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Mixed catechol-hydroxamate and catechol-(*o*-hydroxy)phenacyl siderophores: synthesis and uptake studies with receptor-deficient *Escherichia coli* mutants

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Abstract

Biscatechol-hydroxamate and catechol-bishydroxamate chelators of similar architecture were synthesized by a small set of acylation reactions, Michael additions, and domino Wittig alkenations and they were tested for siderophoric activity in various receptor-deficient mutants of *Escherichia coli* under iron starvation. Growth promotion occurred only in mutants featuring catecholate recognizing receptors. Simplified mimics of the natural siderophores parabactin and agrobactin, carrying an *o*-hydroxybenzamide instead of the 2-(*o*-hydroxyphenyl)oxazoline ligand, were prepared analogously. Unlike the originals these mimics are fully functional siderophores in *E. coli*.

Keywords: Siderophores; Receptor specificity; Wittig alkenation; Michael addition

1. Introduction

Most bacteria under aerobic conditions excrete specific chelators, so-called siderophores, to sequester and transport Fe(III), which is essential for them.[1–5] Bacterial siderophores feature tris-bidentate ligands of the catechol, hydroxamate, or carboxylate types aptly positioned for octahedral coordination of the metal. Cognate outer membrane receptors then recognize the resulting siderophore–iron complexes and initiate their internalization.[4,5] A few bacteria do not produce siderophores of their own but rely entirely on alien ones, e.g., on hydroxamates from fungi.[6] Some competitors in the chemical warfare of germs have adapted to this peculiarity by producing conjugates of siderophores with antibiotic effectors (e.g., sideromycins).[2,7–9] Synthetic siderophores with various types and combinations of ligands and with simplified structures were also frequently employed as carriers for antibiotics.[3,7,10–12] Their rational design requires information on the uptake pathways, receptor specificities, and essential structural features as well as a short reliable synthetic strategy.

In a preceding paper[13] we reported on such a short synthesis of triscatechol and biscatechol-hydroxamate siderophores. Herein we extend this methodology to the synthesis of other mixed-ligand siderophores with hydroxamate, catechol, and (*o*-hydroxy)phenacyl groups and we also elucidate their uptake pathways in growth promotion experiments with receptor-deficient mutants of *Escherichia coli*.

2. Results and discussion

2.1. Synthesis of mixed catechol-hydroxamate chelators

A series of tris-bidentate chelators with different combinations of catechol, hydroxamate, and (*o*-hydroxy)phenacyl ligands were prepared for receptor specificity and siderophoric activity studies. They are all capable of strain-free octahedral coordination of Fe(III) cations as to MM2 force field calculations. The biscatechol-hydroxamate **7** was available from benzyl acrylate **1** in five steps (Scheme 1; top). Michael addition

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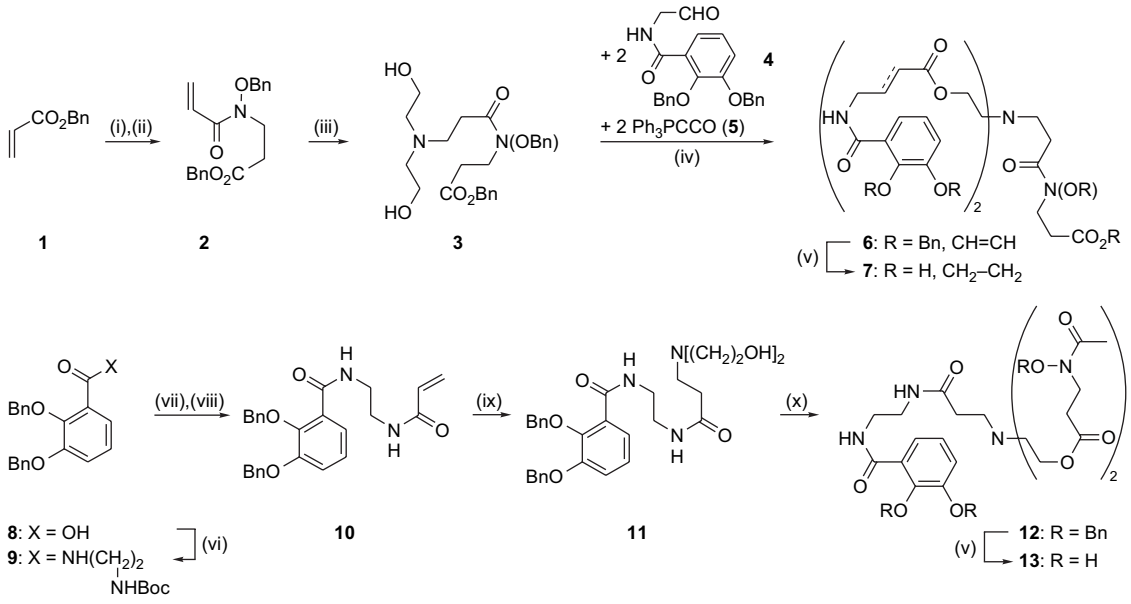


Scheme 1. Reagents and conditions: (i) H_2NOBn, EtOH, rt, 24 h, 63%; (ii) acryl chloride, NEt_3, CH_2Cl_2, 0 °C to rt, 30 min, 85%; (iii) $[HO(CH_2)_2]_2NH$, EtOH, rt, 24 h, 60%; (iv) THF, cat. benzoic acid, rt, 8 h, 55%; (v) 5% Pd/C, H_2 (1 bar), MeOH/dioxane, rt, 7 h, 95%; (vi) (a) $SOCl_2$, $H_2N(CH_2)_2NHBoc$, CH_2Cl_2, rt, 1.5 h, 100%, (b) NEt_3, CH_2Cl_2, 0 °C to rt, 30 min, 83%; (vii) 3 M HCl, THF, 0 °C to rt, 2–3 h, then aq NaOH/CH_2Cl_2, 90%; (viii) acryl chloride, NEt_3, CH_2Cl_2, 0 °C to rt, 30 min, 93%; (ix) $[HO(CH_2)_2]_2NH$, EtOH, reflux, 48 h, 68%; (x) AcN(OBn)$(CH_2)_2CO_2H$ and $ClCO_2Et$ premixed, CH_2Cl_2, NEt_3, 0 °C, 30 min, then added to **11**, CH_2Cl_2, 0 °C to rt, 5 h, 56%.

of benzoxamine afforded benzyl 3-(*N*-benzoxamino)propionate, which was immediately acylated with acryl chloride to give benzoxamate **2**. A further Michael addition of diethanolamine yielded the diol **3**, which was bis-esterified in a three-component domino reaction with aldehyde **4**[13] and ylide **5**[14] to give **6**. In this domino process[15] the hydroxy groups of **3** added across the C=C bond of ylide **5** to form a stabilized bisester ylide, which in turn Wittig-alkenated the aldehyde group of **4**. Subsequent catalytic hydrogenation left chelator **7** as a colorless foamy solid. A catechol-bishydroxamate **13** was also built up on a route based upon acylation and Michael addition reactions starting from dibenzoxybenzoic acid **8**[16] (Scheme 1; bottom). This was coupled with mono-Boc-protected ethylenediamine to give compound **9**. Cleavage of the Boc group followed by acylation with acryl chloride afforded acryl amide **10**, which was treated with diethanolamine. The resulting diol **11** was bis-acylated with 3-[*N*-(benzoxy)acetamido]propionic acid[17] via mixed-anhydride coupling furnishing the benzyl protected precursor **12**. Hydrogenolysis produced the colorless solid chelator **13**.

A third type of catechol chelator in which an oxazoline heteroatom in a formal benzyl position can take part in ligating is found in parabactin (**14a**) and agrobactin (**14b**), as produced by *Paracoccus denitrificans* and *Agrobacterium tumefaciens*, respectively.[18] The natural enantiomers of **14** are derived from L-threonine and form Λ-configured[19a] Fe(III) complexes, which are not recognized by the receptors of *E. coli*.[19b] We now prepared achiral analogs with longer spacers between the phenyl rings and with either an imidazoline (e.g., **15**) or a salicylamide (e.g., **16**) as a potential donor group (Fig. 1). They were tested for siderophoric activity in an *E. coli* mutant strain devoid of own siderophores.

The synthesis of the imidazolines **15** started from the 2-(*o*-benzoxy)phenylimidazolines **18**, which were readily accessible by oxidative cyclization of (di)benzoxybenzaldehyde **17a**[20] or **17b**,[21] respectively, with ethylenediamine and *N*-bromosuccinimide (NBS)[22] (Scheme 2; top). N-acylation of **18** with acryl chloride gave the corresponding acryl amides **19**, which were in turn reacted with diethanolamine to furnish the diols **20**. These were submitted to the three-component reaction with ylide **5** and aldehyde **4** as described above affording the pre-chelators **21**. Their hydrogenation eventually left the ligands **15** as pale yellow solids. The route to chelators **16** was based on a two-fold three-component reaction with ylide **5**. The first one with aldehyde **4** and *N*-Boc-diethanolamine **22**[23] afforded the biscatechol fragment **23a**. Cleavage of its Boc group liberated amine **23b**, which was submitted

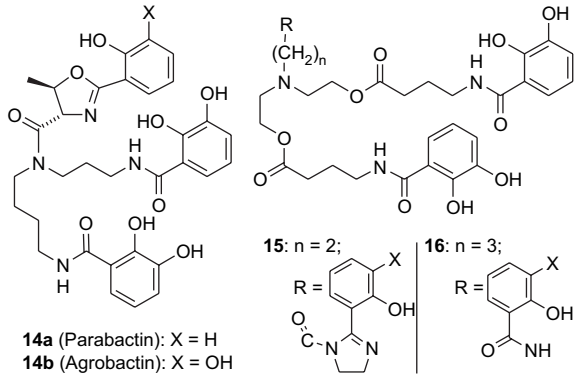
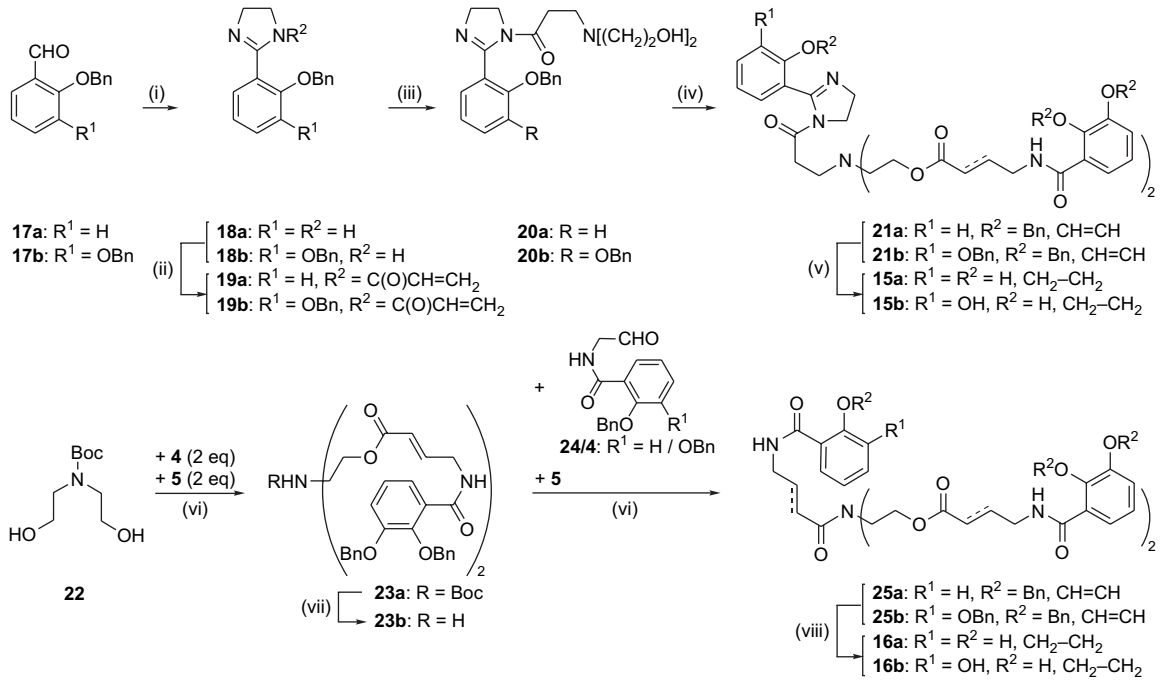


Figure 1. Triscatechol and biscatechol-(*o*-hydroxy)phenacyl chelators: the natural siderophores parabactin (**14a**) and agrobactin (**14b**), and synthetical mimics **15** and **16**.



Scheme 2. Reagents and conditions: (i) (a) $H_2N(CH_2)_2NH_2$, CH_2Cl_2, 0 °C, 20 min, (b) NBS, 0 °C to rt, 16 h, c) satd aq $NaHCO_3$, 90/88% (**a/b**); (ii) acryl chloride, NEt_3, CH_2Cl_2, 0 °C to rt, 30 min, 68/63% (**a/b**); (iii) $[HO(CH_2)_2]_2NH$, EtOH, rt, 24 h, 65/54% (a/b); (iv) **5** (2 equiv), **4** (2 equiv), THF, cat. benzoic acid, rt, 8 h, 60/55% (**a/b**); (v) 10% Pd/C, H_2 (1 bar), MeOH/dioxane, rt, 36 h, 95%; (vi) THF, cat. benzoic acid, rt, 8 h, 58/66/58% (**23a/25a/25b**); (vii) 3 M HCl, THF, 0 °C to rt, 2–3 h, then satd $NaHCO_3/CH_2Cl_2$, 84%; (viii) 5% Pd/C, H_2 (1 bar), MeOH/dioxane, rt, 16 h, 90/92% (**16a/16b**).

to the second three-component reaction with **5** and either aldehyde **4** or **24** to give the respective biscatechol-salicylamide **25a** or triscatechol **25b**. A concluding hydrogenolysis provided the potential siderophores **16**.

2.2. Biological evaluation

2.2.1. Receptor specificity

The mixed-ligand chelators **7** and **13** were tested for siderophore properties on three *E. coli* mutants with defunct production of species-own enterobactin and with defined limitations of their receptor machinery. The strain HK97 (=H5093) lacks receptors of types FhuA and FhuE, which recognize exogenous hydroxamate siderophores such as fungi-produced ferrichromes and coprogens.[24] The strain H5596, like the wild type progenitor, owns receptors for both hydroxamate and catechol siderophores.[25] In strain H5085[25] the catecholate receptors of types Fep, Cir, and Fiu are defective or missing. Overnight cultures of these strains were plated on agar, optionally containing the synthetic iron chelator EDDA [ethylene diamine bis(*o*-hydroxyphenyl)acetic acid] as a moderately effective iron sequestering agent to create iron starvation conditions. Sterile cellulose filter discs imbued with different concentrations of **7** or **13** were placed on the dishes in sets of four and the diameters of the growth zones resulting upon incubation at 36 °C for 48 h were determined and taken as a measure for siderophoric acivity.[26] Table 1 summarizes the results. In the presence of EDDA, compound **7** promoted the growth of *E. coli* mutants HK97 and H5596 in a concentration-dependent manner (Fig. 2; left panel), while virtually no growth was observed of the strain H5085. Similar observations, albeit with less pronounced growth promotion,

Table 1
Growth influencing effects of chelators **7/13** on siderophore-deficient *E. coli* mutants

Amount applied (μg)	HK97[a] +EDDA	HK97 no EDDA	H5596[b] +EDDA	H5596 no EDDA	H5085[c] +EDDA	H5085 no EDDA
150	23/19	++[d]/++	22/14	++/++	− −[d]/− −	−23/++
75	20/15	++/++	18/11	++/++	− −/− −	−20/++
38	17/12	++/++	16/8	++/++	− −/− −	−17/++
15	15/8	++/++	13/− −	++/++	− −/− −	−15/++

Agar plates (Mueller–Hinton II medium, optionally 100 μg mL^{-1} EDDA) inoculated with 100 μL of suspensions of the respective *E. coli* strains were covered with 6 mm cellulose discs containing 15 μL of an ethanolic solution (10, 5, 2.5, or 1 mg mL^{-1}) of **7** or **13**, respectively. The diameters (in mm) of the resulting growth (+) or inhibition (−) zones were determined after 48 h of incubation at 36 °C and are cited here.

[a] HK97 (=H5093): defective hydroxamate receptors due to *FhuA*$^-$ and *FhuE*$^-$.

[b] H5596: hydroxamate and catecholate receptors like wild type.

[c] H5085: defective catecholate receptors FepA, Cir, and Fiu.

[d] ++: confluent growth all over the plate; − −: no growth at all.

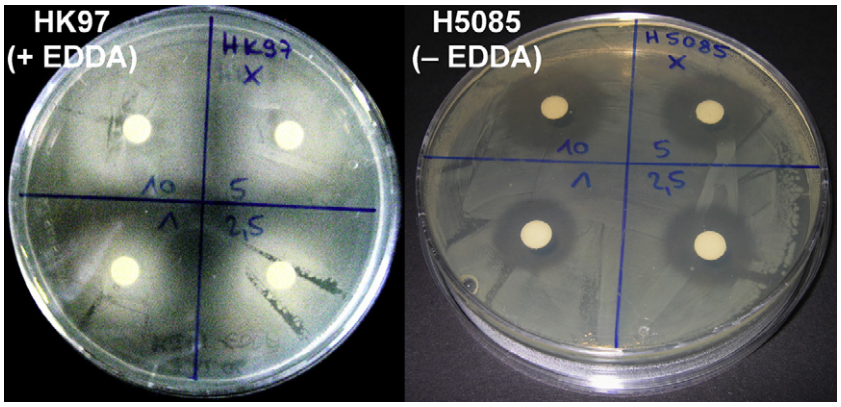


Figure 2. Effects of chelator **7** in *E. coli* strains HK97 featuring catecholate-specific receptors (left panel) and H5085 lacking such receptors (right panel). Conditions as stated in Table 1. Pictures were taken after 48 h of incubation at 36 °C.

were made for ligand **13**. This suggests that Fe(III) complexes of both chelators **7** and **13** were actively internalized only via the catecholate rather than the hydroxamate receptors. In the absence of EDDA, bacteria of strains HK97 and H5596 grew fast and eventually spread all over the dishes, with and without compounds **7** and **13** being present. Strain H5085 grew slowly in the absence of EDDA and only beyond a distinct growth inhibition zone around the cellulose discs impregnated with **7** (Fig. 2; right panel). Formation of chelate complexes not recognized by the receptors of H5085 apparently led to iron depletion in the diffusion zone of **7**, while further afield growth was entertained by passive receptor-independent iron uptake. Interestingly, this inhibitory effect on H5085 was not observed for compound **13**. In brief, chelators **7** and, to a lower extend, **13** are reasonably good siderophores, both binding ferric ions more strongly than EDDA and both being recognized and internalized exclusively by receptors specific for catecholates.

2.2.2. *Siderophoric activity of chelators **15** and **16***

E. coli H5596 bacteria, featuring both catecholate and hydroxamate receptors but lacking own siderophores, were treated in agar diffusion tests under iron starvation with the mimics of parabactin (**15a**, **16a**) and of agrobactin (**15b**, **16b**). Table 2 summarizes the results. In general, the triscatechol chelators (**b**) were more strongly growth promoting than their biscatechol-(monohydroxy)phenyl analogs (**a**) and the hydroxybenzamides **16** exhibited a stronger siderophoric effect than the imidazolines **15**. Chelator **16b** was even as efficacious as the natural siderophore ferrichrome. Apparently, the formation of five-membered chelate rings is favored over that of six-membered ones and that of O,O-chelates over that of O,N-chelates. Hence compounds **15b** and **16b** act as genuine triscatechol ligands. Support for this rationale comes from the observation that the replacement of the benzamide in compound **16a** by a benzoate group results in a complete loss of activity as esters are less good O-donor ligands than amides. The 1:1 stoichiometry of Fe(III) complexes of chelators **16a** and also of **7** (as part of a conjugate with the antibiotic lorabid) was proved spectrophotometrically by measuring at λ_{max} (510 nm) of their visible absorption band according to a method by Bergeron.[27]

Table 2
Promotion of growth of siderophore-deficient *E. coli* mutant H5596 under iron limitation by chelators **15** and **16**

Amount applied (μg)	**15a**	**15b**	**16a**	**16b**
150	9 [0.20][a]	16 [0.19]	17 [0.23]	24 [0.22]
75	0 [0.10]	14 [0.10]	15 [0.12]	21 [0.11]
38	0 [0.05]	12 [0.05]	12 [0.06]	20 [0.06]
15[b]	0 [0.02]	9 [0.02]	9 [0.02]	16 [0.02]

Agar plates (Mueller–Hinton II medium, 100 μg mL^{-1} EDDA) inoculated with 100 μL of an *E. coli* H5596 suspension were covered with 6 mm cellulose discs containing 15 μL of an ethanolic solution (10, 5, 2.5, or 1 mg mL^{-1}) of the respective compound. The diameters (in mm) of the resulting growth zones were determined after 48 h of incubation at 36 °C and are cited here.

[a] The applied quantities in μmol for each compound are given in brackets.

[b] In a reference run 15 μg of natural trishydroxamate siderophore ferrichrome were applied causing a growth zone of 17 mm diameter.

3. Conclusions

We have established short synthetic routes to different triscatechol, biscatechol-hydroxamate, and catechol-bishydroxamate chelators based upon a small set of reliable acylation reactions, Michael additions, and domino Wittig alkenations. The finding that mixed catechol-hydroxamate siderophores are internalized exclusively by catecholate-specific receptors of *E. coli* renders conceivable drug conjugates of them attractive as antibiotic tools for the selection of mutants with limited receptor machineries. The methodology for the conjugation of these siderophores to β-lactam antibiotics under non-aqueous conditions is in place.[13] However, preliminary studies of a loracarbef conjugate revealed an only mediocre antibacterial activity in *E. coli* and *Bacillus subtilis* despite a high uptake. This might be due to an incomplete intracellular breakdown of the drug–siderophore conjugate. Further work is in progress here. Finally, we prepared simplified mimics of the natural siderophores parabactin and agrobactin which, unlike the originals, are fully functional in *E. coli*. The high activity of **16a** means that a catechol ligand as part of an octahedral coordination sphere can be replaced by a salicylamide group.

4. Experimental

4.1. General

Melting points were determined with a Gallenkamp apparatus and are uncorrected. IR-spectra were recorded on a Perkin–Elmer One FTIR spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded under conditions as indicated on a Bruker Avance 300 spectrometer. Chemical shifts (δ) are given in parts per million downfield from TMS as internal standard. Mass spectra were recorded using a Varian MAT 311A (EI). Elemental analyses were carried out with a Perkin–Elmer 2400 CHN elemental analyzer. For column chromatography Merck silica gel 60 (230–400 mesh) was used. Solvents were dried and distilled (THF, diethyl ether, and dioxane over Na/Ph_2CO, CH_3OH over CaO, acetone over K_2CO_3, CH_2Cl_2, and $CHCl_3$ over P_2O_5) and stored under argon. Starting compounds were purchased from the usual sources and were used without further purification.

4.2. Synthesis of biscatechol-hydroxamate chelator 7

4.2.1. N-Benzyloxy-N-benzoxycarbonylethyl acryl amide 2

A solution of benzyl acrylate **1** (1.62 g, 10.0 mmol) and benzoxyamine (1.47 g, 12.0 mmol) in ethanol (30 mL) was stirred at room temperature for 24 h. The solvent was evaporated and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 2:1, R_f 0.58) to give 1.80 g (63%) benzyl 3-(benzoxyamino)propionate as a colorless oil; ν_{max}/cm^{-1} 3274, 3032, 2923, 1733, 1455; δ_H (300 MHz, $CDCl_3$) 2.66 (2H, t, J=6.3 Hz), 3.24 (2H, t, J=6.3 Hz), 4.70 (2H, s), 5.14 (2H, s), 5.84 (1H, br s), 7.25–7.45 (10H, m); δ_C (75 MHz, $CDCl_3$) 32.4, 47.5, 66.3, 76.7, 127.8, 128.0, 128.3, 128.4, 128.6, 128.9, 135.9, 137.8, 172.4; *m/z* (EI, 70 eV) 285 (M^+, 4%), 194 (7%), 181 (52%), 91 (100%). Anal. Calcd for $C_{17}H_{19}NO_3$: C, 71.6; H, 6.7; N, 4.9. Found: C, 71.3; H, 6.9; N, 5.2%. A solution of benzyl 3-(benzoxyamino)propionate (1.75 g, 6.1 mmol) and NEt_3 (1.3 mL) in CH_2Cl_2 (30 mL) was slowly treated at 0 °C with a solution of acryl chloride (0.61 g, 6.7 mmol) in the same solvent (5 mL) by means of a syringe. The mixture was stirred for 30 min and then washed with sat. aq $NaHCO_3$ and water. The organic phase was dried over Na_2SO_4. Evaporation of the volatiles left a yellow oil that upon column chromatography (silica gel 60, cyclohexane/ethyl acetate 2:1, R_f 0.43) yielded **2** (1.76 g, 85%); ν_{max}/cm^{-1} 3033, 2949, 1732, 1660, 1454; δ_H (300 MHz, $CDCl_3$) 2.68 (2H, t, J=6.7 Hz), 4.02 (2H, t, J=6.7 Hz), 4.80 (2H, s), 5.09 (2H, s), 5.70 (1H, dd, J=11.2, 2.0 Hz), 6.38 (1H, dd, J=17.1, 2.0 Hz), 6.67 (1H, dd, J=11.2, 17.1 Hz), 7.20–7.40 (10H, m); δ_C (75 MHz, $CDCl_3$) 32.1, 41.7, 66.5, 76.8, 126.1, 128.0, 128.2, 128.5, 128.6, 129.0, 129.3, 129.4, 134.0, 135.6, 166.9, 171.4; *m/z* (EI, 70 eV) 339 (M^+, 1%), 232 (1%), 181 (24%), 91 (100%). Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.9; H, 6.2; N, 4.1. Found: C, 71.1; H, 6.4; N, 4.0%.

4.2.2. N-Benzyloxy-N-benzoxycarbonylethyl 3-bis(2′-hydroxyethyl)aminopropionamide 3—typical procedure for the Michael addition of amines

A solution of diethanolamine (0.63 g, 6.0 mmol) and acryl amide **2** (1.70 g, 5.0 mmol) in ethanol (20 mL) was stirred at room temperature for 24 h. The solvent was evaporated and the residue filtered through a short plug of silica gel 60 with ethyl acetate/methanol (10:1) and then dried on an oil pump to give **3** (1.33 g, 60%); pale yellow, highly viscous oil; ν_{max}/cm^{-1} 3424, 2948, 1735, 1658, 1455; δ_H (300 MHz, $CDCl_3$) 2.40–2.55 (6H, m), 2.58 (2H, t, J=6.6 Hz), 2.72 (2H, t, J=6.1 Hz), 3.46 (2H, br s), 3.53 (4H, t, J=5.1 Hz), 3.94 (2H, t, J=6.6 Hz), 4.77 (2H, s), 5.08 (2H, s), 7.20–7.40 (10H, m); δ_C (75 MHz, $CDCl_3$) 30.2, 31.9, 41.3, 48.6, 56.0, 59.3, 66.4, 76.1, 128.0, 128.1, 128.3, 128.5, 128.9, 129.2, 134.0, 135.4, 171.3, 174.4; *m/z* (EI, 70 eV, +MSTFA) 588 (M^+, 2%), 499 (62%), 408 (11%), 262 (19%), 146 (14%), 130 (20%), 91 (100%). Anal. Calcd for $C_{24}H_{32}N_2O_6$: C, 64.9; H, 7.3; N, 6.3. Found: C, 65.3; H, 7.1; N, 6.3%.

4.2.3. N-Benzyloxy-N-benzoxycarbonylethyl 3-di-[8′-aza-3′-oxa-4′,9′-dioxo-9′-(2″,3″-dibenzoxyphenyl)non-5′-enyl]-aminopropionamide 6—typical procedure for the three-component domino Wittig alkenation with ylide 5

A solution of Ph_3PCCO (5)[14] (2.11 g, 6.98 mmol), aldehyde **4**[13] (2.40 g, 6.40 mmol), diol **3** (1.29 g, 2.91 mmol), and benzoic acid (85 mg, 0.70 mmol) in dry THF (100 mL) was stirred at room temperature for 8 h. The solvent was removed in vacuo and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:2, R_f 0.38) to give 1.99 g (55%) of **6** as a pale yellow foam; ν_{max}/cm^{-1} 3380, 3033, 2953, 1750, 1721, 1658, 1576, 1526; δ_H (300 MHz, $CDCl_3$) 2.48 (2H, t, J=6.5 Hz), 2.62 (2H, t, J=7.1 Hz), 2.74 (4H, t, J=6.2 Hz), 2.86 (2H, t, J=6.5 Hz), 3.90–4.05 (6H, m), 4.14 (2H, t, J=6.2 Hz), 4.78 (2H, s), 5.01 (2H, s), 5.13 (4H, s), 5.18 (4H, s), 5.82 (2H, dt, J=15.8, 1.7 Hz), 6.78 (2H, dt, J=15.8, 5.1 Hz), 7.15–7.50 (34H, m), 7.65–7.75 (2H, m), 8.18 (2H, t, J=6.7 Hz); δ_C (75 MHz, $CDCl_3$) 30.8, 32.1, 40.3, 50.2, 52.7, 62.6, 66.5, 71.4, 75.8, 76.4, 76.6, 117.3, 119.0, 121.6, 123.4, 124.2, 126.8, 127.3, 127.6, 127.7, 127.9, 128.0, 128.3, 128.5, 128.6, 128.7, 128.9, 129.1, 136.1, 136.4, 144.2, 146.9, 151.7, 164.5, 165.1, 165.8, 171.5. Anal. Calcd for $C_{74}H_{74}N_4O_{14}$: C, 71.5; H, 6.0; N, 4.5. Found: C, 71.1; H, 6.3; N, 4.6%.

4.2.4. N-Hydroxy-N-hydroxycarbonylethyl 3-di-[8′-aza-3′-oxa-4′,9′-dioxo-9′-(2″,3″-dihydroxyphenyl)nonyl]-aminopropionamide 7—typical procedure for the hydrogenolytic debenzylation

Compound **6** (1.93 g, 1.55 mmol) was dissolved in freshly distilled methanol/dioxane (1:1, 10 mL), 5% Pd on charcoal (100 mg) was added and the resulting mixture was purged with and kept under an atmosphere of hydrogen gas (1 bar) for 7 h while stirring. After filtration, the solvent was removed in vacuo. The residue was taken up in methanol (0.5 mL) and re-precipitated by adding ethyl acetate. Upon storage in the

refrigerator colorless compound **7** (1.03 g, 95%) was obtained; mp 87–89 °C; ν_{max}/cm^{-1} 3315, 2976, 1739, 1684, 1635, 1470; δ_H (300 MHz, CD_3OD) 1.80–1.95 (4H, m), 2.42 (4H, t, J=7.2 Hz), 2.57 (2H, t, J=6.8 Hz), 2.78 (2H, t, J=6.7 Hz), 3.05–3.20 (6H, m), 3.41 (4H, t, J=6.6 Hz), 3.84 (2H, t, J=6.8 Hz), 4.22 (4H, t, J=5.8 Hz), 6.69 (2H, t, J=8.1 Hz), 6.93 (2H, dd, J=8.1, 1.5 Hz), 7.21 (2H, dd, J=8.1, 1.5 Hz); δ_C (75 MHz, CD_3OD) 25.7, 29.8, 32.4, 33.1, 39.8, 45.3, 52.1, 53.9, 62.0, 116.8, 118.7, 119.6, 119.7, 147.3, 150.4, 169.7, 171.7, 174.6, 176.1. Anal. Calcd for $C_{32}H_{42}N_4O_{14}$: C, 54.4; H, 6.0; N, 7.9. Found: C, 54.0; H, 6.1; N, 7.7%.

4.3. Synthesis of catechol-bishydroxamate chelator *13*

4.3.1. 2′,3′-Dibenzoxy-N-[2-(tert-butoxycarbonyl-amino)ethyl]benzamide *9*

A solution of dibenzoxybenzoic acid **8**[16] (2.5 g, 7.5 mmol) and thionyl chloride (3.6 g, 30.0 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 1.5 h. All volatiles were removed under reduced pressure and the oily residue was dried on an oil pump to give 2,3-dibenzoxybenzoyl chloride as a pale yellow waxy solid (2.6 g, 7.5 mmol). This was dissolved in dry CH_2Cl_2 (10 mL) and added dropwise by means of a syringe to a stirred solution at 0 °C of Boc-protected ethylenediamine (1.44 g, 9.0 mmol) and NEt_3 (1.2 mL) in the same solvent (30 mL). The mixture was allowed to come to room temperature and washed with satd aq $NaHCO_3$. The organic phase was dried (Na_2SO_4) and evaporated to leave a pale yellow solid, which was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:3, R_f 0.58) yielding **9** (2.96 g, 83%) as a colorless solid of mp 108 °C; ν_{max}/cm^{-1} 3379, 2980, 1705, 1650, 1576, 1531; δ_H (300 MHz, $CDCl_3$) 1.38 (9H, s), 3.05–3.15 (2H, m), 3.30–3.40 (2H, m), 4.82 (1H, br), 5.07 (2H, s), 5.14 (2H, s), 7.00–7.05 (2H, m), 7.25–7.45 (10H, m), 7.65–7.75 (1H, m), 8.02 (1H, t, J=6.1 Hz); δ_C (75 MHz, $CDCl_3$) 28.3, 39.6, 41.0, 71.3, 76.4, 79.9, 117.1, 123.2, 124.4, 127.1, 127.7, 128.2, 128.6, 128.7, 128.8, 129.0, 136.2, 136.4, 146.7, 151.7, 156.1, 165.9; *m/z* (EI, 70 eV) 476 (M^+, 2%), 420 (11%), 329 (11%), 285 (24%), 91 (100%). Anal. Calcd for $C_{28}H_{32}N_2O_5$: C, 70.6; H, 6.8; N, 5.9. Found: C, 70.9; H, 6.9; N, 5.8%.

4.3.2. 2′,3′-Dibenzoxy-N-[2-(acrylamino)ethyl]benzamide *10*

Compound **9** (2.90 g, 6.10 mmol) in THF (10 mL) was treated at 0 °C with 3 M HCl in THF (20 mL). The mixture was stirred while slowly coming to room temperature. After 2–3 h, diethyl ether (100 mL) was added, and the precipitate was filtered off and washed with diethyl ether. It was suspended in CH_2Cl_2 (100 mL) and washed with cold diluted aq NaOH (pH 10). The clear organic phase was separated and dried over Na_2SO_4. Evaporation of all volatiles left 2′,3′-dibenzoxy-*N*-(2-aminoethyl)benzamide (2.06 g, 90%) as a colorless oil, which was used as such; ν_{max}/cm^{-1} 3370, 2963, 1654, 1576, 1533, 1454; δ_H (300 MHz, $CDCl_3$) 1.45 (2H, br s), 2.55–2.65 (2H, m), 3.20–3.30 (2H, m), 5.02 (2H, s), 5.05 (2H, s), 7.00–7.05 (2H, m), 7.25–7.45 (10H, m), 7.65–7.75 (1H, m), 8.02 (1H, t, J=5.8 Hz); δ_C (75 MHz, $CDCl_3$) 41.0, 42.2, 72.8, 75.9, 116.6, 122.7, 124.0, 127.2, 127.3, 127.8, 128.2, 128.4, 136.0, 136.2, 146.4, 151.3, 165.1; *m/z* (EI, 70 eV) 376 (M^+, 2%), 347 (20%), 334 (17%), 285 (28%), 227 (65%), 136 (50%), 91 (100%). It was acylated with acryl chloride analogously to the synthesis of **2**. Yield: 2.20 g (84%, with respect to **9**); colorless solid of mp 95 °C; ν_{max}/cm^{-1} 3376, 2970, 1655, 1571, 1533, 1458; δ_H (300 MHz, $CDCl_3$) 3.20–3.40 (4H, m), 5.07 (2H, s), 5.15 (2H, s), 5.62 (1H, dd, J=10.1, 1.6 Hz), 5.91 (1H, dd, J=17.0, 10.1 Hz), 6.15 (1H, dd, J=17.0, 1.6 Hz), 6.72 (1H, br), 7.00–7.05 (2H, m), 7.25–7.45 (10H, m), 7.65–7.75 (1H, m), 8.20 (1H, d, J=5.6 Hz); δ_C (75 MHz, $CDCl_3$) 38.9, 41.2, 71.3, 76.5, 117.4, 123.0, 124.4, 125.7, 126.8, 127.6, 128.3, 128.7, 128.8, 128.9, 129.0, 131.1, 136.2, 136.3, 146.8, 151.6, 165.9, 166.9; *m/z* (EI, 70 eV) 430 (M^+, 3%), 339 (9%), 225 (10%), 136 (8%), 91 (100%). Anal. Calcd for $C_{26}H_{26}N_2O_4$: C, 72.5; H, 6.1; N, 6.5. Found: C, 72.3; H, 6.1; N, 6.2%.

4.3.3. N-[2-(2′,3′-Dibenzoxybenzamido)ethyl]-3″-bis(2‴-hydroxyethyl)aminopropionamide *11*

Analogously to the synthesis of **3**, compound **11** (1.78 g, 68%) was obtained as a colorless, highly viscous oil from **10** (2.10 g, 4.9 mmol) and diethanolamine (0.62 g, 5.9 mmol). Reflux conditions (48 h) were required for completion; ν_{max}/cm^{-1} 3365, 2947, 1647, 1576, 1542, 1454; δ_H (300 MHz, $CDCl_3$) 2.23 (2H, t, J=5.7 Hz), 2.55 (4H, t, J=5.2 Hz), 2.69 (2H, t, J=5.7 Hz), 3.20–3.35 (4H, m), 3.54 (4H, t, J=5.2 Hz), 3.99 (2H, br s), 5.05 (2H, s), 5.10 (2H, s), 7.05–7.10 (2H, m), 7.25–7.45 (10H, m), 7.60–7.70 (1H, m), 7.85 (1H, d, J=5.2 Hz), 8.22 (1H, d, J=5.6 Hz); δ_C (75 MHz, $CDCl_3$) 34.0, 39.2, 39.9, 51.1, 56.2, 59.5, 71.1, 75.3, 117.1, 122.6, 124.3, 126.9, 127.5, 128.2, 128.5, 128.6, 128.7, 128.9, 136.1, 136.2, 146.6, 151.6, 166.7, 173.1; *m/z* (EI, 70 eV, +MSTFA) 678 (M^+–1, 2%), 664 (6%), 576 (100%), 431 (9%), 262 (15%), 91 (47%). Anal. Calcd for $C_{30}H_{37}N_3O_6$: C, 67.3; H, 7.0; N, 7.8. Found: C, 67.0; H, 7.1; N, 8.0%.

4.3.4. N-[2-(2′,3′-Dibenzoxybenzamido)ethyl]-3″-bis[2‴-(3⁗-N-benzoxyacetamido)propanoyloxyethyl]-aminopropionamide *12*

A stirred mixture of 3-[*N*-(benzoxy)acetamido]propionic acid[17] (0.90 g, 3.80 mmol), NEt_3 (0.63 mL), and dry CH_2Cl_2 (15 mL) was treated at 0 °C with chloroethyl formiate (0.45 g, 4.20 mmol). After 30 min, the mixture was transferred to an ice-cooled solution of diol **11** (0.92 g, 1.72 mmol) in the same solvent (20 mL) by means of syringe. The mixture was stirred at room temperature for 5 h and washed with satd aq $NaHCO_3$ and water. The organic phase was dried (Na_2SO_4) and evaporated to leave a yellowish oil, which was purified by column chromatography (silica gel 60, ethyl acetate/methanol 4:1, R_f 0.33). Yield: 0.94 g (56%), colorless highly viscous oil; ν_{max}/cm^{-1} 3374, 2942, 1723, 1667, 1623, 1562, 1459; δ_H (300 MHz, $CDCl_3$) 2.00 (3H, s), 2.02 (3H, s), 2.25 (2H, t, J=6.2 Hz), 2.50–2.65 (6H, m), 2.70–2.80 (4H, m), 3.20–3.35 (4H, m), 3.55 (2H, t, J=5.0 Hz), 3.86 (4H, t,

J=6.6 Hz), 4.07 (2H, t, J=5.6 Hz), 4.75 (2H, s), 4.77 (2H, s), 5.05 (2H, s), 5.10 (2H, s), 7.05–7.10 (2H, m), 7.25–7.45 (20H, m), 7.55–7.65 (1H, m), 7.73 (1H, d, J=5.3 Hz), 8.20 (1H, d, J=5.9 Hz); δ_C (75 MHz, $CDCl_3$) 20.4, 31.8, 33.2, 38.9, 39.8, 41.2, 50.3, 51.7, 56.1, 58.7, 61.6, 71.1, 76.1, 76.3, 117.0, 122.7, 124.3, 126.9, 127.5, 128.1, 128.3, 128.5, 128.6, 128.7, 128.9, 129.2, 129.3, 134.0, 136.1, 136.2, 146.6, 151.6, 166.3, 171.2, 172.4, 174.5. Anal. Calcd for $C_{54}H_{63}N_5O_{12}$: C, 66.8; H, 6.5; N, 7.1. Found: C, 66.9; H, 6.7; N, 7.0%.

4.3.5. N-[2-(2′,3′-Dihydroxybenzamido)ethyl]-3″-bis[2‴-(3⁗-N-hydroxyacetamido)propanoyloxyethyl]-aminopropionamide ***13***

Analogously to the synthesis of **7**, compound **13** (540 mg, 95%) was obtained from **12** (900 mg, 0.93 mmol) as a colorless foamy solid of mp 86–88 °C; ν_{max}/cm^{-1} 3372, 2897, 1736, 1641, 1621, 1549, 1460; δ_H (300 MHz, CD_3OD) 2.08 (6H, s), 2.58 (2H, t, J=6.7 Hz), 2.69 (2H, t, J=6.6 Hz), 2.78 (2H, t, J=6.1 Hz), 3.25–3.40 (4H, m), 3.45–3.60 (6H, m), 3.80–3.95 (6H, m), 4.33 (2H, t, J=5.3 Hz), 6.71 (1H, t, J=7.9 Hz), 6.93 (1H, dd, J=7.9, 1.1 Hz), 7.25 (1H, dd, J=7.9, 1.1 Hz); δ_C (75 MHz, $CDCl_3$) 20.5, 29.2, 32.5, 39.9, 40.1, 44.9, 52.2, 53.5, 56.3, 56.4, 59.2, 116.5, 118.8, 119.6, 119.7, 147.0, 150.1, 169.9, 172.3, 172.8, 174.4. Anal. Calcd for $C_{26}H_{39}N_5O_{12}$: C, 50.9; H, 6.4; N, 11.4. Found: C, 51.2; H, 6.2; N, 11.0%.

4.4. Synthesis of the imidazoline chelators ***15***

4.4.1. 2-(2′-Benzoxyphenyl)-4,5-dihydroimidazole ***18a***

A solution of 2-benzoxybenzaldehyde **17a**[21] (2.54 g, 12.0 mmol) in CH_2Cl_2 (30 mL) was slowly added to a solution of ethylenediamine (0.76 g, 12.6 mmol) in the same solvent (20 mL), kept at 0 °C. The resulting mixture was stirred for 20 min and then treated with *N*-bromosuccinimide (2.32 g, 13.0 mmol) in small portions. After stirring overnight at room temperature, the hydrobromide of imidazoline **18a** was precipitated by addition of diethyl ether (100 mL) and placement in the refrigerator. The salt was filtered off, washed with diethyl ether, and separated between ethyl acetate and satd aq $NaHCO_3$. The organic phase was dried over Na_2SO_4 and the solvent was evaporated leaving **18a** (2.72 g, 90%) as a colorless solid of mp 52 °C; ν_{max}/cm^{-1} 3431, 2868, 2249, 1602, 1567, 1493; δ_H (300 MHz, $CDCl_3$) 3.66 (4H, s), 5.12 (2H, s), 5.91 (1H, br s), 6.95–7.00 (2H, m), 7.30–7.45 (6H, m), 8.08 (1H, dd, J=7.8, 1.9 Hz); δ_C (75 MHz, $CDCl_3$) 49.3, 71.0, 112.9, 118.8, 121.5, 127.4, 128.3, 128.8, 131.2, 131.8, 136.0, 156.7, 163.6; *m/z* (EI, 70 eV) 252 (M^+, 11%), 161 (74%), 133 (38%), 91 (100%). Anal. Calcd for $C_{16}H_6N_2O$: C, 76.2; H, 6.4; N, 11.1. Found: C, 76.0; H, 6.3; N, 11.3%.

4.4.2. 2-(2′,3′-Dibenzoxyphenyl)-4,5-dihydroimidazole ***18b***

Analogously to **18a**, compound **18b** (2.60 g, 88%) was obtained from **17b**[22] (2.64 g, 8.3 mmol) as a pale yellow solid of mp 58 °C; ν_{max}/cm^{-1} 3435, 2868, 2253, 1599, 1568, 1492, 1454, 1433; δ_H (300 MHz, $CDCl_3$) 3.60 (4H, s), 5.01 (2H, s), 5.12 (2H, s), 5.50 (1H, br s), 7.00–7.05 (2H, m), 7.30–7.50 (10H, m), 7.60–7.65 (1H, m); δ_C (75 MHz, $CDCl_3$) 49.7, 71.1, 76.2, 115.9, 122.6, 124.4, 127.5, 128.1, 128.3, 128.5, 128.6, 128.8, 136.5, 136.8, 147.0, 151.9, 163.4; *m/z* (EI, 70 eV) 358 (M^+, 5%), 267 (66%), 251 (21%), 239 (40%), 91 (100%). Anal. Calcd for $C_{23}H_{22}N_2O_2$: C, 77.1; H, 6.2; N, 7.8. Found: C, 77.4; H, 6.1; N, 7.6%.

4.4.3. 2-(2′-Benzoxyphenyl)-1-propenoyl-4,5-dihydroimidazole ***19a***

Analogously to the synthesis of **2**, compound **19a** (2.06 g, 68%) was obtained from **18a** (2.50 g, 9.9 mmol) and acryl chloride (0.98 g, 10.9 mmol) as a pale yellow oil of R_f 0.48 (cyclohexane/ethyl acetate 1:2). Yield: 2.06 g (68%); ν_{max}/cm^{-1} 3034, 2875, 2247, 1656, 1629, 1597, 1471; δ_H (300 MHz, $CDCl_3$) 3.90–4.05 (4H, m), 5.02 (2H, s), 5.41 (1H, dd, J=10.6, 1.9 Hz), 5.92 (1H, dd, J=16.7, 10.6 Hz), 6.23 (1H, dd, J=16.7, 1.9 Hz), 6.90–6.95 (1H, m), 7.05–7.10 (1H, m), 7.30–7.50 (7H, m); δ_C (75 MHz, $CDCl_3$) 47.8, 53.0, 70.5, 112.5, 121.2, 122.3, 127.9, 128.4, 128.6, 128.8, 128.9, 129.8, 132.7, 136.5, 144.1, 156.6, 162.4; *m/z* (EI, 70 eV) 306 (M^+, 6%), 289 (9%), 251 (63%), 200 (94%), 132 (41%), 91 (100%). Anal. Calcd for $C_{19}H_{18}N_2O_2$: C, 74.5; H, 5.9; N, 9.1. Found: C, 74.3; H, 5.9; N, 9.2%.

4.4.4. 2-(2′,3′-Dibenzoxyphenyl)-1-propenoyl-4,5-dihydroimidazole ***19b***

Analogously to **19a**, compound **19b** (1.74 g, 63%) was obtained from **18b** (2.40 g, 6.7 mmol) as a pale yellow viscous oil of R_f 0.46 (cyclohexane/ethyl acetate 1:2); ν_{max}/cm^{-1} 3032, 2875, 2247, 1656, 1630, 1595, 1473, 1412; δ_H (300 MHz, $CDCl_3$) 3.75–3.85 (4H, m), 5.06 (2H, s), 5.13 (2H, s), 5.36 (1H, dd, J=10.4, 1.6 Hz), 5.83 (1H, dd, J=16.7, 10.4 Hz), 6.18 (1H, dd, J=16.7, 1.6 Hz), 6.95–7.10 (3H, m), 7.20–7.45 (10H, m); δ_C (75 MHz, $CDCl_3$) 47.3, 52.8, 70.9, 75.4, 116.6, 121.5, 124.4, 127.3, 127.6, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.6, 136.4, 137.5, 146.6, 151.6, 156.1, 163.2; *m/z* (EI, 70 eV) 412 (M^+, 4%), 357 (7%), 321 (38%), 267 (22%), 239 (13%), 215 (25%), 91 (100%). Anal. Calcd for $C_{26}H_{24}N_2O_3$: C, 75.7; H, 5.9; N, 6.8. Found: C, 75.9; H, 5.8; N, 6.9%.

4.4.5. 2-(2′-Benzoxyphenyl)-1-[3′-di(2″-hydroxyethyl)amino]-propanoyl-4,5-dihydroimidazole ***20a***

Analogously to the synthesis of **3**, compound **20a** (1.71 g, 65%) was obtained from diethanolamine (735 mg, 7.0 mmol) and **19a** (1.96 g, 6.4 mmol) as a pale yellow viscous oil; ν_{max}/cm^{-1} 3402, 2948, 2876, 2245, 1664, 1626, 1597, 1492; δ_H (300 MHz, $CDCl_3$) 2.08 (2H, t, J=5.8 Hz), 2.26 (4H, t, J=4.9 Hz), 2.53 (2H, t, J=5.8 Hz), 3.31 (4H, t, J=4.9 Hz), 3.55 (2H, br s), 3.80–3.90 (4H, m), 5.00 (2H, s), 6.90–7.00 (2H, m), 7.20–7.40 (7H, m); δ_C (75 MHz, $CDCl_3$) 27.4, 47.2, 49.6, 55.8, 59.2, 67.8, 70.1, 112.2, 120.7, 125.8, 127.0, 128.4, 128.7, 128.9, 131.4, 136.1, 155.8, 156.3, 168.2; *m/z* (EI, 70 eV, +MSTFA) 554 (M^+−1, 3%), 540 (5%), 452 (100%), 265 (15%), 262 (10%), 215 (14%), 91 (55%), 73

(31%). Anal. Calcd for $C_{23}H_{29}N_3O_4$: C, 67.1; H, 7.1; N, 10.2. Found: C, 66.8; H, 7.3; N, 10.4%.

4.4.6. 2-(2′,3′-Dibenzoxyphenyl)-1-[3′-di(2″-hydroxyethyl)-amino]propanoyl-4,5-dihydroimidazole **20b**

Analogously to **20a**, compound **20b** (1.14 g, 54%) was obtained from diethanolamine (473 mg, 4.5 mmol) and **19b** (1.69 g, 4.1 mmol) as a pale yellow foam; ν_{max}/cm^{-1} 3385, 2949, 2877, 2246, 1664, 1626, 1595, 1473; δ_H (300 MHz, $CDCl_3$) 2.11 (2H, t, J=5.9 Hz), 2.32 (4H, t, J=5.1 Hz), 2.62 (2H, t, J=5.9 Hz), 3.45–3.60 (4H, m), 3.75 (2H, br s), 3.80–3.90 (4H, m), 5.01 (2H, s), 5.17 (2H, s), 6.95–7.05 (1H, m), 7.10–7.25 (3H, m), 7.30–7.50 (9H, m); δ_C (75 MHz, $CDCl_3$) 27.7, 47.3, 50.1, 55.9, 59.0, 67.5, 70.2, 74.3, 112.1, 120.9, 124.7, 126.3, 127.5, 127.8, 128.0, 128.2, 128.3, 128.5, 136.2, 136.8, 147.7, 151.6, 156.1, 168.4; *m/z* (EI, 70 eV, +MSTFA) 660 (M^+-1, 1%), 646 (7%), 558 (100%), 413 (22%), 262 (34%), 203 (11%), 91 (87%), 73 (31%). Anal. Calcd for $C_{30}H_{35}N_3O_5$: C, 69.6; H, 6.8; N, 8.1. Found: C, 69.4; H, 7.0; N, 8.0%.

4.4.7. 2-(2′-Benzoxyphenyl)-1-{3′-di-[8″-aza-3″-oxa-4″,9″-dioxo-9″-(2‴,3‴-dibenzoxyphenyl)non-5″-enyl]amino}-propanoyl-4,5-dihydroimidazole **21a**

Analogously to the synthesis of **6**, compound **21a** (1.45 g, 60%) was obtained from ylide **5** (1.45 g, 4.8 mmol), aldehyde **4**[13] (1.65 g, 4.4 mmol), and diol **20a** (0.82 g, 2.0 mmol) as a pale yellow foam; R_f 0.40 (cyclohexane/ethyl acetate 1:4); ν_{max}/cm^{-1} 3384, 3034, 2939, 2251, 1719, 1655, 1578, 1528, 1453; δ_H (300 MHz, $CDCl_3$) 2.16 (2H, t, J=6.8 Hz), 2.55 (4H, t, J=5.4 Hz), 2.72 (2H, t, J=6.8 Hz), 3.80–4.05 (12H, m), 5.06 (2H, s), 5.11 (4H, s), 5.16 (4H, s), 5.76 (2H, dt, J=15.4, 1.6 Hz), 6.75 (2H, dt, J=15.4, 4.5 Hz), 7.10–7.50 (27H, m), 7.60–7.75 (6H, m), 8.0–8.10 (2H, m), 8.19 (2H, t, J=7.0 Hz); δ_C (75 MHz, $CDCl_3$) 33.9, 40.3, 47.4, 50.8, 52.6, 62.3, 70.2, 71.3, 75.8, 117.3, 118.9, 121.4, 123.3, 124.4, 126.9, 127.5, 127.6, 127.9, 128.2, 128.4, 128.5, 128.7, 128.9, 130.1, 130.3, 131.8, 132.0, 132.2, 136.0, 136.2, 144.0, 151.7, 156.2, 164.6, 165.2, 165.7, 170.1; *m/z* (EI, 70 eV) 1208 (M^+-1, 1%), 779 (2%), 417 (4%), 317 (29%), 251 (22%), 235 (31%), 91 (100%). Anal. Calcd for $C_{73}H_{71}N_5O_{12}$: C, 72.4; H, 5.9; N, 5.8. Found: C, 72.7; H, 6.1; N, 5.6%.

4.4.8. 2-(2′,3′-Dibenzoxyphenyl)-1-{3′-di-[8″-aza-3″-oxa-4″,9″-dioxo-9″-(2‴,3‴-dibenzoxyphenyl)-non-5″-enyl]-amino}propanoyl-4,5-dihydroimidazole **21b**

Analogously to **21a**, compound **21b** (723 mg, 55%) was obtained from ylide **5** (725 mg, 2.4 mmol), aldehyde **4**[13] (825 mg, 2.2 mmol), and **20b** (517 mg, 1.0 mmol) as a pale yellow foam; R_f 0.42 (cyclohexane/ethyl acetate 1:4); ν_{max}/cm^{-1} 3380, 3034, 2951, 2250, 1719, 1657, 1578, 1529, 1453; δ_H (300 MHz, $CDCl_3$) 2.12 (2H, t, J=6.5 Hz), 2.53 (4H, t, J=5.2 Hz), 2.71 (2H, t, J=6.5 Hz), 3.79 (4H, t, J=5.2 Hz), 3.85–4.05 (8H, m), 5.02 (6H, s), 5.08 (2H, s), 5.15 (4H, s), 5.71 (2H, dt, J=16.0, 1.8 Hz), 6.74 (2H, dt, J=16.0, 4.6 Hz), 7.10–7.50 (31H, m), 7.65–7.80 (6H, m), 8.0–8.10 (2H, m), 8.21 (2H, t, J=7.1 Hz); δ_C (75 MHz, $CDCl_3$) 34.4, 40.2, 47.2, 50.9, 52.6, 62.3, 70.8, 71.3, 75.9, 76.2, 116.1, 117.3, 121.4, 123.2, 124.4, 126.7, 126.9, 127.3, 127.5, 127.6, 127.8, 128.0, 128.2, 128.8, 129.7, 130.5, 131.4, 132.0, 132.2, 132.7, 136.0, 136.3, 140.1, 143.8, 144.2, 146.2, 151.7, 162.5, 164.8, 165.1, 169.3; *m/z* (EI, 70 eV) 885 (1%), 386 (4%), 358 (7%), 317 (21%), 226 (14%), 91 (100%). Anal. Calcd for $C_{80}H_{77}N_5O_{13}$: C, 73.0; H, 5.9; N, 5.3. Found: C, 72.8; H, 6.2; N, 5.4%.

4.4.9. 2-(2′-Hydroxyphenyl)-1-{3′-di-[8″-aza-3″-oxa-4″,9″-dioxo-9″-(2‴,3‴-dihydroxyphenyl)nonyl]amino}propanoyl-4,5-dihydroimidazole **15a**

Catalytic hydrogenation of **21a** (1.40 g, 1.16 mmol) was performed analogously to the synthesis of **7**, 10% Pd on charcoal catalyst (250 mg) and extended reaction time (36 h) were required to achieve completion. Compound **15a** (827 mg, 95%) was obtained as a colorless foamy solid of mp 136–138 °C; ν_{max}/cm^{-1} 3358, 2941, 2251, 1729, 1648, 1585, 1456; δ_H (300 MHz, CD_3OD) 1.80–1.90 (4H, m), 2.14 (2H, t, J=6.4 Hz), 2.39 (4H, t, J=6.9 Hz), 2.75–2.95 (6H, m), 3.30–3.40 (4H, m), 3.86 (4H, t, J=5.1 Hz), 3.90–4.05 (4H, m), 6.71 (2H, t, J=8.0 Hz), 6.90–7.10 (5H, m), 7.35–7.45 (2H, m), 7.75–7.85 (1H, m); δ_C (75 MHz, CD_3OD) 25.7, 31.4, 33.0, 40.1, 41.3, 47.2, 47.8, 50.3, 62.3, 116.2, 118.7, 120.1, 120.8, 121.4, 121.9, 122.1, 122.6, 147.0, 147.3, 150.1, 156.0, 162.8, 168.8, 171.9, 174.5. Anal. Calcd for $C_{38}H_{45}N_5O_{12}$: C, 60.0; H, 5.9; N, 9.2. Found: C, 60.3; H, 5.6; N, 9.1%.

4.4.10. 2-(2′,3′-Dihydroxyphenyl)-1-{3′-di-[8″-aza-3″-oxa-4″,9″-dioxo-9″-(2‴,3‴-dihydroxyphenyl)-nonyl]amino}-propanoyl-4,5-dihydroimidazole **15b**

Analogously to **15a**, compound **15b** (398 mg, 95%) was obtained from **21b** (710 mg, 0.54 mmol) as a colorless foamy solid of mp 144–146 °C (CH_2Cl_2/ethyl acetate); ν_{max}/cm^{-1} 3355, 2940, 2256, 1731, 1647, 1582, 1457; δ_H (300 MHz, CD_3OD) 1.80–1.90 (4H, m), 2.17 (2H, t, J=6.3 Hz), 2.39 (4H, t, J=6.7 Hz), 2.80–3.00 (6H, m), 3.30–3.40 (4H, m), 3.93 (4H, t, J=5.2 Hz), 3.95–4.10 (4H, m), 6.75 (2H, t, J=8.1 Hz), 6.80–7.00 (4H, m), 7.25–7.35 (3H, m); δ_C (75 MHz, CD_3OD) 25.6, 31.3, 33.0, 40.2, 41.5, 47.3, 47.7, 50.6, 62.2, 114.1, 116.4, 116.8, 118.5, 120.0, 121.2, 121.7, 122.3, 147.2, 147.4, 150.1, 151.2, 162.6, 169.1, 172.0, 174.4. Anal. Calcd for $C_{38}H_{45}N_5O_{13}$: C, 58.5; H, 5.8; N, 9.0. Found: C, 58.7; H, 6.0; N, 8.8%.

4.5. Synthesis of the benzamide chelators **16**

4.5.1. N-(tert-Butoxycarbonyl)-N,N-{di-[8-aza-3-oxa-4,9-dioxo-9-(2′,3′-dibenzoxyphenyl)-non-5-enyl]}amine **23a**

Analogously to the synthesis of **6**, compound **23a** (1.16 g, 58%) was obtained from ylide **5** (1.33 g, 4.4 mmol), aldehyde **4**[13] (1.57 g, 4.2 mmol), and *N*-Boc-diethanolamine **22**[23] (410 mg, 2.0 mmol) as a pale yellow foam of R_f 0.42 (cyclohexane/ethyl acetate 1:4); ν_{max}/cm^{-1} 3358, 2972, 1739, 1706, 1653, 1553; δ_H (300 MHz, $CDCl_3$) 1.42 (9H, s), 3.34

(4H, t, J=5.4 Hz), 3.95–4.05 (4H, m), 4.26 (4H, t, J=5.4 Hz), 5.11 (4H, s), 5.16 (4H, s), 5.79 (2H, dt, J=16.0, 1.9 Hz), 6.81 (2H, dt, J=16.0, 4.5 Hz), 7.15–7.25 (4H, m), 7.30–7.55 (20H, m), 7.70–7.75 (2H, m), 8.18 (2H, t, J=7.1 Hz); δ_C (75 MHz, $CDCl_3$) 26.5, 41.3, 50.8, 61.6, 70.5, 74.7, 80.1, 118.0, 120.5, 121.4, 123.4, 124.9, 126.6, 127.3, 127.5, 127.9, 128.3, 128.7, 136.2, 136.5, 144.2, 147.4, 152.1, 154.6, 165.9, 168.2; *m/z* (EI, 70 eV) 1003 (M^+, 1%), 947 (3%), 903 (2%), 573 (5%), 473 (13%), 286 (17%), 91 (100%). Anal. Calcd for $C_{59}H_{61}N_3O_{12}$: C, 70.6; H, 6.1; N, 4.2. Found: C, 71.0; H, 5.9; N, 4.3%.

4.5.2. N-{4-[3′-Di-(8″-aza-3″-oxa-4″,9″-dioxo-9″-(2‴,3‴-dibenzoxyphenyl)-non-5″-enyl)-amino]-4-oxobut-2-enyl}-2-(benzoxy)benzamide ***25a***

Deprotection of compound **23a** (1.10 g, 1.1 mmol) was carried out as described for compound **9**. Di-[8-aza-3-oxa-4,9-dioxo-9-(2′,3′-dibenzoxyphenyl)-non-5-enyl]amine **23b** (0.83 g, 84%) was obtained as a pale yellow foam and used without further purification; R_f 0.12 (ethyl acetate); ν_{max}/cm^{-1} 3362, 2924, 1736, 1659, 1578, 1463; δ_H (300 MHz, $CDCl_3$) 2.93 (4H, t, J=5.4 Hz), 4.00–4.10 (4H, m), 4.24 (2H, t, J=5.4 Hz), 5.11 (4H, s), 5.16 (4H, s), 5.85 (2H, dt, J=15.6, 1.8 Hz), 6.83 (2H, dt, J=15.6, 4.9 Hz), 7.10–7.20 (4H, m), 7.35–7.55 (20H, m), 7.70–7.75 (2H, m), 8.20 (2H, t, J=6.9 Hz); δ_C (75 MHz, $CDCl_3$) 41.2, 48.4, 61.8, 70.6, 74.7, 118.1, 120.5, 121.3, 123.6, 124.7, 126.1, 127.2, 127.5, 127.7, 128.1, 128.7, 136.1, 136.3, 144.0, 147.2, 151.6, 165.9, 168.2. Compound **23b** (0.40 g, 0.44 mmol) was submitted to a three-component reaction with ylide **5** (160 mg, 0.53 mmol) and aldehyde **24** (130 mg, 0.48 mmol) as described for compound **6**. Yield: 347 mg (66%), pale yellow foam; R_f 0.44 (cyclohexane/ethyl acetate 1:2); ν_{max}/cm^{-1} 3362, 2931, 1733, 1676, 1638, 1577; δ_H (300 MHz, $CDCl_3$) 3.50 (2H, t, J=5.4 Hz), 3.64 (2H, t, J=5.0 Hz), 3.85–3.95 (2H, m), 3.95–4.05 (2H, m), 4.10–4.20 (4H, m), 4.28 (2H, t, J=5.4 Hz), 5.10 (2H, s), 5.11 (2H, s), 5.16 (2H, s), 5.18 (4H, s), 5.68 (1H, d, J=15.5 Hz), 5.83 (1H, d, J=15.5 Hz), 6.33 (1H, dt, J=15.2, 1.7 Hz), 6.68 (1H, dt, J=15.2, 4.4 Hz), 6.83 (2H, dt, J=15.5, 4.7 Hz), 7.00–7.10 (2H, m), 7.15–7.25 (4H, m), 7.30–7.50 (26H, m), 7.65–7.75 (3H, m), 3.10–8.20 (3H, m); δ_C (75 MHz, $CDCl_3$) 40.1, 40.3, 40.7, 46.1, 47.5, 61.8, 62.4, 71.2, 71.3, 76.5, 112.9, 117.3, 120.6, 120.7, 120.9, 121.2, 121.8, 123.2, 123.3, 126.7, 127.4, 127.6, 127.8, 128.4, 128.6, 128.9, 129.0, 129.1, 129.3, 132.0, 132.8, 135.7, 136.2, 136.4, 142.3, 144.8, 145.3, 146.9, 151.7, 156.8, 165.1, 165.3, 165.4, 165.7, 166.5. Anal. Calcd for $C_{72}H_{68}N_4O_{13}$: C, 72.2; H, 5.7; N, 4.7. Found: C, 72.5; H, 5.9; N, 4.5%.

4.5.3. N-{4-[3′-Di-(8″-aza-3″-oxa-4″,9″dioxo-9″-(2‴,3‴-dibenzoxyphenyl)-non-5″-enyl)-amino]-4-oxobut-2-enyl}-2,3-(dibenzoxy)benzamide ***25b***

Analogously to **25a**, compound **25b** (332 mg, 58%) was obtained from amine **23b** (0.40 g, 0.44 mmol), ylide **5** (160 mg, 0.53 mmol), and aldehyde **4** (180 mg, 0.48 mmol) as a pale yellow foam; R_f 0.42 (cyclohexane/ethyl acetate 1:2); ν_{max}/cm^{-1} 3359, 2933, 1733, 1672, 1635, 1578; δ_H (300 MHz, $CDCl_3$) 3.52 (2H, t, J=5.3 Hz), 3.65 (2H, t, J=5.0 Hz), 3.95–4.05 (4H, m), 4.15–4.25 (4H, m), 4.29 (2H, t, J=5.4 Hz), 5.09 (2H, s), 5.11 (4H, s), 5.16 (2H, s), 5.19 (4H, s), 5.69 (1H, d, J=15.6 Hz), 5.81 (1H, d, J=15.6 Hz), 6.29 (1H, dt, J=15.3, 1.8 Hz), 6.66 (1H, dt, J=15.3, 4.4 Hz), 6.83 (2H, dt, J=15.6, 4.6 Hz), 7.10–7.25 (6H, m), 7.30–7.50 (30H, m), 7.65–7.75 (3H, m), 8.10–8.20 (3H, m); δ_C (75 MHz, $CDCl_3$) 40.4, 40.7, 40.8, 46.3, 47.6, 61.8, 62.3, 71.1, 76.3, 117.9, 118.0, 120.6, 121.4, 121.7, 123.3, 123.6, 124.9, 126.2, 127.4, 127.5, 127.7, 128.1, 128.6, 136.3, 136.5, 142.5, 144.6, 145.2, 146.8, 151.7, 165.1, 165.4, 165.8, 166.6. Anal. Calcd for $C_{79}H_{74}N_4O_{14}$: C, 72.8; H, 5.7; N, 4.3. Found: C, 72.5; H, 5.3; N, 4.3%.

4.5.4. N-{4-[3′-Di-(8″-aza-3″-oxa-4″,9″-dioxo-9″-(2‴,3‴-dihydroxyphenyl)-nonyl)amino]-4-oxobutyl}-2-(hydroxy)-benzamide ***16a***

Analogously to the synthesis of **7**, compound **16a** (190 mg, 90%) was obtained from **25a** (335 mg, 0.28 mmol) as a colorless foamy solid of mp 124–126 °C; ν_{max}/cm^{-1} 3378, 2944, 1719, 1644, 1619, 1585; δ_H (300 MHz, CD_3OD) 1.80–1.95 (6H, m), 2.30–2.40 (4H, m), 2.49 (2H, t, J=6.0 Hz), 3.35–3.45 (4H, m), 3.55–3.70 (6H, m), 4.14 (4H, t, J=5.5 Hz), 6.68 (2H, dt, J=8.1, 1.2 Hz), 6.80–6.95 (4H, m), 7.18 (2H, dt, J=8.1, 1.2 Hz), 7.25 (1H, dt, J=7.9, 1.2 Hz), 7.70–7.80 (1H, m); δ_C (75 MHz, CD_3OD) 25.7, 26.1, 31.6, 32.4, 39.7, 39.8, 46.7, 46.8, 63.2, 116.8, 117.0, 118.6, 118.8, 119.0, 119.7, 120.2, 128.8, 134.9, 147.4, 150.5, 160.4, 171.7, 174.6, 174.9, 175.1. Anal. Calcd for $C_{37}H_{44}N_4O_{13}$: C, 59.0; H, 5.9; N, 7.4. Found: C, 59.2; H, 6.0; N, 7.1%.

4.5.5. N-{4-[3′-Di-(8″-aza-3″-oxa-4″,9″-dioxo-9″-(2‴,3‴-dihydroxyphenyl)-nonyl)amino]-4-oxobutyl}-2,3-(dihydroxy)benzamide ***16b***

Analogously to **16a**, compound **16b** (177 mg, 92%) was obtained from **25b** (325 mg, 0.25 mmol) as a colorless foamy solid of mp 133–135 °C; ν_{max}/cm^{-1} 3372, 2948, 1722, 1643, 1625, 1584; δ_H (300 MHz, CD_3OD) 1.80–1.95 (6H, m), 2.30–2.45 (4H, m), 2.51 (2H, t, J=6.0 Hz), 3.35–3.50 (6H, m), 3.55–3.65 (4H, m), 4.16 (4H, t, J=5.3 Hz), 6.66 (3H, t, J=7.9 Hz), 6.91 (3H, dd, J=7.9, 1.1 Hz), 7.18 (3H, dd, J=7.9, 1.1 Hz); δ_C (75 MHz, CD_3OD) 25.8, 26.1, 31.6, 32.4, 39.8, 40.2, 46.7, 46.9, 63.2, 116.8, 118.7, 118.8, 119.8, 147.4, 150.5, 171.3, 174.5, 174.7, 175.0. Anal. Calcd for $C_{37}H_{44}N_4O_{14}$: C, 57.8; H, 5.8; N, 7.3. Found: C, 58.1; H, 5.8; N, 7.4%.

4.6. Agar diffusion assay

Agar plates containing 20 mL of Mueller–Hinton II medium (BD-Diagnostics, Heidelberg) with or without EDDA (LaboTest OHG, Niederschöna; 100 μg mL^{-1}) were inoculated with 100 μL of the respective bacteria suspension in liquid broth (opacity: Mc Farland 0.5) using a Drigalski spatula. Test samples were prepared as solutions of the respective

compound in ethanol at 10, 5, 2.5, and 1 mg mL^{-1}. Each solution (15 μL) was applied onto sterile 6 mm cellulose discs (CT 0998 B, Oxoid, Wesel). The ethanol was allowed to evaporate and the discs were placed upon the inoculated agar plate. The diameters in millimeter of the resulting growth and/or inhibition zones were determined after 48 h of incubation at 36 °C.

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References and notes

1. Drechsel, H.; Jung, G. *J. Pept. Sci.* **1998**, *4*, 147–181.
2. Winkelmann, G.; van der Helm, D.; Neilands, J. B. *Iron Transport in Microbes, Plants and Animals*; VCH: Weinheim, 1987; pp 223–233.
3. Roosenberg, J. M.; Lin, Y.-M.; Yong, L.; Miller, M. J. *Curr. Med. Chem.* **2000**, *7*, 159–197.
4. Faraldo-Gómez, J. D.; Sansom, M. S. P. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 105–116.
5. Braun, V.; Braun, M. *Curr. Opin. Microbiol.* **2002**, *4*, 194–201.
6. Payne, S. M. *CRC Crit. Rev. Microbiol.* **1988**, *16*, 81–111.
7. Braun, V. *Drug Resist. Updates* **1999**, *2*, 363–369.
8. Paulsen, H.; Brieden, M.; Benz, G. *Liebigs Ann. Chem.* **1987**, 565–575.
9. Benz, G.; Schröder, T.; Kurz, J.; Wünsche, C.; Karl, W.; Steffens, G.; Pfitzner, J.; Schmidt, D. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 527–528.
10. Heinisch, L.; Wittmann, S.; Stoiber, T.; Berg, A.; Ankel-Fuchs, D.; Möllmann, U. *J. Med. Chem.* **2002**, *45*, 3032–3040.
11. (a) Ghosh, A.; Ghosh, M.; Niu, C.; Malouin, F.; Möllmann, U.; Miller, M. J. *Chem. Biol.* **1996**, *3*, 1011–1019; (b) Diarra, M. S.; Lavoie, M. C.; Jacques, M.; Darwish, I.; Dolence, E. K.; Dolence, J. A.; Ghosh, A.; Ghosh, M.; Miller, M. J.; Malouin, F. *J. Antimicrob. Agents Chemother.* **1996**, *40*, 2610–2617; (c) Ghosh, M.; Miller, M. J. *Bioorg. Med. Chem.* **1996**, *4*, 43–48.
12. (a) Budzikiewicz, H. *Curr. Top. Med. Chem.* **2001**, *1*, 73–82; (b) Rivault, F.; Liébert, C.; Burger, A.; Hoegy, F.; Abdallah, M. A.; Schalk, I. J.; Mislin, G. L. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 640–644.
13. Schobert, R.; Stangl, A.; Hannemann, K. *Tetrahedron* **2006**, *62*, 7799–7808.
14. Schobert, R.; Boeckman, R. K., Jr.; Pero, J. E. *Org. Synth.* **2005**, *82*, 140–145.
15. Bestmann, H. J.; Schobert, R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 790–791.
16. Gardner, R. A.; Kinkade, R.; Wang, C.; Phanstiel, O. *J. Org. Chem.* **2004**, *69*, 3530–3537.
17. Zhu, J.; Robin, S.; Goasdoué, N.; Loupy, A.; Galons, H. *Synth. Commun.* **1995**, *25*, 2515–2519.
18. Peterson, T.; Neilands, J. B. *Tetrahedron Lett.* **1979**, *20*, 4805–4808.
19. (a) Bergeron, R. J.; Dionis, J. B.; Elliott, G. T.; Kline, S. J. *J. Biol. Chem.* **1985**, *260*, 7936–7944; (b) Neilands, J. B.; Peterson, T.; Leong, S. A. High Affinity Iron Transport in Microorganisms. In *Inorganic Chemistry in Biology and Medicine*; Martell, A. E., Ed.; ACS: Washington, DC, 1980; pp 263–278.
20. Essoy, O.; Fleck, R.; Blanco, M. J.; Masamune, S. *Bioorg. Med. Chem.* **1999**, *7*, 279–286.
21. Veriot, G.; Dutasa, J.-P.; Matouzenko, G.; Collet, A. *Tetrahedron* **1995**, *51*, 389–400.
22. Fujioka, H.; Murai, K.; Ohba, Y.; Hiramatsu, A.; Kita, Y. *Tetrahedron Lett.* **2005**, *46*, 2197–2199.
23. Garrigues, B.; Lazraq, M. *Tetrahedron Lett.* **1986**, *27*, 1685–1686.
24. Killmann, H.; Braun, V. *J. Bacteriol.* **1992**, *174*, 3479–3486.
25. Bred and provided by the Institute of Microbiology, Department of Membrane Physiology, University of Tübingen, Germany.
26. Rabsch, W.; Winkelmann, G. *BioMetals* **1991**, *4*, 244–250.
27. (a) Bergeron, R. J.; Wiegand, J.; Weimar, W. R.; Vinson, J. R. T.; Bussenius, J.; Yao, G. W.; McManis, J. S. *J. Med. Chem.* **1999**, *42*, 95–108; (b) Bergeron, R. J.; Wiegand, J.; Weimar, W. R.; McManis, J.; Smith, R.; Abboud, K. *Chirality* **2003**, *15*, 593–599.