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Shapes, Dynamics, and Stability of β-Ionone and Its Two Mutants Evidenced by High-Resolution Spectroscopy in the Gas Phase

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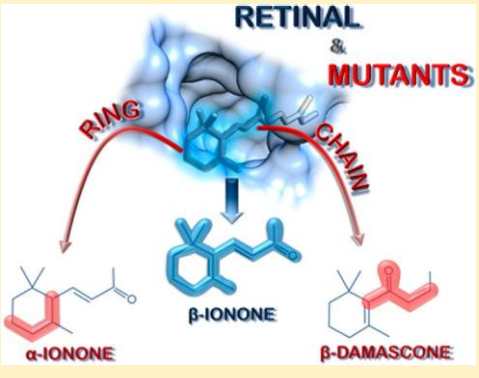
Supporting Information

ABSTRACT: The conformational landscapes of β-ionone and two mutants (α-ionone and β-damascone) have been analyzed by means of state-of-the-art rotational spectroscopy and quantum-chemical calculations. The experiments performed at high resolution and sensitivity have provided a deep insight into their conformational spaces, assigning more than 8000 transitions corresponding to the rotational structures of 54 different species (3 isomers, 14 conformers, and 40 isotopologues). Methyl internal rotation dynamics were also observed and analyzed. The work proved the great flexibility of β-ionone due to its flatter potential energy surface. This feature confers on β-ionone a wider ability to interconvert between conformers with rather similar energies with respect to its mutants, allowing the retinal ligand to better adapt inside the binding pocket.



In the past decade, great interest has been devoted to the conformational analysis of biomolecules. The different factors that govern the stability of peptides, saccharides, nucleic acids, and drugs at a molecular level may be of great importance to the understanding of the mechanisms in which these systems participate in living organisms.[1,2] Structural studies in solution provide ideal media to compare with the biological surroundings. On the contrary, gas-phase studies allow for biomolecules to be probed under isolated conditions, and intrinsic properties of these biosystems can be studied without the interferences of external factors: solvent interactions, crystal packing forces, and so on. Several experimental techniques are available for gas-phase conformational analysis of biomolecules and their clusters. Many of them are based on laser spectroscopy.[2] The most precise approach for gas-phase conformational analysis is the use of rotational spectroscopy in combination with supersonic expansions. The inherently high spectral resolution of this technique allows for the unambiguous identification of isomers,[3] conformers,[4] and isotopologues.[5] Although initially the technique was restricted to rather small molecular systems, recent advances have revolutionized the field and have made it possible to study larger systems[6,7] and also intermolecular complexes.[8−10] The rich conformational landscape of larger biomolecules (15−25 heavy atoms) characterized by several degrees of freedom is nowadays accessible to rotational spectroscopy. The major recent developments achieved by this technique have been further enhanced by the coupling of laser vaporization to microwaves setups, which avoids decomposition processes of biomolecules,[11−14] the application of double-quantum coherence to phase-discriminate the enantiomeric pair of chiral molecules (three-wave mixing),[15] and the Chirp Pulse Fourier Transform (CP-FTMW) technique that has revolutionized the field of microwave spectroscopy.[16,17] Indeed, CP-FTMW instruments allow the recording of broadband rotational spectra in a single acquisition, resulting in shorter spectral acquisition times compared with cavity FTMW experiments. Moreover, millions of rotational spectra can be averaged over time to improve the signal-to-noise ratio.

In this work, the power of the CP-FTMW technique has been exploited recording dense rotational spectra with several thousand lines (more than 8000 lines were assigned), revealing the rotational structure of 54 different species, including isotopologues and methyl internal tunneling states.

This extensive study is aiming at retinal, a very important biochromophore involved in vision processes, which interacts with rhodopsin receptor protein in the form of retinal protonated Schiff-base (RPSB).[18] This linkage is evident from the position of the UV−Vis absorption maximum, which is strongly affected by a subtle interplay of interactions between the protein and the chromophore. In general, there is common agreement that the interactions between the different protein's amino acid residues and the chromophore induce conformational changes in retinal that vary the wavelength absorption

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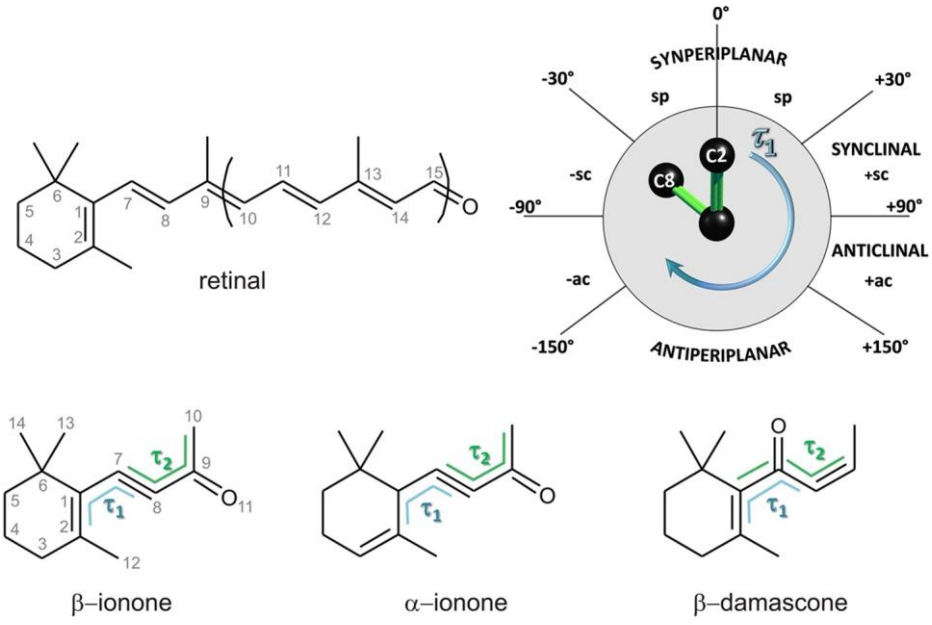
Chart 1. Sketch of the Retinal Structure and Its Analyzed Derivatives, Defining the Corresponding Dihedral Angles (τ_1,τ_2), and the Atom Numbering Used in the Text[a]



[a]IUPAC nomenclature was used. sp, sc, ac, and ap (synperiplanar 0°/±30°, synclinal 30°/90°, anticlinal 90°/150°, and antiperiplanar±150°/180°) forτ_1 angle and C, T (cis/trans~0°/180°) forτ_2 angle. A conformer labelled as−sc/C would indicate aτ_1 (−30°and−90°) andτ_2 with*cis* configuration.

maximum position.[19] However, the details of these interactions are not clear and are still debated in a variety of studies.[20−25]

Some questions arise. Why has nature settled on this particular motif? Might it present biosynthetic advantages? In this work, we seek information about the structures that act as primary factors in nature's choice of retinal in the visual cycle. The main structural features characterizing thisflexible molecule are a six-membered cyclohexene ring and a lateral chain comprising a double bond and a C = O group (see Chart 1). These structural elements have been exhaustively investigated analyzingβ-ionone (β-i), considered to be the simplest prototype of retinal, and two"mutants",α-ionone (α-i) andβ-damascone (β-d), by means of CP-FTMW spectroscopy, supported by theoretical calculations.β-i (the shorter homologue to the biological unit) gives us the opportunity to explore the conformational landscape, the structure, and the main torsions that govern the retinal biomolecule. The two mutants are useful for evaluating the role of these structural elements in comparison with isomerization on the ring (α-i) and on the side chain (β-d). Some previous studies have been performed in the gas phase on ionones, but they did not cover the whole conformational space.[26,27]

It is known that retinal's spectral tuning correlates with the orientation of the alkyl chain with respect to the ring (indicated asτ_1 in Chart 1). In our study, the differentτ_1 arrangements can be explored through the full conformational possibilities of β-i, while the effects of breakingπ-conjugation are analyzed in α-i, where the double bond in the six-membered ring is located in a different position with respect toβ-i, making C_1 hybridized sp^3. The analysis of this feature is interesting because theβ-i moiety in retinal appears quite free to span the whole torsional space at room temperature.[23] The interactions of the retinal methyl regions with the receptor residues are also involved in the switching of the spectral tuning process.[28] Hence,β-d was selected to explore the behavior of the terminal methyl group in position 9 and to probe the effect of the closeness of the carbonyl oxygen on the interaction with the ring methyl groups and thus on the structure. The ability to identify these and other more subtle structural differences, particularly those involving the ring and the side chain, provides a striking illustration of the power of rotational spectroscopy.

The conformational landscape of the three related isomers sharing the sum formula $C_{13}H_{20}O$ can be analyzed systematically with respect to the principal torsions that rule their structural arrangements (see Chart 1):τ_1, the orientation of the alkyl chain with respect to the ring;τ_2, the orientation of the double bond of the alkyl chain with respect to the C_{10} ($\tau_2 \approx 0$ or 180°); and the ring puckering angles, defined by the Cremer−Pople diagram, in which the cyclohexene's minima adopt distorted half-chair configurations (HC, see Figure S4).[29] These three torsions make possible a characterization of the whole conformational space of each compound.

To predict the most stable conformations adopted byβ-i and the two mutants, we calculated the potential energy surfaces (PESs) of each molecule by scanningτ_1 andτ_2. The ring, due to the double bond, assumes only two HC configurations (twist-up and twist-down), with a high inversion barrier close to 20 kJ/mol (see Tables S18−S20), in agreement with data reported in literature.[30] For this reason, the ring was treated as being independent from the side-chain motions. Inβ-i andβ-d, the two positions are equivalent by symmetry ($^5HC_4$ = $^4HC_5$, where the top and bottom numbers identify the atoms that lie above or below the cyclohexene plane, respectively), whereas forα-i, the puckering gives rise to two nonequivalent species ($^5HC_6$ and $^6HC_5$), and hence two PESs were calculated. The PESs are depicted in Figure 1 for the three systems as 2D contour plots. Eight different minima are predicted forα-I (4 $^5HC_6$ and 4 $^6HC_5$), six forβ-i, and four forβ-d, the last with a $^5HC_4$ ring configuration. The labeling chosen for the

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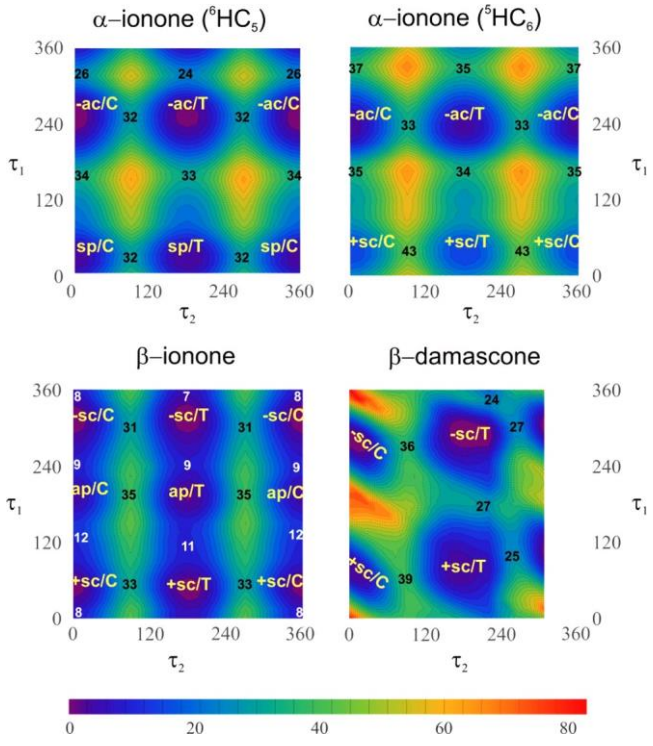

Figure 1.Theoretical 2D conformational PESs (kJ/mol) ofα-ionone, β-ionone, andβ-damascone, exploring theτ 1 (ordinate/degrees) and τ2 (abscissa/degrees) torsions. The numbers reported on the graphics refer to the barrier heights separating the minima. Noβ-damascone data are available forτ 2 > 315°because of the steric hindrance between the carbonyl group and the cyclohexene ring. Note that the energy scale of the four PESs is the same, but each molecule has a different absolute minimum energy offset.

identification of the conformers uses the IUPAC nomenclature (see Chart 1).

The rotational spectra (6−18 GHz) ofβ-i,α-i, andβ-d were collected in three different experiments using the CP-FTMW spectrometer available at the University of the Basque Country.[31] Experimental details are available in the Supporting Information. The three rotational spectra appear very complex and dense, as shown in Figure 2. In the spectrum ofα-i, six

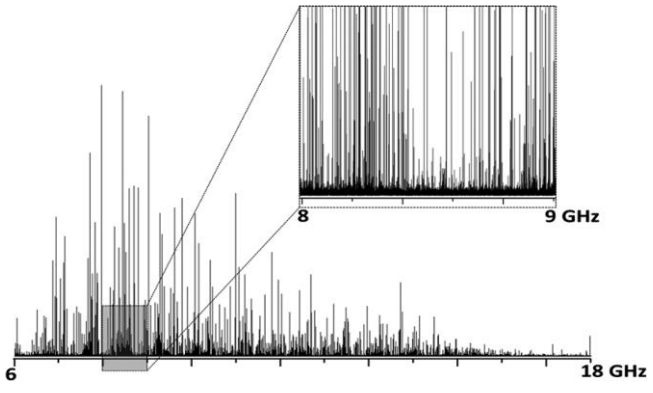

Figure 2.Overview of the CP-FTMW rotational spectrum of β-ionone. The experimental spectrum is the average of 16.25 million accumulated spectra recorded using three nozzles simultaneously. Signal-to-noise ratio of the most intense assigned line ($10_{2,8}$−$9_{2,7}$) is ∼950. The enlarged portion of the spectrum shows the large number of lines present.

different sets of rotational transitions were identified andfit to a semirigid asymmetric rotor Hamiltonian using Watson's S-reduction in the IIIr-representation,[32] yielding the rotational constants*A*,*B*, and*C*reported in Table 1. Subsequent comparison between experimental and predicted values (see Tables S12−S23) proved that the six observed sets of transitions correspond to conformers ac/C and ac/T with both $^5HC_6$ and $^6HC_5$ configurations of the cyclohexene ring and sp/C and sp/T with a $^6HC_5$ configuration of the ring. A similar procedure was used forβ-i andβ-d, where four conformers were identified (sc/C, sc/T, sc/C, and sc/T). Besides these 14 structurally different species, a total of 39 ^{13}C and 1 ^{18}O (α-i) isotopologue were also observed and assigned in natural abundance, illustrating the high sensitivity of the employed technique (see the SI for the isotopologues' results.) The experimental rotational constants, reported in Table 1, validate the calculated values, MP2/6-311++G(d,p) and B3LYP-D3/6-311++G(d,p)[33] (see the complete data set in the SI), showing an excellent agreement. Both methods used are able to characterize the structures with very high precision, giving an average relative error in the rotational constants of δ_{MP2} = 0.15/0.42/0.81% and$\delta_{B3LYP\text{-}D3}$ = 0.48/0.40/0.35% forβ-i,α-i, andβ-d, respectively.

An experimental estimation of the relative energy between the conformers was obtained following established methods for rotational spectroscopy measurements,[34,35] assuming that no conformational relaxation occurs during the supersonic expansion. The estimations show that forβ-i, the two lowest-lying conformers (sc/C and sc/T) are almost isoenergetic. Moreover, the other conformers (sc/C and sc/T) lie also quite close in energy (2.0(9) and 3.9(9) kJ/mol, respectively). For this reason, at room temperature, theβ-i population is distributed over all of them. The two"mutants"display a slightly different behavior with a locked dominant structure, −ac/C forα-i and sc/C forβ-d, and the other conformers lie at much higher energy values. This suggests that forβ-i the population spans a larger number of conformations thanα-i andβ-d, giving greaterflexibility to the system compared with the rigidity of the two mutants (see Tables S1−S3 for the complete relative intensity data).

From the structural point of view, one might think that hyperconjugation inβ-i would induce planarity of the C_2C_1−C_7C_8 fragment. However, the two conformers that approach planarity (ap/T and ap/C) are predicted to lie higher in energy, and hence they are not observed in our rotational spectra. The steric effects between the hydrogen atom attached to C_8 and the hydrogen atoms of the methyl group (C_{12}) apparently prevent these arrangements. Thusτ 1 takes only synclinal orientations ($\tau_1 \approx \pm 52°$), locking this torsion. However,τ 2 takes both values (0 and 180°), and both structures are nearly isoenergetic (see Figure 3). In the mutants, the conformational space changes notably, the most important fact being the observation of a locked dominant structure. Inβ-d the significant difference is in the alkyl chain. Hence, the side chain locksτ 2 instead ofτ 1, and it adopts mainly a*cis* configuration with a preference for a +sc arrangement ofτ 1 with respect to the cyclohexene ring (see Figure 3). On the contrary, inα-i the significant variation is in the ring, obviously causing a change in the puckering of the cyclohexene (see later analysis). However, despite having the same side chain asβ-i,α-i adopts a dominant structure−ac/C that was not observed inβ-i (see Figure 3).

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Table 1. Experimental Rotational Constants (in MHz) and Experimental Methyl Torsional Barriers (in kJ/mol) of the Observed Conformers

	−ac/T $^6HC_5$	−ac/C $^6HC_5$	sp/T $^6HC_5$	sp/C $^6HC_5$	−ac/T $^5HC_6$	−ac/C $^5HC_6$
α-i*A*	1059.1769(7) [a]	1041.5530(5)	980.8933(9)	977.6680(6)	1156.413(1)	1137.4744(9)
B	405.7415(1)	408.06351(7)	450.4025(2)	452.9156(1)	399.7992(1)	402.3243(1)
C	359.6409(1)	363.66898(8)	392.9703(2)	393.5871(1)	341.2269(2)	344.53064(9)
V_3^{chain}	4.3070(9)	5.254(3)	4.30(2)	5.446(7)	4.26(2)	5.276(3)
	−sc/T−sc/C		+sc/T		+sc/C	
β-i*A*	1109.0046(3)		1100.5066(2)	1134.5209(6)	1121.9807(4)	
B	406.02837(8)		408.99572(6)	402.9824(1)	405.98131(9)	
C	338.45550(7)		339.93901(6)	333.3748(1)	335.12030(7)	
V_3^{chain}	4.1064(5)		5.107(2)	4.074(3)	5.231(3)	
β-d*A*	1014.172(2)		860.9633(5)	1059.0938(5)	897.2905(3)	
B	466.4507(6)		541.9859(2)	462.8105(1)	536.2615(1)	
C	401.7351(9)		489.0367(2)	389.8836(2)	472.7732(1)	
V_3^{chain}	6.948(7)		6.950(9)	6.92(8)	7.040(6)	
V_3^{alone}	5.419(2)		6.54(1)	6.49(7)	6.60(1)	

[a]Error in parentheses in units of the last digit.

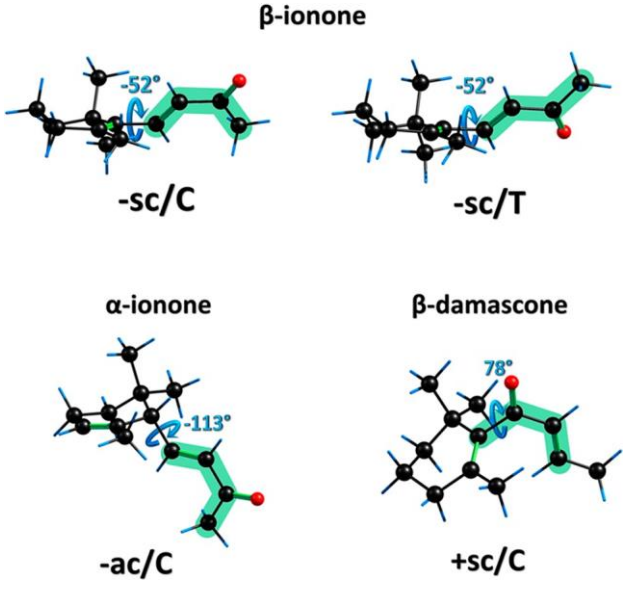

Figure 3.Structures of the observed global minima.β-ionone exhibits two nearly isoenergetic conformations.

To evaluate the changes in the ring puckering properties among the three molecules, we performed a Cremer−Pople analysis,[29] which allows the conformational description of a six-membered ring with three polar coordinates. Each conformation with its corresponding coordinates is located in the spherical visual representation of the Cremer−Pople study in Figure S4. It is clear from the analysis that all three systems share the same structure of the ring□ a distorted HC conformation. Four heavy atoms lie in the same plane, and two heavy atoms stand out of the plane, one atom above and the other below, being on opposite sides of the double bond of the ring. Distinct HC configurations differ in the identity of the atoms that lie out of the plane. All of the conformations thatβ-i andβ-d adopt are located in the same part of the sphere, corresponding to a distorted $^5HC_4$ conformation, equivalent to $^4HC_5$ by symmetry. Inα-i, the mutation was introduced in the ring. Hence,α-i has a different configuration, the symmetry is lost, and the ring adopts distorted $^5HC_6$ and $^6HC_5$ conformations.

Besides very precise structural information, rotational spectroscopy can give insights into the internal dynamics in molecules. For the ionones (α-i andβ-i), spectroscopic signatures of internal motions related to the chain terminal methyl group were observed, as the rotational lines appeared as doublets. On the contrary,β-d presented a more complex pattern due to the internal rotations of the chain terminal methyl group and of the methyl attached to C_2; here the rotational lines appeared as quintuplets. The observed tunneling splittings lead to an experimental determination of the barriers (V_3) for the methyl torsion. TheV_3 methyl barrier is a probe of the electronic and steric environment of the internal rotor. In the present study, the obtained values (shown in Table 1) could be used for a systematic analysis of the local electronic environment in the different molecules and conformers. In particular, the barrier values of the terminal methyl rotors (V_3^{chain}) characterize the conformation of the side chain. In fact, the folded carbon chain arrangements present barriers 25% higher than those of the extended ones (V_3^{cis} ≈6 kJ/mol, V_3^{trans} ≈4 kJ/mol; see Table 1), in which the larger distance to the double bond allows for a more free rotation.β-d reveals a stand-alone behavior; the two detected methyl torsions give rise to similarV_3 values for all four minimum energy conformers (∼7 kJ/mol). The higherV_3 values ofβ-d likely reflect the larger sterical interactions between the carbonyl oxygen and the neighboring methyl regions.

In summary, among the three molecules,β-i appears to be the mostflexible system with a conformational landscape characterized by barriers low enough to be surmounted under biological conditions (see Figure 1). As population is spread over several conformations, this mobility could be the reason why nature choseβ-i to be part of the retinal biochromophore. To compare the conformations detected forβ-i with the structures adopted in the biological environment, a search for the RPSB ligand (RET) was carried out on the Worldwide Protein Data Bank (www.wwpdb.org).[36] The analyzed structures (5FEN, 5F7G, 5FFH, 5F58, 5F6B, 5FAZ)[37] present the retinal alkyl chain oriented in a synclinal arrangement, identical to theβ-i conformations observed under isolated conditions (see Figure 4). The elongation of the side chain

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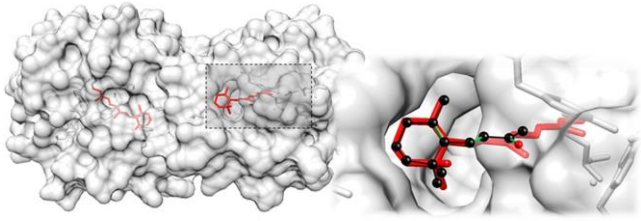

Figure 4.5F6B pdb structure. RET ligand is overlapped with the −sc/Cβ-i conformation.

makes the *trans* configuration the preferred one, maybe also for maximizing the intermolecular interactions. It can be also noted that in some mutated proteins,[19] the RET ligand can be found in an antiperiplanar arrangement (4EXZ, 4EEJ, 4EFG, 4GKC; see Table S45), showing the great flexibility of this moiety to easily overcome the conformational barriers and adopt more fitting structures.

This in-depth gas phase study, supported by comparison between the crystallized and isolated data structures, has revealed that the β-i moiety is the most appropriate to give to the retinal biochromophore the degrees of freedom required to fulfill its biological function during the photoisomerization process.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.8b00256.

(1) Experimental Section: (1.1) Rotational Spectra (Figures S1−S3); (1.2) Relative Energies (Tables S1−S3); (1.3) Experimental versus Ab initio Spectroscopic Parameters (Tables S4−S17). (2) Theoretical Section: (2.1) Cremer−Pople Analysis (Figure S4); (2.2) Ring Inversion Energy Barrier (Tables S18−S20); (2.3) Calculated Spectroscopic Parameters MP2 versus B3LYP-D3 (Tables S21−S23); (2.4) Methyl Internal Rotation Energy Barriers (Tables S24−S26); (2.5) 3D Potential Energy Surfaces (Figure S5); (2.6) Ab initio Geometries (Tables S27−S44). (3) PDB Analysis. (4) Fits of the Observed Conformers and Isotopic Species. (5) References. (PDF)

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Notes

The authors declare no competing financial interest.

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