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New synthetic catecholate-type siderophores based on amino acids and dipeptides

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Abstract

New analogs of bacterial siderophores with one, two or three catecholate moieties were synthesized using various mono- and diamino acid and dipetide scaffolds, respectively. In addition to 2,3-dihydroxybenzoyl siderophore analogs and their acylated derivatives, 3,4-dihydroxybenzoyl derivatives were prepared. Furthermore, the synthesis of a new triscatecholate serving as an intimate model for enterobactin is reported. Most of the new compounds gave a positive CAS-test and were active as siderophores tested by growth promotion assays with a set of siderophore indicator mutants under iron limitation. Structure-activity-correlations have also been studied.

Introduction

A great number of bacterial siderophores contains amino acids or peptides as building blocks forming the backbone for their iron chelating catecholate or hydroxamate groups. The most powerful natural siderophore enterobactin consists of a trilactone ring formed by three *N*-(2,3-dihydroxybenzoyl)-serine molecules. This triscatecholate siderophore is produced by *E.coli* and other enteric bacteria under iron deficient conditions (O'Brien & Gibson 1970; Pollack & Neilands 1970). But even the monomer, *N*-(2,3-dihydroxybenzoyl)-serine, acts as a siderophore (Handtke 1990). Examples of other natural catecholate-type siderophores based on amino acids or peptides are the biscatecholate *N,N′*-bis-(2,3 dihydroxybenzoyl)-L-lysine (Corbin & Bulen 1969), and the monocatecholates *N*-(2,3-dihydroxybenzoyl)-glycine (Ito & Neilands 1958) and chrysobactin (Persmark *et al.* 1989) which is comprised a D-lysyl-serine moiety. Syntheses of these compounds and data on their siderophore activity have been published (Ito & Neilands 1958; Rastetter *et al.* 1981; C. Lu *et al.* 1996). Only a few synthetic siderophore analogs with other amino acids and dipeptides (e.g., L-threonine, lysyl-lysine) as backbone have been prepared (Chimiak & Neilands 1984; Kanai *et al.* 1985) and no systematic investigations on structure-activity-correlations have been performed until now.

In this paper we report on the synthesis of novel mono- bis-, and triscatecholate siderophore analogs with various amino acids and dipeptides as the backbone and a free carboxyl anchor group. The synthesized derivatives can be conjugated directly or via spacers with other biologically active agents like antibiotics designing iron transport-mediated drug delivery. The siderophore activity of the prepared siderophore analogs was investigated by the CAS test and growth promotion tests using siderophore indicator mutants and wild type strains. First results on structure activity correlations are presented.

Materials and methods

Synthesis of the siderophore analogs

^{1}H-NMR spectra were recorded on a 300 and 500 MHz Bruker spectrometer, respectively. The chemical shifts δ are given in ppm related to tetramethyl silane as internal standard. The coupling constants J are reported in Hz. Here only spectra of some representative compounds were given, other ^{1}H- and ^{13}C-NMR spectra were recorded and can be obtained from the authors. High resolution mass spectra were obtained by using fast-atom bombardment (FAB) or electrospray ionisation (ESI) technique. Column chromatography was accomplished using silica gel (Merck 60, 0.040–0.063 mm). Thin layer chromatography was conducted with precoated silica plates (Merck 60 F254) using UV detection. Solvents and reagents used were dried and purified by standard methods (Perrin & Armarego 1988). The following compounds have been synthesized according to published procedures: 2,3-di(methoxycarbonyloxy)benzoyl chloride (**1**), and (8-methoxycarbonyloxy-3,4-dihydro-2,4-dioxo-2H-1,3-benzoxazin-3-yl)-acetic acid (**2a**, Wittmann *et al.* 2000), 2,3-di(benzyloxy)benzoyl chloride (**4**), and N-(2,3-dihydroxybenzoyl)-L-serine (**5a**, Rastetter *et al.* 1981), 2,3-diacetoxybenzoyl chloride (**9**, Bergeron *et al.* 1980), 3,4-di(benzyloxy)benzoyl chloride (**14**, yield 96%, mp 76–79 °C, Barton *et al.* 1965), succinimido 2,3-dihydroxybenzoate (**18**, Bergeron *et al.* 1983), amino acid benzyl ester hydrochlorides (Deimer 1974), N^2-benzyloxycarbonyl-L-2,3-diaminopropionic acid (**20a**, Waki *et al.* 1981).

*N-(2,3-Dihydroxybenzoyl)-glycine (**3a**)*, $C_9H_9NO_5$ (211.2)

To **2a** (295 mg, 1 mmol) suspended in water (3 ml) was added 2M NaOH (3 ml) under N_2 and the mixture was stirred for 2 h. After acidification with 2 M HCl, the formed precipitate was crystallized from water affording **3a** (158 mg, 75%): mp 220–222 °C [Ito & Neilands 1958: mp 210–11 °C]; MS (FAB): 212.1 ($[M+H]^+$), ^{1}H-NMR (500 MHz, DMSO-d_6): 3.85 (d, J = 5.8, 2H NCH_2), 6.76 (dd, J = 8.2, 1H, aromatic H), 7.18 (dd, J_1 = 8.2, J_2 = 2.0, 1H, aromatic H), 7.30 (dd, J_1 = 8.2, J_2 = 2.0, 1H, aromatic H), 9.12 (t, J = 5.8, 1H, NHCO), 9.30 (s, broad, 1H, exchangeable with D_2O, 1H, 3-OH), 12.25 (s, broad, exchangeable with D_2O, 1H, 2-OH).

*N-(2,3-Dihydroxybenzoyl)-glycylglycine (**3b**)*, $C_{11}H_{12}N_2O_6$ (268.2)

3b (yield 50%) was prepared in analogy to **3a**: mp 255–256 °C (water); MS (FAB): 267.0 ($[M+H]^+$).

*N-(2,3-Dihydroxybenzoyl)-p-hydroxy-D(−)-phenylglycine (**3c**)*, $C_{15}H_{13}NO_6$ (303.3)

In an ultrasonic bath 2,3-di(methoxycarbonyloxy)benzoyl chloride **1** (280 mg, 1 mmol) was added with stirring at 0–5 °C to *p*-hydroxy-D(−)-phenylglycine (330 mg, 2 mmol) dissolved in 15 ml of a saturated aqueous $NaHCO_3$-solution and the mixture was stirred for 30 min at this temperature. The reaction mixture was acidified with HCl and the precipitate was separated and hydrolyzed with aqueous NaOH under N_2 as described for **3a**. Yield 47%: m.p. 109–111 °C (water). MS (FAB): 304.2 ($[M+H]^+$).

*N-(2,3-Dihydroxybenzoyl)-L-alanine (**5b**)*, $C_{10}H_{11}NO_5$ (225.20)

(a) Acylation.
A solution of 2,3-di(benzyloxy)benzoyl chloride (1.5 g, 4.25 mmol) in THF (8 ml) was added dropwise with stirring at 0–5 °C to an aqueous solution (8 ml) of L-alanine (379 mg, 4.25 mmol) and NaOH (340 mg, 8.5 mmol). The mixture was stirred for 4 h at ambient temperature, acidified to pH 2 and extracted with ethyl acetate. The organic layer was washed with saturated NaCl solution, dried, filtered and evaporated to dryness. Crystallization of the residue (1.59 g) from ethanol/water gave pure *N*-[2,3-di(benzyloxy)benzoyl]-L-alanine (624 mg, 36%): m.p. 127 °C (Nakonieczna *et al.* 1989: 128–129 °C).

(b) Deprotection.
A solution of *N*-[2,3 dibenzyloxy)benzoyl]-L-alanine (600 mg, 1.48 mmol) in an ethanol/acetic acid mixture (9.5/0.5 ml) was stirred over 10% Pd-C (40 mg) at atmospheric hydrogen pressure and room temperature for 4 h. The reaction mixture was filtered over celite and the solvent was evaporated. The residue was dissolved in ethyl acetate, washed with saturated NaCl solution and evaporated to provide **5b** (321 mg, 96%) as a white solid. (MS (FAB): 226.1 ($[M+H]^+$), ^{1}H-NMR (300 MHz, DMSO-d_6): 1.42 (d, 3H, J = 7.3, CH_3), 4.44 (dq, 1H, J_1 = 7.2, J_2 = 7.4, CH), 6.72 ('t', 1H, J = 8.0, aromatic H), 6.94 (dd, 1H, J = 1.6, aromatic H), 7.40 (dd, J = 1.6, 1H, aromatic H), 8.94 (d, J = 7.1, exchangeable with D_2O, 1H, NHCO), 9.27

(s, broad, exchangeable with D_2O, 1H, 3-OH), 12.28 (s, broad, exchangeable with D_2O, 2H, 2-OH).

*N-(2,3-Dihydroxybenzoyl)-glycyl-L-alanine (**5c**),* $C_{12}H_{14}N_2O_6$ (282.26)

Succinimido 2,3-dihydroxybenzoate (**6**) prepared from 2,3-dihydroxybenzoic acid (770 mg, 5 mmol) and N-hydroxysuccinimide (575 mg, 5 mmol) in dioxane (5 ml) was dissolved in THF (5 ml) and to this solution glycyl-L-alanine (730 mg, 5 mmol) dissolved in THF (5 ml), water (5 ml) and triethylamine (1.2 ml, 85 mmol) was added. The mixture was stirred 10 h at ambient temperature, evaporated, and ethyl acetate/water was added. After acidification, the organic phase was separated, washed with NaCl solution, dried (Na_2SO_4), filtered and evaporated to give **5c** (847 mg, 60%), MS (ESI): 281.2 ($[M\text{-}H]^-$).

*N-(2,3-Dihydroxybenzoyl)-glycyl-L-leucine (**5d**),* C_{15} $H_{120}N_2O_6$ (324.34)

5d (yield 45%) was prepared analogously to **5c**, MS (ESI): 323.3 ($[M\text{-}H]^-$).

*N-(2,3-Dihydroxybenzoyl)-L-threonine (**8**)*

*(a) N-(1-Ethoxycarbonyl-propen-2-yl)-L-threonine potassium salt (**7a**),* $C_{10}H_{16}NO_5$ (269.34).
Ethyl acetoacetate (6.5 ml, 51 mmol) was added to a boiling solution of L- threonine (5.65 g, 47.6 mmol) and KOH (2.86 g, 51 mmol). The mixture was refluxed for 15 min and cooled to O °C to afford **7a** (8.8 g, 73%) as white crystals.

*(b) L-Threonine p-bromophenacyl ester hydrobromide (**7b** $C_{12}H_{14}BrNO_4$x HBr (397.07).*
To **7a** (4.04 g, 15 mmol) in acetonitrile (100 ml) were added *p*-bromophenacyl bromide (4.16 g, 15 mmol) and 18-crown-6 (195 mg) and the mixture was refluxed for 30 min. After cooling, the filtered solution was evaporated and the residue was dissolved in 50 ml of $CHCl_3$, washed with water, dried (Na_2SO_4), and evaporated. Crystallisation of the crude from hexanes/acetone yielded *N*-(1-ethoxycarbonyl-propen-2-yl)-L-threonine p-bromophenacyl ester (3.2 g, 50%) as white crystals. The latter (2.14 g, 0.5 mmol) was stirred for 10 min in 20 ml of 1 M methanolic HCl and evaporated. Crystallization of the residue from isopropanol afforded **7b** (1.2 g 75%) as slightly yellow crystals: mp 140–145 °C.

*(c) N-[2,3-Di-(benzyloxy)benzoyl]-L-threonine-p-bromophenacyl ester (**7c**),*
$C_{33}H_{30}BrNO_7$(632.49).
A solution of **4** (1.6 g, 4.53 mmol) in THF (10 ml) was added dropwise to a solution of **7b** (1.8 g, 4.53 mmol) and diisopropylethylamine (2.33 ml 1.76 mmol) in THF (20 ml). The mixture was stirred for 4 h at ambient temperature and evaporated. The residue was dissolved in ethyl acetate, washed successively with 2 M HCl, saturated $NaHCO_3$ solution, and water, dried and evaporated. Purification of the residue by column chromatography (toluene : AcOEt = 3:1) gave **7c** (1.81 g ,63%) as a white foam: TLC: R_f 0.53 (toluene : AcOEt = 1 : 1).

*(d) N-[2,3-Di (benzyloxy)benzoyl]-L-threonine (**7d**),*
$C_{25}H_{25}NO_6$ (435.46)
Zn-powder (5.6 g, 85.7 mmol) was added to a solution of **7c** (1.5 g, 2.37 mmol) in acetic acid (20 ml) and the mixture was stirred for 2 h at ambient temperature. After removal of the solvent diethyl ether/$NaHCO_3$-solution was added and the aqueous phase washed with ether, acidified with HCl to pH2 and extracted with ethyl acetate. The organic phase was dried (Na_2SO_4), filtered and evaporated to give **7d** (869 mg, 84%) as a white solid, MS (ESI): 434.3($[M\text{-}H]^-$).

*(e) N-(2,3-Dihydroxybenzoyl)-L-threonine (**8**),*
$C_{11}H_{13}NO_6$ (255.22).
A solution of **7d** (813 mg, 1.87 mmol) in ethanol/acetic acid (20:1, 10 ml) was hydrogenolyzed over 10% Pd-C (85 mg) at ambient temperature and atmospheric hydrogen pressure for 6 h. The reaction mixture was filtered over celite and evaporated. The residue was dissolved in ethyl acetate, washed with saturated NaCl solution, dried (Na_2SO_4), filtered and evaporated to provide **8** (454 mg, 95%) as a white solid, MS (ESI): 254.1 ($[M\text{-}H]^-$), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.14 (d, J = 6.3, 3H, CH_3), 4.23 (m, 1H, CH-O), 4.43 (dd, J_1 = 3.4, J_2 = 8.3, 1H, CH-N), 5.05 (s, broad, exchangeable with D_2O, 1H, OH), 6.73 ('t', J = 7.9, 1H, aromatic H), 6.95 (dd, J_1 = 1.5, J_2 = 7.8, 1H, aromatic H), 7.39 (dd, J_1 = 1.5, J_2 = 8.0, 1H, aromatic H), 8.61 (d, J = 8.3, exchangeable with D_2O, 1H, NHCO), 9.48 (s, broad, exchangeable with D_2O, 1H, 3-OH), 11.24 (s, broad, exchangeable with D_2O, 1H, 2-OH).

*N-[2,3-Di(acetoxy)benzoyl]-L-alanine (**10a**),*
$C_{14}H_{15}NO_7$ (309.27)

(a) Acylation.
A solution of **9** (513 mg, 2 mmol) in dichloromethane (10 ml) was added dropwise under N_2 at −30 °C to a solution of L-alanine-benzylester hydrochloride (431 mg, 2 mmol) and diisopropylethylamine (0.68 ml, 4 mmol) in dichloromethane (10 ml). The mixture was stirred 1h at −30 °C and 2 h at ambient temperature, washed with 1 M HCl, saturated $NaHCO_3$ solution, and water, dried (Na_2SO_4), filtered and evaporated. Crystallization of the crude from ethanol afforded *N*-(2,3-diacetoxybenzoyl)-L-alanine-benzylester (689 mg, 86%) as a white solid: mp 94 °C, MS (FAB): 400.2 ($[M+H]^+$).

(b) Deprotection.
A solution of the benzyl ester (365 mg, 0.914 mmol) in ethanol/acetic acid (5:1, 12 ml) was hydrogenolyzed over 10% Pd-C (37 mg) for 2 h as described for **8**. The crude product crystallized after storage for 3 days affording **10a** as colourless crystals (280 mg, 99%): mp 108-111 °C, MS (ESI): 310.3 ($[M+H]^+$), 332.3 ($[M+Na]^+$), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.54 (d, J = 7.2, 3H, CH_3), 2.30 (s, 3H, OCH_3), 2.35 (s, 3H, OCH_3), 4,79 (m, 1H, CH), 7.00 (d, J = 7.1, 1H, NHCO), 7.34 (m, 2H, aromatic H), 7.72 (m, 1H, aromatic H).

*N-[2,3-Di(acetoxy)benzoyl]-L-serine (**10b**),*
$C_{14}H_{15}NO_8$ (325,27)

L-Serine benzylester hydrochloride (1.12 g, 4.83 mmol) was treated with **9** (1.24 g, 4.83 mmol) according to the procedure used for **10a** except using triethylamine (9.66 mmol, 1.34 ml) instead of diisopropylethylamine. Crystallization of the crude from toluene gave *N*-(2,3-diacetoxybenzoyl)-L-serine benzylester (854 mg, 43%) as colourless needles (mp 87–88 °C). Benzyl ester cleavage was performed as described for **8** affording **10b** (80%) as a white solid, MS (FAB): 326.1 ($[M+H]^+$).

*O-Acetyl-N-(2,3-di(acetoxy)benzoyl]-L-serine (**10c**),*
$C_{16}H_{17}NO_9$ (367.31)

(a) Acylation.
Two drops of perchloric acid were added to a solution of *N*-(2,3-diacetoxybenzoyl)-L-serine benzylester (1.4 g, 3.37 mmol) in acetic anhydride (15 ml). The mixture was stirred for 1 h at room temperature, poured onto ice and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and evaporated to dryness. Column chromatography (AcOEt : toluene = 1 : 2) of the crude afforded *O*-acetyl-*N*-(2,3-diacetoxybenzoyl)-L-serine benzylester (629 mg, 41%) as colourless needles: mp 87 °C.

(b) Deprotection.
The benzyl ester was cleaved by hydrogenolysis as described for **8** affording **10c** (99%) as a white foam: MS (FAB): 368.2 ($[M+H]^+$).

*O-Benzyl-N-[2,3-di(acetoxy)benzoyl]-L-serine (**10d**),*
$C_{21}H_{21}NO_8$ (415.39)

O-Benzyl-L-serine benzyl ester hydrochloride (mp 130–132 °C) was prepared from *O*-benzyl-L-serine in analogy to **7a**, and **7b**, respectively. Subsequent acylation of the benzyl ester hydrochloride (1.5 g, 4.66 mmol) with **9** (1.3 g, 5.12 mmol) and diisopropylethylamine (1.6 ml, 9.32 mmol) in dichloromethane (50 ml) was performed as described for **10a** yielding *O*-benzyl-*N*-(2,3-diacetoxybenzoyl)-L-serine benzyl ester (2.07 g, 88%) as a yellow oil. The latter (637 mg, 1.26 mmol) was stirred with 10% Pd-C (640 mg) and cyclohexa-1,4-diene (1.18 ml, 12.6 mmol) in ethanol (30 ml) under an hydrogen atmosphere for 30 min. The reaction mixture was filtered over celite and evaporated. Column chromatography ($CHCl_3$: AcOEt : AcOH = 3 : 1 : 0,3) gave **10d** (355 mg, 68%) as a white foam: TLC: R_f 0.27 ($CHCl_3$: AcOEt : AcOH = 3 : 1 : 0.3), MS (FAB): 416.4 ($[M+H]^+$).

*N-[2,3-Di(acetoxy)benzoyl]-L-β-alanine (**10e**),*
$C_{14}H_{15}NO_7$ 309.27)

To β-alanine (89 mg, 1 mmol) dissolved in aqueous 0,5 M $NaHCO_3$ solution (4 ml, 2 mmol) was added dropwise **9** (257 mg, 1 mmol) in THF at 0–5 °C with stirring. After 1 h the reaction mixture was evaporated and the obtained aqueous solution was acidified at 0 °C with HCl to pH2 and extracted with ethyl acetate. The extract was washed with NaCl solution, dried (Na_2SO_4), filtered and concentrated. Addition of cold benzene gave **10e** (216 mg, 70%), MS (ESI) 308.2 ($[M-H]^-$).

*N-[2,3-Di(acetoxy)benzoyl]-L-phenylglycine (**10f**),* $C_{19}H_{17}NO_9$ (403.35)

The preparation was performed as described for **10e** affording **10f** (290 mg, 20%) after purification by preparative HPLC, MS (ESI): 404.2 ($[M+H]^+$).

*N-[3,4-Di(methoxycarbonyloxy)benzoyl]-glycine (**12a**),* $C_{13}H_{13}NO_9$ (327.25)

To glycine (150 mg, 2 mmol) dissolved in 0,5 M aqueous $NaHCO_3$ solution (4 ml, 2 mmol) was added dropwise **11** (257 mg, 1 mmol) at 0–5 °C with stirring. After 1 h, the reaction mixture was evaporated, the obtained aqueous solution acidified at 0 °C with HCl to pH2 and extracted with ethyl acetate. The extract was washed with NaCl solution, dried (Na_2SO_4), filtered and concentrated. Addition of cold benzene gave **12a** (216 mg, 70%), MS (FAB): 328.1 ($[M+H]^+$), ^{1}H-NMR (300 MHz, DMSO-d_6): 3.85 (2s, 6H, $COOCH_3$), 3.92 (d, J = 8.3, 2H, NCH_2), 7.56 (d, J = 8.4, 1H, aromatic H), 7.84 (d, J = 8.5, 1H, aromatic H), 7.87 ('s', 1H, aromatic H), 8.94 (t, J = 5.8, 1H, NHCO).

*N-[3,4-Di-(methoxycarbonyloxy)benzoyl]-glycyl-glycine (**12b**),* $C_{15}H_{16}N_2O_{10}$ (384.3)

The preparation was performed as described for **12a**, MS (FAB): 385.0 ($[M+H]^+$).

*N-(3,4-Dihydroxybenzoyl)-glycine (**13a**),* $C_9H_9NO_5$ (211.2)

12a (885 mg, 3 mmol) was stirred in 2M aqueous KOH (10 ml) under N_2 for 1 h. The solution was acidified with HCl and extracted with THF. The extract was filtered, dried (Na_2SO_4), filtered and concentrated. Addition of benzine gave **13a** (475 mg, 75%): m. p.220–22 °C, MS (FAB): 212.2 ($[M+H]^+$), ^{1}H-NMR (500 MHz, DMSO-d_6): 3.83 (d, J = 5.8, 2H, NCH_2), 6.76 (d, J = 8.2, aromatic H), 7.18 (dd, J_1 = 8.2, J_2 = 2.0, aromatic H), 7.24 (d, J = 2.0, 1H, aromatic H), 8.42 (t, J = 5.8 ,1H, NHCO), 9.11 (s, broad, 1H, exchangeable with D_2O, catecholic OH), 9.94 (s, broad, 1H, exchangeable with D_2O, catecholic OH).

*N-(3,4-Dihydroxybenzoyl)-glycylglycine (**13b**),* $C_{11}H_{12}N_2O_6$ (268,2)

13b (yield 29%) was prepared from **12b** as described for **12a** except using aqueous NaOH: mp 230–34 °C (water), MS (FAB): 267.0 ($[M-H]^+$).

*N-(3,4-Dihydroxybenzoyl)-4-hydroxy-D(−)-phenyl-glycine (**13c**),* $C_{15}H_{13}NO_6$ (303.3)

3,4-Di(methoxycarbonyloxy)benzoyl chloride (280 mg, 1 mmol) in THF (3 ml) was added at 0 °C to 4-hydroxy-D(−)-phenylglycine (170 mg, 1 mmol) dissolved in aqueous 1 M $KHCO_3$ solution (10 ml). The mixture was stirred 1 h at 0 °C, 1 h at ambient temperature and filtered. The filtrate was acidified with HCl, extracted with ethyl acetate, washed with water, dried (Na_2SO_4) and evaporated. The residue was stirred in 1M aqueous NaOH (20 ml) under N_2 and the solution was acidified with HCl and worked up as described for **13a** affording **13c** (188 mg, 62%), MS (FAB): 304.1 ($[M+H]^+$).

*N-(3,4-Dihydroxybenzoyl)-L-serine (**15**),* $C_{10}H_{11}NO_6$ (241.20)

(a) Acylation.
3,4-Di(benzyloxy)benzoyl chloride (1.2 g, 3.4 mmol) dissolved in THF (8 ml) was added with stirring at 0–5 °C to a solution of O-benzyl-L-serine (664 mg, 3.4 mmol) and NaOH (272 mg, 6.8 mmol) in H_2O (6 ml). The mixture was stirred 1 h at 0–5 °C and 3 h at room temperature and extracted with ethyl acetate. The organic phase was separated, washed with water, dried (Na_2SO_4), filtered and evaporated. Crystallization of the crude white solid (1.56 g) from ethyl acetate/hexene gave *O*-benzyl-*N*-[3,4-di(benzyloxy)benzoyl]-L-serine (1.17 g, 67%) as white crystals: mp 113 °C. MS (ESI): 510.4 ($[M-H]^-$).

(b) Deprotection.
A solution of *O*-benzyl-*N*-[3,4-di(benzyloxy)benzoyl]-L-serine (860 mg, 1.6 mmol) in ethanol/acetic acid (9.5/0.5 ml) was hydrogenolyzed over 10% Pd-C (40 mg) at ambient temperature and atmospheric hydrogen pressure for 24 h. Work-up as described for **8** provided **15** (112 mg, 28%) as a white solid. ^{1}H-NMR (300 MHz, DMSO-d_6): 3.74 (m, 2H, NCH_2), 4.41 (m, 1H, NCH), 6.78 (d, J = 8.2, 1H, aromatic H), 7.24 (dd, J = 2.1, 8.2, 1H, aromatic H), 7.31 (d, J = 2,1, 1H, aromatic H), 7.96 (d, J = 7.7, 1H, NHCO), 9.15 (s, broad, 1H , exchangeable with D_2O, catecholic OH), 9.48 (s, broad, 1H, exchangeable with D_2O, catecholic OH).

*N,N-Bis-[2,3-di(benzyloxy)benzoyl-L-diamino acids **(16a-d)***

General procedure.
A solution of **4** (1.8 g, 5.1 mmol) in THF (10 ml) was added dropwise at 0 °C to a solution of the diamino acid monohydrochloride (2.5 mmol) and NaOH (304 mg, 7.6 mmol) in H_2O (10 ml). The mixture was stirred for 4 h at ambient temperature, acidified to pH 2 with 1M HCl and extracted with ethyl acetate. The organic layer was washed three times with NaCl solution, dried (Na_2SO_4), filtered and evaporated. Purification of the crude by column chromatography ($CHCl_3$: AcOEt : AcOH = 30 : 10 : 1) afforded the corresponding acylated diamino acid **16a-d**.

*N,N′-Bis-[2,3-di(benzyloxy)benzoyl]-L-2,3-diaminopropionic acid **(16a)**,* $C_{45}H_{40}N_2O_8$ (736.79)

Colourless solid; yield: 81%: TLC: R_f 0.21 ($CHCl_3$: AcOEt : AcOH = 30 : 10 : 1), MS (FAB): 737.2 ($[M+H]^+$), ^{1}H-NMR (300 MHz, $CDCl_3$): 3.35 (m, 1H, NCH_2), 3.67 (m, 1H, NCH_2), 4.52 (m, 1H, CH), 5.12 (m, 8H, OCH_2), 7.07–7.46 (m, 24H, aromatic H), 7.64 (m, 2H, aromatic H), 8.60 (t, J = 6.0, 1H, NHCO), 8.88 (d, J = 5.7, 1H, NHCO).

*N,N′-Bis-[2,3-di(benzyloxy)benzoyl]-L-2,4-diaminobutyric acid **16b**,* $C_{46}H_{42}N_2O_8$ (750.85)

Colourless foam; yield: 84%; TLC: R_f 0.25 ($CHCl_3$: AcOEt : AcOH = 30 : 10 : 1); MS (FAB): 751.5 ($[M+H]^+$).

*N,N′-Bis-[2,3-di(benzyloxy)benzoyl]-L-ornithine **(16c)**,* $C_{47}H_{44}N_2O_8$ (764.84)

Colourless solid; yield: 64%; TLC: R_f 0.20 ($CHCl_3$: AcOEt : AcOH = 30 : 10 : 1).

*N,N′-Bis-[2,3-di(benzyloxy)benzoyl]-L-lysine **(16d)**,* $C_{48}H_{46}N_2O_8$ (778.87)

Colourless foam; yield: 53%, MS (ESI) 779.6 ($[M+H]^+$), 800.8 ($[M+Na]^+$).

*N,N-Bis-(2,3-dihydroxybenzoyl)-L-diamino acids **(17a-d)***

General procedure.
A solution of the *N,N′*-bis[2,3 di(benzyloxy)benzoyl]-L-amino acid (1.2 mmol) in an ethanol/acetic acid mixture (10/1 ml) was stirred over 10% Pd-C (100 mg) at atmospheric hydrogen pressure and room temperature for 24 h. The reaction mixture was filtered over celite and the solvent was evaporated. The residue was dissolved in ethyl acetate, washed with saturated NaCl solution and evaporated to provide the corresponding biscatecholate **17a-d**.

*N,N′-Bis-(2,3-dihydroxybenzoyl)-L-2,3-diaminopropionic acid **(17a)**,* $C_{17}H_{16}N_2O_8$ (376.32)

Colourless solid; yield: 99%, MS (FAB): 377.2 ($[M+H]^+$), ^{1}H-NMR (300 MHz, DMSO-d_6): 3.70 (m, 1H, CH_2), 3.85 (m, 1H, CH_2), 4.64 (m, 1H, CH), 6.69 (m, 2H, aromatic H), 6.90 (m, 2H, aromatic H), 7.21 (dd, J = 8.1, 1H, aromatic H), 7.30 (dd, J = 1.3, 8.0, 1H, aromatic H), 8.92 (t, exchangeable with D_2O, 1H, NHCO), 9.00 (d, exchangeable with D_2O, 1H, J = 7.4, NHCO), 9.30 (s, broad, exchangeable with D_2O, 2H, OH), 12.00 (s, broad, exchangeable with D_2O, 2H, OH).

*N,N′-Bis-(2,3-dihydroxybenzoyl)-L-2,4-diaminobutyric acid **(17b)**,* $C_{18}H_{18}N_2O_8$ (390.35)

Colourless foam; yield: 96%, MS (FAB) : 391.3 ($[M+H]^+$).

*N,N′-Bis-(2,3-dihydroxybenzoyl)-L-ornithine **(17c)**,* $C_{19}H_{20}N_2O_8$ (404,37)

Colourless solid; yield: 99%, MS (ESI): 403.3 ($[M-H]^-$).

*N,N′-Bis-(2,3-dihydroxybenzoyl)-L-lysine **(17d)**,* $C_{20}H_{22}N_2O_8$ (418.40)

Colourless solid; yield: 85%, MS (ESI): 417.3 ($[M-H]^-$).

*N,N′-Bis-(2,3-dihydroxybenzoyl)-D-ornithine **(19)**,* $C_{19}H_{20}N_2O_8$ (404.37)

(a) Acylation.
A solution of **18** (2.0 g, 4,64 mmol) in acetone (16 ml) was added to D-ornithine monohydrochloride (317 mg, 1.85 mmol) dissolved in a mixture of H_2O (4.3 ml) and triethylamine (1.3 ml). After stirring for 15 h at ambient temperature the mixture was evaporated and the residue was dissolved in $CHCl_3$ (50 ml), washed with 1M HCl, and water,

dried, filtered and evaporated. Column chromatography ($CHCl_3$: AcOEt : AcOH = 3 : 1 : 0.3) of the crude yielded *N,N′*-bis-[2,3-di(benzyloxy)benzoyl]-D-ornithine (945 mg, 67%) as a colourless foam.

(b) Hydrogenolysis.
The protected ornithine derivative (500 mg, 0.65 mmol) was hydrogenolyzed as described for **17a-c** affording **19** (262 mg, 99%) as a colourless solid, MS (FAB): 405.2 ($[M+H]^+$), ^{1}H-NMR (300 MHz, DMSO-d_6): 1.64 (m, 2H, CH_2), 1.91 (m, 2H, CH_2), 3.33 (m, 2H, NCH_2), 4.46 (m, 1H, NCH), 6.70 (m, 2H, aromatic H), 6.93 (m, 2H, aromatic H), 7.28 (dd, $J_1 = 1.3$, $J_2 = 8.0$, 1H, aromatic H), 7.41 (dd, J = 8.0, 1H, aromatic H) 8.80 (t, exchangeable with D_2O, 1H, NHCO), 8.91 (d, J = 7.3, exchangeable with D_2O, 1H, NHCO), 9.12 (s, broad, catecholic OH).

N^3-[N,N′-Bis-(2,3-dihydroxybenzoyl)]-L-lysyl-N^2-(2,3-dihydroxybenzoyl)-L-2,3-diaminopropionic acid ***(21)****,* $C_{30}H_{32}BrN4O10$ (640.6)

(a) N^2-Benzyloxycarbonyl-N^3-tert-butoxycarbonyl-L-2,3-diaminopropionic acid p-bromophenacylester ***(20b)****,* $C_{24}H_{27}N_2O_7$ (535.39)
To a solution of **20a** (1.51 g, 6.34 mmol) and NaOH (254 mg, 6.34 mmol) in dioxane/water (16/28 ml) was added with ice cooling tert-butyl pyrocarbonate (1.52 g, 6.97 mmol). The solution was stirred 2 h at ambient temperature and evaporated. H_2O (10 ml) and AcOEt (20 ml) were added to the residue and the pH was adjusted between 2 and 3 using 5% aqueous citric acid. The organic layer was separated and the aqueous layer was extracted twice with AcOEt. The AcOEt layers were combined, washed with water, dried (Na_2SO_4) and filtered. Evaporation and drying of the residue yielded N^2-benzyloxycarbonyl-N^3-tert-butoxycarbonyl-L-2,3-diaminopropionic acid (1.76 g, 82%). The latter (1.69 g, 4.99 mmol) was dissolved in dry acetone (60 ml) and *p*-bromophenacyl bromide (1.39 g, 4.99 mmol) and K_2CO_3 (690 mg, 4.99 mmol) were added. After refluxing the mixture for 4 h, the solvent was evaporated and the residue was partitioned between H_2O and AcOEt. The organic layer was separated and the aqueous layer was extracted twice with AcOEt. The AcOEt layers were combined, washed with water, dried (Na_2SO_4), filtered and evaporated. Crystallization of the yellowish residue (2.25 g) from EtOH gave **20b** (1.93 g, 72%) as colourless crystals: mp 125 °C.

(b) N^2-Benzyloxycarbonyl-L-2,3-diaminopropionic acid p-bromophenacylester trifluoroacetate ***(20c)****,* $C_{21}H_{20}BrF_3N_2O_7$ (549.30)
20b (1.70 g, 3.17 mmol) was dissolved in a mixture of CF_3COOH (10 ml) and H_2O (5 ml). After stirring 1 h at ambient temperature the solvent was evaporated under reduced pressure at a temperature below 30 °C. Toluene was added to the residue and the mixture was evaporated again. Trituration of the remaining oil with diethyl ether and drying of the solidified product afforded **20c** (1.66 g, 95%) as a colourless solid.

(c) N^3-[N,N′-Bis-(benzyloxycarbonyl)]-L-lysyl-N^2-(benzyloxycarbonyl)-L-2,3-diaminopropionic acid p-bromophenacyl ester ***(20d)****,* $C_{41}H_{43}BrN_4O_9$ (831.70)
Dicyclohexylcarbodiimide (487 mg, 2.36 mmol) was added at 0 °C under N_2 to a solution of *N,N′*-bis(benzyloxycarbonyl)-L-lysine (932 mg, 2.25 mmol) and 1-hydroxy-benzotriazole (653 mg, 4.72 mmol) in DMF (8 ml). After stirring this mixture for 1 h at 0 °C and 1 h at room temperature, a solution of **20c** (1.235 g, 2.25 mmol) and *N*-methylmorpholine (0.25 ml, 2.26 mmol) in DMF (8 ml) was added and the mixture was stirred overnight at room temperature. The solvent was removed *in vacuo* and AcOEt was added to the residue. After filtration from the urea, the filtrate was washed successively with 2 M HCl, saturated $NaHCO_3$ solution and saturated NaCl solution, dried (Na_2SO_4), filtered and evaporated. The remaining slightly yellow solid was dissolved in $CHCl_3$, treated with AcOEt and cooled to 0 °C. The formed precipitate was collected and dried to afford **20d** (1.34 g, 72%) as a colourless solid, MS (FAB): 831.3 ($[M^+]$).

(d) N^3-(L-Lysyl)-L-2,3-diaminopropionic acid tri(hydroacetate) ***(20e)****,* $C_{15}H_{32}N_4O_9$ (412.45)
Zn powder (5.38 g, 82.3 mmol) was added to a solution of **20d** (1.37 g, 1.647 mmol) in acetic acid (50 ml) and the mixture was stirred 1 h at room temperature. After evaporation of the solvent, the residue was treated with diethyl ether /5% aqueous $NaHCO_3$ solution. The formed precipitate was washed with diethyl ether and treated with 1M HCl/AcOEt. The organic layer was separated, washed with 1M HCl and saturated NaCl solution, and dried (Na_2SO_4). Evaporation of the solvent and drying *in vacuo* afforded N^3-[*N,N′*-bis-(benzyloxycarbonyl)]-L-lysyl-N^2-(benzyloxycarbonyl)-L-2,3-diaminopropionic acid (674 mg, 64%) as a white solid: TLC: R_f 0.67 (toluene

Table 1. Growth promotion of the catechol derivatives on Gram negative siderophore indicator strains. Diameter of growth zone (mm), substance application 5 μg

Compound	Pseud. aerug. PAO 6609	E. coli AB 2847	Salm.typh. enb 7	Salm. typh.2706	Morg. morg. SBK 3
3a	0	21	18	18	8
b	12	12	21	26	0
c	0	19	0	0	0
5a	17	15	25	30	18
b	10	11	31	12	8
c	0	0	0	0	0
d	0	0	18	n.t.	0
8	0	10	12	22	18
10a	10	18	31	12	0
b	10	22	27	20	0
c	0	0	17	n.t.	22
d	0	0	0	n.t.	0
e	0	0	17	n.t.	0
f	0	0	14	n.t.	0
12a	0	0	14	0	0
b	10	0	0	0	0
13a	0	0	10	0	0
b	10	0	25	0	0
c	0	0	16	13	13
15	0	0	0	0	0
17a	14	8	0	n.t.	12
b	14	33	36	40	40
c	20	30	48	50	n.t.
d	28	35	22	20	45
19	20	40	50	40	30
21	48	n.t.	50	50	n.t.
control	41[a]	26[b]	37[c]	30[b]	22[d]

[a]: ferrioxamin E, [b]: enterobactin, [c]: ferrioxamin, G [d]: 2,3-dihydroxybenzylidene-1,3,5-trimethylaniline (Reissbrodt *et al.* 1993).

: AcOEt = 1 : 1); MS (FAB): 635.3 ($[M+H]^+$). Hydrogenolysis of the latter (650 mg, 1.024 mmol) was performed in EtOH/AcOH (1 : 1, 20 ml) with 10% Pd-C (100 mg) for 6 h at ambient temperature and atmospheric hydrogen pressure. The reaction mixture was filtered over celite and the solvent evaporated. Dissolution of the residue in EtOH and evaporation provided **20e** (418 mg, 99%) as a white foam:, MS (ESI): 233.3 ($[M+H]^+$, free base).

(e)
*N^3-{N,N′-Bis-[2,3-di(benzyloxy)benzoyl)]-L-lysyl}-N^2-[2,3-di(benzyloxy)benzoyl]-L-2,3-diaminopropionic acid (**20f**)*, $C_{72}H_{68}N_4O_{12}$ (1181.3)
To a solution of **20e** (400 mg, 0.97 mmol) in H_2O (5 ml) and THF (5 ml) was added dropwise and alternately at 0 °C a solution of NaOH (178 mg, 4.46 mmol) in H_2O (5 ml), and a solution of 4 (1.23 g, 3.49 mmol) in THF (5 ml). The mixture was stirred at room temperature for 3 h, acidified at pH 2 with 2M HCl and extracted with AcOEt. The organic layer was washed with H_2O, dried (Na_2SO_4), filtered and evaporated. Column chromatography (AcOEt : toluene : AcOH = 10 : 10 : 2) of the crude afforded **20f** (797 mg, 70%) as a colourless solid, MS (FAB): 1181.3 ($[M]^+$).

*(f) N^3-[N, N′-Bis-(2,3-dihydroxybenzoyl)-L-lysyl]-N^2-(2,3-dihydroxybenzoyl)-L-2,3-diaminopropionic acid (**21**)*, $C_{30}H_{32}N_4O_{12}$ (640.6)

20f (200 mg, 0.169 mmol) was hydrogenolyzed in EtOH/AcOH (9/1 ml) over 10% Pd/C (40 mg) for 7 h as described for **17a-d**. After filtration from the cata-

Table 2. Growth promotion of the catechol derivatives on Gram negative bacteria under iron limitation. Diameter of growth zone (mm), substance application 5 μg

Compound	Ps. aerug. ATCC 27853	Ps. aerug. SG 137	Ps. aerug. NTCC 10662	Ps. aerug. ATCC 9027	Ps. aerug. K799/WT	E.coli ATCC 25922	CAS assay
3a	0	0	0	0	0	0	+
b	0	15	0	0	14	0	+
c	0	12	0	0	10	12	+
5a	17	20	17	10	17	17	+
b	0	12	0	0	0	0	+
c	0	13	0	0	0	0	+
d	0	16	0	0	0	0	+
8	0	0	12	0	0	0	+
10a	0	0	0	0	0	0	−
b	13	12	0	10	10	15	−
e	0	0	0	0	0	8	−
f	0	0	0	0	0	0	−
12a	0	12	0	0	0	11	−
13b	0	0	0	0	0	13	−
c	0	0	0	0	0	0	−
17a	17	16	15	14	20	25	+++
b	18	17	20	0	17	0	+++
c	22	23	25	24	25	27	+++
d	28	32	25	29	30	37	+++
19	20	31	20	24	25	33	+++
21	30	34	27	35	30	35	+++
control	35[a]	30[a]	30[a]	30[a]	35[a]	33[b]	

[a]: desferrioxamin B, [b]: ferricrocin (2 μg) − no CAS reaction, + weak CAS reaction, + + + strong CAS reaction.

lyst the crude product was dissolved in AcOEt/EtOH. Filtration and evaporation of the solvent yielded **21** (102 mg, 95%) as a colourless solid: HPLC: t_R 5.54 min (Eurospher 100-C_{18}, acetonitrile : H_2O = 35 : 65, 0.1% TFA); MS (FAB): 641.2 ($[M+H]^+$), ^{1}H-NMR (500 MHz, DMSO-d_6 with presaturation): 1.31 (m, 2H, CH_2), 1.48 (m, 2H, CH_2), 1.72 (m, 2H, CH_2), 3.19 (m, 2H, CH_2-N), 3.42–3.71 (m, 2H, CH_2-N), 4.42 (m, 1H, CH), 4.53 (m, 1H, CH), 6.65 (m, 3H, aromatic H), 6.89 (m, 3H, aromatic H), 7.23 (m, 2H, aromatic H), 7.37 (m, 1H, aromatic H), 8.30 (m, 1H, NHCO). 8.35 (t, J = 5.5, 1H, NHCO), 8.71 (m, 2H, NHCO).

Results and discussion

Synthesis of monocatecholate siderophor analogs

In analogy to *N*-(2,3-dihydroxybenzoyl)-L-serine, the monomer of enterobactin, we synthesized monocatecholate siderophore analogs of the *N*-(2,3-dihydroxbenzoyl)-type using different amino acids (glycine, *p*-hydroxy-D-phenylglycine, L-alanine, L-threonine) and dipeptides (glycylglycine, glycyl-L-leucine, glycyl-L-alanine). Furthermore, *N*-[2,3-di(acetoxy)benzoyl] amino acids as well as *N*-[3,4-di(methoxycarbonyloxy)benzoyl] and *N*-(3,4-dihydroxybenzoyl) derivatives of amino acids (glycine, *p*-hydroxy-D-phenylglycine, L-serine) and of glycylglycine have been prepared.

The *N*-(2,3-dihydroxybenzoyl) derivatives of glycine, glycylglycine and *p*-hydroxy-D-phenylglycine **3a-c** were synthesized from the corresponding amino acids and the dipeptide, respectively, and 2,3-di(methoxycarbonyloxy)benzoyl chloride **1** via the benzoxazindione derivatives **2** (Wittmann *et al*. 2000).

N-(2,3-Dihydroxybenzoyl)-L-serine **5a** was prepared by acylation of *O*-benzyl-L-serine with 2,3-di(benzyloxy)benzoyl chloride **4** followed by hydrogenolysis according to Rastetter *et al.* (1981).

Table 3. Growth promotion of the catechol derivatives on test strains and iron transport mutants of mycobacteria.
Diameter of growth zone (mm), substance application 5 μg

Compound	SG 987	M10	mc^2 155	M24	B1	B3	U3
3a	0	0	0	0	0	0	0
b	0	0	0	0	22	0	0
c	10	0	15	0	15	0	0
5a	9	0	0	0	0	0	0
b	0	0	0	0	10	0	0
c	16	0	0	0	15	0	0
d	0	0	0	0	0	0	0
8	8	0	0	0	0	0	0
10a	0	0	10	0	8	0	0
b	0	0	0	0	9	0	0
d	0	0	0	0	0	0	0
e	0	0	19	18	10	0	0
f	0	0	0	0	12	0	0
12a	7	0	9	0	0	0	0
b	0	0	0	0	0	0	0
13a	0	0	0	0	0	0	0
b	0	0	8	0	0	0	0
c	0	0	0	0	0	0	0
17b	0	0	0	0	0	0	0
c	19	15	22	0	20	0	0
d	19	23	29	24	25	27	27
19	19	19	29	20	24	25	25
21	19	0	0	0	20	0	0
mycobactin (2μg)	17	17	19	19	16	16	16

N-(2,3-Dihydroxybenzoyl)-L-alanine **5b** was obtained by the same way or by direct acylation of L-alanine with the hydroxysuccinimide ester of 2,3-dihydroxybenzoic acid. The latter method has also been used for the synthesis of *N*-(2,3-dihydroxybenzoyl)-glycyl-L-alanine (**5c**) and *N*-(2,3-dihydroxybenzoyl)-glycyl-L-leucine (**5d**), respectively. The syntheses are outlined in Figure 1.

N-(2,3-Dihydroxybenzoyl)-L-threonine **8** is known as a building block of vanoxonin, an inhibitor of thymidylate synthetase (F. Kanai *et al.* 1985). We prepared **8** via the *N*-(1-ethoxycarbonyl-propen-2-yl)-L-threonine-potassium salt **7a** and the corresponding *p*-bromophenacyl ester **7b** in analogy to a reported protecting group methodology (Balog *et al.* 1971). Acylation of **7b** with 2,3-di(benzyloxy)benzoyl chloride and subsequent cleavage of the bromophenacyl ester **7c** led to the benzylether protected catechol derivative **7d** which recently has been synthesized in a different route by threonine acylation with the hydroxysuccinimide ester **18** (Nakonieczna *et al.* 1989). Hydrogenolysis of **7d** afforded the desired threonine derivative **8** (Figure 2). *N*-[2,3-Di(acetoxy)benzoyl]-derivatives of L-alanine, L-serine, *O*-acetyl- and *O*-benzyl-L-serine, respectively, **10 a-d** were synthesized from the corresponding amino acid benzyl esters and 2,3-di(acetoxy)benzoyl chloride **9** followed by conventional hydogenolysis (Heinisch *et al.* 1996). In the case of the *O*-benzyl-serine derivative **10c**, selective benzylester cleavage in the presence of the *O*-benzylether moiety was achieved by catalytic transfer hydrogenation following a previously described procedure (Bajwa 1992). The *N*-[2,3-di(acetoxy)benzoyl]-derivatives of β-alanine **10e** and D(−)-phenylglycine **10f** were obtained by direct acylation of the amino acids with **9** (Figure 3).

We synthesized the *N*-(3,4-dihydroxybenzoyl)-derivatives **13a-c** of glycine, glycylglycine and *p*-

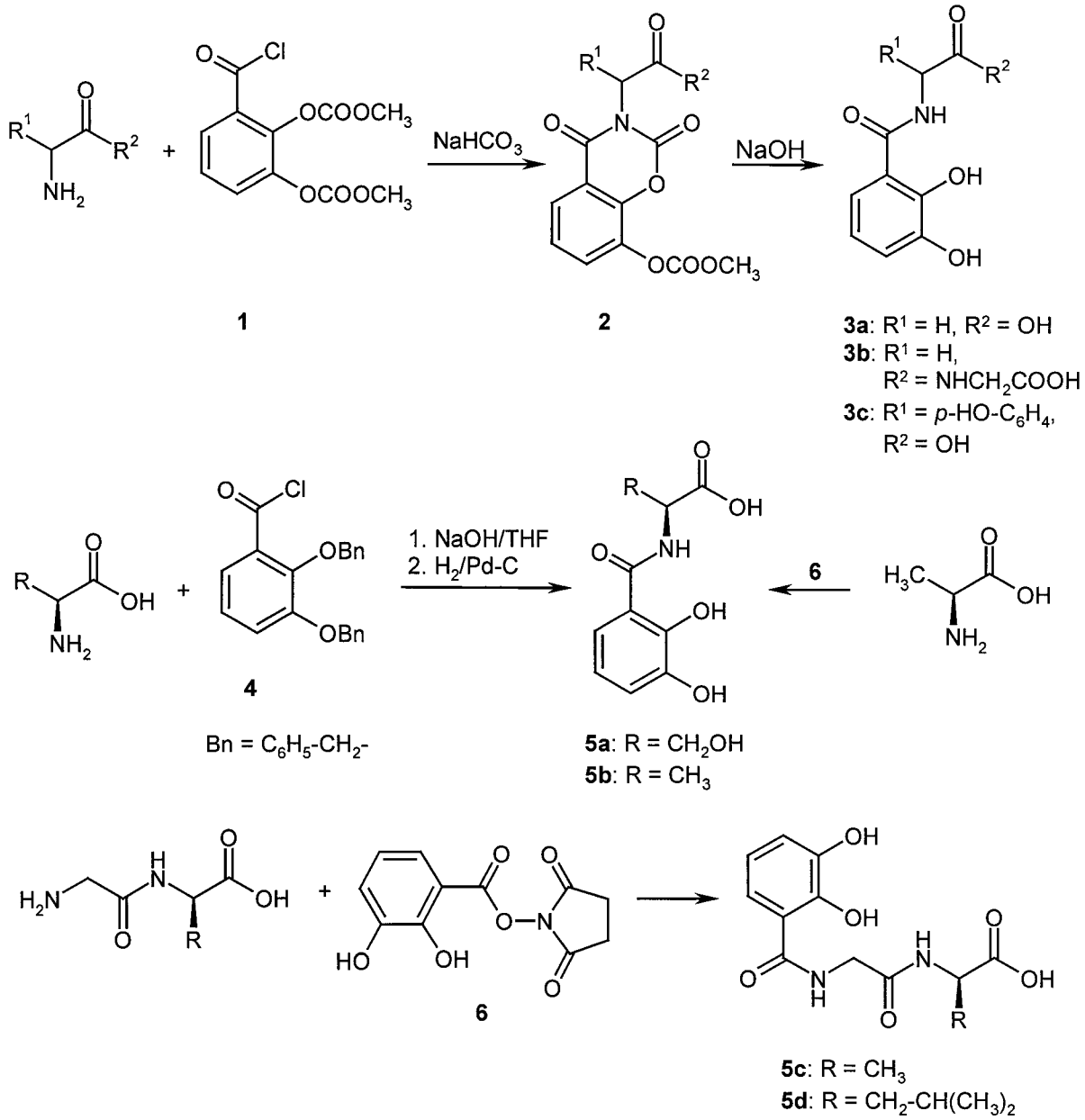

Figure 1. Synthesis of monocatecholate siderophore analogs based on various amino acids and dipeptides, respectively.

hydroxy-D(−)-phenylglycine by reaction of these amino acids with 3,4-di(methoxycarbonyloxy)benzoyl chloride (Heinisch *et al.* 1992) and saponification of the acylated derivatives **12a** and **b**. The 3,4-diacyloxy derivative of *p*-hydroxy-D(−)-phenylglycine was not isolated. *N*-(3,4-Dihydroxybenzoyl)-L-serine **15** was prepared by acylation of *O*-benzyl-L-serine with 3,4 dibenzyloxybenzoyl chloride **14** followed by hydrogenolysis of the tribenzylated intermediate (Figure 4).

Preparation of biscatecholate siderophore analogs

N,N′-Bis-(2,3-dihydroxybenzoyl)-L-lysine (**17d**) acts as a siderophore of *Azotobacter vinelandii* (Corbin & Bulen 1969). Similar to enterobactin, there is a seven atom spacing between the two dihydroxybenzoyl moi-

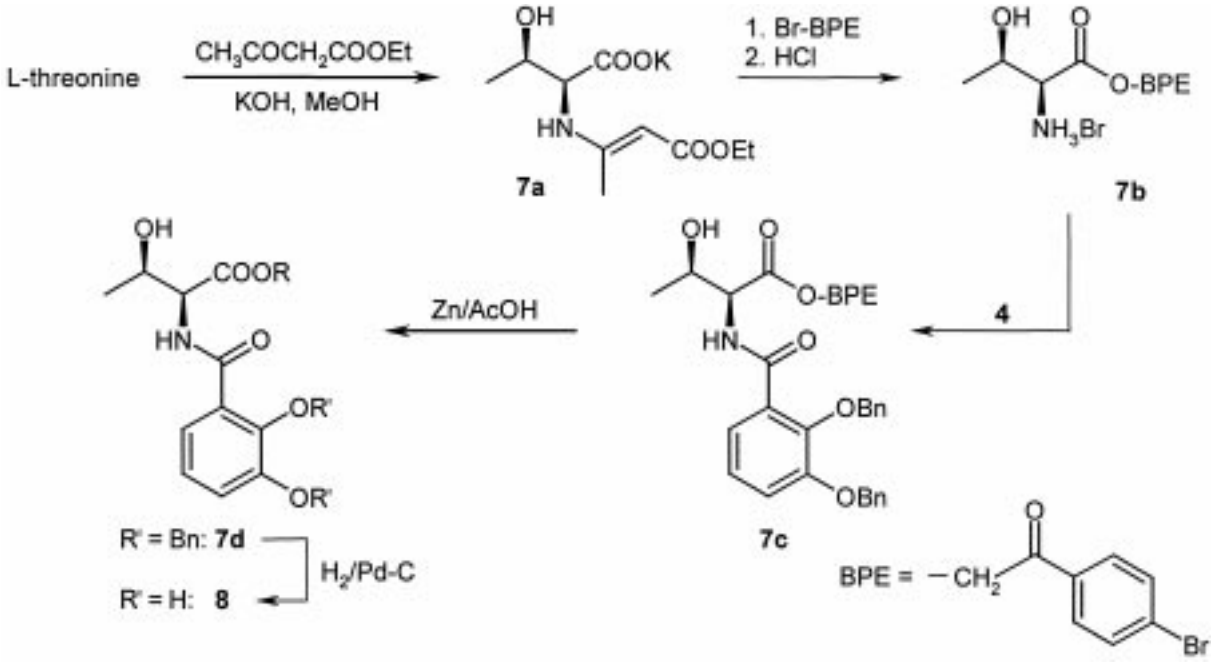

Figure 2. Synthesis of N-(2,3-dihydroxybenzoyl)-L-threonine.

eties allowing the bidentate ligands to coordinate to a central iron atom in an optimum way. In the course of our studies concerning structure activity correlations, we investigated the influence of the atom spacing between the catechol moieties on the siderophore activity.

For this reason we prepared the *N*,*N*′-bis-(2,3-dihydroxybenzoyl)-derivatives of L-diaminopropionic acid (**17a**), L-diaminobutyric acid (**17b**), L-ornithine (**17c**), and L-lysine (**17d**). The biscatecholates were obtained by acylation of the corresponding diamino acids with 2,3-di-(dibenzyloxy)-benzoyl chloride and subsequent hydrogenolysis. To study the stereoisomeric influence of the amino acid scaffold on the siderophore activity, we have also prepared the *N*,*N*′-bis-(2,3-dihydroxybenzoyl)-D-ornithine **19** by acylation of D-ornithine with the active ester **18** followed by hydrogenolysis.

Synthesis of a triscatecholate siderophore analog

It was our intention to synthesize a triscatecholate siderophore containing the same atom spacing between the dihydroxybenzoyl moieties as it can be found in enterobactin.

In addition to known amino acid-based enterobactin analogs (Rastetter *et al.* 1981; Akiyama & Ikeda 1995) our triscatecholate should possess a free carboxyl group to conjugate it with other bioactive agents. Therefore, we decided to use a L-lysyl-L-diaminopropionic acid dipeptide as backbone for our triscatecholate. Z-N^2-L-2,3-diaminopropionic acid (**20a**), which can easily be prepared from Z-*N*-asparagine by a Hofmann-type rearrangement, seemed to be a suitable starting material for this dipeptide. In a first reaction sequence the N^3-amino group of **20a** was temporarily protected by a Boc group and the free carboxyl group was esterified with *p*-bromophenacyl bromide affording the completely protected diamino acid **20b**. After selective removal of the Boc group, the obtained diaminopropionic acid derivative **20c** was coupled with a *N*,*N*′-bis (Z)-protected lysine using the *N*,*N*′-dicyclohexylcarbodiimide/1-hydroxybenzotriazole coupling methodology. The coupling product **20d** was completely deprotected resulting in the desired L-lysyl-L-diaminopropionic acid dipeptide **20e**. Acylation of **20e** with 2,3-(dibenzyloxy)benzoyl chloride and subsequent hydrogenolysis led to the new triscatecholate derivative **21**. Several of the acylated catechol derivatives described here and also some benzoxazine-2,4-diones derived from these catechols we conjugated with β-lactames to produce highly active antibacterial compounds (Heinisch *et al.* 1996; Wittmann *et al.* 2000).

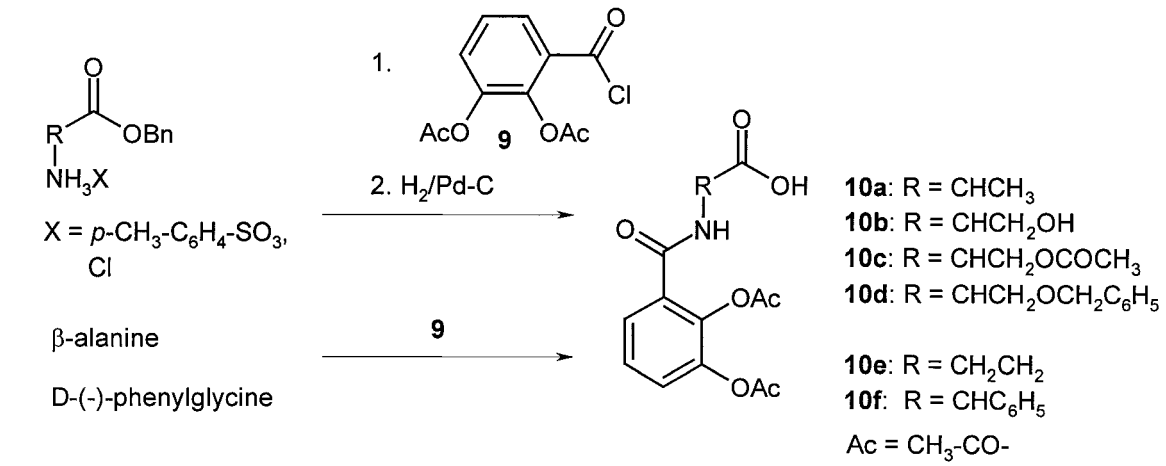

Figure 3. Preparation of N-[2,3-(diacetoxy)-benzoyl]-amino acids.

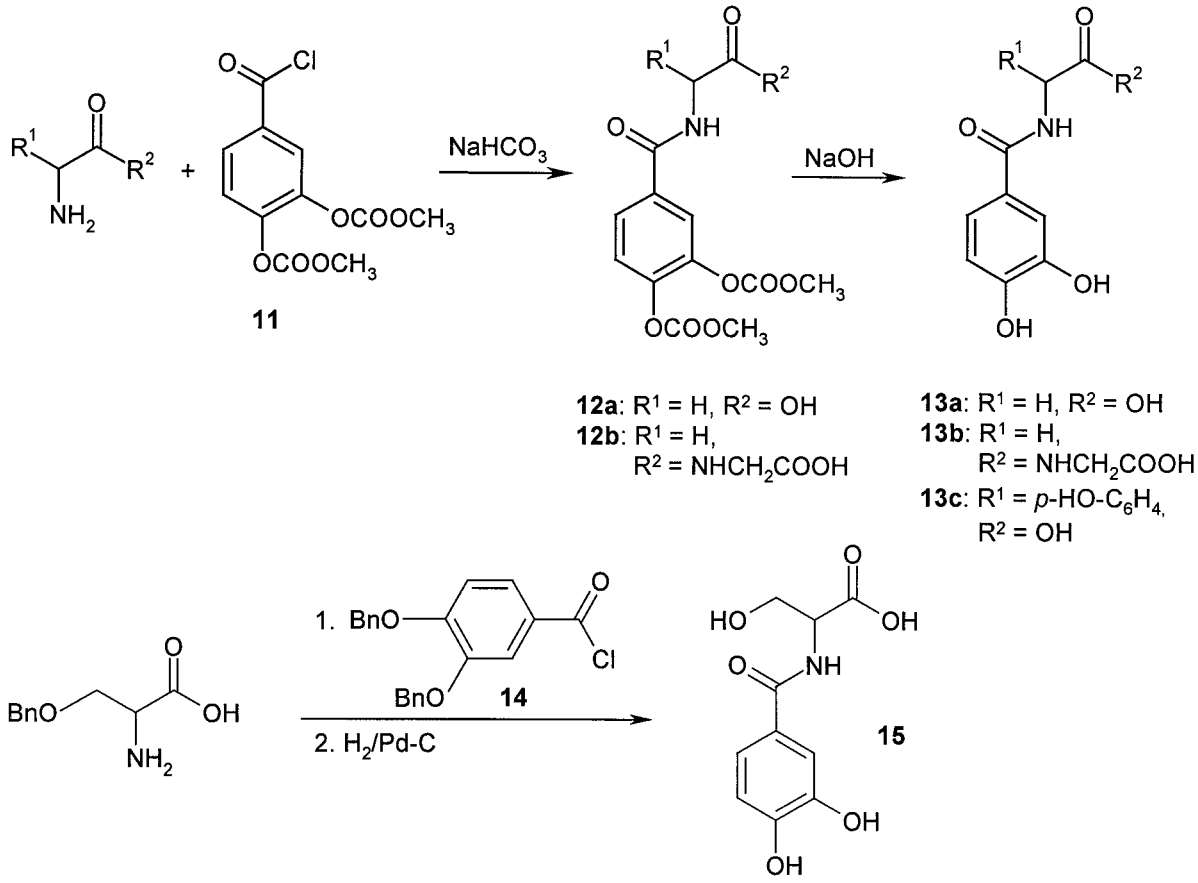

Figure 4. Synthesis of *N*-(3,4-dihydroxybenzoyl)-amino acids.

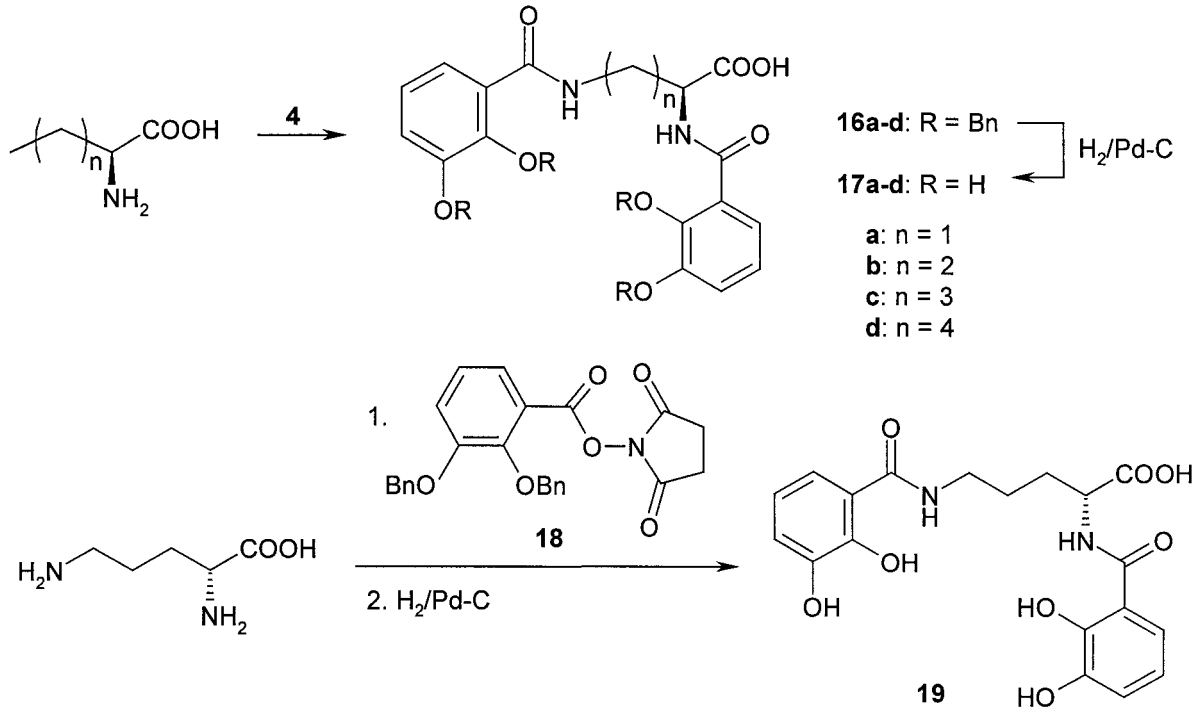

Figure 5. Synthesis of biscatecholate siderophore analogs.

Siderophore activity

The synthesized compounds **3a-c, 5a-d, 8, 10a-f, 12a-b, 13a-c, 15, 17a-d, 19** and **21** were tested for their siderophore activities by growth promotion tests (Reissbrodt *et al.* 1993) with various wild type bacteria and mutants that are well defined in their ability to transport and utilize natural siderophores (siderophore indicator strains). The following indicator strains were used: *Pseudomonas aeruginosa* PAO 6609 (pyoverdin-), *E. coli* AB 2847 (aroB-), *Salmonella typhimurium* enb7 (enterobactin-), *S. typhimurium* 2706 (enterobactin-) and *Morganella morganii* SBK3 (wild type). Furthermore, all compounds were tested with the *E. coli* strain IR112 (tonB-). None of the synthesized derivatives could be utilized by the latter strain indicating an energy-coupled tonB-dependent active transport process. The results of the growth promotion assays are given in Table 1. The monocatecholate derivatives **3–8** more or less stimulated growth of several strains, most efficiently *S. typhimurium*, whereas stimulation of the *Pseudomonas* strain was rather weak. Growth promotion by the the *N*-[2,3-di(acetoxy)benzoyl]-derivatives **10a-f** was most effective with **10a** and **b** as well as on *E. coli* and *S. typimurium*. The siderophore activity of the *N*-[2,3-di(acetoxy)benzoyl-L-serine derivatives **10c-f** was diminished by substitution of the free OH-group of serine. Compared with the *N*-(2,3-dihydroxybenzoyl)-derivatives, the corresponding *N*-(3,4 dihydroxybenzoyl)-derivatives **12a, 12b, 13a-c** and **15** exhibited a lower growth promoting activity except by **13 a-c** for *S. typhimurium* enb7. The biscatecholates **17a-d**, and **19** as well as the triscatecholate **21** were highly active siderophores. Among the biscatecholates **17a-d**, the diaminopropionic acid derivative **17a** having a three atom spacing between the 2,3-dihydroxybenzoyl moieties exhibited the lowest siderophore activity for all tested strains. Obviously the formation of an optimum binding cavity for the ferric ion was more difficult by the very short atom spacing between the catecholates in the diaminopropionic acid derivatives. For *P. aeruginosa* PAO 6609 the siderophore activity increased continuously in the order diaminopropionic acid > diaminobutyric acid > ornithine > lysine biscatecholate. Enterobacterial strains like *S. typhimurium* enb-7 and 2706 showed best stimulation results with the ornithine derivative. The two enantiomeric biscatecholates of L- and D-ornithine (**17c** and **19**, respectively) demonstrated similar activities. As expected most effective growth promotion was obtained by the triscatecholate **21**. For

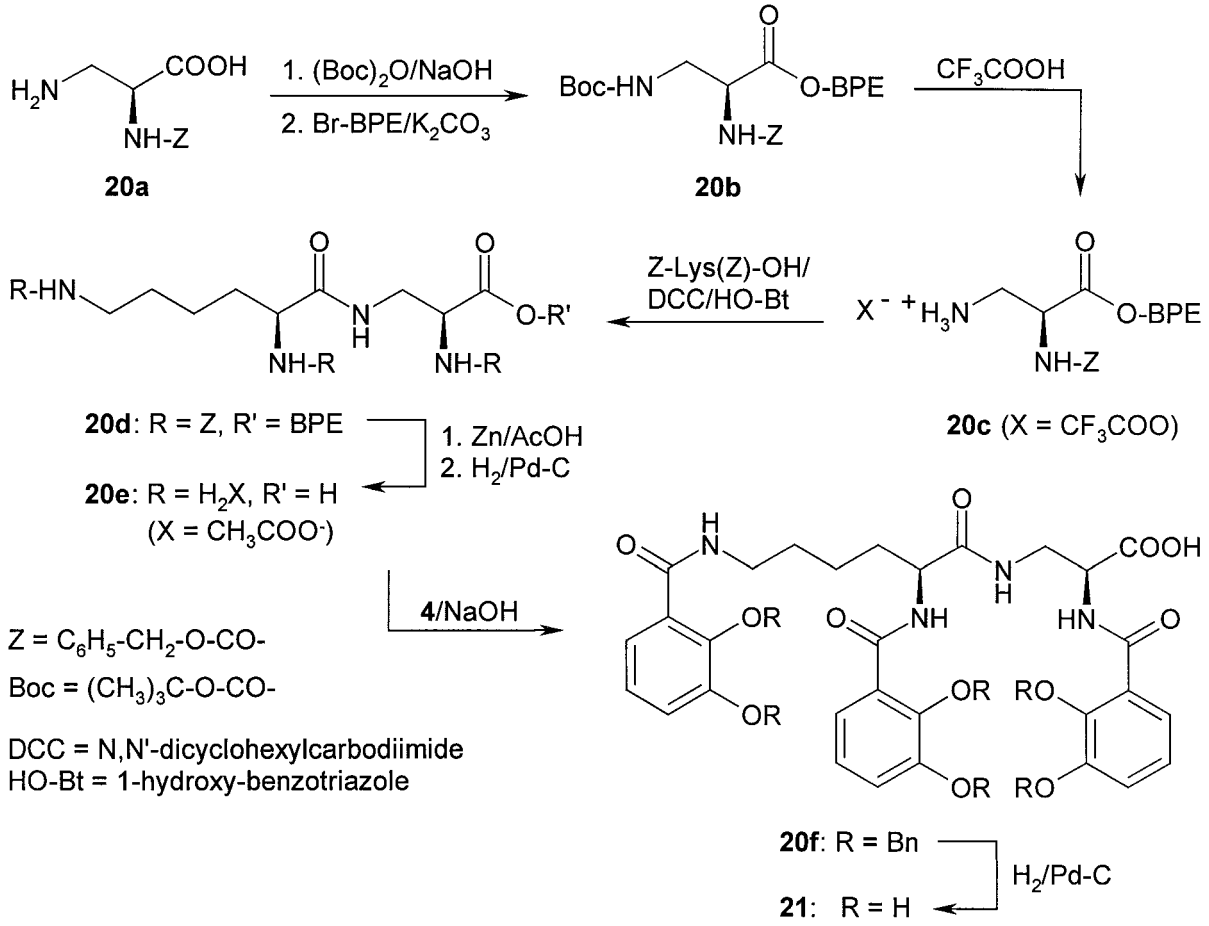

Figure 6. Synthesis of an amino acid-based triscatecholate siderophore analog.

P. aeruginosa PAO 6609 and *S. typhimurium enb-7*, growth promotion by **21** was more efficient than by the control siderophores.

Synthetic siderophore mimetics have to compete *'in vivo'* with the bacterial siderophores for the rare iron and for the siderophore receptors to be transported into the cells. To be efficient there is a prerequisite for a siderophore structure to serve as antibiotic vector. To prove this, we used in a second series of experiments the following Gram-negative wild type strains grown under iron limitation: *P. aeruginosa* ATCC 27853, SG 137, NTCC 10662, ATCC 9027, K799/WT and *E. coli* ATCC 25922 (Table 2). Compounds **10c, 10d, 12b** and **13a** were not tested in these experiments. Results of these tests are given in Table 2. From the monocatechol derivatives only **5a** (monomer of enterobactin) was active in all strains. All other compounds were active in *P. aeruginosa* SG 137 preferrentially. It could be speculated, that these compounds are not able to compete with pyoverdin, the most active siderophore of the pseudomonads and that strain SG 137 is depressed in pyoverdin biosynthesis. There were first hints by fluorescence data of the different strains. The biscatechols **17a-d** and **19** and especially the triscatechol **21** were highly active also in all wild type strains.

Moreover, most of the new compounds we tested also for growth promotion of mycobacteria, such as the wild type strain *M. smegmatis* SG 987 and mc^2 155, mutants *M. smegmatis* M10 (exochelin-) and M24 (mycobactin-), and 3 mutants of *M. smegmatis* mc^2155 generated by gene replacement: B1 (blocked in exochelin biosynthesis), B3 (blocked in mycobactin and exochelin biosynthesis) and U3 (blocked in mycobactin biosynthesis and exochelin uptake) (Schumann et al, 1998). The results are given in Table 3. The biscatechols **17b-d, 19** and the triscatechol **21** were

active, especially **17c, d** and **19** for the mutants B1, B3 and U3 and compound **21** for the mutant B1.

In parallel to the growth promotion assays the relative iron complexing capacity of the siderophore derivatives was checked by the chromazurol-S (CAS) assay according to Schwyn & Neilands (1987). A positive CAS reaction is associated with iron chelation (Table 2). There is striking evidence for a correlation between iron complexing capacity of the compounds and their respective growth promoting activity especially for the monochatechols **3a-c, 5a-d, 8**, with weak CAS and growth promoting activity and the biscatechols **17b-d, 19** and the triscatechol **21** with both strong CAS and strong growth promoting activity.

The negative CAS reaction of compounds **10a-13c** corresponds with the low or missing siderophore activity. Compounds **10a-f** are CAS negative due to acylated catechol groups. Compounds **12a-13c** with 3,4 catechol substitutents seems to be different in iron complexation compared to 2,3 catechol substituted compounds.

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