Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron 62 (2006) 7799–7808
Tetrahedron

Conjugates of methyl 6-aminopenicillanate with biscatechol-hydroxamate chelators: synthesis and siderophoric activity

Rainer Schobert,* Andreas Stangl and Kerstin Hannemann

Organisch-chemisches Laboratorium der Universität, 95440 Bayreuth, Germany

Received 27 March 2006; revised 10 May 2006; accepted 24 May 2006
Available online 15 June 2006

Abstract—A synthesis of biscatechol-hydroxamate and triscatechol chelators and of conjugates of the former with methyl 6-aminopenicillanate is described. It is based on the multiple use of the ylide Ph_3PCCO as a C_2 building block in one-pot coupling reactions between aldehydes and alcohols or amines. These conjugates were actively internalized into siderophore-deficient *Escherichia coli* mutant H5596.

1. Introduction

Resistance of microbial pathogens against β-lactam antibiotics is a haunting problem for the clinician, which partakes of a vicious circle as the main reason for it is an overprescription of antibiotics by physicians and GPs in particular. Mechanistically, β-lactam resistance can arise from various factors. Gram-negative bacteria are especially hard to tackle due to their outer membrane permeability barrier,[1,2] which can be overcome, though, by transporter-mediated active internalization[2,3] of specific compounds such as siderophores. These microbial secondary metabolites are iron chelators excreted to sequester extracellular ferric ions under iron starvation conditions.[2–6] Specific outer membrane receptors recognize the siderophore–iron complex and initiate its transport into the cell. Many natural siderophores are trisbidentate ligands employing catechol, salicylate or hydroxamate groups in iron complexation. On the other hand, this active import of ferrisiderophores also opens a flank in the microbial defence strategy. There are many examples of natural conjugates of siderophores with antibiotic effectors mainly produced by *Streptomyces* (sideromycins),[7] which are actively taken up into bacterial target cells via iron transport systems.

Following this 'trojan horse' strategy new antibiotics against Gram-negative pathogenic bacteria were devised, including covalent β-lactam–siderophore conjugates.[4,7–10] The conjugate of a synthetic hydroxamate-biscatechol siderophore with Lorabid®, for example, was found to be about 2000 times more active in vitro against certain *Acinetobacter* strains than Lorabid® itself.[9] Ampicillin conjugates with natural pyoverdins showed activity against multi-resistant *Pseudomonas aeruginosa*, an opportunistic bacterium responsible for frequently lethal hospital infections.[10]

In this paper we report on the synthesis of new iron triscatechol chelators as mimics of natural siderophores such as protochelin and agrobactin and also of hydroxamate-biscatechol chelators resembling semisynthetic siderophores such as spermexatol **1**[11] (Fig. 1). Those hydroxamate-biscatechol ligands showing siderophoric properties were then covalently linked to methyl 6-aminopenicillanate. The resulting

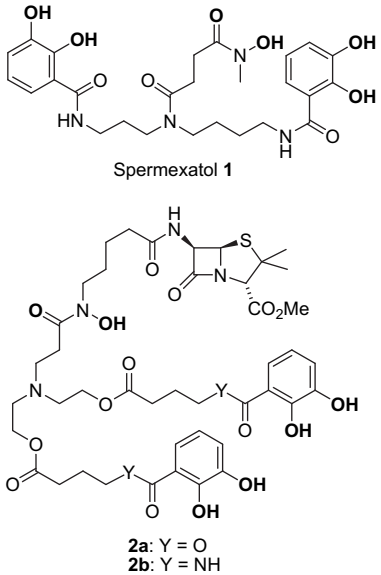


Figure 1.

Keywords: β-Lactams; Siderophores; Phosphorus ylides; Conjugates.
* Corresponding author. Fax: +49 921 552671; e-mail: rainer.schobert@uni-bayreuth.de

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.05.056

conjugates **2** were tested for siderophoric activity. The synthesis of both the siderophore precursors and their conjugates with methyl penicillanate was based on the use of ketenylidenetriphenylphosphorane, Ph_3PCCO **3**[12] as an acylating C_2 building block.

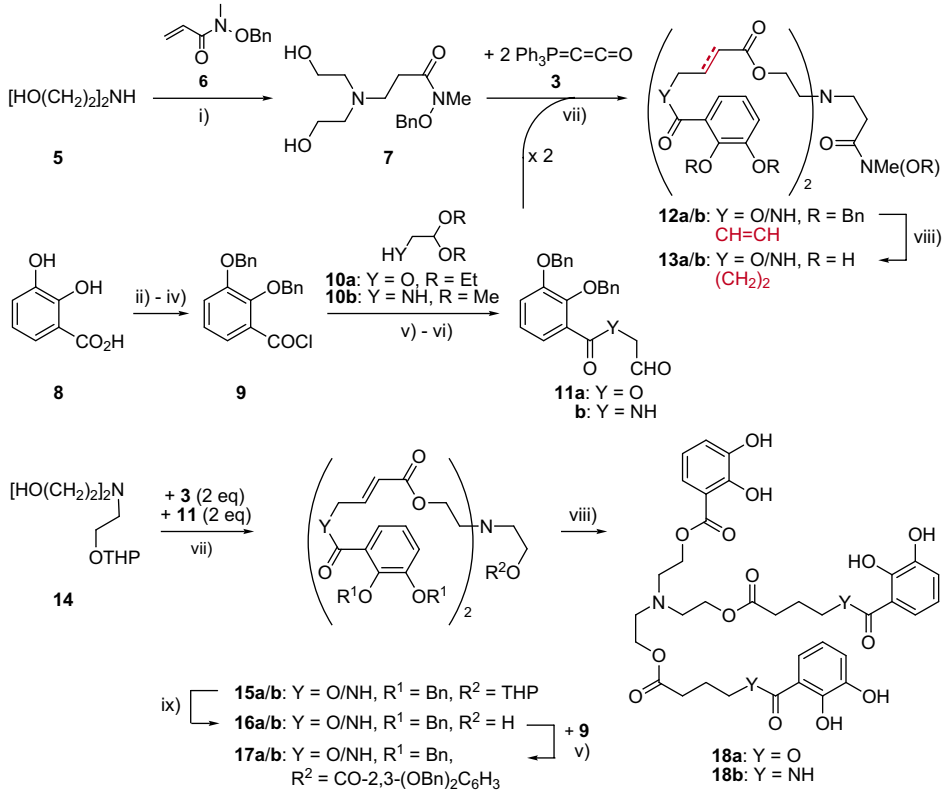
2. Results and discussion

As shown in Figure 1, the siderophores were attached to the 6-amino group of methyl 6-amino-penicillanate [6-APA(OMe)] **4** via an amide bond, which we reckoned would probably survive internalization and enzymatic dismanteling of the siderophore–iron complex. A diethanolamine fragment served as the hub of the siderophore moiety. It carried two catechol appendages optionally linked via ester (Y═O) or amide (Y═NH) bonds and a third chelate ligand (a hydroxamate in case of the conjugates **2**) as part of the tether leading to the penicillin. In modelling studies we ascertained that a strainfree octahedral coordination of Fe^{3+} would be possible for these tris-bidentate ligands. Although it is a well-known fact that a free carboxylic acid functionality at C-3 is required for a penicillin to exhibit antimicrobial activity, we prepared only conjugates of the methyl penicillanate. At this point we just wanted to see whether the ferriconjugate complexes are still being recognized by the receptors, internalized and broken down in order to release growth-promoting ferric ions. The *Escherichia coli* mutant strain H5596 on which we tested all chelators and conjugates lacks own siderophores yet possesses fully functional receptors. If kept under iron limitation, these bacteria depend on external siderophoric substances. Like many *E. coli* strains it is, however, relatively insensitive towards β-lactam antibiotics. Once the siderophoric properties of the herein described siderophore–penicillinate conjugates are proven, tests for antibiotic activity will have to be carried out with the analogues featuring a free carboxylic acid at C-3 of the penicillin on other more susceptible bacteria with similar receptors. Only this would make sure that any growth inhibition originates not from iron starvation but from a genuine penicillin effect.

2.1. Synthesis of the iron chelators

We first prepared chelators that did not provide an anchor for linkage to the penicillin and tested them for siderophoric properties (Scheme 1).

To construct biscatechol-hydroxamate chelators of type **13**, diethanolamine **5** was added to acryl amide **6** to give the monoprotected triol **7**. Compound **6** was obtained from reaction of acryl chloride and *N*-methyl-*O*-benzylhydroxylamine, which in turn was prepared from commercial hydroxylamine hydrochloride as described by Grigg et al.[13] The catechol residues were attached to the diethanolamine hub via an *E*-α,β-unsaturated ester linkage generated by a three-component reaction[14] between diol **7** and 2 equiv each of a suitable aldehyde **11** and the ylide Ph_3PCCO **3**. In this domino process the OH-groups of alcohol **7** added across the central C═C bond of the ylide **3** to form a stabilized ester ylide, which in turn Wittig-alkenated the aldehyde group in compounds **11** to leave the pentabenzylated, bisunsaturated pre-chelators **12**. Then, simultaneously, the olefinic double



Scheme 1. Reagents and conditions: (i) EtOH, rt, 16 h, 77%; (ii) BnBr, K_2CO_3, acetone, reflux, 16 h; (iii) NaOH (2 M), MeOH, rt, 1.5 h, then aq HCl; (iv) $SOCl_2$, CH_2Cl_2, rt, 3 h; (v) CH_2Cl_2, Py, rt, 3 h; (vi) HCl (15%), acetone/THF, rt, 16 h, 80–90%; (vii) THF, rt, 6–12 h, 60–80%; (viii) H_2, Pd/C, MeOH, 6 h, 99%; (ix) *p*-TsOH, toluene, 60 °C, 16 h, ca. 80%.

bonds were saturated and the benzyl protecting groups were removed with hydrogen gas at palladium on charcoal to afford the ligands **13** as colourless or pale yellow solids.

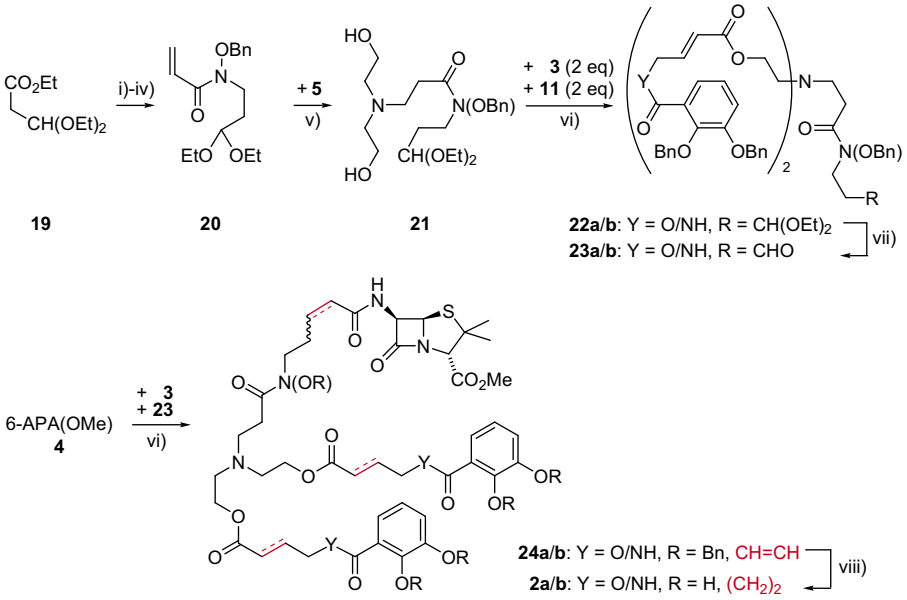
In a similar way we also built up ligands **18** featuring the prominent triscatechol siderophore motif. The mono-THP-protected trisethanolamine **14**[15] was submitted to a three-component reaction with ylide **3** and the appropriate aldehyde **11** to furnish the branched bis-bidentate precursor ligands **15**. Removal of the THP protecting group of **15** with *para*-toluenesulphonic acid in toluene at 60 °C for 16–32 h yielded alcohol **16**, which was subsequently acylated with dibenzoxybenzoyl chloride **9**. Catalytic hydrogenation of **17**, again with palladium (5%) on charcoal, left the free triscatechol compounds **18** as colourless solids. Acid chloride **9** was readily accessible in three steps from dihydroxybenzoic acid **8**, namely by perbenzylation with benzyl bromide, saponification of the intermediate benzyl dibenzoxybenzoate with aqueous NaOH in methanol and treatment of the resulting dibenzoxybenzoic acid with thionyl chloride in dichloromethane. It is worthy of note that conducting the saponification step on a prepurified sample of benzyl dibenzoxybenzoate under less drastic conditions than those described by Gardner et al.[16] (2 M NaOH, 1.5 h, rt instead of 5 M NaOH, 3 h, reflux) improved the yield and purity of the product considerably. Acid chloride **9** was also used to prepare the catechol substituted aldehydes **11** by coupling with the hydroxy or amino substituted acetaldehyde acetals **10** followed by acidic hydrolysis.

2.2. Synthesis of penicillinate–siderophore conjugates

In a previous paper[17] we introduced an expeditious one-pot protocol for the acylation of the 6-amino group of 6-APA or its esters with Ph_3PCCO **3** and different aldehydes. To apply this method to the synthesis of penicillin–siderophore conjugates **2**, the above described chelators **13** had to be fitted at some stage with an additional formyl anchor group. With only slight adaptations to the synthetic pathway outlined in the top half of Scheme 1, we prepared congeners of the biscatechol-hydroxamate ligands **13** that bore a 3-oxopropanyl instead of the methyl group on the hydroxamate nitrogen atom (Scheme 2). Compound **20**, the chain-lengthened analogue of benzoxamate **6** was obtained in four steps from ethyl 3,3-diethoxypropionate **19**. Selective ester reduction with 1 equiv of DIBAL-H[18] in diethyl ether at −78°C furnished the corresponding malonic aldehyde semiacetal, which was transferred into its benzyl protected oxime in an exothermic, quantitative reaction using *O*-benzylhydroxylamine in THF. Reduction[19] with $Na(CN)BH_3$ in dry ethanol/acetic acid gave the corresponding amine in ca. 70% yield, which in turn was added to acryl chloride. The benzoxamate **20** was then linked to diethanolamine in a further Michael-type addition, the resulting diol **21** was fused with the catechol aldehydes **11** in a three-component reaction with ylide **3** as described. The product compounds **22** were treated with aqueous HCl (10%) in acetone to yield the aldehydes **23**. The conjugates **2** were assembled by yet another three-component reaction between ylide **3**, the aldehydes **23** and 6-APA(OMe) **4**, which gave the fully protected conjugates **24** in ca. 60% yield. Their hydrogenolysis (H_2, Pd/charcoal, methanol/dioxane) finally afforded the target conjugates **2a** and **2b** as pale yellow solids.

2.3. Siderophoric activity

The chelators **13** and **18** were screened for siderophoric properties by applying cellulose discs containing small defined doses of them to agar dishes inoculated with *E. coli* strain H5596. This mutant strain lacks own siderophores yet possesses fully functional receptors. As summarized in Table 1, both the hydroxamate-biscatechols **13** and the triscatechols **18** exhibited distinct siderophoric growth-promoting activity with the amides **b** (Y=NH) markedly more so than the esters **a** (Y=O) in either case. The effect is comparable to that of the natural trishydroxamate



Scheme 2. Reagents and conditions: (i) DIBAL-H, Et_2O,−78 °C, 1 h; (ii) H_2NOBn, THF, rt, 2 h; (iii) $Na(CN)BH_3$, EtOH/AcOH, 0 °C to rt, 12 h, 70%; (iv) HC=CHCOCl, pyridine/$CHCl_3$, 0 °C to rt, 1 h; (v) EtOH, rt, 24 h, 85%; (vi) THF, rt, 16 h, 55–62%; (vii) 10% aq HCl, acetone, rt, 1 h, 90%; (viii) H_2, Pd/C (5%), MeOH, rt, 12 h, 60–64%.

Table 1. Promotion of growth of siderophore-deficient *E. coli* mutant H5596 under iron limitation by chelators **13**, **18**, penicillin–chelator conjugates **2** and ferrichrome as a standard[a,b]

Applied quantities (μg)	**13a**	**13b**	**18a**	**18b**	**2a**	**2b**	Ferrichrome
150	25 (0.23)	29 (0.23)	26 (0.21)	30 (0.21)	19 (0.15)	25 (0.15)	—
75	21 (0.12)	26 (0.12)	23 (0.10)	26 (0.10)	16 (0.08)	22 (0.08)	—
38	17 (0.06)	20 (0.06)	19 (0.05)	21 (0.05)	12 (0.04)	17 (0.04)	—
15	12 (0.02)	13 (0.02)	15 (0.02)	15 (0.02)	0 (0.015)	13 (0.015)	17 (0.02)

[a] Agar plates (Mueller–Hinton II medium, 100 μg mL^{-1} EDDA) inoculated with 100 μL of an *E.* coli H5596 suspension were covered with 6 mm cellulose discs containing 15 μL of an ethanolic solution (10, 5, 2.5 or 1 mg mL^{-1}) of the respective compound. The diameters (in mm) of the resulting growth zones were determined after 24 h of incubation at 36 °C and are cited here. The applied quantities in micromolars for each compound are given in parentheses.
[b] Compounds **25** and **26** proved inactive (Ø=0 mm) and are not listed here.

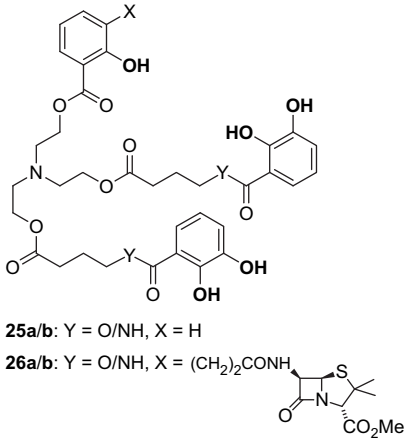


Figure 2. Pentadentate ligands lacking siderophore activity.

siderophore ferrichrome.[4,5] The siderophore–β-lactam ester conjugates **2a** and **2b** also effected normal growth of the bacteria indicating an active transport of iron from the medium into the cells. Interestingly, deleting just one of the six donor atoms required for an octahedral coordination of the metal ion resulted in complete loss of siderophoric activity regardless of whether or not a penicillin is attached. For example, neither the biscatechol-salicylate chelators **25**[20] nor their conjugates **26**[21] were effective in overcoming the iron deprivation of *E. coli* H5596 caused by treatment with ethylenediamine bis(*o*-hydroxyphenyl)acetic acid, EDDA (Fig. 2).

In conclusion we have demonstrated that chelators of the biscatechol-hydroxamate type and conjugates thereof with methyl penicillanate can be prepared in few steps by extensive use of three-component reactions between appropriate aldehydes, amines or alcohols and the cumulated phosphorus ylide Ph_3PCCO. We have also established that the conjugates are still being recognized and internalized by the pertinent receptors and hence exhibit siderophore activity as long as donor atoms are available to maintain the characteristic pattern of an octahedral ligand sphere. We are currently synthesizing the corresponding conjugates featuring a free penicillanic acid moiety in order to test them for antimicrobial activity against susceptible germs.

3. Experimental

3.1. General

Melting points were determined with a Gallenkamp apparatus and are uncorrected. IR-spectra were recorded on spectrophotometers Perkin–Elmer One FTIR and Perkin–Elmer 1600 Series FTIR. Magnetic resonance (NMR) spectra were recorded under conditions as indicated on a Bruker Avance 300 spectrometer. Chemical shifts (δ) are given in parts per million downfield from TMS as internal standard. Mass spectra were recorded using a Varian MAT 311A (EI). Elemental analyses were carried out with a Perkin–Elmer 2400 CHN elemental analyser. Optical rotations were determined at 589 nm with a Perkin–Elmer 343 polarimeter. For column chromatography Merck silica gel 60 (230–400 mesh) was used. Solvents were dried and distilled (THF, diethyl ether and dioxane over Na/Ph_2CO, CH_3OH over CaO, acetone over K_2CO_3, CH_2Cl_2 and $CHCl_3$ over P_2O_5) and stored under argon. Starting compounds were purchased from the usual sources and were used without further purification.

3.1.1. Synthesis of the iron chelators 13 and 18.

3.1.1.1. *N*-Benzyloxy-*N*-methyl 3-[bis-(2′-hydroxyethyl)amino]propionamide 7. A solution of diethanolamine **5** (0.74 g, 7.0 mmol) and *N*-benzoxy-*N*-methylacrylamide **6** (1.34 g, 7.0 mmol), as obtained in 90% yield from *N*-methyl-*O*-benzyl hydroxylamine[13] and acryl chloride in chloroform/pyridine, in ethanol (20 mL) was stirred at room temperature for 16 h. The solvent was evaporated and the residue filtered over silica gel 60 (ethyl acetate/methanol 4:1) and dried on an oil pump to give **7** as a pale yellow viscous oil (1.60 g, 77%); ν_{max}/cm^{-1} 3395, 2941, 1645, 1454, 1389; δ_H (300 MHz; $CDCl_3$) 2.40–2.55 (6H, m, NCH_2C–O, O=CCH_2), 2.71 (2H, t, ^3J=6.3 Hz, NCH_2CC=O), 3.10 (3H, s, CH_3), 3.48 (4H, t, ^3J=5.4 Hz, $NCCH_2OH$), 3.84 (2H, s, OH), 4.76 (2H, s, CH_2Ph), 7.25–7.35 (5H, m); δ_C (75 MHz; $CDCl_3$) 30.1, 33.2, 48.7, 55.9, 59.2, 76.0, 128.5, 128.7, 129.0, 134.1, 174.1. Anal. Calcd for $C_{15}H_{24}N_2O_4$: C, 60.8; H, 8.2; N, 9.5. Found: C, 60.6; H, 8.1; N, 9.5%.

3.1.1.2. 2,3-Dibenzoxybenzoyl chloride 9. Perbenzylation of dihydroxybenzoic acid **8** (10.0 g, 64.9 mmol) was carried out as described in literature[16] yielding crude benzyl 2,3-dibenzoxybenzoate as an orange oil. This was dissolved in diethyl ether and washed with saturated aqueous $NaHCO_3$ and water. Drying (Na_2SO_4) and removal of all volatiles left a yellowish oil, which was extracted with hexane several times to remove excess benzyl bromide. The pure product (26.7 g, 62.9 mmol) was obtained as a colourless solid upon storage at −18 °C over night. Selective saponification with 2 M NaOH for 1–1.5 h at room temperature afforded 2,3-dibenzoxybenzoic acid (20.6 g, 61.6 mmol) as a colourless solid in 95% overall yield. Treating it with thionyl chloride (29.7 g, 250 mmol) in CH_2Cl_2 in the presence of

catalytic amounts of DMF and evaporation of the solvent left **9** (21.7 g, 61.6 mmol) as a pale yellow, waxy solid, which was used for the following reactions without further purification.

3.1.1.3. Formylmethyl 2,3-dibenzoxybenzoate 11a. 2′,2′-Diethoxyethyl 2,3-dibenzoxybenzoate. A solution of **9** (3.70 g, 10.5 mmol) in CH_2Cl_2 (30 mL) was added dropwise to a solution of 2,2-diethoxyethanol **10a** (1.60 g, 11.9 mmol) in CH_2Cl_2 (20 mL) containing pyridine (1.58 g, 20 mmol). The mixture was stirred for 3 h, then poured into cold satd aqueous $NaHCO_3$. The organic phase was washed with satd $NaHCO_3$, aqueous HCl and water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 2:1 v/v, R_f 0.62) giving 2′,2′-diethoxyethyl 2,3-dibenzoxybenzoate as a pale yellow oil (3.64 g, 77%); ν_{max}/cm^{-1} 2976, 1728, 1579, 1474, 1456; δ_H (300 MHz; $CDCl_3$) 1.22 (6H, t, ^3J=7.1 Hz, CH_3), 3.45–3.70 (4H, m, $MeCH_2$), 4.31 (2H, d, ^3J=5.4 Hz, CH_2CO_2), 4.75 (1H, t, ^3J=5.4 Hz, CHO_2), 5.12 (2H, s, CH_2Ph), 5.14 (2H, s, CH_2Ph), 7.00–7.15 (2H, m), 7.25–7.50 (11H, m); δ_C (75 MHz; $CDCl_3$) 15.2, 62.4, 64.4, 71.1, 75.5, 99.6, 118.0, 122.9, 123.9, 126.6, 127.4, 127.8, 128.0, 128.2, 128.7, 136.5, 137.5, 148.3, 152.7, 165.7; *m/z* (EI, 70 eV) 450 (M^+, 3%), 405 (6%), 314 (11%), 242 (33%), 91 (100%).

Formylmethyl 2,3-dibenzoxybenzoate **11a**. 2′,2′-Diethoxyethyl 2,3-dibenzoxybenzoate (3.62 g, 8.0 mmol) dissolved in acetone/THF 3:1 (v/v, 40 mL) was treated with aqueous HCl (15%, 20 mL) for 16–20 h (TLC control). The pH value was adjusted to 7 by addition of aqueous NaOH, most of the solvent was evaporated and ethyl acetate (100 mL) was added. The organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:1 v/v, R_f 0.28) to leave **11a** as a colourless, highly viscous oil (2.49 g, 82%); ν_{max}/cm^{-1} 2934, 2877, 1733, 1721, 1579, 1474; δ_H (300 MHz; $CDCl_3$) 4.74 (2H, s, CH_2CHO), 5.11 (2H, s, CH_2Ph), 5.14 (2H, s, CH_2Ph), 7.10–7.20 (3H, m), 7.25–7.45 (10H, m), 9.60 (1H, s, CHO); δ_C (75 MHz; $CDCl_3$) 68.9, 71.2, 75.7, 118.6, 122.8, 124.1, 125.3, 127.9, 128.1, 128.3, 128.6, 136.4, 137.3, 148.7, 152.8, 165.4, 196.0; *m/z* (EI, 70 eV) 376 (M^+, 6%), 285 (18%), 225 (26%), 181 (33%), 91 (100%). HR-EIMS calcd for $C_{23}H_{20}O_5$: *m/z* 376.1311. Found: 376.1313.

3.1.1.4. *N*-Formylmethyl 2,3-dibenzoxybenzamide 11b. *N*-(2′,2′-Dimethoxyethyl) 2,3-dibenzoxybenzamide. Analogously to 2′,2′-diethoxyethyl 2,3-dibenzoxybenzoate (Section 3.1.1.3), *N*-(2′,2′-dimethoxyethyl) 2,3-dibenzoxybenzamide (3.36 g, 70%) was obtained as a yellowish oil from **9** (4.01 g, 11.4 mmol) and **10b** (1.35 g, 12.9 mmol); R_f 0.54 (cyclohexane/ethyl acetate 1:2 v/v); ν_{max}/cm^{-1} 3384, 2933, 1654, 1576, 1532, 1455; δ_H (300 MHz; $CDCl_3$) 3.25 (6H, s, OMe), 3.48 (2H, t, ^3J=5.5 Hz, NCH_2), 4.36 (1H, t, ^3J=5.5 Hz, CHO_2), 5.07 (2H, s, CH_2Ph), 5.14 (2H, s, CH_2Ph), 7.05–7.15 (2H, m), 7.25–7.45 (11H, m), 7.70–7.80 (1H, m), 8.16 (1H, t, ^3J=5.5 Hz, NH); δ_C (75 MHz; $CDCl_3$) 41.1, 53.9, 71.1, 75.9, 102.3, 116.8, 123.1, 124.3, 127.1, 127.6, 127.7, 128.2, 128.5, 128.7, 129.8, 136.2, 146.6, 151.7, 165.1; *m/z* (EI, 70 eV) 421 (M^+, 8%), 298 (55%), 225 (21%), 208 (58%), 91 (100%).

N-Formylmethyl 2,3-dibenzoxybenzamide **11b**. Analogously to **11a** (Section 3.1.1.3), compound **11b** (2.23 g, 76%) was obtained from the acetal precursor (3.30 g, 7.83 mmol) as a colourless solid of mp 88–89 °C; R_f 0.38 (cyclohexane/ethyl acetate 1:4 v/v); ν_{max}/cm^{-1} 3377, 2928, 2872, 1728, 1654, 1576, 1523; δ_H (300 MHz; $CDCl_3$) 4.03 (2H, d, ^3J=5.2 Hz, NCH_2), 5.08 (2H, s, CH_2Ph), 5.13 (2H, s, CH_2Ph), 7.00–7.10 (2H, m), 7.20–7.50 (11H, m), 7.68 (1H, dd, ^3J=7.2, ^4J=2.5 Hz, H^{ar}), 8.52 (1H, t, ^3J=5.2 Hz, NH), 9.49 (1H, s, CHO); δ_C (75 MHz; $CDCl_3$) 50.2, 71.3, 76.0, 118.4, 124.4, 125.3, 126.7, 128.3, 128.5, 128.7, 128.8, 129.0, 136.3, 137.0, 147.0, 151.7, 165.5, 196.8; *m/z* (EI, 70 eV) 375 (M^+, 7%), 284 (10%), 225 (8%), 181 (11%), 91 (100%). HR-EIMS calcd for $C_{23}H_{21}NO_4$: *m/z* 375.1471. Found: 376.1472.

3.1.1.5. Perbenzylated biscatechol-hydroxamate 12a—typical procedure for the three-component reaction with ylide 3. A solution of **7** (72 mg, 0.24 mmol), **3** (175 mg, 0.58 mmol) and **11a** (200 mg, 0.53 mmol) in dry THF (20 mL) was stirred at room temperature for 6 h. The solvent was removed in vacuum and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:2 v/v, R_f 0.40) to give **12a** as a pale yellow foamy solid (166 mg, 63%); ν_{max}/cm^{-1} 2950, 1731, 1716, 1638, 1577, 1474; δ_H (300 MHz; $CDCl_3$) 2.47 (2H, t, ^3J=7.0 Hz, $OCCH_2$), 2.77 (4H, t, ^3J=6.2 Hz, NCH_2CO), 2.88 (2H, t, ^3J=7.0 Hz, NCH_2CCO), 3.16 (3H, s, CH_3), 4.15 (4H, t, ^3J=6.2 Hz, $NCCH_2O$), 4.79 (2H, s, $NOCH_2Ph$), 4.86 (4H, dd, ^3J=4.5, ^4J=1.8 Hz, $CH_2C{=}C$), 5.11 (4H, s, CH_2Ph), 5.13 (4H, s, CH_2Ph), 6.08 (2H, dt, $^3J_{trans}$=15.9, ^4J=1.8 Hz, $C{=}CHC{=}O$), 6.95 (2H, dt, $^3J_{trans}$=15.9, ^3J=4.5 Hz, $HC{=}CC{=}O$), 7.05–7.20 (4H, m), 7.30–7.50 (27H, m); δ_C (75 MHz; $CDCl_3$) 30.5, 50.1, 52.6, 62.6, 62.9, 71.1, 75.5, 76.1, 118.1, 122.0, 122.7, 123.9, 126.0, 127.3, 127.8, 128.0, 128.1, 128.5, 128.6, 128.9, 129.2, 134.3, 136.4, 137.1, 141.4, 148.3, 152.7, 165.2, 165.5. Anal. Calcd for $C_{65}H_{64}N_2O_{14}$: C, 71.2; H, 5.9; N, 2.6. Found: C, 69.9; H, 6.1; N, 2.7%.

3.1.1.6. Perbenzylated biscatechol-hydroxamate 12b. Analogously to **12a** (Section 3.1.1.5), **12b** (139 mg, 32%) was obtained from **7** (120 mg, 0.40 mmol), **3** (290 mg, 0.96 mmol) and **11b** (330 mg, 0.88 mmol); R_f 0.38 (cyclohexane/ethyl acetate 1:4 v/v); ν_{max}/cm^{-1} 2948, 1733, 1656, 1638, 1471, 1373; δ_H (300 MHz; $CDCl_3$) 2.41 (2H, t, ^3J=6.6 Hz, $OCCH_2$), 2.73 (4H, t, ^3J=6.0 Hz, NCH_2CO), 2.80 (2H, t, ^3J=6.6 Hz, NCH_2CCO), 3.16 (3H, s, CH_3), 3.90–4.00 (4H, m, $CH_2C{=}C$), 4.10 (4H, t, ^3J=6.0 Hz, $NCCH_2O$), 4.77 (2H, s, $NOCH_2Ph$), 5.08 (4H, s, CH_2Ph), 5.15 (4H, s, CH_2Ph), 5.78 (2H, dt, $^3J_{trans}$=16.2, ^4J=1.7 Hz, $C{=}CHCO$), 6.80 (2H, dt, $^3J_{trans}$=16.2, ^3J=5.7 Hz, $HC{=}CCO$), 7.05–7.15 (4H, m), 7.20–7.50 (25H, m), 7.70–7.80 (2H, m), 8.11 (2H, t, ^3J=5.5 Hz, NH); δ_C (75 MHz; $CDCl_3$) 31.0, 36.5, 40.2, 49.9, 52.5, 62.5, 68.4, 71.3, 76.1, 117.4, 123.5, 124.4, 126.7, 127.5, 128.3, 128.7, 128.9, 129.1, 129.3, 130.0, 133.2, 135.8, 136.0, 144.2, 146.9, 151.0, 162.5, 165.7, 169.8. Anal. Calcd for $C_{65}H_{66}N_4O_{12}$: C, 71.3; H, 6.1; N, 5.1. Found: C, 71.1; H, 6.3; N, 5.0%.

3.1.1.7. Biscatechol-THP ether 15a. Analogously to **12a** (Section 3.1.1.5), **15a** (981 mg, 69%) was obtained from **14**[15] (320 mg, 1.38 mmol), **3** (997 mg, 3.30 mmol) and **11a** (1.14 g, 3.03 mmol); R_f 0.54 (cyclohexane/ethyl acetate 1:1 v/v); ν_{max}/cm^{-1} 2944, 1731, 1720, 1579, 1473, 1453; δ_H (300 MHz; $CDCl_3$) 1.45–1.80 (6H, m, CH_2), 2.80–2.95 (6H, m, NCH_2), 3.40–3.55 (2H, m, NCC*H*HOTHP, OC*H*H), 3.70–3.85 (2H, m, NCCH*H*OTHP, OCH*H*), 4.21 (4H, t, ^3J=6.1 Hz, $NCCH_2O$), 4.57 (1H, t, ^3J=2.8 Hz, CH), 4.88 (4H, dd, ^3J=4.7, ^4J=1.8 Hz, $OCH_2C{=}C$), 5.11 (4H, s, CH_2Ph), 5.15 (4H, s, CH_2Ph), 6.05 (2H, dt, $^3J_{trans}$=15.8, ^4J=1.9 Hz, OCCH=C), 6.78 (2H, dt, $^3J_{trans}$=15.8, ^3J=4.7 Hz, OCC=CH), 7.05–7.20 (6H, m), 7.25–7.50 (20H, m); δ_C (75 MHz; $CDCl_3$) 19.4, 25.3, 30.6, 53.4, 54.4, 62.1, 62.7, 63.0, 66.0, 71.1, 75.6, 98.9, 118.2, 122.1, 122.8, 124.0, 126.1, 127.5, 127.9, 128.0, 128.2, 128.5, 136.4, 137.2, 141.3, 148.4, 152.8, 165.3, 165.6; *m/z* (EI, 70 eV) 917 (5%), 602 (10%), 518 (32%), 317 (8%), 225 (12%), 181 (45%), 91 (100%). Anal. Calcd for $C_{61}H_{63}NO_{14}$: C, 70.9; H, 6.1; N, 1.4. Found: C, 70.7; H, 6.3; N, 1.4%.

3.1.1.8. Biscatechol-THP ether 15b. Analogously to **12a** (Section 3.1.1.5), **15b** (953 mg, 60%) was obtained from **14**[15] (360 mg, 1.54 mmol), **3** (1.12 g, 3.70 mmol) and **11b** (1.27 g, 3.39 mmol); R_f 0.28 (cyclohexane/ethyl acetate 1:1 v/v); ν_{max}/cm^{-1} 2945, 1719, 1657, 1576, 1526, 1454; δ_H (300 MHz; $CDCl_3$) 1.40–1.75 (6H, m, CH_2), 2.70–2.90 (6H, m, NCH_2), 3.40–3.55 (2H, m, NCC*H*HOTHP, OC*H*H), 3.70–3.85 (2H, m, NCCH*H*OTHP, OCH*H*), 3.92 (4H, m, NCH_2), 4.18 (4H, t, ^3J=6.0 Hz, $NCCH_2O$), 4.50–4.55 (1H, m, CH), 5.07 (4H, s, CH_2Ph), 5.14 (4H, s, CH_2Ph), 5.81 (2H, dt, $^3J_{trans}$=15.7, ^4J=1.7 Hz, OCCH=C), 6.78 (2H, dt, $^3J_{trans}$=15.7, ^3J=5.2 Hz, OCC=CH), 7.05–7.15 (4H, m), 7.20–7.50 (18H, m), 7.65–7.75 (4H, m), 8.14 (2H, t, ^3J=5.8 Hz, NH); δ_C (75 MHz; $CDCl_3$) 19.2, 25.2, 30.4, 40.1, 53.2, 54.1, 62.0, 62.5, 65.8, 71.1, 76.4, 98.7, 117.1, 121.4, 123.2, 124.3, 126.5, 127.5, 128.1, 128.5, 128.7, 135.9, 136.2, 143.9, 146.7, 151.5, 164.9, 165.6; *m/z* (EI, 70 eV) 916 (4%), 559 (30%), 517 (11%), 399 (14%), 317 (49%), 225 (52%), 91 (100%). Anal. Calcd for $C_{61}H_{65}N_3O_{12}$: C, 71.0; H, 6.4; N, 4.1. Found: C, 70.8; H, 6.6; N, 4.0%.

3.1.1.9. Alcohol 16a. A solution of **15a** (970 mg, 0.94 mmol) and *p*-toluenesulphonic acid hydrate (200 mg) in toluene (30 mL) was stirred at 60 °C for 24 h. Ethyl acetate (50 mL) was added and the mixture was washed with $NaHCO_3$ solution and water. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:1 v/v, R_f 0.23) to yield **16a** as a yellow oil (722 mg, 81%); ν_{max}/cm^{-1} 3495, 2949, 1730, 1715, 1579; δ_H (300 MHz; $CDCl_3$) 2.72 (2H, t, ^3J=5.2 Hz, NCH_2COH), 2.83 (4H, t, ^3J=5.8 Hz, NCH_2COR), 3.51 (2H, t, ^3J=5.2 Hz, $NCCH_2OH$), 4.18 (4H, t, ^3J=5.8 Hz, $NCCH_2OR$), 4.80–4.90 (4H, dd, ^3J=5.3, ^4J=1.8 Hz, $OCH_2C{=}C$), 5.11 (8H, s, CH_2Ph), 6.08 (2H, dt, $^3J_{trans}$=15.9, ^4J=1.8 Hz, OCCH=C), 6.90 (2H, dt, $^3J_{trans}$=15.9, ^3J=5.3 Hz, OCC=CH), 7.10–7.20 (4H, m), 7.25–7.50 (22H, m); δ_C (75 MHz; $CDCl_3$) 52.8, 56.6, 58.8, 62.3, 62.9, 71.1, 75.5, 118.1, 121.8, 122.7, 123.9, 126.0, 127.4, 127.8, 128.0, 128.5, 128.7, 136.3, 137.1, 141.7, 148.3, 152.7, 165.2, 165.5; *m/z* (EI, 70 eV; +MSTFA) 1021 (M^+, 1%), 931 (2%), 918 (13%), 590 (10%), 518 (10%), 181 (11%), 91 (100%). Anal. Calcd for $C_{56}H_{55}NO_{13}$: C, 70.8; H, 5.8; N, 1.5. Found: C, 71.0; H, 5.8; N, 1.4%.

3.1.1.10. Alcohol 16b. Analogously to **16a** (Section 3.1.1.9), **16b** (656 mg, 76%) was obtained from **15b** (940 mg, 0.91 mmol); R_f 0.28 (cyclohexane/ethyl acetate 1:4 v/v); ν_{max}/cm^{-1} 3388, 2952, 1717, 1654, 1576, 1527; δ_H (300 MHz; $CDCl_3$) 2.70 (2H, t, ^3J=5.3 Hz, NCH_2COH), 2.81 (4H, t, ^3J=5.7 Hz, NCH_2COR), 3.49 (2H, t, ^3J=5.3 Hz, $NCCH_2OH$), 3.90–4.00 (4H, m, NCH_2), 4.16 (4H, t, ^3J=5.7 Hz, $NCCH_2OR$), 5.08 (4H, s, CH_2Ph), 5.16 (4H, s, CH_2Ph), 5.79 (2H, dt, $^3J_{trans}$=15.7, ^4J=1.8 Hz, OCCH=C), 6.81 (2H, dt, $^3J_{trans}$=15.7, ^3J=5.2 Hz, OCC=CH), 7.05–7.15 (4H, m), 7.20–7.50 (20H, m), 7.65–7.75 (2H, m), 8.15 (2H, t, ^3J=5.9 Hz, NH); δ_C (75 MHz; $CDCl_3$) 40.2, 52.8, 56.6, 58.9, 62.2, 71.2, 76.5, 117.2, 121.3, 123.2, 124.4, 126.7, 127.5, 128.2, 128.6, 128.7, 128.9, 136.1, 132.3, 144.5, 146.8, 151.6, 165.0, 165.7; *m/z* (EI, 70 eV, +MSTFA) 1019 (M^+, 1%), 929 (1%), 916 (5%), 559 (18%), 17 (11%), 317 (16%), 225 (23%), 181 (18%), 91 (100%). Anal. Calcd for $C_{56}H_{57}N_3O_{11}$: C, 70.9; H, 6.1; N, 4.4. Found: C, 70.6; H, 6.0; N, 4.6%.

3.1.1.11. Triscatechol 17a. A solution of **16a** (370 mg, 0.39 mmol) and pyridine (0.2 mL) in dry CH_2Cl_2 (10 mL) was slowly treated with a solution of **9** (152 mg, 0.43 mmol) in CH_2Cl_2 (5 mL) by means of a syringe. The mixture was stirred for 2–3 h, washed with diluted HCl (pH 3), satd $NaHCO_3$ and water and then dried over Na_2SO_4. The solvent was removed in vacuum and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:1 v/v, R_f 0.41) leaving **17a** as a colourless foamy solid (283 mg, 61%); ν_{max}/cm^{-1} 2957, 1730, 1701, 1577, 1471, 1453; δ_H (300 MHz; $CDCl_3$) 2.75–2.85 (6H, m, NCH_2), 4.15 (4H, t, ^3J=6.0 Hz, $NCCH_2OR$), 4.27 (2H, t, ^3J=6.1 Hz, $NCCH_2OR'$), 4.83 (4H, dd, ^3J=4.3, ^4J=1.9 Hz, $OCH_2C{=}C$), 5.08 (8H, s, CH_2Ph), 5.13 (4H, s, CH_2Ph), 6.05 (2H, dt, $^3J_{trans}$=15.8, ^4J=1.9 Hz, OCCH=C), 6.94 (2H, dt, $^3J_{trans}$=15.8, ^3J=4.3 Hz, OCC=CH), 7.05–7.20 (8H, m), 7.25–7.45 (31H, m); δ_C (75 MHz; $CDCl_3$) 53.1, 62.7, 63.0, 63.1, 67.1, 71.2, 71.3, 75.5, 75.7, 117.9, 118.3, 122.1, 122.7, 123.9, 124.0, 126.1, 126.6, 127.5, 127.7, 127.9, 128.0, 128.2, 128.4, 128.7, 128.9, 129.1, 136.1, 136.5, 137.3, 137.7, 141.5, 146.3, 152.8, 165.0, 165.6, 166.1. Anal. Calcd for $C_{77}H_{71}NO_{16}$: C, 73.0; H, 5.7; N, 1.1. Found: C, 72.7; H, 5.9; N, 1.1%.

3.1.1.12. Triscatechol 17b. Analogously to **17a** (Section 3.1.1.11), **17b** (231 mg, 55%) was obtained from **16b** (315 mg, 0.33 mmol) and **9** (128 mg, 0.36 mmol); R_f 0.42 (cyclohexane/ethyl acetate 1:2 v/v); ν_{max}/cm^{-1} 2952, 1739, 1718, 1652, 1575, 1468; δ_H (300 MHz; $CDCl_3$) 2.75–2.90 (6H, m, NCH_2), 3.90–4.00 (4H, m, $NHCH_2$), 4.14 (4H, t, ^3J=5.9 Hz, $NCCH_2OR$), 4.27 (2H, t, ^3J=6.1 Hz, $NCCH_2OR'$), 5.10 (6H, s, CH_2Ph), 5.13 (2H, s, CH_2Ph), 5.18 (4H, s, CH_2Ph), 5.77 (2H, dt, $^3J_{trans}$=15.9, ^4J=1.6 Hz, OCCH=C), 6.80 (2H, dt, $^3J_{trans}$=15.9, ^3J=5.3 Hz, OCC=CH), 7.00–7.15 (3H, m), 7.20–7.50 (34H, m), 7.65–7.75 (2H, m), 8.10 (2H, t, ^3J=5.8 Hz,

NH); δ_C (75 MHz; $CDCl_3$) 40.2, 53.1, 62.5, 62.9, 71.1, 71.2, 71.4, 75.5, 76.5, 117.2, 117.9, 118.9, 121.4, 122.7, 123.3, 123.9, 124.3, 126.2, 126.7, 127.4, 127.7, 127.8, 128.0, 128.2, 128.3, 128.5, 128.6, 128.7, 128.9, 129.2, 136.1, 136.3, 136.5, 137.5, 144.1, 146.8, 151.6, 152.7, 165.0, 165.7, 165.9. Anal. Calcd for $C_{77}H_{73}N_3O_{14}$: C, 73.1; H, 5.8; N, 3.3. Found: C, 72.9; H, 6.0; N, 3.4%.

3.1.1.13. Biscatechol-hydroxamate 13a—typical procedure for the hydrogenolytic debenzylation. Compound **12a** (145 mg, 0.13 mmol) was dissolved in freshly distilled methanol/dioxane (3:1 v/v, 10 mL), 5% Pd/charcoal catalyst (75 mg) was added and the resulting mixture was flashed with and kept under an atmosphere of hydrogen gas (1 bar) for 6 h while stirring (TLC control). After filtration, the solvent was removed in vacuum. The pale yellow residue was precipitated from CH_2Cl_2 to yield **13a** (77 mg, 90%) as a colourless solid of mp 126–127 °C; ν_{max}/cm^{-1} 3334, 2943, 1745, 1683, 1631, 1468; δ_H (300 MHz; MeOD-d_3) 1.80–1.95 (4H, m, $OCCCH_2C$), 2.46 (4H, t, ^3J=7.0 Hz, $OCCH_2CC$), 2.58 (2H, t, ^3J=6.7 Hz, $OCCH_2$), 2.70–2.85 (6H, m, NCH_2), 3.12 (3H, s, CH_3), 4.10 (4H, t, ^3J=6.1 Hz, $NCCH_2OR$), 4.41 (4H, t, ^3J=5.9 Hz, $OCCCCH_2$), 6.71 (2H, t, ^3J=8.1 Hz, H^{ar}), 6.92 (2H, dd, ^3J=8.1, ^4J=1.6 Hz, H^{ar}), 7.17 (2H, dd, ^3J=8.1, ^4J=1.6 Hz, H^{ar}); δ_C (75 MHz; MeOD-d_3) 26.4, 30.8, 31.9, 36.3, 52.9, 54.2, 62.6, 64.7, 117.9, 118.5, 119.4, 125.7, 147.8, 151.0, 169.2, 171.6, 174.8. Anal. Calcd for $C_{30}H_{38}N_2O_{14}$: C, 55.4; H, 5.9; N, 4.3. Found: C, 55.2; H, 5.9; N, 4.2%.

3.1.1.14. Biscatechol-hydroxamate 13b. Analogously to **13a** (Section 3.1.1.13), **13b** (53 mg, 82%) was obtained from **12b** (110 mg, 0.10 mmol) as a pale yellow solid of mp 131–133 °C; ν_{max}/cm^{-1} 3316, 2941, 1738, 1680, 1633, 1469; δ_H (300 MHz; MeOD-d_3) 1.80–1.95 (4H, m, $NCCH_2$), 2.42 (4H, t, ^3J=7.1 Hz, $NCCCH_2$), 2.57 (2H, t, ^3J=6.7 Hz, $OCCH_2$), 2.77 (4H, t, ^3J=6.0 Hz, NCH_2COR), 2.80 (2H, t, ^3J=6.4 Hz, NCH_2CCO), 3.05–3.20 (7H, m, CH_3, $NHCH_2$), 4.11 (4H, t, ^3J=6.0 Hz, $NCCH_2OR$), 6.71 (2H, t, ^3J=7.8 Hz, H^{ar}), 6.92 (2H, dd, ^3J=7.8, ^4J=1.2 Hz, H^{ar}), 7.18 (2H, dd, ^3J=7.8, ^4J=1.2 Hz, H^{ar}); δ_C (75 MHz; MeOD-d_3) 25.8, 31.7, 32.6, 36.3, 39.9, 52.2, 54.0, 63.9, 116.9, 118.8, 119.5, 119.7, 147.6, 150.7, 171.0, 171.8, 175.1. Anal. Calcd for $C_{30}H_{40}N_4O_{12}$: C, 55.6; H, 6.2; N, 8.6. Found: C, 55.3; H, 6.3; N, 8.4%.

3.1.1.15. Triscatechol 18a. Analogously to **13a** (Section 3.1.1.13), **18a** (142 mg, 90%) was obtained from **17a** (280 mg, 0.22 mmol) as a colourless solid of mp 133–134 °C; ν_{max}/cm^{-1} 3368, 2930, 1738, 1689, 1590, 1468; δ_H (300 MHz; MeOD-d_3) 1.95–2.10 (4H, m, $OCCCH_2$), 2.41 (4H, t, ^3J=7.0 Hz, $OCCH_2C$), 2.75–2.95 (6H, m, NCH_2), 4.08 (4H, t, ^3J=5.5 Hz, $NCCH_2OR$), 4.25–4.40 (6H, m, $NCCH_2OR'$, $OCCCCH_2$), 6.65–6.85 (6H, m, H^{ar}), 6.95–7.10 (3H, m, H^{ar}); δ_C (75 MHz; MeOD-d_3) 26.4, 35.3, 54.3, 54.6, 61.1, 63.2, 63.9, 113.8, 116.7, 117.0, 119.5, 120.0, 125.8, 125.9, 146.8, 147.4, 150.0, 151.6, 170.3, 171.6, 174.2. Anal. Calcd for $C_{35}H_{39}NO_{16}$: C, 57.6; H, 5.4; N, 1.9. Found: C, 57.8; H, 5.2; N, 2.0%.

3.1.1.16. Triscatechol 18b. Analogously to **13a** (Section 3.1.1.13), **18b** (107 mg, 86%) was obtained from **17b** (220 mg, 0.17 mmol) as a colourless solid of mp 138–139 °C; ν_{max}/cm^{-1} 3364, 2932, 1736, 1644, 1586, 1462; δ_H (300 MHz; MeOD-d_3) 1.80–1.90 (4H, m, $OCCCH_2$), 2.38 (4H, t, ^3J=6.9 Hz, $OCCH_2C$), 2.85–3.00 (6H, m, NCH_2), 3.25–3.35 (4H, m, $NHCH_2$), 4.22 (4H, t, ^3J=5.5 Hz, $NCCH_2OR$), 4.53 (2H, t, ^3J=5.4 Hz, $NCCH_2OR'$), 6.70–6.95 (6H, m, H^{ar}), 7.10–7.25 (3H, m, H^{ar}); δ_C (75 MHz; MeOD-d_3) 25.8, 32.4, 39.7, 54.5, 54.7, 62.4, 63.1, 113.9, 116.9, 118.7, 119.8, 120.4, 121.6, 122.1, 147.0, 147.4, 150.3, 151.4, 171.4, 171.8, 174.8. Anal. Calcd for $C_{35}H_{41}N_3O_{14}$: C, 57.8; H, 5.7; N, 5.8. Found: C, 57.8; H, 5.8; N, 5.7%.

3.1.2. Synthesis of the penicillinate–biscatechol-hydroxamate conjugates 24 and 2.

3.1.2.1. *N*-Benzyloxy-*N*-(3,3-diethoxypropyl) acrylamide 20. 3,3-Diethoxypropanal *O*-benzyloxime. To a solution in THF (50 mL) of malonic aldehyde semiacetal (2.63 g, 22.7 mmol), obtained from **19** in 85% according to literature,[18] a solution of *O*-benzyl-hydroxylamine (3.08 g, 25.0 mmol) in THF (10 mL) was added dropwise. The mixture was stirred for 3 h, the solvent was largely evaporated, ethyl acetate (50 mL) was added and the resulting mixture was washed with diluted HCl (pH 3) and water. The organic phase was dried over Na_2SO_4, the solvent was removed in vacuum and the remaining yellow oil was bulb-to-bulb distilled (130–135 °C, 0.1 Torr) to give 3,3-diethoxypropanal *O*-benzyloxime as a colourless liquid (4.86 g, 85%); *E*/*Z* ca. 2:1; ν_{max}/cm^{-1} 2676, 1704, 1496, 1454; δ_H (300 MHz; $CDCl_3$) *E*-isomer: 1.10–1.25 (6H, m, CH_3), 2.53 (2H, t, ^3J=5.8 Hz, CH_2C=N), 3.45–3.75 (6H, m, $MeCH_2$), 4.59 (1H, t, ^3J=5.8 Hz, $CH(OEt)_2$), 5.06 (2H, s, CH_2Ph), 7.20–7.40 (5H, m, H^{ar}), 7.51 (1H, t, ^3J=5.8 Hz, CH=N); *Z*-isomer: 1.10–1.25 (6H, m, CH_3), 2.72 (2H, t, ^3J=5.6 Hz, CH_2C=N), 3.45–3.75 (6H, m, $MeCH_2$), 4.68 (1H, t, ^3J=5.6 Hz, $CH(OEt)_2$), 5.12 (2H, s, CH_2Ph), 6.78 (1 H, t, ^3J=5.6 Hz, CH=N), 7.20–7.40 (5H, H^{ar}); δ_C (75 MHz; $CDCl_3$; mixture of isomers) 15.1, 15.3, 30.9, 34.4, 61.6, 61.7, 75.5, 75.7, 99.7, 100.6, 127.4, 127.6, 127.9, 128.1, 128.7, 129.0, 137.6, 137.9, 147.3, 147.5; *m*/*z* (EI, 70 eV) 251 (M^+, 4%), 206 (14%), 103 (73%), 91 (100%).

3,3-Diethoxypropylbenzoxyamine. 3,3-Diethoxypropanal *O*-benzyloxime (4.80 g, 19.1 mmol) was reduced with $Na(CN)BH_3$ (1.44 g, 22.9 mmol) following a literature procedure;[19] pale yellow oil (3.53 g, 73%) after purification by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:1 v/v, R_f 0.54); ν_{max}/cm^{-1} 2975, 1738, 1453; δ_H (300 MHz; $CDCl_3$) 1.15 (6H, t, ^3J=6.8 Hz, CH_3), 1.80–1.90 (2H, m, CH_2CNH), 2.93 (2H, t, ^3J=6.5 Hz, CH_2N), 3.45–3.70 (4H, m, CH_2Me), 4.53 (1H, t, ^3J=5.6 Hz, CHO_2), 4.65 (2H, CH_2Ph), 5.75 (1H, br, NH), 7.20–7.40 (5H, m, H^{ar}); δ_C (75 MHz; $CDCl_3$) 15.3, 31.2, 47.9, 61.2, 76.0, 101.7, 127.6, 128.2, 128.3, 138.1; *m*/*z* (EI, 70 eV) 253 (M^+, 2%), 207 (18%), 136 (16%), 91 (100%).

N-Benzyloxy-*N*-(3,3-diethoxypropyl) acrylamide **20**. A solution of 3,3-diethoxypropyl-benzoxyamine (3.50 g, 13.8 mmol) and pyridine (1.58 g, 20 mmol) in dry $CHCl_3$ (50 mL) was slowly treated at 0 °C with a solution of acryl chloride (1.37 g, 15.2 mmol) in the same solvent (10 mL) by means of a syringe. The mixture was stirred for 1 h while coming to room temperature, washed with satd $NaHCO_3$, aqueous HCl (pH 3) and water and finally dried over

Na_2SO_4. Removal of the solvent left a yellow oil, which was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 2:1 v/v, R_f 0.32) yielding **20** as a pale yellow liquid (3.47 g, 82%); ν_{max}/cm^{-1} 2974, 1620, 1442, 1413, 1372; δ_H (300 MHz; $CDCl_3$) 1.17 (6H, t, ^3J=7.0 Hz, CH_3), 1.90–2.00 (2H, m, $CH_2C(OEt)_2$), 3.40–3.65 (4H, m, CH_2Me), 3.73 (2H, t, ^3J=7.0 Hz, NCH_2), 4.51 (1H, t, ^3J=5.5 Hz, CHO_2), 4.77 (2H, CH_2Ph), 5.56 (1H, dd, $^3J_{cis}$=10.3, ^2J=2.1 Hz, C=C*H*H), 6.33 (1H, dd, $^3J_{trans}$=17.1, ^2J=2.1 Hz, C=CH*H*), 6.65 (1H, dd, $^3J_{cis}$=10.3, $^3J_{trans}$=17.1 Hz, C*H*=CH_2), 7.25–7.40 (5H, m, H^{ar}); δ_C (75 MHz; $CDCl_3$) 15.1, 31.1, 41.9, 61.3, 76.8, 100.7, 126.2, 128.5, 128.6, 128.8, 129.1, 134.1, 166.3; *m/z* (EI, 70 eV) 307 (M^+, 1%), 262 (16%), 216 (8%), 171 (41%), 154 (23%), 91 (100%). Anal. Calcd for $C_{17}H_{25}NO_4$: C, 66.4; H, 8.2; N, 4.6. Found: C, 66.2; H, 8.1; N, 4.8%.

3.1.2.2. *N*-Benzyloxy-*N*-(3′,3′-diethoxypropyl) 3-[bis-(2″-hydroxyethyl)amino]propionamide 21. Analogously to **7** (Section 3.1.1.1), **21** (3.89 g, 85%) was obtained after 32 h stirring as a pale yellow oil from **5** (1.17 g, 11.1 mmol) and **20** (3.40 g, 11.1 mmol); ν_{max}/cm^{-1} 3412, 2975, 1645, 1453; δ_H (300 MHz; $CDCl_3$) 1.12 (6H, t, ^3J=7.1 Hz, CH_3), 1.80–1.95 (2H, m, $CH_2C(OEt)_2$), 2.40–2.55 (6H, NCH_2), 2.74 (2H, t, ^3J=5.9 Hz, O=CCH_2), 3.30–3.60 (10H, m, CH_2Me, CH_2OH), 3.69 (2H, t, ^3J=6.8 Hz, CH_2NOBn), 4.48 (1H, t, ^3J=5.6 Hz, CHO_2), 4.78 (2H, s, CH_2Ph), 7.30–7.40 (5H, m, H^{ar}); δ_C (75 MHz; $CDCl_3$) 15.2, 30.3, 31.0, 48.7, 56.0, 59.4, 61.3, 76.2, 100.7, 128.5, 128.8, 129.0, 134.2, 173.9. Anal. Calcd for $C_{21}H_{36}N_2O_6$: C, 61.1; H, 8.8; N, 6.8. Found: C, 61.3; H, 8.6; N, 6.9%.

3.1.2.3. Perbenzylated biscatechol-hydroxamate 22a. Analogously to **12a** (Section 3.1.1.5), **22a** (0.85 g, 62%) was obtained as yellowish, highly viscous oil from **21** (0.47 g, 1.15 mmol), **3** (0.83 g, 2.75 mmol) and **11a** (0.95 g, 2.53 mmol); R_f 0.56 (cyclohexane/ethyl acetate 1:2 v/v); ν_{max}/cm^{-1} 2974, 1722, 1663, 1580, 1453; δ_H (300 MHz; $CDCl_3$) 1.15 (6H, t, ^3J=6.5 Hz, CH_3), 1.80–1.95 (2H, m, $CH_2C(OEt)_2$), 2.48 (2H, t, ^3J=6.7 Hz, O=CCH_2), 2.70–2.90 (6H, m, NCH_2), 3.40–3.75 (6H, m, CH_2Me, BnONCH_2), 4.12 (4H, t, ^3J=6.2 Hz, $NCCH_2OR$), 4.47 (1H, t, ^3J=5.5 Hz, CHO_2), 4.77 (2H, s, N–O–CH_2Ph), 4.87 (4H, dd, ^3J=4.6, ^4J=1.8 Hz, CH_2C=C), 5.12 (8H, s, C–O–CH_2Ph), 6.05 (2H, dt, $^3J_{trans}$=15.8, ^4J=1.8 Hz, O=CCH=C), 6.99 (2H, dt, $^3J_{trans}$=15.8, ^3J=4.6 Hz, O=CC=CH), 7.10–7.25 (6H, m), 7.30–7.55 (25H, m); δ_C (75 MHz; $CDCl_3$) 15.2, 30.7, 31.1, 50.2, 52.6, 61.1, 62.6, 63.0, 70.8, 71.2, 75.6, 76.2, 100.8, 118.3, 122.0, 122.7, 124.4, 125.3, 126.1, 127.2, 127.5, 127.7, 128.0, 128.8, 136.4, 137.2, 141.3, 151.3, 152.9, 163.0, 163.7, 165.5. Anal. Calcd for $C_{71}H_{76}N_2O_{16}$: C, 70.3; H, 6.3; N, 2.3. Found: C, 70.4; H, 6.1; N, 2.2%.

3.1.2.4. Perbenzylated biscatechol-hydroxamate 22b. Analogously to **12a** (Section 3.1.1.5), **22b** (0.79 g, 55%) was obtained as a yellowish, highly viscous oil from **21** (0.49 g, 1.20 mmol), **3** (0.86 g, 2.86 mmol) and **11b** (1.00 g, 2.65 mmol); R_f 0.44 (cyclohexane/ethyl acetate 1:4 v/v); ν_{max}/cm^{-1} 2974, 1718, 1659, 1577, 1524, 1454; δ_H (300 MHz; $CDCl_3$) 1.16 (6H, t, ^3J=7.0 Hz, CH_3), 1.80–1.95 (2H, m, $CH_2C(OEt)_2$), 2.46 (2H, t, ^3J=6.6 Hz, O=CCH_2), 2.72 (4H, t, ^3J=6.2 Hz, NCH_2COR), 2.84 (2H, t, ^3J=6.4 Hz, NCH_2CCO), 3.40–3.75 (6H, m, CH_2Me, BnONCH_2), 3.85–3.95 (4H, m, NHCH_2), 4.11 (4H, t, ^3J=6.2 Hz, $NCCH_2OR$), 4.49 (1H, t, ^3J=5.6 Hz, CHO_2), 4.78 (2H, s, N–O–CH_2Ph), 5.08 (4H, s, C–O–CH_2Ph), 5.13 (4H, s, C–O–CH_2Ph), 5.78 (2H, dt, $^3J_{trans}$=15.7, ^4J=1.7 Hz, O=CCH=C), 6.78 (2H, dt, $^3J_{trans}$=15.7, ^3J=4.6 Hz, O=CC=CH), 7.05–7.15 (4H, m), 7.30–7.55 (25H, m), 7.70–7.80 (2H, m), 8.12 (2H, t, ^3J=6.0 Hz, NH); δ_C (75 MHz; $CDCl_3$) 15.3, 29.7, 29.8, 31.1, 40.3, 50.2, 52.7, 61.3, 62.4, 71.3, 76.3, 76.5, 100.9, 117.3, 121.5, 123.4, 124.4, 126.7, 127.7, 128.2, 128.7, 128.9, 129.3, 130.0, 136.1, 136.3, 144.2, 146.9, 151.6, 165.1, 165.7. Anal. Calcd for $C_{71}H_{78}N_4O_{14}$: C, 70.4; H, 6.5; N, 4.6. Found: C, 70.2; H, 6.4; N, 4.7%.

3.1.2.5. Aldehyde 23a. To a solution of **22a** (0.90 g, 0.75 mmol) in acetone (15 mL) at 0 °C aqueous HCl (10 mL, 10%) was added. The mixture was stirred for 3 h while warming up to room temperature. The pH was adjusted to 7 by addition of aqueous NaOH and most of the solvent was removed under reduced pressure. Ethyl acetate (20 mL) was added and the organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:2 v/v, R_f 0.48) to give aldehyde **23a** (0.74 g, 88%) as a pale yellow, highly viscous mass; ν_{max}/cm^{-1} 2931, 1722, 1663, 1581, 1454; δ_H (300 MHz; $CDCl_3$) 2.50 (2H, t, ^3J=6.3 Hz, O=CCH_2), 2.55–2.65 (2H, m, CH_2CHO), 2.75–2.95 (6H, m, NCH_2), 3.89 (2H, t, ^3J=5.8 Hz, BnONCH_2), 4.18 (4H, t, ^3J=6.1 Hz, $NCCH_2OR$), 4.73 (2H, s, N–O–CH_2Ph), 4.86 (4H, dd, ^3J=4.8, ^4J=1.9 Hz, CH_2C=C), 5.11 (8H, s, C–O–CH_2Ph), 6.08 (2H, dt, $^3J_{trans}$=15.9, ^4J=1.9 Hz, O=CCH=C), 7.02 (2H, dt, $^3J_{trans}$=15.9, ^3J=4.8 Hz, O=CC=CH), 7.10–7.30 (6H, m), 7.35–7.55 (25H, m), 9.71 (1H, d, ^3J=2.5 Hz, CHO); δ_C (75 MHz; $CDCl_3$) 30.7, 41.2, 50.0, 52.6, 62.5, 70.8, 71.2, 75.5, 76.3, 118.2, 122.7, 123.8, 125.3, 126.1, 127.2, 127.5, 127.7, 128.0, 128.2, 128.7, 136.3, 137.0, 141.2, 150.8, 152.6, 163.1, 163.9, 165.4, 200.0. Anal. Calcd for $C_{67}H_{66}N_2O_{15}$: C, 70.6; H, 5.8; N, 2.5. Found: C, 70.4; H, 6.0; N, 2.4%.

3.1.2.6. Aldehyde 23b. Analogously to **23a** (Section 3.1.2.5), **23b** (0.63 g, 90%) was obtained as a pale yellow foamy mass from **22b** (0.75 g, 0.63 mmol); R_f 0.34 (cyclohexane/ethyl acetate 1:4 v/v); ν_{max}/cm^{-1} 2957, 1718, 1654, 1576, 1526, 1476; δ_H (300 MHz; $CDCl_3$) 2.55–2.70 (4H, m, CH_2CHO, O=CCH_2,), 2.80–3.05 (6H, m, NCH_2), 3.85 (2H, t, ^3J=6.3 Hz, BnONCH_2), 3.90–4.00 (4H, m, NHCH_2), 4.21 (4H, t, ^3J=6.0 Hz, $NCCH_2OR$), 4.76 (2H, s, N–O–CH_2Ph), 5.07 (4H, s, C–O–CH_2Ph), 5.13 (4H, s, C–O–CH_2Ph), 5.78 (2H, dt, $^3J_{trans}$=15.7, ^4J=1.7 Hz, O=CCH=C), 6.79 (2H, dt, $^3J_{trans}$=15.7, ^3J=4.7 Hz, O=CC=CH), 7.05–7.15 (4H, m), 7.25–7.50 (25H, m), 7.70–7.80 (2H, m), 8.14 (2H, t, ^3J=6.0 Hz, NH), 9.70 (1H, s, CHO); δ_C (75 MHz; $CDCl_3$) 31.2, 40.2, 41.1, 49.9, 52.5, 62.8, 71.2, 76.4, 76.6, 117.2, 123.2, 124.4, 126.6, 127.5, 127.7, 128.0, 128.3, 128.6, 128.8, 129.1, 129.4, 134.0, 136.1, 136.3, 144.3, 147.8, 151.6, 163.8, 165.0, 165.4, 200.0. Anal. Calcd for $C_{67}H_{68}N_4O_{13}$: C, 70.8; H, 6.0; N, 4.9. Found: C, 70.5; H, 5.8; N, 4.8%.

3.1.2.7. Pentabenzylated conjugate 24a. A solution of methyl 6-aminopenicillanate **4** (110 mg, 0.48 mmol), **3** (174 mg, 0.58 mmol), **23a** (705 mg, 0.48 mmol) and a catalytic amount of benzoic acid in THF (20 mL) was stirred at room temperature over night. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:2 v/v, R_f 0.33) to yield **24a** (415 mg, 62%) as a colourless foamy solid; ν_{max}/cm^{-1} 2926, 1785, 1723, 1653, 1581, 1474; δ_H (300 MHz; $CDCl_3$) 1.46 (3H, s, CH_3), 1.61 (3H, s, CH_3'), 2.45–2.55 (4H, m, O=CCH_2, BnONCCH_2), 2.70–2.90 (6H, m, NCH_2), 3.71 (2H, t, ^3J=5.9 Hz, BnO–N–CH_2), 3.77 (3H, s, OCH_3), 4.12 (4H, t, ^3J=6.2 Hz, $NCCH_2OR$), 4.39 (1H, s, CHCO_2Me), 4.76 (2H, s, N–O–CH_2Ph), 4.83 (4H, dd, ^3J=4.8, ^4J=1.8 Hz, OCH_2C=C), 5.06 (4H, s, C–O–CH_2Ph), 5.12 (4H, s, C–O–CH_2Ph), 5.52 (1H, d, ^3J=4.3 Hz, SCH), 5.72 (1H, dd, ^3J=4.3, ^3J=7.1 Hz, SCHCH), 5.85 (1H, d, $^3J_{trans}$=16.0 Hz, NCOCH=C), 6.06 (2H, dt, $^3J_{trans}$=15.9, ^4J=1.8 Hz, OCOCH=C), 6.31 (1H, d, ^3J=7.1 Hz, NH), 6.80 (1H, dt, $^3J_{trans}$=16.0, ^3J=4.6 Hz, NCOC=CH), 6.93 (2H, dt, $^3J_{trans}$=15.9, ^3J=4.8 Hz, OCOC=CH), 7.05–7.20 (6H, m), 7.30–7.55 (25H, m); δ_C (75 MHz; $CDCl_3$) 27.0, 29.6, 30.9, 31.4, 50.3, 52.4, 52.7, 58.6, 62.7, 63.0, 64.8, 68.1, 70.5, 71.0, 71.3, 75.6, 76.4, 118.3, 122.7, 123.8, 125.0, 126.2, 126.8, 127.1, 127.4, 127.7, 128.0, 128.1, 128.5, 136.1, 136.4, 137.0, 141.5, 149.9, 152.8, 163.5, 164.0, 165.6, 168.1, 170.7, 173.8. Anal. Calcd for $C_{78}H_{82}N_4O_{18}S$: C, 67.1; H, 5.9; N, 4.0. Found: C, 66.8; H, 6.1; N, 4.1%.

3.1.2.8. Pentabenzylated conjugate 24b. Analogously to **24a** (Section 3.1.2.7), **24b** (298 mg, 51%) was obtained as a pale yellow foamy solid from **4** (97 mg, 0.42 mmol), **3** (154 mg, 0.51 mmol) and **23b** (610 mg, 0.42 mmol); R_f 0.30 (cyclohexane/ethyl acetate 1:4 v/v); ν_{max}/cm^{-1} 2928, 1783, 1719, 1661, 1576, 1524; δ_H (300 MHz; $CDCl_3$) 1.44 (3H, s, CH_3), 1.62 (3H, s, CH_3'), 2.40–2.55 (4H, m, O=CCH_2, BnONCCH_2), 2.73 (4H, t, ^3J=6.2 Hz, NCH_2COR), 2.86 (2H, t, ^3J=6.4 Hz, NCH_2CCO), 3.67 (2H, t, ^3J=5.8 Hz, BnO–N–CH_2), 3.71 (3H, s, OCH_3), 3.90–4.00 (4H, m, NHCH_2), 4.11 (4H, t, ^3J=6.2 Hz, $NCCH_2OR$), 4.35 (1H, s, CHCO_2Me), 4.74 (2H, s, N–O–CH_2Ph), 5.06 (4H, s, C–O–CH_2Ph), 5.13 (4H, s, C–O–CH_2Ph), 5.51 (1H, d, ^3J=4.2 Hz, SCH), 5.70–5.85 (3H, m, SCHCH, OCOCH=C), 5.79 (1H, d, $^3J_{trans}$=15.8 Hz, NCOCH=C), 6.46 (1H, d, ^3J=7.3 Hz, NH), 6.65–6.80 (3H, m, NCOC=CH, OCOC=CH), 7.05–7.15 (4H, m), 7.25–7.50 (25H, m), 7.90–8.00 (2H, m), 8.13 (2H, t, ^3J=7.1 Hz, NH); δ_C (75 MHz; $CDCl_3$) 27.0, 32.1, 32.3, 34.8, 40.2, 49.7, 52.3, 52.6, 58.1, 58.2, 62.4, 64.6, 68.8, 70.5, 71.3, 76.4, 76.6, 117.3, 121.5, 123.3, 124.3, 126.6, 127.2, 127.5, 128.0, 128.4, 128.6, 128.7, 128.8, 129.0, 136.1, 136.3, 137.5, 144.1, 146.8, 152.6, 161.2, 163.8, 165.1, 165.8, 170.6, 174.0. Anal. Calcd for $C_{78}H_{84}N_6O_{16}S$: C, 67.2; H, 6.1; N, 6.0. Found: C, 66.9; H, 6.2; N, 5.9%.

3.1.2.9. Conjugate 2a. Analogously to **13a** (Section 3.1.1.13), **2a** (161 mg, 60%) was obtained after a 24 h reaction period as a colourless solid of mp 154–156 °C from **24a** (400 mg, 0.29 mmol), $[\alpha]_D^{20}$ 48 (*c* 1.0, EtOH); ν_{max}/cm^{-1} 3358, 2930, 1785, 1738, 1681, 1635; δ_H (300 MHz; MeOD-d_3) 1.38 (3H, s, CH_3), 1.55 (3H, s, CH_3'), 1.55–1.70 (4H, m, $ONCH_2(CH_2)_2$), 2.05–2.25 (6H, m, $OCOCCH_2$, NH(CO)CH_2), 2.65 (2H, t, ^3J=6.1 Hz, ON(CO)CH_2), 2.80–3.00 (6H, m, NCH_2), 3.25 (4H, t, ^3J=5.9 Hz, $OCOCH_2$), 3.67 (2H, t, ^3J=5.8 Hz, $ONCH_2$), 3.70 (3H, s, OCH_3), 4.12 (4H, t, ^3J=6.0 Hz, $NCCH_2OR$), 4.35–4.45 (5H, m, $OCOCH_2$, CHCO_2Me), 5.50 (1H, d, ^3J=4.6 Hz, SCH), 5.70–5.80 (1H, m, SCHCH), 6.73 (2H, t, ^3J=8.1 Hz, H^{ar}), 6.93 (2H, dd, ^3J=8.1, ^4J=1.7 Hz, H^{ar}), 7.20 (2H, dd, ^3J=8.1, ^4J=1.7 Hz, H^{ar}); δ_C (75 MHz; MeOD-d_3) 23.7, 26.3, 27.1, 27.4, 30.8, 31.3, 31.8, 33.9, 48.0, 52.5, 52.7, 54.3, 58.2, 62.8, 63.4, 64.5, 70.4, 76.1, 117.8, 118.6, 119.2, 125.9, 147.8, 151.2, 168.1, 169.2, 171.5, 172.0, 174.6, 175.6. Anal. Calcd for $C_{43}H_{56}N_4O_{18}S$: C, 54.4; H, 6.0; N, 5.9. Found: C, 54.0; H, 5.8; N, 6.1%.

3.1.2.10. Conjugate 2b. Analogously to **13a** (Section 3.1.1.13), **2b** (118 mg, 64%) was obtained after a 24 h reaction period as a faintly yellow solid of mp 160–163 °C from **24b** (280 mg, 0.20 mmol), $[\alpha]_D^{20}$ 46 (*c* 1.0, EtOH); ν_{max}/cm^{-1} 3356, 2928, 1782, 1741, 1680, 1642, 1584; δ_H (300 MHz; MeOD-d_3) 1.40 (3H, s, CH_3), 1.59 (3H, s, CH_3'), 1.60–1.75 (4H, m, $ONC(CH_2)_2$), 2.10–2.25 (4H, m, $NHCH_2CH_2$), 2.24 (2H, t, ^3J=6.1 Hz, $NCOCH_2$), 2.69 (2H, t, ^3J=6.0 Hz, $ONCOCH_2$), 2.80–3.00 (6H, m, NCH_2), 3.23 (4H, t, ^3J=5.8 Hz, $OCOCH_2$), 3.68 (3H, s, OCH_3), 3.70–3.90 (6H, m, NHCH_2, HONCH_2), 4.14 (4H, t, ^3J=6.1 Hz, $NCCH_2OR$), 4.33 (1H, s, CHCO_2Me), 5.48 (1H, d, ^3J=4.4 Hz, SCH), 5.75–5.85 (1H, m, Hz, SCHCH), 6.70 (2H, t, ^3J=8.0 Hz, H^{ar}), 6.92 (2H, dd, ^3J=8.0, ^4J=1.5 Hz, H^{ar}), 7.21 (2H, dd, ^3J=8.0, ^4J=1.5 Hz, H^{ar}), 8.02 (2H, t, ^3J=7.3 Hz, NH); δ_C (75 MHz; MeOD-d_3) 23.5, 25.8, 27.0, 27.2, 31.5, 31.9, 32.4, 33.8, 40.0, 48.3, 52.4, 52.5, 54.0, 58.1, 63.3, 63.6, 71.3, 76.1, 117.3, 118.8, 119.6, 119.9, 148.0, 150.5, 165.7, 168.8, 170.1, 170.7, 172.0, 174.9. Anal. Calcd for $C_{43}H_{58}N_6O_{16}S$: C, 54.5; H, 6.2; N, 8.9. Found: C, 54.7; H, 6.1; N, 8.7%.

3.1.3. Chelators 25[20] and 26[21] lacking siderophore activity.

3.1.3.1. Biscatechol-salicylate 25a. Colourless solid of mp 107–108 °C; ν_{max}/cm^{-1} 3362, 2930, 1734, 1692, 1588; δ_H (300 MHz; MeOD-d_3) 1.95–2.10 (4H, m, $OCOCCH_2$), 2.42 (4H, t, ^3J=7.1 Hz, $OCOCH_2C$), 2.75–2.95 (6H, m, NCH_2), 4.08 (4H, t, ^3J=5.6 Hz, $NCCH_2OR$), 4.25–4.40 (6H, m, $NCCH_2OR'$, $OCCCCH_2$), 6.75–6.85 (2H, m, H^{ar}), 6.90–7.05 (4H, m, H^{ar}), 7.20–7.35 (4H, m, H^{ar}); δ_C (75 MHz; MeOD-d_3) 25.1, 32.8, 54.4, 54.6, 64.1, 64.7, 65.8, 113.8, 119.2, 120.5, 121.4, 128.8, 129.6, 131.2, 136.9, 146.8, 147.2, 151.6, 162.8, 171.7, 174.6, 175.3. Anal. Calcd for $C_{35}H_{39}NO_{15}$: C, 58.9; H, 5.5; N, 2.0. Found: C, 58.6, H, 5.4, N, 2.1%.

3.1.3.2. Biscatechol-salicylate 25b. Colourless solid of mp 111–113 °C; ν_{max}/cm^{-1} 3363, 2928, 1737, 1642, 1583, 1460; δ_H (300 MHz; MeOD-d_3) 1.85–1.95 (4H, m, $OCCCH_2$), 2.39 (4H, t, ^3J=7.0 Hz, $OCCH_2C$), 2.80–2.95 (6H, m, NCH_2), 3.30–3.40 (4H, m, NHCH_2), 4.17 (4H, t, ^3J=5.6 Hz, $NCCH_2OR$), 4.51 (2H, t, ^3J=5.5 Hz, $NCCH_2OR'$), 6.65–6.85 (4H, m, H^{ar}), 7.00–7.30 (6H, m, H^{ar}); δ_C (75 MHz; MeOD-d_3) 25.5, 32.0, 39.6, 54.4, 54.5, 62.8, 63.6, 113.5, 117.1, 118.6, 120.3, 121.4, 121.9, 122.2, 128.0, 136.6, 148.2, 151.4, 161.3, 171.0, 171.7, 174.6. Anal. Calcd for $C_{35}H_{41}N_3O_{13}$: C, 59.1; H, 5.8; N, 5.9. Found: C, 58.7; H, 6.0; N, 5.7%.

3.1.3.3. Conjugate 26a. Pale yellow solid of mp 141–143 °C; $[\alpha]_D^{20}$ 62 (*c* 1.0, EtOH); ν_{max}/cm^{-1} 3365, 2938, 1780, 1732, 1694, 1636, 1453; δ_H (300 MHz; $CDCl_3$) 1.42 (3H, s, CH_3), 1.56 (3H, s, CH_3'), 1.95–2.10 (4H, m, $OCOCCH_2$), 2.35–2.45 (4H, m, $OCOCH_2C$), 2.75–3.00 (8H, m, NCH_2, $NH(CO)CH_2$), 3.11 (2H, t, ^3J=6.5 Hz, $NH(CO)CCH_2$), 3.74 (3H, s, OCH_3), 4.13 (4H, t, ^3J=5.7 Hz, $NCCH_2OR$), 4.30–4.45 (6H, m, $NCCH_2OR'$, $OCCCCH_2$), 4.39 (1H, s, $CHCO_2Me$), 5.48 (1H, d, ^3J=4.3 Hz, SCH), 5.71 (1H, dd, ^3J=4.3 Hz, ^3J=7.0 Hz, SCHCH), 6.32 (1H, d, ^3J=7.0 Hz, NH), 6.80–6.90 (2H, m, H^{ar}), 6.95–7.10 (3H, m, H^{ar}), 7.20–7.40 (4H, m, H^{ar}), 10.90 (5H, br, OH); δ_C (75 MHz; $CDCl_3$) 23.6, 25.2, 26.2, 29.7, 32.5, 35.3, 52.4, 54.5, 54.6, 58.2, 61.8, 63.9, 64.3, 64.7, 65.7, 70.1, 113.8, 116.3, 119.1, 120.3, 126.8, 127.3, 130.0, 131.2, 136.8, 146.6, 151.0, 162.4, 169.2, 171.1, 171.6, 172.1, 174.6, 175.5. Anal. Calcd for $C_{46}H_{55}N_3O_{18}S$: C, 57.0; H, 5.7; N, 4.3. Found: C, 56.6; H, 5.5; N, 4.5%.

3.1.3.4. Conjugate 26b. Pale yellow solid of mp 146–148 °C; $[\alpha]_D^{20}$ 57 (*c* 1.0, EtOH); ν_{max}/cm^{-1} 3360, 2932, 1781, 1741, 1675, 1639, 1585; δ_H (300 MHz; MeOD-d_3) 1.41 (3H, s, CH_3), 1.58 (3H, s, CH_3'), 1.85–1.95 (4H, m, $OCCCH_2$), 2.30–2.40 (4H, m, $OCCH_2C$), 2.80–3.05 (8H, m, NCH_2, $NH(CO)CH_2$), 3.08 (2H, t, ^3J=6.6 Hz, $NH(CO)CCH_2$), 3.30–3.45 (4H, m, $NHCH_2$), 3.72 (3H, s, OCH_3), 4.15 (4H, t, ^3J=5.6 Hz, $NCCH_2OR$), 4.50 (2H, t, ^3J=5.7 Hz, $NCCH_2OR'$), 4.35 (1H, s, $CHCO_2Me$), 5.50 (1H, d, ^3J=4.4 Hz, SCH), 5.70–5.80 (1H, m, SCHCH), 6.80–6.95 (3H, m, H^{ar}), 7.05–7.35 (6H, m, H^{ar}); δ_C (75 MHz; MeOD-d_3) 24.2, 25.6, 26.4, 29.8, 32.0, 35.3, 39.7, 52.2, 54.5, 54.7, 58.1, 62.8, 63.5, 64.1, 65.6, 69.6, 116.3, 118.2, 118.5, 119.6, 120.2, 127.4, 132.5, 135.0, 136.6, 148.2, 151.5, 161.8, 168.9, 171.2, 171.7, 172.1, 174.8, 175.6. Anal. Calcd for $C_{46}H_{57}N_5O_{16}S$: C, 57.1; H, 5.9; N, 7.2. Found: C, 56.8; H, 5.7; N, 7.0%.

3.1.4. Growth promotion assay. Siderophore activities of the chelators **13**, **18** and **25** of the conjugates **2** and **26**, and of ferrichrome were determined by measuring their ability to stimulate growth of bacterial strain *E. coli* H5596 kept under iron limitation by treatment with ethylenediamine bis(*o*-hydroxyphenyl)acetic acid (EDDA).

Agar plates containing 20 mL of Mueller–Hinton II medium (BD-Diagnostics, Heidelberg) with or without EDDA (LaboTest OHG, Niederschöna) at 100 μg mL^{-1} were inoculated with 100 μL of an *E. coli* H5596 suspension in liquid broth (opacity: Mc Farland 0.5) using a Drigalski spatula. Test samples were prepared as solutions of the respective compound in ethanol at 10, 5, 2.5 and 1 mg mL^{-1} and 15 μL of each solution was applied onto sterile 6 mm cellulose discs (CT 0998 B, Oxoid, Wesel). The ethanol was allowed to evaporate and the discs were placed upon the inoculated agar plate. The diameters in millimetre of the resulting growth zones were determined after 24 h of incubation at 36 °C.

Acknowledgements

We are indebted to Professor Klaus Hantke, Institute of Microbiology, University of Tübingen for kindly providing us with various *E. coli* mutant strains and siderophores.

References and notes

1. Hikaido, H. *Science* **1994**, *245*, 388–399.
2. Faraldo-Gómez, J. D.; Sansom, M. S. P. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 105–116.
3. Braun, V.; Braun, M. *Curr. Opin. Microbiol.* **2002**, *4*, 194–201.
4. Roosenberg, J. M.; Lin, Y.-M.; Yong, L.; Miller, M. J. *Curr. Med. Chem.* **2000**, *7*, 159–197.
5. Drechsel, H.; Jung, G. *J. Pept. Sci.* **1998**, *4*, 147–181.
6. Winkelmann, G.; van der Helm, D.; Neilands, J. B. *Iron Transport in Microbes, Plants and Animals*; VCH: Weinheim, 1987; pp 223–233.
7. Braun, V. *Drug Resist. Updat.* **1999**, *2*, 363–369.
8. Heinisch, L.; Wittmann, S.; Stoiber, T.; Berg, A.; Ankel-Fuchs, D.; Möllmann, U. *J. Med. Chem.* **2002**, *45*, 3032–3040.
9. Gosh, A.; Gosh, M.; Niu, C.; Malouin, F.; Möllmann, U.; Miller, M. J. *Chem. Biol.* **1996**, *3*, 1011–1019.
10. Budzikiewicz, H. *Curr. Top. Med. Chem.* **2001**, *1*, 73–82.
11. Sharma, S. K.; Miller, M. J.; Payne, S. M. *J. Med. Chem.* **1989**, *32*, 357–367.
12. Schobert, R.; Boeckman, R. K., Jr.; Pero, J. E. *Org. Synth.* **2005**, *82*, 140–145.
13. Grigg, R.; Rankovic, Z.; Thoroughgood, M. *Tetrahedron* **2000**, *56*, 8025–8032.
14. Bestmann, H. J.; Schobert, R. *Angew. Chem., Int. Ed. Engl* **1985**, *24*, 790–791.
15. Newkome, G. R.; Marston, C. R. *J. Org. Chem.* **1985**, *50*, 4238–4245.
16. Gardner, R. A.; Kinkade, R.; Wang, C.; Phanstiel, O. *J. Org. Chem.* **2004**, *69*, 3530–3537.
17. Schobert, R.; Stangl, A. *Tetrahedron Lett.* **2005**, *46*, 1127–1130.
18. Storer, R.; Moussa, A.; Wang, J. Y.; Chaudhuri, N.; Mathieu, S.; Stewart, A. Patent WO 2,005,003,374, 2005.
19. Gehrer, E.; Klötzer, W.; Singewald, N.; Stadlwieser, J. *Synthesis* **1987**, 633–635.
20. Prepared analogously to **18** by replacing **9** with 2-benzoxybenzoyl chloride; details are available from the authors upon request.
21. Prepared from **16**, which was esterified with 2-benzoxy-3-formylbenzoic acid to give an aldehyde. The latter was reacted with **3** and **4** and the resulting product amide was exhaustively hydrogenated to leave compound **26**. Details are available from the authors upon request.