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13-Demethyl-13-substituted-13,14-dihydroretinols as potential affinity labels of retinol-binding proteins: syntheses and stability studies

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Abstract

13-Demethyl-13-substituted-13,14-dihydroretinols were synthesized and their stability under various conditions was measured in order to evaluate whether they would be useful as affinity labels of retinol binding proteins and retinol metabolizing enzymes. The 13-chloro analog could not be isolated because it eliminated HCl under the Wittig reaction conditions of its preparation. The *trans*- and *cis*-13,14-epoxy analogs are stable in non-protic organic solvents, but undergo an elimination reaction under various chromatographic conditions and in mixtures of organic solvents with water or alcohol. The 13-hydroxy and 13-methoxy analogs are stable in aqueous solutions and are therefore suitable for biological studies.

Keywords: Retinoids; Affinity label; Stability; Epoxide

1. Introduction

Vitamin A and its metabolites have many biological functions. Retinal, the aldehyde derived from vitamin A, is a central player in vision. Retinol and retinyl esters

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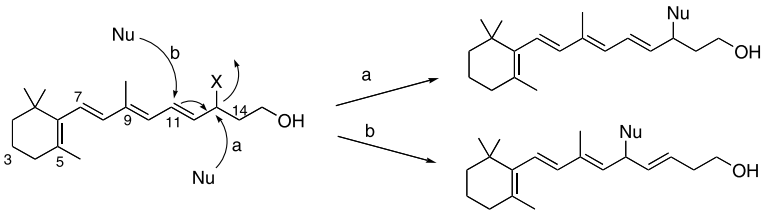
also participate in the visual cycle. The latter is also used for storage of retinoids. Retinoic acids are important for cellular differentiation and morphogenesis. 14-Hydroxy-retro-retinol and anhydroretinol play a role in cell growth, proliferation and death. 13,14-Dihydroxyretinol, 4-oxo-retinol, and 4-oxo-retinal probably also have cellular functions. Retinal is also essential for the proton pumping activity of the bacterial pigment bacteriorhodopsin [1].

Many enzymes and proteins interact with vitamin A and its derivatives. Among these are the various opsins, which combine with retinal to form the visual pigments, dehydrogenases, ester synthases, transferases and hydrolases, trans retinyl ester isomerohydrolase, specific retinoid binding proteins and receptors and bacterioopsin. Some of these proteins have been isolated and (partially) structurally characterized, while others are only poorly characterized. One useful tool in the study of the interaction between these proteins and their retinoid ligands is affinity labeling by retinol/al analogs. Diazo ketones and esters, azides, diazirines, allyl bromides, halomethyl ketones and α-bromo esters are examples of affinity labels that have been used in the study of visual pigments [2], bacteriorhodopsin [2e,3], lecithin:retinol acyltransferase (LRAT) [4] and retinoid binding proteins [5].

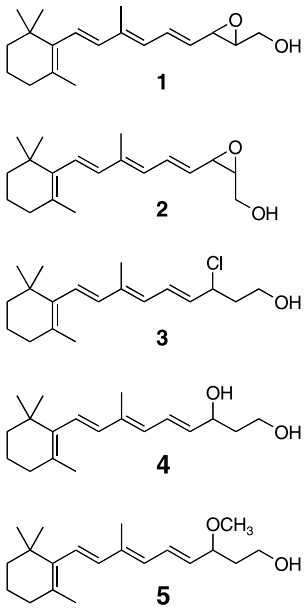
In the present paper we describe the design of a new family of selective affinity labels for enzymes and proteins that interact with retinol. It is based on 13-substituted-13,14-dihydroretinol, which can undergo a nucleophilic displacement at at least two possible carbons (Scheme 1). This paper describes their synthesis, as well as structure–stability correlation studies, which were carried out in order to assess their suitability for affinity labeling experiments.

2. Results and discussion

The design of the potential affinity labels is based on the introduction of a leaving group at the C_{13} sp^3 carbon of a retinol skeleton (Scheme 1). This leaving group can be displaced by nucleophilic residues of the target enzyme/protein. The structure of these compounds confers high versatility since they can interact with nucleophiles that are spatially close to C_{13} (S_N2 reaction) and C_{11} (S_N2' reaction) and even to C_9 and C_7 of the retinol skeleton (though with higher loss



Scheme 1.



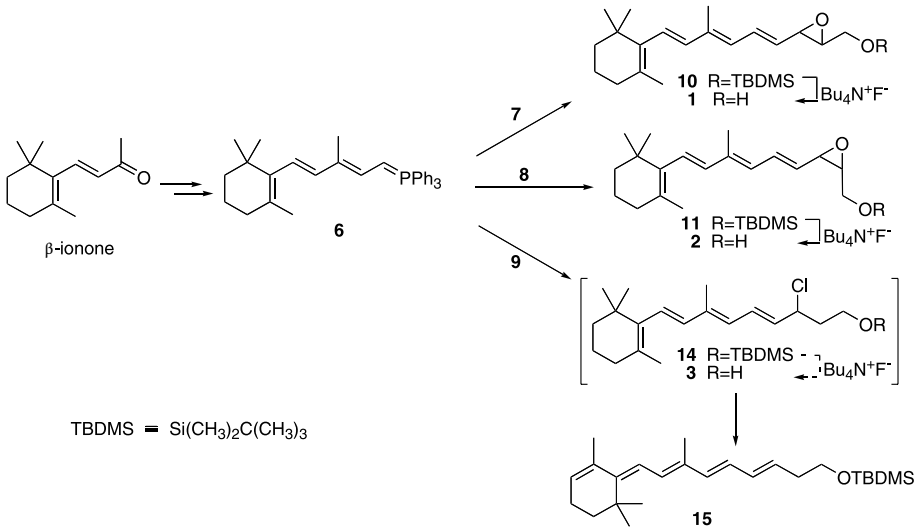
Scheme 2.

of conjugation). On the other hand, these affinity labels will exhibit selectivity only towards retinol binding proteins and enzymes bearing a nucleophilic residue at the appropriate position. In contrast, diazo- or azido-based affinity labels produce reactive intermediates that interact non-selectively with neighbouring residues.

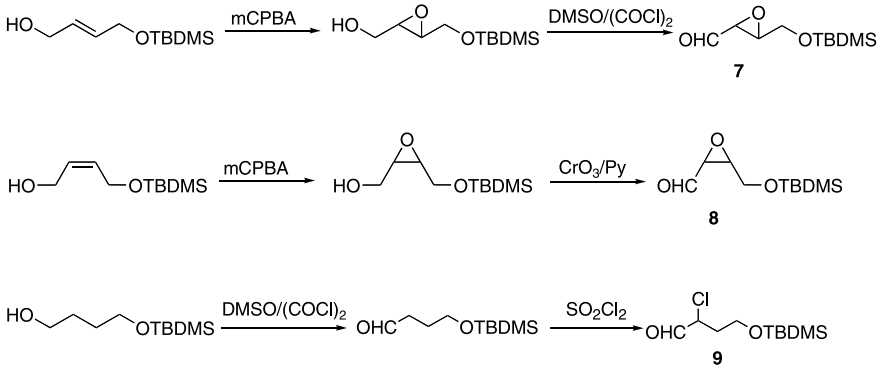
The leaving groups tested were 13,14-epoxy- (*trans* and *cis*), 13-chloro-, 13-hydroxy- and 13-methoxy- of 13-demethyl-13,14-dihydroretinol (compounds **1**–**5**, respectively. Scheme 2). The chlororetinol analog was expected to be the most reactive and the hydroxy- and methoxy analogs to be the least reactive affinity labels. The substituents replace the 13-methyl group of the native retinol, thus avoiding unexpected steric problems from additional substitution.

2.1. Synthesis

A convergent strategy for the synthesis of the 13-substituted retinol analogues **1**–**3** was employed, based on the assembly of two fragments, the all-*trans* isomer of the C15 phosphorane **6** and an appropriate four-carbon chain aldehyde, via the Wittig–Horner condensation (Scheme 3). The C4 aldehydes **7**–**9**, which carry the electrophilic functionalities, were synthesized via a simple functionalization–oxidation or oxidation–functionalization sequence (Scheme 4). An efficient linear approach, based on a key Reformatsky reaction, was employed in the synthesis of the hydroxy- and methoxyretinol analogs **4** and **5** (Scheme 6).



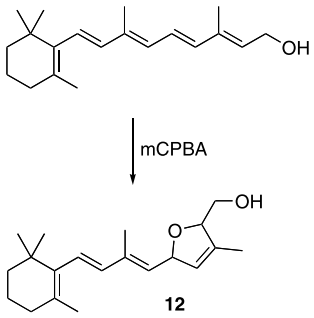
Scheme 3.



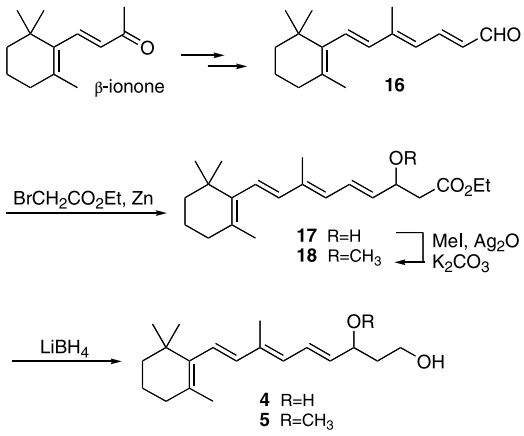
Scheme 4.

2.1.1. Epoxyretinol

The synthesis of 13-demethyl-13,14-*trans*-epoxyretinol **1** and 13-demethyl-13,14-*cis*-epoxyretinol **2** ({3-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5- trienyl]-oxyranyl}methanol) is outlined in Scheme 3 [6]. *trans* and *cis* C4 epoxy aldehydes (**7** and **8**, respectively) were prepared from monosilylated *trans* and *cis* 2-butene-1,4-diol by a short epoxidation and oxidation sequence (Scheme 4) [7]. The readily available C15 phosphorane **6** [8] was condensed with the appropriate C4 epoxy aldehyde (either **7** or **8**), yielding a mixture of all-*trans* and 11-*cis* O-protected epoxyretinol analogs **10** and **11**, respectively. Removal of the silyl protecting group furnished the epoxyretinol analogs **1** and **2** (as an all-*trans*/11-*cis* mixture).



Scheme 5.



Scheme 6.

It should be noted that initially we attempted to synthesize 13,14-epoxyretinol by direct mCPBA epoxidation of retinol. We assumed that the directing effect of the allylic alcohol [9] would lead to preferred epoxidation of the C_{13}=C_{14} double bond over the other more substituted double bonds. Indeed, such a directing effect was observed, but, to our surprise, the major product was 11,14-epoxyretinol **12** (Scheme 5) [10]. Small amounts of a bisepoxy product **13** were also isolated, as determined by MS analysis. Attempts to carry out Sharpless chiral epoxidation yielded starting material only. The same was also true for H_2O_2/OH^- epoxidation of retinal.

2.1.2. Chlororetinol

The same approach was applied to the synthesis of 13-chloro-13,14-dihydroretinol **3** (3-chloro-7-methyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-4,6,8-trien-1-ol) (Scheme 3). The C4 α-chloro aldehyde unit **9** was prepared from butane-1,4-diol by mono silylation, Swern oxidation and chlorination (Scheme 4) [11]. It was then reacted with the same C15 phosphorane **6** used for the synthesis of the epoxyretinols.

No traces of the desired O-protected-13-chlororetinol analog **14** were detected. Instead, the major crude product of the reaction was identified as the 4,13-elimination product **15** (Scheme 3). It exhibited a 328, 344, 360 nm triplet in its UV absorption spectrum (in ether) and its ^{1}H- NMR and MS supported the suggested structure.

We did not apply any other synthetic strategy for the preparation of the chlororetinol analog **3** since it became clear from our stability studies that the putative chlororetinol would be unstable under the normal aqueous conditions of biological manipulations (Section 2.2).

2.1.3. Hydroxy- and methoxyretinol

Reformatsky reaction [12] on the readily available C17 aldehyde **16** [13] yielded ethyl 13-hydroxy-13,14-dihydroretinoate **17** (Scheme 6). The latter could be selectively alkylated on the 13-hydroxy substituent (MeI, Ag_2O, and K_2CO_3 [14]) to the corresponding methoxy ester **18**. Reduction of either hydroxy ester **17** or methoxy ester **18** afforded the corresponding retinol analogs **4** (7-methyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-4,6,8-triene-1,3-diol) and **5** (3-methoxy-7-methyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-4,6,8-trien-1-ol), respectively (Scheme 6).

2.2. Stability

The five 13-substituted-13,14-dihydroretinol analogs (Scheme 2) span a wide range of chemical stability. The 13-chloro-analog **14** is so unstable that it could not be isolated even as a crude product. In fact, only the elimination product, the retro-retinol analog **15** (Scheme 3), was identified in the crude reaction mixture and isolated in good yield. The conditions of the Wittig reaction are probably basic enough to promote the observed 4,13 HCl elimination. The hydroxy- and methoxyretinol analogs (**4** and **5**, respectively; Scheme 6) are stable in organic solvents and in neutral aqueous solution. No elimination product could be detected upon incubation in a water: acetonitrile (1:1) solution for four days. The epoxyretinol analogs **1** and **2** (Scheme 2) exhibited marginal stability. They were obtained in good yield (and spectrally characterized) as crude products, but underwent an elimination reaction under a variety of chromatographic purification protocols. They were stable in organic solvents (CH_2Cl_2, CH_3CN, DMF, and DMSO), but extremely unstable in organic solvent mixtures with protic solvents such as water or alcohols. Thus, the half-life of the 13,14-*trans*-epoxyretinol in wet dichloromethane or ethanol: dichloromethane solution is about 40 min (Fig. 1). It was previously demonstrated that the half-life of a similar compound (the corresponding retinoate) in methanol is only 7 min [6b]. In more polar solvents such as 4:1 acetonitrile: water solution, the half-life of the epoxyretinol analog **1** is much shorter—just a couple of minutes. The *cis* epoxyretinol analog **2**, as well as the two corresponding silyl ether protected analogs **10** and **11** (Scheme 3), exhibited similar elimination kinetics.

It is interesting to note that two different products were obtained under different conditions. Silica gel chromatography of the epoxyretinol analogs, or their incubation in wet dichloromethane or in methanol, afforded the expected 4,13 elimination products that exhibited a 334, 349, 368 nm triplet in the UV absorption spectrum (in

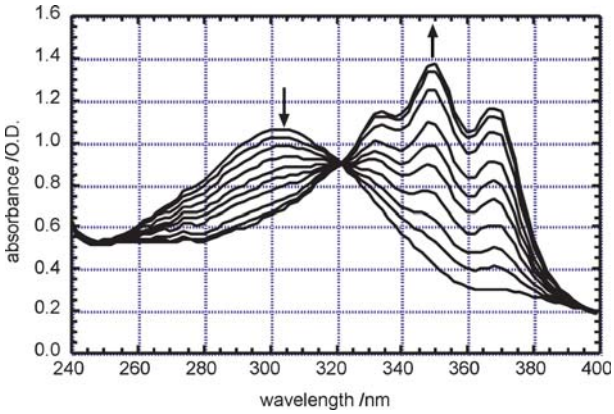


Fig. 1. Stability of epoxyretinol **1**. Time course (10 min intervals) of elimination from the epoxyretinol (λ_{max} 302 nm) to the corresponding retro-retinol (λ_{max} 348 nm) in dichloromethane:ethanol solution.

CH_2Cl_2, see Fig. 1). NMR analysis of one of these products, **19**, derived from compound **10**, confirmed its structure. On the other hand, incubation in acetonitrile: water yielded a product that exhibited a 302, 316, 332 nm triplet in its UV absorption spectrum. This product was not further analyzed.

3. Conclusion

This study describes a structure–stability relationship for 13-substituted-13,14-dihydroretinols. The most reactive analog, 13-chloro-13,14-dihydroretinol, is so unstable it eliminates HCl spontaneously upon its formation via the Wittig reaction. The 13,14-epoxides are stable in non-protic organic solvents, but undergo a facile 4,13 elimination reaction in alcohols or aqueous solutions. In contrast, the 13-hydroxy- and methoxyretinols are stable also in aqueous solutions. This trend correlates well with the chemical reactivity of allylic halides, epoxides, alcohols and ethers.

From a practical standpoint, it is clear that only the hydroxy- and methoxyretinol analogs **4** and **5** could be used for affinity labeling studies. Although they are the least reactive, they are the most stable of the tested 13-substituted-13,14-dihydro-retinol analogs. They are currently under investigation as affinity labels of some retinol metabolizing enzymes.

4. Experimental

4.1. General

^1H, and ^{13}C NMR spectra were recorded at 200, 300 or 600, and 50, 75 or 150 MHz, respectively, in $CDCl_3$ (TMS as an internal standard) or, when specified,

in CD_3OD. ^1H NMR assignments were supported by COSY experiments, while ^{13}C NMR assignments were supported by DEPT and hetero COSY experiments. J values are given in Hz. Mass spectra were recorded in DCI mode with methane or ammonia as the reagent gas, unless otherwise stated. TLC was performed on E. Merck 0.2 mm precoated silica gel F-254 plates, and viewed by UV and vanillin [15]. Chromatography refers to flash column chromatography [16], carried out on silica gel 60 (230–400 mesh ASTM, E. Merck). Anhydrous solvents were dried and freshly distilled (THF and ether from sodium/benzophenone, pyridine and triethylamine from CaH_2, CH_2Cl_2 from $CaCl_2$ and DMF from 4 Å molecular sieves).

4.2. Synthesis

Compound **6** was prepared according to a literature procedure [8]. Compound **7** and compound **8** [17] were prepared according to [18], except a Swern oxidation was used instead of the SO_3-pyridine oxidation of the primary alcohol in the last step [17b]. Compound **16** was prepared as described elsewhere [13].

4.2.1. 4-(t-Butyldimethylsilyloxy)-2-chlorobutanal (9)

A solution of sulfuryl chloride (distilled, 65 mg, 0.5 mmol) in CH_2Cl_2 (2 ml) was added slowly to a solution of 4-(*t*-butyldimethylsilyloxy)butanal [19] (100 mg, 0.5 mmol) in CH_2Cl_2 (5 ml). After stirring for 1 h, the reaction mixture was extracted twice with saturated $NaHCO_3$ solution and then with saturated NaCl solution until the aqueous layer became neutral. The organic phase was dried ($MgSO_4$) and the solvent was removed to provide 76 mg (64% yield) of **9**. ^1H NMR δ 9.53 (1 H, d, J 1.9), 4.41 (1H, ddd, J 7.3, 5.0, 1.9), 3.81 (2H, m), 2.23 (1H, ddt, J 14.5, 7.2, 5.0), 2.05 (1H, dddd, J 14.5, 7.3, 5.9, 4.2), 0.88 (9H, s), 0.06 (3H, s); ^{13}C NMR δ 195.2, 61.5, 58.2, 35.6, 25.8, 18.2, −5.5, −5.5; MS m/z 239, 237 (MH^+), 238, 236.

4.2.2. O-t-butyldimethylsilyl-13-demethyl-13,14-trans-epoxy-13, 14-dihydroretinol (10)

n-BuLi (0.6 ml, 1 M in hexane) was injected to a solution of the phosphonium salt of **6** (350 mg, 0.64 mmol) in dry THF (7 ml) under Ar atmosphere at −78 °C. The reaction mixture became red. After stirring for 20 min at −78 °C, a solution of aldehyde **7** (70 mg, 0.32 mmol) in dry THF (3 ml) was added and the reaction mixture was stirred for additional 1 h. Water was added and the mixture was extracted twice with hexane. The organic phase was dried ($MgSO_4$) and the solvents were removed to give the product as a mixture of 1.3:1 11-*cis*:11-*trans* isomers. The crude product could not be chromatographed due to its instability. *trans* Isomer: ^1H NMR δ 6.75 (1H, dd, J 15.2, 11.4, H-11), 6.14 (1H, d, J 16.1, H-7), 6.02 (1H, d, J 16.1, H-8), 5.99 (1H, d, J 11.4, H-10), 5.37 (1H, dd, J 15.2, 8.3, H-12), 3.77 (1H, dd, J 12.0, 3.2, H-15), 3.74 (1H, m, H-15), 3.32 (1H, dd, J 8.3, 2.1, H-13), 2.99 (1H, m, H-14), 1.97 (2H, br t, J 5.9, CH_2-4), 1.88 (3H, s, Me-19), 1.64 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.00 (6H, s, Me-16,17), 0.87 (9H, s, *t*-Bu), 0.06 (6H, s, $SiMe_2$). *cis* Isomer: ^1H NMR δ 6.57 (1H, dd, J 12.0, 10.2, H-11), 6.37 (1H, d, J 12.0, H-10), 6.19 (1H, d, J 15.8, H-7), 6.08 (1H, d, J 15.8, H-8), 5.07 (1H, dd, J 10.2, 8.0, H-12), 3.85 (1H, dd, J 12.8, 3.1, H-15), 3.68 (2H, m, H-13, H-15),

2.99 (1H, m, H-14), 1.97 (2H, br t, J 5.9, CH_2-4), 1.88 (3H, s, Me-19), 1.67 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.02 (6H, s, Me-16,17), 0.87 (9H, s, *t*-Bu), 0.04 (6H, s, $SiMe_2$); ^{13}C NMR (mixture of *cis* and *trans* isomers) δ 137.3, 137.1, 132.0, 131.8, 130.1, 129.0, 128.1, 127.8, 126.5, 123.6, 63.0 (C-15, *cis*), 63.0 (C-15, *trans*), 60.9 (C-14, *trans*), 60.4 (C-14, *cis*), 56.1 (C-13, *trans*), 51.9 (C-13, *cis*), 39.5, 34.1, 32.9, 28.8, 25.8, 21.6, 19.1, 18.2, 12.5, 12.2, −5.4; λ_{max}(ether) 298 nm; HRMS m/z 402.2950 (M^+. $C_{25}H_{42}SiO_2$ requires 402.2954).

*4.2.3. 13-Demethyl-13,14-trans-epoxy-13,14-dihydroretinol (**1**)*

A solution of tetrabutylamonium fluoride (0.4 ml, 1 M in THF) was added dropwise to a solution of **10** (160 mg, 0.4 mmol) in dry THF (10 ml). After 30 min the mixture was diluted with hexane and extracted twice with water. The organic phase was dried ($MgSO_4$) and the solvents were evaporated to give a 1.6:1 mixture of 11-*cis*:11-*trans* isomers of the product. The crude product could not be chromatographed due to its instability. *cis* Isomer: ^{1}H NMR δ 6.65 (1H, dd, J 12.0, 11.8, H-11), 6.42 (1H, d, J 11.8, H-10), 6.24 (1H, d, J 16.0, H-7), 6.13 (1H, d, J 16.0, H-8), 5.10 (1H, dd, J 12.0, 9.1, H-12), 3.93 (1H, dd, J 12.5, 2.3, H-15), 3.86 (1H, dd, J 9.1, 2.1, H-13), 3.72 (1H, dd, J 12.5, 3.4, H-15), 3.11 (1H, m, H-14), 2.01 (2H, br t, J 6.7, CH_2-4), 1.93 (3H, s, Me-19), 1.71 (3H, s, Me-18), 1.64 (2H, m, CH_2-3), 1.45 (2H, m, CH_2-2), 1.02 (6H, s, Me-16,17); *trans* isomer: ^{1}H NMR δ 6.80 (1H, dd, J 15.2, 11.4, H-11), 6.20 (1H, d, J 16.1, H-7), 6.06 (1H, d, J 16.1, H-8), 6.03 (1H, d, J 11.4, H-10), 5.40 (1H, dd, J 15.2, 8.1, H-12), 3.90 (1H, dd, J 10.9, 2.2, H-15), 3.68 (1H, dd, J 10.9, 3.8, H-15), 3.48 (1H, dd, J 8.1, 2.4, H-13), 3.11 (1H, m, H-14), 2.01 (2H, br t, J 6.7, CH_2-4), 1.93 (3H, s, Me-19), 1.69 (3H, s, Me-18), 1.64 (2H, m, CH_2-3), 1.45 (2H, m, CH_2-2), 1.01 (6H, s, Me-16,17); *cis* isomer: ^{13}C NMR δ 137.3 (C-8), 130.7 (C-11), 128.2 (C-7), 126.0 (C-12), 123.5 (C-10), 61.1 (C-15), 60.4 (C-14), 51.7 (C-13), 39.6 (C-2), 34.2 (C-1), 33.0 (C-4), 28.9 (Me-16,17), 21.7 (Me-18), 19.2 (C-3), 12.3 (Me-19); λ_{max}(ether) 300 nm; HRMS m/z 288.2096 (M^+. $C_{19}H_{28}O_2$ requires 288.2089).

*4.2.4. O-t-butyldimethylsilyl-13-demethyl-13,14-cis-epoxy-13, 14-dihydroretinol (**11**)*

Compound **11** was prepared as described for **10**, yielding a mixture of 1.5:1 11-*cis*:11-*trans* isomers. The crude product could not be chromatographed due to its instability. *cis* Isomer: ^{1}H NMR δ 6.64 (1H, ddd, J 12.0, 10.9, 1.1, H-11), 6.41 (1H, d, J 12.0, H-10), 6.24 (1H, d, J 16.3, H-7), 6.13 (1H, d, J 16.3, H-8), 5.23 (1H, dd, J 10.9, 7.9, H-12), 3.87 (1H, ddd, J 7.9, 4.5, 1.1, H-13), 3.79 (1H, dd, J 11.7, 4.5, H-15), 3.71 (1H, dd, J 11.7, 6.0, H-15), 3.29 (1H, br q, J 4.5, H-14), 2.01 (2H, br t, J 6.0, CH_2-4), 1.93 (3H, s, Me-19), 1.71 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.03 (6H, s, Me-16,17), 0.90 (9H, s, *t*-Bu), 0.10 (6H, s, $SiMe_2$); *trans* isomer: ^{1}H NMR δ 6.80 (1H, dd, J 15.0, 11.3, H-11), 6.21 (1H, d, J 16.2, H-7), 6.08 (1H, d, J 16.2, H-8), 6.05 (1H, d, J 11.3, H-10), 5.51 (1H, dd, J 15.0, 7.7, H-12), 3.80 (1H, dd, J 11.8, 4.6, H-15), 3.72 (1H, dd, J 11.8, 6.0, H-15), 3.55 (1H, dd, J 7.7, 4.4, H-13), 3.28 (1H, m, H-14), 2.01 (2H, br t, J 6.0, CH_2-4), 1.93 (3H, s, Me-19), 1.71 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.02 (6H, s, Me-16,17), 0.91 (9H, s, *t*-Bu), 0.08 (6H, s, $SiMe_2$); ^{13}C NMR (mixture of *cis* and *trans* isomers) δ 137.3, 137.1, 130.6, 128.5, 128.3, 127.9, 127.6, 125.6, 123.7, 123.2, 61.9 (*cis*), 61.6 (*trans*), 59.3 (*trans*),

58.9 (*cis*), 56.7 (*trans*), 52.7 (*cis*), 41.5, 39.5, 34.1, 32.9, 28.8, 25.8, 21.6, 19.1, 18.2, 12.5 (*trans*), 12.1 (*cis*), −5.3, −5.4; λ_{max}(ether) 298 nm; HRMS m/z 402.2940 (M^+. $C_{25}H_{42}SiO_2$ requires 402.2954).

*4.2.5. 13-Demethyl-13,14-cis-epoxy-13,14-dihydroretinol (**2**)*

Compound **2** was prepared as described for **1**, yielding a 3.5:1 mixture of 11-*cis*:11-*trans* isomers of the product. *trans* Isomer: ^{1}H NMR δ 6.81 (1H, dd, J 15.2, 11.6, H-11), 6.06 (1H, d, J 16.0, H-8), 6.04 (1H, d, J 11.6, H-10), 5.53 (1H, dd, J 15.2, 8.2, H-12), 3.59 (1H, dd, J 8.2, 4.4, H-13), 3.36 (1H, obscured, H-14), 2.01 (2H, br t, J 6.4, CH_2-4), 1.93 (3H, s, Me-19), 1.70 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.45 (2H, m, CH_2-2), 1.02 (6H, s, Me-16,17); *cis* Isomer: ^{1}H NMR δ 6.65 (1H, dd, J 12.1, 10.9, H-11), 6.38 (1H, d, J 12.1, H-10), 6.25 (1H, d, J 16.1, H-7), 6.13 (1H, d, J 16.1, H-8), 5.26 (1H, dd, J 10.9, 8.2, H-12), 3.91 (1H, dd, J 8.2, 4.4, H-13), 3.81 (1H, dd, J 12.4, 4.4, H-15), 3.68 (1H, dd, J 12.4, 6.6, H-15), 3.36 (1H, dt, J 6.6, 4.4, H-14), 2.01 (2H, br t, J 6.4, CH_2-4), 1.93 (3H, s, Me-19), 1.71 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.45 (2H, m, CH_2-2), 1.02 (6H, s, Me-16,17); *cis* isomer: ^{13}C NMR δ 137.3 (C-8), 130.7 (C-11), 128.1 (C-7), 126.0 (C-12), 123.5 (C-10), 61.1 (C-15), 58.7 (C-14), 51.7 (C-13), 39.5 (C-2), 34.2 (C-1), 33.0 (C-4), 28.9 (C-16,17), 21.7 (C-18), 19.2 (C-3), 12.2 (C-19); λ_{max}(ether) 300 nm; HRMS m/z 288.2090 (M^+. $C_{19}H_{28}O_2$ requires 288.2089).

*4.2.6. 11,14-Epoxyretinol (**12**)*

mCPBA (27 mg, 0.09 mmol) was added to a mixture of *trans* retinol (30 mg, 0.1 mmol), K_2HPO_4 (27 mg, 0.15 mmol) and water (40 μl) in CH_2Cl_2 (4 ml) at r.t. After 30 min, saturated $NaHCO_3$ solution was added and the mixture was extracted twice with CH_2Cl_2. The organic phase was dried ($MgSO_4$) and evaporated to dryness. Chromatography afforded 9 mg (30% yield) of **12**. A bisepoxy product **13** was also isolated (about 10% yield), as determined by its mass spectrum. ^{1}H NMR δ 6.15 (1H, d, J 16.0, H-7), 5.99 (1H, d, J 16.0, H-8), 5.47 (1H, m, H-10), 5.42 (1H, d, J 11.1, H-12), 4.95 (1H, br d, J 8.1, H-11), 4.03 (1H, dd, J 11.5, 3.7, H-15), 3.90 (1H, m, H-14), 3.62 (1H, dd, J 11.5, 4.6, H-15), 2.00 (2H, br t, J 6.4, CH_2-4), 1.89 (3H, d, J 1.2, CH_3-19), 1.85 (3H, m, CH_3-20), 1.67 (3H, d, J 0.7, CH_3-18), 1.59 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.00 (6H, s, CH_3-16,17); ^{13}C NMR δ 137.5 (C-6), 136.9 (C-8), 129.0 (C-5), 127.3 (CH), 127.2 (C-7), 126.0 (CH), 70.5 (C-11), 67.7 (C-15), 66.1 (C-14), 39.6 (C2), 34.2 (C-1), 32.9 (C-4), 28.9 (C-16,17), 21.6 (C-18), 19.5 (C-20), 19.3 (C-3), 12.8 (C-19); MS m/z 320 (MNH_4^+), 303 (MH^+).

*4.2.7. Bisepoxy retinol (**13**)*

MS m/z 336 (MNH_4^+), 319 (MH^+), 303, 301.

*4.2.8. O-t-butyldimethylsilyl-13-demethyl-retroretinol (**15**)*

Aldehyde **9** (70 mg, 0.3 mmol) was coupled to phosphorane **6** (330 mg, 0.6 mmol) as described for **10**. After drying the organic phase and evaporation of the solvent, the elimination product **15** was obtained. ^{1}H NMR δ 6.75 (1H, d, J 12, H-8), 6.58 (1H, dd, J 15.5, 8.5, H-11), 6.36 (1H, d, J 12, H-7), 6.2 (2H, m, H-10, H-12), 5.76

(1H, br t, *J* 4, H-4), 5.67 (1H, dt, *J* 16, 7, H-13), 3.66 (2H, m, CH_2-15), 2.33 (2H, m, CH_2-14), 2.03 (2H, m, CH_2-3), 1.88 (3H, s, Me-19), 1.4 (2H, m, CH_2-2), 1.24 (3H, s, Me-18), 1.02 (6H, s, Me-16,17), 0.90 (9H, s, *t*-Bu), 0.07 (6H, s, $SiMe_2$); λ_{max}(ether) 328, 344, 366 nm triplet; MS *m*/*z* (EI) 386 (M^+), 255, 201, 185.

*4.2.9. Ethyl 13-demethyl-13-hydroxy-13, 14-dihydroretinoate (**17**)*

Ethyl bromoacetate (1.1 ml, 10 mmol) was added dropwise to a mixture of freshly activated Zinc (0.67 g, 10 mmol) in triethylborate (8 ml) and dry THF (3 ml), under Ar atmosphere. After the addition was completed, the mixture was heated to 55 °C and after a few minutes aldehyde **16** (1 g, 4.1 mmol), dissolved in dry THF (5 ml), was added. After 3 h the reaction mixture was cooled to r.t. and dilute aqueous NH_4OH was added. The mixture was extracted twice with ether and the combined organic phase was washed with water, dried ($MgSO_4$) and concentrated. Chromatography (EtOAc:hexane 1:9) yielded 990 mg (73% yield) of clean **17**. ^{1}H NMR δ 6.66 (1H, ddd, *J* 15.1, 11.2, 1.2, H-11), 6.16 (1H, d, *J* 16.2, H-7), 6.05 (1H, d, *J* 16.2, H-8), 6.00 (1H, d, *J* 11.2, H-10), 5.70 (1H, dd, *J* 15.1, 6.3, H-12), 4.64 (1H, br quint, *J* 5.2, H-13), 4.16 (2H, q, *J* 7.2, OCH_2), 3.12 (1H, d, *J* 4.2, OH), 2.58 (1H, dd, *J* 16.2, 4.2, CH_2-14), 2.54 (1H, dd, *J* 16.2, 8.2, CH_2-14), 2.01 (2H, t, *J* 6.0, CH_2-4), 1.92 (3H, d, *J* 1.2, Me-19), 1.69 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.28 (3H, t, *J* 7.2, Me), 1.01 (6H, s, Me-16,17); ^{13}C NMR δ 172.2 (C-15), 137.7 (C-9), 137.4 (C-8), 136.7 (C-6), 133.1 (C-12), 129.2 (C-5), 128.5 (C-10), 127.4 (C-11), 127.2 (C-7), 68.9 (C-13), 60.8 (OCH_2), 41.7 (C-14), 39.6 (C-2), 34.2 (C-1), 33.0 (C-4), 28.9 (Me-16,17), 21.7 (C-18), 19.3 (C-3), 14.7 (Me), 12.6 (C-19); λ_{max}(CH_2Cl_2) 296 nm; HRMS *m*/*z* 332.2354 (M^+. $C_{21}H_{32}O_3$ requires 332.2351).

*4.2.10. 13-Demethyl-13-hydroxy-13,14-dihydroretinol (**4**)*

Lithium borohydride (85 mg, 3.9 mmol) was added to a solution of **17** (1.3 g, 3.9 mmol) in freshly distilled dry ether (30 ml) under N_2 atmosphere at 0 °C. The resulting mixture was stirred at 0 °C. After 20 min water was added and the mixture was extracted twice with ether. The combined organic phase was dried ($MgSO_4$) and concentrated. Chromatography (EtOAc:hexane 1:1) afforded 645 mg (57% yield) of the product **4**. ^{1}H NMR δ 6.64 (1H, ddd, *J* 15.1, 11.3, 1.3, H-11), 6.17 (1H, d, *J* 16.5, H-7), 6.07 (1H, d, *J* 16.5, H-8), 6.02 (1H, d, *J* 11.3, H-10), 5.75 (1H, dd, *J* 15.1, 6.6, H-12), 4.49 (1H, td, *J* 6.6, 5.7, H-13), 3.89 (1H, dt, *J* 11.0, 5.3, H-15), 3.83 (1H, ddd, *J* 11.0, 6.7, 5.0, H-15), 2.55 (1H, br s, OH), 2.48 (1H, br s, OH), 2.01 (2H, t, *J* 6.2, CH_2-4), 1.93 (3H, d, *J* 1.2, Me-19), 1.82 (2H, m, CH_2-14), 1.70 (3H, d, *J* 1.0, Me-18), 1.61 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.01 (6H, s, Me-16,17); ^{13}C NMR δ 137.8 (C-9), 137.4 (C-8), 136.6 (C-6), 134.9 (C-12), 129.3 (C-5), 128.6 (C-10), 127.2 (C-7), 127.0 (C-11), 72.7 (C-13), 61.2 (C-15), 39.6 (C-2), 38.7 (C-14), 34.2 (C-1), 33.0 (C-4), 28.9 (Me-16,17), 21.7 (C-18), 19.3 (C-3), 12.6 (C-19); λ_{max}(CH_2Cl_2) 294 nm; HRMS *m*/*z* 290.2253 (M^+. $C_{19}H_{30}O_2$ requires 290.2246).

*4.2.11. Ethyl 13-demethyl-13-methoxy-13,14-dihydroretinoate (**18**)*

Ag_2O (1.2 g, 5.18 mmol), K_2CO_3 (1.45 g, 10.5 mmol) and methyliodide (1.75 ml, 28 mmol) were added to hydroxyester **17** (0.9 g, 2.7 mmol) in acetonitrile (14.5 ml).

The reaction mixture was stirred at r.t. for 60 h. The mixture was filtered through celite and concentrated under vacuum. Chromatography (EtOAc:hexane 1:9) afforded 440 mg (47% yield) of the product. ^{1}H NMR δ 6.64 (1H, dd, J 15.0, 11.3, H-11), 6.19 (1H, d, J 16.0, H-7), 6.07 (1H, d, J 16.0, H-8), 6.03 (1H, d, J 11.3, H-10), 5.55 (1H, dd, J 15.0, 8.2, H-12), 4.16 (2H, q, J 7.2, CH_2-O), 4.13 (1H, td, J 8.2, 5.4, H-13), 3.29 (3H, s, MeO-13), 2.62 (1H, dd, J 15.0, 8.2, CH-14), 2.47 (1H, dd, J 15.0, 5.4, CH-14), 2.03 (2H, t, J 6.0, CH_2-4), 1.93 (3H, s, Me-19), 1.70 (3H, s, Me-18), 1.61 (2H, m, CH_2-3), 1.46 (2H, m, CH_2-2), 1.25 (3H, t, J 7.2, Me), 1.01 (6H, s, Me-16,17);^{13}C NMR δ 170.9 (C-15), 137.7 (C-9), 137.3 (C-8), 136.8 (C-6), 131.4 (C-12), 129.7 (C-10), 129.3 (C-5), 128.3 (C-11), 127.5 (C-7), 78.7 (MeO), 60.5 (OCH_2), 56.4 (C-13), 41.4 (C-14), 39.5 (C-2), 34.2 (C-1), 33.0 (C-4), 28.9 (Me-16,17), 21.6 (C-18), 19.2 (C-3), 14.2 (Me), 12.6 (C-19); λ_{max}(CH_2Cl_2) 296 nm; HRMS m/z 346.2517 (M^+. $C_{22}H_{34}O_3$ requires 346.2508).

*4.2.12. 13-Demethyl-13-methoxy-13,14-dihydroretinol (**5**)*

Lithium borohydride (26 mg, 1.2 mmol) was added to a solution of **18** (0.418 g, 1.2 mmol) in freshly distilled dry ether (9 ml) under Ar atmosphere at 0 °C. After 40 min water was added and the mixture was extracted twice with ether. The combined organic phase was dried ($MgSO_4$) and concentrated. Chromatography (EtOAc:hexane 4:1) yielded 255 mg (70% yield) of the product. ^{1}H NMR δ 6.57 (1H, dd, J 15.2, 11.3, 0.6, H-11), 6.17 (1H, d, J 16.1, H-7), 6.06 (1H, d, J 16.1, H-8), 6.02 (1H, d, J 11.3, H-10), 5.55 (1H, dd, J 15.2, 8.1, H-12), 3.89 (1H, dt, J 8.1, 4.5, H-13), 3.75 (1H, ddd, J 10.8, 6.0, 4.8, H-15), 3.72 (1H, ddd, J 10.8, 6.0, 4.8, H-15), 3.28 (3H, s, MeO-13), 1.99 (1H, dd, J 6.0, CH_2-4), 1.92 (3H, d, J 0.9, Me-19), 1.78 (2H, m, CH_2-14), 1.68 (3H, d, J 0.6, Me-18), 1.60 (2H, m, CH_2-3), 1.45 (2H, m, CH_2-2), 1.00 (6H, s, Me-16,17); ^{13}C NMR δ 137.6 (C-9), 137.3 (C-8), 136.4 (C-6), 132.4 (C-12), 129.3 (C-10), 129.1 (C-5), 128.4 (C-11), 127.2 (C-7), 81.8 (C-13), 60.4 (C-15), 56.1 (MeO), 39.4 (C-2), 38.0 (C-14), 34.1 (C-1), 32.9 (C-4), 28.8 (Me-16,17), 21.6 (C-18), 19.1 (C-3), 12.5 (C-19); λ_{max}(CH_2Cl_2) 294 nm; HRMS m/z 304.2395 (M^+. $C_{20}H_{33}O_2$ requires 304.2402).

*4.2.13. O-t-butyldimethylsilyl-13-demethyl-14-hydroxy retroretinol (**19**)*

Epoxyretinol **10** was dissolved in ether (5 ml). Silica (0.5 g) was added and the mixture was stirred for 3 h at r.t. It was then filtered and evaporated to dryness. ^{1}H NMR δ 6.76 (1H, d, J 12.0, H-8), 6.50-6.25 (4H, m, H-7, H-10, H-11, H-12), 5.77 (1H, br t, J 4, H-4), 5.66 (1H, dd, J 15.5, 6.5, H-13), 4.24 (1H, m, H-14), 3.66 (1H, dd, J 10.0, 3.7, H-15), 3.46 (1H, dd, J 10.0, 8.0, H-15), 2.63 (1H, d, J 3.0, OH), 2.12 (2H, m, H-3), 1.87 (3H, s, Me-19), 1.50 (2H, m, H-2), 1.26 (3H, S, Me-18), 0.90 (15H, s, Me-16,17, t-Bu), 0.06 (6H, s, $SiMe_2$).

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