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SYNTHESIS OF OPTICALLY ACTIVE 3-DIAZOACETYLRETINALS WITH TRIISOPROPYLPHENYLSULFONYLHYDRAZONE

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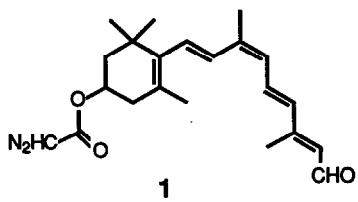
Abstract. An improved synthesis of photoaffinity labeled, optically active retinal derivatives is presented. A stable, easy to handle, glyoxalic acid 2,4,6,-triisopropylphenylsulfonylhydrazone (TIPPS) reacts with 3-hydroxyretinal to give the diazoacetylretinal analog in satisfactory yield.

As part of our continuing studies on retinal proteins[1], we desired to prepare photoaffinity labeled[2] rhodopsins in order to gain experimental data that would be essential in clarifying the tertiary structures of these proteins. The folding pattern of bovine rhodopsin is unknown; in bacteriorhodopsin (bR), despite the fact that electron microscope scattering[3] showed that the transmembrane section consists of 7 α-helices, the folding pattern and location of the retinal chromophore is still very controversial[4,5] despite numerous efforts by neutron diffraction, computation, etc. Khorana and coworkers have successfully carried out photolytic cross-linking studies of bR to completion using the m-diazirinophenyl analog of retinal;[6] however, the involvement of Ser-193 and Glu-194 modified by the photoactivated retinal cannot be reconciled with neutron diffraction results[7].

For these reasons we have been pursuing a different photoaffinity label, namely, the diazoacetyl group, particularly 3-diazoacetoxyretinals[8]. The diazoacetoxyretinals are stable in water, the active carbene intermediate can be readily generated with minimal damage to the protein by 254 nm irradiation (λ_{max} 245 nm) in ice with a medium-pressure tungsten lamp for 20 min, the extent of cross-linking is high (*ca.*30%); furthermore, the labeled bR analog is functional, *i.e.*, it translocates protons[8b]. However, drawbacks in the previous method, in which glyoxalic acid tosylhydrazone[9] was coupled to 3-hydroxyretinal, lay in (i) the handling of the labile (and radioactive) reagent in the presence of unstable retinals, (ii) formation of significant amounts of retinal sulfinate esters which were difficult to separate from the diazoacetoxyretinal, and (iii) low esterification yields of 10-15%.

After many futile attempts, the reagent, glyoxalic acid 2,4,6-triisopropylphenylsulfonylhydrazone **10** was developed. This is very stable and can be stored safely at room temperature for years. Due to the steric bulk of the three isopropyl groups, the side reaction occurs only to a trace extent, and hence the workup and recovery of unreacted hydroxyretinals is greatly facilitated. Even though the yield of esterification is improved only slightly (20%) this is compensated by ready recovery of the retinals. The preparations of

3S-diazoacetoxy-9-*cis*-retinal **1** and 3R-diazoacetoxy-all-*trans*-retinal **2** are described. The visual pigment rhodopsin only binds to one enantiomer[8a,c], which turns out to be the 3S enantiomer. On the other hand, since bR binds equally well to both the 3R and 3S enantiomers[10], separate affinity studies with both enantiomers should lead to self-consistent complementary results.



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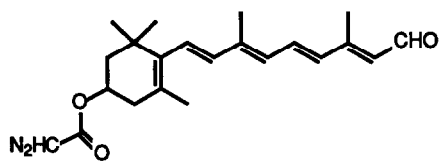

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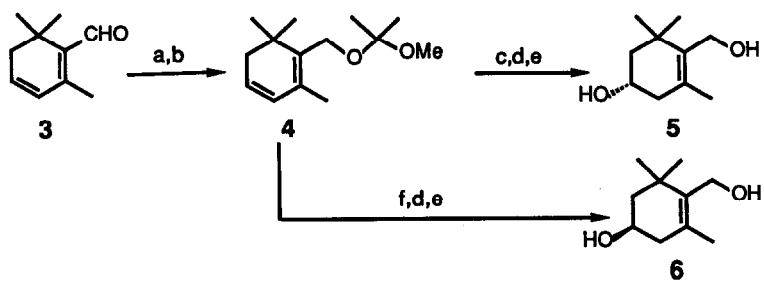
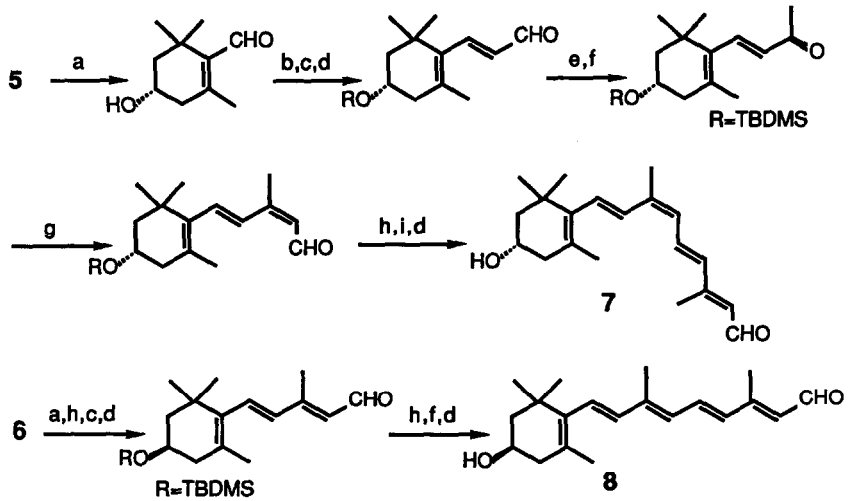
The precursors **5** and **6** of the R- and S-enantiomers of 3-hydroxy retinals were prepared from the readily available safranal **3** by a slight modification of the published method[11]. Sodium borohydride reduction of **3**, followed by protection of the alcohol group gave common intermediate **4** . Acetal **4** was then treated with (-) diisopinocamphenyl borane [(-)-$(IPC)_2BH$][12] to give diol **5**, and with (+)-$(IPC)_2BH$ to give enantiomer **6** in optical yields of 75 % e.e.(52% chemical yield). These compounds were further purified by repeated recrystallization from ethyl acetate to give optically pure forms[13] (Scheme 1).



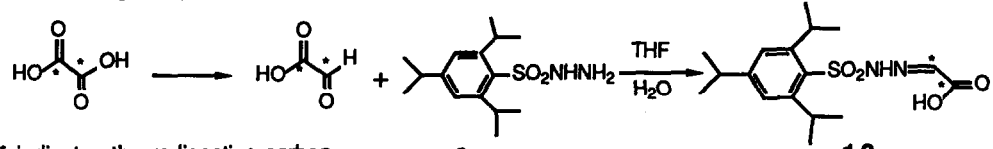
Scheme I. (a) $NaBH_4$, EtOH, 92%; (b) $H_2C(CH_3)COCH_3$, H^+, 98%; (c) (-)-$(IPC)_2BH$; (d) H_2O_2, NaOH; (e) H^+, acetone; (f) (+)-$(IPC)_2BH$

Transformation of **5** to the corresponding 3S-hydroxy-9-*cis* retinal **7**, and **6** to 3R-hydroxy-all-*trans*-retinal **8** was accomplished by known procedures[8,14](Scheme II).

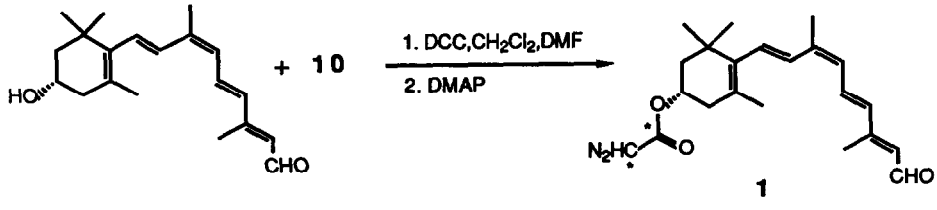


Scheme II,(a) PDC,CH_2Cl_2, dioxane, 50%; (b) $(EtO)_2P(O)CH_2CN$, NaH, 78%; (c) TBDMSCl,DMF, imidazole,95%; (d) DIBAL,70%; (e) CH_3MgBr,-78°,92%; (f) MnO_2, CH_2Cl_2,98%; (g) $TMSCH_2CH(N\text{-}t\text{-}Bu)$, LDA, THF, -78° then H^+, 75%;(h) $(EtO)_2P(O)CH_2C(CH_3)CHCN$, NaH, THF,85%; (i) $(n\text{-}Bu)_4NF$, THF, r.t.,92%.

Triisopropylphenylsulfonylhydrazide **9** was prepared following the method described for the tosylhydrazide[15]. To a chilled solution (-10° C) of triisopropylsulfonyl chloride (3.02 g, 10 mmole) in THF, hydrazine hydrate (1.25 g, 25 mmole) was added dropwise over a period of 30 min. The reaction mixture was warmed to 0° C and stirred an additional 5 hr. Evaporation of the solvent followed by recrystallization (THF: isooctane) gave pure compound as a white powder, m.p. 116-118° C. Preparation of hydrazone**10** followed a route similar to that of glyoxalic acid tosylhydrazone[9]. Glyoxalic acid was generated quantitatively by reducing oxalic acid with sodium amalgam (1.2 %) at pH 1.5- 2 in water and coupled with hydrazide **9** to give hydrazone **10**[16] in 98% yield.



* indicates the radioactive carbon (8,8 mci/mmole)



The following is a typical procedure for the preparation of diazoacetate. A solution of retinal(10 μmole) in 1mL of dry CH_2Cl_2 was added to the solution of hydrazone **10** (10 μmole) in 1mL of 30% DMF in CH_2Cl_2 followed by DCC(10 μmole) in 0.5mL of CH_2Cl_2. Reaction mixture was stirred for 3 h, DMAP(2 mg) was added, and stirring was continued for an additional 30 min. Solvent was removed *in vacuo*, and the residue was purified by prep TLC plate(hexane/EtOAc, 3/1). The band at rf 0.3-0.6 was collected and extracted with ether. Further purification by HPLC (silica column, 4mL/min.,10% EtOAc in hexane l_{max}=380 nm), gave the desired product in 20% yield. ^{14}C-Labeled 3-diazoacetoxyretinals were also prepared by the same procedure using ^{14}C-oxalic acid (8.8 mCi/mmole). Triisopropylphenylsulfonylhydrazone (TIPPS) has greatly facilitated the preparation of practical quantities of radioactive photoaffinity labeled retinals, and has made it feasible to consider mapping the retinal binding site by synthesizing retinal analogs with the diazoacetoxy group at various locations (ongoing).

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10. Unpublished.
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13. Compound **5**: 28% from **3**, m.p. 148° (lit.148-149°); [α]d25 126°(dioxane) [lit.125°(dioxane)].
14. All reactions were carried out under the dim red light. All the compounds were isolated and characterized by ^1H NMR.
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16. Compound **10**: m.p. 154-156°, ^1H NMR(CD_3CN) δ 1.23 (6 Me's); 2.95(m, 1H, CH); 4.10(m, 2H, CH); 7.11 (s, 1H, vinyl); 7.16 (s, 1H, NH); 7.3; (s, 2H, aromatic); 10.23 (br s,1H, COOH).

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