3.22(5)
O32	8175(2)	1137(1)	50181(8)	4.42(4)
O52	4390(2)	232(1)	71992(7)	4.83(4)
Atom	x	y	z	B
	x1000	x1000	x10000	
HN1	600(2)	368(1)	5033(10)	2.0(5)*
H2	649(2)	657(1)	609(9)	1.0**
H4	599(2)	458(1)	1006(8)	1.0**
H6	571(3)	413(1)	3974(9)	2.3(5)*
H8	810(2)	395(1)	1693(10)	1.6(5)*
H9	548(3)	765(2)	2235(11)	3.4(6)*
H10	176(3)	610(2)	2802(12)	4.2(7)*
H11	444(3)	585(2)	3390(10)	2.7(5)*
H12	574(3)	713(1)	3965(9)	1.5(5)*
H31	351(2)	639(1)	714(9)	1.4(5)*
H51	186(3)	463(2)	1990(9)	2.3(5)*

Starred atoms were refined isotropically.
Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as:
(4/3)*[a2*B(1,1)+b2*B(2,2)+c2*B(3,3)+ab(cos gamma)*B(1,2)+ac(cos beta)*B(1,3)+bc(cos alpha)*B(2,3)]

Double starred atoms had the B value fixed, because on refinement the B value became negative.

Table 2. Bond distances and angles for 4-phenyl-1,4-dihydropyridine-3,5-dicarboxaldehyde (15).

N1-C2	1.360(3)	C6-N1-C2	119.3(2)
N1-C6	1.360(3)	N1-C2-C3	122.1(3)
C2-C3	1.344(3)	N1-C6-C5	123.1(2)
C6-C5	1.342(3)	C2-C3-C4	123.2(2)
C3-C4	1.504(3)	C6-C5-C4	122.0(2)
C5-C4	1.514(3)	C2-C3-C31	116.8(2)
C3-C31	1.432(3)	C6-C5-C51	118.4(2)
C5-C51	1.436(4)	C4-C3-C31	120.1(2)
C31-032	1.221(3)	C4-C5-C51	119.6(2)
C51-052	1.212(3)	C3-C4-C5	109.1(2)
C4-C7	1.516(3)	C3-C4-C7	111.7(2)
C7-C8	1.386(3)	C5-C4-C7	110.9(2)
C7-C12	1.383(3)	C3-C31-032	124.4(2)
C8-C9	1.378(4)	C5-C51-052	124.0(3)
C12-C11	1.382(4)	C4-C7-C8	120.9(2)
C9-C10	1.364(4)	C4-C7-C12	121.1(2)
C11-C10	1.374(4)	C8-C7-C12	118.0(2)
C8-C81		C7-C8-C9	120.6(3)
C81-F82A		C7-C12-C11	121.2(3)
C81-F82B		C8-C9-C10	120.7(3)
C81-F82C		C12-C11-C10	119.7(3)
		C9-C10-C11	119.8(3)
C7-C8-C81		C8-C81-F81A	
C9-C8-C81		C8-C81-F81B	
F81A-C81-F81B		C8-C81-F81C	
F81A-C81-F81C		F81B-C81-F81C	
N1-H1	.85(3)	HN1-N1-C2	119(2)
C2-H2	1.02(2)	HN1-N1-C6	120(2)
C6-H6	1.00(3)	H2-C2-N1	116(1)
C31-H31	1.07(3)	H2-C2-C3	121(1)
C51-H51	1.03(3)	H6-C6-N1	118(1)
C4-H4	1.05(3)	H6-C6-C5	119(1)
C8-H8	1.00(3)	H31-C31-C3	116(1)
C12-H12	.98(2)	H51-C51-C5	114(1)
C9-H9	.98(3)	H31-C31-032	119(1)
C11-H11	1.01(3)	H51-C51-052	122(1)
C10-H10	.99(3)	C3-C4-H4	110(1)
		C5-C4-H4	107(1)
		C7-C4-H4	108(1)
C7-C8-H8	118(1)	C8-C9-H9	120(2)
C9-C8-H8	121(1)	C10-C9-H9	121(2)
C9-C10-H10	122(2)	C10-C11-H11	121(2)
C11-C10-H10	118(2)	C12-C11-H11	119(2)
C7-C12-H12	119(1)		
C11-C12-H12	120(1)		

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