SYNTHESIS OF 11Z-8,18-PROPANO- AND METHANO-RETINALS AND THEIR INTERACTION WITH BOVINE OPSIN

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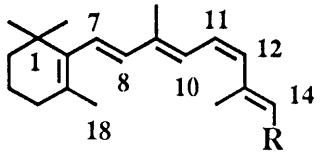
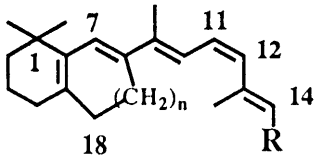
In order to clarify the conformation of chromophore in rhodopsin, especially the torsional angle around the C6-C7 single bond, 8,18-propano- and methano-retinals were prepared from 2,2-dimethylcyclohexanone *via* the palladium-catalyzed coupling reaction of vinyl triflates with methyl *E*-3-trimethylstannyl-2-butenoate, and their interaction with bovine opsin was investigated.

KEY WORDS 8,18-propanoretinal; 8,18-methanoretinal; retinal analog; rhodopsin; opsin shift

In the visual pigment rhodopsin (Rh) **1**, the chromophore 11Z-retinal **2** is bound to the ε–amino group of lysine residue in the apoprotein through a protonated Schiff base (PSB), and exhibits a characteristic circular dichroism (CD) signal at α and β bands in the visible and near-UV region.[1] In the course of studies of a photobleaching process of Rh, very recently, we synthesized 11Z-8,18-ethanoretinal **3**, in which C8 and C18 positions of **2** are connected by an ethylene group, and found that the torsional angle around the 6-7 single bond in Rh is very close to that of 8,18-ethanoretinal **3**.[2] In this paper, we describe the synthesis of 11Z-8,18-propano- and methano-retinals **4** and **5**, which have different methylene groups from **3**, and their interaction with bovine opsin in order to clarify the influence of change in the torsional angle around the 6-7 single bond.





1: R=N$^+$H-opsin
2: R=CHO

3: n=2
4: n=3
5: n=1

The bicyclic ketone **8**,[3] prepared from 2,2-dimethylcyclohexanone **6** *via* Dieckmann condensation of **7** in the same manner as that described for the preparation of **3**[2] in eight steps, was converted to the vinyl triflate **9**. Treatment of **9** with methyl *E*-3-trimethylstannyl-2-butenoate[4] in the presence of palladium diacetate[5] afforded the coupling product **10**[3] and the recovered bicyclic ketone **8** (28%). The structure of **10** was confirmed from the NOE experiment in its NMR spectra. The transformation of **10** to the Wittig salt **11** was accomplished by the sequence of $LiAlH_4$ reduction and treatment with

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triphenylphosphine hydrobromide. The Wittig reaction of **11** with methyl *E*-3-formylcrotonate was achieved using NaOMe as a base to give the ester **12** as an isomeric mixture of double bond. Without isolation of this mixture, the final transformation of **12** to the corresponding aldehyde was achieved according to the usual method by $LiAlH_4$ reduction and MnO_2 oxidation, and 11Z-isomer **4**[3,6] was isolated in pure form by repeating preparative HPLC in the dark (Chart 1).

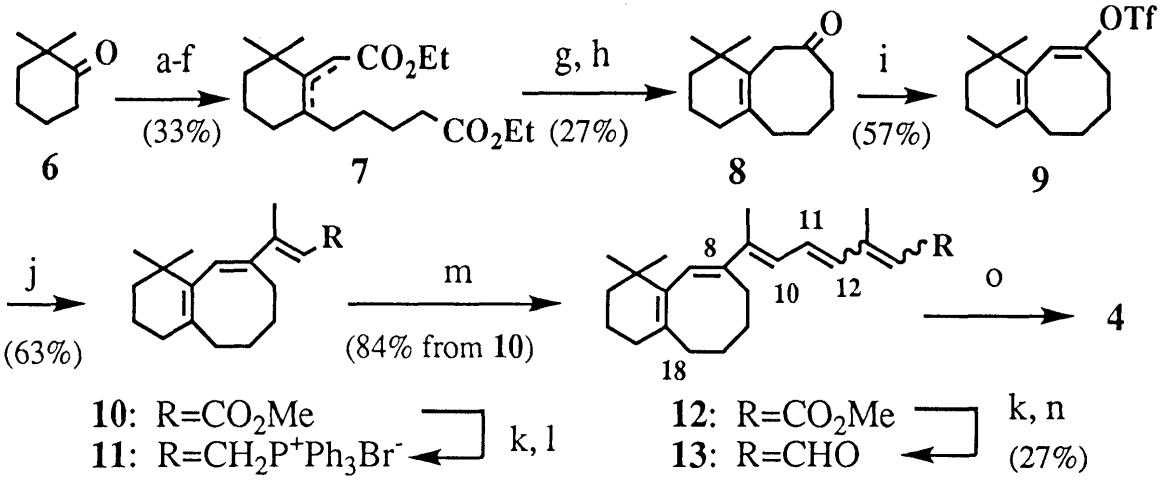

Chart 1

a) NaH, $(MeO)_2CO$; b) NaH, $Br(CH_2)_4CO_2Et$; c) c.HCl; d) c.H_2SO_4, EtOH; e) LDA, AcOEt; f) $SOCl_2$, pyridine; g) *t*-BuOK; h) $MgCl_2 \cdot 6H_2O$; i) LDA, Tf_2NPh; j) $Me_3Sn(Me)C{=}CHCO_2Me$, $Pd(OAc)_2$; k) $LiAlH_4$; l) $Ph_3P \cdot HBr$; m) NaOMe, $OHC(Me)C{=}CHCO_2Me$; n) MnO_2; o) p.HPLC.

In a similar fashion, bicyclic ketone **15**[3] prepared from diallyl ester **14** was converted to the corresponding retinal analog **5**.[3,6,7] It is noteworthy that in the Wittig reaction of **18**, *E*-3-formylcrotononitrile was used instead of the crotonate, and final transformation to the aldehyde **5** was achieved by DIBAL reduction (Chart 2). The present methodology employing the palladium-catalyzed coupling reaction of vinyl triflate with methyl *E*-3-trimethylstannyl-2-butenoate as a key step provides a general and convenient route for the synthesis of various retinal analogs from the bicyclic ketones.

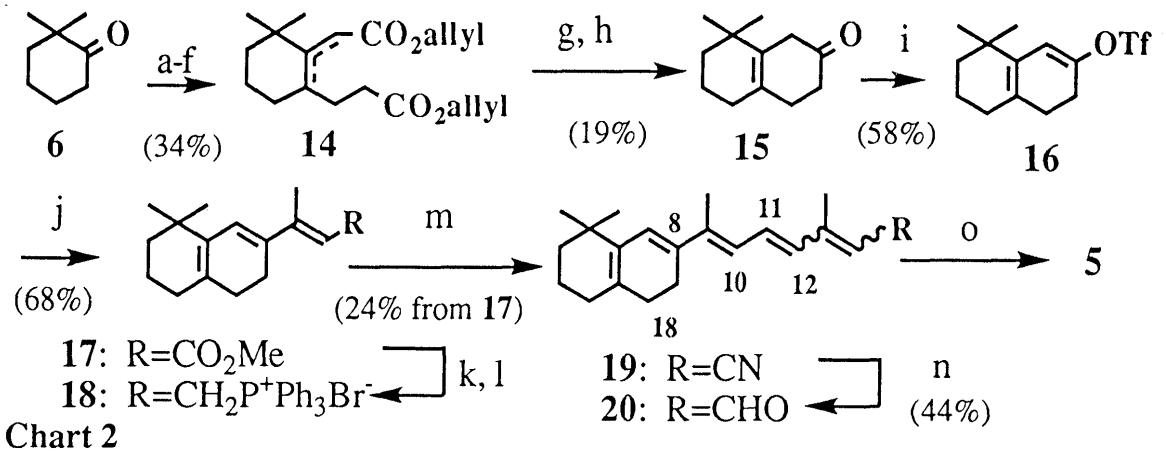

Chart 2

a) NaH, $(MeO)_2CO$; b) NaOMe, $CH_2{=}CHCO_2Et$; c) c.HCl; d) DCC, DMAP, $CH_2{=}CHCH_2OH$; e) LDA, AcOallyl; f) $SOCl_2$, pyridine; g) LiTMP; h) Et_3N, HCO_2H, $Pd(OAc)_2$; i) LDA, Tf_2NPh; j) $Me_3Sn(Me)C{=}CHCO_2Me$, $Pd(OAc)_2$; k) $LiAlH_4$; l) $Ph_3P \cdot HBr$; m) $OHC(Me)C{=}CHCN$, NaOMe; n) DIBAL; o) p.HPLC.

Subsequently, binding experiments of **4** and **5** with bovine opsin were carried out in a CHAPS-PC mixture by the method reported previously[8] to afford the new artificial rhodopsins having the absorption maximum at 483 nm and 546 nm, respectively. The absorption data and opsin shifts of the chromophores and pigments are summarized in the Table 1. These results strongly suggest that the torsional angle around the 6-7 single bond of retinal **2** is very close to that of 8,18-ethanoretinal **3**, and **3** is the most suitable model among the bicyclic retinals for the rhodopsin chromophore.[9]

Table 1. The UV-VIS, CD Data and Opsin Shifts of Rhodopsins

Chromophores	Aldehydes[a]	PSB[b]	Rhodopsins[c]			Opsin shifts
				CD nm (mdeg / absorption)		
	λ max / nm	λ max / nm	λ max / nm	α–band	β–band	Δ ν / cm^{-1}
11Z-Retinal (**2**)	376.5	440	498	489 (+8.7)	332 (+17.50)	2650
11Z-8,18-Ethanoretinal (**3**)	386	457	503	491 (+8.23)	335 (+16.97)	2000
11Z-8,18-Propanoretinal (**4**)	374[b]	440	483	475 (+11.0)	340 (+12.0)	2000
11Z-8,18-Methanoretinal (**5**)	416[b]	496	546	545 (+5.5)	340 (+32.0)	1850

a) In ethanol. b) In methanol. c) In 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate-phosphatidyl choline (CHAPS-PC) mixture.

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3) Satisfactory ^{1}H-NMR, IR and MS spectral data were obtained.
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6) ^{1}H-NMR data for compounds **4** (pale yellow oil) and **5** (pale yellow oil) are as follows, For **4** : (CD_3OD, 500 MHz) δ 0.86 (3H, s), 1.14 (3H, s), 1.06-1.20 (2H, m), 1.48-1.54 (2H, m), 1.57-1.63 (1H, m), 1.63-1.72 (2H, m), 1.72-1.80 (1H, m), 1.86-1.90 (2H, m), 1.96-2.01 (1H, m), 2.01-2.06 (1H, m), 2.05 (3H, s), 2.24-2.30 (1H, m), 2.39 (3H, d, *J* = 1 Hz), 2.51 (1H, dd, *J* = 12.5, 8.5 Hz), 6.02 (1H, br d, *J* = 8.5 Hz), 6.06 (1H, br d, *J* = 10 Hz), 6.44 (1H, br s), 6.83 (1H, d, *J* = 11.5 Hz), 6.86 (1H, dd, *J* = 11.5, 10 Hz), 10.05 (1H, d, *J* = 8.5 Hz, CHO); For **5**: (CD_3OD, 500 MHz) δ 1.05 (6H, s), 1.50-1.54 (2H, m), 1.63-1.68 (2H, m), 2.05 (3H, s), 2.06-2.12 (4H, m), 2.33 (2H, t, *J* = 5.5 Hz), 2.40 (3H, d, *J* = 1 Hz), 6.01 (1H, dq, *J* = 8.5, 1 Hz), 6.02 (1H, br d, *J* = 10.5 Hz), 6.39 (1H, br s), 6.81 (1H, br d, *J* = 11 Hz), 6.83 (1H, t, *J* = 11 Hz), 10.03 (1H, d, *J* = 8.5 Hz, CHO)
7) Synthesis of all-*E*-isomer of **5** has already reported; van der Steen R., Biesheuvel P. L., Erkelens C., Mathies R. A., Lugtenburg J., *Recl. Trav. Chim. Pays-Bas*, **108**, 83 (1989).
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9) A detailed discussion will be published elsewhere.

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