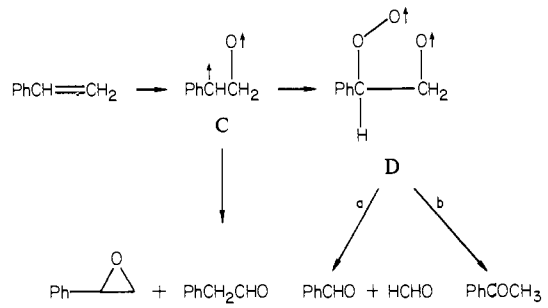
Scheme III



Since *trans*-stilbene (**2**) is a sparingly soluble solid, its oxidation was performed in the powder, resulting in a 15% yield of **4** and **5**, formed in a 2.5:1 ratio. When the oxidation was done in 15% diethylene glycol suspension, these two compounds were formed (25%) in a 1:1 ratio.[10] Triphenylethylene was also oxidized in the solid state, yielding triphenyl ethylene oxide and phenyl benzhydryl ketone in a 4:1 ratio (35% total yield).

Oxidation of *trans*- and *cis*-β-methylstyrene (**6a**) and (**6b**) with $O(^3P)$ atoms produced by O_2 discharge gave after 2 h at −25 °C two epoxides, **7a** and **7b**, and two ketones, **8** and **9**. The product of methyl migration, 2-phenylpropanal, was formed in minute amounts only (<1%). The product ratios (Scheme I) indicated a small preference for O attack on the β-C atom. However, when the source of the O atoms was N_2O/N_2 discharge, this preference was increased (2.5 vs. 1.5 for **6a**).

These differences in the regioselectivity were more distinct in oxidation of styrene. With N_2O/N_2 discharge, the major products were styrene oxide and phenylacetaldehyde while acetophenone and benzaldehyde were formed in small amounts (Table II). The relative abundance of the last two compounds increased when O_2 discharge was used, particularly in neat liquid, suggesting that O_2 also contributes to the formation of these products (Table II).

It is to be emphasized that in all the cases studied no products resulting from oxygen atom attack on the phenyl rings were detected.[11,12]

The suggested pathway for the reaction of $O(^3P)$ atoms with *cis*- and *trans*-stilbenes **1** and **2** involves the intermediacy of triplet diradicals A and B, respectively (Scheme II). The trans-diradical B possesses the geometrical requirements for both cyclization and H migration, giving *trans*-epoxide **4** and the ketone **5** (presumably as a triplet), respectively.[1e] The steric interactions of the phenyl groups in the cis-diradical A lead, in addition, to rotation about the C–C bond forming the *trans*-epoxide **4**.

When the temperature is lowered, the increased viscosity of the liquid slows down the rotation, resulting in a higher relative yield of *cis*-epoxide **3** and lower yields of *trans*-epoxide **4** and benzyl phenyl ketone **5**. It follows that in the solid state the relative yield of **3** will be the highest.

We assume that in the case of styrene, the molecular oxygen present in the O_2 discharge interferes by interaction with the primary diradical C to form peroxy diradical D (Scheme III). The latter undergoes either cleavage of the C–C bond to form benzaldehyde[13] and formaldehyde (path a), or rearranges by H migration with evolution of O_2 to give acetophenone (path b).

Investigations of $O(^3P)$ atom reactions in condensed phases with aromatic and other unsaturated systems are in progress.

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(10) Different products were reported to be formed in the γ radiolysis of *trans*-stilbene in liquid CO_2, ref 3a.

(11) It was claimed, however, that $O(^3P)$ atoms attack both the styrene ring and the styrene side chain: Sloane, T. M.; Brudzynski, R. J. *J. Am. Chem. Soc.* **1979**, *101*, 1495.

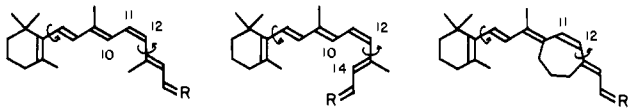
(12) $O(^3P)$ atom reactions with α-methylstyrene, 1,1-diphenylethylene, and 1-phenyl-2-butene resulted in an attack on the double bond only, giving the expected epoxides, ketones, and aldehydes.

(13) The formation of benzaldehyde by ozonolysis is less likely since only negligible amounts of ozone are produced in O_2 discharge: ref 1c and Herron, J. T.; Schiff, H. T. *Can. J. Chem.* **1958**, *36*, 1159. Moreover, we did not detect among the reaction products either styrene ozonide or benzoic acid.

Nonbleachable Rhodopsins Retaining the Full Natural Chromophore

Sir:

The visual pigments rhodopsins[1] consist of 11-*cis*-retinal (I)[2] bound to the ϵ-amino group of the apoprotein lysine moiety through a protonated Schiff base (II, SBH^+).[3,4] The key event



I R:O
II R:N⁺H-lys-OPSIN
III R:N⁺H-n-Bu
IV R:N⁺H-lys-OPSIN
V R:O (s-l)
VI R:N⁺H-lys-OPSIN

in the visual transduction is the photoisomerization of the 11-*cis*-ene to trans geometry,[2] which eventually yields *all-trans*-retinal and opsin ("bleaching"). In this communication, we report the preparation and properties of rhodopsins, e.g., VI, in which this key step is blocked.

We had previously reported that 11,12-dihydrorhodopsin[5] formed from retinal lacking the crucial 11-ene gave a "nonbleachable" pigment.[6] The data on this pigment and other hydrorhodopsins led to the external point-charge model[7] to account for the variance in λ_{max} of visual pigments. In contrast to hydrorhodopsins,[7b] the present pigments retain the full retinal chromophore.

We have shown that of the two possible conformations for 11-*cis*-retinal, 12-*s*-*trans*-II and 12-*s*-*cis*-IV, both of which are nonplanar (indicated by curved arrows) due to the interaction between 10-H/13-Me and 10-H/14-H, the chromophore in rhodopsin adopts the transoid conformer II.[8] In order to mimic the nonplanar 12-*s*-*trans*-retinal, but more importantly to prepare an 11-cis-locked chromophore, we conceived the model retinal V

(1) In view of the recent preparations of various artificial pigments from synthetic model retinals, we propose the use of the generic name "rhodopsin" prefixed by the specific modification carried out with the chromophore, e.g., 11,12-dihydro-9-*cis*-rhodopsin.

(2) Wald, G. *Nature (London)* **1968**, *219*, 800. Hubbard, R.; Kropf, A. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 130.

(3) The protonated Schiff base theory is the one generally accepted, especially on grounds of resonance laser Raman studies: Callender, R.; Honig, B. *Annu. Rev. Biophys. Bioeng.* **1977**, *6*, 33. Eyring, G.; Mathies, R. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 33.

(4) There are, however, still some controversial views regarding the protonated Schiff base theory; see: Favrot, J.; Leclercq, J. M.; Roberge, R.; Sandorfy, C.; Vocelle, D. *Photochem. Photobiol.* **1979**, *29*, 99.

(5) Gawinowicz, M. A.; Balogh-Nair, V.; Sabol, J. S.; Nakanishi, K. *J. Am. Chem. Soc.* **1977**, *99*, 7720, and references cited therein.

(6) The 11,12-dihydrorhodopsin, λ_{max} 315 nm, was not affected by room light, and hence was "nonbleachable" in the classical sense of the terminology. When irradiated with UV light, the pigment underwent irreversible photodecomposition of the chromophore rather than detachment of the chromophore from the opsin.

(7) (a) Nakanishi, K.; Balogh-Nair, V.; Gawinowicz, M. A.; Arnaboldi, M.; Motto, M. G.; Honig, B. *Photochem. Photobiol.* **1979**, *29*, 657. (b) Arnaboldi, M.; Tsujimoto, K.; Balogh-Nair, V.; Nakanishi, K. *J. Am. Chem. Soc.* **1979**, *101*, 7082. (c) Honig, B.; Dinur, V.; Nakanishi, K.; Balogh-Nair, V.; Gawinowicz, M. A.; Arnaboldi, M.; Motto, M. G. *Ibid.* **1979**, *101*, 7084. (d) Sheves, M.; Nakanishi, K.; Honig, B. *Ibid.* **1979**, *101*, 7086.

(8) (a) Chan, W. K.; Nakanishi, K.; Ebrey, T. G.; Honig, B. *J. Am. Chem. Soc.* **1974**, *96*, 3642. (b) Ebrey, T. G.; Govindjee, R.; Honig, B.; Pollock, E.; Chan, W. K.; Crouch, R.; Yudd, A.; Nakanishi, K. *Biochemistry* **1975**, *14*, 3933. (c) Callender, R. H.; Doukas, A.; Crouch, R.; Nakanishi, K. *Ibid.* **1976**, *15*, 1621.

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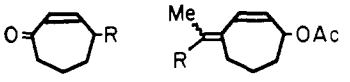
Table I. Absorption and CD Data (in MeOH)

	λ_{max}, nm (ϵ)				
	aldehyde	SB[a]	SBH$^+$[b]	rhodopsin[c]	CD, rhodopsin,[d] λ_{max}, nm (θ)
11-*cis*-retinal	375 (20 000)[e]	350[e]	440[e]	500	340 (+12.6)[f] 490
11-*cis*-**1**	230 (8 000) 265 (9 000) 295 (12 000) 376 (25 000)	355	447	490	330 (+16.9) 488 (+14.3)
11,13-*dicis*-**2**	225 (10 000) 295 (12 000) 371 (22 000)	351	436	488	330 (+15.1) 484 (+15.9)
9,11-*dicis*-**3**	230 (9 000) 295 (11 000) 371 (20 000)	351	441	489	330 (+12.3) 485 (+14.0)
9,11,13-*tricis*-**4**	225 (10 000) 285 (12 000) 366 (16 000)	346	430	483	330 (+12.5) 481 (+14.6)

[a] Schiff base with *n*-BuNH$_2$. [b] Protonated Schiff base with *n*-BuNH$_2$. [c] Data for 2% digitonin, 67 mM phosphate buffer, pH 7.0. [d] Intensities in parentheses represent (θ/A_{max}) × 10^{-3} or ellipticity (in mdeg/absorption). [e] Reference 5. [f] Reference 26.

(**1**). This cycloheptatrienylidene compound is forced into a noncoplanar 9,11,13-triene conformation by virtue of the seven-membered ring, a situation which probably has a direct bearing with the CD of rhodopsin (see below).

We first describe the syntheses of retinals **1**–**4**. Cycloheptenone **5** (5.5 g) was converted into the allyl acetate **6**[9] (4.0 g, 48%) by NBS bromination in CCl_4, treatment with aqueous KOAc under



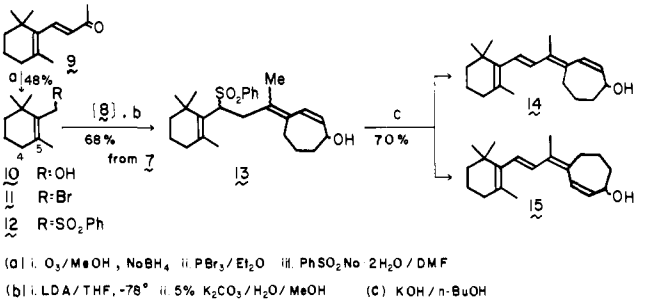
5 R = H **7** R = CHO

6 R = OAc **8** R = CH_2Br

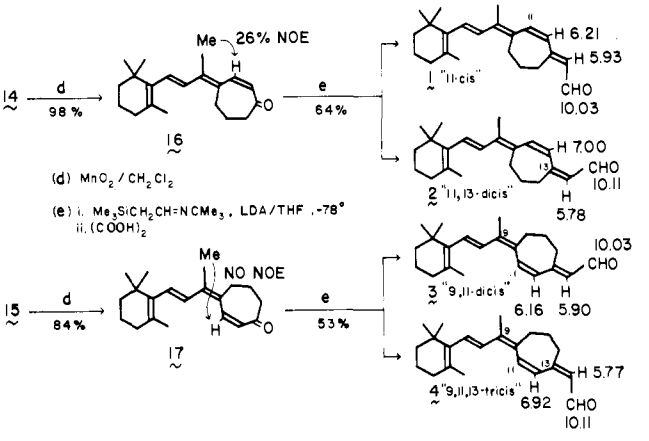
phase-transfer catalytic conditions,[10] and flash chromatography[11] of the crude oil. Reaction of enone acetate **6** (3.4 g) with the anion (LDA) of trimethylsilylated propionaldehyde *tert*-butylimine[12] in THF gave a 1:1 mixture of the two dienals **7** (47%), which could be separated by silica gel chromatography (flash) into the *E* and *Z* isomers having the olefinic proton signals at, respectively, 6.63 and 6.00 ppm (J = 12 Hz) and 7.08 and 5.95 ppm (J = 12 Hz). However, in the actual synthesis, the mixture **7** (104 mg, 0.5 mM) was converted directly into the bromide **8** by $NaBH_4$ reduction followed by bromination with PBr_3 (0.33 molar equiv of reagent in 2 mL of THF, 0 °C, 10 min, under argon). The THF solution of the unstable allyl bromide **8** was immediately treated with 0.5 mM LDA in 1 mL of THF (−78 °C, under argon) in order to neutralize the generated H_3PO_3. This solution of **8** was used directly in subsequent steps.

β-Cyclocitryl alcohol **10** was obtained by ozonolysis[13] of β-ionone **9** (Scheme I) and $NaBH_4$ reduction of the ozonide; treatment with PBr_3 gave the bromide **11**, which was reacted with sodium phenylsulfinate[14] to yield the phenyl sulfone **12**,[15] 48% overall yield from β-ionone. The THF solution of sulfone **12** anion (0.5 mM)[16] was reacted with the above-mentioned THF solution of allyl bromide **8**, and the resulting oily acetate was hydrolyzed with aqueous K_2CO_3 to a ca. 1:1 mixture of the condensed products **13**, 68% overall yield from allyl acetate **7**. Elimination of benzenesulfinic acid from **13** (2.1 g) was carried out with KOH/*n*-BuOH;[17] SiO_2 chromatography of the reaction mixture gave tetraenol **14** [mp 84.5–86 °C (*n*-hexane)] and isomer **15** (oil, total yield 70%).

Scheme I



Scheme II



Oxidation of isomers **14** and **15** gave, respectively, the corresponding tetraenones **16** and **17**, the geometric structures of which were based on measurements of nuclear Overhauser effects (NOE) in the ^1H NMR data (see **16** and **17**) (Scheme II). Tetraenone **16** yielded a mixture of "11-cis"[18] and "11,13-dicis" aldehydes **1** and **2** (64%) when reacted with silylated acetaldehyde *tert*-butylimine;[12] similarly, tetraenone **17** afforded a mixture of "9,11-dicis" and "9,11,13-tricis" aldehydes **3** and **4** (53%). The mixtures **1**/**2** and **3**/**4** were separated into their respective components by high-pressure liquid chromatography (high-pressure LC) (Lichrosorb Si 60, 10 × 250 mm, 10% ether/*n*-hexane). The

(9) The structure of all products was supported by CI-MS (CH_4 carrier gas), IR (CCl_4), UV (MeOH), and ^1H NMR ($CDCl_3$) data.
(10) Toru, T.; Kurozumi, S.; Tanaka, T.; Miura, S.; Kobayashi, K.; Ishimoto, S. *Synthesis* **1974**, 867.
(11) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(12) Corey, E. J.; Enders, D.; Bock, M. G. *Tetrahedron Lett.* **1976**, 7.
(13) Muller, N.; Hoffman, W. *Synthesis* **1975**, 781.
(14) Grieco, P. A.; Masaki, Y. *J. Org. Chem.* **1974**, *39*, 2135.
(15) The sulfone **12** has been synthesized by an alternative method from geraniol, but the synthesis gives a mixture of the 4-ene and 5-ene compounds: Torii, S.; Uneyama, K.; Ishihara, M. *Chem. Lett.* **1975**, 479.
(16) Uneyama, K.; Torii, S. *Chem. Lett.* **1977**, 39.
(17) Manchand, P. S.; Wong, H. S.; Blount, J. F. *J. Org. Chem.* **1978**, *43*, 4769.
(18) The "cis" and "trans" nomenclature refers to the corresponding isomers in regular retinal.

structures of products **1**–**4** are based on the ^1H NMR peaks of 12-H/14-H and observations of NOEs (ca. 40%) between CHO/cisoid CH_2 in **1** and **3** and between CHO/12-H in **2** and **4**.

Bovine opsin was incubated as a suspension with a 5-fold excess of pure chromophores **1**–**4** at pH 7.0 (67 mM phosphate buffer, 25 °C, 20 h in the dark), the suspension was centrifuged, and the pellets were washed five times with hexane at −20 °C to remove excess and decomposed chromophore, and then were solubilized in 2% digitonin.[19,20] As shown in Table I, *all four chromophores yield pigments*. Furthermore, as expected, since the 11-ene is cis locked, the absorption spectra or the orange color of pigment solution did not change when irradiated for 8 h with a 100-W tungsten lamp [>500 nm (filter cutoff)] at 4 °C; namely, *the four pigments are not bleached when exposed to light of wavelengths corresponding to their absorption maxima.*

When the CH_2Cl_2 denaturation–extraction procedure[21] was applied to the 11-*cis*-**1**-derived pigment, in the dark, and also after irradiation with a 100-W tungsten lamp (2 h, 4 °C), the high-pressure LC traces of extracted chromophores were similar; they consisted of 88% of isomer **1** and 12% of a mixture of other isomers. This experiment again shows the nonbleachable nature of the pigment.[22]

The following experiments provide support that the chromophores occupy the same opsin binding site as with natural 11-*cis*-retinal. The hexane-washed pigments were reincubated for a further period with 11-*cis*-retinal, which should bind to the unoccupied binding sites to give rhodopsin, λ_{max} 500 nm. Because of the overlap of the λ_{max} of natural and unnatural rhodopsins, the additional binding was checked by measuring the increase in the 480–500-nm absorption upon reincubation, and subsequent decrease when the pigment was irradiated (only natural rhodopsin is bleached). After 3 h, i.e., when reincubation was complete, the absorbance values showed that reincubation of **1**-, **2**-, **3**-, and **4**-derived pigments produced only 8, 10, 3, and 30%[23] of additional natural rhodopsin.

It is remarkable that in addition to the 11-cis analogue **1**, the 11,13-*dicis*-**2**, 9,11-*dicis*-**3**, and 9,11,13-*tricis*-**4** compounds with 7-membered rings and multiple cis bonds[24] all afford visual pigment analogues. This suggests that the side-chain binding site is rather nondiscriminating as was the case with the ring binding site.[25] The close similarity in the CD curves (Table I) of the four pigments with that of natural rhodopsin, 340 nm (+12.6)/490 nm (+11.7) (in 2% digitonin),[26] should be noted. Since the heptatrienylidene chromophore is constrained into a nonplanar shape, it is likely that a chirally twisted chromophore plays an important role in the CD. Comparisons with the CD spectra of other artificial rhodopsins should clarify the origin of the extrema of natural pigments.[27]

The current studies show that *retinals with fixed 11-cis geometry block the bleaching of visual pigments*; namely, *an 11-cis to 11-trans isomerization is a prerequisite for visual transduction.*

Acknowledgment. We thank the National Institutes of Health (Grant EY 01253) and Hoffmann-La Roche Inc. for financial support. We acknowledge the assistance of John D. Carriker.

(19) Bridges, C. D. B. *Vision Res.* **1977**, *17*, 301.

(20) All four pigments were unstable in Triton X-100 at room temperature in the dark. In 2% digitonin (pH 7.0, 6.7 mM phosphate buffer), only a 10% decrease in λ_{max} intensities was seen when left for 66 h (4 °C) in the dark; in the form of suspension or pellets, the pigments were stable in the dark at 4 °C for at least 3 days. The pigments, however, are not stable to 0.1 M NH_2OH; e.g., with the pigment derived from **1**, half of it was bleached after 30 min, and all had bleached after 24 h in the dark.

(21) (a) Crouch, R.; Purvin, V.; Nakanishi, K.; Ebrey, T. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 1538. (b) Pilkiewicz, F. G.; Pettei, M. J.; Yudd, A. P.; Nakanishi, K. *Exp. Eye Res.* **1979**, *24*, 421.

(22) Bathorhodopsin, the crucial intermediate leading to visual transduction, was not formed from the pigment derived from "11-*cis*"-**1**, as shown by both continuous irradiation at 77 K and flash experiments at 10 °C: Mao, B.; Tsuda, M.; Ebrey, T. G.; Akita, H.; Balogh-Nair, V.; Nakanishi, K., submitted elsewhere for publication.

(23) The binding conditions may not have been optimum for the 9,11,13-tricis chromophore **4**.

(24) Other rhodopsins containing multiple cis bonds in the chromophore are the following. 9,13-Dicis, ref 21. (a) 7,11-Dicis: Kini, A.; Matsumoto, H.; Liu, R. S. H. *J. Am. Chem. Soc.* **1979**, *101*, 5078. 7-Cis, 7,9-dicis, 7,11-dicis, 7,13-dicis, and 7,9,13-tricis: DeGrip, W. J.; Liu, R. S. H.; Ramamurthy, V.; Asato, A. *Nature (London)* **1976**, *262*, 416. Matsumoto, H.; Yoshizawa, T. *Vision Res.* **1978**, *18*, 607.

(25) Nakanishi, K.; Yudd, A. P.; Crouch, R. K.; Olson, G.; Cheung, H.-C.; Govindjee, R.; Ebrey, T. G.; Patel, D. J. *J. Am. Chem. Soc.* **1975**, *98*, 236. Blatchly, R. A.; Carriker, J. D.; Balogh-Nair, V.; Nakanishi, K. *Ibid.* **1980**, *102*, 2495.

(26) Waddell, W. H.; Yudd, A. P.; Nakanishi, K. *J. Am. Chem. Soc.* **1976**, *98*, 238.

(27) Two mechanisms have been proposed. (a) Twisted chromophore: Sperling, W.; Rafferty, C. N. *Nature (London)* **1969**, *224*, 591. Burke, M. J.; Pratt, D. C.; Faulkner, R. R.; Moscowitz, A. *Exp. Eye Res.* **1973**, *17*, 557. Ebrey, T. G.; Yoshizawa, T. *Ibid.* **1973**, *17*, 545. (b) Dipole–dipole interaction: Johnston, E. M.; Zand, R. *Biochem. Biophys. Res. Commun.* **1972**, *47*, 712. Kropf, A.; Whittenberger, P.; Goff, S. P.; Waggoner, A. S. *Exp. Eye Res.* **1973**, *17*, 591. Waggoner, A. S.; Stryer, L. *Biochemistry* **1971**, *10*, 3250.

(28) Fellow in Cancer Research supported by Grant DRG-162F of the Damon Runyon–Walter Winchell Cancer Fund.

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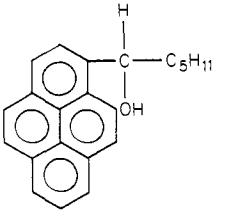
Enhanced Optical Activity Associated with Chiral 1-(1-Hydroxyhexyl)pyrene Excimer Formation

Sir:

Chiroptical techniques are powerful methods for the study of optically active molecules. The relatively new technique of circularly polarized luminescence (CPL) spectroscopy has proved to be exceedingly useful in the study of molecular excited states.[1,2] Although the photophysical processes for excimer formation have been elucidated for some time,[3] investigation of the stereochemistry of optically active excimers has only been initiated recently. Thus, appreciable differences have been found in the kinetic and thermodynamic parameters of excimer formation between the enantiomerically pure *N*-[4-(1-pyrene)butanoyl]-D-tryptophan methyl ester, pyr-D-Trp, and its racemate, *N*-[4-(1-pyrene)butanoyl]-DL-tryptophan methyl ester, pyr-DL-Trp, in methanol and those for pyr-D-Trp between (*R*)-(−)-2-octanol and (*S*)-(+)-2-octanol.[4] In the present work, CPL spectra of (+)-**1**-(1-hydroxyhexyl)pyrene, (+)-**1**, and its enantiomer, (−)-1-(1-hydroxyhexyl)pyrene, (−)-**1**, have been determined in methanol



1(+) or 1(−)

at different concentrations. Dramatically enhanced optical activities associated with the excimer formation of (+)-**1** and (−)-**1** have been observed (eq 1 and 2).

$$(+)\text{-}\mathbf{1} \xrightarrow{h\nu} (+)\text{-}\mathbf{1}^* + (+)\text{-}\mathbf{1} \rightleftharpoons (+)\text{-}\mathbf{1}^*(+)\text{-}\mathbf{1} \quad (1)$$

$$(-)\text{-}\mathbf{1} \xrightarrow{h\nu} (-)\text{-}\mathbf{1}^* + (-)\text{-}\mathbf{1} \rightleftharpoons (-)\text{-}\mathbf{1}^*(-)\text{-}\mathbf{1} \quad (2)$$

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