Recl. Trav. Chim. Pays-Bas **107**, 125–131 (1988) 0165-0513/88/03125-07$2.25

5-Demethylretinal and its 5-^{2}H, 7-^{2}H and 5,7-$^2H_2$ isotopomers. Synthesis, photochemistry and spectroscopy#

M. B. Spijker-Assink, C. Winkel, G. S. Baldwin and J. Lugtenburg

Department of Organic Chemistry, Leiden University, 2300 RA Leiden, The Netherlands
(Received June 24th, 1987)

Abstract. All-*trans*-5-demethylretinal and its 5-^{2}H, 7-^{2}H and 5,7-$^2H_2$ isotopomers have been synthesized by means of a new and simple scheme. Using photochemistry, the 9-, 11- and 13-*cis*, as well as the 9,13- and 11,13-di-*cis* isomers, were obtained. The structures were established by means of ^{1}H NMR spectroscopy.

Introduction

Visual pigments (rhodopsins) contain as their photoreactive chromophore 11-*cis*-retinal (or 3-dehydro-11-*cis*-retinal) bound as a protonated Schiff base to the ε-amino group of a lysine of the peptide chain (Fig. 1a)[1,2]. Upon excitation with light, *cis–trans* isomerization takes place. Bovine rhodopsin's primary photoproduct, bathorhodopsin, has the strained all-*trans* chromophore[3]. The energy of this photoproduct, being 32 kcal/mol higher than that of rhodopsin[4,5], is the driving force for subsequent thermal reactions leading, via intermediates, to free all-*trans*-retinal and opsin with free active site. All-*trans*-retinal is converted into 11-*cis* -retinal which recombines with opsin to regenerate rhodopsin[6].

Dedicated to Prof. *G. J. M. van der Kerk* on the occasion of his 75th birthday.

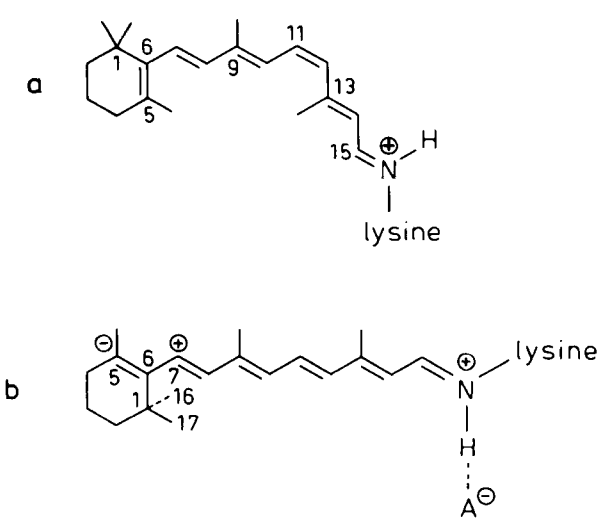


Fig. 1. Chromophoric groups of a) rhodopsin, b) bacteriorhodopsin.

Bacteriorhodopsin, likewise a retinal–protein complex, acts as a light-driven proton pump in the purple membrane of Halobacterium halobium[2,7]. In light-adapted bacteriorhodopsin, the chromophore occurs in the all-*trans* configuration (Fig. 1b)[2,7]. In the primary photoproduct K, the chromophore is in the 13-*cis* configuration, with a 16 kcal/mol higher energy content than in light-adapted bacteriorhodopsin[8]. This energy provides the driving force for the subsequent thermal reactions which lead, via intermediates, to light-adapted bacteriorhodopsin. During this cycle, a proton is taken up from the inside of the bacterial cell wall and expulsed to the outside medium, thus creating the energy of a proton gradient which can be utilised by the bacterium to form ATP and to power its life processes.

In both cases, the intimate interaction of the peptide chain with the chromophore is responsible for the colour of the pigment and the photochemical properties etc. Retinals show in solution a twisted (40°) *cis* conformation around the 6–7 single bond. According to solid-state ^{13}C NMR spectroscopy, the conformation around the 6-bond in bacteriorhodopsin is planar 6-*s-trans*[9], while in rhodopsin it is twisted 6-*s-cis*, just as in unperturbed retinals[10,11]. In the case of bacteriorhodopsin, this has also been established via a bio-organic method: 8,16-methanobacteriorhodopsin, with its chromophore locked in the planar 6-*s-trans* conformation, shows properties virtually identical to those of native bacteriorhodopsin[12].

Since NMR cannot be applied to short-lived intermediates, we use another physical technique to establish the configuration around the 6–7 bond in the intermediates of the

*Fig. 2. The in-phase C(5)D – C(7)D in-plane rock vibration in the a) 6–7-*s*-cis b) 6–7-*s*-trans configuration.*

photocycles. Laser Resonance Raman spectroscopy has proven its value in obtaining detailed structural information on both the stable pigments and their photoproducts[13,14]. The in-phase C(12)D–C(14)D in-plane rock combination of the 12,14-dideuterated chromophore has been used to establish the conformation around the C13–C14 double bond in bacteriorhodopsin and its photo-intermediates. The in-phase combination is at $\approx 910\ cm^{-1}$ in 13-*trans* and at $\approx 940\ cm^{-1}$ in 13-*cis* chromophores. Thus, the chromophore is 13-*cis* in BR_{548}, K_{625}, L_{550} and M_{412}, and 13-*trans* in BR568 and O_{640}[13].

We realised that the in-phase combination of the C(5)D–C(7)D in 5D,7D-5-demethylbacteriorhodopsin and 5D,7D-5-demethylrhodopsin together with their photoproducts can be similarly used to establish the configuration around the 6–7 bond in the chromophores (Fig. 2). The properties of both 5-demethylrhodopsin and 5-demethylbacteriorhodopsin are almost identical with those of the native pigment[3,15]. In order to establish that the peaks around 910 and 940 cm^{-1} are indeed due to in-phase combinations of C–D in-plane rock vibrations, we also require, in addition to 5D,7D-, the 5D-, 7D- and 5,7-non-deutero-5-demethylretinals.

In this paper the synthesis of 5-demethylretinal and its 5D-, 7D- and 5,7-dideutero isotopomers in the all-*trans*, 13-, 11- and 9-*cis* forms (Fig. 3) is described.

Synthesis

In Scheme 1, the synthetic sequence leading to the conversion of 5-demethyl-β-cyclocitral **2** into 5-demethylretinal **1** is shown. In previous publications we described a 4-step ten-carbon extension for the preparation of retinals, some ring-demethylated retinals and open-chain retinal analogues in good yield[16,17]. Similary, **2a-d** can be elongated by twice performing a *Horner–Emmons* coupling with C_5-phosphonate nitrile **3**[18] followed by diisobutylaluminium hydride (Dibal) reduction. 5-Demethylretinals **1a-d** are obtained as mixtures of 9*E*/*Z*, 13*E*/*Z* isomers, with high *E* content, in 51% yield. The isomers are isolated by means of HPLC, giving the all-*trans* and 9-*cis* isomers in a pure state and the 13-*cis* admixed with the 9,13-di-*cis* isomer (see below).

For the preparation of synthons **2a-d**, we have developed an efficient method which allows the introduction of the ^2H label at the required positions using commercially available enriched compounds, *cf.* Scheme 2.

The anion of **5** is condensed with dimethyl carbonate to give, after work-up, **6** in 83% yield[19]. Subsequent ring closure with $SnCl_4$[19] leads, in *ca.* 72% yield, to a 3/1 keto/enol mixture of **7**, which can be quantitatively reduced by $NaBH_4$ or $NaBD_4$ to give the *cis* and *trans* isomers of **8a,b**. Dehydration of the alcohol using $POCl_3$ affords the β-esters **9a,b** in 80% yield. Reduction of the ester function

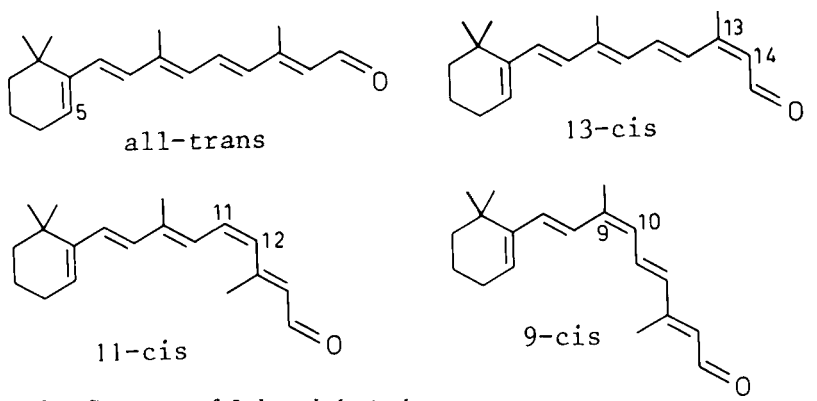
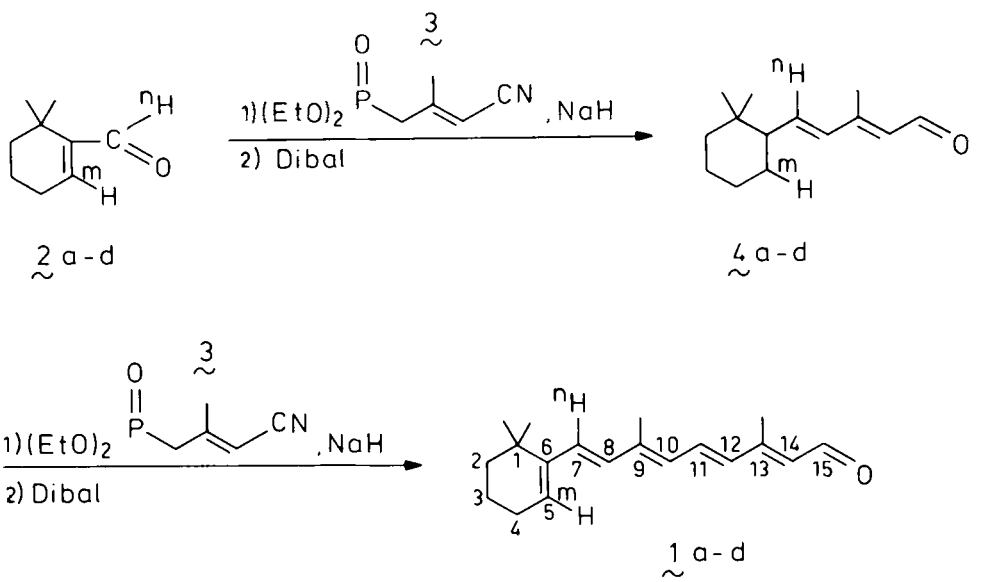
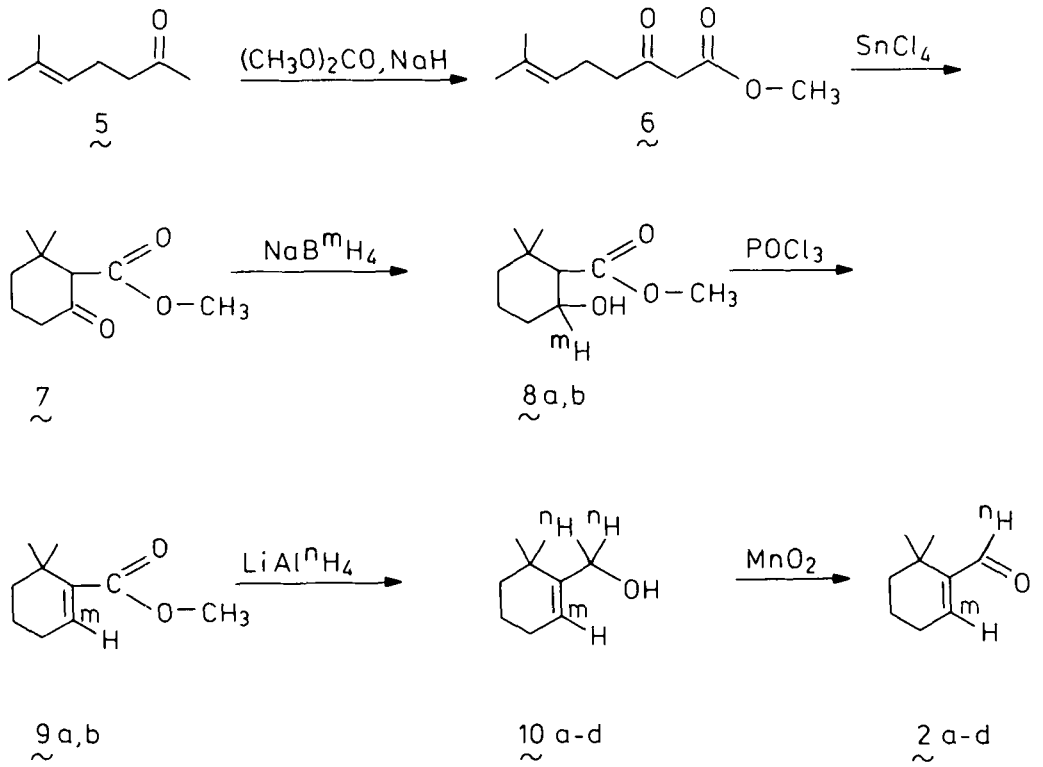


Fig. 3. Structures of 5-demethylretinal.



Scheme 1. Synthesis of 5-demethylretinal (**1a**), *5D-* (**1b**), *7D-* (**1c**) *and 5,7-D_2-5-demethylretinal* (**1d**). *a:* m = *1*, n = *1*; *b:* m = *2*, n = *1*; *c:* m = *1*, n = *2*; *d:* m = *2*, n = *2*.



Scheme 2. Synthesis of synthon **2a-d**.
a: m = *1*, n = *1*; *b:* m = *2*, n = *1*; *c:* m = *1*, n = *2*; d: m = *2*, n = *2*.

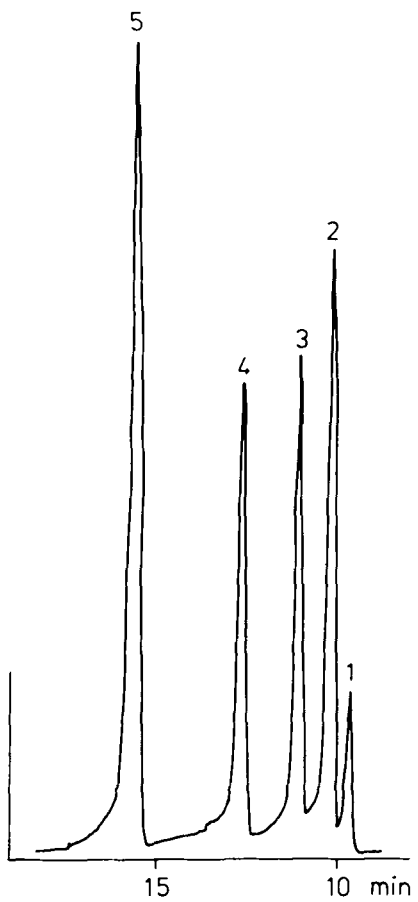


*Fig. 4. HPLC chromatogram (silica gel, 10% ether in pentane, λ_{det} 360 nm) of an irradiated mixture of all-*trans*-5-demethylretinal in CH_3CN.*
*1 = 11,13-di-*cis, *2 = 13-*cis + *9,13-di-*cis, *3 = 11-*cis, *4 = 9-*cis, *5 = all-*trans.

by $LiAlH_4$ or $LiAlD_4$ leads to the alcohols **10a-d**, which can be subsequently oxidized by MnO_2 to give synthons **2a-d** in 42% yield based on **9a,b**.

Photochemistry

Photochemistry of retinals and modified retinals is the method of choice for the preparation of the various geometric isomers in good yield and adequate purity. It is the best way to obtain the 11-*cis* isomer essential for the study of visual pigments[20,21].
A dilute solution of pure all-*trans*-5-demethylretinal was irradiated in acetonitrile for 90 minutes using a tungsten lamp (200 W). In Fig. 4, the HPLC trace of the photostationary state mixture is given. In addition to the peaks 2, 4 and 5 (also observed in the HPLC traces of **1a-d** from the synthesis), two further peaks, 1 and 3, are present in the photomixture. Each of the peaks was isolated by preparative HPLC and characterized by spectroscopic methods.

Spectroscopic characterization

Mass spectrometry

The double-focus mass spectra were determined for **1a,b, c** and **d**. The mass values are resp. 270.2002 (calcd. for $C_{19}H_{26}O$: 270.1983), 271.2046, 271.2049 (calcd. for $C_{19}H_{25}DO$: 271.2046) and 272.2116 (calcd. for $C_{19}H_{24}D_2O$: 272.2109). The ^2H incorporation of **1b, c** and **d**, determined from the single-focus mass spectra, amounts to 97.16%, 98.73% ^2H and 95.84% $^2H_2$, respectively.

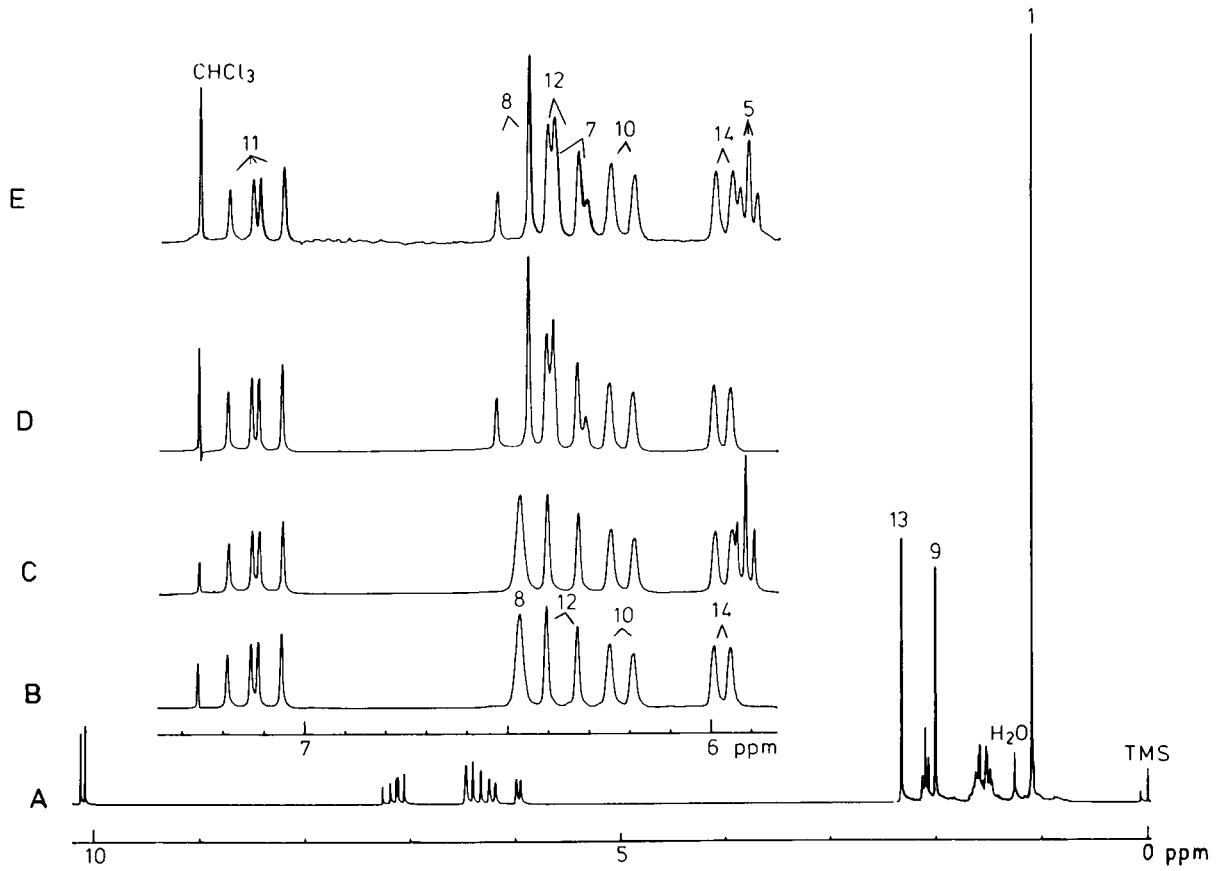


*Fig. 5. A: 200-MHz ^1H NMR spectrum in $CDCl_3$ of all-*trans*-5D,7D-5-demethylretinal. B, C, D, E: vinylic region of the 200-MHz ^1H NMR spectra of the all-*trans* isomers of* **1d**, **c**, **b** *and* **a**, *resp.*

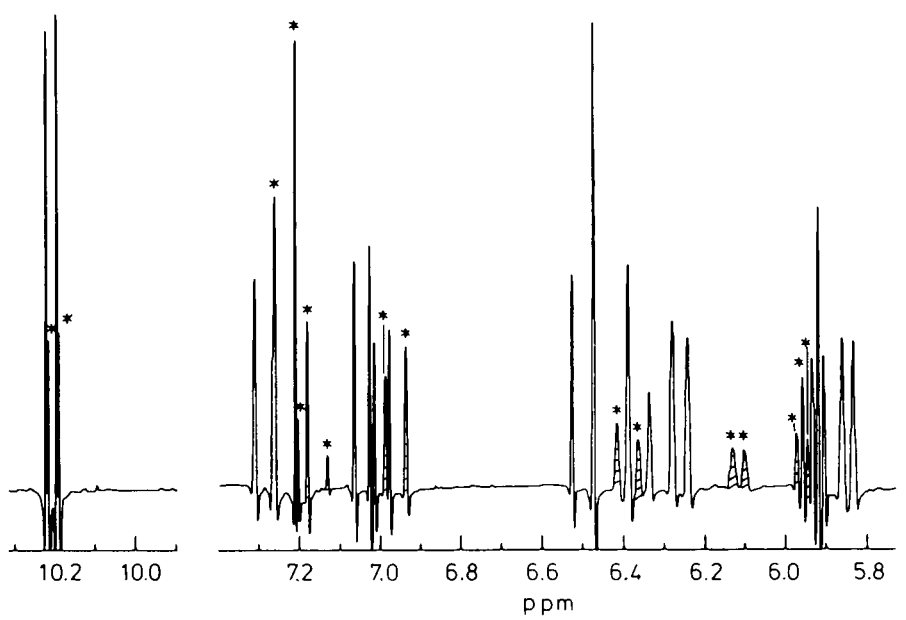


*Fig. 6. Low-field part of the 300-MHz ^1H NMR spectrum of 13-*cis- *and 9,13-di-*cis*-5-demethylretinal. Signals of the 9,13-di-*cis *form are designated with an asterisk.*

^1H NMR spectroscopy

200- and 300-MHz ^1H NMR spectroscopy was used for the determination of the various isomeric structures of **1a-d** and the location of the ^2H isotope.

In Fig. 5A, the 200-MHz ^1H NMR spectrum of all-*trans*--5,7-D_2-5-demethylretinal (**1d**) is shown. The three different signals of the 1,1-dimethyl, 9-methyl and 13-methyl groups, at δ 1.09, 2.00 and 2.32, resp., are readily identified. A four-proton multiplet (1.4–1.7 ppm) corresponds to the 2- and 3-CH_2 group; the triplet at δ 2.11 is due to the 4-CH_2 group. The aldehyde proton H-15 is found at lowest field at 10.11 ppm (J 8.1 Hz). The vinylic proton signals lie at 5.9–7.2 ppm. An expansion of this region is shown in Fig. 5B. Comparison with the same region of the spectrum of all-*trans*-**1a** in Fig. 5E shows that no signals of H-5 (triplet, J 4.2 Hz) at

δ 4.91 nor of H-7 (doublet, *J* 15.8 Hz) at δ 6.35 are present and further that the signal of H-8 at δ 6.47 is a singlet. This proves that the ^2H incorporation in **1d** is >95% at the required positions. This also holds for the 7-D and 5-D compounds (**1c** and **1b**): in the vinylic regions of their all-*trans* isomer spectra, a singlet for H-8 (δ 6.49), absence of H-7 signals and a sharper H-5 triplet (δ 4.91) due to the absence of the small H-5–H-7 coupling are found (Fig. 5C) and no signals for H-5 (Fig. 5D).

Similarly, the ^1H NMR spectra of the other components in the isomeric mixture of **1a-d** and of the irradiation products were analysed. The chemical-shift values and coupling constants are listed in Table I.

The materials from peaks 5, 4 and 3 showed the characteristic data of the all-*trans*, 9-*cis* and 11-*cis* form, respectively.

Typical for the 9-*cis* isomer is the 0.5 ppm shift to lower field for H-8 and the 0.14 ppm shift upfield for H-10 relative to the all-*trans* form. The coupling constants are in agreement with the 9-*cis* structure.

Characteristic for the *cis* geometry around the C(11)=C(12) bond is the J_{11-12} 10.6 Hz for the 11-*cis* isomer.

The 300-MHz ^1H NMR spectrum of peak 2 (Fig. 6) displays two different aldehyde doublets in a 3/1 ratio, demonstrating the presence of two isomers. From the chemical shifts of H-15 and H-14, it is clear that both isomers have a 13-*cis* double bond. The main component has chemical shifts and coupling constants which are in agreement with a 13-*cis* structure. The minor component shows a 0.46 ppm shift to lower field for H-8, indicating that this isomer, in addition to the 13-*cis* double bond, has a 9-*cis* double bond. In particular, the coupling constants and chemical shift values at the tail end are, as expected, in close agreement with those of the corresponding retinal isomers[22]. Furthermore, the δ values for H-7 are about the same as for retinal. For H-8, they are significantly different, *i.e.* about 0.3 ppm shifted to lower field.

Since the isomer belonging to peak 1 appeared to be very labile in $CDCl_3$ solution, where it is converted into 13-*cis*-5-demethylretinal, its 300-MHz spectrum was recorded in C_6D_6. The chemical shifts are in agreement with a 11,13-di-*cis* structure with its characteristic δ(13-CH_3) 1.52 ppm, δ(H-12) at 5.65 and δ(H-14) at 5.88 ppm. The signals for H-7, H-10 and H-11 are close together in the region 6.3–6.5 ppm, δ(H-8) being found at 6.49 ppm.

UV-Vis spectroscopy

The all-*trans*, 13-, 9- and 11-*cis* isomers of **1a-d** were characterized by UV-Vis absorption, as well as by HPLC retention times and ^1H NMR spectroscopy. The λ_{max} values determined in hexane are listed in Table I.

Discussion

All-*trans*-5-demethylretinal and its 5D-, 7D- and 5,7-dideuterated forms are obtained in good yield with high isotope incorporation using the reactions depicted in Schemes 1 and 2. The only reaction step not giving a single product is the $LiAlH_4$ reduction of **9** to **10**. In addition to the reduction of the ester function to the alcohol, subsequent double bond reduction to the cyclohexane derivative takes place. We were unable to find conditions under which this side-reaction did not occur. However, we found that reduction of the ester with diisobutylaluminium hydride leads exclusively to **10**. This is the method of choice for the reactions leading to 5-demethylretinal and its 5-deuterated isotopomer. For the preparation of the 7-D and 5,7-D_2 forms we still had to use $LiAlD_4$. The **10c** and **10d** admixed with the fully reduced forms are oxidized by MnO_2 leading to **2c** and **2b** and the corresponding hydrogenated aldehyde. Interestingly, the saturated primary alcohol is likewise oxidized to the aldehyde[23]. At the stage of the retinals, the 5-demethyl-5,6-dihydroretinals were removed by HPLC.

Table I λ_{max} *and 200-MHz* ^1H *NMR data (*$CDCl_3$*, TMS as reference) of all-*trans-, *9-*cis- *and 11-*cis-5-demethylretinal; 300-MHz* ^1H *NMR data for the 13-*cis *and 9,13-di-*cis *isomer.*

Hydrogen atoms	Chemical shift, δ (ppm)				
	All-*trans*	9-*Cis*	11-*Cis*	13-*Cis*	9,13-Di-*cis*
2-H_2 + 3-H_2	1.4–1.7	1.4–1.7	1.4–1.7	1.4–1.7	1.4-1.7
4-H_2	2.11	2.13	2.10	2.11	2.11
5-H	5.91	5.97	5.92	5.92	5.96
7-H	6.38	6.39	6.37	6.37	6.39
8-H	6.47	6.97	6.47	6.49	6.96
10-H	6.22	6.08	6.57	6.26	6.11
11-H	7.12	7.29	6.69	7.02	7.18
12-H	6.37	6.26	5.93	7.28	7.23
13-H	5.98	5.97	6.08	5.84	5.84
14-H	10.11	10.11	10.09	10.21	10.20
15-H	1.09	1.10	1.08	1.10	1.10
1-CH_3	2.00	2.00	1.96	2.00	2.00
9-CH_3	2.33	2.34	2.36	2.14	2.16
13-CH_3					
	Coupling constants (Hz)				
4–5	4.2	4.2	4.2	4.1	4.2
7–8	15.8	15.8	15.8	15.8	15.8
10–11	11.7	11.7	12.5	11.4	10.5
11–12	15.0	15.0	10.6	14.9	15.0
14–15	8.1	8.1	8.1	8.0	8.1
	λ_{max} (nm) in hexane				
	374	369	370	370	

Upon irradiation of all-*trans*-5-demethylretinal in acetonitrile with visible light, *cis–trans* isomerization occurs easily. Interestingly, even after irradiation for 90 min, no 7-*cis* form is observed, which is in contrast to the case of retinal where the 7-*cis* isomer is prominently present[21]. Also remarkable is the presence of considerably larger amounts of 9,13-di-*cis* and 11,13-di-*cis* isomers. The 9,13-di-*cis* isomer overlaps completely in HPLC with the 13-*cis* form. However, using ^1H NMR spectroscopy, it can be easily identified.

In order to prepare pure 13-*cis*-5-demethylretinal, the *all*-*trans* form has to be irradiated for a short time with light from a tungsten lamp equipped with a 420-nm cut-off filter[24]. In this mixture of photo-isomers, neither the 9,13-di-*cis* nor the 11,13-di-*cis* isomer is present. This result is in agreement with the fact that absorption of a photon leads to isomerization involving only one bond. Both the 9,13- and the 11,13-di-*cis* isomers are the result of two separate photoisomerizations.

Despite the absence of one bathochromic (methyl) group, the λ_{max} values for the 5-demethylretinal isomers (Table I) are 5–7 nm higher than for the corresponding retinals[21]. This points to a more planar conformation around the C6–C7 *s* bond, allowing a better conjugation between the C5–C6 double bond and the rest of the conjugated chain.

Experimental

All experiments were carried out under a nitrogen atmosphere and the purified polyenes were handled in dim red light. Distilled dry solvents were used. Pet. ether refers to low-boiling petroleum ether 40–60°C. Unless otherwise stated, purification was performed by flash chromatography[25] (Merck silica gel 60, 230–400 mesh) using ether/pet. ether mixtures. TLC analyses were performed on Schleicher and Schuell F 1500/LS 254 silica gel plates using ether/pet. ether mixtures. Evaporation of the solvents was carried out *in vacuo* (15 Torr). The ^1H NMR spectra were recorded on a JEOL PS-100, a JEOL FX-200 or a Bruker WM-300 spectrometer, using tetramethylsilane (TMS; 0 ppm) as internal standard. Exact mass and label determinations were carried out using a Kratos MS 9/50 mass spectrometer (source conditions: electron energy 70 eV, *T* 425 K). The IR spectra were obtained using a Pye-Unicam SP 3–200 and the UV-Vis spectra using a Cary 219 spectrophotometer. HPLC separations were performed using a Dupont 830 equipped with a Dupont spectrophotometer (360 nm) and a 25 cm × 22.5 mm Zorbax Sil column. Elution was effected using 10% ether in pentane at a flow rate of ± 20 ml/min. ^1H NMR signals were assigned by comparison with those reported for other retinoids[22]. Spectral signal designations for the C_{14} aldehydes **4a-d** and the 5-demethylretinals **1a-e** were based on the IUPAC retinoid numbering system[26]; those of the other compounds on the IUPAC nomenclature. NaB^2H_4 (99% ^2H) and $LiAl^2H_4$ (99% ^2H) were purchased from Fluka AG. The experimental conditions are described for the non-enriched compounds. For the labelled compounds only the spectral changes relative to the unlabelled compounds are given.

Methyl 7-methyl-3-oxo-6-octenoate (**6**)[19]

Sodium hydride (NaH, 9.6 g, 22 mmol, 55% in mineral oil) was rinsed three times with dry pet. ether to remove the mineral oil and suspended in dry ether. A solution of 0.27 mol of dimethyl carbonate (24 g) in 30 ml of dry ether was added and the stirred suspension was heated to reflux. 0.10 mol (12.6 g) of **5** was added dropwise over 3 h, maintaining a slow hydrogen evolution. The mixture was refluxed for a further 2 h and the solid mass was allowed to stand overnight at room temperature. After cooling to 0°C, a cooled solution of 20 ml of methanol in 100 ml of ether was added and the mixture was stirred vigorously for 2 h. The suspension was poured onto a mixture of ice (160 g) and concentrated HCl (40 ml). The aqueous layer was extracted three times with ether and the combined organic layers were washed with sodium bicarbonate followed by brine, dried over $MgSO_4$ and evaporated. Distillation under reduced pressure (3 Torr, 104–108°C) afforded 83% of **6**. ^1H NMR (100 MHz, $CDCl_3$): δ1.61 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 2.2–2.7 (m, 4H, 4-CH_2 and 5-CH_2), 3.43 (s, 2H, 2-CH_2), 3.71 (s, 3H, O–CH_3), 5.0 (m, 1H, 6-H).

Methyl 2,2-dimethyl-6-oxocyclohexanecarboxylate (**7**)[19]

Stannic chloride, ($SnCl_4$, 14.0 g, 54 mmol) was added dropwise to a cooled (0°C), stirred solution of 36 mmol of **6** (6.7 g) in 125 ml of dichloromethane. After addition, the solution was stirred overnight at room temperature. The mixture was then diluted with 200 ml of ether, washed four times with 100 ml portions of 5% HCl, neutralized and dried over $MgSO_4$. Evaporation of the solvents gave 6.5 g of crude product which was chromatographed (5% ether in pet. ether) in small portions (1–3 g) yielding a 3/1 keto/enol mixture of **7** (65–79%). ^1H NMR (100 MHz, $CDCl_3$): keto: δ 1.02 (s, 3H, CH_3), 1.07 (s, 3H, CH_3), 1.2–2.8 (m, 6H, 4-CH_2, 5-CH_2 and 6-CH_2), 3.19 (s, 1H, 2-H), 3.66 (s, 3H, O–CH_3); enol: δ 1.18 (s, 6H, 2 × CH_3), 1.2–2.8 (m, 6H, 4-CH_2, 5-CH_2 and 6-CH_2), 3.77 (s, 3H, O–CH_3).

Methyl 6-hydroxy-2,2-dimethylcyclohexanecarboxylate (**8a,b**)

Sodium borohydride ($NaBH_4$, 95 mg, 2.5 mmol) was added to a cooled (0°C) and stirred solution of 7.1 mmol (1.3 g) of **7** in 7.5 ml of ethanol and 1.3 ml of H_2O. The solution was stirred for 4 h at room temperature after which time ice-water as added. The mixture was extracted three times with ether and the combined organic layers were washed with water, saturated aqueous NH_4Cl solution and brine and dried over $MgSO_4$. Evaporation of the solvents yielded 1.3 g (100%) of virtually pure **8** as a mixture of *cis* and *trans* isomers.

8a: ^1H NMR (100 MHz, $CDCl_3$): δ 0.91, 0.99, 1.00 and 1.04 (s, 6H, 2 × CH_3), 1.1–1.8 (m, 6H, 4-CH_2, 5-CH_2 and 6-CH_2), 2.14 and 2.44 (d, 1H, 2-H, J_{HH} 10 Hz, 4 Hz), 3.67 and 3.69 (s, 3H, O–CH_3), 4.07 (m, 1H, 1-H). IR (NaCl): 3450 cm^{-1} (O–H stretch).

8b: ^1H NMR (100 MHz, $CDCl_3$): as for **8a** except for the singlet at δ 2.14 and 2.44 (2-H) and absence of the (1-H) signal at δ 4.07.

Methyl 6,6-dimethyl-1-cyclohexenecarboxylate (**9a,b**)

Using a syringe, 23 mmol $POCl_3$ (2.1 ml) was added slowly to a cooled (0°C), stirred solution of 7.0 mmol of **8** (1.3 mg) in 17 ml of pyridine. The solution was then heated to 95°C. After the mixture had been allowed to react for 22 h at this temperature, it was cooled, ice-water was added and the dark solution was extracted six times with ether. The combined organic layers were washed with 1 N HCl, neutralized, washed with brine, dried over $MgSO_4$ and concentrated. The yellow oil (1.0 g) was purified by column chromatography (5% ether in pet. ether) to yield 4.1 mmol (58%) of **9** (0.69 g) and 1.8 mmol (25%) of the corresponding α-compound (0.30 g) as colourless oils. The latter compound could be isomerized to the β-isomer **9** (1.5 mmol, 0.25 g (83%)) by refluxing for 24 h in a solution of $NaOCH_3$ in methanol.

9a: ^1H NMR (100 MHz, $CDCl_3$): δ 1.24 (s, 6H, 2 × CH_3), 1.4–1.8 (m, 4H, 4-CH_2 and 5-CH_2), 2.15 (m, 2H, 6-H), 3.70 (s, 3H, O–CH_3), 6.83 (t, 1H, 1-H, J_{HH} 4 Hz).

9b: ^1H NMR: as for **9a**, except for the triplet (*J* 5 Hz) for the 6-H signal and the absence of the 1-H signal.

α-Isomer of **9a** ^1H NMR (100 MHz, $CDCl_3$): δ 0.93 and 1.08 (s, 6H, 2 × CH_3), 1.1–2.2 (m, 4H, 5-CH_2 and 6-CH_2), 2.88 (m, 1H, 3-H), 3.69 (s, 3H, O–CH_3), 5.57 (m, 1H, 2-H), 5.80 (m, 1H, 1-H).

α-Isomer of **9b**: same NMR data except for a doublet for 3-H (*J* 2 Hz) and absence of the 1-H signal.

6,6-Dimethyl-1-cyclohexenemethanol (**10a-d**)

A solution of 3.8 mmol (0.64 g) of **9** in 10 ml of dry ether was added slowly to a cooled (−40°C), stirred suspension of 3.8 mmol (144 mg) $LiAlH_4$ in 15 ml of dry ether. After warming to 0°C over one hour, half-saturated aqueous NH_4Cl was carefully added. The layers were separated and the aqueous layer extracted with ether. The ethereal extracts were washed with water followed by brine, dried over $MgSO_4$ and evaporated to give 0.53 g of crude **10** which was used without further purification in the next step.

10a: ^1H NMR (100 MHz, $CDCl_3$): δ 1.08 (s, 6H, 2 × CH_3), 1.2–1.8 (m, 4H, 4-CH_2 and 5-CH_2), 2.04 (m, 2H, 3-CH_2), 4.11 (bs, 2H,

1'-CH_2), 5.66 (t, J_{HH} 4 Hz, 1H, 2-H). IR (NaCl): 3340 cm^{-1} (O–H stretch).
10b: ^1H NMR: as for **9a**, except for δ 2.04 (t, J_{HH} 6 Hz) and the absence of a signal at δ 5.66.
10c: ^1H NMR: as for **9a**, except for the absence of a signal at δ 4.11.
10d: ^1H NMR: as for **9b**, except for the absence of a signal at δ 4.11.

6,6-Dimethyl-1-cyclohexenecarboxaldehyde (**2a-d**)

A solution of 0.53 g of **10** in hexane was added to a suspension of 4.2 g of activated MnO_2 in 30 ml hexane and stirred at room temperature for 16 h. The MnO_2 was filtered off through Celite and washed extensively with ether. The filtrate was concentrated, leaving an oil which was chromatographed (7% ether–pet. ether) to give 1.6 mmol (0.22 g, 42% overall yield from **8**) pure crystalline **2**.
2a: ^1H NMR (100 MHz, $CDCl_3$): δ 1.27 (s, 6H, 2 × CH_3), 1.3–1.8 (m, 4H, 4-CH_2 and 5-CH_2), 2.34 (m, 2H, 3-CH_2), 6.68 (t, J_{HH} 4 Hz, 1H, 2-H), 9.27 (s, 1H, aldehyde-H).
2b: ^1H NMR: as for **2a**, except for δ 2.34 (t, J_{HH} 6 Hz) and the absence of a signal at δ 6.68.
2c: ^1H NMR: as for **2a**, except for the absence of a signal at δ 9.27.
2d: ^1H NMR: as for **2b**, except for the absence of a signal at δ 9.27.

5-(6,6-Dimethyl-1-cyclohexenyl)-3-methyl-2,4-pentadienal (**4a-d**)

Sodium hydride (NaH, 91 mg, 2.1 mmol, 55% in mineral oil) was rinsed three times with dry pet. ether to remove the mineral oil and suspended in 10 ml dry THF. A solution of 2.3 mmol (0.50 g) diethyl (3-cyano-2-methyl-2-propenyl)phosphonate **3**[18] in dry THF was added dropwise at 0°C and stirred for 30 min at room temperature. After cooling to 0°C, a solution of 1.6 mmol (0.22 g) of **2** in dry THF was added slowly and the stirred mixture was allowed to warm to room temperature. After 1½ h, the solution was poured into half-saturated NH_4Cl and extracted with ether. The organic layers were washed with water followed by brine, dried ($MgSO_4$) and then concentrated. The residue was chromatographed (5% ether in pet. ether) to give the pure C_{14} nitrile in 78% yield (0.25 g). This nitrile was dissolved in dry pet. ether and the stirred solution cooled to −60°C. A 1-M solution (1.3 equiv, 1.6 ml) of diisobutylaluminium hydride (Dibal) in hexane was added dropwise using a syringe and the mixture was then warmed to −20°C over 1 h. A suspension of 1/5 water/silica gel in 50% ether/pet. ether was added and the mixture stirred at 0°C. After drying with $MgSO_4$, the solids were filtered off and washed with dry ether. Evaporation of the solvents yielded 0.24 g (95%) of virtually pure **4** as a 9-*E*/*Z* mixture.
4a: ^1H NMR (100 MHz, $CDCl_3$) 9-E: δ 1.12 (s, 6H, 2 × CH_3), 1.4–1.8 (m, 4H, 2-CH_2 and 3-CH_2), 2.1 (m, 2H, 4-CH_2), 2.30 (s, 3H, 9-CH_3), 5.8–6.1 (m, 2H, 5-H and 10-H), 6.48 (d, J_{HH} ~ 11 Hz, 1H, 8-H), 6.60 (d, J_{HH} ~ 11 Hz, 1H, 7-H), 10.0 (d, *J* 8 Hz, 1H, 11-H).
4b: ^1H NMR (100 MHz, $CDCl_3$) 9-E: as for **4a**, absence of signal at δ 6.0–6.1, doublet for 10-H, J_{HH} 8 Hz.
4c: ^1H NMR (200 MHz, $CDCl_3$) 9-E: δ 1.10 (s, 6H, 2 × CH_3), 1.4–1.7 (m, 4H, 2-CH_2 and3-CH_2), 2.11 (m, 2H, 4-CH_2), 2.29 (d, J_{allyl} 1.1 Hz, 3H, 9-CH_3), 5.96 (d, J_{HH} 8.3 Hz, 1H, 10-H), 6.03 (t, J_{HH} 4.0 Hz, 1H, 5-H), 6.48 (s, 1H, 8-H), 10.11 (d, J_{HH} 8.3 Hz, 1H, 11-H).
4d: ^1H NMR (200 MHz, $CDCl_3$) 9-E: as for **4c**, except for the absence of a signal at 6.03 ppm.

5-demethylretinal **1a-d** (*all*-trans, *13*-cis, *11*-cis *and 9*-cis)

The C_{14} aldehyde **4** (0.24 g) was converted into 0.21 g (66%) of the corresponding retinal **1** using the same procedure as described for **4**. Larger amounts of pet. ether were required here for the Dibal reaction due to the smaller solvability of the C_{19} nitrile and 5-demethylretinal. The all-*trans* isomer of **1** was purified by means of column chromatography (20% ether/pet. ether) followed by HPLC (10% ether/pentane). A stirred solution of pure all-*trans*-5-demethylretinal in acetonitrile (~0.1 mg/ml) was irradiated under argon using a 200 W tungsten lamp for 1½ h. The solvent was evaporated and the residue dissolved in the HPLC solvent. By means of preparative HPLC separation, the 11-*cis*-, 9-*cis*- and all-*trans*-5-demethylretinals were isolated in a pure state. For the preparation of the 13-*cis* isomer in a pure state, the same procedure was followed except for the fact that in this case a solution of the all-*trans* isomer was irradiated for 5 min using light with λ > 420 nm (Corning CS 3–74 filter plate and 150 W tungsten lamp).
1a: ^1H NMR (200 MHz, $CDCl_3$): see Table I and Fig. 5. Measured $M^{+\bullet}$: 270.2002 (calcd.: 270.1983).
1b: ^1H NMR: absence of the 5-H signal and triplet instead of quartet for 3-CH_2. Measured $M^{+\bullet}$: 271.2046 (calcd.: 271.2046); deuterium incorporation calculated from the mass spectrum: 97.16%.
1c: ^1H NMR: singlet for the 8-H signal, absence of the 7-H signal. Measured $M^{+\bullet}$: 271.2049 (calcd.: 271.2046); deuterium incorporation calculated from the mass spectrum: 98.73%.
1d: ^1H NMR: singlet for the 8-H signal, triplet for 3-CH_2 and absence of the 5-H and 7-H signals. Measured $M^{+\bullet}$: 272.2116 (calcd.: 272.2109); deuterium incorporation calculated from the mass spectrum: 95.84%.

Acknowledgements

The authors wish to thank Mr. *F. Lefeber* for recording the NMR spectra and Mr. *J. J. van Houte* for recording the mass spectra. We are grateful to Mrs. *S. Amadio* for her assistance in preparing the English manuscript.

References

1 *M. Ottolenghi*, Adv. Photochem. **12**, 97 (1980).
2 *R. R. Birge*, Annu. Rev. Biophys. Bioeng. **10**, 315 (1981).
3 *G. Eyring, B. Curry, R. Mathies, R. Fransen, I. Palings* and *J. Lugtenburg*, Biochemistry **19**, 2410 (1980).
4 *G. A. Schick, T. M. Cooper, R. A. Holloway, L. P. Murray* and *R. R. Birge*, Biochemistry **26**, 2556 (1987).
5 *A. Cooper*, Nature **282**, 531 (1979).
6 *G. Wald*, Science **162**, 230 (1968).
7 *W. Stockenius* and *R. A. Bogomolni*, Annu. Rev. Biochem. **52**, 587 (1982).
8 *R. R. Birge* and *T. M. Cooper*, Biophys. J. **42**, 62 (1983).
9 *G. S. Harbison, S. O. Smith, J. A. Pardoen, J. M. L. Courtin, J. Lugtenburg, J. Herzfeld, R. A. Mathies* and *R. G. Griffin*, Biochemistry **24**, 6955 (1985).
10 *S. O. Smith, I. Palings, V. Copié, D. P. Raleigh, J. M. L. Courtin, J. A. Pardoen, J. Lugtenburg, R. A. Mathies* and *R. G. Griffin*, Biochemistry **26**, 1606 (1987).
11 *L. C. P. J. Mollevanger, A. P. M. Kentgens, J. A. Pardoen, J. M. L. Courtin, W. S. Veeman, J. Lugtenburg* and *W. J. de Grip*, Eur. J. Biochem. **163**, 9 (1987).
12 *R. van der Steen, P. L. Biesheuvel, R. A. Mathies* and *J. Lugtenburg*, J. Am. Chem. Soc. **108**, 6410 (1986).
13 *S. O. Smith, J. Lugtenburg* and *R. A. Mathies*, J. Membrane Biol. **85**, 95 (1985) and references cited therein.
14 *S. O. Smith, M. S. Braiman, A. B. Myers, J. A. Pardoen, J. M. L. Courtin, C. Winkel, J. Lugtenburg* and *R. A. Mathies*, J. Am. Chem. Soc. **109**, 3108 (1987).
15 *M. R. Fransen, I. Palings, J. Lugtenburg, P. A. A. Jansen* and *G. W. T. Groenendijk*, Recl. Trav. Chim. Pays-Bas **99**, 384 (1980).
16 *M. Muradin-Szweykowska, J. A. Pardoen, D. Dobbelstein, L. J. P. van Amsterdam* and *J. Lugtenburg*, Eur. J. Biochem. **140**, 173 (1984).
17 *J. M. L. Courtin, L. Verhagen, P. L. Biesheuvel, J. Lugtenburg, R. L. van der Bend* and *K. van Dam*, Recl. Trav. Chim. Pays-Bas **106**, 112 (1987).
18 *K. Fujiwara, H. Takahashi* and *M. Ohta*, Bull. Chem. Soc. Jpn **35**, 1743 (1962).
19 *J. D. White, R. W. Skeean* and *G. L. Trammell*, J. Org. Chem. **50**, 1939 (1985).
20 *M. Denny* and *R. S. H. Liu*, J. Am. Chem. Soc. **99**, 4865 (1977) and references cited therein.
21 *R. S. H. Liu* and A. E. Asato, Tetrahedron **40**, 1931 (1984).
22 *D. J. Patel*, Nature **221**, 825 (1969).
23 *A. J. Fatadi*, Synthesis 65 (1976).
24 *W. Gärtner, P. Towner, H. Hopf* and *D. Oesterhelt*, Biochemistry **22**, 2637 (1983).
25 *W. C. Still, M. Kahn* and *J. Mitra*, J. Org. Chem. **43**, 2923 (1978).
26 IUPAC-IUB Joint Commission on Biochemical Nomenclature, Eur. J. Biochem. **129**, 1 (1982).