SUPPLEMENTARY INFORMATION

# β-O-4 type dilignol compounds and their iron complexes for modeling of iron binding to humic acids: synthesis, characterization, electrochemical studies and algal growth experiments

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## Material and methods

*$^{1}H$ and $^{13}C$ NMR spectroscopy*

$^{1}H$ NMR spectra were recorded on a Bruker Avance III™ 500 MHz spectrometer in DMSO-$d_6$ at 298 K using standard pulse programs at 500.10 MHz for $^{1}H$ experiments and 75 MHz for $^{13}C$ experiments.

*pH-potentiometry and UV–Vis spectrophotometry*

Iron(III) stock solution was prepared by dissolving the appropriate amount of the iron(III) chloride in known amounts of HCl. The concentration was determined by complexometry *via* the EDTA complexes. Accurate strong acid content of the iron(III) stock solution was determined by pH-potentiometric titrations. Ligands **6a**, **6b** and **6c** were dissolved in HCl solutions ($c_{HCl}$ ~ 15 mM) to obtain the acidic stock solutions ($c_L$ ~2 mM, pH ~1.9).The pH-metric measurements for determination of the exact concentrations of HCl and KOH stock solutions were carried out at 25.0 ± 0.1 °C in aqueous solutions at an ionic strength of 0.10 M KCl in order to keep the activity coefficients constant. All the titrations were performed with carbonate-free KOH solutions of known concentration (0.10 M). An Orion 710A pH-meter equipped with a Metrohm combined electrode (type 6.0234.100) and a Metrohm 665 Dosimat burette were used for the pH-metric titrations. The electrode system was calibrated to the pH = -log[$H^+$] scale by means of blank titrations (strong acid *vs.* strong base; HCl *vs.* KOH) according to the method suggested by Irving *et al.*[1] The average water ionization constant, p$K_{water}$, is 13.76 ± 0.01 at 25 °C, which corresponds well to the literature data.[2] Samples were deoxygenated by bubbling purified argon for *ca.* 10 min prior to the measurements and argon was also passed over the solutions during further titrations. The exact concentration of the ligands' stock solutions together with the proton dissociation constants were determined by pH-potentiometric titrations with the use of the computer program HYPERQUAD.[3]

A Hewlett Packard 8452A diode array and Thermo Scientific Evolution 220 spectrophotometers were used to record the UV–Vis spectra in the 200 – 700 nm and 350 – 1000 nm intervals, respectively. The path length was 1 cm. The spectrophotometric measurements employing the batch technique instead of continuous titrations were performed on samples of ligands alone ($c_L$ = 200 μM) or with iron(III) over the pH range between 2 and 11.5 at an ionic strength of 0.10 M (KCl) at 25.0 ± 0.1 °C. In the latter case the concentration of the ligand was usually 1.0 mM and the metal-to-ligand ratios were 1:1, 1:2 and 1:3. UV–Vis spectra for the ligands **6b** and **6c** were also recorded in the pH range from 11 to 13.8 to follow the second deprotonation step, but the constant ionic strength could not be guaranteed.

Proton dissociation constants of ligands, the stability constants of the iron(III) complexes and the molar absorbance spectra of the individual species were calculated by the computer program PSEQUAD.[4] Literature data were used for iron(III) hydroxido species.[5] $\beta$ ($M_pL_qH_r$) is defined for the general equilibrium $p$M + $q$L + $r$H $\rightleftharpoons$ $M_pL_qH_r$ as $\beta(M_pL_qH_r) = [M_pL_qH_r]/[M]^p[L]^q[H]^r$ where M denotes the metal ion and L the completely deprotonated ligand.

The UV–Vis spectra for the stability measurements (1–21 days) were recorded on an Agilent 8453 spectrophotometer and Perkin Elmer lambda 35 with PTP 6 (Peltier Temperature Programmer) and Julabo AWC 100 recirculating cooler in the range of 200–800 nm in both distilled water and seawater at 25 °C. For the measurements over 21 days, the samples were kept at the same conditions as algae cultures (kept at 21°± 0.5 °C by means of a water bath, stirred with 300 rpm and irradiated with a 16:8 h light : dark cycle).

*Electrospray ionization mass spectroscopy*

Electrospray ionization mass spectra were measured with a Bruker maXis ESI-QqTOF spectrometer in the positive and negative mode using acetonitrile/methanol with 1% $H_2O$ as solvent.

*ATR IR spectroscopy*

ATR-IR spectra were measured using a Bruker Vertex 70 Fourier transform IR spectrometer.

*Cyclic voltammetry*

Cyclic voltammograms of the ligands **6b**, **6c** and catechol in aqueous solution in the absence and in the presence of iron(III) were determined at 25.0 ± 0.1 °C over the pH range between 2 and 11.5. The solutions contained 2 mM ligand while the metal-to-ligand ratio was usually 1:2. Ionic strength was 0.10 M (KCl). Measurements were performed on a conventional three-electrode system under argon atmosphere and a PC controlled Autolab-PGSTAT 204 potentiostat. Samples were purged for 15 min with argon before recording the cyclic voltammograms. A platinum electrode was used as working electrode, a platinum electrode as the auxiliary electrode and Ag/AgCl/KCl (3 M) as reference electrode. Electrochemical potentials were converted into the normal hydrogen electrode (NHE) scale by adding 0.21 V.[6] The electrochemical system was calibrated with a solution of $K_3[Fe(CN)_6]$ ($E_{1/2}$ = +0.23 ± 0.01 V *vs.* Ag/AgCl/KCl (3 M) in our setup). Redox potentials were obtained at 100 mV/s scan rate in the range of –0.8 to +1.0 V.

Cyclic voltammograms in dimethylformamide (DMF) were measured in a three-electrode cell using a 2.0 mm and 3.0 mm diameter glassy carbon working electrode, a platinum auxiliary electrode, and an $Ag|Ag^+$ reference electrode containing 0.1 M $AgNO_3$. Measurements were performed at room temperature using an EG & G PARC 273A potentiostat/galvanostat. Deaeration of solutions was accomplished by passing a stream of argon through the solution for 5 min prior to the measurement and then maintaining a blanket atmosphere of argon over the solution during the measurement. The potentials were measured in DMF containing 0.10 M [*n*-$Bu_4N$][$BF_4$] and 2 $^n$M of substance, using [Fe($\eta^5$-$C_5H_5$)2] ($E_{1/2}$ = +0.6-0.68 V *vs* NHE) as internal standard and are quoted relative to the normal hydrogen electrode NHE.[7]

*Elemental analysis*

Elemental analyses were performed at the Microanalytical Laboratory of the University of Vienna with a Perkin-Elmer 2400 CHN Series II elemental analyzer or a Eurovector EA3000 elemental analyzer and are within 0.4% of the calculated values (except for oxygen).

*EPR spectroscopy*

Powder EPR spectra of the ligands, **6a–c** as well as from the iron complexes, **7b** and **7c**, were recorded in dimethylformamide (7 mg/mL). The acquisition parameters of the Bruker Elexsys-II E500 CW-EPR spectrometer were set as follows: microwave frequency, 9.43 GHz; modulation frequency, 100 kHz; center field, 6000 G; sweep width, 12000 G; sweep time, 335.5 s; modulation amplitude, 20.37 G; microwave power, 15 mW; conversion time, 81.92 ms; resolution, 4096 points; averaged scans, 3. Analyses were performed at 90 ± 1 K using a high sensitivity cavity (SHQE1119). A dimethylformamide spectrum was subtracted from all sample spectra. The rhombicity of the complexes were calculated using the software Visual RHOMBO v 1.0 (2009).[8]

*Determination of the distribution coefficients*

Distribution coefficients (*D*) values of the ligands **6a–c** and catechol were determined by the traditional shake-flask method in *n*-octanol–buffered aqueous solution at pH 2.5 (3.2 mM HCl) and 8.3 (20 mM HEPES buffer) in the presence of 0.10 M KCl at 25.0 ± 0.2 °C as described previously.[9] Two parallel experiments were performed for each sample. The ligands were dissolved in 100 μM *n*-octanol pre-saturated buffered aqueous solution. Then these solutions and *n*-octanol (using 1:1 ratio) were gently mixed with 360° vertical rotation (~20 rpm) for 3 h to avoid emulsion formation, and the mixtures were centrifuged at 5000 rpm for 3 min by a temperature controlled centrifuge (Sanyo) at 25 °C. After

separation, UV−Vis spectra of the compounds in the aqueous phase were compared to those of the original aqueous solutions and *D* values were calculated as follows:

[Absorbance (original solution) / Absorbance (aqueous phase after separation) – 1].

Some measurements were performed for the iron(III) – **6b** system as well at pH 8.3 in buffered aqueous solution (20 mM HEPES) and in seawater.

*Algal growth experiments*

Algal growth experiments were carried out with batch cultures of the unicellular chlorophyte species *Chlorella salina,* strain SAG 8.86 and *Prymnesium parvum,* strain SAG 127.79 obtained from the Culture Collection of Algae at Goettingen University. These algae species were chosen because of their widespread occurrence and abundance in the Northern Atlantic Ocean. Experiments were performed in modified sterile *f/2* medium[10], containing EDTA as complexing agent (control samples), prepared with 35‰ salinity artificial seawater as described by Kester[11] at pH 8.2. Cultures were grown in 200 mL Schott flasks kept at 21± 0.5 °C by means of a water bath, stirred with 300 rpm and supplied with filtered air. Plant grow fluorescent lamps with a 16:8 h light : dark cycle were used to provide algae with light at mean intensities, directly measured at the flask surface, of 165 µmol $m^{-2}$ $s^{-1}$. All cultures were carried out in triplicates; for each approach three different control samples were prepared: full *f/2* medium (+ Fe, + EDTA), *f/2* medium without iron (- Fe, + EDTA) and *f/2* medium without EDTA (+ Fe, - EDTA) (see Table S5). As a negative control for our studies, we utilized iron-free samples where we used extra pure sodium chloride for the seawater preparation to avoid any iron contamination. In order to test lignols, the respective iron concentration was added in form of the respective complex or $FeCl_3$ (when only ligands were used) into the *f/2* medium no additional EDTA was used. All the nutrient stock solutions were sterilized by passing through a 0.2 µm capsule filter (Sartorius Sartobran 300). Algae were precultured in full medium, at the beginning of the experiment an inoculum of 2-5 mL was used to obtain an initial concentration of app. $9\times10^4$-$1.5\times10^5$ cells $mL^{-1}$. The concentrations of tested substances were 11.7 µmol $L^{-1}$ and 23.4 µmol $L^{-1}$ depending on experiment. The experiments were carried out over a period of 20 to 30 days (depending on algal growth) and the algae concentration was monitored daily (starting point: day 7 the experiment). The number of cells in the culture was estimated with a Neubauer improved cell counting chamber with a 0.1 mm depth and microscope. Because of the mobility of *P. parvum*, in order to count the cells, 1 mL of each sample was collected and algae were fixed adding 10 µL of 10% formic acid solution. *C. salina* cells were counted without any treatment.

## Synthesis of lignin models

***Tert*-butyl 2-(2-methoxyphenoxy)acetate** (**3**) *Tert*-Butyl chloroacetate (5.57 g, 37 mmol), guaiacol (5 g, 40 mmol), $K_2CO_3$ (10.92 g, 79 mmol) and KI (3.32 g,20 mmol) were stirred in acetone (70 mL) at room temperature for 170 h. The suspension was filtered and the separated solids were washed thoroughly with acetone. The combined filtrates were concentrated in *vacuo*. The residue was dissolved in diethyl ether and washed with brine, 10 % KOH solution (3 x 25 mL) and again with brine containing diluted HCl and then the aqueous layer was neutralized. The organic layer was dried over anhydrous $Na_2SO_4$, filtered and the solvent was evaporated to dryness. The obtained colorless crystalline solid was dried in *vacuo*. Yield: 7.81 g, 33 mmol, 89 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 7.01-6.82 (m, 4 H, $H_{Ar}$), 4.62 (s, 2H, $CH_2$), 3.78 (s, 3 H, $CH_3$), 1.43 (s, 9 H, $CH_3$) ppm.

**3,4-Bis(benzyloxy)benzaldehyde** (**2b**) To a stirred solution of 3,4-dihydroxybenzaldehyde (2.5 g, 18 mmol) in acetone (100 mL), was added potassium carbonate (7.45 g, 39.6 mmol) followed by benzyl bromide (4.7 mL, 39.6 mmol) and refluxed for 12 h. The mixture was filtered, washed with acetone (30 mL), the filtrate was concentrated and dried in *vacuo*. In order to remove the excess of benzyl bromide the crude oil was suspended in petrol ether (30 mL) and sonificated for approximately 5 min. The solvent was decanted (5x) and finally stored for 2 h at 4°C until complete crystallization of the product. The solid was filtered off, washed with petrol ether and dried in *vacuo*. Yield: 5.61 g, 17.6 mmol, 98 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 9.83 (s, 1H, $H_{ald}$), 7.59-7.26 (m, 13 H, $H_{ar}$), 5.29 (s, 2H, $CH_{2bn}$), 5.23 (s, 2H, $CH_{2bn}$) ppm.

**2,3-Bis(benzyloxy)benzaldehyde** (**2c**)The product was prepared following the same procedure as for compound 2b yielding 2,3-bis(benzyloxy)benzaldehyde as a white solid. Yield: 5.61 g, 17.6 mmol, 98 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 10.16 (s, 1H, $H_{ald}$), 7.63-7.03 (m, 13 H, $H_{ar}$), 5.27 (s, 2H, $CH_{2bn}$), 5.18 (s, 2H, $CH_{2bn}$) ppm.

**Tert-butyl 3-(3,4-bis(benzyloxy)phenyl)-3-hydroxy-2-(2-methoxyphenoxy)propanoate** (**4b**) In a dry and argon flushed three-necked flask with an addition funnel, argon inlet and low temperature thermometer, diisopropylamine (0.8 mL, 5.5 mmol) was dissolved in THF (15 mL) and cooled to 0 °C. Then *n*-BuLi (3.7mL, 1.6 M in hexane, 5.8 mmol) was added dropwise within 15 min and the reaction mixture was stirred for 30 min at 0 °C. Then, after cooling to –80 °C, *tert*-butyl 2-(2-methoxyphenoxy)acetate (**3**) (1.19 g, 5 mmol) in THF (15 mL) was added within 80 min, followed by 3,4-

bis(benzyloxy)benzaldehyde (**2b**) (1.66 g, 5 mmol) in THF (15mL) within 30 min. After stirring the reaction mixture for 2 h, water (25 mL) was added. The water phase was extracted with EtOAc (4x30 mL) and the combined organic layers were washed with 1 M HCl, water and brine and dried over $Na_2SO_4$. Then the solvent was removed under *vacuo* to give the crude product as yellow oil. The crude product was purificated *via* column liquid chromatography (hexane/ethyl acetate 5/1 → 0/1) to give the *erythro* diastereoisomer and *erythro/threo* diastereoisomer mixture as colorless oils. Yield: 0.560 g of *erythro* (1 mmol, 20 %) and 1.81 g of *erythro/threo* mixture (3.22 mmol, 66 %). *Erythro*: $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 7.50–7.26 (m, 11 H, $H_{ar}$), 7.03–6.67 (m, 6 H, $H_{ar}$), 5.78 (s, 1H, OH), 5.12 (s, 4H, $CH_{2bn}$), 4.84 (d, *J* = 7 Hz, 1H, $H_{al}$), 4.50 (d, *J* = 7 Hz, 1H, $H_{al}$), 3.70 (s, 3H, $CH_3$), 1.34 (s, 9H, $CH_{3tert}$) ppm. *Threo*: $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 7.50–7.26 (m, 11 H, $H_{ar}$), 7.03-6.67 (m, 6 H, $H_{ar}$), 5.72 (s, 1H, OH), 5.14 (s, 4H, $CH_{2bn}$), 4.85 (d, *J* = 6 Hz, 1H, $H_{al}$), 4.61 (d, *J* = 6 Hz, 1H, $H_{al}$), 3.75 (s, 3H, $CH_3$), 1.15 (s, 9H, $H_{3tert}$) ppm.

**Tert-butyl 3-(2,3-bis(benzyloxy)phenyl)-3-hydroxy-2-(2-methoxyphenoxy)propanoate** (**4c**) The product was prepared following the same procedure as for compound **4b** yielding *erythro* diastereisomer and *erythro/threo* diastereoisomer mixture as colorless oils. Yield: 0.199 g of *erythro* (0.36 mmol, 7 %) and 1.91 g of *erythro/threo* mixture (3.43 mmol, 69 %). *Erythro*: $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 7.55–6.71 (m, 17 H, $H_{ar,}$ OH), 5.80 (d, *J* = 5.5 Hz, 1H, $H_{al}$), 5.43 (t, *J* = 6.5 Hz, 1H, $CH_{al}$), 5.19 (s, 2H, $CH_{2bn}$), 5.14 (d, *J* = 10.5 Hz, 1H, $CH_{2bn}$), 5.02 (d, *J* = 10.5 Hz, 1H, $CH_{2bn}$), 4.71 (d, *J* = 6.5 Hz, 1H, $H_{al}$), 3.60 (s, 3H, $CH_3$), 1.31 (s, 9H, $CH_{3tert}$) ppm. *Threo*: $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 7.55-6.59 (m, 18 H, OH, $H_{ar}$), 5.71 (d, *J* = 6 Hz, 1H, $H_{ar}$), 5.41 (dd, *J* = 7 Hz *J* = 6 Hz, 1H, $H_{al}$), 5.19 (s, 2H, $CH_{2bn}$), 5.16 (d, *J* = 12.5 Hz 1H, $CH_{2bn}$), 4.99 (d, *J* = 12.5 Hz, 1H, $CH_{2bn}$), 4.70 (d, *J* = 7 Hz, 1H, $H_{al}$), 3.68 (s, 3H, $CH_3$), 1.26 (s, 9H, $H_{3tert}$) ppm.

**1-(3,4-Bis(benzyloxy)phenyl)-2-(2-methoxyphenoxy)propane-1,3-diol** (**5b**) In dry and argon flushed three-necked flask with a dropping funnel and condenser, $LiAlH_4$ (0.1 g, 2.5 mmol) was dissolved in THF (11 mL) and cooled to 0 °C. Then **4b** (0.560 g, 1 mmol) dissolved in THF (11 mL), was added *via* dropping funnel over 30 min. After the addition, the reaction mixture was stirred at 60 °C for 3 h, followed by cooling down to 0 °C. In Order to quench the reaction, water (0.6 mL), then aqueous NaOH (0.6 mL) and again water (1.7 mL) were added dropwise. The mixture was stirred for another 30 min at room temperature and then filtered through a pad of celite. The filtered aluminium salts were washed with $CH_2Cl_2$ (4x15 mL) and the filtrate was washed with brine and dried over $Na_2SO_4$. The solvent was

removed in *vacuo* to give the product as colorless oil. Yield: 0.465 g, 0.98 mmol, 98 %. *Erythro*: $^{1}$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 7.49–7.29 (m, 11 H, $H_{ar}$), 7.19 (s, 1 H, OH), 7.01–6.80 (m, 7 H, $H_{ar}$, OH), 5.41 (d, *J* = 5 Hz, 1 H, $H_{al}$), 5.90 (s, 2 H, $CH_{2bn}$), 5.07 (s, 2 H, $CH_{2bn}$), 4.76 (t, *J* = 5 Hz, 1 H, $CH_2$), 4.62 (t, *J* = 5 Hz, 1 H, $CH_2$), 4.31 (m, 1 H, $H_{al}$), 3.70 (s, 3H, $CH_3$) ppm.

**1-(2,3-Bis(benzyloxy)phenyl)-2-(2-methoxyphenoxy)propane-1,3-diol** (**5c**) The product was prepared following the same procedure as for compound **5b** yielding *erythro* diastereisomer as a colorless oil. Yield: 0.164 g, 0.34 mmol, 94 %. *Erythro*: $^{1}$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 7.52–7.01 (m, 14 H, $H_{ar}$), 6.89 (m, 1 H, $H_{ar}$), 6.81 (m, 1 H, $H_{ar}$), 6.53 (m, 1 H, $H_{ar}$), 5.37 (d, *J* = 5 Hz, 1 H, $H_{al}$), 5.28 (t, *J* = 5 Hz, 1 H, $CH_2$), 5.17 (s, 2 H, $CH_{2bn}$), 5.09 (d, *J* = 11 Hz, 1 H, $CH_{2bn}$), 4.93 (d, *J* = 11 Hz, 1 H, $CH_{2bn}$), 4.49 (t, *J* = 5 Hz, 1 H, $CH_2$), 4.41 (m, *J* = 5 Hz, 1 H, $H_{al}$), 3.71 (m, 1 H, $H_{al}$), 3.65 (s, 3H, $CH_3$) ppm.

**4-(1,3-Dihydroxy-2-(2-methoxyphenoxy)propyl)benzene-1,2-diol** (**6b**) A catalytic amount of Pd/C (20 mg, 10 wt. % loading) was added to a solution of **5b** (465 mg, 0.98 mmol) in methanol (dried over mol sieves, 25 mL) under an argon atmosphere. The suspension was put stirred for 16 h under an $H_2$ atmosphere. The solution was filtered off, concentrated and dried in vacuo yielding the product as yellow oil. Yield: 0.258 g, 0.84 mmol, 86 %. *Erythro*: $^{1}$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 8.76 (s, 2 H, OH), 7.01–6.61 (m, 7 H, $H_{ar}$), 5.25 (s, 1 H, OH), 4.72 (d, *J* = 5 Hz, 1 H, $CH_{al}$), 4.52 (s, 1 H, OH), 4.21 (m, 1 H, $CH_{al}$), 3.73 (s, 3 H, $CH_3$), 3.65 (dd, *J* = 12 Hz, *J* = 6 Hz, 1 H, $CH_2$), 3.58 (dd, *J* = 12 Hz, *J* = 4 Hz, 1 H, $CH_2$) ppm. $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): δ = 150.5, 148.6, 145.1, 144.6, 133.9, 121.7, 121.2, 118.1, 117, 115.2, 114.9, 113.3, 84.9, 71.9, 60.4, 56.1 ppm. ESI-MS: *m*/*z* 329 $[M+Na]^+$, 305 $[M-H]^-$; Anal. Calcd for $C_{16}H_{18}O_6 \cdot 0.25\ H_2O$: C, 61.83; H, 6.00; O, 32.17. Found: C, 61.80; H, 6.10; O, 32.75.

**3-(1,3-Dihydroxy-2-(2-methoxyphenoxy)propyl)benzene-1,2-diol** (**6c**) The product was prepared following the same procedure as for the compound **6b** yielding *erythro* diastereisomer as a colorless oil. Yield: 0.101 g, 0.33 mmol, 98 %. *Erythro*: $^{1}$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): δ = 8.58 (s, 1 H, OH), 7.38 (dd, *J* = 8 Hz, *J* = 2 Hz, 1 H, $H_{ar}$), 6.68–6.60 (m, 6 H, $H_{ar}$), 5.26 (s, 1 H, OH), 5.20 (d, *J* = 3 Hz, 1 H, $CH_{al}$), 4.45 (m, 1 H, $CH_{al}$), 4.43 (s, 1 H, OH), 3.77 (s, 3 H, $CH_3$), 3.71 (dd, *J* = 12 Hz, *J* = 7 Hz, 1 H, $CH_2$), 3.54 (dd, *J* = 12 Hz, *J* = 2 Hz, 1 H, $CH_2$) ppm. $^{13}$C NMR (DMSO-$d_6$, 75 MHz, 25 °C): δ = 150.2, 148.7, 145.1, 142.5, 129.4, 121.2 (2C), 119.1, 118.3, 115.9, 114.2, 113.2, 82.7, 67.7, 60.1, 56.1 ppm. ESI-MS: *m*/*z* 329 $[M+Na]^+$, 305 $[M-H]^-$; Anal. Calcd for $C_{16}H_{18}O_6 \cdot 0.25\ H_2O$: C, 61.83; H, 5.99; O, 32.17. Found: C, 61.50; H, 5.93; O, 31.10.

**[$Fe_2$(4-(1,3-Dihydroxy-2-(2-methoxyphenoxy)propyl)benzene-1,2-diolato)$_4$($H_2O$)$_2$]** (**7b**) $FeCl_3$ (53 mg, 0.33 mmol) dissolved in methanol (5 mL) was added to the solution of **6b** (300 mg, 0.96 mmol) and KOH (110 mg, 1.96 mmol) in methanol (10 mL). The dark reddish solution was stirred for 3 h and the formed dark violet precipitate was filtered and suspended in distilled water (3 mL). The suspension was sonicated for 15 min, filtered off, washed with distilled water and the product was dried *in vacuo*. Yield: 30 mg, 0.02 mmol, 7%. ESI-MS: *m/z* 664.22 [Fe+(ligand)$_2$]$^-$; Anal. Calcd for $Fe_2C_{64}H_{70}O_{26}\cdot(H_2O)$: C, 55.50; H, 5.24. Found: C, 55.25; H, 5.60. IR (ATR, selected bands, $\nu_{max}$): 2926, 1592, 1498, 1456, 1252, 1214, 1118, 1115, 1022, 811, 745, 620 cm$^{-1}$.

**[$Fe_2$(3-(1,3-Dihydroxy-2-(2-methoxyphenoxy)propyl)benzene-1,2-diolato)$_4$($H_2O$)$_2$]** (**7c**) $FeCl_3$ (25 mg, 0.15 mmol) dissolved in methanol (2 mL) was added to the solution of **6c** (95.5 mg, 0.31 mmol) and KOH (17.4 mg, 0.31 mmol) in methanol (5 mL). The dark reddish solution was stirred for 3 h and the formed dark violet precipitate was filtered and suspended in distilled water (3 mL). The suspension was sonicated for 15 min, the solid was filtered off, washed with water and acetone. The obtained product was dried *in vacuo*. Yield: 60 mg, 0.04 mmol, 27%. ESI-MS: *m/z* 454 [M+Na]$^+$; Anal. Calcd for $Fe_2C_{64}H_{70}O_{26}\cdot(CH_3COCH_3)_{0.5}$: C, 56.36; H, 5.27.Found: C, 56.76; H, 5.35. IR (ATR, selected bands, $\nu_{max}$): 3358, 1593, 1499, 1456, 1252, 1217, 1119, 1023, 868, 738 cm$^{-1}$.

## Cyclic voltammograms and electrochemical data

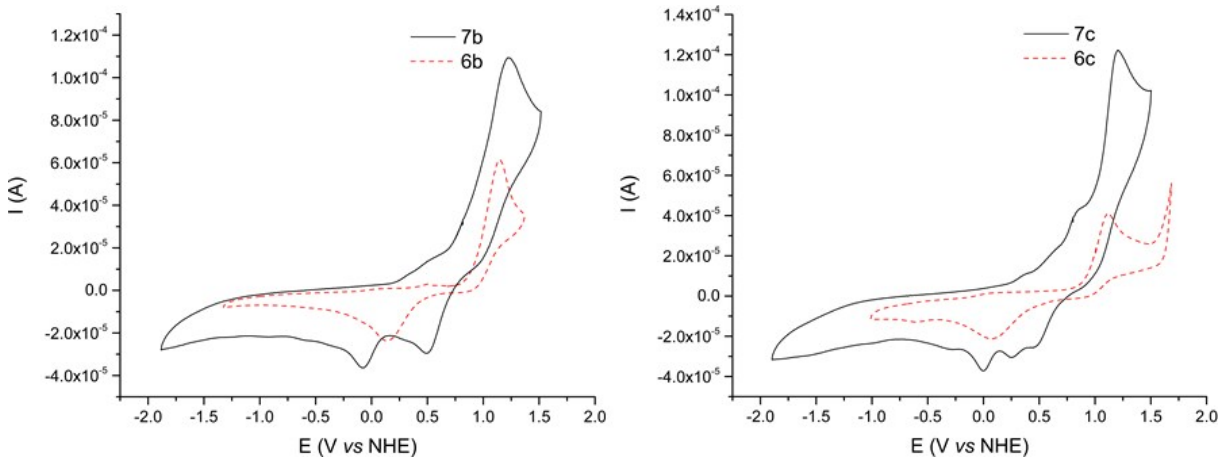


**Figure S1.** Cyclic voltammograms of Fe(III) complexes **7b** and **7c** at 0.20 V s$^{-1}$ in DMF showed at selected potential regions.

**Table S1.** Electrochemical data (anodic ($E_a$), cathodic ($E_c$) peak potentials and their differences ($\Delta E$), half-wave potentials ($E_{1/2}$)) obtained by cyclic voltammetry for ligands **6b**, **6c** and catechol (cat) in the absence and the presence of iron(III) in aqueous solution at various pH values. *(T = 25 °C; I = 0.1 M (KCl); scan rate = 100 mV/s)*

| system | pH | $E_a$ (V) | $E_c$ (V) | $\Delta E$ (V) | $E_{1/2}$(V) |
|---|---|---|---|---|---|
| **6b** | 3.70 | +0.02 | –0.03 | 0.05 | 0.00 |
| | | +0.98 | +0.65 | 0.33 | +0.82 |
| | 6.45 | +0.99 | +0.63 | 0.36 | +0.81 |
| | 8.50 | +0.65 | +1.00 | 0.35 | +0.83 |
| | 8.85 | +1.00 | | | |
| | | +0.50 | | | |
| | 8.16 | +1.01 | | | |
| | | +0.46 | | | |
| | 9.89 | +0.45 | | | |
| | 11.00 | +0.41 | +0.27 | | |
| | 12.19 | | +0.01 | | |
| **iron(III) – 6b** | 1.80 | +0.63 | +0.39 | 0.24 | +0.51 |
| **(1:2)** | 4.96 | +0.76 | +0.42 | 0.34 | +0.59 |
| | 6.24 | +0.71 | | | |
| | 7.94 | +0.65 | | | |
| | 9.03 | +0.62 | –0.14 | | |
| | 10.03 | +0.43 | –0.19 | | |
| | 11.01 | | –0.08 | | |
| **iron(III)** | 2.70 | –0.10 | –0.21 | 0.11 | –0.15 |
| **6c** | 3.48 | –0.14 | –0.20 | 0.06 | –0.17 |
| | | +0.83 | +0.45 | 0.38 | +0.64 |
| | 4.86 | +0.82 | +0.41 | 0.41 | +0.62 |
| | 6.40 | +0.81 | +0.41 | 0.40 | +0.61 |
| | 6.99 | +0.83 | +0.41 | 0.42 | +0.62 |
| | 8.11 | +0.85 | +0.40 | 0.45 | +0.63 |
| | 9.09 | +0.88 | | | |
| | | +0.28 | | | |
| | 10.06 | +0.28 | | | |
| | 11.01 | +0.20 | +0.06 | 0.14 | +0.13 |
| **iron(III) – 6c** | 4.82 | | +0.32 | | |
| **(1:2)** | 5.76 | +0.69 | +0.16 | | |
| | 6.62 | +0.66 | +0.13 | | |
| | 8.36 | +0.58 | –0.15 | | |
| | 9.40 | | –0.17 | | |
| | 9.80 | | –0.18 | | |
| | 10.29 | | –0.05 | | |
| **cat** | 5.54 | +0.84 | +0.47 | 0.37 | +0.66 |
| | 8.00 | +0.79 | +0.47 | 0.32 | +0.63 |
| | | | +0.25 | | |
| **iron(III)-cat (1:2)** | 8.08 | +0.64 | +0.42 | 0.22 | +0.53 |
| **iron(III)-cat (1:3)** | 8.11 | +0.63 | +0.40 | 0.23 | +0.52 |
| | | | +0.11 | | |

## Proton dissociation and complex formation processes

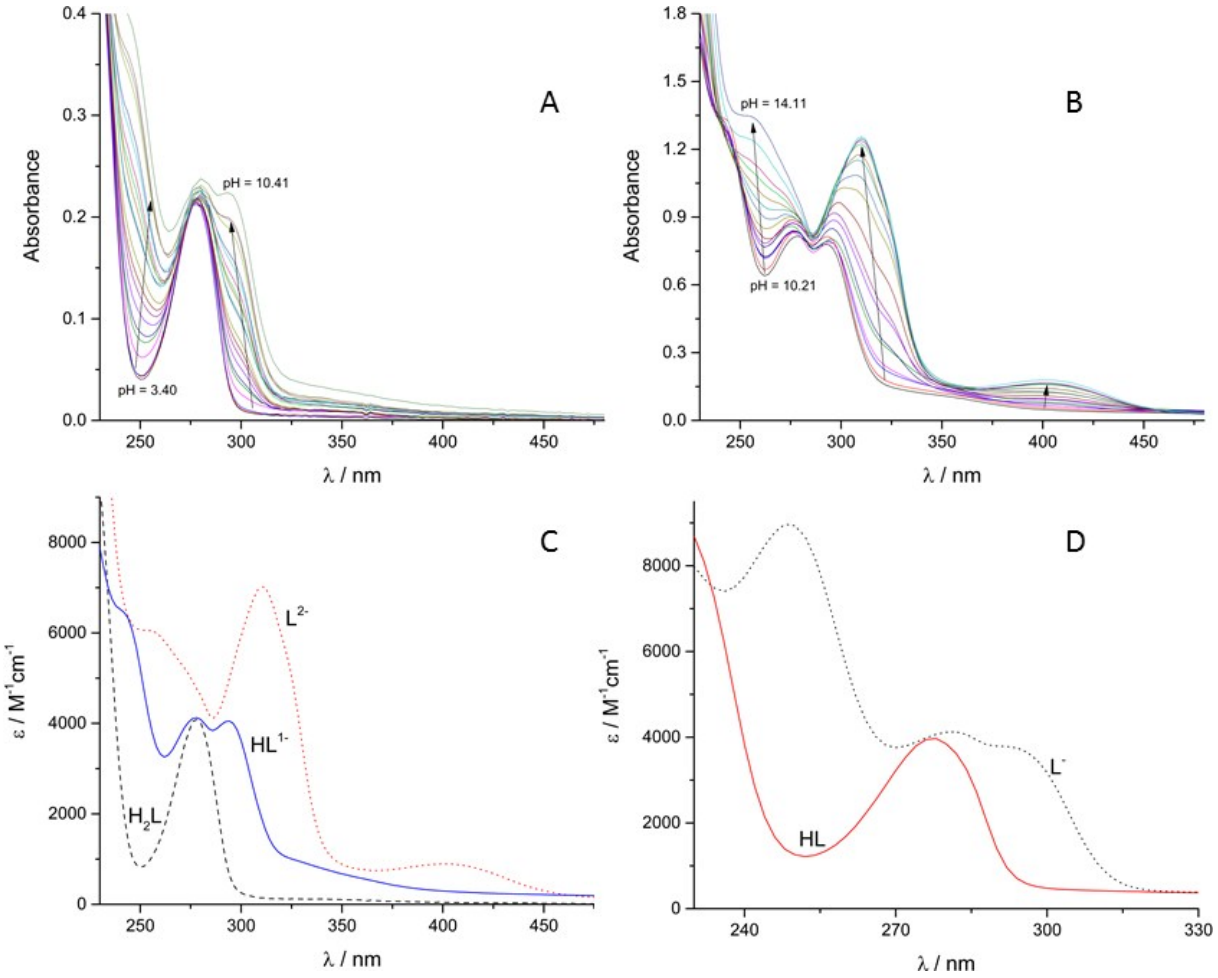


**Figure. S2.** UV–Vis absorbance spectra recorded for ligand **6b** in the pH range from 2 to 10.4 ($c_L$ = *0.05 mM*) (A) and from 10.2 to 14.1 ($c_L$ = *0.200 mM*) (B). Calculated molar absorbance spectra of the individual ligand species of **6b** (C) and **6a** (D). (*I = 0.10 M (KCl); T = 25 ˚C*)

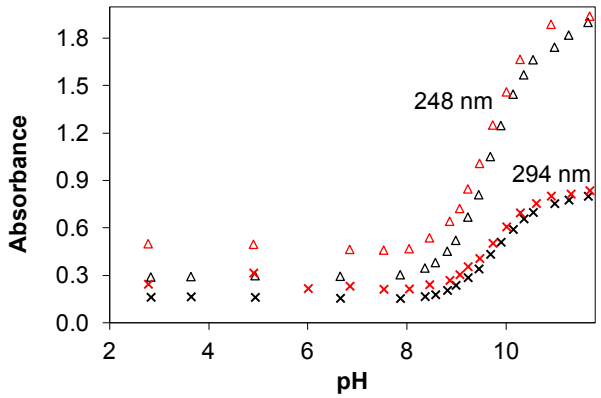

**Figure S3.** Absorbance values obtained at 248 and 294 nm for ligand **6a** (black symbols) and for the iron(III) – **6c** (1:2) (red symbols) system. ($c_L$ = *201 μM; I = 0.10 M (KCl); T = 25 ˚C*)

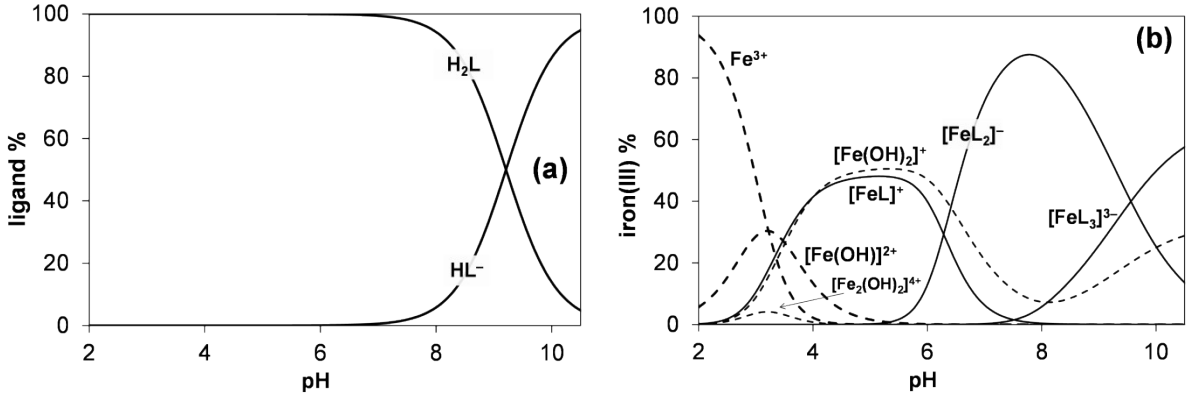


**Figure S4.** Concentration distribution curves for **6b** (a) and the iron(III) – **6b** system (1:2) (b) calculated with the determined equilibrium constants (see data for **6b** in Table 1). ($c_L$ = *2.0 mM; Fe(III):L = 1:2; I = 0.10 M (KCl); T = 25 ˚C*)

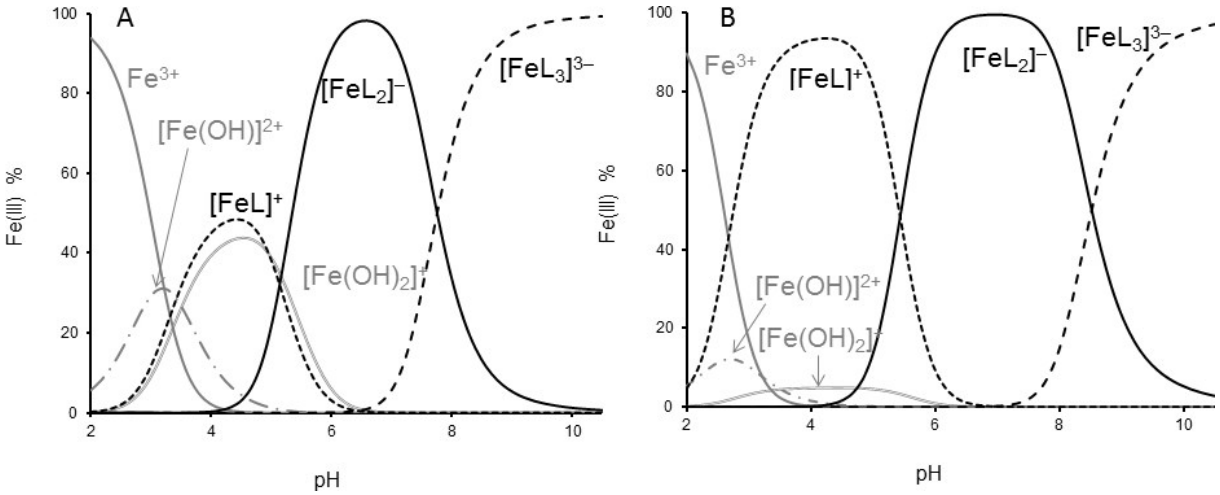


**Figure. S5.** Concentration distribution curves for the iron(III) – **6c** (A) and iron(III) – catechol (B) systems calculated with the determined equilibrium constants (see data for **6c** in Table 1) and references data of catechol.[12] ($c_L$ = *1.0 mM; Fe(III):L = 1:3; I = 0.10 M (KCl); T = 25 ˚C*)

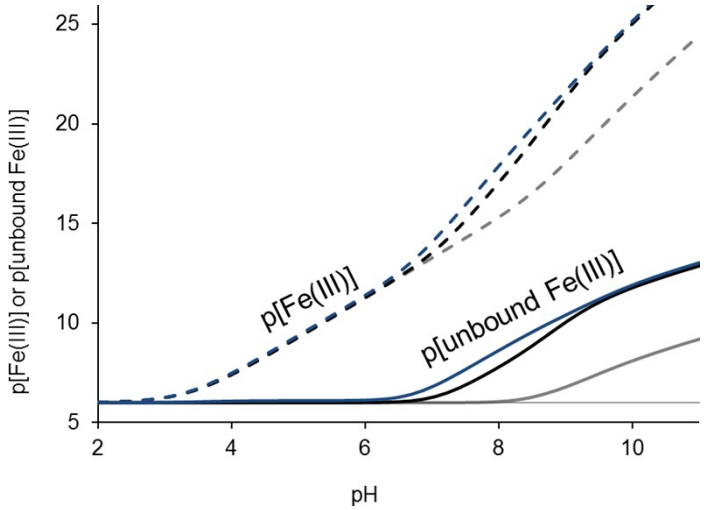


**Figure S6.** Negative logarithm of the equilibrium concentration of iron(III) (p[Fe(III)], dashed lines) and the unbound iron(III) fraction (p[unbound Fe(III) ], solid lines) plotted against the pH for the iron(III) – **6c** (black), iron(III) – **6b** (grey), and iron(III) – catechol (blue) systems calculated with the determined equilibrium constants (see data for **6c**, **6b** in Table 1) and references data of catechol.[12] ($c_{Fe(III)}$ *= 1.0 μM; Fe(III):L = 1:10; I = 0.10 M (KCl); T = 25 ˚C*)

## Liphopilicity

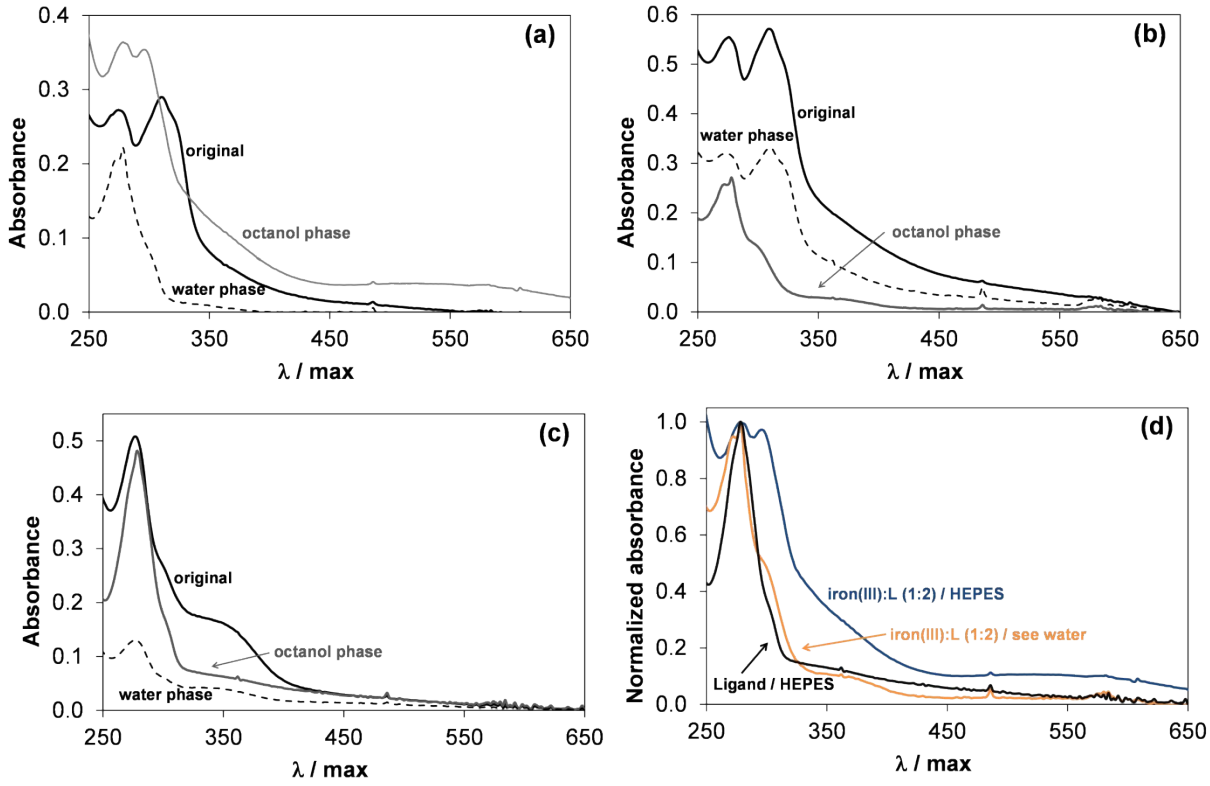


**Figure S7.** UV–Vis absorbance spectra of iron(III) – **6b** (1:2) containing samples recorded for the original solution, in the aqueous and *n*-octanol phases following the separation at pH 8.3 (20 mM HEPES) (a), or using see water (pH 8.3) (b). Spectra obtained for **6b** for comparison at pH 8.3 (20 mM HEPES) (c). Normalized absorbance spectra recorded for the *n*-octanol phases in the case of **6b**, and iron(III) – **6b** (1:2) system (d). (*$c_L$ = 100 μM; Fe(III):L = 1:2; I = 0.10 M (KCl) or I ~0.7 M for the see water; T = 25 ˚C*)

## Time dependent UV–Vis spectra in seawater

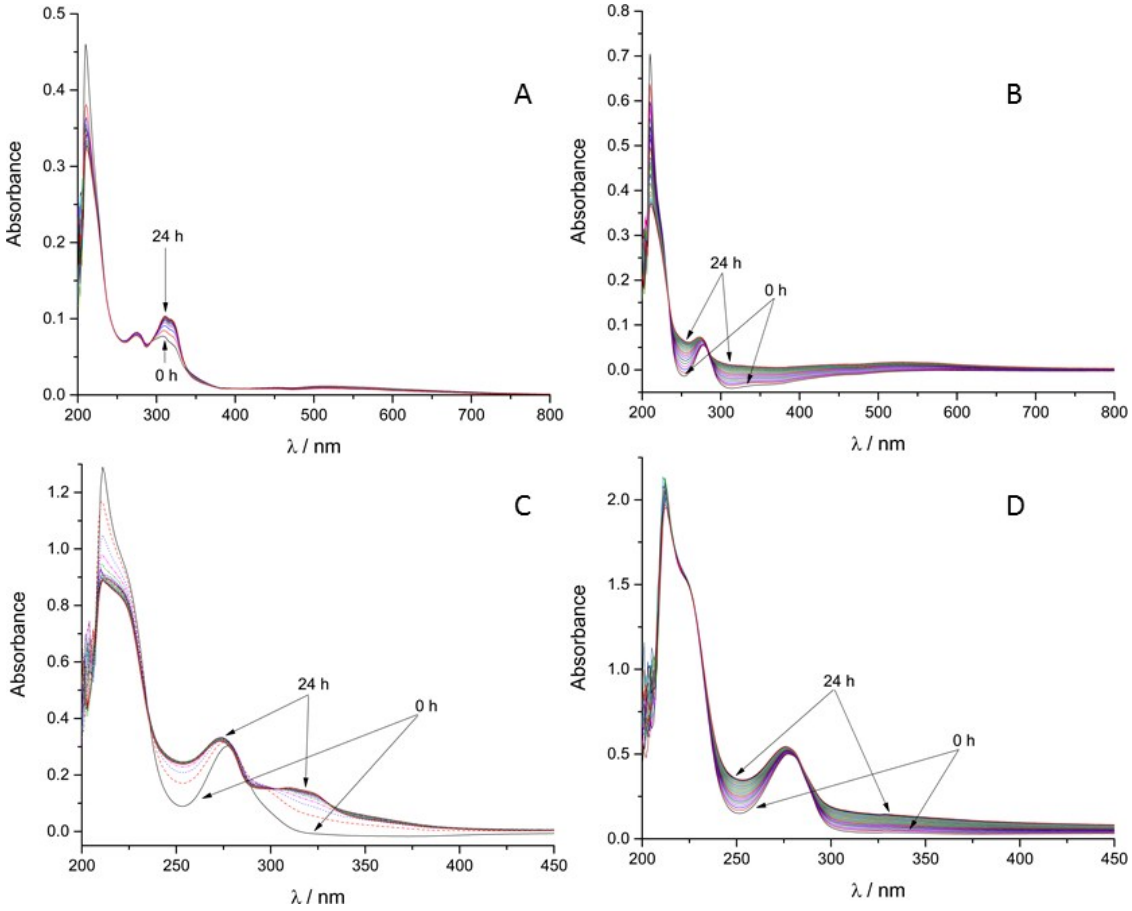


**Figure S8.** Time dependent UV–Vis spectra of **7b,c** (A,B) and **6b,c** (C,D) seawater over 24 hours showing the region where the changes occurred (spectra were measured in an 1 h interval, start and end point of the measurement are indicated, *T = 25 ˚C*).

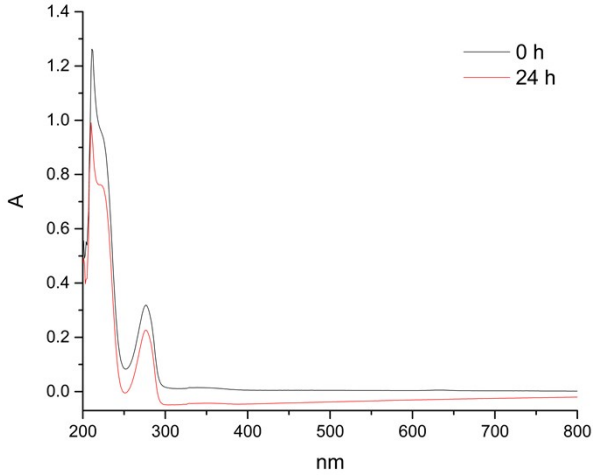


**Figure S9.** Time dependent UV–Vis spectra of **6a** in seawater.

## Time dependent UV–Vis spectra in distilled water

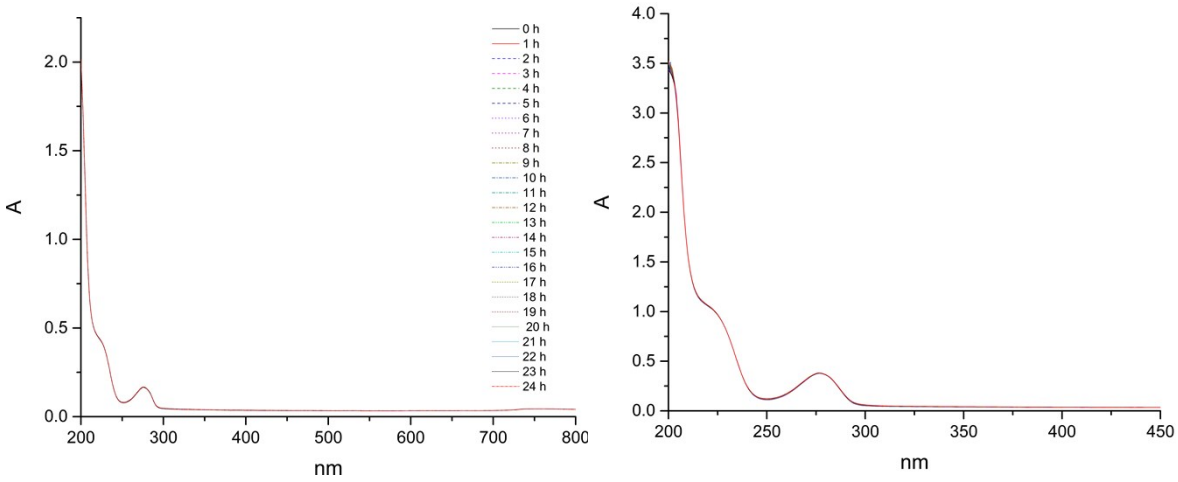


**Figure S10.** Time dependent UV–Vis spectra of **6a** (left) and **6b** (right) in distilled water.

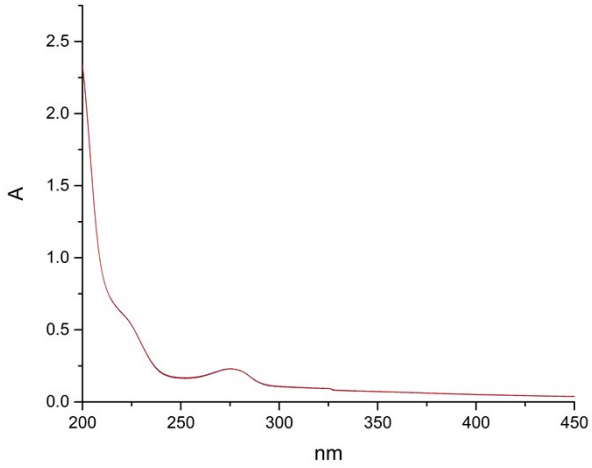


**Figure S11.** Time dependent UV–Vis spectra of **6c** in distilled water.

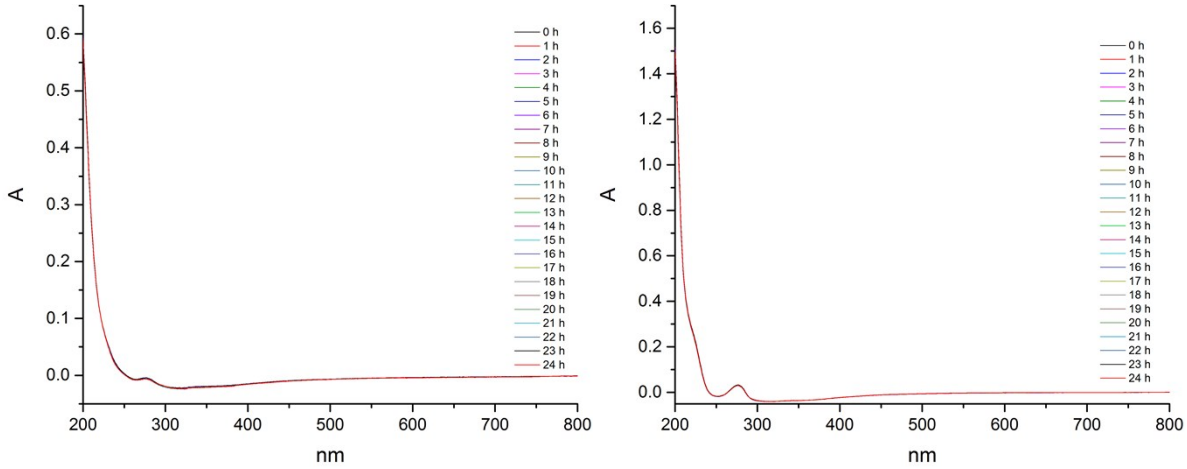


**Figure S12.** Time dependent UV–Vis spectra of **7b** (left) and **7c** (right) in distilled water.

## Time dependent UV–Vis spectra in distilled water at pH 8

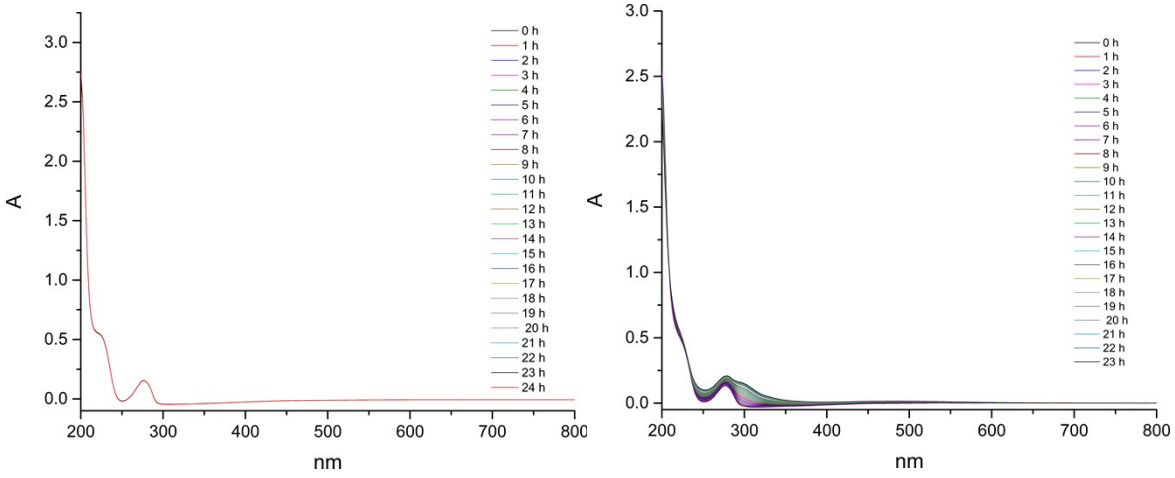


**Figure S13.** Time dependent UV–Vis spectra of **6a** (left) and **6b** (right) in distilled water at pH 8

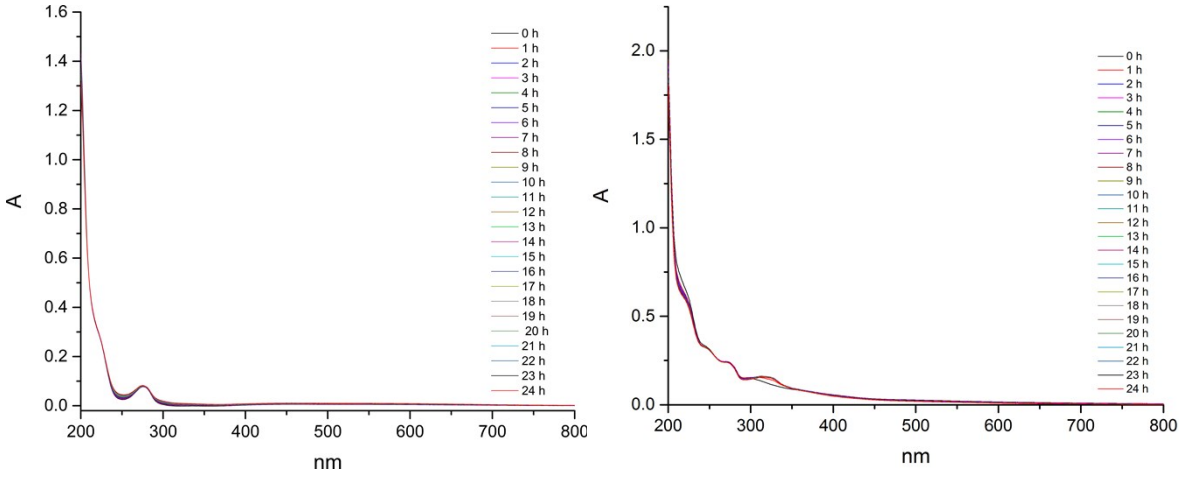


**Figure S14.** Time dependent UV–Vis spectra of **6c** (left) and **7b** (right) in distilled water at pH 8.

## Time dependent UV–Vis spectra in seawater over 21 days

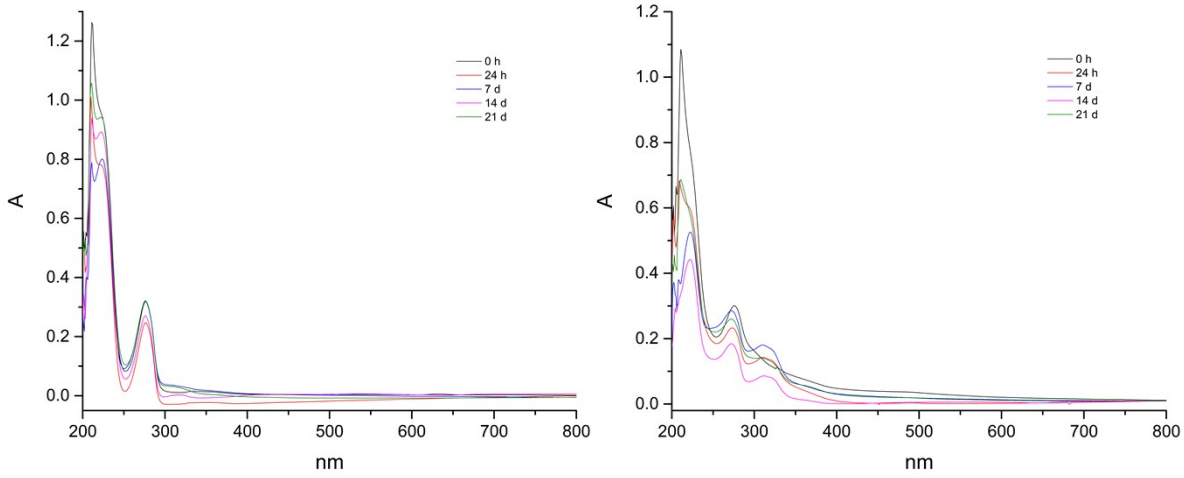


**Figure S15.** Time dependent UV–Vis spectra of **6a** (left) and **6b** (right) in seawater over 21 days.

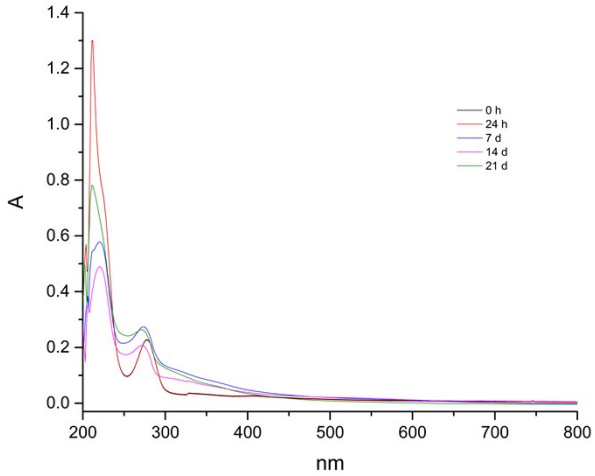


**Figure S16.** Time dependent UV–Vis spectra of **6c** in seawater over 21 days.

## Algal studies

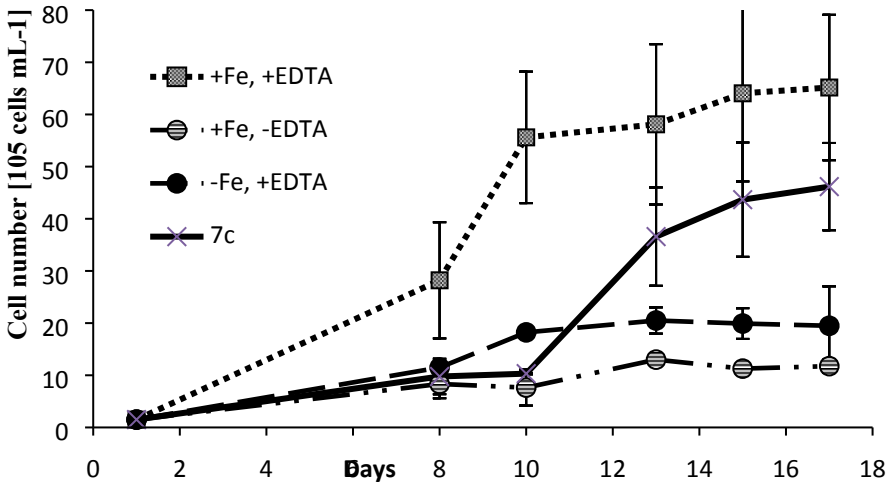


**Figure S17.** Growth curves of *C. salina* (error bars: ± SD) treated with model compound **7c** compared to control samples (+Fe, +EDTA; +Fe, -EDTA; -Fe, +EDTA).

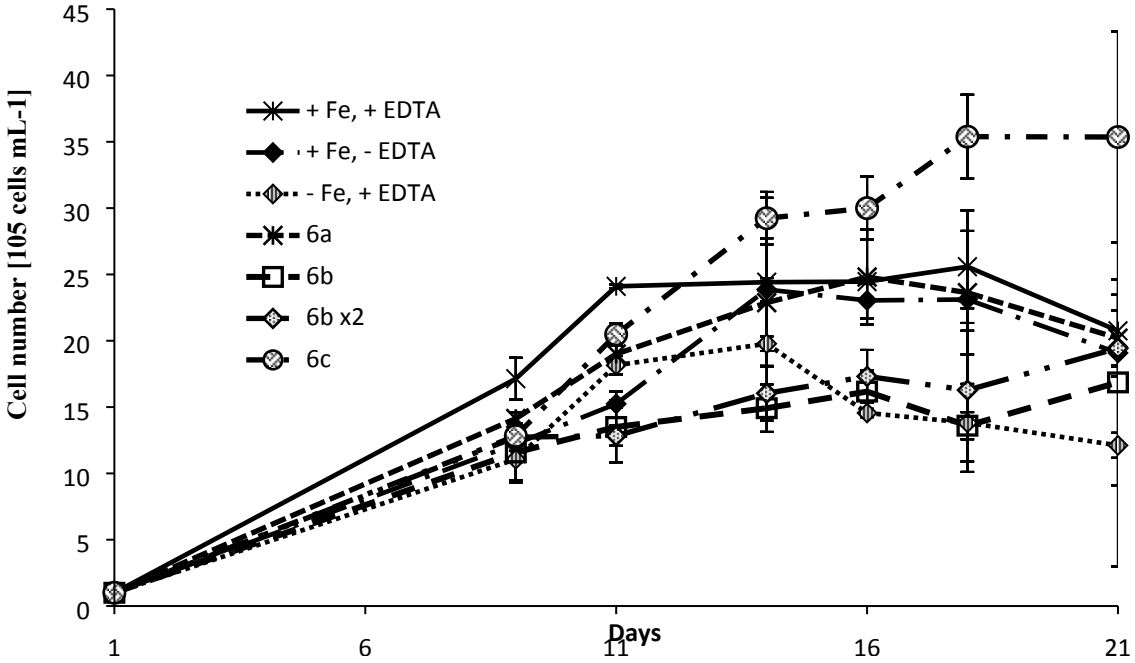


**Figure S18.** Growth curves of *P. parvum* treated with model compounds **6a**, **6b**, **6bx2** and **6c** compared to control samples (+Fe, +EDTA; +Fe, -EDTA; -Fe, +EDTA).

**Table S2.** Algae end-concentration and relation between the control samples to samples treated with **6a**, **6b**, **6c**, **6c x2**, **7b,** and **7c**.

| | **6a** | **6b** | **6c** | **6c x2** | **7b** | **7c** |
|---|---|---|---|---|---|---|
| Algae concentration (cells $mL^{-1}$) | $8.8\times10^5 \pm 2.5\times10^5$ | $7.8\times10^6 \pm 7.8\times10^5$ | $2.7\times10^6 \pm 6.5\times10^5$ | $7.8\times10^6 \pm 2.0\times10^6$ | $7.6\times10^6 \pm 4.1\times10^5$ | $4.6\times10^6 \pm 8\times10^5$ |
| +Fe, +EDTA | 0.15 | 1.4 | 0.5 | 1.4 | 1.3 | 0.7 |
| -Fe, + EDTA | 0.8 | 7.2 | 2.5 | 7.3 | 7 | 2.4 |
| +Fe, - EDTA | 0.3 | 2.6 | 0.9 | 2.6 | 2.5 | 3.9 |

**Table S3.** Specific growth rates of *C. Salina* after 17 days for **6a–c**, **6c** (c = 2× c(EDTA)) and **7b,c**.

| Model compound /control sample | **6a** | **6b** | **6c** | **6c2x** | **7b** | **7c** | **+Fe, +EDTA(1)*** | **+Fe, +EDTA (2)*** | **-Fe, +EDTA (1)*** | **-Fe, +EDTA (2)*** |
|---|---|---|---|---|---|---|---|---|---|---|
| Growth rate of *C. Salina*(after 17 d) | 0.08 | 0.23 | 0.18 | 0.22 | 0.23 | 0.20 | 0.17 | 0.22 | 0.08 | 0.15 |

*1 refers to the first experiment and 2 to the second where **7c** was tested.

**Table S4.** Specific growth rates of *P. Parvum* after 17 days for **6a**, **6b**, **6c** and **6b x2**.

| Model compound /control sample | **6a** | **6b** | **6c** | **6bx2** | **+Fe, +EDTA** | **-Fe, +EDTA** |
|---|---|---|---|---|---|---|
| Growth rate of *P. Parvum* (after 17 d) | 0.19 | 0.16 | 0.20 | 0.17 | 0.19 | 0.15 |

## Composition of enriched seawater medium for algae experiments

**Table S5.** Composition of enriched seawater medium for algae experiments for each sample.

| **Full medium[13]** | **Medium -Fe** | **Medium -EDTA** | **Medium + model compounds** |
|---|---|---|---|
| 200 mL of filtered artificial seawater[11] | 200 mL of filtered artificial seawater | 200 mL of filtered artificial seawater | 200 mL of filtered artificial seawater |
| 0.2 mL of micronutrient solution[10] | 0.2 mL of micronutrient solution | 0.2 mL of micronutrient solution | 0.2 mL of micronutrient solution |
| 0.2 mL of vitamin solution[10] | 0.2 mL of vitamin solution | 0.2 mL of vitamin solution | 0.2 mL of vitamin solution |
| 0.2 mL of 0.88 M $NaNO_3$ | 0.2 mL of 0.88 M $NaNO_3$ | 0.2 mL of 0.88 M $NaNO_3$ | 0.2 mL of 0.88 M $NaNO_3$ |
| 0.2 mL of 0.1 M $Na_2SiO_3*9H_2O$ | 0.2 mL of 0.1 M $Na_2SiO_3*9H_2O$ | 0.2 mL of 0.1 M $Na_2SiO_3*9H_2O$ | 0.2 mL of 0.1 M $Na_2SiO_3*9H_2O$ |
| 0.2 mL of 0.036 M $NaH_2PO_4*H_2O$ | 0.2 mL of 0.036 M $NaH_2PO_4*H_2O$ | 0.2 mL of 0.036 M $NaH_2PO_4*H_2O$ | 0.2 mL of 0.036 M $NaH_2PO_4*H_2O$ |
| - | - | - | 0.007 mM of model compound |

## Chemicals used for the synthesis and preparation of stock solutions and enriched seawater medium

All solvents used for synthesis and characterization of the compounds were of analytical grade and used without further purification. All chemicals used for the synthesis of the compounds as well as for preparation of stock solutions and artificial seawater were purchased from Sigma Aldrich, Alfa, Fluka, Reanal or Acros and used without further purification:

*Tert*-Butyl chloroacetate, Sigma-Aldrich, 186791
Guaiacol, Sigma-Aldrich, G5502
$K_2CO_3$, Merck, 4924
KI, Alfa Aesar, 11601
$Na_2SO_4$, Sigma-Aldrich, 798592
3,4-dihydroxybenzaldehyde, Sigma-Aldrich, D108405
2,3-dihydroxybenzaldehyde, Fisher, 183510050
4-Hydroxy-3-methoxybenzaldehyde, Sigma-Aldrich, V1104
benzyl bromide, Fisher, 105871000
diisopropylamine, Sigma-Aldrich, 471224
*n*-BuLi 1.6 M in hexane, Sigma-Aldrich, 186171
$LiAlH_4$, Fluka, 62420
NaOH, Sigma-Aldrich, 30620
Pd/C (10% Pd basis), Sigma-Aldrich, 75990ferrocene
KOH, Sigma-Aldrich, 60370
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Sigma-Aldrich, H3375
HCl, Reanal, 30715-1-08-65
KCl, Reanal, 18050-1-08-38
$K_3[Fe(CN)_6]$, Sigma-Aldrich, 244023l
$MgSO_4 \cdot 6H_2O$, Sigma Aldrich, 31413 and Alfa Aesar, 10797
$CaCl_2 \cdot 2H_2O$, Fluka, 21100 and Alfa Aesar, 10680
$SrCl_2 \cdot 6H_2O$, Aldrich, 204463
KBr, Fluka, 90737
$Na_2SO_4$, Aldrich, 204447 and Fluka 71962
KCl, Fluka, 05257 and Fluka, 60130
NaCl, Sigma Aldrich, 71379 and Alfa Aesar, 87605 and Sigma Aldrich, 204439
NaF, Aldrich, 450022
$NaHCO_3$, Sigma Aldrich, 31437
$H_3BO_3$, Fluka, 15660
$Na_2EDTA \cdot 2H2O$, Sigma Aldrich, E6635
$FeCl_3 \cdot 6H_2O$, Sigma Aldrich, 31232 and Reanal, 33252-1-08-38
$MgCl_2 \cdot 4H_2O$, Riedel-de Haen, 31422
$NaMoO_4 \cdot H_2O$, Riedel-de Haen, 31439
$CoCl_2 \cdot 6H_2O$, Fluka, 60820
$ZnSO_4 \cdot 7H_2O$, Sigma Aldrich, 31665
$CuSO_4 \cdot 5H_2O$, Fluka, 61245
$NaH_2PO_4 \cdot H_2O$, Sigma Aldrich, 71504
$NaNO_3$, Fluka, 71758

$Na_2SiO_3 \cdot 9H_2O$, Sigma, S4392
Vitamin $B_{12}$, Sigma Aldrich, V2876
Biotin, Sigma Aldrich, B4501
Thiamine hydrochloride, Sigma, T4625


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