Electronic Supplementary Material (ESI) for Analyst.

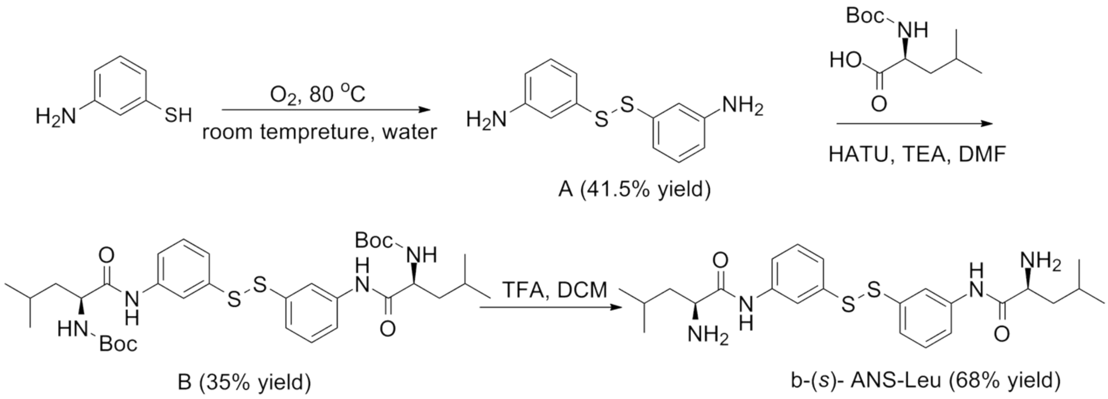
Supporting Information

Detection of Leucine Aminopeptidase Activity in Serum Using Surface-Enhanced Raman Spectroscopy

Dan Guo, Zhen-Fei Gan, Lei Jiang, Mao-Feng Cao, Fato Tano Patrice, Mahmoud Elsayed Hafez and Da-Wei Li*

Key Laboratory for Advanced Materials, *Joint International Laboratory for Precision Chemistry & School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai, 200237, P. R. China.*

**E-mail: daweili@ecust.edu.cn*



Scheme S1. Synthetic route for the SERS probe b-(*s*)-ANS-Leu.

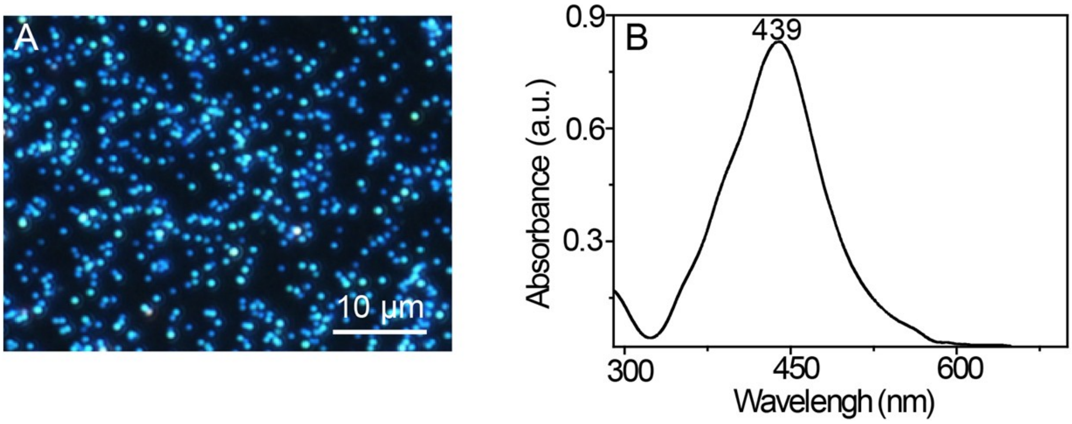


Fig. S1 (A) Dark-field color images and (B) UV–Vis spectrum of the synthesized AgNPs.

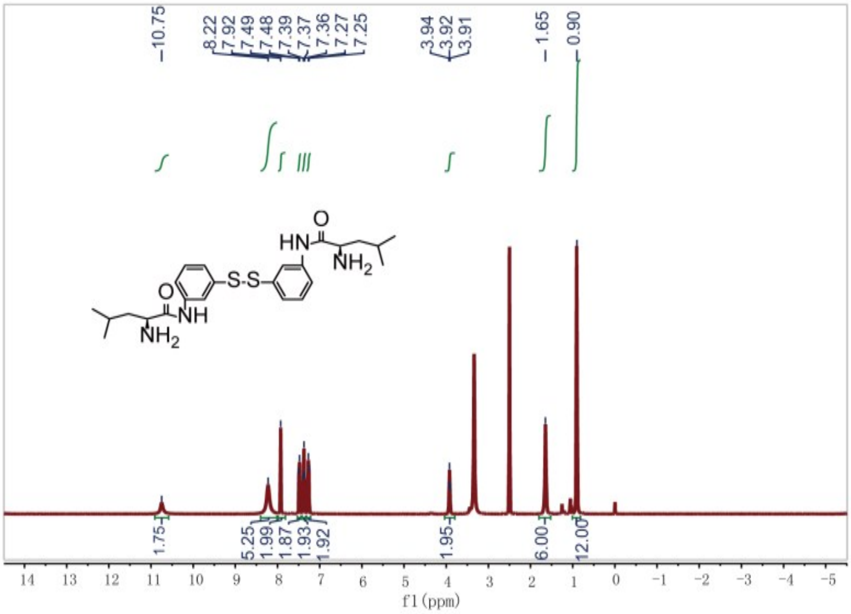


Fig. S2 ^{1}H NMR spectrum of the SERS probe b-(*s*)-ANS-Leu. (500 MHz, DMSO-d^{6}, ppm) δ 10.75 (s, 2H), 8.22 (s, 6H), 7.92 (s, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.37 (t, *J* = 7.9 Hz, 2H), 7.26 (d, *J* = 7.8 Hz, 2H), 3.92 (t, *J* = 6.5 Hz, 2H), 1.65 (s, 6H), 0.90 (s, 12H).

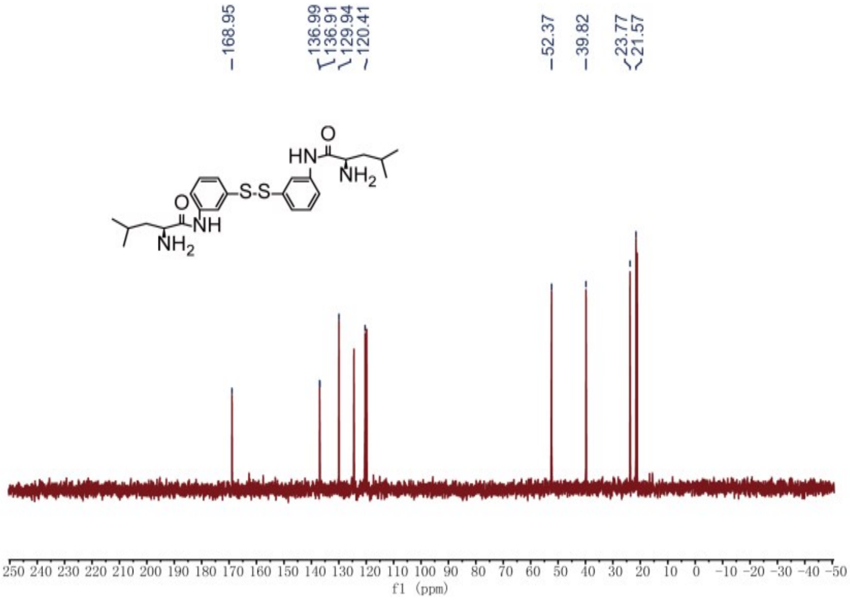


Fig. S3 ^{13}C NMR spectrum of the SERS probe b-(*s*)-ANS-Leu. ^{13}C NMR (500 MHz, DMSO-d^{6}, ppm) δ (168.95, 136.99, 136.91, 129.94, 120.41, 52.37, 39.82, 23.77, 21.57).

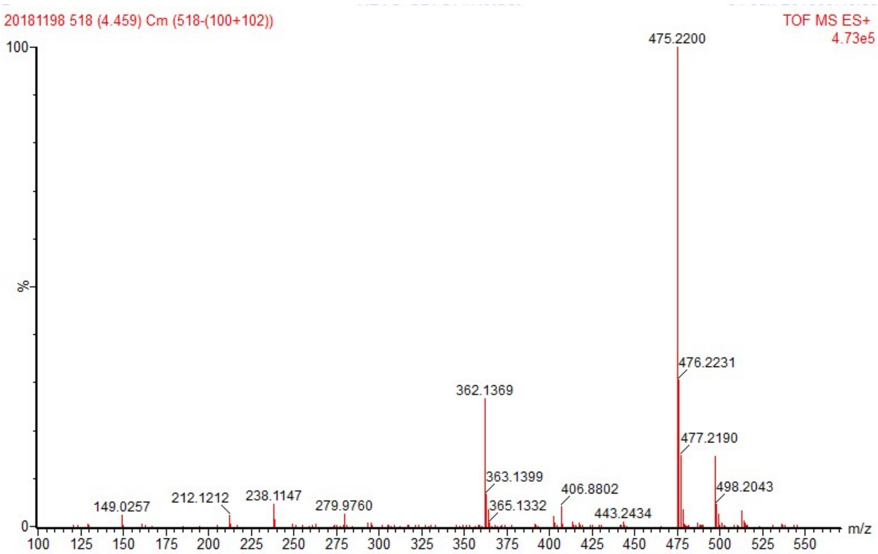


Fig. S4 Mass spectrum of the SERS probe b-(*s*)-ANS-Leu. Mass (MS-ESI): m/z cacld for (M) + $C_{24}H_{34}N_4O_2S_2$ 474.21; found 475.2200 $(M+H)^+$.

Density Functional Theory (DFT) calculations. In order to better understand of the SERS variation of SERS probe b-(*s*)-ANS-Leu and its hydrolysis reaction product (3,3'-disulfanediyldianiline, DSDA) triggered by LAP, quantum chemical calculations at DFT/B3LYP level of theory were conducted.[1] Since Ag surface can induce -S-S- cleavage, the quantum analysis was conducted on the monomer (*s*)-ANS-Leu and 3-aminothiophenol (3-ATP) instead of the probe and DSDA. Vibrational frequencies of the (*s*)-ANS-Leu and 3-aminothiophenol (3-ATP) were performed at 6-311G (d.p) level for C, H, O and S. According to basis set and the utilized function, Raman shifts were revised by multiplying the factor of 0.967.[2] The polarizable continuum model (IEF-PCM)[3] was applied to optimize geometry and calculate the vibrational frequencies taking aqueous circumstance effect into consideration.

Table S1. Theory Raman peaks calculated with DFT for (*s*)-2-amino-N-(3-sulforphenyl)-Leu ((*s*)-ANS-Leu), 3-aminothiophenol (3-ATP), experimental SERS peaks of (*s*)-ANS-Leu before and after reaction with LAP, and the corresponding band assignments.

(*s*)-ANS-Leu			**3-ATP**		
Theory (cm^{-1})	Experimental (cm^{-1})	Assignment	Theory (cm^{-1})	Experimental (cm^{-1})	Assignment
429	426	$\rho(CCC)_{ring}/\delta(CCC)_{leucine}$	394	410	$\upsilon(C\text{-}S)/\rho(CCC)_{ring}$
1012	993	$\upsilon(CCC)_{ring}$	551	529	$\tau(NH_2)/\upsilon(CCC)_{ring}/\upsilon(C\text{-}NH_2)$
1084	1096	$\upsilon(C\text{-}NH_2)/\upsilon(CCC)_{leucine}/\rho(CH_3)$	694	677	$\upsilon(CCC)_{ring}/\delta(HCCH)_{ring}$
			1005	993	$\upsilon(CCC)_{ring}$

υ, stretching; δ, scissoring; ρ, rocking vibration; τ, out-of-plane bending

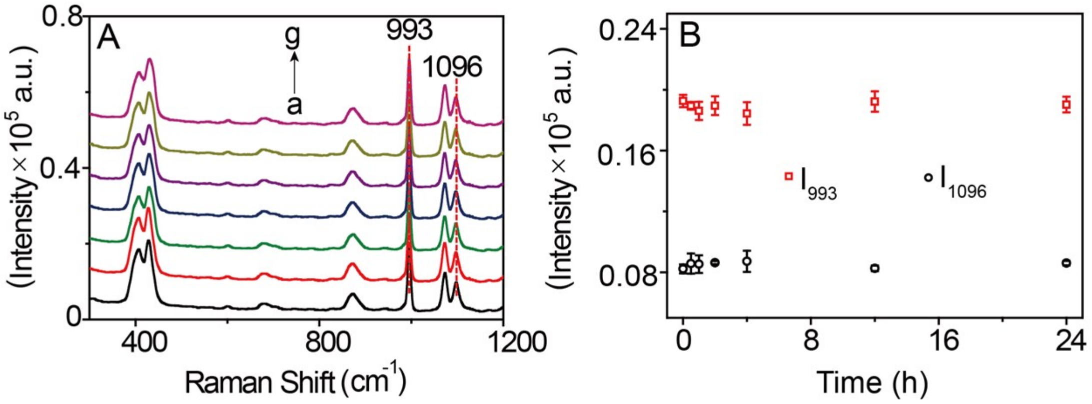


Fig. S5 (A) SERS spectra of the b-(*s*)-ANS-Leu after storage in PBS (pH=7.4, 20 mM) under ambient condition for different time (a-g): 0, 0.5, 1, 2, 4, 12, 24 h. (B) Plot of intensity *versus* storage time based on the measurement at 993 cm^{-1} (red squares) and 1096 cm^{-1} (black circles). Error bars represent ± S.D (n = 3).

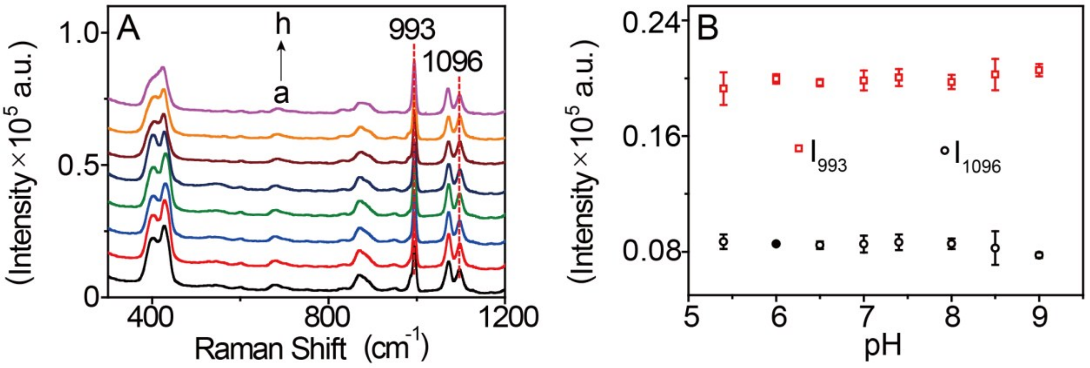


Fig. S6 (A) SERS spectra of the b-(*s*)-ANS-Leu after storage in PBS (20 mM) with different pH (a-h): 5.4, 6.0, 6.5, 7.0, 7.4, 8.0, 8.5, 9.0. (B) Plot of intensity *versus* pH value based on the measurement at 993 cm^{-1} (red squares) and 1096 cm^{-1} (black circles). Error bars represent ± S.D (n = 3).

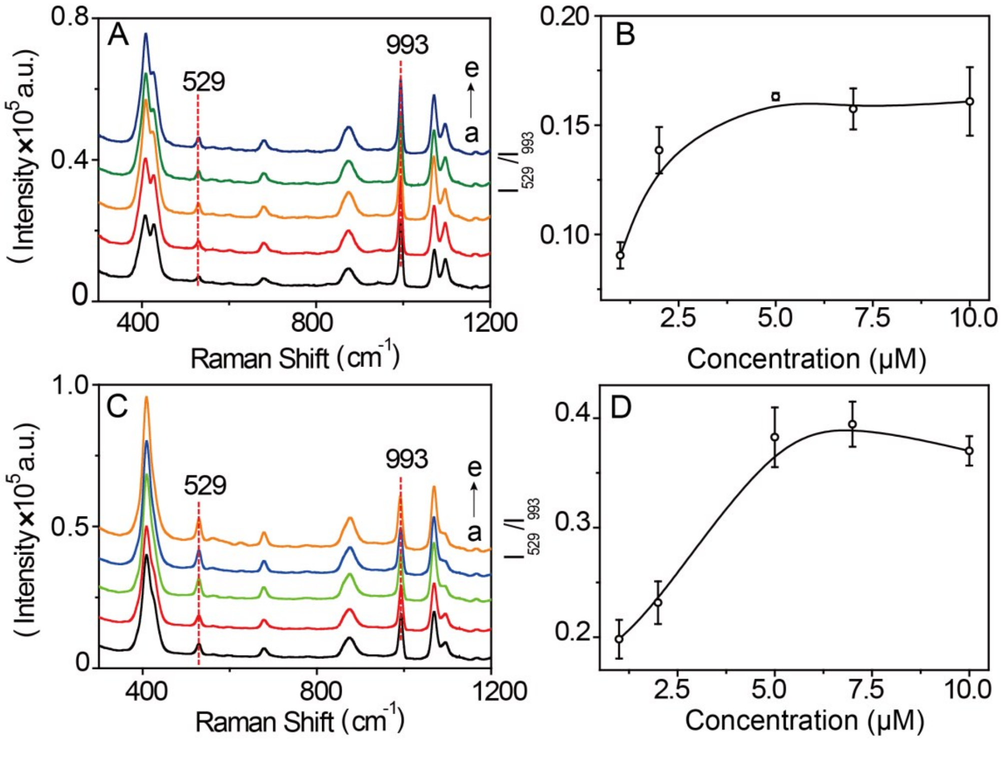


Fig. S7 (A) SERS spectra of b-(*s*)-ANS-Leu at different concentrations (a-e): 1, 2, 5, 7, 10 µM after incubation with 1 mU/mL LAP in PBS (pH=7.4, 20 mM, 37 °C) for 10 min. (B) Ratiometric peak intensities of I_{529}/I_{993} based on the spectra in (A). (C) SERS spectra of b-(*s*)-ANS-Leu at different concentrations (a-e): 1, 2, 5, 7, 10 µM after incubation with 10 mU/mL LAP in PBS (pH=7.4, 20 mM, 37 ^{o}C) for 10 min. (D) Ratiometric peak intensities of I_{529}/I_{993} based on the spectra in (C). Error bars represent ± S.D (n = 3).

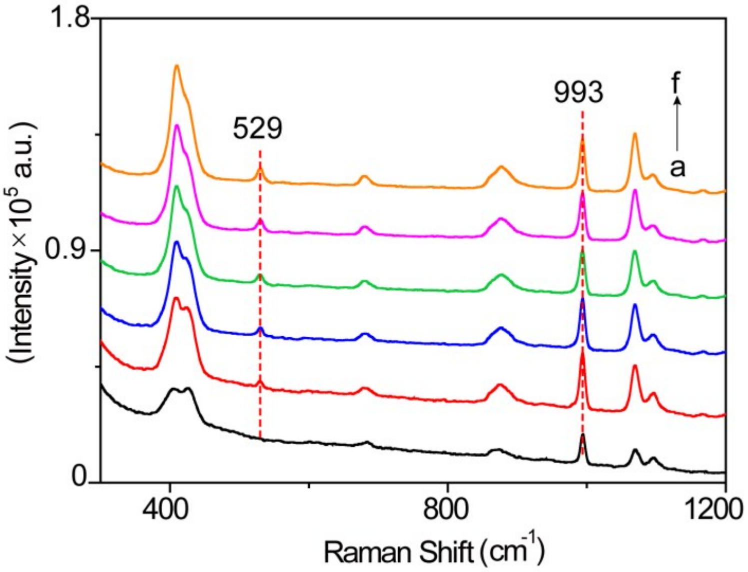


Fig. S8 SERS spectra of 5 μM b-(*s*)-ANS-Leu in FBS serum at 37 ^{o}C for 45 min with addition of different LAP concentrations (a-f): 0, 40, 50, 70, 90, 100 mU/mL.

Table S2. Recovery test of LAP in FBS

Sample	Spiked （mU/mL）	Found （mU/mL）	Recovery （%）	RSD （%）
Fetal bovine serum	0	0.94	-	3.15
	40	41.10	102.75	4.60
	50	50.12	100.24	3.46
	70	70.79	101.12	4.52
	90	92.60	102.88	2.68
	100	107.31	107.31	7.50

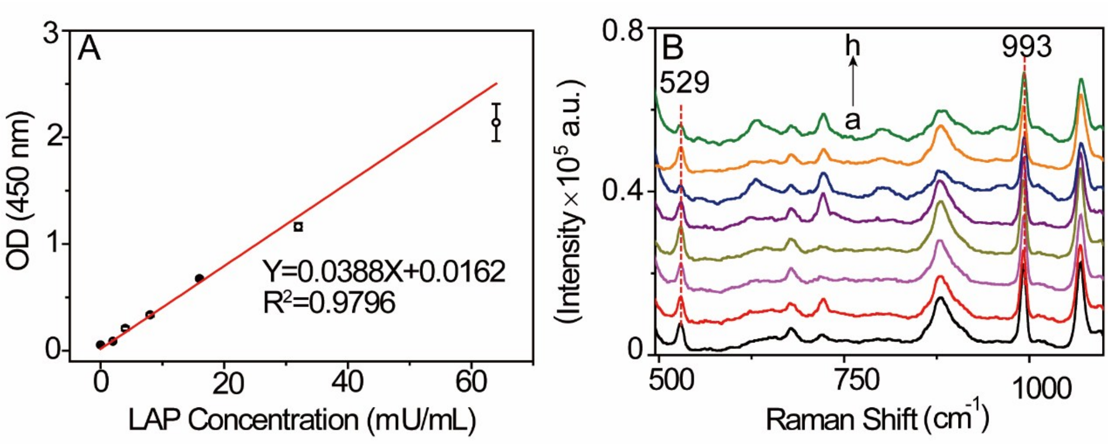


Fig. S9 (A) Calibration plot corresponding to the OD data changes at different LAP concentrations (0, 2, 4, 8, 16, 32, 64 mU/mL) by ELISA Kit. (B) SERS spectra of 5 μM b-(*s*)-ANS-Leu in different human serum samples at 37 °C for 45 min. (a-h) represents the number of samples: 1, 2, 3, 4, 5, 6, 7, 8.

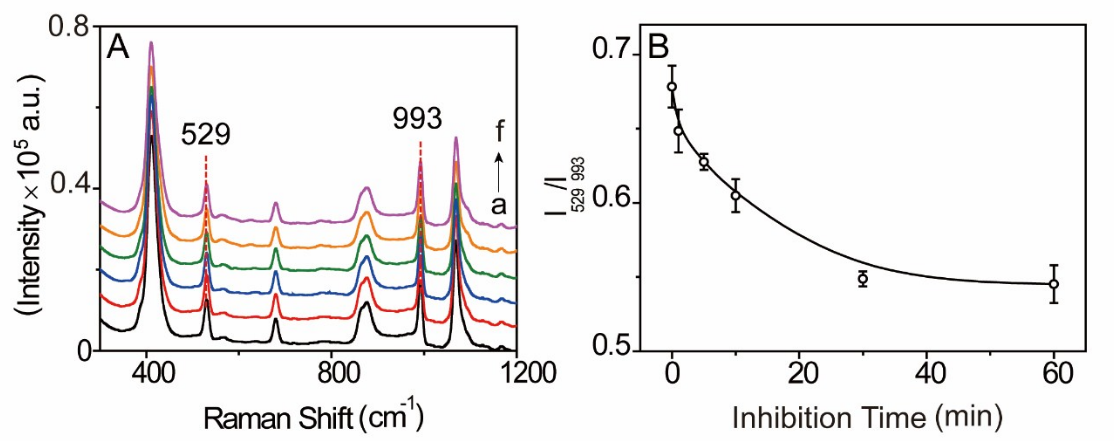


Fig. S10 (A) Inhibition time course of SERS spectra of 5 μM b-(*s*)-ANS-Leu in PBS (pH=7.4, 20 mM) with 100 mU/mL LAP pretreated by 2 μM bestain at different inhibition time (a-f): 0, 1, 5, 10, 30, 60 min. (B) Plot of ratiometric peak intensities of I_{529}/I_{993} *versus* inhibition time.

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[1] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci B, G. A. Petersson, H, Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. E. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Jr. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam , M. Klene, J. E. Knox, 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, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö Farkas, J. B. Foresman, J. V. Ortiz and J. Cioslowski, Fox DJ (2009) Gaussian, Gaussian 09, Revision D.01, Inc., Wallingford CT.

[2] http://cccbdb.nist.gov.

[3] J. Tomasia, B. Mennuccia and E. Cancèsb, *J. MOL. STRUC-THEOCHEM*, 1999, **464**, 211-226.