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Synthesis of new paramagnetic retinal analogues

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

Keywords Nitroxides · Radicals · Terpenoids · Wittig reaction

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 an effort 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. The 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 the 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 had a long-standing interest to synthesize the paramagnetic analogues of amino acids [6], carbohydrates [7], drugs [8, 9], and antioxidants [10] in order to study the receptor binding via 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 part 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**) have been synthesized earlier [1, 2] (Fig. 1), we envisioned that 18-methyl group insertion for compound **4** as well as incorporating a pyrroline ring into the 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

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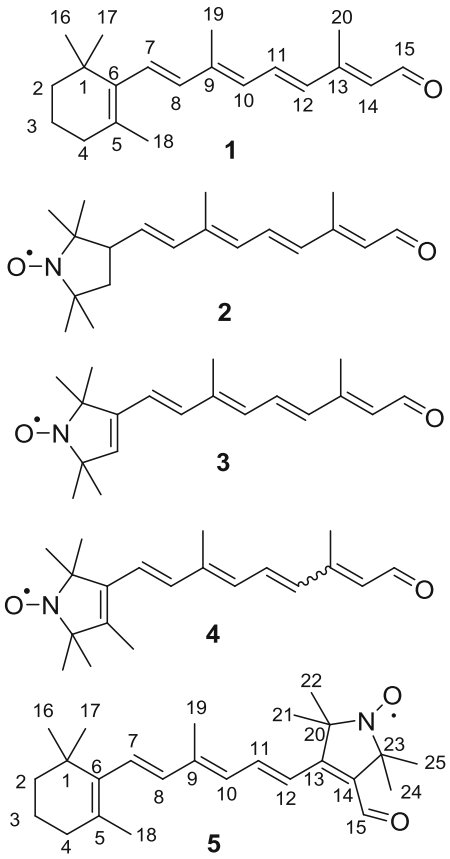

Fig. 1 Retinal (**1**), previously reported (**2**, **3**) and herein reported paramagnetic retinal derivatives (**4**, **5**)

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 utilizing organometallic reagents in the presence of nitroxides and the difficulties of NMR investigation of the 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.

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 via a 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 they were found to be *E*,*E*-isomers. Further elongation of the chain from aldehyde **9** with the 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 % of the 11-*Z*-isomer and 64 % of the all-*E*-isomer, as well as two additional minor isomers in amounts of 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 (Fig. 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 the paramagnetic retinal analogue contains 24 % of the 11-*Z*-isomer and 73 % of the all-*E*-isomer, along with 0.5 and 2 % of another two minor isomers.

To study further the steric constraints 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 the 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 the treatment of aldehyde **13** with ylide, generated from *β*-ionylidenethyltriphenylphosphonium bromide [21] with LDA at −78 °C in THF, gave a 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 achieved 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 a C(13)-C(14) *Z* double bond. Otherwise, the structure of compound **5** was confirmed using HMQC, HMBC, COSY, and NOESY, and revealed the *E* configuration for all three double bonds in question in Fig. 3.

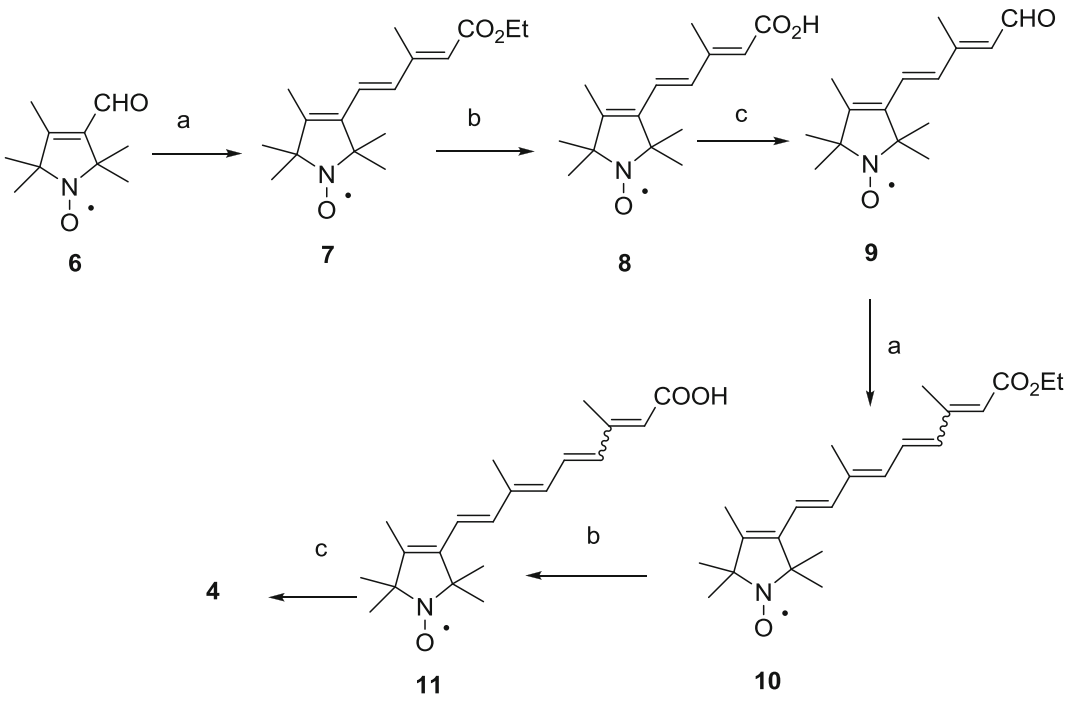

Fig. 2 Reagents and conditions: **a** 4-diethoxyphosphinyl-3-methyl-2-butenoate (1.5 equiv.), BuLi (1.2 equiv), THF, 30 min, −78 °C, then compound **6** or **9** (1.0 equiv.), 30 min, −78 °C, −78 °C → r.t., 8 h, quench with aq. NH_4Cl; 39–48 %; **b** MeOH, 10 % aq. NaOH (excess), 8 h, then H^+ pH 4;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

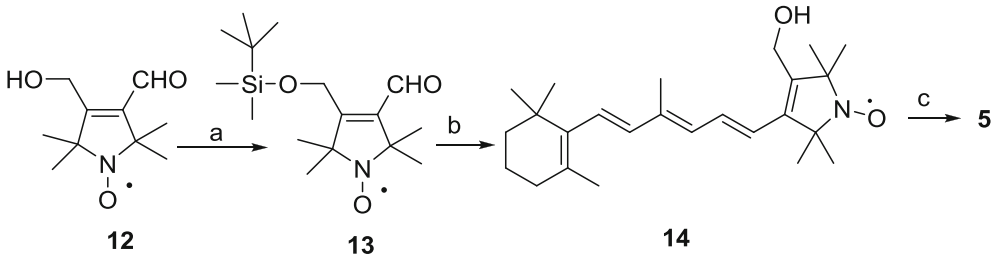

Fig. 3 Reagents and conditions: **a** TBDMSCl (2.0 equiv.), imidazole (3.0 equiv.), DMF, 0 °C → r.t., 24 h; 68 %; **b** β-ionylidenethyltriphenylphosphonium bromide (1.0 equiv.), LDA (1.0 equiv.), THF, −78 °C, 15 min, then compound **13** (1.0 equiv.), −78 °C, 1 h, then −78 °C → r.t., 1 h, work-up, then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

Conclusion

In this study, several new paramagnetic retinal **4**, **5**, and retinoic acid **11** analogues have been synthesized using Horner-Wadsworth-Emmons and Wittig reactions incorporating the nitroxide moiety into the two ends of the retinal structure, respectively. We are confident that the new paramagnetic retinal and retinoic acid analogues reported herein can be utilized in both antioxidant and receptor binding studies, and hopefully the 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 a Fisons EA 1110 CHNS elemental analyzer. The results were found to be in good agreement (±0.3 %) with the calculated values. Mass spectra were recorded on a Thermoquest Automass Multi. ^1H NMR spectra were recorded with a Bruker Avance III Ascend 500. Chemical shifts are referenced to Me_4Si. The paramagnetic compounds were reduced with hydrazobenzene. Measurements were run at a 298 K probe temperature in $CDCl_3$ solution.

ESR spectra were taken on a 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 a Bruker Alpha FT-IR instrument with ATR support on a diamond plate. UV spectra were taken with a Specord 40 instrument (Analytic Jena). The HPLC system was interfaced to a gradient pump Dionex P680 and Dionex PDA-100 detector; the acquisitions were performed with $\lambda = 450$ nm detection at 22 °C. Data acquisitions were performed by Chromeleon 6.70 software. The HPLC separations were carried out on an end-capped column (250 × 4.6 mm i.d.; YMC C30, 3 μm). The eluents consisted of: A: 81 % MeOH, 15 % TBME, 4 % H_2O, and B: 6 % MeOH, 90 % TBME, 4 % H_2O. The linear gradient used was: 0′ 100 % A–15′ 85 % A, 15 % B eluent, and the flow rate was 1.00 cm^3/min. Flash column chromatography was performed on a Merck Kieselgel 60 (0.040–0.063 mm). Qualitative TLC was carried out on commercially available plates (20 × 20 × 0.02 cm) coated with Merck Kieselgel GF254. Compounds **6** [15], **12** [19], ethyl 4-diethoxyphosphinyl-3-methyl-2-butenoate [16], and *β*-ionylidenethyltriphenylphosphonium bromide [21] were prepared according to published procedures and other reagents were purchased from Aldrich.

General procedure for Horner-Wadsworth-Emmons reaction

A solution of 2.4 cm^3 BuLi (6.0 mmol, 2.5 M in hexanes) was added dropwise at −78 °C to a stirred solution of 1.98 g 4-diethoxyphosphinyl-3-methyl-2-butenoate (7.5 mmol) in 20 cm^3 anhydrous THF. The mixture was stirred under N_2 at this temperature for 30 min, then 910 mg aldehyde **6** (5.0 mmol) or 1.24 g aldehyde **9** (5.0 mmol) was added dropwise in 10 cm^3 THF 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 10 cm^3 sat. aq. NH_4Cl solution, 20 cm^3 EtOAc was added and the organic phase was separated. The aqueous phase was extracted with 10 cm^3 EtOAc, 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.

(2E,4E)-Ethyl 5-(1-oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1H-pyrrol-3-yl)-3-methylpenta-2,4-dienoate radical (**7**, $C_{17}H_{26}NO_3$)
Yield: 605 mg (48 %); m.p.: 53 °C; $R_f = 0.40$ (hexane/Et_2O 2:1); IR (neat): $\overline{\nu} = 1{,}701$, 1,645, 1,606 cm^{-1}; UV–Vis (ethanol, $c = 2.36 \times 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 303 (26,400) 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-1H-pyrrol-3-yl)-3,7-dimethylnona-2,4,6,8-tetraenoate radical (**10**, $C_{22}H_{32}NO_3$)
Yield: 698 mg (39 %); m.p.: 98 °C; $R_f = 0.35$ (hexane/Et_2O 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.30$ (s, 6H, CH_3), 1.28 (t, 3H, CH_3), 1.45 (s, 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) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 10.80$ (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) ppm; IR (neat): $\overline{\nu} = 1{,}694$, 1,601, 1,569 cm^{-1}; UV–Vis (ethanol, $c = 2.24 \times 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 361 (52,100), 262 (4,500) 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

Aq. NaOH (10 %, 10 cm^3) was added to a solution of 1.17 g ester **7** (4.0 mmol) or 1.43 g **10** (4.0 mmol) in 20 cm^3 MeOH. 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 × 15 cm^3). 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 × 30 cm^3 fraction and then $CHCl_3$/Et_2O from 10/90 % to 50/50 %).

(2E,4E)-5-(1-Oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1H-pyrrol-3-yl)-3-methylpenta-2,4-dienoic acid radical (**8**, $C_{15}H_{22}NO_3$)
Yield: 443 mg (42 %); m.p.: 152 °C; $R_f = 0.33$ ($CHCl_3$/Et_2O 2:1); IR (neat): $\overline{\nu} = 3{,}034$, 1,674, 1,602, 1,584 cm^{-1}; UV–Vis (ethanol, $c = 2.74 \times 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 299 (25,300) 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-1H-pyrrol-3-yl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid radical (**11**, $C_{20}H_{28}NO_3$)
Yield: 462 mg (35 %); m.p.: 208 °C; $R_f = 0.27$ ($CHCl_3$/Et_2O 2:1); IR (neat): $\overline{\nu} = 3{,}046$, 1,672, 1,596, 1,564 cm^{-1};

UV–Vis (ethanol, $c = 1.68 \times 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 257 (4,000), 357 (51,700) 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 404 mg Et_3N (4.0 mmol) in 20 cm^3 anhydr. Et_2O 217 mg ethyl chloroformate (2.0 mmol) in 5 cm^3 Et_2O was added dropwise at 0 °C. The mixture was stirred at this temperature for 3 h, then the triethylamine hydrochloride was filtered off in a glass sintered funnel, washed with 10 cm^3 Et_2O, and the ether was evaporated in vacuo (<40 °C). The residue was immediately dissolved in 15 cm^3 dry EtOH and 84 mg $NaBH_4$ (2.2 mmol) was added in three 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 (<40 °C), the residue was dissolved in 20 cm^3 $CHCl_3$, washed with 10 cm^3 brine, and the organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was immediately dissolved in 20 cm^3 dry CH_2Cl_2, and 1.72 g activated MnO_2 (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 10 cm^3 CH_2Cl_2, the solvent was evaporated, and the residue was purified by flash column chromatography (gradient: hexane/Et_2O 90/10 % to 75/25 % 10 × 30 cm^3 then hexane/EtOAc 60/40 %) to give aldehydes **9** and **4** as yellow solids.

(2E,4E)-5-(1-Oxyl-2,2,4,5,5-pentamethyl-2,5-dihydro-1H-pyrrol-3-yl)-3-methylpenta-2,4-dienal radical
(**9**, $C_{15}H_{22}NO_2$)
Yield: 153 mg (35 %); m.p.: 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) ppm; ^{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) ppm; IR (neat): $\overline{\nu}$ = 1,649, 1,616, 1,600 cm^{-1}; UV–Vis (ethanol, $c = 2.71 \times 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 323 (32,600) 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-1H-pyrrol-3-yl)-3,7-dimethylnona-2,4,6,8-tetraenal radical
(**4**, $C_{20}H_{28}NO_2$)
Yield: 157 mg (25 %); m.p.: 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) ppm; ^{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) ppm; IR (neat): $\overline{\nu}$ = 1,652, 1,597, 1,567 cm^{-1}; UV–Vis (ethanol, $c = 1.98 \times 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 377 (33,600), 270 (10,800) 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-1H-pyrrol-3-carbaldehyde radical (**13**, $C_{15}H_{30}NO_3Si$)
To a stirred solution of 990 mg alcohol **12** (5.0 mmol) and 1.02 g imidazole (15.0 mmol) in 7 cm^3 dry DMF 1.50 g *t*-butyldimethylchlorosilane 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 a mixture of ice and 50 cm^3 sat. aq. $NaHCO_3$ solution, extracted with Et_2O (3 × 20 cm^3), 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. M.p.: 74 °C; R_f = 0,44 (hexane/Et_2O 2:1); ^{1}H 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) ppm; IR (neat): $\overline{\nu}$ = 1,658, 1,625 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-[(1E,3E,5E)-4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hexa-1,3,5-trien-1-yl]-2,5-dihydro-1H-pyrrole radical (**14**, $C_{25}H_{38}NO_2$)
To a stirred solution of 2.72 g β-ionylidenethyltriphenylphosphonium bromide (5.0 mmol) in 40 cm^3 anhydr. THF, 2.8 cm^3 LDA solution (5.0 mmol in THF/heptane/ethylbenzene) was added dropwise at −78 °C. After stirring the solution for 15 min, 1.56 g of compound **13** (5.0 mmol) dissolved in 10 cm^3 THF was added dropwise at −78 °C to the dark red solution and the stirring was continued for 1 h at −78 °C, then the reaction mixture was allowed to warm to room temperature and was stirred at this temperature overnight. The solution was diluted with 30 cm^3 Et_2O and 10 cm^3 sat. aq. NH_4Cl solution was added. The organic phase was separated, dried ($MgSO_4$), filtered, and evaporated. The residue was dissolved in 20 cm^3 THF, 1.30 g

$Bu_4NF \times H_2O$ (5.0 mmol) was added in one portion, and the reaction mixture was stirred for 15 min at room temperature. Then, 20 cm^3 Et_2O was added, the reaction mixture was washed with 20 cm^3 water, 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 %). M.p.: 106 °C; $R_f = 0.47$ (hexane/EtOAc 2:1); IR (neat): $\overline{\nu} = 3{,}481$, 1,591 cm^{-1}; UV–Vis (ethanol, $c = 1.85 \times 10^{-5}$ 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-[(1E,3E,5E)-4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hexa-1,3,5-trien-1-yl]-2,5-dihydro-1H-pyrrol-3-carbaldehyde radical
(**5**, $C_{25}H_{36}NO_2$)

To a stirred solution of 384 mg alcohol **14** (1.0 mmol) in 10 cm^3 CH_2Cl_2, 860 mg activated MnO_2 (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 5 cm^3 CH_2Cl_2, the solvent was evaporated, and the residue was purified by flash column chromatography with gradient elution (hexane/Et_2O 90/10 % to 60/40 %) to give aldehyde **5** (210 mg, 55 %) as a yellow solid. M.p.: 100 °C; $R_f = 0.57$ (hexane/Et_2O 2:1); ^1H 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) ppm; ^{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) ppm; IR (neat): $\overline{\nu} = 1{,}646$, 1,565, 1,540 cm^{-1}; UV–Vis (ethanol, $c = 1.73 \times 10^{-5}$ mol dm^{-3}): λ_{max} (ε) = 384 (21,300), 258 (8,300) nm (mol^{-1} dm^3 cm^{-1}); MS (70 eV): $m/z = 382$ (M^+, 16), 352 (29), 377 (17), 43 (100).

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