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Photoionization and Dissociative Photoionization Study of Cholesterol by IR Laser Desorption/Tunable Synchrotron VUV Photoionization Mass Spectrometry†

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Elementary cholesterol was analyzed with IR laser desorption/tunable synchrotron vacuum ultraviolet photoionization mass spectrometry. An exclusive molecular ion of cholesterol is observed by near threshold single-photon ionization with high efficiency. Fragments are yielded with the increase of photon energy. The structures of various fragments are determined with commercial electron ionization time-of-flight mass spectrometry. Dominant fragmentation pathways are discussed in detail with the aid of *ab initio* calculations.

Key words: Cholesterol, Mass spectrometry, Laser desorption, Photoionization, *Ab initio*

I. INTRODUCTION

As an essential steroid, cholesterol acts as a major structural component of plasma membranes, and is a precursor of steroid hormones and bile acids. In addition, cholesterol has been reported to be a risk factor for many diseases, such as hyperpiesia, neurodegenerative, and cardiovascular diseases [1,2]. Many mass spectrometry-based methodologies have been attempted for the analysis of cholesterol. Following the introduction of fast atom bombardment (FAB) as a method of ionization of polar molecules, FAB mass spectrometry became widely used in the analysis of conjugated steroids. However, due to the lack of a basic or acidic group, the ionization efficiency of cholesterol by conventional FAB is poor. Low ion yields upon electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI) also result in a relatively unsatisfactory sensitivity [3,4]. Generally, to enhance the ionization efficiency, a series of derivatization methods are introduced before mass spectrometric analysis. Berkel *et al*. converted the alcohol group on C3 position of cholesterol to a ferrocene carbamate ester [5]. Upon ESI process, this compound readily gives molecular ions at m/z=613 (386+227, the former one corresponds to the molecular weight of cholesterol), which further gives rise to ferrocene carbamic acid ion at m/z=245 at low collision energy [5]. In another method, steroids with an alcohol functional group were converted to pentafluobenzyl ethers, which dissociate with the loss of a pentafluorobenzyl radical to produce an alkoxide anion upon electron-capture negative chemical ionization (ECNCI) [6]. This derivatization method actually behave in a similar manner as negative-ion atmospheric pressure chemical ionizatio (APCI) to produce $(M\text{-}H)^-$ ions. Recently, the combination of high performance liquid chromatography (HPLC) with APCI has been proven to be a powerful strategy, but dehydration always appears during ionization which make an incomplete mass spectrum [7]. Atmospheric pressure photoionization (APPI), which typically uses a krypton lamp as a light source to ionize a sample, has also been applied to study steroids [8]. The comparison for the analysis of anabolic steroids by the ionization techniques of ESI, APCI, and APPI has been discussed by Leinonen *et al*. [9].

More recently, we have developed a reliable, simple and fragment-controllable method named infrared laser desorption/vacuum ultraviolet photoionization mass spectrometry (IR LD/VUV PIMS) for the analysis of single and complex organic molecules. Due to tunable wavelength in the VUV range, this method can also offer ionization energies and appearance energies in addition to information of molecular structures [10-13]. In the current research, IR LD/VUV PIMS is attempted to study the major dissociative photoionization behaviors of cholesterol. Moreover, such dissociative mechanism is validated by theoretical calculation.

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II. EXPERIMENTAL AND COMPUTATIONAL DETAILS

A. Experimental methods

The experiments were carried out at the National Synchrotron Radiation Laboratory (NSRL) in Hefei, China. The apparatus has been reported elsewhere [10-13]. In brief, the experiments utilized 1064 nm output of a pulsed Nd:YAG laser (Surelite I-20, Continuum Inc., Santa Clara, CA, USA) with a duration of 7 ns for IR laser desorption. The laser beam was focused onto a stainless steel substrate with a 40-cm focal length lens, and the central spot was kept at around 1 mm in diameter. Laser power density at the surface of substrate was controlled at 6 mJ/pulse to generate intact neutral molecules for near-threshold VUV photoionization. VUV light beam is perpendicular and overlapping with the desorption plume in the photoionization region. The VUV photoionization takes place at a distance of 2-4 mm from the substrate surface, where the plume of molecules formed from the desorption process disperses and is ionized by VUV light. Ions produced by VUV light were analyzed by a home-made reflectron TOF mass spectrometer. A pulsed voltage of 260 V applied to the repeller plates was used to propel ions into the flight tube. In operation, the pulsed voltage with the frequency of 10 kHz works with a delay of 150 μs after the laser fires with the frequency of 10 Hz, which was controlled by a home-made pulse/delay generator. The pressure of photoionization chamber was around 0.1 mPa.

Synchrotron radiation from an undulator beamline of the 800 MeV electron storage ring was monochromatized with a 1 m Seya-Namioka monochromator equipped with a laminar grating (1500 groove/mm, Horiba Jobin Yvon, Longjumeau Cedex, France). The grating covers the photon energy from 7.8 eV to 24 eV. The monochromator was calibrated with the known ionization energies of the inert gases. The energy resolving power ($E/\Delta E$) is about 1000. A gas filter filled with argon or neon was used to eliminate high-order harmonic radiation. The average photon flux can reach the magnitude of 10^{13} photon/s. A silicon photodiode (SXUV-100, international radiation detectors inc., Torrance, CA, USA) was used to monitor the photon flux for normalizing ion signals.

To fulfill the structural assignments and propose the fragmentation pathways, accurate mass measurement for cholesterol was also obtained using Micromass GCT TOF mass spectrometer (Micromass, Manchester, UK) with EI method (electron energy 70 eV, trap current 10 μA). The source temperature was set at 220 °C and the sample was volatilized from a heated insertion probe in the source by direct probe introduction in positive ion mode. The instrument was calibrated at a resolution of 8000 (FWHM) using heptacosafluorotributylamine as an internal reference and single point lock-mass was locked at m/z=219. Sample analysis, exact mass measurement, and elemental composition determination were performed automatically using the OpenLynx software within MassLynx.

Cholesterol was obtained from Fluka. Before the experiment, cholesterol was deposited onto the stainless steel substrate without any preparation and purification. No organic matrix was used for the experiment.

B. Computational methods

Ab initio calculations were carried out using the Gaussian 03 program [14]. The geometry of cholesterol was fully optimized at the hybrid density functional B3LYP/6-31G level. The vibrational frequencies were calculated at the same level for characterizing the nature of structure, which were also used to compute the zero-point energy (ZPE). Further single energies were calculated at the B3LYP/6-31++G(d,p) level with the optimized geometries.

III. RESULTS AND DISCUSSION

A. Photoionization mass spectra

A series of photoionization mass spectra were obtained at some slected photon energies. Figure 1 shows the mass spectrum of cholesterol at the photon energy of 10.0 eV. Due to near-threshold photoionization, only a pure molecular ion at m/z=386 is observed with high intensity. This tunable ionization manner makes it a true "soft" ionization, which is useful to identify the molecular weight of an analyte. In comparison, in the MALDI mass spectrum there was no evidence for the formation of a molecular ion or an $[M+H]^+$ ion, and only dehydrated protonated ion $[M+H\text{-}H_2O]^+$ with low abundance was detected [4]. In another 70 eV electron impact ionization experiment, the mass spectrum

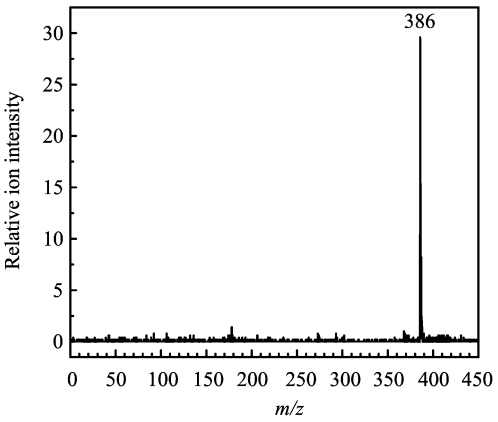

FIG. 1 Photoionization mass spectrum of cholesterol at the photon energy of 10.0 eV.

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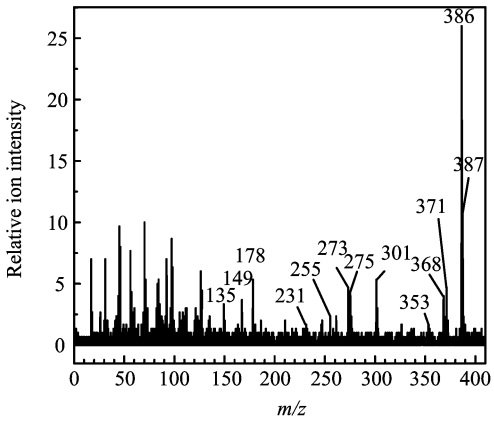


FIG. 2 Photoionization mass spectrum of cholesterol at the photon energy of 12.0 eV.

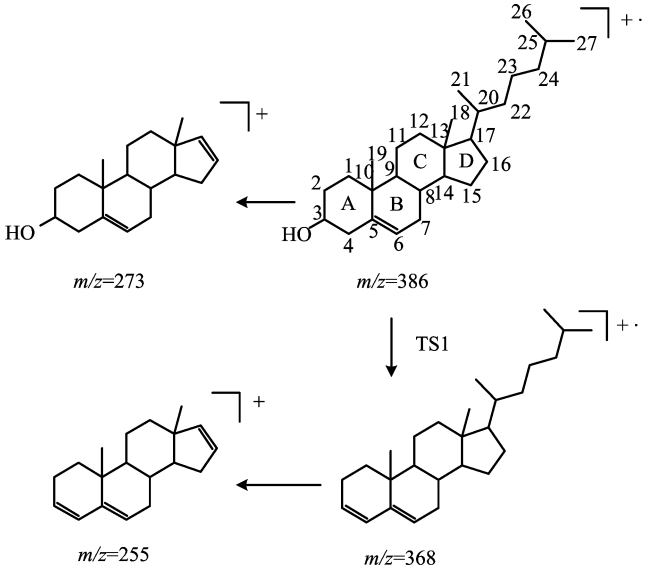
of cholesterol is characterized by a large number of fragment signals (see the supporting information).

Cholesterol is a relatively fragile molecule. By increasing photon energy slightly, many fragments of cholesterol can be formed, as shown in Fig.2. In general, cholesterol and its derivatives exhibit complex fragmentation patterns resulting from dehydration, cleavage of any four rings and side-chain cleavage [3,15]. Some major dissociation channels at the photon energy of 12.0 eV are discussed in this work. For deep insight into the photoinduced dissociative reactions, a computational B3LYP method was implemented to clarify the fragmentation mechanisms.

B. Dehydration and side-chain cleavage reactions

Similar to other sterols containing just an alcohol group on C3 position, dehydration is also a major process upon ionization, yielding a fragment signal at m/z=368. Theoretical calculations indicate that the molecular ion can undergoes C4–H and C3–O(H) bonds fission via a four-membered ring transition state TS1 (9.13 eV relative to neutral cholesterol, which is defined as zero in this work) to form the m/z=368 ion and neutral H_2O, as shown in Scheme 1. At the B3LYP/6-31++G(d,p) level, the barrier for this step is calculated to be 1.52 eV.

Side-chain cleavage is a major fragmentation channel of biologically important sterols. The first fragment ion at m/z=371 in Fig.2 is due to the loss of a CH_3 with C25–C26 or C25–C27 bond fission, while the m/z=353 ion is a characteristic peak originated from consecutive loss of H_2O and CH_3. At the photon energy of 12.0 eV, fragment ions at m/z=273 and m/z=255 formed by side-chain cleavage of the C17–C20 bond are also yielded. The presence of an CH_2OH group causes these two fragment ions to be changed in mass by 18 Da, as shown in Scheme 1.



Scheme 1 Proposed formation pathways for the fragments at m/z=368, 273, and 255.

C. Ring cleavage reactions

The formation pathways of the fragment at m/z=301 are depicted in Scheme 2. This ion may be formed via C22–C23 bond cleavage pattern [15]. Based on theoretical calculation, this direct dealkylation channel is the lowest energy-required pathway. It is also found that the C13–C17 bond of the m/z=301(a) ion is indeed broken (pathway I) to keep more stable. However, accurate mass measurements assign this fragment to $C_{22}H_{37}$, which is formed from ring-opening after dehydration (pathway II). This discrepancy may be due to slow reaction rate of the former dealkylation process, causing the extremely low abundance of the m/z=301(a) ion.

Loss of C_7H_9 after dehydration and subsequent ring-opening reaction result in the fragment at m/z=275. Scheme 3 shows that the m/z=368 ion first undergoes a C9–C10 bond cleavage to form an intermediate (INT1) via TS2 (8.92 eV relative to neutral cholesterol). The newly formed C5–C9 bond will break immediately followed by forming another new bond of C6–C9 (INT2) via TS3 (8.78 eV relative to neutral cholesterol). Then INT2 undergoes C5–C6 bond fission, yielding the fragment at m/z=275 and neutral C_7H_9. The overall barriers of TS2 and TS3 of this pathway are determined to be 1.48 and 0.27 eV, respectively. Careri *et al.* assigned the m/z=275 ion to $[(m/z=273)+2H]^+$ [15], which is different from our theoretical and experimental results.

The fragment at m/z=231 yields a relatively weak peak at the photon energy of 12.0 eV. The major fragmentation to generate this ion is at the two-ring (C and D) junction. Theoretical calculations indicate that C14–C15 bond fission occurs upon ionization, yielding

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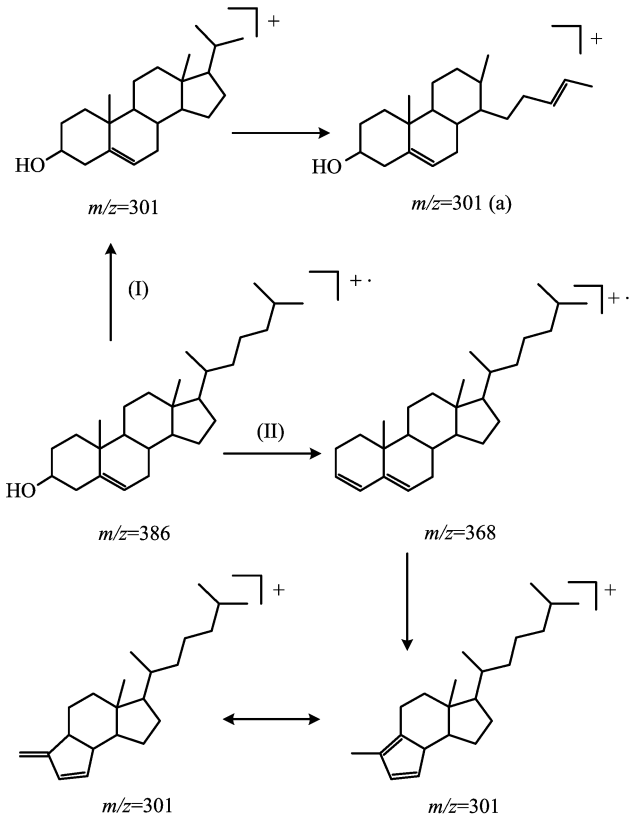
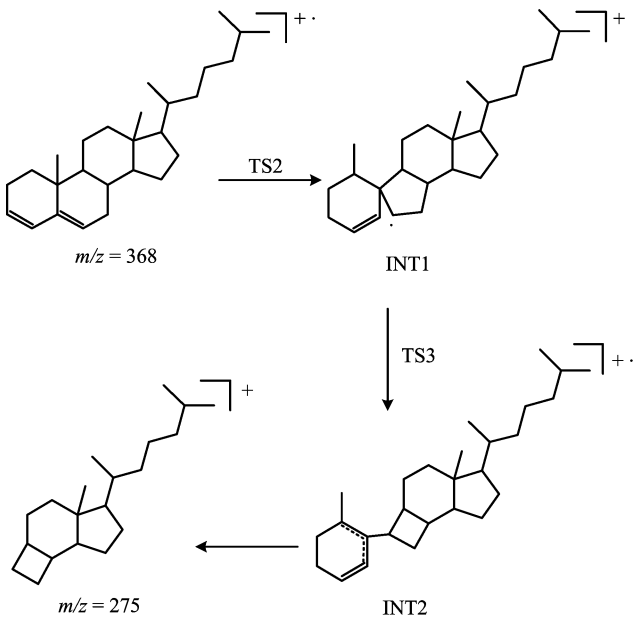


Scheme 2 Proposed formation pathways for the fragment at m/z=301.



Scheme 3 Proposed formation pathway for the fragment at m/z=275.

INT3 via TS4 (9.48 eV relative to neutral cholesterol) with a barrier energy of 1.87 eV. Then the fragment at m/z=231 is produced by the loss of $C_{11}H_{23}$. Further ejection of C_4H_5 from the m/z=231 ion yields the fragment at m/z=178. As demonstrated in Scheme 4, C8–C14 bond cleavage firstly occurs with the m/z=231 ion to form INT4 via TS5 (14.34 eV relative to neutral cholesterol). The barrier energy of this step is 6.21 eV. To stay at lower energy, triangle formation is favored for C12–C13–C14 atoms. Subsequently, INT5 is produced with an elongated C11–C12 bond and a shortened C9–C11 bond. Accompanied by thorough C11–C12 bond cleavage, the fragment at m/z=178(a) is yielded with the loss of C_4H_5. Due to the intramolecular hydrogen transfer or bond rearrangement, the m/z=178(a) ion has two other isomeric products, among which the m/z=178(c) ion is the highest in energy for all minima in potential energy surface. Calculations suggest that the m/z=178(c) ion can convert into the m/z=178 (b) and (a) ions via TS7 and TS8, which are determined to be 1.35 and 1.10 eV, respectively. In addition, if the m/z=301(a) ion formed from side-chain cleavage in Scheme 2 exists, the fragment at m/z=231 will be yielded from this ion via an alternative pathway.

Scheme 4 shows that the m/z=301(a) ion can overcome TS9 (10.55 eV relative to neutral cholesterol) to give an intermediate INT6 at m/z=231 along with the loss of neutral 2-pentene. Subsequently, the m/z=231 ion will be transformed from INT6 via TS10 (11.78 eV relative to neutral cholesterol). The energy barriers for these two steps are 1.17 and 1.1 eV, respectively. Furthermore, after C-ring cleavage the m/z=178(c) ion can also be formed from the fragment at m/z=301. In the low mass range of the spectrum in Fig.2, more complex peaks are observed such as m/z=149 ($C_{11}H_{17}^+$), 135 ($C_{10}H_{15}^+$), 121 ($C_9H_{13}^+$), 92 ($C_7H_8^+$), and 83 ($C_6H_{11}^+$). Most of the fragments are assigned to hydrocarbon products by various bond cleavage patterns.

IV. CONCLUSION

Cholesterol was investigated using IR laser desorption combined with tunable synchrotron VUV photoionization mass spectrometry technique. Mass spectra at two different photon energies are obtained for comparison. Structural assignments of product ions are supported by accurate EI-TOF-MS measurements. Complicated fragmentation channels of cholesterol in low photon energy range are discussed in detail with the help of theoretical calculations. It was found that the fragmentation pathways of cholesterol involve a series of dehydration, side-chain cleavage, and ring cleavage reactions.

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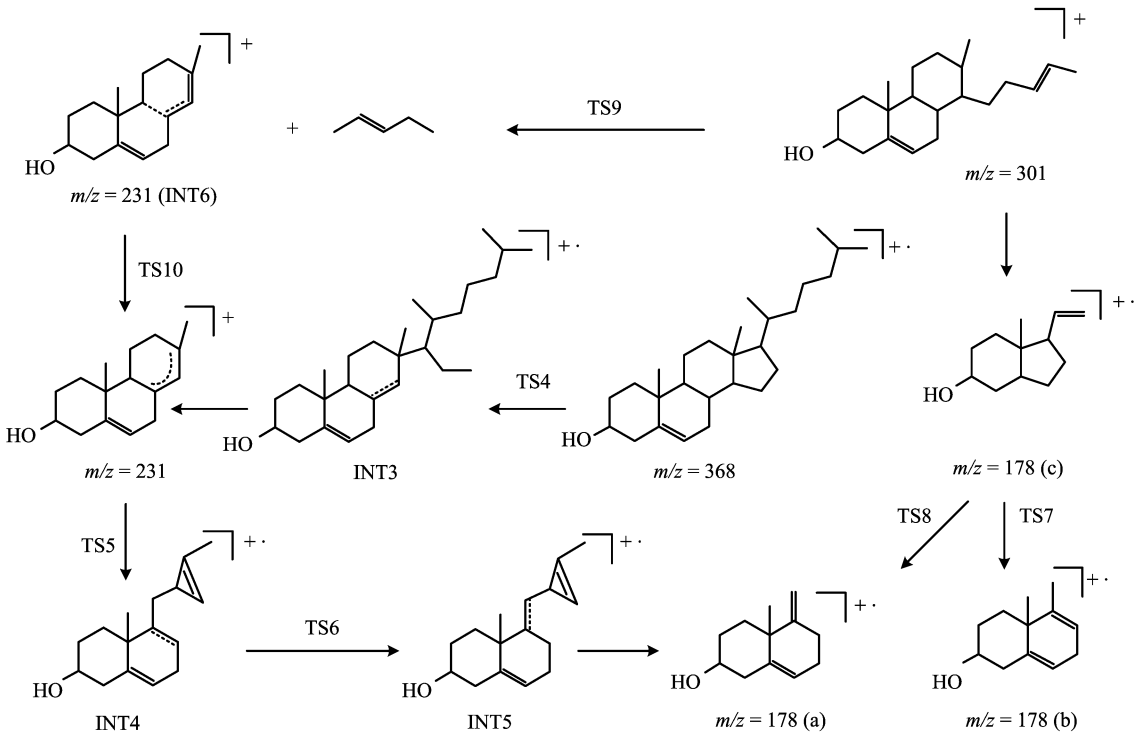

Scheme 4 Proposed formation pathways for the fragments at m/z=231 and 178.

V. ACKNOWLEDGMENTS

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[1] J. M. Dietschy and S. D.Turley, J. Lipid. Res. **45**, 1375 (2004).
[2] C. P. Cannon, Clin. Cornerstone **8**, 11 (2007).
[3] W. J. Griffiths, Mass Spectrom. Rev. **22**, 81 (2003).
[4] Y. Q. Wang, M. Hornshaw, G. Alvelius, K. Bodin, S. Y. Liu, J. Sjovall, and W. J. Griffiths, Anal. Chem. **78**, 164 (2006).
[5] G. J. van Berkel, J. M. E. Quirke, R. A. Tigani, A. S. Dilley, and T. R. Covey, Anal. Chem. **70**, 1544 (1998).
[6] G. Singh, A. Gutierrez, K. Xu, and I. A. Blair, Anal. Chem. **72**, 3007 (2000).
[7] Q. G. Tian, M. L. Failla, T. Bohn, and S. J. Schwartz, Rapid Commun. Mass Spectrom. **20**, 3056 (2006).
[8] M. J. Greig, B. B. olanos, T. Quenzer, and J. M. R. Bylund, Rapid Commun. Mass Spectrom. **17**, 2763 (2003).
[9] A. Leinonen, T. Kuuranne, and R. Kostiainen, J. Mass Spectrom. **37**, 693 (2002).
[10] Y. Pan, T. C. Zhang, X. Hong, Y. W. Zhang, L. S. Sheng, and F. Qi, Rapid Commun. Mass Spectrom. **22**, 1619 (2008).
[11] Y. Pan, T. C. Zhang, H. J. Guo, X. Hong, and F. Qi, J. Mass Spectrom. **43**, 1701 (2008).
[12] Y. Pan, H. Yin, T. C. Zhang, H. J. Guo, L. S. Sheng, and F. Qi, Rapid Commun. Mass Spectrom. **22**, 2515 (2008).
[13] Y. Pan, L. D. Zhang, H. J. Guo, and F. Qi, J. Phys. Chem. A **112**, 10977 (2008).
[14] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. J. Montgomery, 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, and J. A. Pople, *Gaussian 03, Revision C.02*, Wallingford, CT: Gaussian Inc., (2004).
[15] M. Careri, D. Ferretti, P. Manini, and M. Musci, J. Chromatogr. A **794**, 253 (1998).

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