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How many peroxyl radicals can be scavenged by hydroxyl-substituted Schiff bases in the oxidation of linoleic acid?

Zai-Qun Liu[a]* and Di Wu[b]

Eleven hydroxyl-substituted Schiff bases (SchOHs) were synthesized by the reaction between hydroxyl-substituted benzaldehydes and hydroxyl-substituted anilines, and their antioxidant effects on the oxidation of linoleic acid (dissolved in sodium dodecyl sulfate micelle) induced by 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH) were investigated. The relationships between the period of the oxidation inhibited by SchOHs (t_{inh}) and their concentrations ([SchOHs]) were measured firstly, and then treated by a chemical kinetic equation, $t_{inh} = (n/R_i)$[SchOH], to obtain the number (n) of the oxidative chains terminated by one molecule of SchOH. Therefore, the antioxidant activities of SchOHs can be expressed quantitatively by the n value. Finally, the spin-densities (SD) on O atom in the radical of SchOH (SchO$^{\bullet}$) were calculated by quantum chemical method, and, to some extent, SD provided an explanation to the difference of the antioxidant effects among various SchOH. Therefore, the obtained results provided an attempt to bridge the kinetic measurement and quantum calculation in the study on the property of an antioxidant. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: antioxidant; free radical; kinetics; linoleic acid; Schiff base; spin-density

INTRODUCTION

The oxidation of linoleic acid (LH, $CH_3(CH_2)_5CH{=}CHC\mathbf{H}_2CH{=}CH(CH_2)_7COOH$) induced by radical has been investigated by chemical kinetics for a long period,[1] in which 2,2′-azobis-(2-amidinopropane hydrochloride) (AAPH, R—N=N—R, R=$CMe_2C(=NH)NH_2$) serves as the radical-initiator. The radical derived from the decomposition of AAPH combines with oxygen, and then, abstracts the hydrogen atom at the allyl position of LH. So, the radical-initiation is shown in the following equations:

$$R - N = N - R \rightarrow 2R^{\bullet} + N_2 \quad (1)$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad (2)$$

$$ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet} \quad (3)$$

As shown in Eqns (4 and 5), the radical propagation takes place until LH are oxidized completely. Finally, the combination of peroxyl radicals (LOO$^{\bullet}$) terminates the radical reaction as shown in Eqn (6).

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (4)$$

$$LOO^{\bullet} + LH \rightarrow LOOH^{\bullet} + L^{\bullet} \quad (5)$$

$$2\,LOO^{\bullet} \rightarrow non - radical\,product \quad (6)$$

With an antioxidant (AH) added to aforementioned reactions, as shown in Eqn (7), AH forms radical (A$^{\bullet}$) by trapping LOO$^{\bullet}$. So, AH is oxidized in place of LH until AH is consumed thoroughly, resulting in an inhibition period (t_{inh}) eventually. In particular, AH reacts with more than one radical to form a non-radical product, so n in Eqn (7) is *stoichiometric factor* to represent the number of oxidative chains terminated by one molecule of AH.[2]

$$n\,LOO^{\bullet} + AH \rightarrow n\,LOOH + A^{\bullet} \quad (7)$$

Furthermore, t_{inh} is proved to be proportional to the concentration of AH as shown in the following equation:

$$t_{inh} = \frac{n}{R_i}[AH] \quad (8)$$

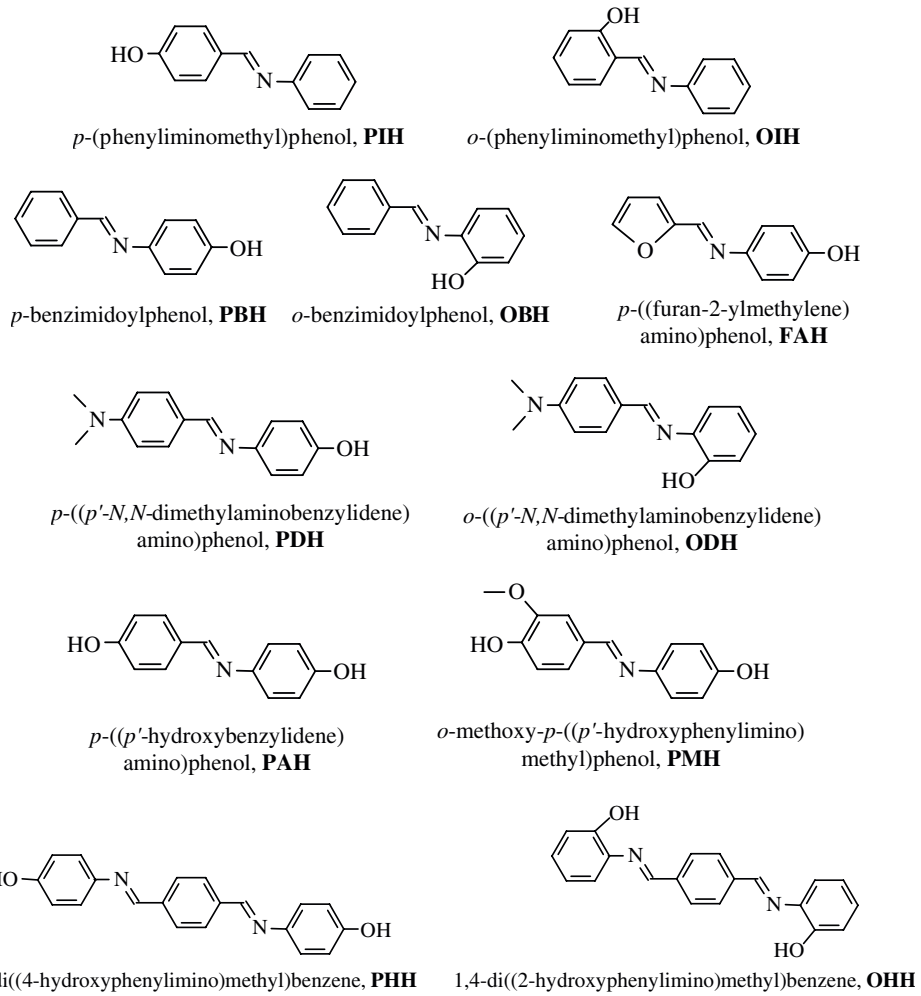
where R_i indicates the initiation rate of the reaction.

We have applied Eqn (8) to treat the relationship between t_{inh} and the concentration of hydroxyl-substituted Schiff bases (SchOHs) in AAPH-induced hemolysis of erythrocytes.[3–6] In addition, SchOHs possess many physiological activities such as antimicrobial[7] and anticancer.[8] To our knowledge, the researches on antioxidant properties of SchOHs by chemical kinetics are not reported frequently. So, we synthesize a series of SchOHs (refer Scheme 1) by the reaction between hydroxyl-substituted benzaldehydes and hydroxyl-substituted anilines, and determine the n values in AAPH-induced oxidation of LH in order to express the antioxidant activities of SchOHs quantitatively. Moreover, in order to reveal the stabilization of SchO$^{\bullet}$, we calculate the spin-densities (SD) on O atom when SchOHs form radicals. Therefore, the aim of this work is to provide a novel pathway to clarify the antioxidant effects of SchOHs.

* *Correspondence to: Z.-Q. Liu, Department of Organic Chemistry, College of Chemistry, Jilin University, No. 2519 Jiefang Road, Changchun 130021, China. E-mail: zaiqun-liu@jlu.edu.cn*

a *Z.-Q. Liu*
Department of Organic Chemistry, College of Chemistry, Jilin University, Changchun 130021, China

b *D. Wu*
State Key Laboratory of Theoretical and Computational Chemistry, Institute of Theoretical Chemistry, Jilin University, Changchun 130021, China



Scheme 1. Structures, nomenclatures and abbreviations of SchOHs used herein

MATERIALS AND METHODS

Materials

LH, AAPH and α-tocopherol (TOH) were purchased from ACROS and used as received. Other reagents were at analytical grade and used without further purification. SchOHs were synthesized by the reaction between the corresponding aromatic aldehyde and aniline, and characterized by element analysis and NMR spectra (since the obtained SchOHs were not novel compounds, data of element analysis and NMR data were not shown herein). AAPH was dissolved in phosphate-buffered solution (PBS: 5.0 mM Na_2HPO_4, 5.0 mM NaH_2PO_4, 10.0 μM EDTA). Sodium dodecyl sulfate (SDS) was dissolved in PBS at a final concentration of 0.1 M. LH was dissolved in the SDS/PBS solution at a final concentration of 11.0 mM under ultrasonic vibrations. SchOHs were dissolved in dimethyl sulfoxide (DMSO) to form stock solutions (1.0 mM).

Measurement of inhibition period of Schiff bases in AAPH-induced oxidation of linoleic acid

The process of AAPH-induced peroxidation of LH was followed *in situ* by a SP-3 oxygen uptake apparatus equipped with a Clark electrode that was sensitive to the variety of oxygen concentration as low as 10^{-8} M (Shanghai Institute of Phytobiology, Chinese Academy of Sciences). The experimental operation followed our previous reports.[9,10] Briefly, the SDS solution of LH was put into a pool with a 37.0 °C water circle by thermostat and stirred for 5 min to reach a saturated concentration of oxygen in the air. Then the pool was sealed by the Clark electrode, and the volume of the SDS solution of LH in the pool was 2 ml. The DMSO solution of SchOH was injected into the pool to a certain concentration. Finally, the PBS solution of AAPH was injected into the pool at a final concentration of 16.0 mM to initiate the oxidation of LH. As shown in Fig. 1 (*vide post*), the rate of oxygen uptake was slow at the beginning of the reaction, and then rapid, leading to a t_{inh}. All the t_{inh} were measured triplicate with the experimental error within 10%. The relationships of t_{inh}–[SchOH] were analysed statistically by one-way ANOVA in Origin Professional software, in which $p < 0.001$ indicated a significant difference.

Quantum calculation for the spin-density on O atom when SchOHs form radicals

The structures of SchO$^{\bullet}$ have been optimized at B3LYP/6-31+G(d) level. With the harmonic vibrational frequencies and zero-point vibrational energies (ZPVE) at the same level,[11] the SD on O atom

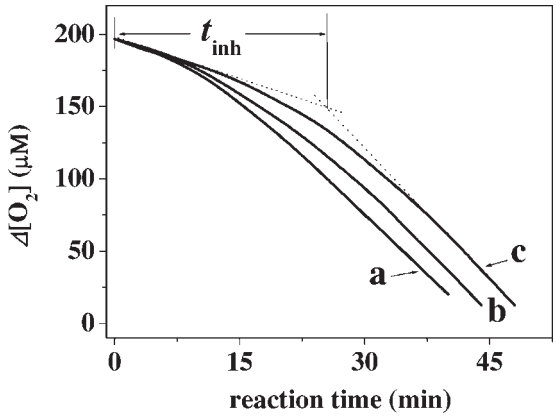


Figure 1. The oxygen exhaustion when 11.0 mM LH (in 0.1 M SDS/PBS micelle) is oxidized by 16.0 mM AAPH and inhibited by 0.42 μM (a), 0.85 μM (b) and 1.69 μM (c) PMH

in SchO$^{\bullet}$ were calculated by using the Gaussian 03 program package.[12]

RESULTS AND DISCUSSION

The measurement of the initiation rate (R_i)

The initiation rate (R_i) is of importance for Eqn (8) to be used to calculate n of an antioxidant. Because it is difficult to measure R_i directly,[13] TOH or its water-soluble analogue (Trolox) is usually designated as the reference antioxidant whose n is taken as 2.00.[14] Thus, R_i can be obtained according to the relationship between the t_{inh} and the concentration of TOH or Trolox when n_{TOH} or $n_{Trolox} = 2.00$. TOH is selected to be the reference antioxidant in this work because all the SchOHs used herein are lipophilic compounds. TOH with a hydrophilic head and a hydrophobic tail has special property in micelle, such as SDS. Although SchOHs are also lipophilic compounds, they do not have the special properties in SDS. So, either TOH or SchOH is dissolved in DMSO so that the distribution status of SchOH is similar to the reference antioxidant, TOH. Then, the t_{inh} generated by different concentrations of TOH have been measured, and the relationship of t_{inh}–[TOH] has been obtained from the following equation:

$$t_{inh} = 748.2\ (\pm 47.4)\ [\mathrm{TOH}] - 41.6\ (\pm 134.3) \quad (9)$$

The coefficient in Eqn (9), 748.2, means $2.00/R_i$, thus, $R_i = 2.67 \times 10^{-9}\ \mathrm{M\,s^{-1}}$.

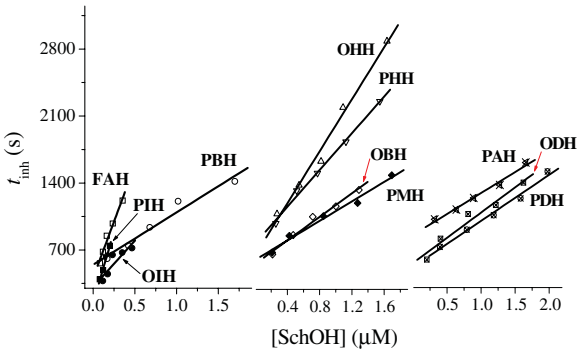


Figure 2. The relationships between t_{inh} and the concentration of SchOHs.

The measurement of n of SchOHs

Figure 1 outlines a representative chart of oxygen exhaustion when 11.0 mM LH is oxidized by 16.0 mM AAPH, and inhibited by various concentrations of PMH. The exhausting rate of oxygen at the beginning of the reaction is not as fast as the following period, indicating that PMH hinders the oxidation of LH. The period from the beginning of the reaction to the cross-point of the tangents (dotted lines) is designated as inhibition period (t_{inh}). So, we measured the t_{inh} in the presence of various concentrations of all SchOHs, and found that t_{inh} is proportional to the concentration of SchOHs. Figure 2 illustrates the relationship between t_{inh} and the concentrations of SchOHs. Moreover, the linear relationships of t_{inh}–[SchOHs] have been expressed quantitatively and involved in Table 1 as well.

Table 1. The relationships of t_{inh}–[SchOHs] and *stoichiometric factor* (n) of SchOHs in protecting LH, together with spin-density (SD) on O atom when SchOH forms radical[a]

SchOHs	t_{inh} (s) = (n/R_i)[concentration (μM)] + B	n	SD on O atom of SchO$^{\bullet}$
PIH	$t_{inh} = 2995.7\ (\pm 185.2)\ [\mathrm{PIH}] + 143.4\ (\pm 27.4)$	8.0 (±0.5)	0.3329
OIH	$t_{inh} = 967.2\ (\pm 42.2)\ [\mathrm{OIH}] + 270.3\ (\pm 12.5)$	2.6 (±0.1)	0.3646
FAH	$t_{inh} = 2347.1\ (\pm 198.5)\ [\mathrm{FAH}] + 408.3\ (\pm 42.4)$	6.3 (±0.5)	0.3195
OBH	$t_{inh} = 645.4\ (\pm 47.6)\ [\mathrm{OBH}] + 579.7\ (\pm 39.5)$	1.7 (±0.1)	0.3404
PBH	$t_{inh} = 546.8\ (\pm 63.2)\ [\mathrm{PBH}] + 545.8\ (\pm 60.6)$	1.5 (±0.2)	0.3417
ODH	$t_{inh} = 539.9\ (\pm 63.0)\ [\mathrm{ODH}] + 564.0\ (\pm 62.9)$	1.4 (±0.2)	0.3286
PDH	$t_{inh} = 493.74\ (\pm 34.9)\ [\mathrm{PDH}] + 520.8(\pm 45.7)$	1.3 (±0.1)	0.3297
PMH	$t_{inh} = 535.2\ (\pm 39.3)\ [\mathrm{PMH}] + 623.3\ (\pm 40.9)$	1.4 (±0.1)	0.3346(aniline side) 0.2956(benzaldehyde side)
PAH	$t_{inh} = 448.5\ (\pm 31.0)\ [\mathrm{PAH}] + 864.6\ (\pm 33.0)$	1.2 (±0.1)	0.3375(aniline side) 0.3143(benzaldehyde side)
OHH	$t_{inh} = 1425.8\ (\pm 91.8)\ [\mathrm{OHH}] + 675.6\ (\pm 90.9)$	3.8 (±0.2)	0.4048
PHH	$t_{inh} = 1008.7\ (\pm 43.0)\ [\mathrm{PHH}] + 803.0\ (\pm 41.2)$	2.7 (±0.1)	0.3342

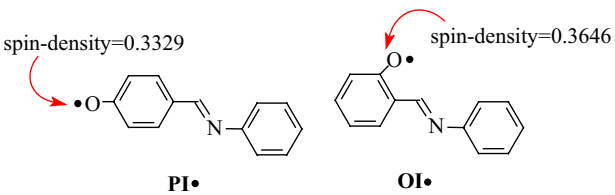
[a] $R_i = 2.67 \times 10^{-9}\mathrm{Ms^{-1}}$, [LH] = 11.0 mM, [AAPH] = 16.0 mM.

According to Eqn (8), the *n* of SchOHs are the product of R_i multiplying the coefficients in the equations in Table 1, and the obtained results are also listed in Table 1. High value of *n* implicates that the ability of the corresponding SchOH to hinder the oxidative chain is stronger than those SchOHs with low values of *n*. It is worthy to note that *n* of SchOHs is a relative value compared with 2.00 of TOH, and the range of concentration used in the experiment is also different among these SchOHs. For example, although the *n* of PIH, FAH and OIH are relatively higher than those of the other SchOHs, the concentrations of these SchOHs to be an antioxidant are just less than 0.5 μM. They will be a pro-oxidant to accelerate the oxidation of LH when the concentration exceeds this limit. Therefore, their *n* values are only available within 0.5 μM under this experimental condition.

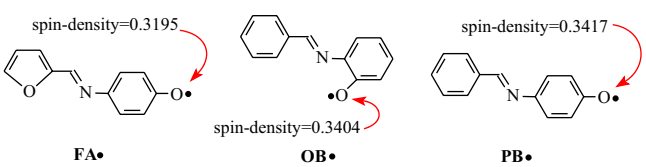
The explanation of the antioxidant activities of SchOHs by quantum chemical calculation

The hydrogen atom of —OH in SchOHs is abstracted by radical to form SchO• when SchOHs serve as antioxidants to protect LH. Two benzene rings are connected with C═N to form a conjugative system in SchOHs, which benefits for the supplementation of electron to the O atom when SchOHs form radicals. So, the corresponding SchO• is relative stable if the SD on O atom in this SchO• is low. The first step to calculate the SD of a SchO• is to optimize the structure of the SchO• at B3LYP/6-31+G(d) level, and to assign the harmonic vibrational frequencies and ZPVE of SchO• at the same level.[11] Then the SD on O atom in SchO• is calculated by using the Gaussian 03 program package,[12] and the results are involved in Table 1 as well. These SD values may help us to understand the difference of the antioxidant activities among SchOHs.

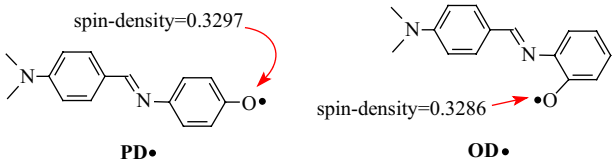
The $n_{PIH} > n_{OIH}$ indicates that antioxidant ability of PIH is higher than that of OIH, which can be understood by the low SD on O atom in PI•. The single electron in PI• can be dispersed to the two benzene rings via C═N. The low antioxidant activity of OIH may also be due to the formation of such an intramolecular hydrogen bond that makes the abstraction of hydrogen atom of —OH in OIH difficult.



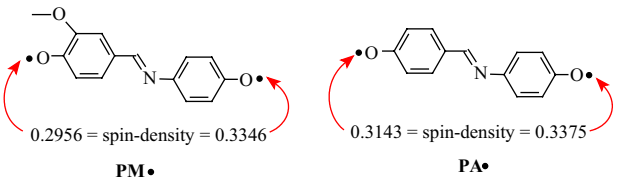
This phenomenon can be also found in other SchO•. For example, the *n* values of FAH, OBH and PDH reveal that the antioxidant activity order is FAH > OBH > PBH. Meanwhile, the SD values in these SchO• give the same sequence. A relative low SD on O atom in FA• implicates that furan ring benefits for the stabilization of FA• more than benzene ring in PB•. Actually, furan ring in FA• exhibits more rich electron than benzene ring in PB•.



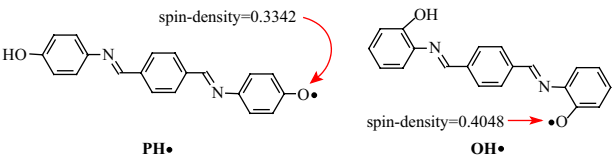
The n_{ODH} is similar to n_{PDH}, indicating that they possess similar antioxidant activities. This can be understood by the similarity of the SD on O atoms in OD• and PD•.



The difference in the structures of PAH and PMH is due to a methoxyl attaching to the *ortho*-position of —OH in PMH. The antioxidant activity of PMH ($n_{PMH} = 1.4$) is a little higher than that of PAH ($n_{PAH} = 1.2$). The SD on O atom derived from the aniline ring is similar, but the SD on O atom derived from benzaldehyde side in PM• (0.2956) is lower than that of PA• (0.3143). This may be due to methoxyl group supplement electron to the radical, resulting in a relatively lower SD on O atom. So, the antioxidant activity of PMH is higher than that of PAH.



Inconsistency is found in the *n* values of PHH and OHH and SD on O atoms in PH• and OH•. The antioxidant activity of OHH is higher than PHH because of $n_{OHH} > n_{PHH}$. However, the SD on O atom in PH• is lower than that of OH•. This may be due to the accuracy of B3LYP/6-31+G(d) level, and UB3LYP/6-31+G(d) level should be necessary to optimize the structure of SchOHs with complicated structures in the future calculation.



CONCLUSION

In conclusion, SchOHs serve as a kind of antioxidant to protect LH against AAPH-induced oxidation. The quantitative relationships of t_{inh}–[SchOHs] lead to obtain *n* value that can give a quantitative comparison of the antioxidant activity. Moreover, the SD values obtained by quantum calculation can help us to understand the result of the antioxidant properties of SchOHs. The combination of experimental measurement and quantum calculation gives a novel idea to investigate the property of an antioxidant.

Acknowledgements

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REFERENCES

[1] L. R. Mahoney, *Angew. Chem. Int. Ed. Eng.* **1969**, *8*, 547–555.
[2] M. C. Foti, *J. Pharm. Pharmacol.* **2007**, *59*, 1673–1685.
[3] Y.-Z. Tang, Z.-Q. Liu, *Cell Biochem. Funct.* **2008**, *26*, 185–191.
[4] Y.-Z. Tang, Z.-Q. Liu, *Cell Biochem. Funct.* **2007**, *25*, 701–710.
[5] Y.-Z. Tang, Z.-Q. Liu, *Cell Biochem. Funct.* **2007**, *25*, 149–158.
[6] X.-Y. Luo, J.-Z. Zhao, Y.-J. Lin, Z.-Q. Liu, *Chem. Res. Chinese Univ.* **2002**, *18*, 287–289.
[7] K. M. Patel, K. N. Patel, N. H. Patel, M. N. Patel, *Synth. React. Inorg. Met. Org. Chem.* **2001**, *31*, 239–246.
[8] K. R. H. Solomons, H. E. Lieberman, P. W. Groundwater, D. E. Hibbs, M. B. Hursthouse, *Anti-Cancer Drug Des.* **1997**, *12*, 635–647.
[9] Z.-Q. Liu, *J. Phys. Chem. A* **2006**, *110*, 6372–6378.
[10] Z.-Q. Liu, *J. Phys. Org. Chem.* **2006**, *19*, 136–142.
[11] G. da. Silva, C.-H. Kim, J. W. Bozzelli, *J. Phys. Chem. A* **2006**, *110*, 7925–7934.
[12] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr. T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian 03, revision B. 03, Gaussian, Inc. Pittsurgh, PA, **2003**.
[13] M. Antolovich, P. D. Prenzler, E. Patsalides, S. McDonald, K. Robards, *Analyst* **2002**, *127*, 183–198.
[14] V. W. Bowry, R. Stocker, *J. Am. Chem. Soc.* **1993**, *115*, 6029–6044.