Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Chlorophyll Breakdown by Bio-mimetic Synthesis**

*Michael Oberhuber, Joachim Berghold, and Bernhard Kräutler**

[*] Dr. M. Oberhuber, Dr. J. Berghold, Prof. Dr. B: Kräutler
Institute of Organic Chemistry &
Center for Molecular Biosciences
University of Innsbruck
Innrain 52a, 6020 Innsbruck (Austria)
fax: 0043/512/507-2892
e-mail: bernhard.kraeutler@uibk.ac.at

Material and methods

General (for abbreviations – see main text): Chemicals and reagents were reagent grade commercials from Sigma-Aldrich-Fluka (Buchs, Switzerland). Dichloromethane and chloroform were filtered over basic aluminum oxide before use, other solvents were distilled. HPLC solvents were from Merck (Darmstadt, Germany). Porcine liver esterase (PLE, 41 U/mg) was from Sigma. Sep-Pak-C18-cartridges were from Waters Associates, Milford (MA). Analytical HPLC: 0.5 ml/min, column: Hypersil ODS 5 µm, 250 x 4.6 mm i.d., preparative HPLC: 5 ml/min, column: Hypersil ODS 5 µm, 250 x 21.2 mm i.d., gradient syntax: min (vol. %) (A/B/C), A: methanol, B:,potassium phosphate buffer (100 mM, pH 7.0) C: water, unless otherwise indicated. HPLC system: Hewlett-Packard 1100 (UV/Vis and fluorescence diode array detector), solvents were degassed by a online vacuum degasser or Gynkotek/Dionex system consisting of analytical pump: Gynkotek M480G with vacuum on-line degasser, Gynkotek diode array detector UVD 340: preparative pump: Gynkotek M300, solvents were degassed by sonication, Hitachi SPD-6AV UV/Vis and Jasco FP-920 fluorescence detector. All chromatograms on the HP system were taken at room temperature and data was processed by HP Chemstation for 3D. All analytical chromatograms on the Gynkotek system were taken at 5°C, all preparative chromatograms on the Gynkotek system at RT and data was processed by Gynkotek HPLC-data system Gynkosoft 5.50. UV/Vis spectra: Hitachi-U3000 spectrophotometer, concentrations were calculated following Lambert-Beer´s Law.. CD spectra: Jasco-J715 Spectropolarimeter. NMR spectra: Varian Unity$_{plus}$ 500, chemical shifts for proton and carbon NMR were referenced in ppm (relative to tetramethylsilane), using residual solvent signals: $\delta(C^1HD_2OD)$ = 3.34 ppm and $\delta(^{13}CD_3OD)$ = 49.9 ppm[S1]; FAB-MS: Finnigan MAT 95-S positive ion mode, caesium gun, 20 keV, positive ion mode, matrix: glycerine or nitrobenzyl alcohol (NOBA).ESI-MS: 3.2 kV, solvent: aqueous methanol.

Synthesis of pFCCs (3 and *epi*-3) by electrochemical reduction of RCC (5)

A solution of RCC (**5**, 1.0 mg, 1.6 µmol), $LiClO_4$ (0.1 M), and phenol (12.8 µmol) in methanol (25 ml) was placed into the cathode compartment of an electrolysis cell and was reduced at a Hg-electrode at -1.3 V vs. 0.1 N calomel electrode in a glove box (N_2, <10 ppm O_2) at room temperature. When the current had decreased to 0.3 mA, a solution containing RCC (**5**, 7.2 mg, 11.2 µmol), $LiClO_4$ (30 µM), and phenol (90 µmol) in methanol (1.5 ml) was added at a rate that kept the current constant between 0.30 and 0.35 mA. Once the addition was complete, stirring was continued for two hours, after which 2.6 C (2.1 F/mol) were consumed, and quantitative conversion was detected by HPLC (Figure S1).

The reaction mixture was diluted with 50 ml of potassium phosphate buffer (0.1 M, pH 6.0) and extracted two times with 100 ml of dichloromethane. The combined organic layers were filtered through a plug of cotton and evaporated in vacuo to dryness at T < 0°C. The crude reaction products were purified by preparative HPLC in several runs (flow: 5 ml/min. gradient: 0.00 min (20/10/70), 1.00 min (60/10/70), 30.00 min (90/1/9), 35.00 min (95/0.5/4.5), 35.10 min (95/0/5), 36.00 min (95/0/5), 36.50 min (100/0/0)). Fractions eluting after 32 (**3**), 34.5 (**3'**), 36 (***epi*-3**), 39 (***epi*-3'**), 44 (**6**), and 46 min (**6'**), respectively, were pooled and diluted 1:1 with cold water. Each fraction was loaded on a Sep-Pak C18 cartridge at 4°C, washed with 20 ml of cold water, eluted with methanol, and evaporated in vacuo to dryness at T < 0°C. Yield: 0.66 mg (8%) of **3**, 0.1 mg (1%) of **3'**, 0.64 mg (8%) of ***epi*-3**, 0.1 mg (1%) of ***epi*-3'**, 1.1 mg (13%) of **6** and 1.1 mg (13%) of **6'**.

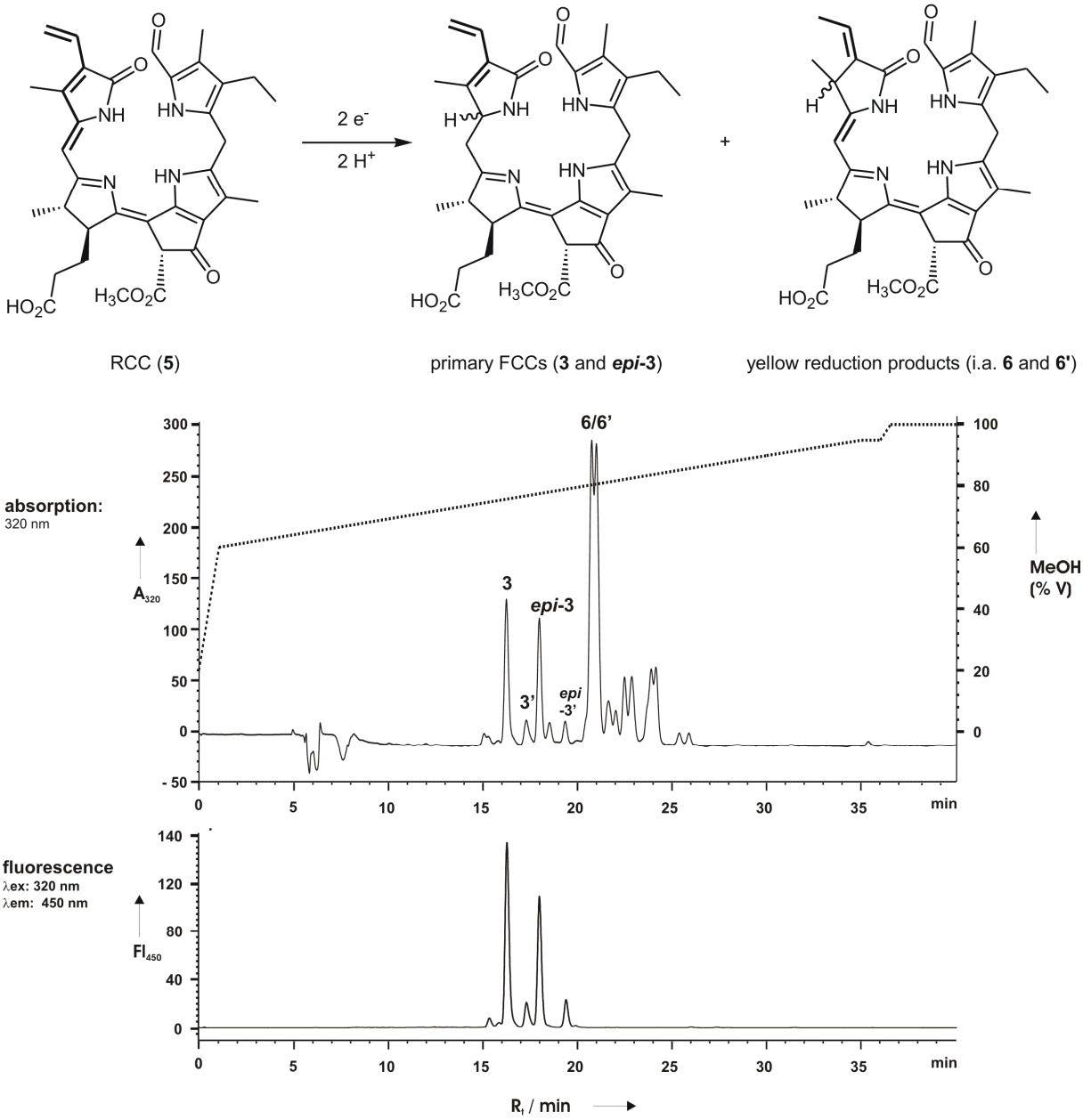


Figure S1: top: Electrochemical reduction of a methanolic solution of RCC (**5**) in presence of phenol and $LiClO_4$; middle: reversed-phase HPLC analysis of the solution after complete reduction: UV/Vis trace at 320 nm; dotted line: vol % of methanol in eluent (flow: 0.5 ml/min, for gradient details, see text); bottom: fluorescence trace (λ_{ex}: 320 nm, λ_{em} : 450 nm)

Spectroscopic data: **3**: UV/Vis (MeOH/potassium phosphate (20 mM, pH 7.0) = 7/3 v/v): λ_{max}(nm)(log ε) = 262 (sh, 4.27), 322 (4.37), 360 (4.17). Fluorescence: emission spectrum: (λ_{ex} = 367 nm) λ_{max}(nm): 441; excitation spectrum (λ_{em} = 441 nm) λ_{max}(nm) (rel abs.) = 247(0.81), 262(0.78), 330(0.82), 367(1.00). ^{1}H-NMR (500 MHz, 1 mM in CD_3OD, -20°C): δ=0.95 (m, 1H; $H_3C(8^2)$), 1.12 (d, $^3J(H,H)$ = 7.2 Hz, 3H; $H_3C(18^1)$), 1.62 (m, 1H; $H_AC(17^1)$), 1.94 (m, 1H; $H_BC(17^1)$), 2.08 (s, 3H; $H_3C(2^1)$), 2.11 (s, 3H; $H_3C(12^1)$), 2.13 (m, 2H; $H_2C(17^2)$), 2.24 (s, 3H; $H_3C(7^1)$), 2.28 (m, 1H; HC(17)); 2.39 (d, J=7.5 Hz; 2H, $H_2C(8^1)$), 2.56 (dd, $^3J(H,H)$ = 8.8 Hz, 17.2 Hz; 1H, $H_AC(20)$), 2.74 (m, 1H; HC(18)), 3.03 (dd, ^3J=2.7 Hz, 17.1 Hz, 1H; $H_BC(20)$), 3.75 (s, 3H; $H_3C(13^5)$), 3.97

(d, J(H,H) = 16.6 Hz, 1H; $H_AC(10)$), 4.02 (d, J(H,H)= 16.6 Hz; 1H, $H_BC(10)$), 4.59 (s, 1H; HC(1)), 5.38 (d, J(H,H) = 11.7 Hz, 1H; $H_AC(3^2)$), 6.24 (d, J(H,H)=17.7 Hz, 1H; $H_BC(3^2)$), 6.53 (dd, J(H,H) = 11.6 Hz, 17.7 Hz, 1H; $HC(3^1)$), 9.32 (s, 1H; HC(5)).

3': UV/Vis (MeOH/potassium phosphate (20 mM, pH 7.0) = 7/3 v/v): λ_{max}(nm) (rel. ε)) = 262 (sh, 0.79), 322 (1.00), 362 (0.69).

***epi*-3**: UV/Vis (MeOH/potassium phosphate (20 mM, pH 7.0) = 7/3 v/v): λ_{max}(nm) (log ε) = 262 (sh, 4.27), 320 (4.37), 360 (4.17). Fluorescence: emission spectrum: (λ_{ex} = 366 nm) λ_{max}(nm): 441; excitation spectrum (λ_{em} = 441 nm) λ_{max}(nm) (rel abs.) = 242 (0.84), 265 (0.79), 329 (0.81), 366 (1.00). ^{1}H-NMR (500 MHz, 1 mM in CD_3OD, -20°C): δ=0.97 (t, J(H,H) = 7.5 Hz, 3H; $H_3C(8^2)$), 1.12 (d, ^{3}J(H,H) = 7.6 Hz, 3H; $H_3C(18^1)$), 1.65 (m, 1H; $H_AC(17^1)$), 1.91 (m, 1H, $H_BC(17^1)$), 2.07 (s, 3H; $H_3C(2^1)$), 2.12 (s, 3H; $H_3C(12^1)$), 2.24 (s, 3H; $H_3C(7^1)$), 2.28 (m, 1H; HC(17)), 2.33 (d, ^{3}J(H,H) = 7.8 Hz, 2H; $H_2C(17^2)$), 2.42 (q, J=7.7 Hz, 2H; $H_2C(8^1)$), 2.62 (dd, J(H,H) = 7.3 Hz, 18.1 Hz, 1H; $H_AC(20)$), 2.71 (q, J(H,H) = 7.0 Hz, 1H; HC(18)), 3.06 (dd, J=4.1 Hz, 18.5 Hz, 1H; $H_BC(20)$), 3.73 (s, 3H, $H_3C(13^5)$), 4.00 (d, J (H,H) = 16.6 Hz, 2H; $H_2C(10)$), 4.55 (s, 2H, $H_2C(1)$); 5.37 (dd, J(H,H)=1.2 Hz, 11.6 Hz, 1H, $H_AC(3^2)$), 6.25 (dd, J=1.3 Hz, 17.6 Hz, 1H; $H_BC(3^2)$), 6.54 (dd, J=11.4 Hz, 17.6 Hz, 1H; $HC(3^1)$), 9.34 (s, 1H; HC(5)).

***epi*-3'**: UV/Vis (MeOH/potassium phosphate (20 mM, pH 7.0) = 7/3 v/v): λ_{max}(nm) rel. ε = 262 (sh, 0.79), 322 (1.00), 362 (0.68).

6: UV/Vis (MeOH/potassium phosphate (20 mM, pH 7.0) = 7.5/2.5 v/v): λ_{max}(nm) (rel. ε) = 232 (0.75), 314 (1.00), 422 (0.58); ^{1}H-NMR (500 MHz, 0.5 mM in CD_3OD, 26°C): δ=0.99 (t, ^{3}J(H,H) = 7.8 Hz, 3H; $H_3C(8^2)$), 1.12 (d, J=6.8 Hz; $H_3C(18^1)$), 1.46 (s, 3H; $H_3C(2^1)$), 1.74 (m, 1H; $H_AC(17^1)$), 1.92 (m, 1H; $H_BC(17^1)$), 2.00 (d, ^{3}J(H,H) = 6.8 Hz, 1H; $HC(3^2)$), 2.02 (s, 3H; $H_3C(12^1)$), 2.12 (m, 2H; $H_2C(17^2)$), 2.26 (s, 3H; $H_3C(7^1)$), 2.35 (dd, ^{3}J(H,H) = 2.9 Hz, 7.8 Hz, 1H; HC(17)), 2.45 (q, ^{3}J(H,H) = 7.8 Hz, 2H; $H_2C(8^1)$), 2.79 (q, ^{3}J(H,H) = 7.2 Hz, 1H; HC(18)), 3.74 (s, 3H; $H_3C(13^5)$), 3.84 (q, broad, ^{3}J(H,H) = 7 Hz, 1H; HC(2)), 4.05 (m, 2H; $H_2C(10)$), 4.57 (s, 1H; $HC(13^2)$), 5.48 (s, 1H; HC(20)), 6.75 (dq, ^{3}J=2.0 Hz, 7.8 Hz, 1H; $HC(3^1)$); 8.55 (s, 1H, HN); 9.39 (s, HC(5)); FAB-MS (m/z (%)): 668.3 (28), 667.3 (49, $[M+K]^+$), 651.3 (21, $[M+Na]^+$), 630.3 (51), 629.3 (100, $[M+H]^+$), 628.3 (49), 597.3 (24, $[M-MeOH+H]^+$), 492.2 (54, [M-ring B+H]$^+$).

6': UV/Vis (MeOH/potassium phosphate (20 mM, pH 7.0) = 7.5/2.5 v/v): λ_{max}(nm) (rel. ε) = 232 (0.75), 314 (1.00), 422 (0.57). ^{1}H-NMR (500 MHz, 0.5 mM in CD_3OD, 26°C): δ=1.00 (t, ^{3}J(H,H) = 7.8 Hz, 3H; $H_3C(8^2)$), 1.13 (d, ^{3}J(H,H) = 6.8 Hz; $H_3C(18^1)$), 1.45 (s, 3H; $H_3C(2^1)$), 1.73 (m, 1H; $H_AC(17^1)$), 1.90 (m, 1H; $H_BC(17^1)$), 1.98 (d, ^{3}J(H,H) = 6.8 Hz, 1H; $HC(3^2)$), 2.02 (s, 3H; $H_3C(12^1)$), 2.12 (m, 2H; $H_2C(17^2)$), 2.28 (s, 3H; $H_3C(7^1)$), 2.36 (dd, ^{3}J(H,H) = 2.9 Hz, 7.8 Hz, 1H; HC(17)), 2.45 (q, ^{3}J(H,H) = 7.8, 2H; $H_2C(8^1)$), 2.79 (q, ^{3}J(H,H) = 7.2, 1H; HC(18)), 3.74 (s, 3H; $H_3C(13^5)$), 3.85 (m, 1H; HC(2)), 4.05 (m, 2H; $H_2C(10)$), 4.58 (s, 1H, $HC(13^2)$), 5.49 (s, 1H; HC(20)), 6.75 (dq, ^{3}J(H,H) = 2.0 Hz, 7.8 Hz, 1H, $HC(3^1)$), 8.55 (s, 1H; HN), 9.39 (s, HC(5)); FAB-MS (m/z (%)): 667.3 (12, $[M+K]^+$), 630.3 (51), 629.3 (100, $[M+H]^+$), 628.3 (53), 597.3 (24, $[M-MeOH+H]^+$), 492.2 (54, [M-ring B+H]$^+$).

Synthesis of NCCs by electrochemical reduction of RCC (5) and direct isomerization of FCCs to NCCs

A solution of RCC (**5**, 1.0 mg, 1.6 µmol), $LiClO_4$ (0.1 M), and phenol (12.8 µmol) in methanol (25 ml) in the cathode compartment of an electrolysis cell was similarly reduced at a Hg-electrode at -1.3 V vs. 0.1 N calomel electrode (N_2, <10 ppm O_2). After the current had decreased to 0.3 mA, a solution containing RCC (**5**, 6.5 mg, 10.4 µmol), $LiClO_4$ (30 µM), and phenol (90 µmol) in methanol (1.5 ml) was added at a rate that kept the current

constant between 0.30 and 0.35 mA. Once the addition was complete, stirring was continued for three hours, after which time about 1.76 C (1.5 F / mol) were consumed, and quantitative conversion was detected by HPLC. The tautomerization reaction was initiated by the adding 20 ml degassed sodium acetate buffer (20 mM, pH 4.9) to the reaction mixture (30 ml). The mixture was stirred in the glove box at room temperature, protected from light. After 17 h, 80% conversion of FCCs to NCCs was detected by HPLC. The solution was again acidified with acetic acid (to pH4.8) and stirring was continued for ca. 100 min, when detection of FCCs by HPLC ceased. The reaction mixture was diluted with 50 ml of potassium phosphate buffer (0.1 M, pH 4.8) and extracted three times with 200 ml of dichloromethane. The combined organic layers were filtered through a plug of cotton and evaporated in vacuo to dryness at T < 0°C.

The crude reaction products were purified by preparative HPLC in several batches (gradient: 0.00 min (20/10/70), 5.00 min (20/10/70), 10.00 min (65/10/25), 36.00 min (65/0/35)). Fractions eluting after 30 (**4**) and 31 min (***epi*-4**), respectively, were pooled and diluted 1:1 with water. Each fraction was loaded on a Sep-Pak C18 cartridge, washed with 20 ml of cold water, eluted with methanol, and evaporated in vacuo to dryness at T < 0°C. Yield: 0.5mg (7%) **4** and 0.5mg (7%) ***epi*-4**.

Spectroscopic data: **4**: UV/Vis (MeOH/potassium phosphate (50 mM, pH 7.0) = 6.5/3.5 v/v): λ_{max}(nm) (rel. ε): 216 (1.00), 232 (0.70), 314 (0.64); ^{1}H-NMR: (500 MHz, 2 mM in CD_3OD, 26 °C): δ=0.99 (m, 3H; $H_3C(8^2)$), 1.89 (s, 3H; $H_3C(2^1)$), 1.94 (s, 3H; $H_3C(18^1)$), 2.08 (s, 3H; $H_3C(12^1)$), 2.25 (s, 3H; $H_3C(7^1)$), 2.34 (m, 2H; $H_2C(17^2)$), 2.42 (dd, ^{3}J (H,H) = 7.8 Hz, 15.6 Hz, 2H; $H_2C(8^1)$), 2.51 (dd, ^{3}J (H,H) = 8.8 Hz, 14.6 Hz, 1H; $H_AC(20)$), 2.66 (m, 1H; $H_BC(17^1)$), 2.73 (m, 1H; $H_BC(17^1)$), 2.79 (dd, ^{3}J (H,H) = 5.9 Hz, 14.6 Hz, 1H; $H_BC(20)$), 3.74 (s, 3H; $H_3C(13^5)$), 3.91 (s, 2H; $H_2C(10)$), 4.07 (dd, ^{3}J (H,H) = 5.9 Hz, 7.8 Hz, 1H; HC(1)), 4.91 (s, 1H; HC(15)), 5.38 (dd, ^{3}J (H,H) = 2.0 Hz, 11.7 Hz, 1H; $H_AC(3^2)$), 6.10 (dd, ^{3}J (H,H) = 17.6 Hz, 1H; $H_BC(3^2)$), 6.44 (dd, ^{3}J (H,H) = 11.7 Hz, 17.6 Hz, 1H; HC(3^1)), 9.36 (s, 1H, HC(5));^{13}C-NMR:(125 MHz, 2 mM in CD_3OD, 26 °C, TMS) from HSQC and HMBC data: δ=8.6 (7^1), 9.0 (12^1), 9.1 (18^1), 12.3 (2^1), 15.1 (8^2), 17.4 (8^1), 21.9 (17^1), 23.5 (10), 30.3 (20), 37.1 (15), 39.6 (17^2), 52.6 (13^5), 61.5 (1), 67.5 (13^2), 111.9 (12), 115.3 (18), 119.4 (3^2), 120.7 (17), 124.2 (16), 124.2 (19), 125.6 (13), 125.9 (8), 127.5 (3^1), 128.4 (3), 129.0 (6), 133.8 (11), 134.4 (7), 137.5 (9), 156.9 (2), 161.4 (14), 171.5 (13^3), 174.5 (4), 181.2 (17^3); FAB-MS (m/z (%)): 667.3 (14,$[M+K]^+$), 651.3 (97, $[M+Na]^+$), 630.3 (49), 629.3 (100, $[M+H]^+$), 597.3 (32, $[M-MeOH+H]^+$) 505.3 (62, [M-ring A+H]$^+$).

***epi*-4**: UV/Vis (MeOH/potassium phosphate (50 mM, pH 7.0) = 6.5/3.5 v/v): λ_{max}(nm) (rel. ε): 216 (1.00), 232 (0.70), 314 (0.64); ^{1}H-NMR: (500 MHz, 26 °C, 2 mM in CD_3OD): δ=0.98 (t, ^{3}J (H,H) = 6.8 Hz, 3H; $H_3C(8^2)$), 1.93 (s, 6H; $H_3C(2^1)$ and $H_3C(18^1)$), 2.07 (s, 3H; $H_3C(12^1)$), 2.24 (s, 3H; $H_3C(7^1)$), 2.34 (m, 2H; $H_2C(17^2)$), 2.42 (dd, ^{3}J (H,H) = 6.8 Hz, 14.6 Hz, 2H; $H_2C(8^1)$), 2.47 (dd, ^{3}J (H,H) = 9.8 Hz, 14.6 Hz, 1H; $H_AC(20)$), 2.64 (m, 1H; $H_BC(17^1)$), 2.77 (m, 1H; $H_BC(17^1)$), 2.83 (dd, ^{3}J (H,H) = 5.9 Hz, 14.6 Hz, 1H; $H_BC(20)$), 3.74 (s, 3H; $H_3C(13^5)$), 3.92 (s, 2H; $H_2C(10)$), 3.97 (dd, ^{3}J (H,H) = 4.9 Hz, 8.8 Hz, 1H; HC(1)), 4.88 (s, 1H; HC(15)), 5.34 (dd, ^{3}J (H,H) = 2.0 Hz, 11.7 Hz, 1H; $H_AC(3^2)$), 6.10 (dd, ^{3}J (H,H) = 2.0 Hz, 17.6 Hz, 1H; $H_BC(3^2)$), 6.44 (dd, ^{3}J (H,H) = 11.7 Hz, 18.6 Hz, 1H; HC(3^1)), 9.34 (s, 1H; HC(5)); ^{13}C-NMR: (125 MHz, 26 °C, 2 mM in CD_3OD) from HSQC spectrum: δ=8.5 (7^1), 9.0 (12^1), 9.1 (18^1), 12.3 (2^1), 15.1 (8^2), 17.4 (8^1), 22.0 (17^1), 23.5 (10), 30.3 (20), 37.1 (15), 39.5 (17^2), 52.6 (13^5), 61.8 (1), 112.0 (12), 115.3 (18), 119.4 (3^2), 120.5 (17), 124.3 (16), 124.4 (19), 125.6 13), 126.0 (8), 127.5 (3^1), 129.0 (6), 133.9 (11), 134.4 (7), 137.5 (9), 155.8 (2), 161.4 (14), 171.5 (13^3), 174.6 (4), 181.2 (17^3); FAB-MS (m/z (%)): 667.3 (19, $[M+K]^+$), 651.3 (14, $[M+Na]^+$), 631.3 (31), 630.3 (45), 629.3 (100, $[M+H]^+$), 597.3 (14, $[M-MeOH+H]^+$), 506.3 (44) [M-ring A+H]$^+$).

Kinetic analysis of tautomerization reaction: FCCs → NCCs

Typical procedure: An aqueous solution containing FCC **3** (3.3 nmol, 0.2 mM; final concentration: 6.2 µM) was added to 500 µl sodium acetate buffer (50 mM, pH 5.0) in a UV/Vis cell (1 cm light path) at room temperature and flushed with argon. The reaction was followed by monitoring UV absorption at 360 nm and by HPLC with detection at 320 nm. The reaction product was identified as NCC **4** by HPLC (see Figure S2) and pseudo-first-order rate constants were calculated.

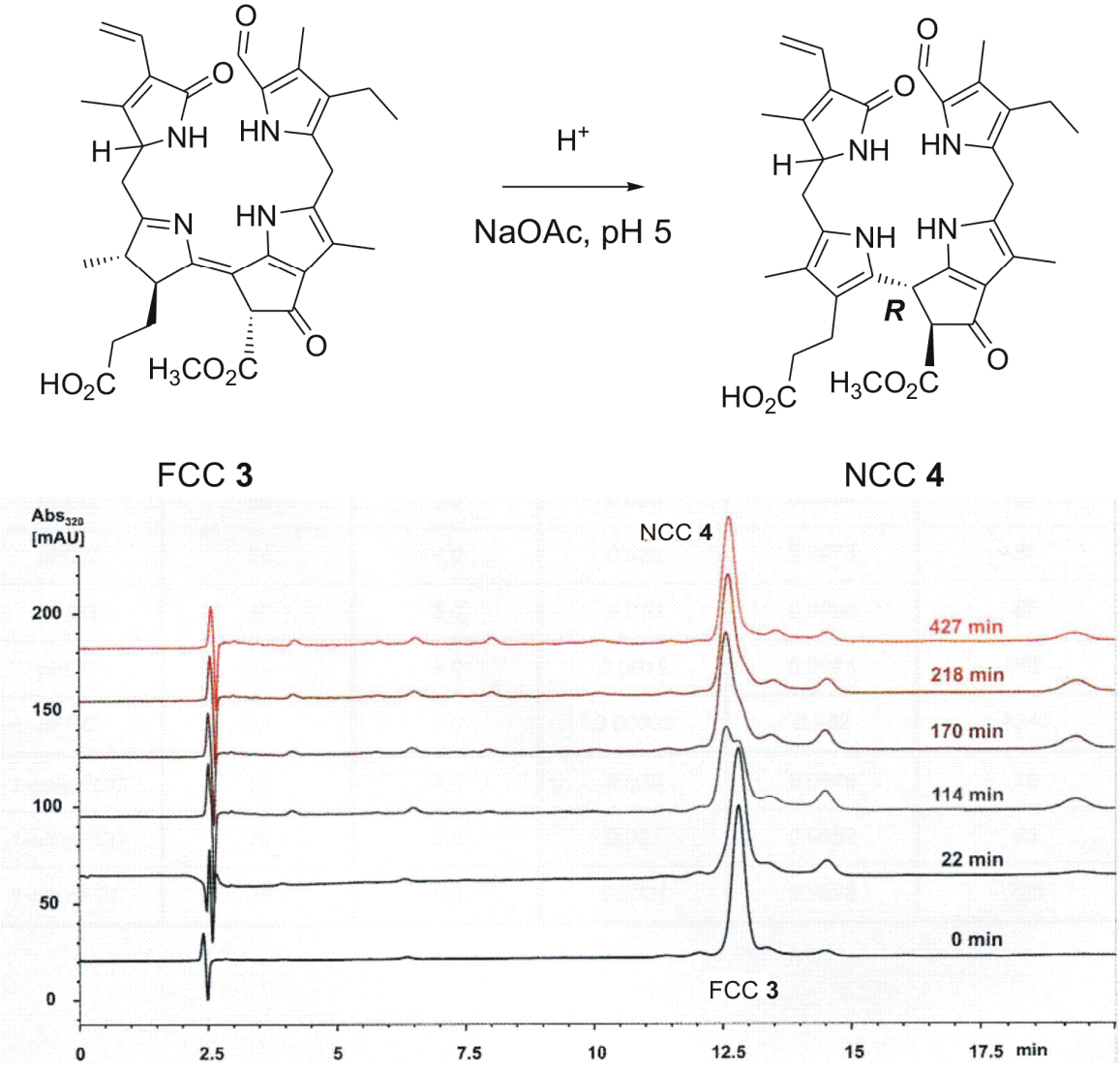


Figure S2: top: Acid-induced isomerization of FCC **3** to the NCC **4.** Bottom: reversed-phase HPLC analysis of reaction progress (UV/vis traces at 320 nm, flow: 0.5 ml/min, gradient: 0.00 min (57/43/0), 20.00 min (66/34/0)).

Table S1. Kinetic analysis of isomerization reactions in aquous solutions (26 °C, specified pH-values)

starting material	pH	obs. rate constant (k) [min^{-1}]	coefficient of determination, r^2	half life, $t_{1/2}$ [min]
3	3.5	0.026	0.9964	27
3	4.0	0.020	0.9975	31
3	5.0	0.010	0.9986	67
3	6.0	0.0019	0.9988	365
3	7.0	0.00032	0.982	2340
***epi*-3**	4.0	0.039	0.9978	18
***epi*-3**	5.0	0.021	0.9952	33
***epi*-3**	6.0	0.0031	0.9828	223

pKa value of proton donor

The apparent pKa of the acid inducing the tautomerization reaction was calculated from a linear regression of 1/k vs. [H^+] according to equation (S1) as pKa (**3**) = 5.0 (± 0.2); pKa(***epi*-3**) = 4.8 (± 0.2)

equation S1: $1/k = 1/k'' + 10^{(pH-pKa)}/k''$, k'' = calc. rate constant for protonated form of acid

Synthesis of NCC methyl esters by tautomerization from FCC methyl esters

A solution of FCC methyl ester **Me-*epi*-3** (1.08 mg, 1.68 µmol) in degassed methanol (20 ml) was saturated with Ar and acidified with trifluoroacetic acid (to a final concentration of 0.31 M). The solution was stirred under an Ar atmosphere at room temperature, protected from light. The reaction was monitored using UV/Vis spectrometry, and after 18h (67% conversion), the reaction mixture was diluted with dichloromethane and extracted three times with potassium phosphate buffer (0.1 M, pH 7). The combined organic layers were filtered through a plug of cotton and evaporated in vacuo to dryness at T < 0°C.

The crude reaction products were purified by preparative HPLC (10 ml min^{-1} MeOH/water= 62/38 v/v). Fractions eluting after 61 (**4a**) and 70 min (**4b**), respectively, were collected and diluted 1:1 with water. Each fraction was loaded on a Sep-Pak C18 cartridge, washed with 20 ml of cold water, eluted with methanol (2 ml), and evaporated in vacuo to dryness at T < 0°C. Yield: 75 µg (7.4%) **4a** and 280 µg (26%) **4b**.

0.77 mg, 1.20 µmol of **Me-3** were converted following the same procedure. After 18h reaction time, 160 µg (21%) **4c** and 90 µg (12%) **4d** were isolated following work-up and HPLC purification.

Spectroscopic data: **4a**. UV/Vis (MeOH): λ_{max} (log ε)=313 (4.18). CD (MeOH): $\lambda_{min/max}$ (Δε)=313 (-0.1), 284 (3.7), 254 (0.2), 247 (0.1), 229 (-2.3); ^{1}H NMR (500 MHz, CD_3OD, 26°C): δ=0.97 (m, 3H, $H_3C(8^2)$); 1.89 (s, 3H, $H_3C(2^1)$); 1.90 (s, 3H, $H_3C(18^1)$); 2.12 (s, 3H, $H_3C(12^1)$); 2.25 (s, 3H, $H_3C(7^1)$); 2.30-2.47 (m, 4H, $H_2C(17^2)$ and $H_2C(8^1)$); 2.53-2.59 (m, 1H, $H_AC(20)$); 2.62-2.67 (m, 2H, $H_2C(17^1)$); 2.80-2.87 (m, 1H, $H_BC(20)$); 3.59 (s, 3H, $H_3C(17^5)$); 3.76 (s, 3H, $H_3C(13^5)$); 3.90 (s, 2H, $H_2C(10)$); 4.04-4.09 (m, 1H, HC(1)); 4.21-4.24 (m, 1H, $HC(13^2)$); 5.31-5.37 (m, 1H, $H_{cis}C(3^2)$); 6.06-6.11 (m, 1H, $H_{trans}C(3^2)$); 6.37-6.46 (m, 1H, $HC(3^1)$); 9.36 (s, 1H, HC(5)); ESI-MS: *m/z* (%)=1307.7 (20) $[2M+Na]^+$; 683.3 (1), 682.3 (3), 681.3 (6) $[M+K]^+$; 667.3 (10), 666.3 (40), 665.3 (100) $[M+Na]^+$; 645.3 (1), 644.3 (2), 643.3 (4) $[M+H]^+$.

4b. UV/Vis (MeOH): λ_{max} (log ε)=313 (4.26), 237.0 (sh, 4.37). CD (MeOH): $\lambda_{min/max}$ (Δε)=315 (8.0), 281 (-17.0), 257 (-6.7), 251 (-6.7), 225 (22.0). ^{1}H NMR (500 MHz, CD_3OD, 26°C): δ=0.99 (t, J=7.9; 3H, $H_3C(8^2)$); 1.90 (s, 3H, $H_3C(18^1)$); 1.94 (s, 3H, $H_3C(2^1)$); 2.11 (s, 3H, $H_3C(12^1)$); 2.25 (s, 3H, $H_3C(7^1)$); 2.28-2.36/2.37-2.44 (2m, 2H, $H_2C(17^2)$); overlapping with 2.41 (q, J=7.9; 2H, $H_2C(8^1)$); 2.58-2.66 (m, 2H, $H_2C(17^1)$); overlapping with 2.62 (dd, J=14.9/7.1; 1H, $H_AC(20)$); 2.84 (dd, J=15.0/6.2; 1H, $H_BC(20)$); 3.60 (s, 3H, $H_3C(17^5)$); 3.76 (s, 3H, $H_3C(13^5)$); 3.89/3.92 (AB spin system, J_{AB}=16.8; 2H, $H_2C(10)$); 4.04 (dd, J=7.1/6.1; 1H, HC(1)); 4.84 (HC(15), from HSQC spectrum); 5.34 (dd, J=11.5/1.8; 1H, $H_{cis}C(3^2)$); 6.08 (dd, J=17.6/1.8; 1H, $H_{trans}C(3^2)$); 6.42 (dd, J=17.6/11.5; 1H, $HC(3^1)$); 9.34 (s, 1H, HC(5)). ^{13}C-NMR (125 MHz, CD_3OD, 26°C) from HSQC spectrum: δ=8.6 (7^1); 9.1 (12^1); 9.1 (18^1); 12.4 (2^1); 15.1 (8^2); 17.4 (8^1); 20.4 (17^1); 23.4 (10); 30.1 (20); 37.3 (15); 51.9 (17^5); 52.7 (13^5); 61.4 (1); 118.9 (3^2); 126.9 (3^1); ESI-MS: *m/z* (%)=1307.7 (30, $[2M+Na]^+$), 683.3 (2), 682.3 (3), 681.3 (7, $[M+K]^+$), 667.3 (10), 666.3 (40), 665.3 (100, $[M+Na]^+$), 645.3 (1), 644.3 (3), 643.3 (7, $[M+H]^+$).

4c. UV/Vis (MeOH): λ_{max} (log ε)=313 (4.27), 237 (sh, 4.39). CD (MeOH): $\lambda_{min/max}$ (Δε)=314 (6.5), 283 (-13.8), 255 (1.1), 244 (2.9), 224 (20.6); ^{1}H NMR (500 MHz, CD_3OD, 26°C): δ=0.98 (t, J=7.5; 3H, $H_3C(8^2)$); 1.89 (s, 3H, $H_3C(2^1)$); 1.90 (s, 3H, $H_3C(18^1)$); 2.12 (s, 3H, $H_3C(12^1)$); 2.25 (s, 3H, $H_3C(7^1)$); 2.31-2.47 (m, 4H, $H_2C(17^2)$ and $H_2C(8^1)$); 2.57 (dd, J=14.6/8.4; 1H, $H_AC(20)$); 2.62-2.67 (m, 2H, $H_2C(17^1)$); 2.82 (dd, J=14.6/5.3; 1H, $H_BC(20)$); 3.60 (s, 3H, $H_3C(17^5)$); 3.77 (s, 3H, $H_3C(13^5)$); 3.90 (s, 2H, $H_2C(10)$); 4.07 (dd, J=8.4/5.4; 1H, HC(1)); 4.85 (HC(15), from HSQC); 5.35 (dd, J=11.5/2.2; 1H, $H_{cis}C(3^2)$); 6.09 (dd, J=17.6/2.2; 1H, $H_{trans}C(3^2)$); 6.42 (dd, J=17.6/11.5; 1H, $HC(3^1)$); 9.36 (s, 1H, HC(5)); 9.60 (b, 1H, HN). ^{13}C-NMR (125 MHz, CD_3OD, 26°C) from HSQC spectrum: δ=8.5 (7^1); 9.0 (18^1); 9.0 (12^1); 12.3 (2^1); 15.0 (8^2); 17.4 (8^1); 20.5 (17^1); 23.5 (10); 30.1 (20); 36.3 (17^2); 37.2 (15); 51.8 (17^5); 52.7 (13^5); 61.7 (1); 119.1 (3^2); 127.1 (3^1). ESI-MS:*m/z* (%)=1307.6 (10) $[2M+Na]^+$; 683.3 (1), 682.3 (4), 681.2 (10) $[M+K]^+$; 667.3 (10), 666.3 (40), 665.3 (100) $[M+Na]^+$; 644.3 (1), 643.3 (2) $[M+H]^+$.

4d. UV/Vis (MeOH): λ_{max} (log ε)=312 (4.23), 237 (sh, 4.34). CD (MeOH): $\lambda_{min/max}$ (Δε)=317 (-1.3), 281 (6.4), 257 (2.8), 252 (2.8), 225 (-5.0); ^{1}H NMR (500 MHz, CD_3OD, 26°C): δ=0.99 (t, J=7.5; 3H, $H_3C(8^2)$); 1.90 (s, 3H, $H_3C(18^1)$); 1.94 (s, 3H, $H_3C(2^1)$); 2.11 (s, 3H, $H_3C(12^1)$); 2.25 (s, 3H, $H_3C(7^1)$); 2.28-2.35/2.37-2.44 (2m, 2H, $H_2C(17^2)$); overlapping with 2.41 (q, J=7.5; 2H, $H_2C(8^1)$); 2.59-2.66 (m, 3H, $H_AC(20)$ and $H_2C(17^1)$); 2.81-2.87 (dd, J=14.6/5.3; 1H, $H_BC(20)$); 3.60 (s, 3H, $H_3C(17^5)$); 3.77 (s, 3H, $H_3C(13^5)$); 3.89/3.93 (AB spin system, J_{AB}=17.2; 2H, $H_2C(10)$); 4.02-4.06 (m, 1H, HC(1)); 5.35 (dd, J=11.5/1.8; 1H, $H_{cis}C(3^2)$); 6.08 (dd, J=17.6/1.8; 1H, $H_{trans}C(3^2)$); 6.42 (dd, J=17.6/11.5; 1H, $HC(3^1)$); 9.35 (s, 1H, HC(5)); ESI-MS:*m/z* (%)=1307.7 (8) $[2M+Na]^+$; 683.3 (4), 682.3 (10), 681.3 (26) $[M+K]^+$; 667.3 (10), 666.3 (40), 665.3 (100) $[M+Na]^+$; 645.3 (1), 644.3 (6), 643.3 (14) $[M+H]^+$.

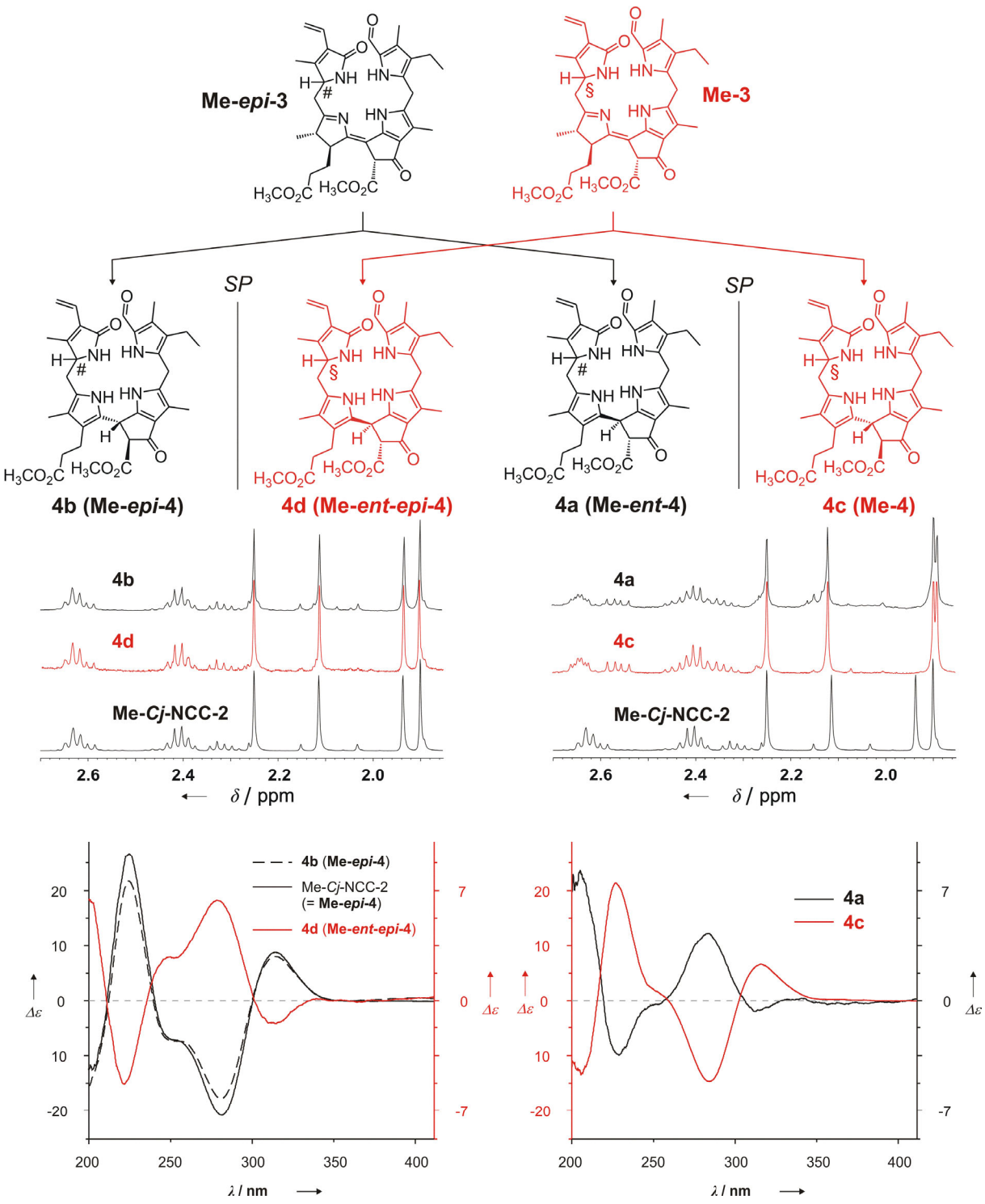


Figure S3: top: Acid-induced isomerization of FCC methyl esters to NCC methyl esters**.** Middle: 1H NMR region with characteristic differences between C1 epimers. Bottom: CD spectra (in methanol) of the synthetic NCC methyl esters **4a**-**4d**, and of the methyl ester of the natural NCC *Cj*-NCC-2 (= **Me-*epi*-4**).). The identical configuration at C-1, shared by **Me-3** and the NCCs **4c** and **4d** is symbolized by §, the opposite common configuration of **Me-*epi*-3** and **4a** and **4b** by the symbol #; SP=symmetry plane.

Synthesis of *Cj*-NCC-2 methyl ester (Me-*Cj*-NCC-2)

33 mg of a crude sample of *Cj*-NCC-2 were obtained from 250 g (fresh weight) of senescent leaves from

Cercidiphyllum japonicum as described[S2, S3], but using chloroform/MeOH/water=95/10/1 v/v as eluent for the preparative TLC (R_f(*Cj*-NCC-2) ca. 0.4).

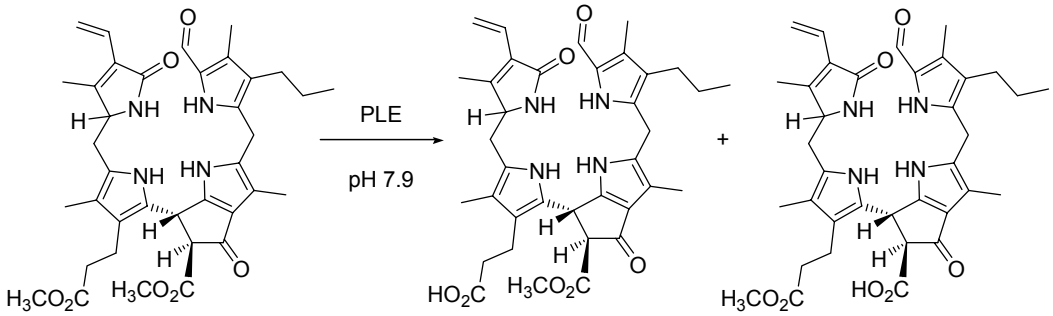
Crude *Cj*-NCC-2 was dissolved in dichloromethane/MeOH 1/2 v/v (15 ml) under Ar, triethylamine (3 µl, 22 µmol) were added, and. the solution was cooled to 0°C. BOP ((benzotriazol-1-yloxy)tris(dimethylamino) phosphonium hexafluorophosphate, 8 mg, 18 µmol) were added under vigorous stirring. The solution was allowed to warm to room temperature and stirring was continued. After 7 h another 5 mg (11 µmol) BOP were added and stirring was continued for 15 h at room temperature. The reaction mixture was diluted with 40 ml dichloromethane and extracted with potassium phosphate buffer (0.1 M, pH 7). The organic layer was filtered through a plug of cotton and evaporated in vacuo to dryness at T < 0°C.

The crude reaction product was purified by preparative HPLC (10 ml min^{-1} MeOH/water = 72/28 v/v). The fraction containing Me-*Cj*-NCC-2 was collected after 19 min and concentrated using a Sep-Pak cartridge as described above. The solvent was evaporated in vacuo at T<0°C to give 4.1 mg (6.4 µmol)] of Me-*Cj*-NCC-2. Spectroscopic data: UV/Vis (MeOH): λ_{max} (log ε)=312 (4.26), 237 (sh, 4.37). CD (MeOH): $\lambda_{min/max}$ (Δε)=314 (8.6), 282 (-20.7), 255 (-7.3), 250 (-7.0), 225 (26.5); ^{1}H NMR (500 MHz, CD_3OD, 26°C): δ=0.99 (t, *J*=7.5; 3H, $H_3C(8^2)$); 1.90 (s, 3H, $H_3C(18^1)$); 1.94 (s, 3H, $H_3C(2^1)$); 2.11 (s, 3H, $H_3C(12^1)$); 2.25 (s, 3H, $H_3C(7^1)$); 2.28-2.36/2.37-2.44 (2m, 2H, $H_2C(17^2)$); overlapping with 2.41 (q, *J*=7.5; 2H, $H_2C(8^1)$); 2.58-2.66 (m, 3H, $H_2C(17^1)$ and $H_AC(20)$); 2.84 (dd, *J*=14.6/5.7; 1H, $H_BC(20)$); 3.60 (s, 3H, $H_3C(17^5)$); 3.76 (s, 3H, $H_3C(13^5)$); 3.89/3.93 (AB spin system, J_{AB}=16.7; 2H, $H_2C(10)$); 4.02-4.06 (m, 1H, HC(1)); 4.84 (HC(15) from HSQC); 5.34 (dd, *J*=11.9/2.2; 1H, $H_{cis}C(3^2)$); 6.08 (dd, *J*=17.6/2.2; 1H, $H_{trans}C(3^2)$); 6.42 (dd, *J*=17.6/11.5; 1H, $HC(3^1)$); 9.34 (s, 1H, HC(5)). ^{13}C-NMR (125 MHz, CD_3OD, 26°C) from HSQC/HMBC spectra: δ=8.5 (7^1); 9.0 (12^1); 9.1 (18^1); 12.4 (2^1); 15.1 (8^2); 17.4 (8^1); 20.4 (17^1); 23.4 (10); 29.9 (20); 36.3 (17^2); 37.2 (15); 51.8 (17^5); 52.7 (13^5); 61.4 (1); 68.3 (13^2); 112.3 (12); 115.4 (18); 119.0 (3^2); 119.6 (17); 124.3 (19); 124.5 (16); 126.0 (8); 126.2 (13); 126.8 (3^1); 128.8 (3); 129.1 (6); 134.1 (11); 134.3 (7); 137.4 (9); 156.6 (2); 160.7 (14); 171.6 (13^3); 175.2 (17^3); 175.2 (4); 177.5 (5); FAB-MS(NOBA): *m/z* (%)=666.5 (20), 665.5 (36) $[M+Na]^+$; 645.3 (16), 644.3 (30), 643.3 (68) $[M+H]^+$; 521.3 (50), 520.3 (100) $[M+H-123]^+$.

Identification of NCC methyl esters by HPLC

Solutions containing approximately equal amounts of tautomerization products **4a**, **4b**, **4c**, and **4d** were analyzed by HPLC (0.5 ml min^{-1} MeOH/water= 72/28 v/v). Each sample was co-injected with a solution containing Me-*Cj*-NCC-2. While **4b** and **4d** co-eluted with Me-*Cj*-NCC-2 after ~ 18 min, **4c** and **4a** eluted after ~ 16 min, clearly separated from Me-*Cj*-NCC-2.

Enzymatic hydrolysis of Me-*Cj*-NCC-2



Me-*Cj*-NCC-2 **7** (=*Cj*-NCC-2) **8**

Me-*Cj*-NCC-2 (4.1 mg, 6.4 µmol) in 0.4 ml dimethylsulfoxide (DMSO) was added to a solution of porcine liver esterase (4.3 U/ml) in 40 ml potassium phosphate buffer (100 mM, pH 7.9) under an Aratmosphere. After stirring at room temperature for 38h, 98 U PLE were added and stirring was continued for another 6 h. The pH of the reaction mixture was adjusted to pH 5.1 by addition of KH_2PO_4, 65 ml ethyl acetate were added, the layers were separated, and the organic layer filtered through a plug of cotton after dilution with 25 ml dichloromethane. The resulting solution was freed from solvents vacuo at T < 0°C and purified by preparative HPLC (10 ml min^{-1} ammonium acetate in MeOH/water = 65/35 v/v (200 mM, pH 6.8)): Fractions were collected after 16 (**8**) and 19.5 min (**7**), respectively, and concentrated using a Sep-Pak cartridge as described above, to give 1.2 mg (30%) of **7** and 0.84 mg (21%) of **8**.

Spectroscopic data: **7**: UV/Vis (MeOH) λ_{max} *(rel. ε)* 238 (sh, 1.00), 315 (0.83). CD (MeOH) $\lambda_{min/max}$ *(Δε)* 225 (26.1), 250 (-8.4), 256 (-8.0), 282 (-19.2), 318 (7.2). ^{1}H NMR (500 MHz, ca. 11 mM in CD_3OD, 26°C) δ=0.99 ($H_3C(8^2)$); 1.92 ($H_3C(18^1)$); 1.94 ($H_3C(2^1)$); 2.09 ($H_3C(12^1)$); 2.24 ($H_3C(7^1)$); 2.26-2.38 ($H_2C(17^2)$); 2.42 ($H_2C(8^1)$); 2.53 ($H_AC(20)$); 2.60-2.72 ($H_2C(17^1)$); 2.84 ($H_BC(20)$); 3.75 (s, 3H, $H_3C(13^5)$); 3,89/3.93 ($H_2C(10)$); 4.00 (HC(1)); 4.86 (HC(15)); 5.34 ($H_{cis}C(3^2)$); 6.08 ($H_{trans}C(3^2)$); 6.43 ($HC(3^1)$); 9.32 (HC(5)); 9.52 (NH). ^{13}C-NMR (125 MHz, ca. 11 mM in CD_3OD, 26°C) from HSQC/HMBC spectra: δ=8.7 (7^1); 9.1 (12^1); 9.3 (18^1); 12.5 (2^1); 15.2 (8^2); 17.6 (8^1); 21.4 (17^1); 23.6 (10); 30.3 (20); 38.0 (17^2); 37.3 (15); 52.8 (13^5); 61.8 (1); 67.7 (13^2); 112.4 (12); 115.4 (18); 119.1 (3^2); 120.2 (17); 124.4 (19); 124.4 (16); 126.1 (8); 126.1 (13); 127.0 (3^1); 128.8 (3); 129.2 (6); 134.1 (11); 134.5 (7); 137.8 (9); 156.8 (2); 161.2 (14); 171.7 (13^3); 174.9 (4); 177.6 (5); 179.2 (17^3); 197.8 (13^1) ; MS (nano-ESI) *m/z (%)* 1257.4 (6, $[2M+H]^+$); 629.1 (100, $[M+H]^+$); 597.1 (5, $[M+H-32]^+$); 506.2 (3, $[M+H-123]^+$).

8: UV/Vis (MeOH) λ_{max} *(rel. ε)* 238 (sh, 1.00), 315 (0.80). CD (MeOH) $\lambda_{min/max}$ *(Δε)* 225 (48.5), 250 (-16.7), 255 (-16.4), 282 (-47.4), 317 (17.2). ^{1}H NMR (500 MHz, CD_3OD, 26°C) δ 1.00 ($H_3C(8^2)$); 1.88 ($H_3C(18^1)$); 1.93 ($H_3C(2^1)$); 2.11 ($H_3C(12^1)$); 2.25 ($H_3C(7^1)$); 2.41 ($H_2C(8^1)$); overlapping with 2.35-2.44 ($H_2C(17^2)$); 2.58 ($H_AC(20)$); 2.60-2.68 ($H_2C(17^1)$); 2.83 ($H_BC(20)$); 3.59 ($H_3C(17^5)$); 3.88/3.92 ($H_2C(10)$); 4.03 (HC(1)); 4.81 (HC(15)); 5.34 ($H_{cis}C(3^2)$); 6.08 ($H_{trans}C(3^2)$); 6.43 ($HC(3^1)$); 9.33 (HC(5)); 9.50 (NH). ^{13}C-NMR (125 MHz, CD_3OD, 26°C) from HSQC/HMBC spectra: δ=8.7 (7^1); 9.2 (12^1); 9.3 (18^1); 12.5 (2^1); 15.3 (8^2); 17.6 (8^1); 20.8 (17^1); 23.6 (10); 30.2 (20); 36.7 (17^2); 38.0 (15); 51.9 (17^5); 61.6 (1); 64.8 (13^2); 112.3 (12); 115.4 (18); 119.1 (3^2); 119.1 (17); 123.7 (19); 126.1 (8); 126.9 (13); 127.1 (3^1); 128.8 (3); 129.3 (6); 133.4 (11); 134.2 (7); 137.7 (9); 156.9 (2); 161.3 (14); 174.8 (4); 177.5 (5); 175.4 (17^3); MS (ESI) *m/z (%)* 1301.9 (29, $[2M-H+2Na]^+$);

1279.9 (47, $[2M+Na]^+$); 673.5 (16, $[M-H+2Na]^+$); 667.5 (40, $[M+K]^+$); 651.4 (100, $[M+Na]^+$); 629.5 (21, $[M+H]^+$); 607.5 (55, $[M+Na-44]^+$).

Identification of 7 with *Cj*-NCC-2 by HPLC

Solutions containing approximately equal amounts of **7** and **8** were both mixed with an authentic sample of *Cj*-NCC-2 obtained from plant extracts and analyzed by HPLC (0.5 ml min^{-1}; 0 min (ammonium actetate (200 mM, pH 6.8) in MeOH/water = 55/45), 40 min (ammonium actetate (200 mM, pH 6.8) in MeOH)). While **7** co-eluted with *Cj*-NCC-2 after ~ 23 min, **8** eluted after ~ 26 min, clearly separated from *Cj*-NCC-2. Based on this analysis and the spectroscopic data, **7** was identified as *Cj*-NCC-2 and **8** as an isomeric 13^2-demethyl-17^5-methyl-NCC.

References

[S1] H. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, 7512.
[S2] C. Curty, N. Engel, *Phytochemistry* **1996**, *42*, 1531.
[S3] M. Oberhuber, J. Berghold, K. Breuker, S. Hörtensteiner, B. Kräutler, *Proc. Nat. Acad. Sci. USA* **2003**, *100*, 6910.