DISSERTATION / DOCTORAL THESIS

Titel der Dissertation /Title of the Doctoral Thesis
„Synthesis, characterization and algal growth studies of iron complexes with aquatic humic acid models“

verfasst von / submitted by
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Betreut von / Supervisor: O. Univ.-Prof. Dr. Dr. Bernhard K. Keppler

Ao. Univ.-Prof. Mag. Dr. Regina Krachler
A scientist in his laboratory is not a mere technician:
he is also a child confronting natural phenomena that
impress him as though they were fairy tales.

(Учёный в своей лаборатории не только техник,
он также ребёнок, смотрящий на природные явления,
покорённые ему как сказка.)

Maria Skłodowska-Curie

Look deep into nature, and then you will understand everything better.

Albert Einstein

Algae? This is a color?

Albert Einstein
Acknowledgment

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My parents and sister for helping and supporting me in every chapter of my life.

Emanuel for his love, patience and faith in me.
And for financial support:

universität
wien

FWF

Der Wissenschaftsfonds.
Several experiments have shown that iron is limiting the productivity of marine ecosystems. In the geographical areas called high-nutrient low-chlorophyll (HNLC) the primary macronutrients like nitrate and phosphate are present in high concentration. However, the vegetation of plankton is poor in comparison to the nutrients concentration. Low bioavailability and solubility of iron is responsible for the inhibition of growth of marine microorganisms. Almost all dissolved iron in the ocean is coordinated to organic ligands. Important natural iron chelators are terrigenous humic substances which originate from decomposition of plant and animal residues. Considering the impact of those natural macromolecules on the environment, especially on the productivity of marine microorganisms and carbon fixation, it is very important to clarify the chemistry behind the iron complexation, its release and uptake as well as the structure of the complexes and ligands. Unfortunately, research on humic substances, especially aquatic humic substances (AHS) is very challenging due to the very low concentration and complex structure of those natural iron chelators. In order to understand the chemistry of AHS, regarding iron binding and postulated release of iron by photoreduction, model systems represent a good approach and may contribute to those findings.

In this work, different ligands and iron complexes were synthesized and investigated as potential models for AHS. Various possible coordination modes in iron complexes were tested with a series of iron-O,O; O,N and O,S chelates. Iron shows a very strong interaction with AHS, even at low pH values, which is similar with the iron in hydroxamic acid complexes. Therefore the suitability of iron hydroxamates as model substances for humic acids was investigated. Besides, catechols are known for their high affinity towards iron and also occur in natural systems. The presence of many phenolic groups in natural AHS suggests that such systems might also represent suitable candidates. Moreover, combining a catechol moiety with β-O-4 type dilignol compounds, which are known building blocks of lignin, results in very promising model compounds possessing both structural and functional properties of AHS. All prepared compounds in this work were characterized by elemental analysis, IR spectroscopy, electrospray ionization mass spectrometry, UV–Vis, cyclic voltammetry and where possible by X-ray diffraction analysis. The electrochemical properties of the compounds related to the postulated release of the iron by reduction in natural humic acid iron complexes have been investigated. The ligand scaffold and the presence of different functional groups have a big influence on the stability and solubility of the compounds. Concerned about the destiny and
reactivity of the complexes in seawater, stability experiments have been performed by means of UV–Vis spectroscopy under different conditions. Gathered information from both UV–Vis and cyclovoltammetric experiments, contributed to the interpretation and elucidation of the algal studies. As the major part of this work, the ability of the established model systems to supply marine algae with iron was investigated in algal batch cultures with unicellular species *Chlorella salina, Diacronema lutheri* and *Prymnesium parvum*, where the growth of each culture was monitored over approximately one month.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHS</td>
<td>aquatic humic substances</td>
</tr>
<tr>
<td>Anal. Calcd.</td>
<td>analysis calculated</td>
</tr>
<tr>
<td>ATR</td>
<td>attenuated total reflection</td>
</tr>
<tr>
<td>conc.</td>
<td>concentrated</td>
</tr>
<tr>
<td>CV</td>
<td>cyclic voltammetry or voltammogramm</td>
</tr>
<tr>
<td>DMF</td>
<td>dimethyl formamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>E</td>
<td>electric potential</td>
</tr>
<tr>
<td>E₁/₂</td>
<td>half-wave potential</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>Eₚ</td>
<td>peak potential</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>electrospray ionisation mass spectroscopy</td>
</tr>
<tr>
<td>EPR</td>
<td>electron paramagnetic resonance</td>
</tr>
<tr>
<td>et al.</td>
<td>et alii</td>
</tr>
<tr>
<td>e.g.</td>
<td>exempli gratia</td>
</tr>
<tr>
<td>EXAFS</td>
<td>extended X-Ray absorption fine structure</td>
</tr>
<tr>
<td>HEPES</td>
<td>4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid</td>
</tr>
<tr>
<td>HNLC</td>
<td>high nutrient low chlorophyll areas</td>
</tr>
<tr>
<td>I</td>
<td>electric current</td>
</tr>
<tr>
<td>IR</td>
<td>infra-red</td>
</tr>
<tr>
<td>λₘₐₓ</td>
<td>wavelength of absorption peak (UV/VIS)</td>
</tr>
<tr>
<td>logβ</td>
<td>overall stability constant</td>
</tr>
<tr>
<td>logD</td>
<td>n-octanol-water distribution coefficient</td>
</tr>
<tr>
<td>logK</td>
<td>stepwise stability constant</td>
</tr>
<tr>
<td>m/z</td>
<td>molecular mass/charge-ratio</td>
</tr>
<tr>
<td>n</td>
<td>molar number</td>
</tr>
<tr>
<td>NHE</td>
<td>standard hydrogen electrode</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>pH</td>
<td>pondus hydrogenii (power of hydrogen)</td>
</tr>
<tr>
<td>pKa</td>
<td>logarithmic acid dissociation constant</td>
</tr>
<tr>
<td>pKₑₐ₃</td>
<td>average water ionization constant</td>
</tr>
<tr>
<td>R. T.</td>
<td>room temperature</td>
</tr>
<tr>
<td>SAG</td>
<td>Sammlung von Algenkulturen der Universität Göttingen</td>
</tr>
<tr>
<td>UV–Vis</td>
<td>ultraviolet-visible spectrophotometry</td>
</tr>
<tr>
<td>VIS</td>
<td>visible</td>
</tr>
<tr>
<td>vₘₐₓ</td>
<td>maximum wavelength</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------</td>
</tr>
<tr>
<td>A</td>
<td>amper</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
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<tr>
<td>h</td>
<td>hour</td>
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<tr>
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<td>liter</td>
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<td>m</td>
<td>meter</td>
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<td>minute</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>M</td>
<td>molar concentration</td>
</tr>
<tr>
<td>V</td>
<td>volt</td>
</tr>
</tbody>
</table>
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Iron is an essential micronutrient for almost all living beings, ranging from archaea to human. As a core of active centers of important enzymes like for example cytochromes and catalases, it is required for a broad range of biological functions. For algae, especially the synthesis of chlorophyll and the maintenance of the proper structure of chloroplasts cannot take place without iron.\(^1\)\(^3\) Moreover, approximately 80 % of the iron in plants is located in the proteins (cytochromes and ferredoxin) of the photosynthetic chain (Figure 1).

Figure 1. Light-dependent reactions of photosynthesis at the thylakoid membrane.

The explanation of wide occurrence of iron in catalytic centers of proteins is the chemistry of iron, which allows relatively easy change in oxidation state and hence significant change of the properties.\(^4\) This in turn enables electron transfer as well as binding of different molecules and their modifications. Furthermore, the bioavailability and occurrence of iron billions years ago contributed to its today's importance for living organisms. At the beginning of life formation on Earth, iron was predominantly present as Fe(II). Its concentrations in primeval ocean where life started to form, were around \(10^{-7}\) M. At this point, for first catalytic reactions and formation of proteins, elements with high bioavailability and matching properties were incorporated into the active centers and one of them was iron.\(^5\)\(^6\) One of the side products of those reactions which
included the photosynthesis, was oxygen. The increasing concentration of oxygen led about 2.4 billion years ago to the dramatic changes is environment called ‘great oxidation event’, where the oxygen concentration reached 1% of the present concentration. This had disastrous consequences for the bioavailability of essential elements as sulfur, copper, zinc, molybdenum and iron, which are sensitive to redox conditions. From anoxic environment poor in zinc, molybdenum and copper, but rich in $\text{H}_2\text{S}$ and Fe(II), the ocean turned to oxic and oxygenated conditions. Thus, the concentration of iron, due to the oxidation of Fe(II) and precipitation of Fe(III) (hydr)oxides, decreased to $10^{-20}$ M. However, the demand for iron still remained high because of iron-containing cofactors. Today, the (buffered) concentration of Fe(II) in cytoplasm is still maintained at $10^{-6}$-$10^{-7}$ M, which is the same concentration as it was in the primeval ocean before. This short overview about the role of iron in evolution and its changing availability is a very good explanation, why this metal is now the third most limiting nutrient for the growth of photoautotrophic organisms. Although, some organisms could overcome iron demand with substitution with other metal ions like manganese, most of them rely on iron supply. Iron chemistry in seawater and its bioavailability for microorganisms has been intensively investigated for a long time. About a 25% of worldwide oceans waters are known as high nutrient low chlorophyll (HNLC) regions. In those areas nutrient concentrations of phosphate and nitrate are high but the primary production is low (Figure 2).

![Figure 2](image_url)

**Figure 2.** Global sea-surface phosphate concentration (left) in comparison to global distribution of chlorophyll concentration (right). Red marked areas stand for high concentrations whereas violet for low concentrations. Presented data is average between January 1998 and December 2006.

The reason for the poor growth of plankton is the very low concentration of iron. The presence of bioavailable iron is crucial for growth and vitality of algae. HNLC areas are not only characterized by low chlorophyll content, but also by the presence of certain species of algae. Especially diatoms with high iron demand cannot grow under iron deficiency
conditions. Thus, the HNLC regions are characterized by small algae, which means iron is not only controlling the total growth of algae and other microorganisms but also selectively influences the development and expansion of several species, which affects the food chain within the whole ecosystem. Since the photoautotrophic marine organisms are responsible for about a half of worldwide photosynthetic activity as well as the regulation of the carbon dioxide concentration, iron biochemistry in seawater needs to be fully understood. Looking into the future, the possibility to enhance vegetation and biomass production in the ocean may have several positive environmental aspects as for example increased oxygen production, carbon dioxide drawdown and an increase in the population of species feeding on algae. This may compensate the environmental imbalance caused due to overfishing and anthropogenic carbon dioxide production. Also growing interest in algae and their use in several economic sectors like for example production of biofuel and several extraction products can profit from the findings concerning iron demand and bioavailability in aqueous systems.

**Figure 3.** Schematic view of CO₂-drawdown due to the algal bloom occurred after release of iron into the surface waters.

HNLC regions represent possible niche, where with proper handling, conditions supporting algae growth can be created. Attempts to create those conditions in HNLC regions have already been undertaken in several large scale iron-enrichment experiments, which led to algal blooms. Smetacek et al. has shown that after fertilization with iron, a large diatom bloom
appeared after four weeks, followed by death and sinking of algae. At least half of the algal biomass sank below 1000 m and therefore the carbon was stored for a long time, which obviously affects the global carbon cycle (Figure 3). Thus, the addition of soluble Fe(III) affects the biological pump and storage of carbon dioxide in HNLC regions. Unfortunately, the artificial fertilization of the ocean has many drawbacks. Fertilization experiments stimulated the production of toxins by algae like for example domoic acid by species *Pseudo-nitzschia* sp. (Figure 4).\(^{33}\) As an example domoic acid accumulates in fish and shellfish without visual symptoms of illness and it is extremely dangerous for mammals and birds. Other side effect of iron fertilization is lower oxygen content in affected deeper waters causing smothering of corals and decreasing aquatic vegetation. In comparison to the artificial iron fertilization, natural processes including iron input into the ocean do not lead to those severe side effects. Planquette et al.\(^{34}\) investigated the natural iron fertilization occurring around the Crozet Islands (Indian Ocean). Due to the iron input, the algae biomass strongly increased, but neither lack of oxygen causing death of organisms at the seafloor nor any toxins produced by algae were detected.

Thus, natural iron fertilization occurring in Southern Ocean, called ‘the island effect’, covers iron demand of algae including species responsible for higher carbon dioxide drawdown and biomass production without leading to destruction and extinction of other marine living beings. The comparison of natural and artificial fertilization leads to the conclusion that more information about the iron speciation in the ocean needs to be obtained in order to carry out
such experiments. Use of synthetic iron chelators which mimic the natural ones may decrease the side effects and lead to better results.

**SOURCES OF IRON**

Before starting with biochemical speciation of iron in aqueous systems, we should take a look on different iron sources and their output (Figure 5). One of the iron sources is the atmosphere. Iron reaches atmosphere due to volcanic eruptions or desert dust and is transported with wind and rain into the surface waters. Moreover, melting of icebergs, hydrothermal vents and upwelling processes contribute to the iron content in seawater.\textsuperscript{35,36} Also rivers represent an important repository of iron. Unfortunately, significant amount of the iron transported with rivers precipitate in estuarine areas due to the major change in ionic strength.\textsuperscript{37-42} Exceptions are rivers draining peat bog areas, which are rich in humic substances. The reason for this is the ability of humic substances to complex iron and thereby keeps the iron in solution in seawater.\textsuperscript{43-45}

![Figure 5. Sources of iron in the ocean.](image-url)
For the living organisms the chemical speciation of iron is more important than its origin since not every reservoir of iron is bioavailable. Oceans biogeochemical cycles of important elements and nutrients like carbon, sulfur and nitrogen are well studied and clarified but in case of iron there are several outstanding questions concerning its transport and uptake. Although iron is a common element in the lithosphere, iron concentrations in the ocean are very low. This is not attributed to the low abundance of iron, but to the chemistry of iron in aquatic systems. There are several factors influencing the bioavailability of iron as well as several possible pathways of iron uptake by marine microorganisms (Figure 6).46

![Figure 6. Schematic view of different transport and uptake mechanism for iron.](image)

The oxidation state of iron plays a crucial role in microbial biochemistry of iron. Iron(III) is the most common oxidation state, due to the oxidative conditions in most environments. More soluble and accessible for algae is Iron(II) but its presence strongly dependents on pH value and oxygen content of the medium.48 Most of the aquatic system, which sustain life, are highly oxygenated and such systems do not facilitate the reduction of Iron(III). Dissolved inorganic Iron(III) is present in very low concentrations in seawater, which is a result of the low solubility of Iron(III) hydroxide (iron hydroxide is a general term describing different iron species: iron
oxide, oxohydroxide and hydroxide).\textsuperscript{49} Only a small part may undergo photoreduction, gaining soluble Iron(II) but its concentration is distant from covering the lowest iron demand. Additionally in seawater, higher ionic strength and pH value contribute to low iron hydroxide solubility.\textsuperscript{49} Studies showed that approximately 99.9% of dissolved iron in the ocean is present as iron complexes with organic ligands.\textsuperscript{16, 17} Without those ligands, the solubility of iron would be too low to sustain the life in the ocean.\textsuperscript{50} The presence of natural iron chelators explains the increased solubility of iron in natural seawater compared to artificial one.\textsuperscript{51, 52} Figure 7 shows the difference between the concentration of dissolved iron in natural seawater and 0.7 M NaCl solution.\textsuperscript{53}

![Figure 7](image_url)

**Figure 7.** A comparison of the solubility of Fe(III) as a function of pH in seawater and 0.7 M NaCl at 25 °C.\textsuperscript{53}

The uptake of iron bound in complexes by the primary producers occurs due to the several processes which are dependent on the complex and ligand stability and their redox behavior. After consumption of iron by primary producers, ligand-metal equilibrium changes, promoting the iron dissociation from the complex. Also the degradation of the ligand leads to the complex breakdown. The reduction of Iron(III) in the complex decreases the affinity of ligands towards iron and induces the release. This process can occur due to the self-reduction/oxidation of the complex which means the oxidation of the ligand with simultaneous reduction of iron.\textsuperscript{54} Especially photo-reductive dissociation seems to have a big impact on the bioavailability of iron.\textsuperscript{55}
Iron chelating molecules have different origin and broad palette of structures. Generally, we can distinguish between iron chelators, which originate mainly from decomposition of organic material, and chelators synthesized by certain organisms. Many bacterial species evolved special mechanisms for the efficient supply of iron. For example the production of low molecular weight siderophores represents an effective way to bind iron. Siderophores are a group of substances with extremely high affinity towards iron. As example, the iron complex with enterobactin has the highest stability constant among all iron chelators. Under iron-stress conditions, the synthesis and release of siderophores takes place. The functional groups responsible for the iron complexation are catecholates, hydroxamates and \( \alpha \)-hydroxycarboxylates. High affinity towards iron allows a complexation at very low iron concentrations which are present in seawater. Once iron is bound, the complexes remain stable in solution and the release of iron can only take place under certain conditions. Some of the gram-negative bacteria have special receptors for siderophores and vitamin B\(_{12}\) located in the outer membrane. There are two proposed intracellular release mechanisms for iron bound to siderophores. One includes the intracellular reduction to Iron(II) which has lower affinity to siderophore and can be easily released from the complex. The second involves the breakdown of the siderophore in the cell and the uptake of Iron(III). Some of the siderophores are able to undergo photoreduction increasing the steady state concentration of Iron(II) in seawater which can be uptaken by other organisms. Still, iron connected to siderophores is accessible almost only for organisms possessing corresponding uptake systems. Siderophores are not the only organic ligands present in the ocean. Dissolved organic matter (DOM) presents alternative source of iron binding molecules of great importance. Dissolved organic matter, also known as humic substances has different iron binding abilities depending on the origin and composition. Especially river derived aquatic humic substances (AHS) are getting increasingly attention regarding their properties as iron transport systems. Iron bound via AHS is highly accessible to algae in contrast to siderophore derived iron.

Dissolution of the iron due to complexation is one factor influencing its bioavailability, but more important are the properties of the resulting complexes which were already mentioned in case of siderophores. The possibility of photo- and bioreduction of those compounds as well as their stability and hydrolysis are playing important role in the iron uptake processes. Depending on the ligand and reduction potential, iron bound in complexes can be reduced by bioreductants present in seawater or more important due to photochemical reactions. Formed Iron(II)
species, usually with low affinity to the ligands binding Iron(III), are not stable and undergo oxidation with O₂ and H₂O₂ (fenton reaction). However, the steady state concentration of Iron(II) is increased and therefore more dissolved bioavailable iron is present in solution.

**HUMIC SUBSTANCES**

**ORIGIN AND CHEMISTRY**

The term humic substances (HS) describes a broad group of substances which originates from decomposition of plants and animal residues. Due to chemical and physical processes as well as transformation by microorganisms, a variety of substances with different properties results. There is no general formula for humic substances, as those substances are very heterogenic. Only the components of them are characterized, but no full revised structure could be determined. Figure 8 shows how humic substance may look like.

![Proposed structure of humic acid, including characteristic building blocks.](image)

The building blocks of humic substances consist mainly of lignin, lignin decomposition products, sugars, aminoacids and polyphenols (Figure 9). Polyphenols and lignin decomposition products can be easily oxidized into quinones and then incorporated in the structure of humic substances. As the humification process includes various components and binding forces including ionic, hydrophobic, hydrophilic and donor-acceptor bindings, structures can differ depending on biological, physical, climatic and geographical conditions. Indeed, the formation of HS is one of the less understood natural processes. Several theories explain the possible pathways. Waksman et al.⁶²-⁶⁴ Postulated that HS are formed from lignin, which is utilized by
microorganisms and the residue forms HS. The modification of lignin includes cleavage of the methoxy groups generating phenols and oxidation of aliphatic hydroxyl groups to acids. The polyphenol theory from Stevenson indicated that aldehydes and polyphenols, released due to the microbial transformations, undergo further enzymatic reactions leading to quinones. Quinones polymerize and form humic acids. This theory is especially for plants with relatively low lignin content plausible. Stevenson also developed the sugar-amine theory where reduced sugars and amino acids, formed as by-products of microbial metabolism, undergo non-enzymatic polymerization, also leading to humic acids precursors. Nowadays, the lignin theory seems to be most likely, because lignin plays important role in the majority of the humification processes. Moreover, it does not necessarily negate the role of polyphenols in origin of HS as polyphenols are products of biodegradation of lignin. On the basis of properties like solubility and polymerization degree, humic substances can be divided in three groups: humins, humic acids and fulvic acids (Scheme 1).

**Figure 9.** Schematic view presenting the formation of humic acids and transport of iron.

Humins are insoluble in water and have the highest polymerization degree and high carbon content. Humic acids can be dissolved under basic conditions together with fulvic acids. The adjustment of the pH value to 1 results in precipitation of humic acids. Despite higher
molecular weight, this separation step represents the most significant difference between fulvic and humic acids. Fulvic acids are the most interesting group among humic substances on the one hand due to the good aqueous solubility on the other hand due to the high oxygen content. Moreover they have relatively small molecular weights (1-2 kDa) and high exchange acidity. Due to the presence of many functional groups, fulvic acids are particularly very good transition metals chelators, especially for iron.

![Diagram of division of humic substances](image)

**Scheme 1.** Scheme of division of humic substances according to their properties.\(^{67}\)

Humic substances consist mainly of C, H, O and N (in some cases also S) and the content of each element varies with the group (humins, HA, FA) and origin. However, the general empirical formula of HS can be described as \(\text{C}_{36}\text{H}_{30}\text{N}_{2}\text{O}_{15}\text{H}_{x}\text{O} (x = 0-15)\).\(^{66}\) Table 1 shows elemental analysis and other characteristics of purified fractions of HS from different regions.

Besides the binding properties of transition metals, other interesting characteristics of HS should be mentioned. In relation to electrochemistry, HS can act as reduction and oxidation agents, but in most cases they are reductants. Relatively high redox potential between 0.3 V and 0.7 V\(^{68,69}\) enable the reduction of several transition metals like for example Fe(III), Hg(II), Sn(IV), Cr(VI) and V(V). Functional groups of HS participating in this reaction are mainly catechol moieties, which can be oxidized to quinones, but also aldehydes and alcohols. Occurring processes involve semiquinone as intermediate and concurrently free radical and they are reversible one-electron transfer steps.
Table 1. Analytical data for purified HAs.\textsuperscript{86}

<table>
<thead>
<tr>
<th>HA Source</th>
<th>Elemental Analysis\textsuperscript{(a)} (%)</th>
<th>Total Acidity/ R-COOH/ Phenolic OH\textsuperscript{(b)}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>PHi</td>
<td>46.6</td>
<td>5.68</td>
</tr>
<tr>
<td>WhI</td>
<td>52.38</td>
<td>5.81</td>
</tr>
<tr>
<td>WhS</td>
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<td>WhR</td>
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<td>5.35</td>
</tr>
<tr>
<td>GhS</td>
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</tr>
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<td>IHa</td>
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<td>O</td>
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</tr>
<tr>
<td>NHa</td>
<td>52.9</td>
<td>5.4</td>
</tr>
<tr>
<td>NNYA</td>
<td>49.8</td>
<td>4.7</td>
</tr>
</tbody>
</table>

\textsuperscript{(a)} On a dry, ash-free basis. \textsuperscript{(b)} Determined by combustion of 100.0 mg HA samples in air 850 °C for 2 h. \textsuperscript{(c)} For method details refer 2. \textsuperscript{(d)} Taken as the difference between total acidity and R-COOH. Data from Paramonova, T. A.; Zech, W. (28, pp 469-474)

This behavior of HS makes their iron complexes bioavailable. Due to UV–Vis irradiation, electrons can be transferred from HS to Iron(III) and the reduction of the metal takes place.\textsuperscript{47} Iron(II) affinity to HS is very low and the ion is released from the complex and can be uptaken by microorganisms till re-oxidation proceeds. Additionally, the redox behavior including formation and stabilization of free radicals make them very good photosensitizers.\textsuperscript{70} HS, similar to their iron complexes, are able to absorb light and form several reactive species (e.g. $3\text{HS}^*$, OH$^*$, $1\text{O}_2$, $2\text{O}_2^*$, and $\text{e}_{\text{aq}}^-$). Those species influence the photodegradation pathways of other organic molecules in aquatic systems. Another interesting behavior of HS is their large sorption capacity for both hydrophobic and hydrophilic substances. This phenomenon can be explained with, similar to proteins, possession of both hydrophilic and hydrophobic groups. Phenolic, carboxylic and catecholic groups provide the solubility and hydrophilic character whereas aliphatic and aromatic groups provide hydrophobic properties. Moreover, the outrageous diversity of absorbed substances can be also owed to the structure flexibility. On the one hand, aliphatic chains provide free rotation and movement and on the other hand, aromatic rings insure the rigid structure. Further characteristics of HS are the high exchange capacity and the possibility to act as pH buffer.\textsuperscript{71} High buffer capacity, which is an important property for the maintenance of soil pH, can be attributed to acidic groups and proton-binding ability of HS.

## APPLICATIONS

Due to the extreme diversity and many different properties of humic substances, they are of growing interest regarding their use. No other natural product is as versatile as humic substances. Application of them in different economical, scientific and medical sectors requires better understanding of their structure and estimation of the components responsible for
certain properties and proportions of those components. Research on HS is as challenging as the great features they are possessing. Although several questions still need to be answered, there are already established applications for those natural compounds. HS are widely used as fuel and there are several power stations using them to produce energy. The biggest plant is Shatura Power Station in Russia, which has the largest peat power capacity in the world. Also HS-based fertilizers are commonly used due to their benefits including breaking up compacted soil, complexing micronutrients and enable their uptake by plants, stimulating the microflora vegetation and enhancing water retention. Beside those standard uses, more specific applications are being developed. One of them is detoxification of soil and water contaminated with toxic chemicals of different origin. Because of to their redox properties, HS are able to reduce toxic metals to their less or non-toxic oxidation states like for example Cr(VI) to Cr(III). Also organic material can undergo reduction by HS. Important use is here the cleavage of halogenated hydrocarbons, which are widely distributed in ground water and soil. Also sorption properties of HS can find application in wastewater treatment for binding heavy metals (iron, nickel, mercury, cadmium and copper) or for the decontamination of soils from hydrophobic contaminants like for example methylated naphthalenes. Moreover, also radionuclides form complexes with HS, giving the opportunity to use HS to discharge contaminated water from nuclear power plants. Generally, HS are able to remove several hazardous substances (herbicides, fungicides, insecticides, nematicides, dioxins, estrogenic compounds) with different properties from environment. Several industrial applications are known for HS, few of them are as additives to control the setting rate of concrete, leather dye, additives to enhance the mechanical strength of unprocessed ceramics, as dyes for coloring Nylon 6 or PVC plastics, hardeners of polyurethane foams or as plasticizer and ingredients for PVC. Besides that, medicine can benefit from HS. HS are known for their antiviral and anti-inflammatory effects as well as interactions with enzymes and effects on blood coagulation. Summarizing, there are already several possible and already established applications of HS and the development and research on HS in less traditional areas as for example environmental chemistry and biomedicine is in progression.

HUMIC ACIDS AS IRON CHELATORS IN THE OCEAN

The properties of AHS allow very effective complexation of iron in aquatic systems. They also prevent iron from precipitation in seawater, where the solubility of iron hydroxide species is
even lower as in riverine water. Main sources of AHS are rivers draining peat bog areas (Figure 10). Their typical brownish color is attributed to high AHS content.

![Figure 10. Typical brown color of the river originating from peat bog areas.](image)

AHS are changing the amount of dissolved iron (Iron oder FE), which riches the ocean. The majority of dissolved Fe (Iron oder FE) in river water exists as small colloid particles. Flocculation of these colloids upon mixing of river water with seawater causes a massive removal of the Fe (Iron oder FE). Whereas iron (Iron oder FE) in AHS poor rivers precipitate almost completely in the mixing zone due to the higher pH and ionic strength of seawater, almost 20% of iron transported with AHS rich rivers remains in solution. This enhances significantly the amount of dissolved iron in the ocean. Klunder et al. showed that a large fraction of the dissolved iron in central Arctic Ocean originates from river water. Arctic rivers are draining tundra regions, which are rich in peat bogs thus they have very high DOM content. Therefore, the concentration of DOM in polar surface water is relatively high reaching 1.5 mg/L. Moreover, the complexes of iron and AHS can reach areas distant from the coast, where the iron concentration is usually very low. AHS itself have no autochthonous source in the ocean, but they were found in entire Atlantic, Arctic and Pacific oceans. Due to the very low concentration of AHS-iron complexes in seawater, the research on them is very challenging. New methods for example cathodic stripping voltammetry are used to investigate the complex stabilities and affinity of AHS towards iron. Lagera and van den Berg
used methods based on cathodic stripping voltammetry to detect and quantify AHS binding iron in seawater. They found coexistence of both iron and AHS in the Irish Sea, which leads to the conclusion that iron is transported into the ocean by AHS originating from peat bog areas. Those results implicate that AHS are important terrigenous iron chelators and play very important role in iron supply for marine organisms.\textsuperscript{95} Also several other researchers have shown that land-derived AHS act as iron chelators in the open ocean.\textsuperscript{96-99} Although, analytical methods provide us information about the concentration of AHS and their bioavailable iron complexes as well as the affinity of AHS towards iron, the chemical speciation remains unclear.\textsuperscript{100} Also the uptake and iron release mechanisms are still being under discussion.\textsuperscript{101}

### MODEL SUBSTANCES

#### MOTIVATION

Due to the challenging problems with the characterization of AHS-iron complexes in seawater, strategies need to be developed to clarify the transport pathway and uptake of iron bound to those natural chelators as well as their structure and coordination motif. The difficulties in investigations of the natural complexes are lying on the one hand in their low concentration in seawater and on the other hand in the enormous diversity and properties of humic acids as well as paramagnetic Iron(III). The complexes can be very variable depending on AHS origin and structure, but fundamentally important is the direct surroundings of bounded iron and its properties. In order to investigate the chemistry and biological importance of AHS-iron binding, we decided to use an indirect approach in form of model compounds. As mentioned before, there are several proposed structures for humic substances. The problem is that most of them are only the result of theoretical studies and never have been synthetized\textsuperscript{62-65, 102, 103} Several publications describe investigation on the Iron(III) binding ability of simple model ligands for humic acids\textsuperscript{104-108} but none of the complexes has been isolated and characterized yet. For example, binding studies of iron with guaiacylglycerol-\(\beta\)-guaiacyl ether precursors (coniferyl alcohol, sinapic acid, ferulic and coumaric acid) and dehydrogenation polymers of coniferyl alcohol have been already carried by Guillon et al. regarding sorption properties of lignin.\textsuperscript{105, 106} More important, the bioavailability of iron in investigated model systems was not examined and compared with natural compounds. The goal of this thesis is to fill in this knowledge gap by synthesizing iron complexes with model aquatic humic acids, including their isolation and characterization as well as biological studies. Those studies will the test of the bioavailability of iron in synthesized complexes, as a substantial property of AHS-iron complexes in ocean, which
can be carried out in form of algae growth experiments. Finding proper models enable the verification of the structure of AHS-iron complexes and therefore gaining more information regarding their chemistry. Furthermore, with proper models, several new investigations are possible ranging from the use of models as reference substances for different measurements to $^{13}$C-labeled ligands and radioactive labeled Fe to verify the iron uptake pathways.

New insights into properties and structure of land-derived AHS enable verification of the importance of those natural iron carriers. Moreover, better understanding of the connection between peat bogs and their ability to sustain life not only in freshwater systems, but also far beyond them in seawater is provided. From the viewpoint of environmental chemistry, it is also essential to examine the effects of climate changing, agriculture and other anthropogenic factors on peatlands and their production of AHS. Due to their impact on marine primary production, changes in their amount and chemistry may have dramatic influence on ecosystems, especially considering the area of more than $3.4 \times 10^6$ km$^2$ which peatlands in Siberia, Canada, Alaska and Northern Europe are covering.$^{109, 110}$

### PROPERTIES AND REQUIREMENTS

Model compounds should provide the core properties of AHS-iron complexes, but still possess less complexity in order to enable characterization. It is not easy to put those two requirements together. Compulsorily crude simplification of the structures is needed and therefore, we decided to focus on different iron coordination possibilities. After finding proper compounds, adjustment of the compounds in relation to functional groups, solubility and patterns present in the natural synthetic pathways of humic substances, can be made. The iron binding ability will be investigated on different types of ligands. It should be kept in mind, that model systems represent a rough simplification of aquatic humic substances (AHS), which have several consequences. Each model refers to one particular iron binding type in AHS, whereas the heterogeneity of the humic acids allows many different coordination modes and sites. Iron can be bound at the same time at different positions, coordinated by different functional groups having different binding strengths. Also the large size of the molecules compared to simple ligands may lead to the conformational changes and aggregate formation, which affects the binding sites. The availability and amount of possible iron binding groups depends on pH and ionic strength. Summarizing, it is not possible to reproduce all of the properties of AHS within one model but it is thoroughly feasible to identify potential coordination motifs and building
blocks responsible for the reversible iron binding and transport in the ocean. The choice of the ligands depends on the current information about the functional groups of natural humic acids, which are among other aromatics, phenols and carboxylic groups. The functional groups mainly responsible for the iron complexation have been tested. Until now, most reliable information about the possible surrounding of iron in AHS-iron complexes is provided by EXAFS studies.\textsuperscript{111, 112} Recent EXAFS studies on humic substances put further requirements for the model compounds together\textsuperscript{112} which include mononuclear iron complexes as predominant form observed in AHS. Dinuclear and polynuclear complexes are present in less soluble fractions of humic substances, but they are also worth investigation due to the chemistry of many natural chelators which form preferable dinuclear complexes like catechol.\textsuperscript{107} As those natural chelators are very effective in iron transporting, similar scaffold could also work for AHS. Another important issue by searching for proper models is the first coordination sphere of iron. The EXAFS studies suggested that oxygen is the most important atom bound to iron, but they cannot clearly distinguish between nitrogen and oxygen. This means those two atoms can theoretically be present in the first coordination sphere of iron.

\textbf{Scheme 2.} Examples of possible coordination modes of Iron(III) in humic substances.

Admittedly, the oxygen content in humic substances is much higher than the nitrogen and also many identified oxygen containing groups are present, so the $O,O$ coordination seems to be
more likely. However, due to the possible incorporation of amino acids in the structure of humic substances, other coordination possibilities as for example $O,N$ and $O,S$ should be also verified. Examples of considerable coordination modes of Iron(III) in humic substances are presented in Scheme 2. As the natural compounds are soluble in freshwater and seawater, a further requirement for the model compounds is aqueous solubility. Also the affinity of the ligand towards iron should be high to enable the isolation of the complex and efficient transport of iron. AHS are found to be capable of transporting iron far away from the coast. Summarizing, criteria and properties shown in Scheme 3 should be considered and investigated by designing model compounds for AHS (Scheme 3). Beside the already described requirements, chosen ligand scaffolds should occur in the similar form in the environment thus the biosynthesis is theoretically possible.

**Scheme 3.** Important criteria for the design of model compounds.

Considering the requirements for the ligands and complexes mentioned above, we can define some examples of potential candidates for the models. Iron shows very strong interaction with humic substances even at low pH values, which is similar with iron in hydroxamic acid compounds. Thus ligands with hydroxamate functional groups seem to be suitable models for humic substances as proposed by Frimmel et al. The strong chelating properties of hydroxamates are exploited by many microorganisms like bacteria which produce and release low molecular siderophores to bind and transport iron. Significant nitrogen content and
possible presence of nitrogen in the first and second coordination sphere of iron in AHS supports our decision to use them as models. Also known coordination of iron with catechol ligands, observed in the natural systems, makes them of potential interest. Furthermore, benzcarboxylic acids, salicylic acid, maltol and their derivatives are simple characterized systems with the required properties. Due to the presence of many carboxylic groups in natural HS, derivatives of benzcarboxylic acid have already been proposed as humic acid models by Milunovic et al. Unfortunately complexation of iron with those ligands leads to polynuclear complexes, which cannot be used as models for AHS because of their low aqueous solubility.

## BIOAVAILABILITY OF IRON DERIVED FROM MODEL SUBSTANCES

Bioavailability of iron for marine microorganisms is one of the most important criteria for the eligibility of the complexes as model compounds for AHS. In order to elucidate this property, biological tests need to be implemented into the characterization of synthesized systems. As the marine unicellular photoautotrophic algae build the base of the food chain, algae growth experiments (with representative species) are suitable for elucidation of the iron bioavailability from synthesized complexes. The growth of algae is strongly inhibited in the absence of iron and several studies in batch cultures have shown poor growth of the cultures under iron-limiting conditions. Indirectly, the monitoring of algae growth after treatment with iron complexes implicates the bioavailability of iron. If the culture does not respond to the treatment with better growth as for the samples lacking iron, the bounded iron is probably not bioavailable or the complex is toxic for algae. Moreover, for the control samples, simple addition of iron to the algae culture is in most cases not sufficient enough to achieve the good growth and chelating substances (in most cases ethylenediaminetetraacetic acid (EDTA)) need to be added in order to keep iron in solution. This fact enables also the possibility to test ligands themselves and their impact on bioavailability of iron. In this case iron can be added as usual and the EDTA can be replaced with the model ligand. At the planning stage of the experimental setup for an algae test, several factors should be taken into consideration. The most important one is the selection of representative organism for the investigation. Ideally, the algae should have wide occurrence in the ocean and be possible to cultivate. Additionally, it is advantageous if there are already publications including biological tests on certain species. This allows the comparison of conditions and expected growth. Of course the requirements for iron are different for diverse groups and species. Thus, the experiments should include two or more species, favorable from different groups (for example green and gold algae). After selection of
the species, cultivation conditions need to be customized. Those conditions include: light/dark cycle, temperature, stirring, container size, cultivation medium, pH, aeration etc. Figure 10 shows an example of the experimental setup throughout this doctoral work.

![Figure 10. Example of an algal growth experiment.](image)

Examples of species suitable for algae growth experiments are *Chlorella salina*, *Diacronema lutheri* and *Prymnesium parvum*. All three algae species have widespread occurrence and abundance in the Northern Atlantic Ocean. Figure 11 shows *C. salina* and *D. lutheri*. Chlorophyte *C. salina* represents green algae and *D. lutheri* is a marine phytoflagellate which is both broadly used in aquaculture industry as food source for larval and juvenile molluscs, crustaceans and fish species.\textsuperscript{122,123} Moreover, they occur in coastal waters of Ireland and Sweden and in estuarine zones of AHS-rich rivers, which makes them interesting for experiments including AHS models. Also several investigations concerning the cultivation of those algae have been published.\textsuperscript{124-128} *P. parvum* is harmful algae species belonging to haptophyta. It produces toxins responsible for massive fish dying after bloom of this species. Also the growth of other algae species is strongly inhibited in the presence of *P. parvum*. Thus, it is an example of algae, which not necessarily contributes to maintenance of the healthy ecosystems. The investigation of the growth of this species under different conditions and use of model compounds can help to find a difference in the uptake pathways of iron and perhaps a way to inhibit the growth of *P. parvum* while other species can growth unhindered.
Figure 10. *Chlorella salina* (left) and *Diacronema lutheri* (right).
REFERENCES


120. J. Zeilinger, University of Vienna, 2011.


RESULTS

The content of this PhD work is based on following two published articles and one manuscript:

**Benzoic hydroxamate-based iron complexes as model compounds for humic substances: synthesis, characterization and algal growth experiments**

**Synthetic iron complexes as models for natural iron-humic compounds: synthesis, characterization and algal growth experiments**

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

The results are presented chronologically and in the publisher’s format or as manuscript.

Additionally, I contributed to the following publications and manuscripts:

**Photoreduction of Terrigenous Fe-Humic Substances Leads to Bioavailable Iron in Oceans**
1. BENZOIC HYDROXAMATE-BASED IRON COMPLEXES AS MODEL COMPOUNDS FOR HUMIC SUBSTANCES: SYNTHESIS, CHARACTERIZATION AND ALGAL GROWTH EXPERIMENTS

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Graphical abstract:

A series of monomeric and dimeric FeIII complexes bearing benzoic hydroxamates as O,O-chelates has been prepared and characterized by elemental analysis, IR spectroscopy, UV–Vis spectroscopy, electrospray ionization mass spectrometry (ESI-MS), cyclic voltammetry, EPR spectroscopy and for some examples by X-ray diffraction analysis. The stability of the synthesized complexes in pure water and seawater was monitored over 24 h by means of UV–Vis spectrometry. The ability to release iron from the synthesized model complexes has been investigated with algal growth experiments.

As the first author, I designed and performed the synthesis of the novel compounds. I also characterized the compounds and carried out algal studies. Furthermore, I made the major contribution to all chapters of the manuscript.
Benzoic hydroxamate-based iron complexes as model compounds for humic substances: synthesis, characterization and algal growth experiments†

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A series of monomeric and dimeric FeIII complexes bearing benzoic hydroxamates as O,O′-chelates has been prepared and characterized by elemental analysis, IR spectroscopy, UV-Vis spectroscopy, electrospray ionization mass spectrometry (ESI-MS), cyclic voltammetry, EPR spectroscopy and for some examples by X-ray diffraction analysis. The stability of the synthesized complexes in pure water and seawater was monitored over 24 h by means of UV-Vis spectrometry. The ability to release iron from the synthesized model complexes has been investigated with algae growth experiments.

Introduction

Iron has been recognized as an essential trace element for all living organisms, ranging from archaea to mammals and is required for the function of many proteins and enzymes. Also photosynthesis as well as nitrogen assimilation is dependent on iron-based enzyme cofactors.1–3 More than 50% of the photosynthetic activity on earth is attributed to phytoplankton, which consists of photoautotrophic microscopic organisms.3–5 They are responsible for a major part of the global biosynthesis of organic compounds from inorganic carbon dioxide dissolved in water and therefore play a key role in the global CO2 cycle. Although iron is a common element on earth, large areas of the ocean show very low iron concentrations.6–8 In so called high-nutrient, low-chlorophyll (HNLC) areas nutrients like nitrate and phosphate are present in high concentrations; however, chlorophyll concentrations, as indicator for phytoplankton density are low.9,10 Evidences have shown that insufficient concentrations of iron in these areas are responsible for the inhibition of growth, development and productivity of marine phytoplankton.11–13 The presence of bioavailable iron in seawater is crucial for a high productivity of phytoplankton.14–20 The observed low iron concentrations can be explained by the chemical speciation of iron in seawater. Thermodynamically stable iron(III) precipitates in oxygenated seawater at pH 8 as ferric hydroxide aggregates.21 In fact, 99.97% of dissolved iron in seawater exists as complexes with organic chelators.22–25 However, the bioavailability of iron does not solely depend on its solubility moreover other parameters such as complex hydrolysis and photo- or bioreductions are essential.26 The accumulated complexed Fe(III) can be reduced by bioreductants or more importantly photochemical reactions. The formed Fe(II) dissociates and is prone to oxidation reactions with O2 or H2O2 (Fenton reaction). This reaction cycle increases the steady-state concentration of dissolved bioavailable iron.27

Until now it was believed that the main sources of iron in the ocean are atmospheric deposition of volcanic dust, terrestrial sand and extraterrestrial dust, the upwelling of iron rich waters as well as hydrothermal vents and ice melting;26,28 however, the role of river water has been mainly neglected. Upon mixing of river water with seawater in the brackish water zone of estuaries the majority of the transported iron gets sequestered into the sediment due to the major change in ionic strength.29–34 Recent investigations in the Mississippi Delta and North Scotland have shown that a significant proportion of iron(III) complexes of aquatic humic substances (AHS) in the river remain in colloidal solution in sea water and can contribute to iron-fertilization of coastal waters.35–37 AHS, resistant against salt-induced flocculation, have been found in the entire Atlantic, Arctic and Pacific oceans. The identification of AHS as important carriers for iron explains the increased bioavailability of iron in natural seawater compared to artificial one.38,39 AHS have been under investigation for a very long time, and their composition has not been clarified in detail. The complex structure of this substance class origins from the incomplete decomposition of organic material, such as lignins which undergo chemical, physical and microbiological transformations.40,41 Furthermore other organic molecules like amino acids, sugars and peptides can be incorporated leading to a broad range of different structural
Unfortunately, the very low concentration of those natural iron chelators in seawater makes the detection and characterization of them challenging. Being aware of the importance of those natural macromolecules for marine microorganisms it is of large interest to find synthetic compounds that are comparable to AHS, regarding their iron binding and transport properties in sea water, as well as their capability of providing bioavailable iron to algae. Comparing the latest EXAFS studies and the current information about AHS we put further requirements for the model compounds together. Overall they include mononuclear Fe(III) complexes, mainly $O,O$ coordination, functional groups such as phenols and aromatic carboxylic groups and good water solubility. Iron shows very strong interaction with AHS even at low pH values, which is similar to iron complexes bearing hydroxamic acid derivatives. Thus ligands with hydroxamate functional groups seemed to be suitable models for humic substances as proposed by Frimmel et al. The strong chelating properties of hydroxamates are exploited by many microorganisms like bacteria which produce and release low molecular siderophores to bind and transport iron. Significant nitrogen content and possible presence of nitrogen in the first and second coordination sphere of iron in AHS supports our decision to use them as models.

Herein we report the synthesis of benzhydroxamic acids and their respective iron(III) coordination compounds. The synthesized complexes were investigated and characterized by standard analytical methods such as elemental analysis, UV-Vis, IR, EPR, cyclic voltammetry, mass spectrometry and X-ray diffractometry. Moreover, the stability of the iron complexes in (sea) water was investigated. Algal growth experiments were performed with the marine single-cell chlorophyte Chlorella salina and haptophyte Diacronema lutheri to elucidate the potential of these iron complexes to act as model compounds for humic substances in regards of their biological function in sea water environment.

**Experimental**

**Materials and methods**

All chemicals were purchased from Sigma Aldrich (salicylic acid, 4-hydroxybenzoic acid, 3,4,5-trimethoxy benzoic acid, 3,4-dihydroxyhydrocinnamic acid, o-coumaric acid, iron(III) chloride hexahydrate, potassium hydroxide, hydroxylamine hydrochloride, palladium on carbon 10 wt% loading), Alfa (2,4-dihydroxybenzoic acid, 2,3-dihydroxybenzoic acid, 3,5-dihydroxybenzoic acid), Fluka (N-methylmorpholine, ethyl chloroformate, 3-hydroxybenzoic acid), Riedl de Haen (iron(III) chloride anhydrous) or Acros (vanillic acid, cinnamic acid, syringic acid, benzyl bromide, sodium bicarbonate) and used without further purification. All solvents were of analytical grade and used without further purification. If not otherwise stated the substances were synthesized and purified according to the general procedure. $^1$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. UV-vis experiments were performed 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. Electrospay ionization mass spectra were measured with a Bruker maXis ESI-QqTOF spectrometer in the positive and negative mode using ACN/Methanol 1% H$_2$O as solvent. 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. ATR-IR spectra were measured using a Bruker Vertex 70 Fourier transform IR spectrometer. Cyclic voltammograms 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$_4$N][BF$_4$], using [Fe($n$-$^5$-C$_2$H$_3$)$_2$] $^{[E_{1/2} = +0.6–0.68}$ V vs. NHE] as internal standard and are quoted relative to the normal hydrogen electrode NHE. Electron paramagnetic resonance (EPR) spectra were acquired on a Bruker Elexys-II E500 CW-EPR spectrometer operating at X-band with 10 kHz modulation frequency. All samples were dissolved in DMF and transferred to a 3 mm inner diameter quartz EPR tube, which was placed into a high-sensitivity cavity (SHQE1119). The spectra of all samples and the solvent alone were recorded at 90 ± 1 K with a variable nitrogen temperature control system using the following settings: center field: 6000 G; sweep width: 12 000 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. The rhombic symmetry of the $S = 5/2$ spin system was determined by the experimentally observed effective $g$-values using visual RHOMBO. X-ray diffraction measurements were performed on a D8 Venture at 100 K experimental parameters of the X-ray diffraction measurements are listed in Table S1–S13.† Distortion parameters for all diffraction measurements are listed in Table S14.† SHELXTL was used to process the data. The structures were solved by direct methods and refined by full-matrix least-squares techniques. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were inserted at calculated positions and refined with a riding model or as free rotating groups. The following computer programs were used: structure solution, SHELXS-97;† refinement, SHELXL-2013 (ref. 55) OLEX2; molecular diagrams. The crystallographic data files of the complexes have been deposited at the Cambridge Crystallographic Database as CCDC 1436734 (6a), 1436733 (6b), 1436732 (6d), 1436731 (6c), 1436730 (6g), 1436729 (6j), respectively.† Algal growth experiments were carried out with batch cultures of the unicellular chlorophyte species C. salina, strain SAG 8.86 and haptophyte D. lutheri, strain SAG 926-1 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 flasks, containing EDTA as a complexing agent, prepared with 35% salinity artificial seawater as described by Kester et al. 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 S15). 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. To test benzoic hydroxamates-based iron complexes, the respective iron concentration was added in form of the respective complex into the f/2 medium and 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 approx. 9 × 10⁵ cells per mL. In the first and second experiment on C. salina, five and three model compounds were tested respectively. The algal growth experiment on D. lutheri was carried out with six model compounds. The concentrations of tested substances were 11.7 μmol L⁻¹. Moreover in each experiment one sample with isolated humic acid fraction and three control samples were (described above) tested. The experiments were carried out over a period of 24 to 30 days (depending on algal growth) and the algae concentration was monitored after 7 days from beginning of experiment and then every other day. 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 D. lutheri, 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 iron complexes

[Fe(eudesmic hydroxamate)] (6a). FeCl₃ (18.5 mg, 0.11 mmol) dissolved in methanol (2 mL) was added to the suspension of eudesmic hydroxamic acid 4a (79 mg, 0.35 mmol) and KOH (20 mg, 0.35 mmol) in methanol (5 mL). The dark red solution was stirred for 2 h, filtered and the solvent was removed in vacuo. The residue was extracted with dichloromethane (20 mL), the solution was filtered and the solvent was removed. The obtained residue was dissolved in a small amount of methanol (2 mL) and stored overnight at 4 °C. The formed red precipitate was filtered off and then dried in vacuo. Yield: 66 mg, 0.09 mmol, 82%. X-ray diffraction quality single crystals were grown in acetone/water (slow evaporation). ESI-MS: m/z 773 [M + K]⁺, 757 [M + Na]⁺, 547 [M - L + K]⁺, anal. calc’d for C₃₀H₃₆Fe₃N₃O₁₅: C, 49.06; H, 4.94; N, 5.72; O, 32.67; found: C, 48.78; H, 4.60; N, 5.60; O, 32.56. IR (ATR, selected bands, r_max): 3220, 1567, 1518, 1489, 1414, 1362, 1298, 1242, 1124, 1077, 1030, 978, 858, 828, 771, 719 cm⁻¹.

[Fe(syringic hydroxamate)] (6b). FeCl₃ (87 mg, 0.53 mmol) dissolved in methanol (2 mL) was added to the suspension of 4b (272 mg, 1.64 mmol) and KOH (89.4 mg, 1.6 mmol) in methanol (10 mL). The dark red solution was stirred for 2 h, filtered and the solvent was removed in vacuo. The residue was extracted with acetone (10 mL) and left in refrigerator overnight. The suspension was filtered and diethyl ether was slowly added yielding to the formation of a red precipitate. The product was filtered off and dried in vacuo. Yield: 181 mg, 0.32 mmol, 61%. X-ray diffraction quality single crystals were grown in ethanol (slow evaporation). ESI-MS: m/z 565 [M + Na]⁺, 380 [M – L + H]⁺; anal. calc’d for C₂₄H₂₄Fe₃N₃O₁₅H₂O: C, 57.87; H, 4.68; N, 7.50; found: C, 57.85; H, 4.66; N, 7.33. IR (ATR, selected bands, r_max): 2983, 1639, 1514, 1449, 1360, 1295, 1208, 1068, 1012, 968, 852, 752, 752, 698, 670, 580 cm⁻¹.

[Fe(salicilyc hydroxamate)] (6c). FeCl₃ (96 mg, 0.59 mmol) dissolved in methanol (5 mL) was added to the suspension of 5c (380 mg, 1.78 mmol) and KOH (100 mg, 1.78 mmol) in methanol (12 mL). The dark red solution was stirred for 2 h, filtered and the solvent was removed in vacuo. The residue was extracted with MeOH/CHCl₃, 1:1 (100 mL) and left in refrigerator overnight. The suspension was filtered and diethyl ether was slowly added yielding to the formation of a red precipitate. The product was filtered off and then dried in vacuo. Yield: 170 mg, 0.23 mmol, 37%. ESI-MS: m/z 731 [M + K]⁺, 715 [M + Na]⁺, 693 [M + H]⁺; anal. calc’d for C₂₇H₃₀Fe₃N₃O₁₅: 0.75CHCl₃HCl: C, 42.63; H, 3.96; N, 5.37; found: C, 42.61; H, 4.22; N, 5.40. IR (ATR, selected bands, r_max): 3270, 1576, 1491, 1424, 1353, 1289, 1251, 1223, 1117, 1073, 979, 832, 776, 724, 571 cm⁻¹.

[Fe(3-hydroxybenzoic hydroxamate)] (6c). FeCl₃ (127 mg, 0.78 mmol) dissolved in methanol (3 mL) was added to the suspension of 5d (352 mg, 2.34 mmol) and KOH (128 mg, 2.34 mmol) in methanol (20 mL). The dark red solution was stirred for 2 h, filtered and the solvent was removed in vacuo. The residue was extracted with methanol (20 mL) and stored at 4 °C overnight. The suspension was filtered and the solvent was evaporated to small volume. Slow diffusion of diethyl ether into the methanolic phase resulted in the formation of dark red precipitate. The product was filtered off and then dried in vacuo. Yield: 199 mg, 0.34 mmol, 43%. X-ray diffraction quality single crystals were grown in acetone (slow evaporation). ESI-MS: m/z 535 [M + Na]⁺; anal. calc’d for C₂₃H₁₈Fe₃N₃O₁₅: C, 49.24; H, 3.54; N, 8.20; found: C, 49.40; H, 3.85; N, 8.11. IR (ATR, selected bands, r_max): 2970, 1603, 1566, 1480, 1385, 1312, 1245, 1154, 1098, 1057, 1034, 919, 858, 748, 666, 578 cm⁻¹.

[Fe(3-hydroxystilbene hydroxamate)] (6e). FeCl₃ (68 mg, 0.42 mmol) dissolved in methanol (3 mL) was added to the suspension of 5e (193 mg, 1.3 mmol) and KOH (71 mg, 1.26 mmol) in methanol (20 mL). The dark red solution was stirred for 2 h, filtered and the solvent was removed in vacuo. The residue was extracted with acetone (20 mL) and stored in refrigerator overnight. The suspension was filtered, the solvent was evaporated and the residue was dissolved in a small
amount of acetone. Slow diffusion of hexane into the acetone solution resulted in the formation of dark red precipitate. The product was filtered off and then dried in vacuo. Yield: 178 mg, 0.29 mmol, 69%. X-ray diffraction quality single crystals were grown in acetone (slow evaporation). ESI-MS: m/z 535 [M + Na]+; m/z 360 [M − Li]+; anal. calcld for C_{21}H_{18}FeN_{3}O_{12}H_{2}O: C, 49.61; H, 4.73; N, 6.81; found: C, 49.3; H, 4.46; N, 6.59. IR (ATR, selected bands, v_max): 3321, 2970, 1595, 1518, 1455, 1382, 1238, 1105, 1069, 997, 907, 825, 717 cm\(^{-1}\).

The precipitate was washed with water and dried in vacuo at 50 °C. Mother liquor was left overnight and second fraction of the product crystallized. Crystals were washed with water and dried in vacuo at 50 °C. Yield: 130 mg, 0.21 mmol, 43%. ESI-MS: m/z 619 [M + Na]+; anal. calcld for C_{21}H_{20}FeN_{3}O_{5}0.5H_{2}O: C, 53.57; H, 5.16; N, 6.94; found: C, 53.53; H, 5.44; N, 6.76. IR (ATR, selected bands, v_max): 3220, 1596, 1512, 1455, 1382, 1238, 1105, 1069, 997, 907, 825, 720 cm\(^{-1}\).

**Isolation of the seawater-soluble humic acid fraction**

Seawater-soluble humic acid fraction was isolated according to the previously published procedure.\(^{31}\) 1 L filtered creek water (Craggie Burn, Scotland UK) was concentrated under reduced pressure to approximately 10 mL. 20 mL of artificial seawater were added and the solution was kept in darkness at 4 °C for 24 h. The sample was filled up to 50 mL with artificial seawater and filtrated through 0.45 µm cellulose-acetate membrane. 3 mL of the precipitate was washed with water and dried in vacuo at 50 °C. Mother liquor was left overnight and second fraction of the product crystallized. Yield: 175 mg, 0.29 mmol, 48%. ESI-MS: m/z 583 [M + Na]+; elemental analysis: calcld for C_{21}H_{24}FeN_{3}O_{12}2H_{2}O: C, 42.3; H, 3.72; N, 7.04; found: C, 41.97; H, 3.56; N, 6.78; O, 37.19. IR (ATR, selected bands, v_max): 3196, 1565, 1487, 1358, 1306, 1210, 1158, 1125, 1063, 1001, 983, 847, 815, 675 cm\(^{-1}\).

**[Fe(p-hydrocoumaric hydroxamato)] (6f).** FeCl_{3}·6H_{2}O (168 mg, 0.62 mmol) dissolved in water (2 mL) was added to the solution of 5j (337 mg, 1.87 mmol) and KOH (105 mg, 1.87 mmol) in water (20 mL) at 50 °C. Dark red precipitate was formed and the suspension was stirred at 50 °C for 2 h and filtered while hot. The precipitate was washed with water and dried in vacuo at 50 °C. Mother liquor was left overnight and second fraction of the product crystallized. Crystals were washed with water and dried in vacuo at 50 °C. Yield: 130 mg, 0.21 mmol, 43%. ESI-MS: m/z 619 [M + Na]+; anal. calcld for C_{21}H_{20}FeN_{3}O_{5}0.5H_{2}O: C, 53.57; H, 5.16; N, 6.94; found: C, 53.53; H, 5.44; N, 6.76. IR (ATR, selected bands, v_max): 3220, 1596, 1512, 1455, 1382, 1238, 1105, 1069, 997, 907, 825, 720 cm\(^{-1}\).

**[Fe(dihydrocaffeic hydroxamato)] (6k).** FeCl_{3} (195.8 mg, 1.2 mmol) dissolved in methanol (5 mL) was added to the suspension of 5k (710 mg, 3.6 mmol) and KOH (201 mg, 3.6 mmol) in methanol (25 mL). The dark brown solution was stirred for 2 h, filtered and the solvent was removed in vacuo. The dry residue was extracted with MeOH/CHCl_{3} 1:1 (60 mL) and left in refrigerator overnight. The suspension was filtered and diethylether was slowly added yielding the formation of a black precipitate. The product was filtered off and then dried in vacuo at r.t. Yield: 168 mg, 0.21 mmol, 18%. ESI-MS: m/z 667 [M + Na]+; m/z 449 [M+2 + H]+; anal. calcld for C_{21}H_{20}FeN_{3}O_{5}0.75CHCl_{3}·0.75MeOH: C, 45.16; H, 4.49; N, 5.54; found: C, 45.23; H, 4.78; N, 5.21. IR (ATR, selected bands, v_max): 3321, 2970, 1595, 1518, 1449, 1359, 1282, 1238, 1195, 1114, 1068, 1032, 1000, 862, 805, 717 cm\(^{-1}\).

**[Fe(vanillic hydroxamato)] (6l).** FeCl_{3}·6H_{2}O (177 mg, 0.65 mmol) dissolved in water (2 mL) was added to the solution of 5l (362 mg, 1.99 mmol) and KOH (110 mg, 1.96 mmol) in water (20 mL) at 50 °C. Red precipitate was formed and the suspension was stirred at 50 °C for 2 h and filtered hot. The precipitate was washed with water and dried in vacuo at 50 °C. Yield: 200 mg, 0.31 mmol, 47%. ESI-MS: m/z 625 [M + Na]+; elemental analysis: calcld for C_{21}H_{24}FeN_{3}O_{5}2H_{2}O: C, 45.16; H, 4.42; N, 6.58; found: C, 45.13; H, 4.41; N, 6.11. IR (ATR, selected bands, v_max): 3202, 1597, 1485, 1339, 1288, 1233, 1121, 1054, 1029, 955, 852, 788, 708, 649 cm\(^{-1}\).
the filtrate were treated with 1 mL of concentrated nitric acid to estimate the iron content with GF-AAS (6.131 mg L\(^{-1}\)). For algal growth experiments, the applied volume of the concentrated humic acid sample contained the same iron amount as the modified sterile f/2 medium (1.17 \times 10^{-5} \text{ mol L}^{-1}).

**Results and discussion**

**Synthesis – general overview**

The hydroxamate ligands were synthesized starting from the respective commercially available benzoic acids, which were converted into the corresponding methyl esters 1c-1l. Methyl esters bearing free phenolic groups were protected with benzyl groups (2c-2l) to avoid undesired side reactions. In the next step the methyl esters were cleaved under alkaline conditions and converted to the corresponding hydroxamic acids in a one-step approach using ethyl chloroformate to generate intermediately the more reactive anhydrides which were treated with hydroxylamine yielding the desired hydroxamic acids (4b-4l).\(^{11}\) Cleavage of the benzyl groups was performed by palladium-catalyzed hydrogenation yielding the desired ligands (5c-5l) in good yields (Scheme 1). 3,4,5-Trimethoxybenzhydroxamic acid 4a was synthesized according to the previously published procedure by reaction of 3,4,5-trimethoxybenzoic acid with thionyl chloride and subsequent conversion with hydroxylamine hydrochloride under alkaline conditions.\(^{46}\) The Fe(III) complexes were synthesized by deprotonation of the ligand with potassium hydroxide and addition of iron chloride in aqueous potassium hydroxide and addition of iron chloride in aqueous or methanolic solution (Scheme 2).

The purification of the complexes were found to be the crucial step of the synthesis, because the complexes were good to highly soluble in protic solvents as the formed by-product KCl. Separation via Sephadex G-10 or LH 20 failed and therefore a broad range of different purification strategies were tested. In some cases (6f, 6h, 6i, 6j and 6l) work up could be facilitated by using water as reaction solvents. However, multiple extraction steps were necessary for complexes 6c and 6k to isolate the salt-free compound explaining the poor yields. Overall, the complexes were isolated in poor to good yields (18-82%) depending on the utilized purification protocol. In order to confirm the formation of the desired products, \(^{1}H\)-NMR spectroscopy, electrospray ionization mass spectrometry (ESI-MS) and elemental analyses were performed for the synthesized ligands (see ESI†). Due to the paramagnetic properties of Fe(III) complexes, \(^{1}H\)-NMR spectroscopy could not be utilized for characterization and therefore other methods were used instead (elemental analysis, ESI-MS, X-ray diffraction analysis). The ESI-MS spectra of the complexes (ESI-MS, positive and negative mode) were measured in methanol or in acetonitrile. Overall the positively charged H\(^+\), Na\(^+\) or K\(^+\) adducts were detected. For example complex 6a showed peaks with m/z values of 773, 757 and 547, which were assigned to the [Fe(C\(_{10}\)H\(_{12}\)NO\(_{5}\)Na\(^+\)], [Fe(C\(_{10}\)H\(_{12}\)NO\(_{5}\)K\(^+\)] and [Fe(C\(_{10}\)H\(_{12}\)NO\(_{5}\)K\(^+\)] species, respectively. In the case of the dinuclear Fe(III) complex 6g a peak at m/z 390, which can be assigned to [Fe(C\(_{7}\)H\(_{8}\)NO\(_{4}\)H\(^+\)) - 2H\(^+\)]. All detected molecular peaks of the complexes showed the typical isotopic iron pattern. Also the electrochemical behaviour of the complexes 6a-l and their corresponding ligands was studied by means of cyclic voltammetry. Due to the redox activity of the ligands and subsequent reactions after irreversible reduction of iron(III) to iron(II), interpretation of the cyclic voltammograms and clear assignment of the iron reduction peak was not clearly achievable. What can be derived is that all complexes (see S20-S31†) showed only irreversible reduction waves. Depending on the ligand scaffold, the possible reduction peaks of iron(II) were found within a broad potential range but in most cases between -1.6 and -0.5 V vs. NHE. However, the measured values are distinctly lower compared to the reported potentials of Fe(II)-EDTA or Fe(II)-SOD (+0.12 and +0.27 V, respectively)\(^{27}\) and therefore more in the region of ferrienterobactin and ferrioxamine (-0.75 and -0.45 V, respectively).\(^{44}\) In this case Fe(III) cannot be reduced by biological reduction agents and the Fenton reaction is not feasible.

**Single-crystal X-ray analysis**

Single crystals suitable for X-ray diffraction analysis were obtained for complexes 6a, 6b, 6d, 6e, 6g, 6j by either precipitation from acetone, water, methanol, ethanol or slow diffusion.
of diethyl ether into acetone or methanol solution (see Experimental section, see Fig. 1 and S14–S19†). The complexes 6j and 6g crystallized in the monoclinic space group P21/n and C2/c, respectively. The complex 6a crystallized in the triclinic centrosymmetric space group P1 and 6e in the orthorombic space group Pbca. In case of monomeric iron complexes, the octahedral Fe(III) center is surrounded by three chelate ligands bound via oxygen atoms of the hydroxamate groups (Table S14†). Compounds 6g and 6b crystallized in the monoclinic space group P21/c (Fig. 1). X-ray diffraction studies of 6g confirmed the formation of a dinuclear Fe(III) complex. Catechol groups are well known for their chelating properties and strong interaction with iron; however, the hydroxamic groups seem to have higher affinity towards iron explaining the observed structure. Important bond lengths between iron and the donor atoms are shown in Table 1.

Comparison with the EXAFS studies of natural humic acids showed the similarity to the model compounds. In all crystallized complexes the Fe–O bond distances were around 2 Å and are in the same range as the distances in humic acids. The bond length indicates the octahedral iron(III) configuration. Complex 6b, with the Fe–O bond length of 1.98 Å showed best agreement with experimental values from EXAFS studies. The Fe···C distance in model compounds was between 2.78 Å and 2.81 Å and significantly shorter as in natural complexes where this bond is about 2.95 Å. Due to the shorter distance to carbon, the Fe–O–C bond angle was around 112° and also smaller as described in the literature namely 125°.

The EXAFS studies of natural humic acids indicate the presence of monomeric iron complexes in the solution which fits to all models with exception of 6g. In 6g the Fe···Fe bond length of 3.28 Å is in agreement with the values measured for the precipitated fulvic acids where the dimeric/polymeric form is predominant. Overall the synthesized hydroxamate-based Fe(III) complexes seem to be appropriate models for humic acid.

EPR spectroscopy
EPR measurements revealed the presence of rhombic high spin Fe(III) in all samples (Fig. 2). The EPR line at g_{eff} = 4.25 is
Stability constants of the complexes with ligand seawater the formation of trihydroxamates is highly preferred. As a completely rhombic system is reached at $\frac{4}{3}$, a weak feature at approximately $5/2$ systems. As a completely rhombic system is reached at $\frac{4}{3}$, the crystal rhombicity ($\eta$) as determined by the zero-field splitting parameters, was calculated to be 0.327 for all the $S = 5/2$ systems. As a completely rhombic system is reached at $\eta = 0.33$, the crystal field at the Fe(III) center shows a strong orthorhombic character.

**Stability studies**

The stability of these model compounds in aqueous systems is important for further biological investigations, because the complexes have to be stable enough to enable cellular iron accumulation and if the complex decomposes too fast, the released iron will precipitate in water. According to literature iron shows high affinity to ligands bearing hydroxamic groups and iron monohydroxamates are, under the condition that no other instable groups are present, in most cases stable in aqueous solutions. At pH regions of our interest, namely 8.2–8.4 (seawater) the formation of trihydroxamates is highly preferred. Stability constants of the complexes with ligand scaffolds similar to our models have been already estimated. The determined values were in the range between 28.8 and 29.7 for the formation of trihydroxamates. Logarithmic stability constants of the complexes with benzoic hydroxamic acid and propanohydroxamic acid, which show structural similarities with our models, were 28.8 and 28.44 respectively. Comparing those values with the stability constants of Fe(III) EDTA which is 25.1, iron trihydroxamates are supposed to be more stable. However it should be considered that EDTA possesses six binding sides and the formation of its complex with iron(III) is thermodynamically favoured in comparison to bidentate hydroxamates. In order to prove this presumption, we investigated the stability of 6e (25 nM) treated with EDTA in distilled water and seawater. After addition of EDTA (25 nM) to the solution of 6e, significant changes in UV-Vis spectra were observed over 24 h (see S12–S13) indicating ligand exchange reactions. Therefore EDTA was not added to the complex solutions used for algal growth experiments. The aqueous stability of the synthesized complexes in seawater at pH = 8.2–8.4 (see Fig. S1–S4†) and distilled water (see Fig. S5–S9†) was monitored over 24 h by means of UV-Vis spectrometry. Complex 6k bearing hydrocaffeic hydroxamate was found to be the most instable over this time range, whereas most of the investigated complexes showed no changes in their UV-Vis spectra under these conditions (Fig. 3). Furthermore, 6k showed an increased stability in distilled water over 24 h. The possible explanation for the changes in the recorded UV-Vis spectra of 6k can be attributed to the presence of a uncoordinated catecholic moiety, which is prone to photo-oxidation in seawater; however, stabilization can be achieved by coordination of iron(III) ions.

The obtained results confirm that the Fe(III) is tightly bound to the hydroxamate and the free catechol group is able to react with other metal ions or undergo oxidation. In seawater, the intensity of the signal at 335 nm increases over 24 h and also a new signal arose at 380 nm over time. In distilled water only the signal at 335 nm increased but no differences in absorbance at 380 nm was observed. Measurements at pH 11 showed the increase in absorbance at 335 nm and also the signal at 380 nm, which appeared directly after pH-adjustment from 7 to 11. On the basis of these results, the signal at 335 nm can be assigned to the oxidation of catecholic moiety, which proceeds more slowly in distilled water. The signal at 380 nm seems to be associated with the deprotonation of 6k which occurs very fast at pH 11 in contrast to pH 8.2–8.4 (seawater). Free catecholic
moieties are also present in the structure of 6g, but in contrast to 6k, this model compound remained stable in solution. This contrary behaviour might be explained by the formation of stable hydrogen bonds between the nitrogen and the oxygen atoms of the ligand scaffold. To investigate the impact of the ligand scaffold on the observed different behaviour of 6g and 6k, the stability of the respective free ligands, namely dihydrocaffic hydroxamic acid and hypogallic hydroxamic acid, were determined in both seawater and distilled water. Both were found to be instable in seawater and probably oxidation occurred over time. Complex 6k was not the only instable compound in seawater. We also saw significant changes in UV-Vis spectra of the complexes 6f, 6i and 6l (Fig. 4). In contrast to the decay in seawater, these compounds were found to be remarkably stable in distilled water. The observed results for 6f, 6i and 6l might be explained by the reaction or interaction with metal ions from seawater. Also the pH value of approximately 8.2–8.4 can contribute to the observed changes. To verify this assertion the stability of those complexes at pH 11 was investigated. In case of the complexes 6f and 6l it is most likely that both of them are deprotonated at higher pH value (see Fig. S10 and S11†). The increasing signal in seawater over 24 h is present in the spectra obtained at pH 11 immediately after base addition. The complex 6i seems to interact with seawater components because no influence of pH change on stability has been observed.

Algal growth experiments

C. salina and D. lutheri showed significantly different growth response depending on the tested model compounds and control samples. Fig. 5 shows the respective growth curves of C. salina batch cultures using different iron sources and chelating agents. Without iron supply the culture can’t grow effectively after its own intracellular reserve is consumed. If our compounds do not supply algae with iron, the culture growth will be comparable or worse. As second negative control we supplied the culture with iron but we waived the chelator EDTA.

Fig. 3 Time dependent UV-Vis spectra of 6k (above) and 6e (below) in seawater over 24 hours (spectra were measured in an 1 h interval, start and end point of the measurement are indicated by an arrow).

Fig. 4 Time dependent UV-Vis spectra of 6f (A, B) and 6l (C, D) in seawater (A, C) and in distilled water (B, D). (The spectra were measured over 24 h in an 1 h interval, start and end point of the measurement are indicated by an arrow in (A) and (C)).

Fig. 5 Growth curves of C. salina (error bars: ±SD) treated with model compounds 6c, 6d, 6e, 6h, 6j and isolated fraction of humic acid compared to control samples (+Fe, +EDTA; +Fe, −EDTA; −Fe, +EDTA).
In this case the bioavailability of iron and other metal ions is decreased. The EDTA complex with iron has good aqueous solubility; however, the metal is bound very strongly to chelating ligand, limiting its bioavailability. Nevertheless the complex is light sensitive, which results in reduction of Fe(III) to Fe(II) and release of iron (lower affinity of EDTA towards Fe(II)).

Thus the growth of *C. salina* without EDTA is expected to be worse than in the ideal medium. This negative control evaluates the impact of the chelator and other metal ions on the culture. It also shows if the algal species can uptake iron and other metal ions at very low concentrations and how far the growth will be inhibited. Samples with ideal f/2 medium without iron showed, as expected, poor growth.

Controls with medium with iron but without the chelator EDTA showed moderate growth rates whereas samples with full medium with iron and EDTA have grown to algae concentration EDTA showed poor growth. Samples with ideal f/2 medium without iron showed, also shows if the algal species can uptake iron and other metal ions under control conditions between several cultures. In the second experiment, the bioavailability of iron. Complexes 6e and 6d with one free hydroxy group were more efficient promoting growth of *C. salina* than the complexes with methoxy groups or aliphatic chains with relative poor water solubility like 6j and 6c. Another aspect of the first experiment was the different growth patterns of the cultures. Samples with HS and iron without EDTA started to grow very soon but we noticed the short delay in growth of the sample with the ideal medium.

This delay was much more significant in case of model compounds. We observed the distinct changes in algae concentration after approximately two weeks. That lead us to the conclusion that the stability of the complexes in water seems to play an important role for the release and bioavailability of iron. Unfortunately it was not possible to monitor the growth of the culture longer than 30 days due to the limited stability of f/2 medium over this time period. Further observation would not provide reliable values due to the changing conditions between several cultures. In the second experiment, three model compounds namely 6b, 6i and 6k were tested. Fig. 6 shows growth curves of *C. salina* batch cultures under different conditions including standard deviation.

### Table 2 Relation between the end algae concentration of control samples to samples treated with 6c, 6d, 6e, 6h, 6j, and isolated humic acid

<table>
<thead>
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<th>6c</th>
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<th>6e</th>
<th>6h</th>
<th>6j</th>
<th>Humic acid</th>
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<td>+Fe, +EDTA in%</td>
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<td>1.6</td>
<td>0.8</td>
<td>0.2</td>
<td>0.3</td>
<td>3</td>
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</table>

Fig. 6 Growth curves of *C. salina* (error bars: ±SD) treated with model compounds 6b, 6i and 6k compared to control samples (+Fe, +EDTA; +Fe, −EDTA; −Fe, +EDTA).
Table 3  Relation between the end algae concentration of control samples to samples treated with 6b, 6i and 6k

<table>
<thead>
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<th>6b</th>
<th>6i</th>
<th>6k</th>
<th>Humic acid</th>
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</thead>
<tbody>
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<td>+Fe, +EDTA in %</td>
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<tr>
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<td>0.1</td>
<td>2.1</td>
<td>3.6</td>
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</table>

The samples kept at ideal conditions reached after 24 days the algae concentration of approximately $4.5 \times 10^5 \pm 8.2 \times 10^4$ cells per mL whereas the growth of cultures lacking EDTA or iron was poor. In contrast to the first experiment, control samples with chelator and without iron reached slightly higher concentration as control samples with iron without chelator. Nevertheless both values were low. Similar to the first experiment, samples containing isolated humic acid fraction had the biggest positive impact on the culture, reaching at the end of the experiment the concentration near $10 \times 10^6 \pm 6.7 \times 10^5$ cells per mL which was better as the end concentration of the ideal medium. Model compounds 6b and 6i did not significantly support the growth of C. salina. In fact, in samples treated with 6b the lowest final algal concentration was observed. This can be explained with hydrophobic character and low solubility of the cinnamic hydroxamic acid in the complex 6b. UV-Vis studies of this compound have shown significant decrease in absorbance after 24 h due to the precipitation. Together with the results from the algal growth experiments, we assume that the iron bound in 6b is not sufficiently bioavailable for the C. salina. Samples containing 6k showed best response to the treatment and reached the concentration of $1.3 \times 10^6 \pm 5.2 \times 10^5$ cells per mL which is 30% of the algal concentration in ideal medium (Table 3). This effect of 6k on algae growth can be explained by the moderate stability of the complex in seawater which was already mentioned in UV-Vis studies. Moreover the ligand in 6k has two possible metal-binding sides. Dihydrocafeeic hydroxamic acid is bound via hydroxamate to iron and the catecholic group is free for coordination which might additionally enhance the bioavailability of other metal ions as for example Zn and Cu.

This is an important factor because it is unlikely to achieve the same growth rate of the culture as under the ideal conditions using model complexes instead of EDTA and iron chloride. EDTA is not only chelator for iron but also for other metals included in micronutrient solution like for example Cu, Zn and Mn. In order to compare the results from the first two experiments with C. salina, we performed the same tests with the photoautotrophic algal species D. lutheri. In this experiment, six model compounds namely 6c, 6d, 6e, 6g, 6j and 6k were investigated. Fig. 7 shows the determined growth curves of D. lutheri batch cultures under different conditions including standard deviation. The culture of D. lutheri reached at all tested conditions significantly lower cell numbers in the growth experiments compared to C. salina. Control samples with ideal medium with iron and EDTA have grown to algae concentration of approximately $2.1 \times 10^6 \pm 7.1 \times 10^5$ cells per mL.

Surprisingly the samples containing medium with iron but without the chelator EDTA had almost the same impact on the culture as the positive control whereas the samples lacking iron reached the end concentration of $1 \times 10^6 \pm 6 \times 10^4$ cells per mL.

These findings might be explained by slower growth due to the lower cell number, higher intracellular iron pool or better iron uptake at low concentrations. As already observed in C. salina, samples treated with soluble humic acid fraction showed the best growth response. None of the investigated model compounds showed a big impact on the culture and the concentrations at the end of the experiment remained under the concentration of samples treated with ideal medium without iron. The complexes 6c and 6j did not support the growth of D. lutheri at all. Those two complexes had also been found to be the less effective in the culture of C. salina. The relation between the concentration of algae in samples treated with model compounds and control samples at the end of the experiment is shown in Table 4.

In all performed algal experiments, both C. salina and D. lutheri showed the typical growth pattern in full medium. In iron-free samples (−Fe, +EDTA) the growth was strongly inhibited, which means, iron deficiency conditions were given. The relative long time between the beginning of each experiment and start of the exponential growth is typical and known.
Conclusions

Herein, we present the synthesis of 11 novel iron(III) complexes as promising model compounds for humic acids. All complexes were characterized by elemental analysis, ESI-MS, IR, EPR, cyclic voltammetry and some of them by X-ray diffraction analysis. Comparison of the bond lengths, angles and distances were characterized by elemental analysis, ESI-MS, IR, EPR, cyclic voltammetry and some of them by X-ray diffraction analysis. The ligand scaffold and the presence of different functional groups seem to have a big influence on the stability and solubility of the compounds, which was monitored in water and seawater over 24 h. We determined the impact of the synthesized model compounds on growth of the algae species *C. salina* and *D. lutheri*. The study revealed that several of our models support the growth of the algae culture confirming that the iron bound in the complexes is bioavailable. The results obtained from algal growth experiments support our proposal that iron complexes with benzoic hydroxamates are suitable models for the research on iron binding ability of humic substances; however, the induced growth upon treatment with the model compounds is still lower compared to ideal conditions and might be explained by the observed low redox potentials of the complexes. Therefore optimization with regard on the algal growth and redox potentials is required and currently ongoing. Furthermore, the synthesized model compounds will be used as reference substances in EXAFS experiments, where the similarity in the nearest surrounding of iron in the natural humic acids will be investigated.

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Notes and references

SUPPLEMENTARY INFORMATION

Benzoic hydroxamates-based iron complexes as models compounds for humic substances: synthesis, characterization and algal growth experiments

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**Synthesis of hydroxamates**

**General procedure for preparation of methyl esters**

The respective \( n \)-hydroxy benzoic acid (1 eq, 32 mmol) was dissolved in MeOH (60 mL), and a catalytic amount of sulfuric acid (3 mL) was added. The reaction mixture was stirred at reflux overnight. The resulting mixture was concentrated under reduced pressure and the residue dissolved in EtOAc. The solution was transferred into a separating funnel, washed with saturated NaHCO\(_3\) solution and brine. The combined organic layers were dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated to give the methyl ester 1c–l in good yield.

**Methyl 4-hydroxy-3,5-dimethoxybenzoate (1c)**

Yield: 3.59 g, 15.9 mmol, 84 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 9.32\) (s, 1 H, OH\(_{Ar}\)), 7.22 (s, 2 H, H\(_{Ar}\)), 3.83 (s, 6 H, OCH\(_3\)Ar), 3.82 (s, 3 H, OCH\(_3\)ester) ppm.

**Methyl 2-hydroxybenzoate (1d)**

Yield: 4.98 g, 32.8 mmol, 84 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 10.55\) (s, 1 H, OH\(_{acid}\)), 7.76 (dd, \(J = 4\) Hz, \(J = 2\) Hz, 1 H, H\(_{Ar}\)), 7.51 (ddd, 1 H, \(J = 6\) Hz, \(J = 2\) Hz, \(J = 1\) Hz, H\(_{Ar}\)), 6.98 (dd, \(J = 8\) Hz, \(J = 1\) Hz, 2 H, H\(_{Ar}\)), 6.92 (ddd, 1 H, \(J = 6\) Hz, \(J = 1\) Hz, H\(_{Ar}\)), 3.89 (s, 3 H, OCH\(_3\)ester) ppm.

**Methyl 3-hydroxybenzoate (1e)**

Yield: 2.24 g, 14.7 mmol, 74 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 9.83\) (s, 1 H, OH\(_{Ar}\)), 7.42–7.29 (m, 3 H, H\(_{Ar}\)), 7.04 (m, 1 H, H\(_{Ar}\)), 3.83 (s, 3 H, OCH\(_3\)ester) ppm.

**Methyl 4-hydroxybenzoate (1f)**

Yield: 4.46 g, 29.3 mmol, 91 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 10.32\) (s, 1 H, OH\(_{Ar}\)), 7.82 (d, \(J = 2\) Hz, 2 H, H\(_{Ar}\)), 6.85 (d, \(J = 9\) Hz, 2 H, H\(_{Ar}\)), 3.78 (s, 3 H, OCH\(_3\)ester) ppm.

**Methyl 2,3-dihydroxybenzoate (1g)**

Yield: 4.9 g, 29.1 mmol, 90 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 9.91\) (s, 2 H, OH\(_{Ar}\)), 7.24 (dd, \(J = 8\) Hz, \(J = 2\) Hz, 1 H, H\(_{Ar}\)), 7.04 (dd, \(J = 8\) Hz, \(J = 2\) Hz, 1 H, H\(_{Ar}\)), 6.76 (t, 1 H, \(J = 8\) Hz, H\(_{Ar}\)), 3.89 (s, 3 H, OCH\(_3\)ester) ppm.

**Methyl 2,4-dihydroxybenzoate (1h)**

Yield: 4.25 g, 25.3 mmol, 78 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 10.72\) (s, 1 H, OH\(_{Ar}\)), 10.46 (s, 1 H, OH\(_{Ar}\)), 7.64 (d, \(J = 9\) Hz, 1 H, H\(_{Ar}\)), 6.38 (dd, \(J = 9\) Hz, \(J = 3\) Hz, 1 H, H\(_{Ar}\)), 6.31 (d, 1 H, \(J = 2\) Hz, H\(_{Ar}\)), 3.84 (s, 3 H, OCH\(_3\)ester) ppm.

**Methyl 3,5-dihydroxybenzoate (1i)**

Yield: 4.26 g, 25.3 mmol, 78 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 10.72\) (s, 1 H, OH\(_{Ar}\)), 10.46 (s, 1 H, OH\(_{Ar}\)), 7.64 (d, \(J = 9\) Hz, 1 H, H\(_{Ar}\)), 6.38 (dd, \(J = 9\) Hz, \(J = 3\) Hz, 1 H, H\(_{Ar}\)), 6.31 (d, 1 H, \(J = 2\) Hz, H\(_{Ar}\)), 3.84 (s, 3 H, OCH\(_3\)ester) ppm.
Yield: 4.32 g, 25.7 mmol, 79 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 9.60\) (s, 2 H, OH\(_{Ar}\)), 6.83 (d, \(J = 3\) Hz, 2 H, H\(_{Ar}\)), 6.46 (t, 1 H, \(J = 2\) Hz, H\(_{Ar}\)), 3.80 (s, 3 H, OCH\(_{ester}\)) ppm.

**Methyl 3-(4-hydroxyphenyl)propanoate (1j)**

Yield: 2.03 g, 11.3 mmol, 74 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 9.18\) (s, 1 H, OH\(_{Ar}\)), 7.01 (d, \(J = 8\) Hz, 2 H, H\(_{Ar}\)), 6.70 (d, \(J = 8\) Hz, 2 H, H\(_{Ar}\)), 3.58 (s, 3 H, OCH\(_{ester}\)), 2.76 (t, \(J = 8\) Hz, 2 H, H\(_{Al}\)), 2.56 (t, \(J = 8\) Hz, 2 H, H\(_{Al}\)) ppm.

**Methyl 3-(3,4-dihydroxyphenyl)propanoate (1k)**

Yield: 2.07 g, 10.6 mmol, 96.5 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 10.68\) (s, 1 H, OH\(_{Ar}\)), 7.63 (d, \(J = 8\) Hz, 1 H, H\(_{Ar}\)), 6.58 (d, \(J = 2\) Hz, 1 H, H\(_{Ar}\)), 6.44 (dd, 1 H, \(J = 8\) Hz, \(J = 2\) Hz, H\(_{Ar}\)), 3.58 (s, 3 H, OCH\(_{ester}\)), 2.67 (t, \(J = 8\) Hz, 2 H, H\(_{Al}\)), 2.53 (t, \(J = 8\) Hz, 2 H, H\(_{Al}\)) ppm.

**Methyl 4-hydroxy-3-methoxybenzoate (1l)**

Yield: 5.3 g, 29.1 mmol, 98 %. \(^1\)H NMR (DMSO-d\(_6\), 500.10 MHz, 25 °C): \(\delta = 9.94\) (s, 1 H, OH\(_{acid}\)), 7.47 (d, \(J = 9\) Hz, 1 H, H\(_{Ac}\)), 7.46 (s, 1 H, H\(_{Ac}\)), 6.88 (d, \(J = 8\) Hz, 1 H, H\(_{Ac}\)), 3.83 (s, 3 H, OCH\(_{3Ar}\)), 3.79 (s, 3 H, OCH\(_{3acid}\)) ppm.
General procedure for preparation of protected methyl esters

To a stirred solution of methyl n-hydroxybenzoate (1eq, 27.6 mmol) in acetone (60 mL), was added potassium carbonate (2.25 eq, 62 mmol or 1.2 eq, 31 mmol) followed by benzyl bromide (2.25 eq, 62 mmol or 1.2 eq, 31 mmol) and refluxed for 10 h. The mixture was filtered, washed with acetone (30 mL), 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 ultrasonificated for approximately 5 min and the solvent was decanted (5x) and finally stored for 2 h at 4°C until a precipitate was formed. The solid was filtered off, washed with petrol ether and dried in vacuo.

Methyl 4-(benzyloxy)-3,5-dimethoxybenzoate (2c)
Yield: 3.30 g, 10.9 mmol, 69 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.48–7.27 (m, 5 H, H₆Ar), 7.25 (s, 2 H, H₆Ar), 5.01 (s, 2H, CH₂bn), 3.86 (s, 3 H, OCH₃ester), 3.84 (s, 6 H, OCH₃Ar) ppm.

Methyl 2-(benzyloxy)benzoate (2d)
Yield: 4.19 g, 17.3 mmol, 53 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.77–6.97 (m, 7 H, H₆Ar), 5.19 (s, 2H, CH₂bn), 3.83 (s, 3 H, OCH₃ester) ppm.

Methyl 3-(benzyloxy)benzoate (2e)
Yield: 2.57 g, 10.6 mmol, 74 %. ¹H NMR (DMSO, 500.10 MHz, 25 °C): δ = 7.59–7.29 (m, 9 H, H₆Ar), 5.18 (s, 2 H, s, 2H, CH₂bn), 3.86 (s, 3 H, OCH₃ester) ppm.

Methyl 4-(benzyloxy)benzoate (2f)
Yield: 6.59 g, 27.2 mmol, 93 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): ¹H NMR (DMSO, 500.10 MHz, 25 °C): δ = 7.97–7.06 (m, 9 H, H₆Ar), 5.18 (s, 2 H, s, 2H, CH₂bn), 3.81 (s, 3 H, OCH₃ester) ppm.

Methyl 2,3-bis(benzyloxy)benzoate (2g)
Yield: 8.12 g, 23.3 mmol, 80 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.55–7.16 (m, 13 H, H₆Ar), 5.22 (s, 2H, CH₂bn), 5.02 (s, 2H, CH₂bn), 3.79 (s, 3 H, OCH₃ester) ppm.

Methyl 2,4-bis(benzyloxy)benzoate (2h)
Yield: 4.46 g, 12.8 mmol, 51 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.62–7.17 (m, 13 H, H₆Ar), 5.24 (s, 2H, CH₂bn), 5.19 (s, 2H, CH₂bn), 3.81 (s, 3 H, OCH₃ester) ppm.

Methyl 3,5-bis(benzyloxy)benzoate (2i)
Yield: 7.64 g, 21.9 mmol, 85 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.56–6.91 (m, 8 H, H₆Ar), 5.15 (s, 4H, CH₂bn), 3.84 (s, 3 H, OCH₃ester) ppm.

Methyl 3-(4-(benzyloxy)phenyl)propanoate (2j)
Yield: 2.44 g, 9 mmol, 80 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta = 7.47–6.90$ (m, 9 H, H$_{Ar}$), 5.07 (s, 2H, CH$_{2bn}$), 3.58 (s, 3 H, OCH$_3$ester), 2.79 (t, $J = 8$ Hz, 2 H, H$_{Al}$), 2.58 (t, $J = 8$ Hz, 2 H, H$_{Al}$) ppm.

**Methyl 3-(3,4-bis(benzyloxy)phenyl)propanoate (2k)**

Yield: 3.71 g, 9.8 mmol, 63 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta = 7.52–6.71$ (m, 13 H, H$_{Ar}$), 5.12 (s, 2 H, CH$_{2bn}$), 5.09 (s, 2 H, CH$_{2bn}$), 2.67 (t, $J = 8$ Hz, 2 H, H$_{Al}$), 2.53 (t, $J = 8$ Hz, 2 H, H$_{Al}$) ppm.

**Methyl 4-(benzyloxy)-3-methoxybenzoate (2l)**

Yield: 6.49 g, 23.9 mmol, 82 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta = 7.62–7.11$ (m, 8 H, H$_{bn}$), 5.17 (s, 2H, CH$_{2bn}$), 3.83 (s, 6 H, OCH$_3$Ar, OCH$_3$acid) ppm.
General procedure for preparation of protected benzoic acids

The methyl esters (20 mmol) were hydrolyzed using 30% (w/v) aq KOH (2.5 mL) solution in MeOH (40 mL) which was heated at 80 °C for 10 h. After completion of the reaction, the solvent was evaporated. Water (40 mL) was added to the residue and acidified by addition of HCl (5 M) to pH 1. The formed precipitate was collected by filtration, washed with water and dried in vacuo to afford the carboxylic acids 3c–f as white powders.

4-(Benzyloxy)-3,5-dimethoxybenzoic acid (3c)
Yield: 3.00 g, 10.4 mmol, 95.5 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 12.94 (s, 1 H, OHacid), 7.49–7.27 (m, 5 H, Hbn), 7.25 (s, 2 H, Hbenz), 5.00 (s, 2H, CH₂bn), 3.84 (s, 6 H, OCH₃Ar) ppm.

2-(Benzyloxy)benzoic acid (3d)
Yield: 1.38 g, 6.05 mmol, 93 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 12.66 (s, 1 H, OHacid), 7.70–6.99 (m, 9 H, HAr), 5.21 (s, 2H, CH₂bn) ppm.

3-(Benzyloxy)benzoic acid (3e)
Yield: 3.00 g, 13.1 mmol, 96 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 13.02 (s, 1 H, OHacid), 7.63–7.19 (m, 9 H, HAr), 5.18 (s, 2 H, s, 2H, CH₂bn) ppm.

4-(Benzyloxy)benzoic acid (3f)
Yield: 3.89 g, 17.1 mmol, 63 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 12.65 (s, 1 H, OHacid), 7.98–7.03 (m, 9 H, HAr), 5.19 (s, 2 H, s, 2H, CH₂bn) ppm.

2,3-Bis(benzyloxy)benzoic acid (3g)
Yield: 7.6 g, 22.7 mmol, 98 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.57–7.10 (m, 13 H, HAr), 5.19 (s, 2H, CH₂bn), 5.02 (s, 2H, CH₂bn) ppm.

2,4-Bis(benzyloxy)benzoic acid (3h)
Yield: 4.26 g, 13.1 mmol, 99.5 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.55–7.19 (m, 13 H, HAr), 5.16 (s, 2H, CH₂bn), 5.04 (s, 2H, CH₂bn) ppm.

3,5-Bis(benzyloxy)benzoic acid (3i)
Yield: 5.9 g, 17.7 mmol, 81 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.58–6.86 (m, 8 H, HAr), 5.14 (s, 4H, CH₂bn) ppm.

3-(4-(Benzyloxy)phenyl)propanoic acid (3j)
Yield: 1.79 g, 7 mmol, 77 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 12.09 (s, 1 H, OHacid), 7.48–6.88 (m, 9 H, HAr), 5.07 (s, 2H, CH₂bn), 2.76 (t, J = 8 Hz, 2 H, HAl), 2.49 (t, J = 8 Hz, 2 H, HAl) ppm.
3-(3,4-Bis(benzyloxy)phenyl)propanoic acid (3k)
Yield: 3.54 g, 9.78 mmol, 99 %. $^1$H NMR (DMSO-d$_6$, 500.10 MHz, 25 °C): δ = 7.49–6.70 (m, 13 H, H$_{Ar}$), 5.10 (s, 2 H, CH$_{2bn}$), 5.08 (s, 2H, CH$_{2bn}$), 2.74 (t, $J$ = 8 Hz, 2 H, H$_{Al}$), 2.49 (t, $J$ = 8 Hz, 2 H, H$_{Al}$) ppm.

4-(Benzyloxy)-3-methoxybenzoic acid (3l)
Yield: 5.49 g, 21.3 mmol, 89 %. $^1$H NMR (DMSO-d$_6$, 500.10 MHz, 25 °C): δ = 7.58–7.07 (m, 8 H, H$_{bn}$), 5.17 (s, 2H, CH$_{2bn}$), 3.81 (s, 3 H, OCH$_{3Ar}$) ppm.
General procedure for preparation of protected hydroxamic acids

To a solution of the benzoic acid (0.88 g, 10 mmol) in diethyl ether (30 mL) ethyl chloroformate (1.3 g, 12 mmol) and $N$-methylmorpholine (1.3 g, 13 mmol) were added at 0 °C and the mixture was stirred for 30 min. The formed solid was filtered off and the filtrate was added to freshly prepared methanolic hydroxylamine (0.5 g, 15 mmol) solution. The reaction mixture was stirred at room temperature for 45 min. The formed white precipitate was filtered off and washed with diethyl ether. The crude hydroxamic acid was recrystallized from EtOAc or EtOAc/PE (8:2) to give the respective hydroxamic acid as white powder.

$N$-Hydroxycinnamamide (4b)
Yield: 0.272 g, 1.7 mmol, 17 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 10.75 (s, 1 H, NH$_{acid}$), 9.07 (s, 1 H, OH$_{acid}$), 7.47 (d, $J$ = 16 Hz, 1 H, H$_{Al}$), 7.60–7.35 (m, 5 H, H$_{Ar}$), 6.48 (d, $J$ = 16 Hz, 1 H, H$_{Al}$) ppm.

4-(Benzyloxy)-$N$-hydroxy-3,5-dimethoxybenzamide (4c)
Yield: 1.10 g, 3.6 mmol, 35 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 11.15 (s, 1 H, NH$_{acid}$), 9.03 (s, 1 H, OH$_{acid}$), 7.49–7.28 (m, 5 H, H$_{bn}$), 7.11 (s, 2 H, H$_{benz}$), 4.96 (s, 2H, CH$_2$bn), 3.83 (s, 6 H, OCH$_3$Ar) ppm.

2-(Benzyloxy)-$N$-hydroxybenzamide (4d)
Yield: 1.38 g, 5.7 mmol, 94 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 11.20 (s, 1 H, NH$_{acid}$), 9.09 (s, 1 H, OH$_{acid}$), 7.56–7.30 (m, 9 H, H$_{Ar}$), 7.16 (t, $J$ = 9 Hz, 1 H, H$_{Ar}$), 7.01 (t, $J$ = 7 Hz 1 H, H$_{Ar}$), 5.23 (s, 2H, CH$_2$bn) ppm.

3-(Benzyloxy)-$N$-hydroxybenzamide (4e)
Yield: 0.779 g, 3.2 mmol, 32 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ =11.10 (s, 1 H, NH$_{acid}$), 9.05 (s, 1 H, OH$_{acid}$), 7.51–7.12 (m, 9 H, H$_{Ar}$), 5.15 (s, 2 H, s, 2H, CH$_2$bn) ppm.

4-(Benzyloxy)-$N$-hydroxybenzamide (4f)
Yield: g, 0.67, 2.8 mmol, 31 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 11.10 (s, 1 H, NH$_{acid}$), 8.91 (s, 1 H, OH$_{acid}$), 7.81–7.00 (m, 9 H, H$_{Ar}$), 5.17 (s, 2H, CH$_2$bn) ppm.

2,3-Bis(benzyloxy)-$N$-hydroxybenzamide (4g)
Yield: g, 2.31, 6.6 mmol, 75 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 10.74 (s, 1 H, NH$_{acid}$), 9.11 (s, 1 H, OH$_{acid}$), 7.56–6.94 (m, 13 H, H$_{Ar}$), 6.51 (s, 2H, CH$_2$bn), 5.02 (s, 4H, CH$_2$bn) ppm.

2,4-Bis(benzyloxy)-$N$-hydroxybenzamide (4h)
Yield: 1.4 g, 4 mmol, 67 %. $^1$H NMR (DMSO-$d_6$, 500.10 MHz, 25 °C): $\delta$ = 10.76 (s, 1 H, NH$_{acid}$), 9.17 (s, 1 H, OH$_{acid}$), 7.55–6.95 (m, 13 H, H$_{Ar}$), 5.21 (s, 2H, CH$_2$bn), 5.02 (s, 4H, CH$_2$bn) ppm.
3,5-Bis(benzyloxy)-N-hydroxybenzamide (4i)
Yield: 1.65 g, 4.7 mmol, 53.6 %. ESI-MS: m/z 196 [L-H]. $^1$H NMR (DMSO-d$_6$, 500.10 MHz, 25 °C): δ = 11.24 (s, 1 H, NH$_\text{acid}$), 9.10 (s, 1 H, OH$_\text{acid}$), 7.49–6.79 (m, 8 H, H$_\text{Ar}$), 5.14 (s, 4H, CH$_2$bn) ppm.

3-(4-(Benzyloxy)phenyl)-N-hydroxypropanamide (4j)
Yield: 1.49 g, 5.5 mmol, 79 %. $^1$H NMR (DMSO-d$_6$, 500.10 MHz, 25 °C): δ = 10.37 (s, 1 H, NH$_\text{acid}$), 8.69 (s, 1 H, OH$_\text{acid}$), 7.46–6.88 (m, 9 H, H$_\text{Ar}$), 5.06 (s, 2H, CH$_2$bn), 2.74 (t, J = 8 Hz, 2 H, H$_\text{Al}$), 2.22 (t, J = 8 Hz, 2 H, H$_\text{Al}$) ppm.

3-(3,4-Bis(benzyloxy)phenyl)-N-methylpropanamide (4k)
Yield: 3.45 g, 9.14 mmol, 93 %. $^1$H NMR (DMSO-d$_6$, 500.10 MHz, 25 °C): δ = 10.38 (s, 1 H, NH$_\text{acid}$), 8.72 (s, 1 H, OH$_\text{acid}$), 7.50–6.69 (m, 13 H, H$_\text{Ar}$), 5.10 (s, 2 H, CH$_2$bn), 5.08 (s, 2 H, CH$_2$bn), 2.74 (t, J = 8 Hz, 2 H, H$_\text{Al}$), 2.23 (t, J = 8 Hz, 2 H, H$_\text{Al}$) ppm.

4-(Benzyloxy)-N-hydroxy-3-methoxybenzamide (4l)
Yield: 0.568 g, 2.1 mmol, 30 %. $^1$H NMR (DMSO-d$_6$, 500.10 MHz, 25 °C): δ = 11.11 (s, 1 H, NH$_\text{acid}$), 8.94 (s, 1 H, OH$_\text{acid}$), 7.48–7.04 (m, 8 H, H$_\text{bn}$), 5.14 (s, 2H, CH$_2$bn), 3.81 (s, 3 H, OCH$_3$Ar) ppm.
General procedure for preparation of hydroxamic acids

A catalytic amount of Pd/C (10 wt. % loading) was added to a solution of protected hydroxamic acid in methanol (dried over mol sieves) under an argon atmosphere. The suspension was put stirred for 4h under an H₂ atmosphere until the hydroxamic acid was completely dissolved. The solution was filtered off, concentrated and dried in vacuo yielding pure hydroxamic acids.

**N,4-Dihydroxy-3,5-dimethoxybenzamide (5c)**
Yield: 0.765 g, 3.6 mmol, 99.5 %. ESI-MS: m/z 163 [L-H]⁻. Anal. Calcd for C₉H₁₁NO₅ · 0.1 C₇H₆: C, 52.38; H, 5.35; N, 6.30; found: C, 52.79; H, 5.73; N, 6.33. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 11.15 (s, 1 H, OHₐr), 8.89 (s, 1 H, OHₐcid), 7.10 (s, 2 H, Hₗ₂), 3.80 (s, 6 H, OCH₃) ppm. ¹³C NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 168.9 (C=O), 145.4 (Cₗ), 143.8 (Cₗ), 125.8 (Cₗ), 119.2 (CHₐr), 116.2 (CHₐr), 115.9 (CHₐr), 34.9 (CH₂), 30.8 (CH₂) ppm.

**N,2-Dihydroxybenzamide (5d)**
Yield: 0.742 g, 4.8 mmol, 85%. ESI-MS: m/z 152 [L-H]⁻.

**N,3-Dihydroxybenzamide (5e)**
Yield: 0.193 g, 1.3 mmol, 39 %. ESI-MS: m/z 176 [L+Na]⁺.

**N,4-Dihydroxybenzamide (5f)**
Yield: 0.420 g, 2.7 mmol, 99.5 %. ESI-MS: m/z 152 [L-H]⁻.

**N,2,3-Trihydroxybenzamide (5g)**
Yield: 0.918 g, 5.4 mmol, 83 %. ESI-MS: m/z 192 [L+Na]⁺.

**N,2,4-Trihydroxybenzamide (5h)**
Yield: 0.673 g, 3.9 mmol, 99.4 %. ESI-MS: m/z 170 [L+H]⁺.
N,3,5-Trihydroxybenzamide (5i)
Yield: 0.718 g, 3.6 mmol, 99.3 %. ESI-MS: m/z 192 [L+Na]+. 1H NMR (DMSO-d6, 500.10 MHz, 25 °C): δ = 9.60 (s, 2 H, OH_Ar), 6.58 (d, J = 2 Hz, 2 H, H_Ar), 6.37 (s, 1 H, H_Ar), ppm.

N-Hydroxy-3-(4-hydroxyphenyl)propanamide (5j)
Yield: 0.699 g, 3.8 mmol, 99.7 %. ESI-MS: m/z 180 [L-H]-. 1H NMR (DMSO-d6, 500.10 MHz, 25 °C): δ = 10.38 (s, 1 H, NH_acid), 9.19 (s, 1 H, OH_acid), 8.69 (s, 1 H, OH_Ar), 6.98 (d, J = 8 Hz, 2 H, H_Ar), 6.67 (d, J = 8 Hz, 2 H, H_Ar), 6.29 (t, J = 8 Hz, 2 H, H_Ar), 2.20 (t, J = 8 Hz, 2 H, H_Ar), ppm.

3-(3,4-dihydroxyphenyl)-N-hydroxypropanamide (5k)
Yield: 0.885 g, 4.5 mmol, 95.7 %. ESI-MS: m/z 196 [L-H]-. Anal. Calcd for C9H11NO4 · 0.5 H2O: C, 52.40; H, 5.87; N, 6.79; found: C, 52.38; H, 5.87; N, 6.41. 1H NMR (DMSO-d6, 500.10 MHz, 25 °C): δ = 10.33 (s, 1 H, NH_acid), 8.67 (s, 2 H, OH_Ar), 6.61 (d, J = 8 Hz, J = 2 Hz, 2 H, H_Ar), 6.57 (d, J = 2 Hz, 1 H, H_Ar), 6.42 (dd, J = 8 Hz, J = 2 Hz, 1 H, H_Ar), 2.63 (t, J = 8 Hz, 2 H, H_Ar), 2.16 (t, J = 8 Hz, 2 H, H_Ar), ppm. 13C NMR (DMSO-d6, 500.10 MHz, 25 °C): δ = 168.9 (C=O), 145.4 (C_q), 143.8 (C_q), 125.8 (C_q), 119.2 (CH_Ar), 116.2 (CH_Ar), 115.9 (CH_Ar), 34.9 (CH2), 30.8 (CH2) ppm.

N,3-Dihydroxy-4-methoxybenzamide (5l)
Yield: 0.362 g, 2 mmol, 95 %. ESI-MS: m/z 182 [L]-. 1H NMR (DMSO-d6, 500.10 MHz, 25 °C): δ = 9.61 (s, 1 H, NH_acid), 7.80 (s, 2 H, OH_acid), 7.35 (d, J = 3 Hz, 1 H, H_Ar), 7.26 (dd, J = 8 Hz, J = 2 Hz, 1 H, H_Ar), 7.09 (s, 1 H, OH_Ar), 6.83 (d, J = 8 Hz, 1 H, H_Ar), 3.80 (s, 3 H, OCH3_Ar) ppm.
Time dependent UV-vis spectra in seawater

Figure S1. Time dependent UV-vis spectra of 6a (left) and 6b (right) in seawater.

Figure S2. Time dependent UV-vis spectra of 6c (left) and 6d (right) in seawater.

Figure S3. Time dependent UV-vis spectra of 6g (left) and 6h (right) in seawater.
Figure S4. Time dependent UV-vis spectra of 6i (left) and 6j (right) in seawater.
Time dependent UV-vis spectra in distilled water

Figure S5. Time dependent UV-vis spectra of 6a (left) and 6b (right) in distilled water.

Figure S6. Time dependent UV-vis spectra of 6c (left) and 6d (right) in distilled water.

Figure S7. Time dependent UV-vis spectra of 6g (left) and 6h (right) in distilled water.
**Figure S8.** Time dependent UV-vis spectra of 6i (left) and 6j (right) in distilled water.

**Figure S9.** Time dependent UV-vis spectra of 6k (left) and 6e (right) in distilled water.
Time dependent UV-vis spectra in distilled water at pH 11

Figure S10. Time dependent UV-vis spectra of 6f (left) and 6l (right) in distilled water at pH 11

Figure S11. Time dependent UV-vis spectra of 6i (left) and 6k (right) in distilled water at pH 11
Time dependent UV-vis spectra of 6e distilled water and seawater with EDTA

**Figure S12.** Time dependent UV-vis spectra of 6e treated with EDTA in distilled water (left – whole spectra, right – enlarged area, pH: distilled water = 5.71, distilled water + 6e = 5.64, distilled water + 6e + EDTA = 4.62). Used concentration of 6e and EDTA was 25 nM. The time dependent measurement was carried out at 25 °C.

**Figure S13.** Time dependent UV-vis spectra of 6e treated with EDTA in seawater (left – whole spectra, right – enlarged area, pH: seawater = 8.15, seawater + 6e = 8.01, distilled water + 6e + EDTA = 7.75). Used concentration of 6e and EDTA was 25 nM. The time dependent measurement was carried out at 25 °C.
Crystallographic parameters and X-ray structures

Experimental parameters and CCDC Codes of the X-ray diffraction measurements are listed in Table S1–S13. Distortion parameters for all diffraction measurements are listed in Table S14.

<table>
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<th>Sample</th>
<th>Machine</th>
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<th>Temp.</th>
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<th>Time/Frame</th>
<th>#Frames</th>
<th>Frame width</th>
<th>CCDC</th>
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<td></td>
<td>[K]</td>
<td>[mm]</td>
<td>[s]</td>
<td>°</td>
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<tr>
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<td>D8</td>
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<td>96</td>
<td>2442</td>
<td>0.4</td>
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<tr>
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<td>Mo</td>
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<td>0.4</td>
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<tr>
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<td>24</td>
<td>2665</td>
<td>0.4</td>
<td>1436729</td>
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</table>

Table S1. Experimental parameters and CCDC-Codes.

1. 6a.

Figure S14. Asymmetric Unit of 6a, drawn with 50% displacement ellipsoids. Co-crystallized water and disorder omitted for clarity. The target molecule could be determined in good quality but apart from some possible to refine located (still generating non confidentially short interactions) water positions, it was not possible to install satisfying solvent models for still available volume. The help of solvent mask (“squeeze”) was required to finalize the crystallographic data. Two voids (each 51.8 Å³, 3.6 respectively 3.5 e⁻) were excluded from the original hkl-file.
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<tr>
<th>Chemical formula</th>
<th>C30H44FeN3O19</th>
<th>Crystal system</th>
<th>triclinic</th>
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<td>Formula weight [g/mol]</td>
<td>806.53</td>
<td>Space group</td>
<td>P-1</td>
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<td>Temperature [K]</td>
<td>100</td>
<td>Z</td>
<td>2</td>
</tr>
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<td>Measurement method</td>
<td>Φ and ω scans</td>
<td>Volume [Å³]</td>
<td>1874.03(18)</td>
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<tr>
<td>Radiation (Wavelength [Å])</td>
<td>MoKα (λ = 0.71073)</td>
<td>Unit cell dimensions [Å] and [°]</td>
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</tr>
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<td>Crystal size [mm³]</td>
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<td>13.2910(7) 78.6474(19)</td>
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<td>Crystal habit</td>
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<td>Absorption coefficient [mm⁻¹]</td>
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<td>Density (calculated) [g/cm³]</td>
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</tr>
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<td>Abs. correction Tmax</td>
<td>1</td>
</tr>
<tr>
<td>Abs. correction type</td>
<td>multi-scan</td>
<td>F(000) [e⁻]</td>
<td>846</td>
</tr>
</tbody>
</table>

Table S2. Sample and crystal data of 6a.

| Index ranges | -14 ≤ h ≤ 14, -16 ≤ k ≤ 16, -16 ≤ l ≤ 16 | Theta range for data collection [°] | 4.678 to 50.7 |
| Reflections number | 18793 | Data / restraints / parameters | 6846/7/499 |
| Refinement method | Least squares | Final R indices | all data |
| Function minimized | Σ w(Fo² - Fc²)² | R1 = 0.0896, wR2 = 0.1622 |
| Goodness-of-fit on F² | 1.042 | I>2σ(I) | R1 = 0.0600, wR2 = 0.1507 |
| Largest diff. peak and hole [e Å⁻³] | 1.32/-0.67 | Weighting scheme | w=1/[σ²(Fo²)+(0.0838P)²+2.2433P] |

Table S3. Data collection and structure refinement of 6a.
Figure S15. Asymmetric Unit of 6b, drawn with 50% displacement ellipsoids. Co-crystallized ethanol, water and disorder omitted for clarity. The percentage of main residue disorder is 22% (solvent disorder 100%).

Table S4. Sample and crystal data of 6b.

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<td>Chemical formula</td>
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<td>Formula weight [g/mol]</td>
<td>591.01</td>
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<tr>
<td>Temperature [K]</td>
<td>100</td>
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<tr>
<td>Crystal system</td>
<td>monoclinic</td>
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<tr>
<td>Space group</td>
<td>P2_1/c</td>
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<tr>
<td>Measurement method</td>
<td>\Φ and \ω scans</td>
</tr>
<tr>
<td>Volume [Å^3]</td>
<td>3211.5(5)</td>
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<tr>
<td>Radiation (Wavelength [Å])</td>
<td>MoKα (λ = 0.71073)</td>
</tr>
<tr>
<td>Unit cell dimensions [Å] and [°]</td>
<td>16.0853(15) 90</td>
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<tr>
<td>[Å] and [°]</td>
<td>28.696(3) 96.758(3)</td>
</tr>
<tr>
<td>Crystal size [mm^3]</td>
<td>0.22 × 0.2 × 0.01</td>
</tr>
<tr>
<td>Crystal habit</td>
<td>clear red plate</td>
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<tr>
<td>Density (calculated) [g/cm^3]</td>
<td>1.222</td>
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<td>Absorption coefficient [mm^-1]</td>
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<td>Abs. correction Tmin</td>
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<td>Abs. correction Tmax</td>
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<td>Abs. correction type</td>
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<td>F(000) [e-]</td>
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Table S5. Data collection and structure refinement of 6b.

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<th>Index ranges</th>
<th>Theta range for data collection [°]</th>
<th>Reflections number</th>
<th>Data / restraints / parameters</th>
<th>Refinement method</th>
<th>Function minimized</th>
<th>Final R indices</th>
<th>Weighting scheme</th>
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<td>-19 ≤ h ≤ 19, -34 ≤ k ≤ 34, -8 ≤ l ≤ 8</td>
<td>3.816 to 50.698</td>
<td>72071</td>
<td>5869/6/414</td>
<td>Least squares</td>
<td>Σ w(Fo^2 - Fc^2)^2</td>
<td>all data</td>
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<td>Goodness-of-fit on F^2</td>
<td>1.105</td>
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<td>l &gt; 2σ(l)</td>
<td>R1 = 0.0729 wR2 = 0.1747</td>
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</tr>
<tr>
<td>Largest diff. peak and hole [e Å^-3]</td>
<td>0.1/-0.66</td>
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<td></td>
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<td></td>
<td></td>
<td>w = 1/[σ^2(Fo^2)+(0.0855P)^2+6.6742P]</td>
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</table>

3. 6d.

Figure S16. Asymmetric Unit of 6d, drawn with 50% displacement ellipsoids. Co-crystallized acetone omitted for clarity. The acetone occupancy is refined with 0.5. The solvent model is fixed with restraints and influences the quality of the refinement as visible in the weighting scheme. Three moderate intramolecular hydrogen bonds, following the classification of Jeffrey, N1A-H...O3A (2.677(6) Å and 128.9°), N1B–H...O3B(2.643(6) Å and 130.8°), N1C–H...O3C (2.648(5) Å and 129.5°) could be tagged.
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<th>Chemical formula</th>
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<td>Temperature [K]</td>
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<td>Measurement method</td>
<td>(\Phi) and (\omega) scans</td>
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<td>Crystal habit</td>
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<td>Density (calculated) / [g/cm(^3)]</td>
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**Table S6.** Sample and crystal data of 6d.

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<td>Least squares</td>
<td>Final R indices</td>
<td>all data</td>
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<td>Function minimized</td>
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<td>(R_1 = 0.1097, wR_2 = 0.2701)</td>
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<td>(I &gt; 2\sigma(I))</td>
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<td>Weighting scheme</td>
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**Table S7.** Data collection and structure refinement of 6d.
Figure S17. Asymmetric Unit of 6e, drawn with 50% displacement ellipsoids. Co-crystalized acetone omitted for clarity. The assembly of strongest Q-peaks and the presence of one solvent accessible void (38 Å³) is a matter of conjecture for small amounts of disordered methanol and water instead of one acetone. In this context the not satisfying low value of second weighting scheme parameter should be mentioned.

Table S8. Sample and crystal data of 6e.

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</tr>
<tr>
<td>Largest diff. peak and hole ([e \AA^{-3}])</td>
<td>0.73/-0.40</td>
<td></td>
<td>(w = 1/[\sigma^2(F_o^2) + (0.0452P)^2 + 9.9934P])</td>
</tr>
</tbody>
</table>

Table S9. Data collection and structure refinement of 6e.

**6g.**

![Molecular structure of 6g](image)

**Figure S18.** Molecular structure of 6g, drawn with 50% displacement ellipsoids. Co-crystalized methanol, diethyl ether and disorder omitted for clarity. The percentage of main residue disorder is 42% (solvent disorder 100%). Two intramolecular hydrogen bonds (N1A-H...O3A, N1B-H...O3B, values because of disorder not listed, but geometric properties tag them as moderate following the classification of Jeffrey in the asymmetric unit are co-responsible to the molecular structure.)
<table>
<thead>
<tr>
<th><strong>Chemical formula</strong></th>
<th>C38H52Cl2Fe2N4O20</th>
<th><strong>Crystal system</strong></th>
<th>monoclinic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formula weight [g/mol]</strong></td>
<td>1067.43</td>
<td><strong>Space group</strong></td>
<td>P2₁/c</td>
</tr>
<tr>
<td><strong>Temperature [K]</strong></td>
<td>100</td>
<td><strong>Z</strong></td>
<td>2</td>
</tr>
<tr>
<td><strong>Measurement method</strong></td>
<td>Φ and ω scans</td>
<td><strong>Volume [Å³]</strong></td>
<td>2417.2(2)</td>
</tr>
<tr>
<td><strong>Radiation (Wavelength [Å])</strong></td>
<td>MoKα (λ = 0.71073)</td>
<td><strong>Unit cell dimensions [Å] and [°]</strong></td>
<td>12.4686(6) 90</td>
</tr>
<tr>
<td><strong>Crystal size [mm³]</strong></td>
<td>0.08 × 0.06 × 0.06</td>
<td><strong>Unit cell dimensions [Å] and [°]</strong></td>
<td>11.1309(5) 94.456(3)</td>
</tr>
<tr>
<td><strong>Crystal habit</strong></td>
<td>clear orange block</td>
<td><strong>Unit cell dimensions [Å] and [°]</strong></td>
<td>17.4694(10) 90</td>
</tr>
<tr>
<td><strong>Density (calculated) [g/cm³]</strong></td>
<td>1.467</td>
<td><strong>Absorption coefficient [mm⁻¹]</strong></td>
<td>0.789</td>
</tr>
<tr>
<td><strong>Abs. correction Tmin</strong></td>
<td>0.6382</td>
<td><strong>Abs. correction Tmax</strong></td>
<td>0.7452</td>
</tr>
<tr>
<td><strong>Abs. correction type</strong></td>
<td>multi-scan</td>
<td><strong>F(000) [e⁻]</strong></td>
<td>1108</td>
</tr>
</tbody>
</table>

Table S10. Sample and crystal data of 6g.

<table>
<thead>
<tr>
<th><strong>Index ranges</strong></th>
<th>-15 ≤ h ≤ 15, -11 ≤ k ≤ 13, -19 ≤ l ≤ 21</th>
<th><strong>Theta range for data collection [°]</strong></th>
<th>4.342 to 50.7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reflections number</strong></td>
<td>10928</td>
<td><strong>Data / restraints / parameters</strong></td>
<td>4359/29/313</td>
</tr>
<tr>
<td><strong>Refinement method</strong></td>
<td>Least squares</td>
<td><strong>Final R indices</strong></td>
<td>all data: R1 = 0.0802, wR2 = 0.1202</td>
</tr>
<tr>
<td><strong>Function minimized</strong></td>
<td>Σ w(Fo² - Fc²)²</td>
<td><strong>L&gt;2σ(l)</strong></td>
<td>R1 = 0.0498, wR2 = 0.1086</td>
</tr>
<tr>
<td><strong>Goodness-of-fit on F²</strong></td>
<td>1.057</td>
<td><strong>Weighting scheme</strong></td>
<td>w=1/[σ²(Fo²)+(0.0419 P)²+3.5548P] where P=(Fo²+2Fc²)/3</td>
</tr>
<tr>
<td><strong>Largest diff. peak and hole [e Å⁻³]</strong></td>
<td>0.53/-0.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table S11. Data collection and structure refinement of 6g.
5. 6j.

Figure S19. Asymmetric Unit of 6j, drawn with 50% displacement ellipsoids. Co-crystalized water and disorder omitted for clarity. The percentage of main residue disorder is 18% (solvent disorder 50%).

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>C27H34FeN3O11</th>
<th>Crystal system</th>
<th>monoclinic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula weight [g/mol]</td>
<td>632.42</td>
<td>Space group</td>
<td>P2₁/n</td>
</tr>
<tr>
<td>Temperature [K]</td>
<td>100</td>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Measurement method</td>
<td>Φ and ω scans</td>
<td>Volume [Å³]</td>
<td>2870.8(2)</td>
</tr>
<tr>
<td>Radiation (Wavelength [Å])</td>
<td>MoKα (λ = 0.71073)</td>
<td>Unit cell dimensions [Å] and [°]</td>
<td>15.9200(7)</td>
</tr>
<tr>
<td>Crystal size [mm³]</td>
<td>0.265 × 0.168 × 0.085</td>
<td>11.6967(5)</td>
<td>104.5149(15)</td>
</tr>
<tr>
<td>Crystal habit</td>
<td>clear red block</td>
<td>15.9254(6)</td>
<td>90</td>
</tr>
<tr>
<td>Density (calculated) [g/cm³]</td>
<td>1.463</td>
<td>Absorption coefficient [mm⁻¹]</td>
<td>0.59</td>
</tr>
<tr>
<td>Abs. correction Tmin</td>
<td>0.6983</td>
<td>1324</td>
<td></td>
</tr>
<tr>
<td>Abs. correction Tmax</td>
<td>Abs. correction type</td>
<td>F(000) [e]</td>
<td></td>
</tr>
</tbody>
</table>

Table S12. Sample and crystal data of 6j.
Index ranges | \(-19 \leq h \leq 19, -14 \leq k \leq 14, -19 \leq l \leq 19\) | Theta range for data collection [°] | 4.18 to 50.692

Reflections number | 100539 | Data / restraints / parameters | 5251/9/402

Refinement method | Least squares | Final R indices | all data: 
- R1 = 0.0462
- wR2 = 0.1097
- l>2σ(l): 
  - R1 = 0.0446
  - wR2 = 0.1087

Function minimized | \(\Sigma w(F_o^2 - F_c^2)^2\) | Weighting scheme | \(w = 1/[σ^2(F_o^2) + (0.0327P)^2 + 5.2426P]\)

Goodness-of-fit on \(F^2\) | 1.183 | 

Largest diff. peak and hole [e Å\(^{-3}\)] | 0.68/-0.34 | 

Table S13. Data collection and structure refinement of 6j.

### 6. Determination of Distortion in Coordination Polyhedra following K. Robinson+2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Geometry</th>
<th>Volume</th>
<th>Quadratic Elongation</th>
<th>Angle Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>Octahedral meridional</td>
<td>10.450</td>
<td>1.030</td>
<td>103.72</td>
</tr>
<tr>
<td>6b</td>
<td>Octahedral meridional</td>
<td>10.594</td>
<td>1.018</td>
<td>61.39</td>
</tr>
<tr>
<td>6d</td>
<td>Octahedral facial</td>
<td>10.461</td>
<td>1.025</td>
<td>85.2</td>
</tr>
<tr>
<td>6e</td>
<td>Octahedral facial</td>
<td>10.460</td>
<td>1.027</td>
<td>93.16</td>
</tr>
<tr>
<td>6g</td>
<td>Octahedral</td>
<td>11.284</td>
<td>1.030</td>
<td>87.01</td>
</tr>
<tr>
<td>6j</td>
<td>Octahedral meridional</td>
<td>10.588</td>
<td>1.020</td>
<td>68.89</td>
</tr>
</tbody>
</table>

Table S14.

Composition of enriched seawater medium for algae experiments

<table>
<thead>
<tr>
<th>Full medium(^3)</th>
<th>Medium -Fe</th>
<th>Medium -EDTA</th>
<th>Medium + model compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 mL of filtered artificial seawater(^1)</td>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
</tr>
<tr>
<td>0.2 mL of micronutrient solution(^3)</td>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
</tr>
<tr>
<td>0.2 mL of vitamin solution(^3)</td>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
</tr>
<tr>
<td>0.2 mL of 0.88 M NaNO(_3)</td>
<td>0.2 mL of 0.88 M NaNO(_3)</td>
<td>0.2 mL of 0.88 M NaNO(_3)</td>
<td>0.2 mL of 0.88 M NaNO(_3)</td>
</tr>
<tr>
<td>0.2 mL of 0.1 M Na(_3)SiO(_4)*9H(_2)O</td>
<td>0.2 mL of 0.1 M Na(_3)SiO(_4)*9H(_2)O</td>
<td>0.2 mL of 0.1 M Na(_3)SiO(_4)*9H(_2)O</td>
<td>0.2 mL of 0.1 M Na(_3)SiO(_4)*9H(_2)O</td>
</tr>
<tr>
<td>0.2 mL of 0.036 M NaH(_2)PO(_4)*H(_2)O</td>
<td>0.2 mL of 0.036 M NaH(_2)PO(_4)*H(_2)O</td>
<td>0.2 mL of 0.036 M NaH(_2)PO(_4)*H(_2)O</td>
<td>0.2 mL of 0.036 M NaH(_2)PO(_4)*H(_2)O</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.007 mM of model compound</td>
</tr>
</tbody>
</table>

Table S15.
**Cyclic voltammograms**

Figure S20. Cyclic voltammograms of complex 6a and ligand 5a in DMF solution containing 0.10 M [n-Bu₄N][BF₄] at a scan rate 0.20 V s⁻¹ using a glassy carbon working electrode.

Figure S21. Cyclic voltammograms of complex 6b and ligand 4b in DMF solution containing 0.10 M [n-Bu₄N][BF₄] at a scan rate 0.20 V s⁻¹ using a glassy carbon working electrode.
**Figure S22.** Cyclic voltammograms of complex 6c and ligand 5c in DMF solution containing 0.10 M [n-Bu4N][BF4] at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.

**Figure S23.** Cyclic voltammograms of complex 6d and ligand 5d in DMF solution containing 0.10 M [n-Bu4N][BF4] at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.
Figure S24. Cyclic voltammograms of complex 6e and ligand 5e in DMF solution containing 0.10 M [n-Bu$_4$N][BF$_4$] at a scan rate 0.20 V s$^{-1}$ using a glassy carbon working electrode.

Figure S25. Cyclic voltammograms of complex 6f in DMF solution containing 0.10 M [n-Bu$_4$N][BF$_4$] at a scan rate 0.20 V s$^{-1}$ using a glassy carbon working electrode.
Figure S26. Cyclic voltammograms of complex 6g and ligand 5g in DMF solution containing 0.10 M n-Bu4N[BF4] at a scan rate 0.20 V s⁻¹ using a glassy carbon working electrode.

Figure S27. Cyclic voltammograms of complex 6h and ligand 5h in DMF solution containing 0.10 M n-Bu4N[BF4] at a scan rate 0.20 V s⁻¹ using a glassy carbon working electrode.
**Figure S28.** Cyclic voltammograms of complex 6i and ligand 5i in DMF solution containing 0.10 M \([n\text{-Bu}_4\text{N}][\text{BF}_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.

**Figure S29.** Cyclic voltammograms of complex 6j and ligand 5j in DMF solution containing 0.10 M \([n\text{-Bu}_4\text{N}][\text{BF}_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.
Figure S30. Cyclic voltammograms of complex 6k and ligand 5k in DMF solution containing 0.10 M [n-Bu$_4$N][BF$_4$] at a scan rate 0.20 V s$^{-1}$ using a glassy carbon working electrode.

Figure S31. Cyclic voltammograms of complex 6l and ligand 5l in DMF solution containing 0.10 M [n-Bu$_4$N][BF$_4$] at a scan rate 0.20 V s$^{-1}$ using a glassy carbon working electrode.
2. SYNTHETIC IRON COMPLEXES AS MODELS FOR NATURAL IRON-HUMIC COMPOUNDS: SYNTHESIS, CHARACTERIZATION AND ALGAL GROWTH EXPERIMENTS

Ewelina Orlowska, Alexander Roller, Marc Pignitter, Franz Jirsa, Regina Krachler, Wolfgang Kandioller, and Bernhard K. Keppler


Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Str. 42, A-1090 Vienna, Austria
Department of Nutritional and Physiological Chemistry, Faculty of Chemistry University of Vienna, Althanstr. 14/UZA II, A-1090 Vienna, Austria
Department of Zoology, University of Johannesburg, Auckland Park, 2006 South Africa

Graphical abstract:

Synthesis and characterization of a series of model complexes for aquatic humic acids providing different coordination motifs were performed. The model compounds were investigated relating to their stability in artificial seawater and redox properties. Furthermore, the impact of the synthesized model compounds on growth of the marine algae species *Chlorella salina* and *Prymnesium parvum* was evaluated.

I performed the synthesis and characterization of the model complexes as well as algal studies. Moreover, I coordinated the collaboration with coauthors and prepared all chapters of the manuscript.
Synthetic iron complexes as models for natural iron-humic compounds: Synthesis, characterization and algal growth experiments

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b Department of Nutritional and Physiological Chemistry, Faculty of Chemistry, University of Vienna, Althanstr. 14/UZA II, A-1090 Vienna, Austria
c Department of Zoology, University of Johannesburg, Auckland Park 2006, South Africa

HIGHLIGHTS
• synthesis and characterization of a series of model complexes for aquatic humic acids providing different coordination motifs.
• characterization of the complexes regarding their stability in artificial seawater and redox properties.
• investigations on the impact of the synthesized model compounds on growth of the marine algae species C. salina and P. parvum.
• iron complex with thiomaltol showed positive impact on the culture of C. salina and P. parvum even though thiomaltol was found to be highly cytotoxic.
• catechol-based iron complexes are found to be excellent models for aquatic humic acids due to their remarkable ability to supply algae with iron.

GRAPHICAL ABSTRACT

A series of monomeric and dimeric FeIII complexes with O,O-, O,N-, O,S-coordination motifs has been prepared and characterized by standard analytical methods in order to elucidate their potential to act as model compounds for aquatic humic acids. Due to the postulated reduction of iron in humic acids and following uptake by microorganisms, the redox behavior of the models was investigated with cyclic voltammetry. Most of the investigated compounds showed iron reduction potentials accessible to biological reducing agents. Additionally, observed reduction processes were predominantly irreversible, suggesting that subsequent reactions can take place after reduction of the iron center. Also the stability of the synthesized complexes in pure water and artificial seawater was monitored from 24 h up to 21 days by means of UV–Vis spectrometry. Several complexes remained stable even after 21 days, showing only partially precipitation but some of them showed changes in UV–Vis spectra already after 24 h which were connected to protonation/deprotonation processes as well as redox processes and degradation of the complexes. The ability to act as an iron source for primary producers was tested in algal growth experiments with two marine algae species Chlorella salina and Prymnesium parvum. Some of the compounds showed effects on the algal cultures, which are comparable with natural humic acids and better as for

Keywords:
Humic acids models
Coordination compounds
Iron complexes
Iron uptake
Algal growth experiments

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1. Introduction

Humic substances are extensively investigated due to their interesting properties, their impact on the environment and potential use in different industrial sectors (Peña-Méndez & Patocka, 2005). Besides many other functions, humic substances play an important role for the bioavailability of transition metal ions including Fe, Mn, Cu, and Zn (Rashid, 1974) due to their chelating properties and high affinity towards those metals; however, the impact of those properties may have been underestimated, especially in case of iron. Iron is essential for all living beings and its bioavailability limits their growth and function. In plants it affects photosynthesis and the formation of chlorophyll and carbohydrates (Osterber, 1974; Egami, 1975; Raven, 1988). The bioavailability of iron in soils is strongly regulated by the pH value and oxygen content. At neutral pH and under aerobic conditions iron occurs mainly as iron oxide (the general therm ‘iron oxide’ describes various forms of iron oxide, oxyhydroxide and amorphous iron hydroxide) which is poorly soluble in water and therefore cannot be taken up by plants and microorganisms. If the oxygen content in soil is low, iron(III) can be reduced to the far more accessible and readily soluble iron(II) form (Lindsay & Schwab, 1982). More problematic is the bioavailability of iron in aquatic systems. Most of these systems are highly oxygenated, at least in the regions where photosynthetic activity is present. Due to this high oxygen content and the low solubility of inorganic iron(III) salts, iron is often the limiting factor for the growth and productivity of primary producers (Worms et al., 2006). Additionally in seawater, higher ionic strength and pH – 8.2 contribute to rapid precipitation of iron (Wu et al., 2001; Liu & Millero, 2002; Liu & Millero, 1999). Huge regions of the ocean have high concentrations of nutrients like phosphate and nitrate, which should result in high photosynthetic productivity of algae; however, growth of microorganisms is rather limited and doesn’t correlate with nutrient concentrations, which can be explained by insufficient iron abundance (Watson et al., 1991; Martin & Fitzwater, 1988; Fitzwater et al., 1996). As those high nutrient low chlorophyll (HNLC) regions make up approximately 30% of the total ocean surface (Pitchford & Brindley, 1999; Edwards et al., 2004) we can probably assume how important the solubility of iron is. Photoautotrophic marine microorganisms are responsible for half of worldwide photosynthetic production and play a crucial role in the carbon cycle (Raven, 1988; Gregg et al., 2002; Field et al., 1998). The iron cycle is linked to the carbon cycle via primary production; iron uptake in algae and its chemical speciation in seawater. It is therefore very important to understand the limitations and environmental aspects of iron bioavailability. The analysis of the dissolved iron in seawater showed that 99.9% is coordinated to organic ligand scaffolds (Johnson et al., 1997; Hutchins et al., 1999). Humic acids belong to those natural chelators and are assumed to be important factors for iron supply of marine organisms (Gledhill & Buck, 2012). For a long time the impact of humic substances on iron bioavailability was underestimated due to the assumption that most of this organic material precipitates in salt water (Sholkovitz, 1976; Eckert & Sholkovitz, 1976; Boyle et al., 1977; Sholkovitz & Copland, 1981; Hunter et al., 1997; Nowostawska et al., 2008). This assumption was found to be incorrect because approximately 20% of aquatic humic substances resist precipitation and remain dissolved in seawater (Kraclher et al., 2005; Kraclher et al., 2012; Kraclher et al., 2010; Jirsa et al., 2013; Rose & Waite, 2003). Humic acid-based iron complexes have been found in the entire Atlantic, Arctic and Pacific oceans. Unfortunately, very low concentrations of those substances make their characterization very challenging (Ibisanmi et al., 2011). Moreover, humic substances are not uniform, they originate from diverse plant and animal residues and have therefore very complex and varying structures (Waksman & Hutchins, 1936). Generally, they are divided in three groups according to their size, properties and solubility (Christl et al., 2000). The group of humic substances with a good aqueous solubility (fulvic acids) is of big interest regarding iron transport and complexation. This group consists of rather small molecules with a molecular mass of around 0.5–2 kDa and relatively high oxygen content (Thurman et al., 1982; Peuravuori, 1996). As mentioned before, the characterization of humic acids iron complexes is challenging; however, it is crucial to understand how iron is bound and released from those complexes, which functional groups are involved and how the uptake of iron by microorganisms works. In this case, the synthesis of model compounds for humic acids can help to answer those questions and contribute to a better understanding of the global iron cycle. Model compounds, due to the uniform and simplified structure motifs enable a glimpse inside the chemistry of those natural chelators. However, it should be kept in mind, that model systems represent rough simplification of aquatic humic substances (AHS). Each model refers to one particular iron binding type in AHS, whereas the heterogeneity of the humic acids allows many different coordination modes and sites. Iron can be bound at the same time at different positions, coordinated by different functional groups having different binding strengths. Also the large size of the molecules compared to simple ligands may lead to the conformational changes and aggregate formation, which affects the binding sites. The availability and amount of possible iron binding groups depends on pH and ionic strength. Summarizing, it is not possible to reproduce all of the properties of AHS within one model but it is thoroughly feasible to identify potential coordination motifs and building blocks responsible for the reversible iron binding and transport in the ocean.

Regarding all the information about aquatic humic acids and their properties, we decided to synthesize simple models and test the different possible coordination modes in iron complexes. The model complexes should include the building blocks of natural humic acids but they should be simple enough to enable full characterization. Those building blocks are mainly aromatic rings, phenolic and carboxylic groups. (Leenheer et al., 1995; Stevenson, 1994) As the stable iron oxidation state in aquatic systems is iron(III), the models should include this oxidation state. Also, the number of iron atoms in the complex has to be considered. Latest EXAFS studies (van Schaik et al., 2008) implicate mononuclear Fe(III) complexes as predominant form but the dinuclear models are also worth consideration due to the chemistry of many natural chelators which form preferable dinuclear complexes, for example catechol. Another important issue is the first coordination sphere of iron. The EXAFS studies cannot clearly distinguish between nitrogen and oxygen which means those two atoms can both occur. Admittedly, the oxygen content in humic substances is much higher than the nitrogen and also many identified oxygen containing groups are present, so the O,O coordination seems to be more likely. Due to the possible incorporation of amino acids in the structure of humic substances (Hagen, 2007; Pressprich & Chambers, 2004), other coordination possibilities such as O,N and O,S should be verified. In our previous work, we synthesized benzoic hydroxamate derivatives and their complexes as model compounds and we found several similarities to humic acid iron complexes (Orłowska et al., 2016). However, the bioavailability of iron in those complexes was not comparable with natural compounds and we decided to elucidate iron complexes based on different ligand coordination motifs.

Herein, we report the synthesis of a series of iron model complexes with different structural characteristics. We have chosen coordination motifs e.g. catechol, hydroxamate, salicylate, maltolate, thiomaltolate,
flavone and picolinic acid providing different sets of donor atoms. The synthesized complexes were characterized by elemental analysis, spectroscopic methods (UV–Vis), mass spectrometry, cyclic voltammetry and EPR spectroscopy. Moreover, the thermodinamic stability of the iron complexes in seawater was investigated. Most importantly, iron bioavailability and uptake from the complexes was monitored with algae growth experiments using two seawater algae species, namely *Chlorella salina* and *Prymnesium parvum*.

2. Experimental

2.1. Materials and methods

All chemicals were purchased from Sigma Aldrich, Alfa, Fluka, Riedel-de Haen or Acros and used as obtained. For the synthesis of the model compounds following chemicals were used: FeCl3 (anhydrous, Riedel-de Haen, 12321), FeCl3 · 6H2O (Riedel-de Haen, 31232), NaOMe (Acros-Fisher, AC17312-1000), NaOH (Sigma Aldrich, 30620), KOH (Sigma Aldrich, 484016), 3-hydroxy-2-methyl-4-pyrene (Acros Fisher, 121,551,000), PPh4Cl (Sigma Aldrich, 88,060), salicylic acid (Sigma Aldrich, 84,210), picolinic acid (Sigma Aldrich, P42800), Lawessons reagent (Acros-Fisher, 10124840). All solvents were of analytical grade and used without further purification. The formation and purity of the compounds was verified with 1H NMR, ESI-MS, elemental analysis, X-ray diffraction studies, EPR and ATR-IR spectroscopy. Specific devices and conditions used for those measurements are listed below.

1H NMR spectra were recorded on a Bruker Avance III™ 500 MHz spectrometer in DMSO-d6 at 298 K using standard pulse programs at 500.10 MHz for 1H experiments.

Electrospray ionization mass spectra were measured with a Bruker maXis ESI-QqTOF spectrometer in the positive and negative mode. Spectra were measured with a Bruker Vertex 70 Fourier transform IR spectrometer. Specific devices and conditions used for those measurements are listed below.

Elemental analyses were performed at the Microanalytical Laboratory of the University of Vienna with a Perkin-Elmer EA3000 elemental analyzer and are within 0.4% of the calculated values. If not otherwise stated the substances were synthesized and purified according to the general procedure.

EPR spectra were acquired on a Bruker Elexys-II E500 CW-EPR spectrometer equipped with a high sensitivity cavity (SHQE1119) operating at 90 ± 1 K. The parameters of the spectrometer were set as follows: microwave frequency, 9.43 GHz; modulation frequency, 100 kHz; center field, 6000 G; sweep width, 12,000 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.

Samples were dissolved in dimethylformamide (7 mg/mL). The EPR conversion time, 81.92 ms; resolution, 4096 points; averaged scans, 3. Overall, four experiments on f2/2 where xo was the initial cell concentration at time t0 and x1 the concentration at the time t2. Overall, four experiments on C. salina and one experiment on P. parvum were carried out. The concentrations of tested substances were 11.7 μmol L−1. The experiments were carried out over a period of 20 to 30 days (depending on algal growth) and the algae concentration was monitored after 6–8 days from beginning of experiment and then every other day. 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% formalin acid solution. C. salina cells were counted without any treatment.

2.2. Synthesis of iron complexes

2.2.1. [Fe(maltolato)2]2+ (Morita et al., 1975)

1FeCl3 (37 mg, 0.23 mmol) dissolved in methanol (2 mL) was added to a solution of maltol (87 mg, 0.70 mmol) and NaOMe (41 mg, 0.77 mmol) in methanol (5 mL). The dark red solution was stirred for 3 h and the solvent was removed in vacuo. The residue was extracted with dichloromethane (20 mL) and the solution was filtered.
Isopropanol was slowly added to the solution resulting in the formation of a red precipitate. The product was filtered off and dried in vacuo. Yield: 99 mg, 0.16 mmol, 71%. ESI-MS: \( m/z \) 454 [M + Na]+. Anal. Calcd for C_{16}H_{12}FeO_{5}: C, 50.14; H, 3.51; O, 33.40. Found: C, 49.95; H, 3.41; O, 33.17. IR (ATR, selected bands, \( \nu_{\max} \)): 2969, 1601, 1562, 1497, 1461, 1295, 1275, 1244, 1205, 1036, 922, 848, 763, 721, 609 cm\(^{-1}\).

2.2.2. [Fe(thiomaltolato)₃] (Lewis et al., 2003)

(2) FeCl₃ (37 mg, 0.23 mmol) dissolved in methanol (2 mL) was added to the suspension of thiomaltol (99 mg, 0.70 mmol) and KOH (58 mg, 1.05 mmol) in MeOH (5 mL). The dark red solution was stirred for 3 h and the solvent was removed in vacuo. The dry residue was extracted with chloroform (15 mL) and the solution was filtered. Isopropanol was slowly added to the solution resulting in the formation of a red precipitate. The product was filtered off, washed with H₂O and then dried in vacuo. Yield: 88 mg, 0.18 mmol, 80%. X-ray diffraction quality single crystals were grown in methanol (slow evaporation). ESI-MS: \( m/z \) 518 [M + K]+. Anal. Calcd for C_{18}H_{12}FeO₂: C, 44.27; H, 3.30; O, 21.29. Found: C, 44.45; H, 3.00; O, 20.92. IR (ATR, selected bands, \( \nu_{\max} \)): 2948, 1571, 1453, 1410, 1364, 1270, 1215, 1180, 1056, 1032, 902, 816, 738, 663, 582 cm\(^{-1}\).

2.2.3. [Fe₂(catechol)₄(H₂O)₂](Ph₄P)₂ (Grillo et al., 1996)

(2) The red precipitate was filtered off, washed with H₂O and dried in vacuo. Yield: 160 mg, 0.18 mmol, 61%. ESI-MS: \( m/z \) 870 [M]⁺. Anal. Calcd for C_{28}H_{18}FeO₁₂·3.5 H₂O: C, 46.63; H, 3.49; O, 34.39. Found: C, 46.66; H, 3.25; O, 34.99. IR (ATR, selected bands, \( \nu_{\max} \)): 2970, 1603, 1553, 1545, 1382, 1323, 1180, 1145, 1056, 1033, 1014, 884, 863, 792, 747, 696, 655, 578 cm\(^{-1}\).

3. Results and discussion

3.1. Synthesis – general overview

Chloroflavone, thiomaltol, 3-hydroxy benzohydroxamic acid and its iron complex (5) were synthesized according to the previously published procedures (Orlowska et al., 2016; Lewis et al., 2003; Litkei et al., 1997). Single crystals suitable for X-ray diffraction analysis were obtained for the chloroflavone ligand by slow evaporation in chloroform (see S16). The Fe(III) complexes were synthesized by deprotonation of the ligand and conversion with iron(II) chloride in aqueous or methanol solution. The purification of the complexes was found to be the crucial step of the synthesis, the complexes were found to be soluble in protic solvents as were the formed by-products. In case of the catechol-based iron complex, pure complex was precipitated by utilization of the more lipophilic counterion, tetraphenylphosphonium. Overall, complexes 1–7 were isolated in moderate to good yields (43–80%) depending on the applied purification protocol. An overview of the synthesized model complexes is shown in scheme 1.

In order to confirm the formation of the desired products, electrospray ionization mass spectrometry (ESI-MS), IR spectroscopy and elemental analysis were performed for the synthesized compounds (see experimental section). Due to the paramagnetic properties of Fe(III) complexes, \(^{1}H\) NMR spectroscopy could not be utilized for characterization and other methods were used instead. The ESI-MS spectra of the complexes (positive and negative mode) were measured in methanol and/or in acetonitrile. For almost all complexes the positively charged \( H^+ \), Na⁺ or K⁺ adducts were detected. For example the peak with \( m/z \) value of 445 in the spectrum of 6 was assigned to the \([Fe(C₈H₄NO₂)₃]Na^+\). In case of the iron complex with catechol a negatively charged peak with \( m/z \) value of 272 was observed, which was assigned to \([Fe(catechol)]_2^-\) and a positively charged peak of \([Ph₄P]^+\) with \( m/z \) value of 339. All detected molecular peaks of the complexes showed the typical isotyping pattern.

3.2. Cyclic voltammetry

Redox potentials of model compounds present important information about possible redox processes in solution. Reduction of iron(II) to iron(II) is broadly believed to be necessary for uptake (Wells et al., 1995). The lower ligand affinity to the softer Fe(II) results in a facilitated interaction about possible redox processes in solution. Reduction of iron(III) to iron(II) is broadly believed to be necessary for uptake (Wells et al., 1995). The lower ligand affinity to the softer Fe(II) results in a facilitated interaction about possible redox processes in solution. Reduction of iron(III) to iron(II) is broadly believed to be necessary for uptake (Wells et al., 1995). The lower ligand affinity to the softer Fe(II) results in a facilitated interaction about possible redox processes in solution. Reduction of iron(III) to iron(II) is broadly believed to be necessary for uptake (Wells et al., 1995). The lower ligand affinity to the softer Fe(II) results in a facilitated interaction about possible redox processes in solution. Reduction of iron(III) to iron(II) is broadly believed to be necessary for uptake (Wells et al., 1995). The lower ligand affinity to the softer Fe(II) results in a facilitated interaction about possible redox processes in solution. Reduction of iron(III) to iron(II) is broadly believed to be necessary for uptake (Wells et al., 1995). The lower ligand affinity to the softer Fe(II) results in a facilitated interaction about possible redox processes in solution.
CVs displaying additional irreversible reduction/oxidation processes, the potential sector is shown where the iron reduction peak can be presumed. Complexes 1 and 6 in DMF show a reversible single-electron reduction wave, Ired assigned to the FeIII/FeII process. The redox potential values for the FeIII/FeII redox couple are −0.62 V for 1 and 0.21 V for 6. On the other hand, thiomaltol-based complex 2 showed different electrochemical processes. An irreversible oxidation occurred at 1.29 V and −0.02 V and an irreversible reduction at 0.02 V and −1.85 V.

Also, a reversible process with E½ = −0.45 V, which can be assigned to iron reduction, was detected. Other signals can be associated in the reduction and oxidation of the thiomaltol ligand. The dimeric iron catechol complex 3 showed three different reduction waves at −1.62, −0.04 and 0.50 V and the oxidation of catechol at 1.18 V. All these redox processes were irreversible or quasi-reversible. Clear assignment of the reduction waves was not possible and therefore free catechol was measured for comparison. The ligand displayed a reduction wave at −0.42 V and an oxidation wave at 0.55. Due to the complexation with iron those values have changed but it can be assumed that one of the three peaks is related to ligand reduction and the other two to Fe(III)/Fe(II) reduction of the two iron centers. Analysis of the cyclic voltammograms of the dimeric salicylic acid complex 4 showed one irreversible oxidation wave at 1.27 V. This process can be connected with salicylic acid and its oxidation. Also, two quasi-reversible reduction peaks at −0.10 V and −0.42 V and an irreversible reduction at −1.40 V were observed. Two quasi-reversible reductions can be assigned to Fe(III)/Fe(II) reduction and confirm the presence of two iron centers in the complex. In case of salicylic hydroxamic complex 5, only irreversible processes were observed. The assignment of the Fe-reduction peak was not possible due to the low intensity of the signals and redox activity of the ligand. The behavior of the free ligand was also studied in order to compare the signals and enable the characterization; however, no clear assignment could be made. All observed signals were in the negative region between −0.50 V and −1.20 V and, therefore, it can be assumed that electrochemical reduction is unlikely under biological conditions. The electrochemical studies of compound 7 revealed several irreversible processes and one reversible one. Two oxidations waves, Iox with the values 1.24 V and 1.41 V, which can be associated with ligand oxidation, were observed. In order to assign the other observed processes, electrochemical studies of uncoordinated chloroflavone have been performed. The ligand showed three irreversible reduction processes at −0.78 V, −1.00 V and −1.30 V. Knowing that the ligand undergoes only irreversible reductions, we assumed that three irreversible reductions observed in the complex can be associated with chloroflavone and the reversible process with E½ = −0.27 V is the Fe(III)/Fe(II) reduction. Those results correlate with the electrochemical studies of 1, where reversible reduction was observed as well and the iron in 1 has a similar coordination mode (bound via a carbonyl oxygen and a hydroxyl group). Summarizing the electrochemical studies, the majority of the measured model complexes showed iron reduction potentials accessible to biological reducing agents (down to −350 mV) (Pierre & Fontecave, 1999). Therefore, iron release by reduction and cleavage of the complex entity can be considered as a possible mode of action. Additional irreversible reduction processes occurred for the complexes

![Scheme 1. Overview of the synthesized model compounds ([Fe(maltolato)]3 (1), [Fe(thiomaltolato)]3 (2), [Fe2(catechol)4(H2O)2](Ph4P)2 (3), [Fe2(salicylic acid)4] (4), [Fe(3-hydroxybenzoic hydroxamate)]3 (5), [Fe(picolinic acid)]3 (6), [Fe(chloroflavone)]3 (7)).](image-url)
2–5 and 7 suggesting that reactions, possibly degradation of the complex, can take place after reduction of the iron center.

Unfortunately it is impossible to measure the redox properties of the complexes under culturing conditions because of the very low concentrations of the compounds in seawater. The concentrations used for cyclic voltammetric studies are in μM range whereas the concentrations in culturing studies are in μM range. Moreover, there are several factors which impact the redox behavior (pH value and its changes during the experiment, light, other ions present, long duration of the experiments) and cannot be included in CV studies. Therefore, cyclic voltammetric studies do not deliver the clear answer if the iron in the complexes can be reduced or not but rather the likelihood of the reduction.

3.3. EPR spectroscopy

EPR spectroscopic analyses showed the characteristic g_{eff} = 4.3 peak for all iron complexes, thereby proving the high spin state of the Fe(III) species (Fig. 2). The high intensity of the EPR line at g_{eff} = 4.3 emerges from the three coinciding g-values for the I = 3/2 > doublet of the S = 5/2 system. According to the g_{eff}-values a rhombicity of 0.31 and 0.19 was calculated for the iron centers in the complexes. Thus, the crystal field at the Fe(III) centers show strong orthorhombic character and only low axial symmetry. As iron complexes 3 and 4 consist of two iron centers, broader signals due to spin-spin interactions between the unpaired electrons of the two iron centers are expected but not obtained. The absence of any detectable interactions between the two paramagnets can be explained by the two identical iron centers yielding signals which cannot be resolved by cw-EPR at 90 K. Although complex 6 is a mononuclear complex, splitting of the g_{eff} = 4.3 peak was obtained. The splitting is caused by superhyperfine interactions of the magnetic nitrogen nuclei with the unpaired electrons of the iron. Thus, the neighboring nitrogen atoms of the iron center in the complex 6 can be identified by means of EPR spectroscopy.

3.4. UV–Vis Stability studies

The possible reduction and cellular uptake of iron is closely related with the stability of the coordination compounds in seawater. On the one hand, iron complexes should be prone to redox processes, degradation and dissociation; however, if decomposition progresses too fast then the released iron, independently of oxidation state, will precipitate in seawater. On the other hand, very inert complexes may also have no influence on the growth of algae because no iron would be released. The stability constants of the formed iron complexes of most ligands used in this study were previously reported. The highest logβ overall stability constants were found for the complexes with catechol (3) and salicylic acid (4) with logβ values of 43.7 and 36.6 respectively (Avdeef et al., 1978; Maqsood & Kazmi, 1993). The stability constants of maltolato (1) and hydroxamato iron(III) (5) complexes were found in the same range but the substitution of oxygen in thiomaltol resulted in lower logβ value for thiomaltolato iron(III) complex 3, namely 23.1 (compared to 29.8 for maltolato complex 1) (Chaves et al., 2012). This can be explained with the higher affinity of sulfur towards soft metal ions. We can assume that the stability of the complex 7 will be comparable with 1 due to the very similar coordination chemistry. Lowest stability constants were found for iron(III) picolinate (6) which is connected to the nitrogen in the first coordination sphere (Lannon et al., 1986). Similar to sulfur, nitrogen also shows higher affinity towards soft metal ions and its iron(II) (softer as iron(III)) complexes are also well known.

In summary, we should expect the highest stability for the catechol complex 3 whereas the complex 6 would be most labile. However, the problem with stability constants found in the literature is that the conditions in seawater medium in algal studies are not the same as those used for the estimation of those constants. It is also not possible to measure logβ values in seawater due to the buffer properties, ionic strength and the presence of other metal ions competing for the ligand. Also the light exposure, pH value and oxygenation contribute to the changes in stability of the metal complexes. Chemistry of catechol shows that those differences should not be left unattended. This ligand is prone to the oxidation in seawater and the reduction of iron(III) in the presence of catechol was observed (Santana-Casiano et al., 2010). We decided to use UV–Vis spectroscopy to monitor changes occurring in the complexes after dissolution in seawater in order to predict the stability of the complexes and evaluate the algal studies. Therefore, the stability of the synthesized complexes in seawater at pH ≈ 8.2–8.4 (see Figs. S1–S4) and distilled water (see Figs. S5–S9) was monitored over 24 h by means of UV–Vis spectrometry. In some cases the stability of the complexes at pH ≈ 8.2 and the ligand itself was evaluated. Maltol complex 1 showed absorbances at 220 and 320 nm with no shift changes over time. In distilled water, a decrease of the absorption at 320 nm followed by increase of absorption at 275 nm with an isosbestic point at 285 nm was observed (Fig. 3). Those changes can be assigned to protonation and deprotonation processes.

Similar to 1 the respective thiomaltol analog 2 was stable over 24 h in both seawater and distilled water but precipitation of the complex resulted in an absorbance decrease over time. Complex 3 (Fig. 4) was found to be unstable independently of the applied experimental setup (distilled water, seawater and water at pH 8.2). In all cases an isosbestic point was found at ca. 300 nm. Also, the absorption of peak at 270 nm was increasing with the time as well as the absorption in the range between 250 and 270 nm. The changes were most distinctive in seawater. This behavior can be explained by both reduction and/or deprotonation of the complex as well as the redox activity of the catechol moiety (Schweigert et al., 2001).

Complexes 4–7 were found to be stable in distilled water and 4–6 also in seawater. Complex 7 showed significantly lower solubility in seawater compared to the other model compounds leading to partial precipitation over 24 h but no spectral changes were observed. The solubility of the complex, however, was sufficient to perform algal studies.

Concerned about the stability and behavior of the complexes during the long term algal studies we performed additionally UV–Vis studies over 21 days (see Figs. S12-S15). We observed slowly partial precipitation of the complexes 1, 4, 5 and 7. Despite the changes during the first 24 h in case of complex 3, no further changes in spectra were observed for 3 and 6 during 21 days. In case of complex 2, after the partial precipitation after 24 h we observed after 7 days significant changes in spectra. Apparently after longer exposure of 2 decomposition of the complex and ligand can be observed.
3.5. Algal growth experiments

The ability of all model compounds to supply marine microorganisms with iron was investigated utilizing unicellular green algae *Chlorella salina*. For the model compounds with the best impact on the culture of *C. salina* an experiment on the haptophyte *Prymnesium parvum* was performed. It is broadly believed that algae uptake iron as unchelated iron rather than as complex with organic ligands (Sutak et al., 2012; Morrissey & Bowler, 2012). Only for the microorganisms producing siderophores, the uptake of iron as siderophore-complex and its reduction in the cell was observed (Hopkinson & Morel, 2009). There are different possible pathways of iron release from the complexes (Hunter & Boyd, 2007). One of them includes simple ligand-metal equilibrium which changes after consumption of iron by primary producers, promoting the iron dissociation from the complex. Another pathway is based on the degradation of the ligand, which also leads to the complex breakdown. Third possibility is the reduction of iron in the complex, which decreases the affinity of ligands towards iron and induces the release. This process can occur due to the self-reduction/oxidation of the complex which means the oxidation of the ligand with simultaneous reduction of iron (Rose & Waite, 2003b). Especially photo-reductive dissociation seems to have a big impact on the bioavailability of iron and is already known as a mechanism of iron release from AHS (Blazevic et al., 2016). Considering those facts, the perfect model ligand should show high affinity towards ferric iron and low towards ferrous iron, and be able to undergo redox processes. Preliminary experiments have shown that *C. salina* is sensitive to iron limitation and displays poor growth in medium without iron (Krachler et al., 2016). Additionally this algae species does not produce any iron chelating molecules which would facilitate the iron uptake. Therefore *C. salina* showed only poor growth in medium with iron but without chelating molecules. It is also important to mention that, in all performed algal experiments, both *C. salina* and *P. parvum* showed the typical growth pattern in full medium (Fogg & Thake, 1987; Miron et al., 2002). In iron-free samples (−Fe, + EDTA) the growth was strongly inhibited for *C. salina* and partially inhibited for *P. parvum*, which means, iron deficiency conditions were given. The long time between the beginning of each experiment and start of the exponential growth for *C. salina* is typical and known from large-scale iron fertilization experiments in HNLC regions (Pitchford & Brindley, 1999; Fujii & Chai, 2009). The pH value was monitored at the beginning of the experiments and during the experiments. In all experiments higher pH values (up to 10.5) were observed in exponential growth phase of the culture, which are connected to the increased CO₂ demand. After the end of the exponential growth and the beginning of the stationary phase the pH decreased to the initial value. In samples, where no growth or poor growth was observed, no significant changes in pH value were observed.

**Fig. 3.** Time dependent UV–Vis spectra of 1 in distilled water (A), water at pH 8 (B) and seawater (C) over 24 h (spectra were measured in an 1 h interval, start and end point of the measurement are indicated in A and B, in C no changes were observed).

**Fig. 4.** Time dependent UV–Vis spectra of 3 (50μM) in distilled water (A) seawater (B) over 24 h showing the region where the changes occurred (spectra were measured in an 1 h interval, start and end point of the measurement are indicated in A and B).
iron reduction and following disintegration of natural humic acid iron complexes. Surprisingly the *C. salina* culture treated with 3 responded in very good growth and reached the highest concentration of near \(8 \times 10^6 \pm 1.1 \times 10^5 \text{cells mL}^{-1}\), which was better than the control sample with EDTA and iron. Catechol is known for its high affinity towards iron and our redox studies confirm also the possibility of the reduction of the complex under biological conditions (Santana-Casiano et al., 2010). The high redox potential of iron also allows the Fenton reaction, which is in line with the properties of natural AHS (Blazevic et al., 2016). Results of the algal growth tests with compounds 1, 2, 5 and 6 are shown in Fig. 6. Compound 1 displayed a similar impact on the culture as the control sample without EDTA, reaching about half of the concentration of the positive control (EDTA and Fe) at the end of the experiment. This means, the iron can be utilized from the complex but the uptake is not

![Fig. 5. Growth curves of *C. salina* (error bars: ±SD) treated with model compounds 3, 4, 7 compared to control samples (+ Fe, + EDTA; + Fe, − EDTA; − Fe, + EDTA).](image1)

![Fig. 6. Growth curves of *C. salina* (error bars: ±SD) treated with model compounds 1, 2, 5, 6, thiomaltol and catechol compared to control samples (+ Fe, + EDTA; + Fe, − EDTA; − Fe, + EDTA).](image2)
as efficient as for the EDTA complex. Complex 5 showed similar effects and the culture reached the end concentration of 2.4 × 10^6 ± 9.6 × 10^5 cells mL^{-1}. According to the cyclovoltammetric studies iron in both complexes (1 and 5) should not be reduced under physiological conditions and the complexes possess high solubility and stability in seawater. Of course, the measured redox potentials are only the implication of the possible redox behavior because the same conditions as in the culture studies cannot be applied for electrochemical measurements. Still, considering the high stability of those complexes we can assume that the reduction is rather unlikely. The possible explanation for iron release can be the partial decomposition due to the very long duration of the experiment. The growth rate of the algae treated with complex 6 at the end of the experiment was better than for samples without iron but worse than the samples with iron but lacking EDTA. Although the end concentration of the cells was 9.3 × 10^6 ± 4.6 × 10^5 cells mL^{-1}, which is higher when compared to 1 and 5, algae in both control samples (Fe and EDTA, Fe without EDTA) grew better. This observation can also be explained with the redox chemistry of the picolinate iron complex. The reduction of 6 is possible under physiological conditions but the reduction is fully reversible, which means that most likely no subsequent reactions and breakdown of the complex takes place. The redox behavior combined with high stability confirmed by UV–Vis experiments can lead to the limited bioavailability of iron in this complex. In contrast to 1, 5 and 6, complex 2 showed a remarkable positive impact on C. salina growth (1.1 × 10^7 ± 1.2 × 10^6 cells mL^{-1}). The final algae concentration is comparable with the results obtained from previous investigations, where the isolated AHS were tested on algae, namely 9 × 10^6 ± 3.6 × 10^6 cells mL^{-1} and 1 × 10^7 ± 6.7 × 10^5 cells L^{-1} (Krachler et al., 2016). It is very surprising, considering that thiomaltol was found to be highly cytotoxic (Hacle, 2016). There is a possibility that C. salina metabolizes the complex and uses it as a source of sulfur and iron or that the complex is not toxic for algae. Though, it is notable that the growth curve of C. salina treated with 2 is different compared to the other model compounds. No significant changes in algal concentration were observed during the first 12 days of the experiment, which shows a strong inhibition in growth of the algae; however, after this initial period, very fast exponential growth of the culture was monitored. This result is in line with our long term UV–Vis stability studies, where the changes in spectra indicate decomposition of complex or/and ligand.

The end-concentrations of the samples treated with model compounds as well as the relation between the end alage concentration of control samples to samples treated with model compounds are shown in Table 1. It is more reliable to compare the values within one experiment because of the different growth stages of the cultures. Even if the applied conditions and the concentrations at the beginning of the experiment were exactly the same, the growth response of the control samples was slightly different. Thus, the algae concentration in control samples at the end of the experiment was different in each experiment. The variances in vitality of the cultures at the beginning of each experiment can be the explanation of those different results. Hence, the relation of the end-concentration of samples treated with model compounds to the end-concentration of control samples provides the more meaningful information. Summarizing the studies on C. salina, two of the seven tested compounds namely 2 and 3 showed positive impact on the culture of C. salina and are potentially suitable models for AHS regarding the bioavailability of iron. Additionally to the experiments with iron complexes, we performed an experiment utilizing only the free ligands from those two compounds by addition of catechol or thiomaltol to culture medium lacking EDTA (Fig. 6). Moreover, the impact of the bulky counter ion (tetraphenylphosphonium, PPh4^+) on the growth of algae after treatment with 3 can be elucidated.

Samples of C. salina containing thiomaltol reached a similar end-concentration as samples treated with 2 and no delay or inhibition of the growth of C. salina as monitored for 2 was observed. One possible explanation could be that structurally different iron-thiomaltol complexes might be formed in seawater, which acts less inhibitory for the growth of C. salina. Moreover, thiomaltol can also react with other metal ions present in medium so that the concentration of the iron complex is much lower in comparison to the samples treated with 2. Cultures containing catechol and iron(III) chloride reached a lower end-concentration compared to the samples with complex 3. The presence of the P-containing bulky counter ion may be the possible explanation for those results. The ligand can represent additional P-source for the algae but the concentration of phosphorus in medium should be sufficient enough to ensure good growth (Klausmeier et al., 2004; Geider & La Roche, 2002); however, more probably the counterion influences the iron uptake. The medium used for the studies has a N:P ratio of 24:1. In some literatures the N:P ratio of 16:1 is postulated to be optimal for the growth of several algae species (Hillebrand & Sommer, 1999). In order to elucidate the impact of the elevated phosphorus concentration in medium, algal studies with N:P ratio 16:1 and 24:1 were performed (see Fig. S17). We did not find significant differences in C. salina growth with regard to higher phosphor concentrations (N:P 16:1).

Additionally to the maximal alage cell concentration, we also calculated the specific growth rate of algae for all tested compounds after 17 days of each experiment which was the duration of the first experiment (Table 2). Similar to the maximal alage cell concentrations, highest specific growth rates were observed for samples treated with complexes 2 and 3 as well as the ligand thiomaltol.

In order to elucidate the effect of the two most promising model compounds on other algal species, which differ in the biology and nutrition requirements, we performed experiments with compounds 2 and 3 on P. parvum. P. parvum is a wide spread alage species, adapted to different salinities as well as changing nutrient conditions. Fig. 7 shows the growth curves of P. parvum treated with 2 and 3 compared to control samples. Both tested compounds positively impacted the growth of P. parvum, reaching the concentrations 4.9 × 10^6 ± 1.2 × 10^5 cells mL^{-1} and 5.8 × 10^6 ± 1.5 × 10^5 cells mL^{-1} for 2 and 3, respectively. The samples treated with 2 were monitored 2 days longer in order to elucidate the maximum end-concentration, whereas in other samples the growth stopped earlier. Analyzing the growth of P. parvum under diverse conditions, several differences when compared to C. salina were observed. Most importantly, the alage strain seems not to be as sensitive to iron

Table 1
Algae end-concentration and relation between the control samples to samples treated with 1, 2, 3, 4, 5, 6 and 7.

<table>
<thead>
<tr>
<th>Algae end-concentration (cells mL^{-1})</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Fe, + EDTA in %</td>
<td>40</td>
<td>193</td>
<td>123</td>
<td>47</td>
<td>31</td>
<td>62</td>
<td>23</td>
</tr>
<tr>
<td>− Fe, + EDTA</td>
<td>63</td>
<td>10.3</td>
<td>4.1</td>
<td>1.6</td>
<td>4.9</td>
<td>1.29</td>
<td>0.77</td>
</tr>
<tr>
<td>+ Fe, − EDTA</td>
<td>6</td>
<td>3.7</td>
<td>6.8</td>
<td>2.6</td>
<td>0.8</td>
<td>0.79</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Table 2
Specific growth rates of C. salina after 17 days for 1, 2, 3, 4, 5, 6, 7, catechol and thiomaltol.

<table>
<thead>
<tr>
<th>Growth rate</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Catechol</th>
<th>Thiomaltol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.16</td>
<td>0.24</td>
<td>0.23</td>
<td>0.17</td>
<td>0.11</td>
<td>0.10</td>
<td>0.13</td>
<td>0.19</td>
<td>0.25</td>
</tr>
</tbody>
</table>
or EDTA deficiency as C. salina. The growth of the culture lacking EDTA was almost the same as the culture cultivated in full medium. Furthermore, the growth without iron was not inhibited to the same degree as for C. salina.

Thus, it is possible that P. parvum can better accumulate and store iron and that the requirements for iron are lower compared to those of C. salina. The physiology of this species differs from C. salina, as those algae are mixotrophic organisms, with possible heterotrophic growth (phagotrophy) (Martin-Cereceda et al., 2003). It is also known that P. parvum can adapt to iron-stress conditions with the expression of several genes (Maki et al., 2008). Overall, the model compounds 2 and 3 showed elevated growth rates of P. parvum compared to the control samples which confirms that the iron bound in the complexes is bioavailable for algae. Growth rates of the cultures after 17 days are listed in Table 3. Highest specific growth rate for P. parvum was observed in samples containing complex 3.

## 4. Conclusions

Herein, we investigated seven iron(III) complexes which could act as model compounds for humic acids with various structural features, different sets of donor atoms and chemical behavior. The simple composition and relatively small size of the selected complexes enabled characterization by elemental analysis and mass spectroscopy. Also, the electrochemical properties of the compounds related to the postulated iron release by reduction in natural humic acid iron complexes have been investigated. The oxidation state of iron could be characterized by EXAFS studies. Furthermore, growth of the culture containing complex 3 and thiomaltolato complex was compared to control samples which confirmed that the iron bound in the complexes is bioavailable for algae. Growth rates of the cultures after 17 days are listed in Table 3. Highest specific growth rate for P. parvum was observed in samples containing complex 3.

Some of the compounds showed effects on the culture, which are comparable with natural humic acids, for example catechol complex 3 and thiomaltolato complex 2. According to previous work and EXAFS studies, it is unlikely that the majority of iron in humic acids is bound via sulfur, so the best fitting model in our studies seems to be the dimeric iron complex containing catechol. Due to the presence of many phenolic moieties in natural AHS, this mode of coordination seems to be convincing. The investigated systems were very simple but the introduction of proper catechol derivatives bearing lipophilic moieties may be the next step for the successful modeling of AHS and a better understanding of their biogeochemistry in the ocean.

## Acknowledgements

We thank the University of Vienna and the Austrian Science Fund (FWF) (P 25840-N19) for financial support.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2016.10.109.

## References


Model iron complexes as tool for the investigation of iron binding in humic acids: synthesis, characterization and algal growth experiments

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Time dependent UV-Vis spectra in seawater

Figure S1. Time dependent UV-Vis spectra of 2 (left) and 3 (right) in seawater.

Figure S2. Time dependent UV-Vis spectra of 4 (left) and 5 (right) in seawater.

Figure S3. Time dependent UV-Vis spectra of 6 (left) and 7 (right) in seawater.
Time dependent UV-Vis spectra in distilled water

Figure S4. Time dependent UV-Vis spectra of 2 (left) and 3 (right) in distilled water.

Figure S5. Time dependent UV-Vis spectra of 4 (left) and 5 (right) in distilled water.

Figure S6. Time dependent UV-Vis spectra of 6 (left) and 7 (right) in distilled water.
Time dependent UV-Vis spectra in distilled water at pH 8

Figure S11. Time dependent UV-Vis spectra of 3 in distilled water at pH 8

Time dependent UV-Vis spectra in seawater medium (21 days)

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Figure S13. Time dependent UV-Vis spectra of 3 and 4 in seawater medium over 21 days

Figure S14. Time dependent UV-Vis spectra of 5 and 6 in seawater medium over 21 days

Figure S15. Time dependent UV-Vis spectra of 7 in seawater medium over 21 days
Crystallographic parameters and X-ray structures

Experimental parameters and CCDC Codes of the X-ray diffraction measurements are listed in Table S1–S3.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Machine</th>
<th>Source</th>
<th>Temp.</th>
<th>Detector Distance</th>
<th>Time/Frame</th>
<th>#Frames</th>
<th>Frame width</th>
<th>CCDC</th>
</tr>
</thead>
<tbody>
<tr>
<td>chloroflavone</td>
<td>D8</td>
<td>Mo</td>
<td>100 (2)</td>
<td>35</td>
<td>12</td>
<td>1265</td>
<td>0.4</td>
<td>1499386</td>
</tr>
</tbody>
</table>

Table S1. Experimental parameters and CCDC-Codes.

1. chloroflavone.

Figure S16. Asymmetric Unit of chloroflavone, drawn with 50% displacement ellipsoids. The two aromatic mean planes [C1-C9][C10-C15] trap the angle of 15.8626(7) degree.
Table S2. Sample and crystal data of chloroflavone.

<table>
<thead>
<tr>
<th>Chemical formula</th>
<th>C15H9ClO3</th>
<th>Crystal system</th>
<th>monoclinic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula weight [g/mol]</td>
<td>272.67</td>
<td>Space group</td>
<td>P21/n</td>
</tr>
<tr>
<td>Temperature [K]</td>
<td>100</td>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Measurement method</td>
<td>Φ and ω scans</td>
<td>Volume [Å³]</td>
<td>1178.80(8)</td>
</tr>
<tr>
<td>Radiation (Wavelength [Å])</td>
<td>MoKα (λ = 0.71073)</td>
<td>Unit cell dimensions [Å] and [°]</td>
<td>13.1740(5) / 90</td>
</tr>
<tr>
<td>Crystal size [mm³]</td>
<td>0.208 × 0.187 × 0.099</td>
<td></td>
<td>5.0737(2) / 109.8027(12)</td>
</tr>
<tr>
<td>Crystal habit</td>
<td>clear yellow block</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density (calculated) / [g/cm³]</td>
<td>1.536</td>
<td>Absorption coefficient / [mm⁻¹]</td>
<td>0.324</td>
</tr>
<tr>
<td>Abs. correction Tmin</td>
<td>0.6785</td>
<td>Abs. correction Tmax</td>
<td>0.7460</td>
</tr>
<tr>
<td>Abs. correction type</td>
<td>multiscan</td>
<td>F(000) [e⁻]</td>
<td>560</td>
</tr>
</tbody>
</table>

Table S3. Data collection and structure refinement of chloroflavone.

| Index ranges | -17 ≤ h ≤ 18, -7 ≤ k ≤ 6, -26 ≤ l ≤ 26 | Theta range for data collection [°] | 3.314 to 60.202 |
| Reflections number | 25178 | Data / restraints / parameters | 3464/0/173 |
| Refinement method | Least squares | Final R indices | all data, R1 = 0.0380, wR2 = 0.0936 |
| Function minimized | Σ w(Fo² - Fc²)² | | I>2σ(I), R1 = 0.0328, wR2 = 0.0895 |
| Goodness-of-fit on F² | 1.078 | Weighting scheme | w=1/[σ²(Fo²)+(0.0483P)²+0.5578P] |
| Largest diff. peak and hole [e Å⁻³] | 0.47/-0.22 | | 

Composition of enriched seawater medium for algae experiments

<table>
<thead>
<tr>
<th>Full medium</th>
<th>Medium -Fe</th>
<th>Medium -EDTA</th>
<th>Medium + model compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
</tr>
<tr>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
</tr>
<tr>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
</tr>
<tr>
<td>0.2 mL of 0.88 M NaNO₃</td>
<td>0.2 mL of 0.88 M NaNO₃</td>
<td>0.2 mL of 0.88 M NaNO₃</td>
<td>0.2 mL of 0.88 M NaNO₃</td>
</tr>
<tr>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
</tr>
<tr>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0023 mM of model compound</td>
</tr>
</tbody>
</table>
Chemical used for the preparation of enriched seawater medium:

MgSO₄·6H₂O, Sigma Aldrich, 31413 and Alfa Aesar, 10797
CaCl₂·2H₂O, Fluka, 21100 and Alfa Aesar, 10680
SrCl₂·6H₂O, Aldrich, 204463
KBr, Fluka, 90737
Na₂SO₄, 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
NaHCO₃, Sigma Aldrich, 31437
H₂BO₃, Fluka, 15660
Na₂EDTA·2H₂O, Sigma Aldrich, E6635
FeCl₃·6H₂O, Sigma Aldrich, 31232
MgCl₂·4H₂O, Riedel-de Haen, 31422
NaMoO₄·H₂O, Riedel-de Haen, 31439
CoCl₂·6H₂O, Fluka, 60820
ZnSO₄·7H₂O, Sigma Aldrich, 31665
CuSO₄·5H₂O, Fluka, 61245
NaH₂PO₄·H₂O, Sigma Aldrich, 71504
NaNO₃, Fluka, 71758
Na₂SiO₃·9H₂O, Sigma, S4392
Vitamin B₁₂, Sigma Aldrich, V2876
Biotin, Sigma Aldrich, B4501
Thiamine hydrochloride, Sigma, T4625

Growth curves of C. salina with different N:P ratios

Figure S17. Growth curves of C. Salina (error bars: ± SD) with different N:P ratios compared to control samples (+Fe, -EDTA; -Fe, +EDTA, -Fe, -EDTA).
Cyclic voltammograms

**Figure S18.** Cyclic voltammograms of complex 1 DMF solution containing 0.10 M \([n\text{-}Bu_4N][BF_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.

**Figure S19.** Cyclic voltammograms of complex 2 and thiomaltol in DMF solution containing 0.10 M \([n\text{-}Bu_4N][BF_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.
Figure S20. Cyclic voltammograms of complex 3 and catechol 5c in DMF solution containing 0.10 M \([n-\text{Bu}_4\text{N}][\text{BF}_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.

Figure S21. Cyclic voltammograms of complex 4 and ligand 5d in DMF solution containing 0.10 M \([n-\text{Bu}_4\text{N}][\text{BF}_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.
Figure S22. Cyclic voltammograms of complex 5 and 3-hydroxybenzhydroxamic acid in DMF solution containing 0.10 M \([n-\text{Bu}_4\text{N}][\text{BF}_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.

Figure S23. Cyclic voltammograms of complex 6 in DMF solution containing 0.10 M \([n-\text{Bu}_4\text{N}][\text{BF}_4]\) at a scan rate 0.20 V s\(^{-1}\) using a glassy carbon working electrode.
Figure S24. Cyclic voltammograms of complex 7 and chloroflavone in DMF solution containing 0.10 M [n-Bu₄N][BF₄] at a scan rate 0.20 V s⁻¹ using a glassy carbon working electrode.

3. B-O-4 TYPE DILIGNOL COMPOUNDS AND THEIR IRON COMPLEXES FOR MODELLING OF IRON BINDING TO HUMIC ACIDS: SYNTHESIS, CHARACTERIZATION, ELECTROCHEMICAL STUDIES AND ALGAL GROWTH EXPERIMENTS

Ewelina Orlowska\textsuperscript{a}, Éva A. Enyedy\textsuperscript{b}, Marc Pignitter\textsuperscript{c}, Franz Jirsa\textsuperscript{a,d}, Regina Krachler\textsuperscript{a}, Wolfgang Kandioller\textsuperscript{a}, and Bernhard K. Keppler\textsuperscript{a}

Submitted manuscript

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Graphical abstract:

Within this manuscript a series of B-O-4 type dilignol compounds and their iron complexes, as model compounds for aquatic humic acids and their complexes, were prepared and characterized. Properties regarding complex stability, lipophilicity, redox behavior as well as the ability to supply microorganisms with iron were evaluated.

As the first author, I performed the entire synthesis and purification of the model compounds. Furthermore, I accomplished the analytical studies including cyclic voltammetry, UV–Vis spectrophotometry and NMR spectroscopy as well as algal studies. I made the major contribution to all chapters of the manuscript.
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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\textit{Keywords}: humic acids models, algal growth experiments, dilignol, iron, coordination compounds.

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E-mail: wolfgang.kandioller@univie.ac.at
ABSTRACT

A series of β-O-4 type dilignols and their iron(III) complexes, designed as model compounds for humic acids, were prepared and characterized by $^1$H-NMR and $^{13}$C-NMR spectroscopy, elemental analysis, EPR spectroscopy, IR spectroscopy, UV–Vis spectroscopy and electrospray ionization mass spectrometry (ESI-MS). Properties regarding iron binding, stability, lipophilicity and bioavailability for microorganisms have been evaluated with cyclic voltammetry, stability studies in water and seawater by means of UV–Vis spectrophotometry and the algae growth assays with seawater algal species Chlorella salina and Prymnesium parvum. Both established ligands and their iron complexes undergo deprotonation processes in seawater whereas no changes in UV–Vis spectra were observed in distilled water. The iron(III) complex formation constants, $pK_a$ values and lipophilicity of the dilignols were in the same range as for the analogous catechol coordination compound. Synthesized dilignols were prone to redox reactions under biological conditions similar to natural aquatic humic acids. Moreover, an increased iron bioavailability was observed for the presented complexes compared to corresponding catechol complexes and comparable to the bioavailability of iron bound to humic acid complexes recovered from Craggie Burn river. Those results confirm that β-O-4 type dilignol compounds are excellent model ligands for aquatic humic acids.
Introduction

Iron chemistry in seawater and its bioavailability for microorganisms has been intensively investigated for a long time.[1-4] Several experiments have shown that iron is limiting the productivity of marine ecosystems in huge areas of the ocean[5-9] but only little is known about the chemical speciation of iron in seawater and its accessibility for phytoplankton. Iron is not only an important factor for growth of algae and other microorganisms but also selectively supporting the development and expansion of several species (with lower iron demand), which impacts the food chain within the whole ecosystem.[4, 10] Analytical methods provide information about the concentration of dissolved and undissolved iron or iron solubility dependent on the presence of organic ligands. However, exact structures of bioavailable iron complexes and uptake mechanisms remain still unclear. There are several factors influencing iron bioavailability for microorganisms. Besides different uptake mechanisms, kinetics and iron requirements for each species, one pressing issue is the chemical form of bioavailable iron. It is known, that inorganic iron displays an extremely low solubility in seawater, which is the result of the low solubility of iron oxide (the general term ‘iron oxide’ describes various forms of iron oxide, oxyhydroxide and amorphous iron hydroxide).[11, 12] Thus, almost all of dissolved iron in the ocean is bound to organic scaffolds.[13-16] Those ligands have a large range of different structures and origins. One group of them are siderophores, which are produced by bacteria particularly in order to complex iron(III).[17-19] Stability constants for iron complexes of these natural chelators are extremely high and special mechanisms have been developed by certain organisms to release the iron from formed complexes.[20] Another big group of important iron chelators are substances which originate from decomposition of organic material, known as humic substances. Their main sources are rivers, especially derived from peat bog regions which are rich in aquatic humic substances (AHS). It has been shown, that AHS are greatly improving the bioavailability of iron in coastal waters.[21-25] Also algal studies on microalgae Chlorella salina and Diacronema lutheri in batch cultures support the assumption of the positive impact of AHS on iron supply.[26] Algal cultures treated with isolated AHS showed increased growth response compared to samples treated with iron(III) chloride and EDTA (ethylenediaminetetraacetate), a complexation agent used for commercial algal cultivation[27]). Knowing the importance of those chelators on phytoplankton growth and vitality, which affect huge amount of other processes like for example the global CO₂ cycle,[28-30] it is of great
interest to clarify the chemistry behind iron complexation, release and uptake as well as the structure of the complexes and ligands. Unfortunately, research on humic substances, especially AHS is very challenging due to their low concentration in seawater[31] and the structural complexity depending on origin, incorporated building blocks and formation processes.[32-35] In order to understand the chemistry of AHS regarding iron binding properties and the postulated release by photoreduction[36] the utilization of model systems represents a highly promising approach. In our previous studies, we synthesized simple iron complexes with different coordination motifs and ligand scaffolds and investigated them for their suitability as model compounds for humic acids iron complexes.[37] The model compounds were characterized and investigated by various analytical techniques (cyclic voltammetry, EPR, EXAFS,[36] UV‒Vis spectroscopy etc.) in addition to algal batch culture studies on chlorophyte and haptophyte unicellular algae species. The results of the latter assays led to the conclusion, that catechol-based ligand systems are excellent scaffolds for modeling of iron–AHS complexes. Therefore catechol-derived ligands and complexes with structural characteristics and molecular weight in range of natural AHS were established. The main components of humic substances are lignin decomposition products, originating from support tissues in plants and guaiacylglycerol-β-guaiacyl ether represents a suitable model for lignin.[38, 39] Binding studies of iron with guaiacylglycerol-β-guaiacyl ether precursors (coniferyl alcohol, sinapic acid, ferulic and coumaric acid) and dehydrogenation polymers of coniferyl alcohol have been already carried by Guillen et al. regarding their sorption properties in comparison to lignin.[40, 41] Although guaiacylglycerol-β-guaiacyl ether seems to be a good model for AHS, formation of stable iron complexes is unlikely. For this reason, we decided to modify the β-O-4 backbone of guaiacylglycerol-β-guaiacyl ether by introduction of a free catecholic moiety.

In this work we report the synthesis of diastereomERICALLY pure lignols designed as model compounds for AHS, bearing free catecholic groups and their respective iron complexes. The synthesized substances were characterized by elemental analysis, spectroscopic methods (IR, EPR, UV–Vis), NMR spectroscopy (in the case of the ligands), mass spectrometry (ESI-MS) and cyclic voltammetry. The The proton dissociation constants (pKₐ), complex formation constants and lipophilicity (as distribution coefficients) were determined. The bioavailability of the respective iron coordination compounds was elucidated in two unicellular algal species, namely chlorophyta Chlorella salina and haptophyte Prymnesium parvum in batch cultures.
Results and discussion

Synthesis – general overview

DiastereomERICALLY pure gUaiacylglycerol-β-guaiaCyl ether 6a was synthesized according to previously published procedure.[42] Two novel β-O-4 type lignol compounds 6b,c were synthesized in a similar approach (Scheme 1, see SI). Compound 3 was prepared according to the procedure described by Nakatsubo et al.[43] using tert-butyl chloroacetate. Aldehydes bearing free phenolic groups were protected with benzyl groups to avoid undesired side reactions (2a–c). Aldol condensation of 3 and 2a–c in the presence of lithium diisopropyl amide at -78°C led to diastereoisomeric mixture of 4a–c (1:1 ratio of erythro and threo, respectively). As described in literature, recrystallization of 4a from EtOAc gave rise to pure erythro diastereoisomer. For the compounds 4b and 4c recrystallization was not successful and column chromatography (n-hexane/EtOAc 5:1 to 1:1) was performed. Due to the very similar elution time, it was difficult to separate both diastereoisomers and only the erythro diastereoisomer was purely separated. The isolated compounds were reduced with lithium aluminum hydride and benzyl groups were cleaved by palladium-catalyzed hydrogenation yielding 6a–c. Formation of the desired dilignols was confirmed by NMR spectroscopy and elemental analysis. The ESI-MS spectra of the ligands were measured in methanol or in acetonitrile. Overall the positively charged Na⁺ adducts, with m/z values of 329 (6a and 6b), were detected. Lignols were isolated in low to moderate yields due to the crucial purification step including the separation of diastereoisomers. Thus in this step the yield of the pure erythro diastereoisomers was between 7% and 20%. The Fe(III) complexes were synthesized by deprotonation of the ligand with potassium hydroxide and addition of iron(III) chloride in methanolic solution (see SI). The complexes 7b and 7c were isolated in low to
moderate yields (7–27%) and characterized by standard analytical methods. We presume that similar to analogous Fe(III) catechol coordination compounds, dinuclear complexes with two lignols bridging two iron centers were obtained. The mass spectra of the complexes display, similar to isolated and characterized catechol complex, one negatively charged fragment with \( m/z \) value of 664, which can be assigned to Fe(lignol)\(_2\)^-. All detected molecular peaks of the complexes showed the expected isotopic iron pattern. Also elemental analysis, electrochemical studies and complex formation studies support our proposed structure.

**Cyclic voltammetry**

The electrochemical behavior of the ligands and complexes was studied in order to estimate the possibility of iron reduction under physiological conditions. The proposed mechanism of iron release in natural humic acid complexes includes the photoreduction of Fe(III) to Fe(II).[36] Fe(II) has low affinity to AHS, dissociates from the complex and can be uptaken by the microorganisms. Although Fe(II) undergoes oxidation in oxygenated seawater, the steady state concentration of Fe(II) remains higher due to this reduction process.[44] Thus redox potentials present important information regarding the bioavailability of iron and cyclic voltammetric measurements of \( 6b \) and \( 6c \) were performed in aqueous solution at various pH values in the absence and in the presence of iron(III) ions. Representative cyclic voltammograms are shown in Figure 1 for \( 6b \) and catechol as reference, and electrochemical data are presented in Table S1. A reversible \( (E_{1/2} = 0.00 \text{ V}) \) and a quasi-reversible process \( (E_{1/2} = +0.82 \text{ V}) \) can be observed at pH 3.7 in the case of \( 6b \). The reversible process might belong to the one-electron transfer between the catechol and the semiquinone radical.[45] On the other hand mostly irreversible oxidation peaks appear at pH > 8.8, which is the pH range where the ligand \( 6b \) is present in its monodeprotonated (HL\(^-\)) form (Figure S4A). The current maximum of the cathodic peak at +0.65 V is decreasing with increasing pH and a novel oxidation peak appears at +0.50 V and becomes dominant at pH 9.89, but disappears at pH >12. In addition a new reduction peak is observed at pH ≥11 at +0.27 V. In the presence of half-equivalent iron(III) the position of the quasi-reversible peak pair is already shifted at pH 1.8 and \( \Delta E \) is somewhat lower \((+0.33 \text{ vs. } +0.24 \text{ V})\) (Fig. 3b), although practically no complex formation takes place under these conditions (Figure S4B), because peaks belong to the unbound iron are observed at a lower potential range \((E_{1/2} = –0.15 \text{ at pH 2.7, not shown here})\). The cathodic peak at +0.42 V is disappearing with increasing pH, while
the oxidation peak still can be observed shifting from +0.76 to +0.42 V. The anodic peak is most probably related to the electrochemical oxidation of the ligand, which is mostly bound to the metal ion (e.g. 95% of the ligand is coordinated at pH 8.) In addition a novel cathodic peak is seen at ca. −0.1 V at pH > 9 where more and more tris-ligand species are assumed to be formed and the reduction process can be described to the iron(III) / iron(II) transition. Notably, only irreversible processes are observed at pH > 5. Overall, 6c behaves very similarly to 6b; however, formal potentials $E_{1/2}$ of the ligand are always lower by 170-180 mV (Table S1). At the same time in the presence of iron(III) ions the observed anodic and cathodic peak positions are just slightly different in the case of the studied ligands. Comparing the recorded voltammograms of 6b, 6c to those of catechol at pH ~ 8 when iron(III) was added to the solution it can be noted that the oxidation peak of catechol (+0.64 V) appears at a similar potential as in the case of the other two ligands, while a cathodic peak with high intensity is seen at +0.42 V and another smaller reduction peak appears at lower potentials (+0.11 V). In the case of catechol, iron(III) is present in the bis complex predominantly at 1:2 metal-to-ligand ratio at pH 8 due to the formation of the higher stability complexes compared to ligands 6b and 6c (c.f. Figure S4B in the case of 6b) based on the determined stability constants (vide infra).

**Figure 1.** Cyclic voltammograms of 6b (A) and the iron(III) – 6b (1:2) (B) system at various pH values in aqueous solution, and for catechol (at pH 5.54 and 8.00) and iron(III) – catechol (1:2, 1:3) system (at pH ~8.1) for comparison (C). ($c_L = 2.0 \text{ mM}; I = 0.10 \text{ M (KCl)}; T = 25 ^\circ \text{C}$)
The isolated Fe(III) complexes 7b,c of the respective dilignols ligands 6b,c were investigated for comparison. However, due to the lower aqueous solubility the experiments were performed in DMF (Figure S1). Under these conditions the two irreversible reduction peaks with values $-0.07$ V and $+0.52$ V and an irreversible oxidation peak at $+1.2$ V were detected for 7b which can be assigned to ligand and iron reduction. Complex 7c showed three irreversible reduction peaks with the values $+0.01$ V, $+0.29$ V and $+0.44$ V and similar to 7b an oxidation peak at $+1.2$ V.

Overall, the observed reduction potentials of model ligands, complexes and in situ prepared iron(III) complexes are lying in the area accessible to photoreduction as well as for natural occurring reducing agents.[46] Those studies confirm that those dilignols can be seen as suitable model compounds for AHS regarding their redox activity.

**EPR spectroscopy**

EPR spectral analyses of the ligands 6a–c, revealed a $g$-factor of 2.0 similar to the free electron (Figure 2). Thus, all ligands might represent organic free radicals. Santana-Casiano et al. showed that catechol-type structures can be readily oxidized in the presence of oxygen yielding stable radicals.[47] Stable radicals were also detected in humic acids.[48, 49] The high stability of the free radicals can be explained by the delocalization of the electron over the phenol ring. The hyperfine splitting of the signal indicates an interaction between the unpaired electron and magnetic hydrogen nuclei. The line pattern of the EPR spectrum suggests that the electron is delocalized over the carbon atoms of the aromatic ring. The EPR spectrum of the ligand, 6a, differs from the other ligands exhibiting a $g$-factor of approximately 1.9. The methoxy group at the aromatic ring might participate in the delocalization of the electron, thereby inducing the appearance of additional signals. The two iron complexes, 7b and 7c, showed a distinct signal at $g = 4.3$ (Figure 2). This isotropic-looking line is characteristic of Fe(III) in the high spin state. By considering all experimental $g$-values for each of the Kramers’ doublets, a rhombicity of 0.32 and 0.11 was determined for both complexes, suggesting low axial symmetry.
Proton dissociation processes and lipophilicity of the ligands

The proton dissociation constants of the studied ligands 6a–c were determined by pH-potentiometric and UV–Vis spectrophotometric titrations in aqueous solution (Table 1). The first deprotonation process in all cases takes place at pH < 11.5, therefore pK\textsubscript{1} values could be determined accurately by the evaluation of the pH-potentiometric titration curves; on the other hand, these titrations were used to obtain the exact concentration of the ligands stock solutions and to check their stability in aqueous solution. Consecutive titrations showed that no ligand decomposition occurred in the pH range studied under an argon atmosphere and the stock solutions (pH ~1.8) were stable over 48 h. The proton dissociation processes could be followed by UV–Vis spectrophotometric titrations as well, since the deprotonation of the hydroxyl functional groups is accompanied by characteristic spectral changes. However, the second step occurs only at pH > 11.5 and spectra had to be recorded at high pH values (pH > 12.6) where the ionic strength of 0.10 M could not be kept constant and the measurement of the pH values became uncertain due to the alkaline error of the glass electrode. Therefore the pK\textsubscript{2} constants have fairly high experimental errors and should be considered as estimated values. The titrations were performed under strictly anaerobic conditions due to the susceptibility of the catechol type ligands towards oxidation. Representative UV–Vis spectra recorded at various pH values are shown for 6b in Figures. S2A, S2B. Proton dissociation constants and the spectra of the individual ligand species H\textsubscript{2}L, HL\textsuperscript{−}, and L\textsuperscript{2−} (Table 1 and Figures S2C, S2D) were calculated on the basis of deconvolution of the measured spectra. The formation of a new band with higher \(\lambda_{\text{max}}\) values (294 nm) can be observed due to the deprotonation of the first OH moiety. While the
completely deprotonated form of the ligands \( (L_2^-) \) in the case of \( 6b \) and \( 6c \) is characterized by a strong band at 308 and 314 nm, and a weaker band at 404 and 400 nm, respectively.

Comparing the pK\(_1\) values of \( 6b \) and \( 6a \) it can be clearly seen that the acidity of \( 6a \) is significantly weaker due to the electron donating effect of the methyl group, while \( 6b \) and \( 6c \) have similar pK\(_a\) values, close to the reported values of catechol (pK\(_1\) = 9.22 and pK\(_2\) = 13.0).[50]

Table 1. Proton dissociation constants (pK\(_a\)) of ligands \( 6a-c \) determined by various methods, λ\(_{\text{max}}\) and molar absorbance values (\( \varepsilon \)) of the ligand species in the different protonates states, distribution (%) and \( n\)-octanol-water distribution coefficients (logD) at pH 2.5 and 8.3. (\( T = 25 \, ^\circ\text{C}; \, I = 0.1 \, M \, (KCl)\)).

<table>
<thead>
<tr>
<th></th>
<th>( 6a )</th>
<th>( 6b )</th>
<th>( 6c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH-potentiometry</td>
<td>pK(_1)</td>
<td>pK(_2)</td>
<td>pK(_1)</td>
</tr>
<tr>
<td>( 6a )</td>
<td>9.70 ±0.09</td>
<td>–</td>
<td>9.21 ±0.02</td>
</tr>
<tr>
<td>( 6b )</td>
<td>9.77 ±0.01</td>
<td>–</td>
<td>9.20 ±0.01</td>
</tr>
<tr>
<td>( 6c )</td>
<td>9.77 ±0.01</td>
<td>–</td>
<td>9.20 ±0.01</td>
</tr>
<tr>
<td>UV–Vis photometry</td>
<td>λ(_{\text{max}}) (nm)</td>
<td>( \varepsilon ) (M(^{-1}) cm(^{-1}))</td>
<td>λ(_{\text{max}}) (nm)</td>
</tr>
<tr>
<td>( H_2L )</td>
<td>278 / 3970(^a)</td>
<td>276 / 4040</td>
<td>276 / 4200</td>
</tr>
<tr>
<td>( HL^- )</td>
<td>248 / 8970(^b)</td>
<td>280 / 4610</td>
<td>280 / 4400</td>
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<tr>
<td>( 282 / 4125(^b) )</td>
<td>294 / 4320</td>
<td>294 / 4540</td>
<td></td>
</tr>
<tr>
<td>( 294 / 3750(^b) )</td>
<td>–</td>
<td>258 / 6000</td>
<td>266 / 8980</td>
</tr>
<tr>
<td>( L_2^- )</td>
<td>308 / 6930</td>
<td>314 / 6900</td>
<td>404 / 890</td>
</tr>
<tr>
<td>( \log D_{2.5} )</td>
<td>+0.57 ±0.10</td>
<td>+0.79 ±0.15</td>
<td>+0.57 ±0.10</td>
</tr>
<tr>
<td>( \log D_{8.3} )</td>
<td>90% ( H_2L ), 10% ( HL^- )</td>
<td>97% ( HL^- ), 3% ( L^- )</td>
<td>90% ( H_2L ), 10% ( HL^- )</td>
</tr>
<tr>
<td>( \log P ) (predicted)</td>
<td>+0.44 ±0.02</td>
<td>+0.73 ±0.14</td>
<td>+0.44 ±0.02</td>
</tr>
<tr>
<td>( +1.51c +1.66d )</td>
<td>( +1.66c +1.92d )</td>
<td>( +1.51c +1.66d )</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) Data for the neutral \( HL \) species. \(^{b}\) Data for the \( L^- \) species. \(^{c}\) MarvinSketch.[51] \(^{d}\) ChemDraw.[52]

The lipo-hydrophilic character (logD) of the ligands \( 6a–c \) was studied at pH 2.5 and 8.3 via the partitioning between \( n\)-octanol and water (Table 1, Figure S7A-B). The ligands are present in their neutral forms at the chosen acidic pH, thus logD\(_{2.5}\) can be considered as logP values. The predicted logP values (with ChemDraw and MarvinSketch programs) for these compounds are \( ca. \) one order of magnitude higher than the experimentally obtained ones; however, they show the same tendency. All results indicate that the methoxy derivative has a more lipophilic character compared to \( 6b,c \), which have moderate hydrophilic character like the reference compound catechol. At the chosen slightly alkaline pH the ligands are partly deprotonated, which causes the increased hydrophilicity. We have made an attempt at determining the lipophilicity of the iron(III)
complexes of the studied compounds, but a precipitate was formed and clogged between the two phases even at lower ligand concentration (100 µM), which hindered the quantitative analysis. Comparing the spectra of the aqueous phase obtained after partitioning for the iron(III) containing samples in buffered solution and in seawater to that of the free ligand, it can be concluded that the unbound ligand-to-complex ratio is significantly changed in the case of the buffered solution. However, in seawater the absorbance values (thus concentration) were significantly decreased in both cases. The spectra recorded for the octanol phases (Fig. S7C-D) show unambiguously the presence of some iron(III) complexes in the organic solvent in addition to the unbound ligand despite the net charge of the complex even in the case of the seawater with the very high salt content.

**Complex formation processes of ligands**

The complex formation processes of the ligand 6b with iron(III) were studied primarily by pH-potentiometry in aqueous solution. However, the complexation was found to be sluggish, especially in the acidic pH-range meaning that the equilibrium was not reached within 10 min as also reported for catecholates.[50] Therefore UV–Vis spectrophotometry was applied to follow the complexation of 6a–c with iron(III) using longer waiting time in the pH range from 2 to 11 with the exclusion of air; and the process was found to be reversible under this condition. Spectra were recorded in the wavelength range 350 – 1000 nm where mostly the strong metal-to-ligand charge transfer (CT) bands can be seen and the non-coordinated ligands do not absorb. A representative spectrum series for the iron(III) – 6c (1:3) system is shown in Figure 3A, which shows characteristic changes upon increasing pH. The $\Delta_{\text{max}}$ values of the main CT band were decreased with increasing pH (Figure 3B) and a well-isolated isosbestic point is observed at 530 nm at pH > 7.5 showing a clean transformation of a complex to another species, most probably due to the equilibrium between the bis- and tris-ligand complexes. The recorded spectra were deconvoluted resulting in the overall stability constants and the molar absorbance spectra of the [FeL]$^+$, [FeL$_2$]$^-$ and [FeL$_3$]$^{3-}$ complexes of 6b and 6c (see data in Table 2, and Figure 3C in the case of 6c). In these complexes most probably the completely deprotonated ligands (L$^{2-}$) coordinate via an (O$^-$,O$^-$) donor set as it was reported for the reference compound, catechol, and for other catecholate derivatives.[50, 53, 54] The coordination of the monoprotonated ligand (HL$^-$) in the metal complexes is very rare and observed only in compounds isolated from organic
solvents;[55, 56] however, it was also suggested for the iron(III) mono-ligand complexes of catechol by R.C. Hider et al.[54] It is noteworthy that intramolecular redox processes between Fe(III)-catecholate and Fe(II)-semiquinone species are known to occur, mostly in the case of the mono complex.[45, 54, 57] In addition existence of dimeric species [Fe₂L₄]²⁻ of catechol in the pH range of the formation of the bis-complex was also suggested based on Mössbauer spectroscopy. This species was suggested to contain two equivalent sites and a bridging hydroxyl group.[54] Spectrophotometry is not an adequate method to distinguish between the formation of [Fe₂L₄]²⁻ and [FeL₂]⁻ complexes with the same metal-to-ligand ratio. Therefore two kinds of models could be calculated for the studied systems using the titration data which consist of the formation of the mononuclear or the dinuclear complex, but the latter model gave somewhat poorer fits between the experimental and calculated absorbance values. The calculated molar absorbance spectra and λₘₐₓ values (Table 2) of the [FeL]⁺, [FeL₂]⁻ and [FeL₃]³⁻ complexes 6b and 6c are in accordance with literature data related to the catechol complexes.[53]

<table>
<thead>
<tr>
<th></th>
<th>[FeL]⁺</th>
<th>[FeL₂]⁻</th>
<th>[FeL₃]³⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>6b</td>
<td>logβ</td>
<td>18.18 ± 0.02</td>
<td>30.67 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>logK</td>
<td>18.18</td>
<td>12.49</td>
</tr>
<tr>
<td></td>
<td>λₘₐₓ (nm) / ε (M⁻¹ cm⁻¹)</td>
<td>614 / 3200</td>
<td>573 / 3550</td>
</tr>
<tr>
<td>6c</td>
<td>logβ</td>
<td>18.32 ± 0.02</td>
<td>33.13 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>logK</td>
<td>18.32</td>
<td>14.81</td>
</tr>
<tr>
<td></td>
<td>λₘₐₓ (nm) / ε (M⁻¹ cm⁻¹)</td>
<td>657 / 2840</td>
<td>567 / 3300</td>
</tr>
<tr>
<td>catechol</td>
<td>logβ</td>
<td>20.11</td>
<td>34.80</td>
</tr>
<tr>
<td></td>
<td>logK</td>
<td>20.11</td>
<td>14.69</td>
</tr>
</tbody>
</table>

a Model with the dimeric complex: logβ [FeL]⁺: 18.17 ± 0.02, logβ [Fe₂L₄]²⁻: 64.43 ± 0.03, logβ [FeL₃]³⁻: 39.45 ± 0.02.  b Model with the dimeric complex: logβ [FeL]⁺: 18.40 ± 0.02, logβ [Fe₂L₄]²⁻: 70.34 ± 0.03, logβ [FeL₃]³⁻: 43.69 ± 0.02.  c Data are taken from Ref[50] (I = ca. 0.15 M; T = 27 °C).
Figure 3. UV–Vis absorbance spectra recorded for the iron(III)–6c (1:3) system at various pH values (A) and the λ_{max} values at 1:3 (●), 1:2 (∗) and 1:1 (∆) metal-to-ligand ratios (B) (c_L = 1.06 mM). Calculated molar absorbance spectra of the individual complexes of 6c (C). Concentration distribution curves for the iron(III)–6c system at various total concentrations of the ligand at a constant concentration of iron(III) and at pH 8.0 (D). (c_L = 0 – 75 µM; c_{Fe(III)} = 15 µM; pH = 8.0; I = 0.10 M (KCl); T = 25 °C)

Since ligand 6a has lower water solubility compared to the other two studied compounds its complexation with iron(III) was studied via the ligand’s bands at lower concentrations (~ 200 µM). The measured absorbance values in the absence and the presence of the metal ion (Fig. S3) were fairly similar suggesting the negligible formation of the iron(III) complexes under these conditions. The coordination of the monodentate phenolate type ligand is very weak similarly to the case of the phenol itself.[58]

Direct comparison of stability constants (Table 2) and concentration distribution curves calculated for the iron(III) complexes formed with 6b, 6c (Figure S5A) and catechol (Figure S5B) shows that the studied ligands form somewhat lower stability complexes than catechol. (Notably, direct comparison of the logβ values in this case is possible due to the similar stoichiometry of the complexes formed and the similar pK_a values of the ligands.) In these cases
the predominant formation of the bis complexes is found at neutral and slightly basic pH values. However, the actual speciation at pH 8 depends on the actual metal-to-ligand ratio (Figure 3D).

In order to compare the metal ion binding abilities of the studied ligands to each other and to catechol or to other iron chelators (such as the catecholate-containing siderophores) pM (p[Fe(III)]) values were calculated and plotted against the pH. p[Fe(III)] is the negative logarithm of the equilibrium concentration of the free metal ion, while p[unbound Fe(III)] values were also computed which show the summed equilibrium concentration of the unbound metal fraction involving the iron(III)-hydroxido species under the conditions employed (c_{Fe(III)} = 1 \mu M, Fe(III):L = 1:10) (Figure S6). These pM–pH curves reveal the following stability trend: 6b < 6c < catechol. p[Fe(III)] values 14.08 and 14.81 were obtained at pH 7.4 for 6b and 6c respectively, which are significantly lower than that of the hexadentate catecholate-type enterobactin (35.5) or the hexadentate hydroxamate-type desferrioxamine B (26.6)[59] representing the much weaker iron(III) binding ability of the studied ligands compared to the naturally occurring siderophores.

**Algal growth experiments**

The understanding of the chemistry of these model compounds in aqueous solution provides useful information for the interpretation of algal studies. In order to evaluate the stability of the iron complexes and dilignol ligands in aqueous solution, time dependent UV–Vis studies in seawater, distilled water and water at pH 8 have been performed. Both complexes 7b and 7c and the ligands 6b and 6c showed changes in their UV–Vis spectra over 24 h in seawater (Figure S8) in contrast to 6a (see S9). The experiments in distilled water revealed no changes in absorbance in all tested compounds (see S10-S12). The time dependent spectra of 7b and 6a–c in water at pH ~8 (see S13-S14), showed also an increase in the same signals as observed in seawater. The same behavior was observed for as time period of 21 days (duration of algal growth tests) and only ligand 6a showed an additional band after 14 days (see S15-S16). Those results confirm that the established compounds are sufficiently stable for algal growth experiments. In order to elucidate the bioavailability of the iron complexes 7b,c as well as the free ligands 6a–c to provide bioavailable iron, algal tests on *C. salina* and *P. parvum* have been performed. Those two species were intentionally chosen due to their wide occurrence and different biology. *C. salina* is a representative for unicellular green algae. The species react very sensitive to iron limitation. It has been recently reported, that cultures cultivated without iron or chelator EDTA showed poor
The second species *P. parvum*, is a unicellular haptophyte and this algae is able to grow under photoautotrophic and heterotrophic conditions. The species is also less sensitive to iron deficiency and can adapt to several environmental limitations. Moreover, *P. parvum* is well known for its toxicity and is a cause of mass fish deaths in coastal waters. Investigating both species may help to understand the alterations in iron uptake and preferences of each algae species towards different iron sources. All algae experiments were carried out in batch cultures, using three different nutrition setups. As full medium, seawater with micronutrient and vitamin solution as well as solution of NaNO$_3$, Na$_2$SiO$_3$, NaH$_2$PO$_4$ as described by Guillard et al.[27], were used (see Table S5). Those are common conditions for marine algae cultivation. The second set of control samples was lacking EDTA, which serves as chelator for iron and other metal ions. Those are useful negative references to study ligands 6a–c because these samples show the growth of the culture containing inorganic iron without chelating molecules. Third control set enables the evaluation of the culture growth without iron but with chelating molecules. With this control, the impact of other metal ions in solution can be elucidated. Theoretically, if the iron bound in complexes 7b and 7c is not bioavailable, the growth of the culture should be worse than the culture without iron. On the other hand if the ligands 6a–c cannot form bioavailable complexes with iron in solution, the growth of the culture should be comparable with the growth of the culture lacking EDTA. The growth response of *C. salina* in the first experiment is shown in Figure 4. As expected samples treated with medium without EDTA and iron showed poor growth. Samples treated with 6a reached similar low algae concentration confirming that this ligand cannot stabilize iron in solution.
This result for 6a is not surprising because former complexation experiments and speciation studies revealed a low affinity of the ligand towards iron. In contrast, 6b, c showed positive impact on the growth of C. salina. In case of 6c two different concentrations were chosen for the experiment. In the standard setup concentrations equal the EDTA concentration were applied. In this case the culture grew better than in samples lacking both EDTA and iron, but worse than in full medium. For the other samples with 6c we used twice higher concentration compared to that of EDTA because one EDTA molecule is able to bind one iron molecule, but at least two dilignols are needed to form a stable Fe(III) complex. For those samples, we observed better growth as for those with standard 6c concentration and even better than in full medium. Although ligand 6b was only used in same concentration (as EDTA), the growth response was similar to algae treated with complex 7b. Those results revealed that 6b is more efficient than the respective complex 7b and ligand 6c. Overall, both ligands 6b and 6c and the complex 7b are able to provide C. salina with bioavailable iron. Treatment with those model compounds showed better growth response than the samples cultivated in full medium. Comparison of the end-algal-concentration in samples treated with 6b, 6c and 7b with our former experiments, where C. salina was treated with isolated AHS, showed similar values. Whereas samples containing AHS grew to $9 \times 10^6 \pm 3.6 \times 10^6$ cells mL$^{-1}$ and $10 \times 10^6 \pm 6.7 \times 10^5$ cells mL$^{-1}$ in each experiment, samples
with model compounds reached approximately $7.7 \times 10^6 \pm 1 \times 10^6$ cells mL$^{-1}$. Algae cultures containing complex 7c as iron source reached only half of the concentration of the culture containing 7b (see Figure S17). Table S2 shows the end concentrations of the samples as well as the relation between the end-algae concentration of control samples to samples treated with model compounds from both experiments with *C. salina*.

Additionally to the maximal algae cell concentration, we also calculated the specific growth rate of algae for all tested compounds after 17 days of each experiment (which was the duration of the algal growth experiment for 7c (Table S3). Similar to the maximal algae cell concentrations, highest specific growth rates were observed for samples treated with 6b and 7b.

The model ligands were also tested on *P. parvum* for they ability to bind and supply iron (Figure S18). The culture of *P. parvum* was not as sensitive as *C. salina* to iron deficiency and we did not observe such pronounced effects on algal growth as for *C. salina*. In fact, the control with full medium reached almost the same concentration as the control without chelator (EDTA). Ligand 6b was tested in two concentrations, (1× and 2× c(EDTA)); however, no impact on the final algae concentration was observed. In contrast to the experiments on *C. salina* samples treated with 6a showed similar growth response as 6b. Ligand 6c displayed the only positive impact on *P. parvum* in comparison to control with full medium. Overall the end concentrations of *P. parvum* in the batch culture were significantly lower as the concentration of *C. salina*. The specific growth rates of algae for all tested compounds after 17 days are shown in Table S4. Similar to the maximal algae cell concentrations, highest specific growth rates were observed for samples treated 6c.

Summarizing the algal studies results, some of our herein presented compounds can be seen as suitable models for humic acids regarding the iron transport and ability to supply microorganisms with iron. In all experiments, the algal growth in samples without iron was strongly inhibited. For *C. salina* typical growth pattern, known from iron enrichment experiments,[61, 62] was observed.[63, 64]

**Acknowledgements**

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**Supplementary Information**

Detailed information about materials, methods and syntheses are provided.


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SUPPLEMENTARY INFORMATION

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

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Material and methods

\textit{1H and \textsuperscript{13}C NMR spectroscopy}

\textsuperscript{1}H NMR spectra were recorded on a Bruker Avance III\textsuperscript{TM} 500 MHz spectrometer in DMSO-\textit{d}_6 at 298 K using standard pulse programs at 500.10 MHz for \textsuperscript{1}H experiments and 75 MHz for \textsuperscript{13}C experiments.

\textit{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} \sim 15 \text{ mM}) to obtain the acidic stock solutions (c_L \sim 2 \text{ mM}, pH \sim 1.9). The pH-metric measurements for determination of the exact concentrations of HCl and KOH stock solutions were carried out at 25.0 \pm 0.1 \textdegree 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\textsuperscript{+}] scale by means of blank titrations (strong acid vs. strong base; HCl vs. KOH) according to the method suggested by Irving et al.\textsuperscript{1} The average water ionization constant, pK\textsubscript{water}, is 13.76 ± 0.01 at 25 \textdegree C, which corresponds well to the literature data.\textsuperscript{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.\textsuperscript{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 \text{ \mu M}) 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 \pm 0.1 \textdegree 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. Literature data were used for iron(III) hydroxido species. $\beta (M_pL_qH_r)$ is defined for the general equilibrium $pM + qL + rH \rightleftharpoons M_pL_qH_r$ 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$_2$O 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. 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 AgNO₃. 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-Bu₄N][BF₄] and 2 mM of substance, using [Fe(η⁵-C₅H₅)₂] (E₁/₂ = +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:

$$\frac{\text{Absorbance (original solution)}}{\text{Absorbance (aqueous phase after separation)}} - 1.$$

Some measurements were performed for the iron(III)$^\text{-6b}$ 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. 9×10$^4$-1.5×10$^5$ 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), K₂CO₃ (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 Na₂SO₄, filtered and the solvent was evaporated to dryness. The obtained colorless crystalline solid was dried in vacuo. Yield: 7.81 g, 33 mmol, 89 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.01-6.82 (m, 4 H, H₄Ar), 4.62 (s, 2H, CH₂), 3.78 (s, 3 H, CH₃), 1.43 (s, 9 H, CH₃) 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 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 9.83 (s, 1H, H₄ald), 7.59-7.26 (m, 13 H, H₄ar), 5.29 (s, 2H, CH₂bn), 5.23 (s, 2H, CH₂bn) 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 %. ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 10.16 (s, 1H, H₄ald), 7.63-7.03 (m, 13 H, H₄ar), 5.27 (s, 2H, CH₂bn), 5.18 (s, 2H, CH₂bn) 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 (15 mL) 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 Na₂SO₄. Then the solvent was removed under vacuo to give the crude product as yellow oil. The crude product was purified 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 %).

**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 diastereoisomer 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: ¹H NMR (DMSO-d₆, 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, CH₂bn), 5.14 (d, J = 10.5 Hz, 1H, CH₂bn), 5.02 (d, J = 10.5 Hz, 1H, CH₂bn), 4.71 (d, J = 6.5 Hz, 1H, H_al), 3.60 (s, 3H, CH₃), 1.31 (s, 9H, CH₃tert) ppm. Threo: ¹H NMR (DMSO-d₆, 500.10 MHz, 25 °C): δ = 7.55–6.59 (m, 18 H, OH, H_ar), 5.71 (d, J = 6 Hz, 1H, H_al), 5.41 (dd, J = 7 Hz J = 6 Hz, 1H, H_al), 5.19 (s, 2H, CH₂bn), 5.16 (d, J = 12.5 Hz 1H, CH₂bn), 4.99 (d, J = 12.5 Hz, 1H, CH₂bn), 4.70 (d, J = 7 Hz, 1H, H_al), 3.68 (s, 3H, CH₃), 1.26 (s, 9H, H₃tert) 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, LiAlH₄ (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 CH₂Cl₂ (4x15 mL) and the filtrate was washed with brine and dried over Na₂SO₄. The solvent was removed in vacuo to give the product as colorless oil. Yield: 0.465 g, 0.98 mmol, 98 %. Erythro: ¹H NMR (DMSO-d₆, 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$_3$), 3.71 (dd, J$_1$ = 12 Hz, J$_2$ = 6 Hz, 1 H, CH$_2$), 3.58 (dd, J$_1$ = 12 Hz, J$_2$ = 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$_6$·0.25 H$_2$O: 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$_1$ = 8 Hz, J$_2$ = 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, 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$_6$·0.25 H$_2$O: C, 61.83; H, 6.00; 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$_2$O)$_2$] (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\(_2\)C\(_{64}\)H\(_{70}\)O\(_{26}\)(H\(_2\)O): C, 55.50; H, 5.24. Found: C, 55.25; H, 5.60. IR (ATR, selected bands, \( \nu_{\text{max}} \)): 2926, 1592, 1498, 1456, 1252, 1214, 1118, 1115, 1022, 811, 745, 620 cm\(^{-1}\).

\[ \text{[Fe}_2\{3-(1,3-Dihydroxy-2-(2-methoxyphenoxy)propyl)benzene-1,2-diolato}\}_4(H_2O)_2\] (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\(_2\)C\(_{64}\)H\(_{70}\)O\(_{26}\)(CH\(_3\)COCH\(_3\))\(_0.5\): C, 56.36; H, 5.27. Found: C, 56.76; H, 5.35. IR (ATR, selected bands, \( \nu_{\text{max}} \)): 3358, 1593, 1499, 1456, 1252, 1217, 1119, 1023, 868, 738 cm\(^{-1}\).

**Cyclic voltammograms and electrochemical data**

![Cyclic voltammograms of Fe(III) complexes 7b and 7c at 0.20 V s\(^{-1}\) in DMF showed at selected potential regions.](image)

*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. ($T = 25^\circ C; I = 0.1 \text{ M } (\text{KCl}); \text{scan rate} = 100 \text{ mV/s}$)

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<td>11.01</td>
<td></td>
<td>-0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iron(III)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.70</td>
<td>-0.10</td>
<td>-0.21</td>
<td>0.11</td>
<td>-0.15</td>
</tr>
<tr>
<td>6c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.48</td>
<td>-0.14</td>
<td>-0.20</td>
<td>0.06</td>
<td>-0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+0.83</td>
<td>+0.45</td>
<td>0.38</td>
<td>+0.64</td>
</tr>
<tr>
<td></td>
<td>4.86</td>
<td>+0.82</td>
<td>+0.41</td>
<td>0.41</td>
<td>+0.62</td>
</tr>
<tr>
<td></td>
<td>6.40</td>
<td>+0.81</td>
<td>+0.41</td>
<td>0.40</td>
<td>+0.61</td>
</tr>
<tr>
<td></td>
<td>6.99</td>
<td>+0.83</td>
<td>+0.41</td>
<td>0.42</td>
<td>+0.62</td>
</tr>
<tr>
<td></td>
<td>8.11</td>
<td>+0.85</td>
<td>+0.40</td>
<td>0.45</td>
<td>+0.63</td>
</tr>
<tr>
<td></td>
<td>9.09</td>
<td>+0.88</td>
<td>+0.28</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>10.06</td>
<td>+0.28</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>11.01</td>
<td>+0.20</td>
<td>+0.06</td>
<td>0.14</td>
<td>+0.13</td>
</tr>
<tr>
<td>iron(III) – 6c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1:2)</td>
<td>4.82</td>
<td></td>
<td>+0.32</td>
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</tr>
<tr>
<td></td>
<td>5.76</td>
<td>+0.69</td>
<td>+0.16</td>
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</tr>
<tr>
<td></td>
<td>6.62</td>
<td>+0.66</td>
<td>+0.13</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>8.36</td>
<td>+0.58</td>
<td>-0.15</td>
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</tr>
<tr>
<td></td>
<td>9.40</td>
<td>-0.17</td>
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<td></td>
<td>9.80</td>
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<tr>
<td></td>
<td>10.29</td>
<td></td>
<td>-0.05</td>
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<td>cat</td>
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<td></td>
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<tr>
<td></td>
<td>5.54</td>
<td>+0.84</td>
<td>+0.47</td>
<td>0.37</td>
<td>+0.66</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>+0.79</td>
<td>+0.47</td>
<td>0.32</td>
<td>+0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+0.25</td>
<td></td>
</tr>
<tr>
<td>iron(III)-cat (1:2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.08</td>
<td>+0.64</td>
<td>+0.42</td>
<td>0.22</td>
<td>+0.53</td>
</tr>
<tr>
<td>iron(III)-cat (1:3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.11</td>
<td>+0.63</td>
<td>+0.40</td>
<td>0.23</td>
<td>+0.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+0.11</td>
<td></td>
</tr>
</tbody>
</table>
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 ($c_L = 0.05 \text{ mM}$) (A) and from 10.2 to 14.1 ($c_L = 0.200 \text{ mM}$) (B). Calculated molar absorbance spectra of the individual ligand species of 6b (C) and 6a (D). ($I = 0.10 \text{ M (KCl)}; T = 25 \degree \text{C}$)

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. ($c_L = 201 \text{ mM}; I = 0.10 \text{ M (KCl)}; T = 25 \degree \text{C}$)
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. (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 \( \text{[c}_{\text{Fe(III)}} = 1.0 \mu \text{M; Fe(III):L = 1:10; } l = 0.10 \text{ M (KCl); } T = 25 ^\circ \text{C}} \)
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). (c₁ = 100 mM; 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, $T = 25^\circ$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.

<table>
<thead>
<tr>
<th>Model compound</th>
<th>6a</th>
<th>6b</th>
<th>6c</th>
<th>6c x2</th>
<th>7b</th>
<th>7c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae concentration (cells mL⁻¹)</td>
<td>8.8×10⁵ ± 2.5×10⁵</td>
<td>7.8×10⁶ ± 2.7×10⁶</td>
<td>7.8×10⁶ ± 7.6×10⁶</td>
<td>4.6×10⁶ ± 7.8×10⁵</td>
<td>2.7×10⁶ ± 6.5×10⁵</td>
<td>7.8×10⁶ ± 2.0×10⁶</td>
</tr>
<tr>
<td>+Fe, +EDTA</td>
<td>0.15</td>
<td>1.4</td>
<td>0.5</td>
<td>1.4</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>-Fe, + EDTA</td>
<td>0.8</td>
<td>7.2</td>
<td>2.5</td>
<td>7.3</td>
<td>7</td>
<td>2.4</td>
</tr>
<tr>
<td>+Fe, - EDTA</td>
<td>0.3</td>
<td>2.6</td>
<td>0.9</td>
<td>2.6</td>
<td>2.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Model compound</th>
<th>6a</th>
<th>6b</th>
<th>6c</th>
<th>6c x2</th>
<th>7b</th>
<th>7c</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Fe, +EDTA (1)*</td>
<td>0.08</td>
<td>0.23</td>
<td>0.18</td>
<td>0.22</td>
<td>0.23</td>
<td>0.17</td>
</tr>
<tr>
<td>+Fe, +EDTA (2)*</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>-Fe, + EDTA (1)*</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>-Fe, + EDTA (2)*</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Model compound</th>
<th>6a</th>
<th>6b</th>
<th>6c</th>
<th>6b x2</th>
<th>+Fe, +EDTA</th>
<th>-Fe, +EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Fe, +EDTA (1)*</td>
<td>0.08</td>
<td>0.16</td>
<td>0.20</td>
<td>0.17</td>
<td>0.19</td>
<td>0.15</td>
</tr>
<tr>
<td>+Fe, +EDTA (2)*</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>-Fe, + EDTA (1)*</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>-Fe, + EDTA (2)*</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
</tbody>
</table>

P. Parvum (after 17 d)
Composition of enriched seawater medium for algae experiments

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

<table>
<thead>
<tr>
<th>Full medium</th>
<th>Medium - Fe</th>
<th>Medium - EDTA</th>
<th>Medium + model compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
<td>200 mL of filtered artificial seawater</td>
</tr>
<tr>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
<td>0.2 mL of micronutrient solution</td>
</tr>
<tr>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
<td>0.2 mL of vitamin solution</td>
</tr>
<tr>
<td>0.2 mL of 0.88 M NaNO₃</td>
<td>0.2 mL of 0.88 M NaNO₃</td>
<td>0.2 mL of 0.88 M NaNO₃</td>
<td>0.2 mL of 0.88 M NaNO₃</td>
</tr>
<tr>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
<td>0.2 mL of 0.1 M Na₂SiO₃·9H₂O</td>
</tr>
<tr>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
<td>0.2 mL of 0.036 M NaH₂PO₄·H₂O</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.007 mM of model compound</td>
</tr>
</tbody>
</table>

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
K₂CO₃, Merck, 4924
KI, Alfa Aesar, 11601
Na₂SO₄, 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
LiAlH₄, Fluka, 62420
NaOH, Sigma-Aldrich, 30620
Pd/C (10% Pd basis), Sigma-Aldrich, 75990ferrocene
KOH, Sigma-Aldrich, 60370
4-([2-hydroxyethyl]-1-piperazinethanesulfonic acid (HEPES), Sigma-Aldrich, H3375
HCl, Reanal, 30715-1-08-65
KCl, Reanal, 18050-1-08-38
K₃[Fe(CN)]₆, Sigma-Aldrich, 244023
MgSO₄·6H₂O, Sigma Aldrich, 31413 and Alfa Aesar, 10797
CaCl₂·2H₂O, Fluka, 21100 and Alfa Aesar, 10680
SrCl₂·6H₂O, Aldrich, 204463
KBr, Fluka, 90737
Na₂SO₄, 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
NaHCO₃, Sigma Aldrich, 31437
H₃BO₃, Fluka, 15660
Na₂EDTA·H₂O, Sigma Aldrich, E6635
FeCl₃·6H₂O, Sigma Aldrich, 31232 and Reanal, 33252-1-08-38
MgCl₂·4H₂O, Riedel-de Haen, 31422
NaMoO₄·H₂O, Riedel-de Haen, 31439
CoCl₂·6H₂O, Fluka, 60820
ZnSO₄·7H₂O, Sigma Aldrich, 31665
CuSO₄·5H₂O, Fluka, 61245
NaH₂PO₄·H₂O, Sigma Aldrich, 71504
NaNO₃, Fluka, 71758
Na₂SiO₃·9H₂O, Sigma, S4392
Vitamin B₁₂, Sigma Aldrich, V2876
Biotin, Sigma Aldrich, B4501
Thiamine hydrochloride, Sigma, T4625

13. R. R. Guillard and J. H. Ryther, Canadian Journal of Microbiology, 1962, 8, 229-.
CONCLUSION

Within this PhD work, the synthesis, characterization and algal studies of (mostly novel) model compounds for aquatic humic substances were realized. The results have been published in two publications and one further manuscript has been submitted on 26th January 2017 to Environmental Science & Technology.

In the first part of the work, a series of 11 novel monomeric and dimeric Fe\textsuperscript{III} complexes bearing hydroxamates has been prepared and extensively characterized by elemental analysis, IR spectroscopy, electrospray ionization mass spectrometry, cyclic voltammetry, UV–Vis and for some examples by X-ray diffraction analysis. Comparison of the bond lengths, angles and distances in evaluated X-ray structures with the EXAFS studies of natural humic acids showed that selected model structures are suitable as AHS model compounds. Algal studies with the complexes showed that several of our models support the growth of the algae confirming that iron bound in the complexes is bioavailable. However, the induced growth upon treatment with the model compounds was lower compared to ideal conditions and isolated natural AHS fractions. This might be explained by the observed low redox potentials of the iron complexes. Therefore, search for more suitable models and optimization with regard on the algal growth and redox potentials was required.

Different possible coordination modes in iron complexes with AHS were investigated in the second part of the work. A series of iron O,O; O,N; O,S-chelates with different structural properties and chemical behavior were synthesized and characterized. Two of the seven compounds, namely catechol and thiomaltolato complex, showed effects on the algal cultures, which are comparable with natural humic acids. According to the previous work, EXAFS studies and elemental analysis of AHS, it was unlikely that the majority of iron in humic acids is bound via sulfur, so the best fitting model from this study was the dimeric catechol-iron-complex. Moreover, due to the presence of many phenolic moieties in natural AHS, this coordination motif seemed to be convincing.

On the basis of those findings, in the third part of the work catechol-based ligands and complexes with structural characteristics and molecular weight in range of natural AHS were developed. Knowing, that lignin derivatives and degradation products are important
components of humic substances, a building block and known model of lignin, namely guaiacylglycerol-β-guaiacyl ether was used as the output molecule for the studies. A series of β-O-4 type dilignol compounds and their iron complexes were prepared and characterized. Cyclovoltammetric measurements showed that all of the compounds were reducible under biological conditions, which was in good accordance with the behavior of AHS. On the basis of EPR measurements, all synthetized ligands might represent organic free radicals, which are stabilized by the delocalization of the electron over the phenol ring. The aqueous chemistry of the model compounds including complex and ligand stability, pKa values, complex formation constants and lipophilicity was intensively investigated using UV–Vis spectrophotometry and potentiometric titrations. Ligands bearing catecholic moiety showed very good properties regarding iron complexation and delivery to the algae. Performed algal studies on the cultures of *C. salina* and *P. parvum* confirmed the bioavailability of iron in complexes and also after addition of the ligands to iron-containing seawater medium. Those results confirm that catecholic moieties play important role by the complexation and dissolution of iron by humic substances. Summarizing, of β-O-4 type dilignol compounds bearing catecholic moieties are excellent models for AHS, having their structural and functional similarities, which enables better understanding of the biochemistry of iron and AHS.

The obtained results significantly extend the knowledge about the structure of humic acids-based iron complexes, the binding mode and strength of the coordination bond between the metal and ligand. In addition, it is the next step for the explanation of the biogeochemical iron and carbon cycle in the marine ecosystems and elucidation of the importance of natural chelates as ion carriers in the ocean.
Scientific Publications


Presentations at conferences (oral&poster)

13th European Biological Inorganic Chemistry Conference – Poster (2016)
13th international Symposium on Applied Bioinorganic Chemistry (ISABC13) – Poster (2015)
"Ich habe mich bemüht, sämtliche Inhaber der Bildrechte ausfindig zu Machen und ihre Zustimmung zur Verwendung der Bilder in dieser eingeholt. Sollte dennoch eine Urheberrechtsverletzung bekannt werden, ersuche ich um Medlungen bei mir:" 

Ewelina Orlowska 
Wien, Januar 2017