prepared previously.[7] ^{1}H NMR: δ 5.78 (s, 1 H), 3.43 (dt, J = 4.3, 10.4 Hz, 1 H), 1.44 (s, 3 H), 1.26 (s, 3 H), 1.21 (s, 9 H), 0.90 (d, J = 6.4 Hz, 3 H), and others.

p-Chlorophenyl Oxathianyl Ketone 2 (R = p-ClC$_6$H$_4$). To 50 mg (0.25 mmol) of oxathiane 1 in 1 mL of dry THF was added dropwise 0.36 mL of 1.39 M n-butyllithium in hexanes under N_2 at −78 °C. The solution was stirred for 0.5 h and then allowed to warm to 0 °C; p-chlorobenzonitrile (103 mg, 0.75 mmol) was added. Stirring was continued for 1.5 h at 0 °C, and then 1 mL of 2 N hydrochloric acid was added. After a few minutes the solution was neutralized with saturated aqueous sodium carbonate and extracted twice with ether (10 mL each). The extract was washed with saturated aqueous sodium chloride (5 mL), dried with magnesium sulfate, and concentrated to yield 133 mg of crude products. The ^{1}H NMR spectrum indicated over 90% conversion of starting oxathiane 1. The unreacted p-chlorobenzonitrile was removed by codistillation with 40 mL of water, and the residue was chromatographed using silica gel and 0–2% ethyl acetate in hexanes to give 53 mg (62%) of a light purple crystalline product: mp 95–95.5 °C. Anal. Calcd for $C_{18}H_{23}ClO_2S$: C, 63.79; H, 6.84. Found: C, 63.84; H, 6.87. ^{1}H NMR: δ 8.03 (td, J = 2.2, 8.6 Hz, 2 H), 7.40 (td, J = 2.2, 8.6 Hz, 2 H), 6.12 (s, 1 H), 3.56 (dt, J = 4.3, 10.4 Hz, 1 H), 1.55 (s, 3 H), 1.30 (s, 3 H), 0.92 (d, J = 6.4 Hz, 3 H), and others. ^{13}C NMR: δ 191.8 (C), 174.3 (C), 132.4 (C), 131.0 (CH), 128.6 (CH), 81.2 (CH), 77.6 (CH), 50.5 (CH), 44.7 (C), 41.5 (CH_2), 34.5 (CH_2), 31.4 (CH), 29.3 (CH_3), 24.3 (CH_2), 22.4 (CH_3), 22.0 (CH_3).

Oxathianyl Furyl Ketone 2 (R = 2-C$_4$H$_3$O). By the procedure described above, only 5 mg of the desired ketone was isolated from the reaction of 50 mg of oxathiane 1 and 70 mg of 2-furonitrile: (MH^+) calcd for $C_{16}H_{23}O_3S$ 295.1367, found 295.1364. ^{1}H NMR : δ 7.64 (dd, J = 0.6, 1.6 Hz, 1 H), 7.52 (dd, J = 0.6, 3.6 Hz, 1 H), 6.52 (dd, J = 1.6, 3.6 Hz, 1 H), 5.98 (s, 1 H), 3.53 (dt, J = 4.3, 10.5 Hz, 1 H), 1.51 (s, 3 H), 1.29 (s, 3 H), 0.92 (d, J = 6.3 Hz, 3 H), and others. ^{13}C NMR: δ 181.7, 149.8, 147.3, 121.5, 112.2, 81.2, 77.6, 50.6, 44.5, 41.6, 34.6, 31.5, 29.3, 24.4, 22.5, 22.0.

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Registry No. 1, 79618-03-4; 2 (R = iPr), 92572-77-5; 2 (R = Et), 79563-61-4; 2 (R = Me), 79563-75-0; 2 (R = t-Bu), 92572-79-7; 2 (R = Ph), 89556-31-0; 2 (R = p-ClC$_6$H$_5$), 127645-44-7; i-PrCN, 78-82-0; H_3CCH_2CN, 107-12-0; MeCN, 75-05-8; t-BuCN, 630-18-2; PhCN, 100-47-0; p-ClC$_6$H$_5$CN, 623-03-0; 2-furanonitrile, 617-90-3.

Synthesis of a New Photoactivatable Analogue of 11-*cis*-Retinal

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Introduction

Rhodopsin, an integral membrane protein, functions as a photoreceptor in vertebrate retina. Bovine rhodopsin consists of a single polypeptide chain of 348 amino acids whose sequence is known.[1-3] Hydropathy analysis[1,2] of the protein sequence and proteolysis and monoclonal antibody studies[4,5] suggest that the protein traverses the lipid bilayer seven times as α-helical segments. One model for the secondary structure of the protein is shown in Figure 1. The chromophore, 11-*cis*-retinal, is linked to Lys-296 in helix G as a Schiff base.[6,7] It is important to understand the nature of the chromophore–protein interactions which give rise to characteristic visible absorbance of rhodopsin with the absorption maximum at 500 nm. For this purpose it would be desirable to determine the orientation of retinal within the protein. One approach to this problem is the use of a retinal analogue that carries a photoactivatable group. The use of photoactivatable ligands to study their three-dimensional interactions with the proteins was introduced by Westheimer and his co-workers.[8] Previously, in this laboratory, the photosensitive analogue of retinal, *all-trans*-(*m*-diazirinylphenyl)retinal, has been used to study the orientation of retinal in bacteriorhodopsin, a seven-helical transmembrane protein.[9] On photoactivation, the analogue crosslinked efficiently to specific sites in bacterio-opsin. The crosslinking sites were identified by fragmentation of the labeled protein and sequence analysis of the appropriate fragments.[9]

We have now developed a similar approach to investigate the orientation of retinal in the visual pigment, bovine rhodopsin. Nakanishi and his co-workers have reported the synthesis of the photosensitive analogue, 3-(diazoacetoxy)-9-*cis*-retinal,[10] and used it in the crosslinking

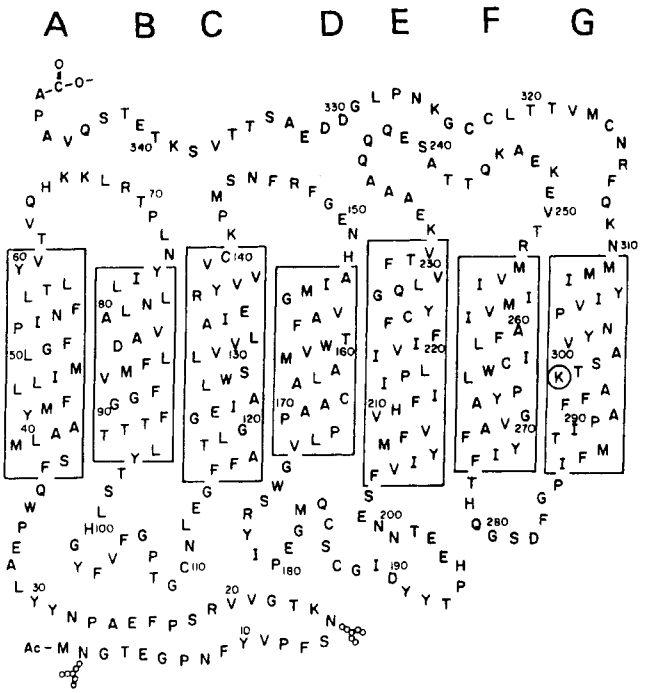

Figure 1. Secondary structure model of bovine rhodopsin (from ref 2). Seven transmembrane helices were designated as A–G. Lysine-296, the attachment site of retinal, is circled. Oligosaccharides (three molecules of each mannose and *N*-acetylglucoseamine) attached to two sites at Asparagine 2 and 15 are indicated by small circles. Amino acids are shown by one-letter codes.

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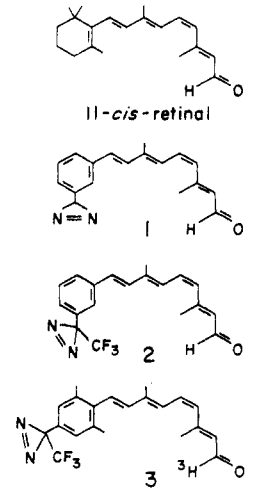

Figure 2. Structures of photoactivatable analogues of 11-*cis*-retinal.

experiment with rhodopsin.[11] While crosslinking with the protein was reported, the sites of crosslinking have not been reported to date. Further, the 9-*cis*-retinal used in the above experiments is an isomer of the natural 11-*cis*-retinal. In addition, diazirines are known to be more reactive in photoactivation than the diazo groups[12] which were used by Nakanishi and colleagues. We have now tested a number of diazirine analogues of 11-*cis*-retinal (1–3, Figure 2) for regeneration of rhodopsin chromophore from the apoprotein. Analogues 1 and 2 did not regenerate rhodopsin whereas analogue 3 regenerated rhodopsin in high yield (~80%). We report here the synthesis of analogue 3 and show its use in efficient regeneration of rhodopsin with the apoprotein, opsin. Photochemical crosslinking studies using rhodopsin regenerated with the retinal analogue 3, and identification of the crosslinking sites are reported elsewhere.[13]

Rationale and the Synthetic Approach

We first prepared 11-cis isomers of photosensitive retinal analogues 1 and 2 (Figure 2). Previously described methods[9,14,15] were used, the all-trans compounds thus obtained were subjected to photoisomerization, and the products were separated by HPLC. The *all-trans*-retinal analogue of 1 was only obtained in low yield; this limited the availability of the corresponding 11-cis isomer (1). All-trans analogue of 2 was obtained in high yield. Attempts for regeneration of rhodopsin with 1 and 2 were unsuccessful. Therefore, analogue 3 with the modified structure was synthesized. The rationale was as follows. Studies using various analogues of retinal have shown that the methyl groups in the phenyl and β-ionone rings are important for the regeneration of rhodopsin unlike bacteriorhodopsin.[16,17] Presumably, the methyl groups introduce a twist in the 6–7 bond, and the ring and the polyene chain become noncoplanar, which is preferred for regeneration of rhodopsin.[16] Further NMR studies using

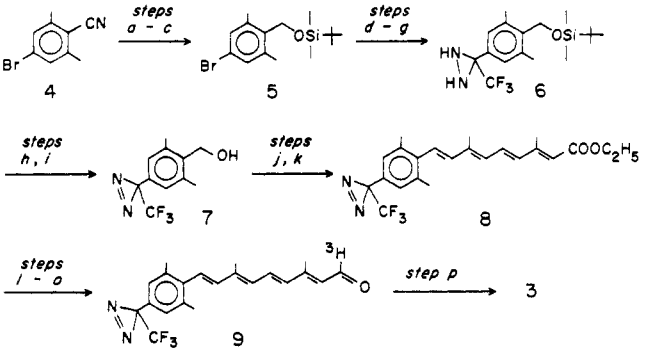

Figure 3. Scheme for the synthesis of the photoactivatable analogue, 11-*cis*-retinal, 3. Reagents and reaction conditions used are: (a) DIBAL, benzene; (b) $LiAlH_4$, THF; (c) $ClSi(Me)_2(t\text{-}Bu)$, DMF; (d) *n*-BuLi, (trifluoroacetyl)piperidene, THF, −40 °C; (e) NH_2OH, pyridine; (f) tosyl chloride, pyridine; (g) NH_3 ether, −40 °C; (h) Ag_2O, ether; (i) HCl, MeOH; (j) pyridinium chlorochromate, CH_2Cl_2; (k) $Br(Ph)_3PCH_2C(CH_3)=CHCH=CHC(CH_3)=CHCOOCH_2CH_5$, THF, (l) DIBAL, ether; (m) MnO_2, CH_2Cl_2; (n) NaB^3H_4, THF–H_2O; (o) MnO_2, CH_2Cl_2; (p) *hν*, >435 nm.

Table I. Summary of Absorption Maxima and Molar Extinction Coefficients of Photoactivatable Retinal Analogues[a]

	all-trans		11-cis	
compound	λ_{max}, nm	ε, cm^{-1} M^{-1}	λ_{max}, nm	ε, cm^{-1} M^{-1}
retinal	383	43 500	380	24 500
analogue 1	390	49 000	385	–
analogue 2	393	32 000	385	20 000
analogue 3	370	46 000	365	22 000

[a] Absorbance was measured in ethanol. ε for 11-cis analogue 1 was not measured since enough material for accurate measurement was not obtained.

^{13}C-labeled retinal shows that the retinal is in the twisted 6-s-cis conformation in rhodopsin,[18] contrary to the planar 6-s-trans conformation observed in bacteriorhodopsin,[19] in agreement with the analogue studies. Thus, two methyl groups in the phenyl ring at positions ortho to the polyene chain were incorporated. In addition, the (trifluoromethyl)diazirine, a highly reactive carbene precursor, was placed at the para position.

The synthetic scheme used in the synthesis of retinal 3 is shown in Figure 3. The starting material was 4-bromo-1-cyano-2,6-dimethylbenzene, 4.[20] (Trifluoromethyl)diazirine was incorporated into the phenyl ring by the methods described by Nassal[14] and Brunner et al.[15] The diaziridine, 6, the precursor of diazirine, was readily formed at −40 °C in ammonia. The yield of the diaziridine 6 was ~90%. The latter was oxidized and deprotected to give 7. The polyene was attached by the Wittig reaction using the C-10 phosphonium bromide in the presence of lithium bis(trimethylsilyl)amide in THF to give 8. The reaction conditions used increased the yield to 63% from ~10% reported previously.[9]

Isomerization of 9 was carried out by the photochemical method described by Zawadski and Ellis.[21] The absorption maximum of 9 when adsorbed onto silica gel suspended in cyclohexane was red-shifted to 400 nm from 365

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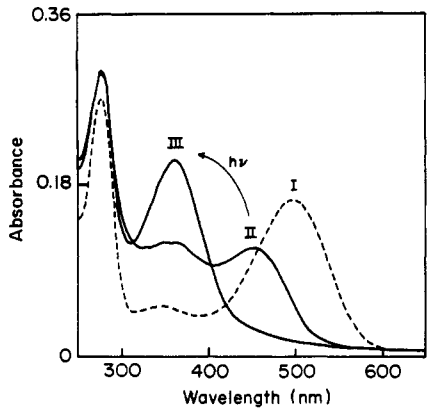

Figure 4. UV/vis spectrum of rhodopsin and rhodopsin analogue. (I) Native 11-*cis*-retinal rhodopsin. (II) Rhodopsin regenerated with the photoactivatable retinal analogue 3. A peak at ~380 nm is unbound excess retinal that could not be removed by washing with bovine serum albumin. (III) Rhodopsin analogue bleached by irradiation with >475-nm light. All spectra were taken in 10 mM Tris·HCl buffer, pH 6.8, containing 1% lauryl maltoside.

nm, its absorption maximum in cyclohexane solution. Irradiation of the suspension at >435 nm led to efficient isomerization to the 11-cis isomer, 3, which was isolated by HPLC. Assignment of major isomers was carried out by ^{1}H NMR analysis as described.[22] The 11-*cis*-retinal analogue, 3, constituted ~30% of the total mixture, and the intactness of the photosensitive diazirine moiety was confirmed by mass spectroscopy. The absorption maxima and the molar extinction coefficients of retinal analogues 1, 2, and 3 are shown in Table I.

Upon incubation of 3 with the apoprotein bovine opsin in rod outer segments, about 80% of opsin regenerated rhodopsin-like chromophore with absorption maximum at 460 nm (Figure 4). The rhodopsin analogue was stable for several weeks at −20 °C in 40% glycerol. The molar extinction coefficient of this rhodopsin analogue was estimated to be 46 000 cm^{-1} M^{-1} by bleaching the rhodopsin analogue.

Experimental Section

General Procedure. All ^{1}H NMR and ^{13}C NMR spectra were recorded at 270 and 400 MHz, respectively. Mass measurements were carried out in the electron-impact mode. Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck Inc.).

4-Bromo-2,6-dimethylbenzyl Dimethyl-*tert*-butylsilyl Ether (5). Diisobutylaluminum hydride (12.5 mmol in hexane) was added to a solution of 4[20] (2.1 g, 10 mmol) in dry benzene (20 mL) at 4 °C. After the mixture was stirred for 1 h at room temperature, 5% sulfuric acid (10 mL) was added at 4 °C. The organic phase was separated, and the product was further extracted with diethyl ether and dried ($MgSO_4$). Purification by flash chromatography with 10% diethyl ether in hexane gave 3.8 g (88%) of 4-bromo-2,6-dimethylbenzaldehyde: ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 6 H), 7.36 (s, 2 H), 10.6 (s, 1 H). The aldehyde (8.5 g, 40 mmol) was added to a suspension of lithium aluminum hydride (1.52 g, 20 mmol) in THF (30 mL) at 4 °C over 10 min. After the mixture was stirred at 4 °C for 1 h, diethyl ether (100 mL) and 1 N NaOH (20 mL) were slowly added to the mixture at 4 °C. The reaction mixture was stirred at room temperature for 10 min, filtered through Celite, and dried ($MgSO_4$). Purification by flash chromatography with 30% diethyl ether in pentane gave 7.5 g (88%) of 4-bromo-2,6-dimethylbenzyl alcohol: ^{1}H NMR ($CDCl_3$) δ 1.56 (br, 1 H), 2.40 (s, 6 H), 4.69 (s, 2 H), 7.20 (s, 2 H).

Imidazole (4.3 g, 63 mmol) and *tert*-butyldimethylsilyl chloride (4.5 g, 30 mmol) in DMF (13 mL) were added to a solution of the preceding benzyl alcohol (5.4 g, 25 mmol) at 4 °C. After the mixture was stirred at room temperature for 15 h, water (250 mL) was added to the reaction mixture at 4 °C. The mixture was extracted five times with 50 mL of diethyl ether. The combined organic phase was washed with brine and dried ($MgSO_4$). Purification by flash chromatography with 5% diethyl ether in pentane gave 10 g (85%) of ether 5: R_f = 0.40 (20% diethyl ether in hexane); ^{1}H NMR ($CDCl_3$) δ 0.1 (s, 6 H), 0.90 (s, 9 H), 2.35 (s, 6 H), 4.63 (s, 2 H), 7.29 (s, 2 H).

2,6-Dimethyl-4-[(trifluoromethyl)diazirinyl]benzyl Alcohol (7). The diazirine moiety was incorporated essentially as described[14,15] for the synthesis of 3-(trifluoromethyl)-3-phenyldiazirine except that the diaziridine (6) formation in ammonia was carried out at −40 °C throughout for 15 h. The diaziridine 6 (2.8 g, 7.8 mmol) was oxidized to diazirine by silver dioxide (7.2 g, 31 mmol), and the protecting group was removed by HCl. Upon removal of silyl alcohol in vacuo, benzyl alcohol 7 was obtained in 93% yield: mp 72–75 °C; R_f = 0.50 (40% diethyl ether in hexane); UV 360 nm; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 1 H), 2.48 (d, 6 H), 4.73 (d, 2 H), 6.85 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 19.36, 58.49, 120.74, 123.49, 126.15, 128.51, 137.94, 138.25.

Retinoic Ester Analogue 8. The benzyl alcohol 7 (1.22 g, 5 mmol) in CH_2Cl_2 (20 mL) was added to a suspension of pyridinium chlorochromate (1.84 g, 8.5 mmol) in CH_2Cl_2 (15 mL) at 4 °C. After being stirred for 2 h at room temperature, the reaction mixture was diluted with 150 mL of diethyl ether and filtered through Celite. Purification by flash chromatography with 10% diethyl ether in pentane gave 1.2 g of the corresponding benzaldehyde: ^{1}H NMR ($CDCl_3$) δ 2.50 (d, 6 H), 6.87 (s, 2 H), 10.02 (s, 1 H). To a suspension of 4.0 g (7.5 mmol) of the C-10 phosphonium bromide in 15 mL of dry THF was added 7.5 mL of 1 M lithium bis(trimethylsilyl)amide (7.5 mmol) in THF at −78 °C. The mixture was warmed up to 4 °C, and 1.2 g (5 mmol) the preceding benzaldehyde in 20 mL of THF was added. After 20 min, 10 mL of saturated aqueous NH_4Cl was added. THF was removed, and crude product was extracted with diethyl ether. The ether solution was washed with brine and dried ($MgSO_4$). Purification by flash column chromatography with 20% diethyl ether in pentane gave a yellow solid ester 8: yield 63%; R_f = 0.40 (50% diethyl ether in hexane); MS m/z 418 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3 H), 2.09 (s, 3 H), 2.32 (s, 6 H), 2.37 (s, 3 H), 5.80 (s, 1 H), 6.23 (d, 1 H), 6.34 (d, 1 H), 6.36 (q, 1 H), 6.40 (d, 1 H), 6.62 (d, 1 H), 6.84 (s, 2 H), 7.00 (q, 1 H).

***all-trans*-[^{3}H]Retinal Analogue 9.** The nonradioactive form of retinal analogue 9 was prepared by standard procedures with diisobutylaluminum hydride and manganese dioxide. Flash column chromatography (10% diethyl ether in pentane) gave a yellow solid: yield 50%; R_f = 0.55 (30% diethyl ether in hexane); mp 60–62 °C; MS m/z 374 (M^+); ^{1}H NMR ($CDCl_3$) δ 2.12 (s, 3 H), 2.32 (s, 6 H), 2.35 (s, 3 H), 6.00 (d, 1 H), 6.27 (d, 1 H), 6.41 (d, 1 H), 6.38 (d, 1 H), 6.70 (d, 1 H), 6.84 (s, 2 H), 7.15 (q, 1 H), 10.13 (d, 1 H); ^{13}C NMR ($CDCl_3$) δ 13.81, 14.32, 20.99, 119.00, 127.55, 128.75, 128.90, 130.59, 130.66, 133.92, 135.89, 135.95, 136.29, 138.00, 139.03, 152.44, 167.10, 194.00. The [^{3}H]-labeled aldehyde 9 was obtained from the preceding nonradioactive analogue by first reducing with NaB^3H_4 (500 mCi/mmol) followed by oxidation as above; yield 60%, specific activity of 9 is 70 mCi/mmol.

11-*cis*-Retinal Analogue 3. Compound 9 was photoisomerized by the procedure described.[21] The *all-trans*-retinal analogue 9 (5 mg) was dissolved in 3 mL of ethyl acetate, and 3 g of silica gel (230–400 mesh) was added. The solvent was removed in vacuo, and the resultant retinal-coated silica was suspended in 3 mL of cyclohexane. This slurry solution was irradiated with a 300-W lamp using a cutoff filter (Schott) at 435 nm for 6–8 min. The retinal analogue was recovered from silica gel by filtering the solution and washing the silica gel with ethyl acetate. The isomers were separated by HPLC (Dynamax-60A silica, 21.4 × 300 mm, Rainin) using 10% diethyl ether/0.08% 2-propanol in hexane. The flow rate was 8 mL/min. Two major peaks in addition to the peak corresponding to the all-trans were found. Each peak was collected, and isomers were characterized by ^{1}H NMR spectroscopy. The peak eluted at 11 min contained 13-cis isomer, and the peak at 12 min contained 11-cis isomer: ^{1}H NMR of 11-cis analogue ($CDCl_3$) δ 2.07 (s, 3 H), 2.31 (s, 6 H), 2.34 (s, 3 H), 6.01 (d, 1 H), 6.06 (d, 1 H), 6.35 (d, 1 H), 6.60 (d, 1 H), 6.66 (d, 1 H), 6.70 (t, $J_{11,12}$ = 11.9 Hz, 1 H), 6.84 (s, 2 H), 10.1 (d, 1 H), HRMS

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m/z calcd for $C_{21}H_{21}O_1N_2F_3$ 374.1607, found 374.1610.

Regeneration of Rhodopsin Analogue. Rod outer segments (ROS) were prepared according to Papermaster and Dryer[23] from frozen bovine retinas. Rhodopsin (20 mg) in ROS was bleached in the presence of 50 mM NH_2OH in 10 mM tris-acetate, pH 7.4, with a 300-W lamp using a cutoff filter of 475 nm. Bleached ROS was washed with 200 mL of 10 mM sodium phosphate, pH 6.5, five times, and incubated with a 3-fold molar excess of [^{3}H]-11-*cis*-retinal analogue **3** in 10 mL of the same buffer in the dark at room temperature overnight. The ROS was washed with 7 mL of 10 mM sodium phosphate, pH 6.5, containing 2% bovine serum albumin, seven times to remove excess unbound analogue (see Figure 4). Approximately 70% of unbound retinal was removed. Regenerated rhodopsin was bleached on illumination.

Acknowledgment. We thank the National Institutes of Health (Grant No. GM28289-09) for financial support, Hoffmann-LaRoche for a gift of the phosphonium bromide, Drs. M. Nassal and T. Sato for very helpful advice on synthesis, Dr. F. D. Greene for comments on the manuscript, and J. Carlin for assistance in preparing this manuscript.

Registry No. **3**, 127685-64-7; **4**, 5757-66-4; **4** aldehyde, 5769-33-5; **5**, 127666-66-4; **5** alcohol, 17100-59-3; **6**, 127666-67-5; **7**, 127666-68-6; **7** aldehyde, 127666-74-4; **7** (*tert*-butyldimethylsilyl ether), 127666-75-5; **8**, 127666-69-7; **9**, 127666-70-0; 4-bromo-2,6-dimethylaniline, 24596-19-8; trifluoroacetylpiperidine, 340-07-8; 3,5-dimethyl-4-[(*tert*-butyldimethylsilyloxy)methyl]-α,α,α-trifluoroacetophenone, 127666-71-1; 3,5-dimethyl-4-[(*tert*-butyldimethylsilyloxy)methyl]-α,α,α-trifluoroacetophenone oxime, 127666-72-2; 3,5-dimethyl-4-[(*tert*-butyldimethylsilyloxy)-methyl]-α,α,α-trifluoroacetophenone tosyloxime, 127666-73-3; (*E,E,E*)-2,6-dimethyl-8-ethoxy-8-oxo-2,4,6-octatrienyltriphenylphosphonium bromide, 75002-35-6.

Supplementary Material Available: Experimental details and ^{1}H NMR spectral data of the intermediates for the synthesis of **3** (3 pages). Ordering information is given on any current masthead page.

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Application of Two-Dimensional NMR Spectroscopy in the Structural Determination of Marine Natural Products. Total Structural Assignment of the Cembranoid Diterpene Eupalmerin Acetate through the Use of Two-Dimensional ^{1}H-^{1}H, ^{1}H-^{13}C, and ^{13}C-^{13}C Chemical Shift Correlation Spectroscopy[1]

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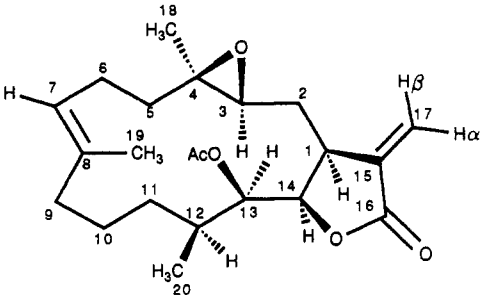
Introduction

Numerous cembranoid diterpenes have been isolated from terrestrial organisms (plants and insects) and especially from marine organisms of the coelenterate phylum.[4] Their carbon skeleton, which is characterized by a 14-membered carbocyclic ring, is the most frequently elaborated by coelenterates. The often dense array of functional groups and stereocenters have made these diterpenoid natural products an attractive target for the natural products chemist.

For the vast majority of cembranoid diterpenes, their structures have been elucidated through the use of either single-crystal X-ray diffraction studies or chemical degradation techniques. However, throughout the last decade, a series of reports have appeared which have described partial or full ^{1}H and ^{13}C NMR spectral assignments in the cembrane series.[5] These studies have helped to establish a data base of sufficient size to be useful in efforts directed at the ^{1}H and ^{13}C NMR based structure elucidation of new cembranoids of unknown structure. However, to the best of our knowledge, only one cembranoid structure has been successfully established using only NMR spectroscopic techniques.[5e] We report here the complete structural assignment of the cembranoid diterpene eupalmerin acetate (**1**), which has been accomplished exclusively on the basis of two-dimensional proton-proton, proton-carbon, and carbon-carbon chemical shift correlation NMR spectroscopy. In the present work, the selection of known cembranolide eupalmerin acetate (**1**) as an appropriate template to probe the effectiveness of modern two-dimensional NMR techniques is based in part on the observation that neither its ^{1}H or ^{13}C NMR spectral data were ever reported following its discovery almost two decades ago.[6] This observation prompted us to initiate a careful and detailed spectral investigation of its molecular structure.



1

Eupalmerin acetate (**1**) was found originally by Ciereszko and co-workers in the Caribbean gorgonian *Eunicea succinea* collected from Florida.[7] Rehm also found it to occur in *Eunicea palmeri* collected near Miami, but not in specimens collected in the Florida Keys.[6] Eupalmerin acetate has also been reported to occur in *Eunicea mammosa* from Puerto Rico.[7] The gross structure of **1** was deduced by Rehm in 1971 primarily on the grounds of

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