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6-Arylmethylidene Penicillin-based Sulfone Inhibitors for Repurposing Antibiotic Efficiency in Priority Pathogens

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KEYWORDS: antimicrobial resistance, β-lactamase inhibitors, carbapenem-hydrolyzing class D β-lactamase enzymes, enzyme covalent adducts, proteomic analysis.

ABSTRACT

The ability of 6-(aryl)methylidene penicillin-based sulfones **1**−**7** to repurpose β-lactam antibiotics activity with bacterial species that carry carbapenem-hydrolyzing class D β-lactamases (OXA-23, OXA-24/40 and OXA-48), as well as with class A (TEM-1, CTX-M-2) and class C (CMY-2, DHA-1) enzymes, is reported. The combinations imipenem/**3** and imipenem/**4** restored almost completely the antibiotic efficacy in OXA-23 and OXA-24/40 carbapenemase-producing *A. baumannii* strains (1 μg mL^{-1}), and also provided good results for OXA-48 carbapenemase-producing *K. pneumoniae* strains (4 μg mL^{-1}). Compounds **2**−**6** in combinations with ceftazidime and ampicillin were also efficient in restoring antibiotic efficacy in *E. coli* strains carrying class C (CMY-2 and DHA-1) and class A (TEM-1 and CTX-M-2) β-lactamase enzymes, respectively. Kinetic and inhibition studies with the OXA-24/40 enzyme, protein mass spectrometry analysis and docking studies allowed us to gain an insight into the inhibition mechanism and the experimentally observed differences between the ligands.

INTRODUCTION

The growing appearance and dissemination worldwide of superbugs is increasingly limiting dangerously the ability of antibiotics to cure bacterial infections.[1,2,3,4] As noted by the World Health Organization (WHO) in February 2017, there is a huge concern about the lack of effective therapies against the Gram-negative pathogens *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*.[5] In this respect, great success has been achieved with combined therapy approaches that block the most relevant resistance mechanism in Gram-negative bacteria, *i.e.,* the enzymatic inactivation of β-lactams by β-lactamases.[6,7,8,9,10,11,12,13] These drugs are around 70% of commonly prescribed antibiotics. At the low concentrations generally used, β-lactamase inhibitors do not prevent bacterial growth, but when co-administered with the antibiotic they enhance the activity of the antibiotic, thus repurposing the existing life-saving drugs in clinical use.[14,15,16,17]

There are four types of β-lactamases (classes A, B, C and D). Among them, the class D enzymes, which are also known as 'oxacillinases (OXA)', are very relevant because they hydrolyze penicillins and narrow-spectrum cephalosporins.[18] Some class D β-lactamases have evolved to inactivate expanded-spectrum cephalosporins and even carbapenems [carbapenem-hydrolyzing class D enzymes (CHDLs)].[19,20] Infections caused by bacteria that produce these last two groups of enzymes are of high concern, because they are frequently found in deadly pathogens such as multidrug-resistant *Acinetobacter baumannii* (*e.g.* OXA-23, OXA-24/40), or *Enterobacteriaceae* (*e.g.* OXA-48).[21,22] The dissemination of these enzymes is seriously compromising the use of carbapenems, which are often considered as the antimicrobials of last resort.[23] The carbapenemase activity of OXA-23 and OXA-24/40 enzymes is improved by the presence of an uncommon and highly hydrophobic "tunnel-like" entrance to the active site and this: (i) limits the entry to only certain ligands; and (ii) fixes the antibiotic in the productive conformation for β-lactam ring opening for a longer time (Figure 1).[24] This architecture of the active site, which is achieved by the side chains of a phenylalanine (OXA-23) or a tyrosine (OXA-24/40) and a methionine, is very rigid and remains largely unchanged during the catalysis. The OXA-48 enzyme, in which the hydrophobic bridge of OXA-23 and OXA-24/40 enzymes is absent, has a similar carbapenemase efficiency to that of the OXA-24/40 enzyme. In this case, the

enzymatic efficacy is achieved by the existence of a large hydrophobic region near the active site that allows the carbapenem to be fixed into an efficient conformation for hydrolysis (Figure 1).[24]

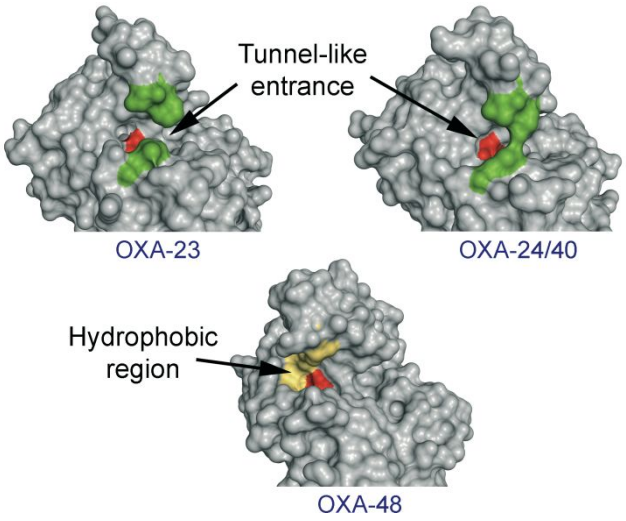


Figure 1. Overall structures of relevant CHDL enzymes: OXA-23 from *A. baumannii* (PDB ID 4K0X[25]), OXA-24/40 from *A. baumannii* (PDB ID 3G4P[26]), and OXA-48 from *K. pneumoniae* (PDB ID 4S2P[27]). The tunnel-like entrance in OXA-23 and OXA-24/40 and the hydrophobic region in OXA-48 are highlighted in green and yellow, respectively. The position of the catalytic serine is shown in red.

The β-lactamase inhibitors clavulanic acid and the penicillin-based sulfones sulbactam and tazobactam, which are widely employed in clinic, are ineffective against the CHDLs enzymes. More recently developed 1,6-diazabicyclo[3,2,1]octane (DBO) β-lactamase inhibitors such as avibactam, which is used in combination with ceftazidime to treat infections caused by strains carrying class A, C and some class D β-lactamases (OXA-48), also proved to be inefficient against OXA-23 and OXA-24/40 (Figure 2A).[28] Relebactam, another DBO inhibitor that was approved in 2019 in combination with imipenem-cilastatin, shows a similar inhibition spectrum. More recently, Durand-Réville *et al*.[29] demonstrated that the rigidification of the six-membered ring of avibactam with a double bond, as well as the introduction of a methyl group, provides a new DBO β-lactamase inhibitor, namely durlobactam (phase III clinical trials in combination with sulbactam), with enhanced inhibitory capacity against bacteria that produce OXA-24/40.

As revealed by the structure of OXA-24/40 inactivated by durlobactam (PDB ID 6MPQ,[30] 1.95 Å), the methyl group of the ligand interacts with the hydrophobic bridge of the active site (Figure 2B).[30] Moreover, the penicillin-based sulfone **1** (LN-1-255), which was developed by Buynak,[31] proved to have a markedly higher efficacy than tazobactam and avibactam against relevant CHDLs in *A. baumannii* (the plasmid encoded OXA-23, OXA-24/40, OXA-58, OXA-143, and OXA-235, and the chromosomally encoded OXA-51) and *K. pneumoniae* OXA-48 (Figure 2C).[28,32] This compound enhances the *in vitro* activity of imipenem by between 32- and 128-fold and also has good therapeutic efficacy *in vivo*.[33] The effectiveness of LN-1-255 lies in its ability to form an indolizine adduct that is resistant to hydrolysis. This aromatic moiety would be formed after dioxothiazolidine ring opening and by nucleophilic attack of the pyridine nitrogen atom on the conjugated initial imine adduct (Figure 2C).[34] The available crystallographic structures of OXA-24/40 from *A. baumannii* covalently modified by this type of (2-pyridyl)methylene penicillin-based sulfone ligand (PDB IDs 3MBZ, 3FYZ, 3FZC and 3FV7)[26] shows numerous favorable lipophilic interactions of the enzyme adduct with the tunnel and its entrance, which involves residues Trp115, Leu168, Met223 and Val130 (Figure 2D). These advantageous interactions would further increase the hydrophobicity of the tunnel-like entrance, thus limiting the approach of the water molecule for the final hydrolysis.

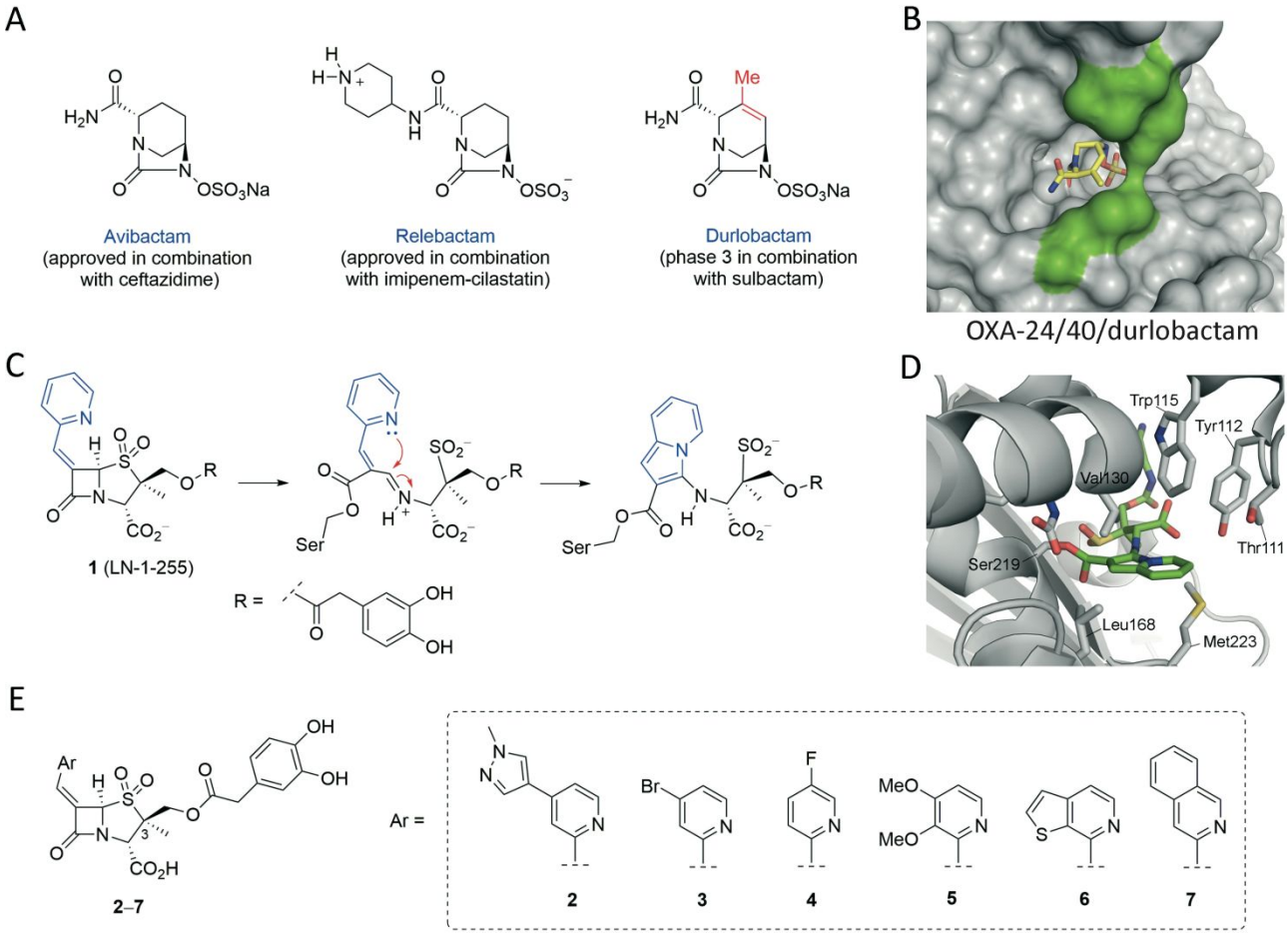


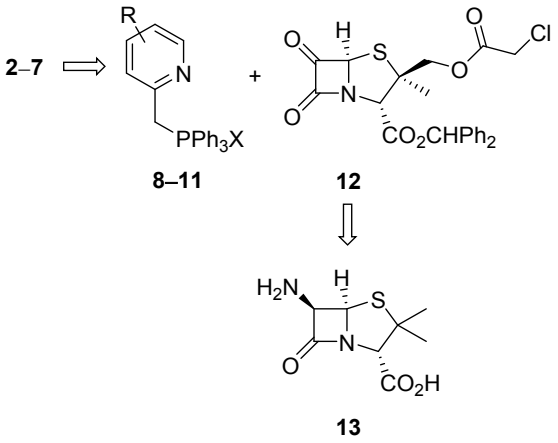
Figure 2. A. Chemical structures of avibactam, relebactam and durlobactam. These DBOs have been either approved by the FDA or are currently under clinical studies in combination with an antibiotic, which is indicated in parenthesis. B. Detailed view of the crystal structure of OXA-24/40 from *A. baumannii* covalently modified by durlobactam (PDB ID 6MPQ[30]). C. Mechanism of inhibition of 6-(2-pyridyl)alkylidene penicillin-based sulfone **1** (LN-1-255). D. Detailed view of the enzyme adduct observed in PDB ID 3FZC[32] (OXA-24/40 from *A. baumannii*). The relevant side chain residues are shown and labelled. E. Target derivatives **2**−**7**.

Given the background outlined above, and in the search for new penicillin-based sulfone inhibitors, we became interested in exploring the effect on the inhibitory potency of **1** against the CHDL enzymes of (i) the incorporation of substituents in the pyridine ring that would change the electron density of this moiety, and (ii) the use of larger pyridine-based heterocycles. To this end, we report here the synthesis of six 6-arylmethylidene penicillin-based sulfones, compounds **2**−**7**, which contain pyridine rings substituted with a

methylpyrazoyl group, a halogen (Br, F) or methoxy groups, a thieno[2,3-c]pyridine or an isoquinoline (Figure 2E). Microbiological studies on antimicrobial susceptibility with previously studied bacterial strains of *Acinetobacter baumannii* harboring OXA-23 or OXA-24/40, and *Klebsiella pneumoniae* carrying OXA-48 are reported. In addition, the capacity of compounds **2**−**7** to restore the antibiotic efficacy in widely distributed bacterial strains of *Escherichia coli* producing β-lactamases of classes A (TEM-1, CTX-M-2) and C (CMY-2, DHA-1) are also included. CMY-2 and DHA-1 are widely distributed plasmidic AmpC enzymes, and TEM-1 and CTX-M-2 [Extended Spectrum Beta-lactamases (ESBLs)] are spread worldwide in pathogenic Gram-negative bacteria, including *Enterobacteriae* and *P. aeruginosa*.[18,35,36] The results of the kinetic studies on the reported analogs against OXA-24/40*,* the detection by mass spectrometry of the enzyme adducts, the monitoring of the β-lactam ring opening, and docking studies, allowed us to gain an insight into the inhibition mechanism and the experimentally observed differences between the ligands.

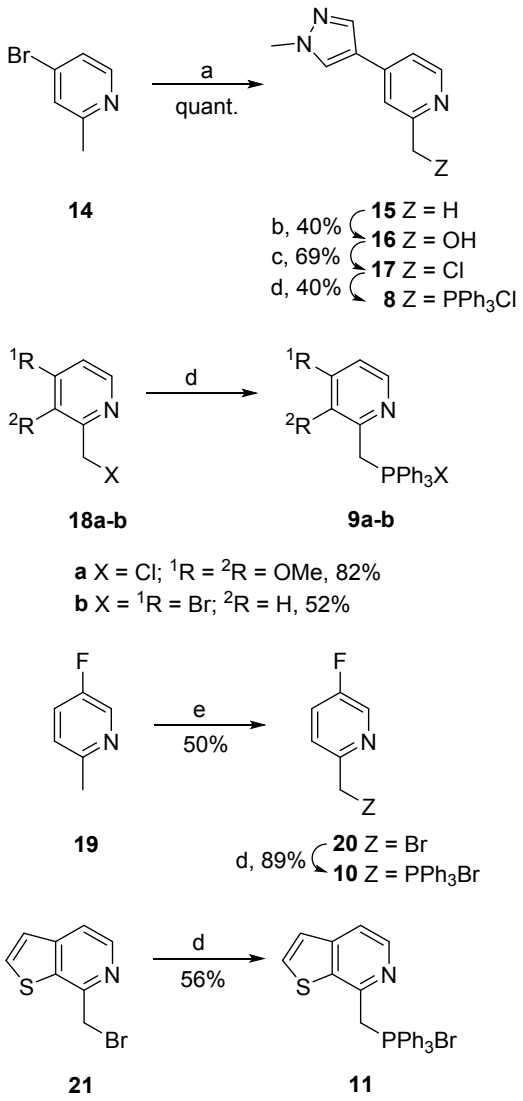
RESULTS AND DISCUSSION

Synthesis of Compounds 2−7 – The synthesis of the target compounds **2**−**7** involved the introduction of the pyridine moiety by a Wittig reaction between the phosphonium salts **8**−**11** and previously reported 3-isoquinolylmethylphosphonium bromide[37] and the ketone **12** as a key step (Scheme 1). The latter compound was prepared according to modified reported protocols from commercially available (+)-6-aminopenicillanic acid (**13**).[38,39,40,41]



Scheme 1. Synthetic approach for compounds **2**−**7**.

The synthesis of the required phosphonium salts **8**, **9a−b**, **10** and **11**, was achieved from commercially available pyridines **14**, **18a−b**, **19** and **21**, respectively, as outlined in Scheme 2. Thus, phosphonium salt **8** was prepared in four steps from 2-methyl-4-bromopyridine (**14**) by Suzuki cross-coupling between the bromide **14** and 1-methylpyrazole-4-boronic acid pinacol ester using $Pd(PPh_3)_4$ as catalyst. Oxidation of the methyl group at position C2 in the resulting pyridine **15** gave alcohol **16**. Conversion of **16** into the chloride **17** by treatment with thionyl chloride and subsequent reaction with triphenylphosphine afforded the desired phosphonium salt **8**. Compounds **9a-b** were prepared by nucleophilic substitution of the halides **18a-b** by treatment with triphenylphosphine. Bromination of 2-methyl-5-fluoropyridine (**19**) with *N*-bromosuccinimide in the presence of catalytic amounts of AIBN gave bromide **20**, which was converted into the phosphonium salt **10** in the same way as derivatives **9** from **18**. Finally, phosphonium salt **11** was synthesized from previously described bromide **21**[42] by treatment with triphenylphosphine.

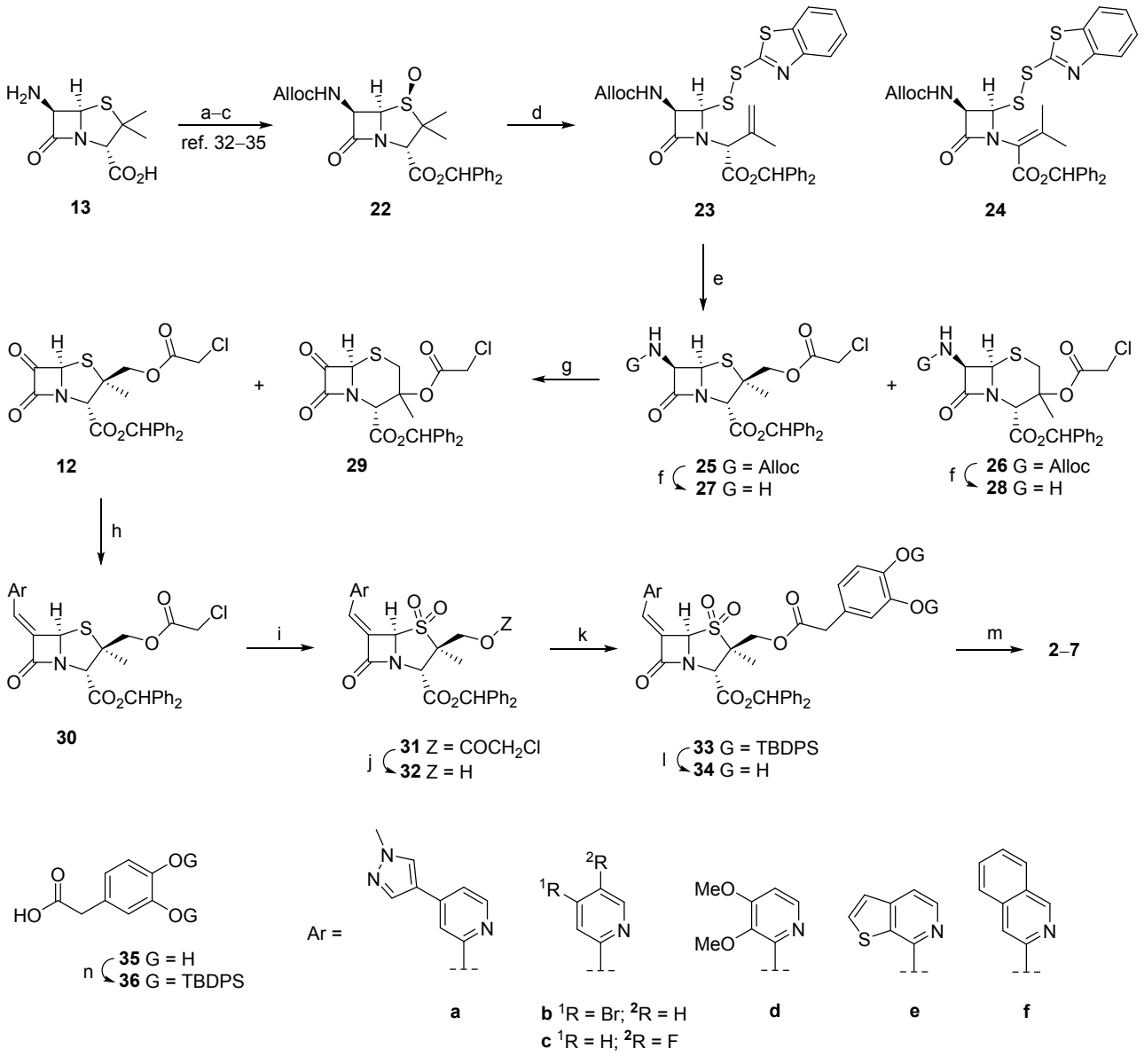


Scheme 2. Synthesis of phosphonium salts **8**–**11**. *Reagents and conditions.* (a) $Pd(PPh_3)_4$, 1-methylpyrazole-4-boronic acid pinacol ester, dioxane, K_3PO_4 (aq), 85 ºC. (b) 1. Ac_2O, 90 ºC; 2. KOH, MeOH, 0 ºC to RT. (c) $SOCl_2$, DCM, 0 ºC to RT. (d) Ph_3P, PhMe, Δ. (e) NBS, AIBN, CCl_4, Δ.

The synthesis of target compounds **2**–**7** was carried out as outlined in Scheme 3. Firstly, sulfoxide **22** was prepared in three steps from commercially available (+)-6-aminopenicillanic acid (**13**) following previously reported protocols that involved: (i) esterification of the carboxylic acid in **13** by treatment with diphenyldiazomethane;[39,40] (ii) protection of the free amino group with allyl chloroformate in pyridine; and (iii) oxidation of the sulfide moiety with peracetic acid.[38] Sulfoxide **22** was then transformed into disulfide **23** following the strategy described by Kamiya *et al*.[43] This transformation involves a thermal sigmatropic

rearrangement of sulfoxide **22** and subsequent trapping of the sulfenic acid intermediate with 2-mercaptobenzothiazole. However, when this reaction was carried out in toluene, as reported, in addition to **23** the formation of the undesired thermodynamic alkene **24** was also observed. Moreover, the use of other solvents and mixtures thereof, as well as different reaction temperatures and reaction times, afforded complex reaction mixtures that were tedious to purify. Fortunately, the formation of the thermodynamic alkene **24** was avoided when the reaction was carried out in dioxane under reflux and in the presence of anhydrous $MgSO_4$. Under these conditions, disulfide **23** was obtained in 91% yield as the only reaction product and without the need for purification. Next, the cyclization of the disulfide **23** for the modification of the pro-*R* methyl group with an ester group was carried out following previously reported protocols by treatment with silver acetate and chloroacetic acid.[38] This provided a chromatographically inseparable mixture of the kinetic (five-membered, compound **25**) and thermodynamic (six-membered, compound **26**) compounds in a 2:1 ratio. Deprotection of the allylcarbamate group in the resulting cyclic compounds **25** and **26** provided a chromatographically inseparable mixture of the amines **27** and **28** in a 2:1 ratio. Buynak *et al*.[38,44] described the transformation of amine **27** (and its six-membered isomer **28**) into the corresponding ketone **12** (and its six-membered isomer **29**) through a sequence of two reactions: (i) the formation of the corresponding diazonium salt by treatment with isopropyl nitrite and catalytic amounts of trifluoroacetic acid; and (ii) oxidation of the crude reaction product with propylene oxide in the presence of catalytic amounts of rhodium octanoate. However, in our hands this protocol was poorly reproducible and in many cases involved the loss of large quantities of product. Fortunately, we found that treatment of the amine **27** (and its six-membered isomer **28**) with triflic anhydride and triethylamine and subsequent hydrolysis by treatment with dilute HCl provided ketone **12** (and its six-membered isomer **29**) in a reproducible manner. In addition, after the Wittig reaction between ketone **12** (and **29**) and the phosphonium salts **8**–**11** and 3-isoquinolylmethylphosphonium bromide it was possible to isolate the desired five-membered analogs **30** from their six-membered counterparts. Under these conditions – and in contrast to previously reported protocols – the subsequent use of mixtures of compounds was avoided in the remaining steps of the synthesis.

Oxidation of **30** with *meta*-chloroperbenzoic acid followed by deprotection of the chloroacetoxy group in the resulting sulfone **31** by treatment with thiourea and pyridine led to alcohols **32**. Esterification of **32** with the protected acid **36** using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) as a coupling agent in the presence of catalytic amounts of 4-*N*,*N*-dimethylaminopyridine provided esters **33** in good yields. Protected acid **36** was prepared in two steps from commercially available 3,4-dihydroxyphenylacetic acid (**35**). Finally, removal of the TBDPS protecting groups in **33** by treatment with TBAF/AcOH, followed by the elimination of the benzhydryl protecting group in the resulting catechols **34** by reaction with *m*-cresol and subsequent treatment with sodium bicarbonate, satisfactorily provided the target compounds **2**–**7**.



Scheme 3. Synthesis of compounds **2**–**7**. *Reagents, conditions and yields.* (a) Ph_2CN_2, CH_2Cl_2/MeOH, RT, 87%. (b) $ClCO_2Allyl$, Py, CH_2Cl_2, –10 °C, 93%. (c) $MeCO_3H$, CH_2Cl_2, –5 °C, 95%. (d) 2-

mercaptobenzothiazole, $MgSO_4$, dioxane, Δ, 91%. (e) $ClCH_2CO_2H$, AgOAc, AcOEt, RT. (f) Bu_3SnH, $Pd(PPh_3)_4$ (cat.), AcOH, DCM, RT, 86% from **23**. (g) 1. Tf_2O, Et_3N, DCM, –78 °C→0 °C. 2. Et_3N, –78 °C. 3. HCl (0.5 M). (h) **8**, **9a**, **9b**, **10**, **11** or (3-isoquinolyl)methyltriphenylphosphonium bromide[36], DCM, –78 °C or RT, 20–49% from a mixture of amines **27** and **28**. (i) MCPBA, DCM, RT, 70–81%. (j) Thiourea, Py, DMF, 0 °C→RT. (k) **36**, EDC, DMAP, DCM, –15 °C→RT, 37–73% from **31**. (l) TBAF, AcOH, THF, 0 °C, 66–88%. (m) 1. *m*-cresol, 50 °C. 2. $NaHCO_3$ or NH_4HCO_3, 27–66%. (n) 1. TBDPSCl, Im, DMF, RT. 2. LiOH (aq), THF, RT. 3. HCl (aq), 64%.

Susceptibility Studies – Seven bacterial strains of *A. baumannii*, *K. pneumoniae*, and *E. coli* were employed. These strains do not have a mechanism of resistance other than that caused by the enzymatic hydrolysis of the β-lactam antibiotic and they were selected because they are widely distributed in priority pathogens. Specifically, in this work three bacterial strains that produce CHDL enzymes (OXA-23, OXA-24/40 and OXA-48), two strains that harbor class A enzymes (TEM-1 and CTX-M-2) and two strains that carry class C enzymes (CMY-2 and DHA-1) were employed. All of the bacterial strains and plasmids used in this study are summarized in Table 1. With the exception of *E. coli* TG1 bacterial strains that harbor TEM-1 and CTX-M-2, which are described herein, all of the strains were coded in previous studies.

Table 1. Bacterial strains and plasmids used in this study

Strains	Description	Reference or source
A. baumannii ATCC 17978	*A. baumannii* reference strain completely sequenced. Carbapenem-susceptible strain (genbank code CP000521.1).	ATCC[a]
A. baumannii ATCC 17978 (pET-RA-KmR+OXA-24/40)	*A. baumannii* ATCC 17978 strain carrying pET-RA+KmR plasmid encoding $bla_{OXA-24/40}$ gene. Carbapenem-resistant strain.	28
A. baumannii ATCC 17978 (pET-RA-KmR+OXA-23)	*A. baumannii* ATCC 17978 strain carrying pET-RA+KmR plasmid encoding bla_{OXA-23} gene.	28

	Carbapenem-resistant strain.	
K. pneumoniae Δ*ompK*35/36	CSUB10R *K. pneumoniae* strain without porins OmpK35 and OmpK36. β-Lactam-resistant strain.	55
K. pneumoniae Δ*ompK*35/36 (pBGS18+OXA-48)	*K. pneumoniae* Δ*ompK35/36* strain carrying pBGS18 plasmid encoding $bla_{\text{OXA-48}}$ gene. Increased β-lactam resistance.	56
E. coli TG1	*E. coli* reference strain completely sequenced. Protease deficient. It is suitable for transformation and protein expression. Antimicrobial-susceptible strain (genbank code U00096.3).	ATCC[a]
E. coli TG1 (pBGS18/TEM-1)	*E. coli* TG1 strain with cloned pBGS18 plasmid encoding bla_{TEM-1} gene. Penicillin-resistant strain.	This study
E. coli TG1 (pBGS18/CTX-M-2)	*E. coli* TG1 strain with cloned pBGS18 plasmid encoding $bla_{\text{CTX-M-2}}$ gene. β-Lactam-resistant strain except to carbapenems.	This study
E. coli TG1 (pBGS18+CMY-2)	*E. coli* TG1 strain with cloned pBGS18 plasmid encoding $bla_{\text{CMY-2}}$ gene. β-Lactam-resistant strain except to carbapenems.	57
E. coli TG1 (pBGS18+DHA-1)	*E. coli* TG1 strain with cloned pBGS18 plasmid encoding $bla_{\text{DHA-1}}$ gene. β-Lactam-resistant strain except to carbapenems.	58
E. coli BL21	*E. coli* reference strain completely sequenced. Antimicrobial-susceptible strain (genbank code CP001509.3).	ATCC[a]
E. coli BL21 (pGEX-6p-1+OXA-24/40)	*E. coli* BL21 strain carrying pGEX-6p-1 plasmid encoding $bla_{OXA-24/40}$ gene. Strain used for expression and purification of OXA-24/40 β-lactamase.	28

Plasmids	Description	Reference or source
pBGS18	Plasmid carrying *E. coli* replication origin. Encodes kanamycin resistance.	59
pET-RA-KmR	Plasmid carrying *A. baumannii* replication origin. Encodes rifampicin and kanamycin resistance.	60
pGEX-6P-1	Plasmid carrying *E. coli* replication origin. Bacterial expression plasmid. GST fusion vector. Encodes ampicillin resistance.	GE Healthcare

[a]ATCC = American Type Culture Collection

The susceptibilities to ampicillin, ceftazidime and imipenem with class A, C and D β-lactamases, respectively, in the absence of and in combination with compounds **2**−**7** at fixed concentrations of 16 μg mL^{-1} were determined. The enhancing effects of the reported inhibitors were also compared with that obtained with compound **1** and two well-known non-penicillin-based sulfone inhibitors, namely the DBOs avibactam and relebactam. The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) of each antibiotic and antibiotic/inhibitor combination, with quoted results being the mean of three independent replicates, following CLSI criteria.[45] MICs were defined as the lowest concentration of each combination that completely inhibited visible growth on plates. The results are summarized in Table 2.

Table 2. MIC values (μg mL^{-1}) for imipenem, ceftazidime and ampicillin with *A. baumannii* ATCC 17978, *K. pneumoniae ΔompK* 35/36 and *E. coli* TG1 pBGS18 carrying representative β-lactamases of classes D (OXA-23, OXA-24/40 and OXA-48), A (TEM-1 and CTX-M-2), and C (CMY-2 and DHA-1), in the presence and absence of β-lactamase inhibitors **1**−7, avibactam and relebactam.[a]

Bacterial strains	*A. baumannii* ATCC17978 + pET-RA-KmR /OXA-23	*A. baumannii* ATCC17978 + pET-RA-KmR /OXA-24/40	*K. pneumoniae ΔompK* 35/36 + pBGS18/OXA-48	*E. coli* TG1 + pBGS18/TEM-1	*E. coli* TG1 + pBGS18/CTX-M-2	*E. coli* TG1 + pBGS18/CMY-2	*E. coli* TG1 + pBGS18/ DHA-1
Antibiotic[b]	IMP	IMP	IMP	AMP	AMP	CTZ	CTZ
No inhibitor	8	64	64	>1024	>1024	32	32
Avibactam[c]	4	16	NA	NA	NA	NA	NA
Relebactam[c]	8	64	8	4	16	≤ 0.25	≤ 0.25
1[c]	0.5	0.5	4	2	16	≤ 0.25	≤ 0.25
2[c]	1	1	8	2	16	≤ 0.25	≤ 0.25
3[c]	1	1	4	2	16	≤ 0.25	≤ 0.25
4[c]	1	1	4	2	32	≤ 0.25	≤ 0.25
5[c]	4	8	64	2	256	≤ 0.25	≤ 0.25
6[c]	2	8	64	2	256	≤ 0.25	≤ 0.25
7[c]	2	2	8	4	256	2	2
Wild-type strains without β-lactamase	0.5	0.5	0.5	2	2	≤ 0.25	≤ 0.25

[a]Data represent the means of three independent experiments. [b]IMP = imipenem; AMP = ampicillin; CTZ = ceftazidime. [c]Inhibitor concentration = 16 μg mL^{-1}. NA = Not applicable (avibactam shows antimicrobial activity against *Enterobacteriaceae*).

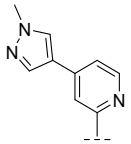
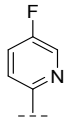
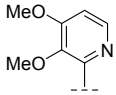
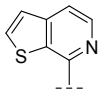
The results of these studies revealed that the combination ceftazidime/compounds **2**−**6** restored completely the efficacy of ceftazidime to levels similar to that of the parent compound **1** and relebactam, and provided MIC values of ≤ 0.25 μg mL^{-1} in *E. coli* producing class C (CMY-2 and DHA-1). Under similar conditions the combination ceftazidime/compound **7** proved to be much less efficient, with a MIC value of 2 μg mL^{-1}. A similar trend was observed with the combination ampicillin/compounds **2**−**6** in *E. coli* producing TEM-1, which recovered completely the ampicillin efficacy (MIC = 2 μg mL^{-1}) to the level of compound **1**, while a two-fold decrease in the *in vitro* activity was observed with the combination ampicillin/compound **7** (MIC = 4 μg mL^{-1}). Moreover, the combinations ampicillin/**2** and ampicillin/**3** in *E. coli* strains producing the CTX-M-2 enzyme provided a 64-fold decreases in the MIC value of the antibiotic, in a similar way to relebactam and compound **1**.

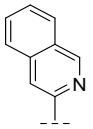
The results of the susceptibility studies against bacterial strains producing CHDLs enzymes (OXA-23, OXA-24/40 and OXA-48) revealed that combinations of imipenem with the reported compounds were in most cases more efficient than those with avibactam and relebactam, which generally did not show a significant antibiotic enhancing effect. The results against *A. baumannii* strains carrying enzymes OXA-23 and OXA-24/40 revealed that the combinations of imipenem with compounds **2**, **3** and **4** restored almost completely the activity of the antibiotic, with MIC values in all cases of 1 μg mL^{-1}. For OXA-48-producing *K. pneumoniae* strains the reported compounds are less efficient than for the *A. baumannii* strains producing OXA-23 and OXA-24/40. The best *in vitro* results were obtained with the combinations of imipenem with compounds **3** and **4**, with MIC values in both cases of 4 μg mL^{-1} −*i.e.* similar to that of the parent compound **1**.

Kinetics and Inhibition Studies – The inhibitory properties of compounds **2**–**7** against OXA-24/40 from the *A. baumannii* enzyme were tested as described previously.[28] The inhibition data are provided in Table 3. The best inhibitor in the series was compound **3**, with an improved efficacy (k_{inact}/K_I) of approximately 1.6 times relative to the parent compound **1** (Table 3, entries 1 *vs* 3). Thus, compound **3** had the highest affinity for the enzyme, which led to an almost two-fold decrease in the inhibition constant (K_I = 140 nM) relative to

1 and the same k_{inact} value as **1** (22 ms^{-1}). Methylpyrazole derivative **2** showed a slightly improved efficacy and this was mainly due to its higher conversion (k_{inact} = 24 ms^{-1}) by the enzyme (Table 3, entries 1 *vs* 2). Compound **4** was the inhibitor that reacted most rapidly with the enzyme (k_{inact} = 25 ms^{-1}) and it had the lowest affinity of the series (K_I = 495 nM). However, compound **4** was also the worst recognized by OXA-24/40. This situation meant that the efficiency of **4** was only half that of the parent compound. The efficacy of compound **5** was approximately 15% lower than that of the parent compound **1**, mainly due to its lower affinity (K_I = 276 nM) for the enzyme but also because of its faster conversion (24 ms^{-1}) (Table 3, entries 1 *vs* 5). The ligands that contained large pyridine-based heterocycles (**6** and **7**) showed quite different behavior (Table 3, entries 6 and 7). For example, whereas compound **6** had good affinity for the enzyme, with a reaction rate around 50% slower than the other analogs of the series, only slow reaction with the enzyme was observed with compound **7**.

Table 3. Inhibition kinetics of OXA-24/40 from *A. baumannii* by inhibitors **1**−**7**

Entry	Inhibitor	Ar	K_I (nM)	k_{inact} (ms^{-1})	k_{inact}/K_I (M^{-1} s^{-1})
1	**1**		234 ± 48	22 ± 2	98225 ± 32157
2	**2**		217 ± 19	24 ± 6	109731 ± 27375
3	**3**		140 ± 26	22 ± 4	159036 ± 1403
4	**4**		495 ± 95	25 ± 11	48903 ± 13560
5	**5**		276 ± 120	24 ± 13	83233 ± 11298
6	**6**		247 ± 167	11 ± 5	47564 ± 15036

7	**7**		ND	ND	ND

ND = not determined.

Taken together, it seems that there is no correlation between the nucleophilicity of the pyridine nitrogen atom and the inactivation rate, since either the introduction of electron-withdrawing (fluoro) or electron-donating (methoxy, heterocycle) groups in the *para*- or *meta*-positions of the ring afforded in all cases higher or equal k_{inact} constants to the parent compound **1**. In contrast, the substitution seems to have a more important role in: (i) the affinity of the ligand for the enzyme, (ii) the formation of the enzyme/inhibitor complex and/or (iii) the stability of the corresponding indolizine adduct. Moreover, comparison of the results for the inhibitory efficacy of ligands **2**−**4** against the enzyme OXA-24/40 from *A. baumannii* with the aforementioned *in vitro* data for the combinations imipenem/**2**−**4**, which afforded MIC values of 1 μg/mL in all cases (64-fold decrease), suggests that the bacterial permeability of compounds **2** and **3** is lower than that of compound **4**. Incubation studies, mass spectrometry detection of the corresponding enzyme adducts and docking studies were performed in order to gain an insight into the ligand mechanism of action and binding interactions responsible for ligands efficiency. The results of these studies are discussed below.

UV-Vis Spectroscopy and Mass Spectrometry Studies – The formation of the indolizine derivatives of the reported ligands was analyzed by monitoring the methanolysis of compounds **2**−**7** by UV-Vis spectroscopy after treatment with 1 equivalent of NaOMe (1 M in MeOH) at 25 ºC for 15 min (Figure S1). At the beginning, the formation of a band centered at 295 nm (for **3**) in the UV spectra was observed and this corresponds to the conjugated imine intermediate (Figure 2C). This band rapidly decreased as two new bands centered at 253 nm and 408 nm (for **3**) appeared due to the formation of the indolizine skeleton as confirmed by the mass spectra of the reaction mixture.[34]

In order to find evidences for the covalent modification mechanism, compounds **2**−**7** were incubated with OXA-48 and OXA-24/40 enzymes in 50 mM TRIS.HCl, 150 mM NaCl, 1 mM EDTA buffer at pH 7.0 and

25 °C for 30 min in a 1:100 enzyme/ligand ratio. During this period, the activity of the enzyme was progressively determined by UV-Vis spectroscopy using aliquots from the incubation samples and the control using nitrocefin as substrate. After incubation, enzymatic activity was barely detected. The samples were filtered, washed (5 mM ammonium bicarbonate) and concentrated using Amicon® centrifugal filters prior to analysis by mass spectrometry (MALDI). The mass spectra revealed the formation of stable inactivation adducts and each contained a peak for the covalently modified enzyme with an increase in mass of between 360 and 502, which corresponds to the indolizine adduct with loss of the catechol group or SO_2 depending on the particular case (Figure S2).

Binding mode of ligands 2−7 with OXA-24/40. **Enzyme/ligand complexes** – The binding modes of compounds **2**−**7** in the active site of the β-lactamase OXA-24/40 from *A. baumannii* were analyzed by docking (GOLD 5.2.2 program) using the available coordinates of the crystallographically determined OXA-24/40 covalently modified by a derivative of **1** in which position 4 of the pyridine moiety is substituted by the -$NHCO_2Me$ group (PDB ID 3FV7,[26] 2.0 Å). With the exception of compound **7**, all of the compounds were anchored to the enzyme active site in a similar arrangement for nucleophilic attack of the catalytic serine. This anchoring involved a set of strong electrostatic and polar interactions similar to those established by the reference compound **1** (Figure 3). In all cases, the catechol group seems to have great flexibility and wide variability of arrangements, as identified by docking. For ligands **2**−**6**, the carbonyl group of the lactam ring is located very close to the catalytic serine, Ser81, and this group was in an appropriate arrangement for nucleophilic attack, which triggers the enzymatic reaction (Figures 3A and 3C). In these cases, the estimated distance was around 2.8 Å, as measured between heavy atoms (carbon atom of the β-lactam carbonyl group and oxygen atom of the side chain of Ser81). In contrast, the β-lactam carbonyl group of ligand **7** was located further from the catalytic serine (~3.4 Å) since its isoquinoline ring was deeply embedded in the tunnel-like entrance of the OXA-24/40 active site − a situation that might explain its lower activity (Figures 3B and 3C).

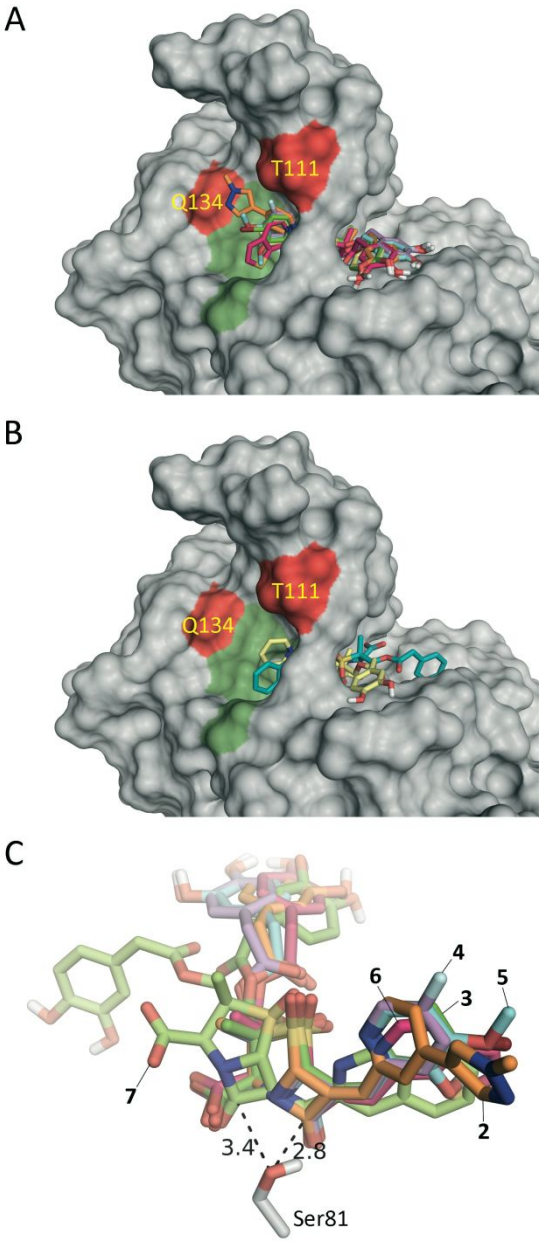


Figure 3. Binding modes of ligands **1**−**7** obtained by docking in the active site of the OXA-24/40 from *A. baumannii* enzyme. A. Comparison of the overall binding mode of ligands **1** (yellow), **2** (orange), **3** (green), **4** (violet), **5** (cyan) and **6** (pink). The pyridyl moieties are located at the entrance of the active center, involving mainly apolar residues (green, Val130, Leu168, Gly224 and Trp115), and flanked by two polar residues (red, Gln134 and Thr111). B. Comparison of the overall binding mode of ligands **1** (yellow) and **7** (dark cyan). C. Comparison of the relative position of ligands **2**−**7** to the catalytic serine (Ser81).

The pocket that forms the entrance to the active site, in which the pyridine-based moieties of ligands **2**−**7** are located, is mainly apolar and specifically involves residues Val130, Leu168, Gly225, and Trp115 (green,

Figures 3A−B). This pocket is flanked by two polar residues that are suitable for hydrogen bonding interactions, namely residues Gln134 and Thr111 (red, Figures 3A−B). A hydrogen-bonding interaction between the 1-methylpyrazole moiety in **2** and the amide side chain of residue Gln134, which is absent from **1**, seems to enhance the binding and fix the arrangement of the pyridine ring in the pocket – a disposition that might explain the lower K_I and higher k_{inact} for **3** than for the parent compound **1** (Figure S3). In contrast, the close proximity of the fluoro-substituent in **4** to the hydroxyl group in the side chain of Thr111 would disfavor the binding, as reflected by the two-fold decrease in affinity of compound **4** *vs* **1** (Figure S3). The binding mode of compound **3** revealed that the substituent of the pyridine ring (Br) does not appear to interact with the enzyme since it is located in the position *para* to the nitrogen atom. However, this electron-withdrawing group would reduce the electron density of the ring and this may enhance the CH-π interactions with the side chains of the apolar residues that surround it, specifically Leu168, Val130, Trp115, Tyr112, Met223 and the methyl group of Thr111 (Figures S3 and S4). This favorable interaction of the aromatic ring with the pocket proved to be very relevant, since the introduction of electron-rich pyridine-based moieties, as in compounds **5** and **6**, led to a decrease in the inhibitor enzyme affinity of up to two-fold.

CONCLUSIONS

6-Arylmethylidene penicillin-based sulfones **2**−**7**, analogs of the known penicillin-based sulfone β-lactamase inhibitor **1**, were synthesized from commercially available (+)-6-aminopenicillanic acid (**13**) by a Wittig reaction between the ketone **12** and arylphosphonium salts **8**−**12** as a key step. The results of the susceptibility studies of ampicillin against *E. coli* strains producing class A β-lactamases (TEM-1, CTX-M-2), ceftazidime against *E. coli* strains producing class C enzymes (CMY-2, DHA-1), imipenem against *A. baumannii* strains harboring CHDL enzymes (OXA-23, OXA-24/40) and *K. pneumoniae* strains expressing OXA-48, in the absence and in the presence of compounds **2**−**7** along with avibactam and relebactam, revealed that: (i) the combinations ceftazidime/**2**-**6** and ampicillin/**2**−**6** restored completely the antibiotic efficacy in strains harboring classes C (CMY-2, DHA-1) and A (TEM-1) enzymes to give similar results as the parent compound **1**; (ii) lower efficacy was obtained with the combination ampicillin/**2**−**6** in strains producing CTX-M-2, in particular, a 64-fold improvement in the antibiotic MIC value was achieved with

inhibitors **2** and **3**, as found for relebactam and compound **1**; (iii) the combination of imipenem and compounds **2**−**7** for bacterial strains carrying the studied CHDL enzymes proved to be, in most cases, more efficient than when using avibactam and relebactam, which in most cases did not show a significant antibiotic enhancing effect. In this latter case, the best *in vitro* results were obtained using compounds **3** and **4**. Mass spectrometry (MALDI) studies carried out on the CHDL enzymes, OXA-24/40 and OXA-48, revealed that the reported penicillin-based sulfone inhibitors **2**−**7** form stable inactivation adducts with these β-lactamase enzymes.

The inhibition data for compounds **2**–**7** with the *A. baumannii* OXA-24/40 enzyme revealed that compound **3** was the best inhibitor of the series, with an improved efficacy of approximately 1.6 times relative to the parent compound **1**, with a K_I of 140 nM and a k_{inact} of 22 ms^{-1}. The kinetic results in combination with docking studies on the corresponding OXA-24/inhibitor binary complexes also suggested that the substitution and the electron density of the pyridine moiety do not appear to have a significant effect on the inactivation rate. On the contrary, the latter two factors would have a more important role in ligand affinity and/or the stability of the corresponding indolizine adduct. Thus, the higher affinity of compound **3** is thought to be due to the reduced electron density of its pyridine ring, which in turn would enhance CH–π interactions with the side chains of the apolar residues of the tunnel-like entrance that surround it.

EXPERIMENTAL SECTION

General. All starting materials and reagents were commercially available and were used without further purification. ^{1}H NMR spectra (250, 300 and 500 MHz), ^{13}C NMR spectra (63, 75 and 125 MHz), ^{31}P NMR spectra (202 MHz) and ^{19}F NMR spectra (282 MHz) were measured in deuterated solvents. *J* values are given in Hertz. NMR assignments were carried out by a combination of 1 D, COSY, and DEPT-135 experiments. FT-IR spectra were recorded in a PerkinElmer Two FTIR spectrometer with attenuated total reference. $[\alpha]_D^{20}$ = values are given in deg mL g^{-1} dm^{-1}. Milli-Q deionized water was used in all the buffers. Melting points were measured in a Büchi M-560 apparatus. The spectroscopic UV-Vis measurements were made on a Varian Cary 100 UV-Vis spectrophotometer with a 1 cm pathlength cell fitted with a Peltier temperature controller. Protein analysis was performed using a MALDI TOF/TOF Mass Spectromether

(4800 Analyzer, AbSciex). The ProteoMass™ MALDI calibration kit (Merck) was employed for calibration and sinapic acid as a matrix. Protein spectra were analyzed using the Data Explorer™ Software. The purity of compounds **2**–**8** was analyzed by HPLC and by NMR. HPLC was performed on a Thermo Dionex UltiMate 3000 apparatus having a Brucker amazon SL mass spectrometry detector, using a Phenomenex kinetex XB-C18 column (particle size = 1.7 μm; dimensions: 50 mm × 2.1 mm, pore size = 100 Å), and eluting at a flow rate of 0.35 mL min^{-1} with a gradient of 5−75% B in 10 min [A = Milli-Q water + 0.1% TFA; B = acetonitrile + 0.1% TFA]. All tested compounds have a purity ≥ 95%.

2-Methyl-4-(1-methyl-1*H*-pyrazol-4-yl)pyridine (15) – A solution of 2-methyl-4-bromopyridine (**14**) (1 g, 5.81 mmol) in dioxane (29 mL) under inert atmosphere, was treated with tetrakis(triphenylphosphine)palladium(0) (134 mg, 0.12 mmol), aqueous solution of potassium phosphate (10.3 mL, 0.8 M), and 1-methylpyrazole-4-boronic acid pinacol ester (1.45 g, 7 mmol). The resulting mixture was heated at 85 °C for 12 h. After cooling to room temperature, the reaction mixture was diluted with a (1:1) dichloromethane/water, the organic layer was separated, dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography, eluting with a gradient of methanol/ethyl acetate [1): (0:100); 2) (10:90)], to give the compound **15** (1.12 g, quant.) as a white foam. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.35 (d, *J* = 5.3 Hz, 1H, ArH), 7.76 (d, *J* = 0.7 Hz, 1H, ArH), 7.65 (s, 1H, ArH), 7.14 (br s, 1H, ArH), 7.07 (dd, *J* = 1.8 and 5.3 Hz, 1H, ArH), 3.87 (s, 3H, NCH_3) and 2.48 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.7 (C), 149.5 (CH), 140.3 (C), 137.0 (CH), 127.9 (CH), 120.6 (C), 119.2 (CH), 117.0 (CH), 39.2 (CH_3), and 24.4 (CH_3) ppm. MS (ESI) *m/z* = 174 (MH^+). HRMS calcd for $C_{10}H_{12}N_3$ (MH^+): 174.1026; found, 174.1025.

[4-(1-Methyl-1*H*-pyrazol-4-yl)pyridin-2-yl]methanol (16) – A solution of compound **15** (655 mg, 3.46 mmol) in acetic anhydride (6.6 mL) was heated at 90 ^{0}C for 12 h. After cooling to room temperature, the solvent was removed under reduced presure and the resulting residue was dissolved in methanol (4.3 mL). The solution was cooled to 0 °C, treated with KOH (291 mg, 5.2 mmol) and stirred at room temperature for 1 h. Ethyl acetate and saturated solution of $NaHCO_3$ was added to the reaction mixture. The organic layer was separated, dried (Na_2SO_4 anh.), and concentrated under reduced pressure. The resulting residue was

purified by flash chromatography on silica gel, eluting with a gradient of methanol/ethyl acetate/hexane: [1) (0:90:10); 2) (0:100:0); 3) (10:90:0)], to give the alcohol **16** (257 mg, 40%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.34 (d, *J* = 5.2 Hz, 1H, ArH), 7.76 (s, 1H, ArH), 7.67 (s, 1H, ArH), 7.37 (s, 1H, ArH), 7.14 (d, *J* = 4.4 Hz, 1H, ArH), 5.12 (br s, 1H, OH), 4.71 (s, 2H, CH_2) and 3.85 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.6 (C), 148.9 (CH), 141.0 (C), 137.1 (CH), 128.2 (CH), 120.4 (C), 118.4 (CH), 116.6 (CH), 64.4 (CH_2), and 39.2 (CH_3) ppm. FTIR (ATR) ν: 3154 (OH) cm^{-1}. MS (ESI) *m/z* = 190 (MH^+). HRMS calcd for $C_{10}H_{12}N_3O$ (MH^+): 190.0975; found, 190.0974.

2-(Chloromethyl)-4-(1-methyl-1*H*-pyrazol-4-yl)pyridine (17) – A solution of the alcohol **16** (394 mg, 2.08 mmol) in dry dichloromethane (10.4 mL), under inert atmosphere and at 0 ^{0}C, was treated with a solution of freshly distilled thionyl chloride in dry dichloromethane (4.7 mL, 1.4 M). The ice bath was removed, and the reaction mixture was stirred at room temperature for 12 h. Saturated aqueous solution of Na_2CO_3 was then added, the organic layer was separated, dried (Na_2SO_4 anh.) and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel, eluting with (5:95) methanol/ethyl acetate, to afford the chloride **17** (297 mg, 69%) as a pink oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.42 (d, *J* = 5.2 Hz, 1H, ArH), 7.79 (s, 1H, ArH), 7.70 (s, 1H, ArH), 7.44 (s, 1H, ArH), 7.21 (d, *J* = 5.2 Hz, 1H, ArH), 4.60 (s, 2H, CH_2) and 3.87 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 156.9 (C), 149.8 (CH), 141.3 (C), 137.1 (CH), 128.1 (CH), 120.1 (C), 119.0 (CH), 118.7 (CH), 46.8 (CH_2) and 39.3 (CH_3) ppm. MS (ESI) *m/z* = 208 (MH^+). HRMS calcd for $C_{10}H_{11}ClN_3$ (MH^+): 208.0636; found, 208.0635.

4-[(1-Methyl-1*H*-pyrazol-4-yl)pyrid-2-yl]methyltriphenylphosphonium chloride (8) – A solution of the chloride **17** (296 mg, 1.43 mmol) in toluene (6.5 mL) was treated with triphenylphosphine (449 mg, 1.71 mmol) and heated under reflux for 12 h. After cooling to room temperature, the solvent was concentrated under reduced pressure, diethyl ether was added, and the resulting precipitate was filtered. The phosphonium salt **8** (245 mg, 40%) was obtained as a pink solid. Mp: 297−298 ^{0}C. ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 8.27 (s, 1H, ArH), 8.24 (d, *J* = 5.3 Hz, 1H, ArH), 7.87−7.82 (m, 10H, 10×ArH), 7.73−7.69 (m, 6H, 6×ArH), 7.60 (s, 1H, ArH), 7.40 (td, *J* = 1.4 and 5.3 Hz, 1H, ArH), 5.54 (d, *J* = 15.5 Hz, 2H, CH_2) and 3.87 (s, 3H, CH_3) ppm. ^{13}C NMR (125 MHz, DMSO-*d6*) δ: 150.9 (d, *J* = 8 Hz, C), 149.4 (CH), 141.0 (C), 136.7 (CH),

134.6 (d, J = 2 Hz, 3×CH), 133.9 (d, J = 10 Hz, 6×CH), 129.8 (d, J = 13 Hz, 6×CH), 129.5 (CH), 121.1 (d, J = 8 Hz, CH), 119.6 (C), 119.0 (C), 118.7 (CH), 118.4 (C), 39.4 (CH_3) and 31.1 (d, J = 51 Hz, CH_2) ppm. MS (ESI) *m/z* = 434 (MH−Cl). HRMS calcd for $C_{28}H_{25}N_3P$ (MH−Cl): 434.1781; found, 434.1781.

(3,4-Dimethoxypyrid-2-yl)methyltriphenylphosphonium chloride (**9a**) – A solution of 2-chloromethyl-3,4-dimethoxypyridine hydrochloride (**18a**) (1 g, 4.50 mmol) in distilled water (15 mL) was treated with potassium carbonate (622 mg, 4.5 mmol) and then stirred at room temperature for 15 min. The aqueous solution was extracted with diethyl ether (×3). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting orange oil was dissolved, under inert atmosphere and at room temperature, in dry toluene (20.5 mL) and treated with triphenylphosphine (1.43 g, 5.4 mmol). The reaction mixture was heated under reflux 24 h. After cooling to room temperature, the solvent was removed under reduced pressure and the resulting solid residue was suspended in diethyl ether and filtered. The phosphonium salt **9a** (1.53 g, 82%) was obtained as a white solid. Mp: 217 °C (dec.). ^{1}H NMR (500 MHz, DMSO-d*6*) δ: 7.86 (d, J = 5.5 Hz, 1H, ArH), 7.84–7.80 (m, 9H, 9×ArH), 7.71–7.67 (m, 6H, 6×ArH), 7.02 (d, J = 5.5 Hz, 1H, ArH), 5.42 (d, J = 14.5 Hz, 2H, CH_2P), 3.84 (s, 3H, OCH_3) and 3.75 (s, 3H, OCH_3) ppm. ^{13}C NMR (125 MHz, DMSO-d*6*) δ: 158.2 (C), 144.7 (CH), 143.7 (C), 143.6 (d, J_{C-P} = 7 Hz, C), 134.3 (d, J_{C-P} = 3 Hz, 3×CH), 133.7 (d, J_{C-P} = 10 Hz, 6×CH), 129.7 (d, J_{C-P} = 13 Hz, 6×CH), 119.9 (d, J_{C-P} = 88 Hz, 3×C), 108.6 (CH), 60.5 (OCH_3), 56.1 (OCH_3) and 26.5 (d, J_{C-P} = 57 Hz, CH_2P) ppm. ^{31}P NMR (202 MHz, DMSO-d*6*) δ: 26.6 (s, 1P) ppm. MS (ESI) *m/z* = 414 (M–Cl). HRMS calcd for $C_{26}H_{25}NO_2P$ (M–Cl): 414.1617; found: 414.1607.

(4-Bromopyrid-2-yl)methyltriphenylphosphonium bromide (9b) – A solution of bromide **18b**[44] (505 mg, 2.02 mmol) in toluene (9.2 mL) was treated with triphenylphosphine (636 mg, 2.42 mmol) and heated under reflux for 12 h. After cooling to room temperature, the solvent was concentrated under reduced pressure, diethyl ether was added and the resulting precipitate was filtered. The phosphonium salt **9b** (534 mg, 52%) was obtained as a pink solid. Mp: 245−247 °C. ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 8.24 (d, J = 5.4 Hz, 1H, ArH), 7.87−7.80 (m, 9H, 9×ArH), 7.74−7.70 (m, 6H, 6×ArH), 7.62 (br s, 1H, ArH), 7.54 (td, J = 1.7 and 5.4

Hz, 1H, ArH) and 5.55 (d, J = 15.5 Hz, 2H, CH_2Ar) ppm. ^{13}C NMR (75 MHz, DMSO-*d6*) δ: 152.2 (d, J = 8 Hz, C), 150.3 (C), 134.8 (3×CH), 133.9 (d, J = 10 Hz, 6×CH), 132.8 (CH), 129.9 (d, J = 13 Hz, 6×CH), 128.6 (d, J = 8 Hz, CH), 126.0 (CH), 118.9 (d, J = 87 Hz, 3×C) and 30.6 (d, J = 51 Hz, CH_2) ppm. MS (ESI) *m/z* = 432 and 434 (M−Br). HRMS calcd for $C_{24}H_{20}NP^{79}Br$ (M−Br): 432.0511; found, 432.0511.

2-(Bromomethyl)-5-fluoropyridine (20) – A solution of 5-fluoro-2-methylpyridine (**19**) (150 mg, 1.35 mmol) in dry carbon tetrachloride (3.1 mL) and under inert atmosphere, was treated with *N*-bromosuccinimide (385 mg, 2.16 mmol) and AIBN (22 mg, 0.14 mmol). The resulting mixture was heated under reflux for 2 h. After cooling to room temperature, the reaction mixture was filtered and concentrated under reduced pressure. The crude residue was purified by flash chromatography on silica gel, eluting with (10:90) diethyl ether/hexane, to give the bromide **20** (129 mg, 50%) as a pink oil. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.39 (d, J = 2.6 Hz, 1H, ArH), 7.45−7.34 (m, 2H, 2×ArH) and 4.52 (s, 2H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.9 (d, $J_{C–F}$ = 265 Hz, C), 152.9 (d, $J_{C–F}$ = 4 Hz, C), 138.0 (d, $J_{C–F}$ = 24 Hz, CH), 124.6 (d, $J_{C–F}$ = 5 Hz, CH), 123.8 (d, $J_{C–F}$ = 19 Hz, CH) and 32.7 (CH_2) ppm. MS (CI) *m/z* = 190 and 192 (M^+). HRMS calcd for $C_6H_5{}^{79}BrNF$ (M^+): 189.9662; found, 189.9663.

(5-Fluoropyrid-2-yl)methyltriphenylphosphonium bromide (**10**) – A solution of bromide **20** (30 mg, 0.16 mmol) in toluene (0.7 mL) was treated with triphenylphosphine (50 mg, 0.2 mmol) and heated under reflux for 12 h. After cooling to room temperature, the solvent was concentrated under reduced pressure. The resulting residue was suspended in diethyl ether and the resulting precipitate was filtered. The phosphonium salt **10** (64 mg, 89%) was obtained as a white solid. ^1H NMR (500 MHz, DMSO-*d6*) δ: 8.38 (d, J = 3.0 Hz, 1H, ArH), 7.87−7.66 (m, 16H, 16×ArH), 7.40 (dd, J = 4.2 and 8.5 Hz, 1H, ArH) and 5.51 (d, J = 15.6 Hz, 2H, CH_2Ar) ppm. ^{13}C NMR (125 MHz, DMSO-*d6*) δ: 158.4 (dd, J = 3 and 253 Hz, C), 146.7 (dd, J = 4 and 9 Hz, C), 137.4 (dd, J = 2 and 24 Hz, CH), 134.8 (d, J = 3 Hz, 3×CH), 133.9 (d, J = 10 Hz, 6×CH), 129.9 (d, J = 13 Hz, 6×CH), 127.2 (dd, J = 5 and 8 Hz, CH), 124.5 (d, J = 19 Hz, CH), 118.9 (d, J = 87 Hz, 3×C) and 30.0 (d, J = 51 Hz, CH_2) ppm. ^{19}F NMR (282 MHz, DMSO-*d6*) δ: −128.0 (t, $J_{F–H}$ = 4 Hz, 1F) ppm. ^{31}P NMR (202 MHz, DMSO-*d6*) δ: 27.0 (s, 1P) ppm. MS (ESI) *m/z* = 372 (M−Br). HRMS calcd for $C_{24}H_{20}NPF$ (M−Br): 372.1312; found, 372.1313.

Triphenyl(thieno[2,3-*c*]pyridin-7-ylmethyl)phosphonium bromide (11) – A solution of the bromide **21**[42] (272 mg, 1.2 mmol) in toluene (5.6 mL) was treated with triphenylphosphine (378 mg, 1.4 mmol) and then heated under reflux for 12 h. After cooling to room temperature, the solvent was concentrated under reduced pressure and diethyl ether was added. The resulting white precipitate was filtered to give the phosphonium salt **11** (327 mg, 56%) as a white solid. Mp: 277–278 ºC. ^{1}H NMR (500 MHz, DMSO-*d6*) δ: 8.21 (d, *J* = 5.4 Hz, 1H, ArH), 8.09 (d, *J* = 5.5 Hz, 1H, ArH), 7.92 (dd, *J* = 7.1 and 14.6 Hz, 6H, 6×ArH), 7.81 (t, *J* = 6.6 Hz, 3H, 3×ArH), 7.75 (d, *J* = 5.5 Hz, 1H, ArH), 7.69 (m, 6H, 6×ArH), 7.59 (d, *J* = 5.3 Hz, 1H, ArH), and 5.99 (d, *J* = 14.8 Hz, 2H, CH_2Ar) ppm. ^{13}C NMR (125 MHz, DMSO-*d6*) δ: 145.6 (C), 145.5 (d, J_{C-P} = 7 Hz, C), 141.5 (CH), 135.4 (d, J_{C-P} = 9 Hz, C), 134.3 (d, J_{C-P} = 2 Hz, 2×CH), 134.0 (CH), 133.8 (d, J_{C-P} = 10 Hz, 6×CH), 129.7 (d, J_{C-P} = 13 Hz, 6×CH), 123.7 (CH), 120.0 (d, J_{C-P} = 88 Hz, 3×C), 117.5 (CH) and 30.3 (d, J_{C-P} = 58 Hz, CH_2) ppm. ^{31}P NMR (202 MHz, DMSO-*d6*) δ: 26.56 (s, 1P) ppm. MS (ESI) *m/z* = 410 (M−Br). HRMS calcd for $C_{26}H_{21}NPS$ (M−Br): 410.1115; found, 410.1127.

Compound 23 – A solution of sulfoxide **22**[38,39,40,41] (1 g, 2.07 mmol) in dry dioxane (8.3 mL) was treated with anhydrous $MgSO_4$ (1 g, 8.31 mmol) and 2-mercaptobenzothiazole (378 mg, 2.26 mmol). The resultant yellow suspension was heated at 110 ºC for 14 h. After cooling to room temperature, the solid was removed by filtration over Celita®. The filtrate and the washings were concentrated under reduced pressure. The resultant residue was dissolved in diethyl ether, treated with activated charcoal and stirred for 30 min. The suspension was filtered through a plug of Celita®. The filtrate and the washings (diethyl ether) were concentrated under reduced pressure to give disulfide **23**[38,44] (1.2 g, 91%), as a pale yellow foam. Compound **23** was converted into amine **27** (and its six-membered analog **28**) without further purification following previously reported protocol.[38,44]

Compound 30a – A solution of the amines **27** and **28** (2:1 molar ratio, respectively) (621 mg, 1.31 mmol) in dry dichloromethane (6.6 mL), under inert atmosphere and at –78 °C, was treated dropwise with dry triethylamine (0.6 mL, 3.93 mmol). After 5 min stirring, trifluoromethanesulfonic anhydride was added dropwise (0.7 mL, 3.93 mmol) during 5 min. The reaction mixture was warmed up to 0 °C during a 1 h period, and then was cooled again to –78 °C. Dry triethylamine (0.6 mL, 3.93 mmol) was added dropwise

during 10 min. The resulting mixture was stirred for 30 min and then treated with cold HCl (20 mL, 0.5 M). After 10 min, the organic layer was separated, and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with cold HCl (3×10 mL, 0.5 M), dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure to give the diketone **12** and its six-membered analog **29** (2:1 molar ratio, respectively). The latter mixture of ketones was used for the subsequent Wittig reaction without further purification. A solution of compound **8** (673 mg, 1.31 mmol) in dry THF (5.2 mL), under argon atmosphere and at room temperature, was treated with KO^{t}Bu (146 mg, 1.31 mmol) and then stirred for 2 h. The resultant solution, at room temperature and under inert atmosphere, was added *via canula* to a solution of the previously obtained diketones **12** and **29** (2:1 molar ratio, respectively) (620 mg, 1.31 mmol) in dry dichloromethane (10 mL). After 35 min stirring, saturated solution of NH_4Cl was added and the resultant suspension was stirred for 10 min. The organic layer was separated, and the aqueous layer was extracted with dichloromethane. The combined organic extracts were successively washed with water and brine, dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography, eluting with ethyl acetate/hexane: 1) (55:45); 2) (70:30), to give the pyridine **30a** (244 mg, 30%) as a yellow foam. $[\alpha]_D^{20}$ = +285.1 (*c*1.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.56 (d, *J* = 5.0 Hz, 1H, ArH), 7.85 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.44−7.28 (m, 12H, 12×ArH), 6.98 (s, 1 H, CH=), 6.94 (s, 1H $CHPh_2$), 6.32 (s, 1H, H5), 4.99 (s, 1H, H2), 4.22 (d, *J* = 11.7 Hz, 1H, OC*H*H), 4.16 (d, *J* = 15.0 Hz, 1H, C*H*HCl), 4.09 (d, *J* = 15.0 Hz, 1H, CH*H*Cl), 3.97 (s, 3H, CH_3), 3.93 (d, *J* = 11.7 Hz, 1H, OCH*H*), and 1.23 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.2 (C), 166.9 (C), 166.7 (C), 152.8 (C), 150.8 (CH), 145.4 (C), 141.2 (C), 139.2 (C), 139.2 (C), 137.2 (CH), 128.7 (4×CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 127.5 (2×CH), 127.2 (2×CH), 124.5 (CH), 122.8 (CH), 120.0 (C), 119.6 (CH), 78.5 (CH), 72.0 (OCH_2), 70.6 (CH), 65.7 (CH), 64.1 (C), 40.8 (CH_2), 39.4 (CH_3) and 20.5 (CH_3) ppm. FTIR (ATR) ν: 1756 (CO) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 629 (MH^+). HRMS calcd for $C_{33}H_{30}N_4ClO_5S$ (MH^+): 629.1620; found, 629.1619.

Compound 30b − It was prepared according to the procedure described for compound **30a** using: (i) for the preparation of the diketone: amines **27** and **28** (2:1 molar ratio, respectively) (220 mg, 0.46 mmol) in dry

dichloromethane (2.3 mL), dry triethylamine (0.19 mL, 1.38 mmol) and trifluoromethanesulfonic anhydride (0.23 mL, 1.38 mmol) in dry triethylamine (0.19 mL, 1.38 mmol) and cold HCl (1 mL, 0.5 N); (ii) for the Wittig reaction: **9a** (236 mg, 0.46 mmol) in dry THF (1.8 mL), KO^{t}Bu (52 mg, 0.46 mmol), diketones **12** and **29** (2:1 molar ratio, respectively) (220 mg, 0.46 mmol) in dry dichloromethane (3.5 mL). Eluent for chromatography: 1) (1:99) ethyl acetate/dichloromethane; 2) (40:60) diethyl ether/hexane. Yield = 125 mg (43%). White solid. $[\alpha]_D^{20}$ = +318.2 (*c*0.9, $CHCl_3$). Mp: 67−68 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.43 (s, 1H, ArH), 7.53 (s, 1H, ArH), 7.41−7.36 (m, 11H, 11×ArH), 6.98 (s, 1H, C*H*Ph_2), 6.86 (s, 1H, CH=), 6.28 (s, 1H, H5), 4.99 (s, 1H, H2), 4.21 (d, *J* = 11.6 Hz, 1H, OC*H*H), 4.09 (m, 2H, CH_2Cl), 3.91 (d, *J* = 11.6 Hz, 1H OCH*H*) and 1.23 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.6 (C), 166.8 (C), 166.7 (C), 153.6 (C), 150.9 (CH), 147.1 (C), 139.2 (C), 139.2 (C), 133.5 (C), 129.5 (CH), 128.7 (4×CH), 128.5 (CH), 128.3 (CH), 127.6 (2×CH), 127.2 (2×CH), 126.8 (CH), 122.9 (CH), 78.6 (CH), 72.0 (OCH_2), 70.5 (CH), 65.8 (CH), 64.3 (C), 40.8 (CH_2) and 20.5 (CH_3) ppm. FTIR (ATR) ν: 1766 (CO) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 627 (MH^+). HRMS calcd for $C_{29}H_{25}N_2BrClO_5S$ (MH^+): 627.0351; found, 627.0353.

Compound 30c − It was prepared according to the procedure described for compound **30a** using: (i) for the preparation of the diketone: amines **27** and **28** (2:1 molar ratio, respectively) (720 mg, 1.53 mmol) in dry dichloromethane (7.8 mL), dry triethylamine (0.63 mL, 4.56 mmol) and trifluoromethanesulfonic anhydride (0.78 mL, 4.56 mmol) in dry triethylamine (0.63 mL, 4.56 mmol) and cold HCl (3 mL, 0.5 N); (ii) for the Wittig reaction: **10** (693 mg, 1.53 mmol) in dry THF (6.0 mL), KO^{t}Bu (171 mg, 1.53 mmol), diketones **12** and **29** (2:1 molar ratio, respectively) (720 mg, 1.53 mmol) in dry dichloromethane (11.7 mL). Eluent for chromatography: 1) (1:99) ethyl acetate/dichloromethane, 2) (50:50) diethyl ether/hexane. Yield = 200 mg (23%). Yellow foam. $[\alpha]_D^{20}$ = +317.3 (*c*1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.50 (s, 1H, ArH), 7.53−7.32 (m, 12H, 12×ArH), 6.98 (s, 1H, C*H*Ph_2), 6.92 (s, 1H, CH=), 6.27 (s, 1H, H5), 4.99 (s, 1H, H2), 4.21 (d, *J* = 11.6 Hz, 1H, OC*H*H), 4.15 (d, *J* = 15.0 Hz, 1H, C*H*HCl), 4.08 (d, *J* = 15.0 Hz, 1H, CH*H*Cl), 3.92 (d, *J* = 11.6 Hz, 1H, OCH*H*) and 1.24 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.1 (C), 167.0 (C), 166.7 (C), 159.2 (d, J_{C-F} = 256 Hz, C), 148.6 (d, J_{C-F}= 4 Hz, C), 145.3 (d, J_{C-F} = 2 Hz, C), 139.5 (C), 139.4 (d, J_{C-F} = 24 Hz, CH), 139.2 (C), 128.7 (3×CH), 128.5 (CH), 128.4 (CH), 127.6 (2×CH), 127.5

(CH), 127.3 (2×CH), 123.3 (d, J_{C-F} = 19 Hz, CH), 123.0 (CH), 78.6 (CH), 72.0 (OCH_2), 70.5 (CH), 65.7 (CH), 64.4 (C), 40.8 (CH_2) and 20.6 (CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ: −124.2 (s, 1F) ppm. FTIR (ATR) ν: 1765 (CO) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 589 (MNa^+). HRMS calcd for $C_{29}H_{24}N_2ClFO_5SNa$ (MNa^+): 589.0971; found, 589.0969.

Compound 30e − It was prepared according to the procedure described for compound **30a** using: (i) for the preparation of the diketone: amines **27** and **28** (2:1 molar ratio, respectively) (227 mg, 0.48 mmol) in dry dichloromethane (2.4 mL), dry triethylamine (0.2 mL, 1.44 mmol), trifluoromethanesulfonic anhydride (0.24 mL, 1.44 mmol) in dry triethylamine (0.2 mL, 1.44 mmol) and cold HCl (1 mL, 0.5N); (ii) for the Wittig reaction: **11** (235 mg, 0.48 mmol) in dry THF (1.9 mL), KO^{t}Bu (54 mg, 0.48 mmol), diketones **12** and **29** (2:1 molar ratio, respectively) (228 mg, 0.48 mmol) in dry dichloromethane (3.7 mL). Eluent for chromatography: 1. (1:98) ethyl acetate/dichloromethane; 2. (50:50) diethyl ether/hexane. Yield = 142 mg (49%). White foam. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.54 (d, *J* = 5.3 Hz, 1H, ArH), 7.71 (d, *J* = 5.4 Hz, 1H, ArH), 7.65 (d, *J* = 5.3 Hz, 1H, ArH), 7.38 (m, 11H, 11×ArH), 7.27 (s, 1H, CH=), 6.98 (s, 1H, C*H*Ph_2), 6.42 (s, 1H, H5), 5.04 (s, 1H, H2), 4.24 (d, *J* = 11.7 Hz, 1H, OC*H*H), 4.16 (d, *J* = 14.5 Hz, 1H, C*H*HCl), 4.10 (d, *J* = 14.5 Hz, 1H, CH*H*Cl), 3.96 (d, *J* = 11.7 Hz, 1H, OCH*H*) and 1.26 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.9 (C), 166.9 (C), 166.7 (C), 146.9 (C), 146.6 (C), 146.1 (C), 143.8 (CH), 139.3 (C), 139.2 (C), 138.0 (C), 131.8 (CH), 128.7 (4×CH), 128.4 (CH), 128.3 (CH), 127.6 (2×CH), 127.3 (2×CH), 123.7 (CH), 121.1 (CH), 118.1 (CH), 78.5 (CH), 72.0 (OCH_2), 70.8 (CH), 65.8 (CH), 64.2 (C), 40.8 (CH_2) and 20.6 (CH_3) ppm. IR (ATR) ν: 1762 (CO) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 605 (MH^+). HRMS calcd for $C_{31}H_{26}N_2ClO_5S_2$ (MH^+): 605.0966; found, 605.0966.

Compound 30f − It was prepared according to the procedure described for compound **30a** using: (i) for the preparation of the diketone: amines **27** and **28** (2:1 molar ratio, respectively) (150 mg, 0.32 mmol) in dry dichloromethane (1.6 mL), dry triethylamine (0.13 mL, 0.96 mmol) and triflic anhydride (0.16 mL, 0.96 mmol) in dry triethylamine (0.13 mL, 0.96 mmol) and cold HCl (1 mL, 0.5 N); (ii) for the Wittig reaction: (3-isoquinolyl)methyltriphenylphosphonium bromide[37] (139 mg, 0.32 mmol) in dry THF (1.3 mL), KO^{t}Bu (36 mg, 0.32 mmol), diketones **12** and **29** (2:1 molar ratio, respectively) (150 mg, 0.32 mmol) in dry

dichloromethane (2.5 mL). Eluent for chromatography: (50:50) diethyl ether/hexane. Yield = 51 mg (27%). Yellow foam. $[\alpha]_D^{20}$ = +383.2 (*c*1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.22 (s, 1H, ArH), 7.98 (d, *J* = 7.8 Hz, 1H, ArH), 7.85 (d, *J* = 8.0 Hz, 1H, ArH), 7.75−7.64 (m, 3H, 3×ArH), 7.46−7.30 (m, 10 H, 10×ArH), 7.09 (s, 1H C*H*Ph_2), 7.00 (br s, 1H, CH=), 6.43 (s, 1H, H5), 5.02 (s, 1H, H2), 4.23 (d, *J* = 11.6 Hz, 1H, OC*H*H), 4.16 (d, *J* = 15.0 Hz, 1H, C*H*HCl), 4.09 (d, *J* = 15.0 Hz, 1H, CH*H*Cl), 3.95 (d, *J* = 11.6 Hz, 1H OCH*H*) and 1.25 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.6 (C), 167.1 (C), 166.7 (C), 153.1 (C), 146.4 (C), 144.1 (C), 139.3 (C), 139.3 (C), 135.8 (C), 131.1 (CH), 128.7 (CH), 128.7 (4×CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 127.8 (CH), 127.5 (2×CH), 127.3 (2×CH), 127.1 (CH), 125.2 (CH), 124.4 (CH), 78.5 (CH), 72.1 (OCH_2), 70.9 (CH), 65.8 (CH), 64.4 (C), 40.8 (CH_2) and 20.6 (CH_3) ppm. FTIR (ATR) ν: 1759 (CO) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 599 (MH^+). HRMS calcd for $C_{33}H_{28}N_2ClO_5S$ (MH^+): 599.1402; found, 599.1399.

Compound 31a – A solution of the compound **30a** (154 mg, 0.24 mmol) in dry dichloromethane (1.6 mL), under inert atmosphere and at room temperature, was treated with *m*-chloroperbenzoic acid (85 mg, 0.49 mmol, 77%) and stirred for 40 min. Saturated aqueous solution of Na_2SO_3 was then added and the reaction mixture was stirred for 5 min. The aqueous layer was separated and the organic layer was succesively washed with saturated $NaHCO_3$, water and brine. The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane: 1) (65:35); 2) (75:25), to afford the sulfone **31a** (87 mg) and the corresponding sulfoxide of **30a** (45 mg). A solution of the latter compound (45 mg) in dry dichloromethane (0.5 mL), under argon and at room temperature, was oxidized to **31a** by treatment with *m*-chloroperbenzoic acid (14 mg, 0.08 mmol). After 12 h stirring, saturated solution of Na_2SO_3 was added. After 5 min stirring, the aqueous layer was separated and the organic layer was washed successively with saturated $NaHCO_3$, water and brine. The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel, eluting with (65:35) ethyl acetate/hexane, to give **31a** (28 mg). Yield = 115 mg (72%). White foam. $[\alpha]_D^{20}$ = +219.4 (*c*1.1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.52 (d, *J* = 5.0 Hz, 1H, ArH), 7.81 (s, 1H, ArH), 7.74 (s, 1H, ArH), 7.38−7.31

(m, 12H, 12×ArH), 7.24 (s, 1 H, CH=), 7.00 (s, 1H $CHPh_2$), 5.78 (s, 1H, H5), 4.79 (s, 1H, H2), 4.71 (d, *J* = 12.2 Hz, 1H, OC*H*H), 4.52 (d, *J* = 12.2 Hz, 1H, OCH*H*), 4.11 (d, *J* = 15.5 Hz, 1H, C*H*HCl), 4.05 (d, *J* = 15.5 Hz, 1H, CH*H*Cl), 3.93 (s, 3H, CH_3) and 1.25 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.6 (C), 166.5 (C), 166.1 (C), 151.3 (C), 150.9 (CH), 141.5 (C), 138.7 (C), 138.6 (C), 137.2 (CH), 133.4 (C), 130.4 (CH), 128.9 (2×CH), 128.8 (3×CH), 128.5 (CH), 128.4 (CH), 127.6 (2×CH), 127.1 (2×CH), 122.7 (CH), 120.7 (CH), 119.8 (C), 79.5 (CH), 73.8 (CH), 66.0 (C), 64.9 (OCH_2), 59.7 (CH), 40.6 (CH_2), 39.4 (CH_3) and 15.8 (CH_3) ppm. FTIR (ATR) ν: 1778 (CO) and 1752 (CO) cm^{-1}. MS (ESI) *m/z* = 661 (MH^+). HRMS calcd for $C_{33}H_{30}N_4ClO_7S$ (MH^+): 661.1518; found, 661.1516.

Compound 31b – It was prepared according to the procedure described for compound **31a** using: (i) for the first oxidation: **30b** (328 mg, 0.52 mmol), *m*-chloroperbenzoic acid (234 mg, 1.05 mmol), dry dichloromethane (3.5 mL). Eluent for chromatography: (50:50) diethyl ether/hexane; (ii) for the oxidation of the obtained sulfoxide of **30b** (116 mg, 0.18 mmol) to sulfone **31b**, *m*-chloroperbenzoic acid (49 mg, 0.22 mmol) in dry dichloromethane (1.2 mL). Eluent for chromatography: (50:50) diethyl ether/hexane. Reaction time = 30 min. Yield = 242 mg (70%). Yellow foam. $[\alpha]_D^{20}$ = +231.9 (*c*1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.49 (d, *J* = 5.1 Hz, 1H, ArH), 7.56 (d, *J* = 1.5 Hz, 1H, ArH), 7.49 (d, *J* = 1.8 and 5.2 Hz, 1H, ArH), 7.38−7.30 (m, 10H, 10×ArH)), 7.20 (d, *J* = 1.2 Hz, 1H, CH=), 7.00 (s, 1H, C*H*Ph_2), 5.74 (d, *J* = 1.3 Hz, 1H, H5), 4.79 (s, 1H, H2), 4.69 (d, *J* = 12.2 Hz, 1H, OC*H*H), 4.50 (d, *J* = 12.2 Hz, 1H, OCH*H*), 4.11 (d, *J* = 15.2 Hz, 1H, C*H*HCl), 4.04 (d, *J* = 15.2 Hz, 1H, CH*H*Cl) and 1.23 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.0 (C), 166.5 (C), 166.0 (C), 152.0 (C), 151.1 (CH), 138.7 (C), 138.6 (C), 135.2 (C), 133.8 (C), 129.4 (CH), 128.9 (2×CH), 128.8 (3×CH), 128.6 (2×CH), 128.2 (2×CH), 127.6 (2×CH), 127.1 (2×CH), 79.5 (CH), 73.8 (CH), 66.0 (C), 65.0 (OCH_2), 59.9 (CH), 40.6 (CH_2) and 15.8 (CH_3) ppm. FTIR (ATR) ν: 1783 (CO), 1755 (CO) and 1748 (CO) cm^{-1}. MS (ESI) *m/z* = 659 and 661 (MH^+). HRMS calcd for $C_{29}H_{25}N_2{}^{79}BrClO_7S$ (MH^+): 659.0249; found, 659.0250.

Compound 31c – It was prepared according to the procedure described for compound **31a** using: (i) for the first oxidation: **30c** (170 mg, 0.30 mmol), *m*-chloroperbenzoic acid (134 mg, 0.60 mmol), dry dichloromethane (2.0 mL). Eluent for chromatography: (60:40) diethyl ether/hexane; (ii) for the oxidation of

the obtained sulfoxide of **30c** (71 mg, 0.12 mmol) to sulfoxide **31c**, *m*-chloroperbenzoic acid (32 mg, 0.14 mmol) in dry dichloromethane (0.8 mL). Eluent for chromatography: (60:40) diethyl ether/hexane. Reaction time = 30 min. Yield = 146 mg (81%). Yellow foam. $[\alpha]_D^{20}$ = +289.8 (*c*0.9, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.54 (d, *J* = 1.8 Hz, 1H, ArH), 7.43–7.32 (m, 12H, 12×ArH), 7.26 (s, 1H, CH=), 7.00 (s, 1H, C*H*Ph_2), 5.72 (d, *J* = 1.3 Hz, 1H, H5), 4.79 (s, 1H, H2), 4.69 (d, *J* = 12.2 Hz, 1H, OC*H*H), 4.51 (d, *J* = 12.2 Hz, 1H, OCH*H*), 4.11 (d, *J* = 15.2 Hz, 1H, C*H*HCl), 4.05 (d, *J* = 15.2 Hz, 1H, CH*H*Cl) and 1.24 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.4 (C), 166.5 (C), 166.1 (C), 160.0 (d, $J_{C–F}$ = 261 Hz, C), 147.1 (d, $J_{C–F}$ = 4 Hz, C), 140.0 (d, $J_{C–F}$ = 25 Hz, CH), 138.7 (C), 138.6 (C), 133.4 (d, $J_{C–F}$ = 3 Hz, C), 128.9 (2×CH), 128.8 (3×CH), 128.6 (2×CH), 127.7 (2×CH), 127.6 (CH), 127.1 (2×CH), 123.6 (d, $J_{C–F}$ = 19 Hz, CH), 79.5 (CH), 73.7 (CH), 66.0 (C), 64.9 (OCH_2), 59.8 (CH), 40.6 (CH_2Cl) and 15.8 (CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ: − 121.9 (t, *J* = 6 Hz, 1F) ppm. FTIR (ATR) ν: 1781 (CO) and 1750 (CO) cm^{-1}. MS (ESI) *m/z* = 599 (MH^+). HRMS calcd for $C_{29}H_{25}N_2ClFO_7S$ (MH^+): 599.1050; found, 599.1046.

Compound 31d – Firstly, compound **30d** was prepared according to the procedure described for compound **30a** using: (i) for the ketone preparation: amines **27** and **28** (2:1 molar ratio, respectively) (3.9 g, 8.23 mmol) in dry dichloromethane (41 mL), triethylamine (2×3.5 mL) and trifluoromethanesulfonic anhydride (4.2 mL); (ii) for the Wittig reaction: the phosphonium salt **9b** (3.7 g, 8.23 mmol) in dry THF (33 mL), KO^{t}Bu (924 mg, 8.23 mmol) in dry dichloromethane (63 mL). Chromatography eluent: (70:30) diethyl ether/hexane. Yield = 1.2 g (20%). Yellow foam. Secondly, compound **30d** was oxidized to **31d** as follows: A solution of **30d** (490 mg, 0.81 mmol) in dry dichloromethane (5.4 mL), under inert atmosphere and at room temperature, was treated with *meta*-chloroperbenzoic acid (363 mg, 1.62 mmol, 77%) and stirred for 5 h. Dichloromethane and saturated solution of Na_2SO_3 was then added. The resultant mixture was stirred for 10 min, the aqueous layer was separated and the organic extract was successively washed with saturated $NaHCO_3$, water and brine. The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography, eluting with (2:98) ethyl acetate/dichloromethane, to give the sulfone **31d** (390 mg, 75%) as a white foam. $\mathbf{[\alpha]_D^{20}}$ = +280.7 (*c*1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.27 (d, *J* = 5.4 Hz, 1H, ArH), 7.70 (d, *J* = 1.5 Hz, 1H, CHAr),

7.41−7.31 (m, 10H, 10×ArH), 7.00 (s, 1H, $CHPh_2$), 6.82 (d, *J* = 5.4 Hz, 1H, ArH), 5.74 (d, *J* = 1.5 Hz, 1H, H5), 4.77 (s, 1H, H2), 4.71 (d, *J* = 12.0 Hz, OC*H*H), 4.50 (d, *J* = 12.0 Hz, OCH*H*), 4.10 (d, *J* = 15.3 Hz, C*H*H), 4.05 (d, *J* = 15.3 Hz, CH*H*), 3.90 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3) and 1.24 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.8 (C), 166.3 (C), 166.0 (C), 158.9 (C), 146.5 (CH), 146.2 (C), 144.5 (C), 138.6 (C), 138.5 (C), 132.9 (C), 128.7 (2×CH), 128.6 (2×CH), 128.3 (CH), 127.4 (2×CH), 126.9 (2×CH), 125.2 (CH), 108.8 (CH), 79.2 (CH), 73.8 (CH), 65.7 (C), 64.7 (CH_2), 61.8 (CH), 59.5 (OCH_3), 55.8 (OCH_3), 40.4 (CH_2) and 15.6 (CH_3) ppm. FTIR (ATR) ν: 1779 (CO), 1744 (CO) and 1773 (CO) cm^{-1}. MS (ESI) *m/z* = 641 (MH^+). HRMS calcd for $C_{31}H_{30}ClN_2O_9S$ (MH^+): 641.1355; found, 641.1355.

Compound 31e – It was prepared according to the procedure described for compound **31a** using: (i) for the first oxidation: **30e** (157 mg, 0.26 mmol), *m*-chloroperbenzoic acid (117 mg, 0.52 mmol) in dry dichloromethane (1.7 mL). Eluent for chromatography: (60:40) diethyl ether/hexane; (ii) for the oxidation of the obtained sulfoxide of **30e** (62 mg, 0.1 mmol) to **31e**, *m*-chloroperbenzoic acid (27 mg, 0.12 mmol) in dry dichloromethane (0.7 mL). Eluent for chromatography: (60:40) diethyl ether/hexane. Reaction time = 30 min. Yield = 128 mg (78%). White foam. $[\alpha]_D^{20}$ = +364.1 (*c*0.7, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.26 (s, 1H, ArH), 7.98 (d, *J* = 8.0 Hz, 1H, ArH), 7.85 (d, *J* = 8.0 Hz, 1H, ArH), 7.77 (s, 1H, ArH), 7.75−7.65 (m, 2H, 2×ArH), 7.43−7.33 (m, 11H, 10×ArH+CH=), 7.02 (s, 1H, C*H*Ph_2), 5.89 (s, 1H, H5), 4.80 (s, 1H, H2), 4.71 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.52 (d, *J* = 12.1 Hz, 1H, OCH*H*), 4.12 (d, *J* = 15.2 Hz, 1H, C*H*HCl), 4.06 (d, *J* = 15.2 Hz, 1H, CH*H*Cl) and 1.28 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.0 (C), 166.5 (C), 166.2 (C), 153.5 (C), 144.9 (C), 138.8 (C), 138.7 (C), 135.8 (C), 132.2 (C), 131.4 (CH), 130.9 (CH), 129.2 (CH), 129.0 (CH), 128.9 (2×CH), 128.8 (2×CH), 128.8 (CH), 128.6 (CH), 128.1 (CH), 127.7 (2×CH), 127.4 (CH), 127.2 (2×CH), 124.8 (CH), 79.5 (CH), 74.2 (CH), 66.0 (C), 65.1 (OCH_2), 59.7 (CH), 40.6 (CH_2Cl) and 15.9 (CH_3) ppm. FTIR (ATR) ν: 1779 (CO) and 1748 (CO) cm^{-1}. MS (ESI) *m/z* = 631 (MH^+). HRMS calcd for $C_{33}H_{28}N_2ClO_7S$ (MH^+): 631.1300; found, 631.1299.

Compound 31f – It was prepared according to the procedure described for compound **31a** using: **30f** (80 mg, 0.13 mmol) in dry dichloromethane (0.9 mL) and *m*-cloroperbenzoic acid (58 mg, 0.26 mmol). Reaction time = 30 min. Eluent for chromatography: (5:95) ethyl acetate/dichloromethane. Yield = 65 mg (78%).

Yellow foam. $[\alpha]_D^{20}$ = +47.5 (*c*1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.58 (d, *J* = 5.3 Hz, 1H, ArH), 7.71 (d, *J* = 5.5 Hz, 1H, ArH), 7.68 (d, *J* = 5.3 Hz, 1H, ArH), 7.59 (d, *J* = 1.3 Hz, 1H, CH=), 7.43–7.30 (m, 11H, 11×ArH), 7.02 (s, 1H, C*H*Ph_2), 5.85 (d, *J* = 1.3 Hz, H5), 4.83 (s, 1H, H2), 4.74 (d, *J* = 12.2 Hz, 1H, OC*H*H), 4.54 (d, *J* = 12.2 Hz, 1H, OCH*H*), 4.12 (d, *J* = 15.2 Hz, 1H, C*H*HCl), 4.06 (d, *J* = 15.1 Hz, 1H, CH*H*Cl) and 1.28 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.3 (C), 166.5 (C), 166.1 (C), 146.4 (C), 145.4 (C), 144.0 (C), 138.7 (C), 138.6 (C), 134.4 (C), 131.9 (CH), 128.9 (3×CH), 128.8 (3×CH), 128.5 (CH), 127.6 (2×CH), 127.1 (2×CH), 127.0 (CH), 123.7 (CH), 119.3 (CH), 79.5 (CH), 73.9 (CH), 66.0 (C), 64.9 (OCH_2), 59.8 (CH), 40.6 (CH_2Cl) and 15.8 (CH_3) ppm. FTIR (ATR) ν: 1777 (CO) and 1746 (CO) cm^{-1}. MS (ESI) *m/z* = 637 (MH^+). HRMS calcd for $C_{31}H_{26}N_2ClO_7S_2$ (MH^+): 637.0864; found, 637.0866.

3,4-Di(*tert*-butyldiphenylsilyloxy)phenylacetic acid (36) – A solution of 3,4-dihydroxyphenylacetic acid (**35**) (2 g, 11.9 mmol), imidazole (3.3 g, 47.6 mmol) and *tert*-butyldiphenylsilyl chloride (12.4 mL, 47.6 mmol) in dry DMF (24 mL), under inert atmosphere and at room temperature, was stirred for 96 h. The reaction mixture was diluted with a (1:4) mixture of ethyl acetate/water, the organic layer was separated and the aqueous layer was extracted with ethyl acetate (×3). The combined organic extracts were washed with HCl (10%), dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel, eluting with (30:70) diethyl ether/hexane, to afford a pink foam (10.4 g). The later foam was diluted in THF (119 mL) and treated with aqueous lithium hydroxide (60 mL, 0.5 M). The resultant solution was stirred at room temperature for 30 min, diluted with Milli-Q water and the THF was concentrated under reduced pressure. The resultant aqueous solution was acidified with HCl (10%) until pH 4 and then extracted with ethyl acetate (×3). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel, eluting with (50:50) diethyl ether/hexane, to give the acid **36**[45] (4.9 g, 64%) as a white foam.

Compound 33a – A solution of the ester **31a** (74 mg, 0.11 mmol) in dry DMF (0.14 mL), under inert atmosphere and at 0 °C, was treated with dry pyridine (0.06 mL, 0.62 mmol) and thiourea (26 mg, 0.34 mmol). The reaction mixture was stirred for 12 h allowing to reach room temperature. The reaction mixture

was diluted with a (1:4) mixture of ethyl acetate/water, the organic layer was separated and the aqueous layer was extracted with ethyl acetate (×5). The combined organic extracts were successively washed with water and brine, dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure to afford the alcohol **32a**, which was used in the next step without further purification. A solution of the acid **36** (72 mg, 0.11 mmol), *N*,*N*-4-dimethylaminopyridine (14 mg, 0.11 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (86 mg, 0.45 mmol) in dry dichloromethane (0.2 mL), under argon and at −15 °C, was treated with a solution of the alcohol **32a** (64 mg, 0.11 mmol) in dry dichloromethane (0.4 mL). The reaction mixture was stirred at this temperature for 30 min, at 0 °C for 1 h and was allowed to rich room temperature for 30 min. Ethyl acetate and H_2SO_4 (0.5 M) were added, the aqueous layer was separated and the organic layer was washed succesively with water and saturated $NaHCO_3$. The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/hexane: [1) (50:50); 2) (70:30)], to afford the compound **33a** (66 mg, 49%), as a pink foam. $[\alpha]_D^{20}$ = +133.3 (*c*1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.58 (d, *J* = 4.8 Hz, 1H, ArH), 7.85−7.76 (m, 10H, 10×ArH), 7.40−7.30 (m, 25H, 24×ArH+CH=), 6.98 (s, 1H, $CHPh_2$), 6.39 (d, *J* = 8.5 Hz, 1H, ArH), 6.36 (s, 1H, ArH), 6.26 (d, *J* = 8.0 Hz, 1H, ArH), 5.73 (s, 1H, H5), 4.63 (s, 1H, H2), 4.48 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.25 (d, *J* = 12.1 Hz, 1H, OCH*H*), 3.96 (s, 3H, CH_3), 3.09 (s, 2H, CH_2Cl), 1.18 (s, 9H, 3×CH_3), 1.15 (s, 9H, 3×CH_3) and 1.05 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.4 (C), 167.7 (C), 166.2 (C), 151.4 (C), 151.0 (C), 146.0 (C), 145.4 (C), 141.5 (C), 138.8 (C), 138.8 (C), 137.3 (C), 135.8 (4×CH), 135.7 (4×CH), 133.6 (C), 133.2 (C), 133.2 (C), 130.2 (CH), 130.0 (2×CH), 129.9 (2×CH), 128.9 (2×CH), 128.8 (2×CH), 128.7 (CH), 128.5 (CH), 128.3 (CH), 127.9 (10×CH), 127.7 (2×CH), 127.1 (2×CH), 125.6 (C), 122.6 (CH), 121.6 (2×CH), 120.8 (CH), 120.4 (CH), 119.9 (C), 79.4 (CH), 73.6 (CH), 66.3 (C), 63.3 (OCH_2), 60.0 (CH), 39.7 (CH_2), 39.5 (CH_3), 27.0 (3×CH_3), 26.8 (3×CH_3), 19.7 (C), 19.6 (C) and 15.6 (CH_3) ppm. FTIR (ATR) ν: 1784 (CO) and 1748 (CO) cm^{-1}. MS (ESI) *m/z* = 1211 (MH^+). HRMS calcd for $C_{71}H_{71}N_4O_9SSi_2$ (MH^+): 1211.4475; found, 1211.4473.

Compound 33b − It was prepared according to the procedure described for compound **33a** using: (i) for the ester hydrolysis: **31b** (242 mg, 0.37 mmol), dry pyridine (0.2 mL), and thiourea (79 mg, 1.1 mmol) in dry

DMF (0.5 mL); (ii) for the esterification: **36** (236 mg, 0.37 mmol), *N*,*N*-4-dimethylaminopyridine (45 mg, 0.37 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (281 mg, 1.5 mmol) in dry dichloromethane (0.6 mL), and **32b** (216 mg, 0.37 mmol) in dry dichloromethane (1.2 mL). Eluent for chromatography: (40:60) diethyl ether/hexane. Yield = 267 mg (60%). Yellow foam. $[\alpha]_D^{20}$ = +131.6 (*c*1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.48 (d, *J* = 5.2 Hz, 1H, ArH), 7.83−7.78 (m, 8H, 8×ArH), 7.54 (d, *J* = 1.5 Hz, 1H, ArH), 7.47−7.17 (m, 23H, 23×ArH), 7.17 (d, *J* = 1.1 Hz, 1H, CH=), 6.97 (s, 1H, C*H*Ph_2), 6.38 (d, *J* = 8.3 Hz, 1H, ArH), 6.35 (d, *J* = 2.0 Hz, 1H, ArH), 6.25 (dd, *J* = 2.1 and 8.3 Hz, 1H, ArH), 5.68 (d, *J* = 1.3 Hz, 1H, H5), 4.64 (s, 1H, H2), 4.45 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.22 (d, *J* = 12.1 Hz, 1H, OCH*H*), 3.01 (s, 2H, CH_2Cl), 1.18 (s, 9H, 3×CH_3), 1.15 (s, 9H, 3×CH_3) and 1.03 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.4 (C), 167.0 (C), 166.0 (C), 152.2 (C), 151.1 (C), 146.0 (C), 145.4 (C), 138.8 (C), 138.7 (C), 135.8 (4×CH), 135.7 (4×CH), 135.4 (C), 133.8 (C), 133.3 (2×C), 133.2 (C), 130.0 (2×CH), 129.9 (2×CH), 129.4 (CH), 128.9 (2×CH), 128.8 (2×CH), 128.8 (CH), 128.5 (CH), 128.4 (CH), 128.1 (CH), 127.9 (9×CH), 127.7 (2×CH), 127.1 (2×CH), 125.6 (C), 121.6 (2×CH), 120.4 (CH), 79.5 (CH), 73.6 (CH), 66.3 (C), 63.4 (OCH_2), 60.1 (CH), 39.7 (CH_2), 27.0 (3×CH_3), 26.8 (3×CH_3), 19.7 (C), 19.6 (C) and 15.6 (CH_3) ppm. FTIR (ATR) ν: 1788 (CO) and 1747 (CO) cm^{-1}. MS (ESI) *m/z* = 1209 and 1211 (MH^+). HRMS calcd for $C_{67}H_{66}N_2{}^{79}BrO_9Si_2S$ (MH^+): 1209.2844; found, 1209.3209.

Compound 33c − It was prepared according to the procedure described for compound **33a** using: (i) for the ester hydrolysis: **31c** (130 mg, 0.22 mmol), dry pyridine (0.1 mL), and thiourea (50 mg, 0.66 mmol) in dry DMF (0.3 mL); (ii) for the esterification: **36** (140 mg, 0.22 mmol), *N*,*N*-4-dimethylaminopyridine (27 mg, 0.22 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (166 mg, 0.87 mmol) in dry dichloromethane (0.5 mL), and **32c** (114 mg, 0.22 mmol) in dry dichloromethane (0.6 mL). Eluent for chromatography: (60:40) diethyl ether/hexane. Yield = 165 mg (66%). Yellow foam. $[\alpha]_D^{20}$ = +142.2 (*c*1.3, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.56 (d, *J* = 2.2 Hz, 1H, ArH), 7.84 (t, *J* = 6.1 Hz, 8H, 8×ArH), 7.47–7.28 (m, 24H, 24×ArH), 7.24 (d, *J* = 1.1 Hz, 1H, CH=), 7.00 (s, 1H, C*H*Ph_2), 6.43–6.39 (m, 2H, 2×ArH), 6.29 (dd, *J* = 2.0 and 8.3 Hz, 1H, ArH), 5.69 (d, *J* = 1.1 Hz, 1H, H5), 4.67 (s, 1H, H2), 4.50 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.26 (d, *J* = 12.1 Hz, 1H, OCH*H*), 3.12 (s, 2H, CH_2Ar), 1.21 (s, 9H, 3×CH_3), 1.18 (s,

9H, 3×CH_3) and 1.07 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.3 (C), 167.4 (C), 166.1 (C), 160.1 (d, $J_{C–F}$ = 260 Hz, C), 147.2 (d, $J_{C–F}$ = 4 Hz, C), 146.0 (2×C), 145.4 (2×C), 139.7 (d, $J_{C–F}$ = 25 Hz, CH), 138.8 (C), 138.7 (C), 135.7 (4×CH), 135.6 (4×CH), 133.6 (d, $J_{C–F}$ = 3 Hz, C), 133.2 (C), 133.1 (C), 130.0 (2×CH), 129.9 (2×CH), 128.9 (2×CH), 128.8 (2×CH), 128.7 (CH), 128.5 (CH), 128.4 (CH), 127.9 (8×CH), 127.6 (2×CH), 127.5 (d, $J_{C–F}$ = 5 Hz, CH), 127.1 (2×CH), 125.6 (C), 123.6 (d, $J_{C–F}$ = 19 Hz, CH), 121.6 (2×CH), 120.4 (CH), 79.4 (CH), 73.5 (CH), 66.3 (C), 63.4 (OCH_2), 60.1 (CH), 39.7 (CH_2Ar), 27.0 (3×CH_3), 26.8 (3×CH_3), 19.7 (C), 19.6 (C) and 15.5 (CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ: − 122.1 (s, 1F) ppm. FTIR (ATR) ν: 1786 (CO) and 1744 (CO) cm^{-1}. MS (ESI) *m/z* = 1149 (MH^+). HRMS calcd for $C_{67}H_{66}N_2FO_9SSi_2$ (MH^+): 1149.4006; found, 1149.4006.

Compound 32d – A solution of ester **31d** (111 mg, 0.17 mmol) in dry DMF (0.21 mL), under inert atmosphere and at 0 °C, was treated with dry pyridine (75 μL, 0.94 mmol) and thiourea (39 mg, 0.51 mmol). The resulting solution was stirred for 16 h at room temperature and then diluted with a mixture of ethyl acetate/water (1:4). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (×3). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure to give the alcohol **32d** (90 mg, 94%) as a yellow oil. $[\alpha]_D^{20}$ = +308.5 (*c*1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.28 (d, *J* = 5.4 Hz, 1H, ArH), 7.70 (d, *J* = 1.2 Hz, 1H, CHAr), 7.39–7.28 (m, 10H, 10×ArH), 7.01 (s, 1H, $CHPh_2$), 6.83 (d, *J* = 5.4 Hz, 1H, ArH), 5.69 (d, *J* = 1.2 Hz, 1H, H5), 5.22 (s, 1H, H2), 4.04 (d, *J* = 12.9 Hz, OC*H*H), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3) 3.79 (d, *J* = 12.9 Hz, OCH*H*), and 1.09 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.0 (C), 166.8 (C), 159.0 (C), 146.5 (CH), 146.2 (C), 144.7 (C), 138.9 (C), 138.7 (C), 133.0 (C), 128.7 (2×CH), 128.6 (2×CH), 128.2 (2×CH), 127.6 (2×CH), 126.8 (2×CH), 125.0 (CH), 108.8 (CH), 78.8 (CH), 73.8 (CH), 68.1 (C), 63.4 (CH_2), 61.8 (CH), 58.0 (OCH_3), 55.8 (OCH_3) and 15.7 (CH_3) ppm. FTIR (ATR) ν: 3351 (OH) and 1773 (CO) cm^{-1}. MS (ESI) *m/z* = 565 (MH^+). HRMS calcd for $C_{29}H_{29}N_2O_8S$ (MH^+): 565.1639; found: 565.1639.

Compound 33d – A solution of the acid **36** (103 mg, 0.16 mmol), *N*,*N*-4-dimethylaminopyridine (20 mg, 0.16 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (123 mg, 0.64 mmol) in dry dichloromethane (0.8 mL), under argon and at −15 °C, was treated with a solution of the alcohol **32d** (90

mg, 0.16 mmol) in dry dichloromethane (0.8 mL). The reaction mixture was stirred at this temperature for 30 min and at 0 °C for 1 h, and it was then allowed to rich room temperature for 30 min. Ethyl acetate was added, the aqueous layer was separated and the organic layer was washed succesively with H_2SO_4 (0.5 M), water and saturated $NaHCO_3$. The organic extract was dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography, eluting with (70:30) diethyl ether/hexane, to afford the compound **33d** (106 mg, 56%) as a yellow oil. $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ = +116.3 (*c*1.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.28 (d, *J* = 5.4 Hz, 1H, ArH), 7.83−7.78 (m, 10H, 10×ArH), 7.70 (d, *J* = 1.2 Hz, 1H, CH=), 7.00 (s, 1H, $CHPh_2$), 6.81 (d, *J* = 5.4 Hz, 1H, ArH), 6.38 (d, *J* = 8.1 Hz, 1H, ArH), 6.35 (d, *J* = 2.1 Hz, 1H, ArH), 6.25 (dd, *J* = 2.1 and 8.1 Hz, 1H, ArH), 5.68 (d, *J* = 1.5 Hz, 1H, H5), 4.61 (s, 1H, H2), 4.46 (d, *J* = 12.3 Hz, 1H, OC*H*H), 4.24 (d, *J* = 12.3 Hz, 1H, OCH*H*), 3.90 (s, 6H, 2×OCH_3), 3.08 (s, 2H, CH_2), 1.17 (s, 9H, $SiC(CH_3)_3$), 1.14 (s, 9H, $SiC(CH_3)_3$), and 1.03 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.2 (C), 168.0 (C), 166.1 (C), 159.0 (C), 146.6 (CH), 146.2 (C), 145.8 (C), 145.2 (C), 144.7 (C), 138.7 (C), 138.6 (C), 135.6 (5×CH), 135.5 (5×CH), 133.1 (2×C), 133.0 (2×C), 129.8 (2×CH), 128.7 (2×CH), 128.6 (2×CH), 128.5 (CH), 128.3 (CH), 127.7 (8×CH), 127.5 (2×CH), 126.9 (2×CH), 125.5 (C), 125.1 (CH), 121.4 (CH), 120.2 (CH), 108.8 (CH), 79.2 (CH), 73.4 (CH), 66.1 (C), 63.2 (CH_2), 61.8 (CH), 59.7 (OCH_3), 55.8 (OCH_3), 39.6 (CH_2) 26.8 ($SiC(CH_3)_3$), 26.6 ($SiC(CH_3)_3$), 19.5 ($SiC(CH_3)_3$), 19.4 ($SiC(CH_3)_3$), and 15.4 (CH_3) ppm. FTIR (ATR) ν: 1786 (CO) and 1748 (CO) cm^{-1}. MS (ESI) *m/z* = 1191 (MH^+). HRMS calcd for $C_{69}H_{71}N_2O_{11}SSi_2$ (MH^+): 1191.4312; found: 1191.4288.

Compound 33e – It was prepared according to the procedure described for compound **32a** using: (i) for the ester hydrolysis: **31e** (157 mg, 0.25 mmol), dry pyridine (0.14 mL), and thiourea (57 mg) in dry DMF (0.3 mL); (ii) for the esterification: **36** (161 mg, 0.25 mmol), *N*,*N*-4-dimethylaminopyridine (31 mg, 0.25 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (191 mg, 1.0 mmol) in dry dichloromethane (0.6 mL), and **32e** (138 mg, 0.25 mmol) in dry dichloromethane (0.7 mL). Eluent for chromatography: (60:40) diethyl ether/hexane. Yield = 216 mg (73%). White foam. $[\boldsymbol{\alpha}]_{\mathbf{D}}^{\mathbf{20}}$ = +176.6 (*c*1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.26 (s, 1H, ArH), 7.96 (d, *J* = 7.9 Hz, 1H, ArH), 7.86−7.64 (m, 12H, 12×ArH), 7.44−7.28 (m, 22H, 22×ArH), 7.26 (s, 1H, CH=), 6.99 (s, 1H, C*H*Ph_2), 6.40−6.36 (m, 2H,

2×ArH), 6.27 (dd, *J* = 1.8 and 8.3 Hz, 1H, ArH), 5.84 (s, 1H, H5), 4.65 (s, 1H, H2), 4.28 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.25 (d, *J* = 12.1 Hz, 1H, OCH*H*), 3.10 (s, 2H, CH_2Ar), 1.18 (s, 9H, 3×CH_3), 1.15 (s, 9H, 3×CH_3) and 1.08 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.4 (C), 168.1 (C), 166.3 (C), 153.5 (C), 146.0 (C), 145.3 (C), 145.0 (C), 138.9 (C), 138.8 (C), 135.8 (C), 135.8 (4×CH), 135.7 (4×CH), 133.2 (2×C), 133.2 (2×C), 132.4 (C), 131.3 (CH), 130.7 (CH), 130.0 (2×CH), 130.0 (2×CH), 129.1 (CH), 129.0 (CH), 128.9 (2×CH), 128.8 (2×CH), 128.7 (CH), 128.5 (CH), 128.1 (CH), 127.9 (8×CH), 127.7 (2×CH), 127.4 (CH), 127.1 (2×CH), 125.7 (C), 124.6 (CH), 121.6 (2×CH), 120.4 (CH), 79.4 (CH), 74.0 (CH), 66.2 (C), 63.5 (OCH_2), 60.0 (CH), 39.7 (CH_2Ar), 27.0 (3×CH_3), 26.8 (3×CH_3), 19.7 (C), 19.6 (C) and 15.6 (CH_3) ppm. FTIR (ATR) ν: 1783 (CO) and 1747 (CO) cm^{-1}. MS (ESI) *m/z* = 1181 (MH^+). HRMS calcd for $C_{71}H_{69}N_2O_9SSi_2$ (MH^+): 1181.4257; found, 1181.4260.

Compound 33f – It was prepared according to the procedure described for compound **33a** using: (i) for the ester hydrolysis: **31f** (572 mg, 0.9 mmol) in dry DMF (1.1 mL), pyridine (0.5 mL, 5.0 mmol) and thiourea (205.2 mg, 2.7 mmol); (ii) for the esterification: **36** (580 mg, 0.9 mmol), *N*,*N*-4-dimethylaminopyridine (110 mg, 0.90 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (690 mg, 3.60 mmol) in dry dichloromethane (2.3 mL), alcohol **32f** (504 mg, 0.9 mmol) in dry dichloromethane (2.2 mL). Eluent for chromatography: gradient of diethyl ether/hexane [1) (25:75); 2) (50:50)]. Yield = 400 mg (37%). White solid. $[\alpha]_D^{20}$ = +130.7 (*c*1.1, $CHCl_3$). Mp: 108–110 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.59 (d, *J* = 5.3 Hz, 1H, ArH), 7.79 (m, 8H, 8×ArH), 7.72 (d, *J* = 5.4 Hz, 1H, ArH), 7.69 (d, *J* = 5.3 Hz, 1H, ArH), 7.59 (d, *J* = 1.3 Hz, 1H, CH=), 7.42–4.24 (m, 23H, 23×ArH), 6.97 (s, 1H, C*H*Ph_2), 6.37 (d, *J* = 8.3 Hz, 1H, ArH), 6.34 (d, *J* = 2.1 Hz, 1H, ArH), 6.24 (dd, *J* = 2.1 and 8.3 Hz, 1H, ArH), 5.79 (d, *J* = 1.3 Hz, 1H, H5), 4.64 (s, 1H, H2), 4.48 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.24 (d, *J* = 12.1 Hz, 1H, OCH*H*), 3.08 (s, 2H, CH_2Ph), 1.16 (s, 9H, 3×CH_3), 1.13 (s, 9H, 3×CH_3) and 1.05 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.4 (C), 167.4 (C), 166.2 (C), 146.5 (C), 146.1 (C), 145.6 (C),145.4 (C), 144.1 (C), 138.8 (C), 138.7 (C), 137.9 (C), 135.8 (6×CH), 135.7 (6×CH), 134,7 (C), 133.3 (C), 133.2 (C), 131.9 (C), 130.0 (2×CH), 129.9 (2×CH), 128.9 (2×CH), 128.8 (2×CH), 128.7 (CH), 128.5 (CH), 127.9 (10×CH), 127.7 (2×CH), 127.1 (2×CH), 126.9 (CH), 125.6 (C), 123.8 (CH), 121.6 (2×CH), 120.4 (CH), 119.3 (CH), 79.5 (CH), 73.7 (CH), 66.4 (C), 63.3 (CH_2),

60.1 (CH), 39.8 (CH_2), 27.0 (3×CH_3), 26.8 (3×CH_3), 19.7 (C), 19.5 (C) and 15.6 (CH_3) ppm. FTIR (ATR) ν: 1784 (CO) and 1747 (CO) cm^{-1}. MS (ESI) *m/z* = 1187 (MH^+). HRMS calcd for $C_{69}H_{67}N_2O_9S_2Si_2$ (MH^+): 1187.3821; found, 1187.3816.

Compound 34a – A solution of compound **33a** (218 mg, 0.18 mmol) and glacial acetic acid (0.24 mL, 4.25 mmol) in dry THF (1.8 mL), under argon and at 0 °C, was treated with tetrabutylammonium fluoride (1.42 mL, 1.42 mmol, *ca* 1 M in THF). The resultant mixture was stirred at 0 ºC for 30 min and at room temperature for 1 h. Ethyl acetate and water were added, the aqueous layer was separated, and the organic layer was washed with saturated $NaHCO_3$, dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography, eluting with ethyl acetate/hexane: 1) (80:20); 2) (90:10), to give the catechol **34a** (99 mg, 75%) as a yellow foam. $[\alpha]_D^{20}$ = +202.1 (*c*0.9, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.47 (d, *J* = 5.1 Hz, 1H, ArH), 7.94 (s, 1H, ArH), 7.75 (s, 1H, ArH), 7.39 (s, 1H, ArH), 7.36−7.24 (m, 11H, 10×ArH+CH=), 7.20 (dd, *J* = 1.2 and 5.0 Hz, 1H, ArH), 7.07 (br s, 1H, OH), 6.94 (s, 1H, $CHPh_2$), 6.86 (d, *J* = 1.8 Hz, 1H, ArH), 6.82 (d, *J* = 8.1 Hz, 1H, ArH), 6.68 (dd, *J* = 1.7 and 8.1 Hz, 1H, ArH), 5.97 (br s, 1H, OH), 5.70 (s, 1H, H5), 4.83 (s, 1H, H2), 4.72 (d, *J* = 12.4 Hz, 1H, OC*H*H), 4.30 (d, *J* = 12.4 Hz, 1H, OCH*H*), 3.97 (s, 3H, CH_3), 3.57 (d, *J* = 15.0 Hz, 1H, C*H*HAr), 3.51 (d, *J* = 15.0 Hz, 1H, CH*H*Ar) and 1.09 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.8 (C), 168.1 (C), 166.1 (C), 151.2 (C), 150.6 (CH), 144.2 (C), 143.9 (C), 141.1 (C), 138.7 (C), 138.6 (C), 137.4 (CH), 132.6 (CH), 130.9 (C), 128.9 (3×CH), 128.8 (3×CH), 128.5 (CH), 127.7 (2×CH), 126.9 (2×CH), 125.4 (C), 122.7 (CH), 121.5 (CH), 120.5 (CH), 119.7 (C), 116.6 (CH), 115.3 (CH), 79.5 (CH), 73.4 (CH), 66.6 (C), 63.0 (OCH_2), 59.3 (CH), 40.6 (CH_2), 39.2 (CH_3) and 15.7 (CH_3) ppm. FTIR (ATR) ν: 3434 (OH), 1780 (CO) and 1744 (CO) cm^{-1}. MS (ESI) *m/z* = 735 (MH^+). HRMS calcd for $C_{39}H_{35}N_4O_9S$ (MH^+): 735.2119; found, 735.2116.

Compound 34b – It was prepared according to the procedure described for compound **34a** using: **33b** (154 mg, 0.13 mmol) in dry THF (1.3 mL), acetic acid (170 µL, 3.1 mmol) and tetrabutylammonium fluoride (1.0 mL). Reaction conditions: 30 min, 0 ºC. Eluent for chromatography: gradient of diethyl ether/hexane [1) (75:25); 2) (90:10)]. Yield = 78 mg (84%). Yellow foam. $[\alpha]_D^{20}$ = +184.5 (*c*1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.45 (d, *J* = 5.1 Hz, 1H, ArH), 7.51 (s, 1H, ArH), 7.44 (dd, *J* = 1.5 and 5.1 Hz, 1H, ArH),

7.32−7.26 (m, 10H, 10×ArH), 7.18 (s, 1H, CH=), 6.94 (s, 1H, C*H*Ph_2), 6.78 (d, *J* = 1.3 Hz, 1H, ArH), 6.75 (d, *J* = 8.3 Hz, 1H, ArH), 6.64 (dd, *J* = 1.3 and 8.0 Hz, 1H, ArH), 6.39 (br s, 1H, OH), 5.81 (br s, 1H, OH), 5.73 (s, 1H, H5), 4.87 (s, 1H, H2), 4.63 (d, *J* = 12.5 Hz, 1H, OC*H*H), 4.28 (d, *J* = 12.5 Hz, 1H, OCH*H*), 3.55 (d, *J* = 15.3 Hz, 1H, C*H*HCl), 3.49 (d, *J* = 15.3 Hz, 1H, CH*H*Cl) and 1.12 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.8 (C), 167.8 (C), 166.1 (C), 152.0 (C), 151.1 (CH), 143.7 (C), 143.6 (C), 138.7 (C), 138.5 (C), 134.6 (C), 133.8 (C), 129.6 (CH), 129.1 (CH), 129.0 (2×CH), 128.8 (3×CH), 128.5 (CH), 128.3 (CH), 127.7 (2×CH), 126.9 (2×CH), 125.5 (C), 122.0 (CH), 116.7 (CH), 115.4 (CH), 79.6 (CH), 73.6 (CH), 66.4 (C), 63.2 (OCH_2), 59.2 (CH), 40.5 (CH_2), and 16.0 (CH_3) ppm. FTIR (ATR) ν: 3427 (OH), 1782 (CO) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 733 and 735 (MH^+). HRMS calcd for $C_{35}H_{30}N_2{}^{79}BrO_9S$ (MH^+): 733.0850; found, 733.0852.

Compound 34c – It was prepared according to the procedure described for compound **34a** using: **33c** (188 mg, 0.16 mmol) in dry THF (1.6 mL), acetic acid (70 μL, 1.3 mmol) and tetrabutylammonium fluoride (1.4 mL). Reaction conditions: 30 min, 0 ºC. Eluent for chromatography: gradient of diethyl ether/hexane [1) (75:25); 2) (100:0)]. Yield = 75 mg (66%). Yellow foam. $[\alpha]_D^{20}$ = +153.4 (*c*1.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.53 (s, 1H, ArH), 7.38−7.26 (m, 13H, 12×ArH+CH=), 6.94 (s, 1H, C*H*Ph_2), 6.80−6.75 (m, 2H, 2×ArH), 6.66 (d, *J* = 8.0 Hz, 1H, ArH), 6.32 (br s, 1H, OH), 5.70 (s, 2H, H5+OH), 4.88 (s, 1H, H2), 4.63 (d, *J* = 12.5 Hz, 1H, OC*H*H), 4.27 (d, *J* = 12.5 Hz, 1H, OCH*H*), 3.56 (d, *J* = 15.5 Hz, 1H, C*H*HAr), 3.50 (d, *J* = 15.5 Hz, 1H, CH*H*Ar) and 1.13 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.7 (C), 168.3 (C), 166.1 (C), 159.8 (d, J_{C-F} = 247 Hz, C), 147.1 (d, J_{C-F} = 4 Hz, C), 143.8 (C), 143.6 (C), 139.8 (d, J_{C-F} = 25 Hz, CH), 138.8 (C), 138.6 (C), 132.8 (C), 129.1 (CH), 129.0 (2×CH), 128.9 (4×CH), 128.5 (CH), 127.7 (2×CH), 126.8 (2×CH), 125.6 (C), 123.7 (d, J_{C-F} = 19 Hz, CH), 122.1 (CH), 116.7 (CH), 115.4 (CH), 79.5 (CH), 73.5 (CH), 66.5 (C), 63.1 (OCH_2), 59.1 (CH), 40.6 (CH_2Ar) and 16.1 (CH_3) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ: – 121.6 (s, 1F) ppm. FTIR (ATR) ν: 3432 (OH), 1782 (CO) and 1744 (CO) cm^{-1}. MS (ESI) *m/z* = 673 (MH^+). HRMS calcd for $C_{35}H_{30}N_2FO_9S$ (MH^+): 673.1651; found, 673.1654.

Compound 34d – It was prepared according to the procedure described for compound **34a** using: **33d** (236 mg, 0.2 mmol) in dry THF (2 mL), glacial acetic acid (80 μL, 1.4 mmol) and tetrabutylammonium fluoride

(1.6 mL). Eluent for chromatography: (90:10) diethyl ether/hexane. Yield = 124 mg (87%). Yellow foam. $[\alpha]_D^{20}$ = +188.8 (*c*1.3, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ: 8.22 (d, *J* = 5.3 Hz, 1H, ArH), 7.70 (d, *J* = 1.0 Hz, 1H, CHAr), 7.34–7.19 (m, 10H, 10×ArH), 6.90 (s, 1H, $CHPh_2$), 6.78 (m, 2H, 2×ArH), 6.73 (d, *J* = 8.0 Hz, 1H, ArH), 6.61 (dd, *J* = 2.0 and 8.0 Hz, 1H, ArH), 5.70 (br s, 1H, H5), 4.84 (s, 1H, H2), 4.60 (d, *J* = 12.5 Hz, 1H, OC*H*H), 4.22 (d, *J* = 12.5 Hz, 1H, OCH*H*), 3.87 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.52 (d, *J* = 15.2 Hz, 1H, C*H*H), 3.45 (d, *J* = 15.2 Hz, 1H, CH*H*) and 1.08 (s, 3H, CH_3) ppm. ^{13}C NMR (63 MHz, $CDCl_3$) δ: 170.6 (C), 168.7 (C), 166.0 (C), 159.1 (C), 146.4 (C), 146.3 (CH), 144.2 (C), 144.1 (C), 143.6 (C), 143.4 (C), 138.6 (C), 138.4 (C), 128.8 (2×CH), 128.7 (3×CH), 128.3 (CH), 127.5 (2×CH), 126.6 (2×CH), 125.6 (CH), 125.3 (C), 121.8 (CH), 116.6 (CH), 115.1 (CH), 109.0 (CH), 79.2 (CH), 73.5 (CH), 66.2 (C), 62.9 (CH_2), 61.9 (CH), 58.7 (OCH_3), 55.9 (OCH_3), 40.4 (CH_2) and 15.9 (CH_3) ppm. FTIR (ATR) ν: 3447 (OH), 1780 (CO) and 1748 (CO) cm^{-1}. MS (ESI) *m/z* = 715 (MH^+). HRMS calcd for $C_{37}H_{35}N_2O_{11}S$ (MH^+): 715.1956; found: 715.1956.

Compound 34e – It was prepared according to the procedure described for compound **34a** using: **33e** (216 mg, 0.18 mmol), glacial acetic acid (78 μL, 1.4 mmol), tetrabutylammonium fluoride (1.47 mL) in dry THF (1.8 mL). Reaction conditions: 30 min, 0 ºC. Eluent for chromatography: diethyl ether/hexane [1) (75:25); 2) (100:0)]. Yield = 93 mg (70%). White foam. $[\alpha]_D^{20}$ = +262.9 (*c*1.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.20 (s, 1H, ArH), 7.91 (d, *J* = 7.8 Hz, 1H, ArH), 7.81 (d, *J* = 7.9 Hz, 1H, ArH), 7.71−7.61 (m, 3H, 3×ArH), 7.40−7.26 (m, 11H, 10×ArH+CH=), 6.95 (s, 1H, C*H*Ph_2), 6.80 (s, 1H, ArH), 6.75 (d, *J* = 8.0 Hz, 1H, ArH), 6.63 (d, *J* = 7.8 Hz, 1H, ArH), 6.43 (br s, 1H, OH), 5.86 (s, 1H, H5), 5.78 (br s, 1H, OH), 4.90 (s, 1H, H2), 4.65 (d, *J* = 12.5 Hz, 1H, OC*H*H), 4.28 (d, *J* = 12.5 Hz, 1H, OCH*H*), 3.54 (d, *J* = 15.4 Hz, 1H, C*H*HAr), 3.47 (d, *J* = 15.4 Hz, 1H, CH*H*Ar) and 1.16 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.7 (C), 169.0 (C), 166.3 (C), 153.5 (C), 144.8 (C), 143.8 (C), 143.6 (C), 138.9 (C), 138.7 (C), 135.7 (C), 131.5 (C), 131.5 (CH), 131.4 (CH), 129.3 (CH), 129.0 (CH), 128.9 (2×CH), 128.8 (3×CH), 128.4 (CH), 128.1 (CH), 127.7 (2×CH), 127.5 (CH), 126.8 (2×CH), 125.5 (C), 125.0 (CH), 122.0 (CH), 116.7 (CH), 115.4 (CH), 79.5 (CH), 73.9 (CH), 66.4 (C), 63.2 (OCH_2), 58.9 (CH), 40.6 (CH_2Ar) and 16.1 (CH_3) ppm. FTIR (ATR) ν:

3426 (OH), 1777 (CO) and 1740 (CO) cm^{-1}. MS (ESI) *m/z* = 705 (MH^+). HRMS calcd for $C_{39}H_{33}N_2O_9S$ (MH^+): 705.1901; found, 705.1900.

Compound 34f – It was prepared according to the procedure described for compound **34a** using: **33f** (50 mg, 0.04 mmol), glacial acetic acid (17 μL, 0.3 mmol), tetrabutylammonium fluoride (0.32 mL) in dry THF (0.4 mL). Reaction conditions: 30 min, 0 ºC. Eluent for chromatography: diethyl ether. Yield = 25 mg (88%). Yellow foam. $[\alpha]_D^{20}$ = +204.8 (*c*1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.55 (d, *J* = 5.3 Hz, 1H, ArH), 7.68 (d, *J* = 5.6 Hz, 1H, ArH), 7.64 (d, *J* = 5.0 Hz, 1H, ArH), 7.59 (s, 1H, CH=), 7.37−7.26 (m, 11H, 11×ArH), 6.96 (s, 1H, C*H*Ph_2), 6.79 (s, 1H, ArH), 6.73 (d, *J* = 7.7 Hz, 1H, ArH), 6.62 (d, *J* = 8.1 Hz, 1H, ArH), 6.37 (br s, 1H, OH), 5.89 (br s, 1H, OH), 5.84 (s, 1H, H5), 4.89 (s, 1H, H2), 4.67 (d, *J* = 12.5 Hz, 1H, OC*H*H), 4.33 (d, *J* = 12.5 Hz, 1H, OCH*H*), 3.53 (d, *J* = 15.4 Hz, 1H, C*H*HPh), 3.47 (d, *J* = 15.4 Hz, 1H, CH*H*Ph) and 1.17 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.9 (C), 168.1 (C), 166.2 (C), 146.4 (C), 145.3 (C), 144.0 (C), 143.6 (C), 138.7 (C), 138.6 (C), 137.9 (C), 133.9 (C), 132.0 (CH), 128.9 (2×CH), 128.8 (4×CH), 128.5 (CH), 127.7 (2×CH), 127.5 (CH), 126.9 (2×CH), 125.5 (C), 123.7 (CH), 121.9 (CH), 119.4 (CH), 116.7 (CH), 115.4 (CH), 79.5 (CH), 73.7 (CH), 66.5 (C), 63.2 (CH_2), 59.3 (CH), 40.5 (CH_2), and 16.0 (CH_3) ppm. FTIR (ATR) ν: 3432 (OH), 1778 (CO), and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 711 (MH^+). HRMS calcd for $C_{37}H_{31}N_2O_9S_2$ (MH^+): 711.1465; found, 711.1462.

Compound 2 – A solution of the ester **34a** (99 mg, 0.14 mmol) in *m*-cresol (0.9 mL) under inert atmosphere was heated at 50 °C for 12 h. After cooling to room temperature, the reaction mixture was diluted with diethyl ether (2 mL) and extrated with aqueous NH_4HCO_3 (7 mL, 0.16 mmol, 23 mM). The aqueous layer was washed with diethyl ether (×2), acidified with HCl (0.1 M) until pH 4, and extracted with ethyl acetate. The organic layer was washed with Milli-Q water, dried (anh. Na_2SO_4) and concentrated under reduced. The acid **3** (24 mg, 30%) was obtained as an orange oil. $[\alpha]_D^{20}$ = +257.5 (*c*0.9, acetone). ^{1}H NMR (300 MHz, acetone-d*6*) δ: 8.57 (d, *J* = 5.0 Hz, 1H, ArH), 8.24 (s, 1H, ArH), 8.00 (d, *J* = 0.6 Hz, 1H, ArH), 7.85 (d, *J* = 1.0 Hz, 1H, ArH), 7.58 (dd, *J* = 1.7 and 5.0 Hz, 1H, ArH), 7.41 (d, *J* = 1.3 Hz, 1H, CH=), 6.82 (d, *J* = 2.0 Hz, 1H, ArH), 6.75 (d, *J* = 8.0 Hz, 1H, ArH), 6.65 (dd, *J* = 2.0 and 8.0 Hz, 1H, ArH), 6.96 (d, *J* = 1.3 Hz, 1H, H5), 4.71 (d, *J* = 12.2 Hz, 1H, OC*H*H), 4.70 (s, 1H, H2), 4.52 (d, *J* = 12.2 Hz, 1H, OCH*H*), 3.96 (s, 3H,

CH_3), 3.54 (s, 2H, CH_2Ph) and 1.58 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, acetone-d*6*) δ: 171.5 (C), 168.6 (C), 168.5 (C), 152.8 (C), 151.6 (CH), 145.8 (C), 145.0 (C), 142.8 (C), 137.8 (CH), 134.6 (C), 130.6 (CH), 129.9 (CH), 126.4 (C), 123.3 (CH), 121.7 (CH), 121.2 (CH), 120.4 (C), 117.4 (CH), 116.0 (CH), 74.1 (CH), 66.7 (C), 64.3 (OCH_2), 60.5 (CH), 40.6 (CH_2), 39.4 (CH_3) and 16.2 (CH_3) ppm. FTIR (ATR) ν: 3410 (OH), 1782 (CO) and 1740 (CO) cm^{-1}. MS (ESI) *m/z* = 569 (MH^+). HRMS calcd for $C_{26}H_{25}N_4O_9S$ (MH^+): 569.1337; found, 569.1335.

Compound 3 − It was prepared according to the procedure described for compound **2** using: **34b** (46 mg, 0.06 mmol) and *m*-cresol (0.4 mL). Extraction with NH_4HCO_3 (3.33 mL, 24 mM). Reaction time = 12 h. Yield = 16 mg (44%). Green foam. $[\alpha]_D^{20}$ = +229.1 (*c*1.0, acetone). ^1H NMR (300 MHz, acetone-d*6*) δ: 8.55 (d, *J* = 5.2 Hz, 1H, ArH), 7.94 (d, *J* = 1.7 Hz, 1H, ArH), 7.71 (dd, *J* = 1.8 and 5.2 Hz, 1H, ArH), 7.47 (d, *J* = 1.0 Hz, 1H, CH=), 6.81 (d, *J* = 1.8 Hz, 1H, ArH), 6.75 (d, *J* = 8.1 Hz, 1H, ArH), 6.65 (dd, *J* = 1.9 and 8.1 Hz, 1H, ArH), 5.95 (d, *J* = 1.1 Hz, 1H, H5), 4.74 (s, 1H, H2), 4.69 (d, *J* = 12.2 Hz, 1H, OC*H*H), 4.51 (d, *J* = 12.2 Hz, 1H, OCH*H*), 3.54 (s, 2H, CH_2Cl) and 1.58 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, acetone-d*6*) δ: 171.5 (C), 168.4 (C), 168.1 (C), 153.8 (C), 152.1 (CH), 145.8 (C), 145.1 (C), 136.5 (C), 134.2 (C), 130.3 (CH), 129.0 (CH), 128.8 (CH), 126.5 (C), 121.8 (CH), 117.4 (CH), 116.1 (CH), 74.1 (CH), 66.8 (C), 64.4 (OCH_2), 60.7 (CH), 40.6 (CH_2), and 16.3 (CH_3) ppm. FTIR (ATR) ν: 3434 (OH), 1779 (CO) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 567 and 569 (MH^+). HRMS calcd for $C_{22}H_{20}N_2{}^{79}BrO_9S$ (MH^+): 567.0067; found, 567.0066.

Compound 4 – It was prepared according to the procedure described for compound **2** using: **34c** (75 mg, 0.11 mmol) and *m*-cresol (0.7 mL). Extraction with NH_4HCO_3 (5.42 mL, 24 mM). Reaction time = 13 h. Yield = 37 mg (66%). Green foam. $[\alpha]_D^{20}$ = +269.8 (*c*1.2, acetone). ^1H NMR (300 MHz, acetone-d*6*) δ: 8.57 (d, *J* = 2.3 Hz, 1H, ArH), 7.81–7.72 (m, 2H, 2×ArH), 7.50 (d, *J* = 1.0 Hz, 1H, CH=), 6.81 (d, *J* = 1.9 Hz, 1H, ArH), 6.75 (d, *J* = 8.1 Hz, 1H, ArH), 6.65 (dd, *J* = 1.9 and 8.0 Hz, 1H, ArH), 5.92 (d, *J* = 1.0 Hz, 1H, H5), 4.70 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.71 (s, 1H, H2), 4.52 (d, *J* = 12.2 Hz, 1H, OCH*H*), 3.54 (s, 2H, CH_2Ph) and 1.58 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, acetone-d*6*) δ: 171.3 (C), 168.2 (2×C), 160.5 (d, J_{C-F} = 258 Hz, C), 148.5 (d, J_{C-F} = 4 Hz, C), 145.5 (C), 144.8 (C), 139.5 (d, J_{C-F} = 25 Hz, CH), 134.2 (d,

$J_{C–F}$ = 2 Hz, C), 128.8 (2×CH), 126.1 (C), 124.5 (d, $J_{C–F}$ = 19 Hz, CH), 121.5 (CH), 117.1 (CH), 115.8 (CH), 73.7 (CH), 66.5 (C), 64.0 (CH_2), 60.3 (CH), 40.3 (CH_2), and 16.0 (CH_3) ppm. ^{19}F NMR (282 MHz, acetone-d*6*) δ: – 119.7 (t, *J* = 5 Hz, 1F) ppm. FTIR (ATR) ν: 3427 (OH), 1772 (CO), 1740 (CO) and 1699 (CO) cm^{-1}. MS (ESI) *m/z* = 507 (MH^+). HRMS calcd for $C_{22}H_{20}FN_2O_9S$ (MH^+): 507.0868; found, 507.0871.

Compound 5 – It was prepared according to the procedure described for compound **2** using: **34d** (48 mg, 0.067 mmol) and *m*-cresol (0.42 mL). Extraction with $NaHCO_3$ (2 mL, 40 mM). Reaction time = 5 h. Yield = 15 mg (41%). Green foam. $[\alpha]_D^{20}$ = +257.6 (*c*1.0, acetone). ^{1}H NMR (300 MHz, acetone-d*6*) δ: 8.28 (d, *J* = 5.4 Hz, 1H, ArH), 7.62 (d, *J* = 0.9 Hz, 1H, CHAr), 7.15 (d, *J* = 5.4 Hz, 1H, ArH), 6.81 (d, *J* = 1.8 Hz, 1H, ArH), 6.75 (d, *J* = 8.1 Hz, 1H, ArH), 6.65 (dd, *J* = 1.8 and 8.1 Hz, 1H, ArH), 5.88 (d, *J* = 0.9 Hz, 1H, H5), 4.69 (d, *J* = 13.5 Hz, 1H, OC*H*H), 4.67 (s, 1H, H2), 4.50 (d, *J* = 12.3 Hz, 1H, OCH*H*), 4.00 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.53 (s, 2H, CH_2) and 1.57 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, acetone-d*6*) δ: 172.6 (C), 169.9 (C), 169.8 (C), 161.1 (C), 148.4 (CH), 147.9 (C), 146.9 (C), 146.4 (C), 146.1 (C), 135.6 (C), 127.1 (C), 125.8 (CH), 122.5 (CH), 118.3 (CH), 116.9 (CH), 111.3 (2×CH), 75.1 (CH), 73.5 (CH), 67.6 (C), 65.2 (CH_2), 63.0 (CH), 61.5 (OCH_3), 57.5 (OCH_3), 41.5 (CH_2) and 17.2 (CH_3) ppm. FTIR (ATR) ν: 3441 (OH), 1772 (CO) and 1737 (CO) cm^{-1}. MS (ESI) *m/z* = 547 (M–H). HRMS calcd for $C_{24}H_{22}N_2O_{11}S$ (M–H): 547.1028; found: 547.1027.

Compound 6 – It was prepared according to the procedure described for compound **2** using: **34e** (113 mg, 0.16 mmol) and *m*-cresol (1 mL). Extraction with NH_4HCO_3 (7.9 mL, 24 mM). Reaction time = 7 h. Yield = 40 mg (46%). Brown solid. $[\alpha]_D^{20}$ = +166.1 (*c*1.1, acetone). ^{1}H NMR (300 MHz, acetone-d*6*) δ: 8.61 (d, *J* = 5.3 Hz, 1H, ArH), 8.12 (d, *J* = 5.4 Hz, 1H, ArH), 7.94 (d, *J* = 5.3 Hz, 1H, ArH), 7.71 (d, *J* = 1.3 Hz, 1H, CH=), 7.64 (d, *J* = 5.4 Hz, 1H, ArH), 6.82 (d, *J* = 1.9 Hz, 1H, ArH), 6.75 (d, *J* = 8.0 Hz, 1H, ArH), 6.66 (dd, *J* = 1.9 and 8.2 Hz, 1H, ArH), 6.04 (d, *J* = 1.3 Hz, 1H, H5), 4.76 (s, 1H, H2), 4.72 (d, *J* = 12.2 Hz, 1H, OC*H*H), 4.53 (d, *J* = 12.2 Hz, 1H, OCH*H*), 3.55 (s, 2H, CH_2Ph) and 1.61 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, acetone-d*6*) δ: 171.5 (C), 168.4 (C), 168.1 (C), 147.7 (C), 146.9 (C), 145.7 (C), 145.0 (C), 144.7 (CH), 138.4 (C), 136.5 (C), 133.7 (CH), 126.8 (CH), 126.4 (C), 124.8 (CH), 121.8 (CH), 121.6 (C), 120.1 (CH), 117.4 (CH), 116.0 (CH), 74.3 (CH_2), 66.7 (CH), 64.2 (CH), 60.6 (CH), 40.6 (CH_2), and 16.3 (CH_3)

ppm. FTIR (ATR) ν: 3438 (OH) and 1739 (CO) cm^{-1}. MS (ESI) *m/z* = 545 (MH^+). HRMS calcd for $C_{24}H_{21}N_2O_9S_2$ (MH^+): 545.0683; found, 545.0680.

Compound 7 − It was prepared according to the procedure described for compound **2** using: **34f** (93 mg, 0.13 mmol) and *m*-cresol (0.8 mL). Extraction with NH_4HCO_3 (6.67 mL, 24 mM). Reaction time = 13 h. Yield = 19 mg (27%). Yellow oil. $[\alpha]_D^{20}$ = +328.8 (*c*1.0, acetone). ^{1}H NMR (300 MHz, acetone-d*6*) δ: 9.33 (s, 1H, ArH), 8.21 (d, *J* = 8.0 Hz, 1H, ArH), 8.11 (s, 1H, ArH), 8.04 (d, *J* = 8.0 Hz, 1H, ArH), 7.88−7.79 (m, 2H, 2×ArH), 7.60 (s, 1H, CH=), 6.81 (d, *J* = 1.5 Hz, 1H, ArH), 6.75 (d, *J* = 8.0 Hz, 1H, ArH), 6.66 (dd, *J* = 1.7 and 8.0 Hz, 1H, ArH), 6.04 (s, 1H, H5), 4.72 (s, 1H, H2), 4.71 (d, *J* = 12.1 Hz, 1H, OC*H*H), 4.52 (d, *J* = 12.1 Hz, 1H, OCH*H*), 3.54 (s, 2H, CH_2Ph) and 1.61 (s, 3H, CH_3) ppm. ^{13}C NMR (75 MHz, acetone-d*6*) δ: 171.5 (C), 168.8 (C), 168.6 (C), 154.0 (CH), 146.2 (C), 145.7 (C), 145.0 (C), 136.7 (C), 133.5 (C), 132.2 (CH), 131.0 (CH), 130.0 (CH), 129.7 (C), 128.9 (CH), 128.3 (CH), 126.3 (C), 125.3 (CH), 121.7 (CH), 117.3 (CH), 115.9 (CH), 74.3 (CH), 66.5 (C), 64.3 (CH_2), 60.4 (CH), 40.5 (CH_2), and 16.2 (CH_3) ppm. FTIR (ATR) ν: 3448 (OH) and 1742 (CO) cm^{-1}. MS (ESI) *m/z* = 539 (MH^+). HRMS calcd for $C_{26}H_{23}N_2O_9S$ (MH^+): 539.1119; found, 539.1116.

Bacterial strains and media – Clinically relevant β-lactamases of classes A, C and D that are well inhibited by the parent compound **1** were included in this study, all of them coded in previously studies, with the exception of *E. coli* TG1 bacterial strains harboring TEM-1 and CTX-M-2 that are described below (Table 1). All strains were grown in Luria-Bertani (LB) broth (10 g mL^{-1} tryptone, 5 g mL^{-1} yeast extract, 10 g mL^{-1} sodium chloride) or in LB agar at 37 °C. When necessary, LB medium was supplemented with ampicillin (50 mg L^{-1}) or kanamycin (50 mg L^{-1}). Bacterial strains were frozen in LB broth with 15% glycerol and were maintained at −80°C until analysis.

Cloning of the TEM-1 and CTX-M-2 genes in *E. coli* TG1 – To perform susceptibility assays bla_{TEM-1} and $bla_{CTX-M-2}$ genes from *E. coli* clinical strains isolated in A Coruña Hospital were cloned into the pBGS18 plasmid, with the previously reported β-lactamase CXT-M-14 gene promoter[46] using the *Bam*HI and *Eco*RI

restriction sites. Recombinant plasmids were then transformed by electroporation in *E. coli* TG1. Transformants were selected with LB agar plates supplemented with ampicillin at 40 mg L^{-1}.

Susceptibility studies – MIC assays were performed by microdilution in Mueller-Hinton II broth (Becton, Dickinson and Company, Sparks, MD) to ampicillin, ceftazidime or imipenem as reporter substrates of β-lactamases of classes A, C and D, respectively, in the presence and in the absence of the reported inhibitors **2**−**7**, according to the standard method recommended by CLSI.[43] MIC values in the presence of **1** and two non-penicillin-based sulfone inhibitors, avibactam and relebactam were also measured to compare the efficacy of **2**−**7**. These studies were carried out as previously described for **1**.[28]

Kinetic and inhibition studies – The class D carbapenemase OXA-24/40 was cloned in p-GEX-6P-1 plasmid and transformed in *E. coli* BL21, then the β-lactamase was purified to homogeneity using the GST Gene Fusion System (Amersham Pharmacia Biotech, Munich, Germany) as previously described (Figure S5).[28] To evaluate the inactivation of OXA-24/40 enzyme from *A. baummanni* by ligands **3**−**8** the parameters k_{inact} and K_I were measured in the presence of nitrocefin as previously published.[28] All the kinetic experiments were performed in triplicate at 25°C in 50 mM sodium phosphate with 20 mM of sodium bicarbonate pH 7.4, using 96 well plates and the purified protein, under steady-state conditions on an Epoch 2 Microplate Spectrophotometer (Biotek, VT, USA). The inhibitor complex inactivation rate (k_{inact}) in the presence of nitrocefin (NCF) (Oxoid, Hampshire, UK) at λ = 482 nm (ε/M^{-1} cm^{-1} 15 900) was measured and K_I determined as previously described.[49,50,51] k_{inact} values indicate the number of molecules of enzyme that are inactivated per second and the K_I value corresponds to the concentration of inhibitor required to reach 50% k_{inact}. The experiments were performed using nitrocefin as a reporter substrate at 100 μM, and increasing concentrations of inhibitors over a 15 minute time course. The k_{obs} values were determined using non-linear least squares fit of the data, employing Graphpad software (La Jolla, Ca, USA) and Equation 1.

$$A = A_0 + v_f \times x + (v_0 - v_f) \times [1 - \exp(-k_{obs} \times x)] / k_{obs} \quad \text{(Eq. 1)}$$

Here, A is absorbance, v_0 is initial velocity, v_f is the final velocity, and x is time. Then, the k_{obs} values were plotted against inhibitor concentrations to determine k_{inact} and K_I using Equation 2. The K_I value was corrected using Equation 3.

$k_{obs} = k_{inact}$ x [I] / (K_I + [I]) (Eq. 2)

K_I(corrected) = K_I(observed) / [1 + ([S] / $K_{m\ NCF}$)] (Eq. 3)

Ultraviolet-Visible Spectroscopy Studies – The formation of the indolizine derivatives of the reported ligands was analyzed by monitoring the methanolysis of the ligands by UV-Vis spectroscopy after treatment with 1 equivalent of NaOMe (1 M in MeOH) at 25 ºC for 15 min. The wavelength range was 600 nm to 200 nm with a spectral band width of 2.0 nm. The spectra were collected every 0.5 min, 1.0 min, 1.5 min and 2.0 min. The reaction mixture was further analyzed by HPLC on a Thermo Dionex UltiMate 3000 apparatus having a Brucker amazon SL mass spectrometry detector, using the conditions indicated in the general section.

Mass Spectrometry Studies – A 100 µL solution of OXA-24/40 from *A. baumannii* (15 µL from a stock protein concentration of 0.9 mg mL^{-1}) and OXA-48 from *K. pneumoniae* (5 µL from a stock protein concentration of 3.9 mg mL^{-1}) in 50 mM TRIS.HCl, 150 mM NaCl and 1 mM EDTA pH 7.0 at 25 °C, was incubated with compounds **2**–**7** for 30 min. A 1/100 enzyme/inhibitor molar ratio was employed. The activity was progressively determined by UV-Vis spectroscopy using aliquots from the incubation samples and the control. The enzyme was assayed by monitoring the increase in absorbance at 482 nm in the UV-Vis spectrum due to the absorbance of the hydrolyzed nitrocefin (ε/M^{-1} cm^{-1} 15 900) in 50 mM sodium phosphate with 20 mM sodium bicarbonate pH 7.4 at 25 ºC. Each assay was initiated by addition of nitrocefin. After 30 min (no activity was observed unless the control), the samples were concentrated and successively washed with 5 mM ammonium bicarbonate for MALDI analysis by centrifugation at 4 °C using Amicon® centrifugal filters (Amicon Ultra-10). The samples were free-dried, diluted with 5 mM ammonium bicarbonate (5 µL) and analyzed by mass spectrometry using a MALDI TOF/TOF Mass Spectromether (4800 Analyzer, AbSciex). The ProteoMass™ MALDI calibration kit (Merck) was employed

for calibration and sanipic acid as a matrix. Spectra were analyzed using the Data Explorer™ Software. The experiments were performed in triplicate.

Docking studies – These studies were carried out using program GOLD 5.2.2.[52] For the no-covalent binding studies, the protein coordinates found in the crystal structure of OXA-24/40 from *A. baumannii* covalently modified by SA4-44 inhibitor (a derivative of compound **1** in which the position 4 of the pyridine moiety is substituted by -$NHCONH_2$ group) (PDB ID 3FV7,[26] 2.0 Å) were employed. For the covalent docking, the crystal structure of the protein coordinates covalently modified by durlobactam (ETX2514) (PDB ID 6MPQ,[30] 1.95 Å) was used. The geometries of ligands **2**−**7** and the corresponding indolizine adducts geometries were minimized using the AM1 Hamiltonian as implemented in the program Gaussian 09[53] and used as MOL2 files. Each ligand was docked in 25 independent genetic algorithm (GA) runs, and for each of these a maximum number of 100000 GA operations were performed on a single population of 50 individuals. Operator weights for crossover, mutation and migration in the entry box were used as default parameters (95, 95, and 10, respectively), as well as the hydrogen bonding (4.0 Å) and van der Waals (2.5 Å) parameters. For the no-covalent docking, the position of the inhibitor present in PDB ID 3FV7[27] was used to define the docking region. For the covalent docking, the atom OG of the catalytic serine (Ser81) was used for the covalent linking and the position of durlobactam was employed to establish the docking region. For both cases, the radius of the selected spheric region was set to 10 Å. All crystallographic water molecules and the aforementioned ligands were removed for docking. The "flip ring corners" flag was switched on, while all the other flags were off. The GOLD scoring function was used to rank the ligands in order to fitness. The molecular graphics program PyMOL was employed for visualization and depicting ligand/protein structures.[54]

ASSOCIATED CONTENT

Supporting Information

Additional figures illustrating UV-Vis spectroscopy and mass spectrometry studies (Fig. S1-S2), docking studies (Fig. S3-S4), OXA-24/40 enzyme purity (Fig. S5), molecular formula strings, HPLC traces for lead compounds and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interests.

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ABBREVIATIONS AND ACRONYMS USED

OXA, oxacillinase; CHDL, carbapenem-hydrolyzing class D β-lactamase enzyme; ESBL, Extended-spectrum β-lactamase; CTX-M, cefotaxime-hydrolyzing β-lactamase enzyme; TEM, Temoneira β-lactamase enzyme; CMY, Cephamycin-hydrolyzing β-lactamase enzyme; DHA, Dhahran hospital β-lactamase enzyme; 1,6-diazabicyclo[3,2,1]octane, DBO; *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide, EDC.

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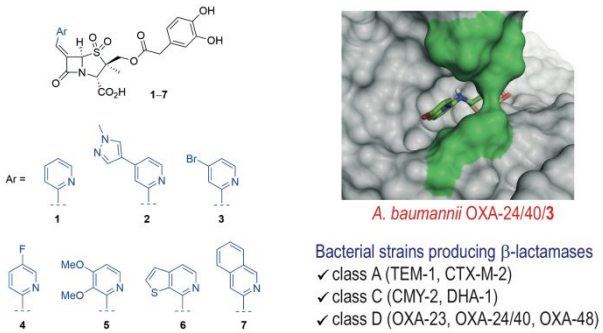
TOC-GRAPHIC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A. baumannii OXA-24/40/3
Bacterial strains producing β-lactamases
✓class A (TEM-1, CTX-M-2)
✓class C (CMY-2, DHA-1)
✓class D (OXA-23, OXA-24/40, OXA-48)