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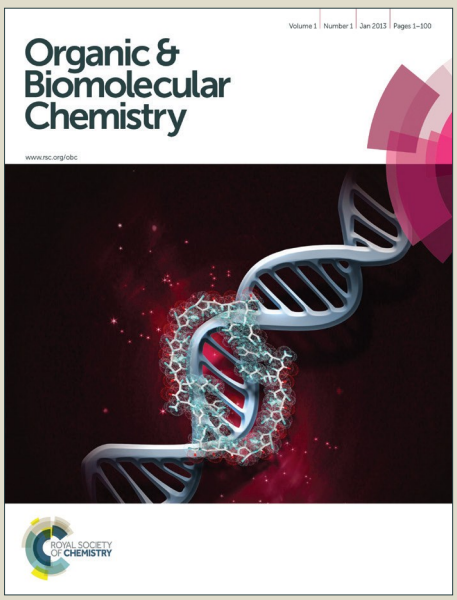

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ARTICLE

Synthesis and antibiotic activity of oxazolidinone-catechol conjugates against *Pseudomonas aeruginosa*†

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Pseudomonas aeruginosa is a Gram-negative pathogenic bacterium responsible for severe infections in which resistance to most of the approved families of antibiotics is increasing. Oxazolidinone antibiotics are active against many Gram-positive bacteria, but are only weakly active against Gram-negative pathogens. We describe the synthesis of conjugates between a catechol moiety and oxazolidinone antibiotics. These conjugates were significantly more active against *P. aeruginosa* (218-1024 µM) than linezolid (MIC >1024 µM), the reference molecule from the oxazolidinone family. Antibiotic activity was slightly higher in medium depleted of iron, suggesting the involvement of a bacterial iron uptake system in this biological activity. The bacterial iron uptake pathway involved in the transport is still to be adressed, but the present data excluded a contribution of the enterobactin transporter PfeA.

Introduction

Antibiotics are among the drugs that have made the greatest contribution to lengthening human lifespan. During the last century, the continual discovery of new biological targets and the synthesis of specific inhibitors led to the regular development of innovative antibiotics in the fight against pathogenic bacteria. However, no new family of broad-spectrum antibiotics has been developed and approved for use since the early 1980s.[1-3] Along with antibiotic misuse, this has led to both sporadic outbreaks of ancient bacterial disease (tuberculosis, syphilis, plague, etc.) and the emergence of highly resistant bacteria.[3] There is therefore an urgent need to discover new and efficient antibiotics.

Bacteria of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa* and *Enterobacter species*) are considered to represent a major health challenge for the forthcoming decades.[2,4] This heterogeneous group contains Gram-positive and Gram-negative bacteria naturally resistant to many bactericidal compounds, with a capacity to acquire additional resistances rapidly. Gram-positive bacteria from the ESKAPE group have been shown to be sensitive to linezolid **1**, [5-10] the sole approved drug among all oxazolidinone candidates developed and evaluated to date.[11-16] Linezolid **1** has been shown to be poorly effective against many Gram-negative bacteria, including *Pseudomonas aeruginosa*.[7,8] The target of this antibiotic is a ribosomal RNA.[9,10] The difference in the activity of this antibiotic between Gram-positive and Gram-negative bacteria seems to be related to both the low permeability and expression of efflux systems in Gram-negative bacteria.[17] By hijacking bacterial nutrients uptake systems, it may be possible to increase the penetration and to promote accumulation of oxazolidinone antibiotics in Gram-negative bacteria.

Iron is a key nutrient in bacteria. Bacterial proliferation requires iron concentrations in the micromolar range.[18] However, the concentrations of iron(III) freely available to pathogens in eukaryotic hosts have been estimated at only 10^{-18} to 10^{-24} M.[19] Various highly efficient uptake strategies providing access to iron(III) have evolved in microorganisms.[20-22] Siderophore-dependent iron acquisition systems are the more widely expressed and are found in the vast majority of bacteria.[20-22] Siderophores are low-molecular weight secondary metabolites (MW < 2000 Da) synthesized by bacteria and excreted into the extracellular medium.[23] These compounds scavenge iron from the environment, or from host tissues, through their chelation properties and their very high affinity for iron(III).[23] In Gram-negative bacteria, specific outer membrane transporters (OMTs) translocate ferric siderophores into the bacterial cell.[24-27] Thus, the conjugation

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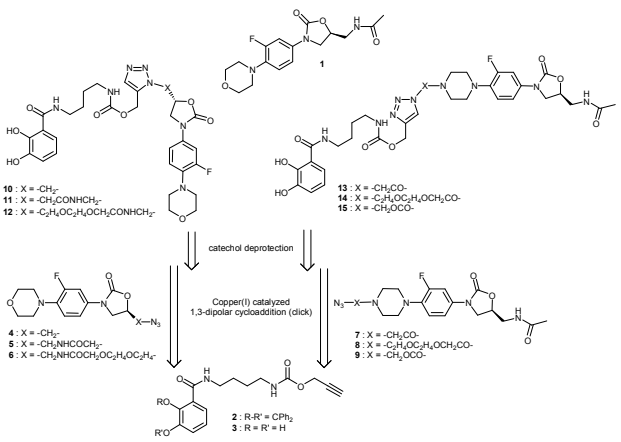
† Electronic Supplementary Information (ESI) available: LC-HRMS of conjugates **10**, **11**, **12**, **13**, **14** and **15**. See DOI: 10.1039/x0xx00000x

of antibiotics to siderophores can efficiently promote the intracellular accumulation of the bactericidal drug through the use of endogenous iron uptake systems.[28-41] However, the efficiency and scope of such a Trojan horse strategy are highly dependent on the siderophores used as vectors. Indeed, once they have crossed the outer membrane via dedicated OMTs, the fate of the chelates is dependent on the siderophore and the bacterial species concerned. For pyoverdine, a siderophore of *P. aeruginosa*, the ferric complex is dissociated in the periplasm and iron is scavenged by periplasmic proteins, promoting further translocation to the cytoplasm via an ABC transporter embedded in the inner membrane.[42,43] Pyoverdine therefore never crosses the inner membrane in its ferric form. This property makes pyoverdine an interesting vector for the delivery of antibiotics with periplasmic targets, such as beta-lactams.[39,40] Attempts to deliver antibiotics from other families via this system have failed.[41] In *Escherichia coli*, the ferric complex of enterobactin, a catechol siderophore, is transported across the outer membrane by the FepA OMT,[25] and further translocated through the inner membrane into the cytoplasm by an ABC transporter (FepBCDG).[44] The dissociation of iron from the siderophore takes place in the cytoplasm.[45]

Enterobactin is used as a siderophore not only by *E. coli,* but also by other pathogenic Gram-negative bacteria, including those from the ESKAPE group.[46-48] *P. aeruginosa* does not produce enterobactin, but it can express a specific OMT, PfeA,[49] to promote enterobactin-dependent iron uptake when this siderophore is present in the extracellular medium. However, the periplasmic and cytoplasmic fates of ferric enterobactin in *P. aeruginosa* remain to be determined. This ability of enterobactin to cross bacterial membranes and to be used as a siderophore by a great number of bacterial species has excited interest in the use of catechol siderophores for the delivery of antibiotics with periplasmic or cytoplasmic targets in various pathogenic bacteria.

Recent results from the groups of Miller and Nolan have demonstrated that tricatechol vectors based, or inspired, on enterobactin can significantly increase the bactericidal activity of beta-lactam antibiotics.[30-32] However, published examples of the efficient vectorization of antibiotics with cytoplasmic targets by catechol siderophores remain rare. In this context, we synthesized conjugates **10** to **15** of a monocatechol moiety and oxazolidinone antibiotics. For this purpose, the precursor **2** of a monocatechol vector (MCV **3**) bearing a terminal propargyl function was connected to oxazolidinone azides **4** to **9** by copper(I)-promoted 1,3-dipolar cycloaddition reactions (click chemistry) (Scheme 1).[50,51]

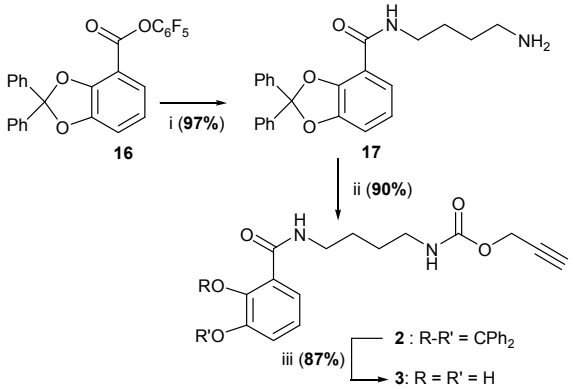
We describe here the synthesis of the monocatechol vector (MCV) **3** and of conjugates **10** to **15**. The minimum inhibitory concentrations (MIC) of these novel oxazolidinone-catechol conjugates against *P. aeruginosa* are also presented.



Scheme 1 Structure of linezolid **1** and retrosynthetic pathways for the synthesis of conjugates **10** to **15**.

Results and discussion

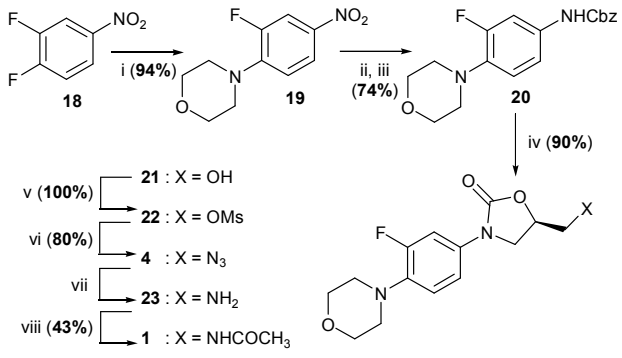
Catechol precursor **16,** which we described in a previous study,[52] was used as the starting point for the synthesis of monocatechol vector (MCV) **3**. The reaction of compound **16** with 1,4-diaminobutane generated amide **17**, which was isolated with a yield of 97%. The amine group of compound **17** was then reacted with propargyl-chloroformate, to generate the protected MCV **2**. When necessary, cleavage of the 2,2-diphenyl-benzo[1,3]dioxole moiety by TFA in the presence of triisopropylsilane (TIS) and ethanol, was carried out to generate the corresponding MCV **3** bearing a free catechol group (Scheme 2).



Scheme 2 Synthesis of the mono-catechol vector (MCV) **3**. i. 1,4-diaminobutane, CH_2Cl_2, 20 °C. ii. propargyl-chloroformate, DIPEA, CH_2Cl_2, 24 °C. iii. TFA/CH_2Cl_2 20%, TIS, EtOH, 22 °C.

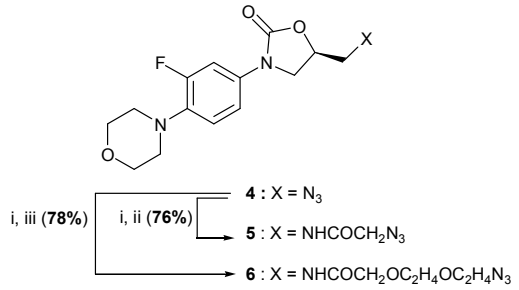
In parallel, the synthesis of oxazolidinones **4** to **9** was based on published protocols for related linezolid analogs.[53-55] In our hands, our alternative synthetic pathways, characterized by the use of reduction reactions promoted by transition metal borides, generated the expected products in higher yields. In these synthetic pathways, 4,5-dinitrofluorobenzene **18** was used as the starting material.

Difluorinated compound **18** was reacted with morpholine in refluxing acetonitrile, and the substitution product **19** was obtained with a yield of 94%. The nitro function was then reduced in the presence of copper boride, obtained by the reaction of sodium borohydride with copper sulfate. The resulting amine was insufficiently stable for purification and was reacted with benzyl-chloroformate. The expected benzyl-carbamate **20** was isolated with a 74% yield, in a two-step process. The deprotonation of compound **20** by *n*-butyl lithium, followed by reaction with (*R*)-glycidyl-butyrate, resulted in the production of the oxazolidinone **21**, which was isolated with a 90% yield. The hydroxyl compound **21** was then converted quantitatively into the corresponding mesylate **22**. This compound was then treated with sodium azide in hot DMF leading to the key azide **4**, isolated with an 80% yield. In the synthesis of linezolid, used as a reference in our biological evaluation, the azide **4** was reduced to an amine **23** with cobalt boride, which was obtained by reacting sodium borohydride with cobalt dichloride. Yields for this step were significantly lower if copper boride was used in place of cobalt boride. The resulting primary amine **23** was not purified and was reacted with acetic anhydride to generate the linezolid **1**. Using this synthetic approach, we were able to isolate linezolid **1** with a 43% yield in two steps, starting from key azide **4** and in 22% yield from fluorinated precursor **18** (Scheme 3).



Scheme 3 Synthesis of oxazolidinone azide **4** and of linezolid **1**. i. Morpholine, CH_3CN, 80°C. ii. $CuSO_4$, $NaBH_4$, MeOH/H_2O, 5°C to 27°C. iii. CbzCl, DIPEA, CH_2Cl_2, 23-27°C. iv. a. *n*BuLi, THF, -78°C to -10°C. b. (*R*)-glycidyl-butyrate, THF, -78°C to 20°C. v. MsCl, DIPEA, CH_2Cl_2, 25°C. vi. NaN_3, DMF, 75°C. vii. $CoCl_2$, $NaBH_4$, MeOH/H_2O, 5°C. viii. Ac_2O, DIPEA, CH_2Cl_2, 18°C.

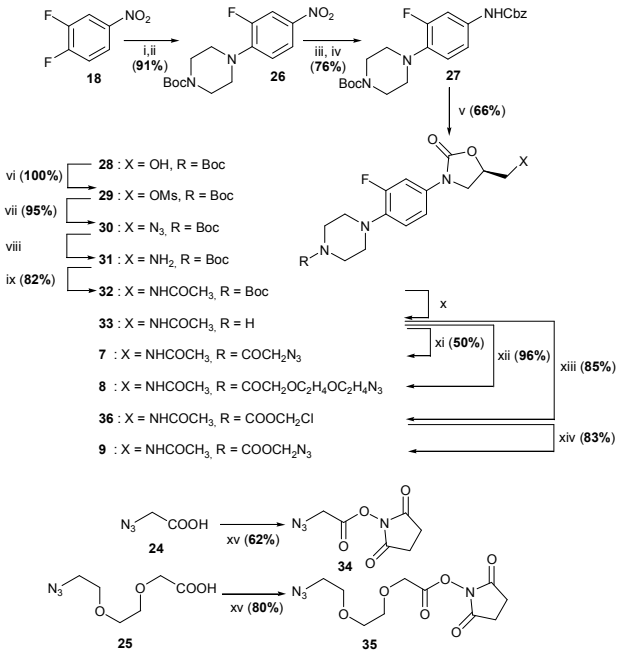
The azide **4** was also reduced into amine **23** to prepare oxazolidinone derivatives **5** and **6**. For this purpose, 2-azidoacetic acid **24** and 2-(2-azidoethoxy)ethoxy)acetic acid **25** were reacted with the amine **23** in the presence of EDCI hydrochloride. Linezolid analogs **5** and **6** were isolated with yields of 76% and 78%, respectively. Using these protocols, we obtained oxazolidinone azides **4**, **5** and **6**, with overall yields from the starting material **18** of 50% (6 steps), 38% (8 steps) and 39% (8 steps), respectively (Scheme 4).



Scheme 4 Syntheses of oxazolidinone azides **5** and **6**. i. $CoCl_2$, $NaBH_4$, MeOH/H_2O, 5°C. ii. N_3CH_2COOH (**24**), EDCI.HCl, CH_2Cl_2, 20°C. ii. $N_3C_2H_4OC_2H_4OCH_2COOH$ (**25**), EDCI.HCl, CH_2Cl_2, 20°C.

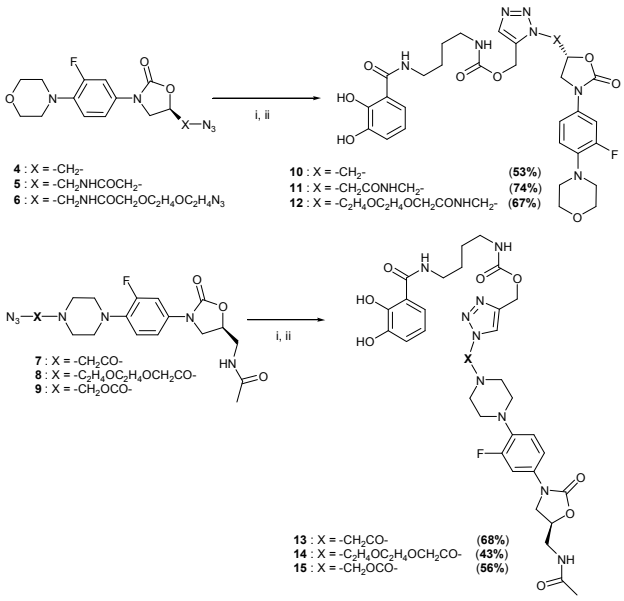
For the synthesis of oxazolidinone derivatives **7**, **8** and **9**, 4,5-dinitrofluorobenzene **18** was reacted with an excess of piperazine. The resulting amine was used without further purification and was protected by a Boc group, through reaction with di-*tert*-butyl-dicarbonate. The expected carbamate **26** was isolated with a yield of 91% over two steps. The subsequent synthetic steps were similar to those described for the synthesis of linezolid **1**. The nitro function of compound **26** was reduced by copper boride and the resulting amine was converted into the dicarbamate **27**, which was isolated with a 76% yield over two steps. This compound was then treated with *n*-butyl-lithium and the resulting anion was reacted with (*R*)-glycidyl-butyrate. The oxazolidinone **28** was isolated with a 66% yield. The hydroxyl group of compound **28** was then activated under the form of the mesylate **29** and further converted into the azide intermediate **30**. The reduction of azide **30** by cobalt boride to generate amine **31**, followed by an acetylation reaction, resulted in the acetylamide **32**, isolated with an 81% yield in two steps. Cleavage of the Boc protecting group with trifluoroacetic acid (TFA) released the key amine **33**. This amine was then reacted with activated esters **34** or **35,** obtained by reacting 2-azidoacetic acid **24** or 2-(2-azidoethoxy)ethoxy)acetic acid **25**, respectively, with *N*-hydroxysuccinimide in the presence of EDCI hydrochloride. The reaction of amine **33** with *N*-hydroxysuccinimide esters **34** or **35**, in the presence of DIPEA resulted in the oxazolidinone derivatives **7** and **8** with yields of 50% and 96%, respectively, over two steps starting from **34**. In the synthesis of oxazolidinone azide **9**, amine **33** was reacted with chloromethyl chloroformate. The resulting chloride **36** was isolated with a yield of 85% and further treated with sodium azide, to generate the expected azide **9** with a yield of

83%. These protocols led to the production of oxazolidinone derivatives **7**, **8** and **9,** obtained with overall yields of 17% (11 steps), 34% (11 steps) and 25% (12 steps), respectively, from 4,5-dinitrofluorobenzene **18** (Scheme 5).



Scheme 5 Synthesis of oxazolidinone azides **7** to **9**. i. piperazine, CH_3CN, 80°C. ii. Boc_2O, $NaHCO_3$, THF/H_2O, 25°C. iii. $CuSO_4$, $NaBH_4$, $MeOH/H_2O$, 5°C. iv. CbzCl, DIPEA, CH_2Cl_2, 25°C. v. a. *n*BuLi, THF, -78 °C to -10 °C. b. (*R*)-glycidyl-butyrate, THF, -78 °C to 25°C. vi. MsCl, DIPEA, CH_2Cl_2, 20°C. vii. NaN_3, DMF, 75°C. viii. $CoCl_2$, $NaBH_4$, $MeOH/H_2O$, 5°C. ix. Ac_2O, DIPEA, CH_2Cl_2, 20 °C. x. TFA, TIS, CH_2Cl_2, 20-22°C. xi. **34**, DIPEA, CH_2Cl_2, 20°C. xii. **35**, DIPEA, CH_2Cl_2, 22°C. xiii. Chloromethyl-chloroformate, DIPEA, CH_2Cl_2, 21°C. xiv. NaN_3, DMF, 75°C. xv. *N*-hydroxysuccinimide, EDCI.HCl, CH_2Cl_2, 18-20°C.

The oxazolidinone azides **4**, **5**, **6**, **7**, **8** and **9** were then conjugated to MCV precursor **2**, by 1,3-dipolar cycloadditions. Click reactions were performed in the presence of copper sulfate and sodium ascorbate. The best results were obtained in a THF/water mixture, even though this solvent system is usually not considered to be the most appropriate for click reactions. In these conditions, expected 1,2,3-triazoles were produced, and further cleavage of the 2,2-diphenyl-benzo[1,3]dioxole moiety with TFA in the presence of TIS and ethanol, generated the corresponding free catechol compounds **10**, **11**, **12**, **13**, **14** and **15**, isolated with yields of 53%, 74%, 67%, 68% 43% and 56%, respectively, over two steps (Scheme 6).



Scheme 6 Synthesis of MCV-oxazolidinone conjugates **10**, **11**, **12**, **13**, **14** and **15**. i. $CuSO_4$, sodium ascorbate, THF/H_2O, 21-23°C. ii. TFA, TIS, EtOH, CH_2Cl_2, 22-25°C.

We then assessed the antibiotic activities of conjugates **10**, **11**, **12**, **13**, **14** and **15**, and of the MCV vector **3** against *P. aeruginosa* PAO1. Experiments were performed in CAA medium, an iron depleted medium and in Mueller-Hinton broth (MHB), which has a higher concentration of iron.[56] Experiments in CAA medium were also performed on a PAO1 strain with defective expression of PfeA, the outer membrane transporter of enterobactin in *P. aeruginosa*. MICs were established by the microtiter broth dilution method.

Linezolid **1**, used as the reference compound in this evaluation, displayed antibiotic activity only at a concentration of 1024 µM in CAA and it had no detectable antibiotic activity at this concentration in MHB. The MCV vector **3** was also tested in CAA medium and MHB, and was found to have MICs of 256 µM and 512 µM, respectively. This antibiotic activity may be connected to iron sequestration by MCV **3**. In CAA medium, compounds **10**, **11**, **12**, **14** and **15** had higher antibiotic activities (MIC=256 µM) than the reference compound **1**. Moreover, conjugate **13,** which had a MIC of 128 µM, appeared to be, at least, eight times more active than linezolid **1** itself in CAA medium. Conjugates **10** to **15** had higher MICs (512-1024 µM) in Mueller-Hinton broth than in iron-depleted medium. These data suggest the involvement of an iron acquisition system in the uptake of conjugates. The *P. aeruginosa* genome encodes at least 12 outer membrane transporters (OMTs) involved in iron uptake pathways, each dependent on a different siderophore.[57] In this context PfeA, an enterobactin OMT, appeared the be a good candidate. A

mutant of *P. aeruginosa* strain PAO1 lacking PfeA (PAO1Δ*pfeA*) was therefore constructed. Similar results were obtained for PAO1 and PAO1Δ*pfeA* in CAA medium, demonstrating that PfeA was not involved in the uptake of compounds **10** to **15** (Table 1).

Table 1 Minimal inhibitory concentration (MIC)s measured in CAA medium and Mueller-Hinton broth (MHB) medium with PAO1 and PAO1Δ*pfeA* strains

	PAO1		PAO1Δ*pfeA*
Compounds	MIC(μM) [a] CAA	MIC (μM) [a] MHB	MIC (μM) [a] CAA
1	1024	>1024	1024
3	256	512	256
10	256	512	256
11	256	512	256
12	256	512	256
13	128	512	128
14	256	512	256
15	256	1024	256

[a]MIC values are means of three independent experiments.

In conjugates **10**, **11**, **12**, **13** and **14**, the drug is connected to the vector via a spacer arm that is stable *in vivo*. In the Trojan horse strategy, the release of the drug from the vector inside the bacteria, after the penetration of the envelope might be advantageous, yielding to potentially higher levels of biological activity. Compound **15** was designed for this purpose. In this conjugate, the triazole moiety is connected to an oxygen atom via a methylene bridge. This methylene could act as a formaldehyde precursor and it has been suggested that similar chemical structures can be cleaved under basic conditions and are thus potentially useful for the design of hydrolyzable linkers.[58] The MICs obtained with this compound in our model indicated that this linker did not increase antibiotic activity in conditions of iron depletion. The higher MIC (1024 μM) observed for **15** in MHB may be associated with extracellular hydrolysis of the linker. Similar results were reported for structurally similar linkers.[35,41]

Conclusions

We describe here the efficient synthesis of six novel conjugates **10**, **11**, **12**, **13**, **14** and **15** of a monocatechol vector with linezolid analogs. One of these compounds (conjugate **13**) was significantly more active than linezolid **1** or the MCV vector **3** alone. However, even oxazolidinone **13** clearly cannot be considered to be a "magic bullet" against *P. aeruginosa.* Nevertheless, this compound is one of the most active oxazolidinones against this pathogen to have been described, particularly in conditions of iron depletion. Molecular mechanisms involved in iron uptake pathways dependent on catechol siderophores have been much less studied in *P. aeruginosa* than in *Escherichia coli*. The design of innovative Trojan horse conjugates of catechol siderophores with antibiotics therefore requires improvements in our understanding of these iron acquisition mechanisms in *P. aeruginosa*. Indeed, the OMT involved and the compartment (periplasm, cytoplasm) in which iron is released are crucial items of information for the design of the siderophore carrier and the choice of antibiotic to be vectorized.[28] Finally, our results again highlight the urgent need to develop dedicated hydrolyzable linkers to increase significantly the scope of Trojan horse antibiotic strategies based on siderophores.

Experimental section

General information

All reactions were carried out under argon purchased from Linde gas. Solvents used for reaction were of analytical grade purity (>99.9%). When necessary, and specified in the protocols, solvents were purchased anhydrous from Aldrich, Acros or Alfa Aesar companies. All chemicals were obtained from commercial suppliers (Aldrich, Acros or Alfa-aesar) and were used as received, unless otherwise stated. 2-(2-azidoethoxy)ethoxy)acetic acid **25** was purchased from MCAT GmbH-Germany. In reduction protocols using metal borides, best yields were obtained using $NaBH_4$ in pellets, crushed extemporaneously in a mortar.

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) or using prepacked silica gel columns on a *Reveleris* chromatographic device (Grace Davison).

Instrumentation

NMR spectra were recorded on a Bruker Avance 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), using the residual non-deuterated solvent as reference. The chemical shifts (δ) and coupling constants (J) are expressed in ppm and Hertz respectively. Multiplicities were indicated as s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). A broad signal is mentioned with br preceding 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.

N-(4-aminobutyl)-2,2-diphenylbenzo[*d*][1,3]dioxole-4-carboxamide (17)

To a solution of 1,4-diaminobutane (311 μL, 273 mg, 3.01 mmol) in anhydrous CH_2Cl_2 (5 mL) at 20°C, was dropwise added a solution of pentafluorophenyl-ester **16**[52] in anhydrous CH_2Cl_2 (5 mL). The resulting solution was stirred at 20 °C for 2 h. The mixture was adsorbed on silica and purified on a silica

gel column (CH_2Cl_2/NH_4OH 99/1 then CH_2Cl_2/EtOH/NH_4OH 90/19/1) to afford compound **17** (233 mg, 0.60 mmol, yield 97%) as an amorphous solid. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.56-7.49 (m, 5H), 7.39-7.36 (m, 6H), 7.19 (dd, *J* = 6.6, 4.5 Hz, 1H), 6.98 (dd, *J* = 1.4, 7.7 Hz, 1H), 6.91 (t, *J* = 7.9 Hz, 1H), 3.51-3.46 (m, 2H), 2.71 (t, *J* = 6.9 Hz, 2H), 1.69-1.62 (m, 2H), 1.56-1.50 (m, 2H). NMR ^{13}C (100 MHz, $CDCl_3$): δ 163.7, 147.3, 144.7, 139.3, 129.8, 128.6, 126.5, 122.6, 122.2, 118.2, 116.2, 111.7, 41.9, 39.7, 31.1, 27.2. HRMS (ESI): $C_{24}H_{24}N_2O_3$: calcd: 388.17869, found: 388.17916.

Prop-2-yn-1-yl (3-(2,2-diphenylbenzo[*d*][1,3]dioxole-4-carboxamido)propyl)carbamate (2)

To a solution of amine **17** (800 mg, 2.06 mmol) in CH_2Cl_2 (10 mL) at 24°C were added successively DIPEA (1 mL, 742 mg, 6.05 mmol) and propargylchloroformate (0.25 mL, 303 mg, 2.47 mmol). The resulting solution was stirred 12 h at 24°C under argon. The reaction mixture was adsorbed on silica gel and purified on a silica gel column (CH_2Cl_2/EtOH 95/5) to afford compound **2** (871 mg, 1.85 mmol, yield 90%) as a white powder. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.49-7.42 (m, 5H), 7.35-7.28 (m, 6H), 7.09 (dd, *J* = 6.2, 4.9 Hz, 1H), 6.91 (dd, *J* = 7.7, 1.4 Hz, 1H), 6.83 (t, *J* = 7.9 Hz, 1H), 5.1 (t, *J* = 6.2 Hz, 1H), 4.54 (d, *J* = 2.5 Hz, 2H), 3.41 (q, *J* = 6.4 Hz, 2H), 3.13 (q, *J* = 6.1 Hz, 2H), 2.43 (t, *J* = 2.4 Hz, 1H), 1.61-1.47 (m, 4H). NMR ^{13}C (100 MHz, $CDCl_3$): δ 163.7, 155.6, 147.2, 144.6, 139.1, 129.7, 128.5, 126.4, 122.5, 122.1, 118.2, 115.9, 111.7, 78.4, 74.5, 52.3, 40.8, 39.3, 27.3, 27.0. HRMS (ESI): $C_{28}H_{26}N_2O_5$: calcd: 470.18417, found: 470.18502.

Prop-2-yn-1-yl (3-(2,3-dihydroxybenzamido)propyl)carbamate (3)

To a solution of catechol **2** (200 mg, 0.42 mmol) in CH_2Cl_2 (2 mL) were added successively TFA (0.5 mL), triisopropylsilane (0.15 mL), and EtOH (0.15 mL). The reaction mixture was stirred 12 h at 22°C then solvents were removed under reduced pressure. The residue was solubilized in THF, and cyclohexane was added until precipitation. Precipitate was filtered to afford the expected compound **3** (113 mg, 0.37 mmol, yield 87%) as a white powder. NMR ^{1}H (400 MHz, $CDCl_3$): δ 13.04 (br s, 1H), 8.04 (br s, 1H), 7.12 (dd, *J* = 1.5, 8.1 Hz, 1H), 6.82 (dd, *J* = 1.4, 7.8 Hz, 1H), 6.57 (t, *J* = 8.0 Hz, 1H), 6.30 (br s, 1H), 4.5 (d, *J* = 2.5 Hz, 2H), 3.31 (q, *J* = 6.3 Hz, 2H), 3.31 (q, *J* = 6.3 Hz, 2H), 2.82 (t, *J* = 2.5 Hz, 1H), 2.72 (s, 1H), 1.57-1.42 (m, 4H). NMR ^{13}C (100 MHz, MeOD): δ 171.6, 158.1, 150.4, 147.4, 119.6, 118.7, 116.9, 79.6, 75.7, 53.1, 41.6, 40.2, 28.3, 27.7. MS (ESI): m/z 307.1 $[M+H]^+$.

4-(2-Fluoro-4-nitrophenyl)-morpholine (19)

A solution of 3,4-difluoronitrobenzene **18** (840 µL, 1232 mg, 7.74 mmol,) in morpholine (6 mL) was heated at 80°C during 2 h. The reaction mixture was then evaporated to dryness under reduced pressure. The residue was then triturated in Et_2O (120 mL). The solid formed was filtered off and the filtrate was diluted in CH_2Cl_2 (100 mL) before being washed with an aqueous solution saturated in K_2CO_3. The organic phase was then collected, dried over $MgSO_4$, filtrated and solvents were evaporated under reduced pressure. The expected compound **19** (1650 mg, 7.30 mmol, yield: 94%) was isolated as a yellow solid. NMR ^{1}H (400 MHz, Acetone-d_6): δ 8.02 (d, *J* = 8.8 Hz, 1H, 7.93 (d, *J* = 13.6 Hz, 1H), 7.19 (d, *J* = 8.8 Hz, 1H), 3.82 (br t, *J* = 4.4 Hz, 4 H), 3.33 (br t, *J* = 4.4 Hz, 4 H). NMR ^{19}F (acetone-d_6): δ -120.4. NMR ^{13}C (acetone-d_6): δ 157.7, 155.3, 154.4, 152.7, 137.9, 136.3, 135.6, 129.4, 129.0, 120.2, 107.9, 107.6, 67.6, 67.0, 52.2. MS (ESI): m/z 227.0 $[M+H]^+$.

4-(4-Benzyloxycarbonylamino-2-fluoro-phenyl)-morpholine (20)

To a solution of compound **19** (1000 mg, 4.42 mmol) in methanol (100 mL), cooled down below 5°C, was added a saturated aqueous solution of $CuSO_4$ (4 mL). The resulting green mixture was vigorously stirred and $NaBH_4$ (1170 mg, 30.97 mmol) was added by portions. The resulting dark suspension was then allowed to reach room temperature (27°C) and an additional amount of $NaBH_4$ (1170 mg, 30.97 mmol) was portion-wise added. After 1 h at 27°C, the mixture was filtrated on celite and the filtrate was diluted with MilliQ water (100 mL) and CH_2Cl_2 (200 mL). After partition and two extractions with CH_2Cl_2, organic layers were collected, washed with brine, dried over Na_2SO_4 and filtered before being evaporated under reduced pressure. The resulting amine, obtained under the form of a light purple solid, was used as it for the next step, without any other purification step. To a solution of the crude amine in CH_2Cl_2 (100 mL) at 27°C, were successively added DIPEA (2 mL) and CbzCl (684 µL, 830 mg, 4.86 mmol). The resulting solution was stirred at room temperature (23-27 °C) during 14 h. The mixture was then adsorbed on silica gel before being purified by chromatography on a silica gel column (CH_2Cl_2 then CH_2Cl_2/AcOEt : 8/2). The expected carbamate **20** (1075 mg, 3.26 mmol, yield: 74% over two steps) was obtained under the form of a white powder. NMR ^{1}H (400 MHz, Acetone-d_6): δ 8.76 (br s, 1H), 7.31-7.49 (m, 6H), 7.21 (d, *J* = 8.8 Hz, 1H), 6.98 (t, *J* = 8.8 Hz, 1H), 5.17 (s, 2H), 3.76 (br t, *J* = 4.4 Hz, 4 H), 2.98 (br t, *J* = 4.4 Hz, 4 H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.9. NMR ^{13}C (100 MHz, $CDCl_3$): δ 154.9, 153.4, 136.1, 128.9, 128.6, 128.5, 119.9, 114.7, 108.3, 104.6, 80.1, 67.4, 51.1, 28.7. MS (ESI): m/z 331.2 $[M+H]^+$.

(3-(3-fluoro-4-morpholinophenyl)-5-(hydroxymethyl)oxazolidin-2-one (21)

To a solution of carbamate **20** (2009 mg, 6.08 mmol) in anhydrous THF (60 mL) at -78°C was added *n*-BuLi (3.42 mL, 2.5 M in hexanes, 8.56 mmol). The reaction mixture was allowed to reach -10°C in 30 minutes. The solution was cooled down to -78°C and (*R*)-glycidyl-butyrate (0.55 mL, 567 mg, 3.95 mmol) was added dropwise. The reaction mixture was then stirred 12 h at 25°C. The reaction mixture was quenched with an aqueous solution saturated in NH_4Cl before being extracted with ethyl acetate. Organic phases were collected, dried over Na_2SO_4, filtered and adsorbed on silica gel. The crude product

was purified on a silica gel column (CH_2Cl_2 then CH_2Cl_2/EtOH : 96/4) leading to the expected alcohol **21** (1049 mg, 3.54 mmol, yield: 90%) isolated as a white solid. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.43 (dd, *J* = 2.8, 14 Hz, 1H), 7.10 (dd, *J* = 1.6, 8.8 Hz, 1H), 6.93 (t, *J* = 9.2 Hz, 1H), 4.74-4.69 (m, 1H), 4.00-3.90 (m, 3H), 3.85 (t, *J* = 4.4 Hz, 4H), 3.75 (br d, *J* = 12 Hz), 3.04 (t, J = 4.8 Hz, 4H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.2. NMR ^{13}C (100 MHz, $CDCl_3$): δ 157.0, 154.7, 154.5, 136.5, 136.4, 133.6, 133.5, 119.2, 114.1, 107.8, 107.6, 72.9, 67.1, 63.0, 51.3, 46.6. MS (ESI): *m/z* 297.0 $[M+H]^+$.

(3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl methanesulfonate (22)

To a solution of alcohol **21** (1000 mg, 3.37 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0°C were added successively Et_3N (0.70 mL, 508 mg, 5.06 mmol) and methanesulfonyl chloride (0.40 mL, 592 mg, 5.06 mmol). The resulting solution was then stirred at 25 °C for 4 h under argon. The reaction mixture was successively washed with water, with an aqueous solution saturated in $NaHCO_3$, and finally with brine. The organic layer was dried over Na_2SO_4, filtered and solvents were removed under reduced pressure leading to the mesylate **22** (1280 mg, 3.37 mmol, yield: 100%) obtained as a white solid. NMR ^{1}H (400 MHz, DMSO-d_6): δ 7.49 (dd, *J* = 2.8, 14 Hz, 1H), 7.20 (dd, *J* = 1.6, 8.8 Hz, 1H), 7.07 (t, *J* = 9.2 Hz, 1H), 5.02-4.96 (m, 1H), 4.48 (dd, *J* = 3.2, 12.0 Hz, 1H), 4.39 (dd, *J* = 5.2, 12.0 Hz, 1H), 4.15 (t, *J* = 9.6 Hz, 1H), 3.80 (dd, *J* = 6.0, 9.2 Hz, 1H), 3.73 (t, *J* = 4.4 Hz, 4H), 3.25 (s, 3H), 2.96 (t, *J* = 4.8 Hz, 4H). NMR ^{19}F (100 MHz, DMSO-d_6): δ -121,2. NMR ^{13}C (100 MHz, DMSO-d_6): δ 155.7, 153.6, 153.3, 135.6, 119.2, 114.1, 106.8, 106.6, 69.9, 69.6, 66.1, 50.6, 45.9, 36.7. MS (ESI): *m/z* 375.0 $[M+H]^+$.

5-(azidomethyl)-3-(3-fluoro-4-morpholinophenyl)oxazolidin-2-one (4)

To a solution of mesylate **22** (715 mg, 1.91 mmol) in DMF (7 mL) was added sodium azide (251 mg, 3.82 mmol). The resulting suspension was heated at 75°C for 6 h. The lukewarm reaction mixture was dropwise poured into a water/ice mixture (1000 mL). The resulting white precipitate was filtered and dried under reduced pressure and was characterized as the pure azide **4** (489 mg, 1.52 mmol, yield: 80%). NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.42 (dd, *J* = 2.6, 14.3 Hz, 1H), 7.12-7.09 (m, 1H), 6.92 (t, *J* = 9.1 Hz, 1H), 4.79-4.72 (m, 1H), 4.03 (t, *J* = 8.9 Hz, 1H), 3.86-3 .84 (m, 4H), 3.8 (dd, *J* = 6.4, 8.9 Hz, 1H), 3.68 (dd, *J* = 4.7, 13.2 Hz, 1H), 3.57 (dd, *J* = 4.4, 13.2 Hz, 1H), 3.05-3.02 (m, 4H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.1. NMR ^{13}C (100 MHz, $CDCl_3$): δ 156.9, 154.5-154.0, 133.1, 119.2, 114.1, 107.8, 107.6, 70.7, 67.1, 53.2, 51.2, 47.7. MS (ESI): *m/z* 322.2 $[M+H]^+$.

Linezolid (1)

To a solution of azide **4** (200 mg, 0.62 mmol) in methanol (100 mL) was added a solution of $CoCl_2.6H_2O$ (296 mg, 1.24 mmol) in H_2O (4 mL). The purple solution was cooled to 5 °C and freshly crushed $NaBH_4$ (130 mg, 3.44 mmol) was added portionwise. The resulting dark suspension was then stirred 1 h at room temperature (18°C). The reaction mixture was filtered on celite and the filtrate was diluted with MilliQ water (50 mL) and CH_2Cl_2 (50 mL). After partition and two extractions with CH_2Cl_2, organic layers were collected, washed with brine, dried over Na_2SO_4 and filtered. Solvents were evaporated under reduced pressure leading to the crude amine **23** used in the next step without further purification. To a solution of amine **23** in CH_2Cl_2 (10 mL) were successively added DIPEA (110 µl, 81 mg, 0.63 mmol) and anhydride acetic (60 µl, 65 mg, 0.63 mmol). The solution was stirred for 3 h at 18°C. The reaction mixture was adsorbed on silica gel and purified on a silica gel column (CH_2Cl_2 then CH_2Cl_2/EtOH : 96/4) leading to linezolid **1** (115 mg, 0.30 mmol, yield: 43%) isolated as a white powder. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.40 (dd, *J* = 2.6, 14.3 Hz, 1H), 7.06-7.03 (m, 1H), 6.89 (dd, *J* = 8.8, 9.4 Hz, 1H), 4.77-4.71 (m, 1H), 3.99 (t, *J* = 9.0 Hz, 1H), 3.85-3.83 (m, 4H), 3.74-3.71 (m, 1H), 3.70-3.56 (m, 2H), 3.03-3.01 (m, 4H), 1.99 (s, 3H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.1. NMR ^{13}C (100 MHz, $CDCl_3$): δ 171.1, 156.7, 154.5, 136.7, 133.0, 119.0, 114.1, 107.8, 107.5, 72.1, 67.1, 52.5, 51.1, 47.8, 43.5, 42.1, 23.2. MS (ESI): *m/z* 379.2 $[M+H]^+$.

2-azido-*N*-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (5)

To a solution of azide **4** (150 mg, 0.47 mmol) in methanol (60 mL) was added a solution of $CoCl_2.6H_2O$ (222 mg, 0.94 mmol) in H_2O (4 mL). The purple solution was cooled to 5 °C and freshly crushed $NaBH_4$ (141 mg, 3.74 mmol) was added portionwise. The resulting dark suspension wad then stirred 1 h at 20°C. The resulting dark suspension was then stirred 1 h at 20°C. The reaction mixture was filtered on celite and the filtrate was diluted with MilliQ water (50 mL) and CH_2Cl_2 (50 mL). After partition and two extractions with CH_2Cl_2, organic layers were collected, washed with brine, dried over Na_2SO_4 and filtered. Solvents were evaporated under reduced pressure. To a solution of amine **23** in CH_2Cl_2 (10 mL) were successively added EDCI.HCl (107 mg, 0.56 mmol) and 2-azidoacetic acid **24** (42 µl, 57 mg, 0.56 mmol) were added. The reaction mixture was then stirred for 3 h at 20°C. The reaction mixture was adsorbed on silica gel and purified on a silica gel column (CH_2Cl_2 then CH_2Cl_2/EtOH : 96/4) to afford the compound **5** (134 mg, 0.34 mmol, yield: 76%) isolated as a white solid. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.38 (dd, *J* = 2.6, 14.3 Hz, 1H), 7.05-7.01 (m, 2H), 6.88 (dd, *J* = 8.8, 9.4 Hz, 1H), 4.78-4.71 (m, 1H), 4.01 (t, *J* = 8.9 Hz, 1H), 3.96 (s, 2H), 3.84-3.81 (m, 4H), 3.75-3.56 (m, 3H), 3.02-3.00 (m, 4H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.0. NMR ^{13}C (100 MHz, $CDCl_3$): δ 168.0, 156.6, 156.8, 136.8, 132.9, 119.0, 114.1, 107.7, 71.7, 67.1, 52.5, 51.1, 47.9, 42.2. MS (ESI): *m/z* 379.2 $[M+H]^+$.

2-(2-(2-azidoethoxy)ethoxy)-*N*-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (6)

To a solution of azide **4** 300 mg, 0.93 mmol) in methanol (120 mL) was added a solution of $CoCl_2.6H_2O$ (444 mg, 1.87 mmol) in H_2O (8 mL). The purple solution was cooled to 5 °C and freshly crushed $NaBH_4$ (265 mg, 7.00 mmol) was added portionwise. The resulting dark suspension wad then stirred 1 h at 20°C. The reaction mixture was filtered on celite and the filtrate was diluted with MilliQ water (70 mL) and CH_2Cl_2 (70 mL). After partition and two extractions with CH_2Cl_2, organic layers were collected, washed with brine, dried over Na_2SO_4 and filtered. Solvents were evaporated under reduced pressure. To a solution of amine **23** in CH_2Cl_2 (50 mL) were successively added EDCI.HCl (197 mg, 1.03 mmol) and and 2-[2-(2-azidoethoxy)ethoxy]acetic acid **25** (177 mg, 0.93 mmol). The reaction mixture was then stirred for 2 h at 20°C. The reaction mixture was adsorbed on silica gel and purified on a silica gel column (CH_2Cl_2 then CH_2Cl_2/EtOH : 90/10). The expected azide **6** (342 mg, 0.73 mmol, yield: 78%) was isolated as an amorphous solid. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.44-7.39 (m, 2H), 7.06-7.03 (m, 1H), 6.90 (t, J = 9.13 Hz, 1H), 4.77-4.74 (m, 1H), 4.04-3.95 (m, 3H), 3.85-3.83 (m, 4H), 3.75-3.62 (m, 9H), 3.52-3.40 (m, 2H), 3.04-3.01 (m, 4H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.2. NMR ^{13}C (100 MHz, $CDCl_3$): δ 171.0, 154.2, 136.6, 133.0, 131.5, 118.9, 113.9, 107.6, 71.7, 71.0, 70.3, 70.2, 70.1, 66.9, 51.0, 50.7, 47.8, 41.5. MS (ESI): 467.2 $[M+H]^+$.

4-(2-Fluoro-4-nitro-phenyl)-piperazine-1-carboxylic acid *tert*-butyl ester (26)

A solution of piperazine (559 mg, 6.50 mmol) in acetonitrile (100 mL) was heated at 70°C. To this solution was added dropwise, over 10 min, a solution of 3,4-difluoronitrobenzene **18** (340 µL, 500 mg, 3.15 mmol) in acetonitrile (25 mL). The mixture was then refluxed 2 h at 80°C. Solvents were evaporated under reduced pressure. The oily residu was triturated into *tert*-butyl-methyl-ether (70 mL). The resulting precipitate was filtered off (excess of piperazine) and the filtrate was adsorbed on silica gel and purified on a silica gel column (CH_2Cl_2 then CH_2Cl_2/EtOH : 9/1). The resulting piperazinyl compound was not further purified and used as it for the next step. To a solution of piperazinyl compound in THF (40 mL), at 25 °C, were added successively di-*tert*-butyl-dicarbonate (867 µL, 824 mg, 3.78 mmol), a saturated aqueous solution of $NaHCO_3$ (15 mL) and MilliQ water (15 mL). The mixture was then stirred at 25°C during 5 h before being extracted twice with CH_2Cl_2. The organic layers were washed with brine, dried over Na_2SO_4, filtered and adsorbed on silica gel. A chromatography on a silica gel column (cyclohexane/Et_2O: 9/1 then cyclohexane/Et_2O: 8/2) led to the expected carbamate **26** (926 mg, 2.85 mmol, yield : 91%) isolated under the form of deep yellow powder. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.96 (ddd, J = 0.8, 2.4, 9.2 Hz, 1H), 7.89 (dd, J = 2.8, 13.2 Hz, 1H), 6.89 (t, J = 8.8 Hz, 1H), 3.58 (t, J = 5.2 Hz, 4H), 3.21 (t, J = 5.0 Hz, 4H), 1.46 (s, 9H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -118.7. NMR ^{13}C (100 MHz, $CDCl_3$): δ 154.6, 145.5, 141.0, 121.0, 117.3, 112.8, 112.5, 80.3, 49.6, 49.5, 28.4. MS (ESI): m/z 226.2 $[M\text{-Boc}]^+$, 270.0 $[M\text{-}t\text{Bu}]^+$.

4-(4-Benzyloxycarbonylamino-2-fluoro-phenyl)-piperazine-1-carboxylic acid *tert*-butyl ester (27)

To a solution of compound **26** (2000 mg, 6.15 mmol) in THF (55 mL) and methanol (150 mL), cooled down below 5°C, was added a saturated aqueous solution of $CuSO_4$ (27 mL). The resulting green mixture was vigorously stirred and $NaBH_4$ (4030 mg, 106.50 mmol) was added by portions. The resulting dark suspension was then allowed to reach room temperature (21°C). After 10 mn at 21°C, the mixture was filtrated on celite and the filtrate was diluted with MilliQ water (200 mL) and CH_2Cl_2 (400 mL). After partition and two extractions with CH_2Cl_2, organic layers were collected, washed with brine, dried over Na_2SO_4 and filtered before being evaporated under reduced pressure. The resulting amine obtained under the form of a brownish oily residue, was used without any further purification step. To a solution of the crude amine in CH_2Cl_2 (100 mL) at 25°C, were successively added (5.41 mL, 6.14 mmol) and CbzCl (1.44 mL, 1731 mg, 10.15 mmol). The resulting solution was stirred at room temperature (20-25 °C) during 3 days. The mixture was then adsorbed on silica gel before being purified by chromatography on a silica gel column (cyclohexane/Et_2O: 9/1). The expected carbamate **27** (2017 mg, 4.70 mmol, yield : 76% over two steps) was obtained under the form of a white powder. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.38-7.24 (m, 6H), 6.94 (dd, J = 1.6, 9.2 Hz, 1H), 6.83 (t, J = 8.8 Hz, 1H), 6.58 (br s, 1H), 5.17 (s, 2H), 3.55 (t, J = 5.0 Hz, 4H), 2.94 (t, J = 4.8 Hz, 4H), 1.46 (s, 9H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.9. NMR ^{13}C (100 MHz, $CDCl_3$): δ 154.9, 153.4, 136.1, 128.9, 128.6, 128.5, 119.9, 114.7, 108.3, 104.6, 80.1, 67.4, 51.1, 28.7. MS (ESI): m/z 330.2 $[M\text{-Boc}]^+$, 374.2 $[M\text{-}t\text{Bu}]^+$, 430.2 $[M+H]^+$.

***tert*-butyl-4-(2-fluoro-4-(5-(hydroxymethyl)-2-oxooxazolidin-3-yl)phenyl)piperazine-1-carboxylate (28)**

To a solution of carbamate **27** (1960 mg, 4.56 mmol) in anhydrous THF (55 mL) at -78 °C, was dropwise added *n*-BuLi (2.00 mL, 2.5 M in hexanes, 5.02 mmol). The reaction mixture was allowed to reach -10°C in 30 minutes. The solution was cooled down to -78°C and (*R*)-glycidyl-butyrate (0.63 mL, 650 mg, 4.56 mmol) was added dropwise. The reaction mixture was then stirred 2 h at 25°C. The reaction mixture was quenched with an aqueous solution saturated in NH_4Cl before being extracted with ethyl acetate. Organic phases were collected, dried over Na_2SO_4, filtered and adsorbed on silica gel. The crude product was purified on a silica gel column (CH_2Cl_2 then CH_2Cl_2/EtOH : 96/4) leading to the resulting alcohol **28** (1200 mg, 3.03 mmol, yield: 66%). NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.43 (dd, J = 2.4, 14.0 Hz, 1H), 7.10 (ddd, J = 0.8, 1.6, 8.8 Hz, 1H), 6.93 (t, J = 9.2 Hz, 1H), 4.75-4.69 (m, 1H), 4.00-3.90 (m, 3H), 3.73 (br d, J = 12.0 Hz, 1H), 3.57 (br t, J = 4.4 Hz, 4H), 2.97 (br t, J = 4.4 Hz, 4H), 1.46 (s, 9H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.2. NMR ^{13}C (100 MHz, $CDCl_3$): δ 157.0, 154.7, 154.5, 119.6, 114.0, 107.8, 107.6, 80.1, 72.9, 63.0, 50.9, 46.6, 28.6. MS (ESI): m/z 296.0 $[M\text{-Boc}]^+$, 340.2 $[M\text{-}t\text{Bu}]^+$, 418.2 $[M+Na]^+$.

tert-butyl-4-(2-fluoro-4-(5-(((methylsulfonyl)oxy)methyl)-2-oxooxazolidin-3-yl)phenyl)piperazine-1-carboxylate (29)

To a solution of alcohol **28** (1850 mg, 4.68 mmol) in anhydrous CH_2Cl_2 (25 mL) at 0°C was added successively Et_3N (1.30 mL, 942 mg, 9.36 mmol) and methanesulfonyl chloride (0.54 mL, 800 mg, 7.02 mmol). The resulting solution was then stirred 12 h at 25°C under argon. The reaction mixture was successively washed with water, with an aqueous solution saturated in $NaHCO_3$, and finally with brine. The organic layer was dried over Na_2SO_4, filtered and solvents were removed under reduced pressure leading to the mesylate **29** (2390 mg, 4.679 mmol, yield: 100%) obtained as a pale yellow solid. NMR ^{1}H (400 MHz, DMSO-d_6): δ 7.43 (dd, J = 2.4, 14 Hz, 1H), 7.07 (dd, J = 1.6, 8.8 Hz, 1H), 6.97 (t, J = 9.2 Hz, 1H), 4.92-4.86 (m, 1H), 4.47 (dd, J = 3.6, 12 Hz, 1H), 4.39 (dd, J = 4.0, 12 Hz, 1H), 4.09 (t, J = 9.2 Hz, 1H), 3.90 (dd, J = 6.0, 9.2 Hz, 1H), 3.59 (br t, J = 4.4 Hz, 4), 3.08 (s, 3H), 2.99 (br t, J = 4.4 Hz, 4H), 1.46 (s, 9H). NMR ^{19}F (100 MHz, DMSO-d_6): δ -119.6. NMR ^{13}C (100 MHz, DMSO-d_6): δ 157.0, 154.7, 153.7, 120.0, 114.2, 108.0, 107.8, 69.6, 68.1, 50.9, 46.6, 38.1, 28.6. MS (ESI): m/z 374.0 $[M\text{-Boc}]^+$, 418.0 $[M\text{-}t\text{Bu}]^+$, 496.2 $[M+Na]^+$.

tert-butyl -4-(4-(5-(azidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-1-carboxylate (30)

To a solution of mesylate **29** (163 mg, 0.34 mmol) in DMF (3 mL) was added sodium azide (90 mg, 1.37 mmol). The resulting suspension was heated at 75°C for 5 h. The lukewarm reaction mixture was dropwise poured into a water/ice mixture (750 mL). The resulting white precipitate was filtered and dried under reduced pressure and was characterized as the pure azide **30** (138 mg, 0.33 mmol, yield 95%). NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.42 (dd, J = 2.4, 14 Hz, 1H), 7.09 (dd, J = 1.6, 8.8 Hz, 1H), 6.93 (t, J = 9.2 Hz, 1H), 4.78-4.72 (m, 1H), 4.02 (t, J = 9.2 Hz, 1H), 3.79 (dd, J = 6.0, 8.8 Hz, 1H), 3.67 (dd, J = 4.8, 13.0 Hz, 1H), 3.59-3.54 (m, 5H), 2.98 (br t, J = 4.4 Hz, 4H), 1.46 (s, 9H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.0. NMR ^{13}C (100 MHz, $CDCl_3$): δ 157.1, 154.9, 154.0, 119.8, 114.2, 107.9, 107.7, 80.2, 70.8, 53.3, 50.9, 47.7, 28.6. MS (ESI): m/z 421.2 $[M+H]^+$.

tert-butyl -4-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-1-carboxylate (32)

To a solution of azide **30** (400 mg, 0.95 mmol) in methanol (70 mL) was added a solution of $CoCl_2.6H_2O$ (453 mg, 1.90 mmol) in H_2O (8 mL). The purple solution was cooled to 5 °C and freshly crushed $NaBH_4$ (180 mg, 4.75 mmol) was added by portion. The resulting dark suspension wad then stirred 1 h at 20°C. The reaction mixture was filtered on celite and the filtrate was diluted with MilliQ water (70 mL) and CH_2Cl_2 (70 mL). After partition and two extractions with CH_2Cl_2, organic layers were collected, washed with brine, dried over Na_2SO_4 and filtered. Solvents were evaporated under reduced pressure leading to the crude amine **31** used without any further purification step. To a solution of amine **31** in CH_2Cl_2 (50 mL) were successively added DIPEA (470 µL, 349 mg, 2.85 mmol) and anhydride acetic (178 µL, 192 mg, 1.90 mmol). The reaction mixture was stirred 16 h at 25°C. The reaction mixture was adsorbed on silica gel and purified on a silica gel column (CH_2Cl_2/EtOH 95/5). The acetamide **32** (338 mg, 0.77 mmol, yield 81%) was isolated as a slight yellow solid. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.36 (dd, J = 14.2, 2.6 Hz, 1H), 7.00-6.97 (m, 1H), 6.85 (t, J = 9.2 Hz, 1H), 6.42 (t, J = 6.1 Hz, 1H), 4.73-4.67 (m, 1H), 3.95 (t, J = 9.0 Hz, 1H), 3.69 (dd, J = 9.1, 6.7 Hz, 1H), 3.60-3.55 (m, 2H), 3.52 (t, J = 4.9 Hz, 4H), 2.92 (dd, J = 5 Hz, 4H), 1.94 (s, 3H), 1.41 (s, 9H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -120.0. NMR ^{13}C (100 MHz, $CDCl_3$): δ 171.37, 156.93, 154.84, 154.5, 136.6, 133.4, 119.6, 114.1, 107.8, 80.1, 72.1, 50.8, 47.8, 42.1, 28.6, 23.2. HRMS: $C_{21}H_{29}FN_4O_5$: calcd: 436.2122 found: 436.21186.

Azidomethyl-4-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazine-1-carboxylate (7)

A solution of carbamate **32** (310 mg, 0.71 mmol) in a mixture of TFA/CH_2Cl_2 (2/8, 5 mL) was stirred for 12 h at 21 °C. Solvents were then removed under reduced pressure. At 21°C, the resulting amine **33** was solubilized in a mixture of CH_2Cl_2 (15 mL) and DIPEA (1 mL). To this solution was added the NHS ester **34** (169 mg, 0.852 mmol). The resulting solution was stirred for 12 h at 21 °C. The reaction mixture was adsorbed on silica gel and purified by flash chromatography (CH_2Cl_2/EtOH : gradient from 96/4 to 90/10). The azide compound **7** (150 mg, 0.36 mmol, yield: 50%) was isolated as a white powder. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.22 (dd, J = 2.6, 14.6 Hz, 1H), 6.87 (dd, J = 2.4, 8.9 Hz, 1H), 6.79 (t, J = 9.2 Hz, 1H), 4.57-4.51 (m, 1H), 3.90-3.86 (m, 3H), 3.52 (dd, J = 6.5, 9.3 Hz, 1H), 3.43-3.40 (m, 2H), 3.32-3.30 (m, 2H), 3.28-3.26 (m, 2H), 2.78-2.71 (m, 4H), 1.81-1.79 (m, 1H), 1.67 (s, 3H). NMR ^{13}C ($CDCl_3$): δ 173.4, 167.3, 157.0, 155.7, 154.6, 136.2, 134.5, 120.5, 115.0, 107.7, 72.7, 51.2, 50.9, 50.7, 48.3, 45.3, 42.4, 42.3, 22.4. HRMS (ESI): $C_{18}H_{22}FN_7O_4$: calcd: 419.17173, found: 419.17121.

***N*-((3-(4-(4-(2-(2-(2-azidoethoxy)ethoxy)acetyl)piperazin-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (8)**

A solution of carbamate **32** (150 mg, 0.34 mmol) in a mixture of TFA/CH_2Cl_2 (2/8, 5 mL) was stirred for 5 h at 22 °C. Solvents were then removed under reduced pressure. At 21°C, the resulting amine **33** was solubilized in a mixture of CH_2Cl_2 (6 mL) and DIPEA (1.5 mL). To this solution was added the NHS ester **35** (111 mg, 0.39 mmol). The resulting solution was stirred for 12 h at 22 °C. The reaction mixture was adsorbed on silica gel and purified by flash chromatography (CH_2Cl_2/EtOH 8/2). The azide compound **8** (167 mg, 0.329 mmol, yield 96%) was isolated as an amorphous colorless solid. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.41 (d, J = 13.9 Hz, 1H), 7.03 (d, J = 8.6 Hz, 1H), 6.87 (t, J = 9.1 Hz, 1H), 6.4 (t, J = 5.6 Hz, 1H), 4.76-4.71 (m, 1H), 4.23 (s, 2H), 3.98 (t, J = 9.0 Hz, 1H), 3.74-3.55 (m, 13H), 3.36 (t, J = 5.3 Hz, 2H), 3.02-2.97 (m, 4H), 1.99 (s, 3H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -75.8, -120.0. NMR ^{13}C (100 MHz, $CDCl_3$): δ 171.3, 167.8, 156.9, 154.5, 154.5, 136.3, 136.2, 133.5, 119.6, 114.0, 107.8, 72.1, 70.9, 70.8, 70.7, 70.2, 51.2, 50.8, 50.6, 47.8, 45.3,

42.1, 42.0, 23.2. HRMS (ESI): $C_{22}H_{30}FN_7O_6$: calcd 507.22416, found 507.22526

_N_-((3-(4-(4-(2-chloroacetyl)piperazin-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (36)

A solution of carbamate **32** (250 mg, 0.57 mmol) in a mixture of TFA/CH_2Cl_2 (2/8, 8 mL) was stirred for 12 h at 20 °C. Solvents were then removed under reduced pressure. At 21°C, the resulting amine **33** was solubilized in a mixture of CH_2Cl_2 (13 mL) and DIPEA (1 mL). To this solution was added chloromethyl-chloroformate (56 µL, 82 mg, 0.63 mmol). The resulting solution was stirred for 4 h at 21 °C. The reaction mixture was adsorbed on silica gel and purified by flash chromatography (CH_2Cl_2 then CH_2Cl_2/EtOH 80/20). The chloride compound **36** (208 mg, 0.48 mmol, yield 85%) was isolated as a white solid. NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.48 (dd, J = 2.6, 14.1 Hz, 1H), 7.11-7.08 (m, 1H), 6.96 (t, J = 9.1 Hz, 1H), 6.12 (t, J = 6.4 Hz, 1H), 5.84 (s, 2H), 4.82-4.76 (m, 1H), 4.04 (t, J = 9.0 Hz, 1H), 3.79-3.60 (m, 7H), 3.09-3.04 (m, 4H), 2.04 (s, 1H). NMR ^{19}F (100 MHz, $CDCl_3$): δ -68.9, -120.4. NMR ^{13}C (100 MHz, $CDCl_3$): δ 171.2, 157.0, 154.5, 154.4, 152.7, 135.8-135.7, 133.9-133.8, 119.9, 114.0, 107.8, 107.6, 72.1, 71.0, 50.6, 47.8, 44.2, 23.2. HRMS (ESI): $C_{18}H_{22}ClFN_4O_5$: calcd: 428.12628, found: 428.12504.

_N_-((3-(4-(4-(2-azidoacetyl)piperazin-1-yl)-3-fluorophenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (9)

To a solution of the chloride compound **36** (75 mg, 0.17 mmol) in DMF (2 mL) was added NaN_3 (34.11 mg, 0.52 mmol). The resulting suspension was heated at 75°C for 24 h. The reaction mixture was diluted in water and extracted several times with CH_2Cl_2. Organic layers were collected, dried over Na_2SO_4, filtered and solvents were removed under reduced pressure leading to the expected azide compound **9** (63 mg, 0.14 mmol, yield: 83%). NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.23 (t, J = 5.9 Hz, 1H), 7.5 (dd, J = 2.6, 14.8 Hz, 1H), 7.18 (dd, J = 2.7, 8.8 Hz, 1H), 7.12-7.05 (m, 1H), 5.26 (s, 2H), 4.73-4.67 (m, 1H), 4.08 (t, J = 9.0 Hz, 1H), 3.7 (dd, J = 6.5, 9.1 Hz, 1H), 3.57 (br s, 4H), 3.4 (t, J = 5.5 Hz, 2H), 2.97 (br s, 4H), 1.84 (s, 3H). NMR ^{19}F (100 MHz, DMSO-d_6): δ -67.9, -121.2. NMR ^{13}C (100 MHz, DMSO-d_6): δ 169.9, 155.8, 153.9, 153.5, 153.3, 135.2, 133.7, 119.8, 114.9, 106.5, 75.5, 71.5, 50.0, 47.2, 43.7, 43.4, 41.4, 22.4. HRMS: $C_{18}H_{22}FN_7O_5$: calcd: 435.16665, found: 435.16703.

General procedure for the synthesis of conjugates (10) to (15)

To a solution of catechol **2** (1 eq.) and azides **4** to **9** (1.1 eq.) in THF (1 mL per 0.1 mmol of **2**) was added successively an 0.8M aqueous solution of copper sulfate (1 eq.) and sodium ascorbate (4.7 eq.). The suspension was sonicated and then stirred under argon at room temperature (21-23°C). The reaction mixture was filtered on a celite pad and purified on a silica gel column (CH_2Cl_2/EtOH mixture) to afford the expected conjugates. For the release of the catechol moiety, conjugates were dissolved in a mixture of CH_2Cl_2/TFA/TIS/EtOH (70/20/5/5, 5 mL per 0.1 mmol of **2**). The reaction mixture was stirred at room temperature (22-25°C) before being evaporated under reduced pressure. The residue was suspended in a minimum of THF and cyclohexane (or pentane) was added until precipitation of expected conjugates **10** to **15**. Precipitates were filtered on a Hirsch funnel.

(1-((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)-1_H_-1,2,3-triazol-4-yl)methyl (4-(2,3-dihydroxybenzamido)butyl)carbamate (10)

White powder (yield 53%). NMR ^{1}H (400 MHz, THF-d_8): δ 13.1 (s, 1H), 8.03 (br s, 1H), 7.91 (s, 1H), 7.48 (dd, J = 2.0, 14.9 Hz), 7.14-7.12 (m, 1H), 7.07 (d, J = 8.3 Hz, 1H), 6.95 (t, J = 9.2 Hz, 1H), 6.84 (d, J = 7.7 Hz, 1H), 6.61 (t, J = 8.3 Hz, 1H), 6.45 (t, J = 5.0 Hz, 1H), 5.10-4.93 (m, 3H), 4.78-4.71 (m, 2H), 4.14 (t, J = 9.5 Hz, 1H), 3.87-3.83 (m, 1H), 3.75-3.73 (m, 4H), 3.39 (q, J = 6.1 Hz, 2H), 3.13 (q, J = 6.1 Hz, 2H), 2.98-2.96 (m, 4H), 1.63-1.51 (m, 4H). NMR ^{19}F NMR (100 MHz, DMSO-d_6): δ -78.1, -123.8. NMR ^{13}C (100 MHz, THF-d_8): δ 168.7, 154.7, 154.3, 152.4, 151.2, 148.4, 145.1, 142.2, 134.3, 131.9, 122.9, 116.8, 116.1, 115.6, 114.0, 112.6, 111.6, 104.9, 104.6, 69.0, 55.5, 50.0, 49.2, 45.4, 38.4, 37.0, 24.9, 24.7, 23.5. HRMS (ESI): $C_{29}H_{34}FN_7O_8$: calcd: 627.24529, found: 627.24484.

(1-(2-(((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)amino)-2-oxoethyl)-1_H_-1,2,3-triazol-4-yl)methyl (4-(2,3-dihydroxybenzamido)butyl)carbamate (11)

White powder (yield 74%). NMR ^{1}H (400 MHz, DMSO-d_6): δ 12.8 (br s, 1H), 9.1 (br s, 1H), 8.78-8.75 (m, 2H), 8.04 (s, 1H), 7.50 (dd, J = 2.5, 15.0 Hz, 1H), 7.28-7.25 (m, 2H), 7.19 (dd, J = 2.5, 8.8 Hz, 1H), 7.08 (t, J = 9.6 Hz, 1H), 6.91 (dd, J = 7.7, 1.2 Hz, 1H), 6.68 (t, J = 7.9 Hz, 1H), 5.15 (s, 2H), 5.03 (s, 2H), 4.75-4.73 (m, 1H), 4.1 (t, J = 9.2 Hz, 1H), 3.75-3.69 (m, 5H), 3.54-3.45 (m, 2H), 3.31-3.25 (m, 2H), 3.04-2.95 (m, 6H), 1.53-1.40 (m, 4H). NMR ^{19}F (100 MHz, DMSO-d_6): δ -73.8, -121.3. NMR ^{13}C (100 MHz, DMSO-d_6): δ 170.2, 166.7, 156.3, 154.4, 153.8, 150.2, 146.7, 142.9, 136.0, 133.8, 126.5, 119.7, 119.2, 118.3, 117.5, 115.4, 114.6, 107.3-107.1, 71.9, 66.6, 57.3, 51.9, 51.2, 47.7, 42.1, 39.1, 27.4, 26.7. HRMS (ESI): $C_{31}H_{37}FN_8O_9$: calcd: 684.26675, found: 684.26885.

(1-(2-(2-(2-(((3-(3-fluoro-4-morpholinophenyl)-2-oxooxazolidin-5-yl)methyl)amino)-2-oxoethoxy)ethoxy)ethyl)-1_H_-1,2,3-triazol-4-yl)methyl (4-(2,3-dihydroxybenzamido)butyl)carbamate (12)

White powder (yield 67%). NMR ^{1}H (400 MHz, DMSO-d_6): δ 12.85 (br s, 1H), 9.10 (br s, 1H), 8.75 (t, J = 5.9 Hz, 1H), 8.07 (s, 1H), 8.01 (t, J = 5.9 Hz, 1H), 7.48 (dd, J = 15.0, 2.5 Hz, 1H), 7.27 (dd, J = 8.1, 1.4 Hz, 1H), 7.23-7.16 (m, 2H), 7.06 (t, J = 9.4 Hz, 1H), 6.90 (dd, J = 7.9, 1.0 Hz, 1H), 6.67 (t, J = 8.0 Hz, 1H), 5.03 (br s, 2H), 4.78-4.72 (m, 1H), 4.53 (t, J = 5.1, 2H), 4.09 (t, J = 9.1Hz, 1H), 3.89 (s, 2H), 3.81 (t, J = 5.2, 2H), 3.78-3.76 (m, 1H), 3.74-3.72 (m, 4H), 3.52 (s, 4H), 3.48 (t, J = 5.7 Hz, 2 H), 3.27 (q, J = 6.2 Hz, 2H), 3.01 (q, J = 6.3 Hz, 2H), 2.97-2.94 (m, 4H), 1.56-1.41 (m, 4H). NMR ^{19}F (400 MHz, DMSO-d_6): δ - 73.3, - 121.3. NMR ^{13}C (100 MHz, DMSO-d_6): δ 169.9, 169.6, 155.8, 154.5 (d,

J = 243.5 Hz, C-F), 153.9, 149.6, 146.1, 142.6, 135.5 (d, *J* = 8.9 Hz), 133.3 (d, *J* = 10.9 Hz), 124.8, 119.2, 119.1, 118.6, 117.7, 117.0, 114.8, 114.0, 106.6 (d, *J* = 26.2 Hz), 71.2, 69.9, 69.7, 69.2, 68.6, 66.1, 56.8, 50.6, 49.2, 47.3, 41.1, 39.9, 38.5, 26.8, 26.1. HRMS (ESI): $C_{35}H_{45}FN_8O_{11}$: calcd: 772.31918, found: 772.31979

(1-(2-(4-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazin-1-yl)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl (4-(2,3-dihydroxybenzamido)butyl)carbamate (13)

White powder (yield 68%). NMR ^{1}H (400 MHz, DMSO-d_6): δ 12.80 (br s, 1H), 9.10 (br s, 1H), 8.76 (t, *J* = 4.9 Hz, 1H), 8.23 (t, *J* = 5.8 Hz, 1H), 8.00 (s, 1H), 7.51 (dd, *J* = 2.6, 14.8 Hz, 1H), 7.28-7.26 (m, 2H), 7.19 (dd, *J* = 2.5, 8.8 Hz, 1H), 7.09 (t, *J* = 9.0 Hz, 1H), 6.89 (dd, *J* = 1.5, 7.7 Hz, 1H), 6.67 (t, *J* = 7.9 Hz, 1H), 5.51 (s, 2H), 5.05 (s, 2H), 4.74-4.76 (m, 1H), 4.09 (t, *J* = 9.0 Hz, 1H), 3.72-3.60 (m, 5H), 3.40 (t, *J* = 5.2 Hz, 2H), 3.27 (q, *J* = 6.4 Hz, 2H), 3.06-2.96 (m, 6H), 1.83 (s, 3H), 1.55-1.39 (m, 4H). NMR ^{19}F (400 MHz, DMSO-d_6): δ -73.4, -121.1. NMR ^{13}C (100 MHz, DMSO-d_6): δ 170.4, 170.2, 164.8, 156.4, 154.5, 150.2, 146.7, 142.8, 135.6, 134.2, 126.7, 120.4, 119.2, 118.3, 117.5, 115.4, 114.6, 107.2-107.0, 72.0, 57.4, 51.1, 51.0-50.6, 47.8, 44.8, 42.1, 41.9, 39.1, 27.4, 26.7, 22.9. HRMS (ESI): $C_{33}H_{40}FN_9O_9$: calcd: 725.2933, found: 725.29308.

(1-(2-(2-(2-(4-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2-fluorophenyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl (4-(2,3-dihydroxybenzamido)butyl)carbamate (14)

White powder (yield 43%). NMR ^{1}H (400 MHz, DMSO-d_6): δ 12.86 (br s, 1H), 9.11 (br s, 1H), 8.76 (t, *J* = 5.8 Hz, 1H), 8.24 (dd, *J* = 6.5, 5.4 Hz, 1H), 8.10 (s, 1H), 7.50 (dd, *J* = 14.9, 2.5 Hz, 1H), 7.28 (dd, *J* = 8.0, 1.3 Hz), 7.22 (t, *J* = 5.7 Hz, 1H), 7.18 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.06 (t, *J* = 9.4 Hz, 1H), 6.91 (dd, *J* = 7.6, 1.2 Hz, 1H), 6.67 (t, *J* = 7.9 Hz, 1H), 5.03 (s, 2H), 4.74-4.68 (m, 1H), 4.53 (t, *J* = 5.1 Hz, 2H), 4.17 (s, 2H), 4.08 (t, *J* = 9.0 Hz, 1H), 3.82 (t, *J* = 5.3 Hz, 2H), 7.71 (dd, *J* = 8.9, 6.4 Hz, 1 H), 3.56-3.48 (m, 8H), 3.41 (t, *J* = 5.4 Hz, 2H), 3.27 (q, *J* = 5.4 Hz, 2H), 3.01 (q, *J* = 6.4, 2H), 2.94 (br s, 4H), 1.84 (s, 3H), 1.56-1.42 (m, 4H). NMR ^{19}F (400 MHz, DMSO-d_6): δ -121.2. NMR ^{13}C (100 MHz, DMSO-d_6): δ 169.9, 169.7, 167.2, 155.8, 154.6 (d, *J* = 244 Hz, C-F), 154.0, 149.6, 146.1, 142.2, 135.2 (d, *J* = 9.4 Hz), 133.7 (d, *J* = 10.5 Hz), 124.8, 119.7, 118.7, 117.8, 117.0, 114.8, 114.0, 106.6 (d, *J* = 26.6 Hz), 71.5, 69.6, 69.3, 69.2, 68.6, 67.0, 56.8, 50.6, 50.3, 49.3, 47.3, 44.3, 41.4, 41.1, 39.9, 38.6, 26.9, 26.2. HRMS (ESI): $C_{37}H_{48}FN_9O_{11}$: calcd: 813.34573, found: 813.34619.

(4-((((4-(2,3-dihydroxybenzamido)butyl)carbamoyl)oxy)methyl)-1*H*-1,2,3-triazol-1-yl)methyl 4-(4-(5-(acetamidomethyl)-2-oxooxazolidin-3-yl)-2)fluorophenyl)piperazine-1-carboxylate (15)

White powder (yield 56%). NMR ^{1}H (400 MHz, DMSO-d_6): δ 12.80 (s, 1H), 9.10 (s, 1H), 8.76 (t, *J* = 5.5 Hz, 1H), 8.24-8.21 (m, 2H), 7.49 (dd, *J* = 2.6, 14.8 Hz, 1H), 7.28-7.25 (m, 2H) , 7.16 (dd, *J* = 2.5, 8.4 Hz, 1H), 7.05 (t, *J* = 9.3 Hz, 1H), 6.90 (dd, *J* = 1.5, 7.8 Hz, 1H), 6.67 (t, *J* = 7.9 Hz, 1H), 6.30 (s, 2H), 5.05 (s, 2H), 4.07 (t, *J* = 9.2 Hz, 1H), 3.69 (dd, *J* = 6.4, 9.2 Hz, 1H), 3.54-3.51 (m, 4H), 3.39 (t, *J* = 5.5 Hz, 2H), 3.27 (q, *J* = 6.4 Hz, 2H), 3.01 (q, *J* = 6.3 Hz, 2H), 2.93 (br s, 4H) , 1.82 (s, 3H), 1.55-1.42 (m, 4H). NMR ^{19}F (100 MHz, DMSO-d_6): δ -73.9, -121.2. NMR ^{13}C (100 MHz, DMSO-d_6): δ 169.9, 169.6, 155.8, 153.9, 153.3, 152.8, 149.6, 146.1, 143.1, 135.1, 133.8, 125.7, 119.8, 118.7, 117.7, 117.0, 114.9, 114.0, 106.7, 71.5, 70.7, 56.6, 50.0, 47.2, 43.7, 43.4, 41.4, 38.5, 26.8, 26.1, 22.4. HRMS (ESI): $C_{33}H_{40}FN_9O_{10}$: calcd: 741.28822, found: 741.28481.

2,5-dioxopyrrolidin-1-yl 2-azidoacetate (34)

To a solution of 2-azidoacetic acid **24** (805 µL, 1087 mg, 10.76 mmol) in CH_2Cl_2 (50 mL) were added EDCI.HCl (2061 mg, 10.76 mmol) and *N*-hydroxysuccinimide (1125 mg, 9.78 mmol). The resulting solution was stirred 48 h at 20 °C. The reaction mixture was washed successively with water and brine, dried over Na_2SO_4, filtered and solvents were removed under reduced pressure leading to NHS ester **34** (1210 mg, 6.107 mmol, yield 62%) obtained as a pale yellow oil which slowly crystallized at 5°C. NMR ^{1}H (400 MHz, $CDCl_3$): δ 4.21 (s, 2H), 2.85 (s, 4H). NMR ^{13}C ($CDCl_3$, 100MHz): 168.5, 164.3, 48.2, 25.7.

2,5-dioxopyrrolidin-1-yl 2-(2-(2-azidoethoxy)ethoxy)acetate (35)

To a solution of 2-(2-(2-azidoethoxy)ethoxy)acetic acid **25** (200 mg, 1.06 mmol) in CH_2Cl_2 (20 mL) were added EDCI.HCl (243 mg, 1.27 mmol) and *N*-hydroxysuccinimide (146 mg, 1.27 mmol). The resulting solution was stirred 1 h at 18 °C. The organic medium was washed successively with water and brine, dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure leading to NHS ester **35** (570 mg, 1.99 mmol, yield 80%) obtained as a pale yellow oil. NMR ^{1}H (400 MHz, $CDCl_3$): δ 4.49 (s, 2H), 3.79-3.79 (m, 2H), 3.69-3.68 (m, 2H), 3.66-3.63 (m, 2H), 3.39-3.36 (m, 2H), 2.82 (br s, 4H). NMR ^{13}C ($CDCl_3$, 100MHz): 168.7, 166.0, 71.5, 70.7, 70.1, 66.7, 50.7, 25.6. HRMS (ESI): $C_{10}H_{14}N_4O_6$: calcd: 286.09133, found: 286.09111.

Preparation of PAO1Δ*pfeA* mutant

PCR fragments flanking *pfeA* were amplified from genomic *P. aeruginosa* PAO1 DNA,[59] using the primer pairs Δ*pfeA*#1/Δ*pfeA*#2 (5'-AACATGAATTCAGCTGGCCCAGGCCCTG-3'/5'-TGAGGACATCGGTGATCTCCG-3'), and Δ*pfeA*#3/Δ*pfeA*#4 (5'-TCACCGATGTCCTCACGCTATACCAGCCTGACCGCGTC-3'/5'-AACATAAGCTTAGGCCGTCGAGCGGCTG-3'). These two fragments were mixed, and a 1400bp fragment was amplified using the primer pairs ΔpfeA#1/ΔpfeA#4 and cloned into the suicide plasmid pME3088,[60] using *Eco*RI and *Hind*III restriction sites, to create pME3088Δ*pfeA*. Deletion of the chromosomal *pfeA* gene of *P. aeruginosa* was generated by transferring pME3088Δ*pfeA* from *E. coli* TOP10 to the PAO1 wild-type strain and integration of the plasmid into the chromosome with selection for tetracycline resistance. A second crossing-over event excising the vector was achieved by enrichment for

tetracycline-sensitive cells to generate the corresponding mutant PAO1 Δ*pfeA*.[61] The deleted strain was verified by PCR and sequencing.

Antimicrobial susceptibility assays

Evaluation of the conjugates activities was carried out in Mueller-Hinton II Broth (MHB) (Cation-Adjusted; Becton Dickinson) and in an iron-deficient CAA medium (casamino acid medium, composition: 5 $g.L^{-1}$ [(low-iron-CAA); Difco], 1.46 $g.L^{-1}$ K_2HPO_4 $3H_2O$, 0.25 g l^{-1} $MgSO_4$ $7H_2O$) by the two-fold serial dilution method with an inoculum of 10^5 bacteria per mL. Data were reported as MIC, which reflects the lowest concentration of antibiotic that inhibits the visible cell growth after an 18 h (MHB) or 48 h (CAA) incubation at 30 ◦C.

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