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Supporting Information

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Blue Luminescence of Ripening Bananas

Supporting Information

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Materials. Commercially available solvents (reagent-grade) were redistilled before use for extractions. HPLC grade methanol (MeOH) was from Merck (Darmstadt, Germany) and Acros Organics (Geel, Belgium). Potassium dihydrogen phosphate puriss. p.a. and potassium phosphate dibasic-anhydrous puriss.p.a. were from Fluka (Buchs, Switzerland). Amberlite IRA-900, Cl-form ion exchange resin was from Acros Organics (Geel, Belgium). Sep-Pak-C18 Cartridges were from Waters Associates. The pH values were measured with a WTW Sentix 21 electrode connected to a WTW pH535 digital pH meter.

Methods. *Analytical HPLC:* Dionex Summit HPLC system with manual sampler, P680 pump, online degasser and diode array detector and Jasco FP-920 fluorescence detector. Injection loop 1 ml (Rheodyne injection valve), a Hypersil ODS 5µm 250 x 4.6mm i.d. column at r.t. protected with a Phenomenex ODS

4 mm x 3 mm i.d. precolumn was used with a flow rate 0.5 $mlmin^{-1}$. Data were collected and processed with Chromeleon V6.50 Solvent A: 100 mM potassium phosphate (pH 7.0), solvent B: MeOH. *Preparative HPLC:* Gynkotek HPLC System with manual sampler, M300 pump, UVD 340 diode array detector and Jasco FP-920 fluorescence detector, a Hypersil ODS 5 μm 250 mm x 21.2 mm i.d. column at r.t. with a flow rate of 5 $mlmin^{-1}$ was used. *Spectroscopy. UV/Vis:* Hitachi U-3000. *Fluorescence:* Varian Cary Eclipse. *CD:* Jasco J715. *NMR:* Bruker UltraShield 600 MHz or Varian Unity Inova 500 MHz spectrometers. *Mass spectrometry:* Finigan MAT 95-S, positive-ion mode, m/z (rel. abundance); ESI: infusion, spray voltage 1.4 kV, solvent water/methanol 1:1 (v/v). FAB-MS: cesium gun, 20 keV, glycerol matrix; High resolution (HR) mass spectra: polyethylene glycole as internal mass standard

Luminescence of intact bananas: The luminescence from intact, commercially available (ethylene-ripened) bananas from a supermarket in New York City was measured directly without removing the peel using a fibre-optics attachment of a Fluorolog 3 spectrometer (Horiba Jobin Yvon) and an excitation wavelength of 350 nm.

Banana-Sampling.

Ethylene-ripened yellow bananas (*Musa cavendish*, supplier 'Dole'). These were bought in the supermarket and were stored at room light and ambient temperature. An extract was produced (see below) from the bananas at day zero, one, two, three, four and five. Unripe green bananas (*Musa cavendish*, supplier 'Dole') were bought from the merchant before ethylene treatment and were extracted likewise.

Extract preparation and analysis. A section (30 cm^2) of the outer peel was frozen in liquid nitrogen and was ground. MeOH (1 ml) was added to the cold mixture, which was ultra filtrated (3 min, 13000 rpm, 2 Viva pure mini-spin columns). From each sample of peels 1.2 to 1.3 ml of liquid extract were obtained. A sample of 20 μl of the extracts was used for analysis by HPLC, with fluorescence tracing at 450 nm. The relative amounts of fluorescent chlorophyll catabolites (FCCs) were determined by integrating the areas of all luminescent FCC-fractions.

Naturally ripened and green unripe bananas (*Musa cavendish*). These were cut from banana trees in the plantation Malpaes Trece in Guachico, Island of Teneriffa, on Feb. 18, 2008. They were transported to Innsbruck (at ambient temperature) and were analyzed by HPLC on Feb. 19 (as described in the preceding section for commercial bananas).

Isolation of the main banana FCC (*Mc*-FCC-56). The peels of 215 yellow bananas (*Musa cavendish*) were worked up in two parallel extractions. The peels were frozen in liquid nitrogen and ground. 2 l of MeOH were added, the mixture was left to warm up to room temperature (during about half an hour) and the pulp was squeezed trough a fruit-squeezer, giving a turbid juice. The filter cake was suspended again twice in 500 ml of MeOH each and extracted, as before. The juice was then filtered through a bed of Celite and most MeOH was removed on a rotary evaporator. The residual mixture was diluted with water and 100 ml of potassium phosphate buffer 100 mM pH7. The preparation was desalted in two parallel batches by passing through 2 SepPak Vac C18 5g cartridges (solvent for elution: MeOH/water 75/25) and concentrated to a volume of 30 ml using a rotary evaporator. Water (40 ml) and 10 ml of buffer (pH7) were added and the extract was loaded onto an anion exchange column with 12.5 ml of solid resin. Elution was carried out with 100 ml of 0.5 M aqueous NaCl and 100 ml 0.5 M aqueous NaCl / MeOH (4:1). From further purification by preparative HPLC, crude samples of *Mc*-FCC-56 were obtained from both batches, which were desalted over SepPak cartridges and purified further by semi-preparative HPLC. A fraction containing *Mc*-FCC-56 and its isomer, *Mc*-FCC-53, was loaded on a cartridge, washed with water and eluted with MeOH/water 9/1. The solvents were evaporated *in vacuo* and 13.1 mg of a 3/1 mixture of *Mc*-FCC-56 and its isomer *Mc*-FCC-53 were obtained. This isomeric mixture was used to record ^{13}C, DEPT, $^{1}H,^{13}C$-HSQC and HMBC spectra (in CD_3OD, 25°C, slow isomerization occurred during time consuming measurements). Subsequent separation of the sample from NMR-analysis by analytical HPLC yielded analytically pure samples: 9.9 mg of *Mc*-FCC-56 and 1.1 mg of *Mc*-FCC-53, used for recording further spectra (in CD_3OD, 25°C, negligible isomerization of *Mc*-FCC-56 to *Mc*-FCC-

53): ^{1}H-NMR, ^{1}H,^{1}H-COSY and ROESY spectra, as well as further ^{1}H,^{13}C-HMBC spectra (see Figure S2).

Citric acid induced isomerization of *Mc*-FCC-56. An aqueous solution (60 µl) of 1M citric acid was added to 3 ml of a solution of *Mc*-FCC-56 (30.9 µM) in MeOH. The mixture was kept under argon atmosphere in the dark at 15°C. 159 h after addition of the acid, 1 ml of water was added. The resulting solution was subjected to analytical HPLC, which indicated it to consist of NCCs mainly. The two most abundant NCC fractions (*Mc*-NCC-55 and *Mc*-NCC-58) were collected, and their UV/Vis- and CD-spectra were recorded (see Figure S3).

Exploratory analysis of a yellow banana leaf. About 2 cm^2 of a yellow banana leaf were ground with liquid nitrogen in a mortar and extracted with 10 ml of methanol. The turbid extract was filtered, concentrated on a rotary evaporator and diluted with about 10 ml water. The mixture was filtered again before desalting with a SepPak cartridge. Elution with 5 ml of methanol gave an extract, which was concentrated to about 1.3 ml on a rotary evaporator, and was ready for analytical HPLC (samples of 20 µl of this aqueous extract were injected, see Figure S5).

Spectroanalytical Data.

***Mc*-FCC-56** (retention time in analytical HPLC (r_t) = 56 min, see main text and Figure S6).

UV-Vis (c = 5.77*10^{-5}M): λ_{max} (log ε) = 356 nm (4.10), 317 (4.29), 237 (4.40).

***Mc*-FCC-53** (r_t = 53 min); UV-Vis: λ_{max} (rel. ε) = 368 nm (0.47), 316 (0.69), 239 (1.00).

MS (ESI): m/z (%) = 869.32 (5, $[M+K]^+$), 854.27 (8), 853.31 (16, $[M+Na]^+$), 833.33 (13), 832,33 (46), 831.27 (100, $[M+H]^+$), 693.25 (4), 645.23 (3, $[M-C_7H_7O_6+H]^+$).

HR-FAB: m/z = 831.309 ($[M+H]^+$), m/z_{calc} ($C_{42}H_{47}N_4O_{14}$) = 831.308.

***Mc*-FCC-49** (r_t = 49 min); UV-Vis: λ_{max} (rel. ε) = 363 (0.51), 318 (0.93), 242 (1.00).

^{1}H-NMR: (600 MHz, CD_3OD): δ =1.13 (d, J = 7.3 Hz, $H_3C(18^1)$), 1.91 (m, $H_2C(17^1)$), 2.05 (s, $H_3C(2^1)$), 2.15 (s, $H_3C(12^1)$), 2.21 (s, $H_3C(7^1)$), 2.33 (m, $H_2C(17^2)$), 2.46 ($H_BC(20)$), superimposed by 2.49 (m, HC (17)), 2.67 (m, $H_2C(8^1)$), 2.78 (m, HC(18)), 3.03 (dd, J = 4.3/18.5, $H_AC(20)$), 3.07 (dd, J = 7.6/8.5 Hz), 3.12 (m), 3.57 (m, $H_2C(8^2)$), 3.66 (m), 3.75 (s, $H_3C(13^5)$), 3.81 (m), 3.89 (d, J = 3.2 Hz), 4.03/4.21 (AB-system, J = 16.8 Hz, H_2C (10)), superimposed by 4.23 (d, J = 7.8 Hz), 4.30 (b, HC(6')), 4.35 (b), 4.39 (dd, J = 4.3/8.3 Hz, HC(1)), 5.40(dd, J ~ 2/12 Hz, $H_AC(3^2)$) superimposed by 5.45 (s (b), HC (5')), 5.56 (s (b), HC (3')), 6.18 (dd, J ~ 2/18 Hz, $H_BC(3^2)$), 6.51 (dd, J = 11.7/17.8 Hz, $HC(3^1)$), 9.33 (s, HC(5)). FAB-MS: m/z (%) = 1033.42 (40), 1032.69 (64), 1031.65 (97, $[M+K]^+$), 995.55 (32), 994.63 (56), 993.57 (100, $[M+H]^+$), 871.59 (49, [M-ring A+H]$^+$).

***Mc*-FCC-46** (r_t = 46 min); UV-Vis ('online' spectrum of an HPLC fraction) λ_{max} (rel. ε) = 368 (0.66), 317 (1.00), 233 (1.00); FAB-MS: m/z (%) = 1033.57 (42), 1032.54 (58), 1031.58 (71, $[M+K]^+$), 1015.58 (47, $[M+Na]^+$), 993.57 (30, $[M+H]^+$), 846.69 (54), 845.73 (100, $[M-C_7H_7O_6+K]^+$), 807.96 (93, $[M-C_7H_7O_6+H]^+$).

***Mc*-NCC-58** (r_t = 58 min); UV-Vis (c = 2.02*10^{-5}M): λ_{max} (rel. ε) = 314 (0.53), 242 (0.71), 212 (1.00); CD (c = 2.02*10^{-5}M): λ_{max}/nm(Δε) = 222 (21.2), 245 (-5.4), 283 (-12.0), 316 (6.3).

MS (ESI): m/z (%) = 1179.73 (8), 945.23 (6, $[M+3K]^+$), 929.30 (7, $[M+2K+Na]^+$), 907.25 (18, $[M+2K]^+$), 891.32 (24, $[M+K+Na]^+$), 871.31 (29), 870.28 (57), 869.31 (100, $[M+K]^+$), 854.32 (23), 853.37 (39

$[M+Na]^+$), 833.28 (14), 832.30 (42), 831.37 (63, $[M+H]^+$), 721.21 (13), 687.21 (18), 429.17 (36), 413.17 (17).

***Mc*-NCC-55** (r_t = 55 min); UV-Vis (c = $1.06*10^{-5}$M): λ_{max}(rel. ε) = 314 (0.57), 242 (0.73), 212 (1.00)**;** CD (c = $1.06*10^{-5}$M): λ_{max}/nm (Δε) = 230 (-16.1), 283 (6.9), 320 (-5.0). MS (ESI): m/z (%) = 891.32 (9, $[M+K+Na]^+$), 869.31 (30, $[M+K]^+$), 853.31 (30 $[M+Na]^+$), 833.39 (19), 832.35 (55), 831.31 (100, $[M+H]^+$), 705.25 (13), 683.25 (13), 667.21 (6, $[M-C_7H_7O_6+Na]^+$).

***Mc*-NCC-42** (r_t = 42 min, for UV-Vis, see ref. [13]):

^{1}H-NMR (500 MHz, CD_3OD): δ [ppm] = 1.93 (s, $H_3C(18^1)$), 2.02 (s, $H_3C(2^1)$), 2.08 (s, $H_3C(12^1)$), 2.26 (s, $H_3C(7^1)$), 2.32 (t, J = 7 Hz, $H_2C(17^2)$), 2.54 (dd, J = 8.5/14.6 Hz, $H_AC(20)$), 2.62 (m, $H_AC(17^1)$) superimposed by 2.63 (dd, $H_2C(8^1)$), 2.72 (m, $H_BC(17^1)$), 2,87 (dd, J = 4.4/14.6 Hz, $H_BC(20)$), 3.47 (m, $H_2C(8^2)$), 3.62 (dd, J = 5.0/11.3 Hz, $H_AC(3^2)$), 3.69 (dd, J = 6.6/11.3 Hz, $H_BC(3^2)$), 3.97 (m, $H_2C(10)$), 4.01 (dd, J = 4.5/8.6 Hz, HC(1)), 4.56 (dd, $HC(3^1)$), 9.36 (s, HC(5)). ^{13}C-NMR (125 MHz, CD_3OD, ^{13}C-signal assignment from HSQC & HMBC experiments): δ = 8.93 (7^1), 9.16 (12^1), 9.31 (18^1), 12.4 (2^1), 21.9 (17^1), 23.8 (10), 28.1 (8^1), 29.7 (20), 37.2 (15), 39.1 (17^1), 52.8 (13^5), 62.4 (1), 62.5 (8^2), 65.9 (3^2), 68.6 (3^1), 113 (12), 115 (18), 120 (17), 121 (8), 124 (16), 124 (19), 126 (13), 129 (4), 132 (3), 135 (7), 135 (11), 139 (9), 159 (2). HR-FAB: m/z = 679.300 ($[M+H]^+$), m/z_{calc} ($C_{35}H_{43}N_4O_{10}$) = 679.297.

Figures.

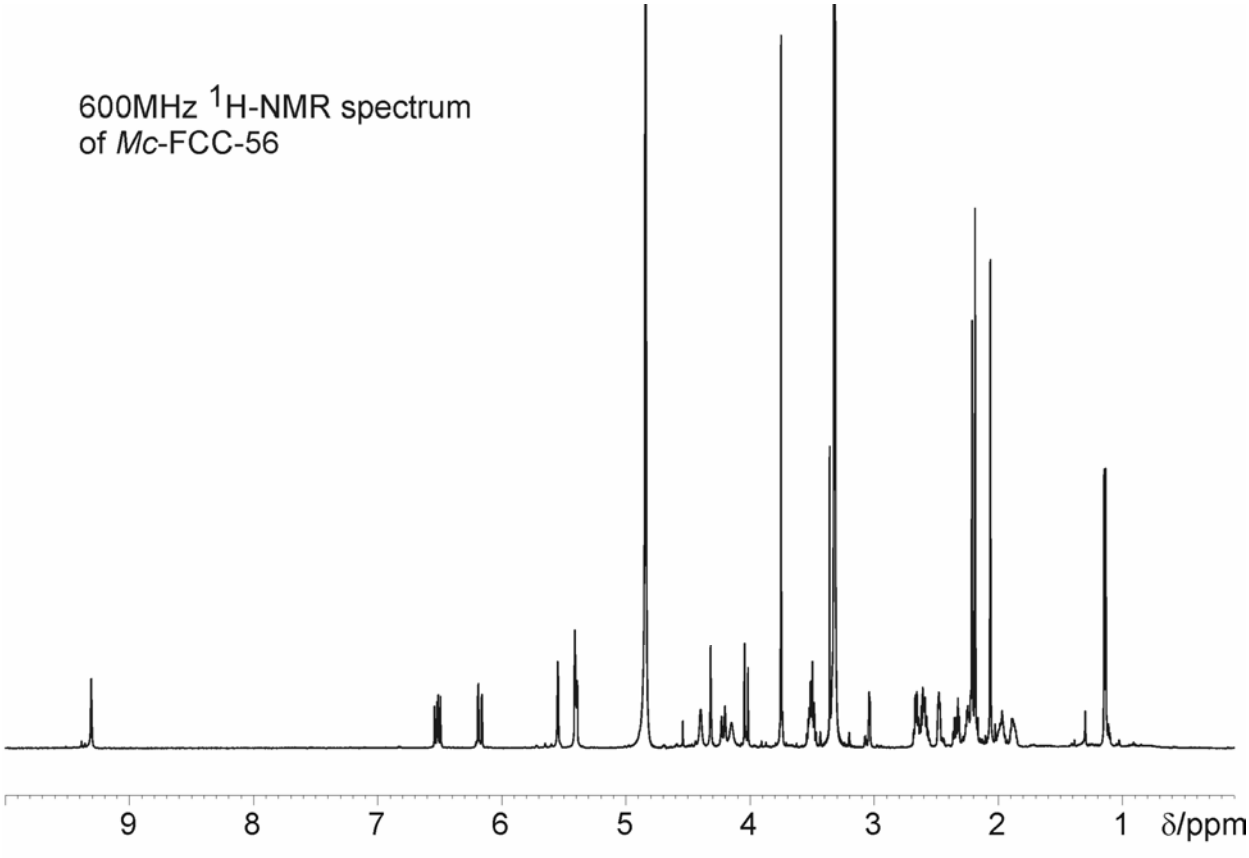

Figure S1. 600 MHz ^{1}H-NMR spectrum of *Mc*-FCC-56 (25 ^{o}C, in CD_3OD)

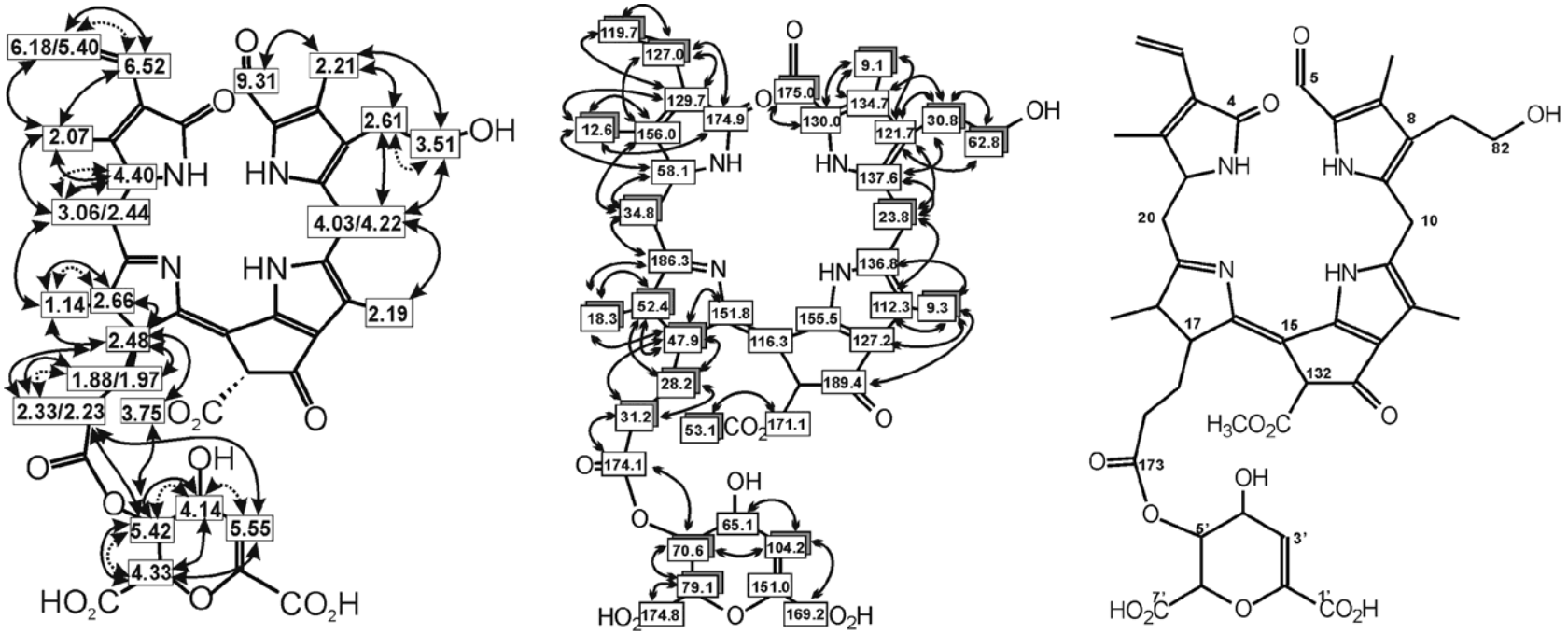

Figure S2: Graphical representations of ^{1}H (left) and ^{13}C (centre) chemical shift data and correlations (in CD_3OD) and deduced constitutional formula (right) of *Mc*-FCC-56; shadowed boxes indicate assignments due to ^{1}J correlations from $^{1}H,^{13}C$-HSQC spectra, arrows indicate long range couplings from $^{1}H,^{13}C$-HMBC spectra.

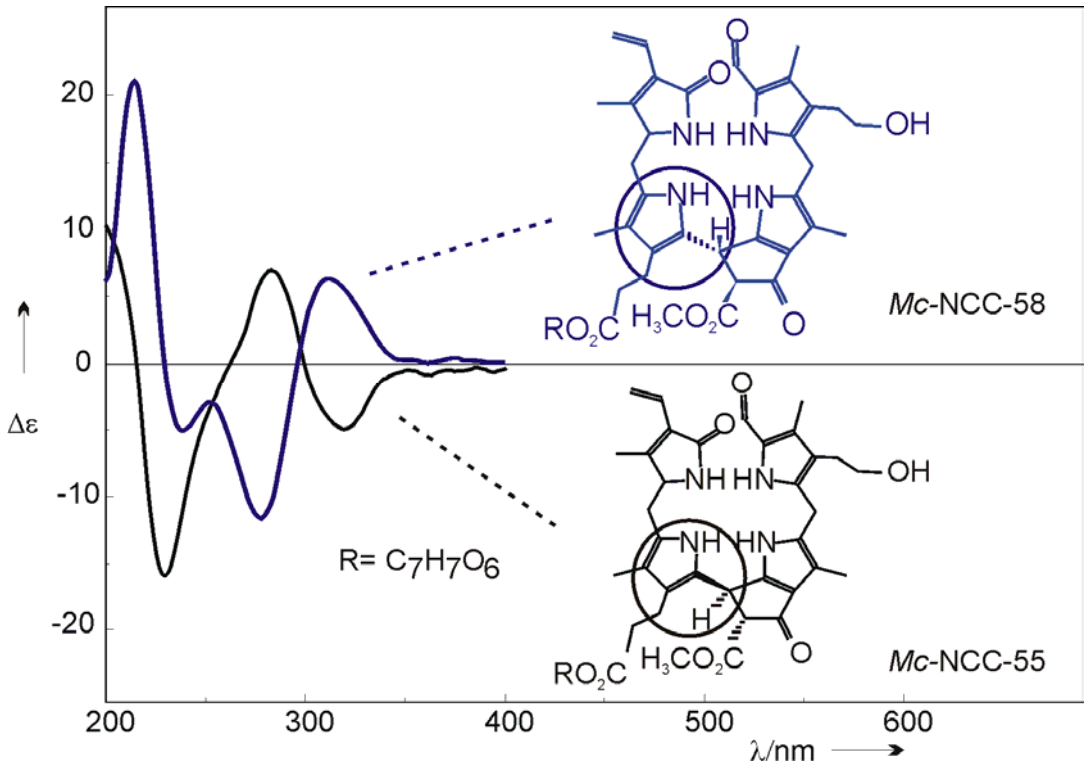

Figure S3: CD-spectra (in methanol) and formulae of *Mc*-NCC-58 (top) and *Mc*-NCC-55 (bottom) obtained from treatment of *Mc*-FCC-56 with dilute citric acid.

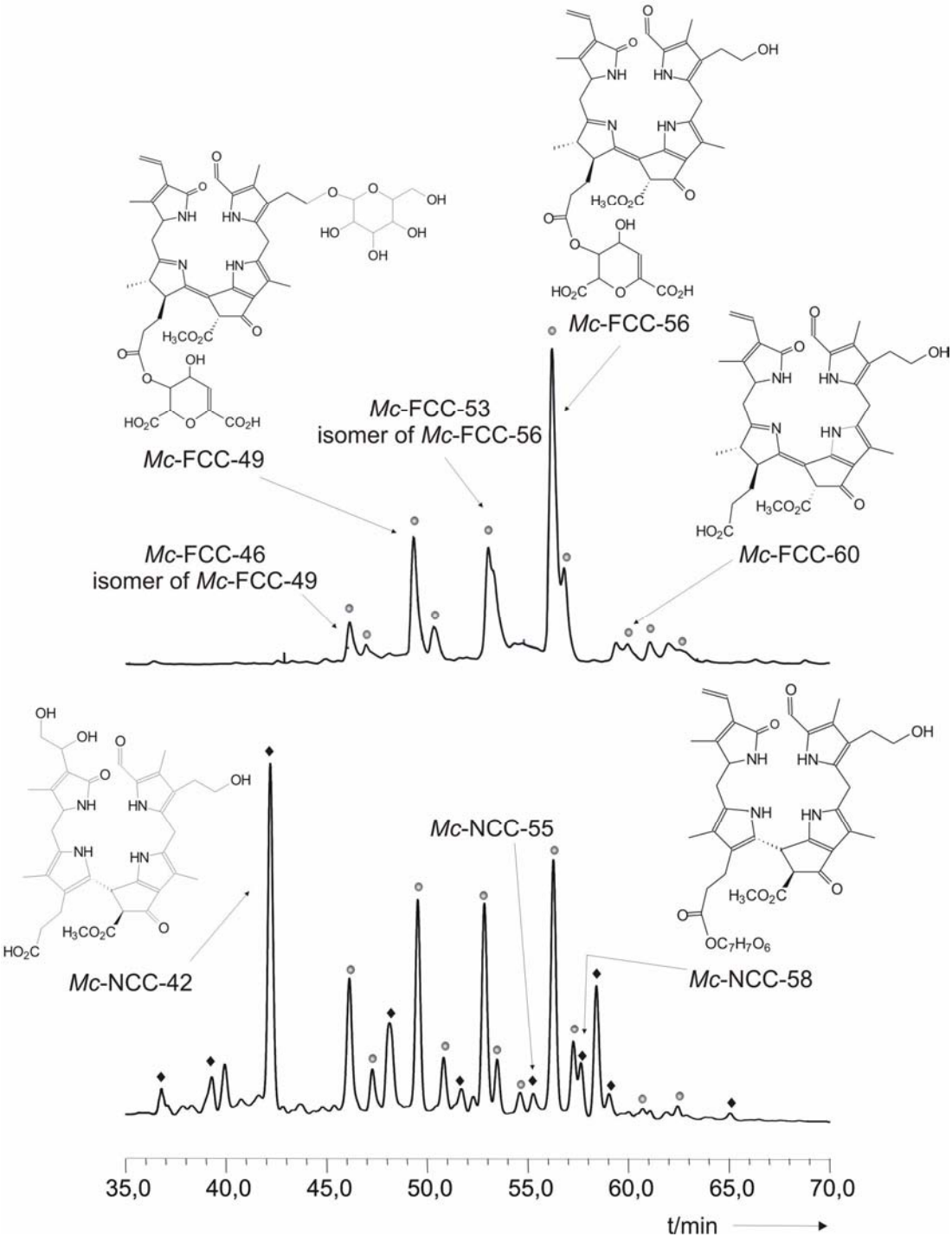

Figure S4: HPLC-analysis of a freshly prepared extract of yellow banana peels (top: trace of luminescence at 450 nm; bottom: trace of absorbance at 320 nm); characteristic fractions are marked as FCC (○) or NCC (♦), based on UV/Vis-& luminescence data; tentative formulae derived from further spectral data (see text in Supp. Inf.).

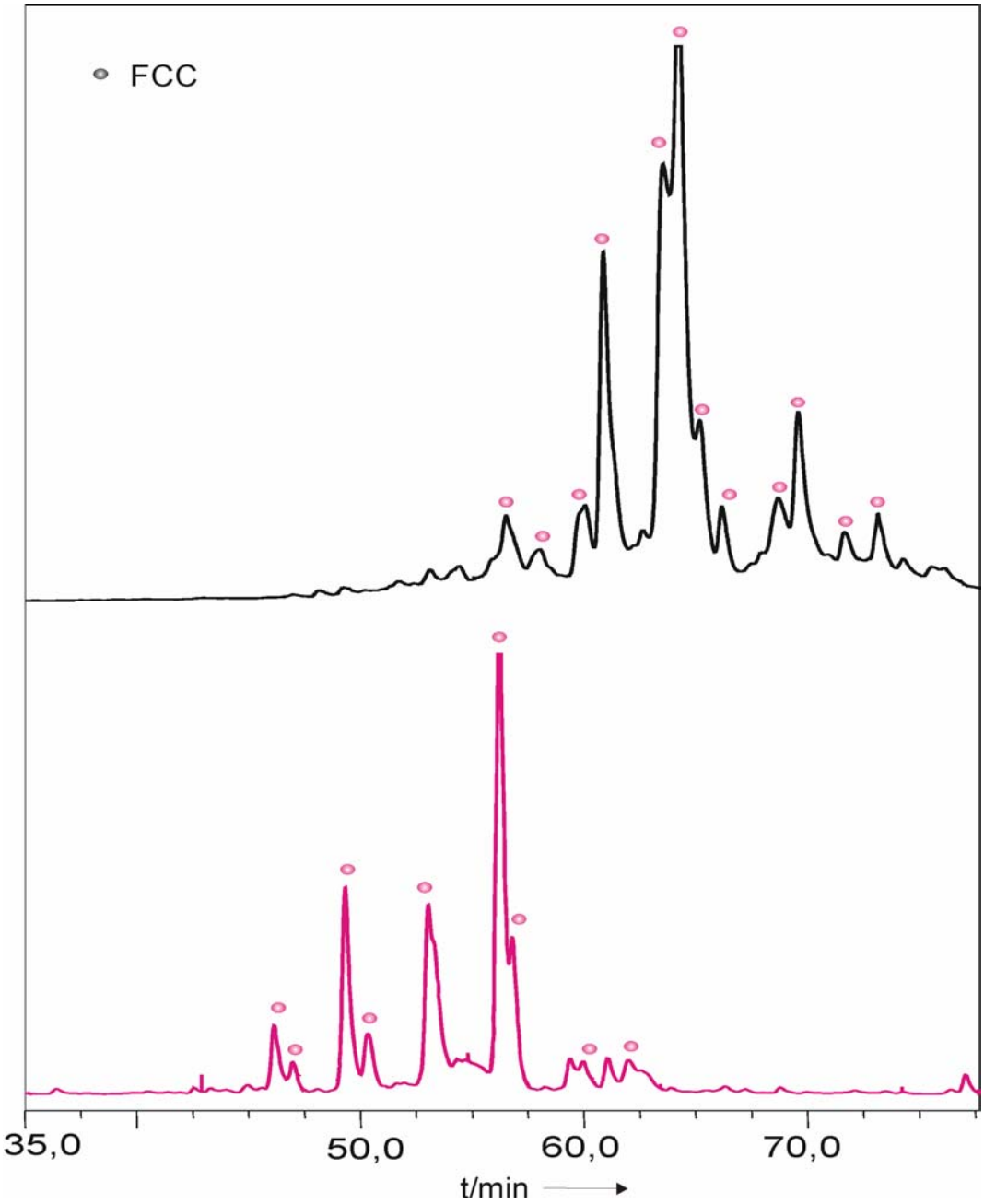

Figure S5. HPLC analyses of extracts of a yellow banana leaf (top) and of a yellow banana peel (bottom), with detection of luminescence at 450 nm; FCC fractions are labelled with a dot (tentative on-line identification by their UV/Vis-spectra).

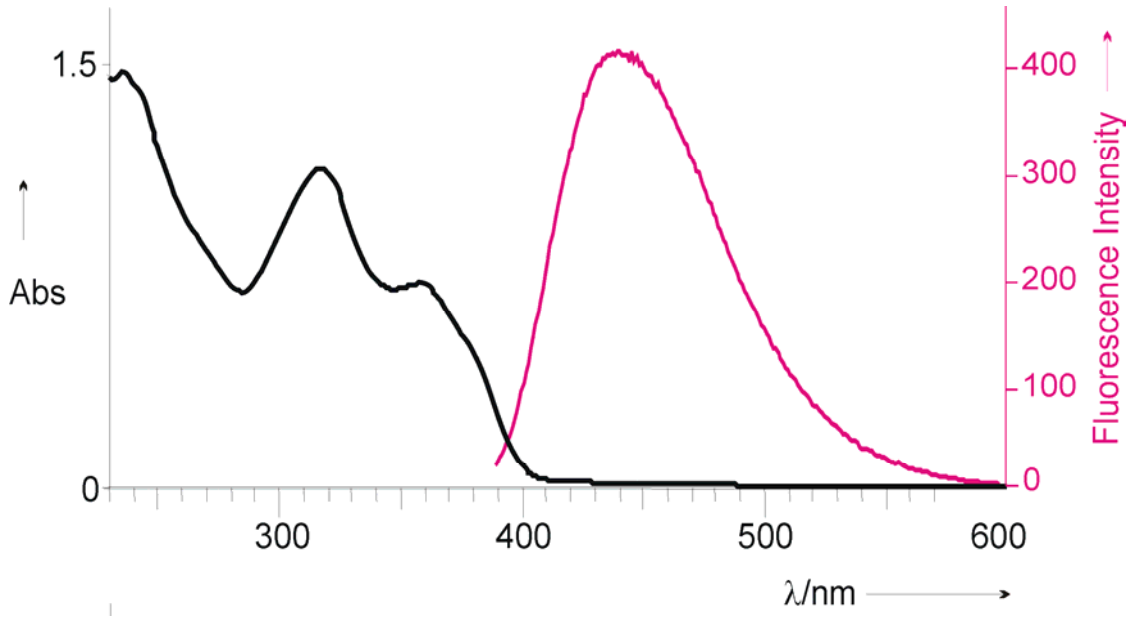

Figure S6: Spectral analysis of *Mc*-FCC-56: UV-Vis spectrum (black, solvents methanol/water 9/1, $5.77*10^{-5}$M) and fluorescence emission spectrum (magenta, in methanol, excitation at 350 nm, $4.21*10^{-6}$ M).