Synthesis of paramagnetic and diamagnetic flavones and flavanones

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Dedicated to Prof. Sándor Antus on the occasion of his 60th birthday
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Abstract
Paramagnetic and diamagnetic flavone and flavanone derivatives modified on the C or B rings were synthesized by condensation, Sonogashira reaction, lithiation, and the Baker-Venkataraman procedure.

Keywords: Antioxidants, carbon-carbon bond formation, flavones, nitroxides, radicals

Introduction

Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Recently, much attention has been paid to different flavonoid derivatives as antioxidants, and dietary intake of these natural compounds has a significant effect on preventing a variety of diseases. Most of the flavonoids are very potent antioxidants because they can chelate metal ions, scavenge oxygen free radicals and prevent the oxidation of low density lipoprotein (LDL).[1] The most effective flavonoid-like antioxidants such as quercetin **1**, sylibin **2**, and luteolin **3** have several phenolic hydroxyl or alkoxyl groups on the A, B and C rings (Figure 1).[2-4]

It is well known that nitroxides exhibit SOD mimicry properties,[5] and their precursors are good protectors against ionizing radiation and H_2O_2 caused stress.[6] From our laboratory we reported the synthesis of compounds **4-7**, substituting the B ring of flavonoid structures with five- and six-membered nitroxides and their precursors.[7,8] We thought this offered a chance of improving the antioxidant properties of flavones and flavanones by combining them with

nitroxides or their precursors. In the present paper we report the synthesis of paramagnetic flavonoid derivatives bearing paramagnetic rings on their original B or C rings in order to diminish alterations in their structure.

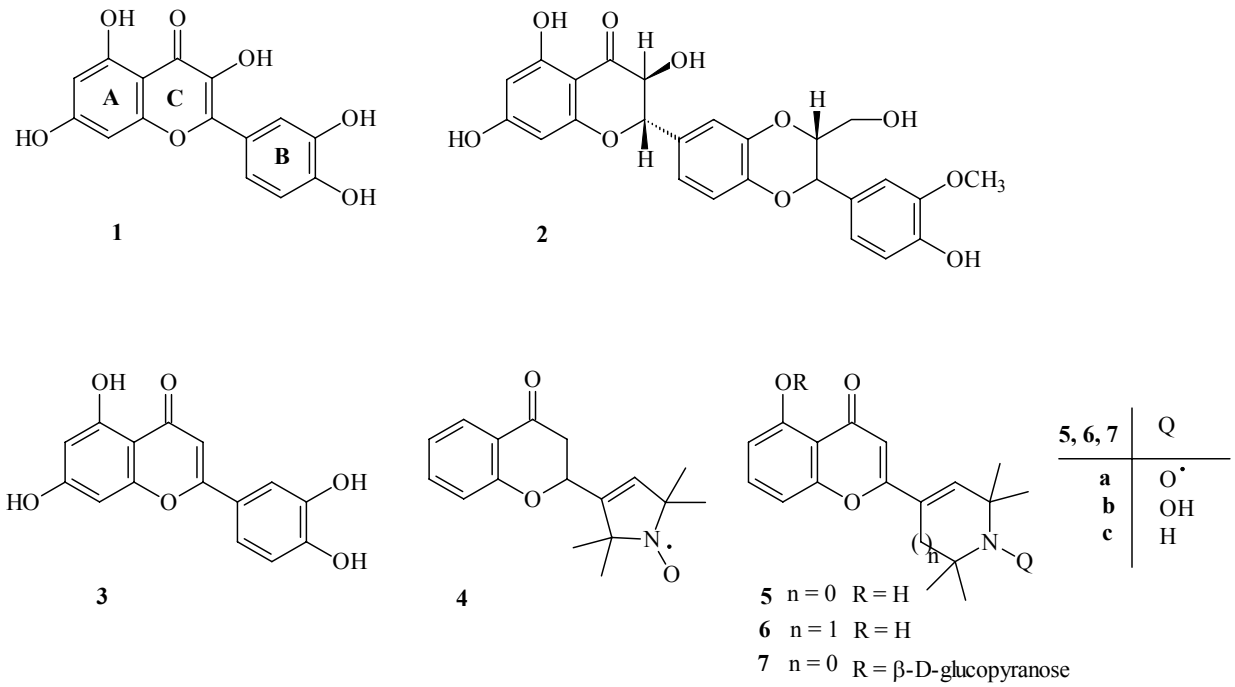

Figure 1. Structure of natural flavone, flavanone derivatives and synthetic benzopyranones.

Results and Discussion

We used a paramagnetic benzaldehyde **8**[9] to synthesize paramagnetic flavone derivatives. This aldehyde was condensed with 2-hydroxyacetophenone in EtOH, in the presence of sodium hydroxide, and the paramagnetic chalcone **9** was cyclised in 50 % acetic acid, in the presence of a catalytic amount of HCl, to yield the paramagnetic flavanone derivative **10a**. The latter was converted to a hydroxylamine compound by ascorbic acid and transformed to the diamagnetic *O*-acetate[10] **10d** for NMR spectroscopic identification. For the synthesis of the paramagnetic flavone derivatives a paramagnetic acid chloride was required. Thus, oxidation of aldehyde **8** with Ag_2O in a basic aqueous medium[11] gave **11**, a paramagnetic benzoic acid, which was converted to the acid chloride by treatment with thionyl chloride in benzene.[12] The moisture sensitive acid chloride was not isolated in pure form, but used immediately in the next reactions.

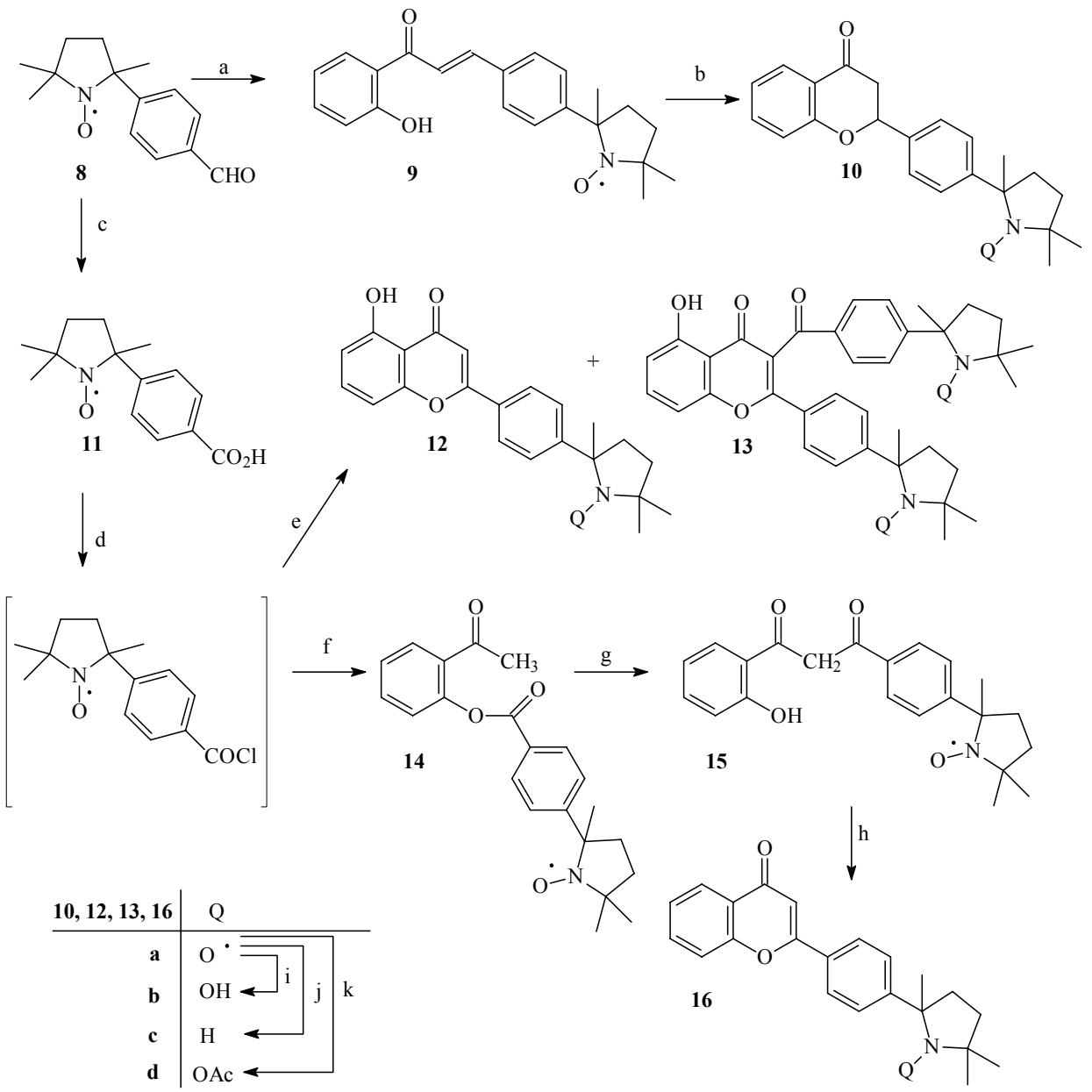

Figure 2. *Reagents and conditions:* a) 2-hydroxyacetophenone (1.0 eq.), NaOH (5.0) eq., $EtOH/H_2O$, 24 h, rt., then H^+, 52 %. b) 50 % aq. AcOH, 18 % aq. HCl, reflux, 12 h, then $NaHCO_3$, extraction with $CHCl_3$, activated MnO_2 (1.0 eq.), O_2, 10 min, 40 %. c) Ag_2O (1.1 eq.), 10 % aq. NaOH/THF, 50 °C, 2 h, then H^+, 48 %. d) $SOCl_2$ (1.5 eq.), pyridine (1.5 eq.), benzene, 0 °C→ rt., 1 h, filtration, evaporation. e) 2,6-dihydroxyacetophenone (1 eq.), K_2CO_3 (5 eq.), acetone, reflux, 24 h, 15-27 %. f) 2-hydroxyacetophenone (1.0 eq.), Et_3N (1.1 eq), CH_2Cl_2, 0 °C→ rt., 1 h, 74 %. g) KOH (1.5 eq.), pyridine, 50 °C, 30 min., then 10 % aq. AcOH, 68 %. h) $AcOH/H_2SO_4$, 100 °C, 30 min, then $NaHCO_3$, MnO_2 (1.0 eq.), O_2, 10 min., 32 %. i) EtOH/HCl,

reflux, 15 min. j) (only for **13**) Fe/AcOH, 70 °C, 1 h, then K_2CO_3, 45 %; k) ascorbic acid (5.0 eq.), dioxane/H_2O, 40 °C, 15 min., then extraction, $CHCl_3$, AcCl (1.1 eq.), Et_3N (1.1 eq.), N_2, 10 °C → rt., 1 h, 27-56 %.

Acylation of 2,6-dihydroxyacetophenone with this acid chloride, in a one-pot procedure, in the presence of K_2CO_3, gave a mixture of the 5-hydroxy-flavone derivative **12a** and biradical **13a.**[8,13]

For spectroscopic identification, these products were converted to diamagnetic derivatives **12d** and **13d** and, for biological study, to hydroxylamines **12b** and **13b**, by refluxing in ethanol saturated by HCl gas,[6] or to diamine **13c**, by reduction with Fe/AcOH.[14] The IR and NMR spectra suggested the 3-acyl structure instead of the 5-*O*-acyl derivative, as well as the findings that treatment of **13a** with NaOH did not gave compound **12a** as a possible hydrolysis product. To study the effect of the absence of OH groups on the **A** ring, a paramagnetic flavone also was synthesized. Acylation of 2-hydroxyacetophenone with paramagnetic benzoyl chloride afforded the keto ester **14** in 74 % yield which in turn underwent a Baker–Venkataraman rearrangement in pyridine in the presence of KOH.[15] This latter compound was cyclised to the paramagnetic flavone **16a** by acid catalysis (Figure 2). Conjugation of a pyrroline nitroxide ring to the C ring of a flavone also was considered. Thus, condensation of 2-hydroxychalcone **17** with aldehyde **18**[16], in the presence of base, gave the paramagnetic flavanone derivative **19a** as a 1:2 mixture of the *Z* and *E* isomers (determined by ^{1}H NMR from the ratio of peaks at 5.40 ppm and 5.34 ppm).[17,18] Lithiation of flavone **20** with LDA in THF gave the 3-lithio derivative,[19] which, upon treatment with paramagnetic aldehyde **18**, gave alcohol **21**. The latter was oxidized to the 1,3-diketone **22** by MnO_2 in $CHCl_3$. Sonogashira reaction of 3-iodo-4'-methoxyflavone **23**[20] with the paramagnetic acetylene compound **24**,[21] in the presence of L-prolinol, CuI and a palladium catalyst, gave a flavone derivative **25**[22] where attachment to the pyrroline ring was from the 3 position of the C ring *via* an ethynyl spacer (Figure 3).

In conclusion, classical synthetic methodologies could be used for the synthesis of paramagnetic flavone and flavanone derivatives containing nitroxides on the B and C rings. The antioxidant activity of the new compounds is under study.

Experimental Section

General Procedures. Melting points were determined with a Boetius micro melting point apparatus and are uncorrected. Elemental analyses (C, H, N, S) were performed on a Fisons EA 1110 CHNS elemental analyser. The IR (Specord 75) spectra were in each case consistent with the assigned structure. Mass spectra were recorded on a VG TRIO-2 instrument in the EI mode. NMR spectra were recorded with Varian Unity Inova 400 WB spectrometer; chemical shifts were referenced to TMS. ESR spectra were taken on Miniscope MS 200 in 10^{-4} M $CHCl_3$ solution and all monoradicals gave triplet line a_N = 14.0-14.4 G, and biradical **13a** gave quintet

line a_{N1} = 14.0 G, a_{N2} = 7.3 G. Flash column chromatography was performed on Merck Kieselgel 60 (0.040-0.063 mm). Qualitative TLC was carried out on commercially prepared plates (20 x 20 x 0.02 cm) coated with Merck Kieselgel GF_{254}. Compound **17** was purchased from Fluka**,** all reagents and compound **20** were purchased from Aldrich, compounds **8,**[9] **18,**[16] **23,**[20] **24**[21] were prepared according to published procedures.

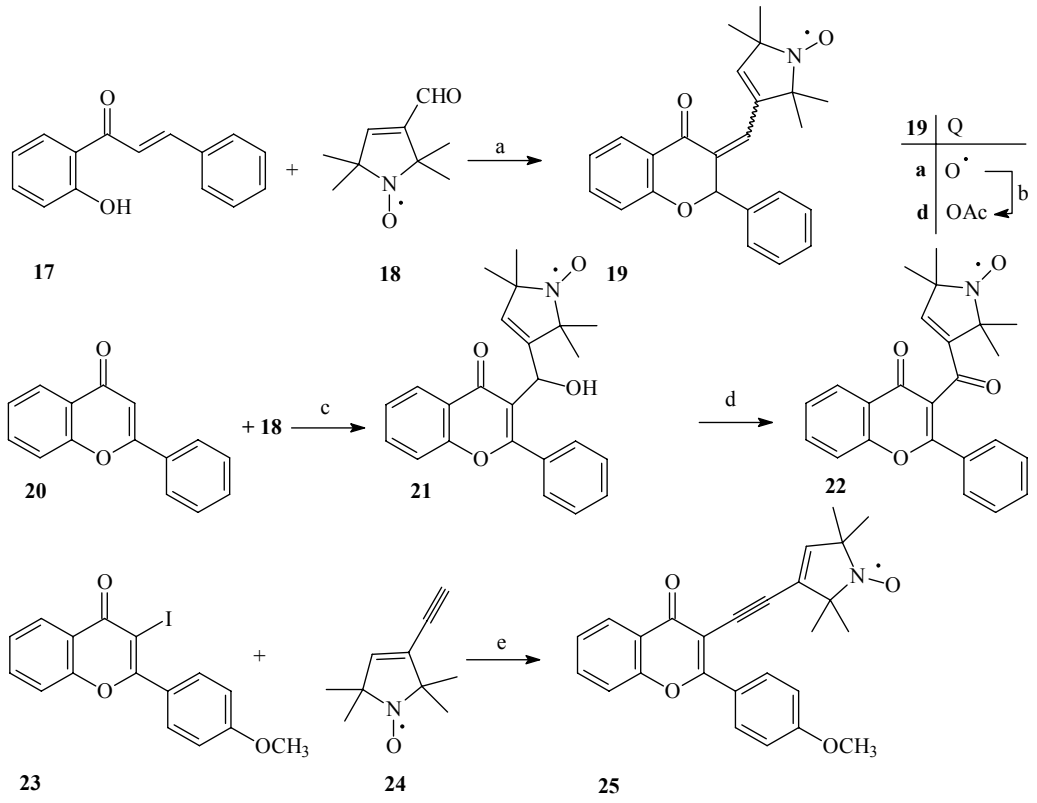

Figure 3. *Reagents and conditions:* a) NaOH (0.2 eq.), MeOH/H_2O, 4 days, rt., 28 %; b) ascorbic acid (5.0 eq.), dioxane/H_2O, 40 °C, 15 min., then extraction, $CHCl_3$, AcCl (1.1 eq), Et_3N (1.1 eq), N_2, 10 °C→ rt. 1 h, 27%; c) LDA, -78 °C, THF, 5 min., then **18** (1.0 eq), 10 min, H_2O, -78 °C→ rt., 52 %; d) activated MnO_2 (4.0 eq.), $CHCl_3$, rt., 12 h, 64 %; e) $Pd(PPh_3)_2Cl_2$ (0.05 eq.), L-prolinol (4.0 eq.), CuI (0.1 eq), DMF/H_2O, rt. 1 h, then **24** (3 eq.), 12 h, 15 %.

1-(2-Hydroxyphenyl)-3-[4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)-phenyl]propenone radical (9). To a mixture of aldehyde **8** (1.16 g, 5.0 mmol) and 2-hydroxyacetophenone (680 mg, 5.0 mmol) in EtOH (10 mL), NaOH (1 g, 25.0 mmol) in water (2 mL) was added and the mixture was allowed to stand overnight at ambient temperature. The EtOH was evaporated and the residue was acidified with 1.0 M aq. HCl solution and extracted with $CHCl_3$ (2 x 30 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (hexane/Et_2O) to give an orange solid 910 mg (52 %), mp 117-118 °C; IR

(nujol) ν: 1630, 1600, 1550 cm^{-1}. Anal calcd. for: $C_{22}H_{24}NO_3$: C 75.40, H 6.90, N 4.00; Found C 75.29, H 6.95, N 3.88. MS (m/z, %): 350 (M^+, 7), 336 (20), 320 (100), 263 (10).

2-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)-phenyl]chroman-4-one radical (10a). To a 50 % AcOH solution (10 mL) of compound **9** (700 mg, 2.0 mmol), aqueous HCl (18 %, 0.1 mL) was added and the mixture was refluxed overnight. After cooling, the mixture was poured into water (30 mL), and the pH was adjusted to 7 by adding solid $NaHCO_3$, followed by extraction with EtOAc (2x 30 mL). The organic phase was dried ($MgSO_4$), MnO_2 (174 mg, 2.0 mmol) was added, O_2 was bubbled through for 10 min., and the mixture was filtered and evaporated. The residue was purified by flash column chromatography (hexane/Et_2O) to give a pale yellow solid 280 mg (40 %), mp 123-125 °C; IR (nujol) ν: 1680, 1590, 1540 cm^{-1}. Anal calcd. for: $C_{22}H_{24}NO_3$: C 75.40, H 6.90, N 4.0; Found C 75.33, H 6.84, N 3.83. MS (m/z, %): 350 (M^+, 7), 336 (11), 320 (100), 218 (55).

General procedure for synthesis of *O*-acetyl derivatives (10d, 12d, 13d, 19d).
To a solution of radical **10a**, **12a**, **13a** or **19a** (5.0 mmol) in dioxane (15 mL), was added a solution of ascorbic acid (4.40 g, 25.0 mmol) in water (10 mL), and the mixture was stirred at 40 ºC for 15 min under N_2. The pale yellow or colorless solution was extracted with $CHCl_3$ (2 x 20 mL) and dried ($MgSO_4$) under N_2. First, acetyl chloride (431 mg, 5.5 mmol or 863 mg, 11.0 mmol in the cases of **12a** and **13a**) and then, slowly, Et_3N (555 mg, 5.5 mmol or 1.11 g, 11.0 mmol in case of **12a** and **13a**) were added, the temperature being kept below 10 ºC. Stirring was continued for 1 h, after which time the mixture was filtered and the filtrate was evaporated in vacuo to dryness. The residue was taken up in brine (10 mL) and extracted with EtOAc (2 x 15 mL). The organic layer was dried ($MgSO_4$), filtered, evaporated and chromatographed (flash, hexane/Et_2O) to give the *O*-acetyl derivatives **10d** or **12d** or **13d** or **19d** in 27-56 % yield. These diamagnetic species were made for NMR study.
10d. white solid, mp 122-123 ºC, ^{1}H NMR ($CDCl_3$) δ_H: 7.92 (d, 1H, aromatic proton), 7.73 (d, 2H, aromatic protons), 7.49 (t, 1H, aromatic proton), 7.41 (d, 2H, aromatic protons), 7.04 (t, 1H, aromatic proton), 7.03 (d, 1H, aromatic proton), 5.45 (dd, *J* = 13.4, 2.8 Hz, 1H), 3.09 (dd, *J* = 16.9, 13.4 Hz, 1H), 2.87 (dd, *J* = 16.9, 2.8 Hz, 1H), 2.08 (t, *J* = 7.1 Hz, 2H), 1.99 (s, 3H), 1.87 (m, 1H), 1.73 (m, 1H), 1.56 (s, 3H), 1.25 (s, 6H). ^{13}C NMR ($CDCl_3$) δ_C: 192.2, 170.3, 161.6, 149.9, 136.3, 136.1, 127.0, 126.4, 126.4, 125.9, 125.9, 121.5, 120.9, 118.1, 79.5, 70.4, 65.7, 44.4, 38.3, 36.2, 29.4, 24.4, 19.3.
12d. yellow amorphous solid, ^{1}H NMR ($CDCl_3$) δ_H: 7.84 (s, 4H, aromatic protons), 7,52 (t, 1H, aromatic proton), 6.97 (d, 1H, aromatic proton), 6.78 (d, 1H, aromatic proton), 6.70 (s, 1H), 2.10 (m, 2H), 2.00 (s, 3H), 1.88 (m, 1H), 1.73 (m, 1H), 1.58 (s, 3H), 1.26 (s, 3H), 1.25 (s, 3H). ^{13}C NMR ($CDCl_3$) δ_C: 138.6, 170.1, 164.8, 160.8, 156.4, 153.7, 135.2, 128.9, 126.8, 126.8, 126.2, 126.2, 111.3, 110.8, 107.0, 105.5, 70.4, 65.9, 38.4, 36.0, 29.2, 24.3, 19.1.
13d. white solid, mp 138-139 ºC, ^{1}H NMR ($CDCl_3$) δ_H: 7.88 (d, 2H, aromatic protons), 7.74 (d, 2H, aromatic protons), 7.66 (d, 2H, aromatic protons), 7.59 (d, 2H, aromatic protons), 7.58 (t,

1H, aromatic proton), 7.00 (d, 1H, aromatic proton), 6.82 (d, 1H, aromatic proton), 2.05 (m, 4H), 1.98 (s, 3H), 1.95 (s, 3H), 1.83 (m, 2H), 1.68 (m, 2H), 1.54 (s, 3H), 1.49 (s, 3H), 1.23 (s, 6H), 1.21 (s, 3H), 1.18 (s, 3H). ^{13}C NMR ($CDCl_3$) δ_C: 192.4, 181.8, 170.1, 163.5, 160.8, 156.2, 135.9, 135.1, 129.4, 129.4, 128.9, 128.4, 128.4, 126.5, 126.5, 126.5, 126.5, 120.9, 111.7, 110.1, 107.0, 70.7, 70.5, 65.9, 65.9, 38.0, 38.0, 36.1, 36.0, 29.2, 29.2, 24.4, 24.4, 24.0, 24.0, 19.3, 19.2.

19d. white solid, mp 149-151 ºC, ^{1}H NMR ($CDCl_3$) δ_H: 7.88 (d, 1H, aromatic proton), 7.40 (t, 1H, aromatic proton), 7.35-7.25 (m, 5H, aromatic protons), 6.94 (t, 1H, aromatic proton), 6.93 (t, 1H, aromatic proton), 6.50 (bs, 1H) and 6.48 (bs, 1H) for *Z* and *E* isomer, 5.40 (bs,1H) and 5.34 (bs, 1H) for *Z* and *E* isomer, 2.13 (s, 3H), 1.41 (s, 3H), 1.25 (s, 3H), 1.24 (s, 3H), 1.18 (s, 3H). ^{13}C NMR ($CDCl_3$) δ_C:181.7, 171.1, 158.8, 139.0, 138.2, 136.6, 136.2, 135.9, 130.7 and 130.4 for *E* and *Z* isomer, 128.8, 128.8, 128.6, 127.5, 127.2, 127.2, 121.7, 118.6, 77.8, 72.0, 69.4, 30.9, 28.8, 28.1, 22.7, 22.6 (for minor *Z* isomer 28.5, 27.5, 23.9, 23.6), 19.1.

4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzoic acid radical (11). To a vigorously stirred suspension of freshly precipitated Ag_2O (2.55 g, 11.0 mmol) in 10 % aq. NaOH solution (20 mL), was added aldehyde **8** (2.32 g, 10.0 mmol) in THF (5 mL) at 50 ºC, and the mixture was stirred for 2 h. After cooling, the solution was filtered through a Celite pad and the residue was washed with MeOH (20 mL). The combined organic solvents were evaporated, the aqueous phase was acidified with 5 % aq. H_2SO_4 to pH = 2, and extracted with $CHCl_3$ (3 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography ($CHCl_3$/Et_2O) to give carboxylic acid **11** 1.19 g, 48 % as a yellow solid, mp 130-131 ºC; IR (nujol) ν: 3100, 1690, 1600, 1560 cm^{-1}. Anal calcd. for: C_{14} $H_{18}NO_3$: C 67.72, H 7.31, N 5.64; Found C 67.66, H 7.30, N 5.52. MS (m/z, %): 248 (M^+, 12), 148 (56), 121 (60), 43 (100).

4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzoyl chloride radical. To a stirred solution of acid **11** (2.48 g, 10.0 mmol) and pyridine (1.2 mL, 14.8 mmol) in dry benzene (15 mL) was added $SOCl_2$ (1.2 mL, 15.3 mmol) dropwise at 0 °C. After stirring at ambient temperature for 1 h, the pyridinum hydrochloride was filtered off, and washed with benzene (5 mL). The benzene was evaporated and the residue was dissolved in dry acetone (8 mL) for **12** and **13**, or dry CH_2Cl_2 (8 mL) for **14**, and used immediately in the next step.

5-Hydroxy-2-[4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)-phenyl]chromen-4-one radical (12a) and 3-[4-(1-Oxyl-2,5,5-trimethyl-pyrrolidin-2-yl)benzoyl]-2-[4-(1-oxyl-2,5,5-trimethyl-pyrrolidin-2-yl)phenyl]chromen-4-one biradical (13a). A solution of 2,6-dihydroxyacetophenone (1.52 g, 10.0 mmol) and K_2CO_3 (6.75 g, 50.0 mmol) in dry acetone (40 mL) was stirred for 10 min. at room temperature whereupon 4-(1-oxyl-2,5,5-trimethyl-pyrrolidin-2-yl)benzoyl chloride radical (made from 10.0 mmol acid, see above) in acetone (8 mL) was added dropwise. The mixture was stirred at reflux for 24 h, then the acetone was evaporated, water (15 mL) was added and the mixture was extracted with EtOAc (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated to yield a residue that was purified by flash column chromatography (hexane/Et_2O, hexane/EtOAc) to give **12a (**546 mg, 15 %) as a yellow solid, mp 149-150 °C, as a first band and **13a (**1.60 g, 27%) as a yellow

solid, mp 120-123 °C, as a second band. Anal. calcd. for **12a** $C_{22}H_{22}NO_4$: C 72.51, H 6.08, N 3.84; Found C 72.45, H 6.01, N 3.71; IR (nujol) ν:1650, 1600, 1570 cm^{-1}. MS (m/z, %): 364 (M^+, 18), 350 (29), 334 (47), 278 (100). Anal. calcd. for **13a** $C_{36}H_{38}N_2O_6$: C 72.71, H 6.44, N 4.71; Found C 72.80, H 6.29, N 4.55; IR (nujol) ν: 1670, 1600, 1600, 1530 cm^{-1}. MS (m/z, %): 594 (M^+, 11), 580 (19), 564 (35), 549 (100).

General procedure for reduction of paramagnetic compounds to diamagnetic hydrochloride salts of *N*-hydroxylamines (12b, 13b, 16b)

Compound **12a**, **13a** or **16a** (0.1 mmol) was dissolved in EtOH (5 mL) previously saturated with HCl gas. The solution was refluxed for 15 min., allowed to cool and the solvent evaporated off. The residue was crystallized from Et_2O or acetone/ Et_2O or EtOAc/hexane to give diamagnetic *N*-hydroxy HCl salts for biological study. **12b:** pale yellow solid, mp 125-126 °C, **13b**: beige solid, mp 158-160 °C, **16b**: off-white solid, mp 149-152 °C, ^{1}H NMR (DMSO-d_6) δ_H: 8.15 (d, 2H, aromatic protons), 8.06 (d, 1H, aromatic proton), 7.85-7.82 (m, 4H, aromatic protons), 7.52 (t, 1H, aromatic proton), 7.10 (s, 1H), 2.57 (m, 1H), 2.41 (m, 1H), 2.19 (m, 1H), 2.0 (m, 1H), 1.65 (bs, 3H), 1.46 (bs, 6H). ^{13}C NMR (DMSO-d_6) δ_C: 177.1, 162.1, 155.7, 134.4, 131.6, 126.2, 126.2, 126.2, 126.2, 125.6, 124.8, 123.3, 118.6, 107.2, 34.7, 31.1, 24.6, 24.6, 22.3.

4-(2,5,5-Trimethylpyrrolidin-2-yl)-benzoic acid 2-[4-(2,5,5-trimethylpyrrolidin-2-yl)-phenyl]-4-oxo-4*H*-chromen-5-yl ester (13c). To a solution of nitroxide **13a** (1.18 g, 2.0 mmol) in AcOH (8 mL), was added Fe powder (560 mg, 10 mmol) and the mixture was warmed to 70 °C until the reaction started. The mixture was stirred at room temperature for 1 h, diluted with water (20 mL), decanted and the decanted aq. solution made alkaline with solid K_2CO_3. The mixture was extracted with $CHCl_3$ (3 x 15 mL) and the combined organic layers were dried ($MgSO_4$), filtered and evaporated. Chromatographic purification ($CHCl_3$ / MeOH) gave the diamine **13c** 508 mg (45 %) as an off-white solid, mp 133-135 °C. Anal. calcd. for **13c** $C_{36}H_{40}N_2O_4$: C 76.57, H 7.14, N 4.96; Found C 76.60, H 7.10, N 4.82; IR (nujol) ν: 1670, 1640, 1600, 1530 cm^{-1}. MS (m/z, %): 564 (M^+, 2), 549 (100), 532 (28), 333 (10).

4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzoic acid 2-acetyl-phenyl ester radical (14). To a stirred solution of 2-hydroxyacetophenone (1.36 g, 10.0 mmol) and Et_3N (1.11 g, 11 mmol) in CH_2Cl_2 (20 mL) was added 4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)benzoyl chloride radical (made from 10.0 mmol acid, see above) in CH_2Cl_2 (8 mL) dropwise at 0 °C. The mixture was stirred at room temperature for 1 h and the organic phase was washed with brine (10 mL), saturated aq. $NaHCO_3$ (30 mL), separated, dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography ($CHCl_3$/Et_2O) to give compound **14** 2.70 g (74 %) as a pale yellow solid, mp 113-115 °C. Anal. calcd. for **14** $C_{22}H_{24}NO_4$: C 72.11, H 6.60, N 3.84; Found C 72.05, H 6.59, N 3.73; IR (nujol) ν: 1720, 1680, 1640, 1600, 1550 cm^{-1}. MS (m/z, %): 366 (M^+, 8), 336 (15), 231 (52), 145 (100).

1-(2-Hydroxyphenyl)-3-[4-(1-oxyl-2,5,5-trimethylpyrrolidin-2-yl)phenyl]propane-1,3-dione radical (15). To a stirred solution of compound **14** (1.88 g, 5.0 mmol) in dry pyridine (7 mL) was added powdered KOH (421 mg, 7.5 mmol) at 50 °C with stirring. After 30 min., the mixture turned brown, was allowed to cool and was poured onto a mixture of ice (30 g) and 10 % aq. AcOH (10 mL). The reaction mixture was extracted with EtOAc (3x 15 mL), the organic phase was washed with sat. aq. $NaHCO_3$ (15 mL), dried ($MgSO_4$), filtered, evaporated and the residue was purified by chromatography (hexane/EtOAc) to give compound **15** 1.27 g (68 %), mp 81-83 °C. Anal. calcd. for $C_{22}H_{24}NO_4$: C 72.11, H 6.60, N 3.84; Found C 72.10, H 6.52, N 3.69; IR (nujol) ν: 3500, 1600, 1580 cm^{-1}. MS (m/z, %): MS (m/z, %): 366 (M^+, 10), 352 (10), 336 (46), 145 (100).

2-[4-(1-Oxyl-2,5,5-trimethylpyrrolidin-2-yl)phenyl]chromen-4-one radical (16a). To a solution of compound **15** (366 mg, 1.0 mmol) in AcOH (10 mL) was added cc. H_2SO_4 (0.1 mL) and the mixture was heated at 100 °C for 30 min. The mixture was poured onto crushed ice (30 g), made basic by adding solid $NaHCO_3$ (intense foaming) and extracted with $CHCl_3$ (2x 20 mL). The organic phase was dried ($MgSO_4$), activated MnO_2 (87 mg, 1.0 mmol) was added and O_2 was bubbled through for 10 min. The mixture was filtered, evaporated and the residue was purified by flash column chromatography (hexane/Et_2O) to give compound **16a** as a yellow solid 111 mg (32 %), mp 115-116 °C; anal. calcd. for $C_{22}H_{22}NO_3$: C 75.84, H 6.36, N 4.02; Found C 75.80, H 6.33, N 3.99; IR (nujol) ν: 1630, 1600, 1550 cm^{-1}. MS (m/z, %): 348 (M^+, 10), 334 (20), 318 (100), 262 (40).

3-[1-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)methylidene]-2-phenyl-chroman-4-one radical (19). To a mixture of 2-hydroxychalcone **17** (1.12 g, 5.0 mmol) and aldehyde **18** (840 mg, 5.0 mmol) in MeOH (20 mL) was added NaOH (40 mg, 1.0 mmol) in water (2 mL). The mixture was allowed to stand for 4 days at rt, then the MeOH was evaporated off, and the residue was taken up in aq. HCl solution (1 M, 15 mL). Extraction with EtOAc (2 x 15 mL), drying of the combined organic phase ($MgSO_4$), filtration and evaporation gave a residue that was purified by flash chromatography (hexane/Et_2O) to give **19** 523 mg (28 %) as a third band (after the starting chalchone and flavanone), mp 152-153 °C, orange solid, anal. calcd. for $C_{24}H_{24}NO_3$: C 76.98, H 6.46, N 3.74; Found C 76.85, H 6.40, N 3.62; IR (nujol) ν: 1640, 1604, 1560 cm^{-1}. MS (m/z, %): 374 (M^+, 10), 360 (43), 344 (100), 329 (27).

3-[Hydroxy-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-yl)-methyl]-2-phenyl-chromen-4-one radical (21). To a stirred solution of flavone **20** (1.11 g, 5.0 mmol) in dry THF (10 mL) was added LDA (3 mL, 5.4 mmol, (1.8 M solution in heptane/THF/ethylbenzene)) at –78 ºC. After 5 min., the aldehyde **18** (840 mg, 5.0 mmol) dissolved in THF (5 mL) was added dropwise and after a further 10 min., water (10 mL) was added and the mixture was allowed to warm to rt. The mixture was diluted with EtOAc (10 mL), the organic phase was separated and the aqueous phase was extracted with EtOAc (10 mL). The combined organic phases were dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography to give compound **21** 1.01g (52 %) as a yellow solid, mp 198-199 °C; anal. calcd for $C_{24}H_{24}NO_4$: C

73.83, H 6.20, N 3.59; found C 73.80, H 6.15, N 3.45; IR (nujol) ν: 3460, 1630, 1550 cm^{-1}. MS (m/z, %): 390 (M^+, 25), 376 (25), 360 (48), 251 (100).

3-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-carbonyl)-2-phenylchromen-4-one radical (22). A solution of compound **21** (195 mg, 0.5 mmol) and activated MnO_2 (348 mg, 4.0 mmol) was stirred in $CHCl_3$ (20 mL) for 12 h at ambient temperature. The MnO_2 was filtered off, washed with $CHCl_3$ (10 mL) and the combined filtrate was evaporated. The residue was crystallized from hexane/Et_2O to give compound **22** as a yellow solid 124 mg (64 %), mp 185-187 °C; anal. calcd. for $C_{24}H_{22}NO_4$: C 74.21, H 5.71, N 3.61; found: C 74.13, H 5.67, N 3.52; IR (nujol) ν: 1670, 1605, 1550 cm^{-1}. MS (m/z, %): 388 (M^+, 3), 374 (4), 358 (15), 249 (100).

3-(1-Oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1*H*-pyrrol-3-ylethynyl)-2-(4-methoxyphenyl)chromen-4-one radical (25). A solution of 3-iodo-4'-methoxyflavone **23** (189 mg, 0.5 mmol), CuI (15 mg, 0.08 mmol), $(PPh_3)_2PdCl_2$ (17 mg, 0.024 mmol) and L-prolinol (202 mg, 2.0 mmol) in DMF (5 mL) / water (1 mL) was stirred at rt for 1 h under N_2. The paramagnetic acetylene **24** (246 mg, 1.5 mmol) dissolved in DMF (1 mL) was added dropwise and the mixture was stirred for a further 12 h at rt. The mixture was poured into water (20 mL), extracted with EtOAc (2 x 15 mL), the organic phase was separated, dried ($MgSO_4$), filtered and evaporated. The residue was purified by flash column chromatography (hexane/EtOAc) to give compound **25** 31 mg (15 %) as a yellow solid mp 118-120 °C. Anal. calcd. for $C_{26}H_{24}NO_4$: C 75.34 H 5.84, N 3.38; Found C 75.30, H 5.82, N 3.41. IR (nujol) ν: 1640, 1600, 1550 cm^{-1}. MS (m/z, %): 414 (M^+, 1), 384 (3), 277 (74), 252 (100).

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