BioMetals 1998, **11**, 243–251

Novel catecholate-type siderophore analogs based on a *myo*-inositol scaffold

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Received 5 March 1998, accepted for publication 20 March 1998

A novel 1,3,5-triamino-*myo*-inositol derivative is presented as a readily available scaffold for the design of tripodal siderophore mimetics. Based on this scaffold, various hexadentate catecholate-type siderophore analogs were synthesized by attaching the catechols to the inositol scaffold via spacer units of different structure and length. The potential to tune the polarity of the inositol containing siderophore analogs has also been demonstrated by varying the protection group strategy. The siderophore activity of the prepared siderophore analogs was examined by cross-feeding tests with various Gram-negative bacteria and mycobacteria.

Keywords: Gram-negative bacteria, inositol, mycobacteria, siderophore analog, synthesis, tricatecholate

Introduction

Microorganisms produce powerful iron (III)-chelating agents called siderophores to acquire iron from the environment. In recent years bacterial siderophores and their synthetic analogs have attracted growing interest as clinically useful iron chelators for the treatment of iron overload (hemosiderosis, Cooley anemia) Dionis *et al.* 1991, Singh 1994) or for the design of iron transport-mediated drugs (Miller & Malouin 1993, Ghosh *et al.* 1996). Furthermore, structurally diverse analogs of natural siderophores are useful tools for the study of the complex recognition phenomena of siderophore-mediated microbial iron (III) uptake (Raymond 1994, Shanzer *et al.* 1996).

Most of the potent natural siderophores possess catechol or hydroxamate groups acting as iron (III) binding sites. The chelating groups are attached to different linear or cyclic scaffolds in such a way as to generate an octahedral binding cavity for the ferric ion. These scaffolds bearing the ligands can be cyclic hexapeptides as in the most ferrichromes, spermidine or nor-spermidine backbones (agrobactin, vibriobactin) or a macrocyclic trilacton composed of L-serine residues as in the case of enterobactin. The latter siderophore exhibits the largest binding constant for the ferric ion among all natural substances. Therefore, various analogs of enterobactin have been synthesized in which the trilacton backbone is substituted by other readily accessible scaffolds e.g. by a *cis*-1,5,9-triaminocyclododecane (Corey & Hurt 1977), a 1,3,5-tris (aminomethyl)benzene (Weitl & Raymond 1979) or various lysine derivatives (Chimiak & Neilands 1984, Akiyama & Ikeda 1995).

Recently, the synthesis of a chiral enterobactin analog based on a *scyllo*-inositol scaffold was reported (Tse & Kishi 1993). This *scyllo*-inositol scaffold was prepared in several steps from *myo*-inositol, which is a commercially available compound. *myo*-Inositol can be readily transformed into an orthoformate in which three hydroxyl groups at the 1, 3 and 5 position are protected temporarily and the remaining three hydroxyl groups are accessible for further modification. From this point of view *myo*-inositol seems to provide unique opportunities for molecular architecture.

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Here we report a simple and efficient procedure for the preparation of a *myo*-inositol based scaffold and its use for the synthesis of structurally varying triscatecholate siderophor analogs. Furthermore, preliminary results on the biological activity of these new siderophore analogs will be presented.

Materials and methods

Synthesis of the siderophore analogs

^{1}H- and ^{13}C-NMR spectra were recorded on a 300 and 500 MHz Bruker spectrometer, resp. The chemical shifts δ are given in ppm related to tetramethyl silane as internal standard. The coupling constants J are reported in Hz. High resolution mass spectra were obtained by using fast-atom bombardment (FAB) or electron-spray ionization (ESI) techniques. The KBr technique was used for performing IR spectroscopy.

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) applying UV detection.

Solvents and reagents used were dried and purified by standard methods (Perrin & Armarego 1988). *myo*-inositol 1,3,5-orthoformate **1** and its 2,4,6-tribenzyl derivative **1** were synthesized according to the literature (Lee & Kishi 1985), using DMF as solvent for the transesterification (Baudin *et al.* 1988, Billington *et al.* 1989). Partially inactivated Raney Ni was freshly prepared as described (Becker *et al.* 1990) The succinimide active esters **6** and **7** were obtained following the procedures of Bergeron *et al.* (1983) and Chimiak & Neilands (1984) respectively.

N-*[2,3-Di(benzyloxy)benzoyl]-β-alanine (8),*
C_{24} H_{23} NO_5 (405.4)

A solution of β-alanine (0.21 g, 2.36 mmol) in water (2 ml) and triethylamine (0.67 ml, 4.8 mmol) was treated with a solution of **7** (1.02 g, 2.36 mmol, Chimiak & Neilands 1984) in acetone (10 ml). After stirring at room temperature for 16 h acetone was removed by distillation. Ethyl acetate and 1 M HCl were added to the residue (pH 2 of the aqueous phase). The organic layer was separated, extracted several times with 1 M HCl and with water and dried (Na_2SO_4). Evaporation of the solvent and recrystallization of the crude from aqueous ethanol afforded **8** (0.77 g, 81%) as a white crystalline solid: melting point (m.p.) 100–101°C; TLC: R_f 0.28 ($CHCl_3$:AcOEt:AcOH = 3:1:0.1); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.50 (t, J = 6.3, 2H, —CH_2), 3.52 (m, 2H, J = 6.2, —CH_2—), 5.09 (s, 2H, benzylic H), 5.14 (s, 2 H, benzylic H), 7.14 (m, 2 H, aromatic H), 7.31–7.46 (m, 10H, aromatic H), 7.70 (m, 1H, aromatic H), 8.28 (t, J = 5.8, 1H, -NH); ^{13}C-NMR (75 MHz, $CDCl_3$): 33.9, 35.0, 71.3, 76.4, 117.3, 123.3, 124.4, 127.0, 127.6, 128.2, 128.6, 128.7, 128.7, 136.3, 136.4, 146.9, 151.7, 165.6, 176.3; MS (FAB): 406.1 ([[M+1]$^+$).

N-*(2,3-Dihydroxybenzoyl)-β-alanine (**9**),*
C_{10} H_{11} NO_5 (225.2)

A solution of **8** (0.2 g, 0.49 mmol) in ethanol (10 ml) was hydrogenolyzed over 10% Pd–C (20 mg) at room temperature and atmospheric hydrogen pressure for 3 h. The reaction mixture was filtered over celite and evaporated at reduced pressure. The residue was dissolved in ethyl acetate, washed with saturated NaCl solution, dried (Na_2SO_4) and evaporated to provide **9** (0.1 g, 91%) as a white solid: ^{1}H-NMR (500 MHz, DMSO-d_6): 2.52 (t, J = 7.0, 2H, —CH_2), 3.47 (m, J = 6.9, 2H, —CH_2), 6.66 ('t', J = 8.0, 1H, aromatic H), 6.89 (dd, 1H, aromatic H), 7.25 (dd, J = 8.0, 1H, aromatic H), 8.79 (t, J = 5.4, 1H, exchange with D_2O, —NH), 9.13 (s, broad, 1H, exchange with D_2O, —OH), 12.31 (s, broad, 1H, exchange with D_2O, —COOH), 12.57 (s, broad, 1H, exchange with D_2O, —OH); ^{13}C-NMR (125 MHz, DMSO-d_6): 33.5, 35.3, 115.0, 117.3, 117.9, 118.8, 146.2, 149.4, 169.6, 172.7; MS (FAB): 226.0 ([M+1]$^+$).

*3-(2,3-Dihydroxyphenyl)propionic acid (**10**),*
$C_9H_{10}O_4$ (182.2)

(a) Demethylation: A solution of *trans*-2,3-dimethoxycinnamic acid (20 mmol, 4.13 g) in dichloromethane (80 ml) was treated at –78°C under nitrogen and constant stirring with 70 ml of a 1 M solution of BBr_3 in dichloromethane. After stirring 5 h at room temperature, the reaction mixture was poured into ice water and stirred for 0.5 h. The white precipitate was collected by filtration and dried under vacuum. Recrystallization of the crude product from water yielded 2.2 g (61%) of *trans*-2,3-dihydroxycinnamic acid.

(b) Hydrogenation: A solution of *trans*-2,3-dihydroxycinnamic (1.56 g, 8. 6 mmol) in ethanol was stirred over Pd–C (10% Pd) at atmospheric hydrogen pressure and room temperature for 3 h. The reaction mixture was filtered over celite and the solvent was evaporated. The crude product was purified by dissolution in diethylether and reprecipitation by careful addition of petrolether. After drying, **10** (1,2 g, 76%) was obtained as a white solid: m.p. 115–117°C (Yarrow *et al.* 1972: 114–115°C); ^{1}H-NMR (300 MHz, DMSO-d_6): 2.52 (t, 2H, —CH_2), 2.73 (t, 2H, —CH_2), 6.54–6.65 (m, 3H, aromatic H), 8.19 (s, 1H, OH), 9.21 (s, 1H, —OH), 12.05 (s, 1H, broad, —COOH); MS (FAB): 183.1 ([M+1]$^+$); EA calcualted for $C_9H_{10}O_4$ (182.17): C 59.33, H 5.53; found: C 58.97, H 5.71.

*3-[2,3-Di(benzyloxy)phenyl]propionic acid **(11)**,*
$C_{23}H_{22}O_4$ (362.4)

A mixture of **10** (1.19 g, 6.5 mmol), benzylbromide (5.57 g, 32.6 mmol), K_2CO_3 (7.0 g, 196,0 mmol) and acetone (25 ml) was refluxed for 16 h. After filtration the liquid components were removed by distillation under reduced pressure. The residue was dissolved in $CHCl_3$ and subsequently washed with saturated $NaHCO_3$ solution and

water. The organic layer was dried (Na_2SO_4) and evaporated to provide 3-[2,3-di(benzyloxy)phenyl]propionic acid benzylester (2.6 g) as an oily liquid. The latter was dissolved in a mixture of dioxane (15 ml) and 2 M aqueous NaOH (11 ml). After stirring at reflux for 1 h the mixture was acidified with 2 M HCl to pH 2 and extracted several times with $CHCl_3$. The organic layer was washed with saturated NaCl solution, dried (Na_2SO_4), and evaporated. The crude was dissolved in boiling ethanol and reprecipitated by addition of water to yield **11** (1.47 g, 62%) as a white solid: m.p. 85–87°C; ^{1}H-NMR (300 MHz, $CDCl_3$): 2.61 (m, 2H, —CH_2), 2.93 (m, 2H, —CH_2), 5.07 (s, 2H, benzylic H), 5.14 07 (s, 2H, benzylic H), 6.82 (dd, 1H, J = 1.8, 7.4, aromatic H), 6.91 (dd, 1H, J = 1.8, 8.1, aromatic H), 6.99 ('t', 1H, J = 7.5, 8.1, aromatic H), 7.26–7.45 (m, 10H, aromatic H); ^{13}C-NMR (75 MHz, $CDCl_3$): 25.5, 34.4, 70.9, 74.7, 112.8, 122.2, 124.0, 127.5, 127.6, 127.8, 127.9, 127.9, 128.3, 128.4, 128.5, 134.6, 137.0, 137.7, 138.3, 146.6, 152.0, 178.4, MS (FAB): 362 ([M^+]).

*1,3,5-Tri(2-cyanoethyl)-2,4,6-tribenzyl-myo-inositol (**2**), $C_{36}H_{39}N_3O_6$ (609.7)*

2,4,6-Tribenzyl-*myo*-inositol (Billington *et al.* 1989, **1**, 0.7 g, 1.55 mmol) was dissolved in acrylnitrile (2 ml) at room temperature. The solution was cooled to 5°C, and 0.5 ml of a freshly prepared aqueous solution of KOH (40%) were added with stirring under N_2. After 5 min at 5°C the solution was warmed up to 40–45°C and stirred for 1 h at this temperature. The reaction mixture was treated with ice water (5 ml), carefully acidified with 2 M HCl and extracted three times with $CHCl_3$ (each 10 ml). The organic layer was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. Recrystallization of the yellow oily residue yielded **2** (0.74 g, 78%) as colorless crystals: m.p. 114–116°C; TLC: R_f 0.21 (hexane:AcOEt = 1:2); ^{1}H-NMR (300 MHz, $CDCl_3$): 2.36 ('t', 2H, J = 6.4, —CH_2—N), 2.44 ('t', J = 6.2, 2 —CH_2—N), 3.20 (m, 3H, J = 2.4, 9.5, inositol-CH), 3.65 (m, J = 6.4, 2H, 2 —CH_2—O), 3.74 (m, 2H, —CH_2—O), 3.87–3.97 (m, J = 6.4, 9.5, 4H, —CH_2-O, inositol—CH), 4.05 (t, J = 2.3, 1H, inositol—CH), 4.79–4.88 (m, 6H, benzylic H), 7.29–7.46 (m, 15 H, aromatic H); ^{13}C-NMR ($CDCl_3$, 75 MHz):18.8, 19.1, 19.2, 65.9, 67.9, 74.6, 74.7, 75.8, 81.1, 81.7, 83.9, 117.8, 117.9, 127.7, 127.8, 127.8, 128.2, 128.3, 128.5, 138.5, 138.6; IR: 3030, 2880, 2245, 1493, 1449, 1397, 1351, 1214, 1127, 1097, 1064, 750; MS (ESI): 632.2 ([M+Na]$^+$); EA calcualted for $C_{36}H_{39}N_3O_6$ (609.70): C 70.91, H 6.45, N 6.89, found: C 70.94, H 6.41, N 7.07.

*1,3,5-Tri(3-aminopropyl)-2,4,6-tribenzyl-myo-inositol (**3**), $C_{36}H_{51}N_3O_6$ (621.8)*

A mixture of **2** (0.3 g, 0.49 mmol), 6.6% ethanolic NaOH (15 ml), and partially inactivated Raney-Ni (300 mg) was stirred in an autoclave at room temperature. under a hydrogen pressure of 10 bar for 16 h. The reaction mixture was filtered over celite and the filtrate was evaporated nearly to dryness. Water was added to the residue until the solution became cloudy. This solution was extracted several times with $CHCl_3$. The organic layer was dried (Na_2SO_4) and evaporated. After drying at 0.05 mbar, **3** (289 mg, 94%) was obtained as a colourless viscous oil: ^{1}H-NMR (500 MHz, $CDCl_3$): 1.21 (s, broad, 6 H, —NH_2), 1.68 (m, J = 6.4, 6.9, 6H, —CH_2—), 2.70 ('t', J = 6.9, 2H, —CH_2—N), 2.77 ('t', J = 6.9, 4H, —CH_2—N), 3.16 (m, J = 2.2, 9.3, 9.8, 3H, inositol—CH), 3.61 (m, J = 6.2, 4H, —CH_2—O), 3.85 (m, J = 6.3, 9.5, 4H, —CH_2—O, inositol—CH), 4.07 ('t', J = 2.2, 1H, inositol—CH), 4.78 (m, 2 H, benzylic H), 4.86 (m, 4H, benzylic H), 7.32–7.44 (m, 15H, aromatic H); ^{13}C-NMR (125 MHz, $CDCl_3$): 33.8, 34.4, 39.3, 39.5, 68.6, 71.6, 74.0, 74.1, 75.4, 81.3, 81.3, 83.4, 127.1, 127.2, 127.4, 127.5, 127.9, 128.1, 128.4, 138.9; IR (KBr): 3435, 2930, 1582, 1469, 1355, 1328, 1305, 1208, 1090, 815, 739; MS (ESI): 622.5 ([M+H]$^+$), 644.5 ([M+Na]$^+$).

*1,3,5-Tris[N-(2,3-dihydroxybenzoyl)-aminopropyl]-2,4,6-tribenzyl-myo-inositol (**12**), $C_{57}H_{63}N_3O_{15}$ (1030.1)*

A solution of **6** (125 mg, 0.56 mmol) in DMF (5 ml) and triethylamine (56 mg, 0.56 mmol) was added to a solution of **3** (115 mg, 0.18) in DMF (5 ml). The mixture was stirred at room temperature for 12 h and the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (20 ml), washed subsequently with water and 1 M HCl and dried (Na_2SO_4). Evaporation of the solvent and column chromatography (short column, AcOEt:EtOH:H_2O = 5:1:0.4) of the crude gave **12** (115 mg, 60%) as a slightly yellow solid: TLC: R_f 0.77 (AcOEt:EtOH:H_2O = 5:1:0.4); ^{1}H-NMR (300 MHz, DMSO-d_6): 1.82 (m, J = 6.5, 6.8, 6H, —CH_2—), 3.16–3.74 (m, 17H, —CH_2—N, —CH_2—O, inositol—CH), 4.17 ('s', 1H, inositol—CH), 4.68–4.78 (m, 6H, benzylic H), 6.64 (dd, J = 7.5, 7.9, 3H, aromatic H), 6.89 (m, J = 1.4, 7.8, 3H, aromatic H), 7.20–7.38 (m, 18H, aromatic H), 8.74 (m, 3H, —NH—CO); ^{13}C-NMR (75 MHz, DMSO-d_6): 18.5, 29.7, 30.0, 36.5, 56.0, 67.7, 70.9, 73.7, 74.4, 74.5, 80.2, 81.1, 82.8, 114.8, 114.9, 117.0, 117.1, 117.7, 118.6, 127.2, 127.3, 127.7, 128.0, 128.0, 138.9, 139.3, 146.2, 149.8, 169.7; MS (FAB): 1031.0 ([M+1]$^+$).

*1,3,5-Tris{N-[2,3-di(benzyloxy)benzoyl]-aminopropyl}-2,4,6-tribenzyl-myo-inositol (**13**), $C_{99}H_{99}N_3O_{15}$ (1570.9)*

Triethylamine (69 mg, 0.68 mmol) was added to a solution of **3** (141 mg, 0.23 mmol) and **7** (294 mg, 0.68 mmol) in DMF (7 ml). The mixture was stirred at room temperature for 16 h. After evaporation of the solvent the residue was dissolved in $CHCl_3$ and washed subsequently with 1 M HCl, saturated $NaHCO_3$ solution and water. The organic phase was dried (Na_2SO_4) and evaporated. Purification of the crude by column chromatography (AcOEt:hexane = 3 :1) gave **13** (246 mg, 69%) as a white foam: TLC: R_f 0.24 (AcOEt:hexane = 3 :1); ^{1}H-NMR (500 MHz, $CDCl_3$): 1.63 (m, J = 6.5, 6H, -CH_2—), 3.01 (dd, J = 2.2, 9.8, 2H, inositol-CH), 3.08 (dd, J = 9.2, 1H, inositol—CH), 3.28 (m, J = 6.6, 4H —CH_2—N), 3.38 (m, J = 6.5, 2H, —CH_2—N), 3.43 (m, J = 6.6, 4H,

—CH_2—O), 3.70 ('t', J = 6.4, 2H, —CH_2—O), 3.78 (t, J = 9.5, 2H, inositol—CH), 3.94 (t, J = 2.2, 1H, inositol—CH), 4.66 (m, 2H, benzylic H), 4.74 (m, 4H, benzylic H), 4.99 (m, 6H, benzylic H), 5.08 (m, 6H, benzylic H), 7.10–7.67 (m, 51H, aromatic H), 7.67 (m, 3H, aromatic H), 7.78 (t, J = 5.6, 1H, —NH—CO), 7.83 (t, J = 5.7, 2H, —NH—CO); ^{13}C-NMR (125 MHz, $CDCl_3$): 30.1, 30.3, 36.9, 37.3, 68.5, 71.3, 71.4, 74.3, 76.2, 76.3, 81.4, 81.5, 83.6, 127.3, 127.4, 127.6, 127.6, 127.7, 127.7, 127.8, 128.1, 128.2, 128.2, 128.3, 128.5, 128.6, 128.6, 128.7, 128.7, 136.4, 136.4, 136.5, 138.9, 139.1, 146.7, 146.7, 151.7, 165.1, 165.2; MS (FAB): 1572.4 ($[M+1]^+$).

*1,3,5-Tris{N-{3-[2,3-di(benzyloxy)phenyl]-propionyl}-aminopropyl}-2,4,6-tribenzyl-myo-inositol (**14**), $C_{105}H_{111}N_3O_{15}$ (1655.0)*

HBTU (255 mg, 0.67 mmol) was added with stirring to a suspension of **3** (139 mg, 0.22 mmol), **11** (244 mg, 0.67 mmol) and triethylamine (136 mg, 1.34 mmol) in dry acetonitrile (20 ml). After some minutes a clear solution was formed. The mixture was stirred for 2 h at room temperature and the solvent was evaporated at reduced pressure. The residue was dissolved in $CHCl_3$ (20 ml), washed subsequently with 1 M HCl, saturated $NaHCO_3$ solution and water, dried (Na_2SO_4) and evaporated. The crude was purified by column chromatography (AcOEt:hexane = 20:1) to provide **14** (237 mg, 64%) as a white solid: TLC: R_f 0.20 (AcOEt:hexane = 20:1); ^{1}H-NMR (500 MHz, $CDCl_3$,): 1.60 (m, J = 6.6, 6H, —CH_2—), 2.16 ('t', J = 7.6, 2H, —CH_2—CO), 2.24 (m, J = 6.5, 4H, —CH_2—CO), 2.83–2.89 (m, J = 9.4, 7H, —CH_2—Ar, CH-inositol), 3.13 (m, J = 5.6, 6.7, 6H, —CH_2—Ar, —CH_2—N), 3.31 (m, J = 6.7, 2H, —CH_2—N), 3.45 (m, J = 6.0, 4H, —CH_2—O), 3.67 ('t', J = 6.0, 2H, —CH_2—O), 3.81 (t, J = 9.5, 2H, CH-inositol), 4.02 (t, 1H, CH—inositol), 4.72 (m, 2H, benzylic H), 4.81 (m, 4H, benzylic H), 5.04 (m, 6H, benzylic H), 5.12 (s, 6H, benzylic H), 5.24 (t, 1H, —NH—CO), 5.45 (t, J = 5.6, 2H, —NH—CO), 6.77 (dd, 3H, aromatic H), 6.88 (m, 3H, aromatic H), 6.96 (m, 3H, aromatic H), 7.29–7.47 (m, 45H, aromatic H); ^{13}C-NMR (125 MHz, $CDCl_3$): 26.4, 29.7, 29.9, 30.0, 36.8, 37.0, 37.1, 38.6, 68.7, 70.8, 71.3, 74.0, 74.3, 74.8, 74.8, 75.3, 77.2, 81.3, 81.4, 83.5, 122.4, 124.0, 124.0, 127.4, 127.5, 127.6, 127.9, 128.2, 128.4, 128.4, 128.5, 135.2, 135.3, 136.9, 137.0, 137.8, 138.8, 138.9, 146.3, 151.9; MS (FAB): 1655.0 ($[M]^+$).

*1,3,5-Tris{N-{N-[2,3-di(benzyloxy)benzoyl]-β-alanyl}-aminopropyl}-2,4,6-tribenzyl-myo-inositol (**15**), $C_{108}H_{114}N_6O_{18}$ (1784.1)*

HBTU (250 mg, 0.66 mmol) was added with stirring to a suspension of **3** (137 mg, 0.22 mmol), **8** (268 mg, 0.66 mmol), and triethylamine (134 mg, 1.32 mmol) in dry acetonitrile (12 ml). The reaction was performed as described for **14**. Purification of the crude product by column chromatography ($CHCl_3$:MeOH = 20:1) afforded **15** (326 mg, 83%) as a white foam: TLC: R_f 0.54 ($CHCl_3$:MeOH = 20:1); ^{1}H-NMR (300 MHz, $CDCl_3$): 1.65 (m, J = 6.3, 6H, —CH_2—), 2.11 (m, 6H, —CH_2—CO), 3.12 (m, J = 2.2, 5.5, 9.2, 6.6, 7H, inositol—CH, —CH_2—N), 3.32 (m, J = 6.5, 2H, —CH_2—N), 3.42–3.52 (m, J = 6.0, 6.4, 10H, —CH_2—N, —CH_2—O), 3.74 ('t', J = 6.0, 2H, —CH_2—O), 3.81 (t, J = 9.5, 2H, inositol—CH), 4.04 (t, J = 2.2, 1H, inositol—CH), 4.71 (m, 2H, benzylic H), 4.80 (m, 4H, benzylic H), 5.05 (s, 6H, benzylic H), 5.11 (s, 6H, benzylic H), 5.76 (t, J = 5.5, 1H, —NH—CO), 6.10 (t, J = 5.6, 2H, —NH—CO), 7.10 (m, 6H, aromatic H), 7.26–7.43 (m, 45H, aromatic H), 7.64 (m, 3H, aromatic H), 8.16 (m, J = 5.6, 3H, —NH—CO); ^{13}C (75 MHz, $CDCl_3$): 29.7, 29.9, 35.7, 35.8, 36.0, 36.9, 38.6, 68.6, 71.3, 71.4, 74.1, 74.3, 75.3, 76.2, 76.2, 81.3, 83.4, 117.1, 123.0, 123.1, 124.2, 124.3, 127.4, 127.4, 127.6, 127.6, 128.2, 128.4, 128.5, 128.6, 128.6, 128.9, 136.3, 136.4, 138.8, 138.9, 146.8, 151.8, 165.4, 165.5, 170.7, 170.8; MS (FAB): 1782.7 ($[M-1]^+$).

*1,3,5-Tris[N-(2,3-dihydroxybenzoyl)-aminopropyl]-myo-inositol (**16**), $C_{36}H_{45}N_3O_{15}$ (759.8)*

A mixture of **13** (244 mg, 0.155 mmol) and $Pd(OH)_2$—C (20% Pd, 120 mg) in THF (10 ml) was hydrogenolyzed at room temperature and atmospheric hydrogen pressure for 3 h. The reaction mixture was filtrated over celite and evaporated at reduced pressure. The residue was chromatographed over a short column (AcOEt:EtOH:H_2O = 5:1:0.4) to yield **16** (117 mg, 99%) as a white solid: TLC: R_f 0.56 (AcOEt:EtOH:H_2O = 5:1:0.4); ^{1}H-NMR (500 MHz, DMSO-d_6): 1.80 (m, J = 6.7, 6H, —CH_2—), 2.89 (t, J = 9.2, 1H, inositol—CH), 3.00 (dd, J = 2.3, 9.8, 2H, inositol—CH), 3.38 (m, J = 6.6, 6 H, —CH_2—N), 3.52 (m, J = 6.2, 2H, —CH_2—O), 3.60 (t, J = 9.7, 2H, inositol—CH), 3.78 (m, J = 6.1, 4H, —CH_2—O), 4.15 (t, 1H, inositol—CH), 6.66 (m, J = 7.9, 3H, aromatic H), 6.90 (m, J = 1.4, 7.8, 3H, aromatic H), 7.28 (m, J = 1.2, 8.1, 3H, aromatic H), 8.74 (m, 3H, —NH—CO); ^{13}C-NMR (75 MHz, DMSO-d_6): 23.5, 28.8, 29.3, 29.5, 36.5, 36.6, 65.4, 71.2, 80.1, 84.2, 106.9, 117.0, 117.1, 117.8, 118.6, 146.2, 149.7, 169.7; MS (FAB): 760.5 ($[M+1]^+$).

*1,3,5-Tris{N-[(2,3-dihydroxyphenyl)-propionyl]-aminopropyl}-myo-inositol (**17**), $C_{42}H_{57}N_3O_{15}$ (843.9)*

A mixture of **14** (208 mg, 0.126 mmol) and $Pd(OH)_2$—C (20% Pd, 105 mg) in THF (10 ml) was hydrogenolyzed as described for **16**. The crude product was chromatographed over a short column (AcOEt:EtOH:H_2O = 5:1:0.4) to yield **17** (94 mg, 88%) as a slightly yellow solid: TLC: R_f 0.34 (AcOEt:EtOH:H_2O = 5:1:0.4); ^{1}H-NMR (500 MHz, DMSO-d_6): 1.60 (m, 6H, —CH_2—), 2.31 (m, 6H, —CH_2—CO), 2.70 (m, 6H, —CH_2—Ar), 2.82 (t, J = 9.2, 1H, inositol—CH), 2.91 (dd, J = 2.2, 9.7, 2H, inositol—CH), 3.12 (m, J = 6.1, 6H, —CH_2—N), 3.39–3.52 (m, J = 9.5, 4H, —CH_2—O, —CH—inositol), 3.65 (m, 2H, —CH_2—O), 3.78 (m, 2H, —CH_2—O), 4.06 (m, 1H, inositol—CH), 6.50 (m, 6H, aromatic H), 6.59 (m, 3H, aromatic H), 7.78 (m, 3H, —NH—CO), 8.20 (s, broad, —OH), 9.04 (s, broad, —OH), 11.67 (s, broad, —OH); ^{13}C-NMR (125 MHz, DMSO-d_6): 23.3, 23.5, 25.7, 28.9, 29.5, 29.8, 33.1, 35.6, 35.9,

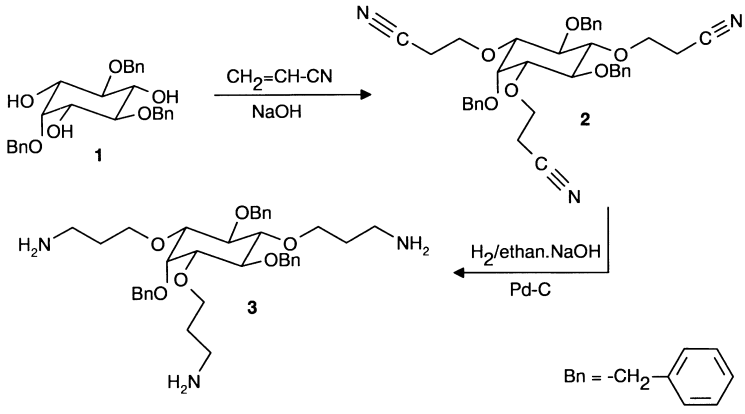

Figure 1. Synthesis of the *myo*-inositol based siderophore scaffold.

65.0, 66.6, 66.7, 68.2, 69.5, 71.1, 80.0, 84.1, 97.2, 107.0, 107.8, 113.2, 118.7, 120.0, 128.3, 143.0, 145.0, 172.0; MS (FAB): 844.3 ($[M]^+$).

*1,3,5-Tris{N-[N-(2,3-dihydroxybenzoyl)-β-alanyl]-aminopropyl}-myo-inositol (**18**),$C_{45}H_{60}N_6O_{18}$ (973.0)*

The hydrogenolysis of **15** (226 mg, 0.127 mmol) in THF (15 ml) over $Pd(OH)_2$—C (20% Pd, 113 mg) was performed as described for **16**. The reaction mixture was filtrated over Celite and evaporated. After drying at 0.05 bar, pure **17** (94 mg, 88%) was obtained as a white solid: TLC: R_f 0.27 ($CHCl_3$:AcOEt:AcOH = 3:1:0.3); ^{1}H-NMR (500 MHz, DMSO-d_6): 1.60 (m, 6H, —CH_2—), 2.37 (m, 6 H, —CH_2—CO), 2.79 (t, J = 9.2, 1H, inositol—CH), 2.87 (dd, J = 2.2, 9.7, 2H, inositol-CH), 3.15 (m, J = 5.2, 6.2, 6H, -CH_2—N), 3.40 (m, J = 6.2, 2H, —CH_2—N), 3.47 (m, 4 H, -CH_2-N), 3.52 (t, J = 9.5, 2 H, inositol—CH), 3.53–3.81 (m, J = 6.0, 6H, —CH_2—O), 4.04 (t, 1H, inositol—CH), 6.65 ('t', J = 7.9, 8.0, 3H, aromatic H), 6.89 (dd, J = 1.3, 7.8, 3H, aromatic H), 7.23 (dd, J = 1.3, 8.1, 3H, aromatic H), 7.85 (m, J = 5.2, 3H, —NH—CO), 8.78 (m, J = 5.4, 3H, -NH-CO); ^{13}C-NMR (125 MHz, DMSO-d_6): 23.3, 23.5, 27.4, 28.9, 29.1, 29.5, 29.8, 35.0, 35.9, 38.2, 64.9, 66.7, 68.2, 69.4, 71.1, 80.0, 84.0, 105.8, 106.9, 115.1, 117.2, 117.9, 118.7, 128.2, 128.9, 140.6, 146.1, 149.4, 169.5, 170.1, 170.2; MS (FAB): 973.7 ($[M+1]^+$).

Results and discussion

Synthesis of the myo-inositol scaffold

myo-Inositol-1,3,5-orthoformate was prepared by the reaction of *myo*-inositol with triethyl orthoformate in DMF following literature procedures (Lee & Kishi 1985, Baudin *et al.* 1988, Billington *et al.* 1989). Subsequent benzylation of the remaining free hydroxyl groups and removal of the orthoformate moiety yielded the 1,3,5-tribenzyl-*myo*-inositol (**1**, Billington *et al.* 1989). The latter compound seemed to be a suitable intermediate for the synthesis of a tri-armed aminogroup containing the siderophore scaffold. Therefore we developed a synthetic route to 1,3,5-tri(3-aminopropyl)-2,4,6-tribenzyl-*myo*-inositol starting from **1** by a two step procedure (Figure 1).

In the first step acrylonitrile was added to **1** by a Michael type addition leading to crystalline 1,3,5-tri(2-cyanoethyl)-2,4,6-tribenzyl-*myo*-inositol (**2**). The following selective reduction of the trinitrile in the presence of the benzyl ethers proved to be a difficult step.

Several metal hydrides including $NaBH_4$/cobalt(II) chloride, which is known to work well for the reduction of cascade-like polynitriles (Buhleier *et al.* 1978) gave only low yields of the desired triamine. Finally, we found out that the reduction of **2** with a partially inactivated Raney nickel catalyst in ethanolic sodium hydroxide solution (Bergeron & Garlich 1984) under a hydrogen pressure of 10 bar afforded the triamine **3** as a colorless oil in a yield of more than 90%.

Preparation of carboxylic acids containing the catechol moiety

2,3-Di(benzyloxy)benzoic acid (**5**) was prepared by benzylation of 2,3-dihydroxybenzaldehyde and subsequent oxidation of the aldehyde with sodium chlorite in the presence of sulfamic acid (Figure 2) in accordance with Rastetter *et al.* (1981). The *N*-hydroxysuccinimide active esters **6** and **7** were synthesized by reaction of the corresponding acid (2,3-dihydroxybenzoic acid, **4** and its *O*-benzyl derivative, **5**, respectively) with N-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC)

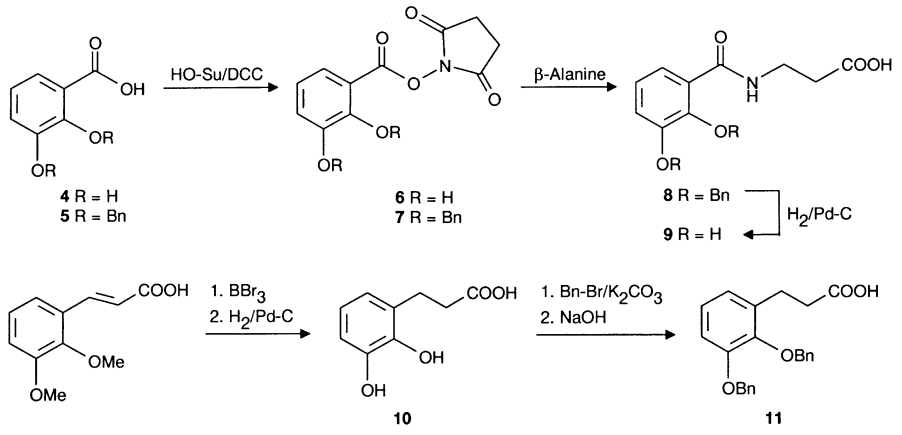

Figure 2. Synthesis of the catechol-containing educts.

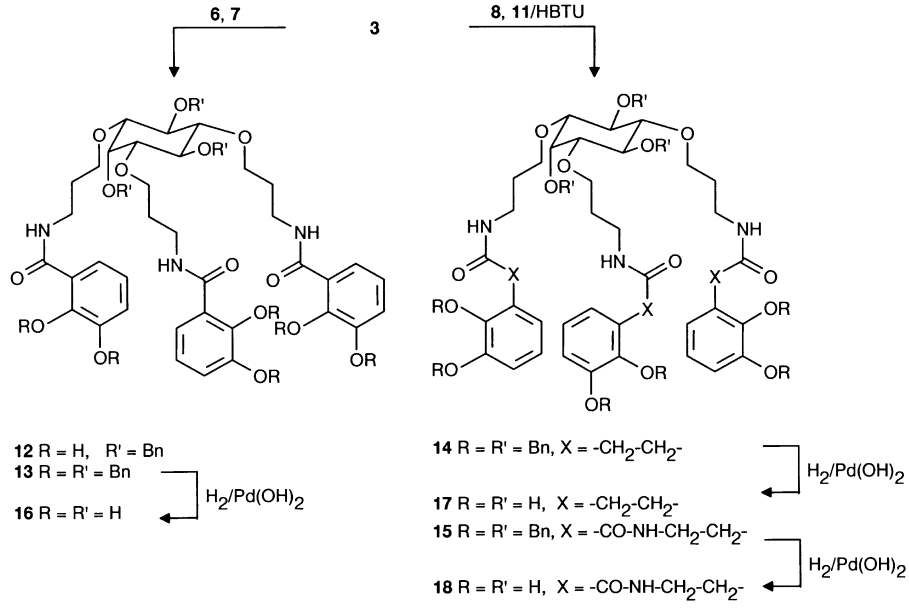

Figure 3. Synthesis of the catechol-type siderophore analogs.

and purified by crystallization (Bergeron *et al* 1983, Chimiak & Neilands 1984).

Coupling of **7** with β-alanine in a mixture of acetone, water and triethylamine yielded *N*-[2,3-di(benzyloxy)benzoyl]-3-aminopropionic acid (**8**). Hydrogenolysis of **8** gave the unprotected catecholic acid **9**. 3-(2,3-Dihydroxyphenyl)propionic acid (**10**) was obtained by demethylation of commercially available *trans*-2,3-dimethoxycinnamic acid with boron tribromide in dichloromethane followed by hydrogenation of the double bond over 10% Pd–C in a hydrogen atmosphere. Benzylation of the resulting acid with benzyl bromide afforded the benzyl ester of the benzylether-protected catechol derivative. The latter was saponified with NaOH in dioxane resulting in the desired 3-[2,3-di(benzyloxy)phenyl]propionic acid (**11**).

Synthesis of the siderophore analogs

The coupling reactions of the catechol containing derivatives **6**, **7**, **8** and **11** with the *myo*-inositol scaffold **3** were performed by two different procedures (Figure 3). The *N*-hydroxysuccinimide esters **6** and **7**, respectively were treated with **3** in DMF in the presence of triethylamine yielding the catechol-containing siderophore analog **12** and the benzylether-protected derivative **13**.

Table 1. Growth promotion tests of the synthesized siderophore analogs and selected educts on Gram-negative bacteria

Indicator strain	Iron related marker	Diameter of growth zone (mm)								
		Compound								
		2	3	9	10	12	16	17	18	C[a)]
S. typhimurium enb-7	ent (class II)	0	0	12	0	0	18	12	18	22
E. coli AB 2847	aroB	0	0	0	0	0	0	0	0	22
E. coli IR 112	aroB, tonB	0	0	0	0	0	0	0	0	26
K. pneumoniae KN 4401	ent, iuc	12	25	inh[c)]	30[b)]	26	inh[c)]	inh[c)]	inh[c)]	27
Y. enterocolitica H 5030	yb	12	0	18	20	0	12	10	10	26
P. aeruginosa PAO 6609	pvd	0	0	0	0	0	0	0	0	34

[a)] Control: ferrioxamine G (*S. typhimurium* enb-7), 2,3-dihydroxybenzoic acid (*E. coli* AB 2847 and *E. coli* IR 112), ferrioxamine E (*K. pneumoniae* KN 4401), ferrichrome (*Y. enterocolitica* H 5030), ferrioxamine E (*P. aeruginosa* PAO 6609).
[b)] Growth inhibition zone in the center of growth promotion zone.
[c)] inh = inhibition

In the second procedure the free carboxylic acids **8** and **11** were attached to **3** using *O*-benzotriazolyl-tetramethyl-isouronium hexafluorophosphate (HBTU) as coupling agent. The reactions were carried out in acetonitrile with triethylamine as a base leading to the benzylether-protected compounds **14** and **15**. After usual work-up all the products were purified by column chromatography.

Hydrogenolysis of **13**, **14** and **15** over 10% Pd–C resulted in mixtures of incompletely deprotected products even at hydrogen pressures of 10–15 bar and reaction times of 24 h. Finally, we succeeded in obtaining the fully deprotected siderophore analogs **16**, **17** and **18** in high purity by using $Pd(OH)_2$ on carbon (Pearlman's catalyst, 20% Pd) as hydrogenation catalyst under atmospheric hydrogen pressure.

The structures of the synthesized siderophore analogs were confirmed by the usual spectroscopic methods (^{1}H- and ^{13}C-NMR, FAB-MS).

Investigations of siderophore activity

The siderophore activity of the synthesized siderophore analogs and of selected educts was examined by growth promotion tests (Reissbrodt *et al*. 1993) with various bacteria that are well defined in their ability to transport and utilize natural siderophores (so called siderophore indicator strains). In a first series of experiments we used the following Gram-negative indicator strains: *Salmonella typhimurium* enb-7, *E. coli* AB 2847, *E. coli* IR 112, *Klebsiella pneumoniae* KN 4401, *Yersinia enterocolitica* H 5030 and *Pseudomonas aeruginosa* PAO 6609. The results of the growth promotion tests are given in Table 1. Both the tested catechol educts N-(2,3-dihydroxybenzoyl)-β-alanine (**9**) and 2,3-(dihydroxyphenyl)propionic acid (**10**) stimulated *Y. enterocolitica* H 5030. *S. typhimurium* enb-7 was also weakly stimulated by **9**. Surprisingly, even the *myo*-inositol educts **2** and **3** bearing no catechol moities showed a weak stimulation of *K. pneumoniae* KN 4401. Additionally, **2** weakly promoted *Y. enterocolitica* H 5030. In spite of a negative CAS assay (chromazurol-S-assay according to Schwyn & Neilands 1987) it can be assumed that **2** and **3** are able to sequester iron to a lower extent by their metal-chelating nitrilo and amino groups, respectively, and act as a weak growth promoters.

The siderophore analog **16** containing three 2,3-dihydroxybenzoyl-moities and three unprotected hydroxyl groups at the inositol ring stimulated both *S. typhimurium* enb-7 and to a lesser extent, *Y. enterocolitica* H 5030 and showed no promotion but some growth inhibition of *K. pneumoniae* KN 4401. The less hydrophilic compound **12,** which differs from **16** only by masking of the hydroxyls with benzyl groups, effectively promoted *K. pneumoniae* KN 4401 but was unable to feed either *S. typhimurium* enb-7 or *Y. enterocolitica* H 5030. Based on the present results an explanation for this behavior may be the different hydrophilicity of the tested siderophore analogs **12** and **16**. The hydrophilicity/lipophilicity ratio of the iron chelators seems to play an important role during the siderophore transport and recognition. Further investigations are necessary for the full understanding of this phenomenon. Both the siderophore analogs **17** and **18** reasonably stimulated the growth of *S. typhimurium* enb-7 and of *Y. enterocolitica* H 5030. Compared with the corresponding monocatecholate educts **10** and **9**, the triscatecholates **17** and **18**, respectively exhibited a significantly higher growth promotion of *S. typhimurium* enb-7. For *Y. enterocolitica* H 5030 the educts **10** and **9** were

Table 2. Growth promotion tests of siderophore analogs on mycobacterial indicator strains

Compound	*M. smegmatis* SG 987	*M. smegmatis* mc^2 155	*M. smegmatis* M 10	*M. phlei* 239	*M. phlei* M 77
12	23	18	17	15	0
16	20	10	14	15	0
17	0	0	0	0	0
18	23	24	19	17	0

more effective growth promotors than were the appropriate triscatecholates. The growth of *K. pneumoniae* KN 4401 can be promoted very effectively by compound **10** but also inhibited by higher concentrations in the center of the growth promotion zone. Only inhibition zones were detected after treatment with compounds **16**, **17** or **18**.

None of the synthesized derivatives could be utilized by *E. coli* AB 2847 and by the tonB mutant *E. coli* IR 112, indicating an energy-coupled tonB-dependent active transport process. The tonB mutant *E. coli* IR 112 is able to grow with 2,3-dihydroxybenzoic acid or its *O*-acetyl derivative (Reissbrodt *et al.* 1993), but not with any of the well known natural catecholate siderophores. *P. aeruginosa* PAO 6609 was also not stimulated by any of the siderophore analogs. This underlines the selectivity of the *myo*-inositol based catecholates.

In a second series of experiments the siderophore activity of the synthesized analogs was tested with selected mycobacterial strains deficient in iron support. The following strains were used: *M. smegmatis* SG 987 (wild strain), *M. smegmatis* mc^2 155, *M. smegmatis* M 10 (mutant of SG 987 with deficiency of exochelin; CAS-negative), *M. phlei* 239 (wild strain, unable to produce exochelin) and *M. phlei* M 77 (mycobactin-negative, mutant of *M. phlei* 239) (Möllmann *et al.* 1997). The results of the growth promotion tests are shown in Table 2.

With the exception of compound **17** all the synthesized triscatecholates acted as siderophores and are able to supply iron for growth of the wild type strain and the exochelin deficient strains but not to the mycobactin deficient strain. This indicates ligand exchange with mycobactin in a way comparable to that assumed for exochelin (Wheeler & Ratledge 1994).

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