

# SUBSTITUENTS AT THE C<sub>13</sub> POSITION OF RETINAL AND THEIR INFLUENCE ON THE FUNCTION OF BACTERIORHODOPSIN

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**ABSTRACT** Retinal analogues in which the 13-methyl group is replaced by H, C<sub>2</sub>H<sub>5</sub>, CF<sub>3</sub>, and OCH<sub>3</sub> residues are studied by means of quantumchemical modified neglect of diatomic overlap-correlated version (MNDOC) calculations. The analogues are suitable to test the stereochemical mechanism of proton pumping in bacteriorhodopsin. The results explain the proton-pumping activities of bacterio-opsin reconstituted with these analogues and elucidate the decisive role of retinal's ground-state intramolecular properties in the pump cycle of bacteriorhodopsin.

## INTRODUCTION

Bacteriorhodopsin (BR) acts in the cell membrane of *Halobacterium halobium* as a light-driven proton pump (1). The light energy absorption and vectorial proton translocation involves BRs chromophore retinal (see Fig. 1), which is bound as a protonated Schiff base to the lysine residue 216 and in the light-adapted state absorbs at 568 nm. Upon light excitation BR<sub>568</sub> enters a reaction cycle in which at least five ground state intermediates of the chromophore have been identified spectroscopically. The primary photophysical reaction entails an all-*trans* → 13-*cis* isomerization of retinal. The subsequent dark phase of the cycle is characterized by a transient deprotonation of the Schiff base and by a thermally activated reisomerization of retinal to the all-*trans* geometry and is accompanied by conformational changes in the protein. A thermal isomerization of retinal around the 13–14 bond is also observed during the dark adaptation of light-adapted BR<sub>568</sub> (2, 3). Hence, this bond and its torsional stability in the ground and the excited state appear to be of crucial importance for the function of retinal in BR. To obtain more insight into BRs pump cycle, one may, therefore, replace retinal by derivatives that exhibit altered stereochemical properties of the 13–14 bond. Replacement of retinal's methyl group at C<sub>13</sub> by various other substituents appears to be most promising in this respect. Substituents like H, C<sub>2</sub>H<sub>5</sub>, CF<sub>3</sub>, or OCH<sub>3</sub> will change retinal's intramolecular steric interactions and its electronic charge density in the region of the 13–14 bond and will modify its interaction with the protein environment as well. In fact, a number of experimental investigations used these substituents (X), i.e., X = H in reference 4, X = CF<sub>3</sub> in reference 5, X = C<sub>2</sub>H<sub>5</sub> (Vogel, J., W. Gärtner, K. Mauer, S. Schneider, and D. Oesterhelt, manuscript in preparation)

and X = OCH<sub>3</sub> (Gärtner, W., and D. Oesterhelt, manuscript in preparation).

Retinal is a polyene dye that exhibits the characteristic structure of alternating single and double  $\pi$ -electron bonds. In the ground state a rotation around double bonds is prevented by very high barriers of activation, typically of ~50 kcal/mol. However, an isomerization around the 13–14 double bond is observed in the dark phase of the BR pump cycle on a time scale of milliseconds and during dark adaptation of BR<sub>568</sub> on a time scale of minutes. A strong perturbation of the polyene-bond pattern is a prerequisite for these reactions. In BR and rhodopsin such a perturbation is induced by the protonation of the Schiff-base nitrogen of the chromophore and by a specific distribution of negative charges in the retinal binding site. The effects of protonation and local electric fields are witnessed by the strong bathochromic shift of the absorption maximum from ~360 nm for the unprotonated Schiff base (6) to values ranging between 430 nm (7) and 610 nm (8) for the protonated chromophore inside the protein (9–12). On the basis of quantumchemical modified neglect of diatomic overlap-correlated version (MNDOC) (13) calculations, we have recently demonstrated (14) that the same interactions that induce spectral shifts of the retinal chromophore also alter its ground-state stereochemical properties in very specific ways. It has been shown that the thermal isomerization barriers in the terminal region of retinal close to the Schiff-base nitrogen are particularly sensitive.

Protonation of a retinal Schiff base reduces, for instance, the rotational barrier of the 13–14 double bond from 47 to 11.5 kcal/mol and it increases the isomerization barrier for the neighboring 14–15 single bond from ~5 to values up to 20 kcal/mol (14, 15). Therefore, protonation can make double-bond isomerizations thermally feasible on the millisecond time scale of the BR pump cycle,

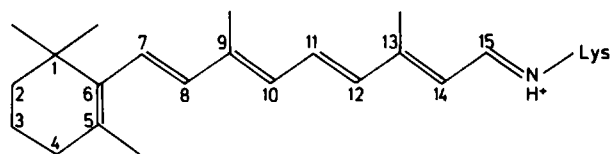


FIGURE 1 The Schiff base of retinal is shown. The chromophore is attached through the terminal nitrogen to a lysine residue of BR.

whereas thermal single-bond isomerizations can be prevented on that time scale. Protonation can also make thermally possible those isomerization processes that involve simultaneously two double bonds. For example, the barrier for an all-*trans*  $\rightarrow$  13,15-di-*cis* isomerization is predicted at 19.5 kcal/mol corresponding to a time scale of minutes for this reaction. Because the 13,15 di-*cis* retinal conformer exhibits only a minimal geometrical difference compared with the light-adapted all-*trans* form, it had been suggested previously (14–16) that the thermal all-*trans*  $\rightarrow$  13-*cis* isomerization observed (2, 3) during dark adaptation of BR<sub>568</sub> should actually comprise an all-*trans*  $\rightarrow$  13,15-di-*cis* reaction. Recently, this conjecture has been verified by nuclear magnetic resonance (NMR) (18) and resonance Raman (19) experiments.

From our calculations (14) it has also become apparent to which extent electrical interactions of a protonated retinal Schiff base (RSBH<sup>+</sup>) with negative charges can modify the magnitude of isomerization barriers. Negative ions near the protonated Schiff-base nitrogen shift barriers in the direction of their conventional polyene values, whereas such ions near the cyclohexene ring have the opposite effect. Positioning, for instance, a negative F<sup>-</sup> ion 3 Å near the proton at the Schiff-base nitrogen produces an all-*trans*  $\rightarrow$  13-*cis* barrier of 25.2 kcal/mol, adding a second ion 3 Å above C<sub>11</sub> lowers the barrier to 10.6 kcal/mol. The latter value is nearly identical to the 11.5 kcal/mol value quoted above for the retinyl cation and suggests that the chromophore structure of a RSBH<sup>+</sup> in the absence of any counterion can model to a certain degree a structure with two counterions in the retinal environment distributed symmetrically at both ends of retinal's polyene chain. In fact, such a two external point charge model has been evoked in reference 17 to explain the shifts of the main absorption maxima of retinal analogues containing shortened chains of conjugated double bonds. However, we prefer in the following the most simple model of BR's chromophore structure that is a RSBH<sup>+</sup> without any counterion. We will also introduce a further simplification and represent the chromophore by the shortened compound shown in Fig. 2, which comprises retinal's polyene part only. Our previous theoretical investigations (14) have shown that this model chromophore provides an excellent description of the properties of the complete chromophore.

Extending our earlier (14) quantumchemical MNDOC (13) studies, we want to demonstrate in this paper how the

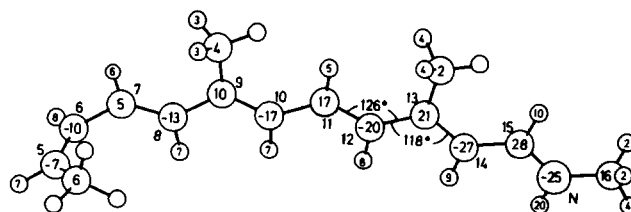


FIGURE 2 The geometry and atomic partial charges of the polyene part of a protonated retinal Schiff base as predicted by the MNDOC method is shown; the charges are given in units of  $10^{-2} e$ ; charges smaller than  $2 \times 10^{-2} e$  are not indicated. The compound in this figure is 13-CH<sub>3</sub>-RET-NH<sub>2</sub><sup>+</sup>. (See footnote 1 for the nomenclature for the various retinal derivatives.)

stereochemistry of a RSBH<sup>+</sup> is altered when the CH<sub>3</sub> group at C<sub>13</sub> is replaced by the residues X = H, C<sub>2</sub>H<sub>5</sub>, CF<sub>3</sub>, and OCH<sub>3</sub> and explain some of the corresponding experimental findings (4, 5; Vogel, J., W. Gärtner, K. Mauer, S. Schneider, and D. Oesterheld, manuscript in preparation, and Gärtner, W., and D. Oesterheld, manuscript in preparation). For this purpose we consider in the Results section how the effects of protonation on equilibrium geometry, charge distribution, and ground-state isomerization barriers of a retinal Schiff base are modified when the 13-methyl group is substituted by the residues X listed above. We will focus the attention on the activation energy for the thermal all-*trans*  $\leftrightarrow$  13-*cis* isomerization. The results are analyzed in the Discussion and a number of the corresponding experimental findings are explained. The paper is concluded by a short summary.

## RESULTS

Table I shows for various substituents X at C<sub>13</sub> the R<sub>13</sub> torsional barriers, i.e., the ground-state activation energies,  $E_a$ , for all-*trans*  $\leftrightarrow$  13-*cis* isomerizations. The data result from MNDOC calculations on the unprotonated and protonated model compounds 13-X-RET-Y.<sup>1</sup> The relative energies  $E_{(t-c)}$  of the all-*trans* and 13-*cis* isomers are also given. Protonation reduces the torsional barrier of the 13–14  $\pi$ -bond from values of  $\sim 50$  kcal/mol, characteristic for a polyene-type double bond, to values between 16.4 kcal/mol for X = H and 4.3 kcal/mol for X = OCH<sub>3</sub>. Thus, our calculations agree with the common observation that the unprotonated retinal derivatives are stable with respect to thermal isomerization. They predict that the ground-state all-*trans*  $\leftrightarrow$  13-*cis* isomerization of the corresponding cations can proceed on time scales between seconds for X = H and nanoseconds for X = OCH<sub>3</sub>. Furthermore, the data in Table I prove the conjecture that the thermal isomerization behavior of the 13–14 bond of a

<sup>1</sup>Throughout this paper the following nomenclature for various retinal derivatives is used: We characterize them as compounds 13-X-RET-Y, where the residues X and Y are appended to the carbon atoms C<sub>13</sub> and C<sub>15</sub>, respectively. The structure called RET of the compound excluding X and Y matches the retinal's polyene part.

TABLE I  
R<sub>13</sub> BARRIERS OF RETINAL DERIVATIVES  
13-X-RET-Y

X	Y	E <sub>a</sub> <sup>*</sup>	E <sub>(t-c)</sub> <sup>‡</sup>
kcal/mol			
H	NH	51.7	-0.9
CH <sub>3</sub>	NH	46.8	0.2
H	NH <sub>2</sub> <sup>+</sup>	16.4	-1.1
CH <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	11.5	1.0
C <sub>2</sub> H <sub>5</sub>	NH <sub>2</sub> <sup>+</sup>	10.3	1.2
CF <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	11.3	2.3
OCH <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	4.3	3.3

MNDOC-BWEN torsional barriers  $E_a$  and relative energies  $E_{(t-c)}$  of *cis* and *trans* isomers for the rotation around the 13-14 bond, which we denote as R<sub>13</sub>, of protonated (Y = NH<sub>2</sub><sup>+</sup>) and unprotonated (Y = NH) model retinal Schiff bases 13-X-RET-Y (see footnote 1 for nomenclature). The computational methods by which these results have been obtained are described in reference 14.

\*The energy  $E_a$  of the conformation twisted by 90° is defined relative to the lower of the two isomers.

‡ $E(\text{trans}) - E(\text{cis})$ .

RSBH<sup>+</sup> depends sensitively on the nature of the substituent X at C<sub>13</sub>.

The strong decrease of the torsional stability of the 13-14 double bond induced by protonation is found to be rather independent of the substituent X. For the two examples given in Table I, the residues X = H and X = CH<sub>3</sub>, this decrease of the activation energy is identical and amounts to an energy difference of 35.3 kcal/mol. The decrease is caused by a partial migration of a  $\pi$ -electron charge to the protonated nitrogen rendering the polyene chain positive. The charge shift is effected through the induction of strong local dipoles between the positively charged odd numbered carbon atoms and the negatively charged even numbered carbon atoms (see Fig. 2). In reference 14 we have shown that the torsional stability of a  $\pi$ -double bond decreases with the strength of the dipole induced by protonation in this bond.

A simple connection exists also between the C—C bond lengths and the magnitude of the isomerization barriers. Table II shows the bond lengths  $L_i$  of the C<sub>*i*</sub>—C<sub>*i+1*</sub> bonds,  $i = 9, \dots, 14$ , obtained for the 13-X-RET-Y molecules. The lengths of the double bonds are collected in Table II A, those of the single bonds in Table II B. The unprotonated compounds (Y = NH) exhibit the characteristic polyene-bond alternation with double-bond lengths of ~1.35 Å and single-bond lengths of ~1.46 Å. In the protonated molecules (Y = NH<sub>2</sub><sup>+</sup>) this bond alternation is much less distinct, a fact that points towards a much more delocalized character of the  $\pi$ -system. The effect of the protonation is strongest in the terminal region of the polyene chain between C<sub>13</sub> and the protonated nitrogen. For most of the protonated compounds the double-bond length  $L_{13}$  is found slightly larger than the single-bond length  $L_{14}$  (see the fifth column of Table II A, B). A comparison of the lengths  $L_{13}$

TABLE II  
C-C BOND LENGTHS OF RETINAL DERIVATIVES  
13-X-RET-Y

A. Double bonds				
X	Y	L <sub>9</sub>	L <sub>11</sub>	L <sub>13</sub>
		Å	Å	Å
H	NH	1.354	1.345	1.342
CH <sub>3</sub>	NH	1.354	1.345	1.354
H	NH <sub>2</sub> <sup>+</sup>	1.374	1.380	1.395
CH <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	1.372	1.376	1.408
C <sub>2</sub> H <sub>5</sub>	NH <sub>2</sub> <sup>+</sup>	1.372	1.376	1.409
CF <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	1.376	1.383	1.403
OCH <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	1.369	1.371	1.431

B. Single bonds				
X	Y	L <sub>10</sub>	L <sub>12</sub>	L <sub>14</sub>
		Å	Å	Å
H	NH	1.456	1.461	1.473
CH <sub>3</sub>	NH	1.461	1.480	1.474
H	NH <sub>2</sub> <sup>+</sup>	1.430	1.411	1.400
CH <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	1.435	1.432	1.399
C <sub>2</sub> H <sub>5</sub>	NH <sub>2</sub> <sup>+</sup>	1.435	1.431	1.399
CF <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	1.430	1.424	1.403
OCH <sub>3</sub>	NH <sub>2</sub> <sup>+</sup>	1.440	1.453	1.384

MNDOC bond lengths  $L_i$ ,  $i = 9, \dots, 14$ , for the model retinal derivatives 13-X-RET-Y.

of the 13-14 double bond with the corresponding activation energies in Table I shows that the height of the isomerization barriers increases almost uniformly with the shortening of the C—C  $\pi$ -bonds.

One can discriminate between three general mechanisms for the effects of substituents at C<sub>13</sub> on the torsional stability of the neighboring 13-14 bond: (a) a large residue can stretch this bond by means of intramolecular steric interactions and, thereby, reduce the isomerization barrier; (b) a highly polar residue can increase or decrease the local dipole by changing the electron density pattern at the polyene backbone and correspondingly weaken or strengthen the bond; and (c) a residue containing lone pair electrons can alter the  $\pi$ -bond by conjugation with the polyene  $\pi$ -system. Depending on the chemical nature of the respective substituent, the variation of the R<sub>13</sub> activation energies exhibited by Table I is caused by a combination of these mechanisms.

The energy difference  $E_{(t-c)}$  of the all-*trans* and 13-*cis* isomers gives a good indication for the relative importance of intramolecular steric interactions (see Table I). Only for the des-methyl compound (X = H) is the all-*trans* state energetically below the 13-*cis* state by ~1 kcal/mol. The reason is the absence of a steric hindrance in the all-*trans* isomer for this derivative, whereas there is a weak repulsion between the hydrogens at C<sub>12</sub> and C<sub>15</sub> in the 13-*cis* configuration. This steric interaction is illustrated in Figs. 3 a and b by means of the C<sub>12</sub>—C<sub>13</sub>—C<sub>14</sub> bond angles. Due to

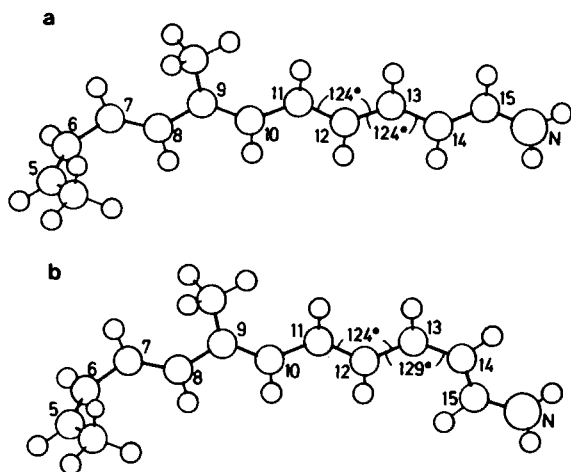


FIGURE 3 The equilibrium geometry of the protonated Schiff-base 13-H-RET-NH<sub>3</sub><sup>+</sup> is drawn to scale as obtained from the MNDOC method; (a) all-*trans*, (b) 13-*cis*.

the repulsion of the two hydrogen atoms, this angle is widened from its all-*trans* value of 124° to a value of 129° in the 13-*cis* isomer. The steric effect of a larger residue at C<sub>13</sub>, however, should be weaker on the 13-*cis* than on the all-*trans* state. This can be inferred by a comparison of Figs. 3 and 4 that show the all-*trans* and 13-*cis* isomers for the 13-desmethyl and 13-methoxy derivatives. Upon introduction of a large residue, the energy of the all-*trans* state is increased relative to that of the 13-*cis* state because in the 13-*cis* state the residue interacts only with the neighboring hydrogen at C<sub>11</sub>, whereas in the all-*trans* state it interacts with two hydrogens, the one at C<sub>11</sub> and the one at C<sub>15</sub>. Due to this interaction, the polyene chain of all-*trans* retinal assumes the well-known (20) banana-type curvature with a compressed bond angle at C<sub>13</sub> and with enlarged angles at C<sub>12</sub> and C<sub>14</sub> (compare Fig. 3 *a* with Figs. 4 *b* and 2). The  $E_{(t-c)}$  values in Table I demonstrate that the increase of intramolecular steric interactions in the all-*trans* state relative to the 13-*cis* state rises in the sequence X = H, CH<sub>3</sub>, C<sub>2</sub>H<sub>5</sub>, CF<sub>3</sub>, OCH<sub>3</sub>. Due to a stretching of the 13–14 bond, the activation energies  $E_a$  decrease and the bond lengths  $L_{13}$  increase when X = H is replaced by more bulky substituents. Bulkiness is defined here as a measure of the lateral extension of the residue in the polyene plane. Hence, one may conclude that increasing the size of the substituent X reduces more and more the barrier for the all-*trans* → 13-*cis* isomerization.

The simple rationalization just introduced is not yet sufficient to understand the data in Table I. For instance, our calculations predict the OCH<sub>3</sub> group to cause the largest steric hindrance in the all-*trans* state although this group is not more voluminous than the C<sub>2</sub>H<sub>5</sub> residue. In the equilibrium geometry the latter substituent minimizes its steric interaction with the polyene chain by sticking out of the polyene plane at a right angle. However, according to our MNDOC description, such a perpendicular orientation is energetically unfavorable for the methoxy residue. The

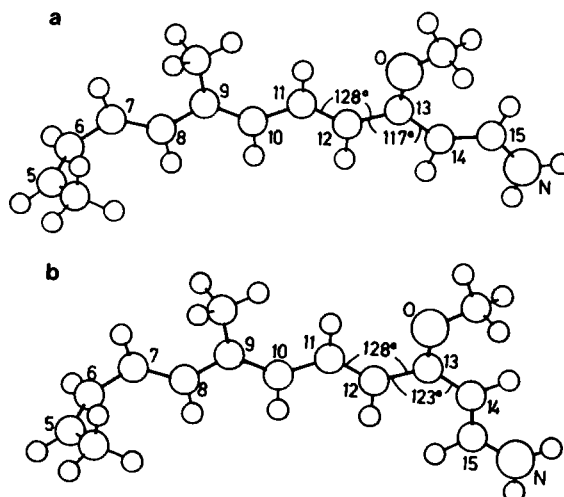


FIGURE 4 MNDOC geometry of 13-OCH<sub>3</sub>-RET-NH<sub>3</sub><sup>+</sup> is shown; (a) all-*trans*, (b) 13-*cis*.

most favorable conformation is the one shown in Fig. 4 *a* in which the OCH<sub>3</sub> group is coplanar with the polyene chain. In this conformation the intramolecular steric interaction attains a maximum value. The corresponding increased nuclear repulsion energy is, however, overcompensated by a gain of  $\pi$ -electron binding energy caused by a conjugation of one of the oxygens lone pairs with retinal's  $\pi$ -system. In the coplanar conformation the oxygen orbitals form an approximate sp<sup>2</sup> hybrid and its doubly occupied p<sub>x</sub>-orbital contributes to two  $\pi$ -molecular orbitals that bind the oxygen atom to the atoms C<sub>11</sub>, C<sub>12</sub>, and C<sub>13</sub> and are anti-bonding in the region between C<sub>13</sub> and C<sub>14</sub>. As a consequence of this added conjugation the electron density increases slightly in the  $\pi$ -system between C<sub>5</sub> and C<sub>12</sub> and the bond alternation becomes more distinct in this region. This is reflected in the data of Table II. Of all protonated compounds the OCH<sub>3</sub> derivative has the shortest double-bond lengths  $L_9$  and  $L_{11}$  and the longest single-bond lengths  $L_{10}$  and  $L_{12}$ . In the terminal region the OCH<sub>3</sub> group produces the opposite effect. The added  $\pi$ -electron conjugation contributes here to the strength of the 14–15 single bond such that  $L_{14}$  assumes its shortest value in the OCH<sub>3</sub> compound.

In the OCH<sub>3</sub> derivative the extreme elongation of  $L_{13}$  to 1.431 Å is explained only in part by the steric repulsion between the CH<sub>3</sub> group and the hydrogen at C<sub>15</sub>. The elongation derives mainly from the increased polarization of the 13–14 bond, which is induced by the oxygen bearing a negative partial charge of  $-16 \times 10^{-2}$  e. The negative charge induces an additional positive partial charge of  $+14 \times 10^{-2}$  e at C<sub>13</sub> and an additional negative partial charge of  $-5 \times 10^{-2}$  e at C<sub>14</sub> as can be seen from Table III. This table shows the changes  $\Delta Q$  of the partial charges at the retinal backbone upon substitution of the 13-methyl group. The extraordinarily low barrier predicted for the all-*trans* → 13-*cis* isomerization of 13-OCH<sub>3</sub>-RET-NH<sub>3</sub><sup>+</sup> can be rationalized mainly through the enhanced local dipole in

TABLE III  
SUBSTITUENT-INDUCED PARTIAL CHARGE  
SHIFTS

Atom	$\Delta Q$				
	$Q$ CH <sub>3</sub>	H	C <sub>2</sub> H <sub>5</sub>	CF <sub>3</sub>	OCH <sub>3</sub>
C <sub>7</sub>	+5	+1	0	+2	-1
C <sub>8</sub>	-13	0	0	-1	0
C <sub>9</sub>	+10	+1	0	+2	-1
C <sub>10</sub>	-17	0	0	0	0
C <sub>11</sub>	+18	+1	0	+1	0
C <sub>12</sub>	-21	0	+1	+1	-1
C <sub>13</sub>	+21	+1	-1	-14	+14
C <sub>14</sub>	-27	0	+1	+2	-5
C <sub>15</sub>	+28	-1	-1	0	-2
N	-23	+1	0	+1	-2

Shifts  $\Delta Q$  of the partial charges  $Q$  at the C-atoms 7–15 and at the terminal nitrogen of 13-X-RET-NH<sub>2</sub><sup>+</sup> upon replacement of the 13-CH<sub>3</sub> group by the residues X = H, C<sub>2</sub>H<sub>5</sub>, CF<sub>3</sub>, and OCH<sub>3</sub>; the partial charges  $Q$  of all-*trans* 13-CH<sub>3</sub>-RET-NH<sub>2</sub><sup>+</sup> are given as a reference; The charge shifts  $\Delta Q$  are defined by  $\Delta Q = Q(\text{CH}_3) - Q(\text{X})$ .

the 13–14 bond. In contrast, the CF<sub>3</sub> substituent reduces this dipole. The carbon atom of this group carries a positive partial charge of  $+48 \times 10^{-2}$  e, attracts electron density towards C<sub>13</sub> and diminishes, therefore, the induced dipole of the 13–14 bond (see Table III). Hence, in this case the increased electron density counteracts the stretching of the bond due to the steric repulsion caused by this rather bulky derivative and leads to the relatively large activation energy of 11.3 kcal/mol for the CF<sub>3</sub> derivative. As shown in Table III, the other substituents at C<sub>13</sub> (H and C<sub>2</sub>H<sub>5</sub>) do not cause significant changes of the partial charge pattern along the retinal backbone such that for these residues the steric interactions appear to be the main source of the predicted variations of the 13–14 isomerization barriers.

To elucidate further the effect of the OCH<sub>3</sub> residue on the adjacent double bond caused by a combination of conjugation, polarization, and steric hindrance, we have also determined the all-*trans*  $\rightleftharpoons$  9-*cis* isomerization barrier for 9-OCH<sub>3</sub>-RET-NH<sub>2</sub><sup>+</sup>. Table IV compares the potential curve for this isomerization with the potential calculated for the compound 9-CH<sub>3</sub>-RET-NH<sub>2</sub><sup>+</sup>. Again, replacement of the methyl by a methoxy group decreases drastically the isomerization barrier of the adjacent bond from a value of 25.6 kcal/mol for the RSBH<sup>+</sup> to 10.4 kcal/mol for the 9-OCH<sub>3</sub> derivative and increases the steric hindrance in the all-*trans* configuration relative to that in the 9-*cis* isomer (compare the  $E_{(t-c)}$  values of the two compounds in Table IV). As in the 13-OCH<sub>3</sub> compound these effects are caused by a coplanar position of the methoxy group leading to a conjugation of its *p*<sub>x</sub>-lone pair with the  $\pi$ -system and by an enhancement of the dipole in the 9–10 double bond.

## DISCUSSION

The data in Table I on the torsional potential curves around the 13–14 bond divide the protonated retinal Schiff

TABLE IV  
R<sub>13</sub> BARRIERS OF RETINAL DERIVATIVES  
9-X-RET-NH<sub>2</sub><sup>+</sup>

X	$E_a^*$	$E_{(t-c)}^*$
	kcal/mol	
CH <sub>3</sub>	25.6	1.5
OCH <sub>3</sub>	10.4	2.8

MNDOC-BWEN torsional barriers  $E_a$  and relative energies  $E_{(t-c)}$  of *cis* and *trans* isomers for the rotation around the 9–10 bond, which we denote as R<sub>9</sub>, of protonated retinal Schiff-bases 9-X-RET-NH<sub>2</sub><sup>+</sup>.

\*The energy  $E_a$  of the conformation twisted by 90° is defined relative to the lower of the two isomers.

† $E_{(trans)} - E_{(cis)}$ .

bases 13-X-RET-NH<sub>2</sub><sup>+</sup> roughly into three classes concerning their thermal isomerization behavior.

The first class is made up of compounds with isomerization barriers very similar to that of retinal and consists of the X = C<sub>2</sub>H<sub>5</sub> and X = CF<sub>3</sub> derivatives. If the intramolecular properties of retinal are decisive for the proton-pumping activities of BR, one can expect that these retinal derivatives, when reconstituted with bacterio-opsin, should exhibit activities close to those of BR. This is, in fact, what has been observed for these two retinal derivatives although the corresponding artificial BR chromophores show quite remarkable spectral shifts in opposite directions. The 13-trifluoromethyl-retinal chromophore absorbs at 624 nm far to the red of BR<sub>568</sub>, whereas the 13-ethyl-retinal chromophore is slightly shifted to the blue absorbing at 560 nm (5; Vogel, J., W. Gärtner, K. Mauer, S. Schneider, and D. Oesterhelt, manuscript in preparation). In spite of these spectral differences, the proton-pumping activity of both derivatives is comparable to that of BR. In the case of the 13-ethyl compound, the photocycle is accelerated because here the M intermediate decays faster (Vogel, J., W. Gärtner, K. Mauer, S. Schneider, and D. Oesterhelt, manuscript in preparation). Because this decay involves a thermal isomerization around the 13–14 double bond, the acceleration is in agreement with our theoretical results that predict a reduction of the R<sub>13</sub> barrier by 1.2 kcal/mol upon ethyl substitution (see Table I).

The second class, characterized by a significantly larger 13–14 isomerization barrier, comprises only the 13-desmethyl derivative. Its calculated R<sub>13</sub> barrier of 16.4 kcal/mol is 4.9 kcal/mol higher than that of retinal and, consequently, the thermal reisomerization of 13-desmethyl-retinal in the pump cycle is predicted to occur on a time scale of seconds instead of the time scale of milliseconds of natural BR. Again, our theoretical description matches the experimental results (4). There are, of course, also observations that cannot be explained on the basis of our quantum-chemical results. Such disagreements may indicate interactions between the protein and the RSBH<sup>+</sup>. An example is the isomeric composition of retinal and 13-desmethyl-retinal in the BR binding site. Dark-adapted BR<sub>548</sub> consists

of roughly 50% all-*trans*, 15-anti retinal, and of 50% of the 13-*cis*, 15-*syn* isomer (18, 19), whereas the energy difference of 1.0 kcal/mol calculated for the isolated all-*trans* and 13-*cis* ground states suggests a 15% all-*trans* and 85% 13-*cis* composition in thermal equilibrium. Similarly, the isomeric composition of 13-desmethyl-retinal in the BR binding site is 15% all-*trans* and 85% 13-*cis* (4) in contrast to our MNDOC result that predicts the reverse ratio. Thus, it becomes clear that the protein-retinal interactions are the main source of the relative stabilization of the all-*trans* and 13-*cis* states inside the protein. Furthermore, the measurements together with our calculations indicate that this stabilization measures not more than ~2 kcal/mol. In view of the small magnitude of these energy values, it cannot be excluded, however, that the disagreements discussed above are in part also due to numerical inaccuracies of our calculations.

The 13-methoxy compound belongs to the third class characterized by a drastically reduced activation energy for isomerization around the 13–14 bond. This reduction is illustrated in Fig. 5, which compares the  $R_{13}$  potential curves of retinal and of the 13-methoxy derivative. Fig. 5 implies, in particular, that the barrier for an all-*trans* → 13-*cis* isomerization is only 1.0 kcal/mol, which corresponds to 1.7 kT at room temperature. Consequently, the thermal equilibration between the two isomers should be completed on a time scale of nanoseconds. Furthermore, the all-*trans* state experiences strong sterical hindrance and is predicted at 3.3 kcal/mol above the 13-*cis* state. A protein-induced stabilization of the all-*trans* configuration relative to the 13-*cis* configuration should not considerably alter this energy difference. One must conclude, therefore, that in thermal equilibrium a (meta-)stable all-*trans* isomer of 13-methoxy-retinal cannot exist in the BR binding site at a noticeable concentration (see Fig. 5). But because only retinal's all-*trans* isomer is capable of pumping protons in BR (21), this conjecture implies that the 13-methoxy derivative cannot perform as an efficient

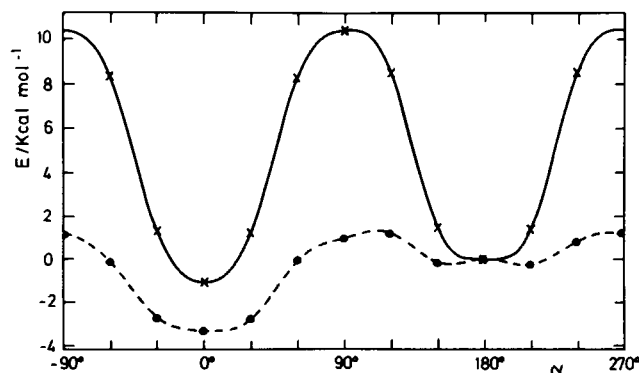


FIGURE 5 The effect of 13-methoxy substitution on  $R_{13}$  isomerization potential curves for the model retinal Schiff-bases 13-X-RET-NH<sub>2</sub><sup>+</sup> is shown; X = CH<sub>3</sub> (—); X = OCH<sub>3</sub> (---); the curves represent a cubic spline interpolation of the numerical values calculated at 30° intervals; all-*trans* is at a torsion angle  $\alpha$  of 180°, 13-*cis* is at 0°.

proton pump. These conclusions have been verified by the observation that 13-methoxy-retinal upon reconstitution with bacterio-opsin forms a chromophore absorbing at 515 nm that, in fact, contains the 13-*cis* isomer<sup>2</sup> and does not exhibit proton-pumping activity.

The explanation of the observations on the artificial BR chromophore reconstituted from 13-methoxy-retinal in terms of the intramolecular properties of the corresponding RSBH<sup>+</sup> is corroborated by chromophore reconstitution experiments with 9-*cis* 9-methoxy-retinal. As is well known, no 9-*cis* isomer of retinal fits into BR's binding site (22). Correspondingly, a mixture of 9-*cis* 9-methoxy-retinal with bacterio-opsin absorbs at 410 nm, a spectral position that indicates that no protonated Schiff-base linkage has been formed. Light-induced isomerization around the 9–10 double bond leads to the formation of a 560-nm chromophore containing the all-*trans* isomer. This chromophore is thermally unstable and decays to the 410-nm mixture containing the 9-*cis* configuration of the derivative (Gärtner, W., and D. Oesterhelt, manuscript in preparation). This behavior is explained by the data in Table IV, which demonstrate that the all-*trans* state of 9-OCH<sub>3</sub>-RET-NH<sub>2</sub><sup>+</sup> is thermally unstable with respect to an all-*trans* → 9-*cis* isomerization in contrast to the properties of the corresponding 9-methyl RSBH<sup>+</sup>.

#### SUMMARY

We have demonstrated on the basis of quantumchemical MNDOC calculations in how far the ground-state intramolecular properties of the protonated retinal Schiff base are decisive for the proton-pumping activity of BR. In particular, the torsional stability of the 13–14 double bond is found to be of cardinal importance. The barrier for the isomerization around this bond is modified upon substitution of the methyl group at C<sub>13</sub> by the residues H, C<sub>2</sub>H<sub>5</sub>, CF<sub>3</sub>, and OCH<sub>3</sub>. The activation energy for a thermal all-*trans* → 13-*cis* isomerization is increased for the 13-desmethyl compound, is found similar to retinal for the CF<sub>3</sub> residue, is slightly smaller for the C<sub>2</sub>H<sub>5</sub> substituent, and is strongly decreased for the 13-methoxy derivative. Depending on the magnitude of the isomerization barrier, BRs proton pump cycle is slowed down, remains unaltered, is accelerated, or is prevented. Our study proves the value of quantumchemical calculations for an understanding of substitution effects and intramolecular chromophore properties on the function of BR.

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<sup>2</sup>Extraction experiments from 13-methoxy-retinal BR revealed the existence of two isomers, 13-*cis* and *g*, 13-di-*cis*. No trace of the all-*trans* isomer could be detected (Gärtner, W., and D. Oesterhelt, manuscript in preparation).

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