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New artificial siderophores based on a monosaccharide scaffold

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Abstract

New artificial catecholate siderophores with methyl α-D-glucopyranoside as scaffold were synthesized. The dihydroxy- or di(acetoxy)benzoyl moieties were attached either directly or via aminopropyl spacer groups, to the carbohydrate scaffold. The siderophore activity of the prepared siderophore analogs was examined by a growth promotion assay using various Gram-negative bacteria and mycobacteria and by the CAS-assay.

Introduction

During the recent years a series of synthetic analogs of bacterial siderophores (Shanzer & Libman 1998) attracted growing interest as clinically useful iron chelators for the treatment of iron overload diseases (Bergeron *et al.* 1999) and for iron transport-mediated antibiotics (Miller *et al.* 1993) or antimicrobial compounds, e.g. with special activity against *Plasmodium* species (Cabantchik 1995; Pradines *et al.* 1996). Various analogs were synthesized from one of the strongest siderophores, enterobactin as a trimer of 2,3-dihydroxybenzoyl serine, which is produced by *Escherichia coli* and other enteric bacteria under iron limitation (O'Brien *et al.* 1970). Thus the cyclic trilactone backbone of enterobactin was replaced by different structures as *cis*-1,5,9-triaminocyclododecane (Corey & Hurt 1977), 1,3,5-tris(aminomethyl)benzene (Weitl & Raymond 1979), various lysine derivatives (Chimiak & Neilands 1984; Akiyama & Ikeda 1995) or cyclitols (Coleman *et al.* 1992; Tse & Kishi 1993). Recently, we prepared enterobactin analogs containing a *myo*-inositol scaffold (Schnabelrauch *et al.* 1998). However, no artificial siderophores were described, which use a carbohydrate backbone for the iron-chelating groups.

Here we report the synthesis and siderophore activities of siderophore analogs based on a methyl α-D-glucopyranoside scaffold. The paper suggests that carbohydrates can substitute advantageously the trilactone structure of enterobactin because of their good accessibility to cells and higher hydrolytic stability. The availability of different stereoisomeric forms and their high functionality render the carbohydrates particularly attractive for the design of siderophore model compounds of varying structure and polarity with an optimum octahedral binding cavity for the ferric ion.

The siderophore activity of the new siderophore analogs was examined by the CAS-assay and by growth promotion assays with various Gram-negative bacteria and mycobacteria.

Material and methods

Synthesis of the siderophore analogs

Analytical methods

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Advance DRX 300 and a Bruker Advance DPX 500 MHz Bruker spectrometer, respectively. The chemical shifts δ are given in ppm. The coupling constants J are reported in Hz. High resolution mass spectra were obtained by a Finnigan MAT 95 XV high resolution mass spectrometer with fast-atom bombardment (FAB) and electrospray ionization (ESI), respectively. Column chromatography was accomplished using silica gel (Merck 60, 0.040–0.063 mm). Purifications of the compounds by preparative HPLC were performed on an Abimed Gilson instruments equipped with an 115 UV detector (254 nm) and a Knauer Vertex reversed phase column (250 × 32 mm or 50 × 20 mm) packed with Eurosper 100-C18 (7 mm). The column was eluated by a gradient of acetonitrile and water, beginning with ratio 30:70 (v/v) and achieving 100:0 (v/v) after a period of 20 min (flux rate 20 ml/min or 10 ml/min). Thin layer chromatography (TLC) was carried out on precoated silica plates (Merck 60 F_{254}). Solvents and reagents used were dried and purified by standard methods.

*Methyl 2,3-bis-O-[2,3-di(acetoxy)benzoyl]-4,6-O-isopropylid ene-α-*D*-glucopyranoside (***2***), $C_{32}H_{34}O_{16}$ (674.6)*

To a solution of methyl 4,6-O-isopropylidene-α-D-glucopyranoside **1** (Debost *et al.*, 1984) (1.2 g, 5 mmol) in anhydrous tetrahydrofurane (50 ml), triethylamine (2 ml, 15 mmol) and *N*,*N*-dimethylaminopyridine (60 mg) were added with stirring under an atmosphere of nitrogen. After cooling to −20 °C a solution of 2,3-diacetoxybenzoyl chloride in anhydrous tetrahydrofurane was added dropwise, stirring was continued for 1 h at −15 °C and 3 h at ambient temperature. Solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. It was washed subsequently with 1 M HCl, saturated $NaHCO_3$, brine and water, dried over Na_2SO_4, filtered and solvent was evaporated *in vacuo*. The resulting colourless foam was purified by HPLC and afforded **2** (2.16 g, 64%, mp: 93–95 °C). $[\alpha]_D^{20}$ + 52.4 (*c* 8.9, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.32 (s, 3H, CCH_3),1.41 (s, 3H, CCH_3), 2.19 (s, 3H, $COCH_3$), 2.20 (s, 6H, $COCH_3$), 2.23 (s, 3H, $COCH_3$), 3.32 (s, 3H, α OCH_3), 3.7-3.85 (m, 3H, H-6, H5), 3.85 (t, J = 3.6, 1H, H4), 4.96 (d, J = 3.6, 1H, H1), 5.03 (dd, J = 10, 3.6, 1H, H-2) 5.67 (dd, J = 1.9, 8.4, 1H, H-3), 7.15–7.29 (m, 4H, aromatic H), 7.66 (dd, J = 1.8, 7.9, 1H, aromatic H), 7.72 (dd, J = 1.8, 7.9, 1H, aromatic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 18.91 ($C\underline{C}H_3$), 20.41, 20.48, 20.51 (2x) (CO-$\underline{C}H_3$), 28.89 ($C\underline{C}H_3$), 55.47 (α $O\underline{C}H_3$), 62.36, 63.48, 69.89, 72.25 72.36, 97.64, 99.94 (C1/C6 and $\underline{C}(CH_3)_2$), 123.78, 124.73, 125.98, 126.22, 127.73, 128.19, 128.48, 129.38, 142.39, 142.84, 143.39, 143.47 (aromatic C), 162.73, 163.21 (-CO-), 167.95, 168.04, 168.12, 168.20 ($\underline{C}O$-CH_3). MS (ESI) m/z 692.1 ($[M+NH_4]^+$), 697.2 ($[M+Na]^+$).

*Methyl 2,3-bis-O-[2,3-di(acetoxy)benzoyl]-α-*D*-glu copyranoside (***3***), $C_{29}H_{30}O_{16}$ (634.6)*

To a solution of **2** (1 g, 1,5 mmol) in dichloromethane (20 ml), acetic acid (0.5 ml) was added. The reaction mixture was stirred at ambient temperature for 1 h until no educt was shown at TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid). Then it was extracted several times with brine and water. The organic layer was dried over Na_2SO_4, filtered and solvent was evaporated *in vacuo*. **3** was precipitated in light petroleum and ether as a colourless solid (860 mg, 91%; mp. 85–87 °C). $[\alpha]_D^{20}$ + 62.7 (*c* 6.5, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 2.28 (s, 6H, $COCH_3$), 2.30 (s, 3H, $COCH_3$), 2.35 (s, 3H, $COCH_3$), 3.43 (s, 3H, α OCH_3), 3.8-3.9 (m, 4H, 2H-6,H-5, H-4), 5.03 (d, J = 3.6, 1H, H-1), 5.13 (dd, J = 3.6, 10.2, H-3), 5.70 (dd, J = 1.8, 10.2, 1H, H-3),7.20–7.37(m, 4H, aromatic H), 7.67 (dd, J = 1.6, 7.8, 1H, aromatic H), 7.85 (dd, J = 1.71, 7.8, H, aromatic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 20.50 (3x $CO\underline{C}H_3$), 20.70(3x $CO\underline{C}H_3$), 55.45 (α $O\underline{C}H_3$), 62.06 (C6), 69.75 (C4), 71.11 (C2), 71.36 (C5), 74.03 (C3), 96.98 (C1), 123.79, 124.85, 126.25, 127.80, 128.31, 129.24, 142.13, 142.92, 143.15, 143.50 (aromatic C), 163.25, 164.49 (-CO-),168.08 (2x), 168.24, 168.82 ($\underline{C}O$-CH_3). MS (ESI) m/z 652.3 ($[M+NH_4]^+$), 657.2 ($[M+Na]^+$).

*Methyl 2,3,4-tri-O-(2-cyanoethyl)-6-O-trityl-α-*D*-glu copyranoside (***5***), $C_{35}H_{37}N_3O_6$ (595.7)*

To a solution of methyl 6-O-trityl-α-D-glucopyranoside **4** (Ho & Wong 1995) (6,6 g, 0.015 mol) in 10% aqueous KOH (5.0 g), acrylonitrile (3 eq/OH, 8,7 ml) was added and the reaction mixture was left in an ultrasonic bath at 40 °C for 2 h. The reaction mixture was cooled and extracted with chloroform. The organic extracts were successively washed with 1 N HCl and water, dried over Na_2SO_4, filtered, and solvent was

evaporated *in vacuo*. The raw product was crystallized from ethanol. Purification by chromatography on silica gel (5:1 chloroform-ethyl acetate) gave **5** (5,2 g, 58%, mp 146 °C). $[\alpha]_D^{20}$ + 71.0 (*c* 7.8, methanol), ^{1}H-NMR (500 MHz, $CDCl_3$): 2.12–2.22 (m, 2H, CH_2-CN), 2.60 (m, 4H, -CH_2-CN), 3.10 (dd, J = 7.3, 3.1, 1H), 3.32 (dt, J = 8.9, 5.9, 3.2, 1H), 3.44 (s, 3H, α-OCH_3), 3.46–3.65 (m, 4H), 3.7 (t, J = 6.3, 2H, O-CH_2), 3.75 (m, 1H), 3.91 (t, J = 6.1, 2H, O-CH_2-), 3.93 (m, 1H), 4.95 (d, 3.4, 1H, H-1), 7.22 -7.49 (m, 15H, aromatic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 18.92, 19.31, 19.39 ($\underline{C}H_2$-CN), 55.1 (α O$\underline{C}H_3$), 61.49 (C6), 65.93 (C4), 66.77, 67.82, 69.83 (O-$\underline{C}H_2$-), 77.87 (C2), 80.96 (C5), 81.0(C3), 86.39 ($\underline{C}Ph_3$), 97.11 (C1), 117.67, 117.72, 118.67 (CH_2-$\underline{C}N$), 127.14, 127.85, 128.65, 143.55 (aromatic C). MS (ESI) m/z 618.4 ($[M+Na]^+$).

*Methyl 2,3,4-tri-O-(3-aminopropyl)-6-O-trityl-α-*D*-glucopyranoside (***6***), $C_{35}H_{49}N_3O_6$ (607.8)*
To a solution of $CoCl_2$ (4.5 g, 0.034 mol) in anhydrous methanol (100 ml), $NaBH_4$ (1,3 g, 0.034 mol) was added under an atmosphere of nitrogen at 0 °C. During half an hour a black solid precipitated. Methyl 2,3,4-tri-O-cyanoethyl-6-O-trityl-α-*D*-glucopyranoside (2,0 g, 3.4 mmol) in anhydrous tetrahydrofurane (10 ml) was added followed by addition of $NaBH_4$ (4 g, 0.1 mol) in rations. After stirring at room temperature for about 2 h until no nitrile was shown at TLC (5:1 chloroform-ethylacetate), the reaction mixture was brought to pH 2-3 with 2 N HCl. Under vigorous stirring the catalyst was destroyed and the solution got a pink colour. Then it was made alkaline with aqueous NH_3 and extracted with chloroform. The organic extracts were dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The resulting residue a colourless foam of **6** (1.9 g, 92%) was used in next reactions without further purification. $[\alpha]_D^{20}$ + 59.6 (*c* 5.3, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.76–1.88 (m, 6H, -CH_2-), 2.45 (m, 2H, CH_2-NH_2), 2.82 (m, 4H, CH_2-NH_2), 3.12 (dd, J = 4.4, 9.6, 1H), 3.29–3.38 (m, 3H), 3.47 (s, 3H, α OCH_3), 3.66 (m, 1H), 3.74 (m, 6H, O-CH_3), 3.82 (m, 1H),4.91 (d, J = 3.5, 1H), 7.21–7.33 (m, 9H, aromatic H), 7.44–7.51 (m, 6H, aromatic H), ^{13}C-NMR (300 MHz,$CDCl_3$): 33.84, 34.06, 34.43 (-CH_2-), 39.41, 39.42, 39.56 ($\underline{C}H_2$-NH_2), 54.84 (α O$\underline{C}H_3$), 62.52 (C6), 69.13 (C4), 70.30, 70.88, 71.47 (O-$\underline{C}H_2$-), 78.50 (C2), 80.72 (C5), 81.61 (C3), 86.26 ($\underline{C}Ph_3$), 97.52 (C1), 127.13, 127.93, 128.77, 143.96 (aromatic C), MS (ESI) m/z 608.3 ($[M+H]^+$), 646.1 ($[M+K]^+$).

*Methyl 2,3,4-tris-O-{N-[2,3-di(acetoxy)benzoyl]-aminopropyl}-6-O-trityl-α-*D*-glucopyranoside* (**7a**), $C_{68}H_{73}N_3O_{21}$ *(1268.4)* To a solution of **6** (2 g, 3.2 mmol), triethylamine (5.4 ml, 39.2 mmol) and *N*,*N*-dimethylamino-pyridine (1.56 g, 9.6 mmol) in anhydrous tetrahydrofurane (50 ml), a solution of 2,3 diacetoxybenzoyl chloride (4.8 g, 18.8 mmol) in anhydrous tetrahydrofurane was added at −15 °C under an atmosphere of nitrogen. The reaction mixture was stirred at 0 °C for 1 h and at r.t. for 4 h. Solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. It was washed subsequently with 1 M HCl, saturated $NaHCO_3$, brine and water, dried over Na_2SO_4, filtered and solvent was evaporated *in vacuo*. The resulting colourless foam was purified by HPLC and afforded **7a** (1.8 g, 49%). TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid, Rf = 0.49). $[\alpha]_D^{20}$ + 25.1 (*c* 4.6, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.46 (m, 2H, -CH_2-), 1,77 (m, J = 6.3, 6.1, 4H, -CH_2-), 2,23-2.28 (m, 18H, CO-CH_3), 3.12 (m, 2H, -CH_2-NH), 3.25-3.77 (m, 15 H, -CH_2-NH, -O-CH_2-, -CH), 4.11 (m, 1H,-CH), 4.91 (d, J = 3.1, 1H,-CH), 6.46 (m, 1H, -NH-CO-), 6.83 (m, 1H, -NH-CO-), 6.89 (m, 1H, -NH-CO-), 7.16–7.48 (m, 24 H, aromatic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 20.44, 20.49 (CO-$\underline{C}H_3$), 29.54, 29.62, 29.86 (-$\underline{C}H_2$-) 37.86, 37.95 (CH_2-NH) 54.64 (α OCH_3) 62.25 (C6), 69.07 (C4), 70.12, 71.06, 71.74 (O-$\underline{C}H_2$), 78.50 (C2), 80.46 (C5), 81.53 (C3), 86.31 ($\underline{C}Ph_3$), 97.03 (C1), 125.44, 126.00, 126.23, 126.35, 127.01, 127,76 127.84, 127.88, 128.68, 130.57, 140.14, 140.34, 142.95, 143.03, 143.78 (aromatic C), 164.81, 165.03, 165.20 ($\underline{C}$O-NH), 167.90, 167.99, 168.1, 168.15, 168.19 ($\underline{C}$O-CH_3), MS (ESI) m/z 1290.3 ($[M+Na]^+$).

*Methyl 2,3,4-tris-O-[N-(2,3-dihydroxybenzoyl)amino propyl]-6-O-trityl-α-*D*-glucopyranoside (***7b***), $C_{56}H_{61}N_3O_{15}$ (1016.1)*
To a solution of sodium hydroxide (192 mg) in water (5 ml), **7a** (1 g, 0.8 mmol) was added under an atmosphere of nitrogen. The reaction mixture was stirred at r.t. for 1 h, then it was neutralized with 1 M HCl and a grey solid precipitated. It was filtered and washed with water. The residue was dissolved in ethyl acetate and extracted with brine and water, dried over Na_2SO_4, filtered and solvent was evaporated in *vacuo*. The resulting solid was purified by HPLC and afforded **7b** (120 mg, 15%) after lyophilization, ^{1}H-NMR (300 MHz, $CDCl_3$): 1.54-1.66 (m, 2H, -CH_2-), 1.76-1.9 (m, 4H, -CH_2-), 2.96-3.51 (m, 2H), 3.2–3.87 (m, 16H), 3.41 (s, 3H, α OCH_3), 4.95 (d, J = 3.0, 1H,

H1), 5.7–5.9 (br, 3H, OH), 6.57-6.69 (m, 3H, benzylic H), 6.73–6.78 (m, 1H, NH), 6.87–6.94 (m, 1H, NH), 6.95–7.02 (m, 3H, benzylic H), 7.18–7.2 (m, 1H, NH), 7.21–7.3 (m, 12H, trityl and benzylic H), 7.4–7.46 (m, 6H, trityl H), 12,6–12.9 (br, 3H, OH), ^{13}C-NMR (300 MHz, $CDCl_3$): 29.032, 29.19, 29.53 (-CH_2-), 38.11, 38.41, 38.76 (-$\underline{C}H_2$-NH-), 54.87 (α O$\underline{C}H_3$), 62.05 (C6), 70.35 (C4), 70.75, 72.26, 72.45 (O-CH_2-), 78.67 (C2), 80.82 (C5), 82.12 (C3), 86.45 ($\underline{C}Ph_3$), 97.12 (C1), 113.9(2C), 114.101, 115.61, 115.76, 116.13, 117.92, 118.07, 118.42, 118.47, 118.68 (aromatic C), 127.13, 127.83, 128.68, 143.64 (trityl C), 145.86 (2C), 145.98, 149.1 (2C), 149.22 (armatic C-OH), 169.54, 169.91 (2C) (CO-NH), MS (ESI) m/z 1014 ([M-H]$^+$).

*Methyl 2,3,4-tris-O-N-[3,4-di(methoxycarbonyloxy) benzoyl]-aminopropyl-6-O-trityl-α-*D*-glucopyrano side (***7c***)* $C_{68}H_{73}N_3O_{27}$ *(1386.7)*

To a solution of **6** (660 mg, 1.1 mmol), triethylamine (3 ml, 21 mmol) and *N,N*-dimethylamino-pyridine (550 mg, 3.3 mmol) in anhydrous THF (50 ml), a solution of 3,4-di(methoxycarbonyloxy)benzoyl chloride (1.3 g, 4.5 mmol) in anhydrous THF was added at −15 °C under an atmosphere of nitrogen. The reaction mixture was stirred at 0 °C for 1 h and at r.t. for 5 h. Solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. It was washed subsequently with 1 M HCl, saturated $NaHCO_3$, brine and water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The resulting colourless foam was purified by HPLC and afforded **7c** (1.2 g, 32%). TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid, Rf = 0.41). $[\alpha]_D^{20}$ +25.1 (*c* 7.5, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.70-1.99 (m. 6H, -CH_2), 3.13 (m, 2H), 3.38–3.91 (m, 15H) 3.40 (s, 3H, α OCH_3), 3.83 (s, 3H. OCH_3), 3.85 (s, 3H. OCH_3), 3.86 (s, 3H. OCH_3), 3.87 (s, 3H. OCH_3), 3.89 (s, 3H. OCH_3), 3.90 (s, 3H. OCH_3), 3.94 (m, 1H), 4.95 (d, J = 3.4, 1H, H1) 6.85 (m, 1H, NH), 6.96 (m, 1H, -NH), 7.2 (m,1H ,NH), 7.26-7.30 (m, 15H, trityl H and benzylic H), 7.45–7.49 (m, 6H, trityl H), 7.60–7.84 (m, 3H, benzylic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 29.4 (2C), 29.68 (-CH_2-), 38.03, 38.19, 39.04 (-CH_2-NH), 54.67 (α OCH_3), 55.87 (OCH_3), 62.33 (C6), 70.37 (C4), 71.25, 71.4, 71.58 (O-CH_2-), 78.57 (C2), 80.76 (C5), 81.32 (C3), 86.37 ($\underline{C}Ph_3$) 97.12 (C1), 122.17, 122.23, 122.26, 122.9, 122.96, 123.05, 125.33, 125.59, 125.77, 127.04, 127.78, 128.69, 133.53 (3C), 142.23(3C), 143.77, 144.58 (3C), (aromatic C), 152.79, 153.05, 153.11(CO-NH), 165.14, 165.18, 165,21, 165.52, 165.67, 165.74 ($\underline{C}O$-OCH_3), MS (ESI) m/z 1386.7 ([M+Na]$^+$).

*2,3-Di(acetoxy)phenoxyacetic acid (***9a***),* $C_{12}H_{12}O_7$ *(268.2)*

To a solution of 2,3-dihydroxy-phenoxyacetic acid (18 g, 0.1 mol) and acetic anhydride (20 g, 0.2 mol) 2 to 3 drops of H_2SO_4 (95%) were added with stirring at r.t. and under an atmosphere of nitrogen. After about 5 to 10 min a colourless solid was precipitated, which was dissolved in dry diethyl ether (140 ml). The reaction mixture was stirred for 16 h, then it was hydrolysed with ice-water (400 g) and extracted 6 times with dichloromethane (100 ml). The organic layers were washed with ice-water, dried over Na_2SO_4, filtered and solvent was evaporated *in vacuo*. The residue was recrystallized from toluene using coal. (15,2 g, 57%, mp 122–125 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.26 (s, 6H, CO$\underline{C}H_3$), 4.70 (s, 2H, OCH_2) 6.85 (d, J = 8.3, 1H, aromatic H), 6.90 (d, J = 7.2, 1H, aromatic H), 7.25 (d, J = 8.3, 1H, aromatic H). MS (FAB) m/z 269.2 ([M+H]$^+$)

*2,3-Di(acetoxy)phenoxyacetyl chloride (***9b***),* $C_{12}H_{11}ClO_6$ *(286.7)*

9a (3.9 g, 14 mmol) and PCl_5 (4.8 g, 23.4 mmol) were suspended in dry CCl_4 (10 ml) and the reaction mixture was stirred at about 50 °C up to complete solution. The solvent was evaporated *in vacuo* and the residue was recrystallized in CCl_4 to obtain **9b** (3.2 g, 71%, mp 86 °C).

*Methyl 2,3,4-tris-O-{N-[2,3-di(acetoxy)phenoxyace tyl]-amino propyl}-6-O-trityl-α-*D*-glucopyranoside (***7d***),* $C_{71}H_{79}N_3O_{24}$ *(1358.4)*

To a solution of **6** (1.7 g, 2.8 mmol), triethylamine (3 ml, 21 mmol) and 4-*N,N*-dimethylaminopyridine (1.36 g, 8.4 mmol) in anhydrous tetrahydrofurane (50 ml), a solution of 2,3-di(acetoxy)phenoxyacetyl chloride **9b** (3.2 g, 10.8 mmol) in anhydrous tetrahydrofurane was added at −15 °C under an atmosphere of nitrogen. The reaction mixture was stirred at 0 °C for 1 h and at r.t. for 4 h. Solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. It was washed subsequently with 1 M HCl, saturated $NaHCO_3$, brine and water, dried over Na_2SO_4, filtered and solvent was evaporated *in vacuo*. The resulting colourless foam was purified by HPLC and afforded **7d** (0.94 g, 25%). TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid, Rf = 0.19). $[\alpha]_D^{20}$ + 28.1 (*c*

6.2, methanol). ^{1}H-NMR (300 MHz, $CDCl_3$): 1.64–1.75 (m 6H, -CH_2-), 2.24–2.33 (m, 18 H, CO-CH_3), 3.0–3.61 (m, 18H), 3.38 (s, 3H, α OCH_3), 4.51 (s, 2H, CO-CH_2-O), 4.53 (s, 2H, CO-CH_2-O), 4.59 (s, 2H, CO-CH_2-O), 4.79 (d, J = 3.4, 1H, H1), 6.75-6.85 (m, 6H, benzylic H), 7.0–7.1 (m, 3H, NH), 7.18-7.30 (m, 12H, trityl and benzylic H), 7.45–7.48 (m, 6H, trityl H). ^{13}C-NMR (300 MHz, $CDCl_3$): 20.45 (CO-$\underline{C}H_3$), 29.62, 29.63, 29.91 (-CH_2-), 36.30, 36.82, 36.96 (CH_2-NH), 54.7 (α OCH_3), 62.33 (C6), 67.38, 67.56, 67.62 (CO-$\underline{C}H_2$-O), 68.32 (O-CH_2), 69.99 (C4), 70.4, 70.97 (O-CH_2-), 78.31 (C2), 80.56 (C5), 81.52 (C3), 86.2 ($\underline{C}Ph_3$), 97.33 (C1), 110.03, 110.38 (2C), 116,29, 116.32, 116.32, 126.12, 126.46, 126.51, 126.51, 126.94, 127.17, 127.21, 127.71, 127.85, 127.89, 128,67, 131.89, 131.95, 132.04, 143.52, 143.55, 143.83, 143.89 (aromatic C), 149.99, 150.04, 150.12, (CO-NH), 167.55, 167.80, 167.85, 168.04, 168.12, 168.25 (-$\underline{C}O$-CH_3), MS (ESI) m/z 1375 ([M+NH_4]$^+$), 1380 ([M+Na]$^+$).

*Methyl 2,3,4-tris-O-[N-(8-methoxycarbonyloxy-3,4-dihydro-2H -1,3-benzoxazin-3-yl-acetyl)-aminopropyl]-6-O-trityl -α-*D*-glucopyranoside (***7e***), $C_{71}H_{70}N_6O_{24}$ (1439.4)*

To a solution of **6** (1.88 g, 3.1 mmol), triethylamine (5.5 ml, 39.4 mmol) and *N*,*N*-dimethylaminopyridine (534 mg, 3.3 mmol) in anhydrous tetrahydrofurane (70 ml), a solution of 8-methoxycarbonyloxy-3,4-dihydro-2H-1,3-benzoxazin-3 -yl-acetyl chloride (3.7, 11.8 mmol) in anhydrous tetrahydrofurane was added at −15 °C under an atmosphere of nitrogen. The reaction mixture was stirred at 0 °C for 1 h and at r.t. for 3 h. Solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. It was washed subsequently with 1 M HCl, saturated $NaHCO_3$, brine and water, dried over Na_2SO_4, filtered and solvent was evaporated *in vacuo*. The resulting yellow foam was purified by HPLC and afforded **7e** (1.3 g, 29%). $[\alpha]_D^{20}$ + 25.1 (*c* 7.5, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.45 (m, 2H, -CH_2-), 1.83 (m, 4H, -CH_2-), 3.30–3.97 (m, 20H), 3.43 (s, 3H, α OCH_3), 3.97 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.5–4.65 (m, 3H), 4.95 (d, J = 3.3, 1H, H1), 5.1 (m, 1H), 6.25 (m, 1H, NH), 6.54 (m, 1H, NH), 6.71 (m, 1H, NH), 7.23- 7.33 (m, 12H, trityl and benzylic H), 7.47–7.53 (m, 9H, trityl and benzylic H), 7.86–8.03 (m, 3H, benzylic H). ^{13}C-NMR (300 MHz, $CDCl_3$): 29.33, 29.47, 29.66 (-CH_2-), 37.49, 37.81, 38.05 (-CH_2-NH), 42.94, 44.52, 44.53 (-CO-$\underline{C}H_2$-N), 54.84 (α OCH_3), 56.12, 56.15, 56.2 (OCH_3), 62.33 (C6), 69.48 (O-CH_2-), 70.29 (C4), 70.91, 71.48 (O-CH_2-), 77.19 (C2), 78.51 (C5), 80.44 (C3), 86.34 ($\underline{C}Ph_3$), 97.17 (C1), 115.18, 115.49, 115.52 125.21, 125.28, 125.66, 127.0, 127,76, 128.71, 129.21, 129.51, 129.63, 136.89, 137.85, 138.0, 143.81, 144.39, 144.65, 144.73 (aromatic C), 146.59, 146.87, 146.96 (CO-NH), 159.27, 159.65, 159.71 (N-$\underline{C}O$-O), 164.72, 165.46, 165.57 ($\underline{C}O$-CH_3), MS (ESI) m/z 1461.7 ([M+Na]$^+$).

*Methyl 2,3,4-tris-O-{N-[N-(2,3-dihydroxybenzoyl)-glycyl]-aminopropyl}-6-O-trityl-α-*D*-glucopyranoside (***7f***), $C_{62}H_{70}N_6O_{18}$ (1187.3)*

To a solution of sodium hydroxide (192 mg) in water (5 ml) **7e** (400 mg, 0.3 mmol) was added under N_2 atmosphere. The reaction mixture was stirred at r.t. for 1 h and neutralized with 1 M HCl. A grey solid was precipitated, which was filtered and washed with water. The residue was dissolved in ethyl acetate and the extract washed with brine and water, dried over Na_2SO_4, filtered and evaporated in *vacuo*. **7f** (200 mg, 15%) was afforded (TLC, 3:1:0.5 chloroform-ethyl acetate-acetic acid, Rf = 0.12), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.54–1.74 (m, 6H, -CH_2-), 2.9–3.9 (m, 24 H), 3.33 (s, 2H, CO-CH_2-NH), 3.34 (s, 2H, CO-CH_2-NH), 3.36 (s, 2H, CO-CH_2-NH), 3.39 (s, α OCH_3), 4.8 (d, J = 3.4, H1), 6.51–6.90 (m, 6H, NH), 6.97-7.23 (m, 18H, trityl and benzylic H), 7.33–7.43 (m, 6H, trityl H), ^{13}C-NMR (300 MHz, $CDCl_3$): 29.96, 30.15, 30.32 (-CH_2-), 37.23, 37.74, 39.31 (-CH_2-NH), 43.58, 43.75, 43.62 (CO-$\underline{C}H_2$-NH), 55.32 (α OCH_3), 62.98 (C6), 70.19 (O-CH_2-), 70.69 (C4), 71.07, 71.23 (O-CH_2-), 76.70 (C2), 77.68 (C5), 78.19 (C3), 86.65 ($\underline{C}Ph_3$), 97.38 (C1), 117.5, 119.02, 119.09, 119.16, 119.32, 119.56, 127.13, 127.87, 128.74, 128.82, 129.21, 129.52, 143.21, 146.13, 146.19, 146.25, 147.35, 149.25, 149.52 (aromatic C), 165.77, 168.33, 168.87 (CH_2-$\underline{C}O$-NH), 170.84, 170.92, 172.84 (CO-NH), MS (ESI) m/z 592 ([M−H]$^{2+}$), 1209.7 ([M+Na]$^+$), 1185.7 ([M−H]$^+$).

*Methyl 2,3,4-tris-O-{N-[2,3-di(acetoxy)benzoyl]aminopropyl}-α-*D*-glucopyranoside (***8a***), $C_{49}H_{59}N_3O_{21}$ (1026.0)*

To a solution of **7a** (250 mg, 0.2 mmol) in dichloromethane boron trifluoride etherate (30 mg, 0.2 mmol) in methanol (0.08 ml, 0.2 mmol), was added at r.t.. The reaction was finished after about 15 min, until no educt was shown at TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid). The reaction

mixture was extracted three times with water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. **8a** was precipitated in light petroleum and diethyl ether as a colourless solid (148 mg, 78%). $[\alpha]_D^{20}$ + 31.2 (*c* 2.1, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.70–1.78 (m, 6H, -CH_2-), 2.18–2.22 (m, 18H, CO-CH_3), 3.10–3.80 (m, 18H), 3.19 (s, 3H, α OCH_3), 4.72 (d, J = 3.5, 1H, H1), 6.63 (t, J = 5.3, 1H, CO-NH), 6.77–6.83 (m, 2H, CO-NH), 7.10–7.28 (m, 6H, benzylic H), 7.36–7.42 (m, 3H, benzylic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 21.58 (CO-CH_3), 30.57, 30.92, 31.16 (-CH_2-), 38.53, 39.02, 39.24 (-CH2-NH), 55.92 (α OCH_3), 62.55 (C6), 70.13, 71.70, (O-CH_2-), 71.78 (C4), 72.80 (O-CH_2-), 78.74 (C2), 81.58 (C5), 82.60 (C3), 98.28 (C1), 126.57, 126.62, 127.12, 127.25, 127.35, 127.54, 131.64, 131.67, 131.77, 140,87, 141.22, 141.42, 144.05, 144.13, 144.28 (aromatic C), 166.12, 166.30, 166.36 (CO-NH), 169.08, 169.12, 169.16, 169.21, 169.27, 169.43 (C<u>O</u>-CH_3), MS (ESI) m/z 1026.8 ($[M+H]^+$), 1048.5 ($[M+Na]^+$).

*Methyl 2,3,4-tris-O-{N-[3,4-di(methoxycarbonyloxy) benzoyl]-aminopropyl}-α-*D*-glucopyranoside (***8c***), $C_{49}H_{59}N_3O_{27}$ (1116.1)*

To a solution of **7c** (600 mg, 0.4 mmol) in dichloromethane, boron trifluoride etherate (60 mg, 0.4 mmol) in methanol (0.16 ml, 0.4 mmol), was added at r.t. The reaction was finished after about 20 min, until no educt was shown at TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid, Rf = 0.1). The reaction mixture was treated as above and **8c** was afforded as colourless foam (336 mg, 75%), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.74-1.85 (m, 6H, -CH_2-), 3.35–3.97 (m, 18H), 3.32 (s, 3H, α OCH_3), 3.9 (6s, 18H, OCH_3), 4.83 (d, J = 3.4, 1H, H1), 7.0–7.07 (m, 2H, NH), 7.1–7.17 (m, 1H, NH), 7.22–7.34 (m, 6H, benzylic H), 7.45–7.47 (m, 3H, benzylic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 29.33, 29.69, 29.86 (-CH_2-), 37.55, 38.39, 38.94 (-CH_2-NH), 54.86 (α OCH_3), 55.89 (COO<u>C</u>H_3), 61.60 (C6), 70.25, 70.65 (O-CH_2-), 70.86 (C4), 71.64 (O-CH_2-), 77.73 (C2), 80.77 (C5), 81.37 (C3), 97.28 (C1), 122.15, 122.20, 122.23, 123.02, 123.05, 112.31, 125.51, 125.58, 125.77, 133.47, 133.52, 133.55, 142.20, 142.25, 142.29, 144.52, 144.56, 144.59 (aromatic C), 152.79, 153.05, 153.11, 165.18 (2C), 165,54 (2C), 165.67 (2C) (O-C<u>O</u>-CH_3), MS (ESI) m/z 1122 [$(M+H)^+$], 1144 [$(M+Na)^+$].

*Methyl 2,3,4-tris-O-{N-[2,3-di(acetoxy)phenoxyace tyl]-amino propyl}-α-*D*-glucopyranoside (***8d***), $C_{52}H_{65}N_3O_{24}$*

To a solution of **7d** (300 mg, 0.22 mmol) in dichloromethane, boron trifluoride etherate (33 mg, 0.22 mmol) in methanol (0.09 ml, 0.22 mmol) was added at r.t. The reaction was finished after about 20 min, until no educt was shown at TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid). The reaction mixture was treated as above and **8d** was afforded as colourless foam (201 mg, 82%). $[\alpha]_D{}^{20}$ + 26.2 (*c* 4.3, methanol), ^{1}H-NMR (300 MHz, $CDCl_3$): 1.7–1.77 (m, 6H, -CH_2-), 2.26–2.33 (m, 18H, CCH_3), 3.10–3.79 (m, 18H), 3.32 (s, 3H, α OCH_3), 4.55 (CO-CH_2-O), 4.58 (CO-CH_2-O),4.58 (CO-CH_2-O), 4.69 (d, J = 3.4, 1H, H1), 6.79–6.87 (m, 3H, benzylic H), 6.96–7.04 (m, 3H, NH), 7.24–7.34 (m, 6H, benzylic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 20.51 (-CH_3), 29.67, 29.99 (2C) (-CH_2-), 36.26, 36.34, 37.09 (CH_2-NH), 54.93 (α OCH_3), 61.46 (C6), 67.53, 67.59, 67.94 (O-C<u>H</u>$_2$-CO), 68.33, 68.87 (O-CH_2-), 70.70 (C4), 71.13 (O-CH_2-), 77.47 (C2), 80.61 (C5), 81.64(C3), 97.58 (C1), 110.01, 110.14, 110.53, 116.30, 116.33, 116.39, 126.50, 126.58, 126.96, 131.95, 131.99, 132.08, 143.51, 143.53, 143.55 (aromatic C), 150.01, 150.11, 150.23 (CO-NH), 167.62, 167.70, 167.85, 168.09, 168.14, 168.22 (C<u>O</u>-CH_3), MS (ESI) m/z 1138 ($[M+Na]^+$).

*Methyl 2,3,4-tris-O-[N-(8-methoxycarbonyloxy-3,4-dihydro-2H-1,3-benzoxazin-3-yl-acetyl)-amino propyl]-α-*D*-glucopyranoside (***8e***), $C_{52}H_{56}N_6O_{27}$ (1197.1)*

To a solution of **7e** (300 mg, 0.21 mmol) in dichloromethane, boron trifluoride etherate (30 mg, 0.21 mmol) in methanol (0.09 ml, 0.21 mmol), was added at r.t.. The reaction was finished after about 20 min, until no educt was shown at TLC (3:1:0.5 chloroform-ethyl acetate-acetic acid). The reaction mixture was treated as above and **8e** was afforded as colourless foam (125 mg, 50%),. ^{1}H-NMR (300 MHz, $CDCl_3$): 1.6–1.75 (m, 6H, -CH_2-), 3.05–3.84 (m, 20H), 3.27 (s, 3H, α OCH_3), 3.79 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.43–4.53 (m, 3H), 4.70 (d, J = 3.2, 1H, H1), 5.0 (m, 1H), 6.9–7.1 (m, 3H, NH), 7.1–7.25 (m, 3H, benzylic H), 7.3–7.5 25 (m, 3H, benzylic H), 7.7–7.9 25 (m, 3H, benzylic H), ^{13}C-NMR (300 MHz, $CDCl_3$): 29.80, 30.06, 30.22 (-CH_2-), 37.46, 37.94, 38.37 (-CH_2-NH), 43.37, 44.90, 44.92 (O-CH_2-N), 53.85 (α OCH_3), 55.42, 56.59, 56.66 (OCH_3), 61.79 (C6), 69.33, 70.81 (O-

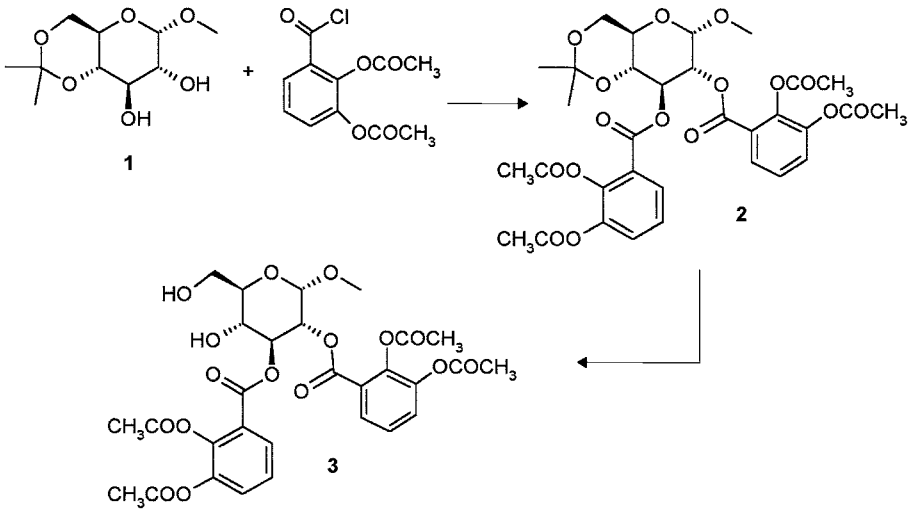

Fig. 1. Synthesis of biscatecholate derivatives.

CH_2-), 71.33 (C4), 71.82 (O-CH_2-), 78.09 (C2), 80.83 (C5), 81.65 (C3), 97.79 (C1), 115.57, 115.83, 115.89, 125.76, 126.06, 126.21, 129.63, 129.93, 130.08, 137.24, 138.20, 138.38, 144.78, 145.09, 145.11 (aromatic C), 147.09, 147.34, 147.46, 159.74, 160.12, 160.18, 164.38, 165.13, 165.26, 166.60, MS (ESI) m/z 1219 ($[M+Na]^+$).

Results and discussion

Synthesis of biscatecholate derivatives

Several biscatecholate derivatives possessing amino acid scaffolds, such as *N*,*N*′- bis-(2,3 dihydroxybenzoyl)-L-lysine are known as bacterial siderophores (Corbin & Bulen 1969). For the synthesis of biscatecholates with a carbohydrate backbone we started from methyl 4,6-isopropylidene-α-D-glucopyranoside **1** which was prepared from methyl α-D-glucopyranoside and dimethoxypropane (Bazin *et al.* 1995). Compound **1** contains two neighbouring free OH-groups with trans-orientation in the 2- and 3-position. From **1** and 2,3-di(acetoxy)benzoyl chloride (Rastetter *et al.* 1981) we obtained the methyl 2,3-di-*O*-(2,3-diacetoxybenzoyl)-4,6-*O*-isopropylidene-α-D-glucopyranoside **2.** Removal of the isopropylidene group by acetic acid (Hanessian *et al.* 1983) furnished methyl 2,3-bis-*O*-[2,3-di(acetoxy)benzoyl]-α-D-glucopyranoside **3** (Figure 1).

Preparation of triscatecholate siderophore analogs

Our approach towards triscatecholate siderophore analogs is based on a carbohydrate backbone (Figure 2). At first, we prepared the methyl 6-O-trityl-α-D glucopyranoside **4** (Bazin *et al.* 1995) containing three free neighbouring OH groups in 2-, 3-, and 4-position. In a Michael type cyanoethylation reaction compound **4** was treated with acrylonitrile and potassium hydroxide to yield methyl 2,3,4-tri-O-(2-cyanoethyl)-6-O-trityl-α-D-glucopyranoside **5**. The latter compound was hydrogenated by Co_2B and $NaBH_4$ affording methyl 2,3,4-tri-O-(3-aminopropyl)-6-O-trityl-α-D-glucopyranoside **6** as the crystalline key intermediate for the attachment of different chelating groups. By reaction of **6** with 2,3-di(acetoxy)benzoyl chloride (Rastetter *et al.* 1981) and 3,4-di-(methoxycarbonyloxy)benzoyl chloride (Heinisch *et al*. 1992), resp., and triethylamine in tetrahydrofurane we obtained the methyl 2,3,4-tris-O-{*N*-[2,3-di(acetoxy)benzoyl]-aminopropyl}-6-O-trityl-α-D-glucopyranoside **7a** and the corresponding 3,4-di(methoxycarbonyloxy)benzoyl compound **7c**. Removal of the trityl group by BF_3-etherate gave the methyl 2,3,4-tris-O-{*N*-[2,3-di(acetoxy)benzoyl]-aminopropyl}-α-D-glucopyranoside **8a** and the corresponding 3,4-di(methoxy-carbonyloxy)benzoyl derivative **8c**. Compound **7a** was deacetylated by 1 M sodium hydroxide solution under N_2 to methyl 2,3,4-tris-O-[*N*-(2,3-dihydroxybenzoyl)-aminopropyl]-6-tri-

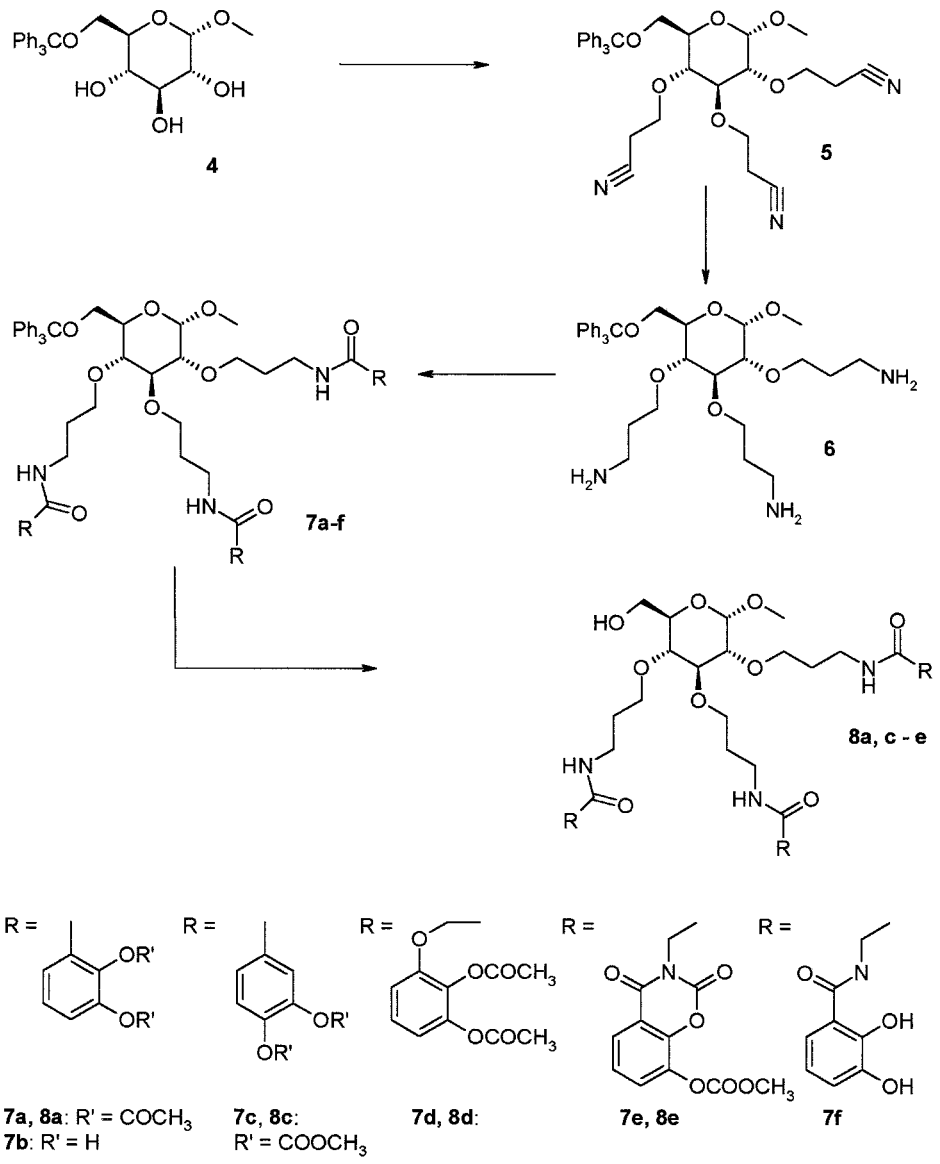

Fig. 2. Synthesis of triscatecholate derivatives.

tyl-α-D-glucopyranoside **7b** containing free catecholic OH-groups.

Siderophore analogs with longer spacer units were synthesized by reaction of **6** with 2,3-di(acetoxy)phenoxyacetyl chloride **9b** (prepared from 2,3-diacetoxyacetic acid **9a** and PCl_5 (Egbe 1995) and with 8-methoxycarbonyloxy-3,4-dihydro-2H-1,3-benzoxazin-3 -yl-acetyl chloride (Wittmann *et al.* 1999) resulting in the O-trityl derivatives **7d** and **7e**. Removal of the trityl groups by BF_3 etherate (Dax *et al.* 1978) afforded the derivatives **8d** and **8e**. Compound **7e** was hydrolysed by 1 M sodium hydroxide under N_2 to give methyl 2,3,4-tris-O-N-[N-(2,3- dihydroxybenzoyl)-glycyl]-3-aminopropyl-6-O-trityl- α-D-glucopyranoside **7f**.

Investigation of siderophore activity

The siderophore activity of the synthesized siderophore analogs and selected educts was examined by the growth promotion assay (Reissbrodt *et al.* 1993) using various bacteria, which have been well defined in their ability to transport and utilize natural siderophores (siderophore indicator strains). In a first series of

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

Indicator strain	Diameter of growth zone (mm), substance application 5 μg Compound												
	2	3	7a	7b	7c	7d	7e	7f	8a	8c	8d	8e	Control
S. typhimurium: enb-7	10	17	15	0	0	0	18	18	32	0	0	0	28[a]
S typhimurium TA 2700	0	0	10	n.d.	n.d.	n.d.	n.d.	n.d.	33	n.d.	n.d.	n.d.	30[b]
E. coli AB 2847	0	10	0	0	0	0	0	0	25	0	0	0	28[c]
M. morganii SBK 3	0	0	0	0	0	0	0	10	10	0	0	24	22[d]
Y. enterocolitica H5030	0	0	0	10	0	10	0	0	0	0	16	0	18[c]
Strains of antibacterial screening													
P. aeruginosa ATCC 27853	0	0	0	0	0	0	0	12	n.d.	0	15	14	35[e]
P. aeruginosa SG 137	0	0	15	0	16	15	11	15	22	16	16	14	30[e]
P. aeruginosa NCTC 10662	0	0	15	0	0	0	14	15	25	15	22	17	30[e]
P. aeruginosa ATCC 9027	0	0	0	0	0	15	0	15	25	0	17	18	35[e]
E. coli ATCC25922	0	0	12	11	0	15	15	19	35	12	22	21	38[f]

[a] ferrioxamin G.
[b] enterobactin.
[c] ferrichrome.
[d] 2,3-dihydroxybenzylidene-1,3,5-trimethylaniline (Reissbrodt *et al.* 1993).
[e] desferal.
[f] ferricrocin (2 μg).

experiments we used the following Gram-negative indicator strains (Iron related marker): *Salmonella typhimurium* enb-7 (enterobactin-) and TA 2700 (enterobactin-, FEB C-), *E. coli* AB 2847 (aromates biosynthesis-), *E. coli* IR 112 (aroB-, tonB-), *Klebsiella pneumoniae* KN 4401(enterobactin-, aerobactin biosynthesis-), *Morganella morganii* SBK 3 (wild type),*Yersinia enterocolitica* H 5030 (yersiniabactin-) and *Pseudomonas aeruginosa* PAO 6609 (pyoverdin-). None of the above compounds stimulated the growth of the indicator strains *P. aeruginosa* PAO 6609, *Klebsiella pneumoniae* KN 4401 and *E. coli* IR 112 missing the protein tonB. This result suggested, that the presence of tonB is necessary for siderophore activity of these compounds. The results of growth promotion tests with the other strains are given in Table 1. All new compounds showed siderophore activity, which was particularly visible with special bacterial strains. The biscatechol derivatives **2** and **3** displayed only a low growth promotion of the indicator strains *S. typhimurium* enb-7 and *E. coli* AB 2847, but the triscatechol derivatives showed different activities. The methyl α-D-glucopyranoside **8a** with three 2,3-di(acetoxy)benzoyl ligands promoted very strongly the growth of indicator strains *S. typhimurium* enb-7, TA 2700 and *E. coli* AB 2847 but not that of the other indicator strains. The corresponding 3,4-di(methoxycarbonyloxy)benzoyl derivative **8c** showed only a minor activity against the strains *P. aeruginosa* ATCC 27853, SG 137 and *E. coli* ATCC 25922. However, compound **8d** promoted *Yersinia enterocolitica* H 5030. Derivatives **7a–f** with a trityl substituent were of lower activity.

In a second series of experiments, we used the following Gram-negative strains of the antibacterial screening, grown under iron limitation: *P. aeruginosa* ATCC 27853, SG 137, NCTC 10662 and *E. coli* ATCC 25922. In these experiments compounds **7f** with trityl group and **8a–e** missing the trityl group were active in growth promotion of the strains but compounds **7a–c** were less active. No activity was found for compounds **2** and **3**.

Moreover, we tested the new compounds for growth promotion of mycobacteria, such as the wild type strain of *M. smegmatis* SG 987 and mc^{2}155, mutants of *M. smegmatis* M10 (exochelin-) and M24 (mycobactin-), a wild type of *M. phlei* 239 (exochelin-) and the mutant B1 of *M. smegmatis* mc^{2}155 generated by gene replacement (blocked in exochelin biosynthesis) (Schumann *et al.* 1998). The results are depicted in Table 2. In this assay the compounds **2** and **3** were inactive. Compounds **7a–f** and **8a, 8c–e** promoted the growth of either one or several of these strains. The most active substances were **7f** (a derivative with elongated spacer) and **8a** (2,3-di(acetoxy)benzoyl derivative).

Table 2. Growth promotion of the synthesized siderophore analogs and selected educts on test strains of mycobacteria

Indicator strain	Diameter of growth zone (mm), substance application 5 μg Compound												
	2	3	7a	7b	7c	7d	7e	7f	8a	8c	8d	8e	mycobactin (2 μg)
SG 987	0	0	9	14	0	13	10	28	23	0	10	10	17
M10	0	0	16	15	13	14	13	20	21	0	0	0	17
M77	0	0	0	0	0	0	0	0	0	10	0	0	17
mc^2 155	0	0	15	15	0	16	12	30	25	13	14	14	19
M24	0	0	0	0	0	0	0	8	26	0	0	0	19
B1	0	0	10	13	10	13	11	25	20	17	15	15	16

In a third series we tested the above compounds for their iron complexing capacity using the chromazurol-S test (CAS-assay) (Schwyn & Neilands 1987). In this assay the compounds **7f**, **8a**, **8c** and **8e** were active visible by the change of the blue colour of the chromazurol S-iron complex to the orange of the iron free chromazurol S and the new iron siderophore complex.

In conclusion, synthesis of monosaccharide based siderophore analogs displaying high activity as siderophores was accomplished for the first time. Such compounds are of interest as penetration vectors for antibiotics or other biological applications involving the iron uptake and metabolism.

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