Long-Chain-Substituted Uric Acid and 5,6-Diaminouracil Derivatives as Novel Agents against Free Radical Processes: Synthesis and in Vitro Activity

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A new series of N-alkylated uric acids (2,6,8-purinetrione) and 5,6-diaminouracils (5,6-diamino-2,4-pyrimidinedione) were synthesized, and their activities against free radicals were evaluated. Long-chain derivatives of both series exhibited a large inhibitory activity against oxygen radical induced lipid peroxidation in bovine heart mitochondria (IC50 lower than 1 μM), compared to the reference antioxidants trolox C or α-tocopherol. This activity appeared related to (i) the ability of these compounds to reduce the stable radical 1,1-diphenyl-2-picrylhydrazyl and (ii) their lipophilicity estimated by log P determination. In order to study the scavenging mechanisms of diaminouracils and urate derivatives against lipid radicals, they were also tested against the azo-initiated peroxidation of either methyl linoleate in organic solvents or a liposomal suspension of dilinoleoylphosphatidylcholine. Urate derivatives reacted moderately with lipid radicals and were slowly consumed, significantly affecting the propagation of the peroxidation. Diaminouracils strongly reduced the propagation rate. They were quickly consumed and were able to deactivate about 1 mol of lipid radical per mole of compound in organic solvent. Dodecyl urates and decyl- and dodecyldiaminouracils were chosen for further in vitro investigation and in vivo evaluation.

Introduction

In the last few years, increasing evidence has been amassed in the literature for the active participation of uncontrolled peroxidation processes in various pathologies, such as inflammatory injury,[1] degenerative disease,[2] ischemia-reperfusion injury and trauma,[3,4] cancer,[5] radiation,[6] and drug intoxication.[7] The major species responsible for these phenomena are active oxygen radicals (OR) generated in vivo under stress conditions:[8] superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and reactive transition metals, especially iron (such as Fenton catalyst[9] and highly reactive iron–oxo complexes[10]). Biological membranes contain highly oxidable structures such as polyunsaturated fatty acids (PUFA) and are particularly sensitive to radical chemistry (chain reactions). Such membrane damage could modify membrane fluidity and permeability and may affect many vital processes such as calcium homeostasis[11] and phospholipase activation, energy production in mitochondria, and controlled release of mediators. The importance of OR in vivo is demonstrated by the sophisticated mechanisms present in cells and extracellular fluids for preventing their formation and repairing oxidative damage.[12,13] This includes (i) enzyme targeted against OR (superoxide dismutase, catalase, glutathion peroxidase),[14–16] (ii) iron transport (transferrin in plasma),[9] oxidation without OR production (ceruloplasmin in plasma),[17] and storage in a nonreactive form (ferritin in cells),[9] and (iii) low molecular weight compounds acting as radical scavengers in the aqueous phase (ascorbic acid, uric acid) or as chain-breaking antioxidants (reacting with chain-propagating radical species) in biological membranes: principally vitamin E and ubiquinols.[18]

It was recently reported that uric acid (UA) plays an important role as antioxidant and particularly in human extracellular fluids.[13,19] This hypothesis is supported by its ability to scavenge hydroxyl radicals,[20] singlet oxygen,[21] heme–oxo oxidant structures[19,20] and water-soluble peroxy radicals.[22] Other reports indicate that UA forms stable coordination complexes with ferric and ferrous iron, making it less active with regard to the initiation of lipid peroxidation.[23] Iron chelation by UA in plasma also protects ascorbic acid from iron-catalyzed oxidation.[24] UA is present at a concentration of approximately 300 μM in human plasma,[19] which approaches maximal aqueous solubility at pH 7.4. UA is also practically insoluble in a hydrophobic environment like biological membranes (see Results).

For all these reasons, we initially decided to design new drugs derived from UA by modifying its lipophilicity in order to protect biological membranes against lipid peroxidation. The combination of lipophilic and hydrophilic molecular fractions should also exert a site-specific antioxidant protection against OR at the lipid/water interface, which is a prime site of superoxide anion formation and free iron location.[25] The molecular mechanism by which UA scavenges free radicals has been studied by several authors[22,26,27] who have shown that some substitutions of the nitrogens were possible without loss of its properties. We introduced aliphatic residues (unbranched chains of 6–16 carbons) on the four nitrogens of the UA structure. From a chemical point of view this often involved the construction of the five-membered ring from the corresponding substituted 5,6-diaminouracil. It turned out that these latter compounds showed powerful activity against biological membrane peroxidation. They were studied along with the urate derivatives.

This paper describes the synthesis, in vitro activity, and structure/activity relationship of two novel series of antioxidants: the N-substituted 5,6-diaminouracils and the N-substituted urate derivatives.

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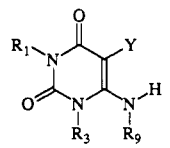
Table I. Physical Data, Lipophilicity, and Reducing Activity of 6-Aminouracil (1), 5,6-Diaminouracil (4), and Urate (5) Derivatives

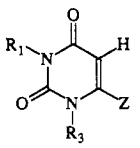
no.	R1[e]	R3[e]	R7[e]	R9[e]	formula	mp, °C[b]	log P_{corr} (log P_{exp})	reducing activity RC10 (μM)[c]
1h	H	H		n-$C_{12}H_{25}$	$C_{16}H_{29}N_3O_2$	>250		inactive
1g	n-$C_{12}H_{25}$	H		H	$C_{16}H_{29}N_3O_2$	242–243		inactive
4a	H	n-C_6H_{13}	H	H	$C_{10}H_{19}N_4O_2Cl$	>250	1.02 (1.01)	6.5
4b	H	n-C_8H_{17}	H	H	$C_{12}H_{23}N_4O_2Cl$	>200	2.03 (2.05)	6.5
4c	H	n-$C_{10}H_{21}$	H	H	$C_{14}H_{27}N_4O_2Cl$	>200	3.05 (3.04)	5.6
4d	H	n-$C_{12}H_{25}$	H	H	$C_{16}H_{31}N_4O_2Cl$	>200	4.07	7.0
4g	n-$C_{12}H_{25}$	H	H	H	$C_{16}H_{31}N_4O_2Cl$	>200	5.03	5.5
4l	H	H	n-$C_{12}H_{25}$	H	$C_{16}H_{31}N_4O_2$	>200	4.4	4.5
4h	H	H	H	n-$C_{12}H_{25}$	$C_{16}H_{31}N_4O_2Cl$	>200	4.4	6.4
4i	H	H	H	n-$C_{16}H_{33}$	$C_{20}H_{39}N_4O_2Cl$	>200	6.5	6.4
4j	CH_3	H	H	n-$C_{18}H_{37}$	$C_{23}H_{45}N_4O_2Cl$	187	>7[d]	6.2
4e	CH_3	n-$C_{12}H_{25}$	H	H	$C_{17}H_{33}N_4O_2$	194–195	5.7	5.0
4k	H	$CH_2C_6H_5$	n-$C_{12}H_{25}$	H	$C_{23}H_{36}N_4O_2$	160–161	>7[d]	6.5
41a	CH_3	H	H	H	*a*		−0.55	6.0
43a	H	CH_3	H	H	*a*		−1.52 (−1.4)	4.5
40a	H	H	H	H	*a*		−1.80 (−1.92)	7.0
5a	H	n-C_6H_{13}	H	H	$C_{11}H_{16}N_4O_3$	>250	−0.01 (−0.02)	4.5
5b	H	n-C_8H_{17}	H	H	$C_{13}H_{20}N_4O_3$	>250	1.01 (1.10)	6.5
5c	H	n-$C_{10}H_{21}$	H	H	$C_{15}H_{24}N_4O_3$	>250	2.03 (2.04)	3.5
5d	H	n-$C_{12}H_{25}$	H	H	$C_{17}H_{28}N_4O_3$	>250	3.05 (2.90)	3.2
5g	n-$C_{12}H_{25}$	H	H	H	$C_{17}H_{28}N_4O_3$	>250	4	5.2
5l	H	H	n-$C_{12}H_{25}$	H	$C_{17}H_{28}N_4O_3$	>250	3.22	27
5h	H	H	H	n-$C_{12}H_{25}$	$C_{17}H_{28}N_4O_3$	>250	3.22	6.2
5i	H	H	H	n-$C_{16}H_{33}$	$C_{21}H_{36}N_4O_3$	>250	5.26	4.5
5e	CH_3	n-$C_{12}H_{25}$	H	H	$C_{18}H_{30}N_4O_3$	>250	3.94	3
5m	CH_3	n-$C_{12}H_{25}$	CH_3	CH_3	$C_{20}H_{34}N_4O_3$	76–77		inactive
urate	H	H	H	H	*a*		−2.80 (−2.91)	4.5
51a	CH_3	H	H	H	*a*		−2.4	4.5
53a	H	CH_3	H	H	*a*			3.5
57a	H	H	CH_3	H	*a*			20
59a	H	H	H	CH_3	*a*			5
513a	CH_3	CH_3	H	H	*a*			3.5
517a	CH_3	H	CH_3	H	*a*			14
537a	H	CH_3	CH_3	H	*a*			inactive
trolox					*a*			3.7
α-tocopherol					*a*			6.0

[a] Commercial compounds. [b] For the large majority of compounds thermal decomposition occurred before the melting point was obtained. [c] Concentration in compound required to scavenge 10 μM 1,1-diphenylpicrylhydrazyl under our experimental conditions and determined from dose/response curves. Each determination was made in duplicate with less than 5% error. [d] For corrected log P over 7, values are generally considered as irrelevant. [e] Substituent position according to Chart I.

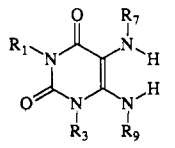
Chart I. General Formulas of Intermediate and Target Molecules (Diaminouracils 4 and Urate Derivatives 5)



1, Y = -H; 2, Y = -NO; 3, Y = -NHCHO
8, Y = NO_2; 9, Y = -N($COOC_2H_5$)$C_{12}H_{25}$



6, Z = -OH; 7, Z = -Cl



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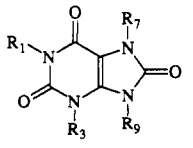

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Chemistry

The synthetic sequence leading to the diaminopyrimidine of general formulas **4a–i** includes condensation of an alkylurea with ethyl cyanoacetate to give the aminopyrimidines **1a–d**,[28] nitrosation,[29] and subsequent reduction of the nitroso function by zinc powder in formic acid, producing formamide **3**[30] which was further hydrolyzed (Chart I).[30] Compound **1e** was prepared by methylation of **1d**. Compounds **1h,i** were obtained by reaction of 6-amino-2,4-pyrimidinedione with an amine according to ref 29, while preparation of **1j** was slightly different as the more reactive 6-chloro-3-methyl-5-nitro-2,4-pyrimidinedione[31] precursor was used in the condensation with a primary amine, the nitro group **8j** being further reduced to a formylamino group, in a similar way as the nitroso compounds.

6-Chloro-3-dodecyl-2,4-pyrimidinedione, **7g**, obtained by reaction of 1-dodecyl-2,4,6-pyrimidinetrione (**6g**) with phosphorus oxychloride,[32] was treated with benzylamine, providing the benzylamino derivative **1f** which was further hydrogenated, affording compound **1g**. This synthetic sequence allows the unambiguous preparation of 1-substituted uric acids.

Compound **4l**, in which R7 is not simply a proton, was prepared by the reaction of dodecylamine with 6-amino-1-benzyl-5-bromo-2,4-pyrimidinedione according to the procedure previously described.[33] The benzyl group was removed by catalytic hydrogenation. The cyclization leading to **5l** was achieved by treatment of the intermediate urethane **9l** with sodium ethoxide in ethanol. The other uric acids **5a–i** were prepared by cyclization of the corresponding diamino derivatives with urea[34] (Chart I). Tetrasubstituted uric acid **5m** was prepared by methylation of **5e** with methyl iodide in DMF in the presence of potassium carbonate.

Biology

As an estimation of the lipophilicity, partition coefficients (P) values were determined by the combination of

Table II. Antioxidant Power and Radical Scavenging Properties of 6-Aminouracil (1), 5,6-Diaminouracil (4), and Urate (5) Derivatives

no.	substituent	position[c]	mitochondria DHF/FeII IC50 (μM)[b] (% *I* at 100 μM)	ML/DMVN in TBA/methanol T_{inh} (s) for 20 μM[a]	liposomes DPLC/DMVN T_{inh} (s) for 20 μM[a]
1g	$C_{12}H_{25}$	1	(1%)	inactive	inactive
1h	$C_{12}H_{25}$	9	(2%)	inactive	inactive
4a	C_6H_{13}	3	3.5	105	2010
4b	C_8H_{17}	3	0.62	114	3350
4c	$C_{10}H_{21}$	3	0.28	114	3700
4d	$C_{12}H_{25}$	3	0.27	102	3350
4g	$C_{12}H_{25}$	1	0.26	144	4200
4l	$C_{12}H_{25}$	7	0.57	84	6000
4h	$C_{12}H_{25}$	9	0.30	105	3500
4i	$C_{16}H_{33}$	9	0.90	150	2020
4j	CH_3, $C_{18}H_{37}$	1, 9	22	114	1750
4e	CH_3, $C_{12}H_{25}$	1, 3	0.24	126	3150
4k	$CH_2C_6H_5$, $C_{12}H_{25}$	3, 7	0.67		
4l	CH_3	1	50		
43	CH_3	3	82	156	800
40			100		
trolox			5.0	195	15000
α-tocopherol			(46%)	190	inactive
				% *I* 100 μM	IC50 μM (% *I* at 100 μM)
5a	C_6H_{13}	3	(26%)	8	200
5b	C_8H_{17}	3	28	15	37
5c	$C_{10}H_{21}$	3	3.7	8	22
5d	$C_{12}H_{25}$	3	1.6	17	20
5g	$C_{12}H_{25}$	1	1.25	25	10
5l	$C_{12}H_{25}$	7	1.6	10	200
5h	$C_{12}H_{25}$	9	1.0	13	11
5i	$C_{16}H_{33}$	9	0.75	10	8
5e	CH_3, $C_{12}H_{25}$	1, 3	0.85	15	18
5m	$3CH_3$, $C_{12}H_{25}$	1, 7, 9, 3	(5%)	0.7	(0.5%)
urate			(5%)	7	(0.5%)
51	CH_3	1	(10%)	13	
53	CH_3	3	(11%)	10	
57	CH_3	7	(20%)		
59	CH_3	9	(9%)		
513	CH_3, CH_3	1, 3	(36%)		
517	CH_3, CH_3	1, 3	(14%)		
537	CH_3, CH_3	3, 7	(5%)		

[a] Values determined from dose–response curves with duplicate experiments for each concentration. [b] Each compound was tested in duplicate at several concentrations. Average value for each concentration was plotted to determine an IC50. [c] Substituent position according to Chart I. DHF, dihydroxyfumaric acid; ML, methyl linoleate; TBA *tert*-butyl alcohol; DLPC, dilinoleoylphosphatidylcholine; DMVN, 2,2′-azobis-2,4-dimethylvaleronitrile.

two methods: experimentally for some compounds in an octanol/water system (P = [compound]^{octanol}/[compound]^{buffer}) and also calculated[35,36] for all the compounds (see the Experimental Section). Calculated log P values were in good correlation with experimental values: $\log P_{calc} = 1.04 \times \log P_{exp} + K$ (with K values of 0.89 and −1.63 for urate and diaminouracil derivatives, respectively). Corrected log P values were determined as $\log P_{corr} = \log P_{calc} - K$. This gave comparable and realistic values for all the compounds studied. These results are summarized in Table I.

The capacity of the different compounds to reduce the stable radical 1,1-diphenyl-2-picrylhydrazyl[37] (DPPH$^{\bullet}$) was determined. Except for compounds **51**, **5m**, **57**, and **537**, the reactions were fast and RC10 values represent the concentrations of compounds corresponding to the scavenging of 10 μM of DPPH$^{\bullet}$. Results are summarized in Table I with commercial methyl derivatives trolox C and α-tocopherol as references.

Evaluation of antioxidant properties of the compounds was performed by formation of thiobarbituric acid reactive substances (TBARS)[38] using heart mitochondria as a support for lipid peroxidation, and iron(II)/dihydroxyfumaric acid (DHF) as initiator. DHF is a rich and sustained source of superoxide radicals in aqueous solution via autoxidation.[39] Under these conditions, incubation of bovine heart mitochondria produced 0.84 ± 0.06 (n = 12) nmol of TBARS/min per mg of protein, whereas without induction no TBARS formation could be detected. The active compounds inhibited TBARS formation during 40 min in a dose-dependent manner from 0 to 100%. For less active compounds, the inhibition percentage determined or the highest concentration studied was given instead of the IC50 value. These results are summarized in Table II.

In order to test the ability of uracils and urate derivatives to scavenge lipid radicals (to act as a real chain-breaking antioxidant), these compounds were evaluated on two systems in which lipid peroxidation involved only propagation by lipid radicals. The incubation medium was either homogeneous (methyl linoleate in organic solvent) or biphasic (a liposomal suspension of dilinoleoylphosphatidylcholine (DLPC) in aqueous buffer). In both cases lipid peroxidation was initiated by thermal degradation of the lipid-soluble azo initiator, 2,2′-azobis-2,4-dimethylvaleronitrile (DMVN). Lipid peroxidation proceeded

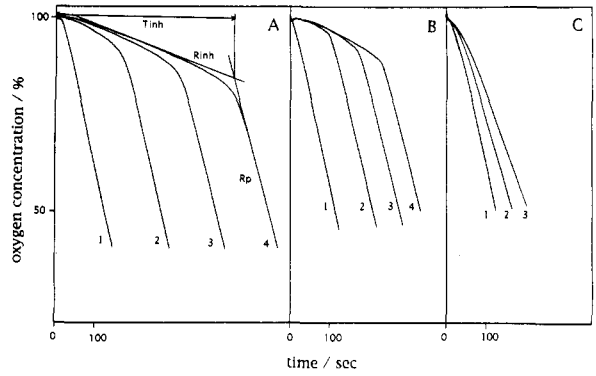

Figure 1. Inhibition of peroxidation of methyl linoleate by α-tocopherol, **4d**, and **5h**. Rate of oxygen uptake during peroxidation of methyl linoleate 2,2′-azobis-2,4-dimethylvaleronitrile (50 mM) in a *tert*-butyl alcohol/methanol medium (4/1 by volume) at 40 °C. (A) α-Tocopherol: 1, control; 2, 20 μM; 3, 40 μM; 4, 60 μM. (B) **4d**: 1, control; 2, 20 μM; 3, 40 μM; 4, 60 μM. (C) **5h**: 1, control; 2, 100 μM; 3, 200 μM. 100% in oxygen concentration corresponds to 1.8 mM. T_{inh}, inhibition time; R_{inh}, propagation rate during inhibition; R_p propagation rate after or without inhibition.

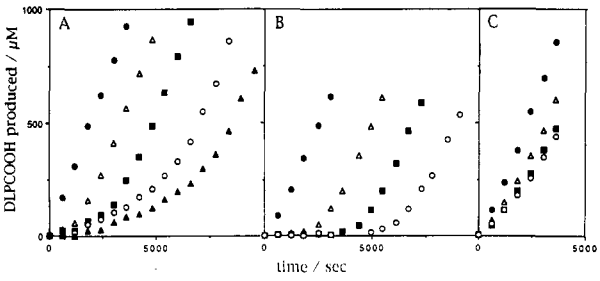

Figure 2. Inhibition of peroxidation of dilinoleoyl phosphatidylcholine by trolox, **4d**, **and 5i**. Production of hydroperoxides from large multilamellar liposomes of dilinoleoyl phosphatidylcholine (20 mM) initiated by 2,2′-azobis-2,4-dimethylvaleronitrile (0.5 mM) at 48 °C. The conjugated diene formation was monitored at 234 nm. (A) (●) control experiment, (△) trolox 2.5 μM, (■) 5 μM, (○) 7.5 μM, (▲) 10 μM; (B) (●) control experiment, (△) **4d** 16.6 μM, (■) 33.3 μM, (○) 50 μM; (C) (●) control experiment, (△) **5i** 5 μM, (■) 10 μM, (○) 15 μM. As on Figure 1, T_{inh}, R_{inh}, and R_p can be estimated.

via a typical radical chain mechanism:[40] propagated by lipid radicals (L•, LOO•) and regulated by termination reactions. Propagation velocity was followed by either oxygen consumption during methyl linoleate peroxidation (Figure 1) or conjugated diene formation resulting from the peroxidation of the linoleoyl residues of DLPC (Figure 2). The concentrations of the compounds were chosen so that they did not interfere with UV detection. This was confirmed by HPLC separation[41] of dilinoleoylphosphatidylcholine hydroperoxide (DLPCOOH) produced during incubation for one compound of each chemical series (not shown). Under these conditions, lipid peroxidation occurred at a constant rate, as described in the control experiments of Figures 1 and 2. Inactive compounds like **5m** had no effect on the propagation rate. Scavengers reacting moderately with lipid radicals compete with termination reactions. They are slowly consumed and durably affect the propagation rate. This was the case for urate derivatives. Kinetic examples are given in Figures 1C (**5h**) and 2C (**5i**). For these compounds an IC50 or an inhibition value of the rate of propagation characterizes their efficacy. Compounds reacting quickly with lipid radicals (like trolox in the two systems or α-tocopherol in monophasic medium, see Figure 1A or 2A) drastically affect the propagation rate and are much more quickly consumed. After their consumption, peroxidation starts again as quickly as in control experiments, giving a two-step kinetics. This was the case for substituted 5,6-diaminouracils. Typical kinetics obtained with compound **4d** are presented in Figures 1B and 2B. The efficacy of these compounds can be better compared by examining their inhibition time (T_{inh}) for a given concentration rather than their propagation rate during inhibition (R_{inh}). Results are summarized in Table II. Under these kinetic conditions, it is important to note that T_{inh} is directly related to the stoichiometric factor n (moles of radical scavenged per mole of antioxidant present)[42] through the equation: $T_{inh}R_i = n[\text{antioxidant}]$, where R_i is the initiation rate of lipid peroxidation. Trolox has been shown to be a reference antioxidant in comparable models[42] with $n = 2$. R_i is calculated using trolox, and then n of an unknown antioxidant can be estimated in comparison.

Discussion

With the exception of some substituted urates (**5l**, **5m**, **57a**, and **537a**), urate and diaminouracil derivatives present a similar ability to scavenge the nitrogen-centered radical 1,1-diphenyl-2-picrylhydrazyl (DPPH•) comparable to trolox or α-tocopherol. Since the reactions were extremely fast, RC10 values between 3 and 7 μM at the end of the reaction showed that the stoichiometry of the reaction was about 2 mol of DPPH• reduced for 1 mol of compound, like trolox or α-tocopherol. Results on diaminouracil derivatives indicate that monoalkylations were possible on each nitrogen atom without any loss in reducing activity. Nevertheless, the 5-amino group appeared necessary for reducing activity since substituted 6-aminouracils (**1g**, **1h**) and the nitroso intermediate **2h** (not shown) were totally inactive. Concerning the urate series the activity of methylated urates against DPPH• has been previously studied.[27] It has been demonstrated that the presence of two protons, one on nitrogen atom 7 and one on nitrogen atom 3 or 9, is essential for a maximal reducing activity. Methylation of the 7-nitrogen drastically decreases the velocity of the reaction without a change in the stoichiometry. Double methylation of the 3,9-, 7,9-, or 3,7-nitrogens also decreases the stoichiometry of the reaction. Our results are consistent with the above work (compounds **5l**, **5m**, **57a**, and **537a**) and demonstrate that substitution can be extended to long-chain alkyl groups without loss in their ability to reduce DPPH•.

Some diaminouracil and urate derivatives exerted a powerful protection of mitochondrial membranes against lipid peroxidation with IC50 values lower than 1 μM. These results should be compared with those of α-tocopherol (slightly active) or its short-chain analog trolox (IC50 = 5 μM). Relations with corrected log P values indicated that for both molecular series, antioxidant activity was extremely dependent on the lipophilicity of the compounds, with maximal activity for urate derivatives occurring when log P_{corr} was greater than 3 (**5d**, **5g**, **5l**, **5h**, **5i**, and **5e**) and for diaminouracils with log P_{corr} between 3 and 6 (**4c**, **4d**, **4g**, **4h**, and **4e**). This corresponded to dodecyl and hexadecyl urate derivatives and decyl- and dodecyldiaminouracil derivatives. For a given alkyl chain length, diaminouracils were more lipophilic and more active than urate derivatives. For a given lipophilicity, diaminouracils were also more active, probably indicating a greater reactivity of the diaminouracil moiety. Uric acid, 5,6-diaminouracil, and their methylated derivatives were unable to protect biological membranes in the micromolar

range, despite an ability to reduce DPPH• equivalent to that of long-chain derivatives. Uric acid has previously been described as an inhibitor of lipid peroxidation in comparable systems[43] but in the concentration range of 200–300 μM. Control compound **5m** was totally inactive even at 100 μM, indicating that inhibition of lipid peroxidation by long-chain substituted derivatives is not due to a simple lipid/compound interaction such as membrane dilution, detergent effect, or disturbance of membrane fluidity, which could also affect the rate of peroxidation. In both series the four possible *N*-dodecyl derivatives had similar antioxidant power (even compound **5l**, despite a limited reactivity against DPPH•). Interestingly, antioxidant power drastically decreased in the diaminouracil series for compounds having an alkyl chain length greater than 12 carbons (corrected log *P* greater than 6). A restriction in concentration at the site of peroxidation, due to a limited incorporation into the phospholipid membranes could be one explanation of this phenomenon. It can be associated with the ineffectiveness of α-tocopherol, in contrast to its short-chain analog trolox; due to its extremely hydrophobic structure, α-tocopherol is poorly incorporated into the phospholipid bilayer when added with a dispersion method and forms clusters. This decreases its efficiency as a radical scavenger.[44] Short-chain analogs such as trolox are also lipophilic despite their relative solubility in water. They have a better transbilayer mobility and are more active antioxidants.[44] The antioxidant activity could be maximized with log P_{corr} values of the diaminouracil series, which were evaluated between 3 and 6.

Different modes of action have been proposed in the literature to explain the antioxidant power of the urate structure (see Introduction), and very little is known about the reactivity of the 5,6-diaminouracil structure, apart from some results on its interaction with metal ions and sensitivity towards molecular oxygen in an aqueous environment.[45,46] The ability of both chemical series to reduce the stable radical DPPH• suggests a radical scavenging potential. We evaluated the two molecular series in two tests involving only lipid radicals in the propagation steps of a peroxidative process, either in an organic medium (homogeneous) or in a lipid bilayer environment (heterogeneous). The results clearly showed that both urate and diaminouracil derivatives indeed acted as chain-breaking antioxidants, and that this activity in the liposomal model (as in biological membranes) depended largely on the location of the compound. However, some kinetic differences were apparent between the two series.

All urate derivatives showed a limited antioxidant power on the monophasic experiments (not related to the lipophilicity of the structures). The highest inhibition was obtained with compound **5h**, and Figure 1C shows that this inhibition was dose-dependent despite its limited amplitude. However, with the exception of compounds **5l** and **5m**, the chain-breaking activity of the urate derivatives seemed highly related to the lipophilicity of the compounds in the liposomal model, with IC50 values between 10 and 20 μM for compounds with log P_{corr} between 3 and 6. These values corresponded to a strong inhibition, since the peroxidation propagation rate was around 15 μM peroxide formed per minute in this model, and the rate of initiation deduced from the inhibition curves obtained with trolox was 0.37 μM/min. As anticipated, the control compound **5m** was totally inactive in the two tests. Similar to the reducing activity against DPPH•, substitution on the 7-nitrogen of the urate structure (compound **5l**) also decreased the chain-breaking activity. These results indicate that the aptitude to scavenge lipid radicals is related to the reducing capacity of DPPH•, and that these properties play a major role in the antioxidant activity of series **5**.

All the 5,6-diaminouracil derivatives inhibited propagation of the peroxidation in the monophasic model, regardless of the alkyl chain length, with inhibition times between 84 s (**4l**) and 156 s (**4j**) at 20 μM. Since the peroxidation initiation rate deduced from the inhibition curves obtained with α-tocopherol was 0.22 μM/s, these data indicated that, under these conditions, diaminouracil derivatives were able to deactivate about 1 mol of lipid radical per mole of compound, compared to a stoichiometry of 2 for 1 in the case of trolox or α-tocopherol. However, the chain-breaking activity of 5,6-diaminouracils in the liposomal model was strongly related to their antioxidant power evaluated on the mitochondrial model. Optimal activity was obtained between the octyl and dodecyl derivatives (log P_{corr} between 2 and 6), and the activity decreased significantly for compounds with higher log P_{corr} (**4e**, **4i**, and **4j**). α-Tocopherol was totally inactive in the liposomal model under these conditions (added in DMSO to preformed LML) despite a strong reactivity in the monophasic model. As in the mitochondrial model, α-tocopherol and probably the long-chain substituted diaminouracils are poorly incorporated in the phospholipid bilayer when added exogenously. This was confirmed for α-tocopherol since it was shown to be as active as trolox when incorporated in the phospholipid solution before the liposome preparation (not shown). 5,6-Diaminouracil derivatives were also less active than trolox in the liposomal model. Despite a much lower inhibition rate (see Figure 1), inhibition times (T_{inh}) were less than half as long for the best compounds. This indicated apparent stoichiometric factors *n* which were proportionately as small, from $n = 0.1$ (**4j**) to $n = 0.8$ (**4l**). Nevertheless, some 5,6-diaminouracil derivatives were much more active as an antioxidant on the mitochondrial membrane than trolox.

Within the two new series presented here, some compounds are extremely efficient in protecting biological membranes against lipid peroxidation in vitro, compared to the reference antioxidant trolox C. One of the major causes of this powerful antioxidant activity is the combination of two properties: the ability to deactivate radicals by giving up electrons, making them inactive, without themselves propagating chain reactions (radical scavengers), and the molecular proximity to the site of the peroxidative processes (lipophilicity). Monoalkylation of 5,6-diaminouracil or urate structures on each nitrogen lead to radical scavengers (with the exception of 7-*N*-urate derivatives), indicating that a large variety of new active compounds could be synthesized.

Although antioxidant properties appear to be highly related to radical scavenging activity in these two chemical series, 7-*N*-dodecyluric acid (**5l**) is effective as an antioxidant despite a low chain-breaking activity. Similarly, the powerful antioxidant activity of diaminouracils compared to that of trolox is difficult to explain within the framework of a simple chain-breaking activity, since this latter compound has a greater radical scavenging activity. We cannot exclude the participation of urate and diami-

nouracil derivatives in other antioxidant mechanisms, such as interactions with metal ions (redox reactions, chelation), as suggested in the literature.[23] The best compounds of the two series, dodecyl urates and decyl- and dodecyldiaminouracils, were chosen for further in vitro investigation and in vivo evaluation.

Experimental Section

Starting materials were purchased from Aldrich Co.

Absolute ethyl alcohol was distilled upon magnesium turnings; *N,N'*-dimethylformamide (DMF) was dried over molecular sieves (4 Å); all other solvents and commercial compounds were of the highest purity available. Column chromatography was carried out on Kieselgel 60 (Merk, 230–400 mesh). Organic extracts were dried over $MgSO_4$ and evaporated in vacuo. Melting points are uncorrected. ^1H NMR spectra were recorded on a Hitachi R24B (60 MHz) or, in which case this will be specified, on a Brucker (AM300) (300-MHz spectrometer with Me_4Si as internal standard). ^{13}C NMR spectra were recorded with a Brucker (AMX500) (500 MHz) spectrometer. All elemental analyses are within ±0.4% of the calculated values unless otherwise specified. Compound **5l** has been previously described.[47] UV detection was performed on a DU70 Beckman UV/visible spectrometer. Fluorimetric measurements of TBARS were performed on a SFM25 Kontron fluorescence spectrometer.

Chemical Methods. General Procedure for the Preparation of 1-Alkyl-2,4-pyrimidinedione. To 100 mL of a 1.7 M solution of sodium ethoxide in absolute ethanol was added 0.15 mol of both ethyl cyanoacetate and alkylurea. The mixture was refluxed for 7 h and then precipitated by addition of concentrated aqueous HCl until acidic, followed by 200 mL of water. The precipitate was isolated by filtration and then recrystallized in ethanol.

6-Amino-1-hexyl-2,4-pyrimidinedione (1a): 69%; mp >250 °C; NMR (DMSO-d_6) δ 0.90 (t, 3H), 1.30 (m, 8H), 3.53 (t, 2H), 4.55 (s, 1H), 6.70 (bs, 2H), 10.15 (s, 1H). Anal. ($C_{10}H_{17}N_3O_2$) C, H, N.

6-Amino-1-octyl-2,4-pyrimidinedione (1b): 68%; mp >250 °C; NMR (DMSO-d_6) δ 0.90 (t, 3H), 1.25 (m, 12H), 3.52 (t, 2H), 4.56 (s, 1H), 6.75 (bs, 2H), 10.10 (s, 1H). Anal. ($C_{12}H_{21}N_3O_2$) C, H, N.

6-Amino-1-decyl-2,4-pyrimidinedione (1c): 67%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H), 1.25–1.52 (m, 16H), 3.73 (t, 2H), 4.61 (s, 1H), 6.77 (s, 2H), 10.31 (s, 1H). Anal. ($C_{14}H_{25}N_3O_2$) C, H, N.

6-Amino-1-dodecyl-2,4-pyrimidinedione (1d): 65%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.87 (t, 3H), 1.26 (m, 18H), 1.52 (m, 2H), 3.73 (t, 2H), 4.58 (s, 1H), 6.69 (bs, 2H), 10.20 (s, 1H). Anal. ($C_{16}H_{29}N_3O_2$) C, H, N, O.

6-Amino-1-dodecyl-3-methyl-2,4-pyrimidinedione (1e). Compound **1d** (10 mmol, 2.95 g) was suspended in a mixture of 10 mL of 1 N aqueous sodium hydroxide and 10 mL of ethanol, and then 1.3 g of dimethyl sulfate was introduced at 60 °C. Heating was maintained until the pH was almost neutral; 1 N aqueous NaOH was introduced until the pH became basic. Extraction with $CHCl_3$, drying ($MgSO_4$), and evaporation afforded **1e** (2.35 g, 76%): mp 73–74 °C; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.22 (m, 18H), 1.49 (m, 2H), 3.07 (s, 3H), 3.71 (t, 2H), 4.67 (s, 1H), 6.68 (bs, 2H).

6-(Dodecylamino)-2,4-pyrimidinedione (1h). A mixture of 7.5 g of 6-aminouracil (59 mmol), 12 g of dodecylamine and 12 g of dodecylamine hydrochloride was stirred at 160 °C for 3 h. After the addition of 100 mL of ethanol and filtration, the solid was suspended for a few minutes in 2 N aqueous NaOH, the precipitate was recrystallized twice in acetic acid, 7 g, 40%: mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.25 (m, 18H), 1.50 (m, 2H), 2.98 (q, 2H), 4.37 (s, 1H), 5.98 (t, 1H), 9.78 (s, 1H), 10.07 (s, 1H). Anal. ($C_{16}H_{29}N_3O_2$) C, H, N, O.

The same procedure was applied for the preparation of **6-(hexadecylamino)-2,4-pyrimidinedione (1i)**: 36%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.83 (t, 3H), 1.23 (m, 28H), 1.50 (m, 2H), 3.00 (q, 2H), 4.36 (s, 1H), 5.96 (t, 1H), 9.80 (s, 1H), 10.10 (s, 1H). Anal. ($C_{20}H_{37}N_3O_2$) C, H, N, O.

Preparation of 1-dodecyl-2,4,6-pyrimidinetrione (6g). Applying the procedure used for the synthesis of **1a–d** and replacing ethyl cyanoacetate by diethyl malonate, we obtained **6g** with 65% yield after crystallization from ethanol: mp 129–130 °C; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.25 (m, 18H), 1.51 (m, 2H), 3.68 (s, 2H), 3.76 (t, 2H), 11.42 (s, 1H). Anal. ($C_{16}H_{28}N_2O_3$) C, H, N, O.

Preparation of 6-chloro-3-dodecyl-2,4-pyrimidinedione (7g). To a mixture of 30 g of **6g** (101 mmol) and 13.5 mL of water was cautiously added 100 mL of $POCl_3$. The mixture was then refluxed for 1 h, and excess phosphorus oxychloride was removed by distillation under reduced pressure. The residue was poured on crushed ice, and the precipitate was washed with water. Recrystallization from a H_2O/MeOH mixture afforded 20.5 g of **6g** (64%): mp 156–157 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.26 (m, 18H), 1.51 (m, 2H), 3.72 (t, 2H), 5.70 (s, 1H), 12.21 (s, 1H). Anal. ($C_{16}H_{27}N_2O_2Cl$) C, H, N.

6-(Benzylamino)-3-dodecyl-2,4-pyrimidinedione (1f). A solution of 30 g (95 mmol) of **6g** in 150 mL of benzylamine was heated at 170 °C for 1 h, and then 100 mL of water were added. The precipitate was collected and washed with methanol. Crystallization from MeOH gave 25.13 g of **1f** (69%): mp 191–192 °C; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.26 (m, 18H), 1.52 (m, 2H), 3.73 (t, 2H), 4.23 (d, 2H), 4.58 (s, 1H), 6.38 (t, 1H), 7.34 (m, 5H), 10.06 (bs, 1H). Anal. ($C_{23}H_{25}N_3O_2$) C, H, N.

6-Amino-3-dodecyl-2,4-pyrimidinedione (1g). **1f** (25 g, 95 mmol) in 400 mL of ethanol was hydrogenated on 4 g of Pd/C (10% Pd) at 80 °C with hydrogen pressure of 3 MPa for 6 h. Filtration of the hot solution and concentration afforded 17.02 g of **1g** (60%): mp 242–243 °C; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.25 (m, 18H), 1.52 (m, 2H), 3.63 (t, 2H), 4.54 (s, 1H), 6.12 (bs, 2H), 10.06 (bs, 1H). Anal. ($C_{16}H_{29}N_3O_2$) Calcd: C, 65.05; H, 9.89; N, 14.22. Found: C, 65.25; H, 10.09; N, 13.86.

General Procedure for Nitrosation of Compounds 1a–i. Isoamyl nitrite (5 mL) was added dropwise to a suspension of 17 mmol of derivative **1** in 120 mL of ethanol; two drops of 12 N HCl was then added. After 3 h of stirring at room temperature, violet crystals were isolated and recrystallized from ethanol.

6-Amino-1-hexyl-5-nitroso-2,4-pyrimidinedione (2a): 73%; mp 195–196 °C; NMR (300 MHz, DMSO-d_6) δ 0.83 (t, 3H), 1.23 (m, 6H), 1.50 (m, 2H), 3.77 (t, 2H), 9.10 (bs, 1H), 11.5 (bs, 1H), 13.32 (s, 1H). Anal. ($C_{10}H_{16}N_4O_3$) C, H, N.

6-Amino-5-nitroso-1-octyl-2,4-pyrimidinedione (2b): 71%; mp 199–200 °C; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.20 (m, 10H), 1.52 (m, 2H), 3.76 (t, 2H), 9.05 (bs, 1H), 11.45 (bs, 1H), 13.30 (s, 1H). Anal. ($C_{12}H_{20}N_4O_3$) C, H, N.

6-Amino-1-decyl-5-nitroso-2,4-pyrimidinedione (2c): 72%; mp 202–203 °C; NMR (300 MHz, DMSO-d_6) δ 0.81 (t, 3H), 1.22 (m, 14H), 1.55 (m, 2H), 3.78 (t, 2H), 9.02 (bs, 1H), 11.43 (bs, 1H), 13.29 (s, 1H). ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 14.15, 22.30, 26.02, 26.52, 28.88, 28.93, 29.09, 29.11, 31.47, 40.48, 138.84, 146.87, 148.94, 160.59. Anal. ($C_{14}H_{24}N_4O_3$) C, H, N.

6-Amino-1-dodecyl-5-nitroso-2,4-pyrimidinedione (2d): 78%; mp 205–206 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.26 (m, 18H), 1.51 (m, 2H), 3.76 (t, 2H), 9.16 (bs, 1H), 11.49 (bs, 1H), 13.28 (bs, 1H). Anal. ($C_{16}H_{28}N_4O_3$) C, H, N.

6-Amino-1-dodecyl-3-methyl-5-nitroso-2,4-pyrimidinedione (2e): 79%; mp 157–158 °C; NMR (300 MHz, DMSO-d_6) δ 0.88 (t, 3H), 1.27 (m, 18H), 1.52 (m, 2H), 3.29 (s, 2H), 3.84 (t, 2H), 9.16 (bs, 1H), 13.21 (bs, 1H). Anal. ($C_{17}H_{30}N_4O_3$) C, H, N.

6-Amino-3-dodecyl-5-nitroso-2,4-pyrimidinedione (2g): 87%; mp 230 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.25 (m, 18H), 1.51 (m, 2H), 3.80 (t, 2H), 7.97 (bs, 1H), 11.36 (bs, 1H), 13.10 (bs, 1H). Anal. ($C_{16}H_{28}N_4O_3$) C, H, N.

6-(Dodecylamino)-5-nitroso-2,4-pyrimidinedione (2h): 78%; mp 172–173 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.26 (m, 18H), 1.50 (m, 2H), 3.43 (q, 2H), 10.81 (s, 1H). Anal. ($C_{16}H_{28}N_4O_3$) C, H, N.

6-(Hexadecylamino)-5-nitroso-2,4-pyrimidinedione (2i): 79%; mp 167–169 °C. Anal. ($C_{20}H_{36}N_4O_3 \cdot 0.5H_2O$) Calcd: C, 61.70; H, 9.51; N, 14.40. Found: C, 61.85; H, 9.60; N, 14.45.

3-Methyl-5-nitro-6-octadecyl-2,4-pyrimidinedione (8j). 6-Chloro-3-methyl-5-nitro-2,4-pyrimidinedione (2.05 g, 10 mmol) was introduced in small portions into a solution of 3 g of octadecylamine (11 mmol) and 1 g of triethylamine (10 mmol) in 20 mL of ethanol. The solution was refluxed for 20 min and

then neutralized by addition of acetic acid. The precipitate was washed with water and recrystallized in ethanol: yield 2.11 g (48%); mp 130 °C; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.22 (m, 30H), 1.54 (m, 2H), 2.78 (m, 2H), 3.12 (s, 3H), 9.21 (t, 1H).

General Procedure for Reduction with Zinc Powder in Formic Acid. Zinc powder (4 g) (Aldrich, <325 mesh) was added portionwise to a refluxing solution of 10 mmol of nitroso or nitro derivatives in 100 mL of formic acid. After 1 h under reflux, the hot mixture was filtered through a Celite pad and the solvent evaporated. Crystallization from methanol afforded the expected compounds.

6-Amino-5-formamido-1-hexyl-2,4-pyrimidinedione (3a): 86%; mp 210–211 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.25 (m, 6H), 1.51 (m, 2H), 3.80 (t, 2H), *E* isomer; 7.02 (s, 2H), 7.71 (d, J = 11.5 Hz, 1H), 8.01 (d, J = 11.5 Hz, 1H), 10.70 (s, 1H), *Z* isomer; 6.67 (s, 2H), 8.08 (s, 1H), 8.55 (s, 1H), 10.62 (s, 1H). Anal. ($C_{11}H_{18}N_4O_3$) C, H, N.

6-Amino-5-formamido-1-octyl-2,4-pyrimidinedione (3b): 86%; mp 214–215 °C; NMR (300 MHz, DMSO-d_6) δ 0.83 (t, 3H), 1.25 (m, 10H), 1.52 (m, 2H), 3.81 (t, 2H), *E* isomer; 7.03 (s, 2H), 7.72 (d, J = 11.5 Hz, 1H), 8.00 (d, J = 1.5 Hz, 1H), 10.68 (s, 1H), *Z* isomer; 6.63 (s, 2H), 8.07 (s, 1H), 8.57 (s, 1H), 10.62 (s, 1H). Anal. ($C_{13}H_{22}N_4O_3$) C, H, N.

6-Amino-1-decyl-5-formamido-2,4-pyrimidinedione (3c): 85%; mp 215–216 °C; NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H), 1.25 (m, 14H), 1.51 (m, 2H), 3.75 (t, 2H), *E* isomer; 6.92 (s, 2H), 7.69 (d, J = 11 Hz, 1H), 7.97 (d, J = 11 Hz, 1H), 10.67 (s, 1H), *Z* isomer; 6.66 (s, 2H), 8.04 (s, 1H), 8.56 (s, 1H), 10.58 (s, 1H). Anal. ($C_{15}H_{26}N_4O_3$) C, H, N.

6-Amino-1-dodecyl-5-formamido-2,4-pyrimidinedione (3d): 90%; mp 215–216 °C. Anal. ($C_{17}H_{30}N_4O_3$) C, H, N, O.

6-Amino-1-dodecyl-5-formamido-3-methyl-2,4-pyrimidinedione (3e): 85%; mp 213–215 °C; NMR (300 MHz, DMSO-d_6) δ 0.88 (t, 3H), 1.27 (m, 18H), 1.54 (m, 2H), 3.16 (s, 3H), 3.85 (t, 2H), *E* isomer; 6.97 (s, 2H), 7.75 (d, J = 11.5 Hz, 1H), 8.07 (d, J = 11.5 Hz, 1H), *Z* isomer; 6.67 (s, 2H), 8.12 (s, 1H), 8.63 (s, 1H). Anal. ($C_{18}H_{32}N_4O_3$) C, H, N.

6-Amino-3-dodecyl-5-formamido-2,4-pyrimidinedione (3g): 73%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.27 (m, 18H), 1.52 (m, 2H), 3.68 (t, 2H), *E* isomer; 6.32 (s, 2H), 7.72 (d, J = 11.5 Hz, 1H), 8.06 (d, J = 11.5 Hz, 1H), 10.42 (s, 1H), *Z* isomer; 6.04 (s, 2H), 8.04 (d, J = 0.9 Hz, 1H), 8.63 (d, J = 0.9 Hz, 1H), 10.36 (s, 1H). Anal. ($C_{17}H_{30}N_4O_3$) C, H, N.

6-(Dodecylamino)-5-formamido-2,4-pyrimidinedione (3h): 81%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.25 (m, 18H), 1.50 (m, 2H), 3.19 (m, 2H), *E* isomer; 6.85 (sm, 1H), 7.68 (d, J = 11.5 Hz, 1H), 8.05 (d, J = 11.5 Hz, 1H), 10.37 (s, 1H), 10.44 (s, 1H), *Z* isomer; 6.49 (m, 1H), 7.90 (d, J = 1 Hz, 1H), 8.46 (d, J = 1 Hz, 1H), 10.37 (s, 1H), 10.44 (s, 1H). Anal. ($C_{17}H_{30}N_4O_3$) C, H, N.

5-Formamido-6-(hexadecylamino)-2,4-pyrimidinedione (3i): 83%; mp >250 °C. Anal. ($C_{21}H_{38}N_4O_3$) Calcd: C, 63.93; H, 9.71; N, 14.20. Found: C, 63.30; H, 9.39; N, 14.10.

5-Formamido-3-methyl-6-(octadecylamino)-2,4-pyrimidinedione (3j): 86%; mp 196–199 °C; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.25 (m, 32H), 3.09 (s, 3H), 3.21 (m, 2H), *E* isomer; 6.71 (t, 1H), 7.56 (d, J = 11 Hz, 1H), 7.87 (d, J = 11 Hz, 1H), 10.54 (bs, 1H); *Z* isomer; 6.34 (t, 1H), 8.10 (s, 1H), 8.49 (s, 1H), 10.50 (bs, 1H). Anal. ($C_{24}H_{44}N_4O_3$) C, H, N.

General Procedure for the Preparation of Diaminouracils (4). Ten millimoles of the formyl derivative was suspended in 100 mL of methanol, and then gaseous HCl was bubbled into the solution for 15 min. The mixture was then refluxed for 3 h. After the mixture was cooled to 0 °C, the precipitate was filtered off and rinsed with methanol. The diaminouracil derivatives tended to turn pink on prolonged exposure to air.

5,6-Diamino-1-hexyl-2,4-pyrimidinedione, hydrochloride (4a): 88%; mp >260 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.25 (m, 6H), 1.52 (m, 2H), 3.81 (t, 2H), 7.80 (bs, 1H), 9.52 (bs, 3H), 11.10 (s, 1H). Anal. ($C_{10}H_{19}N_4O_2Cl$) C, H, N, Cl.

5,6-Diamino-1-octyl-2,4-pyrimidinedione, hydrochloride (4b): 87%; mp >200 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.25 (m, 10H), 1.49 (m, 2H), 3.80 (t, 2H), 7.81 (bs, 2H), 9.46 (bs, 3H), 11.13 (s, 1H). Anal. ($C_{12}H_{23}N_4O_2Cl$) C, H, N, Cl.

1-Decyl-5,6-diamino-2,4-pyrimidinedione, hydrochloride (4c): 85%; mp >200 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.25 (m, 14H), 1.51 (m, 2H), 3.81 (t, 2H), 7.81 (s, 2H), 9.75 (bs, 3H), 11.10 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 13.89, 22.01, 25.75, 27.35, 28.72, 28.79, 28.94, 28.95, 31.22, 41.53, 82.85, 149.45, 149.88, 158.76. Anal. ($C_{14}H_{27}N_4O_2Cl$) C, H, N.

5,6-Diamino-1-dodecyl-2,4-pyrimidinedione, hydrochloride (4d): 85%; mp >200 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.26 (m, 18H), 1.52 (m, 2H), 3.82 (t, 2H), 7.38 (s, 2H), 9.05 (bs, 3H), 11.08 (s, 1H). Anal. ($C_{16}H_{31}N_4O_2Cl$) C, H, N.

5,6-Diamino-1-dodecyl-3-methyl-2,4-pyrimidinedione, hydrochloride (4e): 86%; mp 194–195 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.23 (m, 18H), 1.50 (m, 2H), 3.13 (s, 3H), 3.84 (t, 2H), 7.75 (bs, 2H), 9.45 (bs, 3H). Anal. ($C_{17}H_{33}N_4O_2$-Cl) C, H, N, Cl.

5,6-Diamino-3-dodecyl-2,4-pyrimidinedione, hydrochloride (4g): 82%; mp >200 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.25 (m, 18H), 1.52 (m, 2H), 3.68 (t, 2H), 7.19 (s, 2H), 9.40 (bs, 3H), 11.05 (bs, 1H). Anal. ($C_{16}H_{31}N_4O_2Cl$) C, H, N, Cl.

5-Amino-6-(dodecylamino)-2,4-pyrimidinedione, hydrochloride (4h): 83%; mp >200 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.25 (m, 18H), 1.51 (m, 2H), 3.27 (q, 2H), 7.96 (t, 1H), 9.65 (bs, 3H), 11.90 (s, 1H). Anal. ($C_{16}H_{31}N_4O_2Cl$) C, H, N, Cl.

5-Amino-6-(hexadecylamino)-2,4-pyrimidinedione, hydrochloride (4i): 85%; mp >200 °C dec. Anal. ($C_{20}H_{39}N_4O_2$-Cl) Calcd C, 59.60; H, 9.75; N, 13.90; Cl, 8.79. Found: C, 59.50; H, 10.00; N, 13.67; Cl, 8.65.

5-Amino-3-methyl-6-(octadecylamino)-2,4-pyrimidinedione, hydrochloride (4j): 81%; mp 187 °C dec; NMR (300 MHz, DMSO-d_6) δ 0.89 (t, 3H), 1.27 (m, 30H), 1.55 (m, 2H), 3.15 (s, 3H), 3.32 (m, 2H), 7.86 (m, 1H), 10.40 (bs, 3H). Anal. ($C_{23}H_{45}N_4O_2Cl$) Calcd: C, 62.07; H, 10.19; N, 12.58; Cl, 7.97. Found: C, 62.18; H, 9.96; N, 12.32; Cl, 8.09.

6-Amino-1-benzyl-5-(dodecylamino)-2,4-pyrimidinedione (4k). A suspension of 5.12 g (23.5 mmol) of 6-amino-1-benzyl-5-bromo-2,4-pyrimidinedione and 11.1 g of dodecylamine (60 mmol) in 100 mL of 1-butanol was refluxed for 3 h. The solvent was removed under reduced pressure and the residue taken up in 50 mL of ethanol. Recrystallization of the precipitate from ethanol afforded 4.2 g of **4k**: 45%; mp 160–161 °C; (DMSO–$CDCl_3$) δ 0.87 (m, 3H), 1.25 (m, 20H), 2.95 (m, 2H), 5.04 (s, 2H), 5.85 (bs, 2H), 7.19 (s, 5H). Anal. ($C_{23}H_{36}N_4O_2$) C, H, N.

6-Amino-5-(dodecylamino)-2,4-pyrimidinedione (4l). 4k (1 g) was dissolved in 50 mL of ethanol, 100 mg of Pd/C (10% Pd) were added, and catalytic hydrogenation was performed at 50 °C at atmospheric pressure until the theoretical quantity of H_2 had been consumed. The mixture was filtered while hot and the filtrate concentrated under reduced pressure. The precipitate formed was recrystallized in ethanol: yield 510 mg (66%); mp >200 °C; NMR (300 MHz, DMSO-d_6–$CDCl_3$) δ 0.86 (t, 3H), 1.27 (m, 18H), 1.48 (m, 2H), 2.62 (t, 2H), 3.32 (m, 1H), 5.67 (s, 2H), 9.80 (s, 1H), 10.08 (s, 1H).

General Procedure for the Preparation of Uric Acids 5a–i. A mixture of 2 mmol of diaminouracil **4** and 1 g of urea was heated at 170–180 °C for 1–2 h, and then 20 mL of water was poured into the cooled mixture. The solid was isolated, washed with ethanol, and recrystallized in acetic acid in the presence or decolorizing charcoal.

3-Hexyl-2,6,8-purinetrione (5a): 75%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H), 1.25 (m, 6H), 1.52 (m, 2H), 3.72 (t, 2H), 10.72 (s, 1H), 10.98 (s, 1H), 11.80 (s, 1H). Anal. ($C_{11}H_{16}N_4O_3$) C, H, N.

3-Octyl-2,6,8-purinetrione (5b): 78%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.25 (m, 10H), 1.53 (m, 2H), 3.71 (t, 2H), 10.76 (s, 1H), 11.01 (s, 1H), 11.85 (s, 1H). Anal. ($C_{13}H_{20}N_4O_3$) C, H, N.

3-Decyl-2,6,8-purinetrione (5c): 72%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.25 (m, 14H), 1.51 (m, 2H), 3.81 (t, 2H), 10.71 (s, 1H), 10.98 (s, 1H), 11.82 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6–$CDCl_3$) δ 14.42, 22.61, 26.08, 28.07, 29.06, 29.08, 29.32, 29.34, 31.81, 43.54, 92.93, 137.21, 149.91, 152.83, 153.18. Anal. ($C_{15}H_{24}N_4O_3$) C, H, N.

3-Dodecyl-2,6,8-purinetrione (5d): 71%; mp >250 °C; NMR (300 MHz, DMSO-d_6–$CDCl_3$) δ 0.85 (t, 3H), 1.22 (m, 18H), 1.48

(m, 2H), 3.73 (t, 2H), 10.54 (s, 1H), 11.35 (s, 1H), 12.03 (s, 1H). Anal. ($C_{17}H_{28}N_4O_3$) C, H, N.

3-Dodecyl-1-methyl-2,6,8-purinetrione (5e): 88%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.24 (m, 18H), 1.56 (m, 2H), 3.19 (s, 3H), 3.77 (t, 2H), 10.79 (s, 1H), 11.89 (s, 1H). Anal. ($C_{18}H_{30}N_4O_3$) C, H, N.

1-Dodecyl-2,6,8-purinetrione (5g): 73%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.25 (m, 18H), 1.48 (m, 2H), 3.73 (t, 2H), 10.54 (s, 1H), 11.35 (s, 1H), 12.03 (s, 1H). Anal. ($C_{17}H_{28}N_4O_3$) C, H, N.

9-Dodecyl-2,6,8-purinetrione (5h): 49%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.85 (t, 3H), 1.25 (m, 18H), 1.51 (m, 2H), 3.63 (t, 2H), 10.82 (s, 1H), 10.86 (s, 1H), 12.00 (s, 1H). Anal. ($C_{17}H_{28}N_4O_3$) C, H, N.

9-Hexadecyl-2,6,8-purinetrione (5i): 41%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.84 (t, 3H), 1.22 (m, 26H), 1.5 (m, 2H), 3.62 (t, 2H), 10.78 (s, 1H), 10.81 (s, 1H), 11.98 (s, 1H). Anal. ($C_{21}H_{36}N_4O_3$) C, H, N.

Procedure for the Synthesis of 7-Dodecyl-2,6,8-purinetrione (5l). 6-Amino-1-benzyl-5-(*N*-dodecyl-*N*-(ethoxycarbonyl)amino)-2,4-pyrimidinedione (9k). 4k (2 g, 5 mmol), 1 g of dry K_2CO_3, and 1 g of ethyl chloroformate in 200 mL of dimethylformamide (DMF) were stirred for 2 h at room temperature, and then 200 mL of $CHCl_3$ were introduced. The organic phase was washed with water, dried, and concentrated. The residue was chromatographed, eluting with MeOH/$CHCl_3$ (5% v/v): yield 1.64 g; 69%; mp 196–197 °C; NMR (300 MHz, DMSO-d_6–$CDCl_3$) δ 0.90 (t, 3H), 1.09 (t, 3H), 1.28 (m, 18H), 1.53 (m, 2H), 3.31 (m, 2H), 4.03 (q, 2H), 5.16 (s, 2H), 6.81 (s, 2H), 7.15–7.30 (m, 5H), 10.79 (s, 1H). Anal. ($C_{26}H_{40}N_4O_4$) C, H, N.

6-Amino-5-(*N*-dodecyl-*N*-(ethoxycarbonyl)amino)-2,4-pyrimidinedione (9l). 9k (6 g, 12.7 mmol) and 0.3 g of Pd/C (10% Pd) were suspended in 100 mL of ethanol and 10 mL of concentrated aqueous NH_4OH solution. The mixture was hydrogenated at atmospheric pressure at 50 °C. When the theoretical amount of hydrogen had been consumed, 400 mL of ethanol was added and the catalyst was filtered from the hot solution. Concentration of the solvent and filtration afforded 3.84 g of **9l**: 79%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.82 (t, 3H), 1.10 (t, 3H), 1.25 (m, 18H), 1.52 (m, 2H), 3.20 (m, 2H), 3.97 (q, 2H), 6.21 (s, 2H), 10.25 (bs, 2H). Anal. ($C_{19}H_{34}N_4O_4$) C, H, N.

7-Dodecyl-2,6,8-purinetrione (5l). 9l (3.5 g, 9.1 mmol) was introduced into 100 mL of a 1 M ethanolic sodium ethoxide solution. The suspension was refluxed for 4 days. The solvent was removed, and the residue was dissolved in 20 mL of water and acidified with 12 N HCl. Filtration and subsequent recrystallization in acetic acid afforded **5l**: 1.56 g, 51%; mp >250 °C; NMR (300 MHz, DMSO-d_6) δ 0.86 (t, 3H), 1.27 (m, 18H), 1.53 (m, 18H), 3.78 (t, 2H), 10.43 (s, 1H), 11.28 (bs, 2H). Anal. ($C_{17}H_{28}N_4O_3$) C, H, N, O.

3-Dodecyl-1,7,9-trimethyl-2,6,8-purinetrione (5m). 5e (0.5 g, 1.5 mmol), 0.7 g of dry K_2CO_3, and 1.5 g of methyl iodide were suspended in 10 mL of DMF for 3 h. Extraction with chloroform, washing of the organic phase with water, and subsequent chromatography (eluent MeOH/$CHCl_3$ (1% v/v)), gave 414 mg of the tetrasubstituted derivative **5m**: 73%; mp 76–77 °C; NMR ($CDCl_3$) δ 0.85 (t, 3H), 1.26 (m, 20H), 3.36 (s, 3H), 3.58 (s, 6H), 4.10 (t, 2H). Anal. ($C_{20}H_{34}N_4O_3$) C, H, N.

Biochemical Methods. Determination of Partition Coefficients. Experimental determination for some compounds in an octanol/water system: A 1/1 mixture of octanol and potassium phosphate buffer (10 mM, pH 7.4) was shaken overnight to ensure phase equilibration, and then the phases were separated. The compound to be assayed was dissolved in either the organic ($\log P > 0$) or aqueous ($\log P < 0$) phase at a concentration below 20% of its maximal solubility. The same volume of the other phase was then added and the system shaken for 1 h at 25 °C. The product concentration in each phase was then determined by UV, using the extinction coefficient determined for each compound (urates: $\lambda_{max} = 290$ nm, $\epsilon \sim 14\,000$ M^{-1} cm^{-1}; 5,6-diaminouracils: $\lambda_{max} = 280$ nm, $\epsilon \sim 11\,000$ M^{-1} cm^{-1}). log *P* values were also calculated for the compounds of the two series with the software ClogP3 from Daylight, Chemical Information System, Inc., based on the Hansch and Leo fragments method.[35,36]

Determination of the Reducing Activity[37] of the Stable Radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH·). A water/alcohol medium was chosen in order to solubilize and test all the derivatives under the same conditions. Various concentrations of compounds diluted in DMSO were added to a 1-mL mixture of Tris/HCl (10 mM, pH 7.4) and methanol (2/1, v/v) containing 20 μM DPPH·. The absorbance at 517 nm was measured after a 20-min incubation at room temperature. The concentration of compound necessary to reduce 10 μM DPPH· (RC10) was used as an indicator of its reducing capacity. DMSO (1%) alone had no effect on the test.

Inhibition of Lipid Peroxidation. Bovine heart mitochondria were isolated from slaughterhouse material according to a mechanical method[48] with slight modifications: after mechanical tissue disruption and differential centrifugation, mitochondria were washed twice in an EDTA free medium containing 250 mM sucrose, 10 mM Tris/HCl, pH 7.2. Lipid peroxidation was conducted as follows: mitochondria (0.6 mg/mL) were incubated at 37 °C in KCl 140 mM, Tris/HCl 30 mM, pH 7.2 containing 1 mM dihydroxyfumarate and various concentrations of compounds diluted in DMSO (final concentration in DMSO 0.2%). The reaction started with the addition of 50 μM ferrous ammonium sulfate. After 40 min, lipid peroxidation was assessed by the formation of thiobarbituric acid reactive substances (TBARS).[38] Then 100-μL aliquots were added to a 1.5-mL solution of thiobarbituric acid (0.33% m/v), trichloroacetic acid (13.5% m/v), HCl (0.85 N). Samples were incubated for 15 min at 100 °C and rapidly cooled; 1 mL of trichloroacetic acid (70% m/v) was then added. Samples were centrifuged, and fluorescence in the supernatant was read (excitation 515 nm, emission 553 nm). Inhibition percentages were determined using a control (incubation with DMSO alone) and a blank (incubation without initiation of peroxidation: dihydroxyfumarate and iron). The IC50 was calculated as the test compound concentration that reduced the amount of TBARS to 50% of the control value. DMSO alone had no effect on the test.

Scavenging Activity against Lipid Radicals. Lipid radicals were generated by thermo-dependent azo initiation.

(A) In Homogeneous Experiments.[40] Reactions were conducted at 40 °C in *tert*-butyl alcohol/methanol (4/1 v/v) in the presence of 0.6 M methyl linoleate (ML) and various concentrations of compound diluted in DMSO (final concentration in DMSO 1%). The reaction started with addition of 25 mM 2,2′-azobis-2,4-dimethylvaleronitrile (DMVN) recrystallized from hot methanol. ML hydroperoxide (MLOOH) production was monitored with a Clark type oxygen electrode. Calibration of the electrode was carried out by correlating the oxygen consumption with the amount of diene conjugation monitored at 234 nm, since 96% of MLOOH contains a conjugated dienic structure.[49] DMSO alone had no effect on the test.

(B) In a Liposomal Structure. Large multilamellar liposomes (LML) were prepared as follows: a 16-mL aliquot of a stock solution of dilinoleoylphosphatidylcholine (DLPC, 20 mg/mL in chloroform) free of peroxides was mixed with 0.1 mL of a 100 mM DMVN solution in methanol and 5 mL of methanol. The mixture was dried under vacuum in a rotary evaporator to give a thin homogeneous film. Twenty milliliters of an aqueous solution of NaCl (130 mM), phosphate (20 mM, pH 7.3) was then added, and a suspension of small multilamellar (SML) liposomes was prepared by shaking. LML were obtained by freezing and thawing (liquid nitrogen) the SML suspension five times. Final concentrations were DLPC 20 mM, DMVN 0.5 mM. Incubations were started by adding various concentrations of the compounds diluted in DMSO (final concentration in DMSO 1%) and quickly raising the temperature to 48 °C to induce sufficient thermal degradation of DMVN. High temperature neither affects the liposome structure no impairs the efficacy of antioxidants.[41] Ten-microliter samples were removed at 10-min intervals and were added to 2 mL of cold methanol. Absorbance was read at 234 nm against methanol, and the conjugated diene formation (production of DLPC hydroperoxide) was quantified using an extinction coefficient of 28 000 M^{-1} cm^{-1}. DMSO alone had no effect on the test except for a deductible UV absorbance. Under the same incubation conditions no peroxidation occurred in LML free from DMVN.

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References

(1) Torrielli, M. V.; Dianzani, M. U. Free Radicals in Inflammatory Disease. In *Free Radicals in Molecular Biology, Aging and Disease*; Armstrong, P., Sohal, R. S., Kutler, R. G., Slater, T. F., Eds.; Raven Press: New York, 1984; Vol. 27, 355–379.
(2) Jenner, P. Oxidative Stress as a Cause of Parkinson Disease. *Acta Neurolog. Scand.* **1991**, *84*, S136, 6–15.
(3) Godin, D. V. The Role of Reactive Oxygen Derived Radicals in Ischemic Heart Disease. *Can. J. Cardiol.* **1989**, *5*, 235–238.
(4) Yamamoto, M.; Shima, T.; Uozumi, T.; Sogabe, T.; Yamada, K.; Kawazaki, T. A Possible Role of Lipid Peroxidation in Cellular Damage Caused by Cerebral Ischemia and the Protective Effect of Alpha-tocopherol. *Stroke* **1983**, *14*, 977–982.
(5) Harman, D. Free Radicals and the Origination, Evolution and Present Status of the Free Radical Theory of Aging. In *Free Radicals in Molecular Biology, Aging And Disease*; Armstrong, P., Sohal, R. S., Kutler, R. G., Slater, T. F., Eds.; Raven Press: New York, 1984; Vol. 27, pp 1–12.
(6) Weiss, J. F., Simic, M. G., Eds. In *Perspectives in Radioprotection*; Pergamon Press: New York, 1987; pp 1–407.
(7) Chow, K. C. Vitamin E and Oxidative Stress. *Free Radicals Biol. Med.* **1991**, *11*, 215–232.
(8) Halliwell, B.; Gutteridge, J. M. C. Free Radicals and Antioxidant Protection: Mechanisms and Significance in Toxicology and Disease. *Human Toxicol.* **1988**, *7*, 7–13.
(9) Halliwell, B.; Gutteridge, J. M. C. Oxygen Radicals and Iron in Relation to Biology and Medicine: some Problems and Concepts. *Arch. Biochem. Biophys.* **1986**, *246*, 501–514.
(10) Minotti, G.; Aust, S. D. The Role of Iron in Radical Mediated Lipid Peroxidation. *Chem. Biol. Interact.* **1989**, *71*, 1–19.
(11) Kako, K. J.; Yamagishita, T.; Kato, M.; Kaminishi, T.; Matsuoka, M. Mechanisms of Oxidant-Induced Perturbation of Calcium Homeostasis in Heart Cells. In *Medical, Biochemical and Chemical Aspect of Free Radicals*; Hayiashi, O., Niki, E., Kondo, M., Yoshikawa, T., Eds.; Elsevier: New York, 1989; pp 161–165P.
(12) Sies, H. Biochemistry of Oxidative Stress. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1058–1071.
(13) Halliwell, B.; Gutteridge, J. M. C. The Antioxidants *of Extracellular Fluids. Arch. Biochem. Biophys.* **1990**, *280*, 1–10.
(14) Ursini, F.; Bindoli, A. The Role of Selenium Peroxidases in the Protection Against Oxidative Damage of Membranes. *Chem. Phys. Lipids* **1987**, *44*, 255–276.
(15) Mc cord, J.; Fridovich, I. Superoxide Dismutase. An Enzymic Function for Erythrocuprein. *J. Biol. Chem.* **1969**, *244*, 6049–6055.
(16) Stocker, R.; Frei, B. Endogeneous Antioxidant Defenses in Human Blood Plasma. In *Oxidative Stress*; Sies, H., Ed.; Academic Press: New York, 1991; pp 213–245.
(17) Gutteridge, J. M. C.; Stocks, J. Ceruloplasmin: Physiological and Pathological Perspectives. *CRC Crit. Rev. Clin. Lab. Sci.* **1981**, *14*, 257–329.
(18) Takayanagi, R.; Takeshige, K.; Minakami, S. NADH and NADPH Dependant Lipid Peroxidation in Bovine Heart Submitochondrial Particles. Dependance on the Rate of Electron Flow in the Respiratory Chain and an Antioxidant Role of Ubiquinol. *Biochem. J.* **1980**, *192*, 853–860.
(19) Ames, B. N.; Cathcart, R.; Schwiers, E.; Hochstein, P. Uric Acid Provides an Antioxidant Defense in Humans Against Oxidant and Radical Caused Aging and Cancer: a Hypothesis. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6858–6862.
(20) Howell, R. R.; Wyngaarden, J. B. On the Mechanism of Peroxidation of Uric Acid by Hemoproteins. *J. Biol. Chem.* **1960**, *235*, 3544–3550.
(21) Simon, M. I.; Van Vunakis, H. Dye-sensitized Photoxidation of Purines and Pyrimidine Derivatives. *Arch. Biochem. Biophys.* **1964**, *105*, 197–206.
(22) Simic, M. G.; Jovanovic, S. V. Antioxidation Mechanisms of Uric Acid. *J. Am. Chem. Soc.* **1989**, *111*, 5778–5782.
(23) Davies, K. J. A.; Sevanian, A.; Muakkassah-Kelly, S. F.; Hochstein, P. Uric-Acid-iron Ion Complexes. A New Aspects of the Antioxidant Functions of Uric Acid. *Biochem. J.* **1986**, *235*, 747–754.
(24) Sevanian, A.; Davies, K. J. A.; Hochstein, P. Serum Urate as an Antioxidant For Ascorbic Acid. *Am. J. Clin. Nutr.* **1991**, *54*, 1129S–1134S.
(25) Jamieson, D. Oxygen Toxicity and Reactive Oxygen Metabolites in Mammals. *Free Radicals Biol. Med.* **1989**, *7*, 87–108.
(26) Maples, K. R.; Mason, R. P. Free Radical Metabolites of Uric Acid. *J. Biol. Chem.* **1988**, *263*, 1709–1712.
(27) Smith, R. C.; Reeves, J.; McKee, M. L.; Daron, H. Reactions of Methylated Urates with 1,1-diphenyl-2-picrylhydrazyl. *Free Radicals Biol. Med.* **1987**, *3*, 51–257.
(28) Papesh, V.; Schroeder, E. F. Synthesis of 1-mono- and 1,3-disubstituted 6-aminouracils. Diuretic activity. *J. Org. Chem.* **1951**, *16*, 1879–1890.
(29) Goldner, H.; Dietz, G.; Lartens, E. A New Xanthine Synthesis. *Liebigs Ann. Chem.* **1966**, *691*, 142–158.
(30) Pfleiderer, W.; Nübel, G. Synthesis of 9-Substituted Xanthines. *Liebigs Ann. Chem.* **1961**, *631*, 168–174.
(31) Pfleiderer, W.; Walter, H. Reactions with 4-Chloro and 4-Chloro-5-nitropyrimidines. *Liebigs Ann. Chem.* **1964**, *677*, 113–126.
(32) Pfleiderer, W.; Schündehütte, K. H. Reactions with 1,3-Dimethyl-4-chlorouracile. *Liebigs Ann. Chem.* **1958**, *612*, 158–163.
(33) Hützenlaub, W.; Pfleiderer, W. Easy Syntheses of 7-Methyl- and 1,7-dimethylxanthine and Uric Acid. *Liebigs Ann. Chem.* **1979**, 1847–1854.
(34) Pfleiderer, W. Synthese, Structure and properties of Uric Acid and its N-methylated derivatives. *Liebigs Ann. Chem.* **1974**, 2030–2045.
(35) Hansch, C.; Leo, A. In *Substituant Constants for Correlation Analysis in Chemistry and Biology*; Wiley: New York, 1979; pp 1–339.
(36) Chou, J. T.; Jurs, P. C. Computer Assisted Computation of Partition Coefficients from Molecular Structures Using Fragment Constants. *J. Chem. Inf. Comput. Sci.* **1979**, *19*, 172–178.
(37) Blois, M. S. Antioxidant Determination by the Use of a Stable Free Radical. *Nature (London)* **1958**, *181*, 1199–1200.
(38) Buege, J. A.; Aust, S. D. Microsomal Lipid Peroxidation. In *Methods In Enzymology*; Fleischer, S., Packer, L., Eds.; Academic Press: New York, 1978; pp 302–310.
(39) Halliwell, B. Generation of Hydrogen Peroxide, Superoxide and Hydroxyl Radical during the Oxidation of Dihydroxyfumaric Acid by Peroxidase. *Biochem. J.* **1977**, *1963*, 441–448.
(40) Niki, E.; Saito, T.; Kawakami, A.; Kamiya, Y. Inhibition of Oxidation of Methyl Linoleate in Solution by Vitamin E and Vitamin C. *J. Biol. Chem.* **1984**, *259*, 4177–4182.
(41) Stocker, R.; Peterhans, E. Synergistic Interactions between Vitamin C and the Bile Pigments Bilirubin and Biliverdin. *Biochim. Biophys. Acta* **1989**, *1002*, 238–244.
(42) Burton, G. W.; Ingold, K. U. Autoxidation of Biological Molecules. 1. The Antioxidant Activity of Vitamin E and Related Chain Breaking Phenolic Antioxidants in vitro. *J. Am. Chem. Soc.* **1981**, *103*, 6472–6477.
(43) Smith, R. C.; Lawing, L. Antioxidant Activity of Uric Acid ans 2-N Ribosyluric Acid with Unsaturated Fatty Acids and Erythrocyte Membranes. *Arch. Biochem. Biophys.* **1983**, *223*, 166–172.
(44) Kagan, V. E.; Serbinova, E. A.; Balakova, R. A.; Stoytchev, T. S.; Erin, A. N.; Prilipko, L. L.; Evstigneeva, R. P. Mechanisms of Stabilisation of Biomembranes by Alpha Tocopherol. The Role of The Hydrocarbon Side Chain in the Inhibition of Lipid Peroxidation. *Biochem. Pharmacol.* **1990**, *40*, 2403–2413.
(45) Okamoto, Y.; Ogura, K.; Kurasawa, Y.; Kinoshita, T. A Convenient Synthesis of Pyrimido(5,4)pterinetetrones. *Heterocycles* **1984**, *22*, 1231–1234.
(46) Al-arab, M. M.; Hamilton, G. A. Possible Model Reaction for Some Amine Oxidases Kinetics and Mechanisms of the Copper-II-catalyzed Autoxidation of Some Diaminouracils. *J. Am. Chem. Soc.* **1986**, *108*, 5972–5978.
(47) Kelly, T. R.; Maguire, M. P. A Receptor for the Oriented Binding of Uric Acid Type Molecules. *J. Am. Chem. Soc.* **1987**, *109*, 6549–6551.
(48) Azzone, G. F.; Colonna, R.; Zicke, B. Preparation of Bovine Heart Mitochondria in High Yield. In *Methods In Enzymology*; Fleischer, S., Packer, L., Eds.; Academic Press: New York, 1979; Vol. 55, pp 46–50.
(49) Mörsel, J. Lipid Peroxidation Part 1. Primary Reactions. *Die Nahrung* **1990**, *34*, 1, 3–12.