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Cleavage and Oligomerization of Malondialdehyde

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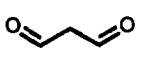
Key Words: Quasi-dimeric malondialdehyde; quasi-trimeric malondiadehyde; 3-substituted 2,4-dihydroxymethyleneglutaraldehydes, 1,4-dihydropyrane-3,5-dicarbaldehydes; 1,4-dihydropyridine-3,5-dicarbaldehydes.

Abstract: Malondialdehyde (MDA) slowly self-condensates in aqueous solution at pH 4.5-6 and room temperature yielding (*E*)-(3'-oxo-1'-propenyl)malondialdehyde and 2,4-dihydroxymethylene-3-(2'-oxoethyl)glutaraldehyde. The latter compound exists in solution in equilibrium with 4-(1,3-dioxopropan-2-yl)-5-formyl-2-hydroxy-3,4-dihydro-2*H*-pyran, the *trans*-form of which can be readily isolated as a crystalline hemipotassium salt. Cleavage of MDA also occurs under these conditions leading to acetaldehyde, which further reacts with the excess of MDA to form 2,4-dihydroxymethylene-3-methylglutaraldehyde.

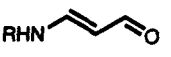
INTRODUCTION

Malondialdehyde (MDA, **1**) is a ubiquitous, natural compound produced in substantial quantity in mammalian tissues as an end product of polyunsaturated lipids peroxidation.[1] MDA is toxic and mutagenic,[2,3] and has been associated with such biological processes as the cross-linking of proteins[4] and DNA[5], cell aging,[6] and the formation of the fluorescent lipofuscin pigments which accumulate in aging organisms as a result of *in vivo* lipid peroxidation.[7] MDA is also considered to be one of the substances responsible for the deterioration of food.[8] The estimation of MDA in oxidized lipids or tissues by the 2-thiobarbituric acid (TBA) method[9] is often used as an index of lipid peroxidation and rancidity. However, the TBA test is nonspecific for MDA, and some of its precursors and/or derivatives have been shown to give a positive test.[10] In spite of its biological implications, the chemistry of MDA is still unclear. Several authors[10,11] have pointed out that MDA is readily transformed into low molecular weight polymers having properties (*e.g.*, the TBA test) similar to those of the parent compound. MDA reacts with amines and amino acids to yield 3-aminoacroleines (**2**), 1-amino-3-iminopropenes (**3**),[6] and fluorescent *N*-substituted 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehydes (**4**).[12] Furthermore, MDA reacts with nucleosides and DNA bases to form oligomeric adducts containing 1, 2 or 3 equiv of MDA.[13] These results suggest the previous formation of oligomers of MDA which further react to afford the final products. In accordance with that, Golding *et al.*[14] have shown that MDA undergoes self-condensation in water to afford a dimer and a trimer

of MDA, isolated as their sodium or potassium salts **5** and **6**, respectively. We report here the results of a study on the stability of MDA under physiological conditions (aqueous solution, room temperature and pH near neutrality). Preliminary accounts of portions of this work have appeared.[15,16]



MDA, **1**



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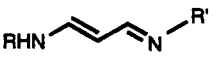

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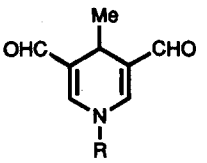

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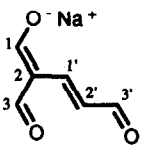

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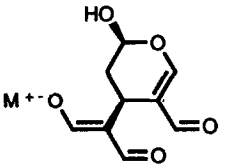

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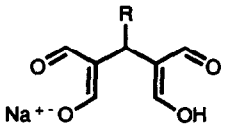
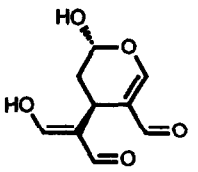
RESULTS AND DISCUSSION

NaMDA was prepared by acid resin (Dowex 50Wx8) catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane (TMP) followed by neutralization (M NaOH, pH 7).[17] TLC of the hydrolyzate revealed the presence of NaMDA and minor amounts of the monosodium salts of 2,4-dihydroxymethylene-3-(2,2-dimethoxyethyl)glutaraldehyde (**7**), 2,4-dihydroxymethylene-3-methylglutaraldehyde (**8**), (E)-(3'-oxo-1'-propenyl)malondialdehyde (**5**), 2,4-dihydroxymethylene-3-(2'-oxoethyl)glutaraldehyde (**9**), and other TBA-reactive compounds. Evaporation of the solvent and treatment of the residue with acetone afforded a product, the analysis of which by gel-exclusion chromatography showed at least six peaks with elution volumes of 60, 74, 86, 96, 103 and 115 mL (peaks 1-6, Fig. 1), respectively. Peaks 3-6 were subsequently shown to be superimposable on the peaks produced by NaMDA, and compounds **9**, **8**, and **5**, respectively. Crystallization from acetone-water and chromatography afforded pure NaMDA (75%), **7** (6%), **8** (*ca.* 1%), and **5** (*ca.* 0.5%). Compound **9** was produced in trace amounts under these conditions, but its yield could be increased up to *ca.* 30% when the pH of the hydrolyzate of TMP, or of a freshly prepared solution of NaMDA, was adjusted at 4.5-5.0 (*i.e.*, approximately the pK_a value of MDA[18]) with M NaOH, and kept at this pH value until TLC indicated the complete consumption of MDA. In similar experiments using M KOH, the product was racemic *trans*-4-(1,3-dioxopropan-2-yl)-5-formyl-2-hydroxy-3,4-dihydro-2*H*-pyran (**12**), which crystallized very readily as its hemipotassium salt **13**. Attempts to obtain compound **6** (M=K) following Golding's procedure, or by treating **13** with KOH, were unsuccessful; the only product isolated, or recovered, was **13**.



7: R= $CH_2CH(OMe)_2$
8: R= CH_3
9: R= CH_2CHO
10: R= *n*-C_3H_7
11: R= *n*-C_5H_{11}



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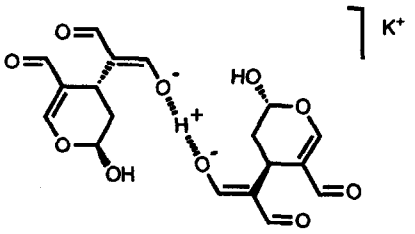

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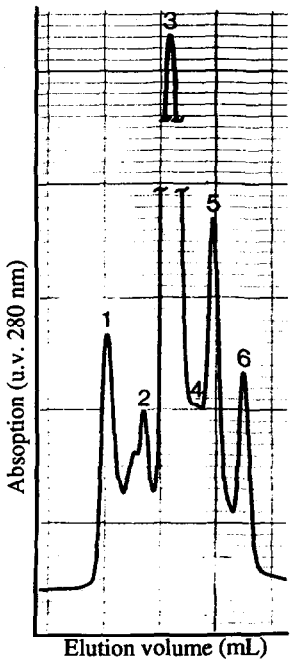
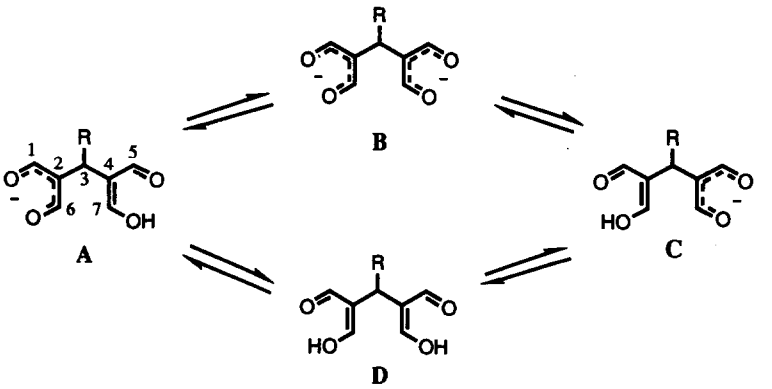


Peak	mL
1	60
2	74
3	86
4	96
5	103
6	115

Figure 1. Gel exclusion chromatogram of a NaMDA sample (before recrystallization).

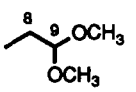
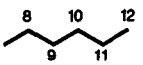
Evidence for structures **5**, **7-9**, and **13** is as follows. The structure of **7** suggests that it arises from the condensation of 3,3-dimethoxypropanal, a known[19] product of the partial hydrolysis of TMP, and two equivalents of MDA, and indeed **7** was readily prepared (72%) by reaction of the acetal and NaMDA. Similarly, **8** could be formed by condensation of acetaldehyde, present (see below) in the hydrolyzate of TMP, and two equivalents of MDA, and support for this view was achieved by the synthesis of **8** in 60% yield from acetaldehyde and NaMDA. This seems to be a general reaction of aliphatic aldehydes and NaMDA as was further exemplified by the synthesis, in good yields, of **10** and **11** from *n*-butanal and *n*-hexanal, respectively. Likewise, compound **9** can be considered to derive from the condensation of MDA, acting as the "aldehyde", and two moles of MDA. Further support for the structure of **9** was obtained by its formation in 90% by hydrolysis (M HCl, pH 1) of its acetal **7**.

Consideration of the spectral properties of **7-11** indicated that these compounds have very similar structures. For comparison purposes data of their ^{1}H and ^{13}C NMR spectra have been collected in Table I. The spectra show that all of these compounds are salts of 3-substituted 2,4-dihydroxymethylene-glutaraldehydes differing in the nature of the substituent R at C(3). The signals at δ_C 99.6 and δ_H 5.45 of compound **9** are considered to be due to the hydrated form of the HC(9)=O group. The simplicity of the spectra, which apparently suggests the presence of a symmetry element in the molecules, can be explained by considering that the anion (form **A**, Scheme 1) of these compounds is in a rapid equilibrium with the protonated form **D** and, through the dianion **B**, with the equivalent anion **C**. The observed spectra are the averaged spectra of **A-D** (however, see below for the more complex equilibrium of compound **9**). Likewise, the UV spectra of **7-11** were very similar showing in water solution a maximum at *ca.* 250 nm and a shoulder at *ca.* 270 nm; in 0.1M HCl solution only the maximum at 250 nm, assigned to the undissociated form **D**, was observed, and in 0.2M NaOH solution the only maximum appearing at *ca.* 270 nm is attributed to the mono anions **A** and **C**, and the dianion **B**. In accordance with the above results, acidification of **8** and titration (0.1006M NaOH, 25°C) of the resulting 2,4-dihydroxymethylene-3-methylglutaraldehyde showed that this compound is a dibasic acid with $pK_{a(1)}=4.93$ and $pK_{a(2)}=8.00$. The IR spectra of **7-11** showed two bands in the ranges 1670-1690 and 1545-1575 cm^{-1} attributable to the C=O and C=C groups of the conjugated -O-C=C-C=O system; compound **9** showed, in addition to this absorption, a sharp band at 1721 cm^{-1} assigned to the non-conjugated CHO.



Scheme 1

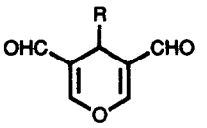
Table 1. ^{1}H and ^{13}C NMR spectral data[a] for compounds **7-11**.

	Compound				
	7	**8**	**9**	**10**	**11**
R=					
Nuclei					
H-1,5,6,7	8.61br s	8.24br s	8.20br s	8.31br s	8.11br s
H-3	4.05t *J* 8.0	4.15q *J* 7.6	3,55t *J* 7.0	3,84t *J* 8.3	3,88t *J* 8.1
H-8	1.95dd *J* 6.0	1.25d	1.82dd *J* 4.5	1.68m	1.55m
H-9	4.16t		5.45t	1.13m	
H-10				0.82m	1.06m
H-11					
H-12					0.68m
OCH_3	3.21s				
C-1,5,6,7	188.6	188.8	188.9	188.9	188.9
C-2,4	124.3	126.6	121.3	125.4	125.4
C-3	21.5	20.0	21.6	25.1[b]	22.7[c]
C-8	33.1	16.8	33.2	32.1	31.6
C-9	105.1		99.6	25.4[b]	25.3[c]
C-10				13.9	27.7[c]
C-11					29.7[c]
C-12					14.1
OCH_3	54.3				

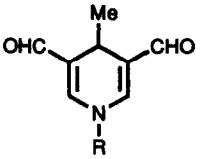
[a]In D_2O solution at 200 and 50.3 MHz. [b,c]The assignments may be interchanged

2,4-Dihydroxymethyleneglutaraldehydes **7-11** cyclized readily upon treatment with HCl/ether or acetic anhydride affording the corresponding 4-substituted 1,4-dihydropyrano-3,5-dicarbaldehyde **14-18**, usually in good yields. The spectral properties of these compounds were in accordance with the assigned structures and had similarities with those of the parent open-chain compounds; thus, **16** showed carbonyl bands at 1720 (non-conjugated CHO) and 1678 cm^{-1} (conjugated CHO), *i.e.*, at almost the same wave-numbers as those of **9**, and its ^{1}H NMR spectra in D_2O-acetone showed a signal at δ 4.94 attributable to the hydrated form of the non-conjugated CHO group in equilibrium with the non-hydrated form. Treatment of **8** with ammonia or the appropriate amine in water solution at room temperature yielded the 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehydes **19-22**.

A spectroscopic and X-ray crystallographic investigation of **13** has been previously reported.[16] The results showed that this compound is formed by two enantiomeric *trans*-4-(1,3-dioxopropan-2-yl)-5-formyl-2-hydroxy-3,4-

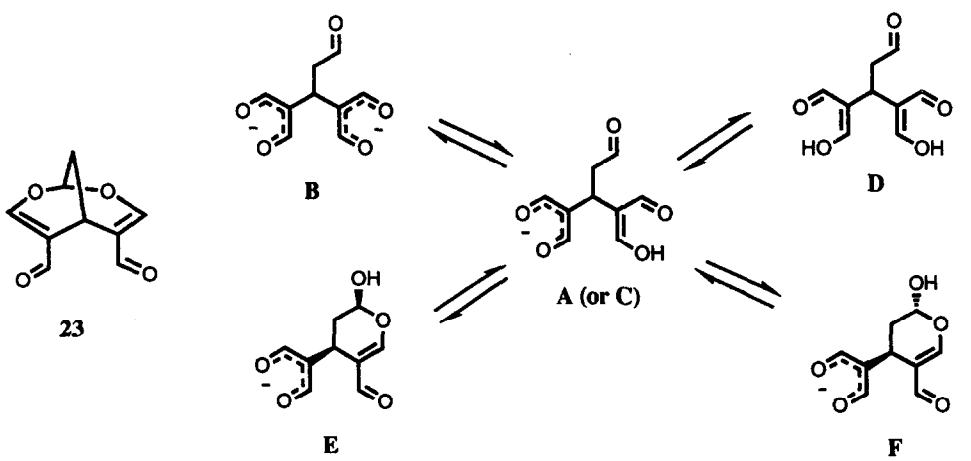


14: R= $CH_2CH(OMe)_2$
15: R= CH_3
16: R= CH_2CHO
17: R= *n* -C_3H_7
18: R= *n* -C_5H_{11}



19: R= H
20: R= CH_3
21: R= *n* -C_3H_7
22: R= *cyclo* -C_6H_{11}

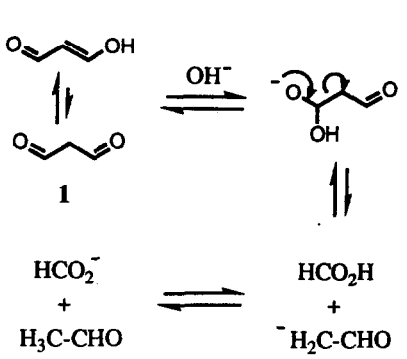
dihydro-2*H*-pyran anions held together by a very short (and strong) symmetrical hydrogen bond, the negative charge of the resulting robust, racemic anion being neutralized by a potassium cation. The c.p.-m.a.s. ^{13}C NMR spectrum of the compound (see ref. 16 and Experimental) was very different from that of **9** in D_2O solution (Table I), and showed distinct signals for eight of its nine carbon atoms, the resonances due to C-5 and C-7 being overlapped. The IR spectrum in the solid state (KBr), showing bands at 1655 and 1618 cm^{-1}, was different from that of **9** and similar to that reported[14] for **6**. On the other hand, the UV and NMR spectra in solution were pD-dependent and, at pD *ca.* 4.4, similar to those of **9**. Treatment of an aqueous solution of **13** with Dowex 50Wx8 (acid form) followed by neutralization with NaOH yielded (82%) **9**. On the other hand, when an aqueous solution of **13** was kept at pH 2 for 12 hr, a complex mixture resulted from which 2,8-dioxabicyclo[3,3,1]nonan-3,6-diene-4,6-dicarbaldehyde (**23**) was isolated in 22% yield. Compound **23** had been previously obtained[14] from **6**, the *cis* stereochemistry of which was assigned on this basis; in the light of the above results this assignment is not valid. All the results can be rationalized by assuming that the 2,4-dihydroxymethylene-3-(2'-oxoethyl)-glutaraldehyde anion (**A,C**) exists in aqueous solution as a complex equilibrium mixture (Scheme 2) involving the *cis*- (**E**) and the *trans*-form (**F**) of the 4-(1,3-dioxopropan-2-yl)-3,4-dihydro-2*H*-pyran anion (**16**), as well as the dianion (**B**) and the protonated form (**D**).



Scheme 2

Structure **5** followed from analytical and spectral data. The position and *E*-configuration of the carbon-carbon double bond was deduced from the presence in the ^{1}H NMR spectrum of signals at δ 7.55 (d, $J_{1',2'}$ 15,4 Hz, 1H, H-1'), 6.97 (dd, $J_{1',2'}$ and $J_{2',3'}$ 8.7 Hz, 1H, H-2'), and 9.26 (d, $J_{2',3'}$, 1H, H-3'), in addition to the two-proton singlet at δ 8.94 due to H-1 and H-3. This compound had the same properties as the compound described by Golding *et al.*[14]

The formation of **8** implies the presence of acetaldehyde in the hydrolyzate of TMP which could arise from the cleavage of MDA or its anion. This was verified by incubating for 15 min. in closed vials freshly prepared solutions of NaMDA, adjusted at pH varying from 3 to 8, and then analyzing the head-space by gas chromatography. Acetaldehyde and MDA were detected in the pH range 4-7, the highest yield of the former compound, and the lowest of the latter, occurring at pH 5.5 (Fig. 2); under these conditions *ca.* 9% of the head-space content was acetaldehyde. This compound was isolated as its 2,4-dinitrophenylhydrazone from a solution of NaMDA stored at pH 5.5 and 35°C for 15 min. The formation of acetaldehyde can be viewed as a hydrolytic cleavage (Scheme 3) of the dicarbonyl form of MDA similar to that undergone by 1,3-diketones under more strongly basic conditions. The liability of MDA to hydrolysis may be due to the greater electrophilicity of the aldehyde carbonyl relative to the carbonyls of 1,3-diketones. The cleavage is restricted to the pH range 4-7 because at higher pH values MDA exists almost exclusively as its anion, and at pH below there is not enough concentration of hydroxyl anions.



Scheme 3

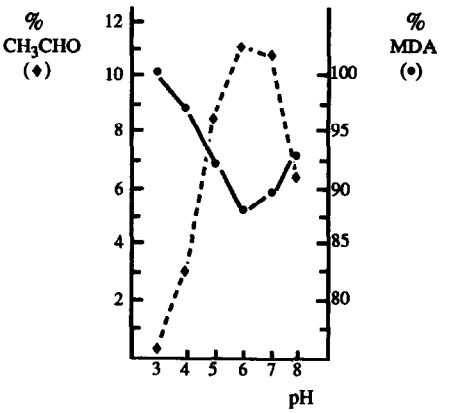
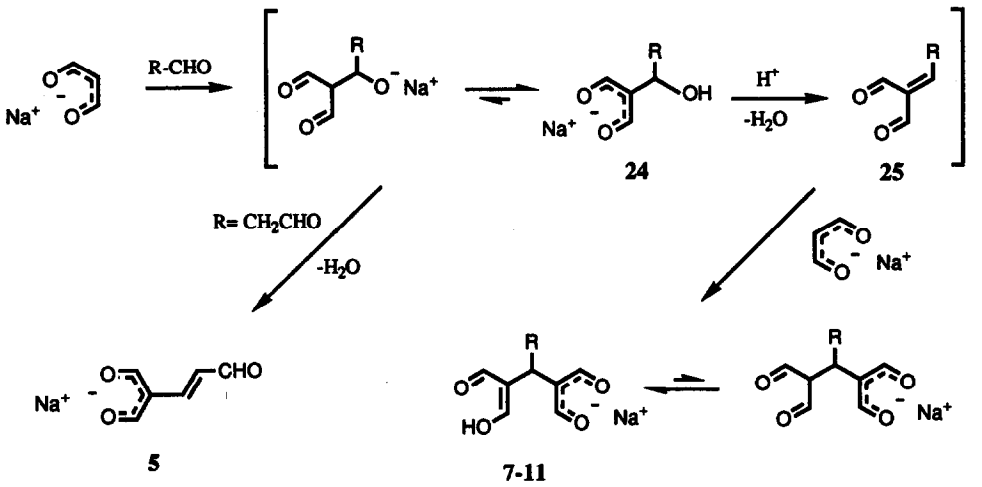


Figure 2

The formation of the 3-substituted 2,4-dihydroxymethyleneglutaraldehydes **7-11** probably takes place by the addition of the MDA anion to the alkylidene-malondialdehydes **25** resulting from the condensation of MDA with itself, with the acetaldehyde present in the reaction medium, or with the added aldehyde (Scheme 4). Compound **5** can be formed by dehydration of intermediate **24** (R=CH_2CHO).

The above results throw some light on some unclear aspects of MDA chemistry. Thus, compounds **5**, **7-11**, and **13** gave a strong colour reaction with TBA with λ_{max} 530 nm (ϵ 51000-200000) [the values for MDA



Scheme 4

are: λ_{max} 530 nm (ϵ 144500)]; **5**, **7**, and **8** showed a second maximum at 626 nm (ϵ 9500-11000). The low-weight polymers of MDA reportedly[10] formed in the hydrolysis by acids of TMP or tetraethoxypropane gave similar colour reactions with TBA; some of these polymers are most probably protonated forms of **5**, **7**, and **9**. The ready formation of **8** from MDA alone and its subsequent transformation into the 1,4-dihydropyridine-3,5-dicarbaldehydes **19-22** by treatment with amines, explains the observation[12] that **19-22** (and compounds of similar structures derived from amino acids and peptides) can be obtained by the one-pot reaction of MDA-aldehyde-amine (or amino acid). 3-Substituted 2,4-diacylglutaraldehydes similar to **7-11** may be intermediates in the Hantzsch 1,4-dihydropyridine synthesis from 1,3-dicarbonyl compounds, aldehydes and ammonia (or amines). From the biological point of view, it should be noted that MDA can coexist in physiological media with alkanals,[19] and therefore compounds similar to **7-11** may be formed in these media, in addition to the derivatives **5**, **8**, and **9** (or tautomeric forms) arising from the cleavage and oligomerization of MDA. These tri- and tetra-aldehydes may act modifying biological macromolecules, as observed for nucleosides and DNA bases.[13]

EXPERIMENTAL

Material and methods

Melting points were determined on a Reichardt 222/27 apparatus and are uncorrected. UV. spectra were recorded with a Hewlett-Packard 8450 A spectrophotometer. FT-IR spectra were recorded in a Bomem-Michelson Mb120 spectrophotometer. Elemental analyses were conducted at the Department of Analytical Chemistry of the University of Seville. ^1H and ^{13}C NMR spectra were recorded at 200 and 50.3 MHz, respectively, in a Varian XL-200 spectrometer. MS were recorded in a AEI MS 30/70 connected to a VG Data System PDP-11/250 and a Kratos MS-80RFA spectrometers. TLC was performed on Alugram Sil G/UV_{254} (Machery-Nagel) plates. Column

and preparative TLC chromatography were performed on Silica gel 60 (70-230 mesh) and Silica Gel F_{254} (Merck) plates (20x20, 0.5 mm), respectively. The following solvent systems were used: A) acetone-water 20:1, B) ethyl acetate-methanol (7:1), C) ethyl acetate-methanol-triethylamine (6:1:1, 3 irrigations), D) ethyl acetate-methanol-triethylamine-water (6:2:1:1), E) chloroform, F) ether-hexane (2:1), G) ether-hexane (1:1), H) chloroform-acetone (2:3), I) ethyl acetate-methanol (16:1). Spots were visualized by quenching of UV fluorescence (λ_{max}=254 nm) or by charring with 0.02M TBA in 50% acetic acid. Solutions were concentrated under diminished pressure at <35°. Gas chromatography was performed on a Perkin-Elmer Sigma 3B instrument fitted with a head space adapter, using a Carbowax 20M (5% Chromosorb G-AW, 80-100 mesh) column. GC conditions were: sample temperature 60°, column temperature 90°, detector temperature 250°, carrier gas (nitrogen) flow rate 20 mL/min. Gel filtration was performed on a column (60x2 cm) of Bio-Gel P2 (fractionation range from 100 to 1800 daltons) by elution with 0.1M phosphate buffer (pH 7.0) containing 1% NaCl at a flow rate of 10 mL/min and detection with UV light at 280 nm. TBA tests were conducted by mixing 0.3 mL of sample solutions with 1.0 mL of 20 mM TBA and 1.0 mL of glacial acetic acid. The mixture was heated at 100° for 15 min and 50-fold diluted with water before recording the absorption spectra (400-600 nm). pK_a measurements were performed by automatic titration of samples (25 mL, c> 0.01M) with 0.25M base with background of KCl (ionic strength around 1) at 25°C.

Hydrolysis of TMP

A) TMP (8.2 g, 50 mmol) was stirred with Dowex 50Wx8 resin (40 g) in water (100 mL) for 1 hr. The resin was filtered off and the filtrate neutralized with 5M NaOH to pH 7. The solution, containing (TLC^{20}) NaMDA (main component), **5**, **7**, **8**, and **9**, was evaporated and the syrupy residue treated with acetone to yield a solid that was filtered off (solid A). The filtrate was concentrated and the residue treated with ethyl acetate and recrystallized from water giving pure compound **5**. Recrystallization of solid A from acetone-water afforded NaMDA. Column chromatography (solvent A), followed by preparative TLC (solvent B) of the mother liquor afforded **7** and **8**.

The gel-exclusion chromatography of solid A showed peaks with elution volumes of 60, 74, 86, 96, 103, and 115 mL (Fig. 1).

NaMDA monohydrate: 3.40 g, 75%, m.p. 240°C (dec.), [lit.[11] 245° (dec.)]; λ_{max} (pH<3) 245 nm (ϵ 13400); λ_{max} (pH>7) 267 nm (ϵ 31800); ^{1}H NMR (D_2O): δ 8.60 (d, 2H, *J* 10.2 Hz, H-1 and H-3) and 5.25 (t, 1 H, *J* 10.2, H-2) [lit.[7] δ 8.65 (d) and 5.30 (t). Anal. calcd. for $C_3H_3NaO_2 \cdot H_2O$: C, 32.15; H, 4.50. Found: C, 31.92; H, 4.47. Elution volume (gel exclusion chromatography) 86 mL. TBA test λ_{max}(H_2O) 530 nm (ϵ 144500).

Sodium (*E*)-(3'-oxo-1'-propenyl)malondialdehyde 5: 57 mg, 1%, m.p. 245°C (dec.); λ_{max}(H_2O) 277 (ϵ 14300) and 352 nm (ϵ 18800); ν_{max}(KBr) 3.470 (OH), 3347 (enol), 1665 (C=O), and 1603 cm^{-1} (C=C enol); ^{1}H NMR (D_2O): δ 6.97 (1H, dd, *J* 15.4 and 8.7, H-2'), 7.55 (1H, d, H-1'), 8.94 (2H, s, 1,3-CHO), and 9.26 (1H, d, 3'-CHO); ^{13}C NMR (D_2O): δ 117.0 (C-2), 122.3 (C-2'), 150.0 (C-1'), 196.3 (C-1, C-3) and 200.6 (C-3'). Anal. calcd. for $C_6H_5NaO_3$: C, 48.66; H, 3.40. Found: C, 48.30; H, 3.13. Elution volume (gel exclusion chromatography) 115 mL. TBA test λ_{max}(H_2O) 530 (ϵ 23600) and 626 nm (ϵ 10400).

Sodium 2,4-dihydroxymethylene-3-(2,2-dimethoxyethyl)glutaraldehyde 7: 0.8 g, 6%, m.p. 140°C (dec.); λ_{max}(0.1M HCl) 251 (ϵ 22000); λ_{max}(0.2M NaOH) 268 nm (ϵ 33000); ν_{max}(KBr) 3470, 3223 (OH, enol),

2834, 2772 (-CHO), 1690 (C=O), and 1548 cm^{-1} (C=C enol); ^1H and ^{13}C NMR: See Table 1. Anal. calcd. for $C_{11}H_{15}NaO_6 \cdot H_2O$: C, 46.48; H, 6.03. Found: C, 46.61; H, 5.78. TBA test $\lambda_{max}(H_2O)$ 530 (ϵ 50500) and 626 nm (ϵ 11000).

Sodium 2,4-diformyl-3-methylglutaraldehyde 8: 50 mg, 0.5%, m.p. 250°C (dec.); λ_{max}(0.1M HCl) 249 (ϵ 14000); λ_{max}(0.2M NaOH) 270 nm (ϵ 32000); ν_{max}(KBr) 3500, 3208 (OH, enol), 1682 (C=O), and 1574 cm^{-1} (C=C enol); ^1H and ^{13}C NMR: See Table 1; $pK_{a(1)}$=4.93 and $pK_{a(2)}$=8.00. Anal. calcd. for $C_8H_9NaO_4$: C, 40.51; H, 5.94. Found: C, 40.28; H, 5.73. Elution volume (gel exclusion chromatography) 103 mL. TBA test $\lambda_{max}(H_2O)$ 530 nm (ϵ 205600).

B) A water solution of MDA (pH 3.4), prepared immediately before use by acid hydrolysis (acid resin Dowex 50Wx8, 40 g) of TMP (8.2 g, 50 mmol), was adjusted to pH 4.5-5.0 with 5M NaOH and maintained at this pH value by periodical additions of acid resin Dowex 50Wx8. After 2 days MDA had been consumed (TLC, solvents C and D).The solution was extracted with ethyl acetate (3 x 20 mL). The pH of the aqueous phase was adjusted to 7 with M NaOH, concentrated to dryness, and the residual syrup treated with acetone. Recrystallization from acetone-water afforded **sodium 2,4-dihydroxymethylene-3-(2'-oxoethyl)glutaraldehyde 9:** 2.0 g, 30%, m.p. 174-176°C; $\lambda_{max}(H_2O)$ 265 (ϵ 27000); λ_{max}(0.1M HCl) 250 (ϵ 14500); λ_{max}(0.2M NaOH) 270 nm (ϵ 24500); ν_{max}(KBr) 3450 (OH, enol), 2827, 2783 (-CHO), 1721 (C=O), 1605 (C=O, enol) and 1550 cm^{-1} (C=C, enol); ^1H and ^{13}C NMR: See Table 1. Anal. calcd. for $C_9H_9NaO_5$: C, 49.09; H, 4.12. Found: C, 49.08; H, 4.23. Elution volume (gel exclusion chromatography) 96 mL. TBA test $\lambda_{max}(H_2O)$ 530 (ϵ 49500) and 626 nm (ϵ 9500).

C) A water solution of MDA was prepared as described above and the pH adjusted to 4.5 with 5M KOH. The solution was stored at room temperature for 48 h and then concentrated *in vacuo* until crystallization of **13** began. The crystallization was completed by adding cold methanol and the product washed with cold methanol and recrystallized from water to afford **potassium hydrogen bis-[*trans*-4-(1,3-dioxopropan-2-yl)-5-formyl-2-hydroxy-3,4-dihydro-2*H*-pyran] 13:** 2.2 g, 32%, m.p. 149°C (dec.); λ_{max} (H_2O, pH 4.5) 260 nm (ϵ 51800); ν_{max}(KBr) *ca.* 2980 (very broad and strong, symmetrical hydrogen bond), 2814, 2741 (-CHO), 1655 (C=O, CHO), 1618 (C=O, enol), *ca.* 1200 (very broad and strong, symm. hydrogen bond), and *ca.* 800 cm^{-1} (very broad and strong, symm. hydrogen bond). ^1H NMR (D_2O): δ 1.90 (2H, m, $-CH_2-$), 3.62 (1H, t, *J* 6.7, H-3), 5.62 (1H, t, *J* 4.5, H-2) and 8.25 (4H, br s, =CH-OH, =CH-O^-, and -CHO); ^{13}C C.p.-m.a.s. NMR: δ 23.34 (C-3), 36.29 (C-4), 95.99 (C-2), 118.80 (C-5, C-2'), 168.45 (C-6), 184.27 (C-1'), 189.79, and 193.58 (-CHO); $pK_{a(1)}$=4.45 and $pK_{a(2)}$=9.5. Anal. calcd. for $C_{18}H_{19}KO_{10}$: C, 49.76; H, 4.40; K, 9.00. Found: C, 49.75; H, 4.43; K, 9.01.

Hydrolysis of acetal 7 to 9

A solution of **7** (54 mg, 0.2 mmol) in water (3 mL) was treated with M HCl until pH 1. The mixture was stored at room temperature until completion of the reaction (1 h, TLC, solvent D) and then extracted with ethyl acetate (4 x 5 mL). The organic phase was dried (Na_2SO_4) and concentrated, and the resulting syrup dissolved in water (3 mL). The solution was neutralized (pH 7) with 0.1M NaOH and concentrated, and the residue treated with acetone to afford **9** (39 mg, 90%), identical with the product described above.

Conversion of 13 into 9

To a solution of **13** (434 mg, 1 mmol) in water (10 mL) was added acid resin Dowex 50Wx8 (3 mL). After stirring for 5 min. the resin was removed by filtration and the pH of the filtrate raised to 7 by addition of M NaOH. Removal of the solvent and treatment with MeOH-acetone afforded **9** (360 mg, 82%), identical with the product described above.

Cleavage of MDA

A) Freshly prepared 0.05M solutions of MDA (TMP, Dowex 50Wx8, as described above) were adjusted to various pH values (3-8) and incubated in closed vials (15 min, 35°C). Head space gas-chromatography revealed the presence of acetaldehyde in the pH range 4-7, the highest concentration (9% of the head-space content) being observed at pH 5.5 (Fig.2).

B) A freshly prepared 0.05M solution of MDA (TMP, Dowex 50Wx8, as described above) was adjusted to pH 5.5, incubated (15 min, 35°C), and then treated with 2,4-dinitrophenylhydrazine (30 mg in 3 mL of 95% EtOH). Acetaldehyde 2,4-dinitrophenylhydrazone (1%) was isolated by preparative TLC (solvent G). M.p. and mixed m.p. 167-168°C (dec).

Sodium salts of 3-substituted 2,4-dihydroxymethyleneglutaraldehydes

Sodium 2,4-dihydroxymethylene-3-(2,2-dimethoxyethyl)glutaraldehyde monohydrate 7: To a solution of NaMDA (1.9 g, 17 mmol) in water (20 mL) was added 3,3-dimethoxypropanal[19b] (1 g, 8.5 mmol) and the mixture was stirred at room temperature for 2 days. The solvent was removed and the residue crystallized from methanol to afford **7** (1.62 g, 72%), identical with the product described above.

Compounds **8**, **10**, and **11** were prepared as follows: to a solution of NaMDA (**1**) (0.22 g, 2 mmol) in water or methanol-water (1:1) (5 mL) was added the appropriate aldehyde (1.5 mmol), and the reaction mixture was kept at room temperature until total consumption of the NaMDA (UV monitoring, λ_{max} 266 → 250 nm). Starting aldehyde, reaction times, isolation procedures, yield and physical, spectroscopic, and analytical data of these products are as follows:

Sodium 2,4-dihydroxymethylene-3-methylglutaraldehyde 8: From acetaldehyde, 24 hours, the product crystallized by diluting the reaction mixture with acetone (5mL) and cooling at 0°C. Recrystallization from methanol afforded **8** (60%), identical with the product described above.

Sodium 2,4-dihydroxymethylene-3-propylglutaraldehyde 10: From *n*-butanal, 5 days, the product was isolated by evaporation of the solvent and treatment of the resulting residue with ethanol. Recrystallization from methanol afforded **10** (0.15 g, 67%), m.p. 255°C (dec.); λ_{max}(0.1M HCl) 248 (ϵ 15500); λ_{max}(0.2M NaOH) 270 nm (ϵ 29000); ν_{max}(KBr) 3287, 3125 (OH, enol), 2872, 2791 (-CHO), 1690 (C=O), and 1568 cm^{-1} (C=C enol); ^1H and ^{13}C NMR: See Table 1. Anal. calcd. for $C_{10}H_{13}NaO_4 \cdot 0.25H_2O$: C, 53.45; H, 6.05. Found: C, 53.66; H, 6.19. TBA test $\lambda_{max}(H_2O)$ 530 nm (ϵ 168800).

Sodium 2,4-dihydroxymethylene-3-pentylglutaraldehyde 11: From *n*-hexanal, 3 days, work-up as for **10**, afforded **11** (0.37 g, 85%), m.p. 270°C (dec.); λ_{max}(0.1M HCl) 250 (ϵ 17500); λ_{max}(0.2M NaOH) 270 nm (ϵ 18000); ν_{max}(KBr) 3378, 3320 (OH, enol), 2832, 2718 (-CHO), 1670 (C=O), and 1568 cm^{-1} (C=C enol);

^1H and ^{13}C NMR: See Table 1. Anal. calcd. for $C_{12}H_{17}NaO_4 \cdot 2.5H_2O$: C, 49.14; H, 7.21. Found: C, 49.17; H, 6.75. TBA test $\lambda_{max}(H_2O)$ 530 nm (ϵ 90900).

4-Substituted 1,4-dihydropyrane-3,5-dicarbaldehydes

A) To a stirred suspension of the appropriate sodium 3-alkyl-2,4-dihydroxymethyleneglutaraldehyde (1 mmol) in dry ether (10 mL) cooled at 0°C was added ether containing 1 equivalent of hydrogen chloride (16 mL). After stirring for 1 h at room temperature, the insoluble materials were filtered and washed with ether. The filtrates were evaporated and the residue chromatographed on preparative plates (solvent E) to afford the products.

B) The appropriate sodium 3-alkyl-2,4-dihydroxymethyleneglutaraldehyde (2 mmol) was suspended in acetic anhydride (5 mL) cooled at 0°C and stirred for 24 h. The mixture was partitioned (water-chloroform 1:1, 50 mL), the organic phase washed with water (2 x 10 mL), dried, concentrated, and the residue purified by column chromatography (solvent F).

Starting sodium 2,4-dihydroxymethyleneglutaraldehyde, yield and physical, spectroscopic, and analytical data of compounds **14-18** are as follows:

4-(2,2-Dimethoxyethyl)-1,4-dihydropyrane-3,5-dicarbaldehyde 14: From **7**, 77% (Method B), m.p. 66-68°C; ν_{max}(KBr) 1678 (C=O), and 1607 cm^{-1} (C=C), ^1H NMR ($CDCl_3$): δ 1.92 (2H, dd, *J* 5.6 and 5.2, -CH_2-), 3.22 (6H, s, 2x -OCH_3), 3.85 (1H, t, *J* 5.2, H-4), 4.32 [1H, t, *J* 5.6, -$CH(OCH_3)_2$], 7.33 (2H, s, H-2, H-6), and 9.46 (2H, s, 2x -CHO); ^{13}C NMR ($CDCl_3$): δ 22.8 (C-4), 35.4 (-CH_2-), 52.4 (2x -OCH_3), 102.1 [-$CH(OCH_3)_2$], 123.4 (C-3, C-5), 157.3 (C-2, C-6), and 188.8 (-CHO). HRMS: m/z calcd. for $C_{11}H_{14}O_5$ 226.0841. Found 226.0843.

4-Methyl-1,4-dihydropyrane-3,5-dicarbaldehyde 15: From **8**, 46% (Method A), 81% (Method B), m.p. 85-87°C; λ_{max}(MeOH) 212 (ϵ 12000), 230 (ϵ 5500), and 295 nm (ϵ 6000); ν_{max}(KBr) 1669 (C=O), and 1605 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 1.99 (3H, d, *J* 6.6, -CH_3), 3.59 (1H, q, H-4), 7.15 (2H, s, H-2, H-6), and 9.38 (2H, s, 2x -CHO); ^{13}C NMR ($CDCl_3$): δ 21.5 and 21.6 (-CH_3, C-4), 125.7 (C-3, C-5), 157.4 (C-2, C-6), and 189.7 (-CHO). Anal. calcd. for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 62.83; H, 5.46.

4-(2-Oxoethyl)-1,4-dihydropyrane-3,5-dicarbaldehyde 16: From **9**, 81% (Method B), m.p. 71-74°C, ν_{max}(KBr) 1720 (-CH_2CHO), 1678 (α,β-unsatd. -CHO), and 1605 cm^{-1} (C=C), ^1H NMR ($CDCl_3$): δ 2.82 (2H, dd, *J* 4.7 and 1.6, -CH_2-), 3.93 (1H, t, *J* 4.7, H-4), 7.36 (2H, s, H-2, H-6), 9.42 (2H, s, 2x α,β-unsatd. CHO), and 9.68 (1H, d, *J* 1.6, satd. -CHO); ^1H NMR (D_2O/acetone-d_6) showed two sets of signals, A and B, corresponding to **16** and its hydrated form, respectively. A: δ 2.67 (2H, dd, *J* 5.0 and 2.3, -CH_2-), 3.92 (1H, t, *J* 5.0, H-4), 7.78 (2H, s, H-2, H-6), 9.49 (2H, s, 2x α,β-unsatd. CHO), and 9.67 (1H, d, *J* 2.3, -CHO); B: δ 1.86 (2H, dd, *J* 5.7 and 5.0, -CH_2-), 3.71 (1H, t, *J* 5.0, H-4), 4.94 [1H, t, *J* 5.7, $CH(OH)_2$], 7.73 (2H, s, H-2, H-6), and 9.49 (2H, s, 2x α,β-unsatd. CHO); ^{13}C NMR ($CDCl_3$): δ 21.8 (C-4), 45.9 (-CH_2-), 122.0 (C-3, C-5), 158.1 (C-2, C-6), 189.0 (α,β-unsatd. -CHO), and 200.1 (-CHO). HRMS: m/z calcd. for $C_9H_8O_4$ 180.0423. Found 180.0436.

4-Propyl-1,4-dihydropyrane-3,5-dicarbaldehyde 17: From **10**, 47% (Method A), m.p. 99-100°C λ_{max}(MeOH) 213 (ϵ 14500), 230 (ϵ 5500), and 295 nm (ϵ 5500); ν_{max}(KBr) 1669 (C=O), and 1605 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, *J* 7.1, -CH_3), 1.15 (2H, m, -CH_2-), 1.58 (2H, m, -CH_2-), 3.81 (1H, t, *J*

4.6, H-4), 7.31 (2H, s, H-2, H-6), and 9.46 (2H, s, 2x -CHO); ^{13}C NMR ($CDCl_3$): δ 13.8 (C-3'), 18.1 (C-2'), 25.9 (C-4), 34.4 (C-1'), 123.5 (C-3, C-5), 157.8 (C-2, C-6), and 189.1 (-CHO). Anal. calcd. for $C_{10}H_{12}O_3$: C, 66.50; H, 6.75. Found: C, 66.63; H, 7.07.

4-Pentyl-1,4-dihydropyrane-3,5-dicarbaldehyde 18: From **11**, 36% (Method A), m.p. 85-86°C; λ_{max}(MeOH) 213 (ϵ 12200), 230 (ϵ 6700), and 295 nm (ϵ 5900); ν_{max}(KBr) 1667 (C=O), and 1605 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 0.83 (3H, m, $-CH_3$), 1.22 (4H, m, 2x $-CH_2-$), 1.56 (2H, m, $-CH_2-$), 3.81 (1H, t, *J* 5.0, H-4), 7.33 (2H, s, H-2, H-6), and 9.66 (2H, s, 2x -CHO); ^{13}C NMR ($CDCl_3$): δ 13.8 (C-5'), 22.3 (C-4), 24.2, 25.7, 31.4, 31.7 (C-1', C-2', C-3', and C-4'), 123.3 (C-3, C-5), 157.7 (C-2, C-6), and 189.0 (-CHO). Anal. calcd. for $C_{12}H_{16}O_3$: C, 69.20; H, 7.74. Found: C, 69.25; H, 7.85.

1,4-Dihydropyridine-3,5-dicarbaldehydes

4-Methyl-1,4-dihydropyridine-3,5-dicarbaldehyde 19: To a solution of **8** (475 mg, 2 mmol) in water (10 mL) was added ammonium acetate (154 mg, 2 mmol) and the mixture was maintained at room temperature until completion of the reaction (TLC, solvent H, 3 days). The solvent was removed and the residue chromatographed on preparative plates (solvent H, R_F 0.62) to afford **19** (48 mg, 16%), m.p. 169-171°C (lit.[21] 155-168°C).

1-Alkyl-4-methyl-1,4-dihydropyridine-3,5-dicarbaldehydes 20-22 (General procedure): To a solution of **8** (475 mg, 2 mmol) in water (10 mL) was added the appropriate amine (2 mmol) and M HCl (2 mL). The mixture was maintained at room temperature until completion of the reaction (TLC, solvent H). Starting amine, reaction time, isolation procedure, yield and physical, spectroscopic, and analytical data of these compounds are as follows:

1,4-Dimethyl-1,4-dihydropyridine-3,5-dicarbaldehyde 20: From 40% methylamine, 3 days, the solution was extracted with $CHCl_3$ (4 x 10 mL), the organic phase dried (Na_2SO_4) and concentrated, and the residue chromatographed on preparative plates (solvent I, R_F 0.56). Yield 58%, m.p. 145-148°C (lit.[22] 144-147°C).

1-*n*-Butyl-4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde 21: From *n*-butylamine, 2 days, work-up as for **20** afforded **21** (209 mg, 51%), m.p. 116-117°C; λ_{max}(H_2O) 238 (ϵ 19900), 266 (ϵ 7100), and 400 nm (ϵ 9600); λ_{max}(Exc) 400 nm and λ_{max}(Em) 465 nm (IRM 1.73); ν_{max}(KBr) 2871, 2737 (-CHO), 1651 (C=O), and 1568 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 1.00 (3H, t, *J* 7.2, $-CH_3$'), 1.11 (3H, d, *J* 6.7, $-CH_3$), 1.4-1.7 (4H, m, 2x $-CH_2-$), 3.46 (2H, t, *J* 7.1, *N*-CH_2-), 3.94 (1H, q, H-4), 6.68 (2H, s, H-2, H-6), and 9.28 (2H, s, 2x -CHO); ^{13}C NMR ($CDCl_3$): δ 13.6, 19.5, 32.1 (C-2' - C-4'), 22.2 and 23.1 ($-CH_3$, C-4), 54.9 (C-1'), 123.7 (C-3, C-5), 146.2 (C-2, C-6), and 188.7 (-CHO). Anal. calcd. for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.38; H, 8.25; N, 6.84.

1-Cycloexyl-4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde 22: From cyclohexylamine, 3 days, the solution was concentrated and the residue crystallized twice from methanol yielding **22** (160 mg, 34%), m.p. 151-152°C; λ_{max}(H_2O) 239 (ϵ 26500), 267 (ϵ 9600), and 402 nm (ϵ 12000); λ_{max}(Exc) 400 nm and λ_{max}(Em) 465 nm (IRM 1.37); ν_{max}(KBr) 2824, 2733 (-CHO), 1649 (C=O), and 1549 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, d, *J* 6.7, $-CH_3$), 1.2-2.1 (10H, m, 5x $-CH_2-$), 3.29 (1H, m, *N*-CH), 3.95 (1H, q, H-4), 6.79 (2H, s, H-2, H-6), and 9.28 (2H, s, 2x -CHO); ^{13}C NMR ($CDCl_3$): δ 21.9 and 23.5 ($-CH_3$, C-4), 24.8, 25.3, 32.3

(C-2' - C-6'), 63.8 (C-1'), 123.6 (C-3, C-5), 144.6 (C-2, C-6), and 188.7 (-CHO). Anal. calcd. for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.11; H, 8.17; N, 5.72.

2,8-Dioxabicyclo[3.3.1]nona-3,6-diene-4,6-dicarbaldehyde 23

To a solution of **13** (217 mg, 0.5 mmol) in water (10 mL) was added M HCl until pH 2. The solution was kept at room temperature for 24 h and then extracted with $CHCl_3$ (3 x 5 mL), the organic phase concentrated and the residue purified by column chromatography (solvent G) to afford crystalline **23** (40 mg, 22%), m.p. 147-148°C (lit.[14] 148°C). ^1H NMR ($CDCl_3$): δ 1.92 (2H, dd, *J* 2.0 and 3.0, H-9), 4.14 (1H, dt, *J* 3.0 and 5.0, H-5), 6.17 (1H, dt, *J* 2.0 and 5.0, H-1), 7.34 (2H, s, H-3, H-7), and 9.32 (2H, s, 2x -CHO).

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