Syntheses of new paramagnetic retinal analogues

Tamás Kálai, Noémi Lazsányi, Gergely Gulyás-Fekete, Kálmán Hideg

Received: ……/Accepted …

Abstract New paramagnetic retinal analogues have been synthesized by Horner-Wadsworth-Emmons and Wittig reactions. In these new analogues the pyrroline nitroxide moiety is situated in the place of β-ionone ring or at the end of the polyene chain.

Keywords Nitroxides ● Radicals ● Terpenoids ● Wittig reaction

T. Kálai
Institute of Organic and Medicinal Chemistry, University of Pécs, H-7624 Pécs, Szigeti st. 12., Hungary
Szentágothai Research Centre, Ifjúság st. 20, 7624 Pécs, Hungary
N. Lazsányi ● K. Hideg (✉)
Institute of Organic and Medicinal Chemistry, University of Pécs, H-7624 Pécs, Szigeti st. 12., Hungary
e-mail: kalman.hideg@aok.pte.hu
G. Gulyás-Fekete
Institute of Biochemistry and Medical Chemistry, University of Pécs, H-7624 Pécs, Szigeti st. 12., Hungary

Introduction

The use of retinal and its synthetic analogues to probe the binding site and the photochemistry of both the visual pigment rhodopsin and bacteriorhodopsin have been well established. Paramagnetic modifications of retinal have also been published earlier [1, 2]. These efforts are part of a tendency to determine the accessibility and penetration of small molecules to specific sites of proteins. The mechanism by which small molecules reach various domains in proteins is of fundamental interest in the study of protein dynamics and enzyme mechanisms [3]. Retinal and its metabolites (retinoids) are essential to the proper function of a number of biological processes. Visual cycle is perhaps the most thoroughly described field, but reproduction, cell growth and differentiation, embryonic development, immune response and intermediacy metabolism are also regulated by all-*E* retinoic acid and 9-*Z*-retinoic acid [4]. The role of retinal and retinoids in antioxidant defense is still controversial: they are used in treatment of diseases associated with oxidative stress, but several studies report that they may increase oxidative stress by impairing mitochondrial function [5].

In our laboratory we have a long-standing interest to synthesize paramagnetic analogues of amino acids [6], carbohydrates [7], drugs [8, 9] and antioxidants [10] to study the receptor binding by EPR spectroscopy

[7-9] and to study their antioxidant properties. Most of these paramagnetically modified molecules exhibited better antioxidant activity than the original biomolecules did [9, 10]. As art of our ongoing interest, in the synthesis of spin labeled biomolecules we have lately focused on the synthesis of paramagnetic analogues of diterpenes such as retinal and paramagnetic retinoic acid. Although paramagnetic analogues **2, 3** of retinal **1** has been synthesized earlier [1, 2] (Figure 1), we envisioned that 18 methyl group insertion for compound **4** as well as incorporating pyrroline ring into aldehyde end of retinal molecule **5** may open up further perspectives and challenges in the study of retinal function and biological activity. To the best of our knowledge, paramagnetic retinal analogues with bulky substituents on the retinal polyene chains have not been synthesized so far, although several diamagnetic analogue syntheses and studies have been published concluding that these modifications block the chromophore binding or slow down the 13-*Z* all-*E* isomerization [11-13]. The challenge of synthesizing carotenoids and retinal derivatives inspired many distinguished organic chemists both in laboratories and in the industry [14], however reports on spin labeled retinal derivatives are still limited [1, 2], probably because of the difficulty of utilization of organometallic reagents in the presence of nitroxides and the difficulties of NMR investigation of paramagnetic species formed. In this paper we report the extension of the

Horner-Wadsworth–Emmons olefination and Wittig reaction-based approach for paramagnetic retinal and retinoic acid synthesis for further biological studies including antioxidant and receptor-binding investigations.

< Fig. 1 >

Results and Discussion

We began our synthesis with 1-oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-carbaldehyde **6** available by Suzuki reaction [15]. For chain elongation compound **6** was treated with the anion of ethyl 4-diethoxyphosphinyl-3-methyl-2-butenoate [16] in THF at -78 °C giving compound **7**. Transformation of the ester group into aldehyde by the previously reported protocols [1, 2] did not give satisfactory results in our hands. Therefore the resulting ester **7** was hydrolyzed to carboxylic acid **8** cautiously in aqueous sodium hydroxide-methanol solution. Compound **8** was converted to mixed anhydride ester with ethyl chloroformate in the presence of Et_3N and this was reduced with 1.1 equivalent sodium borohydride in ethanol to an alcohol [17]. Oxidation of this alcohol with activated MnO_2 provided aldehyde **9**.

The configuration of two double bonds in the chain of **9** was proven by HMQC, HMBC COSY and NOESY measurements and were found to be *E,E*-isomers. Further elongation of the chain from aldehyde **9** with lithium salt of ethyl 4-diethoxyphosphinyl-3-methyl-2-butenoate in THF at -78 °C gave ester **10**. The 2D measurements, ^{1}H NMR and ^{13}C NMR studies of compound **10** suggested the presence of both *Z* and *E* isomers. This was also confirmed by HPLC studies [18] revealing that the product contains 33% 11-*Z*-isomer and 64% all *E*-isomer and further 2 minor isomers in 1 and 2 %. Compound **10** was hydrolyzed with aqueous sodium hydroxide in methanol to the paramagnetic analogue of retinoic acid **11**. This acid was converted to mixed anhydride ester, which was reduced with 1.1 equivalent $NaBH_4$ in ethanol at 0 °C and the alcohol achieved was not isolated, but oxidized immediately to paramagnetic retinal **4** with activated MnO_2 in CH_2Cl_2 at room temperature (Figure 2). The 2D measurements, ^{1}H NMR and ^{13}C NMR studies of compound **4** suggested the presence of both *Z* and *E* isomers also. This was confirmed by HPLC studies as well, confirming that paramagnetic retinal analogue contains of 24% 11-*Z*-isomer and 73% of all *E*-isomer and 0.5% and 2 % of another two minor isomers.

< Figure 2 >

To study further the steric constrainsts in the retinal binding pocket by EPR spectroscopy we envisioned that the paramagnetic 13-*Z*-locked retinal

analogue might be a useful substrate. To incorporate the pyrroline ring into C(13)-C(14) positions of the retinal molecule we used 1-oxyl-4-(hydroxymethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carbaldehyde **12** [19] as a starting material. It was silylated on the hydroxyl group [20] and treatment of aldehyde **13** with ylide, generated from β-ionylidenethyltriphenylphosphonium bromide [21] with LDA at -78 °C in THF, gave the mixture of silylated and desilylated products. After removing the silyl group from the crude product with Bu_4NF in THF during the work-up, we got alcohol **14.** Oxidation of compound **14** with activated MnO_2 in CH_2Cl_2 at room temperature provided aldehyde **5**, a paramagnetic retinal derivative with C(13)-C(14) *Z* double bond. Otherwise structure of compound **5** was confirmed using HMQC, HMBC, COSY, NOESY and revealed the *E* configuration for all three double bond in question.

< Figure 3 >

Conclusion

In this study, some new paramagnetic retinal **4, 5** and retinoic acid **11** analogues have been synthesized by Horner-Wadsworth-Emmons and Wittig reactions incorporating the nitroxide moiety into the two ends of retinal structure, respectively. We are confident that the new paramagnetic retinal and retinoic acid analogues reported herein will find utilization in both antioxidant and receptor binding studies, and hopefully methodologies

described will be applicable in accessing other biomolecules modified by a nitroxide moiety.

Experimental

Melting points were determined with a Boetius micro melting point apparatus. Elemental analyses were performed on Fisons EA 1110 CHNS elemental analyzer. Results were found to be in good agreement (±0.3%) with the calculated values. Mass spectra were recorded on a Thermoquest Automass Multi. ^{1}H NMR spectra were recorded with Bruker Avance 3 Ascend 500. Chemical shifts are referenced to Me_4Si. The paramagnetic compounds were reduced with hydrazobenzene. Measurements were run at 298K probe temperature in $CDCl_3$ solution. ESR spectra were taken on Miniscope MS 200 in 10^{-4} M $CHCl_3$ solution and all monoradicals gave triplet line a_N = 14.4 G. The IR spectra were taken with Bruker Alpha FT-IR instrument with ATR support on diamond plate. UV spectra were taken with Specord 40 instrument (Analytic Jena). The HPLC system was interfaced to a gradient pump Dionex P680 and Dionex PDA-100 detector; the acquisitions was performed λ = 450 nm detection at 22^{o}C. Data acquisitions were performed by Chromeleon 6.70 software. The HPLC separations were carried out on an end-capped column (250 x 4.6 mm i.d.; YMC C30, 3µm). The eluents consisted of: A: 81% MeOH, 15% TBME, 4% H_2O and B: 6% MeOH, 90% TBME, 4% H_2O. Linear gradient was

used: 0’ 100% A – 15’ 85% A, 15% B eluent and flow rate was 1.00 cm^3/min. Flash column chromatography was performed on Merck Kieselgel 60 (0.040-0.063 mm). Qualitative TLC was carried out on commercially available plates (20 x 20 x 0.02 cm) coated with Merck Kieselgel GF254. Compounds **6** [15], **12** [19]**,** ethyl 4-diethoxyphosphinyl-3-methyl-2-butenoate [16], β-ionylidenethyltriphenyl-phosphonium bromide [21] were prepared according to published procedures and other reagents were purchased from Aldrich.

General procedure for Horne –Wadsworth –Emmons reaction

A solution of BuLi (2.4 mL, 6.0 mmol, 2.5M in hexanes) was added dropwise at -78 °C to a stirred solution of 4-diethoxyphosphinyl-3-methyl-2-butenoate (1.98 g, 7.5 mmol) in anhydr. THF (20 mL). The mixture was stirred under N_2 at this temperature for 30 min, then aldehyde **6** (910 mg, 5.0 mmol) or aldehyde **9** (1.24 g, 5.0 mmol) was added dropwise in THF (10 mL) at -78 °C. The mixture was stirred at this temperature for 30 min., and then it was allowed to warm to room temperature and was stirred overnight. Following the addition of sat. aq. NH_4Cl solution (10 mL), EtOAc (20 mL) was added and the organic phase was separated. The aqueous phase was extracted with EtOAc (10 mL), the combined organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was

purified by flash column chromatography with gradient elution (hexane/ether: 90%/10% to 60%/40%) to furnish compounds **7** as a yellow and **10** as deep yellow solids.

(2*E*,4*E*)-Ethyl 5-(1-oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-3-methylpenta-2,4-dienoate Radical $C_{17}H_{26}NO_3$ (**7**)*:* 605 mg (48%), Mp.: 53° C, R_f: 0.40 (hexane/Et_2O 2:1). IR (neat): $\tilde{\nu}$ = 1701, 1645, 1606 cm^{-1}; UV-Vis (ethanol, $c = 2.36 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 303 (26400) nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): *m*/*z* = 292 (M^+, 100), 277 (32), 262 (23), 91 (83).

Ethyl 9-(1-oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-3,7-dimethylnona-2,4,6,8-tetraenoate Radical $C_{22}H_{32}NO_3$ (**10**) *:* 698 mg (39%). Mp.: 98 °C, R_f: 0.35 (hexane/Et_2O 2:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.30 (s, 6H, CH_3), 1.28 (t, 3H, CH_3), 1.45 (, 6H, CH_3), 1.86 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.27 (m, 2H, CH_2), 6.22 (d, 1H, CH), 6.31 (d, 1H, CH), 6.40 (dd, 1H, CH), 6.57 (d, 1H, CH), 6.67 (d, 1H, CH), 7.06 (m, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 10.80 (CH_3), 13.72 (CH_3), 14.25 (CH_3), 23.87 (CH_3), 24.99 (CH_3), 25.60 (CH_3), 59.56 (CH_2), 68.76 (C), 69.50 (C), 121.67 (CH), 130.44 (CH), 130.57 (CH), 131.99 (CH), 133.70 (CH), 135.46 (C), 135.68 (CH), 138.68 (C), 139.26 (C), 142.66 (C), 167.02 (C). IR (neat): $\tilde{\nu}$ = 1694, 1601, 1569 cm^{-1}; UV-Vis

(ethanol, c = 2.24 ·10^{-5} mol dm^{-3}): λ_{max} (ε) = 361 (52100), 262 (4500) nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): m/z = 358 (M^+, 2), 344 (28), 328 (11), 192 (79).

General procedure for ester hydrolysis:

10% aq. NaOH (10 mL) was added to a solution of ester **7** (1.17g, 4.0 mmol) or **10** (1.43 g, 4.0 mmol) in MeOH (20 mL). The mixture was allowed to stand overnight at ambient temperature in the dark. The MeOH was evaporated *in vacuo* (< 40 °C), and the pH was adjusted to 4 by cautious addition of 5% H_2SO_4 at 0 °C. Then the aqueous phase was immediately extracted with $CHCl_3$ (2 x 15 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated. The carboxylic acids **8** and **11** were isolated as yellow solids after flash column chromatography by gradient elution (hexane/EtOAc 66%/33% for 5 x 30 mL fraction and then $CHCl_3$/Et_2O from 10%/90% to 50%/50%).

(2*E*,4*E*)-5-(1-Oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-3-methylpenta-2,4-dienoic acid Radical $C_{15}H_{22}NO_3$ (**8**): 443 mg (42%), Mp.: 152 °C, R_f : 0.33 ($CHCl_3$/Et_2O 2:1). IR (neat): $\bar{\nu}$ = 3034, 1674, 1602, 1584 cm^{-1}; UV-Vis (ethanol, c = 2.74 ·10^{-5} mol dm^{-3}): λ_{max} (ε) = 299 (25300) nm

(mol^{-1} dm^3 cm^{-1}); MS (70 eV): *m*/*z* = 264 (M^+, 13), 249 (14), 234 (8), 43 (100).

9-(1-Oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid Radical $C_{20}H_{28}NO_3$ (**11**): 462 mg (35%). Mp.: 208 °C, R_f 0.27 ($CHCl_3$/Et_2O 2:1). IR (neat): $\overline{\nu}$ = 3046, 1672, 1596, 1564 cm^{-1}; UV-Vis (ethanol, *c* = 1.68 ·10^{-5} mol dm^{-3}): λ_{max} (ε) = 257 (4000), 357 (51700) nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): *m*/*z* = 330 (M^+, 9), 316 (8), 300 (5), 282 (15), 91 (68) 44 (100).

General procedure for conversion of acids to aldehydes

To a stirred solution of carboxylic acids **8** (2.0 mmol) or **11** (2.0 mmol) and Et_3N (404 mg, 4.0 mmol) in anhydr. Et_2O (20 mL) ethyl-chloroformate (217 mg, 2.0 mmol) in Et_2O (5 mL) was added dropwise at 0 °C. The mixture was stirred at this temperature for 3h, then the trierthylamine hydrochloride was filtered off on glass sintered funnel, washed with Et_2O (10 mL) and the ether was evaporated off *in vacuo* (< 40 °C). The residue was immediately dissolved in dry EtOH (15 mL) and $NaBH_4$ (84 mg, 2.2 mmol) was added in 3 portions during 30 min. at 0 °C. After the consumption of the starting mixed anhydride ester, monitored by TLC (~ 90 min.), the EtOH was evaporated off (< 40 °C), the residue was

dissolved in $CHCl_3$ (20 mL), washed with brine (10 mL) and the organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was immediately dissolved in dry CH_2Cl_2 (20 mL), activated MnO_2 (1.72 g, 20.0 mmol) was added in one portion and stirred overnight at room temperature in the dark. Then the reaction mixture was filtered through Celite, washed with CH_2Cl_2 (10 mL), the solvent was evaporated and the residue was purified by flash column chromatography (gradient: hexane/Et_2O 90%/10% to 75%/25% 10 x 30 mL then hexane/EtOAc 60%/40%) to give aldehydes **9** and **4** as yellow solids.

(2*E*,4*E*)-5-(1-Oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-3-methylpenta-2,4-dienal Radical $C_{15}H_{22}NO_2$ (**9**): 153 mg (35%). Mp.: 104 °C, R_f 0.17 (hexane/Et_2O 2:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.31 (s, 6H, CH_3), 1.44 (s, 6H, CH_3), 1.87 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 6.09 (d, 1H, CH), 6.55 (d, 1H, CH), 6.82 (d, 1H, CH), 10.20 (s, 1H, CHO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 11.14 (CH_3), 12.79 (CH_3), 23.97 (CH_3), 25.11 (CH_3), 68.70 (C), 69.78 (C), 128.12 (CH), 129.38 (CH), 131.83 (CH), 135.32 (C), 144.84 (C), 154.82 (C), 191.09 (CHO). IR (neat): $\bar{\nu}$ = 1649, 1616, 1600 cm^{-1}; UV-Vis (ethanol, c = 2.71 ·10^{-5} mol dm^{-3}): λ_{max} (ε) = 323 (32600) nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): m/z = 248 (M^+, 78), 218 (11), 91 (82), 42 (100).

9-(1-Oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-3,7-dimethylnona-2,4,6,8-tetraenal Radical $C_{20}H_{28}NO_2$ (**4**): 157 mg (25%), Mp.: 140 °C, R_f 0.47 (hexane/EtOAc 2:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.30 (s, 6H, CH_3), 1.44 (s, 6H, CH_3), 1.86 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 6.24 (d, 1H, CH), 6.33 (d, 1H, CH), 6.46 (d, 1H, CH), 6.56 (d, 1H, CH), 6.66 (d, 1H, CH), 7.20 (m, 1H, CH), 10.17 (s, 1H, CH); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 10.87 (CH_3), 13.01 (CH_3), 23.80 (CH_3), 24.94 (CH_3), 25.54 (CH_3), 68.88 (C), 69.67 (C), 122.56 (CH), 129.16 (CH), 130.73 (CH), 131.97 (CH), 133.53 (CH), 135.06 (CH), 135.46 (C), 140.59 (C), 140.93 (C), 142.62 (C), 190.97 (CH).

IR (neat): $\tilde{\nu}$ = 1652, 1597, 1567 cm^{-1}; UV-Vis (ethanol, c = 1.98·10^{-5} mol dm^{-3}): λ_{max} (ε) = 377 (33600), 270 (10800) nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): m/z = 314 (M$^+$, 41), 300 (15), 288 (28), 91 (63), 44 (100).

1-Oxyl-4-(*t*-butyldimethylsilyloxymethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carbaldehyde Radical $C_{15}H_{30}NO_3Si$ (**13**) :

To a stirred solution of alcohol **12** (990 mg, 5.0 mmol) and imidazole (1.02 g, 15.0 mmol) in dry DMF (7 mL) *t*-butyl-dimethylchlorosilane (1.50 g) was added in 3-4 portions at 0 °C, then the solution was stirred for 24 h at ambient temperature. The solution was poured onto mixture of ice and sat. aq. $NaHCO_3$ solution (50 mL), extracted with Et_2O (3 x 20 mL), the

organic phase was dried ($MgSO_4$), filtered, evaporated and the residue was purified by flash column chromatography (gradient: hexane/Et_2O 90%/10% to 70/30%) to give the title compound (1.06 g, 68%) as a yellow solid, Mp.: 74 °C, R_f: 0,44 (hexane/Et_2O 2:1). ^1H NMR (500 MHz, $CDCl_3$) δ = 0.12 (s, 3H, $SiCH_3$), 0.13 (s, 3H, $SiCH_3$), 0.94 (s, 9H, $C(CH_3)_3$), 1.34 (s, 6H, CH_3), 1.39 (s, 6H, CH_3), 4.59 (s, 2H, CH_2), 10.27 (s, 1H, CHO) ppm; ^{13}C NMR (125 MHz, $CDCl_3$) δ = -5.64 ($SiCH_3$), 18.08 (SiC), 24.01 (CH_3), 24.22 (CH_3), 25.67 (CH_3), 58.28 (CH_2), 67.95 (C), 69.60 (C), 139.38 (C), 159.96 (C), 189.24 (CHO). IR (neat): $\tilde{\nu}$ = 1658, 1625 cm^{-1}; MS (70 eV): *m*/*z* = 312 (M^+, 2), 240 (12), 183 (27), 75 (100).

1-Oxyl-3-hydroxymethyl-2,2,5,5-tetramethyl-4-[(1*E*,3*E*,5*E*)-4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hexa-1,3,5-trien-1-yl]-2,5-dihydro-1*H*-pyrrole Radical $C_{25}H_{38}NO_2$ (**14**): To a stirred solution of β-ionylidenethyltriphenyl-phosphonium bromide (2.72 g, 5.0 mmol) in anhydr. THF (40 mL), LDA solution (2.8 mL, 5.0 mmol in THF/heptane/ethylbenzene) was added dropwise at -78 °C. After stirring the solution for 15 min., compound **13** (1.56 g, 5.0 mmol) dissolved in THF (10 mL) was added dropwise at -78 °C to the dark red solution and the stirring was continued for 1h at -78 °C, then the reaction mixture was allowed to warm to room temperature and stirred at this temperature

overnight. The solution was diluted with Et_2O (30 mL) and sat. aq. NH_4Cl solution (10 mL) was added. The organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The residue was dissolved in THF (20 mL) $Bu_4NF \cdot xH_2O$ (1.30 g, 5.0 mmol) was added in one portion and the reaction mixture was stirred for 15 min. at room temperature, then Et_2O (20 mL) was added, the reaction mixture was washed with water (20 mL), the organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The chromatographic purification of the crude product (gradient: hexane/EtOAc 90%/10% to 70%/30%) offered compound **14** as a pale yellow solid 691 mg (36%), Mp.: 106 °C; R_f: 0.47 (hexane/EtOAc 2:1). IR (neat): $\tilde{\nu}$ = 3481, 1591 cm^{-1}; UV-Vis (ethanol, $c = 1.85 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 334 (37900), nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): m/z = 348 (M^+, 70), 369 (10), 354 (41), 42 (100).

1-Oxyl-2,2,5,5-tetramethyl-4-[(1*E*,3*E*,5*E*)-4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hexa-1,3,5-trien-1-yl]-2,5-dihydro-1*H*-pyrrol-3-carbaldehyde Radical $C_{25}H_{36}NO_2$ (**5**):

To a stirred solution of **14** alcohol (384 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) activated MnO_2 (860 mg, 10.0 mmol) was added and the mixture was stirred overnight at ambient temperature in the dark. Then the reaction mixture was filtered through Celite, washed with CH_2Cl_2 (5 mL), the

solvent was evaporated off and the residue was purified by flash column chromatography with gradient elution (hexane/Et_2O 90%/10 % to 60%/40%) to give **5** aldehyde 210 mg (55%) as a yellow solid. Mp.: 100 °C, R_f: 0.57 (hexane/Et_2O 2:1). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.10 (s, 6H, CH_3), 1.46 (s, 12H, CH_3), 1.55 (s, 2H, CH_2), 1.69 (s, 2H, CH_2), 1.79 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.09 (m, 2H, CH_2), 6.22 (m, 2H, CH), 6.41 (d, 1H, CH), 6.53 (d, 1H, CH), 7.09 (dd, 1H, CH), 10.02 (s, 1H, CHO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 12.92 (CH_3), 19.06 (CH_2), 21.59 (CH_3), 24.31 (CH_3), 24.80 (CH_3), 28.82 (CH_3), 32.98 (CH_2), 34.12 (C), 39.47 (CH_2), 67.80 (C), 69.58 (C), 120.36 (CH), 129.17 (CH), 129.61 (CH), 130.23 (C), 135.52 (CH), 136.74 (CH), 137.50 (C), 138.52 (C), 140.79 (C), 159.48 (C), 187.62 (CHO). IR (neat): $\bar{\nu}$ = 1646, 1565, 1540 cm^{-1}; UV-Vis (ethanol, c = $1.73 \cdot 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 384 (21300), 258 (8300) nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): m/z = 382 (M^+, 16), 352 (29), 377 (17), 43 (100).

Acknowledgements

We are grateful to Prof. József Deli (Department of Pharmacognosy, University of Pécs) for HPLC measurement and helpful discussions, Viola Csokona for elemental analyses and to the Hungarian National Research Fund (OTKA K81123, K104956) for the financial support.

References

1. Renk GE, Or SY, Crouch RK (1987) J Am Chem Soc 109:6163.
2. Groesbeek M, Lugtenburg J (1995) Rec Travaux Chim Pays-Bas 114:403.
3. Steinhoff HJ, Savitsky A, Wegener C, Pfeiffer M, Plato M, Mobius K (2000) BBA-BIOENERGETICS 1457:253.
4. Wada A, Fukunaga K, Ito M, MizuguchiY, Nakagawa K, Okano T, (2004) Bioorg Med Chem 12:3931.
5. Siems W, Sommerburg O, Schild L, Augustin W, Langhans CD, Wiswedel I. (2002) FASEB J 16:1289.
6. Kálai T, Schindler J, Balog M, Fogassy E, Hideg K (2008) Tetrahedron 64:1094.
7. Zhao M, Kalai T, Hideg K, Altenbach C, Hubbell WL, Kaback HR, (2000) Biochemistry 39:11381.
8. Miyazaki J, Hideg K, Marsh D (1992) BBA 1103:62.
9. Petrlova J, Kálai T, Maezawa, I, Altman R, Harishchandra G, Hong HS, Bricarello DA, Parikh AN, Lorigan GA, Jin LW, Hideg K, Voss JC (2012) Plos One 7:e35443.
10. Kálai T, Borza E, Antus Cs, Radnai B, Gulyás-Fekete G, Fehér A, Sümegi B, Hideg K (2011) Bioorg Med Chem 19:7311.

11. Lewis JW, Pinkas I, Sheves M, Ottolenghi M, Kliger DS (1995) J Am Chem Soc 117:918.

12. Danshina SV, Drachev AL, Drachev LA, Eremin SV, Kaulen AD, Khytrina LV, Mitsner BI (1990) Arch Biochem Biophys 279:225

13. Yan B, Xie A, Nienhaus UG, Katsuta Y, Spudich JL (1993) Biochemistry 32:10224.

14. Britton G, Liaaen-Jensen S, Pfander H. (1996) Carotenoids. vol. 2. Birkhauser, Basel.

15. Kálai T, Balog M, Jekő J, Hubbell WL, Hideg K (2002) Synthesis 34: 2365.

16. Magoulas GE, Bariamis E, Athanassopoulos MC, Haskopoulos A, Dedes PG, Krokidis MG, Karamanos NK, Kletsas D, Papaioannou D, Maroulis G. (2011) Eur J Med Chem 46:721.

17. Hideg K, Hankovszky HO, Lex L, Kulcsár Gy (1980) Synthesis 12: 911.

18. Gulyás-Fekete G, Murillo E, Kurtan T, Papp T, Illyes ZT, Drahos L, Visy J, Agocs A, Turcsi E, Deli J (2013) J Nat Prod 76:607.

19. Kálai T, Jekő J, Hideg K. (2000) Synthesis 32:831.

20. Greene TW, Wuts PG (1999) Protective Groups in Organic Synthesis. Wiley, Hoboken.

21. Tietze LF, Eicher TH (1989) Reactions and syntheses in the organic chemistry laboratory. University Science Books, Mill Valley.

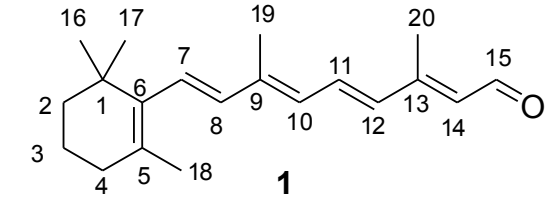
Figure Captions

Fig. 1 Retinal (**1**), previously reported (**2, 3**) and herein reported paramagnetic retinal derivatives (**4, 5**).

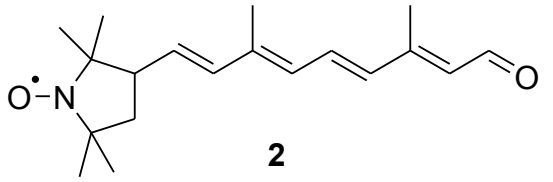
Fig. 2 Reagents and conditions: (a) 4-diethoxyphosphinyl-3-methyl-2-butenoate (1.5 equiv.), BuLi (1.2 equiv), THF, 30 min., -78 °C, then compound **6** or **9** (1.0 equiv.), 30 min., -78 °C, -78 °C →r.t., 8h, quench with aq. NH_4Cl 39-48%; (b) MeOH, 10% aq. NaOH (excess), 8h, then H^+ pH=4, 35- 42%; (c) Et_3N (2.0 equiv), $ClCO_2Et$ (1.0 equiv), Et_2O, 0 °C, 3h, filtration, evaporation the solvent off then 1.1 equiv $NaBH_4$, EtOH, 2h, 0 °C, work-up then activated MnO_2 (10 equiv.), CH_2Cl_2, r.t. 8h, 25-31%.

Fig. 3 Reagents and conditions: (a) TBDMSCl (2.0 equiv.), imidazole (3.0 equiv.), DMF, 0 °C → r.t., 24h, 68 %; (b) β-ionylidenethyltriphenyl-phosphonium bromide (1.0 equiv.), LDA (1.0 equiv.), THF, -78 °C, 15 min., then compound **13** (1.0 equiv.), -78 °C , 1h, then -78 °C→r.t., 1h, work-up, then Bu_4NF (1.0 equiv.), THF, r.t. 15 min. 36%; (c) activated MnO_2 (10.0 equiv.), CH_2Cl_2, r.t. 8h, 55%.

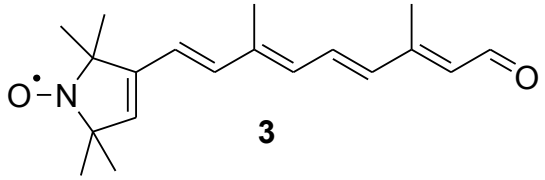
Fig 1


16
17
19
20
7
11
15
6
2
1
9
13
O
8
10
12
14
3
4
5
18

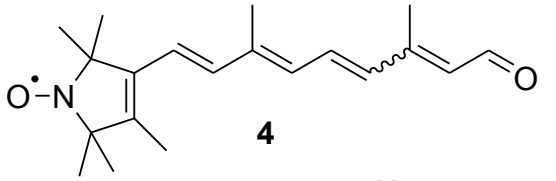
1


O
N
O

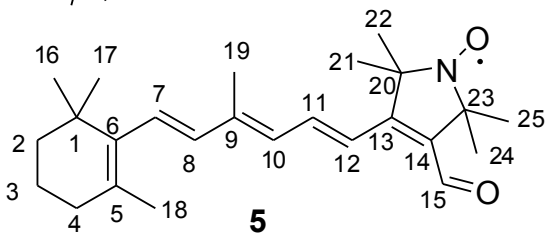
2


O
N
O

3


O
N
O

4


22
16
17
19
21
20
N
O
7
11
23
25
6
2
1
9
13
24
8
10
12
14
3
15
O
4
5
18

5

Fig 2

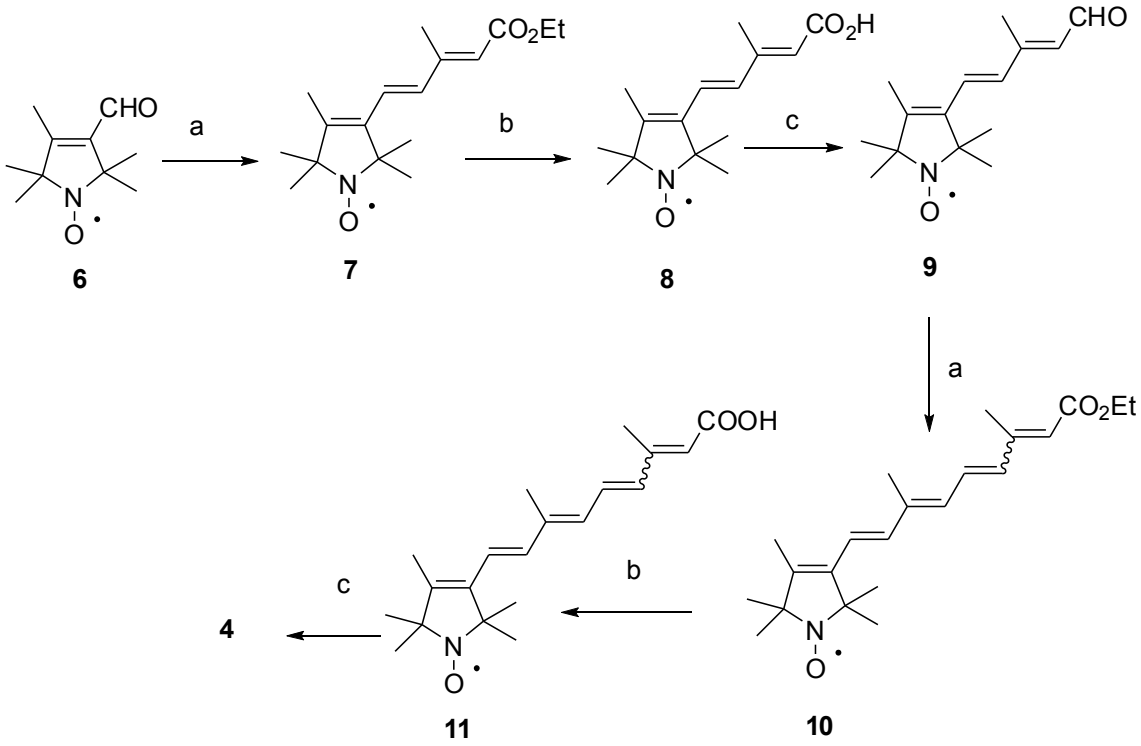
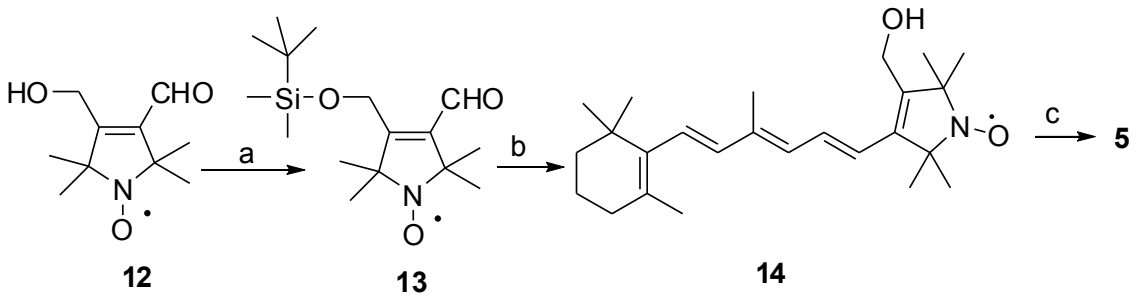


Fig. 3



Graphics for use in the Table of Contents

New paramagnetic retinal analogues have been synthesized by Horner-Wadsworth-Emmons and Wittig reactions. In these new analogues the pyrroline nitroxide moiety is situated in the place of β-ionone ring or at the end of the polyene chain.