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Diphenyl-benzo[1,3]dioxole-4-carboxylic acid pentafluorophenyl ester: a convenient catechol precursor in the synthesis of siderophore vectors suitable for antibiotic Trojan horse strategies†

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Catechols are components of many metal-chelating compounds, including siderophores that are naturally occurring iron(III) chelators excreted by microorganisms. Catechol derivatives are poorly soluble in organic media and the synthesis of catechol-containing molecules requires the use of protected catechol precursors with improved organic solubility. We therefore developed 2,2-diphenyl-benzo[1,3]dioxole-4-carboxylic acid pentafluorophenyl ester. This activated ester reacts with an amine functionalized scaffold to generate chelators in which the catechol functions are protected in the form of diphenyl-benzodioxole moieties. The catechol can subsequently be deprotected, at the end of the synthesis, with trifluoroacetic acid (TFA). This strategy was applied to the synthesis of two catechol compounds functionalized with a terminal propargyl extension. These two compounds were shown to promote iron uptake in *Escherichia coli* and *Pseudomonas aeruginosa*. These two compounds are suitable for use as vectors in antibiotic Trojan horse approaches, as they could be conjugated with azide-functionalized antibiotics using the Huisgen dipolar 1,3-cycloaddition.

Introduction

Although iron is one of the major components of the earth's crust, its bioavailability is drastically limited by the low solubility of iron(III) at physiological pH under aerobic conditions. Indeed, the iron(III) concentration in the environment is estimated to be 10^{-9} M and in human biological fluids it is around 10^{-18} M.[1a] Many microorganisms require an iron(III) concentration in the micromolar range for an optimal proliferation. Under iron limitation, almost all microorganisms synthesize and secrete siderophores so that they can assimilate this essential element.[1] Siderophores chelate iron(III) in the extracellular medium and the resulting ferric–siderophore–iron(III) is then translocated by a multiprotein system through the membranes into the cytoplasm.[2] Siderophores are a broad family of natural compounds with substantial structural diversity, but nevertheless optimized during evolution towards a common purpose: the efficient chelation of iron(III).[1] Although there are some notable exceptions, most siderophores are a combination of bidentate moieties, for example catechol, hydroxamate or hydroxy-acid, attached to a peptide or a peptide-like scaffold. Catechol is the most common bidentate moiety found in the siderophores described to date. Catechol siderophores, and their synthetic analogues, have been used in recent decades for a variety of applications in human health.[3,4] However, the use of catechol siderophores as vectors for antibiotic Trojan horse strategies is probably one of the more striking possibilities.[5] The low permeability of membranes is the first defense of bacteria against antibiotics.[6] Siderophore-dependent iron uptake pathways are gates through this barrier and therefore possible routes for introducing antibiotic compounds into the bacteria.[7] Several groups have described the synthesis of conjugates between siderophores and antibiotics with this aim.[5,8–22] Many of the siderophore vectors used contain, at least, one catechol group to promote iron chelation.[8–11] We have developed the bis-catechol **1** and tris-catechol vector **2** carrying a terminal alkyne to facilitate the subsequent conjugation of azide-functionalized antibiotics using the Huisgen dipolar 1,3-cycloaddition reaction (Fig. 1).

Catechol containing siderophores are generally synthesized by a common pathway. A peptide, or peptidomimetic, scaffold is synthesized first, then catechol precursors are grafted onto amine functions present on the scaffold. The catechol precursors described to date are all derived from the commercially available 2,3-hydroxybenzoic acid **3** (Fig. 2). In these

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†Electronic supplementary information (ESI) available: ^{1}H-NMR and HRMS spectra of compounds **1**, **2** and **7**. ^{19}F-NMR of compound **7**. See DOI: 10.1039/c3ob41990h

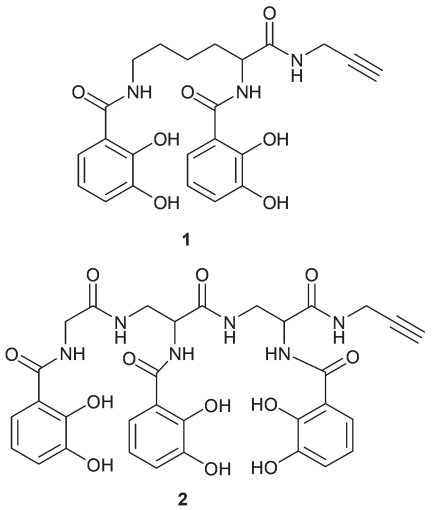


Fig. 1 Structures of bis-catechol and tris-catechol vectors **1** and **2**.

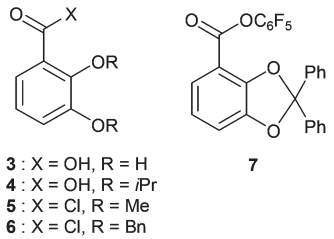


Fig. 2 Structures of 2,3-dihydroxybenzoic acid **3** and of catechol precursors **4** to **7**.

precursors, the carboxylate is usually activated in the form of an acyl-chloride; the two hydroxyl groups are protected. Protecting groups prevent side reactions, and avoid problems of solubility in organic media and tedious purification steps. The last step of the siderophore synthesis is usually the cleavage of these protecting groups to restore the chelation ability of the final compound. Several catechol synthons have been described in recent decades. In most of these compounds, the two hydroxyls of the catechol are protected by alkylation. Synthons **4**, **5** and **6** have been the most widely used for the synthesis of siderophores and their analogues. The methyl and isopropyl protecting groups of synthons **5** and **4** can be cleaved by strong Lewis acids, for example BCl_3 or BBr_3, although in some cases the yields are low.[8,23,24] Also, the use of such acids may affect many other organic functions. The benzyl protecting groups of precursor **6** can be released by catalytic reduction in the presence of hydrogen.[9,10,24,25] However, this cleavage strategy is not compatible with many chemical functions, and especially the alkyne moiety present on the expected chelators **1** and **2**. To avoid these problems, we synthesised diphenyl-benzo[1,3]dioxole-4-carboxylic acid pentafluorophenyl ester **7**, a new catechol precursor; this precursor can be used for the synthesis of chelating molecules that are sensitive to both strong Lewis acids and hydrogenolysis (Fig. 2).

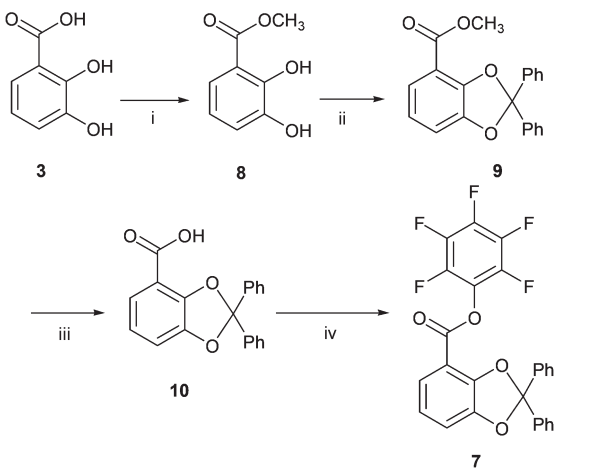
In compound **7**, the catechol moiety is protected in the form of a diphenyl-dioxole. It has been reported that the catechol function can be released from this structure easily under mild acidic conditions.[26] This new catechol precursor, compound **7**, was used in the synthesis of the bis-catechol and tris-catechol compounds **1** and **2**, respectively.

Results and discussion

Organic synthesis

The title compound **7** was prepared from commercially available 2,3-dihydroxybenzoic acid **3**. This acid was first reacted with thionyl chloride and the resulting acyl chloride was esterified by a methanolic solution of triethylamine. The expected ester **8** was isolated with a yield of 99%. The purified methyl-ester **8** was then heated at 160 °C in a slight excess of dichlorodiphenylmethane to produce a dioxole **9**;[26] as this compound cannot easily be purified, the crude mixture was used as it is for the next step. The methylester function of the dioxole **9** was converted into the corresponding carboxylate **10**, using potassium trimethylsilanolate (TMSOK) in refluxing THF.[27] The expected carboxylate **10** was thus obtained from methyl-ester **8** with a yield of 59% over two steps. Other methods, including those described in the literature for similar molecules,[26] were tested but were unsuccessful: in general, they led to partial hydrolysis of the dioxole moiety during the work-up. The carboxylate function of compound **10** was then reacted with pentafluorophenol in the presence of various carbodiimide coupling reagents. The best results were obtained using diisopropyl-carbodiimide (DIC),[28] which gave a yield of 85%. Under these conditions the expected catechol precursor **7** was obtained from the 2,3-dihydroxybenzoic acid **3** by a multigram four-step process with an overall yield of 50% (Scheme 1).

During the development of the precursor **7**, di-(4-methoxyphenyl)-dioxole was tested first as the protecting group for



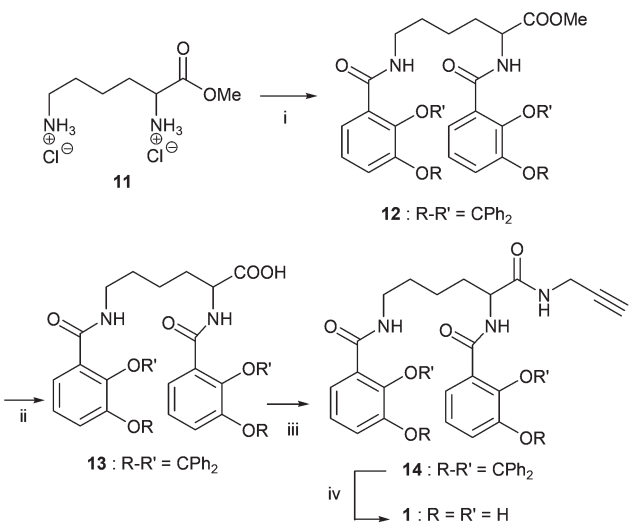
Scheme 1 Synthesis of diphenyl-benzo[1,3]dioxole-4-carboxylic acid pentafluorophenyl ester **7**. i. A. $SOCl_2$ reflux. B. MeOH, NEt_3, 0 °C to 25 °C. ii. Cl_2CPh_2, neat, 160 °C. iii. TMSOK, THF, 70 °C. iv. DIC, C_6F_5OH, acetonitrile–pyridine, 25 °C.

the catechol moiety. However, this protecting group was too unstable and undesired cleavage occurred under many synthetic conditions. The diphenyl-dioxole proved to be a more suitable protecting group for catechol. The activation of the carboxylate in the form of an *N*-hydroxysuccinimide ester was also investigated. The yield of the activation reactions was identical for both *N*-hydroxysuccinimide and pentafluorophenol, but pentafluorophenol was found to be more easily removed from the crude mixture after the reaction between the activated ester and the siderophore scaffold.

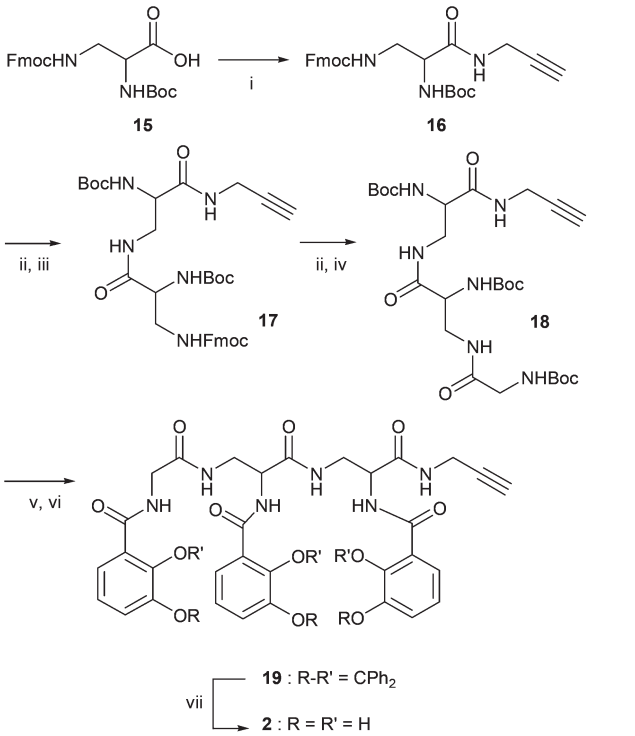
The catechol synthon **7** was then used in the synthesis of the two catechol compounds **1** and **2**. Compound **1** is an analogue of azotochelin, one of the three catechol siderophores of *Azotobacter vinelandii*, a Gram-negative bacterium and a member of the Pseudomonadaceae family.[29–31] The synthesis of this compound starts with the commercially available lysine methylester dihydrochloride **11**: this lysine derivative was treated with the catechol precursor **7** in the presence of DIPEA leading to the expected diamide **12** which was isolated in quantitative yield. The methyl ester function of compound **12** was then quantitatively saponified using the $LiOH–H_2O_2$ system in THF.[32] The corresponding free acid **13** was coupled to propargylamine in the presence of HBTU, leading to the protected vector **14**, isolated with a yield of 87%. The diphenyl-dioxole groups protecting the catechol functions of molecule **14** were cleaved using diluted TFA in the presence of triisopropylsilane (TIS). Using this procedure, the bis-catechol compound **1** was isolated in 95% yield (Scheme 2).

The second target chelator, the tris-catechol compound **2**, was synthesized using commercially available Boc-3-(Fmoc-amino)-L-alanine (Boc-Dap-Fmoc) **15** as the starting material. Boc-Dap-Fmoc **15** was first coupled to propargylamine in the presence of HBTU. The reaction led to the expected amide **16** isolated in 86% yield. Compound **16** was then treated with a solution of diethylamine in methanol to cleave the Fmoc group. The resulting free amine was coupled to another Boc-Dap-Fmoc unit using the conditions described above. The expected molecule **17** was thereby isolated over two combined steps from compound **16** at a yield of 63%. Further treatment of compound **17** with diethylamine, followed by coupling to Boc-*N*-glycine in the presence of HBTU, led to the expected tricarbamate scaffold **18** at a yield of 44%. Treatment of compound **18** with diluted TFA led to the corresponding triamine; this triamine was then reacted with the catechol precursor **7**. The protected tris-catechol compound **19** was isolated, and the yield over two steps from the tricarbamate **18** was 41%. The protecting groups were cleaved by treatment with diluted TFA in the presence of triisopropylsilane (TIS), leading to the expected free tris-catechol molecule **2**. The compound **2** was easily purified by partition in a water–diethyloxide mixture and was thereby isolated with a quantitative yield (Scheme 3).

Catechol compounds **1** and **2** may be suitable for use to vectorize antibiotics as part of a Trojan horse strategy. To evaluate the potential of these two compounds in this type of application, iron uptake experiments were used to assess whether they are recognized as siderophores by target bacteria.



Scheme 2 Synthesis of bis-catechol vector **1**. i. DIPEA, CH_2Cl_2, 20 °C. ii. LiOH, H_2O_2, THF, 24 °C. iii. Propargylamine, HBTU, DIPEA, CH_2Cl_2, 22 °C. iv. TFA–CH_2Cl_2 10%, TIS, 24 °C.



Scheme 3 Synthesis of tris-catechol vector **2**. i. Propargylamine, HBTU, DIPEA, CH_2Cl_2, 25 °C. ii. Diethylamine, MeOH, 0 °C to 25 °C. iii. **15**, HBTU, DIPEA, CH_2Cl_2, 24 °C. iv. Boc-*N*-Glycine, HBTU, DIPEA, CH_2Cl_2, 24 °C. v. TFA–CH_2Cl_2 25%, 24 °C. vi. **7**, DIPEA, THF, 24 °C. vii. TFA–CH_2Cl_2 10%, TIS, 24 °C.

Iron uptake experiments

The ability of catechol compounds **1** and **2** to promote $^{55}Fe(III)$ uptake by *E. coli* and *P. aeruginosa* was tested. To simplify these analyses, strains not producing endogenous siderophores were used for the uptake assays: *E. coli* strain BW25113 *entB*::Kan^{R},[33] defective for the biosynthesis of enterobactin, and *P. aeruginosa* strain PAD07, a pyoverdine and pyochelin-deficient strain.[34] Llamas *et al.* showed that the expression of some TonB-dependent siderophore transporters in *P. aeruginosa* is upregulated by the absence of iron and the presence of the corresponding siderophore in the culture medium.[35,36] Therefore, both strains were grown in the presence of the tested siderophore (catechol **1** or **2**). Afterwards for the iron uptake assays, the bacteria were incubated in the presence of 100 nM enterobactin-^{55}Fe (Fig. 3A and 3B), **1**-^{55}Fe (Fig. 3C and 3D) or **2**-^{55}Fe (Fig. 3E and 3F) and the radioactivity incorporated into the bacteria monitored. Enterobactin was used as a reference siderophore. The experiments were repeated with cells pretreated with the protonophore CCCP: this compound inhibits the proton motive force (PMF) across the bacterial cell membrane and therefore inhibits TonB-dependent iron uptake (Fig. 3).[37]

Enterobactin-^{55}Fe, **1**-^{55}Fe and **2**-^{55}Fe were taken up by both *P. aeruginosa* and *E. coli* (Fig. 3). Moreover, ^{55}Fe incorporation by *P. aeruginosa* was more efficient with catechol compound **1** and that by *E. coli* with catechol compound **2**. The absence of ^{55}Fe accumulation in the presence of CCCP indicates that **1**

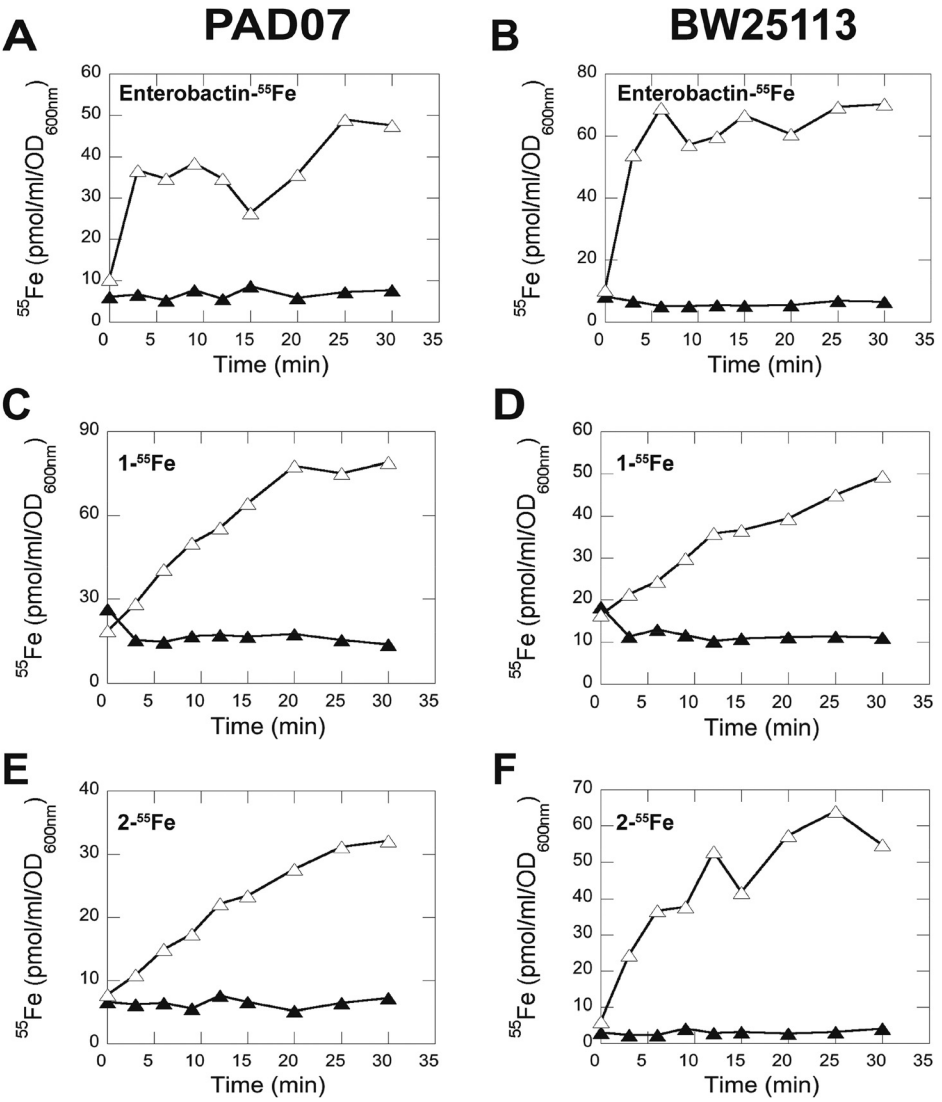


Fig. 3 Enterobactin-^{55}Fe, **1**-^{55}Fe and **2**-^{55}Fe uptake by PAD07 (*P. aeruginosa*) and BW25113*entB*::Kan^{R} (*E. coli*). PAD07 (A, C and E) and BW25113*entB*::Kan^{R} (B, D and F) cells at an OD_{600} of 1 were incubated for 15 min in 50 mM Tris-HCl (pH 8.0) buffer. Transport assays were then started by adding enterobactin-^{55}Fe (A and B), **1**-^{55}Fe (C and D) or **2**-^{55}Fe (E and F). Aliquots (100 μL) of the suspension were removed at various times and filtered, and the radioactivity retained was counted. As controls, similar experiments were repeated in the presence of 200 μM CCCP: △, experiment in the presence of the tested siderophores and no CCCP; ▲ experiment in the presence of the tested siderophores and CCCP. All experiments were repeated three times, with comparable results.

and **2** are transported through one or more TonB-dependent transporters, which are PMF-dependent (Fig. 3). ^{55}Fe uptake occurred for compounds **1** and **2** only when cells were grown in the presence of the tested iron chelator. When the cells were grown in the absence of the tested chelator, no uptake was observed (data not shown) indicating that like many siderophores used by *P. aeruginosa*,[35,36] compounds **1** and **2** induce the expression of the TonB-dependent transporters involved in their uptake.

Conclusions

We report the synthesis of diphenyl-benzo[1,3]dioxole-4-carboxylic acid pentafluorophenyl ester **7**, a new catechol precursor suitable for the synthesis of siderophores and other chelators containing the catechol moiety. Its synthetic versatility was illustrated by the efficient synthesis of the bis-catechol and tris-catechol compounds **14** and **19** in which the catechol moiety is protected in the form of a 2,2-diphenyl-1,3-dioxole. The acetylenic function of these two molecules was not chemically altered during the deprotection of catechol function under acidic conditions. The deprotected bis- and tris-catechol compounds **1** and **2** enabled efficient iron(III) uptake by both *E. coli* and *P. aeruginosa*, two Gram-negative bacterial species. The bis-catechol **1** proved to be more efficient than the tris-catechol **2** for ^{55}Fe incorporation into *P. aeruginosa* whereas tris-catechol **2** was more efficient in *E. coli*. However, the iron acquisition systems involved in the uptake of ferric complexes of **1** and **2** have not yet been identified. Nevertheless, these results pave the way for the use of catechol compounds **1** and **2** as vectors in antibiotic Trojan horse strategies. For this purpose, the terminal alkyne function of protected catechol compounds **14** and **19** will be linked to azide-functionalized antibiotics by regioselective copper(I)-catalyzed 1,3-dipolar cycloaddition.[38,39] This "click" reaction will be followed by treatment with diluted TFA to release the catechol groups. Many antibiotics are resistant to mild conditions of this type, such that their structures should be unaffected. This work is underway, with the aim of developing novel tools for antibiotic therapy against pathogenic *E. coli* and *P. aeruginosa* strains.

Experimental section

General

All reactions were carried out under argon purchased from Air Liquide. Solvents used for reaction were of analytical grade purity (>99.9%). When necessary, and specified in the protocols, solvents were purchased extra-dry from Aldrich, Acros and Alfa-Aesar companies. Amines were distilled and stored on KOH before use. All other chemicals were obtained from commercial suppliers (Aldrich, Acros or Alfa-Aesar) and were used as received, unless otherwise stated. $^{55}FeCl_3$ was obtained from Perkin Elmer Life and Analytical Sciences (Billerica, MA, USA) with a specific activity of 93.76 Ci g^{-1}. This radioactive ^{55}Fe solution was diluted with non-radioactive $FeCl_3$ to 9.4 Ci g^{-1}. Tetracycline hydrochloride was purchased from Euromedex. The protonophore CCCP (carbonyl cyanide *m*-chlorophenylhydrazone), kanamycin, streptomycin sulphate and enterobactin were purchased from Sigma-Aldrich.

Chromatography

All reactions were monitored by thin-layer chromatography (TLC) using Merck aluminium sheets precoated with silica gel 60F^{254} (0.25 mm). Column chromatography purifications were performed using Merck silica gel 60 (63–200 μm). In some protocols, silica should be demetallated prior to use. For this purpose commercial silica was suspended and stirred 10 h in 1 N HCl. Silica gel was filtered and washed several times with bidistillated water, dried under reduced pressure and then in an oven (110 °C) for at least 72 h.[40]

Instrumentation

NMR spectra were recorded on Bruker Avance 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), using the residual non-deuterated solvent as a reference. The chemical shifts (δ) and coupling constants (J) are expressed in ppm and hertz (Hz) respectively. Multiplicities were indicated as s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). A broad signal is mentioned with br preceeding the multiplicity. Mass spectra were recorded in the *Service Commun d'Analyse* (SCA) *de la Faculté de Pharmacie de l'Université de Strasbourg* and were measured after calibration in ES-TOF experiments on a Bruker Daltonic MicroTOF mass spectrometer. Countings of radioactive samples were performed on a Packard TriCarb 2100TR.

Methyl 2,3-dihydroxybenzoate 8. A solution of 2,3-dihydroxybenzoic acid **3** (5.00 g, 32.44 mmol) in thionyl chloride (15 mL) was heated at 78 °C (reflux). When the gases emission stopped, excess thionyl chloride was eliminated under reduced pressure. At 0 °C, the residue was very slowly dissolved in a mixture of methanol (17 mL) and triethylamine (6 mL). The resulting solution was stirred for 16 hours at 25 °C. The reaction mixture was adsorbed on silica gel before being purified by chromatography on a silica gel column (cyclohexane–CH_2Cl_2 1 : 1 then pure CH_2Cl_2). The expected methylester **8** was isolated in the form of white crystals (5.40 g, 32.10 mmol, yield: 99%). R_f 0.41 (cyclohexane–CH_2Cl_2 1 : 1). ^{1}H-NMR (400 MHz, $CDCl_3$, 25 °C): δ 7.34 (d, J = 8.1 Hz, 1H); 7.09 (d, J = 7.9 Hz, 1H); 6.77 (t, J = 8.0 Hz, 1H); 3.93 (s, 3H). ^{13}C-NMR (100 MHz, acetone d_6) δ 171.8, 150.9, 147.0, 121.6, 121.0, 120.0, 113.5, 53.0. MS (ESI) m/z: 169.0 [M + H$^+$].

2,2-Diphenylbenzo[*d*][1,3]dioxole-4-carboxylic acid 10. 2,3-Dihydroxybenzoic acid methyl ester **8** (5.40 g, 32.10 mmol) and dichloro-diphenylmethane (7.4 mL, 9.10 g, 38.50 mmol) were heated neat at 160 °C. When the gases emission stopped, the viscous crude mixture was cooled down to room temperature under argon before being dissolved in ethyl acetate (200 mL). The organic layer was washed three times with an aqueous solution saturated in $NaHCO_3$ and finally with brine. After filtration and solvent removal under reduced pressure the resulting pale yellow powder (compound **9**) was dissolved in THF

(150 mL) and potassium trimethylsilanolate tech. (90%) (21.00 g, 160.60 mmol) was added. The solution was then heated to reflux for 2 hours. THF was then removed under reduced pressure and the resulting residue was diluted in an aqueous solution saturated with citric acid. After several extractions with $CHCl_3$, the organic layers were collected, washed with brine, dried over Na_2SO_4, and filtered before being adsorbed on silica gel. A purification by chromatography on a silica gel column (cyclohexane–CH_2Cl_2 1 : 1 then pure CH_2Cl_2 and finally CH_2Cl_2–EtOH 9 : 1) afforded the acid **10** (6.00 g, 19.00 mmol, yield: 59% over two steps) isolated as a pale yellow powder. R_f 0.40 (CH_2Cl_2–EtOH 96 : 4). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63–7.60 (m, 4H), 7.47 (d, J = 8.2 Hz, 1H), 7.40–7.35 (m, 6H), 7.06 (d, J = 7.7 Hz, 1H), 6.87 (dd, J = 7.7, 8.2 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): 169.5, 149.0, 148.6, 139.8, 129.6, 128.6, 126.6, 123.5, 121.6, 118.5, 113.4, 112.3. MS (ESI) m/z: 319.1 [M + H$^+$], 341.1 [M + Na$^+$]. HRMS (ESI): $C_{20}H_{14}O_4$: calcd 318.0892, found 318.0888.

Pentafluorophenyl 2,2-diphenylbenzo[*d*][1,3]dioxole-4-carboxylate 7. To a solution of the dioxole **10** (1.00 g, 3.14 mmol) in a mixture of acetonitrile (9 mL) and pyridine (1 mL) were successively added pentafluorophenol (726 mg, 3.95 mmol) and diisopropylcarbodiimide (614 μL, 3.95 mmol). The mixture was stirred at 25 °C under argon for 3 hours. The crude mixture was adsorbed on silica gel before being purified by chromatography on a silica gel column (cyclohexane–CH_2Cl_2 1 : 1). The expected catechol synthon **7** (1.29 g, 2.67 mmol, yield: 85%) was isolated as a white solid. R_f 0.50 (cyclohexane–CH_2Cl_2 1 : 1).^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71–7.67 (m, 4H), 7.59 (d, J = 8.3 Hz, 1H), 7.46–7.39 (m, 6H), 7.18 (d, J = 7.8 Hz, 1H), 6.96 (dd, J = 7.7, 8.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.5, 149.6, 148.9, 139.7, 136.9, 129.6, 128.6, 126.5, 123.4, 121.9, 119.0, 114.2, 109.8. ^{19}F NMR (100 MHz, $CDCl_3$): δ −152.0 (d, 2F) −158.1 (t, 1F), −162.4 (m, 2F). MS (ESI): m/z 485.0 [M + H$^+$]. HRMS (ESI): $C_{26}H_{13}F_5O_4$: calcd 484.0734, found 484.0735.

(*R*)-Methyl *N*2,*N*6-bis(2,2-diphenylbenzo[*d*][1,3]dioxole-4-carbonyl)lysinate 12. Lysine methyl ester di-hydrochloride **11** (239 mg, 1.03 mmol) was dissolved in a mixture of DIPEA (2.05 mL, 12.40 mmol) and anhydrous CH_2Cl_2 (10 mL). To this solution was added dropwise a solution of pentafluorophenyl-ester **7** (1000 mg, 2.06 mmol) in anhydrous CH_2Cl_2 (10 mL). The mixture was stirred overnight for 16 hours at 24 °C before being adsorbed on silica gel and further purified by chromatography on a silica gel column (CH_2Cl_2 then CH_2Cl_2–EtOH 9 : 1). The diamide **12** (785 mg, 1.03 mmol, yield: 100%) was isolated as a white foamy solid. R_f 0.35 (CH_2Cl_2–EtOH 96 : 4). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.01 (d, J = 7.4 Hz, 1H), 7.66 (dd, J = 2.0, 7.2 Hz, 2H), 7.6 (dd, J = 2.3, 7.5 Hz, 2H), 7.56–7.50 (m, 6H), 7.43–7.33 (m, 12H), 7.19 (t, J = 5.8 Hz, 1H), 7.02 (dd, J = 7.9, 10.7 Hz, 2H), 6.93 (t, J = 8.0 Hz, 2H), 4.88 (dt, J = 5.5, 6.9 Hz, 1H), 3.75 (s, 3H), 3.53–3.38 (m, 2H), 2.11–2.02 (m, 1H), 1.96–1.87 (m, 1H), 1.71–1.67 (m, 2H), 1.52–1.44 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.8, 164.1, 163.4, 147.5, 147.4, 145.3, 144.9, 139.7, 139.6, 139.2, 129.9, 129.7, 129.6, 128.7, 126.5, 126.2, 122.6, 122.4, 122.3, 118.6, 118.4, 115.9, 115.4, 112.2, 112.0, 52.7, 39.8, 32.6, 29.6, 22.8. MS (ESI) m/z 761.3 [M + H$^+$], 783.3 [M + Na$^+$]. HRMS (ESI): $C_{47}H_{40}N_2O_8$: calcd 760.2785, found 760.2792.

(*R*)-2,6-Bis(2,2-diphenylbenzo[*d*][1,3]dioxole-4-carboxamido)-hexanoic acid 13. To a solution of methyl ester **12** (785 mg, 1.03 mmol) in THF (5 mL) was added, at 0 °C, a solution of LiOOH (5 mL; 2.7 M of LiOH solution in 30% H_2O_2). The mixture was stirred at 24 °C overnight for 16 hours. After completion of the reaction, an aqueous solution saturated in citric acid was added and THF was evaporated under reduced pressure. The aqueous residue was extracted three times with chloroform. Organic layers were collected, dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The expected acid **13** (770 mg, 1.03 mmol, yield: 100%) was isolated as a white foamy solid. R_f 0.50 (CH_2Cl_2–EtOH 92 : 8). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.03 (d, J = 7.3 Hz, 1H), 7.62 (d, J = 8.0 Hz, 2H), 7.57–7.48 (m, 8H), 7.35–7.20 (m, 12H), 7.19 (t, J = 5.8 Hz, 1H), 7.02 (dd, J = 7.5, 13.9 Hz, 2H), 6.9 (td, J = 6.6, 8.0 Hz, 2H), 4.86 (q, J = 6.5 Hz, 1H), 3.50–3.34 (m, 2H), 2.11–2.08 (m, 1H), 1.96–1.94 (m, 2H), 1.68–1.63 (m, 2H), 1.54–1.50 (m, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.6, 163.8, 147.6, 147.4, 145.0, 139.7, 139.6, 139.2, 129.9, 129.7, 129.6, 128.7, 126.6, 126.2, 122.7, 122.4, 122.3, 115.8, 115.4, 112.3, 112.1, 52.8, 40.0, 32.2, 29.6, 22.7. MS (ESI) m/z 747.2 [M + H$^+$], 770.0 [M + Na$^+$].

***N*,*N*′-(6-Oxo-6-(prop-2-yn-1-ylamino)hexane-1,5-diyl)bis(2,2-diphenylbenzo[*d*][1,3]dioxole-4)carboxamide) 14.** To a solution of acid **13** (200 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (5 mL) were added successively HBTU (112 mg, 0.29 mmol), DIPEA (133 μL, 0.80 mmol) and propargylamine (43 μL, 0.67 mmol). The reaction was stirred under argon at 22 °C for 16 hours. The mixture was diluted with CH_2Cl_2 and successively washed with an aqueous solution saturated in $NaHCO_3$, with water and finally with a saturated aqueous citric acid solution. The organic layer was dried over Na_2SO_4, filtrated and adsorbed on silica gel. A purification by chromatography on a silica gel column (CH_2Cl_2 then CH_2Cl_2–EtOH 9 : 1) afforded the propargylamide **14** (183 mg, 0.23 mmol, yield: 87%) isolated as a white solid. R_f 0.33 (cyclohexane–AcOEt 1 : 1) ^{1}H-NMR (400 MHz, acetone d_6): δ 8.33 (d, J = 7.3 Hz, 1H), 8.10 (t, J = 5.2 Hz, 1H) 7.89 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 8.0 Hz, 2H), 7.75–7.70 (m, 4H), 7.67 (t, J = 6.7 Hz, 1H), 7.58–7.43 (m, 14H), 7.25 (d, J = 7.7 Hz, 1H), 7.20 (d, J = 7.6 Hz, 1H), 7.05 (dt, J = 6.5 Hz, 7.7 Hz, 2H), 4.90 (dt, J = 5.1, 7.1 Hz, 1H), 4.18 (br s, 2H), 3.54 (q, J = 7.4 Hz, 2H), 2.75 (s, 1H), 2.20–2.11 (m, 2H), 2.06–1.96 (m, 2H), 1.82–1.74 (m, 2H), 1.68–1.61 (m, 2H). ^{13}C-NMR (100 MHz, acetone d_6): δ 172.2, 171.0, 163.6, 163.0, 148.3, 145.8, 145.6, 140.9, 140.8, 140.5, 130.4, 130.3, 129.5, 129.4, 127.1, 126.9, 126.8, 123.2, 123.0, 122.9, 118.9, 118.8, 117.7, 117.2, 112.6, 112.2, 81.1, 72.4, 53.9, 40.3, 34.1, 30.4, 29.3, 23.4. MS (ESI) m/z 784.2 [M + H$^+$], 807.2 [M + Na$^+$]. HRMS (ESI): $C_{49}H_{41}N_3O_7$: calcd 783.2945, found 783.2960.

***N*,*N*′-(6-Oxo-6-(prop-2-yn-1-ylamino)hexane-1,5-diyl)bis(2,3-dihydroxybenzamide) 1.** Compound **14** (105.5 mg, 0.14 mmol) was dissolved in a mixture of TFA (400 μL, 1.53 mmol) in CH_2Cl_2 (3.6 mL). After 16 hours at 24 °C, the mixture was

evaporated under reduce pressure and diethyl-oxide was added. The gum formed was triturated in diethyl-oxide. The solvent was removed by filtration and the resulting solid was finally dissolved in acetonitrile before being adsorbed on demetellated silica gel and purified by chromatography on a demetalled silica gel column (CH_2Cl_2, then CH_2Cl_2–EtOH 9 : 1). The expected bis-catechol compound **1** (58 mg, 0.13 mmol, yield: 95%) was isolated as a brownish solid. R_f 0.50 (CH_2Cl_2–EtOH 96 : 4). ^{1}H-NMR (400 MHz, CD_3CN): δ 7.49 (d, J = 7.5 Hz, 1H), 7.39–7.35 (m, 1H), 7.19 (dd, J = 1.7, 8.2 Hz, 1H), 7.10 (t, J = 5.9 Hz, 1H), 7.06 (dd, J = 1.6, 8.2 Hz, 1H), 6.96 (ddd, J = 1.6, 7.9, 12.2 Hz, 2H), 6.72 (dt, J = 8.0, 9.6 Hz, 2H), 4.51 4.45 (m, 1H), 3.92 (dd, J = 2.6, 5.9 Hz, 2H), 3.36 (dq, J = 1.9, 7.1 Hz, 2H), 1.97–1.89 (m, 1H), 1.86–1.76 (m, 1H), 1.69–1.56 (m, 2H), 1.51–1.39 (m, 2H), 1.26 (s, 1H). ^{13}C-NMR (100 MHz, CD_3CN): δ 171.9, 171.0, 170.9, 150.1, 150.0, 146.5 (2), 119.3, 119.1, 118.9, 118.8, 118.1, 117.5, 115.2, 114.9, 80.7, 71.5, 54.1, 39.4, 31.7, 29.1, 28.9, 23.4. MS (ESI) m/z 456.0 [M + H^+]. HRMS (ESI): $C_{23}H_{25}N_3O_7$: calcd 455.1693, found 455.1701.

(9*H*-Fluoren-9-yl)methyl *tert*-butyl (3-oxo-3-(prop-2-yn-1-yl-amino)propane-1,2-diyl)dicarbamate 16. Boc-DAP-Fmoc-OH **15** (1000 mg, 2.35 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL). HBTU (978 mg, 2.58 mmol), DIPEA (1.16 mL, 7.03 mmol) and propargylamine (0.15 mL, 2.35 mmol) were added successively to this solution at 24 °C. The reaction mixture was then stirred at 24 °C for 16 hours under argon. The solution was adsorbed on silica gel and a chromatographic purification on a silica gel column (CH_2Cl_2, then CH_2Cl_2–EtOH 96 : 4) afforded propargylamide **16** (940 mg, 2.03 mmol, yield: 86%) isolated as a white powder. R_f 0.50 (CH_2Cl_2–EtOH 96 : 4). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.45 (d, J = 7.6 Hz, 2H), 7.3 (d, J = 7.4 Hz, 2H), 7.08 (t, J = 7.4 Hz, 2H), 6.99 (t, J = 7.4 Hz, 2H), 4.06 (d, J = 6.9 Hz, 2H), 3.89 (t, J = 7.0 Hz, 2H), 3.66 (d, J = 2.5 Hz, 2H), 3.17 (dd, J = 5.0, 13.9 Hz, 1H), 2.04 (t, J = 2.5 Hz, 1H), 1.11 (s, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.5, 141.0 (2), 127.3, 126.7, 124.6, 119.6, 71.1, 66.7, 54.5, 46.8, 42.4, 28.4, 27.5. MS (ESI) m/z 486.2 [M + Na^+]. HRMS (ESI): $C_{26}H_{29}N_3O_5$: calcd 463.2107, found 463.2105.

[1-(2-*tert*-Butoxycarbonylamino-2-prop-2-ynylcarbamoyl-ethyl carbamoyl)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-ethyl]-carbamic acid *tert*-butyl ester 17. The propargylamide **16** (900 mg, 1.94 mmol) was dissolved in MeOH (15 mL). To this solution was added, at 0 °C, diethylamine (1.60 mL, 15.62 mmol). The resulting solution was stirred at 24 °C for 3 hours and then the mixture was evaporated under reduced pressure. Crude amine (*ca.* 470 mg) was used for the next synthetic step without any further purification. The crude product was dissolved in a mixture of DIPEA (966 µL, 5.84 mmol) and anhydrous CH_2Cl_2 (15 mL). To this solution was added, at 24 °C, a solution of HBTU (873 mg, 2.14 mmol) and Boc-DAP-Fmoc-OH **15** (831 mg, 1.95 mmol) in CH_2Cl_2 (20 mL). The mixture was finally stirred for 72 hours at 24 °C under argon. The mixture was adsorbed on silica gel and a purification by chromatography on a silica gel column (CH_2Cl_2 then CH_2Cl_2–EtOH 9 : 1) led to the expected tricarbamate **17** (793 mg, 1.22 mmol, overall yield on two combined steps: 63%) isolated as a white solid. R_f 0.70 (CH_2Cl_2–EtOH 9 : 1). ^{1}H-NMR (400 MHz, DMSO d_6): δ 8.27 (br s, 1H), 7.93 (br s, 1H) 7.89 (d, J = 7.6 Hz, 2H), 7.68 (br s, 2H), 7.41 (dd, J = 6.7, 8.0 Hz, 2H), 7.32 (dd, J = 6.7, 7.7 Hz, 2H), 7.23 (br s, 1H), 6.82 (d, J = 7.4 Hz, 1H), 6.67 (d, J = 7.9 Hz, 1H), 4.28 (br s, 2H), 4.22–4.19 (m, 1H), 4.00 (br s, 1H), 3.83 (br s, 2H), 3.08 (br s, 1H) 1.36 (s, 18H), 1.25 (br s, 2H). ^{13}C-NMR (100 MHz, DMSO d_6): δ 170.5, 170.0, 156.3, 155.1, 143.8, 140.7, 127.6, 127.1, 125.1, 120.1, 80.9, 78.4, 73.0, 65.6, 54.9, 54.2, 53.6, 46.6, 42.2, 40.5, 28.0. MS (ESI) m/z 672.2 [M + Na^+]. HRMS (ESI): $C_{34}H_{43}N_5O_8$: calcd 649.3112, found 649.3111.

{2-[2-*tert*-Butoxycarbonylamino-3-(2-*tert*-butoxycarbonyl amino-acetylamino)-propionylamino]-1-prop-2-ynylcarbamoyl-ethyl}-carbamic acid *tert*-butyl ester 18. To a solution of compound **17** (500 mg, 0.77 mmol) in MeOH (5 mL) at 0 °C was added diethylamine (793 µL, 7.70 mmol). The resulting solution was stirred at 24 °C for 3 hours. The mixture was evaporated under reduced pressure and the crude amine (*ca.* 500 mg) was used as it is, without any further purification, in the following synthetic step. The crude amine was dissolved in a mixture of anhydrous CH_2Cl_2 (8 mL) and DIPEA (580 µL, 3.51 mmol). To this solution was added, at 24 °C, a solution of *N*-Boc-glycine (205 mg, 1.17 mmol) and HBTU (488 mg, 1.29 mmol) in CH_2Cl_2 (5 mL). After 16 hours at 24 °C, the mixture was adsorbed on silica gel and a purification of the crude material by a chromatography on a silica gel column afforded the expected tricarbamate **18** (200 mg, 0.34 mmol, overall yield on two combined steps: 44%) isolated as a pale yellow powder. R_f 0.26 (CH_2Cl_2–EtOH 96 : 4). ^{1}H NMR (400 MHz, MeOD): δ 4.19 (t, J = 5.3 Hz, 1H), 4.11 (t, J = 4.4 Hz, 1H), 3.98–3.96 (m, 2H), 3.72 (d, J = 3.0 Hz, 2H), 3.68–3.65 (m, 1H), 3.52 (d, J = 4.4 Hz, 2H), 3.38–3.34 (m, 1H), 2.6 (t, J = 2.4 Hz, 1H), 1.46 (s, 9H), 1.44 (s, 18H). ^{13}C NMR (100 MHz, MeOD): δ 173.7 (2), 173.4 (2), 172.5 (2), 158.6, 157.6, 81.0, 80.9 (2), 72.5, 57.0, 55.7, 44.8, 42.3, 41.9, 29.7, 28.8, 28.7. MS (ESI) m/z 607.2 [M + Na^+]. HRMS (ESI): $C_{26}H_{44}N_6O_9$: calcd: 584.3170, found 584.3175.

***N*-(1-(2,2-Diphenylbenzo[*d*][1,3]dioxol-4-yl)-11-(2,2-diphenyl benzo[*d*][1,3]dioxole-4-carboxamido)-1,4,8,12-tetraoxo-2,5,9,13-tetraazahexadec-15-yn-7-yl)-2,2-diphenyl benzo[*d*][1,3]dioxole-4-carboxamide 19.** The tricarbamate **18** (100 mg, 0.171 mmol) was dissolved in a mixture of TFA (250 µL, 3.42 mmol) in CH_2Cl_2 (750 µL). This solution was stirred for 16 hours at 24 °C before being evaporated under reduced pressure. The resulting crude triamine was used without any further purification for the next synthetic step. The crude triamine was dissolved in a mixture of DIPEA (360 µL, 2.18 mmol) and anhydrous THF (3 mL). To this solution, at 24 °C, under argon, was added a solution of the pentafluorophenyl-ester **7** (249 mg, 0.51 mmol) in anhydrous THF (5 mL). The mixture was stirred for 3 hours at 24 °C. The reaction mixture was adsorbed on silica gel and purified by chromatography on a silica gel column (CH_2Cl_2, then CH_2Cl_2–EtOH 92 : 8). The expected triamide **19** (84 mg, 0.07 mmol, overall yield over two steps: 41%) was isolated as a white powder. R_f 0.45 (CH_2Cl_2–EtOH 96 : 4). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.58 (d, J = 6.8 Hz,

1H), 8.37 (d, J = 6.5 Hz, 1H), 8.21 (t, J = 5.4 Hz, 1H), 8.04 (t, 1H), 7.93 (t, J = 5.2 Hz, 1H), 7.88 (t, 1H), 7.73 (dt, J = 1.5, 6.7 Hz, 2H), 7.68–7.65 (m, 6H), 7.55 (dt, J = 1.8, 8.0 Hz, 4H), 7.44 (d, J = 8.2 Hz, 1H), 7.40–7.25 (m, 20H), 6.97 (d, J = 7.8 Hz, 2H), 6.9 (d, J = 7.8 Hz, 1H), 6.79 (t, J = 8.0 Hz, 1H), 6.77–6.72 (m, 2H), 4.84 (dt, J = 5.2, 6.7 Hz, 1H), 4.73 (dt, J = 6.8, 5.8 Hz, 1H), 4.17 (d, J = 5.5 Hz, 2H), 4.08–4.07 (m, 2H), 3.96–3.84 (m, 2H), 3.71–3.68 (m, 2H), 2.01 (t, J = 2.5 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$, 25 °C): δ 170.6, 170.1, 169.6, 164.4, 164.0, 163.5, 147.4, 145.3, 139.6, 139.1, 129.5, 128.5, 126.4, 126.1, 122.2, 122.0, 118.4, 115.1, 111.9, 79.3, 71.6, 62.6, 58.4, 54.3, 53.7, 53.4, 45.3, 44.0, 41.7, 29.3. MS (ESI) m/z 1185.2 [M + H$^+$]. HRMS (ESI): $C_{71}H_{56}N_6O_{12}$: calcd 1184.3956, found 1184.3954.

***N*-(7-(2,3-Dihydroxybenzamido)-1-(2,3-dihydroxyphenyl)-1,4,8,12-tetraoxo-2,5,9,13-tetraazahexadec-15-yn-11-yl)-2,3-dihydroxy benzamide 2.** To a solution of protected tris-catechol compound **19** (40 mg, 0.04 mmol) in anhydrous CH_2Cl_2 (900 µL) was added triisopropylsilane (21 µL, 0.10 mmol) and TFA (100 µL). The mixture was stirred for 16 hours at 24 °C before being evaporated under reduced pressure. The resulting residue was suspended in diethyl-oxide leading to the formation of a gummy solid. The diethyl-oxide was removed and this solid was dissolved in water. The lyophilization afforded a slight grey solid which needed to be rinsed several times with diethyl-oxide. The expected tris-catechol compound **2** (23 mg, 0.034 mmol, yield: 100%) was isolated as a white powder. R_f 0.39 (CH_2Cl_2–EtOH 90 : 10). ^{1}H NMR (400 MHz, DMSO d_6): δ 12.24 (s, 1H), 12.05 (s, 1H), 11.98 (s, 1H), 9.22 (br s, 3H), 9.01 (t, J = 5.6 Hz, 1H), 8.80 (d, J = 7.2 Hz, 1H), 8.75 (d, J = 7.2 Hz, 1H), 8.54 (t, J = 5.1 Hz, 1H), 8.36 (t, J = 5.8 Hz, 1H), 8.14 (t, J = 6.3 Hz, 1H), 7.30–7.26 (m, 3H), 6.94–6.91 (m, 3H), 6.71–6.65 (m, 3H), 4.55–4.49 (m, 2H), 3.93–3.80 (m, 4H), 3.60–3.57 (m, 2H), 3.44–3.35 (m, 2H), 3.09 (s, 1H). ^{13}C NMR (100 MHz, DMSO d_6): δ 170.3, 169.6, 169.4, 169.1, 149.2, 149.0, 146.1, 146.0, 118.9, 118.0, 117.7, 115.4, 115.3, 115.2, 80.8, 73.1, 53.8, 53.6, 42.3, 40.4, 30.7, 28.2. MS (ESI) m/z 693.2 [M + H$^+$], 716.0 [M + Na$^+$]. HRMS (ESI): $C_{32}H_{32}N_6O_{12}$: calcd 692.2078, found 692.2091.

Bacterial strains

The pyochelin- and pyoverdine-deficient *Pseudomonas aeruginosa* strain PAD07,[34] and the enterobactin-deficient *Escherichia coli* strain BW25113*entB*::Kan^r,[33] have been described previously.

Culture media and growth conditions

The strains were grown overnight in succinate medium (composition in g L^{-1} is: K_2HPO_4, 6.0; KH_2PO_4, 3.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4{\cdot}7H_2O$, 0.2; sodium succinate, 4.0 and the pH was adjusted to 7.0 by the addition of NaOH) at 30 °C,[41] in the presence of 50 µg mL^{-1} tetracycline and 100 µm mL^{-1} streptomycin for PAD07 and 50 µg mL^{-1} kanamycin and 10% casamino acids for BW25113*entB*::Kan^r. PAD07 and BW25113*entB*::Kan^r cells were grown in the presence of 10 µM of the tested molecules (enterobactin, compound **1** or **2**) to obtain the expression of the corresponding siderophore pathways.

Iron uptake experiments

Iron uptake assays were carried out as previously reported for the FpvA/pyoverdine system.[42] After an overnight culture (20 h), bacteria were prepared in 50 mM Tris-HCl (pH 8.0) at $OD_{600\ nm}$ of 1, and incubated at 37 °C. Transport assays were started by adding 100 nM of enterobactin-^{55}Fe(III), **1**-^{55}Fe(III) or **2**-^{55}Fe(III). Enterobactin-^{55}Fe(III) and the two catecholate-^{55}Fe(III) complexes were prepared at concentrations of 10 µM of ^{55}Fe(III) with a siderophore : iron (mol : mol) ratio of 20 : 1. The solutions were prepared using a 10 mM solution of enterobactin, 1 or 2 (in DMSO). To 2 µL of this solution were added 4 µL of a solution of $^{55}FeCl_3$ (250 µM, 9.4 Ci g^{-1} in HCl 0.5 N), obtained by dilution of the stock solution, plus 96 µL of 50 mM Tris-HCl pH 8.0. To separate siderophore-^{55}Fe transported into *P. aeruginosa* cells from unbound siderophore-^{55}Fe, aliquots (100 µL) of the suspensions were removed at different times, filtered (0.45 µm, cellulose nitrate membrane filters, Whatman), and washed with 5 mL 50 mM Tris-HCl (pH 8.0) and the radioactivity counted. As controls, the experiments were repeated with cells incubated at 0 °C in the presence of 200 µM CCCP.

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Notes and references

1 (*a*) K. N. Raymond and C. J. Carrano, *Acc. Chem. Res.*, 1979, **12**, 183–190; (*b*) R. C. Hider and X.-L. Kong, *Nat. Prod. Rep.*, 2010, **27**, 637–657.
2 I. J. Schalk and L. Guillon, *Amino Acids*, 2013, **44**, 1267–1277.
3 M. J. Kappel, V. L. Pecoraro and K. N. Raymond, *Inorg. Chem.*, 1985, **24**, 2447–2452.
4 C. C. C. R. de Carvalho, M. P. C. Marques and P. Fernandes, *Rec. Pat. Biotechnol.*, 2011, **5**, 183–198.
5 M. G. P. Page, *Ann. New York Acad. Sci.*, 2013, **1277**, 115–126.
6 M. G. P. Page, *Handbook Exp. Pharmacol.*, 2012, **211**, 67–86.

7 M. Ballouche, P. Cornelis and C. Baysse, *Rec. Pat. Anti-Infect. Drug Discov.*, 2009, **4**, 190–205.
8 A. Souto, M. A. Montaos, M. Balado, C. R. Osorio, J. Rodriguez, M. L. Lemos and C. Jimenez, *Bioorg. Med. Chem.*, 2013, **21**, 295–302.
9 C. Ji, P. A. Miller and M. J. Miller, *J. Am. Chem. Soc.*, 2012, **134**, 9898–9901.
10 T. Zheng, J. L. Bullock and E. M. Nolan, *J. Am. Chem. Soc.*, 2012, **134**, 18388–18400.
11 U. Möllmann, L. Heinisch, A. Bauernfeind, T. Köhler and D. Ankel-Fuchs, *Biometals*, 2009, **22**, 615–624.
12 S. J. Milner, A. Seve, A. M. Snelling, G. H. Thomas, K. G. Kerr, A. Routledge and A.-K. Duhme-Klair, *Org. Biomol. Chem.*, 2013, **11**, 3461–3468.
13 T. A. Wencewicz, U. Möllmann, T. E. Long and M. J. Miller, *Biometals*, 2009, **22**, 633–648.
14 M. J. Miller, A. J. Walz, H. Zhu, C. Wu, G. Moraski, U. Möllmann, E. M. Tristani, A. L. Crumbliss, M. T. Ferdig, L. Checkley, R. L. Edwards and H. I. Boshoff, *J. Am. Chem. Soc.*, 2011, **133**, 2076–2079.
15 M. J. Miller, H. Zhu, Y. Xu, C. Wu, A. J. Walz, A. Vergne, J. M. Roosenberg, G. Moraski, A. A. Minnick, J. McKee-Dolence, J. Hu, K. Fennell, E. K. Dolence, L. Dong, S. Franzblau, F. Malouin and U. Möllmann, *BioMetals*, 2009, **22**, 61–75.
16 F. Rivault, C. Liébert, A. Burger, F. Hoegy, M. A. Abdallah, I. J. Schalk and G. L. A. Mislin, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 640–644.
17 O. Kinzel, R. Tappe, I. Gerus and H. Budzikiewicz, *J. Antibiot.*, 1998, **51**, 499–507.
18 C. Hennard, Q. C. Truong, J.-F. Desnottes, J.-M. Paris, N. J. Moreau and M. A. Abdallah, *J. Med. Chem.*, 2001, **44**, 2139–2151.
19 S. Yoganathan, C. S. Sit and J. C. Vederas, *Org. Biomol. Chem.*, 2011, **9**, 2133–2141.
20 H. Budzikiewicz, *Curr. Top. Med. Chem.*, 2011, **1**, 73–82.
21 U. Möllmann, A. Ghosh, E. K. Dolence, J. A. Dolence, M. Ghosh, M. J. Miller and R. Reissbrodt, *Biometals*, 1998, **11**, 1–12.
22 A. Ghosh and M. J. Miller, *J. Org. Chem.*, 1993, **58**, 7652–7659.
23 M. Apostol, P. Baret, G. Serratrice, J. Desbrieres, J.-L. Putaux, M.-J. Stebe, D. Expert and J.-L. Pierre, *Angew. Chem., Int. Ed.*, 2005, **44**, 2580–2582.
24 A. Pinter and G. Haberhauer, *Chem.–Eur. J.*, 2008, **14**, 11061–11068.
25 W. H. Rastetter, T. J. Erickson and M. C. Venuti, *J. Org. Chem.*, 1981, **46**, 3579–3590.
26 R. Paulini, C. Lerner, F. Diederich, R. Jakob-Roetne, G. Zurcher and E. Borroni, *Helv. Chim. Acta*, 2006, **89**, 1856–1887.
27 E. D. Laganis and B. L. Chenard, *Tetrahedron Lett.*, 1984, **25**, 5831–5834.
28 A. T. Ślósarczyk, R. Ramapanicker, T. Norberg and L. Baltzer, *R. Soc. Chem. Adv.*, 2012, **2**, 908–914.
29 J. L. Corbin and W. A. Bulen, *Biochemistry*, 1969, **8**, 757–672.
30 J.-P. Bellenger, F. Arnaud-Neu, Z. Asfari, S. C. B. Myneni, E. I. Stiefel and A. M. L. Kraepiel, *J. Biol. Inorg. Chem.*, 2007, **12**, 367–376.
31 O. Knosp, M. Von Tigerstrom and W. J. Page, *J. Bacteriol.*, 1984, **159**, 341–347.
32 D. L. Boger, D. Yohannes, J. Zhou and M. A. Patane, *J. Am. Chem. Soc.*, 1993, **115**, 3420–3430.
33 T. Baba, T. Ara, M. Hasegawa, Y. Takai, Y. Okumura, M. Baba, K. A. Datsenko, M. Tomita, B. L. Wanner and H. Mori, *Mol. Syst. Biol.*, 2006, **2**, 2006–2008.
34 H. Takase, H. Nitanai, K. Hoshino and T. Otani, *Infect. Immun.*, 2000, **68**, 1834–1839.
35 M. A. Llamas, M. Sparrius, R. Kloet, C. R. Jimenez, C. Vandenbroucke-Grauls and W. Bitter, *J. Bacteriol.*, 2006, **188**, 1882–1891.
36 K. A. Mettrick and I. L. Lamont, *Mol. Microbiol.*, 2009, **74**, 1257–1271.
37 E. Clément, P. J. Mesini, F. Pattus, M. A. Abdallah and I. J. Schalk, *Biochemistry*, 2004, **43**, 7954–7965.
38 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
39 C. W. Tornøe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
40 Z. A. Youard, G. L. Mislin, P. A. Majcherczyk, I. J. Schalk and C. Reimmann, *J. Biol. Chem.*, 2007, **282**, 35546–35553.
41 P. Demange, S. Wendenbaum, C. Linget, C. Mertz, M. T. Cung, A. Dell and M. A. Abdallah, *Biol. Metals*, 1990, **3**, 155–170.
42 I. J. Schalk, C. Hennard, C. Dugave, K. Poole, M. A. Abdallah and F. Pattus, *Mol. Microbiol.*, 2001, **39**, 351–360.