Supplementary Information

Investigating the photophysical properties of rhodamines using a spectroscopic single-molecule fluorescence method

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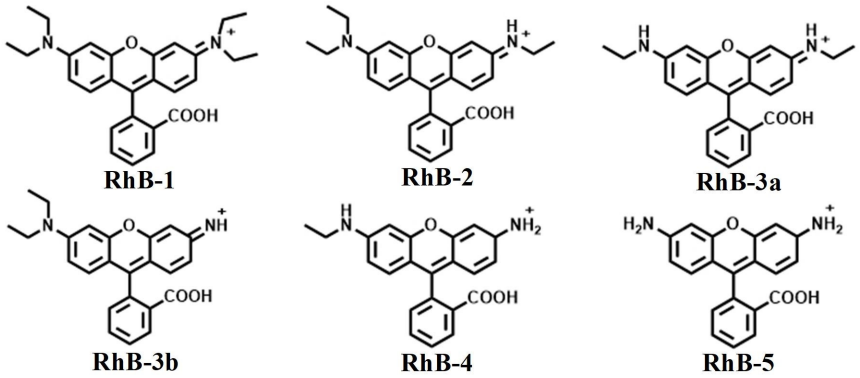

Fig. S1 The molecular structures of different kinds of rhodamine B in their "open" forms.

Table S1 The reported peak wavelengths in the absorption spectra and emission spectra of five kinds of rhodamine B in 0.1% TFA – ethanol.[1]

	RhB-1	RhB-2	RhB-3a	RhB-3b	RhB-4	RhB-5
Absorption peak (nm)	554	540	531	528	519	509
Emission peak (nm)	578	564	556	554	543	531

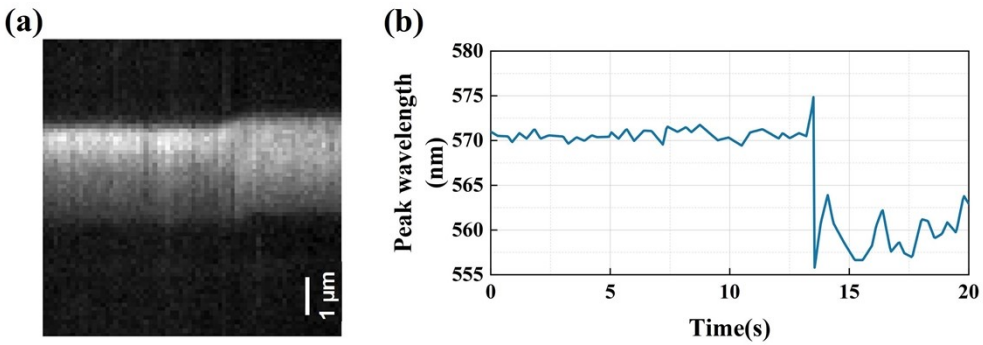

Fig. S2 The blue shift of the emission peak of a single RhB molecule due to the transition to its dealkylated product. (a) Time profile of the blue shift process. (b) The variation of the peak wavelength with time. The peak wavelength shifts from ~ 570 nm to ~ 558 nm after transition at t = 13.5 s.

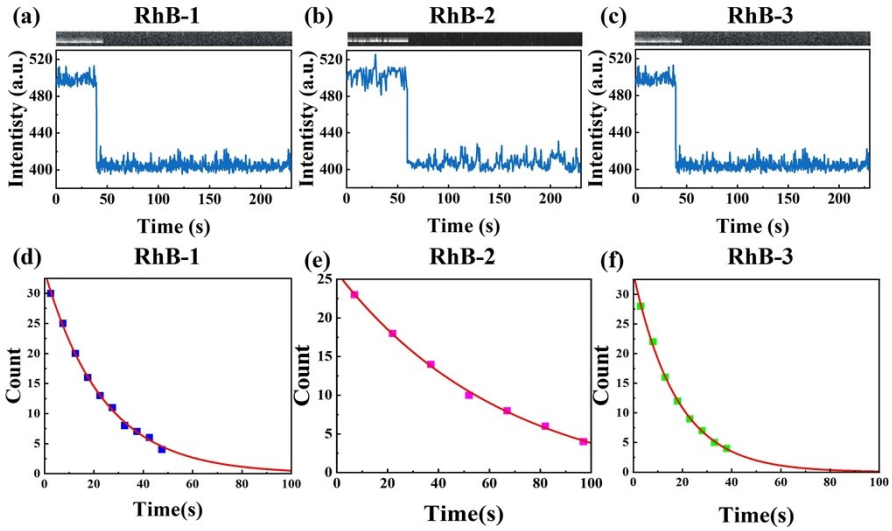

Fig. S3 Single-molecule fluorescence signals and photobleaching lifetimes of three types of rhodamine B molecules in T50 buffer (pH 4.9). (a)-(c) Typical single-molecule fluorescence trajectories measured for RhB-1, RhB-2, and RhB-3 molecules, respectively. (d)-(f) Photobleaching time distributions of RhB-1, RhB-2, and RhB-3 molecules fitted with single exponential functions to generate characteristic photobleaching lifetimes. The photobleaching lifetime of RhB-1 molecules is 32.5±2.1 s, RhB-2 molecules is 66.2±2.5 s, and RhB-3 molecules is 23.6±1.3 s.

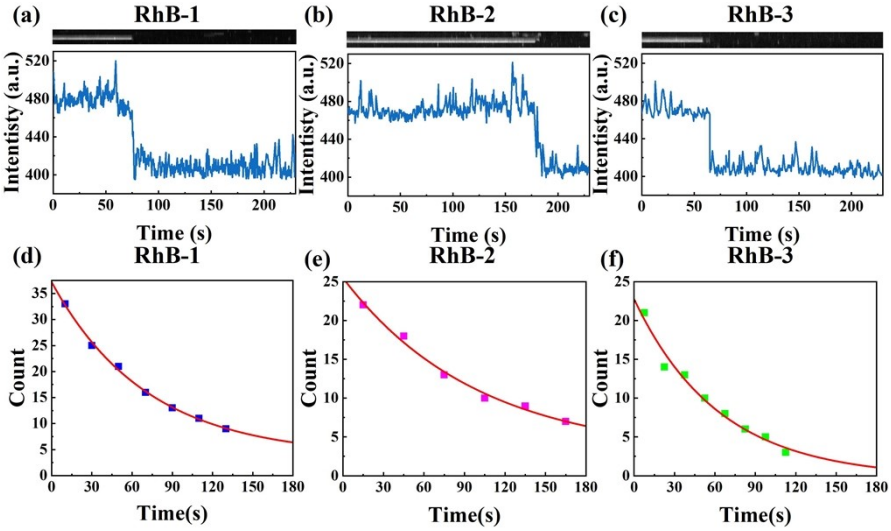

Fig. S4 Single-molecule fluorescence signals and photobleaching lifetimes of three types of rhodamine B molecules in T50 buffer (pH 8.0 with PCA-PCD OSS). (a)-(c) Typical single-molecule fluorescence trajectories measured for RhB-1, RhB-2, and RhB-3 molecules. (d)-(f) Photobleaching time distributions of RhB-1, RhB-2, and RhB-3 molecules fitted with single exponential functions to generate characteristic photobleaching lifetimes. The photobleaching lifetime of RhB-1 molecules is 78.1±3.1 s, RhB-2 molecules is 113.3±4.1 s, and RhB-3 molecules is 62.9±3.3 s.

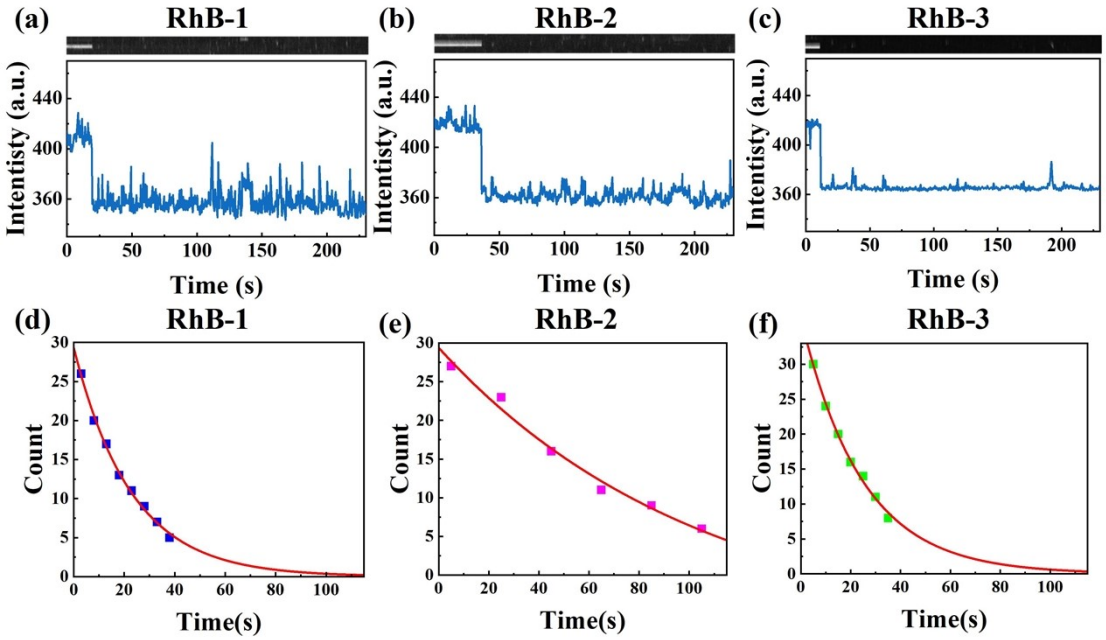

Fig. S5 Single-molecule fluorescence signals and photobleaching lifetimes of three types of rhodamine B molecules in T50 buffer (pH 10.3). (a)-(c) Typical single-molecule fluorescence trajectories measured for RhB-1, RhB-2, and RhB-3 molecules in T50 buffer. (d)-(f) Photobleaching time distributions of RhB-1, RhB-2, and RhB-3 molecules in T50 buffer, fitted with single exponential functions to generate characteristic photobleaching lifetimes. The photobleaching lifetime of RhB-1 molecules is 23.8±1.6 s, RhB-2 molecules is 53.3±1.8 s, and RhB-3 molecules is 18.1±1.5 s.

Table S2 The measured populations of RhB-1, RhB-2, and RhB-3 within the same sample under different T50 buffer conditions (pH 4.9, pH 8.0, pH 10.3).

		T50 (pH 4.9)	T50 (pH 8.0)	T50 (pH 10.3)
Population	RhB-1	42%	43%	44%
	RhB-2	37%	38%	36%
	RhB-3	21%	19%	20%

Computational details

Gaussian 16 program[2] is used for the density functional theory (DFT) calculations[3] in the gas phase geometry optimization where the B3LYP-D3 method[4] is selected using the Gaussian keyword "Empirical Dispersion = GD3" and the C, N, O and H atoms use 6-31G (d,p) basis set.[5] Frequency analysis is performed at the same level to ensure that the optimized geometry is at the minimum point on the potential energy surface without imaginary frequencies. Then the time-dependent DFT (TD-DFT) calculations[6] are preformed to calculate the fluorescent emission wavelengths using the M06 functionals[7] combined with the 6-31+G (d, p) basis set in water solvent with a SMD model.[8]

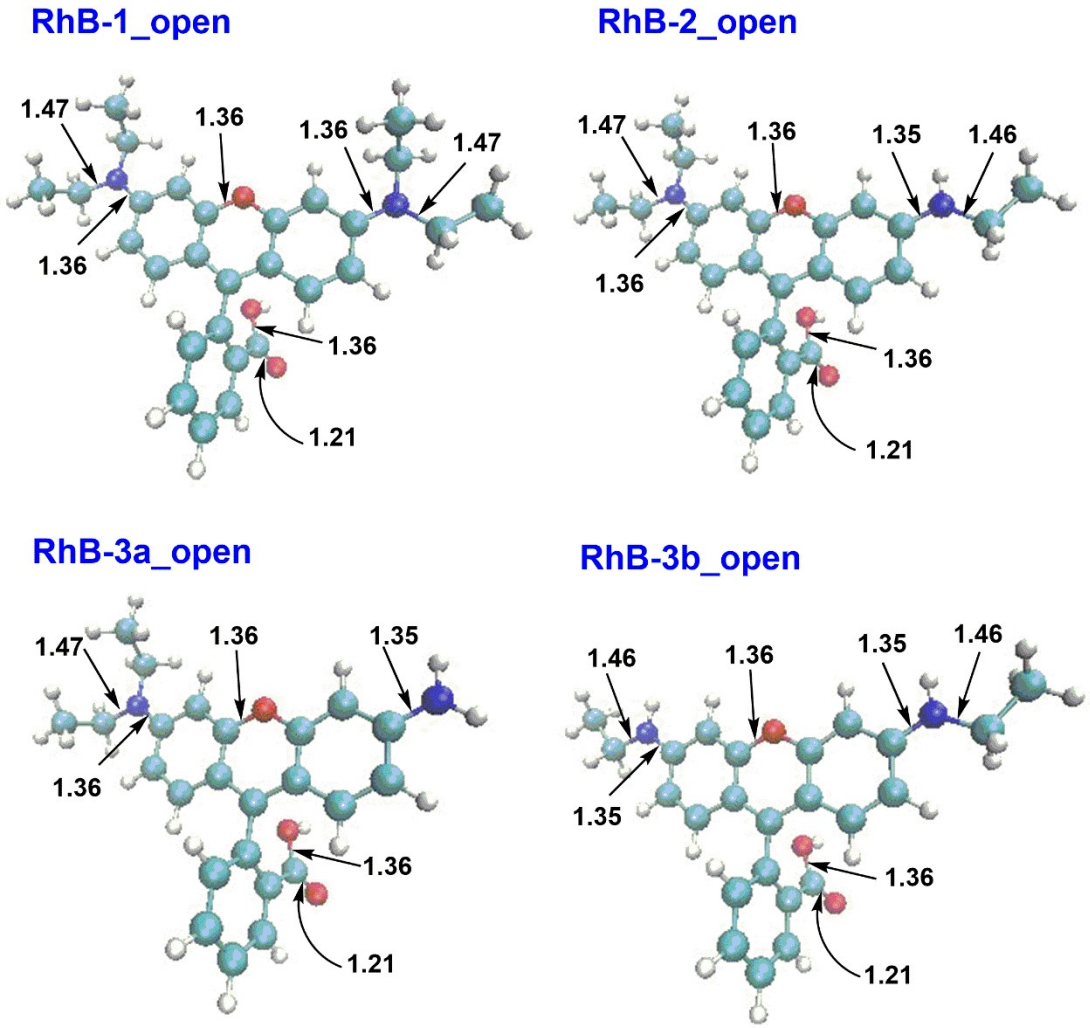

Fig. S6 Fully optimized structures of the "open"/fluorescent form of rhodamine molecules **RhB-1**, **RhB-2**, **RhB-3a**, and **RhB-3b**. (Color scheme: C, cyan; H, white; O, red; N, blue. Distances are given in Å.)

Cartesian coordinates (xyz file) of all molecules

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RhB-1_open (charge=1 multiplicity=1 E= -1263.519283 Hartree)

C	-0.02231200	5.29275000	-1.35492800
C	0.39828900	4.91798100	-0.08213000
C	0.34434200	3.57957200	0.32921200
C	-0.12252500	2.59467200	-0.56832800
C	-0.54657400	2.98621500	-1.84598800
C	-0.50078000	4.32399300	-2.23754900
H	0.02051700	6.33454900	-1.65478600
H	0.77092100	5.65266200	0.62307000
H	-0.89720900	2.22873400	-2.53997000
H	-0.83203400	4.60390500	-3.23248200
C	-0.12251000	1.13637000	-0.26293600
C	0.78291400	3.30719300	1.73253200
O	1.47516900	4.05949400	2.38287200
C	1.08991800	0.41877400	-0.21056600
C	1.06175300	-0.98326100	0.03773200
C	2.37966500	0.99069200	-0.39683200
C	2.19872900	-1.75921500	0.11546000
C	3.52224400	0.24256400	-0.31715500
H	2.45381000	2.05047400	-0.61164900
C	3.48106100	-1.16959800	-0.03752200
H	2.06461000	-2.81647900	0.28965100
H	4.46845600	0.73856600	-0.47611500
C	-1.33382700	0.44229600	-0.08261400
C	-2.62310500	1.04810900	-0.08765000
C	-1.30636100	-0.96278300	0.15479100
C	-3.76463800	0.32084000	0.10353000
H	-2.69174600	2.11803200	-0.24575300
C	-2.44471700	-1.71680300	0.34816300
C	-3.72234000	-1.09972600	0.33680800
H	-4.71281200	0.83884500	0.08498200
H	-2.31670200	-2.77778200	0.50501300
O	-0.12388900	-1.63507700	0.20156400
N	4.62526000	-1.89671600	0.07138300
N	-4.85901100	-1.81036700	0.55621400
C	5.92672000	-1.24622700	-0.21729800
H	6.00695700	-0.35885300	0.41904400
H	5.92468700	-0.89972200	-1.25925100
C	7.16236900	-2.10863700	0.01958800
H	8.04202400	-1.50314100	-0.21524800
H	7.25140700	-2.42463800	1.06247900
H	7.19279400	-2.99164000	-0.62397600

C	-6.19027500	-1.18504200	0.45943400
H	-6.88363100	-1.83137400	1.00154100
H	-6.17966400	-0.23609300	1.00351500
C	-6.67808500	-0.98495600	-0.97896100
H	-6.77940400	-1.94190500	-1.49654400
H	-7.65620500	-0.49496900	-0.97528100
H	-5.98590900	-0.36260500	-1.55270200
C	-4.81443000	-3.26644200	0.76964300
H	-5.73068900	-3.54080400	1.29708700
H	-3.99345000	-3.49653600	1.45545400
C	-4.69064400	-4.08105700	-0.52245700
H	-4.62650600	-5.14708300	-0.28502100
H	-5.55984200	-3.92952500	-1.16698300
H	-3.79760400	-3.80177900	-1.08825100
O	0.30179000	2.14288500	2.23225500
H	0.64072700	2.07655600	3.14135700
C	4.56221300	-3.35656600	0.23875600
H	3.78205300	-3.58886200	0.96790800
H	5.49237300	-3.68609400	0.69749500
C	4.32783800	-4.10207000	-1.07884900
H	3.39448700	-3.78603900	-1.55302800
H	5.14379000	-3.91312700	-1.78244900
H	4.27587900	-5.17991800	-0.89912600

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RhB-2_open (charge=1 multiplicity=1 E= -1263.519283 Hartree)

C	-1.20146700	4.93030200	1.25647900
C	-1.51051200	4.46385300	-0.01768600
C	-1.22814500	3.14229300	-0.38975900
C	-0.63852400	2.26805300	0.54917900
C	-0.32904400	2.75124900	1.82814900
C	-0.60506400	4.07208900	2.18072900
H	-1.42236300	5.95786300	1.52557800
H	-1.97101200	5.11221700	-0.75480500
H	0.11692900	2.07730900	2.55290000
H	-0.35904200	4.42454600	3.17733800
C	-0.37621700	0.82505200	0.28326100
C	-1.57264100	2.77176600	-1.79702400
O	-2.31611100	3.41709800	-2.50328800
C	-1.43880800	-0.10265900	0.27054100
C	-1.15292800	-1.48383800	0.05657900
C	-2.80790100	0.24193600	0.45546200
C	-2.13494600	-2.44984000	0.01685700
C	-3.80080300	-0.70026500	0.41689500
H	-3.06264200	1.28065800	0.63129100

C	-3.48826400	-2.08055800	0.19268100
H	-1.85180100	-3.48350700	-0.14937600
H	-4.83021100	-0.39814500	0.56062000
C	0.93903100	0.36067800	0.10397000
C	2.09464500	1.19389700	0.07907400
C	1.16868300	-1.03128800	-0.09957400
C	3.34847400	0.68460300	-0.11047900
H	1.96606300	2.26131500	0.21560700
C	2.42430400	-1.56791000	-0.28862000
C	3.56655100	-0.72551900	-0.30932700
H	4.18580400	1.36745000	-0.11156600
H	2.49275300	-2.63768900	-0.42080800
O	0.12879500	-1.90978900	-0.11583900
N	-4.45804700	-3.01893400	0.15297700
N	4.81227200	-1.22046800	-0.52402900
C	-5.89190400	-2.77722500	0.31941900
H	-6.23526400	-2.07593200	-0.45175500
H	-6.07093600	-2.30719400	1.29482200
C	-6.66399500	-4.08856000	0.21978000
H	-7.73296900	-3.90269600	0.34520000
H	-6.51743800	-4.55996900	-0.75738500
H	-6.35098400	-4.79051400	0.99932100
C	6.00298400	-0.35186900	-0.49993800
H	6.78642200	-0.87282900	-1.05435100
H	5.79123400	0.55864200	-1.06766100
C	6.49579900	-0.01489000	0.91099100
H	6.79748500	-0.91735300	1.44824200
H	7.36149500	0.65145400	0.85329200
H	5.71872700	0.48331900	1.49739700
C	5.03920700	-2.66876900	-0.66670400
H	5.99612100	-2.79392300	-1.17762000
H	4.28218700	-3.07925500	-1.34215800
C	5.05284600	-3.42880200	0.66404500
H	5.18642700	-4.49870100	0.47895100
H	5.87291100	-3.09021600	1.30200100
H	4.11784400	-3.29059000	1.21353300
H	-4.17515800	-3.97538400	-0.00769900
O	-0.94778400	1.64982300	-2.23139000
H	-1.23950200	1.51521000	-3.14928800

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RhB-3a_open (charge=1 multiplicity=1 E= -1263.519283 Hartree)

C	3.23805800	-3.78472600	1.18961400
C	3.38195100	-3.19241800	-0.06154400
C	2.64950600	-2.04833700	-0.40625700

C	1.76657900	-1.47971300	0.53770000
C	1.62701400	-2.08861900	1.79266100
C	2.35415500	-3.23355600	2.11767300
H	3.80977600	-4.67286800	1.43739100
H	4.05866600	-3.60491300	-0.80161600
H	0.95409100	-1.64751700	2.52133500
H	2.23239400	-3.68724900	3.09607300
C	1.00955600	-0.21725100	0.30450600
C	2.86182600	-1.52902800	-1.79246900
O	3.79796000	-1.84094300	-2.49521800
C	1.67281500	1.02976100	0.34214000
C	0.91566500	2.22251500	0.16012800
C	3.07277000	1.18776400	0.55277300
C	1.48726700	3.48065500	0.17173300
C	3.65871700	2.42419200	0.56566200
H	3.68002400	0.30345600	0.70672800
C	2.87545700	3.60507000	0.37173000
H	0.85643500	4.35043700	0.02725400
H	4.72769900	2.51985500	0.72752400
C	-0.38125600	-0.24505700	0.10914600
C	-1.16554100	-1.43370200	0.03788200
C	-1.08984300	0.98059500	-0.06200600
C	-2.51599300	-1.39615300	-0.16384400
H	-0.66724700	-2.38968000	0.14852100
C	-2.45162500	1.04308000	-0.26377400
C	-3.21963900	-0.14899300	-0.33038300
H	-3.05642500	-2.33105900	-0.20157900
H	-2.89480500	2.02254200	-0.36729800
O	-0.42945500	2.17060600	-0.03169200
N	3.47101200	4.81912300	0.38721700
N	-4.55661100	-0.12224800	-0.55834500
C	-5.36413800	-1.35606400	-0.56675300
H	-6.28044800	-1.13069200	-1.11607100
H	-4.84384200	-2.11830900	-1.15375200
C	-5.70727100	-1.88114200	0.83096700
H	-6.30989700	-1.15829500	1.38618800
H	-6.28057500	-2.80914900	0.74854000
H	-4.80509100	-2.08690900	1.41366500
C	-5.27950900	1.15527700	-0.68571500
H	-6.20513100	0.94631300	-1.22594400
H	-4.70046400	1.82444900	-1.32937900
C	-5.59475000	1.82496500	0.65609200
H	-6.09243400	2.78386800	0.48437100
H	-6.25883600	1.20198000	1.26012200

H	-4.68553500	2.00949800	1.23455100
H	2.94124400	5.66578900	0.25581700
H	4.46385800	4.91122300	0.52989700
O	1.88428000	-0.68844500	-2.21219100
H	2.12283400	-0.42532300	-3.11758900

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RhB-3b_open (charge=1 multiplicity=1 E= -1263.519283 Hartree)

C	0.24270900	4.85858900	-1.14520100
C	0.57344500	4.38772800	0.12214000
C	0.44980700	3.02869100	0.44071500
C	0.00266100	2.12242800	-0.54530400
C	-0.33141100	2.60996800	-1.81656300
C	-0.21558200	3.96725200	-2.11573600
H	0.33923500	5.91495000	-1.37285900
H	0.92826700	5.06096500	0.89458100
H	-0.66739100	1.91278800	-2.57775700
H	-0.47717800	4.32265700	-3.10729000
C	-0.06926000	0.64836900	-0.33769000
C	0.79761700	2.65140100	1.84529400
O	1.45496300	3.34615100	2.58888100
C	1.11033700	-0.12289300	-0.29910800
C	1.01233000	-1.53801600	-0.14222500
C	2.42546800	0.41467300	-0.40397800
C	2.11704100	-2.35896000	-0.08118900
C	3.53753900	-0.38105600	-0.34264200
H	2.54074800	1.48426400	-0.53563200
C	3.41205900	-1.79909900	-0.17561600
H	1.97417800	-3.42742400	0.03817600
H	4.51970300	0.06630700	-0.42555700
C	-1.31572800	0.00022200	-0.24174500
C	-2.57208200	0.67276300	-0.24674500
C	-1.35383600	-1.41997500	-0.09548400
C	-3.75484800	-0.00752900	-0.14041500
H	-2.57916700	1.75282100	-0.33489400
C	-2.53205700	-2.12531000	0.01175200
C	-3.76625200	-1.43531700	-0.01175700
H	-4.68905100	0.53922500	-0.14721600
H	-2.49351000	-3.20389400	0.11845800
O	-0.20300400	-2.14439200	-0.05059500
N	4.50072800	-2.59369800	-0.11351500
N	-4.92556500	-2.11736700	0.09179400
C	-6.26802100	-1.53350800	0.08988400
H	-6.35261000	-0.81422400	0.91433200
H	-6.42352100	-0.98184100	-0.84592600

C	-7.32010900	-2.62764900	0.23737400
H	-7.19330200	-3.17165800	1.17878100
H	-8.31941400	-2.18684600	0.23647500
H	-7.26662900	-3.34096100	-0.59133100
H	4.34793300	-3.58630700	-0.00279600
H	-4.86891100	-3.12220500	0.18157900
C	5.89308600	-2.14965500	-0.19970600
H	6.09448300	-1.42787600	0.60186700
H	6.05232800	-1.63384500	-1.15518700
C	6.83586500	-3.34256900	-0.08203700
H	7.87302100	-3.00624900	-0.14621500
H	6.70687800	-3.85384500	0.87728600
H	6.66509600	-4.06161300	-0.88969200
O	0.27794100	1.45987800	2.23011900
H	0.55881000	1.32589400	3.15151900

References

1. Butkevich, A. N.; Bossi, M. L.; Lukinavičius, G.; Hell, S. W., *J Am Chem Soc **2019**, 141 (2),* 981-989.

2. 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.; MontgomeryJr., J. A.; Peralta, J. E.; Ogliaro, F.; M. Bearpark, Heyd, J. J.; Brothers, E. K.; 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, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; and Fox, D. J. *Gaussian 16, Revision A03;* Gaussian, Inc., Wallingford, CT, **2016**.

3. Hohenberg, P.; Kohn, W., *Phys. Rev.* **1964**, *136*, B864–B871.

4. (a) Becke, A. D., *Phys. Rev. A* **1988**, *38*, 3098–3100. (b) Becke, A. D., *J. Chem. Phys.* **1993**, *98*, 5648–5652. (c) Lee, C.; Yang, W.; Parr, R. G., *Phys. Rev. B* **1988**, *37*, 785–789. (d) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J., *J. Phys. Chem.* **1994**, *98*, 11623–11627. (e) Hertwig, R. H.; Koch, W., *Chem. Phys. Lett.* **1997**, *268*, 345–351. (f) Grimme, S.; Antony, J.; Ehrlich, S.; Krieg, H. *J. Chem. Phys.* **2010**, *132*, 154104/1-20.

5. (a) Krishnan, R.; Binkley, J. S.; Seeger, R.; Pople, J. A., *J. Chem. Phys.* **1980**, *72*, 650–654. (b) Frisch, M. J.; Pople, J. A.; Binkley, J. S., *J. Chem. Phys.* **1984**, *80*, 3265-3269. (c) Lin, X.; Phillips, D. L. *J. Org. Chem.* **2008**, *73*, 3680–3688.

6. Perdew, J. P.; Burke, K.; Ernzerhof, M. *Phys. Rev. Lett.* **1996**, *18*, 3865–3868.

7. (a) Zhao, Y.; Chen, F. *J. Mult. Analy.* **2008**, *99*, 215−231. (b) Zhao, Y.; Truhlar, D. G. *J. Phys. Chem. C* **2008**, *112*, 6860−6868.

8. Marenich, A. V.; Cramer, C. J.; Truhlar, D. G., *J. Phys. Chem. B* **2009**, *113*, 6378−6396.