Pyridinol-Based Antioxidants

6-Amino-3-Pyridinols: Towards Diffusion-Controlled Chain-Breaking Antioxidants**

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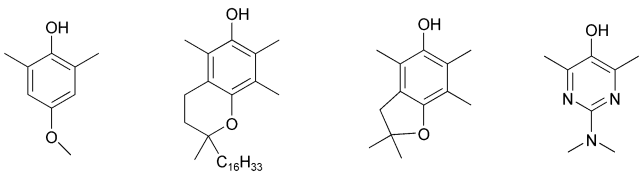
Peroxyl radicals (LOO•) are the chain-propagating species in the rate-determining step of lipid peroxidation [Eq. (1)].

$$LOO^{\cdot} + L{-}H \xrightarrow{k_p} LOOH + L^{\cdot} \quad (1)$$

Chain-breaking antioxidants, most commonly substituted phenols (ArOH) such as **1**–**3**, inhibit peroxidation by transferring their phenolic H atom to the propagating radical [Eq. (2)] at a rate (k_{inh}[ArOH]) faster than that of chain

$$LOO^{\cdot} + ArOH \xrightarrow{k_{inh}} LOOH + ArO^{\cdot} \quad (2)$$

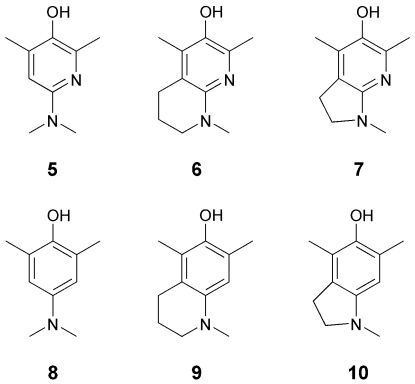
propagation (k_p[L-H]). The best-known example of a phenolic antioxidant is α-tocopherol (α-TOH, **2**), the most potent form of vitamin E, nature's primary defense against radical chain oxidation.[1] Recently, considerable effort has been devoted to the development of peroxyl-radical-trapping antioxidants more effective than α-TOH,[2–5] since the peroxidation of lipids in vivo has been suggested to be a major factor in the development of several degenerative diseases.[6]



1 **2** **3** **4**

The bond dissociation enthalpy (BDE) of the phenolic O–H bond plays a central role in determining antioxidant efficacy; phenols having lower O–H BDEs are generally better antioxidants.[7,8] Substitution with electron-donating (ED) groups at positions *ortho* and *para* to the phenolic OH group leads to compounds having lower BDEs. However, good ED substituents also lead to a decrease in the ionization potential (IP) of the phenol, thereby rendering the compound directly reactive with oxygen. Calculations predicted that incorporation of two nitrogen atoms at the 3- and 5-positions of the phenolic ring significantly raises the IP and greatly improves the stability of the compound in air while only minimally lowering the O–H BDE.[4] Experiments confirmed this prediction. Thus, for example, the 5-pyrimidinol **4** is perfectly stable in air and has a k_{inh} value about twice that of α-TOH.[4]

Calculations also predicted that 3-pyridinol would have a lower O–H BDE than the analogous 5-pyrimidinol and an IP intermediate to those of phenol and 5-pyrimidinol.[4] We therefore expected that 6-amino-3-pyridinols such as **5** would



5 **6** **7**

8 **9** **10**

have lower O–H BDEs than the analogous 5-pyrimidinols (e.g. **4**) and still have better air-stability than the corresponding phenols. Furthermore, the free 5-position in the pyridine skeleton allows for fusion of aliphatic rings (i.e. to give **6** and **7**), structural changes that have been shown to increase k_{inh} significantly in the series **1**–**3** because of improved stereoelectronics.[2,9] Herein we show by theory the effects of substitution on the O–H BDE and IP of a series of 3-pyridinols. We also report here on the synthesis and experimental investigation of three new 3-pyridinol antioxidants that were selected on the basis of our calculations.[10]

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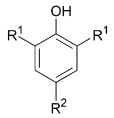
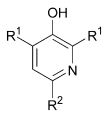
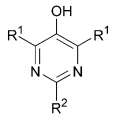
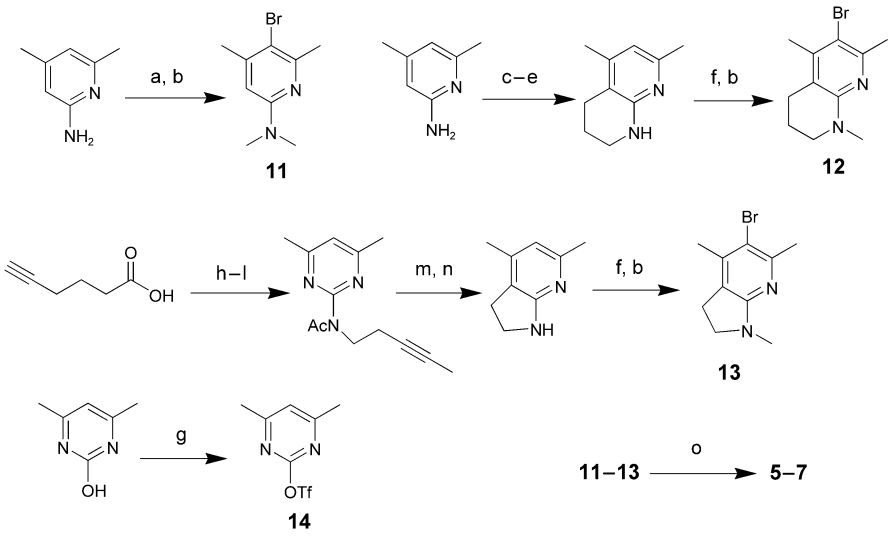
Table 1: Calculated substituent effects on gas-phase O–H BDEs at 298 K and adiabatic IPs at 0 K for substituted phenols, 3-pyridinols, and 5-pyrimidinols.[a]

Substitution	BDE	IP	BDE	IP	BDE	IP
$R^1=H$, $R^2=H$	87.1 (0.0)	195.4 (0.0)	88.2 (0.0)	206.4 (0.0)	89.6 (0.0)	219.7 (0.0)
$R^1=H$, $R^2=CH_3$	84.6 (−2.5)	186.9 (−8.5)	85.4 (−2.8)	196.6 (−9.8)	86.8 (−2.8)	209.3 (−10.4)
$R^1=CH_3$, $R^2=CH_3$	80.4 (−6.7)	178.3 (−17.1)	81.1 (−7.1)	186.3 (−20.1)	83.2 (−6.4)	198.0 (−22.7)
$R^1=H$, $R^2=OCH_3$	81.0 (−6.1)	176.5 (−18.9)	82.0 (−6.2)	186.1 (−20.3)	83.6 (−6.0)	199.1 (−21.6)
$R^1=CH_3$, $R^2=OCH_3$	77.0 (−10.1)	169.2 (−26.2)	78.1 (−10.1)	177.4 (−29.0)	79.8 (−9.8)	188.3 (−31.4)
$R^1=H$, $R^2=N(CH_3)_2$	77.0 (−10.1)	157.7 (−37.7)	77.0 (−11.2)	164.6 (−41.8)	78.3 (−11.3)	174.6 (−45.1)
Compounds						
8, **5**, **4** (monocyclic)	72.3 (−14.8)	152.3 (−43.1)	73.5 (−14.7)	157.7 (−48.7)	74.1 (−15.5)	167.0 (−52.7)
9, **6** (fused 6-ring)	71.2 (−15.9)	148.3 (−47.1)	73.3 (−14.9)	154.6 (−51.8)		
10, **7** (fused 5-ring)	70.5 (−16.6)	145.2 (−50.3)	72.4 (−15.8)	152.3 (−54.1)		
2 (α-TOH)	74.8 (−12.3)	159.3 (−36.1)				

[a] Data for phenols and 5-pyrimidinols are from ref. [4] except for **8**–**10**. All values are in kcal mol^{-1}. Substituent effects (relative to the unsubstituted parent) are in parentheses.

The calculated O–H BDEs and IPs of several substituted phenols, 3-pyridinols, and 5-pyrimidinols were obtained using density functional theory models ((RO)B3LYP/6-311+G(2d,2p)//AM1/AM1 and B3LYP/6-31G(d)//AM1/AM1, respectively) and the values are given in Table 1.[11,12] Clear trends can be seen. For all three classes, the O–H BDE and IP values decrease when the electron density on the aromatic ring is increased. The order for the calculated values of these properties is always phenol < 3-pyridinol < 5-pyrimidinol. The calculations suggest that the highest antioxidant activities in conjunction with reasonable air-stability (IP values comparable to **2**) are expected from pyridinols **5**–**7**. Therefore, we selected these three molecules as targets for synthesis and experimental investigations.



Scheme 1. Synthetic approach to 3-pyridinols **5**–**7**. Reagents and conditions: a) DBDMH, CH_2Cl_2, −40 °C, 40 min, 67%; b) HCOOH, aq H_2CO, reflux, 18 h, 100% for **11**, 77% for **12**, 84% for **13**; c) acrylic acid, pyridine, reflux, 24 h, 30% after crystallization; d) polyphosphoric acid, 125 °C, 40 min, 75%; e) BH_3·THF, THF, reflux, 18 h, 83%; f) DBDMH, CH_2Cl_2, −78 °C, 10–30 min, 93% for **12**, 83% for **13**; g) Tf_2O, Et_3N, CH_2Cl_2, 0 °C, 1 h, 98%; h) 15 M aq KOH, reflux, 4 h, 95%; i) $(PhO)_2PON_3$, *t*BuOH, reflux, 30 h, 79%; j) ethereal HCl, 24 h, 70%; k) **14**, Et_3N, DMF, 1 d, 93%; l) Ac_2O, DMAP, 100 °C, 20 h, 91%; m) Ph_2O, reflux, 10 h, 82%; n) NaOH, MeOH, reflux, 20 h, 100%; o) 1. *n*BuLi, THF, −78 °C, 30 min; 2. dry 2-nitro-*m*-xylene, THF, −78 °C, 1–3 h, 63% for **5**, 25% for **6**, 27% for **7**. DBDMH = dibromodimethylhydantoin, DMAP = dimethylaminopyridine, DMF = dimethylformamide, Tf = trifluoromethanesulfonyl.

There are few synthetic approaches to 6-amino-3-pyridinol structures described in the literature, and **5**–**7** have never been reported.[13] We devised a synthetic sequence in which the reactive OH moiety is introduced at low temperatures in the last step, thus minimizing potential decomposition of products and/or intermediates. Only a brief description of the syntheses will be presented here; a forthcoming paper will report our extensive synthetic efforts in detail. Construction of the appropriate pyridine substructure involved a three-step approach featuring a Friedel–Crafts reaction for **6** and a seven-step sequence with an intramolecular Diels–Alder sequence for **7**. Construction of the pyridine was followed by bromination, methylation, and hydroxylation (Scheme 1). The pyridinols **5**–**7** were obtained as yellow or orange solids.

The O–H BDEs that were measured experimentally by radical-equilibration EPR studies are collected in Table 2.[7b] The absolute experimental values (in benzene) were slightly higher (2–3 kcal mol^{-1}) than the calculated (gas-phase) values (Table 1), an issue already addressed by us in previous works.[4] However, the corresponding ΔBDE (BDE_{PyrOH}–$BDE_{\alpha\text{-}TOH}$) shows good agreement between experimental and

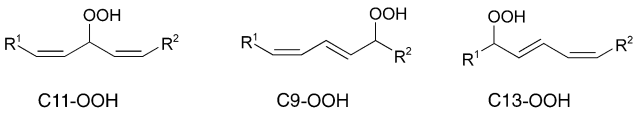
Table 2: Solution-phase O–H BDEs and inhibition rate constants for 3-pyridinols **5**–**7**.

ArOH	O–H BDE [kcal mol^{-1}][a]	Δ(O–H BDE) [kcal mol^{-1}][b]	k_{inh} by clock [$\times 10^7$ M^{-1} s^{-1}][c]	k_{inh} by O_2 uptake [$\times 10^7$ M^{-1} s^{-1}][d]	k_{inh}/k_{inh} (**2**)[e]
5	77.0 ± 0.5	−1.3/−1.3	1.16 ± 0.04	1.6 ± 0.6	5.0
6	76.3 ± 0.6	−2.0/−1.5	n.d.[f]	8.8 ± 3.2	28
7	75.4 ± 0.7	−2.9/−2.4	n.d.[f]	28.0 ± 18[g]	88
4	78.2 ± 0.3	−0.1/−0.7	0.65 ± 0.08	0.86 ± 0.05	2.1
2 (α-TOH)	78.3 ± 0.3	0.0/0.0	0.38[h]	0.32[i]	1.0

[a] From EPR equilibration studies. [b] Experimental BDE (78.3 kcal mol^{-1}) or calculated BDE (74.8 kcal mol^{-1}) for α-TOH **2** as reference. Values are experimental/calculated ΔBDE. [c] In benzene at 37 °C by methyl linoleate radical clock. [d] In chlorobenzene by inhibited styrene autoxidation at 30 °C for **2**, **5**, **6**, **7**; in benzene at 50 °C for **4**. [e] The k_{inh} values from O_2 uptake were used. [f] See text. [g] The rather large error (= 2σ) is the result of the large scatter in the measurements due to the dramatic inhibition by **7**; initiation rates as low as 1×10^{-10} s^{-1} had to be used to obtain measurable rates of oxygen consumption (as low as 1×10^{-9} M^{-1} s^{-1}) with concentrations of **7** as low as 2×10^{-7} M. [h] Value used to calibrate the radical clock. [i] Value from ref. [2], reconfirmed in ref. [4]. The value of 0.41 ± 0.04 at 50 °C is used for comparison with **4**.

calculated substituent effects for the 3-pyridinol series. The stability of the 3-pyridinols towards air was examined by monitoring their typical fluorescence and UV absorbance (see Supporting Information) in aerated solution (0.3 mM in *tert*-butylbenzene) at 37 °C. The simple 3-pyridinol **5** was stable over a 24 h period while **7** showed significant decomposition (ca. 30%) after 6 h. The stability of compound **6** was intermediate to that of **5** and **7**, in qualitative agreement with the calculated IPs for these compounds (Table 1).

Two different experimental approaches were used to determine absolute k_{inh} values for the 3-pyridinols. First, we utilized a peroxyl radical clock based on the antioxidant-dependent trapping of bisallylic (C11-OOH) and conjugated (C9- and C13-OOH) hydroperoxide products in initiator-induced autoxidations of methyl linoleate.[14] At low concentrations of antioxidant (10–100 mM), there is a linear correlation between concentration of antioxidant and ratio of trapping of C11-OO• vs C9- and C13-OO• with the slope of the line directly related to k_{inh} of the antioxidant.[14] Thus, by measuring the ratio of linoleate hydroperoxide products at various concentrations of an antioxidant it is possible to determine its k_{inh}.



C11-OOH C9-OOH C13-OOH

$R^1=C_5H_{11}$, $R^2=(CH_2)_7COOMe$

3-Pyridinol **5** proved more effective than **4** or **2** in trapping C11-OO•, and a value of $k_{inh} = 1.16(\pm0.04)\times10^7$ M^{-1} s^{-1} was determined by this method (see Supporting Information). The total yield of hydroperoxide products formed from linoleate in the presence of **5** was ca. eight times less than that observed for identical oxidations inhibited by **2** (data not shown). Interestingly, **6** and **7** proved to be such good inhibitors that we were unable to obtain reliable k_{inh} values for these compounds by the radical clock method.[15] Controlled styrene autoxidations inhibited by **5**–**7** were well behaved, however,[2] and allowed us to investigate the peroxyl-radical-trapping activity of these compounds (see Supporting Information). The stoichiometric factor *n* (number of chains broken by one molecule of antioxidant) was determined to be 2 for all three compounds, similar to phenols and 5-pyrimidinols.[4] The inhibition rate constants k_{inh} were determined from the slope of the plot of oxygen consumption in the presence of the inhibitor,[2,4,16] and the values obtained are presented in Table 2 together with previous data for **2** and **4**, which are given for comparison.[4,14]

The 6-amino-3-pyridinols described here are a novel class of phenolic antioxidants that are more effective than any other phenolic class reported to date. The vacant 5-position on the pyridine ring of these compounds allows for fusion of an additional aliphatic ring, a substitution that further lowers the O–H BDE. Indeed, the pyridinols **6** and **7** are, to the best of our knowledge, the fastest peroxyl-radical-trapping chain-breaking antioxidants ever reported (previously 3,7-dimethoxyphenothiazine with $k_{inh} = 5.5\times10^7$ M^{-1} s^{-1}).[17,18] The air-stability of **7** is moderate, but its k_{inh} exceeds 10^8 M^{-1} s^{-1}, thus approaching the diffusion-controlled limit for a bimolecular reaction.

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[18] This compound is quite reactive with air, much more so than **6** or **7**. Quantitatively this is indicated by our calculated IP, which is 7.8 and 5.5 kcal mol^{-1} lower than those of **6** and **7**, respectively.

