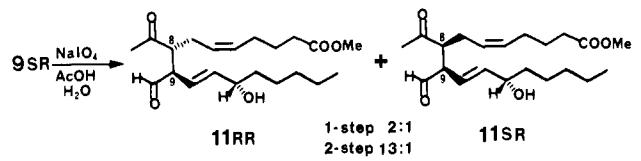
The LGE_2 skeleton was assembled as outlined in Scheme II. Alkylation of (diethylphosphono)acetone (**8**) with methyl 7-bromohept-5(*Z*)-enoate[12] affords **5** whose sodium salt reacts with isopropylidene-L-glyceraldehyde (**6**) to produce the isomeric enones **4**-*Z* and **4**-*E* (1:2.3). Reaction of either isomeric enone with vinyl cuprate **7**,[13] prepared from 1-iodo-3(*S*)-[(*tert*-butyldimethylsilyl)oxy]-1(*E*)-octene[14] afforded identical mixtures of **9**-*RR*[15] (70%), which has the C-8 configuration of LGE_2, and the C-8 epimer **9**-*SR*[15] (30%). Interestingly, Michael addition occurred only in the presence of $MgBr_2$, which presumably serves as a Lewis acid catalyst.[16] That **9**-*RR* and **9**-*SR* are epimeric at position 8 is evident from the observation that saponification of either ester afforded identical mixtures of epimeric acids **10**-*RR* (70%) and **10**-*SR* (30%).

Unexpectedly, treatment of either **9**-*RR* or **9**-*SR* with aqueous acetic acid *followed by sodium periodate* afforded the same 13:1 mixture of LGE_2 methyl ester (**11**-*RR*) and the 8-epi isomer (**11**-*SR*). This remarkably high preference for the required 8*R* configuration contrasts with the 7:3 equilibrium ratio observed for **10**-*RR* and **10**-*SR*. The 1,2-dihydroxyethyl substituent in the intermediate vicinal diol **3** apparently plays a role in the stereoselective epimerization since efficient interception of this intermediate allows conversion of **9**-*SR* to mixtures richer in the **11**-*SR* epimer. Thus, treatment of **9**-*SR* with aqueous acetic acid *in the presence of sodium periodate* yielded a 2:1 mixture of **11**-*RR* and **11**-*SR*. Further discussion of this remarkable stereoselection is



deferred to a full account of this work. The aldehydic ^1H NMR resonance for **11**-*RR* in $CDCl_3$ occurs at δ 9.47 as found for the methyl ester of LGE_2 derived from PGH_2 while the corresponding resonance for **11**-*SR* occurs at δ 9.56. For comparison with LGE_2 methyl ester derived from PGH_2, 8-*epi*-9-*epi*-LGE_2 methyl ester **11**-*SS* was obtained from **9**-*SS* prepared by substituting isopropylidene-D-glyceraldehyde for the L isomer **6** in Scheme II. The ^1H and ^{13}C NMR spectra of **11**-*RR*, **11**-*SS*, and LGE_2 methyl ester obtained from PGH_2 are almost identical. To facilitate correlation, these keto aldehydes were converted to fluorenylidene derivatives **12** by chemoselective Wittig reaction with 9-fluorenylidenetri-*n*-butylphosphorane.[17] The adducts **12** show intense UV absorptions ($\epsilon_{max} \simeq 40\,000$; hexane) at both 258 and 229 nm. Furthermore, in contrast with LGE_2 (**1**), analytically pure fluorenylidene derivatives were readily isolated quantitatively by HPLC on partisil. As expected, **12**-*RR* and the fluorenylidene derivative of LGE_2 methyl ester derived from PGH_2 exhibit identical ORD curves while the curve for **12**-*SS* is virtually a mirror image (Figure 1).

Levuglandin E_2 was prepared in good yield from the ketal **10**-*RR* (or **10**-*SR*) by treatment with aqueous acetic acid followed by sodium periodate. The ^1H NMR spectrum of synthetic LGE_2 is identical with that of the more stable levuglandin obtained from PGH_2 except for tiny absorptions owing to minor impurities in the latter sample which were absent in the synthetic product. The total synthesis now makes LGE_2 readily available. The present results also demonstrate that epimerization of LGE_2 at positions 8 or 9 is not extensive under the conditions of its solvent-induced formation from PGH_2.

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Supplementary Material Available: Physical data and purification of compounds **4**-**6** and **8**-**12** (12 pages). Ordering information is given on any current masthead page.

The Nature of Restrictions in the Binding Site of Rhodopsin. A Model Study

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Continuing the effort of early workers,[1] the research teams at Hawaii and elsewhere have recently completed the synthesis of all 16 possible geometric isomers of vitamin A.[2] Among the 14 stable retinal isomers, 10 have been shown to form visual pigment analogues: 11-cis, 9-cis,[1] 9-cis,13-cis,[1,3] 7-cis, 7-cis,9-cis, 7-cis,13-cis, 7-cis,9-cis,13-cis,[4] 7-cis,11-cis,[5] 9-cis,11-cis,[6] and 7-cis,9-cis,11-cis.[7,8] The notable exceptions are the all-trans and the 13-cis isomers, which do not yield stable analogues. However, it is known that after introduced into the binding site, such geometries can be generated as transient forms, i.e., in lumirhodopsin (or Meta-I)[9] and photo-Meta-II-465.[10]

The failure of these two isomers to form stable pigments has been rationalized on the ground of a longitudinal restriction of the binding site.[11,12] This explanation is quite satisfactory for the all-trans isomer, which has the longest distance between the center of the cyclohexenyl ring and the carbonyl carbon.[11] Since the 13-cis isomer is not uniquely longer than several other isomers, conformational rigidity was also considered important.[12]

An analysis of longitudinal restrictions based on the length of the chromophore overlooks the tetherlike function of the *n*-butyl group of Lys-296.[13] Also, the model implies freedom of in-plane motion of the imino carbon relative to a rigid cyclohexenyl ring. This is only partially correct because within the binding site the relative distance of the primary (the protonated Schiff base) and the secondary binding sites (the hydrophobic pocket) is likely to vary only within the limits allowed by conformational changes of the butyl group and to a smaller extent the chromophore itself. We now present a different approach in analyzing the shape of the binding site on the basis of its behavior toward retinal isomers.

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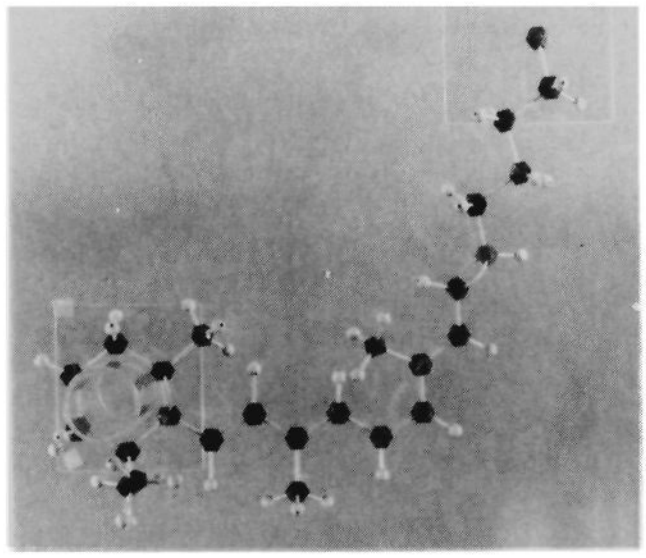

Figure 1. Molecular model of the tethered chromophore of rhodopsin. The butyl group and the polyene chromophore are in the most relaxed conformation. The distance between the α-carbon of Lys-296 and the center of the cyclohexenyl ring is affixed by means of a loose bolt through the α-carbon and a Lucite cylinder in the middle of the ring. The Lucite plates are taped to the supporting surface. In this way, only pivotal motions about both centers are permissible. Structures for the remaining 15 isomers were built from this model.

This analysis provides new information related to two-dimensional restrictions of the binding site.

In Figure 1 is shown a model of the retinyl chromophore of rhodopsin bonded to the butyl group of Lys-296. The distance between the α-carbon of Lys-296 and the center of the cyclohexenyl ring is affixed in a manner as shown. For clarity only single bonds are shown for the middle portion of the polyene chain.

Within this longitudinal restriction between the primary and secondary binding sites, the model is converted to the remaining 15 geometric isomers of the chromophore. In all structures, the more stable s-trans conformation of all single bonds, other than that of the 6,7-bond, is maintained. Such a conformation for the most flexible 12,13-bond has been rigorously established for rhodopsin.[14] On the basis of X-ray crystallographic data a larger ring–chain dihedral angle and a slightly twisted 8,9-bond are assumed for all 7-cis isomers.[12] The model shows that in spite of varying lengths of the isomeric chromophores,[11] all 16 isomers can be constructed within the given limits. However, there is substantial lateral displacement of atoms and change of conformation of the butyl group. This lateral displacement is largely limited to the area defined by the plane of the chromophore. This is the same plane surrounded by and parallel to the axes of the α-helices of the protein,[15] a relatively open space. Therefore, not surprisingly, so many geometric isomers with a diverse molecular shape form pigment analogues.

The inactivity of 13-*cis*- and *all-trans*-retinal must then be due to factors *other than* longitudinal restrictions. When the relative positions of the carbon atoms of the two isomeric chromophores are compared with those of all other stable chromophores of active isomers, a possible reason for their failure emerges. Figure 2 is a carbon population map for all 10 active isomers. The van der Waal radii of the outer carbons of this collection therefore define the maximum perimeter of the binding site of rhodopsin. The corresponding positions of the inactive 13-cis and all-trans isomers are marked separately. The map reveals that only in the shaded area, atoms of the two inactive isomers commonly occupy an area different from those of others. An obvious conclusion is that this region is forbidden to the chromophore, most likely being already occupied by a group protruding from the protein surface, thus providing steric inhibition for the observed negative results. One might further infer from the location of the region and its size, being sufficiently large to contain a three-atom fragment, that this protruding group is a carboxylate anion, i.e., the counterion

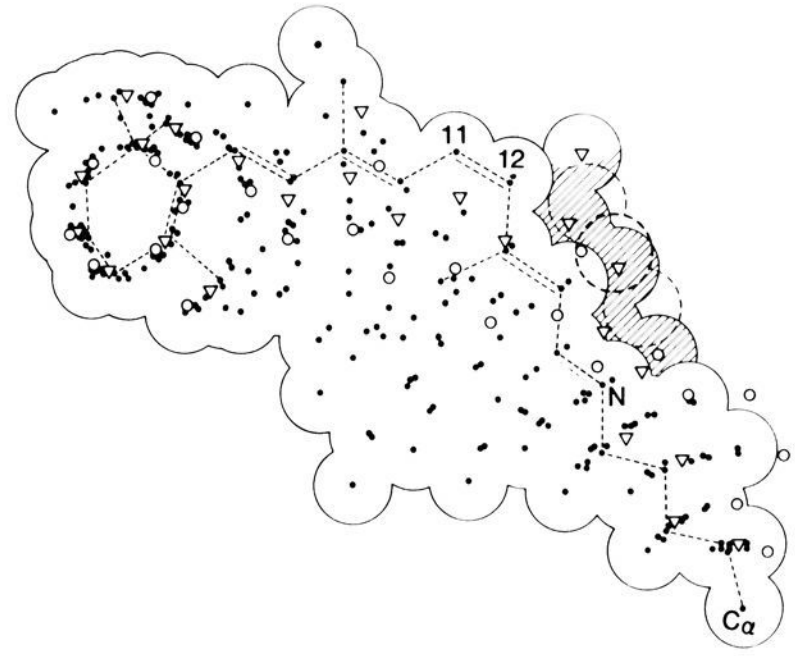

Figure 2. A two-dimensional map of the binding site of opsin: overlay (a population map) of carbon atoms of all 10 active isomers (see text) of the tethered chromophore of rhodospin (solid dots). Superimposed are carbon positions of the inactive isomers: 13-cis (triangles) and all-trans (circles). The dashed straight lines are the carbon framework of the 11-cis isomer. The maximum perimeter of the binding site is defined by the van der Waal radii of the outer carbons of the active isomers. The shaded area is that commonly occupied by only the inactive isomers. The dashed circles represent a hypothetical carboxylate anion, which should point perpendicularly to the chromophore.[16]

Table I. Properties of 9-Cis and 11-Cis Isomers of 12-Substituted Pigment Analogues

substituent isomer	retinal absorptn, λ_{max}, nm	pigment absorptn, λ_{max}(det)[a]	pig. yield
12-H, 11-cis[b]	365	498 (D)	100%
9-cis[b]	363	483 (D)	>90%
12-CH_3, 11-cis	290	489 (D)	2%
		495 (C)	5%
9-cis	359	486 (D)	46%
		487 (C)	78%
12-Cl, 11-cis	290	(D)	<2%
9-cis	370	488 (D)	~50%
12-F, 11-cis[c]	363	507 (D)	>90%
9-cis[c]	362	493 (D)	>80%

[a] Detergent: digitonin (D) or CHAPS (C). [b] Data from ref 2, 8. [c] Data from ref 18.

of the protonated Schiff base. Following the discussion of Hargrave, one is tempted to suggest that this group is Asp-83.[13b] Furthermore, the bidentate nature of the charged group might even qualify it as the "secondary" point charge.[16]

Results from several retinal analogue studies are in support of the conclusion of the location of the binding site restriction. For example, for both 12-methyl- and 12-chlororetinal[17] the surprising results of low yields of pigments from the two 11-cis isomers while relatively high yields of stable pigments from the corresponding 9-cis isomers (Table I) can be readily accounted for by the positions of the substituents relative to the forbidden zone. Hence, with the smaller 12-fluororetinal, both isomers form pigment analogues in high yields.[18] Failure of pigment formation from a ring-fused retinal analogue[19] is also in agreement with the above conclusion.

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In summary, this simple analysis allows one to expand beyond the one-dimensional limitation of the earlier model. The conclusions are consistent with the current knowledge of the binding site. Furthermore, the model successfully explains new, unusual analogue results. Continued effort will be directed toward a better understanding of restrictions in the third dimension of the binding site.

Acknowledgment. The work is supported by a grant from the U.S. Public Health Services (AM-17806).

Note Added in Proof. The pivotal motion of the α,β-bond about the C_α atom probably should be more restricted than what is permitted by the current model, correctly suggested to us by Prof. R. Birge. However, models show that such a refinement has no effect on the conclusions reached in the current study.

Supplementary Material Available: Photographs of molecular models of all remaining 15 isomers of the confined butyl retinylidene chromophore and ^1H NMR data (δ, J, and NOE) of isomers of 12-methyl- and 12-chlororetinal (3 pages). Ordering information is given on any current masthead page.

Control of Olefination Stereochemistry Using Long-Chain Zirconium Alkylidene Analogues of the "Tebbe" Reagent

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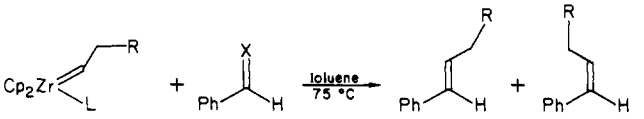
The Tebbe compound,[1] $Cp_2Ti(CH_2)ClAlMe_2$, has been shown to be a reagent of general synthetic utility for methylene group transfer to a range of organic carbonyls.[2] In these reactions, since only CH_2 is transferred, the problem of controlling stereochemistry of olefination does not exist. Using long-chain alkylidene analogues[3,4] of the Tebbe reagent, however, raises this as an important issue. Observations concerning "Wittig-type" reactivity of the Tebbe reagent or its analogues suggested a stepwise olefination process involving an intermediary metallooxetane which then fragments to give the metal oxo complex and the olefinic product.[5] It could be that stereochemical factors leading to the preferential formation of either possible metallooxetane from an *unsymmetrically*-substituted metal carbene complex control the product distribution of olefins thus formed. The oxygen atom in the metallooxetane may not provide much differentiating stereochemical information and, therefore, may not be of much use in controlling formation of either metallooxetane or ultimate product distribution. Replacing (=O) with a (=NR) group, however, might provide one method to control stereochemistry in an olefination sequence: the imine, which contains a sterically significant group on nitrogen, would give rise to an N-substituted metallazetidine structure, and the ring that is preferentially formed may be the one that minimizes steric interactions among all *four groups* of the four-membered ring. With this concept in mind, we have studied reactions between substituted zirconium carbene complexes and imines and find that not only does olefination occur but also that a correlation exists between the E/Z selectivity of olefins formed and the size of the substituent group on imine nitrogen.

Table I

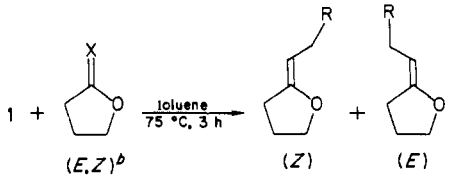


1a. R = *tert*-butyl; L = PPh_3
1b. R = n-C_4H_9; L = PMe_2Ph

carbene	X	"A"[c]	E/Z	h at 75 °C	olefin yield, %[a,b]
1a	=O		0.50	3	76
	$=NCH_3$	1.8	2.06	3	88
	$=NCH(CH_3)_2$	2.1	2.30	4	47
	=NPh	3.1	2.76	3	56
	$=NC(CH_3)_3$	>4.5	4.32	2	16
1b	=O		1.11	5	72
	$=NCH_3$	1.8	1.13	5	85
	$=NCH(CH_3)_2$	2.1	1.67	5	39

[a] Based on carbene; product ratios were determined by VPC analysis; products were isolated by preparative VPC and structures were confirmed by comparison with authentic materials. [b] **1** slowly dimerizes under these conditions;[4] no carbene complex remained; the unreacted imine can be recovered unchanged. [c] For substituents on N.[12]

Table II



carbene	X	E/Z	enol ether yield, %[a]
1a	=O	1.00	71
	=NPh	0.53	72
1b	=O	1.04	80
	=NPh	0.35	91

[a] Based on carbene complex, determined by VPC. [b] 1:2.3 ratio.

In a typical olefination procedure benzaldehyde (1 equiv) was added to a toluene solution of **1a**[4] (0.062 M) at 25 °C; the reaction mixture was then heated to 75 °C for 3 h. A preference for formation of the Z isomer of the olefin was noted as it is in "salt-free" Wittig reactions[6] using triphenylphosphoranes.[6b] In contrast, using the imines shown in Table I this E/Z selectivity can be reversed.[7] In each case, procedures were identical with the one noted for benzaldehyde. For example, *N*-methylbenzylideneimine (1 equiv) was added to a toluene solution of **1a**[4] (0.062 M) at 25 °C, followed by heating to 75 °C for 3 h. A preference for the E isomer of the olefin was noted.

The Tebbe reagent is especially useful in that it can methylenate esters to give enol ethers.[2] We had noted that zirconium carbene complexes likewise react readily with esters.[4] Consistent with the observations described for imines, we observe that imidates[8] also react with zirconium carbene complexes to yield enol ethers. Here, as in the case of the imines described above, by use of an N-substituted imidate, E/Z selectivity of the olefin formed can be controlled (see Table II).

Reactions discovered between zirconium carbene complexes and imines or imidates (and thioketones) expand in scope the nature of substrates that can react with group 4 carbene complexes to give olefins. The fact that a sterically significant group can be utilized in conjunction with a "throwaway" part of the substrate

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