Colorless Chlorophyll Catabolites in Senescent Florets of Broccoli

(Brassica oleracea var. *Italica)*

Supporting Information

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Dedicated to the memory of Paula Enders

Materials and Methods

1 Chemicals and Plant Materials

Chemicals: HPLC grade HiPerSolv CHROMANORM methanol (MeOH) was from VWR PROLABO. Potassium dihydrogen phosphate and potassium phosphate dibasic anhydrous were from Sigma-Aldrich.

Plant Material: Fresh, green broccoli (Brassica oleracea var. Italica), produced by Conzorzio APO-FOGGIA s.c., Italy, was typically harvested five days before it was sold at the store (Spar Supermarket) in Innsbruck.

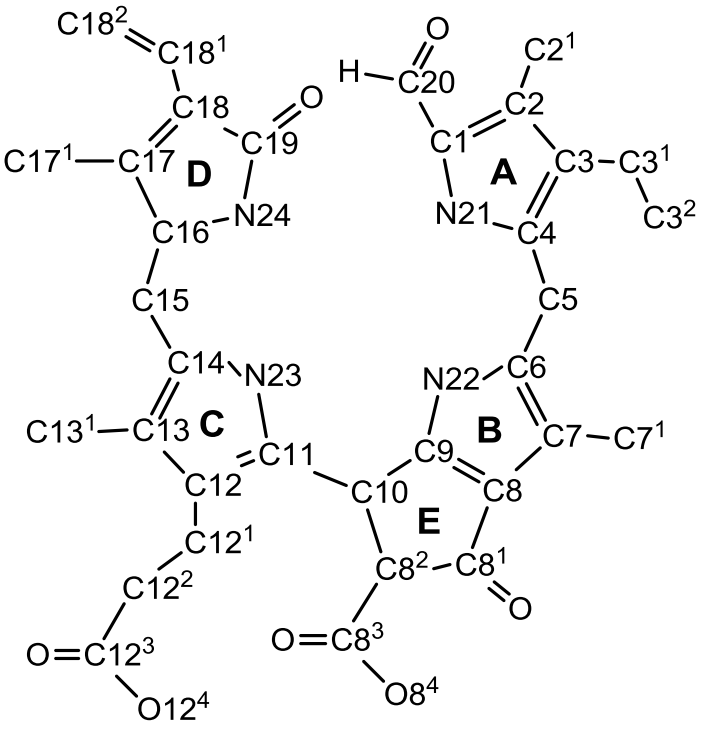
2 Spectroscopy

UV/Vis: Hitachi U3000 spectrophotometer, λ_{max} [nm] (relative ε), in H_2O. CD: Jasco J715 spectropolarimeter, λ_{max} and λ_{min} [nm] (Θ), in H_2O. ^1H nuclear magnetic resonance (NMR): Bruker UltraShield 600 MHz or Varian Unity Inova 500 MHz spectrometers, δ[ppm] with δ(HDO) = 4.79 ppm, in D_2O). Electrospray ionization mass spectrometry (ESI-MS): Finnigan MAT 95S, m/z (rel. intensity), positive-ion mode, 1.4 kV spray voltage; signals with > 5% rel. intensity are listed.

3 High Performance Liquid Chromatography (HPLC)

Dionex P680 HPLC Pump, on-line UV/Vis-spectra: Dionex UVD340U; column: Phenomenex HyperClone 5µ ODS 250mm x 4.6 mm i.d., column at 20 °C, protected with a Phenomenex ODS 4 mm x 3.0 mm i.d. pre-column was used with a flow rate 0.5 ml.min-1. Solvent A: 50 mM aqueous potassium phosphate buffer (pH 7), solvent B: MeOH, HPLC grade; solvent composition 1: 0-5 min: A/B = 80/20; 5-55 min: A/B = 80/20 to 30/70, constant gradient; 55-60 min: 30/70 to 0/100, constant gradient; 60-70 min: A/B = 0/100; 70-75 min: A/B = 0/100 to 80/20, constant gradient. Analytical HPLC: sample size 50 µl, semi-preparative HPLC: sample size 2 ml.

Numbering of relevant atoms of NCCs



Numbering of relevant atoms of DNCCs

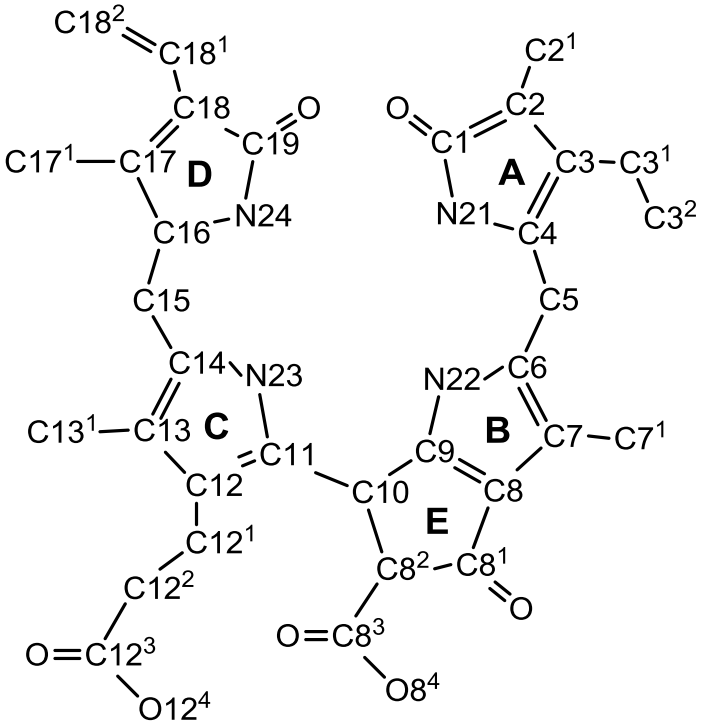


Figure 1S. Numbering of relevant atoms of NCCs (top) and of DNCCs (bottom)

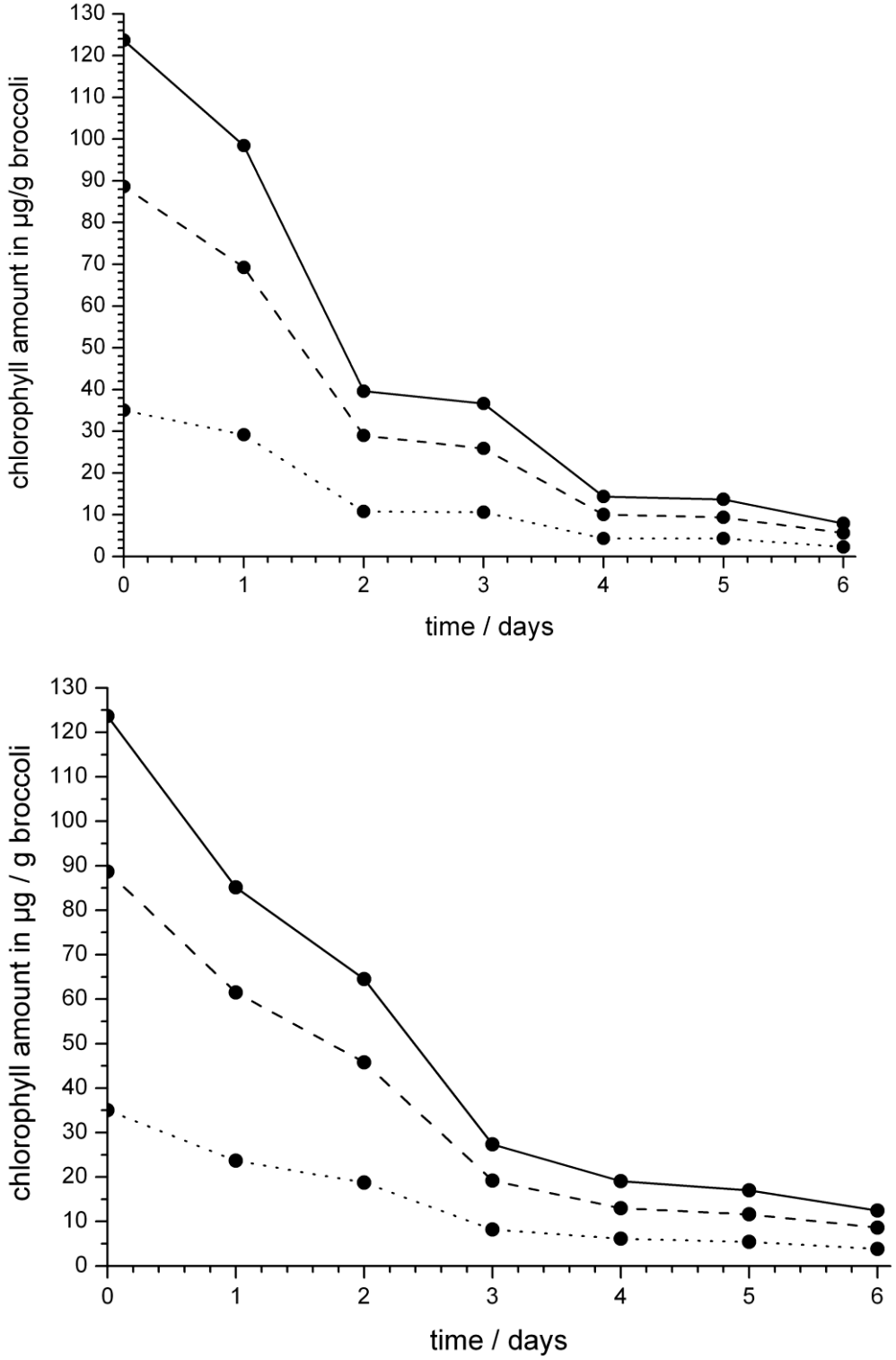


Figure 2S. Qualitative analysis of time dependence of the amounts of chlorophyll in the raw broccoli plant material during the de-greening in the dark (top) and in day light (bottom). Total chlorophyll *a*+*b* (continuous line), chlorophyll *a* (dashed line) and chlorophyll *b* (dotted line) in µg / g raw material

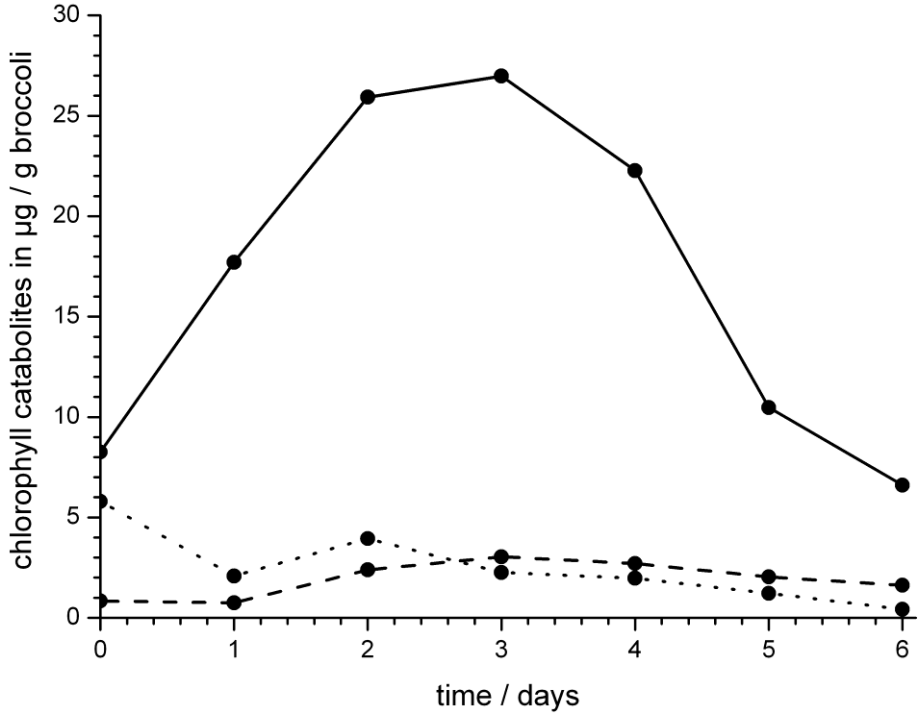


Figure 3S. Qualitative analysis of time dependence of the amounts of colourless chlorophyll catabolites in the raw broccoli during the de-greening in the dark. Amount (in microgram per gram broccoli) of *Bo*-NCC-1 (continuous line), *Bo*-NCC-2 (dotted line) and *Bo*-DNCC (dashed line) in raw material

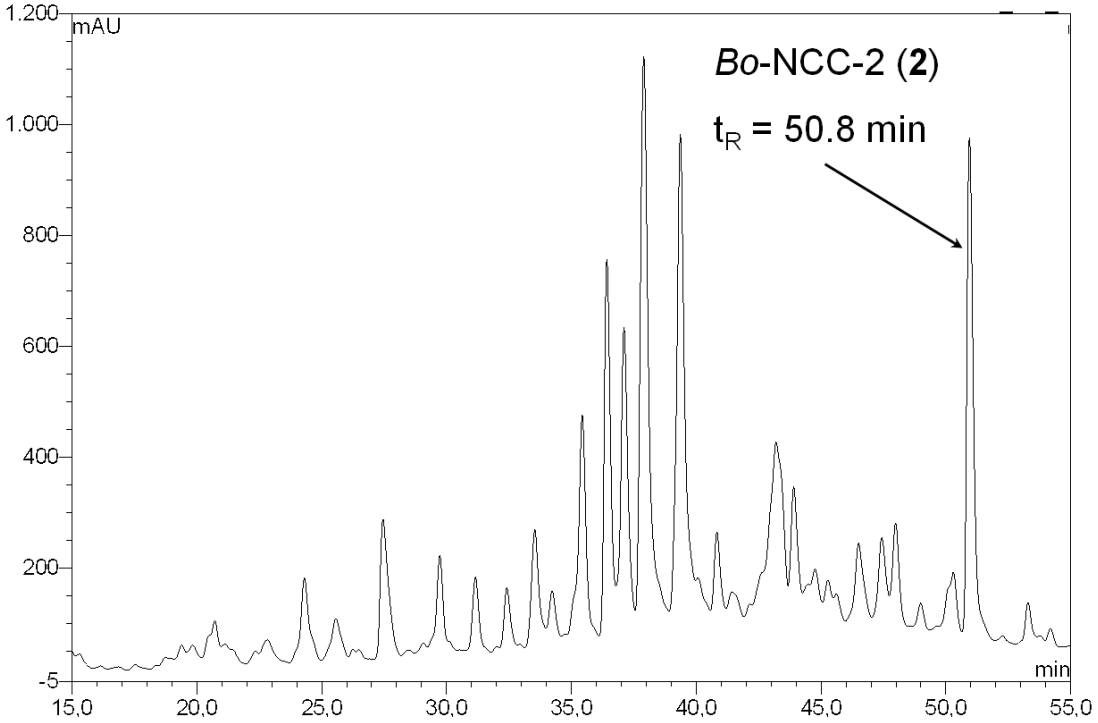


Figure 4S. HPLC-Analysis of the CH_2Cl_2 phase of the broccoli extract (detected by 320nm). The fraction identified as *Bo*-NCC-2 (**2**) is highlighted

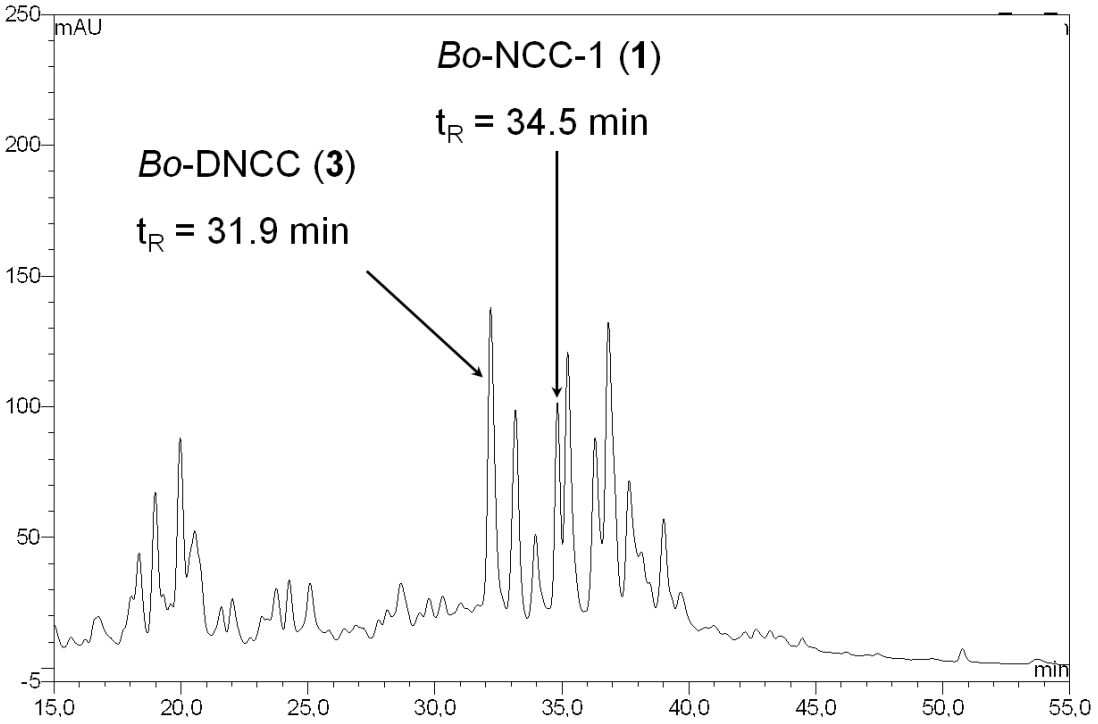


Figure 5S. HPLC-Analysis of the aqueous phase of the broccoli extract (detected by 250nm). The fractions identified as *Bo*-NCC-1 (**1**) and *Bo*-DNCC (**3**) are highlighted

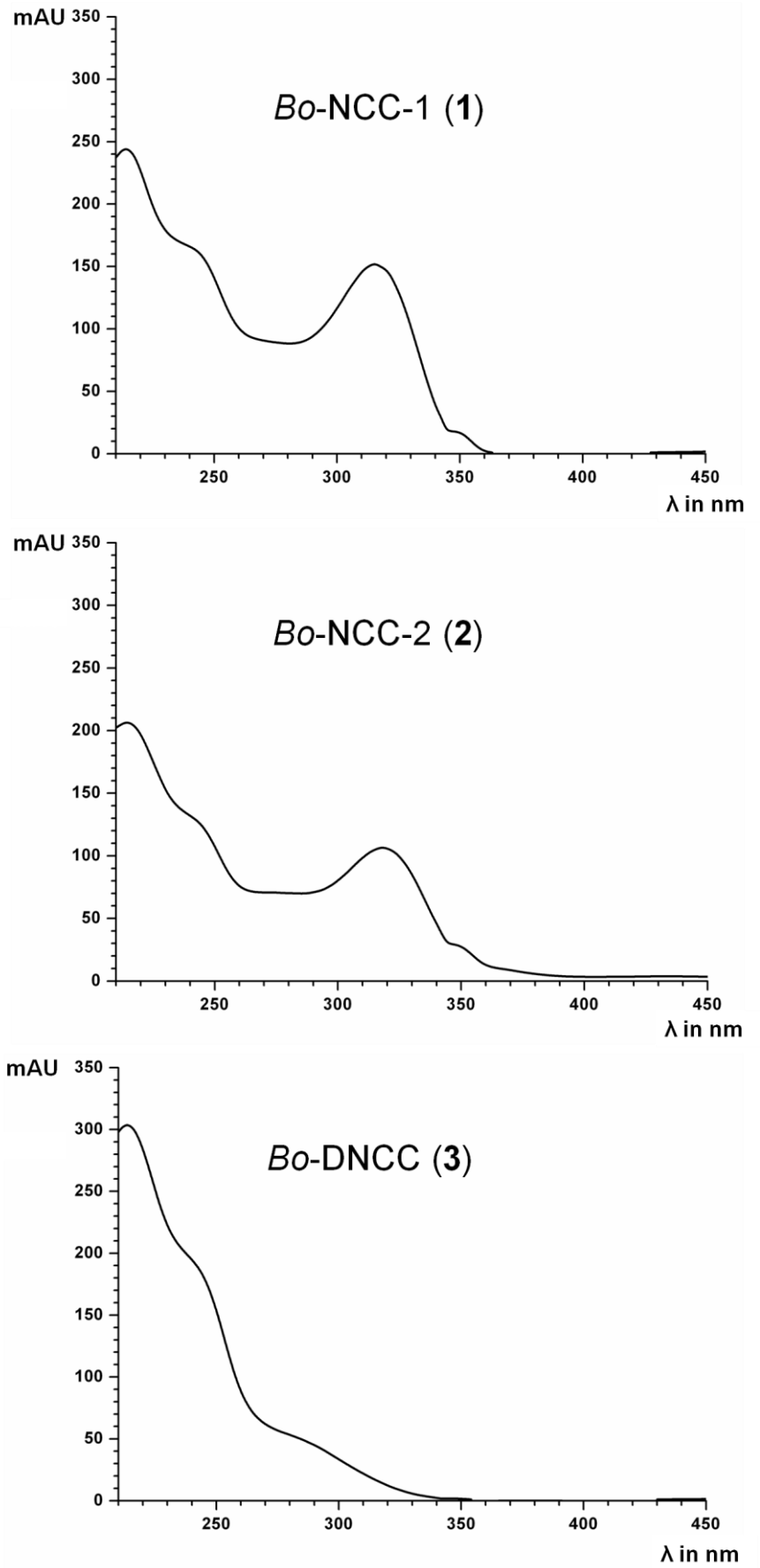


Figure 6S. UV/Vis-online spectra of *Bo*-NCC-1 (**1**, t_R = 34.5 min), of *Bo*-NCC-2 (**2**, t_R = 50.8 min) and of *Bo*-DNCC (**3**, t_R = 31.9 min)

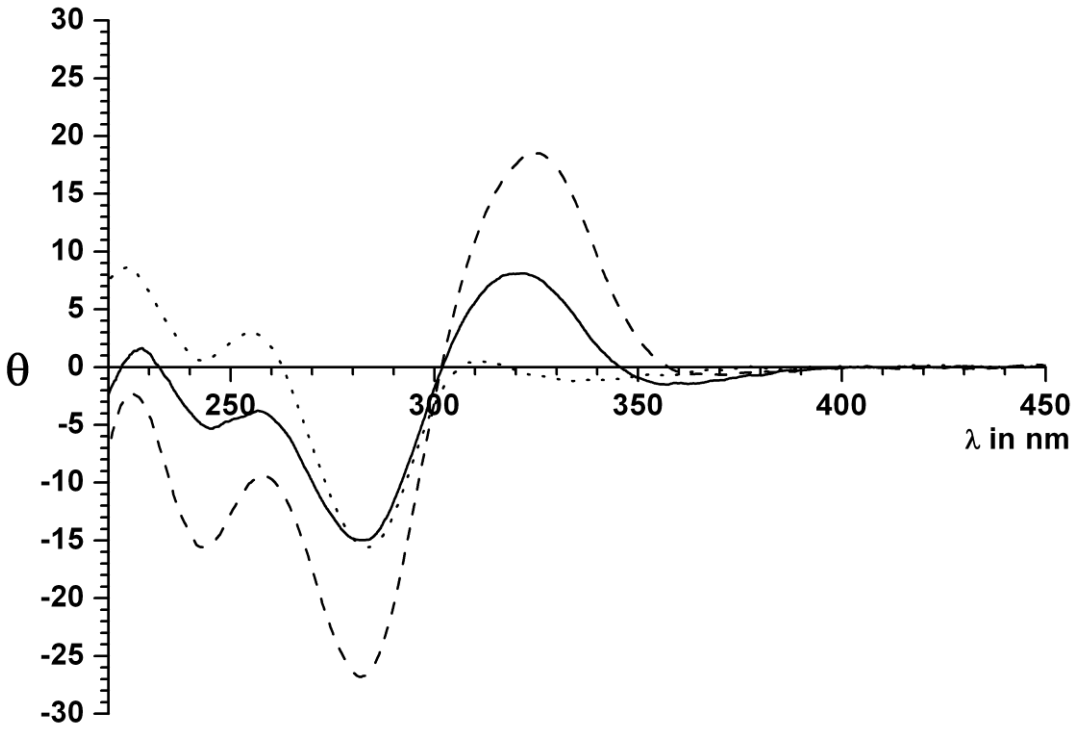


Figure 7S. CD –spectra of *Bo*-NCC-1 (**1**, solid line), of *Bo*-NCC-2 (**2**, dashed line) and of *Bo*-DNCC (**3**, dotted line) in unbuffered aqueous solution

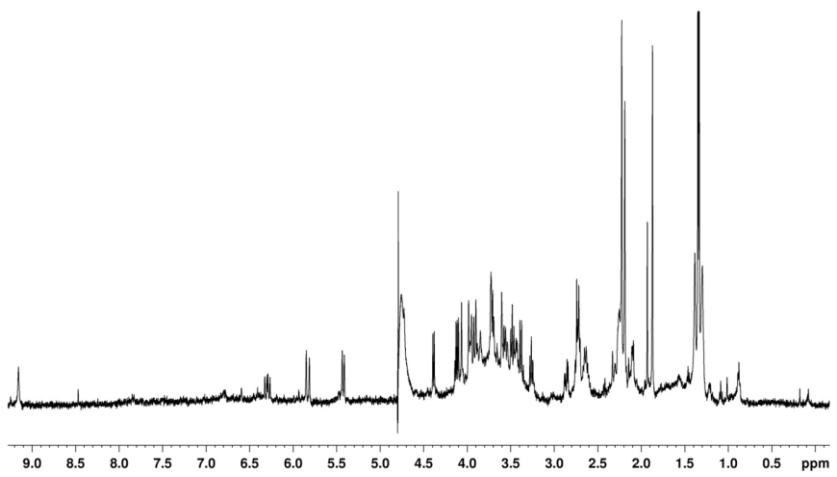


Figure 8S. 500 MHz ^{1}H-NMR-spectrum of *Bo*-NCC-1 (**1**) in D_2O,

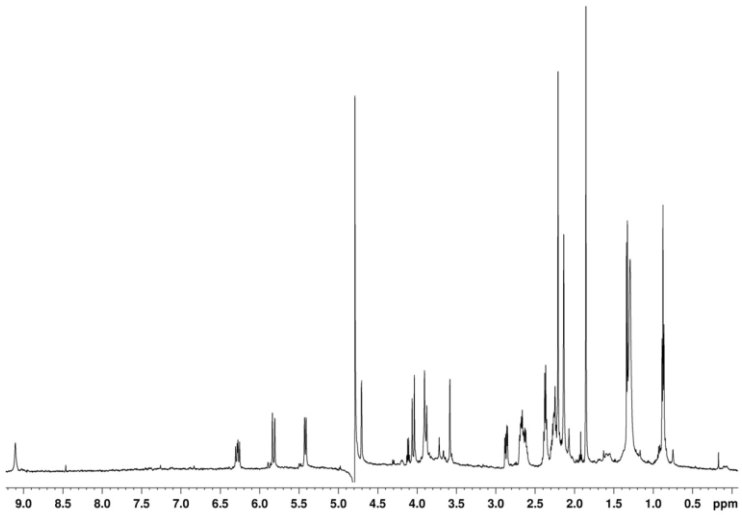


Figure 9S. 600 MHz ^{1}H-NMR-spectrum of *Bo*-NCC-2 (**2**) in D_2O

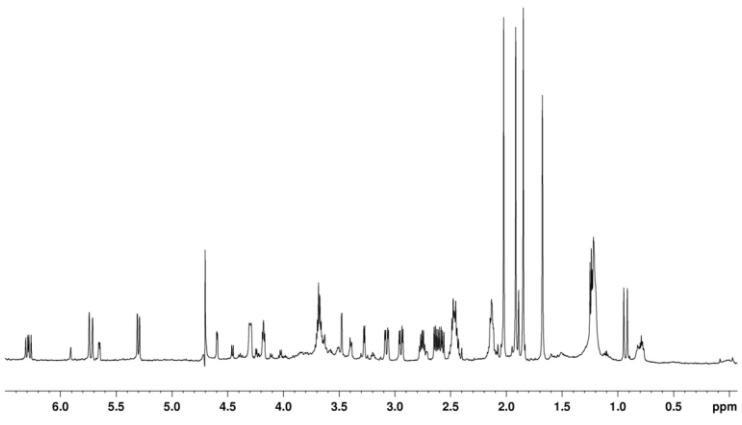


Figure 10S. 600 MHz ^{1}H-NMR-spectrum of *Bo*-DNCC (**3**) in D_2O

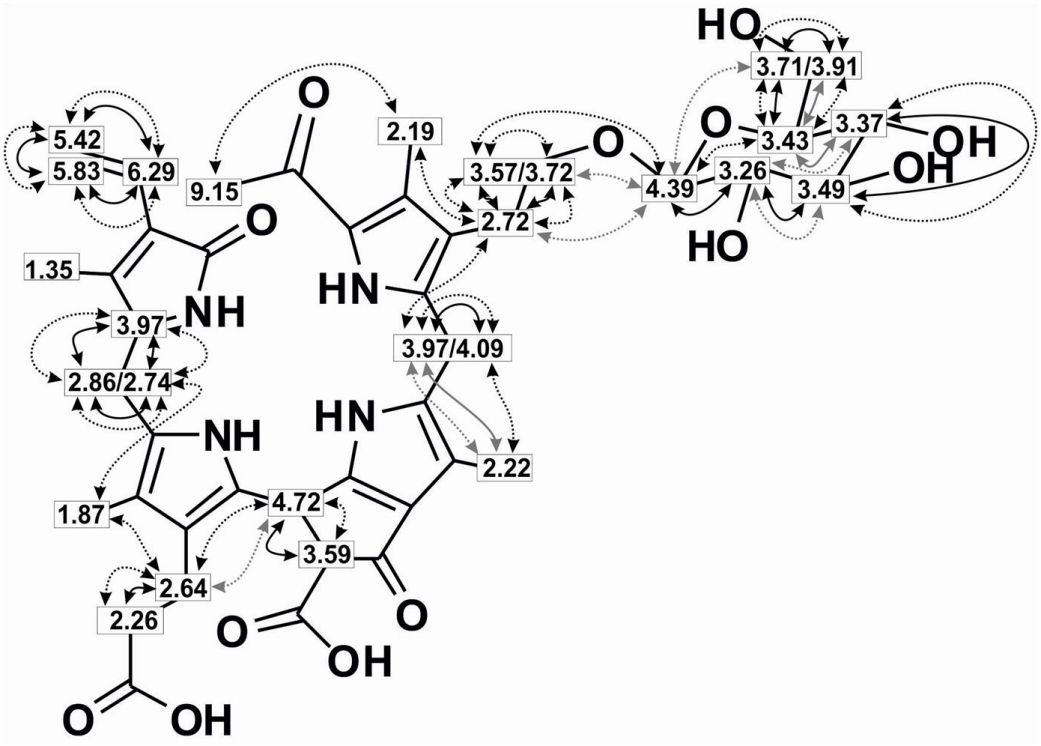


Figure 11S. Graphical representation of ^{1}H-NMR chemical-shift data of *Bo*-NCC-1 (**1**) in D_2O. Assignments of signals in 500 MHz ^{1}H-NMR-spectra of carbon-bound hydrogen atoms (solid arrows represent COSY correlations, dashed arrows represent ROESY correlations)

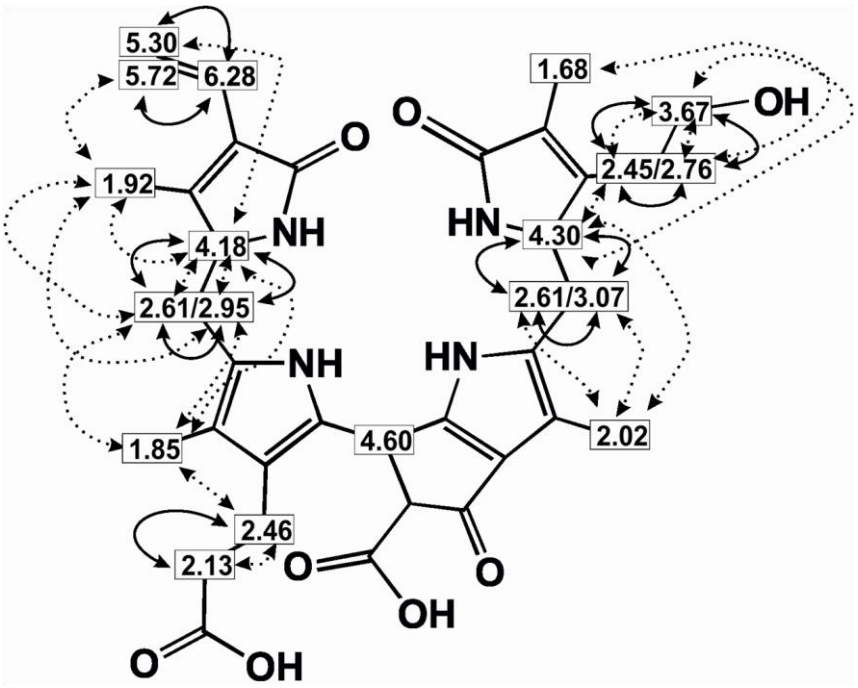


Figure 12S. Graphical representation of ^{1}H-NMR chemical-shift data of *Bo*-DNCC (**3**) in D_2O. Assignments of signals in 600 MHz ^{1}H-NMR-spectra of carbon-bound hydrogen atoms (solid arrows represent COSY correlations, dashed arrows represent ROESY correlations)

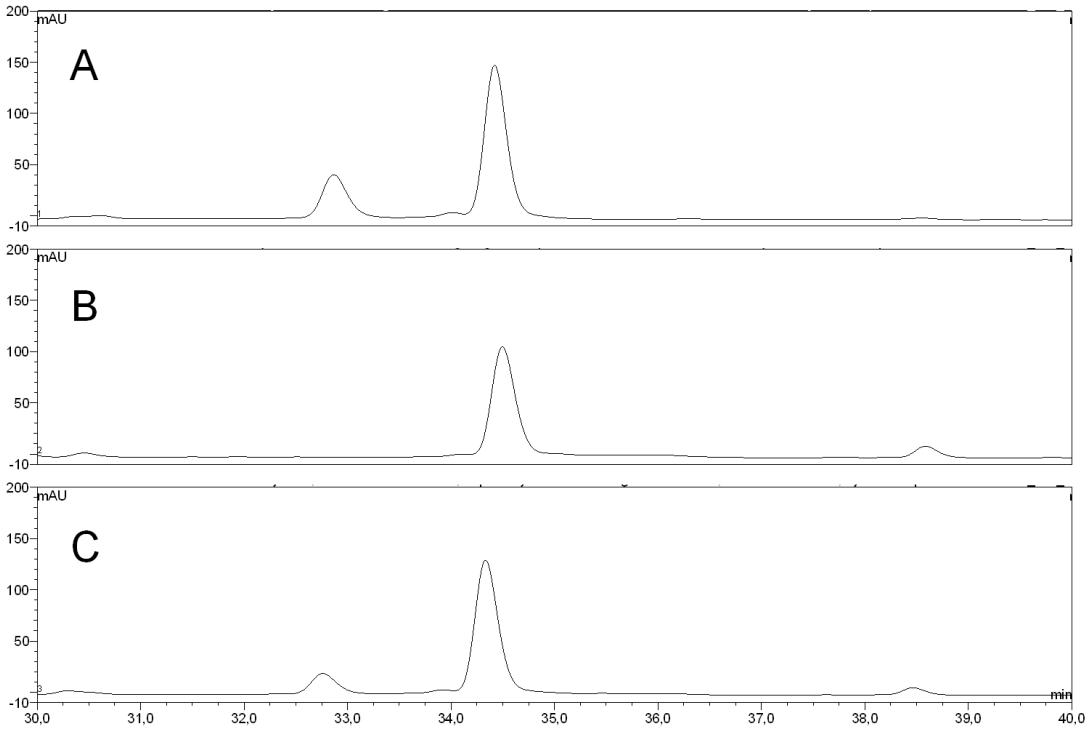


Figure 13S. HPL-chromatograms of *Bo*-NCC-1 (**1**) and *Bn*-NCC-2 (detected at 320nm). **A**) *Bo*-NCC-1 (**1**); **B**) *Bn*-NCC-2; **C**) *Bo*-NCC-1 (**1**) and *Bn*-NCC-2 in a (1:1) co-injection.

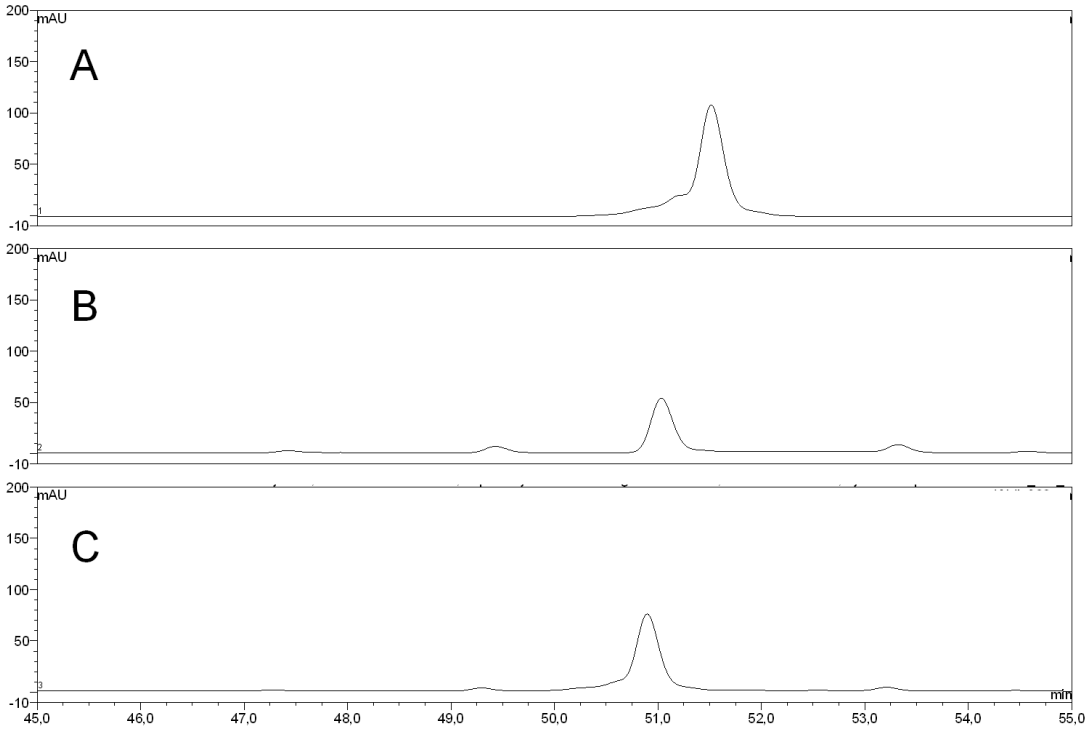


Figure 14S. HPL-chromatograms of *Bo*-NCC-2 (**2**) and *Bn*-NCC-4 (detection at 320nm). **A**) *Bo*-NCC-2 (**2**); **B**) *Bn*-NCC-4; **C**) *Bo*-NCC-2 and *Bn*-NCC-4 in a (1:1) co-injection.

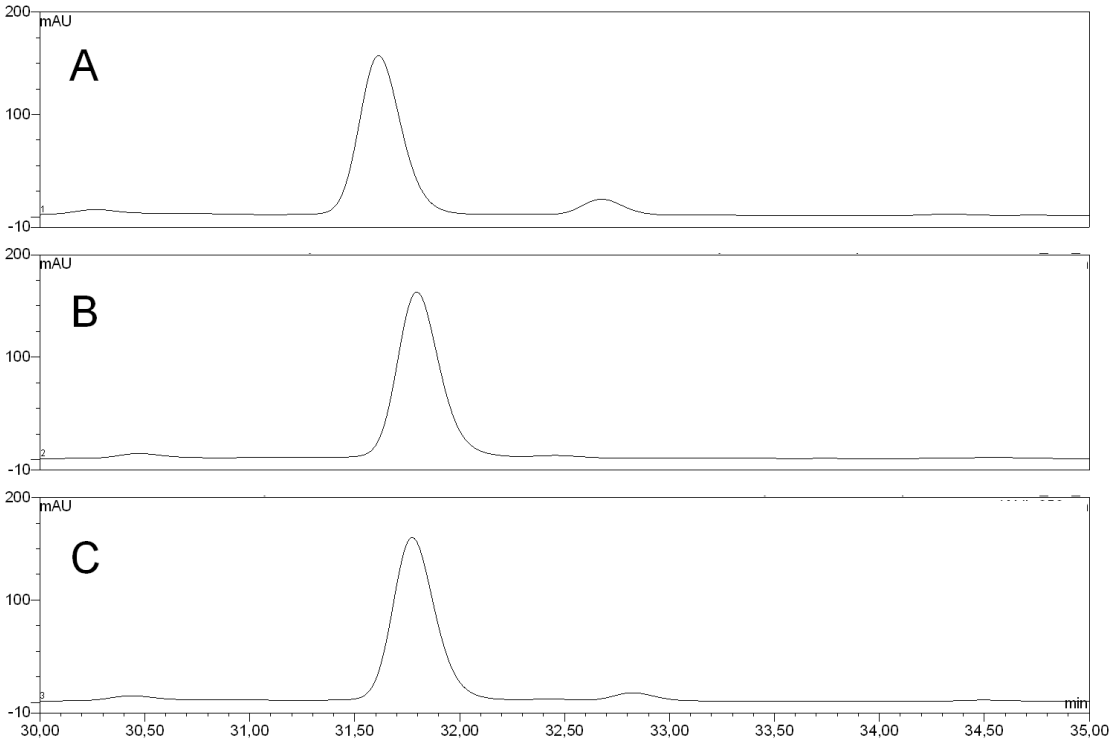


Figure 15S. HPL-chromatograms of *Bo*-DNCC (**3**) and *At*-DNCC-1 (detection at 250nm). **A**) *Bo*-DNCC (**3**); **B**) *At*-DNCC-1; **C**) *Bo*-DNCC-1 and *At*-DNCC-1 in a (1:1) co-injection.