RADICALS DERIVED FROM GUANINE: STRUCTURES AND ENERGETICS

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Dedicated to Professor Josef Paldus on the occasion of his 70th birthday.

The electron affinities (EA) of five radicals derived from guanine by removing a hydrogen atom are predicted by using four carefully calibrated (*Chem. Rev.* **2002**, *102*, 231) density functional methods. The most stable guanine radical arises from the removal of the H^{10b} atom (Fig. 1) from the NH_2 group. The theoretical adiabatic electron affinities (EA_{ad}) for the five possible guanine radicals are substantial, in the range of 2.18–2.99 eV, which is much higher than that for the closed-shell guanine molecule (–0.17 eV). The N^9 dehydrogenated radical has the highest EA_{ad} (2.99 eV), and its related anion is the lowest energy species among those studied in the present research. The energy difference between the two N^{10}-sited (amino group) radicals is 4.8 kcal/mol, indicating that these two structures are not conventional internal-rotation conformers.

Keywords: Purines; Nucleobases; DNA damage; Guanine radicals; Electron affinity; Density functional computations; DFT; *Ab initio* calculations.

Electron trapping on nucleic acid bases is thought to be a crucial step in the radiation-induced damage of DNA [1]. DNA subunits are known to undergo ionization, protonation, deprotonation, hydrogenation, and dehydrogenation, which processes can lead to a permanent alteration of the original bases and to an uncorrected transfer of genetic information[2,3]. Recent studies of dissociative electron attachment (DEA) to gas phase DNA and RNA bases suggest that dehydrogenation from the base radical anions is an important reaction channel[4], i.e., $(B^-)^\bullet \rightarrow (B_{-H})^- + H^\bullet$. The dehydrogenated anions $(B_{-H})^-$ were also found to be major species formed from nucleic acid bases via negative ion chemical mass spectrometry[5].

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It is well known that guanine is the most easily oxidized of the nucleic acid bases[6]. The decay products of the guanine cation G^+ have been identified as the N^{10}-sited deprotonated radicals $(G_{-H})^\bullet$ by ESR studies[7], i.e., $G^+ \rightarrow (G_{-H})^\bullet + H^+$. This observation is important to understanding the direct effects of ionizing radiation on DNA. The 1996 theoretical study of Hutter and Clark predicted that the proton at atom N^1 of guanine (Fig. 1) may shift to cytosine in the guanine-cytosine radical cation[8]. The Hartree–Fock study of Sevilla and coworkers predicted that the N^1 and N^{10} deprotonated forms of guanine are both important[9]. While the adiabatic electron affinities (EA_{ad}) value reported by Sevilla[9] for the dehydrogenated (N^1-sited) guanine radical is 2.16 eV, Chen and coworkers[5] have predicted the EA_{ad} for the dehydrogenated (N^9-sited) guanine radical to be quite different (3.46 eV) using a semiempirical method.

Recent development of carefully calibrated DFT methods[10] allows experiment-consistent EA_{ad} values for the DNA and RNA bases, with the ordering U > T > C ~ G > A [11]. These DFT methods have also been successfully used to predict the neutral-anion energy separation for the radicals of adenine, cytosine, and thymine[12–14]. In the present research, we report reliable electron affinities (EA) for all five possible guanine radicals. Information concerning the structures and energetics of the guanine radicals is important for the understanding of DNA lesions.

THEORETICAL METHODS

Four density functionals: BLYP, BP86, B3LYP, and BHLYP, are used here to predict the total energies, equilibrium structures, and vibrational frequencies for the guanine radicals and anions. The BLYP and BP86 functionals are

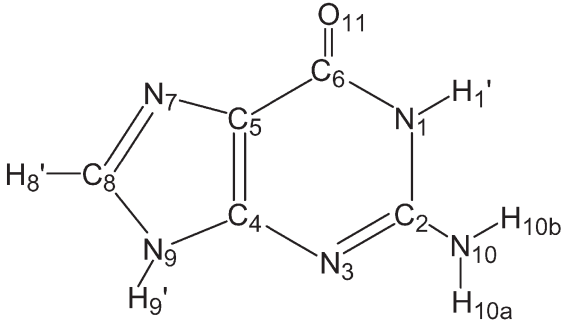


FIG. 1
IUPAC numbering in guanine (**1**)

combinations of the Becke's 1988 pure DFT exchange functional (B)[15] with the dynamical correlation functional of Lee, Yang, and Parr (LYP)[16] or that of Perdew (P86)[17,18]. The hybrid HF/DFT functionals (B3LYP and BHLYP) use Becke's three-parameter functional[19] and the half-and-half hybrid functional[20], respectively.

The basis sets used in this study are of double-quality, with added polarization and diffuse functions, denoted DZP++. The DZP sets are constructed by augmenting the Huzinaga–Dunning[21,22] sets of contracted double-gaussian functions with one set of *p*-type polarization functions for each H atom and one set of five *d*-type polarization functions for the C, N, and O atoms (α_p (H) = 0.75, α_d (C) = 0.75, α_d (N) = 0.80, α_d (O) = 0.85). To complete the DZP++ basis, one even-tempered diffuse *s* function is added to each H atom, while sets of even-tempered *s* and *p* diffuse functions are centered on each heavy atom. The even-tempered orbital exponents were determined by the prescription of Lee and Schaefer[23],

$$\alpha_{\text{diffuse}} = \frac{1}{2}\left[\frac{\alpha_1}{\alpha_2} + \frac{\alpha_2}{\alpha_3}\right]\alpha_1$$

in which α_1, α_2, and α_3, are the three smallest gaussian orbital exponents of the *s*- or *p*-type primitive functions for a given atom ($\alpha_1 < \alpha_2 < \alpha_3$). In the final DZP++ set, six contracted functions per H atom and 19 functions per C, N, or O atom are included. The total number of basis functions for the guanine radicals is 233. The geometry optimizations are performed using analytic gradients with tight convergence criteria. All computations were carried out with the Gaussian 98 program package[24].

The electron affinities were determined in the following manner. The adiabatic electron affinity is defined as the difference between the total energies of the neutral and corresponding anion species at their respective optimized geometries

EA_{ad} = *E*(optimized neutral) – *E*(optimized anion),

the vertical electron affinity (EA_{vert}) of the radical is defined as

EA_{vert} = *E*(optimized neutral) – *E*(anion at optimized neutral geometry),

and the vertical detachment energy (VDE) of the anions is determined via

VDE = *E*(neutral at optimized anion geometry) – *E*(optimized anion).

RESULTS AND DISCUSSION

Radicals

The structure and numbering scheme for guanine are shown in Fig. 1. The optimized bond lengths at the DZP++ B3LYP level of theory for the five radicals are displayed in Fig. 2. More detailed results including the complete optimized geometries, total energies, and the zero-point vibrationally corrected energies for the radicals and anions using all four functionals (B3LYP, BHLYP, BLYP, and BP86) are accessible in the supporting information.

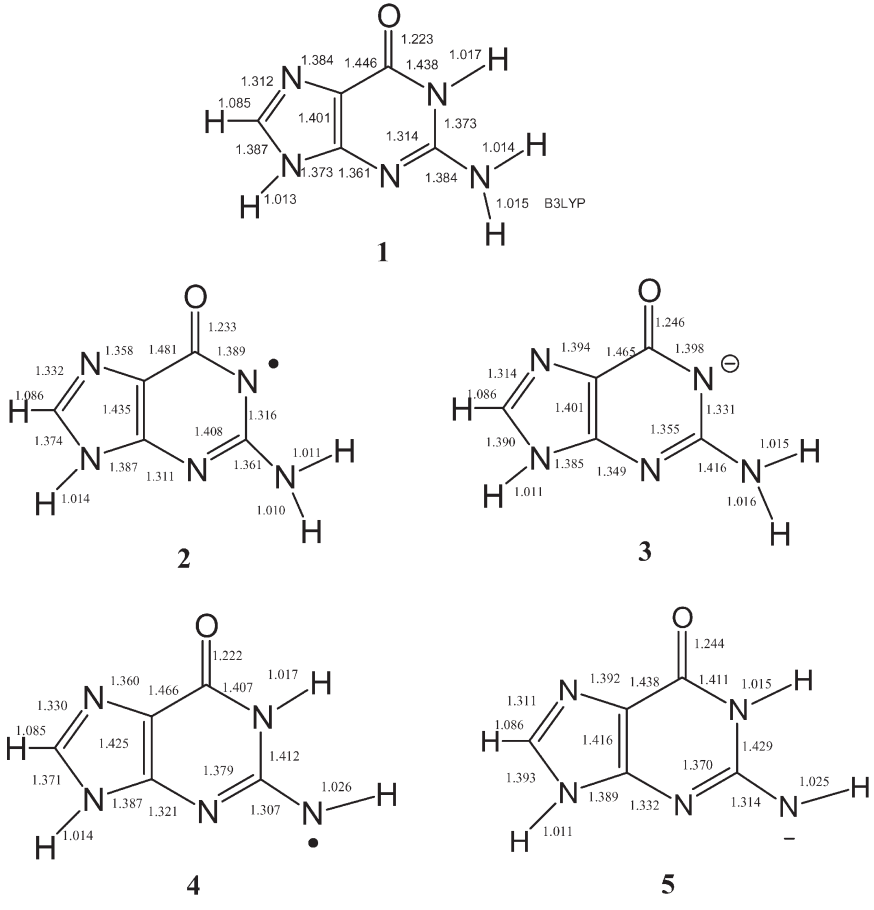
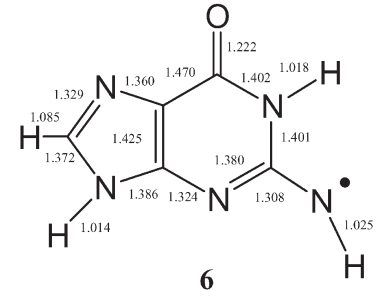


FIG. 2
Bond lenghts in guanine radicals and their corresponding anions at the DZP++ B3LYP level of theory

By removing one H atom from the guanine molecule, there are four nitrogen-sited radicals and one carbon-sited radical. The geometries of the four nitrogen-sited radicals differ significantly from guanine. Radicals **2**, **4**, and **6** are planar, while radical **8** is slightly out of plane. The changes in bond lengths are not exclusively situated around the site where the hydrogen atom is abstracted. In radicals **2**, **4**, and **6**, compared with guanine, large geometry changes of the five-membered ring suggest that the unpaired electron is delocalized. The carbon-sited radical **10** is nonplanar, having the amino group H atoms protruding out of the molecular plane



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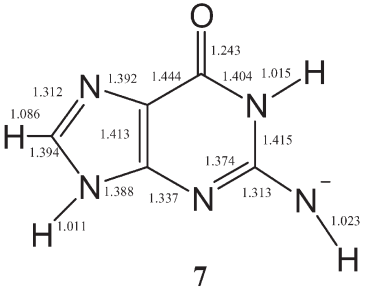

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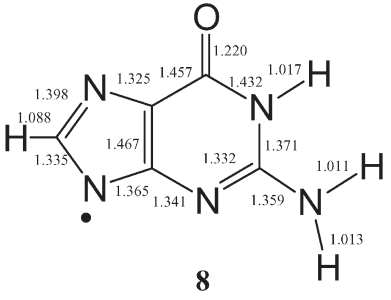

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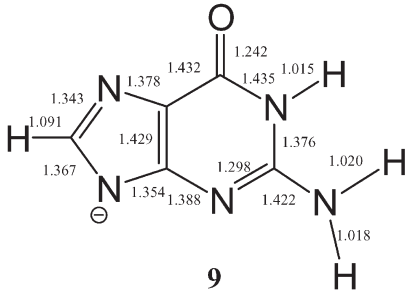

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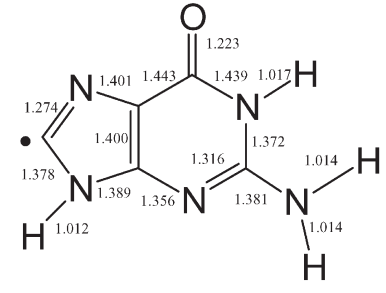

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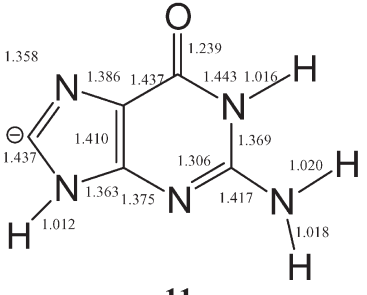

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FIG. 2
(*Continued*)

with bond angles and torsion angles similar to those of the guanine molecule. The deviations of the corresponding bond lengths of the six-membered ring between radical **10** and guanine are less than 0.005 Å.

The total and relative energies for the five radicals, at the DZP++ B3LYP level of theory are reported in Table I. The lowest energy radical is **6**, followed by **8**, **2**, **4**, and **10**. The energies for the four nitrogen-sited radicals are reasonably close (within 5 kcal/mol), while the carbon-sited radical (**10**) lies 27 kcal/mol higher. The same energetic order is predicted by both the BLYP and BP86 functionals, but the DZP++ BHLYP method predicts that radical **4** is lower in energy than radical **2** by 0.24 kcal/mol. Table I shows that the nitrogen-sited radicals (**2**, **4**, **6**, and **8**) are more stable than the carbon-sited radical (**10**), and this is consistent with Evangelista's[21] theoretical studies of the radicals derived from adenine. Evangelista pointed out that two rotational conformers would be generated by removing one of the two hydrogen atoms from the amino group of the adenine molecule[21], and that the geometries and energies of these two conformers are similar to each other, with an energy difference of only 1 kcal/mol. However, for the two analogous conformers (**4** and **6**) of the guanine radical, the energy difference is larger, and some of the geometrical parameters change (more discussion below).

The ESR/ENDOR experiments[7] proposed that the decay product of the guanine cation in crystals at helium temperatures is the dehydrogenated radical at the N^{10} site. This removal of a hydrogen atom from guanine and its derivatives can also be observed in cases where guanosine reacts with radicals in aqueous solution[6]. All the experimental studies confirm that dehydrogenation will occur at the nitrogen centers rather than the carbon center to form stable radicals.

TABLE I
Total and relative energies of radicals and anions derived from guanine (ZPVE-corrected relative energies in parentheses) predicted with the B3LYP method

Structure	Relative energy kcal/mol	Total energy E_h	Structure	Relative energy kcal/mol	Total energy E_h
6	0.00 (0.00)	–542.01025	**9**	0.00 (0.00)	–542.11524
8	2.99 (2.51)	–542.00549	**7**	2.00 (1.79)	–542.11205
2	4.69 (4.06)	–542.00277	**3**	2.60 (2.40)	–542.11110
4	4.93 (4.76)	–542.00240	**5**	7.47 (6.94)	–542.10335
10	26.71 (26.90)	–541.96769	**11**	42.35 (41.63)	–542.04776

In 1992, Sevilla and co-authors[9] predicted the energies of radical **2** and radical **4** (or **6**, not distinguished from **4** in their study) at different levels of theory. In their study the different theoretical levels predicted different energy orderings. The highest level (HF/6-31G*//HF/3-21G) in their study predicted structure **2** to lie below structure **4** by 0.98 kcal/mol, which is roughly consistent with the present results.

In this research, the B3LYP method predicts that the energy difference between structures **4** and **6** is relatively large, 4.8 kcal/mol (with ZPVE corrections). Accordingly, the geometries for the radical rotamers **4** and **6** differ. The N^1–C^2 bond distance, which is adjacent to the radical site, has a notable difference of 0.011 Å between structures **4** and **6**. This indicates that the two hydrogen atoms in the NH_2 group are not equivalent in guanine, because they reside in different chemical environments. This situation is similar to that reported earlier for cytosine[13], but not for adenine[12].

Anions

The optimized geometries of the five deprotonated guanine structures (**3**, **5**, **7**, **9**, and **11**) at the B3LYP level of theory are displayed in Fig. 2, and their energies are reported in Table I. Only the closed-shell singlet states are taken into account for these anions.

Figure 2 shows that significant geometry alterations for the five anions take place upon addition of one electron to the neutral radicals. For example, anion **3** is nonplanar with the amino group hydrogens protruding out of the molecular plane, compared with the planar neutral radical **2**. When comparing radical **10** with its anion **11**, the C^4–N^3, N^7–C^8, and C^8–N^9 bond distances change in the following significant ways: C^4–N^3, 1.356 → 1.375 Å, N^7–C^8, 1.274 → 1.358 Å, and C^8–N^9, 1.378 → 1.437 Å. These substantial variations between the radicals and their related anions indicate that all five anions are of covalent character, with the extra electron being strongly bound. Moreover, for both nitrogen-sited and carbon-sited anions, the structural changes are not limited to the radical sites, and this phenomenon suggests delocalization of the spin density.

It should be noted again that, similar to radicals **4** and **6**, the N^1–C^2 bond distances for the rotamer anions **5** and **7** reflect a significant difference of 0.014 Å. This highlights the fact that the two hydrogen atoms in the NH_2 group of guanine experience rather different environments.

It is apparent from Table I that the nitrogen-sited anions (**3**, **5**, **7**, and **9**) are energetically preferred to the carbon-sited anion (**11**). The energies of the anions are in the same order as that of their corresponding radicals,

except for anions **7** and **9**. The carbon-sited anion **11** lies energetically above the global minimum (structure **9**) by 42.4 kcal/mol, which difference is much larger than the corresponding value (26.7 kcal/mol) for the related radicals (**6** and **10**). An analogous energetic pattern appears in the adenine radicals and their anions[12].

Electron Affinities

Three kinds of neutral-anion energy separations are listed in Tables II–IV. These are the adiabatic electron affinities (EA_{ad}), the vertical electron affinities (EA_{vert}) for the neutral radicals, and the vertical detachment energies (VDE) for the related anions. Substantial positive energy separations are predicted by all four functionals. The values derived from the B3LYP and BP86 methods are very close, while those determined by the BHLYP and BLYP methods are slightly different. Given an overview of the situation, the electron affinities predicted by four functionals are consistent within deviations of less than 0.25 eV, with BP86 predicting the largest EAs and BHLYP predicting the smallest. The B3LYP method is considered to be most reliable[10,11], and it predicts EA_{ad} values for the nitrogen-sited radicals (**2**, **4**, **6**, and **8**) to be in the range of 2.75–2.99 eV, while that (2.18 eV) for the carbon-sited radical (**10**) is much lower. The vertical electron affinities for the nitrogen radicals are in the range of 2.59–2.69 eV (B3LYP, Table III), while the vertical detachment energies for the anions are necessarily

TABLE II
Adiabatic electron affinities of radicals related to guanine, in eV (ZPVE-corrected EA in parentheses)

Structure	B3LYP	BHLYP	BLYP	BP86
2	2.95 (2.93)	2.80 (2.77)	2.75 (2.73)	2.95 (2.94)
4	2.75 (2.76)	2.57 (2.57)	2.57 (2.59)	2.77 (2.79)
6	2.77 (2.78)	2.61 (2.60)	2.58 (2.60)	2.79 (2.80)
8	2.99 (2.97)	2.82 (2.79)	2.80 (2.79)	3.01 (3.00)
10	2.18 (2.22)	1.93 (1.96)	2.10 (2.14)	2.24 (2.28)

higher, in the range of 2.86–3.17 eV (B3LYP, Table IV). The difference between EA_{vert} and VDE for the carbon-sited structures (**10** and **11**) is quite large (0.99 eV), because of the substantial geometry change from the neutral (**10**) to the anion (**11**), particular around the radical site.

Compared with the EA_{ad} values for the deprotonated radicals of other DNA bases[12–14], the electron affinity for the guanine radical is the lowest, i.e., $EA(T_{-H}^*) = 3.74 > EA(A_{-H}^*) = 3.26 > EA(C_{-H}^*) = 3.00 > EA(G_{-H}^*) = 2.99$ eV. This order is qualitatively consistent with that predicted by Chen et al. with their semiempirical method[5]. But their EA_{ad} value for radical **2** is 3.46 eV, much higher than in the present research. The theoretical EA_{ad} value for radical **2** predicted by Sevilla et al. is 2.16 eV from the 6-31+G(d)//HF/3-21G level of theory[9]. The latter EA is lower by 0.79 eV compared to the present B3LYP prediction.

TABLE III
Vertical electron affinities of radicals related to guanine, in eV

Structure	B3LYP	BHLYP	BLYP	BP86
2	2.69	2.50	2.53	2.73
4	2.63	2.43	2.47	2.67
6	2.67	2.48	2.49	2.70
8	2.59	2.38	2.46	2.67
10	1.69	1.39	1.65	1.78

TABLE IV
Vertical detachment energies of anions related to guanine, in eV

Structure	B3LYP	BHLYP	BLYP	BP86
3	3.17	3.06	2.94	3.15
5	2.86	2.70	2.66	2.87
7	2.87	2.72	2.66	2.87
9	3.34	3.22	3.12	3.33
11	2.68	2.46	2.55	2.71

CONCLUSIONS

The theoretical electron affinities of five radicals derived from guanine by hydrogen atom abstraction have been predicted using four density functionals, B3LYP, BHLYP, B3LYP, and BP86 in conjunction with DZP++ basis sets. Previous studies show that these methods lead to reasonable values of electron affinities[10,11].

There are five dehydrogenated radicals for guanine. The most stable radical is the amino-sited (N^{10}) radical (**6**), while its rotational conformer (**4**) lies higher by ca. 5 kcal/mol. Therefore, these two structures should not be treated as conventional rotational conformers. The anion **7** (related to the neutral global minimum **6**) is not the lowest energy anion; the N^9-sited anion (**9**) lies 2 kcal/mol below **7** (Table I).

The EAs for the five radicals predicted by four functionals are substantial. The B3LYP method predicts ZPVE-corrected EA_{ad} values for the five radicals in the range 2.22 to 2.97 eV. The carbon-sited radical (**10**) has the lowest EA_{ad} (2.22 eV with ZPVE correction), while the nitrogen-site radical (**8**) has the highest EA_{ad} value, namely 2.97 eV.

The EA_{vert} and VDE values are quite different from the adiabatic electron affinities, because of the considerable geometrical changes between the radicals and the corresponding anions.

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REFERENCES

1. Huels M. A., Hahndorf I., Illenberger E., Sanche L.: *J. Chem. Phys.* **1998**, *108*, 319.
2. Tanaka N., Hiromi K., Norio I.: *J. Phys. Chem.* **1991**, *95*, 1494.
3. a) Sagstuend E., Hole E. O., Nelson W. H., Close D. M.: *Radiat. Res.* **1996**, *146*, 425; b) Sagstuend E., Hole E. O., Nelson W. H., Close D. M.: *Radiat. Res.* **1998**, *149*, 120.
4. a) Denifl S., Ptasinska S., Cingel M., Matejcik S., Scheier P., Märk T. D.: *Chem. Phys. Lett.* **2003**, *377*, 74; b) Gohlke S., Abdoul-Carime H., Illenberger E.: *Chem. Phys. Lett.* **2003**, *380*, 595.
5. Chen E. S. D., Chen E. C. M., Sane N.: *Biochem. Biophys. Res. Commun.* **1998**, *246*, 228.
6. Steenken S.: *Chem. Rev.* **1989**, *89*, 503.
7. a) Close D. M., Sagstuen E., Nelson W. H.: *J. Chem. Phys.* **1985**, *82*, 4386; b) Hole E. O., Nelson W. H., Close D. M., Sagstuen E.: *J. Chem. Phys.* **1987**, *86*, 5218.
8. Hutter M., Clark T.: *J. Am. Chem. Soc.* **1996**, *118*, 7574.
9. Colson A.-O., Besler B., Close D. M., Sevilla M. D.: *J. Phys. Chem.* **1992**, *96*, 661.
10. Rienstra-Kiracofe J. C., Tschumper G. S., Schaefer H. F., Nandi S., Ellison G. B.: *Chem. Rev.* **2002**, *102*, 231.

11. Wesolowski S. S., Leininger M. L., Pentchev P. N., Schaefer H. F.: *J. Am. Chem. Soc.* **2001**, *123*, 4023.
12. Evangelista F. A., Paul A., Schaefer H. F.: *J. Phys. Chem. A* **2004**, *108*, 3565.
13. Luo Q., Li J., Li Q. S., Kim S., Wheeler S. E., Xie Y., Schaefer H. F.: *Phys. Chem. Chem. Phys.* **2005**, *7*, 861.
14. Profeta L. T. M., Larkin J. D., Schaefer H. F.: *Mol. Phys.* **2003**, *101*, 3277.
15. Beck A. D. *Phys. Rev. A* **1988**, *38*, 3098.
16. Lee C., Yang W., Parr R. G.: *Phys. Rev. B* **1988**, *37*, 785.
17. Perdew J. P.: *Phys. Rev. B* **1986**, *33*, 8822.
18. Perdew J. P.: *Phys. Rev. B* **1986**, *34*, 7406.
19. Becke A. D.: *J. Chem. Phys.* **1993**, *98*, 5648.
20. The BH and HLYP method implemented in the Gaussian programs has the formula, 0.5 × E_x(HF) + 0.5 × E_x(LSDA) + 0.5 × ΔE_x(B88) + E_c(LYP), which is *not* precisely the formulation proposed by Becke A. D.: *J. Chem. Phys.* **1993**, *98*, 1372.
21. Huzinaga S.: *J. Chem. Phys.* **1965**, *42*, 1293.
22. Dunning T. H.: *J. Chem. Phys.* **1970**, *53*, 2823.
23. Lee T. J., Schaefer H. F.: *J. Chem. Phys.* **1985**, *83*, 1784.
24. Frisch M. J., Trucks G. W., Schlegel H. B., Scuseria G. E., Robb M. A., Cheeseman J. R., Zakrzewski V. G., Montgomery J. A., Stratmann R. E., Burant J. C., Dapprich S., Millam J. M., Daniels A. D., Kudin K. N., Strain M. C., Farkas O., Tomasi J., Barone V., Cossi M., Cammi R., Mennucci B., Pomelli C., Adamo C., Clifford S., Ochterski J., Petersson G. A., Ayala P. Y., Cui Q., Morokuma K., Malick D. K., Rabuck A. D., Raghavachari K., Foresman J. B., Cioslowski J., Ortiz J. V., Baboul A. G., Stefanov B. B., Liu G., Liashenko A., Piskorz P., Komaromi I., Gomperts R., Martin R. L., Fox D. J., Keith T., Al-Laham M. A., Peng C. Y., Nanayakkara A., Challacombe M., Gill P. M. W., Johnson B., Chen W., Wong M. W., Andres J. L., Gonzalez C., Head-Gordon M., Replogle E. S., Pople J. A.: *Gaussian 98*. Gaussian, Inc., Pittsburgh (PA) 1998.