

**Anionic carbohydrate polymers for hydrophilization
of poorly soluble antitumor compounds of the colchicine series**

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Methods

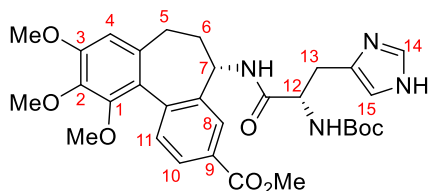
¹H NMR, ¹³C NMR spectra were recorded on Agilent DDR2 400 spectrometer at 25 °C. Chemical shifts (δ) are reported in ppm for the solutions in DMSO-d₆ with internal reference TMS and *J* values in Hertz. Elemental analysis was performed using an Elementar (Vario Micro Cube) apparatus, all the compounds were found to have purity >95%. Column chromatography was performed using Merck Kieselgel 60 (70 – 230 mesh). All reactions were performed with commercially available reagents. Solvents were purified according to standard procedures. Compound **2** was obtained as reported previously [E. S. Shchegravina, D. S. Tretiakova, A. S. Alekseeva, T. R. Galimzyanov, Yu. N. Utkin, Yu. A. Ermakov, E. V. Svirshchevskaya, V. V. Negrebetsky, N. Yu. Karpechenko, V. P. Chernikov, N. R. Onishchenko, E. L. Vodovozova, A. Yu. Fedorov and I. A. Boldyrev, *Bioconjugate Chem.*, 2019, **30**, 1098; <https://doi.org/10.1021/acs.bioconjchem.9b00051>].

The size and zeta potentials of microgels was studied on a Brookhaven-90 Plus analyzer (Brookhaven, United States). Hydrodynamic characteristics were performed in a 0.01 M Tris buffer with pH 7.

Dialysis was performed using dialysis tubes “Serva” (MWCO = 12 kDa). For this aim, 10 ml of solution was placed in the dialysis tube, and dialyzed against 2000 ml of bidistilled water for 24 hours.

Synthetic procedures for compounds 3 and 4

Synthesis of methyl (5*S*)-5-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(1*H*-imidazol-4-yl)propanamido)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*][7]annulene-3-carboxylate **3**.

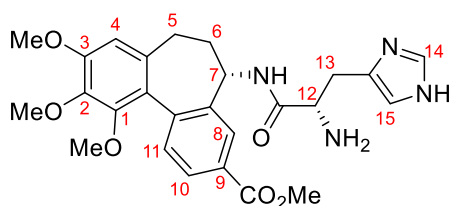


Atomic numeration is given only for NMR assignment!

Compound **2** (1.00 equiv., 0.22 mmol, 80 mg) was treated with *N*-Boc-histidine (1.00 equiv., 0.22 mmol, 56 mg), *N,N'*-diisopropylcarbodiimide (DIC) (1.50 equiv., 0.33 mmol, 42 mg, 51 μ l), *N*-hydroxysuccinimide (1.00 equiv., 0.22 mmol, 25 mg) and triethylamine (3.00 equiv., 0.66 mmol, 67 mg, 92 μ l) in dry dichloromethane under inert atmosphere during 16 hours at room temperature. After completeness of the reaction the solvent was removed under reduced pressure and the product was purified with silica gel column chromatography with eluent petrol ether – ethyl acetate – ethanol (1 : 1 : 1). Compound **3** was obtained as pale-beige solid with 45% yield.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.92 (s, 1H, NH-his), 8.64 (d, *J* = 7.3 Hz, 1H, C7-NH), 7.58 (s, 1H, C14-H), 7.14 (s, 1H, C8-H), 7.10 (d, *J* = 10.6 Hz, 1H, C10-H), 7.02 (d, *J* = 10.9 Hz, 1H, C11-H), 6.84 (d, *J* = 8.2 Hz, 1H, NH-Boc), 6.81 (s, 1H, C15-H), 6.77 (s, 1H, C4-H), 4.31 (dt, *J* = 12.9, 6.8 Hz, 1H, C7-H), 4.22 (td, *J* = 8.4, 4.7 Hz, 1H, C12-H), 3.87 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.53 (s, 3H, OMe), 2.91 – 2.73 (m, 2H, C6-H), 2.62 – 2.57 (m, 1H, C5-H), 2.26 – 2.20 (m, 1H, C5-H), 1.32 (s, 9H, Boc). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 177.86, 170.71, 163.52, 155.09, 152.96, 150.42, 146.67, 140.73, 139.26, 136.68, 135.08, 134.33, 134.17, 130.43, 125.41, 114.25, 112.03, 107.78, 85.03, 78.05, 60.82, 60.69, 56.00, 55.83, 40.67, 28.11, 27.36, 23.29. **Elemental analysis:** for C₃₁H₃₈N₄O₈ calculated: C, 62.61; H, 6.44; found: C, 62.43; H, 6.26.

Synthesis of methyl (5*S*)-5-((*S*)-2-amino-3-(1*H*-imidazol-4-yl)propanamido)-9,10,11-trimethoxy-6,7-dihydro-5*H*-dibenzo[*a,c*][7]annulene-3-carboxylate **4**



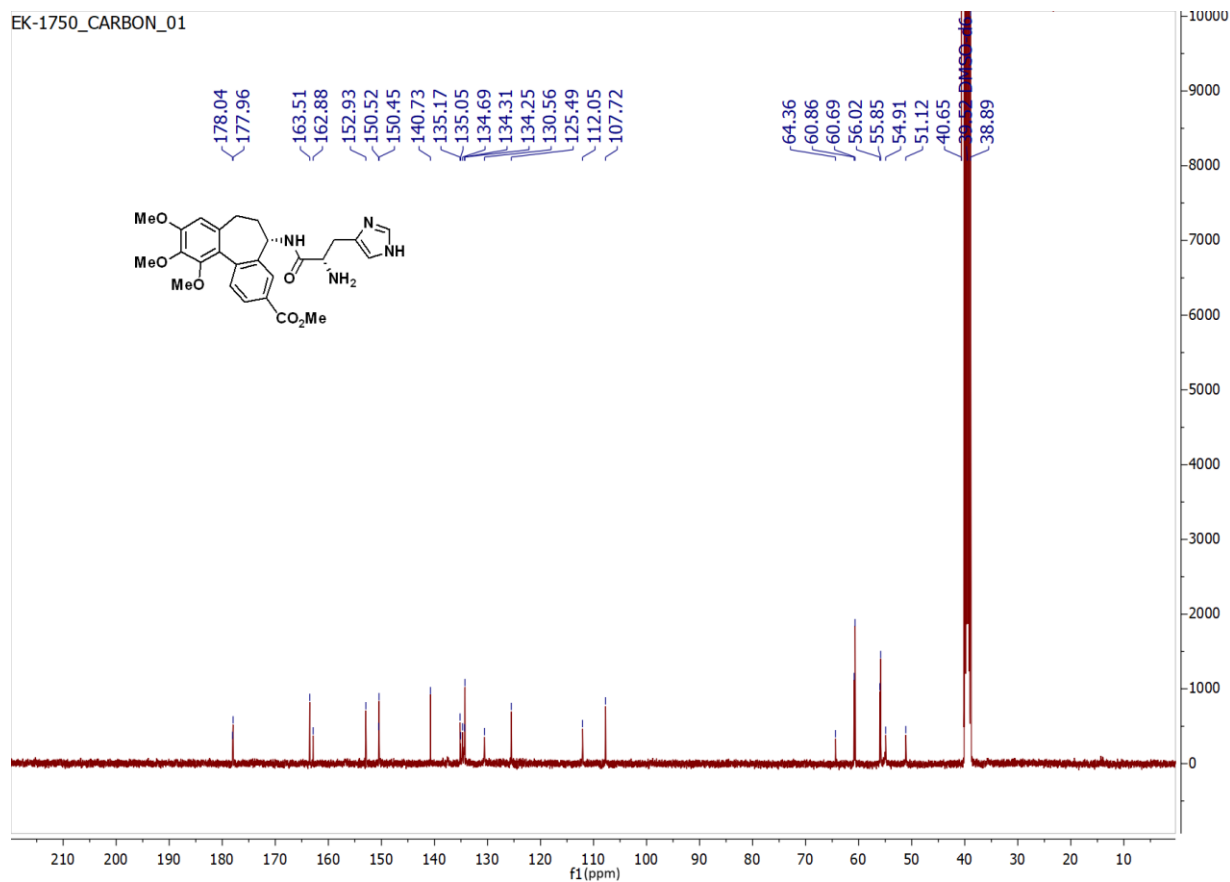
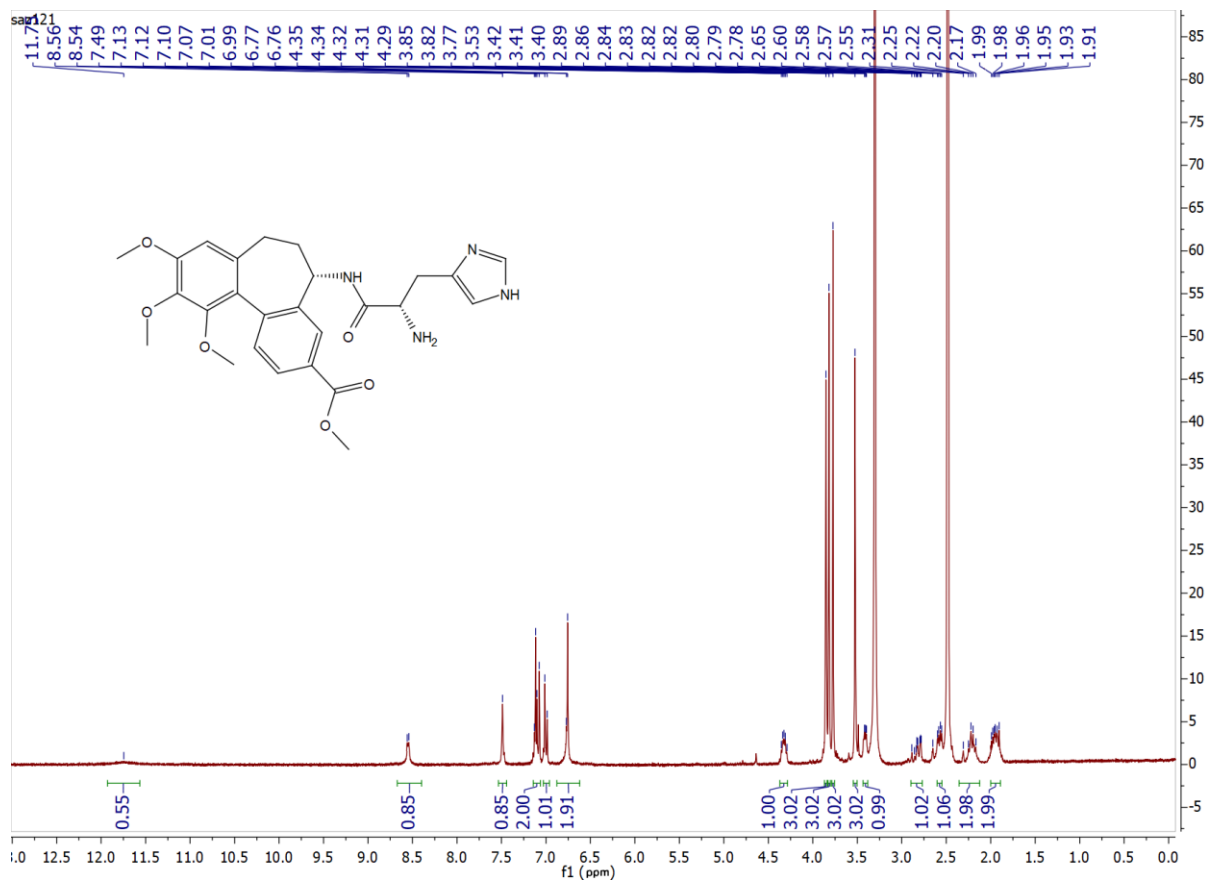
Atomic numeration is given only for NMR assignment!

Starting compound **3** (1.00 equiv., 0.092 mmol, 65 mg) was dissolved in dichloromethane (4.00 ml) and 3M HCl was added dropwise (0.85 ml). After the completeness of the reaction, saturated solution of Na₂CO₃ was added until the pH was 8. The mixture was extracted with dichloromethane, the combined organic layer was dried over Na₂SO₄, the solvent was removed under reduced pressure. The target compound **4** was obtained as beige solid with 45% yield.

¹H NMR (400 MHz, DMSO-*d*₆) δ 11.75 (s, 1H, NH-his), 8.55 (d, *J* = 7.2 Hz, 1H, C7-NH), 7.49 (s, 1H, C14-H), 7.14 – 7.06 (m, 2H, C15-H, C10-H), 7.00 (d, *J* = 10.8 Hz, 1H, C11-H), 6.88 – 6.62 (m, 2H, C8-H, C4-H), 4.32 (dt, *J* = 13.9, 6.9 Hz, 1H, C7-H), 3.85 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.77 (s, 3H, OMe), 3.53 (s, 3H, OMe), 3.44 – 3.38 (m, 1H, C12-H), 2.90 – 2.77 (m, 1H, C13-H), 2.58 (dd, *J* = 12.8, 5.8 Hz, 1H, C13-H), 2.36 – 2.12 (m, 2H, C6-H), 2.00 – 1.89 (m, 2H, C5-H). **¹³C NMR** (101 MHz, DMSO-*d*₆) δ 178.04, 177.96, 163.51, 162.88, 152.93, 150.52, 150.45, 140.73, 135.17, 135.05, 134.69, 134.31, 134.25, 130.56, 125.49, 112.05, 107.72, 64.36, 60.86, 60.69, 56.02, 55.85, 54.91, 51.12, 40.65, 38.89. **Elemental analysis:** for C₂₆H₃₀N₄O₆ calculated: C, 63.15; H, 6.11; found: C, 63.32; H, 6.05.

Chemical structure of compound 115-2 is shown above the ¹H NMR spectrum. The structure is a complex molecule featuring a central benzene ring substituted with a methoxy group and a side chain containing a chiral center, a carbonyl group, and a heterocyclic ring system. The ¹H NMR spectrum (400 MHz, DMSO-d₆) displays peaks corresponding to the protons in the molecule, with chemical shifts ranging from 1.32 to 8.65 ppm. Integration values are provided below the baseline, and peak labels are present above the spectrum.



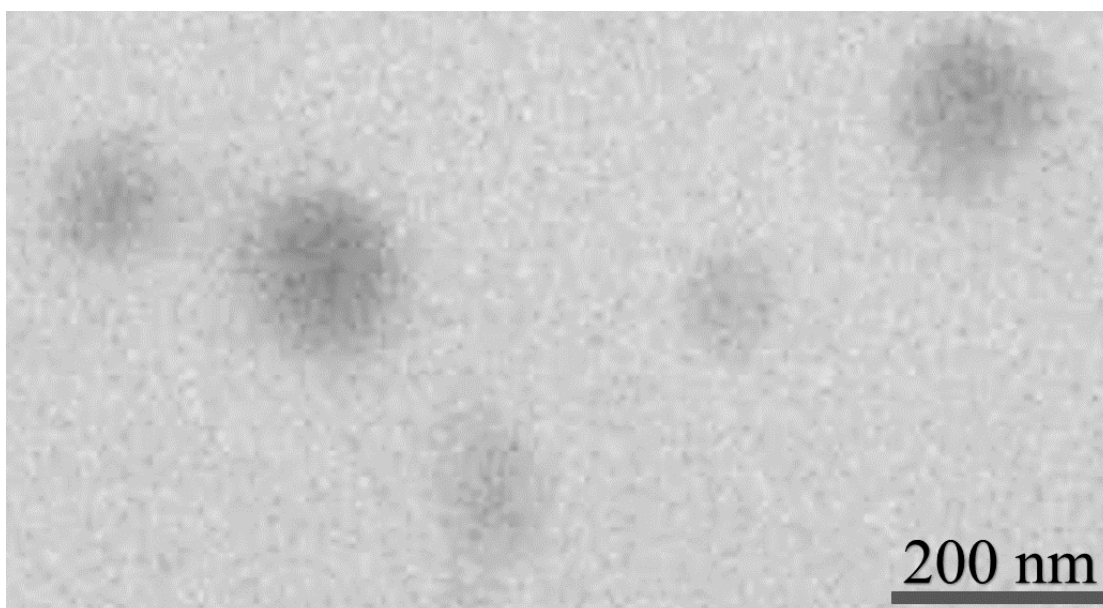


Synthetic procedures for hydrochlorides 2' and 4'

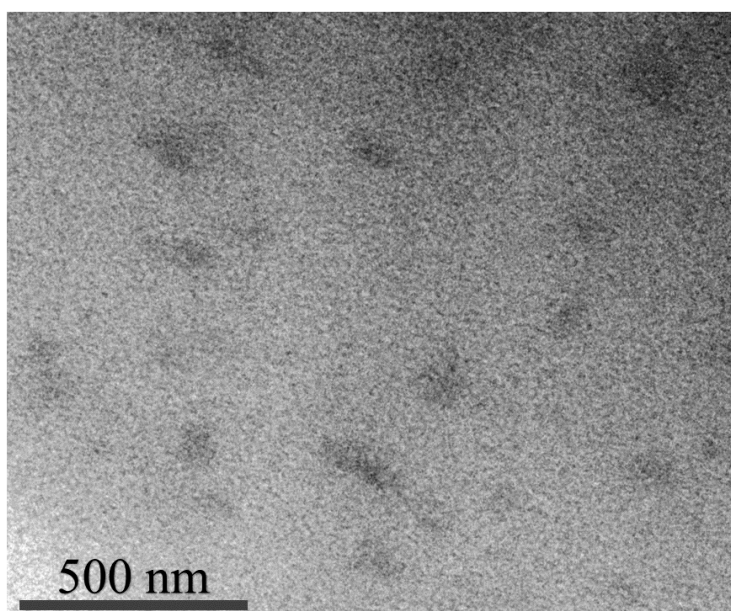
The protonation of amines **2** or **4** was reached *via* adding 50 μ l of concentrated aqueous HCl to 0.5 mg of **2** and 2.5 mg **4**. The resulting suspensions were heated for 15 minutes at 50 °C with stirring until yellow transparent solutions appeared, indicating complete dissolution of **2** or **4** and formation of protonated positively charged salts **2'** and **4'**.

Polysaccharide microgels were synthesized *via* mixing aqueous solutions of anionic CMC and a divalent metal salt, CaCl₂ or MgCl₂. The concentration of anionic CMC units was [CMC] = 4×10^{-3} M and a molar ratio [CMC]/[Me²⁺] was of 10.

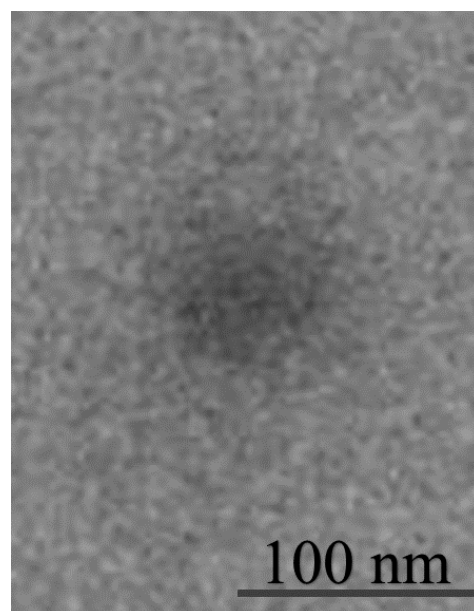
Water soluble forms of colchicines were prepared as follows. Acidic aqueous solution with 0.3 mg of **2**, or 0.4 mg of **4** (50 μ l) was added dropwise to 3 ml of an aqueous solution with 2 mg of CMC-Ca²⁺ or CMC-Mg²⁺ microgel with vigorous stirring. A molar ratio [CMC]/[**2**] = [CMC]/[**4**] was of 10. The resulting mixtures were additionally stirred for 30 minutes, pH was adjusted to 7 with NaOH solution, then the mixtures were placed in dialysis bags and purified for 24 hours by flow dialysis.



(a)



(b)



(c)

Figure S1. TEM images of (a) CMC- Ca^{2+} microgels and (b),(c) CMC- Ca^{2+} -4.

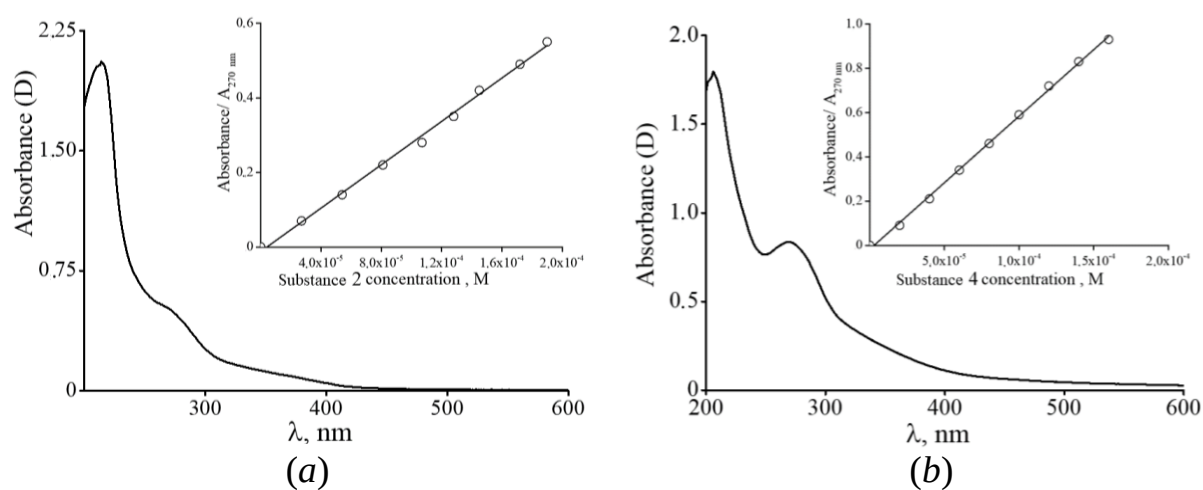


Figure S2. UV spectra of (a) compound **2** and (b) compound **4** solutions in H₂O/DMSO (60/1 v/v). [**2**] = 1.5×10^{-4} M and [**4**] = 1.8×10^{-4} M. In the upper right corners are the dependences of the optical density of solutions at 270 nm vs. **2** and **4** concentrations.

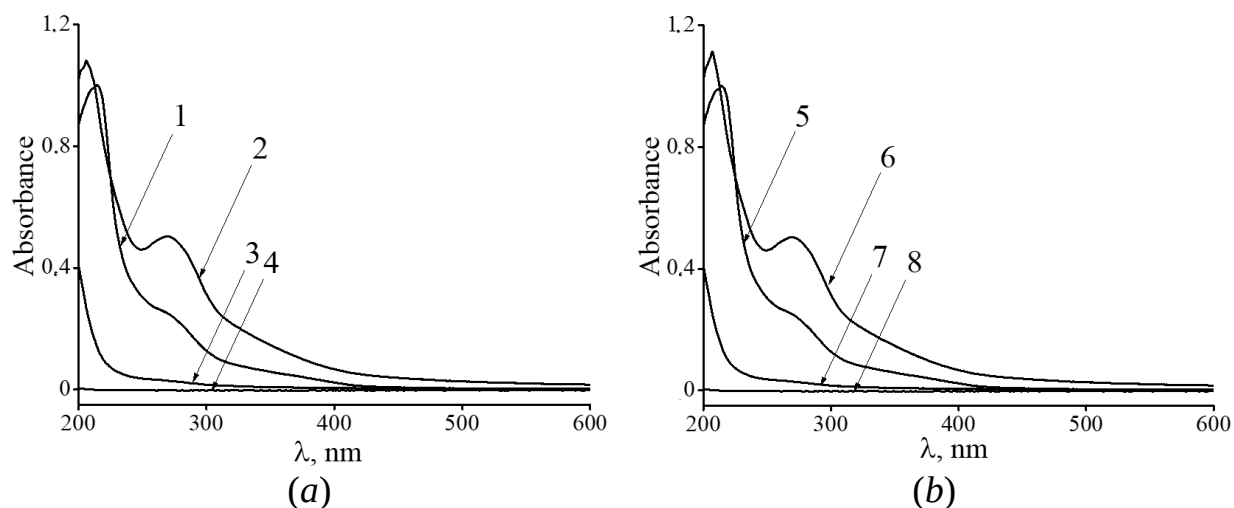


Figure S3. UV spectra of water solutions. (a) Compound **2** immobilized by CMC- Ca^{2+} microgel – **1**; compound **4** immobilized by CMC- Ca^{2+} microgel – **2**; CMC- Ca^{2+} microgel – **3**; internal dialysis solution after purification of CMC- Ca^{2+} -**2** – **4**. (b) Compound **2** immobilized by CMC- Mg^{2+} microgel – **1**; compound **4** immobilized by CMC- Mg^{2+} microgel – **2**; CMC- Mg^{2+} microgel – **3**; internal dialysis solution after purification of CMC- Mg^{2+} -**2** – **4**; the total concentration of CMC- Ca^{2+} -**2**, CMC- Ca^{2+} -**4**, CMC- Mg^{2+} -**2** and CMC- Mg^{2+} -**4** is $c = 0.25 \text{ mg ml}^{-1} (\text{H}_2\text{O})$; the total concentration of CMC- Ca^{2+} and CMC- Mg^{2+} is $c = 0.25 \text{ mg ml}^{-1} (\text{H}_2\text{O})$.