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Synthesis of the First Water-Soluble Hemicryptophane Host: Selective Recognition of Choline in Aqueous Medium

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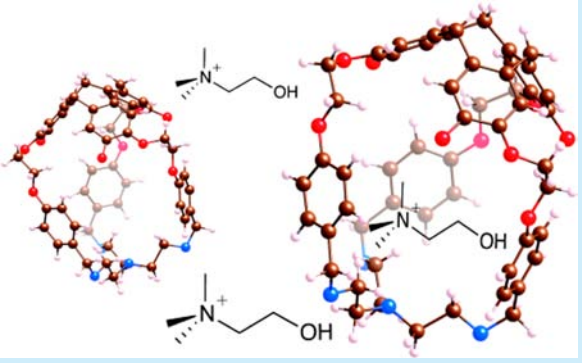
Supporting Information

ABSTRACT: The first water-soluble hemicryptophane cage compound, **4**, was synthesized in seven steps from commercially available products, and its complexation properties were studied. NMR and ITC experiments indicate that **4** is an efficient receptor for choline in water ($\Delta G^\circ = -5.2$ kcal mol^{-1}). High substrate selectivity was achieved since no complexation was observed for its closely related substrates: glycine betaine and betaine aldehyde. Density functional theory calculations were performed to analyze the interactions that are involved in the formation of the inclusion complex.



The design of artificial molecular receptors is very attractive as they can mimic biological systems such as enzymes.[1−3] A large number of host compounds have been designed, and in most cases, their complexation properties have been studied in organic solvents.[4] Nevertheless, most of the recognition events in nature take place in aqueous medium, and to obtain a better understanding and control of biological phenomena, synthetic receptors should be able to selectively complex guests in water.[5] Supramolecular recognition in water is a difficult challenge since, on one hand, the host must be water-soluble, and on the other hand, specific interactions are required to overcome the competitive influence of water. Indeed, the formation of complexes involves interchange between guest−water and guest−host interactions, which is not obviously a favorable process.[6] A prerequisite is that the water-soluble host compound should be able to efficiently discriminate closely related substrates to mimic more accurately the selectivity of biological systems and thus to lead to potential applications.

Choline oxidase is an important enzyme catalyzing the oxidation of choline to glycine betaine via betaine aldehyde as intermediate.[7,8] This enzyme is of great interest for medicinal and biotechnological applications. For instance, intracellular accumulation of glycine betaine improves resistance in pathogenic bacteria and transgenic plants, allowing normal cell function under conditions of hyperosmotic and temperature stress.[9] Furthermore, choline oxidase controls the level of water-soluble organic amines (choline and glycine betaine) in cells, therefore regulating their osmotic equilibrium.[10] Thus, host molecules with the ability to selectively complex choline over its glycine betaine and betaine aldehyde metabolites may find applications as inhibitors or for the selective monitoring of choline concentration. Although selective complexation of choline over glycine betaine or closely related substrates has been reported,[11] to the best of our knowledge, there is no host capable of distinguishing choline from its closely related metabolite betaine aldehyde.

Among different classes of host molecules soluble in water, cryptophanes,[12] adequately substituted with water-solubilizing groups, have been identified as remarkable complexation agents for cesium ion,[13] xenon,[14−19] or ammonium ions. For instance, water-soluble cryptophane-O binds choline efficiently ($\Delta G^\circ = -5.3$ kcal/mol) but without significant selectivity when compared to acetylcholine or trimethylpropylammonium guests.[20] The related hemicryptophane compounds represent a very interesting family of host molecules for the recognition of various substrates.[21,22] Over the past decade, several examples have been reported in the fields of molecular recognition and supramolecular catalysis.[23−37] However, their solubilization in aqueous media is far from trivial, and no water-soluble hemicryptophanes or their recognition properties in water have been reported so far. Herein, we describe the unprecedented synthesis of a water-soluble hemicryptophane host and its use as an efficient receptor for the complexation of choline neurotransmitter in water. The lack of complexation of both glycine betaine and betaine aldehyde under the same conditions highlights the high substrate specificity of this heteroditopic host compound.

A few examples of recognition of ammonium and zwitterionic neurotransmitters by hemicryptophane hosts are

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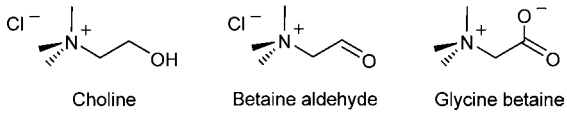


known in the literature.[29−32] We have recently reported the efficient binding properties of hemicryptophane **1** toward alkylammonium derivatives (Scheme 1).[27] Accordingly, its water-soluble counterpart is likely the best suitable host to investigate the recognition of choline guest in aqueous media. The strategy used to synthesize the water-soluble variant of hemicryptophane **1** is presented in Scheme 1 and relies on the deprotection of the methoxy groups of the cyclotribenzylene moiety to restore the phenol functions allowing the solubilization in basic aqueous solution.[12] The deprotection of the methoxy groups in **1** follows the strategy developed for the cryptophane derivatives using Ph_2PLi.[20,37] To avoid the concomitant presence of amine and phenol units in the resulting crude reaction mixture that requires tedious and difficult purification steps, we first Boc-protected the benzylamine groups in **1** giving rise to compound **2**. Then the methoxy groups of **2** were removed using Ph_2PLi to afford the trihydroxy-*N*-Boc protected derivative **3**. Finally, deprotection of the amine functions in **3**, using triflic acid, gave the water-soluble hemicryptophane **4**. Following this synthetic pathway, host **4** was obtained in seven steps from the commercially available products with an overall yield of 8% (Scheme S-1, Supporting Information). We should notice that the use of BBr_3 or TMSI as reactants to obtain compound **4** directly from **1** did not lead to the desired product.

The ^{1}H NMR spectrum of hemicryptophane **4** in D_2O/NaOD confirms the absence of the methoxy groups and displays the expected signals for the cyclotribenzylene unit (Figure 1): two singlets for the aromatic protons and the characteristic AB system for the $ArCH_2$ bridges, two doublets for the H_3−H_4 aromatic protons of the linkers and multiplets for the OCH_2 and NCH_2 groups. At pH = 12, the hydroxy phenol groups in **4** are deprotonated to generate the triphenolate species.

The complexation of choline neurotransmitter (as its chloride salt) by host **4** was first investigated in deuterated water (pD = 12) at 298 K through ^{1}H NMR titration (Figure S-1, Supporting Information). During the experiments, only averaged signals were observed for **4** and choline, indicating fast exchange conditions. Increasing the host/guest ratio induced a significant high-field shift of the guest's protons due to the shielding effect of the aromatic cavity (Figure S-1, Supporting Information). The determination of the binding constant by modeling the titration curve afforded a K_a value of 6.4 × 10^3 M^{-1} (Figure S-2, Supporting Information). However, the guest displays broad NMR signals, preventing an accurate estimation of the host/guest ratio, hence of the binding constant. At lower temperature, slow exchange conditions could be reached and at 270 K we observed a new signal in the high-field region, highlighting the formation of an inclusion complex with the ammonium moiety of the choline located in the shielding region of the cyclotribenzylene unit (Figure 2).

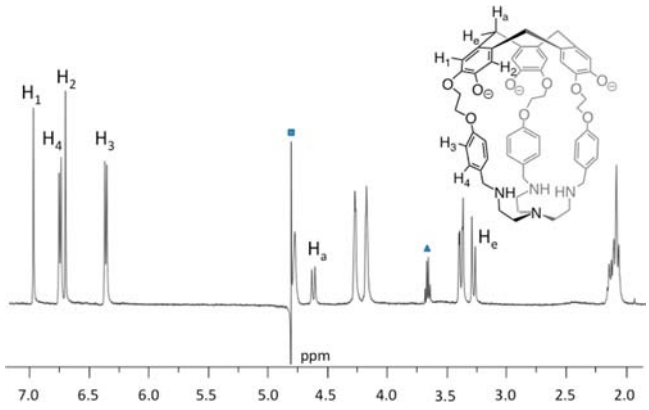


Figure 1. ^{1}H NMR spectrum (500 MHz, D2O, 298 K, pd =12) of hemicryptophane **4** (■, water; ▲, ethanol).

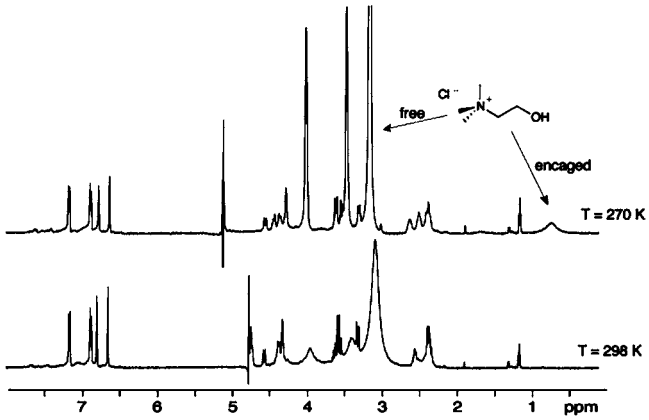
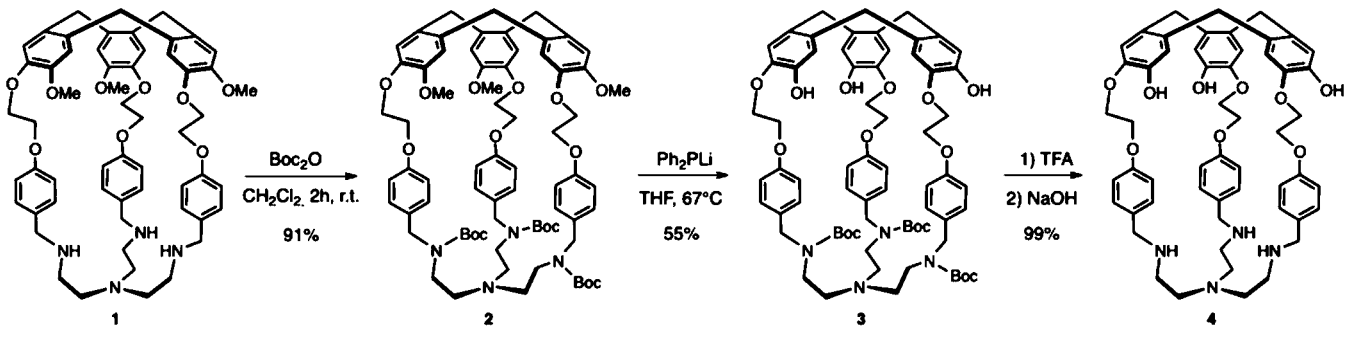


Figure 2. ^{1}H NMR spectra of host **4** with an excess of choline chloride in D_2O/NaOD at different temperatures.

We then used isothermal titration calorimetry (ITC) to determine more accurately the association constant for the choline@**4** complex in water (pH = 12). Addition of successive aliquots of choline chloride (24.03 mM) to **4** (1.12 mM)

Scheme 1. Synthesis of Water-Soluble Hemicryptophane 4



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revealed the formation of a complex with a K_a value of 2300 M^{-1}, which is of the same order of magnitude as that obtained by NMR titration (Figure 3). Furthermore, ITC thermogram is

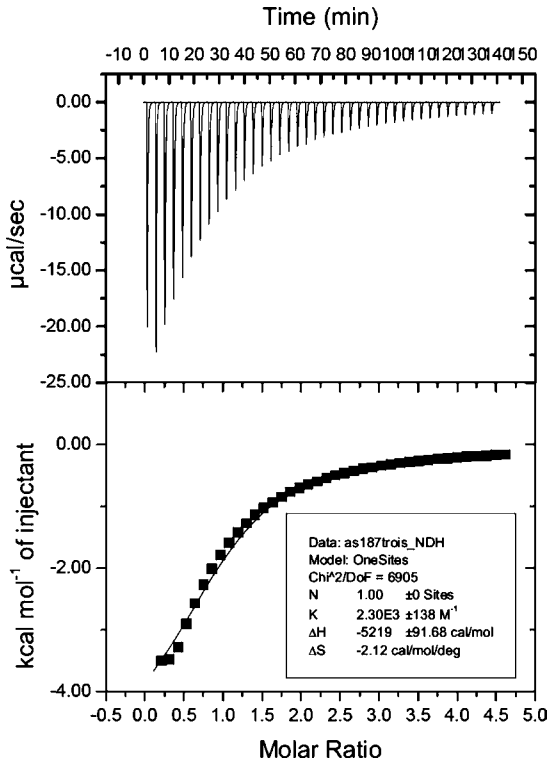

Figure 3. Enthalpogram of an aqueous solution of hemicryptophane **4** (1.12 mM) titrated by choline chloride (24.03 mM) at pH 12 and 298 K.

exothermic, characterized by both a favorable enthalpic term (ΔH = −5.22 kcal·mol^{-1}) and a negative entropy (ΔS = −2.12 cal mol^{-1} K^{-1}, $-T\Delta S$ = +0.63 kcal mol^{-1}), suggesting that the complexation is mainly enthalpy-driven. Favorable interactions of choline with the host cavity of **4** can account for the efficiency of the recognition process.

More information on the selectivity of the host−guest recognition process is accessible by a comparison of the complexation properties of **4** toward choline and its related metabolites betaine aldehyde and glycine betaine. No complexation was observed by ITC for these latter two guests (Figure S-4, Supporting Information), emphasizing the high selectivity of hemicryptophane **4** for choline. This is all most striking given that betaine aldehyde is predominantly under its *gem*-diol hydrate form in aqueous solution (99% at pH 12).[8] Thus, host **4** is able to discriminate choline from its aldehyde, although they only differ by one OH function. It can be noticed that the selectivity observed for choline may be related to the zwitterionic character of betaine aldehyde and choline betaine at pH 12.

Further insight into this recognition process was obtained from density functional theory (DFT) calculations.[38] In the optimized geometry of the complex, choline is encapsulated in the hemicryptophane cavity (Figure 4). The ammonium unit of the guest interacts with both (i) the aromatic rings of the host with several CH···π distances in the range of 3.3 Å and (ii) the negatively charged oxygen atoms (the N^+···O^- distances are 4.4 Å). This is consistent with the high-field shift observed for the methyl protons of the guest in the NMR spectra. Interestingly,

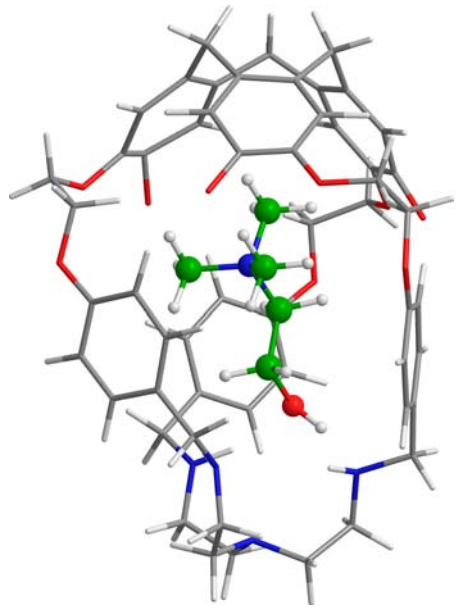

Figure 4. DFT-optimized structure of the choline@**4** complex.

hydrogen bonds occur between the OH unit of the guest and two nitrogen atoms of the tren unit (OH···N distance is 2.7 Å and NH···O distance is 3.0 Å). Thus, both the cyclotribenzylene and tren units contribute to the efficient and selective binding of choline, emphasizing the heteroditopic character of the host.

In summary, we have described the synthesis and charaterization of the first water-soluble hemicryptophane host compound. Efficient complexation of choline in water was then achieved with this receptor. NMR experiments and DFT calculations highlight the formation of an inclusion complex, the ammonium part of the guest being located inside the cavity in the vicinity of the cyclotriveratrylene unit. ITC measurements allowed the determination of the binding constant and the thermodynamic parameters. The combination of such techniques and analysis suggests that **4** is an efficient and selective artificial receptor for the choline neurotransmitter in water since neither betaine aldehyde nor glycine betaine were complexed by this host. This opens the way for the use of hemicryptophane structures for the selective recognition of compounds of biological interest in biological media.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, scheme of the synthesis, and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Kirby, A. J. *Angew. Chem., Int. Ed.* **1996**, *35*, 707−724.
(2) Sanders, J. K. M. *Chem.—Eur. J.* **1998**, *4*, 1378−1383.
(3) Lehn, J.-M. *Rep. Prog. Phys.* **2004**, *67*, 245−249.

(4) Steed, J. W.; Atwood, J. L. In *Supramolecular Chemistry*, 2nd ed.; Wiley-VCH: Weinheim, 2009.
(5) Oshovky, G. V.; Reinhoudt, D. N.; Verboom, W. *Angew. Chem., Int. Ed.* **2007**, *46*, 2366−2393.
(6) Klein, E.; Ferrand, Y.; Barwell, N. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2008**, *47*, 2693−2696.
(7) Fan, F.; Gadda, G. *J. Am. Chem. Soc.* **2005**, *127*, 2067−2074.
(8) Fan, F.; German, M. W.; Gadda, G. *Biochemistry* **2006**, *45*, 1979−1986.
(9) (a) Ko, R.; Tombras Smith, L.; Smith, G. M. *J. Bacteriol.* **1994**, *176*, 426−431. (b) Chen, T. H.; Murata, N. *Plant Cell Environ.* **2011**, *34*, 1−20. (c) Sakamoto, A.; Valverde, R.; Alia; Chen, H. H.; Murata, N. *Plant J.* **2000**, *22*, 449−453.
(10) (a) Peddie, B. A.; Lever, M.; Hayan, C. M.; Randall, K.; Chambers, S. T. *FEMS Microbiol. Lett.* **1994**, *120*, 125−131. (b) Boch, J.; Kempf, B.; Schmid, R.; Bremer, E. *J. Bacteriol.* **1996**, *178*, 5121−5129.
(11) (a) Guo, D.-S.; Uzunova, V. D.; Su, X.; Liu, Y.; Nau, W. M. *Chem. Sci.* **2011**, *2*, 1722−1734. (b) Hof, F.; Trembleau, L.; Ullrich, E. C.; Rebeck, J. *Angew. Chem., Int. Ed.* **2003**, *42*, 3150−3153. (c) Ballester, P.; Sarmentero, M. A. *Org. Lett.* **2006**, *8*, 3477−3480. (d) Ballester, P.; Shivanyuk, A.; Far, A. R.; Rebek, J. *J. Am. Chem. Soc.* **2002**, *124*, 14014−14016.
(12) Brotin, T.; Dutasta, J.-P. *Chem. Rev.* **2009**, *109*, 88−130.
(13) Brotin, T.; Montserret, R.; Bouchet, A.; Cavagnat, D.; Linares, M.; Buffeteau, T. *J. Org. Chem.* **2012**, *77*, 1198−1201.
(14) Huber, J. G.; Brotin, T.; Dubois, L.; Desvaux, H.; Dutasta, J.-P.; Berthault, P. *J. Am. Chem. Soc.* **2006**, *128*, 6239−6246.
(15) Aru Hill, P.; Wei, Q.; Troxler, T.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2009**, *131*, 3069−3077.
(16) Delacour, L.; Kotera, N.; Traoré, T.; Garcia-Argote, S.; Puente, C.; Leteurtre, F.; Gravel, E.; Tassali, N.; Boutin, C.; Léonce, E.; Boulard, Y.; Berthault, P.; Rousseau, B. *Chem.—Eur. J.* **2013**, *19*, 6089−6093.
(17) Fairchild, R. M.; Joseph, A. I.; Holman, K. T.; Fogarty, H. A.; Brotin, T.; Dutasta, J.-P.; Boutin, C.; Huber, G.; Berthault, P. *J. Am. Chem. Soc.* **2010**, *132*, 15505−15507.
(18) Jacobson, D. R.; Khan, N. S.; Collé, R.; Fitzgerald, R.; Laureano-Pérez, L.; Bai, Y.; Dmochowski, I. J. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 10969−10973.
(19) Hill, P. A.; Wei, Q.; Eckenhoff, R.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2007**, *129*, 9262−9263.
(20) Garel, L.; Lozach, B.; Dutasta, J.-P.; Collet, A. *J. Am. Chem. Soc.* **1993**, *115*, 11652−11653.
(21) Gosse, I.; Dutasta, J.-P.; Perrin, M.; Thozet, A. *New J. Chem.* **1999**, *23*, 545−548.
(22) Canceill, J.; Collet, A.; Gabard, J.; Kotzyba-Hibert, F.; Lehn, J.-M. *Helv. Chim. Acta* **1982**, *65*, 1894−1897.
(23) Martinez, A.; Robert, V.; Gornitzka, H.; Dutasta, J.-P. *Chem.—Eur. J.* **2010**, *16*, 520−527.
(24) Le Gac, S.; Jabin, I. *Chem.—Eur. J.* **2008**, *14*, 548−557.
(25) Perraud, O.; Robert, V.; Martinez, A.; Dutasta, J.-P. *Chem.—Eur. J.* **2011**, *17*, 4177−4182.
(26) Perraud, O.; Martinez, A.; Dutasta, J.-P. *Chem. Commun.* **2011**, *47*, 5861−5863.
(27) Perraud, O.; Lefevre, S.; Robert, V.; Martinez, A.; Dutasta, J.-P. *Org. Biomol. Chem.* **2012**, *10*, 1056−1059.
(28) Wang, L.; Wang, G.-T.; Zhao, X.; Jiang, X.-K.; Li, Z.-T. *J. Org. Chem.* **2011**, *76*, 3531−3535.
(29) Perraud, O.; Robert, V.; Gornitzka, H.; Martinez, A.; Dutasta, J.-P. *Angew. Chem., Int. Ed.* **2012**, *51*, 504−508.
(30) Schmitt, A.; Chatelet, B.; Collin, S.; Dutasta, J.-P.; Martinez, A. *Chirality* **2013**, *8*, 475−479.
(31) Cochrane, J. R.; Schmitt, A.; Wille, U.; Hutton, C. A. *Chem. Commun.* **2013**, *49*, 8504−8506.
(32) Perraud, O.; Robert, V.; Martinez, A.; Dutasta, J.-P. *Chem.—Eur. J.* **2011**, *17*, 13405−13408.
(33) Martinez, A.; Dutasta, J.-P. *J. Catal.* **2009**, *267*, 188−192.
(34) Perraud, O.; Sorokin, A. B.; Dutasta, J.-P.; Martinez, A. *Chem. Commun.* **2013**, *49*, 1288−1290.
(35) Makita, Y.; Sugimoto, K.; Furuyosho, K.; Ikeda, K.; Fujiwara, S.-i.; Shin-ike, T.; Ogawa, A. *Inorg. Chem.* **2010**, *49*, 7220−7222.
(36) Makita, Y.; Ikeda, K.; Sugimoto, K.; Fujita, T.; Danno, T.; Bobuatong, K.; Ehara, M.; Jujiwara, S. I.; Ogawa, A. *J. Organomet. Chem.* **2012**, *706*, 26−29.
(37) Canceill, J.; Lacombe, L.; Collet, A. *J. Chem. Soc., Chem. Commun.* **1987**, 219−221.
(38) Gaussian 03: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, O.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc.: Wallingford, CT, 2009.

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